Table III—Typical Assay Results of Commercial Preparations

Sample[a]	Acetaminophen (I)	Guaifenesin (II)	Pseudoephedrine Hydrochloride (III)	Pholcodine (IV)	Methyl Paraben (VI)	Propyl Paraben (VII)
1	100.0	100.1	100.3	100.0	101.5	101.2
2	102.4	98.8	99.2	98.3	100.0	101.8
3	98.9	98.5	101.0	99.5	100.5	97.3
4	100.0	97.0	98.2	101.1	97.7	98.5
Mean Recovery[b]	100.6	98.1	100.0	100.6	99.2	99.9
SD[c]	1.5	1.6	1.5	2.3	1.8	2.1

[a] Recoveries expressed as percent of theoretical. [b] Calculated from nine replicates. [c] Standard deviations of a single determination calculated from nine replicates.

Initially hexanesulfonic acid[11] was chosen with a water–methanol ratio of 70:30, but this resulted not only in a long retention time for VIII, as its retention is not dependent on the ion-pairing agent, but also gave a poor separation of III and V. By using octanesulfonic acid as the ion-pairing agent and changing the water–methanol–acetic acid ratio of the mobile phase to 55:45:2, a satisfactory separation was achieved. Addition of acetic acid reduced tailing of III and peak broadening of IV.

The analytical results demonstrate the ability of ion-pair reverse-phase HPLC to simultaneously assay four actives and four paraben preservatives. A particular advantage of the method is the minimum time required for sample preparation and analysis of the complete separation requiring only 18 min. The method has been successfully used on a routine basis for over 6 months. Special column clean-up procedures have not been required during this time and no significant loss of column performance has been observed.

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Synthesis and Pharmacological Activity of Benzo[*b*]thiophene-3-carboxylic Acid Derivatives

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Received December 7, 1981, from the **Department of Chemistry, College of Pharmacy* and the ‡*Department of Animal Physiology and Pharmacology, School of Veterinary Medicine, Tehran University, Tehran, Iran.* Accepted for publication March 31, 1982.

Abstract □ Several dialkylaminoethyl benzo[*b*]thiophene-3-carboxylates, *N*-(2-dialkylaminoethyl)benzo[*b*]thiophene-3-carboxamides, 2-dialkylaminoethyl benzo[*b*]thiophene-3-carbamates, and substituted ureas with benzo[*b*]thiophene moiety, were prepared and tested for local anesthetic, anticholinergic, and antihistaminic activities. Several of the compounds showed significant activity

Keyphrases □ Benzo[*b*]thiophene-3-carboxylic acid derivatives—synthesis and pharmacological activity, local anesthetic, anticholinergic, and antihistaminic activity □ Anesthetics, local—synthesis and pharmacological activity of benzo[*b*]thiophene-3-carboxylic acid derivatives □ Anticholinergics—synthesis and pharmacological activity of benzo[*b*]thiophene-3-carboxylic acid derivatives □ Antihistamines—synthesis and pharmacological activity of benzo[*b*]thiophene-3-carboxylic acid derivatives

Many of the clinically active local anesthetics are dialkylaminoalkyl esters and dialkylaminoalkylamides of carboxylic acids (1). Some dialkylaminoalkylesters of 2- or 3-benzo[*b*]thiophenecarboxylic acid have been reported to have hypotensive, antiviral, and antifungal activities (2), as have some benzo[*b*]thiophene-2-carboxamides (3). It

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Table I—Physical Constants of 2-Dialkylaminoethyl Benzo[*b*]thiophene-3-carboxylates

Compound	R_1	R_2	R_3	Yield, %	Melting Point[a]	Formula[b]	Analysis, % Calc.	Found
IIIa	H	CH_3	CH_3	85	131–132°	$C_{13}H_{16}ClNO_2S$	C 54.64	54.58
							H 5.60	5.76
							N 4.90	5.08
IIIb	H	C_2H_5	C_2H_5	90	151–152°	$C_{15}H_{20}ClNO_2S$	C 57.42	57.35
							H 6.38	6.19
							N 4.47	4.65
IIIc	H	n-C_4H_9	n-C_4H_9	80	101–102°	$C_{19}H_{28}ClNO_2S$	C 61.71	61.90
							H 7.58	7.76
							N 3.79	3.96
IIId	H	CH_3	$CH_2C_6H_5$	80	194–195°	$C_{19}H_{20}ClNO_2S$	C 63.07	62.91
							H 5.53	5.35
							N 3.87	4.05
IIIe	H	—$(CH_2$—$CH_2)_2O$		70	198–199°	$C_{15}H_{18}ClNO_3S$	C 54.96	55.09
							H 5.50	5.75
							N 4.27	4.45
IIIf	CH_3	CH_3	CH_3	70	170–171°	$C_{15}H_{20}ClNO_2S$	C 57.42	57.26
							H 6.38	6.57
							N 4.47	4.65

[a] All compounds were crystallized as hydrochlorides and the recrystallization solvent was ethanol–ethyl acetate. [b] IR, NMR, and mass spectra of all compounds were consistent with the structural assignment.

Table II—Physical Constants of *N*-(2-Dialkylaminoethyl)benzo[*b*]thiophene-3-carboxamides

Compound	R	Yield, %	Melting Point[a]	Formula[b]	Analysis, % Calc.	Found
IV*a*	CH_3	70.	134–135°	$C_{13}H_{17}ClN_2OS$	C 54.83	54.95
					H 5.98	6.05
					N 9.84	9.98
IV*b*	C_2H_5	65	78–79°	$C_{15}H_{21}ClN_2OS$	C 57.60	57.76
					H 6.72	6.91
					N 8.96	8.79
IV*c*	—$(CH_2)_2O(CH_2)_2$—	75	176–177°	$C_{15}H_{19}ClN_2O_2S$	C 55.13	55.01
					H 5.82	5.95
					N 8.58	8.73
V		70	235–236°	$C_{14}H_{17}ClN_2OS$	C 56.66	56.49
					H 5.73	5.54
					N 9.44	9.62

[a] All compounds were crystallized as hydrochlorides and the recrystallization solvent was ethanol–ethyl acetate. [b] IR, NMR, and mass spectra of all compounds were consistent with the structural assignment.

has been demonstrated that some dialkylaminoalkylesters and dialkylaminoalkylamides of benzo[*b*]thiophene-2-carboxylic acid have local anesthetic activity (4). Recently, antibacterial and antifungal activities of alkyl and polyhalophenyl esters of benzo[*b*]thiophene-3-carbamic acid have been reported (5).

In a continuing effort to find a potent pharmacologically active compound with low toxicity (6), a series of dialkylaminoethyl benzo[*b*]thiophene-3-carboxylates, *N*-(2-dialkylaminoethyl)benzo[*b*]thiophene-3-carboxamide, 2-dialkylaminoethyl benzo[*b*]thiophene-3-carbamates, and substituted ureas with the benzo[*b*]thiophene moiety were prepared and the efficacy was determined.

DISCUSSION

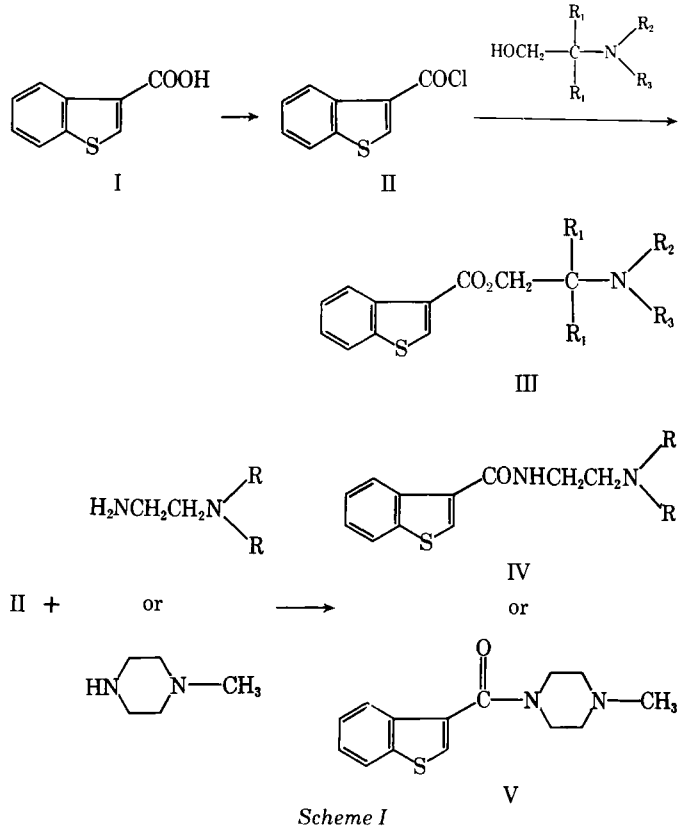
Chemistry—Dialkylaminoethyl benzo[*b*]thiophene-3-carboxylates were synthesized using readily available benzo[*b*]thiophene-3-carboxylic acid (I) (5). Reaction of I with thionyl chloride and the subsequent reaction of the acyl halide with dialkylaminoethanol gave the desired compound III (Scheme I).

Benzo[*b*]thiophene-3-carboxamide derivatives (IV or V) were obtained through the reaction of benzo[*b*]thiophene-3-carbonyl chloride (II) with 2-dialkylaminoethylamine or *N*-methylpiperazine (Scheme I).

2-Dialkylaminoethyl benzo[*b*]thiophene-3-carbamates (VII) were prepared through the reaction of benzo[*b*]thiophene-3-carboxazide (VI) (5) with 2-dialkylaminoethanol (Scheme II).

Substituted ureas with the benzo[*b*]thiophene moiety (VIII or IX) were obtained through the reaction of VI with a dialkylaminoalkylamine or *N*-methylpiperazine (Scheme II).

The physical data for the prepared compounds are summarized in Tables I–IV.



Scheme I

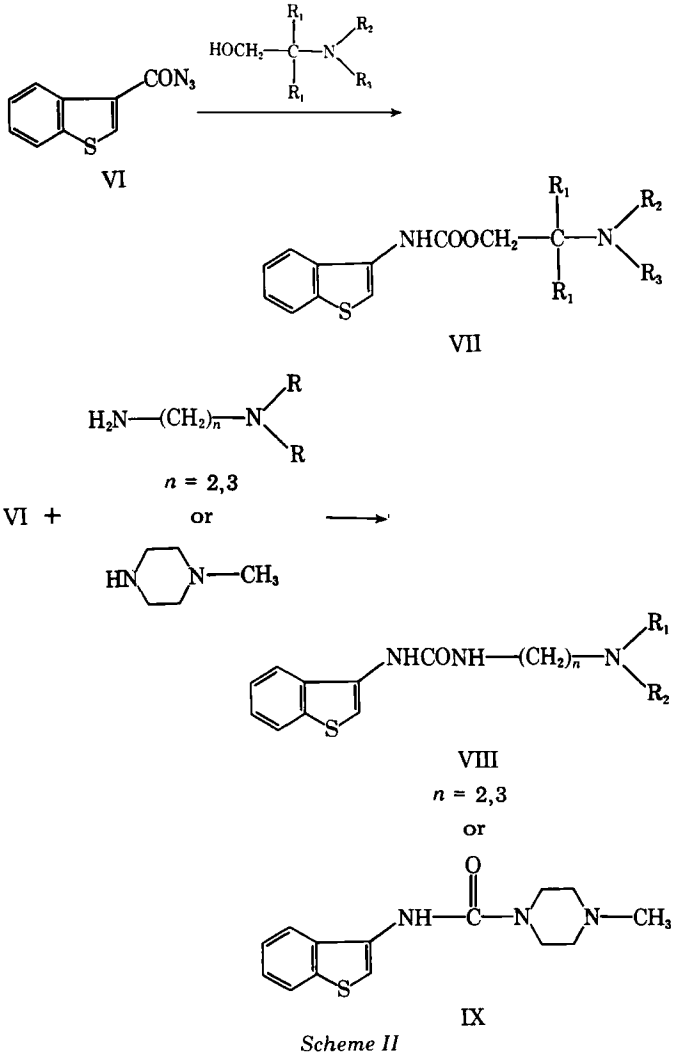
Table III—Physical Constants of 2-Dialkylaminoethyl Benzo[*b*]thiophene-3-carbamates

Compound	R_1	R_2	R_3	Yield, %	Melting Point[a]	Formula[b]	Analysis, % Calc.	Found
VII*a*	H	CH_3	CH_3	78	174–175°	$C_{13}H_{17}ClN_2O_2S$	C 51.91	52.10
							H 5.66	5.47
							N 9.32	9.51
VII*b*	H	C_2H_5	C_2H_5	75	155–156°	$C_{15}H_{21}ClN_2O_2S$	C 54.79	54.96
							H 6.39	6.58
							N 8.52	8.71
VII*c*	H	*n*-C_4H_9	*n*-C_4H_9	70	140–141°	$C_{19}H_{29}ClN_2O_2S$	C 59.30	59.15
							H 7.54	7.36
							N 7.28	7.11
VII*d*	H	$-(CH_2\text{-}CH_2)_2O$		75	161–162°	$C_{15}H_{19}ClN_2O_3S$	C 52.55	52.73
							H 5.55	5.71
							N 8.18	8.06
VII*e*	CH_3	CH_3	CH_3	80	194–195°	$C_{15}H_{21}ClN_2O_2S$	C 54.79	54.61
							H 6.39	6.54
							N 8.52	8.34

[a] All compounds were crystallized as hydrochlorides and the recrystallization solvent was ethanol–ethyl acetate. [b] IR, NMR, and mass spectra of all compounds were consistent with the structural assignments.

Pharmacological Assay—The compounds listed in Tables I–IV were screened for surface anesthetic, anticholinergic, and antihistaminic activities. For surface anesthetic activity, a rabbit conjunctival sac was kept filled with the aqueous solution of the hydrochloric salt of the compounds for 60 sec. The cornea was tested once every minute, and the duration of anesthesia was followed for 18 min. Lidocaine hydrochloride was used for comparison. The results are presented in Table V.

Compounds III*e*, III*f*, IV*b*, and VIII*d* were the most potent. The LD_{50} values of compounds III*e*, III*f*, and IV*b* in mice, estimated by the moving average method (7), were 424.7 (395.9–455.6), 274.2 (255.2–294.8), and 179.5 (158.3–204.2) mg/kg, respectively, when injected intraperitoneally.



Scheme II

Apart from transient irritation, no conjunctival intolerance or corneal opalescene was observed 24 and 48 hr and 1 week after drug application.

Anticholinergic and antihistaminic activities were tested on isolated guinea pig ileum. The results are presented in Table V.

Compounds III*a*, III*f*, IV*b*, VII*c*, and VIII*d* were the most potent as anticholinergics; and compounds III*a*, V, VII*c*, and VIII*d* were the most potent as antihistamines.

EXPERIMENTAL[1]

Benzo[*b*]thiophene-3-carbonyl Chloride (II)—A mixture of the acid, I (17.8 g, 0.1 mole), and thionyl chloride (35 ml) was refluxed for 4 hr. Excess thionyl chloride was removed under reduced pressure, and the residue was fractionated to give 17.7 g (90%) of the desired compound, bp 149–150°; 4 mm Hg [lit. (11) bp 296–298°, 758 mm Hg].

Anal.—Calc. for C_9H_5ClOS: C, 54.96; H, 2.54. Found: C, 55.07; H, 2.39.

2-Dimethylaminoethyl Benzo[*b*]thiophene-3-carboxylate (III*a*)—A solution of 2-dimethylaminoethanol (0.89 g, 0.01 mole) and II (1.965 g, 0.01 mole) in 20 ml of dry benzene was refluxed for 4 hr. The solvent was evaporated, and the residue was crystallized from ethanol–ethyl acetate to give III*a* (2.43 g, 88%), mp 131–132°; IR (potassium bromide): 1710 and 1195 (ester) cm^{-1}; NMR (deuterochloroform, as free base): 8.67 (m, 1H, H_4), 8.43 (s, 1H, H_2), 7.90 (m, 1H, aromatic), 7.50 (m, 2H, aromatic), 4.47 (t, 2H, OCH_2), 2.73 (t, 2H, CH_2N), and 2.33 (s, 6H, NCH_3) ppm; *m/z* 249.

Compounds III*b–f* were prepared similarly (Table I).

***N*-(2-Dimethylaminoethyl)benzo[*b*]thiophene-3-carboxamide (IV*a*)**—A solution of II (1.965 g, 0.01 mole) and 2-dimethylaminoethylamine (0.88 g, 0.01 mole) in 30 ml of dry benzene was refluxed for 2 hr. The solvent was evaporated and the residue was crystallized from ethanol–ethyl acetate to give IV*a* (1.74 g, 70%), mp 134–135°; IR (potassium bromide): 3260 (NH) and 1640 (amide) cm^{-1}; NMR (deuterochloroform, as free base): 8.45 (m, 1H, H_4), 7.95 (s, 1H, H_2), 7.86 (m, 1H, aromatic), 7.45 (m, 2H, aromatic), 6.96 (bs, 1H, NH), 3.56 (q, 2H, $CONCH_2$), 2.46 (q, 2H, CH_2N), and 1.26 (s, 6H, NCH_3) ppm; *m/z* 248.

Compounds IV*b*, IV*c*, and V were prepared similarly (Table II).

2-Dimethylaminoethyl Benzo[*b*]thiophene-3-carbamate (VII*a*)—A solution of benzo[*b*]thiophene-3-carboxazide (VI, 2.03 g, 0.01 mole) (5) in 20 ml of dry benzene was refluxed for 2 hr, 2-dimethylaminoethanol (0.89 g, 0.01 mole) was added, and reflux was continued for an additional 2 hr. The solvent was evaporated and the residue was crystallized as a hydrochloride from ethanol–ethyl acetate to give VII*a* (2.34 g, 78%), mp 174–175°; IR (potassium bromide): 3180 (NH), 1710 (carbonyl) cm^{-1}; NMR (deuterochloroform, as free base): 8.06–7.40 (m, 6H, aromatic and NH), 4.42 (t, 2H, OCH_2), 2.70 (t, 2H, CH_2N), and 2.36 (s, 6H, NCH_3) ppm; *m/z* 264.

Compounds VII*b–e* were prepared similarly (Table III).

***N*$_1$-(Dimethylaminoethyl)-*N*$_3$-(benzo[*b*]thiophene-3-yl)urea (VIII*a*)**—A solution of VI (2.03 g, 0.01 mole) in 20 ml of dry benzene was refluxed for 2 hr, 2-dimethylaminoethylamine (0.88 g, 0.01 mole) was

[1] Melting points were taken on a Kofler hot-stage microscope and are uncorrected. IR spectra were recorded using a Perkin-Elmer model 267 spectrograph. NMR spectra were determined using a Varian T-60 spectrometer and chemical shifts (δ) are in parts per million relative to internal tetramethylsilane. Mass spectra were recorded on a Varian MAT-311 instrument.

Table IV—Physical Constants of N_1-(Dialkylaminoalkyl)-N_3-(benzo[*b*]thiophene-3-yl)ureas

Compound	*n*	R_1	R_2	Yield, %	Melting Point[a]	Formula[b]	Analysis, % Calc.	Found
VIII*a*	2	CH_3	CH_3	75	207–208°	$C_{13}H_{18}ClN_3OS$	C 52.09	51.95
							H 6.01	5.87
							N 14.02	14.15
VIII*b*	3	CH_3	CH_3	80	205–206°	$C_{14}H_{20}ClN_3OS$	C 53.59	53.77
							H 6.38	6.56
							N 13.40	13.25
VIII*c*	3	C_2H_5	C_2H_5	95	209–210°	$C_{16}H_{24}ClN_3OS$	C 56.22	56.04
							H 7.03	6.91
							N 12.30	12.05
VIII*d*	3	n-C_4H_9	n-C_4H_9	80	179–180°	$C_{20}H_{32}ClN_3OS$	C 60.38	60.19
							H 8.05	7.87
							N 10.57	10.38
VIII*e*	2	$-(CH_2\text{-}CH_2)_2O$		90	222–223°	$C_{15}H_{20}ClN_3O_2S$	C 52.71	52.87
							H 5.86	6.04
							N 12.30	12.14
IX				75	235–236°	$C_{14}H_{18}ClN_3OS$	C 53.93	53.99
							H 5.78	5.88
							N 13.48	13.29

[a] All compounds were crystallized as hydrochlorides and the recrystallization solvent was ethanol–ethyl acetate. [b] IR, NMR, and mass spectra of all compounds were consistent with the structural assignments.

Table V—Local Anesthetic, Anticholinergic, and Antihistaminic Activities of Benzo[*b*]thiophene Derivatives

Compound	Local Anesthetic Activity[a] Concentration, %	Potency	Duration, min	Concentration, μg/ml	Anticholinergic[b]	Antihistaminic[b]
III*a*	2	0.55(0.39–0.71)	6–12	1	100	93.7
				5	100	97.2
				10	100	97.2
III*b*	2	0.32(0.2–0.44)	2–8	5	33.3	21.3
				10	33.3	30.3
III*c*	2	0.0	—	5	56.9	34.1
				10	60.8	34.1
III*d*	2	0.41(0.25–0.57)	4–8	5	0.0	49.4
				10	0.0	64.7
III*e*	1	0.67(0.56–0.78)	9–14	5	15.1	13.9
	2	0.83(0.74–0.92)	14–16	10	15.1	13.9
III*f*	1	0.55(0.44–0.66)	9–12	1	94.3	4.5
	2	0.88(0.78–0.98)	15–18	5	100	61.8
				10	100	73.1
IV*a*	2	0.0	—	10	0.0	0.0
IV*b*	1	0.54(0.43–0.65)	7–12	5	63.8	18
	2	0.61(0.50–0.72)	10–14	10	96.6	40.9
IV*c*	2	0.0	—	10	0.0	0.0
V	2	0.17(0.05–0.22)	1–5	5	0.0	85.7
				10	0.0	89.6
VII*a*	2	0.22(0.09–0.35)	2–6	5	9.5	26.4
				10	9.5	63.1
VII*b*	2	0.13(0.04–0.22)	1–4	5	26.5	33.3
				10	26.5	70.8
VII*c*	2	0.22(0.09–0.35)	3–6	1	20.4	77.8
				5	81.5	100
				10	83.3	100
VII*d*	2	0.26(0.15–0.37)	4–6	5	0.0	17.1
				10	0.0	22.0
VII*e*	2	0.15(0.06–0.24)	1–5	1	10.2	0.0
				5	40.5	22.9
				10	57.3	33.7
VIII*a*	2	0.11(0.03–0.19)	1–4	5	14.0	13.6
				10	14.0	17.0
VIII*b*	2	0.0	—	5	0.0	9.1
				10	0.0	10.0
VIII*c*	1	0.05(0.00–0.10)	0–2	5	15.1	33.3
	2	0.13(0.04–0.22)	1–4	10	16.8	56.4
VIII*d*	1	0.50(0.34–0.66)	6–11	5	73.0	97.3
	2	0.77(0.65–0.89)	11–15			
VIII*e*	2	0.38(0.25–0.51)	4–8	5	21.1	0.0
				10	21.1	0.0
IX	2	0.36(0.23–0.49)	3–6	10	0.0	0.0
Lidocaine hydrochloride	1	0.33(0.18–0.48)	3–7			
	2	0.86(0.57–0.97)	15–16			

[a] Surface anesthesia was tested according to a previously described method (8). Anesthetic potency was calculated for the first 18 min (9). A potency of 1.00 indicates an onset of anesthesia in 1 min and a duration of at least 18 min. [b] Reduction of contraction (percent) against acetylcholine (0.01 μg/ml) and histamine dihydrochloride (0.01 μg/ml). Assay was carried out on isolated guinea pig ileum according to a previous method (10).

added, and reflux was continued for an additional 3 hr. The solvent was evaporated, and the residue was crystallized as a hydrochloride from ethanol–ethyl acetate to give VIII*a* (2.25 g, 75%), mp 207–208°; IR (potassium bromide): 3300 (NH), 1685 (carbonyl) cm^{-1}; NMR (deuterochloroform): 8.80 (bs, 1H, NH), 7.87–7.50 (m, 2H, aromatic), 7.57 (s, 1H, H_2), 6.40 (bs, 1H, NH), 3.37 (q, 2H, $CONCH_2$), 2.47 (q, 2H, CH_2N), and 2.13 (s, 6H, NCH_3) ppm; *m/z* 263.

Compounds VIII*b–e* and IX were prepared similarly (Table IV).

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Binding of Several Phenothiazine Neuroleptics to a Common Binding Site of α_1-Acid Glycoprotein, Orosomucoid

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Abstract □ The interaction of several phenothiazine neuroleptics with α_1-acid glycoprotein was investigated using circular dichroism and equilibrium dialysis techniques. For chlorpromazine only, one high-affinity binding site of the protein was found. The binding of the drug to this single site generated typical polyphasic extrinsic Cotton effects. Since several other phenothiazine neuroleptics gave qualitatively comparable extrinsic Cotton effects in the presence of α_1-acid glycoprotein and potently inhibited the binding of chlorpromazine to the single site, it was concluded that all phenothiazine derivatives investigated bound preferentially to only one common binding site of the α_1-acid glycoprotein molecule.

Keyphrases □ Phenothiazines—neuroleptics, binding to a common binding site of α_1-acid glycoprotein, orosomucoid □ Neuroleptic agents—binding of phenothiazines to a common binding site of α_1-acid glycoprotein, orosomucoid □ α_1-Acid glycoproteins—binding of several phenothiazine neuroleptics to a common binding site, orosomucoid □ Orosomucoid—binding of several phenothiazine neuroleptics to a common binding site of α_1-acid glycoprotein

While for most neutral or anionic drugs the predominating role of the albumin fraction as the major binding component in human blood is established, increasing evidence has been presented during recent years that this is not the case for several basic drugs where other proteins also contribute considerably to the plasma binding. Out of these, orosomucoid (α_1-acid glycoprotein) received the most attention because of its possible significance for the pharmacokinetic pattern of basic drugs (1). Thus, large variations in the blood levels of α_1-acid glycoprotein observed in patients suffering from various disease states could have been responsible for similarly large variations of the free plasma levels of some basic drugs measured in the same patients (1–3). Since the average plasma levels of α_1-acid glycoprotein are rather low, usually between 10 and 40 μmole/liter (2, 3), a fairly strong drug binding to this protein has to be assumed if variations of its plasma levels were to contribute considerably to the free fraction of a drug. Some recent work shows that several basic drugs are bound very strongly to α_1-acid glycoprotein (4–8). An example of this is the phenothiazine derivative, perazine, which is bound with very high affinity to mainly one site of the α_1-acid glycoprotein molecule (5–7).

The present study reports similar findings for chlorpromazine. Evidence is presented that a variety of phenothiazine neuroleptics, including perazine and chlorpromazine, are preferentially, if not exclusively, bound to only one common binding site of the α_1-acid glycoprotein molecule.

EXPERIMENTAL

Materials—α_1-Acid glycoprotein[1] (orosomucoid) had an electrophoretic purity >99%. [^{14}C]Chlorpromazine[2] had a specific activity of 80 mCi/mmole and a radiochemical purity >99%. All chlorpromazine derivatives were gifts from the manufacturers[3]. All other chemicals were obtained from commercial suppliers. All solutions were prepared with deionized water.

Circular Dichroism Measurements—Circular dichroism measurements were carried out with a spectropolarimeter[4] calibrated with *d*-camphorsulfonic acid. All spectra were recorded in cylindrical cells with 10-mm path length using a full-scale deflection of 0.02° θ and a spectral band width of 2 nm. All measurements were made in 0.07 *M* phosphate buffer (pH 7.4). Results are expressed as molar ellipticity ($[\theta]$) calculated with reference to the α_1-acid glycoprotein concentration (25 μM).

Equilibrium Dialysis—Binding of [^{14}C]chlorpromazine to α_1-acid glycoprotein was determined by equilibrium dialysis using a protein concentration of 12.5 μM and varying concentrations of the drug. All solutions were prepared in 0.07 *M* phosphate buffer (pH 7.4); 0.9 ml of the protein solution was dialyzed for 16 hr in the dark against 0.9 ml of buffer containing [^{14}C]chlorpromazine. One-milliliter dialysis cells and

[1] Behringwerke, Marburg, West Germany.

[2] Amersham Buchler, Braunschweig, West Germany.

[3] Perazine from Promonta, Hamburg, West Germany, promazine from Wyeth, Münster, West Germany; prothipendyl from Homburg, Frankfurt, West Germany; trifluopromazine from Heyden, Munich, West Germany; acepromazine from Clin-Comar, Paris, France.

[4] Cary 61.

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