Synthesis and Anticonvulsant Activity of Imidooxy Derivatives

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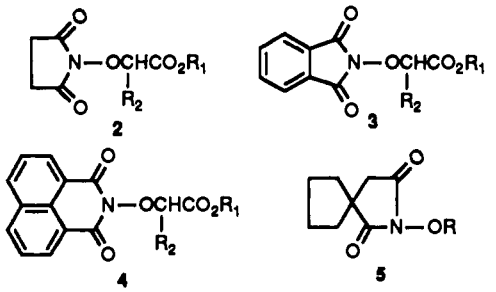
Previous results of anticonvulsant activity in several imidooxy carboxylates related to (aminooxy)acetic acid in young chicks, prompted an in-depth reinvestigation of these analogues in mice. A series of 22 succinimidooxy, phthalimidooxy, and naphthalimidooxy carboxylates were synthesized and evaluated for anticonvulsant activity by the National Institute of Neurological and Communicative Disorders and Stroke (NINCDS). Methyl (succinimidooxy)acetate (**2d**), ethyl (succinimidooxy)acetate (**2e**), methyl (phthalimidooxy)acetate (**3d**), ethyl (phthalimidooxy)acetate (**3e**), and ethyl 2-(phthalimidooxy)propionate (**3g**), which were initially found to be active as anticonvulsants in young chicks were uniformly inactive in the Phase I seizure tests involving maximal electroshock (MES), pentylenetetrazol (scMet), or neurologic toxicity toxicity (Tox). Several newer analogues, ethyl (succinimidooxy)formate (**2c**) and methyl 3-(phthalimidooxy)-2-methylacrylate (**4h**) were found to be active in the scMet (**3a**) or both (**4h**) evaluations. Most interesting was the anticonvulsant results of *N*-(benzyloxy)-2-azaspiro[4.4]nonane-1,3-dione (**5b**), which displayed anti-MES activity and a protective index (TD_{50}/ED_{50}) of >4.5.

γ-Aminobutyric acid (GABA)[1] has been inferred as being a major inhibitory neurotransmitter in the forebrain.[2] A deficiency in GABA has been further implicated in epilepsy, schizophrenia, and Huntington's and Parkinson's diseases.[3] In animal studies, it has been shown that blocking GABA-receptor-mediated events results in a convulsive state.[4] (Aminooxy)acetic acid (AOAA, **1**) was found to be a potent anticonvulsant.[5-10] AOAA was ini-

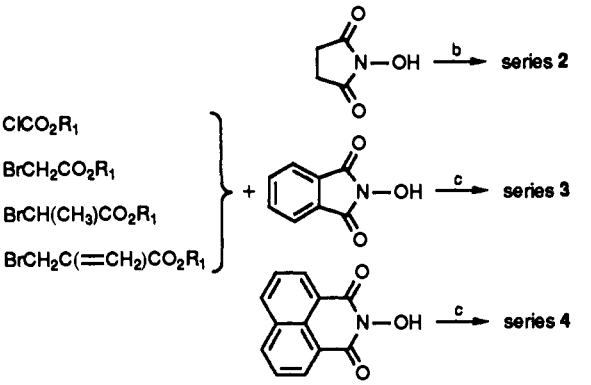
$$H_2NOCH_2CO_2H$$
1

tially thought to act solely by the inhibition of GABA-T, thus raising the brain levels of GABA.[11,12] It was later shown that AOAA exerts a dual anticonvulsant action, one involving GABA metabolism and another not involving GABA, depending upon the convulsant agent employed in the study.[13] Further study has revealed several serious side effects due to the use of AOAA as an anticonvulsant: it can induce convulsions at higher doses, and the anticonvulsant ED_{50} is only slightly lower than the LD_{50}.[14] It was thus hypothesized that molecular modification of the parent structure, **1**, was necessary to determine which portion of the structure was responsible for this lethal effect and to separate it from the anticonvulsant action. Our preliminary studies involved the synthesis and testing of a series of succinimidooxy (type **2**[15,16]) and phthalimidooxy (type **3**[17-19]) analogues of AOAA, in which the amino function is converted into an imido group and the acid group is esterified. In these studies it was shown that



several analogues were indeed active in young chicks, the most notable, ethyl (phthalimidooxy)acetate (ENPA, **3e**), had no convulsant tendency at doses many times higher than the convulsant dose of AOAA. GABA levels in these animals were elevated during the peak anticonvulsant effect of ENPA, similar to that noted with AOAA. We wish to report further studies on the synthesis of additional

Scheme I[a]



[a] R_1 = Me, Et. [b] Et_3N, THF. [c] NaOEt; H_2O/Me_2CO.

succinimidooxy and phthalimidooxy analogues of AOAA. In addition, we wish to extend the series to include na-

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(1) Abbreviations: GABA, γ-aminobutyric acid; AOAA, (aminooxy)acetic acid; GABA-T, 4-aminobutyrate:2-oxoglutarate aminotransferase; ENPA, ethyl (phthalimidooxy)acetate.
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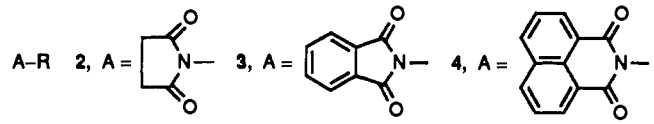
Table I. Physical Properties of Imidooxy Derivatives[a]



compd	R	% yield	mp, °C or bp, °C (mm)	formula	anal.[b]
2a	OH		90[c,d]	$C_4H_5NO_3$	
2b	OCH_3	71	104–106[e]	$C_5H_7NO_3$	C, H, N
2c	$OCO_2C_2H_5$	84	54–56[f]	$C_7H_9NO_5$	C, H, N
2d	$OCH_2CO_2CH_3$	62	131–132[g,h]	$C_7H_9NO_5$	
2e	$OCH_2CO_2C_2H_5$	83	78–80[h,i]	$C_8H_{11}NO_5$	
2f	$OCH(CH_3)CO_2CH_3$	74	136 (0.18)	$C_8H_{11}NO_5$	C, H, N
2g	$OCH(CH_3)CO_2C_2H_5$	73	133–135 (0.20)	$C_9H_{13}NO_5$	C, H, N
2h	$OCH_2C(=CH_2)CO_2CH_3$	67	88–90[e]	$C_9H_{11}NO_5$	C, H, N
3a	OH		240–241[j,k]	$C_8H_5NO_3$	
3b	OCO_2CH_3	74	137–138[h]	$C_{10}H_7NO_5$	C, H, N
3c	$OCO_2C_2H_5$	92	93–95[e]	$C_{11}H_9NO_5$	C, H, N
3d	$OCH_2CO_2CH_3$	72	143–144[e,l]	$C_{11}H_9NO_5$	
3e	$OCH_2CO_2C_2H_5$	80	96–97[h,m]	$C_{12}H_{11}NO_5$	
3f	$OCH(CH_3)CO_2CH_3$	81	84–86[n]	$C_{12}H_{11}NO_5$	C, H, N
3g	$OCH(CH_3)CO_2C_2H_5$	76	79–80[o,p]	$C_{13}H_{13}NO_5$	
3h	$OCH_2C(=CH_2)CO_2CH_3$	72	109–110[q]	$C_{13}H_{13}NO_5$	C, H, N
4a	ONa		>300[r]	$C_{12}H_6NO_3Na$	
4b	OCO_2CH_3	89	210–212[s]	$C_{14}H_9NO_5$	C, H, N
4c	$OCO_2C_2H_5$	94	198–199[t]	$C_{15}H_{11}NO_5$	C, H, N
4d	$OCH_2CO_2CH_3$	92	172–174[t]	$C_{15}H_{11}NO_5$	C, H, N
4e	$OCH_2CO_2C_2H_5$	92	148–149[t]	$C_{16}H_{13}NO_5$	C, H, N
4f	$OCH(CH_3)CO_2CH_3$	70	143–144[h]	$C_{16}H_{13}NO_5$	C, H, N
4g	$OCH(CH_3)CO_2C_2H_5$	77	109–110[h]	$C_{17}H_{15}NO_5$	C, H, N
4h	$OCH_2C(=CH_2)CO_2CH_3$	94	127–128[h]	$C_{17}H_{13}NO_5$	C, H, N
4i	OCH_3	70	210–211[h]	$C_{13}H_9NO_3$	C, H, N
5a	OH	50	117–118[u]	$C_8H_{11}NO_3$	C, H, N
5b	$OCH_2C_6H_5$	76	139–141[d]	$C_{15}H_{17}NO_3$	C, H, N
5c	$CH_2C_6H_5$	85	193–194 (1.5); 63.5[v]	$C_{15}H_{17}NO_2$	C, H, N

[a] The infrared and ^1H NMR spectra were consistent with assigned structures. Recrystallization solvents as indicated. [b] All compounds gave satisfactory C, H, N analyses (±0.4%). [c] Commercially available from Aldrich Chemical Co., Milwaukee, WI 53233. [d] EtOAc. [e] 2-Propanol. [f] 1-Octanol. [g] Reference 15, mp 128–130 °C. [h] EtOH. [i] Reference 15, mp 80–81 °C. [j] Aldrich Chemical Co. reports mp 233 °C dec. [k] EtOAc–Me_2CO. [l] Reference 18, mp 134–136 °C. [m] Reference 27, mp 95–97 °C. [n] Me_2CO–H_2O. [o] Reference 27, mp 79–80 °C. [p] Ligroine (70–90 °C)–Me_2CO. [q] EtOH–ligroine (70–90 °C). [r] Commercially available from Sigma Chemical Co., St. Louis, MO 63178. [s] Me_2CO–MeOH. [t] Me_2CO–EtOH. [u] Toluene. [v] MeOH.

phthalimidooxy and imidooxy analogues of 2-azaspiro-[4.4]nonane-1,3-dione, types **4** and **5**, respectively. We will report the anticonvulsant evaluation of these analogues in mice and evaluate their potential utility as anticonvulsants.

Results and Discussion

Chemistry. The imidooxy derivatives (**2a–h**, **3a–h**, and **4a–i**, Table I) were synthesized according to the procedures shown in Scheme I. As an example, *N*-hydroxysuccinimide, **2a**, was dissolved in an aprotic solvent (THF) in the presence of triethylamine. The haloester was then added and the reaction was allowed to stand at room temperature until completion (2–4 h). In the case of *N*-hydroxyphthalimide (**3a**), however, this compound was converted to its sodium salt before reaction with the halo esters in aqueous media. *N*-Hydroxynaphthalimide (**4a**) was commercially available as its sodium salt and was used in the same manner as **3a**. In addition to the reaction of *N*-hydroxyimides being conducted at room temperature, another advantage involved the use of the sodium salts of **3a** and **4a**, which developed a red color before the addition of the halo ester, the color disappearing at the end of the reaction.[18,19] The IR spectra of the imidoxy carboxylates showed three characteristic peaks between 1680 and 1800 cm^{-1}.

The coupling of the spiro nucleus to pharmacophoric groups has resulted in spiro succinimides with potential anticonvulsant activity;[20–24] thus two precursors of the imidoxy carboxylates were synthesized, the spiro benzoyloxy analogue, **5b**, and the *N*-hydroxy analogue, **5a**. These analogues were obtained from anhydride **6** as shown in Scheme II. The synthesis of **6** was improved over that previously reported in that 1-(cyanomethyl)-1-cyanocyclopentane, obtained by the addition of cyanide to ethyl α-cyclopentylidene-α-cyanoacetate, was isolated to remove it from the excess cyanide and then subjected to acid hydrolysis to convert it to 1-carboxycyclopentane-1-acetic acid, which was converted to its anhydride (**6**). Under controlled conditions, anhydride **6** was treated with benzyloxyamine or hydroxylamine (generated in situ)[25] to yield *N*-benzyloxy spiro succinimide **5b** and *N*-hydroxy spiro succinimide **5a**, respectively. In a manner reported previously,[23] anhydride **6** was reacted with benzylamine in refluxing xylene to provide, after fractional distillation, the desired **5c**.

Pharmacology. Preliminary pharmacological testing

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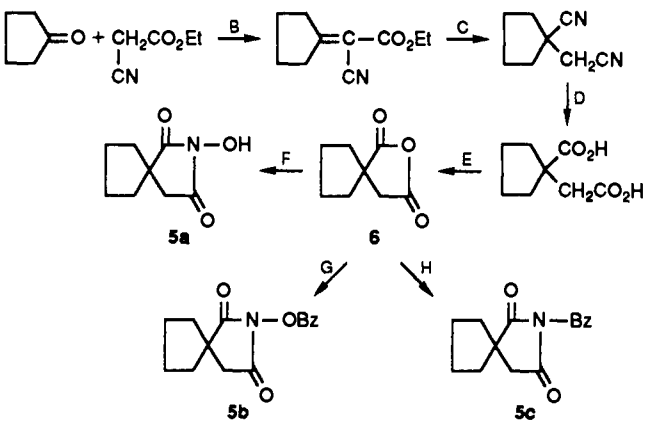
Table II. Anticonvulsant Screening Project (ASP): Phase I Test Results

compd	dose, mg/kg	activity: scMet[a] 30 min	scMet[a] 4 h	MES[b] 30 min	MES[b] 4 h	Tox[c] 30 min	Tox[c] 4 h	ASP classification[d]
2c	30	0/1	0/1	0/1	0/1	0/4	0/2	
	100	0/1	0/1	1/3 (0/4)	0/3	0/8	4/4	1
	300	1/1 (2/4)	–	0/1 (0/4)	–	4/4 (8/8)	2/2[e]	
3a	30	0/1	0/1	0/1	0/1	0/4	0/2	
	100	0/0[f]	–	2/3	–	8/8[g]	4/4[h]	1
	300	–	–	–	–	4/4[h]	–	
3h	30	1/1 (2/4)	1/1	0/1	0/1	0/4 (0/4)	2/2[i]	
	100	1/1 (4/4)	–	0/3	–	0/8 (0/4)	4/4[e]	4
	300	0/0[f]	–	1/1	–	4/4[f]	2/2[e]	
4a	30	0/1	0/1	0/1	0/1	0/4	0/2	
	100	0/1	0/1	0/3	0/3	0/8	0/4	2
	300	1/1 (2/4)	–	0/4[j]	–	3/4 (3/4)	2/2[e]	
5a	30	0/1	0/1	0/1	0/1	0/4	0/2	
	100	0/1	0/1	0/3	0/3	0/8	0/4	3
	300	–	–	–	–	4/4[h]	–	
5b	30	0/1	0/1	0/1	0/1	0/4	0/2	
	100	0/1	0/1	0/3	0/3	0/8	0/4	2
	300	0/1	0/1	1/1	1/1	0/4	0/2	
5c	30	0/1	0/1	0/1	0/1	0/4	0/2	
	100	0/1	0/1	0/3	0/3	0/8	0/4	3
	300	0/1	0/1	0/1	0/1	0/4	0/2	

[a] Subcutaneous pentylenetetrazol test (number of animals protected/number of animals tested). [b] Maximal electroshock test. [c] Toxicity (number of animals exhibiting toxicity/number of animals tested). [d] The classifications are as follows: 1, anticonvulsant activity at 100 mg/kg or less; 2, anticonvulsant activity at doses greater than 100 mg/kg; 3, no anticonvulsant activity at doses up to and including 300 mg/kg; 4, anticonvulsant activity at 30 mg/kg or less. Results in parentheses are the results of a second trial. [e] Died before 4 h. [f] Died without having a seizure. [g] Six anesthesized, two lost righting reflexes. [h] Died from respiratory depression. [i] Unable to hold onto rotorod. [j] Toxic at 30 min.

Scheme II[a]



[a] B = HOAc, NH_4OAc; C = KCN; D = HCl; E = Ac_2O; F = NH_2OH·HCl, Na_2CO_3; G = $BzONH_2$·HCl, $NaHCO_3$; H = $BzNH_2$, molecular sieves.

of the compounds listed in Table I, except **4i**, has been provided by the Antiepileptic Drug Development Program, Epilepsy Branch, Neurological Disorders Program, National Institutes of Neurological and Communicative Disorders and Stroke (NINCDS), by testing procedures that have been described.[26] Phase I results of the active moieties in mice are shown in Table II. The three tests were maximal electroshock seizure (MES), subcutaneous pentylenetetrazol (scMet), and neurologic toxicity (Tox). Although compounds **2d**, **2e**, **3d**, **3e**, and **3g** have been reported to possess anticonvulsant activity in young chicks with an undeveloped blood–brain barrier,[15,16,18,19] there has been no report to confirm this anticonvulsant activity in mice. These compounds, as well as the *N*-hydroxy derivatives **2a**, **3a**, **4a**, and **5a** were also submitted for anticonvulsant screening. The previously active compounds **2d**, **2e**, **3d**, **3e**, and **3g** were uniformly inactive in the Phase I screening procedure. This inactivity may, as previously indicated, have been due to an undeveloped blood–brain barrier in the test animals or the ability of young chicks to biotransform these compounds into active anticonvulsant metabolites. Surprisingly, two of the starting *N*-hydroxy compounds, **3a** and **4a**, were quite potent but also very toxic. *N*-Hydroxyphthalimide (3a) was effective in the MES evaluation at 100 mg/kg at 30 min, but was toxic to all of the animals at that dosage either at 30 min or 4 h after administration. *N*-Hydroxynaphthalimide (**4a**) (as the sodium salt) was effective in the pentylenetetrazol evaluation at 300 mg/kg and like **3a** was quite toxic to the animals at that dosage at 30 min or at 4 h. Ethyl (succinimidooxy)formate (**2c**) displayed potency in the pentylenetetrazol evlauation at 300 mg/kg at 30 min and an initial activity in the MES evaluation; however a second trial indicated an error in the initial result. The 4-h toxicity at 100 and 300 mg/kg was alarming however. Acrylate **3h** proved to be the most potent analogue, displaying activity in the pentylenetetrazol test at 30 mg/kg; however, as with formate **2c** there appeared to be a delayed toxicity at 4 h at 30 and 100 mg/kg. Since none of the naphthalimidooxy derivatives were active, except for the starting material, planarity in this series paralleled the earlier finding of Davis,[21] who found that the out-of-plane spiro dibenzo[*a*,*d*]cycloheptadienyl analogues were more active than the planar derivatives. This was recently reaffirmed by Doukas,[24] who indicated that the planar fluorenyl analogue had limited conformational flexibility when compared to the out-of-plane indanyl analogues that were active. As a result of these tests, starting *N*-hydroxyimides **3a** and **4a** and acrylate **3h** were advanced to Phase II trials for quantification of their anticonvulsant activity and

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Table III. Phase II Quantification Data

compd	ED_{50}[a,b] MES	ED_{50}[a,b] scMet	TD_{50}[a,c]	PI[d] MES	PI[d] scMet	TPE[e] activity	TPE[e] toxicity
4a	-	89.57 (48.93–134.0)	76.03 (53.54–103.4)	-	0.84	0.25	4.00
3a	95.38 (81.62–104.5)	-	94.54 (89.56–99.05)	0.99	-	0.25	0.25
3h	-	25.42 (16.29–36.61)	18.18 (15.67–21.03)	-	0.72	1.00	4.00
5b	111.2 (100.4–122.5)	-	>500	4.50	-	0.25	4.00
phenytoin	9.50 (8.13–10.44)	*f*	65.46 (52.49–72.11)	6.89	-	2.00	2.00
carbamazepine	8.81 (5.45–14.09)	*f*	71.56 (45.91–134.7)	8.12	-	0.25	0.25
phenobarbital	21.78 (14.99–25.52)	13.17 (5.87–15.93)	69.01 (62.84–72.89)	3.17	5.24	1.0	0.5
valproate	271.7 (247.0–337.9)	148.6 (122.6–177.0)	425.8 (368.9–450.4)	1.57	2.87	0.25	0.25
ethosuximide	>1000	130.4 (111.0–150.5)	440.8 (383.1–485.3)	-	3.38	0.5	0.5

[a] ED_{50} and TD_{50} values are in milligrams/kilograms of test drug delivered intraperitoneally (ip). [b] Measured at time of peak effect. [c] Measured at time of peak neurologic deficit. [d] PI = protective index (TD_{50}/ED_{50}). [e] Time of peak effect. TPE for activity determined in the MES test for compounds **3a** and **5b** and in the scMet test for **4a** and **3h**. Numbers in parentheses are 95% confidence limits. [f] Not effective.

Table IV. Delayed Toxicity of Methyl 2-(Phthalimidooxy)acrylate (**2h**)

dose, mg/kg	time, h	activity scMet	activity tox
30	0.25	4/8	0/8
	0.50	4/8	0/8
	1.00	8/8	1/8
	2.00	8/8	8/8
	4.00	-	8/8
	6.00	-	8/8
50	0.50	-	4/8
	1.00	-	7/8
	2.00	-	6/8

neurotoxicity by determining the median effective dose (ED_{50}) and median toxic dose (TD_{50}).

The spiro[4.4]nonane series was highly significant in that the *N*-benzyloxy analogue **5b** was effective against MES at 300 mg/kg and also displayed a remarkable lack of neurologic toxicity which was an important advantage over the previous series. In contrast, the *N*-hydroxy analogue **5a** displayed no seizure protection in the MES evaluation and exhibited a high degree of toxicity at 300 mg/kg in which all of the test animals died of respiratory depression. Of further interest was the fact that **5c**, the *N*-benzyl analogue, proved ineffective as an anticonvulsant in either test, although it displayed no toxicity at 300 mg/kg. Thus, the benzyloxy analogue **5b** was also advanced to Phase II evaluation.

Phase II evaluation data is provided in Table III. Acrylate **3h**, as noted in Phase I data, was active at 30 mg/kg at 30 min without evident toxicity. This fact was also noted at 100 mg/kg at 30 min. However, toxicity was observed at 4 h at each dosage. Phase II data indicates a TD_{50} at 4 h, which is less than the ED_{50} at 15 min, resulting in a negative protective index. This is shown in Figure 1 and Table IV. This consistency of Phase I and Phase II observations indicates a strong possibility of a toxic metabolite being produced during the evaluation. Hydrolysis of the succinimidooxy analogues has been reported,[15,16,27] further substantiating this hypothesis. Both starting *N*-hydroxyimides **3a** and **4a** also proved to be too toxic for further evaluation. Compound **5b**, however, was both safe and effective with an ED_{50} of 111.2 mg/kg and a TD_{50} greater than 500 mg/kg, providing a protective index greater than 4.5. This compares favorably with the currently available anticonvulsants shown in Table III.

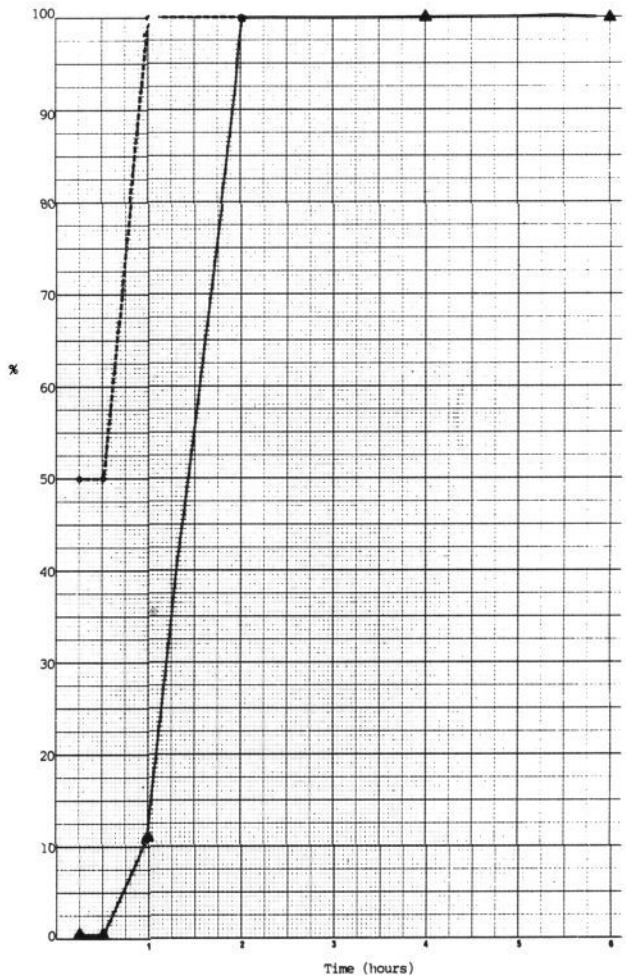

Figure 1. Delayed toxicity of methyl 2-(phthalimidooxy)acrylate (**2h**) at 30 mg/kg (see Table IV): (●) scMet activity, (▲) toxicity, (%) percentage of animals protected or exhibiting toxicity.

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Benzyl substitution on active anticonvulsant pharmacophores[28-30] has provided variable results. Benzoyloxy substitution represents a unique, and hitherto unreported, departure from this well-worn pathway. Further research in this area is continuing in our laboratory and will be reported shortly.

Experimental Section

Chemistry. Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Observed boiling points were also uncorrected. IR spectra were recorded on samples in Nujol, or as diluted chloroform solutions in matched sodium chloride cells or neat with a Perkin-Elmer 1330 spectrophotometer. ^{1}H NMR spectra were recorded on a General Electric QE 300-MHz spectrometer in deuterated solvents using tetramethylsilane as an internal reference. Elemental analyses (C, H, N) were performed by Schwarzkopf Microanalytical Laboratory, Woodside, NY. Where analyses are indicated only by the symbols of the elements, analytical results for the elements were within 0.4% of the theoretical values. Experimental data for all of the imidooxy compounds are provided in Table I. Ethyl α-cyclopentylidene-α-cyanoacetate,[31,32] 1-carboxycyclopentane-1-acetic acid,[33,34] and 1-carboxycyclopentane-1-acetic acid anhydride (**6**)[31] were prepared by literature procedures; however, in the case of anhydride **6**, acetyl chloride was replaced with acetic anhydride with comparable yields. Typical experiments illustrating the general procedures for the preparation of the imidooxy derivatives **2–5** are described below.

Ethyl (Succinimidooxy)formate (2c). A solution of *N*-hydroxysuccinimide (5.7 g, 50 mmol) in dry THF (80 mL) was placed in a 250-mL three-neck flask equipped with a condenser, a magnetic stirrer, and a pressure-equalizing addition funnel. The contents were cooled on an ice bath, and ethyl chloroformate (7.6 g, 70 mmol) was added dropwise over 5 min followed by dry THF (10 mL). The mixture was stirred, and triethylamine (7.6 g, 75 mmol) in dry THF (10 mL) was added dropwise over 10 min with continuous stirring (CAUTION: too rapid addition of the triethylamine without adequate cooling on an ice bath may cause the reaction mixture to erupt). A thick white precipitate was formed and allowed to warm up to room temperature with stirring for 2 h. The reaction mixture was filtered, and the solvent was removed under reduced pressure to give a reddish-brown residue. Recrystallization from 1-octanol afforded 7.9 g (84%) of **2c** as white glassy crystals: mp 54–56 °C; NMR ($CDCl_3$) δ 1.42 (t, 3 H, J = 6 Hz, CH_3), 2.85 (s, 4 H, succinimido ring), 4.40 (q, 2 H, J = 6 Hz, CH_2); IR ($CHCl_3$) 3000 (CH), 1800 (C=O), 1780 (C=O), 1730 (C=O) cm^{-1}.

Methyl 2-(Phthalimidooxy)propionate (3f). To a freshly prepared solution of sodium (2.3 g, 0.10 mol) in absolute ethanol (60 mL) was added a solution of *N*-hydroxyphthalimide (16.3 g, 0.10 mol) in absolute ethanol (350 mL), and the red reaction mixture was stirred at room temperature for 30 min. The brick red precipitate was collected, washed with water, and dried in the oven at 100 °C for 30 min to give 17.45 g (95%) of sodium phthalimide oxide as brick red crystals; mp >300 °C. To the solution of sodium phthalimide oxide (0.92 g, 5 mmol) in water (15 mL) was added acetone (10 mL), followed by a solution of methyl 2-bromopropionate (1.17 g, 7 mmol). The reaction mixture was stirred at room temperature for 16 h, during which the red color disappeared. On standing at room temperature for 48 h, the product solidified in the aqueous mixture and was collected. Recrystallization from acetone–water gave 1.01 g (81%) of **3f** as white crystals: mp 84–86 °C; NMR ($CDCl_3$) δ 1.65 (d, 3 H, J = 6 Hz, CH_3), 3.81 (s, 3 H, OCH_3), 4.90 (q, 1 H, J = 6 Hz, CH), 7.82 (m, 4 H, phthalimido ring); IR ($CHCl_3$) 1770 (C=O), 1730, sh (C=O), 1715 (C=O) cm^{-1}.

Methyl 3-(Naphthalimidooxy)-2-methylacrylate (4h). To a solution of sodium naphthalimide oxide, **4a** (1.18 g, 5 mmol), in water (50 mL), was added methyl 2-(bromomethyl)acrylate (1.25 g, 7 mmol) in acetone (10 mL). The red reaction mixture was stirred at room temperature. The red color disappeared within 5 min and the reaction mixture was filled with a white precipitate. After standing for 4 h, the white precipitate was collected, washed with water, and recrystallized from ethanol to give 1.46 g (94%) of **4h** as pale fluffy crystals: mp 127–128 °C; NMR ($CDCl_3$) δ 3.82 (s, 3 H, OCH_3), 5.04 (s, 2 H, OCH_2), 6.10 (s, 1 H, =CH), 6.50 (s, 1 H, =CH), 7.80–8.70 (m, 6 H, naphthalimido ring); IR ($CHCl_3$) 1718 (C=O), 1684 (C=O), 1588 (C=C) cm^{-1}.

***N*-Hydroxy-2-azaspiro[4.4]nonane-1,3-dione (5a).** The method of Bauer and Miarka was employed.[25] Hydroxylamine hydrochloride (8.60 g, 0.12 mol) was dissolved in water (20 mL) and sodium carbonate (6.72 g, 0.06 mol) was slowly added. When all the solid had dissolved, anhydride **6** (15.4 g, 0.10 mol) was slowly added and the mixture was heated to 60–70 °C for 1 h. The mixture was refrigerated for 16 h. The solid material was separated and washed with cold 5 M HCl (2 × 10 mL). The filtrate yielded additional product on further refrigeration. Recrystallization of the total yield from toluene gave 8.4 g (50%) of **5a** as white crystals: mp 117–118 °C; NMR ($CDCl_3$) δ 1.73–2.20 (m, 8 H, cyclopentane ring), 2.65 (s, 2 H, CH_2), 7.30 (br s, 1 H, OH); IR ($CHCl_3$) 3150 (OH), 2950 (CH), 1760 (C=O), 1705 (C=O) cm^{-1}.

***N*-(Benzyloxy)-2-azaspiro[4.4]nonane-1,3-dione (5b).** (Benzyloxy)hydroxylamine hydrochloride was converted into its free base as follows: (benzyloxy)hydroxylamine hydrochloride (25.0 g, 0.16 mol) was dissolved in water (25 mL) and a 10% $NaHCO_3$ solution (150 mL) was added. After the effervescence ceased, the free base was extracted with EtOAc (100 mL). The organic layer was dried (Na_2SO_4) and distilled at atmospheric pressure to remove the solvent and then under reduced pressure to obtain benzyloxyamine: bp 35–36 °C (1.4 mm). It should be noted that the first 5 mL of distillate should be discarded even though the bp was constant.[35] The condensation reaction followed the method of Groutas and co-workers.[36] Spiro anhydride **6** (6.63 g, 43 mmol) was dissolved in dry toluene (36 mL), and the mixture was stirred and heated to reflux. Benzyloxyamine (5.41 g, 44 mmol) in dry toluene (18 mL) was added over 30 min while a constant reflux temperature was maintained. After the addition, the mixture was refluxed an additional 30 min, the hot solution was filtered through anhydrous Na_2SO_4, and the solvent was removed under reduced pressure. The residue was dissolved in EtOAc (100 mL) and washed with 10% $NaHCO_3$ solution (50 mL). Removal of the solvent under reduced pressure yielded a solid which was recrystallized from EtOAc to give 8.50 g (76%) of **5b** as white crystals: mp 139–141 °C; NMR (CD_3COCD_3) δ 1.72–2.82 (m, 8 H, cyclopentane ring), 2.61 (s, 2 H, CH_2), 5.08 (s, 2 H, OCH_2), 7.39–7.48 (m, 5 H, aromatic); IR (Nujol) 1775 (C=O), 1710 (C=O) cm^{-1}.

***N*-Benzyl-2-azaspiro[4.4]nonane-1,3-dione (5c).** To a mixture of anhydride **6** (10 g, 0.06 mol) in 10 mL of dry xylene containing 4A molecular sieves was added benzylamine (10.72 g, 0.10 mol). The mixture was stirred and refluxed for 4 h and then fractionally distilled under reduced pressure to yield 12.4 g (85%) of **5c**, bp 193–196 °C (1.50 mm), which solidified on standing. The product was recrystallized from MeOH to give an analytical sample, mp 63.5 °C, as white prisms: IR (Nujol) 1780 (C=O), 1715 (C=O) cm^{-1}. Anal. ($C_{15}H_{17}NO_3$) C, H, N.

Pharmacology. The anticonvulsant evaluation was performed by the Antiepileptic Drug Development Program, Epilepsy Branch, Neurological Disorders Program, National Institute of Neurological and Communicative Disorders and Stroke and included Phases I and II testing procedures, which have previously been described.[26] These tests were performed in male Carworth Farms #1 mice. Phase I of the evaluation included three tests:

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maximal electroshock (MES), subcutaneous pentylenetetrazol (scMet), and the rotorod test for neurological toxicity (Tox). Compounds were either dissolved or suspended in 30% polyethylene glycol 400 and were administered by intraperitoneal injection at three dosage levels (30, 100, and 300 mg/kg) with anticonvulsant activity and neurotoxicity noted 30 min and 4 h after administration. Phase II testing quantitated the anticonvulsant activity and neurotoxicity observed for the most promising compounds in Phase I. Thus, **3a**, **3h**, **4a**, and **5b** were evaluated in this development scheme. Data for these compounds is seen in Tables III and IV. Phase II determined the median effective dose (ED_{50}) and median toxic dose (TD_{50}). The ED_{50} and TD_{50} values and their confidence limits were determined at the time of peak effect of each compound by the method of Litchfield and Wilcoxon.[37]

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Synthesis of Chiral and Achiral Pyranenamine Derivatives. Potent Agents with Topical Ocular Antiallergic Activity

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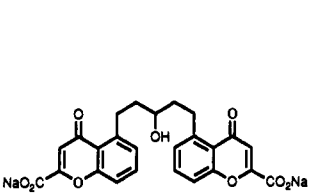
The *SS*, *RR* and meso stereoisomers of pyranenamine SK&F 84210 (**2**) were synthesized stereospecifically starting from commercially available (*R*)-(−)- or (*S*)-(+)-2,2-dimethyl-1,3-dioxolane-4-methanol (**3**). In addition, two achiral pyranenamines **19** and **26** were also synthesized. When evaluated by intravenous and topical routes in the rat passive ocular anaphylaxis (POA) assay, (*SS*)- and *meso*-**2** as well as achiral compounds **19** and **26** were found to be more potent antiallergic agents than (*RR*)-**2**.

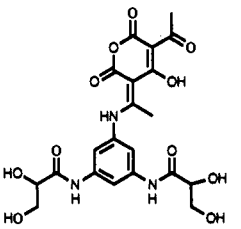
Ocular allergic diseases such as seasonal allergic conjunctivitis, chronic conjunctivitis, giant papillary conjunctivitis, and vernal keratoconjunctivitis are examples of type I immediate hypersensitivity reactions.[1] In individuals afflicted with these diseases, exposure to exogenous antigens (pollen, dust, animal dander, etc.) produces IgE antibodies which bind to the surface of mast cells present in ocular tissue. Degranulation of mast cells releases various inflammatory mediators including histamine, serotonin, platelet-activating factor, leukotrienes, etc. These mediators produce the symptoms of red, itchy, watery eyes, foreign-body sensation, and inflamed tissue, which are characteristic of most types of conjunctivitis.[2] In severe cases (e.g., vernal keratoconjunctivitis) the symptoms can also include photophobia and inflammation or ulceration of the cornea, which can lead to blindness.[3]

The problems associated with traditional therapies for ocular allergic diseases are well-known.[3] Topical antihistamine–vasoconstrictors lack efficacy in all but the mildest cases since they only block release of histamine, just one of the mediators responsible for the symptoms which are observed. Steroids relieve the symptoms of many types of conjunctivitis but also have significant side effects including increased risk of glaucoma, cataract formation, and enhancement of epithelial herpetic keratitis, which preclude their use in many cases.

More recently, the development of agents which may prevent the mast cell from degranulating, and thus prevent the release of inflammatory mediators, has shown potential for treating various allergic diseases.[4] The lead compound in this area, disodium chromoglycate (DSCG, **1**) has been



DSCG
1



SK&F 84210
2

marketed for the treatment of asthma. The recent introduction of DSCG in an ophthalmic formulation has provided an alternative therapy for the prophylactic treatment of allergic ocular diseases.[2] It has also prompted us to investigate the topical efficacy of other, more potent compounds as therapeutic agents for the treatment of ocular allergic diseases.

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