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SOME 6-SUBSTITUTED NICOTINAMIDES: SYNTHESIS AND ANTINEOPLASTIC ACTIVITIES

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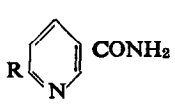
Abstract—The preparation of 6-methoxy-, -*n*-amylamino, -anilino-, -benzylamino-, -phenylhydrazino-, -phenylazo, -(2-hydroxyethylthio)-, and -benzylthio-nicotinamide is described. The results of a preliminary screening of these new compounds and some related 6-substituted nicotinic acids and nicotinamides against the transplanted Walker rat carcinoma and the mouse lymphoid leukaemia, L 1210, are reported. Moderate activity against the leukaemia is shown by 6-chloro-, -methoxy-, -anilino- and phenylazo-nicotinamide.

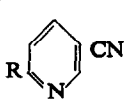
6-AMINONICOTINAMIDE is a potent nicotinamide antagonist which has shown antineoplastic activity.[1, 2] Its activity may be due to the formation of 6-aminonicotinamide adenine dinucleotide *in vivo*. This unnatural cofactor analogue competes with NAD for apoenzymes but cannot undergo hydrogen transfer reactions owing to the influence of the electron releasing 6-substituent.[3] The preparation of new 6-substituted nicotinamides which might have similar properties is now described. Particular attention has been given to the introduction of lipoid solubilizing groups into the substituents since this might be expected to enhance activity against solid tumours. Localization of the cytotoxic action of 6-aminonicotinamide could be achieved by the preparation of a derivative with latent activity (cf. ref. 4). Azo-compounds are reduced *in vivo* to amines and this reduction would be expected to occur more readily in cancer cells which often have a lower redox potential than normal cells.[5] These considerations led to the synthesis of 6-phenylazonicotinamide.

MATERIALS

The chlorine atom in 6-chloronicotinamide can be replaced under vigorous conditions. Thus when the chloroamide is heated in sealed tubes with sodium methoxide in methanol, with aqueous *n*-amylamine, or with aniline in aqueous acetone, 6-methoxy- (I, R = MeO), -*n*-amylamino- (I, R = $C_5H_{11}NH$), and -anilino-nicotinamide (I, R = PhNH) are formed. The more nucleophilic thiol groups in mercaptoethanol and benzyl mercaptan react under milder conditions giving 6-(2-hydroxyethylthio)- (I, R = $HOCH_2CH_2S$) and -benzylthio-nicotinamide (I, R = $PhCH_2S$) respectively.



(I)



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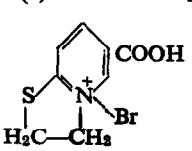


(III)

The chlorine atom in 6-chloronicotinamide is much less reactive than that in 4-chloronicotinamide.[6] Greater reactivity is shown by the chlorine atom in 6-chloronicotinonitrile than in the amide and it is more convenient to obtain some of the required substituted amides by hydrolysis of the readily prepared nitriles (II). 6-Methoxy-, anilino-, and benzylamino-nicotinamide (I, R = $PhCH_2NH$) have been prepared by this method.

On treatment with hydrobromic acid 4-(2-hydroxyethylthio)nicotinic acid gives the bromoethylthio derivative which can be converted into the potentially irreversible antagonist, 4-(2-bromoethylthio)nicotinamide.[6] Similar treatment of 6-(2-hydroxyethylthio)nicotinamide gives a product (III); this facile internal alkylation precludes the synthesis of the desired 6-(2-bromoethylthio) derivative.

Diazotization and coupling reactions are not generally applicable to the preparation of 6-substituted pyridines and so the azo compound was obtained by the following procedure. Phenylhydrazine condenses smoothly with 6-chloronicotinonitrile in dimethylsulphoxide solution—other solvents give inferior results—yielding 6-phenylhydrazinonicotinonitrile (II, R = PhNHNH) and this on oxidation with mercuric oxide affords 6-phenylazonicotinonitrile (II, R = PhN:N). Hydrolysis of this nitrile with IRA-400 resin gives the required 6-phenylazonicotinamide (I, R = PhN:N).

Melting points were determined with a Townson and Mercer heated metal block apparatus and are corrected. The activated alumina used was Spence Type H.

6-Methoxynicotinonitrile

6-Chloronicotinonitrile (1·74 g) was added to a solution of sodium (340 mg) in methanol (50 ml) and the mixture was heated under reflux for 3 hr. After evaporation to dryness under reduced pressure the product was extracted with benzene and passed through a column of activated alumina. Early eluates contained *6-methoxynicotinonitrile* which formed flattened needles, m.p. 95°, from light petroleum (b.p. 60–80°); yield 1·5 g. (Found: C, 63·1%; H, 4·5 %; N, 20·6%. Calc. for $C_7H_6N_2O$: C, 62·7%; H, 4·5%; N, 20·9%.)

6-Methoxynicotinamide

(a) 6-Chloronicotinamide (3 g) and sodium methoxide (1·4 g) in methanol (20 ml) were heated in a sealed glass tube at 150° for 8 hr. The product, obtained by evaporating a hot methanol extract of the contents of the tube, was crystallized from acetone. *6-Methoxynicotinamide* (1·3 g) formed prisms, m.p. 189–190°. (Found: C, 54·9%; H, 5·1%; N, 18·2%. Calc. for $C_7H_8N_2O_2$; C, 55·2%; H, 5·3%; N, 18·4%.)

(b) 6-Methoxynicotinonitrile (5 g), Amberlite IRA-400 resin (OH form, 20 g), water (100 ml) and methanol (40 ml) were heated under reflux for 4 hr. Evaporation of the filtered solution and crystallization of the product from acetone gave the amide (3 g), m.p. 189–190°, identical with that prepared by method (a).

6-n-Amylaminonicotinamide

6-Chloronicotinamide (3·1 g), *n*-amylamine (11·2 ml) and water (8·8 ml) were heated in a sealed tube at 170° for 4 hr. The contents of the tube were extracted with hot methanol and the residue obtained on evaporating the extract was crystallized from water (500 ml). *6-n-Amylaminonicotinamide* (3 g) formed needles, m.p. 170–175°. (Found: C, 63.7%; H, 8·3%; N, 19·8%. Calc. for $C_{11}H_{17}N_3O$: C, 63·7%; H, 8·3%; N, 20·3%).

6-Anilinonicotonic acid

6-Chloronicotinic acid (1 g) and aniline (3 ml) were heated at 100° for 4 hr. Water (20 ml), aqueous NaOH (15 ml, N) and ether (50 ml) were added to the cooled product. Acidification (aq. HCl, N) of the aqueous layer gave *6-anilinonicotinic acid* (500 mg) which formed prisms, m.p. 272–273°, from ethanol. Found: C, 67·4%; H, 4·7%; N, 13·0%. Calc. for $C_{12}H_{10}N_2O_2$: C, 67·2%; H, 4·7%; N, 13·1%.)

6-Anilinonicotinonitrile

6-Chloronicotinonitrile (5 g) and aniline (10 ml) were heated at 120° for 2 hr. The resultant semi-solid mass was ground with an excess of saturated aqueous Na_2CO_3 and extracted with chloroform. The dried (Na_2SO_4) extract was passed through a column of activated alumina which was eluted with chloroform. Early eluates contained unchanged chloronitrile and aniline and later *6-anilinonicotinonitrile* (5 g) was removed. It formed needles, m.p. 179–181°, from methanol or benzene. (Found: C, 73·7%; H, 4·5%; N, 21·6%. Calc. for $C_{12}H_9N_3$: C, 73·8%; H, 4·6%; N, 21·5%.)

6-Anilinonicotinamide

(a) 6-Chloronicotinamide (1·56 g), aniline (6 ml), acetone (4·5 ml) and water (4·5 ml) were heated in a sealed tube at 150° for 8 hr. Excess of aniline was removed by distillation in steam and the residue was crystallized from aqueous methanol giving *6-anilinonicotinamide* (700 mg), needles, m.p. 183–148° (Found: C, 67·7%; H, 5·2%; N, 19·8%. Calc. for $C_{12}H_{11}N_3O$: C, 67·6%; H, 5·2%; N, 19·7%.)

(b) 6-Anilinonicotinonitrile (500 mg) and IRA-400 resin (OH form, 2 g) in water (40 ml) and methanol (5 ml) were heated under reflux for 4 hr. On cooling, the filtered solution deposited prismatic needles, m.p. 184–185°, identical with the product obtained by method (a).

6-Benzylaminonicotinonitrile

6-Chloronicotinonitrile (1 g) and benzylamine (2 g) were heated at 130° for 3 hr. The product was worked up as described for the anilinonitrile. *6-Benzylaminonicotinonitrile* formed flattened needles, m.p. 131–133°, from benzene–light petroleum (b.p. 40–60°); yield 500 mg. (Found: C, 74·5%; H, 5·1%; N, 20·1%. Calc. for $C_{13}H_{11}N_3$: C, 74·6%; H, 5·3%; N, 20·1%.)

6-Benzylaminonicotinamide

Hydrolysis of 6-benzylaminonicotinonitrile (1 g) by the procedure described for the anilinonitrile afforded *6-benzylaminonicotinamide* (600 mg) which formed needles, m.p. 173–174°, from aqueous methanol. (Found: C, 68·8%; H, 5·8%; N, 18·6%. Calc. for $C_{13}H_{13}N_3O$: C, 68·7%; H, 5·8%; N, 18·5%.)

6-Phenylhydrazinonicotinonitrile

6-Chloronicotinonitrile (32·9 g), phenylhydrazine (50 ml) and dimethyl sulphoxide (150 ml) were heated on a steam bath for 8 hr. On dilution with water (1 l.) an oil separated. The solid formed on standing was filtered off and washed with benzene. *6-Phenylhydrazinonicotinonitrile* formed pale yellow plates, m.p. 171–173°, from benzene; yield 29 g. (Found: C, 68·8%; H, 4·9%; N, 26·5%. Calc. for $C_{12}H_{10}N_4$: C, 68·5%; H, 4·8%; N, 26·6%.)

6-Phenylazonicotinonitrile

6-Phenylhydrazinonicotinonitrile (29 g), red mercuric oxide (50 g), dried magnesium sulphate (50 g) and benzene (750 ml) were heated under reflux for 3 hr. The cooled solution was passed through a short column of activated alumina which was further eluted with benzene. Concentration of the combined deeply coloured eluates gave *6-phenylazonicotinonitrile* (23 g) which formed red flattened needles, m.p. 166–168°, from ether. (Found: C, 68·9%; H, 4·0%; N, 27·1%. Calc. for $C_{12}H_8N_4$: C, 69·1%; H, 3·9%; N, 26·9%.)

6-Phenylhydrazinonicotinamide

6-Phenylhydrazinonicotinonitrile (1 g) and IRA-400 resin (OH form, 5 g) in water (50 ml) and methanol (50 ml) were heated under reflux for 4 hr. The filtered solution was evaporated under reduced pressure and the residue was crystallized from acetone. *6-Phenylhydrazinonicotinamide* (600 mg) formed prisms, m.p. 207–209°. (Found: C, 62·6%; H, 5·2%; N, 24·7%. Calc. for $C_{12}H_{12}N_4O$: C, 63·1%; H, 5·3%; N, 24·6%.)

6-Phenylazo-nicotinamide and -nicotinic acid

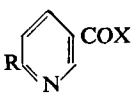
6-Phenylazonicotinonitrile (23 g) and IRA-400 resin (OH form, 50 g) in water (250 ml) and ethanol (250 ml) were heated under reflux for 2½ hr. The hot solution was filtered and the resin was washed with hot ethanol until no more colour was removed. On concentrating the combined extracts under reduced pressure to 300 ml, *6-phenylazonicotinamide* separated as large orange plates, m.p. 261–263°; yield 15 g. (Found: C, 63·5%; H, 4·5%; N, 24·9%. Calc. for $C_{12}H_{10}N_4O$; C, 63·7%; H, 4·5%; N, 24·8%.)

On washing the deep purple resin with hot ethanol (500 ml) containing formic acid (50 ml) and concentrating the extract *6-phenylazonicotinic acid* was obtained as small deep red prisms, m.p. 248–250°; yield 2 g. (Found: C, 63·0%; H, 4·2%; N, 18·0%. Calc. for $C_{12}H_9N_3O_2$: C, 63·4%; H, 4·0%; N, 18·5%.)

6-(2-Hydroxyethylthio)nicotinamide

6-Chloronicotinamide (10 g), 2-mercaptoethanol (16 m) and $NaHCO_3$ (7 g) in water (50 ml were heated under reflux for 1 hr. The residue obtained on evaporating the solution to dryness was extracted with hot methanol (100 ml). This extract was diluted with chloroform (900 ml) and passed down a column of activated alumina. Continued elution of the column with methanol–chloroform

TABLE 1. SCREENING AGAINST WALKER 256 (SUBCUTANEOUS) TUMOR



Compound		Vehicle	Dose (mg/kg)	No. of daily doses	Survivors	C/T
R	X					
OH	OH[9]	Na salt in water	240	6	3/3	0·8
			600	6	3/3	0·7
			1500	6	3/3	0·9
Cl	OH	—	240	6	3/3	0·9
			600	6	3/3	1
			1500	6	3/3	0·9
PhNH	OH[10]	—	100	8	3/3	1
			250	8	3/3	1
			625	8	0/3	—
Cl	NH_2	Arachis oil	240	6	3/3	1
			600	6	3/3	1·4
			1500	6	0/3	—
MeO	NH_2	—	100	5	3/3	1
			200	5	3/3	1
			400	5	0/3	—
NH_2	NH_2	Water	0·32	6	3/3	1·1
			0·8	6	3/3	1·2
			2	6	0/3	—
$C_5H_{11}NH$	NH_2	Arachis oil	1	6	3/3	1
			2	6	3/3	1
			5	6	3/3	1
PhNH	NH_2	—	100	6	3/3	1·1
			250	6	3/3	1
			625	6	1/3	1·1
$PhCH_2NH$	NH_2	—	100	5	3/3	1
			250	5	2/3	2
			625	5	0/3	—
HS	NH_2[11]	—	100	6	3/3	1
			250	6	3/3	1·1
			625	6	0·3	—
Ph : N	NH_2	—	100	5	2/3	0·9
			250	5	2/3	1·1
			625	5	1/3	1

(1 : 9) slowly removed 6-(2-*hydroxyethylthio)nicotinamide* (6 g) which formed prisms, m.p. 136–138°, from water. The product contains water of crystallization which is lost on slow heating; if the specimen is placed in the apparatus at 120° it melts immediately. The analytical specimen was dried at 80°/10mm for 2 hr. (Found: C, 48·3%; H, 5·0%; N, 14·2%; S, 16·3%. Calc. for $C_8H_{10}N_2O_2S$: C, 48·5%; H, 5·1%; N, 14·1%; S, 16·2%.)

Action of hydrobromic acid on 6-(2-hydroxyethylthio)nicotinamide

A solution of 6-(2-hydroxyethylthio)nicotinamide (1·4 g) in concentrated HBr (8 ml, *d* 1·7) was heated under reflux for 1 hr. The cooled solution deposited prisms, decomp. above 320°, which were

collected and washed successively with a little water, acetone and ether. The analytical data indicate that the compound is the *quaternary acid* (III) (Found: C, 36·6%; H, 3·1%; total Br, 30·5%; ionic Br, 29·5%; N, 5·3%; S, 12·2%; equiv. 269. Calc. for $C_8H_8BrNO_2S$: C, 36·6%; H, 3.1%; Br, 30·5%; N, 5·3%; S, 12·2% equiv. 262·1.)

Benzylthionicotinamide

6-Chloronicotinamide (4 g), benzylmercaptan (4 ml) and $NaHCO_3$ (4 g) in water (40 ml and acetone (40 ml) were heated under reflux for 3 hr. The cooled solution yielded the *thio-amide* (3·2 g) which formed plates, m.p. 197°, from ethanol. (Found: C, 63·6%; H, 5·0%; N, 11·8%; S, 13·5%. Calc. for $C_{13}H_{12}N_2OS$: C, 63·9%; H, 5·0%; N, 11·5%; S, 13·1%.)

METHODS

The protocol for testing the compounds as inhibitors of the growth of the transplanted Walker rat carcinoma 256 is that given by Connors *et al.*[7] and the method of assay against the mouse lymphoid leukaemia, L1210, is essentially that already described,[8] except that the C_{57}/DBA2 hybrid strain of mouse was used as host.

TABLE 2. SCREENING AGAINST MOUSE LYMPHOID LEUKEMIA, L1210

Compound R	Compound X	Vehicle	Dose (mg/kg)	No. of daily doses	T/C ratio	Approx. LD_{50} for host mouse (mg/kg) daily
OH	OH[9]	Na salt in water	350	5	103	1400
			700	5	98	
			1400	5	110	
Cl	OH[10]	—	350	5	97	1400
			700	5	101	
			1400	5	101	
PhNH	OH	Arachis oil	18·75	10	92	280
			37·5	10	105	
			75	10	107	
			150	10	90	
			300	2	24	
Cl	NH_2[11]	—	175	5	130	700
			350	5	112	
			700	5	24	
MeO	NH_2	—	100	5	106	400
			200	5	138	
			400	5	124	
NH_2	NH_2	Water	3·9	10	140	16
			7·8	10	124	
			15·6	10	118	
$C_5H_{11}NH$	NH_2	Arachis oil	2·75	5	85	11
			5·5	5	105	
			11	5	115	
PhNH	NH_2	—	175	5	120	700
			350	5	134	
			700	5	94	
$PhCH_2NH$	NH_2	—	70	5	61	280
			140	5	68	
			280	5	66	
HS	NH_2[11]	—	87·5	5	74	350
			175	5	83	
			350	5	35	
$PhCH_2S$	NH_2	—	250	5	87	1000
			500	5	98	
			1000	5	67	
PhN : N	NH_2	—	187·5	5	104	700
			350	5	120	
			700	5	118	

Compounds were administered by daily intraperitoneal injection starting on the day following implantation or inoculation. Results of the Walker tumour test are expressed as C/T ratios, i.e. the weight of tumours in controls/the weight of tumours in treated rats. The T/C ratio shown for the L1210 assay equals [the average survival time of treated mice divided by the average survival time of controls] $\times$ 100. The appropriate LD_{50}s for the host mice were determined for non-inoculated animals —the compounds being administered as ten daily doses.

RESULTS AND DISCUSSION

The results of the carcinostatic assays against the Walker 256 tumour and the lymphoid leukaemia, L1210, together with preliminary toxicity data are given in Tables 1 and 2.

There is a considerable variation in the toxicity of the nicotinamide derivatives. Only 6-*n*-amylaminonicotinamide has a toxicity of the same order as 6-amino-nicotinamide. Substitution in the amino group by benzyl or phenyl groups lowers the toxicity which can be correlated with the basicity of the 6-substituent. The relatively low toxicity of the 6-phenylazo derivative suggests that little reduction to amine occurs in the tissues of the host animal.

No significant activity against the Walker tumor is shown by 6-amino-nicotinamide nor by any of the derivatives now tested with the possible exception of 6-benzyl-aminonicotinamide which produces a 50 per cent inhibition of tumour growth at a near toxic dose. This slight increase in effectiveness could be due to the extra lipoid solubility conferred by the aralkyl substituent.

Moderate activity—of the same order as that shown by 6-aminonicotinamide (30–40 per cent increase in survival time)—is shown by 6-chloro-, -methoxy-, and -anilino-nicotinamide. Hydrolysis *in vivo*, of the chlorine atom to the electron releasing hydroxyl group could account for the somewhat unexpected activity of the chloro derivative. The low activity of the 6-phenylazo derivative may be accounted for by its reduction to 6-aminonicotinamide in leukaemic cells.

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