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Bioorganic & Medicinal Chemistry 14 (2006) 4361–4372
Bioorganic & Medicinal Chemistry

# Synthesis of 7-amino-3a,4-dihydro-3*H*-[1]benzopyrano [4,3-*c*]isoxazole derivatives displaying combined $\alpha_2$-adrenoceptor antagonistic and 5-HT reuptake inhibiting activities

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Received 30 September 2005; revised 19 February 2006; accepted 24 February 2006
Available online 15 March 2006

**Abstract**—Following a program searching for dual 5-HT reuptake inhibitors and $\alpha_2$-adrenoceptor antagonists started at Johnson & Johnson Pharmaceutical Research & Development, we now report on the synthesis of a series of 7-amino-3a,4-dihydro-3*H*-[1]benzopyrano[4,3-*c*]isoxazole derivatives, some of which proved to be the most potent $\alpha_2$-adrenoceptor blockers within this chemical class of tricyclic isoxazolines, while keeping potent 5-HT reuptake inhibiting activity.


## 1. Introduction

Depression is a severe mental disorder characterized by exaggerated and pervasive feelings of sadness, loss of interest, and decreased energy that affect up to 10% of the population. This disorder is linked with a significant mortality and its lifetime prevalence is 19%.[1,2]

The monoaminergic hypothesis for depression assumes that it is mainly caused by a deficit of monoamines, serotonin (5-HT) and norepinephrine (NE), in corticolimbic synaptic clefts[3] and this hypothesis has been used to explain the efficacy of existing antidepressant therapies. Among these available therapies, selective serotonin reuptake inhibitors (SSRIs) have become the standard treatment for depression. However, there are some limitations associated with the use of SSRIs including a delayed onset of action (2–4 weeks), partial treatment response (60–70%), excitation during early treatment response, nausea, and sexual dysfunction.[4–7]

As we have explained in our previous papers,[8–10] combining 5-HT reuptake inhibition with $\alpha_2$-adrenoceptor blockade is expected to be more effective than 5-HT reuptake inhibition alone, by enhancing monoaminergic transmission in the brain. Recent studies suggest that the addition of the $\alpha_2$-antagonist yohimbine to the SSRI fluoxetine hastens the antidepressant response and, furthermore, there is also a trend suggesting an increased percentage of responders to the combined treatment at the end of a 6-week trial.[11] As the pharmacological effect of this approach has not been fully analyzed yet, the optimal balance of both activities remains unknown. In our previous papers, we have described the discovery of a new series of tricyclic isoxazolines displaying this dual activity.[8–10] Amongst the most interesting compounds within that series the methylcinnamyl derivatives **1** and **2** were identified. We now report on the synthesis and primary pharmacological activity of a series of 7-amino-substituted derivatives, of generic formula **I**, within this chemical class (Fig. 1).

## 2. Chemistry

In order to synthesize the 7-amino-3a,4-dihydro-3*H*-[1]benzopyrano[4,3-*c*]isoxazole derivatives, the scaffolds

*Keywords*: Isoxazolines; 5-HT reuptake inhibitors; $\alpha_2$-Antagonists; Antidepressants.
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doi:10.1016/j.bmc.2006.02.043

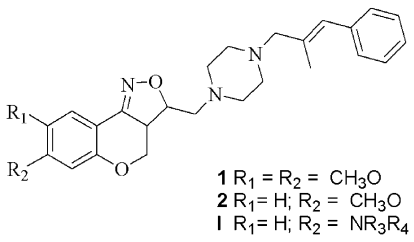



**Figure 1.**

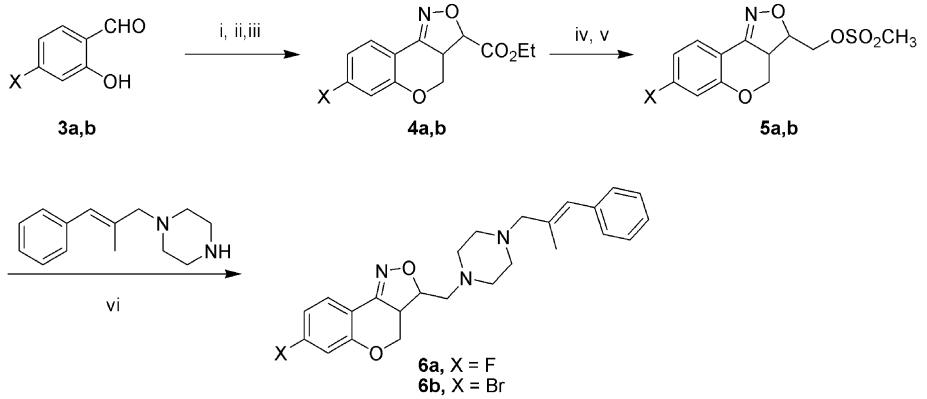
**6a**,**b** bearing fluorine or bromine atoms suitable for further derivation were prepared (Scheme 1). The ester intermediates **4a**,**b** were obtained following essentially the method that we previously described for the synthesis of similar analogues.[10] Thus, alkylation of **3a**,**b**[12] with ethyl 4-bromocrotonate followed by reaction with hydroxylamine gave the corresponding oximes, from which nitrile oxides were prepared using sodium hypochlorite. Subsequent ring closure by intramolecular 1,3-dipolar cycloaddition, in the presence of triethylamine, afforded cycloadducts **4a**,**b**. The stereochemistry of positions 3 and 3a of the tricyclic system was pre-determined by the *trans*-alkene fragment and was unequivocally assigned by NMR. This assignment was based on their respective $^1$H-coupling constants in compounds **4a**,**b** (see Section 5) as well as on the NOE observed between the proton at position 3a and the methylene group of the side chain at position 3, in compounds **5a** and **5b**. Reduction of the ester with sodium borohydride in a mixture of tetrahydrofuran and water yielded the corresponding hydroxymethyl derivatives, which were converted into the mesylates **5a**,**b** by standard procedures. Reaction of these mesylates with excess *N*-(2-methyl-3-phenyl-2(*E*)-propen-1-yl)piperazine afforded the key intermediates **6a**,**b**.

The synthesis of the 7-nitrogenated derivatives **7–10** was accomplished from compounds **6a**,**b** (Scheme 2). Aromatic nucleophilic substitution of the fluorine atom in compound **6a**, by reaction with secondary amines in pyridine or with neat primary amines, gave compounds **7a–f** in moderate yields. Potassium fluoride was used in order to enhance the lower nucleophilic character of primary amines. Preparation of **8** was carried out in two steps for the parallel synthesis of amides and ureas at position 7. Palladium-catalyzed amination of **6b** with *tert*-butyl carbamate in the presence of palladium(II) acetate/Xantphos as catalytic complex, using 1,4-dioxane as solvent and cesium carbonate as base,[13] followed by deprotection of the *tert*-butoxycarbonyl group using a mixture of trifluoroacetic acid in dichloromethane, afforded **8** in 91% overall yield. Acylation of this compound with different acyl chlorides or reaction with the corresponding isocyanates, under standard reaction conditions, yielded the desired final products **9a–m**. Finally, due to the low nucleophilic character of aromatic amines, palladium-catalyzed coupling reactions were also used for the introduction of such moieties. In this case, parallel synthesis of compounds **10a–f** was carried out with palladium(II) acetate/BINAP as catalyst and toluene as solvent.

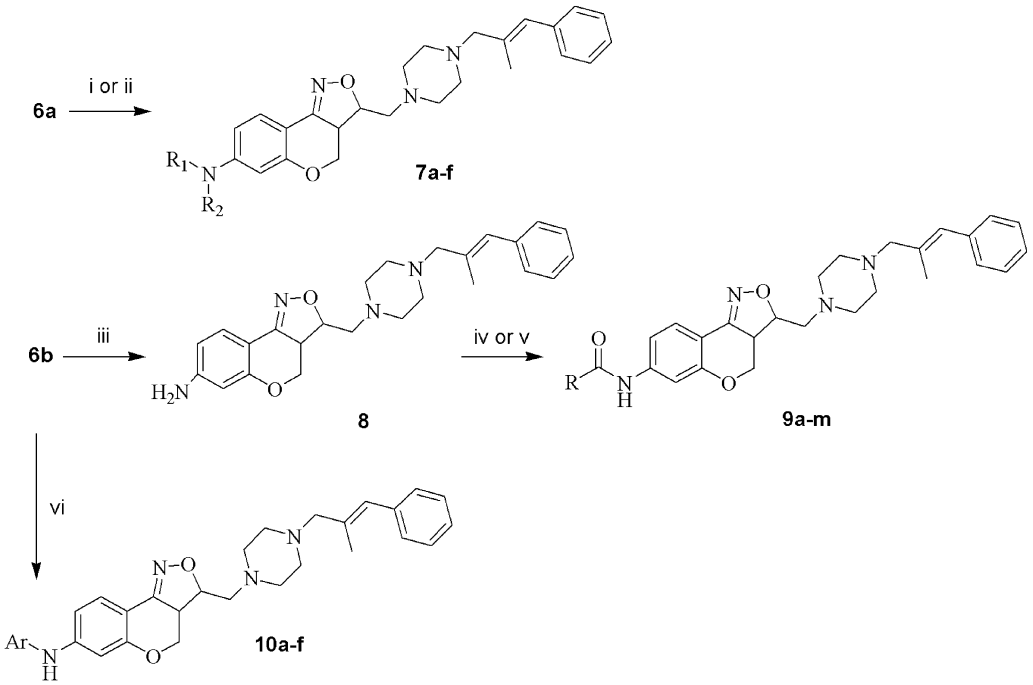
Following the procedures described above, different nitrogenated functions were introduced at position 7 of the 3a,4-dihydro-3*H*-[1]benzopyrano[4,3-*c*]isoxazole scaffold: alkyl and arylamines, alkyl and arylamides, and alkyl and arylureas.

## 3. Results and discussion

Results of the primary pharmacological evaluation of selected compounds are reported in Table 1. In vitro binding affinities at $\alpha_{2A}$- and $\alpha_{2C}$-adrenoceptors, and at 5-HT transporter (5-HTT) site were considered as the primary screening assays. Their affinities for other serotonergic, adrenergic, and dopaminergic receptors, as well as at the dopaminergic and noradrenergic uptake sites, were evaluated as well. None of those compounds showed relevant affinity for any other of those receptors or transporter sites. Antagonism of medetomidine-induced loss of righting and antagonism of *p*-chloroamphetamine (pCA)-induced excitation, in rats, were used as primary assays for their in vivo evaluation as $\alpha_2$-adrenoceptor blockers and 5-HT reuptake inhibitors, respectively.[10]




**Scheme 1.** Reagents and conditions: (i) Ethyl 4-bromocrotonate, $K_2CO_3$, DMF, 0 °C to rt, 5 h; (ii) $NH_2OH{\cdot}HCl$, AcONa, EtOH, 0 °C, 2 h; (iii) (a) NaClO, $CH_2Cl_2$, 0 °C to rt, 2 h; (b) $Et_3N$, rt, 24 h; (iv) $NaBH_4$, THF, $H_2O$, 0 °C to rt, 24 h; (v) $CH_3SO_2Cl$, $Et_3N$, $CH_2Cl_2$, 0 °C, 1 h; (vi) KI, $K_2CO_3$, MIK, reflux, 24 h.




**Scheme 2.** Reagents and conditions: (i) $HNR_1R_2$, pyridine, 150 °C, 24–48 h; (ii) $H_2NR_1$, KF, 150 °C, 4–5 days; (iii) (a) $H_2N$-BOC, $Pd(OAc)_2$, Xantphos, $Cs_2CO_3$, 1,4-dioxane, 100 °C, 24 h; (b) TFA, $CH_2Cl_2$, rt, 5 h; (iv) RCOCl, $Et_3N$, $CHCl_3$, 0 °C to rt, 24 h; (v) RNCO (R = Ar), THF, rt, 24 h or RNCO (R = alk.), toluene, 75 °C, 24 h; (vi) $ArNH_2$, $Pd(OAc)_2$, BINAP, $Cs_2CO_3$, toluene, 100 °C, 24 h.

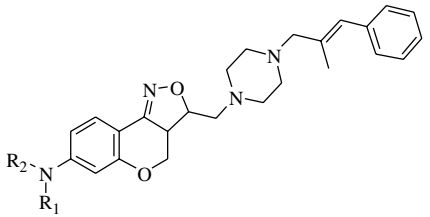
Most of the compounds showed nanomolar or subnanomolar affinities for the $\alpha_2$-adrenoceptors and nanomolar affinity for the 5-HTT site. Compound **7a** and its direct *O*-analogue previously described **2**[8] showed similar in vitro potency. The *N*-ethyl analogue **7b** presented comparable affinity but the two benzyl derivatives **7c** and **7d** proved to decrease binding potency. The presence of a heteroatom, either oxygen or nitrogen, in the alkyl chain afforded very potent derivatives such as **7e** and **7f**. We observed these same features in the 7-*O*-alkyl series described in our previous article.[10] As a matter of fact compound **7f** was the most potent dual-acting compound in vitro identified within this series. The unsubstituted amino analogue **8** was 2-fold less potent than **7a** at the 5-HTT site, although the affinity for $\alpha_2$ receptors of both derivatives was comparable. *N*-Acyl as well as *N*-carbamoyl derivatives showed also quite potent binding affinities for the three targets. Thus, *N*-acyl compounds **9a–f** showed subnanomolar potency at $\alpha_2$-adrenoceptors and affinities at 5-HTT ranging from 3.5 to 19 nM. Interestingly, the aliphatic or aromatic nature of the substituents did not have much influence on potency. Alkylurea derivatives **9g–j** maintained high binding affinities, but arylureas such as **9k** and **9l** showed a dramatic drop in activity at the 5-HTT site. Quite surprisingly, introduction of two methoxy groups in the aromatic ring resulted in high potency at the 5-HTT site again, as can be seen from the in vitro results of compound **9m**. Finally, arylamino derivatives **10a–f** showed lower affinities at both $\alpha_2$-adrenoceptors as well as at the 5-HTT site. The only exception was the 3-cyano derivative **10d** that also showed activity in the nanomolar range.

Although most of the synthesized compounds showed nanomolar or subnanomolar binding values, there was not a clear correlation with their respective in vivo activities. The *N*-methyl derivative **7a** was significantly potent in the medetomidine-induced loss of righting as well as in the pCA-induced excitation assays, at lower doses than its O-substituted analogue **2**. The *N*-ethyl derivative **7b** maintained the in vivo potency as $\alpha_2$ blocker but its activity as 5-HT reuptake inhibitor decreased, although the in vitro affinities for the 5-HTT of both compounds were similar. The in vivo activity of the benzyl derivative **7c** also dropped very significantly.

The introduction of a terminal hydroxy function in the alkyl chain, such as in **7e**, showed a good correlation between in vivo activity and receptor binding values. This derivative was the most potent compound in the medetomidine assay and one of the most potent compounds in the pCA assay. The replacement of the hydroxy function by a dimethylamino group, exemplified by compound **7f**, showed also an interesting combination of activities, affording one of the most potent derivatives in the medetomidine test and the most potent one in the pCA test together with **7a**. Interestingly, although **7e** and **7f** showed comparable binding values for both subtypes of $\alpha_2$ receptors, **7f** was 15-fold less active than **7e** in the medetomidine test.

The aniline derivative **8** showed also in vivo activity as an $\alpha_2$ antagonist but its activity as a 5-HT reuptake inhibitor decreased dramatically, what was in correlation with its lower affinity for the 5-HTT site. Acylation of the nitrogen with small acyl groups, such as in **9a–d**, afforded derivatives showing comparable activities to **8**

**Table 1.** In vitro binding affinities of 7-amino-3a,4-dihydro-3*H*-[1]benzopyrano[4,3-*c*]isoxazole derivatives and their in vivo activities for antagonism of medetomidine-induced loss of righting and pCA-induced excitation



| Compound | $R_1$ | $R_2$ | $\alpha_{2A}$[a] $K_i$ (nM) | $\alpha_{2C}$[a] $K_i$ (nM) | 5-HTT[a] $K_i$ (nM) | Medetomidine (sc)[b] $ED_{50}$ (mg/kg) | pCA (sc)[b] $ED_{50}$ (mg/kg) |
|---|---|---|---|---|---|---|---|
| **1** | — | — | 0.8 | 0.2 | 2.3 | 1.0 | 1.5 |
| **2** | — | — | 0.5 | 0.2 | 19 | 2.5 | >2.5 |
| **7a** | H | $CH_3$ | 0.66 | 0.27 | 17 | 0.32 (0.16–0.62) | 1.3 (0.64–2.5) |
| **7b** | H | $CH_3CH_2$ | 1.4 | 0.11 | 14 | 0.32 (0.16–0.62) | >2.5 |
| **7c** | H | $C_6H_5CH_2$ | 3.4 | 0.68 | 16 | 2.5 (0.96–6.5) | >2.5 |
| **7d** | $CH_3$ | $C_6H_5CH_2$ | 9.3 | 2.9 | 45 | nt | nt |
| **7e** | $CH_3$ | $HOCH_2CH_2$ | 0.31 | 0.10 | 8.5 | 0.04 (0.03–0.06) | 1.8 (0.68–4.6) |
| **7f** | $CH_3$ | $(CH_3)_2NCH_2CH_2$ | 0.27 | 0.09 | 1.6 | 0.63 (0.24–1.6) | 1.3 (0.64–2.5) |
| **8** | H | H | 0.66 | 0.39 | 34 | 0.16 (0.11–0.22) | >2.5 |
| **9a** | H | $CH_3CO$ | 0.68 | 0.24 | 19 | 0.63 (0.24–1.6) | >2.5 |
| **9b** | H | $(CH_3)_3CCO$ | 0.77 | 0.10 | 5.0 | 0.32 (0.16–0.62) | >2.5 |
| **9c** | H | $C_3H_5CO$ | 0.39 | 0.08 | 5.5 | 1.3 (0.35–4.5) | nt |
| **9d** | H | $CH_2{=}CHCO$ | 0.36 | 0.06 | 8.7 | 0.63 (0.24–1.6) | >2.5 |
| **9e** | H | $C_6H_5CO$ | 1.3 | 0.31 | 8.7 | nt | nt |
| **9f** | H | 3-Pyridoyl | 0.48 | 0.12 | 3.5 | $\geqslant$2.5 | >2.5 |
| **9g** | H | Et-NHCO | 2.0 | 0.12 | 3.3 | nt | nt |
| **9h** | H | $(CH_3)_3CNHCO$ | 2.0 | 0.16 | 5.6 | $\geqslant$2.5 | >2.5 |
| **9i** | H | $C_6H_{11}NHCO$ | 6.2 | 0.54 | 11 | >2.5 | >2.5 |
| **9j** | H | $C_2H_5O(CO)CH_2NHCO$ | 1.9 | 0.13 | 9.7 | >2.5 | >2.5 |
| **9k** | H | $C_6H_5NHCO$ | 3.0 | 1.3 | 72 | nt | nt |
| **9l** | H | 3-F-$C_6H_4NHCO$ | 20 | 2.7 | >100 | >2.5 | >2.5 |
| **9m** | H | 3,4-Dimethoxy-$C_6H_4NHCO$ | 3.7 | 0.44 | 11 | >2.5 | >2.5 |
| **10a** | H | Ph | 3.4 | 0.94 | 16 | $\geqslant$2.5 | >2.5 |
| **10b** | $CH_3$ | Ph | 5.5 | 2.2 | 50 | >2.5 | >2.5 |
| **10c** | H | 4-F-$C_6H_4$ | 3.8 | 1.5 | 37 | >2.5 | >2.5 |
| **10d** | H | 3-CN-$C_6H_4$ | 3.7 | 1.6 | 5.0 | $\geqslant$2.5 | >2.5 |
| **10e** | H | 2-Me-$C_6H_4$ | 5.0 | 1.9 | 27 | >2.5 | >2.5 |
| **10f** | H | 3,4-DiMe-$C_6H_4$ | 15 | 3.6 | 97 | nt | nt |

nt, not tested.

[a] The activity of compounds was confirmed in an independent experiment. A difference in $pIC_{50}$ up to 0.6 (SD < 0.5) was considered as reproducible and therefore accepted. SEM values were <0.4.

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

**Table 2.** In vitro binding affinities and in vivo activities for antagonism of medetomidine-induced loss of righting and pCA-induced excitation of compound **7e** and its enantiomers

| Compound | $\alpha_{2A}$[a] $K_i$ (nM) | $\alpha_{2C}$[a] $K_i$ (nM) | 5-HTT[a] $K_i$ (nM) | Medetomidine (sc), $ED_{50}$ (mg/kg)[b] | pCA (sc), $ED_{50}$ (mg/kg)[b] |
|---|---|---|---|---|---|
| **7e** | 0.31 | 0.10 | 8.5 | 0.04 (0.03–0.06) | 1.8 (0.68–4.6) |
| (−)-**7e** | 150 | 3.5 | 3.7 | >2.5 | 0.32 (0.16–0.62) |
| (+)-**7e** | 0.43 | 0.03 | 21 | 0.63 (0.24–1.6) | >2.5 |

[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. SEM values were <0.4.

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

in both in vivo assays. In the case of arylamides, such as derivative **9f**, a drastic decrease in in vivo potency was observed, although binding affinities were in the nanomolar range. Finally, ureas **9g–m** and arylamino derivatives **10a–f** did not show significant in vivo activity in any of both assays.

In view of the results showed above we decided to resynthesize the hydroxyethylamino derivative **7e**, in order to isolate and evaluate its two enantiomers. Surprisingly and in contrast to what we had previously observed with the 7-*O*-substituted derivatives,[10] both enantiomers showed dissociation of activities, as can be deduced from data gathered in Table 2. Thus, compound(−)- **7e** kept the same range of affinity for the 5-HTT as its parent racemate, whereas its affinity for the $\alpha_2$-adrenoceptors dropped dramatically, especially for the $\alpha_{2A}$ subtype. On the contrary, the other enantiomer (+)-**7e** retained the potency as $\alpha_2$ blocker while showing a 3-fold decrease in potency at the 5-HTT site. These findings were confirmed in the in vivo assays. As can be seen in Table 2, compound (−)-**7e** was not active at 2.5 mg/kg in the medetomidine test but was even more potent than its parent racemate in the pCA test. On the other hand, compound (+)-**7e** was active in the medetomidine test, although less potent than the racemate, whereas it did not show activity at 2.5 mg/kg in the pCA assay.

## 4. Conclusions

In conclusion, the introduction of a nitrogen atom at position 7 of the 3a,4-dihydro-3*H*-[1]benzopyrano-[4,3-*c*]isoxazole scaffold was achieved. Diverse substitution patterns and functionalities have been introduced, in order to establish a SAR and to get a more complete picture of this modification. All the compounds synthesized showed higher affinity for the $\alpha_2$ receptor subtypes, especially for the $\alpha_{2C}$ than for the 5-HTT site. From this exploration alkylamino derivatives proved to be the most interesting compounds according to their in vitro and in vivo potencies, especially **7a**, **7e**, and **7f**, which showed different levels of activity as $\alpha_2$-adrenoceptor blockers and 5-HT reuptake inhibitors. The enantiomers of **7e** were isolated and evaluated proving that in this case, in contrast to the 7-*O*-substituted analogues, $\alpha_2$ antagonistic activity and 5-HTT inhibitory activity were dissociated. Further work trying to understand these findings and to evaluate the potential of these compounds with different $\alpha_2$/5-HTT balance is in progress and results will be reported elsewhere.

## 5. Experimental

### 5.1. 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 id 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 10 μm). Optical rotations were measured on a Perkin-Elmer 341 polarimeter with a sodium lamp and reported as follows: $[\alpha]_\lambda^{t\,^\circ C}$ (*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 ($\delta$) 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 id 3.5 μm from Agilent Technologies. Low-resolution mass spectra were obtained on an HPLC-MS system, an Agilent-Micromass HP1100 Platform spectrometer with electrospray ionization (ES). High-resolution mass spectra were recorded on an Agilent-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 chromatography was performed on silica gel, particle size 60 , mesh = 230–400 (Merck).

**5.1.1. Ethyl 7-bromo-3a,4-dihydro-3*H*-[1]benzopyrano[4,3-*c*]isoxazole-3-carboxylate (4b).** A mixture of 5-bromosalicylaldehyde[12] **3b** (3 g, 14.9 mmol), $K_2CO_3$ (4.12 g, 29.8 mmol), and (*E*)-ethyl 4-bromocrotonate (3 mL, 22.3 mmol) in anhydrous dimethylformamide (17 mL) was stirred at room temperature for 4 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 in vacuo. The residue was diluted with water (17 mL) and extracted with dichloromethane (3× 17 mL). The organic phase was dried and concentrated in vacuo. The residue was precipitated with diisopropylether affording 3.11 g (61% yield) of ethyl 4-(2-formyl-5-bromophenoxy)but-2(*E*)-enoate. To a solution of previously prepared ester (3.11 g, 9.9 mmol) in absolute ethanol (25 mL), hydroxylamine hydrochloride (0.83 g, 11.9 mmol) and sodium acetate (1.22 g, 14.8 mmol) were added. After 2 h at room temperature, the TLC analysis showed the absence of starting material. The solvent was evaporated in vacuo, and the residue was dissolved in water (20 mL) and extracted with dichloromethane (3× 30 mL). The organic layer was dried ($Na_2SO_4$) and concentrated at reduced pressure to yield 3.95 g (quantitative yield) of ethyl 4-[2-(hydroxyiminomethyl)-5-bromophenoxy]but-2(*E*)-enoate used in the next reaction step without further purification. To a solution of previously synthesized oxime (3.8 g, 11.6 mmol) in dichloromethane (47 mL), 4% aqueous solution of sodium hypochlorite (40 mL, 23.1 mmol) was added portionwise and the reaction mixture was stirred for 2 h at room temperature. Then, triethylamine (2.4 mL, 17.4 mmol) was added dropwise at 0 °C. The reaction mixture was stirred overnight at room temperature. 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), affording 1.71 g of **4b** as an oil. Yield: 45%. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ ppm 1.36 (t, $J$ = 7.1 Hz, 3H), 4.06 (dt, $J$ = 12.2 and 5.3 Hz, 1H), 4.16 (dd, $J$ = 12.6 and 10.2 Hz, 1H), 4.34 (q, $J$ = 7.3 Hz, 2H), 4.73 (d, $J$ = 11.8 Hz, 1H), 4.75 (dd, $J$ = 9.1 and 5.2 Hz, 1H), 7.14–7.18 (m, 2H), 7.63 (d, $J$ = 8.7 Hz, 1H). MS $m/z$ 327 (MH+).

**5.1.2. Ethyl 7-bromo-3a,4-dihydro-3*H*-[1]benzopyrano[4,3-*c*]isoxazol-3-yl methanesulfonate (5b).** To a solution of **4b** (3.3 g, 10.1 mmol) in THF (77 mL) and water (7 mL) stirred at room temperature, sodium borohydride (0.96 g, 25.3 mmol) was added portionwise and the reaction mixture was stirred for 24 h at room temperature. Then, a saturated solution of ammonium chloride (20 mL) was added and the mixture was extracted with dichloromethane. The organic layer was separated, dried ($Na_2SO_4$), and filtered, and the solvent evaporated, affording 3.01 g of crude alcohol, which was used as such without further purification. To a solution of previously prepared alcohol (3.01 g, 10.1 mmol) and triethylamine (2.1 mL, 15.1 mmol) in dichloromethane (45 mL) stirred at 0 °C under nitrogen atmosphere, methanesulfonyl chloride (0.86 mL, 11.1 mmol) was added dropwise. The reaction mixture was stirred at 0 °C for 2 h. Then, saturated solution of sodium bicarbonate (40 mL) was added. The organic layer was separated, dried with anhydrous $Na_2SO_4$, and filtered, and the solvent evaporated, affording 4.06 g of **5b** as a foam. Yield: 99%. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ ppm 3.27 (s, 3H), 3.83 (dt, $J$ = 12.2 and 6.0 Hz, 1H), 4.25 (dd, $J$ = 12.4 and 10.6 Hz, 1H), 4.51–4.57 (m, 1H), 4.62–4.72 (m, 2H), 4.76 (dd, $J$ = 10.5 and 5.9 Hz, 1H), 7.24 (dd, $J$ = 8.3 and 1.9 Hz, 1H), 7.30 (d, $J$ = 1.9 Hz, 1H), 7.60 (d, $J$ = 8.3 Hz, 1H). MS $m/z$ 349 (MH+).

**5.1.3. 7-Bromo-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 (6b).** To a solution of **5b** (17 g, 46.9 mmol) and *N*-methylcinnamylpiperazine (15.23 g, 70.4 mmol) in methylisobutylketone (220 mL) were added $K_2CO_3$ (6.48 g, 46.9 mmol) and KI (7.79 g, 46.9 mmol). This mixture was refluxed for 24 h. Inorganic salts were then filtered out, and the solvent was removed under reduced pressure. The resulting oil was dissolved in dichloromethane, washed several times with water, and dried ($Na_2SO_4$). The dichloromethane was removed under reduced pressure. The residue was purified by column chromatography (dichloromethane/methanol 99:1) and precipitated with diisopropylether affording 10.4 g of compound **6b** as a foam. Yield: 46%. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ ppm 1.90 (d, $J$ = 1.2 Hz, 3H), 2.47 (br s, 4H), 2.61 (br s, 4H), 2.82 (dd, $J$ = 13.2 and 5.60 Hz, 1H), 2.89 (dd, $J$ = 13.0 and 6.0 Hz, 1H), 3.01 (s, 2H), 3.68 (dt, $J$ = 12.4 and 5.8 Hz, 1H), 4.10 (dd, $J$ = 12.5 and 10.5 Hz, 1H), 4.40–4.48 (m, 1H), 4.65 (dd, $J$ = 10.4 and 6.0 Hz, 1H), 6.42 (s, 1H), 7.14–7.18 (m, 2H), 7.17–7.23 (m, 1H), 7.24–7.36 (m, 4H), 7.63 (d, $J$ = 8.7 Hz, 1H). MS $m/z$ 483 ($MH^+$).

The following compound was prepared analogously starting from 5-fluorosalicylaldehyde **3a**:

**5.1.4. 7-Fluor-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 (6a).** Overall yield: 12%. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ ppm 1.90 (d, $J$ = 1.2 Hz, 3H), 2.47 (br s, 4H), 2.61 (br s, 4H), 2.82 (dd, $J$ = 13.2 and 5.60 Hz, 1H), 2.89 (dd, $J$ = 13.0 and 6.0 Hz, 1H), 3.01 (s, 2H), 3.68 (dt, $J$ = 12.4 and 5.8 Hz, 1H), 4.10 (dd, $J$ = 12.5 and 10.5 Hz, 1H), 4.40–4.48 (m, 1H), 4.65 (dd, $J$ = 10.4 and 6.0 Hz, 1H), 6.42 (s, 1H), 6.66 (dd, $J$ = 9.9 and 2.5 Hz, 1H), 6.73 (dt, $J$ = 8.5 and 2.5 Hz, 1H), 7.17–7.23 (m, 1H), 7.24–7.36 (m, 4H), 7.76 (dd, $J$ = 8.7 and 6.4 Hz, 1H). MS $m/z$ 422 ($MH^+$).

### 5.2. General procedure for the synthesis of compounds 7a–f

#### 5.2.1. Method A (primary amines)

**5.2.1.1. Benzyl-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-ylamine (7c).** A mixture of **6a** (1 g, 2.4 mmol), potassium fluoride (0.14 g, 2.4 mmol), and benzylamine (6.3 mL, 57.6 mmol) was heated at 150 °C for 5 days in a sealed tube. Then, water was added and the mixture was extracted with dichloromethane. The organic layer was separated, dried with anhydrous $Na_2SO_4$, and filtered, and the solvent evaporated. The residue was purified by column chromatography (dichloromethane/ethyl acetate 2:1) and treated with diisopropylether affording 0.89 g of **7c** as a foam. Yield: 73%. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ ppm 1.90 (d, $J$ = 1.0 Hz, 3H), 2.47 (br s, 4H), 2.59 (br s, 2H), 2.63 (br s, 2H), 2.80 (dd, $J$ = 13.1 and 5.4 Hz, 1H), 2.87

(dd, $J$ = 13.1 and 6.2 Hz, 1H), 3.01 (s, 2H), 3.60 (dt, $J$ = 12.5 and 5.7 Hz, 1H), 4.04 (dd, $J$ = 12.4 and 10.4 Hz, 1H), 4.31–4.41 (m, 4H), 4.56 (dd, $J$ = 10.2 and 5.8 Hz, 1H), 6.12 (d, $J$ = 2.3 Hz, 1H), 6.30 (dd, $J$ = 8.5 and 2.3 Hz, 1H), 6.42 (s, 1H), 7.17–7.23 (m, 1H), 7.25–7.39 (m, 9H), 7.56 (d, $J$ = 8.5 Hz, 1H). MS $m/z$ 509 ($MH^+$). Anal. Calcd for $C_{32}H_{36}N_4O_2$: C, 75.56; H, 7.13; N, 11.01. Found: C, 75.40; H, 7.25; N, 10.84.

The following compounds were prepared analogously:

**5.2.1.2. Methyl-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-ylamine (7a).** Crystallized as dihydrochloride salt in 2-propanol. Yield: 19%, mp >300 °C (dec). $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ ppm 1.90 (d, $J$ = 1.2 Hz, 3H), 2.63 (s, 3H), 2.73–3.29 (m, 10H), 3.59 (dt, $J$ = 12.5 and 6.0 Hz, 1H), 3.68 (br s, 2H), 3.99 (dd, $J$ = 12.4 and 10.8 Hz, 1H), 4.32–4.40 (m, 1H), 4.56 (dd, $J$ = 10.6 and 5.6 Hz, 1H), 5.99 (d, $J$ = 2.1 Hz, 1H), 6.27 (dd, $J$ = 8.5 and 2.3 Hz, 1H), 6.67 (s, 1H), 7.23–7.41 (m, 6H), 9.5–10.75 (br s, 1H). MS $m/z$ 433 ($MH^+$).

**5.2.1.3. Ethyl-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-ylamine (7b).** Yield: 27%. Foam. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ ppm 1.26 (t, $J$ = 7.1 Hz, 3H), 1.90 (d, $J$ = 1.2 Hz, 3H), 2.47 (br s, 4H), 2.58 (br s, 2H), 2.63 (br s, 2H), 2.80 (dd, $J$ = 13.3 and 5.4 Hz, 1H), 2.87 (dd, $J$ = 13.3 and 6.2 Hz, 1H), 3.01 (s, 2H), 3.11–3.21 (m, 2H), 3.60 (dt, $J$ = 12.5 and 5.7 Hz, 1H), 3.92 (t, $J$ = 4.9 Hz, 1H), 4.05 (dd, $J$ = 12.4 and 10.4 Hz, 1H), 4.30–4.39 (m, 1H), 4.57 (dd, $J$ = 10.4 and 5.8 Hz, 1H), 6.08 (d, $J$ = 2.3 Hz, 1H), 6.24 (dd, $J$ = 8.5 and 2.3 Hz, 1H), 6.42 (s, 1H), 7.17–7.23 (m, 1H), 7.24–7.36 (m, 4H), 7.55 (d, $J$ = 8.5 Hz, 1H). MS $m/z$ 447 ($MH^+$).

**5.2.2. Method B (secondary amines). 5.2.2.1. Benzylmethyl-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-ylamine (7d).** To a solution of **6a** (0.2 g, 0.5 mmol) in pyridine (2 mL), benzylmethylamine (1.5 mL, 11.4 mmol) was added and the mixture was heated at 150 °C for 4 days in a sealed tube. Then, the solvent was evaporated and the residue was purified by column chromatography (dichloromethane/methanol 99:1) and crystallized as dihydrochloride salt in 2-propanol affording 22 mg of **7d**. Yield: 10%, mp >300 °C (dec). $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ ppm 1.90 (d, $J$ = 0.8 Hz, 3H), 2.95–3.10 (m, 5H), 3.04 (s, 3H), 3.17–3.26 (m, 5H), 3.62 (dt, $J$ = 12.5 and 5.6 Hz, 1H), 3.73 (s, 2H), 3.99 (dd, $J$ = 12.4 and 10.8 Hz, 1H), 4.37–4.44 (m, 1H), 4.51–4.61 (m, 3H), 6.10 (d, $J$ = 2.5 Hz, 1H), 6.45 (dd, $J$ = 9.1 and 2.5 Hz, 1H) 6.68 (s, 1H), 7.12 (d, $J$ = 7.0 Hz, 2H), 7.20 (t, $J$ = 7.3 Hz, 1H), 7.25–7.33 (m, 5H), 7.37 (t, $J$ = 8.5 Hz, 3H). MS $m/z$ 423 ($MH^+$).

The following compounds were prepared analogously:

**5.2.2.2. 2-{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-7ylamino}-ethanol (7e).** Yield: 62%. Foam. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ ppm 1.63 (s, 1H), 1.90 (d, $J$ = 1.2 Hz, 3H), 2.47 (br s, 4H), 2.59 (br s, 2H), 2.63 (br s, 2H), 2.81 (dd, $J$ = 13.1 and 5.2 Hz, 1H), 2.87 (dd, $J$ = 13.1 and 6.2 Hz, 1H), 3.01 (s, 2H), 3.03 (s, 3H), 3.53 (t, $J$ = 5.7 Hz, 2H), 3.60 (dt, $J$ = 12.5 and 5.7 Hz, 1H), 3.83 (t, $J$ = 5.6 Hz, 2H), 4.04 (dd, $J$ = 12.4 and 10.4 Hz, 1H), 4.32–4.39 (m, 1H), 4.57 (dd, $J$ = 10.2 and 5.8 Hz, 1H), 6.22 (d, $J$ = 2.5 Hz, 1H), 6.41–6.46 (m, 2H), 7.18–7.23 (m, 1H), 7.26–7.35 (m, 4H), 7.60 (d, $J$ = 8.9 Hz, 1H). MS $m/z$ 477 ($MH^+$).

**5.2.2.3. *N*,*N*-Dimethyl-*N*′-{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}-ethane-1,2-diamine (7f).** Yield: 76%. Foam. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ ppm 1.90 (d, $J$ = 1.2 Hz, 3H), 2.29 (s, 6H), 2.42–2.53 (m, 6H), 2.58 (br s, 2H), 2.63 (br s, 2H), 2.81 (dd, $J$ = 13.2 and 5.4 Hz, 1H), 2.87 (dd, $J$ = 12.8 and 6.2 Hz, 1H), 2.98 (s, 3H), 3.01 (s, 2H), 3.42–3.51 (m, 2H), 3.60 (dt, $J$ = 12.6 and 5.8 Hz, 1H), 4.05 (dd, $J$ = 12.4 and 10.4 Hz, 1H), 4.31–4.38 (m, 1H), 4.57 (dd, $J$ = 10.4 and 5.8 Hz, 1H), 6.16 (d, $J$ = 2.5 Hz, 1H), 6.38 (dd, $J$ = 9.1 and 2.5 Hz, 1H), 6.42 (s, 1H), 7.17–7.23 (m, 1H), 7.26–7.35 (m, 4H), 7.60 (d, $J$ = 9.1 Hz, 1H). MS $m/z$ 504 ($MH^+$). Anal. Calcd for $C_{30}H_{41}N_5O_2$: C, 71.54; H, 8.20; N, 13.90. Found: C, 71.40; H, 8.35; N, 13.74.

**5.2.3. Resolution of racemic mixture of 7e**

**5.2.3.1. (−)-2-{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-7ylamino}-ethanol ((−)-7e) and (+)-2-{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-7ylamino}-ethanol ((+)-7e).** The racemate 2-{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-7ylamino}-ethanol **7e** (0.83 g, 1.7 mmol) was separated into its enantiomers by preparative HPLC (ethanol/acetonitrile 100:0 to 80:20 at 100 mL/min; column: CHIRALPAK AD 1000 20 μm Daicel). Two pure fractions were collected, the solvents were evaporated yielding 0.48 g of (−)-**7e** as a free base from diisopropylether as a white solid: mp 116.9 °C; 99% ee; $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ ppm 1.7 (br s, 1H), 1.90 (s, 3H), 2.47 (br s, 4H), 2.58 (br s, 2H), 2.63 (br s, 2H), 2.81 (dd, $J$ = 13.1 and 5.2 Hz, 1H), 2.87 (dd, $J$ = 13.3 and 6.2 Hz, 1H), 3.01 (s, 2H), 3.03 (s, 3H), 3.53 (t, $J$ = 5.7 Hz, 2H), 3.60 (dt, $J$ = 12.4 and 5.8 Hz, 1H), 3.83 (t, $J$ = 5.6 Hz, 2H), 4.04 (dd, $J$ = 12.1 and 10.5 Hz, 1H), 4.30–4.39 (m, 1H), 4.57 (dd, $J$ = 10.2 and 5.8 Hz, 1H), 6.22 (d, $J$ = 2.1 Hz, 1H), 6.40–6.47 (m, 2H), 7.20 (t, $J$ = 7.1 Hz, 1H), 7.24–7.36 (m, 4H), 7.60 (d, $J$ = 8.7 Hz, 1H). $[\alpha]_D^{20}$ −45.80 ($c$ 0.44, MeOH); HRMS Calcd for $C_{28}H_{37}N_4O_3$ (M+1): 477.2848. Found: 477.2866 and 0.40 g of (+)-**7e** precipitated as a dihydrochloride acid salt and crystallized in methanol as a light yellow solid: mp >300 °C (dec); 99% ee; $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ ppm 2.05 (s, 3H), 2.96 (s, 3H), 3.39–3.46 (m, 2H), 3.46–3.72 (m, 12H), 3.72–3.82 (m, 2H), 3.92 (s, 2H), 4.07 (dd, $J$ = 12.2 and 10.6 Hz, 1H), 4.63–4.73 (m, 2H), 6.20 (d, $J$ = 2.5 Hz, 1H), 6.47 (dd, $J$ = 9.0 and 2.4 Hz, 1H), 6.82 (s, 1H), 7.29–7.34 (m, 1H), 7.34–7.39

(m, 2H), 7.39–7.46 (m, 2H), 10.5–12.5 (br s, 1H). $[\alpha]_D^{20}$ +71.30 (*c* 0.44, MeOH); HRMS Calcd for $C_{28}H_{37}N_4O_3$ (M+1): 477.2859. Found: 477.2866.

**5.2.3.2. 7-Amino-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 (8).** A dried flask was charged with **6b** (6 g, 12.4 mmol), palladium acetate (0.028 g, 0.12 mmol), Xantphos (0.11 g, 0.19 mmol), *tert*-butyl carbamate (1.75 g, 14.9 mmol), and cesium carbonate (6.06 g, 18.6 mmol) in previously deoxygenated 1,4-dioxane (40 mL). The mixture was stirred at 100 °C for 24 h until the starting aryl bromide had been completely consumed as judged by TLC analysis. The reaction mixture was then cooled to room temperature, diluted with dichloromethane (400 mL), filtered, and concentrated in vacuo. The crude material was purified by column chromatography (dichloromethane/methanol 99:1 and 98:2) yielding 6.43 g of a carbamate intermediate, which was treated with TFA (34 mL) in dichloromethane (170 mL) and stirred at room temperature for 5 h. Then, the solvent was evaporated and the residue was basified with a saturated solution of sodium carbonate (150 mL) and extracted with dichloromethane (3× 150 mL). The organic phase was separated, dried with $Na_2SO_4$, and filtered, and the solvent evaporated. The residue was crystallized with diisopropylether affording 4.72 g of solid **8**. Yield: 91% (two steps), mp 134.1 °C. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ ppm 1.84 (d, $J$ = 1.0 Hz, 3H), 2.37 (br s, 4H), 2.49 (br s, 4H), 2.69 (dd, $J$ = 13.1 and 6.4 Hz, 1H), 2.78 (dd, $J$ = 13.1 and 5.0 Hz, 1H), 2.97 (s, 2H), 3.52 (dt, $J$ = 12.4 and 5.8 Hz, 1H), 4.02 (dd, $J$ = 12.2 and 10.6 Hz, 1H), 4.28 (dt, $J$ = 12.1 and 5.9 Hz, 1H), 4.51 (dd, $J$ = 10.4 and 5.8 Hz, 1H), 5.76 (br s, 2H), 6.08 (d, $J$ = 2.1 Hz, 1H), 6.24 (dd, $J$ = 8.5 and 2.1 Hz, 1H), 6.41 (s, 1H), 7.19–7.24 (m, 1H), 7.26–7.30 (m, 3H), 7.34 (t, $J$ = 7.5 Hz, 2H). MS *m*/*z* 419 ($MH^+$). Anal. Calcd for $C_{25}H_{30}N_4O_2$: C, 71.74; H, 7.22; N, 13.39. Found: C, 71.84; H, 7.36; N, 13.19.

### 5.3. General procedure for the synthesis of compounds 9a–f

**5.3.1. 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*]isoxazole-7-ylamide (9a).** To a solution of **8** (0.1 g, 0.2 mmol) in $CHCl_3$ (3 mL), triethylamine (0.1 mL, 0.9 mmol) and acetyl chloride (0.06 mL, 0.9 mmol) were added. The mixture was stirred at room temperature for 24 h. Then, saturated solution of sodium bicarbonate (3 mL) was added. 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 99:1) and treated with DIPE affording 80 mg of **9a** as a foam. Yield: 73%. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ ppm 1.84 (d, $J$ = 1.2 Hz, 3H), 2.05 (s, 3H), 2.38 (br s, 4H), 2.50 (br s, 4H), 2.72 (dd, $J$ = 13.3 and 6.2 Hz, 1H), 2.81 (dd, $J$ = 12.8 and 5.0 Hz, 1H), 2.97 (s, 2H), 3.66 (dt, $J$ = 12.4 and 5.8 Hz, 1H), 4.14 (dd, $J$ = 12.4 and 10.4 Hz, 1H), 4.38–4.46 (m, 1H), 4.60 (dd, $J$ = 10.6 and 6.0 Hz, 1H), 6.41 (s, 1H), 7.13 (dd, $J$ = 8.7 and 2.1 Hz, 1H), 7.21 (t, $J$ = 7.3 Hz, 1H), 7.26–7.31 (m, 2H), 7.34 (t, $J$ = 7.5 Hz, 2H), 7.40 (d, $J$ = 1.7 Hz, 1H), 7.56 (d, $J$ = 8.7 Hz, 1H), 10.15 (s, 1H). MS *m*/*z* 461 ($MH^+$). Anal. Calcd for $C_{27}H_{32}N_4O_3$: C, 70.41; H, 7.00; N, 12.16. Found: C, 69.98; H, 7.27; N, 11.92.

The following compounds were prepared analogously following a parallel synthetic approach:

**5.3.2. 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*]isoxazole-7-ylamide (9b).** Yield: 62%. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ ppm 1.31 (s, 9H), 1.90 (d, $J$ = 1.0 Hz, 3H), 2.48 (br s, 4H), 2.62 (br s, 4H), 2.81 (dd, $J$ = 13.3 and 5.6 Hz, 1H), 2.89 (dd, $J$ = 13.3 and 6.2 Hz, 1H), 3.02 (s, 2H), 3.66 (dt, $J$ = 12.4 and 5.8 Hz, 1H), 4.07 (dd, $J$ = 12.4 and 10.6 Hz, 1H), 4.38–4.47 (m, 1H), 4.63 (dd, $J$ = 10.4 and 5.8 Hz, 1H), 6.42 (s, 1H), 6.97 (dd, $J$ = 8.5 and 2.1 Hz, 1H), 7.17–7.23 (m, 1H), 7.24–7.38 (m, 5H), 7.45 (d, $J$ = 2.1 Hz, 1H), 7.70 (d, $J$ = 8.5 Hz, 1H). MS *m*/*z* 503 ($MH^+$).

**5.3.3. 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*]isoxazole-7-ylamide (9c).** Yield: 75%. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ ppm 0.87–0.97 (m, 2H), 1.00–1.12 (m, 2H), 1.75–1.84 (m, 1H), 1.91 (d, $J$ = 1.0 Hz, 3H), 2.48 (br s, 4H), 2.65 (br s, 4H), 2.80 (dd, $J$ = 13.3 and 5.4 Hz, 1H), 2.91 (dd, $J$ = 13.3 and 6.2 Hz, 1H), 3.02 (s, 2H), 3.67 (dt, $J$ = 12.4 and 5.7 Hz, 1H), 4.10 (dd, $J$ = 12.4 and 10.6 Hz, 1H), 4.36–4.46 (m, 1H), 4.60 (dd, $J$ = 10.2 and 5.7 Hz, 1H), 6.43 (s, 1H), 7.06 (dd, $J$ = 8.9 and 1.7 Hz, 1H), 7.16–7.30 (m, 5H), 7.37–7.43 (m, 2H), 10.30 (s, 1H). MS *m*/*z* 487 ($MH^+$). Anal. Calcd for $C_{29}H_{34}N_4O_3$: C, 71.58; H, 7.04; N, 11.51. Found: C, 71.26; H, 7.35; N, 11.17.

**5.3.4. 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*]isoxazole-7-ylamide (9d).** Yield: 60%. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ ppm 1.91 (d, $J$ = 1.0 Hz, 3H), 2.48 (br s, 4H), 2.65 (br s, 4H), 2.80 (dd, $J$ = 13.3 and 5.4 Hz, 1H), 2.91 (dd, $J$ = 13.3 and 6.2 Hz, 1H), 3.02 (s, 2H), 3.67 (dt, $J$ = 12.4 and 5.7 Hz, 1H), 4.10 (dd, $J$ = 12.4 and 10.6 Hz, 1H), 4.36–4.46 (m, 1H), 4.60 (dd, $J$ = 10.2 and 5.7 Hz, 1H), 5.77 (dd, $J$ = 10.4 and 2.7 Hz, 1H), 6.23 (dd, $J$ = 16.7 and 2.7 Hz, 1H), 6.43 (s, 1H), 6.83 (dd, $J$ = 16.7 and 10.4 Hz, 1H), 7.2–7.45 (m, 8H), 10.30 (s, 1H). MS *m*/*z* 473 ($MH^+$).

**5.3.5. Benzoic 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*]isoxazole-7-ylamide (9e).** Yield: 80%. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ ppm 1.91 (d, $J$ = 1.0 Hz, 3H), 2.48 (br s, 4H), 2.63 (br s, 4H), 2.82 (dd, $J$ = 13.3 and 5.4 Hz, 1H), 2.89 (dd, $J$ = 13.3 and 6.2 Hz, 1H), 3.01 (s, 2H), 3.66 (dt, $J$ = 12.4 and 5.8 Hz, 1H), 4.09 (dd, $J$ = 12.4 and 10.6 Hz, 1H), 4.36–4.46 (m, 1H), 4.61 (dd, $J$ = 10.2 and 5.7 Hz, 1H), 6.43 (s, 1H), 7.18–7.37 (m, 9H), 7.38–7.43 (m, 2H), 7.48–7.53 (m, 2H), 10.49 (s, 1H). MS *m*/*z* 523 ($MH^+$).

**5.3.6. Nicotinic 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*]isoxazole-7-ylamide (9f).** Yield: 58%. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ ppm 1.90 (d, $J$ = 1.0 Hz, 3H), 2.47 (br s, 4H), 2.64 (br s, 4H), 2.81 (dd, $J$ = 13.3 and 5.4 Hz, 1H), 2.90 (dd, $J$ = 13.3 and 6.2 Hz, 1H), 3.01 (s, 2H), 3.67 (dt, $J$ = 12.4 and 5.8 Hz, 1H), 4.09 (dd, $J$ = 12.4 and 10.6 Hz, 1H), 4.36–4.45 (m, 1H), 4.61 (dd, $J$ = 10.2 and 5.8 Hz, 1H), 6.43 (s, 1H), 7.20–7.45 (m, 8H), 7.50 (dd, $J$ = 7.2 and 4.6 Hz, 1H), 8.21 (dt, $J$ = 7.2 and 1.7 Hz, 1H), 8.65 (dt, $J$ = 4.6 and 1.7 Hz, 1H), 9.03 (br s, 1H), 10.40 (s, 1H). MS *m*/*z* 524 ($MH^+$).

### 5.4. General procedure for the synthesis of compounds 9g–m

#### 5.4.1. Method C (alkylisocyanates)

**5.4.1.1. 1-Ethyl-3-{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-7-yl}-urea (9g).** To a solution of **8** (0.2 g, 0.5 mmol) in toluene (3 mL), ethylisocyanate (0.1 mL, 1.3 mmol) was added and the mixture was stirred at 75 °C for 24 h. After cooling, the solvent was evaporated and the residue was purified by column chromatography (dichloromethane/methanol 99:1 and 98:2) affording 119 mg of **9 g** as a sticky oil. Yield: 51%. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ ppm 1.05 (t, $J$ = 7.1 Hz, 3H), 1.84 (d, $J$ = 1.0 Hz, 3H), 2.39 (br s, 4H), 2.51 (br s, 4H), 2.72 (dd, $J$ = 13.1 and 6.4 Hz, 1H), 2.81 (dd, $J$ = 13.3 and 5.2 Hz, 1H), 2.97 (s, 2H), 3.07–3.14 (m, 2H), 3.63 (dt, $J$ = 12.4 and 6.0 Hz, 1H), 4.12 (dd, $J$ = 12.4 and 10.8 Hz, 1H), 4.39 (dt, $J$ = 12.2 and 5.9 Hz, 1H), 4.59 (dd, $J$ = 10.6 and 6.0 Hz, 1H), 6.21 (t, $J$ = 5.6 Hz, 1H), 6.42 (s, 1H), 6.92 (dd, $J$ = 8.7 and 2.1 Hz, 1H), 7.19–7.26 (m, 2H), 7.26–7.30 (m, 2H), 7.31–7.37 (m, 2H), 7.48 (d, $J$ = 8.5 Hz, 1H), 8.73 (s, 1H). MS *m*/*z* 490 ($MH^+$).

The following compounds were prepared analogously:

**5.4.1.2. 1-Tertbutyl-3-{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-7-yl}-urea (9h).** Yield: 25%. Foam. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ ppm 1.38 (s, 9H), 1.90 (s, 3H), 2.47 (br s, 4H), 2.60 (br s, 4H), 2.80 (dd, $J$ = 13.3 and 5.6 Hz, 1H), 2.87 (dd, $J$ = 13.1 and 6.0 Hz, 1H), 3.01 (s, 2H), 3.62 (dt, $J$ = 12.4 and 5.8 Hz, 1H), 4.03 (dd, $J$ = 12.3 and 10.5 Hz, 1H), 4.35–4.43 (m, 1H), 4.58 (dd, $J$ = 10.4 and 5.8 Hz, 1H), 4.92 (s, 1H), 6.42 (s, 1H), 6.71–6.77 (m, 2H), 7.15 (d, $J$ = 2.1 Hz, 1H), 7.20 (t, $J$ = 7.1 Hz, 1H), 7.24–7.35 (m, 4H), 7.58 (d, $J$ = 8.5 Hz, 1H). MS *m*/*z* 518 ($MH^+$).

**5.4.1.3. 1-Cyclohexyl-3-{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-7-yl}-urea (9i).** Yield: 48%. Syrup. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ ppm 1.06–1.22 (m, 3H), 1.27–1.42 (m, 2H), 1.55–1.65 (m, 1H), 1.65–1.77 (m, 2H), 1.90 (d, $J$ = 1.0 Hz, 3H), 1.92–2.01 (m, 2H), 2.47 (br s, 4H), 2.61 (br s, 4H), 2.80 (dd, $J$ = 13.3 and 5.6 Hz, 1H), 2.87 (dd, $J$ = 13.1 and 6.0 Hz, 1H), 3.00 (s, 2H), 3.57–3.70 (m, 2H), 4.03 (dd, $J$ = 12.4 and 10.6 Hz, 1H), 4.34–4.43 (m, 1H), 4.59 (dd, $J$ = 10.4 and 5.8 Hz, 1H), 5.04 (d, $J$ = 7.9 Hz, 1H), 6.42 (s, 1H), 6.75 (dd, $J$ = 8.5 and 2.1 Hz, 1H), 6.95 (s, 1H), 7.14–7.23 (m, 2H), 7.23–7.36 (m, 4H), 7.59 (d, $J$ = 8.7 Hz, 1H). MS *m*/*z* 544 ($MH^+$).

**5.4.1.4. Ethyl (3-{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-7-yl}-ureido)-acetate (9j).** Yield: 72%. Foam. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ ppm 1.31 (t, $J$ = 7.1 Hz, 3H), 1.90 (d, $J$ = 0.8 Hz, 3H), 2.47 (br s, 4H), 2.61 (br s, 4H), 2.80 (dd, $J$ = 13.2 and 5.6 Hz, 1H), 2.88 (dd, $J$ = 13.3 and 6.2 Hz, 1H), 3.00 (s, 2H), 3.61 (dt, $J$ = 12.4 and 5.8 Hz, 1H), 4.01–4.10 (m, 3H), 4.24 (q, $J$ = 7.1 Hz, 2H), 4.36–4.45 (m, 1H), 4.57 (dd, $J$ = 10.3 and 5.9 Hz, 1H), 5.80 (t, $J$ = 5.5 Hz, 1H), 6.42 (s, 1H), 6.73 (dd, $J$ = 8.6 and 2.0 Hz, 1H), 7.10 (d, $J$ = 2.1 Hz, 1H), 7.16–7.36 (m, 6H), 7.52 (d, $J$ = 8.5 Hz, 1H). MS *m*/*z* 548 ($MH^+$). Anal. Calcd for $C_{30}H_{37}N_5O_5$: C, 65.79; H, 6.81; N, 12.79. Found: C, 65.48; H, 7.03; N, 12.84.

#### 5.4.2. Method D (arylisocyanates)

**5.4.2.1. 1-{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-7-yl}-3-phenylurea (9k).** A mixture of **8** (0.27 g, 0.6 mmol), phenylisocyanate (0.08 mL, 0.7 mmol), and triethylamine (catalytic amount) in THF was stirred at room temperature for 24 h. Then, the solvent was evaporated and the residue was purified by column chromatography (dichloromethane/methanol 98:2 and 96:4) and crystallized with diisopropylether affording 0.16 g of solid **9k**. Yield: 46%, mp 180.3 °C. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ ppm 1.84 (s, 3H), 2.39 (br s, 4H), 2.50 (br s, 4H), 2.73 (dd, $J$ = 13.1 and 6.4 Hz, 1H), 2.82 (dd, $J$ = 13.3 and 5.0 Hz, 1H), 2.97 (s, 2H), 3.66 (dt, $J$ = 12.4 and 5.8 Hz, 1H), 4.15 (dd, $J$ = 12.2 and 10.8 Hz, 1H), 4.37–4.46 (m, 1H), 4.61 (dd, $J$ = 10.5 and 5.9 Hz, 1H), 6.42 (s, 1H), 6.95–7.03 (m, 2H), 7.16–7.39 (m, 8H), 7.45 (d, $J$ = 7.7 Hz, 2H), 7.54 (d, $J$ = 8.5 Hz, 1H), 8.76 (s, 1H), 8.97 (s, 1H). MS *m*/*z* 538 ($MH^+$). Anal. Calcd for $C_{32}H_{35}N_5O_3$: C, 71.49; H, 6.56; N, 13.03. Found: C, 71.40; H, 6.39; N, 12.88.

The following compounds were prepared analogously:

**5.4.2.2. 1-(3-Fluorophenyl)-3-{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-7-yl}-urea (9l).** Yield: 63%, mp 186.8 °C. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ ppm 1.85 (d, $J$ = 1.0 Hz, 3H), 2.39 (br s, 4H), 2.51 (br s, 4H), 2.73 (dd, $J$ = 13.3 and 6.4 Hz, 1H), 2.82 (dd, $J$ = 13.3 and 5.2 Hz, 1H), 2.98 (s, 2H), 3.66 (dt, $J$ = 12.4 and 5.8 Hz, 1H), 4.16 (dd, $J$ = 12.4 and 10.8 Hz, 1H), 4.38–4.47 (m, 1H), 4.62 (dd, $J$ = 10.5 and 5.9 Hz, 1H), 6.42 (s, 1H), 6.81 (dt, $J$ = 8.4 and 2.0 Hz, 1H), 7.01 (dd, $J$ = 8.7 and 2.1 Hz, 1H), 7.14 (dd, $J$ = 8.3 and 1.0 Hz, 1H), 7.19–7.25 (m, 1H), 7.25–7.39 (m, 6H), 7.48 (dt, $J$ = 11.9 and 2.2 Hz, 1H), 7.56 (d, $J$ = 8.5 Hz, 1H), 9.01 (s, 1H), 9.05 (s, 1H). MS *m*/*z* 556 ($MH^+$).

**5.4.2.3. 1-(3,4-Dimethoxyphenyl)-3-{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-7-yl}-urea (9m).** Yield: 73%. Foam. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$

ppm 1.90 (s, 3H), 2.46 (br s, 4H), 2.60 (br s, 4H), 2.79 (dd, $J$ = 13.3 and 5.6 Hz, 1H), 2.88 (dd, $J$ = 13.3 and 6.2 Hz, 1H), 3.00 (s, 2H), 3.61 (dt, $J$ = 12.4 and 5.9 Hz, 1H), 3.84 (s, 3H), 3.85 (s, 3H), 4.01 (dd, $J$ = 12.4 and 10.4 Hz, 1H), 4.35–4.45 (m, 1H), 4.58 (dd, $J$ = 10.4 and 5.8 Hz, 1H), 6.41 (s, 1H), 6.71–6.82 (m, 3H), 6.96–7.07 (m, 2H), 7.15–7.36 (m, 7H), 7.59 (d, $J$ = 8.5 Hz, 1H). MS $m/z$ 598 ($MH^+$).

### 5.4.3. General procedure for the synthesis of compounds 10a–f

**5.4.3.1. 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-7-yl-amino benzonitrile (10d).** A dried flask was charged with **6b** (0.2 g, 0.4 mmol), cesium carbonate (0.19 g, 0.6 mmol), palladium acetate (0.002 g, 0.01 mmol), BINAP (0.011 g, 0.02 mmol), 3-aminobenzonitrile (0.06 g, 0.5 mmol), and toluene (2 mL), and purged with nitrogen. The mixture was stirred at 100 °C for 24 h. The mixture was cooled to room temperature, diluted with dichloromethane, filtered, and concentrated. The crude product was then purified by column chromatography (dichloromethane/methanol 99:1) and treated with diisopropylether affording 0.14 g of **10d** as a foam. Yield: 67%.$^1$H NMR (400 MHz, $CDCl_3$) $\delta$ ppm 1.90 (d, $J$ = 1.0 Hz, 3H), 2.48 (br s, 4H), 2.63 (br s, 4H), 2.82 (dd, $J$ = 13.3 and 5.4 Hz, 1H), 2.89 (dd, $J$ = 13.3 and 6.2 Hz, 1H), 3.01 (s, 2H), 3.66 (dt, $J$ = 12.4 and 5.8 Hz, 1H), 4.09 (dd, $J$ = 12.4 and 10.6 Hz, 1H), 4.36–4.47 (m, 1H), 4.62 (dd, $J$ = 10.2 and 5.8 Hz, 1H), 6.07 (s, 1H), 6.42 (s, 1H), 6.60–6.68 (m, 2H), 7.17–7.43 (m, 9H), 7.69 (d, $J$ = 8.3 Hz, 1H). MS $m/z$ 520 ($MH^+$). Anal. Calcd for $C_{32}H_{33}N_5O_2$: C, 73.96; H, 6.40; N, 13.48. Found: C, 73.61; H, 6.76; N, 13.21.

The following compounds were prepared analogously following a parallel synthetic approach:

**5.4.3.2. 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-7-yl-phenylamine (10a).** Yield: 73%. Oil. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ ppm 1.91 (d, $J$ = 1.0 Hz, 3H), 2.48 (br s, 4H), 2.63 (br s, 4H), 2.82 (dd, $J$ = 13.3 and 5.4 Hz, 1H), 2.89 (dd, $J$ = 13.3 and 6.2 Hz, 1H), 3.01 (s, 2H), 3.66 (dt, $J$ = 12.4 and 5.8 Hz, 1H), 4.09 (dd, $J$ = 12.4 and 10.6 Hz, 1H), 4.36–4.46 (m, 1H), 4.61 (dd, $J$ = 10.2 and 5.7 Hz, 1H), 6.08 (s, 1H), 6.43 (s, 1H), 6.74 (d, $J$ = 2.4 Hz, 1H), 6.86 (dd, $J$ = 8.3 and 2.4 Hz, 1H), 6.91 (t, $J$ = 7.4 Hz, 1H), 7.01 (d, $J$ = 8.3 Hz, 2H), 7.18–7.25 (m, 3H), 7.27–7.35 (m, 4H), 7.49 (d, $J$ = 8.3 Hz, 1H). MS $m/z$ 495 ($MH^+$).

**5.4.3.3. Methyl-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-7-yl-phenylamine (10b).** Yield: 55%. Oil. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ ppm 1.91 (d, $J$ = 1.0 Hz, 3H), 2.48 (br s, 4H), 2.62 (br s, 4H), 2.81 (dd, $J$ = 13.3 and 5.4 Hz, 1H), 2.89 (dd, $J$ = 13.3 and 6.2 Hz, 1H), 3.02 (s, 2H), 3.29 (s, 3H), 3.67 (dt, $J$ = 12.4 and 5.8 Hz, 1H), 4.09 (dd, $J$ = 12.4 and 10.6 Hz, 1H), 4.36–4.47 (m, 1H), 4.61 (dd, $J$ = 10.2 and 5.7 Hz, 1H), 6.43 (s, 1H), 6.75 (d, $J$ = 2.4 Hz, 1H), 6.87 (dd, $J$ = 8.3 and 2.4 Hz, 1H), 6.90 (t, $J$ = 7.4 Hz, 1H), 7.01 (d, $J$ = 8.3 Hz, 2H), 7.17–7.25 (m, 3H), 7.27–7.36 (m, 4H), 7.5 (d, $J$ = 8.3 Hz, 1H). MS $m/z$ 509 ($MH^+$).

**5.4.3.4. 4-Fluorophenyl-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-7-yl-amine (10c).** Yield: 80%. Foam. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ ppm 1.91 (d, $J$ = 1.0 Hz, 3H), 2.48 (br s, 4H), 2.63 (br s, 4H), 2.82 (dd, $J$ = 13.3 and 5.4 Hz, 1H), 2.89 (dd, $J$ = 13.3 and 6.2 Hz, 1H), 3.01 (s, 2H), 3.66 (dt, $J$ = 12.4 and 5.8 Hz, 1H), 4.09 (dd, $J$ = 12.4 and 10.6 Hz, 1H), 4.36–4.46 (m, 1H), 4.61 (dd, $J$ = 10.2 and 5.7 Hz, 1H), 6.08 (s, 1H), 6.43 (s, 1H), 6.74 (d, $J$ = 2.4 Hz, 1H), 6.86 (dd, $J$ = 8.3 and 2.4 Hz, 1H), 7.18–7.39 (m, 9H), 7.49 (d, $J$ = 8.3 Hz, 1H). MS $m/z$ 513 ($MH^+$).

**5.4.3.5. 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-7-yl-2-tolylamine (10e).** Yield: 68%. Foam. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ ppm 1.91 (d, $J$ = 1.0 Hz, 3H), 2.25 (s, 3H), 2.48 (br s, 4H), 2.62 (br s, 4H), 2.81 (dd, $J$ = 13.3 and 5.4 Hz, 1H), 2.89 (dd, $J$ = 13.3 and 6.2 Hz, 1H), 3.02 (s, 2H), 3.68 (dt, $J$ = 12.4 and 5.8 Hz, 1H), 4.10 (dd, $J$ = 12.4 and 10.6 Hz, 1H), 4.36–4.46 (m, 1H), 4.62 (dd, $J$ = 10.2 and 5.7 Hz, 1H), 6.08 (s, 1H), 6.43 (s, 1H), 6.60 (dt, $J$ = 7.7 and 0.8 Hz, 1H), 6.75 (m, 2H), 6.87 (dd, $J$ = 8.3 and 2.4 Hz, 1H), 7.02 (dt, $J$ = 7.8 and 1.3 Hz, 1H), 7.14 (d, $J$ = 7.8 Hz, 1H), 7.17–7.24 (m, 1H), 7.27–7.36 (m, 4H), 7.5 (d, $J$ = 8.3 Hz, 1H). MS $m/z$ 509 ($MH^+$).

**5.4.3.6. 3,4-Dimethylphenyl-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-7-yl-amine (10f).** Yield: 75%. Foam. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ ppm 1.90 (d, $J$ = 1.0 Hz, 3H), 2.25 (s, 3H), 2.35 (s, 3H), 2.48 (br s, 4H), 2.62 (br s, 4H), 2.81 (dd, $J$ = 13.3 and 5.4 Hz, 1H), 2.90 (dd, $J$ = 13.3 and 6.2 Hz, 1H), 3.02 (s, 2H), 3.68 (dt, $J$ = 12.4 and 5.8 Hz, 1H), 4.11 (dd, $J$ = 12.4 and 10.6 Hz, 1H), 4.36–4.47 (m, 1H), 4.61 (dd, $J$ = 10.2 and 5.7 Hz, 1H), 6.08 (s, 1H), 6.43 (s, 1H), 6.55 (d, $J$ = 7.8 and 2.2 Hz, 1H), 6.60 (dt, $J$ = 7.7 and 0.8 Hz, 1H), 6.87 (dd, $J$ = 8.3 and 2.4 Hz, 1H), 6.99 (d, $J$ = 2.2 Hz, 1H), 7.07 (d, $J$ = 7.8 Hz, 1H), 7.19–7.23 (m, 1H), 7.24–7.29 (m, 2H), 7.30–7.36 (m, 2H), 7.5 (d, $J$ = 8.3 Hz, 1H). MS $m/z$ 523 ($MH^+$).

## 5.5. Biology

**5.5.1. Receptor binding assays.** Frozen membranes of CHO cells, stably transfected with either human adrenergic 2A 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 pre-determined 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. Non-specific binding was determined

in the presence of oxymetazoline (1 μM) for the 2A subtype and spiroxatrine (1 μM) for 2C subtype. 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, Maryland, USA) 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). Non-specific binding was determined in the presence of imipramine (1 μM). Filtration was done over pre-soaked 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.[14,15] 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 were incubated under optimized experimental conditions. The reaction was stopped by filtration as above, except for $\alpha_{1A}$ and $D_3$, 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.

### 5.5.2. In vivo pharmacology

**5.5.2.1. 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.

**5.5.2.2. 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.

**5.5.2.3. 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 one to tabulate the $ED_{50}$ and its confidence interval as a function of the slope of the log dose–response curve.[16]

**5.5.2.4. 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), pre-treated with test compound or solvent. Criterion for drug-induced reversal: absence of loss of righting (1.0% false positive controls; $n > 400$).

**5.5.2.5. *p*-Chloroamphetamine-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) pre-treated 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; $n > 200$).

## Acknowledgments

The authors thank Ms. Valle Ancos, Ms. Alcira Del Cerro, and Ms. Encarna Matesanz for the synthesis of some starting materials and Mr. Luis Miguel Font and Ms. Carmen Nieto for their skilful analytical and chromatographic assistance. They also acknowledge Dr. Shirley Pullan and Ms. Ilse Lenaerts for their help and assistance in binding experiments, to Mr. P.C.M. Vermote and Mr. K.A. Hens for performing the pCA and medetomidine tests, respectively, and Dr. Antonio Gómez for his help in management and coordination of all data generated.

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