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Inhibition of PDE5A1 guanosine cyclic monophosphate (cGMP) hydrolysing activity by sildenafil analogues that inhibit cellular cGMP efflux

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Keywords
guanosine cyclic monophosphate; inhibitors; molecular modelling; PDE5; sildenafil analogues

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Abstract

Objectives To determine the ability of 11 sildenafil analogues to discriminate between cyclic nucleotide phosphodiesterases (cnPDEs) and to characterise their inhibitory potencies ($K_i$ values) of PDE5A1-dependent guanosine cyclic monophosphate (cGMP) hydrolysis.

Methods Sildenafil analogues were identified by virtual ligand screening (VLS) and screened for their ability to inhibit adenosine cyclic monophosphate (cAMP) hydrolysis by PDE1A1, PDE1B1, PDE2A1, PDE3A, PDE10A1 and PDE10A2, and cGMP hydrolysis by PDE5A, PDE6C, PDE9A2 for a low (1 nM) and high concentration (10 \mu M). Complete IC\textsubscript{50} plots for all analogues were performed for PDE5A-dependent cGMP hydrolysis. Docking studies and scoring were made using the ICM molecular modelling software.

Key findings The analogues in a low concentration showed no or low inhibition of PDE1A1, PDE1B1, PDE2A1, PDE3A, PDE10A1 and PDE10A2. In contrast, PDE5A and PDE6C were markedly inhibited to a similar extent by the analogues in a low concentration, whereas PDE9A2 was much less inhibited. The analogues showed a relative narrow range of $K_i$ values for PDE5A inhibition (1.2–14 nM). The sildenafil molecule was docked in the structure of PDE5A1 co-crystallised with sildenafil. All the analogues had similar binding poses as sildenafil.

Conclusions Sildenafil analogues that inhibit cellular cGMP efflux are potent inhibitors of PDE5A and PDE6C.

Introduction

Cyclic nucleotide signalling plays an essential role in normal cell physiology and is impaired in many pathological conditions, such as heart disease, pulmonary hypertension, chronic obstructive pulmonary disease, obesity, diabetes and cancer.\cite{1} The family of human phosphodiesterases (PDEs) comprises 11 main forms, from which PDEs 4, 7 and 8 are adenosine cyclic monophosphate (cAMP) selective; PDEs 5, 6 and 9 are guanosine cyclic monophosphate (cGMP) selective; and PDEs 1, 2, 3, 10 and 11 hydrolyse both cAMP and cGMP.\cite{2} However, sildenafil raises cellular cGMP levels by two mechanisms, reduction in cellular efflux by ATP-binding cassette transporter subfamily C, member 5 (ABCC5), previously termed multidrug resistance-associated protein 5 (MRP5),\cite{3} in addition to inhibition of PDE5 activity.\cite{4}

Observations suggest that some binding site resemblance exists between PDE5 and ABCC5. In addition to sildenafil, other compounds with ability to inhibit PDE5 activity also reduce cellular cGMP efflux, such as zaprinast,\cite{3,5,6} dipyridamole,\cite{5,6} vardenafil and tadalafil\cite{6} and trequinsin.\cite{3} In contrast, non-selective PDE inhibitors, such as IBMX (3-isobutyl-1-methyl-xanthine),\cite{6,7} caffeine and theophylline,\cite{6} have much lower affinity for the cGMP efflux pump.

The $K_i$ ratio for sildenafil inhibition of cellular cGMP efflux (ABCC5) and hydrolysis (PDE5) is approximately 1000 : 1. In an attempt to balance the action on ABCC5...
and PDE5 (Kᵢ ratio reduction), we identified a series of 11 high-affinity cGMP transporter inhibitors by virtual ligand screening (VLS).[18] Some of them, IN-01 and IN-02 with Kᵢ values of 75 and 65 ns, respectively, were clearly more potent than sildenafil (Kᵢ of 1200 ns) in their inhibition of cGMP efflux.[18] The present work characterises their selectivity towards other cnPDEs, their interaction with PDE5A determined both by inhibition of cGMP hydrolysis, and docking studies of the analogues into the enzyme-binding site. The possibility of creating dual and balanced inhibitors (of both PDE5 and ABC5) by VLS (virtual ligand screening) represents the novelty of this study.

**Materials and Methods**

**Sildenafil analogues**

The sildenafil analogues (Table 1) were purchased from Ambinter (Greenpharma SAS, Orleans, France) with exception of 4-ethoxy-3-(1-methyl-7-oxo-3-propyl-4H-pyrazolo[4, 3-d]pyrimidine-5-yl)-N-[3-(1-methylpyrrolidin-2-yl)pyridine-2-yl]benzenesulphonamide (I-03) which was unavailable. Consequently, this compound was synthesised at the Department of Medicinal Chemistry, Institute of Pharmacology, Polish Academy of Sciences, Kraków, Poland. The synthesis was achieved, using commercially available 5-(2-ethoxyphenyl)-1-methyl-3,6-dihydro-7H-pyrazolo[4,3-d]-7-pyrimidinone (Sigma-Aldrich, Schnelldorf, Germany), in three-step sequence following procedures reported in the literature.[9] 2-Aminonicotine used in the last step was prepared from (−)-nicotine according to the previously published procedure.[10]

**Phosphodiesterase assay for screening of sildenafil analogues**

The screening was performed by BPS Bioscience Inc. (San Diego, CA, USA) with the following materials: PDE assay buffer (BPS), PDE binding agent (BPS), PDE binding agent diluent for cAMP (BPS), PDE binding agent diluent for cGMP (BPS), Bay 60-7550 was purchased from Cayman Chemicals (Ann Arbor, MI, USA), and cilostamide, sildenafil citrate and papaverine were purchased from Axxora (San Diego, CA, USA). Bay 73-6691 was obtained from Sigma-Aldrich (St. Louis, MO, USA). The assays comprised 10 µs and 1 ns dilutions of the test compound in assay buffer (10% DMSO concentration), and 5 µl of the dilution was added to a 50 µl reaction so that the final concentration of DMSO is 1% in all of reactions. The enzymatic reactions were conducted at room temperature for 60 min in a 50 μl mixture containing PDE assay buffer, 100 nM FAM-cAMP, or 100 nM FAM-cGMP, a cnPDE enzyme and the test compound. Bay 60-7550 (10 μM) was used as a reference compound for PDE1A1, PDE1B, PDE1C and PDE2A1 with respective inhibition of 98%, 97%, 98% and 99%. Cilostamide (10 μM) was used for PDE3A and PDE3B and inhibited 99% and 99% of activity. The reference substance for PDE5 and PDE6C was sildenafil (1 μM) and inhibited both enzymes with 99%. The reference substance for PDE9A was Bay73-6691 (10 μM) which gave an inhibition of 99%. Papaverine (10 μM) was employed for PDE10A1 and PDE10A2 and inhibited 99% of activity. After the enzymatic reaction, 100 µl of a binding solution (1 : 100 dilution of the binding agent with the binding agent diluent) was added to each reaction, and the reaction was performed at room temperature for 60 min. Fluorescence intensity was measured at an excitation of 485 nm and an emission of 528 nm using a Tecan Infinite M1000 microplate reader. PDE activity assays were performed in duplicate at each concentration. Fluorescence intensity was converted to fluorescence polarisation using the Tecan Magellan software. The fluorescence polarisation data were analysed using the computer software, GraphPad Prism (GraphPad Software, San Diego, CA, USA). The fluorescence polarisation (FPb) in absence of the compound in each data set was defined as 100% activity. In the absence of cnPDE and the compound, the value of fluorescent polarisation (FPb) in each data set was defined as 0% activity. The per cent activity in the presence of the compound was calculated according to the following equation: % activity = (FP – FPb)/ (FP – FPb) × 100%, where FP = the fluorescence polarisation in the presence of the compound.

**IC₅₀ assay for PDE5A1 characterisation**

Phosphodiesterase 5A1 human, recombinant, expressed in Sf9 cells, Supelco Discovery SPE (1 ml) with DSC-SAX (100 mg/ml), unlabelled cGMP, crotalus atrox venom and bovine serum albumin were purchased from Sigma-Aldrich. [³H]-cGMP (sp. act 1 mCi/mmol) was obtained from PerkinElmer Inc (Boston, MA, USA). The Kᵢ values of the PDE5A1 cGMP hydrolysis were determined using mixtures of [³H]-cGMP and non-labelled cGMP to achieve total cGMP concentrations from 0.1 to 10 μM. To obtain IC₅₀ values, seven concentrations (0.01 nM–10 μM) of each inhibitor were incubated with 5 μM [³H]-cGMP/unlabelled cGMP. The reaction mixture comprised 20 mM Tris–HCl (pH 7.5), 0.3 mg/ml BSA, 1.5 mM dithiothreitol and 3 mM MgCl₂. Incubation time was 10 min at 30 °C. In all studies, less than 10% of added [³H]-cGMP was hydrolysed to [³H]-GMP during the reaction. The reaction was terminated by transferring the reactant to a water bath (100 °C for 1 min) and cooled on ice (1–2 min). [³H]-GMP was hydrolysed to [³H]-guanosine by adding 2.5 μl 10 mg/ml crotalus atrox snake venom which contains a potent 5’-nucleotidase.[11] The mixture was incubated for 10 min at 30 °C and then

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Table 1  Inhibitors (sildenafil analogues), IUPAC-names, molecular structure and PubChem CID

| Inhibitor | IUPAC name                                                                 | Molecular structure | PubChem CID |
|-----------|-----------------------------------------------------------------------------|---------------------|-------------|
| Sildenafil | 5-[2-ethoxy-5-(4-methylpiperazin-1-yl)sulfonylphenyl]-1-methyl-3-propyl-4H-pyrazolo(4,3-d)pyrimidine-7-one | ![Molecular structure of Sildenafil](image) | 5212        |
| IN-01     | 5-[(3-ethyl-butyl-1-methyl-7-oxo-4H-pyrazolo(4, 3-d)pyrimidin-5-yl)-4-ethoxophenyl]sulfonylamino-2-hydroxybenzoic acid | ![Molecular structure of IN-01](image) | 1598490     |
| IN-02     | 5-[(4-ethoxy-3-(1-methyl-7-oxo-3-propyl-4H-pyrazolo [4, 3-d]pyrimidine-5-yl]phenyl)sulfonylamino]-2-hydroxybenzoic acid | ![Molecular structure of IN-02](image) | 1899750     |
| IN-03     | 4-Ethoxy-3-(1-methyl-7-oxo-3-propyl-4H-pyrazolo [4, 3-d]pyrimidine-5-yl)-N-[3-(1-methylpyrrolidin-2-yl)pyridine-2-yl]benzenesulfonamide | ![Molecular structure of IN-03](image) | 4921527     |
| IN-04     | 4-Ethoxy-N,N-diethyl-3-(1-methyl-7-oxo-3-propyl-4H-pyrazolo [4, 3-d]pyrimidine-5-yl]benzenesulfonamide | ![Molecular structure of IN-04](image) | 1899174     |
| IN-05     | 4-Ethoxy-N-methyl-3-(1-methyl-7-oxo-3-propyl-4H-pyrazolo [4, 3-d]pyrimidine-5-yl]benzenesulfonamide | ![Molecular structure of IN-05](image) | 1896380     |
| IN-06     | 4-Ethoxy-N(2-hydroxyethyl)-3-(1-methyl-7-oxo-3-propyl-4H-pyrazolo[4, 3-d]pyrimidine-5-yl]benzenesulfonamide | ![Molecular structure of IN-06](image) | 1900265     |
| IN-07     | 4-Ethoxy-N(2-Hydroxyethyl)-N-Methyl-3-(1-Methyl-7-Oxo-3-Propyl-4H-Pyrazolo[4,3-D]Pyrimidine-5-Yl]Benzenesulfonamide | ![Molecular structure of IN-07](image) | 1896597     |
| IN-08     | 5-[5-(azepan-1-ylsulfonyl)-2-ethoxyphenyl]-1-methyl-3-propyl-4H-pyrazolo[4,3-d]pyrimidine-7-one | ![Molecular structure of IN-08](image) | 1897952     |
| IN-09     | 5-(2-ethoxy-5-piperidin-1-ylsulfonylphenyl)-1-methyl-3-propyl-4H-pyrazolo[4,3-d]pyrimidine-7-one | ![Molecular structure of IN-09](image) | 1896867     |
| IN-10     | 4-Ethoxy-N,N-dimethyl-3-(1-methyl-7-oxo-3-propyl-4H-pyrazolo [4, 3-d]pyrimidine-5-yl]benzenesulfonamide | ![Molecular structure of IN-10](image) | 1902581     |
| IN-11     | N-Benzyl-4-ethoxy-3-(1-methyl-7-oxo-3-propyl-4H-pyrazolo [4, 3-d]pyrimidine-5-yl]benzenesulfonamide | ![Molecular structure of IN-11](image) | 1896826     |

The compounds were identified by virtual ligand screening (VLS)."
diluted in 250 μl 10 mM Tris/8.2 mM propionic acid (pH 7.5). The samples were applied on a preconditioned/equilibrated DSC-SAX column to separate [3H]-guanosine from [3H]-GMP. The columns were washed with 100 μl 10 mM Tris/8.2 mM propionic acid buffer (pH 7.5) five times. The eluate (400 μl) was transferred to a scintillation vial containing 10-ml scintillation cocktail (Ultima Gold XR; Packard, Groningen, the Netherlands), and radioactivity was quantified in a Packard 1900 TR Liquid Scintillation analyser.

Data analysis and statistics

Values for $K_m$ and $IC_{50}$ were obtained according to Chou[12] and $K_i$ values were calculated according to Cheng and Prusoff.[13] The descriptive statistics is presented as mean value ± SE in text, tables and figures. Kruskal-Wallis test (nonparametric ANOVA) with Dunn’s multiple comparisons post-test was used to compare the $K_i$ values for the analogues with that of sildenafil (InStat, ver. 3.10 for Windows; GraphPad Software).

Molecular modelling

Docking studies and scoring were performed using the ICM molecular modelling software (Molsoft LLC, San Diego, CA, USA).[14] The crystal structure of PDE5A[15] in complex with sildenafil (PDB ID: 2H42) with the resolution 2.3 Å was converted to an ICM object, and receptor maps were calculated based on the pocket defined by position of co-crystallised sildenafil in the crystal structure. Ligands were prepared in the ICM ligand editor and converted to 3D when setting up the ligand during the docking session. Charges were also assigned in this step. The ligands were modelled using the ICM molecular editor and docked into PDE5 using interactive docking. Tautomer sampling was performed, as sildenafil may exist in three tautomeric forms. The docking poses were scored by the ICM scoring function. The scoring function gives a score optimised to rank order the docking hits by their binding affinity.[16] The lower the ICM score, the higher the chance the ligand has a high affinity to the drug target.

Results

Sildenafil analogues and cyclic nucleotide phosphodiesterase selectivity

The sildenafil analogues (Table 1) obtained for inhibition studies of cGMP efflux[8] were screened for their ability to

Table 2  The inhibitors (IN-01–IN-11) listed in Table 1 were screened for their inhibitory activity on a panel of phosphodiesterase (PDE) family members as described in Methods

|               | PDE1A1 | PDE1B | PDE1C | PDE2A1 | PDE3A | PDE3B | PDE5A | PDE6C | PDE9A2 | PDE10A1 | PDE10A2 |
|---------------|---------|-------|-------|---------|-------|-------|-------|-------|--------|----------|---------|
| IN-01         | 93 ± 1.4| 100 ± 2.8| 90 ± 0.7| 97 ± 0.0| 101 ± 3.5| 102 ± 2.1| 41 ± 0.7| 33 ± 1.4| 95 ± 5.7| 99 ± 0.7| 99 ± 0.0|
| IN-02         | 94 ± 2.1| 96 ± 2.1| 97 ± 1.4| 97 ± 0.7| 98 ± 0.0| 100 ± 4.2| 35 ± 1.4| 45 ± 2.8| 92 ± 0.0| 99 ± 0.7| 97 ± 2.1|
| IN-03         | 96 ± 2.8| 98 ± 1.4| 98 ± 2.1| 95 ± 0.0| 97 ± 0.0| 98 ± 0.0| 93 ± 0.7| 69 ± 0.0| 97 ± 5.7| 93 ± 0.0| 99 ± 0.7|
| IN-04         | 92 ± 0.6| 60 ± 0.7| 1.0 ± 0.0| 29 ± 1.4| 51 ± 0.7| 62 ± 1.4| 1.0 ± 0.0| 3.5 ± 0.7| 29 ± 2.8| 2.0 ± 2.8| 7 ± 0.0|
| IN-05         | 94 ± 3.5| 96 ± 0.7| 99 ± 0.0| 0.9 ± 0.0| 98 ± 2.1| 102 ± 0.0| 49 ± 0.7| 49 ± 4.9| 95 ± 0.0| 94 ± 2.8| 98 ± 1.4|
| IN-06         | 95 ± 1.4| 100 ± 1.4| 0 ± 1.4| 46 ± 1.4| 50 ± 0.7| 65 ± 0.7| 1.0 ± 0.0| 4.5 ± 2.1| 29 ± 2.7| 25 ± 2.1| 31 ± 0.0|
| IN-07         | 94 ± 0.0| 99 ± 2.1| 99 ± 0.7| 97 ± 1.4| 96 ± 0.7| 99 ± 1.4| 94 ± 3.5| 81 ± 2.8| 97 ± 3.5| 99 ± 0.7| 94 ± 4.2|
| IN-08         | 95 ± 0.7| 98 ± 3.5| 99 ± 0.7| 99 ± 1.4| 92 ± 0.7| 97 ± 1.4| 98 ± 2.1| 89 ± 2.1| 100 ± 3.5| 95 ± 4.0| 96 ± 0.0|
| IN-09         | 92 ± 0.8| 95 ± 3.5| 98 ± 0.0| 97 ± 1.4| 95 ± 2.1| 98 ± 0.7| 93 ± 2.1| 74 ± 2.1| 101 ± 4.1| 92 ± 2.8| 97 ± 0.7|
| IN-10         | 94 ± 0.7| 92 ± 1.4| 97 ± 0.7| 96 ± 1.4| 101 ± 2.8| 100 ± 0.0| 37 ± 2.4| 44 ± 0.7| 98 ± 5.7| 90 ± 2.8| 99 ± 0.7|
| IN-11         | 92 ± 0.8| 98 ± 2.1| 97 ± 0.7| 96 ± 1.4| 101 ± 2.8| 100 ± 0.0| 37 ± 2.4| 44 ± 0.7| 98 ± 5.7| 90 ± 2.8| 99 ± 0.7|

They were tested in duplicates for two concentrations (1 μM/10 μM). FAM-cAMP (100 μM) was used as substrate for PDE1s, PDE2A1, PDE3s and PDE10s, whereas FAM-cGMP (100 μM) was used for PDE5A, PDE6C and PDE9A2. PDE5A was also tested with 1 μM and 10 μM sildenafil (as a positive control) and reduced the cGMP hydrolysis to 58 ± 1.4% and 1 ± 0% of control, respectively. Results (mean ± SE) are presented as % of control representing two time-independent experiments each in duplicate.
inhibit PDE5A and other members of the cnPDE family (Table 2). As described in methods, the screening was performed with a high and low concentration of the compounds. For the cGMP hydrolysing enzymes, the following order of potency existed; for PDE5A (1 nM inhibitor): IN-02 = IN-08 ≥ IN-01 > IN-04 > IN-09 > IN-11 ≥ IN-03 = IN-07 = IN-05 ≥ IN-06 = IN-10. The respective order for PDE6C (1 nM inhibitor) was IN-01 > IN-08 = IN-02 ≥ IN-04 > IN-11 > IN-09 ≥ IN-03 > IN-07 > IN-05 > IN-06 > IN-10. The inhibitors had low affinity for PDE9A2 with a test concentration of 1 nM. The members of PDE-subfamilies tested for cAMP hydrolysis showed negligible inhibition with 1 nM. Increasing the test concentration to 10 μM gave markedly inhibition of some of the other cnPDEs, including the PDE1, PDE2A1 and the PDE3 (sub-)families. Finally, some of the analogues, in the highest tested concentration, gave virtually complete inhibition of PDE10A1 and PDEA2.

**Characterisation of PDE5A1 inhibition by sildenafil analogues**

The characteristics ($IC_{50}/K_i$ values) of the 11 sildenafil analogues were assessed by full concentration–inhibition curves for their ability to inhibit PDE5A1-mediated cGMP hydrolysis. The $K_i$ value of PDE5A1-mediated cGMP hydrolysis was $1.7 ± 0.4\mu M$. Sildenafil was employed as reference compound for the inhibitors. A $K_i$ value of $3.3 ± 0.9\mu M$ was obtained for sildenafil under the present experimental conditions. All analogues inhibited the PDE5A1-dependent cGMP hydrolysis in a concentration-dependent manner. Figure 1 shows $IC_{50}$ curves for the analogues with sildenafil as reference substance. Table 3 shows both $IC_{50}$ and $K_i$ values. Three analogues were more potent than the rest (Figure 1, panel a), IN-03, IN-08 and IN-09 with $K_i$ values from 1.2 to 1.9 nM (Table 3). Figure 1 (panel b) shows the second group
(IN-01, IN-02 and IN-04) with intermediate affinities ($K_i$ values from 2.6 to 3.0 nM), virtually identical with that of sildenafil (Table 3). The third group (Figure 1, panel c) comprised IN-05 and IN-11 ($K_i$-values were 7.8 and 9.8 nM). The last group (Figure 1, panel d) with the lowest affinities ($K_i$ value range was 12–14 nM) consisted of IN-06, IN-07 and IN-10. The inhibition curves of sildenafil were shifted from the right (Figure 1, panel a) to the left (Figure 1, panel d). Statistical analysis with Kruskal-Wallis test (nonparametric ANOVA) gave a P value $< 0.0001$, considered extremely significant. However, the Dunn’s multiple comparisons post-test showed that only IN-06 and IN-10 had $K_i$-values significantly different from that of sildenafil (Table 3).

### Docking of novel sildenafil analogues to the crystal structure of PDE5A1 catalytic domain

To assess the accuracy of ICM docking procedure, the sildenafil molecule was docked in the structure of PDE5A1 co-crystallised with sildenafil. Self-docking showed that it occupied spatially the same place as sildenafil from crystal structure (Figure 2, panel a). All the analogues had similar binding poses as sildenafil (Figure 2, panel b). The heterocyclic ring system of the sildenafil-like compounds spatially occupied the same position as in the crystal structure of PDE5A1 with sildenafil. Additionally, the salicylic acid moiety of the compounds IN-01 and IN-02 formed hydrogen bonds with Arg667 and Asn661 (Figure 2, panel c).

The screening was succeeded by a thorough characterisation of the inhibitors on PDE5A-mediated cGMP hydrolysis activity. The two different methods employed for screening and PDE5A characterisation gave similar but not identical results. The methods employed for detailed studies on PDE5A were established 4–5 decades ago but is still in use after continuous refinements. The $K_m$ value of PDE5A cGMP hydrolysis was 1.7 μM, virtually identical to that reported (2 μM) by Francis et al. Furthermore, the $K_i$ value (3.3 nM) obtained here for sildenafil inhibition of PDE5A is in close agreement with that (4 nM) considered as typical. The 11 sildenafil analogues were able to inhibit cGMP hydrolysis by PDE5 within a relative narrow range of $K_i$ values (1.2–14 nM). Only two of the analogues had $K_i$-values statistically different from that of sildenafil. This shows that VLS, at least in our hands, is a robust method to predict drug analogues. The molecular modelling employed in this study emphasises the potential of this technology. The inhibitors were recognised by the same binding site as sildenafil and showed an overlapping interaction. The pyrazolopyrimidine group stacked against phenylalanine-820 and the compounds formed two hydrogen bonds with glutamine-817, previously shown to play key role in PDE5 inhibitor binding.

The aim of our work was to identify and characterise sildenafil-like inhibitors with a balanced effect on cGMP hydrolysis and cGMP efflux. In our experimental set-ups,
we found virtually identical values $K_m$ values for PDE5 cGMP hydrolysis (1.7 μM) in the present work and $K_m$ values for high-affinity cGMP transport as reported in previous studies: 2.4, 2.2, and 2.6 μM. On the other hand, the $K_i$ values of sildenafil inhibition of PDE5A1 cGMP hydrolysis and high-affinity cGMP efflux are extremely different. Previously we have reported $K_i$ values of 1.2–3.6 μM for the active cellular extrusion of cGMP. This
means a $K_i$ ratio (transport/hydrolysis) of approximately 1000 : 1. It is intriguing that the $K_i$ ratios were clearly lower for some of the sildenafil analogues, being $\approx 25 : 1$ for both IN-01 and IN-02, and $\approx 100 : 1$ for IN-03. This demonstrates that single molecules may balance action on these two different molecular targets and thereby enhancing the effect of intracellular cGMP. However, the ABC-transporters are multipurpose pumps (‘vacuum cleaners’) with the ability to remove excess of potential harmful endo- and exobiotics. Development of dual and balanced inhibitors should not completely block this vital cellular function.

Conclusions

Virtual ligand screening was employed to identify sildenafil analogues. In previous studies, some of these compounds reduced cellular efflux of cGMP. In the present work, several of the analogues were more potent, equipotent or less potent than sildenafil in their inhibition of PDE5A-mediated cGMP hydrolysis. Taken together, these results demonstrate that it is possible to design inhibitors with dual and balanced action.

Declarations

Conflict of interest

The Authors declare that they have no conflict of interests to disclose.

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