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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: A Novel Series of Potential Antidepressants

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Abstract—The synthesis of a series of novel 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 is described. Their affinity at the three different human α_2-adrenoceptor subtypes and the 5-HT transporter site is reported. The in vivo activity of the compounds was measured in two different assays: (1) inhibition of pCA-induced excitation, which evaluates the ability to block the central 5-HT transporter, and (2) inhibition of xylazine-induced loss of righting, which evaluates the ability to block central α_2-adrenoceptors.

Introduction

The monoaminergic hypothesis for depression, initially put forward to explain the antidepressant effect of tricyclic antidepressants (TCAs) and monoamine oxidase inhibitors (MAOIs), assumes that depression is caused by a functional deficit of monoamines (noradrenaline and/or serotonin) in corticolimbic synaptic clefts.[1] This biogenic amine hypothesis showed that increased noradrenergic and/or serotonergic neurotransmission results in improvement of mood, and was based on observed induction of depressive symptoms reported to occur as a side effect of treatment with the antihypertensive agent reserpine, which results in the depletion of monoaminergic stores.[2,3]

Currently, the most commonly used pharmacological treatments for depression are the selective serotonin (SER) reuptake inhibitors, the SSRIs. Their mood lifting effects are the current gold standard in the treatment of depression. However, limitations of the available treatments include a 2–4-week delay for the onset of action, partial treatment response, excitation during early treatment response, nausea and reduced sexual function. The period of several weeks typically required for the onset of antidepressant action with such agents has been attributed to a feedback inhibition, via presynaptic autoreceptors,[4,5] of further noradrenaline (NE) and/or SER release caused by an acute rise in synaptic neurotransmitter concentrations following inhibition of metabolism or reuptake. Newer drugs also specifically inhibit NE reuptake, have a dual mechanism of action inhibiting both SER and NE reuptake, or inhibit monoamine breakdown by monoamine oxidase inhibition. In addition a number of 'atypical antidepressants' are available on the market (nefazodone, mirtazapine, bupropion), which act by several other mechanisms of action. Enhancement of multiple monoaminergic system

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activities in parallel is expected to increase the efficacy of antidepressant treatment, and broaden the number of parameters on which the therapy will be effective.[6]

Blockade of α_2-adrenoceptors in the brain prevents the negative feedback NE exerts on its own synthesis, neuronal firing and release, resulting in enhanced NEergic neurotransmission.[7,8] α_2-adrenoceptor blockade also increases extracellular dopamine,[9] acetylcholine[10] and SER levels[11] in vivo in the rat and human. Adding α_2-adrenoceptor blocking activity to SER reuptake inhibition is therefore expected to be more effective and to more broadly enhance monoaminergic activity in the brain. The immediate effect on monoamine release of autoreceptor blockade, in contrast to the initial inhibition of activation by reuptake inhibitors through their activation of feedback mechanisms, may reduce the onset of action of such a compound compared to the currently available drugs. In addition, α_2-adrenoceptor antagonism improves sexual function as shown by treatment with the α_2-adrenoceptor antagonist yohimbine,[12] and enhancement of NEergic neurotransmission improves social function more effectively than SSRI's.[13,14] Furthermore, combination therapy of depressive patients with drugs with an α_2-adrenoceptor antagonistic component (mianserin, mirtazapine or yohimbine) in addition to an SSRI, have repeatedly shown increased efficacy and effectiveness on treatment resistant patients.[15–19]

In the past years a few compounds have been described which combine SER reuptake inhibition and α_2-adrenoceptor blockade: Sterling-Winthrop's napamezole (**1**),[20] Abbott's A-80426 (**2**)[21] or Servier's S-34324 (**3**)[22] (Fig. 1). In recent years we started a programme at Johnson & Johnson Pharmaceutical Research & Development, searching for compounds combining both activities. As a result of the biological screening the tricyclic isoxazoline derivative **4** was identified as a hit. The introduction of two methoxy groups in positions 7 and 8 of the tricyclic system slightly enhanced the in vitro potency for the SER transporter, while decreased the affinity at the α_2-receptors. But most interestingly, compound **5** showed more activity than compound **4** in some of our in vivo tests in rats.[23] Both compounds showed much less affinity or no affinity at all for other serotonergic, dopaminergic and adrenergic receptors. We synthesized as well the monomethoxy-substituted derivatives **6** and **7** (Fig. 1). While the 7-methoxy analogue showed comparable in vitro and in vivo activity to that of the dimethoxy compound **5**, the 8-methoxy derivative kept the in vitro activity but exhibited less in vivo potency (see Table 1).

As a part of the subsequent Lead Optimisation programme, we synthesised a large number of compounds with several different moieties replacing the cinnamyl fragment present in our lead **5**. This paper describes the synthesis and structure–activity relationship of only some of those compounds, which were prepared within this series.

Chemistry

The ethyl 3a,4-dihydro-3*H*-[1]benzopyrano[4,3-*c*]isoxazole-3-carboxylates **11a–d** were obtained following essentially the method previously described for the synthesis of the methyl ester analogue of **11a**.[24] Thus, alkylation of salicylaldehydes **8a–d** with ethyl 4-bromocrotonate, in presence of potassium carbonate as base and DMF as solvent, afforded intermediates **9a–d**. These aldehydes were transformed into the oximes **10a–d** by reaction with hydroxylamine. The generation of the required nitrile oxides and subsequent ring closure by intramolecular 1,3-dipolar cycloaddition to the cycloadducts **11a–d**, was carried out using sodium hypochlorite and triethylamine, in yields ranging from 42 to 85% from the corresponding oximes.[25] The stereochemistry of positions 3 and 3a of the tricyclic system was predetermined by the *trans*-alkene fragment and was unequivocally assigned by NMR. Reduction of the four esters with $NaBH_4$ in THF/H_2O as solvent afforded the corresponding hydroxymethyl derivatives in good yields, which were converted into the mesylates **12a–d** by standard procedures. These mesylates were

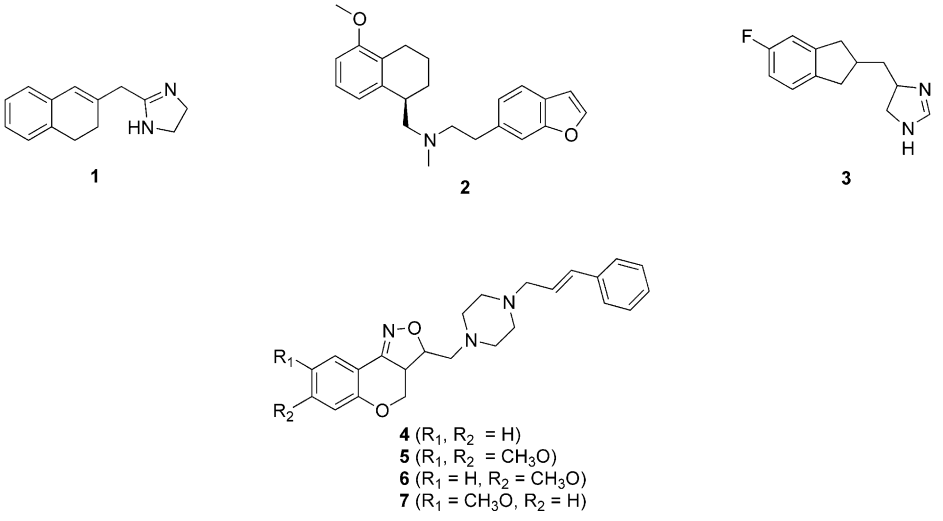
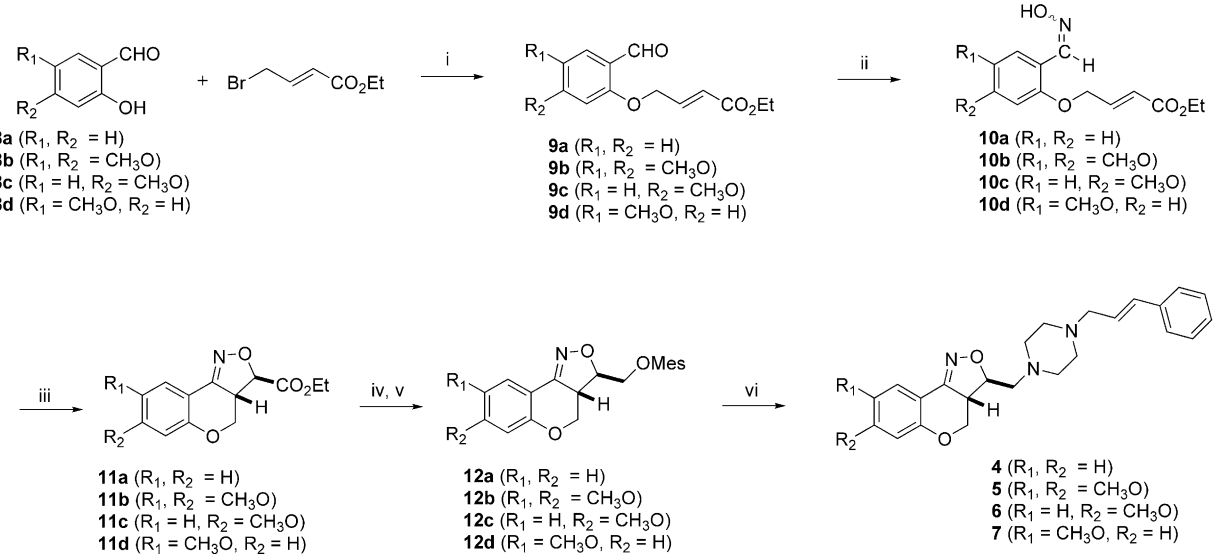


Figure 1.

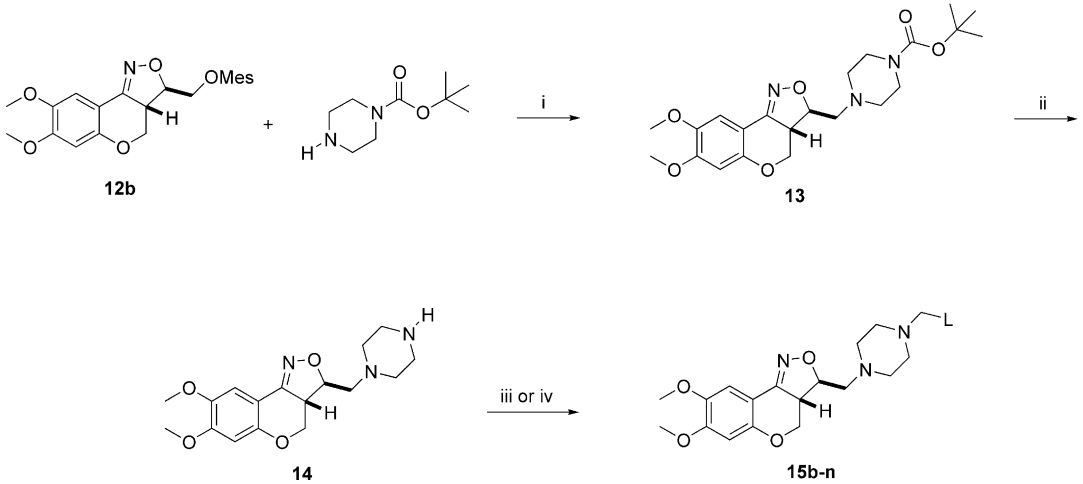
reacted with commercially available cinnamylpiperazine furnishing the target compounds **4**–**7** in 56 to 69% yields (Scheme 1). The relative *cis* configuration between the 3-exocyclic chain and the 3a-hydrogen atom was kept unaltered during these last three steps in the four cases.

Mesylate **12b** served also as starting material for the introduction of other moieties replacing the cinnamyl fragment. In some cases we obtained the target compounds by reaction of this mesylate with the corresponding required piperazine derivatives, which had to be previously synthesized. But for most of the compounds we used a slightly modified and more convergent strategy. Thus, reaction of **12b** with excess *N*-*tert*-butyloxycarbonylpiperazine afforded the *N*-Boc-protected intermediate **13**, which was deprotected with CF_3COOH to furnish the key derivative **14** in 90% yield. Alkylation of this compound with the different required haloalkyl derivatives, or reductive amination with the corresponding aldehydes afforded the desired final products **15b**–**n** (Scheme 2).

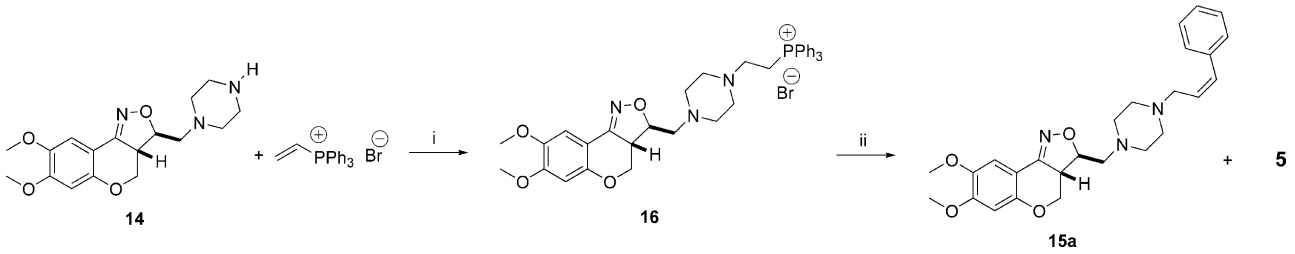
The synthesis of the *cis*-cinnamyl analogue **15a** was achieved by a different procedure. Piperazinyl derivative **14** was reacted with vinyltriphenylphosphonium bromide yielding the phosphonium salt **16**. This intermediate was subjected to a Wittig reaction with benzaldehyde furnishing the mixture of *trans* and *cis* isomers in a 3/1 ratio, which was separated by preparative HPLC affording compound **15a** in 22% yield (Scheme 3). The biological results led us to decide to separate and isolate the enantiomers of four selected compounds for pharmacological screening. The separation and isolation of those enantiomers was performed by preparative chiral HPLC (Chiralpak AD 1000A, 20



Scheme 1. Reagents and conditions: (i) K_2CO_3, DMF, rt, 2–4h; (ii) $NH_2OH2HCl$, pyridine, CH_3CH_2OH, rt, 2 h, 63–75% (two steps); (iii) Et_3N, NaOCl (5%), CH_2Cl_2, rt, overnight, 42–85%; (iv) NaBH4, THF/H2O, rt, 2h, 84–99%; (v) CH_3SO_2Cl, Et_3N, CH_2Cl_2, rt, 1 h, 78–90%; (vi) N-cinnamylpiperazine (2 eq.), neat, 100°C, 2h, 56-69%.



Scheme 2. Reagents and conditions: (i) 1-Boc-piperazine, KI, K_2CO_3, MIK, reflux, overnight, 90%; (ii) CF_3COOH, CH_2Cl_2, rt, 3 h, 100%; (iii) L-CH_2-Hal, K_2CO_3, MIK, reflux, overnight; (iv) L-CHO, $NaBH(AcO)_3$, CH_3COOH (cat.), CH_2Cl_2, rt, 3 h-overnight.



Scheme 3. Reagents and conditions: (i) CH_2Cl_2, rt, overnight, 90%; (ii) (a) PhCHO, MeONa, THF, −60°C, 6 h, then rt, overnight; (b) preparative HPLC separation, 22%.

μm, Diacel, using as eluents mixtures hexane/ethanol or ethanol/acetonitrile with different gradients).

Biological Results and Discussion

Molecules were evaluated in in vitro assays to define their affinity at the three different human α_2-adrenoceptor subtypes and the SER transporter site. Membranes prepared from CHO cells, expressing the human adrenergic α_{2A}-, α_{2B}- or α_{2C}-receptors, were thawed and incubated with [^{3}H]rauwolscine (1 nM final concentration) with or without competitor for 30 min at 25 °C. Human platelet membranes (Oceanix Biosciences Corporation, Hanover, USA) were thawed and incubated with [^{3}H]paroxetine (0.5 nM final concentration) with or without competitor for 60 min at 25 °C as well. The affinities of the compounds for other serotonergic as well as dopaminergic and adrenergic receptors were also measured by standard procedures. Their affinities at the dopaminergic and noradrenergic uptake sites were evaluated as well. None of those compounds showed relevant affinity at any other of those receptors or transporter sites. *p*-Chloroamphetamine (pCA) induces release of serotonin from serotonergic nerve terminals, which results in typical behavioural effects in rats. As pCA produces its biochemical and behavioural effects only after its uptake into the serotonergic neurones by the neuronal SER transporter, compounds that block this transporter mechanism can inhibit its effects. Antagonism of pCA-induced excitation in rats, in the absence of overt sedative effects, is therefore a reliable in vivo index of the ability of test compounds to block the central serotonin transporter.[26] Antagonism of the loss of righting, induced by the α_2-adrenoceptor agonist xylazine in rats, is a reliable index for the central α_2-adrenoceptor blocking activity of test compounds, at least when occurring without behavioural stimulant effects.[27] These two in vivo assays, the inhibition of pCA-induced excitation and the inhibition of xylazine-induced loss of righting, were selected as primary tests to evaluate the in vivo activity of our compounds as SER transporter inhibitors and α_2-adrenoceptor antagonists respectively (Table 1).

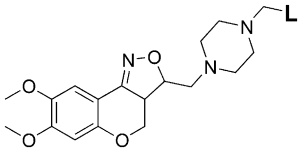
Compound A-80426 showed nanomolar affinities at both α_2-receptors and SER transporter site, but did not show significant in vivo activity in our tests. On the contrary S-34324, which is described as a NE reuptake inhibitor as well,[22] exhibited moderate activity in both pCA and xylazine tests although its in vitro affinity was lower. As can be observed in Table 1, compound **15a**, the corresponding *cis*-analogue of **5**, showed less affinity at both α_2-receptors and SER transporter site and showed less activity in our pCA test. Reduction of the double bond present in the cinnamyl moiety, exemplified by compound **15d**, did not substantially affect the in vitro affinity for both targets, but resulted in a significant decrease of the activity in the pCA test. Shortening of the methylene spacer between the phenyl and the piperazine rings (**15b**,**c**) dramatically decreased the receptor affinities. Replacement of the methylene group adjacent to the phenyl ring in **15d** by a carbonyl group resulted in a compound about 10-fold less active at the SER transporter site (**15e**).

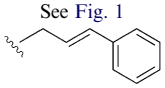
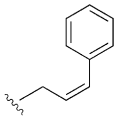
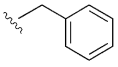
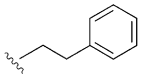
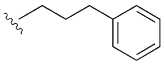
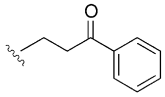
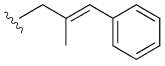
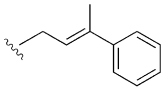
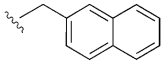
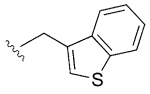
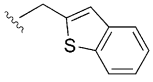
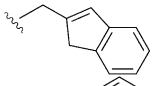
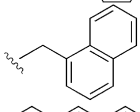
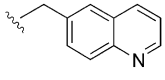
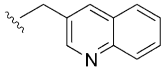
The introduction of a methyl group in either one or the other position of the double bond of the cinnamyl moiety afforded very interesting compounds. Compound **15f** was over 10-fold more potent than **5** at the α_2-receptors and 3-fold more potent at the SER transporter. Even more interesting was its in vivo activity. It showed comparable potency both in the pCA and the xylazine assays. The other methyl-cinnamyl analogue **15g** exhibited also more potency than **5** at the α_2-receptors while both compounds were equipotent at the SER transporter. But interestingly this compound showed 4-fold more potency in the pCA assay and the same potency in the xylazine test when compared to **5**.

The β-naphthyl derivative **15h** was the most potent compound at the SER transporter site and it showed as well quite potent activity in the pCA test, while its affinity for the α_2-receptors was quite similar to that of the cinnamyl lead compound **5**. Some naphthyl-like heterocyclic derivatives were synthesized as well (**15i**, **j**, **m**, **n**) but all of them showed much less affinity at the α_2-receptors, while keeping the affinity for the SER transporter site. The corresponding α-naphthyl analogue **15l** kept as well the affinity at the SER transporter site and the activity in the pCA test, but did not show potent affinity at the α_2-receptors. Compound **15k**, which was a cyclic analogue of the methyl-cinnamyl derivative **15f**, showed also quite remarkable affinity at the SER transporter site and high potency in the pCA assay, but its affinity at the α_2-receptors dropped again showing very low activity in the xylazine test.

The promising results of several compounds shown in Table 1 encouraged us to select four of them for the separation and screening of their corresponding enantiomers. Our first aim was to determine if there was a

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



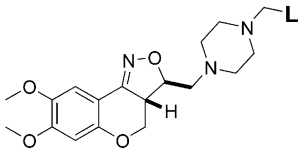
Compd	L	α_{2A}[a] K_i (nM)	α_{2B}[a] K_i (nM)	α_{2C}[a] K_i (nM)	5-HTT[a] K_i (nM)	pCA (sc)[b] ED_{50} (mg/kg)	Xylazine (sc)[b] ED_{50} (mg/kg)
2	A-80426	2.4	11	2.1	4	10	>10
3	S-34324	5.9	23	19	77	2	2.7
4	See Fig. 1	0.9	2	1.7	16	5	2
5		8.8	42	6.2	8.3	0.76	2.5
6	See Fig. 1	0.5	16	1.9	19	1.4	2
7	See Fig. 1	0.8	12	2.3	14	5	2.5
15a		23	n.t.	9.5	35	10	n.t.
15b		186	995	89	158	8	>10
15c		234	>1000	89	199	10	>10
15d		26	42	1.9	10	5	2.5
15e		12	n.t.	5.3	123	n.t.	n.t.
15f		0.8	7.5	0.2	2.3	1.5	1
15g		1.4	31	0.6	10	0.2	2.5
15h		5	49	3.1	1.7	0.79	5
15i		66	370	13	7.2	n.t.	n.t.
15j		98	>1000	89	5	2.5	>10
15k		22	315	4.7	4.1	0.32	10
15l		234	>1000	89	3.9	3.2	>10
15m		117	721	36	8.1	0.63	5
15n		34	>1000	30	14	5	5

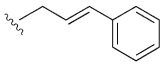
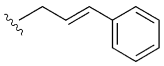
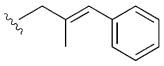
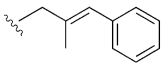
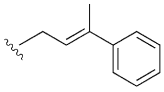
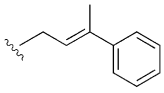
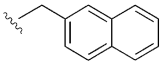
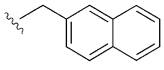
n.t., not tested.

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

[b]ED50 values and corresponding 95% confidence limits were determined according to the modified Spearman–Kaerber estimate using theoretical probabilities instead of empirical ones.[28] This modification allows to tabulate the ED50 and its confidence interval as a function of the slope of the log dose–response curve.

Table 2. Active doses of some selected pure enantiomers for binding to the α_{2A}, α_{2B}, α_{2C} receptors and the 5-HT transporter in vitro and for antagonism of pCA-induced excitation and xylazine-induced loss of righting in vivo (ED_{50} values; mg/kg)



Compd	L	α_{2A}[a] K_i (nM)	α_{2B}[a] K_i (nM)	α_{2C}[a] K_i (nM)	5-HTT[a] K_i (nM)	pCA (sc)[b] ED_{50} (mg/kg)	Xylazine (sc)[b] ED_{50} (mg/kg)
(−)-**5**		25	42	4.8	3.4	2.5	5
(+)-**5**		2.6	14	0.5	1.2	0.51	2.5
(−)-**15f**		16	15	1.4	2.8	>10	>10
(+)**15f**		0.3	3	0.1	5.4	0.82	3.1
(−)-**15g**		30	405	10	10	>10	>10
(+)-**15g**		0.3	14	0.2	4.5	0.5	0.32
(−)-**15 h**		93	95	9.5	1.3	1.5	10
(+)-**15 h**		1.3	14	0.8	1.7	0.08	6.1

[a]The activity of compounds was confirmed in an independent experiment. A difference in pIC_{50} up to 0.6 (S.D. <0.5) was considered as reproducible and therefore accepted.
[b]ED_{50} values and corresponding 95% confidence limits were determined according to the modified Spearman–Kaerber estimate using theoretical probabilities instead of empirical ones.[28] This modification allows to tabulate the ED_{50} and its confidence interval as a function of the slope of the log dose–response curve.

dissociation of affinities between the α_2-adrenoceptors and the SER transporter site. Our second purpose was, in case the activity resided mainly in one of the enantiomers, to biologically characterize the compounds searching for a potential candidate for more in-depth pharmacological evaluation. The selected compounds were the lead **5**, the two methyl-cinnamylpiperazinyl analogues **15f** and **15g** and the naphthylmethylpiperazinyl derivative **15h**. The primary biological results are summarized in Table 2.

As can be deduced from the data shown in Table 2, the affinity at the SER transporter site was comparable for each pair of enantiomers. In the case of the enantiomers of **15g** and **15h** their potencies were very similar to that of the racemate, while the enantiomers of **5** showed higher affinity and those of **15f** showed slightly less affinity than the corresponding racemate. On the contrary, the stereochemical configuration did influence the affinity at the α_2-adrenoceptors. As a matter of fact the four (+)-enantiomers were very significantly more active at those receptors than their corresponding (−)-pairs. Thus, compound (+)-**5** was approximately equipotent at both binding targets, showing higher affinity than racemic **5**. However, this increase was not correlated with an improvement of potency in our xylazine assay. Compound (+)-**15f** showed binding values comparable to its corresponding racemate, while its activity in the pCA and xylazine tests remained quite comparable as well. The methyl-cinnamyl analogue (+)-**15g** showed the best balance of in vivo activities. It showed comparable potency in both assays, keeping the potency in the pCA test but increasing by 8-fold the potency in the xylazine test of its parent racemate. The naphthyl derivative (+)-**15h** exhibited higher affinity at the α_2-adrenoceptors than its parent racemate, showing moderate activity in the xylazine test as well. Although the affinity at the SER transporter site was of the same order, this compound showed the highest potency in our pCA test (ED_{50} = 0.08 mg/kg). It is worthy to note that although the (+) and (−) isomers of **15f** and **15g** showed only slight in vitro difference in their affinities at

the SER uptake site, a large difference in their activity in the pCA test was observed. We have not any convincing explanation for these results. All these compounds are being more in-depth pharmacologically evaluated.

In summary, we have discovered a new series of 3-piperazinylmethyl-3a,4-dihydro-3*H*-[1]benzopyrano[4,3-*c*]isoxazoles as novel dual 5-HT uptake inhibitors and α_2-adrenoceptor antagonists. The combined activity mainly resided in one of the enantiomers. Additional chemical exploration of the most interesting structural modifications is being carried out and will be published elsewhere. The biological evaluation of the most promising compounds as potential new antidepressants will be the subject for further publications.

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27. Xylazine-induced loss of righting in rats: Xylazine (15.0 mg/kg, iv)-induced loss of righting reflex was recorded up to 120 min after injection in overnight-starved, male Wistar rats (200–250 g; internal breeding facilities) pre-treated with test compound or solvent 1 h earlier. Criterion for drug-induced reversal: absence of loss of righting reflex (4.2% false positive controls; $n>300$).
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