A Novel Access to Arylated and Heteroarylated Beta-Carboline Based PDE5 Inhibitors

Nermin S. Ahmed*[,1], Bernard D. Gary[2], Gary A. Piazza[2], Heather N. Tinsley[2], Stefan Laufer[3] and Ashraf H. Abadi[1]

[1]*Department of Pharmaceutical Chemistry, Faculty of Pharmacy and Biotechnology, German University in Cairo, Cairo 11835, Egypt*

[2]*Division of Drug Discovery, Department of Biochemistry and Molecular Biology, Southern Research Institute, The University of Alabama at Birmingham, 2000 Ninth Avenue South, Birmingham, AL 35205, USA*

[3]*Department of Pharmaceutical and Medicinal Chemistry, Eberhard Karls University Töbingen, Auf der Morgenstelle 8, 72076 Töbingen, Germany*

Abstract: Starting from a previously reported lead compound GR30040X (a hydantoin tetrahydro-β-carboline derivative with a 4- pyridinyl ring at C- 5), a series of structurally related tetrahydro-β-carboline derivatives were prepared. The tetrahydro-β-carboline skeleton was fused either to a hydantoin or to a piperazindione ring, the pendant aryl group attached to C-5 or C-6 was changed to a 3, 4-dimethoxyphenyl or a 3-pyridinyl ring; different N-substituents on the terminal ring were introduced, a straight chain ethyl group, a branched *tert.* butyl and *P*-chlorophenyl group rather than *n*-butyl group of the lead compound. All four possible diastereomers of target tetrahydro-β-carboline derivatives were prepared, separated by column chromatography and the significance of these stereochemical manipulations was studied. Synthesized compounds were evaluated for their inhibitory effect versus PDE5. Seven hits were obtained with appreciable inhibitory activity versus PDE5 with IC_{50}s 0.14 - 4.99 μM.

Keywords: 3- pyridine, cGMP, MED, PDE5, tadalafil, tetrahydro-ß-carboline.

1. INTRODUCTION

It is well established that nitric oxide (NO) and natriuretic peptides increase intracellular cGMP levels by stimulation of the soluble and membrane bound guanylate cyclase, respectively. This cGMP formation is the initial step of a crucial biochemical pathway, regulating the cardiovascular, central and peripheral nervous system. Phosphodiesterases (PDEs) are intracellular enzymes that specifically catalyze the hydrolysis of the second messengers cAMP and cGMP to the inactive metabolites AMP and GMP. Various degrees of substrate specificity is reported for this family of enzymes, the cAMP specific PDEs include PDE7, PDE4 and PDE8 and the cGMP specific PDEs include PDE5, PDE6 and PDE9, the others are of dual activity [1]. Thus PDE5 inhibition increases intracellular cGMP levels by preventing its degradation with subsequent decrease in intracellular calcium levels, thereby promoting relaxation of smooth muscle cells and a variety of other calcium-dependent processes [2]. Since inhibitors of PDE5 raise intracellular cGMP levels, the effects will be much more pronounced under conditions when cGMP formation is already increased for example sexual stimulation [3]. Sexual stimulation induces a selective vasorelaxation in penile tissue, which is predominantly mediated by the release of NO in the cavernous nerve endings, the endothelium of penile arteries and the corpora cavernosa [4]. The wide, safe use of PDE5 inhibitors, together with an increasing understanding of cGMP-regulated mechanisms, have triggered a number of attempts to find new applications for these agents. It has been shown that besides corpus cavernosum, PDE5 is also expressed in smooth muscles of the systemic vasculature, prostate, bladder, cardiac tissues, brain and platelets which imply additional target tissues for PDE5 inhibitors [5, 6]. Recently, PDE5 inhibitors have been reported to be effective in the treatment of various disorders such as chronic obstructive pulmonary disease, prostate hyperplasia, hypertension and coronary heart disease [7]. The over expression of PDEs has been described in various cancer pathologies, inhibition of selective PDE isoforms raises the level of intracellular cAMP and /or cGMP which in turn induces apoptosis and cycle arrest in a broad spectrum of tumor cells and regulates the tumor microenvironment, and this may provide a whole new class of antitumor therapy with reduced adverse effects [8, 9].

The superposition of all PDE5A reported inhibitors has revealed with outstanding clarity that there is a highly conserved binding mode among all the inhibitors. All these inhibitors share a core binding site that can be characterized by a planar ring sandwiched by the hydrophobic clamp and the formation of an H bond with an invariant glutamine 817. The substructure of the inhibitors that bind to this core represents

*Address correspondence to this author at the Department of Pharmaceutical Chemistry, Faculty of Pharmacy and Biotechnology, German University in Cairo, Cairo 11835, Egypt; Tel: +20227590623; Fax: +20227581041;
E-mail: nermin.salah@guc.edu.eg

1573-4064/10 $55.00+.00

a framework upon which more potent and selective inhibitors can be developed [10].

Herein, we report about thirty two new tetrahydro-β-carboline derivatives, structurally related to the lead compound (5*R*,11aR)-2-butyl-5-(pyridin-4-yl)-5,6,11,11a-tetrahydro-1*H*imidazo[1',5':1,6]pyrido[3,4-*b*]indole-1,3(2*H*)-dione (GR30040X), by replacing its 4-pyridinyl moiety with 3-pyridinyl, 3,4-dimethoxyphenyl; changing the nature of the substituent on its terminal nitrogen and manipulating its stereochemical aspects and the size of the terminal ring.

2. CHEMISTRY

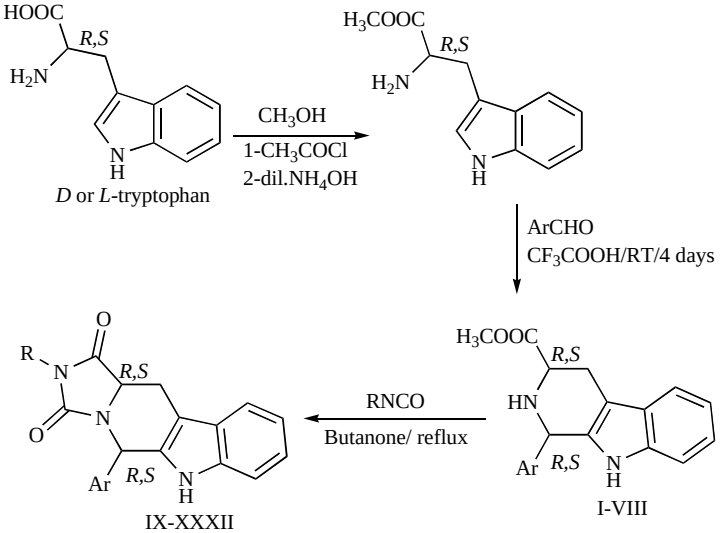
The functional tetrahydro-ß-carboline skeleton can be assembled by the reaction of *D*- or *L*-tryptophane methyl ester (prepared according to a known procedure) [11] with the selected aldehydes (3 pyridinecarboxaldehyde, 3,4 dimethoxybenzaldehyde) under non stereospecific Pictet-Spengler conditions (TFA/ CH_2Cl_2) [12] to furnish the required *cis*- and *trans*- isomers in different yields (**I-VIII**). The diastereomers are then separated using column chromatography. Each of the pure diastereomers is then allowed to react with one of the commercially available isocyanates to allow the fusion of the THBCs to an imidazolinedione ring (Scheme **1**). In order to expand the substrate types, we turned our attention to enlarge the imiadazolidinedione ring to a piperazindione ring; this was accomplished through reaction of the pure diastereomer of THBCs (**V-VIII**) with chloroacetyl chloride to provide the acylated intermediate in almost quantitative yield. Subsequent cyclization of these intermediates with selected amines in methanol provided the piperazindione series (Scheme **2**). The attempt to prepare a piperazindione series from THBCs with a pyridinecarboxaldehyde at position 3 failed, to the best of our knowledge the failure of cyclization may be attributed to intermolecular reaction involving the 3-pyridine ring.

The assignment of *cis- / trans*- stereochemistry for the tetrahydro-ß-carbolines (**I-VIII**) was based on detailed study of ^{13}C-NMR spectroscopy data well established in previous literature [13, 14]. Signals for C-1 and C-3 in the *trans*-THBC isomers appeared at higher field in the carbon spectrum than the analogous carbons of the corresponding *cis*-isomer, probably due to the 1,3-interactions present in the *trans*-isomer. Moreover, the ^{1}H-NMR signals for the proton at C-1 appeared at about 5.5 ppm, on cyclization to the hydantoin and piperazinedione derivatives the same proton which is now attached to C-5 and C-6 respectively makes a remarkable downfield shift compared to its respective THBC, this may be due to the electron withdrawing effect of the neighboring carbonyl. Also, regarding the THBCs a significant difference has been noted between the 2 isomers regarding their melting points and R_f values. A correlation exists between R_f value on TLC and the stereochemistry of the 1, 3-disubstituted tetrahydro-β-carbolines. The *cis*-isomer is systematically less polar than the *trans*-isomer; however, in the hydantoin series, the polarity is reversed, thus, the *cis*-isomer becomes more polar than the *trans*-isomer. The difference in both R_f and melting point values are not as significant in the piperazindione series as it is in the hydantoin series. The presence of a 3-pyridine ring in compounds **(I-IV** & **VIII-XIX**) led to a large chemical shift in the aromatic protons at *ortho* and *para* position; this is due to the deshielding effect of nitrogen on C2 and C4 of the ring.

3. RESULTS AND DISCUSSION

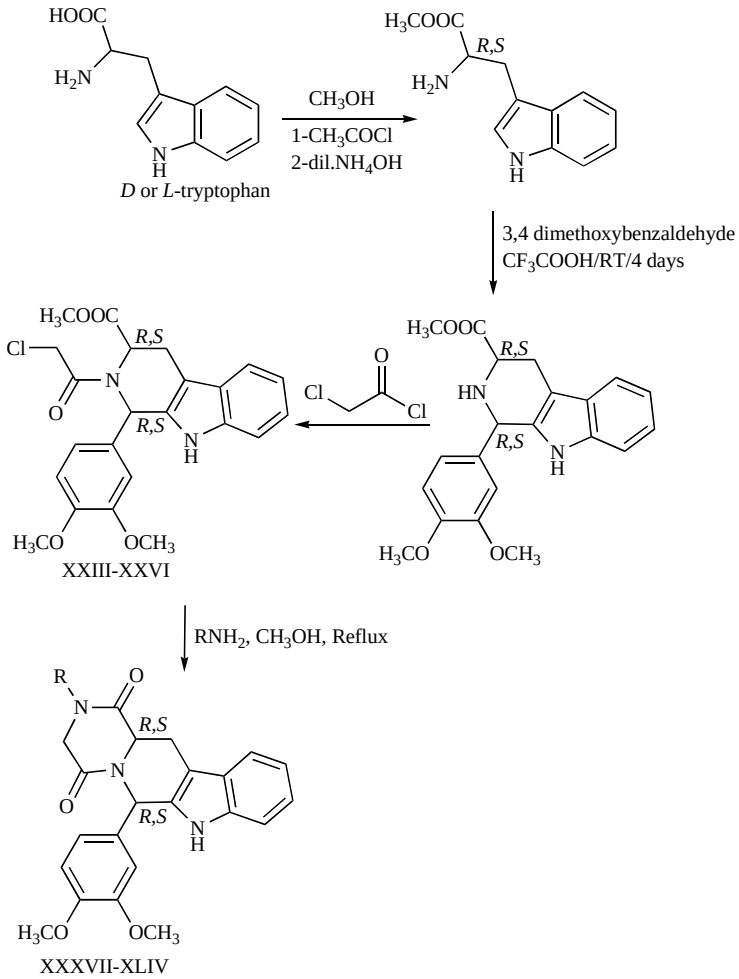
Table **1** shows the reported IC_{50} values for PDE5 inhibition of the reference compound GR30040X compared to other previously synthesized PDE5 inhibitor, the results show that GR30040X PDE5 inhibitory activity is much less than the phenyl congener (**XLV**). The decrease in the activity of GR20040X relative to (**XLV**) was attributed to decrease in the electron density on the pendant pyridine due to the electron withdrawing effect of the N.

Moreover a related structure to (**XLV**) but with pendant 4-methoxyphenyl (**XLVI**) and the structurally related 3,4 benzodioxol derivative (tadalafil) were highly potent PDE5 inhibitors, Thus, we designed our new molecules as



Ar = 3- pyridinyl	I- IV
Ar = 3, 4 dimethoxyphenyl	V-VIII

Scheme 1.



R	Compound
C_2H_5	XXXVII, XXXVIII, XXIX
tert-butyl	XLI, XLII, XLIII, XLIV

Scheme 2.

positional isomers to GR30040X *viz* the 3- pyridinyl congener and by trying the 3, 4-dimethoxyphenyl as the pendant aryl. In the latter case the two methoxy functional groups increase the electron density on the phenyl ring and may lead to more active derivatives. Other structural modifications were: keeping the terminal ring as hydantoin or enlarging it to piperazinedione; variation of the *N*-substituent from *n*-alkyl (ethyl) to branched (*tert*-butyl) and finally to an aromatic (*P*-chlorophenyl) and manipulating the stereochemical aspects of the 2 chiral carbons (C5, C11a in case of hydantoin derivatives) and (C6, C12a in case of piperazindione derivatives).

To our surprise almost all compound with the 3, 4 dimethoxyphenyl substituent were of marginal PDE5 inhibitory activity (5-7 μM) or were totally inactive despite the fact that this substitution is very similar to tadalafil (a potent PDE5 inhibitor with IC_{50}= 7 nM) and **XLVI (**another potent PDE5 inhibitor with IC_{50}= 10 nM), and the pendant aryls in these derivatives have nearly the same electronic properties, The steric compactness of the 3,4 dimethoxy group compared to the conformationally constrained 3,4-methylenedioxy of tadalafil, seems to be a governing factor in activity, possibly suggesting that the presence of this 3,4 dimethoxy free rotating groups prevents the optimal orientation of the phenyl ring, this steric clash is not observed when only 4-methoxyphenyl moeity is present **XLVI**.

Seven of our compounds with the 3-pyridinyl substitution showed PDE5 inhibitory activity with IC_{50}= 0.14 - 4.99 μM (**IX, XII, XIII, XIV, XVI, XVII, XX**). The following SAR conclusions can be withdrawn:

All the active hydantoins were of the *R*- configuration at C-5 but C11a was either *R* or *S*, thus, the stereochemical aspect of the carbon derived from the aldehyde is more important than that of the carbon derived from amino acids e.g. the 5*R*, 11a*R; 5R*, 11a*S* diastereomers were equiactive e.g. **IX** versus **XII** and in other cases *5R, 11a*S was more active than the *5R*, 11a*R* e.g. **XVI** versus **XIII**, the order of activity in the case of **XVI-XIII** was *5R, 11aS > 5R, 11aR >5S, 11aR > 5S, 11aS*. This opens the horizon towards obtaining efficient PDE5 inhibitors derived from cheap commercially available *L*-tryptophan rather than *D*-tryptophan, this may be of an economic advantage.

Regarding the size of substituent on the nitrogen of the terminal ring; *N*-substitution seems to slightly affect the activity, however compounds with ethyl substitution contrib-

utes most to our hit list and those of *P*-chlorophenyl contributes least, but none of these substituent had a deleterious effect on activity, this may encourage the use of less bulky groups in the future. It is apparent that a wide range of *N*-alkyl groups is tolerated the hydantoin series.

Surprisingly, all the piperazinedione derivatives were inactive, thus at least with the respective pendant aryls; the activity is limited to the hydantoin derivatives.

The disubstitued THBCs (**I-VIII**), did not show appreciable inhibition to PDE5 at the screening dose to justify IC_{50} determination. As all the active derivatives were of the THBCs-hydantoin skeleton, thus the PDE5 inhibition is determinately linked to the formation of the respective hydantoin, derivatives. It is worthy to mention that all the chloroethanone derivatives (**XXXIII-XXXVI**) were also among the least active at the screening dose, this confirms that for this class of PDE5 inhibitors an integer tetracyclic part is essential for PDE5 inhibition. The biological screening results are shown in Tables **2-7**.

Docking of **IX** and tadalafil to the PDE5 binding pocket, they showed an interaction slightly different from each other, while like all reported PDE5 inhibitors tadalafil formed a hydrogen bonding with glutamine 817, **IX** showed hydrogen bonding of the oxygen of the carbonyl group of the hydantoin ring with tyrosine 612, the lack of this type of interaction may explain its relative lower potency. The results are displayed in Figs. (**1** and **2**).

Table 1. Reported % PDE5 Inhibition and IC_{50} values Versus PDE5 for some Known PDE5 Inhibitors

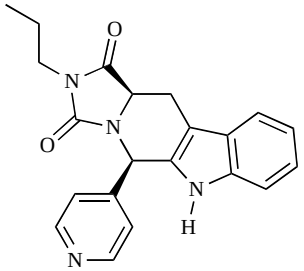
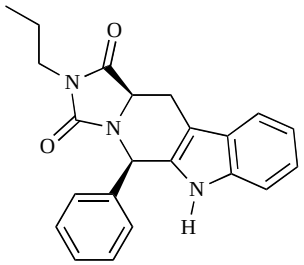
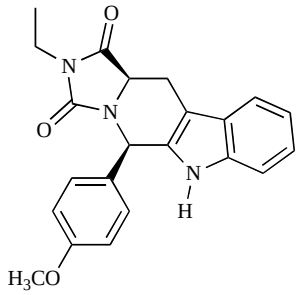
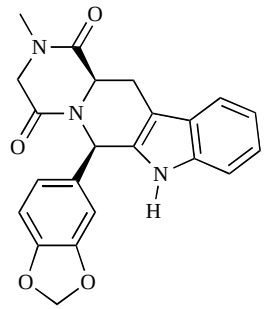
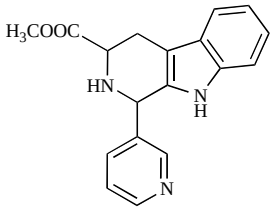
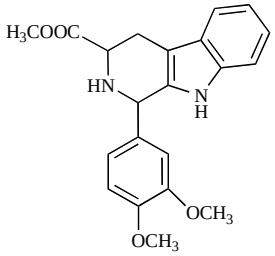
Code	Structure	%PDE5 Inhibition (50 μM)	IC_{50} (μM)
GR30040X		75	0.3
XLV		102	0.06
XLVI		98	0.01
Tadalafil		102	0.007

Table 2. % PDE5 Inhibition and IC_{50} Values of C-3 (3- pyridinyl) tetrahydro-ß-carbolines



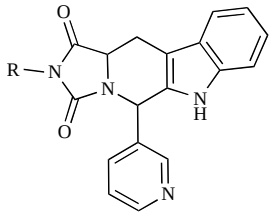
Code	Absolute Stereochemistry	%PDE5 Inhibition (50 µM)	IC_{50} (µM)
I	1*R*, 3*R*	50	*ND
II	1*S*, 3*R*	30	ND
III	1*S*, 3*S*	72	>10
IV	1*R*, 3*S*	41	ND

Table 3. % PDE5 Inhibition and IC_{50} Values of C-3 (3,4- dimethoxyphenyl) tetrahydro-ß-carbolines



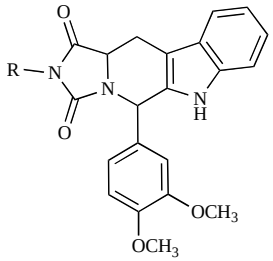
Code	Absolute Stereochemistry	%PDE5 Inhibition (50 µM)	IC_{50} (µM)
V	1*R*, 3*R*	48	ND
VI	1*S*, 3*R*	54	ND
VII	1*S*, 3*S*	8	ND
VIII	1*R*, 3*S*	7	ND

Table 4. % PDE5 Inhibition and IC_{50} Values of C-5 (3- pyridinyl) Hydantoin Derivatives



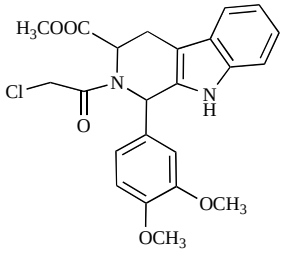
Code	R	Absolute Stereochemistry	%PDE5 Inhibition (50 µM)	IC_{50} (µM)
IX	Ethyl	5*R*, 11a*R*	102	0.14
X	Ethyl	5*S*, 11a*R*	58	ND
XI	Ethyl	5*S*, 11a*S*	24	ND
XII	Ethyl	5*R*, 11a*S*	100	0.15
XIII	*tert*- butyl	5*R*, 11a*R*	90	0.41
XIV	*tert*- butyl	5*S*, 11a*R*	89	4.75
XV	*tert*- butyl	5*S*, 11a*S*	45	ND
XVI	*tert*- butyl	5*R*, 11a*S*	90	0.18
XVII	*P*-chlorophenyl	5*R*, 11a*R*	85	4.99
XVIII	*P*-chlorophenyl	5*S*, 11a*R*	2	ND
XIX	*P*-chlorophenyl	5*S*, 11a*S*	1	ND
XX	*P*-chlorophenyl	5*R*, 11a*S*	87	1.25

Table 5. % PDE5 Inhibition and IC_{50} Values of C-5 (3,4- dimethoxyphenyl) Hydantoin Derivatives



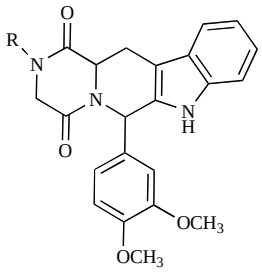
Code	R	Absolute Stereochemistry	% PDE5 Inhibition (50 µM)	IC_{50} (µM)
XXI	Ethyl	5*R*, 11a*R*	78	5.11
XXII	Ethyl	5*S*, 11a*R*	70	7
XXIII	Ethyl	5*S*, 11a*S*	52	ND
XXIV	Ethyl	5*R*, 11a*S*	75	13.73
XXV	*tert*- butyl	5*R*, 11a*R*	54	ND
XXVI	*tert*- butyl	5*S*, 11a*R*	52	ND
XXVII	*tert*- butyl	5*S*, 11a*S*	58	ND
XXVIII	*tert*- butyl	5*R*, 11a*S*	79	6.21
XXIX	*P*-chlorophenyl	5*R*, 11a*R*	22	ND
XXX	*P*-chlorophenyl	5*S*, 11a*R*	20	ND
XXXI	*P*-chlorophenyl	5*S*, 11a*S*	33	ND
XXXII	*P*-chlorophenyl	5*R*, 11a*S*	87	5.36

Table 6. % PDE5 Inhibition and IC_{50} Values of C-3 (3,4- dimethoxyphenyl) Chloroacetyl Derivatives



Code	Absolute Stereochemistry	% PDE5 Inhibition (50 µM)	IC_{50} (µM)
XXXIII	1*R*, 3*R*	70	>10
XXXIV	1*S*, 3*R*	75	>10
XXXV	1*S*, 3*S*	48	ND
XXXVI	1*R*, 3*S*	22	ND

Table 7. % PDE5 Inhibition and IC_{50} Values of C-6 (3,4- dimethoxyphenyl) Piperazinedione Derivatives



Code	R	Absolute Stereochemistry	% PDE5 Inhibition (50 µM)	IC_{50} (µM)
XXXVII	Ethyl	6*R*, 12a*R*	68	4.35
XXXVIII	Ethyl	6*S*, 12a*R*	13	ND
XXXIX	Ethyl	6*S*, 12a*S*	22	ND

Table 7. contd….

Code	R	Absolute Stereochemistry	% PDE5 Inhibition (50 μM)	IC_{50} (μM)
XL	Ethyl	6*R*, 12a*S*	10	ND
XLI	*tert*- butyl	6*R*, 12a*R*	10	ND
XLII	*tert*- butyl	6*S*, 12a*R*	21	ND
XLIII	*tert*- butyl	6*S*, 12a*S*	18	ND
XLIV	*tert*- butyl	6*R*, 12a*S*	55	ND

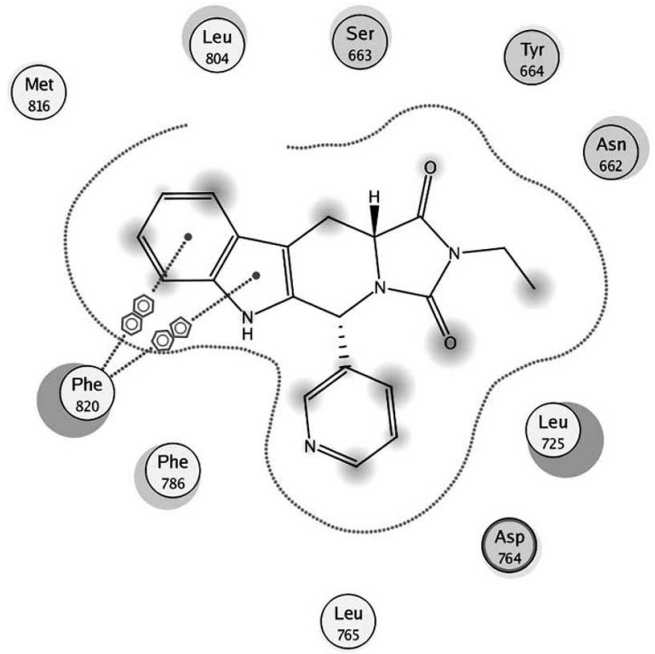


Fig. (1). Detailed mode view showing the docking and interaction of **tadalafil** with human PDE5.

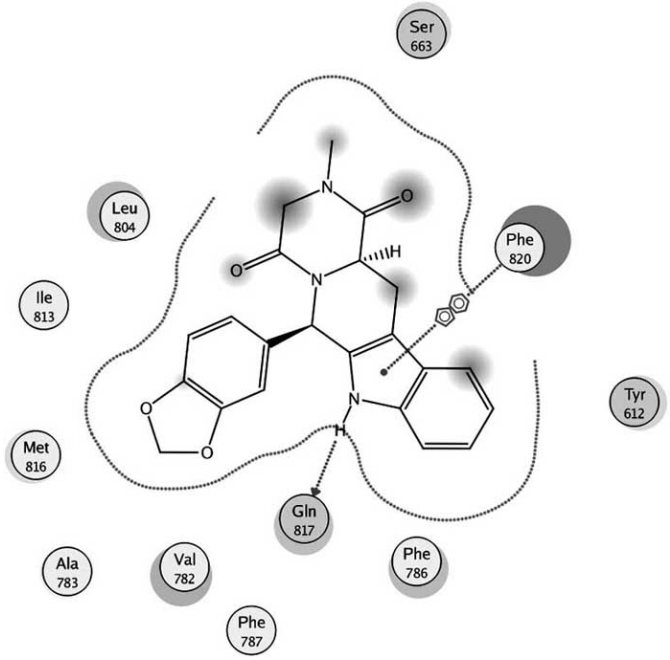


Fig. (2). Detailed mode view showing the docking and interaction of **IX** with human PDE5.

4. EXPERIMENTAL DETAILS

All reactions were carried out under inert gas (nitrogen). Reaction progress was monitored by TLC, performed on precoated silica gel plates (ALUGRAM SIL G/UV254) and detection of the components was made by short UV light. Column chromatography was performed using silica-gel (70-200 μm). Melting points were determined on Buchi Melting Point apparatus and are uncorrected. FTIR spectra were recorded on Nicolet Avatar 380 spectrometer. ^{1}H-NMR spectra were run at 300 MHz and ^{13}C spectra were run at 75.46 MHz in deuterated chloroform ($CDCl_3$) and deuterated dimethylsulfoxide (DMSO), chemical shifts are quoted in (δ) and were related to that of the solvents; chemical shifts (δ) were reported in parts per million (ppm) downfield from TMS; multiplicities are abbreviated as s: singlet; d: doublet; t: triplet; q: quartret; m: multiplet; dd: doublet of doublet; brs: broad. Mass spectra were made on Focus GC/ Polaris MS, model 5890, series II at an ionization potential of 70 ev and the FAB was done on Finnigan TQS 70 by 70 ev in nitrobenzylalcohol –matrix at 30°C. Elemental analysis were performed by the Microanalytical Unit, Faculty of Science, Cairo University; the found values were within ± 0.4% of the theoretical ones, unless otherwise indicated. All starting materials were commercially available and of general purpose pure analytical grade.

General Procedures for the Preparation of Methyl 1-(aryl)-2,3,4,9-tetrahydro-1*H*-*β*-carboline-3-carboxylate (I-VIII)

The appropriate tryptophane methyl ester (10.76 g, 49.3 mmol) and the appropriate aromatic aldehyde namely 3-pyridinecarboxaldehyde or 3, 4-dimehoxybenzaldehyde (54.23 mmol) were dissolved in CH_2Cl_2 (70 mL) and cooled to 0 °C in an ice bath. To this solution was added dropwise TFA (8 mL), and the mixture was stirred at room temperature for 4 days under N_2 atmosphere. The reaction mixture was then basified with dilute NH_4OH solution and extracted with CH_2Cl_2 (3 x 50 m). The organic layer was washed with water, brine, dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The residue was purified and the isomers were separated by column chromatography on silica gel eluting with CH_2Cl_2: CH_3OH in different ratios to give first the appropriate *cis*-isomer followed the *trans*- one.

Methyl (1*R*, 3*R*)-1-(pyridin-3-yl)- 2, 3, 4, 9-tetrahydro-1*H*-β-carboline-3-carboxylate (I)

Orange powder (28%); m.p.: 224-227; °C; R_f = 0.78 (CH_2Cl_2/CH_3OH 90:10); MS (EI): m/z 307 (M^+;100%); IR (cm^{-1}): 3332, 1708; ^{1}H-NMR (DMSO): 9.21 (brs,1H, N*H*), 8.97-8.93 (d,1H, Ar), 8.64 (s,1H, Ar), 7.76-7.74 (d,1H, Ar), 7.53-7.46 (dd,1H, Ar), 7.26-7.24 (d,1H, Ar), 7.11-6.99 (m,3H, Ar), 5.64 (s,1H,C*H*Ph), 4.48-4.37 (m,1H,C*H*C(O)N), 3.79 (s, 3H, OCH_3), 3.74-3.69 (dd, 1H, CH_aH_b),3.13-3.08 (m, 1H, CH_aH$_b$); ^{13}C-NMR: 171.1, 151.0, 150.5, 137.8, 137.6, 137.0, 137.2, 126.2, 124.1, 122.1, 119.4, 118.4, 111.8, 107.3, 56.0 (C1), 55.8 (C3), 52.9, 22.4; Analysis calcd for $C_{18}H_{17}N_3O_2$ C, H, N.

Methyl (1*S*, 3*R*)-1-(pyridin-3-yl)-2, 3, 4, 9-tetrahydro-1*H*-*β* –carboline-3-carboxylate (II)

Yellow powder (13%); m.p.: 180-183 °C; R_f = 0.59 (CH_2Cl_2/CH_3OH 9:1); MS (EI): m/z 307 (M^+; 100%); IR (cm^{-1}): 3232, 1734; ^{1}H-NMR (DMSO): 10.85 (brs,1H, N*H*), 8.61 (s,1H, Ar), 7.68-7.64 (d,1H, Ar), 7.54-7.51 (d,1H, Ar), 7.29-7.27 (d,1H, Ar), 7.14-7.01 (m, 4H, Ar), 5.78 (s,1H, C*H*Ph), 3.96-3.81 (m, 1H, C*H*C(O)N), 3.71 (s, 3H, OCH_3), 3.74-3.69 (dd, 1H, CH_aH_b), 3.15-3.10 (m, 1H, CH_aH$_b$);, ^{13}C-NMR: 171.5, 150.6, 149.7, 137.5, 135.9, 136.9, 126.2, 123.9, 122.1, 121.2, 119.3, 118.5, 111.4, 106.8, 52.8(C1), 52.5(C3), 51.9,23.6; Analysis calcd for $C_{18}H_{17}N_3O_2$ C, H, N.

Methyl (1*S*, 3*S*)-1-(pyridin-3-yl)-2,3,4,9-tetrahydro-1*H*-*β* –carboline-3-carboxylate (III)

Orange powder (18%); m.p.: 225-228 °C; R_f = 0.77 (CH_2Cl_2/CH_3OH 9:1); MS (EI): m/z 307 (M^+;100%); IR (cm^{-1}): 3320, 1727; ^{1}H-NMR (DMSO) : 9.22 (brs,1H, N*H*),8.62 (s, 1H, Ar), 8.46-8.43 (d, 1H, Ar), 8.28-8.25 (d, 1H, Ar), 7.75-7.73 (d, 1H, Ar), 7.69-7.59 (m, 1H, Ar), 7.49-7.43 (dd, 1H, Ar), 7.25-7.22 (d, 1H, Ar), 7.09-7.69 (dd, 1H, Ar), 5.59 (s, 1H, C*H*Ph), 4.67-4.59 (dd, 1H, C*H*C(O)N), 3.77 (s, 3H, OCH_3), 3.74-3.69 (dd, 1H, CH_aH_b), 3.24-2.98 (m, 1H, CH_aH$_b$);^{13}C-NMR: 171.3, 150.7, 150.1, 149.8, 137.5, 136.9, 129.8, 126.4, 124.0, 121.8, 119.2, 118.3, 111.7, 107.4, 56.2 (C1), 55.8(C3), 52.7, 24.3; Analysis calcd for $C_{18}H_{17}N_3O_2$ C, H, N.

Methyl (1*R*,3*S*)-1-(pyridin-3-yl)-2,3,4,9-tetrahydro-1*H*-*β* –carboline-3-carboxylate (IV)

Yellow powder (17%); m.p.: 182-185 °C; R_f = 0.57 (CH_2Cl_2/CH_3OH 9:1); MS (EI): m/z 307 (M^+;100%)**;** IR (cm^{-1}): 3209, 1726; ^{1}H-NMR (DMSO) : 9.22 (brs,1H, N*H*), 8.62 (s,1H, Ar), 8.46-8.43 (d,1H, Ar), 8.28-8.25 (d,1H, Ar), 7.75-7.73 (d,1H, Ar), 7.69-7.59 (m,1H, Ar), 7.49-7.43 (dd,1H, Ar), 7.25-7.22 (d,1H, Ar), 7.09-7.69 (dd,1H, Ar), 5.59 (s, 1H, C*H*Ph), 4.67-4.59 (dd,1H, C*H*C(O)N), 3.77 (s, 3H, OCH_3), 3.74-3.69 (dd,1H, CH_aH_b), 3.24-2.98 (m, 1H, CH_aH$_b$); ^{13}C-NMR: 169.1, 146.3, 142.3, 137.1, 136.9, 132.7, 127.0, 125.7, 125.6, 122.7, 119.6, 118.7, 111.9, 106.9, 53.2(C3), 51.8, 22.1; Analysis calcd for $C_{18}H_{17}N_3O_2$ C, H, N.

Methyl (1*R*, 3*R*)-1-(3,4-dimethoxyphenyl)- 2, 3, 4, 9-tetrahydro-1*H*-β-carboline-3-carboxylate (V)

Orange powder (23 %); m.p.: 174-176 °C; R_f = 0.73 (CH_2Cl_2/CH_3OH 95:5); MS (EI): m/z 366 (M^+; 100%); IR (cm^{-1}): 3341, 1727; ^{1}H-NMR($CDCl_3$): 8.86 (brs, 1H, N*H*), 7.57-7.53 (d, 1H, Ar), 7.25-7.22 (d, 1H, Ar), 6.19-6.92 (m, 5H, Ar), 5.21(s, 1H, C*H*Ph), 4.03-3.96 (dd, 1H, C*H*COO-CH_3), 3.89 (s, 3H, OCH_3), 3.82 (s, 3H, OCH_3), 3.81 (s, 3H, OCH_3), 3.74-3.73 (dd, 1H, CH_aH_b), 3.27-3.03 (m, 1H, CH_aH$_b$); Analysis calcd for $C_{21}H_{22}N_2O_4$ C, H, N.

Methyl (1*S*, 3*R*)-1-(3,4-dimethoxphenyl)-2, 3, 4, 9-tetrahydro-1*H*-*β* –carboline-3-carboxylate (VI)

Yellow powder (14 %); m.p. : 163-165 °C; R_f = 0.54 (CH_2Cl_2/CH_3OH 95:5); MS (EI): m/z 366 (M^+; 100%); IR (cm^{-1}): 3366, 1724; ^{1}H-NMR **(** $CDCl_3$): 7.66 (s, 1H, N*H*),

7.59-7.54 (d, 1H, Ar), 7.25-7.24 (d, 1H, Ar), 7.21-6.82 (m, 5H, Ar), 5.43 (s, 1H, C*H*Ph), 3.92-3.90 (d, 1H, C*H*COO-CH_3), 3.86 (s, 3H, OCH_3), 3.83 (s, 3H, OCH_3),3.74 (s, 3H, OCH_3), 3.72-3.71 (d, 1H, CH_aH_b), 3.33-3.32 (dd, 1H, CH_aH$_b$); Analysis calcd for $C_{21}H_{22}N_2O_4$ C, H, N.

Methyl (1*S*, 3*S*)-1-(3,4-dimethoxyphenyl)-2,3,4,9-tetrahydro-1*H*-*β* –carboline-3-carboxylate (VII)

Orange powder (28%); m.p.: 174-177 °C; R_f = 0.71 (CH_2Cl_2/CH_3OH 95:5); MS (EI): m/z 366 (M^+; 100%); IR (cm^{-1}): 3341, 1727; ^{1}H-NMR ($CDCl_3$): 8.87 (s, 1H, N*H*), 7.57- 7.53 (d, 1H, Ar), 7.25- 7.23 (d, 1H, Ar), 7.18-6.83 (m, 5H, Ar), 5.30 (brs, 1H, C*H*Ph), 4.06-3.96 (dd, 1H, C*H*COO-CH_3), 3.88 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 3.78 (s, 3H, OCH_3), 3.72-3.69 (d, 1H, CH_aH_b), 3.29-3.08 (m, 1H, CH_aH$_b$); Analysis calcd for $C_{21}H_{22}N_2O_4$ C, H, N.

Methyl (1*R*,3*S*)-1-(3,4-dimethoxyphenyl)-2,3,4,9-tetrahydro-1*H*-*β* –carboline-3-carboxylate (VIII)

Yellow powder (12 %); m.p.: 164-167 °C ; R_f= 0.53 (CH_2Cl_2/CH_3OH 95:5) ; MS (EI): m/z 366 (M^+; 100%); IR (cm^{-1}) : 3366 (-NH-), 1724 (C=O); ^{1}H-NMR ($CDCl_3$) :7.69 (brs, 1H, N*H*), 7.57-7.55 (d, 1H, Ar), 7.27-7.24 (d, 1H, Ar),7.19-7.11(m, 4H, Ar), 6.81(s, 1H, Ar),5.40 (brs, 1H, C*H*Ph), 4.06- 4.02 (dd, 1H, C*H*COOCH$_3$), 3.85 (s, 3H, O-CH_3), 3.81 (s, 3H, OCH_3),3.74 (s, 3H, OCH_3), 3.35-3.28 (dd,1H,CH_aH_b), 3.22-3.15 (dd, 1H, CH_aH$_b$); Analysis calcd for $C_{21}H_{22}N_2O_4$ C, H, N.

General procedures for the preparation of 2-Alkyl-5-(pyridin-3-yl)-5,6,11,11a-tetrahydro-1*H* imidazo[1',5':1,6] pyrido[3,4-*b*]indole-1,3(2*H*)-dione

The appropriate isocyanate (1.6 mmol) was added to a well stirred solution of the appropriate beta carboline **I - IV** (0.31 g, 1 mmol) in methyl ethyl ketone (10 ml) and the mixture was stirred at reflux for 16 hours under nitrogen atmosphere. The product was purified using by column chromatography on silica gel eluting with CH_2Cl_2:CH_3OH (99:1).

(5*R*,11a*R*)- 2-ethyl -5-(pyridin-3-yl)-5,6,11,11a-tetrahydro-1*H* imidazo[1',5':1,6]pyrido[3,4-*b*]indole-1,3(2*H*)-dione(IX)

Yellow powder (70%); m.p.: 210-212 °C ; R_f = 0.34 (CH_2Cl_2/MeOH 95:5); MS (EI): m/z 346 (M^+;100%); IR (cm^{-1}): 3153, 1767, 1692 ; ^{1}H-NMR (DMSO)**:** 11.74 (brs, 1H, N*H*), 9.89-9.31 (d, 1H, Ar), 8.94 (s, 1H, Ar), 8.49-8.36 (dd, 1H, Ar), 8.32-8.23 (dd, 1H, Ar), 7.62-7.15 (m, 4H, Ar), 5.92 (brs, 1H, C*H*Ph), 4.38-4.33 (dd, 1H, C*H*C(O)N), 3.58-3.48 (q, 2H, NCH_2), 3.21-3.18 (m, 1H, CH_aCH_b), 3.11-3.01 (m, 1H, CH_aH$_b$), 1.23-1.17 (t, 3H,CH_3); ^{13}C-NMR : 171.2, 166.5, 154.9, 148.8, 141.8, 137.8, 135.8, 134.7, 132.5, 130.5, 125.9, 124.1, 121.8, 120.1, 112.4, 107.1, 57.9 (C5), 54.1 (C11a), 35.4, 22.5, 13.4. Analysis calcd for $C_{20}H_{18}N_4O_2$ C, H, N.

(5*S*,11a*R*)- 2-ethyl -5-(pyridin-3-yl)-5,6,11,11a-tetrahydro-1*H*-imidazo[1',5':1,6]pyrido[3,4-*b*]indole-1,3(2*H*)-dione (X)

Yellowish white powder (40%); m.p.: 248-252 °C , R_f = 0.43 (CH_2Cl_2/MeOH 95:5); MS (EI): m/z 346 (M^+;100%); IR (cm^{-1}): 3333,1760,1620 ; ^{1}H-NMR (DMSO): 9.43 (s, 1H, N*H*), 8.62 (s, 1H, Ar), 8.52-8.51 (d, 1H, Ar), 7.69-7.55 (dd, 1H, Ar), 7.35-7.33 (d, 1H, Ar), 7.30-7.14 (m, 4H, Ar), 6.39 (s, 1H, C*H*Ph), 4.29-4.23 (dd, 1H, C*H*C(O)N), 3.64-3.57 (q, 2H, NCH_2), 3.55-3.48 (dd, 1H, CH_aCH_b), 2.95-2.88 (m, 1H, CH_aH$_b$), 1.14- 1.09 (t, 3H, CH_3).^{13}C-NMR : 172.3, 158.4, 148.9, 136.3, 129.1, 122.9, 119.9, 118.3, 111.4, 108.1, 115.6,114.3,113.0,107.8, 106.4, 53.2 (C5), 49.7 (C11a), 35.2, 23.3,13; Analysis calcd for $C_{20}H_{18}N_4O_2$ C, H, N.

(5*S*,11a*S*)- 2-ethyl -5-(pyridin-3-yl)-5,6,11,11a-tetrahydro-1*H* imidazo[1',5':1,6]pyrido[3,4-*b*]indole-1,3(2*H*)-dione (XI)

Yellow powder (54%); m.p.: 212-214 °C; R_f =0.36 (CH_2Cl_2/MeOH 95:5); MS (EI): m/z 346 (M^+;100%); IR (cm^{-1}): 3318,1737,1679; ^{1}H-NMR (DMSO): 10.75 (s, 1H, N*H*), 8.97 (s, 1H, Ar), 8.82-8.89(d, 1H, Ar),8.83-8.81(dd, 1H, Ar), 8.78-8.76 (d, 1H, Ar), 7.81-6.98 (m, 4H, Ar), 5.65 (brs, 1H, C*H*Ph), 4.57-4.52 (dd, 1H, C*H*C(O)N), 3.41-3.37 (m, 2H, NCH_2), 3.03-2.96 (dd, 1H, CH_aCH_b), 2.51-2.48 (m, 1H, CH_aH$_b$), 1.11-1.07 (t, 3H, CH_3); ^{13}C-NMR : 171.1,157.7, 148.7,148.2,136.7, 135.9, 133.6,125.6,123.2,121.4,120.5, 118.7,118.1, 111.1, 105.1,57.3 (C5), 53.3 (C11a), 33.8, 15.4,12.9; Analysis calcd for $C_{20}H_{18}N_4O_2$ C, H, N.

(5*R*,11a*S*)- 2-ethyl -5-(pyridin-3-yl)-5,6,11,11a-tetrahydro1-*H*-imidazo[1',5':1,6]pyrido[3,4-*b*]indole-1,3(2*H*)-dione (XII)

Yellow powder (20%); m.p.: 246-249 °C; R_f = 0.45 (CH_2Cl_2/MeOH 95:5); MS (EI): m/z 346 (M^+; 100%); IR (cm^{-1}): 3364, 1762, 1703 ; ^{1}H-NMR (DMSO): 10.25 (s, 1H, N*H*), 8.45 (s, 1H, Ar), 8.22- 8.27 (d, 1H, Ar),7.17-7.09 (d,1H, Ar), 7.42-7.30 (m, 1H, Ar), 6.73-6.61 (m, 4H, Ar), 5.93 (brs, 1H, C*H*Ph), 3.92-3.86 (dd, 1H, C*H*C(O)N), 3.70-3.63 (q, 2H, NCH_2), 2.99-2.82 (dd, 1H, CH_aCH_b), 2.35-2.11 (m, 1H, CH_aH$_b$), 1.02-0.75 (t, 3H, CH_3); ^{13}C-NMR (DMSO) : 172.1, 167.2, 154.4, 149.3, 136.8, 135.5, 135.3, 135.4, 129.5 ,125.6, 123.6, 121.9, 117.8, 111.4, 106.8, 52.8 (C5), 49.5 (C11a), 33.3, 22.8, 13.2; Analysis calcd for $C_{20}H_{18}N_4O_2$ C, H, N.

(5*R*,11a*R*)- 2-*tert*-butyl -5-(pyridin-3-yl)-5,6,11,11a-tetrahydro-1*H* imidazo[1',5':1,6]pyrido[3,4-*b*] indole-1,3(2*H*)-dione (XIII)

White powder (50%); m.p.: 275-278 °C; R_f = 0.42 (CH_2Cl_2/MeOH 95:5); MS (EI): m/z 374 (M^+), m/z 317 (100%); IR (cm^{-1}): 3180, 1762,1708; ^{1}H-NMR (DMSO): 9.93 (brs, 1H, N*H*), 8.48 (s, 1H, Ar), 7.84- 7.81 (d, 1H, Ar), 7.56-51 (m, 1H, Ar), 7.35-7.39 (m, 1H, Ar), 7.34-7.13 (m, 4H, Ar), 6.05 (s, 1H, C*H*Ph), 3.76-3.73 (dd, 1H, C*H*C(O)N), 3.39-3.34 (dd, 1H, CH_aCH_b), 2.95-2.86 (m, 1H, CH_aH$_b$), 1.58 (s, 9H, CH_3); Analysis calcd for $C_{22}H_{22}N_4O_2$ C, H, N.

(5*S*,11a*R*)- 2-*tert*-butyl -5-(pyridin-3-yl)-5,6,11,11a-tetrahydro-1*H*-imidazo[1',5':1,6]pyrido[3,4-*b*]indole-1,3(2*H*)-dione (XIV)

Yellow powder (40%); m.p.: 240-242 °C; R_f = 0.58 (CH_2Cl_2/MeOH 95:5); MS (EI): m/z 374 (M^+), m/z 318 (100%); IR (cm^{-1}): 3324, 1761,1726; ^{1}H-NMR (DMSO): 8.75 (brs, 1H, N*H*), 8.53 (s, 1H, Ar), 7.83- 7.81 (d, 1H, Ar), 7.48-7.38 (m, 1H, Ar), 7.33-7.30 (m, 1H, Ar), 7.26-7.17 (m, 4H, Ar), 6.35 (s, 1H, C*H*Ph), 4.10-4.04 (dd, 1H, C*H*C(O)N),

3.47-3.40 (dd, 1H, CH_aCH_b), 2.88-2.75 (m, 1H, CH_aH_b), 1.63 (s, 9H, CH_3); Analysis calcd for $C_{22}H_{22}N_4O_2$ C, H, N.

(5*S*,11a*S*)- 2-*tert*-butyl -5-(pyridin-3-yl)-5,6,11,11a-tetrahydro-1*H*-imidazo[1',5':1,6] pyrido[3,4-*b*]indole-1,3(2*H*)-dione (XV)

White powder (64%); m.p.: 279-282 °C; R_f = 0.42 (CH_2Cl_2/MeOH 95:5); MS (EI): m/z 374 (M^+), m/z 318 (100%); IR (cm^{-1}): 3057, 1762,1692; ^{1}H-NMR (DMSO): 10.79 (brs, 1H, N*H*), 8.62 (s, 1H, Ar), 8.46-8.44 (d, 1H, Ar), 7.64-7.61 (d, 1H, Ar), 7.56-7.54 (d, 1H, Ar), 7.31-7.27 (dd, 1H, Ar),7.23-7.21 (d, 1H, Ar),7.07-6.97 (m, 2H, Ar), 5.89 (s, 1H, C*H*Ph), 4.44-4.39 (dd, 1H, C*H*C(O)N), 3.04-2.95 (m, 1H, CH_aCH_b), 2.51-2.49 (m, 1H, CH_aH_b), 1.50 (s, 9H, CH_3); Analysis calcd for $C_{22}H_{22}N_4O_2$ C, H, N.

(5*R*,11a*S*)- 2-*tert*-butyl -5-(pyridin-3-yl)-5,6,11,11a-tetrahydro-1*H*-imidazo[1',5':1,6]pyrido[3,4-*b*]indole-1,3(2*H*)-dione (XVI)

Yellow powder (57%); m.p.: 240-242 °C; R_f = 0.6 (CH_2Cl_2/MeOH 95:5); MS (EI): m/z 374 (M^+), m/z 318 (100%); IR (cm^{-1}): 3320,1762,1703; NMR (DMSO): 8.72 (s,1H, N*H*), 8.54 (s, 1H, Ar), 7.80- 7.77 (d, 1H, Ar), 7.58-7.56 (d, 1H, Ar), 7.39-7.35 (dd, 1H, Ar), 7.31-7.19 (m, 4H, Ar), 6.34 (s, 1H, C*H*Ph), 4.12-4.06 (dd, 1H, C*H*C(O)N), 3.48-3.41 (dd, 1H, CH_aCH_b), 2.89-2.81 (m, 1H, CH_aH_b),1.63 (s, 9H, CH_3); Analysis calcd for $C_{22}H_{22}N_4O_2$ C, H, N.

General procedures for the preparation of 4-Chlorophenyl-5-(3-pyridinyl)-5,6,11,11a-tetrahydro-1*H*-imidazo [1',5':1,6]pyrido[3,4-*b*]indole-1,3(2*H*)-dione

4-chlorophenyl isocyanate (0.153 g, 1 mmol, 1 equiv.) was added to a well stirred solution of the appropriate beta carboline **I-IV** (0.31 g, 1 mmol, 1 equiv.) in methyl ethyl ketone (10 ml) and the mixture was stirred at reflux for 16 hours under nitrogen atmosphere. The product was purified using by column chromatography on silica gel eluting with CH_2Cl_2:CH_3OH (99:1).

(5*R*,11a*R*)- 4-chlorophenyl -5-(pyridin-3-yl)-5,6,11,11a-tetrahydro-1*H*-imidazo[1',5':1,6]pyrido[3,4-*b*]indole-1,3 (2*H*)-dione (XVII)

Yellow crystals (54%); m.p.: 264-268 °C; R_f = 0.66 (CH_2Cl_2/MeOH 95:5); MS (FAB): m/z 431 (M^++2), m/z 429 (M^+;100%); IR (cm^{-1}): 3292, 1772, 1709; ^{1}H-NMR (DMSO): 10.26 (s, 1H, N*H*), 9.41 (s, 1H, Ar), 8.86-8.77 (d, 1H, Ar), 8.63-8.37 (d, 1H, Ar), 7.56-7.86 (d, 1H, Ar), 7.63-7.06 (m, 8H, Ar), 6.31 (s, 1H, C*H*Ph), 4.44-4.39 (dd, 1H, C*H*C(O)N), 3.62-3.48 (m, 1H, CH_aCH_b), 3.09-2.99 (m, 1H, CH_aH_b); Analysis calcd for $C_{24}H_{17}ClN_4O_2$ C, H, N.

(5*S*,11a*R*)- 4-chlorophenyl -5-(pyridin-3-yl)-5,6,11,11a-tetrahydro-1*H*-imidazo[1',5':1,6]pyrido[3,4-*b*]indole-1,3(2*H*)-dione (XVIII)

Buff crystals (54%); m.p.:229-231 °C; R_f = 0.72 (CH_2Cl_2/MeOH 95:5); MS (FAB): m/z 431 (M^++2), m/z 429 (M^+;100%); IR (cm^{-1}): 3405, 1765, 1698 ; NMR (DMSO) : 10.94 (s, 1H, N*H*), 8.69 (s, 1H, Ar), 8.58-8.56 (d, 1H, Ar), 7.79-7.77 (d, 1H, Ar), 7.60-7.58 (d, 1H, Ar), 7.55-7.49 (dd, 1H, Ar), 7.43-7.02 (m, 7H, Ar), 6.38 (brs, 1H, C*H*Ph), 4.87-4.81 (dd, 1H, C*H*C(O)N), 3.48-3.40 (dd, 1H, CH_aCH_b), 3.12-3.04 (m, 1H, CH_aH_b); Analysis calcd for $C_{24}H_{17}ClN_4O_2$ C, H, N.

(5*S*,11a*S*)- 4-chlorophenyl -5-(pyridin-3-yl)-5,6,11,11a-tetrahydro-1*H* imidazo[1',5':1,6] pyrido [3,4-*b*]indole-1,3(2*H*)-dione (XIX)

Yellow crystals (64%); m.p.: 262-265 °C; R_f = 0.68 (CH_2Cl_2/MeOH 95:5); MS (EI): m/z 431 (M^++2), m/z 429 (M^+;100%); IR (cm^{-1}): 3430,1776,1716; ^{1}H-NMR (DMSO): 10.86 (brs, 1H, N*H*), 8.71 (s, 1H, Ar), 8.47-8.45 (d, 1H, Ar), 7.78-7.79 (d, 1H, Ar), 7.60-7.53 (dd, 1H, Ar),7.43-7.40 (d, 2H, Ar), 7.35-6.99 (m, 6H, Ar), 6.03 (s,1H,C*H*Ph), 4.77-4.72 (dd, 1H, C*H*C(O)N), 3.44-3.36 (dd, 1H, CH_aCH_b), 3.27-3.23 (m, 1H, CH_aH_b); Analysis calcd for $C_{24}H_{17}ClN_4O_2$ C, H, N.

(5*R*,11a*S*)- 4-chlorophenyl -5-(pyridin-3-yl)-5,6,11,11a-tetrahydro-1*H*-imidazo[1',5':1,6]pyrido[3,4-*b*]indole-1,3(2*H*)-dione (XX)

Yellow powder (50%); m.p.: 228-232 °C; R_f =0.74 (CH_2Cl_2/MeOH 95:5); MS (EI): m/z 431 (M^++2), m/z 429 (M^+; 100%); IR (cm^{-1}): 3405, 1776, 1716 ; ^{1}H-NMR (DMSO): 8.68 (brs, 1H, N*H*), 8.62 (s, 1H, Ar), 8.54-8.52 (d, 1H, Ar), 7.72-7.59 (dd, 1H, Ar), 7.42-7.19 (m, 5H, Ar), 7.25-7.24 (d, 2H, Ar), 7.22-7.21 (d, 2H, Ar), 6.43 (s, 1H, C*H*Ph), 4.47-4.42 (dd, 1H, C*H*C(O)N), 3.65-3.58 (dd, 1H, CH_aCH_b), 3.11-3.02 (m, 1H, CH_aH_b); Analysis calcd for $C_{24}H_{17}ClN_4O_2$ C, H, N.

General procedures for the preparation of Ethyl-5-(3,4 dimethoxyphenyl)-5,6,11,11a-tetrahydro-1*H*-imidazo[1',5': 1,6]pyrido[3,4-*b*]indole-1,3(2*H*)-dione

Excess ethyl isocyanate (135 µl, 1.6 mmol) was added to a well stirred solution of the appropriate beta carboline **V-VIII** (0.36 g, 1 mmol) in methyl ethyl ketone (10 ml) and the mixture was stirred at reflux for 16 hours under nitrogen atmosphere. The product was purified using by column chromatography on silica gel eluting with CH_2Cl_2.

(5*R*,11a*R*)- 2-ethyl -5-(3,4 dimethoxyphenyl)-5,6,11,11a-tetrahydro-1*H*-imidazo[1',5':1,6]pyrido[3,4-*b*]indole-1,3 (2*H*)-dione (XXI)

Buff powder (54%); m.p.: 222-225 °C; R_f = 0.43 (CH_2Cl_2/MeOH 99:1); MS (EI): m/z 405 (M^+), m/z 374 (100%); IR (cm^{-1}): 3325, 1767, 1703; ^{1}H-NMR ($CDCl_3$): 11.06 (brs, 1H, N*H*), 7.93-7.90 (d, 1H, Ar), 7.63-7.61 (d, 1H, Ar), 7.46-7.32 (m, 5H, Ar), 6.18 (brs, 1H, C*H*Ph), 4.93-4.86 (dd, 1H, C*H*C(O)N), 3.85 (s, 3H, OCH_3), 3.78 (s, 3H, O-CH_3), 3.58-3.53 (m, 2H, NCH_2), 3.51- 3.48 (m, 1H, CH_a-CH_b), 3.10-3.01 (m, 1H, CH_aH_b), 1.49-1.44 (t, 3H, CH_3); ^{13}C-NMR: 171.4, 154.3,148.8,148.4,136.9, 135.3, 133.5, 126.2, 121.8, 119.6, 119.1, 118.4, 112.0, 111.8,111.5 ,105.2, 58.1 (C5), 55.8 (C11a), 55.9, 55.8, 33.6, 22.5,13.5; Analysis calcd for $C_{23}H_{23}N_3O_4$ C, H, N.

(5*S*,11a*R*)- 2-ethyl -5-(3,4 dimethoxyphenyl)-5,6,11,11a-tetrahydro-imidazo[1',5':1,6] pyrido [3,4-*b*]indole-1,3(2*H*)-dione (XXII)

White powder (26%); m.p.:178-180 °C; R_f = 0.58 (CH_2Cl_2/MeOH 99:1); MS (EI): m/z 405 (M^+), m/z 374

(100%), IR (cm^{-1}): 3338, 1764, 1703; ^{1}H-NMR :11.23 (brs,1H,N*H*), 7.93-7.91 (d, 1H, Ar), 7.69-7.66 (d, 1H, Ar), 7.50- 7.19 (m, 5H, Ar), 6.53 (brs, 1H, C*H*Ph), 5.03-4.97 (dd, 1H, C*H*C(O)N), 4.11 (s, 3H, OCH_3), 3.76 (s, 3H, OCH_3), 3.88-3.79 (q, 2H, NCH_2), 3.23-3.13 (m, 1H, CH_aCH_b), 2.89-2.86 (m, 1H, CH_aH_b), 1.49-1.43 (t, 3H, CH_3); ^{13}C-NMR: 171.8, 154.3, 148.9, 148.8, 136.7, 132.6, 131.6, 125.8, 121.6, 120.1, 118.8, 118.2, 111.9, 111.6, 111.4, 105.9, 55.6 (C5), 53.0 (C11a), 51.5, 55.8, 32.9, 22.6, 13.3; Analysis calcd for $C_{23}H_{23}N_3O_4$ C, H, N.

(5*S*,11a*S*)- 2-ethyl -5-(3,4 dimethoxyphenyl)-5,6,11,11a-tetrahydro-1*H*-imidazo[1',5':1,6] pyrido[3,4-*b*] indole-1,3(2*H*)-dione (XXIII)

Golden yellow powder (65%); m.p.: 224-227 °C; R_f = 0.45 (CH_2Cl_2/MeOH 99:1) ; MS (EI): m/z 405 (M^+), m/z, 374 (100%); IR (cm^{-1}): 3326, 1762, 1708 ; ^{1}H-NMR ($CDCl_3$) : 8.52 (brs, 1H, N*H*), 7.73-6.75 (m, 7H, Ar), 5.76 (brs, 1H, C*H*Ph), 4.24-4.21 (dd, 1H, C*H*C(O)N), 3.85 (s, 3H, OCH_3), 3.77 (s, 3H, OCH_3), 3.70-3.63 (q, 2H, NCH_2), 3.58-3.51 (dd, 1H, CH_aCH_b), 3.10-3.01 (m, 1H, CH_aH_b), 1.23-1.19 (t,3H,CH_3); ^{13}C-NMR: 171.4,154.6,148.7,148.4,136.9, 35.3, 133.5,131.9,126.2, 119.6, 119.1, 118.4, 112.1,111.8, 111.6, 105.2, 58.1(C5), 55.7(C11a), 55.9, 55.8, 33.6, 22.4,13.4; Analysis calcd for $C_{23}H_{23}N_3O_4$ C, H, N.

(5*R*,11a*S*)- 2-ethyl -5-(3,4 dimethoxyphenyl)-5,6,11,11a-tetrahydro-1*H*-imidazo[1',5':1,6]pyrido[3,4-*b*]indole 1,3 (2*H*)-dione (XXIV)

Golden yellow powder (50%); m.p.: 179-182 °C; R_f = 0.56 (CH_2Cl_2/MeOH 99:1); MS (EI): m/z 405 (M^+;100%); IR (cm^{-1}): 3339 ,1764,1703 ; ^{1}H-NMR ($CDCl_3$): 10.84 (brs, 1H, N*H*), 7.56-7.52 (d, 1H, Ar), 7.31-7.27 (d, 1H, Ar), 7.24- 6.81 (m, 5H, Ar), 6.14 (brs, 1H, C*H*Ph), 4.33-4.27 (dd, 1H, C*H*C(O)N), 3.73 (s, 3H, OCH_3), 3.71(s, 3H ,OCH_3), 3.49-3.34 (q, 2H, NCH_2), 3.01-2.95 (m, 1H, CH_aCH_b), 2.50-2.48 (dd, 1H, CH_aH_b),1.13-1.06 (t, 3H, CH_3), ^{13}C-NMR: 172.6, 149.5,136.6,131.8,130.6, 126.3,126.2,126.1,122.9,120.4,120.1, 118.4,111.6,111.3,111.2,108.2, 56.1 (C5), 55.9 (C11a), 53.2, 51.8, 33.7, 23.5,13.5; Analysis calcd for $C_{23}H_{23}N_3O_4$ C, H, N.

General procedures for the preparation of *Tert*-butyl-5-(3,4 dimethoxyphenyl)-5,6,11,11a-tetrahydro-1*H*-imidazo [1',5':1,6]pyrido[3,4-*b*]indole-1,3(2*H*)-dione

Excess tertiary butyl isocyanate (185 µl, 1.6 mmol) was added to a well stirred solution of the appropriate beta carboline **V-VIII** (0.36 g, 1 mmol) in methyl ethyl ketone (10 ml) and the mixture was stirred at reflux for 16 hours under nitrogen atmosphere. The product was purified using column chromatography on silica gel eluting with CH_2Cl_2.

(5*R*,11a*R*)- 2-*tert*-butyl-5-(3,4 dimethoxyphenyl)-5,6,1 1,11a-tetrahydro-1*H* Imidazo[1',5':1,6] pyrido [3,4-*b*]indole-1,3 (2*H*)-dione (XXV)

Buff powder (54%); m.p. 220-223 °C; R_f = 0.67 (CH_2Cl_2/MeOH 99:1); MS (EI): m/z 433 (M^+), m/z 346 (100%); IR (cm^{-1}): 3405, 1765, 1698; ^{1}H-NMR ($CDCl_3$): 7.89 (brs, 1H, N*H*), 7.58-7.56 (d, 1H, Ar), 7.32- 7.29 (d, 1H, Ar), 6.95 (s, 1H, Ar), 6.92-6.71 (m, 4H, Ar), 6.23 (brs, 1H, C*H*Ph), 4.25-4.22 (dd, 1H, C*H*C(O)N), 3.99 (s, 3H, OCH_3), 3.97 (s, 3H, OCH_3), 3.88-3.81 (m, 1H, CH_aCH_b), 3.05-2.83 (m, 1H, CH_aH_b),1.64 (s, 9H, CH_3); Analysis calcd for $C_{25}H_{27}N_3O_4$ C, H, N.

(5*S*,11a*R*)- 2-*tert*-butyl-5-(3,4 dimethoxyphenyl)-5,6,11, 11a-tetrahydro-1*H*-imidazo[1',5':1,6]pyrido[3,4-*b*]indole-1,3(2*H*)-dione (XXVI)

Yellow powder (65%); m.p.: 125-128 °C; R_f = 0.89 (CH_2Cl_2/MeOH 99:1) ; MS (EI): m/z 433 (M^+), m/z 346 (100%); IR (cm^{-1}): 3334,1760,1703; ^{1}H-NMR($CDCl_3$): 7.81 (brs, 1H, N*H*), 7.58-7.56 (d, 1H, Ar), 7.32- 7.29 (d, 1H, Ar), 7.25-6.96 (m, 5H, Ar), 6.81 (brs, 1H, C*H*Ph), 4.21-4.15 (dd, 1H, C*H*C(O)N), 3.86 (s, 3H, OCH_3), 3.84 (s, 3H, OCH_3), 3.51-3.44 (dd, 1H, CH_aCH_b), 2.90-2.80 (m, 1H, CH_aH_b), 1.64 (s, 9H, CH_3); Analysis calcd for $C_{25}H_{27}N_3O_4$ C, H, N.

(5*S*,11a*S*)- 2-*tert*-butyl-5-(3,4 dimethoxyphenyl)-5,6,1 1,11a-tetrahydro-1*H*-imidazo[1',5':1,6] pyrido[3,4-*b*]indole-1,3 (2*H*)-dione (XXVII)

Golden yellow powder (50%); m.p.: 222-225 °C; R_f = 0.65 (CH_2Cl_2/MeOH 99:1); MS (EI): m/z 433 (M^+; 25%), m/z 362 (100%); IR (cm^{-1}): 332, 1722, 1710 ; ^{1}H-NMR ($CDCl_3$): 8.86 (brs, 1H, N*H*), 8.25-8.22 (d, 1H, Ar),7.73-7.71 (d, 1H, Ar),7.64-7.04 (m, 5H, Ar), 5.78 (brs, 1H, C*H*-Ph), 4.25-4.22 (m, 1H, C*H*C(O)N),3.99 (s, 3H, OCH_3), 3.97 (s, 3H, OCH_3), 3.88-3.81 (m, 1H, CH_aCH_b), 3.35-3.27 (m, 1H, CH_aH_b), 1.57 (s, 9H, CH_3); Analysis calcd for $C_{25}H_{27}N_3O_4$ C, H, N.

(5*R*,11a*S*)- 2-*tert*-butyl-5-(3,4 dimethoxyphenyl)-5,6,1 1,11a-tetrahydro-1*H*-imidazo[1',5':1,6]pyrido[3,4-*b*]indole-1,3(2*H*)-dione (XXVIII)

Yellow powder (56%), m.p.:122-125 °C; R_f = 0.90 (CH_2Cl_2/MeOH 99:1); MS (EI): m/z 433 (M^+), m/z 346 (100%); IR (cm^{-1}): 3338, 1760, 1734; ^{1}H-NMR ($CDCl_3$): 7.86 (brs, 1H, N*H*), 7.58-7.56 (d, 1H, Ar), 7.32- 7.29 (d, 1H, Ar), 7.25-6.79 (m, 6H, Ar), 6.22 (brs, 1H, C*H*Ph), 4.21-4.15 (dd, 1H, C*H*C(O)N), 3.87 (s, 3H, OCH_3), 3.83 (s, 3H, OCH_3), 3.51-3.43 (dd, 1H, CH_aCH_b), 2.90-2.80 (m, 1H, CH_aH_b), 1.64 (s, 9H, CH_3); Analysis calcd for $C_{25}H_{27}N_3O_4$ C, H, N.

General procedures for the preparation of 4-Chlorophenyl-5-(3,4 dimethoxyphenyl)-5,6,11,11a- tetrahydro-1*H*-imidazo [1',5':1,6]pyrido[3,4-*b*]indole-1,3(2*H*)-dione

4-chlorophenyl isocyanate (0.153 g,1 mmol, 1 equiv.) was added to a well stirred solution of the appropriate beta carboline **V,VI,VII,VIII** (0.36 g, 1 mmol) in methyl ethyl ketone (10 ml) and the mixture was stirred at reflux for 16 hours under nitrogen atmosphere. The product was purified using column chromatography on silica gel eluting with CH_2Cl_2.

(5*R*,11a*R*)- 4-chlorophenyl-5-(3,4 dimethoxyphenyl)-5,6, 11,11a-tetrahydro-1*H*-imidazo[1',5':1,6]pyrido[3,4-*b*]indole-1,3(2*H*)-dione (XXIX)

Yellow powder (54%); m.p.: 292-295 °C; R_f = 0.71 (CH_2Cl_2/MeOH 99:1); MS (EI): m/z 489 (M^++2), m/z 487 (M^+;100%); IR (cm^{-1}): 3334, 1750, 1721 ; ^{1}H-NMR ($CDCl_3$)

:7.94 (s, 1H, Ar), 7.63-7.60 (d, 1H, Ar), 7.43-7.39 (d, 2H, Ar), 7.24-6.89 (m, 8H, Ar), 6.36 (brs, 1H, C*H*Ph), 4.58-4.47 (m, 1H, C*H*C(O)N), 3.85 (s, 3H, OC*H*$_3$),3.79 (s, 3H, OC*H*$_3$), 3.64-3.58 (dd, 1H, CH_aCH_b), 3.24-3.02 (m, 1H, CH_aH_b), Analysis calcd for $C_{27}H_{22}ClN_3O_4$ C, H, N.

(5*S*,11a*R*)- 4-chlorophenyl-5-(3,4 dimethoxyphenyl)-5,6, 11,11a-tetrahydro-1*H*-imidazo[1',5':1,6]pyrido[3,4-*b*]indole-1,3(2*H*)-dione (XXX)

Yellowish green powder (54%); m.p.: 270-272 °C; R_f = 0.89 (CH_2Cl_2/MeOH 99:1); MS (EI): m/z 489 (M^++2), m/z 487 (M+; 100%); IR (cm $^{-1}$): 3341, 1772, 1710; ^{1}H-NMR ($CDCl_3$): 7.95 (brs, 1H, N*H*), 7.63-7.60 (d, 2H, Ar), 7.35-7.33 (d, 2H, Ar), 7.24-7.02 (d, 7H, Ar), 6.81 (brs, 1H, C*H*-Ph), 4.52-4.46 (dd, 1H, C*H*C(O)N), 3.87 (s, 3H, OC*H*$_3$), 3.84 (s, 3H, OC*H*$_3$), 3.65-3.58 (dd, 1H, CH_aCH_b), 3.10-3.01 (m, 1H, CH_aH_b); Analysis calcd for $C_{27}H_{22}ClN_3O_4$ C, H, N.

(5*S*,11a*S*)- 4-chlorophenyl-5-(3,4 dimethoxyphenyl)-5,6, 11,11a-tetrahydro-1*H*-imidazo[1',5':1,6]pyrido[3,4-*b*]indole-1,3(2*H*)-dione (XXXI)

Yellow powder (54%); m.p.: 295-298 °C; R_f = 0.70 (CH_2Cl_2/MeOH 99:1); MS (EI): m/z 489 (M^++2), m/z 487 (M^+; 100%); IR (cm $^{-1}$): 3285 ,1710, 1624 ; ^{1}H-NMR (CD-Cl_3): 8.82 (brs, 1H, N*H*), 8.24-8.21 (d, 2H, Ar), 7.74-7.71(d, 2H, Ar), 7.64-7.20 (m, 7H, Ar), 6.93 (brs, 1H, C*H*Ph), 4.24-4.21 (dd, 1H, C*H*C(O)N), 3.93 (s, 3H, OC*H*$_3$), 3.89 (s, 3H, OC*H*$_3$), 3.77-3.62 (m, 1H, CH_aCH_b), 3.49-3.45 (m, 1H, CH_aH_b); Analysis calcd for $C_{27}H_{22}ClN_3O_4$ C, H, N.

(5*R*,11a*S*)- 4-chlorophenyl-5-(3,4 dimethoxyphenyl)-5,6, 11,11a-tetrahydro-1*H*-imidazo[1',5':1,6]pyrido[3,4-*b*]indole-1,3(2*H*)-dione (XXXII)

White powder (55%); m.p.:269-272 °C; R_f = 0.90 (CH_2Cl_2/MeOH 99:1); MS (EI): m/z 489 (M^++2), m/z 487 (M^+; 100%); IR (cm $^{-1}$): 3288, 1710, 1628 , ^{1}H-NMR (CD-Cl_3) : 8.01 (brs, 1H, N*H*), 7.65-7.63 (d, 2H, Ar),7.38-7.35 (d, 2H, Ar), 7.22-7.02 (d, 7H, Ar), 6.79 (brs,1H, C*H*Ph), 4.52-4.48 (dd, 1H, C*H*C(O)N), 3.88 (s, 3H, OC*H*$_3$), 3.84 (s, 3H, OC*H*$_3$), 3.69-3.60 (dd, 1H, CH_aCH_b), 3.10-3.01 (m, 1H, CH_aH_b); Analysis calcd for $C_{27}H_{22}ClN_3O_4$ C, H, N.

General Procedures for the Preparation of Methyl -1-(3,4-dimethoxyphenyl)-2-(chloroacetyl)-2,3,4,9-tetrahydro-1*H*-ß-carboline-3-carboxylate

To a well stirred solution of of the appropriate ß-carboline **V-VIII** (2.08 g, 5.7 mmol) and $NaHCO_3$ (0.575 g, 6.89 mmol) in $CHCl_3$ (40 ml) was added dropwise chloroacetylchloride (1.1 ml, 13.69 mmol) at 0°C. The mixture was then stirred under N_2 for 1 hr. The mixture was then diluted with CH_2Cl_2 washed with a solution of $NaHCO_3$, dried over anhydrous Na_2SO_4. The residue was then crystallized from diethyl ether.

Methyl (1*R*, 3*R*)-1-(3,4- dimethoxyphenyl)-2-(chloroacetyl)-2, 3, 4, 9-tetrahydro-1*H*-β-carboline-3-carboxylate (XXXIII)

Yellow powder (92%); m.p.: 179-182 °C; R_f = 0.73 (CH_2Cl_2/MeOH 98:2); MS (EI): m/z 445 (M^++2), m/z 443 (M^+; 100%); IR (cm $^{-1}$): 3334, 1737, 1658 ; ^{1}H-NMR (CD-Cl_3): 7.81 (brs, 1H, N*H*), 7.63-7.61 (d, 1H, Ar), 7.32-7.29 (d, 1H, Ar), 7.22-7.20 (d, 1H, Ar), 7.18-6.72 (m, 4H, Ar), 5.31 (s, 1H, C*H*Ph), 4.24-4.20 (d, 1H, C*H*COOCH$_3$), 3.99- 3.95 (d, 2H, COC*H*$_2$Cl), 3.84 (s, 3H, OC*H*$_3$), 3.78 (s, 3H, OC*H*$_3$), 3.73 (s, 3H, OC*H*$_3$), 3.67-3.60 (m, 1H, CH_aCH_b), 3.62 -3.14 (dd, 1H, CH_aH_b); Analysis calcd for $C_{23}H_{23}ClN_2O_5$ C, H, N.

Methyl (1*S*, 3*R*)-1-(3,4- dimethoxyphenyl)-2-(chloroacetyl)-2, 3, 4, 9-tetrahydro-1*H*-β-carboline-3-carboxylate (XXXIV)

Yellow powder (90%); m.p.: 145-147 °C; R_f = 0.61 (CH_2Cl_2/MeOH 98:2); MS (EI): m/z 445 (M^++2), m/z 443 (M^+; 100%); IR (cm $^{-1}$): 3339, 1740, 1660; ^{1}H-NMR ($CDCl_3$): 7.78 (brs, 1H, N*H*),7.54-7.52 (d, 1H, Ar),7.24-6.82 (m, 6H, Ar), 6.09 (s, 1H, C*H*Ph), 4.15-4.10 (d, 1H, C*H*COOCH$_3$), 3.95- 3.92 (d, 2H, COC*H*$_2$Cl), 3.83 (s, 3H, OC*H*$_3$), 3.72 (s, 3H, OC*H*$_3$), 3.64 (s, 3H, OC*H*$_3$),3.34-3.32 (m, 1H, CH_aCH_b), 2.36-2.34 (m, 1H, CH_aH_b); Analysis calcd for $C_{23}H_{23}ClN_2O_5$ C, H, N.

Methyl (1*S*, 3*S*)-1-(3,4- dimethoxyphenyl)-2-(chloroacetyl)-2, 3, 4, 9-tetrahydro-1*H*-β-carboline-3-carboxylate (XXXV)

Orange powder (97%); m.p.:178-181 °C; R_f = 0.72 (CH_2Cl_2/MeOH 98:2); MS (EI): m/z 445 (M^++2), m/z 443 (M^+; 100%); IR (cm $^{-1}$): 3345, 1737, 1657; ^{1}H-NMR ($CDCl_3$) :7.81 (brs, 1H, N*H*),7.63-7.60 (d, 1H, Ar),7.56-6.73 (m, 6H, Ar),5.31 (s, 1H, C*H*Ph), 4.24-4.20 (d, 1H, C*H*COOCH$_3$), 3.99- 3.96 (d, 2H, COC*H*$_2$Cl), 3.92 (s, 3H, OC*H*$_3$), 3.80 (s, 3H, OC*H*$_3$), 3.78 (s, 3H, OC*H*$_3$), 3.75-3.60 (m, 1H, CH_aCH_b), 3.26-3.18 (m,1H,CH_aH_b); Analysis calcd for $C_{23}H_{23}ClN_2O_5$ C, H, N.

Methyl (1*R*, 3*S*)-1-(3,4- dimethoxyphenyl)-2-(chloroacetyl)-2, 3, 4, 9-tetrahydro-1*H*-β-carboline-3-carboxylate (XXXVI)

Golden yellow powder (94%); m.p.: 142-145 °C; R_f = 0.62 (CH_2Cl_2/MeOH 98:2); MS (EI): m/z 445 (M^++2), m/z 443 (M^+; 100%); IR (cm $^{-1}$) 3339, 1738, 1659, ^{1}H-NMR ($CDCl_3$): 7.68 (brs, 1H, N*H*), 7.55-7.53 (d, 1H, Ar), 7.24-6.81 (m, 6H, Ar), 6.12 (s, 1H, C*H*Ph), 4.15-4.10 (d, 1H, C*H*COOCH$_3$), 3.96- 3.88 (d, 2H, COC*H*$_2$Cl), 3.84 (s, 3H, OC*H*$_3$), 3.74 (s, 3H, OC*H*$_3$), 3.66 (s, 3H, OC*H*$_3$), 3.63-3.59 (m, 1H, CH_aCH_b), 3.33-3.28 (m, 1H, CH_aH_b); Analysis calcd for $C_{23}H_{23}ClN_2O_5$ C, H, N.

General procedures for the preparation of 2-ethyl-6-(3,4 dimethoxyphenyl)-2,3,6,7,12,12a-hexahydropyrazino [1', 2':1,6] pyrido[3,4-b]indole-1,4-dione

A solution of the appropriate chloroacetyl derivative **XXXIII-XXXVI** (0.62 g, 1.4 mmol, 1 equiv) and the ethyl amine (2.8 mmol, 2 equiv) in methanol (25 mL) was heated to reflux under a nitrogen atmosphere for 16 h. The reaction mixture was cooled to room temperature and evaporated to dryness under reduced pressure. The residue was dissolved in CH_2Cl_2, and the organic layer was washed with water, dried over Na_2SO_4, filtered, and concentrated to dryness. The crude product was then purified using column chromatography eluting with CH_2Cl_2: CH_3OH (99.5:0.5). The product was crystallized from ethanol.

(6*R*,12a*R*) -2- Ethyl-6-(3,4-dimethoxyphenyl)-2,3,6,7,12,12a hexahydropyrazino[1',2':1,6] pyrido[3,4-*b*]indole-1,4-dione (XXXVII)

Yellow powder (15%), m.p.: 262-265 °C; R_f = 0.75 (CH_2Cl_2/MeOH 95:5); MS (EI): m/z 419 (M^+; 100%); IR (cm $^{-1}$): 3205, 1666, 1641; ^{1}H-NMR ($CDCl_3$): 7.88 (brs, 1H, N*H*), 7.64-7.62 (d, 1H, Ar), 7.54- 7.44 (d, 1H, Ar), 6.98-6.88 (d, 1H, Ar) ,7.19- 6.72 (m, 4H, Ar), 6.23 (s, 1H, C*H*Ph), 4.33-4.27 (dd, 1H, C*H*C(O)N), 4.14-4.08 (d, 1H, CH_aH$_b$C(O)N), 3.96-3.91 (d, 1H, CH$_a$$H_b$C(O)N), 3.79 (s, 3H, OC$H_3$), 3.78 (s, 3H, OC$H_3$), 3.73-3.66 (q, 2H, NC$H_2$), 3.41-3.35 (dd, 1H, C$H_aCH_b$), 3.29-3.19 (dd, 1H, CH$_a$$H_b$),1.22-1.18 (t, 3H, C$H_3$); Analysis calcd for $C_{24}H_{25}N_3O_4$ C, H, N.

(6*S*,12a*R*) -2- Ethyl-6-(3,4-dimethoxyphenyl) 2,3,6,7,12,12a hexahydropyrazino[1',2':1,6] pyrido[3,4-*b*]indole-1,4-dione (XXXVIII)

Yellow powder (10%); m.p.: 220-223 °C; R_f = 0.79 (CH_2Cl_2/MeOH 95:5); MS (EI): m/z 419 (M^+;100%); IR (cm $^{-1}$): 3205 ,1668,1641; ^{1}H-NMR ($CDCl_3$): 7.85 (brs,1H, N*H*), 7.58-7.54 (d,1H, Ar), 7.25- 6.99 (m, 6H, Ar), 6.74 (brs, 1H, C*H*Ph), 4.39-4.37 (dd, 1H, C*H*C(O)N), 4.18-4.13 (d, 1H, CH_aC(O)N), 4.04-3.99 (d, 1H, CH_bC(O)N), 3.86 (s, 3H, OCH_3),3.84 (s, 3H, OCH_3),3.61-3.56 (q, 2H, NCH_2), 3.44-3.33 (m,1H, CH$_a$CH_b), 3.02-2.99 (m, 1H, CH_aH$_b$), 1.26-1.19 (t, 3H, CH_3); Analysis calcd for $C_{24}H_{25}N_3O_4$ C, H, N.

(6*S*,12a*S*) -2- Ethyl-6-(3,4-dimethoxyphenyl)-2,3,6,7,12,12a hexahydropyrazino[1',2':1,6] pyrido[3,4-*b*]indole-1,4-dione (XXXIX)

Yellow powder; m.p.: 264-267 °C; R_f = 0.74 (CH_2Cl_2/MeOH 95:5); MS (EI): m/z 419 (M^+), m/z 248 (100%); IR (cm $^{-1}$): 3285, 1658, 1650; ^{1}H-NMR ($CDCl_3$): 8.01 (brs, 1H, N*H*), 7.64-7.61 (d, 1H, Ar), 7.55-7.56 (d, 1H, Ar), 7.31- 7.30 (d, 1H, Ar), 7.19-7.18 (d, 1H, Ar), 7.17-7.16 (d, 1H, Ar), 6.90-6.89 (d, 1H, Ar), 6.85-6.82 (dd, 1H, Ar), 6.89 (s, 1H, Ar),6.24 (s, 1H, C*H*Ph), 4.33-4.26 (dd, 1H, C*H*C(O)N), 4.08-4.03 (d, 1H, CH_aC(O)N), 3.95-3.90 (d, 1H, CH_bC(O)N), 3.81 (s, 3H, OCH_3),3.79 (s, 3H, OCH_3), 3.74-3.66 (q, 2H, NCH_2), 3.41-3.33 (dd, 1H, CH$_a$CH_b), 3.29-3.19 (dd, 1H, CH_aH$_b$), 1.22-1.17 (t, 3H, CH_3); Analysis calcd for $C_{24}H_{25}N_3O_4$ C, H, N.

(6*R*,12a*S*) -2- Ethyl-6-(3,4-dimethoxyphenyl)-2,3,6,7,12,12a hexahydropyrazino[1',2':1,6] pyrido[3,4-*b*]indole-1,4-dione (XL)

Orange powder (10%); m.p.: 218-220 °C; R_f = 0.81 (CH_2Cl_2/MeOH 95:5); MS (EI): m/z 419 (M^+; 100 %); IR (cm $^{-1}$): 3280, 1660, 1648; ^{1}H-NMR ($CDCl_3$): 8.29 (brs, 1H, N*H*),7.96-7.94 (d, 1H, Ar), 7.56-7.54 (d, 1H, Ar),7.34-7.31 (d, 1H,Ar), 7.29-7.25 (dd,1H,Ar), 7.17-7.15 (d,1H,Ar), 6.95-6.93 (d,1H,Ar), 6.78 (s,1H,Ar), 6.68 (brs,1H,C*H*Ph), 4.39-4.33 (dd,1H,C*H*C(O)N),3.87 (s,3H,OCH_3),3.83 (s,3H,OCH_3), 3.82-3.73 (q,2H,NCH_2), 3.60-3.53 (m,1H,CH_aC(O)N), 3.42-3.31 (m,1H,CH_bC(O)N), 3.23-3.16 (m,1H,CH$_a$CH_b), 3.00-2.91(m,1H,CH_aH$_b$), 1.22 -1.17 (t,3H,CH_3); Analysis calcd for $C_{24}H_{25}N_3O_4$ C, H, N.

General procedures for the preparation of 2-*tert*- butyl-6-(3,4-dimethoxyphenyl)-2,3,6,7,12,12a-hexahydropyrazino [1',2':1,6] pyrido[3,4-b]indole-1,4-dione

A solution of the of the appropriate chloroacetyl derivative **XXXIII-XXXVI** (0.62 g,1.4 mmol, 1 equiv) and tertiary butylamine (2.8 mmol, 2 equiv) in methanol (25 mL) was heated to reflux under a nitrogen atmosphere for 16 h. The reaction mixture was cooled to room temperature and evaporated to dryness under reduced pressure. The residue was dissolved in CH_2Cl_2, and the organic layer was washed with water, dried over Na_2SO_4, filtered, and concentrated to dryness. The crude product was then purified using column chromatography eluting with CH_2Cl_2: CH_3OH (99:1). The product was crystallized from ethanol.

(6*R*,12a*R*) -2- *tert*-butyl-6-(3,4-dimethoxyphenyl)-2,3,6,7,12, 12ahexahydropyrazino[1',2':1,6] pyrido[3,4-*b*]indole-1,4-dione (XLI)

Buff powder (14%); m.p.:190-192 °C; R_f = 0.4 (CH_2Cl_2/MeOH 92:8); MS (EI): m/z 447 (M^+), m/z 248 (100%); IR (cm $^{-1}$): 3260, 1658,1649; ^{1}H-NMR ($CDCl_3$): 7.76 (brs,1H,N*H*),7.62-7.59 (d,1H,Ar), 7.54- 7.51 (d,1H, Ar), 7.23-7.15 (dd,1H,Ar), 6.93-6.85 (d,1H, Ar), 6.82-6.73 (m, 3H, Ar), 6.67 (s,1H,C*H*Ph), 4.90-4.88 (d,1H,C*H*C(O)N), 4.37-4.31 (d,1H,CH_aH$_b$C(O)N), 3.99-3.95 (d,1H,CH$_a$$H_b$C(O)N), 3.82 (s, 3H, OC$H_3$), 3.77 (s,3H,OC$H_3$), 3.71-3.60 (m,1H,CH$_aCH_b$), 3.47-3.37 (dd,1H, C$H_aH_b$), 1.31 (s, 9H, C$H_3$); Analysis calcd for $C_{26}H_{29}N_3O_4$ C, H, N.

(6*S*,12a*R*) -2- *tert*-butyl-6-(3,4-dimethoxyphenyl)-2,3,6,7,12, 12a hexahydropyrazino [1',2':1,6] pyrido[3,4-*b*] indole-1,4-dione (XLII)

Yellow powder (16%); m.p.: 210-212 °C; R_f = 0.27 (CH_2Cl_2/MeOH 92:8); MS (EI): m/z 447 (M^+), m/z 248 (100%); IR (cm $^{-1}$): 3260,1741,1655; ^{1}H-NMR ($CDCl_3$): 7.90 (brs, 1H, N*H*), 7.82-7.14 (m, 7H, Ar),6.83 (brs,1H,C*H*Ph), 4.90-4.88 (d,1H,C*H*C(O)N), 4.37-4.31(d,1H,CH_aH$_b$C(O)N), 3.99-3.95(d,1H,CH$_a$$H_b$C(O)N), 3.84 (s, 3H, OC$H_3$),3.63 (s, 3H, OC$H_3$),3.71-3.60 (m,1H,CH$_aCH_b$), 3.47-3.37 (dd,1H,C$H_aH_b$), 1.24 (s, 9H, C$H_3$); Analysis calcd for $C_{26}H_{29}N_3O_4$ C, H, N.

(6*S*,12a*S*) -2- *tert*-butyl-6-(3,4-dimethoxyphenyl)-2,3,6,7,12, 12a hexahydropyrazino[1',2':1,6] pyrido[3,4-*b*]indole-1,4-dione (XLIII)

18%, Yellow powder ; m.p.: 192-195 °C; R_f = 0.40 (CH_2Cl_2/MeOH 92:8); MS (EI): m/z 447 (M^+), m/z 248 (100%); IR (cm $^{-1}$): 3262,1699, 1654 ; ^{1}H-NMR ($CDCl_3$): 7.73 (brs, 1H, N*H*), 7.62-7.59 (d, 1H, Ar), 7.56-7.54 (d, 1H, Ar), 7.23- 7.16 (dd, 1H, Ar), 6.88-6.81(d, 1H, Ar), 6.79-6.62 (m, 3H, Ar), 6.33 (s, 1H, C*H*Ph), 4.87-4.85 (d, 1H, C*H*C(O)N), 4.36-4.32 (dd, 1H, CH_aH$_b$C(O)N), 4.10-4.01 (d, 1H, CH$_a$$H_b$C(O)N), 3.81 (s, 3H, OC$H_3$), 3.76 (s, 3H, OC$H_3$), 3.67-3.63 (m, 1H, CH$_aCH_b$), 3.55-3.45 (dd, 1H, C$H_aH_b$), 1.36 (s, 9H, C$H_3$); Analysis calcd for $C_{26}H_{29}N_3O_4$ C, H, N.

(6*R*,12a*S*) -2- *tert*-butyl-6-(3,4-dimethoxyphenyl)-2,3,6,7, 12,12a hexahydropyrazino [1',2':1,6]pyrido[3,4-*b*] indole-1,4-dione (XLIV)

Orange powder (15%); m.p.: 212-214 °C; R_f = 0.17 (CH_2Cl_2/MeOH 92:8); MS (EI): m/z 447 (M^+; 94 %), m/z

248 (100%); IR (cm^{-1}) 3260, 1657, 1641; ^{1}H-NMR ($CDCl_3$): 7.78 (brs, 1H, N*H*), 7.54-7.59 (d, 1H, Ar), 7.34- 6.98 (m, 6H, Ar), 6.82 (s, 1H, C*H*Ph), 4.32-4.27 (d, 1H, C*H*C(O)N), 4.15.-4.09(dd, 1H, CH_aC(O)N), 4.03-3.99 (d, 1H, CH_bC(O)N), 3.87 (s, 3H, OCH_3), 3.83 (s, 3H, OCH_3), 3.75-3.68 (m, 1H, CH_a-CH_b), 3.64 -3.58 (dd,1H, CH_aH$_b$),1.26 (s, 9H, CH_3); Analysis calcd for $C_{26}H_{29}N_3O_4$ C, H, N.

5. Biological evaluation

All the synthesized compounds were evaluated for their inhibitory properties versus recombinant PDE5. Most of compounds were evaluated in 2 steps. The first step was the determination of the percentage inhibition at a screening dose of 50 μM performed in triplicate. For compounds displaying a percentage of inhibition > 65%, the IC_{50} was determined by testing a range of 10 concentrations with at least two replicates per concentration. For all assays, tadalafil (PDE5/PDE11 inhibitor) was used for comparison.

Phosphodiesterase Inhibitory Activity

PDE activity was measured using an adaptation of the IMAP1 fluorescence polarization phosphodiesterase assay (Molecular Devices, Sunnyvale, CA, USA). PDE hydrolysis of the fluorescent- labeled substrate allows it to bind the IMAP1 reagent, which increases fluorescence polarization (FP). The assay used fluorescein (Fl)-cAMP and tetramethylrhodamine (TAMRA) - cGMP as substrates. The different excitation and emission spectra of the substrates (485-530 nm for Fl and 530-590 nm for TAMRA) allowed for simultaneous measurement of cAMPand cGMP hydrolysis in the same well. The assays were performed in 96-well microtiter plates using a reaction buffer containing 10 mM Tris-HCl (pH 7.2) 10 mM $MgCl_2$, 0.05% NaN_3 and 0.1% phosphate-free bovine serumalbumin as the carrier. Each well contained 20 μL of recombinant enzyme (BPS Biosciences, San Diego, CA, USA) and 10 μL inhibitor. The reaction was initiated by the addition of 10 μL of a substrate solution containing 50 nM Fl-cAMP and/or TAMRA-cGMP. After incubating at room temperature for 60 min. the reaction was terminated by adding 120 μL of binding solution. FP was measured with a BioTek Synergy 4 (BioTek Instruments, Winooski, VM, USA) [15].

6. *IN SILICO* STUDY

To reveal the mode of interaction of **IX** with the residues lining the binding pocket of PDE5, a docking experiment was implemented to dock **IX** into the active site of PDE5 with the program MOE version 2007.09. All ligand atoms but no protein atoms were allowed to move during the docking simulation. Before the docking procedure, water molecules, old co-crystallized ligand (tadalafil) and heteroatoms were removed from the protein (PDB ID code 1UDU) crystal structure, To ensure more accurate docking procedures, tadalafil as an example of potent PDE5 inhibitor was redocked to the binding pocket under the same MOE settings as compound **IX**; the interaction of tadalafil is displayed (Fig. **1**) meanwhile the interaction of **IX** is also displayed and the difference in mode of binding is observed (Fig. **2**).

ACKNOWLEDGMENTS

The authors are indebted to the Faculty of Postgraduate Studies, The German University in Cairo for partial finance of the project. Also, some of the instruments used were donated by The Alexander von Humboldt Foundation, Germany.

REFERENCES

[1] Conti, M. Phosphodiesterases and cyclic nucleotide signalling in endocrine signalling in endocrine cells. *Mol. Endocrinol.*, **2000**, *14*, 1317- 1327.

[2] Lucas, K. A.; Pitari, G. M.; Kazerounian, S.; Ruiz-Stewart, I.; Park, J.; Schulz, S. Guanylyl cyclases an signaling by cGMP. *Pharmacol. Rev.*, **2000**, *52*, 375- 414.

[3] Corbin, J. D.; Francis, S. H.; Webb, D. J. Phosphodiesterase type 5 as a pharmacologic target in erectile dysfunction. *Urology*, **2002**, *60*, 4-11.

[4] Champion, H. C.; Bivalacqua, T. J.; Takimoto, E.; Kass, D. A.; Burnett, A. L. Phosphodiesterase-5A dysregulation in penile erectile tissue is a mechanism of priapism. *Proc. Nat.l Acad. Sci. USA,* **2005**, *102*(5), 1661-1666.

[5] Stacey, P.; Rulten, S.; Dapling, A.; Phillips, S. C. Molecular cloning and expression of human cGMP-binding cGMP-specific phosphodiesterase (PDE5). *Biochem. Biophys. Res. Commun.*, **1998**, *247*(2), 249-254.

[6] Loughney, K.; Hill, T. R.; Florio, V. A.; Uher, L.; Rosman, G. J.; Wolda, S. L.; Jones, B. A.; Howard, M. L.; McAllister-Lucas, L. M.; Sonnenburg, W. K.; Francis, S. H.; Corbin, J. D.; Beavo, J. A.; Ferguson, K. Isolation and characterization of cDNAs encoding PDE5A, a human cGMP-binding, cGMP-specific 3',5'-cyclic nucleotide phosphodiesterase. *Gene*, **1998**, *216*(1), 139-147.

[7] Van Driel, M. F. Phosphodiesterase inhibitors: effectiveness and new applications. *Ned. Tijdschr. Geneeskd.*, **2006**, *150*(29), 1613-1616.

[8] Bos, J. Epac protiens: multi-purpose cAMP targets. *Trends Biochem. Sci.*, **2006**, *31*, 680-686.

[9] Hou, Y.; Gupta, N.; Schoenlein, P.; Wong, E.; Worner, R.; Martindale, R.; Ganapathy, V. An anti-tumor role for cGMP -dependant protien kinase. *Cancer Lett.*, **2006**, *240*, 60-68.

[10] Zhang, K. Y.; Card, G. L.; Suzuki, Y.; Artis, D. R.; Fong, D.; Gillette, S.; Hsieh, D.; Neiman, J.; West, B. L.; Zhang, C.; Milbum, M. V.; Kim, S. V.; J., S.; Bollag, G. A glutamine switch mechanism for nucleotide selectivity by phosphodiesterases. *Mol. Cell*, **2004**, *11*, 865-873.

[11] Orme, M. W.; Martinelli, M. J.; Doecke, C. W.; Pawlak, J.M. Modified Pictet-Spengler reaction and products prepared thereform. U.S. Patent 7, 550, 479, June, 23, **2009**.

[12] Whaley W.M.; Govindachari T.R. The Pictet-Spengler synthesis of tetrahydroisoquinolines and related compounds. *Org. React.*, **1951**, *6*, 151-190.

[13] Ungemach, F.; Soerens, D.; Weber, R.; DiPierro, M.; Campos, O.; Mokry, P.; Cook, J. M.; Silverston, J. V. General method for the assignement of stereochemistry of 1,3- disubstituted 1,2,3,4 -tetrahydro-ß-carbolines by Carbon -13 spectroscopy. *J. Am. Chem. Soc.,* **1980**, *102*, 6976-6984.

[14] Sandrin, J.; Soerens, D.; Cook, J. M. Carbon-13 NMR of 1,3-disubstituted 1,2,3,4-tetrahydro-ß-carbolines *Heterocycles*, **1976**, *4*, 1249-1255.

[15] Huang, W.; Zhang, Y.; Sportsman, J. R. A fluorescence polarization assay for cyclic nucleotide phosphodiesterases. *J. Biomol. Screen.*, **2002**, *7*, 215-219.

Received: August 15, 2010 Revised: September 29, 2010 Accepted: October 04, 2010