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Bioorganic & Medicinal Chemistry 12 (2004) 3135–3139
Bioorganic & Medicinal Chemistry

Synthesis and in vitro antimicrobial activities of 2-hydroxy-6-methyl-7-(arylamino)-1,7-dihydropurin-8-ones

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Received 25 February 2004; revised 7 April 2004; accepted 8 April 2004

Abstract—A number of 2-hydroxy-6-methyl-7-(arylamino)-1,7-dihydropurin-8-ones have been synthesized. 3-Oxo-2-(arylhydrazono)butyric acid ethyl ester were acetylated and treated with triethyl amine and formamide in presence of 1,4-dioxane to yield *N*-(5-acetyl-4-ethoxy-2-oxo-2,5-dihydro-imidazol-1-yl)-*N*-arylacetamide, which on refluxation with urea and freshly prepared sodium ethoxide yielded the title compound. All the newly synthesized compounds have been characterized by spectroscopic and elemental analysis data. The synthesized compounds were screened against a representative panel of susceptible and resistant Gram-positive and Gram-negative bacteria using a standard antibiotic drug purinthol as control. Quantitative structure–activity relationship has also been interpreted in terms of correlation of biological activity with molecular refractive index parameters (M_R) and Hammett substituent constant (σ).

1. Introduction

Purines are reported to have a wide range of biological activities.[1–4] The application of purine and its derivatives is found in the research areas as cyclin-dependent kinase inhibitors,[5] antitumour,[6] antimycobacterial,[7,8] corticotropin releasing hormone receptor antagonist,[9] anti-rhinovirus,[10] xanthine oxidase inhibitor[11] and anticonvulsant agents.[12] They appear in nucleic acids and cofactors that play an essential role in the modulation of protein function and signal transduction.[13] These and other findings have resulted in large number of synthetic investigations.[14–17]

Resistance of various bacteria against commercially available antibiotics is increasing day-by-day.[18] As a result need for new antibiotic agents to combat this problem is also increasing. These threats have rekindled our interest to search for new types of lead compounds.

In the present context and in continuation of our previous work on heterocyclic compounds[19–21] we herein report the synthesis and in vitro antibacterial behaviour 2-hydroxy-6-methyl-7-(arylamino)-1,7-dihydropurin-8-ones. 3-Oxo-2-(arylhydrazono)butyric acid ethyl ester[22] **1a**–**k** was acetylated[23] to yield 2-(acetyl arylhydrazono)-3-oxo butyric acid ethyl ester **2a**–**k**, which in turn was treated with triethyl amine and formamide in presence of 1,4-dioxane for 4 h to give *N*-(5-acetyl-4-ethoxy-2-oxo-2,5-dihydro-imidazol-1-yl)-*N*-arylacetamide **3a**–**k**. In order to extend the synthesis to obtain the desired purine, it was refluxed with urea in freshly prepared sodium ethoxide for 3 h and then subjected to acid hydrolysis[23] to yield 2-hydroxy-6-methyl-7-(arylamino)-1,7-dihydropurin-8-ones **4a**–**k**. Structures of these synthesized compounds have been elucidated on the basis of IR, NMR and elemental analysis data and purity was ascertained by TLC (methanol and toluene, 4:6 v/v).

2. Chemistry

The synthesis of novel purine derivatives having different substituents is shown in Scheme 1. The key intermediate 3-oxo-2-(arylhydrazono) butyric acid ethyl ester **1a**–**k** was prepared according to the developed method.[22] This requisite starting material was acetylated[23] in order to protect –NH moiety. This acetylated compound **2a**–**k** was refluxed with formamide in presence of

Keywords: 2-Hydroxy-6-methyl-7-(arylamino)-1,7-dihydropurin-8-ones; Molecular refractive index parameters (M_R); Hammett substituent constant (σ).
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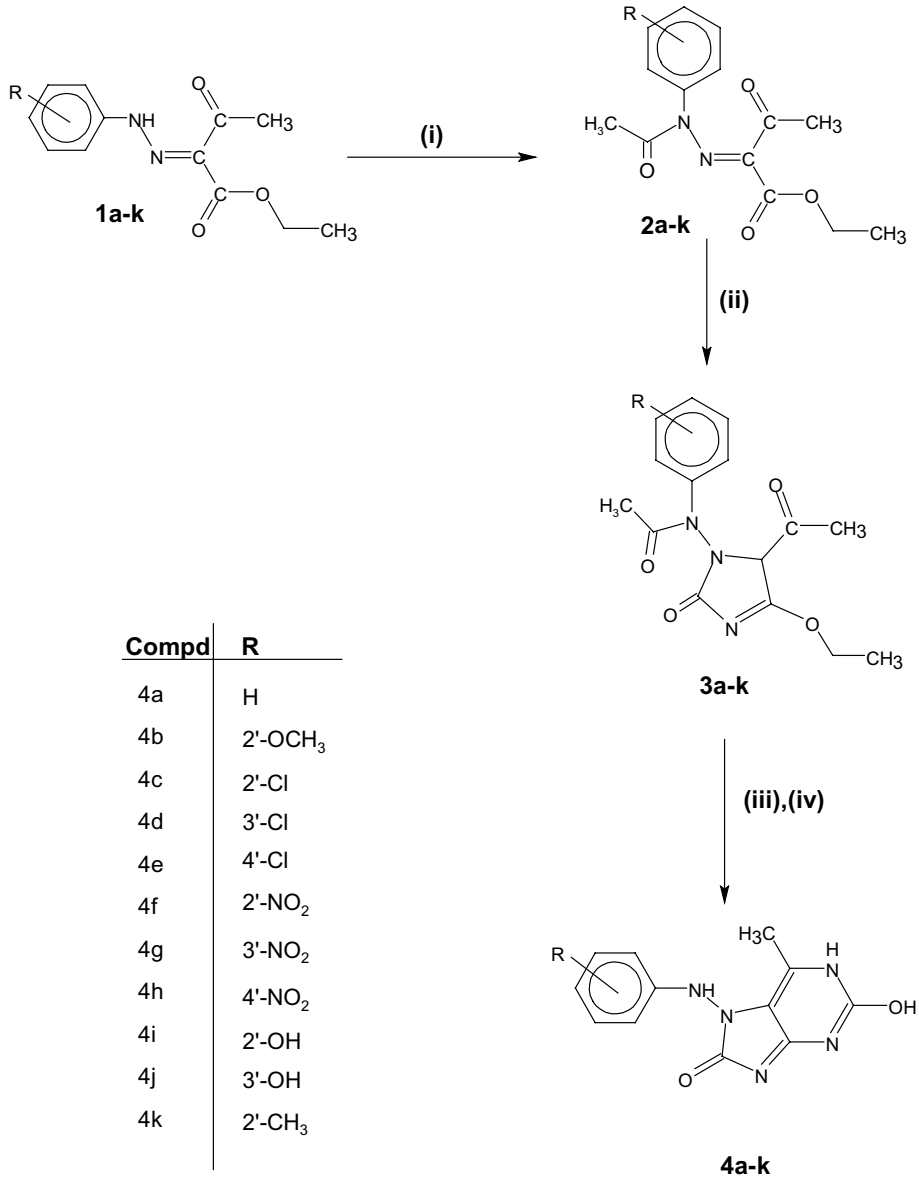
doi:10.1016/j.bmc.2004.04.007



Compd	R
4a	H
4b	2'-OCH_3
4c	2'-Cl
4d	3'-Cl
4e	4'-Cl
4f	2'-NO_2
4g	3'-NO_2
4h	4'-NO_2
4i	2'-OH
4j	3'-OH
4k	2'-CH_3

Scheme 1. Reagents and conditions: (i) $(CH_3CO)_2O$; (ii) $(C_2H_5)_3N$, $HCONH_2$, 1,4-dioxane, reflux 4 h; (iii) $(NH_2)_2CO$, C_2H_5ONa, reflux 3 h; (iv) concd H_2SO_4.

Table 1. Analytical data for compound **3a**,**c**,**f**

Compd	Yield (%)	MP (°C)	Mol. formula	Micro analytical data (%) found (calcd)
3a	74	171–173	$C_{15}H_{17}O_4N_3$	C, 59.35 (59.40); H, 5.62 (5.65); N, 13.80 (13.85)
3c	76	165–167	$C_{15}H_{16}O_4N_3Cl$	C, 53.30 (53.34); H, 4.73 (4.77); N, 12.40 (12.44)
3f	71	160–161	$C_{15}H_{16}O_6N_4$	C, 51.65 (51.72); H, 4.58 (4.63); N, 16.05 (16.09)

triethylamine and 1,4-dioxane for 4 h to afford the corresponding *N*-(5-acetyl-4-ethoxy-2-oxo-2,5-dihydroimidazol-1-yl)-*N*-arylacetamide **3a**–**k** in 70–80% yield as depicted in Table 1.

The reaction of **3a**–**k** with urea in freshly prepared sodium ethoxide solution followed by acidic hydrolysis[23] yielded 2-hydroxy-6-methyl-7-(arylamino)-1,7-dihydropurin-8-ones **4a**–**k**. Hence, pyrimidine ring, which was fused with imidazole ring to yield purine skeleton was synthesized by condensation–cyclization of N–C–N fragment with a C–C–C fragment. The N–C–N fragment employed in the course of study was generated from urea and the reaction has been carried out in the presence of freshly prepared sodium ethoxide solution.

All the synthesized compounds **4a**–**k** exhibited satisfactory elemental analysis and spectral data consistent with the structures.

Table 2. In vitro antibacterial activity of **4a–k** (zone of inhibition, %)

Compd	R	M_R	Bacteria tested[a]				
			Ag	At	Ec	Pd	Bs
4a	H	48.878	40	−9	12	3	26
4b	2′-OCH_3	55.313	−20	−34	−24	−30	−21
4c	2′-Cl	53.745	7	−26	−12	−17	−5
4d	3′-Cl	53.745	13	−20	−8	−13	5
4e	4′-Cl	53.745	53	3	20	13	37
4f	2′-NO_2	55.078	−13	−32	−20	−27	−16
4g	3′-NO_2	55.078	−7	−29	−16	−20	−11
4h	4′-NO_2	55.078	47	−6	16	7	32
4i	2′-OH	50.403	27	−14	4	−7	16
4j	3′-OH	50.403	33	−11	8	−3	21
4k	2′-CH_3	53.670	20	−17	−4	−10	10

[a] Ag *A. globiformis*; At *A. tumefaciens*; Ec *E. coli*; Pd *P. diminuata*; Bs *B. subtilis*.

3. Biological activities

We had conducted antibacterial screening studies versus several clinically relevant Gram-positive and Gram-negative bacteria in an effort to unravel interesting leads from synthesized compounds. Nutrient agar media was prepared for bacterial growth. Stock solutions of tested compound were prepared in dimethylformamide (DMF). Inocula containing approximately 10^7 CFU/mL of bacteria were prepared from broth culture in log phase growth. Filter paper disc method by Rapper[24] was employed to determine the antibacterial activity of synthesized compounds. The inhibition zone of each test solution was measured in mm and reported in terms of % inhibition using the formula inhibition $(\%) = [(\alpha - \beta)/\alpha] \times 100$[25] and presented in Table 2, where α and β stands for zone of inhibition of control drug and synthesized compounds, respectively.

All the newly synthesized compounds were assayed for their antimicrobial activity (zone of inhibition %) against *Arthobacter globiformis*, *Bacillus subtilis* as Gram-positive bacteria and *Agrobacterium tumefaciens*, *Escherichia coli*, *Pseudomonas diminuata* as Gram-negative bacteria. An overview of the antibacterial data in Table 2 shows that synthesized compounds gives promising results when tested against these bacteria in vitro compared to the reference drug purinthol. The screening data revealed that compound exhibit promising activity against *A. tumefaciens*, while least against *A. globiformis*. According to systemic perusal of the data presented in the Table 2, compound **4b** exhibited the best in vitro profile in our abbreviated testing panel. The data pertaining to Table 2 reveals that the activity shown by synthesized purine follows the pattern *A. tumefaciens* > *P. diminuata* > *E. coli* > *B. subtilis* > *A. globiformis*.

Interestingly in a view to establish a quantitative structure–activity relationship (QSAR), molecular refractive index (M_R) values, determined by the method of Dreisbach,[26] has been correlated with the biological activity and reported in Table 2.

A perusal of graphical representation (Figs. 1 and 2) reveals the linearity of drug activity with molecular refractive index (M_R) data. It is interpreted that the activity of 2-hydroxy-6-methyl-7-(arylamino)-1,7-dihydropurin-8-ones has been observed to be highest against *A. tumefaciens* and least against *A. globiformis* as has been justified on the basis of increase in molecular refractive index (M_R) values.

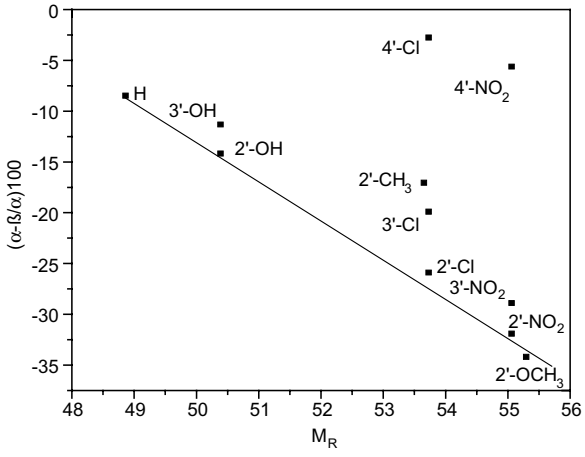


Figure 1. Plot of biological activity versus molecular refractive index (M_R) screened against *A. tumefaciens*.

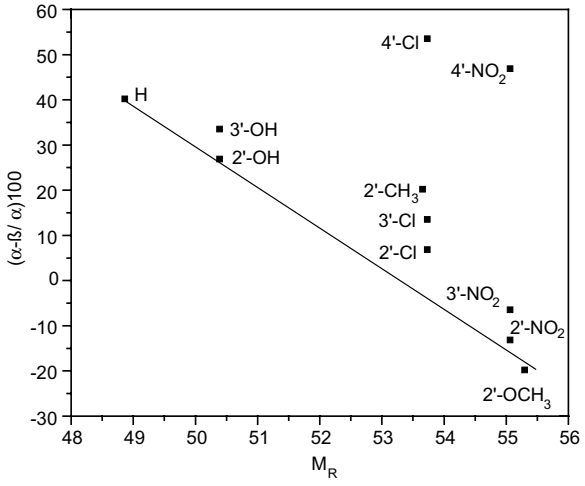


Figure 2. Plot of biological activity versus molecular refractive index (M_R) screened against *A. globiformis*.

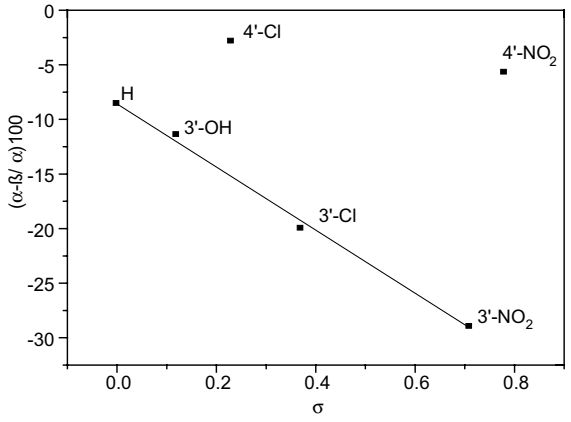


Figure 3. Plot of biological activity versus Hammett substituent constant (σ) screened against *A. tumefaciens*.

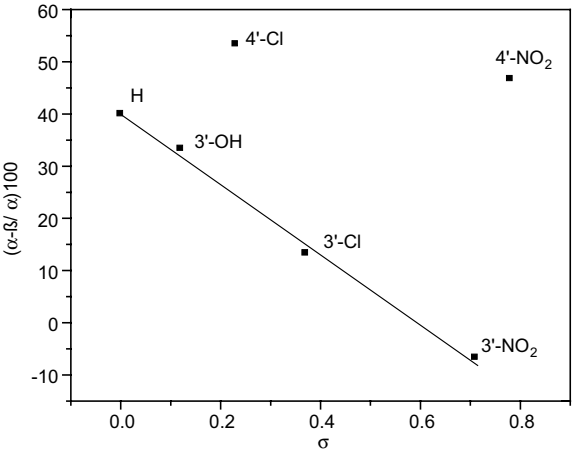


Figure 4. Plot of biological activity versus Hammett substituent constant (σ) screened against *A. globiformis*.

Likewise, drug activity has also been correlated with Hammett substituent constant (σ)[27] (Figs. 3 and 4). Again, it can inferred that drug activity follows the pattern of linearity with (σ) values. All the synthesized compounds bearing substituents viz. H, 3′-OH, 3′-Cl and 3′-NO_2 have been observed to fit in regression line whereas, 4′-Cl, 4′-NO_2 deviate from it indicating that compounds having aromatic ring incorporating electron attracting groups at *para* position decreases the activity but if they are present on *meta*/*ortho* position they tend to increase the activity to a greater extent. Furthermore, insertion of hydroxyl group also increases the activity of the synthesized compounds. In general it has been concluded that the antibacterial results follow the pattern

4b > 4f > 4g > 4c > 4d > 4k > 4i > 4j > 4a > 4h > 4e.

4. Experimental

^{1}H NMR spectra were recorded at 300 MHz Bruker advance DRX 300 instrument using TMS as an internal standard. IR spectra were run on Perkin Elmer model 377 spectrophotometer in KBr pellets. Analytical thin layer chromatography was performed using E. Merck silica gel G 0.50 mm plate Merck no 5700. Melting points were determined in open capillary tubes using an electric melting point apparatus. All the melting points were reported are uncorrected.

4.1. Synthesis of *N*-(5-acetyl-4-ethoxy-2-oxo-2,5-dihydroimidazol-1-yl)-*N*-arylacetamide 3a–k

In a 250 round bottom flask 3-oxo-2- (arylhydrazono) butyric acid ethyl ester[22] **1a–k** (0.02 M), was taken and subjected to acetylation using literature procedure.[23] To this triethylamine (2.4 mL, 0.02 M), formamide (1 mL, 0.02 M) and measured quantity of 1,4-dioxane (50 mL) were added and all the content were refluxed for 4 h to give **3a–k**.

4.2. Synthesis of 2-hydroxy-6-methyl-7-(arylamino)-1,7-dihydropurin-8-ones 4a–k

Compound **3a–k** (0.02 M) was refluxed with urea (1.2 g, 0.02 M) in freshly prepared sodium ethoxide solution (6 g of sodium metal and 50 mL of absolute ethanol) for 3 h. On cooling the product so formed was hydrolyzed[23] using sulfuric acid and the compound obtained was recrystallized from the solution of ethanol and DMF (1:1 v/v). The purity of synthesized compound was ascertained by TLC (methanol + toluene, 4:6 v/v). Structures of the synthesized compounds have been ascertained on the basis of spectroanalytical data.[28–31] Synthetic pathway of all the sequential steps is depicted in Scheme 1.

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28. Analytical data for compound **4a**: mp (°C) 177–179; IR (KBr) (cm^{-1}) 3761 (OH, enolized), 3473 (NH, bonded), 3217 (N–H ring), 1672 (C═O), 1595 (C═C/C═N), 1522, 1475, 1400 (C⁼⁼C ring str.), 1302 (C–N), 1234 (C═O), 1017, 947 (C–H in plane bending, phenyl ring), 845, 771 (C–H out of plane bending, monosubstituted benzene ring); ^1H NMR (δ ppm) 1.21 (s, 1H, OH), 2.50 (s, 3H, CH_3), 7.40 (s, 5H, C_6H_5), 8.21, 9.50 (s, 2H, 2×NH). Anal. Calcd for $C_{12}H_{11}O_2N_5$: C, 56.03; H, 4.28; N, 27.24. Found: C, 55.95; H, 4.20; N, 27.21.
29. Analytical data for compound **4c**: mp (°C) 176–178; IR (KBr) (cm^{-1}) 3780 (OH, enolized), 3470 (NH, bonded), 3210 (N–H ring), 1675 (C═O), 1590 (C═C/C═N), 1520, 1473, 1410 (C⁼⁼C ring str.), 1300 (C–N), 1230 (C═O), 1012 (C–H in plane bending), 840, 740 (C–H out of plane bending, 1,2-disubstituted benzene ring), 660 (C–Cl); ^1H NMR (δ ppm) 1.30 (s, 1H, OH), 2.50 (s, 3H, CH_3), 7.42 (d, Ar–H_6), 8.25, 9.20 (s, 2H, 2×NH). Anal. Calcd for $C_{12}H_{10}O_2N_5Cl$: C, 49.40; H, 3.43; N, 24.01. Found: C, 49.31; H, 3.33; N, 23.90.
30. Analytical data for compound **4g**: mp (°C) 172–174; IR (KBr) (cm^{-1}) 3760 (OH, enolized), 3453 (NH, bonded), 3200 (N–H ring), 1680 (C═O), 1595 (C═C/C═N), 1500, 1472, 1400 (C⁼⁼C ring str.), 1361 (NO_2), 775 (C–H out of plane bending, 1,3-disubstituted benzene ring); ^1H NMR (δ ppm) 1.57 (s, 1H, OH), 2.50 (s, 3H, CH_3), 7.08–7.17 (dd, Ar–H_4), 7.22 (t, Ar–H_5), 7.28 (d, Ar–H_6) 7.93 (s, 1H, Ar–H_2), 8.23, 9.52 (s, 2H, 2×NH). Anal. Calcd for $C_{12}H_{10}O_4N_6$: C, 47.68; H, 3.31; N, 27.81. Found: C, 47.60; H, 3.25; N, 27.73.
31. Analytical data for compound **4k**: mp (°C) 170–172; IR (KBr) (cm^{-1}) 3771 (OH, enolized), 3460 (NH, bonded), 3215 (N–H ring), 1675 (C═O), 1595 (C═C/C═N), 1530, 1480, 1430 (C⁼⁼C ring str.), 745 (C–H out of plane bending, 1,2-disubstituted benzene ring); ^1H NMR (δ ppm) 0.09 (s, 3H, CH_3), 1.40 (s, 1H, OH), 2.51 (s, 3H, CH_3), 7.05 (d, Ar–H_3), 7.57 (d, Ar–H_6), 7.73–7.80 (t, Ar–H_4, Ar–H_5), 8.20, 9.52 (s, 2H, 2×NH). Anal. Calcd for $C_{13}H_{13}O_2N_5$: C, 57.56; H, 4.80; N, 25.83. Found: C, 57.48; H, 4.75; N, 25.70.