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Synthesis and α_2-adrenergic activity of 2-[(methyleneamino)oxy]-*N*-(guanidino)ethaneimines. A bioisosteric replacement of the aryl of guanabenz-type benzylideneaminoguanidine α_2-agonists with the [(methyleneamino)oxy]methyl moiety (MAOMM)

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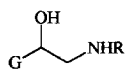
Summary — Some 2-[(methyleneamino)oxy]-*N*-(guanidino)ethaneimines (**10a–g**) were synthesized as analogs of guanabenz-type benzylideneaminoguanidine α_2-agonists (**9**) in which the aryl portion (Ar) is substituted by the [(methyleneamino)oxy]methyl moiety (MAOMM). The α_2-adrenergic activity of compounds **10a–g** was evaluated by functional tests on guinea-pig ileum. The MAOM-derivatives **10a–g** exhibited an α_2-adrenergic stimulating activity fairly similar to that of the benzylideneaminoguanidine reference drug guanabenz, thus supporting the hypothesis of the existence of a bioisoster-like relationship between the MAOMM and the Ar in the class of guanabenz-type α_2-adrenergic agonists.

α_2-adrenergic agonist / aminoguanidine adrenergic drug / guanabenz analog / 2-[(methyleneamino)oxy]-*N*-(guanidino)ethaneimine

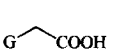
In previous papers from this laboratory, we have demonstrated that an aryl group (Ar) present in drug molecules of different pharmacological classes can be replaced by a [(methyleneamino)oxy]methyl moiety ($C{=}NOCH_2$, MAOMM) with the retention of a similar biological activity [1–7]. Thus the MAOM-analogs (**2**) of arylethanolamine β-adrenergic-blocking agents (**1**) have been found to be β-adrenergic antagonists [1–3], whereas the MAOM-analogs (**4**) of arylacetic non-steroidal antiinflammatory agents (**3**) exhibit a good antiinflammatory activity [4–6], and the MAOM-analogs (**6**) of arylacetyl-substituted penicillins and cephalosporins (**5**) possess an appreciable antimicrobial activity [7]. (The introduction of an oximethereal group into biologically active β-adrenergic drugs was originally reported by G Leclerc et al [8].)

The similar biological activities found between these analogs (**2**, **4**, **6**) and the Ar-substituted compounds (**1**, **3**, **5**) have been ascribed to the existence of a bioisosteric relationship between the MAOMM and the Ar in the drugs of the classes studied. However, the recent observation that the replacement of the Ar of neuroleptic benzamides **7** with the MAOMM leads to compounds **8** that are practically inactive [9], indicates that this replacement may be unsuccessful in some cases.

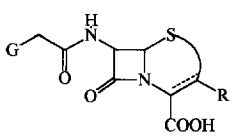
In order to extend the exploration of this MAOMM-Ar equivalency to new classes of drugs, we decided to study compounds of type **10**, which can be viewed as analogs of the benzylideneaminoguanidine α_2-adrenergic agonists **9** [10], in which the Ar is substituted by the MAOMM.



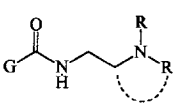
1, G = Ar
2, G = $R_1R_2C{=}NOCH_2$



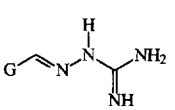
3, G = Ar
4, G = $R_1R_2C{=}NOCH_2$



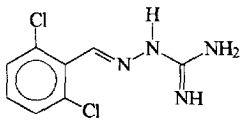
5, G = Ar
6, G = $R_1R_2C{=}NOCH_2$



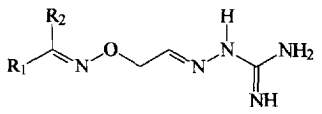
7, G = Ar
8, G = $R_1R_2C{=}NOCH_2$



9, G = Ar
10, G = $R_1R_2C{=}NOCH_2$



Guanabenz



10a-g

This work describes the synthesis of compounds **10a–g** (see table I) and the comparison of their α_2-adrenergic activity with that of guanabenz [10–12], one of the best-known compounds of type **9**. In compounds **10a–g**, at least one of the R_1 and R_2 substituents is an aromatic group. This choice was suggested by the fact that in the various classes of drugs previously studied, an aromatic substituent linked to the oxime carbon of the MAOMM, while not essential for the activity, appeared at times to be capable of improving it [4, 5].

Chemistry

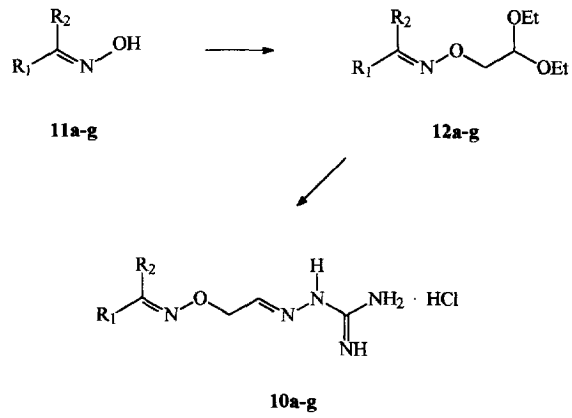
Compounds **10a–g** were synthesized as shown in scheme 1. Base-catalyzed (KOH) reaction of oximes **11a–g** with 2-bromo-1,1-diethoxyethane in DMSO afforded the corresponding 2-[(methyleneamino)oxy]-1,1-diethoxyethane derivatives **12a–g**. Treatment of the acetal derivatives **12a–g** in acetone with Dowex-50W (H^+) resin followed by the addition of aminoguanidine hydrochloride yielded the corresponding hydrochloride salts of the aminoguanidine compounds **10a–g** (table I).

The geometry around the oxime double bond of both the acetal compounds **12a-e** and the final products **10a-e**, for which the *cis–trans* isomerism is possible, was assumed on the basis of the configuration of the starting oximes (**11a–e**), bearing in mind that the latter have been proved to be configurationally stable under the reaction conditions that lead from **11a–e** to **12a–e**, and then to **10a–e**.

Results and discussion

The α_2-adrenergic activities of the aminoguanidine derivatives **10a–g** and the reference drug guanabenz were evaluated as their ability to inhibit acetylcholine release evoked by electrical stimulation of guinea-pig ileum nerve fibres [13]. All the new compounds **10a–g** showed an appreciable α_2-stimulating activity, with pD_2 values approximately 1 to 1.5 units lower than that of guanabenz (table I).

Among the new compounds **10a–g**, **10e** and **10f** showed the highest pD_2 values, while **10c** and **10g** exhibited the lowest ones.



11a-g **12a-g**

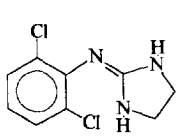
10a-g

Scheme 1.

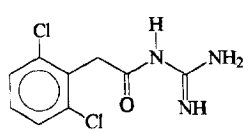
As far as the intrinsic activity (ia) is concerned, all the new aminoguanidine compounds elicited the same maximal response as norepinephrine and guanabenz (ia = 1.00), with the exception of **10b** and **10c**, which exhibited a slightly lower ia value (0.90).

The involvement of α_2-adrenoceptors in the action mechanism of compounds **10a–g** was confirmed by the fact that for the most active compound **10f**, its biological effect is antagonized by the selective α_2-adrenoceptor antagonist rauwolscine, with a pK_b value of 8.27 ± 0.21 (see *Experimental protocols*).

The results shown in table I indicate that the substitution of the Ar of type **9** α_2-adrenergic agonists with an MAOMM bearing an aryl moiety on the oxime carbon, leads to compounds **10a–g** that still possess an α_2-adrenergic agonistic activity, with activity indices not much lower than those of one of the most active compounds of type **9**, guanabenz. It may be noted that in this compound, the phenyl ring presents a type of substitution (ie, two chlorine atoms in the 2,6 positions), which is also present in other known α_2-adrenergic drugs, like clonidine [14] and guanfacine [14], and which would therefore appear to be particularly suitable for the interaction of these drugs with α_2-adrenergic receptors.



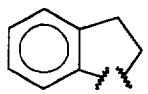
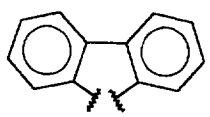
Clonidine



Guanfacine

As regards the influence on the activity of the different substituents linked to the oxime carbon of **10a-g**, the modest differences in their pD_2 and ia values do

Table I. Chemical data and α_2-adrenergic activity of compounds **10a–g**.

Compound	R_1	R_2	*Mp (°C)*	*Recrystallization solvent*	*Formula*[a]	*Yield (%)*[b]	*α_2-Adrenergic activity on isolated guinea pig-ileum*[c]	
							pD_2	*ia*[d]
a	Ph	Me	135–137	EtOH/Et_2O	$C_{11}H_{16}ClN_5O$	63	5.24 ± 0.01	1.00
b	*p*-Cl-Ph	Me	126-128	EtOH/Et_2O	$C_{11}H_{15}Cl_2N_5O$	70	5.21 ± 0.06	0.90
c	*p*-MeO-Ph	Me	67-69	$CHCl_3$/Et_2O	$C_{12}H_{18}ClN_5O_2$	48	4.84 ± 0.15	0.90
d	Ph	Et	159-160	$CHCl_3$/Et_2O	$C_{12}H_{18}ClN_5O$	62	5.18 ± 0.19	1.00
e			97–99	$CHCl_3$/Et_2O	$C_{12}H_{16}ClN_5O$	55	5.56 ± 0.23	1.00
f	Ph	Ph	183–185	*i*-PrOH/Hexane	$C_{16}H_{18}ClN_5O$	43	5.58 ± 0.20	1.00
g			180–181	$CHCl_3$/Et_2O	$C_{16}H_{16}ClN_5O$	49	4.96 ± 0.28	1.00
Guanabenz							6.51 ± 0.06	1.00

[a]Anal C, H, N. [b]For the reaction of the acetal derivative **12** with aminoguanidine. No efforts were made to optimize yields. [c]The values represent the means of four to six experiments for each drug ± standard error. [d]Intrinsic activity, ie, the ratio between the maximal response elicited by the compound under test and that elicited by the full agonist, norepinephrine.

not make it possible to advance any hypothesis about structure–activity relationships for these compounds.

In conclusion, the similar pharmacological activity found to exist between a benzylideneaminoguanidine compound of type **9** like guanabenz and the MAOM-analogs of type **10** (**10a–g**) may indicate that also in the class of aminoguanidine α_2-adrenergic agonists, the MAOMM is to be considered as a bioisoster of aryl groups.

However, the fact that in the compounds examined (**10a–g**), an aromatic group linked to the oxime carbon of the MAOMM is present, does not allow us to exclude the possibility that, in this case, this aromatic group may participate directly in the expression of the activity.

Experimental protocols

Chemistry

Melting points were determined on a Kofler hot-stage apparatus and are uncorrected. IR spectra for comparison of compounds were taken with an FT-IR Mattson 1000 Unicam spectrometer, as liquid films for the oils, or as paraffin oil mulls for the solids. ^1H NMR spectra of all compounds were routinely detected with a Varian CFT-20 instrument operating at 80 MHz in a ca 2% solution of $CDCl_3$ (for the neutral compounds) or DMSO-d_6 (for the salts), using Me_4Si as the internal standard. Oximes of symmetric (**11f,g**) and *E*-oximes of asymmetric ketones (**11a–e**) were prepared by the usual methods [15] and their physical constants were in agreement with those reported in the literature.

Evaporations were undertaken in vacuo, and $MgSO_4$ was always used as the drying agent. Elemental analyses were performed by our analytical laboratory and agreed with the theoretical values to within ±0.4%.

*2-[(Methyleneamino)oxy]-1,1-diethoxyethane derivatives **12a–g***

Solid KOH (10.2 mmol) was added at room temperature in a single portion to a stirred solution of the appropriate oxime **11a–g** (10.2 mmol) and 2-bromo-1,1-diethoxyethane (10.2 mmol) in anhydrous DMSO (30 mL). The mixture was kept in this condition for 2 h and then diluted with H_2O (50 mL) and extracted three times with $CHCl_3$. The organic phases were washed with brine, filtered, and evaporated in vacuo to yield an oily residue consisting almost exclusively of the desired 2-methyleneaminoxy-1,1-diethoxyethane derivatives **12a–g**, which were used for the subsequent reaction without further purification. **12a** (92%): ^1H NMR ($CDCl_3$) δ 1.20 (t, 6H, J = 7.2 Hz), 2.22 (s, 3H), 3.56 and 3.63 (2q, 4H, J = 7.2 Hz), 4.12 (d, 2H, J = 5.6 Hz), 4.75 (t, H, J = 5.6 Hz), 7.23–7.80 (m, 5H). Anal $C_{14}H_{21}NO_3$ (C, H, N). **12b** (96%): ^1H NMR ($CDCl_3$) δ 1.17 (t, 6H, J = 7.3 Hz), 2.16 (s, 3H), 3.49 and 3.57 (2q, 4H, J = 7.3 Hz), 4.13 (d, 2H, J = 5.4 Hz), 4.74 (t, H, J = 5.4 Hz), 7.23 and 7.49 (2d, 4H, J = 8.8 Hz). Anal $C_{14}H_{20}NO_3Cl$ (C, H, N). **12c** (90%): ^1H NMR ($CDCl_3$) δ 1.23 (t, 6H, J = 7.2 Hz), 2.22 (s, 3H), 3.50 and 3.72 (2q, 4H, J = 7.2 Hz), 3.81 (s, 3H), 4.22 (d, 2H, J = 5.6 Hz), 4.85 (t, H, J = 5.6 Hz), 6.90 and 7.65 (2d, 4H, J = 9.0 Hz). Anal $C_{15}H_{23}NO_4$ (C, H, N). **12d** (89%): ^1H NMR ($CDCl_3$) δ 1.13 (t, 6H, J = 7.0 Hz), 1.23 (t, 6H, J = 7.3 Hz), 2.78 (q, 2H, J = 7.0 Hz), 3.52 and 3.70 (2q, 4H, J = 7.3 Hz), 4.22 (d, 2H, J = 5.6 Hz), 4.86 (t, H, J = 5.6 Hz), 7.20–7.80 (m, 5H). Anal $C_{15}H_{23}NO_3$ (C, H, N). **12e** (88%): ^1H NMR ($CDCl_3$) δ 1.23 (t, 6H, J = 7.0 Hz), 2.98 (m, 4H), 3.67 and 3.73 (2q, 4H, J = 7.0 Hz), 4.22 (d, 2H, J = 5.4 Hz), 4.88 (t, H, J = 5.4 Hz), 7.16–7.83 (m, 4H). Anal $C_{15}H_{21}NO_3$ (C, H, N).

12f (91%): ^{1}H NMR ($CDCl_3$) δ 1.15 (t, 6H, J = 7.2 Hz), 3.53 and 3.59 (2q, 4H, J = 7.2 Hz), 4.12 (d, 2H, J = 5.6 Hz), 4.78 (t, H, J = 5.6 Hz), 7.20–7.60 (m, 10H). Anal $C_{19}H_{23}NO_3$ (C, H, N). **12g** (70%): ^{1}H NMR ($CDCl_3$) δ 1.23 (t, 6H, J = 7.2 Hz), 3.67 and 3.75 (2q, 4H, J = 7.2 Hz), 4.45 (d, 2H, J = 5.6 Hz), 4.97 (t, H, J = 5.6 Hz), 7.00–7.91 and 8.28–8.50 (2m, 8H). Anal $C_{19}H_{21}NO_3$ (C, H, N).

The configurational stability of **11a–e** was tested by treating the appropriate oxime with KOH in anhydrous DMSO under the conditions described above. The mixture was then diluted with H_2O, neutralized with aqueous 5% HCl and extracted with $CHCl_3$. Evaporation of the washed (H_2O) and filtered extracts yielded the starting compound practically pure.

*2-[(Methyleneamino)oxy]-N-(guanidino)ethaneimine hydrochloride derivatives **10a–g***

A vigorously stirred solution of the appropriate diethylacetal derivative **12a–g** (1.93 mmol) in anhydrous acetone (50 mL) was treated at room temperature with Dowex-50W (H^+) resin (0.9 g) for 2 h. The resulting mixture was filtered and evaporated in vacuo to give an oily residue, which was redissolved in acetone (50 mL) and treated as above with Dowex resin (0.9 g) for another 2 h. The mixture was filtered and evaporated in vacuo, and the resulting oily residue was dissolved in anhydrous ethanol (30 mL) and stirred with solid aminoguanidine hydrochloride (1.81 mmol) for 2 h. The resulting mixture was evaporated in vacuo to give a solid residue, consisting almost exclusively of the desired hydrochloride derivatives **11a–g**, which were crystallized from the appropriate solvent (see table I). **10a**: ^{1}H NMR (DMSO-d_6) δ 2.23 (s, 3H), 4.77 (d, 2H, J = 5.6 Hz), 7.32–7.72 (m, 10H). **10b**: ^{1}H NMR (DMSO-d_6) δ 2.30 (s, 3H), 4.87 (d, 2H, J = 5.6 Hz), 7.45–7.75 (m, 9H). **10c**: ^{1}H NMR (DMSO-d_6) δ 2.26 (s, 3H), 3.84 (s, 3H), 4.83 (d, 2H, J = 4.8 Hz), 7.00 and 7.64 (2d, 4H, J = 8.8 Hz), 7.65–8.00 (m, 5H). **10d**: ^{1}H NMR (DMSO-d_6) δ 1.07 (t, 3H, J = 7.2 Hz), 2.75 (q, 2H, J = 7.2 Hz), 4.77 (d, 2H, J = 4.8 Hz), 7.25–7.85 (m, 10H). **10e**: ^{1}H NMR (DMSO-d_6) δ 2.90–3.30 (m, 2H), 4.84 (d, 2H, J = 4.8 Hz), 7.18–8.15 (m, 9H). **10f**: ^{1}H NMR (DMSO-d_6) δ 4.84 (d, 2H, J = 4.8 Hz), 7.15–8.15 (m, 15H). **10g**: ^{1}H NMR (DMSO-d_6) δ 5.15 (d, 2H, J = 5.6 Hz), 7.25–8.30 (m, 13H). For chemical and analytical data, see table I.

The configurational stability of oximes **11a–e** was tested by treating the appropriate oxime with Dowex-50W (H^+) resin in anhydrous acetone under the conditions described above. The mixture was then filtered and evaporated in vacuo, and the resulting oily residue was dissolved in anhydrous ethanol and treated, as above for the preparation of **10a–e**, with solid aminoguanidine hydrochloride. Evaporation of the solvent gave a semisolid residue which was diluted with H_2O, extracted with $CHCl_3$, filtered and evaporated to yield the starting compound practically pure.

Pharmacological methods

Guinea-pig ileum

The assays were conducted in accordance with the legislation of the Italian Authorities (DL 27/01/92, No 116) concerning animal experimentation. Under Et_2O anaesthesia, Dunkin–Hartley male guinea pigs weighing 250–300 g, deprived of food intake for 24 h before the experiments, were killed by cervical dislocation and bled. The abdominal cavity was opened by a midline incision, and portions of ileum 2–3 cm in length, about 10 cm distal to the ileocecal valve, were carefully dissected; subsequently, they were freed from the surrounding mesenteric tissue, attached with a thread to the organ holder and to the recording system by opposite sides of their open ends, and suspended in a 10 mL organ bath containing Tyrode solution [composition (mM): NaCl (136.8); KCl (2.95); $CaCl_2$ (1.80); $MgSO_4 \cdot 7H_2O$ (1.05); NaH_2PO_4 (0.41); $NaHCO_3$ (11.9); Glucose (5.5)] at 37 °C, gassed with carbogen (95% O_2; 5% CO_2). The ileum preparations were placed between two platinum electrodes (4 × 45 mm) set at a distance of 7 mm in the bath. The tissues were preloaded with a tension of 0.5 g and left to stabilize for 45–60 min before beginning electrical stimulation, which was carried out with a digital stimulator (Biomedica Mangoni Model BM-ST3) using the following parameters: single rectangular pulses, 0.1 Hz frequency, 0.3 ms pulse width, 12 V supramaximal voltage. The activity of the drugs under examination on α_2-adrenoceptors was evaluated as their ability to inhibit acetylcholine release evoked by electrical stimulation of nerve fibers. The effects of the mediator released on intestinal smooth muscle were recorded as longitudinal contractions by an isotonic transducer (Basile Model 7006) connected with a unirecord microdynamometer (Basile Model 7050). Agonist activity was expressed in terms of pD_2 values ($-\log ED_{50}$) and intrinsic activity (the ratio between the maximal response of a test compound and that of the reference agonist, *l*-norepinephrine.

Experiments to demonstrate the involvement of α_2-adrenoceptors in the mechanism of action of compounds **10a–g**, were performed by evaluating the ability of rauwolscine to shift to the right the concentration–response curve of the most active compound **10f**. The antagonism of rauwolscine against compound **10f** was evaluated following Gaddum's equation [16], and was expressed as pK_b.

l-Norepinephrine was used as a bitartrate, while rauwolscine, compounds **10a–g** and guanabenz were used as hydrochlorides.

Acknowledgment

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