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Spiranes 6. Ring A homologues of *N*-benzyloxy-2-azaspiro[4.4]nonane-1,3-dione. Synthesis, X-ray analysis and anticonvulsant evaluation

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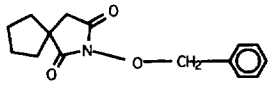
Summary — A series of spirosuccinimides was synthesized and evaluated for anticonvulsant activity. The study was designed to determine the effect of varying the carbocyclic (ring A) nucleus, while maintaining the heterocyclic ring constant, on anticonvulsant activity. Results indicate that maximum activity was obtained with the ring A comprised of a six-membered spiro ring system, **2a**, one methylene group greater than that previously reported for *N*-(benzyloxy)-2-azaspiro[4.4]nonane-1,3-dione, **1**, the prototype analogue. Compound **2a** was active in the MES test providing protection at 100 mg/kg, as was the spirododecane analog **2g**. X-ray analysis revealed significant differences between active **2a**, and the inactive spirooctane analogue, **2f**. However these differences could not explain the unexpected activity demonstrated by the spirododecane analog **2g**.

spirosuccinimide / anticonvulsant activity / X-ray crystallography / molecular modeling / structure–activity relationship

Introduction

As part of a continuing study of the introduction of the spiro nucleus into pharmacophoric groups [1–5], the investigation of spirosuccinimides as potential anticonvulsants was initiated [2, 4, 5]. We had previously reported [4] the anticonvulsant activity of *N*-(benzyloxy)-2-azaspiro[4.4]nonane-1,3-dione **1**, which displayed protection against MES, providing a protective index (TD_{50}/ED_{50}) of > 4.5. In addition, we also



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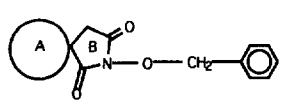
reported that substitution on the aromatic ring of **1** enhanced anticonvulsant activity. It was noted in a subsequent study in our laboratory that the disubstituted 2,4-dichloro; the *para*-substituted trifluoromethyl, the *meta*-chloro and -fluoro, and the *ortho*-bromo and -fluoro analogues each possessed significant anti-MES and/or protection against PTZ activity over the parent compound [5]. This report will provide further structure–activity relationship data on the benzyloxy azaspiro system in which the carbocyclic ring (ring A, **2**) is varied, while maintaining the



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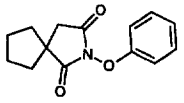
Abbreviations. MES: maximal electroshock; TD_{50}: toxic dose for 50% of test animals; ED_{50}: effective dose for 50% of test animals; PTZ: subcutaneous pentylenetetrazol; Tox: neurologic toxicity; ip: intraperitoneal; ADD: antiepileptic drug development; NINDS: National Institutes of Neurological Disorders and Stroke; TTE: threshold tonic extension; [^{3}H]BTX-B: [^{3}H]-batrachotoxinin A 20-benzoate; IC_{50}: 50% in vitro inhibition of binding of [^{3}H]BTX-B to sodium channels in rat brain synaptoneurosomes; CF1: Carworth Farms No 1; MP: melting point; BP: boiling point.

constant succinimide ring (ring B, **2**), with the unsubstituted *N*-benzyloxy moiety, and anticonvulsant activity, both MES as well as PTZ, being evaluated. These analogues were synthesized as shown in scheme 1.

The requirement for the methylene bridge linking the spiro system to the aromatic nuclei will also be investigated. Accordingly, *N*-phenoxy-2-azaspiro[4.4]-nonane-1,3-dione **7** was prepared and evaluated for activity. The latter synthesis involved the modification of the one-step procedure of Beringer and coworkers [6], using *N*-hydroxy-2-azaspiro-[4.4]nonane-1,3-dione [4] **6** (A = $CH_2)_4$) and diphenyliodonium chloride (scheme 2).



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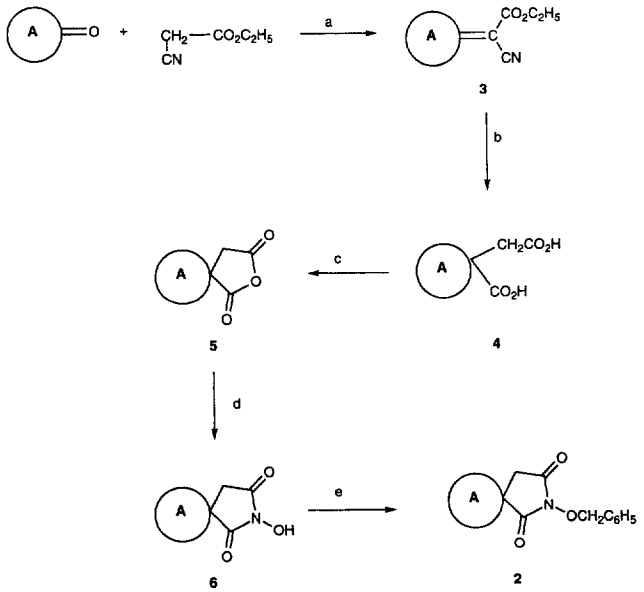
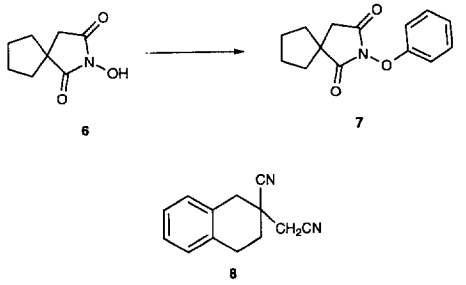
Chemistry

The synthesis of analogues of compound **2** followed our previously reported scheme (scheme 1) [2, 5, 7]. The appropriate ketone was treated with ethyl cyanoacetate in a Knoevenagel reaction [8]. The product, a cycloalkylidene **3** was treated with potassium cyanide, and after hydrolysis of the intermediate cyano ester [3], the diacid **4** was formed [9]. The diacid **4**



Scheme 1. Reaction conditions: a = AcOH, NH_4OAc; b = KCN, HCl; c = Ac_2O; d = NH_2OH·HCl, Na_2CO_3; e = NaoMe, $C_6H_5CH_2Cl$.



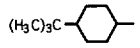
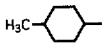
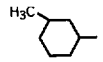
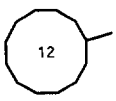
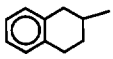
Scheme 2. Reaction conditions: $(C_6H_5)_2ICl$, K_2CO_3, DMSO.

was converted into anhydride **5** [10], which, under controlled conditions, was subsequently treated with hydroxylamine (generated in situ) [4, 11] to produce the respective *N*-hydroxyspirosuccinimide **6**. The final product, the *N*-(benzyloxy)spirosuccinimide **2** was produced by the 'one-pot' method of Lange and coworkers [12] which involved conversion of **6** to its sodio derivative (in situ), followed by addition of benzyl chloride and refluxing overnight. Alternatively, the ring A diacid **4** as previously indicated by Rice et al [13] could be isolated in crude form, and converted into the crude anhydride **5**, which provided the final succinimides in yields comparable to the step-wise isolation of each intermediate. We now employed this modification in our current work. Thus, the crude anhydride **5** was treated with hydroxylamine and the isolated *N*-hydroxyspiro succinimide **6** was subsequently treated with benzyl chloride to provide the desired *N*-benzyloxyspiro succinimide, **2**. The percent yield data in table I is based on the crude anhydride. Due to inherent steric problems, the β-tetralone series (**2h**) led to lower yields than with the other analogues.

X-ray analysis

In an attempt to correlate the activity of analogs of compound **2** with their molecular structure, the X-ray structural analysis of the highly active **2a**, and the inactive spirooctane analog **2f**, was planned. Due to the difficulties in obtaining suitable single crystals of **2a**, the structure of this compound was modeled with Chem-X [14] on the basis of the previous determination of the crystal structure of **2b**, the 4-*tert*-butyl-substituted analogue. The X-ray analysis of **2f** was determined to ascertain whether the difference in anticonvulsant activity was related to the three-dimensional aspects of these compounds. The summary of the intensity data collection and structure refinement is given in table II, and the atomic coordinates of **2b** and

Table I. Ring A 2-azaspiro-1,3-dione analogues **2**[a].

Compound	*Ring A*	*% yield*	*Mp (°C)*	*Formula*	*^{1}H NMR data (δ ppm)*[b]
2a		51.0	149–141	$C_{16}H_{19}NO_3$	1.15–1.82 (10H, m, cyclohexane ring); 2.41 (2H, S, CH_2 of pyrrolidino ring); 5.13 (2H, s, OCH_2); 7.30–7.50 (5H, m, C_6H_5)
2b		78.6	172.5–173.5	$C_{20}H_{27}NO_3$	0.84 (9H, s, *tert*-butyl); 1.06–1.85 (9H, m, cyclohexane ring); 2.39 (2H, s, CH_2 of pyrrolidino ring); 5.14 (2H, s, OCH_2); 7.30–7.51 (5H, m, C_6H_5)
2c		17.9	150–151.5	$C_{17}H_{21}NO_3$	0.91 (3H, s, CH_3); 0.81–1.86 (9H, m, cyclohexane ring); 2.39 (2H, s, CH_2 of pyrrolidino rings); 5.13 (2H, s, OCH_2); 7.34–7.51 (5H, m, C_6H_5)
2d		65.4	142–143	$C_{17}H_{21}NO_3$	0.91 (3H, s, CH_3); 0.89–1.82 (9H, m, cyclohexane ring); 2.41 (2H, s, CH_2 of pyrrolidino ring); 5.14 (2H, s, OCH_2; 7.35–7.51 (5H, m, C_6H_5)
2e		60.0	112–113	$C_{17}H_{21}NO_3$	1.30–1.98 (12H, m, cycloheptane ring); 2.36 (2H, s, CH_2 of pyrrolidino ring); 5.13 (2H, s, OCH_2); 7.28–7.50 (5H, m, C_6H_5)
2f		53.4	113–114	$C_{18}H_{23}NO_3$	1.29–1.92 (14H, m, cyclooctane ring); 2.41 (2H, s, CH_2 of pyrrolidino ring); 5.13 (2H, s, OCH_2); 7.31–7.51 (5H, m, C_6H_5)
2g		17.7	96–97	$C_{19}H_{25}NO_3$	0.87–1.78 (22H, m, cyclododecane ring); 2.41 (2H, s, CH_2 of pyrrolidino ring); 5.14 (2H, s, OCH_2); 7.34–7.52 (5H, m, C_6H_5)
2h		65.4	142–143	$C_{17}H_{21}NO_3$	2.02–3.30 (8H, m, 4 × CH_2); 5.14 (2H, OCH_2); 7.00–7.60 (9H, m, C_6H_4 + C_6H_5)

All compounds gave satisfactory C, H, N analyses (± 0.4%). [a]The infrared spectra were consistent with assigned structures. [b]Recorded on a 300 MHz spectrometer (see the *Experimental protocols*). All compounds determined in $CDCl_3$.

2f are provided in tables III and IV, respectively. The two molecules present in an asymmetric unit of **2b** are the two enantiomeric forms and do not differ significantly either in shape (fig 1) or in the values of the corresponding bond lengths. The overall shape of these molecules are similar to that of compound **2f**, shown in figure 2, except for the difference in the spiro-substituted carbocyclic rings. A significant difference in activity of these molecules may be related to the three-dimensional space occupied by the cyclooctane portion of **2f** in comparison to **2a.** The region of dissimilarity is best illustrated in figure 3 obtained with Chem-X [14] by superimposition of the heterocyclic five-membered rings of **2a** and **2f** and it corresponds to the exclusive van-der-Waals volume of carbocyclic rings which is equal to 36.2 Å^3 [14]. Both rings, however, have the same C_5 symmetry, with a pseudo-mirror plane through atoms C_{12} and C_{16} in the cyclooctane ring, and through atoms C_{10} and C_{14} (following the numbering scheme for compound **2b**) in the cyclohexane ring, which has a chair conformation. It is also worth noting that the van-der-Waals volume of the active **2a** is equal to 202.6 Å^3 [14], which is considerably smaller than those of the inactive **2b** or **2f**, which are equal to 253.4 Å^3 [14], and 227.3 Å^3 [14], respectively.

Pharmacology

Anticonvulsant evaluation has been provided by the ADD Program, Epilepsy Branch, Neurological Disorders Program, NINDS. These testing procedures have been described previously [15–17]. Phase I results in mice are shown in table V. The three tests were: MES, PTZ, and Tox. As previously noted [4], **1**,

Table II. Crystallographic data and summary of data collection and structure refinement for compounds **2b** and **2f**.

	2b	*2f*
Crystal data		
Molecular formula	$C_{20}H_{27}NO_3$	$C_{18}H_{23}NO_3$
Molecular wt	329.43	301.37
Space group (*Z*)	$P2_1/n$ (8)	$P2_1/a$ (4)
a (esd) (Å)	12.499 (6)	9.100 (1)
b (esd) (Å)	9.089 (2)	12.561 (1)
c (esd) (Å)	32.208 (6)	14.675 (1)
β (esd) (°)	92.67 (8)	10652 (1)
(Å^3)	3655.0 (2)	1608.1 (2)
Density obs (g cm^{-3})	1.19	1.24
Density calc (g cm^{-3})	1.197	1.245
Crystal shape and color	Colorless plates	Colorless prisms
Crystal size (mm)	0.3 × 0.3 × 0.03	0.24 × 0.24 × 0.36
Crystallization solvent	Methanol	Methanol
Intensity measurement[a]		
Diffractometer: Enraf-Nonius, FAST, $M_0K\alpha$, λ = 0.71069 Å		
Settings: DET (mm)	48.784	48.896
$^{\theta}$**D** (°)	−19.973	−19.973
Width (mm)	0.15	0.15
Maximum θ (°)	25	25
No of reflections measured	12528	6457
No of observed unique reflections with $I > 2\ (I)$	1298	1921
Structure refinement		
Refinement method	Full-matrix-block	Full-matrix
Least squares on F^2		
$R(F)$ $[I > 2\ (I)]$	0.0425	0.0454
$wR(F^2)$	0.0811	0.1085
S	0.536	0.969
Parameters refined	433	199
Non-H atoms	Positional and anisotropic thermal	
H atoms	allowed to ride on carbon atoms at a distance of 0.96 Å	
Weighting scheme	$w = 1[\sigma^2\ (F^2_0) + (0.0666P)^2$ where $P = (\text{Max}\ (F^2_0) + 2F^2_c)/3$	
Largest diff peak and hole in (e Å^{-3})	0.174 and −0.148	0.276 and −0.198

[a]Intensity data collection procedure see ref [31].

the prototypic benzyloxy spiro analogue was active in the MES evaluation. Data for the current analogues revealed a similar protection spectra. Compounds **2a**, **2d** and **2g** provided MES protection at 100, 300 and 100 mg/kg, respectively. Compound **2a**, the higher homologue of **1**, was superior to **1** in providing a longer (4 h) protective threshold, while **2g**, the dodecane analogue, provided both short-term (30 min)

Table III. Fractional coordinates (× 10^4) and equivalent isotropic displacement parameters (Å^2 × 10^3) with esds of compound **2b**.

Atom	*x/a*	*y/b*	*z/c*	u_{eq}
N (1)	1453 (3)	5997 (3)	2175 (1)	28 (1)
O (1)	1792 (2)	5568 (3)	1786 (1)	38 (1)
O (2)	2397 (2)	4263 (3)	2558 (1)	55 (1)
O (3)	338 (2)	7830 (3)	1917 (1)	43 (1)
C (1)	2966 (4)	6493 (4)	358 (1)	51 (1)
C (2)	3835 (4)	6344 (5)	616 (1)	81 (1)
C (3)	3731 (4)	6322 (5)	1042 (1)	55 (1)
C (4)	2762 (4)	6432 (4)	1211 (1)	33 (1)
C (5)	1892 (4)	6572 (5)	948 (1)	82 (2)
C (6)	2000 (4)	6555 (6)	519 (1)	81 (1)
C (7)	2670 (3)	6502 (4)	1668 (1)	36 (1)
C (8)	737 (3)	7152 (5)	2211 (1)	34 (1)
C (9)	549 (3)	7272 (4)	2668 (1)	35 (1)
C (10)	1177 (3)	6017 (4)	2886 (1)	26 (1)
C (11)	1778 (3)	5289 (5)	2540 (1)	34 (1)
C (12)	448 (3)	4854 (4)	3074 (1)	37 (1)
C (13)	−58 (3)	5448 (4)	3462 (1)	39 (1)
C (14)	755 (3)	5959 (4)	3799 (1)	38 (1)
C (15)	1461 (3)	7104 (4)	3601 (1)	39 (1)
C (16)	2003 (3)	6538 (4)	3222 (1)	37 (1)
C (17)	258 (4)	6438 (5)	4203 (1)	52 (1)
C (18)	1151 (4)	6848 (5)	4526 (1)	93 (2)
C (19)	−370 (4)	5149 (5)	4387 (1)	78 (2)
C (20)	−530 (4)	7751 (5)	4144 (1)	69 (2)
N (1')	6451 (3)	7169 (3)	2187 (1)	28 (1)
O (1')	6809 (2)	7577 (3)	1807 (1)	33 (1)
O (2')	7439 (2)	8785 (3)	2586 (1)	44 (1)
O (3')	5286 (2)	5410 (3)	1924 (1)	43 (1)
C (1')	7948 (4)	6438 (5)	383 (1)	50 (1)
C (2')	8845 (4)	6685 (4)	638 (1)	49 (1)
C (3')	8766 (4)	6779 (4)	1069 (1)	45 (1)
C (4')	7789 (4)	6600 (4)	1240 (1)	35 (1)
C (5')	6891 (4)	6324 (5)	982 (1)	69 (2)
C (6')	6992 (4)	6234 (5)	559 (1)	74 (2)
C (7')	7690 (3)	6609 (4)	1706 (1)	39 (1)
C (8')	5678 (3)	6081 (4)	2224 (1)	31 (1)
C (9')	5475 (3)	5944 (4)	2675 (1)	38 (1)
C (10')	6177 (3)	7113 (4)	2904 (1)	28 (1)
C (11')	6789 (3)	7810 (4)	2559 (1)	31 (1)
C (12')	5506 (3)	8237 (4)	3119 (1)	37 (1)
C (13')	4982 (3)	7635 (4)	3506 (1)	44 (1)
C (14')	5808 (3)	7019 (4)	3818 (1)	34 (1)
C (15')	6454 (3)	5839 (4)	3604 (1)	38 (1)
C (16')	6983 (3)	6444 (4)	3220 (1)	33 (1)
C (17')	5333 (4)	6512 (5)	4229 (1)	48 (1)
C (18')	6231 (3)	5901 (5)	4525 (1)	71 (2)
C (19')	4813 (4)	7802 (5)	11 (1)	72 (2)
C (20')	4493 (3)	5303 (5)	4155 (1)	58 (1)

Numbers in parentheses are the esds for the non-hydrogen atoms. $U_{eq} = (1/3)\ \Sigma_i\Sigma_j U_{ij} a^*_i a^*_j a_i a_j$.

Table IV. Fractional coordinates ($\times 10^4$) and equivalent isotropic displacement parameters ($Å^2 \times 10^3$) with esds of compound **2b**.

Atom	*x/a*	*y/b*	*z/c*	u_{eq}
N (1)	609 (2)	1513 (1)	5412 (1)	25 (1)
O (1)	–13 (1)	2004 (1)	4545 (1)	29 (1)
O (2)	2136 (1)	346 (1)	4888 (1)	37 (1)
O (3)	–761 (1)	2457 (1)	6227 (1)	38 (1)
C (1)	–441 (3)	4071 (2)	1648 (2)	68 (1)
C (2)	618 (3)	3256 (2)	1929 (2)	55 (1)
C (3)	1001 (2)	2900 (1)	2851 (1)	35 (1)
C (4)	344 (2)	3356 (1)	3498 (1)	26 (1)
C (5)	–720 (2)	4157 (1)	3200 (2)	42 (1)
C (6)	–1101 (2)	4507 (2)	2284 (2)	63 (1)
C (7)	799 (3)	2998 (2)	4512 (1)	51 (1)
C (8)	1639 (2)	680 (1)	5515 (1)	26 (1)
C (9)	1929 (2)	298 (1)	6517 (1)	29 (1)
C (10)	1076 (2)	1066 (1)	7022 (1)	23 (1)
C (11)	167 (2)	1771 (1)	6211 (1)	26 (13
C (12)	–64 (2)	487 (1)	7439 (1)	26 (1)
C (13)	594 (2)	–329 (1)	8223 (1)	32 (1)
C (14)	1635 (2)	76 (1)	9172 (1)	34 (1)
C (15)	1094 (2)	1089 (1)	9561 (1)	37 (1)
C (16)	1870 (2)	2121 (1)	9399 (1)	37 (1)
C (17)	1611 (2)	2504 (1)	8381 (1)	32 (1)
C (18)	2240 (2)	1789 (1)	7731 (1)	27 (1)

Numbers in parentheses are the esds for the non-hydrogen atoms. $U_{eq} = (1/3)\ \Sigma_i\Sigma_j U_{ij} a^*_i a^*_j a_i a_j$.

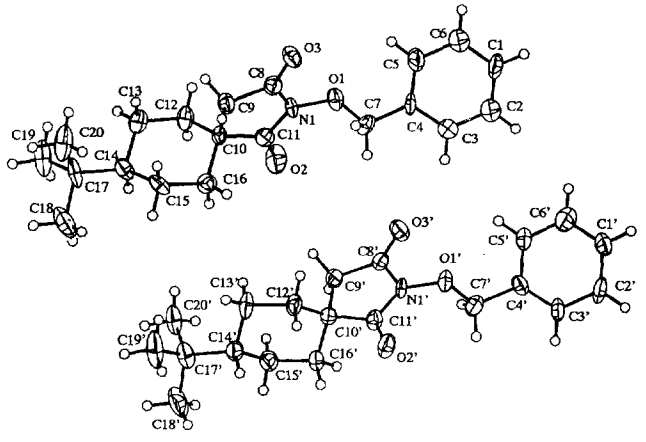


Fig 1. The molecular structure and atom-numbering scheme of **2b**, drawn with SNOOPI [32]. Thermal ellipsoids enclose 20% probability.

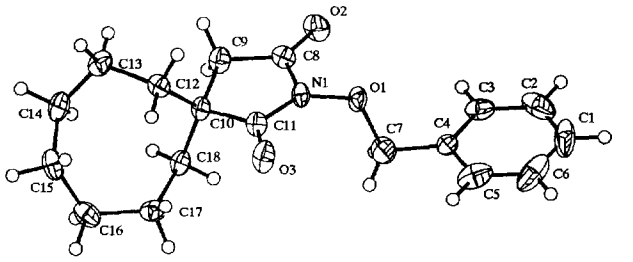


Fig 2. The molecular structure and atom-numbering scheme of **2g**, drawn with SNOOPI [32]. Thermal ellipsoids enclose 20% probability.

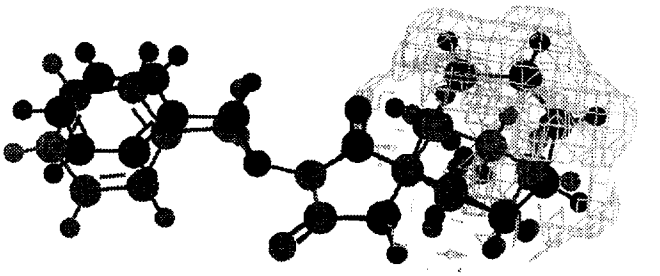


Fig 3. Structure of **2a** (darker print) and **2f** (lighter print) superimposed by fitting the heterocyclic five-membered rings; the exclusive van-der-Waals volumes of the carbocyclic rings are visible.

and long-term (4 h) protection in the MES evaluation. The evaluation showed the following with respect to MES protection: cyclopentyl, **1**, class 2; cyclohexyl, **2a**, class 1; cycloheptyl, **2e**, class 2; cyclooctyl, **2f**, class 3; and cyclododecane, **2g**, class 1. Of further interest and speculation was the dual activity displayed by **7**, the phenoxy analogue, being active in the PTZ evaluation at 300 mg/kg at 4 h and in the MES evaluation at the same dose at 30 min. Compounds **2e** and **7** were further evaluated at 100 mg/kg ip in mice. Both were inactive at 15 min and 1 h, while **2e** was found to be inactive at 2 and 3 h as well. Compound **2a** was further tested for oral activity in the rat (Phase VIa). This data is shown in table VI, and as noted **2a** was active at 50 mg/kg at 15 min with no toxicity to the animals, but was devoid of activity after that period with motor impairment noted in 25% of the animals at 1 and 2 h, respectively. The TTE test [18], a newly developed test by the ADD program, is a clinically nonselective, electroconvulsive seizure model that identifies compounds that raise seizure threshold as well as those that prevent seizure spread. In addition, this test can identify certain compounds that are inactive in both the MES and the PTZ tests. The test is similar to the MES screen but uses a lower level of

Table V. Anticonvulsant screening project (ASP). Phase I test results.

Compound	*Dose (mg/kg)*	*PTZ[a], 30 min*	*PTZ[a], 4 h*	*MES[b], 30 min*	*MES[b], 4 h*	*Tox[c], 30 min*	*Tox[c], 4 h*	*ASP classification[d]*
1[e]								2
2a	100	0/1	0/1	0/3	1/3	0/8	0/4	1
	300	0/1	0/1	0/1	1/1	0/4	0/2	
2b	300	0/1	0/1	0/1	0/1	0/4	0/2	3
2c	300	0/1	0/1	0/1	0/1	1/4	0/2	3
2d	300	0/1	0/1	0/1	1/1	0/4	0/2	2
2e	300	0/1	0/1	0/1	1/1	0/4	0/2	2
2f	300	0/1	0/1	0//1	0/1	0/4	0/2	3
2g	100	0/1	0/1	1/7	1/3	1/8	0/4	1
	300	0/1	0/1	0/1	1/1	2/4	2/2	
2h	300	0/1	0/1	0/1	0/1	0/4	0/2	3
7	300	0/1	1/5	1/1	0/1	0/4	0/2	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: 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. [e]See ref [1d].

electrical current. The lower current makes the TTE test more sensitive, but less discriminate than the MES screen. This ability makes the model attractive because it allows for the identification of compounds that may have been omitted by the standard identification screen. If a compound was found to possess significant activity in the TTE test while remaining inactive in the MES rescreen, it becomes a candidate for more advanced testing. These TTE active compounds may represent compounds acting by novel mechanisms. These compounds will be evaluated further. Thus, **2c** and **2h**, compounds which were inactive in the Phase I evaluation (table V), were candidates for the TTE evaluation. Data for this test are found in table VII. Both compounds were inactive in this test.

In order to elucidate the mechanism of action of these analogues, **1** was evaluated in the sodium channel binding assay. This assay measured the ability of the test compound to inhibit the specific binding of [^{3}H]BTX-B to neurotoxin site 2 of the voltage-dependent sodium channel [19–24]. During normal neuronal activity, this channel cycles through resting, open (active), and inactive states, but the binding of [^{3}H]BTX-B causes persistent channel inactivation. Local anesthetics, class I antiarrhythmics, and class I anticonvulsants (and possibly others) bind at pharmacologically relevant concentrations to a site (or sites) on the sodium channel that is allosterically linked to neurotoxin site 2, resulting in the inhibition of binding of [^{3}H]BTX-B. Electrophysiological studies for many of these drugs also reveal a frequency and voltage-

Table VI. Phase VIa oral rat data. MES test results for **2a** (50 mg/kg).

Time (h)	*MES[a]*	*Tox[b]*
0.25	2/4	0/4
0.5	0/4	0/4
1	0/4	1/4
2	0/4	1/4
4	0/4	0/4

[a]Maximal electroshock test (refer to table V for definition); [b]rotorod toxicity (refer to table V for definition).

Table VII. Threshold tonic extension (TTE) test results ip in mice[a].

Compound	*Dose (mg/kg)*	*Time (h)[b]*				
		0.25	*0.5*	*1*	*2*	*4*
2c	100	0/4	0/4	0/4	–	–
2h	100	0/4	0/4	0/4	1/4	0/4

[a]Refer to *Results and discussion* for information. [b]Number of animals protected/number of animals tested.

dependent block of sodium channel conductance, allowing for an explanation of the selective effects of class I anticonvulsants on hyperactive versus normal neurons [22, 23]. Among the commonly used anticonvulsants, only three (which are designated class I anticonvulsants) [23] appear to cause their anticonvulsant effects by binding to the voltage-dependent sodium channel. These are phenytoin (IC_{50} = 40 μM) [23], carbamazepine (IC_{50} = 131 μM) [23], and lamotrigine (IC_{50} = 114 μM) [24], which are relatively narrow spectrum anticonvulsants that exhibit activities against partial and grand mal seizures. Preliminary screening data is shown in table VIII. As noted, **1** provided minimal inhibition of [^{3}H]BIX-B binding compared to phenytoin and was therefore not subsequently screened for an IC_{50} value.

Results and discussion

Data from table V indicates that the most active compound in the series was the unsubstituted cyclohexyl analog, **2a**. Compound **2a** was more active in mice and rats than **1**, and was classified as most active (class 1) in the MES evaluation in mice, while protecting half the rats at 15 min at 50 mg/kg (table VI). Alkyl substitution on the cyclohexyl ring, either with the bulky 4-*tert*-butyl group (**2b**), or methyl groups in the 4- (**2c**) or 3-position (**2d**) either decreased activity (**2c**), or resulted in the loss of activity (**2b**, **2d**). As noted in table V, increasing the size of ring A to cycloheptyl (**2e**) decreased activity, while the cyclooctane (**2f**) and the β-tetralone analogues (**2h**) were inactive. However, it was observed, most surprisingly, that the cyclododecane compound (**2g**) is highly active. An inactive molecule may be inactive for many reasons, one of which may be that it will not sterically fit into the active site. It is possible that the exclusive van-der-Waals volume showing the region of dissimilarity between active **2a** and inactive **2f** (fig 3) indicates the 'bulk' which cannot be accommodated by the receptor.

Table VIII. Sodium channel binding assay results for **1**[a].

Compound	*Concentration (μM)*[b]	*% Inhibition*
1	500	20
	250	16
Phenytoin[c]	40	46

[a]Refer to *Results and discussion* for information. [b]Concentration of compound employed in the assay. [c]Data from ref [13d].

Of further interest was the activity noted with *N*-(phenoxy)-2-azaspiro[4.4]nonane-1,3-dione **7**. This compound provided equivalent MES protection to **1**, the comparable benzyloxy analogue in mice. This initial result suggests that the methylene (CH_2) bridge in compound **1** may not be necessary for anticonvulsant activity, as equivalent qualitative results were obtained with the *N*-(phenoxy) analogue **7**. It is presumed that the increased flexibility provided by the methylene bridge in the higher homologues, cyclohexyl (**2a**) and cyclododecane (**2g**) may contribute to an increased activity in these analogues. Further quantitative (Phase II) results for these analogues are being undertaken to provide a definitive evaluation of these initial results. Additionally, the synthesis and evaluation of supplemental ring A benzyloxy analogues from less accessible ketones (C_9, C_{10}, C_{11}) as well as the synthesis of comparable *N*-(phenoxy) analogues of the active compounds will be undertaken. To further elucidate the mechanism by which these spiranes act, additional molecular modeling studies will be undertaken. Modeling of **2g** will be done and superimposed with **2a** and **2f** to determine regions of complementarity.

Experimental protocols

Chemistry

Mp were determined on a Thomas-Hoover capillary MP apparatus and are uncorrected. Observed bp were also uncorrected. IR spectra were recorded on samples in Nujol, 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. ^{1}H-NMR data as provided for the *N*-benzyloxy spirosuccinimides is found in table I. Elemental analyses (C, H and N) were performed by Schwarzkopf Microanalytical Laboratory (Woodside, NY). Analyses indicated by the symbols of the elements or functions were within ±0.4% of the theoretical values. Experimental data for the azaspiro benzyloxy compounds are provided in table I. Cyclohexane-1-carboxy-1-acetic acid **4a** [7, 9, 10, 25, 26], 4-methylcyclohexane-1-carboxy-1-acetic acid **4c** [7], cycloheptane-1-carboxy-1-acetic acid **4e** [10, 26], cyclooctane-1-carboxy-1-acetic acid **4f** [27], cyclohexane-1-carboxy-1-acetic anhydride **5a** [7, 9, 10], 4-*tert*-butylcyclohexane-1-carboxy-1-acetic acid anhydride **5b** [13], methyl-cyclohexane-1-carboxy-1-acetic anhydride **5c** [7], cycloheptane-1-carboxy-1-acetic acid anhydride **5e** [10], and *N*-hydroxy-2-azaspiro[4.4]nonane-1,3-dione **6** [4] were prepared by literature methods. Diphenyliodonium chloride was obtained from Aldrich Chemical Company, Milwaukee, WI, USA, and used without further purification. TLC analysis employed a butanol/acetic acid/water (5:4:1) or (5:1:4, upper layer) elution solvent mixture and 5 × 10-cm fluorescent plates (Whatman silica gel 60A).

*4-tert-Butylcyclohexane-1-carboxy-1-acetic acid **4b***

4-*tert*-Butylcyclohexanone (103.7 g, 0.67 mol), ethyl cyanoacetate (63.4 g, 0.56 mol), ammonium acetate (5.2 g, 0.08 mol),

and glacial acetic acid (8.1 g, 0.13 mol) were added to 100 mL benzene in a 500 mL flask attached to a Dean-Stark water separator, and the mixture stirred and refluxed for 12 h. The mixture was washed with three 100 mL portions of water. The washings were extracted with two 100 mL portions of Et_2O and the extracts combined and dried (Na_2SO_4). The solvents were removed under reduced pressure and the residue distilled, bp 132–142 °C (0.35 mm) (lit [13], 119-121 °C (0.25 mm) yield: 119.2 g (71.2%). The alkylidene **3b** (119.2 g, 0.48 mol) was dissolved in 450 mL EtOH and transferred to a 2 L Erlenmeyer flask. Potassium cyanide (63.3 g, 0.97 mol) was separately dissolved in 250 mL H_2O and then added to the flask, with stirring. The solution was allowed to stand for 5 days. After the first 24 h, a crystalline solid was formed which absorbed the solvent. Dilution with an additional nixture of 1:1 EtOH/H_2O (500 mL) redissolved the intermediate. Evaporation of the solvents in vacuo and acidification with HCl (500 mL) and refluxing for 18 h provided the crude product which was recrystallized from MeOH, mp 144–145 °C. Anal $C_{13}H_{21}O_4$ (C, H). (±) 3'-Methylcyclohexane-1-carboxy-1-acetic acid **4d**.

Using a previously reported procedure [7, 10], ethyl (±)-α-(3'-methyl)cyclohexylidene-cyanoacetate **3d** (prepared by the addition of ethyl cyanoacetate to (±) 3-methylcyclohexanone, bp 96–102 °C (0.50 mm): Anal $C_{12}H_{17}NO$ (C, H, N), 38 g (0.18 mol), dissolved in 295 mL EtOH and 0.36 mol of potassium cyanide, dissolved in 195 mL H_2O were mixed, and after standing for 48 h at room temperature, the solvents were removed in vacuo, the residue suspended in 200 mL H_2O and extracted with CH_2Cl_2 (3 × 150 mL). The extract was dried over Na_2SO_4 and after solvent removal in vacuo the residue was reacted with HCl (200 mL) and the mixture stirred and refluxed for 24 h. On cooling to room temperature, a gummy mass was obtained. The HCl was decanted and the semisolid dissolved in a boiling saturated solution of $KHCO_3$. A portion (ca 5 g) did not redissolve. The hot solution was filtered into a flask containing 1 L of HCl and the mixture stirred overnight. The solid diacid **4d**, contaminated with an inorganic coprecipitate of KCl, was filtered and washed with cold H_2O, which removed a large portion of the crystalline mass. The remainder was air-dried and recrystallized from MeOH, mp 164–165 °C, with effervescence. Anal $C_{10}H_{16}O_4$ (C, H). The HCl and the aqueous washings were combined and extracted continuously in a liquid/liquid extractor with Et_2O for 24 h to recover additional **4d**. Yield: 22.74 g (61.9%).

Cyclododecane-1-carboxy-1-acetic acid ***4g***

Cyclododecanone (56.1 g, 0.50 mol), ethyl cyanoacetate (55.6 g, 0.50 mol), ammonium acetate (1.9 g, 0.03 mol) and glacial acetic acid (5.9 g, 0.10 mol) were added to 100 mL benzene and the mixture stirred and refluxed for 12 h using a Dean–Stark water separator. The mixture was washed twice with 100 mL H_2O, and the aqueous extract treated with 100 mL fresh benzene and the combined extracts dried over anhydrous Na_2SO_4. Removal of the solvent under reduced pressure and distillation (bp 171–175 °C (0.25 mm)), provided the alkylidene **3g**, yield: 78.11 g (56.4%). The sample was dissolved in 220 mL EtOH and KCN (36.5 g, 0.56 mol), dissolved in 140 mL H_2O added, mixed vigorously, stoppered and allowed to stand at room temperature for 48 h. The solution was evaporated under reduced pressure and the residue extracted with CH_2Cl_2 (3 × 100 mL), dried (Na_2SO_4) and after evaporation, the residue was acidified with HCl (200 mL) and the mixture refluxed for 14 h. The title compound, **2g**, was recovered in crude form by the previous isolation method, mp 144–160 °C and used without further purification in the subsequent steps. Yield: 33.6 g (44.1%).

3,4-Dihydro-1(2H)naphthalene-1-carboxy-1-acetic acid ***4h***

β-Tetralone (3,4-dihydro-1(2*H*)naphthalenone, 50 g, 0.34 mol), ethyl cyanoacetate (32.2 g, 0.29 mol), ammonium acetate (4.4 g, 0.057 mol) and acetic acid (13.9 g, 0.23 mol) were added to 100 mL benzene and the mixture stirred and refluxed for 12 h under Dean–Stark conditions. Work-up as previously indicated provide the alkylidene **3h** (bp 174–180 °C (0.175 mm)), yield: 47.4 g (68%). The alkylidene (0.18 mol) and 0.36 mol of potassium cyanide were reacted for 48 h at room temperature and after evaporation of the solvents, the product was extracted with CH_2Cl_2, dried (Na_2SO_4) and evaporated to yield a dark-brown oil which was distilled, yielding two fractions, bp 84–90 °C (0.20 mm) and bp 89–94 °C (0.20 mm). The first fraction was redistilled and produced the same boiling point range as the second fraction which was identified as 1-cyano-3,4-dihydro-1(2*H*)-naphthalen-1-acetonitrile **8**. Anal $C_{13}H_{12}N_2$ (C, H, N), yield: 14.4 g (40.7%). This intermediate was transferred to a 500 mL flask and acidified with 300 mL HCl, and the two-phase mixture stirred and refluxed for 14 h. Workup in the usual manner produced the diacid **4h**, mp 234–235 °C (MeOH). Anal $C_{13}H_{14}O_4$ (C, H). Yield: 3.78 g (22.2%).

(±)-3-Methylcyclohexane-1-carboxy-1-acetic acid anhydride ***5d***

Using a previous procedure [7], the title compound was quantitatively prepared, bp 118–121 °C (2.25 mm).

General procedure for the preparation of the N-benzyloxyspirosuccinimides ***2***

Hydroxylamine hydrochloride (8.6 g, 0.12 mol) was dissolved in 20 mL of water in a 100 mL flask and Na_2CO_3 (6.72 g, 0.06 mol) was slowly added. When all of the solid had dissolved, the anhydride **5** (0.10 mol) was slowly added and the mixture heated between 60 and 70 °C for 1 h. The mixture was refrigerated for 16 h. The solid material was separated and washed twice with 10 mL cold 5 N HCl. The filtrate yielded additional compound **6** on further refrigeration. Sodium (2.3 g, 0.10 mol) was added to 50 mL absolute EtOH, and after the reaction, the crude *N*-hydroxyspiro compound **6** (0.10 mol), dissolved in 60 mL absolute EtOH was added. After stirring at room temperature for 15 min, the mixture was heated to reflux for 30 min. Compound **6** (0.10 mol), dissolved in 35 mL of absolute EtOH, was added as rapidly as possible and the mixture refluxed for 1 h. Benzyl chloride (0.1 mol) was added via a syringe over 5 min, and the mixture was refluxed an additional 5 h. The mixture was filtered to remove NaCl, and the filtrate was evaporated under reduced pressure to provide a solid, which on recrystallization twice from the appropriate solvent was sufficient to provide an analytical sample.

N-Phenoxy-2-azaspiro[4.4]nonane-1,3-dione ***7***

To a mixture containing 1.84 g (0.013 mol) of anhydrous K_2CO_3, 3.89 g (0.023 mol) of *N*-hydroxy-2-azaspiro[4.4]nonane-1,3-dione **6** (A = $(CH_2)_4$) [4], and 100 mL DMSO was added 10 g (0.032 mol) of diphenyliodonium chloride. The reaction was allowed to stir at room temperature for 24 h, after which the mixture was poured onto 250 g of ice, and the solid precipitate which formed after 1 h was separated, dried and recrystallized from 2-PrOH (yield: 3.70 g, 65.4%, mp 106–108 °C). Anal $C_{14}H_{15}NO_3$ (C, H, N). ^1H NMR ($CDCl_3$): δ 1.57–2.72 (m, 10H, spirononane ring), 7.01–7.39 (m, 5H, aromatic ring); IR spectrum (Nujol): 1785 and 1724 cm^{-1} (C=O stretches).

X-ray crystal analysis and molecular modeling

All experimental details related to the structural analysis of compounds **2b** and **2f** are provided in table II. The structures

were solved by direct methods with the ShelX86 [28] program and refined with ShelXL93 [29]. Modeling of structure **2a** (on the basis of the X-ray determination of structure **2b**) as well as the calculation of van-der-Waals volume maps were done with Chem-X [14]. The method used to calculate the maps is described extensively in the Chem-X reference manual [30]. The maps obtained for **2a** and **2f** were combined by logical (ie, nonarithmetic) 'exclusive or' operation to obtain the region of dissimilarity.

Pharmacology

Initial evaluations for anticonvulsant activity were performed by the ADD Program, Epilepsy Branch, Neurological Disorders Program, NINDS and included Phases I, II, VIA, and VIB test procedures which have been described [15–17]. These tests were performed either in male CF1 mice or male Sprague-Dawley rats. Phase I, a qualitative anticonvulsant ip evaluation in mice included three tests: MES, PTZ, and the rotorod test for Tox. Compounds were suspended in 0.5% aqueous methylcellulose and administered at three dosage levels (30, 100, and 300 mg/kg) with anticonvulsant activity and motor impairment noted 30 min and 4 h after administration. These data are shown in table V. Phase VIA was a similar qualitative evaluation administered orally in rats utilizing the three tests noted previously. The Phase II and Phase VIB tests quantitated the anticonvulsant activity and motor impairment observed for the most promising compounds in Phase I and Phase VIA, respectively. Phase II quantified data in CF1 mice by ip administration, while Phase VIB provided oral rat data comparable to Phase II ip data in mice. The TTE evaluation [18] is performed as follows. Twenty mice were pretreated with 100 mg/kg of the test compound. At several time intervals (15 min, 30 min, 1 h, 2 h and 4 h) post treatment with the test compound, four mice at each time point were challenged with 12.5 mA of electrical current for 0.2 s via corneal electrodes. This stimulation produced a TTE seizure in animals. For each time interval results are expressed as a ratio of the number of animals protected over the number of animals tested. Phase VIB for **2a** is shown in table VI, while TTE evaluations are provided in table VII. Preliminary [^{3}H]BTX-B inhibition data [19] is shown in table VIII.

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Supplemental information

Additional X-ray crystal data is provided for **2b** (2 tables, 17 pages) and for **2f** (2 tables, 11 pages) and is available from the authors.

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