Synthesis of Isomeric Thienobenzothiazines and Their Effect on Dopamine Metabolism in Rat Brain

Cor J. Grol,* Hans Rollema, Durk Dijkstra, and Ben H. C. Westerink

Department of Medicinal Chemistry, State University, Groningen, The Netherlands. *Received July 30, 1979*

The synthesis of the thieno[2,3-*b*]-, thieno[3,4-*b*]-, and thieno[3,2-*b*]benzothiazines with a hydroxyethylpiperazinylpropyl side chain and various 2 substituents is described. The influence of these neuroleptic compounds on dopamine metabolism in vivo is quantitated by determining the rise in homovanillic acid concentrations in rat corpus striatum. Notable differences in activity were found among the isomers, which were useful for structure-activity correlations in the phenothiazine neuroleptics.

Since the emergence of the phenothiazines in the treatment of schizophrenia, there has been substantial interest in the unique physicochemical properties of these neuroleptics. Due to the electron-donating ability of the phenothiazine ring system, radical cations are easily formed in aqueous solution by chemical,[1] electrochemical,[2] enzymatic,[3] and photochemical oxidation.[4] Observations that these radical cations are intermediates in various reactions with physiologically occurring species like protein,[5] DNA,[6] and biological membranes[7] have prompted speculation concerning their possible participation in the biological effects of the phenothiazines. Because of this potential importance, the properties of the phenothiazine radical cations have been studied extensively.[8] Attempts to correlate properties such as radical lifetime[9] with clinical potency, however, are hampered by the fact that the compounds studied usually had different side chains and substituents which might result in a different pharmacokinetic behavior, as well as in differences in interactions with receptors. In our investigations, we intend to study only variations in the electronic structure of the tricyclic moiety while other properties are kept constant. We planned to do this by synthesizing compounds with isosteric variations in the aromatic structure but with the same side chain and 2 substituent. In our previous publication,[10] we described the synthesis of the thienobenzothiazines with a dimethylaminopropyl side chain and the preliminary results of their neuroleptic evaluation.

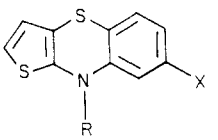
In this paper, the synthesis and neuroleptic activity of the more potent hydroxyethylpiperazinylpropyl derivatives are described. As a measure of neuroleptic activity, the potency to raise HVA levels in the striatum of rat brain was determined.

Chemistry. Starting with the appropriate thienobenzothiazines, the substitution with a hydroxyethylpiperazinylpropyl side chain was accomplished as outlined in Scheme I.

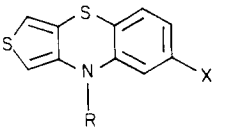
Alkylation of the thiazines with 1-bromo-3-chloropropane and sodium amide gave good yields of the *N*-(chloropropyl) derivatives. Some of these compounds were difficult to purify due to their sensitivity to heat and light. Iodide-catalyzed nucleophilic substitution of the alkyl chlorides with 1-(2-hydroxyethyl)piperazine by refluxing with K_2CO_3 in methyl ethyl ketone afforded the bases, which were converted to their hydrochloric acid salts (Table I).

Pharmacology. A great variety of investigations strongly indicate that the therapeutic action of phenothiazine drugs involves blockade of central dopamine receptor sites.[11] In a previous publication,[12] we have shown a correlation between the increase in dopamine turnover in rat striatum induced by neuroleptics and their clinical efficacy. This effect on dopamine metabolism was therefore used to obtain quantitative data on the neuroleptic activities for a good comparison of the potencies of the various thienobenzothiazines. Enhanced dopamine turnover was detected by measuring the dose-dependent increases in homovanillic acid (HVA) levels in rat corpus striatum, nucleus accumbens, and tuberculum olfactorium.[13]

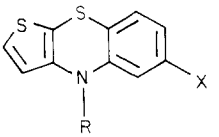
For one set of thienobenzothiazine isomers, the trifluoromethylpromazine analogues **2**, **5**, and **8**, the increase



1, X = Cl
2, X = CF_3



3, X = H
4, X = Cl
5, X = CF_3



6, X = H
7, X = Cl
8, X = CF_3

R = $-CH_2CH_2CH_2N(CH_3)_2$

* Address correspondence to the Laboratorium voor Farmaceutische en Analytische Chemie, Ant. Deusinglaan 2, Groningen, The Netherlands.

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Scheme I

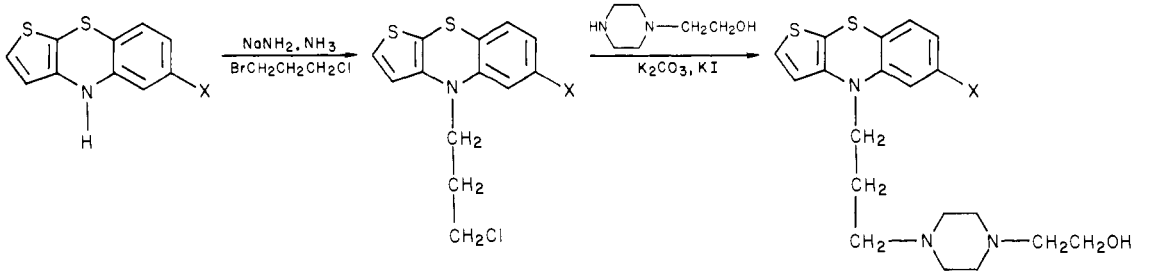
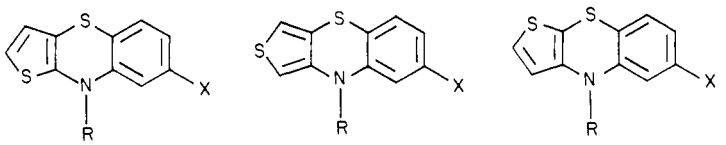


Table I. *N*-(3-Chloropropyl)thieno[1,4]benzothiazines and *N*-[3-[4-(2-Hydroxyethyl)-1-piperazinyl]propyl]thieno[1,4]benzothiazines



9-10, 17-18 11-13, 19-21 14-16, 22-24

no.[d]	X	mp or bp (mm), °C	yield, %	anal.	no.[e]	X	mp, °C (HCl salts)	yield, %	anal.
9	Cl	46-47	73	C, H, N, Cl	17	Cl	229.5-231 (2HCl)	78	C, H, N, S, Cl
10	CF_3	liq	72		18	CF_3	188-190 (1HCl)	41	C, H, N, S, Cl, F[c]
11	H	88.5-90	79	C, H, N, S, Cl	19	H	207-209[d] (2HCl)	55	C, H, N, S, Cl
12	Cl	92.7-93.6	55	C, H, N, S, Cl[a]	20	Cl	142-145 (1HCl)	43	C, H, N, S, Cl
13	CF_3	105-106	71	C, H, N, S, Cl	21	CF_3	230-233 (2HCl·0.5H_2O)	48	C, H, N, S, Cl, F
14	H	58.5-59.5	79	C, H, N, S, Cl	22	H	197-199 (1HCl)	40	C, H, N, S, Cl
15	Cl	54.5-55.5	45	C, H, N, S, Cl	23	Cl	220 dec (2HCl)	62	C, H, N, S, Cl
16	CF_3	133-136 (0.8)	44	C, H, N, S, Cl[b]	24	CF_3	178-181 (2HCl)	52	C, H, N, S, Cl

[a] Cl: calcd, 22.4; found, 21.8. [b] C: calcd, 48.1; found, 48.9. Cl: calcd, 10.1; found, 9.0. S: calcd, 18.3; found, 17.8. [c] Cl: calcd, 7.4; found, 8.1. [d] R = $CH_2CH_2CH_2Cl$. [e] R = $-CH_2CH_2CH_2$-c-$N(CH_2CH_2)_2N$-CH_2CH_2OH.

Table II. Thienobenzothiazine-Induced Increase of HVA Levels in Corpus Striatum and Limbic Areas of the Rat

no.	dose, μmol/kg	% HVA of control levels: striatum	nucleus accumbens	tuberculum olfactorium
8	10	320	250	180
	20	410	350	240
	30	430	360	280
	50	510	510	300
5	5	140	125	100
	10	310	185	160
	15	340	260	210
	25	445	390	270
2	15	130	100	100
	50	310	180	150
	75	440	375	200

in HVA concentrations in the three mentioned brain areas were measured. Table II indicates that these compounds show the same pattern of HVA response as the phenothiazine neuroleptics.

Time–response curves were studied with another set of isomers (**18**, **21**, and **24**) and the parent phenothiazine fluphenazine by measurement of HVA concentrations in corpus striatum. From the results (Figure 1) it appears that the thienobenzothiazines resemble the phenothiazines in the time course of action on dopamine metabolites too and that observed differences in the potencies of the isomers are not due to pharmacokinetic differences.

Finally, doses of all the thienobenzothiazines causing striatal HVA concentrations three times the control value were obtained from log dose–response curves. The results are summarized in Table III.

From the obtained data we can draw the conclusion that the thienobenzothiazines differ quantitatively, but not qualitatively, from the phenothiazines in their effect on dopamine metabolism. Although they are somewhat less active, the thienobenzothiazines fulfill our preset requirement of obtaining isosteric congeners of the phenothiazine neuroleptics useful for structure–activity correlations and which can be utilized in radical-cation studies.

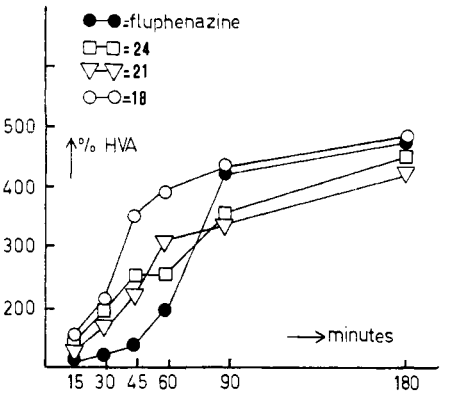


Figure 1. Time–response curves of 18, 21, 24, and the parent phenothiazine fluphenazine by measurement of HVA concentrations in corpus striatum.

Experimental Section

Melting points were taken on a Tottoli apparatus and are uncorrected. Analytical results for the elements indicated were within ±0.4% of the theoretical value unless stated otherwise. NMR spectra were obtained with a Perkin-Elmer Model R-24 spectrometer using Me_4Si as internal standard. The IR spectra were obtained on a Beckman IR-33 spectrophotometer.

***N*-(Chloropropyl)thieno[1,4]benzothiazines 9–16.** The thieno[1,4]benzothiazine (0.01 mol) was stirred during 0.5 h with 0.6 g of powdered $NaNH_2$ in liquid ammonia. 1-Bromo-3-chloropropane (0.01 mol) was added and the stirring continued. When the reaction was completed (TLC), the ammonia was removed by addition of ether and evaporation. The ether layer was

Table III. Potencies of Thienobenzothiazines to Increase HVA Levels in Rat Striatum

no.	ED_{300}[a]	95% CL
6	480	362-636
3	460	347-610
7	36.0	28.2-46.0
4	19.2	14.5-25.4
1	350	264-464
8	6.1	4.8-7.8
5	9.2	7.4-11.4
2	30.1	24.6-36.7
22	18.9	14.8-24.1
19	13.7	10.7-17.4
23	3.27	2.5-4.1
20	1.5	1.2-1.9
17	20.5	16.1-26.2
24	0.75	0.59-0.96
21	0.54	0.42-0.69
18	4.0	3.2-5.2

[a] Dose (μmol/kg) causing a HVA concentration threefold the control level.

washed with H_2O and dried over Na_2SO_4, and the ether was evaporated. The resulting oils were purified by column chromatography (10 g of Al_2O_3), vacuum distillation, or recrystallization from ethanol.

N-[3-[4-(2-Hydroxyethyl)-1-piperazinyl]propyl]thieno[1,4]benzothiazines 17-24. N-(3-Chloropropyl)thieno[1,4]-benzothiazine (1.5 mmol) was dissolved in 15 mL of methyl ethyl ketone, and 130 mg of K_2CO_3, 65 mg of KI, and 1.5 mmol of 1-(2-hydroxyethyl)piperazine were added. The mixture was refluxed until a substantial amount of product was formed (TLC), which took sometimes 2 days. Even then unreacted starting material was still present. After water was added to the mixture, the bases were extracted with ether. Washing, drying over Na_2SO_4, and evaporation of the ether yielded yellow-brown sluggish oils, which were distilled in vacuo and converted into their HCl salts by the dropwise addition of a solution of HCl in ether. The precipitates were recrystallized from ethanol/ether.

Pharmacology. Materials and Methods. The compounds were dissolved in 0.9% NaCl immediately before use. The solutions were protected from light, and a small amount of ascorbic acid was added to prevent oxidation of the phenothiazines. Male albino Wistar rats, weighing 180–250 g (TNO, Zeist, The Netherlands), were injected intraperitoneally with 0.3–0.8 mL of the drug solution under investigation. Controls received an injection with saline–ascorbic acid solution. The rats were decapitated 2 h after the injection (or after various time intervals when time–effect curves were studied), and the corpus striatum was dissected.[14] HVA was assayed by a semiautomatic fluorometric method.[15] For each compound, four to six dose levels were studied and three to six rats were used for each dose. HVA in left and right striata was measured separately, as a control for the analytical procedure, and levels given are the mean of the bilateral parts. Doses, causing a threefold increase in HVA concentrations, were calculated from log dose-response curves.

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Synthesis and Anticancer Activity of Nitrosourea Derivatives of Phensuximide

A. Michael Crider,* Thomas M. Kolczynski, and Kathleen M. Yates

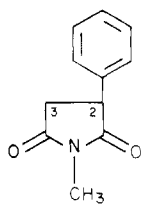
College of Pharmacy, University of Toledo, Toledo, Ohio 43606. *Received October 11, 1979*

Nitrosourea derivatives **9-13** which utilize phensuximide (1) as the carrier were synthesized as potential central nervous system antitumor agents. The N-(2-chloroethyl)-N-nitrosourea **13** was active in the mouse ependymoblastoma brain-tumor system, as well as the intraperitoneal L1210 leukemia system.

The difficulty in the treatment of primary tumors of the central nervous system (CNS) and solid tumors metastasizing to the CNS from a variety of other primary sites, such as the breast and lungs, has been discussed.[1-4] However, some degree of success has been achieved with the development of new antitumor drugs with CNS activity. Several nitrosourea derivatives have been reported to exhibit activity against murine leukemia L1210 implanted intracerebrally.[5-7]

Driscoll and co-workers[2] have proposed that in the search for new antitumor drugs having CNS activity emphasis should be placed on structural types which penetrate the blood–brain barrier in significant concentrations and have antitumor activity. These workers have described the use of phenothiazines[3,4] and hydantoins[2] as carriers which penetrate the CNS to attach alkylating nitrogen mustard groups.

Our approach to the design of nitrosoureas capable of exhibiting CNS antitumor activity involved the use of phensuximide (1) as the carrier. The N-(2-chloroethyl)-



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N-nitrosoureas have shown superior antitumor activity to other alkylnitrosoureas.[8] It was anticipated that the succinimide carrier group would penetrate the CNS. Therefore, less reactive alkylnitrosoureas could possibly

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