Eur. J. Med. Chem. **22** (1987) 273—276

Original paper

Synthesis and α-blocking activity of some analogues of idazoxan*

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(Received May 5, 1986, accepted January 20, 1987)

Summary — Several analogues of idazoxan **1** and fenmetazole **2** were prepared as potential α_2-adrenoreceptor antagonists. Their blocking activity and selectivity on α_1- and α_2-adrenoreceptors were evaluated in isolated rat vas deferens. Although all the drugs displayed a significantly lower activity compared to the parent compounds **1** and **2**, with **3** being the only exception, the present results might help in elucidating the role of ring oxygens of **1** in drug—receptor interaction.

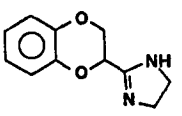
Résumé — **Synthèse et activité α-bloquante de quelques analogues de l'idazoxan.** *Quelques analogues de l'idazoxan (1) et du fenmetazole (2) ont été préparés comme antagonistes potentiels des récepteurs α_2-adrénergiques, leur activité bloquante et la sélectivité vis-à-vis des récepteurs α_1- et α_2-adrénergiques ont été évaluées sur le vas deferens isolé du rat. Bien que tous les composés révèlent une activité significativement inférieure à celle des composés apparentés 1 et 2 à l'exception seulement du dérivé 3, les résultats peuvent aider à élucider le rôle des oxygènes du cycle dans le composé 1 sur l'interaction drogue—récepteur.*

idazoxan / fenmetazole / α_1- and α_2-adrenoreceptors / α-blocking activity / rat vas deferens / structure—activity relationships

Introduction

In addition to the classical α_1-adrenoreceptor which initiates the response of the effector organ, it has been clearly demonstrated that there are α_2-adrenoreceptors located at both pre- and postsynaptic sites. It is now understood that presynaptic α_2-adrenoreceptors regulate transmitter release through a negative feedback mechanism, whereas the role of postsynaptic α_2-adrenoreceptors has not been completely clarified [1—6]. Activation of presynaptic α_2-adrenoreceptors results in a decrease in the amount of noradrenaline released per nerve impulse and it has been proposed that antagonism at presynaptic α_2-adrenoreceptors could be an effective and novel treatment of depression [7].

Recently, idazoxan (**1**, RX 781094) has been described as a selective α_2-adrenoreceptor blocking agent [8]. Extensive structure—activity relationship studies have been carried out on **1** to determine the effect of substituents on both aromatic and imidazoline rings, as well as of structural modifications of these moieties [7, 9—11]. However, the electronic effects of oxygen at positions 1 and 4 of the dioxan ring were not exhaustively explored. To this end, we describe here the synthesis and the pharmacological profile of several compounds related to **1**. Furthermore, since fenmetazole **2**, an α_2-antagonist [7], can be considered as a ring-opened deoxa analogue of **1**, we included in this study the thioanalogue **7** and its derivatives **8** and **9** to investigate the role of oxygen in drug—receptor interaction.



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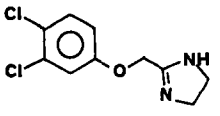

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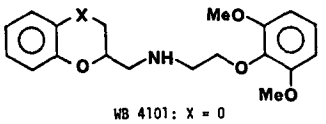

WB 4101: X = O

Benoxathian: X = S

Chemistry

All the compounds were characterized by ^{1}H NMR, IR and elemental analysis and were synthesized by standard

* Some aspects of this work were presented in preliminary form at the 3rd Noordwijkerhout Symposium on Innovative Approaches in Drug Research, September 3—6, 1985, Noordwijkerhout, The Netherlands, Abstr. P11.

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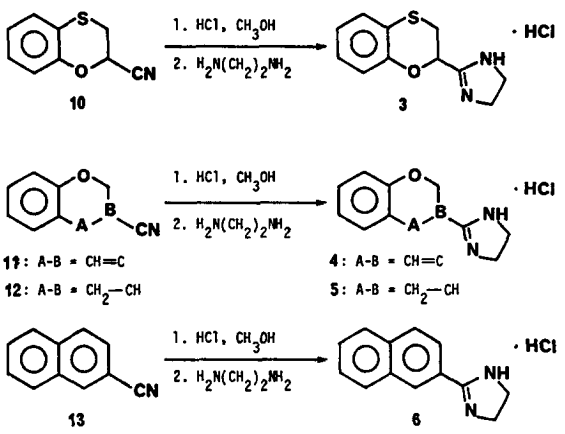
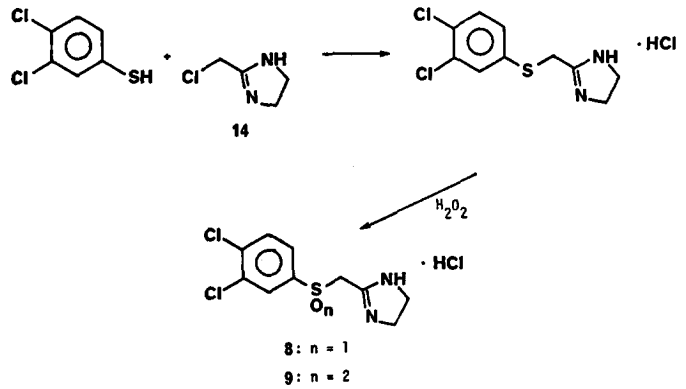
procedures as shown in Schemes 1 and 2. Thus, reaction of **10** [12—14] with HCl gas in MeOH followed by treatment with ethylenediamine gave **3**. Similarly **4**, **5** [10] and **6** were obtained from the corresponding nitriles **11** [15], **12** [10] and **13**, respectively (Scheme 1).



Scheme 1.

Alkylation of 3,4-dichlorothiophenol with 2-chloromethylimidazoline **14** [16] gave **7**. Oxidation of **7** with H_2O_2 in AcOH gave the sulfoxide **8** and the sulfone **9** (Scheme 2).



Scheme 2.

Pharmacology

The biological profile of the compounds listed in Table I at α_1- and α_2-adrenoreceptors was assessed on epididymal and prostatic portions, respectively, of isolated rat vas deferens using standard testing procedures [17, 18]. The potency of the drugs was expressed as pA_2 calculated according to Arunlakshana and Schild [19] or van Rossum [20].

Results and Discussion

In the present study, the α_1- and α_2-adrenoreceptor biological properties of **1** (idazoxan) and **2** (fenmetazole) [7, 21] were also studied and compared with those of **3**—**9**. The results obtained are shown in Table I. It can be seen that all the compounds studied displayed biological profiles significantly different from those of the parent compounds **1** and **2**.

Table I. α_1- and α_2-adrenoreceptor pA_2 values in the isolated rat vas deferens[a].

drug	α_1 pA_2 value against methoxamine	α_2 pA_2 value against BHT 933	α_1/α_2 selectivity ratio[b]
1	5.99 ± 0.10	8.02 ± 0.08	118
2	partial agonist	7.14 ± 0.18	
3	7.22 ± 0.13	8.00 ± 0.05	6
4	5.85 ± 0.14	6.54 ± 0.17	5
5	6.09 ± 0.15	7.04 ± 0.09	9
6	6.56 ± 0.13	5.75 ± 0.11	0.15
7	5.75 ± 0.09	5.82 ± 0.15	1
8	inactive[c]	4.86 ± 0.19	
9	inactive[c]	4.34 ± 0.16[d]	

[a] pA_2 values ± SEE were calculated according to Arunlakshana and Schild [19] unless otherwise specified. pA_2 is defined as the negative logarithm to the base 10 of that dose that requires a doubling of the agonist dose to compensate for the action of the antagonist.
[b] The α_1/α_2 selectivity ratio is the antilog of the difference between the pA_2 values at α_2- and α_1-adrenoreceptors.
[c] Inactive up to a concentration of 100 μM.
[d] Calculated according to van Rossum [20].

The displacement of the ring oxygen at position 4 of the dioxan ring of **1** with a sulfur atom giving **3** did not modify α_2-blocking activity, whereas it did affect the biological profile at the α_1-site (Table I). It appears that the ring hetero atom is more important at the α_1- than at the α_2-adrenoreceptor. In fact, recent findings indicate that the benzodioxanyl moiety might play a different role at α_1- and α_2-sites. For example, it has been reported that substitution of the benzodioxan ring in **1** with a dihydrobenzofuran ring did not modify the α_2-activity [10], whereas the corresponding substitution in WB 4101, which is the prototype of α_1-antagonists bearing a benzodioxan nucleus, caused a significant decrease, of two orders of magnitude or more, in α_1-blocking activity [22].

We recently suggested that the position 4 of α_1-antagonists bearing a benzodioxan or a benzoxathian nucleus could interact with the receptor, either increasing the electron density of the phenyl ring by way of an electron-releasing effect or giving rise to dipole—dipole interaction [17]. Due to the high activity displayed by the dihydrobenzofuran analogue of **1**, which lacks the ring hetero atom at position 4 [10], it is unlikely that this position plays the same role at the α_2-site. Rather it would seem that the ring hetero atom as such is not essential for the binding. Thus, one would expect that the isomeric chroman to **5** (*i.e.*, no oxygen at position 4) retains a potency similar to that of **1**. The

observation that it was only weakly active as an α_2-antagonist [10] could allow the conclusion that the oxygen at position 4 of **1** does play a role in receptor binding in contrast with the above reasoning. This contradiction might perhaps be apparent only in the sense that the chroman nucleus could stabilize a conformation different from those stabilized by both **1** and its dihydrobenzofuran analogue. Thus, the oxygen at position 4 of **1**, although not directly involved in receptor binding, may be important in stabilizing an optimal conformation for the drug—receptor interaction mechanism. In this context, it might be interesting to note that fenmetazole **2** can be regarded as an opened analogue structurally related to **1** which lacks the ring hetero atom at position 4 of the dioxan ring. Displacement of the ether oxygen of **2** with a sulfur atom gave **7** which was a weaker antagonist than the parent compound at α_2-adrenoreceptors. Oxidation of **7** to the corresponding sulfoxide **8** and sulfone **9** resulted in a further decrease in activity. A similar pattern was observed for the substitution of the ring oxygen at position 1 of **1** with less polar groups lacking the lone pairs (*n* electrons) such as methylene. Since **5**, compared to **1**, gave a significant decrease of α_2-blocking activity, compounds **4** and **6** were investigated to evaluate a possible role of π electrons in drug—receptor interaction. It is evident that optimum activity is associated with an oxygen atom. These results might indicate that the oxygen in **2**, as well as that at position 1 of **1**, plays a role in receptor binding either through hydrogen bond formation or electronic effects. However, the possibility that the oxygen at position 1 of **1** may stabilize a conformation displaying a higher affinity towards α_2-adrenoreceptors than that of the conformations stabilized by **4**—**6** cannot be excluded.

Experimental protocols

Chemistry

Melting points were taken in glass capillary tubes on a Buchi SMP-20 apparatus and are uncorrected. IR and NMR spectra were recorded on Perkin—Elmer 297 and Varian EM-390 instruments, respectively. Although the IR and NMR spectra data are not included (because of the lack of unusual features), they were obtained for all compounds reported and were consistent with the assigned structures. The microanalyses were performed by the Microanalytical Laboratory of our department and the elemental compositions of the compounds agreed to within ± 0.4% of the calculated value. Chromatographic separations were performed on silica gel columns (Kieselgel 40, 0.063—0.200 mm, Merck). The term 'dried' refers to the use of anhydrous sodium sulfate.

General procedure for the synthesis of 3—6

The procedure adopted for the synthesis of **4** is described.

HCl gas was bubbled through a stirred and cooled (0°C) solution of **11** [15] (5.0 g, 31.0 mmol) and MeOH (2.2 ml, 60.0 mmol) in dry chloroform (50 ml) for 45 min. After 12 h at 0°C, dry ether was added to the reaction mixture to give an intermediate imidate, which was filtered. 0.5 g (2.2 mmol) of this solid was added to a cooled (0°C) and stirred solution of ethylenediamine (0.2 ml, 2.7 mmol) in abs. EtOH (5 ml). After 1 h, concentrated HCl (0.1 ml) in abs. EtOH (2.0 ml) was added to the reaction mixture which was stored overnight in the refrigerator and then it was diluted with abs. EtOH (5 ml) and heated at 62°C for 2 h. After cooling, the solid was collected and discarded and the filtrate was concentrated and filtered again. The filtrate was evaporated to dryness to give a residue which was taken up in chloroform (50 ml) and washed with 2 N NaOH. Removal of dried solvents gave **4** as the free base which was transformed into the hydrochloride salt with HCl gas in EtOH. The solid was washed with ether and then recrystallized.

Similarly **3**, **5** [10], and **6** [23] were obtained from **10** [12—14], **12** [10], and **13**, respectively (Scheme 1).

The physical characteristics of **3**—**6** are reported in Table II.

Table II.

Compd	mp,[a] °C	yield %	recrystn solvent	formula[b]
3	275-279[c]	48	EtOH-Et_2O	$C_{11}H_{13}ClN_2OS$
4	242-244	62	EtOH-Et_2O	$C_{12}H_{13}ClN_2O$
5	204-205	75	EtOH-Et_2O	$C_{12}H_{15}ClN_2O$
6	280	60	EtOH	$C_{13}H_{13}ClN_2$
7	215-216	90	EtOH	$C_{10}H_{11}Cl_3N_2S$
8	190-191	63	EtOH-Et_2O	$C_{10}H_{11}Cl_3N_2OS$
9	212-214	60	EtOH	$C_{10}H_{11}Cl_3N_2O_2S$

[a] The heating rate was 1°C/min.
[b] Analyses for C, H and N were within ± 0.4% of the theoretical value required.
[c] Decomposition began at 250°C.

2-(3,4-Dichlorothiophenoxy)methyl-2-imidazoline hydrochloride 7

3,4-Dichlorothiophenol (1.79 g, 10.0 mmol) was added to a solution of Na (0.46 g) in abs. EtOH (15 ml). After 1 h, imidazoline **14** [16] as hydrochloride (1.55 g, 10.0 mmol) was added to the reaction mixture which was heated to reflux for 2 h under vigorous stirring. The solution was evaporated to dryness to give a residue which was taken up in chloroform (100 ml) and washed with water (25 ml), 1 N NaOH (2 × 25 ml) and water (25 ml). Removal of dried solvents gave 1.8 g of **7** as the free base which was transformed into the hydrochloride salt with HCl gas in abs. EtOH and purified by recrystallization (Table II).

2-(3,4-Dichlorothiophenoxy)methyl-2-imidazoline S-oxide hydrochloride 8

36% hydrogen peroxide (1.0 ml) was added to a solution of **7** (0.3 g) in AcOH (1.5 ml). After standing 3 days, the solution was diluted with water (10 ml), made basic with 2 N KOH (25 ml) and extracted with chloroform (3 × 30 ml). Removal of dried solvents gave 0.2 g of **8** as the free base which was transformed into the hydrochloride salt with HCl gas in abs. EtOH and purified by recrystallization (Table II).

2-(3,4-Dichlorothiophenoxy)methyl-2-imidazoline S,S-dioxide hydrochloride 9

36% hydrogen peroxide (1.0 ml) was added to a solution of **7** (0.3 g) in AcOH (1.5 ml). After standing 20 days at room temperature, the solution was worked up to give **9** (Table II) as described for **8**.

Pharmacology

General considerations

Male albino rats (175—200 g) were killed by a sharp blow on the head and both vasa deferentia were isolated, freed of adhering connective tissue and transversely bisected. Prostatic, 12 mm in length, and epididymal portions, 14 mm in length, were prepared and mounted individually in baths of 20 ml working volume containing Krebs solution of the following composition (mM): NaCl, 118.4; KCl, 4.7; $CaCl_2$, 2.52; $MgSO_4 \cdot 7H_2O$, 1.2; KH_2PO_4, 1.2; $NaHCO_3$, 25.0; glucose, 11.1. The $MgSO_4 \cdot 7H_2O$ concentration was reduced to 0.6 mM when twitch response to field stimulation was studied. The medium was maintained at 37°C and gassed with 95% O_2—5% CO_2. The loading tension

used to assess α_1- or α_2-blocking activities was 0.4 g or 0.5—0.8 g, respectively, and the contractions were recorded by means of force transducers connected to a two channel Gemini 7070 polygraph.

Field stimulation of the tissue was carried out by means of two platinum electrodes, placed near the top and bottom of the vas deferens, at 0.1 Hz using square pulses of 3 ms duration at a voltage of 10—15 V. The stimulation voltage was fixed throughout the experiments.

α_1-Adrenoreceptor blocking activity

Postsynaptic α_1-adrenoreceptor blocking activity was determined on the epididymal portion of the vas deferens. The tissues were allowed to equilibrate for at least 1 h before the addition of any drug. Methoxamine dose—response curves were obtained cumulatively, the first one being discarded and the second one taken as a control. After incubation with the antagonist for 30 min, a third dose—response curve was obtained. Responses were expressed as a percentage of the maximal response obtained in the control curve. Parallel experiments, in which tissues did not receive any antagonist, were run in order to correct for time-dependent changes in agonist sensitivity [24]. It was generally verified that the third dose—response curve was identical to the second because the change in the dose—ratio is less than 2, which usually represents a minimal correction.

The antagonist potency of compounds **1** and **3**—**6** at α_1-adrenoreceptors was expressed in terms of their pA_2 values according to Arunlakshana and Schild [19]. These values were calculated from the ratio of the doses (DR) of agonist causing 50% of the maximal response in the presence and in the absence of the test compound. The log ($DR - 1$) was calculated at three antagonist concentrations and each concentration was tested at least five times. Compounds **8** and **9** were inactive up to a concentration of 100 μM. Compound **2** behaved as a weak partial agonist.

α_2-Adrenoreceptor blocking activity

This was assessed on the prostatic portion of the vas deferens by antagonism to an α_2-adrenoreceptor agonist, BHT 933 (azepexole). BHT 933 inhibits twitch responses of the field-stimulated vas deferens in a dose dependent manner by acting on the presynaptic α_2-adrenoreceptor and its action is reversed by yohimbine [25] and idazoxan (present investigation), selective α_2-adrenoreceptor antagonists.

The procedure was substantially the same as that described for unstimulated vasa, with the only exception being that two dose—response curves to BHT 933 were constructed and the first one was taken as a control. Dose—ratio (DR) values were determined from the concentration causing 50% inhibition of the twitch response in the absence and in the presence of antagonist. Each antagonist was tested at three different concentrations and each concentration was investigated at least five times. Compound **9**, however, was tested at only two concentrations because of its very low potency.

The results are expressed as pA_2 values calculated according to Arunlakshana and Schild [19] for **1**—**8** whereas for **9**, the method of van Rossum [20] was followed.

Statistical evaluation

Student's t test was used to assess the significance of the experimental results.

Acknowledgements

Supported by the Ministry of Public Education.

A generous gift of BHT 933 from Boehringer Ingelheim (Florence) is gratefully acknowledged.

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