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6-Oxo and 6-thio purine analogs as antimycobacterial agents

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ABSTRACT

6-Oxo and 6-thio analogs of purine were prepared based on the initial activity screening of a small, diverse purine library against *Mycobacterium tuberculosis* (Mtb). Certain 6-oxo and 6-thio-substituted purine analogs described herein showed moderate to good inhibitory activity. N^9-substitution apparently enhances the anti-mycobacterial activity in the purine series described herein. Several 2-amino and 2-chloro purine analogs were also synthesized that showed moderate inhibitory activity against Mtb.

1. Introduction

In spite of the availability of highly active anti-tubercular agents, tuberculosis has remained one of the primary causes of human death and suffering worldwide. It is estimated that approximately one third of the world's population is infected with the bacteria that causes tuberculosis, 2–3 million people die worldwide each year from the disease, and an additional 8 million people become ill with tuberculosis annually.[1] *Mycobacterium tuberculosis* (Mtb) is a facultative intracellular pathogen that persists primarily within macrophages in the human host, and these cells are involved in propagation of infection.[2] Intracellularly sequestered bacilli are considered more resistant to treatment and clearance due to limited access of drugs and the immune system to bacteria within macrophages, necessitating chronic treatment with high therapeutic doses for effective control and treatment of the disease.[3] Additionally, AIDS patients and others with compromised immune systems are susceptible to other opportunistic mycobacteria including *Mycobacterium avium* and *Mycobacterium kansaii*, resulting in further morbidity and a high mortality.[4] It is not surprising that drug resistance, from single drug resistant (SDR) up to totally drug resistant (TDR) strains,[5] is now becoming commonplace considering the fact that virtually the same drug regimens have been in place and poorly deployed worldwide for over half a century. Treatment of highly resistant forms of *Mtb* is both difficult and expensive, and for these more intractable forms, few treatment options are available. Although newer drugs are now in clinical trials, these issues critically underscore the need for continued emphasis on the discovery of newer drugs with novel mechanisms of action.[6]

Phenotypic screening of diverse drug-like compound libraries against Mtb has been more recently implemented in order to respond to this need and discover compounds that are active against whole Mtb bacilli,[7] potentially circumventing issues with antibacterial drug discovery using specific target based screens.[8]

Through similar screens of the Southern Research proprietary library, it has been found that several 9-benzylpurines with a variety of substitutions in the 2-, 6- and/or 8-positions exhibit inhibitory activity against Mtb.[9] High inhibitory activity was found for 9-benzylpurines containing a phenylethynyl-, *trans*-styryl or aryl substituent in the 6-position, and generally chlorine in the 2-position tends to increase activity (compounds **1**–**4**, Fig. 1). Several 6-arylpurines carrying a variety of substituents in the 9-position were prepared by Stille coupling between appropriately substituted 6-chloropurines and aryl(tributyl)tin, and the compounds were screened for antibacterial activity against Mtb $H_{37}Rv$.[10] One of the derivatives, 9-benzyl-2-chloro-6-(2-furyl)purine (**2**), showed a MIC value of 0.78 μg/mL and also showed relatively low cytotoxicity against several singly drug-resistant strains. A series of 9-sulfonylated/sulfenylated-6-mercapto purines (**3** and **4**) has been prepared by reaction of 6-mercaptopurine with sulfonyl/sulfenyl halides.[11] These compounds constitute a new class of potent antimycobacterial agents, possessing excellent MIC values against Mtb $H_{37}Rv$, as well as appreciable activity against *M. avium*. A few compounds in this series have exhibited activity against several drug resistant strains of *Mtb* (e.g., compound **4**). Currently, beyond the broad phenotypic activity, no target(s) has/have been identified.

We have also synthesized a small library of 6-thioalkyl/aryl/benzyl purine analogs to generate a modest structure–activity

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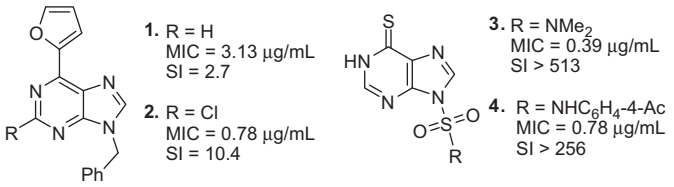
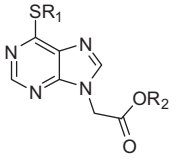


Figure. 1.

relationship (SAR). All the purine derivatives synthesized were screened for their activity against two strains of Mtb ($H_{37}Rv$ & $H_{37}Ra$) and three strains of *M. avium* (MAC NJ211, NJ168, NJ3404). Most of these derivatives were inactive against *M. avium*. In particular, (6-decylsulfanyl-purin-9-yl)-acetic acid ethyl ester **5** and the dodecyl derivative **6**, and (6-decylsulfanyl-purin-9-yl)-acetic acid *tert*-butyl ester **7** exhibited MIC values of 1.56, 0.78 and 3.13 μg/mL respectively against the Mtb $H_{37}Rv$ strain (Fig. 2).[12] Concurrent with the determination of MICs, analogs **5**–**7** were also tested for cytotoxicity (CC_{50}) in a cell proliferation assay for VERO cells at concentrations less than or equal to 62.5 μg/mL or 10 times the MIC for Mtb $H_{37}Rv$: selectivity is assigned by calculating the selectivity index (SI) ratio CC_{50}/MIC.

Very little is known about the mechanism of action of these purine analogs. Purine salvage pathways are predicted to be present from the genome sequence of Mtb, and the metabolism in mycobacteria is similar to that in humans and other organisms.[13] Information about the substrate preferences of the mycobacterial enzymes involved with purine metabolism is still unknown. The Mtb *deoD* gene encodes a presumptive purine nucleoside phosphorylase (PNP) and the gene was cloned, expressed, purified, and found to exhibit PNP activity.[14] Modest biochemical work has been pursued on purine nucleosides with anti-mycobacterial activity, especially 2-methyladenosine that showed potent activity (99% inhibition, MIC = 3 μg/mL, IC_{50} (VERO Cells = 1000 μg/mL, SI >1000).[15] 2-Methyladenosine has demonstrated selective activity against Mtb, suggesting differences in the substrate preferences between mycobacterial and human adenosine kinases that might be exploited to develop novel nucleoside-based drugs for the treatment of mycobacterial diseases. Beyond the purine salvage pathways, ATP binding proteins and kinases are also being interrogated as new drug targets in Mtb.[16]

Based upon our previously synthesized purine series,[12] we planned to generate more diversified 6-substituted mercaptopurines analogs with substitutions at C-2 position along with 6-oxo substituted series for antimycobacterial screening. Compounds were prepared from a diversity perspective in order to further probe specific active scaffolds, but also to explore other purines such as the 2-amino and 2-chloropurine scaffolds.



5. $R_1 = C_{10}H_{21}$, R_2 = Et; MIC = 1.56 μg/mL, CC_{50} VERO Cells = >62.5 μg/mL; SI = >40
6. $R_1 = C_{12}H_{25}$, R_2 = Et; MIC = 0.78 μg/mL, CC_{50} VERO Cells = >10 μg/mL; SI = >12.8
7. $R_1 = C_{10}H_{21}$, $R_2 = C(CH_3)_3$; MIC = 3.13 μg/mL, CC_{50} VERO Cells = >10 μg/mL; SI = >3.19

Figure 2.

2. Results and discussion

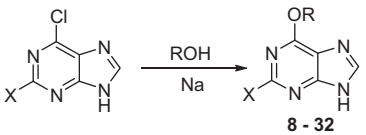
2.1. Chemistry

In the first target set, synthesis of several new 6-oxoaryl/benzyl/aryl purines and their 2-amino or 2-chloro purine analogs (**8**–**32**) was carried out as shown in Scheme 1 by reacting a suitable 6-chloropurine analog with alcohols in the presence of sodium metal. Neutralization with acetic acid followed by the standard workup and column chromatographic purification on silica gel G produced pure products. A total of six analogs of 6-oxopurine, 10 analogs of 2-amino-6-oxopurine and nine analogs of 2-chloro-6-oxopurine were prepared in the initial phase to determine their antibacterial activity. A total of nine analogs have shown >50% inhibitory activity against Mtb $H_{37}Rv$ and are discussed herein (Table 1).

As previously described by us,[12] substitution at the N^9-position enhances antimycobacterial activity, and we have synthesized and screened four analogs **33**–**36** starting from active 6-oxopurine analogs **9**, **10**, **15** and **16** (Scheme 2). The synthesis of these analogs was carried out by reacting the appropriate commercially available purine analog with ethylbromoacetate and K_2CO_3 in anhydrous DMSO at room temperature.

Further analoging of compounds **34** and **35** was achieved by treating these with different D- and L-amino acids. These compounds can utilize bacterial dipeptide transporters to enhance transport via membrane amino acids beyond the fact that adding amino acids at the end increases diversity for probing activity. It is sometimes the case that adding specific chirality can improve activity and only one stereoisomer shows activity. This result is, however, a gross generality as that is not always the case and sometimes both enantiomers are inactive or can show similar activity. Compounds **40**–**50** (Scheme 3) which contain D- or L-amino acid chains were synthesized from commercially available starting material, 2-amino-6-chloro-9*H*-purine-9-acetic acid (**37**). The 6-decyloxy derivative **38** was synthesized by reacting 37 with 1-decanol in the presence of sodium metal. The 6-decylthio derivative **39** was synthesized from **37** by the reaction with 1-decylthiol and $(CH_3)_3COK$ as previously described.[12] Analoging of **38** and **39** was achieved by treating with different D- or L-amino acids using the coupling reagent benzotriazole-1-yl-oxy-trispyrrolidino-phosphonium hexafluorophosphate (PyBOP) in the presence of base Et_3N.

Alternatively, for diversity point of view, compounds **51**–**69** were prepared from easily accessible starting materials. 6-Decylmercaptopurine analogs **51**–**54** were prepared starting from 6-chloropurine analogs by the reaction with 1-decylthiol and $(CH_3)_3COK$ under reflux as previously described (Scheme 4A).[12,17] However, 6-decylmercapto purine analogs **55**–**69** were prepared



8. X=H; R = $CH(CH_3)_2$
9. X=H; R = $(CH_2)_9CH_3$
10. X=H; R = $(CH_2)_{11}CH_3$
11. X=H; R = C_6H_4-4-Cl
12. X=H; R = CH_2-C_6H_{11}
13. X=H; R = CH_2-C_6H_3-3-Cl,4-Cl
14. X=NH_2; R = $CH(CH_3)_2$
15. X=NH_2; R = $(CH_2)_9CH_3$
16. X=NH_2; R = $(CH_2)_{11}CH_3$
17. X= NH_2; R = C_6H_5
18. X= NH_2; R = C_6H_4-4-Cl
19. X= NH_2; R = C_6H_4-4-CH_3
20. X= NH_2; R = C_6H_4-3-CH_3
21. X=NH_2; R = CH_2-C_6H_{11}
22. X=NH_2; R = CH_2-C_6H_5
23. X=NH_2; R = CH_2-C_6H_3-3-Cl,4-Cl
24. X=Cl; R = CH_3
25. X=Cl; R = $CH(CH_3)_2$
26. X=Cl; R = $(CH_2)_9CH_3$
27. X=Cl; R = $(CH_2)_{11}CH_3$
28. X=Cl; R = C_6H_4-4-Cl
29. X=Cl; R = C_6H_4-4-CH_3
30. X=Cl; R = C_6H_4-3-CH_3
31. X=Cl; R = CH_2-C_6H_{11}
32. X=Cl; R = CH_2-C_6H_3-3-Cl,4-Cl

Scheme 1.

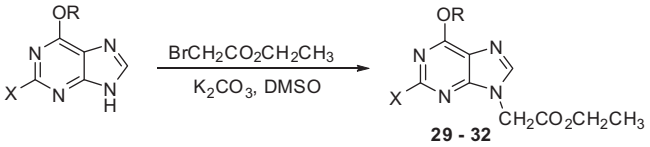
Table 1
Antimycobacterial activity against Mtb and MAC of substituted purines

Compd no.	% Inhib. Mtb $H_{37}Rv$	MIC_{90} Mtb $H_{37}Rv$	MIC_{99} Mtb $H_{37}Ra$	MIC_{99} MAC NJ211	CC_{50} VERO cells	Selectivity index (SI) Mtb $H_{37}Rv$
8	23	>6.25	>12.8	>12.8	n.d.	—
9	96	<6.25	>12.8	>12.8	n.d.	—
10	56	>6.25	>12.8	>12.8	n.d.	—
12	6	>6.25	>12.8	>12.8	n.d.	—
13	21	>6.25	>12.8	>12.8	n.d.	—
14	23	>6.25	>12.8	>12.8	n.d.	—
15	91	3.13	>12.8	>12.8	n.d.	—
16	94	0.78	>6.4	>6.4	>10	>12.8
18	20	>6.25	>12.8	>12.8	n.d.	—
21	93	>6.25	>12.8	>12.8	n.d.	—
23	24	>6.25	>12.8	>12.8	n.d.	—
24	24	>6.25	>12.8	>12.8	n.d.	—
25	23	>6.25	>12.8	>12.8	n.d.	—
26	55	>6.25	>12.8	>12.8	n.d.	—
27	59	>6.25	>12.8	>12.8	n.d.	—
28	8	>6.25	>12.8	>12.8	n.d.	—
32	35	>6.25	>12.8	>12.8	n.d.	—
33	100	1.56	>12.8(P)	>12.8	>62.5	>40.1
34	98	0.78	32	>128	>10	>12.8
35	14	>6.25	>12.8	>12.8	n.d.	—
36	100	6.25	16	>32	>10	>1.6
44	7	>6.25	>12.8	>12.8	n.d.	—
47	16	>6.25	>12.8	>12.8	n.d.	—
48	5	>6.25	>12.8	>12.8	n.d.	—
51	35	>6.25	>12.8	>12.8	n.d.	—
52	49	>6.25	>12.8	>12.8	n.d.	—
53	82	>6.25	>12.8	>12.8	n.d.	—
55	97	6.25	>12.8	>12.8	12.32	2.0
56	84	>6.25	>12.8	>12.8	n.d.	—
57	81	>6.25	>12.8	>12.8	n.d.	—
58	93	6.25	>12.8	>12.8	n.d.	—
59	96	6.25	>12.8	>12.8	n.d.	—
60	97	1.56	>12.8	>12.8	n.d.	—
61	74	>6.25	>12.8	>12.8	n.d.	—
62	89	>6.25	>12.8	>12.8	n.d.	—
63	86	>6.25	>12.8	>12.8	n.d.	—
64	90	>6.25	>12.8	>12.8	n.d.	—
65	92	6.25	>12.8	>12.8	8.04	1.3
66	98	6.25	>12.8	>12.8	n.d.	—
67	96	6.25	>12.8	>12.8	n.d.	—
68	92	>6.25	>12.8	>12.8	n.d.	—
69	85	>6.25	>12.8	>12.8	n.d.	—

% Inhibition was performed at 6.25 μg/mL concentration; compounds not listed here showed no inihibition at 6.25 μg/mL; MIC's are in μg/mL; CC_{50} was determined in μg/mL; SI (Mtb $H_{37}Rv$) = CC_{50}/MIC_{90}.

from their corresponding 6-purinethiones by treatment with 1-chlorodecane/anhydrous K_2CO_3 as reported earlier (Scheme 4B).[18]

7-Deaza compound **70** was synthesized in three steps starting from commercially available 6-chloro-7-deazapurine **71** as shown in Scheme 5. The compound **71** was first converted to 6-thiomethyl-7-deaza purine **72** in excellent yield. Compound **72** was then reacted with ethylbromoacetate/K_2CO_3 followed by ester hydrolysis using 1 N NaOH gave compound **73**. Finally, coupling of **73** with 3,4-diemthylaniline, HATU and DIEA produced the desired product **70**.



33. X=H; R = $(CH_2)_9CH_3$
34. X=H; R = $(CH_2)_{11}CH_3$
35. X=NH_2; R = $(CH_2)_9CH_3$
36. X=NH_2; R = $(CH_2)_{11}CH_3$

Scheme 2.

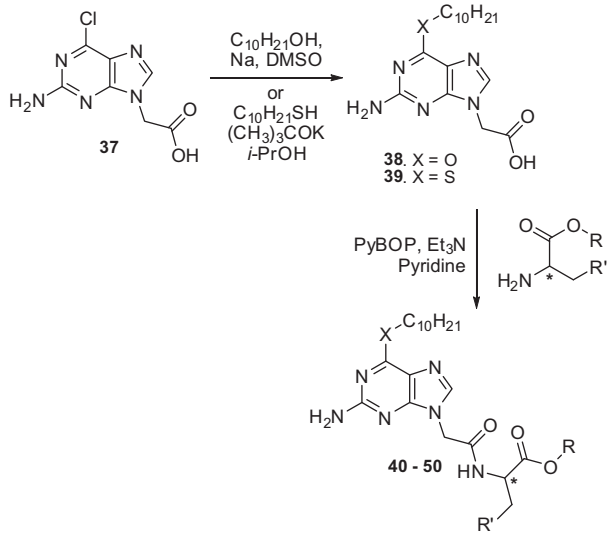
2.2. Anti-mycobacterial activity

All new compounds were screened for their in vitro activity against strains Mtb $H_{37}Ra$ and $H_{37}Rv$ as well as one strain of *M. avium* (NJ211) and are described below. Compounds demonstrating at least 90% inhibition against $H_{37}Rv$ strain at 6.25 μg/mL were retested to determine the MIC_{90} in a BACTEC assay. Active compounds were then screened for cytotoxicity against mammalian VERO cells to determine their selectivity towards bacteria.

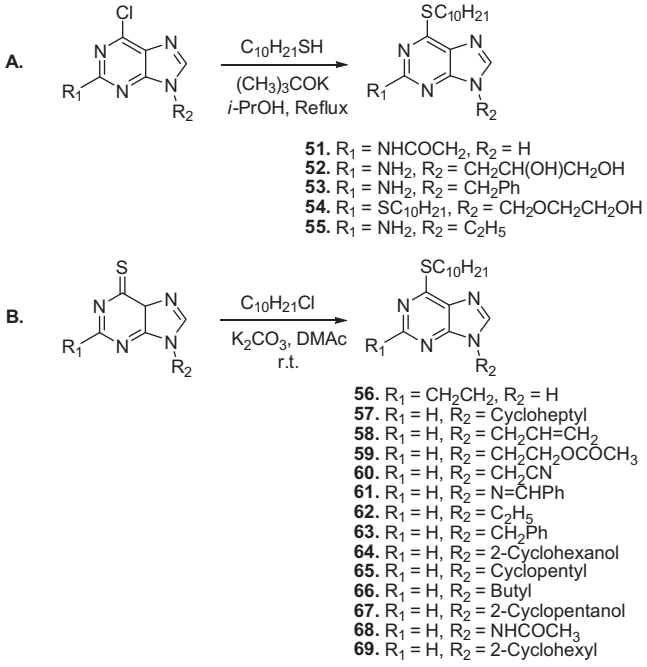
2.2.1. In vitro cell studies

The minimum inhibitory concentration (MIC_{99}, the lowest concentration that completely inhibits growth) of all compounds for Mtb $H_{37}Ra$ were determined using a colorimetric (Alamar blue) microdilution broth assay reported previously.[19] All the compounds were also screened against Mtb $H_{37}Rv$ to determine the MIC_{90} (the minimum concentration that inhibits 90% of growth). Out of 59 purine analogs synthesized, 26 analogs gave >50% inhibition against $H_{37}Rv$ and are shown in Table 1 (compounds not incuded in Table 1 showed no inhibition against Mtb $H_{37}Rv$).

The antimycobacterial purine analogs **16**, **33**, **34**, **36**, **55** and **65** were examined for toxicity against mammalian cells (VERO cells),[20] and their cytotoxicity (CC_{50}) values are reported in Table 1. Their selectivity is assigned by calculating the selectivity index (SI) ratio CC_{50}/MIC. One of the more promising inhibitors of Mtb growth, compound **33**, gave a SI >40.

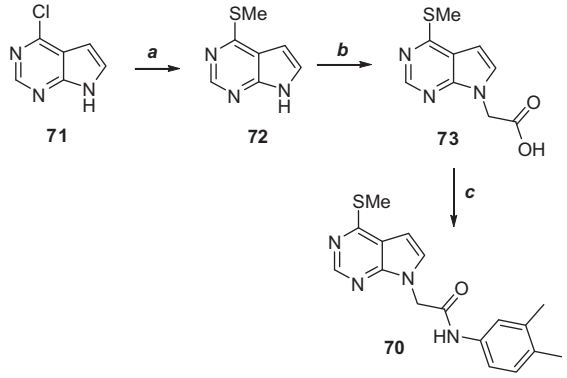


Scheme 3.



Scheme 4.

A 2-thiomethylpurine analog **74** (PubChem ID 726045, Fig. 3) commercially available from ChemBridge showed very potent activity (IC_{90} <0.1 μg/mL) with a selectivity index (SI) >150 against Mtb $H_{37}Rv$ (PubChem Assay ID 1949). Unfortunately, this analog as well as the general class show only relatively poor bioavailablity and antitubercular activity in vivo.[12] Purines are potential substrates for a number of metabolic enzymes in vivo that include adenosine deaminase and xanthine oxidase that can alter biological properties and activity; a typical alteration that can modify metabolism involves preparation of 7-deaza analogs that can potentially circumvent metabolism and retain potent biological



Scheme 5. Reagents and conditions: (a) $NaSCH_3$, MeOH, rt, overnight, 90%; (b) (i) $BrCH_2CO_2Et$, K_2CO_3, DMF, rt, overnight, (ii) 1 N NaOH, MeOH, rt, overnight, 82% in two steps; (c) 3,4-dimethylaniline, HATU, DIEA, DMF, rt, 6 h, 54%.

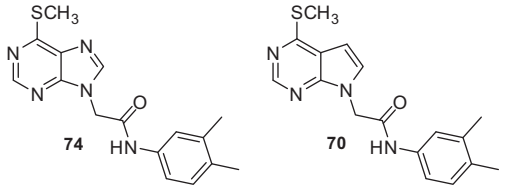


Figure 3.

activity. Purine analogs modified in the five-membered ring have been synthesized and examined for antibacterial activity against Mtb $H_{37}Rv$ in vitro.[21] The 9-deaza analogs were only found to be weak inhibitors, but the 8-aza-, 7-deaza- and 8-aza-7-deazapurine analogs studied displayed excellent antimycobacterial activities, some even substantially better than the parent purine. Hence, the 7-deazapurine analog **70** of purine **74** was prepared and screened against Mtb. Interestingly, this modest alteration led to complete abolition of the whole cell antitubercular activity (IC_{90} >100 μM) in our case for compound **70**. It is also notable that **70** (PubChem CID 44144249) showed modest inhibition (IC_{90} = 7.31 μM) of Mtb $H_{37}Rv$ in the MLSCN screen (Assay ID 485340) when screened in the presence of 6.5 μM (non-lethal, sub-inhibitory dose to bacteria) of the β-lactam antibiotic Meropenem. These data suggest that damage to the cell wall may allow uptake of **70** and activity against the/a molecular target. Speculatively, the purine analog **74** may be actively uptaken and inhibit a target or targets, but the 7-deaza analog **70** may lose the ability to penetrate the mycobacterial cell wall until the peptidoglycan is compromised by the β-lactam. This hypothesis, however, remains unproven.

The two active purines **5** and **6** synthesized previously[12] were examined against a panel of Mtb strains resistant to currently used anti-TB drugs; CIP (ciprofloxacin), INH (isoniazid), KM (kanamycin), EMB (ethambutol), RMP (rifampin), PAS (*p*-aminosalicylic acid), and TAC (thiacetazone) (Table 2). All compounds examined retained their activity against all the drug-resistant strains applied in this study.

2.2.2. In vivo animal studies[22]

Concurrently with the SAR studies, both compounds **5** and **6** were selected for further evaluation in vivo in order to determine cytotoxicity and efficacy in a murine Mtb model to evaluate these scaffolds as further candidates for antitubercular drug discovery. The lead compounds of the series, compounds **6** and **16**, were initially evaluated for in vivo toxicity using an acute toxicity mouse model. These compounds did not show any lethality at 100, 300 and 500 mg/kg. In the short term GKO mouse model, **6** showed

Table 2
MIC of compounds **5** and **6** with $H_{37}Rv$, Erdman and single-drug resistant strains of *Mtb* in the Alamar blue assay

Compd	MIC_{90} (μg/mL) against drug resistance strains in Alamar blue assay								
	$H_{37}Rv$	Erdman	CIP	INH	KM	EMB	RMP	PAS	TAC
5	6.25	12.5	12.5	12.5	6.25	6.25	6.25	—	—
6	3.13	3.13	1.56	1.56	—	3.13	3.13	3.13	3.13

MIC of **5** and **6** were observed 1.56 and 0.78 μg/mL, respectively against $H_{37}Rv$ in Bactec assay. CIP (ciprofloxacin), INH (isoniazid), KM (kanamycin), EMB (ethambutol), RMP (rifampin), PAS (*p*-aminosalicylic acid), and TAC (thiacetazone).

poor efficacy in the lungs by reducing the bacterial load by 0.46 Log_{10}CFU (mean CFU = 8.16) whereas the compound did not show any significant activity in the spleen (0.2 Log_{10}CFU reduction, mean CFU = 6.83). For compound **16**, there was no in vivo activity observed in the lungs or spleens in the GKO mouse model [−0.141 Log_{10}CFU (mean CFU = 8.82) in lungs, and −0.05 Log_{10}CFU (mean CFU = 7.11)].

2.3. Conclusions

In the 6-oxo series, compound **9**, **15** and **16** showed good activity against Mtb $H_{37}Rv$ and possess a decyl/dodecyl chain at the C-6 position of purine. Based on their activity, compounds **29**–**34** possessing N^9 substitution were synthesized and screened. Out of these four compounds, however, compound **35** significantly lost antimycobacterial activity over the comparator compound, **15**. Structural diversity using D- and L-amino acids in compounds **34** and **35** did not increase the activity significantly. The diversity analogs **51**–**69** showed significant high % inhibition but also produced high MIC_{90} values in the Mtb $H_{37}Rv$ screen. In conclusion, small sets of diverse 6-substituted purine analogs were synthesized and screened against Mtb strains. Certain purine analogs synthesized showed interesting antimycobacterial activity against Mtb $H_{37}Rv$. At this point, however, no definitive structure–activity relationship has been determined.

3. Experimental

3.1. Synthesis

Purifications by column chromatography were done with silica gel (Merck, 60 A, 230–400 mesh). Target compounds were dried in vacuo at 25 °C and 78 °C over P_2O_5. Analytical results indicated by element symbols were within ±0.4% of the theoretical values, unless otherwise noted. In such noted cases, a high-resolution mass spectrum was obtained and the result listed. Where solvents are indicated in the formula, their presence was confirmed by ^{1}H NMR. The ^{1}H NMR spectra were determined with a Nicolet NMC 300 NB spectrometer using TMS as internal reference and chemical shifts (δ) are in ppm. ^{1}H NMR spectra were recorded in DMSO-d_6 as a solvent. NOE's were performed on certain intermediates and target compounds to confirm structures. Mass spectra were recorded on a Varian MAT 311A mass spectrometer in the fast-atom-bombardment (FAB) mode. High-resolution mass spectra were obtained on a Bruker BIOTOF II spectrometer. Microanalyses were performed on a Perkin–Elmer 2400 CHN analyzer. All starting materials and reagents were purchased from commercial sources and used as such. Chemical yields have not been optimized and represent the result of a single preparation. All target compounds were stored under argon at −20 °C until used for screening. General synthetic procedures are described as below.

3.1.1. General procedure for synthesis of 6-oxyalkyl/aryl/benzyl purines (8–32)

To a solution of Na (1.36 equiv) in the appropriate alcohol (20 equiv) was added the appropriately substituted purine under an argon atmosphere. The reaction mixture was stirred at reflux for 2–6 h and then allowed to stir at room temperature overnight under argon. Deionized water (10–20 mL) was added and the reaction mixture was neutralized by addition of acetic acid. The neutralized solution was then extracted with diethyl ether. The ethereal layers were pooled, dried over Na_2SO_4, concentrated, and dried in vacuum over P_2O_5 at 25 °C. In most cases, the crude material was purified by column chromatography (CC) over silica gel G.

3.1.1.1. 6-[(Isopropyl)oxy]purine (8). $CHCl_3$–MeOH (99:1) with 0.5% NH_4OH was used as eluent for column chromatography (CC), off-white solid, yield 45%. ^{1}H NMR: δ 13.37 (1H, br s, NH), 8.46 (1H, s, H-2), 8.34 (1H, s, H-8), 5.58 (1H, m, OCH), 1.40 (6H, d, J = 6.2 Hz, 2×CH_3). FABMS *m*/*z*: 179 $(M+H)^+$. ESI-MS: Found 179.0932, calcd for $C_8H_{10}N_4O$ 179.0927. Anal. ($C_8H_{10}N_4O$) Found: C, 54.24; H, 5.77; N, 30.35. Calcd C, 53.92; H, 5.66; N, 31.44.

3.1.1.2. 6-(Decyloxy)purine (9). $CHCl_3$ was used as eluent for CC, off-white solid, yield 51%. ^{1}H NMR: δ 13.50 (1H, br s, NH), 8.47 (1H, s, $^1J_{CH}$ = 203.0 Hz, H-2), 8.36 (1H, s, $^1J_{CH}$ = 210.9 Hz, H-8), 4.52 (2H, t, J = 6.6 Hz, OCH_2), 1.80 (2H, m, CH_2), 1.34 (14H, m, 7× CH_2), 0.85 (3H, m, CH_3). FABMS *m*/*z*: 277 $(M+H)^+$. Anal. ($C_{15}H_{24}N_4O$) Found: C, 64.86; H, 9.01; N, 19.90. Calcd C, 65.19; H, 8.75; N, 20.27.

3.1.1.3. 6-(Dodecyloxy)purine (10). $CHCl_3$–MeOH (98:2) was used for CC, off-white solid, yield 63%. ^{1}H NMR: δ 13.40 (1H, br s, NH), 8.47 (1H, s, H-2), 8.36 (1H, s, H-8), 4.52 (2H, dd, J = 6.6, 6.7 Hz, OCH_2), 1.79 (2H, m, CH_2), 1.29 (18H, m, 9× CH_2), 0.85 (3H, m, CH_3). FABMS *m*/*z*: 305 $(M+H)^+$. Anal. ($C_{17}H_{28}N_4O$) Found: C, 67.26; H, 9.02; N, 18.02. Calcd C, 67.07; H, 9.27; N, 18.40.

3.1.1.4. 6-[(4-Chlorophenyl)oxy]purine (11). $CHCl_3$–MeOH (98:2) was used for CC, off-white solid, yield 84%. ^{1}H NMR: δ 13.61 (1H, br s, NH), 8.53 (1H, s, H-2); 8.44 (1H, s, H-8), 7.54 (2H, d, J = 8.9 Hz, H-3′, H-5′), 7.36 (2H, d, J = 8.9 Hz, H-2′, H-6′). FABMS *m*/*z*: 247 $(M+H)^+$. Anal. ($C_{11}H_7N_4ClO$) Found: C, 53.51; H, 2.83; N 22.51. Calcd C, 53.56; H, 2.86; N, 22.71.

3.1.1.5. 6-[(Cyclohexylmethyl)oxy]purine (12)[23]. $CHCl_3$–MeOH (98:2) was used for CC, off-white solid, yield 32%. ^{1}H NMR: δ 13.39 (1H, br s, NH), 8.43 (1H, s, H-2); 8.33 (1H, s, H-8), 4.31 (2H, d, J = 6.3 Hz, OCH_2), 1.50 (11H, m, C_6H_{11}). FABMS *m*/*z*: 233 $(M+H)^+$. Anal. ($C_{12}H_{16}N_4O{\cdot}0.1H_2O$) Found: C, 61.59; H, 7.01; N, 23.60. Calcd C, 61.57; H, 6.98; N, 23.93.

3.1.1.6. 6-[(3,4-Dichlorobenzyl)oxy]purine (13). $CHCl_3$–MeOH (99:1) with 0.5% NH_4OH was used for CC, off-white solid, yield 35%. ^{1}H NMR: δ 13.51 (1H, br s, NH), 8.52 (1H, s, H-2); 8.42 (1H, s, H-8), 7.82 (1H, d, J = 1.8 Hz, H-2′), 7.69 (1H, d, J = 8.2 Hz, H-5′), 7.53 (1H, dd, J = 1.8, 2.1 Hz, H-6′), 5.62 (2H, s, OCH_2). FABMS *m*/*z*: 295 $(M+H)^+$. Anal. ($C_{12}H_8N_4Cl_2O{\cdot}0.45CH_3OH$) Found: C, 48.63; H, 2.94; N, 17.73. Calcd C, 48.31; H, 3.19; N, 18.10.

3.1.1.7. 2-Amino-6-[(isopropyl)oxy]purine (14). Off-white solid, yield 59%. ^{1}H NMR: δ 7.78 (1H, s, H-8), 6.14 (2H, br s, NH_2),

5.48 (1H, ddd, J = 6.2, 6.3, 6.3 Hz, OCH), 1.34 (6H, d, J = 6.2 Hz, 2× CH_3). FABMS m/z: 194 $(M+H)^+$. Anal. ($C_8H_{11}N_5O$) Found: C, 49.91; H, 5.83; N, 35.86. Calcd C, 49.73; H, 5.74; N, 36.25.

3.1.1.8. 2-Amino-6-(decyloxy)purine (15). $CHCl_3$–MeOH (20:1) was used for CC, off-white solid, yield 62%. ^1H NMR: δ 12.4 (1H, br s, NH), 7.81 (1H, s, H-8), 6.19 (2H, br s, NH_2), 4.37 (2H, dd, J = 6.6, 6.7 Hz, OCH_2), 1.74 (2H, m, CH_2), 1.33 (14H, m, 7× CH_2), 0.85 (3H, s, CH_3). FABMS m/z: 292 $(M+H)^+$. Anal. ($C_{15}H_{25}N_5O$) Found: C, 61.73; H, 8.58; N, 23.81. Calcd C, 61.83; H, 8.65; N, 24.03.

3.1.1.9. 2-Amino-6-(dodecyloxy)purine (16). $CHCl_3$–MeOH (20:1) was used for CC, off-white solid, yield 59%. ^1H NMR: δ 12.4 (1H, br s, NH), 7.80 (1H, s, H-8), 6.19 (2H, br s, NH_2), 4.37 (2H, dd, J = 6.6, 6.7 Hz, OCH_2), 1.73 (2H, m, CH_2), 1.30 (18H, m, 9× CH_2), 0.85 (3H, m, CH_3). FABMS m/z: 320 $(M+H)^+$. Anal. ($C_{17}H_{29}N_5O{\cdot}0.5H_2O$) Found: C, 61.92; H, 8.97; N, 21.24. Calcd C, 62.17; H, 9.21; N, 21.32.

3.1.1.10. 2-Amino-6-(benzyloxy)purine (17). $CHCl_3$–MeOH (20:1) was used for CC, off-white solid, yield 45%. ^1H NMR: δ 12.6 (1H, br s, NH), 7.95 (1H, s, H-8), 7.44 (2H, m, H-2′, H-6′), 7.24 (3H, m, H-3′, H-4′, H-5′), 6.25 (2H, br s, NH_2). FABMS m/z: 228 $(M+H)^+$. Anal. ($C_{11}H_9N_5O{\cdot}0.2CH_3OH$); Found: C, 57.74; H, 4.36; N, 29.83. Calcd C, 57.58; H, 4.23; N, 29.98.

3.1.1.11. 2-Amino-6-[(4-chlorophenyl)oxy]purine (18). $CHCl_3$–MeOH (20:1) was used for CC, off-white solid, yield 83%. ^1H NMR: δ 12.6 (1H, br s, NH), 7.94 (1H, s, H-8), 7.49 (2H, m, H-2′, H-6′), 7.29 (2H, m, H-3′, H-5′), 6.30 (2H, br s, NH_2). FABMS m/z: 262 $(M+H)^+$. Anal. ($C_{11}H_8N_5ClO{\cdot}0.4H_2O$) Found: C, 49.12; H, 3.66; N, 25.76. Calcd C, 49.14; H, 3.30; N, 26.05.

3.1.1.12. 2-Amino-6-[(4-methylphenyl)oxy]purine (19). $CHCl_3$–MeOH (20:1) was used for CC, yellow solid, yield 83%. ^1H NMR: δ 12.5 (1H, br s, NH), 7.93 (1H, br s, H-8), 7.22 (2H, d, J = 8.2 Hz, H-3′, H-5′), 7.10 (2H, d, J = 8.4 Hz, H-2′, H-6′), 6.22 (2H, br s, NH_2), 2.32 (3H, s, CH_3). FABMS m/z: 242 $(M+H)^+$. Anal. ($C_{12}H_{11}N_5O{\cdot}0.4H_2O$) Found: C, 58.03; H, 4.91; N, 28.26. Calcd C, 58.01; H, 4.79; N, 28.19.

3.1.1.13. 2-Amino-6-[(3-methylphenyl)oxy]purine (20). $CHCl_3$–MeOH (20:1) was used for CC, light brown solid, yield 85%. ^1H NMR: δ 12.56 (1H, br s, NH), 7.94 (1H, br s, H-8), 7.31 (1H, dd, J = 7.9, 8.2 Hz, H-5′), 7.03 (3H, m, H-2′, H-4′, H-6′), 6.26 (2H, br s, NH_2), 2.33 (3H, s, CH_3). FABMS m/z: 242 $(M+H)^+$. Anal. ($C_{12}H_{11}N_5O{\cdot}0.45H_2O$) Found: C, 58.03; H, 4.93; N, 27.82. Calcd C, 57.80; H, 4.81; N, 28.09.

3.1.1.14. 2-Amino-6-[(cyclohexylmethyl)oxy]purine (21)[23]. $CHCl_3$–MeOH (20:1) was used for CC, off-white solid, yield 66%. ^1H NMR: δ 12.38 (1H, br s, NH), 7.79 (1H, s, H-8), 6.21 (2H, br s, NH_2), 4.20 (2H, d, J = 6.2 Hz, OCH_2), 1.50 (11H, m, C_6H_{11}). FABMS m/z: 248 $(M+H)^+$. Anal. ($C_{12}H_{17}N_5O{\cdot}0.3CH_3OH$) Found: C, 57.25; H, 7.03; N, 27.09. Calcd C, 57.50; H, 7.14; N, 27.26.

3.1.1.15. 2-Amino-6-(benzyloxy)purine (22)[24]. $CHCl_3$–MeOH (9:1) was used for CC, off-white solid, yield 41%. ^1H NMR: δ 12.44 (1H, br s, NH), 7.83 (1H, br s, H-8), 7.50 (2H, m, H-2′, H-6′), 7.38 (3H, m, H-3′, H-4′, H-5′), 6.29 (2H, br s, NH_2), 5.48 (2H, s, OCH_2). FABMS m/z: 242 $(M+H)^+$. Anal. ($C_{12}H_{11}N_5O{\cdot}0.4CH_3OH$) Found: C, 58.84; H, 4.89; N, 27.27. Calcd C, 58.62; H, 5.00; N, 27.56.

3.1.1.16. 2-Amino-6-[(3,4-dichlorobenzyl)oxy]purine (23). $CHCl_3$–MeOH (99:1) with 0.5% NH_4OH was used for CC, off-white solid, yield 57%. ^1H NMR: δ 12.5 (1H, br s, NH), 7.85 (1H, br s, H-8), 7.79 (1H, d, J = 1.9 Hz, H-2′), 7.67 (1H, d, J = 8.4 Hz, H-5′), 7.51 (1H, dd, J = 2.1, 2.1 Hz, H-6′), 6.32 (2H, br s, NH_2), 5.48 (2H, s, OCH_2). FABMS m/z: 310 $(M+H)^+$. Anal. ($C_{12}H_9Cl_2N_5O$) Found: C, 46.48; H, 3.19; N, 22.34. Calcd C, 46.47; H, 2.92; N, 22.58.

3.1.1.17. 2-Chloro-6-(methoxy)purine (24). $CHCl_3$–MeOH (98:2) was used for CC, off-white solid, yield 49%. ^1H NMR: δ 13.67 (1H, br s, NH), 8.41 (1H, br s, H-8), 4.10 (3H, s, CH_3). FABMS m/z: 185 $(M+H)^+$. ESI-MS: Found 185.0221, Calcd for $C_6H_5ClN_4O$ 185.0225. Anal. ($C_6H_5ClN_4O$) Found: C, 37.36; H, 2.51; N, 28.77. Calcd C, 37.23; H, 3.12; N, 28.94.

3.1.1.18. 2-Chloro-6-[(isopropyl)oxy]purine (25). $CHCl_3$–MeOH (99:1) with 0.5% NH_4OH was used for CC, white solid, yield 62%. ^1H NMR: δ 8.40 (1H, s, H-8), 5.52 (1H, septet, J = 6.2 Hz, OCH), 1.40 (6H, d, J = 6.2 Hz, 2× CH_3). FABMS m/z: 213 $(M+H)^+$. Anal. ($C_8H_9ClN_4O$) Found: C, 45.15; H, 4.34; N, 26.31. Calcd C, 45.19; H, 4.27; N, 26.35.

3.1.1.19. 2-Chloro-6-(decyloxy)purine (26). Cyclohexane–EtOAc (5:1) was used for CC, white solid, yield 62%. ^1H NMR: δ 8.41 (1H, s, H-8), 4.51 (2H, t, OCH_2), 1.81 (2H, m, CH_2), 1.21 (14H, m, 7× CH_2), 0.86 (3H, m, CH_3). FABMS m/z: 311 $(M+H)^+$. Anal. ($C_{15}H_{23}ClN_4O$) Found: C, 57.85; H, 7.41; N, 17.98. Calcd C, 57.96; H, 7.46; N, 18.02.

3.1.1.20. 2-Chloro-6-(dodecyloxy)purine (27). Cyclohexane–EtOAc (5:1) was used for CC, white solid, yield 71%. ^1H NMR: δ 13.62 (1H, br s, NH), 8.41 (1H, s, H-8), 4.51 (2H, t, J = 6.6 Hz, OCH_2), 1.79 (2H, m, CH_2), 1.28 (18H, m, 9× CH_2), 0.85 (3H, m, CH_3). FABMS m/z: 339 $(M+H)^+$. Anal. ($C_{17}H_{27}ClN_4O$) Found: C, 60.28; H, 8.09; N, 16.17. Calcd C, 60.25; H, 8.03; N, 16.53.

3.1.1.21. 2-Chloro-6-[(4-chlorophenyl)oxy]purine (28). $CHCl_3$–MeOH (98:2) was used for CC, off-white solid, yield 56%. ^1H NMR: δ 13.86 (1H, br s, NH), 8.58 (1H, s, H-8), 7.57 (2H, m, H-2′, H-6′), 7.40 (2H, m, H-3′, H-5′). FABMS m/z: 281 $(M+H)^+$. Anal. ($C_{11}H_6Cl_2N_4O$) Found: C, 47.34; H, 2.32; N, 19.80. Calcd C, 47.00; H, 2.15; N, 19.93.

3.1.1.22. 2-Chloro-6-[(4-methylphenyl)oxy]purine (29). $CHCl_3$–MeOH (98:2) was used for CC, off-white solid, yield 91%. ^1H NMR: δ 13.82 (1H, br s, NH), 8.55 (1H, s, H-8), 7.29 (2H, d, J = 8.2 Hz, H-3′, H-5′), 7.20 (2H, dd, J = 2.3, 8.6 Hz, H-2′, H-6′), 2.36 (3H, s, CH_3). FABMS m/z: 261 $(M+H)^+$. Anal. ($C_{12}H_9ClN_4O$) Found: C, 55.15; H, 3.56; N, 21.36. Calcd C, 55.29; H, 3.48; N, 21.49.

3.1.1.23. 2-Chloro-6-[(3-methylphenyl)oxy]purine (30). $CHCl_3$–MeOH (98:2) was used for CC, off-white solid, yield 58%. ^1H NMR: δ 13.8 (1H, br s, NH), 8.56 (1H, s, H-8), 7.38 (1H, dd, J = 7.9, 8.1 Hz, H-5′), 7.14 (3H, m, H-2′, H-4′, H-6′), 2.36 (3H, s, CH_3). FABMS m/z: 261 $(M+H)^+$. Anal. ($C_{12}H_9ClN_4O$) Found: C, 55.47; H, 3.74; N, 21.14. Calcd C, 55.29; H, 3.48; N, 21.49.

3.1.1.24. 2-Chloro-6-[(cyclohexylmethyl)oxy]purine (31). $CHCl_3$–MeOH (98:2) used for CC, off-white solid, yield 76%. ^1H NMR: δ 13.64 (1H, br s, NH), 8.43 (1H, s, H-8), 4.33 (2H, d, J = 6.3 Hz, OCH_2), 1.5 (11H, m, C_6H_{11}). FABMS m/z: 267 $(M+H)^+$. Anal. ($C_{12}H_{15}ClN_4O$) Found: C, 53.91; H, 5.70; N, 20.94. Calcd C, 54.04; H, 5.67; N, 21.01.

3.1.1.25. 2-Chloro-6-[(3,4-dichlorobenzyl)oxy]purine (32). Cyclohexane–EtOAc (5:1) was used for CC, off-white solid, yield 84%. ^1H NMR: δ 13.73 (1H, br s, NH), 8.47 (1H, s, H-8), 7.85

(1H, d, J = 1.8 Hz, H-2′), 7.71 (1H, d, J = 8.2 Hz, H-5′), 7.50 (1H, ddd, J = 1.9, 2.1, 8.2 Hz, H-6′), 5.59 (2H, s, OCH_2). FABMS m/z: 329 $(M+H)^+$. Anal. ($C_{12}H_7Cl_3N_4O{\cdot}0.15C_4H_8O_2$) Found: C, 44.32; H, 2.49; N, 16.17. Calcd C, 44.15; H, 2.41; N, 16.34.

3.1.2. General procedure for synthesis of N^9-substituted purines (33–36)

To a suspension of the appropriate substituted purine, dry K_2CO_3 (1 equiv), and anhydrous DMSO, was added ethylbromoacetate (1.04 equiv) dropwise at 0 °C under an argon atmosphere. The reaction mixture was allowed to stir at 0 °C for 4 to 6 h and then allowed to stir at room temperature overnight under argon. Deionized water (10–20 mL) was added and the reaction mixture was extracted with diethyl ether. The ethereal layers were pooled, dried over Na_2SO_4, concentrated, and dried in vacuo over P_2O_5 at 25 °C. The crude material was purified by column chromatography (CC) over silica gel G (230–400 mesh).

3.1.2.1. 9-Purine acetic acid, 6-(decyloxy) ethyl ester (33). $CHCl_3$ (100%) was used for CC, off-white solid, yield 42%. ^{1}H NMR: δ 8.50 (1H, s, H-2), 8.35 (1H, s, H-8), 5.18 (2H, s, NCH_2), 4.54 (2H, dd, J = 6.6, 6.7 Hz, OCH_2), 4.17 (2H, ddd, J = 7.0, 7.1, 7.2 Hz, OCH_2), 1.80 (2H, m, CH_2), 1.40 (2H, m, CH_2), 1.22 (15H, m, 6× CH_2, CH_3), 0.85 (3H, m, CH_3). FABMS m/z: 363 $(M+H)^+$. Anal. ($C_{19}H_{30}N_4O_3$) Found: C, 62.72; H, 8.11; N, 15.16. Calcd. C, 62.96; H, 8.34; N, 15.46.

3.1.2.2. 9-Purine acetic acid, 6-(dodecyloxy) ethyl ester (34). $CHCl_3$ (100%) was used for CC, off-white solid, yield 77%. ^{1}H NMR: δ 8.50 (1H, s, H-2 or H-8), 8.35 (1H, s, H-2 or H-8), 5.18 (2H, s, NCH_2), 4.54 (2H, dd, J = 6.5, 6.8 Hz, OCH_2), 4.17 (2H, ddd, J = 7.0, 7.1, 7.1 Hz, OCH_2), 1.80 (2H, m, CH_2), 1.3 (21H, m, 9× CH_2, CH_3), 0.85 (3H, m, CH_3). FABMS m/z: 391 $(M+H)^+$. ESI-MS: Found 391.2700, Calcd for $C_{21}H_{34}N_4O_3$ 391.2704. Anal. ($C_{21}H_{34}N_4O_3{\cdot}0.5H_2O$) Found: C, 63.16; H, 8.46; N, 12.98. Calcd C, 63.13; H, 8.83; N, 14.02.

3.1.2.3. 9-Purine acetic acid, 2-amino-6-(decyloxy) ethyl ester (35). $CHCl_3$ (100%) was used for CC, off-white solid, yield 51%. ^{1}H NMR: δ 7.82 (1H, s, H-8), 6.43 (2H, br s, NH_2), 4.91 (2H, s, NCH_2), 4.39 (2H, dd, J = 6.6, 6.7 Hz, OCH_2), 4.15 (2H, ddd, J = 7.1, 7.1, 7.1 Hz, OCH_2), 1.76 (2H, m, CH_2), 1.24 (17H, m, 7× CH_2, CH_3), 0.85 (3H, m, CH_3). FABMS m/z: 378 $(M+H)^+$. ESI-MS: Found 378.2491, Calcd for $C_{19}H_{31}N_5O_3$ 378.2500.Anal. ($C_{19}H_{31}N_5O_3{\cdot}0.3H_2O$) Found: C, 59.39; H, 7.81; N, 17.95. Calcd C, 59.60; H, 8.32; N, 18.29.

3.1.2.4. 9-Purine acetic acid, 2-amino-6-(dodecyloxy) ethyl ester (36). $CHCl_3$ (100%) was used for CC, off-white solid, yield 31%. ^{1}H NMR: δ 7.82 (1H, s, H-8), 6.43 (2H, br s, NH_2), 4.91 (2H, s, NCH_2), 4.39 (2H, dd, J = 6.6, 6.8 Hz, OCH_2), 4.15 (2H, ddd, J = 7.0, 7.1, 7.1 Hz, OCH_2), 1.74 (2H, m, CH_2), 1.24 (21H, m, 5× CH_2, CH_3), 0.85 (3H, m, CH_3). FABMS m/z: 406 $(M+H)^+$. Anal. ($C_{21}H_{35}N_5O_3$) Found: C, 61.82; H, 8.42; N, 16.99. Calcd. C, 62.20; H, 8.70; N, 17.27.

3.1.3. General procedure for synthesis of 6-decyloxy (38) and 6-decylthio (39) substituted purine intermediates

The starting material, 2-amino-6-chloro-9*H*-purine-9-acetic acid (**37**), for the synthesis of **38** and **39** was purchased commercially and used as such. The 6-decyloxy derivative **38** was synthesized in the same manner as described above for compound **8**. The 6-decylthio derivative **39** was synthesized by the method previously described by us.[12]

3.1.3.1. 2-Amino-6-decyloxy-9H-purine-9-acetic acid (38). $CHCl_3$–MeOH (3:1) was used for CC, off-white solid, yield 65%. ^{1}H NMR: δ 7.69 (1H, s, H-8), 6.20 (2H, br s, NH_2), 4.38 (2H, t, J = 6.6 Hz, OCH_2), 4.28 (2H, s, NCH_2), 1.74 (2H, m, CH_2), 1.37 (14H, m, 7× CH_2), 0.85 (3H, m, CH_3). ESI-MS m/z: Found 350.2204 $(M+H)^+$, Calcd for $C_{17}H_{27}N_5O_3$ 350.2187.

3.1.3.2. 2-Amino-6-decylthio-9*H*-purine-9-acetic acid (39). $CHCl_3$–MeOH (7:1) was used for CC, off-white solid, yield 64%, ^{1}H NMR: δ 7.81 (1H, s, H-8), 6.31 (2H, br s, NH_2), 4.40 (2H, br s, NCH_2), 3.26 (2H, t, J = 7.1 Hz, SCH_2), 1.65 (2H, m, CH_2), 1.37 (14H, m, 7× CH_2), 0.85 (3H, m, CH_3). ESI-MS m/z: Found 366.1959 $(M+H)^+$, Calcd for $C_{17}H_{27}N_5O_2S$ 366.1958. Anal. ($C_{17}H_{27}N_5O_2S{\cdot}2.25H_2O$) Found: C, 50.68; H, 6.54; N, 16.86. Calcd C, 50.29; H, 7.82; N, 16.86.

3.1.4. General procedure for synthesis of 6-decyloxy or 6-decylthio-9-L-Phe/or Ala-substituted purine derivatives (39–50)

To a solution of the appropriate intermediate (**38** or **39**) in anhydrous pyridine (5 mL) under argon, was added the appropriate amino acid ester HCl (1.5 equiv) followed by triethylamine (1.5 equiv). The resulting solution was chilled slightly and PyBOP (1.5 equiv) was added. The reaction solution was allowed to stir under argon overnight at room temperature. Deionized water (20 mL) was added and extracted with $CHCl_3$ (2 × 350 mL). The combined organic layers were washed with 1 N HCl (50 mL), d. H_2O (50 mL), dried over Na_2SO_4, concentrated, re-dissolved in toluene and co-evaporated to remove excess pyridine. The resulting residue was dried in vacuo over P_2O_5 at 25 °C. The crude material was then purified by column chromatography (CC) over silica gel G (230–400 mesh).

3.1.4.1. L-Alanine, *N*-[2-(2-amino-6-decyloxy-9*H*-purin-9-yl)-1-oxoethyl]-, methyl ester (40). $CHCl_3$–MeOH (95:5) was used for CC, off-white solid, yield 57%. ^{1}H NMR: δ 8.74 (1H, d, J = 7.0 Hz, N*H*CH), 7.76 (1H, s, H-8), 6.36 (2H, br s, NH_2), 4.74 (2H, br s, NCH_2), 4.39 (2H, t, J = 6.6 Hz, OCH_2), 4.29 (1H, m, NHC*H*), 3.63 (3H, m, CO_2CH_3), 1.74 (2H, m, CH_2), 1.25 (17H, m, CH_3, 7× CH_2), 0.85 (3H, m, CH_3). ESI-MS m/z: Found 435.2721 $(M+H)^+$, Calcd for $C_{21}H_{34}N_6O_4$ 435.2714. Anal. ($C_{21}H_{34}N_6O_4$) Found: C, 53.87; H, 7.23; N, 16.49.

3.1.4.2. L-Phenylalanine, *N*-[2-(2-amino-6-decyloxy-9*H*-purin-9-yl)-1-oxoethyl]-, methyl ester (41). $CHCl_3$–MeOH (99:1) was used for CC, off-white solid, yield 51%. ^{1}H NMR: δ 8.76 (1H, d, J = 7.5 Hz, N*H*CH), 7.69 (1H, s, H-8), 7.25 (5H, m, Ar), 6.33 (2H, br s, NH_2), 4.71 (2H, dd, NCH_2), 4.48 (1H, m, NHC*H*), 4.38 (2H, t, J = 6.5 Hz, OCH_2), 3.59 (3H, s, CO_2CH_3), 2.98 (2H, m, CH_2Ph), 1.73 (2H, m, CH_2), 1.25 (14H, m, 7× CH_2), 0.85 (3H, m, CH_3). FABMS m/z: 511 $(M+H)^+$. Anal. ($C_{27}H_{38}N_6O_4$) Found: C, 63.41; H, 7.23; N, 16.44. Calcd C, 63.51; H, 7.50; N, 16.46.

3.1.4.3. L-Phenylalanine, *N*-[2-(2-amino-6-decyloxy-9*H*-purin-9-yl)-1-oxoethyl]-, ethyl ester (42). $CHCl_3$–MeOH (99:1) was used for CC, off-white solid, yield 59%. ^{1}H NMR: δ 8.76 (1H, d, J = 7.5 Hz, N*H*CH), 7.70 (1H, s, H-8), 7.25 (5H, m, Ar), 6.33 (2H, br s, NH_2), 4.72 (2H, dd, NCH_2), 4.48 (1H, m, NHC*H*), 4.39 (2H, t, J = 6.4 Hz, OCH_2), 4.03 (2H, dd, CO_2CH_2), 2.98 (2H, m, CH_2Ph), 1.74 (2H, m, CH_2), 1.24 (14H, m, 7× CH_2), 1.07 (3H, t, J = 7.0 Hz, CH_3), 0.85 (3H, m, CH_3). FABMS m/z: 525 $(M+H)^+$. Anal. ($C_{28}H_{40}N_6O_4{\cdot}0.8H_2O$) Found: C, 62.63; H, 7.38; N, 15.30. Calcd C, 62.39; H, 7.78; N, 15.59. 20982.

3.1.4.4. L-Alanine, *N*-[2-(2-amino-6-decylthio-9*H*-purin-9-yl)-1-oxoethyl]-, methyl ester (43). Petroleum ether–EtOAc (1:4) was used for CC, off-white solid, yield 66%. ^{1}H NMR: δ 8.76 (1H, d, J = 7.0 Hz, N*H*CH), 7.84 (1H, s, H-8), 6.44 (2H, br s, NH_2), 4.75 (2H, s, NCH_2), 4.31 (1H, m, NHC*H*), 3.63 (3H, s, CO_2CH_3), 3.26 (2H, t, J = 7.0 Hz, SCH_2), 1.64 (2H, m, CH_2), 1.40 (2H, m, CH_2), 1.29

(15H, m, CH_3, 6× CH_2), 0.85 (3H, m, CH_3). ESI-MS *m*/*z*: Found 451.2487 $(M+H)^+$, Calcd for $C_{21}H_{34}N_6O_3S$ 451.2486. Anal. ($C_{21}H_{34}N_6O_3S$) Found: C, 56.98; H, 7.64; N, 17.34. Calcd. C, 55.98; H, 7.61; N, 18.65.

3.1.4.5. D-Alanine, *N*-[2-(2-amino-6-decylthio-9*H*-purin-9-yl)-1-oxoethyl]-, methyl ester (44). $CHCl_3$–MeOH (98:2) was used for CC, off-white solid, yield 57% yield. ^1H NMR: δ 8.75 (1H, d, *J* = 6.9 Hz, NH), 7.84 (1H, s, H-8), 6.45 (2H, s, NH_2), 4.76 (2H, m, NCH_2), 4.31 (1H, m, CH), 3.63 (3H, s, CH_3), 3.27 (2H, m, SCH_2), 1.64 (2H, m, CH_2), 1.37 (2H, m, CH_2), 1.31 (3H, d, *J* = 7.2 Hz, CH_3), 1.24 (12H, m, 6× CH_2), 0.85 (3H, m, CH_3). ESI-MS *m*/*z*: found 451.2481 $(M+H)^+$, Calcd 451.2485 for $C_{21}H_{34}N_6O_3S$. Anal. ($C_{21}H_{34}N_6O_3S$) Found: C, 56.98; H, 7.64; N, 17.34. Calcd C, 55.98; H, 7.61; N, 18.65.

3.1.4.6. L-Phenylalanine, *N*-[2-(2-amino-6-decylthio-9*H*-purin-9-yl)-1-oxoethyl]-, methyl ester (45). $CHCl_3$–MeOH (99:1) was used for CC, off-white solid, yield 69%. ^1H NMR: δ 8.77 (1H, d, *J* = 7.5 Hz, N*H*CH), 7.77 (1H, s, H-8), 7.22 (5H, m, Ar), 6.40 (2H, br s, NH_2), 4.72 (2H, dd, NCH_2), 4.48 (1H, m, NHC*H*), 3.59 (3H, s, CO_2CH_3), 3.26 (2H, m, SCH_2), 2.99 (2H, m, CH_2Ph), 1.65 (2H, m, CH_2), 1.24 (14H, m, 7× CH_2), 0.85 (3H, m, CH_3). FABMS *m*/*z*: 527 $(M+H)^+$. Anal. ($C_{27}H_{38}N_6O_3S$) Found: C, 61.39; H, 7.23; N, 15.76. Calcd C, 61.57; H, 7.26; N, 15.96.

3.1.4.7. D-Phenylalanine, *N*-[2-(2-amino-6-decylthio-9*H*-purin-9-yl)-1-oxoethyl]-, methyl ester (46). $CHCl_3$–MeOH (98:2) was used for CC, off-white solid, yield 87%. ^1H NMR: δ 8.79 (1H, d, *J* = 7.5 Hz, N*H*CH), 7.77 (1H, s, H-8), 7.22 (5H, m, Ar), 6.42 (2H, br s, NH_2), 4.73 (2H, m, NCH_2), 4.48 (1H, m, NHC*H*), 3.59 (3H, s, CO_2CH_3), 3.26 (2H, t, *J* = 7.1 Hz, SCH_2), 3.03 (1H, dd, *J* = 5.9, 13.6 Hz, CH_2Ph), 2.93 (1H, dd, *J* = 8.4, 13.6 Hz, CH_2Ph), 1.65 (2H, m, CH_2), 1.39 (2H, m, CH_2), 1.24 (12H, m, 6× CH_2), 0.85 (3H, m, CH_3). FABMS *m*/*z*: 527 $(M+H)^+$. Anal. ($C_{27}H_{38}N_6O_3S$) Found: C, 61.51; H, 7.24; N, 15.88. Calcd C, 61.57; H, 7.26; N, 15.96.

3.1.4.8. L-Phenylalanine, *N*-[2-(2-amino-6-decylthio-9*H*-purin-9-yl)-1-oxoethyl]-, ethyl ester (47). Petroleum ether–EtOAc (1:2) was used for CC, off-white solid, yield 60%. ^1H NMR: δ 8.78 (1H, d, *J* = 7.5 Hz, N*H*CH), 7.78 (1H, s, H-8), 7.23 (5H, m, Ar), 6.41 (2H, br s, NH_2), 4.75 (1H, d, NCH_2), 4.72 (1H, d, NCH_2), 4.48 (1H, m, NHC*H*), 4.02 (2H, m, OCH_2CH_3), 3.26 (2H, m, SCH_2), 2.98 (2H, m, CH_2Ph), 1.65 (2H, m, CH_2), 1.29 (14H, m, 7× CH_2), 1.07 (3H, t, *J* = 7.0 Hz, $CO_2CH_2CH_3$), 0.85 (3H, m, CH_3). ESI-MS *m*/*z*: Found 541.2976 $(M+H)^+$, Calcd for $C_{28}H_{40}N_6O_3S$ 541.2955. Anal. ($C_{28}H_{40}N_6O_3S \cdot 0.5H_2O$) Found: C, 62.80; H, 7.72; N, 15.03. Calcd C, 61.18; H, 7.52; N, 15.29.

3.1.4.9. L-Serine, *N*-[2-(2-amino-6-decylthio-9*H*-purin-9-yl)-1-oxoethyl]-, methyl ester (48). $CHCl_3$–MeOH (9:1) was used for CC, off-white solid, yield 53%. ^1H NMR: δ 8.74 (1H, d, *J* = 7.8 Hz, N*H*CH), 7.84 (1H, s, H-8), 6.44 (2H, br s, NH_2), 5.16 (1H, t, *J* = 5.4 Hz, OH), 4.81 (2H, m, NCH_2), 4.38 (1H, m, NHC*H*), 3.73 (1H, m, CH_2), 3.64 (3H, s, CH_3), 3.62 (1H, m, CH_2), 3.26 (2H, t, *J* = 7.1 Hz, SCH_2), 1.65 (2H, m, CH_2), 1.40 (2H, m, CH_2), 1.24 (12H, m, 6× CH_2), 0.85 (3H, m, CH_3). FABMS *m*/*z*: 467 $(M+H)^+$. Anal. ($C_{21}H_{34}N_4O_4S \cdot 0.15H_2O$) Found: C, 53.80; H, 7.10; N, 17.51. Calcd C, 53.75; H, 7.37; N, 17.91.

3.1.4.10. D-Serine, *N*-[2-(2-amino-6-decylthio-9*H*-purin-9-yl)-1-oxoethyl]-, methyl ester (49). $CHCl_3$–MeOH (9:1) was used for CC, off-white solid, yield 59%. ^1H NMR: δ 8.74 (1H, d, *J* = 7.8 Hz, N*H*CH), 7.84 (1H, s, H-8), 6.44 (2H, br s, NH_2), 5.17 (1H, t, *J* = 5.4 Hz, OH), 4.80 (2H, m, NCH_2), 4.38 (1H, m, NHC*H*), 3.73 (1H, m, CH_2), 3.64 (3H, s, CH_3), 3.62 (1H, m, CH_2), 3.26 (2H, m, SCH_2), 1.65 (2H, m, CH_2), 1.40 (2H, m. CH_2), 1.24 (12H, m, 6× CH_2), 0.85 (3H, m, CH_3). FABMS *m*/*z*: 467 $(M+H)^+$. Anal. ($C_{21}H_{34}N_6O_4S$) Found: C, 54.04; H, 7.09; N, 18.01. Calcd C, 54.06; H, 7.34; N, 18.00.

3.1.4.11. L-Serine, *N*-[2-(2-amino-6-decylthio-9*H*-purin-9-yl)-1-oxoethyl]-, ethyl ester (50). $CHCl_3$–MeOH (95:5) was used for CC, off-white solid, yield 83%. ^1H NMR: δ 8.70 (1H, d, *J* = 7.8 Hz, N*H*CH), 7.84 (1H, s, H-8), 6.44 (2H, br s, NH_2), 5.15 (1H, t, *J* = 5.6 Hz, OH), 4.80 (2H, m, NCH_2), 4.35 (1H, m, NHC*H*), 4.10 (2H, dd, *J* = 7.1, 14.2 Hz, OCH_2), 3.73 (1H, m, CH_2OH), 3.63 (1H, m, CH_2OH), 3.26 (2H, t, *J* = 7.1 Hz, SCH_2), 1.18 (3H, t, *J* = 7.1 Hz, CH_3), 1.65 (2H, m, CH_2), 1.40 (2H, m, CH_2), 1.24 (12H, m, 6× CH_2), 0.85 (3H, m, CH_3). FABMS *m*/*z*: 481 $(M+H)^+$. Anal. ($C_{22}H_{36}N_6O_4S$) Found: C, 55.39; H, 7.43; N, 17.25. Calcd C, 54.98; H, 7.55; N, 17.49.

3.1.5. General procedure for synthesis of purine analogs 51–54

The 6-decylmercapto purine analogs **51**–**54** were prepared starting from 6-chloropurine analogs by treating overnight with 1-decanethiol in presence of $(CH_3)_3CO$–K^+ in isopropanol (3 mL) under reflux condition. The reactions were cooled to room temperature, 2 mL of deionized water were added and the mixture acidified with acetic acid (0.5 mL). Solid was collected by filtration and washed with water followed by petroleum ether. The resulting material was dried under vacuum at 78 °C overnight and analyzed.

3.1.5.1. *N*-(6-(Decylthio)-9*H*-purin-2-yl)acetamide (51). Starting from *N*-(6-chloro-6,9-dihydro-1*H*-purin-2-yl)acetamide[17] (100 mg, 0.47 mmol), 106 mg (yield 64%). ^1H NMR: δ 10.33 (1H, s, NH), 8.25 (1H, s, H-8), 3.33 (2H, m, SCH_2), 2.20 (3H, s, $COCH_3$), 1.69 (2H, m, CH_2), 1.42 (2H, m, CH_2), 1.24 (12H, m, 6× CH_2), 0.85 (3H, m, CH_3). ESI-MS *m*/*z*: 350 $(M+H)^+$. Anal. ($C_{17}H_{27}N_5OS$); Found: C, 58.26; H, 7.97; N, 19.87. Calcd C, 58.42; H, 7.79; N, 20.04.

3.1.5.2. 3-(2-Amino-6-(decylthio)-9*H*-purin-9-yl)propane-1,2-diol (52). Starting from 3-(2-amino-6-chloro-1*H*-purin-9(6*H*)-yl)propane-1,2-diol[17] (100 mg, 0.41 mmol), 82 mg (yield 52%). ^1H NMR: δ 7.82 (1H, s, H-8), 6.44 (2H, br s, NH_2), 5.07 (1H, d, *J* = 5.1 Hz, CHO*H*), 4.16 (1H, dd, *J* = 3.3, 13.5 Hz, NCH_2), 3.85 (1H, dd, *J* = 7.9, 13.5 Hz, NCH_2), 3.77 (1H, m, C*H*OH) 3.37 (2H, m, CH_2OH), 3.26 (2H, dd, *J* = 6.9, 7.2 Hz, SCH_2), 1.65 (2H, m, CH_2), 1.38 (2H, m, CH_2), 1.24 (12H, m, 6× CH_2), 0.85 (3H, m, CH_3). ESI-MS *m*/*z*: 382 $(M+H)^+$. Anal. ($C_{18}H_{31}N_5O_2S$); Found: C, 56.59; H, 8.04; N, 18.39. Calcd C, 56.66; H, 8.19; N, 18.36.

3.1.5.3. 9-Benzyl-6-(decylthio)-9*H*-purin-2-amine (53). Starting from 9-benzyl-6-chloro-6,9-dihydro-1*H*-purin-2-amine[17] (100 mg, 0.38 mmol), 135 mg (yield 88%). ^1H NMR: δ 8.02 (1H, s, H-8), 7.31 (3H, m, Ar), 7.22 (2H, m, Ar), 6.48 (2H, br s, NH_2), 5.25 (2H, s, NCH_2), 3.26 (2H, dd, *J* = 6.9, 7.2 Hz, SCH_2), 1.65 (2H, m, CH_2), 1.38 (2H, m, CH_2), 1.24 (12H, m, 6× CH_2), 0.85 (3H, m, CH_3). ESI-MS *m*/*z*: 398 $(M+H)^+$. Anal. ($C_{22}H_{31}N_5S$); Found: C, 66.50 H, 7.89; N, 17.42. Calcd C, 66.46; H, 7.86; N, 17.61.

3.1.5.4. 2-((2,6-Bis(decylthio)-9*H*-purin-9-yl)methoxy)ethanol (54). Starting from 2-((2,6-dichloro-1*H*-purin-9(6*H*)-yl)methoxy)ethanol[17] (100 mg, 0.38 mmol), 40 mg (yield 26%). ^1H NMR: δ 8.43 (1H, s, H-8), 5.57 (2H, s, NCH_2), 4.66 (1H, t, *J* = 5.3 Hz, OH), 3.51 (2H, m, OCH_2), 3.45 (2H, m, CH_2OH), 3.27 (2H, dd, *J* = 4.5, 7.8 Hz, SCH_2), 3.18 (2H, dd, *J* = 7.2, 7.5 Hz, SCH_2), 1.70 (4H, m, 2× CH_2), 1.41 (4H, m, 2× CH_2), 1.24 (24H, m, 12× CH_2), 0.84 (6H, m, 2× CH_3). ESI-MS *m*/*z*: 539 $(M+H)^+$. Anal. ($C_{28}H_{50}N_4O_2S_2$); Found: C, 62.53; H, 9.46; N, 10.22. Calcd C, 62.41; H, 9.35; N, 10.40.

3.1.6. General procedure for synthesis of purine analogs 55–69

The 6-decylmercapto purine analogs **55–69** were prepared starting from 6-purinethiones which were treated overnight with 1-chlorodecane/anhydrous K_2CO_3 in DMAc (3 mL) at room temperature. Deionized water (2 mL) was added and the mixture was acidified with acetic acid (0.2 mL). Solid was collected by filtration and washed with water followed by petroleum ether. The material was dried under vacuum at 78 °C overnight and analyzed.

3.1.6.1. 6-(Decylthio)-9-ethyl-9*H*-purin-2-amine (55). Starting from 2-amino-9-ethyl-1*H*-purine-6(9*H*)-thione[25] (100 mg, 0.51 mmol), 80 mg (yield 47%). ^{1}H NMR: δ 7.94 (1H, s, H-8), 6.42 (2H, br s, NH_2), 4.03 (2H, dd, J = 7.2, 14.4 Hz, NCH_2), 3.26 (2H, t, J = 7.2 Hz, SCH_2), 1.64 (2H, m, CH_2), 1.34 (3H, t, J = 7.2 Hz, CH_3), 1.40 (2H, m, CH_2), 1.24 (12H, m, 6× CH_2), 0.85 (3H, m, CH_3). ESI-MS m/z: 336 $(M+H)^+$. Anal. ($C_{17}H_{29}N_5S{\cdot}0.1H_2O$); Found: C, 60.47; H, 8.75; N, 20.54. Calcd C, 60.53; H, 8.73; N, 20.76.

3.1.6.2. 6-(Decylthio)-2-ethyl-9*H*-purine (56). Starting from 2-ethyl-1*H*-purine-6(9*H*)-thione[26] (100 mg, 0.55 mmol), 56 mg (yield 31%). ^{1}H NMR: δ 13.2 (1H, br s, NH), 8.31 (1H, s, H-8), 3.33 (2H, dd, J = 6.9, 7.2 Hz, SCH_2), 2.89 (2H, dd, J = 7.5, 15 Hz, CH_2), 1.70 (2H, m, CH_2), 1.41 (2H, m, CH_2), 1.31 (3H, t, J = 7.5 Hz, CH_3), 1.23 (12H, m, 6× CH_2), 0.84 (3H, m, CH_3). ESI-MS m/z: 321 $(M+H)^+$. Anal. ($C_{17}H_{28}N_4S.0.2H_2O$); Found: C, 63.14; H, 8.70; N, 17.29. Calcd C, 63.00; H, 8.83; N, 17.28.

3.1.6.3. 9-Cycloheptyl-6-(decylthio)-9*H*-purine (57). Starting from 9-cycloheptyl-1*H*-purine-6(9*H*)-thione[27] (100 mg, 0.40 mmol), 124 mg (yield 79%). ^{1}H NMR: δ 8.68 (1H, s, H-2), 8.54 (1H, s, H-8), 4.64 (1H, m, NCH), 3.32 (2H, dd, J = 7.2, 7.5 Hz, SCH_2), 2.08 (6H, m, 3× CH_2), 1.65 (8H, m, 4× CH_2), 1.41 (2H, m, CH_2), 1.23 (12H, m, 6× CH_2), 0.84 (3H, m, CH_3). ESI-MS m/z: 389 $(M+H)^+$. Anal. ($C_{22}H_{36}N_4S.0.1H_2O$); Found: C, 67.77; H, 9.37; N, 14.34. Calcd C, 67.68; H, 9.35; N, 14.35.

3.1.6.4. 9-Allyl-6-(decylthio)-9*H*-purine (58). Starting from 9-allyl-1*H*-purine-6(9*H*)-thione (100 mg, 0.52 mmol), 72 mg (yield 42%). ^{1}H NMR: δ 8.70 (1H, s, H-2), 8.44 (1H, s, H-8), 6.08 (1H, m, CH), 5.21 (1H, m, CH_{2a}), 5.06 (1H, m, CH_{2b}), 4.89 (2H, m, NCH_2), 3.35 (2H, dd, J = 7.2, 7.5 Hz, SCH_2), 1.70 (2H, m, CH_2), 1.41 (2H, m, CH_2), 1.23 (12H, m, 6× CH_2), 0.84 (3H, m, CH_3). ESI-MS m/z: 333 $(M+H)^+$. Anal. ($C_{18}H_{28}N_4S.0.1H_2O$); Found: C, 64.74; H, 8.55; N, 16.72. Calcd C, 64.67; H, 8.50; N, 16.76.

3.1.6.5. 2-(6-(Decylthio)-9*H*-purin-9-yl)ethyl acetate (59). Starting from 2-(6-thioxo-1*H*-purin-9(6*H*)-yl)ethyl acetate (100 mg, 0.42 mmol), 146 mg (yield 92%). ^{1}H NMR: δ 8.71 (1H, s, H-2), 8.47 (1H, s, H-8), 4.50 (2H, m, NCH_2), 4.41 (2H, m, OCH_2), 3.33 (2H, dd, J = 7.2, 7.5 Hz, SCH_2), 2.93 (3H, s, CH_3), 1.70 (2H, m, CH_2), 1.41 (2H, m, CH_2), 1.24 (12H, m, 6× CH_2), 0.85 (3H, m, CH_3). ESI-MS m/z: 379 $(M+H)^+$. Anal. ($C_{19}H_{30}N_4O_2S.0.15H_2O$); Found: C, 59.69; H, 7.88; N, 14.61. Calcd C, 59.86; H, 8.01; N, 14.69.

3.1.6.6. 2-(6-(Decylthio)-1*H*-purin-9(6*H*)-yl)acetonitrile (60). Starting from 2-(6-thioxo-1*H*-purin-9(6*H*)-yl)acetonitrile (100 mg, 0.52 mmol), 75 mg (yield 43%). ^{1}H NMR: δ 8.79 (1H, s, H-2), 8.51 (1H, s, H-8), 5.55 (2H, m, NCH_2), 3.36 (2H, dd, J = 7.2, 7.5 Hz, SCH_2), 1.71 (2H, m, CH_2), 1.41 (2H, m, CH_2), 1.24 (12H, m, 6× CH_2), 0.85 (3H, m, CH_3). ESI-MS m/z: 332 $(M+H)^+$. Anal. ($C_{17}H_{25}N_5S$); Found: C, 61.48; H, 7.36; N, 20.99. Calcd C, 61.60; H, 7.60; N, 21.13.

3.1.6.7. (*E*)-*N*-Benzylidene-6-(decylthio)-9*H*-purin-9-amine (61). Starting from (*E*)-9-(benzylideneamino)-1*H*-purine-6(9*H*)-thione[28] (100 mg, 0.39 mmol), 82 mg (yield 53%). ^{1}H NMR: δ 9.78 (1H, s, CH), 8.90 (1H, s, H-2), 8.82 (1H, s, H-8), 7.95 (2H, m, Ar), 7.60 (3H, m, Ar), 3.37 (2H, dd, J = 7.2, 7.5 Hz, SCH_2), 1.71 (2H, m, CH_2), 1.41 (2H, m, CH_2), 1.24 (12H, m, 6× CH_2), 0.85 (3H, m, CH_3). ESI-MS m/z: 396 $(M+H)^+$. Anal. ($C_{22}H_{29}N_5S.0.1H_2O$); Found: C, 66.52; H, 7.07; N, 17.62. Calcd C, 66.50; H, 7.36; N, 17.62.

3.1.6.8. 6-(Decylthio)-9-ethyl-9*H*-purine (62). Starting from 9-ethyl-1*H*-purine-6(9*H*)-thione[29] (100 mg, 0.55 mmol), 160 mg (yield 90%). ^{1}H NMR: δ 8.70 (1H, s, H-2), 8.48 (1H, s, H-8), 4.27 (2H, dd, J = 7.2, 14.4 Hz, NCH_2), 3.34 (2H, dd, J = 7.2, 7.5 Hz, SCH_2), 1.70 (2H, m, CH_2), 1.43 (3H, t, J = 7.2 Hz, CH_3), 1.40 (2H, m, CH_2), 1.24 (12H, m, 6× CH_2), 0.85 (3H, m, CH_3). ESI-MS m/z: 321 $(M+H)^+$. Anal. ($C_{17}H_{28}N_4S$); Found: C, 63.72; H, 8.94; N, 17.44. Calcd C, 63.71; H, 8.81; N, 17.48.

3.1.6.9. 9-Benzyl-6-(decylthio)-9*H*-purine (63). Starting from 9-benzyl-1*H*-purine-6(9*H*)-thione[17] (100 mg, 0.41 mmol), 59 mg (yield 37%). ^{1}H NMR: δ 8.71 (1H, s, H-2), 8.59 (1H, s, H-8), 7.33 (5H, m, Ar), 5.47 (2H, s, NCH_2), 3.35 (2H, m, SCH_2), 1.70 (2H, m, CH_2), 1.40 (2H, m, CH_2), 1.24 (12H, m, 6× CH_2), 0.85 (3H, m, CH_3). ESI-MS m/z: 383 $(M+H)^+$. Anal. ($C_{22}H_{30}N_4S$); Found: C, 69.05; H, 7.83; N, 14.66. Calcd C, 69.07; H, 7.90; N, 14.65.

3.1.6.10. 2-(6-(Decylthio)-9*H*-purin-9-yl)cyclohexanol (64). Starting from 9-(2-hydroxycyclohexyl)-1*H*-purine-6(9*H*)-thione[18d] (100 mg, 0.40 mmol), 80 mg (yield 51%). ^{1}H NMR: δ 8.68 (1H, s, H-2), 8.42 (1H, s, H-8), 4.99 (1H, d, J = 4.2 Hz, OH), 4.55 (1H, m, NCH), 3.97 (1H, br s, CHOH), 3.34 (2H, m, SCH_2), 2.30 (1H, m, CH_2), 1.74 (6H, m, CH_2), 1.42 (5H, m, CH_2), 1.24 (12H, m, 6× CH_2), 0.85 (3H, m, CH_3). ESI-MS m/z: 391 $(M+H)^+$. Anal. ($C_{21}H_{34}N_4OS$); Found: C, 64.45; H, 8.83; N, 14.34. Calcd C, 64.58; H, 8.77; N, 14.34.

3.1.6.11. 9-Cyclopentyl-6-(decylthio)-9*H*-purine (65). Starting from 9-cyclopentyl-1*H*-purine-6(9*H*)-thione[18a] (100 mg, 0.48 mmol), 134 mg (yield 82%). ^{1}H NMR: δ 8.69 (1H, s, H-2), 8.52 (1H, s, H-8), 4.93 (1H, m, NCH), 3.33 (2H, m, SCH_2), 2.16 (2H, m, CH_2), 2.03 (2H, m, CH_2), 1.89 (2H, m, CH_2), 1.70 (2H, m, CH_2), 1.40 (2H, m, CH_2), 1.23 (12H, m, 6× CH_2), 0.84 (3H, m, CH_3). ESI-MS m/z: 361 $(M+H)^+$. Anal. ($C_{30}H_{32}N_4S.0.1H_2O$); Found: C, 66.06; H, 9.10; N, 15.89. Calcd C, 66.29; H, 8.96; N, 15.46.

3.1.6.12. 9-Butyl-6-(decylthio)-9*H*-purine (66). Starting from 9-butyl-1*H*-purine-6(9*H*)-thione[18a] (100 mg, 0.48 mmol), 124 mg (yield 74%). ^{1}H NMR: δ 8.69 (1H, s, H-2), 8.47 (1H, s, H-8), 4.24 (2H, dd, J = 6.9, 7.2 Hz, NCH_2), 3.34 (2H, t, J = 7.2 Hz, SCH_2), 1.81 (2H, m, CH_2), 1.69 (2H, m, CH_2), 1.39 (2H, m, CH_2), 1.23 (14H, m, 7× CH_2), 0.89 (3H, t, J = 7.2 Hz, CH_3), 0.84 (3H, m, CH_3). ESI-MS m/z: 349 $(M+H)^+$. Anal. ($C_{19}H_{32}N_4S.0.2H_2O$); Found: C, 64.88; H, 9.28; N, 15.89. Calcd C, 64.80; H, 9.27; N, 15.90.

3.1.6.13. 2-(6-(Decylthio)-9*H*-purin-9-yl)cyclopentanol (67). Starting from 9-(2-hydroxycyclopentyl)-1*H*-purine-6(9*H*)-thione[18c] (100 mg, 0.42 mmol), 94 mg (yield 59%). ^{1}H NMR: δ 8.68 (1H, s, H-2), 8.45 (1H, s, H-8), 4.92 (1H, d, J = 4.2 Hz, OH), 4.78 (1H, m, NCH), 4.18 (1H, m, CHOH), 3.34 (2H, m, SCH_2), 2.29 (2H, m, CH_2), 2.11 (2H, m, CH_2), 1.94 (2H, m, CH_2), 1.70 (2H, m, CH_2), 1.40 (2H, m, CH_2), 1.24 (12H, m, 6× CH_2), 0.85 (3H, m, CH_3). ESI-MS m/z: 377 $(M+H)^+$. Anal. ($C_{20}H_{32}N_4OS$); Found: C, 63.88; H, 8.56; N, 14.75. Calcd C, 63.79; H, 8.57; N, 14.88.

3.1.6.14. *N*-(6-(Decylthio)-9*H*-purin-9-yl)acetamide (68). Starting from *N*-(6-thioxo-1*H*-purin-9(6*H*)-yl)acetamide[18b] (100 mg, 0.48 mmol), 40 mg (yield 24%). ^{1}H NMR: δ 8.69 (1H, s, H-2), 8.48 (1H, s, H-8), 3.35 (2H, m, SCH_2), 2.10 (3H, s, CH_3), 1.71 (2H, m, CH_2), 1.40 (2H, m, CH_2), 1.24 (12H, m, 6×

CH_2), 0.85 (3H, m, CH_3). ESI-MS *m*/*z*: 350 $(M+H)^+$. Anal. ($C_{17}H_{27}N_5OS.0.1H_2O$); Found: C, 58.01; H, 8.03; N, 19.80. Calcd C, 58.12; H, 7.80; N, 19.93.

3.1.6.15. 9-Cyclohexyl-6-(decylthio)-9*H*-purine (69). Starting from 9-cyclohexyl-1*H*-purine-6(9*H*)-thione[18a] (100 mg, 0.43 mmol), 93 mg (yield 58%). ^{1}H NMR: δ 8.68 (1H, s, H-2), 8.54 (1H, s, H-8), 4.45 (1H, m, NCH), 3.33 (2H, m, SCH_2), 1.91 (8H, m, 4× CH_2), 1.69 (2H, m, CH_2), 1.41 (4H, m, 2× CH_2), 1.24 (12H, m, 6× CH_2), 0.85 (3H, m, CH_3). ESI-MS *m*/*z*: 375 $(M+H)^+$. Anal. ($C_{21}H_{34}N_4S.0.1H_2O$); Found: C, 66.97; H, 9.19; N, 14.85. Calcd C, 67.01; H, 9.16; N, 14.89.

3.1.7. *N*-(3,4-Dimethylphenyl)-2-(4-(methylthio)-7*H*-pyrrolo[2,3-*d*]pyrimidin-7-yl)acetamide (70)

Compound **73** (110 mg, 0.58 mmol) was dissolved in dry DMF (5 mL) under argon and 3,4-dimethylaniline (106 mg, 0.87 mmol) was added followed by the addition of HATU (265 mg, 0.70 mmol) and DIEA (0.16 mL, 0.16 mmol). The reaction mixture was stirred at room temperature overnight. The resulting solid was filtered and washed with ether to give pure **70** (86 mg, yield 54%) after drying overnight under vacuum over P_2O_5 at 78 °C. Mp: 203–205 °C. ^{1}H NMR: δ 10.26 (1H, s, CONH), 8.60 (1H, s), 7.54 (1H, d, *J* = 3.6 Hz), 7.35 (1H, s), 7.27 (1H, d, *J* = 2.0, 11.0 Hz), 7.05 (1H, d, *J* = 8.1 Hz), 6.54 (1H, d, *J* = 3.3 Hz), 5.11 (2H, s), 2.66 (3H, s), 2.17 (3H, s), 2.16 (3H, s). ESI-MS: *m*/*z* calcd for $C_{17}H_{18}N_4OS$ $(M+H)^+$: 327.1274, found: 327.1278.

3.1.8. 4-(Methylthio)-7*H*-pyrrolo[2,3-*d*]pyrimidine (72)

To a methanol (15 mL) solution of commercially available 4-chloro-7*H*-pyrrolo[2,3-*d*]pyrimidine (400 mg, 2.60 mmol) was added $NaSCH_3$ (272 mg, 3.90 mmol), and the reaction mixture was stirred overnight at room temperature. The mixture was concentrated and the crude material was purified by column chromatography using 5% $CHCl_3$–MeOH to yield pure product **68** (386 mg, yield 90%). ^{1}H NMR: δ 12.08 (1H, br s, NH), 8.58 (1H, s, H-2), 7.45 (1H, d, *J* = 3.4 Hz, H-8), 6.47 (1H, d, *J* = 3.5 Hz, H-7), 2.64 (3H, s, SCH_3). ESI-MS: *m*/*z* calcd for $C_7H_7N_3S$ $(M+H)^+$: 166.0433, found: 166.0432.

3.1.9. 2-(4-(Methylthio)-7*H*-pyrrolo[2,3-*d*]pyrimidin-7-yl)acetic acid (73)

Compound **74** (100 mg, 0.61 mmol) was dissolved in dry DMF (3 mL) under argon and K_2CO_3 (100 mg, 0.73 mmol) was added followed by ethylbromoacetate (0.061 mL, 0.73 mmol). The reaction mixture was stirred overnight at room temperature. Solvent was removed, and the residue was dissolved in $CHCl_3$ (25 mL) and washed with water (2 × 15 mL). The chloroform layers were dried over Na_2SO_4 and concentrated. The crude material was purified by column chromatography over silica gel G (230–400 mesh) using 10% $CHCl_3$–MeOH as the eluant to yield the pure ethyl ester of **73** (120 mg). This material was then dissolved in dry MeOH (3 mL) and 1 N NaOH (1 mL) was added. The reaction mixture was stirred overnight at room temperature. The resulting solid was filtered, washed with water and dried at 78 °C under vacuum to give pure **73** (110 mg, yield 82% in two steps). ^{1}H NMR: δ 8.55 (1H, s, H-2), 7.40 (1H, d, *J* = 3.5 Hz, H-8), 6.41 (1H, d, *J* = 3.5 Hz, H-7), 4.56 (1H, s, NCH_2), 2.65 (3H, s, SCH_3). ESI-MS: *m*/*z* calcd for $C_9H_9N_3O_2S$ $(M+H)^+$: 224.0488, found: 224.0490.

3.2. Biology

3.2.1. Activity against *Mtb* H_{37}Ra and MAC strain

All compounds were tested for their inhibitory activity against *Mtb* H_{37}Ra (ATCC 25177) and MAC NJ211 strains. The screening was performed at 1.28 and 12.8 μg/mL in Middlebrook 7H9 broth supplemented with 0.2% glycerol and ADC enrichment using a colorimetric (Alamar blue) microdilution broth assay.[30] The active compounds (⩽12.8 μg/mL) were re-tested using twofold dilutions to obtain the actual MIC_{99}. The MIC_{99} was recorded as the lowest drug concentration that inhibited the growth completely.

3.2.2. Activity against H_{37}Rv[20]

The primary screen was conducted at 6.25 μg/mL against Mtb. H_{37}Rv (ATCC 27294) using radiometric BACTEC assay in a 12B medium by Tuberculosis Antimicrobial Coordinating Facility (TAACF). Compounds demonstrating at least 90% inhibition at 6.25 μg/mL were re-tested to determine the MIC_{90}, defined as the lowest concentrating inhibiting growth by 90% or higher.

3.2.3. Cytotoxicity against VERO cells and selectivity index (SI)[20]

Concurrent with the determination of MIC_{90}'s, compounds **16**, **33**, **34**, **36**, **55** and **65** were tested for cytotoxicity (CC_{50}) in VERO cells using Promega non-radioactive cell proliferation assay kits by TAACF. The selectivity index is defined as the ratio of the measured CC_{50} in VERO cells to the MIC_{90} (against Mtb H_{37}Rv).

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