Guanidine Sulfates.—The following example is illustrative of the general method used to prepare the guanidine sulfates listed in Tables III and V.

2-(2,6-Xylyloxy)ethylguanidine Sulfate.—2-Methyl-2-thiopseudourea sulfate (157.7 g., 1.21 moles) was dissolved in water (300 ml.) and 2-(2,6-xylyloxy)ethylamine (200 g., 1.21 moles) was added. A vigorous reaction occurred, accompanied by the formation of methyl mercaptan (trapped in a cooled mixture of aqueous sodium hydroxide and hydrogen peroxide.). The aqueous solution was boiled for 2 hr. and then cooled. The solid which separated was filtered, washed with ice-cold water and dried. Two recrystallizations from methanol–isopropyl alcohol followed by drying *in vacuo* at 125° for 6 hr., yielded 195 g. of pure anhydrous salt, m.p. 234–236°.

2-[2-(2,6-Xylyloxy)ethyl]amino-4,5-dihydroimidazole Hydriodide.—2-(2,6-Xylyloxy)ethylamine (15.0 g., 0.091 mole) was added to a solution of 2-methylmercapto-4,5-dihydroimidazole hydriodide[26] (22.2 g., 0.091 mole) in ethanol (100 ml.). The solution was heated under reflux for 2 hr., the evolution of methyl mercaptan being completed during this time. After cooling, the solution was concentrated to low bulk and left at −10° for 48 hr. The crystals deposited (16.1 g.) were recrystallized twice from isopropyl alcohol–ether, affording 11.6 g. of product, m.p. 116–119°.

N-[2-(2,6-Xylyloxy)ethyl]-N′,N″-diphenylguanidine Hydrochloride.—2-(2,6-Xylyloxy)ethylamine (3.8 g., 0.023 mole) was added to a solution of N,N′-diphenyl-S-methylisothiourea[27] (5.5 g., 0.023 mole) in xylene (30 ml.). The mixture was heated under reflux for 24 hr. and then evaporated to dryness *in vacuo*. The residue crystallized slowly at 0° and was finally recrystallized from petroleum ether (60–80°) affording 3.6 g. of material, m.p. 90–94°.

The hydrochloride was prepared using isopropyl alcoholic hydrogen chloride and was recrystallized from isopropyl alcohol–ether, *m.p.* 163–166°.

N-[2-(2,6-Xylyloxy)ethyl]-N′-benzoylguanidine. A.—2-(2,6-Xylyloxy)ethylamine (8.5 g., 0.052 mole) was added to a solution of N-benzoyl-S-methylisothiourea[28] (10.0 g., 0.052 mole) in chlorobenzene (100 ml.). The solution was heated under reflux for 24 hr. and concentrated to an oil which gradually crystallized. Recrystallization from aqueous ethanol followed by recrystallization from benzene–petroleum ether afforded 7.5 g. of the guanidine, m.p. 111–114°.

Anal. Calcd. for $C_{18}H_{21}N_3O_4$: C, 69.42; H, 6.80; N, 13.49. Found: C, 69.26; H, 6.91; N, 13.34.

The hydrochloride was prepared using isopropyl alcoholic hydrogen chloride, and was recrystallized from isopropyl alcohol–ether, m.p. 162–164°.

(26) S. R. Aspinall and E. J. Bianco, *J. Am. Chem. Soc.*, **73**, 602 (1951).
(27) W. Will, *Ber.*, **14**, 1489 (1881).
(28) G. Ito, *Chem. Pharm. Bull.* (Tokyo), **9**, 245 (1961).

B.—2-(2,6-Xylyloxy)ethylguanidine sulfate (20.0 g., 0.04 mole) was suspended in ethanol (500 ml.) and an ethanolic solution of sodium ethoxide, prepared from 1.8 g. of sodium, was added with stirring. The mixture was stirred for 1 hr., filtered, and then concentrated *in vacuo* to yield the free base, 2-(2,6-xylyloxy)ethylguanidine (17.0 g.) as a viscous yellow oil. Ethyl benzoate (13.0 g., 0.085 mole) was added and the mixture heated on the steam bath for 45 min. The product was dissolved in benzene, filtered, and diluted with petroleum ether (60–80°), yielding the crude monobenzoyl derivative which was recrystallized twice from aqueous ethanol, finally affording 2.8 g., m.p. 113.5–115.5°. The melting point was undepressed on admixture with a sample from the first preparation and identical infrared and ultraviolet spectra (λ_{max} 262 mμ (ϵ_{max} 27,673)) were obtained.

N-[2-(2,6-Xylyloxy)ethyl]-N′,N″-dibenzylguanidine. A.—2-(2,6-Xylyloxy)ethylamine (3.3 g., 0.02 mole) was added to a solution of N,N′-dibenzoyl-S-methylisothiourea[28] (6.0 g., 0.02 mole) in xylene (20 ml.). The solution was heated under reflux for 8 hr. and then cooled. Crystals were deposited which were filtered and recrystallized from benzene–petroleum ether (60–80°) affording 5.1 g. of product, m.p. 147–148°.

B.—2-(2,6-Xylyloxy)ethylguanidine sulfate (10.0 g., 0.02 mole) was suspended in 20 ml. of 10% sodium hydroxide solution and then rapidly stirred during the addition of 5.5 g. (0.04 mole) of benzoyl chloride. Stirring was continued for 1 hr. and the solid formed was recrystallized from ethanol followed by further recrystallization from benzene–petroleum ether (60–80°), finally affording 2.9 g. of the product, m.p. 147–149°. The melting point was undepressed on admixture with a sample from the previous experiment and identical infrared and ultraviolet (λ_{max} 253 mμ (ϵ_{max} 31,035), 277 mμ (ϵ_{max} 26,272)) spectra were obtained.

In a previous experiment in which an excess of sodium hydroxide was used, the major product from the Schotten–Baumann reaction was the benzoate salt of 2-(2,6-xylyloxy)ethylguanidine, m.p. 147–148°.

Anal. Calcd. for $C_{11}H_{17}N_3O \cdot C_6H_5COOH$: C, 65.63; H, 7.04; N, 12.76. Found: C, 65.4; H, 6.77; N, 12.65.

An authentic sample of 2-(2,6-xylyloxy)ethylguanidine benzoate which was prepared from 2-(2,6-xylyloxy)ethylguanidine free base (made from sulfate and sodium ethoxide in ethanol) and benzoic acid, had m.p. 148–149°. There was no depression of melting point on admixture of these samples and infrared and ultraviolet (λ_{max} 262 mμ (ϵ_{max} 22,673)) spectra were identical.

Acknowledgments.—Analytical data was kindly supplied by Mr. J. Cross and staff of the Microanalytical department. The assistance of Mrs. J. Gilder, Mr. J. Gormley, and Mr. G. Perry in the synthetic work is also gratefully acknowledged.

Benzo[*b*]thiophene Derivatives. IV.[1] The Preparation and Pharmacological Activity of Compounds Related to Serotonin and Gramine

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A number of derivatives of benzo[*b*]thiophene related to serotonin and gramine have been prepared and tested for pharmacological activity on a variety of smooth muscle preparations and on intact animals. Antihistamine, antiacetylcholine, antiserotonin, and in some cases spasmogenic properties have been demonstrated. The replacement of the indole ring system by the benzo[*b*]thiophene system in the compounds studied leads to a reduction in agonistic activity and to the emergence of variable nonspecific antagonistic properties to serotonin, acetylcholine, and histamine.

The application of the concept of bioisosterism to the preparation of pharmacologically active substances, which antagonize or mimic the active parent compounds, has proved fruitful in several fields of pharmacological endeavor.[2] While the isosteric replacement

(1) Part III: M. Martin-Smith and S. T. Reid, *J. Chem. Soc.*, 938 (1960).

(2) H. L. Friedman in "Influence of Isosteric Replacements upon Biological Activity," Symposium on Chemical-Biological Correlation, Natl. Acad. Sci., Natl. Research Council, Publication No. 206, Washington, D. C., 1951, p. 295.

of thiophene for benzene has been extensively studied,[3] little attention has been devoted to the substitution of the thiophene ring for the pyrrole moiety in naturally occurring indole derivatives, although the benzo[*b*]thiophene isosteres of 3-indole acetic acid,[4] tryptophan,[5] and tryptamine[6] have been reported. Interest in the pharmacological properties of serotonin and related indole compounds[7a–e] has been maintained *in* recent years despite the lack of a precisely defined physiological role in the mammalian organism. The preparation and pharmacological investigation of simple benzo[*b*]thiophene bases related to serotonin and gramine, which is the subject of the present investigation were thus undertaken in the hope of shedding further light on the configuration and specificity of the receptors involved in the action of serotonin and related indole derivatives.

Chemistry

3-Aminomethylbenzo[*b*]thiophene (I) was obtained from 3-chloromethylbenzo[*b*]thiophene[5] by condensation with potassium phthalimide in dimethylformamide, followed by hydrolysis of the substituted phthalimide employing the conditions described by Ing and Manske.[8]

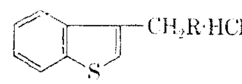
3-Dimethylaminomethylbenzo[*b*]thiophene (II), the benzo[*b*]thiophene isostere of gramine, was prepared from the free amine I by dimethylation with a mixture of aqueous formaldehyde and formic acid. The other tertiary amines (III–VII) were prepared directly by condensation of 3-chloromethylbenzo[*b*]thiophene with the appropriate amine in toluene in the presence of sodamide (see Table I). The crystalline methiodides (VIII–XI) of certain of the amines were also prepared (see Table II).

2-(2′-Aminoethyl)-5-hydroxybenzo[*b*]thiophene (XII) and 2-(2′-aminopropyl)-5-hydroxybenzo[*b*]thiophene (XIII) were synthesized from 5-hydroxybenzo[*b*]thiophene-2-aldehyde, obtained from the known 5-hydroxybenzo[*b*]thiophene-2-carboxylic acid[9] by the method of McFadyen and Stevens.[10] The aldehyde was condensed with either nitromethane or nitroethane in the presence of ammonium acetate, and on reduction of the resulting nitrovinyl compounds with lithium aluminum hydride, the amines XII and XIII, respectively, were obtained (see Table III).

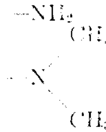
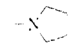
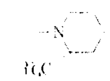
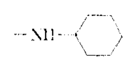
Finally, compounds XIV–XVII were prepared from the known 5-nitrobenzo[*b*]thiophene-2-carboxylic acid.[11a–c] The acid chloride, formed by the action of thionyl chloride, was condensed with the appropriate amine to give the amide. Reduction of the 5-nitro to the amino group was accomplished with hydrazine hydrate and Raney nickel.[12] The diamines XIV–XVII were then obtained by reduction of the amide function

TABLE I

GROUP A



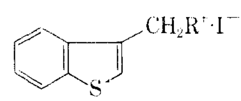
I–VII

Compound	R	M.p., °C.	% Calcd. C	% Calcd. H	% Found C	% Found H
I	$-NH_2$	259–260	54.1	5.1	54.1	5.2
II		218–220	58.0	6.2	57.5	6.0
III		225	57.9	6.0	57.8	5.8
IV		190–191	61.5	6.4	61.0	6.4
V[a]		194–195	50.7	5.5	50.6	5.5
VI		212	63.9	7.2	63.4	7.0
VII		198	63.9	7.2	64.2	7.0

[a] Characterized as the perchlorate.

TABLE II

GROUP B



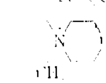
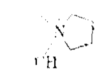
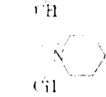
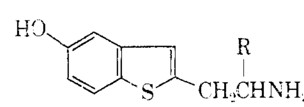
Compound	R	M.p., °C.
VIII	$-N^{+}(CH_3)_3$	192–193
IX		207–209
X		153
XI		195–196

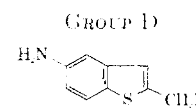
TABLE III

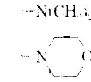
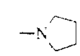
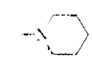
GROUP C



Compound	R
XII	H
XIII	CH_3

GROUP D



Compound	R
XIV	$-N(CH_3)_2$
XV	
XVI	
XVII	

(3) M. Martin-Smith and S. T. Reid, *J. Med. Pharm. Chem.*, **1**, 507 (1959).

(4) E. M. Crook and W. Davies, *J. Chem. Soc.*, 1697 (1937).

(5) S. Avakian, J. Moss, and G. J. Martin, *J. Am. Chem. Soc.*, **70**, 3075 (1948).

(6) W. Herz, *ibid.*, **72**, 4999 (1950).

(7) (a) B. B. Brodie, A. Pletscher, and P. A. Shore, *J. Pharmacol. Exptl. Therap.*, **116**, 9 (1956); (b) D. W. Woolley and E. Shaw, *ibid.*, **121** 13 (1957); (c) E. Shaw and D. W. Woolley, *ibid.*, **111**, 43 (1954); (d) P. B. Barlow and I. Khan, *Brit. J. Pharmacol.*, **14**, 553 (1959); (e) V. Erspamer in "Progress in Drug Research," Vol. 3, E. Jucker, Ed., Birkhauser Verlag, Basle, Switzerland, 1961.

(8) H. R. Ing and R. H. F. Manske, *J. Chem. Soc.*, 2348 (1926).

(9) M. Martin-Smith and M. Gates, *J. Am. Chem. Soc.*, **78**, 5351 (1956).

(10) J. S. McFadyen and T. S. Stevens, *J. Chem. Soc.*, 584 (1936).

(11) (a) K. Fries, H. Herring, K. Hemmecke, and G. Siebert, *Ann.*, **527**, 83 (1936); (b) L. F. Fieser and R. G. Kennelly, *J. Am. Chem. Soc.* **57**, 1611 (1935); (c) F. G. Bordwell and C. J. Albisetti, *ibid.*, **70**, 1955 (1948).

(12) D. Balcom and A. Furst, *ibid.*, **75**, 4334 (1953).

with lithium aluminum hydride. The products were characterized as the N-benzoyl derivatives. The dihydrobromides were employed in the pharmacological tests.

Experimental

Melting points were taken on a Kofler block.

3-Aminomethylbenzo[b]thiophene Hydrochloride (I).—3-Chloromethylbenzo[b]thiophene[5] (4.53 g., 0.025 mole) was dissolved in dimethylformamide (70 ml.) and heated under reflux with potassium phthalimide (4.59 g., 0.025 mole) for 3 hr. The condensation product was precipitated by the addition of water and gave white crystals (5.30 g., 73%) with m.p. 162° (lit.[13] 163°), from ethyl acetate. A slight excess of hydrazine hydrate (5 ml., 98–100%) was added to the substituted phthalimide (5.0 g.) in ethanol (250 ml.), and the solution was heated under reflux for 20 min. A white solid separated from the hot solution and after further refluxing in the presence of dilute hydrochloric acid (6 *N*, 50 ml.) for 30 min., the solution obtained by filtration was made basic with dilute sodium hydroxide solution and extracted with ether to give 3-aminomethylbenzo[b]thiophene (2.47 g., 89%) as a pale yellow oil. Conversion into the hydrochloride gave colorless cubes, m.p. 259–260°, from ethanol (analytical figures in Table I).

3-(Dimethylaminomethyl)-benzo[b]thiophene Hydrochloride (II).—3-Aminomethylbenzo[b]thiophene (1.0 g.), 36% formaldehyde solution (4 ml.), formic acid (4 ml.), and water (8 ml.) were gently heated on the steam bath for 4 hr. Excess of hydrochloric acid (6 *N*) was added and the solvent removed by distillation under reduced pressure leaving **II** as a white solid; colorless prisms (0.99 g., 71%), from ethanol; m.p. 218–222°.

3-(Morpholinomethyl)-benzo[b]thiophene Hydrochloride (III).—Sodamide (3.7 g., 0.095 mole) was added to 3-chloromethylbenzo[b]thiophene (6.0 g., 0.033 mole) and morpholine (2.86 g., 0.033 mole) in dry toluene (70 ml.) and the mixture was heated vigorously under reflux for 24 hr. A darkening in color was observed. Water was added to decompose the excess sodamide, and the organic layer separated. Distillation of the toluene under reduced pressure afforded the amine as a faintly colored oil, b.p. 208–210° (18 mm.). **III** was obtained when dry hydrogen chloride was passed into an ethereal solution of the amine and formed white needles (4.66 g., 52%), m.p. 225°, from chloroform.

Compounds IV–VII.—These compounds were prepared by substitution of the appropriate amine for morpholine in the procedure described for the preparation of **III**.

Compounds VIII–XI.—These compounds were prepared from the corresponding tertiary bases by the addition of excess methyl iodide to the amine dissolved in ethanol, and purified by crystallization from ethanol.

Methyl 5-Hydroxybenzo[b]thiophene-2-carboxylate.—Excess diazomethane (0.1 mole) in ether was added to a solution of 5-hydroxybenzo[b]thiophene-2-carboxylic acid (3.51 g., 0.018 mole) in ethanol. After 24 hr. the solvent was removed under reduced pressure and the ester crystallized from benzene. Colorless plates resulted (3.60 g., 96%), m.p. 162–163°.

Anal. Calcd. for $C_{10}H_8O_3S$: C, 57.7; H, 3.9. Found: C, 57.9; H, 3.9.

1-(5-Hydroxybenzo[b]thiophene-2-yl-carbonyl)-2-*p*-toluenesulfonyl Hydrazine.—Methyl 5-hydroxybenzo[b]thiophene-2-carboxylate (4.0 g.) was dissolved in methanol (80 ml.); hydrazine hydrate (10 ml.) was added, and the solution was heated under reflux for 4 hr. The acid hydrazide which crystallized from the hot solution formed pale yellow needles (3.64 g., 91%), m.p. 282–284°, from acetic acid. *p*-Toluenesulfonyl chloride (2.58 g., 0.014 mole) in dry pyridine (30 ml.) was added dropwise to an ice-cold solution of the hydrazide (2.84 g., 0.014 mole) in dry pyridine (50 ml.) over a period of 30 min. The solution was allowed to stand for a further 30 min. at room temperature and poured into water (1500 ml.). The toluenesulfonyl hydrazine was obtained by filtration and formed pale yellow prisms (3.39 g., 69%), m.p. 215°, from ethyl acetate.

Anal. Calcd. for $C_{16}H_{14}N_2O_4S_2$: C, 53.0; H, 3.9. Found: C, 53.0; H, 4.3.

5-Hydroxybenzo[b]thiophene-2-aldehyde.—1-(5-Hydroxybenzo[b]thiophene-2-yl-carbonyl)-2-*p*-toluenesulfonyl hydrazine (1.86 g., 5.15 mmoles) in ethylene glycol (8 ml.) was heated in an oil bath at 160° and anhydrous sodium carbonate (1.36 g., 12.8 mmoles) was added. Vigorous effervescence occurred and after 90 sec. the reaction mixture was poured into water. The aldehyde was extracted with ether and formed pale yellow needles (0.43 g., 47%), m.p. 193–196°, from ethyl acetate.

Anal. Calcd. for $C_9H_6O_2S$: C, 60.7; H, 3.4. Found: C, 60.7; H, 3.6.

5-Hydroxy-2-(2-nitrovinyl)-benzo[b]thiophene.—To a solution of 5-hydroxybenzo[b]thiophene-2-aldehyde (0.79 g.) in nitromethane (6 ml.) was added ammonium acetate (0.25 g.). The mixture was heated gently on a steam bath for 30 min. On cooling, crystals separated and were collected and washed with hot water. Red cubes (0.60 g., 61%) from ethyl acetate resulted, m.p. 220°.

Anal. Calcd. for $C_{10}H_7NO_3S$: C, 54.3; H, 3.2. Found: C, 54.7; H, 3.3.

5-Hydroxy-2-(2-nitropropenyl)-benzo[b]thiophene.—This compound was prepared by the preceding procedure, using nitroethane in place of nitromethane. The product crystallized as yellow cubes (62%) from ethyl acetate, m.p. 223.5–224°.

Anal. Calcd. for $C_{11}H_9NO_3S$: C, 56.2; H, 3.8. Found: C, 56.1; H, 3.5.

2-(2-Aminoethyl)-5-hydroxybenzo[b]thiophene Hydrochloride (XII).—Lithium aluminum hydride (0.5 g.) was added to dry tetrahydrofuran (150 ml.) in the flask of a Soxhlet extractor, and 5-hydroxy-2-(2-nitrovinyl)-benzo[b]thiophene (0.23 g.) was extracted from the thimble for 3 hr. After the excess lithium aluminum hydride had been decomposed by the careful addition of water, 2 *N* sodium hydroxide solution (100 ml.) was added and the tetrahydrofuran removed by distillation. The basic solution was filtered, saturated with carbon dioxide (pH 8.3), and extracted continuously with ether for 24 hr. Dry hydrogen chloride was passed into the ether solution and white plates precipitated (0.12 g., 52%), m.p. 296°, from methanol.

Anal. Calcd. for $C_{10}H_{12}ClNOS$: C, 52.3; H, 5.3. Found: C, 52.8; H, 5.5.

2-(2-Aminopropyl)-5-hydroxybenzo[b]thiophene Hydrochloride (XIII).—This was prepared by applying the preceding procedure to 5-hydroxy-2-(2-nitropropenyl)-benzo[b]thiophene. The product formed white needles from methanol, m.p. 267–270°.

Anal. Calcd. for $C_{11}H_{14}ClNOS$: C, 54.2; H, 5.6. Found: C, 54.2; H, 5.8.

5-Nitrobenzo[b]thiophene-2-carboxylic Acid Chloride.—5-Nitrobenzo[b]thiophene-2-carboxylic acid (5.0 g.) was dissolved in thionyl chloride (60 ml.) and heated under reflux for 2 hr. The acid chloride obtained after distillation of the excess thionyl chloride formed pale yellow needles (5.1 g., 95%), m.p. 160°, from ethyl acetate.

Anal. Calcd. for $C_9H_4ClNO_3S$: C, 44.7; H, 1.7. Found: C, 45.0; H, 1.7.

2-(Morpholinocarbonyl)-5-nitrobenzo[b]thiophene.—To the preceding acid chloride (5.0 g., 0.022 mole) dissolved in dry benzene (120 ml.), morpholine (3.2 ml., 0.044 mole) was added gradually with shaking at 10° and the mixture then was heated under reflux for 1 hr. On cooling, the morpholine hydrochloride which separated was removed and the filtrate taken to dryness under reduced pressure. The pale yellow plates (4.55 g., 75%) had m.p. 189–190°, from ethanol.

Anal. Calcd. for $C_{13}H_{12}N_2O_4S$: C, 53.4; H, 4.1. Found: C, 53.6; H, 4.4.

The following compounds were similarly prepared.

2-(Dimethylaminocarbonyl)-5-nitrobenzo[b]thiophene, m.p. 141–142°. *Anal.* Calcd. for $C_{11}H_{10}N_2O_3S$: C, 52.8; H, 4.0. Found: C, 52.7; H, 3.8.

2-(Pyrrolidinocarbonyl)-5-nitrobenzo[b]thiophene, m.p. 194–195°. *Anal.* Calcd. for $C_{13}H_{12}N_2O_3S$: C, 56.5; H, 4.4. Found: C, 56.5; H, 4.5.

2-(Piperidinocarbonyl)-5-nitrobenzo[b]thiophene, m.p. 132°. *Anal.* Calcd. for $C_{14}H_{14}N_2O_3S$: C, 57.9; H, 4.9. Found: C, 57.9; H, 5.0.

5-Amino-2-(morpholinomethyl)-benzo[b]thiophene (XV).—To 2-(morpholinocarbonyl)-5-nitrobenzo[b]thiophene (4.5 g.) suspended in ethanol (150 ml.), 98% hydrazine hydrate solution (5 ml.) and Raney nickel (2 g.) were added, and the mixture heated gently on the steam bath for 1 hr. The hot solution was filtered and on cooling 5-amino-2-(morpholinocarbonyl)-benzo[b]thiophene, 3.63 g. (90%), separated from the filtrate; fine needles, from methanol, m.p. 189°.

(13) W. Voegtli, U. S. Patent 2,806,034 (1953); *Chem. Abstr.*, **52**, 2931 (1958).

The recrystallized amine (1.0 g.) together with lithium aluminum hydride (3.0 g.) was dissolved in tetrahydrofuran (90 ml.), and heated under reflux for 48 hr. Then water was carefully added to decompose the excess lithium aluminum hydride and the solution rendered basic with aqueous sodium hydroxide solution. The tetrahydrofuran was removed by distillation and 5-amino-2-(morpholinomethyl)-benzo[*b*]thiophene was isolated by ether extraction of the residue; yellow plates (0.59 g., 65%), from ethyl acetate, m.p. 147°.

Anal. Calcd. for $C_{13}H_{16}N_2OS$: C, 62.9; H, 6.5. Found: C, 62.5; H, 6.3.

Similarly were prepared **5-amino-2-(dimethylaminomethyl)-benzo[*b*]thiophene (XIV), 5-amino-2-(pyrrolidinomethyl)-benzo-[*b*]-thiophene (XVI), and 5-amino-2-(piperidinomethyl)-benzo-[*b*]thiophene** (XVII). These compounds were submitted for analysis as the 5-N-benzoyl derivatives after crystallization from ethanol.

5-N-Benzamido-2-(dimethylaminomethyl)-benzo[*b*]thiophene, m.p. 210–211°. *Anal.* Calcd. for $C_{18}H_{18}N_2OS$: C, 69.6; H, 5.9. Found: C, 69.6; H, 5.9.

5-N-Benzamido-2-(pyrrolidinomethyl)-benzo[*b*]thiophene, m.p. 207.5°. *Anal.* Calcd. for $C_{20}H_{20}N_2OS$: C, 71.4; H, 6.0. Found: C, 71.1; H, 6.3.

5-N-Benzamido-2-(piperidinomethyl)-benzo[*b*]thiophene, m.p. 200°. *Anal.* Calcd. for $C_{21}H_{22}N_2OS$: C, 72.0; H, 6.3. Found: C, 71.9; H, 5.9

Pharmacology

Methods. (a) The Rat Uterus.—Using the method of Amin, Crawford, and Gaddum,[14] reproducible submaximal contractions to serotonin (0.01–0.5 γ/ml.) acting for 30 sec. were obtained; the drug under test was added 30 sec. prior to the next addition of serotonin and the contractions were recorded.

(b) The Rat Fundus Strip.—Vane's method[15] was used. Hyoscine hydrobromide (0.1 γ/ml. in Tyrode's solution) was employed routinely to prevent acetylcholine-like effects. Serotonin (0.01–5 γ/ml.) was added to the bath, allowed to act for 90 sec. and then was washed out. The muscle was stretched simultaneously (60 sec., 0.5 g.) and allowed a further 2.5 min. to recover. The test drug was added 30 sec. before the next addition of serotonin.

(c) The Guinea Pig Ileum.—Reproducible, submaximal contractions to serotonin (0.01–0.5 γ/ml.) and histamine (0.001–0.5 γ/ml.), each acting for 30 sec., were recorded. Test drugs were added 30 sec. prior to the next addition of spasmogen.

(d) The Isolated Perfused Rat Hindquarters.—The method used was that of Burn.[16] Serotonin, epinephrine, or norepinephrine was injected into the abdominal aorta until a reproducible response was obtained. The test-drug was injected 30 sec. before the next dose of serotonin, epinephrine, or norepinephrine, and the effect on the outflow was recorded.

(e) The Isolated Rat Jejunum.—The technique of Van Rossum and Ariëns[17] was used. Reproducible cumulative log dose–response curves for serotonin were recorded by adding the solution to the bath in the dose sequence—a, a, 2a, and so on. A given concentration of serotonin was left in contact with the tissue until a steady state was attained (1 min.). When the maximum effect was recorded, the tissue was washed for 5–10 min., and 20 min. was allowed for recovery. After obtaining reproducible, cumulative log dose–response curves for serotonin the drug under investigation was added to the bath 1 min. before the next addition of serotonin, and the effect recorded.

(f) The Blood Pressure of the Anesthetized Cat and Rat.—Cats were anesthetized by intraperitoneal injection of sodium pentobarbital (50–60 mg./kg.) and rats by the subcutaneous injection of urethane (175–200 mg./100 g.). Blood pressure recordings were made from the common carotid artery. When reproducible pressor responses to epinephrine and norepinephrine were obtained, the drug under test was injected into the jugular vein and the influence on the blood pressure level and the pressor responses to epinephrine and norepinephrine observed.

(g) Toxic Effects on Mice.—Drugs (25–150 mg./kg.) were injected intraperitoneally into groups of 5 mice. The animals were observed for up to 8 hr. and compared with control groups injected with normal saline.

Results

(a) The Rat Uterus.—At dose levels of 2.5–200 γ/ml. none of the compounds exerted a direct stimulant effect but all antagonized serotonin-induced contractions (serotonin, 0.01–0.05 γ/ml). Compound VI exhibited variable activity. In two experiments, concentrations of 50 and 100 γ/ml. potentiated the stimulant response to serotonin and induced spontaneous activity. In another two, the same doses incompletely inhibited the stimulant response to serotonin but still induced spontaneous activity.

(b) The Rat Fundus Strip.—With the exception of compounds VII, XII, and XV, all the compounds, in dose levels ranging from 0.1–100 γ/ml., caused stimulation.

Compound XIII was the most potent, causing powerful contractions of the tissue and greatly enhancing muscle tone at doses of 0.1–20 γ/ml. The tissue did not regain its original length after washing at 5-min. intervals, for 1.5 hr. with Tyrode's solution. At dose levels of from 1–5 γ/ml., with the exception of VII, XII, and XV, all the compounds potentiated the stimulant response to serotonin. At dose levels of from 40–80 γ/ml. XII and XV inhibited the stimulant response to serotonin. Compound II showed variable activity. At dose levels of from 2–5 γ/ml. it incompletely inhibited the stimulant response to serotonin in two experiments. In a third test at 5–20 γ/ml. it exerted a direct stimulant action. In a fourth at 1–2 γ/ml. it potentiated the action of serotonin.

(c) The Guinea Pig Ileum.—With the exception of I, III, and XV, dose levels ranging from 1–200 γ/ml. of the remaining compounds caused stimulation of varying degrees of intensity. In many cases the responses to a second and higher dose were considerably less than to the first. When suitable contractions were obtained, the effects of lysergic acid diethylamide, mepyramine, and atropine were investigated. The compounds of Group B (1–4 γ/ml.) exerted powerful stimulant effects on the tissue but those of Groups C and D had only weak stimulant actions and the effects upon these of lysergic acid diethylamide, mepyramine, and atropine were therefore not investigated. The contractions produced by II were antagonized by atropine (0.5 γ/ml.) and mepyramine (0.5 γ/ml.). The contractions produced by V were inhibited by mepyramine (50 γ/ml.) but not by atropine (50 γ/ml.) or lysergic acid diethylamide (0.05 γ/ml.). Higher concentrations of lysergic acid diethylamide (2.5–5 γ/ml.) had a direct stimulant action on the tissue. The contractions produced by VIII, IX, X, and XI were antagonized by atropine and mepyramine (0.5–50 γ/ml.). Lysergic acid diethylamide (0.001–0.50 γ/ml.) did not always completely antagonize these stimulant responses.

With the exception of VI, IX, and X, all the compounds antagonized the stimulant response to serotonin (0.01–0.5 γ/ml.). The compounds of Group A (1–20 γ/ml.) were the most potent. Next in order were the members of Group C (20–40 γ/ml.) and those of Group D were the weakest (100–200 γ/ml.). At higher con-

(14) A. H. Amin, T. B. B. Crawford, and J. H. Gaddum, *J. Physiol.*, **126**, 596 (1954).

(15) J. R. Vane, *Brit. J. Pharmacol.*, **14**, 87 (1959).

(16) J. H. Burn in "Practical Pharmacology," Blackwell Scientific Publications, Oxford, 1952, p. 65.

(17) J. M. Van Rossum and E. J. Ariëns, *Arch. Intern. Pharmacodyn.*, **118** 418 (1959).

centrations than those which inhibited the stimulant response to serotonin, the compounds themselves exerted a direct stimulant effect.

All the compounds (0.01–500 γ/ml.) inhibited the stimulant response to histamine (0.001–0.5 γ/ml.) though not all did so completely. Compounds II, IV, V, VI, X, and XI were the most potent acting in the dose range of 0.01–2 γ/ml.

With the exception of VIII, all the compounds (5–500 γ/ml.) antagonized the stimulant response to acetylcholine (0.001–0.5 γ/ml.) but not all did so completely.

In general, the benzo[*b*]thiophene compounds were more potent in antagonizing the stimulant responses to histamine than those to serotonin.

(**d**) **The Rat Jejunum.**—In no case was there evidence of a purely agonistic effect and consequently, attempts to classify the compounds were made on the basis of their interaction with serotonin. Where the cumulative serotonin log dose–response curve, in the presence of a compound under test, was shifted along the abscissa and the maximum height reduced, in comparison with the curve for serotonin alone, evidence of a noncompetitive antagonism was assumed,[18] *e.g.*, II, III, VII, VIII, X, all the compounds of Group C, and compound XV in Group D. Compounds I, VI, XI, XVI, and XVII exerted a partially agonistic effect, an initial increase in the response to serotonin being observed prior to the onset of a noncompetitive antagonism. Other compounds exerted nonreproducible activity, appearing to act in a purely noncompetitively antagonistic fashion in several experiments, but exhibiting both an agonistic and an antagonistic noncompetitive effect in others. For example, V exhibited a purely noncompetitive antagonism in two experiments, but in another two it showed both agonistic properties and a noncompetitive antagonism towards serotonin.

(**e**) **The Isolated, Perfused Rat Hindquarters.**—None of the compounds (0.1–0.5 mg.) exerted a direct vasodilator or vasoconstrictor effect. In this dose range VI, VII, VIII, and IX of Group C and the compounds of Group D had no effect on serotonin-vasoconstriction, while the others partly or completely prevented it. They all partially or completely inhibited the vasoconstriction caused by epinephrine or norepinephrine.

(**f**) **Blood Pressure of the Anesthetized Cat and Rat.**—The compounds of Group A produced a fall in blood pressure in the cat (1–5 mg./kg.) and rat (0.3–1.5 mg./kg.) but did not antagonize the pressor effects to epinephrine (2.5 γ/kg.). The compounds of Group B (1–2 mg./kg.) exerted a hypertensive effect on the cat but, in doses of 0.3–1.5 mg./kg., exhibited either hypertensive or hypotensive activity in the rat. Compounds XII and XIII (Group C) produced a direct pressor effect on both species, reversible in the cat by prior administration of 2-N-*m*-hydroxyphenyl-*p*-toluidinomethylimidazoline (phentolamine). The compounds of Group D (0.3–1.5 mg./kg.) exerted a slight hypertensive effect on the rat only.

(**g**) **Toxic Effects on Mice.**—At dose levels of up to 150 mg./kg. by intraperitoneal injection, the compounds in Groups A, C, D, and compound IX in group B had no apparent toxic effects. Within 30 min. of the injection of compounds VIII, X, and XI, there was hyperpnoea, followed by convulsive movements, after which the animals died. The approximate LD_{50} of XI was 57.5 mg./kg.; of X, 62.5 mg./kg.; and of VIII, 67.5 mg./kg.

Discussion

The compounds investigated upon isolated tissues and intact animals have shown such a lack of selectivity as to require discussion of their properties in groups according to their chemical structure.

Of the compounds in Group A, II is the isostere of gramine. Gramine itself possesses convulsant, parasympathomimetic,[19] antiepinephrine,[20] serotonin-like, and antiserotonin properties.[21a–f]

Compound II had weak but detectable agonistic activity on the guinea pig ileum but on the same tissue antagonized the responses to acetylcholine, histamine, and serotonin. The reduced agonistic activity in certain benzo[*b*]thiophene compounds, as compared with their indole isosteres is emphasized when compounds III, V, and VI (Group A) and XII (Group C) are compared, respectively, with 3-(morpholinomethyl)indole, 3-(piperidinomethyl)indole, 3-(2-methylpiperidinomethyl)indole, and 2-(2-aminoethyl)-5-hydroxyindole. Thus, while 3-(morpholinomethyl)indole and 3-(2-methylpiperidinomethyl)indole stimulated the isolated rat uterus and constricted the blood vessels of the isolated rat hindquarters,[21f] the corresponding benzo[*b*]thiophene compounds had no stimulant activity on similar preparations.

A reduction in antagonistic properties and the emergence of stimulant activity, especially on the guinea pig ileum, characterize the quaternary compounds of Group B. Thus, compound IX produced a contraction of the guinea pig ileum, whereas the corresponding tertiary amine had no stimulant action but antagonized the stimulant response to serotonin.

The observation that the stimulant effects of compounds in Group B were antagonized by atropine and mepyramine, but not by lysergic acid diethylamide, suggests interaction with acetylcholine and histamine receptors rather than with a serotonin receptor system. Furthermore, in contrast to the tertiary amines (Group A), the quaternary compounds of Group B raised the blood pressure of anesthetized cats and rats and were toxic to mice.

The compounds of Group C can be regarded as serotonin analogs, incorporating an isomerization of the side chain to the 2-position. Compound XII, unlike its isostere 2-(2-aminoethyl)-5-hydroxyindole, exerts no stimulant effects upon the isolated rat uterus. This supports the general conclusion that replacement of the

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(21) (a) C. E. Powell, E. R. Swanson, and K. K. Chen, *J. Am. Pharm. Assoc., Sci. Ed.*, **44**, 399 (1955); (b) J. H. Gaddum and K. A. Hameed, *Brit. J. Pharmacol.*, **9**, 240 (1954); (c) J. H. Gaddum, K. A. Hameed, D. E. Hathway, and F. F. Stephens, *Quart. J. Exptl. Physiol.*, **40**, 49 (1955); (d) V. Erspamer, *Ric. Sci.*, **23**, 1203 (1953); (e) V. Erspamer, *Science*, **121**, 369 (1955); (f) G. Bertaccini and P. Zamboni, *Arch. Intern. Pharmacodyn.*, **133**, 138 (1961).

pyrrole ring by the thiophene ring is associated with a fall in stimulant activity. While XII inhibits the stimulant response to serotonin on the isolated rat fundus strip, the 2-aminopropyl compound XIII produced powerful contractions of the same tissue.

The presence of a 5-amino group and the side chain in the 2-position characterize the compounds of Group D which show very weak antagonism towards serotonin, histamine, and acetylcholine. With the exception of compound XV, serotonin-induced vasoconstriction was not antagonized but there was partial inhibition of epinephrine and norepinephrine vasoconstriction on the isolated perfused rat hindquarters.

The compounds of Group A exhibit the variability and nonselectivity in pharmacological properties characteristic of the corresponding indole derivatives.[21f,22] These features, the existence of both agonistic and antagonistic activity in the same compounds and the observed auto-inhibitory phenomena, make it difficult to draw any inferences concerning the influence on biological activity of replacing the indole by the benzo[*b*]thiophene ring system. These difficulties are further underlined by the lack of conclusive evidence that any specific serotonin receptor exists. An attempt to establish the nature of the interaction between the benzo[*b*]thiophene compounds and the hypothetical serotonin receptor, using Ariëns' technique on the rat jejunum, showed that I, VI, XI, XIV, XVI, and XVII possessed both agonistic and noncompetitively antagonistic properties, while the others exerted only a noncompetitive antagonism.[15] It can be suggested, therefore, that the replacement of the –NH– group of the indole ring by sulfur leads to a reduction in the intrinsic activity.[23] The ability of the benzo[*b*]thiophene compounds to antagonize acetylcholine, histamine, and serotonin, and the ability of atropine, mepyramine, and lysergic acid diethylamide to antagonize the agonistic component of action of these compounds, where it exists, may have its explanation in a nonspecific affinity for additional receptor fields.[24]

The phenomenon of auto-inhibition was frequently observed in the compounds of Groups A and C, in which the response to the second and higher dose on the guinea pig ileum was observed to be less than that to the first. Moreover, larger doses stimulated the tissue but then rendered it insensitive to further additions of the drug. This effect has also been observed with acetylcholine, histamine, and serotonin.[25a,b]

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Synthesis and Pharmacological Activity of Fluorinated Tryptamine Derivatives

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The synthesis of several fluorinated tryptamine derivatives in which the fluorine atom occupies the 5- or 6-position is reported. The 5-fluoro and unsubstituted tryptamines are more active than the 6-substituted derivatives in inducing spontaneous locomotion when administered to reserpinized white mice. Both 6-fluoro-N,N-diethyltryptamine and N,N-diethyltryptamine exerted peripheral activity when tested in humans, but only the fluorine-free compound seemed to bear hallucinogenic properties. These results may be explained by change in the metabolic pathway of the tryptamines with substituted 6-position in the indole nucleus.

The 6-hydroxylation of various tryptamine derivatives was established in this laboratory as an important metabolic pathway.[3] Evidence was presented, using animal behavioral tests and human experiments, that this pathway might be important in producing pharmacologically active metabolites.[4,5] For further pharmacological and psychological studies it was of interest to prepare and compare the activity of structural isomers blocked in the 6-position, as this may render additional evidence for the proposed biological mechanism.

The fluorinated derivatives seemed to be particularly appropriate for this kind of study because of their stability and the availability of the starting 5- and 6-fluoroindoles.[6,7] Attempts were made to improve the synthesis of 6-fluoroindole since the oxidation of 4-fluoro-2-nitrotoluene (I) to the corresponding aldehyde was cumbersome. Addition of bromine to I at elevated temperature resulted in the introduction of two atoms of the halogen into the molecule, but subsequent hydrolysis yielded a bromine-containing organic acid. Nitration of *p*-fluorobenzyl chloride, alcohol, or acetate in acetic anhydride–nitric acid did not yield the expected nitro derivatives and in all cases only the acetate was recovered. The presence of sulfuric acid in this reaction brought about polymerization.

Nitration of *p*-fluorobenzyl cyanide did not yield either of the two expected isomers. The only compound isolated did not contain fluorine. The analytical data of this substance and derivatives support the

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