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Heterocyclic Amplifiers of Phleomycin. V* Thiadiazolylpyridines and Related Compounds; Preliminary Antitumour Results

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Abstract

Syntheses are described for *N,N*-dimethyl-2-[5′-(pyridin-2″-yl)-1′,3′,4′-thiadiazol-2′-ylthio]ethylamine; the homologous propylamine; the *N,N*-diethyl homologue; the 5′-phenyl analogue; and some substituted phenyl, pyrazinyl and pyrimidinyl analogues.

Unlike the pyridin-4″-yl isomer previously described, these compounds proved but mediocre amplifiers of phleomycin-G *in vitro* against *Escherichia coli*. Testing *in vivo* against Ehrlich's tumour in mice was therefore confined to the pyridin-4″-yl compound and to *N,N*-dimethyl-2-(6′-methyl-2′-phenylpyrimidin-4′-ylthio)ethylamine: the first showed considerable amplifying power towards two phleomycins and a bleomycin; the second, only marginally less amplification towards the same phleomycins.

In view of the high activity of *N,N*-dimethyl-2-[5′-pyridin-4″-yl)-1′,3′,4′-thiadiazol-2′-ylthio]ethylamine (1a) as an amplifier of phleomycin-G *in vitro* against *Escherichia coli*,[1] it seemed advisable to test its efficacy with phleomycins against tumours *in vivo* and to prepare some close analogues for subsequent evaluation in both systems. Moreover, the improved *in vitro* activities evident in some phenylpyrimidines, as compared with corresponding pyridinylpyrimidines or bipyrimidines,[2] suggested the inclusion of a phenylpyrimidine (2) and several phenylthiadiazoles, e.g. (1b), in the present studies. Accordingly, we now report the synthesis of an isomer (1c) and two analogues, (3a) and (3b), of the amplifier (1a); the synthesis of the phenylthiadiazole (1b) and four substituted phenyl derivatives (1d–g); the synthesis of pyrazinyl (3c) and pyrimidinyl (1h) analogues; the *in vitro* evaluation of the above compounds against *E. coli*; and the *in vivo* evaluation of compounds (1a) and (2) as amplifiers of phleomycins against the Ehrlich's tumour in mice, for comparison with recently published data[3,4] on other fused and unfused heterobicyclic amplifiers.

* Part IV, *Aust. J. Chem.*, 1984, **37**, 2093.

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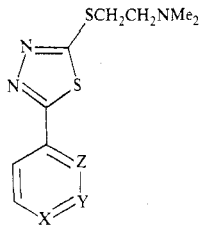
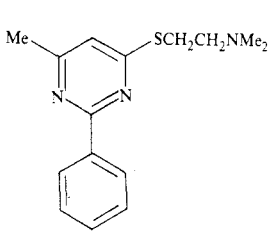
Syntheses

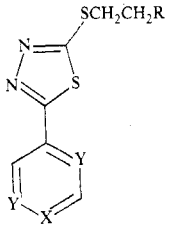
The amplifier (1c) was prepared by *S*-alkylation of the known[5] thione (4a) with 2-chloro-*N*,*N*-dimethylethylamine. Likewise, the thione[1] (4b) gave the amplifiers (3a) and (3b) by alkylation with 2-chloro-*N*,*N*-diethylethylamine and 3-chloro-*N*,*N*-dimethylpropylamine, respectively; and the phenylthiadiazolethione (4c),[5] its substituted phenyl analogues (4d) and (4e),[6] and the pyrazinylthiadiazolethione[7] (5) afforded the amplifiers (1b), (1d), (1f) and (3c), respectively. Treatment of *p*-toluohydrazide or *m*-chlorobenzohydrazide with carbon disulfide in ethanolic potassium hydroxide gave good yields of the unstable potassium dithiocarbazates (6a) and (6b), which underwent cyclization in concentrated sulfuric acid to the thiones (4f) and (4g); alkylation of these gave the amplifiers (1e) and (1g), respectively. In a rather different way, 2-dimethylaminopyrimidine-4-carbonitrile[8] was converted by methanolic hydrazine into 2-dimethylaminopyrimidine-4-carboxamidrazone (7) and thence by methanolic carbon disulfide into the thione (4h) which underwent *S*-alkylation to the amplifier (1h).



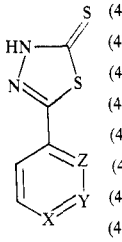
	X	Y	Z
(1a)	N	CH	CH
(1b)	CH	CH	CH
(1c)	CH	CH	N
(1d)	COMe	CH	CH
(1e)	CMe	CH	CH
(1f)	CCl	CH	CH
(1g)	CH	CCl	CH
(1h)	N	$CNMe_2$	N



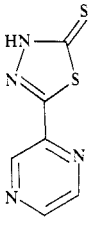
(2)



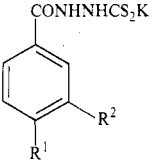
	R	X	Y
(3a)	NEt_2	N	CH
(3b)	CH_2NMe_2	N	CH
(3c)	NMe_2	CH	N



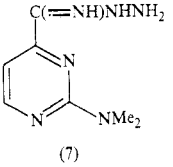
	X	Y	Z
(4a)	CH	CH	N
(4b)	N	CH	CH
(4c)	CH	CH	CH
(4d)	COMe	CH	CH
(4e)	CCl	CH	CH
(4f)	CMe	CH	CH
(4g)	CH	CCl	CH
(4h)	N	$CNMe_2$	N



(5)



	R^1	R^2
(6a)	Me	H
(6b)	H	Cl



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Activities as Amplifiers

When evaluated *in vitro* as amplifiers of phleomycin-G (see ref.[4]) against *Escherichia coli*,[9] the new amplifiers described above proved disappointing. Thus the four-star activity[1] of the parent pyridin-4-ylthiadiazole (1a) was reduced (Table 1) to three-star in its homologues (3a) and (3b); to two-star in its pyridin-2-yl isomer (1c); and also to two-star in its pyrimidinyl (1h) and pyrazinyl (3c) analogues. Likewise, despite the improved activity of phenylpyrimidines, e.g. (2), compared with their bipyrimidine and related analogues,[2,10] replacement of the pyridine ring in amplifier (1a) by a benzene or substituted benzene ring to give the phenylthiadiazoles (1b) and (1d–g) resulted in a reduction to two- or three-star activity. Accordingly, the highly active amplifiers (1a) and (2) were chosen for preliminary *in vivo* evaluation with two phleomycins against the Ehrlich tumour in mice. The technique used for such testing is outlined briefly in the Experimental and broadly followed that reported recently[3] for ten other heterocyclic amplifiers.

The results summarized in Table 2 indicate very significant amplification by the pyridinylthiadiazole (1a) of phleomycin-PEP [side chain:[3] $-CONH(CH_2)_3NHCHMePh$] and phleomycin-CHP [$-CONH(CH_2)_3NH(c\text{-}C_6H_{11})$]: in each case, the ratio of the mean survival times for phleomycin-treated mice, with and without amplifier, proved better than 400% at optimal dosage levels. On the limited data available, the phenylpyrimidine (2) also showed considerable amplification of both phleomycins, with T/C ratios of between 200 and 300%. For comparison, the classical amplifier, caffeine, improved the activity of phleomycin-PEP by some 250% under comparable conditions. Also of interest is the fact that the compound (1a) amplified bleomycin-B_2 [side chain:[11] $-CONH(CH_2)_4NHC(=NH)NH_2$; one of the major constituents of bleomycin sulfate U.S.P.] to the order of 300%.

Table 1. Activities as amplifiers of phleomycin-G *in vitro*

Measured at 2 mM; for details see ref. 9

Compound	Activity	Compound	Activity	Compound	Activity
(1a)	****[A]	(1e)	***[B]	(2)	*****[E]
(1b)	***	(1f)	**[B]	(3a)	***
(1c)	**	(1g)	—[C]	(3b)	***
(1d)	**[B]	(1h)	**[D]	(3c)	**

[A] From ref. 1.
[B] At $<0{\cdot}5$ mM (saturated) against caffeine at $0{\cdot}5$ mM.
[C] Intrinsic antibacterial activity precluded measurement.
[D] At $0{\cdot}5$ mM.
[E] From ref. 2.

Experimental

Analyses were done by the Australian National University Analytical Services Unit. The n.m.r. spectra (chemical shifts in δ) were measured at 90 MHz and 30° against tetramethylsilane in $CDCl_3$.

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Table 2. Amplification of phleomycins against Ehrlich's tumour in mice

For details see Experimental

Phleomycin or bleomycin[A]	Total dose (mg/kg)	Amplifier	Total dose (mmol/kg)	T/C (%)
Phleomycin-PEP	2·5	(1a)	0·25	135
	2·5		1·25	>277
	5·0		1·25	292
	10·0		0·25	141
	10·0		1·25	>450
	20·0		1·25	>312
Phleomycin-CHP	2·5	(1a)	1·25	222
	5·0		1·25	>416
	10·0		1·25	>217
	20·0		1·25	>370
Phleomycin-PEP	5·0	(2)	0·50	>242
	5·0		1·00	>300
Phleomycin-CHP	5·0	(2)	0·50	>217
	5·0		1·00	>217
Phleomycin-PEP	5·0	caffeine	1·00	>234
Bleomycin-B_2	5·0	(1a)	1·25	>288
	10·0		1·25	>131
	20·0		1·25	<100

[A] For structures see ref. 4 and 11.

N,N-*Dimethyl-2-[5'-(pyridin-2''-yl)-1',3',4'-thiadiazol-2'-ylthio]ethylamine (1c)*

5-(Pyridin-2'-yl)-1,3,4-thiadiazole-2(3*H*)-thione[5] (0·78 g) was dissolved in water (10 ml) by addition of the minimal volume of 2 M sodium hydroxide. After adding 2-chloro-*N,N*-dimethylethylamine hydrochloride (0·65 g), the mixture was adjusted to pH 9 and stirred at 60° for 20 min, after which it was adjusted to pH 12 with 10 M sodium hydroxide and extracted with chloroform (4×20 ml). The residue from evaporation of the dehydrated extracts was weighed and dissolved in ethanol (15 ml). Addition of fresh ethanolic hydrogen bromide (1 equivalent) gave the product (1c) as its *hydrobromide* (61%), m.p. 176–177° (from ethanol) (Found: C, 38·0; H, 4·3; N, 15·8. $C_{11}H_{15}BrN_4S_2$ requires C, 38·0; H, 4·4; N, 16·1%). N.m.r. (base) 8·77–7·23, m, H3''–5''; 3·56, t, H2; 2·76, t, H1; 2·33, s, NMe_2.

N,N-*Diethyl-2-[5'-(pyridin-4''-yl)-1',3',4'-thiadiazol-2'-ylthio]ethylamine (3a)*

5-(Pyridin-4'-yl)-1,3,4-thiadiazole-2(3*H*)-thione[1] (0·78 g) and 2-chloro-*N,N*-diethylethylamine hydrochloride (0·76 g) likewise gave the product (3a), isolated (by addition of two equivalents of hydrogen bromide) as its *dihydrobromide* (82%), m.p. 211–212° (from ethanol) (Found: C, 34·4; H, 4·4; N, 12·2. $C_{13}H_{20}Br_2N_4S_2$ requires C, 34·2; H, 4·4; N, 12·3%).

N,N-*Dimethyl-3-[5'-(pyridin-4''-yl)-1',3',4'-thiadiazol-2'-ylthio]propylamine (3b)*

The same thione (0·78 g) and 3-chloro-*N,N*-dimethylpropylamine hydrochloride (0·70 g) similarly gave the amine (3b) as *dihydrobromide* (21%), m.p. 237–238° (from ethanol) (Found: C, 32·8; H, 4·3; N, 12·6. $C_{12}H_{18}Br_2N_4S_2$ requires C, 32·6; H, 4·1; N, 12·7%). N.m.r. (base) 8·73, m, H2'',6''; 7·74, m, H3'',5''; 3·49, t, H3; 2·45, t, H1; 2·23, s, NMe_2; 2·02, m, H2.

N,N-*Dimethyl-2-(5'-phenyl-1',3',4'-thiadiazol-2'-ylthio)ethylamine (1b)*

5-Phenyl-1,3,4-thiadiazole-2(3*H*)-thione[5] (0·78 g) and 2-chloro-*N,N*-dimethylethylamine hydrochloride (0·65 g) were treated as for the pyridinyl analogue (1c) to give the product (1b) as *hydrobromide* (43%), m.p. 189–190° (from methanol) (Found: C, 41·5; H, 4·6; N, 12·0. $C_{12}H_{16}BrN_3S_2$ requires C, 41·6; H, 4·7; N, 12·1%). N.m.r. (base) 7·65, m, Ph; 3·52, t, H2; 2·74, t, H1; 2·31, s, NMe_2.

2-(5′-p-Methoxyphenyl-1′,3′,4′-thiadiazol-2′-ylthio)-N,N-dimethylethylamine (1d)

5-*p*-Methoxyphenyl-1,3,4-thiadiazole-2(3*H*)-thione[6] (0·90 g) was treated likewise to give the product (1d) as *hydrobromide* (53%), m.p. 227–228° (from methanol) (Found: C, 41·5; H, 4·9; N, 11·2. $C_{13}H_{18}BrN_3OS_2$ requires C, 41·5; H, 4·8; N, 11·2%). N.m.r. (base) 7·80, m, H3″,5″; 6·97, m, H2″,6″; 3·87, s, OMe; 3·55, t, H2; 2·73, t, H1; 2·30, s, NMe_2.

N,N-*Dimethyl-2-(5′-p-tolyl-1′,3′,4′-thiadiazol-2′-ylthio)ethylamine (1e)*

To a stirred solution of potassium hydroxide (*c.* 85%, 1·55 g) in ethanol (25 ml) was added *p*-toluohydrazide[12] (3·50 g) followed by carbon disulfide (1·83 g) in ethanol (3·0 ml). After the mixture was stirred for 30 min longer, filtration gave crude potassium 3-*p*-toluoyldithiocarbazate (80%) which was dried immediately in a vacuum. This (fresh) salt (4·0 g) was added little by little with stirring to concentrated sulfuric acid (17·0 ml) at 0–5°. After the mixture was stirred for a further 5 min, the solution was poured onto crushed ice (*c.* 160 g) with thorough mixing. The resulting solid was filtered off and washed with water to give *5-p-tolyl-1,3,4-thiadiazole-2(3*H*)-thione* (4f) (46%), m.p. 205–206° (from methanol) (Found: C, 52·1; H, 4·0; N, 13·6. $C_9H_8N_2S_2$ requires C, 51·9; H, 3·9; N, 13·5%). This was converted as above into the product (1e) as its *hydrobromide* (61%), m.p. 214–215° (from ethanol) (Found: C, 43·5; H, 4·9; N, 11·7. $C_{13}H_{18}BrN_3S_2$ requires C, 43·3; H, 5·0; N, 11·7%). N.m.r. (base) 7·76, m, H2″,6″; 7·25, m, H3″,5″; 3·53, t, H2; 2·75, t, H1; 2·40, s, 4″-Me; 2·31, s, NMe_2.

2-(5′-p-Chlorophenyl-1′,3′,4′-thiadiazol-2′-ylthio)-N,N-dimethylethylamine (1f)

5-*p*-Chlorophenyl-1,3,4-thiadiazole-2(3*H*)-thione[6] (0·91 g) and 2-chloro-*N,N*-dimethylethylamine hydrochloride (0·65 g) similarly gave the product (1f) as *hydrobromide* (53%), m.p. 229–230° (from methanol) (Found: C, 37·7; H, 3·9; N, 10·8. $C_{12}H_{15}BrClN_3S_2$ requires C, 37·9; H, 4·0; N, 11·0%). N.m.r. (base) 7·81, m, H2″,6″; 7·43, m, H3″,5″; 3·54, t, H2; 2·75, t, H1; 2·32, s, NMe_2.

2-(5′-m-Chlorophenyl-1′,3′,4′-thiadiazol-2′-ylthio)-N,N-dimethylethylamine (1g)

m-Chlorobenzohydrazide[13] (4·0 g) was treated with carbon disulfide in alkali (as for the tolyl analogue above) to give potassium 3-*m*-chlorobenzoyldithiocarbazate (94%) and thence *5-(m-chlorophenyl)-1,3,4-thiadiazole-2(3*H*)-thione* (4g) (26%), m.p. 216–218° (from methanol) (Found: C, 42·2; H, 2·3; N, 11·9. $C_8H_5ClN_2S_2$ requires C, 42·0; H, 2·2; N, 12·2%), which underwent *S*-alkylation as above to afford the product (1g) as its *hydrobromide* (47%), m.p. 166–167° (from methanol) (Found: C, 37·7; H, 3·9; N, 11·0. $C_{12}H_{15}BrClN_3S_2$ requires C, 37·9; H, 4·0; N, 11·0%). N.m.r. (base) 7·60, m, C2″,4″–6″; 3·53, t, H2; 2·74, t, H1; 2·30, s, NMe_2.

N,N-*Dimethyl-2-[5′-(pyrazin-2″-yl)-1′,3′,4′-thiadiazol-2′-ylthio]ethylamine (3c)*

5-(Pyrazin-2′-yl)-1,3,4-thiadiazole-2(3*H*)-thione[7] (0·78 g) underwent alkylation as for the pyridinyl analogue (1c) to give the product (3c) as *hydrobromide* (49%), m.p. 238–240° (from methanol) (Found: C, 34·3; H, 4·0; N, 19·8. $C_{10}H_{14}BrN_5S_2$ requires C, 34·5; H, 4·0; N, 20·1%). N.m.r. (base) 9·49, m, H6″; 8·65, m, H4″,5″; 3·58, t, H2; 2·78, t, H1; 2·33, s, NMe_2.

2-[5′-(2″-Dimethylaminopyrimidin-4″-yl)-1′,3′,4′-thiadiazol-2′-ylthio]-N,N-dimethylethylamine (1h)

2-Dimethylaminopyrimidine-4-carbonitrile[8] (1·0 g) was stirred with methanol (2·5 ml) and hydrazine (95%: 1·5 ml) for 2 h. The residue from evaporation under reduced pressure was diluted with methanol (8·0 ml) and then stirred with carbon disulfide (1·0 g) for 8 h. Filtration gave *5-(2′-dimethylaminopyrimidin-4′-yl)-1,3,4-thiadiazole-2(3*H*)-thione* (4h) (84%), m.p. 263–264° (from methanol) (Found: C, 39·8; H, 3·8; N, 29·3. $C_8H_9N_5S_2$ requires C, 40·2; H, 3·8; N, 29·3%). N.m.r. 8·53, d, H6′; 7·07, d, H5′; 3·13, s, NMe_2. The above thione (0·96 g) was treated with 2-chloro-*N,N*-dimethylethylamine hydrochloride (0·65 g) as for the analogue (1c) except that 2 equivalents of hydrogen bromide were used to give the product (1h) as *dihydrobromide* (81%),

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m.p. 237–240° (from ethanol) (Found: C, 30·6; H, 4·3; N, 17·6. $C_{12}H_{20}Br_2N_6S_2$ requires C, 30·5; H, 4·3; N, 17·8%). N.m.r. (base) 8·34, d, H6″; 7·20, d, H5″; 3·45, t, H2; 3·10, s, 2″-NMe_2; 2·65, t, H1; 2·21, s, 1-NMe_2.

Evaluation of Amplifiers in Mice

Ehrlich's tumours were induced in month-old male Swiss mice as reported previously.[3] Total doses of antibiotic (within the range 2·5–20·0 mg/kg of body weight) and amplifier (0·25–1·25 mmol/kg) were administered by intraperitoneal injection in 10 equal portions over the first five days of treatment; appropriate controls (antibiotic alone, amplifier alone, and phosphate buffered saline) were included and procedures were based on those generally accepted.[14] Amplification was measured as a T/C ratio, i.e. the median survival time for the group of mice given antibiotic plus phleomycin over that of mice given a similar dose of antibiotic alone, expressed as a percentage. All groups of mice were retained until their median survival time had been passed or for 100 days, whichever was the shorter period. As customary,[14] a reproducible T/C value of > 125% was considered significantly positive.

The Phleomycins and Bleomycin

Phleomycin-PEP and CHP,[15] phleomycin-G[16] and bleomycin-B_2[17] were prepared, purified and characterized by Dr J. A. Lamberton (Division of Applied Organic Chemistry, CSIRO), Dr A. Lane (Division of Food Research, CSIRO) and their colleagues using methods based on those indicated. Prior to use, each batch of antibiotic was checked for homogeneity by extensive t.l.c. and for identity by ultraviolet and n.m.r. spectra, analysis of copper content, and hydrolysis followed by chromatographic identification of the side-chain amine against authentic material.

Acknowledgments

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