Synthesis and Analgesic Activities of Some (4-Substituted phenyl-1-piperazinyl)alkyl 2-Aminobenzoates and 2-Aminonicotinates

Philippe M. Manoury,* André P. Dumas, Henry Najer,

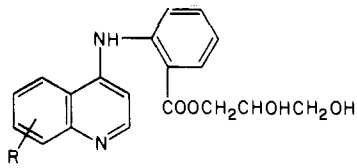
Chemical Research Department

Dan Branceni, Monique Prouteau, and Françoise M. Lefevre-Borg

Biological Research Department, Synthelabo, L.E.R.S., Paris, France. *Received March 20, 1978*

A series of (4-substituted phenyl-1-piperazinyl)alkyl 2-aminobenzoates and 2-aminonicotinates has been prepared and screened for analgesic and antiinflammatory properties in mice and rats. The tabulated results reveal several 2-(4-substituted phenyl-1-piperazinyl)ethyl 2-(7- or 8-substituted 4-quinolinylamino)benzoates to be six to nine times more potent analgesics than the reference compounds (glafenine and aminopyrine) and to possess minor antiinflammatory activity. Compound **45**, 2-[4-[3-(trifluoromethyl)phenyl]-1-piperazinyl]ethyl 2-[[7-(trifluoromethyl)-4-quinolinyl]amino]benzoate (antrafenine), showed marked analgesic activity, long duration of action, and excellent tolerance in pharmacological and toxicological studies, as well as in clinical trials.

Arylanthranilic acids have been shown to be useful analgesic and antiinflammatory compounds. Quinoline analogues of these products[1a,b] [glafenine (1) and flocta-



1, R = 7-Cl
2, R = 8-CF_3

fenine (2)] have been introduced on to the European market.

As a result of an earlier study[2] on the analgesic properties of 4-substituted phenyl-1-piperazinoalkanols, we decided to combine these alcohols with the acid moieties of glafenine and its analogues. We hoped that the resulting esters would be analgesics with reduced antiinflammatory activity and would be less ulcerogenic and better absorbed by virtue of their increased liposolubility.

In addition, the corresponding esters of 2-(phenylamino)benzoic acid and 2-(phenylamino)nicotinic acid were prepared with a view to studying the effect on activity caused by replacement, inter alia, of the quinoline ring by a phenyl moiety.

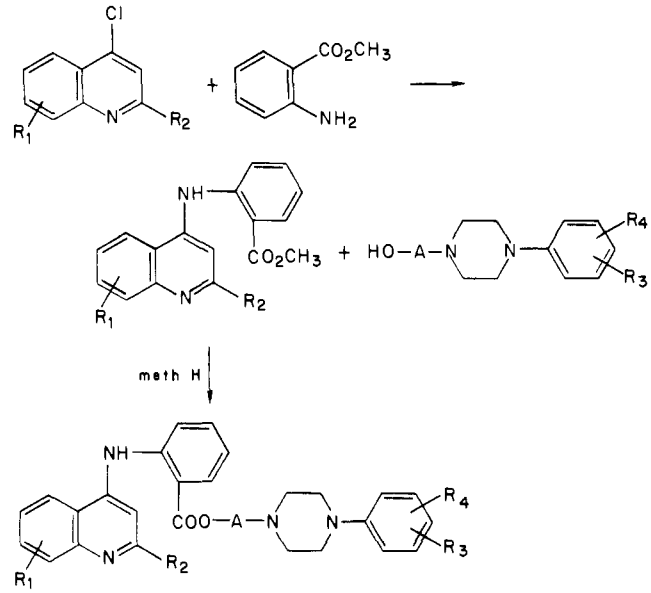
Chemistry. *N*-Phenylpiperazines were prepared by acid-catalyzed condensation of the corresponding anilines with diethanolamine at 240 °C (method A). Subsequent condensation with haloalkanols yielded the 4-substituted phenyl-1-piperazinoalkanols (Table I) (method B). The 2-(phenylamino)benzoates or 2-(phenylamino)nicotinates were obtained by reaction of methyl 2-chloronicotinate or 2-chlorobenzoic acid with suitably substituted anilines (Table II; methods C and D).

The final compounds were synthesized by two methods: base-catalyzed transesterification of methyl 2-(4-quinolinylamino)benzoates with the corresponding 4-phenyl-1-piperazinoalkanol (method H, as outlined in Scheme I; Table V). This reaction was also applied to the synthesis of the alkyl 2-(phenylamino)benzoates and 2-(phenylamino)nicotinates (Table IV).

Alternatively, 4-phenyl-1-piperazinoalkanols were either reacted with isatoic anhydride (method E) or transesterified with methyl anthranilate (method F; Table III), followed by condensation in an acidic medium of the resulting amino esters (method C) with the substituted 4-chloroquinolines (Scheme II; Table V).

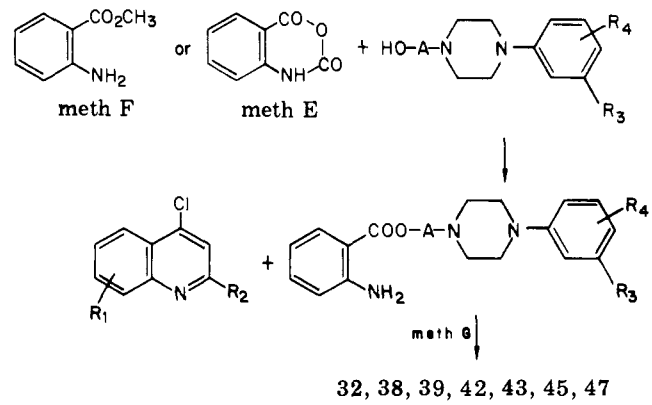
Biological Results. Compounds **22** to **59** were tested for analgesic activity, antiinflammatory activity, and acute

Scheme I



30, 31, 33-37, 40, 41, 44, 46, 48-59

Scheme II



32, 38, 39, 42, 43, 45, 47

toxicities by the methods described under the Experimental Section.

The results of the biological screening tests are summarized in Tables IV and V. Table IV shows that 2-(phenylamino)benzoates (and nicotinates) possessed very low analgesic activity in comparison with the reference compounds. On the other hand, several compounds, as shown in Table V, displayed an interesting level of potency.

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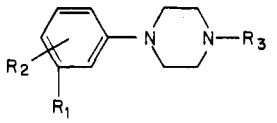
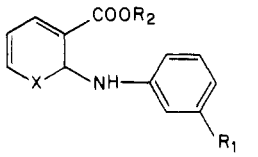
Table I. *N*-(Substituted phenyl)piperazines



no.	R_1	R_2	R_3	formula[a]	method	yield,[b] %	mp or bp (mm), °C
3	SCF_3	H	H	$C_{11}H_{13}F_3N_2S$	A	33	118–121 (3)
4	OCF_3	H	H	$C_{11}H_{13}F_3N_2O$	A	40	135 (1.5)
5	3-Cl	2-CH_3	H	$C_{11}H_{15}ClN_2$	A	46	125 (0.01)
6	CF_3	H	$-CH_2CH_2OH$	$C_{13}H_{17}F_3N_2O$	B	80	142 (0.01)
7	SCF_3	H	$-CH_2CH_2OH$	$C_{13}H_{17}F_3N_2OS$	B	88	182 (0.01)
8	OCF_3	H	$-CH_2CH_2OH$	$C_{13}H_{17}F_3N_2O_2$	B	70	156 (0.01)
9	Cl	2-CH_3	$-CH_2CH_2OH$	$C_{13}H_{19}ClN_2O$	B	73	166 (0.01)
10	CH_3	2-CH_3	$-CH_2CH_2OH$	$C_{14}H_{22}N_2O$	B	67	98[c]
11	CF_3	5-CF_3	$-CH_2CH_2OH$	$C_{14}H_{16}F_6N_2O$	B	80	294[d]

[a] Analyzed for C, H, N; analytical results were within ±0.4% of the theoretical values. [b] No effort was made to optimize yields. [c] Base. [d] Monohydrochloride.

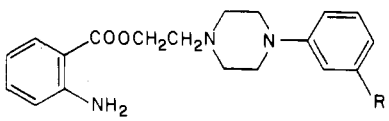
Table II. 2-(Substituted phenylamino)benzoic and 2-(Substituted phenylamino)nicotinic Acids and Esters



no.	X	R_1	R_2	formula[a]	method	yield,[b] %	mp or bp (mm), °C
12	CH	OCF_3	H	$C_{14}H_{10}F_3NO_3$	D	40	109
13	CH	OCF_3	CH_3	$C_{15}H_{12}F_3NO_3$	D	70	144–145 (0.1)
14	N	OCF_3	CH_3	$C_{14}H_{11}F_3N_2O_3$	C	64	142 (0.15)
15	CH	SCF_3	H	$C_{14}H_{10}F_3NO_2S$	D	35	114–116
16	CH	SCF_3	CH_3	$C_{15}H_{12}F_3NO_2S$	D	72	144–146 (0.1)
17	N	SCF_3	CH_3	$C_{14}H_{11}F_3N_2O_2S$	C	78	178–180 (0.1)

[a] Analyzed for C, H, N; analytical results were within ±0.4% of the theoretical values. [b] No effort was made to optimize yields.

Table III. 2-[4-(Substituted phenyl)-1-piperazinyl]ethyl 2-Aminobenzoates



no.	R	formula	anal.[a]	method	yield, %	mp, °C
18	H	$C_{19}H_{23}N_3O_2$	C, H, N	E	89	106
19	Cl	$C_{19}H_{22}ClN_3O_2$	C, H, N	F	73	76
20	CF_3	$C_{20}H_{22}F_3N_3O_2$	C, H, N	E	70	73
21	SCF_3	$C_{20}H_{22}F_3N_3O_2S$	C, H, N	E	78	83

[a] Analyzed as base; analytical results were within ±0.4% of the theoretical values.

The acute toxicity of these products in mice, determined as LD_{50}, was between 2.5 and 4 g/kg po, except for **54** and **56** which were more toxic.

In the acetic acid writhing test, several compounds (**38**, **44**, **45**, **47**, **53**, and **54**) were five to nine times more analgesic than the reference compounds. However, their antiinflammatory activity was always weaker than that of glafenine and aminopyrine.

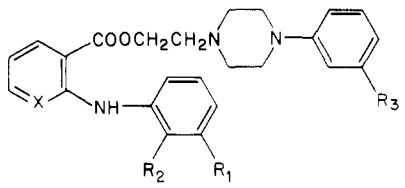
Furthermore, some compounds (**38**, **39**, **44**, **45**, **52**, and **58**) showed moderate but significant analgesic activity in the hot plate test, at lower doses than, for instance, aminopyrine (glafenine is not active in this test).

Structure–Activity Relationships. Increasing the length of chain A (**31**) or branching with a methyl group (**30**) had only a weak influence on the analgesic activity; on the other hand, introduction of a methyl group in the 2 position of the quinoline ring decreased the activity (e.g., **33** is 1.5 times less active than **32**). The introduction of electron-withdrawing groups, such as Cl, CF_3, OCF_3, and SCF_3, in the meta position of the phenyl ring generally increased analgesic potency (**38** and **39** were more active than **32** and **44–47** more than **43**). Notwithstanding, compounds **40** and **41** with *m*-OCF_3 and *m*-SCF_3 were less potent than **32**, while compound **54**, which has no substituent, was very active when compared with **55**, which bears a *m*-SCF_3 group.

Two para-substituted products (**34** and **37**) displayed weak potency. Compound **34** (*p*-OMe, electron donor) was 2.5 times less active than **37** (*p*-Cl), which was itself eight times less active than **38** (*m*-Cl).

In the quinoline ring, replacement of the Cl in the 7 position by a 7-CF_3, 7-SCF_3, 7-OCF_3, or 8-CF_3 group resulted in a general increase in analgesic activity; e.g., compounds **43**, **50**, **54**, and **56** were more potent than compound **32**. Products with a 7-CF_3 group (**44**, **45**, and **47**) were the most active, though compounds **38** (7-Cl), **53**

Table IV. 2-(4-Phenyl-1-piperazinyl)ethyl 2-Phenylaminobenzoates and -nicotinates



compd	X	R_1	R_2	R_3	formula[a]	yield, %	mp, °C[b]	acute tox:[c] LD_{50}, mg/kg po	analgesic act.:[d] ED_{50}, mg/kg po	antiinfl act.:[e] ED_{40}, mg/kg po
aminopyrine								800	40	60
22	CH	CF_3	H	H	$C_{26}H_{27}ClF_3N_3O_2$	58	186	>2000	400	100
23	CH	CH_3	CH_3	H	$C_{27}H_{26}ClF_6N_3O_2$	58	190	4000	90	>50
24	CH	OCF_3	H	H	$C_{26}H_{26}ClF_3N_3O_3$	63	191	2000	>400	150
25	CH	SCF_3	H	Cl	$C_{26}H_{26}Cl_2F_3N_3O_2S$	72	200	>4000	>400	>50
26	CH	SCF_3	H	CF_3	$C_{27}H_{26}ClF_6N_3O_2S$	83	212	>4000	300	>50
27	N	SCF_3	H	CF_3	$C_{26}H_{25}ClF_6N_4O_2S$	59	224	>4000	250	>50
28	N	SCF_3	H	Cl	$C_{25}H_{25}Cl_2F_3N_4O_2S$	77	220	>4000	100	>300
29	N	Cl	CH_3	CF_3	$C_{26}H_{26}ClF_3N_4O_2$	58	91	>4000	200	>100

[a] Analyzed for C, H, N; analytical results were within ±0.4% of the theoretical values. [b] Monohydrochloride. [c] Acute toxicity in mice. [d] Acetic acid writhing test. [e] Carrageenin edema. See Experimental Section.

(8-CF_3), and **54** (7-SCF_3) were very potent.

Discussion

It would be reasonable to expect that in vivo hydrolysis of the described esters would afford the corresponding 2-(4-quinolinylamino)benzoic acids and 4-phenyl-1-piperazinoalkanols which could be independently and simultaneously active. Pharmacokinetic studies of **45** in rats demonstrated that this compound was largely hydrolyzed as expected. The order of the analgesic potency (in the writhing test in mice) of the 2-(4-quinolinylamino)benzoic acids in relation to the substituent on the quinoline ring was as follows: acids 7-CF_3 > 8-CF_3 > 7-OCF_3 > 7-SCF_3 > 7-Cl, ED_{50} (mg/kg) = 18, 30, 65, 90, > 200, while that of the 4-phenyl-1-piperazinoalkanols as a function of the *m*-phenyl substituent was: alcohols CF_3 > Cl > H > OCF_3 > SCF_3, ED_{50} (mg/kg) = 8, 15, 26, 28, 75.

It could be assumed that the analgesic activity of the esters would be maximal when the activity of each potential metabolite was maximal. Only **44**, **45**, and **55** correlated with the prediction. The high potencies of **53** and **54** and low potency of **51**, which were not in accordance with this concept, were noteworthy deviations. Table VI gives, in decreasing order, the analgesic activity in the writhing test of some *m*-phenyl-substituted compounds.

In fact, the measured activity probably depends on at least three parameters: (1) absorption and distribution factors, linked to a great extent with the lipophilicity of the molecule and its partition coefficient; (2) the activity of the ester and its metabolites; (3) and the rate of hydrolysis, which is a function of the nature of the substituents on the two rings.

The analgesic activity of the 7-chloro- and 7-[(trifluoromethyl)thio]-2-(4-quinolinylamino)benzoic acids was increased by esterification with 4-phenylpiperazinoethanols, possibly by virtue of the high lipophilicity and resulting better absorption (see esters **35**, **36**, **38**, and **54**); **55**, however, which has very high lipophilicity, was weakly active. The esters which bear a CF_3 group in position 7 of the quinoline ring were generally active as the acid, and the activity was not in relation either with the lipophilicity of the substituent on the phenyl ring or with the activity of the corresponding (4-(meta-substituted phenyl)-piperazinoethanol.

In the case of the esters derived from 4-phenylpiperazinoethanol itself, the activity increased linearly with the lipophilicity of the substituent on the quinoline ring (**32**, **43**, **54**, and **56**). This relationship did not hold for esters derived from *m*-chloro- or *m*-trifluoromethyl-4-phenylpiperazinoethanol.

As a result of primary screening, five compounds (**44**, **45**, **47**, **53**, and **54**) were retained for further evaluation.

Compound **45** (antrafenine) was selected for development. In pharmacological and toxicological studies,[3] as well as in clinical trials, it showed marked analgesic activity, long duration of action, and low toxicity. The main acute toxicity and analgesic activity data for this compound and for its two components are given in Table VII.

Experimental Section

Melting points were taken on a Tottoli apparatus and are uncorrected. Analytical results for elements indicated were within ±0.4% of the theoretical values. Similarly IR and NMR spectra support the structural assignments.

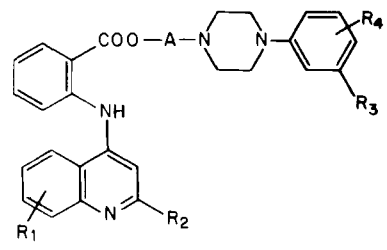
We have prepared according to the literature the 7-(trifluoromethyl)-,[4] 7-[(trifluoromethyl)thio]-,[5] 7-(trifluoromethoxy)-,[2] 8-chloro-,[2] 8-(trifluoromethyl)-,[2] and 4-chloroquinolines; the 3-(trifluoromethyl)phenyl- and 3,5-bis(trifluoromethyl)phenylpiperazines;[6] and the methyl 2-(4-quinolylamino)benzoates.[1,2]

Method A. 1-[3-[(Trifluoromethyl)thio]phenyl]piperazine (3). In a 500-mL three-neck flask equipped with a thermometer, a gas bubbler, and a reflux condenser, 38.6 g (0.2 mol) of 3-[(trifluoromethyl)thio]aniline[7] and 21 g (0.2 mol) of freshly distilled diethanolamine were introduced.

Hydrogen chloride was bubbled into the mixture for about 40 min. The reaction mixture was heated for 1 h at 180 °C. Hydrogen chloride was again introduced for 30 min, the temperature being maintained at 200 °C. The reaction was completed by heating for 1.5 h at 240 °C. The mixture was allowed to cool and then was poured into 100 mL of water. The red solution was basified with 40% NaOH and extracted three times with 100 mL of chloroform. The chloroform extracts were washed with water and dried over $CaCl_2$. After removal of the solvent from the solution, the oily residue was rapidly distilled, the product boiling at 80–130 °C (4 mm); this was redistilled to yield 17.4 g (33%): bp 118–121 °C (3 mm). Anal. ($C_{11}H_{13}F_3N_2S$) C, H, N.

Method B. 4-[3-[(Trifluoromethyl)thio]phenyl]-1-piperazinoethanol (7). 1-[3-[(Trifluoromethyl)thio]phenyl]-piperazine (26.3 g, 0.10 mol), 2-chloroethanol (12.2 g, 0.15 mol), Na_2CO_3 (15.9 g, 0.15 mol), and ethanol (300 mL) were refluxed and stirred together for 6 h. The boiling reaction mixture was filtered and the filtrate was evaporated. The residue was taken

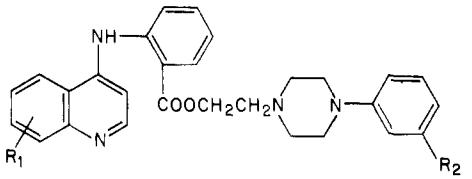
Table V. (4-Phenyl-1-piperazinyl)alkyl 2-[(4-Quinolyl)amino]benzoates



no.	R_1	R_2	R_3	R_4	A	formula[a]	meth	yield, %	mp, °C base	mp, °C salt[b]	acute tox: LD_{50}, mg/kg po	analgesic act. writh test: ED_{50}, mg/kg po	analgesic act. hot plate: ED_{50}, mg/kg po	antiinflam act.,[f] carr edema: ED_{40}, mg/kg po
30	7-Cl	H	H	H	$-CH(CH_3)CH_2$	$C_{29}H_{29}ClN_4O_2$	H	79.7		212	>2000	63	200	300
31	7-Cl	H	H	H	$-(CH_2)_3$	$C_{29}H_{29}ClN_4O_2$	H	72.5	110	192	2000	60	250	50
32	7-Cl	H	H	H	$-(CH_2)_2$	$C_{28}H_{27}ClN_4O_2$	G	88.9	134	202	4250	75	200	120
33	7-Cl	CH_3	H	H	$-(CH_2)_2$	$C_{29}H_{29}ClN_4O_2$	H	77.5	134	242	4000	110	300	>200
34	7-Cl	H	H	*p*-OCH_3	$-(CH_2)_2$	$C_{29}H_{29}ClN_4O_2$	H	91	146	238		200	>300	45
35	7-Cl	H	CH_3	*o*-CH_3	$-(CH_2)_2$	$C_{30}H_{31}ClN_4O_2$	H	87.9	126	220	4000	22	300	120
36	7-Cl	H	Cl	*o*-CH_3	$-(CH_2)_2$	$C_{29}H_{28}Cl_2N_4O_2$	H	81	122	210	>4000	15	>300	30
37	7-Cl	H	H	*p*-Cl	$-(CH_2)_2$	$C_{28}H_{26}Cl_2N_4O_2$	H	60	145	240	>2000	80	>300	100
38	7-Cl	H	Cl	H	$-(CH_2)_2$	$C_{28}H_{26}Cl_2N_4O_2$	G	83	122	216	4000	10	60	>100
39	7-Cl	H	CF_3	H	$-(CH_2)_2$	$C_{29}H_{26}ClF_3N_4O_2$	G	80	100-102	217	2500	24	150	40
40	7-Cl	H	OCF_3	H	$-(CH_2)_2$	$C_{29}H_{26}ClF_3N_4O_3$	H	74.5		228	4000	120	>300	50
41	7-Cl	H	SCF_3	H	$-(CH_2)_2$	$C_{29}H_{26}ClF_3N_4O_2S$	H	73.6		230	3000	120	200	30
42	8-Cl	H	H	H	$-(CH_2)_2$	$C_{28}H_{27}ClN_4O_2$	G	68.7	164	214	>4000	80	>250	300
43	7-CF_3	H	H	H	$-(CH_2)_2$	$C_{29}H_{27}F_3N_4O_2$	G	79	130	210	2500	30	300	40
44	7-CF_3	H	Cl	H	$-(CH_2)_2$	$C_{29}H_{26}ClF_3N_4O_2$	H	65	122	240	>4000	9	150	30
45	7-CF_3	H	CF_3	H	$-(CH_2)_2$	$C_{30}H_{26}F_6N_4O_2$	G	57	88-90	230	4000	6	30	40
46	7-CF_3	H	OCF_3	H	$-(CH_2)_2$	$C_{30}H_{26}F_6N_4O_2$	H	95		270	3500	20	>300	50
47	7-CF_3	H	SCF_3	H	$-(CH_2)_2$	$C_{30}H_{26}F_6N_4O_2S$	G	86		220	3750	8	>300	30
48	7-CF_3	H	CF_3	*m*-CF_3	$-(CH_2)_2$	$C_{31}H_{25}F_9N_4O_2$	H	85	114	238	4000	20	>300	80
49	7-CF_3	H	Cl	*o*-CH_3	$-(CH_2)_2$	$C_{30}H_{28}ClF_3N_4O_2$	H	92	130	230	3000	20	>300	65
50	8-CF_3	H	H	H	$-(CH_2)_2$	$C_{29}H_{27}F_3N_4O_2$	H	86	147		3000	35	>300	64
51	8-CF_3	H	Cl	H	$-(CH_2)_2$	$C_{29}H_{26}ClF_3N_4O_2$	H	81	140		>3000	18	>300	100
52	8-CF_3	H	CF_3	H	$-(CH_2)_2$	$C_{30}H_{26}F_6N_4O_2$	H	81	141		>3000	40	100	120
53	8-CF_3	H	SCF_3	H	$-(CH_2)_2$	$C_{30}H_{26}F_6N_4O_2S$	H	86	128		>3000	6	200	>100
54	7-SCF_3	H	H	H	$-(CH_2)_2$	$C_{29}H_{27}F_3N_4O_2S$	H	78	126	230-232	1200	9	300	100
55	7-SCF_3	H	SCF_3	H	$-(CH_2)_2$	$C_{30}H_{26}F_6N_4O_2S_2$	H	81	90	220	2200	60	200	180
56	7-OCF_3	H	H	H	$-(CH_2)_2$	$C_{29}H_{27}F_3N_4O_3$	H	73.8	80	202	1300	24	300	40
57	7-OCF_3	H	Cl	H	$-(CH_2)_2$	$C_{29}H_{26}ClF_3N_4O_3$	H	58.5		224-226	4000	20	300	85
58	7-OCF_3	H	CF_3	H	$-(CH_2)_2$	$C_{30}H_{28}Cl_2F_6N_4O_3$	H	81	70	226	1500	20	150	43
59	7-OCF_3	H	OCF_3	H	$-(CH_2)_2$	$C_{30}H_{26}F_6N_4O_4$	H	62		247-248	3500	70	>300	75
glafenine											3500	56	>600	30
aminopyrine											850	50	250	80

[a] Analyzed for C, H, N; analytical results were within ±0.4% of the theoretical values. [b] Dihydrochloride. [c] Acute toxicity in mice. [d] Acetic acid writhing test in mice. [e] Hot plate test in mice. [f] Carrageenin edema in rats. For footnotes *c–f*, see Experimental Section.

Table VI. Comparative Analgesic Activities of Some Derivatives



compd	R_1	R_2	analg act.:[a] ED_{50}, mg/kg po
45	7-CF_3	*m*-CF_3	6
53	8-CF_3	*m*-SCF_3	6
47	7-CF_3	*m*-SCF_3	8
54	7-SCF_3	H	9
44	7-CF_3	*m*-Cl	9
38	7-Cl	*m*-Cl	10
51	8-CF_3	*m*-Cl	18
39	7-Cl	*m*-CF_3	24
52	8-CF_3	*m*-CF_3	40
55	7-SCF_3	*m*-SCF_3	60
glafenine			56

[a] Writhing test in mice.

Table VII. Comparative Acute Toxicities and Analgesic Activities of Compound 45 (Antrafenine) and Its Two Components

compd	acute tox: LD_{50}, mg/kg po	writhing test: ED_{50}, mg/kg po	hot-plate test: ED_2, mg/kg po
antrafenine (45)	4000	6	30
TQBA[a]	1000	18	>600
TPPE[a]	740	8	6

[a] Abbreviations used are: TQBA, 7-(trifluoromethyl)-2-(4-quinolinylamino)benzoic acid; TPPE, *m*-trifluoromethyl-4-phenyl-1-piperazinoethanol.

up in ether, a slight amount of insoluble matter was filtered, and the solution was washed with water and dried over $MgSO_4$. After removal of solvent from the solution, the product was distilled to yield 26.85 g (87%), bp 182 °C (0.01 mm). Anal. ($C_{13}H_{17}F_3N_2O$) C, H, N.

Method C. Methyl 2-[[3-[(Trifluoromethyl)thio]phenyl]amino]nicotinate (17). A mixture of 13.3 g (0.077 mol) of methyl 2-chloronicotinate and of 29.9 g (0.155 mol) of 3-[(trifluoromethyl)thio]aniline was heated for 10 min at 200–210 °C. The reaction mixture was allowed to cool and poured into 200 mL of ether. The precipitate, 3-[(trifluoromethyl)thio]aniline hydrochloride, was filtered and the filtrate evaporated to dryness. The oily residue was distilled to yield 19.5 g (78%), bp 178–180 °C (0.1 mm), of compound which solidified in the receiver, mp 50–52 °C. Anal. ($C_{14}H_{11}F_3N_2O_2S$) C, H, N.

Method D. Methyl 2-[[3-[(Trifluoromethyl)thio]phenyl]amino]benzoate (16). (a) 2-[[3-[(Trifluoromethyl)thio]phenyl]amino]benzoic Acid (15). A mixture of 12.9 g (0.054 mol) of potassium 2-bromobenzoate, 25 mL of bis(2-methoxyethyl) ether, 5.8 g (0.05 mol) of 4-ethylmorpholine, 9.7 g (0.05 mol) of 3-[(trifluoromethyl)thio]aniline, and 0.5 g of cupric acetate was gradually heated to 140–145 °C while stirring for 1.5 h. Four milliliters of concentrated hydrochloric acid and 30 mL of water were added; the mixture was extracted with chloroform. The chloroform extracts were washed with water; the organic phase was dried over $MgSO_4$. After removal of the solvent from the mixture, the residue was triturated with water. The solid obtained was recrystallized from cyclohexane to yield 5.5 g (35%), mp 114–116 °C. Anal. ($C_{14}H_{10}F_3NO_2S$) C, H, N.

(b) Methyl 2-[[3-[(Trifluoromethyl)thio]phenyl]amino]benzoate (16). Hydrogen chloride was bubbled for 5 h into a solution of 15.7 g (0.05 mol) of the above compound in 150 mL of methanol. The reaction mixture was refluxed for 10 h. The solvent was evaporated, and the residue was taken up in diethyl ether, washed with water and $NaHCO_3$, and dried. The oil obtained after evaporation was distilled to yield 11.8 g (72%) of the expected ester: bp 144–146 °C (0.1 mm); mp 52 °C. Anal. ($C_{15}H_{13}F_3NO_2S$) C, H, N.

Method E. 2-[4-[3-(Trifluoromethyl)phenyl]-1-piperazinyl]ethyl 2-Aminobenzoate (20). A mixture of 22 g (0.08 mol) of 2-[4-[3-(trifluoromethyl)phenyl]-1-piperazinyl]ethanol and 17.5 g (0.107 mol) of isatoic anhydride in 200 mL of toluene was refluxed for 3 h. The hot solution was treated with charcoal, filtered, evaporated, and taken up in ether. A little insoluble isatoic anhydride was filtered. After removal of solvent from the solution and trituration of the residue in petroleum ether, the product was filtered and recrystallized from isopropyl alcohol to yield 23 g (73%) of the compound, mp 73 °C. Anal. ($C_{20}H_{22}F_3N_3O_2$) C, H, N.

Method F. 2-[4-(3-Chlorophenyl)-1-piperazinyl]ethyl Anthranilate (19). Methyl anthranilate (10.6 g, 0.07 mol), 2-[4-(3-chlorophenyl)-1-piperazinyl]ethanol (12.1 g, 0.05 mol), dry toluene (150 mL), and sodium (0.09 g) were transesterified. When the reaction was finished, the toluene solution was filtered and evaporated. The residue was triturated in petroleum ether, filtered, and recrystallized from isopropyl alcohol to yield 13 g (72%), mp 77 °C. Anal. ($C_{19}H_{22}ClN_3O_2$) C, H, N.

Method G. 2-[4-[3-(Trifluoromethyl)phenyl]-1-piperazinyl]ethyl 2-[[7-(Trifluoromethyl)-4-quinolinyl]amino]benzoate (45). A mixture of 9.23 g (0.0235 mol) of 2-[4-[3-(trifluoromethyl)phenyl]-1-piperazinyl]ethyl 2-aminobenzoate, 7 g (0.03 mol) of 4-chloro-7-(trifluoromethyl)quinoline, 100 mL of water, and 20 mL of 2 N hydrochloric acid was refluxed for 2 h. The reaction mixture was neutralized with $NaHCO_3$ and extracted with methylene chloride. After removal of the solvent from the mixture, the residual solid was triturated in petroleum ether, filtered, and recrystallized from isopropyl alcohol to yield 10.95 g (79%), mp 88 °C. Anal. ($C_{30}H_{26}F_6N_4O_2$) C, H, N.

Method H. 2-(4-Phenyl-1-piperazinyl)ethyl 2-[[7-(Trifluoromethyl)-4-quinolinyl]amino]benzoate (54). A mixture of 18.9 g (0.05 mol) of methyl 2-[[7-[(trifluoromethyl)thio]-4-quinolinyl]amino]benzoate [prepared from 7-[(trifluoromethyl)thio]-4-chloroquinoline and methyl anthranilate], 15.95 g (0.075 mol) of 2-(4-phenyl-1-piperazinyl)ethanol, 0.025 g of sodium, and 100 mL anhydrous toluene was refluxed for 5 h, while the methanol formed during the reaction was slowly distilled. After removal of solvent from the mixture, the residual product was dissolved in methylene chloride, and the solution was washed with water and dried. After removal of solvent, from the solution, the oily product obtained was dissolved in boiling 2-propanol. The precipitate obtained after cooling was filtered, washed with isopropyl alcohol, and dried in vacuo to yield 20.2 g (73%) of the base, mp 120 °C.

The dihydrochloride was prepared by bubbling hydrogen chloride into a solution of 11.05 g (0.02 mol) of base in 70 mL of methylene chloride. The salt was filtered, washed with water, dried, and recrystallized from 250 mL of ethanol to yield 9.5 g (76%), mp 230–232 °C. Anal. ($C_{29}H_{29}Cl_2F_3N_4O_2S$) C, H, N.

Biological Methods. All compounds were tested as dichlorhydrates, except **45**, **50**, and **53** which were studied as bases. They were prepared as aqueous solutions or suspensions (vehicle: "Tween 80", 1% in distilled water) and administered orally in a volume of 10 mL/kg of body weight for mice and 5 mL/kg for rats.

Acute Toxicity. Oral LD_{50} values were determined in male CD1 (Charles River, France) mice, weighing 20 ± 2 g (using at least 10 animals per dose level and a 7-day observation period) according to the method of Litchfield and Wilcoxon.[8]

Acetic Acid Writhing Test. This test was performed according to a modification[9] of the method of Koster et al.[10] CD1 male mice weighing 20 ± 2 g were divided into random groups, and the compounds were given orally at several dose levels. At least two groups of six mice were used per dose. Thirty minutes after treatment, all animals received an ip injection of 10 mL/kg of 0.6% acetic acid in water. The number of writhing movements was counted over a 15-min period after the acetic acid injection. The ED_{50} (dose reducing writhes by 50%) was determined graphically.

Hot Plate Test. The method used was a variant of that described by Eddy et al.[11] OF1 (Iffa Credo, France) male mice

weighing 20 ± 2 g were divided into random groups, and the compounds were given orally at several dose levels, using at least 10 animals per dose. Thirty minutes after treatment, each animal was individually placed on a metal plate heated to 56 °C by boiling acetone. The paw-licking reaction time was noted, and the ED_2 (dose doubling this time) was determined graphically.

Carrageenin Rat Paw Edema. The procedure employed was a modification of the method of Winter et al.[12] CD1 (Charles River, France) male rats weighing about 150 g were arranged in groups of 8 or 10, and the compounds were given orally at several dose levels. One hour after treatment they received a subplantar injection of 1 mg of carrageenin in 0.1 mL of water in the left hind paw. Paw volume was measured prior and 3 h after the carrageenin injection. The ED_{40} (dose inhibiting by 40% the increase in paw volume) was determined graphically.

In this screening program, compounds were usually given at three dose levels, using a control group and a one-dose reference compound group (glafenine or aminopyrine). If activity of the reference compound proved to be abnormal, the assay was discarded. Standard error of the mean and significance compared to the controls (Dunnett's "t" test)[13] were computed in the carrageenin rat paw edema and in the hot-plate test but not in the writhing test (since writhing counts were not done individually but on six animal groups). These figures do not appear in the tables because of lack of space.

References and Notes

(1) (a) A. Allais, G. Rousseau, P. Girault, J. Mathieu, M. Peterfalvi, D. Branceni, G. Azadian-Boulanger, L. Chifflot, and R. Jequier, *Chim. Ther.*, **1**, 65 (1966); (b) A. Allais, G. Rousseau, J. Meier, G. Nominé, M. Peterfalvi, R. Deraedt, L. Chifflot, J. Benzoni, and R. Fournex, *ibid.*, **8**, 154 (1973).
(2) B. Bucher, Synthélabo, 1971, unpublished results.
(3) D. Branceni, M. Prouteau, P. Manoury, H. Najer, and R. Giudicelli, *Proc. Int. Congr. Pharmacol., 6th, 1975*, 794, abstr (1975).
(4) H. R. Snyder, H. E. Freier, P. Kavacic and E. M. Van Heyningen, *J. Am. Chem. Soc.*, **69**, 371 (1947).
(5) Société d'Etudes Scientifiques et Industrielles de l'Ile de France, French Patent 1 600 535; *Chem. Abstr.*, **74**, 99905 (1971).
(6) May and Baker, British Patent 948 766; *Chem. Abstr.*, **60** 12028 (1964).
(7) L. M. Yagupol'skii and M. S. Marenets, *J. Gen. Chem. USSR (Engl. Transl.)*, **26**, 99 (1956); *Chem. Abstr.*, **50**, 13793 (1956).
(8) J. T. Litchfield and F. Wilcoxon, *J. Pharmacol. Exp. Ther.*, **99**, 96 (1949).
(9) M. Peterfalvi, D. Branceni, G. Azadian-Boulanger, L. Chifflot, and R. Jequier, *Med. Pharmacol. Exp.*, **15**, 254 (1966).
(10) R. Koster, M. Anderson, and E. J. de Beer, *Fed. Proc., Fed. Am. Soc. Exp. Biol.*, 18, 412 (1959).
(11) N. B. Eddy and Leimbach, *J. Pharmacol. Exp. Ther.*, **107**, 385 (1953).
(12) C. A. Winter, E. D. Risley, and G. W. Nuss, *Proc. Soc. Exp. Biol. Med.*, 111, 544 (1962).
(13) C. W. Dunnett, *J. Am. Stat. Assoc.*, **50**, 1096 (1955).

Synthesis and Antibacterial Activity of 2'-Substituted Chelocardin Analogues

David L. Garmaise,*[1] Daniel T. W. Chu,[1] Edith Bernstein, Makoto Inaba,

Department of Research, Abbott Laboratories, Limited, Montreal, Quebec H3C 3K6

and John M. Stamm

Department of Microbial Research, Abbott Laboratories, North Chicago, Illinois 60064.

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Chelocardin (1) was condensed with numerous hydrazines, hydrazides, and anilines, yielding 2'-substituted derivatives with antibacterial spectra similar to the parent antibiotic. The hydrazone derivatives **9** and **10** and the two anilino derivatives **42** and **44** had more in vivo antibacterial activity than chelocardin.

Chelocardin, a potent broad-spectrum antibiotic produced by *Nocardia sulphurea* (NRRL-2822), was isolated in 1956. Its structure was established by Mitscher et al. as 1.[2] The antibacterial spectrum was shown to differ substantially from that of tetracycline, since it is more active against Gram-negative than Gram-positive bacteria.[3]

Despite having the opposite configuration to tetracycline at carbon-4 and being in the anhydrotetracycline series, chelocardin possesses remarkable antibacterial activity. The 4-epi isomer, which has the same configuration as in the tetracycline series, is virtually inactive. Hence, the structure–activity relationship known to be associated with tetracycline antibiotics[4] could not be assumed to apply to chelocardin analogues. In this paper, we report the preparation and antibacterial activity of three classes of 2'-substituted chelocardin analogues.

Chemistry. The 2'-substituted chelocardin derivatives were synthesized as indicated in Scheme I. Chelocardin (1) hydrochloride reacted readily with a slight molar excess of substituted hydrazines, **2**, in aqueous tetrahydrofuran to give 2'-substituted hydrazones of chelocardin, **3**. These compounds, described in Table I, were isolated as crystalline hydrochloride salts in yields varying from 12 to 98%.

The 2'-substituted acylhydrazone derivatives of chelocardin, **5** (described in Table II), were prepared by condensation of chelocardin hydrochloride with a variety of hydrazides, **4**, and were also isolated as crystalline hydrochloride salts.

The 2'-substituted anilino compounds, **7** (described in Table III), were prepared by reacting chelocardin hydrochloride with the corresponding aniline **6** in aqueous tetrahydrofuran in the presence of acetic acid.

All the derivatives reported here were characterized and tested as the hydrochloride salts. It is interesting to note that all these compounds show an additional adsorption at approximately 307–312 nm in their UV spectra, indicating the formation of a vinylogous imide system.[5] Hence, the alternative tautomeric structures for **3**, **5**, and **7** represented by structures **3a**, **5a**, and **7a**, respectively, cannot be excluded. The position of substitution, at the 2'-carbonyl rather than at the 1- or 3-carbonyl groups, was

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