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Original paper

Synthesis and anti-viral activity of 6-amino- and 6-dimethylamino-9-(aminoacylamidobenzyl)purines

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Summary — Several aminoacylamido derivatives of 9-benzyladenine and of 9-benzyl-6-dimethylaminopurine were synthesized for evaluation in anti-viral and anti-bacterial screens and in tests for inhibition of protein synthesis. The 9-(aminoacylamidobenzyl)purines were synthesized in two steps from the appropriate 9-(aminobenzyl)adenine **3** or 9-(aminobenzyl)-6-dimethylaminopurine **4.** Amines **3** and **4** were acylated with an *N*-carbobenzoxyamino acid *via* the mixed anhydride method to give the 9-(*N*-carbobenzoxyaminoacylamidobenzyl)purines **5—10.** The *N*-carbobenzoxy groups were removed by catalytic hydrogenolysis or with hydrogen bromide in acetic acid to give 9-(aminoacylamidobenzyl)purines **11—16.** Against rhinovirus serotype 1B, 6-dimethylamino-9-(3-phenylalanylamidobenzyl)-9*H*-purine **16b** had *in vitro* activity with an $IC_{50} = 17\ \mu M$.

Résumé — **Synthèse et activité anti-virale d'amino-6 et 9 de diméthylamino-6 (aminoacylamidobenzyl)-9 purines.** *Plusieurs aminoacylamides dérivés de la benzyl-9 adénine et de la benzyl-9 diméthylamino-6 purine ont été synthétisés pour évaluer leur activité anti-virale ou anti-bactérienne ou inhibitrice de la synthèse protéique. Les acylamidobenzyl-9 purines ont été synthétisées en deux étapes à partir de l'aminobenzyl-9 adénine **3** ou de l'aminobenzyl-9 diméthylamino-6 purine **4,** selon le cas. Les amines **3** et **4** ont été acylées avec un acide* N-*carbobenzoxyaminique par la méthode de l'anhydride mixte pour produire les* N-*carbobenzoxyaminoacylamidobenzyl-9 purines, **5—10**. Les radicaux* N-*carbobenzoxyle ont été supprimés par hydrogénolyse catalytique ou grâce à de l'acide bromhydrique dans de l'acide acétique pour produire les aminoacyl-9 amidobenzyl-9 purines **11—16**. La diméthylamino-6 (phényl-3 alanylamidobenzyl)-9, 9*H*-purine **16b** a démontré une activité* in vitro *vis-à-vis du rhinovirus sérotype 1B avec* $CI_{50} = 17\ \mu M$.

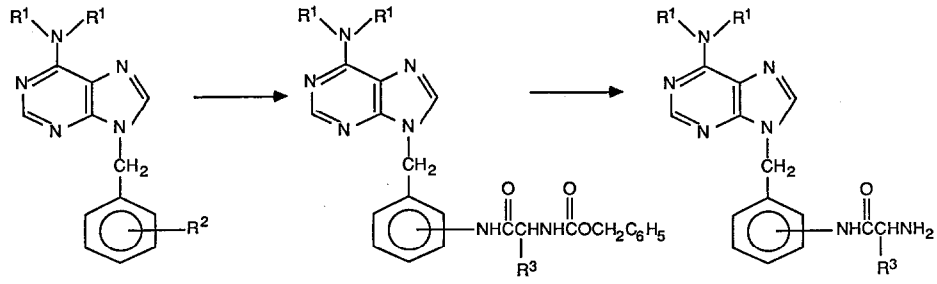
benzylpurine / purine / anti-viral / rhinovirus / adenine

Introduction

Inhibition of adenosine deaminase by substituted derivatives of 9-benzyladenine has been reported previously [1—4]. The benzyl moiety of these compounds was reported to bind to a hydrophobic area on the enzyme [4]. If the aromatic substituent was a bromoacetamide, irreversible enzyme inhibition occurred [1—3]. In light of this interesting enzyme inhibiting activity, analogs of 9-benzyladenine were prepared for evaluation in anti-viral and anti-bacterial screens and in tests for inhibition of protein synthesis. Preparation of compounds in which benzyladenine was substituted with an aminoacylamide moiety was prompted by the frequent occurrence of antibiotics containing bases or nucleosides covalently linked to amino acids [5, 6]. The synthesis and biological evaluation of these compounds are reported herein.

Chemistry

The 9-(aminobenzyl)adenines **3a, b** were prepared from **1a, b** as previously described [1, 2] (Scheme 1). Catalytic reduction of **2a—c** [1—3] gave the 6-dimethylaminopurines **4a—c**. Acylation of the aromatic amines **3** and **4** with the appropriate *N*-carbobenzoxyamino acid by the mixed anhydride method [7] gave the amides **5—10** in good yields (Tables I and II). That acylation had occurred on the aromatic amine in **3a, b** was evident from a negative Bratton—Marshall test [8, 9]. The amides also exhibited ultraviolet spectra with maxima at essentially the same wavelengths as were found for **3a, b** rather than at 273 nm as would be expected for *N*(6)-acylated products [1, 10]. Deblocking of **5—10** to give **11—16** was accomplished by catalytic hydrogenolysis or with hydrogen bromide in



1 $R^1 = H, R^2 = NO_2$
2 $R^1 = CH_3, R^2 = NO_2$
3 $R^1 = H, R^2 = NH_2$
4 $R^1 = CH_3, R^2 = NH_2$

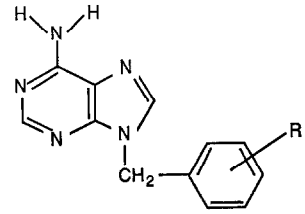
5 $R^1 = H, R^3 = H$
6 $R^1 = H, R^3 = CH_2CH(CH_3)_2$
7 $R^1 = H, R^3 = CH_2C_6H_5$
8 $R^1 = CH_3, R^3 = H$
9 $R^1 = CH_3, R^3 = CH_2CH(CH_3)_2$
10 $R^1 = CH_3, R^3 = CH_2C_6H_5$

11 $R^1 = H, R^3 = H$
12 $R^1 = H, R^3 = CH_2CH(CH_3)_2$
13 $R^1 = H, R^3 = CH_2C_6H_5$
14 $R^1 = CH_3, R^3 = H$
15 $R^1 = CH_3, R^3 = CH_2CH(CH_3)_2$
16 $R^1 = CH_3, R^3 = CH_2C_6H_5$

a = para, b = meta, c = ortho

Scheme 1.

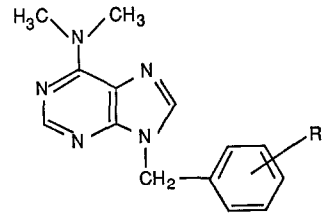
Table I. Derivatives of 9-benzyladenine.



Compd.	R	Method	Yield (%)	mp °C	Formula[a]
5a	*p*-$NHCOCH_2NHCOOCH_2C_6H_5$	C[b]	51	228—231[c]	$C_{22}H_{21}N_7O_3$
5b	*m*-$NHCOCH_2NHCOOCH_2C_6H_5$	C[d]	57	225—227[e,f]	$C_{22}H_{21}N_7O_3 \cdot CH_3OH$[g]
6a	*p*-$NHCOCH[CH_2CH(CH_3)_2]NHCOOCH_2C_6H_5$	C[b,h]	68	220—222[f]	$C_{26}H_{29}N_7O_3$
6b	*m*-$NHCOCH[CH_2CH(CH_3)_2]NHCOOCH_2C_6H_5$	B[d,i,j]	85	209—212(eff)[k]	$C_{26}H_{29}N_7O_3 \cdot HCl$
7a	*p*-$NHCOCH(CH_2C_6H_5)NHCOOCH_2C_6H_5$	C[b]	81	235—236[c]	$C_{29}H_{27}N_7O_3$
7b	*m*-$NHCOCH(CH_2C_6H_5)NHCOOCH_2C_6H_5$	B[d,i,j]	81	174—176(eff)[l]	$C_{29}H_{27}N_7O_3 \cdot HCl$
11a	*p*-$NHCOCH_2NH_2$	D[m,n]	42[o]	222—224	$C_{14}H_{15}N_7O$
11b	*m*-$NHCOCH_2NH_2$	D[m,n]	72	179—185[p]	$C_{14}H_{15}N_7O$
12a	*p*-$NHCOCH[CH_2CH(CH_3)_2]NH_2$	D[m,n]	91	213—214[q]	$C_{18}H_{23}N_7O$
13a	*p*-$NHCOCH(CH_2C_6H_5)NH_2$	D[m,n]	71[q]	221—223	$C_{21}H_{21}N_7O$
13b	*m*-$NHCOCH(CH_2C_6H_5)NH_2$	E	88	293—294(dec)[r]	$C_{21}H_{21}N_7O \cdot 2\ HCl$

[a]All compounds were analyzed for C, H, N and were within ±0.4% of the theoretical values.
[b]For the starting amine see [1].
[c]Recrystallized from 2-methoxyethanol.
[d]For the starting amine see [2].
[e]Melted 177—179°C and resolidified.
[f]Recrystallized from methanol.
[g]Presence of methanol was supported by the 60 mc NMR.
[h]The residual solid was treated with aqueous sodium bicarbonate and an aqueous wash as in Method B.
[i]The starting amine was added dissolved in acetic acid.
[j]The crude solid or syrup was dissolved in hydrogen chloride saturated ethanol and then spin evaporated *in vacuo* to form the hydrochloride salt.
[k]Recrystallized from ethanol.
[l]Recrystallized from propanol.
[m]The residual syrup was dissolved in water and basified with aqueous sodium hydroxide.
[n]Uptake of hydrogen was slow and required 12—18 h.
[o]Recrystallized from 2-methoxyethanol—methanol.
[p]Recrystallized from methanol—benzene.
[q]Recrystallized from 2-propanol.
[r]Recrystallized from ethanol—water.

Table II. Derivatives of 9-benzyl-6-dimethylaminopurine.



Compd.	R	Method	Yield (%)	mp °C	Formula[a]
4a	*p*-NH_2	A[b]	92	194—195[c]	$C_{14}H_{16}N_6$
4b	*m*-NH_2	A	94	156—157[c]	$C_{14}H_{16}N_6$
4c	*o*-NH_2	A[d]	96	198—199[c]	$C_{14}H_{16}N_6$
8a	*p*-$NHCOCH_2NHCOOCH_2C_6H_5$	C	71	181—182[e]	$C_{24}H_{25}N_7O_3$
8c	*o*-$NHCOCH_2NHCOOCH_2C_6H_5$	B[f]	56	217—218[g]	$C_{24}H_{25}N_7O_3$
9a	*p*-$NHCOCH[CH_2CH(CH_3)_2]NHCOOCH_2C_6H_5$	C	52[h]	108—109[i]	$C_{28}H_{33}N_7O_3$
10a	*p*-$NHCOCH(CH_2C_6H_5)NHCOOCH_2C_6H_5$	B[j]	82[g]	187—188	$C_{31}H_{31}N_7O_3$
10b	*m*-$NHCOCH(CH_2C_6H_5)NHCOOCH_2C_6H_5$	B	88	196—197[k]	$C_{31}H_{31}N_7O_3$
10c	*o*-$NHCOCH(CH_2C_6H_5)NHCOOCH_2C_6H_5$	B[f,l]	82[g]	181—182	$C_{31}H_{31}N_7O_3$
14a	*p*-$NHCOCH_2NH_2$	D[m,n]	91	171—173[e]	$C_{16}H_{19}N_7O \cdot CH_3CO_2H$
14c	*o*-$NHCOCH_2NH_2$	D[o]	85[p]	253—255	$C_{16}H_{19}N_7O \cdot HCl$[q]
15a	*p*-$NHCOCH[CH_2CH(CH_3)_2]NH_2$	D[o]	82	207—208(eff)[r]	$C_{20}H_{27}N_7O \cdot 2\ HCl$[s]
16a	*p*-$NHCOCH(CH_2C_6H_5)NH_2$	D[t]	30	175—178[u]	$C_{23}H_{25}N_7O$
16b	*m*-$NHCOCH(CH_2C_6H_5)NH_2$	D	92	282—283(dec)[u]	$C_{23}H_{25}N_7O \cdot 2\ HCl$
16c	*o*-$NHCOCH(CH_2C_6H_5)NH_2$	D[m,t,v]	38[c]	139—141(eff)	$C_{23}H_{25}N_7O$[w,x]

[a]All compounds were analyzed for C, H, N and were within ±0.4% of the theoretical values.
[b]For the starting nitrobenzylpurine see [1].
[c]Recrystallized from ethanol.
[d]For the starting nitrobenzylpurine see [3].
[e]Recrystallized from ethyl acetate—methanol.
[f]The starting amine was added dissolved in acetic acid.
[g]Recrystallized from 2-propanol.
[h]Yield of oil which was used in the next reaction.
[i]Recrystallized from ethanol—water.
[j]Crude product digested with 2-propanol.
[k]Recrystallized from ethanol—2-methoxyethanol.
[l]The bicarbonate and aqueous washes were omitted.
[m]Hydrogenation carried out in methanol—acetic acid, 1/1.
[n]Residual syrup crystallized from ethyl acetate.
[o]Hygroscopic.
[p]Recrystallized from methanol—ether.
[q]Calc'd. for Cl: 9.83: found; 9.97.
[r]Recrystallized from ethanol—ether.
[s]Calc'd. for Cl: 15.6; found 15.7.
[t]The residual syrup was dissolved in water, basified with 5% aqueous sodium bicarbonate, extracted with chloroform and evaporated.
[u]Recrystallized from methanol.
[v]Residual syrup dissolved in hot ethanol and 0.6 eq of fumaric acid was added.
[w]Half fumarate hydrate.
[x]Calc'd. for O : 13.02: found: 13.06.

acetic acid. Hydrogenolysis of the 6-aminopurines was variable and slow, which made the hydrogen bromide method more attractive for **11**—**13**.

Results and Discussion

Nine of these compounds (**4a**, **10a**, **10c**, **13b**, **14c**, **15a**, **16a**, **16b** and **16c**) were tested for *in vitro* anti-bacterial activity [11] against *Streptococcus pyrogenes* (CN10), *Streptococcus faecalis* (CN478), *Staphylococcus aureas* (CN491), *Escherichia coli* (CN314), *Salmonella typhosa* (CN512), *Shigella dysenterie* (CN1513), *Klebsiella pneumoniae* (CN3632), *Enterobacter aerogenes* (2200/86), *Enterobacter cloacae* (2200/87), *Citrobacteri freundii* (2200/77), *Proteus vulgaris* (CN329), *Proteus mirabilis* (S2409), *Pseudomonas aeruginosa* (CN200) and *Candida albicans* (CN1863). None of the compounds was inhibitory at 100 μg/ml. Thirteen of the compounds (**4a**, **4b**, **4c**, **6b**, **7b**, **10a**, **10c**, **13b**, **14c**, **15a**, **16a**, **16b** and **16c**) were tested against an *Escherichia*

coli-f_2—RNA protein-synthesizing system [12] and were inactive at 10^{-4} M. Under the same conditions, puromycin showed 50% inhibition at 10^{-6} M.

The compounds in Table III were tested by means of

Table III. Activity against rhinovirus 1B[a].

Compd.	Plaque inhibition activity[b]	IC_{50} (μM)
4a	—	
4b	± T	50
6b	—	
7b	—	
10a	—	
10b	—	
10c	—	
11a	—	
11b	—	
12a	—	
13a	—	
13b	—	
14c	—	
15a	—	
16a	—	
16b	+	17
16c	—	
BW 683C[c]	+	0.007

[a]For methodology, see [13—15].
[b]T = toxic; — = inactive at 50 μg/disc; ± = slight activity; + = active.
[c]For structure, see [16].

plaque inhibition tests against three DNA viruses (vaccinia virus, adenovirus type 5, herpes simplex virus type 1) and against a range of RNA viruses comprising rhinovirus 1B, measles, corona, respiratory syncytial virus and the NWS strain of influenza virus [13—15]. The only compound that was active at 50 μg/disc was **16b**. In plaque reduction assays, **16b** had an IC_{50} = 17 μM against rhinovirus 1B under assay conditions where BW 683C had an IC_{50} = 0.007 μM [16]. Compound **16b** exhibited no toxicity towards the cell monolayer at this concentration. Removal of the methyl substituents on the 6-amino group as in **13b** or substitution of the phenylalanylamido moiety at the *para*- or *ortho*-positions as in **16a** and **16c** led to inactive compounds. Removal of the aminoacyl moiety of **16b** gave **4b** which was one third as active with an IC_{50} = 50 μM.

Discovery of the anti-rhinovirus 1B activity of **16b** has generated a novel lead in the search for agents with potential clinical utility against the rhinoviruses, which are recognized as the most important causative agents of the common cold [17, 18]. Although **16b** is a relatively weak anti-viral compound, further studies on the effect of different aryl substituents may lead to compounds with improved anti-viral activity.

Experimental protocols

Chemistry

Melting points were taken in capillary tubes on a Mel-Temp block and are uncorrected. Each analytical sample had spectral data compatible with its assigned structure and moved as a single spot on thin—layer chromatography (TLC). Analyses indicated by elemental symbols were within ±0.4% of the theoretical values. NMR data were recorded on a Varian XL-100-15-FT or T-60 spectrometer, using tetramethylsilane as an internal standard. UV data were obtained on a Unicam SP 800 spectrophotometer.

6-Chloro-9-(3-nitrobenzyl)-9H-purine ***17***
A mixture of 34.0 g (0.22 mol) of 6-chloropurine, 30.2 g (0.22 mol) of anhydrous potassium carbonate, and 34.3 g (0.20 mol) of α-chloro-3-nitrotoluene in 400 ml of dry dimethylformamide (DMF) was stirred at ambient temperature for 64 h. The reaction mixture was poured into 2 l of ice water with stirring and the white solid that precipitated was collected by filtration. This material was dissolved in 600 ml of hot chloroform and applied to a column (45 × 7.5 cm) of silica gel (900 g, 70—325 mesh) in $CHCl_3$. After the column had cooled it was eluted with chloroform. The first 2 l of eluate which contained some α-chloro-3-nitrotoluene were discarded and elution with 2% ethanol in chloroform began. The subsequent fractions totaling 10 l of eluate which contained the 9-isomer were pooled and spin evaporated *in vacuo*; yield, 40.9 g (70%), mp: 138—140°C. Recrystallization of a portion from ethanol afforded an analytical sample of unchanged mp. [Lit. [2] mp: 141—142°C]: UV λ_{max} (10% EtOH in 0.1 N HCl) 266 nm (ε 16 500), UV λ_{max} (10% EtOH in 0.1 N NaOH) 266 nm (ε 16 500); NMR (DMSO-d_6) δ 8.94 (s, 1H, purine H); 8.81 (s, 1H, purine H); 8.44—7.53 (complex m, 4H, Ar); 5.76 (s, 2H, CH_2Ar). Anal. $C_{12}H_8ClN_5O_2$ (C, H, N).

6-Dimethylamino-9-(3-nitrobenzyl)-9H-purine ***2b***
A mixture of 35.40 g (122 mmol) of **17**, 100 ml of ethanol and 100 ml of 40% aqueous dimethylamine was stirred at ambient temperature for 17 h. The reaction mixture was spin evaporated *in vacuo* to afford a yellow solid which was dispersed with stirring in water, collected by filtration, washed (water) and dried; yield, 36.4 g (99%), mp: 156—158°C. (Lit. [2] mp: 156—157°C prepared at 90°C in a stell bomb.) Recrystallization of a sample from et hanol afforded the analytical sample in a dimorphic form, mp: 166—167°C. UV λ_{max} (10% EtOH in 0.1 N HCl) 270 nm (ε 25 300), UV λ_{max} (10% EtOH in 0.1 N NaOH) 276 nm (ε 24 800). Anal. $C_{14}H_{14}N_6O_2$ (C, H, N).

Method A: 9-(3-aminobenzyl)-6-dimethylamino-9H-purine ***4b***
A mixture of 10.0 g (33.5 mmol) of **2b** dissolved in 200 ml of acetic acid and 0.50 g of 10% palladium on carbon was shaken in the presence of hydrogen at 2—3 atm for 1.5 h. The mixture was filtered and spin evaporated *in vacuo*, then redissolved in ethanol and reevaporated. The crystalline solid was collected and washed with hexanes; yield, 8.44 g (94%), mp: 154—156°C. The analytical sample was recrystallized from ethanol, mp: 156—157°C. UV λ_{max} (10% EtOH in 0.1 N HCl) 270 nm (ε 19 000); UV λ_{max} (10% EtOH in 0.1 N NaOH) 279 nm (ε 20 500); NMR (DMSO-d_6) δ 8.23 (s, 1H, purine H); 8.18 (s, 1H, purine H); 7.11—6.33 (m, 4H, Ar); 5.23 (s, 2H, CH_2Ar); 5.07 (s, 2H, NH_2); 3.47 (s, 6H, $N(CH_3)_2$).

*Method B: 9-(3-*N*-carbobenzoxyphenylalanylamidobenzyl)-6-dimethylamino-9H-purine* ***10b***
To a stirred, ice bath-cooled solution of 19.55 g (65.3 mmol) of DL-*N*-carbobenzoxyphenylalanine and 9.0 ml (64.3 mmol) of triethylamine in 200 ml of dry tetrahydrofuran (THF) were added 6.90 g (63.6 mmol) of ethyl chloroformate and 10 ml of THF. After 10 min, a solution of 8.45 g (31.5 mmol) of **4b** in 300 ml of THF was added and the reaction was stirred at ambient temperature for 15 h within which the solution gave a negative Bratton—Marshall test [8, 9] for aromatic amine. The reaction mixture was diluted with 30 ml of water and spin evaporated *in vacuo*, then dissolved in methanol and reevaporated. The residual solid was dissolved in 600 ml of chloroform and washed with three 100 ml portions of water, four 100 ml portions of 5% aqueous sodium bicarbonate, 50 ml of water, brine, dried and spin evaporated

in vacuo. The residual solid was then digested with 300 ml of ethanol, cooled and collected; yield, 15.31 g (88%), mp: 194—195°C. Recrystallization of a portion from ethanol—2-methoxyethanol afforded the analytical sample, mp: 196—197°C. UV λ_{max} (20% EtOH in 0.1 N HCl) 268 nm (ε 21 000), UV λ_{max} (20% EtOH in H_2O) 276 nm (ε 19 500); NMR (DMSO-d_6) δ 10.05 (s, 1H, NHAr); 8.27 (s, 2H, purine C-2 and C-8); 7.77—6.94 (s over m, 15H, N*H*CH and ArH); 5.40 (s, 2H, NCH_2Ar); 4.98 (s, 2H, OCH_2Ar); 4.47 (m, 1H, C*H*NH); 3.47 (s, 6H, $N(CH_3)_2$); 3.07—2.80 (m, 2H, CH_2CH).

Method C: 9-(4-N-carbobenzoxyglycylamidobenzyl)-6-dimethylamino-9H-purine ***8a***

To a stirred, ice bath-cooled solution of 6.30 g (30.1 mmol) of *N*-carbobenzoxyglycine and 3.02 g (30.1 mmol) of triethylamine in 100 ml of THF were added 3.20 g (29.6 mmol) of ethyl chloroformate in 10 ml of THF. The precipitate of triethylamine hydrochloride was removed by filtration and washed with solvent. To the combined filtrate and washes, stirred and cooled on an ice bath, were added a solution of 4.00 g (14.9 mmol) of **4a** in 50 ml of THF and 25 ml of 10% aqueous acetic acid. After 2 h at ambient temperature, the reaction was diluted with 25 ml of methanol and spin evaporated *in vacuo*. The residual syrup was crystallized from 100 ml of ethyl acetate; yield, 4.90 g (71%), mp: 176—178°C. Recrystallization from ethyl acetate—methanol afforded analytically pure material; yield, 3.95 g (57%), mp: 181—182°C.

Method D: 6-dimethylamino-9-(3-phenylalanylamidobenzyl)-9H-purine dihydrochloride ***16b***

A mixture of 6.25 g (0.114 mmol) of **10b** dissolved in 200 ml of acetic acid and 0.30 g of 10% palladium on carbon was shaken in the presence of hydrogen at 2—3 atm for 3.5 h. The mixture was filtered and spin evaporated *in vacuo*. The residual syrup was dissolved in 100 ml of ethanol, diluted to 500 ml with ether and then diluted with 25 ml of hydrogen chloride-saturated ethanol to afford a flocculent, white solid; yield, 5.15 g (92%), mp: 260°C (dec) (one spot on TLC). Recrystallization from methanol gave analytically pure material; yield, 3.64 g (65%), mp: 282—283°C (dec) (preheat to 270°C). UV λ_{max} (0.1 N HCl) 268 nm (ε 21 000), UV λ_{max} (0.1 N NaOH and H_2O) 276 nm (ε 19 400); NMR (D_2O) δ 8.32 (s, 2H, purine C-2 and C-8); 7.45—7.10 (s over m, 9H, ArH); 5.44 (s, 2H, NCH_2Ar); 4.34 (m, 1H, C*H*NH_2); 3.63 (br s, 6H, $N(CH_3)_2$); 3.30—3.18 (m, 2H, CH_2CH).

Method E: 9-(3-phenylalanylamidobenzyl)adenine dihydrochloride ***13b***

To a stirred solution of 5.18 g (9.27 mmol) of **7b** in 200 ml of glacial acetic acid were added 30 ml of hydrogen bromide (30—32% in acetic acid). After 21 h at ambient temperature, the reaction was diluted with 200 ml of THF. After 1 h, the orange solvent was decanted from the precipitate which was washed with ether and then dissolved in 100 ml of water. The free base was formed using strong base ion exchange resin (Amberlite IRA-400) by the batch method. The filtered aqueous solution was then spin evaporated *in vacuo*, the foam was dissolved in hydrogen chloride-saturated ethanol, and the resultant solid was collected; 3.80 g (88%), mp: 285—290°C (dec). Recrystallization from ethanol—water gave analytically pure material, mp: 293—294°C (dec); UV λ_{max} (0.1 N HCl) 256 nm (ε 21 500); UV λ_{max} (0.1 N NaOH and H_2O) 256 nm (ε 20 100).

Biological methods

Anti-bacterial activity

The *in vitro* anti-bacterial activity was evaluated by the method previously described [11]. Compounds were tested at 100 μg/ml.

Inhibition of protein synthesis

The method used for detecting inhibitors of protein synthesis was described in a previous paper [12].

Anti-rhinovirus assays

The compounds were tested initially in a plaque inhibition assay that gives a qualitative indication of anti-viral activity [14, 15]. Monolayers of M-HeLa cells [19] in 50 mm diameter plastic Petri dishes were infected with rhinovirus type 1B. After adsorption of virus, excess supernatant was decanted and replaced with 10 ml of 0.5% nutrient agarose (Indubiose A37) containing 10% Eagle's minimum essential medium, 2% fetal bovine serum, 4% tryptose phosphate broth, 5% of 4% sodium bicarbonate solution, 0.5% of 3 M magnesium chloride solution [19] and appropriate antibiotics. 6 mm diameter filter paper discs (punched from 3 M paper) were impregnated with 0.01 ml of a solution containing 50 μg of the test compound and placed on the center of the agarose gel. After incubation for 3 days at 33°C, the cultures were fixed with formalized phosphate buffered saline and the plaques were visualized with methyl violet. The compounds diffused from the disc toward the periphery of the dish. No plaques were seen in which there was an adequate anti-viral concentration. If a compound had toxic properties, this appeared as a zone of dead cells in the center of the dish. Thus, a single dish could indicate activity and toxicity in a qualitative manner and serve as a control. These monolayers were not actively growing cultures, but were sustained by maintenance medium.

If the compound was active or slightly active, the 50% inhibitory concentration (IC_{50}) was measured with the plaque reduction assay. These assays were prepared as above except that, instead of introducing the compounds on paper discs, they were incorporated as doubling dilutions in the agarose overlay. At the end of the experiment, the plaques were counted and expressed as a percentage of the control plaque count and plotted against the logarithm of the compound concentration. The IC_{50} could then be estimated readily. A subjective view of compound toxicity could be obtained in this assay. There would be evidence of cell death from the reduced dye color. This would usually suggest toxicity at a known concentration in a maintained monolayer.

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