Bioorganic & Medicinal Chemistry Letters xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Design, synthesis and evaluation of N^2,N^4-diaminoquinazoline based inhibitors of phosphodiesterase type 5

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ARTICLE INFO

Keywords:
Drug design
Phosphodiesterase type 5
Quinazoline analogs
Solubility

ABSTRACT

We describe the design, synthesis and evaluation of a series of N^2,N^4-diaminoquinazoline analogs as PDE5 inhibitors. Twenty compounds were prepared and these were assessed in terms of their PDE5 and PDE6 activity, ex-vivo vasodilation response, mammalian cytotoxicity and aqueous solubility. Molecular docking was used to determine the binding mode of the series and this was demonstrated to be consistent with the observed SAR. Compound **15** was the most active PDE5 inhibitor (IC_{50} = 0.072 ± 0.008 μM) and exhibited 4.6-fold selectivity over PDE6. Ex-vivo assessment of **15** and **22** in a rat pulmonary artery vasodilation model demonstrated EC_{50}s of 1.63 ± 0.72 μM and 2.28 ± 0.74 μM respectively.

Approximately 17.7 million people die each year as a result of cardiovascular diseases (CVDs) according to the World Health Organization (WHO).[1] CVDs are disorders of the heart and blood vessels that can result from factors including a poor diet, high blood pressure, high blood cholesterol, diabetes, etc. There exists a huge healthcare market which includes erectile dysfunction (ED)[2–5] and pulmonary arterial hypertension (PAH).[6] PDE5 is an enzyme that is highly distributed in smooth muscle tissue located in the heart, lung, corpus cavernosum, liver, brain, and stomach. The enzyme catalyzes the hydrolysis of cyclic guanosine monophosphate (cGMP)[7] and has emerged as a key target to treat both CVDs and ED[8–11] with drugs including Sildenafil (Viagra®) and Tadalafil (Cialis®).[12–16] Nevertheless, a number of common side effects exist including headaches, abnormal vision, muscle pain and diarrhea.[3,4] Given the effectiveness of the target, there is continuing interest in the discovery of novel PDE5 inhibitors.

In this study we report our efforts to profile the activity of previously unreported activity of N^2,N^4-diaminoquinazolines derivatives at PDE5. We utilized molecular docking to PDE5 active site and scaffold similarity to known PDE5 inhibitors (Fig. 1) to facilitate the design and modification of new compounds (Fig. 2).[17–20] We investigated the incorporation of a range of substituents at N^2-,N^4-position and quinazoline ring. Twenty compounds were synthesized according to methods described in Scheme 1. The compounds were evaluated against PDE5 and PDE6, their vasodilation effect was assessed in an ex-vivo rat pulmonary artery model and mammalian cytotoxicity and phosphate buffer solubility were determined.

Substitution of 2,4-dichloroquinazoline with amines was achieved using triethylamine (TEA) in tetrahydrofuran (THF) giving compounds **1–10** following the general procedures reported elsewhere.[21–23] Substitution at the 2-position of intermediates **1–10** was performed under acidic condition and/or heat to give compounds **11–30** as solids, with yields ranging from 15% to 90%.[21,23] The purity of compounds were confirmed as > 95% by HPLC analysis. The ^{1}H-, ^{13}C-NMR and mass spectra of all compounds were obtained and are in agreement with the proposed structures (see supporting information).

A radioactive assay adapted from Sonnengburg et al. was used to determine the inhibitory activity of compounds towards PDE5 and 6 (see supporting information).[24] Reported are the concentrations required to inhibit the enzymes by 50% (IC_{50}) and the percent inhibition at the highest concentration tested (Table 1).[25,26] The cytotoxicity of

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https://doi.org/10.1016/j.bmcl.2018.11.043
Received 10 September 2018; Received in revised form 19 October 2018; Accepted 20 November 2018

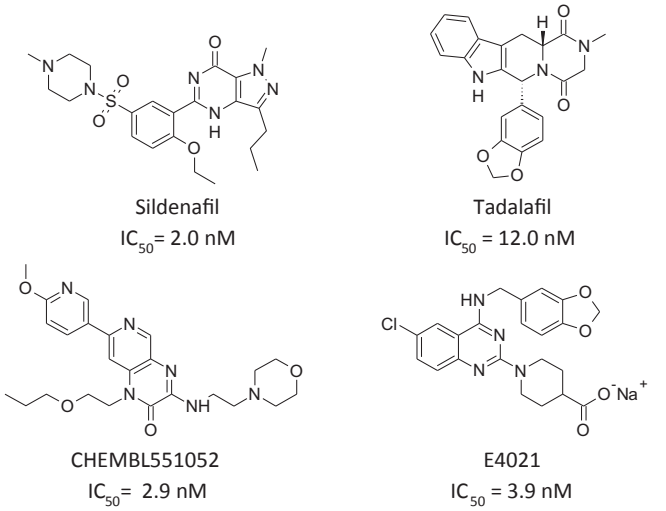


Fig. 1. Structures of PDE5 inhibitors marketed drugs (Sildenafil and Tadalafil), CHEMBL551052[19] and E4021.[17]

the compounds against adenocarcinomic human alveolar basal epithelial cells (A549 cells, ATCC CCL-185) were assessed using an MTT based assay and are reported as an IC_{50} (Table 2). Compounds **11–30** showed affinities for PDE5 in range of 0.07 to > 10 μM (Table 1). Compounds **15** and **22** were found to be the most potent of this series showing IC_{50} values against PDE5 of 0.072 (± 0.008) and 0.089 (± 0.011) μM respectively. Sildenafil, which was used as standard, displayed an IC_{50} of 0.002 (± 0.0008) μM. At the R^1 position, the furfurylamino and 2-thiophenemethylamino substituents displayed comparable if slightly weaker activity than the corresponding benzyl amino derivatives (compounds **22** vs **24** and **28** vs **29**). The benzylamino derivatives also exhibited better PDE5 activity than the corresponding 3-methoxybenzylamino compounds by between 2 and 20-fold (**15** vs **16** and **26** vs **27**). Compound **25**, containing 3-amino-5-methylpyrazole, was found to be inactive (IC_{50} = 3.706 ± 0.642 μM) suggesting a methylene linker to the aromatic ring may be critical. The SAR associated with the R^2 position showed greater variability. Introduction of small polar substituents such as amide and sulfonamide (**14–16** & **20–29**) were preferable over larger substituents such as the 4-morpholino and 4-phenylurea exemplars (**12**, **13**, **18** & **19**). Compounds lacking substituents on the phenyl ring (**11** & **17**) displayed weak to no PDE5 inhibitory activity. Addition of a methoxy substituents to the 6- and 7-position of the quinazoline ring had no dramatic effect on the activity. However, the use of the 5-chloropyrimidine scaffold led to the abolishment of all PDE5 activity suggesting the bicyclic system is critical for activity. Additional selectivity data against PDE6 was obtained (Table 2). Sildenafil was shown to have a 6.5-fold selectivity over PDE6 compared to compound **15** which shows 4.6-fold selectivity and compound **22**, which shows 4.0 fold selectivity.

We undertook computational docking of compounds to PDE5 (PDB code: 3HC8)[19] using GOLD5.1 to help rationalize the observed SAR. Briefly, cofactors and solvent were initially removed from the crystal structure, and docking was performed using default settings. A H-bond between the Gln817 H-bond donor and substrate acceptor was a requirement for a valid solution. Illustrated in Fig. 2 is the docked solution of compound **15** along with the experimental solutions for Sildenafil (PDB code: 1UDT)[10] and CHEMBL551052 (PDB code: 3HC8).[19] The guanidinyl core of Sildenafil makes two H-bond interactions to the amide sidechain of Gln817, two pi-stacking interactions with Phe786 and Phe820 and multiple H-bonds to active site water molecules though its sulfonamide moiety. The quinazoline ring of CHEMBL551052 makes comparable pi-stacking interactions with Phe786 and Phe820, interacts with Gln817 with its pyridyl group and forms multiple solvent interactions.

The quinazoline ring of compound **15** docks in a conformation that sees the overlap of the quinazoline with the bicyclic ring system of CHEMBL551052. The former is found to interact with Gln817 via the 2-position nitrogen H-bond acceptor and make the required π-stacking

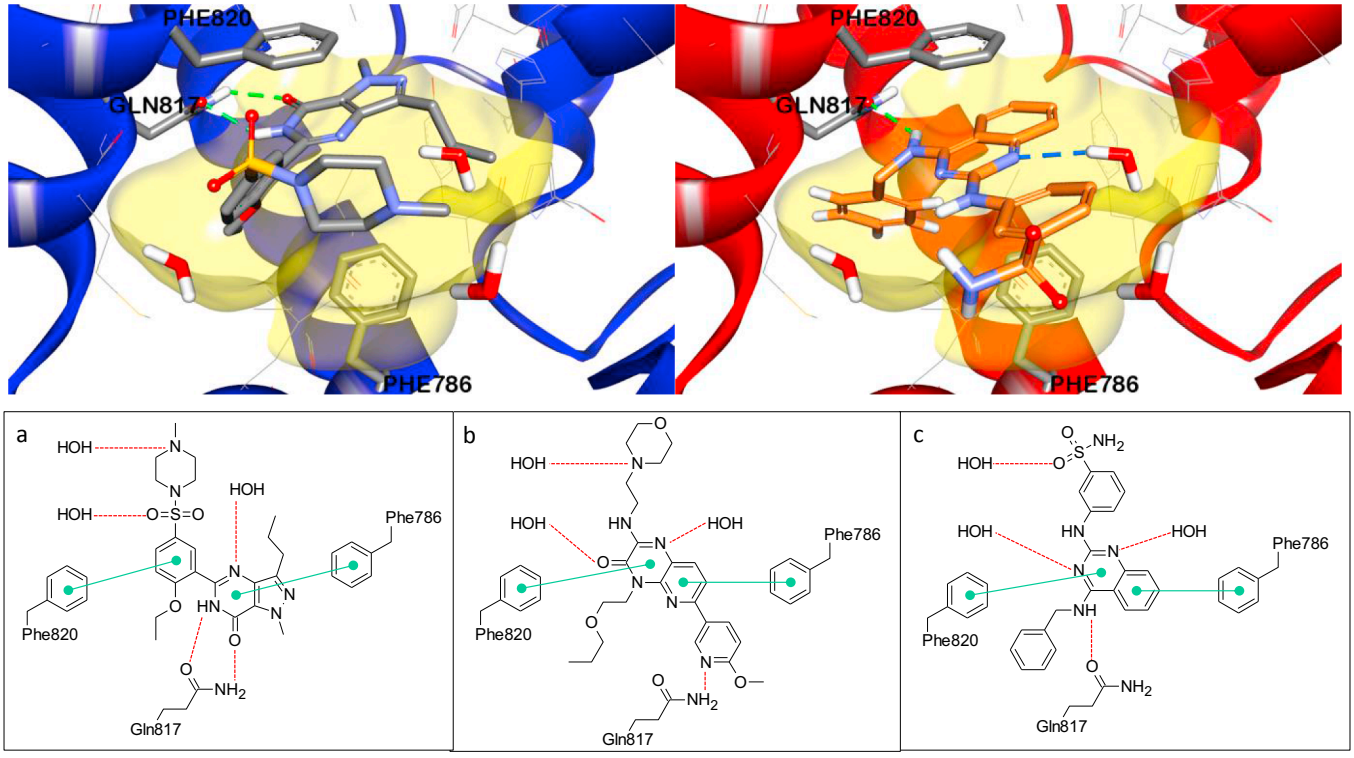
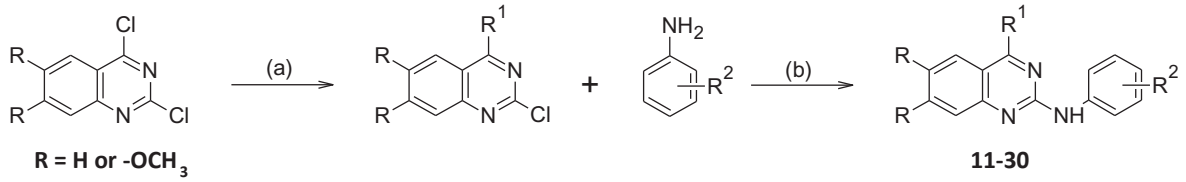


Fig. 2. Sildenafil (top left, PDB code: 1UDT) and compound **15** (top right) bound to PDE5 enzyme. Shown in the bottom panels are 2D ligand interactions diagrams showing the interactions between PDE5 and Sildenafil (a), CHEMBL551052 (b) and compound **15** (c).



Scheme 1. Reagents and conditions used to synthetic routes: (a) amine, Et_3N in THF at room temperature, (b) 1 M HCl, isopropanol, 90 °C, overnight.

Table 1
Molecular weight (M.W.), c log P, inhibitory activity toward PDE5 and PDE6 of compound **11–30**. nd indicated not determined.

ID	Core structure	R^1	R^2	M.W.[a]	c log P[a]	PDE5 IC_{50} μM (SE)	PDE6 IC_{50} μM (SE)
11	Quinazoline	Benzylamino	H	326.4	5.3	1.376 (0.169)	0.890 (0.100)
12		Benzylamino	4-Morpholino	411.5	5.2	0.525 (0.066)	0.450 (0.120)
13		Benzylamino	4-$SO_2N(CH_3)_2$	433.5	4.4	1.260 (0.092)	nd
14		Benzylamino	4-SO_2NH_2	405.5	3.9	0.946 (0.035)	nd
15		Benzylamino	3-SO_2NH_2	405.5	3.9	0.072 (0.008)	0.332 (0.047)
16		3-Methoxybenzylamino	3-SO_2NH_2	435.5	3.8	0.350 (0.045)	nd
17		2-Thiophenemethylamino	H	332.4	5.2	1.440 (0.170)	nd
18		2-Thiophenemethylamino	4-NHCONHPh	466.6	6.4	0.775 (0.101)	nd
19		2-Thiophenemethylamino	4-morpholino	417.5	5.1	0.514 (0.021)	0.610 (0.090)
20		2-Thiophenemethylamino	4-SO_2NH_2	411.5	3.9	0.224 (0.038)	nd
21		2-Thiophenemethylamino	4-$CONH_2$	375.5	4.1	0.177 (0.016)	nd
22		2-Thiophenemethylamino	3-SO_2NH_2	411.5	3.9	0.089 (0.011)	0.360 (0.120)
23		2-Thiophenemethylamino	3-$CONH_2$	375.5	4.1	0.290 (0.098)	nd
24		Furfurylamino	3-SO_2NH_2	395.4	3.0	0.116 (0.023)	0.190 (0.020)
25		3-Amino-5-methylpyrazolo	3-SO_2NH_2	395.4	3.3	3.706 (0.642)	nd
26	6,7-Dimethoxyquinazoline	Benzylamino	3-SO_2NH_2	465.5	3.6	0.218 (0.074)	nd
27		3-Methoxybenzylamino	3-SO_2NH_2	495.6	3.5	0.443 (0.124)	nd
28		2-Thiophenemethylamino	3-SO_2NH_2	471.6	3.5	0.555 (0.120)	nd
29		Furfurylamino	3-SO_2NH_2	455.5	2.7	0.284 (0.122)	nd
30	5-Chloropyrimidine	Benzylamino	H	310.8	4.6	> 10	33.500 (1.940)
Sildenafil				474.6	1.2	0.002 (0.0008)	0.013 (0.001)
Sodium nitroprusside				261.9	0.1	nd	nd

[a] M.W. and c log P was calculated using JChem Version 14.9.100.70.

Table 2
Biological activities, selectivity index (SI), vasodilation effects and solubility at $pH_{7.4}$.

ID	PDE5 IC_{50} μM (SE)	PDE6 IC_{50} μM (SE)	SI PDE6/PDE5	A549 IC_{50} μM (SE)	SI A549/PDE5	Vaso EC_{50} μM (SE)	Sol. $pH_{7.4}$ mg/ml (μM)
11	1.376 (0.169)	0.890 (0.100)	0.65	12.29 (1.20)	9	nd	0.13 (0.41)
12	0.525 (0.066)	0.450 (0.120)	0.86	12.10 (3.60)	23	nd	0.25 (0.60)
15	0.072 (0.008)	0.332 (0.047)	4.61	11.15 (1.22)	155	1.63 (0.72)	0.56 (1.37)
19	0.514 (0.021)	0.610 (0.090)	1.19	11.37 (3.07)	22	nd	0.20 (0.48)
22	0.089 (0.011)	0.360 (0.120)	4.04	15.04 (4.44)	169	2.28 (0.74)	0.14 (0.34)
24	0.116 (0.023)	0.190 (0.020)	1.64	26.92 (2.16)	232	nd	0.15 (0.38)
Sildenafil	0.002 (0.0008)	0.013 (0.001)	6.50	nd	nd	0.14 (0.05)	nd
Nitroprusside	nd	nd	nd	nd	nd	0.019 (0.01)	nd

interactions with Phe820 and Phe786. The sulfonamide substituent adopts a position similar to that found in Sildenafil. The benzyl/furfuryl groups at the 4-position of quinazoline bind within the pocket holding the ethoxy group of Sildenafil and the longer ether chain of CHEMBL551052. The weaker activity of the methoxy substituted phenyl ring is consistent with the fact that it is a rather small pocket. The predicted binding mode is also consistent with the fact that compound **30**, which has a 5-chloropyrimidine core, has negligible affinity. The compound is not as effective at π-stacking with Phe820 and Phe786 in particular.

The general cytotoxicity of the compounds was assessed using the human alveolar basal epithelial cell line (ATCC CCL-185)[27] with IC_{50}s range from 10 to 30 μM. Compound **15**, which showed the greatest activity towards PDE5, also possessed the highest cytotoxicity with an IC_{50} of 11.1 μM (± 1.22). Nevertheless, this still corresponds to a selectivity of over 155-fold for PDE5. Compound **22** demonstrated a selectivity of 169-fold for PDE5. An ex-vivo vasodilation model was then employed to assess the efficacy of **11** and **22** (Table 2).[28] Sildenafil and nitroprusside were used as standards leading to vasodilation responses (EC_{50}) of 0.14 (± 0.05) and 0.019 (± 0.01) μM, respectively. Compounds **15** and **22** displayed EC_{50} values of 1.63 (± 0.72) and 2.28 (± 0.74) μM, respectively. Sildenafil is > 4-fold more active while nitroprusside is > 30-fold more active indicating additional effort is needed to identify further analogs with improved affinity towards PDE5. Finally, we assessed the solubility of a subset of compounds in phosphate buffer at $pH_{7.4}$ using an equilibrium solubility protocol (Table 2).[29] The compounds displayed solubilities in the range of 0.10–0.56 mg/ml (0.22–1.37 μM). Compound **15** was identified as being the most soluble of all compounds.

In conclusion, we report the inhibition of PDE5 by N^2,N^4-quinazolinediamines derivatives – previously unreported PDE5 inhibitors. Compounds in this class are reported to have anti-malarial activity[30]

which could be consistent with this type of activity.[31,32] The binding mode has been determined via molecular docking and is consistent with the observed SAR. Compounds **15** and **22** exhibited high PDE5 inhibitory activities (IC_{50}) of 0.072 and 0.089 μM, respectively and confirmed activity in an ex-vivo rat vasodilation model. Selectivity over the PDE6 isoform was found to be comparable to Sildenafil (4 vs 6-fold). The compounds also displayed good selectivity in terms of their mammalian cytotoxicity.

Acknowledgments

The authors are grateful for financial support provided by the Thailand Research Fund (RSA6100073, DBG5980001 and IRN61W0005), King Mongkut's Institute of Technology Ladkrabang, Kasetsart University Research and Development Institute (KURDI) and the Development and Promotion of Science and Technology Talents Project (DPST).

Appendix A. Supplementary data

Supplementary data (Experimental details and spectra of compounds (NMR, HRMS and HPLC–UV)) to this article can be found online at https://doi.org/10.1016/j.bmcl.2018.11.043.

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