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5-O-CARBOXYMETHYL PIPERAZIDE DERIVATIVES OF SEROTONIN : A NEW CLASS OF POTENT AND SELECTIVE 5-HT_{1D} RECEPTOR AGONISTS

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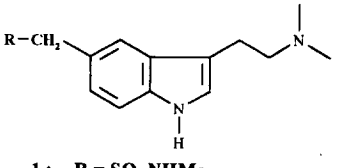
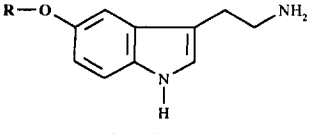
Abstract: New 5-O-carboxymethyl piperazide derivatives of tryptamine (**6a-d**) have been prepared in 4 steps from serotonin as 5-HT_{1D} receptors agonists. Binding studies with cloned human 5-$HT_{1D\alpha}$ and 5-$HT_{1D\beta}$ receptors demonstrate that these derivatives are high affinity ligands at both receptor subtypes and inhibition of forskolin mediated cyclase studies with human 5-$HT_{1D\beta}$ and 5-HT_{1A} receptors show that these compounds are very efficient and selective 5-$HT_{1D\beta}$ agonists.

The design of potent and selective agonists or antagonists at serotonin receptor subtypes has been a subject of intense study during the last fifteen years, especially in the field of drug discovery[1]. Serotonin (5-HT) receptors have been classified[2] into seven families (5-HT_{1-7}), the 5-HT_1 family being the most heterogeneous with five major subtypes (5-$HT_{1A, 1B, 1D, 1E, 1F}$). The 5-HT_{1D} receptors have been divided into two distinct receptor subtypes both negatively coupled to adenylate cyclase and identified in human tissues as 5-$HT_{1D\alpha}$ and 5-$HT_{1D\beta}$ on the basis of molecular biology and cloning studies[3]. To date, no clear pharmacological distinction between 5-$HT_{1D\alpha}$ and 5-$HT_{1D\beta}$ receptor subtypes has been reported and their respective physiological role remains to be determined[4,5].

However, the recent discovery of the 5-HT_{1D} receptor agonist sumatriptan (**1**) as an anti-migraine drug[6] with proven clinical efficacy has stimulated a strong interest and important efforts in the fields of chemical synthesis, pharmacological characterisation and clinical evaluation of new potent 5-HT_{1D} receptor agonists[7]. Among them, MK 462 (**2**) and 311C90 (**3**) are examples of the most advanced derivatives in clinical trials[8].



1 : R = SO_2NHMe
2 : R = 1, 2-4-triazolyl
3 : R = 2-oxo-1,3-oxazolidin-4-yl



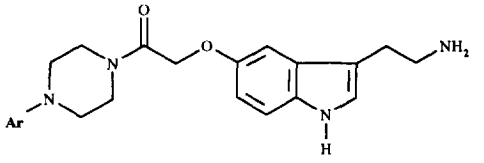
4 : R = CH_2CO-Gly-Tyr-NH_2
5 : R = n C_9H_{19}

It is interesting to note that compounds **1**, **2** and **3** (as well as many other 5-HT_{1D} agonists[9]) have in common a tryptamine moiety which is linked through a carbon-carbon bond to a specific distinct structural subunit. In contrast, the peptide derivative **4** (S-CM-$GTNH_2$)[10] is an O-substituted tryptamine derivative

known as a selective 5-HT_{1D} ligand (IC_{50} = 60 nM for the guinea pig 5-HT_{1D} receptor) although less efficient than sumatriptan in the model of meningeal vascular constriction[11].

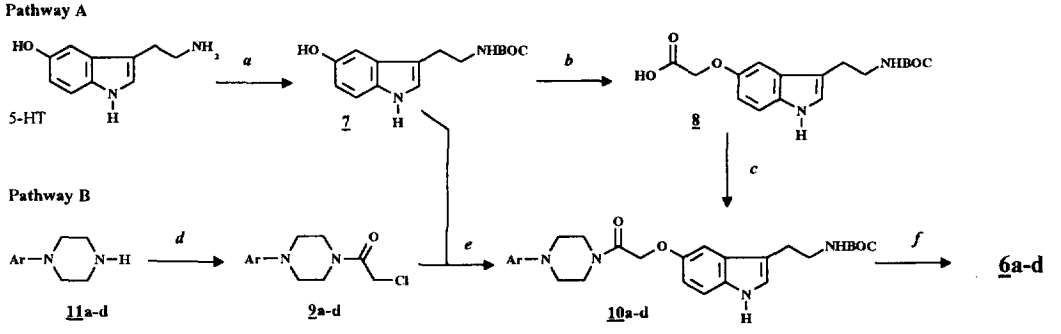
More recently, Glennon and co-workers have reported[12] a non-peptide O-alkylated tryptamine derivative (**5**) termed "NOT" as a potent, selective 5-$HT_{1D\beta}$ agonist. When compared to the 5-C-alkylated tryptamine derivatives **1-3** and their congeners, such types of compounds have the advantage of being easily prepared from serotonin itself.

This result prompted us to report our preliminary work regarding the synthesis, affinity and intrinsic activity at human 5-HT_{1D} and 5-HT_{1A} receptors of a new class of O-substituted tryptamine derivatives of formula **6**.



6a : Ar = C_6H_5
6b : Ar = o-$CH_3C_6H_4$
6c : Ar = m-$OCH_3C_6H_4$
6d : Ar = 1-Naphtyl

The synthesis of the piperazide derivatives of serotonin of formula **6a-d** can be easily achieved by two different routes according to scheme 1. Both of them allow the preparation of the desired compounds **6a-d** in 4 steps starting from serotonin itself by taking advantage of the regioselective alkylation of the phenol oxygen of N-BOC-serotonin **7**. Thus, the synthesis starts with the protection of the amino group of 5-HT by treating the creatinine salt of serotonin with $(BOC)_2O$ (1.5 eq) in the presence of NaOH in water to afford the intermediate **7** in 89 % yield. According to pathway A, this intermediate has been transformed into N-BOC O-carboxymethyl-serotonin **8**[13] by reaction with methyl bromoacetate followed by ester hydrolysis using potassium hydroxyde. Formation of the amide bond by condensation of the carboxylic acid **8** with the appropriate aryl-piperazine **11a-d** is then achieved by using ethyl chloroformate (1.1 eq) at - 15°C and excess N-methyl morpholine in dichloromethane. The intermediate aryl piperazides **10a-d** are then isolated in 76 to 84 % yields after flash chromatography purification on silica gel[14].



Reagents and conditions: a) $(BOC)_2O$ (1.5eq), NaOH, H_2O, 25°C, 89%; b) i- $MeOOCCH_2Br$ (1.8 eq), MEK, K_2CO_3 (2.5 eq), KI (0.1 eq), reflux, 5 h, 91%; ii- KOH (4 eq), EtOH, 25°C, 90%; c) ClCOOEt (1.1 eq), NMM (1.1 eq), CH_2Cl_2, -15°C, 10 min then **11a-d** (1.8 eq), -10°C to 25°C, 2-3 h, 76-84%; d) $ClCOCH_2Cl$ (1.2 eq), MEK, $CaCO_3$ (1.5 eq), 0°C, 74-97%; e) MEK, K_2CO_3 (2.5 eq), KI (0.1 eq), reflux, 5 h, 78%; f) TFA (excess), toluene, 25°C, 1-2 h, 64-80%

Scheme 1

These key intermediates **10a-d** can also be easily prepared in another very efficient way (pathway B) by condensing N-BOC-serotonin **7** with the α-chloro-methyl amides **9a-d** in the presence of potassium carbonate and potassium iodide in methyl ethyl ketone at refluxing temperature (70-80 % yield after flash chromatography purification on silica gel). This synthetic pathway is particularly interesting since the intermediates **9a-d** are almost quantitatively prepared by condensing an aryl piperazine **11a-d** with chloroacetyl chloride in the presence of calcium carbonate in methyl ethyl ketone. It is noteworthy that tentatives to condense chloro acetyl chloride with one equivalent of N(o-tolyl)-piperazine **11b** followed by N-BOC-serotonin in "one pot" give only moderate yields of the expected product **10b** together with unidentified side-products.

Finally, the hydrochloride salts of the aryl piperazide derivatives of serotonin **6a-d** suitable for biological evaluation have been isolated after removal of the BOC protecting group of compounds **10a-d** upon reaction with excess TFA in toluene at 25°C and subsequent treatment of the free amine with HCl in dichloromethane.

The binding affinities of compounds **6a-d** and sumatriptan **1** have been determined at cloned human 5-$HT_{1D\alpha}$, 5-$HT_{1D\beta}$ and 5-HT_{1A} receptors[15,16] . The intrinsic activity of these compounds was assessed as their ability to inhibit the forskolin-stimulated activity of adenylate cyclase coupled to human 5-$HT_{1D\beta}$ receptors in CHO-K_1 cells and to human 5-HT_{1A} receptors in HeLa cells[16]. The results obtained are summarized in table 1 :

TABLE 1[19]

Cpd	Ki (nM) ± SEM			EC_{50} (nM)	
	5-$HT_{1D\alpha}$	5-$HT_{1D\beta}$	5-HT_{1A}	5-$HT_{1D\beta}$	5-HT_{1A}
6a	1.2	0.6	11.4	0.45	50-550
6b	2.1 ± 0.5	0.87 ± 0.46	7.7 ± 1.3	0.6-2.0	110-250
6c	2.0 ± 0.7	0.4 ± 0.08	9.8 ± 0.3	0.24-0.55	85-370
6d	0.95 ± 0.01	0.52 ± 0.23	8.9 ± 0.5	2.8-6.5	95-320
Sumatriptan	8.5 ± 3.5	23.1 ± 3.7	440 ± 90	30-70	> 1000

Compounds **6a-d** are very potent ligands at human 5-HT_{1D} receptors especially when compared to sumatriptan (table 1). By contrast to NOT, which has been reported[12] to be selective for the 5-$HT_{1D\beta}$ human receptor, compounds **6a-d** are almost equipotent at both 5-$HT_{1D\alpha}$ and 5-$HT_{1D\beta}$ receptors. The subnanomolar Ki values obtained for the naphtyl piperazide derivative of serotonin **6d** are among the highest affinities reported to date for ligands binding at 5-HT_{1D} receptor subtypes. Interestingly, all four aryl piperazide derivatives of serotonin **6a-d** have also been identified as high efficacy 5-$HT_{1D\beta}$ receptor agonists with EC_{50} values in inhibiting forskolin stimulated adenylate cyclase in the nanomolar range. In this respect, compounds **6a** and **6c**, with subnanomolar EC_{50} values appear to be much more potent agonists than sumatriptan (EC_{50} : 40 nM) and NOT (reported EC_{50} value : 68 nM)[12]. By contrast, compounds **6a-d** are weak 5-HT_{1A} agonists when considering the EC_{50} values obtained at the second messenger level in HeLa cells. Thus, despite a low selectivity ratio at the binding level, compounds **6a-d** show an interesting selectivity

pattern when comparing their agonist efficacies in 5-$HT_{1D\beta}$ and 5-HT_{1A} receptors[17]. The agonist potency of compounds **6a-d** has been confirmed in the rabbit isolated saphenous vein preparation[18]. For example, compound **6a** (pD_2 = 7.36) was far more potent in contracting the rabbit saphenous vein than sumatriptan (pD_2 = 5.75).

Collectively, the results obtained in human cloned receptors in cellular systems demonstrate that the new aryl-piperazide derivatives of serotonin **6a-d** which can be easily prepared in 4 steps from serotonin are very potent and selective 5-HT_{1D} receptor agonists. Further studies are in progress to determine the potency and selectivity of these new series of 5-HT_{1D} agonists under physiological conditions, details of which will be reported in a forthcoming full paper.

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