Discovery of a New Series of Centrally Active Tricyclic Isoxazoles Combining Serotonin (5-HT) Reuptake Inhibition with α_2-Adrenoceptor Blocking Activity

J. Ignacio Andrés,*,† Jesús Alcázar,† José M. Alonso,† Rosa M. Alvarez,† Margot H. Bakker,§,‡ Ilse Biesmans,§ José M. Cid,† Ana I. De Lucas,† Javier Fernández,† Luis M. Font,† Koen A. Hens,§ Laura Iturrino,† Ilse Lenaerts,§ Sonia Martínez,† Anton A. Megens,§ Joaquín Pastor,† Patrick C. M. Vermote,§ and Thomas Steckler§

Johnson & Johnson Pharmaceutical Research & Development, Division of Janssen-Cilag, Medicinal Chemistry dept., Jarama s/n, 45007 Toledo, Spain, and Johnson & Johnson Pharmaceutical Research & Development, Division of Janssen Pharmaceutica, Turnhoutseweg 30, B2340 Beerse, Belgium

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The synthesis and pharmacology of a new series of 3-piperazinylmethyl-3a,4-dihydro-3*H*-[1]-benzopyrano[4,3-*c*]isoxazoles that combine central serotonin (5-HT) reuptake inhibition with α_2-adrenoceptor blocking activity is described as potential antidepressants. Four compounds were selected for further evaluation, and the combination of both activities was found to be stereoselective, residing mainly in one enantiomer. Reversal of the loss of righting induced by the α_2-agonist medetomidine in rats confirmed the α_2-adrenoceptor blocking activity in vivo and also demonstrated CNS penetration. Antagonism of *p*-chloroamphetamine (pCA)-induced excitation as well as blockade of the neuronal 5-HT depletion induced by p-CA administration in rats confirmed their ability to block the central 5-HTT, even after oral administration. Replacement of the oxygen atom at the 5-position of the tricyclic scaffold by a nitrogen or a carbon atom, as well as O-substitution at position 7, led also to active compounds, both in vitro and in vivo.

Introduction

Major depressive disorder (MDD) affects an estimated 18 million people in the US and 340 million worldwide. In the US and Europe, MDD has a lifetime prevalence of ∼17%. By 2020, MDD is expected to be the second leading cause of disease or injury in the world.[1,2] The monoaminergic hypothesis of depression assumes that depression is caused by the dysfunction of the serotonin (5-HT, SER), norepinephrine (NE) and/or dopamine (DA) neurotransmitter systems in corticolimbic synaptic clefts.[3] Based on this hypothesis, the currently most commonly used pharmacological treatments for depression are the selective serotonin reuptake inhibitors, the SSRIs. Their mood lifting effects are the current gold standard in the treatment of depression. However, they show also some limitations such as a 2−4 week delay for the onset of action, partial treatment response, excitation during early treatment response, nausea, and reduced sexual function.[4] 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,[5,6] of noradrenaline (NE) and/or 5-HT release that counteracts the initial 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 5-HT 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 (Chart 1). Enhancement of multiple monoaminergic system activities in parallel is expected to increase the efficacy of antidepressant treatment and broaden the therapeutic profile.[7] However, to date a convincing proof of early onset of action has not been demonstrated for any single antidepressant drug.[8]

α_2-Adrenoceptors have an important role in the regulation of neurotransmitter release.[9] First of all, release-regulating α_2-adrenoceptors are present on the terminals of noradrenergic neurons. NE released into the synaptic cleft is thought to stimulate these presynaptic autoreceptors, triggering a negative feedback mechanism that acts to inhibit subsequent neurotransmitter release. In addition to their presynaptic location, α_2-autoreceptors are found on the cell bodies of locus coeruleus neurons. Stimulation of these "somatodendritic" receptors also results in a depression of NE release in terminal regions, in this case via a reduction in the firing rate of locus coeruleus projection neurons. 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 noradrenergic neurotransmission.[10,11] α_2-Adrenoceptor blockade also increases extracellular dopamine,[12] acetylcholine,[13] and 5-HT levels[14] in vivo in the rat and human. Thus, it is thought that a 5-HT reuptake inhibitor with associated α_2-adrenoceptor antagonistic activity might be a new type of antidepressant, with a dual action on the central noradrenergic and serotonergic neuronal systems. The immediate effect on

* Corresponding author. Fax: + 34 925 24 57 71; e-mail: iandres@prdes.jnj.com.
† Division of Janssen-Cilag.
§ Division of Janssen Pharmaceutica.
‡ Present address, Abbott GmbH & Co. KG, D-67008 Ludwigshafen, Germany.

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Chart 1. Set of "Atypical Antidepressants" and α_2 Antagonists

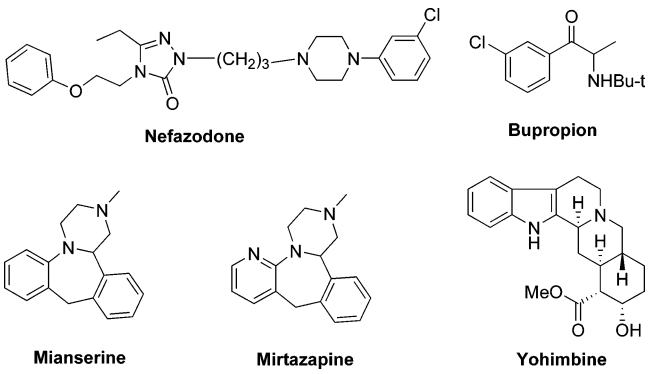
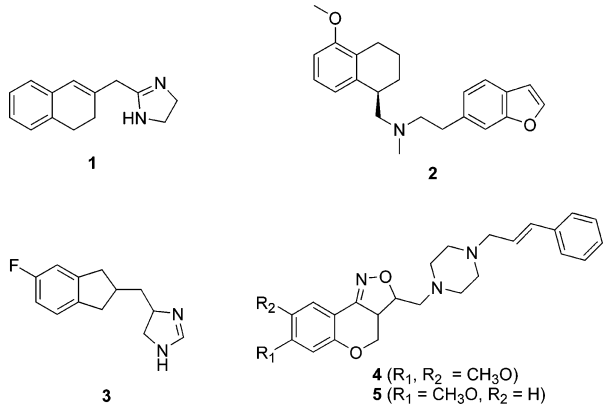


Chart 2. Combined 5-HTT Inhibitors and α_2 Antagonists



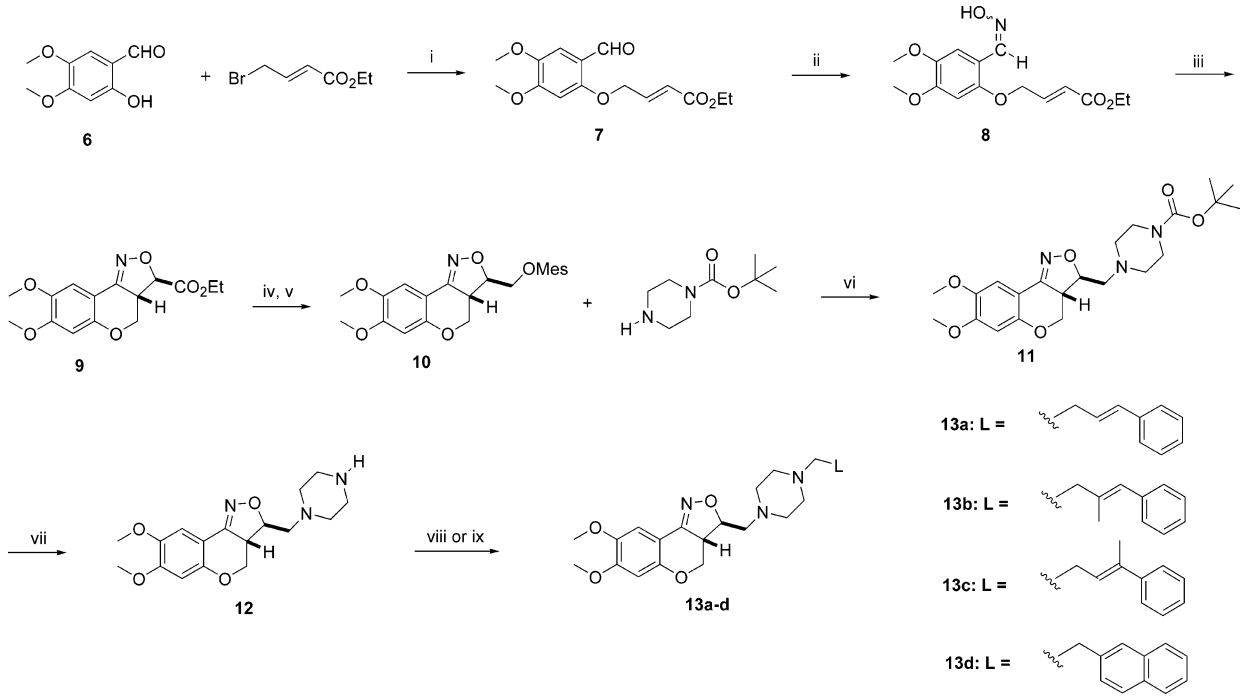
monoamine release of autoreceptor blockade may accelerate the onset of action of such a compound, compared with currently available drugs that require desensitization of the autoreceptors involved in the feedback mechanism in order to become fully effective. In addition, α_2-adrenoceptor antagonism improves sexual function as shown by treatment with the α_2-adrenoceptor antagonist yohimbine,[15] thereby potentially reducing one of the side effects related to 5-HT uptake inhibition and enhancement of NEergic neurotransmission improves social function more effectively than SSRIs.[16,17] Furthermore, combination therapy of depressive patients with drugs with an α_2-adrenoceptor antagonistic component such as mianserin, mirtazapine or yohimbine (Chart 1) in addition to an SSRI have repeatedly shown increased efficacy and effectiveness on treatment of resistant patients.[18−22] In the past years a few compounds have been described which combine 5-HT reuptake inhibition and α_2-adrenoceptor blockade: Sterling-Winthrop's napamezole (**1**),[23] Abbott's A-80426 (**2**)[24] or Servier's S-34324 (**3**)[25] (Chart 2).

In recent years we started a program searching for compounds combining 5-HT reuptake inhibition and α_2-adrenoceptor blockade. In a previous communication we have reported the discovery by random screening of a series of 3-piperazinylmethyl-3a,4-dihydro-3*H*-[1]benzopyrano[4,3-*c*]isoxazole derivatives as novel dual 5-HT reuptake inhibitors and α_2-adrenoceptor antagonists.[26] The identification of the cinnamylpiperazinyl derivatives **4** and **5** (Chart 2) prompted us to start a broad Lead Optimization program. First we synthesized a large number of compounds with several different moieties replacing the cinnamyl fragment. We identified some "cinnamyl-like" fragments that increased the activity at both or at least one of the targets, and we also identified the most potent enantiomers within this series of compounds.[26] We have also recently described a further optimization program focused on the exploration of the aromatic ring present on the cinnamyl moiety.[27] In this article we describe the synthetic methods used to prepare the most promising compounds in this series, as well as primary pharmacological evaluation of the most active enantiomers. We also report the synthesis and primary pharmacological activity of novel 3,3a,4,5-tetrahydroquinolino[4,3-*c*]isoxazoles and 3,3a,4,5-tetrahydronaphthaleno[4,3-*c*]isoxazoles in order to determine whether the presence of a nitrogen or carbon atom at 5-position of the tricyclic core structure would play a relevant role in the biological activity profile of these derivatives. Finally, we report our preliminary findings on how the replacement of the methoxy group in position 7 of the tricyclic isoxazoline scaffold, by a variety of *O*-alkyl and *O*-acyl substituents, influenced the affinity at both targets.

Chemistry

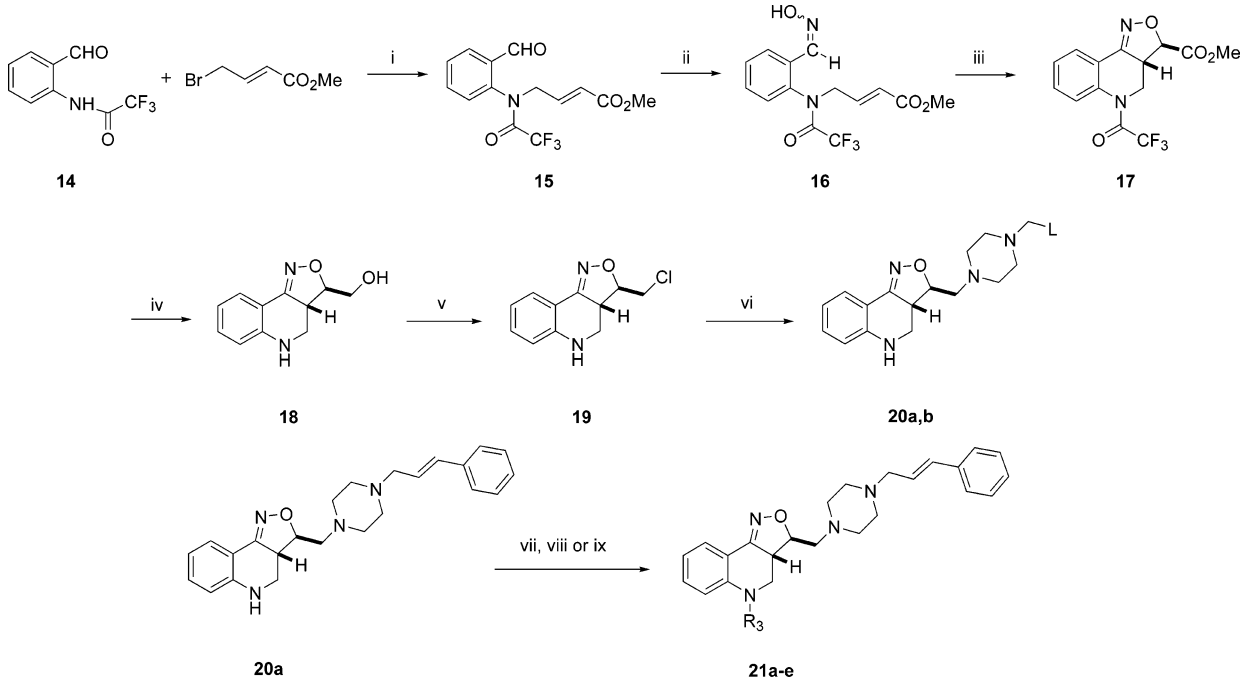
The synthesis of the final 3-piperazinylmethyl-3a,4-dihydro-3*H*-[1]benzopyrano[4,3-*c*]isoxazole derivatives is depicted in Scheme 1. Mesylate **10** was a key intermediate to prepare the target compounds. Its precursor, the ethyl 3a,4-dihydro-3*H*-[1]benzopyrano[4,3-*c*]isoxazole-3-carboxylate derivative **9**, was obtained following essentially the method previously described for the synthesis of the methyl ester analogue without methoxy substituents.[28] Thus, alkylation of the dimethoxysalicylaldehyde **6**[29] with ethyl 4-bromocrotonate, in the presence of potassium carbonate as base and DMF as solvent, afforded intermediate **7**, which was transformed into the oxime **8** by reaction with hydroxylamine. The generation of the required nitrile oxide and subsequent ring closure by intramolecular 1,3-dipolar cycloaddition to the cycloadduct **9** was carried out using sodium hypochlorite and triethylamine, in 85% yield from the corresponding oxime.[30] 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 ester with $NaBH_4$ in THF/H_2O as solvent afforded the corresponding hydroxymethyl derivative in good yield, which was converted into the mesylate **10** by standard procedure. Reaction of this mesylate with excess *N*-*tert*-butyloxycarbonylpiperazine afforded the N-Boc-protected intermediate **11**, which was deprotected with trifluoroacetic acid to provide the key piperazinyl derivative **12** in 90% yield. Alkylation of this compound with the different required haloalkyl derivatives or reductive amination with the corresponding aldehydes, depending on their commercial availability, afforded the desired final products **13a**−**d**. The relative cis configuration between the 3-exocyclic chain and the 3a-hydrogen atom was kept unaltered during the different synthesis steps (Scheme 1). The biological results led us to decide to separate and isolate the enantiomers of four selected compounds for further pharmacological screening. The separation and isolation of those enantiomers was performed by preparative chiral HPLC (CHIRALPAK AD 1000A, 20

Scheme 1. Synthesis of 3-Piperazinylmethyl-3a,4-dihydro-3*H*-[1]benzopyrano[4,3-*c*]isoxazole Derivatives[a]



[a] Reagents and conditions: (i) K_2CO_3, DMF; (ii) $NH_2OH{\cdot}HCl$, pyridine, CH_3CH_2OH; (iii) Et_3N, NaOCl (5%), CH_2Cl_2; (iv) $NaBH_4$, THF/H_2O; (v) CH_3SO_2Cl, Et_3N, CH_2Cl_2; (vi) KI, K_2CO_3, MIK; (vii) CF_3COOH, CH_2Cl_2; (viii) **L**-CH_2−Hal, K_2CO_3, MIK; (ix) **L**-CHO, $NaBH(AcO)_3$, CH_3COOH (cat.), CH_2Cl_2.

Scheme 2. Synthesis of Unsubstituted 3,3a,4,5-Tetrahydroquinolino[4,3-*c*]isoxazoles[a]

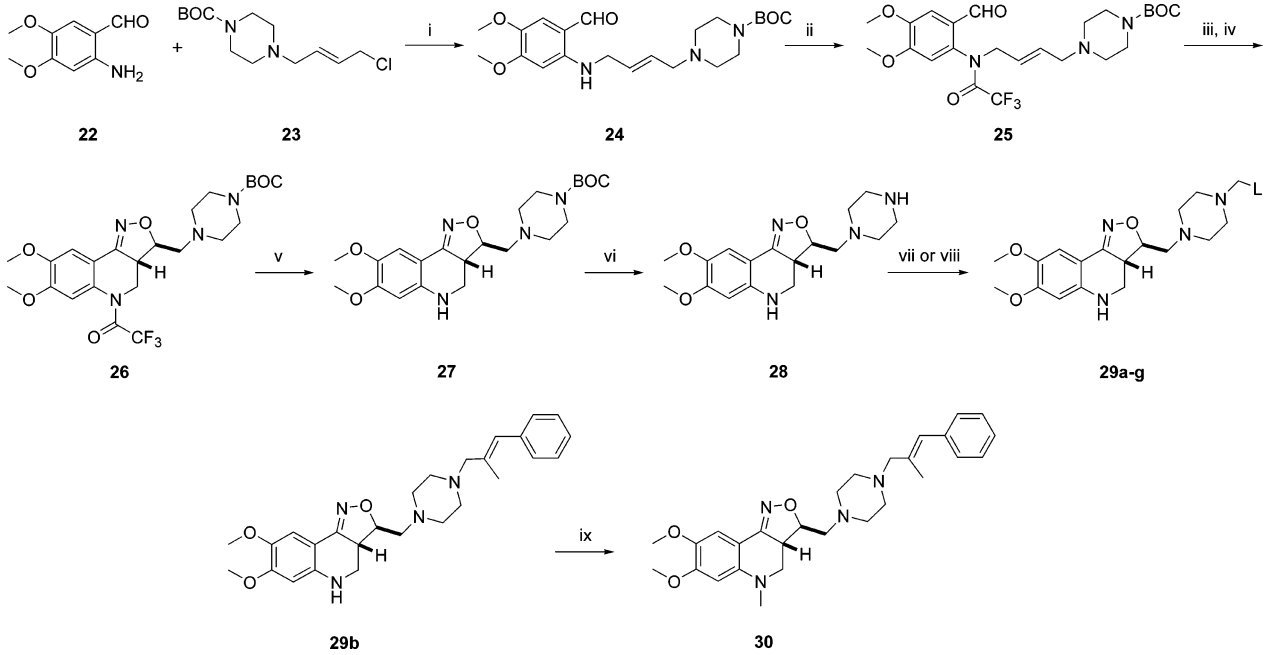


[a] Reagents and conditions: (i) NaH, 18-crown-6, DMF (ii) $NH_2OH{\cdot}HCl$, pyridine, CH_3CH_2OH; (iii) chloramine-T, CH_3CH_2OH; (iv) $NaBH_4$, THF/H_2O; (v) CCl_4, Ph_3P, THF; (vi) **L**-CH_2-piperazine, KI, Dioxane; (vii) HCHO, $NaCNBH_3$, $ZnBr_2$, CH_3OH; (viii) CH_3CH_2NCO or $OC(OCH_2CH_3)_2$, BuLi, THF; (ix) $ClCOCH_3$ or $(CF_3CO)_2O$, NaH, 18-crown-6, THF.

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

The unsubstituted 3,3a,4,5-tetrahydroquinolino[4,3-*c*]isoxazoles **20a**,**b** were synthesized applying some modifications to the synthesis pathway described above (Scheme 2). Thus, starting from the aminobenzaldehyde **14**,[31] alkylation with methyl 4-bromocrotonate, followed by treatment with hydroxylamine yielded oxime **16**. Transformation of this oxime in the corresponding nitrile oxide and subsequent cyclization to afford **17** was carried out using chloramine-T.[30] As mentioned above, the stereochemistry of positions 3 and 3a of the tricyclic system was predetermined by the *trans*-alkene fragment and was unequivocally assigned by NMR. One-pot reduction and deprotection of **17** with $NaBH_4$ in THF/H_2O afforded the hydroxymethyl derivative **18** in quan-

Scheme 3. Synthesis of 7,8-Dimethoxy-Substituted 3,3a,4,5-Tetrahydroquinolino[4,3-*c*]isoxazoles[a]



[a] Reagents and conditions: (i) NaH, 18-crown-6, THF; (ii) $(CF_3CO)_2O$, NaH, 18-crown-6, THF; (iii) $NH_2OH{\cdot}HCl$, pyridine, CH_3CH_2OH; (iv) NCS, Et_3N, CH_2Cl_2; (v) LiOH, H_2O, dioxane; (vi) CF_3COOH, CH_2Cl_2; (vii) **L**-CH_2−Hal, K_2CO_3, DMF; (viii) **L**-CH_2−Hal, K_2CO_3, MIK; (ix) HCHO, $NaCNBH_3$, $ZnBr_2$, CH_3OH.

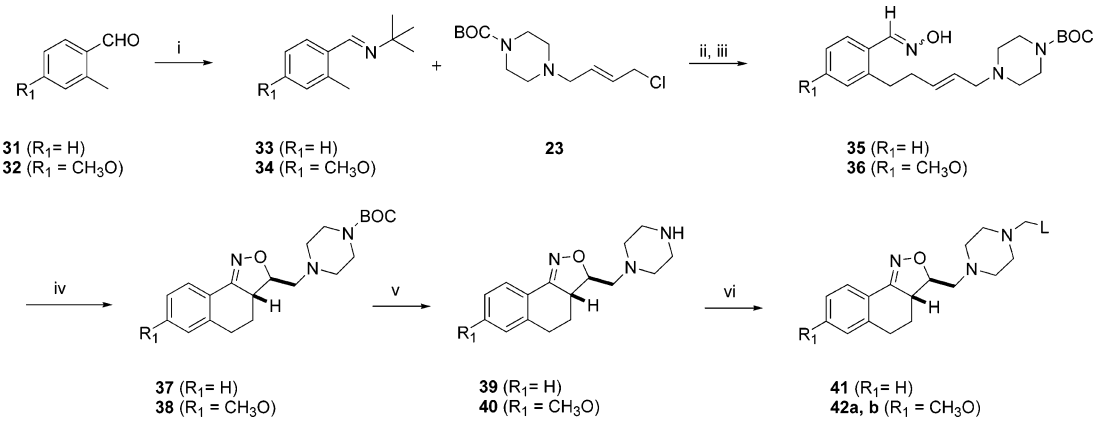
titative yield, which was converted into the chloromethyl derivative **19** by reaction with CCl_4/Ph_3P. Intermediate **19** was reacted with commercially available cinnamylpiperazine and β-naphthylmethylpiperazine[32] yielding respectively the target compounds **20a** and **20b**. Compound **20a** was used as precursor for the synthesis of the 5-substituted compounds **21a**−**e**. Thus, the methylated compound **21a** was prepared by reductive amination of **20a** with formaldehyde in the presence of $NaCNBH_3$. Reaction of **20a** with ethyl isocyanate or diethyl carbonate, in the presence of butyllithium as base, yielded compounds **21b** and **21c**, respectively. Finally, the acylated compounds **21d** and **21e** were synthesized by treatment of **20a** with sodium hydride as base and acetyl chloride or trifluoracetic anhydride, respectively (Scheme 2).

For the synthesis of the dimethoxy derivatives **29a**−**g,** a new synthesis procedure was envisaged (Scheme 3) introducing the piperazine moiety at the first reaction step. Thus, 2-aminobenzaldehyde derivative **22**[33] was reacted with the in-house-available intermediate **23**, affording the N-substituted-2-aminobenzaldehyde intermediate **24**. Reaction of this compound with trifluoracetic anhydride yielded the trifluoroacetyl derivative **25**, which was converted into the tetrahydroquinolinoisoxazole compound **26** by transformation into the corresponding oxime followed by treatment with *N*-chlorosuccinimide.[30] Sequential double deprotection of the tricyclic isoxazoline derivative **26** by using lithium hydroxide and trifluoroacetic acid provided the key derivative **28**. The desired final products **29a**−**g** were obtained by alkylation of **28** with the required haloalkyl reagents. Finally, reductive amination of **29b** with formaldehyde afforded the 5-*N*-methyl derivative **30** (Scheme 3).

The 3,3a,4,5-tetrahydronaphthaleno[4,3-*c*]isoxazole derivatives were prepared as described in Scheme 4. The strategy followed for the synthesis of the final compounds **41** and **42a**,**b** was based on a previously reported method for the alkylation of *o*-methyl benzylidenimines.[34] Thus, the commercially available 2-methyl benzaldehydes **31** and **32** were converted into the corresponding *tert*-butylimines **33** and **34** under standard conditions. Metalation of the *o*-methyl group of these intermediates with butyllithium, in the presence of a catalytic amount of 2,2,6,6-tetramethylpiperidine, afforded the corresponding lithium salts that were alkylated with **23**. The corresponding BOC-protected intermediates were reacted with hydroxylamine, affording the oxime derivatives **35** and **36**. Cyclization of both compounds by treatment with sodium hypochlorite yielded the tricyclic isoxazoline adducts **37** and **38**, which were deprotected using trifluoroacetic acid. Finally, the key intermediates **39** and **40** were alkylated with the required haloalkyl reagents yielding the target compounds **41** and **42a**,**b** (Scheme 4). The relative cis configurations between the 3-exocyclic chain and the 3a-hydrogen atom, for both the 3,3a,4,5-tetrahydroquinolino[4,3-*c*]isoxazole and the 3,3a,4,5-tetrahydronaphthaleno[4,3-*c*]-isoxazole series, were unequivocally assigned by NMR.

We decided as well to explore the influence on activity of the replacement of the methoxy group in position 7 of the tricyclic isoxazoline scaffold, by a variety of *O*-alkyl and *O*-acyl substituents. For that purpose we selected the methylcinnamyl moiety as the fixed fragment, to get structure−activity relationships from those modifications. Scheme 5 illustrates the synthetic procedure used to introduce modifications at position 7 of the tricyclic system, keeping unsubstituted the position 8 of the scaffold. The 7-methoxy derivative **43**, a close analogue of compound **5**[26] that was synthesized following the same method as that described in Scheme 1, was treated with an excess of boron tribromide in dichlo-

Scheme 4. Synthesis of 3,3a,4,5-Tetrahydronaphthaleno[4,3-*c*]isoxazole Derivatives[a]



31 (R_1= H)
32 (R_1 = CH_3O)

33 (R_1= H)
34 (R_1 = CH_3O)

23

35 (R_1= H)
36 (R_1 = CH_3O)

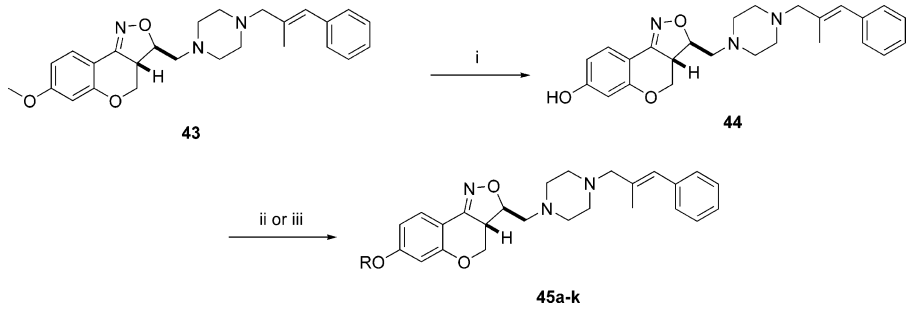
37 (R_1= H)
38 (R_1 = CH_3O)

39 (R_1= H)
40 (R_1 = CH_3O)

41 (R_1= H)
42a, b (R_1 = CH_3O)

[a] Reagents and conditions: (i) $(CH_3)_3CNH_2$, toluene; (ii) BuLi, 2,2,6,6-tetramethylpiperidine, THF; (iii) $NH_2OH{\cdot}HCl$, $NaHCO_3$, CH_3CH_2OH; (iv), Et_3N, NaClO (5%), CH_2Cl_2; (v) CF_3COOH, CH_2Cl_2; (vi) **L**-CH_2-Hal, $NaHCO_3$, $CHCl_3$.

Scheme 5. Synthesis of 7-O-Substituted 3a,4-Dihydro-3*H*-[1]benzopyrano[4,3-*c*]isoxazole Derivatives[a]



43

44

45a-k

[a] Reagents and conditions: (i) BBr_3 (excess), CH_2Cl_2; (ii) RCOCl, Et_3N, CH_2Cl_2; (iii) ROH, DEAD, PPh_3 (polymer bound), THF.

Table 1. Binding Affinities of the Four Enantiomers (+)-**13a**−**d** and Two Reference Compounds for a Set of Receptors and Transporter Uptake Sites

	K_i (nM)[a]														
compd	α_{2A}	α_{2B}	α_{2C}	5-HTT	α_1	5-HT_{2A}	5-HT_{2C}	5-HT_{1A}	5-HT_7	H_1	D_2	D_3	D_4	DAT	NET
2	2.5	11	2.1	4.0	>1000	>1000	>1000	>1000	n.t.[b]	>1000	>1000	n.t.[b]	234	>1000	>1000
3	5.9	23	19	77	>1000	>1000	>1000	770	>1000	>1000	>1000	n.t.[b]	n.t.[b]	>1000	365
(+)-**13a**	2.6	14	0.5	1.2	>1000	>1000	>1000	>1000	>1000	>1000	959	270	>1000	515	132
(+)-**13b**	0.3	3.0	0.1	5.4	380	>1000	>1000	475	>1000	113	230	433	>1000	>1000	177
(+)-**13c**	0.3	14	0.2	4.5	>1000	>1000	>1000	>1000	>1000	>1000	>1000	611	>1000	368	220
(+)-**13d**	1.3	14	0.8	1.7	>1000	>1000	>1000	>1000	>1000	460	>1000	107	>1000	246	33

[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. The K_i values represent the concentration giving half-maximal inhibition of [^{3}H]rauwolscine (α_{2A}, α_{2B} and α_{2C}), [^{3}H]paroxetine (5-HTT), [^{3}H]prazosin (α_1), [^{125}I]R91150 (5-HT_{2A}), [^{3}H]mesulergine (5-HT_{2C}), [^{3}H]8OH-DPAT (5-HT_{1A}), [^{3}H]5-HT (5-HT_7), [^{3}H]pyrilamine (H_1), [^{3}H]spiperone (D_2 and D_4), [^{125}I]iodosulpride, (D_3), [^{3}H]WIN35428 (DAT), [^{3}H]nisoxetine (NET), binding to cloned human receptors or (for DAT and NET) to rat tissue. [b] n.t., not tested.

romethane at room temperature yielding the corresponding 7-hydroxy compound **44** in moderate yield. Acylation of this compound with different acid chlorides under standard reaction conditions, or application of Mitsunobu reactions with the corresponding alcohols, furnished the desired final products **45a**−**k** (Scheme 5).

Pharmacological Results and Discussion

The primary pharmacological results of compounds **13a**−**d** and their corresponding enantiomers were described in our previous communication. Thus, their affinity at the three different human α_2-adrenoceptor subtypes and the 5-HT transporter (5-HTT) site was reported. In addition, the in vivo activity of those compounds was measured in two different assays: (1) inhibition of *p*-chloroamphetamine (pCA)-induced excitation, which evaluates the ability to block the central 5-HTT, and (2) inhibition of xylazine-induced loss of righting, which evaluates the ability to block central α_2-adrenoceptors.[26] We have reported that the affinity at the 5-HTT was comparable for each pair of enantiomers but, on the other hand, the stereochemical configuration influenced the affinity at the α_2-adrenoceptors, the (+)-enantiomers being very more active than their corresponding (−)-enantiomers. The affinities of the four compounds (+)-**13a**−**d** for other serotonergic, adrenergic, and dopaminergic receptors, as well as at the dopaminergic and noradrenergic uptake sites were evaluated in the present study. Results of their in vitro binding affinities are shown in Table 1. These compounds proved to be very selective for both targets as can be deduced from the data shown. The only compound that showed a significant affinity for any other receptor or transporter site was the naphthylmethyl analogue (+)-**13d**, which exhibited a moderate activity at the NE transporter site (K_i = 33 nM). This property

Table 2. Active Doses of the Four Enantiomers (+)-**13a**−**d** for Reversal of Medetomidine-Induced Loss of Righting, Antagonism of pCA-Induced Excitation and PCA-Induced 5-HT Depletion in Vivo, after Subcutaneous (sc) and Oral (po) Administration

	ED_{50} (mg/kg)[a]					
compd	medetomidine (sc)	medetomidine (p.o.)	pCA (sc)	pCA (po)	5-HT depletion (sc)	5-HT depletion (po)
2 (A-80426)	>10	not tested	≥10	>10	not tested	not tested
3 (S-34324)	2.7 (2.0−3.6)	not tested	2.0 (1.4−3.0)	not tested	not tested	not tested
(+)-**13a**	0.63 (0.24−1.6)	1.3 (0.45−3.6)	0.39 (0.24−0.63)	5.0 (2.9−8.8)	0.17 (0.13−0.21)	1.1 (0.83−1.5)
(+)-**13b**	1.2 (0.73−1.9)	0.63 (0.24−1.6)	0.80 (0.28−2.3)	≥10	0.33 (0.26−0.43)	3.8 (2.9−4.9)
(+)-**13c**	0.32 (0.15−0.63)	5.0 (2.9−8.7)	0.50 (0.23−1.1)	≥2.5	0.24 (0.16−0.35)	3.5 (1.8−6.7)
(+)-**13d**	6.2 (3.8−10)	7.1 (3.6−14)	0.08 (0.05−0.14)	5.0 (2.5−9.9)	0.25 (0.12−0.54)	2.6 (2.2−3.2)

[a] 95% confidence limits are shown in parentheses.

might be even an added value for the potential antidepressant activity of this derivative. Compounds showed a weak affinity for a few other tested receptors and transporters, but potencies were more than 100-fold lower (K_i > 100 nM) than those exhibited for the α_2-adrenoceptors and 5-HTT, which were in the nanomolar range (Table 1).

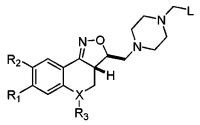
The experimental compounds (+)-**13a**−**d** were tested both subcutaneously and orally in the pCA and medetomidine tests. Reference compounds **2** and **3** were tested subcutaneously (compound **2** also orally in the pCA test). pCA induces release of 5-HT from serotonergic nerve terminals, which results in neuronal 5-HT depletion and typical behavioral effects in rats. As pCA produces its biochemical and behavioral effects only after its uptake into the serotonergic neurons by the neuronal 5-HTT, compounds that block this transporter mechanism also inhibit the pCA-induced effects. Antagonism of pCA-induced 5-HT depletion and, in the absence of overt sedative effects, antagonism of pCA-induced excitation in rats are therefore reliable in vivo indices of the ability of test compounds to block the central 5-HTT. Antagonism of the loss of righting in rats, induced by the α_2-adrenoceptor agonist medetomidine (or xylazine as described in our previous communication), is a reliable index for the central α_2-adrenoceptor blocking activity of test compounds, at least when occurring without behavioral stimulant effects. Experimental details of both assays are described in the Experimental Section. Results of these tests are shown in Table 2. Compound **2** (A-80426) did not show consistent activity in the medetomidine and pCA tests. In contrast, compound **3** (S-34324) exhibited activity in both assays. After subcutaneous administration, three out of our four experimental compounds were active in both assays. Compounds (+)-**13a**, (+)-**13b**, and especially (+)-**13d** were more potent in the pCA test than in the medetomidine assay. Compound (+)-**13c** showed the best balance of in vivo activities while the naphthylmethyl derivative (+)-**13d** showed the highest potency in our pCA test, reflecting potent serotonin reuptake inhibition. There were substantial differences in the activities when the compounds were administered orally. In the medetomidine test, the compounds were about equipotent after both routes of administration. In the pCA test, however, all compounds showed much lower activity after oral administration than after subcutaneous injection, suggesting the generation of metabolites devoid of effect on 5-HT uptake, at least in rats (Table 2).

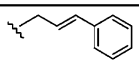
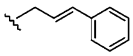
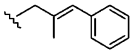
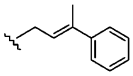
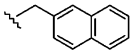
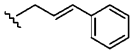
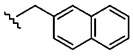
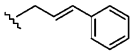
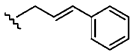
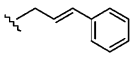
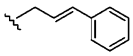
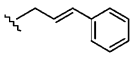
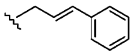
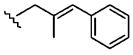
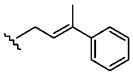
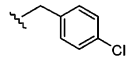
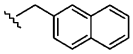
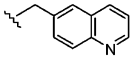
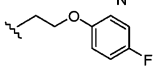
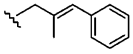
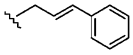
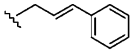
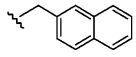
The in vivo activity of compounds (+)-**13a**−**d** was also evaluated against pCA-induced 5-HT depletion. In the 5-HT depletion test, the compounds also showed a lower activity after oral than after subcutaneous administration. After subcutaneous treatment, ED_{50}'s were comparable between the four compounds, ranging between 0.17 and 0.33 mg/kg, indicating potent 5-HT transporter blocking effects. Likewise, results obtained in the 5-HT depletion test after oral administration revealed that all four compounds inhibited the 5-HT transporter as well, although their potency was lower than after subcutaneous injection (Table 2).

For the primary pharmacological evaluation of the series of novel 3,3a,4,5-tetrahydroquinolino[4,3-*c*]isoxazoles, 3,3a,4,5-tetrahydronaphthaleno[4,3-*c*]isoxazoles and 7-O-substituted 3a,4-dihydro-3*H*-[1]benzopyrano-[4,3-*c*]isoxazoles, we first evaluated the in vitro binding affinity at the α_{2A}- and α_{2C}-adrenoceptor subtypes and the 5-HTT site. 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.[35,36] 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.[37] On the contrary, the function of the α_{2B}-adrenoceptor subtype seems to be opposite to the function of the α_{2A}-adrenoceptor in the regulation of blood pressure.[35] Structure and binding data of the compounds are shown in Tables 3 and 5.

Regarding the 3,3a,4,5-tetrahydroquinolino[4,3-c]-isoxazoles (Table 3), the 7,8-unsubstituted compound **20a** was 10-fold less potent at the α_2 receptors and 5-fold less potent at the 5-HTT than the reference analogue **46**.[26] Interestingly, the introduction of a β-naphthyl residue at the piperazine significantly increased the potency for both targets. Thus, compound **20b** showed subnanomolar affinity for the 5-HTT and nanomolar affinity for α_2-adrenoceptors. This preference of β-naphthyl derivatives for 5-HTT was also observed in the 3a,4-dihydro-3*H*-[1]benzopyrano[4,3-*c*]isoxazole series, as exemplified with derivative **13d**. Compounds having different substituents at the 5-position (**21a**−**e**) showed, in general terms, a better profile than the parent compound **20a**. In particular, the methyl- substituted compound **21a** exhibited the best balance of in vitro activities within this quinolino-isoxazole series (Table 3). The introduction of two methoxy groups at positions 7 and 8 of the tricyclic system led to several compounds showing nanomolar affinities for both targets, exemplified by **29a**−**c**, which exhibited similar receptor binding affinities than reference benzopyrano-isoxazole analogues **13a**, **13b**, and **13c**. Further exploration of the piperazine substitution pattern resulted in less active compounds (**29d**−**g**). Only the β-naphthyl derivative **29e** presented potent binding at the 5-HTT site, but 3-fold lower than compound **20b**. Additional substitution on

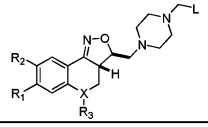
Table 3. In Vitro Binding Affinities of 3,3a,4,5-Tetrahydroquinolino[4,3-*c*]isoxazoles and 3,3a,4,5-Tetrahydronaphthaleno[4,3-*c*]isoxazoles

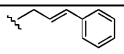
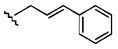
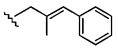
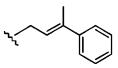
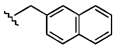
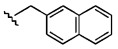
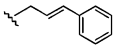
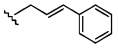
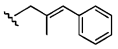
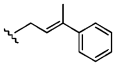
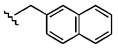


						K_i (nM)[a]		
Compd	X	R_1	R_2	R_3	L	α_{2A}	α_{2C}	5-HTT
46[26]	O	H	H	----		0.9	1.7	16
13a	O	OMe	OMe	----		8.8	6.2	8.3
13b	O	OMe	OMe	----		0.8	0.2	2.3
13c	O	OMe	OMe	----		1.4	0.6	10
13d	O	OMe	OMe	---		5	3.1	1.7
20a	N	H	H	H		20	15	84
20b	N	H	H	H		2.4	9.6	0.5
21a	N	H	H	Me		1.6	4.5	16
21b	N	H	H			20	2.7	23
21c	N	H	H			33	9.8	2.6
21d	N	H	H			47	11	61
21e	N	H	H			26	6.6	15
29a	N	OMe	OMe	H		7.9	5.4	9.7
29b	N	OMe	OMe	H		2.4	0.1	8.9
29c	N	OMe	OMe	H		6.5	3.6	15
29d	N	OMe	OMe	H		>1000	23	141
29e	N	OMe	OMe	H		74	12	1.5
29f	N	OMe	OMe	H		>1000	>1000	22
29g	N	OMe	OMe	H		9.1	1.6	154
30	N	OMe	OMe	Me		11	4	104
41	CH	H	H	H		43	112	106
42a	CH	OMe	H	H		1.8	3.9	7.3
42b	CH	OMe	H	H		1.8	6.3	1.4

[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.

Table 4. Active Doses of 3,3a,4,5-Tetrahydroquinolino[4,3-*c*]isoxazoles and 3,3a,4,5-Tetrahydronaphthaleno[4,3-*c*]isoxazoles for Antagonism of pCA-Induced Excitation and Medetomidine-Induced Loss of Righting in Vivo



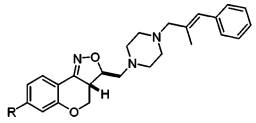
						ED_{50} (mg/kg)[a]	
Compd	X	R_1	R_2	R_3	L	Medetomidine (s.c.)	pCA (s.c.)
46	O	H	H	----		2.0 (0.91-4.4)	5.0 (1.8-14)
13a	O	OMe	OMe	----		2.5 (1.0-6.5)	0.76 (0.51-1.15)
13b	O	OMe	OMe	----		1.0 (0.63-1.6)	1.53 (1.0-2.3)
13c	O	OMe	OMe	----		2.5 (0.96-6.5)	0.20 (0.09-0.44)
13d	O	OMe	OMe	----		5.0 (2.5-9.9)	0.79 (0.36-1.7)
20b	N	H	H	H		2.5 (0.96-6.5)	2.2 (0.97-4.9)
21a	N	H	H	Me		5.0 (2.5-9.9)	>10
29a	N	OMe	OMe	H		5.0 (2.5-9.9)	0.63 (0.24-1.6)
29b	N	OMe	OMe	H		0.20 (0.13-0.29)	2.2 (1.2-4.0)
29c	N	OMe	OMe	H		2.5 (0.96-6.5)	7.9 (3.6-17)
42b	CH	OMe	H	H		>10	0.50 (0.18-1.4)

[a] 95% confidence limits are shown in parentheses.

the nitrogen at the 5-position by a methyl group (compound **30**) was detrimental for both activities, especially for 5-HTT, in contrast to the result observed for the 7,8-unsubstituted analogue previously mentioned **21a**. It is noteworthy that most of the compounds within the 3,3a,4,5-tetrahydroquinolino[4,3-c]isoxazoles series presented preferential affinity for α_{2C} versus α_{2A} adrenoceptor subtypes. With respect to the 3,3a,4,5-tetrahydronaphthaleno[4,3-*c*]isoxazole derivatives, the 7,8-unsubstituted compound **41** showed also much lower binding values than the reference compound **46**. In this series, significant differences between 7-methoxy- and 7-unsubstituted derivatives were observed. Thus, compound **42a** exhibited higher binding affinity than its parent compound **41** for both 5-HTT, and α_2-adrenoceptors. Similarly to the benzopyrano-isoxazole and quinolino-isoxazole series, the β-naphthyl derivative **42b** showed higher potency for the 5-HTT than its parent compound **41** (Table 3).

With these receptor binding results in mind, compounds **20b**, **21a**, **29a**, **29b**, **29c**, and **42b** were selected for secondary in vivo assays to investigate their behavior in models for α_2-adrenoceptor blockade and 5-HTT inhibition. Results obtained in these assays are summarized in Table 4. As can be deduced from the data shown in the table, all the compounds were active in both tests except **21a** in the pCA test and compound **42b**, which was very potent in the pCA test but devoid of activity after subcutaneous injection at 10 mg/kg in the medetomidine test. Regarding the other compounds tested, some of them showed less in vivo potency than expected from their affinity binding data. For instance, compound **20b** was active only at relatively high doses, compound **21a** was at least 2-fold less active than compound **46** in both tests, while **29c** showed 40-fold less potency than compound **13c** in the pCA test, although K_i values for the three compounds were in the nanomolar range. We have not yet found a convincing

Table 5. Active Doses of 7-Substituted Tricyclic Isoxazolines for Binding to the α_{2A} and α_{2C} Adrenoceptors and 5-HTT in Vitro and for Antagonism of pCA-Induced Excitation and Medetomidine-Induced Loss of Righting in Vivo (ED_{50} values; mg/kg)



		K_i (nM)[a]			ED_{50} (mg/kg)[b]	
Compd	R	α_{2A}	α_{2C}	5-HTT	Medetomidine (s.c.)	pCA (s.c.)
43[26]	CH_3O	0.5	0.2	19	2.5 (0.96-6.5)	>10
44	HO	0.2	0.1	3.6	1.2 (0.63-2.5)	>10
45a	$CH_3O(CH_2)_2O$	0.1	0.03	4.5	2.5 (0.96-6.5)	>10
45b	$CH_3CH_2O(CH_2)_2O(CH_2)_2O$	1.3	0.2	17	1.6 (0.90-3.0)	>10
45c		1.3	0.5	56	1.2 (0.63-2.5)	>10
45d	$(CH_3)_2N(CH_2)_2O$	0.1	0.1	3.5	2.5 (0.96-6.5)	1.2 (0.63-2.5)
45e	$CH_3(C=O)O$	0.4	0.2	7.7	0.31 (0.15-0.63)	1.2 (0.63-2.5)
45f	$CH_3CH_2(C=O)O$	0.6	0.2	21	0.63 (0.24-1.6)	>10
45g	$CH_3OCH_2(C=O)O$	0.7	0.2	5.7	0.63 (0.24-1.6)	>10
45h		0.9	0.3	26	0.32 (0.16-0.63)	>10
45i		0.9	0.5	41	0.63 (0.24-1.6)	>10
45j		4.7	1.6	12	1.25 (0.63-2.5)	>10
45k		1.0	0.5	13	0.63 (0.24-1.6)	7.9 (3.6-17)

[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] 95% confidence limits are shown in parentheses.

explanation for this disconnection between the in vitro and in vivo activity in these quinolino-isoxazole derivatives, compared to their benzopyrano-isoxazole analogues. On the contrary, compounds **29a** and **29b** showed similar or even slightly better in vivo potency than their benzopyran pairs **13a** and **13b**. Thus, compound **29b** was equipotent to **13b** in the pCA test while showing 5-fold more potency than **13b** in the medetomidine test. Moreover, compound **29b** was identified as the most active one in the latter test, indicating a potent in vivo α_2-adrenoceptor blockade combined with a potent 5-HT reuptake inhibition, making this compound a good candidate for further enantiomeric separation and pharmacological screening of the isolated enantiomers.

Regarding the 7-O-substituted 3a,4-dihydro-3*H*-[1]-benzopyrano[4,3-*c*]isoxazoles, Table 5 shows that in general these compounds displayed higher affinity for the α_2-adrenoceptors than for the 5-HTT. Looking at the in vivo data this conclusion may be even more clear, as several of the compounds were not active at doses of 10 mg/kg in the pCA test, while on the contrary all these compounds were active in the medetomidine test at doses ranging from ED_{50}'s of 0.3 to 2.5 mg/kg. It is noteworthy that all kind of substitutions, including the methoxy, hydroxy, a variety of *O*-alkyl moieties, and several different *O*-acyl groups resulted in quite comparable affinity at α_2-adrenoceptors. Thus, the methoxyethyloxy derivative **45a** and the dimethylaminoethyloxy derivative **45d** were the most potent compounds in vitro, with K_i values in the sub-nanomolar range. This potency was not exactly correlated with their respective in vivo activities in the medetomidine assay, as both

compounds showed a moderate ED_{50} of 2.5 mg/kg. The 7-hydroxy derivative **44** showed 2-fold higher affinity at α_2-adrenoceptors than its parent methoxy analogue **43**, what was correlated with its activity in the medetomidine test. However, none of both compounds showed activity in the pCA test. The *O*-alkyl derivatives **45a**−**d** showed comparable activity in the medetomidine assay (1.2 to 2.5 mg/kg), but only **45d**, having a nitrogen atom in its structure, proved to be rather potent in the pCA assay. These results suggest that the nature of a hydrogen bond acceptor atom at a certain distance of the oxygen linked to the tricycle in position 7 does not crucially influence the in vitro binding affinity at the 5-HTT, but on the contrary the in vivo activity is indeed influenced. Thus, compound **45d** containing a nitrogen atom, which is not only a hydrogen bond acceptor but a positive ionisable atom as well, was the only compound within this series showing central in vivo 5-HT reuptake blockade.

All the *O*-acyl derivatives, regardless of the substitution pattern, showed in general terms quite comparable in vitro binding affinities at the α_2-adrenoceptors. The two most potent compounds at 5-HTT were **45e** and **45g**. As a matter of fact, **45e** was the most active derivative in the medetomidine test and was active in the pCA assay as well. Surprisingly, **45g** was not active in this in vivo 5-HTT assay despite its nanomolar binding affinity (Table 5). We wondered whether the in vivo activity/inactivity of these *O*-acyl derivatives might actually originate from the corresponding hydroxyl derivative **44**, after hydrolysis of the acyl groups. One could speculate that the *O*-acyl compounds might behave as prodrugs of the hydroxyl derivative, which could not easily pass the blood-brain barrier. This hypothesis might explain the activity of some of them in the pCA test and the inactivity of **44** in this same assay, as well as the general better activity than **44** in the medetomidine test. However, this hypothesis cannot convincingly explain the high activity of the acetyl derivative **45e** when compared with the other analogues. On the other hand, the 7-*O*-acyl derivatives proved to be chemically quite stable, and potential metabolic instability of these compounds could eventually influence their activity after oral administration, but to a less extent after subcutaneous administration when first-pass metabolism is not directly involved. Further studies are in progress trying to better understand the in vivo behavior of these *O*-acyl derivatives.

Conclusion

We have discovered a new series of 3-piperazinylmethyl-3a,4-dihydro-3*H*-[1]benzopyrano[4,3-*c*]isoxazoles as novel dual and selective 5-HT reuptake inhibitors and α_2-adrenoceptor antagonists, with potential to be considered as a new class of antidepressant agents. The combined activity mainly resided in one of the enantiomers. The four selected compounds showed in vivo activity in two different tests, antagonism of pCA-induced excitation and antagonism of medetomidine-induced loss of righting in rats, proving their ability to block the central 5-HTT and the α_2-adrenoceptors, respectively. In addition, they also showed in vivo activity in the pCA-induced 5-HT depletion assay, confirming their potency in inhibiting 5-HT reuptake. Furthermore they showed potent to moderate oral activity in the three assays. Among these compounds the methylcinnamyl derivative (+)-**13c** showed the best balance of in vivo activities. These compounds are being pharmacologically evaluated as potential antidepressants. The replacement of the oxygen atom at 5-position of the tricyclic scaffold by a nitrogen or a carbon atom led to active compounds as well, both in vitro and in vivo. Especially the replacement by a nitrogen atom led to several promising 7,8-dimethoxy-3-piperazin-1-ylmethyl-3,3a,4,5-tetrahydroquinolino[4,3-*c*]isoxazoles, which showed potent in vivo activity in the pCA and medetomidine tests. One of these derivatives, **29b**, presented the highest potency as central α_2-adrenoceptor blocker. This in combination with its high potency for the 5-HTT made it a serious candidate for further pharmacological characterization. Separation and evaluation of the enantiomers of **29b** is already ongoing and further studies on this compound will be published elsewhere. We have also explored the influence of O-substitution in position 7 of the tricyclic scaffold of our novel series of 3-piperazinylmethyl-3a,4-dihydro-3*H*-[1]benzopyrano[4,3-*c*]isoxazoles. The combined activity as dual 5-HT reuptake inhibitors and α_2-adrenoceptor antagonists was maintained or even improved when compared with the first leads described in our previous paper. The enantiomeric separation of a few selected 7-O-substituted derivatives and their further biological evaluation are in progress as well.

Experimental Section

Chemistry. Melting points were determined in open capillary tubes on a Mettler FP62 apparatus and are uncorrected. Elemental analyses are within ±0.4% of the theoretical values. Chiral preparative chromatography was performed on a Waters Delta Prep 4000 with a 5 cm i.d. Prochrom D.A.C. column. The enantiomeric excess was determined by HPLC using a Waters Alliance 2690 instrument with chiral columns (Chiralcel OD, Chiralcel OJ, Chiralpak AD and Chiralpak AS, Daicel 10μm). Optical rotations were measured on a Perkin-Elmer 341 polarimeter with a sodium lamp and reported as follows: $[\alpha]_\lambda^{t°C}$ (*c* g/100 mL, solvent). ^{1}H NMR spectra were recorded on a Bruker DPX-400 and on a Bruker AC-200 spectrometer with standard pulse sequences, operating at 400 and 200 MHz, respectively. Chemical shifts (δ) are reported in parts per million (ppm) downfield from tetramethylsilane (TMS), which was used as internal standard. HPLC-MS analyses were done with an Agilent Technologies 1100 series consisting of a quaternary pump with degasser, autosampler, column oven and DAD detector. A generic gradient: 80/10/10 $AcONH_4$ 0.05%/MeOH/CH_3CN to 50/50 CH_3CN/MeOH in 6 min to 100% CH_3CN in 1.5 min was performed on a Zorbax XDB C-18 30 × 4.6 mm i.d. 3.5 μm from Agilent Technologies. Low-resolution mass spectra were recorded on a single quadrupole Micromass Platform series II mass spectrometer with electrospray ionization (ES). High-resolution mass spectra were recorded on a Micromass LCT Time-of-Flight mass spectrometer with electrospray ionization and Lockmass device for mass calibration. Thin-layer chromatography (TLC) was carried out on silica gel 60 F_{254} plates (Merck) using reagent grade solvents. Flash column chromatography was performed on silica gel, particle size 60 Å, mesh = 230−400 (Merck).

4-(2-Formyl-4,5-dimethoxyphenoxy)but-2(*E*)-enoic Acid Ethyl Ester (7). A mixture of 4,5-dimethoxysalicylaldehyde **6** (24 g, 131.4 mmol), K_2CO_3 (20 g, 144.5 mmol) and (*E*)-ethyl 4-bromocrotonate (25 mL, 144.5 mmol) in anhydrous dimethylformamide (30 mL) was stirred at room temperature for 18 h. When the TLC analysis showed the disappearance of starting material, the crude reaction mixture was filtered through a Celite pad, and the filtrate was concentrated under

reduced pressure. The residue was diluted with water (30 mL) and extracted with ethyl acetate (3 × 30 mL). The organic phase was dried over anhydrous Na_2SO_4 and concentrated to give **7** (33 g, 85%) as an oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.32 (t, J = 7.1 Hz, 3 H) 3.89 (s, 3 H) 3.95 (s, 3 H) 4.24 (q, J = 7.1 Hz, 2 H) 4.82 (dd, J = 4.2, 2.1 Hz, 2 H) 6.23 (dt, J = 15.8, 2.1 Hz, 1 H) 6.46 (s, 1 H) 7.10 (dt, J = 15.8, 4.0 Hz, 1 H) 7.34 (s, 1 H) 10.40 (s, 1 H). MS *m/z* 295 (MH^+).

4-[2-(Hydroxyiminomethyl)-4,5-dimethoxyphenoxy]-but-2(E)-enoic Acid Ethyl Ester (8). To a solution of 4-(2-formyl-4,5-dimethoxyphenoxy)but-2(*E*)-enoic acid ethyl ester **7** (13 g, 49.1 mmol) in absolute ethanol (130 mL) were added hydroxylamine hydrochloride (4 g, 59 mmol) and pyridine (6 mL, 73.6 mmol). The reaction mixture was stirred for 2 h at room temperature until the TLC analysis showed the absence of starting material. The solvent was evaporated under reduced pressure, and the residue was dissolved in water (20 mL) and extracted with ethyl acetate (3 × 30 mL). The organic layer was dried (Na_2SO_4) and concentrated at reduced pressure, and the residue was purified by column chromatography (ethyl acetate), affording 12 g (92%) of **8** as an oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.31 (t, J = 7.1 Hz, 3 H) 3.88 (s, 3 H) 3.96 (s, 3 H) 4.22 (q, J = 7.1 Hz, 2 H) 4.80 (dd, J = 4.2, 2.1 Hz, 2 H) 6.22 (dt, J = 15.8, 2.1 Hz, 1 H) 6.44 (s, 1 H) 7.11 (dt, J = 15.8, 4.0 Hz, 1 H) 7.33 (s, 1 H) 8.55 (s, 1 H) 8.98 (br s, 1 H). MS *m/z* 310 (MH^+).

7,8-Dimethoxy-3a,4-dihydro-3*H*-[1]benzopyrano[4,3-*c*]-isoxazole-3-carboxylic Acid Ethyl Ester (9). To a solution of 4-[2-(hydroxyiminomethyl)-4,5-dimethoxyphenoxy]but-2(*E*)-enoic acid ethyl ester **8** (6.6 g, 21.4 mmol) in dichloromethane (30 mL) was added a 4% aqueous sodium hypochlorite solution (90 mL, 53.5 mmol) portionwise. The mixture was stirred for 5 h at room temperature, and then triethylamine (6 mL, 42.9 mmol) was added dropwise at 0 °C. The reaction was stirred overnight at room temperature, the organic layer was separated and dried over anhydrous Na_2SO_4 and filtered and the solvent evaporated. The residue was purified by column chromatography (2-propanone/dichloromethane 1/9) to provide **9** (3.2 g, 48%) as a sticky oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.35 (t, J = 7.1 Hz, 3 H) 3.13 (s, 3 H) 3.83 (m, 1 H) 3.86 (s, 3 H) 3.88 (s, 3 H) 4.05 (m, 1 H) 4.18 (m, 1 H) 4.35 (q, J = 7.1 Hz, 2 H) 4.72 (m, 2 H) 6.47 (s, 1 H) 7.13 (s, 1 H). MS *m/z* 308 (MH^+).

Methanesulfonic Acid 7,8-Dimethoxy-3a,4-dihydro-3*H*-[1]benzopyrano[4,3-*c*]isoxazol-3-ylmethyl Ester (10). To a solution of 7,8-dimethoxy-3a,4-dihydro-3*H*-[1]benzopyrano-[4,3-*c*]isoxazole-3-carboxylic acid ethyl ester **9** (6.6 g, 21.6 mmol) in 75 mL of tetrahydrofuran and 30 mL of water was added sodium borohydride (2 g, 54 mmol) portionwise, and the reaction was stirred for 2 h at room temperature. After this time, 2-propanone was added while the mixture was stirred for an additional 30 min, and then it was washed with water and extracted with dichloromethane. The organic layer was separated, dried (Na_2SO_4), filtered, and concentrated, affording 3.4 g of crude alcohol that was used as such without further purification. To a solution of the previously prepared alcohol (3.4 g, 12.6 mmol) and triethylamine (2.7 mL, 18.9 mmol) in dichloromethane, (37 mL) stirred at 0 °C under nitrogen atmosphere, was added methanesulfonyl chloride (1.1 mL, 13.9 mmol) dropwise. The mixture was stirred at 0 °C for 30 min, it was quenched with a saturated aqueous solution of $NaHCO_3$, the organic layer was separated, dried over anhydrous Na_2SO_4 and filtered and the solvent was evaporated, yielding **10** (3.4 g, 78%) as a white foam: ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 3.13 (s, 3 H) 3.83 (m, 1 H) 3.86 (s, 3 H) 3.88 (s, 3 H) 4.11 (dd, J = 12.6, 10.4 Hz, 1 H) 4.53 (m, 2 H) 4.62 (m, 2 H) 6.47 (s, 1 H) 7.13 (s, 1 H). MS *m/z* 344 (MH^+).

4-(7,8-Dimethoxy-3a,4-dihydro-3*H*-[1]benzopyrano[4,3-*c*]isoxazol-3-ylmethyl)piperazine-1-carboxylic Acid *tert*-Butyl Ester (11). To a solution of methanesulfonic acid 7,8-dimethoxy-3a,4-dihydro-3*H*-[1]benzopyrano[4,3-*c*]isoxazol-3-ylmethyl ester **10** (4.8 g, 14 mmol) and piperazine-1-carboxylic acid *tert*-butyl ester (5.23 g, 28 mmol) in methylisobutyl ketone (100 mL) were added K_2CO_3 (1.92 g, 14 mmol) and KI (2.31 g, 14 mmol), and the mixture was refluxed for 16 h. Inorganic salts were filtered out, and the solvent was removed under reduced pressure, giving an oil that was dissolved in dichloromethane, washed three times with brine, and dried (Na_2SO_4). The solvent was removed under reduced pressure, and the oily residue obtained was purified by column chromatography (dichloromethane/methanol 97/3), affording 5.5 g (90%) of **11** as a white foam: ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.48 (s, 9 H) 2.38 (m, 4 H) 2.82 (dd, J = 12.9, 5.4 Hz, 1 H) 2.89 (dd, J = 12.9, 6.2 Hz, 1 H) 3.45 (m, 4 H) 3.66 (td, J = 12.5, 5.6 Hz, 1 H) 3.86 (s, 3 H) 3.87 (s, 3 H) 4.07 (dd, J = 12.6, 10.4 Hz, 1 H) 4.40 (m, 1 H) 4.58 (dd, J = 10.4, 5.6 Hz, 1 H) 6.46 (s, 1 H) 7.16 (s, 1 H). MS *m/z* 434 (MH^+).

7,8-Dimethoxy-3-piperazin-1-ylmethyl-3a,4-dihydro-3*H*-[1]benzopyrano[4,3-*c*]isoxazole (12). A 100 mL round-bottom flask was charged with 4-(7,8-dimethoxy-3a,4-dihydro-3*H*-[1]benzopyrano[4,3-*c*]isoxazol-3-ylmethyl)piperazine-1-carboxylic acid *tert*-butyl ester **11** (5.4 g, 12.4 mmol) and dichloromethane (60 mL). The solution was cooled at 0 °C, trifluoroacetic acid (19.1 mL, 24.8 mmol) was added, and the reaction mixture was stirred for 4 h at room temperature. After quenching with a saturated aqueous solution of $NaHCO_3$, the organic layer was separated, dried over anhydrous Na_2SO_4, filtered, and concentrated, yielding **12** (4.1 g, 100%) as a light-brown foam: ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 2.66 (m, 4 H) 2.78 (dd, J = 13.4, 6.3 Hz, 1 H) 2.87 (dd, J = 13.4, 4.6 Hz, 1 H) 3.03 (t, J = 4.8 Hz, 4 H) 3.66 (td, J = 12.5, 5.8 Hz, 1 H) 3.74 (s, 3 H) 3.78 (s, 3 H) 4.08 (m, 1 H) 4.40 (m, 1 H) 4.62 (dd, J = 10.4, 5.8 Hz, 1 H) 6.59 (s, 1 H) 7.02 (s, 1 H). MS *m/z* 334 (MH^+).

General Procedure for the Preparation of Compounds 13a−d. 7,8-Dimethoxy-3-[4-(3-phenyl-2(*E*)-propen-1-yl)-piperazin-1-ylmethyl]-3a,4-dihydro-3*H*-[1]benzopyrano-[4,3-*c*]isoxazole (13a). To a solution of 7,8-dimethoxy-3-piperazin-1-ylmethyl-3a,4-dihydro-3*H*-[1]benzopyrano[4,3-*c*]isoxazole **12** (3.8 g, 11.5 mmol) in methylisobutyl ketone (50 mL) were subsequently added K_2CO_3 (2 g, 14.4 mmol) and cinnamyl chloride (1.6 mL, 11.5 mmol). The mixture was refluxed for 16 h, inorganic salts were filtered out, and the solvent was removed under reduced pressure. The resulting oil was dissolved in dichloromethane, washed three times with brine, and dried (Na_2SO_4), and the solvent was removed under reduced pressure. The residue was purified by column chromatography (dichloromethane/methanol 98/2) to provide **13a** (3.4 g, 66%) as a white solid after recrystallization from diisopropyl ether: mp 143.3 °C (dec); ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 2.61 (br s, 8 H) 2.82 (dd, J = 13.3, 5.4 Hz, 1 H) 2.89 (dd, J = 13.2, 6.2 Hz, 1 H) 3.17 (dd, J = 6.7, 0.93 Hz, 2 H) 3.66 (td, J = 12.5, 5.8 Hz, 1 H) 3.86 (s, 3 H) 3.87 (s, 3 H) 4.06 (dd, J = 12.5, 10.2 Hz, 1 H) 4.40 (m, 1 H) 4.58 (dd, J = 10.2, 5.8 Hz, 1 H) 6.28 (dt, J = 15.8, 6.7 Hz, 1 H) 6.46 (s, 1 H) 6.53 (d, J = 15.8 Hz, 1 H) 7.16 (s, 1 H) 7.23 (m, 1 H) 7.31 (t, J = 7.5 Hz, 2 H) 7.38 (m, 2 H). HRMS Calcd for $C_{26}H_{32}N_3O_4$ (M + 1): 450.2393. Found: 450.2397. Anal. ($C_{26}H_{31}N_3O_4$) C, H, N.

The following compounds were prepared analogously.

7,8-Dimethoxy-3-[4-(2-methyl-3-phenyl-2(*E*)-propen-1-yl)piperazin-1-ylmethyl]-3a,4-dihydro-3*H*-[1]benzopyrano-[4,3-*c*]isoxazole (13b). White solid from diisopropyl ether: yield 23%; mp 97.1 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.90 (d, J = 1.2 Hz, 3 H) 2.48 (br s, 4 H) 2.62 (br s, 4 H) 2.82 (dd, J = 13.2, 5.5 Hz, 1 H) 2.89 (dd, J = 13.2, 6.2 Hz, 1 H) 3.01 (s, 2 H) 3.66 (td, J = 12.5, 5.8 Hz, 1 H) 3.86 (s, 3 H) 3.87 (s, 3 H) 4.06 (dd, J = 12.5, 10.2 Hz, 1 H) 4.41 (m, 1 H) 4.59 (dd, J = 10.2, 5.8 Hz, 1 H) 6.42 (br s, 1 H) 6.46 (s, 1 H) 7.17 (s, 1 H) 7.21 (m, 1 H) 7.31 (m, 4 H). Anal. ($C_{27}H_{33}N_3O_4$) C, H, N.

7,8-Dimethoxy-3-[4-(3-methyl-3-phenyl-2(*E*)-propen-1-yl)-piperazin-1-ylmethyl]-3a,4-dihydro-3*H*-[1]benzopyrano[4,3-*c*]isoxazole (13c). Light yellow solid from diisopropyl ether: yield 44%; mp 130.6 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 2.07 (s, 3 H) 2.64 (br s, 8 H) 2.82 (dd, J = 13.3, 5.2 Hz, 1 H) 2.89 (dd, J = 13.3, 6.2 Hz, 1 H) 3.20 (d, J = 6.8 Hz, 2 H) 3.67 (td, J = 12.6, 5.8 Hz, 1 H) 3.86 (s, 3 H) 3.88 (s, 3 H) 4.07 (dd, J = 12.6, 10.2 Hz, 1 H) 4.41 (m, 1 H) 4.59 (dd, J = 10.2,

5.8 Hz, 1 H) 5.89 (m, 1 H) 6.46 (s, 1 H) 7.17 (s, 1 H) 7.24 (m, 1 H) 7.32 (m, 2 H) 7.41 (m, 2 H). MS *m/z* 464 (MH^+). Anal. ($C_{27}H_{33}N_3O_4$) C, H, N.

7,8-Dimethoxy-3-[4-(naphthalen-2-ylmethyl)-piperazin-1-ylmethyl]-3a,4-dihydro-3*H*-[1]benzopyrano[4,3-*c*]isoxazole (13d). White solid from diisopropyl ether: yield 11%; mp 83.7 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 2.54 (br s, 4 H) 2.62 (br s, 4 H) 2.81 (dd, J = 13.3, 5.4 Hz, 1 H) 2.88 (dd, J = 13.3, 6.2 Hz, 1 H) 3.66 (m, 1 H) 3.85 (s, 2 H) 3.87 (s, 3 H) 4.05 (dd, J = 12.4, 10.4 Hz, 1 H) 4.40 (m, 1 H) 4.58 (dd, J = 10.4, 5.8 Hz, 1 H) 6.45 (s, 1 H) 7.16 (s, 1 H) 7.47 (m, 3 H) 7.73 (s, 1 H) 7.81 (m, 3 H). HRMS Calcd for $C_{28}H_{32}N_3O_4$ (M + 1): 474.2393. Found: 474.2393. Anal. ($C_{28}H_{31}N_3O_4$) C, H, N.

General Procedure for Resolution of Racemic Mixtures. (−)-7,8-Dimethoxy-3-[4-(3-phenyl-2(*E*)-propen-1-yl)piperazin-1-ylmethyl]-3a,4-dihydro-3*H*-[1]benzopyrano-[4,3-*c*]isoxazole ((−)-13a) and (+)-7,8-Dimethoxy-3-[4-(3-phenyl-2(*E*)-propen-1-yl)piperazin-1-ylmethyl]-3a,4-dihydro-3*H*-[1]benzopyrano[4,3-*c*]isoxazole ((+)-13a). The racemate 7,8-dimethoxy-3-[4-(3-phenyl-2(*E*)-propen-1-yl)piperazin-1-ylmethyl]-3a,4-dihydro-3*H*-[1]benzopyrano[4,3-*c*]isoxazole **13a** (3.0 g, 6.7 mmol) was separated into its enantiomers by preparative HPLC (ethanol/acetonitrile 100/0 to 80/20 at 110 mL/min; column: CHIRALPAK AD 1000 Å 20 μm Daicel). Two pure fractions were collected, the solvents were evaporated, and the residues were crystallized as dihydrochloride acid salts in methanol, yielding 1.08 g of (−)-**13a** as a white solid: mp 247.1 °C (dec); 99% ee; $[\alpha]^{20}_D$: −69.29° (c = 0.13, DMF); HRMS Calcd for $C_{26}H_{32}N_3O_4$ (M + 1): 450.2393. Found: 450.2383, and 0.54 g of (+)-**13a** as a light yellow solid: mp 250.7 °C (dec); 99% ee; $[\alpha]^{20}_D$: +55° (c = 0.3, DMF); HRMS Calcd for $C_{26}H_{32}N_3O_4$ (M + 1): 450.2393. Found: 450.2361.

The following compounds were separated analogously.

(−)-7,8-Dimethoxy-3-[4-(2-methyl-3-phenyl-2(*E*)-propen-1-yl)piperazin-1-ylmethyl]-3a,4-dihydro-3*H*-[1]benzopyrano[4,3-*c*]isoxazole ((−)-13b) and (+)-7,8-Dimethoxy-3-[4-(2-methyl-3-phenyl-2(*E*)-propen-1-yl)piperazin-1-ylmethyl]-3a,4-dihydro-3*H*-[1]benzopyrano[4,3-*c*]isoxazole ((+)-13b). White solid (−)-**13b**: mp 243.8 °C (dec); 99% ee; $[\alpha]^{20}_D$: −78.01° (c = 0.106, THF/H_2O 4/1 v/v); HRMS Calcd for $C_{27}H_{34}N_3O_4$ (M + 1): 464.2549. Found: 464.2563. White solid (+)-**13b**: mp 247.3 °C (dec); 99% ee; $[\alpha]^{20}_D$: +84.43° (c = 0.106, THF/H_2O 4/1 v/v); HRMS Calcd for $C_{27}H_{34}N_3O_4$ (M + 1): 464.2549 Found: 464.2536.

(−)-7,8-Dimethoxy-3-[4-(3-methyl-3-phenyl-2(*E*)-propen-1-yl)piperazin-1-ylmethyl]-3a,4-dihydro-3*H*-[1]benzopyrano[4,3-*c*]isoxazole ((−)-13c) and (+)-7,8-Dimethoxy-3-[4-(3-methyl-3-phenyl-2(*E*)-propen-1-yl)piperazin-1-ylmethyl]-3a,4-dihydro-3*H*-[1]benzopyrano[4,3-*c*]isoxazole ((+)-13c). Light brown solid as free base from diisopropyl ether (−)-**13c**: mp 95.7 °C; 99% ee; $[\alpha]^{20}_D$: −92.5° (c = 0.59, DMF); HRMS Calcd for $C_{27}H_{34}N_3O_4$ (M + 1): 464.2549. Found: 464.2556. Light yellow solid as free base from diisopropyl ether (+)-**13c**: mp 93.5 °C; 99% ee; $[\alpha]^{20}_D$: +92.6° (c = 0.55, DMF); HRMS Calcd for $C_{27}H_{34}N_3O_4$ (M + 1): 464.2549 Found: 464.2538. Anal. ($C_{27}H_{33}N_3O_4$) C, H, N.

(−)-7,8-Dimethoxy-3-[4-(naphthalen-2-ylmethyl)piperazin-1-ylmethyl]-3a,4-dihydro-3*H*-[1]benzopyrano[4,3-*c*]isoxazole ((−)-13d) and (+)-7,8-Dimethoxy-3-[4-(naphthalen-2-ylmethyl)piperazin-1-ylmethyl]-3a,4-dihydro-3*H*-[1]benzopyrano[4,3-*c*]isoxazole ((+)-13d). White solid (−)-**13d**: mp 248.5 °C (dec); 99% ee; $[\alpha]^{20}_D$: −79° (c = 0.14, MeOH); HRMS Calcd for $C_{28}H_{32}N_3O_4$ (M + 1): 474.2393. Found: 474.2414. White solid (+)-**13d**: mp 266.0 °C (dec); 99% ee; $[\alpha]^{20}_D$: +60° (c = 0.06, DMF); HRMS Calcd for $C_{28}H_{32}N_3O_4$ (M + 1): 474.2393. Found: 474.2386.

4-[(2-Formylphenyl)−(2,2,2-trifluoroacetyl)amino]but-2(*E*)-enoic Acid Methyl Ester (15). To a solution of sodium hydride (0.42 g, 10.6 mmol) and 18-crown-6 (0.05 mL) in anhydrous dimethylformamide (10 mL) stirred at 0 °C and under nitrogen atmosphere was added a solution of 2,2,2-trifluoro-*N*-(2-formylphenyl)acetamide **14**[31] (2.3 g, 10.6 mmol) in anhydrous dimethylformamide (10 mL) dropwise. After stirring for 20 min at room temperature, a solution of methyl 4-bromocrotonate (2.2 mL, 15.8 mmol) in 10 mL of anhydrous dimethylformamide was added dropwise, and the reaction mixture was stirred for 4 h at 60 °C. The solvent was evaporated, and the residue was partitioned between water and dichloromethane. The organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated, affording **15** (2.0 g) as an oil, which was used without further purification. MS *m/z* 315 (MH^+).

4-{[2-(Hydroxyiminomethyl)phenyl]-(2,2,2-trifluoroacetyl)amino}but-2(*E*)-enoic Acid Methyl Ester (16). To a solution of 4-[(2-formylphenyl)−(2,2,2-trifluoroacetyl)amino]-but-2(*E*)-enoic acid methyl ester **15** (2.0 g, 6.3 mmol) obtained in the previous reaction in ethanol (20 mL) were added hydroxylamine hydrochloride (0.52 g, 7.5 mmol) and pyridine (0.76 mL, 9.4 mmol). After stirring the mixture for 2 h at room temperature, the solvent was evaporated, and the residue was taken up in dichloromethane and washed with a 10% aqueous solution of citric acid. The organic layer was separated, dried over anhydrous Na_2SO_4, and filtered, and the solvent was evaporated, yielding a residue that was purified by column chromatography (dichloromethane/acetone, 9/1) to provide **16** (2.0 g, 96% two steps) as an oil: ^{1}H NMR (200 MHz, $CDCl_3$) δ ppm 3.73 (s, 3 H) 3.74 (dd, J = 16.5, 8.5 Hz, 1 H) 4.94 (dd, J = 16.5, 5.7 Hz, 1 H) 5.90 (d, J = 17.1 Hz, 1 H) 6.90 (m, 1 H) 7.21 (br d, J = 8.5 Hz, 1 H) 7.43 (m, 2 H) 7.68 (dd, J = 7.1, 2.8 Hz, 1 H) 8.15 (s, 1 H) 9.20 (br s, 1 H). MS *m/z* 331 (MH^+).

5-(2,2,2-Trifluoroacetyl)-3,3a,4,5-tetrahydroquinolino-[4,3-*c*]isoxazole-3-carboxylic Acid Methyl Ester (17). To a solution of 4-{[2-(hydroxyiminomethyl)phenyl]-(2,2,2-trifluoroacetyl)amino}but-2(*E*)-enoic acid methyl ester **16** (2.0 g, 6.3 mmol) in ethanol (20 mL) was added chloramine-T hydrate (1.4 g, 6.3 mmol), and the reaction mixture was stirred for 2 h at 80 °C. After cooling to room temperature, the mixture was filtered through a Celite pad and the filtrate was evaporated to dryness. The residue was partitioned between dichloromethane and water, the organic layer was separated, dried (Na_2SO_4), and filtered, and the solvent was evaporated. The resulting residue was purified by column chromatography (dichloromethane), affording **17** (1.2 g, 60%) as a brown foam: ^{1}H NMR (200 MHz, $CDCl_3$) δ ppm 3.67 (t, J = 12.8 Hz, 1 H) 3.90 (s, 3 H) 4.01 (td, J = 12.8, 5.1 Hz, 1 H) 4.71 (dd, J = 12.8, 5.1 Hz, 1 H) 4.80 (d, J = 12.8 Hz, 1 H) 7.33 (t, J = 7.7 Hz, 1 H) 7.49 (td, J = 7.7, 2.5 Hz, 1 H) 7.76 (br d, J = 7.6 Hz, 1 H) 8.01 (dd, J = 7.7, 2.5 Hz, 1H). MS *m/z* 329 (MH^+).

3-Hydroxymethyl-3,3a,4,5-tetrahydroquinolino[4,3-*c*]-isoxazole (18). To a solution of 5-(2,2,2-trifluoroacetyl)-3,3a,4,5-tetrahydroquinolino[4,3-*c*]isoxazole-3-carboxylic acid methyl ester **17** (1.2 g, 3.6 mmol) in a mixture of tetrahydrofuran/water 1/1 (15 mL) cooled at 0 °C was added sodium borohydride (340 mg, 9 mmol). The reaction was allowed to reach room temperature and was stirred overnight. After this time it was quenched with a 10% aqueous solution of NH_4Cl and extracted with ethyl acetate. The organic layer was separated, dried over anhydrous Na_2SO_4, filtered, and concentrated, providing **18** (0.7 g), which was used in the next step without further purification. ^{1}H NMR (200 MHz, DMSO-d_6) δ ppm 3.11 (m, 1 H) 3.50 (m, 2 H) 3.70 (m, 2 H) 4.25 (td, J = 12.0, 4.8 Hz, 1 H) 6.60 (m, 2 H) 7.10 (td, J = 7.6, 2.6 Hz, 1 H) 7.40 (dd, J = 7.6, 2.6 Hz, 1 H). MS *m/z* 205 (MH^+).

3-Chloromethyl-3,3a,4,5-tetrahydroquinolino[4,3-*c*]-isoxazole (19). Triphenylphosphine (1.2 g, 4.7 mmol) was added to a solution of 3-hydroxymethyl-3,3a,4,5-tetrahydroquinolino[4,3-*c*]isoxazole **18** (0.7 g, 3.4 mmol) in tetrahydrofuran (5 mL) and carbon tetrachloride (6 mL). The reaction mixture was refluxed for 3 h, and after cooling to room temperature, the solvent was removed, affording a residue, which was purified by column chromatography (dichloromethane) to provide 0.64 g of **19** (0.64 g, 80% two steps) as a brown oil: ^{1}H NMR (200 MHz, $CDCl_3$) δ ppm 3.42 (m, 1 H) 3.70 (m, 3 H) 3.92 (dd, J = 11.4, 5.1 Hz, 1 H) 4.20 (br s, 1 H) 4.52 (m, 1 H) 6.60 (dd, J = 8.6, 1.1 Hz, 1 H) 6.75 (td, J = 8.5, 1.1 Hz, 1 H) 7.20 (td, J = 8.5, 1.5 Hz, 1 H) 7.68 (dd, J = 8.5, 1.5 Hz, 1 H). MS *m/z* 223 (MH^+).

General Procedure for the Preparation of Compounds 20a,b. 3-[4-(3-Phenyl-2(*E*)-propen-1-yl)piperazin-1-ylmethyl]-3,3a,4,5-tetrahydroquinolino[4,3-*c*]isoxazole (20a). 1-Cinnamylpiperazine (1.81 g, 8.98 mmol) was added to a solution of 3-chloromethyl-3,3a,4,5-tetrahydroquinolino[4,3-*c*]-isoxazole **19** (1.0 g, 4.5 mmol) in *n*-butanol (5 mL), and the mixture was stirred for 8 h at 120 °C. After cooling to room temperature, the solvent was evaporated, and the resulting residue was purified by column chromatography (dichloromethane/methanol 96/4), yielding **20a** (0.77 g, 44%) as a light yellow solid after recrystallization from diisopropyl ether: mp 186.5 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ ppm 2.55 (br s, 6 H) 2.65 (br s, 2 H) 2.82 (m, 2 H) 3.18 (d, J = 6.8 Hz, 2 H) 3.38 (m, 1 H) 3.60 (m, 2 H) 4.14 (d, J = 2.0 Hz, 1 H) 4.48 (m, 1 H) 6.29 (dt, J = 16.8, 6.5 Hz, 1 H) 6.52 (d, J = 16.8 Hz, 1 H) 6.60 (d, J = 8.5 Hz, 1 H) 6.72 (t, J = 8.5 Hz, 1 H) 7.25 (m, 6 H) 7.70 (dd, J = 7.7, 1.0 Hz, 1 H). Anal. ($C_{24}H_{28}N_4O$) C, H N.

The following compound was prepared analogously.

3-[4-(Naphthalen-2-ylmethyl-piperazin-1-ylmethyl)]-3,3a,4,5-tetrahydroquinolino[4,3-*c*]isoxazole (20b). Light yellow foam: yield 30%; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 2.40−2.80 (br s, 8 H) 2.82 (m, 2 H) 3.35 (m, 1 H) 3.60 (m, 2 H) 3.66 (s, 2 H) 4.10 (d, J = 2.0 Hz, 1 H) 4.48 (m, 1 H) 6.60 (d, J = 8.7 Hz, 1 H) 6.72 (ddd, J = 7.7, 7.7 1.0 Hz, 1 H) 7.16 (ddd, J = 8.7, 8.6 1.0 Hz, 1 H) 7.46 (m, 3 H) 7.70 (dd, J = 7.7, 1.0 Hz, 1 H) 7.72 (s, 1 H) 7.80 (m, 3 H). HRMS Calcd for $C_{26}H_{29}N_4O$ (M + 1): 413.2341. Found: 413.2336.

General Procedure for the Preparation of Compounds 21a and 30. 5-Methyl-3-[4-(3-phenyl-2(*E*)-propen-1-yl)-piperazin-1-ylmethyl]-3,3a,4,5-tetrahydroquinolino[4,3-*c*]isoxazole (21a). To a solution of 3-[4-(3-phenyl-2(*E*)-propen-1-yl)piperazin-1-ylmethyl]-3,3a,4,5-tetrahydroquinolino[4,3-*c*]isoxazole **20a** (0.7 g, 1.8 mmol) and formaldehyde (0.14 mL, 1.8 mmol) in methanol (20 mL) was added $ZnBr_2$ (0.2 g, 0.9 mmol). After stirring for a few minutes, sodium cyanoborohydride (0.16 g, 2.7 mmol) was added, and the reaction was stirred for 24 h at reflux temperature. After cooling to room temperature, dichloromethane was added, and the mixture was washed with brine. The organic layer was separated, dried over anhydrous Na_2SO_4, filtered, and concentrated. The resulting residue was purified by column chromatography (dichloromethane/methanol 98/2) yielding **21a** (0.1 g, 14%) as a white solid after crystallization from diisopropyl ether: mp 209.1 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 2.60 (br s, 8 H) 2.84 (m, 2 H) 2.94 (s, 3 H) 3.17 (d, J = 6.8 Hz, 2 H) 3.35 (m, 1 H) 3.47 (m, 1 H) 3.64 (td, J = 12.5, 5.9 Hz, 1 H) 4.49 (m, 1 H) 6.28 (dt, J = 16.0, 6.8 Hz, 1 H) 6.53 (d, J = 16.0 Hz, 1 H) 6.68 (d, J = 8.7 Hz, 1 H) 6.74 (t, J = 7.5 Hz, 1 H) 7.27 (m, 4 H) 7.38 (m, 2 H) 7.75 (d, J = 7.7 Hz, 1 H). MS *m/z* 403 (MH^+). Anal. ($C_{25}H_{30}N_4O$) C, H N.

General Procedure for the Preparation of Compounds 21b,c. 3-[4-(3-Phenyl-2(*E*)-propen-1-yl)piperazin-1-ylmethyl]-3,3a,4,5-tetrahydroquinolino[4,3-*c*]isoxazole-5-carboxylic Acid Ethylamide (21b). To a solution of 3-[4-(3-phenyl-2(*E*)-propen-1-yl)piperazin-1-ylmethyl]-3,3a,4,5-tetrahydroquinolino[4,3-*c*]isoxazole **20a** (0.5 g, 1.2 mmol) in anhydrous tetrahydrofuran (10 mL) was added *n*-butyllithium (1.6 M in hexanes) (0.87 mL, 1.4 mmol) dropwise at −78 °C under nitrogen atmosphere. The reaction mixture was stirred for 0.5 h at −78 °C, and then ethyl isocyanate (0.1 mL, 1.4 mmol) was added dropwise. The mixture was allowed to reach room temperature and was stirred overnight. Dichloromethane was added, and the reaction was quenched with a 10% aqueous solution of NH_4Cl, the organic layer was separated, dried over anhydrous Na_2SO_4, and filtered, and the solvent was evaporated, affording a residue that was purified by column chromatography (dichloromethane/methanol 98/2) to provide **21b** (0.27 g, 49%) as a foam: ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.16 (t, J = 7.2 Hz, 3 H) 2.60 (br s, 8H) 2.85 (dd, J = 13.6, 4.0 Hz, 1 H) 2.93 (dd, J = 13.6, 7.0 Hz, 1 H) 3.31 (m, 6 H) 4.47 (ddd, J = 12.7, 6.8, 3.9 Hz, 1 H) 4.95 (dd, J = 12.7, 5.2 Hz, 1 H) 5.20 (t, J = 5.4 Hz, 1 H) 6.28 (dt, J = 15.8, 6.8 Hz, 1 H) 6.53 (d, J = 15.8 Hz, 1 H) 7.17 (m, 1 H) 7.23 (t, J = 7.3 Hz, 1 H) 7.34 (m, 6 H) 7.99 (m, 1 H). HRMS Calcd for $C_{27}H_{34}N_5O_2$ (M + 1): 460.2699. Found: 460.2707.

The following compound was prepared analogously.

3-[4-(3-phenyl-2(*E*)-propen-1-yl)piperazin-1-ylmethyl]-3,3a,4,5-tetrahydroquinolino[4,3-*c*]isoxazole-5-carboxylic Acid ethyl Ester (21c). Light brown solid from diisopropyl ether: yield 29%; mp 108.7 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.35 (t, J = 7.2 Hz, 3 H) 2.60 (br s, 4 H) 2.70 (br s, 4 H) 2.90 (d, J = 5.8 Hz, 2 H) 3.17 (d, J = 6.5 Hz, 2 H) 3.28 (t, J = 12.5 Hz, 1 H) 3.52 (td, J = 12.5, 5.0 Hz, 1 H) 4.28 (m, 2 H) 4.46 (m, 1 H) 4.85 (dd, J = 12.5, 5.0 Hz, 1 H) 6.28 (dt, J = 15.8, 6.5 Hz, 1 H) 6.53 (d, J = 15.8 Hz, 1 H) 7.15 (m, 1 H) 7.30 (m, 7 H) 7.72 (d, J = 8.5 Hz, 1 H) 7.93 (dd, J = 7.9, 1.4 Hz, 1 H). MS *m/z* 461 (MH^+). Anal. ($C_{27}H_{32}N_4O_3$) C, H, N.

General Procedure for the Preparation of Compounds 21d−e. 5-Acetyl-3-[4-(3-phenyl-2(*E*)-propen-1-yl)piperazin-1-ylmethyl]-3,3a,4,5-tetrahydroquinolino[4,3-*c*]isoxazole (21d). 3-[4-(3-Phenyl-2(*E*)-propen-1-yl)piperazin-1-ylmethyl]-3,3a,4,5-tetrahydroquinolino[4,3-*c*]isoxazole **20a** (0.5 g, 1.2 mmol) dissolved in anhydrous tetrahydrofuran (8 mL) was added dropwise at 0 °C and under nitrogen atmosphere to a mixture of sodium hydride (0.072 g, 1.8 mmol) and 18-crown-6 (0.05 mL) in anhydrous tetrahydrofuran (2 mL). After stirring for 0.5 h at room temperature, acetyl chloride (0.09 mL, 1.4 mmol) was added dropwise, and the reaction mixture was stirred for 3 h at room temperature. The mixture was then quenched with a 10% aqueous solution of NH_4Cl, extracted with dichloromethane, and the organic layer was separated, dried (Na_2SO_4), filtered, and concentrated, affording a residue that was purified by column chromatography (dichloromethane/methanol 98/2). The final solid residue was recrystallized from diisopropyl ether yielding **21d** (0.2 g, 40%) as a yellow solid: mp 127 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 2.30 (s, 3 H) 2.58 (br s, 4 H) 2.65 (br s, 4 H) 2.90 (d, J = 5.6 Hz, 2 H) 3.17 (m, 3 H) 3.48 (td, J = 12.9, 5.2 Hz, 1 H) 4.46 (m, 1 H) 5.06 (br s, 1 H) 6.28 (dt, J = 15.8, 6.9 Hz, 1 H) 6.53 (d, J = 15.8 Hz, 1 H) 7.31(m, 8 H) 7.98 (d, J = 7.5 Hz, 1 H). MS *m/z* 431 (MH^+). Anal. ($C_{26}H_{30}N_4O_2$) C, H, N.

The following compound was prepared analogously.

5-(2,2,2-Trifluoroacetyl)-3-[4-(3-phenyl-2(*E*)-propen-1-yl)piperazin-1-ylmethyl]-3,3a,4,5-tetrahydroquinolino-[4,3-*c*]isoxazole (21e). Light yellow solid from diisopropyl ether: yield 32%; mp 115.6 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 2.41 (br s, 4 H) 2.52 (br s, 4H) 2.76 (m, 2 H) 3.06 (d, J = 6.4 Hz, 2 H) 3.77 (m, 2 H) 4.43 (br s, 1 H) 4.50 (m, 1 H) 6.27 (dt, J = 16.0, 6.6 Hz, 1 H) 6.52 (d, J = 16.0 Hz, 1 H) 7.20 (t, J = 7.3 Hz, 1 H) 7.29 (m, 2 H) 7.36 (m, 1 H) 7.41 (d, J = 7.3 Hz, 2 H) 7.51 (m, 1 H) 7.74 (d, J = 6.8 Hz, 1 H) 7.88 (dd, J = 7.7, 1.4 Hz, 1 H). MS *m/z* 485 (MH^+). Anal. ($C_{26}H_{27}F_3N_4O_2$) C, H, N.

4-(4-Chloro-2(*E*)-buten-1-yl)piperazine-1-carboxylic Acid *tert*-Butyl Ester (23). To a solution of 1,4-dichloro-2(*E*)-butene (93.0 g, 500 mmol) in chloroform (1 L) was added another solution of piperazine-1-carboxylic acid *tert*-butyl ester (54 mL, 500 mmol) in chloroform (500 mL) dropwise. The reaction was heated for 6 h at reflux temperature. After cooling to room temperature, a saturated solution of $NaHCO_3$ was added, and the organic layer was separated, dried over anhydrous Na_2SO_4, filtered, and concentrated, leading to a residue that was purified by column chromatography (ethyl acetate), affording **23** (50.0 g, 36%) as a yellow oil: ^{1}H NMR (200 MHz, $CDCl_3$) δ ppm 1.46 (s, 9 H) 2.40 (t, J = 5.0 Hz, 4 H) 3.02 (d, J = 5.1 Hz, 2 H) 3.45 (t, J = 5.0 Hz, 4 H) 4.07 (d, J = 6.0 Hz, 2 H) 5.81(m, 2 H). MS *m/z* 275 (MH^+).

4-[4-(2-Formyl-4,5-dimethoxy-phenylamino)-2(*E*)-buten-1-yl]piperazine-1-carboxylic Acid *tert*-Butyl Ester (24). To a mixture of sodium hydride (6.1 g, 150 mmol) and 18-crown-6 (0.1 mL) in anhydrous tetrahydrofuran (100 mL) under nitrogen atmosphere, a solution of 2-amino-4,5-dimethoxybenzaldehyde **22**[33] (28 g, 150 mmol) in anhydrous tetrahydrofuran (150 mL) was added dropwise and the reaction mixture was stirred for 30 min at room temperature. Then, a solution of **23** (28.3 g, 100 mmol) in anhydrous tetrahydrofuran

(100 mL) was added dropwise. The reaction mixture was stirred for 3 days at room temperature. After that time, the mixture was quenched with a 10% aqueous solution of NH_4Cl, extracted with dichloromethane and the organic layer was separated, dried (Na_2SO_4), filtered and the solvent evaporated yielding **24** (21.6 g) as an oil, which was used in the next step without further purification. MS *m/z* 420 (MH^+).

4-{4-[(2-Formyl-4,5-dimethoxy-phenyl)−(2,2,2-trifluoroacetyl)amino]but-2-enyl}piperazine-1-carboxylic Acid *tert*-Butyl Ester (25). To a mixture of sodium hydride (2.8 g, 70 mmol) and 18-crown-6 (0.1 mL) in anhydrous tetrahydrofuran (50 mL) under nitrogen atmosphere was added a solution of 4-[4-(2-formyl-4,5-dimethoxy-phenylamino)-2(*E*)-buten-1-yl]-piperazine-1-carboxylic acid *tert*-butyl ester **24** (20 g, 40 mmol) in anhydrous tetrahydrofuran (200 mL) dropwise at room temperature. After stirring for 20 min, trifluoroacetic anhydride (7.4 mL, 50 mmol) was added dropwise, and the reaction mixture was stirred for 2 h at room temperature. The reaction was quenched with a 10% aqueous solution of NH_4Cl and extracted with dichloromethane, the organic layer was separated, dried over anhydrous Na_2SO_4, and filtered, and the solvent was evaporated, affording **25** (15.8 g) as a brown oil, which was used in the next step without further purification. MS *m/z* 516 (MH^+).

4-[7,8-Dimethoxy-5-(2,2,2-trifluoro-acetyl)-3,3a,4,5-tetrahydroquinolino[4,3-*c*]isoxazolin-3-ylmethyl]piperazine-1-carboxylic Acid *tert*-Butyl Ester (26). A mixture of 4-{4-[(2-formyl-4,5-dimethoxy-phenyl)−(2,2,2-trifluoroacetyl) amino]-but-2-enyl}piperazine-1-carboxylic acid *tert*-butyl ester **25** (15.8 g, 30 mmol), hydroxylamine hydrochloride (2.3 g, 33 mmol), and $NaHCO_3$ (5.1 g, 62 mmol) in ethanol (300 mL) was stirred at room temperature overnight. The solid was filtered off, and the filtrate was evaporated to dryness, affording the corresponding oxime, which was used without further purification. This oxime was dissolved in dichloromethane (300 mL), and *N*-chlorosuccinimide (9.8 g, 0.074 mol) was added portionwise. The resulting solution was stirred for 2 h at room temperature, and then triethylamine (10.3 mL, 0.073 mol) was added dropwise. The reaction mixture was stirred for 24 h at room temperature, and it was quenched with a 10% aqueous solution of K_2CO_3. The organic layer was separated, washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated, yielding **26** (18 g) as a yellow oil, which was used in the next step without further purification. MS *m/z* 529 (MH^+).

4-(7,8-Dimethoxy-3,3a,4,5-tetrahydroquinolino[4,3-*c*]-isoxazolin-3-ylmethyl)piperazine-1-carboxylic Acid *tert*-Butyl Ester (27). To a solution of 4-[7,8-dimethoxy-5-(2,2,2-trifluoro-acetyl)-3,3a,4,5-tetrahydroquinolino[4,3-*c*]isoxazolin-3-ylmethyl]piperazine-1-carboxylic acid *tert*-butyl ester **26** (15.5 g, 29 mmol) in a mixture of dioxane/water 3/1 (200 mL) was added lithium hydroxide (0.77 g, 32 mmol), and the reaction mixture was stirred for 3 h at room temperature. After that time, dichloromethane was added, the organic layer was separated, dried over anhydrous Na_2SO_4, and filtered, and the solvent was evaporated, affording **27** (5.8 g) as a yellow oil, which was used in the next step without further purification. MS *m/z* 433 (MH^+).

7,8-Dimethoxy-3-piperazin-1-ylmethyl-3,3a,4,5-tetrahydroquinolino[4,3-*c*]isoxazole (28). To a solution of 4-(7,8-dimethoxy-3,3a,4,5-tetrahydroquinolino[4,3-*c*]isoxazolin-3-ylmethyl)piperazine-1-carboxylic acid *tert*-butyl ester **27** (5.8 g, 13 mmol) in dichloromethane (100 mL) was added trifluoroacetic acid (22.5 mL, 300 mmol) dropwise. The reaction mixture was stirred for 1 h at room temperature. After cooling at 0 °C, it was basified with a 50% aqueous solution of NaOH and extracted with dichloromethane. The organic layer was separated, dried over anhydrous Na_2SO_4, and filtered and the solvent evaporated. The residue was purified by column chromatography (dichloromethane/methanol saturated with ammonia 95/5 and 9/1) to provide **28** (3.8 g, 10% five steps) as a brown foam: ^1H NMR (400 MHz, DMSO-d_6) δ ppm 2.66 (m, 4 H) 2.78 (dd, J = 13.4, 6.3 Hz, 1 H) 2.87 (dd, J = 13.4, 4.6 Hz, 1 H) 3.03 (t, J = 4.8 Hz, 4 H) 3.12 (m, 1 H) 3.50 (m, 2 H) 3.74 (s, 3 H) 3.78 (s, 3 H) 4.20 (br s, 1 H) 4.25 (td, J = 12.0, 4.7 Hz, 1 H) 6.59 (s, 1 H) 7.02 (s, 1 H). MS *m/z* 333 (MH^+).

General Procedure for the Preparation of Compounds 29a−g. 7,8-Dimethoxy-3-[4-(3-phenyl-2(*E*)-propen-1-yl)-piperazin-1-ylmethyl]-3,3a,4,5-tetrahydroquinolino[4,3-*c*]isoxazole (29a). To a solution of 7,8-dimethoxy-3-piperazin-1-ylmethyl-3,3a,4,5-tetrahydroquinolino[4,3-*c*]isoxazole **28** (1.5 g, 4.5 mmol) in dimethylformamide (15 mL) were subsequently added K_2CO_3 (0.51 g, 3.7 mmol) and cinnamyl chloride (0.51 mL, 3.7 mmol). The mixture was stirred for 4 h at 70 °C. After cooling to room temperature, dichloromethane was added, and the mixture was washed several times with water. The organic layer was separated, dried over anhydrous Na_2SO_4, and filtered and the solvent evaporated. The residue was purified by column chromatography (dichloromethane/methanol saturated with ammonia, 98/2), affording **29a** (0.5 g, 25%) as a white foam: ^1H NMR (400 MHz, $CDCl_3$) δ ppm 2.71 (br s, 8 H) 2.86 (d, J = 5.4 Hz, 2 H) 3.31 (m, 3 H) 3.56 (m, 2 H) 3.82 (s, 3 H) 3.84 (s, 3 H) 3.93 (br s, 1 H) 4.45 (m, 1 H) 6.14 (s, 1 H) 6.31 (m, 1 H) 6.56 (d, J = 16.0 Hz, 1 H) 7.14 (s, 1 H) 7.24 (m, 1 H) 7.32 (t, J = 7.3 Hz, 2 H) 7.39 (d, J = 7.3 Hz, 2 H). HRMS Calcd for $C_{26}H_{33}N_4O_3$ (M + 1): 449.2539. Found: 449.2547.

The following compounds were prepared analogously

7,8-Dimethoxy-3-[4-(2-methyl-3-phenyl-2(*E*)-propen-1-yl)piperazin-1-ylmethyl]-3,3a,4,5-tetrahydroquinolino-[4,3-*c*]isoxazole (29b). White foam: yield 20%; ^1H NMR (400 MHz, $CDCl_3$) δ ppm 1.90 (d, J = 1.0 Hz, 3 H) 2.48 (br s, 4 H) 2.59 (br s, 2 H) 2.64 (br s, 2 H) 2.84 (m, 2 H) 3.01 (s, 2 H) 3.32 (m, 1 H) 3.58 (m, 2 H) 3.82 (s, 3 H) 3.84 (s, 3 H) 3.92 (br s, 1 H) 4.46 (m, 1 H) 6.14 (s, 1 H) 6.42 (s, 1 H) 7.15 (s, 1 H) 7.20 (t, J = 7.1 Hz, 1 H) 7.30 (m, 4 H). Anal. ($C_{27}H_{34}N_4O_3$) C, H, N.

7,8-Dimethoxy-3-[4-(3-methyl-3-phenyl-2(*E*)-propen-1-yl)piperazin-1-ylmethyl]-3,3a,4,5-tetrahydroquinolino-[4,3-*c*]isoxazole (29c). Light yellow foam: yield 25%; ^1H NMR (400 MHz, $CDCl_3$) δ ppm 2.07 (s, 3 H) 2.65 (br s, 8 H) 2.85 (m, 2 H) 3.22 (d, J = 6.6 Hz, 2 H) 3.34 (m, 1 H) 3.59 (m, 2 H) 3.82 (s, 3 H) 3.84 (s, 3 H) 3.90 (br s, 1 H) 4.45 (m, 1 H) 5.90 (m, 1 H) 6.14 (s, 1 H) 7.15 (s, 1 H) 7.23 (m, 1 H) 7.32 (m, 2 H) 7.41 (m, 2 H). HRMS Calcd for $C_{27}H_{35}N_4O_3$ (M + 1): 463.2709. Found: 463.2704.

3-[4-(4-Chlorobenzyl)piperazin-1-ylmethyl]-7,8-dimethoxy-3,3a,4,5-tetrahydroquinolino[4,3-*c*]isoxazole (29d). White foam: yield 28%; ^1H NMR (400 MHz, $CDCl_3$) δ ppm 2.47 (br s, 4 H) 2.58 (br s, 2 H) 2.62 (br s, 2 H) 2.83 (m, 2 H) 3.32 (m, 1 H) 3.47 (s, 2 H) 3.56 (m, 2 H) 3.82 (s, 3 H) 3.84 (s, 3 H) 3.89 (br s, 1 H) 4.44 (m, 1 H) 6.13 (s, 1 H) 7.14 (s, 1 H) 7.27 (m, 4 H). MS 457 *m/z* (MH^+). Anal. ($C_{24}H_{29}ClN_4O_3$) C, H, N.

7,8-Dimethoxy-3-[4-(naphthalen-2-ylmethyl)piperazin-1-ylmethyl]-3,3a,4,5-tetrahydroquinolino[4,3-*c*]isoxazole (29e). Light yellow foam: yield 23%; ^1H NMR (400 MHz, $CDCl_3$) δ ppm 2.55 (br s, 6 H) 2.65 (br s, 2 H) 2.83 (m, 2 H) 3.31 (m, 1 H) 3.56 (m, 2 H) 3.67 (s, 2 H) 3.82 (s, 3 H) 3.84 (s, 3 H) 3.89 (d, J = 2.1 Hz, 1 H) 4.44 (m, 1 H) 6.13 (s, 1 H) 7.14 (s, 1 H) 7.47 (m, 3 H) 7.74 (s, 1 H) 7.81 (m, 3 H). MS *m/z* 473 (MH^+). Anal. ($C_{28}H_{32}N_4O_3$) C, H, N.

7,8-Dimethoxy-3-[4-(quinolin-6-ylmethyl)piperazin-1-ylmethyl]-3,3a,4,5-tetrahydroquinolino[4,3-*c*]isoxazole (29f). Light brown foam: yield 20%; ^1H NMR (400 MHz, $CDCl_3$) δ ppm 2.56 (br s, 4 H) 2.62 (br s, 2 H) 2.67 (br s, 2 H) 2.86 (m, 2 H) 3.33 (m, 1 H) 3.58 (m, 2 H) 3.70 (s, 2H) 3.82 (s, 3 H) 3.84 (s, 3 H) 3.90 (br s, 1 H) 4.45 (m, 1 H) 6.13 (s, 1 H) 7.15 (s, 1 H) 7.40 (dd, J = 8.3, 4.1 Hz, 1 H) 7.73 (m, 2 H) 8.06 (d, J = 9.1 Hz, 1 H) 8.14 (dd, J = 8.3, 0.8 Hz, 1 H) 8.89 (dd, J = 4.1, 1.7 Hz, 1 H). HRMS Calcd for $C_{27}H_{32}N_5O_3$ (M + 1): 474.2492. Found: 474.2500.

7,8-Dimethoxy-3-[4-(2-phenoxyethyl)piperazin-1-ylmethyl]-3,3a,4,5-tetrahydroquinolino[4,3-*c*]isoxazole (29g). White foam: yield 55%; ^1H NMR (400 MHz, $CDCl_3$) δ ppm 2.63 (br s, 8 H) 2.84 (m, 4 H) 3.33 (m, 1 H) 3.56 (m, 2 H) 3.82 (s, 3 H) 3.84 (s, 3 H) 3.90 (br s, 1 H) 4.07 (t, J = 5.6 Hz, 2 H) 4.45 (m, 1 H) 6.14 (s, 1 H) 6.84 (m, 2 H) 6.96 (m, 2 H) 7.15 (s, 1 H). MS *m/z* 471 (MH^+). Anal. ($C_{25}H_{31}FN_4O_4$) C, H, N.

The following compound was prepared using the procedure described above for **21a**

7,8-Dimethoxy-5-methyl-3-[4-(2-methyl-3-phenyl-2(*E*)-propen-1-yl)piperazin-1-ylmethyl]-3,3a,4,5-tetrahydroquinolino[4,3-*c*]isoxazole (30). White foam: yield 48%; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.84 (s, 3 H) 2.38 (br s, 4 H) 2.51 (br s, 4 H) 2.70 (dd, J = 13.2, 6.2 Hz, 1 H) 2.78 (dd, J = 13.2, 4.8 Hz, 1 H) 2.89 (s, 3 H) 2.97 (s, 2 H) 3.18 (m, 1 H) 3.52 (m, 2 H) 3.67 (s, 3 H) 3.81 (s, 3 H) 4.37 (m, 1 H) 6.36 (s, 1 H) 6.41 (s, 1 H) 7.01 (s, 1 H) 7.21 (m, 1 H) 7.28 (d, J = 7.1 Hz, 2 H) 7.34 (m, 2 H). MS *m/z* 477 (MH^+). Anal. ($C_{28}H_{36}N_4O_3$) C, H, N.

General Procedure for the Preparation of Compounds 41 and 42a,b. *tert*-Butyl-(2-methylbenzylidene)amine (33). To a solution of 2-methylbenzaldehyde **31** (24.3 mL, 0.21 mmol) in toluene (250 mL) was added *tert*-butylamine (40 mL, 0.38 mmol), and the reaction mixture was heated to reflux for 24 h with azetropic distillation. After cooling the solution, the solvent was evaporated, and the residue was distilled (boiling point 70−73 °C at 0.6 mmHg), affording **33** (29. 3 g, 80%) as a colorless liquid: ^{1}H NMR (200 MHz, $CDCl_3$) δ ppm 1.30 (s, 9 H) 2.49 (s, 3 H) 7.20 (m, 3 H) 7.82 (dd, J = 7.6, 2.6 Hz, 1 H) 8.57 (s, 1 H). MS *m/z* 473 (MH^+).

4-{5-[2-(Hydroxyiminomethyl)phenyl]pent-2-enyl}-piperazine-1-carboxylic Acid *tert*-Butyl Ester (35). To a solution of *tert*-butyl-(2-methylbenzylidene)amine **33** (1.3 g, 7.5 mmol) and 2,2,6,6-tetramethylpiperidine (127 μL, 0.75 mmol) in anhydrous tetrahydrofuran (25 mL) was added a 2.5 M solution of *n*-butyllithium (3.6 mL, 9.0 mmol) dropwise at −10 °C and under nitrogen atmosphere. The reaction mixture was stirred for 30 min at −10 °C, and then it was cooled to −78 °C followed by the dropwise addition of a solution of compound **23** (2.2 g, 8.0 mmol) in anhydrous tetrahydrofuran (5 mL). The mixture was allowed to reach room temperature and was stirred overnight. After quenching with a 10% aqueous solution of NH_4Cl, the mixture was extracted with diethyl ether, the organic layer was separated, dried (Na_2SO_4), and filtered, and the solvent was evaporated, affording 3.5 g of the corresponding imine, which was used in the next step without further purification. To a solution of the freshly prepared imine (3.5 g, 7.5 mmol) in ethanol (25 mL), hydroxylamine hydrochloride (0.60 g, 8.3 mmol) and $NaHCO_3$ (0.95 g, 11.3 mmol) were added. The mixture was stirred for 24 h at room temperature, the solid was filtered off, and the solvent was evaporated giving a residue that was purified by column chromatography (ethyl acetate), to provide **35** (0.75 g, 27%) as a colorless oil: ^{1}H NMR (200 MHz, $CDCl_3$) δ ppm 1.45 (s, 9 H) 2.35 (m, 6 H) 2.82 (m, 2 H) 2.95 (d, J = 6.0 Hz, 2 H) 3.46 (m, 4 H) 5.55 (m, 2 H) 7.20 (m, 3 H) 7.7 (dd, J = 7.6, 2.7 Hz, 1 H). MS *m/z* 374 (MH^+).

4-(3,3a,4,5-Tetrahydronaphtho[4,3-*c*]isoxazol-3-ylmethyl)piperazine-1-carboxylic Acid *tert*-Butyl Ester (37). To a solution of 4-{5-[2-(hydroxyiminomethyl)-phenyl]-pent-2-enyl}-piperazine-1-carboxylic acid *tert*-butyl ester **35** (0.75 g, 2.0 mmol) in dichloromethane (5 mL) a 4% aqueous solution of sodium hypochlorite (8.5 mL, 5.0 mmol) was added portionwise, and the mixture was stirred for 4 h at room temperature. After that time, triethylamine (0.56 mL, 4.0 mmol) was added dropwise at 0 °C, and the reaction mixture was stirred overnight at room temperature. The organic layer was separated, washed with brine, dried over anhydrous Na_2SO_4, and filtered, and the solvent was evaporated. The residue was purified by column chromatography (2-propanone/dichloromethane 1/9), affording **37** (0.62 g, 84%) as a light brown foam: ^{1}H NMR (200 MHz, $CDCl_3$) δ ppm 1.45 (s, 9 H) 1.82 (m, 1 H) 2.22 (m, 1 H) 2.55 (m, 4 H) 2.96 (m, 4 H) 3.25 (td, J = 12.8, 5.0 Hz, 1 H) 3.48 (t, J = 10.0 Hz, 4 H) 4.41 (m, 1 H) 7.27 (m, 3 H) 7.92 (dd, J = 7.9, 1.2 Hz, 1 H). MS *m/z* 372 (MH^+).

3-Piperazin-1-ylmethyl-3,3a,4,5-tetrahydronaphtho-[4,3-*c*]isoxazole (39). To a solution of 4-(3,3a,4,5-tetrahydronaphtho[4,3-*c*]isoxazol-3-ylmethyl)piperazine-1-carboxylic acid *tert*-butyl ester **37** (0.62 g, 1.7 mmol) in dichloromethane (15 mL), cooled at 0 °C, was added trifluoroacetic acid (2.6 mL, 34 mmol). The reaction mixture was stirred for 4 h at room temperature, and then it was quenched with an aqueous saturated solution of $NaHCO_3$. The organic layer was separated, dried (Na_2SO_4), filtered, and concentrated to afford **39** (0.4 g) as an oil, which was used in the next step without further purification. MS *m/z* 272 (MH^+).

3-[4-(3-Phenyl-2(*E*)-propen-1-yl)piperazin-1-ylmethyl]-3,3a,4,5-tetrahydronaphtho[4,3-*c*]isoxazole (41). Freshly prepared 3-piperazin-1-ylmethyl-3,3a,4,5-tetrahydronaphtho-[4,3-c]isoxazole **39** (0.40 g, 1.5 mmol) was dissolved in chloroform (10 mL) and, to the resulting solution, were subsequently added solid $NaHCO_3$ (0.13 g, 1.5 mmol) and cinnamyl chloride (0.21 mL, 1.5 mmol). The reaction mixture was stirred for 48 h at room temperature. After quenching with water, the organic layer was separated, washed with brine, dried over anhydrous Na_2SO_4, and filtered, and the solvent was evaporated. The resulting residue was purified by column chromatography (2-propanone), affording a solid that was recrystallized from diisopropyl ether, to provide **41** (0.25 g, 37% two steps) as a white solid: mp 93.7 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.83 (m, 1 H) 2.22 (m, 1 H) 2.61 (br s, 6 H) 2.71 (br s, 2 H) 2.86 (m, 2 H) 2.96 (m, 2 H) 3.23 (m, 3 H) 4.42 (m, 1 H) 6.29 (dt, J = 16.0, 7.1 Hz, 1 H) 6.53 (d, J = 16.0 Hz, 1 H) 7.23 (m, 3 H) 7.31 (m, 3 H) 7.38 (m, 2 H) 7.93 (dd, J = 7.9, 1.2 Hz, 1 H). Anal. (C25H29N3O) C, H, N.

The following compounds were prepared analogously

7-Methoxy-3-[4-(3-phenyl-2(*E*)-propen-1-yl)piperazin-1-ylmethyl]-3,3a,4,5-tetrahydronaphtho[4,3-*c*]isoxazole (42a). White solid crystallized as dihydrochloride salt from 2-propanone: overall yield 8%; mp 258.3 °C (dec); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.68 (m, 1 H) 2.22 (m, 1 H) 2.89 (br s, 2 H) 3.25−3.60 (m, 9 H) 3.61 (d, J = 13.7 Hz, 2 H) 3.74 (s, 3 H) 3.94 (d, J = 7.1 Hz, 2 H) 4.49 (m, 1 H) 6.29 (m, 1 H) 6.87 (m, 3 H) 7.35 (m, 3 H) 7.50 (d, J = 7.3 Hz, 2 H) 7.67 (d, J = 9.3 Hz, 1 H). MS *m/z* 418 (MH^+). Anal. ($C_{26}H_{31}N_3O_2$·2HCl) C, H, N.

7-Methoxy-3-[4-(naphthalen-2-ylmethyl)piperazin-1-ylmethyl]-3,3a,4,5-tetrahydronaphtho[4,3-*c*]isoxazole (42b). White foam: overall yield 3%; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.68 (m, 1 H) 2.22 (m, 1 H) 2.89 (br s, 2 H) 3.43 (m, 9 H) 3.61 (d, J = 13.7 Hz, 2 H) 3.74 (s, 3 H) 3.94 (d, J = 7.1 Hz, 2 H) 4.49 (m, 1 H) 6.87 (m, 2 H) 7.44 (m, 3 H) 7.70 (s, 1 H) 7.67 (d, J = 9.3 Hz, 1 H) 7.77 (m, 3 H). HRMS Calcd for $C_{28}H_{32}N_3O_2$ (M + 1): 442.2495. Found: 442.2489.

7-Hydroxy-3-[4-(2-methyl-3-phenyl-2(*E*)-propen-1-yl)-piperazin-1-ylmethyl]-3a,4-dihydro-3*H*-[1]benzopyrano-[4,3-*c*]isoxazole (44). To a solution of 7-methoxy-3-[4-(2-methyl-3-phenyl-2(*E*)-propen-1-yl)piperazin-1-ylmethyl]-3a,4-dihydro-3*H*-[1]benzopyrano[4,3-*c*]isoxazole **43** (3.0 g, 6.9 mmol) in dichloromethane (80 mL) stirred at 0 °C, was added boron tribromide (6.5 mL, 6.9 mmol) dropwise. The reaction mixture was allowed to reach room temperature and stirred overnight. The mixture was poured into water and basified with a saturated aqueous solution of $NaHCO_3$, the organic layer was separated, dried over anhydrous Na_2SO_4, and filtered, and the solvent was evaporated. The residue was purified by column chromatography (dichloromethane/methanol 98/2 and 95/5), affording **44** (1.0 g, 36%) as a yellow solid, after crystallization from diisopropyl ether: mp 114.4 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.90 (br s, 3 H) 2.51 (br s, 4 H) 2.62 (br s, 2 H) 2.67 (br s, 2 H) 2.86 (m, 2 H) 3.03 (s, 2 H) 3.61 (td, J = 12.4, 5.8 Hz, 1 H) 4.03 (dd, J = 12.4, 10.4 Hz, 1 H) 4.39 (m, 1 H) 4.56 (dd, J = 10.4, 5.8 Hz, 1 H) 6.39 (d, J = 2.5 Hz, 1 H) 6.42 (s, 1 H) 6.48 (dd, J = 8.7, 2.5 Hz, 1 H) 7.20 (t, J = 7.1 Hz, 1 H) 7.29 (m, 4 H) 7.62 (d, J = 8.7 Hz, 1 H). HRMS Calcd for $C_{25}H_{30}N_3O_3$ (M + 1): 420.2287. Found: 420.2281.

General Procedure for the Preparation of Compounds 45a−d. 7-(2-Methoxyethoxy)-3-[4-(2-methyl-3-phenyl-2(*E*)-propen-1-yl)piperazin-1-ymethyl]-3a,4-dihydro-3*H*-[1]benzopyrano[4,3-*c*]isoxazole (45a). To a solution of 7-hydroxy-3-[4-(2-methyl-3-phenyl-2(*E*)-propen-1-yl)piperazin-1-ylmethyl]-3a,4-dihydro-3*H*-[1]benzopyrano[4,3-*c*]isoxazole **44** (0.5 g, 1.1 mmol), 2-methoxyethanol (0.14 mL, 1.8 mmol), and triphenylphosphine (1.2 g, 2.4 mmol) in tetrahydrofuran (10 mL) was added diethylazodicarboxylate (0.28 mL, 1.8 mmol) dropwise

under nitrogen atmosphere. After stirring the reaction mixture at room temperature overnight, it was filtered off, and the solids were washed with dichloromethane and methanol. The filtrate was evaporated till dryness, and the residue was purified by column chromatography (dichloromethane/ethyl acetate 2/1 and dichloromethane/methanol 97/3), affording **45a** (0.15 g, 28%) as a white solid: mp >300 °C (dec); ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.91 (s, 3 H) 2.49 (br s, 4 H) 2.64 (br s, 4 H) 2.82 (dd, J = 13.3, 5.5 Hz, 1 H) 2.88 (dd, J = 13.3, 6.0 Hz, 1 H) 3.02 (s, 2 H) 3.45 (s, 3 H) 3.65 (td, J = 12.4, 5.8 Hz, 1 H) 3.75 (m, 2 H) 4.08 (dd, J = 12.4, 10.4 Hz, 1 H) 4.11 (m, 2 H) 4.41 (m, 1 H) 4.61 (dd, J = 10.4, 5.8 Hz, 1 H) 6.43 (s, 1 H) 6.47 (d, J = 2.3 Hz, 1 H) 6.62 (dd, J = 8.7, 2.3 Hz, 1 H) 7.20 (t, J = 7.1 Hz, 1 H) 7.31 (m, 4 H) 7.68 (d, J = 8.7 Hz, 1 H). HRMS Calcd for $C_{28}H_{36}N_3O_4$ (M + 1): 478.2706. Found: 478.2725. Anal. ($C_{28}H_{35}N_3O_4$) C, H, N.

The following compounds were prepared analogously

7-[2-(2-Ethoxyethoxy)ethoxy]-3-[4-(2-methyl-3-phenyl-2(*E*)-propen-1-yl)piperazin-1-ylmethyl]-3a,4-dihydro-3*H*-[1]benzopyrano[4,3-*c*]isoxazole (45b). White foam: yield 32%; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.09 (t, J = 7.1 Hz, 3 H) 2.06 (s, 3 H) 3.43 (q, J = 7.1 Hz, 2 H) 3.53 (m, 14 H) 3.73 (m, 2 H) 3.86 (td, J = 12.4, 5.8 Hz, 1 H) 3.92 (s, 2 H) 4.12 (m, 2 H) 4.16 (dd, J = 12.4, 10.4 Hz, 1 H) 4.77 (m, 2 H) 6.59 (d, J = 2.5 Hz, 1 H) 6.67 (dd, J = 8.7, 2.5 Hz, 1 H) 6.82 (s, 1 H) 7.31 (t, J = 7.1 Hz, 1 H) 7.39 (m, 4 H) 7.57 (d, J = 8.7 Hz, 1 H). Anal. ($C_{31}H_{41}N_3O_5$) C, H, N.

7-Cyclopentyloxy-3-[4-(2-methyl-3-phenyl-2(*E*)-propen-1-yl)piperazin-1-ylmethyl]-3a,4-dihydro-3*H*-[1]benzopyrano[4,3-*c*]isoxazole (45c). White foam: yield 19%; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.62 (m, 2 H) 1.79 (m, 2 H) 1.86 (m, 2 H) 1.90 (d, J = 1.2 Hz, 2 H) 1.91 (m, 2 H) 2.47 (br s, 4 H) 2.63 (br s, 4 H) 2.81 (dd, J = 13.3, 5.4 Hz, 1 H) 2.88 (dd, J = 13.3, 6.2 Hz, 1 H) 3.01 (s, 2 H) 3.64 (td, J = 12.4, 5.8 Hz, 1 H) 4.08 (dd, J = 12.4, 10.4 Hz, 1 H) 4.40 (m, 1 H) 4.60 (dd, J = 10.2, 5.8 Hz, 1 H) 4.74 (m, 1 H) 6.41 (d, J = 2.5 Hz, 1 H) 6.42 (s, 1 H) 6.54 (dd, J = 8.7, 2.5 Hz, 1 H) 7.20 (t, J = 7.2 Hz, 1 H) 7.27 (m, 2 H) 7.33 (m, 2 H) 7.66 (d, J = 8.7 Hz, 1 H). MS *m/z* 488 (MH^+). Anal. ($C_{30}H_{37}N_3O_5$) C, H, N.

7-(2-Dimethylaminoethoxy)-3-[4-(2-methyl-3-phenyl-2(*E*)-propen-1-yl)piperazin-1-ylmethyl]-3a,4-dihydro-3*H*-[1]benzopyrano[4,3-*c*]isoxazole (45d). Light yellow solid from diisopropyl ether: yield 24%; mp 174.9 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.91 (s, 3 H) 2.35 (s, 6 H) 2.48 (br s, 4 H) 2.63 (br s, 4 H) 2.75 (t, J = 5.6 Hz, 2 H) 2.82 (dd, J = 13.2, 5.5 Hz, 1 H) 2.88 (dd, J = 13.2, 6.0 Hz, 1 H) 3.01 (s, 2 H) 3.65 (td, J = 12.4, 5.9 Hz, 1 H) 4.07 (m, 3 H) 4.40 (m, 1 H) 4.61 (dd, J = 10.3, 5.9 Hz, 1 H) 6.42 (s, 1 H) 6.46 (d, J = 2.5 Hz, 1 H) 6.60 (dd, J = 8.7, 2.5 Hz, 1 H) 7.20 (t, J = 7.2 Hz, 1 H) 7.31 (m, 4 H) 7.68 (d, J = 8.7 Hz, 1 H). HRMS Calcd for $C_{29}H_{39}N_4O_3$: 491.3022. Found: 491.3003. Anal. ($C_{29}H_{38}N_4O_3$) C, H, N.

General Procedure for the Preparation of Compounds 45e−k. Acetic Acid 3-[4-(2-Methyl-3-phenyl-2(*E*)-propen-1-yl)piperazin-1-ylmethyl]-3a,4-dihydro-3*H*-[1]benzopyrano[4,3-*c*]isoxazol-7-yl Ester (45e). To a solution of 7-hydroxy-3-[4-(2-methyl-3-phenyl-2(*E*)-propen-1-yl)piperazin-1-ylmethyl]-3a,4-dihydro-3*H*-[1]benzopyrano[4,3-*c*]isoxazole **44** (0.1 g, 0.24 mmol) and triethylamine (0.05 mL, 0.36 mmol) in dichloromethane (4 mL) stirred at 0 °C, was added acetyl chloride (0.02 mL, 0.28 mmol) dropwise. After stirring for 3 h at room temperature, a saturated aqueous $NaHCO_3$ solution was added, the organic layer was separated, dried (Na_2SO_4), and filtered, and the solvent was evaporated. The residue was purified by column chromatography (dichloromethane/methanol 98/2), affording **45e** (0.07 g, 63%) as a yellow solid after recrystallization from diisopropyl ether: mp 125.5 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.90 (s, 3 H) 2.30 (s, 3 H) 2.46 (br s, 4 H) 2.62 (br s, 4 H) 2.82 (dd, J = 13.1, 5.6 Hz, 1 H) 2.89 (dd, J = 13.1, 6.0 Hz, 1 H) 3.01 (s, 2 H) 3.68 (td, J = 12.4, 5.9 Hz, 1 H) 4.10 (dd, J = 12.4, 10.4 Hz, 1 H) 4.45 (m, 1 H) 4.64 (dd, J = 10.4, 5.9 Hz, 1 H) 6.42 (s, 1 H) 6.73 (d, J = 2.3 Hz, 1 H) 6.75 (dd, J = 8.3, 2.3 Hz, 1 H) 7.20 (t, J = 7.2 Hz, 1 H) 7.31 (m, 4 H) 7.79 (d, J = 8.3 Hz, 1 H). HRMS Calcd for $C_{27}H_{32}N_3O_4$ (M + 1): 462.2393. Found: 462.2376.

The following compounds were prepared analogously.

Propionic Acid 3-[4-(2-Methyl-3-phenyl-2(*E*)-propen-1-yl)piperazin-1-ylmethyl]-3a,4-dihydro-3*H*-[1]benzopyrano[4,3-*c*]isoxazol-7-yl Ester (45f). Light yellow foam: yield 23%; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.26 (t, J = 7.5 Hz, 3 H) 1.91 (d, J = 1.2 Hz, 3 H) 2.49 (br s, 4 H) 2.59 (q, J = 7.5 Hz, 2 H) 2.64 (br s, 4 H) 2.83 (dd, J = 13.3, 5.8 Hz, 1 H) 2.89 (dd, J = 13.3, 6.2 Hz, 1 H) 3.02 (s, 2 H) 3.68 (td, J = 12.4, 5.8 Hz, 1 H) 4.10 (dd, J = 12.4, 10.4 Hz, 1 H) 4.45 (m, 1 H) 4.64 (dd, J = 10.4, 5.8 Hz, 1 H) 6.43 (s, 1 H) 6.73 (d, J = 2.5 Hz, 1 H) 6.74 (dd, J = 8.3, 2.5 Hz, 1 H) 7.20 (t, J = 7.3 Hz, 1 H) 7.30 (m, 4 H) 7.78 (d, J = 8.3 Hz, 1 H). MS *m/z* 476 (MH^+). Anal. ($C_{28}H_{33}N_3O_4$) C, H, N.

Methoxyacetic Acid 3-[4-(2-Methyl-3-phenyl-2(E)-propen-1-yl)piperazin-1-ylmethyl]-3a,4-dihydro-3H-[1]benzopyrano[4,3-*c*]isoxazol-7-yl Ester (45g). White solid from diisopropyl ether: yield 41%; mp 140.5 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.90 (d, J = 1.2 Hz, 3 H) 2.47 (br s, 4 H) 2.61 (br s, 4 H) 2.82 (dd, J = 13.3, 5.6 Hz, 1 H) 2.89 (dd, J = 13.3, 6.0 Hz, 1 H) 3.01 (s, 2 H) 3.54 (s, 3 H) 3.69 (td, J = 12.4, 5.8 Hz, 1 H) 4.11 (dd, J = 12.4, 10.5 Hz, 1 H) 4.28 (s, 2 H) 4.45 (m, 1 H) 4.65 (dd, J = 10.4, 5.8 Hz, 1 H) 6.42 (s, 1 H) 6.77 (d, J = 2.3 Hz, 1 H) 6.78 (dd, J = 7.3, 2.3 Hz, 2 H) 7.20 (t, J = 7.3 Hz, 1 H) 7.31 (m, 4 H) 7.80 (d, J = 7.3 Hz, 1 H). MS *m/z* 492 (MH^+). Anal. ($C_{28}H_{33}N_3O_5$) H, N; C: calcd, 68.41; found, 67.98

Cyclopropanecarboxylic Acid 3-[4-(2-Methyl-3-phenyl-2(*E*)-propen-1-yl)piperazin-1-ylmethyl]-3a,4-dihydro-3*H*-[1]benzopyrano[4,3-*c*]isoxazol-7-yl Ester (45h). White foam: yield 46%; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.04 (m, 2 H) 1.16 (m, 2 H) 1.83 (m, 1 H) 1.90 (s, 3 H) 2.48 (br s, 4 H) 2.62 (br s, 4 H) 2.82 (dd, J = 13.3, 5.6 Hz, 1 H) 2.89 (dd, J = 13.3, 6.0 Hz, 1 H) 3.01 (s, 2 H) 3.67 (td, J = 12.4, 5.9 Hz, 1 H) 4.09 (dd, J = 12.4, 10.4 Hz, 1 H) 4.44 (m, 1 H) 4.63 (dd, J = 10.4, 5.9 Hz, 1 H) 6.42 (s, 1 H) 6.73 (d, J = 2.1 Hz, 1 H) 6.75 (dd, J = 8.5, 2.1 Hz, 1 H) 7.20 (t, J = 7.1 Hz, 1 H) 7.30 (m, 4 H) 7.77 (d, J = 8.5 Hz, 1 H). MS *m/z* 488 (MH^+). Anal. ($C_{29}H_{33}N_3O_4$) H, N; C: calcd, 71.44; found, 71.02.

Acrylic Acid 3-[4-(2-Methyl-3-phenyl-2(*E*)-propen-1-yl)-piperazin-1-ylmethyl]-3a,4-dihydro-3*H*-[1]benzopyrano-[4,3-*c*]isoxazol-7-yl Ester (45i). Light brown foam: yield 30%; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.90 (d, J = 1.2 Hz, 3 H) 2.48 (br s, 4 H) 2.62 (br s, 4 H) 2.82 (dd, J = 13.3, 5.7 Hz, 1 H) 2.90 (dd, J = 13.3, 6.0 Hz, 1 H) 3.01 (s, 2 H) 3.69 (td, J = 12.4, 5.8 Hz, 1 H) 4.11 (dd, J = 12.4, 10.4 Hz, 1 H) 4.46 (m, 1 H) 4.65 (dd, J = 10.4, 5.8 Hz, 1 H) 6.04 (dd, J = 10.4, 1.1 Hz, 1 H) 6.31 (dd, J = 17.3, 10.4 Hz, 1 H) 6.42 (s, 1 H) 6.62 (dd, J = 17.3, 1.1 Hz, 1 H) 6.78 (s, 1 H) 6.80 (dd, J = 7.9, 2.3 Hz, 1 H) 7.20 (t, J = 7.2 Hz, 1 H) 7.31 (m, 4 H) 7.80 (dd, J = 7.9, 0.8 Hz, 1 H). MS *m/z* 474 (MH^+). Anal. ($C_{28}H_{31}N_3O_4$) C, H, N.

2,2-Dimethyl-propionic Acid 3-[4-(2-Methyl-3-phenyl-2(*E*)-propen-1-yl)piperazin-1-ylmethyl]-3a,4-dihydro-3*H*-[1]benzopyrano[4,3-*c*]isoxazol-7-yl Ester (45j). White foam: yield 67%; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.35 (s, 9 H) 1.90 (d, J = 1.0 Hz, 3 H) 2.48 (br s, 4 H) 2.62 (br s, 4 H) 2.82 (dd, J = 13.3, 5.6 Hz, 1 H) 2.89 (dd, J = 13.3, 6.0 Hz, 1 H) 3.01 (s, 2 H) 3.68 (td, J = 12.4, 5.9 Hz, 1 H) 4.10 (dd, J = 12.4, 10.4 Hz, 1 H) 4.45 (m, 1 H) 4.64 (dd, J = 10.4, 5.8 Hz, 1 H) 6.42 (s, 1 H) 6.69 (d, J = 2.3 Hz, 1 H) 6.71 (dd, J = 8.5, 2.3 Hz, 1 H) 7.20 (t, J = 7.2 Hz, 1 H) 7.31 (m, 4 H) 7.78 (d, J = 8.5 Hz, 1 H). MS *m/z* 504 (MH^+). Anal. ($C_{30}H_{37}N_3O_4$) C, H, N.

Isonicotinic Acid 3-[4-(2-Methyl-3-phenyl-2(*E*)-propen-1-yl)piperazin-1-ylmethyl]-3a,4-dihydro-3*H*-[1]benzopyrano[4,3-*c*]isoxazol-7-yl Ester (45k). White foam: yield 24%; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.91 (d, J = 1.0 Hz, 3 H) 2.48 (br s, 4 H) 2.63 (br s, 4 H) 2.84 (dd, J = 13.3, 5.7 Hz, 1 H) 2.91 (dd, J = 13.3, 6.2 Hz, 1 H) 3.02 (s, 2 H) 3.72 (td, J = 12.4, 5.9 Hz, 1 H) 4.14 (dd, J = 12.4, 10.4 Hz, 1 H) 4.48 (m, 1 H) 4.68 (dd, J = 10.4, 5.9 Hz, 1 H) 6.43 (s, 1 H) 6.88 (s, 1 H) 6.89 (dd, J = 7.9, 2.3 Hz, 1 H) 7.21 (t, J = 7.2 Hz, 1 H) 7.31 (m, 4 H) 7.86 (dd, J = 7.9, 1.2 Hz, 1 H) 7.99 (dd, J = 6.0, 1.7 Hz, 2 H) 8.88 (dd, J = 6.0, 1.5 Hz, 2 H). MS *m/z* 525 (MH^+). Anal. ($C_{31}H_{32}N_4O_4$) C, H, N.

Receptor Binding Assays. Frozen membranes of CHO cells, stably transfected with either human adrenergic 2A, 2B, or 2C receptors, were thawed on ice, briefly homogenized with an Ultra Turrax homogenizer, and then suspended in glycylglycine buffer (25 mM pH 7.6) at an appropriate predetermined protein concentration (5−10 μg protein per incubation mixture). The reaction was started by adding the membrane suspension to the reaction tube that contained the compound of interest together with [^{3}H]rauwolscine (1 nM) in a total volume of 500 μL. The mixture was incubated for 30 min at 25 °C. Nonspecific binding was determined in the presence of oxymetazoline (1 μM) for the 2A subtype and spiroxatrine (1 μM) for 2B and 2C subtypes. Free radioligand was separated from the radioligand−receptor complex by means of rapid vacuum filtration over GF/B unifilter plates with a Packard Harvester Filtration Unit. Filter plates were washed with ice-cold Tris-HCl buffer (50 mM, pH 8.0) and dried overnight. Bound counts were measured in a Topcount Scintillation Counter in the presence of Microscint O. For 5-HTT binding, frozen membranes of human platelets (Novascreen) were thawed on ice, briefly homogenized, and resuspended in Tris-HCl buffer (50 mM, pH 7.4) supplemented with NaCl (120 mM) and KCl (5 mM) at a concentration of 50 μg protein per incubation mixture. The membrane suspension was added to the compound of interest together with [^{3}H]paroxetine (0.5 nM) in a total volume of 250 μL and incubated (60 min, 25 °C). Nonspecific binding was determined in the presence of imipramine (1 μM). Filtration was done over presoaked GF/B unifilters (0.1%PEI) and washed as above with the Tris salt buffer used for the incubation. Specific binding was calculated, and sigmoidal curves were plotted by an internally developed software program based on S-plus software. K_i values were calculated using the Cheng−Prusoff equation.

The affinity of the compounds for the remaining target receptors and transporters was also determined by means of several radioligand competition binding experiments. In general, the compound of interest (or control blank) together with the appropriate tritiated or iodinated radioligand and a membrane suspension with abundant target receptor/transporter was incubated under optimized experimental conditions. The reaction was stopped by filtration as above, except for α1A and D3, where a SPA-based assay was used (scintillation proximity assay). All assays were carried out with membranes from cell lines transfected with the human target, except for DAT and NET, which were done with the rat striatum and the rat cortex, respectively.

In Vivo Pharmacology. Animals. Male Wistar rats (Charles River Breeding Facilities) were used. They were housed in individual cages in air-conditioned laboratories (21 ± 2 °C; 65 ± 15% relative humidity). They were fasted overnight, but tap water remained available ad libitum except during the test period. **Test Compounds**. Test compounds were prepared as solutions in distilled water or 10% hydroxypropyl-β-cyclodextrin after acidification with tartaric acid if necessary. They were stored at room temperature in closed containers protected from light. The solutions were subcutaneously (sc) or orally (po) administered in a volume of 10 mL/kg. **General Procedure and Statistics**. All experiments were performed by unbiased trained technicians using coded solutions. Doses were selected from the geometrical series 0.00063−0.00125−0.0025...10−20−40 mg/kg. Animals were tested in separate daily experimental sessions in order to account for day-to-day variability and to minimize systematic errors. Control injections of solvent were included in each experimental session. All-or-none criteria for significant ($p < 0.05$) effects were defined by analyzing a frequency distribution of a large series of historical control data. On the basis of the thus obtained criteria, ED_{50} values and corresponding 95% confidence limits were determined according to the modified Spearman−Kaerber estimate using theoretical probabilities instead of empirical ones. This modification allows tabulation of the ED_{50} and its confidence interval as a function of the slope of the log dose−response curve.[38] **Tests: Medetomidine-Induced Loss of Righting in Rats.** Medetomidine (0.10 mg/kg, iv)-induced loss of righting was recorded in overnight-starved rats (200−250 g), pretreated with test compound or solvent. Criterion for drug-induced reversal: absence of loss of righting (1.0% false positive controls; $n > 400$). ***p*-Chloroamphetamine (pCA)-induced behavior in rats.** *p*-Chloroamphetamine (pCA; 5 mg/10 mL/kg, sc)-induced excitation was scored (0, 1, 2, or 3) over a 15-min interval starting 45 min after the pCA injection in male rats (200−250 g) pretreated with test compound or solvent. The following all-or-nothing criteria were selected to assess drug-induced inhibition: score for excitation < 2 (0.5% false positives; $n > 200$).

pCA-Induced 5-HT Depletion Assay. In the present experiments we investigated the central 5-HTT blockade activity of test compounds in male Wiga Wistar rats by evaluating their ability to prevent pCA-induced 5-HT depletion when preadministered. pCA is taken up into serotonergic nerve terminals by the 5-HT transporter, after which it is further accumulated in the synaptic vesicles. During the latter step it replaces the 5-HT stored in the vesicles which is subsequently degraded, resulting in depletion of tissue 5-HT stores. Blockade of the neuronal 5-HT transporter prevents the uptake of pCA into the serotonergic terminals and thus the 5-HT depletion induced by pCA administration. **Materials and Methods.** To identify and characterize the in vivo central 5-HTT blockade activity, the test compound was administered subcutaneous (sc) or orally (po) (10 mL/kg) to healthy male Wiga Wistar rats (body weight 200 ± 20 g, $n = 6$ per treatment condition). Thirty five minutes later, pCA was administered to the animals (5 mg/kg, 1 mL/kg, i.p.). Four hours following pCA administration, animals were decapitated, the frontal cortex was dissected from the brain, and the left and right hemispheres were separated for storage. The tissue was snap frozen in liquid nitrogen and stored at −80 °C until extraction. After a perchloric acid/sodium metabisulfite extraction, an HPLC system (LC-14AD*VP*, Shimadzu) coupled to an electrochemical detector (Procédé, Shimadzu) was used to quantify 5-HT levels (pmol/mL) in the supernatant. 5-HT levels were quantified by comparison to a standard curve (six concentrations ranging between 25 nM and 400 nM) after normalization for injection volume and extraction efficiency, using the internal standard dihydroxybenzylamine (200 nM). Protein content was measured in an aliquot of the extract, using a colorimetric assay based on the Lowry method. The 5-HT levels were expressed per amount of protein in the sample (mg/mL). Analysis was performed by Statserver 2000. The ED_{50} is the dose at which the compound has 50% of its maximal inhibitory effect on pCA-induced 5-HT depletion in the prefrontal cortex. The absolute 5-HT concentrations are expressed as % effect. 0% effect is defined as the 5-HT levels after pCA + vehicle treatment, and 100% effect is defined as the 5-HT levels after vehicle + vehicle. Both control levels were determined in each experiment. When different doses were tested in more than 1 experiment % effect of test compound was determined based on the control levels in the same experiment. A sigmoidal dose−response curve was fitted to the % effect levels at the log-transformed tested doses and the ED_{50} was derived from the fitted curve.

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Supporting Information Available: Table of purity determination for HRMS, HPLC, ^{1}H NMR, and combustion analyses. This material is available free of charge via the Internet at http://pubs.acs.org.

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