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DESIGN AND SYNTHESIS OF 2-[4-[4-(*m*-(ETHYLSULFONAMIDO)-PHENYL)PIPERAZIN-1-YL]BUTYL]-1,3-DIOXOPERHYDROPYRROLO[1,2-*c*]IMIDAZOLE (EF-7412) USING NEURAL NETWORKS. A SELECTIVE DERIVATIVE WITH MIXED 5-HT$_{1A}$/D$_2$ ANTAGONIST PROPERTIES

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Abstract: A test series of 32 phenylpiperazines **III** with affinity for 5-HT$_{1A}$ and α_1 receptors was subjected to QSAR analysis using artificial neural networks (ANNs), in order to get insight into the structural requirements that are responsible for 5-HT$_{1A}$ /α_1 selectivity. Good models and predictive power were obtained for 5-HT$_{1A}$ and α_1 receptors. A comparison of these models gives information for the design of the new ligand **EF-7412** (5-HT$_{1A}$: K_i (nM)= 27; α_1: K_i (nM) > 1000). This derivative displayed affinity for dopamine D$_2$ receptor (K_i= 22 nM) and is selective for all other receptor examined (5-HT$_{2A}$, 5-HT$_3$, 5-HT$_4$ and Bz). **EF-7412** acts an antagonist *in vivo* in pre- and postsynaptic 5-HT$_{1A}$ receptor sites and as an antagonist in dopamine D$_2$ receptor.

The discovery of multiple serotonin (5-HT) receptor subtypes in recent years has been accompanied by a parallel explosion in the development of drugs that alter 5-HT neurotransmission. The 5-HT$_{1A}$ receptor is the most intensively studied, as a result of it is involved in a variety of physiological and pathophysiological processes.[1-3] The 5-HT$_{1A}$ receptor belong to the class of G-protein coupled receptors (GPCRs) and the members of this class have a number of characteristic amino acid patterns in common, in spite of their different pharmacology. In particular, the transmembrane amino acid sequence of the 5-HT$_{1A}$ subtype is noteworthy for its high degree of homology to α_1-adrenergic receptor subtypes,[4] so, a great number of ligands display affinity for both receptors. In this way, we have reported recently a new series of bicyclohydantoin- and imide-arylpiperazines **I,II**,[5-9] in which we have undertaken a systematic research of the structural factors that are responsible for 5-HT$_{1A}$/α_1 selectivity. The study of the alkyl chain and the amide substructure have allowed us to suggest some important differences between the no-pharmacophoric sites of both receptors.[9] Regarding the aromatic ring substitution[7] it seems that *ortho*- and *meta*-positions are favorable for the affinity at both receptors, while *para*-substituted derivatives are practically inactive.

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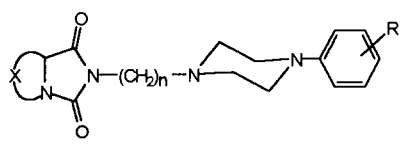

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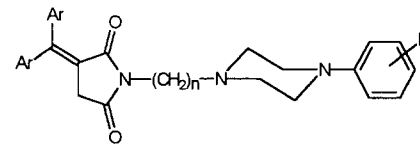
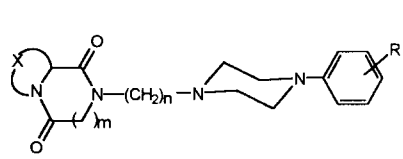
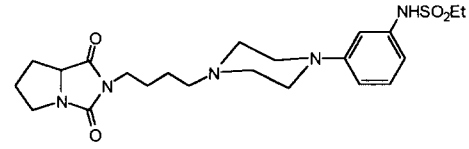


II

In order to gain insight into the physicochemical influence of the 5-HT_{1A}/α_1 receptor pharmacophores we have designed a training set of 32 compounds of general structure **III**. The amide moiety is a bicyclohydantoin or a diketopiperazine (X = $-(CH_2)_3-$, $-(CH_2)_4-$; m = 0, 1); the spacer length is 3 or 4 methylene units, which are the optimum values for both receptors, and the aromatic substituent R occupies the *ortho-* or *meta-* positions and it has been selected from a data base of 387 substituents using the EDISFAR program.[10] After the synthesis of the compounds and the evaluation of their affinities for 5-HT_{1A} and α_1 receptors (most of the compounds showed high affinity for 5-HT_{1A} and α_1 receptor binding site), we have carried out quantitative structure-activity relationships using artificial neural networks.[11]



III (1-32)



EF-7412

X = $-(CH_2)_3-$, $-(CH_2)_4-$; m = 0, 1; n = 3, 4
R = *o*-CH_3, *o*-OCH_3, *o*-OBu, *o*-COOPr, *o*-CONHPr, *o*-CN, *m*-CF_3, *m*-NH_2, *m*-NHCOPr, *m*-Br (selected by EDISFAR program)

The data set used was the *in vitro* 5-HT_{1A} and α_1 receptor affinities (expressed as pK_i values). Each compound was parametrized with six physicochemical descriptors (F, R, V_o, V_m, π_o, π_m) and three indicator variables (I_A = 1 or 0 for X = $-(CH_2)_4-$ or $-(CH_2)_3-$, I_B = 1 or 0 for m = 1 or 0, I_n = 1 or 0 for n = 4 or n = 3).The neural network employed for this modeling was a fully connected three layer network (input, hidden, output) trained by back-propagation of error. Initially the number of neurons in the input layer was equal to the number of molecular descriptors and the indicator variables, whereas the output layer had only one neuron. The number of neurons in the hidden layer was determined by trial and error. The best ANN models are shown in Table I. The dependence of biological activity on the physicochemical parameters was illustrated in 3-D diagramans. On the basis of the obtained plots, the 5-HT_{1A} affinity has a nonlinear dependence with F, V_o, V_m and π_o, nevertheless the nonlinear relationship is not far from the planar one. The α_1 affinity has a clear nonlinear dependence with F, V_o, V_m, π_o and π_m. A comparison of both analysis gives an additional understanding for 5-HT_{1A}/α_1 selectivity: (a) High F values increase the binding affinity for 5-HT_{1A} receptors and decrease the affinity for α_1 sites; (b) The lipophilicity at the *meta* position has only influence for the α_1 receptor; (c) The *meta* position seems to be implicated in the 5-HT_{1A}/α_1 selectivity. While the 5-HT_{1A} receptor is able to accomodate bulky substituents (about 60 $Å^3$) in the region of

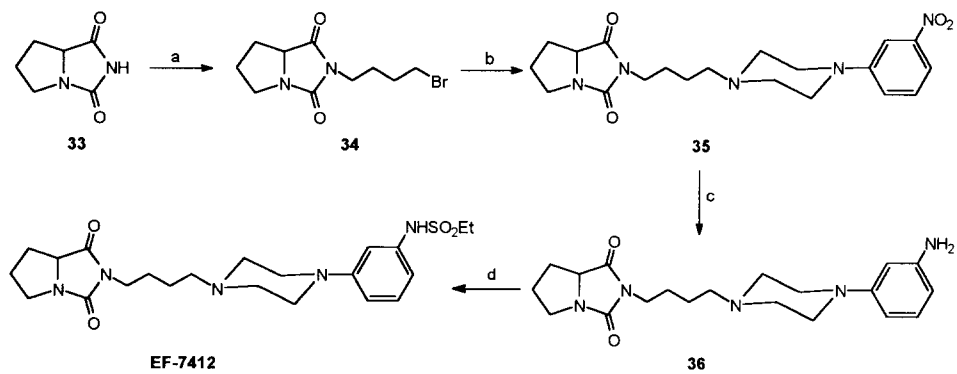
its active site, the steric requirements of the α_1 receptor at this position are more restricted (between 0-22 Å^3). A good way to improve 5-HT$_{1A}$/ α_1 selectivity would be the synthesis of long chain derivatives bearing bulky substituents with high F values and low π values at the *meta* position. Among the different groups that fulfill these requirements the *m*-NHSO$_2$Et was chosen (F = 0.419, π_m = -0.64, V_m = 65.31). On this basis, the new ligand **EF-7412** (X = -(CH$_2$)$_3$-, m = 0, n = 4, R = *m*-NHSO$_2$Et) was designed and synthesized. This compound bound at 5-HT$_{1A}$ sites [$K_{i\ obsd}$ (nM) = 27 ± 6; $K_{i\ calcd}$ (nM) = 36] and showed high selectivity over the α_1 receptor [$K_{i\ obsd}$ (nM) >1000; $K_{i\ calcd}$ (nM) = 2745].

Table I. ANN Models

Receptor	Non-significant Parameters	Architecture	r	r^2	s
5-HT$_{1A}$	R, π_m	7-2-1	0.983	0.966	0.149
α_1	R	8-2-1	0.991	0.982	0.136

Compound **EF-7412** was prepared as shown in Scheme 1. The reaction of the hydantoin **33**[12] with 1,4-dibromobutane in the presence of NaH in *N,N*-dimethylformamide (DMF) afforded the intermediate **34**.[6] Treatment of **34** with 1-(*m*-nitrophenyl)piperazine[13] yielded the nitroderivative **35**,[7] which was reduced to the amino compound **36**[7] by catalytic hydrogenation. The desired compound **EF-7412** was obtained by reaction of **36** with ethylsulfonyl chloride in the presence of pyridine in anhydrous acetone as solvent. Derivative **EF-7412** was characterized by IR and ^{1}H- and ^{13}C-NMR spectroscopy and gave satisfactory combustion analysis (C, H, N).[14] Hydrochloride salt of **EF-7412** was prepared as sample for biological assays.

On the other hand, binding affinities show that compound **EF-7412** possessed an appreciable affinity for D$_2$ receptor subtype (K_i = 22 nM) and is selective for all other receptors examined (5-HT$_{2A}$, 5-HT$_3$, 5-HT$_4$ and benzodiazepine Bz; K_i >1000 nM).



Reagents: (a) NaH, DMF, N_2, 60 °C, 1 h, then Br(CH$_2$)$_4$Br, 110 °C, 2 h; (b) 1-(*m*-nitrophenyl)piperazine, Et$_3$N, acetonitrile, 60 °C, 20 h; (c) H$_2$/10% Pd(C), MeOH, 2 h; (d) EtSO$_2$Cl, pyridine, acetone, N_2, rt, overnight.

Scheme 1

Pharmacological evaluation of the activity of **EF-7412** on 5-HT_{1A} receptor function shows that subcutaneous administration of this compound did not alter rectal temperature neither the serotonergic syndrome (flat body posture and lower lip retraction) or plasma corticosterone levels. In addition, **EF-7412** increased 5-HIAA/5HT and DOPAC/DA ratios in the mouse hypothalamus and slightly decreased spontaneous motor activity in the open field test. However, this compound blocked the hypothermia, the serotonergic syndrome, the enhancement of corticosterone secretion and the increase on 5-HT neuronal activity induced by 8-OH-DPAT. These results suggest that **EF-7412** acts as an antagonist *in vivo* in pre- and postsynaptic 5-HT_{1A} receptor sites. Furthermore, the fact that **EF-7412** increased DA neuronal activity in the mouse hypothalamus and slighlty decreased motor activity in rats (open field test) suggests that this compound may be acting as an antagonist in DA D_2 receptors.

To our knowledge, in this communication we describe the first selective derivative (**EF-7412**) with mixed 5-HT_{1A}/D_2 antagonist properties and this derivative could be useful as a pharmacological tool.

Acknowledgment

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14. Data of **EF-7412**: 82% yield; mp 187-190 °C (methanol/ethyl ether); IR ($CHCl_3$, cm^{-1}) 3200, 1770, 1700; ^1H NMR ($CDCl_3$) δ 1.35 (t, J = 7.5 Hz, 3H, CH_3), 1.50-1.72 (m, 5H, -$(CH_2)_2$-, H_7), 2.05-2.13 (m, 2H, H_6), 2.23-2.27 (m, 1H, H_7), 2.41 (t, J = 7.5 Hz, 2H, CH_2-Npip), 2.57 (t, J = 4.8 Hz, 4H, $2CH_2$-pip), 3.12 (q, J = 7.5 Hz, 2H, SO_2CH_2), 3.20 (t, J = 5.4 Hz, 4H, $2CH_2$-pip), 3.23-3.29 (m, 1H, H_5), 3.50 (t, J = 7.2 Hz, 2H, NCH_2), 3.69 (dt, J = 11.1, 7.2 Hz, 1H, H_5), 4.09 (dd, J = 9.0, 7.5 Hz, 1H, H_{7a}), 6.67-6.71 (m, 2H, H_4- and H_6-phenyl), 6.82 (t, J = 2.1 Hz, 1H, H_2-phenyl), 7.18 (t, J = 8.4 Hz, 1H, H_5-phenyl); ^{13}C NMR ($CDCl_3$) δ 8.2 (CH_3), 23.6, 25.9 (-$(CH_2)_2$-), 26.9 (C_6), 27.5 (C_7), 38.6 (NCH_2), 45.4 (C_5, SO_2CH_2), 48.4 ($2CH_2$-pip), 52.9 ($2CH_2$-pip), 57.7 (CH_2-Npip), 63.3 (C_{7a}), 107.5 (C_2-phenyl), 111.0, 112.1 (C_4- and C_6-phenyl), 130.0 (C_5-phenyl), 137.9 (C_3-phenyl), 152.2 (C_1-phenyl), 160.8 (C_3), 174.0 (C_1). Anal. ($C_{22}H_{33}N_5O_4S.2HCl.1/2H_2O$) C, H, N.