Table I—Time–Dissolution Comparison for Sodium Salicylate Tablets by Three Methods[a]

Minutes	Concentration, m*M*		
	SIE	Atomic Absorption	UV Spectrophotometer
2	0.300	0.300	0.350
4	0.580	0.600	0.625
7	0.850	0.860	0.830
10	0.95	1.00	1.15
14	1.25	1.30	1.34
22	1.20	1.40	1.37

[a] All assays carried out with phosphate buffer at pH 9 and at 37°.

The equilibration time of electrode response to changes in sodium-ion concentration was determined by pumping a concentrated sodium salicylate solution into the beaker under conditions of tablet dissolution. It was found that the electrode-recording system follows the change in sodium concentration even at rates more than twice those observed during actual tablet dissolution.

To control extraneous sodium content, the initial tablet dissolutions were carried out using compressed tablets manufactured in these laboratories. Identical tablet dissolutions were recorded in systems buffered at pH 7.5 and 9.0, and no significant change in drug release was noted.

Figure 1 shows the change in electrode potential for the dissolution of a sodium salicylate tablet. Since the tableting procedure introduces some inherent variation in the individual characteristics of each tablet, it was necessary to ascertain that the recorded variation was indeed due to the tablet and not to the analytical method. This was accomplished by correlation with results from atomic absorption spectrophotometry and UV spectrophotometric analysis. Table I shows the values obtained by each of the three assay methods. Note the agreement of molar concentrations determined by the SIE system and the atomic absorption method. Both of these methods assay for sodium ions. The UV spectrophotometric method assays for salicylate content and, within experimental variation, confirms the sodium-ion data.

In summary, the SIE with attached recording devices provides a means of measuring tablet dissolution that is accurate and relatively inexpensive. It also provides a continuous direct readout of drug appearance without the disadvantages incurred by repeated sampling and was shown to be as reproducible and accurate as either of the spectrophotometric methods used.

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Synthesis and Pharmacological Evaluation of 1,4-Dihydro-2*H*-3,1-benzoxazin-2-one Derivatives

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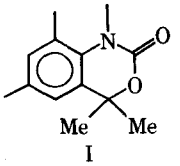
Abstract □ Some 1,4-dihydro-2*H*-3,1-benzoxazin-2-one derivatives were synthesized and tested for pharmacological activity. 4-Phenyl-6-chloro-1,4-dihydro-2*H*-3,1-benzoxazin-2-one (Compound 4) exhibited anticonvulsant activity against chemically and electrically induced seizures and low acute toxicity in mice. Structure–activity relationships are discussed.

Keyphrases □ 1,4-Dihydro-2*H*-3,1-benzoxazin-2-one derivatives—synthesis as possible anticonvulsants, screened for pharmacological activity, structure–activity relationships □ Anticonvulsants, potential—synthesis and pharmacological screening of 1,4-dihydro-2*H*-3,1-benzoxazin-2-one derivatives, structure–activity relationships □ 3,1-Benzoxazin-2-one derivatives—synthesis and pharmacological screening as possible anticonvulsants, structure–activity relationships

3,1-Benzoxazin-2-ones are relatively new potential therapeutic agents. A number of derivatives of Structure I were recently reported by Bernardi *et al.* (1) to have anticonvulsant activity. A series of structurally related 1,3-benzoxazin-2-ones was described by the same authors (2), among which 2,3-dihydro-4*H*-1,3-benzoxazin-2-one-3-acetamide (II) showed antireserpine activity (3–5). Some 4-phenyl-substituted-3,1-benzoxazin-2-ones were also synthesized (6, 7), but apparently no pharmacological data were reported. The present paper deals with the synthesis and pharmacological evaluation of the 1,4-dihydro-2*H*-3,1-benzoxazin-2-one derivatives listed in Table I.

CHEMISTRY

N-Alkylation of the known 1,4-dihydro-2*H*-3,1-benzoxazin-2-one with 2-bromoacetamide in the presence of sodium hydride gave Compound 1. Benzoxazinones 2, 4, and 8 were more conveniently obtained by sodium borohydride reductive cyclization of the corresponding 2-trichloroacetamidobenzophenones (8) than through basic cyclization of 2-trichloroacetamidobenzhydrols (8, 9) or by reaction of 2-aminobenzhydrols with phosgene (6, 7). *N*-Methylation of Compounds 2, 4, and 8 with methyl iodide in the presence of sodium hydride gave benzoxazinones 3, 5, and 9, respectively. Similarly, Compounds 6 and 7 were obtained from Compound 4 by alkylation with 2-bromoacetamide or ethyl bromoacetate.



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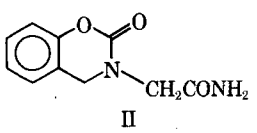

II

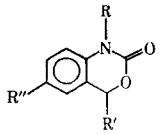

Table I—1,4-Dihydro-2*H*-3,1-benzoxazin-2-ones

Compound	R	R′	R″	Crystallization Solvent[a]	Yield, %[b]	Melting Point[c]	Formula	Analysis, %[d]	Calc.	Found
1	CH_2CONH_2	H	H	A	60	218–220°	$C_{10}H_{10}N_2O_3$	C	58.25	58.03
								H	4.89	4.86
								N	13.58	13.32
2	H	C_6H_5	H	B	63	179–181° dec.	$C_{14}H_{11}NO_2$	C	74.65	74.72
								H	4.92	4.85
								N	6.22	6.13
3	CH_3	C_6H_5	H	C	62	134–136°	$C_{15}H_{13}NO_2$	C	75.30	75.25
								H	5.48	5.61
								N	5.85	5.77
4	H	C_6H_5	Cl	D	65	191–193° dec.	$C_{14}H_{10}ClNO_2$		—	—
5	CH_3	C_6H_5	Cl	D	60	185–187° dec.	$C_{15}H_{12}ClNO_2$		—	—
6	CH_2CONH_2	C_6H_5	Cl	E–F	58	191–193° dec.	$C_{16}H_{13}ClN_2O_3$	C	60.67	60.84
								H	4.14	4.34
								Cl	11.18	11.60
								N	8.84	8.51
7	$CH_2COOC_2H_5$	C_6H_5	Cl	B–G	60	129–130°	$C_{18}H_{16}ClNO_4$	C	62.52	62.61
								H	4.66	4.65
								Cl	10.25	10.35
								N	4.05	3.74
8	H	C_6H_5	NO_2	H–I	53	225–227° dec.	$C_{14}H_{10}N_2O_4$		—	—
9	CH_3	C_6H_5	NO_2	J	63	165–167°	$C_{15}H_{12}N_2O_4$	C	63.38	63.68
								H	4.26	4.41
								N	9.86	9.76

[a] A, methanol; B, benzene; C, isopropyl alcohol; D, ethanol; E, water; F, acetone; G, cyclohexane; H, dioxane; I, hexane; and J, toluene. [b] Yields are of pure products. [c] Melting points were taken in capillary tubes sealed at one end with a partial immersion thermometer and are uncorrected. [d] Where no analytical data are reported, the synthesis of compounds was described elsewhere (8, 9).

PHARMACOLOGY

The experiments were performed on mice (male albino Swiss) or rats (male Wistar); animals were always treated intraperitoneally with benzoxazinones suspended in olive oil–5% acacia gum (1:1) (2.0 ml./100 g. body weight). The following dose levels were chosen on a logarithmic scale: 50, 100, and 200 mg./kg. Behavioral observations were carried out in mice according to the general screening procedure described by Irwin (10). The antagonism of the derivatives against chemically and electrically induced seizures in mice was studied. Strychnine (3 mg./kg. i.p.) and pentylenetetrazole (80 mg./kg. i.p.) were employed as chemical agonists. The activity of the tested compounds was evaluated as antagonism against strychnine death or inhibition of the clonic convulsions induced by pentylenetetrazole.

The maximal electroshock test was performed by an a.c. constant-current stimulator. A 150-v. current of 0.2-sec. duration was employed to deliver the shock. Prevention of tonic limb extension was considered as an index of anticonvulsant activity. The benzoxazinones were given 60 min. before the agonists. The ED_{50} was determined according to Miller and Tainter (11). Motor activity was evaluated in mice with a modified Dews apparatus (12). The animals were treated 30 min. before the recording, and motility was registered continuously for 7 hr. Analgesic and anti-inflammatory activities were evaluated by the hot-plate method (13, 14) (mice) and on carrageenin-induced edema in the rat hind paw (15). The acute toxicity was evaluated in mice, the observation being prolonged for 48 hr. The obtained results are summarized in Table II.

Benzoxazinones 2–5, 8, and 9 induced a moderate depression of the CNS, without influencing the rectal temperature in mice. The other derivatives were inactive. Tested for anticonvulsant properties, Compounds 2 and 4 antagonized both chemically and electrically induced seizures, the most active being Compound 4. Benzoxazinone 3 showed a detectable anticonvulsant activity against electroshock only. Compounds 4, 5, 8, and 9 slightly decreased spontaneous motor activity. The depression of motility was most pronounced with Compound 4. None showed analgesic or anti-inflammatory properties. Furthermore, a low acute toxicity of benzoxazinones resulted after intraperitoneal administration.

As regards the structure–anticonvulsant activity relationships, the last seems to be linked to the structure of 4-phenyl-1,4-dihydro-2*H*-3,1-benzoxazin-2-one (Compound 2); 6-substitution with a chlorine atom (Compound 4) enhanced the activity, while its replacement with a nitro group (Compound 8) abolished it. *N*-Alkylation is detrimental for anticonvulsant action, because *N*-methyl derivatives 5 and 9, as well as the acetamide 6 and the ethyl ester 7, were practically ineffective against chemically or electrically induced convulsions; Compound 3 showed antielectroshock activity only. Moreover, in these experiments, Compound 1, the isomer of 1,3-benzoxazin-2-one (II) previously described (3–5), seemed to be devoid of any antireserpine activity, which is a peculiar feature of II.

Table II—Pharmacological Activities of 1,4-Dihydro-2*H*-3,1-benzoxazin-2-ones

Compound	Depression of CNS	Anticonvulsant Activity, ED_{50}, mg./kg. i.p. Antistrychnine	Anti-pentylenetetrazole	Antimax-shock	Motor Activity, mg./kg. i.p.	Acute Toxicity, LD_{50}, mg./kg. i.p.
1	I[a]	I	>200	I	I	>800
2	++[b]	140	105	135	I	>800
3	+[b]	>200	>200	143	I	>800
4	++	80	78	52	100↓[c]	>800
5	+	>200	>200	200	200↓	>800
6	I	>200	>200	200	I	>800
7	I	I	I	I	I	>800
8	+	I	>200	200	200↓	>800
9	+	I	>200	200	200↓	>800

[a] I = inactive. [b] ++ or + = more or less severe depression. [c] ↓ = decreased motility.

EXPERIMENTAL

Crystallization solvents, yields, melting points, and analyses of the benzoxazinones 1–9 are reported in Table I. IR spectra were recorded on a spectrophotometer[1]. Each analytical sample gave a single spot on TLC (silica gel G); the solvent systems used were: benzene–methanol (95:5 and 4:1), and chloroform–ethyl acetate–diethylamine (7:1:1 and 7:2:1).

1,4-Dihydro-2*H*-3,1-benzoxazin-2-one-1-acetamide (Compound 1)—To 6.0 g. (0.125 mole) of sodium hydride (50% mineral oil dispersion) in 600 ml. of anhydrous dimethylformamide, 18.6 g. (0.125 mole) of 1,4-dihydro-2*H*-3,1-benzoxazin-2-one (8) was added; the mixture was stirred 1.5 hr. at room temperature until a clear solution was obtained. After adding 20.0 g. (0.145 mole) of 2-bromoacetamide and stirring for 2 hr. at room temperature, the reaction mixture was extracted with hexane to remove the mineral oil, evaporated to dryness under reduced pressure, diluted with 1000 ml. of chloroform, washed with water, and dried (Na_2SO_4). Evaporation of the solvents gave crude Compound 1, which was purified by crystallization; IR (mineral oil) 3405, 3315, 3220 (NH_2), 1705 (NHCOO), and 1670 cm.$^{-1}$ ($CONH_2$).

2-Trichloroacetamidobenzophenone—To a stirred solution of 59.2 g. (0.30 mole) of 2-aminobenzophenone and 37.4 g. (0.37 mole) of triethylamine in 800 ml. of anhydrous benzene, 67.3 g. (0.37 mole) of trichloroacetyl chloride was added dropwise; the resulting solution was refluxed for 45 min. The reaction mixture was cooled; washed with 0.5 *N* NaOH, 0.5 *N* HCl, and water; and dried ($CaCl_2$). After removal of the solvent *in vacuo*, 88 5 g. (86%) of product was obtained and employed without further purification for cyclization to Compound 2. A pure sample, m.p. 99–101°, was obtained by crystallization from methanol; IR (mineral oil) 3275 (NH), 1718 ($NHCOCCl_3$), and 1640 cm.$^{-1}$ (CO).

Anal.—Calc. for $C_{15}H_{10}Cl_3NO_2$: C, 52.60; H, 2.94; Cl, 31.03; N, 4.09. Found: C, 52.72; H, 3.01; Cl, 31.11; N, 4.05

4-Phenyl-1,4-dihydro-2*H*-3,1-benzoxazin-2-one (Compound 2)—To a stirred solution of 82.1 g. (0.24 mole) of 2-trichloroacetamidobenzophenone in 330 ml. of anhydrous dioxane, 11.4 g. (0.30 mole) of sodium borohydride was added; the suspension was stirred at room temperature for 6 hr. The reaction mixture was cooled to 0°, slightly acidified with 1 *N* HCl, and diluted with water to 2000 ml. The separated benzoxazinone, Compound 2, was washed with water, dried, and crystallized; IR (mineral oil) 3145, 3090 (NH), and 1703 cm.$^{-1}$ (CO).

4-Phenyl-6-chloro-1,4-dihydro-2*H*-3,1-benzoxazin-2-one (Compound 4) and 4-Phenyl-6-nitro-1,4-dihydro-2*H*-3,1-benzoxazin-2-one (Compound 8)—The products were synthesized substantially as described for Compound 2; a detailed description of their preparation was reported elsewhere (8).

4-Phenyl-1-methyl-1,4-dihydro-2*H*-3,1-benzoxazin-2-one (Compound 3)—To a stirred solution of 20.0 g. (0.09 mole) of Compound 2 in 55 ml. of anhydrous dimethylformamide, 4.8 g. (0.10 mole) of sodium hydride (50% mineral oil dispersion) and 20.1 g. (0.14 mole) of methyl iodide were slowly added at 30–40°. After 45 min. at reflux, the reaction mixture was extracted twice with 100 ml. of warm isopropyl ether–hexane (1:1) and finally with 200 ml. of warm isopropyl ether–acetone (4:1). The solid which separated from isopropyl ether–hexane extracts and the residue obtained after evaporation of the solvents of the final extraction were combined and crystallized; IR (mineral oil) 1698 cm.$^{-1}$ (CO).

[1] Perkin-Elmer model 257.

4-Phenyl-6-chloro-1-methyl-1,4-dihydro-2*H*-3,1-benzoxazin-2-one (Compound 5) and 4-Phenyl-6-nitro-1-methyl-1,4-dihydro-2*H*-3,1-benzoxazin-2-one (Compound 9)—The method of synthesis was substantially the same as described for Compound 3; a detailed description of the preparation of Compound 5 was reported elsewhere (9).

4-Phenyl-6-chloro-1,4-dihydro-2*H*-3,1-benzoxazin-2-one-1-acetamide (Compound 6)—The product was synthesized substantially as was Compound 1, starting from benzoxazinone 4.

4-Phenyl-6-chloro-1,4-dihydro-2*H*-3,1-benzoxazin-2-one-1-acetic Acid Ethylester (Compound 7)—A solution of 41.4 g. (0.16 mole) of Compound 4 in 120 ml. of anhydrous dimethylformamide was treated with 8.6 g. (0.18 mole) of sodium hydride (50% mineral oil dispersion) and, dropwise, with 44.0 g. (0.26 mole) of ethyl bromoacetate. After 45 min. at reflux and extraction with hexane to remove the mineral oil, 300 ml. of chloroform and 150 ml. of water were added; the organic layer was separated, dried, and concentrated *in vacuo*. The oily residue, by treatment with ether, gave crude Compound 7, which was purified by crystallization; IR (mineral oil), 1752 (COOEt) and 1723 cm.$^{-1}$ (NHCOO).

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