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"The design and synthesis of novel *N*-heterocyclic compounds, and their evaluation of anti-cancer and anti-viral activity"

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CERTIFICATE

This is to certifiy that the work incorporated in the thesis entitled **"THE DESIGN AND SYNTHESIS OF NOVEL *N*-HETEROCYCLIC COMPOUNDS, AND THEIR EVALUATION OF ANTI-CANCER AND ANTI-VIRAL ACTIVITY".** which is being submitted to the University of Salerno for the award of **Doctor of Philosophy in Organic and Medicinal Chemistry** by **Mr. Vijaykumar S. More** was carried out by him under my supervision at the **Depatment of Pharmaceutical Science, University of Salerno, Italy**. Such material as has been obtained from other sources has been duly acknowledged in the thesis.

Prof. Paolo de Caprariis
(Research Supervisor)

DECLARATION

I hereby declare that the thesis entitled **"THE DESIGN AND SYNTHESIS OF NOVEL *N*-HETEROCYCLIC COMPOUNDS, AND THEIR EVALUATION OF ANTI-CANCER AND ANTI-VIRAL ACTIVITY"** submitted for the award of degree of **Doctor of Philosophy** in Organic and Medicinal Chemistry to the University of Salerno, Italy (SA) has not been submitted by me to any other university or institution. This work was carried out by me at the University of Salerno, Italy (SA).

(**Vijaykumar S. More**) **February, 2012**

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Vijaykumar S. More

ABSTRACT

The thesis entitled “The design and synthesis of novel *N*-heterocyclic compounds, and their evaluation of anti-cancer and anti-viral activity" is divided into three chapters.

The title of the thesis clearly reflects the importance of nitrogen heterocycles compounds: in fact they are extremely pivotal structural motifs responsible for eliciting various biological activities in natural products and synthetic medicines. This has attracted the medicinal chemists towards the synthesis of various compounds having nitrogen heterocycles as useful medicines to treat various diseases. It is also evident by a large number of marketed pharmaceutical products possess nitrogen heterocycles. In each chapter different heterocycle moieties belonging to β-carboline, indoles and isoindolinones nucleus respectively are presented.

In the first chapter we presented β-carboline derivatives and their anticancer activity on different cell lines. Our *lead compound* was harmine, which is the most representative β-carboline alkaloid endowed with antitumor properties showing high cytotoxicity both *in vitro* against different human tumor cell lines and *in vivo*. It also exhibited remarkable DNA intercalation capacity and significant Topo I inhibition activity. We designed and synthesized novel β-carbolines derivatives with the aim to evaluate their antiproliferative properties, to acquire more information about the structural requirements for the possible improvement of the cytotoxic potential and to elucidate SARs between substituent properties and antitumor activities. Most of the compounds were evaluated for Topo I inhibitory activity and compaired to harmine. Almost all compounds demonstrated interesting cytotoxic activities in particular against prostate cancer cells PC-3 with IC_{50} in low micromolar range. Compound X was found to be the most potent one with IC_{50} value of 8 μM.

In the second chapter we reported the design and synthesis of new arbidol derivatives as antiviral agents: arbidol is an indole compound launched in the Russian Federation for the prophylaxis and treatment of influenza A and B and other acute respiratory viral infections, but due to its relatively high CC_{50} value, a clinical application is forbidden. So in order to reduce its toxicity and improve its

antiviral properties, we carried out some structural modifications at position 2, 4, 5 and 6 on indole nucleus and we evaluated their effect *in vitro,* on anti- influenza virus (HA), hepatitis C virus (HCV) and chikungunya virus (CHIKV) activities. Viral infections are in fact the most common illnesses experienced by people of all ages and they are also one of the major causes of morbidity and mortality in elderly people and young children throughout the world. Currently, treatments are limited and the increasing prevalence of drug-resistant pathogens highlighted the need for new anti-viral drugs with novel mechanisms of action. Biological evaluation led us to discovery of a new potent influenza virus replication inhibitor, identified in compound **15**. Particularly it showed activity against all the tested viruses, both A and B type; moreover it seemed to lead to a better inhibition of some viruses in comparison to Arbidol. This compound was also found to be a promising *lead compound* for the design of new HCV virus replication inhibitors. Actually, biological studies are in course to better study mechanism underwent the action of compound **15**, which could act non only as virus replication inhibitor but also as fusion inhibitor. Then a focused analysis on the interaction of this compound with the HA protein will be carried out.

The third chapter is divided into two sections.

Section A provided a brief introduction about aldol addition to 1,3-dicarbonyl compounds and described a simple and effective multicomponent regioselective one pot aldol addition/protection reaction of β-ketoesters to a series of aldehydes in the presence of Me_3SiCl and *i*-Pr_2EtN. The analysis of the scope and application of reaction revealed a dramatic dependence of the reactivity on the used substrates.

Section B described a simple and general access to a series of new phthalimidines derivatives, (mentioned in Section A) in the presence of tertiary amines under very mild conditions exploiting the aldol addition of readily enolizable 1,3 dicarbonyl compounds to 2-cyanobenzaldehyde. Recently, it has been recognized that 3-substituted isoindolinones possess a variety of biological activities, consequently, considerable effort has been devoted to the synthesis of this nitrogen heterocycle, which also act as useful synthetic building blocks and intermediates in organic synthesis.

The obtained 3-substituted isoindolinones were preliminary tested on two different virus strains (HCV and CHIKV virus) to evaluate potential activity on

the virus replication, but unfortunately it was found that all compounds were not active. However further studies are desirable focusing on activities like hypnothic, anti-schizophrenia etc, for which isoindolinone moiety also shows important applications.

CHAPTER-I

Synthesis and Cytotoxic Activity of New β-Carboline Derivatives

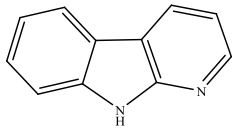
1.1 INTRODUCTION

In medicine, biotechnology and pharmacology drug discovery is the process by which drugs are discovered and/or designed. In the past most drugs have been discovered either by identifying the active ingredient from traditional remedies or by serendipitous discovery. The discovery, development and exploitation of antibiotics were one of the most significant advances in medicine in the 20^{th} century. Early drug discovery involves several phases from target identification to preclinical development. The majority of pharmaceutical drug discovery programs currently, begins with a known macromolecular target, and seeks to indentify a suitable small molecule modulator. The process of optimizing the lead molecule into a candidate drug is usually the longest and most expensive stage in the drug discovery. The identification of small molecule modulators of protein function and the process of transforming these into high-content lead series are key activities in modern drug discovery.[1]

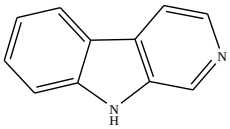
The first step of drug discovery involves the identification of new active compounds, often called "hits", which are typically found by screening many compounds for the desired biological properties. These hits can come from natural sources, such as plants, animals or fungi. More often, hits can come from synthetic sources, such as historical compound collections and combinatorial chemistry. Another step in drug discovery involves further chemical modifications in order to improve the biological and physiochemical properties of a given candidates compounds library. Despite advances in technology and understanding of biological system, drug discovery is still a long process with low rate of new therapeutic discovery. The idea that effects of drug in human body are mediated by specific interactions of the drug molecule with biological macromolecules, (proteins or nucleic acid in most cases) led scientists to the conclusion that individual chemicals are required for the biological activity of the drug. This made for the beginning of the modern era in pharmacology, as pure chemicals, instead of crude extracts, became the standard drug. Organic chemistry also leads to the synthesis of many chemicals isolated from biological sources. Despite the rise of combinatorial chemistry as an integral part of lead discovery process, the natural products still play a major role as starting material for drug discovery.

A report was published in 2007, the 974 small molecules, new chemical entities, 63% were natural derived or semisynthetic derivatives of natural products. For certain therapy areas, such as antimicrobials, antineoplastics, antihypertensive and anti-inflammatory drugs, the numbers were higher.

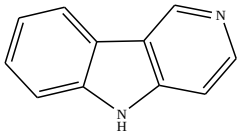
In literature synthetic approaches to a number of carboline derivatives having antitumor activity are described. Several carboline derivatives show activity at nanomolar concentrations and so are of keen interest for possible development into clinically useful antitumor agents. Carbolines are pyridoindoles and are classified as *α, β, γ* or *δ* according to the mode of ring fusion, as illustrated below (Fig. 1).



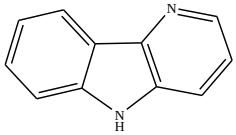
α-Carboline



β-Carboline



γ-Carboline



δ-Carboline

Figure 1

1.2 *β*-CARBOLINE

The *β*-carboline alkaloids are a large group of natural and synthetic indole alkaloids that possess a common tricyclic pyrido[3,4-*b*]indole ring structure,[2,3] have attracted attention regarding several aspects of medicinal chemistry as illustrated below (Fig. 2). Numerous reports also disclosed that other simple and complicated β-carboline alkaloids were extensively presented in extracts from the leaves, barks and roots of a variety of plants.

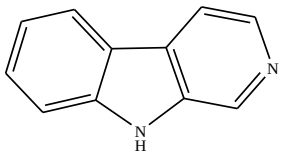


Figure 2: *β*-carboline skeleton

The *β*-carboline alkaloids were originally isolated from *Peganum harmala* (*Zygophillaceae*, *Syrian Rue*), which is being used as a traditional herbal drug as an emmenagogue and abortifacient in the Middle East and North Africa.[4] In the Amazon basin plants containing *β*-carbolines were widely used as hallucinogenic drinks or snuffs. Besides, the extracts of the seeds of *Peganum harmala* have been traditionally used for hundreds of years to treat the alimentary tract cancers and malaria in Northwest China.[5]

Figure 3. Peganum harmala

These molecules can be categorized according to the saturation of their *N*-containing, six membered ring. Unsaturated members are named as fully aromatic *β*-carboline (βCs), whereas the partially or completely saturated ones are known as dihydro *β*-carboline (DHβCs) and tetrahydro-*β*-carbolines (THβCs), respectively. The pyridinic nitrogen is more basic then the pyrrolic one, while its basicity increases upon excitation [6, 7] and is affected by the substitutions presence in the structure.[8] Depending upon pH and solvent, *β*-carboline can exist in four forms the cation, the neutral form, a zwitterions (or an alternative quinine type canonical forms), and an anion.[9]

These compounds possess a wide diversity of important biochemical effects and pharmacological properties. Numerous previous reports investigated the effects of *β*-carboline alkaloids on the central nervous system (CNS), such as their affinity with benzodiazepine receptors (BZRs), 5-HT_{2A} and 5-HT_{2C} receptors.[10-12] Recent interest in these alkaloids has been focused on their potent antitumor

activity. Several investigations[13-25] on the synthesis of a variety of *β*-carboline derivatives and the evaluation of their antitumor activities unraveled that *β*-carbolines demonstrated potent antitumor activities and the activity was correlated to both the planarity of the molecule and the presence of the ring substituents. Recently it is discovered that *β*-carboline derivatives may function their antitumor activity through multiple mechanisms, such as intercalating into DNA[26], inhibiting Topoisomerase I and II[27], CDK[28-29], and IKK (IkB kinase complex).[30]

Harmine (Figure 4) is the most representative β-carboline alkaloid endowed with antitumor properties. It showed high cytotoxicity both *in vitro* against different human tumor cell lines and *in vivo* in mice bearing both Lewis lung cancer and Sarcoma 180. It exhibited remarkable DNA intercalation capacity and significant Topo I inhibition activity.[19,22] Tetracyclic systems in which another ring has been fused to the *β*-carboline nucleus have also shown promising antitumor activity.

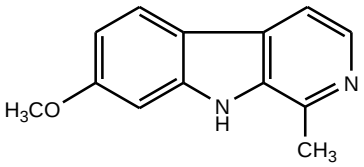


Figure 4. Chemical structure of Harmine

In particular, 1-methoxycanthin-6-one (Fig. 5), a natural product isolated from the medicinal plant *Ailanthus altissima*, showed interesting antiproliferative properties, suppressing the growth of a panel of human tumor cell lines, including epiderimoid carcinoma of the nasopharynx (KB), ileocecal carcinoma (HCT-8), renal cancer (CAK-1), breast cancer (MCF-7) and melanoma (SK-MEL-2).[31]

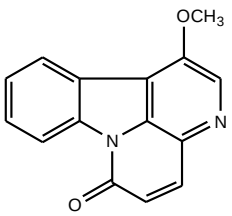


Fig. 5. 1-methoxycanthin-6-one

Ammirante *et al.* demonstrated that 1-methoxycanthin-6-one induced apoptosis *via* a JNK-dependent mechanism. c-Jun NH_2-terminal kinase (JNK), a member of the mitogen activated protein kinase (MAPK) family, is a key regulator of apoptosis. Modulation of its activity can either promote or inhibit apoptotic processes, depending on cell system and contexts. Indeed, the kinase acts on a variety of targets, including, in addition to c-Jun, other transcription factors (ATF2, Elk-1, p53, and c-Myc) and proapoptotic and anti-apoptotic members of the Bcl-2 family (Bcl-2, Bcl-xL, Bim, and BAD), thereby influencing levels and activities of molecules that variously participate in cell death mechanisms. Furthermore, the compound synergized with human recombinant tumor necrosis factor (TNF)–related apoptosis-inducing ligand (hrTRAIL) in apoptosis induction. TRAIL, a member of the TNF gene superfamily, interacts with a complex system of receptors consisting of two proapoptotic death receptors (TRAIL-R1 and TRAIL-R2) and three decoy receptors (TRAIL-R3, TRAIL-R4, and osteoprotegerin). As a stable soluble trimer, the cytokine selectively induces apoptosis in many transformed cells but not in normal cells; differential expression levels of decoy versus proapoptotic receptors and other mechanisms seem to account for normal cell resistance to TRAIL induced cell death. TRAIL action involves the formation of a death inducing signaling complex (DISC) and activation of caspase-8; the apoptotic processes then follow two pathways: the mitochondrial independent activation of caspase-3 and mitochondrial-dependent apoptosis due to cleavage of BID by caspase-8. The JNK-activating and proapoptotic properties of 1-methoxycanthin-6-one render this molecule a candidate for *in vivo* studies of its activity in monotherapies or combined antineoplastic therapies.[32]

1.3 PRESENT WORK

In the present investigation, we designed and synthesized novel 1, 4-disubstituted, 1,4,9-trisubstituted *β*-carbolines and 1-methoxycanthin-6-one derivatives (Figure 6).

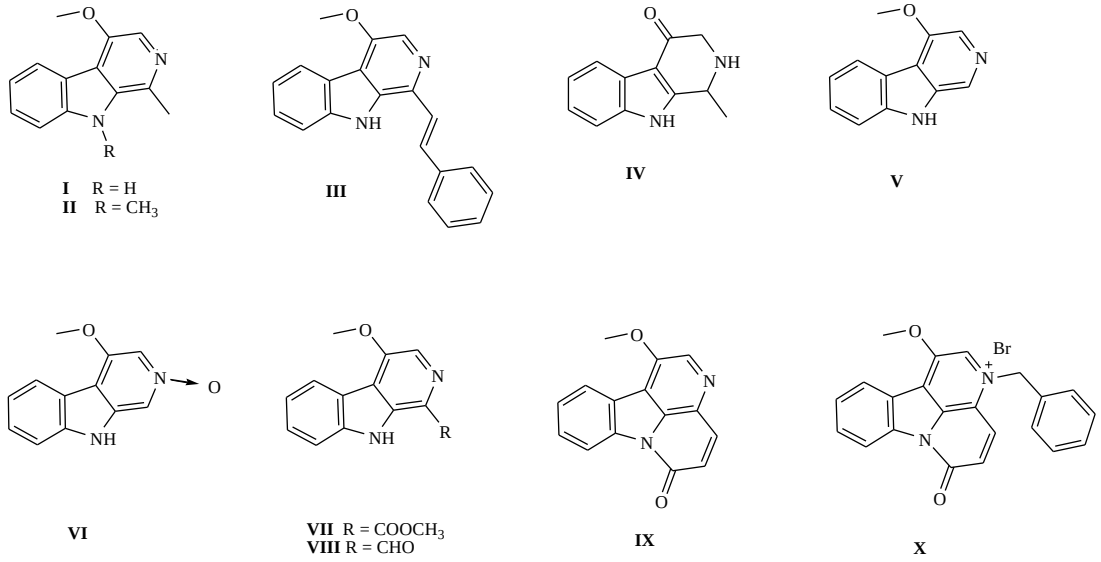


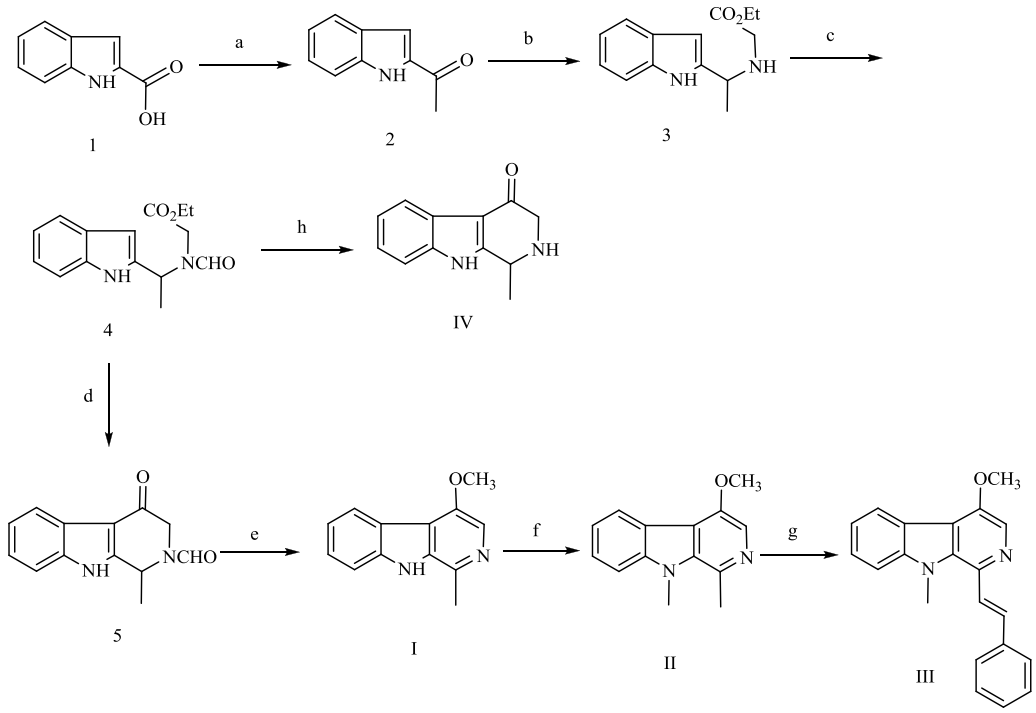
Figure 6: Chemical structure of 1,4-disubstituted, 1,4,9-trisubstituted *β*-carbolines and tetracyclic derivatives.

The purpose of this study is to evaluate the antiproliferative properties of these compounds in order to acquire more information about the structural requirements for the possible improvement of the cytotoxic potential and to elucidate SARs between substituent properties and antitumor activities.

1.3.1 RESULTS AND DISCUSSION

Chemistry

The preparation of derivatives **I-IV** is summarized in Scheme 1.



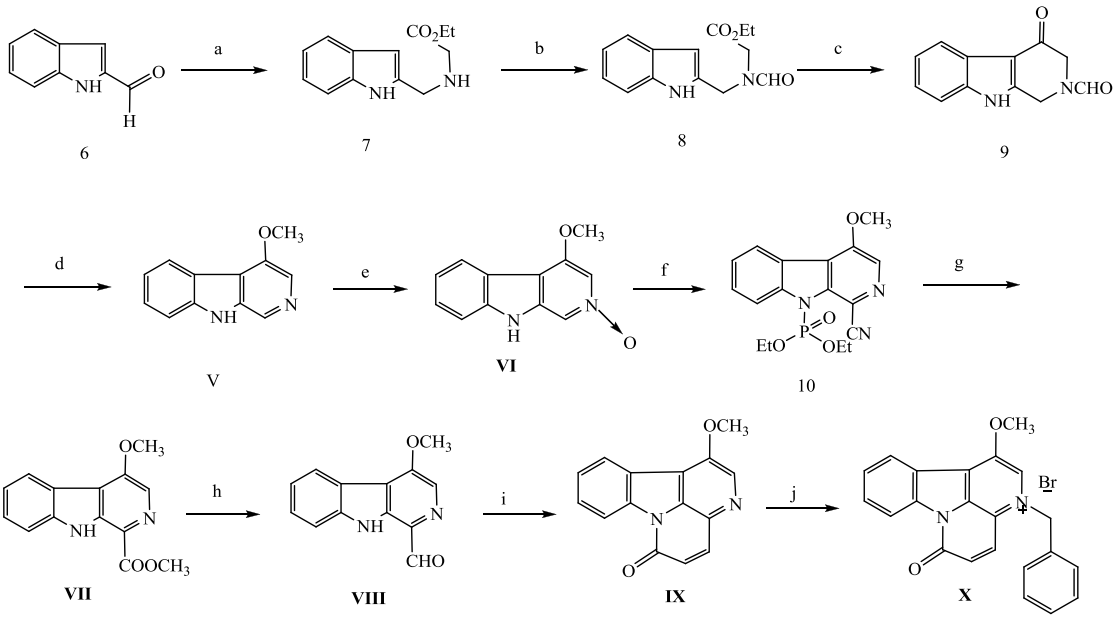
Scheme 1. Synthesis of compounds **I-IV**

Reagents and conditins: a) MeLi 1.6M in ether, THF, rt, o.n., 84%; b) glycine ethyl ester hydrochloride, $NaBH(OAc)_3$, TEA, AcOH, dry 1,2-dichloroethane, rt, 24 h, 89%; c) HCO_2Et, HCOOH, rt, 12 h, 94%; d) $MeSO_3H$, 70°C, 1h, 96%; e) $Me_2C(OMe)_2$, *p*-TsOH, benzene; then *p*-chloranil, rt, 12h, 50%; f) , CH_3I, NaH, THF-DMF, rt, 30', 80%; g) $(CH_3CO)_2O$, C_6H_5CHO, reflux, 24h, 67%; h) $MeSO_3$,H, 70% in H_2O, 70°C, 1h, 95%.

Treatment of indole-2-carboxylic acid (**1**) with a solution of methyl lithium in ether afforded 2-acetylindole (**2)** in very good yield. **2** was converted to the glycine derivative (**3**) *via* reductive amination, using sodium triacetoxyborohydride as reducing agent in presence of triethylamine and acetic acid. Ethyl 2-[1-(1*H*-indol-2-yl)ethylamino]acetate (**3)** was treated with ethylformate and formic acid to furnish the key intermediate **4**. Cyclization of **4** with methanesulfonic acid gave intermediate **5** in excellent yield.

4-Methoxy-1-methyl-*β*-carboline (**I**) was obtained in a one-step conversion. This reaction was achieved by treatment of **5** with 2, 2-dimethoxypropane, dehydrogenation of intermediate with chloranil, followed by ready methanolysis of *N*-formyl pyridinium intermediate. Methylation of the *N*-9 position of compound **I** has been achieved by the action of sodium hydride in a mixture of anhydrous DMF/THF followed by addition of methyl iodide, affording compound **II** in good yield. 1-Benzylidine substituted *β*-carboline **III** was readily prepared by reaction of 1,9-dimethyl-4-methoxy *β*-carboline **II** with benzaldehyde in refluxing acetic anhydride. Treating **4** with methanesulfonic acid 70% in water we obtained compound **IV** in which cyclization and deformylation were accomplished simultaneously.

Compounds **V, VI, VII, VIII**, and 1-methoxycanthin-6-one (**IX**) were prepared according to the literature procedures.[33, 34] (Scheme-2).

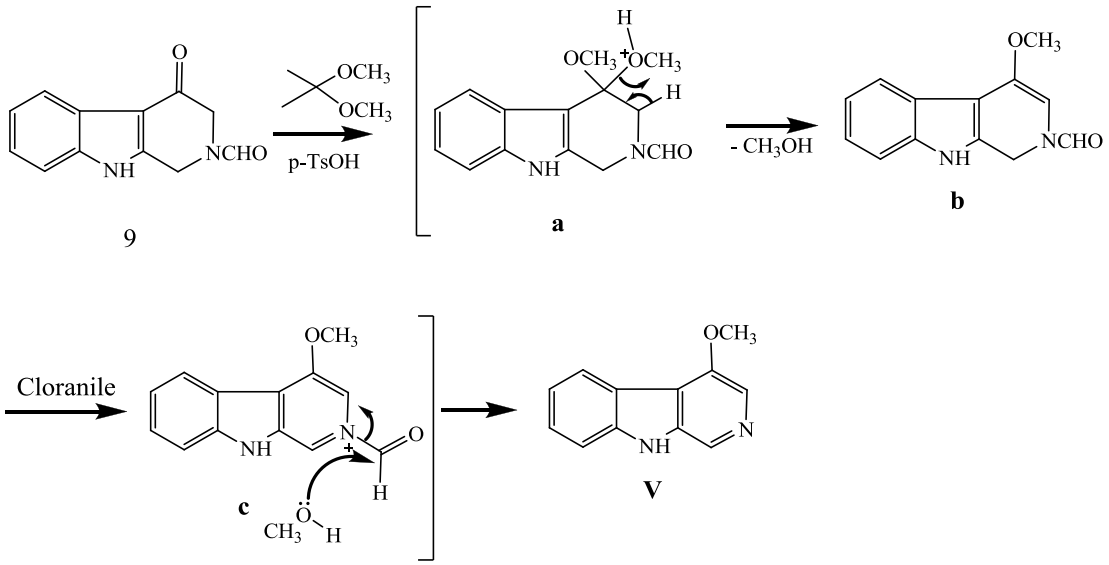


Scheme 2: Synthesis of compound (**V-X**)

Reagents and condtions: a) glycine ethyl ester hydrochloride, $NaBH_3CN$, EtOH, 14h 0 °C ,89%; b) HCO_2Et, HCOOH, rt, 12 h, 79%; c) $MeSO_3H$, 70 °C, 1h, 75%; d) $Me_2C(OMe)_2$, *p*-TsOH, benzene; *p*-chloranil, rt, 12h, 72%; e) *m*-CPBA, CH_2Cl_2, rt, 24h, 70%; f) DEPC, TEA, CH_3CN, 70 °C, 5h, 45%;g) HCl-MEOH , CH_2Cl_2, 24h, rt -22h reflux 84%; h) DIBAL-H, CH_2Cl_2, -40 °C, 5', 70%; i) LiHMDS, THF, 15', -78 °C, 88%; j) BnBr, EtOAc, reflux, 7h, 67%.

The indole-2-carboxaldehyde was subjected to reductive amination, by treatment with ethyl glycinate hydrochloride in the presence of sodium cyanoborohydride providing the intermediate **7** with a yield of 89%, after purification by flash chromatography. This intermediate was first formylated and then subjected to cyclization in the presence of methanesulfonic acid to give the compound **9** with a yield of 59%.

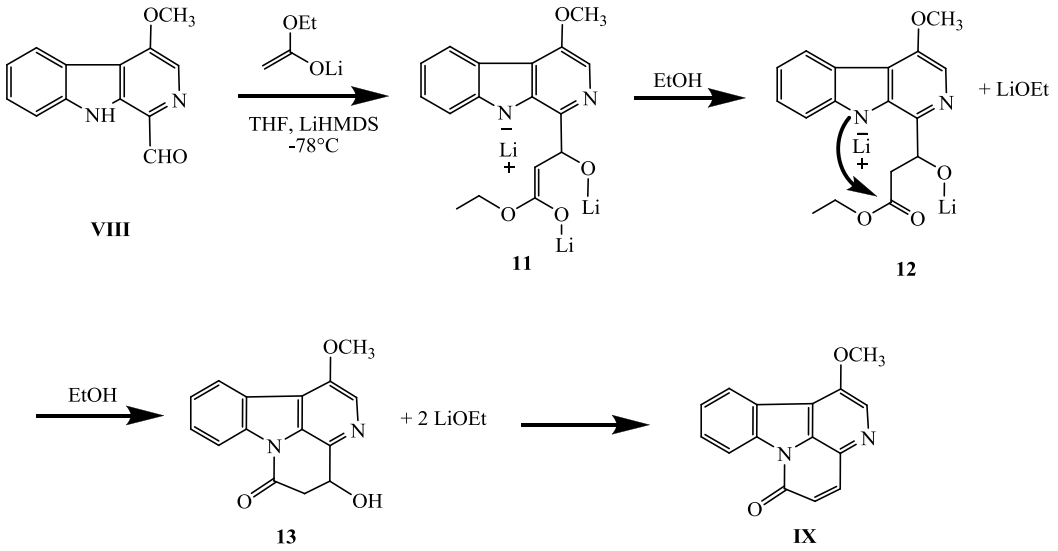
Aromatization and following formation of 4-methoxy-*β*-carboline core were performed in the presence of 2,2-dimethoxypropane and chloranil as oxidizing agent (Scheme 2a). Subsequently rapid methanolysis of the intermediate *N*-formyl pyridinium (**c**) gave the compound **V** with a yield of 72%. Acidic treatment of the intermediate **V** with *m*-chloroperbenzoic acid afforded the corresponding *N*-oxide (**VI**) in 70% yield. Then cyano group was introduced into position 1 of the *β*-carboline ring. In our synthetic scheme we used a modified Reissert-Henze reaction[35] which involves the use of diethyl phosphorocyanidate in an aprotic solvent such as acetonitrile. The reaction was maintained at 70 °C for 5 h giving the intermediate **10** with a yield of 45%, after purification by column chromatography.



Scheme 2a

This intermediate was then subjected to acid-catalyzed methanolysis to afford the desired compound (**VII**) in excellent yields. The reduction of the ester group to aldehyde was carried out using a solution of 1M DIBAL-H in dichloromethane

and maintaining the solution at -40 °C for 5 minutes. The 4-methoxy-*β*-carboline-1-carbaldehyde (**VIII**) was isolated by flash chromatography with a yield of 70 %. The cyclization and formation of the tetracyclic nucleus of 1-methoxy-6-cantin-one **(IX**) was performed using a cyclization-aldol condensation reaction carried out at low temperature on 4-methoxy-*β*-carboline-1-carbaldehyde (Scheme 2b).



Scheme 2b

This reaction involves the use of lithium hexamethyldisilazide (LiHMDS) as base, generated *in situ* by adding 1,1,1,3,3,3-hexamethyldisilazane (HMDS) and *n*-BuLi in THF at -78 °C under inert atmosphere. Addition of ethyl acetate to the solution led to formation of lithium 1-ethoxyenolate, which reacts with 4-methoxy-*β*-carboline-1-carbaldehyde giving lithium enolate (**11**). This intermediate is treated with ethanol to generate the ester (**12**) which rearranges to form the 4, 5-dihydro-4-hydro-4-hydroxycanthin-one (**13).** This compound in the presence of lithium ethoxide, generated *in situ*, undergoes dehydration to form 1-methoxycanthin-6-one.

3-benzyl-1-methoxycanthin-6-onium bromide **(X)** was synthesized from 1-methoxycanthin-6-one by the simple quaternarization with benzyl bromide in refluxing ethyl acetate**.**

1.4 BIOLOGICAL ACTIVITY

1.4.1 ANTI-PROLIFERATIVE ACTIVITY

The cytotoxic potential of all synthesized compounds was evaluated *in vitro* against a panel of human tumor cell lines. The tumor cell line panel consisted of human T-cell lymphoblast-like cells (Jurkat), human breast cancer (MCF-7), human colon carcinoma (HT-29), human lung adenocarcinoma epithelial (A549), human prostate cancer (PC3), human melanoma (M14), human anaplastic thyroid carcinoma (ARO), and human glioblastoma (T98G). IC_{50} values for **I-X** and harmine (for comparison) are reported in Table 1.

Table 1. ***In vitro*** Antitumor Activity of Derivatives **I-X**

Compd	**IC50 (μM)[a]**							
	M14	**MCF-7**	**HT-29**	**A549**	**PC-3**	**Jurkat**	**ARO**	**T98G**
Harmine	ns	ns	79.4±0.9	ns	22±0.8	ns	26.5±0.7	35±1.1
I	ns	ns	50±1.5	80±0.5	20±1.2	ns	50±0.8	ns
II	36±0.6	32±0.1	27±0.2	50±0.4	22±1.2	65±0.8	30±0.5	40±1.2
III	ns	ns	ns	ns	60±0.9	ns	ns	ns
IV	ns	ns	ns	ns	24±0.8	ns	18±0.6	ns
V	ns	ns	ns	ns	ns	ns	64±1.2	ns
VI	ns	ns	ns	ns	ns	ns	ns	ns
VII	ns	ns	ns	ns	50±	ns	ns	ns
VII	38.9±0.1	35±0.45	90±0.3	45±1.2	25±0.2	28.8±0.9	39±0.4	ns
IX	50±1.1	50±0.3	31±0.1	ns	26±0.1	50±0.2	15±0.6	90±0.7
X	37±0.3	46±0.75	72±0.4	ns	8±0.9	ns	28±1.0	ns

[a]IC_{50}= compound concentration required to inhibit tumor cell proliferation by 50 %; n.s. not significant. Data represent the mean values of three independent determinations performed in triplicate.

In this study we evaluated cytotoxic effects of β-carboline derivatives and compared with harmine, a natural alkaloid highly cytotoxic against human tumor cell lines. In addition, 1-methoxycanthin-6-one (**IX**) was tested for cytotoxicity against a panel of several tumor cells. Our experiments confirmed the cytotoxic

effect of 1-methoxycanthin-6-one (**IX**) and, for the first time, we demonstrated the potential cytotoxic activity of compounds **I-VIII** and **X** in different cancer cell lines. From the above data, the following conclusions were drawn.

The shift of methoxy group from position 7 to 4 (**harmine *vs* I**) led to an increase of activity against HT-29 and A549 cell lines while no significant activities were detected in the other cell lines. The introduction of methyl group into position-9 of compound **I** led to compound **II** which demonstrated an improvement of activity to all tumor cell lines. When methyl group in position 1 was replaced with benzylidine substituent there is a loss of activity. Compounds **V** and **VI**, with no substituent at position-1, were inactive to all tumor cell lines. Interestingly, the compound bearing a carboxyaldehyde substituent in position 1 (**VIII**) of β-carboline ring system displayed a strong anti-proliferative effect against almost all tumor cell lines. In addition, the compound with a carboxylate (**VII**) substituent in position-1 was inactive to all tumor cell lines except to PC-3. Tetrahydro-β-carboline derivative **IV** showed good activity only against PC-3 and ARO cells.

As almost of the synthesized compounds of the present study showed interesting anticancer activity especially against PC-3 and ARO cell lines, oral bioavailability was considered to play an important role for the development of bioactive molecules as therapeutic agents. Therefore, a computational study for prediction of ADME properties of the molecules was performed by determination of lipophilicity, topological polar surface area (TPSA), absorption (% ABS) and simple molecular descriptors used by Lipinski in formulating his ‘‘rule of five.’’[36]

Table 2. Predicted ADME, Lipinski Parameters and Molecular Properties of the Synthesized Compounds I-X and Harmine[a]

Compds	% ABS	TPSA	n-ROTB	n-OHNH donors	n-ON accept.	mi LogP	Mol. Wt	*n* violations
Harmine	95	37.92	1	1	3	2.626	212.25	0
I	95	37.92	1	1	3	2.796	212.25	0
II	99	27.06	1	0	3	2.864	226.28	0
III	99	27.06	3	0	3	5.192	314.39	1
IV	94	44.89	0	2	3	1.829	200.24	0
V	96	37.92	1	1	3	2.575	198.23	0
VI	92	50.48	1	1	4	0.943	214.22	0
VII	87	64.22	3	1	5	2.646	256.26	0
VIII	90	54.99	2	1	4	2.604	226.24	0
IX	94	43.61	1	0	4	2.891	250.26	0
X	97	34.61	3	0	4	0.816	341.39	0

[a] % ABS: Percentage of absorption, TPSA: topological polar surface area, n-ON: number of hydrogen bond acceptors, n-OHNH: number of hydrogen bond donors, n-ROTB: number of rotatable bonds. Calculations were performed using *Molinspiration online property calculation toolkit* (http://www.molinspiration.com).

Calculations were performed using Molinspiration online property calculation toolkit.[37] Table 2 represents a calculated percentage of absorption (% ABS), topological polar surface area (TPSA) and Lipinski parameters of the synthesized compounds. Percentage of absorption (% ABS) was estimated using the equation: % ABS = 109 - 0.345 x TPSA, according to Zhao *et al*.[38] TPSA was also calculated using Molinspiration online property calculation toolkit[37] according to the fragment-based method of Ertl *et al*.[39]

Polar surface area, together with lipophilicity, is an important property of a molecule in transport across biological membranes. Too high TPSA values give rise to a poor bioavailability and absorption of a drug. According to the above criterions, calculated percentages of absorption for compounds ranged between 87 and 99%.

As a whole, the present results showed that all the compounds studied, except **V** and **VI**, showed a very selective activity against prostate cancer cells PC-3 at

IC_{50} values nearly to 20 μM. In particular, X demonstrated an important anti-proliferative effect at low concentration (IC_{50} 8 μM). Moreover the most active compounds **II**, **VIII**, **IX** and **X** appear to be suitable as leads for further anticancer molecules development efforts on the fact their size and chemical properties are appropriate to classify them as drug-like compounds as they follow all the Lipinski's rule of five.

1.4.2 TOPOISOMERASE-I ACTIVITY

Topoisomerase-targeting agents have long been considered an attractive target for the design of cancer chemotherapeutics because they can cause permanent DNA damage that triggers a series of cellular events, inducing apoptosis and finally causing cell death.[40] DNA topoisomerases have been shown to be the molecular target of many anticancer drugs, including known DNA intercalators.

Selected compounds **(I, II, IV, VIII, IX** and **X)** have been subjected to further studies to investigate their mechanism of antiproliferative activity. Especially, they have been evaluated for their ability to inhibit Topoisomerase I.

They have been tested for their intercalation, inhibition of relaxation activity, and poisoning at the concentration of 312 μM. Compounds **I**, **II** and **X** demonstrated intercalation and inhibition of relaxation activity (IC_{50} are reported in Table 3).

Table 3. Inhibition activity of compounds against DNA Topoisomerases I

Compound	**Topoisomerase I activity (IC_{50}) (μM)**
I	61.44±9.63
II	117.75±21.41
IV	(-)
VIII	(-)
IX	(-)
X	17.77±2.75
Harmine	65.25±14.09

Note: (-) indicates that the compound is inactive (negative at 312 μM or with IC_{50}>250 μM)

Other three compounds do not show any activity. Compound **X** exhibits strong Topoisomerase I inhibition, **I** and **II** show respectively moderate and weak activity. It appears of interest that: i) the regioisomer of harmine (compound **I**) maintains the same activity of the lead harmine (61.44 μM *vs* 65 μM); ii) the substitution of methyl group in position 1 with a carboxyaldehyde (**VIII**) is not effective; iii) N-9-methylation of β-carboline nucleus (compound **II**) is detrimental for the activity; iv) the loss of aromaticity of pyridine ring (**IV**) leads to an inactive compound; v) 1-methoxycanthin-6-one (**IX**) is unable to inhibit Topo I, while its N-2-benzylated analog (**X**) shows to be the most potent inhibitor of the series, better than the harmine.

1.5 CONCLUSION

We systematically designed and synthesized a number of novel *β*-carboline derivatives by efficient synthetic routes, and were proved to be selective and potent agent against prostate cancer. This important first step will allow us to determine preliminary relationship between structure and cytotoxic activities. The *in vitro* experiments revealed that the shift of methoxy group in position 4 of *β*-carboline ring of harmine led to enhanced cytotoxic activity, substituents at position-1 were essential for high activity towards specific tumor types. From the above data we have also demonstrated that substitution at the position 3 of 1-methoxycanthin-6-one with benzyl increased activity against most of the cell lines tested. The presented data suggest that several derivatives are potential candidates for clinical development with models of prostate cancer.

Most of the compounds were evaluated for Topo I inhibitory activity and compared to harmine. The compounds (**I, II** and **X**) demonstrated intercalation and inhibition of relaxation activity. It was observed that the N-2 benzylated analog (**X**) exhibited strong Topoisomerase I inhibition, better than the harmine. As well as compound **I** and **II** showed respectively moderate and weak activity.

1.6 EXPERIMENTAL SECTION

a) Biology

i) Cell Cultures

Human melanoma (M14), breast (MCF-7), glioblastoma (T98G), lung (A549), colon (HT-29) cancer cells were cultured in DMEM medium supplemented with 2 mM L-glutamine, 10% heat-inactivated fetal bovine serum (FBS), 1% penicillin/streptomycin (all from Cambrex Bioscience, Verviers, Belgium) and human T-cell lymphoblast-like cells (Jurkat), anaplastic thyroid (ARO), prostate (PC3) cancer cells were cultured in RPMI medium supplemented with 2 mM L-glutamine, 10% FBS, 1% penicillin/streptomycin at 37 °C in an atmosphere of 95% O_2 and 5% CO_2. The cells were used up to a maximum of 10 passages.

ii) MTT Bioassay

Human cancer cells (3×10^3) were plated in 96-well culture plates in 90 μL of culture medium and incubated at 37 °C in humidified 5% $C0_2$. The day after, 10 μL aliquot of serial dilutions of compounds (1–50 μM) was added to the cells and incubated for 48 h. Cell viability was assessed through MTT assay [1]. Briefly, 25 μL of MTT (5 mg/mL) were added and the cells were incubated for an additional 3 h. Thereafter, cells were lysed and the dark blue crystals solubilized with 100 μL of a solution containing 50% (v:v) *N*,*N*-dimethylformamide, 20 % (w:v) SDS with an adjusted pH of 4.5. The optical density (OD) of each well was measured with a microplate spectrophotometer (Titertek Multiskan MCC/340) equipped with a 620 nm filter. Cells viability in response to treatment was calculated as percentage of control cells treated with solvent DMSO at the final concentration 0.1 % : % viable cells = (100 x OD treated cells)/ OD control cells.

iii) Chemicals and Reagents:

Supercoiled pHOT-1 DNA, 10 × TGS buffer, and Human Topo I enzyme were obtained from Topo GEN, Inc. (Port Orange, FL). pHOT-1 DNA was purchased at a concentration of 250 ng/μL in 10 mM Tris-HCl buffer (pH 7.5) containing 1 mM EDTA and was used as is after dilution in 1 x TGS buffer. Human Topo I was purchased in solution at 2 U/μL (where 1 U is the amount of

enzyme needed to relax 0.25 µg of pHOT-1 DNA in 30 min at 37 ºC). Proteinase K was obtained from Promega Corp. (Madison, WI). Agarose (molecular biology grade) was purchased from Midwest Scientific (St. Louis, MO) and sodium dodecyl sulfate (SDS) was purchased from EMD Biosciences (San Diego, CA). Dimethyl sulfoxide (DMSO; ACS grade), bromophenol blue (electrophoresis grade), and ethidium bromide (EtBr; electrophoresis grade) were purchased from Fisher Scientific (Fair Lawn, NJ).

b) Chemistry

All reactions were performed in oven-dried (140 °C) or flame-dried glassware under dry N_2. CH_2Cl_2 was reagent grade and was dried and distilled immediately from CaH_2 before use. Column chromatographic purification of products was carried out using silica gel 60 (70–230 mesh, Merck). The reagents (Aldrich and Fluka) were used without further purification. The NMR spectra were recorded on Bruker DRX 400, 300, 250 spectrometers [400 MHz, 300 MHz, 250 MHz (^{1}H); 100 MHz, 75 MHz, 62.5 MHz (^{13}C)]. Spectra were referenced to residual $CHCl_3$ [δ = 7.26 (^{1}H), 77.23 (^{13}C)]. Yields are given for isolated products showing one spot on a TLC plate and no impurities detectable in the NMR spectrum. Mass spectral analyses were carried out using an electrospray spectrometer, Waters 4 micro quadrupole. Elemental analyses were performed with FLASHEA 1112 series-Thermo Scientific for CHNS-O apparatus.

c) Procedures and Characterization data

2-Acetylindole (2)

To a solution of indole-2-carboxylic acid (0.5 g, 3 mmol) in anhydrous THF (5 mL) at 0 °C was added methyl lithium (1.6 M in diethyl ether, 7.68 mL, 12 mmol) under nitrogen atmosphere in a dropwise manner. After addition, the reaction mixture was allowed to slowly warm to room temperature, stirred overnight, and treated with a saturated aqueous ammonium chloride solution. Ethyl acetate (400 mL) was added and organic layer was separated. The aqueous layer was extracted with ethyl acetate (3x200 mL). The combined organic layers were dried (Mg_2SO_4)

and concentrated. The residue was purified by a flash chromatography on silica gel (CH_2Cl_2) to afford the title compound (411 mg, 84%).

^{1}HNMR (300 MHz; $CDCl_3$): δ 9.01 (br s, 1H), 7.69 (d, *J* = 8 Hz, 1H), 7.45 (d, *J* = 8 Hz, 1H), 7.00-7.35 (m, 3H), 2.59 (s, 3H).

MS (ESI) = m/z 160.73 ($[M+H]^+$).

Anal. Calcd for $C_{10}H_9NO$: C, 75.45; H, 5.70; N, 8.80. Found: C, 75.25; H, 5.83; N, 8.56.

Ethyl 2-[1-(1*H*-indol-2-yl)ethylamino]acetate (3)

The mixture solution of ethyl glycinate (3.299 g, 24 mmol) and Et_3N (3.327 mL, 24 mmol) in dry 1,2-dichloroethane (40 mL) was stirred at room temperature for 0.5 h, the 1-(1*H*-indol-2-yl)ethanone (1.9 g, 12 mmol) was added, treated with sodium triacetoxyborohydride (6.59 g, 30 mmol) and HOAc (1.78 mL, 30 mmol). The mixture was stirred at room temperature for 24 h. The reaction was quenched with saturated $NaHCO_3$ and extracted with CH_2Cl_2. The combined organic layers were dried (Mg_2SO_4) and concentrated. The residue was purified by flash chromatography using petroleum ether/ethyl acetate 1:1 to give desired compound (2.6 g, 89 % yield).

^{1}H NMR (300 MHz; $CDCl_3$): δ 8.84 (br s, 1H), 7.54 (d, *J* = 7.9 Hz, 1H), 7.32 (d, *J* = 7.9 Hz, 1H), 7.14 (t, *J* = 7.9 Hz, 1H), 7.05 (t, *J* = 7.9 Hz, 1H), 6.34 (s, 1H), 4.15 (m, 3H), 3.34 (dd, *J* = 6.7 Hz, 12 Hz, 2H), 2.31 (br s, 1H), 1.75 (d, *J* = 6.7 Hz, 3H), 1.62 (t, *J* = 7.2 Hz, 3H).

MS (ESI) = m/z 246.81 ($[M+H]^+$); MS (ESI) m/z 269.12 ($[M+Na]^+$).

Anal. Calcd for $C_{14}H_{18}N_2O_2$: C, 68.27; H, 7.37; N, 11.37. Found: C, 68.44; H, 7.34; N, 11.56.

Ethyl 2-[1-(1*H*-indol-2-yl)ethyl]-*N*-formylaminoacetate (4)

Ethyl 2-[1-(1*H*-indol-2-yl)ethylamino]acetate (2.4 g, 9.6 mmol) was dissolved in a mixture of ethylformate (31.2 mL) and formic acid (0.336 mL), and stirred at 55 °C for 9h. The organic layer was diluted with AcOEt, washed with aqueous $NaHCO_3$, dried over anhydrous $MgSO_4$ and evaporated to dryness in vacuo. The

residue was purified by flash chromatography using petroleum ether/ethyl acetate 1:1 to give the title compound (2.5 g, 94%).

^{1}HNMR (300 MHz; $CDCl_3$): δ 9.50 (brs, 1H), 8.19 and 8.26 (each s, lH), 7.01-7.72 (m, 4H), 6.43 and 6.48 (each br s, 1H), 4.15-4.30 (m, 3H), 3.36 (dd, *J* = 6.7 Hz & 12 Hz, 2H), 1.62 and 1.72 (each d, *J* = 7.9 Hz, 3H), 1.05 and 1.25 (each t, *J* = 8.0 Hz, 3H).

MS (ESI) = m/z 275.81 ($[M+H]^+$).

Anal. Calcd for $C_{15}H_{18}N_2O_3$: C, 65.68; H, 6.61; N, 10.21. Found: C, 65.88; H, 6.24; N, 10.96.

2-Formyl-1-methyl-4-oxo-1,2,3,4-tetrahydro β-carboline (5)

A mixture of ethyl 2-[1-(1*H*-indol-2-yl)ethyl]-*N*-formylaminoacetate **(**2.2 g, 7.9 mmol) and methanesulfonic acid (20.7 mL) was stirred at 70 °C for 1 h. The reaction mixture was poured into ice-water, and neutralized with 10% aqueous $NaHCO_3$, and extracted with AcOEt. The organic layer was washed with saturated aqueous $NaHCO_3$ and brine, dried over anhydrous $MgSO_4$ and evaporated to dryness in vacuo. The residue was subjected to flash chromatography using ethyl acetate: hexane (6:4) to give the title compound (1.73g, 96%).

^{1}HNMR (300 MHz; DMSO-d_6): δ 8.19 and 8.26 (each s, lH), 7.92 (d, *J* = 7.9 Hz, 1H), 7.42 (d, *J* = 7.9 Hz, 1H), 7.01-7.32 (m, 2H), 5.35 and 5.60 (m, 1H), 3.80 and 4.60 (each d, 2H), 1.45 and 1.72 (each d, *J* = 7.9 Hz, 3H).

MS (ESI) = m/z 229.31 ($[M+H]^+$).

Anal. Calcd for $C_{13}H_{12}N_2O_2$: C, 68.41; H, 5.30; N, 12.27. Found: C, 68.28; H, 5.24; N, 12.19.

4-Methoxy-1-methyl β-carboline (I)

A solution of *p*-toluenesulfonic acid monohydrate (0.76 g, 3.9 mmol) in benzene (25 mL) was heated under azeotropic conditions for 1h. After cooling, 2-formyl-1-methyl-4-oxo-1,2,3,4-tetrahydro-*β*-carboline (0.9 g, 3.9 mmol) and dimethoxypropane (1.47 mL, 0.85 mmol) was added to the benzene solution, and the mixture was stirred at room temperature for 1h. Chloranil (1.94 g, 7.80 mmol) was then added, and the whole was stirred at room temperature for 12h. The

reaction mixture was poured into 5% aqueous NaOH, and extracted with CH_2Cl_2. The organic layer was washed with brine, dried over anhydrous $MgSO_4$, and evaporated to dryness in vacuo. The residue was subjected to flash-chromatography using $CHCl_3$/MeOH (9:1) to give the title compound (0.42 g, 50%).

^{1}H NMR (300 MHz; $CDCl_3$): δ 10.22 (br s, 1H), 8.35 (d, *J* = 7.9 Hz, 1H), 8.01 (s, 1H), 7.47-7.51 (m, 2H), 7.22-7.33 (m, 1H), 4.1 (s, 3H), 2.7 (s, 3H).

^{13}C-NMR (75 MHz; $CDCl_3$): 19.7, 56.1, 111.0, 117.8, 120.1, 120.3, 121.2, 124.3, 127.0, 135.1, 135.5, 139.5, 150.7.

MS (ESI) = m/z 213.33 ($[M+H]^+$).

Anal. Calcd for $C_{13}H_{12}N_2O$: C, 68.41; H, 5.30; N, 12.27. Found: C, 68.18; H, 5.14; N, 12.98.

1,9-Dimethyl-4-methoxy-β-carboline (II)

A mixture of 4-methoxy-1-methyl-β-carboline (2.12 g, 10 mmol), anhydrous DMF (50 mL), and anhydrous THF (50 mL) was stirred at rt until clear, and then 60% NaH (0.6 g, 15 mmol) and iodomethane (2 mL, 30 mmol) were added and stirred at rt for 30 min. Later the mixture was evaporated in reduced pressure. The resulting solution was poured into H_2O (100 mL), and extracted with ethyl acetate (3 x 150 mL). The organic phase was washed with water and brine, then dried over anhydrous sodium sulfate, filtered, and evaporated. The oil obtained was purified by silica column chromatography with ethyl acetate as the eluent. Upon recrystallization, white crystals of compound were obtained (1.8 g, 80 %),

^{1}H NMR (300 MHz; $CDCl_3$): δ 8.39 (d, *J* = 7.9 Hz, 1H), 8.04 (s, 1H), 7.44-7.53 (m, 2H), 7.24-7.32 (m, 1H), 4.22 (s, 3H), 4.14 (s, 3H), 2.73 (s, 3H).

^{13}C NMR (100 MHz; $CDCl_3$): δ 19.4, 34.3, 56.1, 111.0, 117.8, 120.0, 120.3, 121.2, 124.3, 127.1, 135.1, 135.7, 139.4, 150.8.

MS (ESI) = m/z 227.87 ($[M+H]^+$).

Anal. Calcd for $C_{14}H_{14}N_2O$: C, 74.31; H, 6.24; N, 12.38. Found: C, 74.45; H, 6.03; N, 11.98.

4-Methoxy-9-methyl-1-styryl-*β*-carboline (III)

A mixture of 4-methoxy-1,9-dimethyl-β-carboline (2.26 g, 10 mmol), acetic anhydride (50 mL) and benzaldehyde (5.25 g, 50 mmol) was refluxed for 24 h. After completion of the reaction as indicated by TLC, the solution was poured into ice-water (200 mL) and made basic with sodium bicarbonate. The aqueous mixture was extracted with ethyl acetate, and the organic phase was washed with water and brine and then dried over anhydrous sodium sulfate. The solvent was removed under vacuum, and the residue was dissolved in ethanol and made acidic with concentrated hydrochloric acid. The solvent was evaporated in reduced pressure and the resulting oil was crystallized from acetone to give yellow solid. The solid was dissolved in water and made basic with sodium bicarbonate, and the aqueous mixture was extracted with ethyl acetate. The organic phase was washed with water and brine, dried over anhydrous sodium sulfate, concentrated under vacuum. The oil residue was crystallized to give desired compound in 67 % yield.

^{1}H NMR (300 MHz; $CDCl_3$): δ 8.39 (d, *J* = 7.9 Hz, 1H), 8.04 (s, 1H), 7.44-7.53 (m, 2H), 7.15-7.32 (m, 6H), 6.94-6.95 (d, *J* = 8.5 Hz, 2H), 4.14 (s, 3H), 2.73 (s, 3H).

^{13}C NMR (75MHz; $CDCl_3$): δ 37.3, 56.1, 111.0, 117.8, 120.0, 120.3, 121.2, 124.3, 127.1, 127.4, 127.9, 128.5, 128.6, 135.1, 135.7, 137.2, 139.4, 150.8.

MS (ESI) = m/z 315.33 ($[M+H]^+$).

Anal. Calcd for $C_{21}H_{18}N_2O$: C, 80.23; H, 5.77; N, 8.91. Found: C, 80.04; H, 6.01; N, 7.98.

1-Methyl-4-oxo-1,2,3,4-tetrahydro *β*-carboline (IV)

A mixture of ethyl 2-[1-(1*H*-indol-2-yl)ethyl]-*N*-formylaminoacetate **(**2.2 g, 7.9 mmol) and methanesulfonic acid 70 % in water (22.7 mL) was stirred at 70 °C for 1 h. The reaction mixture was poured into ice-water, and neutralized with 10 % aqueous $NaHCO_3$, and extracted with AcOEt. The organic layer was washed with saturated aqueous $NaHCO_3$ and brine, dried over anhydrous $MgSO_4$ and evaporated to dryness in vacuo. The residue was subjected to flash chromatography using $CHCl_3$/MeOH (9:1) to give the title compound (1.53 g, 95 %).

^{1}H NMR (300 MHz ; CD_3OD): δ 7.92 (d, *J* = 7.8 Hz, 1H), 7.42 (d, *J* = 7.8 Hz, 1H), 7.01-7.32 (m, 2H), 5.35 and 5.60 (m, 1H), 3.80 (dd, *J* = 6.7 Hz & 12 Hz, 2H), 1.72 (d, *J* = 7.9 Hz, 3H).

^{13}C NMR (75 MHz ; CD_3OD): δ 20.2, 54.3, 58.6, 109.7, 111.3, 119.8, 121.6, 121.8, 126.3, 134.4, 135.3, 196.5.

MS (ESI) = m/z 201.31 ($[M+H]^+$).

Anal. Calcd for $C_{12}H_{12}N_2O$: C, 71.98; H, 6.04; N, 13.99. Found: C, 71.17; H, 5.84; N, 13.87.

Ethyl 2-[(1*H*-indol-2-yl)methylamino]acetate(7):

Indole -2-carboxaldehyde (4.8 g, 1.0 eq, 32.736 mmol) was treated with Et_3N (5.056 ml, 1.1 eq, 36.01 mmol), $NaBH_3CN$ (12.992 g, 6.0 eq, 196.41 mmol) and ethyl aminoacetatate HCl (13.846 g, 3.0 eq, 98.20 mmol) in ethanol (110 ml) was added at 0 °C and stirred rt for 4 days. The reaction was monitored by TLC. Then the reaction mixture was poured into ice water and extracted with ethyl acetate. The organic layer was washed with brine, dried over anhy. $MgSO_4$, and evaporate to dryness in vaccum. The residue was subjected to column chromatography using hexane-ethyl acetate (1:1) to give pure product (5.800 g, 76%).

Nature of compound: pale brown solid; mp 72-74 °C

IR (KBr): 3320, 331O (NH), 1720 (CO) cm^{-1}

^{1}H NMR (400 MHz, $CDCl_3$): 8.68 (br s, 1H), 6.92-7.69 (m, 4H), 6.30 (br s, 1H), 4.14 (q, *J* = 7 Hz, 2H), 3.35 and 9.92 (each s, 2H), 2.04 (br s, 1H), 1.21 (t, *J* = 7 Hz, 3H).

MS (ESI) = m/z-233.57 $[(M+H)^+]$.

Anal. Calcd for $C_{13}H_{16}N_2O_2$,: C, 67.22: H, 6.94; N, 12.06. Found: C, 67.20; H, 7.00; N, 11.92.

Ethyl *N*-[(indol-2-yl)methyl]-*N*-formylaminoacetate (8)

The mixture of amine (5.700 g, 1.0 eq, 24.29 mmol), ethyl formate 1.2 eq. of 95% formic acid (1.158 ml, 1.2 eq, 29.15 mmol) in toluene (200 ml) was heated under reflux using a dean stark trap for overnight. The progress of the reaction was monitored by TLC, and starting material disappeared. The reaction mixture was evaporated to give the crude product. The evaporation residue was purified

by column chromatography using ethyl acetate: hexane (3:7) to give pure product (6.075 g, 96%).

IR (KBr): 3420, 328O (NH), 1738, 1664 (C=O) cm^{-1}

^{1}H NMR (400 MHz; $CDCl_3$): δ 9.18 and 9.61 (s, lH), 8.08 and 8.28 (each s, lH), 7.01-7.72 (m, 4H), 6.40 and 6.45 (each br s, lH), 4.13 (q, *J* = 8 Hz, 2H), 3.88, 4.01, 4.61 and 4.69 (each s, 4H), 1.17 and 1.27 (each t, *J* = 8 Hz, 3H),

MS (ESI) = m/z 259.09 $[(M+H)^+]$.

Anal. Calcd for $C_{14}H_{16}N_2O_3$: C, 64.60; H, 6.20; N, 10.76. Found: C, 64.53; H, 6.14; N, 1.87.

3, 4-Dihydro-4-oxo-1*H*-pyrido[3,4-b]indole-2(9*H*)-carbaldehyde (9)

Ethyl *N*-[(indol-2-yl)methyl]-*N*-formylaminoacetate (500 mg, 1.0 eq, 1.93 mmol) was treated with methanesulfonic acid (5 mL) at 70 °C for 3.5 h. The reaction mixture was poured into ice-water, and neutralized with 10% aqueous $NaHCO_3$ and extracted with ethyl acetate. The organic layer was washed with sat. aqueous $NaHCO_3$ and brine, dried over anhy. $MgSO_4$ and evaporated to dryness in vacuum. The residue was subjected to column chromatography using ethyl acetate:hexane (2:1) to give the title compound (310 mg, 75 %).

Nature of compound: Solid; mp 224-225 °C.

IR (KBr): 3200, 1660, 1640 cm^{-1}

^{1}H-NMR (400 MHz; DMSO-d_6): δ 12.18 (br s, lH), 8.21 and 8.31 (each s, lH), 7.93 (dif. d, *J* = 7.5 Hz, lH), 7.52 (dif. d, *J* = 7.5 Hz, lH), 7.24 (dif. t, *J* = 7.5 Hz, lH), 7.21 (dif. t, *J* = 7.5 Hz, lH), 4.20, 4.24, 4.91, and 4.94 (each s, 4H).

MS (ESI) = m/z-215.09 $[(M+H)^+]$.

Anal. Calcd for $C_{12}H_{10}N_2O_2$: C, 67.28; H, 4.71; N, 13.08. Found: C, 67.37; H, 4.64; N, 13.01.

4-Methoxy-9*H*-pyrido[3,4-*b*]indole (V)

A solution of *p*-toluenesulfonic acid monohydrate (1.033 g, 1.0 eq, 5.321 mmol) in benzene (100 mL) was heated under azeotropic condition for 1 h. After cooling, initial compound (9) (1.0 eq, 5.321 mmol) and dimethoxypropane (2.003 ml, 3.0 eq, 15.963 mmol) was added to a benzene solution, and mixture was

stirred at rt for 1 h. Chloranil (2.643 g, 2.0 eq, 10.642 mmol) was added and whole reaction mixture was stirred at rt for overnight. The reaction mixture was poured into 5 % NaOH, and extracted with CH_2Cl_2. The organic layer was washed with brine, dried over anhy. $MgSO_4$ and evaporated to dryness in vacuum to give a title product (925 mg, 72%).

Nature of compound: Solid; mp: 250-252 °C.

IR (KBr): 3110 (NH) cm^{-1}

^{1}H NMR (400 MHz; $CDCl_3$): δ 11.63 (br s, lH), 8.56 (s, lH), 8.17 (dif. d, *J* = 7.3 Hz, lH), 8.06 (s, lH), 7.59 (dif. d, *J* = 7.3 Hz, lH), 7.51 (dif. t, *J* = 7.3 Hz, lH), 7.24 (dif. t, *J* = 7.3 Hz, lH), 6: 4.12 (S, 3H).

MS (ESI) = m/z 199.56 [$(M+H)^+$].

Anal. Calcd for $C_{12}H_{10}N_2O$: C, 72.71; H, 5.08; N, 14.13; O, 8.07. Found: C, 72.77; H, 5.04; N, 14.17; O, 8.13.

4-Methoxy-β-carboline-*N*-oxide monohydrate (VI)

m-CPBA (250 mg, 1.5 eq, 1.437 mmol) was added to a suspension of 4-methoxy-β-carboline (200 mg, 1.0 eq, 0.958 mmol) in CH_2Cl_2 at rt and then stirred at rt for 24 h. Hexane was added to the mixture. The precipitate was collected by filtration, then residue was extracted with CH_2Cl_2, washed with 10 % aq. K_2CO_3 and water and dried over sodium sulphate and under reduced pressure to give the product. The solid residue was dissolved in CH_2Cl_2 and washed with 10% K_2CO_3 and water then dried over sodium sulphate and solvent evaporated under reduced pressure to give title product (145 mg, 70%).

Nature of compound: Solid; mp: 273-276 °C

IR (KBr): 3500–2600 (NH and H_2O) cm^{-1}

^{1}H-NMR (400 MHz; DMSO-d_6): δ 11.11 (br s. 1H), 8.25 (d, *J* = 1.5 Hz, lH), 8.00 (dif. d, *J* = 7 Hz, lH), 7.84 (d, *J* = 1.5 Hz, lH), 6.99-7.60 (m, 3H), 4.01 (s, 3H,).

MS (ESI): *m*/*z* = 215 [$M + H^+$].

Anal. Calcd for $C_{12}H_{10}N_2O_2$. H_2O: C, 62.06; H, 5.21; N, 12.06. Found: C, 61.84; H, 5.17; N, 11.99.

9-Diethoxyphophoryl-1-cyano-4-methoxy- *β*-carboline (10)

….4-methoxy β-carboline-*N*-oxide monohydrate (268 mg, 1.0 eq, 1.238 mmol) was treated with DEPC (1.116 mL, 5.4 eq, 6.710 mmol) and Et_3N (0.376 mL, 2.1 eq, 2.674 mmol) in CH_3CN (22 mL) at 70 °C for 3 h. The mixture was poured into ice water and extracted with CH_2Cl_2. The organic layer was washed with brine, dried over anhy. $MgSO_4$, and evaporated to dryness in vacuum. The residue was subjected to column chromatography using ethyl acetate: hexane (3:7) to give pure title product (45%).

Nature of compound: Solid; mp: 139-142°C

IR (KBr): 2240 (CN) cm^{-1}

^{1}H NMR (400 MHz; $CDCl_3$): δ 8.24 (s, 1H), 8.10-8.59 (m, 2H), 7.15-7.71 (m, 2H), 4.02-4.55 (m, 4H), 4.20 (s, 3H,), 1.34 (t, *J* = 7 Hz, 6H).

MS (ESI) m/z 360.1 $[(M+H)^+]$.

Anal. Calcd for $C_{17}H_{18}N_3O_4P$: C, 56.83; H, 5.05; N, 11.69. Found: C, 56.71; H, 5.00; N. 11.60.

4-Methoxy-1-methoxycarbonyl- β-carboline (VII)

The substrate of (100 mg, 1.0 eq, 0.276 mmol) was treated in methanol (10 ml) saturated with dry, HCl gas at rt for 24 h, and then heated under reflux for 22 h. Solvent was evaporated in vacuum. Saturated aq. $NaHCO_3$ was added to the residue, and the mixture was extracted with ethyl acetate. The organic layer was washed with saturated aq. $NaHCO_3$ and brine, dried over anhy. sodium sulphate, and evaporated to dryness in vacuum. The residue was subjected to column chromatography using ethyl acetate: hexane (7:3) to give title product (59 mg, 84 %).

Nature of compound: Solid; mp: 196-197°C.

IR (KBr): 3340 (NH), 1670 (CO) cm^{-1}

^{1}H NMR (400 MHz; CD_3OD): δ 11.60 (br s, 1H), 8.23 (S, 1H), 8.21 (dif. d. *J* = 7.8 Hz. lH), 7.79 (dif. d, *J* = 7.8 Hz, lH), 7.57 (dif. t, *J* = 7.8 Hz, lH), 7.30 (dif. t, *J* = 7.8 Hz, lH), 4.25 (s, 3H), 3.97 (s, 3H).

MS (ESI) = m/z 257.56 $[(M+H)^+]$.

Anal. Calcd for $C_{14}H_{12}N_2O_3$: C, 65.62; H, 4.72; N, 10.93. Found: C, 65.77; H, 4.64; N, 10.83.

4-Methoxy- β-carboline-1-carbaldehyde (VIII)

To a stirred solution of compound (**VII**) (52 mg, 1.0 eq, 0.201 mmol) in CH_2Cl_2 (3.0 ml) was added DIBAL (142.22 ml, 6.0 eq, 1.206 mmol) solution at -40 °C under nitrogen atmosphere. The reaction mixture was stirred at -40 °C for 15 min. and then quenched by the sequential addition of MeOH (0.3 ml) and 10% NaOH (0.3 ml) at -40 °C. Then mixture was stirred at rt for an additional 30 min. Then reaction mixture was diluted with $CHCl_3$, dried over anhy. $MgSO_4$, and then evaporated to vacuum. The residue was subjected to column chromatography using ethyl acetate: hexane (1:1) to give a title product (29 mg, 70%).

Nature of compound: Colourless Solid; mp: 209-210 °C.

IR (KBr): 3350, 1428 cm^{-1}

^{1}H NMR (400 MHz; DMSO-d_6): δ 12.08 (br s, 1H), 10.24 (s, 1H), 8.41 (s, 1H), 8.21 (br d, *J* = 8.0 Hz, 1H), 7.79 (br d, *J* = 8.0 Hz, 1H), 7.57 (br t, *J* = 8.0 Hz, 1H), 7.32 (br t, *J* = 8.0 Hz, 1H), 4.38 (s, 3H).

MS (ESI) = m/z 227.36 [$(M+H)^+$].

Anal. Calcd for $C_{13}H_{10}N_2O_2$: C, 69.02; H, 4.45; N, 12.38. Found: C, 68.94; H, 4.39; N, 12.48.

1-Methoxycanthin-6-one (IX)

To a stirred mixture of HMDS (0.199 ml, 8.0 eq, 0.994 mmol) in THF, n-BuLi (2M in hexane) (0.283 mL, 6.0 eq, 0.708 mmol) was added at -78 °C under nitrogen atmosphere. The mixture was warmed to rt within 30 min. with stirring and then recooled to -78 °C. Ethyl acetate (0.054 mL, 4.6 eq, 0.543 mmol) in THF (2.8 mL) was added to the solution and then stirred for 15 min. at -78 °C. To this solution, compound **VIII** (27 mg, 1.0 eq. 0.118 mmol) in THF was added and then stirred for 15 min. at -78 °C. To the reaction mixture, ethanol (1.8 mL) was added and then stirred for 30 min at rt. The whole reaction mixture was poured into aq. sat. NH_4Cl (9.2 ml), extracted with ethyl acetate, washed with brine, dried

over anhy. sodium sulphate and evaporated in vacuum. The crude solid was purified by silica gel column chromatography using ethyl acetate: hexane (7:3) to give the colorless title product (29 mg, 88%).

Nature of compound: white solid; mp: 258-259 °C.

IR (KBr): 1671cm^{-1}

^{1}H NMR (400 MHz; $CDCl_3$): δ 8.70 (br d, *J* = 8.0 Hz, 1H), 8.51 (s, 1H), 8.24 (br d, *J* = 8.0 Hz, 1H), 7.98 (d, *J* = 10 Hz, 1H), 7.68 (br t, *J* = 8 Hz, 1H), 7.53 (br t, *J* = 8 Hz, 1H), 6.86 (d, *J* = 10 Hz, 1H), 4.27 (s, 3H).

^{13}C NMR (75 MHz; $CDCl_3$): δ 55.1, 115.6, 118.6, 121.8, 122.7, 124.1, 126.6, 127.1, 127.9, 128.1, 129.3, 137.6, 138.2, 150.2, 158.3.

MS (ESI) m/z 251.08 [$(M+H)^+$]

Anal. Calcd for $C_{15}H_{10}N_2O_2$: C, 71.99; H, 4.03; N, 11.19. Found: C, 71.91; H, 4.09; N, 11.13.

3-Benzyl-1-methoxycanthin-6-onium bromide (X)

A mixture of 1-methoxycanthin-6-one (30 mg, 0.120 mmol) and benzyl bromide (0.106 mL, 0.9 mmol) in ethyl acetate (5 ml) was refluxed for 7h. After completion of the reaction as indicated by TLC, the solution was cooled and filtered and crystallized to afford **X** in 67% yield.

^{1}H NMR (300 MHz; CD_3OD): δ 9.1 (s, 1H), 8.51 (br d, *J* = 8 Hz, 1H), 8.24 (br d, *J* = 8 Hz, 1H), 7.98 (d, *J* = 10 Hz, 1H), 7.68 (br t, *J* = 8 Hz, 1H), 7.53 (br t, *J* = 8 Hz, 1H), 7.48 (br m, 5H), 6.86 (d, *J* = 10 Hz, 1H), 6.36 (s, 2H), 4.47 (s, 3H).

^{13}C NMR (100 MHz; CD_3OD): δ 56.8, 58.2, 116.8, 117.1, 123.5, 124.3, 125.5, 125.7, 128.6, 129.0. 129.4, 130.1, 130.5, 132.9, 134.2, 138.2, 138.8, 151.9, 160.0.

MS (ESI)) m/z 422.40 ($[M+H]^+$).

Anal. Calcd for $C_{22}H_{17}BrN_2O_2$: C, 62.72; H, 4.07; Br, 18.97; N, 6.65. Found: C, 63.07; H, 4.81; Br, 19.56, N, 5.66.

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CHAPTER-II

Synthesis of new indoles arbidol like derivatives as anti-viral agents

2.1. INTRODUCTION

The field of antiviral drugs is expanding rapidly and antiviral drugs are becoming important and vital agents in medicine, in particular since new human viruses have appeared. Thus the effort to develop effective drugs to combat these new threats has been intensified and these concerns led to national and global surveillance activities as well as to specific and detailed research studies. In addition increasing prevalence of drug-resistant pathogens highlights the need for new antimicrobial drugs. Among a number of different biological active heterocyclic moieties, the indole nucleus constitutes an important block in numerous natural or synthetic alkaloids in medicinal chemistry. Due to the existence of a vast array of structurally diverse and biologically active indoles, it is not surprising that the synthesis and reactivity of indole derivatives have been a topic of research interest for well over a century.

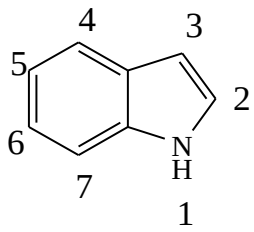


Figure 1: Skeleton structure of Indole

In this chapter the development of new indole structure based inhibitors against influenza virus (HA), hepatitis C virus (HCV) and chikungunya virus (CHIKV) are described

2.1.1. INFLUENZA VIRUS

Influenza viral respiratory infections are the most common illnesses experienced by people of all ages. They are also one of the major causes of morbidity and mortality in elderly people and young children throughout the world.[1-3] Approximately 200 viral respiratory pathogens, the most important are influenza and respiratory syncytial viruses (RSV): other viruses include rhinoviruses, parainfluenza viruses, coxsackie viruses, and adenoviruses.[4]

Influenza is caused by RNA viruses of the family Orthomyxoviridae and they are classified in:

a. Influenza virus A
b. Influenza virus B
c. Influenza virus C

Influenza B is less common than influenza A and influenza C is less common than the other types and usually only causes mild disease in children. Wild aquatic birds are the natural hosts for a large variety of influenza A. Occasionally; viruses are transmitted to other species and may then cause devastating outbreaks in domestic poultry or give rise to human influenza pandemics. The type A viruses are the most virulent human pathogens among the three influenza types and cause the most severe disease.

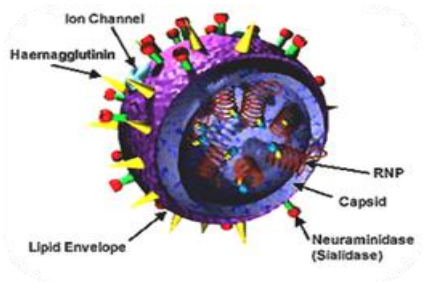


Figure 2. Structure of Influenza virus

Hemagglutinin (HA) and neuraminidase (NA) are the two large glycoproteins on the outside of the viral particles (Figure 2). HA is a lectin that mediates binding of the virus to target cells and entry of the viral genome into the target cell, while NA is involved in the release of progeny virus from infected cells, by cleaving sugars that bind the mature viral particles.[5] Thus, these proteins are targets for antiviral drugs. [6] Furthermore, they are antigens to which antibodies can be raised. Influenza A viruses are classified into subtypes based on antibody responses to HA and NA: [7]

- H1N1, which caused Spanish Flu in 1918, and Swine Flu in 2009
- H2N2, which caused Asian Flu in 1957
- H3N2, which caused Hong Kong Flu in 1968
- H5N1, which caused Bird Flu in 2004
- H7N7, which has unusual zoonotic potential
- H1N2, endemic in humans, pigs and birds
- H9N2
- H7N2
- H7N3
- H10N7

Worldwide influenza pandemics have occurred at irregular and unpredictable intervals throughout history and the impact of pandemic influenza is substantial in terms of morbidity, mortality and economic cost. The chemotherapy options were limited to admantidine or rimantidine, which are only effective against influenza A and often cause side-effects and rapid viral resistance. Recently the launch of the neuraminidase inhibitors zanamivir and oseltamivir give a new option: Nevertheless, the improvement of these options still remains need.[8]

2.1.2. HEPATITIS C VIRUS

Hepatitis C virus is the cause of hepatitis C in humans: this infection currently affects approximately 170 million people worldwide and is resolved in only a minority of patients.[9] The chronic viral infection frequently progresses to end-stage liver disease, cirrhosis and in some cases, to the development of hepatocellular carcinoma. Therefore, hepatitis C is now the most frequent indication for liver transplantation. Hepatitis C virus (HCV) is a small enveloped single-stranded RNA virus of the family Flaviviridae: the genetic material (RNA) is surrounded by an icosahedral protective shell of protein, and further encased in a lipid (fatty) envelope of cellular origin. In the lipid envelope are embedded two viral envelope glycoproteins E_1 and E_2 (Figure 3).

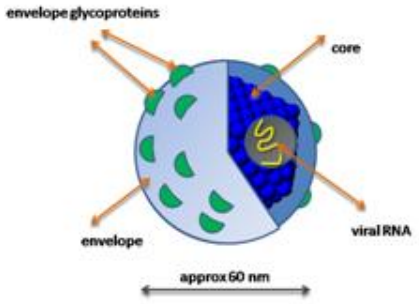


Figure 3. Structure of Hepatitis C virus

There is no therapeutic or prophylactic vaccine available for HCV, and the only effective antiviral therapy, pegylated recombinant interferon a (IFN-a) and ribavirin, produces sustained viral clearance in less than 50% of treated patients.[10] New anti-HCV drugs with novel mechanisms of action are needed.

2.1.3. CHIKUNGUNYA VIRUS

Chikungunya infection is commonly an acute disease marked by febrile arthralgia and a frequent rash: persisting arthralgia has been reported in a significant number of cases.[11] Chikungunya virus (CHIKV) is an arthropod-borne viral disease, this is usually found in tropics and hence the reason why Chikungunya is predominantly seen in asian countries. First described in Tanzania in 1952 which has reemerged since 2005 in Eastern Africa, the Indian Ocean, India and South-East Asia and even reached Europe in 2007.[12] From 2005, this new variant has been responsible for millions of cases of CHIKV disease. Lethal infections are rare but severe cases have been described including neurological presentations and neonatal contaminations which were documented during the outbreak in Reunion Island.[13] Current treatments of Chikungunya fever are for symptoms with no effective licensed vaccine nor specific antiviral drug available. The utilization of the antimalarial chloroquine proved to be poorly active *in vivo* despite it's in cellulo antiviral effect on CHIKV infection.[14,15] Similarly, it has been shown that the combination of interferon-alpha and ribavirin is effective on

CHIKV replication *in vitro* but these compounds have not been tested in animal models and/or clinical trials.[16]

2.1.4. ARBIDOL: A BROAD-SPECTRUM ANTI-VIRAL INDOLE COMPOUND

Arbidol (Fig. 4) is an anti-influenza therapeutic developed in the Russian Research Chemical-Pharmaceutical Institute about 20 years ago[17] and since 1990 this drug has been used in Russia for prophylaxis and treatment of acute respiratory infections.

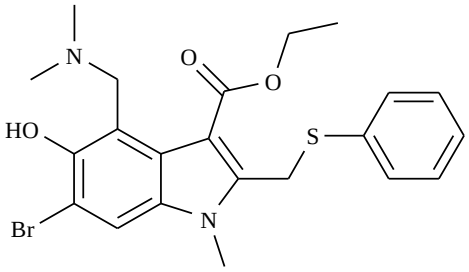


Figure 4. Structure of Arbidol

Until now, it has been shown that ARB exhibits a wide range of activity against a number of RNA, DNA, enveloped and non-enveloped viruses.[18] It shows a broad and potent antiviral activity against a large number of viruses including influenza A, B and C virus,[19] adenovirus,[20] hepatitis B[21] and hepatitis C virus[22] and Chikungunya virus.[23]

The broad-spectrum antiviral activity demonstrated by Arbidol against a number of enveloped and non-enveloped viruses could in part be to due its membrane tropism. Indole derivatives have been shown to exhibit a preference for membrane interfaces due to the flat rigid structure of these molecules, and to their aromaticity which allows them to establish cation-π interactions with the positively-charged quaternary ammonium lipid head groups. The S-phenyl groups of ARB could also interact with the hydrophobic fatty acid chains of phospholipids inside the bilayer. The amino groups could bond the phosphate

moieties of phospholipids, and establish a salt bridge between two adjacent phospholipid molecules as an ion-pair complex.[24] Indeed, it has been shown that:

a) ARB was an entry inhibitor of influenza virus infection by stabilizing the influenza HA and preventing the endosomal membrane fusion.
b) Similarly, ARB proved to be active *in vitro* against HCV virus and the antiviral mechanism was related to inhibition of the HCV glycoprotein conformational changes needed for the membrane fusion process. Arbidol also exerted a clear inhibitory effect on HCV replication, with loss of protein expression and decline in RNA level. One can therefore speculate that ARB-induced inhibition of HCV non-structural protein interactions with organelle membranes that are required for HCV replication might also contribute to ARB's anti HCV actions.[25]
c) Recently ARB was found to be a potent inhibitor against *in vitro* CHIKV virus and this result gave some tracks to understand the molecular basis of its activity. [23]

2.2. PRESENT WORK

a) Arbidol derivatives

By its tropism for membranes and its inhibitory effect on viruses entry, fusion and replication, Arbidol opens promising perspectives in the search for new and efficient antiviral compounds,[24] but since it has a relatively high CC_{50} value and it means toxic, its clinical application is forbidden.[22] So with the aim to reduce its toxicity and improve its antiviral properties a series of novel arbidol derivatives were designed and synthesised focusing our attention on different positions of the indole nucleus (Figure 5).

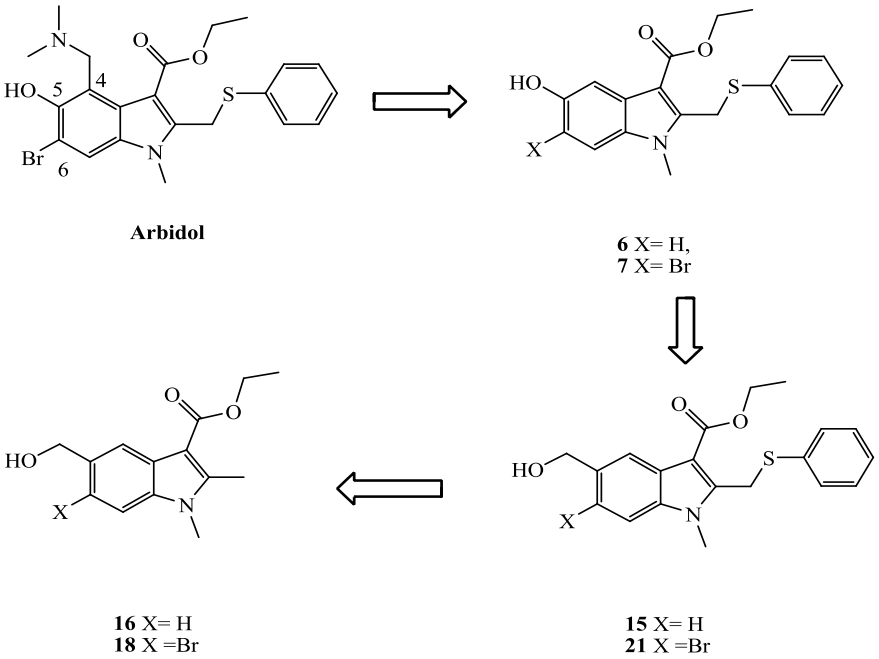
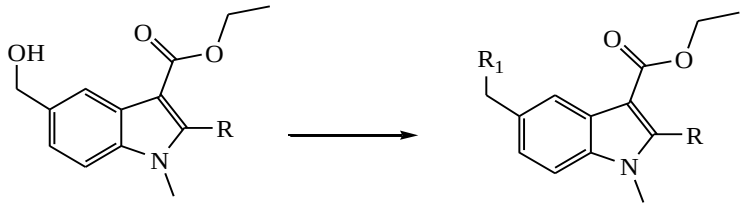


Figure 5. Structural modification on Arbidol, chosen as our *lead compound*

First of all the *N,N*-dimethylamino methyl group in position 4 was removed (compound **6** and **7**). Then phenolic OH in position 5 was homologated in hydroxyl methyl group (compounds **15** and **21**) and subsequently with the aim to investigate the role of phenyl sulfanyl methyl group on the antiviral activity, we introduced a methyl group at the position 2 (compounds **16** and **18**). In addition, to evaluate the role of hydroxy group of compound **15** and **16** in position 5, a new library of compounds were prepared replacing this group with several aliphatic amines and cyclic amines (Table 1).



15 R =CH_2SPh
16 R =CH_3

28 a-h
23 a-n
25, 29, 30

Figure 6

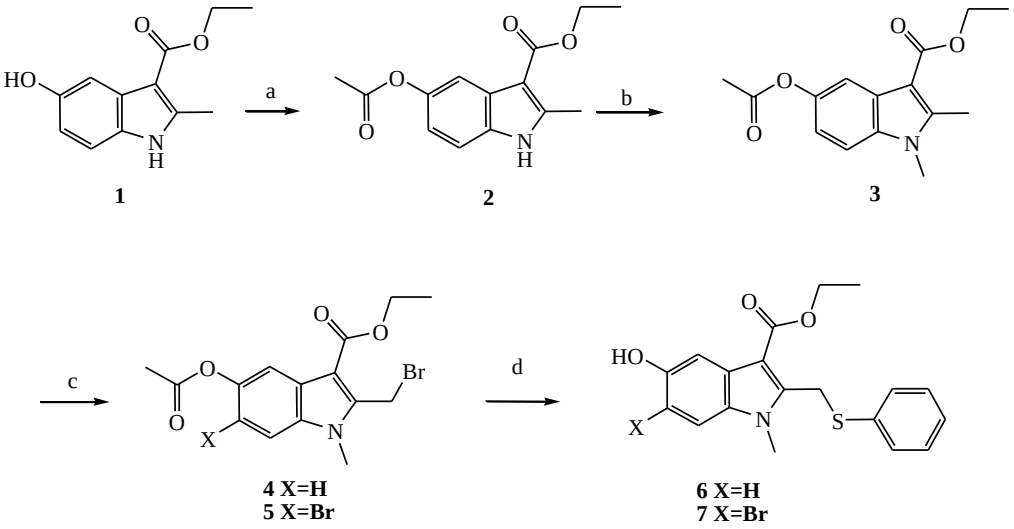
Table 1. Amine indoles derivatives synthesized

Compound	R	R_1
23a	-CH_3	-$N(CH_3)_2$
23b	-CH_3	-$N(CH_2CH_3)_2$
23c	-CH_3	-$N(CH_3)CH_2CH_2CH_2N(CH_2CH_3)_2$
23d	-CH_3	-pyrrolidine
23e	-CH_3	-*N*-methyl piperazine
23f	-CH_3	2-piperazin-1-ylethylamino
23g	CH_3	-$N(CH_3)CH_2CH_2N(CH_2CH_3)_2$
23h	-CH_3	-$N(CH_2CH_3)CH_2CH_2N(CH_2CH_3)_2$
23i	-CH_3	$NHCH_2CH_2NHCH_3$
23j	-CH_3	-$NHCH_2CH_2N$-COO*t*-Bu
23k	-CH_3	-$NHCH_2CH_2N(CH_3)_2$
23l	-CH_3	-$NHCH_2CH_2OH$
23m	-CH_3	-$NHCH_2CH_2NHCH_2CH_2OH$
25	-CH_3	-NH_2
28a	-CH_2SPh	-$N(CH_3)_2$
28b	-CH_2SPh	-$N(CH_2CH_3)_2$
28c	-CH_2SPh	-$N(CH_3)CH_2CH_2CH_2N(CH_2CH_3)_2$
28d	-CH_2SPh	-pyrrolidine
28e	-CH_2SPh	-*N*-methyl piperazine
28f	-CH_2SPh	-*N*-tert-butoxycarbonyl piperazine
28g	-CH_2SPh	-*N*-(2,4 difluoro)phenyl piperazine
28h	-CH_2SPh	-morpholine
29	-CH_3	-diazepan
30	-CH_2SPh	-*N*-methyl-pyrrolidine iodide

Alkyl amines with different chains length and some constrained amines (pyrrolidine, morpholine, piperazine) were used. Bromine was removed from of all amines derivatives since preliminary results showed that bromine was not essential for biological activity.

2.2.1. RESULTS AND DISCUSSION

The desired products were obtained following different schemes. Compound **6** and compound **7** were synthesized according to Scheme 1. Protection of commercially available, ethyl 5-hydroxy-2-methyl-1*H*-indole-3-carboxylate **1** was carried out using acetic anhydride in pyridine under reflux for 1h to give **2**, which was submitted to *N*-methylation dissolving the derivative in DMF, adding NaH 60% and methyl iodide for 1h at room temperature.[26] Bromination of compound **3** in CCl_4 using *N*-Bromosuccinimide gave **4** and **5**,which were easily separated by silica gel column chromatography.[27] In last step nucleophilic displacement of bromine of bromo mrthyl at position 2 by thiophenol in MeOH, using KOH as base gave the final products 6 and 7.[27]

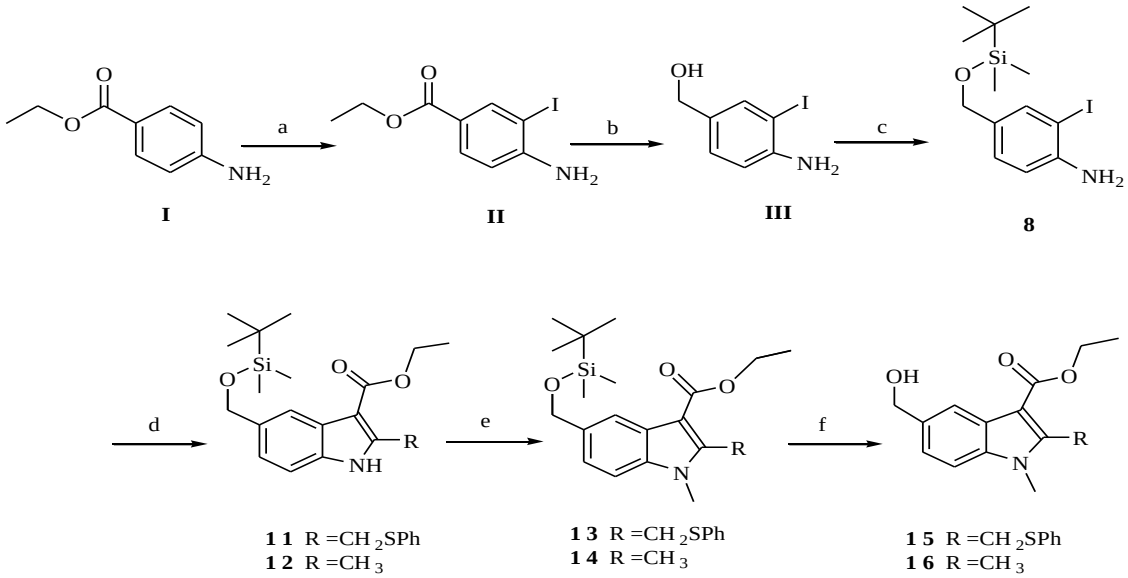


Scheme 1

Reagents and conditions: a) acetic anhydride, pyridine, 1h, reflux. b) CH_3I, NaH 60%, DMF, 1h, rt c) NBS, benzoyl peroxide, CCl_4, overnight, reflux. d) Thiophenol, KOH, CH_3OH, 3h, rt.

Compound **15** and **16** were synthesized according to Scheme 2. Intermediate **8 was** prepared as reported in literature starting from 4-amino ethyl benzoate **I**. [28] This was submitted to a copper-catalyzed (Ullmann-type) reaction using two different *β*-ketoesters (ethyl-3-oxo-4 (phenylthio) butanoate **9**[29] or ethyl acetoacetate **10** to give indoles cyclization products **11** and **12**,[30] in moderate yields. Then they were methylated under similar condition reported in Scheme 1.

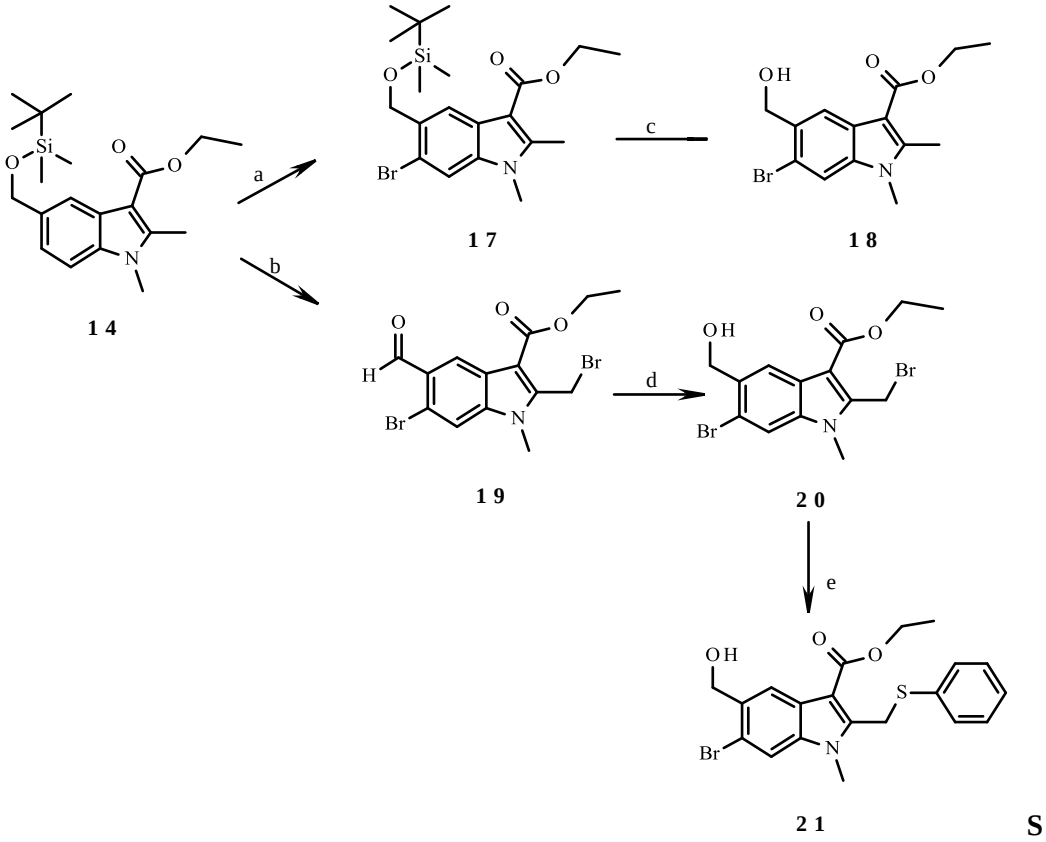
Removal of dimethyltert-butylsilyl group was achieved using tetrabutylamonium fluoride in THF at 0 °C.



Scheme 2

Reagents and conditions: a) $NaIO_4$, KI, NaCl, Acetic acid/water, r.t; b) DIBAL-H 1M CH_2Cl_2, anhy. THF, 0 °C; c) TBDMSiCl, imidazole, DMF, r.t; d) ethyl-3-oxo-4 (phenylthio)butanoate **9** or ethyl acetoacetate **10**, CuI, BINOL, Cs_2CO_3, anhy. DMSO, 50 °C; e) CH_3I, KOH, Acetone, r.t; f) Tetrabutylammonium fluoride, THF, 0 °C.

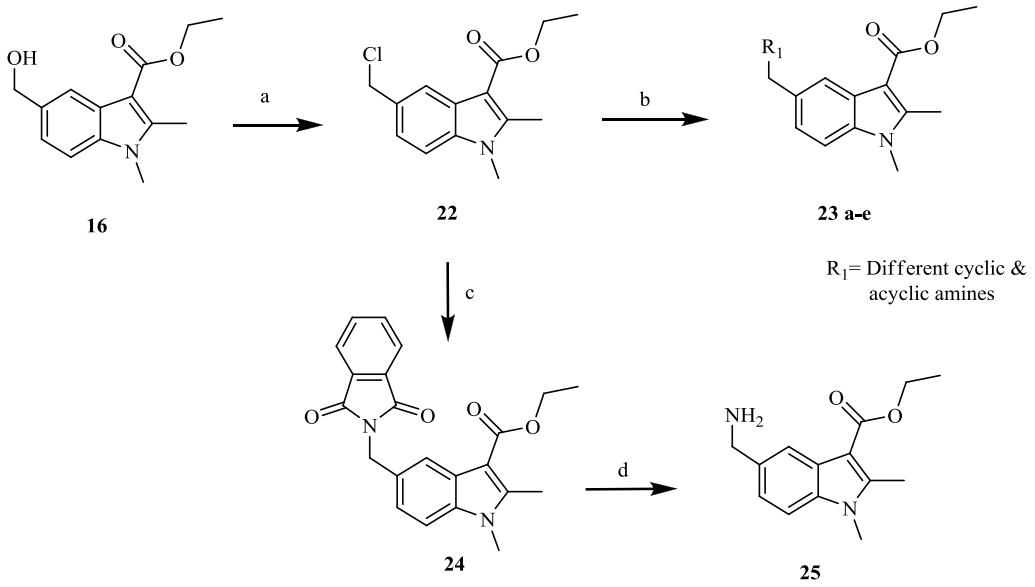
Compound **18** and **21** with bromine in position 6 were synthesized according to Scheme 3 starting from intermediate **14**. Bromination using 1 equivalent of *N*-bromosuccinimide as previously reported gave derivative **17** with bromine in position 6 of indole nucleus. Removal of dimethyltert-butylsilyl group as previously reported was achieved using tetrabutylamonium fluoride in THF 0 °C to give **18**. Bromination using 2 equivalent of *N*-bromosuccinimide gave a mixture complex in which aldehyde **19** was isolated**.** This intermediate was then submitted to a selective reduction of carbonyl function using $NaBH_4$ in THF/MeOH and in the last step nucleophilic displacement of bromine bromomethyl group at position 2 by thiophenol in EtOH, using KOH as base gave the final compound.[27]



cheme 3

Reagents and conditions: a) *N*-Bromosuccinimide (1 equiv.), dibenzoylperoxide, CCl_4, 2h, reflux; b) *N*-Bromosuccinimide (2 equiv.), dibenzoylperoxide, CCl_4, 2h, reflux; c) Tetrabutylammonium fluoride, THF, 1h, 0 °C; d) $NaBH_4$, THF/MeOH, 1h, 0 °C; e) PhSH, KOH, EtOH, r.t, 3h.

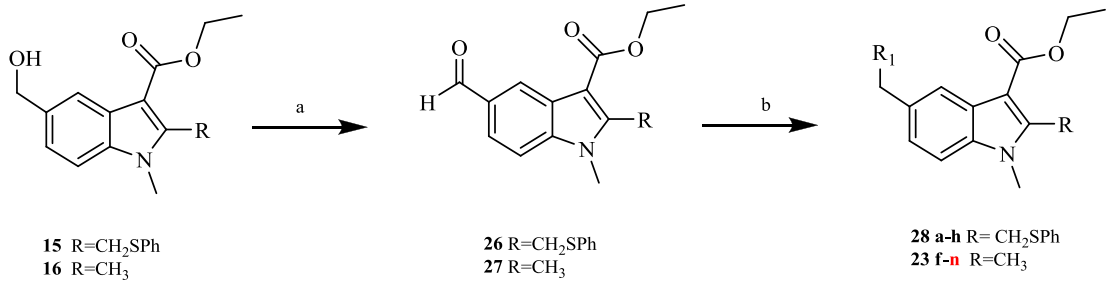
To synthesize amines derivatives two different routes were employed. Amines **23a-e** and **25** were prepared as reported in Scheme 4 starting from intermediate **16**. Hydroxyl group of **16** was converted into chloride **22** using triphenyl phosphine in CCl_4/DMF for 16 h at room temperature.[31] Then nucleophilic displacement of halide by aliphatic amines in DMF in the presence of Cs_2CO_3 under reflux afforded the desired products in moderate yields. When potassium phtalimide was used, intermediate **24** was obtained in quantitative yield according to Gabriel synthesis and following deprotection of subjected to using hydrazine hydrate gave the amine **25**.[32]



Scheme 4

Reagents and conditions: a) PPh_3, CCl_4/DMF, 16h, r.t; b) Aliphatic amines, Cs_2CO_3, DMF, overnight, reflux; c) Potassium phtalimide, DMF, 2h, reflux; d) Hydrazine, EtOH, 2h, reflux.

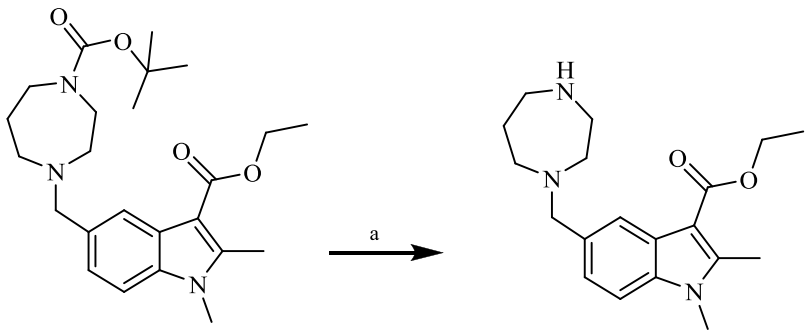
The relative low yield obtained with the previously described method led us to modify our synthetic approach. All the other amines derivatives (**23 f-n, 28 a-h**) were prepared as reported in Scheme 5 starting from intermediate **15** and **16** respectively, which were oxidized to aldehydes using a solution of pyridinium dichromate in CH_2Cl_2. Then reductive amination was carried out in dry 1,2 dichloroethane, in the presence of acetic acid, and $NaBH(OAc)_3$ as reducing agent.



Scheme 5

Reagents and conditions: a) Pyridinium dicromate, CH_2Cl_2, 1h, r.t; b) Aliphatic amines, Acetic acid, $NaBH(OAc)_3$, dry 1,2 dichloroethane, r.t.

Deprotection of amine **23n** prepared as reported in Scheme 5 was achieved using CF_3COOH in dichloromethane at room temperature (Scheme 6).

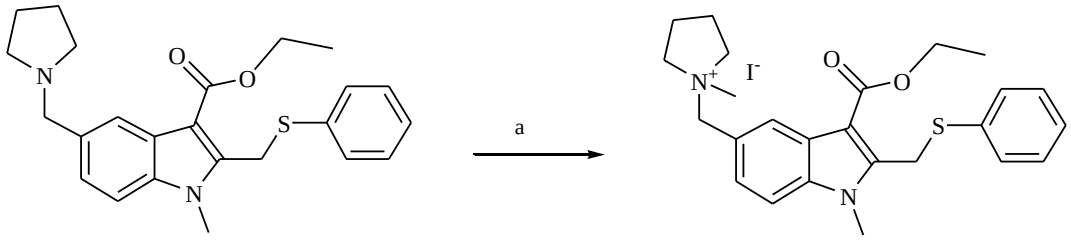


23 n **29**

Scheme 6

Reagents and conditions: a) CF_3COOH, CH_2Cl_2,

Treatment of amine **28d** with methyl iodide gave compound **30** (Scheme 7).



28 d **30**

Scheme 7

Reagents and conditions: a) CH_3I, ethyl acetate, 3h, r.t.

2.3. BIOLOGICAL ACTIVITY OF SYNTHESIZED COMPUNDS

The compounds described herein were tested against influenza virus (HA), hepatitis C virus (HCV) and chikungunya virus (CHIKV). Cytotoxicity assays were also performed to evaluate the toxicity of synthesized compounds.

2.3.1. INFLUENZA VIRUS

The 10 used influenza virus strains were from the World Influenza Centre stock at NIMR, Mill Hill, London and they are reported in Table 2.

Table 2: Virus strains tested

Virus name	Subtype
1. A/California/7/2009	H1N1v
2. A/Brisbane/59/2007	H1N1
3. A/PR8/34 (Puertorico)	H1N1
4. A/Wuhan/359/1995	H3N2
5. AX31	H3N2
6. A/duck/czech/56	H4N6
7. A/Turkey/Italy/214 845	H7N3
8. A/Ty/Ontario/6118	H8N4
9. A/Dk/Memphis/546/74	H11N9
10. B/Brisbane/60/06	B/Vic

The cytotoxicity assay were performed in MDCK cell line exposed to different concentration of Arbidol and its analogues at 24 and 48h of treatment**.** Virus growth and titration were evaluated by hemagglutination assay. Modified enzyme-linked immunoassay (ELISA) (Belshe *et al*., 1988; Grambas and Hay, 1992) was used to measure the inhibition of virus replication by Arbidol and its analogues. CC_{50} of compounds of alcoholic series and amines series are reported in Table 3 and Table 4 respectively.

Table 3. Results of cytotoxicity assay of compounds belonging to alcoholic series

Neutral red assay CC_{50}		
	24h	**48h**
ARBIDOL	115.2μM	
6	125	31.25
7	LC	LC
15	LC	LC
16	LC	LC
18	LC	LC
21	LC	LC

Table 4. Results of cytotoxicity assay of compounds belonging to amine series

Neutral red assay CC_{50}					
	24h	**48h**		**24h**	**48h**
ARBIDOL	115.2μM				
23a	LC	LC	**28a**	42.56μM	50.44μM
23b	LC	LC	**28b**	30.52μM	18.9μM
23c	52.03μM	49.67μM	**28c**	High cytotoxicity	
23d	NT	NT	**28d**	49.3μM	90.3μM
23	NT	NT	**28e**	24.6μM	71.8μM
23f	179.4μM	89.5μM	**28f**	LC	175μM
23g	239μM	183μM	**28g**	LC	225μM
23h	239μM	186.3μM	**28h**	LC	147μM
23i	LC	LC	**29**	LC	193μM
23j	LC	LC	**30**	LC	LC
23k	LC	LC			
23l	LC	LC			
23m	LC	LC			
25	LC	LC			

As is possible to note, the most part of the tested compounds showed a low cytotoxicity (LC) at the highest tested concentration. Compund **6** has CC_{50} value similar to that of Arbidol and it was not tested, while **23c** and **28c** showed an high cytotoxicity.

All the Arbidol derivatives were tested at the different concentration (from 3.9 to 125 μM) for their ability to reduce the plaques number induced by different influenza virus strains in MDCK cells. The data obtained are showed in the Tables **5-7**, reported below.

Table 5. Results of antiviral activity of compounds belonging to alcoholic series

	A/Brisb .59/07 H1N1	A/PR8/ 34 H1N1	A/Califor 7/2009 H1N1-v	A/Wuh3 59/19 H3N2	A/X-31 H3N2	A/Dk/Cze c H4N6	A/TK/ Italy H7N3	A/Ty/Ont 6118 H8N4	A/Dk/ Memph. H11N9	Virus Brisban e/ B
ARB	11.75	15.6	23.41	15.6	7.8	31.25	15.6	15.6	-	31.2
7	31.25	62.5	31.25	31.25	31.2	62.5	-	-	62.25	
15	15.6	31.2	15.6	15.6	3.9	7.8	125	31.25	31.25	62.5
16	-	-	-	-	-	125	-	-	125	-
18	7.9	125	7.9	-	-	7.9	31.25	7.9	7.9	-
21	-	-	-	-	-	125	-	-	125	-

Table 6. Results of antiviral activity of amines with methyl group in position 2

	A/Brisb 59/07 H1N1	A/PR8/ 34 H1N1	A/Califor 7/2009 H1N1-v	A/Wuh3 59/19 H3N2	A/X-31 H3N2	A/Dk/ Czec H4N6	A/TK/ Italy H7	A/Ty/Ont 6118 H8N4	A/Dk/ Memp. H11N9	Virus Briban B
ARB	11.75	15.6	23.41	15.6	7.8	31.2	15.6	15.6	-	31.2
23a	125	62.5	-	125	-	-	-	-	-	
23b	62.5	125	125	15.6	125	-	-	125	125	125
23c	7.9	7.9	7.9	15.6	7.9	15.6	15.6	31.25	15.6	125
23d	7.9	125	-	31.50	62.5	>125	>125	-	-	-
23e	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
23f	31.25	-	31.25	-	62.5	-	-	-	-	-
23g	31.25	-	31.25	-	-	-	-	-	-	-
23h	31.25	-	31.25	-	-	-	-	-	-	-
23i	31.25	-	31.25	-	62.5	-	-	-	-	-
23j	31.25	-	31.25	-	62.5	-	-	-	-	-
23k	31.25	-	31.25	-	62.5	-	-	-	-	-
23l	31.25	-	31.25	-	62.5	-	-	-	-	-
23m	31.25	-	31.25	-	15.6	-	-	-	-	-
25	-	-	-	-	-	-	-	-	-	-
29	-	-	-	-	-	-	125	-	-	-

NT= not tested.

Table 7. Results of antiviral activity of amines with thiophenol group in position 2

	A/Brisb 59/07 H1N1	A/PR8/ 34 H1N1	A/Califor 7/2009 H1N1-v	A/Wuh3 59/19 H3N2	A/X-31 H3N2	A/Dk/Czec H4N6	A/TK/ Italy H7N3	A/Ty/On t 6118 H8N4	A/Dk/ Memp. H11N9	Virus Briban B
ARB	11.75	15.6	23.41	15.6	7.8	31.25	15.6	15.6	-	31.2
28a	7.9	15.6	7.9	7.9	3.9	7.9	31.25	7.9	7.9	31.25
28b	3.9	15.6	3.9	7.9	3.9	7.9	31.25	7.9	7.9	31.25
28c	3.9	3.45	3.9	7.9	15.6	7.9	7.9	7.9	7.9	15.6
28d	7.9	31.25	7.9	7.9	15.6	7.9	46..8	15.6	7.9	125
28e	7.9	31.25	7.9	7.9	15.6	7.9	31.25	15.6	7.9	-
28f	31.25	-	31.25	-	62.5	-	-	-	-	-
28g	31.25	-	31.25	-	62.5	-	-	-	-	-
28h	31.25	-	31.25	-	62.5	-	-	-	-	-
30	31.25	NT	31.25	NT	62.5	NT	NT	NT	NT	NT

NT= not tested.

Safety Index is the ratio between the CC_{50} and the IC_{50}.

It has been calculated only for the compounds with the lowest cytotoxicity and the highest capability to reduce the number of plaques. The data of TI are reported in the Tables **8-10** below.

Table 8. Safety Index values of compounds belonging to alcoholic series

	A/Brisb. 59/07 H1N1	A/PR 8/34 H1N1	A/Califor 7/2009 H1N1-v	A/Wuh35 9/19 H3N2	A/X-31 H3N2	A/Dk/C zec H4N6	A/TK/ Italy H7N3	A/Ty/On 6118 H8N4	A/Dk/ Memph. H11N9	Virus Brisbane / B
ARB	7.38	7.38	7.38	7.38	14.7	14.6	7.38	1.84	-	3.68
7	8	4	8	8	8	4	-	-	4	-
15	16.02	8	16.02	16.02	32.05	32.05	2	8	32.05	4
16	-	-	-	-	-	2	-	-	2	-
18	31.64	2	31.64	-	-	31.64	8	31.64	31.64	-
21	-	-	-	-	4	2	-	2	2	-

Table 9. Safety Index values of amines with methyl group in position 2

	A/Brisb 59/07 H1N1	A/PR8/ 34 H1N1	A/Califor 7/2009 H1N1-v	A/Wuh3 59/19 H3N2	A/X-31 H3N2	A/Dk/ Czec H4N6	A/TK/ Italy H7N3	A/Ty/Ont 6118 H8N4	A/Dk/ Memp. H11N9	Virus Briba ne/ B
ARB	7.38	7.38	7.38	7.38	14.7	14.5	7.38	1.84	-	3.68
23a	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
23b	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
23c	-	6.58	6.58	3.33	6.58	3.33	3.33	1.66	4.42	1.66
23d	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
23e	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
23f	8	-	8	-	4	-	-	-	-	-
23g	7.6	-	7.6	-	3.82	-	-	-	-	-
23h	7.6	-	7.6	-	3.82	-	-	-	-	-
23i	8	-	8	-	4	-	-	-	-	-
23j	8	-	8	-	4	-	-	-	-	-
23k	8	-	8	-	4	-	-	-	-	-
23l	8	-	8	-	4	-	-	-	-	-
23m	8	-	8	-	4	-	-	-	-	-
25	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
29	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

ND= not determined. NT= not tested.

Table 10. Safety Index values of amines with thiophenol group in position 2

	A/Brisb 59/07 H1N1	A/PR8/ 34 H1N1	A/Califor 7/2009 H1N1-v	A/Wuh3 59/19 H3N2	A/X-31 H3N2	A/Dk/ Czec H4N6	A/TK/ Italy H7N3	A/Ty/Ont 6118 H8N4	A/Dk/ Memp. H11N9	Virus Briban B
ARB	7.38	7.38	7.38	7.38	14.7	14.58	7.38	1.84	-	3.68
28a	5.38	2.72	5.38	5.38	10.9	5.38	1.36	5.38	5.38	1.36
28b	8.03	15.6	8.03	3.33	8.03	3.86	0.97	3.86	3.86	0.97
28c	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
28d	62.4	31.25	62.4	62.4	3.16	62.4	1.05	3.16	62.4	0.39
28e	3.11	0.78	3.11	3.11	1.57	3.11	0.78	-	3.11	-
28f	8	-	8	-	4	-	-	-	-	-
28g	8	-	8	-	4	-	-	-	-	-
28h	8	-	8	-	4	-	-	-	-	-
30	8	ND	8	ND	4	ND	ND	ND	ND	ND

ND= not determined.

In the alcohol series the elimination of *N*,*N*-dimethylamino methyl group in postion 4 of Arbidol structure as well as the bromine removal led to compound **6**

with lower antiviral activity, suggesting the importance of the *N*,*N*-dimethylamino methyl and bromine groups into 5-hydroxy indole derivatives. Homologation into 5-hydroxymethyl indole derivative **15** led to a potent replication inhibition, which is comparable to Arbidol, but no cytotoxic effect was observed, as confirmed by good value of safety index (SI = 19.9). The removal of thiophenol moiety was detrimental, since compound **16** was not active at all. Surprisingly the introduction of bromine in position 6 (compound **18** and **21**) led to substancial increase of activity in compound **16** but no in compound **15**. In summary, among these analogues compound **15** showed activity against all the tested viruses, both A and B type; moreover it seems to lead to a better inhibition of some viruses in comparison to Arbidol. It showed a better safety index for the most part of viruses tested compared to Arbidol and the others analogues except for compound **18**. But, **18** was not efficient on all viruses tested and particularly didn't show any effect on B Virus.

For the compounds belonging to the amine series, aliphatic amines possessing different length chains and substitutions were chosen as well as some constrained amines (pyrrolidine, morpholine, piperazine). In particular compounds **23c** and **28c** in which it is possible to recognise the same amine of cloroquine showed very good antiviral activity in comparison with Arbidol, but unfortunately they were found to be cytotoxic.

2.3.2. HEPATITIS C VIRUS

Arbidol and its analogues were tested to evaluate their effect on the replication of Hepatitis C virus (type 1b, Con1 strain) in Cell Huh 7 cells containing subgenomic HCV replicons (Huh 5-2). The cytotoxicity assay were performed in the same cell line exposed to different concentrations. The results are reported in Table 11.

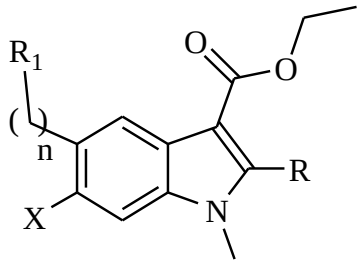


Table 11: Anti-HCV activities and cytotoxicity of synthesized compounds in vitro

Comps	X	n	R	R_1	CC_{50}	EC_{50}	SI
Arbidol					9.94	4.13	2.41
6	Br	0	$-CH_2SPh$	-OH	>146	146	ND
7	H	0	$-CH_2SPh$	-OH	>119	50	2.39
15	H	1	$-CH_2SPh$	-OH	132	6.64	19.9
16	H	1	$-CH_3$	-OH	102	50	2.04
18	Br	1	$-CH_3$	-OH	NT	NT	NT
21	Br	1	$-CH_2SPh$	-OH	115	25.6	4.37
23a	H	1	$-CH_3$	$-N(CH_3)_2$	17.2	3.9	4.39
23b	H	1	$-CH_3$	$-N(CH_2CH_3)_2$	16.8	3.8	4.42
23c	H	1	$-CH_3$	$-N(CH_3)CH_2CH_2CH_2N(CH_2CH_3)_2$	4.1	1.03	3.98
23d	H	1	$-CH_3$	-pyrrolidine	NT	NT	NT
23e)	H	1	$-CH_3$	-*N*-methyl piperazine	32.5	15.2	NT
23f	H	1	$-CH_3$	2-piperazin-1-ylethylamino	>139	61.9	2.25
23g	H	1	CH_3	$-N(CH_3)CH_2CH_2N(CH_2CH_3)_2$	15.3	2.95	5.19
23h	H	1	$-CH_3$	$-N(CH_2CH_3)CH_2CH_2N(CH_2CH_3)_2$	17.6	5.97	2.95
23i	H	1	$-CH_3$	$NHCH_2CH_2NHCH_3$	NT	NT	NT
23j	H	1	$-CH_3$	$-NHCH_2CH_2$Ntert-butoxy carbonyl	NT	NT	NT
23k	H	1	$-CH_3$	$-NHCH_2CH_2N(CH_3)_2$	NT	NT	NT
23l	H	1	$-CH_3$	$-NHCH_2CH_2OH$	NT	NT	NT
23m	H	1	$-CH_3$	$-NHCH_2CH_2NHCH_2CH_2OH$	NT	NT	NT
25	H	1	$-CH_3$	$-NH_2$	ND	>406	ND
28a	H	1	$-CH_2SPh$	$-N(CH_3)_2$	0.609	0.298	2.05
28b	H	1	$-CH_2SPh$	$-N(CH_2CH_3)_2$	2.29	2.12	1.08
28c	H	1	$-CH_2SPh$	$-N(CH_3)CH_2CH_2CH_2N(CH_2CH_3)_2$	<0.80	<0.80	ND

28d	H	1	$-CH_2SPh$	-pyrrolidine	1.94	1	1
28e	H	1	$-CH_2SPh$	-*N*-methyl piperazine	NT	NT	NT
28f	H	1	$-CH_2SPh$	-*N*-tert-butoxycarbonyl piperazine	24.8	11	1.94
28g	H	1	$-CH_2SPh$	-*N*-(2,4 difluoro)phenyl piperazine	36.2	5.17	6.99
28h	H	1	$-CH_2SPh$	-morpholine	2.05	<0.94	2.18
29	H	1	$-CH_3$	-diazepan	NT	NT	NT
30	H	1	$-CH_2SPh$	-*N*-methyl-pyrrolidine iodide	NT	NT	NT

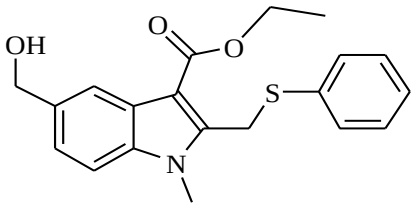
ND= not determined. NT= not tested. CC_{50} (mg/ml) = 50% Cytostatic/Cytotoxic Concentration (concentration at which 50% adverse effect is observed in the host cells. EC_{50} (mg/ml) = Effective Concentration (concentration at which 50% inhibition of virus replication is observed. SI= safety index (CC_{50}/EC_{50}).

Significant inhibitory effect on HCV virus replication was observed for most of the compounds.

In the alcohol series the elimination of dimethylamino group in postion 4 of Arbidol structure as well as the bromine removal were detrimental for antiviral activity since compound **6** and **7** have EC_{50}=146 mg/mL and 50 mg/mL respectively. This suggest the importance of the dimethylamino and bromine groups into 5-hydroxy indole derivatives.

Further homologation of both compounds gave important elucidation about structure activity relationship into 5-hydroxymethyl indole derivatives, because even if compound **15** showed a replication inhibitory effect, comparable to Arbidol however it was no cytotoxic as confirmed by good value of safety index (SI = 19.9).

As observed in the case of influenza virus, the introduction of bromine in position 6 (compound **21**) didn't lead to an increase of activity, while the replacement of thiophenol moiety in position 2 by a methyl was detrimental (compound **16**). In any case compound **15** could be considered a good *lead compound* for further investigations.



15

In the amines series all the compounds were highly cytotoxic even though they showed significant inhibitory effect on HCV virus replication. Thus structural modifications could be desirable to try to reduce toxicity, for example converting compounds **28a-h** in sulfoxides: infact it know that the oxidation of sulfide into sulfinyl reduced the cellular toxicities.[21]

2.3.3. CHIKUNGUNYA VIRUS

The effect on the replication of Chikungunya virus of Arbidol and its analogues were tested *in vero* cells. The cytotoxicity assay were performed in the same cell line. The results are reported in Table 12.

Most of the compounds didn't show any inhibitory effect on Chikungunya virus replication**.** In the alcohol series, no activity was found and only compound **15** showed EC_{50} = 56 mg/mL, higher than our lead compound. Four compounds in the amine series (**28 b-d** and **28 f**) showed an interesting activity when compared to Arbidol, but unfortunately they have very high cytotoxicity in the host cells. It is worthy to note that all these compounds possess a thiophenol moiety in position 2 of the indole nucleus, and when we compared the same compounds in which thiophenol moiety was removed (for example compounds **28 c** and **23 c**) it was possible to note absence of effect and cytotoxicity.

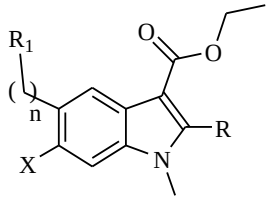


Fig. 9

Table 11: Anti-CHIKV activities and cytotoxicity of synthesized compounds in vero cells

Comps	X	n	R	R_1	CC_{50}	EC_{50}	SI
Arbidol					83	18.4	4.52
6	Br	0	$-CH_2SPh$	-OH	>238	152	1.57
7	H	0	$-CH_2SPh$	-OH	ND	>293	ND
15	H	1	$-CH_2SPh$	-OH	ND	>56	ND
16	H	1	$-CH_3$	-OH	ND	>404	ND
18	Br	1	$-CH_3$	-OH	NT	NT	NT
21	Br	1	$-CH_2SPh$	-OH	ND	>231	ND
23a	H	1	$-CH_3$	$-N(CH_3)_2$	ND	>364	ND
23b	H	1	$-CH_3$	$-N(CH_2CH_3)_2$	119	36	3.27
23c	H	1	$-CH_3$	$-N(CH_3)CH_2CH_2CH_2N(CH_2CH_3)_2$	ND	>258	ND
23d	H	1	$-CH_3$	-pyrrolidine	NT	NT	T
23e	H	1	$-CH_3$	-*N*-methyl piperazine	ND	304	D
23f	H	1	$-CH_3$	2-piperazin-1-ylethylamino	ND	>364	ND
23g	H	1	CH_3	$-N(CH_3)CH_2CH_2N(CH_2CH_3)_2$	ND	>278	ND
23h	H	1	$-CH_3$	$-N(CH_2CH_3)CH_2CH_2N(CH_2CH_3)_2$	ND	ND	ND
23i	H	1	$-CH_3$	$NHCH_2CH_2NHCH_3$	NT	NT	NT
23j	H	1	$-CH_3$	$-NHCH_2CH_2N$tert-butoxy carbonyl	NT	NT	NT
23k	H	1	$-CH_3$	$-NHCH_2CH_2N(CH_3)_2$	NT	NT	NT
23l	H	1	$-CH_3$	$-NHCH_2CH_2OH$	NT	NT	NT
23m	H	1	$-CH_3$	$-NHCH_2CH_2NHCH_2CH_2OH$	NT	NT	NT
25	H	1	$-CH_3$	$-NH_2$	ND	406	ND
28a	H	1	$-CH_2SPh$	$-N(CH_3)_2$	ND	ND	ND
28b	H	1	$-CH_2SPh$	$-N(CH_2CH_3)_2$	21.6	4.16	5.18
28c	H	1	$-CH_2SPh$	$-N(CH_3)CH_2CH_2CH_2N(CH_2CH_3)_2$	2.93	<1.61	1.81
28d	H	1	$-CH_2SPh$	-pyrrolidine	16.6	5.41	3.07
28e	H	1	$-CH_2SPh$	-*N*-methyl piperazine	NT	NT	NT
28f	H	1	$-CH_2SPh$	-*N*-tert-butoxycarbonyl piperazine	15.5	4.3	3.61
28g	H	1	$-CH_2SPh$	-*N*-(2,4 difluoro)phenyl piperazine	ND	ND	ND
28h	H	1	$-CH_2SPh$	-morpholine	ND	>236	ND
29	H	1	$-CH_3$	-diazepan	NT	NT	NT
30	H	1	$-CH_2SPh$	-*N*-methyl-pyrrolidine iodide	NT	NT	NT

ND= not determined. NT= not tested. CC_{50} (mg/ml) = 50% Cytostatic/Cytotoxic Concentration (concentration at which 50% adverse effect is observed in the host cells. EC_{50} (mg/ml) = Effective Concentration (concentration at which 50% inhibition of virus replication is observed. SI= safety index (CC_{50}/EC_{50}).

This suggested that the introduction of thiophenol moiety is essential for the activity on virus replication but also responsible of cytotoxicity in the host cells. Thus structural modifications are desiderable to converting them in sulfoxides as previously reported.[21]

2.4. CONCLUSION

In conclusion a library of indole Arbidol like derivatives have been designed and synthesized for testing their antiviral activity on three different virus (influenza virus (HA), hepatitis C virus (HCV) and chikungunya virus (CHIKV)). Biological evaluation led us to discovery of a new potent influenza virus replication, identified in compound **15**. This compound was also found to be a promising *lead compound* for the design of new HCV virus replication inhibitors. Thus further structural modifications are planning by focusing on the thiophenol ring (elettronrich, elettronpoor substituents or bulky groups could be introduced) or replacing the methyl group on the nitrogen atom of indole nucleus with other moieties. Currently biological study are in course to better study mechanism underwent the action of compound **15**, which could act non only as virus replication inhibitor but also as fusion inhibitor. Then a focused analysis on the interaction of this compound with the HA protein will be carried out.

2.5. EXPERIMENTAL SECTION

a) Biology

i) Cell line and virus

Madin Darby Canine Kidney (MDCK) cells were grown in Dulbecco's Modified Eagle Medium (Sigma D6429), supplemented with 10% foetal calf serum (FCS) inactivated 56 ^{o}C for 1h, Penicillin (100 μg/ml) and streptomycin (100 μg/mL) (Sigma P0781) in a humidified atmosphere at 5% CO_2. The influenza virus strains tested were from the World Influenza Centre stock at NIMR, Mill Hill, London. Virus stocks were grown in the allantoic cavities of 11-day-old embryonated hen

eggs for use in all the experiments. The allantoic fluid was cleared by low-speed centrifugation and stored at 80 °C.

ii) Virus titration

Virus growth and titre were evaluated by hemagglutination assay. Briefly: to 50 µl of virus dilution (2-fold from 1:2 to 1:4096) in PBS, 50 µl of 1% for guinea pig red blood cells (TCS Biosciences PB031) suspension were added and incubated for 60 min at room temperature. The same protocol was performed using 50 µl of 0.75% Turkey red blood cells too (from HPA, Colindale, London) and incubated for 30 minutes at RT. The results obtained using the two different red blood cells are then compared. After the incubation, the agglutinated RBCs settle as a diffuse layer in contrast to the control RBCs which form a compact button on the bottom of the wells. Haemagglutination was determined by tilting the plates and noting the absence of tear-shaped streaming of erythrocytes, which flow at the same rate as RBC controls. The highest dilution of virus that causes complete haemagglutination is considered the HA titration end point. The HA titre is the reciprocal of the dilution of virus in the last well with complete haemagglutination.

iii) Cytotoxicity assay: evaluation of cell viability with neutral red assay

The neutral red assay determines the accumulation of the neutral red dye in the lysosomes of viable (uninjured) cells after incubation with test agents. MDCK cells were plated in 96-well and incubated for 24 h in a humidified atmosphere at 5% CO_2. The cells were exposed to the compounds, dissolved in DMEM with 5% FCS at different concentration (250 µM, 125 µM, 62.5 µM, 31.25 µM, 15.6 µM, 7.8 µM and 3.9 µM), and incubated for 24h and 48h.

Then, cells were washed with PBS and incubated for 2 h with neutral red dye (3-amino-7-dimethylamino-2-methyl-phenazine hydrochloride-Sigma, N4638) dissolved in serum free medium (DMEM) at a final concentration of 40 μg/mL. Cells were then washed again with a PBS solution, to remove the dead cells. The dye was then extracted from the intact cells with a 1% glacial acetic acid in 50% ethanol destaining solution (Glacial acetic acid Sigma, 537020; Ethanol Riedel-de Haen, 32294). The absorbance of the solution was read at spectrofluorimeter with

excitation and emission wavelengths of 530 and 645 nm, respectively. The absorbance recorded is related to the concentration of viable cells. Three independent experiments were performed and the CC_{50} (drug concentration required to reduce cell growth by 50%) was calculated at 24h and 48h.

iv) Plaque reduction assay

A modified enzyme-linked immunoassay (ELISA) (Belshe *et al*., 1988; Grambas and Hay, 1992) was used to measure the inhibition of virus replication by Arbidol and its analogues. Briefly, MDCK cells $3x10^3$ cells per well were seeded in 96-well plates in DMEM containing 10% FBS, 100U/ml penicillin, 100 μg/mL streptomycin sulphate. Cells were incubated at 37 °C at 5% CO_2 until 90% cell confluence was reached, then the cells were washed four times with fresh DMEM with pen/strep. Each micro-titre plate included uninfected control wells, virus-infected control wells and virus-infected wells to which ARB or its derivatives were added. Each compound was two-fold serial diluted to obtain different concentration from 125 to 3.9 μM and added to the MDCK monolayer. After incubation for 30 min at 37 °C, 100 μl of virus-containing allantoic fluid (approximately 0.1 PFU/cell) was added to each well, except uninfected control cells. After 3h of infection, cells were overlaid with DMEM medium supplemented with 0.2% Bovin Serum Albumin, Avicell 2.5 g/l, and 2.5 μg/mL TPCK-trypsin (Sigma T-1426).

The antiviral assays and cytostatic determination assays for HCV virus and chikungunya virus (CHIKV)) were performed on the basis of the procedures reported by Neyts.[33]

b) <u>Chemistry</u>

All reagents were analytical grade and purchased from Sigma–Aldrich (Milano, Italy). Flash chromatography was performed on Carlo Erba silica gel 60 (230–400 mesh; CarloErba, Milan, Italy). TLC was carried out using plates coated with silica gel 60F 254 nm purchased from Merck (Darmstadt, Germany). ^1H and ^{13}C NMR spectra were registered on a Brucker AC 300. Chemical shifts are reported in ppm. The abbreviations used are follows: s, singlet; d, doublet; dd double

doublet; bs, broad signal. MS spectrometry analysis ESI-MS was carried out on a Finnigan LCQ Deca ion trap instrument. Microanalyses were carried out on a Carlo Erba 1106 elemental analyzer. Melting points were performed by Stuart melting point SMP30 and are uncorrected.

d) Procedures and characterization data

Ethyl 5-acetoxy-2-methyl-1*H*-indole-3-carboxylate (2)

A mixture of ethyl 5-hydroxy-2-methyl-1*H*-indole-3-carboxylate (**1**) (3 g, 13.55 mmol), acetic anhydride (26 mL, 270 mmol) and pyridine (3.32 mL, 40.65 mmol) was refluxed for 1h. The cooled reaction medium was then poured into saturated sodium bicarbonate solution. After extraction with ethyl acetate, the combined organic layers were washed with water, dried over sodium sulfate anhydrous and then concentrated. The product was purified by flash chromatography using hexane/ethyl acetate (7/3).

Nature of compound: White powder; Yield: 74%.

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.32 (N-H), 7.75 (d, *J* = 1.76 Hz, 1H), 7.24 (d, *J* = 8.79 Hz, 1H), 6.90 (dd, *J* = 8.79, 1.76 Hz, 1H), 4.38 (q, *J* = 7.1 Hz, 2H), 2.73 (s, 3H), 2.34 (s, 3H), 1.43 (t, *J* = 7.1 Hz, 3H).

MS (ESI): *m*/*z* = 262 [M + H^+].

Anal. Calcd for $C_{14}H_{15}NO_4$: C, 64.36; H, 5.79; N, 5.36. Found: C, 64.52; H, 5.30; N, 6.68.

Ethyl 5-acetoxy-1, 2-dimethyl-1*H*-indole-3-carboxylate (3)

A mixture of ethyl 5-acetoxy-2-methyl-1*H*-indole-3-carboxylate (**2**) (1.86 g, 6.96 mmol) and anhydrous DMF (30 mL) was stirred at rt until clear, and then 60% NaH (0.255 g, 10.44 mmol) and iodomethane (1.31 mL, 20.37 mmol) were added. The mixture was stirred at rt for 1 h, The resulting mixture was poured into H_2O, and extracted with ethyl acetate. The organic phase was washed with water and brine, then dried over anhydrous sodium sulfate, filtered and evaporated. The residue obtained was purified by silica column chromatography with hexane/ethyl acetate (8/2) as an eluent.

Nature of compound: White powder; Yield: 70%.

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.77 (d, *J* = 1.76 Hz, 1H), 7.25 (d, *J* = 8.79 Hz, 1H), 6.93 (dd, *J* = 8.79, 1.76 Hz, 1H), 4.37 (q, *J* = 7.1 Hz, 2H), 3.67 (s, 3H), 2.75 (s, 3H), 2.32 (s, 3H), 1.42 (t, *J* = 7.1 Hz, 3 H).

MS (ESI): *m*/*z* = 298 [M + Na^+].

Anal. Calcd for $C_{15}H_{17}NO_4$: C, 65.44; H, 6.22; N, 5.09. Found: C, 65.27; H, 6.65; N, 5.43.

Ethyl 5-acetoxy-2-(bromomethyl)-1-methyl-1*H*-indole-3-carboxylate (4) and ethyl 5-acetoxy-6-bromo-2-(bromomethyl)-1-methyl-1*H*-indole-3-carboxylate (5)

Ethyl 5-acetoxy-1, 2-dimethyl-1*H*-indole-3-carboxylate (**3**) (0.814 g, 2.89 mmol) and *N*-bromosuccinimide (0.521 g, 2.89 mmol) were dissolved in degassed CCl_4 (100 mL). Benzoyl peroxide (50 mg, 0.20 mmol) was added and the reaction was refluxed over night. The solution was cooled to rt and diluted with CH_2Cl_2. The organic phase was washed with water, brine and dried over anhydrous Na_2SO_4. The product was purified by flash chromatography using toluene/ethyl acetate (95/5) as an eluent.

Nature of compound: Pale yellow powder; Yield: 46%.

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.84 (d, *J* = 1.76 Hz, 1H), 7.34 (d, *J* = 8.79 Hz, 1H), 7.04 (dd, *J* = 8.79, 1.76 Hz, 1H), 5.16 (s, 2H), 4.44 (q, *J* = 7.1 Hz, 2H), 3.82 (s, 3H), 2.34 (s, 3H), 1.46 (t, *J* = 7.1 Hz, 3H).

MS (ESI): *m*/*z* = 355 [M + H^+].

Anal. Calcd for $C_{15}H_{16}BrNO_4$: C, 50.86; H, 4.55; N, 3.95. Found: C, 50.47; H, 4.65; N, 3.43.

From this purification we recovered also ethyl 5-acetoxy-6-bromo-2-(bromomethyl)-1-methyl-1*H*-indole-3-carboxylate (**5).**

Nature of compound: Pale yellow powder; Yield: 6%.

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.87 (s, 1H), 7.55 (s, 1H), 5.08 (s, 2H), 4.42 (q, *J* = 7.1 Hz, 2H), 3.74 (s, 3H), 2.39 (s, 3H), 1.43 (t, *J* = 7.1 Hz, 3H).

General procedures for (6 and 7)

To a solution containing KOH (3.0 eq.) in 1 mL of methanol at room temperature different thiophenols were added (1 eq). After 15 minutes, **4** or **5** (1 eq.) was also added. The mixture was allowed to stay to room temperature for 3 h followed by neutralizing with a diluted acetic acid. The mixture was evaporated and the product was purified by flash chromatography.

Ethyl 5-hydroxy-1-methyl-2-(phenylthiomethyl)-1*H*-indole-3-carboxylate (6)

Elution with hexane/ethyl acetate (7.5/2.5).

Nature of compound: White powder; Yield: 93 %; mp. 168°C-172 °C.

^{1}H NMR (DMSO-d_6): 9.02 (s, OH), 7.19-7.39 (m, 7H), 6.73 (dd, *J* = 8.79, 1.76 Hz, 1H), 4.82 (s, 2H), 4.20 (q, *J* = 7.1 Hz, 2H), 3.67 (s, 3H), 1.28 (t, *J* = 7.1 Hz, 3H).

MS (ESI):m/z = 364.42 [M + Na^+].

Anal. calcd for $C_{19}H_{19}NO_3S$: C, 66.84; H, 5.61; N, 4.10; S, 9.39, Found: C, 66.79; H, 5.55; N, 4.04; S, 9.44

Ethyl 6-bromo-5-hydroxy-1-methyl-2-(phenylthiomethyl)-1*H*-indole-3-carboxylate (7)

Elution with hexane/ethyl acetate (9/1)

Nature of compound : Pale yellow powder; Yield: 41%. mp. 165-169 °C.

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.72 (s, 1H), 7.48 (s, 1H), 7.28-7.43 (m, 5H), 4.72 (s, 2H), 4.42 (q, *J* = 7.1 Hz, 2H), 3.64 (s, 3H), 1.43 (t, *J* = 7.1 Hz, 3H).

MS (ESI): m/z = 443.32 [M + Na^+].

Anal. calcd for $C_{19}H_{18}BrNO_3S$: C, 54.29; H, 4.32; Br, 19.01; N, 3.33; S, 7.63, Found: C, 54.34; H, 4.22; Br, 19.07; N, 3.30; S, 7.55.

General procedure for the synthesis of compounds (11 and 12)

A mixture of amine **8** (4.17 mmol), ethyl-3-oxo-4 (phenylthio) butanoate (**9)** or ethylacetoacetate (**10)** (4.58 mmol), CuI (0.42 mmol), BINOL (0.500 mmol) and Cs_2CO_3 (4.17 mmol) in dry DMSO (15 mL) was stirred at 50 °C for 7 h under an atmosphere of nitrogen. The mixture was partitioned between ethyl acetate and saturated NH_4Cl, the organic layer was washed with brine, dried over anhy.

$MgSO_4$ and concentrated on vacuum, affording a dark oil. The residue was purified by flash chromatography (hexane/ethyl acetate 9/1).

Ethyl 2-(phenylsulphanylmethyl) 5-((tert-butyldimethylsilyloxy)methyl)-1*H*-indole-3 carboxylate (11)

Nature of compound: White solid; Yield: (0.700 g) 37%.

^{1}H NMR (300 MHz, $CDCl_3$): δ 8.98 (s, 1H), 8.03 (s, 1H), 7.28-7.34 (m, 7H), 4.80 (s, 2H), 4.72 (s, 2H), 4.41 (q, *J* = 7.2 Hz, 2H), 1.46 (t, *J* = 7.2 Hz, 3H), 0.97 (s, 9H), 0.13 (s, 6H).

MS (ESI): m/z = 478.20 [M + Na^+].

Anal. calcd for $C_{25}H_{33}NO_3SSi$: C, 65.89; H, 7.30; N, 3.07; S, 7.04, Found: C, 65.34; H, 7.22; N, 3.30; S, 7.55.

Ethyl 2-methyl 5-((*tert*-butyldimethylsilyloxy)methyl)-1*H*-indole-3 carboxylate (12)

Nature of compound : White solid; Yield: (0.235 g). 56%.

^{1}H NMR (300 MHz, $CDCl_3$): δ 8.10 (s, 1H), 8.08 (s, 1H), 7.28 (br s, 2H), 4.88 (s, 2H), 4.41 (q, *J* = 7.2 Hz, 2H), 2.79 (s, 3H), 1.46 (t, *J*= 7.2 Hz, 3H), 0.97 (s, 9H), 0.13 (s, 6H).

MS (ESI): m/z = 370.19 [M + Na^+].

Anal. calcd for $C_{19}H_{29}NO_3Si$: C, 65.67; H, 8.41; N, 4.03. Found: C, 65.34; H, 8.22; N, 4.30.

General procedure for the synthesis of compounds (13 and 14)

To a solution of compound **11** or **12** (1.52 mmol) in acetone (20 mL), KOH pellets (7.65 mmol) was added. After 30 minutes, CH_3I (191 µL, 3.04 mmol) was added dropwise and the mixture was allowed to stirred for 1h. The solvent was removed, the residue taken up with ethyl acetate and washed with brine. The organic layer was dried over anhy. Na_2SO_4 and concentrated on rota to afford pure product.

Ethyl 1-methyl 2-(phenysulphanyl)methyl) 5-((*tert*-butyldimethylsilyloxy)methyl)-1*H*-indole-3 carboxylate (13)

Nature of compound : White solid; Yield: (0.702 g) 98%.

^{1}H NMR (300 MHz, $CDCl_3$): δ 8.03 (s, 1H) 7.28-7.34 (m, 7H), 4.80 (s, 2H), 4.72 (s, 2H), 4.41 (q, *J* = 7.2 Hz, 2H), 3.79 (s, 3H), 1.46 (t, *J*= 7.2 Hz, 3H), 0.97 (s, 9H), 0.13 (s, 6H).

MS (ESI): m/z = 492.21 [M + Na^+].

Anal. calcd for $C_{26}H_{35}NO_3SSi$: C, 66.48; H, 7.51; N, 2.98; S, 6.83, Found: C, 66.34; H, 7.72; N, 2.40; S, 6.25.

Ethyl 1, 2-dimethyl 5-((tert-butyldimethylsilyloxy) methyl)-1*H*-indole-3 carboxylate (14)

Nature of compound : White solid; Yield: (0.380 g). 82%.

^{1}H NMR (300 MHz, $CDCl_3$): δ 8.08 (s, 1H), 7.28 (br s, 2H), 4.88 (s, 2H), 4.41 (q, *J* = 7.2 Hz, 2H), 3.72 (s, 3H), 2.79 (s, 3H), 1.46 (t, *J* = 7.2 Hz, 3H), 0.97 (s, 9H), 0.13 (s, 6H).

MS (ESI): m/z = 362.55 [M + H^+].

Anal. calcd for $C_{20}H_{31}NO_3Si$: C, 66.44; H, 8.64; N, 3.87. Found: C, 66.67; H, 8.97; N, 3.30.

General procedure for the synthesis of compounds (15 and 16)

A solution of compound **13** or **14** (0.895 mmol) in THF (8 mL) was chilled at 0 °C and tetrabutylammonium fluoride (1.0 ml, 1.0 mmol, 1 M in THF) was added keeping the reaction under magnetic stirring for 2 h. The reaction was quenched by the addition of saturated aqueous solution of $NaHCO_3$ (15 ml). Then water (10 mL) was added and the product was extracted with ethyl acetate (3 × 5 mL). The combined organic layer were dried over anhydrous $MgSO_4$ and evaporation of the solvent under reduced pressure gave almost pure product.

Ethyl-5-(hydroxymethyl)-1-methyl-2-(phenysulphanylmethyl)-1*H*-indole-3-carboxylate (15)

Nature of compound: White solid; Yield: (0.702 g) 98%. Mp: 111-112 °C.

^{1}H NMR (300 MHz, $CDCl_3$): δ 8.08 (s, 1H), 7.25-7.35 (m, 7H), 4.81 (s, 2H), 4.68 (s, 2H), 4.27 (q, *J* = 7.2 Hz, 2H), 3.81 (s, 3H), 1.39 (t, *J* = 7.2 Hz, 3H).

MS (ESI): m/z = 356.12 $[M + H^+]$.

Anal. calcd for $C_{20}H_{21}NO_3S$: C, 67.58; H, 5.95; N, 3.94; S, 9.02, Found: C, 68.34; H, 5.12; N, 3.30; S, 8.55.

Ethyl 1, 2-dimethyl-5-(hydroxymethyl)-1*H*-indole-3-carboxylate (16)

Nature of compound : White solid; Yield**:** (0.700 g) 93%.

^{1}H NMR (300 MHz, $CDCl_3$): δ 8.13 (s, 1H), 7.31 (br s, 2H), 4.82 (s, 2H), 4.43 (q, *J* = 7.2 Hz, 2H), 3.74 (s, 3H), 2.80 (s, 3H), 1.47 (t, *J* = 7.2 Hz, 3H).

MS (ESI): m/z = 270.12 $[M + Na^+]$.

Anal. calcd for $C_{14}H_{17}NO_3$: C, 68.00; H, 6.93; N, 5.66, Found: C, 67.34; H, 6.12; N, 5.10.

Ethyl-6-bromo-1,2-dimethyl-5-((tert-butyldimethylsilyloxy)methyl)-1*H*-indole-3 carboxylate (17)

A solution of **14** (0.110 g, 0.301 mmol) in CCl_4 (5 mL) was brought to reflux: *N*-bromosuccinimide (0.54 g, 0.301 mmol) and dibenzoyl peroxide (0.04 g, 0.015 mmol) were then added. The mixture was stirred at reflux temperature for 2h. The solid was filtered off, the filtrate concentrated and then purified by flash chromatography (toluene/acetone 98/2) recovering a 0.021 g of a white solid.

Nature of compound: White solid; Yield: (0.040 g) 34%

^{1}H NMR (300 MHz, $CDCl_3$): δ 8.08 (s, 1H), 7.32 (s, 1H), 4.88 (s, 2H), 4.41 (q, *J* = 7.2 Hz, 2H), 3.72 (s, 3H), 2.79 (s, 3H), 1.46 (t, *J* = 7.2 Hz, 3H), 0.97 (s, 9H), 0.13 (s, 6H).

MS (ESI): m/z = 462.12 $[M + Na^+]$.

Anal. calcd for $C_{20}H_{30}BrNO_3Si$: C, 54.54; H, 6.87; Br, 18.14; N, 3.18, Found: C, 54.03; H, 6.22; Br, 17.07; N, 3.30.

Ethyl 6-bromo-1,2-dimethyl-5-(hydroxymethyl)-1*H*-indole-3-carboxylate (18)

Prepared as described for compounds **15** and **16**.

Nature of compound: White solid; Yield**:** (0.094 g) 90%.

^{1}H NMR (300 MHz, $CDCl_3$): δ 8.17 (s, 1H), 7.51 (s, 1H), 4.82 (s, 2H), 4.43 (q, *J* = 7.2 Hz, 2H), 3.75 (s, 3H), 2.81 (s, 3H), 1.45 (t, *J* = 7.2 Hz, 3H).

MS (ESI): m/z = 326.03 $[M + H^+]$.

Anal. calcd for $C_{14}H_{16}BrNO_3$: C, 51.55; H, 4.94; Br, 24.50; N, 4.29, Found: C, 52.13; H, 3.72; Br, 23.67; N, 3.90.

Ethyl 6-bromo 5-formyl 2-(bromomethyl)-1-methyl-1*H*-indole-3 carboxylate (19)

Prepared as described for compound **17** using 2.25 equivalents of *N*-bromosuccinimide. Purified by flash chromatography (toluene/acetone 98/2) recovering a 0.021 g of a white solid.

Nature of compound: White solid; Yield**:** 15%.

^{1}H NMR (300 MHz, $CDCl_3$): δ 10.4 (s, 1H), 8.74 (s, 1H), 7.62 (s, 1H), 5.15 (s, 2H), 4.49 (q, *J* = 7.2 Hz, 2H), 3.85 (s, 3H), 1.51 (t, *J* = 7.2 Hz, 3H).

MS (ESI): m/z = 423.93 $[M + Na^+]$.

Anal. calcd for $C_{14}H_{13}Br_2NO_3$: C, 41.72; H, 3.25; Br, 39.65; N, 3.48, Found: C, 40.37; H, 3.01; Br, 38.07; N, 2.90.

Ethyl-6-bromo-2-(bromomethyl)-5-(hydroxymethyl)-1-methyl-1*H*-indole-3 carboxylate (20)

To an ice-cooled solution of the aldehyde **19** (0.021 g, 0.052 mmol) in THF (1 mL) was added $NaBH_4$ (0.003 g, 0.062 mmol) in methanol (500 µL). Then, the resulting mixture was stirred at rt for 1h. After complete conversion, the solvent was distilled off under reduced pressure to afford almost pure product (0.020 g).

Nature of compound: Oil; Yield: (20 mg) 99%.

^{1}H NMR (300 MHz, $CDCl_3$): δ 8.21 (s, 1H), 7.56 (s, 1H), 5.12 (s, 2H), 4.84 (s, 2H), 4.42 (q, *J* = 7.2 Hz, 2H), 3.78 (s, 3H), 1.44 (t, *J* = 7.2 Hz, 3H).

MS (ESI): m/z = 403.94 $[M + H^+]$.

Anal. calcd for $C_{14}H_{15}Br_2NO_3$: C, 41.51; H, 3.73; Br, 39.45; N, 3.46, Found: C, 41.01; H, 2.92; Br, 38.77; N, 2.80.

Ethyl-6-bromo-5-(hydroxymethyl)-2-(phenylsulphanylmethyl)-1-methyl-1*H*-indole-3 carboxylate (21)

Prepared as described for compound **6** and **7** using ethanol as solvent. Purified using toluene/ethyl acetate (8/2) recovering a white solid (12 mg).

Nature of compound: White solid; Yield**:** (12 mg) 56%.

^{1}H NMR (300 MHz, $CDCl_3$): δ 8.18 (s, 1H), 7.55 (s, 1H), 4.86 (s, 2H), 4.71 (s, 2H), 4.29 (q, *J* = 7.2 Hz, 2H), 3.66 (s, 3H), 1.38 (t, *J* = 7.2 Hz, 3H).

MS (ESI): m/z = 456.03 $[M + Na^+]$.

Anal. calcd for $C_{20}H_{20}BrNO_3S$: C, 55.30; H, 4.64; Br, 18.40; N, 3.22; S, 7.12, Found: C, 55.03; H, 4.27; Br, 18.07; N, 3.50; S, 7.76.

Ethyl 5-(chloromethyl)-1,2-dimethyl-1*H*-indole-3-carboxylate (22)

Compound **16** (0.400 g, 1.60 mmol) was dissolved in CCl_4 and triphenylphosphine was added, followed by the addition of 3 mL of DMF. The mixture was stirred for 16h, then the solvent was removed and the residue taken up with ethyl acetate, washed with water. The organic layer was dried over anhydrous $MgSO_4$ to afford oil, purified by chromatography (hexane/ethyl acetate 6/4).

Nature of compound: Yellow oil; Yield: (0.280 g) 66%.

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.13 (s, 1H), 7.31 (s, 2H), 4.98 (s, 2H), 4.41 (q, *J* = 7.2 Hz, 2H), 3.69 (s, 3H), 2.73 (s, 3H), 1.47 (t, *J* = 7.2 Hz, 3H).

MS (ESI): m/z = 288.09 $[M + Na^+]$.

Anal. calcd for $C_{14}H_{16}ClNO_2$: C, 63.28; H, 6.07; Cl, 13.34; N, 5.27, Found: C, 62.73; H, 5.97; Cl, 12.87; N, 4.90.

General procedure for the synthesis of amines (23a-e)

A solution of compound **22** (1 equiv.), amines (2 equiv.) and cesium carbonate (0.1 equiv.) in dry DMF (2 mL) was magnetically stirred under reflux overnight. The mixture was diluted with ethyl acetate and washed twice with a solution of saturated aqueous solution of $NaHCO_3$: evaporation of the solvent of the organic layer gave a dark oil purified by flash chromatography.

Ethyl-5-((dimethylamino)methyl)-1,2-(dimethyl)-1*H*-indole-3-carboxylate (23a)

Purified with $CHCl_3$/MeOH 9.5/0.5.

Nature of compound: Yellow oil; Yield: (0.084 g) 63%.

^{1}H NMR (300 MHz, $CDCl_3$): δ 8.03 (s, 1H), 7.28 (s, 2H), 4.43 (q, *J* = 7.2 Hz, 2H), 3.71 (s, 3H), 3.58 (s, 2H), 2.79 (s, 3H), 2.29 (s, 6H), 1.48 (t, *J* = 7.2 Hz, 3H).

^{13}C NMR (75 MHz, $CDCl_3$): δ 166.7, 143.9, 136.3, 124.8, 123.9, 120.3, 118.7, 111.4, 103.7, 65.8, 45.3, 35.8, 15.3, 6.1.

MS (ESI): m/z = 297.17 $[M + Na^+]$.

Anal. calcd for $C_{16}H_{22}N_2O_2$: C, 70.04; H, 8.08; N, 10.21, Found: C, 69.73; H, 7.97; N, 9.90.

Ethyl 5-((diethylamino)methyl)-1,2-(dimethyl)-1*H*-indole-3-carboxylate (23b)

Purified with $CHCl_3$/MeOH 9.5/0.5.

Nature of compound: Yellow oil; Yield: (0.068 g) 67%.

^{1}H NMR (300 MHz, $CDCl_3$): δ 8.07 (s, 1H), 7.63 (d, *J* = 8.4 Hz, 1H), 7.32 (d, *J* = 8.4 Hz, 1H), 4.36 (q, *J* = 7.2 Hz, 2H), 4.19 (s, 2H), 3.68 (s, 3H), 2.96 (q, *J* = 7.2 Hz, 4H), 2.73 (s, 3H), 1.36-1.43 (m, 9H).

^{13}C NMR (75 MHz, $CDCl_3$): δ 166.3, 141.7, 136.7, 124.3, 123.7, 120.1, 118.3, 111.4, 103.5, 61.8, 60.3, 49.3, 35.8, 14.3, 13.4, 5.5.

MS (ESI): m/z = 303.21 $[M + H^+]$.

Anal. calcd for $C_{18}H_{26}N_2O_2$: C, 71.49; H, 8.67; N, 9.26, Found: C, 70.83; H, 8.97; N, 9.90.

Ethyl-5-[[[4-(diethylamino)-1-methyl-butyl]amino]methyl]-1-methyl-2-methyl-1*H*-indole-3-carboxylate (23c)

Elution with Hexane/Ethyl acetate 7/3.

Nature of compound: Yellow oil; Yield: (0.080 g) 96%.

^{1}H NMR (300 MHz, $CDCl_3$): δ 7.99 (s, 1H), 7.21 (s, 2H), 4.35 (q, *J* = 7.2 Hz, 2H), 3.89 (app q, *J* = 11.7 Hz, 2H, AB system), 2.70 (s, 3H), 3.62 (s, 3H), 2.36-2.48 (m, *J* = 7.2 Hz, 7H), 1.36-1.50 (m, 5H), 1.11 (d, *J*= 5 Hz, 3H,), 0.99 (t, *J* = 7.4 Hz, 6H).

^{13}C NMR (75 MHz, $CDCl_3$): δ 166.2, 140.3, 136.2, 124.4, 123.1, 120.7, 118.7, 111.3, 103.2, 62.8, 61.1, 54.2, 51.8, 49.1, 43.3, 35.6, 25.2, 14.1, 13.4, 5.3.

MS (ESI): m/z = 374.87 [M + H^+].

Anal. calcd for $C_{22}H_{35}N_3O_2$: C, 70.74; H, 9.44; N, 11.25, Found: C, 70.93; H, 9.77; N, 10.97.

Ethyl- 1,2-dimethyl-5-(pyrrolidin-1-ylmethyl)-1*H*-indole-3-carboxylate (23d)

White powder (0.139 g). Yield: 62%. ^{1}H NMR data are in agreement with those reported in literature.[10]

Ethyl -1,2-dimethyl-5-(4-methyl)piperazin-1-ylmethyl)-1*H*-indole-3-carboxylate (23e)

Elution with hexane/ethyl acetate 7/3.

Nature of compound: Yellow oil; Yield: (0.090 g) 7%.

^{1}H NMR (300 MHz, $CDCl_3$): δ 8.04 (s, 1H), 7.27 (s, 2H), 4.32 (q, *J* = 7.2 Hz, 2H), 3.69 (s, 3H), 3.58 (s, 2H), 2.79 (s, 3H), 2.41 (s, 8H), 2.33 (s, 3H), 1.41 (t, *J* = 7.2 Hz, 3H).

^{13}C NMR (75 MHz, $CDCl_3$): δ 166.4, 140.7, 136.5, 124.7, 123.3, 120.4, 118.6, 111.3, 103.2, 61.9, 60.3, 55.2, 52.7, 43.1, 35.9, 14.3, 5.7.

MS (ESI): m/z = 330.47 [M + H^+].

Anal. calcd for $C_{19}H_{27}N_3O_2$: C, 69.27; H, 8.26; N, 12.76, Found: C, 68.93; H, 8.83; N, 11.47.

Ethyl-1, 2-dimethyl-5-((1, 3-dioxoisoindolin-2-yl)methyl)-1*H*-indole-3-carboxylate (24)

A solution of compound **22** (0.135 g, 0.503 mmol) and potassium phtalimide **(**0.095 g, 0.508 mmol) in DMF (2 mL) was stirred under reflux for 2 h. Ethyl acetate was added to the mixture and the organic layer washed with 10% HCl, was dried over anhydrous $MgSO_4$ to give a white solid purified by chromatography (petroleum ether/ethyl acetate 8/2).

Nature of compound: White solid; Yield: (0.165 g) 87%.

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.13 (s, 1H), 7.31 (s, 6H), 4.90 (s, 2H), 4.41 (q, *J* = 7.2 Hz, 2H), 3.69 (s, 3H), 2.73 (s, 3H), 1.47 (t, *J* = 7.2 Hz, 3H).

^{13}C NMR (75 MHz, $CDCl_3$): δ 169.2, 166.9, 141.3, 135.3, 133.3, 133.1, 132.2, 128.6, 127.1, 119.2, 117.4, 111.9, 104.3, 61.4, 43.6, 35.8, 14.9, 6.1.

MS (ESI): m/z = 399.33 $[M + Na^+]$.

Anal. calcd for $C_{22}H_{20}N_2O_4$: C, 70.20; H, 5.36; N, 7.44, Found: C, 70.03; H, 4.97; N, 6.90.

Ethyl 5-(aminomethyl)-1,2-dimethyl-1*H*-indole-3-carboxylate (25)

To a stirred solution of **24** (0.132 g, 0.347 mmol) in absolute EtOH (2 mL) and hydrazine (61 µL, 0.69 mmol) were added and the mixture was kept under reflux for 2h. The solid residue was filtered off. After evaporation of the filtrate, the residue was taken up with EtOAc and extracted with 2N HCl; then, the aqueous phase was treated with 2N NaOH and extracted several times with EtOAc. The combined organic layers were dried (Na_2SO_4) and concentrated under vacuum to give pure product.

Nature of compound: slightly yellowish oil; Yield: (0.065 g) 75%.

^{1}H NMR (250 MHz, CD_3OD): δ 7.99 (s, 1H), 7.31 (br s, 2H), 4.43 (q, *J* = 7.2 Hz, 2H), 3.97 (s, 2H), 3.74 (s, 3 H), 2.70 (s, 3H), 1.42 (t, *J* = 7.2 Hz, 3H).

^{13}C NMR (75 MHz, $CDCl_3$): δ 167.6, 141.2, 1136.6, 1129.9, 124.7, 118.6, 109.1, 103.8, 59.6, 52.2, 35.2, 14.7, 5.8.

MS (ESI): m/z = 269.13 $[M + Na^+]$.

Anal. calcd for $C_{14}H_{18}N_2O_2$: C, 68.27; H, 7.37; N, 11.37, Found: C, 68.03; H, 7.27; N, 10.98.

General procedure for the synthesis of aldehydes (26) and (27)

To a solution of pyridinium dicromate (6.68 mmol) in dichloromethane (50 mL), compound **15, 16** (1.671 mmol) previously dissolved in dichloromethane (5 mL) was added and the mixture was allowed to stirred for 1 h at room temperature. Evaporation of the solvent under reduced pressure gave an oil, which was purified by chromatography (hexane/ethyl acetate 7/3).

Ethyl -5-formyl-1-methyl-2 (phenyl sulphanylmethyl)-1*H*-indole-3 carboxylate (26)

Nature of compound: White solid; Yield: (0.500 g) 86%.

^{1}H NMR (250 MHz, $CDCl_3$): δ 10.07 (s, 1H), 8.61 (s, 1H), 7.86 (d, *J* = 8.1 Hz, 1H,), 7.39 (d, *J* = 8.1 Hz, 1H,), 7.25-7.35 (m, 5H), 4.74 (s, 2H), 4.27 (q, *J* = 7.2 Hz, 2H), 3.75 (s, 3H), 1.37 (t, *J* = 7.2 Hz, 3H).

^{13}C NMR (75 MHz, $CDCl_3$): δ 192.0, 166.7, 142.7, 140.8, 136.5, 135.9, 128.7, 126.7, 125.3, 121.9, 113.7, 111.6, 102.7, 60.9, 35.3, 22.7, 14.7.

MS (ESI): m/z = 376.53 $[M + Na^+]$.

Anal. calcd for $C_{20}H_{19}NO_3S$: C, 67.97; H, 5.42; N, 3.96, S, 9.07, Found: C, 67.03; H, 5.97; N, 3.90; S, 8.86.

Ethyl 1, 2-dimethyl-5-formyl-1*H*-indole-3-carboxylate (27)[10]

White solid (0.139 g). Yield: 86%. ^{1}H NMR data are in agreement with those reported in literature.[10]

General procedure for the synthesis of amines derivatives 28a-f and 23f-n

Under nitrogen atmosphere aldehyde **26** or **27** (1.0 equiv.) was dissolved in dry dichloroethane (5 mL) and under magnetic stirring amines (4.0 equiv.), acetic acid (1.05 equiv.) and $NaBH(OAc)_3$ (6.0 equiv.) were added respectively. After completion of the reaction as monitored by thin layer chromatography (TLC), a solution of saturated aqueous solution of $NaHCO_3$ (15 ml) was added and the product was extracted with ethyl acetate (3 × 5 mL). The combined organic layer was dried over anhydrous $MgSO_4$ and evaporation of the solvent under reduced pressure gave a mixture purified by flash chromatography.

Ethyl-5-(dimethylaminomethyl)-1-methyl-2-(phenylsulfanylmethyl)-1*H*-indole-3-carboxylate (28a)

Purified using $CHCl_3$/MeOH/NH_3 1/1/0.1.

Nature of compound: Beige solid; Yield: (0.140 g) 97%.

^{1}H NMR (250 MHz, $CDCl_3$): δ 8.00 (s, 1H), 7.19-7.34 (m, 7H), 4.67 (s, 2H), 4.29 (q, *J* = 7 Hz, 2H), 3.60 (s, 3H), 3.57 (s, 2H), 2.27 (s, 6H), 1.36 (t, *J* = 7.1 Hz, 3H).

^{13}C NMR (75 MHz, $CDCl_3$): δ 167.0, 141.9, 137.1, 129.5, 127.3, 126.2, 125.9, 120.4, 111.3, 103.4, 65.7, 61.4, 46.7, 35.8, 23.1, 14.3.

MS (ESI): m/z = 383.51 [M + H^+].

Anal. calcd for $C_{22}H_{26}N_2O_2S$: C, 69.08; H, 6.85; N, 7.32; S, 8.38, Found: C, 68.97; H, 6.73; N, 7.18; S, 8.23.

Ethyl-5-(diethylaminomethyl)-1-methyl-2-(phenylsulfanylmethyl)-1*H*-indole-3-carboxylate (28b)

Purified using Ethyl acetate/MeOH/NH_3 1/1/0.1.

Nature of compound: Dark oil; Yield: (83 mg) 90%.

^{1}H NMR (300 MHz, $CDCl_3$): δ 8.12 (s, 1H), 7.17-7.47 (m, 7H), 4.75 (s, 2H), 4.32 (q, *J* = 7.2 Hz, 2H), 3.76 (s, 2H), 3.68 (s, 3H), 2.60 (q, *J* = 7.2 Hz, 4H), 1.40 (t, *J* = 7.2 Hz, 3H), 1.12 (t, *J* = 7.2 Hz, 6H).

^{13}C NMR (75 MHz, $CDCl_3$): δ 166.3, 141.9, 137.1, 135.3, 130.1, 127.6, 127.1, 126.1, 125.9, 120.8, 119.0, 111.3, 104.3, 61.3, 60.8, 49.8, 35.9, 23.3, 15.1, 13.8.

MS (ESI): m/z = 411.63 [M + H^+].

Anal. calcd for $C_{24}H_{30}N_2O_2S$: C, 70.21; H, 7.36; N, 6.82; S, 7.81, Found: C, 70.14; H, 7.23; N, 6.78; S, 7.73.

Ethyl-5-[4-(diethylamino)-1-methyl-butyl]amino]methyl]-1-methyl-2-(phenylsulfanylmethyl) 1*H*-indole-3-carboxylate (28c)

Purified using $CHCl_3$/MeOH/NH_3 1/1/0.3

Nature of compound: Yellow oil; Yield: (0.080 g) 96%.

^{1}H NMR (300 MHz, $CDCl_3$): δ 8.02 (s, 1H), 7.32-7.36 (m, 2H), 7.21-7.25 (m, 5H), 4.71 (s, 2H), 4.29 (q, *J* = 6.75 Hz, 2H), 3.89 (app q, *J* = 11.7 Hz, 2H, AB system), 3.64 (s, 3H), 2.69-2.76 (m, 1H), 2.50 (q, *J* = 7.5 Hz, 4H), 2.36-2.42 (m, 2H), 1.41-1.48 (m, 3H), 1.36 (t, *J* = 6.95 Hz, 3H), 1.11 (d, *J* = 6.2 Hz, 3H), 0.99 (t, *J* = 7 Hz, 6H).

^{13}C NMR (75 MHz, $CDCl_3$): δ 166.7, 140.9, 137.1, 135.4, 129.9, 127.3, 127.1, 126.1, 125.9, 121.1, 119.3, 111.9, 104.3, 64.3, 61.4, 55.2, 52.8, 50.5, 44.3, 36.2, 26.1, 23.6, 15.1, 13.4.

MS (ESI): m/z = 482.74 [M + H^+].

Anal. calcd for $C_{28}H_{39}N_3O_2S$: C, 69.82; H, 8.16; N, 8.72; S, 6.66, Found: C, 69.74; H, 8.10; N, 8.66; S, 6.55.

Ethyl-1-methyl-2-(phenylsulfanylmethyl)-5-(pyrrolidin-1-ylmethyl)-1*H*-indole-3-carboxylate (28d)

Purified using $CHCl_3$/MeOH/NH_3 1/1/0.2

Nature of compound: Yellow solid; Yield: (0.098 g) 95%.

^{1}H NMR (300 MHz, $CDCl_3$): δ 8.05 (s, 1H), 7.33-7.38 (m, 3H), 7.27-7.29 (m, 4H), 4.75 (s, 2H), 4.31 (q, *J* = 7.2 Hz, 2H), 3.81 (s, 2H), 3.69 (s, 3H), 2.62 (app s, 4H), 1.82 (app s, 4H), 1.39 (t, *J* = 7.2 Hz, 3H).

^{13}C NMR (75 MHz, $CDCl_3$): δ 166.1, 140.9, 136.7, 135.3, 129.9, 127.1, 126.9, 125.5, 125.1, 120.3, 119.1, 110.8, 103.9, 61.3, 60.7, 57.8, 35.2, 25.9, 22.7, 14.1.

MS (ESI): m/z = 409.51 [M + H^+].

Anal. calcd for $C_{24}H_{28}N_2O_2S$: C, 70.55; H, 6.91; N, 6.86; S, 7.85, Found: C, 70.41; H, 6.87; N, 6.78; S, 7.79.

Ethyl-1-methyl-2-(phenylsulfanylmethyl)-5-(4-methyl)piperazin-1-ylmethyl)-1*H*-indole-3-carboxylate (28e)

Purified using ethyl acetate/MeOH (1/1).

Nature of compound: Beige solid; Yield: (0.083 g) 84%.

^{1}H NMR (300 MHz, $CDCl_3$): δ 8.08 (s, 1H), 7.27-7.41 (m, 7H), 4.76 (s, 2H), 4.32 (q, *J* = 7.2 Hz, 2H), 3.69 (s, 5H), 2.41 (s, 8H), 2.33 (s, 3H), 1.41 (t, *J* = 7.2 Hz, 3H).

^{13}C NMR (75 MHz, $CDCl_3$): δ 166.1, 141.3, 136.5, 134.9, 129.3, 126.7, 126.1, 125.7, 125.3, 120.3, 118.6, 110.9, 104.6, 61.1, 60.7, 55.8, 52.4, 43.3, 35.4, 22.5, 14.3.

MS (ESI): m/z = 460.51 [M + Na^+].

Anal. calcd for $C_{25}H_{31}N_3O_2S$: C, 68.62; H, 7.14; N, 9.60; S, 7.33, Found: C, 68.57; H, 7.05; N, 9.51; S, 7.26.

Ethyl-1-methyl-2-(phenylsulfanylmethyl)-5-((4-(*tert*-butoxycarbonyl)piperazin-1-ylmethyl)-1*H*-indole-3-carboxylate (28f)

Purified using ethyl acetate/hexane (3/7).

Nature of compound: Pale oil; Yield: (0.040 g) 90%.

^{1}H NMR (250 MHz, $CDCl_3$): δ 8.02 (s, 1H), 7.33-7.38 (m, 2H), 7.22-7.29 (m, 5H), 4.73 (s, 2H), 4.30 (q, *J* = 7.1 Hz, 2H), 3.67 (s, 5H), 3.45 (app t, *J* = 4.3 Hz, 4H), 2.46 (app t, *J* = 4.3 Hz, 4H), 1.46 (s, 9H,), 1.39 (t, 3H, overlapped to s at 1.46 ppm, *J* = 7.4 Hz).

^{13}C NMR (75 MHz, $CDCl_3$): δ 166.1, 154.3, 140.9, 136.7, 134.9, 130.0, 127.6, 127.1, 126.5, 126.1, 121.0, 119.3, 110.9, 104.2, 80.3, 61.2, 60.5, 52.9, 50.1, 35.6, 29.1, 23.6, 14.7.

MS (ESI): m/z = 546.73 [M + Na^+].

Anal. calcd for $C_{29}H_{37}N_3O_4S$: C, 66.51; H, 7.12; N, 8.02; S, 6.12, Found: C, 66.45; H, 7.07; N, 7.96; S, 6.04.

Ethyl-1-methyl-2-(phenylsulfanylmethyl)-5-((4-(2,4-difluoro)phenyl)piperazin-1-ylmethyl)-1*H*-indole-3-carboxylate (28g)

Purified using ethyl acetate/hexane (4/6).

Nature of compound: Yellow oil; Yield: (0.040 g) 88%.

^{1}H NMR (250 MHz, $CDCl_3$): δ 8.01 (s, 1H), 7.24-7.40 (m, 7H), 6.74-6.90 (m, 3H), 4.74 (s, 2H), 4.30 (q, *J* = 7.1 Hz, 2H), 3.72 (s, 2H), 3.68 (s, 3H), 3.05 (app t, *J* = 4.3 Hz, 4H), 2.68 (app t, *J* = 4.3 Hz, 4H), 1.38 (t, *J* = 7.1 Hz, 3H).

^{13}C NMR (75 MHz, $CDCl_3$): δ 166.1, 157.5, 154.3, 141.0, 137.4, 135.2, 133.2, 129.7, 126.9, 126.3, 125.9, 125.7, 120.3, 119.2, 118.5, 112.7, 111.2, 106.2, 104.1, 60.9, 60.6, 53.1, 51.0, 36.1, 23.1, 14.1.

MS (ESI): m/z = 536.81 [M + H^+].

Anal. calcd for $C_{30}H_{31}F_2N_3O_2S$: C, 67.27; H, 5.83; F, 7.09; N, 7.84; S, 5.99, Found: C, 67.23; H, 5.79; F, 6.99; N, 7.91; S, 6.03.

Ethyl-1-methyl-5-(morpholinomethyl)-2-(phenylsulfanylmethyl)-1*H*-indole-3-carboxylate (28h)

Nature of compound: Yellow oil; Yield: (0.034 mg) 98%.

^{1}H NMR (250 MHz, $CDCl_3$): δ 8.04 (s, 1H), 7.33-7.39 (m, 2H), 7.22-7.30 (m, 5H), 4.73 (s, 2H), 4.30 (q, *J* = 7.1 Hz, 2H), 3.72 (app t, *J* = 4.3 Hz, 4H), 3.67 (s, 3H), 3.64 (s, 2H), 2.49 (app t, *J* = 4.3 Hz, 4H), 1.38 (t, *J* = 7.1 Hz, 3H).

^{13}C NMR (75 MHz, $CDCl_3$): δ 166.0, 141.1, 136.4, 134.8, 129.8, 126.8, 126.1, 125.3, 125.1, 121.1, 118.4, 111.3, 103.7, 67.1, 61.1, 60.8, 53.3, 35.3, 22.7, 14.2.

MS (ESI): m/z = 425.67 [M + H^+].

Anal. calcd for $C_{24}H_{28}N_2O_3S$: C, 67.90; H, 6.65; N, 6.60; S, 7.55, Found: C, 67.87; H, 6.76; N, 6.67; S, 7.43.

Ethyl 1,2-dimethyl-5-[(2-piperazin-1-ylethylamino)methyl])-1*H*-indole-3-carboxylate (23f)

Purified using dichloromethane/methanol (9/1).

Nature of compound: Yellow oil; Yield: (0.060 g) 88%.

^{1}H NMR (300 MHz, $CDCl_3$): δ 8.04 (s, 1H), 7.25-7.26 (m, 2H), 4.40 (q, *J* = 7.2 Hz, 2H), 3.97 (s, 2H), 3.69 (s, 3H), 2.92 (t, *J* = 6.2 Hz, 2H), 2.77 (s, 3H), 2.52 (t, *J*= 6.2 Hz, 3H), 2.56- 2.59 (m, 8H), 1.46 (t, *J* = 7.2 Hz, 3H).

^{13}C NMR (75 MHz, $CDCl_3$): δ 166.1, 141.2, 134.9, 126.8, 126.1, 120.1, 117.3, 111.3, 103.2, 61.1, 55.3, 53.7, 47.1, 46.3, 35.6, 14.1, 5.3.

MS (ESI): m/z = 359.16 [M + H^+].

Anal. calcd for $C_{20}H_{30}N_4O_2$: C, 67.01; H, 8.44; N, 15.63, Found: C, 66.97; H, 8.13; N, 15.18.

Ethyl-1,2-dimethyl-5-[[[1-(methylamino)-2(diethylamino)]ethylamino]methyl]-1*H*-indole-3-carboxylate (23g)

Purified using dichloromethane/methanol (9/1).

Nature of compound: Yellow oil; Yield: (0.038 g) 51%.

^{1}H NMR (300 MHz, $CDCl_3$): δ 8.04 (s, 1H), 7.25-7.28 (m, 2H), 4.41 (q, *J* = 7.2 Hz, 2H), 3.74 (s, 2H), 3.69 (s, 3H), 2.77 (s, 3H), 2.66 (s, 3H), 2.56- 2.59 (m, 8H), 1.46 (t, *J* = 7.2 Hz, 3H), 1.02-1.05 (m, 6H).

^{13}C NMR (75 MHz, $CDCl_3$): δ 166.1, 141.3, 136.1, 124.8, 123.4, 120.4, 118, 7, 111.2, 103,1, 62.6, 60.9, 53.7, 51.3, 49.2, 43.2, 35.6, 14.1, 13.4, 5.4.

MS (ESI): m/z = 360.51 [M + H^+].

Anal. calcd for $C_{21}H_{33}N_3O_2$: C, 70.16; H, 9.25; N, 11.69, Found: C, 69.97; H, 9.17; N, 11.16.

Ethyl-1,2-dimethyl-5-[[[1-(diethylamino)-2(diethylamino)]ethylamino]methyl]-1*H*-indole-3-carboxylate (23h)

Nature of compound: Yellow oil; Yield: (0.058 g) 77%.

^{1}H NMR (250 MHz, $CDCl_3$): δ 8.03 (s, 1H), 7.25 (d, *J* = 3.4 Hz, 2H), 4.41 (q, *J* = 7 Hz, 2H), 3.63-3.74 (m, 7H), 2.78-2.85 (m, 8H), 2.75 (s, 3H), 1.43 (t, *J* = 7 Hz, 3H), 1.06-1.08 (m, 9H).

^{13}C NMR (75 MHz, $CDCl_3$): δ 166.0, 142.1, 136.3, 124.8, 123.1, 120.5, 118.7, 111.3, 103.5, 60.9, 60.3, 51.3, 51.2, 49.7, 35.6, 14.1, 13.4, 5.3.

MS (ESI): m/z = 374.53 [M + H^+].

Anal. calcd for $C_{22}H_{35}N_3O_2$: C, 70.74; H, 9.44; N, 11.25, Found: C, 70.67; H, 9.26; N, 11.19.

Ethyl-1,2-dimethyl-5-[[2-(methylamino)-ethylamino]methyl]-1*H*-indole-3-carboxylate (23i)

Nature of compound: Yellow oil; Yield: (0.050 g) 81%.

^{1}H NMR (300 MHz, $CDCl_3$): δ 7.99 (s, 1H), 7.22 (d, *J* = 5.07 Hz, 2H), 4.36 (q, *J* = 7.1 Hz, 2H), 3.89 (s, 2H), 3.65 (s, 3H), 2.49-2.44 (m, 4H), 2.79 (s, 3H), 2.35 (s, 3H), 1.42 (t, *J* = 7.1 Hz, 3H).

^{13}C NMR (75 MHz, $CDCl_3$): δ 166.0, 141.1, 134.8, 126.9, 126.1, 119.5, 117.4, 110.9, 104.0, 60.9, 54.9, 51.6, 48.7, 35.6, 14.1, 5.3.

MS (ESI): m/z = 304.13 [M + H^+].

Anal. calcd for $C_{17}H_{25}N_3O_2$: C, 67.30; H, 8.31; N, 13.85, Found: C, 67.35; H, 8.34; N, 13.78.

Ethyl-1,2-dimethyl-5-[[2-(tert-butoxycarbonylamino)-ethylamino]methyl]-1*H*-indole-3-carboxylate (23j)

Nature of compound: Yellow oil; Yield: (0.042 g) 89%.

^{1}H NMR (300 MHz, $CDCl_3$): δ 8.15 (s, 1H), 7.33 (s, 2H), 4.85 (s, 2H), 4.45 (q, *J* = 7.3 Hz, 2H), 3.74 (s, 5H), 2.82 (s, 5H), 1.51 (s, 9H), 1.32 (t, *J* = 7.3 Hz, 3H).

^{13}C NMR (75 MHz, $CDCl_3$): δ 166.6, 156.3, 140.3, 134.7, 126.2, 126.0, 119.2, 117.4, 110.2, 103.4, 79.5, 60.9, 55.1, 48.7, 42.6, 35.7, 28.7, 14.3, 5.4.

MS (ESI): m/z = 390.13 [M + H^+].

Anal. calcd for $C_{21}H_{31}N_3O_4$: C, 64.76; H, 8.02; N, 10.79; O, 16.43, Found: C, 64.35; H, 8.34; N, 10.58; O, 10.60.

Ethyl-1,2-dimethyl-5-[[2-(diethylamino)-ethylamino]methyl]-1*H*-indole-3-carboxylate (23k)

Purified using dichloromethane/methanol/NH_3 9.5/0.5/0.1.

Nature of compound: Yellow oil; Yield: (0.025 g) 99%.

^{1}H NMR (250 MHz, $CDCl_3$): δ 8.03 (s, 1H), 7.26 (s, 2H), 4.40 (q, *J* = 7.1 Hz, 2H), 3.96 (s, 2H), 3.69 (s, 3H), 2.75-2.78 (m, 5H), 2.47 (t, *J* = 6.4 Hz, 2H), 2.20 (s, 6H), 1.44 (t, *J* = 7.1 Hz, 3H).

^{13}C NMR (75 MHz, $CDCl_3$): δ 166.1, 141.3, 136.1, 124.6, 123.9, 119.5, 118.8, 110.2, 103.0, 60.9, 58.5, 54.5, 46.2, 46.1, 35.6, 14.1, 5.4.

MS (ESI): m/z = 318.43 [M + H^+].

Anal. calcd for $C_{18}H_{27}N_3O_2$: C, 68.11; H, 8.57; N, 13.24, Found: C, 67.87; H, 8.23; N, 12.98.

Ethyl-1,2-dimethyl-5-[[2-(hydroxy)-ethylamino]methyl]-1*H*-indole-3-carboxylate (23l)

Purified using dichloromethane/methanol/NH_3 9/1/0.1.

Nature of compound: Yellow oil; Yield: (0.020 g) 87%.

^{1}H NMR (250 MHz, $CDCl_3$): δ 8.04 (s, 1H), 7.24 (d, *J* = 2.7 Hz, 2H), 4.40 (q, *J* = 7.1 Hz, 2H), 3.93 (s, 2H), 3.64-3.68 (m, 5H), 2.83 (t, *J* = 5.2 Hz, 2H), 2.75 (s, 3H), 1.44 (t, *J* = 7.1 Hz, 3H).

^{13}C NMR (75 MHz, $CDCl_3$): δ 166.7, 1403, 136.7, 124.9, 124.1, 120.3, 119.1, 110.2, 103.1, 61.6, 60.9, 54.8, 52.1, 35.6, 14.3, 5.3.

MS (ESI): m/z = 313.36 [M + Na^+].

Anal. calcd for $C_{16}H_{22}N_2O_3$: C, 66.18; H, 7.64; N, 9.65, Found: C, 65.97; H, 7.33; N, 9.18.

Ethy-1,2-dimethyl-5-[[2-(hydroxyethylamino)-ethylamino]methyl]-1*H*-indole-3-carboxylate (23m)

Purified using dichloromethane/methanol/NH_3 9/1/0.2.

Nature of compound: Yellow oil; Yield: (0.018 g) 67%.

^{1}H NMR (250 MHz, $CDCl_3$): δ 8.05(s, 1H), 7.26 (s, 2H), 4.40 (q, *J* = 7.1 Hz, 2H), 3.94 (s, 2H), 3.63-3.68 (s overlapped m, 7H), 2.81 (app s, 2H), 2.78 (s, 3H), 2.33 (app s, 4H), 1.44 (t, *J* = 7.1 Hz, 3H).

^{13}C NMR (75 MHz, $CDCl_3$): δ 166.2, 140.8, 134.7, 126.7, 126.3, 120.3, 117.8, 111.5, 103.3, 61.7, 60.9, 55.3, 52.9, 50.1, 49.6, 35.8, 14.1, 5.3.

MS (ESI): m/z = 334.76 [M + H^+].

Anal. calcd for $C_{18}H_{27}N_3O_3$: C, 64.84; H, 8.16; N, 12.60, Found: C, 65.17; H, 7.83; N, 11.18.

Ethyl-5-((4-(tert-butoxy carbonyl)-1,4-diazepan-1-yl)methyl)-1,2-dimethyl-1*H*-indole-3-carboxylate (23n)

Prepared using 1-BOC homopiperazine.[10]

Nature of compound: Yellow solid; Yield: (0.054 g) 69%.

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.08 (s, 1H), 7.23-7.31 (m, 2H), 4.39 (q, *J* = 7.4 Hz, 2H), 3.79 (s, 2H), 3.67 (s, 3H), 3.45 (m, 6H), 2.73 (s, 3H), 2.63-2.65 (m, 4H), 1.45 (t, *J* = 7.4 Hz, 12H).

^{13}C NMR (75 MHz, $CDCl_3$): δ 166.4, 154.3, 141.3, 134.8, 126.5, 125.3, 120.3, 118.3, 110.8, 103.7, 79.8, 60.9, 60.8, 56.4, 53.6, 48.4, 47.0, 35.6, 28.5, 26.6, 14.1, 5.5.

MS (ESI): m/z = 430.51 [M + H^+].

Anal. calcd for $C_{24}H_{35}N_3O_4$: C, 67.11; H, 8.21; N, 9.78, Found: C, 67.01; H, 8.13; N, 9.38.

Ethyl-5-(1,4-diazepan-1-ylmethyl)-1,2-dimethyl-1*H*-indole-3-carboxylate (29)

Compound **23n** (0.054 g, 0.12 mmol**)** was dissolved in dichloromethane (1 mL) and trifluoroacetic acid (13 µL, 12 mmol)) were added dropwise and the mixture stirred till starting material was consumed. The solvent was removed under reduced pressure, the crude treated with 10% NaOH till pH-12 and the residue extracted with ethyl acetate twice, recovering a yellow oil (0.040 g).

Nature of compound: Yellow oil; Yield: (40 mg) 99%.

^{1}H NMR (250 MHz, $CDCl_3$): δ 8.08 (s, 1H), 7.23-7.31 (m, 2H), 4.37 (q, *J* = 7.1 Hz, 2H), 3.82 (s, 2H), 3.68 (s, 3H), 3.45 (m, 6H), 2.75 (s, 3H), 2.68 (br s, 2H), 1.86 (app t, *J* = 5.4 Hz, 2H), 1.45 (t, *J* = 7.1 Hz, 3H).

^{13}C NMR (75 MHz, $CDCl_3$): δ 166.1, 141.4, 134.8, 126.7, 125.3, 120.9, 118.3, 110.5, 103.4, 61.1, 60.8, 53.6, 48.6, 47.1, 35.6, 29.4, 14.1, 5.3.

MS (ESI): m/z = 352.44 [M + Na^+].

Anal. calcd for $C_{19}H_{27}N_3O_2$: C, 69.27; H, 8.26; N, 12.76, Found: C, 68.97; H, 7.93; N, 12.18.

Ethyl-1-methyl-5-[(1-methylpyrrolidin-1-ium-1-yl) methyl]-2-(phenylsulfanylmethyl) indole-3-carboxylate iodide (30)

A solution of compound **28d** (0.015 g, 0.036 mmol) in ethyl acetate (4 mL) was stirred with iodomethane (10 µL, 0.14 mmol) for 3h. Purified using $CHCl_3$/MeOH/NH_3 1/1/0.2.

Nature of compound: White solid; Yield: (0.020 g) 99%.

^{1}H NMR (300 MHz, CD_3OD): δ 8.26 (s, 1H), 7.62 (s, *J* = 8.2 Hz, 1H,), 7.33-7.38 (m, 6H), 7.24-7.63 (m, 4H), 4.81 (s, 6H), 4.67 (s, 2H), 4.27 (q, *J* = 7.2 Hz, 2H), 3.81 (s, 2H), 3.81 (s, 3H), 3.07 (s, 3H), 2.28 (app s, 4H), 1.34 (t, *J* = 7.2 Hz, 3H).

^{13}C NMR (75 MHz, $CDCl_3$): δ 166.3, 141.5, 136.4, 133.8, 131.7, 129.8, 129.6, 126.8, 126.2, 125.2, 120.2, 118.5, 111.0, 103.5, 69.7, 66.3, 60.9, 49.3, 35.2, 22.6, 22.1, 14.3.

MS (ESI): m/z = 573.05 [M + Na^+].

Anal. calcd for $C_{25}H_{31}IN_2O_2S$: C, 54.54; H, 5.68; N, 5.09; S, 5.82, Found: C, 54.47; H, 5.63; N, 4.98; S, 5.73.

2.6. REFERENCES

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CHAPTER-III

SECTION-A

Multicomponent one-pot aldol addition/protection reaction of β-ketoesters in the presence of Me_3SiCl and *i*-Pr_2EtN: scope and applications

3.1.1 INTRODUCTION

The aldol addition is one of the most powerful synthetic tools for carbon-carbon bond formation in a large variety of organic syntheses due to its broad utility.[1] Aldol structural units are found in many important molecules, whether naturally occurring or synthetic,[2] it has been the subject of intense research in recent years. Aldol products (and especially hydroxymethyl derivatives) have extensively been used for the synthesis of important biologically active compounds,[3] and represents a valuable tool with regard to chiral economy. Asymmetric syn and anti aldol reactions have been used for synthesis of the natural products containing lactone moieties.

"Aldol" is an abbreviation of aldehyde and alcohol. When the enolate of an aldehyde or a ketone reacts at the α-carbon with the carbonyl of another molecule under basic or acidic conditions to obtain β-hydroxy aldehyde or ketone, this reaction is called Aldol Reaction.

The aldol addition of aldehyde to readily enolizable 1,3-dicarbonyl compounds still remains a important challenge in organic chemistry, even though the products are valuable intermediates in the total synthesis of natural products.[4] In the past very few effective methods have been developed with a limited scope [5,6] but even under very mild conditions (i.e., in the presence of weak base or weak acid or in a mixture of both, in metal and organocatalysed conditions, etc.), variable mixtures of regioisomers, dehydration products and aldol adducts have been observed.[5,7] For example, in the presence of the trimethylsilyl enol ether of 1,3-pentanedione and without any catalysts, benzaldehyde gave the aldol adduct in 40% yield, while other aldehydes failed.[7a]

The main difficulties of this reaction can be attributed to the scarce stability of the adducts and to the inadequacy of the current methodologies that favour condensation (Knoevenagel reaction) or retro-aldol process instead of the aldol reaction. For these reasons different strategies and multi-steps non-flexible synthesis have been developed in some cases.[8] In particular β-keto esters and 2,4-diketones have been successfully used as nucleophiles in the addition to acetals in the presence of Lewis acids to yield protected *O*-alkyl aldol adducts.[8b] Recently

Sodeoka reported the first enantioselective addition of β-keto esters to allylic acetals in the presence of chiral palladium catalyst to give again protected *O*-alkyl compounds in good yields and ees.[8c] Chiral *O*-protected β-hydroxy malonates have been also obtained in diastereoselective oxy-Michael reactions of alkylidene malonates using enantiopure alkoxides.[8d]

However very recently a multicomponent one-pot aldol addition/protection reaction (MCR) of malonates to aldehydes for the obtaining of a large class of new aldol adducts in the presence of Me_3SiCl and *i*-Pr_2EtN was developed by Massa and coworkers.[9]

Multi-component reactions[10] are a powerful tool for the generation of collections of molecules; they are extremely convergent and produce a remarkably high increase of molecular complexity in just one step. Sequential transformations and multicomponent one-pot reactions are always resource effective and environmentally acceptable and thus greener as compared to multi-step reactions.[11] They offer significant advantages over conventional linear step syntheses, by reducing time, saving money, energy and raw-materials thus resulting in both economical and environmental benefits. At the same time, diversity can be achieved for building up libraries by simply varying each component.[12]

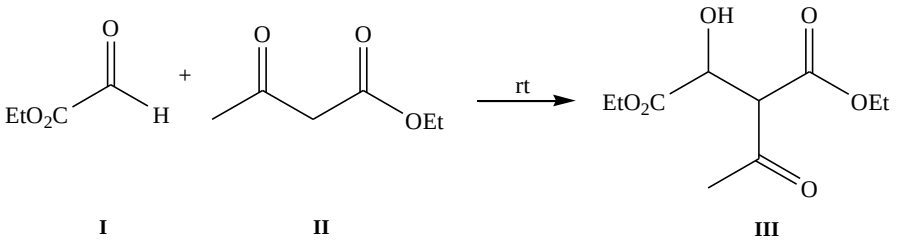
In these studies it was clearly demonstrated that the success of this challenging aldol addition is mainly related to the *in situ* trapping of the unstable aldol adducts by chlorosilanes reagents[9a,b] or by their intramolecular trapping.[9c] In this way a series of aromatic, hetero-aromatic and even simple aliphatic aldehydes were reacted with malonates to give protected aldol adducts [9a,b]. However, under this MCR conditions, β-ketoesters revealed almost completely unreactive.[9b]

3.1.2 REVIEW OF LITERATURE

The most well known and explored route to the trimethylsilyl-protected aldol product in a mild one-pot aldol addition/protection reaction of active methylene compounds with aldehydes in the presence of Me_3SiCl and *i*-Pr_2EtN. In recent years, several important improved procedures have been reported to one-pot aldol addition, some of the representative methods are discussed below.

a) Mahrwald's approach[5]

More recently, Mahrwald *et al*. reported the room temperature aldol addition of ethyl glyoxylate to acetoacetic ethyl ester without any solvent and also described a catalyst-free aldol addition of several 1,3-dicarbonyl compounds to activated aldehydes like ethyl glyoxylate in good to high yields. Even if this can be considered the most effective procedure ever reported, aromatic aldehydes were completely unreactive while inactivated aliphatic were not tested (scheme 1).

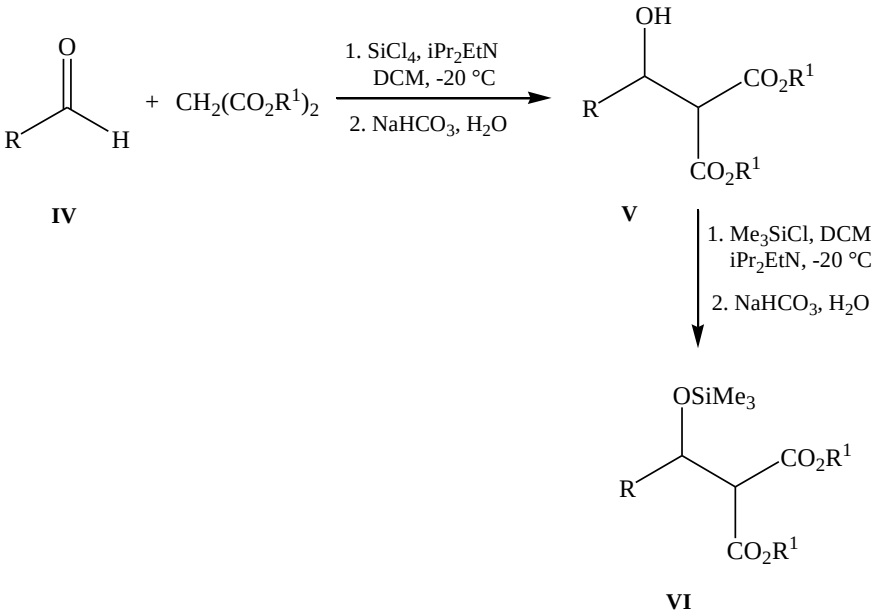


I **II** **III**

Scheme 1

b) Massa's approach[9b]

Massa *et al.* have reported the two step-reaction aldol addition followed by protection reaction in the presence of $SiCl_4$ (scheme 2).



IV **V** **VI**

Scheme 2

The success of this difficult aldol addition is reasonably related to the *in situ* trapping of the aldol adduct, it is possible to obtain directly the trimethylsilyl-protected aldol product in a mild one-pot aldol addition/protection reaction of active methylene compounds with aldehydes in the presence of Me_3SiCl and *i*-Pr_2EtN in dry dichloromethane at -20 °C as shown in Scheme 3.

VII **VIII**

Scheme 3

3.1.3 PRESENT WORK

As part of a program toward a general procedure for the aldol addition of active methylene compounds, my work was focused on the efforts to develop a multi-component one-pot aldol addition/protection reactions to *β*-ketoesters, since the branched aldol adducts derived from β-ketoesters have been obtained only in few cases with a very limited aldehyde scope.[5,9b]

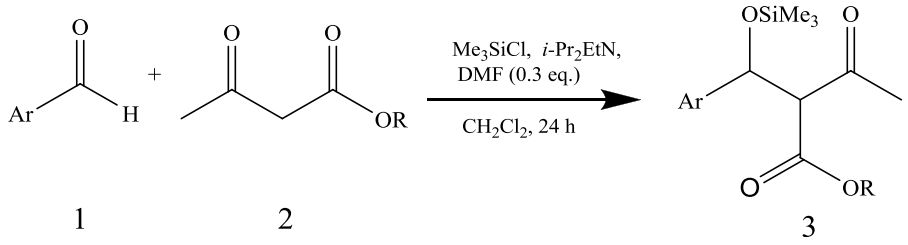
In addition the usefulness of the obtained adducts was explored in the synthesis of substituted diols and alkylated products.

During our studies we also found out that when 2-cyanobenzaldehyde was reacted in the presence of *β*-ketoesters and others 1,3-dicarbonyl compounds, a number of novel 3-substituted isoindolinones were obtained, as we will describe separately.

3.1.4 MULTI-COMPONENT ONE-POT ALDOL ADDITION/PROTECTION REACTION OF *β*-KETOESTERS

Under the conditions used for the one-pot aldol addition/protection reaction of malonates at -20 °C, *β*-ketoesters revealed themselves to be almost completely unreactive. Only *p*-nitro benzaldehyde in the presence of *t*-butyl acetoacetate **2a**

and (-)-menthyl acetoacetate **2b** gave the silylated adducts in about a 30% yield at -20 °C (Scheme 4, Table 1 entries 1-3).



1 2 3

Scheme 4

Table 1. Multicomponent aldol addition/protection reaction of *t*-butyl acetoacetate **2a** with aldehydes in the presence of Me_3SiCl and *i*-Pr_2EtN at -20 °C

Entry	Aldehyde (Ar)	DMF (eq)	Product (3)	Yield (%)[a]	d.r.[b]
1	Ph	0	**3a**	No reac.	
2	4-$NO_2C_6H_4$	0	**3b**	31	1/1
3[c]	4-$NO_2C_6H_4$	0	**3c**	35	1/2/1
4	4-$NO_2C_6H_4$	0.3	**3b**	75	2.5/1
5	Ph	0.3	--	No reac.	
6	4-CNC_6H_4	0.3	**3d**	79	2.5/1
7	4-CNC_6H_4	-	**3d**	55	2.5/1
8	2-CNC_6H_4	0.3	**3e**	80	2/1
9	2-CNC_6H_4	-	**3e**	58	
10	4-ClC_6H_4	0.3	**3f**	20	2/1
11	4-$OMeC_6H_4$	0.3	--	No reac.	
12	3-Phenyl proprionaldehyde	0.3	--	No reac.	

[a] Yields refer to chromatographically pure compounds
[b] Determined by ^1H NMR analysis of crude
[c] Reaction performed in the presence of (-)-menthyl acetoacetate **2b**

In order to overcome this disappointing situation several conditions and strategies were explored. A significant increase of the reactivity was observed when a catalytic amount of DMF was used (Table 1, entry 4). Thus, we were pleased to observe high yields of the silylated aldol adducts in the presence of several aromatic aldehydes bearing strong electron-withdrawing groups like nitro and

cyano both in para and ortho position (Table 1, entries 4, 6 and 8), while without DMF a significant lower yield was observed (Table 1, cfr entries 7 and 9). The presence of a chlorine substituent, strongly decreased the reactivity, while benzaldehyde and nonactivated aldehydes did not react at all (Table 1, entries 5, 11 and 12). As could have been expected, this MCR guarantees high regioselectivity in all the cases for the attack of the aldehydes at the methylene group only, even if **3** were obtained with rather low diastereoselectivity.

The use of DMF was suggested by the well-know effect of activation of trimethylsilyl nucleophiles, like silyl enol ethers, by Lewis bases in a number of C-C bond forming reactions.[13,14] ^{1}H NMR experiments at room temperature in $CDCl_3$ of mixture of TMS-Cl, *t*-butyl acetoacetate and *i*-Pr_2EtN revealed the formation of silyl enol ether intermediates in 1/1 ratio with unreacted starting materials after only 2 h at room temperature, while similar experiments in the presence of di-*t*-butyl malonate left the starting materials unreacted. Probably the improvement of the reaction rate is a consequence of DMF activation of these silyl enol ether intermediates.

In order to improve the reactivity of less reactive aldehydes, different reaction temperature, higher concentrated reaction medium, different solvents and even in solvent-free conditions, different amines and bases in combination with Me_3SiCl were tried. The most relevant results are summarized in Table 2 and 3.

Starting from the conditions of Scheme 1, it can be seen that the modification of three parameters leads to a sufficient reactivity. In the presence of 0.3 eq of DMF, at room temperature and after 72 h, we obtained a 51% yield for 3a (Table 2, entries 1-4). In these new conditions, the effect of DMF is still evident for benzaldehyde (Table 2, cfr entries 3 and 4) and other aldehydes.

For example when *p*-chloro benzaldehyde and the aliphatic 3-phenyl proprionaldehyde were used, we observed 72% and 66% yields respectively, whereas without the Lewis base the yields were lower (cfr entries 7-8 and 9-10 of Table 2). The result of the aliphatic aldehyde is particular interesting because self-condensation products have not been observed, while also under these conditions, *p*-methoxy benzaldehyde was not reactive (Table 2, Entries 5-6).

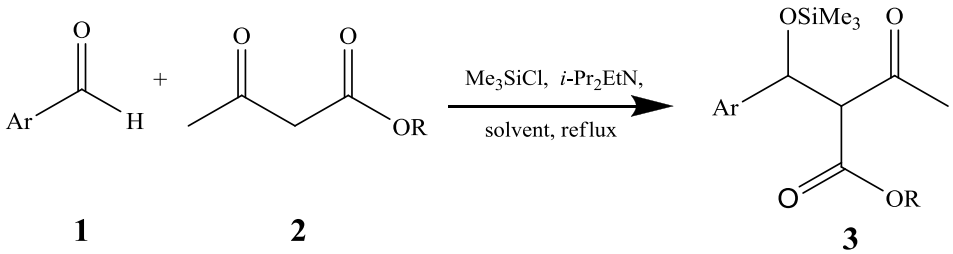
Moreover another disappointing result was observed with methyl acetoacetate 2c for which the aldol adduct 3h was obtained in a 10% yield only (Table 2, Entry 11).

Table 2. Multicomponent aldol addition/protection reaction of *tert*-butyl acetoacetate with aldehydes in the presence of Me_3SiCl and *i*-Pr_2EtN

Entry	Aldehyde (Ar)	Temp. °C	DMF (eq)	Time (hr)	Product (3)	Yield (%)[a]	d.r.[b]
1	Ph	-20°C	0.3	48	**3a**	No reac.	
2	Ph	0°C	-	48	**3a**	No reac.	
3	Ph	rt	-	72	**3a**	42 %	1/1
4	Ph	rt	0.3	72	**3a**	51 %	2/1
5	4-$MeOC_6H_4$	rt	-	72	--	No reac.	
6	4-$MeOC_6H_4$	rt	0.3	72	--	No reac.	
7	4-ClC_6H_4	rt	-	72	**3f**	60 %	1.5/1
8	4-ClC_6H_4	rt	0.3	72	**3f**	72 %	1.6/1
9	3-Ph proprionaldehyde	rt	-	72	**3g**	45 %	1.5/1
10	3-Ph proprionaldehyde	rt	0.3	72	**3g**	66 %	1.7/1
11[c]	Ph	rt	0.3	72	**3h**	10 %	1.3/1

a Yields refer to chromatographically pure compounds
b Determined by 1H NMR analysis of crude
c Methyl acetoacetate **2c** was used instead of of *tert*-butyl acetoacetate

Since a positive effect on the reaction rate was exerted by the increasing of the temperature, we used higher boiling solvents like 1,2-dichloroethane and THF at refluxing conditions (Scheme 5, Table 3).



1 **2** **3**

Scheme 5

Focusing on less reactive substrates, a good yield was obtained with methyl acetoacetate and benzaldehyde (Entry 1, Table 3).

Table 3. Multicomponent aldol addition/protection reaction of *tert*-butyl acetoacetate with aldehydes in the presence of Me_3SiCl and *i*-Pr_2EtN

Entry	Aldehyde (Ar)	R	Solvent	Time (hr)	Prod. (3)	Yield (%)[a]	d.r.[b]
1	Ph	Me	1,2 dichloroethane	8	**3h**	55	1.3/1
2[c]	Ph	Me	1,2 dichloroethane	8	**3h**	50	1.5/1
3	Ph	Me	1,2 dichloroethane	18	**3h**	32	1.3/1
4	Ph	*t*-Bu	1,2 dichloroethane	8	**3a**	57	1.2/1
5	*p*-$OMeC_6H_4$	*t*-Bu	1,2 dichloroethane	8	**3i**	42	1.3/1
6	*p*-ClC_6H_4	*t*-Bu	1,2 dichloroethane	8	**3f**	84	1.4/1
7	*p*-BrC_6H_4	*t*-Bu	1,2 dichloroethane	8	**3l**	78	1.5/1
8	2-furfural	*t*-Bu	1,2 dichloroethane	8	**3m**	68	1.6/1
9	3-Phenyl proprionaldehyde	*t*-Bu	1,2 dichloroethane	8	**3g**	56	1.3/1
10	Ph	*t*-Bu	THF	18	**3a**	62	1.3/1
11	*p*-$OMeC_6H_4$	*t*-Bu	THF	18	**3i**	35	1.2/1
12	Ph	Me	THF	18	**3h**	39	1/1

a Yields refer to chromatographically pure compounds
b Determined by 1H NMR analysis of crude
c Reaction performed with 0.3 eq of DMF.

However the use of DMF did not give any significant improvement to the reactivity and for this reason all the experiments of table 3 were performed without this additive (Table 3, entry 2). Longer reaction time led to the disappearance of the starting materials with the concomitant decrease of the yield due to the formation of decomposition products (Table 3, entry 3). Among the tested aldehydes, we observed a moderate reactivity even with the poorly reactive *p*-methoxybenzaldehyde (Table 3, Entry 5). Furfural, *p*-chlorobenzaldehyde and *p*-bromobenzaldehyde showed good results (Table 3, entries 6-8), while only in

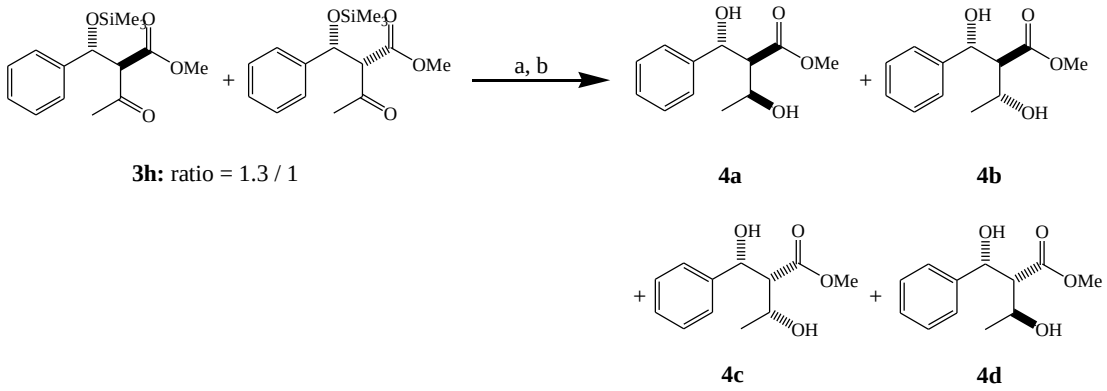
the presence of 3-phenyl propionaldehyde we observed a lower yield for the formation of decomposition products (cfr entry 9 of Table 3 with entry 11 of Table 2) The use of THF led to an increase of the yield in the presence of benzaldehyde and *t*-butyl acetoacetate (Entry 10), while we did not observe any improvement when methyl acetoacetate and *p*-methoxybenzaldehyde were used (Table 3, entries 11 and 12).

3.1.5 SYNTHETIC APPLICATIONS OF ALDOL ADDUCTS

All the developed methods can be easily employed for gram scale synthesis of the aldol adducts, a feature that is particularly useful for preparative purposes. In fact the adducts **3** show very interesting functionalities that could be subjected to different sets of transformations to give valuable compounds. For example, according to scheme 6, the versatility and the stability of **3** was firstly tested treating **3h** with $NaBH_4$ and with TBAF for the deprotection of the resulting crude mixture. This sequence easily provided the valuable known diols **4**,[15a,d] that otherwise require longer synthesis with the use of toxic metal reagents, but that can be readily converted into *β*-lactams and *β*-lactones.[15]

According to ^{1}H NMR analysis on the crude, all the 4 diastereomers were detected in comparison with those reported by Fleming *et al*[15a] and the two major isomers **4a** and **4c** were easily isolated by chromatography. From the comparison of the diastereomeric ratios of **3h** and **4**, we can confidently attribute the stereochemistry of the isomers of **3h** as those described in Scheme 6.

This stereochemical outcome can be generalised to all the adducts **3** for the analogy of ^{1}H NMR spectra. All the aldols **3** have usually been obtained as an inseparable mixture of diastereomers. However the minor isomer of **3e** has been isolated by chromatography and easily crystallised in CH_2Cl_2.



3h: ratio = 1.3 / 1

ratio **4a** / **4b** / **4c** / **4d** = 4.7 / 1.3 / 4 / 1

4 95 % yield from **3h**

Scheme 6

Reagents and conditions: (a) $NaBH_4$, MeOH, 0 °C, 20 min. (b) TBAF (1M solution in THF), THF, 0 °C, 30 min.

Fortunately these crystals were suitable for X-ray structural analysis, the obtained structure is reported in Figure 1.

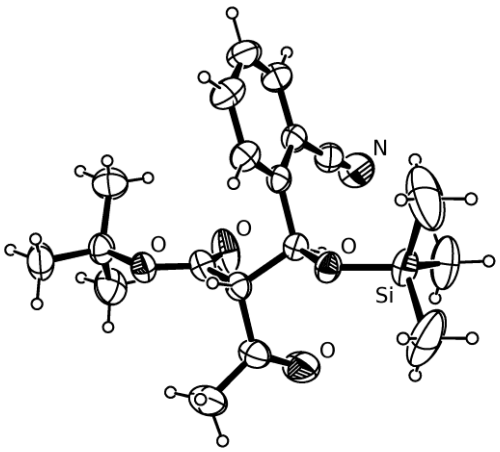
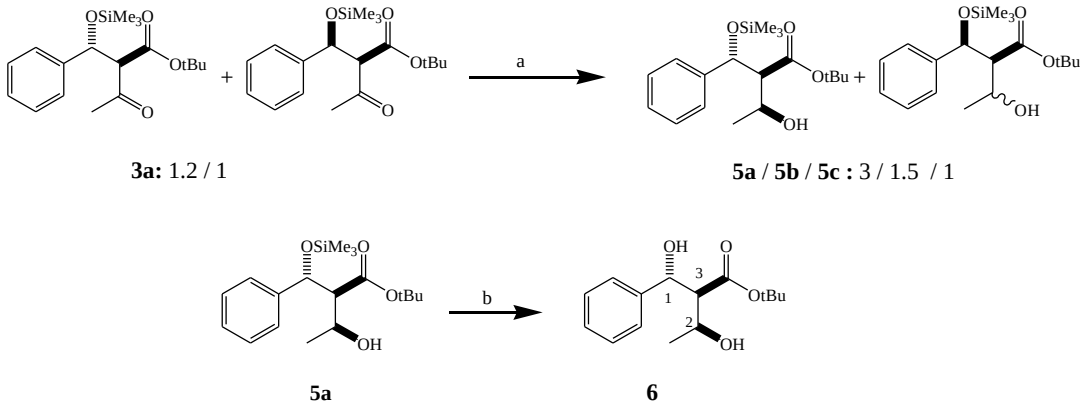


Figure 1: ORTEP structure of 3e

As it can be seen, the relative configuration of the minor isomer of **3e** (*SR,RS*) is in agreement with what was previously supposed for **3h** in scheme 6 even though with a different ester group.

Moreover, when we submitted a 1.3/1 mixture of the two diastereomers **3a** with the supposed configuration as shown in scheme 7, to $NaBH_4$ reduction, we obtained quantitatively a 3/1.5/1 mixture for the three main diastereomers of **5**, as observed by carefully analysis of ^{1}H-NMR spectrum.



3a: 1.2 / 1

5a / **5b** / **5c :** 3 / 1.5 / 1

5a **6**

Scheme 7

Reagents and conditions: (a) $NaBH_4$, MeOH, 0 °C, 20 min., 90%. (b) TBAF (1M solution in THF), THF, 0 °C, 30 min., 95%

The major diastereomer **5a** was isolated by chromatography and treated with TBAF to give the deprotected diol **6** quantitatively.

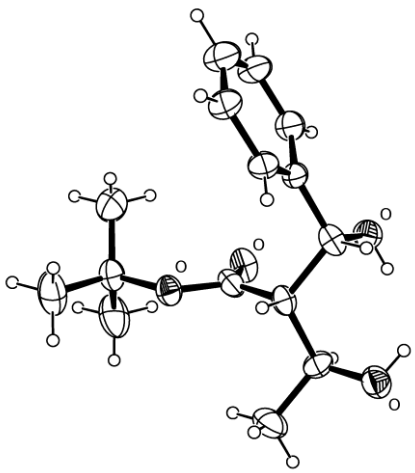
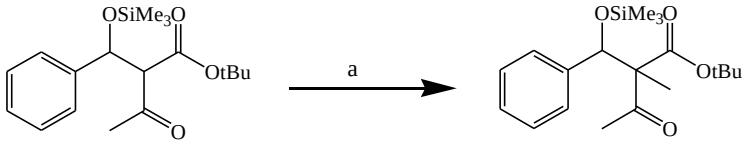


Figure 2: ORTEP structure of 6.

This compound was crystallised again by slow evaporation of CH_2Cl_2 solution and the X-ray scattering gave the structure reported in figure 2. The configuration of the three stereocentres 1'*RS*, 2*SR*, 3*SR*, confirmed what previously supposed by simple analogy.

The aldol addition of ethyl 2-methyl acetoacetate to benzaldehyde so as to give an aldol adduct with contiguous tertiary and quaternary stereocenters was unsuccessful under conditions of both Scheme 4 and 5. However, in order to further expand the range of applications of the obtained aldols **3**, we examined the reactivity of **3a** as nucleophile under alkylation conditions in the presence of CH_3I and K_2CO_3 in DMF (Scheme 8).



3a: 1.3 / 1 **7**

Scheme -8

Reagents and conditions: (a) K_2CO_3, CH_3I, DMF, rt, 48 h, 75%, (d.r. = 95/5).

Very interestingly, employing the usual 1.3/1 mixture of the two diastereomers, we obtained the alkylated product **7** in the enriched 95/5 ratio in 75% yield in 48 h. Shorter reaction time (24 h) gave **7** in rather low yield (35%). However in this case the unreacted **3a** was recovered in 35% yield with an enriched 70/30 diastereomeric ratio. Unfortunately **7** was obtained as an oil and we were not able to crystallise it for X-ray analysis for a rationalization of the stereochemical outcome. Considering the importance in the construction of quaternary centers in stereoselective manner and the fact that, at the best of our knowledge, similar diastereoselective alkylations are rare,[16] further studies are in course to expand the scope of the aldols in this field.

3.1.6 CONCLUSION

In conclusion we have developed a multicomponent one-pot aldol addition/protection reactions of β-ketoesters with a wide range of aldehydes to give protected aldol adducts in the presence of Me_3SiCl and *i*-Pr_2EtN. The use of catalytic amounts of DMF led to an increase in the reactivity with aldehydes bearing electron withdrawing groups, while less reactive aldehydes required different solvents and refluxing conditions. Subsequently, the investigation of the formation of silyl enol ethers of *β*-ketoesters also gave important mechanistic insights into the role that DMF can play. Finally, as examples of the possibilities of further functionalization of the obtained aldols, **3a** and **3h** were submitted to a sequence of reduction and deprotection reactions to give valuable diols, while alkylation reactions afforded quaternary stereocentre with high diastereoselectivity. Considering the variety of versions and reaction conditions that this MCR of *β*-ketoesters can tolerate, we think that this work can give important perspective for a general approach to the aldol addition of other readily enolizable active methylene compounds. Since the class of active methylene compounds is very large and presents different structural motifs, in future studies particular emphasis will be given to other applications of this methodology and of the obtained aldol adducts in the synthesis of valuable compounds. Other studies are also in course for the development of enantioselective versions of these difficult aldol additions.

3.1.7 EXPERIMENTAL SECTION

i) Procedures

a) Procedure for one pot aldol-silylation reaction in the presence of Me_3SiCl and *i*-Pr_2EtN (Scheme 4).

In a flame-dried, 2-necked, round-bottom flask, aldehyde (0.40 mmol) was added to a solution of *i*-Pr_2EtN (2.3 eq., 0.92 mmol), *β*−ketoester (1.5 eq., 0.60 mmol) and Me_3SiCl (2.0 eq., 0.80 mmol) in dry dichloromethane (2.0 mL) under nitrogen at -20 °C. At the end of the reaction, the mixture was quenched with saturated aqueous $NaHCO_3$ (5 mL), extracted with 15x3 mL of CH_2Cl_2 and dried over anhydrous Na_2SO_4. After removing the solvent under reduced pressure, the crude oil was purified by flash chromatography from hexane to 95/5 hexane / AcOEt mixture to afford the pure products **3**.

b) Procedure for one pot aldol-silylation reaction in the presence of Me_3SiCl and *i*-Pr_2EtN (Scheme 5).

In a flame-dried, 2-necked, round-bottom flask equipped with a condenser, aldehyde (0.80 mmol) was added to a solution of *i*-Pr_2EtN (2.0 eq., 1.60 mmol), *β*−ketoester (1.1 eq., 0.88 mmol) and Me_3SiCl (2.0eq., 1.60 mmol) in dry 1,2-dichloroethane/THF (4.0 mL) under nitrogen at reflux. At the end of the reaction, the mixture was quenched with saturated aqueous $NaHCO_3$ (5 mL), extracted with 15x3 mL of CH_2Cl_2 and dried over anhydrous Na_2SO_4. After removing the solvent under reduced pressure, the crude oil was purified by flash chromatography from hexane to 95/5 hexane/AcOEt mixture to afford the pure products **3**.

c) Procedure for conversion of 3a into 5 (Scheme 7).

$NaBH_4$ (0.27 mmol) was added to a solution of **3a** (0.25 mmol) in methanol (1.0 mL), at 0°C and reacted for 20 min. Then the reaction was quenched with saturated aqueous $NaHCO_3$ (5 mL), extracted with 15x3 mL of CH_2Cl_2 and dried over anhydrous Na_2SO_4. After removing the solvent under reduced pressure, the crude oil was purified by flash chromatography with hexane/AcOEt mixture from 90/10 to 1/1 to afford the pure products **5.**

d) Procedure for conversion of 5a into 6 (Scheme 7)

5a was dissolved in THF (1.0 mL) and TBAF (25 µl of 1.0 M solution in THF) was added at 0 °C and reacted for 30 min. Then the mixture was treated with saturated aqueous NaCl (5 mL), extracted with 15x3 mL of CH_2Cl_2 and dried over anhydrous Na_2SO_4. After removing the solvent under reduced pressure, the crude oil was purified by flash chromatography with 1/1 mixtures of hexane and AcOEt to afford the pure product **6**.

e) Procedure for alkylation of 3a (Scheme 8)

K_2CO_3 (0.27 mmol) was added to a solution of **3a** (0.11 mmol) and CH_3I (0.33 mmol) in DMF (1.0 mL), at r.t. and reacted for 48 h. Then ethyl acetate (20 mL) was added and the mixture was extracted with brine (10x3 mL) and dried over anhydrous Na_2SO_4. After removing the solvent under reduced pressure, the crude oil was purified by flash chromatography with hexane/AcOEt mixture from 95/5 to 90/10 to afford the pure products **7.**

e) General procedure for ^{1}H-NMR experiment about formation of silyl enol ethers:

In a flame-dried, 2-necked, round-bottom flask, Me_3SiCl (0.20 mmol) was added to a solution of *i*-Pr_2EtN (1.0 eq.) and *t*-butyl aceoacetate (1.0 eq.) in 0.8 mL of anhydrous $CDCl_3$ at r.t. The resulting solution was transferred into a dry NMR tube and ^{1}HNMR spectra were recorded.

ii) <u>Charaterization data</u>

Note: Compounds **3a-d, 3f** and **3h** are known and were characterised comparing their spectroscopic data with those previously reported in literature[9b] and the diols **4a**, **4b**, **4c** and **4d** are known and were characterised comparing the spectroscopic data with those previously published.[15a]

2-[(2-Cyano-phenyl)-trimethylsilanyloxy-methyl]-3-oxo-butyric acid *tert*-butyl ester(3e) (mixture of two diastereomers)

IR (KBr): 871, 1130, 1250, 1714, 1738, 2222, 2927 cm^{-1}

^{1}H NMR ($CDCl_3$; 300 MHz): δ 7.76 (m, 1+1H), 7,64-7.53 (m, 2+2H), 7.37 (m,1+1H), 5.58 (d, *J* = 9.0 Hz, 1H), 5.54 (d, *J* = 9.0 Hz, 1H, major diastereomer), 4.04 (d, *J* = 9.2 Hz, 1H, major diastereomer,), 3.98 (d, *J* = 9.0 Hz, 1H), 2.33 (s, 3H), 2.05 (s, 3H), 1.47 (s, 9H), 1.21 (s, 9H), -0.02 (s, 9H), -0.04 (s, 9H).

^{13}C NMR ($CDCl_3$; 75 MHz): δ 202.8, 202.2, 165.8, 165.1, 145.5, 144.9, 134.1, 134.0, 133.1, 132.5, 129.5, 128.8, 128.4, 128.2, 117.4, 117.3, 111.3, 111.1, 82.3, 82.1, 71.9, 71.0, 68.9, 67.5, 30.7, 29.5, 27.8, 27.4, -0.25, -0.34.

MS (ESI): *m*/*z* = 384 ($M + Na^+$).

Anal. Calcd for $C_{19}H_{27}NO_4Si$: C, 63.13; H, 7.53; N, 3.87 Found: C, 63.28; H, 7.44; N, 3.75.

2(*RS*)-3-Oxo-2-[1'(*SR*)-(2-cyanophenyl)-trimethylsilanyloxy-methyl)-butyric acid *tert*-butyl ester (3e) (minor diastereomer)

The minor diastereomer was separated by chromatography and was obtained as a solid (recrystallised in CH_2Cl_2). M.p.: 49-52 °C.

IR (KBr): 870, 1132, 1255, 1715, 1739, 2221, 2923 cm^{-1} .

^{1}H NMR ($CDCl_3$; 300 MHz): δ 7.65 (d, *J* = 6.0 Hz, 1H), 7,57-7.52 (m, 2H), 7.39 (m,1H), 5.56 (d, *J* = 9.0 Hz, 1H), 4.06 (d, *J* = 9.0 Hz, 1H), 2.35 (s, 3H), 1.23 (s, 9H), -0.02 (s, 9H).

^{13}C NMR ($CDCl_3$; 75 MHz): δ 200.9, 165.1, 145.0, 133.1, 132.5, 128.8, 128.4, 117.5, 111.4, 82.3, 71.9, 67.5, 30.7, 27.5, -0.32.

MS (ESI): *m*/*z* = 384 ($M + Na^+$).

Anal. Calcd for $C_{19}H_{27}NO_4Si$: C, 63.13; H, 7.53; N, 3.87 Found: C, 63.29; H, 7.61; N, 3.99.

2-Acetyl-5-phenyl-3-trimethylsilanyloxy-pentanoic acid *tert*-butyl ester (3g)

The title compounds were obtained as Oil.

IR (KBr): 660, 840, 1157, 1258, 1461, 1697, 1730, 2930 cm^{-1} .

^{1}H NMR ($CDCl_3$; 300 MHz): δ 7.28-7.16 (m, 5+5H), 4.45-4.43 (m, 2H), 3.64 (d, *J* = 3 Hz,1H, major diastereomer), 3.61 (d, *J* = 3 Hz,1H), 2.97 (bt, 1H), 2.81 (bt, 1H), 2.72-2.64 (m, 4H), 2.27 (s, 3H), 2.20 (s, 3 H), 1.91-1.79 (m, 4H), 1.47 (s, 9H), 1.45 (s, 9 H, major diastereomer), 0.16(s, 9 H), 0.13 (s, 9 H, major diastereomer).

^{13}C NMR ($CDCl_3$; 75 MHz): δ 202.9, 202.2, 166.9, 166.7, 141.8, 128.5, 128.3, 128.2, 126.2, 125.7, 81.9, 81.7, 71.4, 70.5, 66.8, 65.7, 37.3, 36.7, 31.8, 31.1, 30.9, 30.2, 29.6, 29.2, 27.9, 27.8, 0.41, 0.31.

MS (ESI): *m*/*z* = 387.50 ($M + Na^+$).

Anal. Calcd for $C_{20}H_{32}O_4Si$: C, 65.89; H, 8.85. Found: C, 65.71; H, 8.76.

2-[(4-Methoxy-phenyl)-trimethylsilanyloxy-methyl]-3-oxo-butyric acid *tert*-butyl ester (3i)

The title compounds were obtained as Oil.

IR (KBr): 665, 852, 1151, 1260, 1450, 1610, 1699, 1720, 2920 $cm^{-1.}$

^{1}H NMR ($CDCl_3$; 300 MHz): δ 7.27 (d, *J*=9.0 Hz, 2H), 6.81 (d, *J*=9.0 Hz, 2H), 5.18 (d, *J*=9.0 Hz, 1H), 3.76 (d, *J*=9.0 Hz, 1H), 3.73 (s, 3H), 2.31 (s, 3H), 1.20 (s, 9H), 0.08 (s, 9H).

^{13}C NMR ($CDCl_3$; 75 MHz): δ 202.1, 165.7, 159.2, 133.6, 128.3, 113.3, 81.6, 74.0, 69.4, 55.1, 30.4, 27.5, -0.1.

MS (ESI): *m*/*z* = 389.33 ($M + Na^+$).

Anal. Calcd for $C_{19}H_{30}O_5$ Si: C, 62.26; H, 8.25. Found: C, 62.40; H, 8.13.

2-(Furan-2-yl-trimethylsilanyloxy-methyl)-3-oxo-butyric acid *tert*-butyl ester (3m)

The title compounds were obtained as Oil.

IR (KBr): 659, 836, 1161, 1256, 1442, 1692, 1719, 2936 $cm^{-1.}$

^{1}H NMR ($CDCl_3$; 250 MHz): δ 7.35-7.33 (t, 2H), 6.30-6.27 (m, 2H), 6.24-6.22 (m, 2H), 5.27 (d, J=9.0 Hz, 1+1H), 4.10 (d, J=9.0 Hz, 1H), 4.05 (d, J=12.0 Hz, 1H, major diastereomer), 2.31 (s, 3H, major diastereomer), 2.03 (s, 3H), 1.46 (s, 9H), 1.28 (s, 9H, major diastereomer), -0.01 (s, 9H), -0.02 (s, 9H, major diastereomer).

^{13}C NMR ($CDCl_3$; 75 MHz): δ 201.5, 200.6, 165.8, 165.4, 153.5, 153.2, 142.0, 141.9, 110.2, 110.1, 107.9, 107.6, 81.9, 81.8, 67.0, 66.9, 66.6, 65.5, 30.8, 29.8, 27.8, 27.5, -0.40, -0.46.

MS (ES)-: *m*/*z* = 349.15 ($M + Na^+$).

Anal. Calcd for $C_{16}H_{26}O_5$ Si: C, 58.87; H, 8.03. Found: C, 58.76; H, 8.11.

2-[(4-Bromo-phenyl)-trimethylsilanyloxy-methyl]-3-oxo-butyric acid *tert*-butyl ester (3l)

The title compounds were obtained as Oil.

IR (KBr): 693, 760, 1081, 1145, 1256, 1461, 1699, 1719, 2994 cm^{-1} .

^{1}H NMR ($CDCl_3$; 300 MHz): δ 7.46-7.41 (m, 2+2 H), 7.24-7.22 (m, 2+2 H), 5.22-5.19 (d, *J* = 9 Hz, 1+1H), 3.82 (d, *J* = 9 Hz, 1H), 3.75 (d, J=9 Hz, 1H, major diastereomer) 2.31 (s, 3H, major diastereomer) 1.92 (s, 3H), 1.48 (s, 9H), 1.22 (s, 9H, major diastereomer) -0.04 (s, 9H), -0.06 (s, 9H, major diastereomer).

^{13}C NMR ($CDCl_3$; 75 MHz): δ 201.4, 200.9, 165.9, 165.5, 140.9, 140.7, 132.0, 131.3, 131.1, 130.8, 128.8, 128.7, 121.7, 82.0, 81.8, 73.6, 73.0, 70.1, 69.0, 30.7, 29.5, 27.8, 27.5, -0.08, -0.15.

MS (ESI): *m*/*z* = 437.09 ($M + Na^+$).

Anal. Calcd for $C_{18}H_{27}BrO_4Si$: C, 52.05; H, 6.55. Found: C, 52.00; H, 6.69.

Methyl (2*SR*,3*SR*)-3-hydroxy-2-(1'(*RS*)-hydroxybenzyl)-butanoate (4a) [15a]

^{1}H NMR (250 MHz; CDCl3): δ 7.44–7.25 (m, 5H), 5.17 (dd, *J* =7.5 and 4.5 Hz, 1 H), 4.20 (br sextet, *J* = 6.5, 1 H), 3.73 (d, *J* = 7.5, 1 H), 3.56 (s, OMe, 3 H), 2.84 (dd, *J* = 6.5 and 4.5, 1 H), 2.72 (bd, *J* = 5.5, 1 H), 1.27 (d, *J* = 6.5, 3 H).

Methyl (2*RS*,3*RS*,)-3-hydroxy-2-(1'(*RS*)-hydroxybenzyl)-butanoate (4c) [15a]

^{1}H NMR (250 MHz; CDCl3) δ 7.35–7.28 (m, 5 H), 5.10 (d, *J*= 9.0 Hz, 1 H), 4.34 (quintept, *J* = 7.0 Hz, 1 H,), 3.55 (2H, bs), 3.38 (s, 3 H), 2.81 (t, *J* = 9.0 Hz, 1 H), 1.25 (d, *J* = 6.0 Hz, 3 H).

(2*SR*,3*SR*)-3-Hydroxy-2-(1'(*RS*)-phenyl-trimethylsilanyloxy-methyl)-butyric acid *tert* butyl ester (5a)

The title compounds were obtained as Oil.

IR (KBr): 693, 760, 1081, 1145, 1256, 1461, 1699, 1719, 2994, 3260.

^{1}H NMR (250 MHz; $CDCl_3$): δ 7.33-7.29 (m, 5H), 4.99 (d, *J*=7.5 Hz, 1H), 3.83-3.78 (m, 1H), 2.78 (dd, *J* = 7.5 and 6.4 Hz, 1 H), 2.29 (bs, 1H), 1.44 (s, 9H), 1.10 (d, *J* = 5 Hz), 4H), -0.03 (s, 9 H).

^{13}C NMR (75 MHz; $CDCl_3$): δ 171.2, 141.9, 128.3, 127.9, 126.9, 80.8, 74.6, 66.1, 61.7, 27.9, 20.2, -0.09.

MS (ESI): *m*/*z* = 361.42 ($M + Na^+$).

Anal. Calcd for $C_{18}H_{30}O_4Si$: C, 63.87; H, 8.93. Found: C, 63.70; H, 8.80.

(2*SR*,3*SR*)-3-Hydroxy-2-(1'(*RS*)-hydroxy-phenyl-methyl)-butyric acid *tert* butyl ester (6)

The title compounds were obtained as solid.

IR (KBr): 690, 765, 1090, 1140, 1451, 1690, 1729, 2984, 3320.

^{1}H NMR (400 MHz; $CDCl_3$): δ 7.34-7.24 (m, 5H), 5.20-5.17 (m, 1H), 4.25-4.23 (m, 1H), 3.94 (bd, *J*=4 Hz), 2.77 (bs, 1H), 2.71 (dd, *J*= 7.0 and 3.9 Hz, 1 H), 1.31 (d, *J*=4 Hz, 3H), 1.24 (s, 9H).

^{13}C NMR (75 MHz; $CDCl_3$): δ 172.7, 141.8, 128.1, 127.1, 125.4, 81.9, 71.1, 66.7, 59.1, 27.6, 21.0.

MS (ESI): *m*/*z* = 289.15 ($M + Na^+$).

Anal. Calcd for $C_{15}H_{22}O_4$: C, 67.64; H, 8.33. Found: C, 67.50; H, 8.25.

2-Methyl-3-oxo-2-(phenyl-trimethylsilanyloxy-methyl)-butyric acid *tert*-butyl ester (7)

The title compounds were obtained as an oil.

IR (KBr): 680, 770, 1073, 1131, 1250, 1442, 1693, 1709, 2971.

^{1}H NMR (400 MHz; $CDCl_3$): δ 7.34-7.24 (m, 5H), 5.45 (s, 1H), 2.36 (s, 3H), 1.40 (s, 9 H), 1.16 (s, 3H), -0.02 (s, 9H).

^{13}C NMR (75 MHz; $CDCl_3$): δ 204.8, 170.2, 139.9, 127.8, 127.5, 127.4, 81.7, 66.3, 28.6, 27.8, 27.1, 15.7, -0.07.

MS (ESI): *m*/*z* = 351.19 ($M + H^+$).

Anal. Calcd for $C_{19}H_{30}O_4Si$: C, 65,10; H, 8,63. Found: C, 65,25; H, 8,73.

iii) Crystal data and refinement details for structures

Single crystal diffraction data were collected on a Oxford Xcalibur CCD area detector diffractometer, using graphite monochromatic Mo Kα (λ = 0.71069Å) radiation. Data reduction and absorption correction were performed using CrysAlis RED 1.171.26 (Oxford Diffraction). The structure was solved by direct methods using SIR2008[3] and refined by full-matrix least squares using SHELX-

97.[4] Hydrogen atoms were generated in calculated position using SHELX-97 (Table 4).

Table 4. Selective X-Ray crystallographic data for compound 3e and compound 6.

	Comp. 3e	**Comp. 6**
Empirical formula	$C_{19}N_1O_4Si_1H_{25}$	$C_{25}O_4H_{21}$
Formula weight	359	265
Temperature	293(2) K	
Wavelength	0.71073 Å	
Crystal system	Monoclinic	Triclinic
Space group	P 21/n	P-1
Unit cell dimensions	a = 9.8157(4) Å b = 15.5367(5) Å b= 98.289(4)° c = 14.1068(7) Å	a = 6.155(5) Å a= 80.620(5)° b = 9.280(5) Å b= 86.561(5)° c = 14.021(5) Å g = 70.941(5)°
Volume	2128.86(15) $Å^3$	746.8(8) $Å^3$
Z	8	2
Density (calculated)	1.262 Mg/m^3	0.581 Mg/m^3
Absorption coefficient	0.191 mm^{-1}	0.042 mm^{-1}
F(000)	848	139
Theta range for data collection	4.20 to 30.38°	4.35 to 29.78°

Index ranges	-13<=h<=13 -22<=k<=21 -19<=l<=19	-8<=h<=6 -12<=k<=12 -19<=l<=19
Reflections collected	29674	6039
Independent reflections	5512 [R(int) = 0.0488]	3684 [R(int) = 0.1379]
Completeness	85.9 % (to theta=30.38°)	86.3 % (to theta = 29.78°)
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	5512 / 0 / 226	3684 / 0 / 172
Goodness-of-fit on F^2	0.978	0.917
Final R indices [I>2sigma(I)]	R1 = 0.0647, wR2 = 0.1751	R1 = 0.1148, wR2 = 0.2118
R indices (all data)	R1 = 0.1271, wR2 = 0.2241	R1 = 0.3540, wR2 = 0.3341
Largest diff. peak and hole	0.403 and -0.249 e.$Å^{-3}$	0.368 and -0.245 e.$Å^{-3}$

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SECTION-B

The Aldol Addition of Readily Enolizable 1,3-Dicarbonyl Compounds to 2-Cyanobenzaldehyde in the Synthesis of Novel 3-Substituted Isoindolinones

3.2.1 INTRODUCTION

The chemistry and reactivity of the isoindolinone ring system is currently an area of interest for many research groups due to its biological activity and its large number of applications in therapeutic activities.[1] 2,3-Dihydro-1*H*-isoindol-1-ones are known under different names in the literature such as isoindolinones or phthalimidines and are found as a core unit in various natural products.

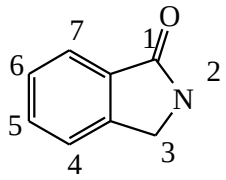


Fig- 1. Skeleton structure of Isoindolinone

In this case, should be specifically mentioned taliscanine,[2a] which occurs in the rhizomes of Aristolochia taliscanina, enterocarpam II,[2b] isolated from the stem bark of Orophea enterocarpa (Annoniacae), or vellutina[2c] extracts of leaves and twigs of *Goniothalamus velutinus* as shown below fig. 2.[3]

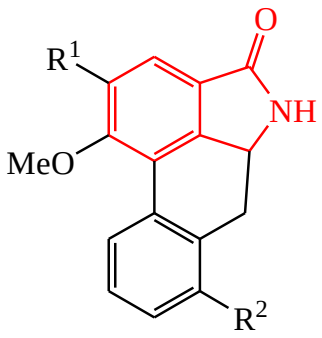


Fig-2.

Taluscanine (R^1 = R^2 = OMe)

Enterocarpam II (R^1 = OMe, R^2 =OH)

Valutinam (R^1 = OH, R^2 = OMe)

Recently, it has been recognized that 3-substituted isoindolinones possess anxiolytic activity: in addition in this class of structures, vasodilatory,[4a] anti-inflammatory,[4b] antihypertensive[4c], antiulcer,[4d] antipsychotics,[5] anesthetic,[6]

antiviral,[7] antileukemic,[8a] and platelet aggregation inhibitory[8b] properties have also been observed.

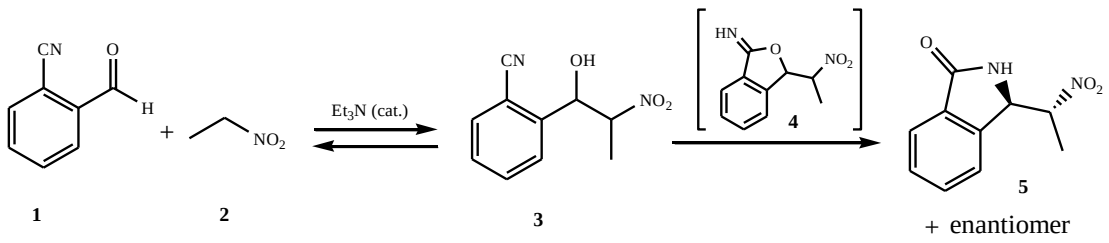
Consequently, considerable effort has been devoted to the synthesis of this benzoannelated nitrogen heterocycle which also act as useful synthetic building blocks and intermediates in organic synthesis.

3.2.2 REVIEW OF LITERATURE

Several traditional methods are available for the synthesis of isoindolinones,[9] based on the use of Grignard reagents,[10a] Diels–Alder reactions,[10b] Wittig reagents,[10c] reduction processes,[10d,e] rearrangement processes[10f] and photochemical reactions.[10g,h] In recent years several new approaches have been developed for the synthesis of substituted isoindolines, of which the most generally useful involve palladium catalysed reactions[11] or lithiation procedures.[12] However, such methods generally require multiple reaction steps, inflexible strategies for the construction of the heterocyclic ring[13,14] and are unsatisfactory both in yield and generality. For these reasons the interest for the development of new and simple methods for the construction of 3-substituted isoindolinones has grown over the past decades.

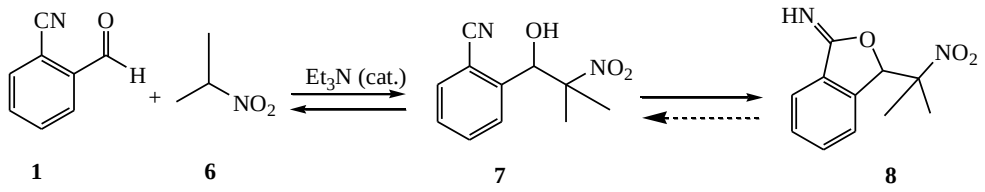
Ramström approach

Ramström *et al* have very recently proposed a useful one-pot tandem nitroaldol (Henry reaction)–cyclization–rearrangement reaction for the synthesis of 3-substituted isoindolinones by reaction of 2-cyanobenzaldehyde with nitroalkanes in the presence of tertiary base.[15] (Scheme 1).



Scheme 1. Synthesis of Isoindolinone in a Diastereoselective One-pot Tandem Reaction-Cystallization Protocol.

This process was possible thanks to the relatively acidic protons of nitro compounds: in fact by using secondary nitroalkane the most acidic proton was eliminated from the molecule and the reaction could be halted at intermediate iminophthalan, which was isolated and characterized by X-ray crystallography.

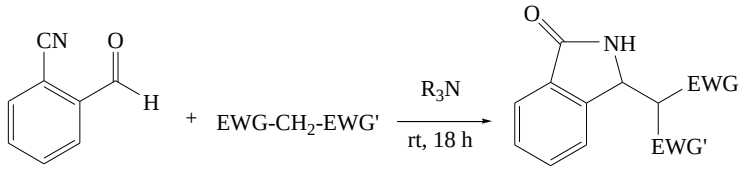


Scheme 2. Synthesis of Intermediate analogue

3.2.3 PRESENT WORK

Based on Ramstrom approach, our idea is related to the development of a more general approach which exploits the aldol addition of readily enolizable 1,3-dicarbonyl compounds to 2-cyanobenzaldehyde. It is important to note that the aldol addition of readily enolizable 1,3-dicarbonyl compounds, such as β-keto esters and malonates, to aldehydes is very difficult to achieve, and very few applications are available, whereas the nitroaldol is a well-established procedure.

Recently we demonstrated that trapping of these unstable aldol adducts in the presence of chlorosilane reagents is a straightforward strategy to drive these difficult aldol additions to completion.[16] Thus, based on this concept, the intramolecular trapping of the intermediate aldol adducts by irreversible cyclization at the cyano group would allow the synthesis of different types of 3-substituted isoindolinones. On the basis of these considerations, according to Scheme 3, a series of readily enolizable 1,3-dicarbonyl compounds (**9)** reacted with 2-cyanobenzaldehyde (**1**) in the presence of triethylamine, and the results are reported in Table 1.

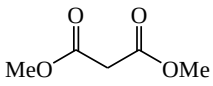
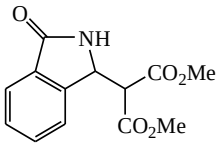
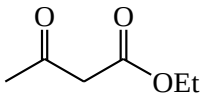
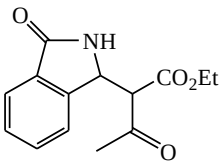
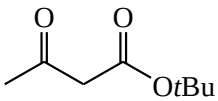
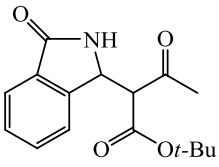


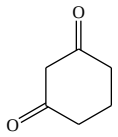
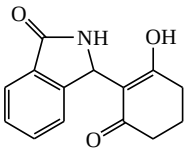
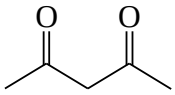
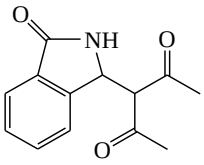
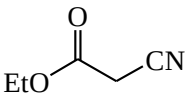
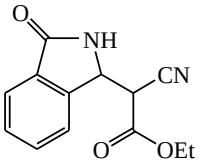
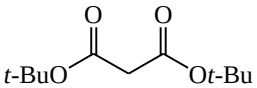
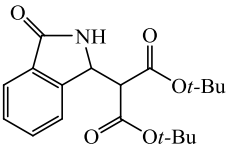
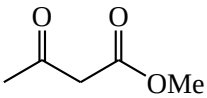
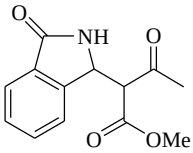
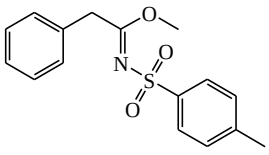
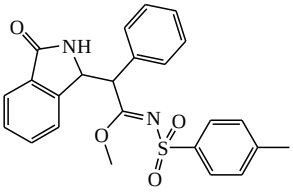
1 **9** **10**

Scheme 3

Under optimized conditions we were pleased to observed that all the model nucleophiles, representing the classes of malonates, β-ketoesters, and 1,3-diketones, gave the new 3-substituted isoindolinones **10** in good to high yields. (Table 1).

Table 1: Synthesised 3-substitued isoindolinones

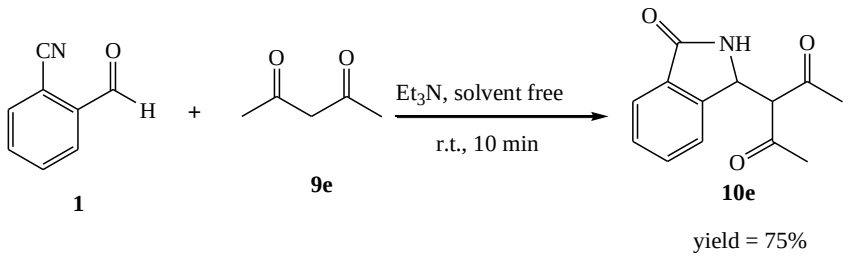
Entry	Adduct 9	Product 10	Yield[a] (%)	dr[b]
1	 **9a**	 **10a**	87	
2	 **9b**	 **10b**	67	1:1
3	 **9c**	 **10c**	87	1.5:1

4	 **9d**	 **10d**	64	
5	 **9e**	 **10e**	25	
6	 **9f**	 **10f**	12	
7	 **9g**	 **10g**	27	
8	 **9h**	 **10h**	75	
9	 **9i**	 **10i**	25	

[a] Yields ref chromatographically pure compounds. [b] The diastereomeric ratio were calculated on the basis of ^{1}H NMR spectrum of the crude product.

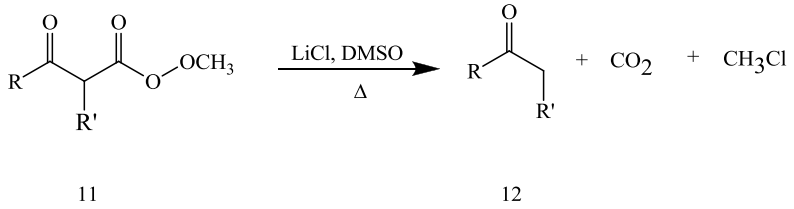
Only acetylacetone, under the same conditions, showed significantly lower reactivity (entry 5).

However, a good yield was observed in a very short time (10 min) when it was reacted under solvent-free conditions. (Scheme 4).



Scheme 4

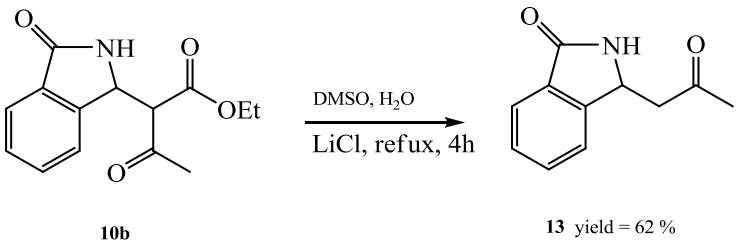
It is worthy to note that the obtained isoindolinones **10** show very interesting functionalities and they could be very useful for further transformations in the synthesis of valuable compounds. For example, according to Scheme 5, the decarboxylation of the β-keto ester derivative **10b**, was carried out by employing a modification of the mild Krapcho procedure.[17]



Scheme 5: Krapcho decarboxylation approach

The Krapcho decarboxylation is the chemical reaction of esters with halide anions. The ester must contain an electron-withdrawing group in the β-position, such as β-ketoesters, malonic esters, α-cyanoesters, or α-sulfonylesters. It works best with methyl esters, since it is basically a S_N2-reaction at carbon. It is driven by the entropy of the overall reaction, as the by-products chloromethane and CO_2 are lost as gases.

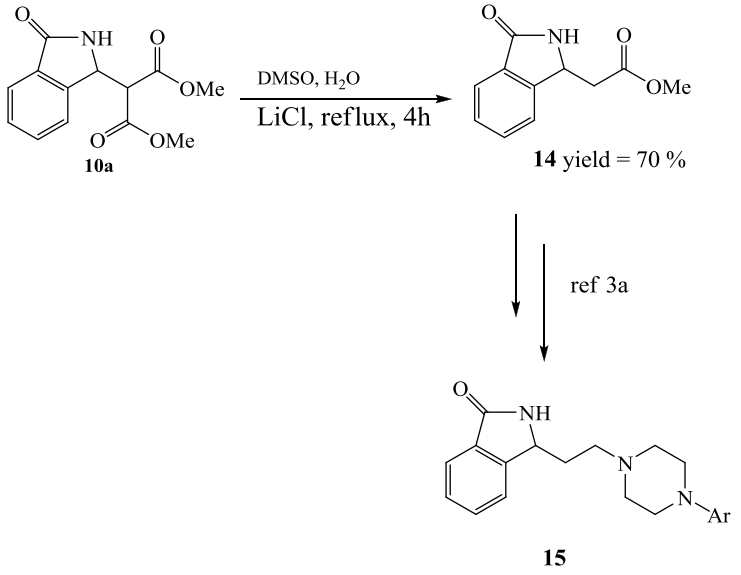
This approach led to known compound **13** (Scheme 6), previously obtained from by less effective synthesis employing a cobalt(II) catalyst.[11] The decarboxylation of compound **10b** reacted with LiCl, DMSO and H_2O at reflux for 4 hr to formed compound **13** as a 62% yield.



10b **13** yield = 62 %

Scheme 6

By using the same decarboxylation procedure a well-known intermediate (compound **14**)[12a] in the synthesis of a series of achiral and chiral piperazine derivatives **15** for dopamine D_4 receptor, a good target for the treatment of schizophrenia, that otherwise required a longer and more tedious method of synthesis[12a] was obtained (Scheme 7).



10a **14** yield = 70 %

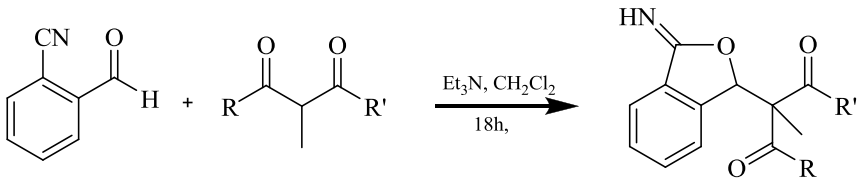
15

Scheme 7

The decarboxylation of compound **10a** gave compound 1**4** in 70 % yield.

As reported by Ramström, we demonstrated that the presence of two acidic protons in the α-position of 1,3-dicarbonyl compounds is the fundamental requisite that allows the isoindolinone ring to be obtained.[15] In fact, when ethyl 2-methylacetoacetate (**16**) or dimethyl methylmalonate (**17**) were reacted under the conditions of Scheme 3, new products were obtained in significantly lower yields.

The structure of these compounds was attributed to the iminoisobenzofurans **18** and **19**, respectively, by comparison with spectroscopic data of reported analogues (Scheme 8).[16,18]



1

16: R=CH_3, R'=OEt

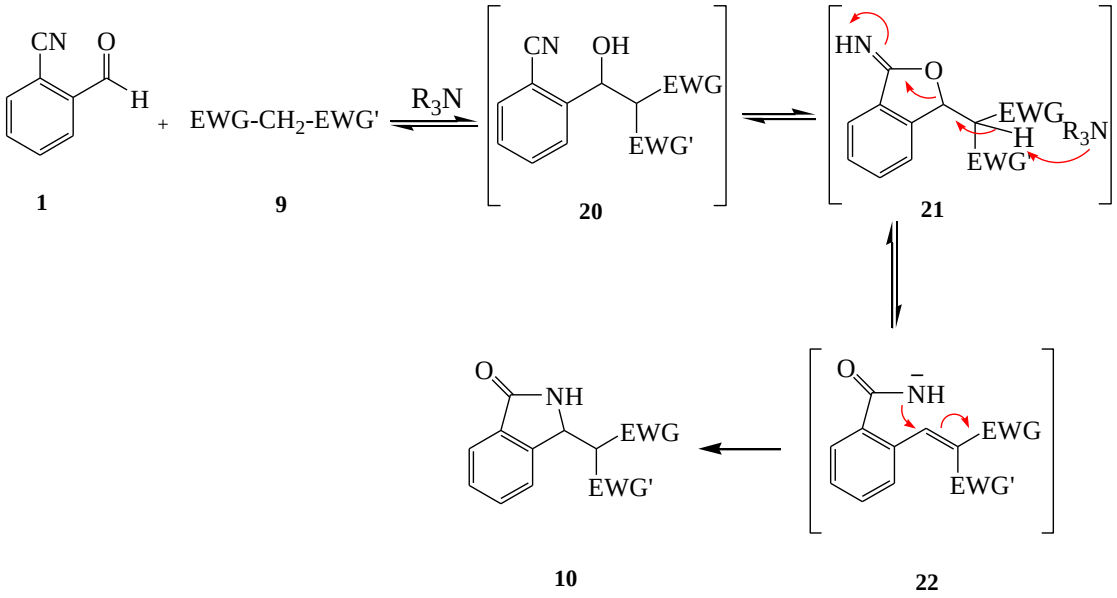
17: R=R'=OCH_3

18: yield = 46%, d.r. = 6/1

19: yield = 35 %

Scheme-8

Thus, on the basis of the obtained results, a general pathway can reasonably be proposed for the series of readily enolizable 1,3-dicarbonyl compounds (Scheme 9).



1 **9** **20** **21** **22** **10**

Scheme-9

After the reversible deprotonation of **9** and its subsequent nucleophilic addition to the aldehyde **1**, the unstable adduct **20** gives the iminoisobenzofuran **21** by cyclization at the cyano group. Deprotonation of **21** in the presence of a base and rearrangement gives the acyclic intermediate **22**. Finally, the intramolecular conjugated addition of **22** leads to the title isoindolinones **10**.

3.2.4 BIOLOGICAL ACTIVITY

The obtained 3-substituted isoindolinones were preliminary screened on two different virus (Hepatitis C Virus (HCV) and Chikungunya virus (CHIKV)) to evaluate potential activity on the virus replication in Hepatoma Human Cells (Huh 5-2 containing 1b genotype) and *Vero cells* respectively. CC_{50}, EC_{50} and EC_{90} values of the compounds comparing with arbidol are shown in Table 2

Table-2. *In vitro* antiviral activity of 3-substituted isoindolines derivatives

Compounds	HCV			CHIKV		
	CC_{50}	**EC_{50}**	**EC_{90}**	**CC_{50}**	**EC_{50}**	**EC_{90}**
10a	>190	ND	ND	ND	>380	>380
10c	>173	ND	ND	>346	184	346
10d	>206	>206	>206	ND	>411	>411
10e	>216	ND	ND	ND	>432	>432
10f	>205	ND	ND	ND	>409	>409
10g	>144	85.5	ND	>288	181	>288
10h	>202	>202	>202	ND	>404	>404
10i	53.9	22.8	ND	>230	112	220
18	115	17.1	ND	>363	323	>363
19	>180	>180	>180	ND	>361	>361
ARBIDOL	9.94	4.13	ND	83.1	19.4	35.2

ND = not determined
CC_{50} = 50 % Cytostatic/Cytotoxic Concentration (concentration at which 50 % adverse effect is observed on the host cell)
EC_{50} = 50 % Effective Concentration (concentration at which 50 % inhibition of virus replication is observed)
EC_{90} = 90 % Effective Concentration (concentration at which 90 % inhibition of virus replication is observed)

Unfortunately it was found that all compounds were not active: however further explore diverse biological activity could be explored by High-Throughput Screening. In addition because of the simplicity of the method, other studies could be desiderable to functionalize this scaffold for example by introduction of different substituents on the aromatic ring (starting from substituted 2-cyanobenzaldeydes) or by alkylation of the amidic nitrogen or both as in Pagoclone and (S)-PD 172938, two example of pharmacologically active compounds.[19]

3.2.5 CONCLUSION:

In conclusion, a study of the aldol addition of readily enolizable 1,3-dicarbonyl compounds to 2-cyanobenzaldehyde gave us the possibility to develop a simple and general access to a series of new 3-substituted isoindolinones in the presence of tertiary amines under very mild conditions.

We synthesized a series of new 3-substituted isoindolinones derivatives and examined their anti-HCV activities and Chikungunya virus. Unfortunately it was found that all compounds were not active.

3.2.6 EXPERIMENTAL SECTION:

i) General remarks

All reactions were performed in oven-dried (140 °C) or flame-dried glassware under dry N_2. Dichloromethane was reagent grade and was dried and distilled immediately from CaH_2 before use. Column chromatographic purification of products was carried out using silica gel 60 (70–230 mesh, Merck). The reagents (Aldrich and Fluka) were used without further purification. The NMR spectra were recorded on Bruker DRX 400, 300, 250 spectrometers (400 MHz, 300MHz, 250MHz, ^{1}H; 100 MHz, 75MHz, 62,5MHz ^{13}C). Spectra were referenced to residual $CHCl_3$ (7.26 ppm, ^{1}H, 77.23 ppm, ^{13}C). Coupling constants *J* are reported in Hz. Yields are given for isolated products showing one spot on a TLC plate and no impurities detectable in the NMR spectrum. Mass spectral analyses were carried out using an electrospray spectrometer, Waters 4 micro quadrupole.

Elemental analyses were performed with FLASHEA 1112 series-Thermo Scientific for CHNS-O apparatus.

ii) General Procedures

a) Dihydro-1*H*-isoindol-1-ones (10)

2-Cyanobenzaldehyde **1**, (0.40 mmol) was added to a solution of 1,3- dicarbonyl compound **9** (0.44 mmol) and Et_3N (0.40 mmol) in CH_2Cl_2 (2.0 mL). At the end of the reaction (TLC monitoring), the mixture was diluted with EtOAc (2 mL) and the solvent evaporated under reduced pressure. Then the crude product was purified by flash chromatography (hexane–EtOAc, 8:2 to 1:1) to afford pure products.

b) Decarboxylation Reaction To Give 13 or 14

Compound **10a** or **10b** (0.22 mmol), DMSO (4.0 mL), H2O (0.30 mL), and LiCl (0.50 mmol) were placed in a round-bottom flask equipped with a magnetic bar and a reflux condenser. The solution was heated to refluxing using an oil bath with stirring for 4 h. After cooling to r.t., the solution was added to EtOAc (60 mL), extracted with brine (3 × 20 mL), and dried (anhydrous Na_2SO_4). Then the solvent was removed under reduced pressure and the crude product was purified by chromatography (hexane:EtOAc, 8:2 to 1:1).

c) 1,3-Dihydroisobenzofuran-1-imines 18 and 19;

2-Cyanobenzaldehyde (**1**, 46 mg, 0.40 mmol) was added to a solution of ethyl 2-methylacetoacetate **16**, (62 mL, 0.44 mmol) or dimethyl methylmalonate **17**, (58 mL, 0.44 mmol) and Et_3N (61 mL, 0.44 mmol) in CH_2Cl_2 (1.0 mL) at r.t. At the end of the reaction (TLC monitoring), the mixture was diluted with EtOAc (2 mL) and the solvent evaporated under reduced pressure. Then the crude was purified by flash chromatography (hexane:EtOAc, 8:2 to 1:1) to give pure **18** and **19**.

iii) Characterization data

Note: Compound **10h** is known and was characterised comparing its spectroscopic data with those previously reported in literature.[11]

Dimethyl 2-(3-Oxo-2,3-dihydro-1*H*-isoindol-1-yl)malonate (10a)

Nature of compound: Solid; yield: 91 mg (87 %); mp 154–155 °C.

IR (KBr): 3182, 3051, 1742, 1696, 1434, 1263, 1143, 965, 769, 751 cm^{-1}.

^{1}H NMR (250 MHz, $CDCl_3$): δ 7.82 (d, *J* = 7.5 Hz, 1H), 7.53–7.47 (m, 2H), 7.32 (m, 2H), 5.18 (d, *J* = 7.5 Hz, 1H), 3.81 (s, 3H), 3.67 (s, 3H), 3.62 (d, *J* = 7.5 Hz, 1H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 171.3, 168.8, 168.3, 144.9, 133.3, 133.2, 130.2, 125.3, 124.1, 57.1, 56.1, 54.3, 53.2.

MS (ESI): *m*/*z* = 286 [M + Na^+].

Anal. Calcd for $C_{13}H_{13}NO5$: C, 59.31; H, 4.98; N, 5.32. Found: 59.45; H, 5.04; N, 5.15

Ethyl 3-Oxo-2-(3-oxo-2,3-dihydro-1*H*-isoindol-1-yl)butanoate (10b)

Nature of compound: Solid; yield: 70 mg (67 %); mixture of diastereoisomers.

IR (KBr): 3237, 2982, 2925, 2854, 1701, 1616, 1470, 1359, 1308, 1204, 1096, 1017, 751, 695 cm^{-1}.

^{1}H NMR (250 MHz, $CDCl_3$): δ 7.84 (d, *J* = 6.60 Hz, 2H), 7.54–7.46 (m, 4H), 7.34–7.26 (m, 2H), 6.86–6.83 (m, 2H), 5.24 (d, *J* =8.02 Hz, 2H), 4.36–4.27 (q, 2H), 4.16–4.08 (q, 2H), 3.81 (d, *J* =7.65 Hz, 1H), 3.65 (d, *J* = 8.37 Hz, 1H), 2.34 (s, 3H), 2.18 (s, 3H), 1.34–1.28 (t, 3H), 1.15–1.10 (t, 3H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 202.9, 201.9, 171.0, 170.9, 168.4,167.8, 145.4, 145.4, 133.3, 133.3, 133.2, 130.2, 130.1, 125.5, 125.4, 124.1, 65.4, 64.1, 63.5, 63.4, 55.7, 55.6, 32.4, 30.9, 15.2, 15.0.

MS (ESI): *m*/*z* = 284 [M + Na^+].

Anal. Calcd for $C_{14}H_{15}NO_4$: C, 66.36; H, 5.79; N, 5.36. Found: C, 66.47; H, 5.65; N, 5.43.

***tert*-Butyl 3-Oxo-2-(3-oxo-2,3-dihydro-1*H*-isoindol-1-yl)butanoate(10c)**

Nature of compound: Solid; yield: 101 mg (87 %); mixture of diastereomers.

^{1}H NMR (300 MHz; $CDCl_3$): δ 7.86 (br d, *J* = 6.0 Hz, 1 + 1H), 7.56–7.49 (m, 3 + 2H), 7.31 (d, *J* = 6.0 Hz, 1H), 7.12 (br s, 1H), 6.68 (br s, 1H), 5.18 (d, *J* = 8.0 Hz, 1H), 5.14 (d, *J* = 6.0 Hz, 1H), 3.78 (d, *J* = 6.0 Hz, 1H), 3.60 (d, *J* = 8.0 Hz, 1H), 2.35 (s, 3H), 2.17 (s, 3H), 1.42 (s, 9H), 1.29 (s, 9H).

^{13}C NMR (100 MHz; $CDCl_3$): δ 202.3, 201.1, 169.9, 169.8, 166.1, 165.5, 144.4, 144.3, 132.1, 132.0, 131.9, 131.8, 128.7, 128.6, 123.9, 123.3, 122.8, 83.4, 83.3, 64.8, 63.3, 54.5, 54.4, 30.7, 29.5, 27.7, 27.5.

MS (ESI): *m*/*z* = 290 [M + H^+].

Anal. Calcd for $C_{16}H_{19}NO_4$: C, 66.42; H, 6.62; N, 4.84. Found: C, 66.35; H, 6.53; N, 4.90.

2-(3-Oxo-2,3-dihydro-1*H*-isoindol-1-yl)cyclohexane-1,3-dione (10d)

Nature of compound: Solid; yield: 63 mg (65%); mp 210 °C (dec).

IR (KBr): 3286, 2931, 1631, 1389, 1302, 995, 683 cm^{-1}.

^{1}H NMR (400 MHz; CD_3OD): δ 7.71 (d, *J* = 8.0 Hz, 1 H), 7.50–7.46 (t, 1 H), 7.39–7.35 (t, 1 H), 7.25 (d, *J* = 8.0 Hz, 1 H), 5.96 (s, 1H), 2.40–2.29 (m, 4 H), 1.96–1.91 (q, 2 H).

^{13}C NMR (100 MHz; CD_3OD): δ 191.5, 173.7, 150.8, 133.4, 132.4, 127.7, 123.4, 122.9, 110.5, 53.1, 35.1, 21.8.

MS (ESI): *m*/*z* = 244 [M + H^+].

Anal. Calcd for $C_{14}H_{13}NO_3$: C, 69.12; H, 5.39; N, 5.76. Found: 69.31; H, 5.28; N, 5.67.

3-(3-Oxo-2,3-dihydro-1*H*-isoindol-1-yl)pentane-2,4-dione (10e)

Nature of compound: Solid; yield: 70 mg (76%); mp 146.7–147.8 °C.

IR (KBr): 3226, 3081, 3025, 2919, 2849, 1697, 1417, 1358, 1311, 1238, 1138, 749, 696cm^{-1}.

^{1}H NMR (250 MHz; $CDCl_3$): δ 7.81 (d, *J* = 5.0 Hz, 1 H), 7.56–7.46 (m, 4 H), 7.30 (d, *J* = 7.5 Hz, 1 H), 5.64 (s, 1 H, enol form), 5.25 (d, *J* = 5.0 Hz, 1 H), 4.06

(d, *J* = 7.5 Hz, 1 H), 2.40 (s, 3 H, enol form), 2.26 (s, 3 H), 2.08 (s, 3 H), 1.41 (s, 3 H, enol form).

^{13}C NMR (100 MHz, CDCl3): δ 203.5, 202.3, 198.4, 190.6, 172.9, 171.5, 148.3, 145.4, 133.8, 133.5, 133.3, 133.2, 130.2, 129.8, 125.5, 125.2, 124.1, 123.6, 108.0, 72.3, 56.2, 32.7, 31.8, 25.6, 24.1.

MS (ESI): *m*/*z* = 254 [M + Na^+].

Anal. Calcd for $C_{13}H_{13}NO_3$: C, 67.52; H, 5.67; N, 6.06. Found: C, 67.45; H, 5.78; N, 6.12.

Ethyl 2-cyano-2-(1-oxoisoindolin-3-yl)acetate(10f):

Nature of compound: solid, yield = (11 mg) 12 %. Mp 164.2-165.9 °C.

^{1}H NMR (250 MHz; $CDCl_3$): δ 7.68-7.65 (t, 1+1H), 7.64 (d, *J*=2.5 Hz, 1H), 7.62-7.55 (m, 3+2H), 7.49 (d, *J*=7.5 Hz, 1H), 7.40 (s, 1H), 5.20 (d, *J*=2.5 Hz, 1H), 5.15 (d, *J*=7.5 Hz, 1H), 4.42-4.32 (m, 2+2H), 4.14 (d, *J*=2.5 Hz, 1H), 3.76 (d, *J*=10 Hz, 1H), 1.38-131 (m, 3+3H).

^{13}C NMR (100 MHz; $CDCl_3$): δ 171.3, 171.1, 165.4, 143.4, 143.2, 134.0, 133.9, 133.3, 132.8, 131.1, 125.9, 125.6, 124.8, 123.4, 115.1, 113.6, 65.2, 55.8, 55.5, 43.8, 43.7, 31.4, 30.9, 15.1. **MS** (ESI): *m*/*z* = 267.41 (M + Na^+).

Anal. Calcd for $C_{16}H_{19}NO_4$: C, 63.93; H, 4.95; N, 11.47. Found: C, 63.32; H, 4.83; N, 11.24.

Di-*tert*-butyl 2-(1-oxoisoindolin-3-yl)malonate (10g):

Nature of compound: solid, yield = (38 mg) 27 %.

^{1}H NMR (300 MHz; $CDCl_3$): δ 7.84 (d, *J*=8.0 Hz, 1H), 7.56-7.46 (m, 2 H), 7.43 (d, *J*=8.0 Hz, 1H), 6.82 (s, 1H), 5.08 (d, *J*=8.0 Hz, 1H), 3.52 (d, *J*=4.0 Hz, 1H), 1.50 (s, 9H), 1.29 (s, 9H).

^{13}C NMR (100 MHz; $CDCl_3$): δ 169.8, 166.7, 165.6, 144.0, 132.1, 131.1, 128.6, 123.7, 123.1, 82.9, 82.7, 57.5, 54.7, 27.7, 27.5.

MS (ESI): *m*/*z* = 348 (M + H^+).

Anal. Calcd for $C_{19}H_{25}NO_5$: C, 65.69; H, 7.25; N, 4.03. Found: C, 65.64; H, 7.28; N, 4.01.

Methyl (1*Z*)-*N*-[(4-methylbenzyl)sulfonyl]-2-phenylethanimidoate (10j)

Nature of compound: solid, yield = (45 mg) 25 %, Mp 141.6-142.7 °C.

^{1}H NMR (400 MHz; $CDCl_3$): δ 7.84 (d, *J* = 7.24 Hz, 1H), 7.74 (d, *J*=8.12 Hz, 2H), 7.54 (s, 3H), 7.42 (s, 5H), 7.26-7.21 (m, 4H), 7.16 (d, *J* = 8.0 Hz, 1H), 6.07 (dd, *J*=7.68 Hz & 10.56 Hz, 2H), 5.27 (d, *J*=7.2 Hz, 1H),5.12 (d, *J*=10.4 Hz, 1H), 3.91 (s, 3H), 3.90 (s, 3H), 2.40 (s, 3H), 2.37 (s, 3H).

^{13}C NMR (100 MHz; $CDCl_3$): δ 172.6, 172.5, 168.1, 145.6, 145.4, 144.7, 139.8, 139.4, 134.2, 133.8, 133.4, 132.8, 131.2, 130.9, 130.6, 130.5, 130.3, 130.2, 129.8, 129.5, 128.0, 127.9, 125.2, 123.9, 123.8, 83.8, 83.4, 57.4, 57.2, 54.9, 54.8, 30.9, 22.7.

MS (ESI): *m*/*z* = 435 ($M + H^+$).

Anal. Calcd for $C_{16}H_{19}NO_4$: C, 66.34; H, 5.10; N, 6.45. Found: C, 66.31; H, 5.10; N, 6.41.

3-(2-Oxopropyl)-2,3-dihydro-1*H*-isoindol-1-one (13)[3b]

Nature of compound: Solid; yield: 26 mg (62%); mp 132.7–134.1 °C.

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.86 (d, *J* = 7.3 Hz, 1H), 7.65–7.36 (m, 2H), 7.38 (d, *J* = 7.3 Hz, 1H), 6.68 (br s, 1H), 4.94 (br d, *J* = 7.8 Hz, 1H), 3.21 (dd, *J* = 3.2, 18.5 Hz, 1H), 2.59 (dd, *J* = 10.2, 18.5 Hz, 1H), 2.24 (s, 3H).

MS (ESI): *m*/*z* = 190 [$M + H^+$].

Anal. Calcd for $C_{11}H_{11}NO_2$: C, 69.83; H, 5.86; N, 7.40. Found: C, 69.62; H, 5.70; N, 7.68.

Methyl (3-Oxo-2,3-dihydro-1*H*-isoindol-1-yl)acetate (14)

Nature of compound: Solid; yield: 32 mg (70 %); mp 145.6–157.7 °C.

^{1}H NMR (250 MHz, $CDCl_3$): δ 7.86 (d, *J* = 7.1 Hz, 1H), 7.59–7.40 (m, 3H), 6.93 (br s, 1H), 4.93 (dd, *J* = 10.2, 3.3 Hz, 1H), 3.78 (s, 3H), 3.03 (dd, *J* = 3.4, 17.1 Hz, 1H), 2.46 (dd, *J* = 10.2, 17.2 Hz, 1H), 2.24 (s, 3H).

^{13}C NMR (75 MHz, $CDCl_3$): δ 171.6, 170.0, 145.6, 132.0, 131.7, 128.6, 124.1, 122.2, 52.6, 52.2, 39.2.

MS (ESI): *m*/*z* = 206 [$M + H^+$].

Anal. Calcd for $C_{11}H_{11}NO_3$: C, 64.38; H, 5.40; N, 6.83. Found: C, 64.52; H, 5.30; N, 6.68.

Ethyl 2-(3-Imino-1,3-dihydroisobenzofuran-1-yl)-2-methyl-3-oxobutanoate (18)

Nature of compound: Oil; yield: 51 mg (46 %); mixture of diastereomers.

IR (KBr): 3296, 2984, 1716, 1693, 1468, 1357, 1252, 1158, 1116, 1021, 984, 839, 779, 609 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.83 (d, *J* = 7.47 Hz, 1H), 7.51–7.45 (m, 2H), 7.35 (d, *J* = 6.90 Hz, 1H), 6.26 (s, 1H), 6.24 (s, 1H, minor), 4.28–4.15 (m, 2H), 2.33 (s, 3H), 2.17 (s, 3H, minor), 1.30–1.12 (m, 3H), 1.09 (s, 3H), 1.06 (s, 3H, minor).

^{13}C NMR (100 MHz, $CDCl_3$): δ 203.5 (minor), 202.0, 169.4, 168.9 (minor), 166.7, 143.5, 143.3 (minor), 132.2, 130.4 (minor), 129.9, 129.3, 123.8, 123.7, 123.2, 83.7 (minor), 83.3, 64.3 (minor), 63.8, 62.2 (minor), 61.9, 29.5, 26.6, 26.4 (minor), 13.7 (minor), 13.6.

MS (ESI): *m*/*z* = 276 [M + H^+].

Anal. Calcd for $C_{15}H_{17}NO_4$: C, 65.44; H, 6.22; N, 5.09. Found: C, 65.53; H, 6.17; N, 5.01.

Dimethyl 2-(3-Imino-1,3-dihydroisobenzofuran-1-yl)-2-methylmalonate (19)

Nature of compound: Oil; yield: 39 mg (35 %).

IR (KBr): 3298, 2954, 1732, 1694, 1468, 1435, 1355, 1268, 1157, 1112, 1034, 978, 839, 779, 751, 662 cm–1.

^{1}H NMR (250 MHz, $CDCl_3$): δ 7.55 (d, *J* = 5.75 Hz, 1H), 7.53–7.41 (m, 3H), 6.20 (s, 1H), 3.82 (s, 3H), 3.78 (s, 3H), 1.13 (s, 3H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 169.59, 169.16, 166.7, 143.0, 132.2, 130.2, 129.3, 123.8, 123.2, 83.4, 57.9, 53.1, 52.8, 14.0.

MS (ESI): *m*/*z* = 278 [M + H^+].

Anal. Calcd for $C_{14}H_{15}NO_5$: C, 60.64; H, 5.45; N, 5.05. Found: C, 60.75; H, 5.49; N, 5.12.

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Publications:

1. Multi-component, regio-selective aldol addition of β-ketoesters to aldehydes: scope and applications

   Vijaykumar More, Antonia Di Mola, Gianluca Croce, Consiglia Tedesco, Carmen Petronzi, Antonella Peduto, Paolo De Caprariis, Rosanna Filosa, and Antonio Massa*

   Organic & Biomolecular Chemistry, 2011, *9*, 8483-8488.

2. The Aldol addition of readily enolizable 1,3-dicarbonyl compounds to 2-cyanobenzaldehyde in the synthesis of novel 3-substituted isoindolinones

   Vijaykumar More, Antonia Di Mola, Milena Perillo, Paolo De Caprariis, Rosanna Filosa, Antonella Peduto, and Antonio Massa*

   Synthesis, 2011, *18*, 3027-3031.

3. Synthesis and cytotoxic activity of new β-carboline derivatives

   A. Peduto* **V. More**, P. De Caprariis, M. Festa, A. Capasso, S. Piacente, L. De Martino, V. De Feo and R. Filosa

   Mini-Reviews in Medicinal Chemistry, 2011, *11*, 486-491.

4. Design of Influenza virus inhibitor with membrane fusion targeting mechanism: Synthesis, structure-Activity relationship and *in vitro* anti-viral activity of novel indole series. (Manuscript Submitted).