Synthesis and Antimicrobial Activities of N^4-(2-Acetoxyethoxymethyl)thiosemicarbazones and N^3-(2-Acetoxyethoxymethyl)thioureas

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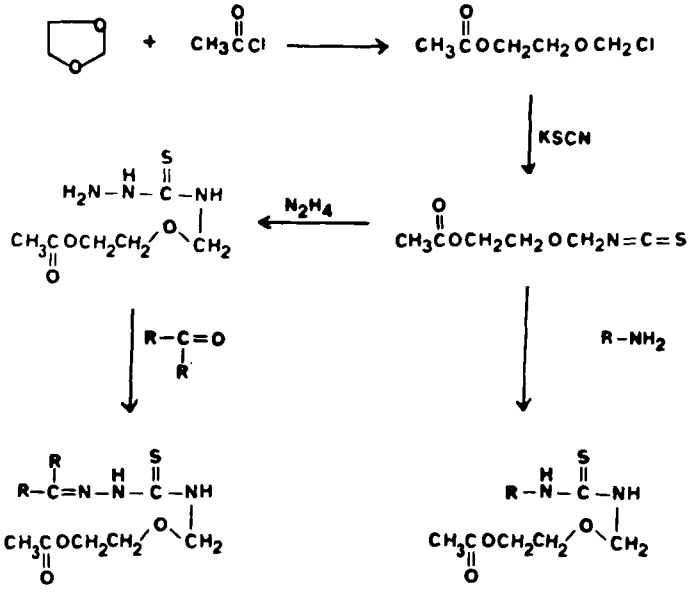
Abstract □ A series of thiosemicarbazones and thioureas having an open-chain analogue of the ribosyl group, the 2-acetoxyethoxymethyl moiety, has been synthesized. Significant growth inhibitory activity versus gram-positive and gram-negative organisms, a yeast, and a mold has been found with the 2-acetoxyethoxymethyl derivatives of *N*-alkyl-, aryl-, and heteroaryl-thiosemicarbazones and thioureas. The molecules may function as inhibitors of ribonucleotide reductase or in utilization of the carbamyl group in pyrimidine biosynthesis.

Heterocyclic aldehyde and ketone thiosemicarbazones have shown activity as antitubercular,[1] antiviral,[2] anticancer,[3] antimalarial,[4] and antimicrobial[5] agents. 1-Formylpyridine and 1-formylisoquinoline thiosemicarbazones, and their hydroxyl derivatives, inhibited the incorporation of [^{3}H]thymidine into DNA of Sarcoma 180 ascites tumor cells and also inhibited the reduction of cytidine diphosphate to the deoxyribonucleotide.[6] The mechanism by which these inhibitions take place is not certain, but metal binding has been implicated.[7] *Escherichia coli* (*E. coli*) ribonucleotide reductase depends on an iron-containing subunit for activity,[8] although an oxidation–reduction reaction involving the iron is apparently not involved. The possibility that the activity of thiosemicarbazones depends on DNA binding suggests that an additional binding feature should enhance their activity. Accordingly, a series of aromatic and heteroaromatic thiosemicarbazones with a simulated ribose function, the *N*-acetoxyethoxymethyl[9] derivative, was synthesized. A corresponding series of thioureas, having only weak metal-binding ability compared with that of the thiosemicarbazones, was also prepared for comparison of activities. It is also possible that a thiosemicarbazone or thiourea function attached to a ribose simulator can function as an inhibitor of pyrimidine nucleotide formation.

The compounds prepared were tested against a representative series of micro-organisms, including both gram-positive and gram-negative bacteria, a yeast, and a mold. A comparison of antimicrobial activities of the thiosemicarbazones and thioureas could indicate whether or not metal binding is of importance in the activity of thiosemicarbazones; similar activities would argue against this possibility. Those compounds showing the best antimicrobial activity were tested for antileukemic activity, and a couple were screened as antimalarial agents.

Chemistry—N^4-(2-Acetoxyethoxymethyl)thiosemicarbazide was prepared in three steps. Chloromethoxyethyl acetate was prepared readily by the reaction of 1,3-dioxolane and acetyl chloride at refluxing temperature by a modification of the method of Gresham.[10] It was then converted to the isothiocyanate derivative by reaction with potassium thiocyanate in refluxing toluene. In this way, 2-(acetoxyethoxy)-methyl isothiocyanate was obtained as a marginally stable,



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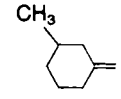
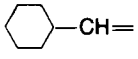
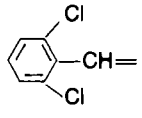
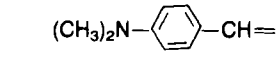
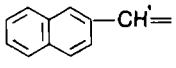
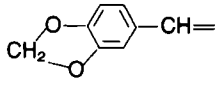
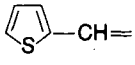
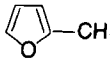
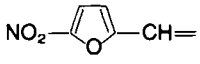
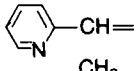
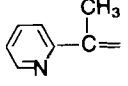
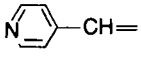
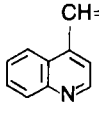
colorless liquid in fairly good yield. The formation of this compound was confirmed by the appearance of a strong band in the IR spectrum of the neat sample at 2200–2000 cm^{-1}, corresponding to the –N=C=S stretching mode, and also by a change in the chemical shift of the methylene group adjacent to –N=C=S to ~0.5 ppm upfield relative to $-CH_2Cl$ in the NMR spectrum of the compound. 2-(Acetoxyethoxy)methyl isothiocyanate was converted to its thiosemicarbazide through a reaction with hydrazine hydrate in methanol, at 0 °C, in excellent yield.

A series of N^4-(2-acetoxyethoxymethyl)thiosemicarbazones was prepared by the reaction between an aldehyde or a ketone with N^4-(2-acetoxyethoxymethyl)thiosemicarbazide in ethyl alcohol. The formation of these compounds was corroborated by IR and NMR analysis. The most characteristic IR peaks of these compounds were the N–H stretching

Table I—Physical Properties of N^4-(2-Acetoxyethoxymethyl)thiosemicarbazones

$CH_3COCH_2CH_2OCH_2NHCNHNR$ (O and S as shown)

Compd. No.	R	Melting Point, °C	Yield, %	Formula[a]
1	H_2[b]	110–111	85	$C_6H_{13}N_3O_3S$
2	$(CH_3)_2C=$	69–70	73	$C_9H_{17}N_3O_3S$
3	$(CH_3O)_2CHCCH_3$	79–81	80	$C_{11}H_{21}N_3O_5S$
4		69–70	67	$C_{13}H_{23}N_3O_3S$
5	$-(CH_2)_5-$[b]	125–126	65	$C_{11}H_{21}N_3O_3S$
6	$-(CH_2)_6-$[b]	99–100	71	$C_{12}H_{23}N_3O_3S$
7		101–102	50	$C_{13}H_{23}N_3O_3S$
8	$C_6H_5CH=$	116–117	80	$C_{13}H_{17}N_3O_3S$
9		132–133	78	$C_{13}H_{15}CL_2N_3O_3S$
10		122–124	70	$C_{15}H_{22}N_4O_3S$
11		133–134	87	$C_{17}H_{19}N_3O_3S$
12		132–133	59	$C_{14}H_{17}N_3O_5S$
13	$O(CH_2CH_2)_2$[b]	143–144	72	$C_{10}H_{19}N_3O_4S$
14		145–146	80	$C_{11}H_{15}N_3O_3S_2$
15		129–130	77	$C_{11}H_{15}N_3O_4S$
16		153–155	76	$C_{11}H_{14}N_4O_6S$
17		142–143	64	$C_{12}H_{16}N_4O_3S$
18		134–135	77	$C_{13}H_{18}N_4O_3S$
19		153–155	76	$C_{12}H_{16}N_4O_3S \cdot HCl$
20		157–158	66	$C_{16}H_{18}N_4O_3S$

[a] Satisfactory analytical data (±0.4% for C, H, N) were found for all new compounds listed, with the exception of compound **20**, calcd. C, 55.48; found C, 54.97. [b] A thiosemicarbazide.

and bending vibrations at 3350–3150 cm^{-1} and 1550–1520 cm^{-1}, respectively, C=S at 1260–1220 cm^{-1}, C=O at 1640–1600 cm^{-1}, and C=O of the ester type at 1740–1730 cm^{-1}. The most characteristic ^1H NMR peak of the N^4-(2-acetoxyethoxymethyl)thiosemicarbazones was the singlet at δ 8.40–8.0 ppm due to the single proton of the methinyl (-CH=) group. The other significant peaks were the acetyl peak (sharp singlet) at δ 2.05 ppm, the two triplets at δ 3.70 and 4.10 ppm, corresponding to the two adjacent methylene groups attached to methoxy and acetoxy groups, respectively, and a doublet (J = 7 Hz) at 5.20 ppm, corresponding to the methylene group next to NH. The peaks for the two NH groups were quite distinguishable. One is coupled to its adjacent CH_2 and appeared as a broad triplet (J = 7 Hz) at δ 9.0 ppm, while the other NH showed a singlet at δ 11.70–12.20 ppm, before treatment with D_2O. The compounds synthesized are listed in Table I.

Reaction of 2-(acetoxyethoxy)methyl isothiocyanate with various amines, including alkyl, aryl, and heteroaryl, gave the thiourea derivatives in good yield. Use of alcohols as reaction solvents sometimes resulted in transetherification to give the N-alkoxymethylthioureas. Deacetylation to give the N-(2-hydroxyethoxymethyl)thioureas was accomplished with dry ammonia in methanol.

The ^1H NMR spectra of the thioureas showed the NH adjacent to CH_2 as a broad triplet in the δ 6.8–9.2 ppm region, whereas the N–H adjacent to an aromatic ring appeared at δ 9.2–9.7 ppm. When the N–H was adjacent to 2-pyridyl, the N–H proton was found in the deshielded region of δ 11.5–12.1 ppm. This may be attributed to an intramolecular H-bond forming a 6-membered ring between N–H and pyridyl nitrogen atoms. The thioureas prepared are listed in Table II.

A few compounds (**5, 6, 13**) were also prepared in which N^1 of the 2-(acetoxyethoxymethyl)thiosemicarbazide forms a part of a heterocyclic ring. These compounds, which may be considered thiosemicarbazides, were prepared by a direct reaction between 2-(acetoxyethoxy)methyl isothiocyanate and a substituted hydrazine in methanol at room temperature.

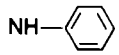
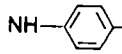
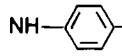
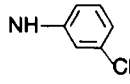
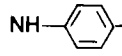
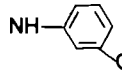
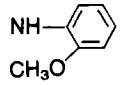
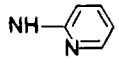
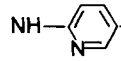
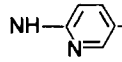
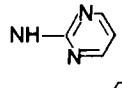
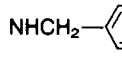
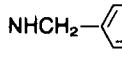
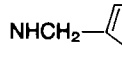
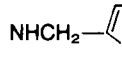
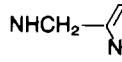
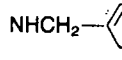
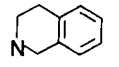
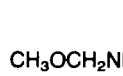
Results

A majority of the thiosemicarbazones tested show inhibitory activity against *Candida albicans* and *Aspergillus niger* (Table III). N^4-(2-Acetoxyethoxymethyl)thiosemicarbazide and N^4-(2-acetoxyethoxymethyl)thiosemicarbazones of 2-propanone, 2-formylthiophene, and 2-acetylpyridine showed the highest inhibitory activity at a minimal inhibitory concentration (MIC) of 10^{-4} M against *Aspergillus niger*. The highest MIC activity against *C. albicans* was observed with N^4-(2-acetoxyethoxymethyl)-5-nitro-2-formyl- and -2-acetylpyridinethiosemicarbazones at 10^{-3} M.

The N^4-(2-acetoxyethoxymethyl)thiosemicarbazones containing heterocycles in compounds **13, 16, 17,** and **18** showed inhibitory effects against *Pseudomonas aeruginosa*. The most active compounds, however, were the derivatives of 2-formyl- and 2-acetylpyridine with MIC values of 10^{-3} M against this species. Unlike the heteroarylidine thiosemicarbazones, the arylidene derivatives did not show any activity against the organisms tested. A fairly large number of compounds exhibited inhibitory activities against *Staphylococcus aureus* and *Escherichia coli*. In most cases these were the thiosemicarbazones derived from heterocyclic and small alkylidene compounds.

Comparison of the overall antimicrobial activities of N^4-(2-acetoxyethoxymethyl)thiosemicarbazones with that of the standard, propylparaben, revealed greater minimal inhibitory concentrations with the former compounds. The minimal inhibitory concentrations for 5-fluorodeoxyuridine are also listed in Table III for comparison. Calculated values for propylparaben from published data are also included.[11] It is apparent, however, that inclusion of the 2-acetoxyethoxymethyl moiety lowered the antibacterial activities of the thiosemicarbazones, in comparison with those of the substituted 2-acetylpyridine thiosemicarbazones reported by Dobek et al.[5]

Table II—Physical Properties of N^3-(2-Acetoxyethoxymethyl)thioureas

Cmpd. No.	R	Melting Point, °C	Yield, %	Formula[a]
21		109–110	77	$C_{12}H_{16}N_2O_3S$
22		141–142	85	$C_{12}H_{15}IN_2O_3S$
23		99.5–100.5	79	$C_{13}H_{18}N_2O_3S$
24		90–92	64	$C_{13}H_{18}N_2O_3S$
25		107–108	89	$C_{13}H_{18}N_2O_4S$
26		64–65	69	$C_{13}H_{18}N_2O_4S$
27		96–97	64	$C_{13}H_{18}N_2O_4S$
28		73–75	74	$C_{11}H_{15}N_3O_3S$
29		96–97	35	$C_{11}H_{14}ClN_3O_3S$
30		99–100	91	$C_{12}H_{17}N_3O_3S$
31		115–116	15	$C_{10}H_{14}N_4O_3S$
32		89–90	91	$C_{13}H_{18}N_2O_3S$
33		83–85	82	$C_{15}H_{22}N_2O_5S$
34		91–93	97	$C_{11}H_{16}N_2O_4S$
35		88–89	91	$C_{11}H_{16}N_2O_3S_2$
36		67–68	90	$C_{12}H_{17}N_3O_3S$
37		69–70	80	$C_{12}H_{17}N_3O_3S$
38		84–85	76	$C_{15}H_{20}N_2O_3S$
39	$NHCH_3$	46–47	79	$C_7H_{14}N_2O_3S$
40	NH_2	55–58	81	$C_6H_{12}N_2O_3S$
41		135–137		$C_9H_{12}N_2OS$

[a] Satisfactory analytical data (±0.4% for C, H, N) were found for all new compounds.

Table III—Antimicrobial Activities of N^4-(2-Acetoxyethoxymethyl)thiosemicarbazones and Thiosemicarbazides

Cmpd. No.	Minimum Inhibitory Concentration, M (μg/mL)[a]				
	S. aureus	*E. coli*	*P. aeruginosa*	*C. albicans*	*A. niger*
1	10^{-2} (2072)	10^{-2} (2072)	—	10^{-2} (2072)	10^{-4} (20.7)
2	10^{-2} (2475)	10^{-2} (2475)	—	10^{-2} (2475)	10^{-4} (24.7)
4	—	—	—	10^{-2} (3015)	10^{-2} (3015)
5	10^{-2} (2754)	10^{-2} (2754)	—	10^{-2} (2754)	10^{-2} (2754)
6	10^{-2} (2894)	10^{-2} (2894)	—	10^{-2} (2894)	—
13	10^{-2} (2773)	10^{-2} (2773)	10^{-2} (2773)	—	10^{-3} (277)
14	—	—	—	—	10^{-4} (29.5)
16	10^{-2} (3305)	10^{-2} (3305)	10^{-2} (3305)	10^{-3} (330)	—
17	10^{-2} (2963)	10^{-3} (296)	10^{-3} (296)	10^{-2} (2963)	10^{-3} (296)
18	10^{-3} (310)	10^{-3} (310)	10^{-3} (310)	10^{-3} (310)	10^{-4} (31.0)
19	—	—	—	10^{-2} (2963)	10^{-3} (296)
Propylparaben	10^{-3} (180)	10^{-2} (1802)	10^{-2} (1802)	10^{-2} (1802)	10^{-3} (180)
Propylparaben[b]	2.8×10^{-3} (504)	5.6×10^{-3} (1009)	—	—	1.1×10^{-3} (198)
5-Fluoro-deoxyuridine	10^{-2} (2462)	10^{-3} (246)	—	10^{-3} (246)	10^{-2} (2462)

[a] A blank indicates no inhibition at $\leq 10^{-2}$ M concentrations. [b] Ref 11.

The N^3-(2-acetoxyethoxymethyl)thioureas were generally less inhibitory than the thiosemicarbazone derivatives (Table IV). A slightly larger number of thioureas were active against *P. aeruginosa*, however. These had phenyl, *p*-methylphenyl, 2-pyrimidyl, 2-furylmethyl, 2-thienylmethyl, and methyl substituents. All of the active thioureas had some activity against *A. niger*, with the 2-furylmethyl derivative being the most active.

N-(2-Hydroxyethoxymethyl)thiourea did not show any activity against these organisms, whereas the acetylated (on the hydroxy) compound was active against *C. albicans* and *A. niger*. The thioureas having a methyl or substituted methyl substituent generally inhibited the growth of most of the organisms. *N*-Methylthiourea, however, was inactive in these tests, whereas N^3-(2-acetoxyethoxymethyl)-N^1-methylthiourea inhibited growth of all organisms tested.

Activity of both the thiosemicarbazones and thioureas is comparable to or greater than that of propylparaben, a commonly used preservative agent for cosmetic preparations. A comparison of the inhibitory activities of thiosemicarbazones and thioureas shows somewhat better levels and spectra of activity for the thiosemicarbazones. A somewhat greater percentage of these exhibited some activity, as well.

Compound 17, the 2-pyridylaldehyde thiosemicarbazone derivative, showed activity in the P388 leukemia screen, giving a T/C% (survival times of test/control animals) of 131 at a dose of 120 mg/kg in mice. Compounds 1, 13, 34, and 39 were inactive, however. Tests were conducted according to the published protocol.[12] Compound 18 was active in an in vitro antimalarial test[13] versus W-2 Indochina *Plasmodium falciparum* resistant to chloroquine but susceptible to mefloquine. Compound 18 had an IC_{50} (50% inhibitory concentration) of 26.34 ng/mL, compared with 91.96 ng/mL for chloroquine and 0.996 ng/mL for mefloquine. Against D-6 African *Plasmodium falciparum* susceptible to chloroquine but resistant to mefloquine, 18 had an IC_{50} of 33.58 ng/mL, compared with 1.79 ng/mL for chloroquine and 8.28 ng/mL for mefloquine. Compound 17 had only slight activity.[14]

Experimental Section

Melting points were determined in capillaries with a Mel-Temp block and are uncorrected. The ^{1}H NMR spectra were obtained with a Varian T-60 spectrometer, using tetramethylsilane as the internal standard. The IR spectra were obtained with a Perkin-Elmer model 457A spectrophotometer using KBr pellets. Elemental analyses were done by Multi Chem Laboratories, Lowell, MA, and are within ±0.4% of theoretical values. Thin-layer chromatography was carried out using silica gel plates with fluorescent indicator. Organic reagents were supplied by Aldrich Chemical Co. or Eastman Organic Chemicals.

Chloromethoxyethyl Acetate—Freshly redistilled acetyl chloride (78.5 g, 1.0 mol) was added in a dropwise manner to 1,3-dioxolane (111 g, 1.5 mol) with stirring. After one-third of the acetyl chloride was added, the temperature was raised to reflux (70 °C), and the remaining acetyl chloride was added during a 45-min period. The solution was refluxed for 8 h and fractionally distilled to give 108 g (71%) of colorless liquid bp 74–76 °C/5.0 torr: ^{1}H NMR ($CDCl_3$): δ 2.05 (3, s, CH_3CO), 3.80 (2, m, CH_2O), 4.20 (2, m, $AcOCH_2$), and 5.50 ppm (2, s, OCH_2Cl); IR (neat): ν 2900 (C–H), 1740 (C=O), 1460 (CH_2), 1370 (CH_3) cm^{-1}.

2-Acetoxyethoxymethyl Isothiocyanate—To a solution of chloromethoxyethyl acetate (152.5 g, 1 mol) in 300 mL of toluene was added potassium thiocyanate (106.4 g, 1.2 mol). The temperature was raised to reflux for 10 h. The pale yellow solution was filtered and the filtrate was fractionally distilled to give 106.5 g (61%) of marginally stable colorless liquid at 93 °C/0.65 torr: ^{1}H NMR ($CDCl_3$): δ 2.05 (3, s, CH_3CO), 3.80 (2, m, CH_2O), 4.20 (2, m, $AcOCH_2$), and 5.0 ppm (2, s, OCH_2N); IR (neat): ν 2900 (C–H), 2100–2030 (–N=C=S), 1735 (C=O), 1130 (C–O–C) cm^{-1}.

N^4-(2-Acetoxyethoxymethyl)thiosemicarbazide—To a cold solution of 2-acetoxyethoxymethyl isothiocyanate (17.5 g, 0.1 mol) in 100 mL of methanol was added anhydrous hydrazine (3.2 g, 0.1 mol). The mixture was stirred in an ice bath (0 °C) for 10 min. The white precipitate which formed was filtered and dried at 60 °C under reduced pressure, and recrystallized from ethanol, yielding 1.80 g (85%) of white crystals: mp 110–111 °C; ^{1}H NMR ($CDCl_3$): δ 2.05 (3, s, CH_3CO), 3.70 (2, m, CH_2O), 4.15 (2, m, $AcOCH_2$), 4.55 (2, bs, NH_2), 5.03 (2, d, J = 7 Hz, OCH_2N), 8.73 (1, bm, NH), and 9.25 ppm (1, bs, NH); IR (KBr): ν 3350–3150 (N–H), 2900 (C–H), 1735 (C=O), 1640, 1540 (N–H), 1260 (C=S, C–O), 1060 (C–O–C) cm^{-1}. Anal. ($C_6H_{13}N_3O_3S$) C, H, N.

N^4-(2-Acetoxyethoxymethyl)-2-formylpyridine Thiosemicarbazone—The following procedure is representative of the formation of the N^4-(2-acetoxyethoxymethyl)thiosemicarbazones. N^4-(2-Acetoxyethoxymethyl)thiosemicarbazide (1.04 g, 5 mmol) was added to a solution of 2-pyridinecarboxaldehyde (0.53 g, 5 mmol) in 10 mL of ethanol. The mixture was stirred at reflux temperature for 30 min, and then cooled in an ice bath. The precipitate was removed by filtration, recrystallized from ethanol, and dried at 60 °C under reduced pressure, giving 0.95 g (64%): mp 142–143 °C, ^{1}H NMR (Me_2SO-d_6): δ 2.05 (3, s, CH_3CO), 3.75 (2, m, CH_2O), 4.17 (2, m, $AcOCH_2$), 5.15 (2, d, J = 7 Hz, OCH_2N), 7.3–8.8 (4, m, Ar), 8.20 (1, s, CH), 9.5 (1, bt, J = 7 Hz, NH), and 12.05 ppm (1, bs, NH); IR(KBr): ν 3150 (N–H), 1735 (C=O), 1540 (CNH), 1240 (C=S) cm^{-1}. Anal. ($C_{12}H_{16}N_4O_3S$) C, H, N.

N^3-(2-Acetoxyethoxymethyl)-N^1-(1-morpholinyl)thiourea—The following procedures are representative of the formation of the N^3-(2-acetoxyethoxymethyl)thioureas. Freshly prepared 2-acetoxyethoxymethyl isothiocyanate (1.75 g, 10 mmol) was added to a solution of *N*-

Table IV—Antimicrobial Activities of N^3-(2-Acetoxyethoxymethyl)thioureas

Cmpd. No.	Minimum Inhibitory Concentration, M (μg/mL)[a]				
	S. aureus	*E. coli*	*P. aeruginosa*	*C. albicans*	*A. niger*
21	—	—	10^{-2} (2683)	—	10^{-2} (2683)
23	—	10^{-2} (2824)	10^{-2} (2824)	10^{-2} (2824)	10^{-2} (2824)
25	—	10^{-2} (2984)	—	10^{-2} (2984)	10^{-2} (2984)
31	—	10^{-2} (2703)	10^{-2} (2703)	10^{-2} (2703)	10^{-2} (2703)
32	10^{-2} (2824)	10^{-2} (2824)	—	10^{-3} (282)	10^{-2} (2824)
34	10^{-2} (2723)	10^{-2} (2723)	10^{-2} (2723)	10^{-2} (2723)	10^{-3} (272)
35	—	10^{-2} (2884)	10^{-2} (2884)	10^{-2} (2884)	10^{-2} (2884)
39	10^{-2} (2063)	10^{-2} (2063)	10^{-2} (2063)	10^{-2} (2063)	10^{-2} (2063)

[a] A blank indicates no inhibition at $\leq 10^{-2}$ M concentrations.

aminomorpholine (1.02 g, 10 mmol) in 20 mL of methanol. The mixture was stirred at room temperature for 1 h. The precipitate was removed by filtration recrystallized from methanol, and dried under reduced pressure at 60 °C, giving 2.0 g (72%) of white crystals: mp 143–144 °C; ^{1}H NMR ($CDCl_3$): δ 2.05 (3, s, CH_3CO), 2.90 (4, bm, $2CH_2$), 3.75 (4, bm, $2CH_2$), 3.85 (2, m, CH_2O), 4.25 (2, m, $AcOCH_2$), and 8.0 ppm (1, bt, J = 7, NH); IR (KBr): ν 3250–3170 (N–H), 1735 (C=O), 1550–1510 (CNH), 1250 (C=S) cm^{-1}. Anal. ($C_{10}H_{19}N_3O_4S$) C, H, N.

N^3-(2-Acetoxyethoxymethyl)-N^1-methylthiourea—2-Acetoxyethoxymethyl isothiocyanate (3.5 g, 20 mmol) was dissolved in 20 mL of dry acetonitrile and cooled in an ice bath. Dry methylamine was passed slowly through the cold solution for 40 min, the mixture was filtered, and acetonitrile was removed under reduced pressure to give a pale yellow syrup. Solution in ethyl ether (15 mL) and refrigeration overnight gave colorless crystals (3.25 g, 79%) which were recrystallized from ethyl acetate: mp 46–47 °C, ^{1}H NMR (Me_2SO-d_6): δ 2.04 (3, s, CH_3CO), 2.84 (3, d, J = 5 Hz, NCH_3), 3.50–4.20 (4, m, $O(CH_2)_2O$), 4.80 (2, d, J = 6 Hz, OCH_2N), 7.60 (1, bm, $NHCH_3$), and 8.04 ppm (1, bt, $NHCH_2$); IR(KBr): ν 3350, 3205 (N–H), 1720 (C=O), 1565 (CNH), 1225 (C=S), 1080 (COC) cm^{-1}. Anal. ($C_7H_{14}N_2O_3S$) C, H, N.

N-(2-Hydroxyethoxymethyl)thiourea—2-Acetoxyethoxymethyl isothiocyanate (2.02 g, 10.5 mmol) was dissolved in 100 mL of methanol saturated with dry ammonia. The solution was placed in a low pressure bomb, stirred at room temperature for 18 h, filtered, and the methanol was removed from the filtrate at 40 °C, giving a pale yellow syrup. Acetone (10 mL) was added, the solution was refrigerated overnight, and crystals were collected, yielding 1.19 g (75%). Recrystallization from 95% ethyl alcohol gave colorless prisms: mp 105–107 °C; ^{1}H NMR (Me_2SO-d_6): δ 3.46, 3.50 (4, 2s, $O(CH_2)_2O$), 4.76 (2, d, J = 6 Hz, OCH_2N), 7.30 (2, br, NH_2), and 8.20 ppm (2, bt, NCH_2); IR (KBr): ν 3500–3100 (OH, NH), 1620 (N–H), 1565 (CNH), 1160 (C=S), 1100 (COC) cm^{-1}. Anal. ($C_4H_{10}N_2O_2S$) C, H, N.

Antimicrobial Activity—Antimicrobial activities were observed first by the agar plate screening method using *Staphylococcus aureus* (A.T.C.C. 6538), *Escherichia coli* (A.T.C.C. 8739), *Pseudomonas aeruginosa* (A.T.C.C. 9027), *Candida albicans* (A.T.C.C. 10231), and *Aspergillus niger* (A.T.C.C. 16404). These organisms represent a gram-positive and two gram-negative bacteria, a yeast, and a mold, respectively. Approximately 10–15 mg of each compound was placed on the inoculated plates. Trypticase Soy Agar was used for bacteria, and Fluid Sabouraud Medium for the yeast and mold. The plates inoculated with *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* were incubated at 37 °C for 24–48 h, and those incubated with *Candida albicans* and *Aspergillus niger* were incubated at 25 °C for 48–72 h. Inocula from the cultures were used without dilution. A zone of inhibition indicated susceptibility of the organism to the compound. The very active compounds had a zone of inhibition >30 mm. A compound was considered quite active if the zone of inhibition was between 20 and 30 mm. A zone of inhibition <20 mm was indicative of slight activity.

Quantitative evaluation was made for the compounds which showed some inhibitory activities in the primary agar plate screening test against any of the five organisms. The serial tube dilution method[15] was chosen for the determination of minimal inhibitory concentrations (Tables III, IV).

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