suspended in 0.05 M Tris-HCl buffer, pH 7.5, containing NaCl (0.05 M) and EDTA (0.001 M). The samples were frozen and stored at −20 °C until the last day. All the samples were then treated together for fluorometric estimation of the DNA concentration according to the method described by Karsten and Wollenberger.[46] Fluorescence measurements were done on a single photon counting apparatus built in the laboratory of one of us (J.B.L.). The ethidium bromide fluorescence was calibrated with use of DNA extracted from untreated MCF-7 cells (~3 × 10^4 cells). Then the DNA content of the samples was compared to that of untreated cells stopped at the zero time of the experiments.

Acknowledgment. We are very grateful to Dr. B. Gaugain for helpful discussion, to Dr. D. Coulaud for electron microscopy measurements, and to A. Bouju for typing the manuscript. The technical assistance of J. Couprie and S. Guerineau is gratefully acknowledged. We greatly acknowledge one of the reviewers for his helpful comments and extended suggestions to improve the manuscript. This research was supported by grants from the Institut National de la Santé et de la Recherche Médicale, the Centre National de la Recherche Scientifique, the Ministère de l'Industrie et de la Recherche, and the Université René Descartes (PARIS V).

(46) Karsten, V.; Wollenberger, A. *Anal. Biochem.* **1977**, *77*, 464.

Registry No. 1, 1137-42-4; **2**, 38459-64-2; **3**, 95764-33-3; (*Z*)-**4**, 95764-34-4; (*E*)-**4**, 95764-35-5; (*Z*)-**5**, 95764-36-6; (*E*)-**5**, 80867-18-1; **6**, 95764-37-7; **7**, 95764-38-8; **8**, 95764-39-9; **9**, 95784-19-3; **9′**, 95764-40-2; **10**, 95764-41-3; **11**, 95764-42-4; **12**, 95764-43-5; **13**, 95764-44-6; **14**, 95764-45-7; **14**·HCl, 95764-46-8; **15**, 95764-47-9; **16**, 95764-48-0; **16′**, 95764-49-1; **17**, 95764-50-4; **17′**, 95764-56-0; **17′**·HCl, 95764-51-5; **20**, 53-16-7; **21**, 95764-52-6; **22**, 95764-53-7; **23**, 95764-54-8; **24**, 95764-55-9; 1,2-dibromoethane, 106-93-4; benzyl chloride, 100-44-7; 5-(benzyloxycarboxamido)pentylamine, 69747-36-0; chloroacetyl chloride, 79-04-9; 9-methoxyellipticine, 10371-86-5; di-*tert*-butyl carbonate, 34619-03-9; ethyl bromoacetate, 105-36-2; 2-bromoethylamine hydrobromide, 2576-47-8; ellipticine, 519-23-3; 9-hydroxyellipticine, 51131-85-2.

Synthesis and Neuroleptic Activity of 3-(1-Substituted-4-piperidinyl)-1,2-benzisoxazoles[1]

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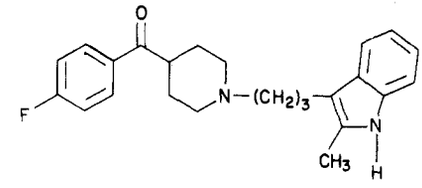
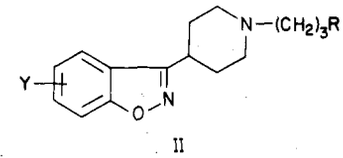
The synthesis of a series of 3-(1-substituted-4-piperidinyl)-1,2-benzisoxazoles is described. The neuroleptic activity of the series was evaluated by utilizing the climbing mice assay and inhibition of [^{3}H]spiroperidol binding. Structure–activity relationships were studied by variation of the substituent on the benzisoxazole ring with concomitant variation of four different 1-piperidinyl substituents. Maximum neuroleptic activity was realized when there was a 6-fluoro substituent on the benzisoxazole ring. The 1-piperidinyl substituent appeared less significant, although in most cases, the (1,3-dihydro-2-oxo-2*H*-benzimidazol-1-yl)propyl group imparted maximum potency. The most potent compound in both assays was 6-fluoro-3-[1-[3-(1,3-dihydro-2-oxo-2*H*-benzimidazol-1-yl)propyl]-4-piperidinyl]-1,2-benzisoxazole (**11b**).

The synthesis in our laboratory of the 4-benzoylpiperidine[3] I (HP 291) and the subsequent discovery of its interesting neuroleptic profile[4] provided the impetus to study structural variants of this system. It is apparent that HP291 can be viewed as a butyrophenone in which the 2-, 3-, and 4-carbons are constrained in a six-membered ring. Since this modification had led to an altered and perhaps more desirable profile with respect to the butyrophenones, it was of interest to study the introduction of further rigidity into the system. One interesting possibility was replacement of the 4-benzoyl group of the piperidine with a bicyclic system such as a 1,2-benzisoxazole to afford II. This system would have one less degree of rotational freedom than a benzoylpiperidine; additionally, it would maintain a somewhat similar conjugative interaction as manifested by the carbonyl–aryl relationship of the benzoyl group. Precedent exists, albeit in another therapeutic area, for such a bioisosteric relationship between benzoyl and



I



II

the 1,2-benzisoxazole moiety.[5] Since such an interchange might alter other factors, structure–activity relationships were studied by the variation of the benzisoxazole substituent (Y) in combination with four different substituents at the piperidine nitrogen.

Chemistry. The stepwise process to the 1,2-benzisoxazoles began with the synthesis of 4-aroylpiperidines, all of which possessed a 2-halo or 2-hydroxy substituent in the aromatic ring (Scheme I, Table I). Thus, 4-chloro-*N*-methylpiperidine (**1**) was reacted, utilizing Grignard chemistry, with 2-halobenzonitriles to yield the desired ketones **3a** and **3d** (method A). Alternatively, an

(1) The paper was presented in part at the 183rd National Meeting of the American Chemical Society, Las Vegas, NV, Mar 1982; MEDI 64.
(2) (a) Chemical Research Department. (b) Visiting scientist, Hoechst AG, Frankfurt, West Germany. (c) Deceased. (d) Department of Biochemistry. (e) Department of Pharmacology.
(3) Helsley, G. C.; Strupczewski, J. T.; Gardner, B. A. U.S. Patent 4 327 108, 1982.
(4) Fielding, S.; Novick, W. J.; Geyer, H. M.; Wilker, J. C.; Kitzen, J. M.; Meyerson, L. R.; Ellis, D. B.; Cornfeldt, M. L. *Fed. Proc., Fed. Am. Soc. Exp. Biol.* **1980**, *39*, 752, Abstr. 2559.
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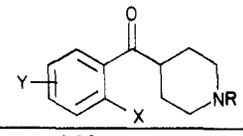
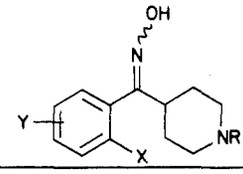
Table I. 4-Benzoylpiperidines



no.	R	X	Y	method	yield,[a] %	mp,[b] °C	recryst[c] solvent	formula	anal.[d]
3a	CH_3	F	H	A	73[e]	167–169	A–I	$C_{13}H_{16}FNO\cdot HCl$	C, H, N, F
3b	CHO	F	4-F	B	32	74–75	*g*	$C_{13}H_{13}F_2NO$	C, H, N
3c	$COCH_3$	Cl	4-Cl	B	54	oil	*h*	$C_{14}H_{15}ClNO_2$	C, H, N, Cl
3d	CH_3	F	6-F	A	9	160–162	C	$C_{13}H_{15}F_2NO\cdot C_2H_2O_2$	C, H, N
3e	$COCH_3$	OH	5-F	C	53	121–123	B–J	$C_{14}H_{16}FNO_3$	C, H, N
3f	$COCH_3$	OH	5-OCH_3	C	90[f]	93–94	K	$C_{15}H_{19}NO_4$	C, H, N
3g	$COCH_3$	OH	4-OCH_3	B	46[f]	139–141	C	$C_{15}H_{19}NO_4$	C, H, N
3h	$COCH_3$	OH	4,5-OCH_3	C	55[f]	163–165	E	$C_{16}H_{21}NO_5$	C, H, N

[a] Yield of analytically pure material unless otherwise stated. [b] Melting points are uncorrected. [c] A = EtOH, B = toluene, C = EtOAc, D = MeOH, E = 2-PrOH, F = DMF, G = CH_3CN, H = acetone, I = Et_2O, J = cyclohexane, K = $(2\text{-Pr})_2O$, L = hexane, M = H_2O. [d] Analyses were within ±0.4% of theoretical values unless otherwise noted. [e] Yield based on distilled product. [f] Crude yield. [g] The crude product was washed with Et_2O. [h] Purified by chromatography on an alumina column with CH_2Cl_2 as eluent.

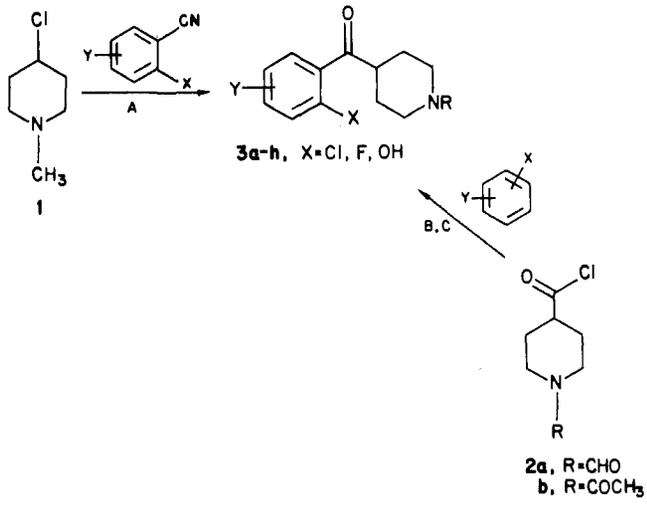
Table II. 4-Benzoylpiperidine Oximes[e]



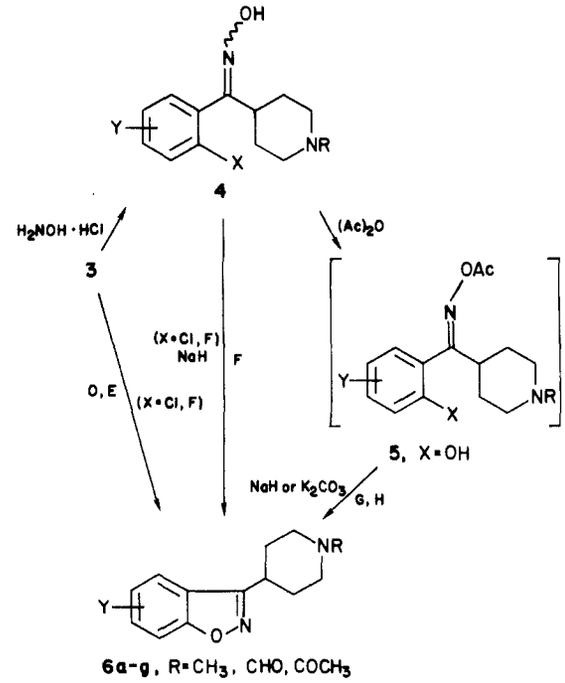
no.	R	X	Y	yield,[a] %	mp,[b] °C	recryst[c] solvent	formula	anal.[d]
4a	CHO	F	4-F	66	184–185	A–M	$C_{13}H_{14}F_2N_2O$	C, H, N
4b	CHO	Cl	4-Cl	14	149–150	H–I	$C_{13}H_{14}Cl_2N_2O$	C, H, N
4c	$COCH_3$	OH	5-F	65	182–184	C	$C_{14}H_{17}FN_2O_3$	C, H, N
4d	$COCH_3$	OH	5-OCH_3	17	191–193	G	$C_{15}H_{20}N_2O_4$	C, H, N
4e	$CO_2CH_2C_6H_5$	OH	4-OCH_3	49	155–156[f]	C–L	$C_{21}H_{24}N_2O_5$	C, H, N
4f	$COCH_3$	OH	4,5-OCH_3	21	151–153	C	$C_{16}H_{22}N_2O_5$	C, H, N

[a–d] See corresponding footnotes to Table I. [e] Data are for mixture of *E* and *Z* isomers, except where noted. [f] Exclusively the *Z* isomer.

Scheme I



Scheme II

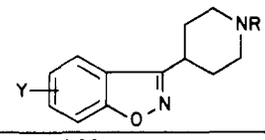


isonipecotoyl chloride **2** was reacted with an appropriately substituted benzene in a Friedel–Crafts reaction either neat (method B) or in the presence of 1,2-dichloroethane (method C) to give the 4-aroylpiperidines **3b**, **3c**, **3e–h**. During the Friedel–Crafts reaction the 2-methoxy group was cleaved to the required 2-hydroxy moiety by the presence of excess aluminum chloride.

By use of chemistry that has been well described,[6] the 4-aroylpiperidines could readily be converted to the 3-(4-piperidinyl)-1,2-benzisoxazoles **6** by one of the methods illustrated in Scheme II. If the aroylpiperidine **3** possessed a 2-halo substituent (fluoro or chloro), synthesis of the benzisoxazole was accomplished by intramolecular displacement of the halogen by the oxime. This could be effected in a one-pot procedure by refluxing the ketone **3**

(6) (a) Wünsch, K.-H.; Boulton, A. J. *Adv. Heterocycl. Chem.* **1967**, *8*, 277. (b) Smalley, R. K. *Adv. Heterocycl. Chem.* **1981**, *29*, 2.

Table III. 3-(4-Piperidinyl)-1,2-benzisoxazole Intermediates



no.	R	Y	method	yield,[a] %	mp,[b] °C	recryst[c] solvent	formula	anal.[d]
6a	CH_3	H	D	47	250–252	A–I	$C_{13}H_{16}N_2O\cdot HCl$	C, H, N
6b	CHO	6-Cl	F	67	114–115	E–I	$C_{13}H_{13}ClN_2O_2$	C, H, N
6c	$COCH_3$	5-F	G	63	158–160	B–L	$C_{14}H_{15}FN_2O_2$	C, H, N
6d	CH_3	4-F	E	14	221–223	C	$C_{13}H_{15}FN_2O\cdot HCl$	C, H, N
6e	$COCH_3$	5-OCH_3	G	36	103–105	K	$C_{15}H_{18}N_2O_3$	C, H, N
6f	$CO_2CH_2C_6H_5$	6-OCH_3	H	38	66–68	K	$C_{21}H_{22}N_2O_4$	C, H, N
6g	$COCH_3$	5,6-OCH_3	H	68	195–197	C	$C_{16}H_{20}N_2O_4$	C, H, N
7a	H	H	K	57	313–315 dec	D–I	$C_{12}H_{14}N_2O\cdot HCl$	C, H, N
7b	H	6-F	J	32	293–295	E–D–I	$C_{12}H_{13}FN_2O\cdot HCl$	C, H, N[e]
7c	H	6-Cl	J	74	228–229	A–I	$C_{12}H_{13}ClN_2O\cdot HCl$	C, H, N
7d	H	5-F	I	83[g]	297–299 dec	D–F–I	$C_{12}H_{13}FN_2O\cdot HCl$	C, H, N
7e	H	5-OCH_3	I	23	274–277 dec	D–I	$C_{13}H_{16}N_2O_2\cdot HCl$	C, H, N
7f	H	6-OCH_3	*f*	80[g]	258–260	D	$C_{13}H_{16}N_2O_2\cdot HBr$	C, H, N
7g	H	5,6-OCH_3	I	40	235–237 dec	A–I	$C_{14}H_{18}N_2O_2\cdot HBr$	C, H, N
7h	H	5-OH	L	51	293–295	D–I	$C_{12}H_{14}N_2O_2\cdot HBr$	C, H, N
7i	H	5,6-OH	L	83	295–298 dec	D–I	$C_{12}H_{14}N_2O_3\cdot HBr$	C, H, N

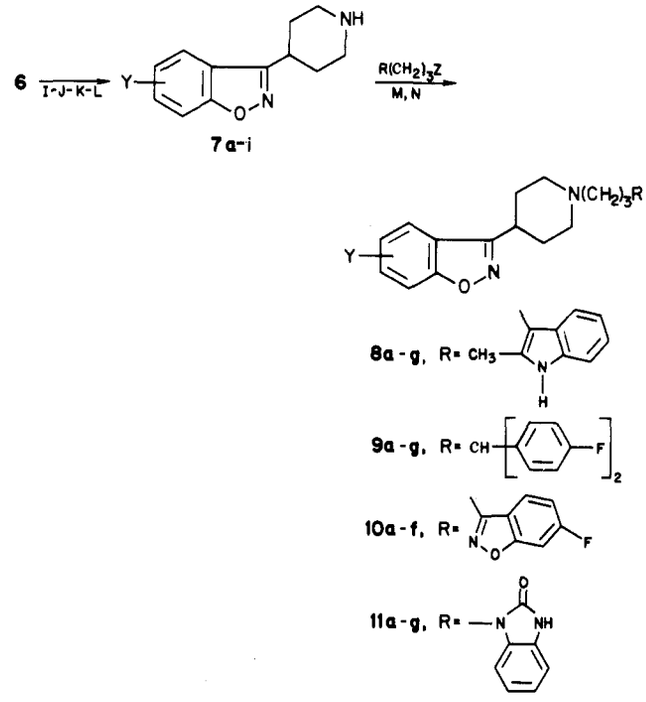
[a–d] See corresponding footnotes to Table I. [e] C: calcd, 56.15; found, 56.99. [f] See Experimental Section for details of synthesis. [g] Crude yield.

with potassium hydroxide, hydroxylamine hydrochloride, and ethoxyethanol–water (method D) or 2-propanol (method E) to yield the desired heterocycle **6** (Table III). In other cases it was more desirable to first generate the oxime **4** (Table II) under standard conditions and then cyclize to **6** with sodium hydride in dimethylformamide–tetrahydrofuran at 90 °C (method F). A mixture of *E* and *Z* oxime isomers was used in this method, although only one isomer (*Z*) will effect the displacement.

If the 4-aroylpiperidine **3** possessed a 2-hydroxy substituent, synthesis of the benzisoxazole was accomplished by intramolecular displacement of acetate from an appropriate oxime acetate. Generation of the oxime **4** produced the expected mixture of *E* and *Z* isomers. In this synthetic sequence the hydroxyl oxygen effects a nucleophilic displacement upon the oxime nitrogen and only the *E* isomer will react. In some cases it was found to be more efficient to separate the oxime isomers by preparative HPLC before continuing. The *E*-rich oxime was then treated with acetic anhydride at 60 °C or at ambient temperature in methylene chloride to yield the (*E*)-*O*-acetyloxime **5**. The crude *O*-acetyloxime was then treated with sodium hydride in dimethylformamide at ambient temperature (method G) or with potassium carbonate in refluxing methanol (method H) to yield the 3-(4-piperidinyl)-1,2-benzisoxazoles **6** (Table III).

The final sequence to the target compounds is depicted in Scheme III. Generation of the secondary amine **7** (Table III) was accomplished, when the amine substituent R was acetyl, with refluxing 6 N hydrochloric acid (method I) and with refluxing 3 N hydrochloric acid–ethanol when it was formyl (method J). For the case where the amine substituent was methyl, cleavage was accomplished by treatment of **6** with phenyl chloroformate to generate an intermediate carbamate, which was then hydrolyzed with base to yield **7** (method K). The hydroxy-substituted benzisoxazoles **7h** and **7i** were prepared by treatment of the methoxy compounds **6e** and **6g** with refluxing hydrobromic acid (method L). The secondary amine **7** was then alkylated with four different pharmacophores in dimethylformamide–potassium carbonate (method M) or in refluxing 4-methyl-2-pentanone–sodium carbonate (method N) to give the target structures **8** (Table IV), **9** (Table V), **10** (Table VI), and **11** (Table VII).

Scheme III

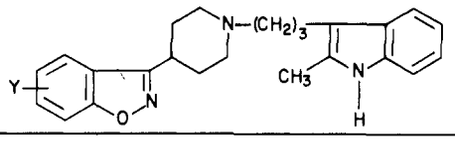


Biological Results and Discussion

For clarity, the pertinent structures have been divided into separate tables on the basis of the substituent on the piperidine nitrogen. The compounds were evaluated for neuroleptic activity by testing in vivo their ability to antagonize climbing in apomorphine-dosed mice and in vitro for their ability to inhibit the binding of [^{3}H]spiroperidol in rat striatal tissue. The results are summarized in Tables IV–VII.

In Table IV the 2-methyl-3-propylindole derivatives most closely related to HP291 are tabulated. Compound **8b**, possessing the 6-fluoro substituent, is the most potent, being essentially equivalent to haloperidol in the in vivo assay and slightly more potent in the in vitro assay, while its isomeric 5-F analogue **8f** is quite weak. The unsubstituted **8a** is next in potency, followed by the 6-chloro derivative **8c**. The other compounds were not particularly

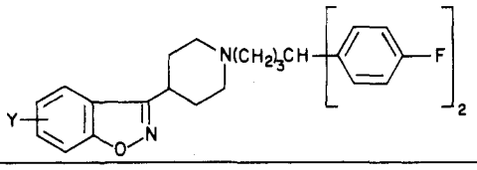
Table IV. 3-[3-[4-(1,2-Benzisoxazol-3-yl)-1-piperidinyl]propyl]-2-methylindoles



no.	Y	method	time, h	temp, °C	yield,[a] %	mp,[b] °C	recryst[c] solvent	formula	anal.[d]	climbing mice assay:[e] ED_{50}, mg/kg, ip	inhibn of [^{3}H]spiroperidol binding:[f] IC_{50}, nM
8a	H	M	4	90	15	110–112	A–M	$C_{24}H_{27}N_3O$	C, H, N	1.1 (1.0–1.2)	42
8b	6-F	N	24	117[h]	32	208–210	H	$C_{24}H_{26}FN_3O\cdot HCl$	C, H, N	0.16 (0.14–0.18)	12
8c	6-Cl	N	6	117	31	108–110	B	$C_{24}H_{26}ClN_3O$	C, H, N	4.1[g]	88
8d	5-OCH_3	N	16	117	33	130–132	G	$C_{25}H_{29}N_3O_2\cdot HCl$	C, H, N	>10	1100
8e	5-OH	M	4	90	34	230–232	A–I	$C_{24}H_{27}N_3O_2\cdot HCl$	C, H, N	>10	340
8f	5-F	M	14	90	43	144–146	A–M	$C_{24}H_{23}FN_3O$	C, H, N	>10	190
8g	5,6-OCH_3	M	6	90	24	176–178	A–M	$C_{26}H_{31}N_3O_3$	C, H, N[i]	>10	2800
haloperidol										0.11 (0.10–0.13)	41
chlor-proma-zine										1.3 (1.1–1.6)	77
HP 291										1.3 (1.1–1.4)	78

[a–d] See corresponding footnotes to Table I. [e] See Experimental Section for testing methodology. The designation >10 indicates that the compound was active at this screening dose but was not potent enough to warrant a dose response. The 95% confidence intervals are given in parentheses. [f] See Experimental Section for testing methodology. [g] Estimated ED_{50}. [h] Reflux temperature. [i] N: calcd, 9.70; found, 8.91.

Table V. 3-[1-[4,4-Bis(4-fluorophenyl)butyl]-4-piperidinyl]-1,2-benzisoxazoles



no.	Y	method	time, h	temp, °C	yield,[a] %	mp,[b] °C	recryst[c] solvent	formula	anal.[d]	climbing mice assay:[e] ED_{50}, mg/kg, ip	inhibn of [^{3}H]spiroperidol binding:[f] IC_{50}, nm
9a	H	M	8	90	30	156–157	C–I	$C_{28}H_{28}F_2N_2O\cdot HCl$	C, H, N	1.6[g]	150
9b	6-F	M	8	100	27	202–204	A–I	$C_{28}H_{27}F_3N_2O\cdot HBr$	C, H, N	0.58 (0.52–0.65)	20
9c	6-Cl	M	5	75	31	205–207	A–I	$C_{28}H_{27}ClF_2N_2O\cdot HCl$	C, H, N	6.4 (5.8–7.0)	200
9d	5-OCH_3	M	8	90	39	214–216	D–I	$C_{29}H_{30}F_2N_2O_2\cdot HBr$	C, H, N	>10	420
9e	5-OH	M	8	90	40	235–236	A–I	$C_{28}H_{28}F_2N_2O_2\cdot HCl$	C, H, N	>10	270
9f	5-F	M	8	90	32	190–192	A	$C_{28}H_{27}F_3N_2O\cdot HCl$	C, H, N[h]	1.6 (1.5–1.8)	140
9g	5,6-OCH_3	M	8	90	48	195–197	B–I	$C_{30}H_{32}F_2N_2O_3\cdot HBr$	C, H, N	>10	420
halo-peridol										0.11 (0.10–0.13)	41
chlor-proma-zine										1.3 (1.1–1.6)	77
HP 291										1.3 (1.1–1.4)	78

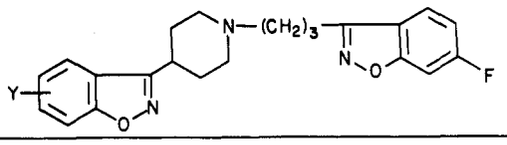
[a–d] See corresponding footnotes in Table I. [e–f] See corresponding footnotes in Table IV. [g] Estimated ED_{50}. [h] C: calcd, 67.12; found, 66.45.

active. It is interesting to note that **8b**, which can be considered the cyclic analogue of HP291, is significantly more potent than the latter in both the in vivo and in vitro assays.

The 4,4-bis(4-fluorophenyl)butyl compounds are illustrated in Table V. The most potent compound in this series was again the 6-fluoro derivative **9b**. It was somewhat less potent than haloperidol in the climbing mice assay and slightly more potent in inhibiting spiroperidol binding. Compounds **9a** and **9f**, the unsubstituted and 5-fluoro derivatives, respectively, were essentially equipotent in the two assays but considerably less potent than **9b**. The 6-chloro compound **9c** showed still weaker activity.

The compounds bearing the 6-fluoro-3-propyl-1,2-benzisoxazole moiety are listed in Table VI. As before, the compound bearing a 6-fluoro substituent, **10b**, was the most active, being somewhat more potent than haloperidol

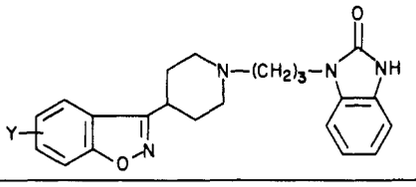
Table VI. 3-[1-[3-(6-Fluoro-1,2-benzisoxazol-3-yl)propyl]-4-piperidinyl]-1,2-benzisoxazoles



no.	Y	method	time, h	temp, °C	yield,[a] %	mp,[b] °C	recryst[c] solvent	formula	anal.[d]	climbing mice assay:[e] ED_{50}, mg/kg, ip	inhibn of [^{3}H]spiroperidol binding:[f] IC_{50}, nm
10a	H	M	8	90	50	242–244	D-I	$C_{22}H_{22}FN_3O_2 \cdot HBr$	C, H, N	1.4 (1.1–1.6)	47
10b	6-F	M	2	90	36	219–220	E-I	$C_{22}H_{21}F_2N_3O_2 \cdot HCl$	C, H, N[h]	0.08 (0.07–0.09)	13
10c	6-Cl	M	5	80	32	220–222	A-I	$C_{22}H_{21}ClFN_3O_2 \cdot HCl$	C, H, N	2.0[g]	70
10d	5-OCH_3	M	8	100	41	192–194	A	$C_{23}H_{24}FN_3O_3 \cdot HBr$	C, H, N	7.2 (6.1–8.4)	150
10e	5-F	M	4	100	31	244–245	F	$C_{22}H_{21}F_2N_3O_2 \cdot HCl$	C, H, N	3.4 (2.9–4.0)	69
10f	5,6-OCH_3	M	6	90	32	149–151	A-M	$C_{24}H_{26}FN_3O_4$	C, H, N	>10	580
haloperidol										0.11 (0.10–0.13)	41
chlorpromazine										1.3 (1.1–1.6)	77
HP 291										1.3 (1.1–1.4)	78

[a–d] See corresponding footnotes to Table I. [e–f] See corresponding footnotes to Table IV. [g] Estimated ED_{50}. [h] C: calcd, 60.89; found, 60.33.

Table VII. 3-[1-[3-(1,3-Dihydro-2-oxo-2*H*-benzimidazol-1-yl)propyl]-4-piperidinyl]-1,2-benzisoxazoles



no.	Y	method	time, h	temp, °C	yield,[a] %	mp,[b] °C	recryst[c] solvent	formula	anal.[d]	climbing mice assay:[e] ED_{50}, mg/kg ip	inhibn of [^{3}H]spiroperidol binding:[f] IC_{50}, nM
11a	H	N	6	117[j]	23	184–186	C	$C_{22}H_{24}N_4O_2$	C, H, N	0.36 (0.28–0.43)	*i*
11b	6-F	N	12	117	27	175–177	A	$C_{22}H_{23}FN_4O_2$	C, H, N	0.09 (0.08–0.10)	6.7
11c	6-Cl	N	6	117	19	233–237 dec	F-A	$C_{22}H_{23}ClN_4O_2 \cdot HBr$	C, H, N	0.8 (0.7–0.10)	25
11d	5-F	N	8	117	36	202–204	D-F-M	$C_{22}H_{23}FN_4O_2$	C, H, N	>10	140
11e	4-F	M	6	90	26	174–175	E-M	$C_{22}H_{23}FN_4O_2$	C, H, N[g]	5.3[h]	68
11f	6-OCH_3	N	16	117	37	176–178	B	$C_{23}H_{26}N_4O_3$	C, H, N	>10	2800
11g	5,6-OCH_3	N	6	117	28	147–149	B-I	$C_{24}H_{28}N_4O_4$	C, H, N	>10	3800
haloperidol										0.11 (0.10–0.13)	41
chlorpromazine										1.3 (1.1–1.6)	77
HP 291										1.3 (1.1–1.4)	78

[a–d] See corresponding footnotes to Table I. [e–f] See corresponding footnotes to Table IV. [g] C: calcd, 66.99; found, 66.50. [h] Estimated ED_{50}. Solubility problems were encountered in attempting to perform this assay. [j] Reflux temperature.

in our assays. Compounds **10a** (no nuclear substituent), **10c** (6-chloro), and **10e** (5-fluoro) also showed good activity, especially in the in vitro assay where they demonstrated somewhat weaker or equivalent potency to chlorpromazine and HP291. In contrast to the two former 5-methoxy analogues **8d** and **9d**, the 5-methoxy compound **10d** in this series was much more active. However, as before, the 5,6-dimethoxy analogue **10f** was uninteresting.

The 1,3-dihydro-2-oxobenzimidazole series of Table VII produced some of the most potent compounds of the study. The compounds **11a**, **11b**, and **11c** all produced ED_{50}'s below 1.0 mg/kg in the climbing mice assay. The 6-fluoro derivative **11b** was the most potent of any of the compounds synthesized when both the in vivo and in vitro

assays are considered. The compounds **11a** (unsubstituted) and **11c** (6-chloro) followed **11b** in potency, although an accurate IC_{50} value could not be determined for **11a**. In this series two new derivatives were tested, compound **11e**, which had a 4-fluoro substituent, and compound **11f**, which had a 6-methoxy substituent. Compound **11e** was more active than chlorpromazine and HP291 in the binding assay. Compound **11f** was only very weakly active.

In analyzing the above discussions, some trends as far as structure–activity relationships become apparent. Clearly, for any given 1-piperidinyl substituent, a fluorine in the 6-position of the benzisoxazole nucleus results in maximum activity. It also appears that the presence of methoxy or hydroxy groups greatly diminishes activity, with the 5,6-dimethoxy compounds being quite weak. Intermediate in potency were the unsubstituted analogues and the 6-chloro derivatives, with the nuclear unsubstituted compounds being somewhat more potent. The two compounds bearing a 5-fluoro substituent in Tables V and VI showed activity similar to that of their 6-chloro counterparts. Enigmatically, this phenomenon was not manifested in the series illustrated in Tables IV and VII.

The pharmacophore at the nitrogen of the piperidine ring also had an effect on relative potency. By holding the benzisoxazole ring substituent constant, the following general trend appears: 1,3-dihydro-2-oxobenzimidazoles (**11a–g**) ≥ 6-fluoro-3-propyl-1,2-benzisoxazoles (**10a–f**) > 2-methyl-3-propylindoles (**8a–g**) > 4,4-bis(4-fluorophenyl)butyls (**9a–g**). The 5-fluoro derivatives **8f**, **9f**, **10e**, and **11d**, however, did not follow the trend.

In conclusion, the data strongly suggest that, within this series, neuroleptic-type activity is closely linked to the nuclear substituent in the benzisoxazole ring, with a weaker, although contributing, link to the 1-piperidinyl substituent. Further, it has been demonstrated that, within the limits of our study, the benzoyl and 1,2-benzisoxazol-3-yl groups are bioisosteric.

Experimental Section

The structures of all compounds were supported by their IR (Perkin-Elmer 457) and ^1H NMR (JEOL C6OHL; tetramethylsilane) spectra. For some compounds additional structural corroboration was obtained by ^{13}C NMR (JEOL FX60; tetramethylsilane) and mass spectral (Finnigan Model 4000 GC–MS equipped with a INCOS data system) analysis. Preparative HPLC was performed on a Waters Associates Prep LC System 500 using silica gel columns. Column chromatography was done with silica gel 60 (70–230 mesh). Melting points were obtained on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Elemental analyses were performed by Micro-Tech Laboratories, Skokie, IL. Unless noted, all starting materials are commercially available.

4-(2-Fluorobenzoyl)-1-methylpiperidine Hydrochloride (3a). Method A. To a stirred suspension, under N_2, of Mg (55.7 g, 2.3 mol) in dry THF (250 mL) was added a few milliliters of bromoethane, followed by the slow addition of freshly distilled 4-chloro-*N*-methylpiperidine (245 g, 1.8 mol) in THF (800 mL). The reaction was initiated by a high-intensity heat gun, and the rate of reaction was controlled by the addition rate. After complete addition of the piperidine, the reaction was refluxed for 1 h, the heating was removed, and 2-fluorobenzonitrile (222 g, 1.8 mol) was added in THF (440 mL). The reaction mixture was refluxed for 3 h and stirred at ambient temperature for 13 h. It was then poured into a solution of NH_4Cl (700 g)–H_2O (2.3 L), and the mixture was heated on the steam bath for 2 h. The cooled mixture was then extracted with Et_2O, and after evaporation of the solvent, there remained 324 g of a brown oil. The oil was vacuum distilled and the desired compound collected at 124 °C (0.45 mm), 224 g. The compound was characterized as a hydrochloride salt, **3a**.

4-(2,4-Difluorobenzoyl)-1-formylpiperidine (3b). Method B. To a stirred, ice-cooled solution of acetic–formic anhydride (from 666 mL of Ac_2O and 334 mL of HCO_2H[7]) was slowly added isonipecotic acid (129.2 g, 1.0 mol) at such a rate that the temperature did not rise above 20 °C. After complete addition of the acid, cooling was continued for an additional hour, and the reaction was then stirred at ambient temperature for 16 h. The reaction medium was distilled under vacuum at 80 °C and the residue diluted with isopropyl ether. The resulting white solid was collected and recrystallized from 2-PrOH–$(2\text{-Pr})_2O$ to yield 120 g (76%) of 1-formylisonipecotic acid.

Thionyl chloride (30 mL) was stirred and cooled to 0 °C, and 1-formylisonipecotic acid (30.0 g, 0.20 mol) was added portionwise. Dimethylformamide (2 mL) was added, and the reaction was stirred at ambient temperature for 3 h. Petroleum ether (400 mL, bp 30–60 °C) was added and 1-formylisonipecotoyl chloride (**2a**) was collected, 33.5 g (dried over P_2O_5). The presence of the acid chloride was corroborated by IR spectroscopy and the compound was used for the next step.

To a stirred suspension of $AlCl_3$ (39.0 g, 0.29 mol) in 1,3-difluorobenzene (120 mL) was added, portionwise, **2a** (30.0 g, 0.17 mol). The reaction was refluxed for 2 h and then poured into ice–H_2O. The aqueous mixture was extracted with $CHCl_3$, and the $CHCl_3$ extract was washed (H_2O), dried ($MgSO_4$), and concentrated to an oil. The oil was triturated with petroleum ether (bp 30–60 °C), and the resulting white solid was collected and washed with cold Et_2O (0 °C) to yield 14.0 g of **3b**.

1-Acetyl-4-(5-fluoro-2-hydroxybenzoyl)piperidine (3e). Method C. To a stirred mixture of 1-acetylisonipecotic acid[8] (20.0 g, 0.12 mol) and 1,2-dichloroethane (90 mL) at 40 °C was added, dropwise, $SOCl_2$ (9 mL) dissolved in 1,2-dichloroethane (27 mL). After 50% of the $SOCl_2$ was added, the temperature was raised to 65 °C and held there through 1 h past complete addition. After the mixture cooled to ambient temperature, 4-fluoroanisole (14.8 g, 0.12 mol) was added, followed by the slow addition of $AlCl_3$ (31.2 g, 0.23 mol). The brown solution was refluxed for 4 h and then poured into ice–H_2O. The mixture was extracted with $CHCl_3$, and the extract was washed with H_2O, dried ($MgSO_4$), and concentrated to yield a brown solid. The solid was triturated with ether, and 31.0 g of the compound was collected. Recrystallization from toluene–cyclohexane afforded 16.5 g of off-white solid **3e**.

3-(1-Methyl-4-piperidinyl)-1,2-benzisoxazole Hydrochloride (6a). Method D. To a solution of **3a** (28.2 g, 0.13 mol) and $H_2NOH{\cdot}HCl$ (19.2 g, 0.28 mol) in ethoxyethanol (400 mL)–H_2O (200 mL) was added a solution of 85% KOH (80.8 g, 1.2 mol) in H_2O (200 mL). The reaction was refluxed under N_2 for 5 h, cooled, and poured into H_2O and the aqueous solution extracted with Et_2O. The extract was washed (H_2O), dried (K_2CO_3), and concentrated to give a colorless oil. The oil was converted to a hydrochloride salt (21.7 g), the salt dissolved in H_2O, and the solution made basic with dilute aqueous NaOH. The free base was then extracted into petroleum ether (bp 30–60 °C), and the extract was dried (K_2CO_3) and subsequently concentrated to yield 16.9 g of a colorless oil. The oil was converted to a hydrochloride salt with ethereal HCl, and the salt was recrystallized from EtOH–Et_2O to yield 15.4 g of **6a**.

4-Fluoro-3-(1-methyl-4-piperidinyl)-1,2-benzisoxazole Hydrochloride (6d). Method E. A mixture of **3d** (209.0 g, 0.63 mol), 85% KOH (400 g, 6.0 mol), $NH_2OH{\cdot}HCl$ (102.0 g, 1.5 mol), 2-PrOH (1.2 L), and H_2O (1.2 L) was stirred and refluxed for 10 h. The brown mixture was poured into H_2O and extracted with hexane. The hexane extract was dried ($MgSO_4$) and the solvent removed under reduced pressure to yield 45.0 g of an orange solid, which was chromatographed on a silica gel column (15:1) using 1% MeOH in CH_2Cl_2 as eluent to yield 30.0 g of a yellow solid. The solid was dissolved in Et_2O and a saturated solution of HCl–Et_2O was added to precipitate 24.7 g of **6d** as a hydrochloride salt.

6-Fluoro-3-(4-piperidinyl)-1,2-benzisoxazole Hydrochloride (7b). Methods F and J. To a stirred suspension, under N_2, of NaH (27.9 g, 0.58 mol, 50% oil dispersion, ether washed) in THF (885 mL) was added, dropwise, **4a** (93.0 g, 0.34 mol)

(7) Huffman, C. W. *J. Org. Chem.* **1958**, *23*, 727.
(8) Duncan, R. L., Jr.; Helsley, G. C.; Welstead, W. J., Jr.; Da Vanzo, J. P.; Funderburk, W. H.; Lundsford, C. D. *J. Med. Chem.* **1970**, *13*, 1. This paper also describes the synthesis of the acid chloride **2b**.

dissolved in DMF (485 mL). The reaction was heated at 70–75 °C for 4 h and then poured into H_2O. The aqueous mixture was extracted with EtOAc, and the extract was washed (H_2O), dried ($MgSO_4$), and concentrated to an orange oil (62.9 g, 73%) of 6-fluoro-3-(1-formyl-4-piperidinyl)-1,2-benzisoxazole. The structure of the compound was verified spectrally and used crude for the next step.

A solution of 6-fluoro-3-(1-formyl-4-piperidinyl)-1,2-benzisoxazole (3.8 g, 0.015 mol), 95% EtOH (45 mL), and 3 N HCl (45 mL) was refluxed for 3 h. The reaction was diluted with H_2O, made basic with aqueous K_2CO_3, and extracted with CH_2Cl_2. The extract was washed (H_2O), dried ($MgSO_4$), and concentrated to yield 3.7 g of a brown oil. The oil was dissolved in MeOH (30 mL), concentrated HCl (1 mL) added, the MeOH evaporated, and the residue crystallized by the addition of Et_2O. After two recrystallizations from 2-PrOH–MeOH–Et_2O, 1.3 g of **7b** was obtained.

1-Acetyl-4-(5-methoxy-1,2-benzisoxazol-3-yl)piperidine (6e). Method G. A mixture of predominately (*E*)-1-acetyl-4-(2-hydroxy-5-methoxybenzoyl)piperidine oxime (10.0 g, 0.034 mol) and acetic anhydride (4.5 mL) was heated at 60 °C for 1.5 h. The resulting solid was stirred with ether to yield the oxime acetate, 7.0 g.

The oxime acetate (7.0 g, 0.021 mol) was added to a stirred suspension of ether-washed NaH (1.1 g, 0.023 mol, of a 50% oil dispersion) in 80 mL of DMF. The reaction was stirred at ambient temperature for 16 h and poured into H_2O (300 mL). The aqueous solution was extracted with EtOAc and the extract was washed with H_2O, brine, and dried ($MgSO_4$). Evaporation of the solvent yielded 6.3 g of a light brown oil. The oil crystallized upon trituration with ether and 3.7 g of a white solid was collected. The solid was recrystallized from toluene–cyclohexane and then from $(2\text{-Pr})_2O$ to yield 2.1 g of **6e**.

Phenylmethyl 4-(6-Methoxy-1,2-benzisoxazol-3-yl)-1-piperidinecarboxylate (6f). Method H. A mixture of **3g** (25.0 g, 0.09 mol) and 6 N HCl (148 mL) was stirred and refluxed for 6 h and then stirred at ambient temperature for 16 h. The hydrochloride salt that precipiated from solution was collected, washed with acetone, and dried to yield 17.7 g. The compound was recrystallized twice from EtOH to yield 16.7 g (68%) of 4-(2-hydroxy-4-methoxybenzoyl)piperidine hydrochloride, mp 264–266 °C.

To a stirred, ice-cooled mixture of 4-(2-hydroxy-4-methoxybenzoyl)piperidine (36.1 g, 0.15 mol), $NaHCO_3$ (16.3 g), and CH_2Cl_2 (25 mL) was added, dropwise, benzyl chloroformate (22.9 mL, 0.15 mol, 95% pure) in CH_2Cl_2 (25 mL). After addition was complete, the reaction was stirred at ambient temperature for 1.5 h and then filtered and the filtrate concentrated to an oil. Upon standing 48 h, the oil solidified and the solid was collected to yield 42.4 g (76%) of phenylmethyl 4-(2-hydroxy-4-methoxybenzoyl)-1-piperidinecarboxylate.

A solution of phenylmethyl 4-(2-hydroxy-4-methoxybenzoyl)-1-piperidinecarboxylate (39.0 g, 0.11 mol), $H_2NOH{\cdot}HCl$ (10.9 g, 0.16 mol), NH_4OAc (18.2 g, 0.24 mol), and EtOH–H_2O (600 + 180 mL) was refluxed for 24 h. Most of the EtOH was evaporated under reduced pressure to yield a biphasic mixture. The mixture was extracted with CH_2Cl_2, and the extract was washed (H_2O), dried ($MgSO_4$), and concentrated to yield an oil. The oil was dissolved in ether and a white solid precipitated from solution, 19.9 g. This solid corresponded to one of the oxime isomers; concentration of the filtrate yielded an oil that was richer in the other oxime isomer. The solid was recrystallized from EtOAc–hexane to yield the *Z* isomer 4e,[9] mp 155–156 °C.

A solution of the oil that corresponded to the *E*-rich isomer of **4e** (13.3 g, 0.034 mol), acetic anhydride (3.6 mL), and CH_2Cl_2 (100 mL) was stirred at ambient temperature for 16 h. The CH_2Cl_2 was evaporated, leaving 16.4 g of the *O*-acetyloxime as a thick, brown oil. The crude *O*-acetyloxime was then stirred and refluxed with K_2CO_3 (7.9 g) and MeOH (30 mL) for 2 h. The reaction was poured into H_2O and the aqueous mixture extracted with EtOAc. The extract was washed (brine), dried ($MgSO_4$), and concentrated to yield a brown oil, 13.2 g. The oil (8.7 g) was chromatographed by preparative HPLC using silica gel columns and CH_2Cl_2–MeOH (1%) as eluent. Evaporation of the appropriate fractions yielded 2.9 g of product. Recrystallization from $(2\text{-Pr})_2O$ gave 2.1 g of **6f**.

5-Fluoro-3-(4-piperidinyl)-1,2-benzisoxazole Hydrochloride (7d). Method I. A mixture of **6c** (17.5 g, 0.07 mol) and 6 N HCl (110 mL) was stirred and refluxed for 6 h. After the mixture stood at ambient temperature for 12 h, a white solid formed and was collected to yield 14.2 g of **7d**. An analytical sample was obtained by recrystallization from CH_3OH–DMF–Et_2O.

3-(4-Piperidinyl)-1,2-benzisoxazole Hydrochloride (7a). Method K. A solution of **6a** (12.3 g, 0.049 mol) in H_2O was made basic with aqueous NaOH, and the basic mixture was extracted with toluene (200 mL). The toluene extract was dried ($MgSO_4$) and filtered and phenyl chloroformate (8.8 g, 0.056 mol) was added. The reaction was stirred, refluxed under N_2 for 16 h, cooled, and filtered, and the filtrate was concentrated to an oil. The oil was dissolved in Et_2O, and petroleum ether (bp 30–60 °C) was added to precipitate 12.3 g (78%) of phenyl 4-(1,2-benzisoxazol-3-yl)-1-piperidinecarboxylate, mp 96–98 °C.

A mixture of phenyl 4-(1,2-benzisoxazol-3-yl)-1-piperidinecarboxylate (5.0 g, 0.015 mol), EtOH (100 mL), and aqueous KOH (5 g of 85% KOH in 50 mL of H_2O) was stirred and refluxed under N_2 for 16 h. Most of the EtOH was evaporated and the aqueous mixture was extracted to yield 2.8 g of a brown oil. The oil was converted to a hydrochloride salt with ethereal HCl and recrystallized from MeOH–Et_2O to yield 2.0 g of **7a**.

5-Hydroxy-3-(4-piperidinyl)-1,2-benzisoxazole Hydrobromide (7h). Method L. A mixture of **6e** (5.4 g, 0.02 mol) and 48% aqueous HBr (25 mL) was stirred and refluxed for 4 h. The reaction was allowed to stand at ambient temperature for 10 h, during which time the hydrobromide salt precipitated from solution. The salt (4.7 g) was recrystallized from MeOH–Et_2O to yield 3.0 g of **7h**.

1-Acetyl-4-(2-hydroxy-5-methoxybenzoyl)piperidine Oxime (4d). A solution of **3f** (15.5 g, 0.055 mol), $H_2NOH{\cdot}HCl$ (7.0 g, 0.1 mol), NH_4OAc (9.5 g), and EtOH–H_2O (250 + 75 mL) was refluxed for 20 h. The reaction was poured into H_2O and extracted with CH_2Cl_2. After washing (H_2O) and drying ($MgSO_4$), the extract was concentrated to yield a brown oil. The oil was triturated with hot toluene, and the toluene was decanted from the residual oil. The oil was then triturated with Et_2O to yield 2.1 g of the oxime. The toluene triturant, upon standing 3 days, deposited an additional 8.1 g more of the compound. The two samples were combined and recrystallized from CH_3CN to yield 2.8 g of **4d** as a mixture of *E* and *Z* isomers.

In a typical separation of the isomers, a 14.0-g mixture of the *E* and *Z* oximes was chromatographed on a Prep LC System 500 using two silica gel columns and eluting with CH_2Cl_2–MeOH (4%). The desired *E* isomer of **4d** eluted first, and the useful fractions amounted to 6.0 g (fractions with some overlapping *Z* isomer were used).

3-[1-[4,4-Bis(4-fluorophenyl)butyl]-4-piperidinyl]-5-hydroxy-1,2-benzisoxazole Hydrochloride (9e). Method M. A mixture of **7h** (1.5 g, 0.005 mol), 4-chloro-1,1-bis(4-fluorophenyl)butane[10] (1.4 g, 0.005 mol), $NaHCO_3$ (0.84 g), a few crystals of KI, and DMF (25 mL) was stirred at 90 °C for 8 h. After cooling to ambient temperature, the reaction mixture was poured into H_2O and extracted with EtOAc. The organic extract was washed with H_2O and brine, dried ($MgSO_4$), and concentrated to yield a dark brown solid. The solid was triturated with Et_2O and then filtered to yield 2.2 g of the product. The compound was partially dissolved in EtOH, a saturated solution of MeOH–HCl added, and the mixture warmed to effect solution. The hydrochloride salt was precipitated by the addition of Et_2O to yield 2.2 g. Recrystallization from EtOH–Et_2O (twice) afforded 1.0 g of **9e**.

3-[3-[4-(5-Fluoro-1,2-benzisoxazol-3-yl)-1-piperidinyl]-propyl]-2-methylindole (8f). Method M. A mixture of **7d** (5.0 g, 0.019 mol), 2-methyl-3-[3-(phenylsulfonyl)propyl]indole[11] (7.0 g, 0.022 mol), and DMF (40 mL), under N_2, was stirred at 90 °C for 14 h. The brown reaction mixture was poured into H_2O and

(9) The stereochemistry could be corroborated by ^{13}C NMR; see: Levy, G. C.; Nelson, G. L. *J. Am. Chem. Soc.* **1972**, *94*, 4897.

(10) Janssen, P. A. J. U.S. Patent 3 238 216, 1966.

(11) Strupczewski, J. T.; Allen, R. C. U.S. Patent 4 352 811, 1982.

extracted with EtOAc. The organic extract was washed with H_2O and brine, dried ($MgSO_4$), and concentrated to yield a beige solid. The solid was triturated with ether and collected to yield 4.0 g of product. Recrystallization (twice) from EtOH–Et_2O gave 3.3 g of **8f**.

3-[1-[3-(6-Fluoro-1,2-benzisoxazol-3-yl)propyl]-4-piperidinyl]-5-methoxy-1,2-benzisoxazole Hydrobromide (10d). Method M. A mixture of **7e** (2.5 g, 0.009 mol), 3-(3-chloropropyl)-6-fluoro-1,2-benzisoxazole[12] (2.2 g, 0.01 mol), K_2CO_3 (2.8 g), a few crystals of KI, and DMF (40 mL) was stirred, under N_2, at 100 °C for 8 h. After cooling to ambient temperature, the reaction was poured into H_2O and extracted with Et_2O. The organic extract was washed with H_2O and brine, dried ($MgSO_4$), and concentrated to yield 4.2 g of a red oil. The oil was chromatographed on silica gel (128 g) with CH_2Cl_2–MeOH (2%) as eluent, resulting in 3.5 g of a purple oil. The oil was dissolved in anhydrous Et_2O and a saturated solution of Et_2O–HBr was added to precipitate 2.3 g of the hydrobromide salt. Recrystallization from EtOH gave 1.8 g of **10d**.

4-Fluoro-3-[1-[3-(1,3-dihydro-2-oxo-2*H*-benzimidazol-1-yl)propyl]-4-piperidinyl]-1,2-benzisoxazole (11e). Method M. A stirred mixture of **6d** (4.4 g, 0.019 mol), CNBr (2.4 g, 0.023 mol), K_2CO_3 (3.0 g, 0.022 mol), and $CHCl_3$ (100 mL) was refluxed for 4 h. The reaction mixture was diluted with H_2O, and the organic layer was separated, washed (H_2O), dried ($MgSO_4$), and concentrated to yield 3.5 g of a brown solid. The solid was chromatographed on a silica gel column (15:1) using CH_2Cl_2–MeOH (1%) as eluent and, following evaporation of the solvent, recrystallized from 2-PrOH–H_2O to give 2.0 g (43%) of 3-(1-cyano-4-piperidinyl)-4-fluoro-1,2-benzisoxazole, mp 90–92 °C.

To a stirred mixture, under N_2, of LAH (2.5 g, 0.07 mol) in THF (200 mL) was added dropwise 3-(1-cyano-4-piperidinyl)-4-fluoro-1,2-benzisoxazole (3.4 g, 0.013 mol). The reaction was stirred and refluxed for 1.5 h and cooled in an icebath, and H_2O was added dropwise. The reaction mixture was filtered and the filtrate was extracted with Et_2O. The extract was washed (H_2O), dried ($MgSO_4$), and concentrated to a yellow oil. The oil was converted to a hydrochloride salt with ethereal HCl and recrystallized from CH_3CN to give 2.0 g of 4-fluoro-3-(4-piperidinyl)-1,2-benzisoxazole hydrochloride. The compound was characterized by spectral analysis and converted to its free base, and the procedure described to synthesize **10d** was used to synthesize **11e**, except that 1-(3-chloropropyl)-1,3-dihydro-2*H*-benzimidazol-2-one was the alkylating agent.

6-Chloro-3-[1-[3-(1,3-dihydro-2-oxo-2*H*-benzimidazol-1-yl)propyl]-4-piperidinyl]-1,2-benzisoxazole Hydrobromide (11c). Method N. A stirred mixture, under N_2, of **7c** (18.9 g, 0.08 mol), 1-(3-chloropropyl)-1,3-dihydro-2*H*-benzimidazol-2-one[13] (16.0 g, 0.08 mol), Na_2CO_3 (17.0 g), KI (1.3 g), and 4-methyl-2-pentanone (1 L) was refluxed for 6 h. The solvent was evaporated under reduced pressure and the residue partitioned between CH_2Cl_2 and H_2O. The organic phase was separated, washed with brine, and dried (Na_2SO_4). The solvent was evaporated under reduced pressure to yield the product as an oil. The oil (23 g) was dissolved in EtOH (150 mL), the solution was cooled and stirred, and 50 mL of a saturated EtOH–HBr solution was added over a 5-min period. After an additional hour of stirring, the hydrobromide salt, 16.0 g, was collected and purified by recrystallized from DMF–EtOH (twice) to yield 5.3 g of **11c**.

Biological Methods. Climbing Mice Assay. The assay is based upon the protocol of Protais[14] and Costall.[15] Mice are individually placed in wire mesh stick cages and are allowed 1 h for adaption and exploration of the new environment. Apomorphine is injected sc at 1.5 mg/kg, a dose causing climbing in all subjects for 0.5 h. Compounds to be tested for neuroleptic activity are injected ip at a screening dose of 10 mg/kg 0.5 h prior to the apomorphine challenge. For evaluation of climbing, three readings are taken at 10, 20, and 30 min after apomorphine administration. The climbing scores are individually totaled, and the total score of the control group is set to 100%. ED_{50} values with 95% confidence limits are calculated by a linear regression analysis.

[^{3}H]Spiroperidol Binding to Rat Striatal Membranes. The assay is based upon the method of Fields.[16] Male Wistar rats (100–150 g) were decapitated and their brains immediately removed. The corpus striatum was rapidly dissected, weighed, and homogenized in 50 volumes of ice-cold Tris-HCl buffer (pH 7.7 at 25 °C) with a Tekmar homogenizer. The homogenate was centrifuged twice at 40000*g* for 10 min with rehomogenization of the intermediate pellet in fresh Tris-HCl buffer. The final pellet was resuspended in 100 volumes of cold 50 mM Tris-HCl buffer (pH 7.6 at 25 °C) containing 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 0.1% ascorbic acid, and 10 μM pargyline. The tissue preparation was then preincubated at 37 °C for 10 min and returned to ice.

To assay [^{3}H]spiroperidol binding, 1 mL of the final tissue suspension was incubated at 37 °C for 10 min in the presence of 0.4 nM [^{3}H]spiroperidol (specific activity of 20 Ci/mmol, obtained from New England Nuclear) and various concentrations of test drugs. Specific [^{3}H]spiroperidol binding was experimentally determined by incubating triplicate samples containing 1 μM (+)-butaclamol in parallel assays. The incubation was stopped by rapid filtration through Whatman GF/B filters under vacuum. Each filter was immediately washed with three 5-mL rinses of cold Tris-HCl buffer and counted by liquid scintillation spectrometry in 10 mL of scintillation cocktail. Specific [^{3}H]-spiroperidol binding was calculated as the difference between total radioactivity bound and the nonspecific radioactivity bound in the presence of 1 μM (+)-butaclamol. The concentration of test drugs causing a 50% inhibition of specific [^{3}H]spiroperidol binding (IC_{50}) was determined by linear regression analysis.

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Registry No. 1, 5570-77-4; **2a**, 84163-43-9; **2b**, 59084-16-1; **3a**, 64671-30-3; **3a** (free base), 84163-45-1; **3b**, 84162-80-1; **3c**, 84162-85-6; **3c** (R = CHO), 84163-44-0; **3d**, 84163-69-9; **3e**, 84162-84-5; **3f**, 84162-81-2; **3g**, 64671-18-7; **3h**, 84162-83-4; (*E*)-**4a**, 95742-11-3; (*Z*)-**4a**, 95742-12-4; (*E*)-**4b**, 95742-13-5; (*Z*)-4b, 95742-14-6; (*E*)-**4c**, 84162-97-0; (*Z*)-**4c**, 84163-60-0; (*E*)-**4d**, 84163-49-5; (*Z*)-**4d**, 84162-95-8; (*E*)-**4e**, 84163-47-3; (*Z*)-**4e**, 84163-00-8; (*E*)-4e acetyl oxime, 84163-48-4; (*E*)-**4f**, 84162-96-9; (*Z*)-**4f**, 84163-58-6; **6a**, 84163-12-2; **6a** (free base), 84163-55-3; **6b**, 84163-11-1; **6c**, 84163-10-0; **6d**, 84163-05-3; **6d** (free base), 84163-59-7; **6e**, 84163-08-6; **6f**, 84163-07-5; **6g**, 84163-09-7; **7a**, 84163-22-4; **7a** (free base), 84163-68-8; **7b**, 84163-13-3; **7b** (free base), 84163-77-9; **7c**, 84163-18-8; **7d**, 84163-16-6; **7e**, 84163-14-4; **7f**, 84163-17-7; **7g**, 84163-15-5; **7h**, 84163-20-2; **7i**, 95742-15-7; **8a**, 84231-40-3; **8b** (free base), 84231-41-4; **8b**, 84231-42-5; **8c**, 95742-16-8; **8d** (free base), 84231-44-7; **8d**, 84231-45-8; **8e** (free base), 84231-47-0; **8e**, 84231-48-1; **8f**, 84243-06-1; **8g**, 84243-15-2; **9a** (free base), 84231-24-3; **9a**, 84231-25-4; **9b** (free base), 84231-26-5; **9b**, 84231-28-7; **9c** (free base), 84231-35-6; **9c**, 84231-36-7; **9d** (free base), 84231-31-2; **9d**, 84231-32-3; **9e** (free base), 84231-33-4; **9e**, 84231-34-5; **9f** (free base), 84231-29-8; **9f**, 84231-30-1; **9g** (free base), 84231-37-8; **9g**, 84231-38-9; **10a** (free base), 84243-03-8; **10a**, 84269-53-4; **10b** (free base), 84243-07-2; **10b**, 84243-08-3; **10c** (free base), 84243-11-8; **10c**, 84243-12-9; **10d** (free base), 84243-09-4; **10d**, 84243-10-7; **10e** (free base), 84243-04-9; **10e**, 84243-05-0; **10f**, 84243-14-1; **11a**, 84231-49-2; **11b**, 84243-00-5; **11c** (free base), 84231-52-7; **11c**, 84231-53-8; **11d**, 84242-99-9; **11e**, 84243-18-5; **11f**, 84243-01-6; **11g**, 84243-16-3; 1,2,4-trimethoxybenzene, 135-77-3; 1-formylisonipecotic acid, 84163-42-8; 1-acetylisonipecotic acid, 25503-90-6; 6-fluoro-3-(1-formyl-4-piperidinyl)-1,2-benzoisoxazole, 84163-41-7; (*E*)-1-acetyl-4-(2-

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hydroxy-5-methoxybenzoyl)piperidine oxime acetate, 95742-17-9; 4-(2-hydroxy-4-methoxybenzoyl)piperidine hydrochloride, 84162-88-9; 4-(2-hydroxy-4-methoxybenzoyl)piperidine, 64671-19-8; phenylmethyl 4-(2-hydroxy-4-methoxybenzoyl)-1-piperidinecarboxylate, 84162-91-4; phenyl 4-(1,2-benzisoxazol-3-yl)-1-piperidinecarboxylate, 84163-21-3; 4-chloro-1,1-bis(4-fluorophenyl)butane, 3312-04-7; 2-methyl-3-[3-(phenylsulfonyl)propyl]indole, 92933-14-7; 3-(3-chloropropyl)-6-fluoro-1,2-benzisoxazole, 84243-02-7; 3-(1-cyano-4-piperidinyl)-4-fluoro-1,2-benzisoxazole, 95742-18-0; 4-fluoro-3-(4-piperidinyl)-1,2-benzisoxazole hydrochloride, 95742-19-1; 4-fluoro-3-(4-piperidinyl)-1,2-benzisoxazole, 95742-20-4; 1-(3-chloropropyl)-1,3-dihydro-(2*H*)-benzimidazol-2-one, 62780-89-6; 2-fluorobenzonitrile, 394-47-8; 2,6-difluorobenzonitrile, 1897-52-5; 1,3-difluorobenzene, 372-18-9; 1,3-dichlorobenzene, 541-73-1; 4-fluoroanisole, 459-60-9; 1,4-dimethoxybenzene, 150-78-7; 1,3-dimethoxybenzene, 151-10-0; acetic formic anhydride, 2258-42-6; isonipecotic acid, 498-94-2; hydroxylamine hydrochloride, 5470-11-1; benzyl chloroformate, 501-53-1; phenyl chloroformate, 1885-14-9.

Synthesis and Pharmacological Activity of Partially Modified Retro-Inverso Dermorphin Tetrapeptides†

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We studied the effect of partial retro-inverso modification of selected peptide bonds of N-terminal tetrapeptide analogues of dermorphin (H-Tyr-D-Ala-Phe-Gly-Tyr-Pro-Ser-NH_2). Among the 14 compounds synthesized and tested for opioid activity, some tetrapeptides have the C-terminus carrying different amide moieties; retromodifications concern the Phe–Gly bond (Ia–f) and/or the C-terminal carboxamide function (IIIa–d, IIa–d). All pseudotetrapeptide derivatives showed opioid activity in vitro and in vivo. The most potent compounds (II) have a biological potency comparable with that of the original tetrapeptides in the guinea pig ileum preparation and in the mouse tail-flick test after intracerebral or subcutaneous administration.

Dermorphins are opioid heptapeptides isolated from the skin of some South American frogs.[1] Their sequences were established to be the following:[2,3]

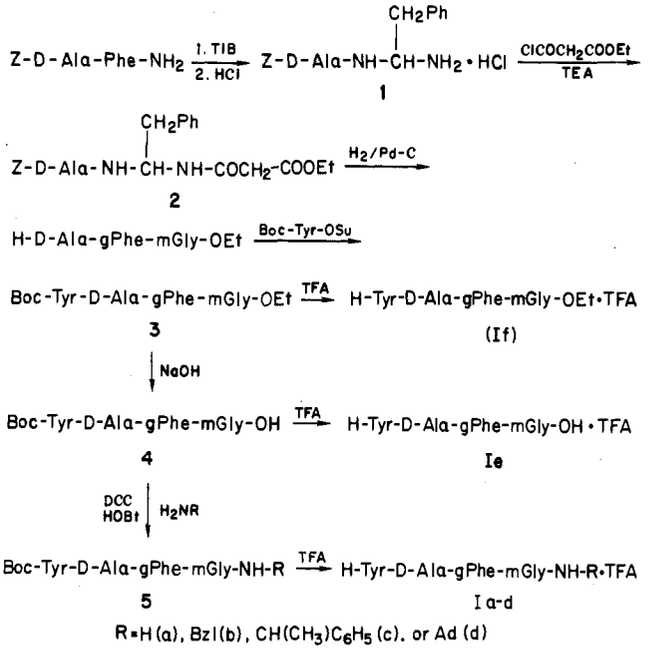
H-Tyr-D-Ala-Phe-Gly-Tyr-Pro-Ser-NH_2 (D)
H——————————Hyp——NH_2 (HD)

Synthetic dermorphins, identical with the natural peptides have been prepared by various methods, using solution[4] or solid-phase[5] techniques. Opioid peptides D and HD possess peripheral and central activity: morphine-like effects in rats,[6] mice[4b,5,7] cell-line preparations,[8] and man[9] have been reported. Our earlier investigation on D analogues concerned the synthesis and study of structure–activity relationships[10] of 60 "small dermorphins" and the synthesis of tetrapeptides (H-Tyr-D-Ala-Phe-Gly-NH-Y), which are very potent analgesics after intracerebroventricular (icv) injection but not following subcutaneous (sc) administration.[11]

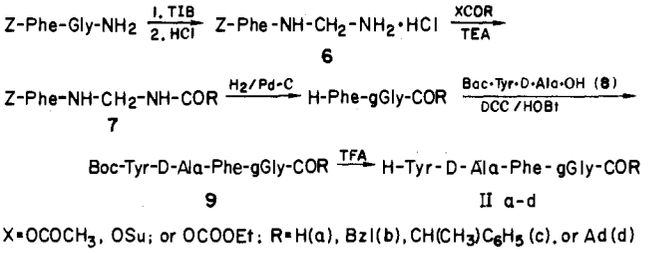
This drawback may result from unfavorable pharmacokinetic or transport parameters and/or low stability in plasma; cleavage of Phe–Gly and Gly–Tyr bonds was postulated to be the main biodegradation pathway of D.[12]

We undertook the synthesis of new D tetrapeptide analogues in which one or more amide bonds are reversed, in the expectation that these partially modified retro-inverso (PMRI)[13] isomers would exhibit enhanced stability toward enzymic degradation. Furthermore, the new

Scheme I. Synthesis of PMRI Analogues Ia–l



Scheme II. Synthesis of PMRI Analogues IIa–d



† Abbreviations according to the IUPAC–IUB Commission, *Biochemistry*, 1972, *11*, 1726–1732, and Specialist Periodical Reports, "Amino Acids, Peptides and Proteins", The Chemical Society, London, 1980, Vol. 11, are used throughout. The following special abbreviations for the partially modified retro-inverso peptides are used: the standard three-letter notation for amino acid residues preceded by prefix g represents the *gem*-diaminoalkyl residue derived from the specified amino acid. The prefix m represents the malonic acid residue derived from the amino acid specified by the three-letter notation. Configurational designation of the retro-inverso residues follows those of the amino acids.

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