Benzo[b]thiophene Derivatives. XII. Synthesis of Some 3-Benzo[b]thienylalkylamines and Comparison of Their Central Nervous System Activity with Tryptamine Isosteres[1a,b]

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The synthesis of a series of benzo[b]thiophene isosteres of substituted tryptamines is described. The CNS stimulatory activity of these 3-(β-dialkylaminoethyl)benzo[b]thiophenes was compared to that of the isosteric indole compounds by degree of reversal of mild pentobarbital sedation in rabbits as measured by a quantitative electroencephalographic technique. The benzo[b]thiophenes were found to be stimulants with approximately the same order of activity as their indole isosteres. This supports the hypothesis that the indole ring N–H group does not participate significantly in interaction with CNS tryptamine receptors.

Following the initial studies of Woolley and Shaw on pharmacologically active analogs of serotonin (5-hydroxytryptamine, 5-HT),[2] great interest has been shown in the preparation of biologically active tryptamine derivatives.[3] Much of this work was directed to prepare 5-HT-like substances with the hope of obtaining a compound more penetrable to the brain after peripheral administration and possessing a greater duration of action than 5-HT itself.[4] It has been established that 5-HT does not readily cross the blood–brain barrier.[5] Indoles such as 5-HT have frequently been suggested as being involved in cardiovascular and neural function,[6] and abnormal indole metabolites have been claimed to be present in the urine of schizophrenic patients.[7] The tryptamine moiety in a number of compounds with hallucinogenic activity has stimulated speculations about the relationship of these or similar indoles to abnormal mental processes.[8]

Despite extensive studies, the role of 5-HT in the central nervous system (CNS) is not yet known.[6] Gaddum[6b] has argued that tryptamine and 5-HT share common receptors in the gut while Woolley and Shaw[6c] directed attention to evidence which indicates that at least in some peripheral tissues, receptors for 5-HT and for tryptamines are not the same. Tedeschi, *et al.*,[6d] have advanced the hypothesis that CNS receptors for tryptamines and 5-HT are shared in common and Vane, *et al.*,[6e] present indirect evidence strongly supporting this concept. Studies on receptors for 5-HT and tryptamine using the rat stomach preparation[9] have led Vane[10] to propose a two-point attachment of 5-HT to its receptor site: the 5-hydroxyl group and the terminal amino group at the 3 position. One of the areas of tryptamine molecules which might be important for the interaction with the receptor is the N–H group in the 1 position. Taborsky, *et al.*,[11] have prepared a series of 1-methylindoles and found that, in general, 1-methylation of indoles did not produce profound changes in pharmacological activity of the parent compound. This suggested that the 1-nitrogen is not functionally involved in indole receptors or enzyme sites.

The concept of bioisosterism has been applied with considerable success to the preparation of pharmacologically active substances.[12] While the isosteric replacement of thiophene for benzene has been extensively studied,[13] only recently has attention been devoted to the substitution of the thiophene ring for the

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pyrrole moiety in naturally occuring indole derivatives.[14] A facile procedure for the preparation of hydroxyl- and alkoxyl-substituted benzo[*b*]thiophenes developed in our laboratory[15] has been applied to the preparation of sulfur isosteres of 5-HT[16] and other tryptamines of interest. It was postulated that the greater hydrophobic nature of benzo[*b*]thiophene compared to indole might provide derivatives with greater ability to localize in CNS tissues than their indole isosteres. The covalent radius of divalent sulfur is 1.04 Å, while that for trivalent nitrogen is only 0.7 Å.[17] Since the covalent radius of hydrogen is 0.3 Å, the bulk of the indole N–H group approximates that of the sulfur atom in benzo[*b*]thiophene. It was therefore expected that the benzo[*b*]thiophene isosteres of tryptamines would have great affinity for CNS tissues and receptor affinity and intrinsic activity comparable to that of the indoles.

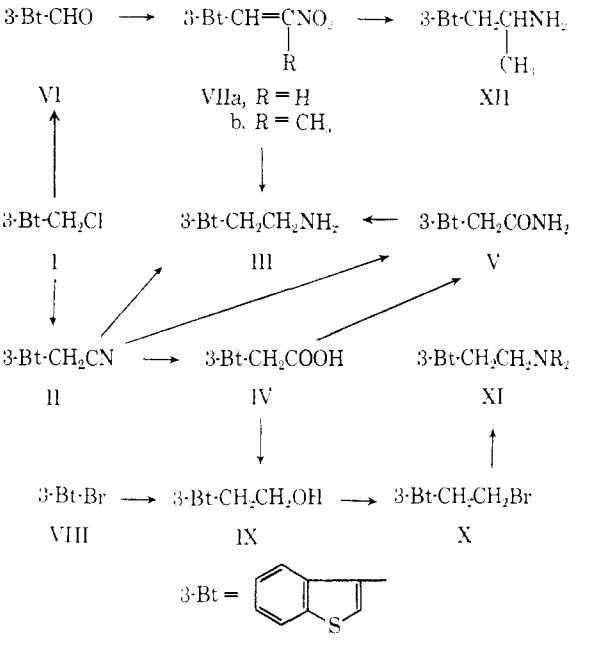
Except for our preliminary reports on the agonistic CNS properties of the 5-HT isostere,[16] no other detailed studies have been published on the CNS activity of benzo[*b*]thiophene derivatives. Lewis, *et al.*,[14g] prepared a number of benzo[*b*]thiophene derivatives related to 5-HT and gramine and found, in general, a reduction in agonistic activity and variable nonspecific antagonistic properties to 5-HT, acetylcholine, and histamine when treated on a variety of smooth muscle preparations. On the other hand, Winter, *et al.*,[18] tested a number of 3-indenealkylamines, 3-benzo[*b*]-thienylalkylamines, and tryptamines in the rat stomach fundus and found the majority of these derivatives to have a greater intrinsic activity than 5-HT but none with higher affinity.

In the course of our work with alkoxyl- and hydroxyl-substituted benzo[*b*]thiophene analogs of CNS-active indoles it was necessary to prepare the isostere of tryptamine and some of its derivatives for a comparative study of CNS effects. This paper reports the synthesis of these compounds and their CNS activity as measured by their ability to reverse pentobarbital sedation in rabbits.

Chemistry.—The syntheses of the desired 3-(β-aminoethyl)benzo[*b*]thiophenes are outlined in Chart I. Although several of these compounds have been previously reported, the desirability of optimizing yields led us to study several synthetic pathways for some of the key compounds. Herz first reported the preparation of 3-(β-aminoethyl)benzo[*b*]thiophene (III) by the $LiAlH_4$ reduction of 3-cyanomethylbenzo[*b*]thiophene (II) in 32% yield. Pharmacological studies were not reported for III and its was prepared in this present work for such evaluation. A detailed study of the preparation of II from I has been reported[19] based on previous work.[14d,20,21] Because of the low yield in the metal hydride reduction of II, other methods of preparing III were investigated as indicated in Chart I.

CHART I

SYNTHESIS OF 3-(β-AMINOETHYL)- AND 3-(β-DIALKYLAMINOETHYL)BENZO[*b*]THIOPHENES



II was readily hydrolyzed to IV which in turn can be converted to the amide V, but V can be easily formed in high yield directly from II. Reduction of V led to III in 60% yield. A convenient synthesis of the aldehyde VI[22] provides the path for the nitrovinyl derivatives VII, and these in turn gave equally good yields of the β-aminoethyl derivatives III and XII. Thus two improved pathways to the desired amines are reported. Compound XII has been prepared by an alternate method also.[23] The Eschweiler–Clarke variant of the Leuckart reaction[24] was used for the preparation of 3-(β-dimethylaminoethyl)benzo[*b*]thiophene (XIII) (66%) from III. Compound XIII has been prepared since this work by the reaction of 3-benzo[*b*]thienylmagnesium bromide and 2-dimethylaminoethyl chloride in 4% yield.[25]

Five other tertiary amine derivatives (XI) were prepared and are listed in Table I. The synthesis of these tertiary amines started with 3-bromobenzo[*b*]-thiophene (VIII), previously reported.[26–28] It is difficult to form a Grignard reagent from VIII in the ordinary way,[29] but it can be done in fair yields by Grig-

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(16) E. Campaigne, T. Bosin, and E. S. Neiss, *J. Med. Chem.*, **10**, 270 (1967).

(17) L. Pauling, "The Nature of the Chemical Bond," 3rd ed. Cornell University Press, Ithaca, N. Y., 1960.

(18) J. C. Winter, P. K. Gessner, and D. D. Godse, *J. Med. Chem.*, **10**, 856 (1967). The benzo[*b*]thiophene derivatives whose intrinsic activity is reported in this publication were prepared in this laboratory and their syntheses are presented in this present paper. We thank Professor P. K. Gessner for providing his results before publication.

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(29) G. Kumpa and S. Weckmann, *J. Prakt. Chem.*, **135**, 109 (1933).

TABLE I
3-(2-DIALKYLAMINOETHYL)BENZO[b]THIOPHENES (XI)

No.	N(R)$_2$	Bp.[a] °C (mm)	Mp.[d] °C	Yield.[e] %	Formula[g,h]
XIII	N(CH$_3$)$_2$[b]	...	260–261	66	$C_{12}H_{16}ClNS$
XIV	N(C$_3$H$_7$)$_2$	160–165 (1)	154–155	45	$C_{16}H_{24}ClNS$
XV		164–168 (1)	200–201	40	$C_{14}H_{18}ClNS$
XVI		149–152 (0.3)	279–280	64	$C_{15}H_{20}ClNS$
XVII		178–183 (2)	213–214	49	$C_{14}H_{18}ClNOS$
XVIII		187–195 (3)	267–268[f]	51	$C_{15}H_{22}Cl_2N_2S$

[a] Boiling points are for the free amines as distilled from the reaction mixture of X and secondary amines. [b] Prepared by the Clarke–Eschweiler reaction. [c] Also obtained in 66% yield by reaction of III and 1,4-dibromobutane. [d] Melting points are of the amine hydrochlorides and are corrected. [e] Yield is of pure product after recrystallization of the hydrochlorides from chloroform–hexane or absolute ethanol–ether. [f] Sublimes. [g] As hydrochloride salts. [h] All analyzed for C, H, N.

nard's entrainment technique.[30] By the use of equimolar amounts of VIII and methyl iodide the mixed Grignard reagents were formed and treated with ethylene oxide to form 3-(2-hydroxyethyl)benzo[b]thiophene (IX), also formed, but in lower yield, by the $LiAlH_4$ reduction of the acid IV.[19] The alcohol IX was converted to a variety of 3-(β-aminoethyl)benzo[b]thiophenes by amination of its corresponding bromide, and these are listed in Table I.

Pharmacology.—Pharmacological investigation of these compounds and some indole isosteres involved a study of their effects on CNS activity as measured by a quantitative technique of electroencephalography. Drug effect on cerebrocortical bioelectrical activity was evaluated in terms of degree of reversal of a mild sedation serving as an initial baseline physiological state.

Quantitation of the electroencephalogram (EEG) was obtained by means of the electronic integrator of Drohocki.[31] This device successively rectifies the brain waves, scans the area subtended by the waves, and continuously records this amplitude measurement as a series of pulses proportionate to the level of activity of the brain (mean energy content, MEC). The integrator is calibrated so that for a given time period a set number of pulses is recorded for a standard microvolt output. The mean number of these integration pulses per minute during any specific control or experimental time period can then be used for statistical analyses. The mean number of pulses during the control or other baseline period may be equaled to 100, and the mean values obtained during experimental (drug-administered) periods are expressed indicially, that is, as per cent change in brain electrical activity with respect to the designated baseline state. A thorough discussion of amplitude analysis of the EEG is presented in a recent review.[32]

Adult, male albino rabbits (3.5–4.5 kg) were equipped with permanently implanted electrodes in the outer table of the calvarium so that frontoparietal brain areas could be continuously recorded for brain activity by means of a Grass Model D polygraph. All drugs were administered intravenously through an indwelling catheter in the marginal vein of the ear, permitting remote injection of any solution with negligible disturbance to the animal by the injection. Although confined to an appropriately desiged open box during the recording session, the rabbits are otherwise unrestrained and unanesthetized, having been previously trained to the entire experimental procedure in a partially dimmed, sound-attenuated room so that the control predrug behavioral activity was minimal.

In each individual experiment the EEG of the rabbit was continuously recorded for a 10-min control period, a 5-min period following administration of only 3 mg/kg of sodium pentobarbital (producing a steady, nonspecific sedated baseline state), and a 25-min period following injection of the test compound or saline. In this study, however, only the first 10 min of the latter drug period were evaluated. Usually, three rabbits were subjected to a specific dose level, and at least three dose levels were administered, utilizing a minimum of nine rabbits per test compound.

To illustrate the dependence of response on dose level, Figure 1 diagrammatically represents the relationships which obtain following indicial transformation of the integrator data expressed as mean number of pulses per minute for a particular experimental period. Designating the mean of the control period as 100, 3 mg/kg of pentobarbital effects an index of about 330% or a greater than 200% increase over control values. It is to be emphasized that the shift from a control state of wakefulness to a state of sedation is reflected in an increase in the number of integration pulses, whereas the shift from a sedated to a wakeful (or stimulated) state produces a decrease in the number of pulses. Increasing doses of tryptamine result in correspondingly

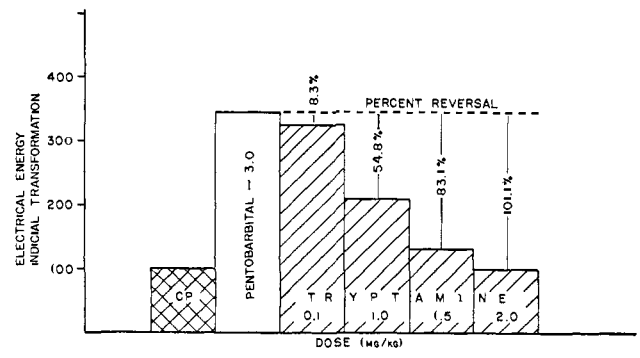

Figure 1.—Effect of pentobarbital on the electrical energy of the rabbit brain as measured by quantitative EEG and reversal of this effect by tryptamine. Only one dose level of tryptamine was administered to a rabbit in each experiment.

(30) V. Grignard, *Compt. Rend.*, **198**, 625 (1934).
(31) Z. Drohocki, *Rev. Neurol.*, **80**, 619 (1948).
(32) L. Goldstein and R. A. Beck, *Intern. Rev. Neurobiol.*, **8**, 265 (1965).

decreasing indices, illustrating dose-related stimulant activity.

More comprehensive and informative than the indicial transformation of the integrator data per individual experimental time period is a mathematical method applying such data to the following formula. The

$$\%R = \left[\frac{(MEC_p - MEC_{cp}) - (MEC_a - MEC_{cp})}{MEC_p - MEC_{cp}}\right]100$$

absolute values of mean electrical energy content (MEC) provide directly a means of expressing brain activity in drug-treated rabbits as the per cent change with respect to activity in a population of saline-treated rabbits (MEC_{cp}), both animal groups having been identically dosed with sodium pentobarbital (MEC_p), and their EEG activities were measured and compared in corresponding drug (MEC_a) and saline time periods. The stimulant effect of any specific dose level of drug may be expressed as the per cent reversal ($\%R$) of pentobarbital sedation.

From such data, dose–effect curves were plotted as illustrated in Figure 2. For each drug the configuration

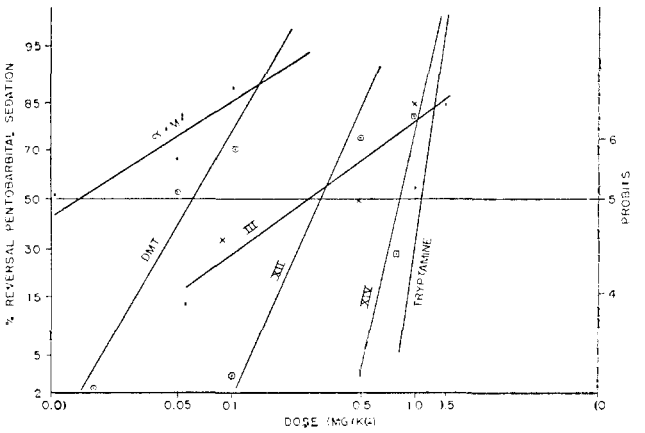

Figure 2.—Dose–response curves of reversal of pentobarbital sedation as measured by quantitative EEG. α-MT is α-methyltryptamine; DMT is dimethyltryptamine.

of the curve indicates direct relationship between the stimulant activity of the compound and the dose level administered. The slope of each curve reflects the specificity or dose dependency of each compound. Finally, the relative positions of the curves indicate the relative intensities of activity with α-methyltryptamine (α-MT) obviously the most active of the series.

The 50% reversal dose, determined for each compound from these curves, was chosen for comparison and is listed in Table II. α-MT and tryptamine differ very significantly in potency, illustrating the dramatic effect of α methylation in the 3-aminoethylindole series; however, III and XII do not differ significantly. Zeller has evaluated the activity of III and XII toward monoamine oxidase (MAO).[33] Compound XII was shown not to be a substrate of beef liver MAO. When XII (1×10^{-3} *M*) was added to the MAO system containing tyramine hydrochloride (1×10^{-2} *M*), it first activated, then reduced, oxygen consumption to 60% after 5 min and −18% after 30 min. Structure III, however, was found to be about 60% as effective as tryptamine as a

(33) E. A. Zeller, personal communication, Department of Biochemistry, Northwestern University Medical School, Chicago, Ill.

Table II

Quantitative EEG Analysis of Tryptamine Derivatives and Benzo[*b*]thiophene Isosteres

Compd	50% reversal dose,[a] mg/kg	% reversal after 1 mg/kg
Tryptamine[c]	0.91	54.2
III	0.26	85.8
α-MT[c]	0.114	100
XII	0.38	100
DMT[c]	0.051	100
XIII	0.01[d]	[e]
DMI[c]	0.12	92.8
XIV	0.82	78.8
XV		76.1
XVI		75.8
5-HTP[c,f]	0.14	
SAS[c,f]	0.16	

[a] Abbreviations: α-MT, α-methyltryptamine; DMT, dimethyltryptamine; DMI, 3-(β-dimethylaminoethyl)indene; 5-HTP, 5-hydroxytryptophan; SAS, sulfur analog of serotonin, 3-(β-aminoethyl)-5-hydroxybenzo[*b*]thiophene. [b] Previously reported [E. Campaigne, E. S. Neiss, and T. Bosin, *J. Med. Chem.*, **10**, 270 (1967)] and provided for comparison. [c] Obtained from the curves in Figure 2. Nonlinear dose–response curve; 0.01 mg/kg gave 46% reversal. [e] This compound had a biphasic response and was agonistic to pentobarbital at this dose.

substrate in this MAO preparation. α-MT is reported to 50% inhibit the oxidation of 10^{-2} *M* tyramine at a concentration of 10^{-2} *M*.[34] In view of the concentration range of active inhibitors of MAO, 10^{-3} to 10^{-7} *M*, it appears that XII is a weak inhibitor of this enzyme comparable to α-MT. While III and XIII are somewhat more active than their isosteres, α methylation changes this situation as XII is considerably less active than α-MT. It may be that III, XII, and tryptamine act similarly at the CNS tryptamine receptors. Since the central excitatory effects of α-MT and XII are apparent soon after injection, it is unlikely that this effect is due to inhibition of MAO. It is of interest that 5-hydroxytryptophan (5-HTP), which is known to be transported into rabbit brain and there decarboxylated to 5-HT,[35] has one-tenth the activity of α-MT while the sulfur analog of serotonin[16] has similar potency as 5-HTP.

Compound XIII had a biphasic response. It was a stimulant at a low dose but augmented the pentobarbital sedation at 1 mg/kg. In comparison, dimethyltryptamine (DMT) was a less potent stimulant at low dose but maintained its stimulant activity up to 1 mg/kg. 3-(β-*N*,*N*-Dimethylaminoethyl)indene[36] was less active than both DMT and XIII at low dose, but unlike XIII maintained a stimulant effect at 1 mg/kg. The biphasic response of XIII appears to be typical of most of the compounds studied when dosage is sufficiently increased. This emphasizes the critical nature of the dose level selected to define the pharmacological activity of any compound, involving as it may different mechanisms of action with different biological effects, whether physiological or approaching toxic.

Dose–effect curves were not determined for compounds XV–XVIII. In contrast to XIII, the tertiary

(34) C. L. Zirkle and C. Kaiser in "Psychopharmacological Agents," Vol. I, M. Gordon, Ed., Academic Press Inc., New York, N. Y., p 515.

(35) (a) B. B. Brodie, E. G. Tomich, R. Kuntzman, and P. A. Shore, *J. Pharmacol. Exptl. Therap.*, **119**, 461 (1957); (b) S. Udenfriend, D. F. Bogdanski, and H. Weissbach, *Fed. Proc.*, **15**, 493 (1956).

(36) We are indebted to Professor P. K. Gessner for a gift of this compound.

amines XIV, XV, and XVI were stimulant at 1 mg/kg with similar potencies. Although less stimulant than DMT they were more effective at pentobarbital reversal than was tryptamine.

The data in Table II indicate that the benzo[*b*]-thiophene analogs are generally as active or more active than their indole isosteres. The exception appears to be XII which is not as potent as α-MT, but which is still more stimulant than tryptamine. The difference between XII and α-MT cannot be explained by the present studies but is in accord with the results of Winter, *et al.*,[18] who demonstrate XII to have about half the relative intrinsic activity of α-MT on the isolated rat stomach preparation. In that study it was shown that the replacement of the ring N–H group in some indolealkylamines by either $-CH_2-$ or –S– leads to parallel changes in both affinity and intrinsic activity and that these substitutions do not appreciably alter their ability to cause muscle contraction. In the present study it appears that the benzo[*b*]thiophene isosteres and tryptamines act similarly in the CNS as measured by quantitative EEG technique. This supports the findings of Winter, *et al.*,[18] on peripheral tissues, and of Taborsky, *et al.*,[11] on both peripheral and CNS tissues, that the ring N–H group of tested tryptamines is not a significant binding site at tryptamine receptors. The quantitative differences in reversal of pentobarbital sedation may be a reflection of the greater lipid solubility and hence greater cerebral tissue concentration of the benzo[*b*]thiophene derivatives. Such an interpretation must be regarded only as a suggestion until further evidence is obtained.

Experimental Section[37]

Benzo[*b*]thiophene-3-acetic Acid (IV).—Using the acid hydrolysis procedure of Wenner,[38] 186 g (1.075 moles) of melted II[19] was added with vigorous stirring to 800 ml of concentrated HCl maintained at 40–45°. After 2 hr, 800 ml of H_2O was added to the white slurry, and the mixture was refluxed until the white slurry became a yellow oily dispersion (6 hr). The mixture was transferred to a beaker and cooled overnight. The solid which formed was collected, dissolved in 1 l. of 10% NaOH, treated with Norit, filtered, and acidified with 6 *N* HCl to produce 187 g (90%) of IV melting at 97–102°. Twice recrystallized from H_2O, it melted at 110–111° (lit.[20] mp 110–111°); ir (KBr), 3.3–4.0 (bonded OH), 5.92 (C=O) μ. Hydrolysis of II by the alkaline procedure of Blicke and Sheets[20] gave only 52% of IV, as these authors reported. *Anal.* ($C_{10}H_8O_2S$) C, H, S.

N-Methylbenzo[*b*]thiophene-3-acetamide.—A solution of 16.2 g (0.084 mole) of IV in 100 ml of dry C_6H_6 was treated with excess $SOCl_2$. After refluxing 2 hr, benzene and excess $SOCl_2$ were removed under reduced pressure, and fresh dry C_6H_6 added. The benzene solution was then added dropwise to 80 ml of $MeNH_2$ with stirring and cooled in a Dry Ice–acetone bath. When addition was complete, the mixture was heated on a water bath to remove excess $MeNH_2$, then ether was added and the solution was washed (dilute HCl, H_2O, 10% $NaHCO_3$) and dried (Na_2SO_4). The residue remaining after removal of solvent recrystallized from water to produce 15.5 g (90%) of fluffy white needles: mp 110–111° (depressed on mixing with IV); ir (KBr), 3.05 (NH), 3.26 (*sec*-amide) 3.42 (CH_2), and 6.10 (amide C=O) μ. *Anal.* ($C_{11}H_{11}NOS$) C, H, S.

Benzo[*b*]thiophene-3-acetamide (V).—A small sample of the crude acid chloride of IV, prepared as above, was added to concentrated NH_4OH, and the resultant amide was recrystallized twice from H_2O: mp 173–174° (lit.[20] 171–173°); ir (KBr), 2.98, 3.15 (NH), and 6.03 (C=O) μ. *Anal.* ($C_{10}H_9NOS$) C, H, N.

A more convenient preparation resulted from hydration of the nitrile II. Using the Wenner[38] procedure for the preparation IV (above), 93 g (0.537 mole) of II was added to 400 ml of concentrated HCl at 40–45°. After 400 ml of cold H_2O was added, the white slurry was cooled and filtered, the crude product was stirred in 10% $NaHCO_3$, and undissolved solid was collected and crystallized (H_2O) giving 82 g (80%) of V. Acidification of the alkaline wash produced 6 g (5.7%) of IV.

1-(3-Benzo[*b*]thienyl)-2-nitroethene (VIIa).—A solution of 20 g (0.122 mole) of VI[22] in 100 ml of $MeNO_2$ containing 6 g of ammonium acetate was heated on a steam bath for 2 hr with occasional stirring, then cooled overnight, and the dark precipitate collected. Two crystallizations (95% C_6H_5OH) produced 19 g (76%) of chunky gold crystals of VIIa: mp 112–113°; ir (KBr), 6.15 (conj C=C), 6.61 (NO_2), and 7.75 (conj NO_2) μ. *Anal.* ($C_{10}H_7NO_2S$) S.

1-(3-Benzo[*b*]thienyl)-2-nitropropene (VIIb).—Treatment of 20 g of VI in 100 ml of $EtNO_2$, as above, resulted in 17 g (63%) of yellow needles of VIIb, recrystallized from dioxane–H_2O; mp 110–111°; ir (KBr), 3.25 (Ar-CH), 6.09 (conj C=C), 6.70 (NO_2), and 7.60 (conj NO_2) μ. *Anal.* ($C_{11}H_9NO_2S$) C, H, S, N.

3-(β-Aminoethyl)benzo[*b*]thiophene (III).—In a separatory funnel with a pressure equalizer side arm, fitted with a condenser and drying tube and attached to a 2-l. flask fitted with a stirrer and containing 600 ml of dry ether and 15 g of $LiAlH_4$, was placed 20.1 g (0.1 mole) of VIIa. The ether was heated on the steam bath with stirring and the refluxing solvent was allowed to extract the slightly soluble nitro compound over a period of 8 hr. After allowing the reaction mixture to stand at room temperature overnight, the excess $LiAlH_4$ was decomposed by the dropwise addition of 200 ml of EtOAc followed by the dropwise addition of 200 ml of 2.5 *N* NaOH. The ether phase was separated and the solid residue was washed several times with ether. The combined ether phase was washed with salt water and dried (Na_2SO_4). Removal of the solvent and fractionation of the residue gave 9.9 g (56%) of an almost colorless liquid which distilled at 134–136° (1 mm); ir (liquid film), 2.88, 3.04 (NH_2), 3.30 (Ar-CH), 3.45, 3.54 (CH_2), 6.30 (N–H), 9.25 (C–N) μ. *Anal.* ($C_{10}H_{11}NS$) C, H, S.

Reduction of 9.55 g (0.05 mole) of the amide V with 6 g $LiAlH_4$ in ether, as above, produced 5.3 g (60%) of the identical amine. Reduction of the nitrile II gave only 38% in our hands, in agreement with Herz, who reported 30–35% of the amine by this reaction.[14e]

The hydrochloride of III precipitated from an ether solution and was recrystallized from absolute EtOH as white crystals: mp 220–221°; ir (KBr), 3.28–3.33 (NH_3^+), 6.29 (NH_3^+), 6.72 (CN_2) μ. *Anal.* ($C_{10}H_{12}ClNS$) C, H, N.

***dl*-3-(β-Aminopropyl)benzo[*b*]thiophene Hydrochloride (XII Hydrochloride).**—Using exactly the same procedure as described for the reduction of VIIa (above), 30 g (0.137 mole) of VIIb was reduced with 16 g of $LiAlH_4$. Removal of the solvent and fractionation of the residue gave 11.5 g (44%) of a colorless liquid which distilled at 150–155° (1 mm); ir (film), 2.90, 3.04 (N–H), 3.28 (Ar-CH), 3.40–3.51 (CH_2, CH_3), 6.04 (N–H) μ. The amine was characterized as its hydrochloride and was recrystallized from $CHCl_3$–hexane as white needles: mp 207–208°; ir (KBr), 3.25–3.55 (Ar-CH, CH_2, NH_3^+), 6.28, 7.65, 12.80 (NH_3^+) μ. *Anal.* ($C_{11}H_{14}ClNS$) C, H, N, S.

3-(β-Hydroxyethyl)benzo[*b*]thiophene (IX).—The procedure of Cagniant[14h] required 3-bromobenzo[*b*]thiophene (VIII) in quantity. Direct bromination of benzo[*b*]thiophene, as described by Szmuskovicz and Modest,[26] gave 76% of VIII as a pale yellow oil boiling at 92–93° (1 mm), and showing only one peak on glpc. The use of N-bromosuccinimide in $CHCl_3$ as described by Buu-Hoi and Lecocq,[28] produced 87% of the same material. A mixture of 71 g (0.5 mole) of MeI and 106.6 g (0.5 mole) of VIII in 350 ml of dry ether was added dropwise with stirring to 26 g (1.07 moles) of Mg turnings suspended in 500 ml of dry ether. After slight warming, the reaction started and the ether refluxed during the addition without additional heating. After the addition, the reaction mixture was stirred and allowed to reflux for 6 hr by which time most of the Mg had reacted. The mixture was cooled to 0° and 44 g (1.0 mole) of ethylene oxide dissolved in 100 ml of dry ether was cautiously added dropwise. A vigorous

(37) Melting points were measured on a Mel-Temp capillary melting point apparatus and are corrected. Infrared spectra were determined with a Perkin-Elmer Model 137 Infracord. The microanalyses were performed by Midwest Microlabs, Inc., Indianapolis, Ind. Where analyses are indicated only by symbols of the elements, analytical results obtained for these elements were within ±0.4% of the theoretical values.

(38) W. Wenner in "Organic Syntheses," Coll. Vol. IV, John Wiley and Sons, Inc., New York, N. Y., 1963, p 760.

reaction accompanied each drop. After the addition, the reaction mixture was allowed to warm to room temperature and stirred 1 hr, 400 ml of dry C_6H_6 was added, and the ether was removed by heating on a water bath. Ice and then 200 ml of 10% HCl was added to the suspended salt, the organic layer was separated, the residue was washed with two 100-ml portions of benzene, and the combined organic layer was washed (100 ml of salt water, two 100-ml portions of 5% Na_2CO_3, salt water). After drying (Na_2SO_4), the solvent was removed and the product distilled to give 50.5 g (57%) of a clear yellow liquid: bp 133–135° (0.5 mm); ir (film), 3.0 (bonded OH), 3.3 (Ar-CH), 3.45, 3.50 (CH_3), 9.6 (C–O) μ. *Anal.* ($C_{10}H_{10}OS$) S.

This compound was identical with that prepared in 42% yield by $LiAlH_4$ reduction of IV.[19]

3-(β-Bromoethyl)benzo[*b*]thiophene (X).—Following the procedure of Cagniant,[14b] 30 g (0.168 mole) of IX was treated with 16 g of PBr_3 in 200 ml of dry $CHCl_3$ containing 1 g pyridine. After standing overnight, the mixture was heated to 50° for 1 hr, cooled, and poured into 200 ml of cold H_2O. The $CHCl_3$ layer was separated, washed (H_2O, 10% HCl, twice with 10% Na_2CO_3, H_2O), and dried (Na_2SO_4). Distillation gave 25 g (65%) of pale yellow oil: bp 134–137° (1 mm); ir (film), 3.27 (Ar-CH), 3.40 (CH_2), 14.85 (C–Br) μ. The oil was used without further purification, as it rapidly became cloudy and decomposed.

3-(β-Aminoethyl)benzo[*b*]thiophenes (XI).—All of the derivatives listed in Table I, except XIII, were prepared as follows: 5 g (0.021 mole) of X was added to each of five batches of 200 ml of anhydrous MeOH. To each flask was added 50 g of a secondary amine, di-*n*-propylamine, pyrrolidine, piperidine, morpholine, and N-methylpiperazine, respectively. Upon the addition of these amines, each reaction mixture became warm. The homogeneous solutions were allowed to remain at room temperature for 15 days, then solvent and excess amines were removed by distillation, and the residue was treated with 50 ml of 10% NaOH and extracted with three 50-ml portions of ether. The extracts were dried (Na_2SO_4), solvent was removed, and the amine derivatives were distilled.

3-(β-Dimethylaminoethyl)benzo[*b*]thiophene Hydrochloride (XIII).—The Eschweiler-Clarke variant of the Leuckart reaction was used in this preparation.[21] III (5 g, 0.0282 mole), 20 ml of 37% CH_2O, 20 ml of 99% HCOOH, and 16 ml of H_2O were heated gently on the steam bath for 4 hr, then 30 ml of 6 *N* HCl was added to the cool reaction mixture and the solvent was distilled at reduced pressure. The tan semisolid that remained was dried at 78° (1 mm), for 16 hr, washed with dry ether, and recrystallized twice from absolute EtOH, to yield 4.5 g (66%) of white crystals: mp 260–261°; ir (KBr), 3.41 (CH_2, CH_3), 3.73 (N–CH_3), 4.0 (R_3NH^+) μ.

Irreversible Enzyme Inhibitors. CXXXII.[1,2] Proteolytic Enzymes. VI.[3] Tolerance for Polar Groups on the Phenoxyacetanilide Type of Inhibitor of α-Chymotrypsin

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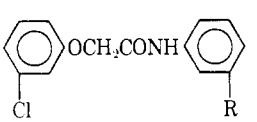
Candidate irreversible inhibitors derived from phenoxyacetanilide (**1**), such as N-[*m*-(*m*-fluorosulfonylphenylureido)phenyl]-3-chlorophenoxyacetamide (**3**), are too insoluble in water for enzymatic evaluation; therefore, a study was conducted on where to position polar groups on phenoxyacetanilide (**1**) that would not interfere with complex formation. Three useful classes of compounds emerged. The first class of compounds consisted of introduction of $RCOO^-$ or $CH_2NH_2^+$ groups on the N-phenyl moiety; this N-phenyl moiety is apparently complexed to a polar region of α-chymotrypsin since no binding was lost. The second class derived from **1** consisted of introduction of a COO^- group on the phenoxy moiety, which is complexed in a hydrophobic region. An *o*-COO^- group (**13**) was well tolerated in the complex, and inhibition could be further enhanced by introduction of a 4- or 5-chloro or 4-bromo atom. The third class consisted of a replacement of the phenoxymethyl moiety of **1** by a quaternized pyridylvinyl or pyridylethyl moiety; only N-methyl-2-pyridylacrylanilide (**28**) in this class was satisfactory, being complexed to the enzyme about one-third as well as **1**. The 2-carboxy-4-chlorophenoxy group of **19** was shown to be a suitable replacement for the 3-chlorophenoxy group of **3** in order to increase solubility; not only was **19** about 100 times as soluble as **3**, but irreversible inhibition was readily detected with **19** at 15% of its maximum solubility.

One of the goals in this laboratory has been the design and synthesis of active-site-directed irreversible inhibitors[5] of proteolytic enzymes[6] that operate by the exo mechanism, that is, the inhibitor forms a covalent bond outside of the active site of the enzyme.[7] α-Chymotrypsin is rapidly inactivated by the irreversible inhibitor **2**, and, in addition, α-chymotrypsin can catalytically hydrolyze the SO_2F group of **2** to the irreversible inert sulfonic acid;[8] neither reaction was seen between **2** and bovine serum albumin. A series of fifteen candidate irreversible inhibitors related to **2** were then synthesized which placed the SO_2F further from the CONH linkage of **2** which is believed to complex its CONH linkage to the catalytic part of the active site;[9] an example is **3**. Most of these compounds were too insoluble to be evaluated. Therefore a program was



1, R = H
2, R = SO_2F
3, R = $NHCONHC_6H_4SO_2F$-*m*

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(2) For the previous paper in this series see B. R. Baker and J. L. Kelley, *J. Med. Chem.*, **11**, 686 (1968).

(3) For the previous paper on proteolytic enzymes see B. R. Baker and E. H. Erickson, *ibid.*, **11**, 245 (1968).

(4) N.D.E.A. predoctoral fellow.

(5) B. R. Baker, "Design of Active-Site-Directed Irreversible Enzyme Inhibitors. The Organic Chemistry of the Enzyme Active-Site," John Wiley and Sons, Inc., New York, N. Y., 1967.

(6) For a discussion of the chemotherapeutic utility of selective irreversible inhibitors of serum proteases in the cardiovascular disease and organ transplantation area see B. R. Baker and E. H. Erickson, *J. Med. Chem.*, **10**, 1123 (1967).

(7) The exo type of irreversible inhibitor can have an extra dimension of specificity not present in reversible inhibitors, the bridge principle of specificity; see ref 5, Chapter IX, for a detailed discussion of this principle.

(8) B. R. Baker and J. A. Hurlbut, *J. Med. Chem.*, **11**, 233 (1968), paper CXIII of this series.

(9) B. R. Baker and J. A. Hurlbut, *ibid.*, **11**, 241 (1968), paper CXIV of this series.