Acknowledgment. The authors are grateful to Payton King for his assistance in performing the baboon experiments and to David Schlyer, Robert MacGregor, Yu-Shin Ding, Bernard Bendriem, and John Gatley for their advice and help with these studies. They are also grateful to the National Institutes of Health (Grant No. NS-15380) and to the Department of Energy, Office of Health and Environmental Research for financial support.

1,2-Dihydro-1-oxopyrrolo[3,2,1-*kl*]phenothiazine-2-carboxamides and Congeners, Dual Cyclooxygenase/5-Lipoxygenase Inhibitors with Antiinflammatory Activity

Banavara L. Mylari,* Thomas J. Carty, Peter F. Moore, and William J. Zembrowski

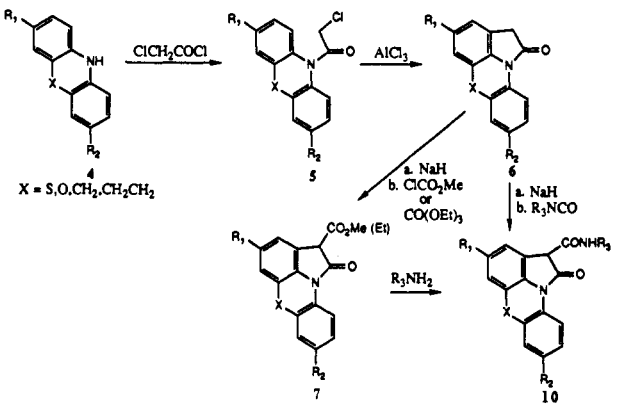
Pfizer Central Research, Groton, Connecticut 06340. *Received October 30, 1989*

A series of 1,2-dihydro-1-oxopyrrolo[3,2,1-*kl*]phenothiazine, 1,2-dihydro-1-oxopyrrolo[3,2,1-*kl*]phenoxazine, and 1,2-dihydro-1-oxopyrrolo[3,2,1-*de*]acridine-2-carboxamides were prepared by reaction of 1,2-dihydro-1-oxopyrrolo[3,2,1-*kl*]phenothiazine or other corresponding phenoxazine and acridan ethyl or methyl esters with appropriate amines. Several members of this family were found to be potent, dual inhibitors of cyclooxygenase and 5-lipoxygenase pathways of arachidonic acid metabolism and to have in vivo antiinflammatory activity in the rat foot edema assay. Structure–activity relationships within this family of compounds are described. 1,2-Dihydro-*N*-(2-thiazolyl)-1-oxopyrrolo[3,2,1-*kl*]phenothiazine-1-carboxamide (**34**) was found to be one of the best compounds to display potent cyclooxygenase/5-lipoxygenase inhibition of arachidonic acid metabolism. Its IC_{50}s against the enzymes sourced from rat basophillic leukemia-1 (RBL-1) cells were 0.07 and 1.4 μM, respectively. It was active in the rat foot edema test for antiinflammatory effect (48% inhibition at 33 mg/kg po) and in the mouse phenylbenzoquinone induced writhing test for analgesic effect (93% inhibition at 32 mg/kg po).

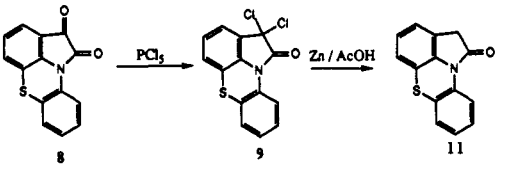
Currently available non-steroidal, cyclooxygenase-inhibiting antiinflammatory drugs, which block arachidonic acid (AA) metabolism to prostaglandins, provide relief to arthritic patients by virtue of their analgesic and antiedema properties. The discovery of the 5-LO pathway of AA metabolism[1] and the participation of the LO metabolite leukotriene B_4[1] as a mediator in the inflammatory response[2-5] offers an opportunity to explore dual CO/LO inhibitors as potentially superior drugs for treatment of inflammatory diseases. Already several dual inhibitors have been discovered,[6-10] some of which are undergoing clinical evaluation.

The work of Kadin,[11] Lombardino,[12] and McManus[13] has established amide structures with pK_a's equal to or lower than typical carboxylic acids as a rich source of antiinflammatory agents. Upon establishing a convenient assay for cellular CO/LO activity, it was found that oxindolecarboxamide **1**[13] was a dual inhibitor of AA metabolism. This lead was pursued within our Central Research Laboratories with the objective of obtaining sufficiently potent and safe dual inhibitors with in vivo rat foot edema (RFE) activity for clinical investigation. One avenue of pursuit has already yielded a novel clinical candidate, **2**, now designated tenidap.[14] We describe here our efforts along another chemical approach leading to a new series of

Scheme I

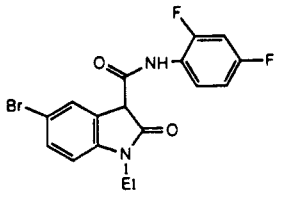


Scheme II

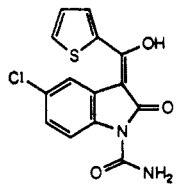


(1) Borgeat, P.; Samuelsson, B. *J. Biol. Chem.* **1979**, *254*, 7865.
(2) Klickstein, L. B.; Shapleigh, C.; Goetzl, E. J. *J. Clin. Invest.* **1980**, *66*, 1166.
(3) Goetzl, E. J.; Pickett, W. C. *J. Exp. Med.* **1981**, *153*, 482.
(4) Myers, R. F.; Siegel, M. L. *Biochem. Biophys. Res. Commun.* **1983**, *112*, 586.
(5) Tichler, A.; Bailey, P.; Dallob, A.; Witzel, B.; Dunette, P.; Rupprecht, D. A.; Dougherty, H.; Humes, J.; Ham, E.; Booney, R.; Egan, R.; Gallagher, T.; Miller, D.; Goldberg, M. *Adv. Prostaglandin, Thromboxane, Leudotriene Res.* **1986**, *16*, 63.
(6) Moore, G. G. I.; Swingle, K. F. *Agents Actions* **1982**, *12*, 674.
(7) Hidaka, T.; Hosoe, K.; Ariki, Y.; Takeo, K.; Yamashita, T.; Katsumi, I.; Kondo, H.; Yamashita, K.; Watanabe, K. *Jpn. J. Pharmacol.* **1984**, *36*, 77.
(8) Ikuta, H.; Shirota, H.; Kobayashi, Y.; Yamagishi, K.; Yamada, I.; Yamotsu, I.; Katayama, K. *J. Med. Chem.* **1987**, *30*, 1995.
(9) Lazer, E. S.; Wong, H-C; Possanza, G. J.; Graham, A. G.; Farina, P. R. *J. Med. Chem.* **1989**, *32*, 100.
(10) Dimartino, J. J.; Griswold, D. E.; Berkowitz, G. P.; Hanna, N. *Agents Actions* **1987**, *20*, 113.
(11) Kadin, S. B.; Wiseman, E. H. *Nature* **1969**, *222*, 275.
(12) Lombardino, J. G.; Wiseman, E. H. *Med. Res. Rev.* **1982**, *2*, 127.
(13) Wiseman, E. H.; Chiani, J.; McManus, J. M. *J. Med. Chem.* **1973**, *16*, 131.
(14) Carty, T. J.; Showell, H. J.; Loose, L. D.; Kadin, S. B. 52nd Annual Meeting of the American Rheumatism Association, Houston, TX; Abstract C54.

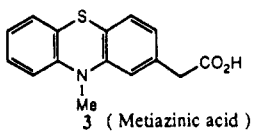
0022-2623/90/1833-2019$02.50/0



1



2 (Tenidap)



3 (Metiazinic acid)

tetracyclic carboxamides **10**, which also are potent dual inhibitors with RFE activity.

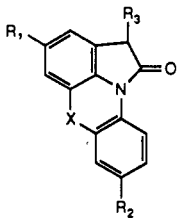
Chemistry

Scheme I outlines the synthetic route employed to prepare the target carboxamides **10**. The tetracyclic lactam backbones **6** were prepared by literature procedures[15a,b] by melting chloroacetyl compounds **5** in the presence of anhydrous aluminum chloride (Table I). Carboxamides **10** were prepared by condensation of **6** with appropriate isocyanates, where available, in the presence of sodium hydride in DMF. Alternatively, a more convenient and versatile procedure consisted of exposing oxindoles **6** to sodium hydride in DMF followed by reaction with methyl chloroformate or diethyl carbonate to prepare lactam esters **7** (Table I) and effecting ester–amide exchange with requisite amines in refluxing toluene or xylene. When we needed a large quantity of **11**, it was more efficient to prepare it starting from isatin **8**[16] by following the sequence outlined in Scheme II. The physical–chemical data for the lactams and the lactam esters, prepared essentially according to Scheme I, are listed in Table II.

Results and Discussion

As mentioned earlier, compound **1** inhibited the 5-LO as well as CO pathway of AA metabolism and showed attractive RFE activity. Our objective was to design more potent compounds related to **1** which could have a greater therapeutic index. One important design consideration was focused on blocking in vivo N-dealkylation of oxindoles (cf. ref 13) by elaborating the *N*-substituent into a ring. Our initial choice of targets (X = S, O, CH_2) was based on the following. The phenothiazine antipsychotic chlorpromazine and its congeners were reported to be inhibitors of prostaglandin synthesis[17–20] although one report contradicted that finding.[21] The pioneering work of Michaelis[22] had established that phenothiazine undergoes a one-electron oxidation to form a highly reactive cation radical. It was also known that, in common with phenothiazine, both phenoxazine and acridan are easily susceptible to oxidation. Antioxidant structures such as phenols inhibit AA metabolism.[9] Modest RFE activity had already

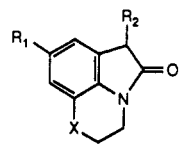
Table I. Physicochemical Data for Pyrrolophenothiazinones and Congeners



compd	X	R_1	R_2	R_3	formula[a]	mp, °C
11	S	H	H	H	$C_{14}H_9NOS$	184 (lit. 181)[b]
12	O	H	H	H	$C_{14}H_9NO_2$	182–183
13	CH_2	H	H	H	$C_{15}H_{11}NO$	175–176
14	CH_2	F	H	H	$C_{15}H_{10}FNO$	134–138
15	CH_2	F	F	H	$C_{15}H_9F_2NO$	206–208
16	CH_2CH_2	H	H	H	$C_{16}H_{13}NO$[b]	110 (lit. 108)[c]
17	S	H	H	CO_2CH_3	$C_{16}H_{11}NO_3S$	144–145
18	O	H	H	CO_2CH_3	$C_{16}H_{11}NO_4$	124–127
19	CH_2	H	H	CO_2CH_3	$C_{17}H_{13}NO_3$	89–92
20	CH_2	F	H	$CO_2C_2H_5$	$C_{18}H_{14}FNO_3$	115–118
21	CH_2	F	F	$CO_2C_2H_5$	$C_{18}H_{13}F_2NO_3$	134–136
22	CH_2CH_2	H	H	CO_2CH_3	$C_{19}H_{15}NO_3$	121–123

[a] Molecular formula of new intermediates was confirmed by high-resolution NMR and mass spectrometry. [b] Acad. Sci. Paris 1967, *265*, 758. [c] British Patent 897,052.

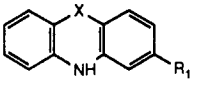
Table II. Physicochemical Data for Pyrroloquinolinones and Pyrrolobenzothienones



compd	X	R_1	R_2	formula[a]	mp, °C
23	S	H	H	$C_{10}H_9NOS$	85–87
24	CH_2	OCH_3	H	$C_{12}H_{13}NO_2$[b]	
25	CH_2	F	H	$C_{11}H_{10}FNO$[b]	
26	S	H	$CO_2C_2H_5$	$C_{13}H_{13}NO_3S$	oil
27	CH_2	OCH_3	$CO_2C_2H_5$	$C_{15}H_{17}NO_4$	83–84
28	CH_2	F	$CO_2C_2H_5$	$C_{14}H_{14}FNO_3$	62–64

[a] Molecular formula of new intermediates was confirmed by high-resolution NMR and mass spectrometry. [b] British Patent 1,394,374.

Table III. CO/LO Inhibition Data for Phenothiazines and Related Compounds

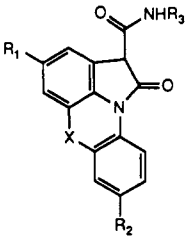


compd	X	R_1	IC_{50},[a] μM	
			5-LO	CO
29	S	H	1.4	0.94
30	S	OCH_3	0.02	N[b]
31	O	H	0.32	0.35
32	CH_2	H	1.6	1.9
33	CH_2CH_2	H	17	N

[a] Means of three determinations, standard deviation no greater than ±25%. [b] N = not measured.

(15) (a) Messer, M.; Farge, D. *Acad. Sci. Paris* **1967**, *265*, 758. (b) British Patent 897,052, 1962; *Chem. Abstr.* **1962**, *57*, 13744d.
(16) Boekelheide, V.; Hollins, R. A. *J. Org. Chem.* **1971**, *36*, 2437.
(17) Krupp, P. *Experientia* **1975**, *31*, 330.
(18) Kunze, H.; Vogt, W. *Biochem. Biophys. Acta* **1974**, *360*, 260.
(19) Kunze, H.; Bahrke, G. J. *J. Pharm. Pharmacol.* **1975**, *27*, 880.
(20) Vanderhoek, J. Y.; Feinstein, M. B. *Mol. Pharmacol.* **1979**, *16*, 171.
(21) Rigas, V. A.; Vunakis, V. H.; Levine, L. *Prostaglandins Med.* **1981**, *7*, 183.
(22) Michaelis, L.; Granick, S.; Schubert, M. P. *J. Am. Chem. Soc.* **1941**, *30*, 2130.

Table IV. Physical and Biological Data for Oxopyrrolophenothiazinecarboxamides and Congeners



compd	X	R_1	R_2	R_3	formula	mp, °C	IC_{50},[a] μM CO	IC_{50},[a] μM LO	RFE % inhibition at 33 mg/kg po
34	S	H	H	2-thiazolyl	$C_{18}H_{11}N_3O_2S_2$	203–205	0.07	1.4	48
35	S	H	H	5-CF_3-2-thiadiazolyl	$C_{18}H_9F_3N_4O_2S_2$	186–188	2.4	1.2	29
36	S	H	H	5-CH_3-2-thiazolyl	$C_{19}H_{13}N_3O_2S_2$	218d	0.48	1.3	44
37	S	H	H	3-CH_3-5-isothiazolyl	$C_{19}H_{13}N_3O_2S_2$	228–231	2.6	1.6	7
38	S	H	H	2-pyridyl	$C_{20}H_{13}N_3O_2S$	228–229	NT[b]	NT	34
39	S	H	H	2,4-difluorophenyl	$C_{21}H_{12}F_2N_2O_2S$	208–209	0.2	1.6	52
40	S	H	H	2,5-difluorophenyl	$C_{21}H_{12}F_2N_2O_2S$	207–208	0.4	1.0	22
41	S	H	H	2,6-difluorophenyl	$C_{21}H_{12}F_2N_2O_2S$	237–241	5.0	3.0	9
42	S	H	H	phenyl	$C_{21}H_{14}N_2O_2S$	236–239	0.7	0.8	36
43	O	H	H	2-thiazolyl	$C_{18}H_{11}N_3O_3S$	206–208	0.02	1.5	42
44	O	H	H	5-CH_3-3-isoxazolyl	$C_{19}H_{13}N_3O_4$	197–198	0.7	1.9	5
45	O	H	H	2-pyridyl	$C_{20}H_{13}N_3O_3$	224–225	1.5	0.6	48
46	O	H	H	2,4-difluorophenyl	$C_{21}H_{12}F_2N_2O_3$	238–239	0.2	1.3	25
47	O	H	H	4-chlorophenyl	$C_{21}H_{13}ClN_2O_3$	268–270	0.7	0.9	8
48	O	H	H	4-fluorophenyl	$C_{21}H_{13}FN_2O_3$	254–256	0.5	0.6	10
49	O	H	H	phenyl	$C_{21}H_{14}N_2O_3$	254	NT	NT	46
50	CH_2	H	F	[1,3,4]-thiadiazol-2-yl	$C_{19}H_{11}FN_4O_2S$	208–210	14	4	15
51	CH_2	F	F	2-thiazolyl	$C_{19}H_{11}F_2N_3O_2S$	199–200	0.7	0.3	25
52	CH_2	H	F	2-thiazolyl	$C_{19}H_{12}FN_3O_2S$	168–174d	0.2	0.7	45
53	CH_2	H	H	5-CF_3-[1,3,4]-thiadiazol-2-yl	$C_{19}H_{11}F_3N_4O_2S$	204–205d	2.4	2.5	27
54	CH_2	H	H	2-thiazolyl	$C_{19}H_{13}N_3O_2S$	231–234	0.5	0.3	40
55	CH_2	H	H	5-pyrazolyl	$C_{19}H_{14}N_4O_2$	248–250	6.2	20	NT
56	CH_2	H	H	2-imidazolyl	$C_{19}H_{14}N_4O_2$	268d	>100	24	NT
57	CH_2	F	F	5-CH_3-isothiazolyl	$C_{20}H_{13}F_2N_3O_2S$	233d	3.5	1.4	19
58	CH_2	F	F	5-CH_3-2-thiazolyl	$C_{20}H_{13}F_2N_3O_2S$	223d	0.1	0.9	19
59	CH_2	H	F	3-CH_3-5-isothiazolyl	$C_{20}H_{14}FN_3O_2S$	203–205	2.8	0.7	45
60	CH_2	H	F	5-CH_3-2-thiazolyl	$C_{20}H_{14}FN_3O_2S$	204–207d	0.8	0.5	42
61	CH_2	H	F	5-CH_3-3-isoxazolyl	$C_{20}H_{14}FN_3O_3$	198–199	7.2	2.8	43
62	CH_2	H	H	2-pyrimidyl	$C_{20}H_{14}N_4O_2$	219–221	>100	>100	NT
63	CH_2	H	H	5-CH_3-2-thiazolyl	$C_{20}H_{14}N_3O_2S$	231–232	1.2	2.6	15
64	CH_2	H	H	3-CH_3-5-isothiazolyl	$C_{20}H_{15}N_3O_2S$	211–213d	3.9	0.7	58
65	CH_2	H	H	5-CH_3-3-isoxazolyl	$C_{20}H_{15}N_3O_3$	198d	6.4	1.2	45
66	CH_2	F	F	2-pyridyl	$C_{21}H_{13}F_2N_3O_2$	246–248	2.2	1.9	27
67	CH_2	H	H	2-pyridyl	$C_{21}H_{15}N_3O_2$	209	9.1	5.9	4
68	CH_2	H	F	2,5-difluorophenyl	$C_{22}H_{13}F_3N_2O_2$	184–186	0.8	1.1	36
69	CH_2	H	F	2,4-difluorophenyl	$C_{22}H_{13}F_3N_2O_2$	223–223	NT	NT	37
70	CH_2	H	H	2,4-difluorophenyl	$C_{22}H_{14}F_2N_2O_2$	186	0.8	2.1	42
71	CH_2	H	H	2,6-difluorophenyl	$C_{22}H_{14}F_2N_2O_2$	264–265	6.9	2.7	18
72	CH_2	H	H	4-chlorophenyl	$C_{22}H_{15}ClN_2O_2$	235–237	NT	NT	33
73	CH_2	H	H	4-fluorophenyl	$C_{22}H_{15}FN_2O_2$	224–226	5.7	0.8	31
74	CH_2	H	H	phenyl	$C_{22}H_{16}N_2O_2$	209	2.9	2.8	30
75	CH_2	H	H	6-CH_3-2-pyridyl	$C_{22}H_{17}N_3O_2$	232–233	>100	0.4	NT
76	CH_2	H	F	3-phenyl-[1,2,4]-thiadiazol-5-yl	$C_{24}H_{15}FN_4O_2S$	211–214	3.3	1.9	19
77	CH_2CH_2	H	H	2-thiazolyl	$C_{20}H_{15}N_3O_2S$	248–249	0.7	21	22
78	CH_2CH_2	H	H	3-methylthioethyl	$C_{20}H_{20}N_2O_2S$	158–159	25	9	2
79	CH_2CH_2	H	H	5-CH_3-2-thiazolyl	$C_{21}H_{17}N_3O_2S$	234–236	3.6	2.3	30
80	CH_2CH_2	H	H	5-CH_3-3-isoxazolyl	$C_{21}H_{17}N_3O_3$	168–169	7.5	13	14
81	CH_2CH_2	H	H	2-pyridyl	$C_{22}H_{17}N_3O_2$	202–204	5.7	>100	41
82	CH_2CH_2	H	H	4,5-dimethyl-2-thiazolyl	$C_{22}H_{19}N_3O_2S$	261–262	2.0	>100	28
83	CH_2CH_2	H	H	2,4-difluorophenyl	$C_{23}H_{16}F_2N_2O_2$	217–218	0.5	0.3	11
84	CH_2CH_2	H	H	4-chlorophenyl	$C_{23}H_{17}ClN_2O_2$	194–196	0.2	0.1	9
85	CH_2CH_2	H	H	4-fluorophenyl	$C_{23}H_{17}FN_2O_2$	224–225	0.8	0.4	10
86	CH_2CH_2	H	H	phenyl	$C_{23}H_{18}N_2O_2$	224–225	5.1	0.5	21
87	CH_2CH_2	H	H	2-benzothiazolyl	$C_{24}H_{17}N_3O_2S$	167–168	1.0	4.4	42
2	tenidap						0.01	9.0	see Table V

[a] Means of three determinations, standard deviation no greater than ±25%. [b] NT = not tested.

been demonstrated with metiazinic acid.[23] Most importantly, we were highly encouraged by the finding, very early in our effort, that the building blocks shown in Table III were potent dual CO/LO inhibitors. Just after we completed this work, Rejeen and co-workers (Merck Frosst Canada) disclosed in a patent publication[24] that certain

(23) Messer, P. M.; Farge, D.; Guyonnet, J. C.; Jeanmart, C.; Julou, L. *Arzneim.-Forsch. (Drug Res.)* **1969**, *19*, 1207.

(24) European Patent Publication 138,481; *Chem. Abstr.* **1986**, *104*, 15081s.

Table V. Antiinflammatory Activity of Tenidap and Standard Agents in the Rat Foot Edema Test

compd	dose range, mg/kg po	no. of tests (five rats/dose)	ED_{50}[a]	95% confidence interval
2, tenidap	1–18	75	13.6	11.8–16.1
indomethacin	1.0–10	18	7.3	6.0–9.4
piroxicam	0.32–10	640	4.5	4.1–5.0
naproxen	3.2–100	18	53.7	41.4–78.5
ibuprofen	3.2–100	28	61.8	50.2–80.4

[a] ED_{50} as determined by linear-regression analysis.

N-alkyl- and *N*-acylphenothiazines are potent leukotriene synthesis inhibitors. In addition, 2-methoxyphenothiazine was found to be active in the RFE assay (46% inhibition at 33 mg/kg po). From the outset, the choice of R_3 side chains was influenced by the earlier experience of Lombardino[12] and McManus.[13] We were gratified by these design choices when one of the very first compounds prepared, **15**, was found to be a potent dual CO/LO inhibitor with RFE activity.

Dual CO/LO inhibition and RFE activity data for four series of oxindolecarboxamides are included in Table IV. Our experience in the RFE assay with tenidap and a few clinically important antiinflammatory agents is included in Table V. Structure–activity studies encompassed amide side chains, oxindole backbones, and substituents on the backbones. In general, compounds with amide side chains (R_3) featuring 2,4-difluorophenyl, isothiazolyl, isoxazolyl, pyridyl, and thiazolyl showed consistently good activity, in both tests, across the four series. The RFE data presented here is similar the the experience in the oxicam series[25] and strongly suggests priority selection of such side chains in approaches to antiinflammatory compounds based on acidic carboxamides. The steric environment around the amide moiety appears to exert a significant effect on enzyme inhibition, as illustrated by compounds **41**, **71**, and **75**. Among the targets with heterocyclic side chains, those containing two nitrogen atoms were the least active; in fact, compounds with either a pyrimidine or a imidazole side chain were devoid of activity. The lower activity of **78** with an acyclic amide side chain points out the critical role of planar aromatic and heterocyclic amides. The expected lower acidity of **78** may have also contributed to its lower activity. Several carboxamides in the subseries with X = CH_2CH_2 were significantly less active than their O, S, and CH_2 counterparts, suggesting that deviation from rigid and nearly planar structures is less conducive to biological activity. This observation prompted us to examine the effect of deleting one of the aromatic rings. So we prepared two series of tricyclic compounds, which are less rigid than the tetracyclic series. The screening data compiled in Table VI shows that members of this series are the least potent inhibitors. However, the influence of other factors including overall shape and lipophilicity cannot be discounted. Because of low in vitro activity, very few compounds from this series were tested for RFE activity. SAR relative to R_2 and R_3 was restricted to the series with X = CH_2. Electron-withdrawing small substituent fluorine did not significantly influence enzyme inhibition activity (e.g. **51** vs **54**).

CO inhibition was found to be necessary but not sufficient for expression of RFE activity. A comparison of **39** and **46** illustrates the point. Other than surmising that there could be differences in absorption, pharmacokinetics, and metabolic disposition, we have no tangible explanation for the observation.

Overall, **34** was one of the best compounds from this program. It was a potent dual CO/LO inhibitor of AA metabolism with the IC_{50} of 0.07 and 1.4 μM, respectively. It showed the desired analgesic[25] and antiinflammatory properties, but was less potent than tenidap.[14] Its pK_a, 4.3, is about $^1/_2$ pK_a unit lower than that of compound **1** in the same solvent system (2:1, dioxane–water). This finding is consistent with the experience in the oxicam series of antiinflammatory compounds wherein N-heterocyclic carboxamides were generally more acidic than *N*-arylcarboxamides.[26] While we do not know the nature of the ionized species in biological fluids (e.g. blood), the amide structure depicted here is consistent with the recently obtained single-crystal X-ray data on **1**[27] (see ORTEP drawing).

Experimental Section

Melting points were taken on a Thomas-Hoover melting point apparatus and are uncorrected. ^1H NMR spectra were all consistent with the molecular structures. Satisfactory elemental analysis was obtained for all new compounds. Molecular formulas of compounds **43**, **51**, **54**, **60**, **92**, and **96** were confirmed by high-resolution FAB mass spectrometry. X-ray data are available as supplementary material.

2-Fluoro-*N*-(chloroacetyl)acridan (5; R_1 = H, R_2 = F). 2-Fluoroacridan was refluxed with excess chloroacetyl chloride in benzene or toluene and the desired product was recovered, according to standard procedures, in nearly quantitative yield; mp 128–131 °C.

Similarly prepared were the following: 2,7-difluoro-*N*-(chloroacetyl)acridan (R_1 = R_2 = F) (mp 128–131 °C); 1,2,3,4-tetrahydro-6-fluoro-*N*-(chloroacetyl)quinoline (X = CH_2, R = F) [^1H NMR ($CDCl_3$, 60 MHz) δ 2.0 (m, 2 H), 2.7 (m, 2 H), 3.7 (m, 2 H), 4.2 (s, 2 H), 6.7–7.2 (m, 3 H)]; *N*-(chloroacetyl)-1,4-dihydrobenzothiazine (X = S, R = H) (mp 85–87 °C).

Pyrrolo[3,2,1-*kl*]phenothiazin-1(2*H*)-one (11). A mixture of isatin 8[15] (50 g, 0.20 mmol), PCl_5 (167 g, 1.2 mol), and benzene (500 mL) was refluxed for 10 h, then cooled to room temperature, and poured onto water (750 mL). The precipitated yellow solid was collected and air-dried. This crude product (48 g), a mixture of **7** and additionally chlorinated product, was not characterized but was dissolved in acetic acid (500 mL) and zinc dust (50 g, 0.8 mol) was gradually added in portions to keep the exothermic reaction under control. The reaction mixture was cooled, concentrated under reduced pressure, and then quenched with water (600 mL). The resulting gummy material was extracted with $CHCl_3$; the organic layer was dried and evaporated to a tan solid. This was chromatographed over silica and eluted with toluene to obtain the desired product (25.5%); mp 184 °C (lit.[15a] mp 181 °C).

All other lactams were prepared from the precursor *N*-chloroacetyl compounds according to the reference listed in footnote b in Table I and the data for the compounds are listed both in Tables I and II.

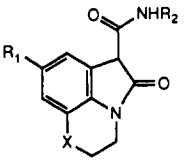
Methyl 1,2-Dihydro-1-oxopyrrolo[3,2,1-*kl*]phenoxazine-2-carboxylate (18). To a slurry of sodium hydride (0.288 g, 6 mmol) in 20 mL of DMF was added pyrrolo[3,2,1-*kl*]phenoxazine (1.12 g, 5 mmol) and the resulting dark red solution was stirred at room temperature for 30 min. To this solution was added methyl chloroformate (0.95 g, 10 mmol) dropwise over 10 min and then heated at 80 °C for 1 h. The reaction mixture was then acidified to pH 2.0 with concentrated CCl and the mixture was then extracted with CH_2Cl_2 (200 mL). The extract was washed with water, the organic layer was dried and evaporated to dryness to yield 0.74 g of the title compound as a solid (53%); mp 124–127 °C.

(25) This compound at 32 mg/kg po showed 93% inhibition of writhing in the standard phenylbenzoquinone writhing assay. We thank Dr. A. Weissman for this data.

(26) Lombardino, J. G.; Wiseman, E. H. *J. Med. Chem.* **1972**, *15*, 848.

(27) We thank Dr. J. Bordner of Pfizer X-ray laboratory for this data.

Table VI. Physical and CO/LO Inhibition Data for Pyrrolobenzothiazenones and Oxopyrroloquinolinecarboxamides



compd	X	R_1	R_2	formula	mp, °C	IC_{50},[a] μM CO	IC_{50},[a] μM LO
88[b]	S	H	2-thiazolyl	$C_{14}H_{11}N_3O_2S_2$	202–204	18	23
89	S	H	5-CF_3-2-[1,2,4]thiadiazolyl	$C_{15}H_{10}F_4N_4O_2S$	223	14	17
90	S	H	3-CH_3-5-isothiazolyl	$C_{15}H_{13}N_3O_2S_2$	232d	>100	14.2
91	S	H	2,4-difluorophenyl	$C_{17}H_{12}F_2N_2O_2S$	206–207	27	7.2
92[c]	CH_2	F	2-thiazolyl	$C_{15}H_{12}FN_3O_2S$	213–214	5.4	14.5
93	CH_2	F	3-CH_3-5-isothiazolyl	$C_{16}H_{14}FN_3O_2S$	246d	>100	26
94	CH_2	F	5-CH_3-2-thiazolyl	$C_{16}H_{14}FN_3O_2S$	242–243d	37	40
95	CH_2	F	2,4-difluorophenyl	$C_{18}H_{13}F_3N_2O_2$	185–187	15.0	3.0
96	CH_2	F	2-benzothiazolyl	$C_{19}H_{14}FN_3O_2S$	235	30.0	18.0
97	CH_2	OCH_3	2,5-difluorophenyl	$C_{19}H_{16}F_2N_2O_3$	172–173	3.4	9.3
98	CH_2	F	3-phenyl-5-[1,2,4]-thiadiazolyl	$C_{20}H_{15}FN_4O_2S$	223–224	5	17
99	CH_2	OCH_3	2-benzothiazolyl	$C_{20}H_{17}N_3O_3S$	199–200	13	14

[a] Means of three determinations, standard deviation no greater than ±25%. [b] 35% inhibition in RFE at 33 mg/kg, p.o. [c] 39% inhibition in RFE at 33 mg/kg, p.o.

Methyl 1,2-Dihydro-1-oxo-6*H*-pyrrolo[3,2,1-*de*]acridan-10-carboxylate (19). To a freshly prepared solution of sodium ethoxide in ethanol (from 0.35 g sodium metal and 10 mL ethanol) was added 6*H*-pyrrolo[3,2,1-*de*]acridan-1(2*H*)-one (1.1 g, 5 mmol) portionwise over 10 min. To the resulting dark red solution was slowly added dimethyl carbonate (1.77 g, 20 mmol) and the solution was then refluxed for 3 h. The reaction mixture was cooled, acidified to pH 2 with concentrated HCl, and then extracted with methylene chloride. The organic extract was washed with water, collected, dried, and evaporated to dryness to obtain a light amber solid (0.61 g, 45%); mp 89–92 °C.

1,2-Dihydro-*N*-(2,4-difluorophenyl)-1-oxopyrrolo[3,2,1-*kl*]phenothiazine-2-carboxamide (39). To a suspension of sodium hydride (3 mmol) in DMF (10 mL) was added pyrrolo[3,2,1-*kl*]phenothiazine-1-one (2 mmol) and to the resulting solution was slowly added 2,4-difluorophenyl isocyanate (2 mmol). The reaction mixture was stirred for 12 h and then poured onto ice–water (50 mL). The resulting solution was acidified to pH 2.0 with 6 N HCl and the precipitated solid was collected. This crude solid was crystallized from methylene chloride to isolate the product (36%); mp 208–209 °C.

1,2-Dihydro-*N*-(2-thiazolyl)-1-oxopyrrolo[3,2,1-*kl*]phenothiazine-1-carboxamide (34). A mixture of methyl 1,2-dihydro-1-oxopyrrolo[3,2,1-*kl*]phenothiazine-2-carboxylate (0.44 g, 1.5 mmol), 2-aminothiazole (0.17 g, 1.7 mmol), and toluene (8 mL) was refluxed for 30 min. As soon as a solution resulted, a yellow solid precipitated. Upon cooling the solid was collected and crystallized from methanol (0.22 g, 46%); mp 203–205 °C.

Single Crystal X-ray Analysis of 1. A colorless crystal of 1 ($C_{17}H_{13}BrF_2N_2O_2$, M_W = 395.2, D_c, X-ray = 1.64 cm^{-3}) with appropriate dimensions of 0.04 × 0.09 × 0.22 mm was surveyed and a 1-Å data set (maximum sin θ/λ = 0.05) was collected on a Nicolet R3m/± diffractometer. Atomic scattering factors were taken from the International Tables for X-ray crystallography.[28] All crystallographic calculations were facilitated by the SHELXTL[29] system. All diffractometer data were collected at room temperature. Crystal parameters were as follows: cell dimensions, a = 4.660 (1) Å, b = 25.173 (7) Å, c = 13.670 (4) Å, α = 90.00°, β = 92.3 (2)°, γ = 90.00°; space group, $P2_1/c$; molecules/unit cell, 4; and linear absorption factor = 38.30 cm^{-1}. Refinement parameters were as follows: number of reflections, 1641; nonzero reflections ($I > 3.0\sigma$), 1231; R index, 0.057; GOF, 1.74; scale factor, 1.564 (4); secondary extinction factor, none. A trial structure was obtained by direct methods. This trial structure refined routinely.

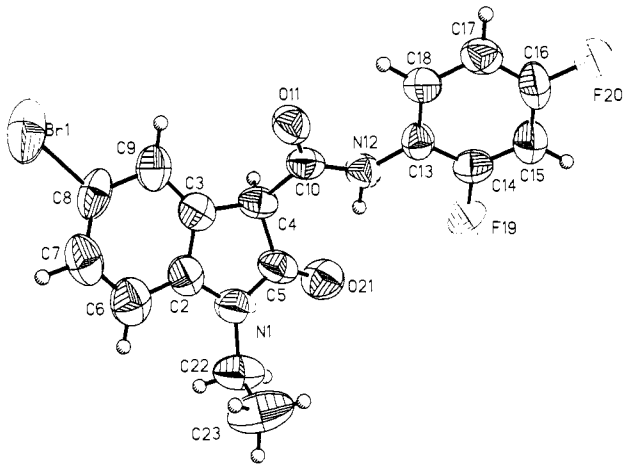


Figure 1. ORTEP drawing of 1.

Hydrogen positions were calculated wherever possible. The methyl hydrogens and the hydrogens on nitrogen and oxygen were located by difference Fourier techniques. The hydrogen parameters were added to the structure factor calculations but were not refined. The shifts calculated in the final cycle of least-squares refinement were all less than 0.1 of their corresponding standard deviations. The final R index was 0.057. A final difference Fourier revealed no missing or misplaced electron density. The refined structure was plotted by using the SHELXTL plotting package (Figure 1). Coordinates, anisotropic temperature factors, distances, and angles are available as supplementary material (Tables S1–S5).

5-Lipoxygenase (5-LO)/Cyclooxygenase (CO) Pathway Activity in Vitro. RBL-1 cells, maintained in monolayer, were grown for 1 day in spinner culture using minimum essential medium (Eagle) with Earle's salts plus 15% fetal bovine serum supplemented with antibiotic/antimycotic solution (GIBCO) according to the method of Jakschik et al.[30,31] The cells were washed once with RPMI 1640 (GIBCO) and resuspended in RPMI 1640 at a cell density of 2 × 10^6 cells/mL. A 0.5-mL aliquot of cell suspension was preincubated at 30 °C for 10 min with a 1-μL dimethyl sulfoxide (DMSO) solution of drug. The incubation was started by simultaneous addition of 5 μL of an ethanolic solution of [^{14}C]arachidonic acid (specific radioactivity, 50–55 mCi/mmol)

(28) International Tables for X-ray Crystallography; Kynoch Press: New York, 1974, Vol. IV.
(29) Sheldrick, G. M. *SHELXTL User Manual*; Nicolet Instrument Co. 1981.
(30) Jakschik, B. A.; Lee, L. H.; Shuffer, F.; Parker, C. W. *Prostaglandin* **1978**, *16*, 733.
(31) Jakschik, B. A.; Lee, L. H. *Nature* **1980**, *287*, 51.

and 2 μL of a DMSO solution of calcimycin (A23187) to give final concentrations of 5 and 7.6 μM, respectively. Five minutes later, the incubation was terminated by the addition of a 0.27-mL volume of CH_3CN/AcOH (100:3). The mixture was then clarified by precipitated protein by centrifugation. Analysis of 5-LO/CO pathway products was performed by HPLC. A 100-μL volume of clarified sample was injected onto a Radial Pak RP-18, OD 032 column (2.6 mm, i.d., Brownlee), and developed with a mobile phase of CH_3CN/H_2O/AcOH over a linear gradient from 35 to 65% acetonitrile for 1 min at a flow rate of 2 mL/min. The developing solvent was continued at 65% acetonitrile for another 4 min before being recycled to original conditions. Detection of product radioactivity was performed with the aid of a Berthold 504 radioactivity monitor equipped with an 800-μL flow cell mixing 2.4 mL/min OMNIFLUOR (New England Nuclear) with column effluent. Integration of peak areas was performed by a SP-4200 computing integrator (Spectra Physics). The radiolabeled product profile contained four major peaks. In order of elution, they were prostaglandin D_2, dihydroxy fatty acids, 5-HETE, and arachidonic acid. The area under the curve (AUC) as measured in integration units for each product was compared to the average AUC value for non-drug-treated samples. The results were expressed as "percent of control" and were plotted versus the log of drug concentration. The results (IC_{50}) in Tables III-VI are presented as the means of three determinations. In no case did the standard deviation exceed ±25%.

Carrageenan-Induced Rat Foot Edema. The standard procedure of Winter et al.[32,33] was employed to measure the inhibition of carrageenan-induced foot edema in rats. A 5-mL volume of drug solubilized in 0.1 M meglumine was administered orally to fasted male Sprague-Dawley rats (~200 g) in groups of five or six. One hour later, 0.05 mL of a carrageenan suspension (1% in water) was administered by subplantar injection into the hind paw. Paw volume was measured by mercury displacement immediately after injection and again at 3 h. The average foot swelling in a group of drug-treated animals ($n = 5$) was compared to that of a group of vehicle-treated animals ($n = 10$) and expressed as percent inhibition. When appropriate this was then plotted versus the log of drug concentration and the half-maximal (ED_{50}) value was estimated by linear-regression analysis.

Acknowledgment. We acknowledge Patricia Joseph for assistance in the CO/LO assay and Balys Kondratas for the RFE testing. We especially thank Deborah Sanford of our Document Preparation Center for her patience in processing this document.

Supplementary Material Available: Tables S1-S5 listing atomic coordinates, isotropic and anisotropic thermal parameters, bond lengths and angles, and H atom coordinates (5 pages). Ordering information is given on any current masthead page.

(32) Winter, C. A.; Risley, E. A.; Nuss, G. W. *Proc. Soc. Exp. Biol. Med.* **1962**, *111*, 544.
(33) Otterness, I. G.; Bliven, M. L. *Nonsteroidal Antiinflammatory Drugs*; Lombardino, J. G., Ed.; John Wiley and Sons: New York, 1985; p 111.

Synthesis of 3-Arylecgonine Analogues as Inhibitors of Cocaine Binding and Dopamine Uptake

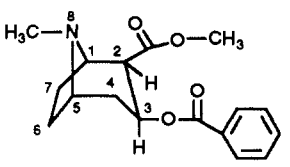
Richard H. Kline, Jr., Jeremy Wright,* Kristine M. Fox, and Mohyee E. Eldefrawi

Department of Biomedicinal Chemistry and Department of Pharmacology and Experimental Therapeutics, University of Maryland, Baltimore, Maryland 21201. *Received September 5, 1989*

3-Arylecgonine analogues were synthesized and characterized by ^1H and ^{13}C NMR, IR, and MS. The compounds were synthesized as racemates from cycloheptatriene-7-carboxylic acid or enantiomerically from (-)-cocaine. These analogues were tested for their ability to inhibit [^3H]cocaine binding to bovine striatal tissue and to inhibit [^3H]dopamine uptake into striatal synaptosomes. Methyl (1*RS*-2-*exo*-3-*exo*)-8-methyl-3-phenyl-8-azabicyclo[3.2.1]octane-2-carboxylate was the most potent analogue. IC_{50} values for inhibition of cocaine binding and dopamine uptake were 20 and 100 nM, respectively. The racemates and the 1*R* isomers were equally potent inhibitors of binding and uptake. Methyl (1*RS*-2-*endo*-3-*exo*)-3-(2,4-dinitrophenyl)-8-methyl-8-azabicyclo[3.2.1]octane-2-carboxylate was the least potent. IC_{50} for inhibition of both binding and uptake was 40 μM.

Introduction

Cocaine abuse has become a serious social problem in the U.S.[1] Initially believed to be nonaddictive, it is now accepted that cocaine is an additive drug.[2] Addiction to cocaine is driven by the powerful positive reinforcing properties of the drug.[3] There is strong evidence that the mesocorticolimbic dopamine (DA) system of the brain is the target site for cocaine and possibly other addictive drugs.[4-7] Cocaine (1) inhibits uptake of DA into the dopaminergic nerve terminals and has a DA-like effect on the firing rate of the mesocorticolimbic dopaminergic neurones.[8,9] Accordingly, one would expect to find cocaine receptors, which would bind [^3H]cocaine with high affinity, associated with that system. This has been verified by several groups.[10-12] One would also expect that cocaine analogues should show a behavioral order of potency which should closely approximate the binding order of potency. Actually, both the binding and uptake assays were used to evaluate the high-affinity binding site of brain striatum as the cocaine receptor responsible for the addictive properties of the drug.[13] For further pharmacological



1

(1) Adams, E. H.; Gfoerer, J. C. *Psychiatr. Ann.* **1988**, 18, 325.
(2) Bouknight, L. G.; Bouknight, R. R. *Postgrad. Med.-Cocaine* **1988**, *83*, 115.
(3) Fishman, M. W.; Schuster, C. R. *Fed. Proc.* **1982**, *41*, 241.
(4) Koob, G. F.; Bloom, F. E. *Science* **1988**, *242*, 715.
(5) Wise, R. A. *Pharmacol. Ther.* **1987**, *35*, 227.
(6) Galloway, M. P. *TIPS* **1988**, *9*, 451.
(7) Di Chiara, G.; Imperato, A. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 5274.
(8) Einhorn, L. C.; Johansen, P. A.; White, F. J. *J. Neurosci.* **1988**, *8*, 100.
(9) Grace, A. A. *Ann. N. Y. Acad. Sci.* **1988**, *537*, 51.
(10) Calligaro, D. O.; Eldefrawi, M. E. *J. Pharmacol. Exp. Ther.* **1987**, *243*, 61.
(11) Kennedy, L. T.; Hanbauer, I. *J. Neurochem.* **1983**, *41*, 172.
(12) Schoemaker, H.; Pimoule, C.; Arbilla, S.; Scatton, B.; Javoy-Agio, F.; Langer, S. Z. *Arch. Pharmacol.* **1985**, *329*, 227.

0022-2623/90/1833-2024$02.50/0