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Synthesis of 3a,4-dihydro-3*H*-[1]benzopyrano[4,3-*c*]isoxazoles, displaying combined 5-HT uptake inhibiting and α_2-adrenoceptor antagonistic activities. Part 2: Further exploration on the cinnamyl moiety ☆

Joaquín Pastor,[a] Jesús Alcázar,[a] Rosa M. Alvarez,[a] J. Ignacio Andrés,[a,*] José M. Cid,[a] Ana I. De Lucas,[a] Adolfo Díaz,[a] Javier Fernández,[a] Luis M. Font,[a] Laura Iturrino,[a] Celia Lafuente,[a] Sonia Martínez,[a] Margot H. Bakker,[b,†] Ilse Biesmans,[b] Lieve I. Heylen[b] and Anton A. Megens[b]

[a]*Johnson & Johnson Pharmaceutical Research & Development, Division of Janssen-Cilag, Medicinal Chemistry Department, Jarama s/n, 45007 Toledo, Spain*
[b]*Johnson & Johnson Pharmaceutical Research & Development, Division of Janssen Pharmaceutica N.V., Turnhoutseweg 30, B2340 Beerse, Belgium*

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Abstract—In our previous paper we have described the synthesis of a series of 3-piperazinylmethyl-3a,4-dihydro-3*H*-[1]benzopyrano[4,3-*c*]isoxazoles, as novel dual 5-HT reuptake inhibitors and α_2-adrenoceptor antagonists. That investigation led to the identification of the cinnamyl fragment as the most suitable moiety for combined activity. This paper outlines a further optimisation programme, focused on the exploration of the aromatic ring present on the cinnamyl moiety of compounds **1**, **2** and **3**.

1. Introduction

Depression is reported to affect up to 10% of the population, with a lifetime prevalence of 19% and is linked with a significant mortality.[1] The development of new pharmacotherapies for the treatment of depression remains an area of active research.[2,3]

In recent years we started a programme at Johnson & Johnson Pharmaceutical Research & Development, searching for compounds that combine serotonin (5-HT) reuptake inhibition and α_2-adrenoceptor blockade. This investigation led to the synthesis and pharmacological evaluation of several interesting series of tricyclic isoxazoline derivatives, such as compounds **1**, **2** and **3** (compound **I** in Scheme 1). Those compounds showed high affinity for α_2 receptors and 5-HT transporter (5-HTT) and in vivo activity in our medetomidine and pCA tests.[4]

Therefore, we were encouraged to carry out a further exploration to evaluate the influence of aromatic ring substitutions, as well as the replacement of phenyl ring by heteroaryl, described as general compound **I**. We herein report the methods used in the synthesis of the compounds and the determination of their binding affinity at the α_2 receptors and 5-HTT. The most promising analogues based on the in vitro data were subjected to our in vivo medetomidine and pCA assays.

2. Chemistry

The synthetic strategy for the exploration of hits **1**–**3** cited above, is depicted in Scheme 1. It is based on a 'Wittig type' olefination reaction as a key step, coupled

Keywords: Isoxazolines; 5-HT reuptake inhibitors; α_2 Antagonists.
☆ For Part 1, see Ref. 4.
* Corresponding author. Tel.: +34-925-24-57-80; fax: +34-925-24-57-71; e-mail: iandres@prdes.jnj.com
† Present address: Abbott GmbH & Co. KG, D-67008 Ludwigshafen, Germany.

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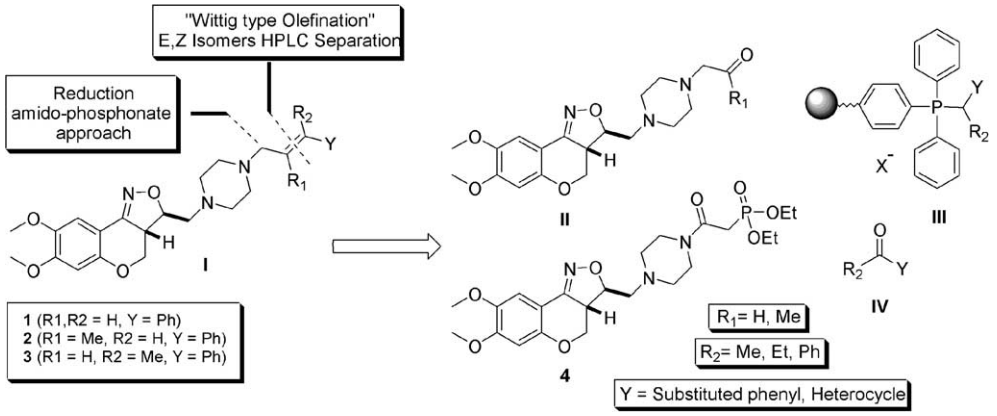
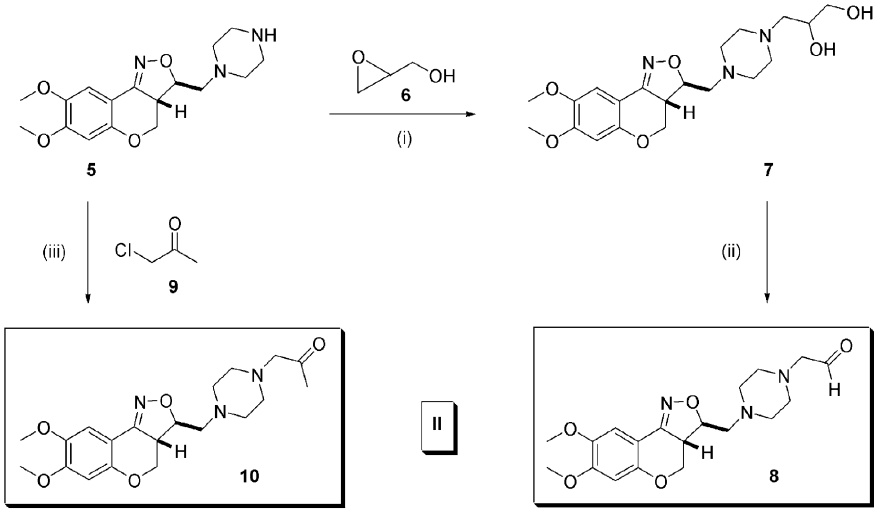


Scheme 1. Identified leads (**1**–**3**) and retrosynthetic analysis for their exploration.

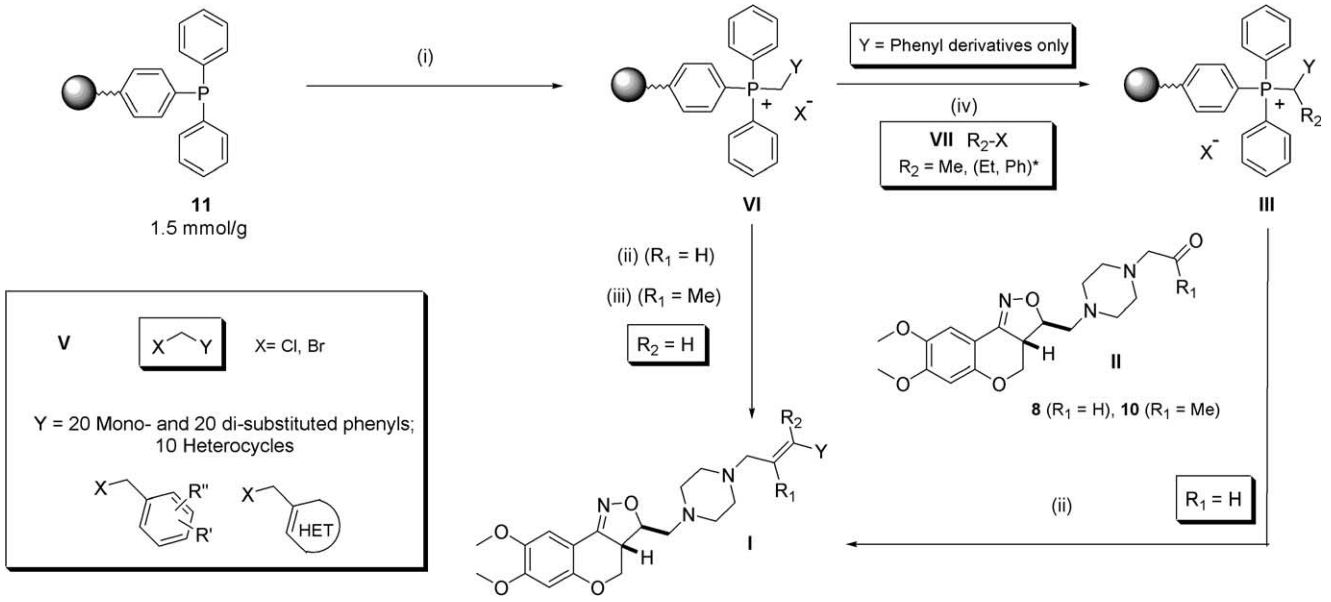
with preparative HPLC/MS separation of the corresponding *E*,*Z*-mixtures of isomers. Thus, the main approach presented herein, consisted in the solid-phase reaction of 3a,4-dihydro-3*H*-[1]benzopyrano[4,3-*c*]isoxazole scaffold-derived carbonyl compounds **II** with polymer supported phosphonium salts **III**.[5] Alternatively, we used a synthetic route based on the HWE reaction of isoxazole scaffold-derived amido-phosphonate **4** with suitable carbonyl derivatives **IV** to obtain some interesting compounds.[6]

The preparation of carbonyl derivatives **II** was achieved starting from compound **5** (Scheme 2).[4] Thus, treatment with an excess of hydroxymethyl-epoxide **6** under basic conditions afforded diol **7**, which was used as crude material in the next step. The oxidative cleavage of **7** was carried out using sodium periodate, giving aldehyde **8** in 95% yield over the two steps. On the other hand, piperazine derivative **5** was transformed into methyl ketone **10** by treatment with α-chloroacetone (**9**), using potassium carbonate as base in acetonitrile.

The solid-phase synthetic approach for the exploration of previously identified hits (**1**–**3**) is depicted in Scheme 3. Thus, reaction of commercially available polymer bound triphenylphosphine **11** with a set of mono- and di-substituted benzyl halides, as well as halomethyl heterocycles from commercial and/or 'in house' sources produced the polymeric phosphonium salts **VI**. This reaction was carried out with sets of 20 samples in an Argonaut's Quest 210 synthesiser, using an excess of the electrophiles in DMF as a solvent and under thermal conditions.[5d] After removal of excess of reagents by filtration and washing, the resins **VI** were resuspended in THF and treated with butyl lithium to produce the corresponding solid supported phosphonium ylides. Addition of aldehyde **8** afforded olefins **I** ($R_1 = R_2 = H$) as *E*,*Z*-mixtures in high purity.[7] Finally, both isomers were separated by preparative HPLC/MS.[8] In a similar way, the series of olefins **I** ($R_1 = Me$, $R_2 = H$) was prepared by reaction of ylides, derived from phosphonium salts **VI**, with ketone **10** in toluene at 100 °C. In summary, the series ($R_1 = R_2 = H$) were prepared using three diverse sets of building blocks, comprising 20 mono- and 20 di-substituted phenyls and 10 heterocycles. Every set of reagents was also used for preparation of series derived from ketone **10** ($R_1 = Me$, $R_2 = H$). In order to explore the influence of R_2, both sets of 20 polymer



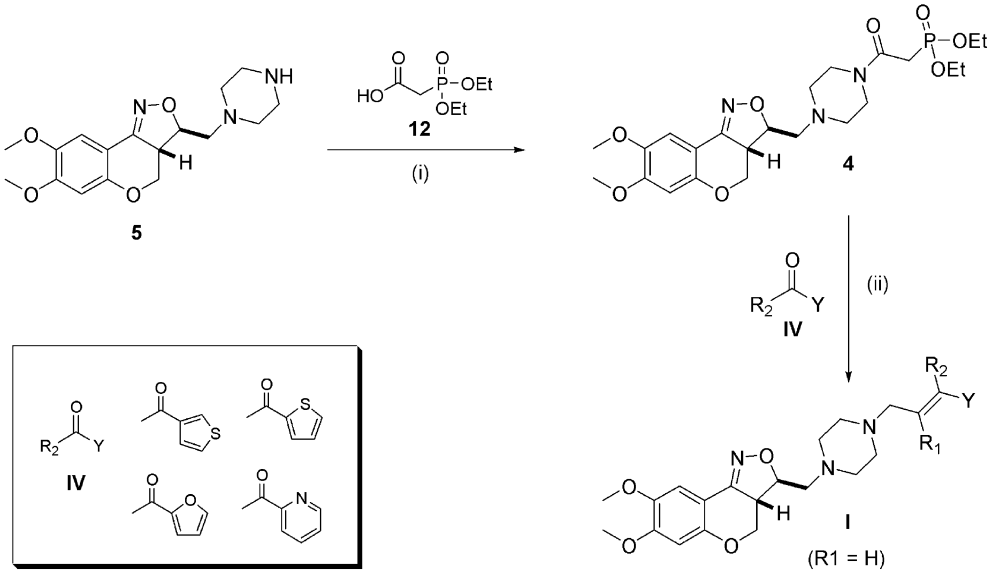
Scheme 2. Reagents and conditions: (i) **5** (5.0 equiv), K_2CO_3 (5.0 equiv), DMF, 80 °C, 48 h; (ii) $NaIO_4$ (1.2 equiv), $NaHCO_3$ (cat.), CH_2Cl_2–H_2O (9:1), rt, 4 h, 95% over two steps; (iii) **9** (1.2 equiv), K_2CO_3 (2.0 equiv), CH_3CN, rt, 18 h, 89%.



Scheme 3. Reagents and conditions: (i) **V** (5.0 equiv), DMF, 90 °C, 20 h; (ii) (a) BuLi 2.5 M in hexanes (0.9 equiv), THF, rt, 1 h; (b) **8** (0.9 equiv), 65 °C, 2 h; (c) preparative HPLC separation; (iii) (a) BuLi 2.5 M in hexanes (0.9 equiv), THF, rt, 1 h; (b) filtration and washing with anhyd THF; (c) **10** (0.9 equiv), toluene, 100 °C, 16 h; (d) preparative HPLC separation; (iv) (a) BuLi 2.5 M in hexanes (2.0 equiv), THF, rt, 30 min; (b) filtration and washings with anhyd THF; (c) **VII** (2.0 equiv), THF, rt, 2 h; (d) filtration and washings. $R_2 = Ph$, single compound prepared by direct alkylation of **11** with diphenylmethyl bromide under condition (i). $R_2 = Et$, single compound prepared from **VI** (Y = Ph) by alkylation with ethyl bromide under condition (iv).

bound phosphonium salts **VI** (mono- and di-substituted phenyls) were further alkylated with MeI to give compounds **III** ($R_2 = Me$). Similarly, reaction of these salts with aldehyde **8** gave the final *E*,*Z*-mixtures of olefins, which were separated by preparative HPLC/MS to afford compounds **I** ($R_1 = H$, $R_2 = Me$). Additionally, two compounds were prepared in order to further explore such position. Compound **I** ($R_1 = H$, $Y = Ph$, $R_2 = Et$) was synthesised following Scheme 3 by alkylation with ethyl bromide, while compound **I** ($R_1 = H$, $Y = R_2 = Ph$) was obtained from its corresponding polymeric phosphonium salt, which was synthesised directly by alkylation of **11** with diphenylmethyl bromide.

As a result of interesting biological data provided by compounds obtained by solid-phase synthesis exploration, four compounds were additionally designed and synthesised ($R_1 = H$, Y = heterocycle, $R_2 = Me$) (Scheme 4). Thus, piperazine derivative **5** was allowed to react



Scheme 4. Reagents and conditions: (i) **12** (1.5 equiv), CDI (1.5 equiv), CH_2Cl_2, rt, 16 h, 94%; (ii) (a) **IV** (1.2 equiv), NaH (60% in mineral oil, 1.5 equiv), 15-crown-6 (cat.), THF, rt, 16 h; (b) $LiAlH_4$ (1.0 M in THF, 1.5 equiv), rt, 2 h; (c) preparative HPLC separation.

with carboxy-phosphonate **12**, using CDI as coupling agent in dichloromethane to give amido-phosphonate **4** in good yield after purification by flash chromatography. Then, compound **4** was subjected to a HWE reaction with ketones **IV** and subsequent reduction with lithium aluminium hydride in a one-pot fashion affording the corresponding olefines, as *E*,*Z* mixtures. Finally, the predominant *E*-isomers were separated by HPLC/MS.[8]

In summary, 146 compounds were prepared as *E*,*Z*-mixtures by solid-phase parallel synthesis (Scheme 3) and the route depicted in Scheme 4. All of them were separated into their *E*,*Z*-isomers by HPLC/MS techniques and evaluated biologically.

3. Biological results and discussion

The α_{2A}-adrenoceptor subtype appears to mediate most of the classical functions attributed to the α_2-adrenoceptors, including its potential upregulation in depression.[9,10] The α_{2C}-adrenoceptor is thought to play a role exclusively within the CNS, being a potential therapeutic target for the treatment of various neuropsychiatric disorders.[9,11] On the contrary, the α_{2B}-adrenoceptor subtype appears to merely counteract the effects of α_{2A}-adrenoceptor and mediate hypertension.[9] These are the reasons why we decided to perform the in vitro primary screening routinely only for the α_{2A} and α_{2C} subtypes. Thus, molecules were evaluated in in vitro assays to define their affinity at the two different human α_{2A}- and α_{2C}-adrenoceptor subtypes and the 5-HT transporter site. The standard procedures used for the in vitro and in vivo evaluation of the compounds have been previously reported.[4]

After preliminary screening in α_{2A} and α_{2C} adrenoceptors and 5-HTT we concluded that, in general, the *Z*-isomers showed less potency than the *E*-isomers (see Table 1, Ref). Most promising analogues were evaluated also in vivo in both medetomidine and pCA assays (results are reported in Table 1).

Modifications of Y while keeping R_1, $R_2 = H$ showed that the introduction of halogen on the phenyl ring was the most favourable substitution. Thus, comparable or better in vitro affinity for all the receptors was observed for compounds **1a**–**c** (*o*, *m* and *p*-F, respectively) and **1d** (*p*-Cl). Even more interesting was their in vivo activity. The *o*-fluoro substituted derivative **1a** showed a slight reduction of potency whereas the *m*- and *p*-fluoro analogues **1b** and **1c** resulted 2.5-fold and 8-fold, respectively, more potent than the unsubstituted compound **1** in the pCA test. An important loss of activity in this pCA assay was observed when the fluorine atom was replaced by chlorine (compound **1d**). Regarding the medetomidine test, the activity was comparable for **1a** while compounds **1b**, **1c** and **1d** were slightly less potent than **1**.

Modifications of Y while keeping $R_1 = Me$, $R_2 = H$ showed that the introduction of halogen, represented by **2a** (*m*-F), **2b** (*p*-F), **2c** (2,5′-diF) and **2d** (2,3′-diF) or electron donor substituents as **2e** (*o*-MeO) led to compounds with comparable or slightly increased in vitro affinities for the three biological targets. Replacement of benzene by heterocycles as furan (**2f**), thiophene (**2g**,**h**) or pyridine (**2i**) were also tolerated for the in vitro activity. The activity in the pCA test was improved 2- to 16-fold, when compared with the unsubstituted compound **2**. Only the di-fluoro compound **2d** and some heterocycles as **2f**, **2g** and **2i** were somewhat less potent in this pCA test. In the medetomidine assay, significant improvement was only disclosed for compounds **2a** and **2g** (2-fold more potent). Compounds **2e** and **2h** exhibited similar potency and compounds **2c** and **2i** were found less potent. Of particular note is the decreased potency in this medetomidine test found for compounds **2b**, **2d** and **2f** despite their acceptable binding affinity at α_2-adrenoceptors.

Modifications of Y while keeping $R_1 = H$, $R_2 = Me$ showed that the introduction of either halogen represented by **3a** (*p*-F) and **3d** (2′,4′-F) or electron-donor groups by **3b** (*o*-OMe) and **3c** (*p*-OH) led to compounds with comparable or even better in vitro affinities. The replacement of benzene by some heterocycles (**3e**, **3f** and **3g**) generally maintained the affinities for α_2-adrenoreceptors but tended to suffer some loss in affinity at the 5-HTT. The high affinity that these compounds showed for α_2-adrenoreceptors was not confirmed in the medetomidine test when compared to **3**. Only **3a** (*p*-F) and the heterocyclic analogues **3e**–**g** disclosed comparable potency. In the pCA test, higher potency was found for compounds **3a** (about 8-fold), **3d** (2-fold) and **3e** (4-fold). Comparable activity was obtained with compound **3f**, while compounds **3b**, **3c** and **3g** were found somewhat less potent.

In summary, we were able to explore the cinnamyl moiety within the 3-piperazinylmethyl-3a,4-dihydro-3*H*-[1]benzopyrano[4,3-*c*]isoxazoline series. In general more potent compounds were obtained either by introduction of a fluorine atom or replacement of the phenyl ring by different heterocycles. Further developments of this series will be reported in due course.

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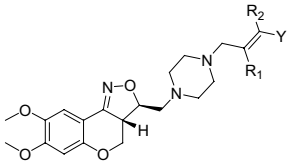
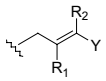
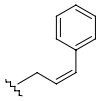
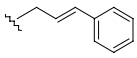
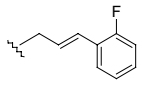
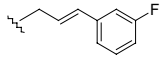
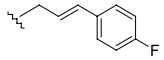
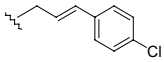
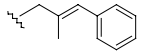
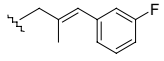
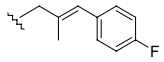
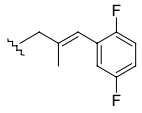
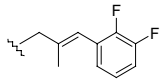
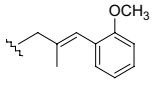
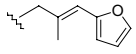
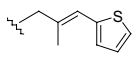
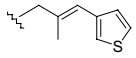
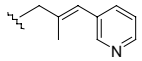
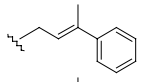
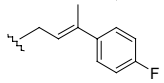
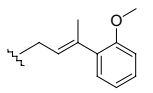
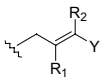
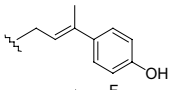
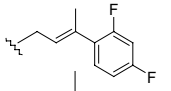
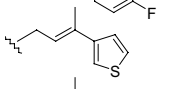
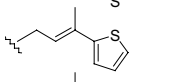
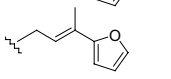
Table 1. Active doses of tricyclic isoxazolines for binding to the α_{2A}, α_{2C} receptors and 5-HT transporter in vitro (K_i values, nM) and for antagonism of pCA-induced excitation and medetomidine-induced loss of righting in vivo (ED_{50} values; mg/kg)



Compd		K_i (nM)			ED_{50} (mg/kg)	
		α_{2A}[a]	α_{2C}[a]	**5**-HTT[a]	pCA (sc)[b]	Medet (sc)[b]
Ref.		23	9.5	35	10	>10
1		8.8	6.2	8.3	0.75 (0.5–1.2)	2.5 (1–6.5)
1a		2.9	2.8	13	1.25 (0.4–3.6)	2.5 (1.6–5.3)
1b		5.9	0.7	2.5	0.3 (0.1–0.7)	5 (2.5–9.9)
1c		9	23	5.6	0.1 (0.06–0.3)	4.7 (1.7–8.5)
1d		69	10	1.3	2.5 (1–6.5)	10
2		0.8	0.2	2.3	1.5 (1.1–2.1)	1.3 (0.7–1.5)
2a		1.1	0.2	0.6	0.16	0.63
2b		3.4	0.7	1.2	0.1 (0.04–0.16)	10
2c		0.3	0.2	3.2	1.25 (0.4–3.6)	2.5
2d		3	0.7	7.1	2.8 (1.4–6.9)	10
2e		1	0.4	1.6	0.8 (0.4–1.7)	2 (0.9–4.4)
2f		1.7	0.5	21	5 (3.6–17)	5 (2.5–10)
2g		1.7	0.2	2.7	5 (1.4–7)	0.63
2h		1.1	0.2	1.9	0.2 (0.1–0.4)	1.25 (0.6–2.5)
2i		22	2.1	63	3.2 (1.1–9)	3.1 (1.6–5.3)
3		0.3	0.2	4.5	0.65 (0.2–1.1)	0.3 (0.16–0.63)
3a		2.9	0.3	1.3	0.08 (0.04–0.15)	1.25 (0.5–2.6)
3b		5.2	0.9	51	1.25 (0.6–2.5)	>2.5

(*continued on next page*)

Table 1 (*continued*)

Compd		K_i (nM)			ED_{50} (mg/kg)	
		α_{2A}[a]	α_{2C}[a]	**5**-HTT[a]	pCA (sc)[b]	Medet (sc)[b]
3c		1.9	0.6	7.6	2 (0.9–4.3)	>2.5
3d		9.3	0.8	5.4	0.3 (0.1–0.5)	>2.5
3e		1.4	0.3	4.8	0.15 (0.04–0.6)	1.25 (0.6–2.5)
3f		0.3	0.2	74	0.63	0.63
3g		0.5	0.4	22	1.25 (0.6–2.5)	0.16

[a] The activity of compounds was confirmed in an independent experiment. A difference in pIC_{50} between experiments up to 0.6 (SD < 0.5) was considered as reproducible and therefore accepted.

[b] ED_{50} and corresponding 95% of confidence limits (shown between brackets) were determined according to the modified Spearman–Kaerber estimate using theoretical probabilities instead of empirical ones.[12] This modification allows to tabulate the ED_{50} and its confidence interval as a function of the slope of the log dose–response curve.

References and notes

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7. The purity of crude samples was >85% as sum of isomers, analysed by HPLC-DAD-ESI-MS, calculating purity by the DAD trace. A generic gradient (80/10/10 0.5% $AcONH_4$/CH_3CN/MeOH to 50/50 CH_3CN/MeOH in 9 min) in a Hypersil BDS C-18 100 mm × 4.6 mm i.d. × 3.5 μm was used.
8. *E*/*Z* isomers were isolated by normal phase HPLC-DAD-ESI-MS, using the MW to collect selectively the fractions of interest. A generic gradient (100% CH_2Cl_2 to 10% MeOH in 15 min) in a Kromasil 60 silica 150 mm × 21 mm i.d. × 10 μm was used.
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