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Comparison of different heterocyclic scaffolds as substrate analog PDE5 inhibitors

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Abstract—Several different heterocyclic systems were compared as PDE5 inhibitor scaffolds. In addition to the known 3*H*-imidazo[5,1-*f*][1,2,4]triazin-4-ones and pyrazolopyrimidinones, isomeric imidazo[1,5-*a*][1,3,5]triazin-4(3*H*)-ones were also shown to be potent and selective PDE inhibitor scaffolds with in vivo activity. SAR trends were elucidated for sulfonamide derivatives with generality across different scaffolds.

Heterocyclic systems of the purinone type play an important role in cellular biochemistry. Isosteric heterocycles have been used as drugs to treat pathological situations where purinones are involved in various indications, e.g., antivirals, metabolic disorders (gout) and cancer.[1a–c]

Intracellular levels of purine second messengers cAMP (**1**) and cGMP (**2**) (Fig. 1) are regulated via synthesis and degradation to AMP and GMP, respectively. Degradative enzymes for these second messengers are phosphodiesterases (PDEs). PDEs constitute an enzyme superfamily with at least 11 members and various isoforms differing by their substrate acceptance (either cAMP, cGMP or both).

Previously we have disclosed potent and selective PDE5 inhibitors of the 3*H*-imidazo[5,1-*f*][1,2,4]triazin-4-one type.[2] It has been demonstrated that PDE5 inhibitors carrying this structural fragment are consistently more potent than congeners of the pyrazolopyrimidine type, e.g., Sildenafil®. This finding cannot be explained satisfactorily on the basis of X-ray crystallograhpic data, since both PDE5 inhibitors, Vardenafil® (**3**) and Sildenafil® (**4**), show a similar binding pattern in the published X-ray structure[3], whereas at least the tenfold difference in potency has been shown to be due to the different heterocyclic systems.[4]

Therefore, we were interested in bringing out a more comprehensive comparison of different heterocyclic systems as PDE inhibitors. Herein, we present our results with different heterocyclic scaffolds as PDE inhibitors, as well as some fundamental substituent effects on inhibitory potency and selectivity (Fig. 1).

Figure 2 gives an overview of the investigated heterocyclic scaffolds.

Syntheses of 3*H*-imidazo[5,1-*f*][1,2,4]triazin-4-ones as PDE inhibitors have been published.[5] The synthesis of the corresponding purinone systems was carried out, as previously reported (Scheme 1).[6,7]

Pyrimidinone ring closure is effected by amino carboxamidoimidazoles after acylation with benzoic acid chlorides under basic conditions. The corresponding 2-amino-3-cyanoimidazoles can be used also, in these instances cyclisation is best performed in the presence of hydrogen peroxide. Alternately, cyclisation can be achieved from the amino-carboxamido heterocycle and benzoic acid esters using potassium *tert*-butoxide.[8,9]

There are many examples of pyrazolopyrimidine systems as PDE inhibitors or kinase inhibitors reported in the literature.[10–14] In brief, 2-amino-3-carbamoylpyrazoles can be generated from hydrazines and alkoxyethylidenemalonodinitrile during a Thorpe cyclisation and subsequent hydrolysis of the nitrile to the amide (Scheme 2). Again, acylation and ring closure are achieved under similar conditions as for the purinones.

Keywords: PDE5 inhibition; Heterocycles.
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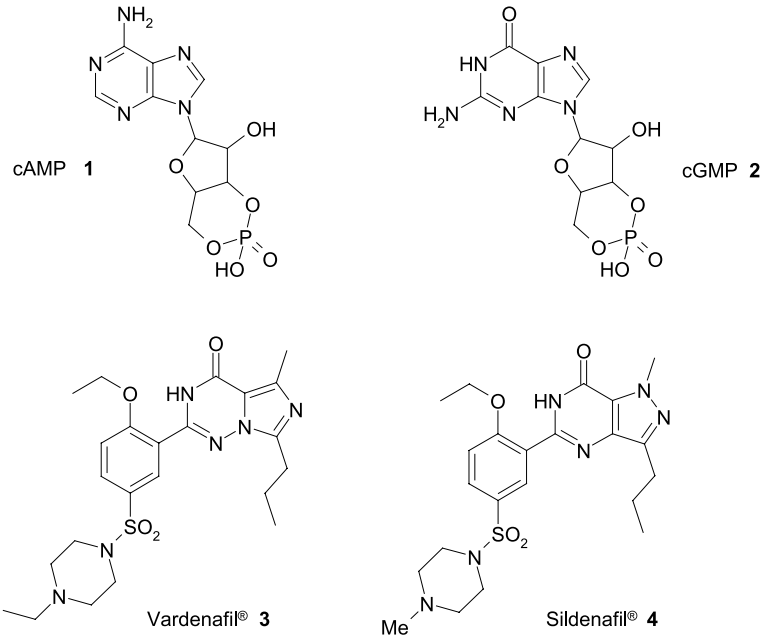


Figure 1. cAMP (**1**), cGMP (**2**), Vardenafil® (**3**), and Sildenafil® (**4**).

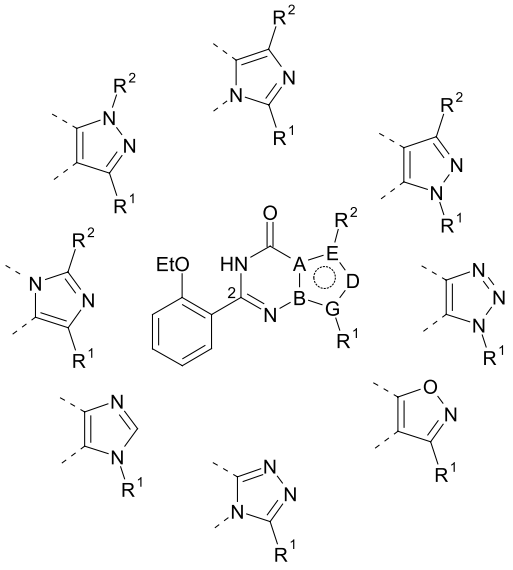


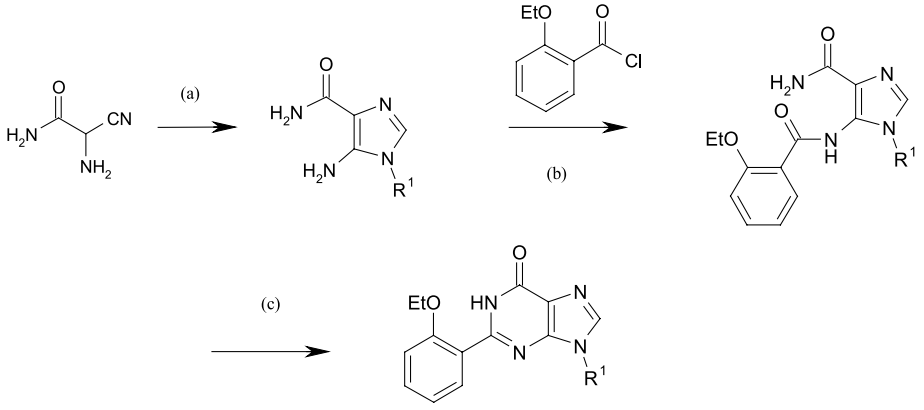
Figure 2. Overview of investigated heterocyclic PDE inhibitor scaffolds.

The isomeric pyrazolopyrimidinones, as found in Sildenafil®, were prepared as previously described (Scheme 3).[15,16]

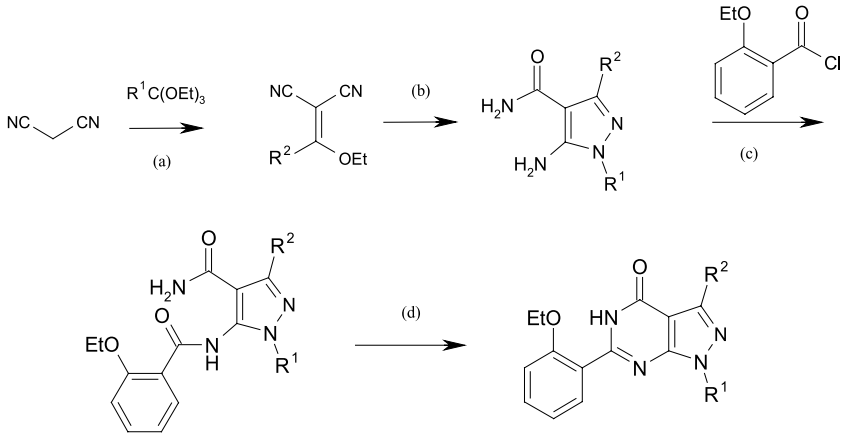
Continuing in the series of heterocyclic scaffolds with two nitrogen atoms in the five-membered part, imidazo[1,5-*a*][1,3,5]triazin-4(3*H*)-ones also were prepared (Scheme 4).

Imidazo[1,5-*a*][1,3,5]triazin-4(3*H*)-ones have been described in the literature in the context of purinone isosteres, albeit only with either hydrogen or heteroatom substitution at the 2-position.[17]

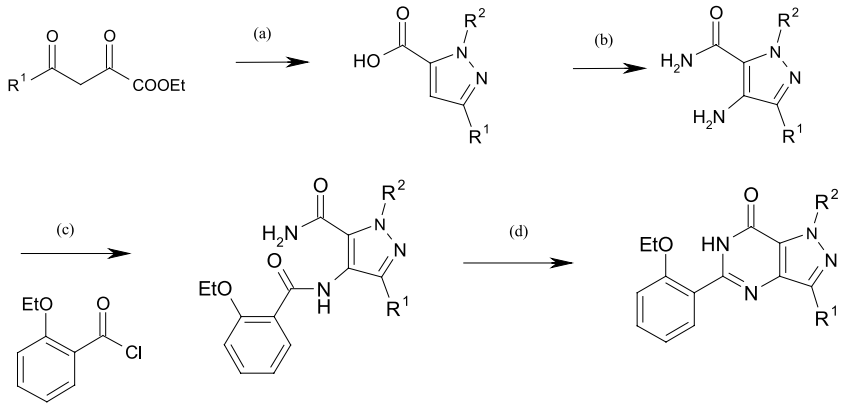
However, the previously reported synthesis proved to be largely applicable to 2-aryl substituted cases as well. Alkylation of 2-acylamino-ethylcyanoacetate, followed by reaction with aryl amidines, delivers an aminopyrimidinone that undergoes an interesting rearrangement to yield the desired heterocyclic scaffold.[18]



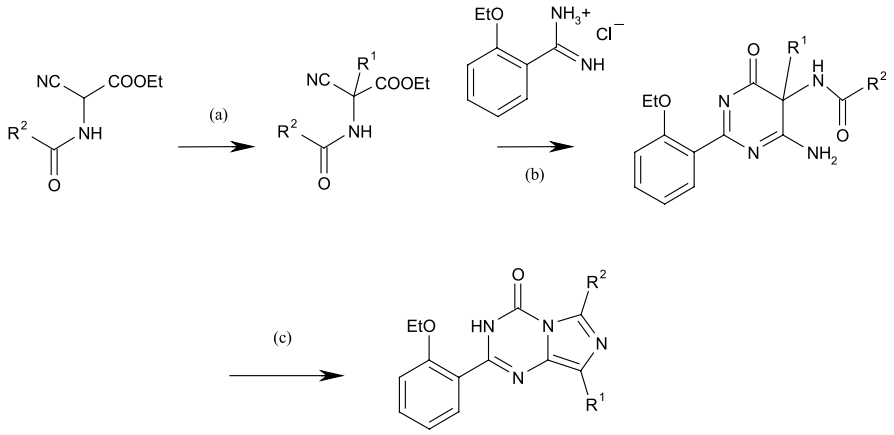
Scheme 1. Synthesis of alkoxyphenylpurinones. Reagents and conditions: (a) i. $HC(OEt)_3$, AcCN, RF, ii. R^1NH_2, RT; (b) Py, toluene, DMAP cat. or NaH, THF, RT; (c) MeOH, NaOH or K_2CO_3.



Scheme 2. Synthesis of phenylpyrazolopyrimidinones. Reagents and conditions: (a) RF, neat; (b) i. MeOH, NH_2NHR^1, RF, ii. NH_3, H_2O_2, EtOH, 48 h; (c) Py, toluene, DMAP cat.; (d) MeOH, NaOH, RF.



Scheme 3. Synthesis of phenylpyrazolopyrimidinones. Reagents and conditions: (a) i. N_2H_4, ii. alkylation, e.g., $Me_2(SO_4)$, iii. NaOH; (b) i. HNO_3, H_2SO_4, ii. NH_3, iii. $SnCl_2$; (c) DCM, NEt_3, DMAP; (d) NaOH, EtOH.

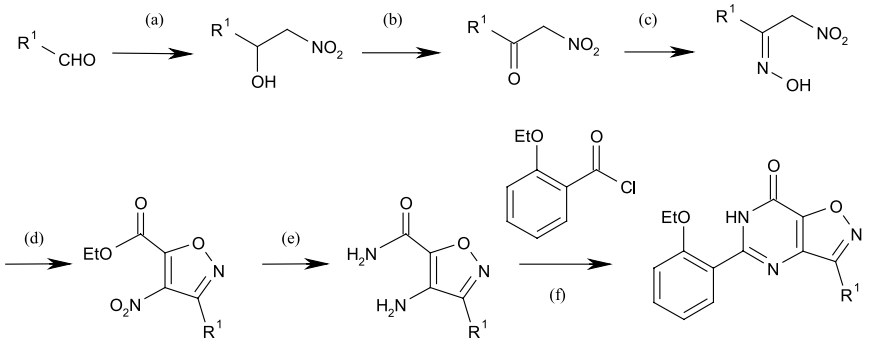


Scheme 4. Synthesis of imidazo[1,5-*a*][1,3,5]triazin-4(3*H*)-ones. Reagents and conditions: (a) R^1Br, NaOEt; (b) NaOEt; (c) i. Py, $TMSC_l$, RT ii. HMDS, RF.

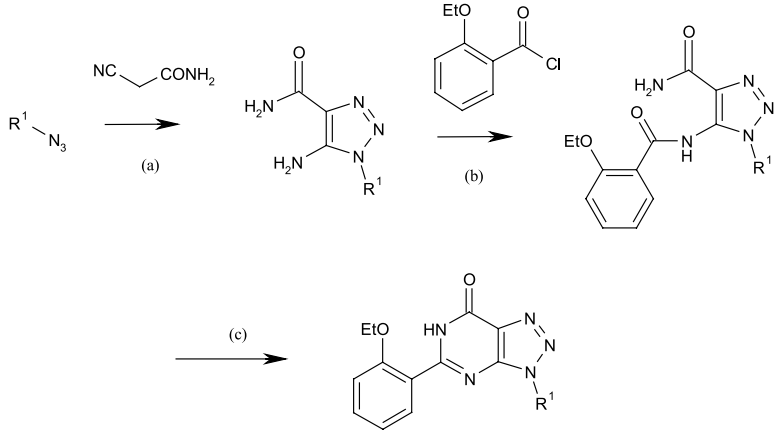
Recently, cycloalkyl substituted imidazo[1,5-*a*][1,3,5] triazin-4(3*H*)-ones have been reported as PDE7 inhibitors in the patent literature.[19]

Members of the isoxazolo[4,5-*d*]pyrimidin-7(6*H*)-ones also were prepared as purinone isosteres with two heteroatoms in the five-membered ring (Scheme 5).[20]

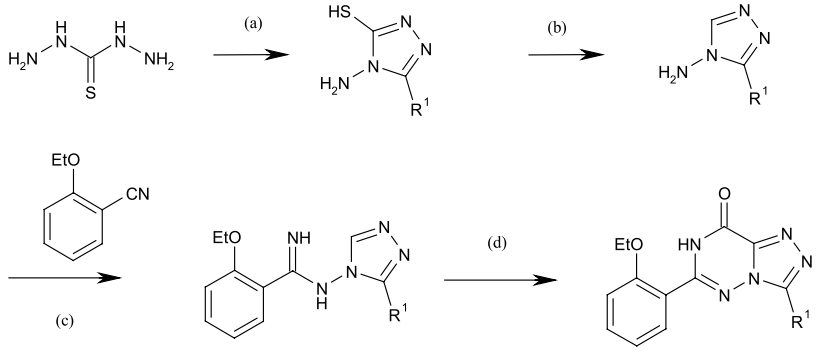
Finally, two heterocyclic scaffolds with three nitrogen atoms in the five-membered ring were prepared: 3-alkyl-3,6-dihydro-7*H*-[1,2,3]triazolo[4,5-*d*]pyrimidin-7-ones, carrying the heterocyclic core of Zaprinast®, were prepared either by alkylation of Zaprinast® or by treating an appropriately substituted azide with carbamoylmalononitrile in the presence of base



Scheme 5. Synthesis of phenyl-isoxazolo[4,5-*d*]pyrimidin-7(6*H*)-ones. Reagents and conditions: (a) CH_3NO_2, KF, propanol; (b) $K_2Cr_2O_7$, NBu_4HSO_4, CH_2Cl_2; (c) $NH_2OH.H_2SO_4$, Tol/EtOH, RF; (d) $ClCOCO_2Et$, NEt3, Et_2O; (e) i. NH_3 in MeOH, RT, ii.Zn, NH_4Cl, water; (f) i. Py, DMAP cat., 60 °C, ii. MeOH, Na_2CO_3, 4d RF.



Scheme 6. Synthesis of 3-alkyl-3,6-dihydro-7*H*-[1,2,3]triazolo[4,5-*d*]pyrimidin-7-ones. Reagents and conditions: (a) i. NaOEt; (b) Py, cat. DMAP; (c) NaOH, EtOH.



Scheme 7. Synthesis of [1,2,4]triazolo[3,4-*f*][1,2,4]triazin-8-(7*H*)-ones. Reagents and conditions: (a) R^1COOH, 165 °C, neat; (b) H_2O_2, HOAc, RF; (c) NaH, dioxane, 90 °C, 16 h; (d) i. NaH, $CO(OEt)_2$, 90 °C, 16 h ii. 2-(OEt)-Ethanol, RF, 16 h.

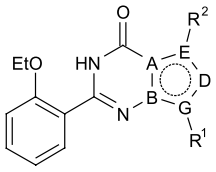
(Scheme 6), followed by acylation and ring closure.

Examples of [1,2,4]triazolo[3,4-*f*][1,2,4]triazin-8-(7*H*)-ones have been described in the literature but mostly with heteroatom substitution in position 2.[21] They were prepared according to Scheme 7.[22]

Thiocarbonohydrazide is condensed with the corresponding acid to yield a 4-amino-5-alkyl-4*H*-1,2,4-triazole-3-thiol that in turn is treated with a 2-alkoxy benzoic acid nitrile after desulfurisation. Thermal ring closure is achieved after acylation with diethylcarbonate. This scaffold constitutes an overlay of the nitrogen atom pattern of Vardenafil® and Sildenafil®.

For the different heterocyclic systems, only activity data were generated, no attempt was made to add in vitro PK parameters to these SAR studies. Table 1 gives PDE inhibitory activity of several different heterocyclic scaf-

Table 1. IC_{50} data[23] for 2-ethoxyphenyl PDE5 inhibitor heterocyclic scaffolds



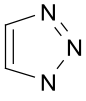
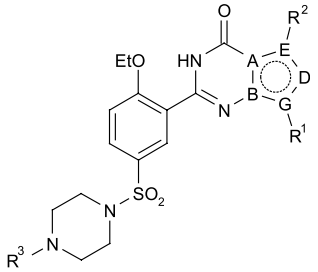
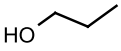
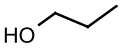
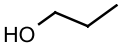
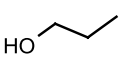
Compound	Heterocycle	R^1	R^2	PDE1 (IC_{50}, nM)	PDE5 (IC_{50}, nM)
6		Propyl	—	10[2]	
7		*c*-Propyl	—	1000	300
8		*c*-Butyl	—	1000	100
9		*c*-Pentyl	—	500	50
10		Propyl	Me	—	1[10]
11		Propyl	Me	300	5
12		*c*-Pentyl	Me	30	5
13		Me	Me	1000	200
14		Propyl	Me	790[15a]	27[15a]
15		Propyl	H	—	50
16		Propyl	Me	200	40
17		*c*-Pentyl	Me	40	20
18		Propyl	—	—	300
19		*c*-Pentyl	—	800	500

Table 1 (*continued*)

Compound	Heterocycle	R^1	R^2	PDE1 (IC_{50}, nM)	PDE5 (IC_{50}, nM)
20		*c*-Pentyl	—	50	200
21		*c*-Pentyl	—	200	20

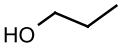
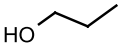
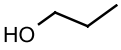
Table 2. IC_{50} data for sulfonamide PDE5 inhibitors



Compound	Heterocycle	R^1	R^2	R^3	PDE1 (IC_{50}, nM)	PDE5 (IC_{50}, nM)
22		Propyl	—	Me	—	10[2]
23		Propyl	Me	Me	300[2]	2[28]
24		Propyl	Et	Me	>1000	8
25		Propyl	Me		20	5
26		Me	Me		1000	10
27		*c*-Pentyl	Me	Me	10	1
28		*c*-Pentyl	Me		10	1
29		*c*-Pentyl	Et	Me	>1000	7
30		Propyl	Me	Me	500	10
31		*c*-Pentyl	Me		30	10

(*continued on next page*)

Table 2 (*continued*)

Compound	Heterocycle	R^1	R^2	R^3	PDE1 (IC_{50}, nM)	PDE5 (IC_{50}, nM)
32		Propyl	Me	Me	1000	8
33		Propyl	Et	Me	>1000	20
34		*c*-Pentyl	Et	Me	>1000	40
35		Propyl	Me	Me	400[15a]	6.6[15a]
36		*c*-Pentyl	Me		10	5
37		*c*-Pentyl	—		50	50
38		*c*-Pentyl	—		30	10

folds. 3*H*-imidazo[5,1-*f*][1,2,4]triazin-4-ones prove to be the most potent scaffolds (**11**–**13**), followed by pyrazolopyrimidinones (**10** and **14**, **15**). Heterocycles with three heteroatoms in the five- membered part were less active (**18**–**20**). Cyclopentyl substitution in R1 leads to a higher PDE1 inhibitory activity (**12**, **17**, **20**), a trend that is also valid for sulfonamide derivatives (vide infra). Selectivity varies only slightly between the different unfunctionalized scaffolds, with the notable exception of [1,2,4]triazolo[3,4-*f*][1,2,4]triazin-8-(7*H*)-ones, which show a higher inhibitory activity for PDE1.

Only the most potent scaffolds were selected for further derivatisation to evaluate substituent effects on activity and selectivity. Table 2 depicts SAR trends for arylsulfonamide derivatives—obtained by chloro-sulfonation and subsequent reaction with an amine—of several heterocycles. As previously observed, 3*H*-imidazo[5,1-*f*][1,2,4]triazin-4-ones are the most potent PDE5 inhibitors in our studies (**23**–**29**). The isomeric imidazo[1,5-*a*][1,3,5]triazin-4(3*H*)-ones that are less potent (**30**) however demonstrate in vitro activity comparable to those of purinones (**22**) and pyrazolopyrimidinones (**32, 35**) and show in vivo oral efficacy in a rabbit model of erectile dysfunction.[24]

The potency of [1,2,4]triazolo[3,4-*f*][1,2,4]triazin-8-(7*H*)-ones (**37**) and isoxazolopyrimidinones (**38**) did not improve upon addition of sulfonamide substituents.

Several interesting substituent effects were observed that seem to constitute general SAR trends for distinct scaffolds.

Ethyl substitution the R^2 position consistently diminishes PDE1 activity and to a lesser extent PDE5 activity (**24**, **29**, **33**, and **34**).

Cyclopentyl at the R^1 position (**27**, **31**, **36**, **37**, and **38**) increases PDE1 inhibitory activity however, in combination with ethyl as R^2 (**29**, **33**), this effect is overcome by the influence of the ethyl group. This effect is reminiscent of the effect of an ethyl group in a recently published purine PDE5 inhibitor series.[25]

The reduction in PDE1 and PDE5 inhibition seen with the ethyl analogues can be explained examining the published X-ray structures for PDE1B[26] and PDE5A.[27] Docking the R^2 ethyl compounds (e.g., **29**) into PDE5A shows potential steric clashes between the R^2 substituent and Ala767. In addition, the H-bonding network around Tyr612 can become perturbed, leading to a slight overall loss in activity. In human PDE1A, B and C and bovine PDE1A, and B the amino acid corresponding to PDE5-Ala767 is a histidine (PDB1B-His373). The R^2 ethyl compounds (e.g., **29**) potentially intrude about 2 Å less deep into the binding pocket than Vardenafil® to avoid steric clashes with this histidine. The associated loss of contacts (e.g., to Gln421) explains

the drastically reduced binding affinity of the R^2-ethyl derivatives to PDE1.

Methyl as R^1 has a similar effect as ethyl substitution in R^2, PDE1 and PDE5 activities are reduced (**26**) putatively due to a loss in hydrophobic interactions, e.g., with PDE5A-Leu725 and Phe786 and PDE1B-Met336 and Phe392.

However, the interpretation of binding potency or inhibitory activity on the basis of structural data has to be done with caution especially for closely related derivatives that do not differ by orders of magnitude in their respective biological activity.

Overall these sulfonamide derivatives followed the same potency order as the unsubstituted scaffolds. Activity in most cases is increased with polar sulfonamide residues on the aromatic ring. Selectivity can be tuned with both parameters: substitution pattern and heterocyclic scaffold.

A new PDE5 inhibitor class with oral efficacy—imidazo[1,5-*a*][1,3,5]triazin-4(3*H*)-ones—was identified during these comparative studies.

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