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# Design and synthesis of new potent, silent 5-HT$_{1A}$ antagonists by covalent coupling of aminopropanol derivatives with selective serotonin reuptake inhibitors

M. Perez[a**], P.J. Pauwels[b], I. Pallard-Sigogneau[a], C. Fourrier[a], P. Chopin[c], C. Palmier[b], V. Colovray[d], S. Halazy[a*]

*Medicinal Chemistry[a], Cellular and Molecular Biology[b], Neurobiology[c], Analytical Chemistry[d]*
*Centre de Recherche Pierre Fabre, 17, Avenue Jean Moulin, 81106 Castres Cédex, France*

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**Abstract:** Hybrid molecules built up by covalent coupling of aminopropanol derivatives (especially pindolol) with antidepressant drugs like fluoxetine, paroxetine or milnacipran were found to be potent and silent 5-HT$_{1A}$ antagonists ($K_B$ < 1 nM for **7c** and **9a**). © 1998 Elsevier Science Ltd. All rights reserved.

Pindolol **1**, a mixed 5-HT$_{1A}$/β-adrenoceptor antagonist, has recently been shown to potentiate the antidepressant effect of Selective Serotonin Reuptake Inhibitors (SSRI's) since their association in the treatment of patients with major depression markedly reduces the latency of the antidepressant response in previously untreated patients and induces a rapid improvement in treatment-resistant patients.[1,2]

The beneficial effect of pindolol on the onset of action of SSRI's may probably be explained by its 5-HT$_{1A}$ antagonist character.[3] Indeed, among the different hypotheses proposed to explain the delayed onset of the therapeutic action of SSRI's, the down-regulation of cell body 5-HT$_{1A}$ receptors appears to be particularly well documented for example by recent findings from *in vivo* microdialysis studies in different animal species.[4,5]

Thus, in order to evaluate molecules which would concomitantly antagonize the cell body 5-HT$_{1A}$ receptor and block the serotonin transporter system, we have designed and prepared a new series of molecules build up by hybridization of known 5-HT$_{1A}$ antagonists of the aminopropanol family[6] (pindolol **1**, propranolol **2** and penbutolol **3**) with SSRI's[7] like fluoxetine **4a** or paroxetine **5** but also with the new antidepressant drug milnacipran **6** which is a novel Serotonin Noradrenaline Reuptake Inhibitor.[8]

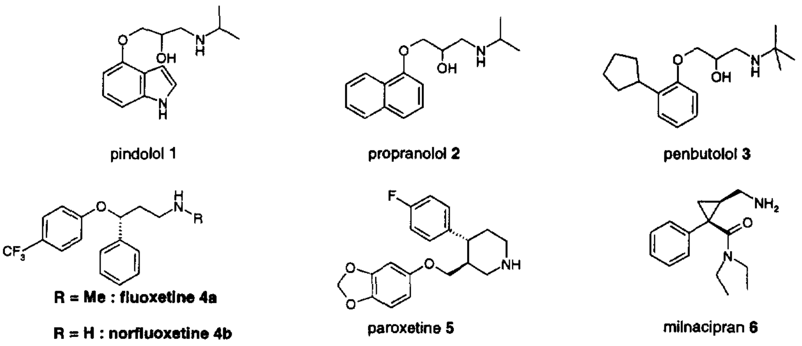
pindolol 1
propranolol 2
penbutolol 3
R = Me : fluoxetine 4a
R = H : norfluoxetine 4b
paroxetine 5
milnacipran 6

**Figure 1**

Our chemical strategy of constructing hybrid molecules from 5-HT$_{1A}$ antagonists and serotonin reuptake inhibitors depicted in figure 1 involved both conceivable approaches recently identified[9] as "the overlapping type" and the "joint type" (figure 2).

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** To whom correspondence should be addressed
E-mail: michel.perez@pierre-fabre.com Fax: (33).5.63.71.43.63

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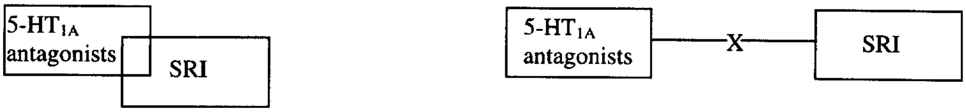


The overlapping type

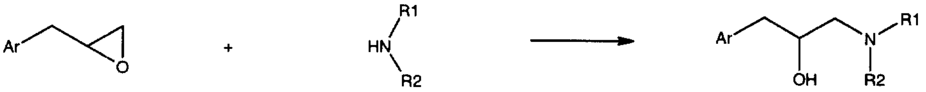
The joint type

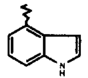
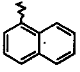
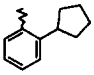
**Figure 2**

Hybrid molecules have been designed by taking advantage of the simultaneous presence in both type of compounds (5-HT$_{1A}$ antagonists and SRI) of a basic nitrogen which has been used as the central common structural unit for the overlapping type of hybrid molecules (compounds **7-11**) and as the point of attachment of the linker X in the case of the joint type of heterodimers **12-14** (table 1). These new heterodimers reported here as well as (-)pindolol have been evaluated at human cloned 5-HT$_{1A}$ receptors (affinity and antagonism potency) and some of them have been tested as serotonin reuptake inhibitors.

## Chemistry

The synthesis of overlapping type molecules **7-11** as well as joint type molecules **12-14** is based on a similar approach implicating the opening of an intermediate epoxide with an amine derivative (Scheme 1).



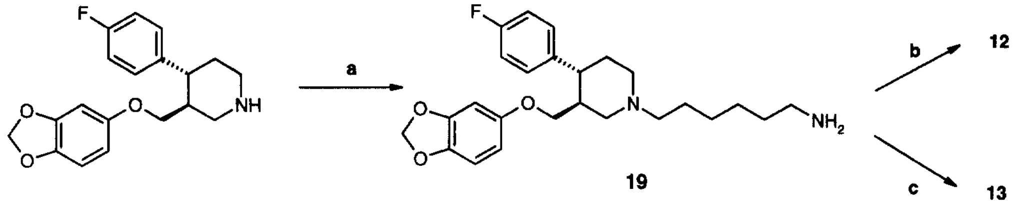
| Ar | epoxides | R1-NH-R2 |
|---|---|---|
|  | **15a (R), 15b (S)** | **norfluoxetine 4b** |
|  | **16a (R), 16b (S)** | **milnacipran 6** |
|  | **17** | **paroxetine 5** |

**Scheme 1**

The racemic mixtures of epoxides were obtained upon reaction of epichlorhydrine with the corresponding phenols, in EtOH, with NaOH as a base. The chiral epoxides were obtained by reaction of glycidyl tosylate[10], with the same phenols, in DMF, with NaH as a base (85-90% ee).

Reaction of norfluoxetine or (S)(+)-norfluoxetine[11] with epoxide intermediates **15-17** in MeOH at reflux overnight led to compounds **7a-c**, **10a,b** and **11** in 23 to 34% yield.[12] Similarly, compound **8** was obtained by reaction of milnacipran[13] with epoxide **15** (43% yield) and compounds **9a,b** upon reaction of paroxetine with epoxides **15a,b** (55 and 66% yield). Moderate yields in the case of norfluoxetine and milnacipran derivatives were partly due to the dialkylation of the primary amine function of these compounds.

Compounds **12** and **13** were prepared according to scheme 2. Thus, the synthesis starts with the preparation of the intermediate amine **19** obtained in 2 steps from paroxetine (80% overall yield). Then, reaction of this amine with epoxides **15** and **16** afforded respectively compound **12** (25% yield) and **13** (35% yield) using the same conditions as described previously.



Reagents and conditions : a) i- NaH (1.3eq), DMF, 0 to 25°C, 1h ; ii- $Br(CH_2)_5CN$ (1.2eq), 25°C, 18 h ; iii- $LAH/Et_2O$ (2eq), $Et_2O$, 0 to 25°C, 30min ; b) **15** (1eq), MeOH, reflux, O.N. ; c) **16** (1eq), MeOH, reflux, O.N.

**Scheme 2**

To prepare compound **14** we used a similar procedure but it was first necessary to protect the primary amine function of norfluoxetine with a Teoc (2-trimethylsilyl)ethyl-4-nitrophenyl carbonate) group (96% yield), then reaction with 5-bromovaleronitrile followed by reduction of the cyano group led to an intermediate amine (46% overall yield). The reaction of this amine with epoxide **16** (43% yield) and subsequent removal of the Teoc group with $NBu_4F$ (93% yield) afforded compound **14**. All the compounds were purified by chromatography on silica gel and subsequent treatment of the free amines with fumaric acid afforded the salts of the compounds[14] suitable for biological evaluation.

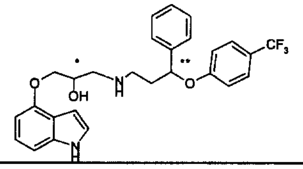
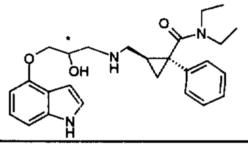
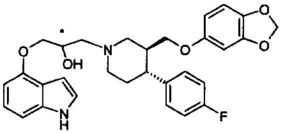
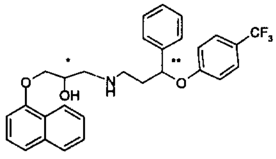
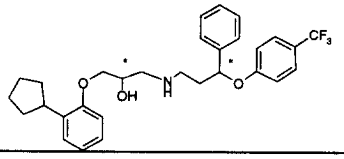
## Biological Results and Discussion

Binding affinity (displacement of $^3H$-8-OH-DPAT) and antagonist potency (antagonism of 5-HT stimulated $[^{35}S]GTP\gamma S$ binding) of compounds **7-11** and (-)pindolol **1** at human cloned $5\text{-}HT_{1A}$ receptors have been determined according to recently published procedures.[15,16] Results reported in table 1 show that, in all cases reported, "overlapping type" of dimers in which pindolol has been hybridized with norfluoxetine (compounds **7a-c**), milnacipran (compound **8**) or paroxetine (compounds **9a-b**) have a better affinity for the human cloned $5\text{-}HT_{1A}$ receptor than pindolol itself and, more interestingly, are also better $5\text{-}HT_{1A}$ antagonists since all 5 of them have a lower $K_B$ value when compared to (-)pindolol. Moreover, and this is probably an important observation, contrary to pindolol which is a partial agonist at $5\text{-}HT_{1A}$ receptors,[17] hybrid molecules **7-10** have no intrinsic activity even at µM concentrations and therefore can be qualified as silent antagonists. At the level of antagonism potency, compounds **7c** ($K_B = 0.2$ nM) and **9a** ($K_B = 0.7$ nM) can be compared to the well-known silent $5\text{-}HT_{1A}$ antagonist, WAY-100,635,[18] which, under the same experimental conditions was found to have a $K_B$ value of 0.34 nM. Interestingly enough, the absolute stereochemistry of the asymmetric carbon atom of the pindolol plays a critical role in the interaction with $5\text{-}HT_{1A}$ receptors since hybrid molecules build up from (S)-pindolol have higher affinity and superior antagonist potency than their corresponding epimers build up from

(R)-pindolol (compare **7c** with **7b** and **9b** with **9a**). These results are in agreement with data reported previously concerning both enantiomers of pindolol since S(-)pindolol is known to bind to 5-HT$_{1A}$ receptors with a much greater affinity than R(+)pindolol.[19] This analogy strongly suggests that pindolol itself and hybrid molecules **7-9** have common binding interactions with the 5-HT$_{1A}$ receptor.

Replacement of the pindolol moiety in hybrid molecules derived from norfluoxetine (compounds **7a-c**) by propranolol (as in compounds **10a-b**) or by penbutolol (as in compound **11**) also leads to 5-HT$_{1A}$ antagonists. However, these hybrid molecules appear to be clearly less potent antagonists than the above-described pindolol derivatives **7** or **9**, in agreement with a concomitant decrease in affinity (Table 1).

**Table 1**

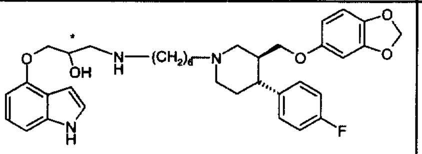
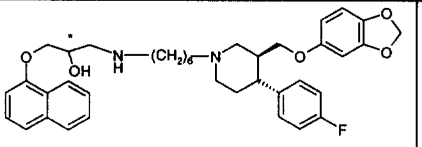
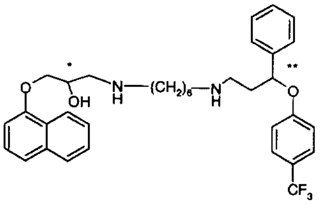
| | | **Stereochemistry** | | **5-HT$_{1A}$** | |
|---|---|---|---|---|---|
| | | * | ** | **Ki**[a] | **K$_B$**[a] |
|  | **7a** | (R,S) | (R,S) | 18.7 | 2.2 |
| | **7b** | R | S | 60.3 | 1.4 |
| | **7c** | S | S | 7.8 | 0.2 |
|  | **8** | (R,S) | NA | 45.4 | 14.8 |
|  | **9a** | S | NA | 5.9 | 0.7 |
| | **9b** | R | NA | 15.7 | 7.5 |
|  | **10a** | S | S | 32.9 | 37.9 |
| | **10b** | R | S | 142 | 388 |
|  | **11** | (R,S) | (R,S) | 36.4 | 15.2 |
| (-)pindolol | **1** | S | NA | 110 | 27.1 |

[a] Ki and KB values are given in nM as a mean values of two or three experiments, each performed in duplicate, typically with individual values within 10-20% of the mean; NA: not applicable

In the case of the "joint type" heterodimers **12-14** (Table 2), the results obtained at 5-HT$_{1A}$ receptors show that bridging pindolol with paroxetine through an hexamethylene linker also leads to the identification of a new potent 5-HT$_{1A}$ antagonist (K$_B$ of 4.3 nM for compound **12** compared to 27 nM for (-) pindolol). However, similar heterodimers in which propranolol has been coupled to paroxetine (compound **13**) or fluoxetine (compound **14**) were found to bind to 5-HT$_{1A}$ receptors with much lower affinity while retaining antagonist properties.

The affinity of the new hybrid molecules **7-14** has also been evaluated at the 5-HT reuptake site, in order to identify molecules with a dual mechanism of action. Unfortunately, the new hybrid molecules were found to interact moderately with the 5-HT uptake site. Better results were obtained with paroxetine derivative **9b** (IC$_{50}$ = 85 nM compared to 2.8 nM for paroxetine) and with milnacipran derivative **8** (IC$_{50}$ = 120 nM compared to 100 nM for milnacipran). Interestingly enough, this particular compound was also found to bind to the noradrenaline uptake site (IC$_{50}$ = 500 nM) and when tested *in vivo*, in the forced swimming test model in mice,[20] it displayed an antidepressant-like activity but with a bell-shaped curve (maximum effect of 36% compared to 88% for milnacipran). Thus, compound **8**, which is an "overlapping type" of hybrid molecule build up from milnacipran and pindolol possesses the cumulated properties of both monomers (inhibition of 5-HT and noradrenaline uptake sites together with antagonism at 5-HT$_{1A}$ receptors) although with a relatively modest potency.

**Table 2**

| | | **Stereochemistry** | | **5-HT$_{1A}$** | |
|---|---|---|---|---|---|
| | | * | ** | **Ki**[a] | **K$_B$**[a] |
|  | **12** | (R,S) | NA | 6.7 | 4.3 |
|  | **13** | (R,S) | NA | 156 | 188 |
|  | **14** | (R,S) | (R,S) | 391 | 1730 |

[a] Ki and KB values are given in nM as a mean values of two or three experiments, each performed in duplicate, typically with individual values within 10-20% of the mean; NA: not applicable

In conclusion, the covalent coupling of aminopropanol derivatives (especially pindolol) with selective serotonin reuptake inhibitors gives new, silent 5-HT$_{1A}$ antagonists, which are clearly more potent than the parent aminopropanol derivatives. However, with the noticeable exception of compound **8** resulting from coupling milnacipran with pindolol, none of this compound binds to the serotonin reuptake site. Work is in progress to evaluate the *in vivo* pharmacological properties of the hybrid molecules reported herein, especially in animal models of depression.

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