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5-HT_{1A} AND 5-HT_{2A} LIGANDS WITH ANXIOLYTIC AND ANTIPANIC-LIKE PROPERTIES

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Abstract. A series of new benzothiazolin-2-one, benzoxazolin-2-one and benzoxazin-3-one derivatives were synthesized and their binding profile at 5-HT_{1A}, 5-HT_{2A}, 5-HT_{2C} as well as D_2 and α_1 receptors was determined. All studied compounds displayed high to moderate affinity for both 5-HT_{1A} and 5-HT_{2A} receptor subtypes. Among these, one compound (**29**) emerged since it exhibited potent antagonist activities at 5-HT_{1A}, 5-HT_{2A}, D_2 and α_1 central receptors and showed anxiolytic and antipanic-like effects in mice. **29** is currently undergoing preclinical evaluation. © 1997 Elsevier Science Ltd.

Introduction

Over the last decade, with the discovery of the multiplicity of serotonin (5-HT) receptors, considerable interest developed around the physiopathological role of central serotoninergic system in CNS disturbances such as depression, anxiety, schizophrenia and panic disorders. Along this line, 5-HT_{1A} and 5-HT_{2A} receptor subtypes were the targets for medicinal chemistry developments.[1-7] Buspirone acts as a partial agonist at presynaptic 5-HT_{1A} autoreceptors and as an antagonist at the postsynaptic level.[8] Clinical trials evidenced that buspirone was effective in the management of anxiety.[3] On the other hand, ritanserin *(Scheme 1)*, a potent and selective 5-HT_{2A} central antagonist, was also found to improve the symptoms of generalized anxiety disorders.[9,10] Recent studies established that in the search of anxiolytics with reduced side-effects, combination of 5-HT_{1A} and 5-HT_{2A} central antagonism could lead to a new therapeutic concept. Several works in this field confirm this hypothesis.[11,12]

Our previous works concerned with central serotoninergic agents led to the design, synthesis and pharmacological evaluation of a series of benzothiazolin-2-one, benzoxazolin-2-one and benzoxazin-3-one derivatives containing various phenylpiperazine fragments (*Scheme 1*, structure A). These compounds displayed high and selective 5-HT_{1A} affinity combined with moderate D_2 affinity.[13] Several arylpiperazine derivatives were described as potent ligands for the various 5-HT receptors.[14-16] The association of these central serotonin (5-HT) and anti-dopamine properties led as expected to important antipsychotic activities with reduced extrapyramidal side-effects (EPS). In the quest of new anxiolytics with low level of side-effects, we therefore decided to introduce in the compounds of general structure A a supplementary 5-HT_{2A} pharmacophoric pattern using the 2-[4-(4-fluorobenzoyl)piperidin-1-yl]ethyl and 2-[4-(bis(4-fluorophenyl)methylen)-piperidin-1-yl]ethyl moieties already present in ketanserin and ritanserin, respectively *(Scheme 1)*

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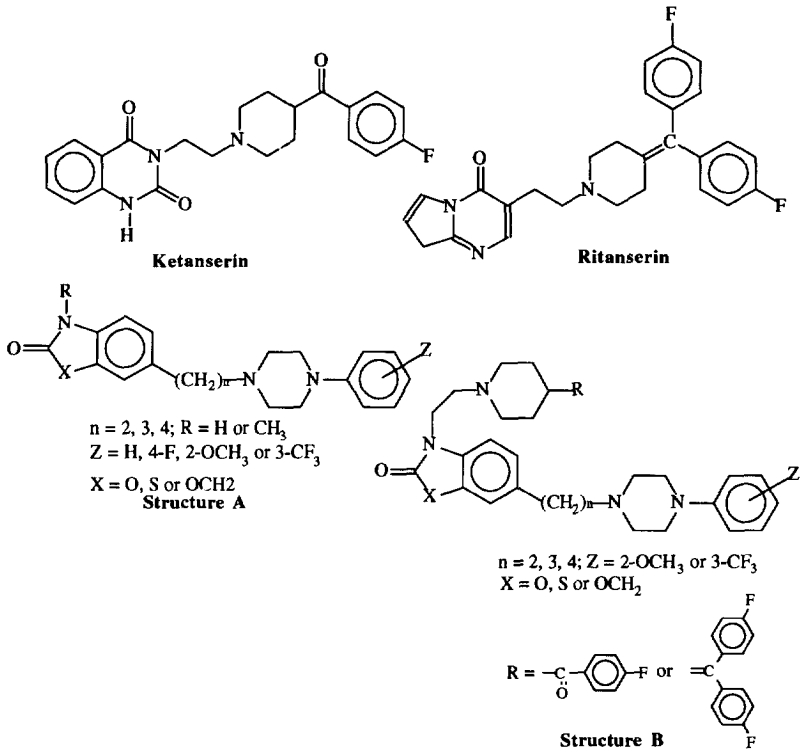
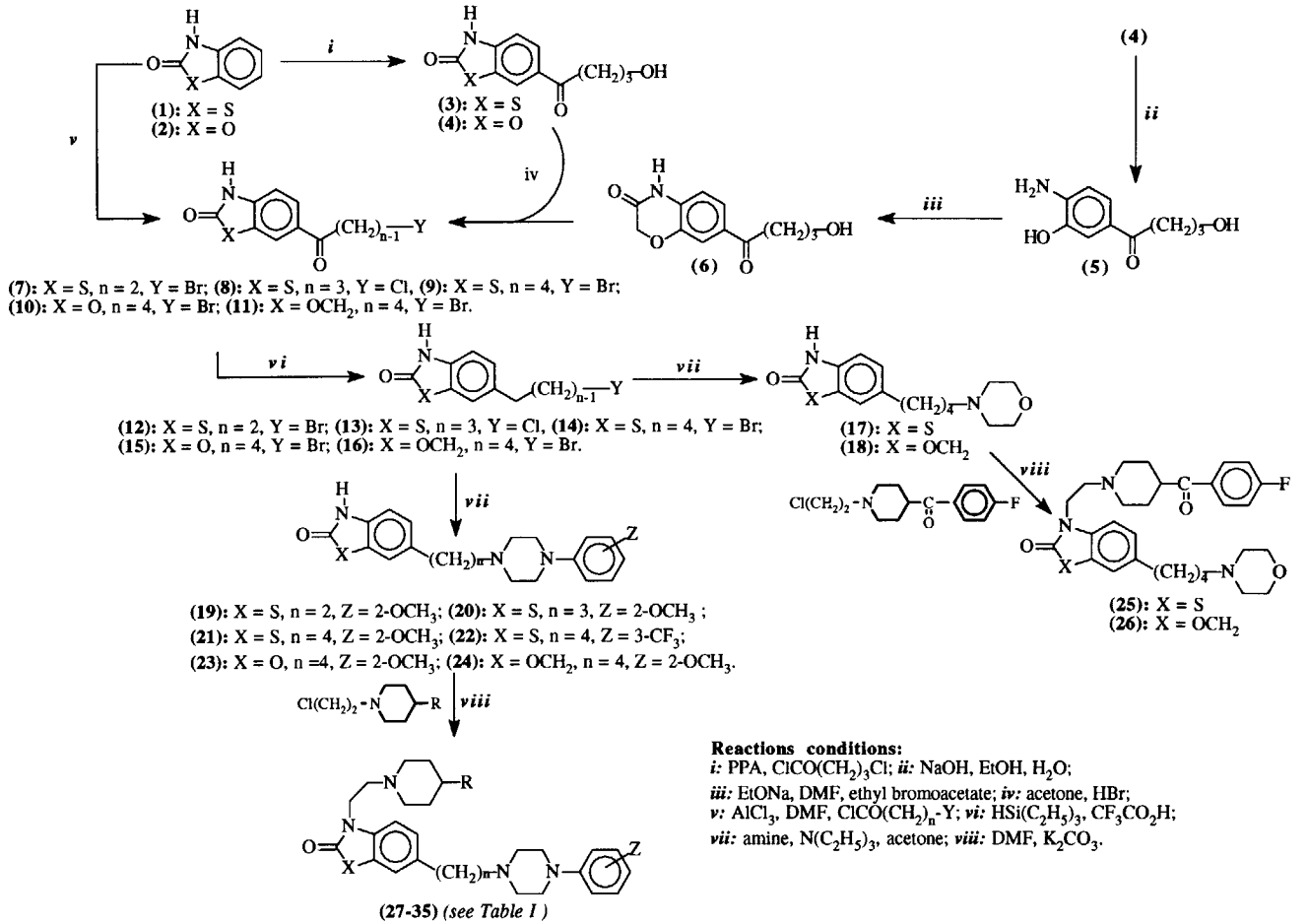
This concept led to compounds of general structure B *(Scheme 1* and Table I*)*.[17] We also investigated the influence of the phenylpiperazine moiety by replacing it with a morpholine ring (*Scheme 2*, compounds **25** and **26**). This paper reports the design and the pharmacological results obtained with this new series of benzoxazolin-2-one, benzothiazolin-2-one and benzoxazin-3-one derivatives among which compound **29** showed high and selective anxiolytic and antipanic-like properties in animal models[18] and was selected for preclinical development.



Scheme 1

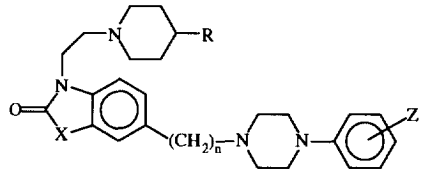
Chemistry

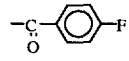
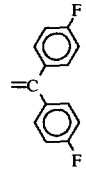
Scheme 2 illustrates the procedures used to synthesize benzothiazolin-2-one, benzoxazolin-2-one and benzoxazin-3-one derivatives **17-24**. From benzothiazolin-2-one (**1**) or benzoxazolin-2-one (**2**), 6-halogenoacyl derivatives **7-11** were obtained as previously described. [19-23] Reduction of the ketone carbonyl group of compounds **7-11** was carried out with the triethylsilane - trifluoroacetic acid reagent as described for the benzothiazolin-2-one analogs. [20] This ketone carbonyl reduction led to compounds **12-16** which were then reacted with the suitable amines in dry acetone in the presence of triethylamine to give the alkylamino derivatives **17-24**. Derivatives **17-24** were transformed to the final compounds **25-35** by condensation with the appropriate N-(2-chloroethyl)piperidinyl moiety in dry DMF in the presence of potassium carbonate. The heterocycle cleavage of the benzoxazolin-2-one derivative **4** was carried out in aqueous medium in the presence of NaOH and led to the *ortho*-aminophenol **5**. A one-pot reaction procedure using sodium ethoxide, dimethylsulfoxide and ethyl bromoacetate gave compound **6**, which was then transformed into its bromo analogue (**11**) using HBr in acetone.


(1): X = S
(2): X = O
(3): X = S
(4): X = O
(4)
(5)
(6)
(7): X = S, n = 2, Y = Br; (8): X = S, n = 3, Y = Cl, (9): X = S, n = 4, Y = Br;
(10): X = O, n = 4, Y = Br; (11): X = OCH2, n = 4, Y = Br.
(12): X = S, n = 2, Y = Br; (13): X = S, n = 3, Y = Cl, (14): X = S, n = 4, Y = Br;
(15): X = O, n = 4, Y = Br; (16): X = OCH2, n = 4, Y = Br.
(17): X = S
(18): X = OCH2
(19): X = S, n = 2, Z = 2-OCH3; (20): X = S, n = 3, Z = 2-OCH3 ;
(21): X = S, n = 4, Z = 2-OCH3; (22): X = S, n = 4, Z = 3-CF3;
(23): X = O, n =4, Z = 2-OCH3; (24): X = OCH2, n = 4, Z = 2-OCH3.
(25): X = S
(26): X = OCH2
(27-35) (see Table I)
Reactions conditions:
i: PPA, ClCO(CH2)3Cl; ii: NaOH, EtOH, H2O;
iii: EtONa, DMF, ethyl bromoacetate; iv: acetone, HBr;
v: AlCl3, DMF, ClCO(CH2)n-Y; vi: HSi(C2H5)3, CF3CO2H;
vii: amine, N(C2H5)3, acetone; viii: DMF, K2CO3.

Scheme 2

Table I: Chemical structures of compounds of general structure B (27-35)



Compds	X	n	Z	R
27	S	2	2-OCH_3	
28	S	3	2-OCH_3	
29	S	4	2-OCH_3	
30	S	4	3-CF_3	
31	O	4	2-OCH_3	
32	OCH_2	4	2-OCH_3	
33	S	4	2-OCH_3	
34	O	4	2-OCH_3	
35	OCH_2	4	2-OCH_3	

Pharmacological studies and discussion

In vivo studies

Affinities (Ki, nM) for 5-HT_{1A}, 5-HT_{2A}, 5-HT_{2C}, D_2 and α_1 receptors were determined in this series. The binding parameters are reported in Table II. The most interesting compounds concerning the 5-HT_{1A} and 5-HT_{2A} binding potencies are derivatives **28**, **29**, **31**, and **33**.

Table II: Binding parameters of compounds 25-35 (Ki in nM)

compds	5-HT_{1A} (a)	5-HT_{2A} (b)	5-HT_{2C} (c)	D_2 (d)	α_1 (e)
25	700±17	0.6±0.16	20±1.5	100±16	40±5
26	930±15	6±1	60±6	100±6.5	70±10
27	6000±335	20±2.1	50±10	5±0.2	1±0.2
28	3±0.2	2±0.3	70±5.8	2±0.1	-
29	7±0.1	2±0.4	240±10	1.2±0.3	5±0.6
30	70±2.5	50±1.4	>10000	30±4.1	1000±76
31	2±0.1	16±0.9	75±6	0.5±0.03	-
32	18±1.4	14±2.3	30±4.2	0.06±0.002	2±0.9
33	5±0.3	7±0.09	110±10	5±0.14	5±0.5
34	98±11	335±24	460±35	3±0.6	5±1.3
35	49±4	120±7.4	250±10	1.4±0.4	13±2.3

Radioligands and tissue preparation for affinity determination. (a): [^{3}H] 8-OH-DPAT and rat hippocampus; (b): [^{3}H] ketanserine and rat cortex; (c) [^{3}H] N-Methylmesulergine and rat choroid plexus; (d) [^{3}H] Raclopride and rat striatum; (e): [^{3}H] Prazosin and rat cortex.

According to these binding results, some structure-affinity relationships can be established:

- All compounds with high affinity for 5-HT_{1A} and 5-HT_{2A} receptors contain an *ortho*-methoxyphenylpiperazine pharmacophore which appears more important for the 5-HT_{1A} affinity than the 5-HT_{2A} one. Introduction of a *meta*-trifluoromethyl substituent (**30**) induces a decrease of the affinity for 5-HT_{1A}, 5-HT_{2A} and α_1 receptors combined with an increase of the D_2 affinity. However, **30** displays the highest 5-HT_{2A}/5-HT_{2C} selectivity in this series. Compounds bearing a morpholinoalkyl side-chain (**25** and **26**) possess high 5-HT_{2A} affinity (0.6 and 6 nM) and selectivity combined with low 5-HT_{1A} receptor affinity (700 and 930 nM respectively).
- Elongation of the methylene side-chain separating the heterocyclic pivotal template and the phenylpiperazine system appears to have important relevance on the binding affinity at 5-HT_{1A} receptors. Indeed, the three and four methylene unit derivatives (**28** and **29** respectively) are about 1000 fold more potent at these receptors as their ethyl homologue **27**. The 5-HT_{2A} and D_2 affinities are conserved.
- Replacement of the benzothiazolin-2-one heterocycle with its oxygen bioisostere, i.e. benzoxazolin-2-one (**31**), leads to compounds as potent at 5-HT_{1A} and D_2 receptors as the selected member (**29**). Compound **31** exhibits however lower 5-HT_{2A} affinity than **29** (10 fold). When the benzoxazin-3-one heterocycle is used as a pivotal template (**32**), this leads to a decrease of the expected 5-H_{1A} and 5-HT_{2A} affinities (about 10 fold) combined with very high affinity at D_2 receptors.
- The introduction of a 4-(bis(4-fluorophenyl)methylen)piperidine one (**33-35**) completely changes the receptor binding profile, except for the sulfur analog **33**. Indeed, compounds **33** and **34** display very high affinities at D_2 and α_1 receptors (in the nanomolar range) combined with moderate to low affinities for the 5-HT receptors. When compared with derivative **29**, compounds **34** and **35** are about 10 fold and 100 fold less potent at 5-HT_{1A} and 5-HT_{2A} receptors respectively. These compounds however can be regarded as useful tools in the field of dopamine pharmacology.

In vivo studies

Two of the most potent ligands for the 5-HT_{2A} receptors, i.e. compounds **25** and **29** were tested *in vivo*.

- Central 5-HT_2 antagonist activity was assessed by the ability of these compounds to antagonise 5-HTP induced head twitches and mescaline induced scratching in mice. While compound **25** did not exert 5-HT_2 antagonist activity at the doses of 4 and 16 mg/kg IP in mice, compound **29** showed potent *in vivo* activity: it dose dependently blocked the 5-HTP induced head twitches (50% and 90% of the antagonism at 45 and 128 mg/kg PO, $p<0.001$) and blocked the mescaline induced scratching at 8 and 32 mg/kg PO (respectively 86% and 70% antagonism, $p<0.01$).
- In the light/dark box test in mice, predictive of anxiolytic activity, compounds **25** and **29** showed potent activity.

Both compounds were tested at the doses of 0.25, 1 and 4 mg/kg PO. Compound **25** was active at the lowest doses: it significantly increased the time spent in the light box (+190% and +241%, $p<0.01$) respectively for the doses of 0.25 and 1 mg/kg and the number of transitions between the two compartments (+160% and +265%, $p<0.01$ respectively). Compound **25** was sedative at the dose of 4mg/kg PO.

Compound **29** showed potent anxiolytic-like activity, without any sedative effect at the three tested doses: it increased dose-dependently the time spent in the light box (from +141% to +300%) and the number of transitions (from +200% to +300%).

Conclusion. We have designed a series of mixed 5-HT_{1A} and 5-HT_{2A} ligands in which several compounds can serve as pharmacological tools in the dopamine and serotonin receptor pharmacology. Among these heterocyclic derivatives, one compound **(29)** emerged as it displayed potent and selective antagonist properties at both 5-HT_{1A} and 5-HT_{2A} central receptors. Although, its high *in vitro* affinity for D_2 receptors, **29** was devoid of marked behavioral signs resulting from the stimulation of the blockade of these dopaminergic receptor subtype (i.e. stimulation and sedation respectively). Moreover, **29** showed anxiolytic and antipanic-like activities in mice without any sedative effects at the three tested doses and was selected for preclinical studies.[18]

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