under reflux for 36 h. The water formed was azeotropically removed in a Dean–Stark separator. The reaction mixture was allowed to cool to room temperature. The product thus obtained was filtered and recrystallized from ethanol, and then from benzene to give **28** in 57% yield: mp 195–196 °C; IR (KBr) 3160, 3100, 3040, 2960, 2910, 2880, 1710, 1610, 1470, 1440, 1280, 1230, 1185, 1120, 1040 cm^{-1}. Anal. ($C_{10}H_8BrNOS_2$) C, H.

1′-(Piperidinomethyl)spiro[1,3-dithiolane-2,3′-indolin]-2′-one (29). Following the procedure as described for **13** but with **27** (1.12 g, 0.005 mol), piperidine (0.43 g, 0.005 mol), and 37% formaldehyde (0.45 g) gave, after recrystallization from absolute ethanol, 78% of **29**: mp 145–146 °C; IR (KBr) 2960, 2875, 2810, 1715, 1600, 1480, 1465, 1345, 1300, 1240, 1165, 1120, 1040 cm^{-1}. Anal. ($C_{16}H_{20}N_2OS_2$) C, H.

1′-(Morpholinomethyl)spiro[1,3-dithiolane-2,3′-indolin]-2′-one (30). Following the procedure as described for **13** but with **27** (1.12 g, 0.005 mol), morpholine (0.45 g, 0.005 mol), and 37% formaldehyde (0.45 g) gave, after recrystallization from ethanol, 81% of **30**: mp 143–144 °C; IR (KBr) 3000, 2960, 2880, 2845, 1720, 1605, 1480, 1460, 1420, 1340, 1305, 1290, 1260, 1230, 1155, 1120, 1000, 860 cm^{-1}. Anal. ($C_{15}H_{18}N_2O_2S_2$) C, H.

3,3-Dimethoxyindolin-2-one (31) was prepared as reported. Compound **31** had mp 92–93 °C (lit.[10] mp 94 °C).

Spiro[1,3-dioxane-2,3′-indolin]-2′-one (32). A mixture of 1 (2.94 g, 0.02 mol), 1,3-propanediol (3.04 g, 0.04 mol), and *p*-toluenesulfonic acid (0.02 g) in 80 mL of toluene was heated under reflux for 13 h. The water formed was azeotropically removed in a Dean–Stark separator. The reaction mixture was allowed to cool to room temperature, decanted, and evaporated in vacuo. The product thus obtained was recrystallized from ethanol to give **32** in 48% yield: mp 150–151 °C; IR (KBr) 3200, 3120, 3010, 2925, 2875, 1700, 1620, 1460, 1325, 1275, 1245, 1210, 1115, 1080, 900, 845 cm^{-1}. Anal. ($C_{11}H_{11}NO_3$) C, H.

4-(Chloromethyl)spiro[1,3-dioxolane-2,3′-indolin]-2′-one (33). A mixture of 1 (2.94 g, 0.02 mol), 3-chloro-1,2-propanediol (2.61 g, 0.02 mol), and *p*-toluenesulfonic acid (0.02) in 80 mL of toluene was heated at reflux. After 12 h the solvent was removed in vacuo. The product thus obtained was recrystallized from benzene to give 0.77 g (16%) of **33**: mp 158–159 °C; IR (KBr) 3200, 3150, 2960, 2940, 1730, 1620, 1465, 1320, 1225, 1120, 1065, 1000, 930, 850 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 7.73–7.03 (aromatic, 4 H), 5.05–4.4 (multiplets, 3 H, CH_2O, CH), 4.27 (d, 2 H, CH_2Cl, J = 6 Hz). Anal. ($C_{11}H_{10}ClNO_3$) C, H.

Pharmacological Evaluation.[7,19,20] Anticonvulsant screenings were carried out through the Antiepileptic Drug Development Program, National Institute of Neurological and Communicative Disorders and Stroke (NINCDS), NIH. Each compound submitted was tested for its ability to protect mice (male Carworth Farm No. 1) against electrically and chemically induced seizures. This evaluation (phase I) was performed at three dose levels viz. 30, 100, and 300 mg/kg. The compounds were administered ip, generally in PEG. The maximal electroshock seizure test evaluates the ability of a compound to prevent seizure spread through neural tissue. Maximal electroshock seizures are elicited with 60 cycle alternating current of 50-mA intensity delivered for 0.2 s via corneal electrodes. Protection in the MES test was defined as the abolition of the hind limb tonic extension component of the seizure. Pentylenetetrazole (85 mg/kg) is administered as a 0.5% solution subcutaneously. The subcutaneous pentylenetetrazole test estimates the ability to raise seizure threshold for excitation of neural tissue. Protection in the sc-Met test was defined as the failure to observe even a single episode of clonic spasms of at least 5-s duration (threshold seizure). All compounds were tested for neurotoxicity. The rotorod test was used to evaluate central nervous system toxicity. Neurologic toxicity was defined as the failure of the dosed animal to remain on a 1-in. diameter knurled plastic rod, rotating at 6 rpm, for 1 min. Compounds exhibiting anticonvulsant activity at 100 mg/kg or less are usually advanced to phase II (quantitative) testing. Phase II determines the time of peak anticonvulsant and toxic effect. The TD_{50} and ED_{50} are then determined (the tests being performed at the time of peak effect).

Acknowledgment. We thank the Epilepsy Branch, National Institute of Neurological and Communicative Disorders and Stroke, NIH, for the anticonvulsant screening results. A Sigma Xi Grant-in-Aid of research (to M.R.) is also acknowledged.

Registry No. 1, 91-56-5; **2**, 87-48-9; **3**, 17630-76-1; **4**, 6341-92-0; **5**, 6344-05-4; **6**, 611-09-6; **7**, 2058-74-4; **8**, 391-12-8; **9**, 608-05-9; **10**, 20780-76-1; 11, 6714-68-7; **12**, 75822-54-7; **13**, 113549-01-2; **14**, 113549-02-3; **15**, 75822-57-0; **16**, 113549-03-4; **17**, 113549-04-5; **18**, 113549-05-6; **19**, 75822-55-8; **20**, 63707-29-9; **21**, 113549-06-7; **22**, 34058-27-0; **23**, 75822-58-1; **24**, 113549-07-8; **25**, 113549-08-9; **26**, 113549-09-0; **27**, 38168-18-2; **28**, 113549-10-3; **29**, 113549-11-4; **30**, 113549-12-5; **31**, 66346-69-8; **32**, 113549-13-6; **33**, 113549-14-7; ethylene glycol, 107-21-1; morpholine, 110-91-8; formaldehyde, 50-00-0; piperidine, 110-89-4; pyrrolidine, 123-75-1; 2-mercaptoethanol, 60-24-2; 1,2-ethanedithiol, 540-63-6; 1,3-propanediol, 504-63-2; 3-chloro-1,2-propanediol, 96-24-2.

(19) For a description of the NINCDS screening protocol, see: (a) Reference 15. (b) Pharmacology section of Conley, J. D.; Kohn, H. *J. Med. Chem.* **1987**, *30*, 567. (c) Clark, C. R.; Davenport, T. W. *J. Med. Chem.* **1987**, *30*, 1214.

(20) Information obtained from ADD Program, NINCDS, NIH.

9-(2-Fluorobenzyl)-6-(alkylamino)-9*H*-purines. A New Class of Anticonvulsant Agents

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Several substituted aryl and 6-alkylamino analogues of the anticonvulsant purine 9-(2-fluorobenzyl)-6-(methylamino)-9*H*-purine (1) were synthesized and tested for anticonvulsant activity against maximal electroshock-induced seizures (MES) in rats. Derivatives with a second fluoro substituent in the 5- or 6-position of the aryl moiety were very active with ip ED_{50}'s that ranged from 2 to 4 mg/kg. Congeners in which the purine 6-substituent was varied among a number of alkylamino groups possessed potent activity against MES that was comparable to or several times better than phenytoin.

Although several drugs are used in the treatment of epilepsy, many patients fail to experience satisfactory seizure control with them, or they do so at the expense of significant side effects.[1,2] Due to the need for new im-

† Organic Chemistry Department.
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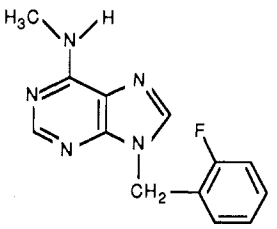
(1) Krall, R. L.; Penry, J. K.; White, B. G.; Kupferberg, H. J.; Swinyard, E. A. *Epilepsia (N.Y.)* **1978**, *19*, 409.

0022-2623/88/1831-1005$01.50/0

proved antiepileptic drugs, a program was initiated to develop an agent with improved properties.[3-6] From this program emerged 9-(2-fluorobenzyl)-6-(methylamino)-9*H*-purine (BW A78U (1)), a novel, orally active anticonvulsant with potent activity against maximal electroshock-induced seizures (MES) in rats and mice.[5] The structure–activity relationships associated with the initial development of 9-benzylpurines with anticonvulsant activity were reported,[6] and methods for synthesis of 1 were published.[7] This paper describes the synthesis and anticonvulsant activity of new analogues of 1.



BW A78U (1)

Chemistry

Most of the 6-(alkylamino)-9-(substituted benzyl)purines in Table I were prepared by amination of the appropriate purine.[6,7] The 6-chloro-9-substituted-purines were prepared in two steps from 5-amino-4,6-dichloropyrimidine[6,8-11] or by alkylation of 6-chloropurine.[6,12] The 9-(3-hydroxybenzyl)purine **6** was made from **7** by treatment with HBr in AcOH. The 9-(4-aminobenzyl)purine **5** was prepared from **4** by catalytic hydrogenation. Reduction of 2,6-difluorobenzonitrile with diborane gave 2,6-difluorobenzylamine (**30**),[13] which was used in the preparation of **11** and **12**. For preparation of **15**, 2,3-difluorobenzyl bromide (**34**) was prepared in three steps from 1,2-difluorobenzene via 2,3-difluorobenzoic acid[14] as described in the Experimental Section.

Biological Results and Discussion

Structure–activity studies show that potent anticonvulsant activity against MES in rats resides in the 6-(dimethylamino)- and 6-(methylamino)-9-benzylpurines **36** and **37** (Table II).[6] Para substitution on the phenyl ring of **36** leads, almost completely, to loss of activity. However, lipophilic, electron-withdrawing meta substituents give compounds that retain substantial anticonvulsant activity, but these analogues are more toxic than the parent **36**.[6]

With the intention of further exploring the effect of aryl substituents on anticonvulsant activity, a fluorine substituent was substituted in the phenyl ring of **37**, a 6-(methylamino)purine (Table II). Substitution in the para and meta positions gave **2** and **3**, which had less anticonvulsant activity. The *o*-fluoro analogue **1** was more active than **36** or **37** with an ip ED_{50} = 1.7 mg/kg and an oral ED_{50} = 2.5 mg/kg in rats.[5] The 2-CF_3 (**8**), 2-Cl (**9**), and 2-CH_3 (**10**) analogues were also anticonvulsants but only at substantially higher doses.

The effect of a second fluorine substituent was investigated. The 2,6-difluoro (**11** and **12**) and 2,5-difluoro (**13**) aryl substitution pattern gave analogues with potent anticonvulsant activity and ip ED_{50}'s that ranged from 2 to 4 mg/kg. However, a 2,4-difluoro (**14**) or 2,3-difluoro (**15**) aryl substitution pattern resulted in a 10-fold loss in activity. The 6-chloro analogue (**16**) of **1** was about 7-fold less active. Thus, substitution of a 2-fluoro group in **37** to give **1** resulted in an agent with potent ip and oral anticonvulsant activity. A second fluorine in the 6- or 5-position of **1** gave compounds that were also highly active, but other substitution patterns resulted in a substantial loss of activity.

The effect of varying the 6-methylamino substituent of **1** was investigated (Table III). Other 6-monoalkylamino purines such as **18** ($NHCH_2CH_3$), **19** ($NHCH_2CH_2CH_3$), **20** ($NHCH(CH_3)_2$), **22** ($NHCH_2$-c-C_3H_5), **24** (NH-c-C_5H_9), and **40** (NH-c-C_3H_5) were active as anticonvulsants with ip ED_{50}'s that ranged from 4.3 to 8 mg/kg. Of nine 6-monoalkylamino purines, only the *tert*-butylamino (**21**) and cyclobutylamino (**23**) congeners were weak or inactive as anticonvulsants. Addition of a second *N*-methyl group on **1** gave **38**, which was about 5-fold less active by the ip route of administration. Other disubstituted aminopurines such as **25–27**, **29**, and **41** were active with ip ED_{50}'s that ranged from 2.9 to 12.5 mg/kg. Only the 6-azetidinyl analogue **28** was inactive with an ED_{50} > 50 mg/kg. Thus, a number of 6-(alkylamino)-9-(2-fluorobenzyl)-9*H*-purines possessed potent activity against MES that was comparable to or several times better than that of phenytoin, the established clinical standard.

Compound **1** and a number of analogues represent a new class of orally active anticonvulsant agents with potent activity against MES in rats. There was only limited specificity for substituents on the 6-amino group, and potent activity was retained when a second fluorine substituent was introduced in the 5- or 6-position of the phenyl ring. This new class of anticonvulsant agents provides a novel lead for the development of compounds that may be useful in the treatment of seizure disorders for which phenytoin is presently indicated.[5]

Experimental Section

Melting points were taken in capillary tubes on a Mel-Temp block or a Thomas-Hoover Unimelt and are uncorrected. NMR spectra were recorded on a Varian XL-100-15-FT, a Varian FT-80A, a Varian T-60, or a Hitachi Perkin-Elmer R-24 spectrometer with Me_4Si as an internal standard. UV absorption spectra were measured on a Unicam SP 800 or Cary 118 UV–vis spectrophotometer. Each analytical sample had spectral data compatible with its assigned structure and moved as a single spot on TLC. TLC's were developed on Whatman 200 μm MK6F plates of silica gel with fluorescent indicator. Preparative flash chromatography[15] was performed on silica gel 60 (40–63 μm, E. Merck No. 9385). The analytical samples gave combustion values for C, H, N within 0.4% of theoretical. Elemental analyses were performed by Atlantic Microlab, Inc.

Method B. 9-(2-Fluorobenzyl)-6-(methylamino)-9*H*-purine (1) Hydrochloride. To a solution of 9-(2-fluoro-

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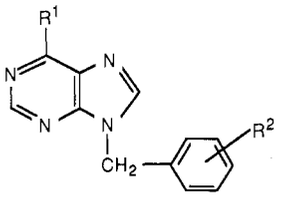
Table I. Physical Properties of 9-Benzylpurines



no.	R^1	R^2	methods[a]	% yield	mp, °C	formula[b]
1	$NHCH_3$	2-F	B[c]	90[d]	255–259	$C_{13}H_{12}FN_5$·HCl
2	$NHCH_3$	4-F	A[e]	65[f]	172–174	$C_{13}H_{12}FN_5$
3	$NHCH_3$	3-F	A[e]	73[g]	120–121	$C_{13}H_{12}FN_5$
4	$NHCH_3$	4-NO_2	A[h]	87[i]	246–247[j]	$C_{13}H_{12}N_6O_2$
5	$NHCH_3$	4-NH_2	C	72[k]	200–202	$C_{13}H_{14}N_6$
6	$NHCH_3$	3-OH	D	67[k]	145–146	$C_{13}H_{13}N_5O$·HBr
7	$NHCH_3$	3-$OCH_2C_6H_5$	A[e]	62[l]	134–135	$C_{20}H_{19}N_5O$
8	$NHCH_3$	2-CF_3	E	30[d]	270–273	$C_{14}H_{12}F_3N_5$·HCl
9	$NHCH_3$	2-Cl	E	16[d]	253–256	$C_{13}H_{12}ClN_5$·HCl
10	$NHCH_3$	2-CH_3	E	42[d]	247–250	$C_{14}H_{15}N_5$·HCl
11	$NHCH_3$	2,6-F_2	E, exp	81[d]	282–285	$C_{13}H_{11}F_2N_5$·HCl
12	$N(CH_3)_2$	2,6-F_2	E, exp	73[m]	220–225	$C_{14}H_{13}F_2N_5$·HCl
13	$NHCH_3$	2,5-F_2	E	26[d]	265–268	$C_{13}H_{11}F_2N_5$·HCl
14	$NHCH_3$	2,4-F_2	E	8[d]	270–273	$C_{13}H_{11}F_2N_5$·HCl
15	$NHCH_3$	2,3-F_2	E, exp	44[l]	135–136.5	$C_{13}H_{11}F_2N_5$
16	$NHCH_3$	2-F, 6-Cl	E	34[d]	280–283[n]	$C_{13}H_{11}ClFN_5$·HCl
17	$N(CH_3)_2$	2-F, 6-Cl	E	35[k]	218–220[o]	$C_{14}H_{13}ClFN_5$·HCl
18	$NHCH_2CH_3$	2-F	A[c,p]	84[q]	143–144	$C_{14}H_{14}FN_5$
19	$NHCH_2CH_2CH_3$	2-F	A,[c,p] B	64[f]	200–204	$C_{15}H_{16}FN_5$·HCl
20	$NHCH(CH_3)_2$	2-F	A[p,f]	59[q]	98–100	$C_{15}H_{16}FN_5$
21	$NHC(CH_3)_3$	2-F	A,[c,p,r] B	57	175–179[f]	$C_{16}H_{18}FN_5$·HCl
22	$NHCH_2C_3H_5$[u]	2-F	A,[c,p,s] B	45[t]	198–201	$C_{16}H_{16}FN_5$·HCl
23	NHC_4H_7[v]	2-F	A[c,p]	74[q]	143–145	$C_{16}H_{16}FN_5$
24	NHC_5H_9[w]	2-F	A,[c,p] B	55[f]	160–163	$C_{17}H_{18}FN_5$·HCl
25	$N(CH_3)CH_2CH_3$	2-F	A,[c,p] B	50[t]	153–156	$C_{15}H_{16}FN_5$·HCl
26	$N(CH_2CH_3)_2$	2-F	A,[c,p] B	26[f]	168–171	$C_{16}H_{18}FN_5$·HCl[x]
27	$N(CH_3)C_3H_5$[u]	2-F	A[c,p,s]	52[q]	100–103	$C_{16}H_{16}FN_5$
28	$N(CH_2)_3$	2-F	A[c,p]	78[g]	181–183	$C_{15}H_{14}FN_5$
29	$N(CH_2)_4$	2-F	A,[c,p] B	62[f]	223–226	$C_{16}H_{16}FN_5$·HCl

[a] A: see preparation of compound 1, "Methylamination of 4", in ref 7. C: see method G in ref 6. D: see preparation of compound 80 in ref 6. [b] All compounds were analyzed for C, H, and N. [c] For the starting 6-chloropurine see ref 6. [d] Recrystallized from EtOAc–EtOH. [e] Recrystallized from cyclohexane–EtOAc. [f] For the starting material see ref 7. [g] Recrystallized from EtOH–H_2O. [h] For the starting material, see: Schaeffer, H. J.; Odin, E. *J. Med. Chem.* **1966**, *9*, 576. [i] Recrystallized from MeOH–$CHCl_3$. [j] Mp 248 °C reported for this compound by Schaeffer, H. J.; Odin, E. *J. Med. Chem.* **1966**, *9*, 576. [k] Recrystallized from EtOH. [l] Recrystallized from EtOAc–hexanes. [m] Recrystallized from EtOH–EtOAc. [n] Mp 189–190 °C reported for this compound by Imai, K.; Seo, T. *Eur. J. Med. Chem.* **1980**, *15*, 207. [o] Mp 134–135 °C reported for the free base of this compound by Matsuno, T.; Imai, K. U.S. Patent 4 189 485, 1980. [p] Excess neat alkylamine in aqueous EtOH was used. [q] Recrystallized from cyclohexane. [r] The crude product was purified by flash chromatography as in method E. [s] The alkylamine or its amine hydrochloride and excess triethylamine were used. [t] Recrystallized from EtOAc. [u] Cyclopropyl substituent. [v] Cyclobutyl substituent. [w] Cyclopentyl substituent. [x] N: calcd, 20.86; found, 20.44.

benzyl)-6-(methylamino)-9*H*-purine[7] (36.0 g, 140 mmol) in warm EtOH (500 mL) was added 12 M HCl (15 mL) in EtOH (50 mL). The resultant mixture was spin evaporated in vacuo to remove the volatiles. The white residue was recrystallized from EtOH–H_2O to give 37.11 g (90%) of 1·HCl: mp 255–259 °C (partial melting at approximately 245 °C); UV (0.1 N HCl + 10% EtOH) λ_{max} 263.5 nm; UV (0.1 N NaOH + 10% EtOH) λ_{max} 268 nm; NMR (Me_2SO-d_6) δ 9.0 (br m, 1 H, NH), 8.44 (s, 1 H, purine H), 8.39 (s, 1 H, purine H), 7.0–7.5 (complex m, 4 H, Ar), 5.52 (s, 2 H, CH_2), 3.06 (br s, 3 H, CH_3). Anal. ($C_{13}H_{13}ClFN_5$) C, H, N.

Method E. 9-(2,5-Difluorobenzyl)-6-(methylamino)-9*H*-purine (13) Hydrochloride. A mixture of 6-chloropurine (5.00 g, 32.3 mmol), DMSO (75 mL), anhydrous K_2CO_3 (5.00 g, 36.2 mmol), and 2,5-difluorobenzyl bromide (prepared in a conventional way[16] from 2,5-difluorotoluene and *N*-bromosuccinimide) (6.47 g, 31.2 mmol) was stirred at ambient temperature for 39 h. The reaction solution was decanted from the solids, poured into ice H_2O, and acidified to pH 5 with AcOH (0.5 mL). The mixture was extracted with CH_2Cl_2 (4 × 100 mL), and the combined extracts were washed with H_2O (5 × 50 mL), dried ($MgSO_4$), and spin evaporated in vacuo. The residue was dissolved in CH_2Cl_2 (50 mL) and added to silica gel 60 (50 g). This mixture was spin evaporated in vacuo, and the residual solids were introduced onto a column of silica gel 60 wetted with ethyl acetate–cyclohexane, 1:1. The column was eluted with ethyl acetate–cyclohexane, 1:2, by the flash chromatography technique. The fractions containing the higher R_f major component were combined and spin evaporated in vacuo to give 3.50 g (40%) of 6-chloro-9-(2,5-difluorobenzyl)-9*H*-purine, which was a single spot on TLC.

A solution of 6-chloro-9-(2,5-difluorobenzyl)-9*H*-purine (3.50 g, 12.5 mmol), EtOH (50 mL), and 40% aqueous $MeNH_2$ (20 mL) was stirred at ambient temperature for 15 h. The reaction mixture was spin evaporated to remove the volatiles. The residue was dispersed in H_2O (70 mL), and the solids were collected. This product was dissolved in warm EtOH (50 mL), and 12 M HCl (15 mL) in EtOH (5 mL) was added. The resultant mixture was spin evaporated in vacuo to remove the volatiles. The white residue was recrystallized from EtOH–H_2O to give 2.62 g (67%) of 13·HCl: mp 265–268 °C; UV (0.1 N HCl + 10% EtOH) λ_{max} 264 nm; UV (0.1 N NaOH + 10% EtOH) λ_{max} 268 nm; NMR (Me_2SO-d_6) δ 9.31 (br s, 1 H, NH), 8.48 (s, 1 H, purine H), 8.41 (s, 1 H, purine H), 7.0–7.5 (complex m, 4 H, Ar and NH), 5.53 (s, 2 H, CH_2), 3.09 (br s, 3 H, CH_3). Anal. ($C_{13}H_{12}ClF_2N_5$) C, H, N.

2,6-Difluorobenzylamine (30) Hydrochloride. A solution of diborane in THF (1 M, 359 mL) was added dropwise to 2,6-difluorobenzonitrile (50 g) in dry THF (80 mL) at 60 °C under a nitrogen atmosphere. After addition was complete (1 h), the reaction was refluxed for 18 h. The reaction was cooled to 30 °C,

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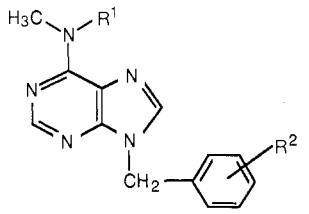
Table II. Anticonvulsant Activity of 9-Benzylpurines against Maximal Electroshock-Induced Seizures[a]



no.	R^1	R^2	MES ED_{50}, mg/kg[b-d] ip	po
1[e]	H	2-F	1.7 ± 0.4	2.5 ± 0.2
2	H	4-F	(33%)	
3	H	3-F	7.0	
4	H	4-NO_2	(0%)	
5	H	4-NH_2	25	
6	H	3-OH	(0%)	
7	H	3-$OCH_2C_6H_5$	(17%)	
8	H	2-CF_3	20	
9	H	2-Cl	6.2	12.5
10	H	2-CH_3	10	25
11	H	2,6-F_2	2.0 ± 0.1	2.7 ± 0.1
12	CH_3	2,6-F_2	2.2 ± 0.2	3.8 ± 0.6
13	H	2,5-F_2	4.0 ± 0.6	7.0 ± 1.0
14	H	2,4-F_2	20	
15	H	2,3-F_2	18	>50
16	H	2-F, 6-Cl	12.5	
17	CH_3	2-F, 6-Cl	5.0	10
36[f]	CH_3	H	6 ± 1	12 ± 3
37[f]	H	H	5 ± 1	12 ± 2
phenytoin			9.6 ± 2.0	20 ± 3

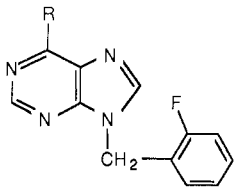
[a] The compounds were tested for their ability to protect Sprague–Dawley male rats against maximal electroshock-induced seizures as described in ref 4. The ED_{50} was the dose needed to protect 50% of the animals against the hind-limb extensor component and were calculated by the method of Miller, L. C.; Tainter, M. L. *Proc. Soc. Exp. Biol. Med.* 1944, *57*, 261. [b] The compounds were administered as solutions or fine dispersions in water or 5% methylcellulose. Samples that were not completely soluble were micronized to enhance the uniformity of sample delivery. [c] Values in parentheses are percent inhibition at 25 mg/kg. [d] Where ED_{50} values are presented with a standard error, a minimum of 12 animals were used per dose level with four doses per compound. ED_{50} values without standard error were determined by using three doses of compound with six animals per point. [e] Synthesis: see ref 7. [f] Synthesis: see ref 6.

and MeOH (500 mL) was added dropwise over 0.5 h. The reaction was cooled on an ice bath, and HCl gas was bubbled through the solution for 0.5 h. The reaction was refluxed for 1 h and then stirred at ambient temperature for 18 h. The reaction was spin evaporated in vacuo, and MeOH (500 mL) was added to the residue. The volatiles were removed by spin evaporation in vacuo, and the solid residue was stirred with CH_2Cl_2 (500 mL) for 1 h. The solids were collected and dried to give 60.9 g (94%) of crude **30** hydrochloride. Recrystallization of 2.0 g from EtOH–EtOAc gave 0.75 g (38% recovery) of analytically pure material: mp 199–200 °C (lit.[13] mp 196–197 °C by a different method); NMR (Me_2SO-d_6) δ 9.2 (br s, 3 H, NH_3), 7.0–8.0 (m, 3 H, Ar), 4.1 (s, 2 H, CH_2). Anal. ($C_7H_7F_2N$·HCl) C, H, N.

5-Amino-4-chloro-6-[(2,6-difluorobenzyl)amino]pyrimidine (31). This compound was prepared from 5-amino-4,6-dichloropyrimidine and **30** as described[7] for the 2-fluoro analogue; the yield was 52.1 g (95%), and the compound was a single spot on TLC. The analytical sample was recrystallized from EtOAc–EtOH: mp 248–250 °C; UV (0.1 N HCl + 10% EtOH) λ_{max} 304 nm; UV (0.1 N NaOH + 10% EtOH) λ_{max} 262, 291 nm; NMR (Me_2SO-d_6) δ 7.81 (s, 1 H, pyrimidine H-2), 6.8–7.7 (complex m, 4 H, Ar and NH), 5.08 (br s, 2 H, NH_2), 4.67 (br d, 2 H, CH_2). Anal. ($C_{11}H_9ClF_2N_4$) C, H, N.

6-Chloro-9-(2,6-difluorobenzyl)-9*H*-purine (32). This compound was prepared from **31** as described[7] for the 2-fluoro analogue except the reaction was also refluxed for 1 h. The yield was 45.2 g (96%); it contained a trace of higher R_f intermediate.

Table III. Anticonvulsant Activity of 9-(2-Fluorobenzyl)purines against Maximal Electroshock-Induced Seizures[a]



no.	R	MES ED_{50}, mg/kg[b-d] ip	po
1[e]	$NHCH_3$	1.7 ± 0.4	2.5 ± 0.2
18	$NHCH_2CH_3$	4.9 ± 0.5	
19	$NHCH_2CH_2CH_3$	5.1 ± 0.5	4.6 ± 1.0
20	$NHCH(CH_3)_2$	5.8 ± 0.6	
21	$NHC(CH_3)_3$	>50	
22	$NHCH_2C_3H_5$[f]	4.3 ± 0.9	6.3 ± 1.0
23	NHC_4H_7[g]	33 ± 3	
24	NHC_5H_9[h]	8 ± 1	
25	$N(CH_3)CH_2CH_3$	2.9 ± 0.3	6.0 ± 0.5
26	$N(CH_2CH_3)_2$	4.2 ± 0.5	5.9 ± 0.6
27	$N(CH_3)C_3H_5$[f]	6.8 ± 0.8	
28	$N(CH_2)_3$	>50	
29	$N(CH_2)_4$	12.5	35
38[e]	$N(CH_3)_2$	8.8 ± 1.7	
39[e]	NH_2	4.5 ± 1.0	12 ± 0.4
40[e]	NHC_3H_5[f]	5.0 ± 0.4	7.8 ± 0.8
41[e]	$N(CH_3)CHO$	11	25
phenytoin		9.6 ± 2.0	20 ± 3

[a-d] See footnotes *a–d* in Table II. [e] Synthesis: see ref 7. [f] Cyclopropyl substituent. [g] Cyclobutyl substituent. [h] Cyclopentyl substituent.

The analytical sample was recrystallized from cyclohexane–EtOAc: mp 142–143 °C; UV (0.1 N HCl + 10% EtOH) λ_{max} 265 nm; UV (0.1 N NaOH + 10% EtOH) λ_{max} 265 nm; NMR (Me_2SO-d_6) δ 8.77 (s, 2 H, purine H), 6.85–7.81 (complex m, 3 H, Ar), 5.68 (s, 2 H, CH_2). Anal. ($C_{12}H_7ClF_2N_4$) C, H, N.

2,3-Difluorobenzyl Alcohol (33). A diborane–THF solution (1 M, 59 mL, 59 mmol) was added dropwise to an ice bath cooled solution of 2,3-difluorobenzoic acid[14] (7.00 g, 44.3 mmol) in dry THF (20 mL) under a nitrogen atmosphere. The reaction mixture was stirred for 1.5 h as it warmed to ambient temperature. A mixture of THF–H_2O, 1:1 (25 mL), was cautiously added to the solution to quench excess diborane. Potassium carbonate (9.5 g) was added, and the mixture was stirred for 1 h and filtered, and the residual paste was washed with THF (3 × 30 mL). The combined filtrate and wash were spin evaporated in vacuo and coevaporated with CH_2Cl_2 (50 mL). The residue was partitioned between Et_2O–H_2O (200 mL), and the organic phase was dried (Na_2SO_4) and then spin evaporated to give 2.30 g of an oil. An additional 0.78 g of oil was recovered from the potassium carbonate paste by partitioning it between Et_2O–H_2O (150 mL:100 mL) followed by evaporation of the organic phase. The combined oils gave 3.08 g (40%) of **33** that was contaminated with 18% by weight of THF and was used without further purification in the next step: NMR (Me_2SO-d_6) δ 6.9–7.3 (m, 3 H, Ar), 5.2 (t, 1 H, OH), 4.5 (d, 2 H, CH_2).

2,3-Difluorobenzyl Bromide (34). Phosphorous tribromide (1.46 g, 5.39 mmol) was added dropwise to a stirred solution of crude **33** (2.12 g, 14.7 mmol) in toluene (25 mL). The solution was heated at 100 °C for 1 h. The reaction solution was decanted from the solids and concentrated to an oil. The oil was partitioned between Et_2O–H_2O (150 mL:100 mL), and the organic layer was dried (Na_2SO_4). The solution was concentrated to an oil under reduced pressure to give 2.90 g of **34** (61%), which was contaminated with 12% (by weight) of Et_2O and 24% (by weight) of toluene. This material was used without further purification in the next step; NMR (Me_2SO-d_6) δ 6.9–7.5 (m, 3 H, Ar), 4.65 (s, 2 H, CH_2).

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N-Terminal Requirements of Small Peptide Anticoagulants Based on Hirudin$_{54-65}$

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C-terminal fragment analogues of the leech anticoagulant peptide hirudin represent a unique class of thrombin inhibitors that blocks thrombin's cleavage of fibrinogen but does not block the catalytic site of thrombin. In this paper, a series of synthetic peptides were prepared by solid-phase methodology to determine the optimal N-terminal and position 56 functionalities for these C-terminal fragment analogues of hirudin. Inhibition of fibrin clot formation by thrombin in vitro was used as a measure of anticoagulant activity. In the minimal C-terminal sequence necessary for anticoagulant activity, hirudin$_{56-64}$, an L aromatic amino acid is required at position 56. Phe^{56} → Tyr substitution retained potency, whereas *p*-Cl-Phe^{56} and phenylglycine^{56} substitutions resulted in decreased potencies. Removal of the cationic amino functionality from the vicinity of Asp^{55} results in increased potency (e.g., hirudin$_{54-65}$, Ac-hirudin$_{55-65}$) and [desNH$_2$-Asp^{55}]hirudin$_{55-65}$ has a marked increase in potency over hirudin$_{55-65}$. [DesNH$_2$-Phe^{56}]hirudin$_{56-65}$ and related analogues show no detectable anticoagulant activity. The sensitivity of position 56 to modification demonstrates the significance of this residue in the interaction between the C-terminal region of hirudin and thrombin.

Bloodsucking animals produce various anticoagulant materials in order to maintain the fluidity of their meal.[1] In particular, the medicinal leech (*Hirudo medicinalis*) secretes a 65 amino acid peptide, hirudin, from its salivary glands (Figure 1). Hirudin is able to maintain the fluidity of blood for the 200 days over which the meal is digested by the symbiotic bacteria, *Pseudomonas hirudinis*.[2] Hirudin's anticoagulant activity results from its being the most potent known inhibitor of thrombin. It binds to thrombin with a reported dissociation constant of 0.8×10^{-10} M (ref 3) to 2.0×10^{-14} M (ref 4), depending on the method of determination that was used. Upon binding to thrombin the cleavage of fibrinogen and subsequent fibrin clot formation is prevented in addition to other reported functions of thrombin.[3,13] Hirudin has no known activity other than its inhibition of thrombin and is excreted from the body unmetabolized in the urine.[5,6]

Recently we reported that a C-terminal fragment of hirudin, unsulfated N^{α}-acetylhirudin$_{45-65}$, inhibited fibrin clot formation without inhibiting the amidase activity of thrombin toward the small peptide substrate D-Phe-Pip-Arg-pNA, S-2238.[7] Hirudin blocks S-2238 hydrolysis by thrombin. Thus, the inhibition of fibrin clot formation by the C-terminal fragment analogue is thought to occur by binding to a noncatalytic site on thrombin. This is similar to fibrin hydrolysates that bind thrombin but do not inhibit S-2238 hydrolysis.[8] Previously, thrombin inhibitors have been active site directed; thus peptides based on hirudin's C-terminus represent a new class of potential antithrombic drugs.

Further studies have determined the minimal C-terminal sequence for inhibition of fibrin clot formation as hirudin$_{56-64}$.[9] A series of single residue substitutions has shown that position 56 requires an L aromatic amino acid for activity and Glu^{57}, Ile^{59}, Pro^{60}, and Leu^{64} are also critical for maintaining potency.[10,11] These data led us to explore the optimal N-terminal and position 56 functionalities of these peptides. Here we report a series of synthetic analogues designed to increase anticoagulant potency of these hirudin fragment analogues.

Table I. Fibrin Clot Inhibition

	relative potency[a]	IC$_{50}$,[b] μM
1, N^{α}-acetylhirudin$_{54-65}$	70	5.3
2, hirudin$_{54-65}$	100	3.7[c]
3, N^{α}-acetylhirudin$_{55-65}$	72	5.2
4, hirudin$_{55-65}$	53	7.1[c]
5, [desNH$_2$-Asp^{55}]hirudin$_{55-65}$	219	1.7
6, hirudin$_{56-65}$	12	34.0
7, hirudin$_{55-64}$amide	16	23.4
8, [desNH$_2$-Phe^{56}]hirudin$_{56-64}$amide	<1.4	>250
9, N^{α}-*trans*-cinnamoylhirudin$_{57-64}$amide	<1.4	>250
10, N^{α}-benzoylhirudin$_{57-64}$amide	<1.4	>250
11, [Tyr^{56}]hirudin$_{54-65}$	112	3.3
12, [*p*-Cl-Phe^{56}]hirudin$_{54-65}$	29	12.6
13, [Pgl^{56}]hirudin$_{54-65}$	<1.4	>250

[a] Relative potency with hirudin$_{54-65}$ = 100. [b] IC$_{50}$ = molar dose of peptide that results in 50% inhibition of fibrin clot formation relative to a blank control after thrombin addition to plasma. [c] Reference 5.

Results and Discussion

All peptides in this report are unsulfated. Sulfation of Tyr^{63} in hirudin resulted in only a 2-fold increase in potency.[12] Hirudin analogues **1**, **3**, **5–13** (see Table I) were

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