Antiallergic Activity of Tetracyclic Derivatives of Quinoline-2-carboxylic Acid. 2. Some Benzothienoquinolinecarboxylic Acids

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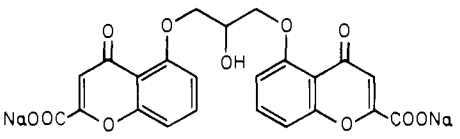
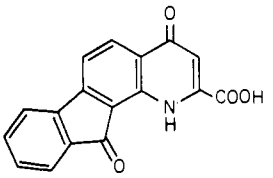
Some benzothienoquinolinecarboxylic acids were synthesized and tested in the rat passive cutaneous anaphylaxis (PCA) assay as potential antiallergic agents. Many of the compounds showed activity comparable to that shown by disodium cromoglycate (DSCG); two of them, 1,4-dihydro-4,6,6-trioxo-5-chloro[1]benzothieno[2,3-*g*]quinoline-2-carboxylic acid and 1,4-dihydro-1,7-dioxo[1]benzothieno[3,2-*f*]quinoline-3-carboxylic acid, showed potency approximately eightfold greater than that of DSCG in the PCA assay.

Disodium cromoglycate (DSCG, 1) has been shown to



1, DSCG



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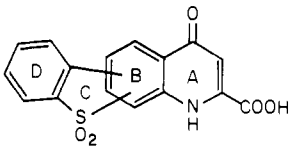
be useful for the prophylactic treatment of the allergic disease state.[1] The passive cutaneous anaphylaxis (PCA) model in rats provides a convenient method for measuring the potential antiallergic activity of compounds which may act like DSCG.[2] Inhibition of the PCA reaction by substituted 1,4-dihydroquinoline-2-carboxylic acids has been described by other investigators as well as by our group.[3-5] In the previous report from our laboratories, we described the enhancement of this PCA inhibitory activity by substitution of the quinoline ring system with acetyl, benzoyl, and phenylsulfonyl moieties and particularly by the incorporation of the benzoyl moiety into a tetracyclic structure, **2**.[5]

We have now extended our work to include compounds which feature the incorporation of the phenylsulfonyl moiety into a tetracyclic structure **3**, in which the con-



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formation of the phenylsulfonyl substituent is effectively fixed. At the outset of this work we were particularly interested in the effect of the shape of the tetracyclic molecule on the PCA activity of the system. Therefore, one of our initial goals was to synthesize all six of the possible isomeric benzothienoquinoline systems, as designated in the heading for Table I. The six structures vary greatly in shape, from the extremely bent structure of type A to the oppositely bent structure of type F. It seemed possible that some of these compounds might be more potent than others due to better accommodation by a receptor site, and an investigation of this possibility was of interest to us. As our work progressed, it also became of interest to prepare and test derivatives of two of the six possible isomeric structural types, types C and F.

The synthesis and PCA inhibitory activity of the six isomeric benzothienoquinoline-2-carboxylic acid systems, as well as the synthesis and PCA inhibitory activity of some derivatives of two of these systems, are the subjects of this report.

Chemistry. The reactions of various anilines with dimethyl acetylenedicarboxylate (DMAD), followed by thermal ring closure to methyl 1,4-dihydro-4-oxoquinolinecarboxylates, have been previously reported,[6] and we used this method as a general route to our compounds. Scheme I shows how this general method was applied to the four isomeric aminodibenzothiophenes in order to prepare four of the six possible isomeric tetracyclic ring systems. Thus reaction of the appropriate aminodibenzothiophene **4**, **10**, **15**, or **21** with DMAD in methanol solution readily gave the corresponding diester, which could be cyclized to the tetracyclic ester at 240 °C in Dowtherm A or diphenyl ether. Oxidation of the sulfur atom to a sulfone was readily accomplished with 30% hydrogen peroxide in refluxing acetic acid. Finally, hydrolysis of the ester to the acid was carried out by reaction with aqueous NaOH solution, followed by acidification with HCl.

In the case of 1- or 4-aminodibenzothiophene (Scheme I, a or b), the cyclization step (**5** to **6** or **11** to **12**) can only lead to a single isomer of type A or B, respectively, since there is only one adjacent ring position open for cyclization. In the case of 2- or 3-aminodibenzothiophene, however, (Scheme I, c or d), there are two such ring positions open during the cyclization step (**16** to **17** or **22** to **23**), so that the isolation of two isomers, an "angular" one and a "linear" one, is conceivable in each case. In fact, we found that for each cyclization reaction we isolated only the single isomer resulting from ring closure to the 1 position (in the case of **16** to **17**, structure type F) or the 4 position (in the case of **22** to **23**, structure type E). Thus the "angular" isomers were obtained in each case. This fact is clearly indicated by the ^{1}H NMR data (see Table V) since (a) the ring B aromatic protons appear as a set of doublets with a coupling constant of ~8 Hz in each case and (b) in the case of compound **17a** ($R_1 = R_2 = H$), the proton in the 11 position appears as a one-proton multiplet which has been shifted downfield about 2 ppm from the rest of the aromatic protons, presumably because of its proximity to the carbonyl oxygen in ring A.

In contrast, the cyclizations of the corresponding diesters from 2- or 3-aminodibenzothiophene 5,5-dioxide took a completely different route. Thus modifying the above sequence as shown in Scheme II, starting with an aminodibenzothiophene 5,5-dioxide (**27** or **33**) led to isolation in each case of the single isomer resulting from ring closure to the 3 position (**29**, structure type C) or the 2 position

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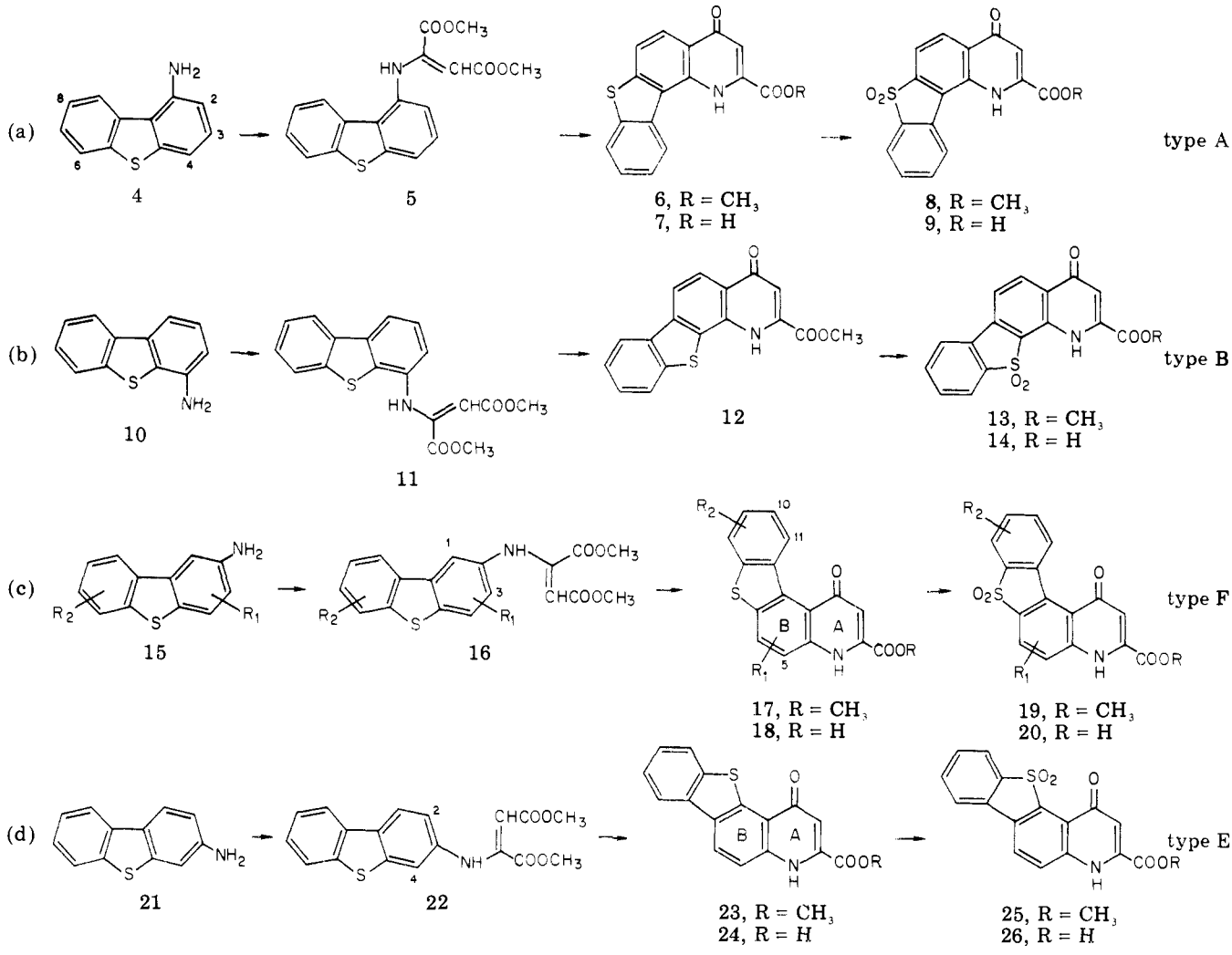
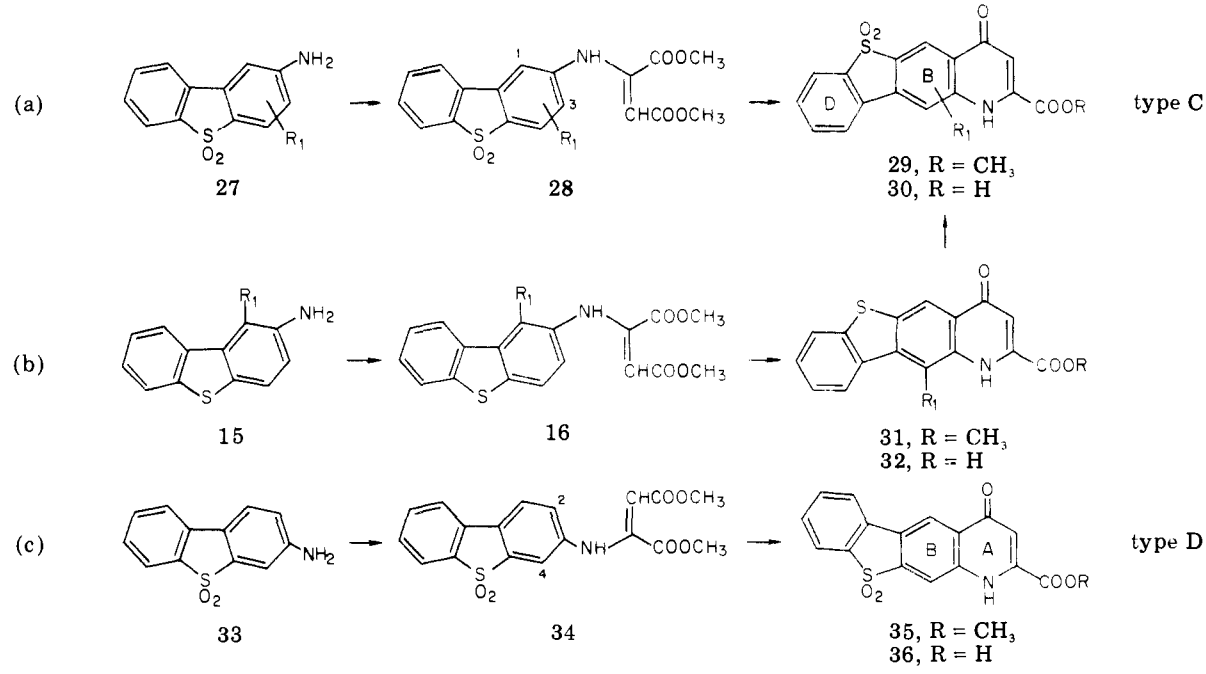
Scheme I

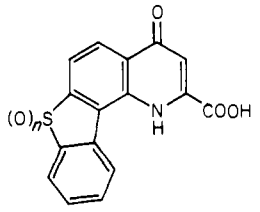


Scheme II

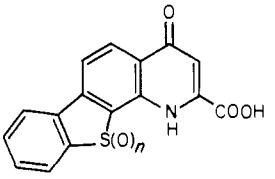


(**35**, structure type D). Again, the structures of these "linear" isomers are indicated by the ^{1}H NMR data (Table V), since (a) the ring B aromatic protons appear as singlets and (b) in the case of compound **29a** (R_1 = H), the ring D aromatic protons appear as a complex 4-proton multiplet. Thus by appropriate choice of the starting aminodibenzothiophene it is possible to prepare all six of the possible benzothienoquinoline ring systems, types A–F.

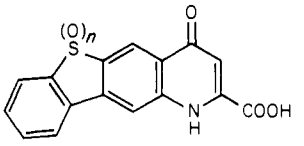
Table I. Benzothienoquinolinecarboxylic Acids[a]



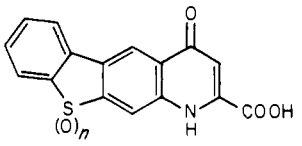
type A, [2,3-*h*]



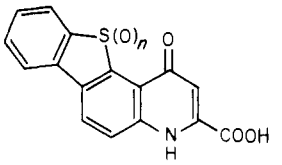
type B, [3,2-*h*]



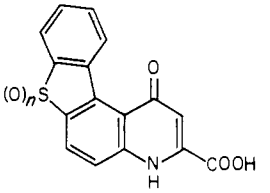
type C, [2,3-*g*]



type D, [3,2-*g*]



type E, [2,3-*f*]



type F, [3,2-*f*]

compd	type	*n*	formula[b]	mp, °C	yield,[c] %	recrystn solvent	MED,[d] mg/kg ip
7	A	0	$C_{16}H_9NO_3S \cdot 0.25H_2O$	290 dec	53	dil HCl[e]	>10
9	A	2	$C_{16}H_9NO_5S \cdot 0.5H_2O$	269-270	12	DMF-H_2O	5
14	B	2	$C_{16}H_9NO_5S \cdot 0.5DMF$	250 dec	11	DMF-H_2O	>25
18a	F	0	$C_{16}H_9NO_3S \cdot 0.25H_2O$	>285	27	H_2O[e]	5
20a	F	2	$C_{16}H_9NO_5S \cdot 0.75H_2O$	285-290	39	DMF	1.3 (0.9-1.7)[f]
24	E	0	$C_{16}H_9NO_3S \cdot {}^1/_3H_2O$	>300	16	DMF	>10
26	E	2	$C_{16}H_9NO_5S \cdot 0.5H_2O$	>300	51	H_2O[e]	>10
30a	C	2	$C_{16}H_9NO_5S \cdot 1.25H_2O$	>360	48	H_2O[e]	2.5
36	D	2	$C_{16}H_9NO_5S \cdot {}^4/_3H_2O$	>300	48	dil HCl[e]	2.5
37a	F	1	$C_{16}H_9NO_4S$	320-322	91	DMF-EtOH	0.3 (0.2-0.4)[g]
42a	C	1	$C_{16}H_9NO_4S \cdot {}^4/_3H_2O$	255-260	11	H_2O[e]	1.25

[a] Prepared by general methods B-D or B and D. See the Experimental Section. [b] The results of C, H, and N analyses are within ±0.4% of the theoretical values. [c] Overall yields are, with one exception, for two steps: cyclization and saponification from the diester (Table IV) or oxidation and saponification from the ester (Table VI). The yield for 37a is for one step (oxidation of 18a). [d] See Biological Methods section of the text for a definition of MED. [e] Not recrystallized; washed with the indicated solvent. [f] This value is an ID_{50} (95% confidence limits), determined on the basis of 36 experiments using six animals each. [g] This value is an ID_{50} (95% confidence limits), determined on the basis of 14 experiments using six animals each.

A series of compounds of type C was prepared either by the above route from substituted 2-aminodibenzothiophene 5,5-dioxides (**27**) or alternatively by starting with a 1-substituted 2-aminodibenzothiophene, in which case eventual cyclization is forced to go to the 3 position (Scheme II, b). A series of compounds of type F was prepared as illustrated in Scheme I (c) by starting with a series of substituted 2-aminodibenzothiophenes.

It was also of interest to prepare the corresponding sulfoxides of types C and F for biological testing, and this was accomplished as shown in Scheme III. Oxidation of the acid **18a** with sodium metaperiodate in basic aqueous DMF led to the sulfoxide **37a**. However, this reaction was extremely inconsistent in our hands, perhaps for solubility reasons, and was not useful as a general method of synthesis. Attempts to prepare the sulfoxides by other oxidative methods were generally unsuccessful since large amounts of the corresponding sulfones were also obtained. When the general reaction sequence of DMAD condensation and cyclization was applied using 2-amino-3-bromodibenzothiophene 5-oxide (**38n**, Scheme III, b) as the starting material, the corresponding type F tetracyclic ester **40n** resulted. Similarly, use of 2-amino-1-chlorodibenzothiophene 5-oxide (**38o**) led to the corresponding type C tetracyclic ester **41o**. In the case of **38a**, however, in which both positions 1 and 3 are open, DMAD condensation and cyclization resulted in a mixture of the two isomers **40a** and **41a**. These isomers were separable by fractional crystallization from DMF, and once again their structures were established by the ^1H NMR spectra (Table V).

The requisite starting amines were made by methods reported in the literature or as described in the Experimental Section. Several of the previously unreported

Scheme III

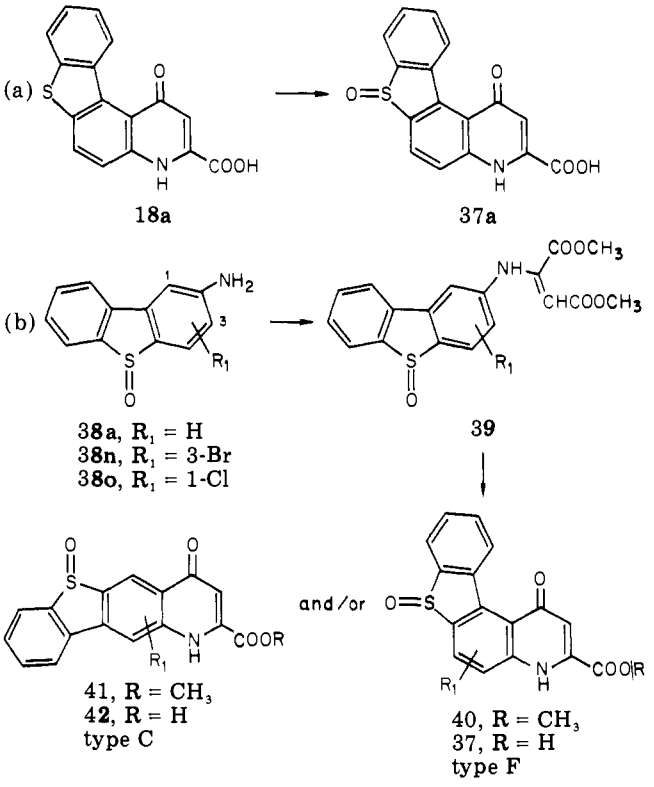
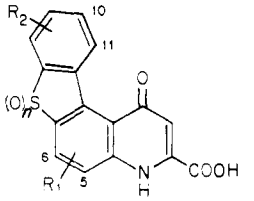


Table II. 1,4-Dihydro-1-oxo[1]benzothieno[3,2-*f*]quinoline-3-carboxylic Acid Derivatives (Type F)[a]



compd	*n*	R_1	R_2	formula[b]	mp, °C	yield,[c] %	MED,[d] mg/kg ip
18a	0	H	H	$C_{16}H_9NO_3S \cdot 0.25H_2O$	>285	27	5
18b	0	H	10-Br	$C_{16}H_8NO_3SBr \cdot 0.5H_2O$	316 dec	40	>10
18c	0	H	10-Cl	$C_{16}H_8NO_3SCl \cdot 0.25H_2O$	308-309 dec	54	>10
18d	0	H	10-CH_3	$C_{17}H_{11}NO_3S \cdot 0.25H_2O$	310-312 dec	57	>10
18e	0	H	10-OCH_3	$C_{17}H_{11}NO_4S \cdot {}^1/_3H_2O$	305-307 dec	55	>5
18g	0	H	10-F	$C_{16}H_8NO_3SF \cdot 0.25H_2O$	208 dec	73	>5
18h	0	H	8-Cl	$C_{16}H_8NO_3SCl \cdot 0.5H_2O$	350 dec	44	>10
18i	0	H	8-OCH_3	$C_{17}H_{11}NO_4S \cdot 0.75H_2O$	305-306 dec	65	>5
18j	0	H	8-CH_3	$C_{17}H_{11}NO_3S \cdot H_2O$[e]	299-300 dec	66	>5
18k	0	6-Cl	H	$C_{16}H_8NO_3SCl \cdot H_2O$	300-302 dec	60	>10
18l	0	6-OCH_3	H	$C_{17}H_{11}NO_4S \cdot {}^4/_3H_2O$	301-302 dec	25	>10
20a	2	H	H	$C_{16}H_9NO_5S \cdot 0.75H_2O$	285-290	39	1.3 (0.9-1.7)[f]
20b	2	H	10-Br	$C_{16}H_8NO_5SBr \cdot H_2O$	310-311 dec	27	>10
20c	2	H	10-Cl	$C_{16}H_8NO_5SCl \cdot 0.5H_2O$	307-308 dec	73	>10
20d	2	H	10-CH_3	$C_{17}H_{11}NO_5S \cdot 0.5H_2O$	302-304 dec	14	10
20e	2	H	10-OCH_3	$C_{17}H_{11}NO_6S \cdot H_2O$	296-298 dec	48	2.5
20f	2	H	10-(n-$C_6H_{13}O$)	$C_{22}H_{21}NO_6S \cdot H_2O$	288-289 dec	69	>10
20g	2	H	10-F	$C_{16}H_8NO_5SF \cdot H_2O$	289-291 dec	33	5
20h	2	H	8-Cl	$C_{16}H_8NO_5SCl \cdot 1.5H_2O$	293-295 dec	65	5
20i	2	H	8-OCH_3	$C_{17}H_{11}NO_6S \cdot 1.5H_2O$	285-287 dec	69	2.5
20j	2	H	8-CH_3	$C_{17}H_{11}NO_5S \cdot 1.5H_2O$	309-310 dec	67	2.5
20k	2	6-Cl	H	$C_{16}H_8NO_5SCl \cdot H_2O$	308-309 dec	57	2.5
20l	2	6-OCH_3	H	$C_{17}H_{11}NO_6S \cdot {}^1/_3H_2O$	297-298 dec	50	1.25
20m	2	5-OCH_3	H	$C_{17}H_{11}NO_6S$	293-295 dec	56	1.25
20n	2	5-Br	H	$C_{16}H_8NO_5SBr \cdot 0.75H_2O$	283-285 dec	27	2.5
37a	1	H	H	$C_{16}H_9NO_4S$	320-322 dec	91	0.3 (0.2-0.4)[g]
37n	1	5-Br	H	$C_{16}H_8NO_4SBr$	289-290 dec	67	2.5

[a] Prepared by general methods B-D or B and D. See the Experimental Section. [b] The results of C, H, and N analyses are within ±0.4% of the theoretical values except where noted. [c] See footnote *c*, Table I. [d] See footnote *d*, Table I. [e] H: calcd, 4.0; found, 3.4. [f] See footnote *f*, Table I. [g] See footnote *g*, Table I.

2-aminodibenzothiophenes were prepared by adapting the procedure of Shen et al.[7] who condensed 4-substituted thiophenols with 2-chloro-5-nitroaniline to obtain, after diazo coupling and reduction of the nitro group, 8-substituted 2-aminodibenzothiophenes (Scheme IV, a). In the present work, this method was extended to the reaction of 2-substituted thiophenols with 2-chloro-5-nitroanilines and to the reaction of 2-aminothiophenol with substituted 4-chloronitrobenzenes (Scheme IV, b). The overall yields were low for these reactions due to the poor diazo coupling reaction, but no attempts were made to optimize the reaction conditions.

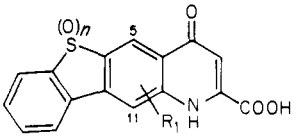
Biological Methods. The quinoline-2-carboxylic acids, as well as some of the esters, were tested for their ability to inhibit the passive cutaneous anaphylaxis (PCA) reaction in Sprague–Dawley rats by a method substantially the same as that described by others.[2,3] Thus antibody for passive sensitization was obtained from rats which were sensitized to egg albumin using *Bordetulla pertusis* as an adjuvant. The antibody was injected intradermally into the middorsal region of the rat, and sensitivity was allowed to develop for 24 h. Compounds to be tested were injected intraperitoneally 5 min before intravenous challenge by egg albumin and Evans blue dye, using six animals at each dose level. The average area of the resulting blue weals for the six animals in each experiment was compared to that resulting on nontreated animals, and the percent inhibition of the PCA reaction was calculated. Oral dosing of some of the compounds 15 min prior to antigen challenge demonstrated no activity for any of the compounds tested and is not reported here.

Compounds were initially tested by ip injection at doses of 25, 10, or 5 mg/kg. Active compounds were then tested at 10, 5, 2.5, 1.25, 0.625, 0.312, and/or 0.156 mg/kg ip, using six animals at each dose level. The lowest such dose which showed greater than 50% inhibition with a $p \leq 0.05$ (Student's t test) is recorded in the tables as the minimum effective dose (MED). For DSCG, as well as several of our benzothienoquinolinecarboxylic acids, sufficient information was obtained to determine an ID_{50} value. These values, and their 95% confidence limits, are noted in the tables. For DSCG in our assay, the ID_{50} value with its 95% confidence range is 2.6 (1.9–3.3) mg/kg ip, based on 44 experiments using six animals each.

Biological Results and Discussion. Tables I–III show the PCA results for the benzothienoquinolinecarboxylic acids which we studied. Tables VI–VIII show the microanalytical data, melting points, yields, and PCA test results for the corresponding intermediate esters. (For Tables VI–VIII, see paragraph at end of paper regarding supplementary material.) Throughout our study, the esters were observed to be relatively less active than their corresponding acids, and as our work progressed we often chose not to test some of the new esters which we prepared and characterized.

Table I shows the results obtained with the isomeric unsubstituted benzothienoquinolinecarboxylic acids. Activity at ip doses ≤5 mg/kg was observed with compounds from four of the six possible isomeric systems, type A (**9**), type C (**30a**, **42a**), type D (**36**), and type F (**18a**, **20a**, **37a**). Interestingly, these are the compounds which have the sulfone (or sulfoxide) at the 6 or 7 position of the

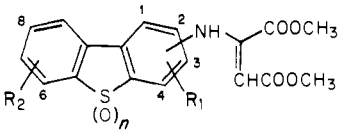
Table III. 1,4-Dihydro-4-oxo[1]benzothieno[2,3-*g*]quinoline-2-carboxylic Acid Derivatives (Type C)[a]



compd	*n*	R_1	formula[b]	mp, °C	yield, %	MED,[c] mg/kg ip
30a	2	H	$C_{16}H_9NO_5S \cdot 1.25H_2O$	>360	91	2.5
30k	2	5-Cl	$C_{16}H_8NO_5SCl$	>320	75	0.312
30o	2	11-Cl	$C_{16}H_8NO_5SCl \cdot 0.75H_2O$	>350	95	0.625[d]
30p	2	11-Br	$C_{16}H_8NO_5SBr \cdot {}^1/_3H_2O$	>350	85	1.25
30q	2	5,11-Cl_2	$C_{16}H_7NO_5SCl_2 \cdot 0.75H_2O$	268-269 dec	91	1.25
30s	2	11-CH_3	$C_{17}H_{11}NO_5S \cdot H_2O$	277-280 dec	80	1.25
32o	0	11-Cl	$C_{16}H_8NO_3SCl \cdot {}^1/_3H_2O$	>330	90	>10
32p	0	11-Br	$C_{16}H_8NO_3SBr \cdot 0.5H_2O$	275-277 dec	89	>10
32r	0	11-CH_2SCH_3	$C_{18}H_{13}NO_3S_2 \cdot 0.25H_2O$	264-268 dec	79	>5
42a	1	H	$C_{16}H_9NO_4S \cdot {}^4/_3H_2O$	255-260 dec	58	1.25
42o	1	11-Cl	$C_{16}H_8NO_4SCl \cdot {}^2/_3H_2O$	283-285	65	1.25

[a] Prepared by general methods B-D or B and D. See the Experimental Section. [b] The results of C, H, and N analyses are within ±0.4% of the theoretical values. [c] See footnote *d*, Table I. [d] Based on an average determined from four experiments (24 animals).

Table IV. Dibenzothiopheneaminobutenedioates[a]



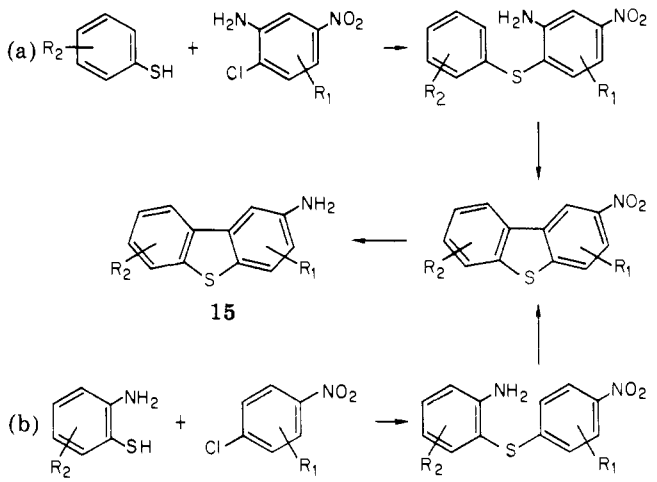
compd	*n*	position of amine subst	R_1	R_2	formula[b]	mp, °C	yield, %
5	0	1	H	H	$C_{18}H_{15}NO_4S$	115-117	89
16a	0	2	H	H	$C_{18}H_{15}NO_4S$	130-133	75
16b	0	2	H	8-Br	$C_{18}H_{14}NO_4SBr$	153-166	84
16c	0	2	H	8-Cl	$C_{18}H_{14}NO_4SCl$	158-163	65
16d	0	2	H	8-CH_3	$C_{19}H_{17}NO_4S$	136-138	78
16e	0	2	H	8-OCH_3	$C_{19}H_{17}NO_5S$	120-128	68
16g	0	2	H	8-F	$C_{18}H_{14}NO_4SF$	129-132	81
16h	0	2	H	6-Cl	$C_{18}H_{14}NO_4SCl$	103	38
16i	0	2	H	6-OCH_3	$C_{19}H_{17}NO_5S$	159-161	79
16j	0	2	H	6-CH_3	$C_{19}H_{17}NO_4S$	126-128	71
16k	0	2	4-Cl	H	$C_{18}H_{14}NO_4SCl$	158-164	78
16l	0	2	4-OCH_3	H	$C_{19}H_{17}NO_5S$	144-155	62
16m	0	2	3-OCH_3	H	$C_{19}H_{17}NO_5S$	103-105	43
16o	0	2	1-Cl	H	$C_{18}H_{14}NO_4SCl$	153-155	95
16p	0	2	1-Br	H	$C_{18}H_{14}NO_4SBr$	158-162	95
16q	0	2	1,4-Cl_2	H	$C_{18}H_{13}NO_4SCl_2$	196-197	87
16r	0	2	1-CH_2SCH_3	H	$C_{20}H_{19}NO_4S_2$	142-143	79
16s	0	2	1-CH_3	H	$C_{19}H_{17}NO_4S$	121-123	61
22	0	3	H	H	$C_{18}H_{15}NO_4S$	103-106	
28a	2	2	H	H	$C_{18}H_{15}NO_6S$	186-188	59
28k	2	2	4-Cl	H	$C_{18}H_{14}NO_6SCl$	226-227	86
34	2	3	H	H	$C_{18}H_{15}NO_6S$	198-199	66
39a	1	2	H	H	$C_{18}H_{15}NO_5S$	147-152	15
39n	1	2	3-Br	H	$C_{18}H_{14}NO_5SBr$	205-208	40
39o	1	2	1-Cl	H	$C_{18}H_{14}NO_5SCl$	185-195	39

[a] Prepared by general method A. See the Experimental Section. Compounds 11 and 16f were prepared but not characterized before subsequent reactions and, therefore, are omitted from the table. [b] The compounds were generally used in subsequent reactions without elemental analysis. Their identity was indicated by spectral data, as well as by spectral and microanalytical data of subsequent reaction products.

Table V. ^{1}H NMR Data for Isomeric Methyl Benzothienoquinolinecarboxylates

compd	structure type	solvent	^{1}H NMR data, δ (*J* values in hertz)
17a	F	Me_2SO-d_6	9.75 (m, 1), 8.40 (d, 1, *J* = 8), 8.08 (d superimposed on m, 2, *J* = 8 for the d), 7.5 (m, 2), 6.85 (s, 1), 4.02 (s, 3)
23	E	CF_3COOD	8.59 (d, 1, *J* = 8), 8.03 (d, 1, *J* = 8), 7.9-7.5 (m, 5), 4.14 (s, 3)
29a	C	CF_3COOD	9.20 (s, 1), 8.88 (s, 1), 8.4-7.9 (m, 5), 4.35 (s, 3)
35	D	CF_3COOD	9.12 (s, 1), 9.02 (s, 1), 8.3-7.9 (m, 5), 4.35 (s, 3)
40a	F	CF_3COOD	8.95 (m, 1), 8.75 (d, 1, *J* = 8), 8.55 (d, 1, *J* = 8), 8.3-7.7 (m, 4), 4.40 (s, 3)
41a	C	CF_3COOD	9.29 (s, 1), 8.77 (s, 1), 8.4-7.7 (m, 5), 4.37 (s, 3)

Scheme IV



quinoline system; when the sulfone is at the 5 position (**26**, type E) or the 8 position (**14**, type B), the acids are less potent. This contrasts with the results obtained with the open phenylsulfonylquinolinecarboxylic acids, where the 8-substituted compound is the most potent one.[5] Since this compound is still less potent than our four tetracyclic compounds, however, it is difficult to speculate on the possible reasons for differences in the order of activity.

The results obtained here are also in contrast to those observed with the indenoquinolinecarboxylic acids, where the compound with the carbonyl at the 8 position of the quinoline system (**2**) is more potent than the isomeric tetracyclic compound with the carbonyl at the 7 position.[5] Thus the shape of the tetracyclic system does not seem to be particularly important for activity in these systems. In fact, with our benzothienoquinolinecarboxylic acids, there is quite a wide range of relative molecular shapes having quite similar levels of activity.

We chose to follow up on two of these structural types by preparing and evaluating some derivatives of types F and C. Table II shows the results obtained with compounds of type F. The PCA results indicate that the oxidation state of the sulfur has an important effect on the relative potency of type F acids. Although many of the sulfide acids are inactive at the doses tested, the corresponding sulfones generally show activity at a dose range of 1.25–5 mg/kg. Only two sulfoxides were made, so no general conclusions can be drawn about the effect of that oxidation state on activity. One of the sulfoxides (**37n**) is approximately equipotent with its corresponding sulfone (**20n**), but the other sulfoxide, the unsubstituted one (**37a**), is significantly more potent than the corresponding sulfone (**20a**) and approximately eightfold more potent than DSCG.

Compounds substituted in the D ring in our study show no advantage over the parent compound and, in fact, are usually less active. For compounds made in our study, substitution in the B ring seems more effective than D-ring substitution but is still not particularly advantageous compared to the parent compound.

Table III shows the results obtained with acids of type C. Because of our experience with esters of the preceeding series, which show little or no PCA activity, the esters of type C were generally not tested, and because of the methods of synthesis, the sulfides were generally not available. In the two cases where both the sulfide and the sulfone were available (**32o** and **30o**; **32p** and **30p**), the sulfones are more potent than the corresponding sulfides, as in the series of type F. Two sulfoxides were prepared (**42a** and **42o**) and each shows little or no potency advantage over the corresponding sulfones (**30a** and **30o**). Substitution in this ring system was done in the B ring and two compounds, the 5-chloro analogue **30k** and the 11-chloro analogue **30o**, are four- to eightfold more potent than the parent compound **30a** or DSCG.

In summary, the work reported here indicates that the fusion of a dioxidized benzothieno moiety to the quinolinecarboxylic acid ring system can give tetracyclic compounds which have PCA inhibitory activity in the rat comparable to or somewhat greater than that of DSCG and that these compounds may therefore be useful in the treatment of allergic diseases. The results of our studies of other ring fusions to the quinolinecarboxylic acid system will be reported at a later date.

Experimental Section

The melting points were obtained on a Mel-Temp block or a Uni-melt apparatus and are uncorrected. The IR spectra were obtained, using Nujol mulls, on a Perkin-Elmer Infracord spectrophotometer; ^{1}H NMR spectra were measured on a Varian T-60, a Varian A-60, or a Varian XL-100 spectrometer. The spectral data are consistent with the assigned structures in all cases. The results of elemental analyses, except where noted, are within ±0.4% of the theoretical values.

The following aminodibenzothiophenes were prepared according to the cited references: 1-aminodibenzothiophene (**4**),[8] 4-aminodibenzothiophene (**10**),[9] 2-aminodibenzothiophene (**15a**),[10] 2-amino-8-bromodibenzothiophene (**15b**),[11] 2-amino-8-chlorodibenzothiophene (**15c**),[7] 2-amino-8-methyldibenzothiophene (**15d**),[7] 2-amino-8-methoxydibenzothiophene (**15e**),[7] 2-amino-8-fluorodibenzothiophene (**15g**),[7] 3-aminodibenzothiophene (**21**),[12] 2-aminodibenzothiophene 5,5-dioxide (**27a**),[13] and 3-aminodibenzothiophene 5,5-dioxide (**33**).[14] Other aminodibenzothiophene starting materials were prepared as described below without attempting to maximize the yields and were generally used without extensive purification.

2-Amino-8-(*n*-hexyloxy)dibenzothiophene (15f). Demethylation of 8-methoxy-2-nitrodibenzothiophene[7] was accomplished by reaction with pyridine hydrochloride at 220 °C for 20 min. The reaction mixture was poured carefully onto ice; the solid was collected by filtration and recrystallized (EtOH) to give a yellow solid, mp 200–203 °C. This material was alkylated in glyme by treatment first with NaH and then with *n*-hexyl bromide, refluxing 4 h. The cooled reaction mixture was filtered; the filtrate was diluted with H_2O to precipitate a solid which was collected by filtration and recrystallized (charcoaled) from benzene to give a yellow solid, mp 99–100 °C. Catalytic reduction of this nitro compound with Raney nickel in EtOH yielded the product, in 25% overall yield, as an oil which was used without purification in subsequent reactions.

2-Amino-6-chlorodibenzothiophene (15h). A mixture of 4-acetamidothiophenol (25.0 g, 0.150 mol), 2,3-dichloronitrobenzene (28.6 g, 0.150 mol), and KOH (8.60 g, 0.16 mol) in 250 mL of 95% EtOH was refluxed 2 h and then cooled, and the solid was filtered to give 48.5 g (93%) of a yellow solid, mp 150–151 °C. This material was reduced catalytically with Raney nickel in EtOH to give 37.0 g (91%) of white solid: mp 149–152 °C; HCl salt, mp 225–230 °C. This HCl salt (33.0 g, 0.100 mol) was suspended in 250 mL of glacial HOAc at ~18 °C while butyl nitrite (13.4 g, 0.130 mol) was added dropwise over 20 min. The mixture was stirred at 20 °C until solution was complete (30 min), and then 100 g of ice was added, followed by 20 g of Cu powder in small portions over 15 min while cooling in an ice bath. The mixture was gradually warmed to 40 °C, where gas evolution became vigorous, and then ceased after about 30 min. The mixture was allowed to stand at room temperature overnight. The brown solid was filtered, washed (H_2O), taken up in hot DMF–H_2O, and filtered while hot through supercel to eliminate the Cu powder. Water was added to the cooled filtrate to precipitate a product which was filtered, washed (H_2O), and suspended in 300 mL of 95% EtOH. NaOH pellets (4.0 g, 0.10 mol) were added and the mixture was refluxed for 18 h, then concentrated in vacuo to 150 mL, cooled, and diluted with H_2O to precipitate a light brown

solid which was filtered and washed (H_2O) to give 13.0 g (56%, 37% overall) of solid, mp 140–150 °C.

The following compounds were prepared from the appropriate starting materials using the method of Shen et al.[7]

2-Amino-6-methoxydibenzothiophene (15i): yield 3%; mp 180–185 °C.

2-Amino-6-methyldibenzothiophene (15j): yield 9%; mp 120–123 °C.

2-Amino-4-chlorodibenzothiophene (15k): yield 8%; mp 86–90 °C.

2-Amino-4-methoxydibenzothiophene (15l): yield 3%; mp 138–143 °C.

2-Amino-3-methoxydibenzothiophene (15m): yield 4%.

2-Amino-1-chlorodibenzothiophene (15o) and 2-Amino-1-bromodibenzothiophene (15p). These amines were the actual products obtained by chlorination or bromination of 2-acetamidodibenzothiophene and subsequent hydrolysis according to the procedure of Gilman and Avakian,[9] who misassigned their structures as 2-amino-3-chlorodibenzothiophene and 2-amino-3-bromodibenzothiophene, respectively. Gilman later corrected their assignment for the case of the chloro compound[15] but not for the bromo compound. The correct structures can be assigned on the basis of the ^1H NMR spectra. Thus ^1H NMR (**15o**) in $CDCl_3 + D_2O$: δ 8.62 (m, 1, H-9), 7.7–6.9 (m, 4), 6.64 (d, 1, J = 9 Hz, H-3). ^1H NMR (**15p**) in $CDCl_3 + D_2O$: δ 8.75 (m, 1, H-9), 7.7–6.9 (m, 4), 6.64 (d, 1, J = 9 Hz, H-3).

2-Acetamido-4-chlorodibenzothiophene. Acetic anhydride (19 mL) was added to a solution of **15k** (7.10 g, 30.4 mmol) in 200 mL of benzene. After stirring overnight at room temperature, the solid was collected by filtration and washed with benzene to give 7.50 g (90%) of white solid, mp 241–243 °C.

2-Amino-1,4-dichlorodibenzothiophene (15q). A solution of 2-acetamido-4-chlorodibenzothiophene (7.50 g, 27.2 mmol) in 200 mL of $CHCl_3$ was treated slowly over 2 h with SO_2Cl_2 (6.3 g, 47 mmol). After stirring for 5 h at room temperature the solid was collected by filtration and suspended in a mixture of 150 mL of EtOH and 150 mL of concentrated HCl, and the reaction mixture was refluxed 17 h. The cooled mixture was poured into 500 mL of cold 30% NaOH; the solid was collected by filtration and recrystallized (EtOH–H_2O) to give 4.78 g (65%) of a white solid, mp 153–155 °C.

2-Amino-1-methylthiomethyldibenzothiophene (15r). This compound was prepared by the general procedure of Gassman,[16] from 2-aminodibenzothiophene, in 52% yield after chromatography on Florisil: mp (EtOH–H_2O) 109–111 °C.

2-Amino-1-methyldibenzothiophene (15s). Raney nickel (2 g) was added to a solution of **15r** (0.75 g, 2.90 mmol) in 200 mL of EtOH, the mixture was shaken for 5 min, the Raney nickel was removed by filtration, and the filtrate was concentrated in vacuo to a colorless oil which was used without further purification or characterization in subsequent reactions.

2-Amino-4-chlorodibenzothiophene 5,5-Dioxide (27k). To a solution of 2-acetamido-4-chlorodibenzothiophene (2.87 g, 10.4 mmol) in 50 mL of glacial HOAc was added 9 mL of 30% H_2O_2. The mixture was refluxed for 3 h, poured into 400 mL of ice–water, and filtered to give 2.80 g of tan solid, mp >280 °C. This material was added to a solution of 100 mL of concentrated HCl in 100 mL of EtOH, refluxed for 2 h, then cooled, and poured into 500 mL of 10% NaOH. The resulting solid was filtered, washed (H_2O), and dried to give 2.27 g (82%) of the amine, mp (methoxyethanol–water) >280 °C.

2-Bromodibenzothiophene 5-Oxide. Chlorine gas (15 g, 0.21 mol) was bubbled slowly over 30 min into a cold (0–5 °C) mixture of 2-bromodibenzothiophene[10] (50.0 g, 0.190 mol) in 500 mL of CCl_4. The mixture was stirred 15 min at 0 °C, then poured onto 750 mL of ice–water, and stirred vigorously to give a heavy white precipitate which was collected by filtration, washed (H_2O), dried, and recrystallized (benzene) to give 29.1 g (55%) of white solid, mp 170–172 °C (lit.[17] mp 171–172 °C).

2-Aminodibenzothiophene 5-Oxide (38a). A mixture of 2-bromodibenzothiophene 5-oxide (150 g, 0.538 mol), 160 g of CuBr, and 1700 mL of concentrated NH_4OH was heated at 200 °C in a pressure reactor for 12 h. The mixture was cooled, and the solid was collected by filtration and washed with H_2O, then benzene, and then warm $CHCl_3$ to give 83 g of brown solid. This solid was taken up in hot MeOH and filtered to remove 35 g of brown solid. The filtrate was concentrated in vacuo and the residue recrystallized (EtOH) to give 33.4 g (28%) of the desired compound, mp 196–199 °C.

2-Amino-1-chlorodibenzothiophene 5-Oxide (38o). A mixture of 2-acetamido-1-chlorodibenzothiophene[9] (10.0 g, 36.3 mmol) and *m*-chloroperbenzoic acid (80.0 g, 46.5 mmol) in 200 mL of $CHCl_3$ was stirred 18 h. The precipitate was filtered and washed ($CHCl_3$) to give 3.5 g (33%) of white solid, mp 240–242 °C dec. This material was added to a solution of 50 mL of 10% NaOH in 50 mL of EtOH, and the mixture was refluxed 30 min. The EtOH was removed in vacuo and the resulting solid was filtered and washed (H_2O, then EtOH, and then CH_2Cl_2) to give 1.7 g (58%) of yellow solid, mp 194–198 °C.

2-Amino-3-bromodibenzothiophene 5-Oxide (39n). Bromine (17 g, 95 mmol) was added dropwise at room temperature to a mixture of **38a** in 75 mL of glacial HOAc. The mixture was stirred overnight, then diluted with H_2O to precipitate a solid which was filtered, and washed (H_2O) to give 11.0 g (40%) of tan solid, mp 195–205 °C.

1,4-Dihydro-1,7-dioxo[1]benzothieno[3,2-*f*]quinoline-3-carboxylic Acid (37a). To a solution of 100 mL of H_2O and 2 mL of 10% NaOH was added **18a** (0.50 g, 1.7 mmol). To this mixture was added 50 mL of DMF and 2.0 g (6.2 mmol) of sodium metaperiodate. The mixture was stirred 72 h and then filtered. The filtrate was diluted with H_2O and acidified with concentrated HCl. The resulting solid was collected by filtration, washed (H_2O), and dried to give **37a**: 0.50 g (92%); mp 299–301 °C. Anal. ($C_{16}H_9NO_4S \cdot {}^2/_3H_2O$) C, H, N. Karl Fischer H_2O analysis: calcd, 3.85; found, 3.7.

In our hands this procedure was difficult to reproduce.

Dimethyl Dibenzothiopheneaminobutenedioates. General Method A. Table V. A mixture of the appropriate amine (10 mmol) in 50 mL of MeOH was treated dropwise over 10 min with 1.8 g (12.7 mmol) of DMAD. The reaction mixture was stirred overnight at room temperature and the desired product was collected by filtration or by removal of the solvent in vacuo. The diesters were generally used in subsequent reactions without further purification.

Methyl Benzothienoquinolinecarboxylates. General Method B. Tables VI–VIII. A mixture of the butenedioate (10 mmol) in 50 mL of diphenyl ether or 50 mL of Dowtherm A was heated at boiling in an open flask for 10–20 min, then allowed to cool, and diluted with hexanes, if necessary, to precipitate a solid which was collected by filtration and recrystallized to give the desired tetracyclic ester.

Methyl Benzothienoquinolinecarboxylate Dioxides. General Method C. Tables VI–VIII. A mixture of the tetracyclic ester (10 mmol) and 20 mL of 30% H_2O_2 in 80 mL of glacial acetic acid was refluxed for 3 h. The reaction mixture was poured into ice–water and the solid product collected by filtration and recrystallized, if necessary, to give the pure ester.

Benzothienoquinolinecarboxylic Acids. General Method D. Tables I–III. A mixture of the ester (5.0 mmol) in 100 mL of H_2O was treated with 10 mL of 10% NaOH and refluxed until solution was complete. The reaction mixture was filtered, cooled, and acidified with concentrated HCl to precipitate a solid which was collected by filtration. The filtration was often made easier by heating the acidic mixture at boiling for a few minutes and then filtering while hot. If necessary the solid was recrystallized, but often it was obtained in analytically pure form without further purification.

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Supplementary Material Available: Tables VI–VIII which contain microanalytical data, melting points, yields, and PCA test results for methyl benzothienoquinolinecarboxylates (6 pages). Ordering information is given on any current masthead page.

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Reactions of Chlorpromazine Cation Radical with Physiologically Occurring Nucleophiles

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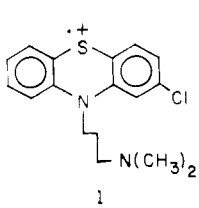
The reactions between chlorpromazine cation radical and a variety of physiologically occurring nucleophiles, which involve formation of a covalent, yet reversible bond, have been examined. As reported earlier, this reaction does not involve disproportionation of the radical but, rather, direct reaction between radical and nucleophile. The resulting adduct further reacts to form chlorpromazine sulfoxide or hydroxylated derivatives, and the original nucleophile is regenerated. The products and kinetics of the reaction depend strongly on the identity of the nucleophile, with the sulfhydryl group being the fastest and water being the slowest of the nucleophiles studied. The likely involvement of these reactions in the metabolism of chlorpromazine is discussed. In addition, it is proposed that the radical/nucleophile interaction is a reasonable model reaction for the effects of chlorpromazine radical on neuronal enzymes and receptor sites.

Since the emergence of the phenothiazine major tranquilizers such as chlorpromazine as tools of major importance in the treatment of schizophrenia, there has been substantial interest in the involvement of their cation radicals in their activity. While the importance of the radical to the antipsychotic effects of chlorpromazine remains a point of controversy, several arguments exist for radical involvement in the metabolism and activity of the drug. First, chlorpromazine cation radical (1) is easily



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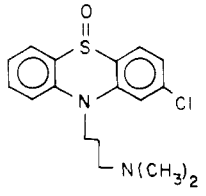

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formed in aqueous solutions by chemical,[1,2] electrochemical,[3,4] enzymatic,[5] and photochemical[6] oxidations and has been reported to have a half-life of a few seconds at physiological pH.[5] Thus the radical would be expected to be present in vivo and would be more reactive than its reduced precursor. Second, the radical is a likely intermediate in the metabolism of chlorpromazine to its sulfoxide (2) and hydroxylated metabolites, although the latter reaction has not been demonstrated in vitro.[5,7,8] Third, 1 affects the functions of several neuronal enzymes much more strongly than chlorpromazine itself, and an interaction between the radical and protein sulfhydryl groups was suggested as the source of the effects.[6,9,10] Fourth, 1 is known to bind strongly to macromolecules, particularly DNA, although the nature of the binding is not understood.[11] Finally, several other effects of the radical on biological membranes have been examined.[13] These observations have prompted several workers[5,7,11,12] to suggest that the radical is the active form of chlorpromazine in vivo, a hypothesis which has been neither proven nor refuted.

Because of its potential importance to drug activity, the chemistry of the radical of chlorpromazine has been studied extensively in vitro. The stability of 1 has been compared to other phenothiazine radicals in strong acid, and no correlation between radical lifetime and therapeutic potency was found.[1] The decay of radical was second order, and the authors proposed a disproportionation of radical to starting material and sulfoxide to explain the kinetics. Other workers have also proposed a disproportionation route and pointed out that the decay was very pH dependent, being faster in less acidic media.[5,14] In a study carried out in a near-neutral pH range, it was reported[5] that CPZ^+ interacted directly with the enzyme peroxidase. Unfortunately, the large majority of reports on radical chemistry has been based on experiments in strong acid, where the radical is more stable, but the results are of unknown physiological importance.

Our laboratory has carried out a detailed examination of the chemistry of chlorpromazine cation radical in aqueous solutions in the pH region from 2 to 7.[8] In an initial report, it was demonstrated that the radical did not disproportionate but rather reacted directly with solution nucleophiles, the buffer in our case. The radical/buffer

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