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Promiscuous enzyme-catalyzed regioselective Michael addition of purine derivatives to α,β-unsaturated carbonyl compounds in organic solvent

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ABSTRACT

Regioselective Michael addition of purine derivatives to α,β-unsaturated carbonyl compounds could be catalyzed by D-aminoacylase amano (DA) in DMSO. The influence of reaction conditions on the Michael addition, including solvent, temperature, and enzyme concentration was systematically investigated. Then we extended this methodology to six structurally diverse purine derivatives and a variety of α,β-unsaturated carbonyl compounds. 21 Michael adducts were selectively synthesized in moderate to high yields. It is the first report on enzyme-catalyzed Michael addition for the preparation of purine derivatives.

1. Introduction

Synthesis of nucleoside derivatives has gained more attention in the past decades because of their antiviral and antitumor ability.[1] However, rare study has been focused on purine derivatives due to their low reactivity and low solubility. Moreover, regioselective reaction is difficult to undergo when purine derivatives are used as the substrates since the alkylation of 2-aminopurines usually gives rise to mixtures of N-9 and N-7 materials.[2] Hence, control of the regioselectivity is crucial during the synthetic procedure. Geen[3] reported Michael addition with 2-aminopurines catalyzed by potassium carbonate in *N*,*N*-dimethylformamide at room temperature, by extending the reaction time and increasing the quantity of Michael acceptors to obtain the exclusive N-9 alkylated products. In another way, Stephane Guillarme[4] adopted protected or deaza natural bases as the Michael donor for the synthesis of several acyclic nucleosides. Recently, Microwave irradiation technique was introduced to the Michael addition of purine derivatives, exclusive N-9 alkylated products could be obtained in a short time.[5] However, these methods always need special equipment or unrecycling catalysts. Recently, the emphasis of science and technology has diverted into environmentally friendly and sustainable resources and processes. Therefore, direct utilization of natural materials for catalytic application is a very charming strategy.

Enzymes are efficient catalysts in organic and bioorganic synthesis. Besides the natural catalytic ability to catalyze a primary reaction, many enzymes can also catalyze secondary reaction at an active site, which is termed 'catalytic promiscuity'.[6] For example, lipase can catalyze the formation of C–C,[7a] C–N,[7b] C–O,[7c] and C–S[7d] through Michael addition, arylmalonate decarboxylase can catalyze aldol additions,[7e] racemase can catalyze PLP-dependent aldol additions.[7f] Recently, enzyme promiscuity was also discovered at alternate-site besides the known natural catalytically active site.[8]

In our former works, we have demonstrated the promiscuous enzyme-catalyzed Michael addition of imidazole and pyrimidine.[9a–e] Taking the low reactivity of purine derivatives and their antiviral and antitumor ability, and the highly regioselectivity of enzyme as catalyst into account, it is very interesting and meaningful for us to exploit the catalytic capability of enzyme on the synthesis of purine derivatives .In this paper, we reported the enzyme-catalyzed Michael addition of purine derivatives (Fig. 1) to α,β-unsaturated carbonyl compound catalyzed by D-aminoacylase. Then the reaction was optimized by investigating the influence of reaction conditions on the Michael addition, including solvent, temperature, and enzyme concentration. This methodology was extended to a variety of structurally diverse purine derivatives and α,β-unsaturated carbonyl compound. 21 products were obtained in moderate to high yield under the catalysis of D-aminoacylase in DMSO at 50 °C successfully.

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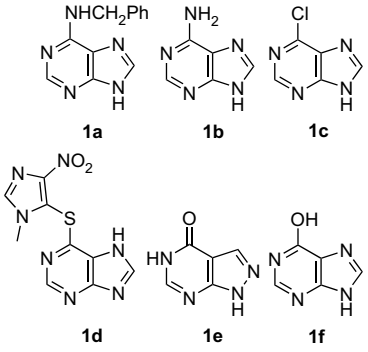


Figure 1. The structure of purine derivatives.

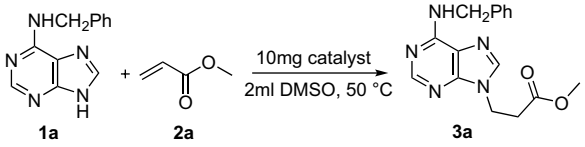
2. Results and discussion

In order to demonstrate the specific catalytic effect of catalysts, we performed some control experiments. The reaction of 6-benzylaminopurine **1a** with methyl acrylate **2a** in the absence of enzyme led to low yield adduct (8%) in 72 h. In contrast, the reaction in the presence of DA and AA is up to 50.7-fold and 28-fold faster (entries 2 and 6, Table 1). Besides, the initial reaction rate is practically proportional to the enzyme amount, also suggesting the catalytic effect of the enzyme (entries 2 and 3, Table 1). When the reactants were incubated with denatured D-aminoacylase (pre-treated with urea at 100 °C for 24 h) the rate is practically equal to the bovine serum albumin (BSA), ruling out the possibility that the polymeric support or the similar amino acid distribution on the protein surface has promoted the process. All these results suggest that the specific active sites and the tertiary structure of D-aminoacylase are responsible for the Michael addition reaction. Three widely used hydrolases, Candida antarctica lipase B (CAL-B), Lipase from *porcine pancreas* (PPL), and alkaline protease from Bacillus subtilis (PA), can only accelerate the reaction by 2.1, 7.3, and 10.7-fold, respectively. Among all the selected catalysts, D-aminoacylase (DA) was the best catalyst for this Michael addition. Thus, DA was selected for the further study.

Then the reaction conditions were optimized by using the Michael addition of 6-benzylaminopurine **1a** to methyl acrylate **2**a as model reaction. As indicated in Table 2, the enzyme-catalyzed Michael addition reaction could only be performed in some polar solvents such as DMSO and DMF (entries 1 and 2, Table 2). In other solvents including dioxane, pyridine, chloroform, and cyclohexane, only trace product was detected, which may be ascribed to the low solubility of 6-benzylaminopurine in those solvents (entries 3–6, Table 2). Then the influence of enzyme concentration on the reaction was examined. As shown in Table 2, when the enzyme concentration increased from 5 mg/ml to 20 mg/ml (entries 1 and 7–9, Table 2), the yield decreased lightly. The optimal enzyme concentration was 5 mg/ml. Next, the influence of reaction temperature on the enzymatic Michael addition reaction was also considered. It was found that the yield decreased with the decrease of temperature (entries 1, 10, and 11, Table 2). The yield was only 8% at 25 °C. The highest yield was obtained at 50 °C. All these studies promoted us to use DMSO as the solvent in the presence of 5 mg/ml enzyme concentration at 50 °C to probe the generality of the conjugated addition processes.

Having the optimal conditions in hand, we applied this method to other Michael acceptors and the results were shown in Table 3. Examination of the results of the different acrylate reveals that the chain length of the ester plays a minimal role in governing the reactivity of the conjugate addition. As the carbon chain of alcohol moiety increased, slight decrease in yield was observed (entries 1–3, Table 3). The reactions of 6-benzylaminopurine (**1a**) with methyl vinyl ketone or acrylonitrile could also provide good yields (entries 4 and 5, Table 3). Compared with acrylate and acrylonitrile, methyl vinyl ketone presented the highest activity. Then a series of sterically hindered Michael acceptors with either α-methyl or β-methyl group were studied under the same conditions (entries

Table 1
Initial rates (V_o) of the Michael addition of 6-benzylaminopurine **1a** to methyl acrylate **2a** in the presence of different catalysts



Entry	Catalyst	Amount (mg)	Yield[a] (%)	V_0 (mM h^{-1})	V_r[b]
1	—	—	8	0.15	1.0
2	D-Aminoacylase	10	92	7.6	50.7
3	D-Aminoacylase	5	88	3.9	26.0
4	DA denatured[c]	10	10	0.22	1.5
5	BSA	10	11	0.25	1.7
6	Acylase 'amano'	10	81	4.2	28.0
7	CAL-B	10	15	0.31	2.1
8	PPL	10	37	1.1	7.3
9	PA	10	58	1.6	10.7

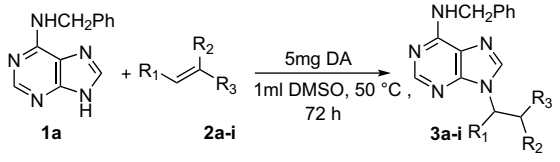
[a] Experimental conditions: 0.2 M 6-benzylaminopurine, 0.4 M methyl acrylate, 10 mg enzyme, 2 ml DMSO, 50 °C, 72 h. All yields were detected by HPLC.
[b] Relative initial rate to the reaction in absence of enzyme.
[c] Enzyme predenatured with urea at 100 °C for 24 h.

Table 2
Screen of reaction conditions of DA-catalyzed Michael addition of 6-benzylaminopurine **1a** to methyl acrylate **2a**[a]

Entry	Amount of catalyst (mg)	Solvent	Temperature (°C)	Yield[b] (%)
1	5	DMSO	50	48
2	5	DMF	50	14
3	5	Dioxane	50	<1
4	5	Pyridine	50	4
5	5	Chloroform	50	<1
6	5	Cyclohexane	50	<1
7	10	DMSO	50	44
8	15	DMSO	50	44
9	20	DMSO	50	42
10	5	DMSO	40	23
11	5	DMSO	25	8

[a] Experimental conditions: 0.5 M 6-benzylaminopurine, 2.5 M methyl acrylate, 5 mg enzyme, 1 ml solvent, 50 °C, 24 h.
[b] All yields were detected by HPLC.

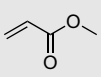
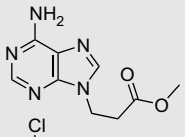
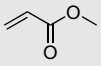
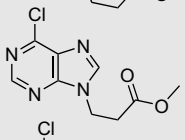
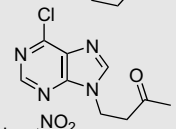
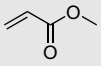
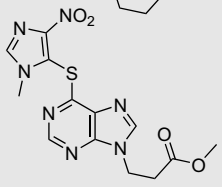
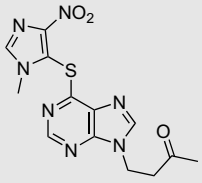
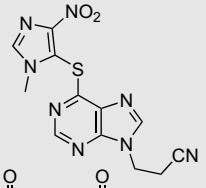
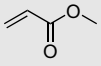
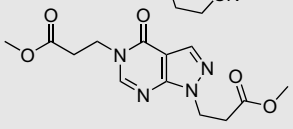
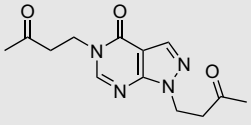
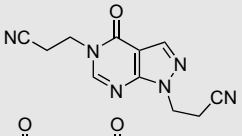
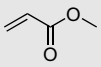
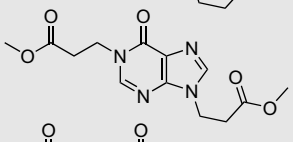
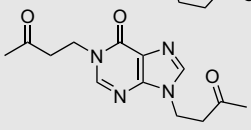
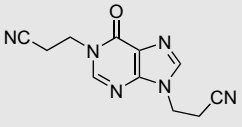
Table 3
Michael addition of 6-benzylaminopurine to various Michael acceptors



Entry	Time (h)	R_1	R_2	R_3	Yield[a] (%)
1	72	H	H	$COOCH_3$	(**3a**) 92
2	72	H	H	$COOCH_2CH_3$	(**3b**) 81
3	72	H	H	$COO(CH_2)_3CH_3$	(**3c**) 78
4	48	H	H	$COCH_3$	(**3d**) 90
5	72	H	H	CN	(**3e**) 84
6	72	H	CH_3	$COOCH_3$	(**3f**) 42
7	72	CH_3	H	$COOCH_3$	(**3g**) 39
8	48	CH_3	H	$COOCH{=}CH_2$	(**3h**) 67
9	48	H	CH_3	$COOCH{=}CH_2$	(**3i**) 62

[a] All yields were detected by HPLC.

Table 4
Michael addition of other purine derivatives to α,β-unsaturated carbonyl compound promoted by DA[a]

Entry	Nucleophile	Acceptor	Time (h)	Product	Yield[b] (%)
1	**1b**		72		(**3j**) 59
2	**1c**		72		(**3k**) 80
3	**1c**		48		(**3l**) 94
4	**1d**		48		(**3m**) 95
5	**1d**		48		(**3n**) 91
6	**1d**		72		(**3o**) 81
7	**1e**		48		(**3p**) 97
8	**1e**		48		(**3q**) 98
9	**1e**		48		(**3r**) 77
10	**1f**		48		(**3s**) 98
11	**1f**		48		(**3t**) 98
12	**1f**		48		(**3u**) 70

[a] Reaction conditions: purine derivatives (0.2 mmol), α,β-carbonyl compound (1 mmol) in 1 mL DMSO at 50 °C.
[b] All yields were detected by HPLC.

6–9, Table 3). All the reactions reached the equilibrium after 48 or 72 h and only provide moderate yield. Compared with substituted methyl acrylates, the substituted vinyl acrylates showed good reactivity and higher yields were observed (entries 6 and 9; 7 and 8, Table 3). It is worthwhile to mention that all of the products were alkylated at the N-9 position according to the ^{1}H NMR and ^{13}C NMR spectral analysis. HMBC spectra also ascertained that the site of alkylation was N-9 rather than N-7 or another position.

The structure of the purine derivatives also affected the results of the Michael addition reaction. Six kinds of structurally diverse purine derivatives underwent Michael addition with methyl acrylate, methyl vinyl ketone or acrylonitrile favorably and the results were summarized in Table 4. As shown in Table 4, the Michael addition of adenine with methyl acrylate only provided the corresponding adduct in 59% yield after 72 h (entry 1, Table 4). The reactivity of adenine was relatively lower than that of 6-benzylaminopurine, probably due to the poor solubility of adenine in DMSO. Then the Michael additions of 6-chloropurine, azathiopurine with methyl acrylate, methyl vinyl ketone, and acrylonitrile were examined (entries 2–6, Table 4). It was found that all the reactions could generate the corresponding mono Michael adducts highly selectively at the N-9 position in good yields after 48–72 h. When allopurinol and 6-hydroxypurine were tested in Michael addition with methyl acrylate, methyl vinyl ketone, and acrylonitrile, bis-adducts were favored in good yields without mono adduct products unexpectedly (entries 7–12, Table 4). According to the ^{1}H NMR and ^{13}C NMR spectral analysis, both the N-2 and N-9 positions of allopurinol or 6-hydroxypurine were alkylated. HMBC spectra also ascertained that the sites of alkylation were N-2 and N-9 rather than N-2 and N-7 or another position. The Michael addition of allopurinol formed bis-adducts because allopurinol has two active NH functionalities. A similar explanation could be elucidated for 6-hydroxypurine due to that 6-hydroxypurine can be converted to the similar structure of allopurinol in equilibrium.

3. Conclusion

An efficient and highly regioselective enzymatic Michael addition for the preparation of purine derivatives was developed. The influence of reaction conditions on the Michael addition, including solvent, temperature, and enzyme concentration was investigated. Moderate to high yields were obtained when 5 mg/ml DA was used to catalyze the Michael addition in DMSO at 50 °C. This strategy works with a broad range of structurally diverse purine derivatives. Further study on the promiscuity of DA was under way.

4. Experimental

4.1. General remarks

Alkaline protease from *Bacillus subtilis* (10 U/mg, 1 U corresponds to the amount of enzyme, which liberates 1 μmol folin-positive amino acids and peptides per minute at pH 7.5 and 37 °C) was obtained from Wuxi Enzyme Co. Ltd. (Wuxi, PR China). Lipase immobilized on acrylic resin from *Candida antarctica* (≥10,000 U/g, recombinant, expressed in *Aspergillus oryzae*) and Lipase from *porcin pancreas* (PPL) (30–90 U/mg protein, one unit will hydrolyze 1.0 μequiv of triacetin in 1 h at pH 7.7 at 37 °C) were purchased from Sigma (Steinheim, Germany). D-Aminoacylase from *Escherichia coli* (10,000 U/mg, 1 U is defined as enzyme quantity, which produces 1 μmol of D-amino acid per 30 min) and acylase 'amano' (AA) from *A. oryzae* (≥30,000 U/g, 1 U is defined as enzyme quantity, which produces 1 μmol of L-amino acid per 30 min) were purchased from Amano Enzyme Inc (Japan). All solvents were analytical grade and were dried by storing over activated 3 Å molecular sieves for 24 h prior to use. All other reagents were used as-received. ^{1}H and ^{13}C NMR spectra were recorded on Bruker AVANCE DMX-500 spectrometer at 500 MHz and 125 MHz in $CDCl_3$ or DMSO-d_6, respectively. Chemical shifts are reported in parts per million (δ), relative to the internal standard of tetramethylsilane (TMS). IR spectra were measured with a Nicolet Nexus FTIR 670 spectrophotometer. Mass spectrometry data were obtained on Brucker Esquire-LC for electro-spray (MS-ES) measurements (solvents: methanol; positive mode). Melting points were determined using XT-4 apparatus and were not corrected.

4.2. Typical procedure

Purine derivatives (0.5 mmol) and α,β-unsaturated carbonyl compounds (2.5 mmol) were added to a 10 mL conical flask containing 1 ml DMSO and the mixture was shaken at ambient temperature for a period of time. The reaction was terminated by filtering off the enzyme and the DMSO was evaporated in reduced pressure. The product was isolated by silica gel column chromatography with an eluent consisting of petrol ether/ethyl acetate (1/1 v/v).

*4.2.1. 3-(6-Benzylamino-purin-1-yl)-propionic acid methyl ester (**3a**)*

Yellow solid, mp 124–125 °C; IR (KBr): 3366, 3097, 1724, 1613 cm^{-1}; ^{1}H NMR ($CDCl_3$, δ, ppm): 8.52 (s, 1H), 7.87 (s, 1H), 7.41–7.27 (m, 5H), 6.45 (s, 1H), 4.85 (s, 2H), 4.59 (t, 2H, *J* 6.0 Hz), 3.63 (s, 3H), 2.92 (t, 2H, *J* 6.0 Hz); ^{13}C NMR ($CDCl_3$, δ, ppm): 171.6, 155.0, 153.4, 140.7, 138.8, 128.9, 128.0, 127.7, 127.4, 121.0, 52.3, 42.0, 39.6, 34.2; ESI-MS (*m*/*z*): 312 (M+H).

*4.2.2. 3-(6-Benzylamino-purin-9-yl)-propionic acid ethyl ester (**3b**)*

White solid, mp 67–68 °C; IR (KBr): 3264, 3059, 1729, 1614 cm^{-1}; ^{1}H NMR ($CDCl_3$, δ, ppm): 8.39 (s, 1H), 7.74 (s, 1H), 7.35–7.25 (m, 5H), 6.54 (s, 1H), 4.86 (s, 2H), 4.45 (t, 2H, *J* 6.0 Hz), 4.13–4.08 (m, 2H), 2.89 (t, 2H, *J* 6.0 Hz), 1.20 (t, 3H, *J* 6.8 Hz); ^{13}C NMR ($CDCl_3$, δ, ppm): 170.8, 154.6, 153.0, 140.5, 138.5, 128.6, 128.5, 127.6, 127.4, 119.6, 61.0, 39.3, 34.2, 14.0; ESI-MS (*m*/*z*): 326 (M+H).

*4.2.3. 3-(6-Benzylamino-purin-9-yl)-propionic acid butyl ester (**3c**)*

Yellow solid, mp 65–66 °C; IR (KBr): 3265, 1733, 1616 cm^{-1}; ^{1}H NMR ($CDCl_3$, δ, ppm): 8.39 (s, 1H), 7.75 (s, 1H), 7.37–7.25 (m, 5H), 6.45 (s, 1H), 4.86 (s, 2H), 4.45 (t, 2H, *J* 6.4 Hz), 4.13 (t, 2H, *J* 6.8 Hz), 2.90 (t, 2H, *J* 6.4 Hz), 1.58–1.51 (m, 2H), 1.32–1.24 (m, 2H), 0.88 (t, 3H, *J* 7.2 Hz); ^{13}C NMR ($CDCl_3$, δ, ppm): 170.9, 154.6, 153.0, 140.5, 138.4, 130.4, 128.6, 127.7, 127.4, 119.6, 65.0, 39.4, 34.1, 30.6, 30.4, 19.1, 13.6; ESI-MS (*m*/*z*): 354 (M+H).

*4.2.4. 4-(6-Benzylamino-purin-9-yl)-butan-2-one (**3d**)*

White solid, mp 106–107 °C; IR (KBr): 3267, 1717, 1626 cm^{-1}; ^{1}H NMR (DMSO-d_6, δ, ppm): 8.31 (s, 1H), 8.25 (s, 1H), 8.19 (s, 1H), 7.35–7.20 (m, 5H), 4.70 (s, 2H), 4.31 (t, 2H, *J* 6.4 Hz), 3.10 (t, 2H, *J* 6.8 Hz), 2.10 (s, 3H); ^{13}C NMR (DMSO-d_6, δ, ppm): 206.7, 154.7, 152.6, 141.3, 140.6, 128.5, 127.5, 126.9, 122.3, 42.5, 38.4, 30.1; ESI-MS (*m*/*z*): 296 (M+H); HRMS (ESI) *m*/*z* calcd for [M+H] $C_{16}H_{18}N_5O$ 296.1433, found 296.1441.

*4.2.5. 3-(6-Benzylamino-purin-9-yl)-propionitrile (**3e**)*

White solid, mp 160–161 °C; IR (KBr): 3279, 3099, 1620 cm^{-1}; ^{1}H NMR (DMSO-d_6, δ, ppm): 8.39 (s, 1H), 7.64 (s, 1H), 7.38–7.34 (m, 5H), 6.67 (s, 1H), 4.87 (s, 2H), 4.42 (t, 2H, *J* 6.8 Hz), 2.99 (t, 2H, *J* 6.8 Hz); ^{13}C NMR (DMSO-d_6, δ, ppm): 154.8, 153.4, 139.2, 138.3, 128.6, 127.6, 127.4, 119.8, 116.6, 39.7, 29.7, 18.8; ESI-MS (*m*/*z*): 279 (M+H).

*4.2.6. 3-(6-Benzylamino-purin-9-yl)-2-methyl-propionic acid methyl ester (**3f**)*

Colorless oil; IR (KBr): 3259, 3068, 1736, 1622 cm^{-1}; ^1H NMR ($CDCl_3$, δ, ppm): 8.41 (s, 1H), 7.75 (s, 1H), 7.39–7.26 (m, 5H), 6.29 (s, 1H), 4.86 (s, 2H), 4.46–4.40 (m, 1H), 4.28–4.24 (m, 1H), 3.65 (s, 3H), 3.22–3.13 (m, 1H), 0.86 (d, 3H, *J* 7.2 Hz); ^{13}C NMR ($CDCl_3$, δ, ppm): 171.1, 154.6, 153.1, 140.7, 140.3, 138.3, 128.5, 127.5, 127.3, 119.4, 45.5, 39.6, 29.5, 14.8; ESI-MS (*m*/*z*): 325 (M+H); HRMS (ESI) *m*/*z* calcd for [M+H] $C_{17}H_{20}N_5O_2$ 326.1539, found 326.1545.

*4.2.7. 3-(6-Benzylamino-purin-9-yl)-butyric acid methyl ester (**3g**)*

Colorless oil; IR (KBr): 3270, 3031, 1736, 1620 cm^{-1}; ^1H NMR ($CDCl_3$, δ, ppm): 8.39 (s, 1H), 7.63 (s, 1H), 7.39–7.25 (m, 5H), 6.70 (s, 1H), 5.05–4.96 (m, 1H), 4.87 (s, 2H), 3.62 (s, 3H), 3.23–3.17 (m, 1H), 2.91–2.85 (m, 1H), 1.68 (d, 3H, *J* 6.8 Hz); ^{13}C NMR ($CDCl_3$, δ, ppm): 170.6, 154.6, 152.7, 138.7, 138.5, 128.5, 127.6, 127.3, 120.1, 51.8, 48.6, 39.3, 20.0; ESI-MS (*m*/*z*): 326 (M+H); HRMS (ESI) *m*/*z* calcd for [M+H] $C_{17}H_{20}N_5O_2$ 326.1539, found 326.1531.

*4.2.8. 3-(6-Benzylamino-purin-9-yl)-butyric acid vinyl ester (**3h**)*

Yellow oil; IR (KBr): 3273, 3031, 1753, 1619 cm^{-1}; ^1H NMR (DMSO-d_6, δ, ppm): 8.31 (s, 1H), 8.25 (s, 1H), 8.19 (s, 1H), 7.35–7.21 (m, 5H), 7.13–7.08 (m, 1H), 5.06–4.97 (m, 1H), 4.84–4.80 (m, 1H), 4.68 (s, 1H), 4.63–4.61 (m, 1H), 3.20–3.14 (m, 2H), 1.57 (d, 3H, *J* 6.8 Hz); ^{13}C NMR (DMSO-d_6, δ, ppm): 168.1, 152.5, 141.4, 140.6, 140.0, 128.5, 127.5, 126.9, 121.2, 99.0, 47.9, 20.6; ESI-MS (*m*/*z*): 338 (M+H); HRMS (ESI) *m*/*z* calcd for [M+H] $C_{18}H_{20}N_5O_2$ 338.1539, found 338.1564.

*4.2.9. 3-(6-Benzylamino-purin-9-yl)-2-methyl-propionic acid vinyl ester (**3i**)*

White solid, mp 62–63 °C; IR (KBr): 3273, 3032, 1748, 1619 cm^{-1}; ^1H NMR ($CDCl_3$, δ, ppm): 8.37 (s, 1H), 7.65 (s, 1H), 7.35–7.25 (m, 5H), 7.20–7.15 (m, 1H), 4.84 (s, 2H), 4.86–4.82 (m, 1H), 4.58–4.56 (m, 1H), 4.44–4.39 (m, 1H), 4.27–4.22 (m, 1H), 3.26–3.17 (m, 1H), 1.28 (d, 3H, *J* 7.6 Hz); ^{13}C NMR ($CDCl_3$, δ, ppm): 171.1, 154.6, 153.1, 140.7, 140.3, 138.4, 128.5, 127.5, 127.3, 119.3, 98.5, 45.5, 39.5, 29.5, 14.8; ESI-MS (*m*/*z*): 338 (M+H); HRMS (ESI) *m*/*z* calcd for [M+H] $C_{18}H_{20}N_5O_2$ 338.1539, found 338.1564.

*4.2.10. 3-(6-Amino-purin-9-yl)-propionic acid methyl ester (**3j**)*

White solid, IR (KBr): 3291, 3130, 1727, 1607 cm^{-1}; ^1H NMR (DMSO-d_6, δ, ppm): 8.12 (s, 1H), 8.08 (s, 1H), 7.20 (s, 2H), 4.36 (t, 2H, *J* 6.4 Hz), 3.56 (s, 3H), 2.94 (t, 2H, *J* 6.4 Hz); ^{13}C NMR ($CDCl_3$, δ, ppm): 171.4, 156.3, 152.8, 149.8, 141.3, 119.1, 52.0, 33.8; ESI-MS (*m*/*z*): 222 (M+H).

*4.2.11. 3-(6-Chloro-purin-9-yl)-propionic acid methyl ester (**3k**)*

White solid, mp 99–100 °C; IR (KBr): 3126, 1725, 1509 cm^{-1}; ^1H NMR (DMSO-d_6, δ, ppm): 8.77 (s, 1H), 8.68 (s, 1H), 4.53 (t, 2H, *J* 6.8 Hz), 3.57 (s, 3H), 3.03 (t, 2H, *J* 6.8 Hz); ^{13}C NMR (DMSO-d_6, δ, ppm): 171.3, 152.3, 151.8, 149.3, 148.1, 131.2, 52.0, 33.3; ESI-MS (*m*/*z*): 241 (M+H).

*4.2.12. 4-(6-Chloro-purin-9-yl)-butan-2-one (**3l**)*

White solid, mp 183–184 °C; IR (KBr): 3103, 1604 cm^{-1}; ^1H NMR (DMSO-d_6, δ, ppm): 8.77 (s, 1H), 8.73 (s, 1H), 4.63 (t, 2H, *J* 6.8 Hz), 3.17 (t, 2H, *J* 6.8 Hz), 2.10 (s, 3H); ^{13}C NMR (DMSO-d_6, δ, ppm): 206.5, 161.9, 151.9, 151.8, 151.7, 142.5, 122.5, 43.6, 41.6, 30.2; ESI-MS (*m*/*z*): 225 (M+H); HRMS (ESI) *m*/*z* calcd for [M+H] $C_9H_{10}ClN_4O$ 225.0465, found 326.0455.

*4.2.13. 3-[6-(3-Methyl-5-nitro-3H-imidazol-4-ylsulfanyl)-purin-9-yl]-propionic acid methyl ester (**3m**)*

Yellow solid, IR (KBr): 3447, 3115, 1735, 1566, 1534 cm^{-1}; ^1H NMR ($CDCl_3$, δ, ppm): 8.55 (s, 1H), 8.15 (s, 1H), 7.76 (s, 1H), 4.55 (t, 2H, *J* 6.8 Hz), 3.75 (s, 3H), 3.66 (s, 3H), 2.96 (t, 2H, *J* 6.8 Hz); ^{13}C NMR ($CDCl_3$, δ, ppm): 171.0, 156.1, 151.7, 150.6, 149.7, 145.0, 137.9, 131.0, 116.7, 52.0, 40.0, 33.4, 33.1; ESI-MS (*m*/*z*): 364 (M+H); HRMS (ESI) *m*/*z* calcd for [M+H] $C_{13}H_{14}N_7O_4S$ 364.0750, found 364.0824.

*4.2.14. 4-[6-(3-Methyl-5-nitro-3H-imidazol-4-ylsulfanyl)-purin-9-yl]-butan-2-one (**3n**)*

Yellow solid, mp 37–38 °C; IR (KBr): 3451, 3115, 3076, 1713, 1627, 1567, 1536 cm^{-1}; ^1H NMR ($CDCl_3$, δ, ppm): 8.53 (s, 1H), 8.15 (s, 1H), 7.75 (s, 1H), 4.50 (t, 2H, *J* 6.0 Hz), 3.75 (s, 3H), 3.09 (t, 2H, *J* 6.0 Hz), 2.10 (s, 3H); ^{13}C NMR ($CDCl_3$, δ, ppm): 205.4, 156.0, 151.6, 150.5, 149.7, 145.4, 137.9, 131.0, 116.8, 42.1, 38.4, 33.1, 29.9; ESI-MS (*m*/*z*): 370 (M+Na); HRMS (ESI) *m*/*z* calcd for [M+H] $C_{13}H_{14}N_7O_3S$ 348.0801, found 348.0811.

*4.2.15. 4-[6-(3-Methyl-5-nitro-3H-imidazol-4-ylsulfanyl)-purin-9-yl]-butyronitrile (**3o**)*

Yellow solid, mp 187–188 °C; IR (KBr): 3447, 3123, 1569,1533 cm^{-1}; ^1H NMR ($CDCl_3$, δ, ppm): 8.48 (s, 1H), 8.17 (s, 1H), 7.75 (s, 1H), 4.55 (t, 2H, *J* 6.8 Hz), 3.70 (s, 3H), 3.03 (t, 2H, *J* 6.8 Hz); ^{13}C NMR ($CDCl_3$, δ, ppm): 156.5, 151.9, 150.4, 149.5, 144.1, 138.1, 130.9, 116.5, 40.7, 39.8, 33.1, 18.5; ESI-MS (*m*/*z*): 331 (M+H); HRMS (ESI) *m*/*z* calcd for [M+H] $C_{12}H_{11}N_8O_2S$ 331.0647, found 331.0719.

*4.2.16. 3-[5-(2-Methoxycarbonyl-ethyl)-4-oxo-4,5-dihydro-pyrazolo[3,4-d]pyrimidin-1-yl]-propionic acid methyl ester (**3p**)*

White solid, mp 117–118 °C; IR (KBr): 3173, 3068, 1724, 1613 cm^{-1}; ^1H NMR ($CDCl_3$, δ, ppm): 8.15 (s, 1H), 8.08 (s, 1H), 4.56 (t, 2H, *J* 6.4 Hz), 4.18 (t, 2H, *J* 6.4 Hz), 3.66 (s, 6H), 3.01 (t, 2H, *J* 6.4 Hz), 2.82 (t, 2H, *J* 6.4 Hz); ^{13}C NMR ($CDCl_3$, δ, ppm): 171.5, 170.9, 158.9, 158.3, 149.5, 128.9, 106.7, 52.0, 51.9, 48.9, 42.6, 33.8, 33.0; ESI-MS (*m*/*z*): 309 (M+H); HRMS (ESI) *m*/*z* calcd for [M+H] $C_{13}H_{17}N_4O_5$ 309.1121, found 309.1141.

*4.2.17. 1,5-Bis-(3-oxo-butyl)-1,5-dihydro-pyrazolo[3,4-d]-pyrimidin-4-one (**3q**)*

White solid, mp 111–112 °C; IR (KBr): 3086, 1710,1676 cm^{-1}; ^1H NMR (DMSO-d_6, δ, ppm): 8.39 (s, 1H), 8.05 (s, 1H), 4.43 (t, 2H, *J* 6.8 Hz), 4.10 (t, 2H, *J* 6.4 Hz), 3.06 (t, 2H, *J* 6.8 Hz), 2.94 (t, 2H, *J* 6.4 Hz), 2.12 (s, 3H), 2.09 (s, 3H); ^{13}C NMR (DMSO-d_6, δ, ppm): 206.7, 152.6, 141.3, 128.5, 127.5, 126.9, 42.5, 38.4, 30.1; ESI-MS (*m*/*z*): 277 (M+H); HRMS (ESI) *m*/*z* calcd for [M+H] $C_{13}H_{17}N_4O_3$ 277.1222, found 277.1223.

*4.2.18. 3-[1-(2-Cyano-ethyl)-4-oxo-1,4-dihydro-pyrazolo[3,4-d]pyrimidin-5-yl]-propionitrile (**3r**)*

White solid, mp 161–162 °C; IR (KBr): 3114, 1704 cm^{-1}; ^1H NMR (DMSO-d_6, δ, ppm): 8.51 (s, 1H), 8.22 (s, 1H), 4.57 (t, 2H, *J* 6.8 Hz), 4.27 (t, 2H, *J* 6.8 Hz), 3.14 (t, 2H, *J* 6.8 Hz), 3.02 (t, 2H, *J* 6.8 Hz); ^{13}C NMR (DMSO-d_6, δ, ppm): 156.5, 151.9, 151.2, 135.6, 118.6, 105.4, 42.8, 41.6, 18.2, 17.7; ESI-MS (*m*/*z*): 243 (M+H); HRMS (ESI) *m*/*z* calcd for [M+H] $C_{11}H_{11}N_6O$ 243.0916, found 243.0951.

*4.2.19. 3-[1-(2-Methoxycarbonyl-ethyl)-6-oxo-1,6-dihydro-purin-9-yl]-propionic acid methyl ester (**3s**)*

White solid, mp 116–117 °C; IR (KBr): 3100, 1736, 1687 cm^{-1}; ^1H NMR (DMSO-d_6, δ, ppm): 8.39 (s, 0.5H), 8.30 (s, 0.5H), 8.22 (s, 0.5H), 8.09 (s, 0.5H), 4.54 (t, 1H, *J* 6.4 Hz), 4.39 (t, 1H, *J* 6.4 Hz), 4.22 (t, 2H, *J* 5.6 Hz), 3.60 (s, 3H), 3.59 (s, 3H), 2.99 (m, 2H), 2.82 (t, 2H, *J* 5.6 Hz); ^{13}C NMR (DMSO-d_6, δ, ppm): 171.7, 171.3, 157.1, 156.4, 149.0, 123.6, 114.5, 52.0, 42.7, 42.4, 35.1, 34.0, 33.1; ESI-MS (*m*/*z*): 309 (M+H); HRMS (ESI) *m*/*z* calcd for [M+H] $C_{13}H_{17}N_4O_5$ 309.1121, found 309.1130.

*4.2.20. 1,9-Bis-(3-oxo-butyl)-1,9-dihydro-purin-6-one (**3t**)*

White solid, IR (KBr): 3005, 1712, 1685 cm^{-1}; ^1H NMR (DMSO-d_6, δ, ppm): 8.27 (s, 1H), 8.16 (s, 1H), 4.45 (t, 2H, *J* 6.4 Hz), 4.13 (t, 2H, *J* 6.8 Hz), 3.09 (t, 2H, *J* 6.8 Hz), 2.95 (t, 2H, *J* 6.4 Hz), 2.10 (s, 3H), 2.09 (s, 3H); ^{13}C NMR (DMSO-d_6, δ, ppm): 207.1, 206.6, 158.9, 154.0, 148.0, 144.9, 114.5, 72.6, 60.6, 43.8, 30.2; ESI-MS (*m*/*z*): 277 (M+H); HRMS (ESI) *m*/*z* calcd for [M+H] $C_{13}H_{16}N_4O_3$ 277.1222, found 277.1240.

*4.2.21. 3-[9-(2-Cyano-ethyl)-6-oxo-6,9-dihydro-purin-1-yl]-propionitrile (**3u**)*

White solid, mp 218–219 °C; IR (KBr): 1712, 1697 cm^{-1}; ^1H NMR (DMSO-d_6, δ, ppm): 8.39 (s, 1H), 8.36 (s, 1H), 4.60 (t, 2H, *J* 6.0 Hz), 4.28 (t, 2H, *J* 6.4 Hz), 3.18 (t, 2H, *J* 6.4 Hz), 3.02 (t, 2H, *J* 6.0 Hz); ^{13}C NMR (DMSO-d_6, δ, ppm): 157.2, 153.8, 147.9, 145.1, 118.7, 114.4, 72.6, 60.6, 19.9, 17.6; ESI-MS (*m*/*z*): 243 (M+H); HRMS (ESI) *m*/*z* calcd for [M+H] $C_{11}H_{11}N_6O$ 243.0916, found 243.0930.

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