Synthesis of 3-Substituted Thieno[2,3-*d*]pyrimidin-4(3*H*)-one-2-mercaptoacetic Acids and Their Ethyl Esters for Pharmacological Screening

M. B. DEVANI *x, C. J. SHISHOO *, U. S. PATHAK *, S. H. PARIKH ‡, G. F. SHAH ‡, and A. C. PADHYA ‡

Abstract □ 3-Substituted thieno[2,3-*d*]pyrimidin-4(3*H*)-one-2-mercaptoacetic acids and their ethyl esters were synthesized from 2-mercaptothieno[2,3-*d*]pyrimidin-4(3*H*)-ones, which were obtained by cyclization of thienylthioureas in acidic medium. Analgesic, anti-inflammatory, and anticonvulsant activities were found in some of these compounds. Significant antimicrobial activity was exhibited by thienylthioureas.

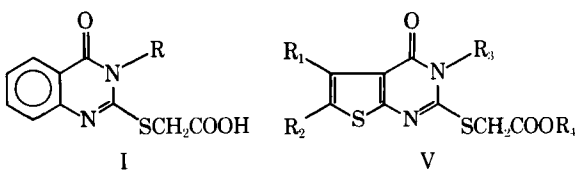
Keyphrases □ Thienopyrimidinonemercaptoacetic acids and ethyl esters—synthesized, screened for analgesic, anti-inflammatory, anticonvulsant, and antimicrobial activity □ Structure–activity relationships—thienopyrimidinonemercaptoacetic acids and ethyl esters, screened for analgesic, anti-inflammatory, anticonvulsant, and antimicrobial activity □ Analgesic agents, potential—thienopyrimidinonemercaptoacetic acids and ethyl esters screened □ Anti-inflammatory agents, potential—thienopyrimidinonemercaptoacetic acids and ethyl esters screened □ Anticonvulsant agents, potential—thienopyrimidinonemercaptoacetic acids and ethyl esters screened □ Antimicrobial agents, potential—thienopyrimidinonemercaptoacetic acids and ethyl esters, screened

Pharmacological activities of 4-quinazolones have been extensively reviewed (1). The isosteric properties of benzene and thiophene are of interest to medicinal chemists. The concept of bioisosterism has been fully exploited and justified in the synthesis of thiophene analogs of established drug molecules (2, 3). Recently, 4-quinazolon-2-mercaptoacetic acids (I) were reported to possess significant anticonvulsant activity (4). Hypocholesterolemic, sedative, and antitussive properties were found in 2-mercapto-3,4-dihydrothieno[2,3-*d*]pyrimidin-4-one (5). Therefore, it was of interest to prepare the analog (V) of I for pharmacological screening.

DISCUSSION

Chemistry—3-Substituted thieno[2,3-*d*]pyrimidin-4(3*H*)-one-2-mercaptoacetic acid derivatives were synthesized starting with 2-amino-3-carbethoxythiophenes (II) (Scheme I). Compound II was readily available through the reaction of Gewald *et al.* (6). On reacting II with an alkyl or aryl isothiocyanate, a thiourea analog (III) was obtained. Compound III cyclized smoothly in ethanol saturated with dry hydrochloric acid to form 2-mercapto-3-substituted thieno[2,3-*d*]pyrimidin-4(3*H*)-one (IV). Refluxing IV with chloroacetic acid or its ethyl ester in the presence of potassium hydroxide yielded the corresponding 2-mercaptoacetic acid derivative (V).

When allylthioureas (XII and XXIII, Table I) were refluxed in ethanol saturated with dry hydrochloric acid, VI was obtained instead of the expected product (IV) (Scheme II).



I V

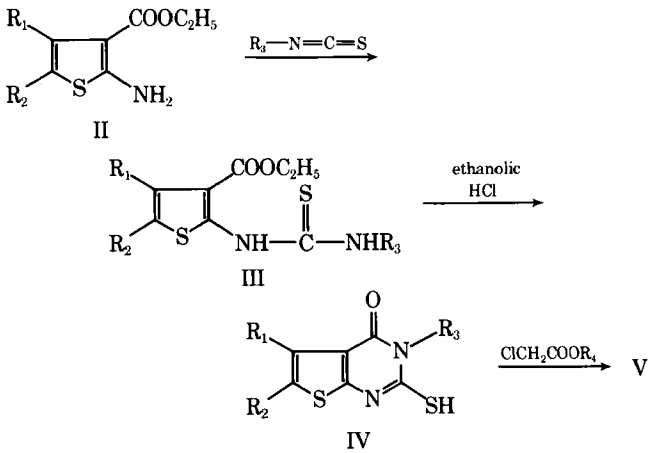
Biological Activity—*Antimicrobial Study*—Thienylthioureas (Table I) were tested for antibacterial and antifungal activities. None of the compounds, at a concentration of 50 μg/ml, showed any appreciable inhibition of the growth of *Escherichia coli* (B-3-4), *Shigella shigae* (NCTC 8217), *Salmonella typhi* H901 (NCTC 3111), *Proteus ox* 19 (B-26-3), *Pseudomonas aeruginosa* (B-27-2), *Mycobacterium tuberculosis* (H37 Rv), *Candida albicans*[1], *Phialophora jeanselmei*[1], *Nocardia asteroides*[1], and *Aspergillus fumigatus*[1]. Compound IX was the most effective antibacterial agent against the Gram-positive organisms (Table II). In addition to antibacterial properties, VII, XII, XX, and XXVIII showed antifungal activity.

In general, thioureas having the terminal nitrogen substituted by a single short chain alkyl (C_1–C_4) or aralkyl group showed maximum activity. Replacement of a terminal alkyl group by an aryl group resulted in the loss of activity. Similar structure–activity relationships were observed with phenyl analogs (7).

Pharmacological Screening—Compounds VII, XIII, XVIII, XIX, XXVII, XXIX, XXXI, XXXIV, XXXVI, XXXVIII–LVII, and LIX were subjected to preliminary pharmacological screening for analgesic, anticonvulsant, and anti-inflammatory activities (Table III). Only two compounds (XLIX and LIV) showed protection (37.5%) against electric shock. None of the compounds had any appreciable protection against pentylenetetrazol-induced convulsions. Both *N*-benzylthiourea (XXIX) and its corresponding thienopyrimidine (XLIV) exhibited significant analgesic and anti-inflammatory activities. Maximum anti-inflammatory activity was found with thiazolothienopyrimidine (LIX).

EXPERIMENTAL

Synthesis—Crystallization solvents, yields, melting points, and analyses of the compounds synthesized are reported in Tables I, IV, and V. Melting points are uncorrected.



II III IV

Scheme I

[1] All India Institute of Medical Sciences Culture Collection.

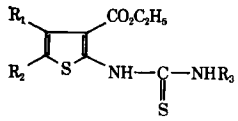
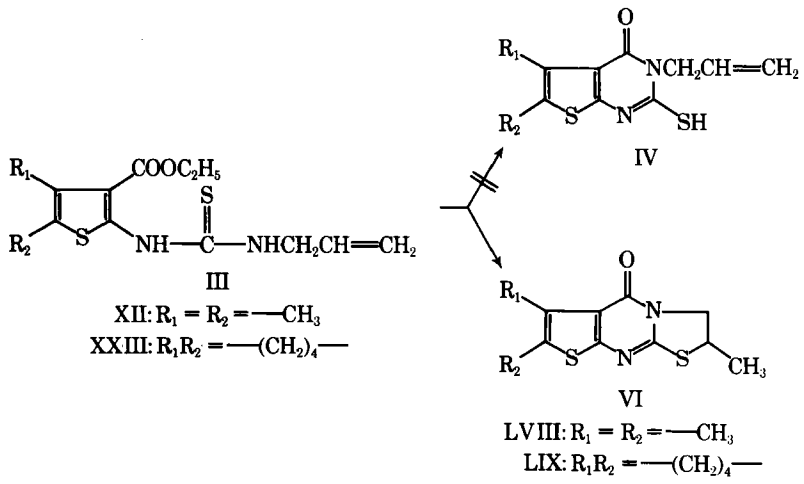


Table I—Physical Properties of Thienylthioureas

Compound	R_1	R_2	R_3	Melting Point	Yield, %	Crystallization Solvent	Molecular Formula	Analysis, % Calc.	Found
VII	CH_3	CH_3	CH_3	182–184°	80	Ethanol–chloroform	$C_{11}H_{16}N_2O_2S_2$	C 48.53	48.90
								H 5.88	6.10
VIII	CH_3	CH_3	C_2H_5	164–166°	82	Ethanol–chloroform	$C_{12}H_{18}N_2O_2S_2$	C 53.35	53.51
								H 6.29	6.12
IX	CH_3	CH_3	n-C_4H_9	128–130°	67	Ethanol	$C_{14}H_{22}N_2O_2S_2$	C 53.50	53.20
								H 7.00	6.90
X	CH_3	CH_3	n-C_6H_{13}	92–94°	78	Ethanol	$C_{16}H_{26}N_2O_2S_2$	C 56.14	56.00
								H 7.60	7.41
XI	CH_3	CH_3	cyclo-C_6H_{11}	179–181°	75	Ethanol	$C_{16}H_{24}N_2O_2S_2$	C 56.47	56.35
								H 7.06	7.27
XII	CH_3	CH_3	CH_2=$CHCH_2$	133–135°	70	Ethanol–chloroform	$C_{13}H_{18}N_2O_2S_2$	C 52.35	52.56
								H 6.04	6.31
XIII	CH_3	CH_3	$C_6H_5CH_2$	146–148°	65	Ethanol–chloroform	$C_{17}H_{20}N_2O_2S_2$	C 58.62	58.90
								H 5.75	6.00
XIV	CH_3	CH_3	C_6H_5	169–172°	74	Ethanol	$C_{16}H_{18}N_2O_2S_2$	C 57.48	57.22
								H 5.39	5.73
XV	CH_3	CH_3	2-$CH_3C_6H_4$	164–165°	70	Ethanol	$C_{17}H_{20}N_2O_2S_2$	C 58.62	58.85
								H 5.75	5.90
XVI	CH_3	CH_3	4-$CH_3C_6H_4$	169–171°	80	Ethanol	$C_{17}H_{20}N_2O_2S_2$	C 58.62	58.95
								H 5.75	6.05
								N 8.05	7.88
XVII	—$(CH_2)_3$—		C_2H_5	200–202°	72	Ethanol	$C_{13}H_{18}N_2O_2S_2$	C 52.35	52.52
								H 6.04	6.32
XVIII	—$(CH_2)_3$—		C_6H_5	183–185°	65	Ethanol–chloroform	$C_{17}H_{18}N_2O_2S_2$	C 58.96	59.20
								H 5.20	5.50
XIX	—$(CH_2)_3$—		4-$CH_3C_6H_4$	198–200°	60	Ethanol–chloroform	$C_{18}H_{20}N_2O_2S_2$	C 60.00	60.20
								H 5.55	5.87
XX	—$(CH_2)_4$—		CH_3	193–195°	80	Ethanol–chloroform	$C_{13}H_{18}N_2O_2S_2$	C 52.35	52.52
								H 6.04	6.25
XXI	—$(CH_2)_4$—		C_2H_5	161–164°	82	Ethanol–chloroform	$C_{14}H_{20}N_2O_2S_2$	C 53.85	53.91
								H 6.41	6.49
XXII	—$(CH_2)_4$—		n-C_6H_{13}	87–89°	73	Ethanol	$C_{18}H_{28}N_2O_2S_2$	C 58.70	58.79
								H 7.61	7.88
XXIII	—$(CH_2)_4$—		CH_2=$CHCH_2$	154–156°	85	Ethanol–chloroform	$C_{15}H_{20}N_2O_2S_2$	C 55.56	55.15
								H 6.17	6.26
								N 8.64	8.56
XXIV	—$(CH_2)_4$—		$C_6H_5CH_2$	136–138°	87	Ethanol–chloroform	$C_{19}H_{22}N_2O_2S_2$	C 60.96	61.15
								H 5.88	6.18
XXV	—$(CH_2)_4$—		C_6H_5	184–187°	82	Ethanol–chloroform	$C_{18}H_{20}N_2O_2S_2$	C 60.00	60.02
								H 5.55	5.87
XXVI	—$(CH_2)_4$—		3-$CH_3C_6H_4$	176–178°	84	Ethanol–chloroform	$C_{19}H_{22}N_2O_2S_2$	C 60.96	60.95
								H 5.88	5.92
XXVII	—$(CH_2)_4$—		4-$CH_3C_6H_4$	165–167°	80	Ethanol–chloroform	$C_{19}H_{22}N_2O_2S_2$	C 60.96	60.91
								H 5.88	5.71
XXVIII	—$(CH_2)_5$—		CH_2=$CHCH_2$	114–116°	76	Ethanol–chloroform	$C_{16}H_{22}N_2O_2S_2$	C 56.80	56.65
								H 6.51	6.64
XXIX	—$(CH_2)_5$—		$C_6H_5CH_2$	129–131°	70	Ethanol	$C_{20}H_{24}N_2O_2S_2$	C 61.86	61.59
								H 6.19	6.47
XXX	—$(CH_2)_5$—		4-$CH_3C_6H_4$	149–151°	70	Ethanol–chloroform	$C_{20}H_{24}N_2O_2S_2$	C 61.86	61.60
								H 6.19	6.49
								N 7.22	7.25



Scheme II

Table II—Antimicrobial Activity of Thienylthioureas (Minimum Inhibitory Concentration Values in Micrograms per Milliliter)

Compound	*Staphylococcus aureus* (NCTC 6571)	*Staphylococcus aureus*[a] (A/R)	*Sarcina lutea* (ATCC 9341)	*Bacillus subtilis* (NCTC 8236)	*Trychophyton mentagrophytes*[b]	*Microsporum gypseum*[b]
VII	50	25	25–50	50	50	50
IX	3.125	6.25	3.125	3.125	—	—
XII	50	50	50	50	50	50
XIII	25	50	25	50	—	—
XX	3.125	6.25	12.5	12.5	50	50
XXI	12.5	12.5	12.5	12.5	—	—
XXIII	12.5	12.5	12.5	25	—	—
XXIV	50	50	12.5	12.5	—	—
XXVIII	12.5	12.5	12.5	12.5	50	50

[a] Local isolate, Gram positive, coagulase positive, yellow pigment on potato medium, resistant to penicillin at 50 units. [b] All India Institute of Medical Sciences Culture Collection.

Preparation of N[1]*-4,5-Substituted Thienyl-*N[2]*-alkyl-(or aryl)-thiourea* (VII–XXX)—2-Amino-3-carbethoxy-4,5-substituted thiophene (6) (0.1 mole) was dissolved in hot ethanol (100 ml), and the appropriate isothiocyanate (0.11 mole) was added dropwise with stirring. The reaction mixture was refluxed on a steam bath for 5–6 hr. The mixture was cooled overnight, and the crude solid which separated was filtered and washed with ethanol. The product was dried and recrystallized from a suitable solvent (Table I).

*Preparation of 2-Mercapto-3,5,6-substituted Thieno[2,3-*d*]pyrimidin-4(*3H*)-one* (XXXI–XLVI)—A suspension of the appropriate N^1,N^2-substituted thiourea (0.05 mole) in ethanol saturated with dry hydrochloric acid (250 ml) was refluxed on a steam bath for 12 hr. The reaction mixture was cooled overnight in an ice box. The precipitated solid mass was filtered and washed with ethanol, and the dried product was recrystallized from an appropriate solvent (Table IV).

*Preparation of 3,5,6-Substituted Thieno[2,3-*d*]pyrimidin-4(*3H*)-one-2-mercaptoacetic Acid* (XLVII–LI)—A mixture of 2-mercapto-3,5,6-substituted thieno[2,3-*d*]pyrimidin-4(3*H*)-one (0.02 mole) and potassium hydroxide (0.04 mole) in ethanol (180 ml) was heated gently to obtain a clear solution. Chloroacetic acid (0.02 mole) dissolved in ethanol (10 ml) was added to the hot solution. The reaction mixture was refluxed for 6 hr on a steam bath and concentrated under vacuum. Dilute hydrochloric acid (10%) was added to obtain pH 5–6. The solid which precipitated was filtered, washed with water, and dried. Then the product was recrystallized from a suitable solvent (Table V).

*Preparation of Ethyl 3,5,6-Substituted Thieno[2,3-*d*]pyrimidin-4(*3H*)-one-2-mercaptoacetate* (LII–LVII)—To a mixture of 2-mercapto-3,5,6-substituted thieno[2,3-*d*]pyrimidin-4(3*H*)-one (0.02 mole) and potassium hydroxide (0.02 mole) in ethanol (180 ml) was added ethyl chloroacetate (0.02 mole) dissolved in ethanol (10 ml). The mixture was then refluxed for 8 hr. After cooling the reaction mixture overnight, the solid obtained was filtered, washed with water, and dried. Then the product was recrystallized from a suitable solvent (Table V).

*Preparation of 2,3-Dihydro-2,6,7-trimethylthieno[2,3-*d*]thiazolo[3,2-*a*]pyrimidin-5-one* (*LVIII*)—N^2-Allyl-N^1-[2-(3-carbethoxy-4,5-dimethylthienyl)]thiourea (14.9 g, 0.05 mole) in ethanol saturated with dry hydrochloric acid (250 ml) was prepared as described for XXXI–XLVI. When the product was recrystallized from ethanol, 7.8 g (70%) of crystalline material was obtained, mp 150–151°; IR ($CHCl_3$): 1660 cm^{-1} [C(=O)N]; UV: λ_{max} (ethanol) 210 (log ϵ 4.32), 274 (3.90), and 319 (4.10) nm; NMR ($CDCl_3$): δ 1.6 (d, 3H, C-2 methyl), 2.4 (s, 2H, C-6 methyl), 2.3 (s, 3H, C-7 methyl), and 3.9–4.6 (m, 3H, C-2 CH and C-3 CH_2).

Anal.—Calc. for $C_{11}H_{12}N_2OS_2$: C, 52.38; H, 4.76. Found: C, 52.60; H, 5.09.

*Preparation of 2-Methyl-2,3,6,7,8,9-hexahydrobenzo[*b*]thieno[2,3-*d*]thiazolo[3,2-*a*]pyrimidin-5-one* (*LIX*)—N^2-Allyl-N^1-[2-(3-carbethoxy-4,5,6,7-tetrahydrobenzo[*b*]thienyl)]thiourea (16.2 g, 0.05 mole) in ethanol saturated with dry hydrochloric acid (250 ml) was prepared as described for XXXI–XLVI. The product was recrystallized from ethanol to yield 11.15 g (77%) of crystalline material, mp 149–150°; IR ($CHCl_3$): 1675 cm^{-1} [C(=O)N]; UV: λ_{max} (ethanol): 211 (log ϵ 4.32), 275 (3.88), and 320 (4.14) nm; NMR ($CDCl_3$): δ 1.5 (d, 3H, C-2 methyl), 1.9 (t, 4H, C-7 CH_2 and C-8 CH_2), 2.7 (m, 4H, C-6 CH_2 and C-9 CH_2), and 4–4.7 (m, 3H, C-2 CH and C-3 CH_2).

Anal.—Calc. for $C_{13}H_{14}N_2OS_2$: C, 56.11; H, 5.03. Found: C, 56.22; H, 5.36.

Biological Screening—*Antimicrobial Testing*—The *in vitro* antibacterial and antifungal activities of VII–XVII, XX–XXVIII, and XXX were studied by a serial dilution technique (8), using dimethylformamide as the solvent. Dilutions were made so that in no instance was there more than 1% dimethylformamide in the test medium. At such concentrations, dimethylformamide has no inhibitory effect on the organisms.

Anticonvulsant Activity (9)—The following tests were performed on a group of eight mice of both sexes:

1. Maximum electric shock (induced using corneal electrode and a rectangular pulse of 50 mamp for 0.2 sec).

2. Pentylenetetrazol convulsions (compounds were administered orally at the dose level of 200 mg/kg followed by 100 mg/kg pentylenetetrazol[2] subcutaneously 1 hr later).

Analgesic Effect (10) (*Writhing Test, Acetic Acid*)—Oral doses (200 mg/kg) of the test compounds were administered to groups of female mice. After 15 min, the mice were injected with 0.1 ml/10 g of a 3% solution of acetic acid intraperitoneally; the number of writhing movements was recorded for 20 min.

Anti-Inflammatory Activity (11) (*Rat Paw Edema*)—Male rats (120–140 g) were divided into groups of four and fasted overnight. They were fed orally with the test compounds (200 mg/kg) as suspensions in 1% gum tragacanth. One hour later, 0.05 ml of 1% carrageenan in sterile saline was injected into the planter surface of one hindpaw in each animal. The volume of the paw was determined immediately and then 3 hr after the injection. The volume

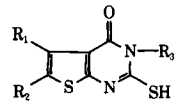
Table III—Results of Pharmacological Screening[a]

Compound	Analgesia, % Inhibition	Anti-Inflammatory Activity, % Inhibition
XIII	20.0	—
XIX	—	14.0
XVI	—	13.6
XXIX	45.4	23.9
XXXI	—	23.5
XXXIV	—	25.0
XXXVI	—	16.6
XLI	—	15.9
XLIII	—	16.0
XLIV	45.0	16.6
LI	23.2	19.4
LIII	23.6	—
LIV	—	21.7
LVI	22.8	16.1
LIX	12.1	30.5
Aspirin (200 mg/kg)	55.0	—
Phenylbutazone (100 mg/kg)	—	60.0

[a] All compounds showed an LD_{50} of more than 1000 mg/kg po.

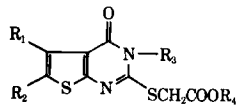
[2] Metrazol.

Table IV—Physical Properties of 2-Mercaptothieno[2,3-*d*]pyrimidin-4-ones



Compound	R_1	R_2	R_3	Melting Point	Yield, %	Crystallization Solvent	Molecular Formula	Analysis, % Calc.	Found
XXXI	CH_3	CH_3	CH_3	270–274°	80	Acetic acid	$C_9H_{10}N_2OS_2$	C 47.79 H 4.42 N 12.39	47.79 4.56 12.21
XXXII	CH_3	CH_3	CH_3CH_2	255–257°	81	Ethyl acetate	$C_{10}H_{12}N_2OS_2$	C 50.00 H 5.00	50.11 5.35
XXXIII	CH_3	CH_3	*n*-C_4H_9	201–202°	80	Ethanol	$C_{12}H_{16}N_2OS_2$	C 53.73 H 5.97	53.74 6.30
XXXIV	CH_3	CH_3	*n*-C_6H_{13}	172–175°	77	Ethanol–chloroform	$C_{14}H_{20}N_2OS_2$	C 56.76 H 6.76	57.04 6.95
XXXV	CH_3	CH_3	$C_6H_5CH_2$	235–240°	74	Acetic acid	$C_{15}H_{14}N_2OS_2$	C 59.60 H 4.64	59.58 4.95
XXXVI	CH_3	CH_3	C_6H_5	308–310°	72	Acetic acid	$C_{14}H_{12}N_2OS_2$	C 58.33 H 4.17	58.01 4.36
XXXVII	CH_3	CH_3	4-$CH_3C_6H_4$	220° dec.	71	Acetic acid	$C_{15}H_{14}N_2OS_2$	C 59.60 H 4.64 N 9.27	59.90 4.91 9.18
XXXVIII	—$(CH_2)_4$—		CH_3	298–301°	83	Acetic acid	$C_{11}H_{12}N_2OS_2$	C 52.38 H 4.76 N 11.11	52.32 5.04 10.86
XXXIX	—$(CH_2)_4$—		C_2H_5	259–261°	77	Acetic acid	$C_{12}H_{14}N_2OS_2$	C 54.14 H 5.26	53.95 5.43
XL	—$(CH_2)_4$—		*n*-C_6H_{13}	194–196°	69	Acetic acid	$C_{16}H_{22}N_2OS_2$	C 59.63 H 6.83 N 8.70	59.75 7.12 9.00
XLI	—$(CH_2)_4$—		$C_6H_5CH_2$	244–246°	67	2-Propanol–chloroform	$C_{17}H_{16}N_2OS_2$	C 62.20 H 4.88	62.49 5.13
XLII	—$(CH_2)_4$—		C_6H_5	315° dec.	72	Acetic acid	$C_{16}H_{14}N_2OS_2$	C 61.15 H 4.46 N 8.92	61.04 4.72 8.82
XLIII	—$(CH_2)_4$—		4-$CH_3C_6H_4$	280–283°	70	Acetic acid	$C_{17}H_{16}N_2OS_2$	C 62.20 H 4.88	61.98 5.10
XLIV	—$(CH_2)_5$—		$C_6H_5CH_2$	255–257°	71	Acetic acid	$C_{18}H_{18}N_2OS_2$	C 63.16 H 5.26	63.25 5.51
XLV	—$(CH_2)_5$—		C_6H_5	250–253°	72	Dioxane–ethanol	$C_{17}H_{16}N_2OS_2 \cdot 0.5H_2O$	C 60.53 H 5.04 N 8.31	60.44 4.99 8.32
XLVI	—$(CH_2)_5$—		3-$CH_3C_6H_4$	305° dec.	69	Acetic acid	$C_{18}H_{18}N_2OS_2$	C 63.16 H 5.26	63.20 5.49

Table V—Physical Properties of Thieno[2,3-*d*]pyrimidin-4-one-2-mercaptoacetic Acids and Ethyl Esters



Compound	R_1	R_2	R_3	R_4	Melting Point	Yield, %	Crystallization Solvent	Molecular Formula	Analysis, % Calc.	Found
XLVII	CH_3	CH_3	CH_3	H	192–195°	87	Ethanol	$C_{11}H_{12}N_2O_3S_2$	C 46.48 H 4.23	46.81 4.52
XLVIII	—$(CH_2)_4$—		CH_3	H	187–190°	81	Ethanol	$C_{13}H_{14}N_2O_3S_2$	C 50.32 H 4.52	50.61 4.90
XLIX	—$(CH_2)_4$—		C_2H_5	H	188–190°	77	Ethanol	$C_{14}H_{16}N_2O_3S_2$	C 51.85 H 4.94 N 8.64	51.80 5.20 8.52
L	—$(CH_2)_4$—		C_6H_5	H	239–241°	50	Ethanol–chloroform	$C_{18}H_{16}N_2O_3S_2$	C 58.06 H 4.30	58.05 4.62
LI	—$(CH_2)_4$—		4-$CH_3C_6H_4$	H	245–247°	71	Ethanol–chloroform	$C_{19}H_{18}N_2O_3S_2$	C 59.07 H 4.66	58.98 4.93
LII	CH_3	CH_3	CH_3	C_2H_5	124–127°	65	Ethanol	$C_{13}H_{16}N_2O_3S_2$	C 50.00 H 5.13	50.00 5.30
LIII	—$(CH_2)_4$—		CH_3	C_2H_5	137–138°	68	Ethanol	$C_{15}H_{18}N_2O_3S_2$	C 53.25 H 5.33 N 8.28	53.61 5.69 8.45
LIV	—$(CH_2)_4$—		$C_6H_5CH_2$	C_2H_5	160–162°	90	Ethanol–chloroform	$C_{21}H_{22}N_2O_3S_2$	C 60.87 H 5.31 N 6.76	61.10 5.62 6.91
LV	—$(CH_2)_4$—		C_6H_5	C_2H_5	202–204°	55	Ethanol	$C_{20}H_{20}N_2O_3S_2$	C 60.00 H 5.00	60.01 5.19
LVI	—$(CH_2)_4$—		3-$CH_3C_6H_4$	C_2H_5	196–198°	85	Ethanol	$C_{21}H_{22}N_2O_3S_2$	C 60.87 H 5.31	60.93 5.63
LVII	—$(CH_2)_4$—		4-$CH_3C_6H_4$	C_2H_5	189–191°	78	Ethanol–chloroform	$C_{21}H_{22}N_2O_3S_2$	C 60.87 H 5.31	60.89 5.63

difference was calculated, and the anti-inflammatory effect was established from the results obtained.

Acute Toxicity—Mice of both sexes (15–20 g) were fasted for 2 hr. Suspensions (1% gum tragacanth) of the test compounds were administered orally, 0.1 ml/10 g of body weight. The LD_{50} was calculated after observation for 1 week (Table III).

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Rapid and Sensitive Colorimetric Determination of Cobalt(II)

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Abstract □ A highly selective and sensitive spectrophotometric determination of cobalt(II) was developed. 7-Nitroso-8-hydroxyquinoline-5-sulfonic acid sodium salt was used as the chromogenic reagent for color development. Although other metals form colored chelates with the ligand, it was possible to develop a selective method using McIlvaine's pH 8 citric acid–phosphate buffer. Under these conditions, iron(II), iron(III), copper(II), zinc(II), and manganese(II), minerals likely to be compounded with cobalt(II) in geriatric formulations, do not interfere with the precision of the method or the color development. Calcium(II) and magnesium(II) do not form colored chelates with the used ligand. Hormones, vitamins, and additives likely to be present along with the cobalt ion in pharmaceutical formulations do not interfere. The sensitivity is 0.37 μg of cobalt(II)/ml of sample solution.

Keyphrases □ Cobalt(II)—colorimetric analysis, pharmaceutical formulations □ Colorimetry—analysis, cobalt(II) in pharmaceutical formulations □ Chelating agents—7-nitroso-8-hydroxyquinoline-5-sulfonic acid sodium salt used in colorimetric analysis of cobalt(II) in pharmaceutical formulations

Cobalt(II) salts are common ingredients in geriatric preparations along with other mineral salts, vitamins, and hormones. Various analytical procedures for the determination of cobalt(II) are available including volumetric (1), polarographic (2), colorimetric (3–7), fluorometric (8, 9), atomic absorption (10), ion-exchange chromatographic (11), and other miscellaneous (12) methods. Nevertheless, the analysis of cobalt(II) in multicomponent pharmaceutical preparations has not been frequently reported.

In a previous study (13), the chelating properties of 7-nitroso-8-hydroxyquinoline-5-sulfonic acid sodium salt were successfully used to develop a highly sensitive method for the determination of iron(II). Accordingly, it was decided to investigate the cobalt(II) complexing properties of this chelating agent to develop an analytical procedure for cobalt(II) that would not necessitate its prior separation from other mineral ions, vitamins, and hormones present in geriatric formulations.

EXPERIMENTAL

Apparatus—A recording spectrophotometer was used to determine the absorbance and obtain the spectra.

Materials—7-Nitroso-8-hydroxyquinoline-5-sulfonic acid sodium salt was prepared according to a reported method (14).

Cobalt sulfate, ferrous sulfate, copper sulfate, zinc sulfate, manganese sulfate, magnesium sulfate, calcium chloride, potassium chloride, hydrochloric acid, citric acid, disodium hydrogen phosphate, sodium acetate, acetic acid, and sodium carbonate were analytical grades.

Ethinyl estradiol, methyltestosterone, thiamine hydrochloride, riboflavin, pyridoxine hydrochloride, folic acid, cyanocobalamin, nicotinamide, ascorbic acid, starch, lactose, and glucose were pharmacopeial grades.

Reagents and Solutions—The following solution were used: ligand solution, 0.0025 *M* in double-distilled water; acetic acid–sodium acetate buffer, pH 3.42–5.89 (15); Clark and Lub's potassium chloride–hydrochloric acid buffer, pH 1.0–2.2 (16); McIlvaine's citric acid–phosphate buffer, pH 2.2–8.0 (16); and Sorensen's phosphate buffer, pH 5.0–8.0 (16). Double-distilled water was used throughout.

Standard Solution of Cobalt Sulfate ($CoSO_4 \cdot 7H_2O$, 0.005 M)—About 0.15 g of cobalt sulfate, accurately weighed, was placed in a 100-ml volumetric flask and dissolved and diluted to volume with water. Appropriate dilutions were made from this stock solution.