Aust. J. Chem., 1981, **34**, 2423–9

Unfused Heterobicycles as Amplifiers of Phleomycin. III† Thiazolylpyridines and Bipyrimidines with Strongly Basic Side Chains

Desmond J. Brown,[A] *Bonnie B. Buttler,*[A] *William B. Cowden,*[A] *Geoffrey W. Grigg,*[B] *Diana Kavulak*[B] *and Denis M. Podger*[B]

[A] John Curtin School of Medical Research,
P.O. Box 334, Canberra City, A.C.T. 2601.
[B] Molecular and Cellular Biology Unit, CSIRO,
P.O. Box 184, North Ryde, N.S.W. 2113.

Abstract

Syntheses are described for 4-(thiazol-4′-yl)pyridines, each 2′-substituted by a dialkylaminoalkyl, dialkylaminoalkylthio or dialkylaminoalkylamino side chain; for 2- and 3-(thiazol-4′-yl)pyridines with a 2′-dimethylaminoethylthio substituent; for 4-(thiazol-2′-yl)pyridines with either a 4′- or a 5′-dialkylaminoalkylcarbamoyl substituent; and for some analogous compounds. The above and similarly substituted 2,4′-, 4,5′- and 5,5′-bipyrimidines have been screened for activity as amplifiers of phleomycin-G against *Escherichia coli B* by an improved *in vitro* procedure. The results are tabulated and discussed.

Introduction

Several systems of unfused heterobicycles, which bore alkyl, alkylthio or amino substituents, have shown encouraging *in vitro* activities as amplifiers of the tumorostatic antibiotic, phleomycin.[1] In order to improve aqueous solubility, a prerequisite for effective testing, and to mimic more closely the important bithiazole portion[2] of phleomycin in its amplifiers, we have now prepared a variety of thiazolylpyridines with dialkylaminoalkyl side chains attached either directly or through a sulfide, secondary amino or amide linkage. The amplifying activities of these, and of some related bipyrimidines recently prepared,[3,4] have been determined against *in vitro* cultures of *Escherichia coli* by a new procedure, simpler and less time-consuming than that[5] used previously.

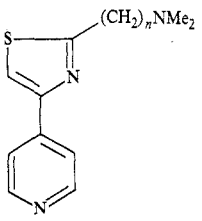
Syntheses

Thiazolylpyridines with a directly attached basic side chain were made by primary synthesis of the thiazole ring from fragments with the pyridine and side chain already attached. Thus 4-bromoacetylpyridinium bromide and 2-dimethylamino(thioacetamide) gave the required amine (1; $n = 1$) and its homologues (1; $n = 2$ or 3)

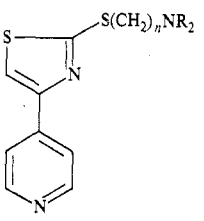
† Part II, *Aust. J. Chem.*, 1981, **34**, 1353.

[1] Brown, D. J., Cowden, W. B., Grigg, G. W., and Kavulak, D., *Aust. J. Chem.*, 1980, **33**, 2291.
[2] Takita, T., Muraoka, Y., Nakatani, T., Fujii, A., Umezawa, Y., Naganawa, H., and Umezawa, H., *J. Antibiot.*, 1978, **31**, 801; Grollman, A. P., and Takeshita, M., *Adv. Enzyme Regul.*, 1980, **18**, 67.
[3] Brown, D. J., Cowden, W. B., and Strekowski, L., *Aust. J. Chem.*, 1981, **34**, 1353.
[4] Brown, D. J., and Strekowski, L., *Aust. J. Chem.*, 1981, **34**, 1157.
[5] Angyal, A. M., Grigg, G. W., Badger, R. J., Brown, D. J., and Lister, J. H., *J. Gen. Microbiol.*, 1974, **85**, 163.

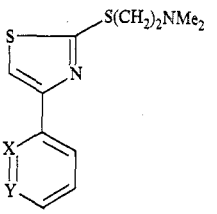
were made similarly from the corresponding thiopropionamide and thiobutyramide, respectively.



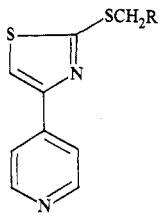
(1)



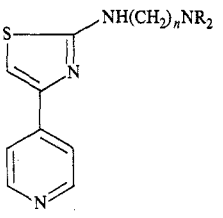
(2)



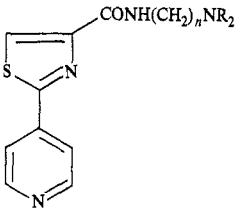
(3)



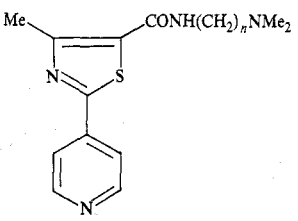
(4)



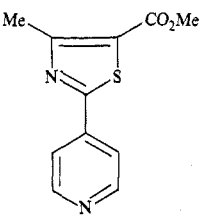
(5)



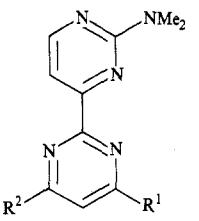
(6)



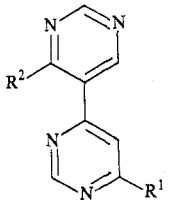
(7)



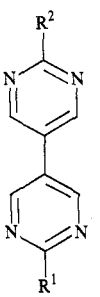
(8)



(9)



(10)



(11)

Analogues with the side chain attached through sulfur were prepared by alkylation of appropriate heterobicyclic thiones: 4-(pyridin-4′-yl)thiazole-2(3*H*)-thione[1] underwent *S*-alkylation by 2-chloro-*N*,*N*-dimethylethylamine to give the product (2; $n = 2$, R = Me) and similar reactions gave the isomers (3; X = N, Y = CH) and (3; X = CH, Y = N), the homologues (2; $n = 2$, R = Et or H) and (2; $n = 3$, R = Me), and the analogues (3; X = Y = CH) and (4; R = CH_2OH or CO_2H).

To prepare examples with the side chain attached through an NH linkage, aminolysis of 4-(pyridin-4′-yl)thiazol-2(3*H*)-thione or its *S*-methyl derivative proved ineffective. However, oxidation of the latter to the corresponding sulfone provided a satisfactory substrate for reactions with dialkylaminoalkylamines to furnish the amplifiers (5; $n = 2$, R = Me or Et) and (5; $n = 3$, R = Me).

Two series of compounds with amide-linked side chains were prepared. The known[6] ethyl 2-(pyridin-4′-yl)thiazole-4-carboxylate was converted with 2-(dimethylamino)ethylamine or related amines into the corresponding 4-carboxamides (6; $n = 2$, R = Me or Et) and (6; $n = 3$, R = Et). Similar 5-carboxamides (7; $n = 2$ or 3) were made by condensation of pyridine-4-carbothioamide with methyl 2-chloroacetoacetate to yield methyl 4-methyl-2-(pyridin-4′-yl)thiazole-5-carboxylate (8) followed by aminolyses.

Biological Activities

Because of an excessive time requirement and other practical difficulties, our former method,[5] for evaluating activity as a phleomycin amplifier, has been replaced by a more simple and reliable procedure (see Experimental). Thus the activity of each compound as an amplifier of phleomycin was measured by a growth inhibition assay of wild-type bacterial cells of *Escherichia coli B*. A small volume of phleomycin solution placed at the centre of an agar plate diffused radially toward the edge and a concentration gradient was so established. Growth of cells placed on the agar surface occurred in the region of sub-toxic levels of phleomycin but were inhibited at a concentration sufficient to prevent cell division or cause cell death: inhibition was observed as a circular zone centred at the point of phleomycin application and the area of the zone gave a measure of the toxicity of phleomycin. The addition of compounds which had amplifier activity, inhibited cell growth at a lower phleomycin concentration than phleomycin alone and thus gave a larger zone of inhibition. To avoid confusion, results in Table 1 have been given in stars (*) as distinct from the pluses (+) used hitherto.

Unlike their simpler analogues,[1] the 4-(2′-dialkylaminoalkylthiothiazol-4′-yl)pyridines (2; R = Me or Et) showed 5-star activity which was reduced to 3- or 4-star when the terminal alkyl groups were removed (2; $n = 2$, R = H). A similar reduction was observed when the pyridine ring was attached at its 2- or 3-position (3; X or Y = N), or when the pyridine was replaced by a benzene ring (3; X = Y = CH). When the terminal amino group was replaced by an hydroxy (4; R = CH_2OH) or a carboxy group (4; R = CO_2H), a reduction to 2- and 1-star activity, respectively, resulted.

The two available analogues with side chain attached through an NH linkage (5; $n = 2$ or 3, R = Me), showed 5- and 4-star activity, respectively, but when an amide linkage was used, activities were 3- or 4-star according to whether attachment was to the 4′-position (6; $n = 2$ or 3) or to the 5′-position (7; $n = 2$ or 3). Surprisingly, the simple ester (8) showed 4-star activity although its survival *in vivo* would be very doubtful. Attachment of the dialkylaminoalkyl chain directly to the thiazole ring (1; $n = 1$–3) produced a progressive increase from 1- to 3-star activity as the chain was lengthened.

[6] Benko, A., and Levente, A., *Justus Liebigs Ann. Chem.*, 1968, **717**, 148.

The simple bipyrimidine derivatives (12)–(15) (for identities see Table 1, footnote[B]) proved virtually inactive. Attachment of dimethylaminoethylamino side chains caused an improvement, marginal in the 5,5′-bipyrimidine (11) system (1- or 2-star activity) but more marked in the 2,4′-bipyrimidine (9) and 4,5′-bipyrimidine (10) systems (2- or 3-star activity). A dramatic rise to 5-star activity was achieved only by the insertion of two sulfide-linked groups [10; $R^1 = R^2 = S(CH_2)_2NMe_2$].

Table 1. Activities as amplifiers of phleomycin

Measured at 3·3 mM; for details see Experimental section. Activities in square brackets were determined by the old method (see ref.[5]). Syntheses of the compounds: (1)–(8), this paper; (9)–(13), ref.[3]; (14) and (15), ref.[4]

Compound	Activity	Compound	Activity
(1; $n = 1$)	**	(6); $n = 3$, R = Me)	***
(1; $n = 2$)	***	(7; $n = 2$)	****
(1; $n = 3$)	****	(7; $n = 3$)	****
(2; $n = 2$, R = H)	****	(8)	****
(2; $n = 2$, R = Me)	*****	(9; $R^1 = NH(CH_2)_2NMe_2$, $R^2 = NMe_2$)	***
(2; $n = 2$, R = Et)	*****	(9; $R^1 = S(CH_2)_2NMe_2$, R^2 = Me)	[**]
(2; $n = 3$, R = Me)	*****	(10; $R^1 = R^2 = S(CH_2)_2NMe_2$)	*****
(3; X = N, Y = CH)	***	(10; $R^1 = R^2 = NH(CH_2)_2NMe_2$)	**
(3; X = CH, Y = N)	****	(10; R^1 = SMe, $R^2 = NH(CH_2)_2NMe_2$)	***
(3; X = Y = CH)	****	(11; $R^1 = R^2 = NH(CH_2)_2NMe_2$)	[**]
(4; R = CH_2OH)	[**]	(11; $R^1 = NMe_2$, $R^2 = NH(CH_2)_2NMe_2$)	[*]
(4; R = CO_2H)	[*]	(11; $R^1 = NH(CH_2)_2NMe_2$, R^2 = SMe)	[*]
(5; $n = 2$, R = Me)	*****	(11; $R^1 = NH(CH_2)_2NMe_2$, R^2 = OMe)	*
(5; $n = 2$, R = Et)	—[A]	(12)[B]	[*]
(5; $n = 3$, R = Me)	****	(13)[B]	[0]
(6; $n = 2$, R = Me)	***	(14)[B]	[0]
(6; $n = 2$, R = Et)	***	(15)[B]	[*]

[A] Solubility too low for measurement.

[B] Compound (12) = 2′-methoxy-1,3-dimethyl-2-oxodihydro-5,5′-bipyrimidinium iodide; (13) = *N*,*N*-dimethyl-2-(5′-bromopyrimidin-4′-ylthio)ethylamine; (14) = 2,4′,6-trimethoxy-1′-methyl-4,5′-bipyrimidin-2′(1′*H*)-one; and (15) = 1,1′,3,3′-tetramethyl-4,5′-bipyrimidine-2,2′,4′,6(1*H*,1′*H*,3*H*,3′*H*)-tetraone.

Experimental

Analyses were done in the Australian National University Analytical Services Unit. Melting points were uncorrected. Unless otherwise indicated, n.m.r. spectra (chemical shifts in δ; J values in Hz) were measured at 60 MHz and 35° in $CDCl_3$ against Me_4Si as internal standard.

N,N-*Dimethyl[4-(pyridin-4′-yl)thiazol-2-yl]methylamine* (*1*; n = 1) *and Homologues*

4-Bromoacetylpyridinium bromide[7] (4·22 g), 2-dimethylamino(thioacetamide) hydrochloride[8] (2·31 g) and methanol (30 ml) were heated under reflux for 8 h. The residue from evaporation was triturated with ethanol (20 ml) and the remaining solid was added to 2 M sodium hydroxide (15 ml). Extraction with ether (3 × 30 ml), followed by evaporation of the dehydrated extract, gave a solid base which was dissolved in ethanol (10 ml) and mixed with 5% ethanol/hydrogen bromide (10·5 ml): after warming the mixture, refrigeration gave the *product* (1; $n = 1$) as *dihydrobromide* (16%), m.p. 260–261° (from methanol) (Found: C, 34·5; H, 4·1; N, 10·8; S, 8·1. $C_{11}H_{15}Br_2N_3S$ requires C, 34·7; H, 4·0; N, 11·0; S, 8·4%).

[7] Schultz, O.-E., and Weber, H., *Arch. Pharm.* (*Weinheim, Ger.*), 1972, **305**, 248.

[8] Sallay, S., U.S. Pat. 3,474,100 (1969) (*Chem. Abstr.*, 1970, **72**, 3506).

Similar treatment of the 4-bromoacetylpyridinium bromide (15 mmol) with an equimolar quantity of 3-dimethylamino(thiopropionamide) hydrochloride[8] or 4-dimethylamino(thiobutyramide) hydrochloride (preparation below) gave, respectively, N,N-*dimethyl-2-[4′-(pyridin-4″-yl)thiazol-2′-yl]ethylamine* (1; $n = 2$) as *dihydrobromide* (41%), m.p. 206–208° (Found: C, 36·6; H, 4·3; N, 10·5; S, 8·2. $C_{12}H_{17}Br_2N_3S$ requires C, 36·5; H, 4·3; N, 10·6; S, 8·1%) [n.m.r. 8·61, m, H2″,6″; 7·70, m, H3″,5″; 7·50, s, H5′; 3·22, t, 2-CH_2; 2·69, t, 1-CH_2; 2·30, s, Me_2] and N,N-*dimethyl-3-[4′-(pyridin-4″-yl)thiazol-2′-yl]propylamine* (1; $n = 3$) as *dihydrobromide* (49%), m.p. 274–276° (from methanol/ethanol) (Found: C, 38·2; H, 4·7; N, 10·2; S, 7·9. $C_{13}H_{19}Br_2N_3S$ requires C, 38·2; H, 4·7; N, 10·3; S, 7·8%).

4-Dimethylamino(thiobutyramide) Hydrochloride

4-Dimethylaminobutyronitrile[9] (5·6 g), thioacetamide (7·5 g) and dimethylformamide (30 ml: presaturated with hydrogen chloride) were heated on the steam bath for 45 min. After cooling, the mixture was agitated vigorously with ether (6 × 50 ml) and the semi-solid residue was triturated with a little propan-2-ol to induce crystallization. The *thiocarboxamide hydrochloride* (57%) had m.p. 115–117° (from ethanol) (Found: C, 39·3; H, 8·2; N, 15·3; S, 17·4. $C_6H_{15}ClN_2S$ requires C, 39·4; H, 8·3; N, 15·3; S, 17·6%) (cf. general method[8]).

N,N-*Dimethyl-2-[4′-(pyridin-4″yl)thiazol-2′-ylthio]ethylamine* (*2*; n = 2, R = Me), *Isomers and Analogues*

4-(Pyridin-4′-yl)thiazol-2(3*H*)-thione hydrobromide[1] (10 mmol), dissolved in the minimal volume of 2 M sodium hydroxide, was stirred at 20° while commercial 2-chloro-*N,N*-dimethylethylamine hydrochloride (12 mmol) was added. After adjusting the solution to pH 9–10 with more sodium hydroxide, it was stirred at 20° for a further 10 min and then at *c.* 40° for 20 min. The cooled solution was adjusted to pH 11–12 prior to ether extraction (3 × 40 ml). Evaporation of the dehydrated extracts gave an oily base which was weighed, dissolved in ethanol (20 ml), and added to hydrogen bromide (2 equiv.) in ethanol (15 ml). The mixture was warmed to attain homogeneity and then chilled to yield the *product* (2; $n = 2$, R = Me) as *dihydrobromide* (59%), m.p. 223–225° (from propan-2-ol/methanol) (Found: C, 33·7; H, 4·0; N, 9·8; S, 15·2. $C_{12}H_{17}Br_2N_3S_2$ requires C, 33·7; H, 4·0; N, 9·8; S, 15·0%). N.m.r. (free base) 8·58, m, H2″,6″; 7·69, m, H3″,5″; 7·54, s, H5′; 3·45, t, SCH_2; 2·73, t, NCH_2; 2·33, s, Me_2.

A similar procedure, using appropriate thiones[1] and chloroalkylamines, gave N,N-*dimethyl-2-[4′-(pyridin-2″-yl)thiazol-2′-ylthio]ethylamine* (3; X = N, Y = CH) as *dihydrobromide* (37%), m.p. 215–217° (from ethanol) (Found: C, 33·7; H, 4·1; N, 9·6; S, 15·3%); N,N-*dimethyl-2-[4′-(pyridin-3″-yl)thiazol-2′-ylthio]ethylamine* (3; X = CH, Y = N) as *dihydrobromide* (75%), m.p. 217–218° (from ethanol) (Found: C, 33·8; H, 4·0; N, 9·7; S, 15·2%); N,N-*diethyl-2-[4′-(pyridin-4″-yl)thiazol-2′-ylthio]ethylamine* (2; $n = 2$, R = Et) as *dihydrobromide* (70%, after evaporation to half volume), m.p. 209–211° (from ethanol) (Found: C, 36·6; H, 4·7; N, 9·0; S, 14·1. $C_{14}H_{21}Br_2N_3S_2$ requires C, 36·9; H, 4·6; N, 9·2; S, 14·1%); *2-[4′-pyridin-4″-yl)thiazol-2′-ylthio]ethylamine* (2; $n = 2$, R = H) as *dihydrobromide* (70%), m.p. 264–265° (from ethanol) (Found: C, 30·3; H, 3·4; N, 10·4; S, 15·8. $C_{10}H_{13}Br_2N_3S_2$ requires C, 30·1; H, 3·3; N, 10·5; S, 16·1%); and N,N-*dimethyl-3-[4′-(pyridin-4″-yl)thiazol-2′-ylthio]propylamine* (2; $n = 3$, R = Me) as *dihydrobromide* (28%), m.p. 226–228° (from propan-2-ol) (Found: C, 35·4; H, 4·3; N, 9·3; S, 14·6. $C_{13}H_{19}Br_2N_3S_2$ requires C, 35·4; H, 4·3; N, 9·5; S, 14·5%).

Likewise, 4-phenylthiazole-2(3*H*)-thione[10] gave the oily base (3; X = Y = CH) (87%) (n.m.r. 7·53, m, Ph; 7·15, s, H5′; 3·35, t, SCH_2; 2·63, t, NCH_2; 2·19, s, Me_2), which was converted by treatment with ethanolic hydrogen bromide (1 equiv.) into N,N-*dimethyl-2-(4′-phenylthiazol-2′-ylthio)ethylamine hydrobromide*, m.p. 158–159° (from ethanol) (Found: C, 45·1; H, 4·9; N, 8·0; S, 18·5. $C_{13}H_{17}BrN_2S_2$ requires C, 45·2; H, 5·0; N, 8·1; S, 18·6%).

2-[4′-(Pyridin-4″-yl)thiazol-2′-ylthio]ethanol (*4*; $R = CH_2OH$)

2-Bromoethanol (0·88 g) was added to a solution of 4-(pyridin-4′-yl)thiazole-2(3*H*)-thione hydrobromide[1] (1·37 g) in the minimal volume of 2 M sodium hydroxide and the mixture was stirred

[9] Lespagnol, A., Cheymol, J., Cuingnet, E., Debaert, M., Adolphe, M., and Adolphe, C., *Congr. Sci. Pharm.*, 1959, 194 (*Chem. Abstr.*, 1962, **56**, 5830).
[10] Emerson, W. S., and Patrick, T. M., *J. Org. Chem.*, 1948, **13**, 722.

at *c.* 20° for 1 h. Filtration gave the *alcohol* (67%), m.p. 129–130° (from aqueous methanol) (Found: C, 50·6; H, 4·2; N, 11·7; S, 26·9. $C_{10}H_{10}N_2OS_2$ requires C, 50·4; H, 4·2; N, 11·8; S, 26·9%). N.m.r. 8·59, m, H 2″,6″; 7·65, m, H 3″,5″; 7·55, s, H 5′; 4·04, t, OCH_2; 3·79, s, OH; 3·46, t, SCH_2.

2-['4-(Pyridin-4″-yl')thiazol-2′-ylthio]acetic Acid (4; R = CO_2H)

Chloroacetic acid (0·60 g) was added to a solution of 4-(pyridin-4′-yl)thiazole-2(3*H*)-thione hydrobromide[1] (1·37 g) in the minimal volume of 1 M sodium hydroxide. After re-establishing solution with a little more alkali, the mixture was maintained at 60° for 20 min and then cooled. Acidification to pH 3 gave the *carboxylic acid* (63%), m.p. 256–258° (dec.) (from methanol) (Found: C, 47·7; H, 3·3; N, 10·8. $C_{10}H_8N_2O_2S_2$ requires C, 47·6; H, 3·2; N, 11·1%).

4-(2′-Methylsulfonylthiazol-4′-yl)pyridine

Adjustment of a concentrated aqueous solution of its hydrobromide[1] to pH 8 gave *4-(2′-methylthiothiazol-4′-yl)pyridine*, m.p. 55° (from cyclohexane) (Found: C, 52·0; H, 3·9; N, 13·7; S, 30·0. $C_9H_8N_2S_2$ requires C, 51·9; H, 3·9; N, 13·5; S, 30·7%).

Hydrogen peroxide (27% w/w; 0·75 g) was added during 30 min to a stirred solution of the above base (0·62 g) in trifluoroacetic acid (10 ml) at 25° and stirring was continued for a further 1 h. Then more hydrogen peroxide (0·40 g) was added as before and stirring was continued for 2 h. The residue from evaporation under reduced pressure was diluted with water (5 ml) and adjusted to pH 7–8 with saturated aqueous sodium hydrogen carbonate. Filtration gave the *sulfone* (86%), m.p. 168–169° (from methanol) (Found: C, 45·1; H, 3·6; N, 11·5; S, 26·8. $C_9H_8N_2O_2S_2$ requires C, 45·0; H, 3·4; N, 11·7; S, 26·8%). N.m.r. 8·71, m, H 2,6; 7·78, m, H 3,5; 8·02, s, H 5′; 3·39, s, Me.

N-(2″-Dimethylaminoethyl)-4-(pyridin-4′-yl)thiazol-2-amine (5; n = 2, R = Me) and Homologues

The above sulfone (0·72 g) and 2-(dimethylamino)ethylamine (5 ml) were heated under reflux for 3 h. The residue from evaporation under reduced pressure was suspended in water (10 ml). Adjustment to pH 11, extraction with ether (3 × 20 ml), and evaporation of the dehydrated extract gave the *amine* (5; *n* = 2, R = Me) (74%), m.p. 92–93° (from cyclohexane) (Found: C, 58·1; H, 6·6; N, 22·8; S, 12·7. $C_{12}H_{16}N_4S$ requires C, 58·0; H, 6·5; N, 22·6; S, 12·9%). N.m.r. 8·55, m, H 2′,6′; 7·64, m, H 3′,5′; 6·90, s, H 5; 5·92, s, br, NH; 3·41, q, 1″-CH_2; 2·56, t, 2″-CH_2; 2·27, Me_2.

Similarly, by using appropriate dialkylaminoalkylamines, were prepared *N-(2″-diethylaminoethyl)-4-(pyridin-4′-yl)thiazol-2-amine* (5; *n* = 2, R = Et) (54%), m.p. 81° (from light petroleum) (Found: C, 60·6; H, 7·1; N, 20·2; S, 11·4. $C_{14}H_{20}N_4S$ requires C, 60·8; H, 7·3; N, 20·3; S, 11·6%) and *N-(3″-dimethylaminopropyl)-4-(pyridin-4′-yl)thiazol-2-amine* (5; *n* = 3, R = Me) (76%), m.p. 91–92° (from cyclohexane) (Found: C, 59·4; H, 7·0; N, 21·5; S, 12·0. $C_{13}H_{18}N_4S$ requires C, 59·5; H, 6·9; N, 21·4; S, 12·2%).

N-(2″-Dimethylaminoethyl)-2-(pyridin-4′-yl)thiazol-4-carboxamide (6; n = 2, R = Me) and Homologues

Ethyl 2-(pyridin-4′-yl)thiazole-4-carboxylate[6] (1·0 g) and 2-(dimethylamino)ethylamine (5 ml) were heated under reflux for 6 h. The (weighed) residue from evaporation was dissolved in ethanol (10 ml) and mixed with hydrogen bromide (2 equiv.) in ethanol (10 ml). The mixture was warmed and then chilled to give the *carboxamide* (6; *n* = 2, R = Me) as *dihydrobromide* (53%), m.p. 275–277° (from methanol) (Found: C, 35·5; H, 4·5; N, 12·5; S, 7·2. $C_{13}H_{20}Br_2N_4OS$ requires C, 35·4; H, 4·6; N, 12·7; S, 7·3%). N.m.r. (base in $CDCl_3$ with D_2O) 8·72, m, H 2′,6′; 7·79, m, H 3′,5′; 8·20, s, H 5; 3·51, t, 1″-CH_2; 2·52, t, 2″-CH_2; 2·32, s, Me_2.

Similar treatment of the same ester with appropriate diamines gave *N-(2″-diethylaminoethyl)-2-(pyridin-4′-yl)thiazole-4-carboxamide* (6; *n* = 2, R = Et) as *dihydrobromide* (50%), m.p. 256–258° (from methanol) (Found: C, 38·7; H, 4·9; N, 11·7; S, 7·1. $C_{15}H_{24}Br_2N_4OS$ requires C, 38·5; H, 5·2; N, 12·0; S, 6·9%) and *N-(3″-dimethylaminopropyl)-2-(pyridin-4′-yl)thiazole-4-carboxamide* (6; *n* = 3, R = Me) as *dihydrobromide* (77%), m.p. 284–286° (Found: C, 37·2; H, 4·7; N, 12·2; S, 7·2. $C_{14}H_{22}Br_2N_4OS$ requires C, 37·0; H, 4·9; N, 12·3; S, 7·1%).

Methyl 4-Methyl-2-(pyridin-4'-yl)thiazole-5-carboxylate (*8*)

Pyridine-4-carbothioamide[11] (8·0 g), methyl 2-chloroacetoacetate (21·0 g) and ethanol (60 ml) were heated under reflux for 8 h. After concentration in an open vessel, chilling gave the *thiazole ester hydrochloride* (74%), m.p. 205–206° (from ethanol) (Found: C, 49·3; H, 4·4; N, 10·3. $C_{11}H_{11}ClN_2O_2S$ requires C, 48·8; H, 4·1; N, 10·3%). N.m.r. [$(CD_3)_2SO$] 8·89, m, H2′,6′; 8·30, m, H3′,5′; 3·80, s, OMe; 2·70, s, 4-Me.

N-(*2″-Dimethylaminoethyl*)- *and* N-(*3″-Dimethylaminopropyl*)-*4-methyl-2-(pyridin-4′-yl)thiazole-5-carboxamide* (*7*; n = *2 or 3*)

The above ester hydrochloride (4·0 g) and 2-(dimethylamino)ethylamine (30 ml) were boiled under reflux for 4 h. Evaporation gave a thick oil which was extracted with boiling cyclohexane: concentration and chilling gave the *carboxamide* (7; $n = 2$) (70%), m.p. 95–97° (from cyclohexane) (Found: C, 58·1; H, 6·4; N, 19·2. $C_{14}H_{18}N_4OS$ requires C, 57·9; H, 6·3; N, 19·3%). N.m.r. 8·55, m, H2′,6′; 7·63, m, H3′,5′; 6·83, s, br, NH; 3·50, m, 1″-CH_2; 2·68, s, 4′-Me; 2·52, t, 2″-CH_2; 2·26, s, NMe_2.

A similar reaction with 3-(dimethylamino)propylamine gave the *carboxamide* (7; $n = 3$) (64%), m.p. 106–108° (from cyclohexane) (Found: C, 58·9; H, 6·5; N, 18·2. $C_{15}H_{20}N_4OS$ requires C, 59·2; H, 6·6; N, 18·4%). N.m.r. 8·59, m, H2′,6′; 7·72, m, H3′,5′; 3·53, m, 1″-CH_2; 2·66, s, 4-Me; 2·42, t, 3″-CH_2; 2·26, s, NMe_2; 1·75, m, 2″-CH_2.

Biological Evaluation

Concentration gradients of phleomycin-G[12] were set up on blood–agar–base plates thus: A well (5 mm diameter) was sucked out of the centre of each plate and each well was plugged with soft overlay agar (0·6% agar in normal saline; 20 μl) to seal the interface between plastic dish and agar. When the soft agar had solidified, phleomycin-G [100 μg/ml in a glucose-salts (GT) buffer, pH 7·4; 40 μl] was dispensed into each well and the plates were kept at 37° for 18 h.

Test tubes, each containing molten soft overlay agar (6 ml), were allowed to reach 46°. Into each tube were dispensed (i) an appropriate concentration of the potential amplifier under test (200 μl) and (ii) a stationary phase culture of *Escherichia coli B* grown in 'Difco' nutrient broth (120 μl). After thoroughly mixing the contents of each tube, a sample (5 ml) from each was pipetted onto the surface of each plate: by commencing at the perimeter and allowing the mixture to drain slowly from the pipette while spiralling gradually to the central region of the plate, it proved possible to obtain a level overlay without upsetting the phleomycin concentration gradient.

Plates were then incubated overnight at 37° and the area of each zone of inhibition was measured on an 'Artek' automatic colony counter.

The ratio, inhibition area with amplifier to inhibition area without amplifier, indicated the effectiveness of amplification. With 3·3 mM amplifiers, the ratio varied from *c.* 1·0 (slight or no amplification) to *c.* 3·5 (powerful amplification). In the present paper, these ratios (1·0–1·5 = *; 1·5–2·0 = **; etc.) have been used to rank the amplifiers tested. [It was evident from such results for a variety of amplifiers already screened by the old method,[5] that the number of stars (*) corresponded, albeit but approximately, to the number of pluses (+) previously used[5] to assist in visualizing degrees of activity.]

Acknowledgments

We thank Drs G. B. Barlin and M. D. Fenn for helpful discussion and the A.N.U. for supporting B.B.B. as a Vacation Scholar.

Manuscript received 1 July 1981

[11] Gardner, T. S., Wenis, E., and Lee, J., *J. Org. Chem.*, 1954, **19**, 753.
[12] Hart, N. K., Hofmann, A., Lamberton, J. A., and Galbraith, M. N., *Heterocycles*, 1979, **13**, 271.