butyricum suspended in paraffin oil into the left hindpaw footpad of male Lewis rats (160–180 g). Hindpaw volumes were measured by a mercury displacement method.[49] The inflammation induced in the injected paw on day 3 is designated as the primary lesion. The inflammation of the noninjected paw on day 16 is designated as the secondary lesion. Unless otherwise indicated, test compounds were homogenized in aqueous 0.5% gum tragacanth and administered po in a volume of 10 mL/kg of body weight, on days 0–10. For the purpose of these studies, antiarthritic activity was measured on the secondary lesion (day 16) in the treated animals relative to the controls (non drug treated). In this model spirogermanium's activity ranged from 50 to 100% activity (mean of 15 experiments—68%) and in the results section is designated as 1.00 (at 30 mg/kg). Statistically significant activity in the biological assays was determined using the Student's *t* test.

Suppressor Cell Coculture Assay. Spleen cells from azaspirane analogue treated or control animals were established in RPMI with 10% fetal bovine serum (RPMI-10) at 5×10^6/mL. Coculture experiments were carried out by first adding varying numbers of the putative suppressor cells (0.15 to 5×10^5) to wells of 96-well round-bottomed microtiter plates (Linbro, Flow Laboratories) in 100 μL of RPMI-10. These were then irradiated (2000 rad) in a γ cell 40 with a Cesium-137 source. To these cultures were added 5×10^5 normal cells and an optimal concentration of Con A (5 μg/mL), and the final volume was adjusted to 200 μL. Cell cultures were incubated for 72 h at 37 °C in a 5% CO_2 atmosphere and pulsed with 0.5 μCi [^{3}H]thymidine (specific activity 1.9 μCi/mmol; Schwarz/Mann, Orangeburg, NY) for the last 18 h of culture. The cells were harvested on an automated multiple sample harvester and cell-associated radioactivity was counted in a Beckman liquid-scintillation counter. Suppressor cell activity is determined by calculating the percent inhibition of proliferation compared to control cultures. For comparison of azaspirane analogues, a program was developed that could be used to compare suppressor cells generated in different experiments and the activities of different compounds. This was calculated in the following manner. A plot of percent suppression at different cell concentrations (dependent variable) vs the logarithm (base *e*) of the number of suppressor cells (independent variable) was generated and the area under the curve (AUC) represented by the data points of this plot was determined by the trapezoidal rule. The trapezoidal rule provides AUC by means of the summation of the areas of the trapezoids whose vertices are located at adjacent values of the independent variable and the corresponding values of the dependent variable. Spirogermanium (1) at 30 mg/kg for 11 days, administered po, assayed on day 16 gives an average value of 100 units by this method (mean value derived from three experiments). All unit values for analogues were standardized to the activity of spirogermanium. On the basis of our experience with these compounds we do not consider an AUC of less than 70 for suppressor cells to have meaningful biological activity, whereas values above this correspond to activity in the AA rat (and other autoimmune disease models). In addition, we consider that it requires a change of approximately 70 units in suppressor cell activity (AUC) to result in a meaningful change in the immune profile of treated animals.

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Supplementary Material Available: A table of the bp/mp of novel cyclohexenone and anhydride derivatives is included (2 pages). Ordering information is given on any current masthead page.

Synthesis, Biological Evaluation, and Quantitative Structure–Activity Relationship Analysis of [β-(Aroylamino)ethyl]piperazines and -piperidines and [2-[(Arylamino)carbonyl]ethyl]piperazines, -piperidines, -pyrazinopyridoindoles, and -pyrazinoisoquinolines. A New Class of Potent H_1 Antagonists[1]

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Some [β-(Aroylamino)ethyl]piperazines and -piperidines and [2-[(Arylamino)carbonyl]ethyl]piperazines, -piperidines, -pyrazinopyridoindoles, and -pyrazinoisoquinolines have been synthesized and their H1-antagonistic activity studied in isolated guinea pig ileum. Quantitative structure–activity relationship analysis indicates that the hydrophobicity of the side chain of these compounds plays a major role in their activity while steric and electronic factors are of secondary importance. All these compounds act on a common receptor and appear to interact similarly with the receptor.

Earlier work in this laboratory on 2-substituted 1,2,3,4,6,7,12,12a-octahydropyrazino[2′,1′:6,1]pyrido[3,4-*b*]indoles (1)[2,3] had shown that the presence of a β-(aroylamino)ethyl side chain at the 2-position results in potent H_1-antagonistic activity. The contributions of the substructure $(CH_2)_2$ NHCOAr and of hydrophobic interactions to the activity were quantified. It was suggested that a hydrophobic substituent at the ortho (π_o) or para (π_p) positions of 1 contributes more to the activity than a substituent at the meta (π_m) position and that the activity is enhanced by a bulky substituent at the ortho position of the side-chain phenyl ring. This effect was similar to the effect of a bulky substituent in one of the phenyl rings of diphenhydramine (2, R = H) observed by Kutter and Hansch.[4] Since 1 and 2 produce a similar biological re-

(1) Communication No. 4515 from Central Drug Research Institute Lucknow.
(2) Saxena, A. K.; Dhaon, M. K.; Ram, S.; Saxena, M.; Jain, P. C.; Patnaik, G. K.; Anand, N. *Indian J. Chem.* **1983**, *22B*, 1224.
(3) Saxena, A. K.; Ram, S. *Prog. Drug Res.* **1979**, *23*, 199.
(4) Kutter, E.; Hansch, C. *J. Med. Chem.* **1969**, *12*, 647.

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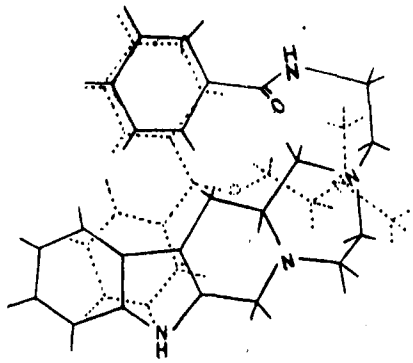


Figure 1. Complimentarity between 1 (R = $-(CH_2)_2NHCOC_6H_5$) (thick line) and **2** (dotted line).

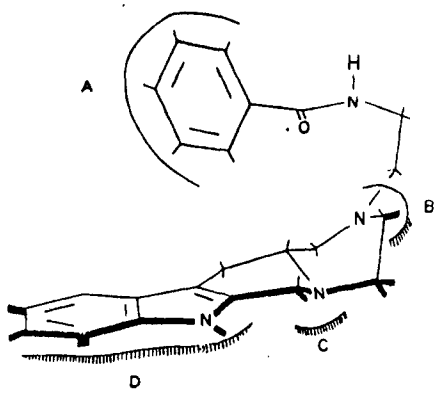
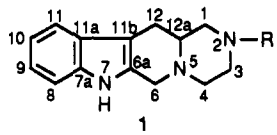
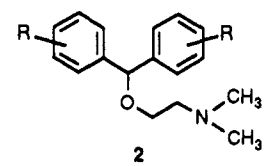


Figure 2. Possible subsites on H_1 receptor involved in interactions with 1 (R = $-(CH_2)_2NHCOAr$).

sponse, the aromatic rings and the tertiary N in the 2-position of 1 probably occupy the same receptor site as the phenyl rings and tertiary N of **2**. This is possible only if the side chain in 1 exists in a folded conformation at the receptor site (Figure 1). Electronic factors do not seem to make any significant contribution to the activity in either case. However, hydrophobicity was of major importance in the case of 1, but not in **2**. This difference may be due to greater parallelism between hydrophobicity and the steric parameter E_s in the latter case as compared to the former.



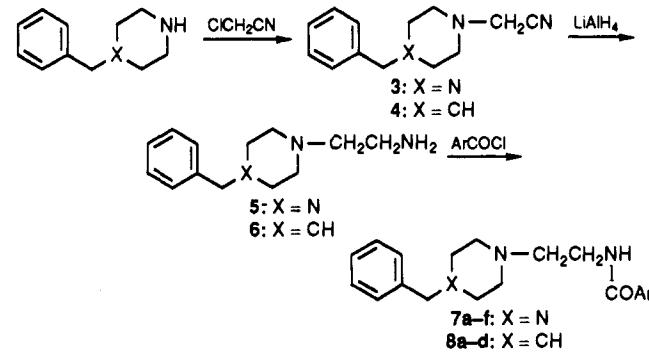
1



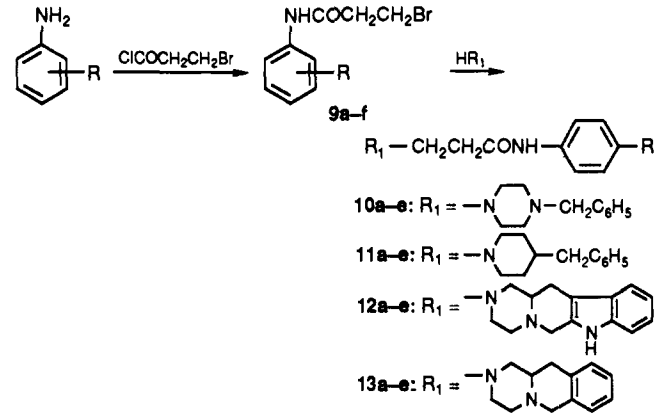
2

Based on the above observations and on structure–activity relationships studies on some semirigid analogs of diphenhydramine, a model of the H_1 receptor having at least four subsites A, B, C, and D was visualized (Figure 2); it was proposed that A and D interact with electron-rich hydrophobic groups, that B is responsible for ionic binding, and that C interacts electrostatically with an electron-rich group.[5,6] These studies also indicated that: (i) antihistaminics of types 1 and **2** act on a common receptor, (ii) ortho substitution in the phenyl ring of the side chain of 1 is likely to have a conformational effect by making the

Scheme I



Scheme II



phenyl ring noncoplanar, (iii) hydrophobic interactions are ·e important at the A subsite than electronic and steric ....ractions, (iv) the distance of the subsite B from subsite A is of prime importance for the activity, and (v) high electron density at the C subsite also contributes to the activity.

With a view to explore further the above model of the H_1 rece··r, the compounds **7a–f**, **8a–d**, **10a–e**, **11a–e**, **12a–e**, ... **13a–d**, which incorporate the above suggested essential structural requirements for H_1-antagonistic activity, have been synthesized and their quantitative structure–activity relationship (QSAR) analysis is described in this paper.

Chemistry

Condensation of benzylpiperazine or benzylpiperidine with chloroacetonitrile yielded the corresponding 1-(cyanomethyl)-4-benzylpiperazine[7] or -piperidine[8] (**3** and **4**, respectively), which upon $LiAlH_4$ reduction afforded **5** and **6**, respectively.[7,8] Treatment of the latter compounds with the appropriate aroyl chloride in the presence of Et_3N gave 1-(β-aroylamino)ethyl-4-benzylpiperazines and -piperidines (**7a–f** and **8a–d**, respectively) (Scheme I).

Reaction of β-bromopropionyl chloride with the appropriate arylamines in the presence of Et_3N gave the β-bromo-*N*-arylpropionamides (**9a–f**) which upon condensation with the appropriate amine yielded the desired 1-[2-[(arylamino)carbonyl]ethyl]-4-benzylpiperazine or -piperidine (**10a–e** and **11a–e**, respectively), 2-[2-[(arylamino)carbonyl]ethyl]-1,2,3,4,6,7,12,12a-octahydro-

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(7) Robert, P. M.; Renal, H. M.; Mary, R. D.; Mary, E. E. *J. Med. Pharm. Chem.* **1962**, *5*, 944.

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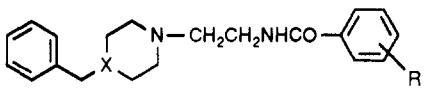
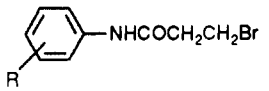
Table I. Physical Properties and Antihistaminic Activity of 1-[β-(Aroylamino)ethyl]-4-benzylpiperazines and -piperidines



compd	R	X	molecular formula	yield, %	mp, °C	solvent	anal.	I_{50},[a] μg/mL
7a	2-I	N	$C_{20}H_{24}IN_3O\cdot 2HCl$	45	260	abs EtOH/ether	CHN	0.18 ± 0.02
7b	2-Br	N	$C_{20}H_{24}BrN_3O\cdot 2HCl$	45	255	-do-	CHN	0.20 ± 0.02
7c	2-Cl	N	$C_{20}H_{24}ClN_3O\cdot 2HCl$	42	188	-do-	CHN	0.30 ± 0.05
7d	2-NO_2	N	$C_{20}H_{24}N_4O_3\cdot 2HCl$	40	88–90	abs EtOH	CHN	0.58 ± 0.05
7e	2-CH_3	N	$C_{21}H_{27}N_3O$	44	oil	-	CHN	2.40 ± 0.60
7f	H	N	$C_{20}H_{25}N_3O\cdot 2HCl$	45	100–2	abs EtOH	CHN	2.75 ± 0.50
8a	2-Br	CH	$C_{21}H_{25}BrN_2O$	40	oil	-	CHN	0.27 ± 0.02
8b	2-Cl	CH	$C_{21}H_{25}ClN_2O$	42	oil	-	CHN	0.28 ± 0.02
8c	2-CH_3	CH	$C_{22}H_{25}N_2O\cdot HCl$	43	160–62	abs EtOH	CHN	0.40 ± 0.04
8d	H	CH	$C_{21}H_{26}N_2O\cdot HCl$	45	81	abs EtOH/ether	CHN	0.56 ± 0.05
2								0.001 ± 0.0001

[a] Inhibitory concentration for 50% block of histamine.

Table II. Physical Properties of β-Bromo-*N*-arylpropionamides



compd	R	molecular formula	yield, %	mp, °C	solvent	anal.
9a	H	$C_9H_{10}BrNO$	79	119–20 (lit.[9] 124–26)	C_6H_6	CHN
9b	2-F	C_9H_9BrFNO	75	83–84 (lit.[10] 84.5–85)	C_6H_6	CHN
9c	2-Cl	$C_9H_9BrClNO$	60	85–86	C_6H_6	CHN
9d	2-C_2H_5	$C_{11}H_{14}BrNO$	80	85 (lit.[10] 87.5–88)	hexane	CHN
9e	2-NO_2	$C_9H_9BrN_2O_3$	80	87–88	C_6H_6	CHN
9f	2-OCH_3	$C_{10}H_{12}BrNO_2$	75	oil	-	CHN

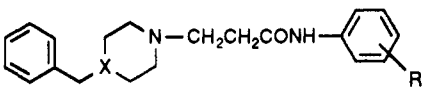
pyrazino[2′,1′:6,1]pyrido[3,4-*b*]indoles (**12a–e**) and 2-[2-[(arylamino)carbonyl]ethyl]-1,3,4,6,11,11a-hexahydro-2*H*-pyrazino[1,2-*b*]isoquinolines (**13a–d**) (Scheme II).

Results

Biological Study. The antihistaminic (H_1) activities (I_{50}) of the compounds are given in Tables I, III–V. These compounds failed to block the AcH response except for **7e** and **7f**, whose I_{50} values were identical with that of histamine. None of the compounds showed significant CNS depressant activity in mice at a dose of 10 mg/kg ip.

Quantitative Structure-Activity Relationships. Physicochemical parameters of 32 compounds were correlated with their I_{50} values expressed as μmol/L. Hydrophobicity values of these compounds, expressed in terms of the hydrophobic substituent constant (π) were taken from the literture;[14] some of the values were ex-

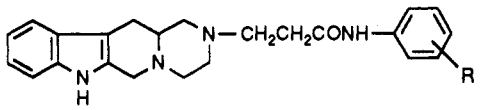
Table III. Physical Properties and Antihistaminic Activity of 1-[[(Arylamino)carbonyl]ethyl]-4-benzylpiperazines and -piperidines



compd	R	X	molecular formula	yield, %	mp, °C	solvent	anal.	I_{50},[a] μg/mL
10a	2-C_2H_5	N	$C_{22}H_{29}N_3O\cdot 2HCl$	40	249–50	abs EtOH/ether	CHN	0.33 ± 0.03
10b	2-Cl	N	$C_{20}H_{24}ClN_3O\cdot 2HCl$	94	236–37	-do-	CHN	0.36 ± 0.06
10c	2-F	N	$C_{20}H_{24}FN_3O\cdot 2HCl$	31	240–41	-do-	CHN	0.31 ± 0.03
10d	H	N	$C_{20}H_{25}N_3O$	75	94–95	MeOH	CHN	0.71 ± 0.10
10e	2-NO_2	N	$C_{20}H_{24}N_4O_3\cdot 2HCl$	90	220–21	abs EtOH/ether	CHN	0.75 ± 0.06
11a	2-C_2H_5	CH	$C_{23}H_{30}N_2O$	84	130–31	MeOH	CHN	0.17 ± 0.01
11b	2-Cl	CH	$C_{21}H_{25}ClN_2O$	70	195–96	MeOH	CHN	0.20 ± 0.02
11c	2-F	CH	$C_{21}H_{25}FN_2O$	65	oil	-	CHN	0.28 ± 0.03
11d	H	CH	$C_{21}H_{26}N_2O$	75	162–63	MeOH	CHN	0.49 ± 0.08
11e	2-OCH_3	CH	$C_{22}H_{28}N_2O_2$	65	105–6	MeOH	CHN	0.34 ± 0.04
2								0.001 ± 0.0001

[a] Inhibitory concentration for 50% block of histamine.

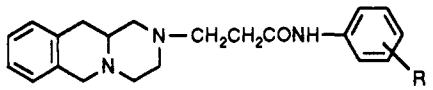
Table IV. Physical Properties and Antihistaminic Activities of 2-[[(Arylamino)carbonyl]ethyl]-1,2,3,4,6,7,12,12a-octahydropyrazino[2′,1′:6,1]pyrido[3,4-*b*]indole



compd	R	molecular formula	yield, %	mp, °C	solvent	anal.	I_{50},[a] μg/mL
12a	2-C_2H_5	$C_{25}H_{29}N_4O$	78	130–32	EtOH	CHN	0.26 ± 0.02
12b	2-Cl	$C_{23}H_{24}N_4OCl$	98	129–30	EtOH	CHN	0.4 ± 0.06
12c	2-F	$C_{23}H_{24}N_4OF$	98	165	EtOH	CHN	0.5 ± 0.06
12d	H	$C_{22}H_{25}N_4O$	45	198–99	EtOH	CHN	0.6 ± 0.07
12e	2-NO_2	$C_{23}H_{24}N_5O_3$	62	185	EtOAc	CHN	0.73 ± 0.1
2							0.001 ± 0.0001

[a] Inhibitory concentration for 50% block of histamine.

Table V. Physical Properties and Antihistaminic Activity of 2-[[(Arylamino)carbonyl]ethyl]-1,3,4,6,11,11a-hexahydro-2*H*-pyrazino[1,2-*b*]isoquinoline



compd	R	molecular formula	yield, %	mp, °C	solvent	anal.	I_{50},[a] μg/mL
13a	2-C_2H_5	$C_{23}H_{28}N_3O$	69	92–93	EtOH	CHN	0.19 ± 0.01
13b	2-Cl	$C_{21}H_{24}N_3OCl$	70	184–85	EtOH	CHN	0.25 ± 0.01
13c	H	$C_{21}H_{25}N_3O$	75	oil	-	CHN	0.45 ± 0.06
13d	2-NO_2	$C_{21}H_{24}N_4O_3$	70	183–84	EtOH	CHN	0.5 ± 0.06
2							0.001 ± 0.0001

[a] Inhibitory concentration for 50% block of histamine.

Table VI. Antihistaminic Activities and Physicochemical Parameters for the Compounds Used in QSAR

compd	structure R[a]	I_{50}[b] μmol/L	log $1/I_{50}$ obs	log $1/I_{50}$ calcd	π	σ	MR	IA
1a	Cl	0.206	0.686	0.683	0.71	0.47	6.03	1.0
1b	CH_3	0.283	0.548	0.560	0.56	-0.01	5.65	1.0
1c	H	0.535	0.272	0.317	0.00	0.00	1.03	1.0
1d	OCH_3	0.364	0.439	0.443	-0.02	0.30	7.87	1.0
1e	NO_2	0.375	0.426	0.404	-0.28	0.67	7.36	1.0
1f	NH_2	0.925	0.034	-0.004	-1.23	0.17	5.42	1.0
7a	I	0.345	0.462	0.378	1.12	0.40	13.94	0.0
7b	Br	0.421	0.376	0.237	0.86	0.47	8.88	0.0
7c	Cl	0.696	0.157	0.150	0.71	0.47	6.03	0.0
7d	NO_2	1.318	-0.120	-0.128	-0.28	0.67	7.36	0.0
7e	CH_3[d]	7.12	-0.852	0.027	0.56	-0.01	5.65	0.0
7f	H[d]	6.94	-0.841	-0.216	0.00	0.00	1.03	0.0
8a	Br	0.661	0.180	0.237	0.86	0.47	8.88	0.0
8b	Cl	0.799	0.097	0.150	0.71	0.47	6.03	0.0
8c	CH_3	1.071	-0.030	0.027	0.56	-0.01	5.65	0.0
8d	H	1.548	-0.190	-0.215	0.00	0.00	1.03	0.0
10a	C_2H_5	0.778	0.109	0.239	1.02	-0.01	10.30	0.0
10b	Cl	0.836	0.078	0.150	0.71	0.47	6.03	0.0
10c	F	1.318	-0.120	-0.093	0.14	0.54	0.92	0.0
10d	H	2.167	-0.336	-0.215	0.00	0.00	1.03	0.0
10e	NO_2	1.701	-0.231	-0.128	-0.28	0.67	7.36	0.0
11a	C_2H_5	0.477	0.321	0.239	1.02	-0.01	10.30	0.0
11b	Cl	0.561	0.251	0.150	0.71	0.47	6.03	0.0
11c	F	0.832	0.080	-0.093	0.14	0.54	0.92	0.0
11d	H	1.513	-0.180	-0.215	0.00	0.00	1.03	0.0
11e	OCH_3	0.963	0.016	-0.089	-0.02	0.30	7.87	0.0
12a	C_2H_5	0.634	0.198	0.239	1.02	-0.01	10.30	0.0
12b	Cl	0.979	0.009	0.150	0.71	0.47	6.03	0.0
12c	F	1.276	-0.106	-0.093	0.14	0.54	0.92	0.0
12d	H	1.604	-0.205	-0.215	0.00	0.00	1.03	0.0
12e	NO_2	1.74	-0.240	-0.128	-0.28	0.67	7.36	0.0
13a	C_2H_5	0.525	0.280	0.239	1.02	-0.01	10.30	0.0
13b	Cl	0.677	0.169	0.150	0.71	0.47	6.03	0.0
13c	H	1.342	-0.128	-0.215	0.00	0.00	1.03	0.0
13d	NO_2	1.316	-0.119	-0.128	-0.28	0.67	7.36	0.0

[a] The value of R corresponds to the type of structure specified by the numerical value in compound number, e.g. in the compd. **1a** and **7a** the structure type is 1 and 7, respectively. [b] The values for all the compounds have been calculated from I_{50} in μg/mL (Table I–V), except for 1a–e, for which the values have been taken from ref 2. [c] The values for all compounds **1a–f**, **7a–f**, **8a–d**, **11a–e**, **12a–e**, and **13a–d** have been calculated by using eq 20. [d] Excluded from analysis as these were nonspecific and blocked AcH also, hence calculated and observed log I_{50} values are also not in agreement.

perimentally verified in octanol/water. Electronic and steric effects were expressed in terms of the Hammet constant (σ) and molar refractivity (MR), respectively, and their values were taken from the literature.[14]

The compounds were grouped into seven different molecular types, A (**1a–f**), B (**7a–f**), C (**8a–d**), D (**10a–e**), E (**11a–e**), F (**12a–e**), and G (**13a–d**) for QSAR analysis. The data for compounds of group A have been taken from earlier work[2] in this laboratory. Since hydrophobicity is an important parameter, its effect on the activity was analyzed separately in each group, and the regression equations 1–7 (Table VII) were derived which show a correlation between log $1/I_{50}$ and the hydrophobicity of the side-chain phenyl group. Equations 8–16 describe similar correlations for compounds in certain combinations of groups, namely, BC, DE, DF, EG, FG, DFG, DEFG, AG, and BG, respectively. Equations 17–18 show the correlations between log $1/I_{50}$ for different types of compounds having a similar set of substituents.

It was evident from our earlier QSAR studies on 2-substituted pyrazinopyridoindoles[2] that the hydrophobicity of the side chain plays an important role, whereas the effects of electronic and steric parameters are secondary. In order to see if the side chain phenyl ring of these prototypes (groups A–G) undergoes similar biomolecular interactions in terms of hydrophobic, electronic, and steric effects, three equations, 19, 20, 22, were derived which describe the correlations between log $1/I_{50}$ and the parameters π, MR, and σ taken together for the groups A, B–G, and A–G, respectively. In order to check the sta-

Table VII. Equations Derived by Regression Analyses*

no.	equation	N	R	S	F
1	$\log 1/I_{50(A)} = 0.304\pi$ (±0.060) + 0.414 (±0.038)	6	0.928	0.094	25.19
2	$\log 1/I_{50(B)} = 0.408\pi$ (±0.082) - 0.027 (±0.006)	4	0.961	0.088	24.51
3	$\log 1/I_{50(C)} = 0.417\pi$ (±0.071) - 0.208 (±0.045)	4	0.972	0.046	33.97
4	$\log 1/I_{50(D)} = 0.328\pi$ (±0.087) - 0.204 (±0.05)	5	0.908	0.093	14.16
5	$\log 1/I_{50(E)} = 0.381\pi$ (±0.105) - 0.046 (±0.006)	5	0.903	0.098	13.20
6	$\log 1/I_{50(F)} = 0.326\pi$ (±0.042) - 0.172 (±0.024)	5	0.976	0.045	60.76
7	$\log 1/I_{50(G)} = 0.334\pi$ (±0.05) - 0.071 (±0.003)	4	0.798	0.052	45.03
8	$\log 1/I_{50(BC)} = 0.426\pi$ (±0.093) - 0.125 (±0.067)	8	0.882	0.116	20.90
9	$\log 1/I_{50(DE)} = 0.363\pi$ (±0.092) - 0.126 (±0.052)	10	0.813	0.131	15.63
10	$\log 1/K_{50(DF)} = 0.327\pi$ (±0.043) - 0.188 (±0.025)	10	0.936	0.065	56.70
11	$\log 1/K_{50(EG)} = 0.355\pi$ (±0.054) - 0.054 (±0.003)	9	0.928	0.076	43.52
12	$\log 1/I_{50(FG)} = 0.334\pi$ (±0.048) - 0.128 (±0.029)	9	0.935	0.071	48.99
13	$\log 1/I_{50(DFG)} = 0.333\pi$ (±0.045) - 0.156 (±0.026)	14	0.907	0.082	55.68
14	$\log 1/I_{50(DEFG)} = 0.34\pi$ (±0.049) - 0.127 (±0.028)	19	0.865	0.10	50.41
15	$\log 1/I_{50(A-G)} = 0.357\pi$ (±0.035) + 0.531A (±0.049) - 0.11 (±0.024)	33	0.921	0.102	83.72
16	$\log 1/I_{50(B-G)} = 0.378\pi$ (±0.042) - 0.125 (±0.0261)	27	0.873	0.104	80.11
17	$\log 1/I_{50(D)} = 0.988(\log 1/I_{50(F)})$ (±0.258) - 0.032 (±0.004)	5	0.911	0.092	14.67
18	$\log 1/I_{50(D)} = 0.919(\log 1/I_{50(E)})$ (± 0.057) - 0.176 (±0.013)	4	0.996	0.022	257.49
19	$\log 1/I_{50(A)} = 0.304\pi$ (±0.014) + 0.164σ (±0.046) + 0.019MR (±0.005) + 0.262 (±0.024)	6	0.998	0.021	188.40
20	$\log 1/I_{50(B-G)} = 0.347\pi$ (±0.049) + 0.144σ (±0.072) + 0.011MR (±0.006) - 0.224 (±0.038)	27	0.915	0.090	39.30
21	$\log 1/I_{50(A-G)} = 0.301\pi$ (±0.035) + 0.017MR (±0.005) + 0.514IA (±0.043) - 0.194 (±0.032)	33	0.942	0.090	76.78
22	$\log 1/I_{50(A-G)} = 0.328\pi$ (±0.034) + 0.143σ (±0.059) + 0.013MR (±0.005) + 0.53IA (±0.041) - 0.229 (±0.003)	33	0.953	0.083	68.70

* The values in parentheses indicate the standard error of the regression coefficient.

tistical justification of the inclusion of additional parameters in eq 15 to obtain eq 22, eq 21 has been derived.

Discussion

QSAR. Quantitative Structure-Activity Relationships. The QSAR of compounds **1a-f**, **7a-f**, **8a-d**, **10a-e**, **11a-e**. **12a-e**, and **13a-d**, which incorporate all the essential structural requirements suggested in the proposed model of a H_1 receptor antagonist, have been studied. In view of the observation that compounds of type I having an ortho substituent in the aryl part of the side chain showed better activity than those with a meta or a para substituent, only ortho-substituted compounds were studied (Tables VI and VII).

It is evident from eqs 2-7 that in all these compounds hydrophobicity plays a major role similar to that in **1a-f**. The slope values given by the equation are almost identical (0.375 ± 0.045) for different molecular prototypes (groups A-G). which indicates that the change in activity due to the same substructural variation in the side-chain phenyl ring is similar in all prototypes. This would in turn suggest that the side-chain phenyl ring in all these compounds occupy the same receptor site. The low significance (<97.5%) of the eqs 2, 3, 5, and 6 may be due to the small number of compounds in the set, because statistically significant results were obtained (eqs 8-14) in the case of the combined groups BC, DE, DF, EG, FG, DFG, and DEFG, having a larger number of compounds in the set. All the compounds may be grouped together if the indicator variable IA is used to show the presence of the pyrazinopyridoindole skeleton (presence of IA = 1, absence of IA = 0). The three-dimensional plot of the data is shown in Figure 3, from which it is clear that the plane bounded by the dotted line correlates well the antihistaminic activity with π and IA and eq 15 describing this plane has a highly statistically significant correlation coefficient (R = 0.92), as shown by the F test [$F_{(2,30)}$ = 83.72; $F_{(2,30 \propto 0.001)}$ = 8.77], with low standard deviation. The regression coefficients were also 100% significant. The equations describing the interrelationship between the observed activities in the different groups for the same set of substituents are linear, with the regression coefficient values close to 1 (eq 17-18). The differences observed in the order of activity of different groups among β-(aroylamino)ethyl derivatives (A > B > C) and among β-arylpropionamide derivatives (E > F > D) would indicate that either the subsite C (Figure 2) is not essential or its requirement of electrons is being provided by the side chain carbonyl, particularly in the case of 4-benzylpiperidines (group E). The difference in the intercept values given by the eqs 1 and 4 suggests that the orientation of the -NHCO- fragment modulates the activity.

Equation 19, with only three variables and six data points, though not acceptable, was derived only to compare the change in antihistaminic activity with substructural variation in terms of all the three effects, viz. hydrophobic (π), steric (MR), and electronic (σ) in pyrazinopyridoindoles, while the corresponding eq 20, for all the other compounds, which is acceptable, describes the correlation of the activity with all these parameters. The identical slope values 0.325 ± 0.021, 0.154 ± 0.01, and 0.0115 ± 0.004 shown by both these equations for π, σ, and MR, respectively, indicate that the side-chain phenyl ring in all these molecules occupies the same receptor site and suggest that at subsite A all these molecules experience the same kind of biomolecular interactions. The general equation (22) for all the compounds has been derived by inclusion of the indicator variable IA for the pyrazinopyridoindole skeleton in eq 20 or by stepwise inclusion in eq 15, of the MR parameter leading to eq 21, followed by inclusion of the σ parameter. The independent parameters used in deriving eq 22 do not intercorrelate, as shown by the correlation matrix (Table VIII). The equation is statistically significant and predicts well the observed activity (Table VI). This equation (22) is a significant improvement over eq 15 as shown by the comparative F test ($F_{(2,28)}$ = 9.0; $F_{(2,28 \propto 0.001)}$ = 8.93).

In conclusion, these compounds appear to act on a common receptor and the essential structural requirements for the molecule to exhibit H_1 antihistaminic activity are the presence of substructures which can interact at the subsites A, B, and D, while interaction at the subsite C and orientation of the -NHCO- fragment modulate the activity. These studies indicate that the application and predictive value of classical QSAR (physicochemical approach) are not limited to prototypes and that it can be used for the mapping of receptor sites provided the substructures competing for the same receptor sites have been

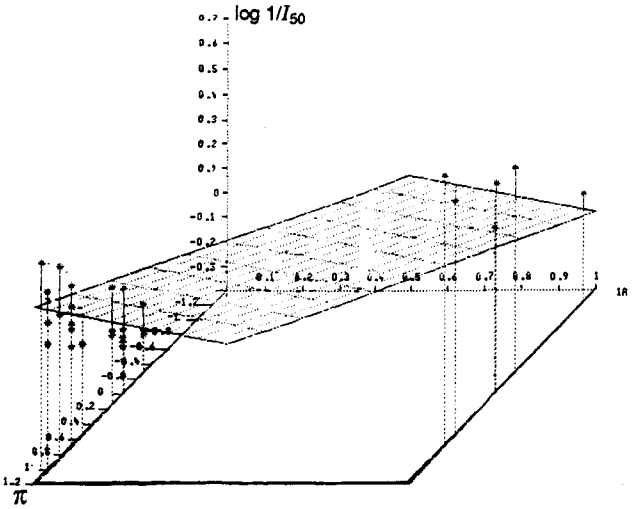


Figure 3. Three dimensional plot describing correlation of antihistaminic activity log $1/I_{50}$ with hydrophobic π and indicator IA parameters.

identified in different prototypes.

Experimental Section

All melting points were determined in an electrically heated apparatus (Tempo and Toshniwal) and are uncorrected. The compounds were routinely checked for purity by TLC on silica gel or alumina plates and their structures were verified by their IR spectra measured on Perkin-Elmer 137 or 157 spectrophotometers (γ max in cm^{-1}), ^1H NMR spectra on a Varian EM-360L (CA) (60 MHz) or a Perkin-Elmer R-32 (90 MHz) spectrometer with TMS as internal reference (chemical shifts in δ ppm), and mass spectra on a JEOL-JMS D-300 spectrometer. Elemental analyses were performed by the microanalysis section of this institute and were within ±0.4% of the calculated values.

1-(Cyanomethyl)-4-benzylpiperazine (3). Chloroacetonitrile (8.3 g, 0.11 mol) was added dropwise to a stirred mixture of 4-benzylpiperazine (17.6 g, 0.1 mol) and Na_2CO_3 (11.66 g, 0.11 mol) in acetone (50 mL) at 0 °C. The reaction mixture was stirred for 2 h at ambient temperature and then evaporated in vacuo. The solid obtained was triturated with water (15 mL) and extracted with ether (2 × 40 mL). The organic layer was washed with water (2 × 10 mL), dried over anhydrous Na_2SO_4, and evaporated to dryness to give **3** as an oil, (lit. bp 152–55 °C/0.2 mm) yield 20.1 g (93.5%).

1-(Cyanomethyl)-4-benzylpiperidine (4). It was prepared from 4-benzylpiperidine (17.5 g, 0.1 mol) by the procedure described for **3**. The compound **4** was crystallized from ether/hexane: yield 15 g (70%); mp 65 °C (lit.[8] mp 68 °C).

1-(β-Aminoethyl)-4-benzylpiperazine (5). A solution of **3** (10.75 g, 0.05 mol) in dry tetrahydrofuran (50 mL) was added dropwise to a stirred suspension of $LiAlH_4$ (5 g) in dry tetrahydrofuran (150 mL). The reaction mixture was refluxed with stirring for 24 h and cooled. The excess of $LiAlH_4$ was decomposed by addition of water and 10% aqueous NaOH, and the solution was filtered. The filtrate was evaporated to give **5** as an oil (lit.[6] bp 180–87 °C/13 mm), yield 10 g (91%).

1-(β-Aminoethyl)-4-benzylpiperidine (6). The reduction of **4** (10.7 g, 0.05 mol) was carried out with $LiAlH_4$ (5 g) under similar experimental conditions as described for **5**. The compound **6** was obtained as an oil (lit.[7] bp 121–130 °C/0.04 mm), yield 10.7 g (98%).

1-(β-Benzamidoethyl)-4-benzylpiperazine (7f). Benzoyl chloride (0.84 g, 0.006 mol) was added dropwise to a stirred solution of **5** (1.095 g, 0.005 mol) and Et_3N (1 mL) in dry tetrahydrofuran (30 mL) at 0 °C. The reaction mixture was stirred for 5 h. It was concentrated and diluted with water (10 mL). The resulting mixture was extracted with $CHCl_3$ (2 × 20 mL). The combined chloroform extracts were washed first with 10% aqueous NaOH solution followed by water and then dried over anhydrous Na_2SO_4. The chloroform was removed to give **7f** as an oil which was converted to its hydrochloride by treatment with ethereal HCl. The **7f** HCl was crystallized from absolute ethanol/dry ether mixture: yield 0.9 g (45%); mp 100–102 °C.

Compounds **7a–e** were prepared in a similar manner and are described in Table I.

1-(β-Benzamidoethyl)-4-benzylpiperidine (8d). It was prepared from benzoyl chloride (0.84 g, 0.006 mol) and **6** (1.09 g, 0.005 mol) by a similar method as described for **7f** and crystallized from ethanol: yield 0.9 g (45%); mp 81 °C.

Compounds **8a–c** were prepared in a similar manner (Table I).

β-Bromo-*N*-phenylpropionamide (9a). β-Bromopropionyl chloride (1.715 g, 0.001 mol) was added dropwise to a stirred solution of aniline (0.93 g, 0.001 mol) and Et_3N (1 mL) in dry benzene (50 mL) at 5 °C. The reaction mixture was stirred for 4 h. It was concentrated and triturated with water (10 mL). The separated solid was filtered and crystallized from benzene to give **9a**: yield 1.7 g (75%); mp 119–120 °C (lit.[9] mp 119–120 °C).

Compounds **9b–f** were made similarly from substituted anilines. The physical data of these compounds are described in Table II.

1-[2-[(Phenylamino)carbonyl]ethyl]-4-benzylpiperazine (10d). A solution of **9a** (1.14 g, 0.005 mol) in dry DMF (25 mL) was added slowly to a stirred suspension of 4-benzylpiperazine (0.88 g, 0.005 mol) and dry Na_2CO_3 (0.265 g, 0.0025 mol) in dry DMF (5 mL). The reaction mixture was stirred at 70 °C for 24 h and thereafter water (15 mL) was added to it. The separated semisolid was extracted into $CHCl_3$ (3 × 10 mL). The combined $CHCl_3$ extracts were washed with water (2 × 5 mL) and dried over anhydrous Na_2SO_4. Concentration of the extract gave **10d** which was crystallized from methanol: yield 1.32 g (81%), mp 94–95 °C.

Compounds **10a–c**, **10e**, and **11a–e** were synthesized similarly (Table III).

2-[2-[(Phenylamino)carbonyl]ethyl]-1,2,3,4,6,7,12,12a-octahydropyrazino[2′,1′:6,1]pyrido[3,4-*b*]indole (12a). A solution of **9d** (1.28 g, 0.0051 mol) in dry DMF (20 mL) was added to a stirred suspension of 1,2,3,4,6,7,12,12a-octahydropyrazino-[2′,1′:6,1]pyrido[3,4-*b*]indole[12] (1.13 g, 0.005 mol) and Na_2CO_3 (0.265 g, 0.0025 mol) in dry DMF (5 mL). The reaction mixture was stirred for 36 h at 60 °C and then cooled and worked up as described for **10d**: yield 1.56 g (70%), mp 130–32 °C.

The compounds **12b–e** were synthesized in a similar manner (Table IV).

2-[2-[(Phenylamino)carbonyl]ethyl]-1,3,4,6,11,11a-hexahydro-2*H*-pyrazino[1,2-*b*]isoquinoline (13a). Condensation of 1,3,4,6,11,11a-hexahydro-2*H*-pyrazino-[1,2-*b*]isoquinoline[13] (0.94 g, 0.005 mol) and **9f** (1.28 g, 0.0051 mol) in the presence of Na_2CO_3 (0.265 g, 0.0025 mol) in DMF (5 mL) under similar conditions as described for **10d** gave **13a**: yield 1.25 g (69%), mp 92–93 °C.

Compounds **13b–d** were made similarly (Table V).

Antihistaminic Activity. Antihistaminic activity (H1) was measured on the isolated terminal part of guinea pig ileum (5-cm long) suspended in an organ bath containing aerated Tyrode solution (20 mL) at 35 °C and spasm of the ileum was induced with 3×10^{-8} g/mL of histamine. The percentage of inhibition was plotted against different concentrations of the compound and the concentration causing 50% inhibition (I_{50}) was calculated.

Partition Coefficient and QSAR Analysis. The log *P* values of compounds **1c**, **1e**, **12d**, **10b**, and **10d** were measured by using the method described earlier[2] and found to be 1.80 ± 0.11, 1.52 ± 0.1, 1.82 ± 0.13, 3.41 ± 0.28, and 2.7 ± 0.18, respectively.

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Table VIII. Correlation Matrix

	π	σ	MR	IA	log $1/I_{50}$
π	1.00				
σ	-0.16	1.00			
MR	0.48	0.16	1.00		
IA	-0.32	-0.08	-0.04	1.00	
log $1/I_{50}$	0.50	0.00	0.51	0.57	1.00

The experimental values for NO_2 and Cl, namely -0.28 and 0.71, obtained by subtracting log P of **1e** from that of **1c** and log P of **10b** from that of **10d**, respectively, were found to be in agreement with the corresponding values in the literature.[14] The values for the other substituents were taken from the literature.[14] Different equations were generated by using standard multiparameter regression analysis program on Wang/IBM PC XT computers.

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Chemistry and Pharmacology of the Non-Benzodiazepine Anxiolytic Enciprazine and Related Compounds

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In the course of studies on tranquilizers, new non-benzodiazepine-like compounds were synthesized. These are 1-(3,4,5-trimethoxyphenoxy)-3-[4-(2-methoxyphenyl)piperazinyl]propan-2-ol (INN: enciprazine) and derivatives thereof which were screened pharmacologically in order to evaluate their central nervous system activity. Compounds with marked antiaggressive and anxiolytic properties but without dependence potential could be detected. Enciprazine was selected for clinical investigations.

Introduction

During recent years the synthesis and biological evaluation of 1-(alkoxyphenoxy)-3-(*N*-arylpiperazinyl)propan-2-ols and related compounds have been under investigation in our laboratories.[1] In the course of our studies on tranquilizers we tried to synthesize new non-benzodiazepine-like compounds by combining the β_2-blocker moiety—characteristic of anxiolytics[2] —with phenylpiperazines.

A variety of biological activities of (phenoxyphenylpiperazinyl)propanol derivatives, e.g. local-anaesthetic, hypotensive, and cardiovascular properties, have been reported.[3] We were especially interested in derivatives acting on the central nervous system (CNS), as the tranquilizing activities of this class of compounds appeared to be very promising.[4a,b]

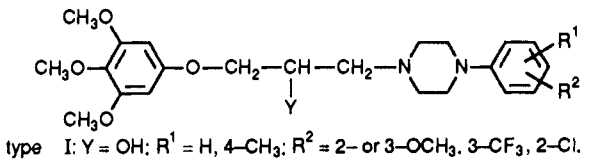
The great interest in the development of non-benzodiazepine-like tranquilizers like buspirone[4c] led us to study the contribution of substituents in the phenylpiperazine moiety with regard to CNS activities.

This paper deals with the synthesis, the pharmacological screening, and the evaluation of the dependence potential of 1-(3,4,5-trimethoxyphenoxy)-3-(4-phenylpiperazinyl)-propan-2-ols of type I, II, III, and IV (Chart I).

Chemistry

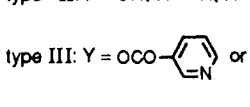
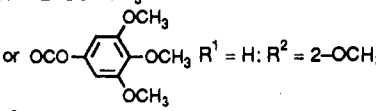
According to the synthetic sequence for β-antagonists, condensation of 1-(3,4,5-trimethoxyphenoxy)-2,3-epoxypropane with appropriate phenylpiperazines in 2-propanol led to the synthesis of compounds of type I. The corresponding epoxy compound was prepared by reacting commercially available 3,4,5-trimethoxyphenol (antiarol) with epichlorohydrin in the presence of NaOH. The acylated *o*-hydroxy compounds of type II were obtained by treatment of 1-benzyl-4-(2-hydroxyphenyl)piperazine with acid chlorides in pyridine and subsequent condensation of the hydrogenolytically deprotected *N*-phenylpiperazines as described for compounds of type I.

Chart I



type I: Y = OH; R^1 = H, 4-CH_3; R^2 = 2- or 3-OCH_3, 3-CF_3, 2-Cl, 2-OC_2H_5, 2-CF_3, 2-SCH_3, 2-NH_2, 2-OH

type II: Y = OH; R^1 = H; R^2 = 2-$OCOCH_3$

type III: Y =  or  R^1 = H; R^2 = 2-OCH_3

type IV: Y = OH; R^1 = H; R^2 = 2-$NHCOCH_3$

Compounds of type III were synthesized by reaction of 1-(3,4,5-trimethoxyphenoxy)-3-[4-(2-methoxyphenyl)-piperazinyl]propan-2-ol with the corresponding (hetero)-aromatic acid chloride in pyridine.

Starting with 1-benzyl-4-(2-nitrophenyl)piperazine, reduction and debenzylation yielded the corresponding aminophenylpiperazine. After condensation according to the

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