Debenzylative cycloetherification as synthetic tool in the diastereoselective synthesis of 3,6-disubstituted hexahydro-2H-furo[3,2-b]pyrroles, PDE1 enzyme inhibitors with antiproliferative effect on melanoma cells

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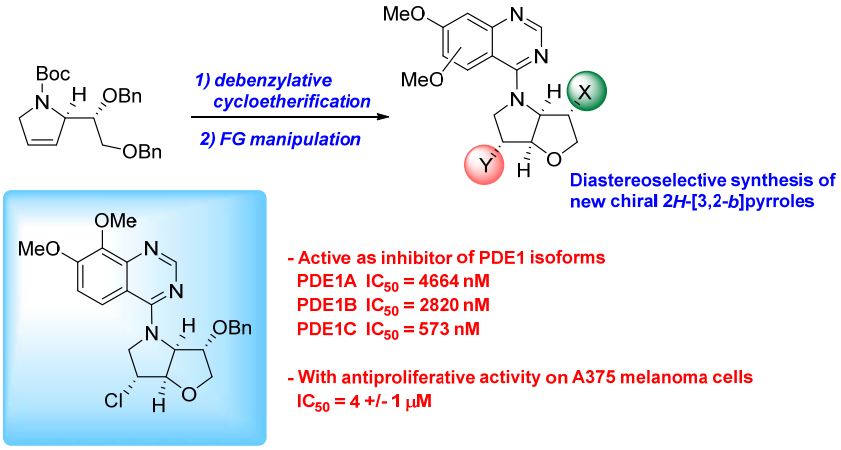
Abstract



Two series of novel chiral hexahydro-2*H*-furo[3,2-*b*]pyrroles, 4-(7,8-dimethoxyquinazolin-4-yl) series A and 4-(6,7- dimethoxyquinazolin-4-yl) series B, were synthesized in enantiomerically pure form and evaluated for their inhibitory effects upon phosphodiesterase 1 (PDE1) and phosphodiesterase 4 (PDE4) as well as for their inhibitory activity on cell proliferation in A375 melanoma and 3T3-fibroblast cells *in vitro*. Key steps of synthesis were i) diastereoselective nucleophilic addition of vinylmagnesium bromide to *N*-allylimine derived from conveniently protected D-glyceraldehyde, ii) ring closing metathesis, iii) debenzylative cycloetherification and iv) aromatic nucleophilic substitution. Some of the obtained compounds were proven to be active as inhibitors of PDE1 isoforms, with IC50 values in the high nanomolar/low micromolar concentration range, and showed antiproliferative activity on A375 melanoma cells.

INTRODUCTION

3',5'-Cyclic adenosine monophosphate (cAMP) and 3',5'-cyclic guanosine monophosphate (cGMP) act as second messengers in hormone-regulated

processes and play an important role in signal transduction, synaptic transmission and other biological processes.[1] Phosphodiesterases (PDEs) are the only enzymes that catalyse the hydrolysis of cyclic nucleotides. They are grouped into 11 broad families, PDE1–PDE11, which in turn are further divided into isoforms on the basis of encoding gene (e.g., PDE4A-D) and splicing isoforms (e.g., PDE4D1 – PDE4D9); so that more than 100 PDE isoforms can be distinguished. Some are cAMP specific (PDE4, 7, and 8), other specifically metabolize cGMP (PDE5, 6, and 9), and some possess dual specificity (PDE1, 2, 3, 10, and 11).[2] Cyclic nucleotides play an important role in a variety of cellular mechanisms and, therefore, regulation of PDEs hydrolytic activity is a very important matter as its alteration can affect multiple cellular processes. That is why there has been an increasing interest in the development of phosphodiesterase inhibitors as tools for specific manipulation of cyclic nucleotide signalling for therapeutic use.[3] In this context, the development of PDE inhibitors, which are selective for PDE families, isoforms and splicing isoforms, could be lead to novel specific therapeutic strategies for various pathologies.

Several studies have revealed that the levels of PDE activity in a variety of tumours is altered, which affects the ratio of cGMP to cAMP and, thereby, their regulatory roles. The observed effects of PDE inhibition in *in vitro* and *in vivo* studies in tumour models suggest a potential role for PDE inhibitors as anticancer drugs.[4]

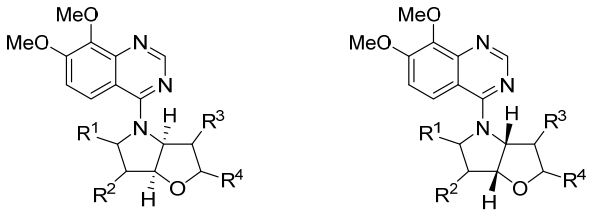
The calcium-dependent PDE1 family was first isolated in 1970 from rat brain[5] and bovine brain[6] and consists of several isoforms that are mainly expressed in brain, cardiac tissues, and smooth muscles.[3e] Their inhibition may provide new therapeutic strategies for various diseases.[4c,7] Moreover PDE1 has been characterized in

melanoma cell lines and it has been shown that PDE1A inhibition exerts anti-proliferative effects.[8]

It has been reported that compounds with an hexahydro-2*H*-furo[3,2-*b*]pyrrole core (Figure 1) are PDE1 inhibitors that are of potential utility for the treatment of neurodegenerative and psychiatric disorders.[9] When $R^1 = R^2 = R^3 = R^4 = H$ compound with 3a*S*,6a*S* configuration showed a higher ability to inhibit PDE1 isoforms than compound with 3a*R*,6a*R* configuration (IC_{50} (nM) ranging from 120 to 160 versus IC_{50} (nM) ranging from 2800 to 3700, depending on the subtype).

All these findings prompted us to develop a stereoselective synthesis of novel compounds with a (3a*S*,6a*S*)-hexahydro-2*H*-furo[3,2-*b*]pyrrole core and to evaluate their activity as PDE1 inhibitors and as anticancer agents.

Figure 1. General structure of PDE1 enzyme inhibitors with the hexahydro-2*H*-furo[3,2-*b*]pyrrole core.[9]

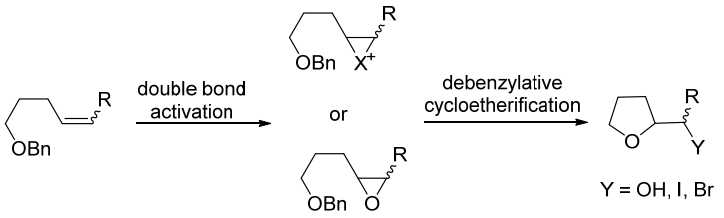


3a*S*,6a*S* 3a*R*,6a*R*

RESULTS AND DISCUSSION

Among the different synthetic strategies to build the tetrahydrofurane ring[10] debenzylative cycloetherification[10c] is an underexploited approach for the synthesis of tetrahydrofuran-containing molecules. In this context, the activation of alkenes in

δ position with respect to the benzyl ether by halogenation (usually iodination and occasionally bromination) or epoxidation leads to tetrahydrofurans through debenzylative cycloetherification (Scheme 1).

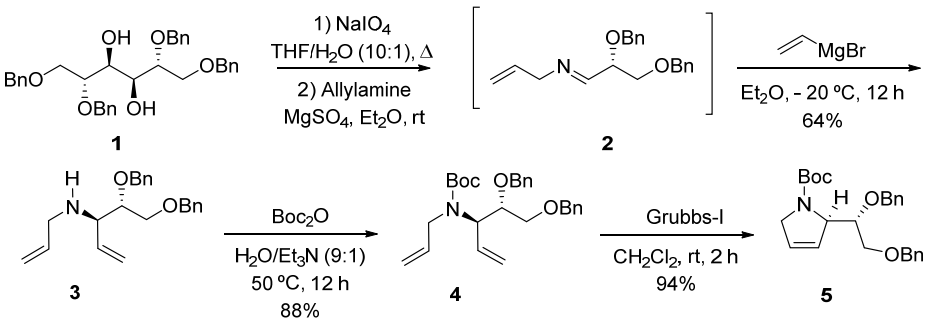


Scheme 1. Debenzylative cycloetherification of δ benzyloxyalkenes

During our the development of versatile synthetic methodologies for the synthesis of chiral nitrogen-containing heterocycles using chiral imines that are readily available from renewable sources as starting materials, we have found[11] that ring closing metathesis of diallylic amines obtained from 2,3-di-*O*-benzylglyceraldehyde *N*-benzylimines provides 2,5-dihydropyrroles, in which the alkene moiety is in the δ position with respect to the primary benzyloxy group. This has provided the basis for the development of a new synthetic procedure to prepare key intermediates in the synthesis of new potential PDE1 inhibitors with the hexahydro-2*H*-furo[3,2-*b*]pyrrole core.

Synthesis of 2,5-dihydropyrrole **5** was performed as outlined in scheme 2**.** To gain access to diallylamine **3**, we added vinylmagnesium bromide to *N*-allylimine **2** - obtained *in situ* by oxidative cleavage of 1,2,5,6-tetra-*O*-benzyl-D-mannitol **1** with sodium periodate and performed a subsequent reaction with allylamine. When the imine **2** reacted with vinylmagnesium bromide in ether at -20 ºC, the desired

diallylamine **3** with *syn* configuration was obtained with total diastereoselectivity after 12 h reaction.[12] This stereochemical outcome is in accordance with previous results for the addition of organometallic reagents to *N*-benzyl imines derived from glyceraldehyde with benzyl ether as hydroxyl protecting group.[11-13] As performance of ring closing methathesis (RCM) using secondary amines as substrates is generally improved by *N*-protection,[14] diallylamine **3** was converted into its corresponding *N*-*tert*-butylcarbamate. Addition of excess of di-*tert*-butyl dicarbonate to a methanol/triethylamine (9:1) solution of **3** at 50 ºC led to *N*-Boc diallylamine **4** in 88% isolated yield. Then 2,5-dihydropyrrole **5** was cleanly obtained in 94% yield from a solution of compound **4** in dichloromethane at room temperature using first generation Grubbs Catalyst (10% mol) to promote ring closing methathesis.

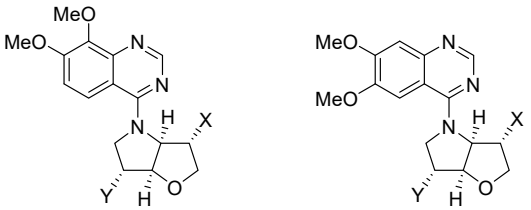


Scheme 2. Synthesis of 2,5-dihydropyrrole **5**.

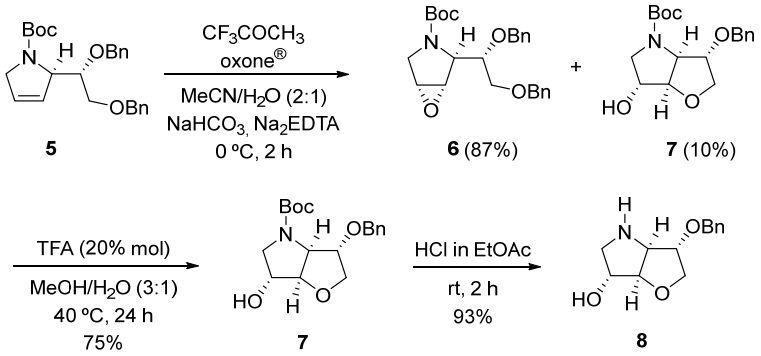
With 2,5-dihydropyrrole **5** in hand and taking into account previous results on biological activity[9], we continued with the synthesis of two series of novel hexahydro-2*H*-furo[3,2-*b*]pyrrole derivatives, *N*-7,8-dimethoxyquinazolin-4-yl (series A) and *N*-6,7-dimethoxyquinazolin-4-yl (series B), of 3a*S*,6a*R* configuration with different substituents at C3 and C6 position (Figure 2). The construction of the required

hexahydro-2*H*-furo[3,2-*b*]pyrrole core was performed by debenzylative cycloetherification procedures using different C=C activation modes depending on the nature of the heteroatom to be introduced at C6 position.

Figure 2. General structure of hexahydro-2*H*-furo[3,2-*b*]pyrrole derivatives of series A and B

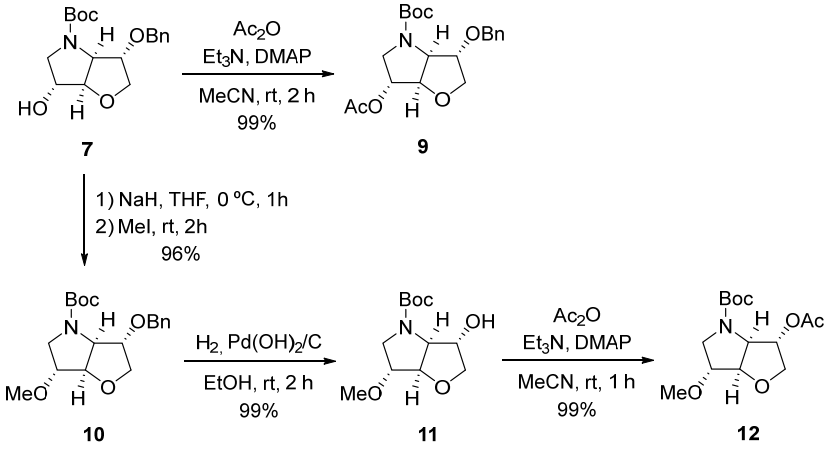


First we performed dioxirane epoxidation of **5** using 3-methyl-3-(trifluoromethyl)dimethyldioxirane,[15] generated *in situ* by oxidation of 1,1,1-trifluoroacetone with oxone®. The reaction in acetonitrile/water (2:1) at 0 ºC provided the desired epoxide **6** derived from the addition of oxygen to the double bond on the side opposite to the substituent at C2 with total diastereoselectivity in 87% isolated yield. We also observed the formation of a small amount of compound **7** derived from debenzylative cycloetherification. Upon treatment with a catalytic amount of trifluoroacetic acid in methanol/water (3:1) at 40 ºC, compound **6** evolved to the formation of **7**, which was isolated in 75% yield. Hydrolysis of **7** with hydrogen chloride in ethyl acetate at room temperature yielded 3-benzyloxy-6-hydroxy hexahydro-2*H*-furo[3,2-*b*]pyrrole **8** with a 93% isolated yield. (Scheme 3)



Scheme 3. Synthesis of 3-benzyloxy-6-hydroxy hexahydro-2*H*-furo[3,2-*b*]pyrroles.

From compound **7** we obtained other hexahydro-2*H*-furo[3,2-*b*]pyrroles with oxygenated substituents on C-3 and C-6 according to Scheme 4.

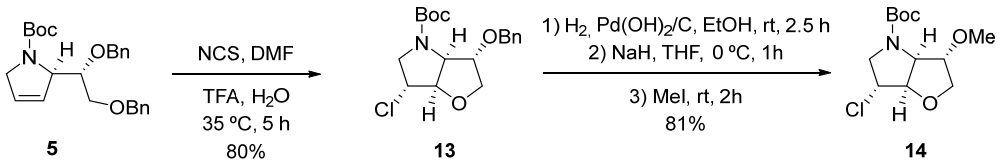


Scheme 4. Synthesis of 3,6-dihydroxy hexahydro-2H-furo[3,2-b]pyrrole derivatives.

Chlorine is present in a significant number of bioactive compounds and nowadays plays a prominent role in drug design.[16] This prompted us to explore activation of the C=C bond by chlorination. In this way, a chlorine atom is installed at C6. This position

in the bicyclic system is essentially unreactive towards nucleophilic substitution, which prevents any behaviour of the compound as alkylating agent.

Reaction of compound **5** with *N*-chlorosuccinimide in acetonitrile at room temperature led to the desired 6-chloro hexahydro-2*H*-furo[3,2-*b*]pyrrole **13** but conversion was incomplete. When the reaction was performed in dimethylformamide at 35 ºC and in the presence of trifluoroacetic acid as an additive, we observed total conversion of the starting material and compound **13** was obtained in 80% yield. As far as we know, this is the first report in the literature on the synthesis of a 2-chloromethyl tetrahydrofuran by debenzylative cycloetherification. Next hexahydro-2*H*-furo[3,2-*b*]pyrrole **14** was obtained from compound **13** according to Scheme 5.

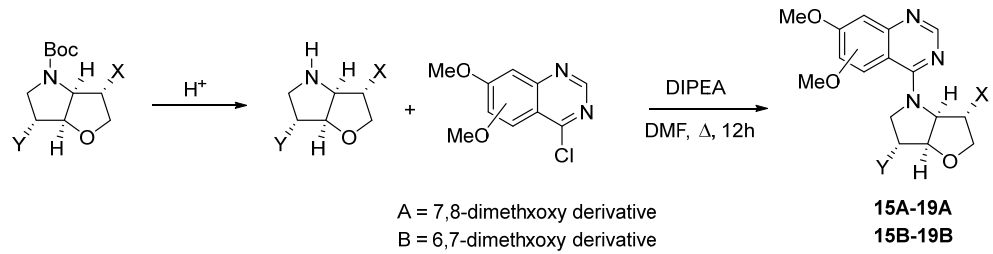


Scheme 5. Synthesis of 3-hydroxy-6-choro hexahydro-2H-furo[3,2-b]pyrrole derivatives.

N-Boc hexahydro-2H-furo[3,2-b]pyrroles **7**, **9**, **12**, **13** and **14** were hydrolysed and coupled with 4-chloro-7,8-dimethoxyquinazoline (A) and 4-chloro-6,7-dimethoxyquinazoline (B) to prepare the corresponding compounds of series A and B, respectively. The nucleophilic aromatic substitution reaction was performed by heating the amine and the chloroquinazoline at the appropriate temperature in the presence of diisopropylethylamine and using dimethylformamide as solvent (Table 1). As a general trend, we observed that 4-chloro-7,8-dimethoxyquinazoline was

more reactive than 4-chloro-6,7-dimethoxyquinazoline providing higher yields working at lower temperatures.

Table 1. Synthesis of compounds of the series A and B



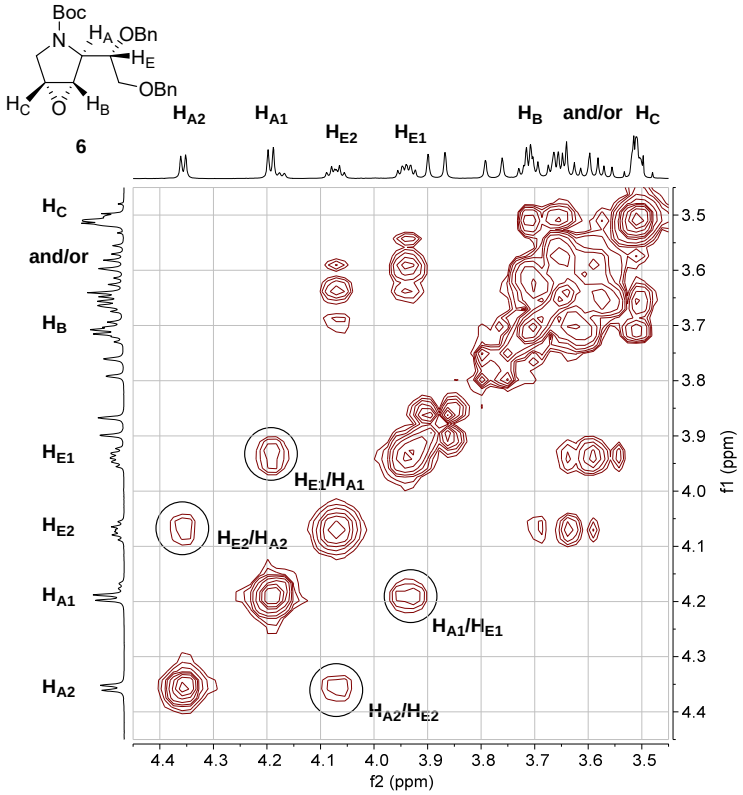
Substrate	X	Y	Hydrolysis conditions [a]	quinazoline	T [ºC]	Product	Overall Yield [%]
7	BnO	OH	I	A	60	**15A**	93
7	BnO	OH	I	B	80	**15B**	70
9	BnO	OAc	II	A	60	**16A**	96
9	BnO	OAc	II	B	80	**16B**	71
12	AcO	OMe	III	A	45	**17A**	86
12	AcO	OMe	II	B	80	**17B**	49
13	BnO	Cl	III	A	45	**18A**	91
13	BnO	Cl	III	B	70	**18B**	55

14	MeO	Cl	III	A	45	**19A**	77
14	MeO	Cl	III	B	70	**19B**	42

[a] Hydrolysis conditions: I, HCl in EtOAc, rt; II, TFA, CH_2Cl_2, rt, III, HCl in EtOAc, 0 ºC

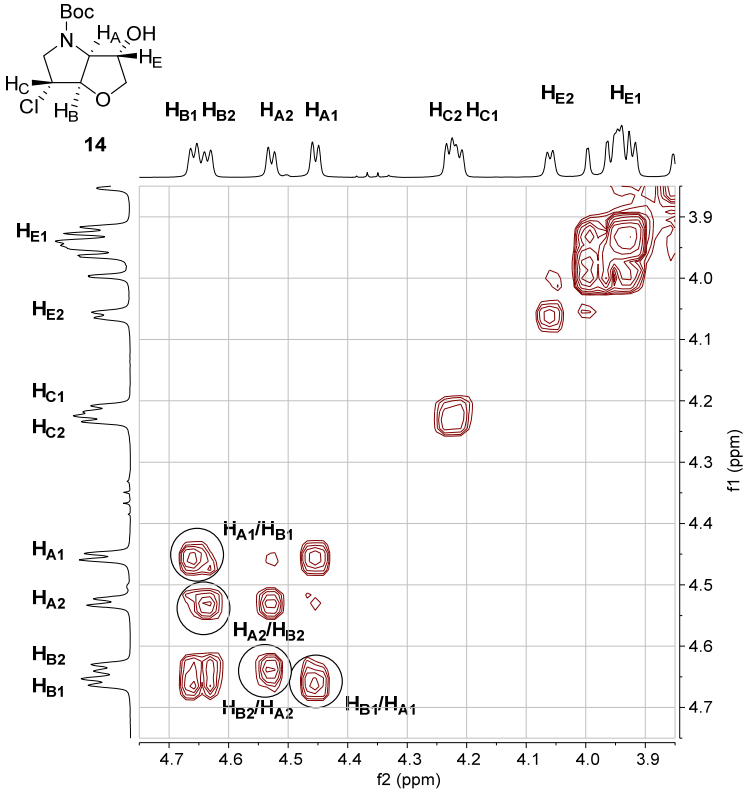
The structures and stereochemistries of epoxide **6** and compounds obtained by debenzylative cycloetherification process were unambiguously established on the basis of ^{1}H-^{1}H NMR coupling interactions and X-ray diffraction analysis. In epoxide **6**, the H_A resonance of both rotamers[17] appears as a doublet at 4.19 and 4.36 ppm with a coupling constant of 3.6 and 4.0 Hz respectively corresponding the $^{3}J_{H\text{-}H}$ coupling with benzylic proton H_E as determined in the COSY spectrum. The absence of coupling between vicinal nuclei ($^{3}J_{H\text{-}H} \approx 0$ Hz) across a single bond (Figure 3) is due to a spatial disposition with protons with a 90° dihedral angle which in compound **6** is a clear indicative of the *trans* disposition between H_A and H_B.

Figure 3. DQF-COSY of compound **6**



For compound **14** (obtained from **13**), H_A and H_B resonances of both rotamers appear as doublets due to its mutual $^3J_{H\text{-}H}$ coupling as determined in the COSY spectra. The absence of vicinal coupling in signals corresponding to H_B and H_C nuclei in compounds **14** (Figure 4) is again a clear indicative of the *trans* disposition between both protons with a 90° dihedral angle.

Figure 4. DQF-COSY of compound **14**



The structure of compound **7** was unambiguously determined by X-ray crystallography.

We evaluated compounds **15A**-**19A** and **15B**-**19B** for their ability to inhibit PDE1 and PDE4. Furthermore, we tested most compounds of series A and series B for their ability to inhibit cell proliferation in A375 melanoma cell lines and 3T3 fibroblast cells. Inhibition of PDE1 and PDE 4 enzymatic activities was initially evaluated as a single point at a concentration of 10 μM using standard *in vitro* enzymatic assays.[18] Compounds with high inhibitory activity were selected to determine their IC_{50} values. As can be seen from the data in Table 2, most of the assayed hexahydro-2*H*-furo[3,2-*b*]pirroles were more potent against PDE1 isoenzyme than against PDE4 isoenzyme with **18A** being the most active compound. The potency of **18A** in inhibiting PDE1C isoform is higher than in inhibiting PDE1A and PDE1B isoforms. In addition compounds **17B** and **19B** preferentially inhibit the PDE1C isoform of PDE1.

Table 2. Inhibitory activity of compounds of the series A and B

Compound	PDE1A (% inh @ 10 μM)	PDE1A (IC_{50}, nM)	PDE1B (% inh @ 10 μM)	PDE1B (IC_{50}, nM)	PDE1C (% inh @ 10 μM)	PDE1C (IC_{50}, nM)	PDE4 (% inh @ 10 μM)
15A	32		22		68		0
15B	22		11		19		14
16A	22		19		36		11
16B	8		–2		12		9
17A	14		16		12		0
17B	48		39		87	2167	0
18A	70	4664	72	2820	97	573	27
18B	28		22		56		24
19A	41		31		56		13
19B	30		40		83	2367	3

With the exception of compound **17A**[19], we tested the compounds for anti-proliferative activity in the 3T3 fibroblast cell line and the A375 melanoma cell line, using the Janus-Green *in vitro* assay.[20] Cell proliferation and survival depicted in Figure 5A and 5B show that none of the compounds tested at 10 μM were cytotoxic *per se* in both lines. However, in 3T3 fibroblasts, **18A** virtually abolished cell proliferation, while **18B** had a lower anti-proliferative activity (Figure 5A, left panel). **15A**, **15B**, **16A**, **16B**, **19A** and **19B** had no statistically significant activity (Figure 5A, both panels). In A375 melanoma cells, we saw the same activity profile, but potencies were generally higher and **18A** virtually abolished cell proliferation, while **18B** had a lower anti-proliferative activity. Like in 3T3 fibroblasts, **19A** and **19B** had no statistically significant activity. The most active compound **18A** was assayed over a concentration range of 100 nM - 50 μM and we found significant anti-proliferative activity in A375 melanoma cells at 1 μM, again, a strong anti-proliferative activity at 10 μM, and a profound anti-proliferative activity and overall lower survival at 50 μM, the highest concentration tested. We calculated an IC_{50} of 4 +/- 1 μM (Figure 5C).

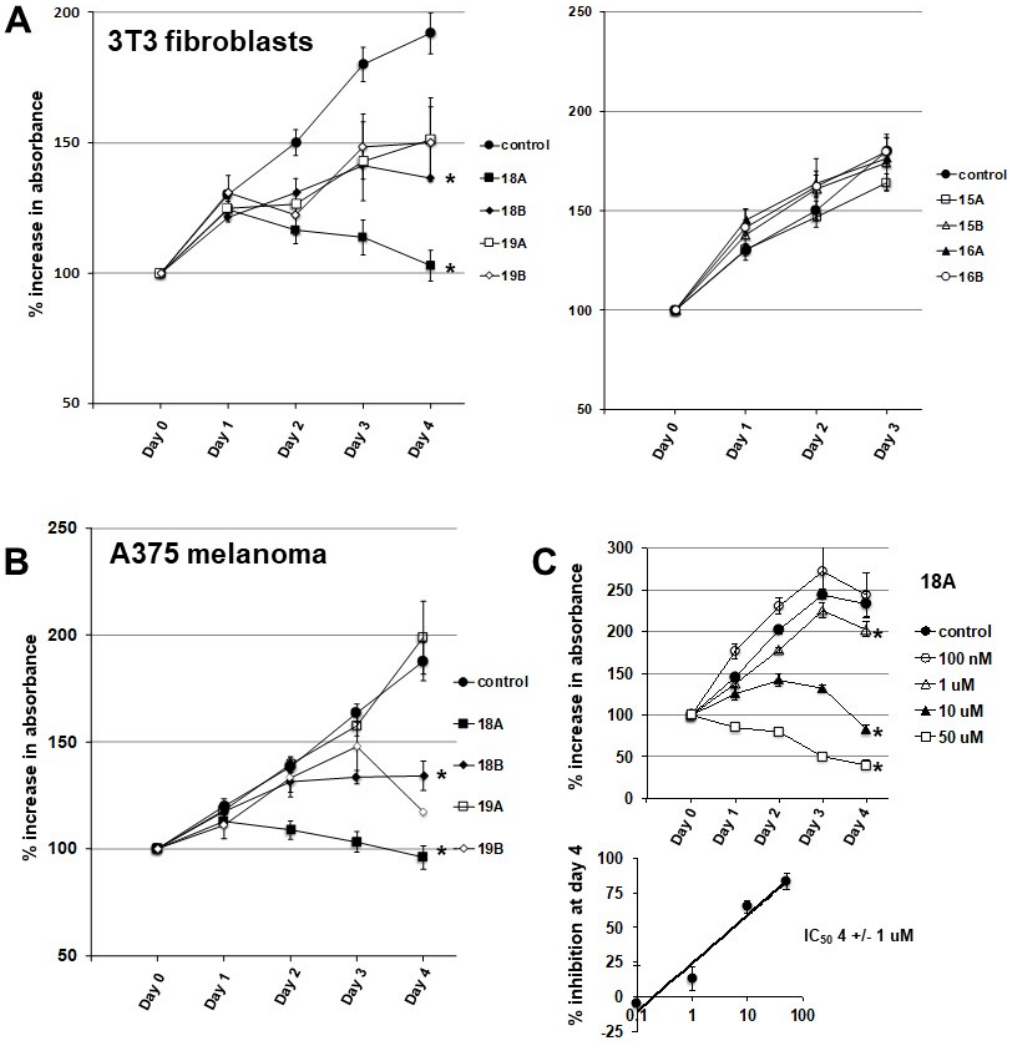


Figure 5. *In vitro* anti-proliferative activity of hexahydrofuropyrroles: A) Time course of inhibition of proliferation in 3T3 fibroblasts. B) Time course of inhibition of cell proliferation in A375 melamoma cells. C) Concentration-dependence of **18A** and IC_{50}. Note that at 50 uM absorbance value below initial values (day 0) indicate a reduced survival. Data are mean +/- SEM; n=4-30 measurements from 1-5 independent experiments. *P <0.05 vs. control (0.1 % DMSO as vehicle), Student´s T test.

In summary, we have shown debenzylative cycloetherification is a powerful synthetic tool in the highly diastereoselective synthesis of 3,6-disubstituted hexahydro-2*H*-furo[3,2-*b*]pyrroles with four stereocenters. This structural motif is present in compounds with inhibitory activity against PDE1 enzyme with potential use as medicaments for the treatment of neurodegenerative and psychiatric disorders.[9] Optimization of the reactions conditions allowed the development of a new and efficient activation of the alkene in δ position with respect to the benzyl ether that allows the introduction of a chlorine atom in 3 position of the bicyclic core. Some of the obtained compounds were proven to act as inhibitors of PDE1 isoforms being more active against PDE1C and revealed antiproliferative activity on A375 melanoma cells.

EXPERIMENTAL SECTION

General Details. Unless otherwise specified, all reagents were obtained from commercial suppliers and were used without purification. For anhydrous conditions, reactions were carried out under Ar in solvents dried using a Solvent Purification System (SPS). Whenever possible, the reactions were monitored by TLC. TLC analysis was performed on precoated silica gel polyester plates with an F_{254} indicator and products were visualized using UV light (254 nm) and ninhydrin, anisaldehyde, potassium permanganate or ethanolic phosphomolybdic acid solutions followed by heating. Column chromatography was performed on silica gel (60, 40−63 μm) with air pressure.

Melting points were determined in open capillary tubes and are not corrected. FT-IR spectra of oils were recorded as thin films on NaCl plates and FT-IR spectra of solids were recorded on pressed KBr pellets, ν_{max} values expressed in cm^{-1} are given for the main absorption bands. Optical rotations were measured on a digital polarimeter at λ 589 nm and 25 ºC in cells with 1 or 10 cm path length, $[\alpha]_D$ values are given in 10^{-1} deg·cm^2·g^{-1} and concentrations are given in g/100 mL. ^{1}H NMR and ^{13}C NMR spectra were acquired in deuterated solvent at room temperature unless otherwise stated at 400 and 100 MHz, respectively using a 5-mm probe. All chemical shifts (δ) are reported in parts per million (ppm) with the solvent resonance as the internal standard,[20] and coupling constants (J) in hertz (Hz). High-resolution mass spectra were recorded from methanolic solutions on a MICROTOF-Q (quadrupole time-of-flight) micro instrument using the positive electrospray ionization mode (ESI+). The

X-ray diffraction data were collected at room temperature on a four-circle diffractometer, using graphite-monochromated Mo-$K\alpha$ radiation (λ = 0.71073 Å).

(*3R,4S*)-*N*-Allyl-4,5-bis(benzyloxy)pent-1-en-3-amine (3)

Solid $NaIO_4$ (3.15 g, 14,74 mmol) and water (3.7 mL) were added successively to a stirred solution of 1,2,5,6-tetra-*O*-benzyl-D-mannitol (4.0 g, 7.37 mmol) in THF (35 ml) and the resulting mixture was vigorously stirred at room temperature for 1–2 h. The reaction mixture was filtered and the filtrate was concentrated *in vacuo*. The obtained crude was immediately dissolved in anhydrous diethyl ether (25 mL) and anhydrous $MgSO_4$ (3.55 g, 29.5 mmol) and allylamine (842 mg, 14.74 mmol) were successively added to the resulting solution. The reaction mixture was stirred at room temperature for 2 h, filtered and concentrated *in vacuo*. The resulting crude imine was dissolved in anhydrous diethyl ether (65 mL), cooled to –20 ºC, and then slowly added under an argon atmosphere to a stirred 1.0 M solution of vinylmagnesium bromide in THF (29.5 mL, 29.5 mmol) at –20 ºC. Stirring was continued for 12 h at the same temperature. The reaction mixture was quenched at 0 ºC by slow addition of water (40 mL) and filtered through Celite® pad. The organic layer was separated and the aqueous layer was extracted with ethyl ether (2 x 80 mL). The combined organic layers were dried over anhydrous $MgSO_4$, filtered and evaporated *in vacuo*. Purification of the residue by flash chromatography (eluent: diethyl ether/hexane: 2/3 containing Et_3N (1% *v*/*v*) yielded diastereomerically pure **3** (2.30 g, 68% yield) as a yellowish oil. $[\alpha]_D^{25}$ = –11.0 (*c* = 1.02 in $CHCl_3$); IR (neat) 3332, 1642 cm^{-1}; ^1H HMR (400 MHz, $CDCl_3$) δ 1.95 (bs, 1H), 3.08 (dddd, *J* = 14.2,

6,6, 1.2, 1.2 Hz, 1H), 3.28–3.35 (m, 2H), 3.60–3.65 (m, 2H), 3.74–3.80 (m, 1H), 4.55 (d, *J* = 12.0 Hz, 1H), 4.5 (d, *J* = 12.0 Hz, 1H), 4.62 (d, *J* = 11.5 Hz, 1H), 4.84 (d, *J* = 11.5 Hz, 1H), 5.10 (dddd, *J* = 10.2, 1.4, 1.4, 1.4 Hz, 1H), 5.17 (ddd, *J* = 17.2, 1.7, 1.7, 1.7 Hz, 1H), 5.20–5.26(m, 1H), 5.62–5.72 (m, 1H), 5.90 (dddd, *J* = 17.2, 10.3, 6.6, 5,2 Hz, 1H), 7.29–7.42 (m, 10H); ^{13}C-APT{1H} NMR (100 MHz, $CDCl_3$) δ 49.6, 62.5, 70.6, 73.1, 73.4, 81.0, 115.7, 118.6, 127.7, 127.7, 127.7, 128.0, 128.4, 128.5, 137.0, 137.7, 138.4, 138.6; HRMS (ESI+) *m/z* $[M+H]^+$ calcd for $C_{22}H_{28}NO_2$ 338.2115, found 338.2120.

tert-**Butyl allyl[(3*R*,4*S*)-4,5-bis(benzyloxy)pent-1-en-3-yl]carbamate (4)**

Di-*tert*-butyl dicarbonate (4.98 g, 22.82 mmol) was added to a solution of compound **3** (3.35 g, 9.93 mmol) in methanol/triethylamine 9/1 (30 mL) at room temperature and the reaction mixture was stirred at 50 ºC for 12 h and then evaporated *in vacuo*. Purification of the residue by flash chromatography (1st eluent: diethyl ether/hexane: 1/7, 2nd eluent: diethyl ether/hexane: 1/5) yielded diastereomerically pure **4** (3.82 g, 88% yield) as a colourless oil. $[\alpha]_D^{25}$ = 4.3 (*c* = 1.02 in $CHCl_3$); IR (neat) 1691, 1642 cm^{-1}; ^{1}H HMR (300 MHz, $CDCl_3$, 333K) δ 1.49 (s, 9H), 3.59 (dd, *J* = 10.6, 5.3 Hz, 1H), 3.71 (dd, *J* = 10.6, 3.3 Hz, 1H), 3.84 (dddd, *J* = 16.0, 6.2, 1.5, 1.5 Hz, 1H), 3.95–4.14 (m, 2H), 4.39 (dd, *J* = 7.6, 7.6 Hz, 1H), 4.53 (d, *J* = 12.0 Hz, 1H), 4.58 (d, *J* = 12.0 Hz, 1H), 4.61 (d, *J* = 11.6 Hz, 1H), 4.79 (d, *J* = 11.6 Hz, 1H), 5.06 (dddd, *J* = 10.2, 1.5, 1.5, 1.5 Hz, 1H) 5.12 (dddd, *J* = 17.2, 1.6, 1.6, 1.6 Hz, 1H), 5.15–5.28 (m, 2H), 5.86 (dddd, *J* = 17.1, 10.2, 5.8, 5.8 Hz, 1H), 6.08 (ddd, *J* = 17.7, 10.4, 7.7 Hz, 1H), 7.24–7.40 (m, 10H); ^{13}C-APT{1H} NMR (75 MHz, $CDCl_3$, 333K) δ 28.7, 50.4, 62.0, 71.4, 73.2, 73.7, 79.6, 79.8, 115.8, 118.2, 127.5, 127.6, 127.8, 127.8, 128.3,

128.4, 135.2, 136.1, 138.7, 139.2, 155.6; HRMS (ESI+) *m/z* $[M+Na]^+$ calcd for $C_{27}H_{35}NNaO_4$ 460.2458, found 460.2454.

tert-Butyl (*R*)-2-[(*S*)-1,2-bis(benzyloxy)ethyl]-2,5-dihydro-1*H*-pyrrole-1-carboxylate (5)

A solution of compound **4** (2.19 g, 5.0 mmol) in dichloromethane (25 mL) was added to a solution of Grubbs first generation catalyst (412 mg, 0.5 mmol) in dichloromethane (25 mL) at room temperature and the resulting solution was stirred for 2 h at the same temperature and then evaporated *in vacuo*. Purification of the residue by flash chromatography (eluent: diethyl ether/hexane: 1/4) yielded diastereomerically pure **5** (1.99 g, 97% yield) as a colourless oil. $[\alpha]_D^{25}$ = 121.6 (*c* = 1.04 in $CHCl_3$); IR (neat) 1698, 1624 cm^{-1}; ^1H HMR (400 MHz, $CDCl_3$, 333K) δ 1.51 (s, 9H), 3.50–3.58 (m, 2H), 3.98 (dddd, *J* = 15.6, 5.6, 2.1, 2.1 Hz, 1H), 4.16–4.38 (m, 2H), 4.52 (d, *J* = 12.1 Hz, 1H), 4.54 (d, *J* = 12.1 Hz, 1H), 4.74–4.88 (m, 3H), 5.79–5.85 (m, 1H), 5.86–5.92 (m, 1H), 7.25–7.44 (m, 10H); ^{13}C-APT{1H} NMR (100 MHz, $CDCl_3$, 333K) δ 28.7, 54.3, 65.3, 71.4, 73.1, 73.5, 79.2, 79.9, 126.7, 127.4, 127.5, 127.6, 127.7, 128.4, 128.4, 138.8, 139.3, 154.4; HRMS (ESI+) *m/z* $[M+Na]^+$ calcd for $C_{25}H_{31}NNaO_4$ 432.2145, found 432.2163.

tert-Butyl (1*S*,2*S*,5*R*)-2-[(*S*)-1,2-bis(benzyloxy)ethyl]-6-oxa-3-azabicyclo[3.1.0]hexane-3-carboxylate (6)

0.1 M Aqueous solution of Na_2EDTA (504 μL), 1,1,1-trifluoroacetone (3.05 g, 27.22 mmol) and water (16 mL) were added successively to a stirred solution of compound **5** (1.03 g, 2.52 mmol) in acetonitrile (30 mL) at 0 ºC. Then a mixture of oxone® (7.90 g, 12.85 mmol) and $NaHCO_3$ (1.48 g, 17.62 mmol) as a solid was added slowly in

small portions over a period of 1 h at 0 ºC. After being stirred for 2 h at 0 ºC, the solid material was removed by filtration and the filtrate was diluted with water (10 mL) and extracted with dichloromethane (3 x 40 mL). The combined organic layers were dried over anhydrous $MgSO_4$, filtered and evaporated *in vacuo*. Purification of the residue by flash chromatography (eluent: diethyl ether/hexane: 1/2 yielded diastereomerically pure **6** (933 mg, 87% yield) as a white solid. M.p. = 66.4–67.6 ºC; $[\alpha]_D^{25}$ = 63.5 (*c* = 1.0 in $CHCl_3$); IR (KBr) 1679, 1109 cm^{-1}; ^1H HMR (400 MHz, $CDCl_3$) (mixture of rotamers) δ 1.40 and 1.46 (s, 9H), 3.21 and 3.24 (dd, *J* = 12.8, 1.2 Hz and *J* = 12.4, 1.2 Hz, 1H), 3.48–3.53 (m, 1H), 3.55–3.63 (m, 1H), 3.63–3.67 (m, 1H), 3.69–3.73 (m, 1H), 3.78 and 3.88 (d, *J* = 12.4 Hz and *J* = 12.8 Hz, 1H), 3.94 and 4.07 (ddd, *J* = 6.4, 3.6, 3.6 and *J* = 7.2, 3.6, 3.6 Hz, 1H), 4.19 and 4.36 (d, *J* = 4.0 Hz and *J* = 3.6 Hz, 1H), 4.51–4.61 (m, 2H), 4.68 and 4.71 (d, *J* = 12.0 Hz and *J* = 12.0 Hz, 1H), 4.80 and 4.81 (d, *J* = 12.0 Hz and *J* = 12.0 Hz, 1H), 7.28–7.40 (m, 10H); ^{13}C-APT{1H} NMR (100 MHz, $CDCl_3$) (mixture of rotamers) δ 28.4 and 28.5, 47.9 and 48.5, 55.0 and 55.6, 57.1 and 57.4, 59.0 and 59.5, 70.3 and 70.7, 72.8 and 73.1, 73.7 and 73.7, 77.6 and 77.7, 80.1 and 80.4, 127.7 and 127.7, 127.8 and 127.9, 128.4 and 128.5, 128.5 and 128.5, 138.0 and 138.2, 138.4 and138.6, 154.9 and 155.2; HRMS (ESI+) *m/z* $[M+Na]^+$ calcd for $C_{25}H_{31}NNaO_5$ 448.2094, found 448.2081.

tert-Butyl (3*S*,3a*S*,6*R*,6a*R*)-3-(benzyloxy)-6-hydroxyhexahydro-4*H*-furo[3,2-*b*]pyrrole-4-carboxylate (7)

Trifluoroacetic acid (50 mg, 0.44 mmol) was added to a stirred dispersion of compound **6** (910 mg, 2.14 mmol) in methanol/water 3/1 (18 mL) and the mixture

was stirred at 40 ºC for 24 h and then evaporated *in vacuo*. The residue was diluted with water (15 mL) and extracted with dichloromethane (3 x 30 mL). The combined organic layers were dried over anhydrous $MgSO_4$, filtered and evaporated *in vacuo*. Purification of the residue by flash chromatography (1st eluent: diethyl ether/hexane: 1/1, 2nd eluent: diethyl ether) yielded diastereomerically pure **7** (538 mg, 75% yield) as a white solid. M.p. = 124.3–128.5 ºC; $[\alpha]_D^{25}$ = 44.1 (*c* = 0.63 in $CHCl_3$); IR (KBr) 3340, 1680 cm^{-1}; ^1H HMR (400 MHz, $CDCl_3$) (mixture of rotamers) δ 1.49 (s, 9H), 3.01 (bs, 1H), 3.27–3.33 (m, 1H), 3.61 and 3.72 (d, *J* = 12.2 Hz and *J* = 12.6 Hz, 1H), 3.76–3.83 (m, 1H), 3.92 and 4.00 (d, *J* = 10.0 Hz and *J* = 10.1 Hz, 1H), 4.16–4.22 (m, 1H), 4.16–4.22 and 4.28–4.31 (m, 1H), 4.47–4.56 (m, 2H), 4.59 and 4.67 (d, *J* = 11.8 Hz and *J* = 10.6 Hz, 1H), 4.70 and 4.82 (d, *J* = 11.5 Hz and *J* = 11.8 Hz, 1H), 7.26–7.40 (m, 5H); ^{13}C-APT{1H} NMR (100 MHz, $CDCl_3$) (mixture of rotamers) δ 28.5 and 28.6, 53.2 and 53.5, 65.1, 71.4 and 71.5, 72.5 and 73.0, 72.7 and 73.4, 80.2 and 80.8, 82.4 and 83.2, 86.4 and 87.0, 127.5 and 127.7, 127.9, 128.4 and 128.6, 137.8 and 138.2, 154.4 and 154.6; HRMS (ESI+) *m/z* $[M+Na]^+$ calcd for $C_{18}H_{25}NNaO_5$ 358.1625, found 358.1636.

(3*S*,3a*S*,6*R*,6a*R*)-3-(Benzyloxy)hexahydro-2*H*-furo[3,2-*b*]pyrrol-6-ol (8)

A solution of compound **7** (167 mg, 0.50 mmol) in 4M HCl in ethyl acetate (1 mL) was stirred at room temperature for 2 h and then evaporated *in vacuo*. The residue was diluted with 1 M aqueous solution of NaOH (8 mL) and extracted with dichloromethane (3 x 15 mL). The combined organic layers were dried over anhydrous $MgSO_4$, filtered and evaporated *in vacuo* to give compound **8** (109 mg, 93% yield) as a yellowish solid. M.p. = 112.1–115.3 ºC; $[\alpha]_D^{25}$ = –41.1 (*c* = 1.0 in

$CHCl_3$); IR (KBr) 3298, 3113, 1118, 1097, 1080, 1043 cm^{-1}; ^1H HMR (400 MHz, $CDCl_3$) δ 2.76 (bs, 2H), 2.85 (dd, *J* = 12.4, 3.2 Hz, 1H), 2.91 (d, *J* = 12.4 Hz, 1H), 3.80 (dd, *J* = 10.0, 4.0 Hz, 1H), 3.86 (dd, *J* = 10.0, 2.4 Hz, 1H), 3.92–3.94 (m, 1H), 4.01 (d, *J* = 5.2 Hz, 1H), 4.16–4.18 (m, 1H), 4.43 (d, *J* = 5.2 Hz, 1H), 4.55 (d, *J* = 11.8 Hz, 1H), 4.60 (d, *J* = 11.8 Hz, 1H), 7.27–7.38 (m, 5H); ^{13}C-APT{1H} NMR (100 MHz, $CDCl_3$) δ 53.2, 66.7, 71.5, 72.0, 76.3, 85.3, 88.4, 127.8, 127.9, 128.6, 137.9; HRMS (ESI+) *m/z* $[M+H]^+$ calcd for $C_{13}H_{18}NO_3$ 236.1281, found 236.1288.

tert-Butyl (3*S*,3a*S*,6*R*,6a*R*)-6-acetoxy-3-(benzyloxy)hexahydro-4*H*-furo[3,2-*b*]pyrrole-4-carboxylate (9)

Acetic anhydride (384 mg, 3.76 mmol) was added to a stirred solution of compound **7** (315 mg, 0.94 mmol), DMAP (57.4 mg, 0.47 mmol) and triethylamine (380 mg, 3.76 mmol) in acetonitrile (3 mL) at 0 ºC and the mixture was stirred at 0 ºC for 5 min first and then at room temperature for 2 h. The reaction mixture was diluted with dichloromethane (30 mL) and water (15 mL). The aqueous layer was then extracted with dichloromethane (2 x 20 mL). The combined organic extracts were washed with brine (15 mL), dried over anhydrous $MgSO_4$, filtered and evaporated *in vacuo*. Purification of the residue by flash chromatography (eluent: diethyl ether/hexane: 1/2) yielded diastereomerically pure **9** (350 mg, 99% yield) as a yellowish solid. M.p. = 78.1–80.5 ºC; $[\alpha]_D^{25}$ = 16.7 (*c* = 1.0 in $CHCl_3$); IR (KBr) 1736, 1699, 1239, 1114 cm^{-1}; ^1H HMR (400 MHz, $CDCl_3$) (mixture of rotamers) δ 1.47 and 1.50 (s, 9H), 2.03 and 2.04 (s, 3H), 3.38 and 3.41 (dd, *J* = 13.0, 3.9 Hz and *J* = 12.8, 4.0 Hz, 1H), 3.62 and 3.78 (d, *J* = 13.0 Hz and *J* = 12.8 Hz, 1H), 3.76–3.85 (m, 1H), 3.92 and 4.08 (d, *J* = 10.0 Hz and *J* = 10.0 Hz, 1H), 4.20 and 4.30 (d, *J* = 3.9 Hz and *J* = 4.0 Hz, 1H),

4.40 and 4.48 (d, *J* = 4.5 Hz and *J* = 4.6 Hz, 1H), 4.56 and 4.60 (d, *J* = 4.5 Hz and *J* = 5.1 Hz, 1H), 4.59 and 4.65 (d, *J* = 11.8 Hz and *J* = 11.8 Hz, 1H), 4.67 and 4.80 (d, *J* = 11.8 Hz and *J* = 11.8 Hz, 1H), 5.08 and 5.11 (d, *J* = 4.0 Hz and *J* = 3.9 Hz, 1H), 7.23–7.37 (m, 5H); ^{13}C-APT{1H} NMR (100 MHz, $CDCl_3$) (mixture of rotamers) δ 21.0, 28.5 and 28.6, 50.9 and 51.3, 65.5 and 65.5, 71.4 and 71.4, 72.4 and 73.0, 74.5 and 75.2, 80.4 and 80.7, 82.4 and 83.2, 84.0 and 84.8, 127.4 and 127.8, 127.6 and 127.9, 128.4 and 128.5, 137.7 and 138.1, 153.8 and 154.2, 169.7 and 169.9; HRMS (ESI+) *m/z* $[M+Na]^+$ calcd for $C_{20}H_{27}NNaO_6$ 400.1731, found 400.1745.

tert-Butyl (3*S*,3a*S*,6*R*,6a*R*)-3-(benzyloxy)-6-methoxyhexahydro-4*H*-furo[3,2-*b*]pyrrole-4-carboxylate (10)

A 60% dispersion of sodium hydride in mineral oil (90 mg, 2.24 mmol) was added in a single portion under an Ar atmosphere at 0 ºC to a solution of compound **7** (300 mg, 0.89 mmol) in anhydrous THF (12 mL). After being stirred for 1 h at 0 ºC, methyl iodide (765 mg, 5.39 mmol) was added and stirring was continued under an Ar atmosphere at room temperature for additional 2 h. The reaction was quenched at 0 ºC with water (7.5 mL) and saturated $NaHCO_3$ (7.5 mL) aqueous solution and then extracted with dichloromethane (3 x 30 mL). The combined organic extracts were washed with brine (10 mL), dried over anhydrous $MgSO_4$, filtered and evaporated *in vacuo*. Purification of the residue by flash chromatography (eluent: diethyl ether/hexane: 3/2) yielded diastereomerically pure **10** (301 mg, 96% yield) as a colourless oil. $[\alpha]_D^{25}$ = 35.9 (*c* = 1.0 in $CHCl_3$); IR (neat) 1702, 1699, 1100 cm^{-1}; ^{1}H HMR (400 MHz, $CDCl_3$) (mixture of rotamers) δ 1.50 (s, 9H), 3.22 and 3.27 (dd, *J* = 12.6, 3.8 Hz and *J* = 12.7, 4.3 Hz, 1H), 3.37 (s, 3H), 3.69 and 3.86 (d, *J* = 12.6 Hz

and *J* = 12.7 Hz, 1H), 3.75–3.85 (m, 2H), 3.93 and 4.00 (d, *J* = 10.0 Hz and *J* = 10.0 Hz, 1H), 4.20 and 4.31 (d, *J* = 3.5 Hz and *J* = 4.0 Hz, 1H), 4.41 and 4.48 (d, *J* = 4.5 Hz and *J* = 4.6 Hz, 1H), 4.60 and 4.84 (d, *J* = 12.0 Hz and *J* = 11.8 Hz, 1H), 4.63–4.65 (m, 1H), 4.69 and 4.71 (d, *J* = 11.8 Hz and *J* = 12.0 Hz, 1H), 7.26–7.40 (m, 5H); ^{13}C-APT{1H} NMR (100 MHz, $CDCl_3$) (mixture of rotamers) δ 28.5 and 28.6, 49.8 and 50.7, 57.0, 65.1 and 65.3, 71.4 and 71.5, 72.4 and 72.9, 80.0 and 80.5, 81.9 and 82.5, 82.5 and 83.4, 83.5 and 84.7, 127.5 and 127.6, 127.9 and 127.9, 128.4 and 128.6, 137.8 and 138.3, 154.0 and 154.3; HRMS (ESI+) *m/z* $[M+Na]^+$ calcd for $C_{19}H_{27}NNaO_5$ 372.1871, found 372.1789.

tert-Butyl (3*S*,3a*S*,6*R*,6a*R*)-3-hydroxy-6-methoxyhexahydro-4*H*-furo[3,2-*b*]pyrrole-4-carboxylate (11)

A solution of compound **10** (298 mg, 0.85 mmol) in ethanol (10 mL) was hydrogenated with molecular hydrogen for 2 h at atmospheric pressure and room temperature and in the presence of 20% $Pd(OH)_2/C$ (74,5 mg) as a catalyst. The catalyst was removed by filtration through a Celite® path and the solvent evaporated *in vacuo*. Purification of the residue by flash chromatography (1st eluent: diethyl ether/hexane: 2/1, 2nd eluent: diethyl ether) yielded diastereomerically pure **11** (220 mg, 99% yield) as a colourless oil. $[\alpha]_D^{25}$ = 51.9 (*c* = 1.0 in $CHCl_3$); IR (neat) 3437, 1700, 1168, 1102 cm^{-1}; ^{1}H HMR (400 MHz, $CDCl_3$) (mixture of rotamers) δ 1.44 and 1.47 (s, 9H), 3.20 and 3.26 (dd, *J* = 12.5, 3.9 Hz and *J* = 12.4, 4.3 Hz, 1H), 3.34 (s, 3H), 3.59 and 3.75–3.81 (d, *J* = 12.4 Hz and m, 1H), 3.73 (d, *J* = 4.0 Hz, 1H), 3.75–3.81 (m, 1H), 3.75–3.81 and 3.85 (m and dd, *J* = 9.8, 4.3 Hz, 1H), 4.17–4.21 (m, 1H), 4.35–4.38 and 4.41–4.43 (m, 1H), 4.58–4.61 (m, 1H); ^{13}C-APT{1H} NMR (100

MHz, $CDCl_3$) (mixture of rotamers) δ 28.5 and 28.6, 49.7 and 50.8, 57.1, 68.1 and 68.8, 74.2 and 74.3, 75.6 and 76.5, 80.4 and 80.6, 81.9 and 82.5, 83.4 and 84.6, 154.9 and 155.1; HRMS (ESI+) *m/z* $[M+Na]^+$ calcd for $C_{12}H_{21}NNaO_5$ 282.1312, found 282.1318.

tert-Butyl (3*S*,3a*S*,6*R*,6a*R*)-3-acetoxy-6-methoxyhexahydro-4*H*-furo[3,2-*b*]pyrrole-4-carboxylate (12)

Acetic anhydride (150 mg, 1.47 mmol) was added to a stirred solution of compound **11** (95,5 mg, 0.37 mmol), DMAP (22.5 mg, 0.18 mmol) and triethylamine (149 mg, 1.47 mmol) in acetonitrile (1 mL) at 0 ºC and the mixture was stirred at 0 ºC for 5 min first and then at room temperature for 1 h. The reaction mixture was diluted with dichloromethane (12 mL) and water (5 mL). The aqueous layer was then extracted with dichloromethane (2 x 6 mL). The combined organic extracts were washed with brine (6 mL), dried over anhydrous $MgSO_4$, filtered and evaporated *in vacuo*. Purification of the residue by flash chromatography (eluent: diethyl ether/hexane: 2/1) yielded diastereomerically pure **12** (110 mg, 99% yield) as a colourless oil. $[\alpha]_D^{25}$ = 16.7 (*c* = 1.0 in $CHCl_3$); IR (neat) 1747, 1699, 1233, 1102 cm^{-1}; ^1H HMR (400 MHz, $CDCl_3$) (mixture of rotamers) δ 1.41 (s, 9H), 2.02 (s, 3H), 3.15–3.21 (m, 1H), 3.30 (s, 3H), 3.61 and 3.70–3.81 (d, *J* = 12.4 Hz and m, 1H), 3.70–3.81 (m, 2H), 3.85 (dd, *J* = 10.7, 3.7 Hz, 1H), 4.19 and 4.31 (d, *J* = 3.7 Hz and *J* = 3.6 Hz, 1H), 4.49–4.54 (m, 1H), 5.25–5.30 (m, 1H); ^{13}C-APT{1H} NMR (100 MHz, $CDCl_3$) (mixture of rotamers) δ 20.9, 28.4, 49.5 and 50.5, 57.0, 65.5 and 65.5, 72.5 and 72.9, 77.3 and 77.6, 80.4 and 80.6, 81.5 and 82.4, 83.7 and 84.9, 153.9 and 154.1, 169.7 and 170.0; HRMS (ESI+) *m/z* $[M+Na]^+$ calcd for $C_{14}H_{23}NNaO_6$ 324.1418, found 324.1423.

tert-Butyl (3*S*,3a*S*,6*R*,6a*R*)-3-(benzyloxy)-6-chlorohexahydro-4*H*-furo[3,2-*b*]pyrrole-4-carboxylate (13)

N-Chorosuccinimide (80 mg, 0.60 mmol), trifluoroacetic acid (40 mg, 0.35 mmol) and water (120 μL) were added successively to a stirred solution of compound **5** (123 mg, 0.30 mmol) in dimethylformamide (1.5 mL) at room temperature. The resulting mixture was stirred at 35 ºC until the complete disappearance of the starting material. The progress of the reaction was monitored by TLC and additional portions of *N*-chorosuccinimide were added if reaction stopped before completion. Reaction was complete in about 5 h, which was followed by removal of the solvent *in vacuo*. Purification of the residue by flash chromatography (eluent: ethyl acetate/hexane: 1/9 yielded diastereomerically pure **13** (85 mg, 80% yield) as a white solid. M.p. = 57.8–59.5 ºC; $[\alpha]_D^{25}$ = 43.2 (*c* = 1 in $CHCl_3$); IR (KBr) 1702, 1164, 1115 cm^{-1}; ^1H HMR (400 MHz, $CDCl_3$) (mixture of rotamers) δ 1.52 and 1.53 (s, 9H), 3.54 and 3.56 (dd, *J* = 13.0, 3.8 Hz and *J* = 13.0, 3.8 Hz, 1H), 3.83 and 3.85–3.89 (dd, *J* = 10.4, 3.6 Hz and m, 1H), 3.85–3.89 and 4.02 (m and d, *J* = 13.2 Hz, 1H), 3.99 and 4.07 (d, *J* = 10.0 Hz and *J* = 10.4 Hz, 1H), 4.21 and 4.31 (d, *J* = 3.4 Hz and *J* = 3.4 Hz, 1H), 4.25 and 4.26 (d, *J* = 3.8 Hz and *J* = 3.8 Hz, 1H), 4.56 and 4.66 (d, *J* = 4.0 Hz and *J* = 4.0 Hz, 1H), 4.62 and 4.68 (d, *J* = 12.0 Hz and *J* = 12.0 Hz, 1H), 4.71 and 4.84 (d, *J* = 11.6 Hz and *J* = 12.0 Hz, 1H), 4.73 and 4.76 (d, *J* = 4.0 Hz and *J* = 4.0 Hz, 1H), 4.27–4.41 (m, 5H); ^{13}C-APT{1H} NMR (100 MHz, $CDCl_3$) (mixture of rotamers) δ 28.4 and 28.5, 53.2 and 53.8, 58.3 and 58.8, 64.9, 71.4 and 71.5, 73.4 and 73.9, 80.6 and 80.9, 82.1 and 82.8, 86.9 and 87.8, 127.5 and 127.8, 127.7 and

127.9, 128.4 and 128.6, 137.6 and 138.0, 153.9 and 154.2; HRMS (ESI+) *m/z* $[M+H]^+$ calcd for $C_{18}H_{24}ClNNaO_4$ 376.1286, found 376.1292.

tert-Butyl (3S,3aS,6R,6aR)-6-chloro-3-methoxyhexahydro-4H-furo[3,2-b]pyrrole-4-carboxylate (14)

A solution of compound **13** (78 mg, 0.22 mmol) in ethanol (2.6 mL) was hydrogenated with molecular hydrogen for 2.5 h at atmospheric pressure and room temperature and in the presence of 20% $Pd(OH)_2/C$ (19,4 mg) as a catalyst. The catalyst was removed by filtration through a Celite® path and the solvent evaporated *in vacuo*. The obtained residue was dissolved in anhydrous THF (1.4 mL) and a 60% dispersion of sodium hydride in mineral oil (13.4 mg, 0.33 mmol) was added in a single portion under an Ar atmosphere at 0 ºC. After being stirred for 1 h at 0 ºC, methyl iodide (185 mg, 1.30 mmol) was added and stirring was continued under an Ar atmosphere at room temperature for additional 2 h. The reaction was quenched at 0 ºC with water (3 mL) and saturated $NaHCO_3$ (2 mL) aqueous solution and then extracted with dichloromethane (3 x 10 mL). The combined organic extracts were washed with brine (10 mL), dried over anhydrous $MgSO_4$, filtered and evaporated *in vacuo*. Purification of the residue by flash chromatography (eluent: diethyl ether/hexane: 2/3) yielded diastereomerically pure **14** (49.5 mg, 81% yield) as a yellowish solid. M.p. = 73.1–74.5 ºC; $[\alpha]_D^{25}$ = 89.7 (*c* = 1.0 in $CHCl_3$); IR (KBr) 1693, 1116, 1076 cm^{-1}; ^1H HMR (400 MHz, $CDCl_3$) (mixture of rotamers) δ 1.47 and 1.52 (s, 9H), 3.44 and 3.48 (s, 3H), 3.50 and 3.53 (dd, *J* = 12.8, 4.0 Hz and *J* = 12.8,, 4.0 Hz, 1H), 3.78 and 3.91–3.95 (dd, *J* = 10.4, 3.6 Hz and m,, 1H), 3.79 and 3.91–3.95 (d, *J* = 10.4 Hz and m, 1H), 3.84 and 3.98 (d, *J* = 13.2 Hz and *J* = 13.2 Hz, 1H), 3.91–

3.95 and 4.06 (m and d, *J* = 3.6 Hz, 1H), 4.21 and 4.23 (d, *J* = 4.0 Hz and *J* = 4.0 Hz, 1H), 4.45 and 4.53 (d, *J* = 4.0 Hz and *J* = 4.0 Hz, 1H), 4.63 and 4.66 (d, *J* = 4.4 Hz and *J* = 4.4 Hz, 1H); ^{13}C-APT{1H} NMR (100 MHz, $CDCl_3$) (mixture of rotamers) δ 28.5 and 28.6, 53.2 and 53.9, 57.3 and 57.4, 58.4 and 58.9, 64.1 and 64.4, 73.5, 80.6 and 80.9, 83.8 and 84.7, 86.9 and 87.8, 153.9 and 154.2; HRMS (ESI+) *m/z* $[M+Na]^+$ calcd for $C_{12}H_{20}ClNNaO_4$ 300.0973, found 300.0980.

(3*S*,3a*S*,6*R*,6a*R*)-3-(Benzyloxy)-4-(7,8-dimethoxyquinazolin-4-yl)hexahydro-2*H*-furo[3,2-*b*]pyrrol-6-ol (15A)

A solution of compound **7** (40 mg, 0.12 mmol) in 4M HCl in ethyl acetate (0.75 mL) was stirred at room temperature for 2 h and then evaporated *in vacuo*. The residue was diluted with 1 M aqueous solution of NaOH (4 mL) and extracted with dichloromethane (3 x 8 mL). The combined organic layers were dried over anhydrous $MgSO_4$, filtered and evaporated *in vacuo*. The residue (≈ 26 mg) was dissolved in anhydrous DMF (1 mL) and DIPEA (75 mg, 0.58 mmol) and 4-chloro-7,8-dimethoxyquinazoline (34 mg, 0.15 mmol) were added successively. The reaction mixture was stirred at 60 ºC for 12 h and then evaporated *in vacuo*. Purification of the residue by flash chromatography (1st eluent: ethyl acetate/dichloromethane: 1/1, 2nd eluent: ethyl acetate/dichloromethane/ ethanol: 5/5/1) yielded diastereomerically pure **15A** (47 mg, 93% yield) as a white solid. M.p. = 178.8–180.2 ºC; $[\alpha]_D^{25}$ = 100.0 (*c* = 0.25 in $CHCl_3$); IR (KBr) 3245, 1494, 1102 cm^{-1}; ^{1}H HMR (400 MHz, $CDCl_3$) δ 3.81 (s, 3H), 3.90–3.94 (m, 1H), 3.93 (s, 1H), 4.02 (d, *J* = 10.0 Hz, 1H), 4.09 (dd, *J* = 12.0, 2.8 Hz, 1H), 4.14 (d, *J* = 3.6 Hz, 1H), 4.57 (d, *J* = 2.8 Hz, 1H), 4.80 (d, *J* = 4.4 Hz, 1H), 4.82 (d, *J* = 12.0 Hz, 1H), 4.86 (d, *J* =

12.6 Hz, 1H), 4.98 (d, *J* = 12.6 Hz, 1H), 5.39 (d, *J* = 4.4 Hz, 1H), 6.97 (d, *J* = 9.4 Hz, 1H), 7.28 (d, *J* = 7.2 Hz, 1H), 7.35 (dd, *J* = 7.4, 7.4 Hz, 1H), 7.43 (d, *J* = 7.2 Hz, 1H), 7.67 (d, *J* = 9.4 Hz, 1H), 8.27 (s, 1H); ^{13}C-APT{1H} NMR (100 MHz, $CDCl_3$) δ 56.2, 58.3, 61.6, 68.5, 71.4, 73.7, 74.2, 82.2, 84.9, 110.2, 110.7, 122.5, 127.8, 127.9, 128.6, 138.3, 139.3, 153.0, 154.0, 160.0; HRMS (ESI+) *m/z* $[M+H]^+$ calcd for $C_{23}H_{26}N_3O_5$ 424.1867, found 424.1871.

(3*S*,3a*S*,6*R*,6a*R*)-3-(Benzyloxy)-4-(7,8-dimethoxyquinazolin-4-yl)hexahydro-2*H*-furo[3,2-*b*]pyrrol-6-yl acetate (16A)

A solution of compound **9** (64 mg, 0.17 mmol) in dichloromethane/ trifluoroacetic acid 5/1 (1.2 mL) was stirred at room temperature for 3 h and then evaporated *in vacuo*. The residue was diluted with 0.6 M potassium carbonate/bicarbonate buffer (4 mL) and extracted with dichloromethane (3 x 8 mL). The combined organic extracts were dried over anhydrous $MgSO_4$, filtered and evaporated under reduced pressure. The residue (≈ 45 mg) was dissolved in anhydrous DMF (1.3 mL) and DIPEA (112 mg, 0.87 mmol) and 4-chloro-7,8-dimethoxyquinazoline (49 mg, 0.22 mmol) were added successively. The reaction mixture was stirred at 60 ºC for 12 h and then evaporated *in vacuo*. Purification of the residue by flash chromatography (eluent: ethyl acetate/ethanol: 9/1 containing Et_3N (1% v/v)) yielded diastereomerically pure **16A** (76 mg, 96% yield) as a white solid. M.p. = 81.3–84.7 ºC; $[\alpha]_D^{25}$ = 78.8 (*c* = 1.0 in $CHCl_3$); IR (KBr) 1750, 1492, 1098, 1046 cm^{-1}; ^{1}H HMR (400 MHz, $CDCl_3$) δ 1.97 (s, 3H), 3.94 (dd, *J* = 10.2, 4.2 Hz, 1H), 3.98–4.01 (m, 1H), 3.99 (s, 3H), 4.03 (d, *J* = 12.0 Hz, 1H), 4.05 (s, 3H), 4.23–4.27 (m, 2H), 4.67 (d, *J* = 4.0 Hz, 1H), 4.83 (d, *J* = 12.4 Hz, 1H), 4.93 (d, *J* = 12.4 Hz, 1H), 5.31 (d, *J* = 3.6 Hz,

1H), 4.34 (d, *J* = 4.0 Hz, 1H), 7.13 (d, *J* = 9.6 Hz, 1H), 7.23–7.28 (m, 1H), 7.30–7.35 (m, 2H), 7.39–7.41 (m, 2H), 7.74 (d, *J* = 9.6 Hz, 1H), 8.71 (s, 1H); ^{13}C-APT{1H} NMR (100 MHz, $CDCl_3$) δ 21.0, 56.4, 56.5, 61.7, 68.2, 71.3, 73.7, 75.5, 81.9, 82.1, 111.7, 112.0, 121.3, 127.8, 127.9, 128.5, 138.1, 142.2, 146.9, 154.1, 154.2, 159.9, 170.0; HRMS (ESI+) *m/z* $[M+Na]^+$ calcd for $C_{25}H_{27}N_3NaO_6$ 488.1792, found 488.1770.

(3*S*,3a*S*,6*R*,6a*R*)-4-(7,8-Dimethoxyquinazolin-4-yl)-6-methoxyhexahydro-2*H*-furo[3,2-*b*]pyrrol-3-yl acetate (17A)

A solution of compound **12** (51 mg, 0.17 mmol) in 4M HCl in ethyl acetate (1 mL) was stirred at 0 ºC for 3 h and then evaporated *in vacuo*. The residue was dissolved in anhydrous DMF (1,4 mL) and DIPEA (145 mg, 1.12 mmol) and 4-chloro-7,8-dimethoxyquinazoline (50 mg, 0.22 mmol) were added successively. The reaction mixture was stirred at 45 ºC for 2.5 h and then evaporated *in vacuo*. Purification of the residue by flash chromatography (1st eluent: ethyl acetate/dichloromethane: 1/1, 2nd eluent: ethyl acetate/dichloromethane/ ethanol: 9/9/2) yielded diastereomerically pure **17A** (57 mg, 86% yield) as a white solid. M.p. = 180.1–181.6 ºC; $[\alpha]_D^{25}$ = 88.4 (*c* = 1.0 in $CHCl_3$); IR (KBr) 1731, 1485, 1244, 1111 cm^{-1}; ^{1}H HMR (400 MHz, $CDCl_3$) δ 2.11 (s, 3H), 3.29 (s, 3H), 3.89 (d, *J* = 10.8 Hz, 1H), 3.94-3.97 (m, 1H), 3.96 (s, 3H), 4.00 (dd, *J* = 10.8, 3.6 Hz, 1H), 4.00 (s, 3H), 4.02–4.07 (m, 2H), 4.66 (d, *J* = 4.4 Hz, 1H), 5.21 (d, *J* = 4.4 Hz, 1H), 5.26 (d, *J* = 3.6 Hz, 1H), 7.11 (d, *J* = 9.4 Hz, 1H), 7.77 (d, *J* = 9.4 Hz, 1H), 8.63 (s, 1H); ^{13}C-APT{1H} NMR (100 MHz, $CDCl_3$) δ 21.1, 55.9, 56.5, 57.2, 61.7, 67.4, 73.5, 77.4, 82.0, 82.5, 111.8, 111.9, 121.3, 142.2, 146.9, 154.1, 154.1, 160.2, 170.1; HRMS (ESI+) *m/z* $[M+H]^+$ calcd for $C_{19}H_{24}N_3O_6$ 390.1660, found 390.1663.

(3*S*,3a*S*,6*R*,6a*R*)-3-(Benzyloxy)-6-chloro-4-(7,8-dimethoxyquinazolin-4-yl)hexahydro-2*H*-furo[3,2-*b*]pyrrole (18A)

A solution of compound **13** (50 mg, 0.14 mmol) in 4M HCl in ethyl acetate (0.75 mL) was stirred at 0 ºC for 3 h and then evaporated *in vacuo*. The residue was diluted with 1 M aqueous solution of NaOH (4 mL) and extracted with dichloromethane (3 x 8 mL). The combined organic layers were dried over anhydrous $MgSO_4$, filtered and evaporated *in vacuo*. The residue (≈ 36 mg) was dissolved in anhydrous DMF (1 mL) and DIPEA (98 mg, 0.76 mmol) and 4-chloro-7,8-dimethoxyquinazoline (43 mg, 0.19 mmol) were added successively. The reaction mixture was stirred at 45 ºC for 12 h and then evaporated *in vacuo*. Purification of the residue by flash chromatography (1st eluent: diethyl ether, 2nd eluent: ethyl acetate containing Et_3N (0.5% v/v)) yielded diastereomerically pure **18A** (57 mg, 91% yield) as a yellowish solid. M.p. = 49.6–52.3 ºC; $[\alpha]_D^{25}$ = 125.7 (*c* = 0.5 in $CHCl_3$); IR (KBr) 1492, 1104, 1041 cm^{-1}; ^1H HMR (400 MHz, $CDCl_3$) δ 3.97 (dd, *J* = 10.4, 4.0 Hz, 1H), 4.00 (s, 3H), 4.05 (d, *J* = 10.4 Hz, 1H), 4.07 (s, 3H), 4.21–4.23 (m, 1H), 4.23 (d, *J* = 11.6 Hz, 1H), 4.41 (dd, *J* = 11.6, 3.6 Hz, 1H), 4.47 (d, *J* = 3.6 Hz, 1H), 4.82 (d, *J* = 12.2 Hz, 1H), 4.83 (d, *J* = 4.6 Hz, 1H), 4.92 (d, *J* = 12.2 Hz, 1H), 5.53 (d, *J* = 4.0 Hz, 1H), 7.14 (d, *J* = 9.4 Hz, 1H), 7.25–7.29 (m, 1H), 7.31–7.36 (m, 2H), 7.38–7.42 (m, 2H), 7.70 (d, *J* = 9.4 Hz, 1H), 8.74 (s, 1H); ^{13}C-APT{1H} NMR (100 MHz, $CDCl_3$) δ 56.6, 58.9, 59.0, 61.8, 67.5, 71.4, 74.6, 81.6, 85.4, 111.6, 112.2, 121.1, 127.9, 128.0, 128.6, 138.0, 142.3, 146.8, 154.1, 154.3, 160.1; HRMS (ESI+) *m/z* $[M+H]^+$ calcd for $C_{23}H_{25}ClN_3O_4$ 442.1528, found 442.1549.

(3*S*,3a*S*,6*R*,6a*R*)-6-Chloro-4-(7,8-dimethoxyquinazolin-4-yl)-3-methoxyhexahydro-2*H*-furo[3,2-*b*]pyrrole (19A)

A solution of compound **14** (48 mg, 0.17 mmol) in 4M HCl in ethyl acetate (0.75 mL) was stirred at 0 ºC for 5 h and then evaporated *in vacuo*. The residue was diluted with 1 M aqueous solution of NaOH (4 mL) and extracted with dichloromethane (3 x 8 mL). The combined organic layers were dried over anhydrous $MgSO_4$, filtered and evaporated *in vacuo*. The residue (≈ 29 mg) was dissolved in anhydrous DMF (1 mL) and DIPEA (112 mg, 0.87 mmol) and 4-chloro-7,8-dimethoxyquinazoline (49 mg, 0.22 mmol) were added successively. The reaction mixture was stirred at 45 ºC for 12 h and then evaporated *in vacuo*. Purification of the residue by flash chromatography (1st eluent: diethyl ether, 2nd eluent: ethyl acetate/ethanol: 9/1 containing Et_3N (0.5% v/v)) yielded diastereomerically pure **19A** (49 mg, 77% yield) as a yellowish solid. M.p. = 224.7–226.9 ºC; $[\alpha]_D^{25}$ = 156.5 (*c* = 0.5 in $CHCl_3$); IR (KBr) 1492, 1105 cm^{-1}; ^1H HMR (400 MHz, $CDCl_3$) δ 3.56 (s, 3H), 3.93 (dd, *J* = 10.0, 4.0 Hz, 1H), 3.99–4.02 (m, 1H), 4.00 (s, 3H), 4.01 (d, *J* = 4.0 Hz, 1H), 4.05 (s, 3H), 4.20 (d, *J* = 12.0 Hz, 1H), 4.38 (dd, *J* = 12.0, 3.8 Hz, 1H), 4.43 (d, *J* = 3.8 Hz, 1H), 4.74 (d, *J* = 4.0 Hz, 1H), 5.44 (d, *J* = 4.0 Hz, 1H), 7.16 (d, *J* = 9.4 Hz, 1H), 7.71 (d, *J* = 9.4 Hz, 1H), 8.69 (s, 1H); ^{13}C-APT{1H} NMR (100 MHz, $CDCl_3$) δ 56.6, 57.6, 58.9, 59.0, 61.8, 66.9, 74.1, 84.1, 85.4, 111.7, 112.2, 121.1, 142.4, 147.1, 154.2, 154.2, 160.2; HRMS (ESI+) *m/z* $[M+H]^+$ calcd for $C_{17}H_{21}ClN_3O_4$ 366.1215, found 366.1206.

(3*S*,3a*S*,6*R*,6a*R*)-3-(Benzyloxy)-4-(6,7-dimethoxyquinazolin-4-yl)hexahydro-2*H*-furo[3,2-*b*]pyrrol-6-ol (15B)

A solution of compound **7** (64 mg, 0.19 mmol) in 4M HCl in ethyl acetate (0.75 mL) was stirred at room temperature for 2 h and then evaporated *in vacuo*. The residue was diluted with 1 M aqueous solution of NaOH (4 mL) and extracted with dichloromethane (3 x 8 mL). The combined organic layers were dried over anhydrous $MgSO_4$, filtered and evaporated *in vacuo*. The residue (≈ 42 mg) was dissolved in anhydrous DMF (1.3 mL) and DIPEA (125 mg, 0.97 mmol) and 4-chloro-6,7-dimethoxyquinazoline (54 mg, 0.24 mmol) were added successively. The reaction mixture was stirred at 80 ºC for 12 h and then evaporated *in vacuo*. Purification of the residue by flash chromatography (1st eluent: ethyl acetate, 2nd eluent: ethyl acetate/ethanol: 9/1) yielded diastereomerically pure **15B** (57 mg, 70% yield) as a white solid. M.p. = 163.7–164.9 ºC; $[\alpha]_D^{25}$ = 92.2 (*c* = 0.5 in $CHCl_3$); IR (KBr) 3361, 1510, 1077, 1003 cm^{-1}; ^1H HMR (400 MHz, $CDCl_3$) δ 3.92 (s, 3H), 3.95 (s, 3H), 3.95–3.99 (m, 1H), 4.01 (dd, *J* = 10.2, 1.0 Hz, 1H), 4.14–4.18 (m, 2H), 4.24 (d, *J* = 11.2 Hz, 1H), 4.54 (d, *J* = 3.2 Hz, 1H), 4.74 (d, *J* = 4.2 Hz, 1H), 4.79 (d, *J* = 12.2 Hz, 1H), 4.89 (d, *J* = 12.2 Hz, 1H), 5.36 (d, *J* = 4.2 Hz, 1H), 7.03 (s, 1H), 7.21 (s, 1H), 7.25–7.40 (m, 5H), 8.31 (s, 1H); ^{13}C-APT{1H} NMR (100 MHz, $CDCl_3$) δ 56.3, 56.3, 58.3, 68.1, 71.5, 73.5, 74.1, 82.3, 85.1, 104.2, 105.9, 109.7, 127.9, 127.9, 128.6, 138.2, 147.0, 147.8, 151.9, 154.3, 158.8; HRMS (ESI+) *m/z* $[M+H]^+$ calcd for $C_{23}H_{26}N_3O_5$ 424.1867, found 424.1872.

(3*S*,3a*S*,6*R*,6a*R*)-3-(Benzyloxy)-4-(6,7-dimethoxyquinazolin-4-yl)hexahydro-2*H*-furo[3,2-*b*]pyrrol-6-yl acetate (16B)

A solution of compound **9** (46.5 mg, 0.12 mmol) in dichloromethane/ trifluoroacetic acid 5/1 (1.2 mL) was stirred at room temperature for 3 h and then evaporated *in vacuo*. The residue was diluted with 0.6 M potassium carbonate/bicarbonate buffer (4 mL) and extracted with dichloromethane (3 x 8 mL). The combined organic extracts were dried over anhydrous $MgSO_4$, filtered and evaporated under reduced pressure. The residue (≈ 33 mg) was dissolved in anhydrous DMF (1 mL) and DIPEA (80 mg, 0.62 mmol) and 4-chloro-6,7-dimethoxyquinazoline (36 mg, 0.16 mmol) were added successively. The reaction mixture was stirred at 80 ºC for 12 h and then evaporated *in vacuo*. Purification of the residue by flash chromatography (eluent: ethyl acetate) yielded diastereomerically pure **16B** (41 mg, 71% yield) as a white solid. M.p. = 170.1–172.0 ºC; $[\alpha]_D^{25}$ = 104.0 (*c* = 0.05 in $CHCl_3$); IR (KBr) 1760, 1504, 1229, 1110, 1061 cm^{-1}; ^1H HMR (400 MHz, $CDCl_3$) δ 2.00 (s, 3H), 3.95 (s, 3H), 3.97–4.03 (m, 2H), 4.02 (s, 3H), 4.04 (d, *J* = 12.0 Hz, 1H), 4.21–4.23 (m, 1H), 4.28 (dd, *J* = 12.0, 4.0 Hz, 1H), 4.70 (d, *J* = 4.4 Hz, 1H), 4.81 (d, *J* = 12.2 Hz, 1H), 4.90 (d, *J* = 12.2 Hz, 1H), 5.35 (d, *J* = 4.0 Hz, 1H), 5.36 (d, *J* = 4.4 Hz, 1H), 7.25 (s, 1H), 7.27 (s, 1H), 7.26–7.29 (m, 1H), 7.31–7.36 (m, 2H), 7.37–7.40 (m, 2H), 8.62 (s, 1H); ^{13}C-APT{1H} NMR (100 MHz, $CDCl_3$) δ 21.1, 56.1, 56.3, 56.4, 68.2, 71.5, 73.7, 75.4, 82.2, 82.6, 103.7, 107.4, 110.3, 127.9, 127.9, 128.6, 138.1, 148.2, 148.8, 152.8, 154.5, 159.0, 170.0; HRMS (ESI+) *m/z* $[M+H]^+$ calcd for $C_{25}H_{28}N_3O_6$ 466.1973, found 244.1970.

(3*S*,3a*S*,6*R*,6a*R*)-4-(6,7-Dimethoxyquinazolin-4-yl)-6-methoxyhexahydro-2*H*-furo[3,2-*b*]pyrrol-3-yl acetate (17B)

A solution of compound **12** (85 mg, 0.28 mmol) in dichloromethane/ trifluoroacetic acid 2.5/1 (2.5 mL) was stirred at 0 ºC for 10 min first and then at room temperature for 2 h. The reaction mixture was evaporated *in vacuo* and the residue was diluted with 0.6 M potassium carbonate/bicarbonate buffer (9 mL) and extracted with dichloromethane (3 x 15 mL). The combined organic extracts were dried over anhydrous $MgSO_4$, filtered and evaporated under reduced pressure. The residue (≈ 28 mg) was dissolved in anhydrous DMF (1 mL) and DIPEA (94 mg, 0.73 mmol) and 4-chloro-6,7-dimethoxyquinazoline (43 mg, 0.19 mmol) were added successively. The reaction mixture was stirred at 80 ºC for 12 h and then evaporated *in vacuo*. Purification of the residue by flash chromatography (1st eluent: ethyl acetate/dichloromethane: 1/1, 2nd eluent: ethyl acetate/dichloromethane/ethanol: 10/10/1) yielded diastereomerically pure **17B** (54 mg, 49% yield) as a white solid. M.p. = 65.2–66.5 ºC; $[\alpha]_D^{25}$ = 58.7 (*c* = 0.25 in $CHCl_3$); IR (KBr) 1736, 1509, 1240, 1094 cm^{-1}; ^1H HMR (400 MHz, $CDCl_3$) δ 2.13 (s, 3H), 3.35 (s, 3H), 3.91 (d, *J* = 10.8 Hz, 1H), 3.97 (s, 3H), 3.99–4.01 (m, 1H), 4.00 (s, 3H), 4.04–4.12 (m, 3H), 4.71 (d, *J* = 4.2 Hz, 1H), 5.24 (d, *J* = 3.6 Hz, 1H), 5.25 (d, *J* = 4.6 Hz, 1H), 7.27 (s, 1H), 7.28 (s, 1H), 8.54 (s, 1H); ^{13}C-APT{1H} NMR (100 MHz, $CDCl_3$) δ 21.2, 55.7, 56.2, 56.4, 57.3, 67.5, 73.6, 77.6, 82.4, 82.5, 103.9, 107.0, 110.2, 148.1, 152.5, 154.5, 159.3, 170.2; HRMS (ESI+) *m/z* $[M+Na]^+$ calcd for $C_{19}H_{23}N_3NaO_6$ 412.1479, found 412.1476.

(3*S*,3a*S*,6*R*,6a*R*)-3-(Benzyloxy)-6-chloro-4-(6,7-dimethoxyquinazolin-4-yl)hexahydro-2*H*-furo[3,2-*b*]pyrrole (18B)

A solution of compound **13** (67 mg, 0.19 mmol) in 4M HCl in ethyl acetate (0.75 mL) was stirred at 0 ºC for 3 h and then evaporated *in vacuo*. The residue was diluted with 1 M aqueous solution of NaOH (4 mL) and extracted with dichloromethane (3 x 8 mL). The combined organic layers were dried over anhydrous $MgSO_4$, filtered and evaporated *in vacuo*. The residue (≈ 48 mg) was dissolved in anhydrous DMF (1.1 mL) and DIPEA (129 mg, 1.0 mmol) and 4-chloro-6,7-dimethoxyquinazoline (56 mg, 0.25 mmol) were added successively. The reaction mixture was stirred at 70 ºC for 12 h and then evaporated *in vacuo*. Purification of the residue by flash chromatography (eluent: ethyl acetate/hexanes: 3/1) yielded diastereomerically pure **18B** (46 mg, 55% yield) as a yellowish solid. M.p. = 62.0–63.0 ºC; $[\alpha]_D^{25}$ = 70.8 (*c* = 0.15 in $CHCl_3$); IR (KBr) 1509, 1219, 1104 cm^{-1}; ^1H HMR (400 MHz, $CDCl_3$) δ 3.95 (s, 3H), 3.99 (dd, *J* = 10.4, 4.0 Hz, 1H), 4.03 (s, 3H), 4.06 (d, *J* = 10.0 Hz, 1H), 4.18 (d, *J* = 3.6 Hz, 1H), 4.24 (d, *J* = 11.6 Hz, 1H), 4.42 (dd, *J* = 11.6, 4.0 Hz, 1H), 4.48 (d, *J* = 4.0 Hz, 1H), 4.78 (d, *J* = 12.0 Hz, 1H), 4.84–4.86 (m, 1H), 4.87 (d, *J* = 12.0 Hz, 1H), 5.53 (d, *J* = 4.0 Hz, 1H), 7.20 (s, 1H), 7.25–7.39 (m, 6H), 8.63 (s, 1H); ^{13}C-APT{1H} NMR (100 MHz, $CDCl_3$) δ 56.3, 56.4, 58.5, 58.9, 67.5, 71.5, 74.5, 81.8, 85.8, 103.6, 107.3, 110.2, 128.0, 128.0, 128.6, 137.9, 148.3, 148.6, 152.7, 154.6, 159.1; HRMS (ESI+) *m/z* $[M+H]^+$ calcd for $C_{23}H_{25}ClN_3O_4$ 442.1528, found 442.1539.

(3*S*,3a*S*,6*R*,6a*R*)-6-Chloro-4-(6,7-dimethoxyquinazolin-4-yl)-3-methoxyhexahydro-2*H*-furo[3,2-*b*]pyrrole (19B)

A solution of compound **14** (48 mg, 0.17 mmol) in 4M HCl in ethyl acetate (0.75 mL) was stirred at 0 ºC for 5 h and then evaporated *in vacuo*. The residue was diluted with 1 M aqueous solution of NaOH (4 mL) and extracted with dichloromethane (3 x 8 mL). The combined organic layers were dried over anhydrous $MgSO_4$, filtered and evaporated *in vacuo*. The residue (≈ 29 mg) was dissolved in anhydrous DMF (1 mL) and DIPEA (112 mg, 0.87 mmol) and 4-chloro-6,7-dimethoxyquinazoline (49 mg, 0.22 mmol) were added successively. The reaction mixture was stirred at 70 ºC for 12 h and then evaporated *in vacuo*. Purification of the residue by flash chromatography (1st eluent: ethyl acetate/dichloromethane: 1/1, 2nd eluent: ethyl acetate/dichloromethane/ethanol: 10/10/1) yielded diastereomerically pure **19B** (27 mg, 43% yield) as a yellowish solid. M.p. = 106.8–108.4 ºC; $[\alpha]_D^{25}$ = 161.3 (*c* = 0.5 in $CHCl_3$); IR (KBr) 1511, 1249, 1109, 1002 cm^{-1}; ^1H HMR (400 MHz, $CDCl_3$) δ 3.51 (s, 3H), 3.93 (dd, *J* = 10.0, 4.0 Hz, 1H), 3.97–3.99 (m, 1H), 3.98 (s, 3H), 4.01 (s, 3H), 4.02 (d, *J* = 9.6 Hz, 1H), 4.24 (d, *J* = 12.0 Hz, 1H), 4.36 (dd, *J* = 12.0, 3.6 Hz, 1H), 4.44 (d, *J* = 3.6 Hz, 1H), 4.79 (d, *J* = 4.0 Hz, 1H), 5.43 (d, *J* = 4.0 Hz, 1H), 7.25 (s, 1H), 7.27 (s, 1H), 8.58 (s, 1H); ^{13}C-APT{1H} NMR (100 MHz, $CDCl_3$) δ 56.2, 56.4, 57.5, 58.4, 58.7, 67.1, 73.7, 84.2, 86.0, 103.5, 107.4, 110.2, 148.2, 148.7, 152.8, 154.5, 159.0; HRMS (ESI+) *m/z* $[M+H]^+$ calcd for $C_{17}H_{21}ClN_3O_4$ 366.1215, found 366.1227.

Inhibition assays

PDE1A, PDE1B and PDE1C inhibition assays were performed in 60 µL samples containing a fixed amount of the PDE1 enzyme (sufficient to convert 20–25% of the tritiated substrate), a buffer (50 mM HEPES, pH 7.6; 10 mM $MgCl_2$; 0.02% Tween20), 0.1 mg/mL BSA, [^{3}H]-cAMP to a final concentration of 15 nM and varying amounts of inhibitors. Reactions were initiated by addition of [^{3}H]-cAMP, and reactions were allowed to proceed for 1 h at room temperature before being terminated through mixing with 20 µL (0.2 mg) yttrium silicate SPA beads (PerkinElmer, Waltham, MA, USA). The beads were allowed to settle for 1 h in the dark before the plates were counted in a Wallac 1450 Microbeta counter (PerkinElmer). The measured signals were converted to activity relative to an uninhibited control (100%), and IC50 values were calculated using XlFit (IDBS) extension to excel. PDE4 inhibition assay was performed in a similar fashion using [^{3}H]-labelled cAMP.

Cell proliferation assays

Cell proliferation/survival was spectrophotometrically assessed using the Janus Green B green assay as described previously with some modifications.[20] Briefly, cells (1500 cells/well) were seeded in 96-well plates and the compound(s) or the vehicle, DMSO, were added. Vehicle concentrations were kept the same for all concentrations of compounds. Cells were formalin-fixed at day 0 (immediately after addition of compounds),1,2, 3, and 4. Fixed cells were stained for 5 min with 50 µl/well of 0.3% Janus B Green dye (Acros Organics, Belgium) at room temperature with continuous stirring followed by a washing step with water. The dye was eluted

with 200 µl/well of 0.5 M HCl of hydrochloric acid and top-read measurements of absorbance were performed in a microplate reader (Sinergy HT, Biotek, USA) at 595 nm. Data in figures are presented as % of control (DMSO). We used absorption values for statistical comparisons and, for calculation of IC50 values, we fitted data points using the concentration-response equation: $y=A2+ (A1–A2)/(1+ (x/x0)^p$.

ASSOCIATED CONTENT

Supporting Information

The supporting information is available free of charge via the internet at http://pubs.acs.org.

Copies ^{1}H NMR and ^{13}C NMR spectra of products and X-ray crystallographic data (ORTEP) for compound **7** (PDF).

X-ray crystallographic data for compound **7** (CIF).

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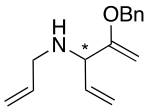

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