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Original article

Identification and experimental confirmation of novel cGMP efflux inhibitors by virtual ligand screening of vardenafil-analogues

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ABSTRACT

Background: Clinical studies have reported overexpression of PDE5 and elevation of intracellular cyclic GMP in various types of cancer cells. ABCC5 transports cGMP out of the cells with high affinity. PDE5 inhibitors prevent both cellular metabolism and cGMP efflux by inhibiting ABCC5 as well as PDE5. Increasing intracellular cGMP is hypothesized to promote apoptosis and growth restriction in tumor cells and also has potential for clinical use in treatment of cardiovascular disease and erectile dysfunction. Vardenafil is a potent inhibitor of both PDE5 and ABCC5-mediated cGMP cellular efflux. Nineteen novel vardenafil analogs that have been predicted as potent inhibitors by VLS were chosen for tests of their ability to inhibit ATP- dependent transport of cGMP by measuring the accumulation of cyclic GMP in inside-out vesicles.

Aim: In this study, we investigated the ability of nineteen new compounds to inhibit ABCC5- mediated cGMP transport. We also determined the Ki values of the six most potent compounds.

Methods: Preparation of human erythrocyte inside out vesicles and transport assay.

Results: Ki values for six of nineteen compounds that showed more than 50 % inhibition of cGMP transport in the screening test were determined and ranged from 1.1 to 23.1 μM. One compound was significantly more potent than the positive control, sildenafil.

Conclusion: Our findings show that computational screening correctly identified vardenafil-analogues that potently inhibit cGMP efflux-pumps from cytosol and could have substantial clinical potential in treatment of patients with diverse disorders.

1. Introduction

Movement of ions and most other polar or charged molecules across the plasma membrane depends on specialized membrane transport proteins. After binding of molecules, they undergo a conformational change in the process of transporting the solute. Among these proteins, ATP-binding cassette (ABC) transporters are a large and functionally diverse class of membrane transporters. ABC transporters have been classified into seven families according to sequence homologies (ABCA through ABCG) [1]. They are targeted in studies exploring experimental treatment of a wide range of conditions, like multidrug resistant *Escherichia coli* infection and cancer therapy [2–4]. The human ABCC subfamily; multi resistance proteins (MRP) is capable to export multiple types of anti-cancer drugs out of the cytoplasm and cause drug resistance to cancer chemotherapy [5,6].

The cyclic nucleotides cAMP and cGMP are involved in physiological processes, crucial for normal cellular function. Intracellular concentrations are regulated by phosphodiesterase enzymes (PDE) and export across the cell membrane by ABC transporters [7]. Elimination of cytosolic cGMP is dependent on PDE5-mediated enzymatic hydrolysis and ABCC5-mediated efflux from the cell. Several PDE5-inhibitors like sildenafil and vardenafil are in clinical use due to their ability to increase intracellular cGMP. These PDE5 inhibitors also impede the activity of ABCC5, giving a dual cGMP-elevating effect. This is foundation for their therapeutic potential to treat several conditions from erectile dysfunction, to various types of cancer.

In the present study, we tested 19 novel compounds chosen by their potential to inhibit ABCC5. Selection was made based on their structural similarity to vardenafil and their predicted affinity for the ABCC5 transporter, by molecular modeling and virtual ligand scanning, a

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Table 1
Interactions of the amino acids in the binding site and Vardenafil, Compound 8 and Compound 16.

TMH	AA	Vardenafil	Compound #8	Compound #16
1	Gln190	Piperazine ring	–	Methoxyphenyl ring
	Phe194	Piperazine ring	–	Methoxyphenyl ring
4	Tyr330	–	Cyclopentane ring	Bromophenyl ring
5	Val411	Propyl chain of guanosine-like moiety	Cyclopentane ring	Bromophenyl ring
6	Phe440	Etoxyphenyl group	Cyclopentane ring	Bromophenyl ring
	Asn441	Piperazine ring	Piperazine ring	Bromophenyl ring
	Thr444	–	Cyclopentane ring	Bromophenyl ring
	Lys448	Approximately 10 Å above the binding area of the ligand	Approximately 10 Å above the binding area of the ligand	Approximately 10 Å above the binding area of the ligand
7	Ser872	Guanine-like moiety	Guanine-like moiety	Guanine-like moiety
	Trp879	Piperazine ring	Guanine-like moiety	Guanine-like moiety
8	Ser921	Propyl chain of guanosine-like moiety	–	–
12	Tyr1135	Guanine-like moiety	Guanine-like moiety	Guanine-like moiety
	Gln1138	Guanine-like moiety	Guanine-like moiety	Guanine-like moiety
	Phe1145	Guanine-like moiety	–	–

method used for various studies aiming to detect novel pharmacological substances [8–11]. Through testing their ability to inhibit ABCC5, we aimed to detect new pharmacological substances with therapeutic potential.

2. Materials and methods

2.1. Software

The ICM program [12] version 3.6-1e, was used for homology modeling, compound docking and vardenafil substructure search. The ICM program package included the ICM VLS add-on and access to Molcart, a database of chemical structures for ~4 M of commercially available compounds. The ICM virtual ligand screening technology provides good tools for accurate individual ligand-protein docking, and the program has been extensively validated both as a virtual screening tool, in prediction of ligand pose, docking and screening accuracy [8–10].

2.2. Homology modeling

To the best of our knowledge, there is no published crystal structure of the ABCC5 transporter. A homology model of ABCC5 [13], which was based on the X-ray crystal structure of the *Mus musculus* ABCB1 [14] (template is available at: https://www.rcsb.org/structure/3g60 - PDB code 3G60), complexed with the ligand cyclic-tris-(R)-valineselenazole (QZ59-RRR), was used for the present study. (The ABCC5 model was refined [13] by globally optimizing side chain positions and annealing the backbone using the RefineModel macro of ICM, followed by the "Regul" option of ICM, and finally energy minimized using the leaprc.ff03 force field of the AMBER 9 program package [15].

2.3. 4D VLS docking

Energy-based torsional sampling was used to generate additional conformations of the ligand binding area of ABCC5 in order to investigate putative ligand binding modes in the highly flexible transporter protein. This computational technique, called "fumigation" [16], is aimed at generating more "druggable" conformations of ligand binding pockets. The technique is based on torsional sampling of the binding pocket side chains in the presence of a repulsive density representing a generic ligand, using the ICM biased probability Monte Carlo sampling procedure. The ligand skin mesh of QZ59-RRR from the template [14] formed the basis for the pocket used for torsional sampling.

The Molcart chemical management system was used to retrieve compounds with a common substructure as in vardenafil. A database of vardenafil-like compounds was obtained and used for a 4D VLS docking into the ABCC5 transporter.

Ligands were prepared in the ICM ligand editor, assigned charges and converted to 3D when setting up the ligand during the docking session. A 4D docking procedure was used employing the binding pocket conformational ensembles, where the pocket ensemble conformations are used as an extra, fourth dimension of the ligand sampling space, allowing ligand docking to the multiple binding pocket conformations in a single docking simulation [17]. The three lowest energy binding pocket conformations were used in the 4D docking procedure.

2.4. Vardenafil analogues

Docking score was calculated from interaction energy, where lower scores corresponds to higher potency. Hits with scores below the docking score of Vardenafil -24.54 were selected based on druglikeness and ordered for in vitro testing. The ICM druglikeness score is predicted based on 5000 marketed drugs from the World Drug Index WDI positives and 10,000 nondrug compounds negatives. Accordingly, a total of 19 compounds Table 1) were purchased from Enamine (Riga, Latvia) and eMolecules (San Diego, CA, USA). PubChem (https://pubchem.ncbi.nlm.nih.gov/) and ChEMBL (https://www.ebi.ac.uk/chembl/) databases were used to retrieve information about any bioactivity studies on the two most potent compounds (#8 and #16). For comparison, bioactivity studies on Vardenafil and Sildenafil were also investigated. In additionSwissADME (http://www.swissadme.ch/) was used to run ADME on compounds #8, #16, vardenafil and sildenafil. ICM (https://onlinelibrary.wiley.com/doi/abs/10.1002/jcc.540150503) was used to calculate toxicity and drug likeness on the molecules. The ICM Tox Score is calculated based on known compounds associated with toxicity/reactivity with scores based on their perceived toxicity and frequency of appearance in approved drugs. A toxscore > = 1. indicates likely toxicity based on substructure match. The ICM drug-likeness score is predicted based on 5000 marketed drugs from the World Drug Index (WDI) (positives) and 10000 non-drug compounds (negatives), and the score is better if it is positive.

2.5. Preparation of IOV

In the present study, a modified version of the Steck IOV preparation [[18]] was used. Fresh human EDTA blood was used to produce IOVs from human erythrocytes. All steps after collecting the blood were performed at 0–4 °C. The cells were sedimented by centrifugation 2300 *g* for 15 min. Plasma and buffycoat were discarded, and the red blood cells were washed 3 times by centrifugation at 1000 *g* 5 mM Tris•HCl, 113 mM KCl, pH = 8.1. Cells were lysed in 10 volumes of

5 mM TrisHCl, 0.5 mM EGTA, 4 mM KCl, pH = 8.1 and washed by repeated centrifugation at 20,000 *g* for 20 min and resuspension in the same buffer until ghosts were milky white. Vesiculation was initiated by adding 39 volumes of 500 nM TrisHCl, pH = 8.2 to one volume of cell suspension. The vesiculation was completed by homogenization of vesicles and unsealed ghosts by passing the suspension five times through a 27 G cannula. IOVs, right-side out vesicles and unsealed vesicles and ghosts were separated by ultracentrifugation (100.000 × g) over night using a density gradient from 1,048 *g*/ml to 1146 *g*/ml Histodenz (Sigma-Aldrich, St. Louis, MO, USA) in 5 mM Tris, 3 mM KCl, 0.3 mM EGTA. The uppermost band was collected, washed and resuspended in 1.47 mM KH_2PO_4, 81 mM K_2HPO_4 and 140 mM KCl, pH 7.6. Sidedness was verified using acetylcholinesterase accessibility.

2.6. Transport assay

cGMP is transported out of cells via ABCC5 with a K_m value of 2.6 μM [19]. In the present study cGMP uptake into IOVs was determined for an inhibitor concentration range of 10^{-3} – 10^{-7} M. IOVs were incubated for 60 min with or without 2.0 mM ATP in a mixture containing 20 mM Tris•HCl, 10 mM $MgCl_2$, 1 mM EGTA, 2 μM [^{3}H]-labeled cGMP, 121 mM KCl, pH = 8.0 at 37°, and inhibitor in increasing concentrations except for in control samples. The transport process was stopped with addition of ice-cold 1.47 mM KH_2PO_4, 8,1 mM K_2HPO_4 and 140 mM KCl, pH 7.6. The IOVs were separated from the incubation medium by filtration (nitrocellulose membrane, 0.22 μm GSWP, Millipore, Billerica, MA, USA). The radioactivity on the filters was quantified by liquid scintillation (Ultima Gold XR, Packard, Groningen, The Netherlands) in a Packard 1900 TR Liquid Scintillation analyzer.

2.7. Determination of k_i-values

The IC_{50}-values were determined according to Chou [20] and transformed to K_i-values according to Cheng and Prusoff [21].

2.8. Statistical analysis

We performed statistical comparison of potency of all inhibitors that inhibited more than 50 % of [3 H]-cGMP uptake by ABCC5 transport into inside-out vesicles (IOV). Normality and equal variance of data were confirmed by Shapiro-Wilks test and Brown-Forsythe test. This was followed by a One Way Analysis of Variance (ANOVA) multiple comparison versus sildenafil as control substance, using the Holm-Sidak method. Sildenafil was chosen as control substance over vardenafil due to higher potency in inhibiting ABCC5 mediated cGMP transport (Table 3).

3. Results

3.1. In silico (Figs. 1 and 2, Tables 1 and 2)

"Fig. 1 shows Vardenafil docked into the binding site. Amino acid residues involved with ligand binding included Gln190 and Phe194 (TMH1), Tyr330 (TMH4), Val411 (TMH5), Phe440, Asn441, Thr444 and Lys448 (TMH6), Ser872 and Trp879 (TMH7), Ser921 (TMH8), and Tyr1135, Gln1138, and Phe1145 (TMH12). The docking score of Vardenafil was -24.5, and this value was used as a threshold score for the VLS. Table 1 shows interactions of the amino acids in the binding site and Vardenafil, Compound 8 and Compound 16. The guanine-like moieties of these 3 ligands had the same tendency of interacting with TMHs 7 and 12. The ligands where posed in the the same spacial orientation except for the orientation of the guanine-like ring of Vardenafil, which was oriented towards TMH8 (Figs. 1 and 2). Lys448 was located approximately 10 Å towards the entrance of ABCC5 with its side chain pointing directly into the transport area.

Table 2 shows the vardenafil-like compounds that were obtained and used for 4D VLS docking into the ABCC5 transporter. 6 compounds were found to have potentially high ABCC5 inhibition and were ordered from eMolecules and Enamine."

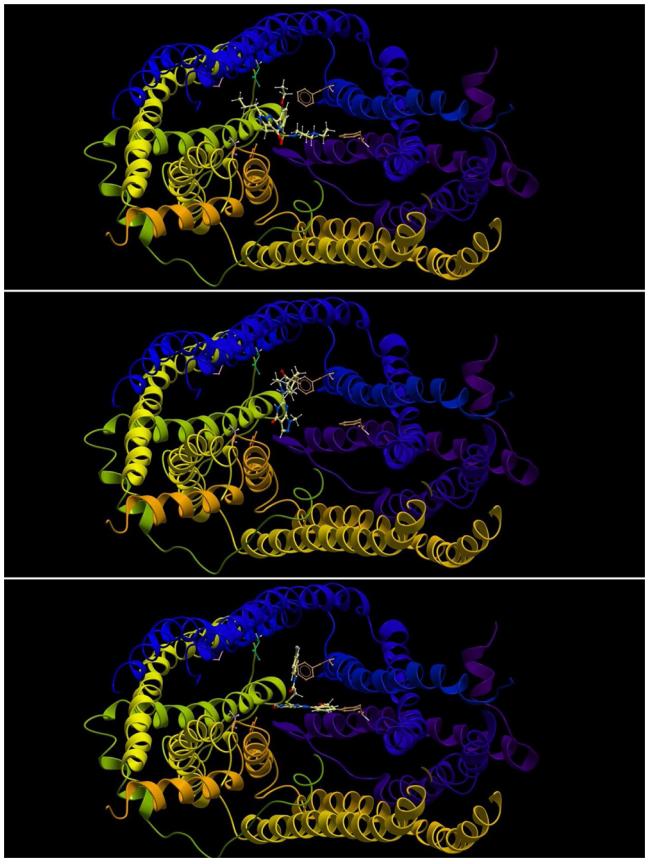


Fig. 1. (PRINT IN COLOR) Vardenafil (top), compound #8 and compound #16 docked into the binding site of the TMHs of ABCC5 viewed as a section from the extracellular side. Amino acids with hydrogen bond interactions with sildenafil are displayed as sticks colored according to atom type (C = light yellow; H = gray; O = red; N = blue; sulfur = yellow) : Gln190 (TMH1), Asn441 (TMH6), and Gln1138 (TMH12). TMHs are shown as ribbons and are spectrum color-coded, from purple (TMH1) to red (TMH12).

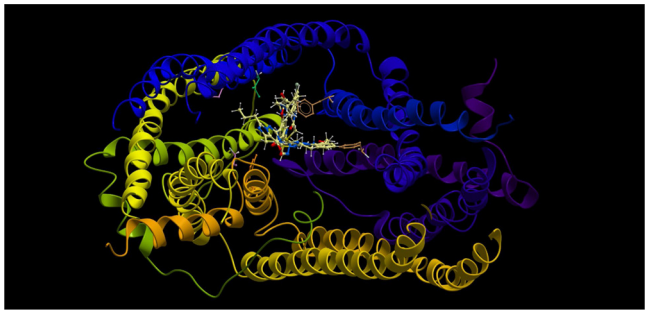
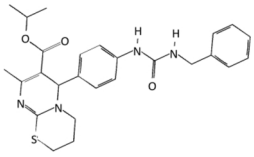
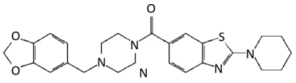
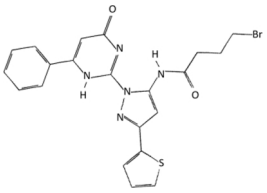
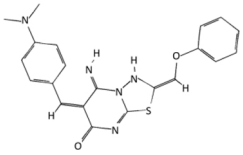
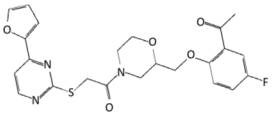
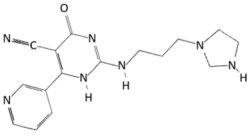
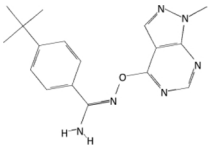
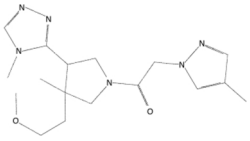
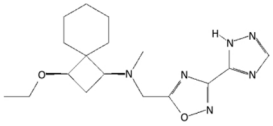
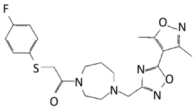
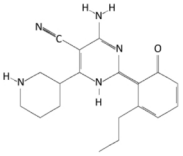
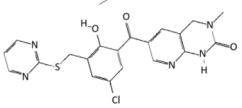


Fig. 2. (PRINT IN COLOR) Vardenafil, compound #8 and compound #16 displayed togheter in the binding site of the TMHs of ABCC5 viewed as a section from the extracellular side. The guanosine parts of the molecules appeared to bind with high affinity. Amino acids with hydrogen bond interactions with sildenafil are displayed as sticks colored according to atom type (C = light yellow; H = gray; O = red; N = blue; sulfur = yellow) : Gln190 (TMH1), Asn441 (TMH6), and Gln1138 (TMH12). TMHs are shown as ribbons and are spectrum color-coded, from purple (TMH1) to red (TMH12).

The ADME, Tox and drug likeness data are shown in Table 4. All compounds were shown to have a high GI absorption theoretically. All compounds except Compound 16 were predicted to be P-glycoprotein

Table 2
Vardenafil analogues.

Compound	Formula	Molecular weight (g/mol)	Structure	Supplier	Compound ID
#1	C26H30N4O3S *CAS: 1080812-22-1*	478.60		Enamine	Z131669058
#2	C25H28N4O3S *CAS: 1190894-94-0*	464.57		Enamine	Z131675110
#3	C21H18BrN5O2S *CAS: 314290-59-0*	484.36		Enamine	Z15383694
#4	C21H19N5O2S *CAS: 331244-89-4*	405.47		Enamine	Z15383727
#5	C23H22FN3O5S *CAS: 16690-24-7*	471.50		Enamine	Z131699428
#6	C16H19N7O *CAS: 1137476-32-4*	325.36		Enamine	Z802694028
#7	C17H20N6O *CAS: 1628210-26-3*	324.38		Enamine	Z729878740
#8	C17H24N6O2 *CAS: 1280995-43-8*	346.41		Enamine	Z1102995434
#9	C17H26N6O2 *CAS: 1311601-55-4*	346.42		Enamine	Z1083966246
#10	C21H24FN5O3S *CAS: 1353528-67-2*	445.51		Enamine	Z218155582
#11	C19H23N5O *CAS: 62337-66-0*	337.41		Enamine	Z1103000948
#12	C20H16ClN5O3S *CAS: 189250-11-1*	441.891		eMolecules	C365-0139

(*continued on next page*)

Table 2 (*continued*)

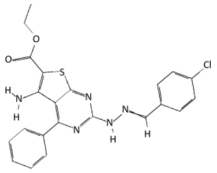
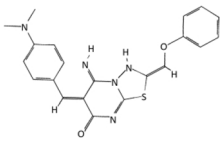
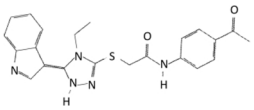
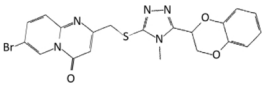
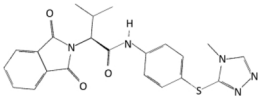
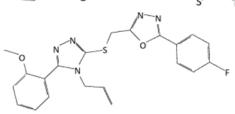
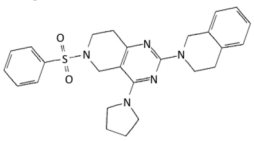
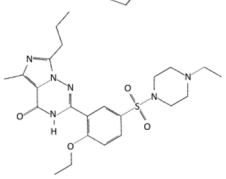
Compound	Formula	Molecular weight (g/mol)	Structure	Supplier	Compound ID
#13	C22H18ClN5O2S *CAS: 57353-08-9*	451.929		eMolecules	C365-0133
#14	C21H19N5O2S *CAS: 189343-71-3*	405.473		eMolecules	C365-0215
#15	C22H21N5O2S *CAS: 194666-84-7*	419.499		eMolecules	C365-0300
#16	C20H16BrN5O3S *CAS: 108667-91-0*	486.342		eMolecules	G873-0200
#17	C22H21N5O3S *CAS: 332869-93-9*	435.499		eMolecules	G873-0190
#18	C21H18FN5O2S *CAS: 852154-45-1*	423.463		eMolecules	C099-0347
#19	C26H29N5O2S *CAS: 1138472-98-6*	475.606		eMolecules	E960-0870
Vardenafil	C23H32N6O4S *CAS: 224785-90-4*	488.60		eMolecules	NC1641891

Table 3
IC50-values ± SEM and Ki-values of six most potent inhibitors and sildenafil. Order of potency: INH 16 > INH 8 > Sildenafil > Vardenafil > INH 9 > INH 17 > INH 6 > INH 11.

Compound	IC50 value (μM)	Ki value (μM)
# 6	18.0 ± 1.9	10.2 ± 1.4
# 8	3.7 ± 1.9	2.1 ± 0.3
# 9	13.2 ± 1.5	7.5 ± 2.0
# 11	40.9 ± 1.6	23.1 ± 5.5
# 16	2.0 ± 1.3	1.1 ± 0.2
# 17	13.5 ± 1.8	7.7 ± 2.5
Sildenafil	6.4 ± 1.9	3.6 ± 0.7
Vardenafil	7.6 ± 1.8	4.3 ± 1.0

substrates, and CYP inhibition varied. All 4 compounds got a ICM TOx Score of 0, and Compound 8 had a high drug likeness score, when compared to Vardenafil and Sildenafil.

3.2. In vitro (Figs. 3–5, Tables 3 and 4)

A single concentration (10 μM) of the 19 identified vardenafil analogues were tested for their ability to inhibit [^{3}H]-cGMP uptake by ABCC5 transport into inside-out vesicles (IOV). Sildenafil was introduced as a reference inhibitor and inhibited 83 % of cGMP uptake. In addition to sildenafil, a total of six compounds inhibited more that 50 % of cGMP uptake. These were compounds #6 (51 %), #8 (94 %), #9 (75 %), #11 (80 %), #16 (100 %) and #17(69 %). Thus, two of the compounds brought forward by VLS (#8 and #16) showed even higher potency than sildenafil. Compound #16 blocked the transport completely and was significantly ($p < 0.05$) more potent than sildenafil.

K_i-values of the 6 inhibitors achieving a 50 % or higher reduction of cGMP transport were calculated according to Cheng and Prusoff [21]; using IC50-values, substrate concentration of cGMP (2.0 μM) and Km of 2.6 μM from Orvoll et al. [19]. Sildenafil, which was used as reference inhibitor, showed a K_i-value of 3.6 μM, while the corresponding values of compounds 8 and 16 were 2.1 μM and 1.1 μM, respectively.

Table 4 shows information about bioactivity studies on compound 8, compound 16, Vardenafil and Sildenafil. No bioactivity studies were found on compounds 8 and 16, whereas the publications on bioassays studies on Vardenafil and Sildenafil were above hundreds.

4. Discussion

In the present study, we used molecular modeling techniques to construct an ABCC5 model and identify interactions with vardenafil analogues. This allowed us to determine potency and specificity of candidate drugs, of which the most promising were selected for further in vitro studies after synthesizing.

Table 4
Bioactivity studies on Compound 8, Compound 16, Vardenafil and Sildenafil. No bioactivity studies were found on Compounds 8 and 16.

Molecule	IUPAC	4 (Bioactivities)		6 (ADME-Tox)		Drug likeness
		Pubchem	ChEMBL	SwissADME	ICM Tox Score	
Compound 8	3-((4-(cyclopentyl-formyl)-piperazin-1-yl)-methyl)-9-methyl-2,4,8,9-tetraaza-bicyclo[4.3.0]nona-1(6),2,7-trien-5-one	No	No	GI absorption: High P-gp substrate: Yes No CYP inhibition	0	1.53409
Compound 16	3-(2-(4-bromo-phenylamino)-2-oxo-ethylsulfanyl)-9-(2-methoxy-phenyl)-2,4,8,9-tetraaza-bicyclo[4.3.0]nona-1(6),2,7-trien-5-one	No	No	GI absorption: High P-gp substrate: No CYP1A2 inhibitor CYP2C19 inhibitor CYP2C9 inhibitor CYP3A4 inhibitor	0	0.392151
Vardenafil	2-[2-ethoxy-5-(4-ethylpiperazin-1-yl)sulfonylphenyl]-5-methyl-7-propyl-3H-imidazo[5,1-f][1,2,4]triazin-4-one	418 bioassays 93 clinical trials	> 100	GI absorption: High P-gp substrate: Yes CYP2C9 inhibitor CYP3A4 inhibitor	0	0.909018
Sildenafil	5-[2-ethoxy-5-(4-methylpiperazin-1-yl)sulfonylphenyl]-1-methyl-3-propyl-6*H*-pyrazolo[4,3-d]pyrimidin-7-one	394 bioassays 229 clinical trials	> 100	GI absorption: High P-gp substrate: Yes CYP2C9 inhibitor CYP3A4 inhibitor	0	0.942503

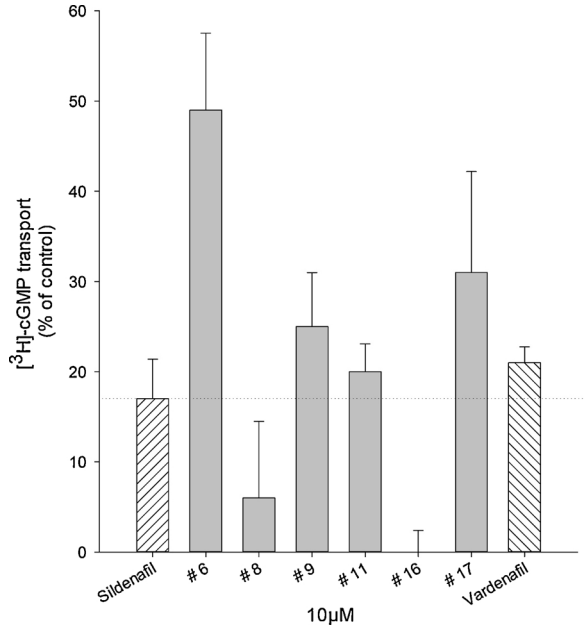


Fig. 3. Showing the ability of 10 μM of the most potent vardenafil analogues to inhibit [^{3}H]-cGMP uptake by ABCC5 transport in inside-out vesicles (IOV). Two of the compounds brought forward by VLS (#8 and #16) showed even higher potency than the reference compound sildenafil. Compound #16 blocked the transport completely and was significantly ($p < 0.05$) more potent than sildenafil.

Modeling of membrane transporters often implies low homology [22,23]. Further, the template quality must be considered, both in regard to low resolution, and the amphiphilic nature of membrane proteins that cause difficulties in experimental structure determination. Structural flexibility was accounted for when performing docking and VLS on our ABCC5 model. A crystal structure of a transporter may not be a realistic representation of the transporter in its native form, and transporters may undergo substantial conformational changes during the transport cycle. Large ranges of motion, changing the accessibility of the transporter from a cytoplasmic facing to an extracellular facing conformation, have been revealed from X-ray crystal structures of the bacterial ABC transporter lipid flippase, MsbA [24]. Induced-fit, demonstrated in a study of substrate-induced changes in ABCB1 [25], and conformational changes due to transport, may be an important part of ligand recognition. The energy-based torsional sampling ("fumigation") generated additional conformations of the ligand binding area of ABCC5, with lower energies than the starting model.

Insight into structural changes of the drug target for yielding a lower energy drug - drug target complex may elucidate how the conformation of the binding site contributes to the adoption of an energetically favorable complex. Ideally, these observations can aid to predict how a designed drug will fit into the drug target.

The VLS add-on to the Internal Coordinate Mechanics (ICM) program [12] (ICM-VLS) has previously been applied to identify new leads for a number of targets [26,27]. In the present study, the VLS docking correctly predicted six ligands as having a similar or higher binding affinity to ABCC5 compared to sildenafil. The most potent vardenafil analogues, compounds #8 and #16, showed K_i-values of 1.0–2.5 μM, lower than sildenafil (3.6 μM) and vardenafil (4.3 μM).

The high potency of compound #8 and particularly compound #16 suggest that they could have potential for use in clinical treatment. The primary indication for administering vardenafil is to treat erectile dysfunction, by reducing PDE5-mediated cGMP elimination in smooth muscle cells. Further elevation of intracellular cGMP is achieved with simultaneous inhibition of ABCC5. Several studies have therefore suggested that inhibition of ABC-transporters could be a valuable strategy for erectile dysfunction treatment [28,29].

In the present experiment, we used a human erythrocyte model to investigate cellular efflux of cGMP by ABC-transporters. This is a well-established model for estimating pharmacological modulation of cGMP efflux from human cells and show that systemic effect beyond impact on smooth muscle in the genitourinary system could be expected by ABCC5 inhibition. We therefore expect that the potent inhibition of cGMP efflux by compounds #8 and #16 will have systemic effects, giving treatment potential beyond alleviating erectile dysfunction.

High intracellular levels of cGMP promotes apoptosis and slows cell growth. This is underlined by that PDE5 and ABCC5 are increased in many cancer cells, suggesting a selection for increased export and degradation of cGMP in cancer cells [2,3]. Accordingly, the therapeutic potential of PDE5 and ABCC5-inhibition for anti-cancer treatment lies in the ability to increase intracellular cGMP levels, promoting apoptosis and slow cell growth in cancer cells.

In vitro studies indicate that ABCC5 is an important regulator of NO/cGMP signaling in cardiomyocytes, regulating intracellular cGMP levels together with PDE-mediated degradation [30]. Elevation of cGMP in cardiomyocytes is associated with a negative inotropic effect and protection against ischemia / reperfusion injury [31]. High expression of ABCC5 in vascular endothelial and smooth muscle cells is well known, where cGMP levels are important in regulating relaxation

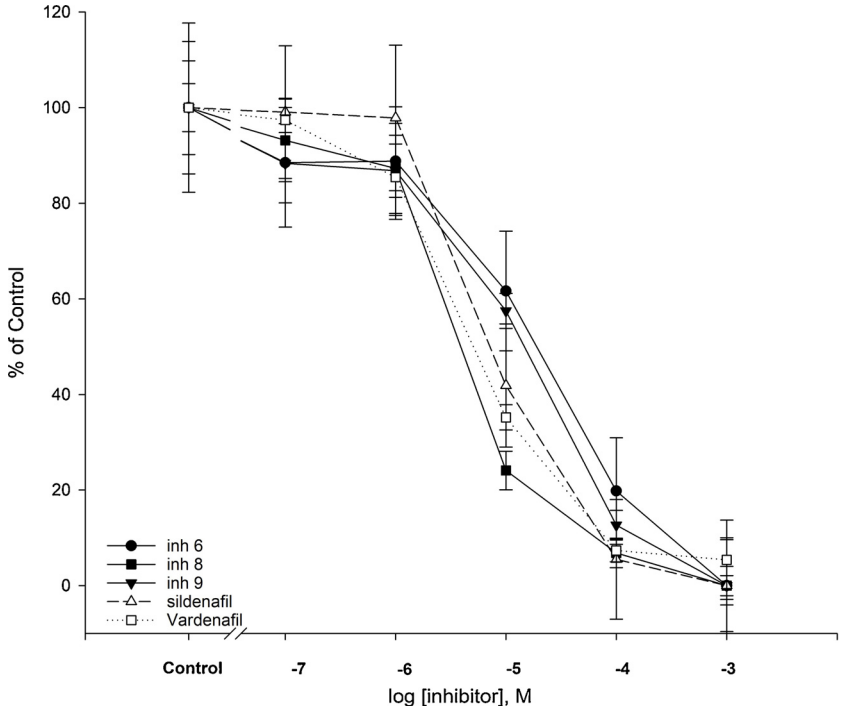


Fig. 4. IC50-curve for INH 6, 8 and 9, showing inhibition of ATP-dependent [3 H]-cyclic nucleotide accumulation in IOV. Inhibitor concentrations are in the range between 10-3 and 10-7 M. Control samples with no inhibitor added represent the maximum transport (100 % activity). The results are presented as mean values ± SEM from three separate experiments with a total of 9 parallels for each concentration of the inhibitors.

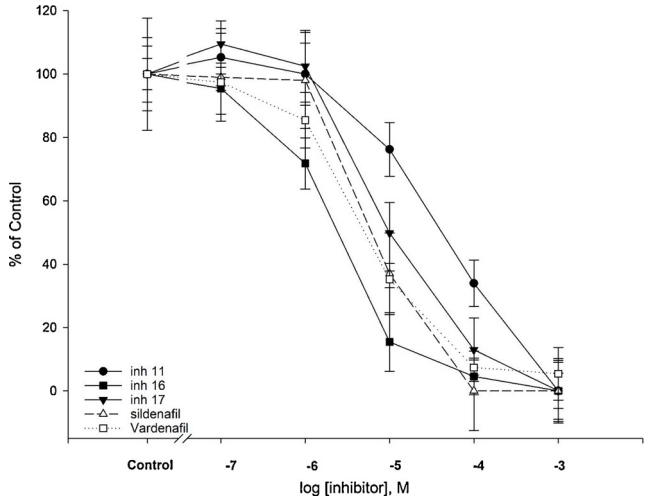


Fig. 5. IC50-curve for INH 11, 16 and 17, showing inhibition of ATP-dependent [3 H]-cyclic nucleotide accumulation in IOV. Inhibitor concentrations are in the range between 10-3 and 10-7 M. Control samples with no inhibitor added represent the maximum transport (100 % activity). The results are presented as mean values ± SEM from three separate experiments with a total of 9 parallels for each concentration of the inhibitors.

and thus arterial dilatation [30]. Potent ABCC5 inhibitors like compound #8 and #16 could therefore have a potential in treatment of cardiovascular disorders. In healthy athletes, vardenafil and sildenafil reduce systolic pulmonary pressure and enhance cardiac output during exercise [32] and in patients with chronic systolic heart failure, sildenafil improves cardiac index [33].

5. Conclusion

Our findings show that computational screening correctly identified vardenafil-analogues that proved to be potent inhibitors of cGMP efflux-pumps from cytosol. Compounds #8 and #16 provided a more efficient cGMP efflux inhibition than sildenafil and vardenafil, known as potent ABCC5 inhibitors. Both compounds could have a substantial clinical potential. Our results advocate further investigation of their pharmacokinetic and pharmacodynamic properties.

Declaration of Competing Interest

The authors have no conflict of interests.

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