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PDE5 inhibitors: An original access to novel potent arylated analogues of tadalafil

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Abstract—A method to access totally new analogues of tadalafil was explored. The Buchwald reaction was adapted and used to replace the methyl group of tadalafil by various aryl groups. Inhibition potencies on PDE5 of these analogues were determined and proved to be comparable to the one of tadalafil. Using the same route, compounds with the same level of activity but improved water solubility were produced by introducing a pyridine or a pyrimidine ring. This original route also opens access to new unpatented compounds.

Phosphodiesterases (PDEs) affect the cellular levels of the cyclic nucleotides cAMP and cGMP, which are involved in many physiological processes such as immunity, cardiac- and smooth-muscle contraction, apoptosis, ion-channel conductance and growth control. Inhibiting these enzymes is therefore an attractive strategy in the development of smooth-muscle relaxants and drugs to treat inflammatory diseases, asthma, depression and many other diseases.[1] The cGMP-specific PDE5 is the major PDE isozyme in the *corpus cavernosum*, and controls penile erection. PDE5 inhibitors used in the clinic amplify the NO-cCMP pathway and enhance the normal process leading to penile erection. The launch of Viagra™ (sildenafil, **1**) (Figure 1)[2] as the first oral treatment for male erectile dysfunction revolutionized the treatment of this condition. Today, Levitra™ (vardenafil, **2**), a closely related structural analogue of sildenafil, and Cialis™ (tadalafil, **3**), a tetrahydro-β-carboline derivative,[3,4] are also available (Fig. 1).

These three PDE5 inhibitors exhibit different PDE selectivity profiles, in particular in their relative affinities for PDE5, PDE6 (found in photoreceptor cells) and PDE11 (of yet unclear biological function). Since tadalafil was launched, multiple analogues were synthesized in order to optimize the selectivity profile against these PDEs.[5] The *N*-methyl group of tadalafil is directed toward a large unoccupied pocket of the active site of PDE5. Our objective was to enlarge the structural diversity of this tadalafil-derived family through the replacement of the *N*2-methyl group by novel aryl substituants, and to evaluate the compatibility of these modifications with PDE5 inhibitory activity.

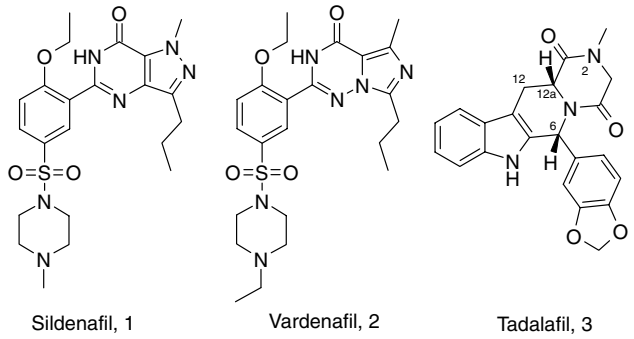

Figure 1. Structure of sildenafil, vardenafil and tadalafil.

Although the *N*2-phenyl derivative **6a** (Scheme 1) displays a significant inhibitory activity against PDE5,[6] no other arylated analogues of tadalafil were reported. This important gap in the structural space surrounding tadalafil can be explained by synthetic difficulties. In the only synthetic route to tadalafil analogs described

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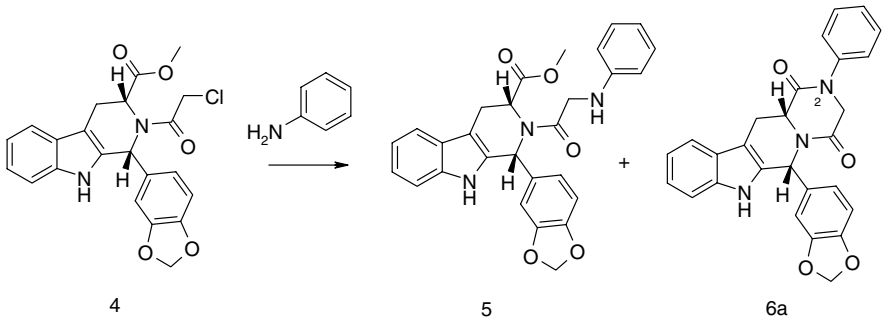
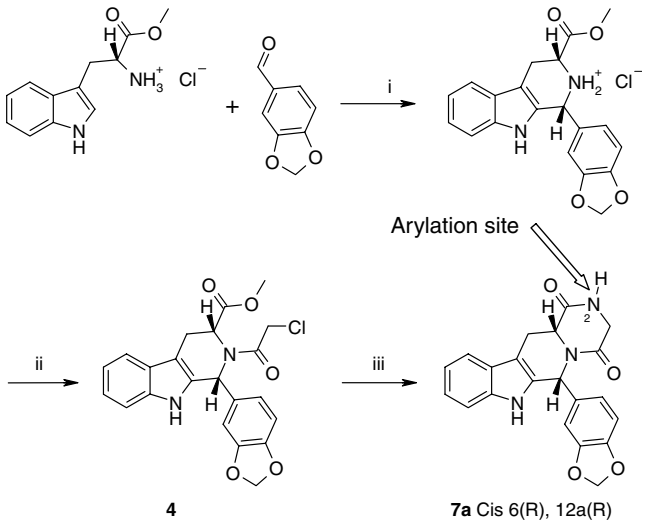


Scheme 1. Reagents and conditions: (i) MeOH reflux, NEt_3, 10 days, <5% yield.

so far, the terminal step is the formation of the diketopiperazine ring from an N-substituted glycyltetrahydrocarboline generated in situ (Scheme 1). This cyclisation is very efficient when N2 is not substituted or alkylated, whereas *N*-phenylglycyltetrahydrocarbolin **5** is only very slowly converted to the desired compound **6a** (only 5% yield after 10 days of reaction).

To fill the diversity gap in this family, we propose to introduce the N2 substituent at the last step of the synthesis, using the mild conditions described by Buchwald et al. for the arylation of amides and lactams.[7] We would thus close the diketopiperazine ring to form Nortadalafil **7a** in high yield, and introduce the aromatic substituent afterward, tapping in a large array of commercially available aryl or heteroaryl halides. Our starting material Nortadalafil **7a** (in the 6(*R*), 12a(*R*) cis configuration) was easily prepared in two steps from D-tryptophane methyl ester, piperonal, chloroacetylchloride and ammonia (Scheme 2).

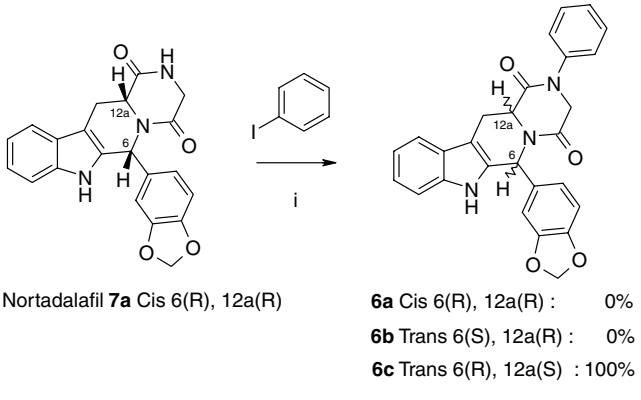
The procedures described by Buchwald et al. for the arylation of amides, carbamates and lactams typically use 0.2–10 mol% of cuprous iodide, 5–20 mol% of a diamine ligand and a mineral base such as K_3PO_4, K_2CO_3 or Cs_2CO_3. The nature of the base is important and the best results are described with K_3PO_4. Reactions are best performed in toluene, DMF, THF or dioxane, at temperatures ranging from 25 to 110 °C. In such conditions, lactams are arylated in good yields.[7]



Scheme 2. Reagents and conditions: (i) isopropanol reflux, 92%; (ii) chloroacetylchloride, chloroform, triethylamine, −10 °C, 85%; (iii) NH_3, methanol, 45 °C, 70%.

These conditions were explored using iodobenzene as a model arylating reagent. It is known that only the cis isomers significantly inhibit PDE5[4] in this family. Therefore, a particular attention has been paid to the configuration of the tetrahydrocarboline system. Indeed, upon heating in basic conditions, one can expect the formation of the thermodynamically favored trans isomer by epimerization of one of the chiral centers of Nortadalafil **7a**, leading to compound **6b** or **6c** represented in Scheme 3. The conditions described by Buchwald gave in quantitative yield a product arylated exclusively at the targeted N2 position, leaving untouched the indolic nitrogen, described as potentially reactive.[8] However, NMR analyses revealed that the product isolated was in the *trans* configuration. Measurement of optical rotation showed that the product formed was **6c** and not **6b** (Scheme 3).

In order to avoid the deleterious isomerisation, we lowered the temperature and the amount of base while using a higher stoichiometry of catalyst. The reaction mixtures were analyzed by NMR after simple workup in ammo-



Scheme 3. Arylation of Nortadalafil. Reagent and conditions: Nortadalafil (1 equiv, 133 mM), CuI 10 mol%, (±)-*trans*-1,2-diaminocyclohexane 20 mol%, iodobenzene (1 equiv), K_3PO_4 (3 equiv), dioxane, at 110 °C.

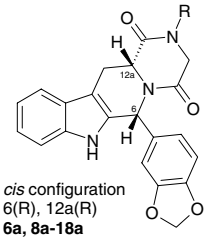
nia, removing the base and the copper complexes. *cis*:*trans* ratios displayed in Table 1 were evaluated directly from NMR spectra.

Table 1. Reaction conditions

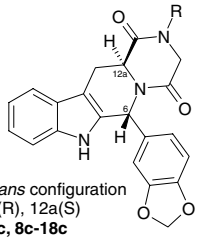
Compound	Temp. (°C)	Catalyst equiv[a]	% Conversion	Reaction time	**6a** (*cis*)/ **6c**(*trans*)
1	110	0.1	100	3 h	0/100
2	28	1.6	90	5 days	50/50
3	14	2.0	80	15 days	95/5

[a] Ratio ligand/CuI = 2.

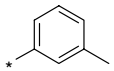
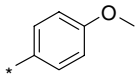
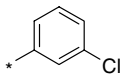
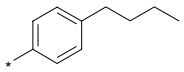
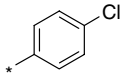
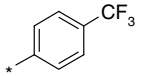
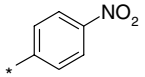
Table 2. Yields of *cis* and *trans* arylated Nortadalafil derivatives



cis configuration
6(R), 12a(R)
6a, 8a-18a



trans configuration
6(R), 12a(S)
6c, 8c-18c

Compound	R	Isolated yield (%) cis	Isolated yield (%) trans	*cis*:*trans* ratio
6		80	0	—
8		52	0	—
9		47	5	9.4
10		65	16	4.0
11		60	17	3.5
12		48	15	3.2
13		28	15	1.9
14		43	23	1.9
15		30	20	1.5
16		25	18	1.4
17		17	18	0.9
18		11	49	0,2

Decreasing temperature allowed a reduction of epimerization but still half of the final product was epimerized leading to a 50:50 mixture of *cis*:*trans* products. Even if long time reaction were required, a further decrease of the temperature to 14 °C (solidification of dioxane) proved to be a good compromise to prepare arylated analogs of tadalafil in the cis configuration.

Conditions (3)[9] from Table 1 were used to prepare a set of 11 analogues of tadalafil. Arylated compounds were isolated in moderate to high yields (35–80%), after thick layer chromatography (Table 2).

Depending on aryl group introduced, various *cis*:-*trans* ratio were obtained. Interestingly, epimerization was reduced with electron-donating rings and increased significantly with electron-withdrawing rings such as 4-nitrophenyl **18**, 3,5-pyrimidyl **17**, 4-trifluoromethyl **15**, and in lesser extend 3-pyridyl **14** (*cis*:-*trans* ratio, respectively, 0.2; 0.9; 1.5 and 1.9). This observation is consistent with an epimerization of carbon 12a (and not 6) which acidity is increased in function of the electron-withdrawing property of the aryl ring.

All these new unpatented tadalafil analogues with cis configuration were evaluated for PDE5 inhibition in a competitive immunoassay using bovine PDE5[10] (Table 3).

Our results show that aryl substituents are globally well tolerated (IC_{50}: 18–377 nM). Both electron-donating and -withdrawing groups are tolerated at meta or para positions. Hydrophobic substituents seem to be unfavorable (*para*-trifluoromethyl **15a**, *para*-chloro **13a**, *para*-butyl **12a**). Interestingly, heterocycles can advantageously replace the benzene ring. Indeed a 5-fold increase in activity is observed when benzene is replaced by thiophene in compound **11a**. Compounds bearing a pyridine or pyrimidine (respectively **14a** and **17a**) are also more active that the parent compound **6a**. Although the best activity is obtained with the para-methoxyphenyl substituent (**9a**, 16 nM), the series of more polar heteroaryl-substituted compounds appears more interesting, in particular **14a** and **17a**, both more

Table 3. Inhibitory activity for compounds **6a** and **8a**–**18a**

Compound	IC_{50} PDE5 nM ± standard error	
Tadalafil **3**	3.3	±0.6
6a	91.0	±10
8a	68.0	±18
9a	16.0	±4
10a	141.0	±30
11a	18.0	±4
12a	152.0	±52
13a	134.0	±43
14a	28.0	±5
15a	377.0	±55
16a	57.0	±9
17a	53.0	±7
18a	122.0	±55

Table 4. Kinetic aqueous solubility of some heteroarylated compounds compared to that of tadalafil and *N*-phenylnortadalafil

Compound	Aqueous solubility PBS buffer pH 7.4 (μM)
Tadalafil	9.7
6a	2.4
14a	33
17a	25

potent than the parent compound and more soluble[11] (Table 4).

In summary, we have adapted a method using a copper-I catalyzed arylation to prepare novel analogues of tadalafil where the methyl group is replaced by various aryl or heteroaryl groups. Depending on the nature of the aryl substituent, the unwanted epimerization of the tetrahydrocarboline system was partially or totally avoided by lowering both temperature and the amount of base and increasing the amount of catalyst. The synthesized compounds were evaluated as PDE5 inhibitors and displayed significant activities comparable to that of tadalafil although weaker. This route can be used to access unpatented analogues of tadalafil and could be crucial for the identification of more selective, more soluble PDE5 inhibitors in a competitive context.

Acknowledgments

We are grateful to the institutions that support our laboratory (Inserm, Université de Lille2 and Institut Pasteur de Lille). This work was funded by 'Région Nord-Pas-de-Calais' and EC. We acknowledge the expert support of the 'Laboratoire d'Application RMN (LARMN)' team (U.Lille2) for NMR spectroscopy. We thank André Tartar and Hélène Gras-Masse for thoughtful comments and discussions.

Supplementary data

Supporting information available: Measurement of optical rotation of compounds **6b**, **6c** and compound from Scheme 3. Experimental procedures and analysis of compounds **4**–**17**. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2006.10.069.

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9. *General procedure for arylation of Nortadalafil:* (6*R*,12a*R*)-6-Benzo[1,3]dioxol-5-yl-2,3,6,7,12,12a-hexahydro-pyrazino[1′, 2′:1,6]-pyrido[3,4-*b*]indole-1,4-dione (Nortadalafil, **6a**) (133 mM, 1 equiv), CuI (2 equiv), K_3PO_4 (2 equiv), (±)-*trans*-1,2-diaminocyclohexane (4.0 equiv) and iodo derivatives (1 equiv) were dissolved in anhydrous dioxane in inerted vessels and stirred at 14 °C for 14 days. The reaction mixture was diluted with ethyl acetate and washed with NH_3/water (5/95) and water. The organic layer was dried over $MgSO_4$, evaporated to dryness and purified by TLC.
10. Compounds were evaluated with a time-resolved fluorescence resonance energy transfer-based assay (HTRF® technology) using the cGMP quantification kit (Cisbio international # 62GMPPEB) and bovine cGMP-specific PDE5 (Calbiochem # 524715). This quantitation method relies on the competition between free cGMP and a conjugate cGMP-fluorophore for the binding to a cGMP-specific antibody labelled with europium cryptate. Fixed amounts of bovine PDE5 (2 μL) and cGMP (150 nM) were incubated 24 h at 37 °C in the presence of varying inhibitors concentrations. Theses assays were performed in black half area 96-well microplates (Corning # 3694). Assay volume was 36 μL and the assay buffer contained 50 mM Tris/HCl, pH 7.4, and 6 mM $MgCl_2$. At the end of the incubation, the detection reagents were added according to the manufacturer's protocol. After 1 h incubation at room temperature in the dark, the HTRF® signals were later read using a Victor3V (Wallac 1420 Multilabel Counter; Perkin-Elmer). Each inhibition assay was performed in duplicate and each well read twice. IC_{50} values were obtained using the curve fitting software XLfit4.2 (IDBS).
11. Kinetic solubilities were determined as previously described in: Poulain, R.; Horvath, D.; Bonnet, B.; Eckhoff, C.; Chapelain, B.; Bodinier, MC.; Deprez, B. *J. Med. Chem.* **2001**, *44*, 3378.