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Original article

Indole amide derivatives: synthesis, structure–activity relationships and molecular modelling studies of a new series of histamine H_1-receptor antagonists

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Abstract – A number of indole amide derivatives bearing a basic side chain, in which the indole ring replaces the isoster benzimidazole nucleus typical of some well-known antihistamines, were prepared and tested for their H_1-antihistaminic activity. The 1-benzyl-3-indolecarboxamides **32**–**42** showed antihistaminic (H_1) activity (pA_2 6–8); the 3-indolylglyoxylylamides **7**–**16** and the 2-indolecarboxamides **48**–**56** showed little or no activity. Insertion of the basic side chain of the active 3-indolecarboxamide derivatives into a piperazine ring (compounds **57**–**59**) led to a dramatic loss of activity. All the active compounds proved to be competitive antagonists, since the values of the regression slope were not statistically different from 1. The most active compounds, **32**, **33**, **38**–**41**, were also tested both in vitro for their anticholinergic activity and in vivo for their ability to antagonize histamine-induced cutaneous vascular permeability in rats. The biological results and the structure–activity relationships of the novel compounds are discussed in the light of molecular modelling studies, taking the molecule of astemizole as a model, and referring to proposed H_1-receptor pharmacophore models. © Elsevier, Paris

indole derivatives / non-classic H_1-antagonists / astemizole / structure–activity relationships

1. Introduction

H_1-antagonists can be classified into two major groups: classic and non-classic [1]. Classic H_1-antagonists all share the following basic structure **I** *(figure 1)*, which consists of a basic nitrogen atom, predominantly protonated at physiological pH, and two aromatic groups, connected via a linking group, which can be of different chemical nature.

As side effects (anticholinergic activity and CNS depression such as sedation, hypnosis, etc.) commonly occur with all classic antihistamines, extensive studies have been carried out with the aim of developing antihistamine drugs with minimal side effects [2–10]. Two approaches have been followed to reach this goal. In the first place hydrophilic substituent groups have been introduced in classic compounds to reduce their blood–brain barrier penetration and, consequently, their sedative effects. Examples of these compounds are terfenadine, cetirizine, loratadine etc. On the other hand, stucturally completely different compounds devoid of the unwanted side-effects of classic H_1-antagonists have been developed. Because of their different chemical structure these compounds were called non-classic compounds. Astemizole *(figure 1)* is one of the few non-classic compounds known to be used therapeutically [11–14].

For these non-classic derivatives of 2-aminobenzimidazole, a model of the binding site has been proposed by Iemura et al. [15]. It consists of an anionic site which binds the positively-charged piperazinyl nitrogen atom, a slit-shaped cavity occupied by the 1-substituent group, and a site perpendicular to the previous one which interacts with the benzimidazole nucleus.

Although the non-classic antihistamines seem to possess new structural features, they present elements of the general structure **I** [1, 16].

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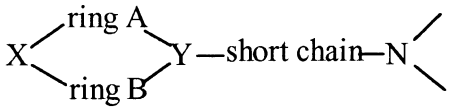
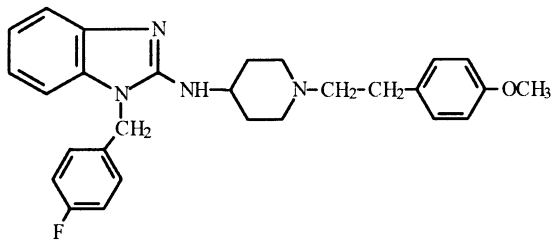


I



Astemizole

Figure 1.

In a previous paper [17], we reported the synthesis and the antihistaminic activity of some 1-substituted 2-benzylaminobenzimidazole derivatives, whose general structure presents few similarities to that of classic antihistamines **I**, even if some common elements can be discerned.

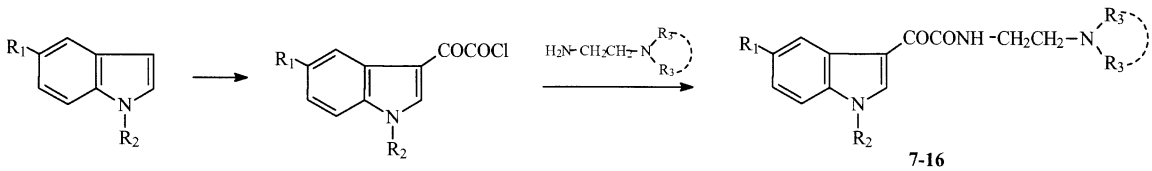
In the present paper we report the synthesis and the evaluation of the antihistaminic activity of a series of amide derivatives bearing a basic chain, in which the benzimidazole nucleus has been replaced by the isoster indole nucleus. In the initially prepared compounds, the dialkylaminoethyl chain was linked at position 3 of the indole system by a COCONH bridge (compounds **7**–**16**), as the indolylglyoxylylamide moiety proved to be a suitable substructure for many compounds synthesized by us, which showed a good antidepressant [18] and anti gastric secretory [19] activity, and interacted with a high affinity at the benzodiazepine receptor [20]. Indeed, a preliminary study had shown a very good superimposition of the groups thought to be responsible for antihistaminic activity in the structure of these substituted 1-benzylindole derivatives and that of astemizole, taken as the model compound (see Section 5).

For a better and wider definition of structure–activity relationships (SAR), we also considered compounds in which the distance between the aromatic moiety (indole nucleus) and the cationic centre was varied by substitution of the oxalylamide group COCONH with a carboxyamide residue CONH, bound at position 3 (compounds **29**–**43**) or 2 (compounds **48**–**56**) of the indole system. It is interesting to note that there are a number of examples in literature of non-classic H_1-antagonists, in which one of the aromatic groups is separated from the cationic centre by means of an intermediate chain CONH$(CH_2)_2$ [21, 22]. Derivatives in which the aminoethylamide chain at position 3 of the indole nucleus was inserted into a piperazine ring (compounds **57**–**59**) were also prepared.

The SARs (antihistaminic) of the indole amides are discussed in the light of molecular modelling studies by comparing the structures of selected derivatives with that of the related astemizole, taken as the reference model.

2. Chemistry

Compounds **7**–**16** were obtained by reacting under mild conditions the indolylglyoxylyl chlorides **1**–**6** [19] with the appropriate amine in the presence of triethylamine in benzene solution *(figure 2)*. The



1-5: R_1 = H, Cl, Br, NO_2, OCH_3; R_2 = H
6: R_1 = H; R_2 = $CH_2C_6H_5$

R_3NR_3 = $N(CH_3)_2$, piperidine, pyrrolidine, N-methylpiperazine, morpholine.

Figure 2.

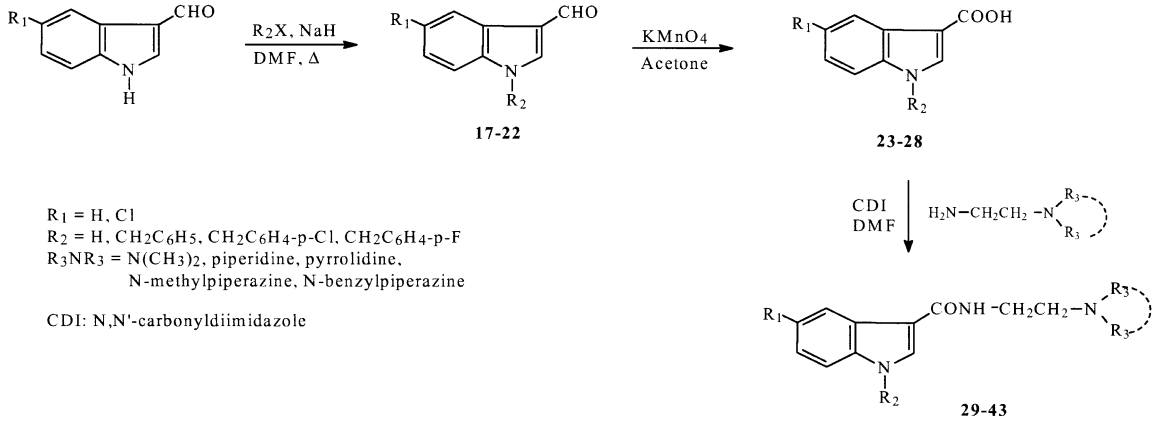


Figure 3.

1-benzylindole-3-ylglyoxylyl chloride **6**, never described in literature, was prepared starting from 1-benzylindole [23] essentially following the experimental procedure used for the synthesis of the other indolylglyoxylyl chlorides.

The synthesis of the N-(3-indolecarbonyl)amine derivatives **29**–**43** involved the preparation of the appropriate 3-indolecarboxylic acids **23**–**28**. These were obtained, with the exception of the commercial unsubstituted acid, by alkylation of indole-3-carbaldehyde and 5-chloroindole-3-carbaldehyde [24], and successive oxidation with potassium permanganate in acetone, essentially in accordance with a literature procedure [25] *(figure 3)*. The target compounds **29**–**43** were prepared from the acids and the appropriate amines, in anhydrous DMF, under a nitrogen atmosphere, using N,N'-carbonyldiimidazole (CDI) as the condensing agent *(figure 3)*.

This experimental procedure was also used for the synthesis of the N-(2-indolecarbonyl)amine derivatives **48**–**56** *(figure 4)*. The 1-benzyl-5-chloro-2-indolecarboxylic acid **47**, never described in literature, was prepared by Fisher indole synthesis starting from ethyl pyruvate and 1-benzyl-1-(4-chlorophenyl)hydrazine.

N,N'-carbonyldiimidazole in DMF was also employed as the condensing agent to obtain the N-(1-benzyl-3-indolecarbonyl)piperazine derivatives **57** and **58** from the 1-benzylindole-3-carboxylic acid **24** and the appropriate N-alkylpiperazine. The 1-benzylindole-3-carboxylic acid **24**, after conversion into its chloride by treatment with excess thionyl chloride in ethyl ether, was condensed with N-(β-hydroxyethyl)piperazine in anhydrous THF, in the presence of triethylamine, to give the target compound **59**.

All products were purified by recrystallization from the appropriate solvent, after a first purification, when nec-

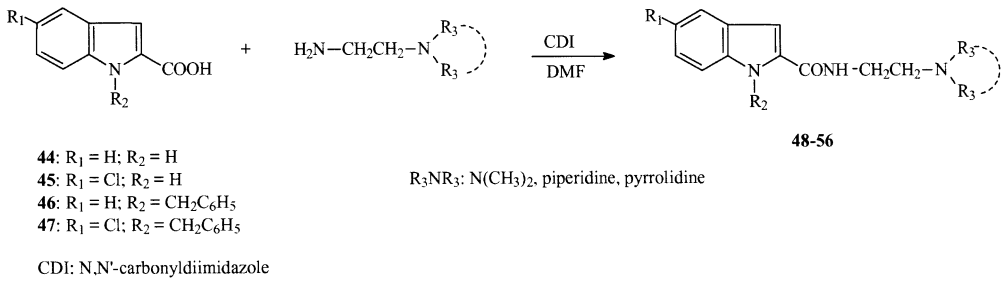
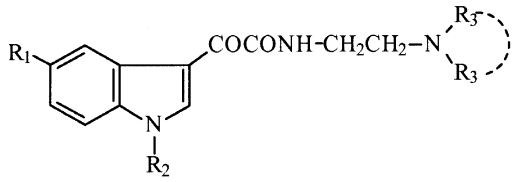


Figure 4.

Table I. N-(Indol-3-ylglyoxylyl)amine derivatives **7–16**: physical properties and histamine antagonist activity.



Compound	R_1	R_2	R_3–N–R_3	Yield (%)	Recryst. solvent	M.p. (°C)	Formula [a]	pA_2 [b] (slope)
7	H	H	CH_3 / CH_3	47	Benzene	174–175	$C_{14}H_{17}N_3O_2$	NA
8	Cl	H	CH_3 / CH_3	55	Methanol	220–221	$C_{14}H_{16}ClN_3O_2$	NA
9	Br	H	CH_3 / CH_3	73	Methanol	221–223	$C_{14}H_{16}BrN_3O_2$	NA
10	NO_2	H	CH_3 / CH_3	72	Methanol	228–230	$C_{14}H_{16}N_4O_4$	NA
11	OCH_3	H	CH_3 / CH_3	48	Benzene	194–195	$C_{15}H_{19}N_3O_3$	NA
12	H	$CH_2C_6H_5$	CH_3 / CH_3	40	Pet. ether 60–80°	102–104	$C_{21}H_{23}N_3O_2$	6.01 ± 0.25 (0.91 ± 0.18)
13	H	$CH_2C_6H_5$	Pyrrolidine	46	Pet. ether 60–80°	120–122	$C_{23}H_{25}N_3O_2$	NA
14	H	$CH_2C_6H_5$	Piperidine	47	Pet. ether 100–140°	131–132	$C_{24}H_{27}N_3O_2$	NA
15	H	$CH_2C_6H_5$	N-methylpiperazine	39	Pet. ether 60–80°	132–133	$C_{24}H_{28}N_4O_2$	NA
16	H	$CH_2C_6H_5$	Morpholine	47	Pet. ether 60–80°	119–121	$C_{23}H_{25}N_3O_3$	NA
Astemizole								8.61 ± 0.14 (1.22 ± 0.28)
Mepyramine								9.50 ± 0.41 (0.90 ± 0.19)

[a] All values for C, H, N were within ±0.4% of calculated values; [b] NA = not active at a concentration lower than 10^{-6} M.

essary, on a silica gel column. Their structure was confirmed by IR, ^{1}H-NMR, MS spectral data and elemental analyses *(table I–III)*.

3. Pharmacology

All compounds synthesized were assessed in vitro for their ability to antagonize histamine-induced contraction of guinea-pig ileum as previously described [17] *(table I–III)*, using mepyramine and astemizole [26, 27], selective anti-H_1 drugs, as the reference standards. This test is a reliable in vitro measure of H_1-antagonist activity. Those products which showed a significant inhibiting effect at the starting concentration of 10^{-6} M, were then tested with lower doses to determine their pA_2 values.

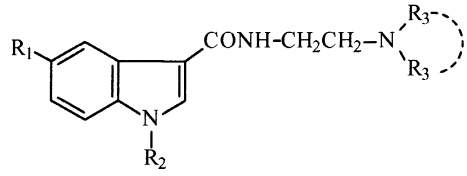
Selected compounds (**32**, **33**, **38**–**41**) which demonstrated significant in vitro inhibition in the guinea-pig ileum assay ($pA_2 > 7$) were then tested in vivo for their ability to antagonize histamine-induced cutaneous vascular permeability in rats [28] *(table IV)*. Moreover, with the aim to evaluate their potential side effects, they were also assayed in vitro for anticholinergic activity.

4. Results

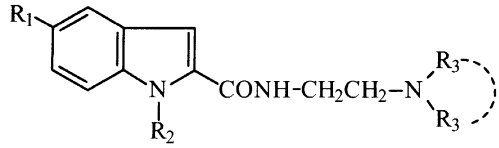
Table I shows the in vitro data of the histamine antagonist activity of indolylglyoxylylamide derivatives **7**–**16**. Compounds **7**–**11** did not exhibit any antagonist activity up to a concentration of 10^{-6} M. The presence of a substituent at position 5 of the indole nucleus with varying electronic and lipophilic characteristics did not give any significant activity improvement. Compound **12**, bearing a benzyl group at the indole nitrogen atom, proved to be a weak H_1-antagonist, showing a pA_2 value of 6.01, with a regression slope not statistically different from 1 (slope = 0.91 ± 0.18). Further studies regarding the basic nitrogen substitution in the 1-benzyl series showed that cyclic amines were not well tolerated (compounds **13**–**16** compared with **12**).

The 3-indolecarboxamide derivatives **29**–**43** showed, in general, a good antihistaminic activity, with pA_2 values ranging from 8.23 to 5.94 and slopes not statistically different from 1 *(table II)*. Also in this series of compounds, the presence of a benzyl substituent at position 1 of the indole moiety was beneficial for the activity (compare compounds **29**, **30** versus **32**, **33**). The introduction of a halo substituent, i.e. fluoro and chloro, in the para position of the N-benzyl moiety did not significantly

Table II. N-(3-Indolecarbonyl)-2-dialkylaminoethylamine derivatives **29–43** and N-(2-indolecarbonyl)-2-dialkylaminoethylamine derivatives **48–56**: physical properties and histamine antagonist activity.



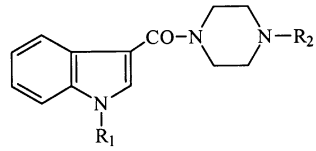
Compound	R_1	R_2	R_3–N–R_3	Yield (%)	Recryst. solvent	M.p. (°C)	Formula [a]	pA_2 [b] (slope)
29	H	H	CH_3 / CH_3	45	Benzene	116–118	$C_{13}H_{17}N_3O$	NA
30	H	H	Piperidine	70	Pet. ether 60–80°	136–137	$C_{16}H_{21}N_3O$	NA
31	H	H	Pyrrolidine	59	Benzene	122–124	$C_{15}H_{19}N_3O$	NA
32	H	$CH_2C_6H_5$	CH_3 / CH_3	68	Benzene	138–140	$C_{20}H_{23}N_3O$	7.03 ± 0.22 (0.97 ± 0.20)
33	H	$CH_2C_6H_5$	Piperidine	35	Benzene/Pet. ether 60–80°	130–131	$C_{23}H_{27}N_3O$	8.23 ± 0.34 (0.80 ± 0.28)
34	H	$CH_2C_6H_5$	N-methylpiperazine	36	Benzene	126–128	$C_{23}H_{28}N_4O$	6.36 ± 0.21 (1.02 ± 0.16)
35	H	$CH_2C_6H_5$	N-benzylpiperazine	32	Toluene	120–122	$C_{29}H_{32}N_4O$	NA
36	Cl	$CH_2C_6H_5$	CH_3 / CH_3	43	Benzene	126–128	$C_{20}H_{22}ClN_3O$	6.23 ± 0.53 (1.00 ± 0.34)
37	Cl	$CH_2C_6H_5$	Piperidine	36	Toluene	138–140	$C_{23}H_{26}ClN_3O$	NA
38	H	$CH_2C_6H_4$-p-F	CH_3 / CH_3	32	Toluene	168–170	$C_{20}H_{22}FN_3O$	7.05 ± 0.13 (0.89 ± 0.16)
39	H	$CH_2C_6H_4$-p-F	Piperidine	39	Toluene	151–152	$C_{23}H_{26}FN_3O$	8.00 ± 0.27 (0.91 ± 0.13)
40	H	$CH_2C_6H_4$-p-Cl	CH_3 / CH_3	69	Toluene	185–186	$C_{20}H_{22}ClN_3O$	7.75 ± 0.37 (0.79 ± 0.13)
41	H	$CH_2C_6H_4$-p-Cl	Piperidine	76	Toluene/Pet. ether 60–80°	158–159	$C_{23}H_{26}ClN_3O$	7.13 ± 0.20 (0.88 ± 0.11)
42	Cl	$CH_2C_6H_4$-p-Cl	CH_3 / CH_3	32	Benzene	132–134	$C_{20}H_{11}Cl_2N_3O$	5.94 ± 0.21 (1.19 ± 0.33)
43	Cl	$CH_2C_6H_4$-p-Cl	Piperidine	43	Benzene	115–117	$C_{23}H_{25}Cl_2N_3O$	NA



48	H	H	CH_3 / CH_3	74	Benzene	137–138	$C_{13}H_{17}N_3O$	NA
49	H	H	Piperidine	81	Benzene	159–161	$C_{16}H_{21}N_3O$	NA
50	Cl	H	CH_3 / CH_3	91	Benzene	203–205	$C_{13}H_{16}ClN_3O$	NA
51	Cl	H	Piperidine	73	Benzene	219–220	$C_{16}H_{20}ClN_3O$	6.53 ± 0.22 (0.93 ± 0.23)
52	Cl	H	Pyrrolidine	77	Benzene	204–206	$C_{15}H_{18}ClN_3O$	5.90 ± 0.33 (0.93 ± 0.31)
53	H	$CH_2C_6H_5$	CH_3 / CH_3	32	Benzene	74–76	$C_{20}H_{23}N_3O$	NA
54	H	$CH_2C_6H_5$	Piperidine	36	Benzene/Pet. ether 60–80°	123–125	$C_{23}H_{27}N_3O$	6.42 ± 0.28 (1.08 ± 0.22)
55	Cl	$CH_2C_6H_5$	CH_3 / CH_3	38	Benzene	118–120	$C_{20}H_{22}ClN_3O$	6.50 ± 0.31 (1.10 ± 0.28)
56	Cl	$CH_2C_6H_5$	Piperidine	25	Benzene/Pet. ether 60–80°	127–128	$C_{23}H_{26}ClN_3O$	6.22 ± 0.38 (1.01 ± 0.30)
Astemizole								8.61 ± 0.14 (1.22 ± 0.28)
Mepyramine								9.50 ± 0.41 (0.90 ± 0.19)

[a] All values for C, H, N were within ±0.4% of calculated values; [b] NA = not active at a concentration lower than 10^{-6} M.

Table III. 4-Substituted-1-(1-benzyl-3-indolecarbonyl)piperazine derivatives **57–59**: physical properties and histamine antagonist activity.



Compound	R_1	R_2	Yield (%)	Recryst. solvent	M.p. (°C)	Formula [a]	pA_2 [b]
57	$CH_2C_6H_5$	CH_3	27		Oil	$C_{21}H_{23}N_3O$	NA
58	$CH_2C_6H_5$	$CH_2C_6H_5$	30	Toluene	127–129	$C_{27}H_{27}N_3O$	NA
59	$CH_2C_6H_5$	CH_2CH_2OH	42	Toluene	145–146	$C_{22}H_{25}N_3O_2$	NA

[a] All values for C, H, N were within ±0.4% of calculated values; [b] NA = not active at a concentration lower than 10^{-6} M.

alter the overall activity (compounds **32**, **33** compared with **38**, **39**, **40**, **41**). The presence of a chlorine atom at position 5 of the indole moiety resulted in compounds with a lower activity (see for example compounds **32**, **33**, **40**, **41** compared with **36**, **37**, **42**, **43**). Also in this series of compounds, the substitution pattern of the basic head was considered. The piperidine derivative **33** was found to be the most interesting compound, showing a pA_2 value of 8.23, one order of magnitude more potent than that of the dimethylamino compound **32**, and comparable with that of astemizole taken as the reference standard. Bulky amines, such as N-methyl and N-benzylpiperazine, gave compounds **34**, **35**, respectively, with a dramatic reduction in the H_1-antagonist activity.

The 2-indolecarboxamide derivatives **48**–**56** showed, in general, little or no H_1-antagonist activity, with pA_2 values ranging between 6.53 and 5.90 *(table II)*. In this series of compounds, the combination of the most interesting moieties, i.e. 1-benzyl group and piperidine as basic head, selected from previous SAR analysis, did not give positive results (compare compound **54** with respect to **33**).

The incorporation of the aminoethyl side chain of the 3-indole substituted derivatives into a piperazine ring resulted in compounds **57**–**59**, which did not show any activity *(table III)*.

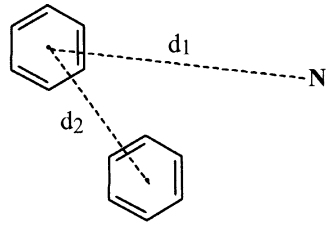
The most active compounds (**32**, **33**, **38**–**41** with $pA_2 >$ 7 in the guinea-pig ileum assay), tested for their anticholinergic activity, did not show any effect up to a concentration of 10^{-4} M. These compounds were also assayed in vivo for their ability to antagonize histamine-induced cutaneous vascular permeability in rats. The data reported in *table IV* show that only compounds **38**–**41** possessed any significant activity (i.e. ca. 50% of inhibition at 10 mg/kg s.c.). Moreover, it has to be pointed out that compounds **40** and **41** presented a pharmacodynamic profile similar to that observed for the reference antagonist mepyramine, as they inhibited dose-dependently the histamine-induced effect.

Table IV. Effects of selected compounds and reference drugs on cutaneous permeability in rats.

Compound	Inhib. (%) [a] (mg/kg s.c.)					ED_{50} [b] (mg/kg s.c.)	Potency ratio [c]
	2	3	4.5	6.7	10		
32					14.0 ± 4.1		
33					37.1 ± 6.7		
38					48.0 ± 7.8		
39					45.2 ± 3.7		
40		13.8 ± 0.8		32.7 ± 3.9	58.5 ± 2.6	8.9 ± 1.2	0.57
41		15.1 ± 1.5		41.8 ± 2.8	63.0 ± 1.9	7.5 ± 1.1	0.68
Mepyramine	12.4 ± 10		35.6 ± 6.7		77.5 ± 5.5	5.1 ± 1.1	

[a] Percent of inhibition vs. control; [b] each compound was tested with three doses ranging between 2 and 10 mg/kg. Each dose was tested s.c. in triplicate; [c] ED_{50}(mepyramine)/ED_{50}(compound).

Table V. Atomic distances (Å) of selected minimum conformations of interest used for superimposition studies. d_1 is the distance between the protonated nitrogen and the centre of gravity of the benzene ring of the indole or benzimidazole nucleus; d_2 is the distance between the centres of gravity of the two unsaturated moieties of the molecule. Root mean square (RMS) values for each superimposition with the two conformations considered for astemizole vs. the compounds described in this studies are also reported.



Compound	d_1	d_2	RMS vs. conf. 1	RMS vs. conf. 2
Astemizole (conf. 1)	8.21	5.58		
Astemizole (conf. 2)	6.77	5.18		
12	8.26	5.27	0.145	0.753
32	6.53	5.20	1.111	0.374
53	7.71	5.89	1.553	2.043
57	7.51	5.19	1.117	0.684

5. Discussion

The 3-indolylglyoxylylamides **12**–**16** have a molecular framework very similar to that of non-classic antihistamines, such as astemizole. Moreover, they fulfil all the requirements reported by Iemura et al. [15] to interact with the H_1-receptor binding site: a positively-charged nitrogen atom, a planar lipophilic area, and a perpendicular substituent at the 1-position entering a slit-shaped cavity. *Figure 5* shows the superimposition of compound **12**, selected as a model for the indolylglyoxylylamide class, in its absolute minimum energy conformation, on the reference compound astemizole. The conformation of astemizole was obtained after minimization of the original X-ray structure, while a molecular model of **12** was generated by the MacroModel/BatchMin V 5.0 programme (computational details are summarized in the Experimental Section). As can be seen, the groups considered essential for binding with the H_1 receptor, i.e. two aromatic rings and the basic nitrogen, lie for both products in the same spatial region. The presence of a strong electrostatic interaction between the oxygen of the amide function and the protonated basic nitrogen limited the conformational freedom of the side chain of compound **12**, thus providing a good fit with astemizole, as demonstrated by the low value of the root mean square (RMS) of the distances between the selected groups thought to be responsible for the activity (RMS = 0.145) *(table V)*. However, in vitro characterization in the guinea-pig ileum assay showed only a weak H_1-antagonist potency for compound **12** (pA_2 = 6.01) *(table I)*. Nevertheless, in view of its competitive antagonist activity vs. histamine (demonstrated by the value of the regression slope not statistically different from 1) this compound was considered to be a good tool to optimize the overall activity of this new class of compounds. As the low H_1-antagonist activity of compound **12** could be due to bad steric and/or electrostatic interactions of the oxalyl bridge with the receptor site, optimization studies were focused on the modification of the COCONH residue. A first attempt was made by removing one of the carbonyl groups of the glyoxyl moiety, leaving the resulting CONH bridge linked to position 3 of the indole ring. The compounds obtained, **29**–**43**, proved to be very interesting. In particular, compound **32** *(table II)* was 10 times more potent as an antagonist than the parent compound **12**.

Molecular modelling studies were undertaken on compound **32** to rationalize the activity of this new class of derivatives. A first comparison of the astemizole molecule with a model of compound **32** in its minimum energy conformation *(figure 6)* showed a different spatial arrangement with a high value of the RMS of 1.111 *(table V)* between the selected moieties supposed to be involved in the receptor interaction. As the conformational freedom of the side chain of **32** is limited by the strong electrostatic interaction between the oxygen of the amide function and the cationic head, a better fit (RMS = 0.374) *(figure 7)* was obtained considering a different astemizole conformation (conf. 2), still accessible for receptor interaction ($E_{\text{conf. 2}} - E_{\text{min}} \leq 5$ kcal/mol). It may be noted that in this conformation (conf. 2), the distance between the protonated nitrogen and the lipophilic area

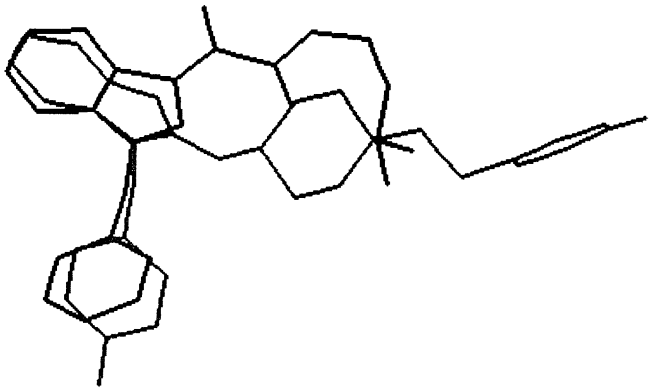


Figure 5. Superimposition of astemizole (gray) and **12** (black). Points used were the basic nitrogens and the centroids of the benzene rings of the indole and benzyl moieties (RMS = 0.145).

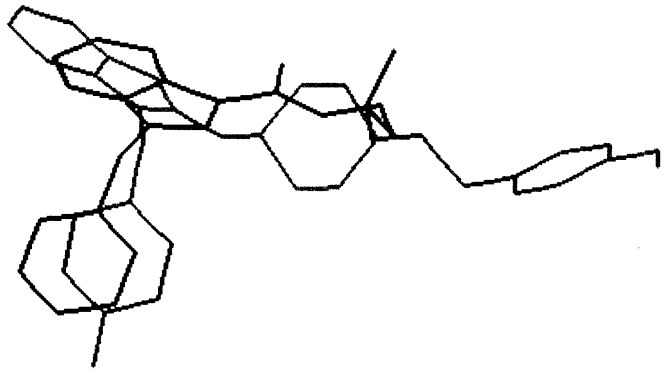


Figure 6. Superimposition of astemizole (minimum energy conformation, gray) and **32** (black). Points used were the basic nitrogens and the benzene rings of the indole and benzyl moieties (RMS = 1.111).

represented by the benzene moiety of the benzimidazole ring (d_1) was shorter than in the minimum energy conformation (conf. 1) *(table V)*, in better agreement with those originally reported by Naruto [29] and Borea [30] in their proposed pharmacophore models (i.e. d_1 about 6.5 Å and 6.2 Å, respectively). In the light of these results, it can be speculated that astemizole could not interact with the H_1-receptor site in its minimum energy conformation (conf. 1), but instead in another accessible conformation (conf. 2) which fulfils the H_1-pharmacophore requirements, as discussed above. This hypothesis could account for the inactivity of compounds **7**–**16**, as the superimposition of **12** on astemizole in its proposed active conformation (conf. 2) did not show a good fit between the groups responsible for the activity (RMS of 0.753 vs. conf. 1 RMS of 0.145).

Different results were obtained when the same amidic side chain was linked at position 2 of the indole moiety. Compounds **48**–**56** showed little or no activity in the guinea-pig ileum bioassay. It was found that none of the accessible conformations of compound **53**, taken as a model for this class of compounds, fits with either of the conformations of astemizole considered in this study. This is reflected by the higher d_1 distance showed by the minimum energy conformation of compound **53** compared with that of compound **32** *(table V)*. It is also worth noting that in this case the presence of a strong electrostatic interaction with the protonated basic nitrogen and the amidic carbonyl group greatly limits the conformational freedom of the molecule. A similar situation occurs in the case of the piperazine derivative **57**. Its greater d_1 value of 7.51 Å may explain its poor activity as an H_1-antagonist.

In conclusion, a good H_1-antagonist activity was shown by the derivatives in which the basic chain was linked at position 3 of the indole system by means of a carboxamide bridge, with a benzyl group at the indole nitrogen atom, which can fit into an appropriate cavity on the receptor site. The molecular modelling results seem to suggest that the pharmacologically active conformation of astemizole could be similar to that indicated as conf. 2

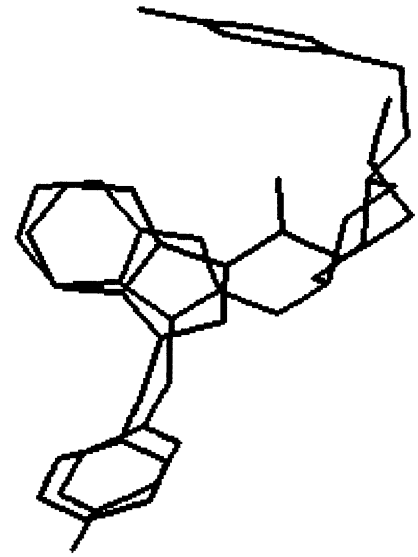


Figure 7. Superimposition of astemizole (conformation 2, gray) and **32** (black). Points used were the same as those in *figure 6* (RMS = 0.374).

(table V, figure 7), even though this conformation is not the minimum energy one, but it is still accessible for receptor interaction. This conformation possesses the right distances between the protonated nitrogen and the centroid of at least one of the aromatic rings for potent H_1-antagonist activity.

6. Experimental protocols

6.1. Chemistry

Melting points were determined on a Köfler hot-stage apparatus and are uncorrected. IR spectra were recorded with a Pye Unicam Infracord Model PU 9516 in Nujol mulls. Routine ^{1}H NMR spectra were determined on a Varian CFT 20 spectrometer operating at 80 MHz, using tetramethylsilane (TMS) as the internal standard. DMSO-d_6 was used as the solvent, unless otherwise indicated. Mass spectra were obtained on a Hewlett-Packard 5988 A spectrometer using a direct injection probe and an electron beam energy of 70 eV. Magnesium sulfate was always used as the drying agent. Evaporations were made in vacuo (rotating evaporator). Analytical TLC was carried out on Merck 0.2 mm precoated silica gel (60 F-254) aluminium sheets, with visualization by irradiation with a UV lamp. Silica gel 60 (70–230 mesh) was used for column chromatography and silica gel 60 (230–400 mesh) was used for flash chromatography. Elemental analyses were performed by our Analytical Laboratory and agreed with theoretical values to within ±0.4%.

The following products were prepared according to literature: 1-methyl-4-(β-aminoethyl)piperazine, b.p. 70–72 °C/4 mmHg (lit. [31] b.p. 61–62 °C/1 mmHg); 1-benzyl-4-(β-aminoethyl)piperazine, b.p. 170–173 °C/5 mmHg (lit. [22] b.p. 180–187 °C/13 mmHg); 1-benzyl-1-phenylhydrazine, m.p. 167–169 °C (lit. [32] m.p. 169–171 °C); 1-benzylindole, m.p. 44–45 °C (lit. [23] m.p. 43–45 °C); 1-benzylindole-2-carboxylic acid, m.p. 199–200 °C (lit. [33] m.p. 198–201 °C); 5-chloroindole-3-carbaldehyde, m.p. 214–215 °C (lit. [24] m.p. 215–216 °C); 1-benzylindole-3-carbaldehyde, m.p. 110–112 °C (lit. [34] m.p. 113–114 °C); 1-(4-chlorobenzyl)indole-3-carbaldehyde, m.p. 120–121 °C (lit. [35] m.p. 121–122 °C); 1-benzylindole-3-carboxylic acid, m.p. 199–200 °C (lit. [25] m.p. 198–201 °C); 1-(4-chlorobenzyl)indole-3-carboxylic acid, m.p. 209–211 °C (lit. [25] m.p. 207–209 °C).

*6.1.1. 1-Benzylindole-3-ylglyoxylyl chloride **6***

A solution of oxalyl chloride (1.72 mL, 17.9 mmol) in 3 mL of anhydrous ethyl ether was added, dropwise at 0 °C, to a solution of 2.02 g (9.8 mmol) of 1-benzylindole in 35 mL of the same solvent. The reaction mixture was left to warm at room temperature, stirred for 4 h and then cooled again at 0 °C. The precipitate formed was collected, washed with small portions of anhydrous ethyl ether and dried under vacuum to give pure **6** as a yellow microcrystalline solid (1.85 g). M.p. 104–105 °C, yield 64%. IR ν cm^{-1}: 1800, 1670, 1220, 1000, 750. ^{1}H-NMR: δ 5.58 (s, 2H, CH_2); 7.12–8.63 (m, 10H, ArH). MS: *m/e* 297 $(M)^+$, 91, base. Anal. ($C_{17}H_{12}ClNO_2$) C, H, N.

*6.1.2. General procedure for the synthesis of N-[(1- or 5-substituted indole-3-yl)glyoxylyl]-2-dialkylaminoethylamine derivatives **7–16***

A solution of the appropriate amine (10 mmol) in 20 mL of anhydrous benzene was added, dropwise, at 0 °C, to a solution of the appropriately substituted indolylglyoxylyl chloride **1–6** (9.4 mmol) and triethylamine (1.5 mL, 11 mmol) in 120 mL of anhydrous benzene. The reaction mixture was left to stir at room temperature for 48–72 h, monitoring the reaction by TLC analysis. The precipitate present was filtered off and the filtrate was concentrated to dryness. The oily residue was solubilized in chloroform, washed with saturated sodium hydrogen carbonate aqueous solution and water, and concentrated again. The crude product obtained was purified by recrystallization from the appropriate solvent. Yields, recrystallization solvents and melting points are listed in *table I*. The spectrometric data for **12**, which is representative of the title compounds, are listed below.

*6.1.2.1. N-[(1-Benzylindole-3-yl)glyoxylyl]-2-dimethylaminoethylamine **12**:*

IR ν cm^{-1}: 3300, 1660, 1625, 1500, 1170, 750. ^{1}H-NMR: δ 2.20 (s, 6H, $(CH_3)_2N$); 2.40 (t, 2H, $(CH_3)_2NCH_2$); 3.33 (q, 2H, $NHCH_2CH_2$); 5.62 (s, 2H, CH_2C_6); 7.83–9.16 (m, 10H, ArH); 8.63 (br t, 1H, NH, exch. with D_2O). MS: *m/e* 349 $(M)^+$, 58, base.

*6.1.3. General procedure for the synthesis of 5-substituted 1-(4-substituted benzyl)indole-3-carbaldehydes **17–22***

Sodium hydride (0.576 g, 24 mmol, 50% dispersion in mineral oil) was added, in small portions, to a solution of the appropriately substituted indole-3-carbaldehyde (20 mmol) in 33 mL of freshly distilled DMF, then, dropwise, the appropriate benzylchloride (24 mmol). The reaction mixture was heated at 90 °C for 2.5–3 h (TLC analysis), and then, after cooling, was poured into ice. After acidification to pH 6 with 2 N hydrochloric acid, the solid formed was collected, washed with water and purified by recrystallization, after filtration, when necessary, on a silica gel column. Yields, recrystallization

solvents, melting points and spectrometric data for the newly synthesized compounds are listed below.

*6.1.3.1. 1-Benzyl-5-chloroindole-3-carbaldehyde **19***:
M.p. 141–143 °C (ethanol); yield 72%. IR ν cm^{-1}: 1640, 1170, 1050, 800. ^{1}H-NMR: δ 5.51 (s, 2H, CH_2); 7.15–8.41 (m, 9H, ArH); 9.89 (s, 1H, CHO). MS: *m/e* 269 $(M)^+$, 91, base. Anal. ($C_{16}H_{12}ClNO$) C, H, N.

*6.1.3.2. 1-(4-Fluorobenzyl)indole-3-carbaldehyde **20***:
M.p. 106–108 °C (ethanol); yield 80%. IR ν cm^{-1}: 1670, 1600, 1290, 1160, 770. ^{1}H-NMR: δ 5.50 (s, 2H, CH_2); 7.13–8.45 (m, 9H, ArH); 9.89 (s, 1H, CHO). MS: *m/e* 253 $(M)^+$, 95, base. Anal. ($C_{16}H_{12}FNO$) C, H, N.

*6.1.3.3. 1-(4-Chlorobenzyl)-5-chloroindole-3-carbaldehyde **22***:
M.p. 135–137 °C (ethanol); yield 52%. IR ν cm^{-1}: 1630, 1510, 1150, 790. ^{1}H-NMR: δ 5.52 (s, 2H, CH_2); 7.15–8.45 (m, 8H, ArH); 9.89 (s, 1H, CHO). MS: *m/e* 303 $(M)^+$, 125, base. Anal. ($C_{16}H_{11}Cl_2NO$) C, H, N.

*6.1.4. General procedure for the synthesis of 5-substituted 1-(4-substituted benzyl)indole-3-carboxylic acids **23**–**28***
A solution of 2.99 g (19 mmol) of potassium permanganate in 60 mL of water was added, dropwise, to a solution of the appropriate indole-3-carbaldehyde **17**–**22** (10 mmol) in 150 mL of acetone. The reaction mixture was left to stir, in the dark, at room temperature, for 16 h. After cooling at 0 °C, the mixture was decolorized by a 10% aqueous solution of hydrogen peroxide and then filtered. The resulting solution was concentrated to half volume and acidified to pH 3 with 2 N hydrochloric acid. The precipitate formed was collected, washed with water, and purified by recrystallization. Yields, recrystallization solvents, melting points and spectrometric data for the newly synthesized compounds are listed below.

*6.1.4.1. 1-Benzyl-5-chloroindole-3-carboxylic acid **25***:
M.p. 196–197 °C (toluene); yield 50%. IR ν cm^{-1}: 3150–2400, 1650, 1550, 1300, 1230, 1170, 760. ^{1}H-NMR: δ 5.53 (s, 2H, CH_2); 7.30–8.33 (m, 9H, ArH); 8.83 (s, 1H, COOH, exch. with D_2O). MS: *m/e* 285 $(M)^+$, 91, base. Anal. ($C_{16}H_{12}ClNO_2$) C, H, N.

*6.1.4.2. 1-(4-Fluorobenzyl)indole-3-carboxylic acid **26***:
M.p. 190–192 °C (toluene); yield 45%. IR ν cm^{-1}: 3100–2400, 1630, 1500, 1250, 1190, 1060, 850, 740. ^{1}H-NMR: δ 5.45 (s, 2H, CH_2); 6.90–8.16 (m, 9H, ArH); 12.00 (br s, 1H, COOH, exch. with D_2O). MS: *m/e* 269 $(M)^+$, 109, base. Anal. ($C_{16}H_{12}FNO_2$) C, H, N.

*6.1.4.3. 1-(4-Chlorobenzyl)-5-chloroindole-3-carboxylic acid **28***:
M.p. 212–214 °C (toluene); yield 62%. IR ν cm^{-1}: 3150–2400, 1640, 1520, 1200, 1080. ^{1}H-NMR: δ 5.48 (s, 2H, CH_2); 7.10–8.40 (m, 8H, ArH); 12.00 (br s, 1H, COOH, exch. with D_2O). MS: *m/e* 319 $(M)^+$, 125, base. Anal. ($C_{16}H_{11}Cl_2NO_2$) C, H, N.

*6.1.5. 5-Chloro-1-benzylindole-2-carboxylic acid **47***
The acid **47** was prepared starting from ethyl pyruvate N-benzyl-N-(4-chlorophenyl)hydrazone. This intermediate was obtained by refluxing for 1 h a mixture of ethyl pyruvate (1.2 mL, 11 mmol) and N-benzyl-N-(4-chlorophenyl)hydrazine (2.3 g, 10 mmol). The crude product obtained was purified by flash chromatography (eluting system: petroleum ether 60–80°/dichloromethane = 95:5), yielding 2.4 g of pure hydrazone as an oil. Yield 72%. IR ν cm^{-1}: 2995, 1710, 1490, 1270, 1140, 730. ^{1}H-NMR ($CDCl_3$): δ 1.38 (t, 3H, CH_2CH_3); 1.93 (s, 3H, CH_3); 4.33 (q, 2H, CH_2CH_3); 5.00 (s, 2H, CH_2C_6); 6.86–7.66 (m, 9H, ArH). MS: *m/e* 330 $(M)^+$, 91, base. Anal. ($C_{18}H_{19}ClN_2O_2$) C, H, N.

A mixture of 1.65 g (5 mmol) of the hydrazone obtained in 5 mL of ethanol and 15 mL of 12% hydrochloric acid was refluxed for 2.5 h. After cooling, the precipitate formed was collected, dried under vacuum and purified by recrystallization from benzene, giving 0.45 g of pure acid. M.p. 151–153 °C; yield 32%. IR ν cm^{-1}: 3000–2400, 1650, 1500, 1240, 1180. ^{1}H-NMR: δ 4.42 (s, 2H, CH_2); 7.15–7.54 (m, 9H, ArH); 11.55 (br s, 1H, COOH, exch. with D_2O). MS: *m/e* 285 $(M)^+$, 44, base. Anal. ($C_{16}H_{12}ClNO_2$) C, H, N.

*6.1.6. General procedure for the synthesis of N-(1,5-substituted 3-indolecarbonyl)-2-dialkylaminoethylamine derivatives **29–43**, N-(1,5-substituted 2-indolecarbonyl)-2-dialkylaminoethylamine derivatives **48–56** and 4-substituted N-(1-benzyl-3-indolecarbonyl)piperazine derivatives **57, 58***
N,N'-carbonyldiimidazole (0.405 g, 2.5 mmol) was added, under stirring in a nitrogen atmosphere, to a solution of the appropriate indolecarboxylic acid **23**–**28**, **44**–**47** (2.5 mmol) in 4 mL of anhydrous DMF. After carbon dioxide evolution had ceased, a solution of the appropriate amine (2.5 mmol) in 2 mL of anhydrous DMF was added to the reaction mixture, which was left to stir at room temperature for 2.5–4 h (TLC analysis). The resulting solution was concentrated to dryness, and the oily residue, dissolved in chloroform, was washed with saturated sodium hydrogen carbonate aqueous solution, and then with water. After drying, the chloroform solution was concentrated to dryness, and the crude

product was purified by flash chromatography (eluting system: dichloromethane/methanol/ammonia conc. = 96:5:0.5 – 79:15:1) and recrystallization *(table II* and *III)*. The spectrometric data for **32**, **55** and **58**, which are representative of the title compounds, are listed below.

*6.1.6.1. N-(1-Benzyl-3-indolecarbonyl)-2-dimethylaminoethylamine **32***:

IR ν cm^{-1}: 3375, 1620, 1540, 1185, 730. ^{1}H-NMR: δ 2.24 (s, 6H, $(CH_3)_2N$); 2.48 (t, 2H, $(CH_3)_2NCH_2$); 2.48 (q, 2H, $NHCH_2$); 5.43 (s, 2H, CH_2C_6); 7.10–8.18 (m, 10H, ArH); 7.73 (br t, 1H, NH, exch. with D_2O). MS: *m/e* 277 $(M - (CH_3)_2N)^+$, 58, base.

*6.1.6.2. N-(1-Benzyl-5-chloro-2-indolecarbonyl)-2-dimethylaminoethylamine **55***:

IR ν cm^{-1}: 3310, 1630, 1545, 1275, 720. ^{1}H-NMR: δ 2.22 (s, 6H, $(CH_3)_2N$); 2.45 (t, 2H, $(CH_3)_2NCH_2$); 3.36 (q, 2H, $NHCH_2$); 5.86 (s, 2H, CH_2C_6); 7.00–7.83 (m, 9H, ArH); 8.60 (br t, 1H, NH, exch. with D_2O). MS: *m/e* 355 $(M)^+$, 58, base.

*6.1.6.3. N-(1-Benzyl-3-indolecarbonyl)-4-benzylpiperazine **58***:

IR ν cm^{-1}: 1620, 1535, 1190, 1000, 745. ^{1}H-NMR: δ 2.38–2.54 (m, 4H, $(CH_2)_2NCH_2$); 3.45–3.71 (m, 4H, $(CH_2)_2NCO$); 3.53 (s, 2H, $(CH_2)_2NCH_2$); 5.45 (s, 2H, NCH_2C_6); 7.06–7.85 (m, 15H, ArH). MS: *m/e* 409 $(M)^+$, 91, base.

*6.1.7. N-(1-Benzyl-3-indolecarbonyl)-4-(β-hydroxyethyl)piperazine **59***

Compound **59** was obtained essentially using an experimental procedure similar to that employed for the above-reported indolylglyoxylylamine derivatives. 1-Benzylindole-3-carbonyl chloride was obtained by adding, dropwise, at 0 °C, thionyl chloride (0.53 mL, 7.3 mmol) to a well-stirred suspension of 0.65 g (2.6 mmol) of the acid **24**. The reaction mixture, after stirring at room temperature for 2.5 h, was concentrated to dryness giving a solid, which was washed repeatedly with anhydrous ethyl ether to yield 0.59 g of pure acid chloride. M.p. 95–97 °C; yield 84%. IR ν cm^{-1}: 1730, 1510, 1390, 1250, 1030, 730. ^{1}H-NMR: δ 5.46 (s, 2H, CH_2); 7.03–8.13 (m, 10H, ArH). MS: *m/e* 269 $(M)^+$, 91, base. Anal. ($C_{16}H_{12}ClNO$) C, H, N.

The acid chloride obtained was reacted with 4-(2-hydroxyethyl)piperazine in anhydrous THF. After the usual work-up, the crude product was purified by flash chromatography (eluting system: dichloromethane/methanol/ammonia conc. = 90:7:0.5) and recrystallization *(table III)*.

IR ν cm^{-1}: 3950, 1600, 1540, 1000, 745. ^{1}H-NMR; δ 2.37–2.52 (m, 8H, $(CH_2)_2NCH_2CH_2OH$); 3.43–3.68 (m, 4H, $(CH_2)_2NCO$); 4.35 (br t, 1H, OH, exch. with D_2O); 5.43 (s, 2H, CH_2C_6); 7.04–7.82 (m, 10H, ArH). MS: *m/e* 364 $(MH)^+$, 91, base.

6.2. Molecular modelling

Models of compounds **12**, **32**, **53** and **57** were constructed with standard bond lengths and angles from the fragment database in MacroModel V 5.0 (Columbia University, New York, NY 10027) using a Silicon Graphics workstation (Indigo II). A starting model of astemizole was built using its X-ray coordinates (Cambridge Structural Database reference code ZENREP 0001). All compounds were modelled as protonated bases and minimized by the MacroModel/BatchMin V 5.0 programme using the MM2 force field. In order to perform an extensive conformational search, a MonteCarlo/Energy minimization [36] was carried out for all the compounds considered in the study ($E_i - E_{min} \leq 5$ kcal/mol). Representative minimum energy conformations of each compound were used to perform superimposition studies (see figure captions).

6.3. Pharmacology

6.3.1. Materials and methods

Experiments were carried out using male or female Durkin–Hartley guinea-pigs, 250–300 g b.w., and male Sprague Dawley albino rats, 120–140 g b.w., supplied by Charles River (Calco, Italy). Reference compounds were obtained from Sigma Chemical Co. (St. Louis, MO, USA).

6.3.2. In vitro test – Histamine H_1 and acetylcholine antagonism: guinea-pig ileum

In accordance with the method described by Magnus [37], segments of pre-terminal ileum, about 2 cm long, were promptly excised from guinea-pigs of either sex and placed under a resting tension of 1.0 g in a 10 mL bath with Tyrode's solution, kept at 37 °C and gassed with 5% CO_2 in O_2. After 1 h equilibration time, graded concentrations of histamine or acetylcholine were added to the bath to obtain cumulative dose–response curves, in accordance with the method reported by Van Rossum [38, 39]. Concentrations in response to histamine or acetylcholine were measured as changes in isometric tension by a force transducer.

H_1-histamine or cholinergic antagonism was assessed by adding to the bath a solution (0.1 mL) at various concentrations of the compound under test and, after 3 min incubation, the cumulative dose–response curves

were constructed. Mepyramine and astemizole [27, 28] were employed as a reference drugs for H_1-histamine antagonism, and atropine was used as standard for acetylcholine antagonism.

The pA_2 values for the competitive H_1-antagonists were calculated in accordance with the method described by Schild [40, 41], using the calculation program reported by Tallarida and Murray [42]. The Student's *t*-test was used to estimate statistical significance.

6.3.3. In vivo test – Histamine-induced coutaneous vascular permeability

The ability of compounds under investigation to inhibit histamine-induced skin weal formation was investigated.

Anaesthetized male Sprague Dawley rats, weighing approximately 150 g, were used. The products were given as aqueous solutions, at a constant volume of 5 mL /kg subcutaneously, in doses from 2 to 10 mg/kg. The same volume of the vehicle was administered to control animals. The agents to be assayed were administered 45 min before an intravenous injection of 0.30 mL 0.9% sodium chloride containing 3.75 mg Evans' blue dye. This treatment was immediately followed by two distinct intracutaneous injections on the shaved backs of 50 µL saline-buffered solution containing 10 µg histamine. The reaction was evaluated after 30 min.

Animals were sacrificed, then the skin was removed and biopsies were taken around the injection sites. The dye contained in each cutaneous fragment was extracted and, after centrifugation, the optical density of the supernatant was measured at 620 nm. Quantization of Evans' blue dye was calculated by standard calibration curves obtained by injecting the dye intradermally at various concentrations in other animals.

The amount of dye that leaked into the tissue was considered the parameter of increased vascular permeability.

The effects of test drugs were expressed as percentage inhibition of the dye amounts extracted from the control group.

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