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Certain 8-amino-9-(benzyl)guanines as potential purine nucleoside phosphorylase inhibitors

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Summary — Several 8-amino-9-(benzyl)guanines were selected for synthesis as potential purine nucleoside phosphorylase (PNP) inhibitors on the basis of the Topliss decision tree. These compounds were prepared by the treatment of oxazolo[5,4-*d*]pyrimidine intermediates with potassium carbonate in a 1-pot reaction. All compounds were evaluated for PNP inhibitory activity using an *in vitro* enzyme inhibition assay. The extent of binding to PNP appeared to be influenced by the presence of electron donating substituents on the phenyl ring of the benzyl group. None of the tested compounds were more active than the parent compound, 8-amino-9-benzylguanine. The inhibitory activity seems to be most likely $-\sigma$-dependent.

purine nucleoside phosphorylase / structure–activity relationships / enzyme inhibition / 8-aminoguanine / 8-amino-9-(benzyl)guanines

Introduction

Human erythrocytic purine nucleoside phosphorylase (PNP, nucleoside phosphorylase, purine nucleoside, orthophosphate ribosyltransferase, EC 2.4.2.1) is an enzyme in the purine salvage pathway which catalyzes the reversible phosphorolysis of guanosine, inosine, xanthosine, their 2'-deoxyribonucleoside congeners, and many closely related analogs [1]. The development of a PNP inhibitor as a chemotherapeutic target became of interest with the discovery that patients with T-cell-related immunodeficiency diseases demonstrated an associated lack of PNP in their erythrocytes [2]. This rare syndrome suggested that suppression of PNP may result in a selective suppression of cellular immunity which may have therapeutic benefits. For example, a successful PNP inhibitor might be used to treat T-cell leukemia; to counter autoimmune diseases without destroying the patient's humoral immunity; to treat tissue rejection after organ transplantation [3]; or, to treat parasitic diseases [4]. A number of PNP inhibitors which resemble purine bases or their nucleosides have been identified [5–7]. Among these, 8-aminoguanine **1** (K_i = 0.8 μM), and 8-aminoguanosine **2** (K_i = 8 μM) [8], were the most potent inhibitors of PNP (see fig 1). Cysteine [9], histidine [10] and arginine [11] have been identified and implicated as participants in the catalytic mechanism of mammalian PNP. It has been proposed that the cysteinyl residue protonates the N-7 of the base moiety [12]. The PNP inhibitory activity of **1** and **2** suggested that the C-8 amino group in these inhibitors might act to increase the electron density in the ring system and thus increase the basicity at N-7 for the subsequent protonation by a cysteinyl residue at the active site of PNP. Based on this, we further assumed that the

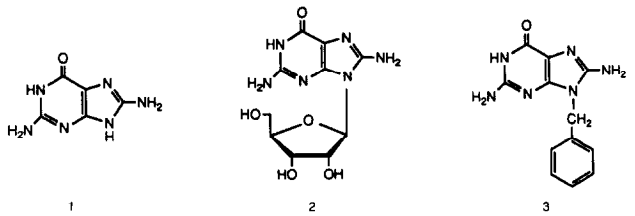

Fig 1. Structures of 8-aminoguanine **1**, 8-aminoguanosine **2**, and 8-amino-9-benzylguanine **3**.

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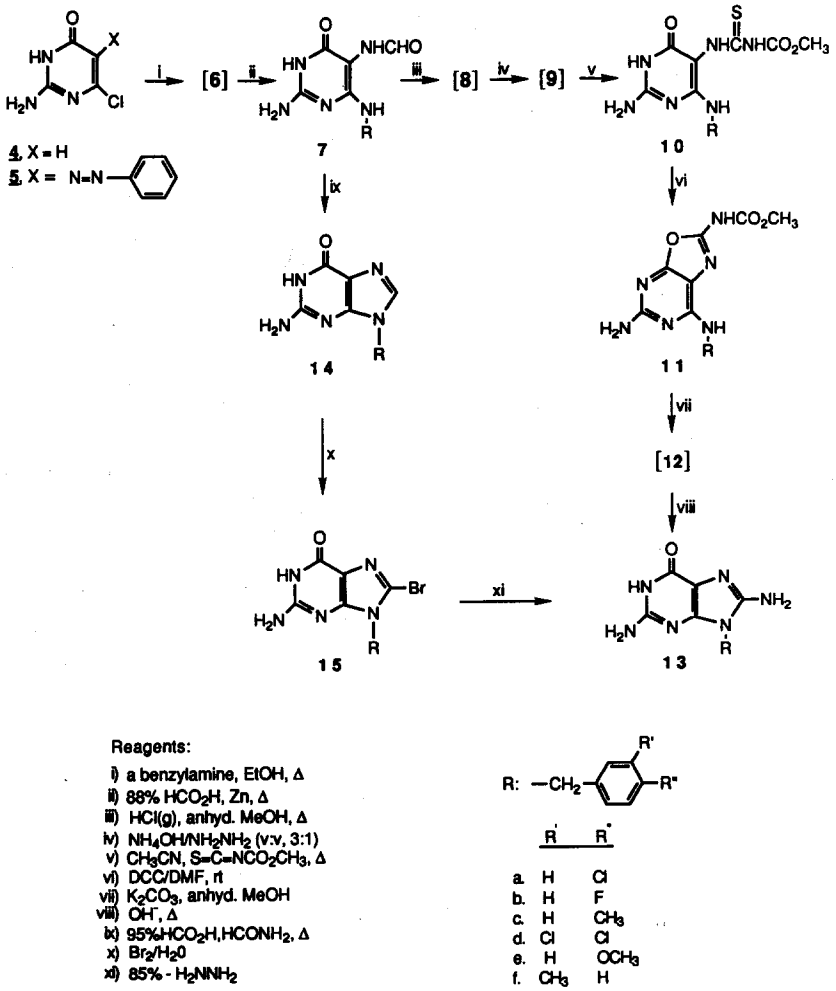
8-aminoguanine nucleus plays an important role in the recognition and in the binding of the molecule to the active site of the enzyme and that at the active site the heterocycle of guanosine or 8-aminoguanosine most likely occupies a pocket in the enzyme with the ribose moiety oriented exterior to the pocket. To determine if a substituent other than a sugar moiety at N-9 would be recognized by PNP, we prepared 8-amino-9-benzylguanine **3**, 8-ABG [8], as a potential inhibitor of PNP. Compound **3** was found to be a potent PNP inhibitor with a K_i value of 0.22 μM, 4-fold more active than 8-aminoguanine. It has been shown in *in vitro* experimental studies that 8-ABG potentiates the cytotoxic effect of 2'-deoxyguanosine.

This current study was designed to determine if a substituent on the benzyl ring might possibly maximize the PNP inhibitory activity demonstrated by **3**. To guide our selection of an appropriate electron-donating or withdrawing substituent to place on the phenyl ring, we chose the methodology designed by Topliss [13–15] which allows one to prepare a limited number of derivatives containing substituents which would give good discrimination between hydrophobic (σ), electronic (π) and steric (E_s) effects and aid in identifying the class of derivatives with the highest probability of enhanced potency.

Chemistry

The target compounds were prepared by a multi-step synthesis, as outlined in scheme 1. Treatment of 2-amino-4-chloropyrimidin-6-one **4** with phenyldiazonium chloride furnished 2-amino-4-chloro-5-phenylazopyrimidin-6-one **5** [16]. Condensation of **5** with the appropriate benzylamines afforded a series of 2-amino-4-arylmethylamino-5-phenylazopyrimidin-6-ones **6a–f**. These compounds were unstable and decomposed on attempts to purify them. However,



Scheme 1.

after being washed thoroughly with alcohol and ether, the crude compounds **6a–f** were observed as a single spot on TLC and were of sufficient purity for the next reaction.

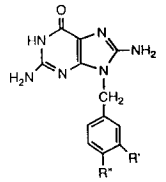
Reductive formylation of the crude 5-diazopyrimidines **6a–f** with zinc dust in formic acid [17] gave the 5-formamidopyrimidines **7a–f** in good yield. The 360-MHz spectra of **7a–f** in DMSO–d_6 revealed multiple signals for the proton of the 5-formamido group indicating that these compounds most likely exist as a tautomeric pair (both the *trans* (*E*) (70%) and *cis* (*Z*) (30%) conformers) similar to previous observations with other *N*-substituted amides [18, 19].

Heating compounds **7a–f** in methanol saturated with hydrogen chloride at reflux temperature effected deformylation and formation of the hydrochloride salt of the 2,5-diaminopyrimidine derivatives **8a–f** [20]. Without isolation compounds **8a–f** were neutralized with ammonium hydroxide and hydrazine (v/v, 3:1) to afford the free bases **9a–f**. These derivatives were immediately condensed with methoxycarbonyl isothiocyanate [21] to afford the thiourea derivatives **10a–f** in 70 to 88% overall yield from **7a–f**. Compound **10e** was isolated in 31% yield. Treatment of the thiourea derivatives **10a–f** with dicyclohexylcarbodiimide (DCC) in DMF at room temperature furnished the oxazolo[5,4-*d*]pyrimidine intermediates **11a–f** in good yield. In a 2-step reaction sequence compounds **11a–f** were treated with 2 equivalents of potassium carbonate to effect a ring opening and rearrangement to give the 8-methoxycarbamoylguanines **12a–f**. TLC analysis indicated that the products **12a–f** were accompanied by a small amount of the 8-aminoguanine derivatives **13a–f**. As a result of this contamination, the derivatives **12a–f** were not isolated but were converted directly to **13a–f** by removal of the reaction solvent *in vacuo* followed by suspension in water and heating the mixture at reflux for 24 h. The reaction mixture was then neutralized with glacial acetic acid to obtain the target 8-aminoguanines **13a–f** in good yield. This approach provided a clean reaction with a relatively easy isolation and purification of the target compounds **13a–f**. Compound **13a** was also prepared in a more conventional manner. In this procedure, compound **7a** was cyclized by treatment with formic acid and formamide to furnish the guanine **14a**. Treatment of **14a** with saturated bromine water solution afforded 8-bromo-9-(*p*-chlorobenzyl)-guanine **15a** in good yield [22]. However, when **15a** was treated at reflux with aqueous hydrazine [23], 8-amino-9-(*p*-chlorobenzyl)guanine **13a** was obtained in only 24% yield.

Biological activity

The 8-amino-9-(benzyl)guanine derivatives **13a–f** were evaluated for PNP inhibitory activity using an *in vitro* enzyme inhibition assay [9]. Double reciprocal plots of the initial velocity data indicated that these compounds inhibited PNP in a competitive manner (with respect to inosine). K_i values were estimated from a replot of the slopes *versus* inhibitor concentrations. These results have been summarized in table I. None of these derivatives showed an increase in inhibitory activity of PNP over the parent benzyl derivative **3** with a K_i value of 0.22 μM. However, all of the derivatives except **13d** were superior PNP inhibitors compared to 8-aminoguanosine (K_i = 8 μM). The selection of the substituents on the benzyl ring was made based upon the Topliss selection tree [13–15]. No obvious correlation between substituent and activity could be delineated from this study. The *p*-chloro derivative **13a** was found to be 10-fold less potent than compound **3**, thus indicating a possible unfavorable effect from a *para* substituent for steric reasons, or that the decreased activity is a result of $-\sigma$ or $-\pi$ effects by the 4-Cl substituent. That the effect

Table I. Inhibition constants of some 8-amino-9-(benzyl)-guanines for purine nucleoside phosphorylase from T-lymphoblasts.



Compd	K_i (μM)	*Rank order* Obsd[c]	*Rank order* Calcd[c] for $-\sigma$
13d 3,4-Cl_2	> 100 (2)	5	5
13a 4-Cl	3.06 (2)[a]	4	4
13c 4-CH_3	1.22 (2)	3	2
13e 4-OCH_3	0.74 (2)	2	1
3	0.22 (2)	1	3
8-Aminoguanosine	7.0 ± 3[b] (4)		
Second compound group			
13f 3-CH_3	1.67 (2)		
13b 4-F	1.11 (2)		

[a]Numbers in parentheses, number of experimental determinations of each kinetic constant; [b]mean ± SD; [c]see [13, 14].

is not enhanced by $+\pi$ or $+\sigma$ substitution is apparent from the total lack of inhibition of PNP by the 3,4-dichlorobenzyl derivative **13d**. The 4-methoxybenzyl derivative **13e**, a second-tier derivative on the Topliss tree, was more active than the 4-chlorobenzyl compound **13a**. This observation supported the preparation of other compounds which possess a $-\sigma$ effect, and, on this basis the 3-methylbenzyl derivative **13f** and 4-fluorobenzyl derivative **13b** were prepared. Although these compounds were active, neither demonstrated increased activity compared to **3**. Thus, although a number of the $-\sigma$-directed derivatives of **3** demonstrated potent inhibition of PNP, a definitive structure–activity relationship due to these changes was not apparent. It can be concluded from this study that the 8-aminoguanine nucleus must play the primary role in binding the molecule to the active site and that the enzyme can tolerate a bulky group such as a benzyl group at N-9. Substituents on the benzyl moiety are well tolerated and do not affect, significantly, the activity demonstrated by this class of compounds.

Experimental protocols

General methods

Melting points were determined using a Thomas–Hoover capillary melting apparatus and are uncorrected. ^{1}H-NMR spectra were obtained at 360 MHz using a Bruker Wm 360 spectrometer. The chemical shifts are reported in ppm (δ) downfield from internal Me_4Si. IR spectra were recorded using a Perkin–Elmer Model 281 infrared spectrophotometer and the values are expressed in cm^{-1}. Analytical samples were dried at 78°C in the presence of P_2O_5 for at least 12 h. TLC was performed on silica-gel GHLF TLC plates (250 microns) and were visualized by UV. Microanalyses were performed by M-H-W Laboratories, Phoenix, AZ. All compounds were analyzed for C, H, N. Analytical results were within ± 0.4% of the theoretical values.

*2-Amino-4-(p-chlorobenzyl)amino-5-formamidopyrimidin-6-one **7a***

4-Chlorobenzylamine (3.0 ml, 25 mmol) was added to a suspension of 2-amino-4-chloro-5-phenylazopyrimidin-6-one **5** [10] (5.0 g, 20 mmol) in absolute EtOH (50 ml). The mixture was heated at reflux in an oil bath for 6 h. Water (100 ml) was added to the mixture and after standing at 25°C for 18 h the solid which had formed was collected by filtration, washed with H_2O (5 ml) and then ether (15 ml) to furnish 2-amino-4-(*p*-chlorobenzyl)amino-5-phenylazopyrimidin-6-one **6a** (5.7 g, 91%). To a mixture of crude **6a** in 88% formic acid (40 ml) was added zinc dust in small portions until the initially orange-colored mixture became colorless. The mixture was heated on a steam bath for 30 min, filtered and washed with 88% formic acid (10 ml). Upon addition of H_2O (100 ml) to the filtrate, a solid formed which was collected by filtration, washed first with H_2O (15 ml), and then with ether (10 ml) to afford compound **7a**. Recrystallization from a mixture of EtOH and H_2O (v:v, 1:1) gave pure compound **7a** (2.83 g, 82%), mp: 270–271°C; UV λ_{max} nm (ε x 10^4): (MeOH) 275 (1.5); (pH 1) 272 (1.9); (pH 11) 267 (1.4). ^{1}H-NMR (DMSO–d_6): isomer A: (60%), δ 4.43 (pseudotriplet, 2H, CH_2), 6.25 (s, 2H, NH_2), 6.65 (t, 1H, NH), 7.30 (m, 4H, Ar-H), 8.03 (s, 1H, CHO), 8.49 (s, 1H, NH), 10.02 (s, 1H, NH); isomer B: (40%), δ, 4.43 (pseudotriplet, 2H, CH_2), 6.33 (s, 2H, NH_2), 6.97 (t, 1H, NH), 7.30 (m, 4H, Ar-H), 7.70 (d, 1H, CHO), 7.90 (d, 1H, NH), 10.02 (s, 1H, NH). Anal $C_{12}H_{12}ClN_5O_2$ (C, H, N).

*2-Amino-4-(p-fluorobenzyl)amino-5-formamidopyrimidin-6-one **7b***

Compound **7b** was prepared in 42% yield using a procedure similar to that which afforded compound **7a**. An analytical sample was prepared by recrystallization from a mixture of DMF and water (v:v, 1:1), mp: 242–244°C; IR (KBr): 3500, 3340, 1700–1570, 1480, 1390, 1220, 815, 760 cm^{-1}. ^{1}H-NMR (360 MHz, DMSO–d_6): isomer A: (60%), δ 4.45 (pseudotriplet, 2H, CH_2), 6.29 (s, 2H, NH_2), 6.65 (t, 1H, NH), 7.07–7.12 (m, 2H, Ar-H), 7.30–7.33 (m, 2H, Ar-H), 8.05 (s, 1H, CHO), 8.51 (s, 1H, NH), 10.05 (s, 1H, NH); isomer B: (40%), δ 4.45 (pseudotriplet, 2H, CH_2), 6.37 (s, 2H, NH_2), 6.97 (t, 1H, NH), 7.07–7.12 (m, 2H, Ar-H), 7.30–7.33 (m, 2H, Ar-H), 7.72 (d, 1H, J = 11.6 Hz, CHO), 7.92 (d, 1H, J = 11.6 Hz, NH), 10.06 (s, 1H, NH). Anal $C_{12}H_{12}FN_5O_2$ (C, H, N).

*2-Amino-4-(p-methylbenzyl)amino-5-formamidopyrimidin-6-one **7c***

Compound **7c** was prepared in 71% yield using a procedure similar to that which afforded compound **7a**. An analytical sample was prepared by recrystallization from a mixture of MeOH and H_2O (v:v, 1:1), mp: 253–254°C; IR (KBr): 3480, 3420, 3320, 3000, 2910, 2730, 1680–1580, 1490, 1380, 1340, 1180, 1100, 760 cm^{-1}. ^{1}H-NMR (DMSO–d_6): isomer A: (60%), δ 2.24 (s, 3H, CH_3), 4.41 (pseudotriplet, 2H, CH_2), 6.24 (s, 2H, NH_2), 6.52 (t, 1H, NH), 7.08 (m, 2H, Ar-H), 7.16 (m, 2H, Ar-H), 8.03 (s, 1H, CHO), 8.40 (s, 1H, NH), 10.01 (s, 1H, NH): isomer B: (40%), δ 2.24 (s, 3H, CH_3), 4.41 (pseudotriplet, 2H, CH_2), 6.32 (s, 2H, NH_2), 6.84 (t, 1H, NH), 7.08 (m, 2H, Ar-H), 7.16 (m, 2H, Ar-H), 7.70 (d, 1H, J = 11.2 Hz, CHO), 7.88 (d, 1H, J = 11.2 Hz, NH), 10.01 (s, 1H, NH). Anal $C_{13}H_{15}N_5O_2 \cdot 1/3\ H_2O$ (C, H, N).

*2-Amino-4-(3,4-dichlorobenzyl)amino-5-formamidopyrimidin-6-one **7d***

Compound **7d** was prepared in 69% yield using a procedure similar to that which afforded compound **7a**. An analytical sample was prepared by recrystallization from a mixture of DMF and water (v:v, 1:1), mp: 264–265°C; IR (KBr): 3500, 3350, 3080, 2880, 2740, 1715, 1690–1570, 1390, 1130, 1030, 765 cm^{-1}. ^{1}H-NMR (DMSO–d_6): isomer A: (60%), δ 4.43 (pseudotriplet, 2H, CH_2), 6.27 (s, 2H, NH_2), 6.70 (t, 1H, NH), 7.22 (d, 1H, Ar-H), 7.50–7.55 (m, 2H, Ar-H), 8.05 (s, 1H, CHO), 8.53 (s, 1H, NH), 10.07 (s, 1H, NH): isomer B: (40%), δ 4.43 (pseudotriplet, 2H, CH_2), 6.36 (s, 2H, NH_2), 7.03 (t, 1H, NH), 7.22 (d, 1H, Ar-H), 7.50–7.55 (m, 2H, Ar-H), 7.71 (d, 1H, J = 11.2 Hz, CHO), 7.93 (d, 1H, J = 11.2 Hz, NH), 10.07 (s, 1H, NH). Anal $C_{12}H_{11}Cl_2N_5O_2$ (C, H, N).

*2-Amino-4-(4-methoxybenzyl)amino-5-formamidopyrimidin-6-one **7e***

Compound **7e** was prepared in 81% yield using a procedure similar to that which afforded compound **7a**. An analytical sample was prepared by recrystallization from a mixture of DMF and water (v:v, 1:1), mp: 256–257°C; IR (KBr): 3370, 3210, 3070, 2910, 2780, 1705, 1660, 1640, 1580, 1510, 1240, 1175, 1030 cm^{-1}. ^{1}H-NMR (DMSO–d_6): 3.71 (s, 3H, OCH_3),

4.40 (d, 2H, CH_2), 6.30 (s, 2H, NH_2), 6.37 (s, 2H, NH_2), 6.54 (t, H, NH), 6.85 (d, 2H, J = 8.2 Hz), 7.21 (d, 2H, J = 7.54 Hz), 7.72 (d, 1H, J = 12 Hz), 7.91 (d, 1H, J = 12 Hz), 8.05 (s, 1H, CHO), 8.51 (s, 1H, CHO); 10.07 (s, 1H, NH). Anal $C_{13}H_{15}N_5O_3$ (C, H, N).

2-Amino-4-(p-chlorobenzyl)amino-5-[1-(3-methoxycarbonyl)-thioureido]pyrimidin-6-one ***10a***

A suspension of **7a** (2.0 g, 6.81 mmol) in anhydrous MeOH (30 ml) was heated at reflux while dry HCl was passed through the mixture. The mixture became a solution within a few min and then became a suspension again. After 2 h heating, the mixture was cooled in an ice bath for 30 min. The solid was collected by filtration, washed with anhydrous ether (10 ml) and air dried for 6 h to obtain 2,5-diamino-4-(*p*-chlorobenzyl)aminopyrimidin-6-one hydrochloride **8a** (1.75 g, 77%). This crude product **8a** was suspended in H_2O (30 ml) and the pH of the mixture was adjusted to 8 with a mixture of aqueous NH_4OH and hydrazine (v:v, 3:1). The mixture was stirred at rt for 1 h, then the white solid was collected by filtration and washed with H_2O (5 ml). The solid was then mixed with 3 equivalents of methoxycarbonyl isothiocyanate (prepared by adding methyl chloroformate dropwise to a suspension of potassium thiocyanate in acetonitrile) and water (10 ml). This mixture was heated at reflux for 5 h. The mixture was cooled to rt, the solid collected by filtration and then washed with water (20 ml) to furnish **10a** (1.89 g, 73%). An analytical sample was prepared by recrystallization from a mixture of DMF and water (v:v, 1:1), mp: 248–250°C; IR (KBr): 3500, 3400, 3320, 3180, 3020, 2950, 2840, 2700, 1740, 1670, 1640, 1590, 1490, 1240, 1190, 1045 cm^{-1}; UV λ_{max} nm (ε x 10^4): (MeOH/DMF, v:v, 9:1) 268 (2.3); (pH 1) 278 (2.7); (pH 11) 267 (2.4); ^{1}H-NMR (DMSO–d_6): d 3.70 (s, 3H, CH_3), 4.45 (d, 2H, CH_2), 6.32 (s, 2H, NH_2), 6.92 (t, 1H, NH), 7.33 (q, 4H, Ar-H), 10.04 (s, 1H, NH), 10.13 (s, 1H, NH), 11.19 (s, 1H, NH). Anal $C_{14}H_{15}ClN_6O_3S$ (C, H, N).

2-Amino-4-(p-fluorobenzyl)amino-5-[1-(3-methoxycarbonyl)-thioureido]pyrimidin-6-one ***10b***

Compound **10b** was prepared in 72% yield using a procedure similar to that which afforded **10a**. An analytical sample was prepared by recrystallization from a mixture of DMF/H_2O (v:v, 1:9), mp: 244–245°C; IR (KBr): 3510, 3390, 3330, 3200, 2860, 2700, 1740, 1660, 1645, 1590, 1500, 1255, 1190, 1040 cm^{-1}; UV λ_{max} nm (ε x 10^4): (MeOH/DMF, v:v, 9:1) 266 (2.3); (pH 1) 266 (2.5); (pH 11) 266 (2.3). ^{1}H-NMR (DMSO–d_6): δ 3.69 (s, 3H, OCH_3), 4.44 (d, 2H, J = 5.7 Hz, CH_2), 6.31 (s, 2H, NH_2), 6.89 (t, 1H, NH), 7.07 (t, 2H, Ar-H), 7.36 (q, 2H, Ar-H), 10.03 (s, 1H, NH), 10.12 (s, 1H, NH), 11.17 (s, 1H, NH). Anal $C_{14}H_{15}FN_6O_3S$ (C, H, N).

2-Amino-4-(p-methylbenzyl)amino-5-[1-(3-methoxycarbonyl)-thioureido]pyrimidin-6-one ***10c***

Compound **10c** was prepared in 75% yield using a procedure similar to that which afforded **10a**. An analytical sample was prepared by recrystallization from a mixture of DMF/H_2O (v:v, 1:3), mp: 250–251°C; IR (KBr): 3500, 3390, 3340, 3230, 3180, 3020, 2720, 1740, 1660, 1640, 1345, 1235, 1190, 1050 cm^{-1}; UV λ_{max} nm (ε x 10^4): (MeOH/DMF, v:v, 9:1) 268 (2.3); (pH 1) 268 (2.7); (pH 11) 267 (2.3); ^{1}H-NMR (DMSO–d_6): d 2.25 (s, 3H, CH_3), 3.70 (s, 3H, OCH_3), 4.43 (d, 2H, J = 5.7 Hz, CH_2), 6.30 (s, 2H, NH_2), 6.83 (t, 1H, NH), 7.07 (d, 2H, J = 7.8 Hz, Ar-H), 7.20 (d, 2H, J = 7.8 Hz, Ar-H), 10.01 (s, 1H, NH), 10.12 (s, 1H, NH), 11.17 (s, 1H, NH). Anal $C_{15}H_{18}N_6O_3S$ (C, H, N).

2-Amino-4-(3,4-dichlorobenzyl)amino-5-[1-(3-methoxycarbonyl)-thioureido]pyrimidin-6-one ***10d***

Compound **10d** was prepared in 85% yield using a procedure similar to that which afforded **10d**. An analytical sample was prepared by recrystallization from a mixture of DMF/H_2O (v:v, 1:1), mp: 247–248°C; ^{1}H-NMR (DMSO–d_6): δ 3.70 (s, 3H, OCH_3), 4.45 (d, 2H, CH_2), 6.32 (s, 2H, NH_2), 6.64 (s, 1H, Ar-H), 6.96 (t, 1H, NH), 7.29 (d, 1H, J = 8.2 Hz, Ar-H), 7.51 (d, 1H, J = 8.2 Hz, Ar-H), 10.07 (s, 1H, NH), 10.13 (s, 1H, NH), 11.22 (s, 1H, NH). Anal $C_{14}H_{14}Cl_2N_6O_3S$ (C, H, N).

2-Amino-4-(p-methoxybenzyl)amino-5-[1-(3-methoxycarbonyl)-thioureido]pyrimidin-6-one ***10e***

Compound **10e** was prepared in 31% yield using a procedure similar to that which afforded **10a**. An analytical sample was prepared by recrystallization from a mixture of DMF/H_2O (v:v, 1:3), mp: 234–235°C; IR (KBr): 3480, 3380, 3370, 3200, 3020, 2950, 2840, 2700, 1745, 1660, 1640, 1580, 1550, 1500, 1390, 1340, 1050 cm^{-1}; UV λ_{max} nm (ε x 10^4): (MeOH/DMF, v:v, 9:1) 269 (2.3); (pH 1) 268 (2.7); (pH 11) 267 (2.4); ^{1}H-NMR (DMSO–d_6): d 3.68 (s, 3H, OCH_3), 3.69 (s, 3H, CH_3), 4.40 (d, 2H, CH_2), 6.30 (s, 2H, NH_2), 6.77 (t, 1H, NH), 6.81 (d, 2H, J = 8.6 Hz, Ar-H), 7.23 (d, 2H, J = 8.6 Hz, Ar-H), 10.00 (s, 1H, NH), 10.11 (s, 1H, NH), 11.14 (s, 1H, NH). Anal $C_{15}H_{18}N_6O_4S$ (C, H, N).

2-Amino-4-(m-methylbenzyl)amino-5-[1-(3-methoxycarbonyl)-thioureido]pyrimidin-6-one ***10f***

Compound **10f** was prepared from **5** without isolation of the intermediates **6f** and **7f**. 3-Methylbenzylamine (1.5 ml, 12 mmol) was added to a suspension of **5** (2.84 g, 10 mmol) in absolute EtOH (50 ml). The mixture was heated at reflux for 6 h then water (100 ml) was added. After standing at rt for 18 h the solid was collected, washed with H_2O (10 ml) and ether (15 ml) to give crude **6f**. Without further purification Zn dust was added to a suspension of **6f** in 88% formic acid until the solution became colorless. After filtration to remove the unreacted Zn, water (100 ml) was added to the filtrate. The resulting solid **7f** was collected and washed with water (15 ml) then ether (10 ml). Crude **7f** was converted to compound **10f** in 88% yield using a procedure similar to that which afforded **10a**. An analytical sample was prepared by recrystallization from a mixture of DMF/H_2O (v:v, 1:3), mp: 254–256°C; IR (KBr): 3505, 3400, 3315, 3210, 3190, 3010, 2950, 2900, 2700, 1740, 1660, 1640, 1500, 1390, 1340, 1250, 1190, 1045 cm^{-1}; UV λ_{max} nm (ε x 10^4): (MeOH/DMF, v:v, 9:1) 267 (2.4); (pH 1) 267 (2.7); (pH 11) 266 (2.4); ^{1}H-NMR (DMSO–d_6): d 2.29 (s, 3H, CH_3), 3.70 (s, 3H, CH_3), 4.45 (d, 2H, CH_2), 6.33 (s, 2H, NH_2), 6.93–6.96 (m, 3H, Ar-H + NH), 7.07–7.17 (m, 2H, Ar-H), 10.03 (s, 1H, NH), 10.14 (s, 1H, NH), 11.20 (s, 1H, NH). Anal $C_{15}H_{18}N_6O_3S$ (C, H, N).

Methyl 6-amino-4-(p-chlorobenzyl)aminooxazolo[5,4-d]pyrimidine-2-carbamate ***11a***

A mixture of **10a** (1.85 g, 4.83 mmol) and dicyclohexylcarbodiimide (3.0 g, 14.56 mmol) in DMF (30 ml) was allowed to stir at rt until all of the starting material had reacted (≈ 48 h). The solvent was evaporated *in vacuo* (oil pump) at 60°C to afford a residual solid which was suspended in boiling toluene (25 ml) and stirred for 30 min. The solid which formed was collected by filtration from the hot mixture, washed with boiling toluene (50 ml) and then ether (10 ml) to afford **11a** (1.5 g, 89%). An analytical sample was prepared by recrystallization from a mixture of DMF/H_2O (v:v, 3:7), mp: 289–290°C; IR (KBr): 3500, 3420, 3200, 3100, 2950, 2860, 1770, 1730, 1670–1660, 1460, 1360, 1300, 1215, 1070, 1015 cm^{-1};

UV λ_{max} nm (ε x 10^4): (MeOH) 284 (2.1); (pH 1) 268 (2.1), 306 (1.4); (pH 11) 298 (2.4); ^{1}H-NMR (DMSO–d_6): d 3.69 (s, 3H, CH_3), 4.57 (brs, 2H, CH_2), 6.22 (s, 2H, NH_2), 7.34 (s, 4H, Ar-H), 7.97 (br s, 1H, NH). Anal $C_{14}H_{13}ClN_6O_3$ (C, H, N).

Methyl 6-amino-4-(p-fluorobenzyl)aminooxazolo[5,4-d]pyrimidine-2-carbamate ***11b***

Compound **11b** was prepared in 80% yield using a procedure similar to that which afforded **11a**. An analytical sample was prepared by recrystallization from a mixture of DMF/H_2O (v:v, 2:8), mp: 278–280°C (210°C changed color); IR (Kr): 3940, 3420, 3310, 3190, 3080, 2950, 1770, 1740, 1670, 1650–1600, 1510, 1470, 1350, 1320, 1230, 1070, 780, 755 cm^{-1}; UV λ_{max} nm (ε x 10^4): (MeOH) 284 (2.0); (pH 1) 268 (2.1), 306 (1.3); (pH 11) 298 (2.2); ^{1}H-NMR (DMSO–d_6): δ 3.69 (s, 3H, CH_3), 4.57 (brs, 2H, CH_2), 6.22 (s, 2H, NH_2), 7.10 (t, 2H, Ar-H), 7.37 (q, 2H, Ar-H), 7.94 (br s, 1H, NH), 11.03 (s, 1H, NH). Anal $C_{14}H_{13}FN_6O_3 \cdot 1/4H_2O$ (C, H, N).

Methyl 6-amino-4-(p-methylbenzyl)aminooxazolo[5,4-d]pyrimidine-2-carbamate ***11c***

Compound **11c** was prepared in 87% yield using a procedure similar to that which afforded **11a**. An analytical sample was prepared by recrystallization from a mixture of DMF/H_2O (v:v, 2:8), mp: 280–281°C; IR (KBr): 3500, 3340, 3310, 3220, 3010, 2960, 2860, 1730, 1670, 1730, 1610, 1465, 1445, 1335, 1065 cm^{-1}; UV λ_{max} nm (ε x 10^4): (MeOH) 284 (2.0); (pH 1) 268 (2.0), 305 (1.3); (pH 11) 298 (2.2); ^{1}H-NMR (DMSO–d_6): δ 2.24 (s, 3H, CH_3), 3.72 (s, 3H, OCH_3), 4.55 (brs, 2H, CH_2), 6.20 (s, 2H, NH_2), 7.08 (d, 2H, J = 7.9 Hz, Ar-H), 7.20 (d, 2H, J = 7.9 Hz, Ar-H), 7.87 (br s, 1H, NH), 11.01 (s, 1H, NH). Anal $C_{15}H_{16}N_6O_3$ (C, H, N).

Methyl 6-amino-4-(3,4-dichlorobenzyl)aminooxazolo[5,4-d]pyrimidine-2-carbamate ***11d***

Compound **11d** was prepared in 87% yield using a procedure similar to that which afforded **11a**. An analytical sample was prepared by recrystallization from a mixture of DMF/H_2O (v:v, 3:7), mp: 294–295°C; IR (KBr): 3500, 3310, 3210, 2870, 1725, 1665, 1610, 1360, 1060 cm^{-1}; UV λ_{max} nm (ε x 10^4): (MeOH) 284 (2.1); (pH 1) 267 (2.1), 307 (1.4); (pH 11) 299 (2.4); ^{1}H-NMR (DMSO–d_6): d 3.69 (s, 3H, OCH_3), 4.57 (brs, 2H, CH_2), 6.26 (s, 2H, NH_2), 7.32 (d, 1H, J = 8.4 Hz, Ar-H), 7.53 (s, 1H, Ar-H), 7.56 (s, 1H, Ar-H), 8.00 (br s, 1H, NH), 11.07 (s, 1H, NH). Anal $C_{14}H_{12}Cl_2N_6O_3$ (C, H, N).

Methyl 6-amino-4-(p-methoxybenzyl)aminooxazolo[5,4-d]pyrimidine-2-carbamate ***11e***

Compound **11e** was prepared in 84% yield using a procedure similar to that which afforded **11a**. An analytical sample was prepared by recrystallization from a mixture of DMF/H_2O (v:v, 3:7), mp: 284–285°C; IR (KBr): 3500, 3320, 3200, 3010, 2960, 2860, 1725, 1670–1610, 1510, 1470, 1440, 1350, 1240, 1070 cm^{-1}; UV λ_{max} nm (ε x 10^4): (MeOH) 283 (2.1); (pH 1) 268 (2.0), 306 (1.3); (pH 11) 298 (2.1); ^{1}H-NMR (DMSO–d_6): δ 3.68 (s, 3H, CH_3), 3.69 (s, 3H, CH_3) 4.55 (brs, 2H, CH_2), 6.19 (s, 2H, NH_2), 6.84 (d, 2H, J = 8.4 Hz, Ar-H), 7.26 (d, 2H, J = 8.4 Hz, Ar-H), 7.83 (br s, 1H, NH), 11.00 (s, 1H, NH). Anal $C_{15}H_{16}N_6O_4$ (C, H, N).

Methyl 6-amino-4-(m-methylbenzyl)aminooxazolo[5,4-d]pyrimidine-2-carbamate ***11f***

Compound **11f** was prepared in 87% yield using a procedure similar to that which afforded **11a**. An analytical sample was prepared by recrystallization from a mixture of methanol/H_2O (v:v, 3:7), mp: 274–275°C; IR (KBr): 3500, 3415, 3185, 2955, 1720, 1665, 1640, 1620, 1460, 1440, 1340, 1080 cm^{-1}; UV λ_{max} nm (ε x 10^4): (MeOH) 283 (2.1); (pH 1) 268 (2.2), 306 (1.4); (pH 11) 299 (2.4); ^{1}H-NMR (DMSO–d_6): δ 2.25 (s, 3H, CH_3), 3.69 (s, 3H, OCH_3), 4.56 (brs, 2H, CH_2), 6.21 (s, 2H, NH_2), 7.00–7.18 (m, 4H, Ar-H), 7.87 (br s, 1H, NH), 11.02 (s, 1H, NH). Anal $C_{15}H_{16}N_6O_3$ (C, H, N).

8-Amino-9-(p-chlorobenzyl)guanine ***13a***

Method 1. A mixture of **11a** (0.60 g, 1.7 mmol) and K_2CO_3 (0.5 g, 3.6 mmol) in anhydrous MeOH (20 ml) was heated at reflux for 4 h. The mixture was evaporated to dryness *in vacuo* (oil pump) at 60°C and the residue was dissolved in H_2O (30 ml). The solution was heated at reflux for 48 h. The mixture was cooled to rt and the pH adjusted to 5 with glacial acetic acid. The solid was collected by filtration and washed with H_2O (50 ml). The crude product was dissolved in 0.1 N aqueous NaOH and reprecipitated with glacial acetic acid to give pure **13a** (0.43 g, 82%), mp: > 300°C; UV λ_{max} nm (ε x 10^4): (methanol/DMF, v:v, 9:1) 294 (1.0); (pH 1) 289 (1.0); (pH 11) 257 (1.5), 274 (1.4); ^{1}H-NMR (DMSO–d_6): δ 5.03 (s, 2H, CH_2), 5.99 (s, 2H, NH_2), 6.25 (s, 2H, NH_2), 7.39 (d, 2H, J = 8.4 Hz, Ar-H), 7.19 (d, 2H, J = 8.4 Hz, Ar-H), 10.59 (s, 1H, NH). Anal $C_{12}H_{11}ClN_6O$ (C, H, N).

Method 2. A mixture of **15a** (0.7 g, 1.97 mmol) and 85% hydrazine (30 ml) was heated at reflux. After 12 h, the mixture had become a fine suspension. After 48 h, the mixture was cooled to rt, the solid was collected by filtration and washed first with H_2O (10 ml) and then MeOH (10 ml). The crude product was recrystallized from a mixture of DMF/MeOH (v:v, 1:1) to furnish **13a** (0.14 g, 24%). This material was identical in all respects to **13a** prepared in *Method 1*.

8-Amino-9-(p-fluorobenzyl)guanine ***13b***

A mixture of **11b** (0.57 g, 1.7 mmol) and K_2CO_3 (0.5 g, 3.6 mmol) in anhydrous MeOH (20 ml) was heated at reflux for 4 h. The mixture was evaporated to dryness *in vacuo* (oil pump) at 60°C and the residue was dissolved in H_2O (30 ml). The solution was heated at reflux for 48 h. The mixture was cooled to rt and the pH adjusted to 5 with glacial acetic acid. The solid was collected by filtration and washed with H_2O (50 ml). The crude product was dissolved in 0.1 N aqueous NaOH and reprecipitated with glacial acetic acid to give pure **13b** (0.39 g, 82%), mp: > 300°C; IR (KBr): 3490, 3300, 3140, 2900, 2750, 1690, 1630, 1610, 1560, 1510, 1370, 1220 cm^{-1}; UV λ_{max} nm (ε x 10^4): (MeOH/DMF, v:v, 1:1) 292 (0.8); (pH 1) 254 (1.4), 288 (0.9); (pH 11) 263 (1.2), 270 (1.2); ^{1}H-NMR (DMSO–d_6): δ 5.01 (s, 2H, CH_2), 6.05 (s, 2H, NH_2), 6.31 (s, 2H, NH_2), 7.50 (t, 2H, Ar-H), 7.22 (q, 2H, Ar-H), 10.76 (s, 1H, NH). Anal $C_{12}H_{11}FN_6O$ (C, H, N).

8-Amino-9-(p-methylbenzyl)guanine ***13c***

A mixture of **11c** (0.94 g, 2.86 mmol) and K_2CO_3 (0.79 g, 5.7 mmol) in anhydrous MeOH (30 ml) was heated at reflux temperature for 4 h. The mixture was evaporated to dryness *in vacuo* (oil pump) at 60°C and the resulting solid was dissolved in H_2O (30 ml). Adjusting the pH of the mixture to 7 with glacial acetic acid effected the precipitation of a solid which was collected by filtration and washed with H_2O (20 ml) to obtain a crude product which contained **12c** as the major product and **13c** as the minor product. These crude products were treated with NaOH (0.9 g, 22.5 mmol) in H_2O (30 ml) and the mixture was heated at reflux for 48 h. The pH of the mixture was adjusted to 7 with glacial acetic acid and the resulting solid was collected by filtration and washed with H_2O (20 ml) to afford pure **13c** (0.56 g, 79%), mp: > 300°C; IR (KBr): 3480, 3380, 3290, 3140, 3020, 2890, 2720, 1690, 1640,

1630, 1610, 1560, 1460, 1410, 1370 cm^{-1}; UV λ_{max} nm (ε x 10^4): (MeOH/DMF, v:v, 9:1) 262 (1.3), 294 (0.8); (pH 1) 254 (1.3) 289 (1.0); (pH 11) 261 (1.2), 272 (1.2). ^{1}H-NMR (DMSO–d_6): δ 2.24 (s, 3H, CH_3), 4.98 (s, 2H, CH_2), 6.02 (s, 2H, NH_2), 7.08 (q, 4H, Ar-H), 10.83 (s, 1H, NH). Anal $C_{13}H_{14}N_6O \cdot 1/2H_2O$ (C, H, N).

8-Amino-9-(3,4-dichlorobenzyl)guanine ***13d***

Compound **13d** was prepared in 81% yield using a procedure similar to that which afforded **13c**. An analytical sample was prepared by recrystallization from a mixture of DMF/H_2O (v:v, 1:1), mp: > 300°C; IR (KBr): 3480, 3300, 3160, 2880, 2840, 2740, 1705, 1690, 1630, 1565, 1465, 1390, 1300 cm^{-1}; UV λ_{max} nm (ε x 10^4): (MeOH/DMF, v:v, 9:1) 264 (1.2), 281 (0.7), 294 (0.8); (pH 1) 255 (1.5); (pH 11) 260 (1.3), 272 (1.1); ^{1}H-NMR (DMSO–d_6): δ 5.03 (s, 2H, CH_2), 6.09 (s, 2H, NH_2), 6.32 (s, 2H, NH_2), 7.12 (dd, 1H, J = 1.98 Hz, J = 8.32 Hz, Ar-H), 7.44 (d, 1H, J = 1.95 Hz, Ar-H), 7.59 (d, 1H, J = 8.31 Hz, Ar-H), 10.75 (s, 1H, NH). Anal $C_{12}H_{11}Cl_2N_6O$ (C, H, N).

8-Amino-9-(p-methoxybenzyl)guanine ***13e***

Compound **13e** was prepared in 84% yield using a procedure similar to that which afforded **13c**. An analytical sample was prepared by dissolving a sample in 0.1 N aqueous NaOH and reprecipitating with glacial acetic acid, mp: > 300°C; UV λ_{max} nm (ε x 10^4): (MeOH/DMF, v:v, 9:1) 264 (1.1), 283 (0.7), 293 (0.7); (pH 1) 254 (1.3), 288 (1.0); (pH 11) 259 (1.3), 274 (1.3); ^{1}H-NMR (DMSO–d_6): δ 3.69 (s, 3H, CH_3), 4.95 (s, 2H, CH_2), 6.02 (s, 2H, NH_2), 6.32 (s, 2H, NH_2), 6.86 (d, 2H, J = 8.6 Hz, Ar-H), 7.15 (d, 2H, J = 8.6 Hz, Ar-H), 10.80 (s, 1H, NH). Anal $C_{13}H_{14}N_6O_2$ (C, H, N).

8-Amino-9-(m-methylbenzyl)guanine ***13f***

Compound **13f** was prepared in 89% yield using a procedure similar to that which afforded **13c**. An analytical sample was prepared by recrystallization from a mixture of DMF/H_2O (v:v, 1:1), mp: > 300°C; IR (KBr): 3490, 3390, 3290, 3150, 2890, 2740, 1690, 1620, 1560, 1460, 1400, 1375 cm^{-1}; UV λ_{max} nm (ε x 10^4): (MeOH/DMF, v:v, 9:1) 261 (1.4), 294 (0.8); (pH 1) 254 (1.3), 289 (1.0); (pH 11) 261 (1.2), 272 (1.2); ^{1}H-NMR (DMSO–d_6): δ 2.24 (s, 3H, CH_3), 5.00 (s, 2H, CH_2), 6.13 (s, 2H, NH_2), 6.30 (s, 2H, NH_2), 6.94 (d, 1H, J = 7.61 Hz, Ar-H), 6.99 (s, 1H, Ar-H), 7 05 (d, 1H, J = 7.61 Hz, Ar-H), 7.19 (t, 1H, Ar-H), 10.69 (s, 1H, NH). Anal $C_{13}H_{14}N_6O \cdot 1/4H_2O$ (C, H, N).

9-(p-Chlorobenzyl)guanine ***14a***

A mixture of **7a** (0.88 g, 3 mmol) in 95% formic acid (5 ml) and formamide (25 ml) was heated at reflux for 5 h. The mixture was then poured onto ice/H_2O (150 ml). The resulting solid was collected by filtration and then washed with H_2O (15 ml). Recrystallization of the solid from a mixture of DMF/water (v:v, 1:1) furnished **14a** (0.76 g, 92%), mp: 336–338°C; UV λ_{max} nm (ε x 10^4): (MeOH) 256 (1.4); (pH 1) 255 (1.3), 277 (0.9); (pH 11) 269 (1.2); ^{1}H-NMR (DMSO–d_6): d 5.16 (s, 2H, CH_2), 6.46 (s, 2H, NH_2), 7.22 (d, 2H, J = 8.42 Hz, Ar-H), 7.39 (d, 2H, J = 8.46 Hz, Ar-H), 7.77 (s, 1H, H-8), 10.60 (s, 1H, NH). Anal $C_{12}H_{10}ClN_5O$ (C, H, N).

8-Bromo-9-(p-chlorobenzyl)guanine ***15a***

Bromine (1 ml) was added to a suspension of **14a** (1.12 g, 3.16 mmol) in H_2O (50 ml). The mixture was allowed to stir at rt for 48 h. The excess Br_2 was removed in the fume hood. The red solid was collected by filtration and washed with water (10 ml). The crude product was dissolved in a 10% aqueous NaOH solution and reprecipitated by the addition of glacial acetic acid to afford **15a** (0.7 g, 49%), mp: > 300°C; UV λ_{max} nm (ε x 10^4): (MeOH) 262 (1.6); (pH 1) 260 (1.6); (pH 11) 273 (1.4); ^{1}H-NMR (DMSO–d_6): δ 5.16 (s, 2H, CH_2), 6.62 (s, 2H, NH_2), 7.20 (d, 2H, J = 8.4 Hz, Ar-H), 7.42 (d, 2H, J = 8.4 Hz, Ar-H), 10.74 (s, 1H, NH). Anal $C_{12}H_9BrClN_5O$ (C, H, N).

PNP inhibitory assay [9]

Substrate [8-^{14}C] inosine was used at a specific activity of 22.5 mCi/mmol. For K_m determinations, a fixed amount of diluted cell extract was incubated with variable amounts of radiolabeled substrate (12.5–100 μM) and inorganic phosphate (50 μM). K_i determinations were performed with variable radiolabeled inosine concentrations (12.5–100 μM), fixed inorganic phosphate (50 μM) and variable inhibitor concentrations (0.03–1.0 μM). All reactions were incubated for 10 min at 37°C. Substrate and product (inosine and hypoxanthine, or guanosine and guanine, respectively) were separated by high voltage paper electrophoresis. The radiolabeled product of the reaction was visualized by UV light (290 nm), cut from the paper and counted in a toluene-based scintillation fluid in a Packard Tri-carb liquid scintillation spectrometer. Enzyme-free blank reactions were used as controls for all reactions. In all initial velocity determinations with or without inhibitor, not more than 15% of the substrate was converted to product. Double reciprocal plots of the initial velocity values *versus* the substrate concentrations were linear.

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