# Heterocyclic Substituted Ureas. II. Immunosuppressive and Antiviral Activity of Benzothiazole- and Benzoxazoleureas

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The structure–activity relationship of the previously reported immunosuppressive and antiviral benzimidazoleureas has been extended to the analogous benzothiazole- and benzoxazoleureas. The most potent series of immunosuppressant compounds was the 1-(2-naphtho[2,1-*d*]thiazolyl)-3-substituted ureas, one member of which was 250 times as active as azathioprine in the sheep erythrocyte test in mice.

Benzimidazoleureas are potent, nontoxic immunosuppressives as determined by the suppression of the primary immune response to sheep erythrocytes in mice. They also protect mice from several different experimental virus infections.[1] We wish to report an extension of this work to the oxygen and sulfur heterocycles.

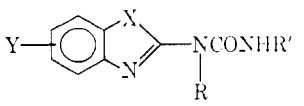
Benzothiazoleureas have been previously reported to be local anesthetics,[2] potential hypoglycemic agents,[3] and antibacterials.[4] Benzoxazolethioureas have been reported as being active against *Mycobacterium tuberculosis in vitro*.[5]

**Chemistry.**—The starting 2-aminobenzothiazoles were either acquired commercially or prepared by cyclization of the corresponding thiourea, using the method of Hugershoff.[6] Tetrahydro-2-aminobenzothiazole was prepared from cyclohexanone and thiourea by the method of Erlenmeyer and Schoenauer.[7] The N-alkyl-substituted 2-aminobenzothiazoles were prepared by autoclaving the appropriate primary amine with 2-chlorobenzothiazole. The 2-aminobenzoxazoles were either acquired commercially or synthesized by cyclization of the appropriate *o*-aminophenol with BrCN.[8]

The 2-aminoheterocycles were treated in an aprotic solvent with an isocyanate or carbamoyl chloride to yield the desired urea.

**Biological Testing.**—The compounds were tested for immunosuppression in the sheep erythrocyte assay in mice and for antiviral activity against Coxsackie A21 (Coe) virus infections in mice, as previously described.[1]

**Immunosuppressive Structure–Activity Relationship.**—The structure–activity relationship here is similar in many ways to that found for the benzimidazoleureas.[1] The inactivity of alkyl derivatives (**16**–**18**, Table I) indicates that R′ must be an aryl group to be active. When Y is H, the best aryl groups for R′ are those that are halogen-substituted, *e.g.*, *p*-chlorophenyl, *m*-chlorophenyl, and *o*-fluorophenyl (**3**, **4**, **9**).




Optimal activity was obtained when substituents were located in the 4 position on the benzothiazole ring; for example, **22**, which has a 4-Cl, is even active when R′ is cyclohexyl.

Conversion of R from H to $CH_3$ decreases the potency to the 50-mg/kg range (**51**–**54**). Reduction of the benzothiazole ring to the tetrahydro compound decreases activity (**55**–**57**). Conversion of X from S to O causes a decrease in activity (**1**, **58**; **2**, **59**; **3**, **60**).

The most potent series results from derivatives in which an additional benzene ring is fused to the benzothiazole ring in the 4,5 or 6,7 position to yield naphthothiazoles (Table III).

**Antiviral Structure–Activity Relationships.**—The antiviral activity appears to be dependent primarily on the nature of R′; if, for example, R′ is 1-naphthyl, the compounds are quite active, with a wide range of substituents for X (**2**, **20**, **24**, **37**, **56**, **62**, **79**). One exception is **52** in which R is changed from H to $CH_3$. This relative lack of activity adds an additional requirement, that R = H.

The antiviral activity seems to require that R′ be an aryl group since practically all aliphatics tested were inactive (**16**, **17**, **18**, **22**, **35**, **50**, **54**, **60**) with the exceptions of the naphthothiazole series in which the aliphatic groups of cyclohexyl (**76**, **81**) and adamantyl (**77**) were active. The naphthothiazoles were the most active group of compounds.

## Experimental Section

Melting points were taken on a Mel-Temp apparatus and are uncorrected. Ir bands, nmr, and titrations were consistent for the proposed structures. All compounds were analyzed for C, H, N and gave results within ±0.4% of the theoretical values. The melting points and formulas are given in Tables I–III found on the following pages.

Generally, the starting amine was checked for solubility in THF, PhMe, or $Me_2CO$. The reaction was usually run in the solvent in which the amine was most soluble. The product was usually recrystallized from mixtures of this solvent and Skelly B until one spot by silica gel tlc in EtOAc.

**1-(2-Benzothiazolyl)-3-phenylurea.**—A solution of 200 ml of dry PhMe containing 10 g (0.067 mole) of 2-aminobenzothiazole and 7.95 g (0.067 mole) of phenyl isocyanate was refluxed and stirred for 4 hr. The cooled solution was filtered to remove the product, which was washed with additional toluene and dried; mp 333–335°, yield 15.3 g.

**1-(4,5,6,7-Tetrahydro-2-benzothiazolyl)-3-cyclohexylurea.**—A mixture of 6.25 g (0.05 mole) of cyclohexyl isocyanate, 5.5 g of $Et_3N$, 9.53 g of 2-amino-4,5,6,7-tetrahydrobenzothiazole hydrochloride, and 200 ml of THF was refluxed and stirred 5 hr. The THF was removed *in vacuo*, and the remaining oil was washed twice with 100 ml of $H_2O$, whereupon it crystallized; the solid was recrystallized from $Me_2CO$–hexane; mp 218–219°, yield 9.0 g.

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(7) H. Erlenmeyer and W. Schoenauer, *Helv. Chim. Acta*, **24**, 1777E (1941).

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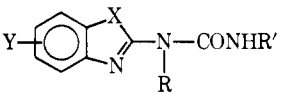
TABLE I

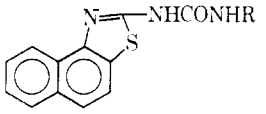
1-(BENZOTHIAZOL-2-YL)- AND 1-(BENZOXAZOL-2-YL)-3-SUBSTITUTED UREAS



| No. | X | Y | R | R′ | Mp, °C | Formula | Coe virus $ED_{50}$, mg/kg | Drug level (mg/kg × 3) for fourfold or greater immunosuppression |
|---|---|---|---|---|---|---|---|---|
| 1 | S | H | H | $C_6H_5$ | 333–335 | $C_{14}H_{11}N_3OS$ | >115 | 6.2 |
| 2 | S | H | H | 1-$C_{10}H_7$ | 278–280 | $C_{18}H_{13}N_3OS$ | 28 | 50.0 |
| 3 | S | H | H | 4-$ClC_6H_4$ | 315–316 | $C_{14}H_{10}ClN_3OS$ | >100 | 6.2 |
| 4 | S | H | H | 2,5-$Cl_2C_6H_3$ | 329–330 | $C_{14}H_{10}Cl_2N_3OS$ | >60 | 1.6 |
| 5 | S | H | H | 2-$ClC_6H_4$ | 335–336 | $C_{14}H_{10}ClN_3OS$ | >100 | 50.0 |
| 6 | S | H | H | 3,4-$Cl_2C_6H_3$ | 316–317 | $C_{14}H_9Cl_2N_3OS$ | 49 | 12.5 |
| 7 | S | H | H | 2,5-$Cl_2C_6H_3$ | 327–328 | $C_{14}H_9Cl_2N_3OS$ | <16 | 12.5 |
| 8 | S | H | H | 4-$FC_6H_4$ | 341–342 | $C_{14}H_{10}FN_3OS$ | 83 | 25.0 |
| 9 | S | H | H | 2-$FC_6H_4$ | 333–333.5 | $C_{14}H_{10}FN_3OS$ | 38 | 6.2 |
| 10 | S | H | H | 4-$NO_2C_6H_4$ | 290–291 | $C_{14}H_{10}N_4O_3S$ | 19 | >100.0 |
| 11 | S | H | H | 3-$NO_2C_6H_4$ | 309–310 | $C_{14}H_{10}N_4O_3S$ | 32 | 50.0 |
| 12 | S | H | H | 2-$NO_2C_6H_4$ | >400 | $C_{14}H_{10}N_4O_3S$ | 120 | 6.2 |
| 13 | S | H | H | 2-$CH_3C_6H_4$ | 335–336 | $C_{15}H_{13}N_3OS$ | 45 | 50.0 |
| 14 | S | H | H | 4-$CH_3C_6H_4$ | 327–328 | $C_{15}H_{13}N_3OS$ | 64 | 100.0 |
| 15 | S | H | H | 3-$CF_3C_6H_4$ | 328–329 | $C_{15}H_{10}F_3N_3OS$ | 52 | 50.0 |
| 16 | S | H | H | $CH_3$ | 272 | $C_9H_9N_3OS$ | >128 | >100.0 |
| 17 | S | H | H | $C_2H_5$ | 331–332 | $C_{10}H_{11}N_3OS$ | 64 | >100.0 |
| 18 | S | H | H | $C_6H_{11}$ | 319–320 | $C_{14}H_{17}N_3OS$ | 159 | 100.0 |
| 19 | S | 4-Cl | H | $C_6H_5$ | 268–270 | $C_{14}H_{10}ClN_3OS$ | 87 | 12.5 |
| 20 | S | 4-Cl | H | 1-$C_{10}H_7$ | 256–258 | $C_{18}H_{12}ClN_3OS$ | <16 | 12.5 |
| 21 | S | 4-Cl | H | 3-$ClC_6H_4$ | 266–268 | $C_{14}H_9Cl_2N_3OS$ | 71 | 50.0 |
| 22 | S | 4-Cl | H | $C_6H_{11}$ | 219–220 | $C_{14}H_{16}ClN_3OS$ | 128 | 1.6 |
| 23 | S | 6-$OCH_3$ | H | $C_6H_5$ | 321–322 | $C_{15}H_{13}N_3O_2S$ | >128 | 25.0 |
| 24 | S | 6-$OCH_3$ | H | 1-$C_{10}H_7$ | 297–298 | $C_{19}H_{15}N_3O_2S$ | 20 | 50.0 |
| 25 | S | 6-$OCH_3$ | H | 4-$ClC_6H_4$ | 310–311 | $C_{15}H_{12}ClN_3O_2S$ | 23 | 50.0 |
| 26 | S | 6-$OCH_3$ | H | 3-$ClC_6H_4$ | 306–307 | $C_{15}H_{12}ClN_3O_2S$ | <16 | 12.5 |
| 27 | S | 6-$OCH_3$ | H | 2-$ClC_6H_4$ | 314–315 | $C_{15}H_{12}ClN_3O_2S$ | 28 | 50.0 |
| 28 | S | 6-$OCH_3$ | H | 2-$FC_6H_4$ | 313–314 | $C_{15}H_{12}FN_3O_2S$ | 26 | 3.1 |
| 29 | S | 6-$OCH_3$ | H | 3-$NO_2C_6H_4$ | 270–272 | $C_{15}H_{12}N_4O_4S$ | 22 | 6.2 |
| 30 | S | 6-$OCH_3$ | H | 2-$NO_2C_6H_4$ | 299–301 | $C_{15}H_{12}N_4O_4S$ | 73 | 6.2 |
| 31 | S | 6-$OCH_3$ | H | 4-$NO_2C_6H_4$ | 278–279 | $C_{14}H_{12}N_4O_4S$ | 54 | 12.5 |
| 32 | S | 6-$OCH_3$ | H | 4-$CH_3C_6H_4$ | 297–298 | $C_{16}H_{15}N_3O_2S$ | 108 | 100.0 |
| 33 | S | 6-$OCH_3$ | H | 3-$CH_3C_6H_4$ | 209–210 | $C_{16}H_{15}N_3O_2S$ | 56 | 50.0 |
| 34 | S | 6-$OCH_3$ | H | 2-$CH_3C_6H_4$ | 311–312 | $C_{16}H_{15}N_3O_2S$ | 19 | 25.0 |
| 35 | S | 6-$OCH_3$ | H | $C_6H_{11}$ | 304–306 | $C_{15}H_{19}N_3O_2S$ | 82 | >100.0 |
| 36 | S | 5,6-$(CH_3)_2$ | H | $C_6H_5$ | 370–372 | $C_{16}H_{15}N_3OS$ | >100 | 100.0 |
| 37 | S | 5,6-$(CH_3)_2$ | H | 1-$C_{10}H_7$ | 355–356 | $C_{20}H_{17}N_3OS$ | 19 | >100.0 |
| 38 | S | 5,6-$(CH_3)_2$ | H | 4-$ClC_6H_4$ | 259–260 | $C_{16}H_{14}ClN_3OS$ | 60 | >100.0 |
| 39 | S | 5,6-$(CH_3)_2$ | H | 3-$ClC_6H_4$ | 363 | $C_{16}H_{14}ClN_3OS$ | 27 | >100.0 |
| 40 | S | 5,6-$(CH_3)_2$ | H | 2-$ClC_6H_4$ | 353–354 | $C_{16}H_{14}ClN_3OS$ | 88 | 100.0 |
| 41 | S | 5,6-$(CH_3)_2$ | H | 3,4-$Cl_2C_6H_3$ | 331–332 | $C_{16}H_{13}Cl_2N_3OS$ | 81 | 50.0 |
| 42 | S | 5,6-$(CH_3)_2$ | H | 4-$FC_6H_4$ | 353–354 | $C_{16}H_{14}FN_3OS$ | 68 | >100.0 |
| 43 | S | 5,6-$(CH_3)_2$ | H | 2-$FC_6H_4$ | 355–356 | $C_{16}H_{14}FN_3OS$ | 28 | 1.6 |
| 44 | S | 5,6-$(CH_3)_2$ | H | 4-$NO_2C_6H_4$ | 324–325 | $C_{16}H_{14}N_4O_3S$ | 27 | 100.0 |
| 45 | S | 5,6-$(CH_3)_2$ | H | 2-$NO_2C_6H_4$ | 328–329 | $C_{16}H_{14}N_4O_3S$ | 64 | 1.6 |
| 46 | S | 5,6-$(CH_3)_2$ | H | 4-$CH_3C_6H_4$ | 349–350 | $C_{17}H_{17}N_3OS$ | 36 | 100.0 |
| 47 | S | 5,6-$(CH_3)_2$ | H | 3-$CH_3C_6H_4$ | 349–350 | $C_{17}H_{17}N_3OS$ | 22 | 25.0 |
| 48 | S | 5,6-$(CH_3)_2$ | H | 2-$CH_3C_6H_4$ | 340–341 | $C_{17}H_{17}N_3OS$ | 19 | 25.0 |
| 49 | S | 2,5-$(CH_3)_2$ | H | 2,5-$Cl_2C_6H_3$ | 360–361 | $C_{16}H_{13}Cl_2N_3OS$ | 17 | 12.5 |
| 50 | S | 5,6-$(CH_3)_2$ | H | $C_6H_{11}$ | 351–352 | $C_{16}H_{21}N_3OS$ | 193 | 100.0 |
| 51 | S | H | $CH_3$ | $C_6H_5$ | 95 | $C_{15}H_{13}N_3OS$ | >128 | 50.0 |
| 52 | S | H | $CH_3$ | 1-$C_{10}H_7$ | 174–175 | $C_{19}H_{15}N_3OS$ | >128 | 50.0 |
| 53 | S | H | $CH_3$ | 4-$ClC_6H_4$ | 140–141 | $C_{15}H_{12}ClN_3OS$ | 85 | 50.0 |
| 54 | S | H | $CH_3$ | $C_6H_{11}$ | 118–119 | $C_{15}H_{19}N_3OS$ | >128 | 50.0 |
| 55 | S | Tetrahydro | H | $C_6H_5$ | 318–319 | $C_{14}H_{15}N_3OS$ | >128 | >100.0 |
| 56 | S | Tetrahydro | H | 1-$C_{10}H_7$ | 257–258 | $C_{18}H_{17}N_3OS$ | 32 | 50.0 |
| 57 | S | Tetrahydro | H | $C_6H_{11}$ | 218–219 | $C_{14}H_{21}N_3OS$ | >128 | 50.0 |
| 58 | O | H | H | $C_6H_5$ | 192–193 | $C_{14}H_{11}N_3O_2$ | 31 | 50.0 |
| 59 | O | H | H | 1-$C_{10}H_7$ | 232–235 | $C_{18}H_{13}N_3O_2$ | 20 | >100.0 |
| 60 | O | H | H | $C_6H_{11}$ | 162–163 | $C_{14}H_{17}N_3O_2$ | 95 | >100.0 |

TABLE II

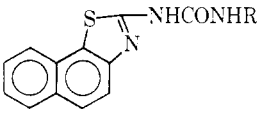
1-(2-NAPHTHO[1,2-*d*]THIAZOLYL)-3-SUBSTITUTED UREAS



| No. | R | Mp, °C | Formula | Cor virus $ED_{50}$, mg/kg | Drug level (mg/kg × 3) for fourfold or greater immunosuppression |
|---|---|---|---|---|---|
| 61 | $C_6H_5$ | 320–322 | $C_{18}H_{13}N_3OS$ | >100 | 12.5 |
| 62 | 1-$C_{10}H_7$ | 269–270 | $C_{22}H_{15}N_3OS$ | 42 | 3.1 |
| 63 | 4-$ClC_6H_4$ | 310–311 | $C_{18}H_{12}ClN_3OS$ | 28 | 100.0 |
| 64 | 3-$ClC_6H_4$ | 314–315 | $C_{18}H_{12}ClN_3OS$ | 21 | 1.6 |
| 65 | 2-$ClC_6H_4$ | 321–322 | $C_{18}H_{12}ClN_3OS$ | 49 | 6.2 |
| 66 | 2,5-$Cl_2C_6H_3$ | 315–316 | $C_{18}H_{11}Cl_2N_3OS$ | 55 | 100.0 |
| 67 | 4-$FC_6H_4$ | 308–309 | $C_{18}H_{12}FN_3OS$ | 16 | 12.5 |
| 68 | 2-$FC_6H_4$ | 318–319 | $C_{18}H_{12}FN_3OS$ | <16 | 3.1 |
| 69 | 4-$NO_2C_6H_4$ | 293–294 | $C_{18}H_{12}N_4O_3S$ | 17 | 50.0 |
| 70 | 3-$NO_2C_6H_4$ | 264–265 | $C_{18}H_{12}N_4O_3S$ | <16 | 3.1 |
| 71 | 2-$NO_2C_6H_4$ | 303–304 | $C_{18}H_{12}N_4O_3S$ | <16 | 3.1 |
| 72 | 3,4-$Cl_2C_6H_3$ | 305–306 | $C_{18}H_{11}Cl_2N_3OS$ | 18 | 3.1 |
| 73 | 4-$CH_3C_6H_4$ | 307–308 | $C_{19}H_{15}N_3OS$ | 17 | 6.2 |
| 74 | 3-$CH_3C_6H_4$ | 317–318 | $C_{19}H_{15}N_3OS$ | 23 | 3.1 |
| 75 | 2-$CH_3C_6H_4$ | 319–320 | $C_{19}H_{15}N_3OS$ | 22 | 1.6 |
| 76 | $C_6H_{11}$ | 245–246 | $C_{18}H_{19}N_3OS$ | 34 | 3.1 |
| 77 | Adamantyl | 242–243 | $C_{22}H_{23}N_3OS$ | 18 | 3.2 |

TABLE III

1-(2-NAPHTHO[2,1-*d*]THIAZOLYL)-3-SUBSTITUTED UREAS



| No. | R | Mp, °C | Formula | Cor virus $ED_{50}$, mg/kg | Drug level (mg/kg × 3) for fourfold or greater immunosuppression |
|---|---|---|---|---|---|
| 78 | $C_6H_5$ | 353–354 | $C_{18}H_{13}N_3OS$ | 23 | 0.8 |
| 79 | 1-$C_{10}H_7$ | 346–347 | $C_{22}H_{15}N_3OS$ | <16 | 0.4 |
| 80 | 4-$CH_3C_6H_4$ | 369–370 | $C_{19}H_{15}N_3OS$ | <16 | >25.0 |
| 81 | $C_6H_{11}$ | 354–355 | $C_{18}H_{19}N_3OS$ | 27 | >12.5 |

# Potential Coenzyme Inhibitors. III.[1] Some Reactions of Substituted Nicotinamide and Dihydronicotinamide Derivatives

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The reaction between KCN and substituted nicotinamide derivatives was examined, and a number of cyanide derivatives have been isolated. The spectroscopic evidence shows that $CN^-$ addition occurs at the 4 position of the pyridine ring. Equilibrium constants for these reactions have been calculated from the absorption spectra, and the influence exerted by the 4-Me substituent upon the rate of addition is discussed. The H-transfer reactions between 2,6-dichlorophenolindophenol and some substituted dihydronicotinamide derivatives were examined by visible absorption spectroscopy. Rate constants for the oxidation reactions at different $H^+$ concentrations were calculated. The reaction rates have been related to the effects of the substituents attached to the nicotinamide ring.

The glycolytic pathway of carbohydrate metabolism involves an oxidative step in which glyceraldehyde phosphate is converted into diphosphoglyceric acid. In this reaction the pyridine ring of the cofactor (NAD, I) accepts an H atom in the $\beta$ configuration giving NADH (II).[2] Cancer cells are relatively deficient in NAD and this coenzyme must be regenerated from NADH if continuous energy production is to be maintained. This is achieved by the reduction of pyruvate

(1) Previous paper in this series: A. C. Lovesey and W. C. J. Ross, *J. Chem. Soc., B*, 192 (1969).

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