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DIMERS OF $5HT_1$ LIGANDS PREFERENTIALLY BIND TO $5HT_{1B/1D}$ RECEPTOR SUBTYPES

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Abstract. New dimers of known $5HT_1$ ligands (5HT, 1-NP or 8-OH-DPAT) have been prepared and evaluated at human cloned $5HT_{1B}$, $5HT_{1D}$ and $5HT_{1A}$ receptors. Binding experiments show that all these dimers have better affinities at $5HT_{1B/1D}$ receptors than their corresponding monomeric ligands. Studies of inhibition of the forskolin-stimulated c-AMP formation mediated by the human $5HT_{1B}$ receptor show that hetero-bivalent ligands [combining an agonist (5HT) with an antagonist (1-NP)] behave as partial agonists while the intrinsic activity of bivalent antagonists (combining two 1-NP residues) was found to be spacer dependent. Surprisingly enough, the dimer of 8-OH-DPAT **6** binds to $5HT_{1A}$, $5HT_{1B}$ and $5HT_{1D}$ receptors with similar high affinity.

Among the large family of serotonin (5-HT) receptors, the $5HT_1$ subfamily has received considerable attention in the field of drug design and discovery.[1] The $5HT_1$ receptors appear to have the highest multiplicity in human with five receptor subtypes cloned to date ($5HT_{1A}$, $5HT_{1B}$, $5HT_{1D}$, $5HT_{1E}$ and $5HT_{1F}$). Human $5HT_{1B}$ and $5HT_{1D}$ receptor subtypes differ from each other on the basis of molecular biology studies and on the basis of recent pharmacological characterizations.[2-5] To date, functional distinction between $5HT_{1B}$ and $5HT_{1D}$ receptor subtypes is under extensive investigations, but recent results suggest that the $5HT_{1B}$ receptor is probably the subtype involved in vasoconstriction[6] as well as in the control of serotonin release in terminal brain areas.[7] $5HT_{1B}$ agonists are under extensive investigations as potential new anti-migraine drugs[8] and the search for $5HT_{1B}$ antagonists has recently been identified as a new promising route in the field of antidepressant therapy.[9]

Among the different methods currently available for medicinal chemists to design potent and selective receptor subtype ligands, the so-called "bivalent ligand" approach appears very promising since many examples of molecules including two pharmacophores in a single ligand have been found[10-12] to have enhanced activity and selectivity over their respective monomer counterparts. We have recently reported[12] dimerization of serotonin **1** as a very efficient method to design potent and selective $5HT_{1B/1D}$ agonists (especially when compared to serotonin itself). Results obtained in that study support the hypothesis that the important increase in potency of the serotonin dimers can be attributed to the presence of two 5-HT pharmacophores in the same molecule, while the enhanced selectivity for $5HT_{1B/1D}$ receptor subtypes could be due to the choice of the position of the spacer attachment to serotonin.

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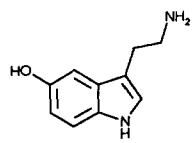
In this paper, we wish to report about our recent investigations concerning the evaluation of the scope of the bivalent ligand approach as a general method to design potent and selective $5HT_{1B/1D}$ ligands. For that purpose, we have prepared and evaluated bivalent ligands of formula **3** which result from the dimerization of the known[13] non-selective $5HT_1$ antagonist, 1-naphtyl-piperazine (1-NP) **2**, mixed dimers **4**, in which 5HT and 1-NP have been covalently linked together and finally, the synthesis and properties of compound **6**, a dimer of the well-known $5HT_{1A}$ agonist,[1] 8-OH-DPAT (**5**) will also be presented and discussed. The nature of the linkers in compounds **3**, **4**, **6** and the position of the linker attachment to each monomer have been determined by analogy with our previously reported study on serotonin dimers.[12]

Scheme 1



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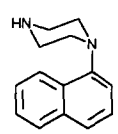

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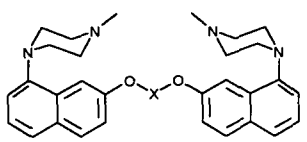

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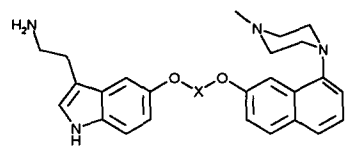

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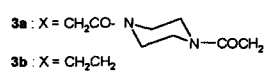
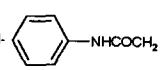
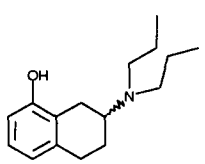


3a : X = CH_2CO-

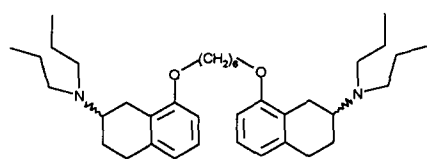
3b : X = CH_2CH_2

4a : X = CH_2CH_2

4b : X = CH_2CONH- 



5

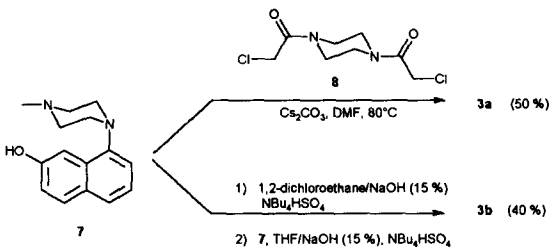


6

Chemistry[14]

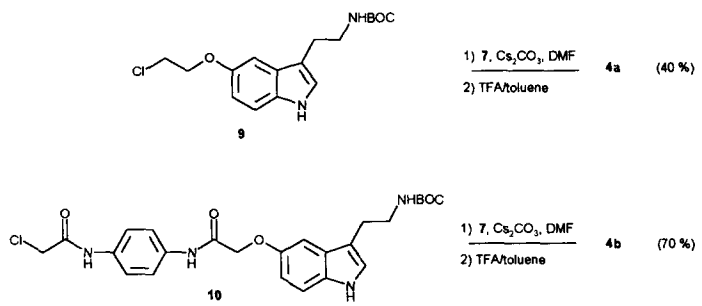
Dimer of 1-NP **3a** (mp: 220°C) has been prepared by condensation of two equivalents of N-methyl-(7-hydroxy naphth.1yl) piperazin 1-yl **7**[15] with N,N'-(bis-chloro-acetyl)piperazine **8**[12] in the presence of cesium carbonate in DMF (Scheme 2). Dimer **3b** (mp: 210°C) was obtained by phase transfer catalysis with tetrabutylammonium hydrogenosulfate in two steps. The chloroethyl derivative was first isolated using 1,2-dichloroethane as solvent. The crude was then reacted with a second equivalent of naphtol 7 in THF (Scheme 2).

Scheme 2



The hybrid molecules **4a** (mp: 180°C) and **4b** (mp: 126°C) in which serotonin and 1-NP are covalently linked together have been prepared by condensation of the 7-hydroxy-naphtyl-piperazine derivative 7 with the electrophilic serotonin derivatives **9** or **10** respectively, according to Scheme 3.

Scheme 3



Intermediate **9** has been prepared as previously described[16] and the chloroacetyl derivative **10** has been prepared by condensation of the previously reported corresponding free aniline[17] with chloroacetyl chloride (K_2CO_3, methyl-ethyl-ketone; 80 % yield).

Finally, the 8-OH-DPAT dimer **6** (mp: 180°C) is easily obtained upon condensation of racemic 8-OH-DPAT with 1,6-dibromo-hexane in the presence of cesium carbonate in DMF (85 % yield). It should be pointed out here that compound **6** is a mixture of stereoisomers. So far, efforts to separate the diastereoisomers of **6** by HPLC were unsuccessfull. Work is in progress to prepare individual isomers by using both enantiomers of 8-OH-DPAT as starting material.

Results and discussion

The binding affinities of bivalent ligands **3a-b**, **4a-b** and **6** as well as 5HT (**1**), 1-NP (**2**) and 8-OH-DPAT (**5**) included for comparison purposes have been determined at recombinant human $5HT_{1A}$, $5HT_{1B}$ and $5HT_{1D}$ receptors according to previously reported procedures.[5,18] The intrinsic activity of these compounds has been assessed as their ability to inhibit forskolin-stimulated c-AMP formation mediated by cloned $5HT_{1B}$ receptors in a CHO cell line.[19] Table 1 summarizes the results obtained with compounds **1-6**. In all cases reported, compounds having two pharmacophoric residues in the same molecule bind to $5HT_{1B/1D}$ receptor subtypes with a much better

affinity compared to their respective monomer. The two dimers of formula **3** in which two naphtylpiperazine moieties are linked together through a 7-hydroxy residue have similar higher affinity for $5HT_{1B/1D}$ receptors than 1-NP itself, although spacers differ considerably. The affinity of naphthylpiperazine dimer **3a** for $5HT_{1A}$ receptors is only slightly improved when compared to 1-NP itself. Hetero-bivalent ligands **4a** and **4b** bind to $5HT_{1B/1D}$ receptors with subnanomolar affinities and therefore with higher affinity than both monomers, serotonin or 1-NP. In the case of compound **4a**, affinity for the $5HT_{1A}$ receptor is also clearly improved thus resulting in a compound with low binding selectivity ratio between $5HT_{1B/1D}$ and $5HT_{1A}$ receptor subtypes. More spectacular is the case of 8-OH-DPAT dimerization: 8-OH-DPAT (**5**) is known as a selective $5HT_{1A}$ agonist, as confirmed in our hands since (±) 8-OH-DPAT binds to the $5HT_{1A}$ receptor with nM affinity (Ki = 1.5 nM) but recognizes $5HT_{1B/1D}$ receptor subtypes with much lower affinity (see Table 1). Dimerization of (±) 8-OH-DPAT through the 8-hydroxy residue by using a n-hexyl linker as found in compound **6** provides a high-affinity $5HT_{1A/1B/1D}$ ligand since this new compound which, to our knowledge, represents the first example of a 8-OH-DPAT dimer binds to the three receptor subtypes with similar nanomolar affinities. Comparison of the binding affinities of compounds **5** and **6** at the $5HT_{1B}$ receptor subtype indicates that the dimerization of 8-OH-DPAT allows the improvement of binding affinity almost by 2 log units. In summary, binding results obtained with bivalent ligands in which two naphthylpiperazine residues have been linked together, with bivalent hetero ligands in which serotonin and 1-NP have been covalently linked together and with a bivalent ligand build from the selective $5HT_{1A}$ agonist 8-OH-DPAT show that dimerization of a known $5HT_1$ ligand clearly improves its binding affinity for $5HT_{1B/1D}$ receptor subtype, to a larger extend than for the $5HT_{1A}$ receptor, thus resulting in the design of new, more potent and (in some cases) more selective $5HT_{1B/1D}$ ligands.

Table 1

	Ki (nM)*			
	$5HT_{1B}$	$5HT_{1D}$	$5HT_{1A}$	$5HT_{1B}$ Intrinsic activity**
5-HT (**1**)	6.76 ± 1.19	5.06 ± 0.99	2.48 ± 0.26	Full agonist (EC_{50} = 4.67 ± 2.33 nM)
1-NP (**2**)	10.6 ± 0.04	6.18 ± 3.38	9.23 ± 0.33	Antagonist (EC_{50} > 10000 nM)
3a	2.56 ± 0.04	1.24 ± 0.28	4.0 ± 1.4	Partial agonist
3b	2.10 ± 0.65	1.71 ± 1.17	14.0 ± 6.2	Antagonist (EC_{50} > 1000 nM)
4a	0.76 ± 0.51	0.40 ± 0.22	0.96 ± 0.14	Partial agonist
4b	0.70 ± 0.56	0.50 ± 0.29	5.8 ± 6.0	Partial agonist
8-OH-DPAT (**5**)	559 ± 327	115 ± 32	1.49 ± 0.83	260 ± 141 nM
6	6.40 ± 0.60	3.90 ± 0.96	6.80 ± 0.44	50 nM

* Values are given as the mean value of two or three experiments each performed in duplicate; [^{3}H]-5CT and [^{3}H]-8-OH-DPAT were used as radioligands respectively at $5\text{-}HT_{1B/1D}$ and $5\text{-}HT_{1A}$ receptors.
** as assessed by inhibition of forskolin-stimulated c-AMP formation

We have previously reported[12] that dimers of serotonin were not only potent $5HT_{1B/1D}$ ligands but were also very potent $5HT_{1B/1D}$ agonists since almost all reported examples of serotonin dimers were characterized as full agonists with subnanomolar EC_{50} values (inhibition of forskolin-stimulated c-AMP formation). 1-NP (**2**) has previously been characterized as another non-selective $5HT_{1B/1D}$ ligand[13] but which differs from serotonin by its intrinsic activity since 1-NP has been characterized as a $5HT_{1B/1D}$ antagonist[15,20] as confirmed by our results at the cyclase level coupled to human cloned receptors in a CHO cell line (Table 1). Interestingly enough, under the same conditions, 1-NP dimer **3b** was also identified as a silent $5HT_{1B}$ receptor antagonist but 1-NP dimer **3a**, in which the linker between both 7-hydroxy-naphthylpiperazine residues is an ethylene bridge appears as a partial agonist since this particular compound is able to inhibit forskolin-stimulated c-AMP formation in CHO cells expressing human $5HT_{1B}$ receptors (EC_{50} = 21 nM). These results show that at least in the case of 1-NP, dimerization of an antagonist can lead to bivalent ligands with different intrinsic activity profiles depending on the nature of the linker. In comparison, hybrid bivalent molecules **4a** and **4b** in which an agonist (5-HT) and an antagonist (1-NP) have been covalently linked together by two different linkers were found to be partial agonists at $5HT_{1B}$ receptors. Interestingly enough, hetero-bivalent ligands **4a** and **4b** bind to $5HT_{1B}$ receptors with similar affinities than previously reported serotonin dimers having the same spacers but differ on what intrinsic activity is concerned since corresponding 5-HT dimers are potent full agonists at $5HT_{1B}$ receptor subtype while heterodimers **4a** and **4b** are partial agonists. The results reported here with these heterodimers in which an agonist (5-HT) and an antagonist (1-NP) (having roughly the same affinity for $5HT_{1B}$ receptor) suggest that these compounds are able to simultaneously stabilize the active (GTP bound) and the inactive conformations of the $5HT_{1B}$ receptor, according to the ternary allosteric model.[21]

In conclusion, results disclosed in this paper with new bivalent ligands show that, in all cases reported, dimerization of known $5HT_1$ ligands appears as a general way to improve binding affinity and selectivity for $5HT_{1B/1D}$ receptors. This effect is particular spectacular in the case of 8-OH-DPAT dimer **6** which binds to $5HT_{1B}$ receptors with an affinity 100 times greater than 8-OH-DPAT itself. However, for what intrinsic activity is concerned, no general conclusion can be reached. In the case of $5HT_{1B}$ receptors, we have previously reported that dimerization of an agonist is a useful method to generate more potent agonists; results reported here show that hetero-bivalent ligands combining an agonist (5-HT) with an antagonist (1-NP) generate partial agonists but the intrinsic activity of bivalent ligands in which two antagonist pharmacophores have been linked together is dependant on the nature of the spacer.

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