Tetrahydrothiadiazoloisoquinolines: Synthesis and Inhibition of Phenylethanolamine-*N*-methyltransferase

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A series of 7,8-fused heterocyclic tetrahydroisoquinolines were synthesized and tested as inhibitors of rabbit adrenal phenylethanolamine-*N*-methyltransferase (PNMT). 6,7,8,9-Tetrahydro[1,2,3]thiadiazolo[5,4-*h*]isoquinoline **5** (SK&F 86607) was found to be a potent inhibitor of PNMT with an IC_{50} similar to that of 7,8-dichloro-1,2,3,4-tetrahydroisoquinoline (**1**, SK&F 64139). The isomeric tetrahydro[1,2,3]thiadiazolo[4,5-*h*]- and tetrahydro[1,2,5]thiadiazolo[3,4-*h*]isoquinolines, **13** and **20**, were also potent PNMT inhibitors. However, substitution of Cl at position 5 (**17**) resulted in loss of potency similar to the loss observed in the 5-chloro analogue of **1**. The 1,2,5 isomer **20** showed only a small drop in activity at 10^{-6} M. All of the thiadiazoles were more potent than the 7,8-benzo-fused analogue **36**. Fusion of other five-membered heterocyclic ring systems at the 7,8-position, e.g. triazole **22** and imidazoles **24** and **26**, resulted in a decrease of PNMT inhibition. The α-adrenoreceptor affinities of **1** and **5** were also compared.

Epinephrine, a hormone synthesized in the adrenal medulla, is released into the blood stream in response to stress and produces profound physiological changes which serve to prepare the animal to cope with the stressful situation. For example, epinephrine produces anxiety, an increase in blood pressure, acceleration of heart rate, and an increase in cardiac output. These changes are detrimental in individuals with certain disease conditions such as angina pectoris, myocardial infarction, and anxiety neuroses.

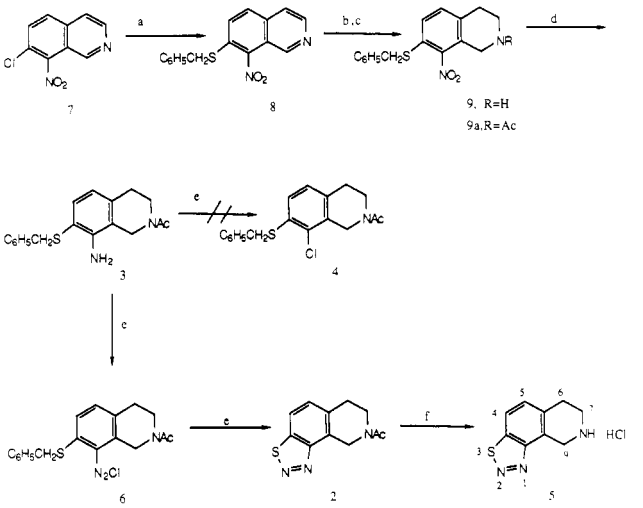
Phenylethanolamine-*N*-methyltransferase (PNMT) catalyzes the final step in the biosynthesis of epinephrine, i.e., the transfer of a methyl group from *S*-adenosylmethionine to norepinephrine (NE) to produce epinephrine (E). Inhibition of PNMT may reduce the formation of epinephrine and therefore be useful in situations where there is an overproduction of epinephrine or where epinephrine production is detrimental. Although an unambiguous definition of their functional significance remains to be determined,[1] such inhibitors might have potential therapeutic effects, especially in the treatment of cardiovascular disease.

In the course of a search for inhibitors of PNMT, we earlier identified tetrahydroisoquinolines (THIQ) as a class of compounds active against this enzyme. Among these, 7,8-dichlorotetrahydroisoquinoline (SK&F 64139, **1**) was found to be the most potent inhibitor of PNMT.[2,3] These compounds have also been the subject of quantitative structure activity relationship studies as inhibitors of PNMT.[4] In an effort to identify other congeners of **1** with increased potency, a novel tricyclic thiadiazole (**2**) (Scheme I) was obtained from diazotization of 2-acetyl-8-amino-7-(benzylthio)-1,2,3,4-tetrahydroisoquinoline (**3**) instead of the expected 8-chloro product **4**. Removal of the *N*-acetyl group gave the tetrahydrothiadiazoloisoquinoline **5** (Scheme I). The in vitro PNMT inhibitory activity of **5** (94% at 10^{-6} M) encouraged us to pursue the synthesis of a series of thiadiazoles and related tricyclic analogues as a new class of PNMT inhibitors[5] and to investigate the thiadiazole group as a 7,8-dichloro bioisostere. The results of this investigation are now described.

Chemistry

The synthesis of the lead structure **5** is outlined in Scheme I. This compound was encountered in an attempt to prepare 2-acetyl-8-chloro-7-(benzylthio)-1,2,3,4-tetrahydroisoquinoline (**4**) from its 8-amino congener by diazotization followed by treatment with cuprous chloride and concentrated HCl at 60 °C for several hours. Neutralization with aqueous ammonia and extraction with CH_2Cl_2 resulted in the isolation of only the intermediate diazonium salt **6**. Additional heating of **6** with CuCl and concentrated HCl gave a new component, which was assigned the thiadiazole structure **2** on the basis of its mass spectrum and ^1H NMR spectrum (see the Experimental Section), which showed loss of benzyl and retention of acetyl. Additional evidence in support of the assigned structure came from the hydrolysis of **2** in refluxing 10% HCl, which gave the amine **5**. Comparison of the NMR spectra of **2** and **5** suggests that the amide **2** exists in two different rotomeric forms. The C_9 protons in the ^1H NMR spectrum of

Scheme I[a]



[a] Procedures: (a) $C_6H_5CH_2SH$, KOH; (b) B_2H_6; (c) Ac_2O; (d) $Na_2S_2O_4$; (e) $NaNO_2$, HCl, CuCl, HOAc; (f) HCl.

† Department of Medicinal Chemistry.
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(5) Bondinell, W. E.; Girard, G. R. U.S. Patent 4,258,049, March 24, 1981.

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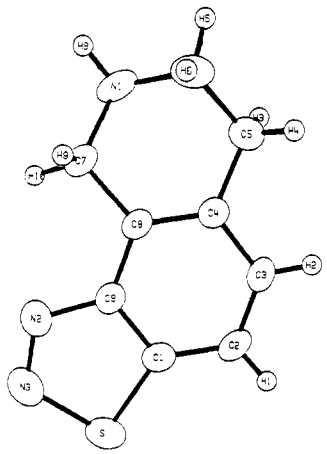
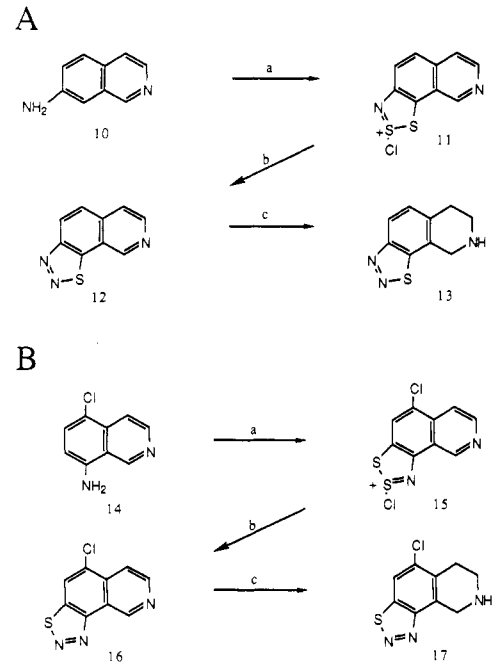


Figure 1.

Scheme II[a]

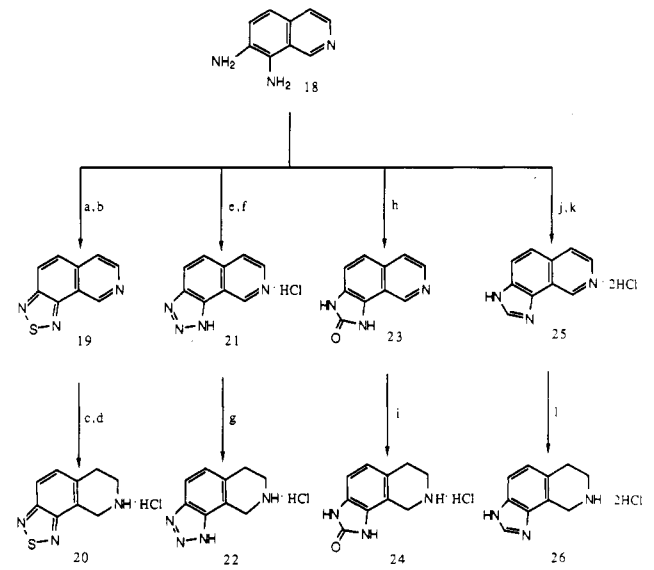


[a] Procedures: (a) S_2Cl_2, HOAc, (b) $NaNO_2$, 50% H_2SO_4; (c) $NaBH_3CN$.

2 appear as two singlets and the C_6 and C_7 protons are well-separated multiplets ($\Delta\delta$ = 0.85 ppm). In the ^1H NMR spectrum of **5** the C_9 protons are a singlet. The C_6 and C_7 proton multiplets are only separated by 0.2 ppm. The structure of **5** was confirmed by X-ray crystallography. The thiadiazole ring is a planar skewed five-membered ring attached to the aromatic portion of **5** while the piperidino ring is puckered into a twist-chair form with N_1 and the adjacent C_8 atoms on opposite sides of the C_7–C_8–C_4–C_5 plane (see Figure 1; the atom numbering is not according to Chemical Abstracts numbering system). The thiadiazole bond distances and angles are in good agreement with those found in similar thiadiazoles such as 1,2,3-benzothiadiazole.[6]

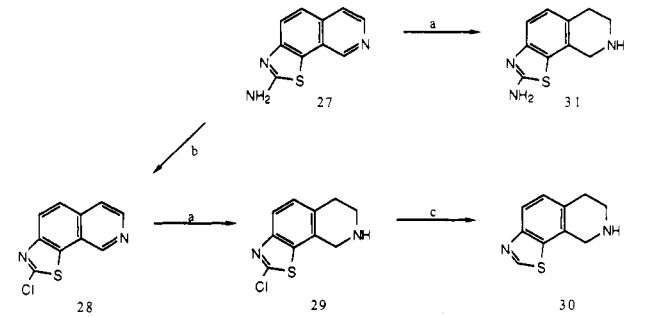
As shown in Scheme II (part A) the Hertz reaction[7] was utilized to prepare compound **13**, the regioisomer of **5**. Similarly, Scheme II (part B) outlines the synthesis of a chloro analogue of **5**. The aminoisoquinolines **10** and **14**

Scheme III[a]



[a] Procedures: (a) $SOCl_2$; (b) NH_4OH; (c) $NaBH_3CN$; (d) HCl; (e) $NaNO_2$, HOAc; (f) HCl; (g) H_2, PtO_2; (h) $(NH_2)_2CO$; (i) H_2, PtO_2, HCl; (j) HCOOH; (k) HCl; (l) H_2, Rh/C.

Scheme IV[a]



[a] Procedures: (a) $NaBH_3CN$; (b) HONO, CuCl; (d) P_4, 47% HI.

were treated with sulfur monochloride to obtain the Hertz compounds **11** and **15**, respectively, followed by nitrous acid treatment to give the thiadiazole isoquinolines **12** and **16**. Reduction of **12** and **16** with $NaBH_3CN$ gave **13** and **17**, respectively. Alternatively, **5** can also be prepared by this method with 8-aminoisoquinoline as the starting material.[5]

7,8-Diaminoisoquinoline (**18**) served as the starting material for compounds **20**, **22**, **24**, and **26** (Scheme III). Treatment of **18** with $SOCl_2$ gave the thiadiazoloisoquinoline **19**, which was reduced with $NaBH_3CN$ to give **20**. The triazole **22** was prepared by treatment[8] of **18** with nitrous acid followed by reduction of the resulting thiazoloisoquinoline **21**. Synthesis of the cyclic urea **24** was accomplished via condensation of **18** with urea[9] to obtain the 2-oxoimidazoloisoquinoline **23**, followed by catalytic reduction. The method of Lebenstedt and Schunack[10] was used to prepare compound **26**.

Preparation of **27** and **28** was previously described by Taurins and Kang-Chuan Hsia.[11] Reduction of **27** with $NaBH_3CN$ gave **31** (Scheme IV). The 2-chlorothiazole **28**

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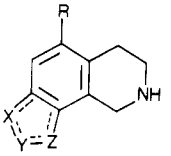
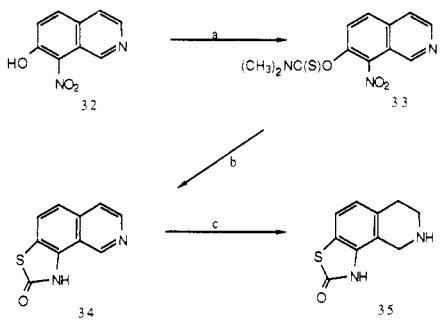
Table I. Tetrahydroisoquinolines



no.	R	X	Y	Z	mp, °C	% yield	recryst solv[a]	formula	in vitro PNMT inhibn 10^{-4} M	in vitro PNMT inhibn 10^{-6} M
1	H	Cl		Cl	225–227	97	AD	$C_9H_9Cl_2N$·HCl	99	96[c]
5	H	S	N	N	285–286	96	AD	$C_9H_9N_3S$·HCl	99	94[d]
13	H	N	N	S	288–290	42	CD	$C_9H_9N_3S$·HCl	99	91
17	Cl	S	N	N	310	44	CD	$C_9H_8ClN_3S$·HCl	98	51
20	H	N	S	N	330 dec	67	D	$C_9H_9N_3S$·HCl	99	79
22	H	N	N	NH	298	65	A	$C_9H_{10}N_4$·HCl	91	20
24	H	NH	C=O	NH	316–320 dec	23	A	$C_{10}H_{11}N_3O$·HCl	4	1
26	H	NH	CH	N	336–340 dec	30	AD	$C_{10}H_{11}N_3$·2HCl	45	0
30	H	N	CH	S	85–87	42	B	$C_{10}H_{10}N_2S$	92	24
31	H	N	CNH_2	S	244–246	15	A	$C_{10}H_{11}N_3S$	41	15
35	H	S	C=O	NH	272–274	38	A	$C_{10}H_{10}N_2OS$	37	3
36[b]	H	CH	CH=CH	CH	223–224			$C_{13}H_{13}N$	96	14

[a] A = MeOH, B = EtOAc, C = CH_2Cl_2, D = Et_2O. [b] Received from Prof. C. F. Koelsch. [c] $IC_{50} = 1 \times 10^{-8}$ M. [d] $IC_{50} = 3.3 \times 10^{-8}$ M.

Scheme V[a]



[a] Procedures: (a) $(CH_3)_2NC(S)Cl$, DABCO; (b) $SnCl_2 \cdot 2H_2O$, HCl, HOAc; (c) $NaBH_3CN$.

was reduced to **29**. Reduction of **29** with red phosphorus in 48% HI led to elimination of the 2-chlorine and formation of the double bond in **30**.

The *O*-aryl dimethylthiocarbamate **33** was prepared from **32** (Scheme V). Newman–Kwart rearrangement[12] under reductive conditions gave the 2-oxothiazole **34**, which was reduced to **35**. Target compounds were converted to their hydrochloride salts and tested for their PNMT inhibitory activity.

Results and Discussion

The results of testing tetrahydroisoquinolines **1**, **5**, **13**, **17**, **20**, **22**, **24**, **26**, **30**, **31**, **35**, and **36** at 10^{-4} and 10^{-6} M in an in vitro test for inhibition of rabbit adrenal PNMT catalyzed conversion of NE to E[13] are tabulated in Table I. The thiadiazole **5** and its isomer **13** had inhibitory activity against PNMT comparable to the potent PNMT inhibitor **1**. The IC_{50} value of **5** was similar to that of **1** (see footnotes *c* and *d*, Table I). Substitution of chlorine at position 5 of the THIQ ring in **5** resulted in a loss of potency. Similar results[2,3] were obtained for **1** and its 5-chloro analogue, suggesting that these inhibitors bind to PNMT with similar orientations. Placement of S between N in compound **20** gave comparable activity to **1** at 10^{-4} M, but it was reduced at 10^{-6} M. All of the thiadiazoles were potent PNMT inhibitors at 10^{-4} M, while two of them, **17** and **20**, had reduced activity at 10^{-6} M. These results suggest that the thiadiazole ring system may be useful as a 7,8-dichloro bioisostere.

Table II. K_i Values and in Vivo PNMT Inhibition Data for Compounds **1** and **5**

no.	K_i, nM[a]	% inhibn of [^{3}H]E biosynthesis, 3 mg/kg po, t.i.d., rats[b]	determined from decrease in adrenal E/NE ratio, mg/kg, po, b.i.d., mice[c] MAD[d]	MID[e]
1	3	50	5.0	2.5
5	8	87	12.5	6.25

[a–c] See ref 3. [d] Minimum active dose ($p < 0.05$, by Student's *t* test). [e] Maximum inactive dose (not significantly different from control, by Student's *t* test).

Substitution of the thiadiazole ring with other ring systems resulted in loss of potency. Replacement of the S in **5** or **13** with N to give triazole **22** or with CH in **13** to give thiazole **30** resulted in loss of activity at 10^{-6} M. Replacement of the thiadiazole ring with imidazoles in compounds **24** and **26** and with 2-substituted thiazoles, compounds **31** and **35**, resulted in no activity at either concentration.

Vicinal disubstitution with substituents other than chlorine could potentially give compounds having PNMT activity similar to **1** for either steric or electronic reasons. One compound considered was the 7,8-benzo-fused analogue **36**, which because of its aromatic nature might also mimic the thiadiazole ring system of **5**. Compound **36** had 96% inhibition at 10^{-4} M but was inactive at 10^{-6} M. This lack of potency may be due to electronic effects rather than steric effects since both rings can be viewed as being approximately the same size.

Because of its in vitro potency, compound **5** was tested in vivo and found to inhibit PNMT following oral administration in two different tests (Table II). These tests measure the inhibition of adrenal [^{3}H]epinephrine biosynthesis and decreases in the endogenous adrenal epinephrine/norepinephrine ratio, respectively, and have been previously described.[3] The inhibition of [^{3}H]epinephrine biosynthesis test measures the ability of a compound to inhibit conversion of [^{3}H]NE to [^{3}H]E in the adrenal medulla of rats. In this assay, **5** had greater potency than

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1. In the adrenal epinephrine/norepinephrine ratio test, the content of E and NE in mouse adrenal glands is determined after chronic oral administration of the test compound. A significant decrease ($p < 0.05$, by Student's t test) in the E/NE ratio is indicative of PNMT inhibition. Compound 1 was 2.5 times more potent than compound 5.

Since α-adrenoreceptors, like PNMT, bind NE, the interaction of 1 and 5 on these receptors were compared.[14] Compound 1 is an antagonist at α_1- and α_2-adrenoceptors with $K_B = 6$ and 0.3 μM, respectively. Compound 5 was also an antagonist with $K_B = 10$ and 0.2 μM, respectively. Replacement of the dichloro substituents of 1 with the thiadiazole moiety (5) had a small effect on α_2 potency and decreased α_1 potency.

Experimental Section

Melting points were determined in open capillary tubes using a Thomas-Hoover Uni-Melt apparatus and are uncorrected. Elemental analyses were performed by the the Analytical and Physical Chemistry Section of Smith Kline & French Laboratories. Where analyses are reported by symbols of elements, results were within ±0.4% of calculated value. Mass spectra were obtained on a Hitachi Perkin-Elmer RMU-6E spectrometer. NMR spectra were recorded with either a Perkin-Elmer R-24 or R-32 spectrometer (Me_4Si), and all J values are in hertz. IR and NMR spectral data were obtained for all numbered or named compounds and were judged to be consistent with the assigned structures. X-ray structure determination of compound 5 was carried out by Molecular Structure Corp., College Station, TX.

7-(Benzylthio)-8-nitroisoquinoline (8). A mixture of 0.52 g (0.0025 mol) of 7[15] and 0.317 g (0.00256 mol) of benzyl mercaptan in 5 mL of degassed 2-propanol under argon at 0 °C was treated with 0.16 g (0.0025 mol) of 86% KOH in 2 mL of EtOH dropwise over 15 min. The mixture was stirred for 1 h at 25 °C and filtered. The collected product was washed with H_2O and EtOH and then dried, affording 0.49 g (69%) of 8: mp 151–153 °C.

7-(Benzylthio)-8-nitro-1,2,3,4-tetrahydroisoquinoline Hydrochloride (9). A solution of 15 g (0.051 mol) of 8 in 100 mL of THF was added to 210 mL of 1 M BH_3–THF (0.21 mol). The mixture was stirred and refluxed for 5 h. MeOH was added and the solvent was evaporated in vacuo. The residue was treated with refluxing 12 N HCl for 1.5 h and then evaporated to dryness. The resulting solid was recrystallized from MeOH–Et_2O to give 13.5 g (80%) of 9: mp 253 °C dec.

2-Acetyl-7-(benzylthio)-8-nitro-1,2,3,4-tetrahydroisoquinoline (9a). A mixture of 17.5 g (0.052 mol) of 9, 15 mL of Ac_2O, and 4.5 g (0.055 mol) of NaOAc in 150 mL of HOAc was heated on a steam bath for 1 h and then evaporated. H_2O was added to the residue, followed by NH_4OH until the mixture became basic. The mixture was extracted with CH_2Cl_2. The extracts were combined, washed with H_2O, 10% HCl, and 5% $NaHCO_3$, dried (Na_2SO_4), and concentrated. Chromatography of the residue on silica (EtOAc) afforded 10.5 g (59%) of 9a: mp 94–95 °C.

2-Acetyl-8-amino-7-(benzylthio)-1,2,3,4-tetrahydroisoquinoline (3). A solution of 1.2 g (0.0035 mol) of 9a in 15 mL of EtOH and 10 mL of H_2O was treated with 4.2 g (0.23 mol) of $Na_2S_2O_4$. The mixture was refluxed for 3 h and then made basic with NH_4OH and extracted with CH_2Cl_2. The organic extracts were combined, washed with H_2O, dried (Na_2SO_4), and concentrated to give 0.93 g (85%) of 3.

8-Acetyl-6,7,8,9-tetrahydro[1,2,3]thiadiazolo[5,4-*h*]isoquinoline (2). To a solution of 1.5 g (0.0048 mol) of 3 in 15 mL of 12 N HCl and 6 mL of HOAc at −10 °C under argon was added 0.52 g (0.0075 mol) of $NaNO_2$ in 3 mL of H_2O. The mixture was stirred for 5 min and then added rapidly to 2.3 g of CuCl in 20 mL of 12 N HCl and heated to 60 °C for 3 h. The mixture was poured over ice, made basic with NH_4OH, and extracted with CH_2Cl_2. The CH_2Cl_2 extracts were combined, washed with H_2O, dried, and concentrated to give the corresponding diazonium salt 6: NMR ($CDCl_3$) δ 7.73 (1 H, d, J = 9, 6CH), 7.23 (1 H, d, 5CH), 7.13 (5 H, s, C_6H_5), 5.25 (2 H, 2s, $1CH_2$), 4.43 (2 H, s, $C_6H_5CH_2$), 3.78 (2 H, m, $4CH_2$), 2.92 (2 H, m, $3CH_2$), 2.19 (3 H, s, $COCH_3$).

The diazonium salt was heated with 2.2 g of CuCl and 40 mL of 12 N HCl for 5 h at 60 °C, followed by workup as above to yield 0.92 g (82%) of 2: mp 133–136 °C; NMR ($CDCl_3$) δ 7.85 (1 H, d, J = 8, 4CH), 7.4 (1 H, d, J = 8, 5CH), 5.4 (2 H, 2s, $9CH_2$), 3.92 (2 H, m, $6CH_2$), 3.07 (2 H, m, $7CH_2$), 2.28 (3 H, s, $COCH_3$); mass spectrum, m/e 233 (M^+). Anal. ($C_{11}H_{11}N_3OS$) C, H, N.

6,7,8,9-Tetrahydro[1,2,3]thiadiazolo[5,4-*h*]isoquinoline Hydrochloride (5). A mixture of 0.15 g (0.0006 mol) of 2 and 5 mL of 10% HCl was refluxed for 3 h and the solvent was evaporated. The residue was dissolved in H_2O and extracted with CH_2Cl_2 (discarded) and the aqueous phase was made basic with NH_4OH and extracted with CH_2Cl_2. The CH_2Cl_2 extracts were combined, dried, and evaporated to give 5: NMR ($CDCl_3$) δ 7.58 (1 H, d, J = 8 , 4CH), 7.16 (1 H, d, J = 8, 5CH), 4.66 (2 H, s, $9CH_2$), 3.15 (2 H, m, 6CH), 2.95 (2 H, m, $7CH_2$), 2.07 (3 H, s, $COCH_3$). The residue was dissolved in MeOH, acidified with HCl–Et_2O followed by dilution with Et_2O. The precipitate was recrystallized from MeOH–Et_2O to give 0.14 g (96%) of 5 as its hydrochloride: mp 285–286 °C. Anal. ($C_9H_9N_3S$·HCl) C, H, N.

[1,2,3]Dithiazolo[4,5-*h*]isoquinolin-2-ium Chloride Hydrochloride (11). A solution of 2.0 g (0.014 mol) of 10 in 50 mL of HOAc was added to 80 mL of cold sulfur monochloride and stirred for 24 h. The mixture was filtered and the orange solid was washed with Et_2O and dried in vacuo to give 3.5 g (92%) of 11: mp 227–237 °C. Anal. ($C_9H_5ClN_2S_2$·HCl·$^1/_8H_2O$) C, H, N.

[1,2,3]Thiadiazolo[4,5-*h*]isoquinoline (12). A solution of 3.5 g (0.0126 mol) of 11 in 73 mL of 50% aqueous H_2SO_4 was cooled to 0 °C and treated with a solution of 1.4 g (0.020 mol) of $NaNO_2$ in 10 mL of H_2O. The mixture was stirred at 0 °C for 2 h, poured into ice–H_2O, treated with charcoal, filtered, made basic with NH_4OH, and extracted with Et_2O. The combined Et_2O extracts were dried, treated with charcoal, filtered, and concentrated to yield 1.0 g (42%) of 12: mp 150–153 °C. Anal. ($C_9H_5N_3S$) C, H, N.

6,7,8,9-Tetrahydro[1,2,3]thiadiazolo[4,5-*h*]isoquinoline Hydrochloride (13). A solution of 0.5 g (0.003 mol) of 12 in 50 mL of MeOH was treated with 1 g (0.016 mol) of $NaBH_3CN$ and the resulting solution was stirred for 24 h. The pH was maintained at 4 by the addition of MeOH–HCl. The mixture was then treated with excess MeOH–HCl and concentrated on a steam bath. The residue was dissolved in H_2O, treated with charcoal, filtered, and made basic with NH_4OH. The basic solution was extracted with Et_2O, and the combined extracts were dried, treated with charcoal, and filtered. Treatment with HCl–Et_2O gave 0.31 g (51%) of 13: mp 284.5 °C. Anal. ($C_9H_9N_3S$·HCl) C, H, N.

5-Chloro[1,2,3]dithiazolo[5,4-*h*]isoquinolin-2-ium Chloride Hydrochloride (15). To 93 mL of cold sulfur monochloride was added a solution of 4.3 g (0.0298 mol) of 14 in 58 mL of HOAc dropwise with stirring. After 18 h at room temperature, the mixture was filtered and the resulting dark brown solid was washed with CH_2Cl_2 and dried, yielding 9.3 g (~100%) of 15: mp 220 °C.

5-Chloro[1,2,3]thiadiazolo[5,4-*h*]isoquinoline (16). To a solution of 3.1 g (0.01 mol) of 15 in 90 mL of 50% H_2SO_4 at 0 °C was added 1.12 g (0.0163 mol) of $NaNO_2$ in 20 mL of H_2O. The mixture was stirred for 2 h at 0 °C, poured into ice–H_2O, and then filtered. The aqueous solution was treated with charcoal, filtered, made basic with NH_4OH, and then extracted with EtOAc. The extracts were combined, dried ($MgSO_4$), decolorized, filtered, and concentrated to give 16 as a dark yellow solid weighing 0.4 g (20%): mp 225–227 °C. Anal. ($C_9H_4ClN_3S$) C, H, N.

5-Chloro-6,7,8,9-tetrahydro[1,2,3]thiadiazolo[5,4-*h*]isoquinoline (17). A solution of 0.5 g (0.0024 mol) of 16 in 50 mL of HOAc was treated with 0.5 g of $NaBH_3CN$. The mixture was stirred for 2 h at room temperature, poured over ice, made basic with 40% NaOH, and extracted with CH_2Cl_2. The combined CH_2Cl_2 extracts were washed with H_2O, dried ($MgSO_4$), and concentrated. The residue was dissolved in a minimum amount of CH_2Cl_2, acidified with HCl–Et_2O, and diluted with Et_2O to obtain 17 as a white solid weighing 0.28 g (44%): mp 310 °C. Anal. ($C_9H_8ClN_3S$·HCl) C, H, N.

7,8-Diaminoisoquinoline (18). A mixture of 5 g (0.264 mol) of 7-amino-8-nitroisoquinoline[10] and 0.9 g of 10% Pd|C in 150

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mL of 2 N HCl was hydrogenated on a Parr shaker at 60 psi until H_2 uptake had ceased. The mixture was degassed, filtered, and concentrated. The residue was dissolved in H_2O and made basic with NH_4OH. The precipitated product was collected by filtration, washed with H_2O, and dried to give 3.0 g (71%) of **18**: mp 157–160 °C. **18** was used without further purification.

[1,2,5]Thiadiazolo[3,4-*h*]isoquinoline (19). To 10 mL of $SOCl_2$ at 0 °C was added 0.2 g (0.001 26 mol) of **18**. The mixture was stirred at 0 °C until solution began to occur and then warmed to room temperature. Stirring was continued until a dark red solution was obtained. The solution was concentrated in vacuo and the solid residue was dissolved in H_2O. Insoluble material was removed and the filtrate was treated with charcoal, filtered, and made alkaline with concentrated NH_4OH. The white precipitate was collected and dissolved in a large volume of Et_2O. The Et_2O was treated with charcoal, filtered, and concentrated to give 0.07 g (30%) of **19**, mp 105 °C. Anal. ($C_9H_5N_3S$) C, H, N.

6,7,8,9-Tetrahydro[1,2,5]thiadiazolo[3,4-*h*]isoquinoline Hydrochloride (20). A solution of 0.7 g (0.003 74 mol) of **19** in 50 mL of MeOH was stirred with 1.4 g (0.0224 mol) of $NaBH_3CN$. The pH was maintained at 4 by the addition of HCl–MeOH. After 24 h, the mixture was treated with excess HCl–MeOH and concentrated in vacuo. The residue was dissolved in H_2O, treated with charcoal, filtered, and made basic with NH_4OH. The basic solution was extracted with Et_2O and the combined extracts were dried ($MgSO_4$) and filtered and then treated with excess HCl–Et_2O to give 0.48 g (67%) of **20** as a white crystalline product, mp 330 °C. Anal. ($C_9H_9N_3S$·HCl) C, H, N.

[1,2,3]Triazolo[4,5-*h*]isoquinoline Hydrochloride (21). A solution of 1.59 g (0.01 mol) of **18** in 1.2 mL of HOAc and 3 mL of H_2O was cooled to 5 °C and treated with 0.75 g (0.011 mol) of $NaNO_2$ in 1.2 mL of H_2O. The mixture was stirred at 5 °C for 20 min and then at room temperature for 1 h and then concentrated in vacuo. The residue was treated with 20 mL of H_2O and an insoluble solid was collected, dissolved in 30 mL of MeOH, treated with charcoal, and filtered, and the filtrate was treated with HCl–Et_2O. The white salt was collected and dried to give 0.9 g (43%) of **21**: mp 290 °C. Anal. ($C_9H_6N_4$·HCl) C, H, N.

6,7,8,9-Tetrahydro[1,2,3]triazolo[4,5-*h*]isoquinoline Hydrochloride (22). A solution of 0.5 g (0.0024 mol) of **21** and 0.1 g of PtO_2 in 50 mL of MeOH was hydrogenated on a Parr shaker at 60 psi until H_2 uptake had ceased. The mixture was degassed, filtered, and concentrated. The solid residue was recrystallized from MeOH to give 0.33 g (65%) of **22**: mp 298 °C. Anal. ($C_9H_{10}N_4$·HCl) C, H, N.

2,3-Dihydro-2-oxoimidazo[2,5-*h*]isoquinoline Hydrochloride (23). A mixture of 0.5 g (0.003 14 mol) of **18** and 0.58 g (0.009 66 mol) of urea was heated under N_2 at 170–180 °C for 3 h. The mixture was treated with hot H_2O and an insoluble brown solid was collected and dissolved in hot MeOH and filtered. The filtrate was treated with charcoal, dried ($MgSO_4$), and then partly concentrated to give 0.11 g (19%) of **23** as a yellow solid: mp >340 °C. Anal. ($C_{10}H_7N_3O$·$^1/_4H_2O$) C, H, N.

2,3-Dihydro-2-oxo-6,7,8,9-tetrahydroimidazo[4,5-*h*]isoquinoline Hydrochloride (24). A solution of 0.5 g (0.002 26 mol) of the hydrochloride of **23** and 0.1 g of PtO_2 in 50 mL of MeOH was hydrogenated on a Parr shaker at 60 psi until H_2 uptake ceased. The mixture was degassed, filtered, and concentrated. The solid residue was recrystallized from MeOH to give 0.12 g (23%) of the hydrochloride **24**: mp 316–320 °C. Anal. ($C_{10}H_{11}N_3O$·HCl·$^1/_4H_2O$) C, H, N.

Imidazo[4,5-*h*]isoquinoline Dihydrochloride (25). A mixture of 4.9 g (0.03 mol) of **18** and 15 mL of HCOOH was stirred and refluxed for 1 h. Excess HCOOH was removed in vacuo and the solid residue was dissolved in MeOH, acidified with gaseous HCl, and precipitated with Et_2O. The resulting dihydrochloride was recrystallized from MeOH–Et_2O to give 4.5 g (63%) of **25**: mp 325–327 °C. Anal. ($C_{10}H_9Cl_2N_3$) C, H; N: calcd, 17.36; found, 17.82.

6,7,8,9-Tetrahydroimidazo[4,5-*h*]isoquinoline Dihydrochloride (26). A mixture of 2 g (0.0083 mol) of **25** and 1 g of Rh/C in 20 mL of 2 N HCl was shaken with H_2 on a Parr shaker at 60 psi until H_2 uptake ceased. The catalyst was removed by filtration and washed with H_2O. The filtrate was concentrated to dryness and the residue was recrystallized from MeOH–Et_2O to give 0.7 g (30%) of **26**: mp 336–340 °C. Anal. ($C_{10}H_{11}N_3$·2HCl) C, H, N.

2-Chloro-1,2,3,4-tetrahydrothiazolo[4,5-*h*]isoquinoline (29). A solution of 3.2 g (0.015 mol) of **28** in MeOH was reduced with 4.5 g (0.073 mol) of $NaBH_3CN$ as described above. Workup afforded 2.3 g (70%) of **29**: mp >300 °C. Anal. ($C_{10}H_9ClN_2S$) C, H, N.

1,2,3,4-Tetrahydrothiazolo[4,5-*h*]isoquinoline (30). A mixture of 2.3 g (0.0102 mol) of **29**, 2.0 g (0.0162 mol) of red phosphorus, 60 mL of 47% HI, and 26 mL of HOAc was heated with stirring for 3 h and cooled and the precipitated solid was collected and dissolved in H_2O. The aqueous solution was made basic with NH_4OH and extracted with EtOAc. The EtOAc extracts were dried ($MgSO_4$), decolorized, filtered, and concentrated to give 0.8 g (42%) of **30**: mp 85–87 °C. Anal. ($C_{10}H_{10}N_2S$·$^1/_8H_2O$) C, H, N.

2-Amino-1,2,3,4-tetrahydrothiazolo[4,5-*h*]isoquinoline (31). A solution of 0.5 g (0.0025 mol) of **27** in 150 mL of MeOH was reduced with 0.6 g (0.0095 mol) of $NaBH_3CN$ as previously described. Workup afforded 0.08 g (15%) of **31** as a white solid: mp 244–246 °C. Anal. ($C_{10}H_{11}N_3S$) C, H, N.

Dimethylthiocarbamic Acid, *O*-(8-Nitro-7-isoquinolyl) Ester (33). A mixture of 5 g (0.02 mol) of **32**,[10] 3.5 g (0.028 mol) of dimethylthiocarbamoyl chloride, and 11 g (0.098 mol) of DABCO in 100 mL of DMF was stirred at room temperature for 24 h and then poured into ice–water (200 mL). The precipitated yellow solid was collected, washed with H_2O, and then dissolved in CH_2Cl_2, dried ($MgSO_4$), and concentrated to give 5 g (90%) of **33** as a yellow solid: mp 143–145 °C. Anal. ($C_{12}H_{11}N_3O_3S$) C, H, N.

2-Oxothiazolo[5,4-*h*]isoquinoline (34). A solution of 1.3 g (0.0047 mol) of **33** in 30 mL of HOAc was treated with 10.6 g (0.047 mol) of stannous chloride dihydrate in 10 mL of 12 N HCl. The mixture was heated on a steam bath for 0.5 h. The precipitated yellow solid was separated, treated with 5% Na_2CO_3, and filtered. The mixture was neutralized with 12 N HCl and the solid was collected and then washed with H_2O and acetone to obtain 0.9 g (95%) of **34**: mp 304–306 °C. Anal. ($C_{10}H_6N_2OS$) C, H, N.

2-Oxo-6,7,8,9-tetrahydrothiazolo[5,4-*h*]isoquinoline (35). A solution of 0.5 g (0.0025 mol) of **34** in 50 mL of MeOH was treated with 1.6 g (0.025 mol) of $NaBH_3CN$, keeping the pH at 4. After 24 h at room temperature, the mixture was treated with excess HCl–MeOH and concentrated. H_2O was added to the residue, and insolubles were removed by filtration. The filtrate was adjusted to pH 8 with NH_4OH, precipitating a yellow solid, which was recrystallized from MeOH to give 0.2 g (38%) of **35**: mp 272–274 °C. Anal. ($C_{10}H_{10}N_2OS$) C, H, N.

Pharmacology. In Vitro PNMT Inhibition Test Primary Assay. This test was performed as previously described.[13,16] Lyophilized, partially purified rabbit adrenal PNMT was obtained commercially from Gallard-Schlesinger Co., where it was prepared by previously described methods.[17] The enzyme was solubilized in potassium phosphate buffer and the reaction was run in 300 μL constituted as follows: PNMT, 280 μg; phosphate buffer (pH 7.4), 50 nmol; (−)NE, 9 nmol; and [*methyl*-^{14}C]SAM (ca. 20 000 dpm), 9 nmol. The reaction was run for 15 or 30 min at 37 °C and then it was terminated by the addition of 1 N HCl (200 μL). Approximately 1 g of solid NaCl was then added and the solution was extracted with 6 mL of acid-washed NaCl-saturated butanol. One milliliter of the butanol layer, containing labeled E, was then added to 10 mL of an aqueous 2,5-bis(5′-*tert*-butyl-2′-benzoxazolyl)thiophene (BBOT) phosphor, counted in a Tricarb liquid scintillation spectrometer for 10 min, and quantitated in terms of nanomoles of E produced. Inhibition values were obtained by comparing the decreased E production in samples containing concentrations of 10^{-4} and 10^{-6} M test compound with controls and are the average of four determinations.

Acknowledgment. We express appreciation to our colleagues at Smith Kline & French Laboratories for contributions to this work: E. A. Reich (elemental anal-

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ysis), G. D. Roberts and S. Rottschaefer (mass spectra), P. J. Hieble (biological data), and C. Kaiser for helpful discussions.

Registry No. 1, 57987-77-6; 1 (free base), 61563-24-4; 2, 78104-37-7; 3, 78104-35-5; 5, 83903-85-9; 5 (free base), 78104-49-1; 6, 78104-36-6; 7, 78104-31-1; 8, 78104-32-2; 9, 78104-33-3; 9a, 78104-34-4; 10, 23707-37-1; 11, 120546-57-8; 12, 78104-40-2; 13, 78234-22-7; 13 (free base), 78104-50-4; 14, 55766-82-0; 15, 120546-58-9; 16, 78104-43-5; 17, 120546-59-0; 17 (free base), 120546-72-7; 18, 120546-60-3; 19, 109909-64-0; 20, 120546-61-4; 20 (free base), 109544-44-7; 21, 120546-62-5; 22, 120546-63-6; 22 (free base), 120546-73-8; 23, 120546-64-7; 24, 120546-65-8; 24 (free base), 120546-74-9; 25, 56623-96-2; 26, 56623-97-3; 26 (free base), 56623-99-5; 27, 35317-80-7; 28, 35317-81-8; 29, 120546-66-9; 30, 120546-67-0; 31, 120546-68-1; 32, 56623-93-9; 33, 120546-69-2; 34, 120546-70-5; 35, 120546-71-6; 36, 109220-61-3; PNMT, 9037-68-7; benzyl mercaptan, 100-53-8; 7-amino-8-nitroisoquinoline, 56623-94-0; dimethylthiocarbamoyl chloride, 16420-13-6.

Supplementary Material Available: Tables of positional and thermal parameters, general temperature factor expressions, B's, bond distances, bond angles, and torsional angles (5 pages). Ordering information is given on any current masthead page.

Synthesis of the Novel π-(Benzyloxymethyl)-Protected Histidine Analogue of Statine. Inhibition of Penicillopepsin by Pepstatin-Derived Peptides Containing Different Statine Side-Chain Derivatives[1]

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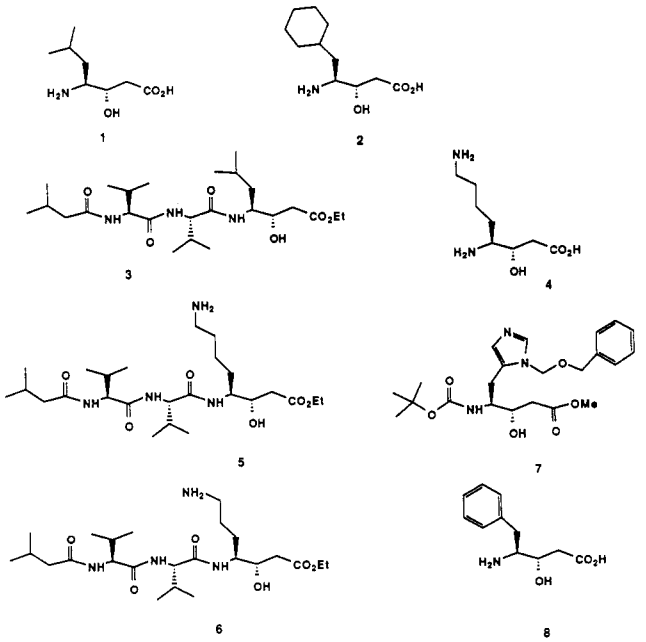
Received August 8, 1988

The synthesis of aspartic proteinase inhibitors derived from a new histidine side-chain analogue of statine (Sta), (3*S*,4*S*)-4-amino-3-hydroxy-5-(imidazol-4-yl)pentanoic acid (HiSta, **20**), is reported. Boc-HiSta(BOM)-OMe (**7**) was prepared in 16% overall yield from Boc-His(π-BOM)-OH via formation of the tetramic acid derivative **11** and stereoselective cis reduction with $NaBH_4$ to the 4-hydroxy lactam **12**. Removal of the Boc group from ester **7** (enantiomeric purity ee = 88–90%) and coupling to the tripeptide segment Iva-Val-Val-OH (**13**) by the DCC/HOBt preactivation method followed by hydrogenolytic removal of the π-BOM group over $Pd(OH)_2$ on carbon gave Iva-Val-Val-HiSta-OMe (**16**). This new peptide **16** is a very potent inhibitor of the fungal aspartic proteinase penicillopepsin ($K_i = 4.5 \times 10^{-9}$ M) that is 10 times more active than the comparable Sta-containing inhibitor **3** and 2–3 times more potent than the new (3*S*,4*S*)-4-amino-3-hydroxy-5-phenylpentanoic acid (AHPPA) analogue **17** ($K_i = 1.5 \times 10^{-8}$ M). However, compound **16**, which has an imidazole residue at the P_1 position, is a significantly weaker inhibitor of the enzyme than the corresponding analogues with the lysine (**5**) and ornithine (**6**) side chains at P_1. Considerations that led to the synthesis of **16** and the results of the enzyme kinetics are discussed in detail.

In recent years, numerous analogues of the natural product inhibitor pepstatin[2] and substrate-derived peptides containing statine [(3*S*,4*S*)-4-amino-3-hydroxy-6-methylheptanoic acid (Sta, **1**)] have been developed as



potent inhibitors of pepsin,[3–7] penicillopepsin,[6,8] renin,[9,10] and other fungal and mammalian aspartic proteinases.[8,11,12] X-ray crystal structures of statine-containing inhibitors bound to penicillopepsin,[13,14] *Rhizopus chinensis* pepsin,[15]

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(1) Abbreviations used follow the IUPAC–IUB commission on Biochemical Nomenclature recommendations. Additional abbreviations are as follows: Boc, *tert*-butyloxycarbonyl; π-BOM, π-(benzyloxymethyl); DCC, dicyclohexylcarbodiimide; HOBt, 1-hydroxybenzotriazole; Iva, isovaleryl; Nph, 4′-nitrophenylalanine; ACHPA, (3*S*,4*S*)-4-amino-3-hydroxy-5-cyclohexylpentanoic acid; AHPPA, (3*S*,4*S*)-4-amino-3-hydroxy-5-phenylpentanoic acid; HiSta, (3*S*,4*S*)-4-amino-3-hydroxy-5-(imidazol-4-yl)pentanoic acid; LySta, (3*S*,4*S*)-4,8-diamino-3-hydroxyoctanoic acid; OrnSta, (3*S*,4*S*)-4,7-diamino-3-hydroxyheptanoic acid; Sta, (3*S*,4*S*)-4-amino-3-hydroxy-6-methylheptanoic acid.

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0022-2623/89/1832-1571$01.50/0