low-affinity state (labeled by [^{3}H]ketanserin), whereas 5-HT antagonists compete with equal affinity for the high- and low-affinity states of the receptor.[2,4,10] Ketanserin, spiperone, and cinanserin competed for [^{125}I]DOI binding with high affinity, produced Hill coefficients of 0.75–0.87, and competed for 65% of total [^{125}I]DOI binding. Serotonin and the putative 5-HT agonists R(−)DOB and DOI also competed for [^{125}I]DOI with high affinity, produced competition curves with Hill coefficients of 0.84–0.90, and competed for 65% of total [^{125}I]DOI binding (Table I). (Representative competition curves are shown in Figure 1.) The affinities (K_i values) of the six agents examined parallel the results from studies where [^{3}H]DOB was employed as the radioligand (Table I). The observations that all competing ligands reduced [^{125}I]DOI binding to the same extent and produced similar Hill coefficients indicate that in this tissue preparation [^{125}I]DOI is principally labeling one site but that there is a minor amount of some other site being labeled. This slight contamination with a second labeled site should not significantly deter from the utility of [^{125}I]DOI.

The results described herein indicate that [^{125}I]DOI, like [^{3}H]DOB, labels the agonist high-affinity state of 5-HT$_2$ receptors (i.e., 5-HT$_{2H}$ receptors) in a saturable, displaceable, and specific manner. We have found the signal to be stable and reliable (presumably due to the very high affinity of the radioiodinated ligand for the receptor). We anticipate that the greater specific activity of [^{125}I]DOI relative to [^{3}H]DOB (i.e., 1625 vs 16–40 Ci/mmol) should result in [^{125}I]DOI being a useful radioligand for subsequent binding and autoradiographic studies of the agonist high-affinity state of 5-HT$_2$ receptors.

Registry No. 1 (R = ^{125}I), 111381-00-1; 1 (R = ^{125}I)·HCl, 111381-06-7; 1 (R = T)·HCl, 42203-78-1; **2**, 2801-68-5; **3**, 67460-68-8; **4**, 111381-01-2; **5**, 111381-02-3; **6**, 111381-03-4; **7**, 111381-04-5; [^{125}I]-**7**, 111381-05-6; **8**, 79315-43-8; (5-HT), 50-67-9.

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Articles

Substituted 1,3,4-Thiadiazoles with Anticonvulsant Activity. 4. Amidines

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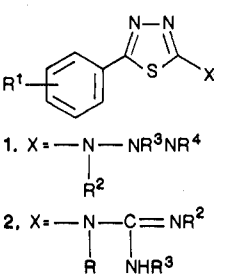
Two different structural types of 2-aryl-1,3,4-thiadiazole amidines were synthesized and evaluated for anticonvulsant activity. Enhancement of the inherent anticonvulsant activity therein and separation of this activity from the accompanying sedative action of these compounds were attempted. The most potent compounds occurred in the 2-(trifluoromethyl)phenyl series of type **3** amidines, but they also possessed a relatively high level of neurotoxicity and sedation as demonstrated in the rotorod test.

Previous papers[1,2] in this series have described 2-aryl-5-hydrazino- and 2-aryl-5-guanidino-1,3,4-thiadiazoles (**1**, **2**) as potential anticonvulsants. The most encouraging results were obtained within the hydrazine series, but it was recognized that the presence of a hydrazine group in these compounds was potentially undesirable because of the side effects associated with the bioisosterically related compound hydralazine.[3] It was felt, therefore, that the closely related amidines could perhaps offer an attractive alternative series to the hydrazines **1**.[1]



The thiadizole amidines studied can be divided into two structural types, **3** and **4**. Although a range of amidines of type **3** have been disclosed previously[4] and claimed to possess herbicidal and fungicidal properties, the derivatives

(1) Chapleo, C. B.; Myers, M.; Myers, P. L.; Saville, J. F.; Smith, A. C. B.; Stillings, M. R.; Tulloch, I. F.; Walter, D. S.; Welbourn, A. P. *J. Med. Chem.* **1986**, *29*, 2273.
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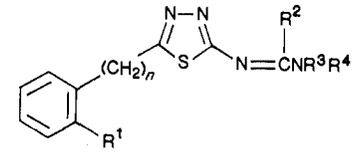
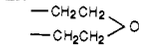
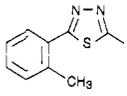
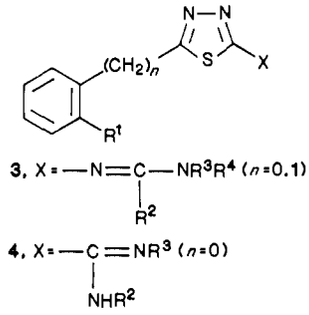
Table I. Type 3 Amidines



no.	R^1	R^2	R^3	R^4	n	recrystn solvent	mp, °C	formula	ED_{50},[a] mg/kg (limits) MMS[b]	ED_{50},[a] mg/kg (limits) MES[c]	TD_{50},[a] mg/kg, rotorod[d]
3a	H	CH_3	H	H	1	EtOAc	91–94	$C_{11}H_{12}N_4S$	50 (39–64)	31 (20–40)	200
3b	H	$PhCH_2$	H	H	1	EtOAc	140–144	$C_{17}H_{16}N_4S$	45 (30–84)	86 (27–151)	>500
3c	CH_3	CH_3	H	H	0		235–238	$C_{11}H_{12}N_4S$·HCl	78 (23–128)	200	sedative at 100 mg/kg
3d	CH_3	CH_3	*n*-Bu	H	0		196–198	$C_{15}H_{20}N_4S$·HCl	46 (24–63)	48 (33–75)	>100
3e	CH_3	CH_3	$PhCH_2$	H	0	cyclohexane	84–87	$C_{18}H_{18}N_4S$	102 (32–233)	86 (INF)	>200
3f	CH_3	CH_3	Et	Et	0	EtOAc	94–96	$C_{15}H_{20}N_4S$	84 (29–115)	81 (30–145)	>200
3g	CH_3	CH_3			0	*i*-ProH	103–104	$C_{15}H_{18}N_4OS$	148 (INF)	108 (68–183)	sedative at 142 mg/kg
3h	CH_3	H		H	0	Me_2CO	183–187	$C_{19}H_{16}N_6S_2$	>200	>200	NT
3i	CF_3	CH_3	H	H	0	Et_2O/petroleum ether	95–97	$C_{11}H_9F_3N_4S$	19 (6–25)	18 (INF)	~80
3j	CF_3	*n*-C_3H_7	H	H	0	EtOAc/hexane	90–92	$C_{13}H_{13}F_3N_4S$	60 (17–125)	63 (47–77)	150
3k	CF_3	CH_3	*n*-Bu	H	0		86–92	$C_{15}H_{17}F_3N_4S$·HCl	21 (20–21)	33 (17–52)	~70
3l	CF_3	$PhCH_2$	*n*-Bu	H	0	cyclohexane	84–86	$C_{21}H_{21}F_3N_4S$	>100	>200	NT
3m	Ph	CH_3	H	H	0	EtOAc	141–144	$C_{16}H_{14}N_4S$	NT	78 (47–86)	>100
3n	Ph	CH_3	*n*-Bu	H	0	EtOAc	87–89	$C_{20}H_{22}N_4S$	90 (45–129)	121 (19–169)	>200
3o	Ph	Me_2CH	*n*-Bu	H	0	cyclohexane	93–94	$C_{22}H_{26}N_4S$	>100	>200	NT

[a] INF = infinity, NT = not tested. Limits obtained from a statistical analysis of the test result (Bliss computer assay[13]). [b] MMS = maximal metrazol seizures[10] (mouse); 1 h after dosing (po). [c] MES = maximal electroshock test[11] (mouse); 1 h after dosing (po). [d] Rotorod[12] (mouse) = rotating rod; 1 h after dosing (po).

described herein are novel; amidines of type 4 also appear to be novel.



We report here the synthesis and pharmacological evaluation of both amidine structural types.

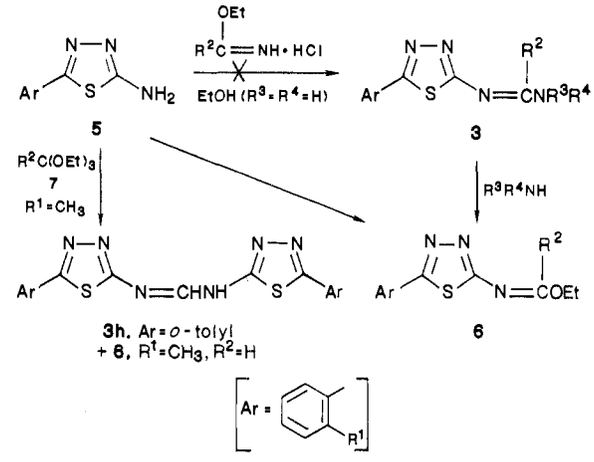
Chemistry

Type 3 Amidines (Table I). The starting material used for the preparation of the amidines of type **3** was the thiadiazole amine **5**.[1] It was considered that amine **5** would react with an ethyl carboximidate salt to give an amidine **3** where $R^3 = R^4 = H$.

However, the product obtained from this reaction was found to be the corresponding imidate **6**; thus a "transimidation" process had occurred involving the loss of ammonium chloride (Scheme I). A reaction of this type is not without precedent[5] and in this particular example is probably a reflection of the base strength of the amine **5**. However, this reaction proved to be of considerable advantage in the synthesis of the required amidines. Imidate **6** served as a useful intermediate reacting with substituted amines to give the amidines **3** as shown.

The versatility of this reaction sequence led to the adoption of this route for the preparation of all amidines of type **3** in preference to the previously published synthetic procedures.[4] One of the byproducts formed during

Scheme I



the formation of imidate **6** is thought to be the corresponding ortho ester **7** arising from the known solvolysis[6] of carboximidate esters. Fortunately the impurities from this reaction did not interfere with the second step of the sequence. As expected,[7] it was found that ortho esters **7** themselves react with amines **5** to give the same imidate **6**, although higher temperatures were required to enhance the reaction. In one example, with amine **5** ($R^1 = CH_3$) and ethyl orthoformate **7** ($R^2 = H$), incomplete reaction occurred under refluxing conditions in ethanol. However, when solvent was excluded from the reaction, two products were formed, which proved to be amidine **3h** and a second compound (which remained in the filtrate and could not be isolated), which mass spectral evidence suggests is the required imidate **6** (Scheme I) (see Experimental Section).

Type 4 Amidines (Table II). The key precursor for amidines of type 4 was the 2-cyanothiadiazole **8**, which was

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(6) McElvain, S. M.; Nelson, J. W. *J. Am. Chem. Soc.* **1942**, *64*, 1825.

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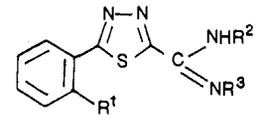
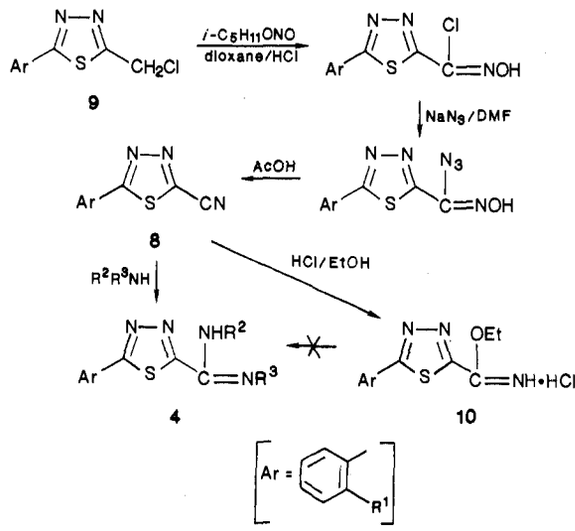
Table II. Type 4 Amidines



no.	R^1	R^2	R^3	recrystn solvent	mp, °C	formula	ED_{50},[a] mg/kg (limits) MMS[b]	ED_{50},[a] mg/kg (limits) MES[c]	TD_{50},[a] mg/kg, rotorod[d]
4a	CH_3	*n*-Bu	H	$Me_2CO/CHCl_3$	192–194	$C_{14}H_{18}N_4S \cdot HCl$	~142	>200	toxic at 200 mg/kg
4b	CH_3	$-(CH_2)_2-$			113–114	$C_{12}H_{12}N_4S$	>200	>100	NT
4c	Ph	$-(CH_2)_2-$			244–247	$C_{17}H_{14}N_4S$	77 (17–121)	103 (INF)	LD_{30} = 200 mg/kg

[a-d] Same as for Table I.

Scheme II



prepared from the corresponding (chloromethyl)thiadiazole **9**[8] in an overall yield of approximately 60% by utilizing a recently described method[9] for the preparation of cyano heterocycles (Scheme II). Difficulties were encountered in the conversion of the nitrile **8** to the amidines **4**, and only three examples were actually characterized. Attempts to prepare the unsubstituted amidine **4** ($R^2 = R^3 = H$) from **8** and ammonia were unsuccessful. Reaction of **8** with butylamine gave the monobutylated product **4a**, but under certain conditions, formation of a dibutylated product **4** ($R^2 = R^3 = n$-Bu) (not isolated; evidence based on mass spectrum) complicated the recovery of **4a**. Imidazolines **4b,c** were obtained from the reaction of **8** with ethylenediamine.

The cyano compound **8** appeared to give the imidate **10** ($R^1 = CH_3$) when treated with HCl and ethanol in ether. It was hoped that **10** could be used as an intermediate to amidines **4**, but in an attempt to obtain **4b** by reacting **10** with ethylenediamine, several products were formed; although there was evidence for the formation of **4b**, it could not be isolated.

Pharmacological Results and Discussion

Structures and selected properties of the synthesized compounds are summarized in Tables I and II. Anticonvulsant activity was assessed in the metrazol[10] and electroshock[11] tests. The rotorod test[12] was used to evaluate the level of neurotoxicity exhibited by the compounds.

Type 3 Amidines (Table I). The majority of the compounds synthesized possessed significant anticonvulsant activity, blocking both electrically and chemically induced seizures. The most potent compounds occurred in the 5-[2-(trifluoromethyl)phenyl] series, especially **3i**, but the level of sedation was also correspondingly high. In the 2-methyl series it was found that the *N*-butyl analogue **3d** possessed higher anticonvulsant activity and lesser neurotoxic effects than the parent amidine **3c**. The N-substituted analogues **3e–g** are also more active in the MES test than **3c**, but less so than **3d**. However, in the 2-trifluoromethyl series, the parent amidine **3i** possessed slightly higher anticonvulsant activity than the *N*-butyl analogue **3k**, although both compounds possessed a similar level of neurotoxicity. A similar finding was observed in the 2-phenyl series **3m,n** (MES test). Reasonable activity was also demonstrated in the benzyl series as exemplified by **3a**, but again a similar level of neurotoxicity was seen when compared with the other series examined.

It can be concluded that enhancement of the anticonvulsant activity and separation of this from the sedative action of such compounds were unfortunately not achieved, within the albeit small number of compounds investigated, by either changing the substitution on the amidine moiety (R^2, R^3, R^4) or by altering the aromatic substituent (R^1).

Type 4 Amidines (Table II). Preparation of amidines of structural type **4** proved to be a more difficult task, and only three compounds were obtained. The level of anticonvulsant activity was very low in two examples, **4a** and **4c**, while the third, **4b**, proved to be inactive. However, both **4a** and **4c** were also found to be toxic as demonstrated in the rotorod test.

Experimental Section

Melting points were determined in a Büchi apparatus in glass capillary tubes and are uncorrected. IR, NMR, and mass spectra were recorded on Perkin-Elmer 700, Varian Associates T-60, and LKB-2091 instruments, respectively, and were consistent with assigned structures. Where analyses are indicated only by symbols of the elements, results obtained were within ±0.4% of the theoretical values. The aminothiadiazole **5**[1] and ethyl acetimidate hydrochloride[6] were prepared by following procedures already described. Representative examples of the two structural types of amidines are given.

Type 3 Amidines. *N*'-[5-[2-(Trifluoromethyl)phenyl]-1,3,4-thiadiazol-2-yl]acetamidine (3i). A suspension of 2-amino-5-[2-(trifluoromethyl)phenyl]-1,3,4-thiadiazole (**5**, $R^1 = CF_3$) (21 g, 85.7 mmol) in ethanol (1300 mL) was treated with ethyl acetimidate hydrochloride (51 g, 414 mmol) at room temperature. Precipitation of NH_4Cl occurred within 3 h. Stirring was con-

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(10) Soaje-Echaque, E.; Lim, R. K. S. *J. Pharmacol. Exp. Ther.* **1962**, *138*, 224. Desmedt, L. K. C.; Niemegeers, C. J. E.; Lewi, P. J.; Janssen, P. A. J. *Arzneim.-Forsch. (Drug Res.)* **1976**, *26*, 1592.
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tinued for 5 days, and the solid was then filtered off. Evaporation of the solvent gave an oily residue (40 g), which was extracted with hexane to remove the remaining NH_4Cl. Evaporation of the extracts provided the slightly impure ethyl *N'*-[5-[2-(trifluoromethyl)phenyl]-1,3,4-thiadiazol-2-yl]acetimidate (**6**, $R^1 = CF_3$, $R^2 = CH_3$) as a yellow oil: yield 18.5 g (~68%); IR (film) ν_{max} 1640 cm^{-1}. Ammonia was then bubbled through the stirred crude imidate (18.5 g) with cooling for 2 h. After a further 16 h at room temperature, CH_2Cl_2 was added and the solid was removed by filtration. The organic phase was evaporated to leave a red oil (17 g), which partly dissolved in cyclohexane (700 mL). Undissolved material was removed by filtration and the solution evaporated to dryness to leave a solid (9.9 g), which was recrystallized from Et_2O/petroleum ether to afford **3i**: yield 5.4 g (23% from **5**); mp 95–97 °C. Anal. ($C_{11}H_9F_3N_4S$) C, H, N.

Compounds **3a** (18%), **3c** (69%), and **3m** (14%) were also prepared by the method described above.

The above procedure was used to prepare compounds **3b** (14%) and **3j** (59%) by replacing ethyl acetimidate hydrochloride by the appropriate ethyl carboximidate hydrochloride; compounds **3d** (39%), **3e** (41%), **3f** (24%), **3g** (44%), **3k** (44%), **3l** (14%), **3n** (36%), and **3o** (23%) were prepared by replacing ammonia with the appropriate amine. Although primary amines reacted satisfactorily at room temperature, the secondary amines required a temperature of 60–80 °C before reaction occurred. Anal. (**3l**) ($C_{21}H_{21}F_3N_4S$) H, N; C: calcd, 60.27; found, 60.76.

***N,N'*-Bis[5-(2-methylphenyl)-1,3,4-thiadiazol-2-yl]formamidine (3h).** A mixture of 2-amino-5-(2-methylphenyl)-1,3,4-thiadiazole (**5**, $R^1 = CH_3$) (1.74 g, 9.1 mmol) and ethyl orthoformate (7 mL, 42 mmol) was stirred for 4 days at room temperature. Anhydrous ether (15 mL) was added, and the crude product (1.5 g) was collected by filtration. Recrystallization from acetone gave **3h**: yield 1.2 g (34%); mp 183–187 °C; MS, 392 (M^+). Anal. ($C_{19}H_{16}N_6S_2$) C, H, N.

TLC (silica) of the reaction mixture indicated that a second product was formed, which could not be isolated. The mass spectrum revealed a signal at 247, which corresponds to M^+ for the imdidate **6** ($R^1 = CH_3$, $R^2 = H$).

Type 4 Amidines. 4,5-Dihydro-2-[5-(2-methylphenyl)-1,3,4-thiadiazol-2-yl]imidazole (4b). (a) 2-Cyano-5-(2-methylphenyl)-1,3,4-thiadiazole (8). Gaseous hydrogen chloride was bubbled through a solution of 2-(chloromethyl)-5-(2-methylphenyl)-1,3,4-thiadiazole[8] (**9**) (0.9 g, 4.02 mmol) and isoamyl nitrite (0.47 g, 4.02 mmol) in anhydrous dioxane (5.5 mL), with the temperature maintained below 50 °C. After 0.5 h, the gas stream was stopped, and after a further 1 h, the resulting 5-(2-methylphenyl)-1,3,4-thiadiazole-2-hydroxamic acid chloride was collected by filtration and washed with *n*-hexane: yield 0.46 g (45%). A mixture of this acid chloride (0.46 g, 1.82 mmol) and sodium azide (0.11 g, 1.7 mmol) in anhydrous DMF (3.2 mL) was stirred at room temperature for 6 h. The mixture was poured into water (10 mL), and the solid was collected by filtration and then dried to leave 5-(2-methylphenyl)-1,3,4-thiadiazole-2-hydroxamic acid azide: yield 0.43 g (91%). A mixture of the azide (0.33 g, 1.27 mmol) in glacial acetic acid (8 mL) was stirred at room temperature for 3 days. After filtration, the solution was evaporated to dryness and the residue was treated with water (10 mL). The resulting solid was collected and dried to leave the nitrile **8**: yield 0.2 g (60%; overall yield from **9** 25%); mp 92–94 °C; MS, 201 (M^+). Anal. ($C_{10}H_7N_3S$) H, N, C: calcd, 59.68; found, 59.21.

(b) 4,5-Dihydro-2-[5-(2-methylphenyl)-1,3,4-thiadiazol-2-yl]imidazole (4b). A mixture of **8** (1.3 g, 6.5 mmol) and ethylenediamine (0.43 g, 7.3 mmol) in ethanol (60 mL) was heated under reflux for 2 h. Solvent was removed under vacuum, and the residue was dissolved in $CHCl_3$ (18 mL) and treated with ethereal HCl. The solid (1.3 g) was collected and dissolved in water (120 mL). After filtration, the filtrate was basified with $NaHCO_3$ and was extracted with ether. The combined extracts were dried and evaporated to give **4b**: yield 0.9 g (57%); mp 113–114 °C; MS, 244 (M^+). Anal. ($C_{12}H_{12}N_4S$) H, N, C: calcd, 58.99; found, 58.45.

Compound **4c** (49%) was also prepared by the above procedure.

Attempt To Prepare 4b via Imidate 10. Gaseous hydrogen chloride was bubbled through a stirred solution of nitrile **8** (1 g, 4.98 mmol) and ethanol (0.27 g, 5.87 mmol) in ether (20 mL) with cooling at 0–10 °C for 0.5 h. The precipitate was collected and washed with ether to leave the imidate hydrochloride **10** (hygroscopic); yield 0.9 g (64%). TLC (silica; chloroform) indicated complete conversion of **8** (R_f 0.6) to **10** (R_f 0.4). A solution of **10** (0.9 g, 3.2 mmol) in ethanol (15 mL) was treated with ethylenediamine (0.27 g, 4.5 mmol) at 0–5 °C. After 48 h at this temperature, the mixture was filtered and the filtrate evaporated to dryness. The residue was dissolved in a minimum of ethanol and treated with ethereal HCl followed by the addition of more ether. The precipitate was collected and dried to leave a white powder (0.36 g).

TLC indicated a mixture of products that could not be isolated, although one appeared to be the required **4b** by comparison with an authentic sample. The mass spectrum also showed a peak at 244 (M^+ for **4b**).

***N*-Butyl-5-(2-methylphenyl)-1,3,4-thiadiazole-2-carboxamidine Hydrochloride (4a).** A mixture of **8** (1.54 g, 7.7 mmol) and *n*-butylamine (0.56 g, 7.7 mmol) in CH_2Cl_2 (24 mL) was stirred at room temperature for 3 days. Addition of ethereal HCl and crystallization of the precipitate from $(CH_3)_2CO/CHCl_3$ gave **4a**: yield 1.07 g (45%); mp 192–194 °C; MS, 274 (M^+). Anal. ($C_{14}H_{18}N_4S$) H, N; C: calcd, 54.10; found, 52.95.

Initially the above reaction was carried out at 100 °C for 6 h. TLC of the reaction product indicated the presence of two compounds, one of which corresponded to **4a**. The mass spectrum revealed peaks at 274 (M^+ for **4a**) and 331. The latter represents M^+ for the dibutylated product **4** ($R^2 = R^3 = n$-Bu).

Pharmacology. General Methods. Male mice (BKW, LACA origin) in the weight range 18–22 g were used in the mouse anticonvulsant and rotorod tests. Compounds were dissolved or suspended in a 2% mixture of Tween 80 in distilled water and injected intraperitoneally (ip) or per os (po). The vehicle was inactive in all the test procedures.

Maximal Electroshock Seizure Test. Maximal seizures were induced by application of an electric current across the brain via corneal electrodes.[11] The stimulus parameters for mice were 4-ms pulses of 50 Hz and 80 V for 0.3 s (SRI Ltd., square stimulator 6052 apparatus). The dose at which the hind limb tonic seizure was blocked in 50% of the animals (ED_{50} value) was determined by probit analysis; the computerized method of Bliss, described by Finney,[13] was used.

Maximal Metrazol Seizure Test. For the mouse metrazol test,[10] 120 mg/kg (leptazol) was injected intraperitoneally 1 h after oral dosage of the anticonvulsant drug.

Rotorod Test. Separate groups of mice were trained to stay on a rotorod that rotated at 16 rpm. The drum diameter was 3 cm. With trained animals, the ability to stay on the rod for the required duration was retained for up to 48 h.[12] Trained animals were dosed with the test compound or the standard drugs or drug vehicle and were tested at timed intervals to measure the effects of the drug on motor performance. The dose at which 50% of the animals fell off the rotorod (TD_{50} value) was determined by probit analysis.

Neurotoxicity. In addition to the scoring of neurotoxicity by the rotorod test, visual observation of sedation, ataxia, loss of righting reflex, and death after oral dosage of the anticonvulsant drugs were performed and noted prior to testing for anticonvulsant activity.

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Registry No. **3a**, 110718-29-1; **3b**, 110718-30-4; **3c**, 110718-31-5; **3c**·HCl, 110718-46-2; **3d**, 110718-32-6; **3d**·HCl, 110718-47-3; **3e**, 110718-33-7; **3f**, 110718-34-8; **3g**, 110718-35-9; **3h**, 110718-36-0; **3i**, 110718-37-1; **3j**, 110718-38-2; **3k**, 110718-39-3; **3k**·HCl, 110718-48-4; **3l**, 110718-40-6; **3m**, 110718-41-7; **3n**, 110718-42-8; **3o**, 110718-43-9; **4** ($R^2 = R^3 = n$-Bu), 110718-53-1; **4a**, 110718-54-2; **4a**·HCl, 110718-52-0; **4b**, 110718-56-4; **4b**·HCl, 110718-55-3; **4c**, 110718-58-6; **5** ($R^2 = H$), 2002-03-1; **5** ($R^1 = CH_3$), 59565-54-7; **5** ($R^1 = CF_3$), 10445-00-8; **5** ($R^1 = Ph$), 110718-44-0; **6** ($R^1 = CF_3$,

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$R^2 = CH_3$), 110718-45-1; 8 ($R^1 = CH_3$), 110718-51-9; 8 (R^1 = Ph), 110718-59-7; 9, 110718-49-5; 10 ($R^1 = CH_3$), 110718-57-5; $CH_3C(OEt)$=NH·HCl, 2208-07-3; $PhCH_2C(OEt)$=NH·HCl, 5442-34-2; $CH_3CH_2CH_2C(OEt)$=NH·HCl, 2208-08-4; $(CH_3)_2CHC(OEt)$=NH·HCl, 52070-18-5; ethyl ortho formate, 122-51-0; 5-(2-methylphenyl)-1,3,4-thiadiazole-2-hydroxaminic acid chloride, 110825-31-5; 5-(2-methylphenyl)-1,3,4-thiadiazole-2-hydroxamine acid azide, 110718-50-8; butylamine, 109-73-9; benzylamine, 100-46-9; diethylamine, 109-89-7; morpholine, 110-91-8; ethylenediamine, 107-15-3.

Specific Sequestering Agents for the Actinides. 16. Synthesis and Initial Biological Testing of Polydentate Oxohydroxypyridinecarboxylate Ligands[1]

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Chemical and biological similarities of plutonium(IV) and iron(III) suggested that octadentate ligands containing hydroxamate or catecholate functional groups, which are found in microbial iron chelating agents (siderophores), would be effective and relatively selective complexing agents for actinide(IV) ions. However, their usefulness for in vivo chelation of actinide(IV) is limited, because catechol and hydroxamate are such weak acids that the potential for octadentate binding of actinide(IV) cannot be achieved at physiological pH. The structurally similar monoprotic and more acidic 1-hydroxy-2(1*H*)-pyridinone (1,2-HOPO) group was, therefore, incorporated into multidentate ligands. Treatment of 1,2-dihydro-1-hydroxy-2-oxopyridine-6-carboxylic acid (**5**) with phosgene in THF solution gives the active ester poly[1,2-dihydro-1,2-dioxopyridine-6-carboxylate], which upon treatment with excess anhydrous dimethylamine gave a 60% yield of *N,N*-dimethyl-1,2-dihydro-1-hydroxy-2-oxopyridine-6-carboxamide (**6**). A similarly reactive intermediate was prepared from **5** and an equimolar amount of phosgene in *N,N*-dimethylacetamide. Combined in situ with 1,3-propanediamine, benzylamine, spermine, spermidine, 1,3,5-tris(aminomethyl)benzene, or desferrioxamine B and excess triethylamine, the latter intermediate gave the corresponding amides in isolated yields ranging from 16% to 60%. The free ligands, their Zn(II) complexes, and the ferric complex of 3,4,3-LIHOPO were administered to mice [30 μmol/kg intraperitoneally 1 h after Pu(IV)-238 citrate, kill at 24 h]. Net Pu removal [Pu excretion (treated) − Pu excretion (control)], expressed as percent of injected Pu, was as follows: Na salts and Zn(II) complexes, respectively, of 3-LIHOPO (54, 56), 3,4-LIHOPO (58, 60), 3,4,3-LIHOPO (73, 76); Na salts of MEHOPO (46), DFO-HOPO (78); Fe(III) complex of 3,4,3-LIHOPO (79). DFO-HOPO and 3,4,3-LIHOPO and its Zn(II) and Fe(III) complexes promoted significantly more Pu excretion than $CaNa_3$-DTPA (61% of injected Pu). Preliminary findings on the acute toxicity of the poly(HOPO) ligands and HOPO monomers are presented in an appendix. The biological data indicate strongly that the aqueous solubility and relatively high acidity of the octadentate HOPO ligands 3,4,3-LIHOPO and DFO-HOPO allow them to form complete eight-coordinate complexes with Pu(IV) ion.

The affinity and specificity of the siderophores [Fe(III) chelators produced by microorganisms[2,3]], prompted us[4-7] and others[8] to synthesize macrochelating agents composed of their binding groups, hydroxamic acid or catechol. Such ligands should be medically useful for removal and carrying of metal ions with similar charge radius ratios [e/r for Fe(III), Pu(IV), Ga(III), and In(III) is 0.465, 0.484, 0.375 and 0.417 e nm^{-1}, respectively[9]]. The coordination behavior of the tetravalent actinides [actinide(IV)] and Fe(III) are so similar that Pu(IV) can fit into the Fe(III) positions in mammalian iron transport and storage systems.[10,11] We have exploited that similarity by using the siderophore functional groups to construct sequestering agents specific for actinide(IV).[5,6,12-16]

The first such agents were based on enterobactin, a hexadentate ligand that coordinates to Fe(III) through the

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