European Journal of Medicinal Chemistry 45 (2010) 1278–1286

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Synthesis, molecular modeling and biological evaluation of novel tadalafil analogues as phosphodiesterase 5 and colon tumor cell growth inhibitors, new stereochemical perspective

Ashraf H. Abadi [a,*], Bernard D. Gary [b], Heather N. Tinsley [b,c], Gary A. Piazza [b,c], Mohammad Abdel-Halim [a]

[a] Department of Pharmaceutical Chemistry, Faculty of Pharmacy and Biotechnology, German University in Cairo, Cairo 11835, Egypt
[b] Division of Drug Discovery, Department of Biochemistry and Molecular Biology, Southern Research Institute, Birmingham AL 35205, USA
[c] Department of Pharmacology and Toxicology, The University of Alabama at Birmingham, 2000 Ninth Avenue South, Birmingham, AL 35205, USA

ARTICLE INFO

Article history:
Received 11 July 2009
Received in revised form
25 September 2009
Accepted 22 October 2009

Keywords:
β-Carboline
PDE5 inhibition
Stereochemistry

ABSTRACT

The synthesis of novel tadalafil analogues in which the benzodioxole moiety is replaced by 2-bromophenyl; the chiral carbons swing from *R,R* to *R,S*, *S,R* and *S,S*; the piperazinedione ring is maintained or reduced to the 5-membered imidazolidinedione or thioxoimidazolinone is described. The prepared analogues were evaluated for their capacity to inhibit the cyclic guanosine monophosphate (cGMP) selective phosphodiesterase 5 (PDE5) isozyme and the growth of human HT-29 colon adenocarcinoma cells. The *R* absolute configuration of C-5 in the β-carboline-hydantoin and C-6 in the β-carboline-piperazinedione derivatives was found to be essential for the PDE5 inhibition. In addition, tadalafil analogues that were synthesized from L-tryptophan were more active than those derived from D-tryptophan, which is of economic value and expands the horizon for the discovery of new carbolines as PDE5 inhibitors. While some analogues displayed potent tumor cell growth inhibitory activity, there was no apparent correlation with their PDE5 inhibitory activity, which leads us to conclude that other PDE isozymes or PDE5 splice variants may be involved.

1. Introduction

Phosphodiesterase type 5 (PDE5) is a key enzyme involved in the regulation of cGMP-specific signaling pathways by catalyzing the hydrolysis of the cyclic nucleotide cGMP into 5′ guanosine monophosphates. It's an essential regulator in normal physiological processes such as smooth muscle contraction and relaxation and it is the major PDE isozyme in penile *corpus cavernosum* tissue that plays a key role in the control of penile erection [1,2]. Tadalafil (Fig. 1) is an orally active tetrahydro-β-carboline (THBC) derivative with PDE5 inhibitory properties that is marketed for the treatment of male erectile dysfunction. Its local vasodilatation action is mediated through high levels of cGMP in male *corpus cavernosum* [3].

Recent studies showed that PDE5 and other cGMP-PDEs are over expressed in a variety of cancers like colon, pancreatic, lung and bladder cancers relative to normal tissues. Moreover, inhibition and consequent high levels of cGMP may be associated with anticancer and apoptotic activities [4,5]. Exisulind (Fig. 1) and its analogues (CP78, CP 461, CP 248) have been shown to selectively induce apoptosis in cell lines derived from many cancers including colon, bladder, prostate, breast and lung. They maintained similar rank orders of apoptosis induction, growth inhibition and PDE5 and PDE2 inhibition. They also caused sustained intracellular cGMP increase in the colon tumor cells; thus it is proposed that the cGMP mediated the mechanism underlying the actions of exisulind and its analogues on apoptosis in neoplastic cells [4–6]. These effects in neoplastic cells are not solely dependent on the specific inhibition of PDE5, rather, it is related to non-selective inhibition of the cGMP-PDEs, which may explain why highly selective PDE5 inhibitors do not induce apoptosis in tumor cell lines. Accordingly, it is apparently important to maintain cross-reactivity among PDE isoforms [7].

In the present work we report the synthesis of novel tadalafil related analogues, evaluate the activity of these compounds as PDE5 inhibitors as well as growth inhibitory agents and determine the correlation between these two activities.

2. Chemistry

The general synthesis of the target β-carboline-hydantoin and -thiohydantoin derivatives is illustrated in Schemes 1 and 2; meanwhile, the synthesis of the β-carbolines-piperazinedione derivatives is illustrated in Scheme 3.

* Corresponding author. Tel.: +202 27590716; fax: +202 27581041.
E-mail address: ashraf.abadi@guc.edu.eg (A.H. Abadi).

0223-5234/$ – see front matter © 2009 Elsevier Masson SAS. All rights reserved.
doi:10.1016/j.ejmech.2009.10.046

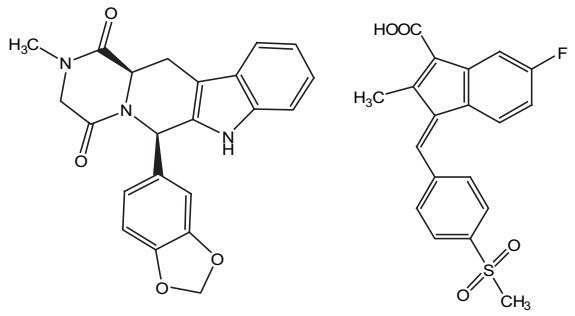
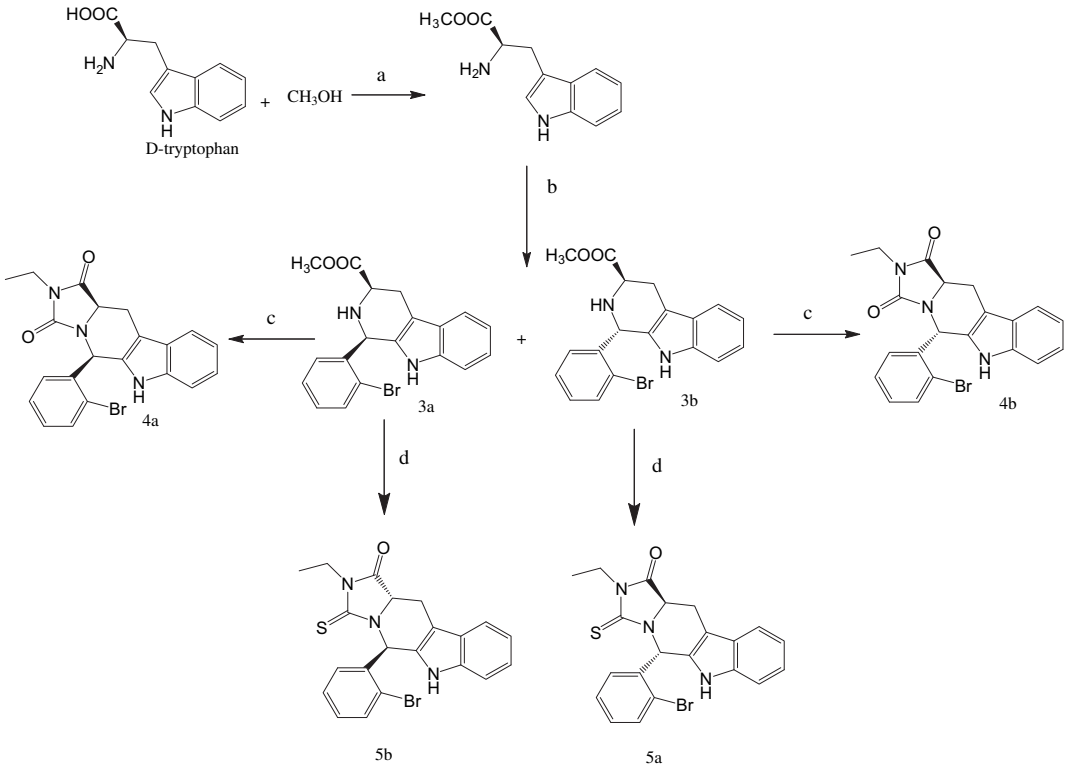


Fig. 1. Chemical structure of the specific PDE5 inhibitor tadalafil (left) and the non-specific PDE5 inhibitor exisulind (right).

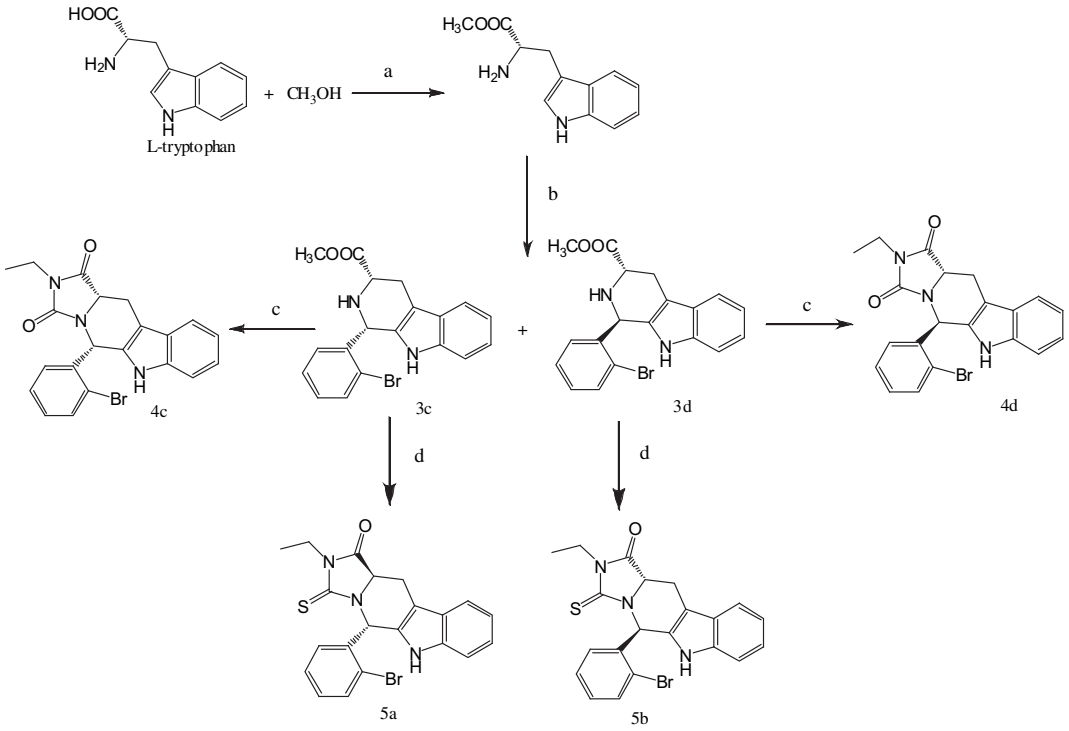
Both D-tryptophan and L-tryptophan methyl esters were synthesized by a general synthetic procedure for amino acid esters [8]. The D- and L-Tryptophan methyl esters and 2-bromobenzaldehyde were subjected to Pictet–Spengler reaction under non-stereo specific conditions to give the corresponding *cis*- and *trans*-1,3-disubstituted THBCs (**3a**–**d**). The produced *cis*- and *trans*-diastereomers of the 1,3-disubistituted THBC were separated by column chromatography using CH_2Cl_2 as an eluent. The respective pure *cis*- or *trans*-isomer was reacted with commercially available ethyl isocyanate to produce the desired *cis*- and *trans*-hydantoins (**4a**–**d**) meanwhile reaction with ethyl isothiocyanate gave only the corresponding thiohydantoins of the *trans*-configuration (**5a**, **5b**). Reaction of the respective THBC with chloroacetyl chloride provided the corresponding amide derivative (**6a**–**d**). The β-carboline-piperazinedione derivatives with the respective *N*-alkyl or *N*-benzyl substituents (**7a**–**10d**) were obtained by ring closure of the open amides **6a**–**d** in the presence of primary amines, namely methylamine, ethylamine, butylamine and benzylamine in refluxing methanol.

The *cis*/*trans* stereochemistry for the THBCs (**3a**–**d**) was assigned depending on the comprehensive study of ^{13}C NMR spectroscopy data established in previous literature, the signals for C-1 and C-3 in the *trans*-diastereomers are clearly upfield from those of the corresponding *cis*-isomers in the carbon spectrum. This is probably due to the 1,3 interactions present in the *trans*-isomer [9–11]. For the THBCs (**3a**–**d**), only the signal of C-1 in the *trans*-isomer appeared at higher field compared to that of the *cis*-isomer, with $\Delta\delta \approx 2$–3, no analogous difference was detected with C-3. Regarding the ^1H NMR signals for the proton at C-1 of **3a**–**d**, they appeared at δ 5.5–5.7. On cyclization to the hydantoin, thiohydantoin or piperazinedione derivatives the same proton makes a huge downfield shift to $\approx\delta$ 6.7. This deshielding effect can be explained from the energy minimized form of the particular THBC and its respective hydantoin derivative where in the former case the 2-bromo substituent on the phenyl ring is at the same direction and close to the proton at C-1 (distance ≈2.6 Å), formation of the new ring causes the pendant phenyl to rotate around its axis orienting the 2-bromo substituent in different plane far from the respective proton (distance ≈4.2 Å), Fig. 2.

Moreover, a correlation exists between R_f value on TLC and the stereochemistry of the 1,3-disubstituted THBC **3a**–**d**, where *cis*-isomer is systematically less polar than the *trans*-isomer while in the hydantoin series, the polarity is reversed, thus, the *cis*-isomer becomes more polar than the *trans*-isomer [12]. This is clear from the R_f values of the 1*R*, 3*R*; 1*S*, 3*R*; 1*S*, 3*S* and 1*R*, 3*S* of the THBC derivatives **3a**–**d** equal to 0.38; 0.15; 0.39 and 0.14, respectively, meanwhile the R_f value of the corresponding hydantoins **4a**–**d** were 0.38, 0.46, 0.39 and 0.45, respectively. In addition, it was found that the R_f values of β-carboline-piperazinedione derivatives increase



Scheme 1. Conditions: (a) CH_3COC l, reflux, neutralization; (b) 2-bromobenzaldehyde, CF_3COOH, Room temperature; (c)C_2H_5NCO, 2-butanone, reflux; (d)C_2H_5NCS, 2-butanoe, reflux.



Scheme 2. Conditions: (a) CH_3COC l, reflux, neutralization; (b) 2-bromobenzaldehyde, CF_3COOH, Room temperature; (c) C_2H_5NCO, 2-butanone, reflux; (c) C_2H_5NCS, 2-butanone, reflux.

with increasing of size of the N-substituent, this may be due to increased lipophilicity.

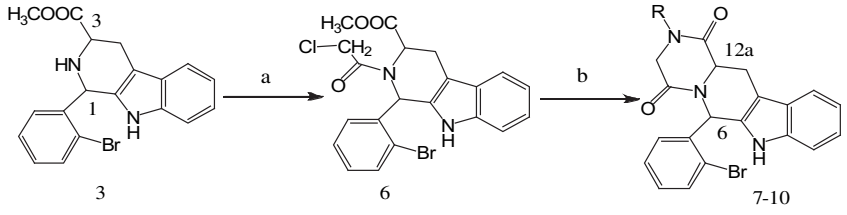
During the attempt to synthesize the thiohydantoin series, only the *trans*-isomers were obtained (**5a**, **5b**). The ^{13}C NMR, ^{1}H NMR spectra, R_f and m.p. for the thiohydantoins obtained on treating the *cis*- and *trans*-THBC derived from D-tryptophan with ethyl isothiocyanate, were completely matching with those derived from the *trans*- and *cis*-THBCs isomers derived from L-Tryptophan. This observation has been previously reported by others and demonstrates the enantiomeric nature of the two products [11,13,14].

Mass spectrometry to all derivatives showed the molecular ion peaks at m/z M^+ and $M^+ + 2$ due to the isotopic nature of bromine atom. Moreover, the 1.3-disubstituted THBC derivatives **3a–d** showed molecular ion peaks that were also the base peaks indicating their stable nature. Mass spectrometry for most hydantoin, thiohydantoin and piperazinedione derivatives showed a base peak at m/z $M^+ - 80$ indicating that the bromine atom was the most liable fragment to be lost on electron bombardment.

The infrared spectra of all derivatives showed bands at $\approx 3400\ cm^{-1}$ for the indole N–H stretching. Compounds **3 a–d** showed peaks at $\approx 1750\ cm^{-1}$ for the ester carbonyl stretching. On the other hand, the β-carboline-hydantoin derivatives **4a–d** showed 2 carbonyl stretching peaks at ≈ 1760 and $1700\ Cm^{-1}$, as one of the carbonyls is flanked between 2 nitrogen atoms, meanwhile the other is flanked between an N and a C, respectively. The β-carboline-piperazinedione derivatives showed 2 carbonyl stretching peaks at ≈ 1660 and $1650\ cm^{-1}$. The relatively lower stretching values of the carbonyls of the 6 membered derivatives relative to the 5-membered derivatives may be explained by the higher ring strain of the smaller ring, leading to higher double bond character of the carbonyl groups.

3. Biological results and discussion

All compounds and their intermediates were tested for their *in vitro* tumor cell growth inhibitory activity using the human HT-29



Cpd. #	R	Stereochemistry	Cpd. #	R	Stereochemistry
7a	Me	6*R*,12a*R*	**9a**	Bu	6*R*,12a*R*
7b	Me	6*S*,12a*R*	**9b**	Bu	6*S*,12a*R*
7c	Me	6*S*,12a*S*	**9c**	Bu	6*S*,12a*S*
7d	Me	6*R*,12a*S*	**9d**	Bu	6*R*,12a*S*
8a	Et	6*R*,12a*R*	**10a**	Benzyl	6*R*,12a*R*
8b	Et	6*S*,12a*R*	**10b**	Benzyl	6*S*,12a*R*
8c	Et	6*S*,12a*S*	**10c**	Benzyl	6*S*,12a*S*
8d	Et	6*R*,12a*S*	**10d**	Benzyl	6*R*,12a*S*

Scheme 3. Conditions: (a) $ClCH_2COCl$, $NaHCO_3$, Room temperature; (b) R-NH_2, MeOH, Reflux.

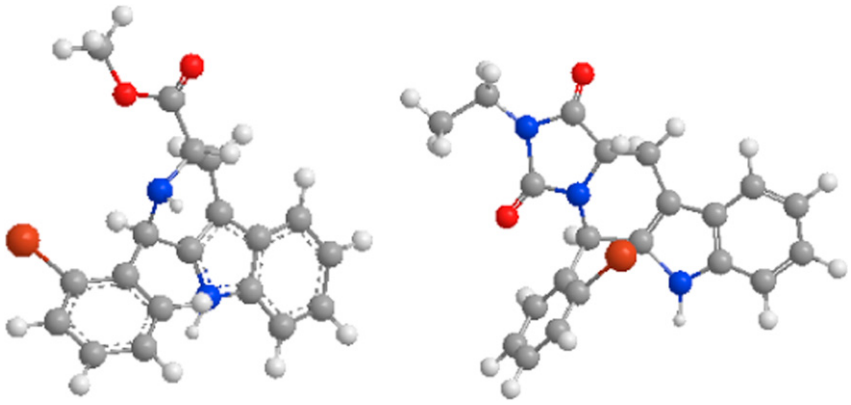


Fig. 2. The energy minimized forms of **3d** (left) and **4d** (right) showing orientations of the bromine substituent relative to the hydrogen on the chiral carbon derived from the aldehyde.

colon tumor cell line as well as their inhibitory properties *vs* recombinant human PDE5 enzyme.

HT-29 tumor cell line is also known to express PDE5 and other cGMP degrading PDE isozymes. Most of compounds were evaluated in 2 steps, first, the percentage inhibition at a screening dose of 100 μM was performed in triplicate, then for compounds displaying a percentage of inhibition >70% were evaluated by testing a range of 10 concentrations with at least two replicates per concentration to calculate an IC_{50} value. For a few compounds, the IC_{50} was initially determined. The results are summarized in Table 1.

From these data we conclude that the stereochemistry of C-5 of the β-carboline-hydantoin and β-carboline-thiohydantoin derivatives and C-6 of the β-carboline-piperazinedione derivatives are the most crucial factor for the PDE5 inhibitory activity. In the β-carboline-hydantoins **4a–d**, the order of activity is 5*R*, 11a*S* (**4d**) > 5*R*, 11a*R* (**4a**) > 5*S*, 11a*S* (**4c**) >5*S*, 11a*R* (**4b**) and for the piperazinediones, the order of activity is 6*R*, 12*aS* > 6*R*, 12*aR* > 6*S*, 12*aS* > 6*S*, 12*aR*. This can be observed by the THBC precursors (**3a–d**) where the order of their PDE5 inhibitory potency is matched with this stereochemistry. These results are in marked contrast to the results obtained with tadalafil isomers whereby the order of activity is 6*R*, 12a*R* > 6*R*, 12a*S* > 6*S*, 12a*R* > 6*S*, 12a*S* [15]. This leads to the assumption that the scope and limitations of such class of compounds are still to be explored. Meanwhile, the effect of the stereochemistry of the chiral carbon derived from the amino acid is of lower impact. Interestingly, the chiral center derived from amino acid with the *S* configuration (L-tryptophan) may lead to more active isomers than those derived from amino acid with the *R* configuration (D-tryptophan). This suggests the feasibility of synthesizing active compounds derived from L-tryptophan rather than from D-tryptophan and offers a highly economic advantage as L-tryptophan is much cheaper than D-tryptophan.

β-Carboline-thiohydantoins are markedly less potent than their corresponding hydantoin congeners, which may be due to the lower electro-negativity of sulfur relative to oxygen, **5a** *vs* **4b** and **5b** *vs* **4d**.

For the impact of the terminal ring size, *viz* hydantoins *vs* piperazinediones, compounds are almost of the same activity. It is apparent that a wide range of *N*-alkyl or *N*-aryl groups and possibly other lipophilic functions can be tolerated on the piperazinedione, given that their impact upon activity is almost the same.

Bromine – a bulky and electronegative substituent – at C-2 of the pendant phenyl still produces highly active compounds in the contrary to what has been previously reported for the 2-methoxy substituent in the β-carboline-hydantoin derivatives [15]. This confirms the necessity to explore the scope and limitations of this class of compounds.

Molecular modeling studies were performed to dock compound **4d** to the human PDE5 using the MOE software [16]. Detailed view showing compounds **4d** is able to dock to the active pocket of PDE5 and interact with the side chain of Gln 817 which forms a single, not bidentate, hydrogen bond with the indole NH group of the respective compound. Compounds **4d** did not interact with the L-region of the protein and the H pocket is filled with the

Table 1
Inhibitory effect of the synthesized compounds on HT29 cells and PDE5.

Cpd.#	% Growth Inhibition at 100 μM	Growth inhibition IC_{50} μM	%PDE5 inhibition at 100 μM	PDE5 inhibition IC_{50} μM	Cpd.#	% Growth Inhibition at 100 μM	Growth inhibition IC_{50} μM	%PDE5 inhibition at 100 μM	PDE5 inhibition IC_{50} μM
3a	89.04	48	85.52	5.6	**7b**	61.73	ND	75.54	88
3b	85.02	78	21.50	ND[a]	**7c**	76.14	27	92.74	2.8
3c	92.75	26	45.97	ND	**7d**	ND	25.5	97.58	0.06
3d	57.46	ND	91.13	3.1	**8a**	ND	>100	97.04	0.32
4a	79.54	21	98.65	0.29	**8b**	65.61	ND	91.67	41
4b	65.14	ND	93.01	4.0	**8c**	ND	32	90.32	32
4c	77.86	24	89.51	0.64	**8d**	47.25	ND	89.25	0.05
4d	69.76	20	99.462	0.038	**9a**	55.74	ND	99.73	0.26
5a	ND	>100	82.80	74	**9b**	50.20	ND	66.66	ND
5b	ND	22.5	93.55	6.1	**9c**	ND	>100	69.09	ND
6a	ND	1.6	86.02	29	**9d**	ND	>100	93.81	0.05
6b	ND	>100	47.85	ND	**10a**	34.20	ND	91.94	3.4
6c	ND	4.1	35.75	ND	**10b**	ND	>100	69.36	ND
6d	ND	1.6	47.85	ND	**10c**	ND	>100	48.66	ND
7a	50.40	ND	95.97	0.1	**10d**	ND	>100	99.99	0.04
					Tadalafil	ND	>100	99.99	0.004

[a] ND = not determined.

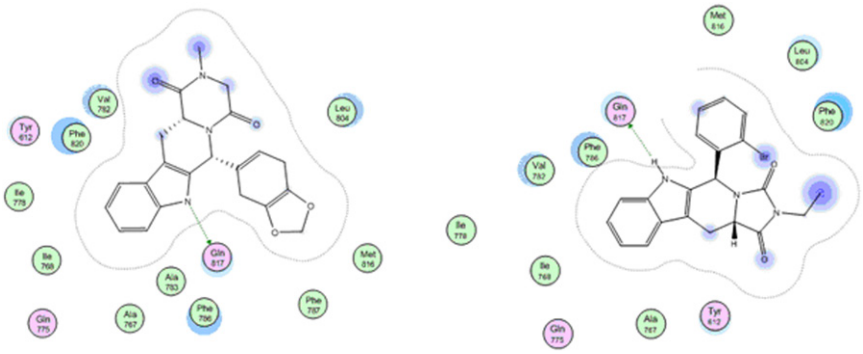


Fig. 3. Detailed view showing the docking and interaction of tadalfil (left), compound **4d** (right) with human PDE5(1UDU).

2-bromphenyl moiety. The more extensive interactions with the H pocket may be one of the reasons that compound **4d** could maintain high affinity to PDE5 without binding to the L region. Interestingly, tadalafil interacts in a very similar fashion, Fig. 3.

As per the anticancer activity, compounds (**6a**, **6c** and **6d**) out of the four open chain amides obtained by the acylation of the NH of the THBC derivatives with chloroacetyl chloride were found to be among the most active growth inhibitory agents with IC_{50} values of 1.6, 4.1 and 1.6 μM, respectively. However, this activity may be due to the alkyl halide nature and expected toxicity of the respective compounds.

For this class of compounds, there does not appear to be a correlation between the PDE5 inhibition and tumor cell growth inhibitory activity, as compound **4d** the most potent PDE5 inhibitor is not the most potent as compound **6d**, which was the most potent compound that inhibited HT-29 tumor cell growth, but did not show PDE5 inhibitory activity. This leads us to hypothesize that other PDE isozymes or possibly PDE5 splice variants may be involved.

4. Conclusion

Compound **4d** derived from L-Tryptophan was the most potent PDE5 inhibitor ($IC_{50} = 0.038$ μM) in a series of tadalafil analogues that were synthesized. The results suggest a new direction for further research to synthesize new PDE5 inhibitors with lower cost. The correlation between the PDE5 inhibitory activity and their tumor cell growth inhibitory activity needs further study on other PDE isozymes and splice variants.

5. Experimental section

5.1. Chemistry

All starting materials were commercially available and used without further purification. All reactions were carried out with the use of standard techniques under an inert atmosphere (N_2). The analytical thin-layer chromatography (TLC) was carried out on E. Merck 60-F_{254} precoated silica gel plates and components were usually visualized using UV light, Flash column chromatography was performed on silica gel 60 (E. Merck, 230–400 mesh). Melting points were determined on Buchi Melting Point apparatus and are uncorrected. Proton NMR (^{1}H NMR) and carbon NMR (^{13}C NMR) spectra were recorded at ambient temperature on Varian Mercury VX-300 MHz spectrometer using tetramethylsilane as internal standard, and proton chemical shifts are expressed in ppm in the indicated solvent. The following abbreviations are used for multiplicity of NMR signals: (s) singlet, (d) doublet, (t) triplet, (q) quadruplet, (dd) double doublet, (m) multiplet. The elemental analyses were performed by the Microanalytical Unit, Faculty of Science, Cairo University; and are within 0.4% of the theoretical value, unless stated otherwise.

5.1.1. Methyl 1-(2-bromophenyl)-2,3,4,9-tetrahydro-1H-β-carboline-3-carboxylate (**3a**–**d**)

The appropriate tryptophan methyl ester (3.42 g, 15.7 mmol) and 2-bromobenzaldehyde (3.19 g, 17.25 mmol) were dissolved in CH_2Cl_2 (10 mL) and cooled to 0 °C in an ice bath. To this solution was added dropwise TFA (1 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 × 10 mL). The organic layer was washed with water, brine, dried over anhydrous 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, to give first the appropriate *cis*-isomer followed by the *trans*-one.

5.1.2. (1R,3R) Methyl 1-(2-bromophenyl)-2,3,4,9-tetrahydro-1H-β-carboline-3-carboxylate (**3a**)

White solid; 33%; mp 105–106 °C; ^{1}H NMR (DMSO-d_6): δ 10.46 (s, 1H, N*H*), 7.7–7.68 (d, 1H, Ar), 7.46–7.43 (d, 1H, Ar), 7.33–7.20 (m, 4H, Ar), 7.04–6.93 (m, 2H, Ar), 5.64(s, 1H, C*H*Ph), 3.95–3.91(m, 1H, C*H*COOCH$_3$), 3.68 (s, 3H, OC*H*$_3$), 3.07–3.03 (dd, 1H, CH$_a$*H*$_b$), 2.89–2,75 (m, 1H, C*H*$_a$H$_b$); ^{13}C NMR (DMSO-d_6): δ 172.88, 140.75, 136.36, 134.16, 132.69, 130.74, 128.02, 126.44, 124.02, 120.94, 118.54, 117.70, 111.32, 107.65, 56.03 (C1), 51.89(C3), 51.85, 25.26; *m*/*z* 386 ($M^+ + 2$), *m*/*z* 384 (M^+, 100%); Anal. ($C_{19}H_{17}BrN_2O_2$) C: 59.23, H:4.45, N: 7.27; found: C:59.75, H:4.51, N:7.10.

5.1.3. (1S,3R) Methyl 1-(2-bromophenyl)-2,3,4,9-tetrahydro-1H-β-carboline-3-carboxylate (**3b**)

White solid; 41%; mp 104–105 °C; ^{1}H NMR (DMSO-d_6): δ 10.73 (s, 1H, N*H*), 7.69–7.62 (m, 1H, Ar), 7.49–7.47 (d, 1H, Ar), 7.25–7.22 (m, 4H, Ar), 7.05–6.98 (m, 2H, Ar), 5.67(s, 1H, C*H*Ph), 3.68–3.63 (m, 4H, C*H*COOCH$_3$ + OC*H*$_3$), 3.14–3.08 (m, 1H, CH$_a$*H*$_b$), 2.90–2,87 (dd, 1H, C*H*$_a$H$_b$); ^{13}C NMR (DMSO-d_6): δ 173.53, 141.11, 136.2, 132.93, 130.37, 129.53, 127.47, 126.35, 124.09, 121.12, 118.51, 117.81, 111.19, 107.86, 53.69 (C1), 51.83(C3), 51.36, 24.89; *m*/*z* 386 ($M^+ + 2$), *m*/*z* 384 (M^+, 100%); Anal. ($C_{19}H_{17}BrN_2O_2$) C: 59.23, H:4.45, N: 7.27; found; C:59.50, H:4.20, N:7.05.

5.1.4. (1S,3S) Methyl 1-(2-bromophenyl)-2,3,4,9-tetrahydro-1H-β-carboline-3-carboxylate (**3c**)

White solid; 41%; mp 104–105 °C; ^{1}H NMR (DMSO-d_6): δ 10.46 (s, 1H, N*H*),7.70–7.67 (d, 1H, Ar), 7.46–7.43 (d, 1H, Ar),7.33–7.20 (m, 5H, Ar + NH),7.04–6.93 (m, 2H, Ar),5.64 (s,1H, C*H*Ph), 3.93(m, 1H, C*H*COOCH$_3$), 3.68 (s, 3H, OC*H*$_3$), 3.07–3.03 (dd, 1H, CH$_a$*H*$_b$), 2.90–2.80 (m, 1H, C*H*$_a$H$_b$); ^{13}C NMR (DMSO-d_6): δ 172.80, 136.36, 134.07,

132.64, 130.74, 129.83, 128.01,126.43, 124.02, 120.93, 118.52, 117.67, 115.92, 111.30, 107.63, 56.61(C1), 51.85(C3), 25.20; *m/z* 386 ($M^+ + 2$), *m/z* 384.13 (M^+, 100%); Anal. ($C_{19}H_{17}BrN_2O_2$) C: 59.23, H:4.45, N: 7.27; found: C:59.02, H:4.50, N:7.09.

*5.1.5. (1R,3S) Methyl 1-(2-bromophenyl)-2,3,4,9-tetrahydro-1H-β-carboline-3-carboxylate (**3d**)*

White solid; 28%; mp 193–195 °C; ^{1}H NMR (DMSO-d_6): δ 10.71 (s, 1H, N*H*),7.69–7.67 (t, 1H, Ar), 7.48–7.46 (d, 1H, Ar), 7.24–7.21 (d, 3H, Ar), 7.07–6.95(m, 2H, Ar), 6.76–6.5(m, 1H, Ar), 5.66(s, 1H, C*H*Ph), 3.67–3.62(m, 4H, C*H*COOCH_3 + OCH_3), 3.13–3.07 (m, 1H, CH$_a$*H*$_b$), 2.89–2.80 (dd, 1H, C*H*$_a$H$_b$); ^{13}C NMR (DMSO-d_6): δ 173.51, 141.10, 136.19, 132.91, 130.35, 129.50, 127.45, 126.34, 124.06, 121.09, 118.48, 117.77, 111.16, 107.83, 53.64(C1), 51.79(C3), 51.35, 24.89; *m/z* 386 ($M^+ + 2$), *m/z* 384 (M^+, 100%); Anal. ($C_{19}H_{17}BrN_2O_2$) C: 59.23, H:4.45, N: 7.27; found: C: 58.94, H:4.58, N:7.11.

*5.1.6. 5-(2-Bromophenyl)-2-ethyl-5,6,11,11a-tetrahydro-1H-imidazo[1′,5′:1,6]pyrido[3,4-b]indole-1,3(2H)-dione (**4a**–**d**)*

Excess Ethyl isocyanate (135 μl, 1.6 mmol) was added to a well stirred solution of the appropriate beta-carboline **3a**–**d** (0.38 g, 1 mmol) in methyl ethyl ketone (10 mL) and the mixture was stirred at reflux for 16 h under a nitrogen atmosphere. The solvent was evaporated under reduced pressure, the residue was purified using preparative TLC and developed with CH_2Cl_2, the required band was stripped and dissolved in CH_2Cl_2 The solution was then filtered to remove the undesired silica, the filtrate was evaporated under reduced pressure to obtain pure powder of desired product.

*5.1.7. (5R,11aR)-5-(2-Bromophenyl)-2-ethyl-5,6,11,11a-tetrahydro-1H-imidazo[1′,5′:1,6] pyrido[3,4-b]indole-1, 3(2H)-dione (**4a**)*

Buff solid; 55%; mp 210–213 °C; ^{1}H NMR ($CDCl_3$): δ 7.85 (s, 1H, N*H*), 7.66–7.63 (m, 1H, Ar), 7.57–7.54 (m, 1H, Ar),7.25–7.06 (m, 6H, Ar), 6.49(s, 1H, C*H*Ph), 4.41–4.37(dd, 1H, C*H*C(O)N), 3.57–3.51 (m, 3H, NCH_2 + CH$_a$*H*$_b$), 3.10–3.01(m, 1H, C*H*$_a$H$_b$), 1.23–1.18 (t, 3H, CH_3); *m/z* 425 ($M^+ + 2$), *m/z* 423 (M^+), *m/z* 344 (100%); Anal. ($C_{21}H_{18}BrN_3O_2$) C:59.45, H:4.28, N:9.90; found: C:59.69, H:4.34, N:9.96.

*5.1.8. (5S,11aR)-5-(2-Bromophenyl)-2-ethyl-5,6,11,11a-tetrahydro-1H-imidazo[1′,5′:1,6] pyrido[3,4-b]indole-1,3(2H)-dione (**4b**)*

yellow solid; 52%; mp 137–138 °C; ^{1}H NMR ($CDCl_3$): δ 7.96 (s, 1H, N*H*), 7.67–7.65 (m, 1H, Ar), 7.64–7.55(m, 1H, Ar), 7.28–7.14(m, 6H, Ar), 6.74(s, 1H, C*H*Ph), 4.60–4.55(dd, 1H, C*H*C(O)N), 3.63–3.51(m, 3H, NCH_2 + CH$_a$*H*$_b$), 2.93–2.83 (m, 1H, C*H*$_a$H$_b$), 1.26–1,21 (t, 3H, CH_3); *m/z* 425 ($M^+ + 2$), *m/z* 423 (M^+), *m/z* 344 (100%); Anal. ($C_{21}H_{18}BrN_3O_2$) C:59.45, H:4.28, N:9.90; found: C:59.19, H:4.34, N:9.92.

*5.1.9. (5S,11aS)-5-(2-Bromophenyl)-2-ethyl-5,6,11,11a-tetrahydro-1H-imidazo[1′,5′:1,6] pyrido[3,4-b]indole-1, 3(2H)-dione (**4c**)*

Yellowish white solid; 63%; mp 213–214 °C; ^{1}H NMR ($CDCl_3$): δ 7.86 (s, 1H, N*H*), 7.63–7.54 (m, 2H, Ar), 7.27–7.03(m, 6H, Ar), 6.49(s, 1H, C*H*Ph), 4.41–4.37(dd, 1H, C*H*C(O)N), 3.57–3.49(m, 3H, NCH_2 + CH$_a$*H*$_b$), 3.10–3.01(m, 1H, C*H*$_a$H$_b$), 1.23–1,18 (t, 3H, CH_3); *m/z* 425 ($M^+ + 2$), *m/z* 423 (M^+), *m/z* 344 (100%); Anal. ($C_{21}H_{18}BrN_3O_2$) C:59.45, H:4.28, N:9.90; found: C:59.35, H:4.34, N:10.01.

*5.1.10. (5R,11aS)-5-(2-Bromophenyl)-2-ethyl-5,6,11,11a-tetrahydro-1H-imidazo[1′,5′:1,6] pyrido[3,4-b]indole-1, 3(2H)-dione (**4d**)*

White solid; 50%; mp 140–141 °C; ^{1}H NMR ($CDCl_3$): δ 7.90 (s, 1H, N*H*), 7.68–7.66 (m, 1H, Ar), 7.53–7.52 (m, 1H, Ar),7.28–7.14 (m, 6H, Ar), 6.74 (s, 1H, C*H*Ph), 4.61–4.56(dd, 1H, C*H*C(O)N), 3.63–3.52 (m, 3H, NCH_2 + CH$_a$*H*$_b$), 2.93–2.84 (m, 1H, C*H*$_a$H$_b$), 1.27–1,22 (t, 3H, CH_3); *m/z* 425 ($M^+ + 2$), *m/z* 423 (M^+), *m/z*, 344 (100%); Anal. ($C_{21}H_{18}BrN_3O_2$) C:59.45, H:4.28, N:9.90; found: C:59.39, H:4.19, N:9.88.

*5.1.11. 5-(2-Bromophenyl)-2-ethyl-1-oxo-5,6,11,11a-tetrahydro-1H-imidazo[1′,5′:1,6] pyrido[3,4-b]indole-3(2H)-thione (**5a**, **5b**)*

Excess Ethyl isothiocyanate (135 μl, 1.5 mmol) was added to a well stirred solution of the appropriate beta-carboline **3a**–**d** (0.38 g, 1 mmol) in methyl ethyl ketone (10 mL) and the mixture was stirred at reflux for 16 h under a nitrogen atmosphere. The solvent was evaporated under reduced pressure, the residue was purified using preparative TLC and developed with CH_2Cl_2, the required band was stripped and dissolved in CH_2Cl_2 The solution was then filtered to remove the undesired silica, the filtrate was evaporated under reduced pressure to obtain pure powder of desired product.

*5.1.12. (5S,11aR)-5-(2-Bromophenyl)-2-ethyl-1-oxo-5,6,11,11a-tetrahydro-1H-imidazo [1′,5′:1,6]pyrido[3,4-b]indole-3(2H)-thione (**5a**)*

Yellow white solid; 56%; mp 228–229 °C; ^{1}H NMR ($CDCl_3$): δ 8.10 (s, 1H, N*H*), 7.72–7.70 (d, 1H, Ar), 7.54–7.51 (d, 1H, Ar), 7.33–7.13 (m, 7H, Ar + C*H*Ph), 4.82–4.76 (dd, 1H, C*H*C(O)N), 4.01–3.94 (m, 2H, NCH_2), 3.59–3.53 (m,1H CH$_a$*H*$_b$), 2.95–2.91 (m, 1H, C*H*$_a$H$_b$), 1.32–1.28 (t, 3H, CH_3); ^{13}C NMR (DMSO-d_6): δ 180.65, 172.82, 138.27, 137.03, 132.96, 131.00, 129.90, 129.57, 128.23, 125.48, 123.53, 121.75, 119.01, 118.20, 111.76, 105.09, 58.03, 55.91, 35.84, 22.47, 12.71; *m/z* 441 ($M^+ + 2$), 439 (M^+), *m/z* 360 (100%); Anal. ($C_{21}H_{18}BrN_3OS$) C:57.28, H:4.12, N:9.54; found: C:57.19, H:4.22, N:9.46.

*5.1.13. (5R-11aS)-5-(2-Bromophenyl)-2-ethyl-1-oxo-5,6,11,11a-tetrahydro-1H-imidazo [1′,5′:1,6]pyrido[3,4-b]indole-3(2H)-thione (**5b**)*

Yellowish white solid; 45%; mp 226–227 °C; ^{1}H NMR ($CDCl_3$): δ 8.09 (s, 1H, N*H*), 7.72–7.70 (d, 1H, Ar), 7.54–7.52 (d, 1H, Ar), 7.30–7.13 (m, 7H, Ar + C*H*Ph), 4.82–4.77 (dd, 1H, C*H*C(O)N), 4.01–3.94 (m, 2H, NCH_2), 3.61–3.49 (m,1H, CH$_a$*H*$_b$), 2.95–2.91 (m, 1H, C*H*$_a$H$_b$), 1.32–1,27 (t, 3H, CH_3); ^{13}C NMR (DMSO-d_6): δ d 180.65, 172.82, 138.29, 137.04, 132.95, 131.00, 129.89, 129.58, 128.22, 125.48, 123.54, 121.74, 119.00, 118.20, 111.76, 105.09, 58.03, 55.91, 35.84, 22.48, 12.72; *m/z* 441 ($M^+ + 2$), 439.04 (M^+), *m/z* 360 (100%); Anal. ($C_{21}H_{18}BrN_3OS$) C:57.28, H:4.12,N: 9.54; found: C:57.18, H:4.20, N:9.50.

*5.1.14. General procedure for methyl 1-(2-Bromophenyl)-2-(2-chloroacetyl)-2,3,4,9-tetrahydro-1H-β-carboline-3-carboxylate (**6a**–**d**)*

To a stirred solution of the appropriate β-carboline **3a**–**d** (2.195 g, 5.7 mmol) and $NaHCO_3$ (0.575 g, 6.89 mmol) in $CHCl_3$ (40 mL) was added dropwise chloroacetyl chloride (1.09 mL, 13.69 mmol) under ice cooling. The mixture was then stirred at room temperature under a nitrogen atmosphere for 1 h. The mixture was diluted with CH_2Cl_2, washed with a solution of $NaHCO_3$, brine, dried over Na_2SO_4, and evaporated under reduced pressure. The residue was then crystallized from diethyl ether.

*5.1.15. Methyl (1R,3R)-1-(2-bromophenyl)-2-(2-chloroacetyl)-2,3,4,9-tetrahydro-1H-β-carboline-3-carboxylate (**6a**)*

White solid; 88%; mp 113–115 °C; ^{1}H NMR ($CDCl_3$): δ 8.00 (br s, 1H, N*H*), 7.88–7.85 (d, 1H, Ar), 7.57–7.54 (d, 1H, Ar), 7.31–7.14 (m, 6H, Ar), 6.73 (s,1H, C*H*Ph), 5.18–5.14 (m, 1H, C*H*COOCH$_3$), 4.19–4.15(m, 2H, COCH_2Cl), 3.64 (br s, 3H, OCH_3), 3.37–3.28 (m, 2H, CH_2); *m/z* 462 ($M^+ + 2$), *m/z* 460(M^+), *m/z* 385 (100%); Anal. ($C_{21}H_{18}BrClN_2O_3$). C:54.63, H:3.93, N:6.07; found: C: 54.16, H:4.02, N:6.06.

*5.1.16. Methyl (1S,3R)-1-(2-bromophenyl)-2-(2-chloroacetyl)-2,3,4,9-tetrahydro-1H-β-carboline-3-carboxylate (**6b**)*

White solid; 74%; mp 132–135 °C; ^{1}H NMR ($CDCl_3$): δ 8.22 (s, 1H, N*H*), 7.61–7.50 (m, 2H, Ar), 7.28–7.08 (m, 6H, Ar), 6.65 (s,1H, C*H*Ph),

5.31 (br s, 1H, C*H*COOCH$_3$), 4.19–4.06 (m, 2H, COC*H*$_2$Cl), 3.82–3.72 (m, 5H, OC*H*$_3$ + C*H*$_2$); *m/z* 462 ($M^+ + 2$), *m/z* 460(M^+), *m/z* 383 (100%); Anal. ($C_{21}H_{18}BrClN_2O_3$). C:54.63, H:3.93, N:6.07; found: C:54.33, H:3.92, N:5.99.

*5.1.17. Methyl (1S,3S)-1-(2-bromophenyl)-2-(2-chloroacetyl)-2,3,4,9-tetrahydro-1H-β-carboline-3-carboxylate (**6c**)*

White solid; 83%; mp 112–113 °C; ^{1}H NMR ($CDCl_3$): δ 7.97 (br s, 1H, N*H*), 7.68–7.65 (d, 1H, Ar), 7.57–7.54 (d, 1H, Ar),7.31–7.12 (m, 6H, Ar), 6.73 (s,1H, C*H*Ph), 5.18–5.14(m,1H, C*H*COOCH$_3$), 4.19–4.15 (m, 2H, COC*H*$_2$Cl), 3.64 (br s, 3H, OC*H*$_3$), 3.37–3.26 (m, 2H, C*H*$_2$); *m/z* 462 ($M^+ + 2$), *m/z* 460(M^+), *m/z* 383 (100%); Anal. ($C_{21}H_{18}BrClN_2O_3$). C:54.63, H:3.93, N:6.07; found: C:54.66, H:3.89, N:6.03.

*5.1.18. Methyl (1R,3S)-1-(2-bromophenyl)-2-(2-chloroacetyl)-2,3,4,9-tetrahydro-1H-β-carboline-3-carboxylate (**6d**)*

White solid; 81%; mp 132–133 °C; ^{1}H NMR ($CDCl_3$): δ 8.22 (s, 1H, N*H*), 7.62–7.50 (m, 2H, Ar), 7.29–7.08 (m, 6H, Ar), 6.65 (s,1H, C*H*Ph), 5.31 (br s,1H, C*H*COOCH$_3$), 4.19–4.06 (m, 2H, COC*H*$_2$Cl), 3.74–3.65 (m, 5H, OC*H*$_3$ + C*H*$_2$); *m/z* 462 ($M^+ + 2$), *m/z* 460(M^+), *m/z* 385 (100%); Anal. ($C_{21}H_{18}BrClN_2O_3$). C:54.63, H:3.93, N:6.07; found: C: 54.55, H:3.90, N:6.13.

*5.1.19. Preparation of 2-alkyl or 2-benzyl-6-(2-Bromophenyl)-2,3,6,7,12,12a-hexahydropyrazino [1′,2′:1,6]pyrido[3,4-b]indole-1,4-dione (**7a**–**10d**)*

A solution of the of the appropriate chloroacetyl derivative **6a**–**d** (1.4 mmol, 1 equiv) and the appropriate amine, namely methylamine (**7a**–**d**); ethylamine (**8a**–**d**), butylamine (**9a**–**d**); benzylamine (**10a**–**d**) (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, using CH_2Cl_2 as an eluent.

*5.1.20. (6R,12aR)-6-(2-Bromophenyl)-2-methyl-2,3,6,7,12,12a-hexahydropyrazino [1′,2′:1,6]pyrido[3,4-b]indole-1,4-dione (**7a**)*

Yellowish white solid; 34%; mp 175–178 °C; ^{1}H NMR ($CDCl_3$): δ 8.27 (s, 1H, N*H*), 7.59–7.56 (m, 2H, Ar), 7.31–7.13 (m, 6H, Ar), 6.72(s, 1H, C*H*Ph), 4.43–4.34(m, 1H, C*H*C(O)N), 4.11–3.92 (m, 3H, C*H*$_2$C(O)N + CH$_a$*H*$_b$), 3.32–3.22(m, 1H, C*H*$_a$H$_b$), 3.09(s,3H, NC*H*$_3$); *m/z* 425 ($M^+ + 2$), *m/z* 423 (M^+), *m/z* 344 (100%) *m/z* 423; Anal. ($C_{21}H_{18}BrN_3O_2$). C:59.45, H:4.28, N:9.90; found: C:59.44, H:4.19, N:9.82.

*5.1.21. (6S,12aR)-6-(2-Bromophenyl)-2-methyl-2,3,6,7,12,12a-hexahydropyrazino [1′,2′:1,6]pyrido[3,4-b]indole-1,4-dione (**7b**)*

White solid; 29%; mp 202–204 °C; ^{1}H NMR ($CDCl_3$): δ 8.15 (s, 1H, N*H*), 7.66–7.63 (m, 1H, Ar), 7.54–7.52 (d, 1H, Ar), 7.33–7.13 (m, 6H, Ar), 6.82 (brs,1H, C*H*PhAr), 4.36–4.31 (dd, 1H, C*H*C(O)N), 4.14–4.01 (m, 3H, C*H*$_2$C(O)N + CH$_a$*H*$_b$), 3.52–3.46 (dd, 1H, C*H*$_a$H$_b$), 3.01 (s, 3H, NC*H*$_3$); *m/z* 425 ($M^+ + 2$), *m/z* 423 (M^+), *m/z* 344 (100%); Anal. ($C_{21}H_{18}BrN_3O_2$) C:59.45, H:4.28, N:9.90; found: C:59.61, H:4.29, N:9.82.

*5.1.22. (6S,12aS)-6-(2-Bromophenyl)-2-methyl-2,3,6,7,12,12a-hexahydropyrazino [1′,2′:1,6]pyrido[3,4-b]indole-1,4-dione (**7c**)*

White powder; 32%; mp 177–178 °C; ^{1}H NMR ($CDCl_3$): δ 8.24 (s, 1H, N*H*), 7.58–7.54 (m, 2H, Ar), 7.30–7.04 (m, 6H, Ar), 6.70 (s, 1H, C*H*Ph), 4.41–4.36 (m, 1H, C*H*C(O)N), 4.10–3.91 (m, 3H, C*H*$_2$C(O)N + CH$_a$*H*$_b$), 3.31–3.21 (m, 1H, C*H*$_a$H$_b$), 3.08 (s, 3H, NC*H*$_3$); *m/z* 425 ($M^+ + 2$), *m/z* 423 (M^+), *m/z* 344 (100%); Anal. ($C_{21}H_{18}BrN_3O_2$) C:59.45, H:4.28, N:9.90; found: C:59.44, H:4.27, N:9.79.

*5.1.23. (6R,12aS)-6-(2-Bromophenyl)-2-methyl -2,3,6,7,12,12a-hexahydropyrazino [1′,2′:1,6]pyrido[3,4-b]indole-1,4-dione (**7d**)*

Greenish white solid; 37%; mp 201–204 °C; ^{1}H NMR ($CDCl_3$): δ 8.07 (s, 1H, N*H*), 7.63–7.52 (m, 2H, Ar), 7.27–7.14 (m,6H, Ar), 6.85 (brs,1H, C*H*PhAr), 4.37–4.32 (dd,1H, C*H*C(O)N), 4.14–4.01 (m, 3H, C*H*$_2$C(O)N + CH$_a$*H*$_b$), 3.53–3.46 (dd, 1H, C*H*$_a$H$_b$), 3.01 (s, 3H, NC*H*$_3$); *m/z* 425 ($M^+ + 2$), *m/z* 423 (M^+), *m/z* 344 (100%); Anal. ($C_{21}H_{18}BrN_3O_2$) C:59.45, H:4.28, N:9.90; found: C:59.31, H:4.32, N:9.96.

*5.1.24. (6R,12aR)-6-(2-Bromophenyl)-2-ethyl-2,3,6,7,12,12a-hexahydropyrazino [1′,2′:1,6]pyrido[3,4-b]indole-1,4-dione (**8a**)*

Buff solid; 30%; mp 121–122 °C; ^{1}H NMR ($CDCl_3$): δ 8.28 (s, 1H, N*H*), 7.58–7.55 (m, 2H, Ar), 7.31–7.05 (m,6H, Ar), 6.73 (s, 1H, C*H*Ph), 4.42–4.32 (m, 1H, C*H*C(O)N), 4.16–3.96 (m, 3H, C*H*$_2$C(O)N + CH$_a$*H*$_b$), 3.46–3.32 (m, 1H, C*H*$_a$H$_b$), 3.03–2.93 (m, 2H, NC*H*$_2$), 1.26–1.21 (t, 3H, C*H*$_3$); *m/z* 439 ($M^+ + 2$), *m/z* 437 (M^+), *m/z* 358 (100%) *m/z* 437; Anal. ($C_{22}H_{20}BrN_3O_2$) C: 60.28, H:4.60, N:9.59; found: C:60.40,H:4.57, N:9.48.

*5.1.25. (6S,12aR)-6-(2-Bromophenyl)-2-ethyl -2,3,6,7,12,12a-hexahydropyrazino[1′,2′:1,6]pyrido[3,4-b]indole-1,4-dione (**8b**)*

White solid; 28%; mp 180–182 °C; ^{1}H NMR (DMSO-d_6): δ 7.95 (s, 1H, N*H*), 7.68–7.65 (m, 1H, Ar), 7.55–7.53 (d, 1H, Ar), 7.34–7.15 (m, 6H, Ar), 6.85 (s, 1H, C*H*Ph), 4.37–4.32 (dd, 1H, C*H*C(O)N), 4.15–4.01 (m, 3H, C*H*$_2$C(O)N + CH$_a$*H*$_b$), 3.52–3.47 (dd, 1H, C*H*$_a$H$_b$), 3.03–2.93 (m, 2H, NC*H*$_2$), 1.23–1.19 (t, 3H, C*H*$_3$); *m/z* 439 ($M^+ + 2$), *m/z* 437 (M^+), *m/z* 358 (100%); Anal. ($C_{22}H_{20}BrN_3O_2$). C: 60.28, H:4.60, N:9.59; found: C:60.15, H:4.50, N:9.62.

*5.1.26. (6S,12aS)-6-(2-Bromophenyl)-2-ethyl -2,3,6,7,12,12a-hexahydropyrazino[1′,2′:1,6]pyrido[3,4-b]indole-1,4-dione (**8c**)*

Greenish white solid; 25%; mp 125–127 °C; ^{1}H NMR ($CDCl_3$): δ 8.25 (s, 1H, N*H*), 7.64–7.52(m, 2H, Ar), 7.25–7.11(m,6H, Ar),6.72 (s,1H, C*H*Ph),4.41–4.35 (dd,1H, C*H*C(O)N),4.15–4.01 (m,3H, C*H*$_2$C(O)N + CH$_a$*H*$_b$),3.52–3.47 (dd, 1H, C*H*$_a$H$_b$), 3.03–2.93 (m, 2H, NC*H*$_2$), 1.24–1.19 (t, 3H, C*H*$_3$); *m/z* 439 ($M^+ + 2$), *m/z* 437 (M^+), *m/z* 358 (100%) *m/z* 437; Anal. ($C_{22}H_{20}BrN_3O_2$). C: 60.28, H:4.60, N:9.59; found: C:60.51, H:4.61, N:9.56.

*5.1.27. (6R,12aS)-6-(2-Bromophenyl)-2-ethyl -2,3,6,7,12,12a-hexahydropyrazino[1′,2′:1,6]pyrido[3,4-b]indole-1,4-dione (**8d**)*

Yellowish white solid; 30%; mp 179–180 °C; ^{1}H NMR ($CDCl_3$): δ 7.99 (s, 1H, N*H*), 7.66–7.51 (m, 2H, Ar), 7.32–7.13 (m, 6H, Ar), 6.86 (s,1H, C*H*Ph), 4.35–4.29 (dd, 1H, C*H*C(O)N), 4.12–3.98 (m, 3H, C*H*$_2$C(O)N + CH$_a$*H*$_b$), 3.51–3.44 (dd, 1H, C*H*$_a$H$_b$), 3.00–2.91 (m, 2H, NC*H*$_2$), 1.23–1.17 (t, 3H, C*H*$_3$); *m/z* 439 ($M^+ + 2$), *m/z* 437 (M^+), *m/z* 358 (100%) *m/z* 437; Anal. ($C_{22}H_{20}BrN_3O_2$) C: 60.28, H:4.60, N:9.59; found: C:60.40,H:4.62, N:9.60.

*5.1.28. (6R,12aR)-6-(2-Bromophenyl)-2-butyl-2,3,6,7,12,12a-hexahydropyrazino [1′,2′:1,6]pyrido[3,4-b]indole-1,4-dione (**9a**)*

Greenish white solid; 37%; mp 129–131 °C; ^{1}H NMR ($CDCl_3$): δ 8.27 (s, 1H, N*H*), 7.56–7.53 (m, 2H, Ar), 7.30–7.11(m, 6H, Ar), 6.72 (s, 1H, C*H*Ph), 4.41–4.36 (dd,1H, C*H*C(O)N), 4.14–4.04 (m, 2H, C*H*$_2$C(O)N), 3.81–3.74 (dd, 1H, CHa*H*$_b$), 3.55–3.46 (m, 1H, C*H*$_a$H$_b$), 3.31–3.22 (t, 2H, NC*H*$_2$), 1.64–1.52 (m, 2H, C*H*$_2$CH$_2$CH$_3$), 1.42–1.33 (m, 2H, C*H*$_2$CH$_3$), 0.98–0.94 (t, 3H, C*H*$_3$); *m/z* 467 ($M^+ + 2$), *m/z* 465 (M^+), *m/z* 386 (100%) *m/z* 465; Anal. ($C_{24}H_{24}BrN_3O_2$) C:61.81, H:5.19, N:9.01; found: C:62.01, H:5.20, N:9.04.

*5.1.29. (6S,12aR)-2-Butyl-6-(2-bromophenyl)-2,3,6,7,12,12a-hexahydropyrazino[1′,2′:1,6]pyrido[3,4-b]indole-1,4-dione (**9b**)*

Yellowish white solid; 36%; mp 135–136 °C; ^{1}H NMR ($CDCl_3$): δ 7.99 (s, 1H, N*H*), 7.65–7.62 (m, 1H, Ar), 7.53–7.51 (d, 1H, Ar) 7.32–7.13 (m, 6H, Ar), 6.82 (s, 1H, C*H*Ph), 4.33–4.39 (dd, 1H, C*H*C(O)N), 4.12–

3.98 (m, 2H, CH_2C(O)N), 3.54–3.44 (m, 2H, NCH_2), 3.32–3.22 (m, 1H, CH$_a$$H_b$), 3.00–2.91 (m, 1H, C$H_aH_b$), 1.60–1.52 (m, 2H, C$H_2CH_2CH_3$), 1.38–1.25 (m, 2H, C$H_2CH_3$), 0.97–0.93 (t, 3H, C$H_3$); *m/z* 467($M^+ + 2$), *m/z* 465($M^+$), *m/z* 217 (100%); Anal. ($C_{24}H_{24}BrN_3O_2$) C:61.81, H:5.19, N:9.01; found: C:61.91, H:5.19, N:9.06.

*5.1.30. (6S,12aS)-6-(2-Bromophenyl)-2-butyl-2,3,6,7,12,12a-hexahydropyrazino [1′,2′:1,6]pyrido[3,4-b]indole-1,4-dione (**9c**)*

Yellowish white solid; 39%; mp127–130 °C; ^{1}H NMR ($CDCl_3$): δ 8.27 (s, 1H, N*H*), 7.57–7.54 (m, 2H, Ar), 7.28–7.11 (m, 6H, Ar),6.72 (s, 1H, C*H*Ph), 4.41–4.38 (dd, 1H, C*H*C(O)N), 4.14–3.94 (m, 2H, CH_2C(O)N), 3.81–3.44(m, 2H, NCH_2), 3.46–3.36(m, 1H, CH$_a$$H_b$),3.28–3.20 (m, 1H, C$H_aH_b$), 1.64–1.54 (m, 2H, C$H_2CH_2CH_3$), 1.40–1.33 (m, 2H, C$H_2CH_3$), 0.99–0.94 (t, 3H, C$H_3$); *m/z* 467($M^+ + 2$), *m/z* 465($M^+$), *m/z* 217 (100%); Anal. ($C_{24}H_{24}BrN_3O_2$) C:61.81, H:5.19, N:9.01; found: C:61.80, H:5.14, N:8.97.

*5.1.31. (6R,12aS)-6-(2-Bromophenyl)-2-butyl-2,3,6,7,12,12a-hexahydropyrazino [1′,2′:1,6]pyrido[3,4-b]indole-1,4-dione (**9d**)*

Yellowish white solid; 31%; mp 135–137 °C; ^{1}H NMR ($CDCl_3$): δ 7.98 (s, 1H, N*H*), 7.71–7.53 (m, 2H, Ar), 7.29–7.13 (m, 6H, Ar), 6.82 (s,1H, C*H*Ph), 4.34–4.29 (dd, 1H, C*H*C(O)N), 4.12–3.97 (m, 2H, CH_2C(O)N), 3.61–3.44 (m, 2H, NCH_2), 3.34–3.21 (m, 1H, CH$_a$$H_b$), 3.00–2.90 (m, 1H, C$H_aH_b$), 1.57–1.47 (m, 2H, C$H_2CH_2CH_3$), 1.35–1.32 (m, 2H, C$H_2CH_3$), 0.98–0.91 (t, 3H, C$H_3$); *m/z* 467 ($M^+ + 2$), *m/z* 465($M^+$), *m/z* 386 (100%); Anal. ($C_{24}H_{24}BrN_3O_2$) C:61.81, H:5.19, N:9.01; found: C:61.77, H:5.23, N:9.06.

*5.1.32. (6R,12aR)-2-Benzyl-6-(2-bromophenyl)-2,3,6,7,12,12a-hexahydropyrazino [1′,2′:1,6]pyrido[3,4-b]indole-1,4-dione (**10a**)*

White solid; 23%; mp 135–137 °C; ^{1}H NMR ($CDCl_3$): δ 8.26 (s, 1H, N*H*), 7.59–7.56 (m, 2H, Ar), 7.56–7.21 (m, 11H, Ar), 6.71 (s, 1H, C*H*Ph), 4.91–4.83 (m, 1H, C*H*C(O)N), 4.55–4.50 (m, 2H, CH_2Ph), 3.95–3.81 (m, 3H, CH_2C(O)N + CH$_a$$H_b$), 3.38–3.28 (m, 1H, C$H_aH_b$); *m/z* 501 ($M^+ + 2$), *m/z* 499($M^+$), *m/z* 420 (100%); Anal. ($C_{27}H_{22}BrN_3O_2$) C:64.81, H:4.43, N:8.40; found: C:64.98, H:4.52, N:8.49.

*5.1.33. (6S,12aR)-2-Benzyl-6-(2-bromophenyl)-2,3,6,7,12,12a-hexahydropyrazino [1′,2′:1,6]pyrido[3,4-b]indole-1,4-dione (**10b**)*

Yellowish white solid; 34%; mp 124–127 °C; ^{1}H NMR ($CDCl_3$): δ 8.03 (s, 1H, N*H*), 7.67–7.54 (m, 2H, Ar), 7.38–7.14(m, 11H, Ar), 6.84 (s, 1H, C*H*Ph), 4.90–4.85 (m, 1H, CHC(O)N), 4.43–4.38 (m, 2H, CH_2Ph), 3.95–3.94 (m, 2H, CH_2–C(O)N), 3.57–3.51 (dd, 1H, CH$_a$$H_b$), 3.04–2.94 (m, 1H, CH$_aH_b$); MS: *m/z* 501 ($M^+ + 2$), *m/z* 499 ($M^+$), *m/z* 420 (100%); Anal. ($C_{27}H_{22}BrN_3O_2$). C:64.81, H:4.43, N:8.40; found: C:64.98, H:4.57, N:8.38.

*5.1.34. (6S,12aS)-2-Benzyl-6-(2-bromophenyl)-2,3,6,7,12,12a-hexahydropyrazino [1′,2′:1,6]pyrido[3,4-b]indole-1,4-dione (**10c**)*

Yellowish white solid; 32%; mp 138–140 °C; ^{1}H NMR ($CDCl_3$): δ 8.26 (s, 1H, N*H*), 7.59–7.50 (m, 2H, Ar), 7.16–7.03 (m, 11H, Ar), 6.70 (s, 1H, C*H*Ph), 4.93–4.86 (m, 1H, C*H*C(O)N), 4.55–4.42(m, 2H, CH_2Ph), 4.01–3.81 (m, 3H, CH_2C(O)N + CH$_a$$H_b$), 3.38–3.28 (m, 1H, C$H_aH_b$); *m/z* 501 ($M^+ + 2$), *m/z* 499 ($M^+$), *m/z* 420 (100%); Anal. ($C_{27}H_{22}BrN_3O_2$). C:64.81, H:4.43, N:8.40; found: C:65.08, H:4.32, N:8.40.

*5.1.35. (6R,12aS)-2-Benzyl-6-(2-bromophenyl)-2,3,6,7,12,12a-hexahydropyrazino [1′,2′:1,6]pyrido[3,4-b]indole-1,4-dione (**10d**)*

Yellowish white solid; 25%; mp 128–130 °C; ^{1}H NMR ($CDCl_3$): δ 7.95 (s, 1H, N*H*), 7.63–7.60 (m, 2H, Ar), 7.34–7.13 (m, 11H, Ar), 6.82 (s, 1H, C*H*Ph), 4.89–4.84 (m, 1H, C*H*C(O)N), 4.56–4.33 (m, 2H, CH_2Ph), 4.01–3.87 (m, 2H, CH_2C(O)N), 3.71–3.67 (dd, 1H, CHaH_b), 3.35–3.28 (m, 1H, CH_aH$_b$); *m/z* 501 ($M^+ + 2$), *m/z* 499 (M^+), *m/z* 420 (100%); Anal. ($C_{27}H_{22}BrN_3O_2$) C:64.81, H:4.43, N:8.40; found: C:64.78, H:4.42, N:8.44.

5.2. Biology

5.2.1. Cell cultures

HT-29 tumor cells were obtained from ATCC. They were grown under standard cell culture conditions at 37 °C in a humidified atmosphere with 5% CO_2. Cells were grown in RPMI 1640 supplemented with 5% fetal bovine serum. 2 mM glutamine, 100 units/mL penicillin, 100 units/mL streptomycin, and 0.25 μg/mL amphotericin. Cells were harvested at 70–90% confluence with trypsin/EDTA and used immediately Cell count and viability was determined by Trypan blue staining followed by hemocytometry. Only cultures displaying >95% cell viability were used for experiments [6].

5.2.2. Growth assays

Tissue culture treated microtiter 96-well plates were seeded at a density of 5000 cells/well. The plates were incubated for 18–24 h prior to any treatment. All test compounds were solubilized in 100% DMSO and diluted with media to obtain a final DMSO concentration of 0.1%. Cells were dosed. Cell viability was measured 72 h after treatment by the Cell Titer Glo Assay (Promega), which is a luminescent assay that is an indicator of live cells as a function of metabolic activity and ATP content. The assay was performed according to manufacturer's specifications. Luminescence was measured by a Perkin Elmer Victor® multi-label plate reader [6].

5.2.3. Phosphodiesterase assay

5 μ/mL of purified PDE 5 (BPS Biosciences) was added to the wells of black 96-well non-binding plates. Immediately, the protein was treated with compound or vehicle control and 50 nM TAMRA-cGMP (Molecular Devices) were added to each assay well. The plates were incubated for 1.5 h at 30 °C.After incubation, IMAP FP Phosphodiesterase Evaluation Assay (Molecular Devices) binding reagent was added to each well and the plates were incubated for an additional 30 min at 30 °C. FP was measured according to manufacturer's specifications using a Biotek Synergy 4 plate reader.

5.2.4. Experimental design and data analysis

Drug effects on tumor cell growth and PDE activity were measured and potency was expressed by an IC_{50} value (50% inhibitory concentration).For growth assays, the IC_{50} value was determined by testing a range of 8 concentrations with at least four replicates per concentration. For enzyme assays, the IC_{50} value was determined by testing a range of 10 concentrations with at least two replicates per concentration. Dose–response curves were analyzed using PrismTM 4 software (GraphPad) to calculate IC_{50} values using a four parameter logistic equation. All *in vitro* experiments involved dose–response analysis, which was repeated at least twice to confirm reproducibility of IC_{50} values.

5.3. Molecular modeling

Compound **4d** was drawn and subjected to energy minimization by the AM1 procedure, the partial charges are also calculated. The human PDE5 X-ray crystal structure complexed with tadalafil (PDB code: 1UDU) was used for docking experiments. One of the protein chain, metals and the old ligands were deleted. The docking was done with the default settings of the MOE-DOCK [16] as follow: The option: Rotate Bonds was selected to give flexible ligand-rigid receptor docking. The scoring function was London dG with a replacement of Alpha Triangle.

Acknowledgment

This work benefited from partial financial support from the Faculty of Postgraduate Studies, the German University in Cairo.

The first author is grateful to the Alexander von Humboldt foundation, Germany for sponsoring a postdoctoral fellowship that helps in accomplishing this work.

References

[1] C. Lugnier, Pharmacol. Ther. 109 (2006) 366–398.
[2] A.T. Bender, J.A. Beavo, Pharmacol. Rev. 58 (2006) 488–520.
[3] G.V. Frajese, F. Pozzi, J. Endocrinol. Invest. 28 (2005) 45–50.
[4] G.A. Piazza, W.J. Thompson, R. Pamukcu, H.W. Alila, C.M. Whitehead, L. Liu, J. Fetter, W.E. Gresh Jr., A.J. Klein-Szanto, D.R. Farnell, I. Eto, C.J. Grubbs, Cancer Res. 61 (2001) 3961–3968.
[5] B. Zhu, L. Vemavarapu, W.J. Thompson, S.J. Strada, J. Cell. Biochem. 94 (2005) 336–350.
[6] W.J. Thompson, G.A. Piazza, H. Li, L. Liu, J. Fetter, B. Zhu, G. Sperl, D. Ahnen, R. Pamukcu, Cancer Res. 60 (2000) 3338–3342.
[7] L. Liu, H. Li, T. Underwood, M. Lloyd, M. David, G. Sperl, R. Pamukcu, W.J. Thompson, J. Pharmacol. Exp. Ther. 299 (2001) 583–592.
[8] A. Donodoni, D. Perrone, Org. Synth. 10 (2004) 320.
[9] F. Ungemach, D. Soerens, R. Weber, M. Dipierro, O. Campos, P. Mokry, J.M. Cook, J. Am. Chem. Soc. 102 (1980) 6976–6984.
[10] J. Sandrin, D. Soerens, J.M. Cook, Heterocycles 4 (1976) 1249–1255.
[11] N. Sunder-Plassmann, V. Sarli, M. Gartner, M. Utz, J. Seiler, S. Huemmer, T.U. Mayer, T. Surrey, A. Giannis, Bioorg. Med. Chem. 13 (2005) 6094–6111.
[12] A. Daugan, P. Grondin, C. Ruault, A.C. Le Monnier de Gouville, H. Coste, J. Kirilovsky, F. Hyafil, R. Labaudiniere, J. Med. Chem. 46 (2003) 4525–4532.
[13] M.L. Lopez-Rodrlguez, M.J. Morcillo, M. Garrido, B. Benhamtu, P. Perez, J.G. de la Campa, J. Org. Chem. 59 (1994) 1583–1585.
[14] M.L. Lopez Rodriguez, M.J. Morcillo, F. Benito, B. Benhamu, E. Fernandez, M. Garrido, L. Orensanz, Chem. Pharm. Bull. (Tokyo) 42 (1994) 2108–2112.
[15] A. Daugan, P. Grondin, C. Ruault, A.C. Le Monnier de Gouville, H. Coste, J.M. Linget, J. Kirilovsky, F. Hyafil, R. Labaudiniere, J. Med. Chem. 46 (2003) 4533–4542.
[16] MOE, Chemical Computing Group Inc.: Montreal, Available at: http://www.chemcomp/com.