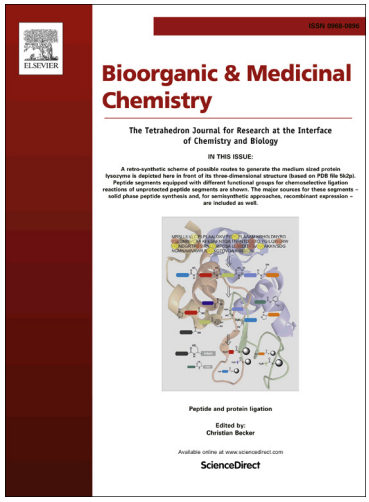
Accepted Manuscript



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Modifications of flexible nonyl chain and nucleobase head group of (+)-erythro-9-(2′s-hydroxy-3′s-nonyl)adenine [(+)-EHNA] as adenosine deaminase inhibitors

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Abstract:

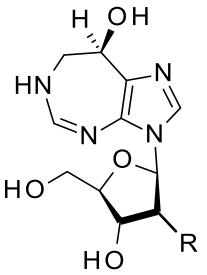
A series of terminal nonyl chain and nucleobase modified analogues of (+)-EHNA (**III**) were synthesized and evaluated for their ability to inhibit adenosine deaminase (ADA). The constrained carbon analogues of (+)-EHNA, **7a-7h, 10a-c**, **12**, **13, 14** and **17a-c** appeared very potent with *Ki* values in the low nanomolar range. *Thio*-analogues of (+)-EHNA **24a-e** wherein 5′C of nonyl chain replaced by sulfur atom found to be less potent compared to (+)-EHNA. Docking of the representative compounds into the active site of ADA was performed to understand structure-activity relationships. Compounds **7a** (*Ki*: 1.1 nM) **7b** (*Ki*: 5.2 nM) and **26a** (*Ki*: 5.9 nM) showed suitable balance of potency, microsomal stability and demonstrated better pharmacokinetic properties as compared to (+)-EHNA and therefore may have therapeutic potential for various inflammatory diseases, hypertension and cancer.

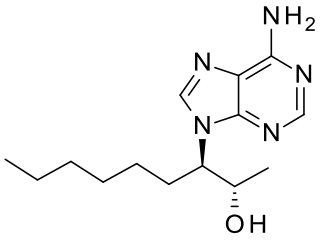
Keywords: ADA; EHNA; Nonyl chain modification; Constrained analogues; Docking, Pharmacokinetics

1. Introduction

Hydrolytic irreversible deamination of adenosine and 2-deoxyadenosine to inosine and 2-deoxyinosine by adenosine deaminase (adenosine aminohydrolase, ADA, EC3.5.4.4) is an important enzymatic reaction in purine salvage pathway.[1] Both adenosine and 2-deoxyadenosine are biologically active purines having profound effect on cellular physiology. The enzymatic activity of ADA helps in the regulation of extracellular and intracellular concentration of these substrates. Extracellular levels of adenosine regulate many physiological responses by activating four G-protein coupled adenosine receptors namely A_1, A_{2A}, A_{2B} and A_3.[2] Adenosine also acts as a sensor and provides information to the immune system about the tissue damage or acute inflammatory changes occurring in the vicinity of the immune system.[3] While 2′-deoxyadenosine behaves as a cytotoxic metabolite and is generally considered the primary cause of lymphotoxicity in ADA deficient severe combined immunodeficiency (SCID) by impairment of the development and function of both T and B cells.[4] Several lines of evidence support a complex regulatory role played by ADA in different immune cell functions as well as a significant involvement of this catabolic enzyme in the pathophysiology of several inflammatory diseases. Indeed, abnormalities in ADA activity have also been reported in a variety of other diseases such as AIDS,[5] rheumatoid arthritis,[6] coronary artery disease,[7] anemia,[8] lymphomas and leukemia.[9] Therefore inhibition of ADA activity has special indication against inflammatory diseases, hypertension, and ischemic injury. In addition ADA inhibition potentiates 2′-deoxyadenosine mediated lymphotoxicity in the treatment of some leukemias[10] and could block the inactivation of cytotoxic and antiviral adenosine analogues that are substrates of ADA and thereby increasing their potency.[11]

The involvement of ADA in various health disorders triggered numerous attempts to develop ADA inhibitors as a potential therapeutic agent. Several structurally unrelated natural and synthetic compounds with various degree of potency have been reported as ADA inhibitors.[12] Among them 2′-deoxycoformycin (**I**, dcf or pentostatin) and coformycin (**II**) are naturally occurring transition state ADA inhibitors with pico-molar affinity.[13-15] The very potent inhibitory activity of transition state analogue inhibitors (dcf and analogues) make its binding to enzyme almost irreversible resulting into severe toxicity,[16] which limits their use as a chemotherapeutic agents.





I R = H, 2'-Deoxycoformycin, K_i = 2.5 pM
II R = OH, Coformycin, K_i = 10 pM

III (+)-EHNA, K_i = 1.13 nM

Figure 1. Known inhibitors of Adenosine deaminase

Inhibitors like (+)-EHNA, **III** (K_i: 1–3 nM) and its analogues are semi-tight competitive inhibitor of ADA, categorized in the class of ground state inhibitors.[17] The use of EHNA as a therapeutic agent is limited due to low *in vivo* metabolic stability (poor pharmacokinetic properties) as it is rapidly metabolized to several hydroxylated derivatives and excreted out of body.[18] Palle *et. al.* reported synthesis and biological evaluation of 8′-hydroxy, 9′-hydroxy and 8′, 9′-dihydroxy derivatives of (+)-EHNA believed to be putative metabolites in (+)-EHNA metabolism.[19] These hydroxylated analogues were less potent than (+)-EHNA confirming the importance of hydrophobic nature of nonyl chain. Subsequently same group have studied introduction of unsaturation by having double and triple bond at the 5',6'-position in nonyl chain.[20] The potency data revealed that *cis*-isomer maintains the potency where as *trans*-isomer and acetylene derivatives are less active compared to (+)-EHNA. Further SAR was expanded wherein *cis*-geometry was maintained by introducing phenyl ring in terminal nonyl side chain and these confimationally restricted analogues showed similar potency to (+)-EHNA.[20] However, very limited SAR was explored and all reported analogues were profiled only for potency. Hence ADA inhibitors with reduced toxicity and improved pharmacokinetic profile are eagerly craved.

In the present study, it was aimed to identify a novel EHNA analogue with improved *in vitro* and *in vivo* metabolic stability while retaining nanomolar potency. Recently the crystal structure of EHNA-ADA complex is reported wherein (+)-EHNA tightly binds in the active site of ADA by several hydrogen bonds, van der Walls and hydrophobic interactions.[21] The critical hydrogen bond interactions of (+)-EHNA with ADA resulting from the nucleoside base and C-2′ hydroxy group where as nonyl chain is responsible for hydrophobic interactions. Hence we thought of modifying EHNA structure to impart desired properties with minimal impact on key interactions at active sites. Accordingly, EHNA structure was fragmented into three main segments P1, P2 and P3 based on their binding position in active

site of ADA (**Figure 2**). Our SAR investigation employed a strategy of point modification in these segments which may help to improve metabolic stability while retaining potency. The chemistry and biological results of this series of compounds are presented in the following sections.

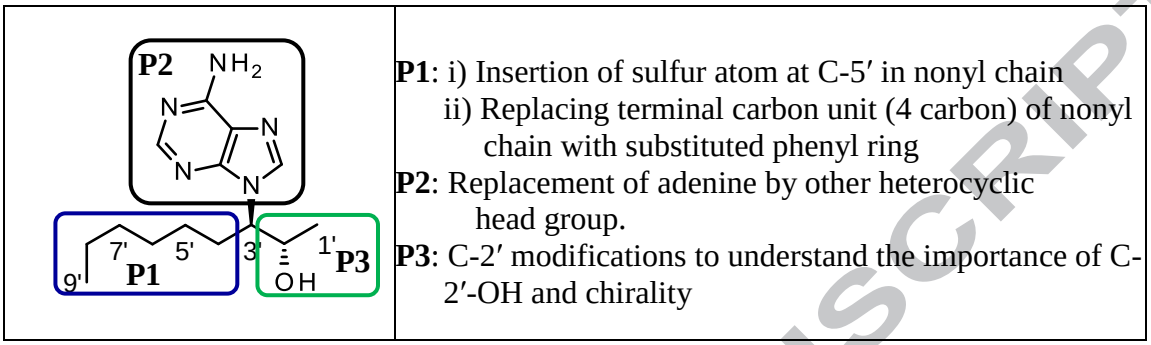
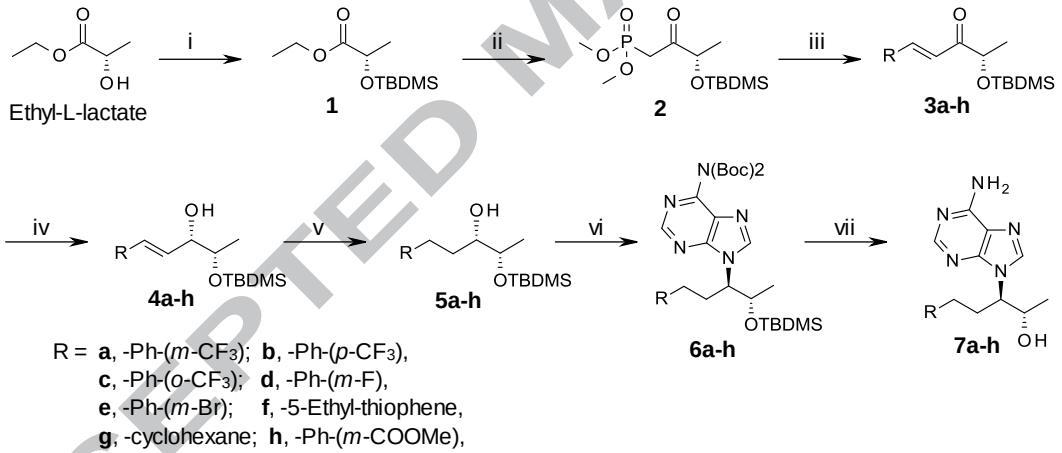


Figure 2. Modification strategy of EHNA analogues.

2. Results and Discussion

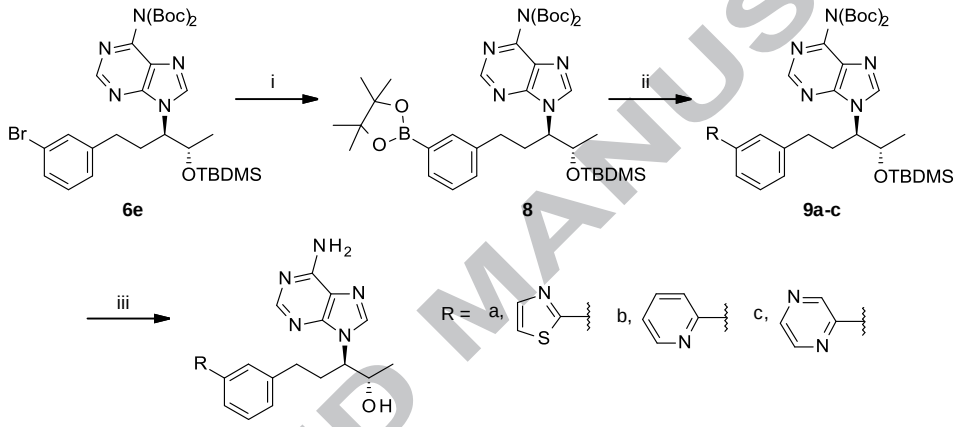
2.1 Chemistry



Scheme 1. Reagents and conditions: (i) TBDMSCl, DMAP, triethylamine, THF, rt, 15 h; (ii) $(MeO)_2P(O)CH_3$, *n*-BuLi, THF, –78 °C, 2.5 h; (iii) R-CHO, *n*-BuLi, –50°C to rt, 16 h; or 3-bromobenzaldehyde, NaH, THF, 0 °C, 3 h; (iv) $CeCl_3.7H_2O$, $NaBH_4$, MeOH, –78°C, 1 h; (v) H_2/Pd-C EtOAc, rt, 1 h or $RhCl(PPh_3)_3$, H_2, THF/*tert*-butyl alcohol, (1:1), rt, 16 h; (vi) N6,N6-Diboc adenine, DEAD, PPh_3, THF, 0 °C to rt, 16 h (vii) Methanolic HCl, Methanol, rt 5 h.

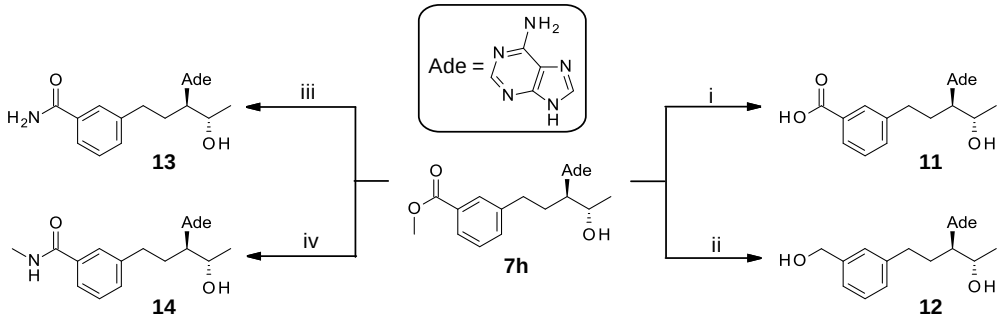
To probe the role of nonyl chain on potency and metabolic stability (P1 segment modification, Figure 2), various *meta*- substituted phenyl analogues were synthesized *via* the general route outlined in **Scheme 1**. Various hydrophobic, hydrophilic and heteroaryls groups were selected on *meta*- position of phenyl group in anticipation of gaining additional H-bonding or van-der Walls contact to retain potency of these analogues. The synthesis started from commercially available ethyl-L-lactate where α-hydroxy group was protected with *tert*-

butyl dimethyl silane to afford **1**.[22] Condensation of **1** with lithium methyldimethyl phosphonate in THF furnished the phosphonate **2** which was then subjected to Wittig-Horner reaction with appropriate aldehyde to provide olefine derivatives **3a-h**.[23] Stereoselective reduction of ketone (**3a-h)** using cerium chloride and sodium borohydride in methanol furnished alcohol (**4a-h)**[24] followed by reduction of double bond with H_2(g) and Pd/C or Wilkinson's catalyst afforded compounds **5a-h**, These modified nonyl analogues were coupled with nucleobase, N6,N6-Diboc adenine [25a] using Mitsunobu reaction to provide compounds **6a-h**[25b]. Deprotection of **6a-h** using methanolic HCl afforded the (+)-EHNA analogues **7a-h** having desired 2′*S*, 3′*R* configuration.



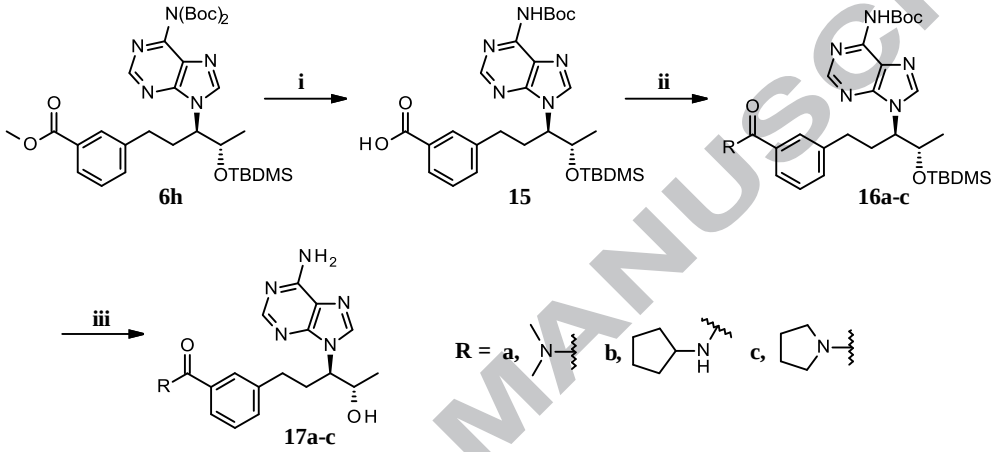
Scheme 2. Reagents and conditions: (i) Bis(pinacolato)diboron, Pd(dppf)Cl_2.CH_2Cl_2, CH_3COOK, dioxane, 80 °C, 3h; (ii) Heteroarylhalides, Pd(dppf)Cl_2.CH_2Cl_2, K_2CO_3, dioxane, 80 °C, 2–4 h; (iii) 4M HCl in dioxane, dioxane, rt, 5–10 h.

Synthesis of biaryl derivatives (**10a-c**) wherein heteroaryl ring substituted at *meta*-position on phenyl ring, is outlined in **Scheme 2**. Intermediate **6e** was converted to boronate ester **8**, which was treated with commercially available heteroaryl halides under typical Suzuki reaction condition to obtain corresponding biaryl derivatives **9a–c** in good yields. Finally, deprotection of **9a–c** with 4M HCl in dioxane at rt provided target compounds **10a–c** in 50–70% yields.

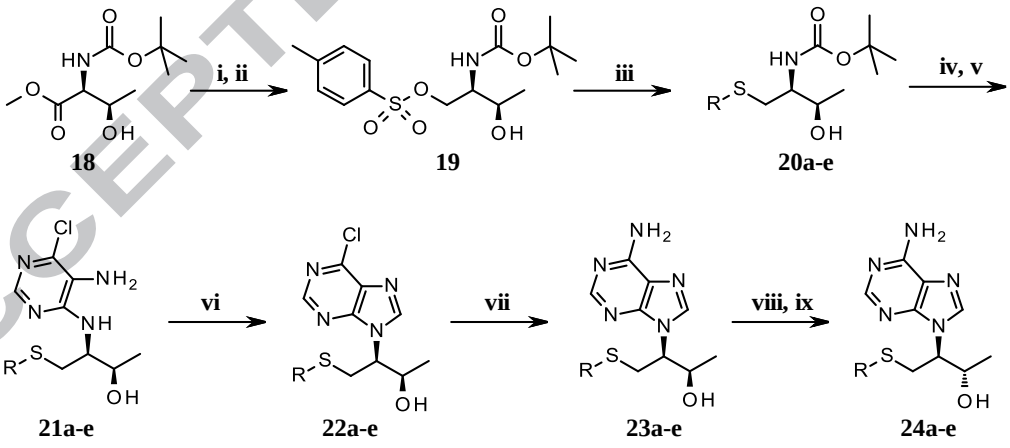


Scheme 3. Reagents and conditions: (**i**) $LiOH.H_2O$, THF:MeOH:H_2O (3:1:1), rt, 5h; (**ii**) LAH, THF, Reflux, 16h; (**iii**) Mg_3N_2, MeOH, 80 °C, 48h; (**iv**) 40 % aqueous Methylamine, 80 °C, 10h

Next, we synthesized analogues having negatively charged or neutral polar substitution on phenyl ring as decribed in **Scheme 3** and **Scheme 4**. Methyl ester of intermediate **7h** was converted to acid **11**, alcohol **12**, amides **13** and **14**. Additional amide derivatives **17a–c** were synthesized by converting the ester **6h** to acid **15**, followed by reacting with respective amines under amide coupling condition and subsequent deprotection.



Scheme 4. Reagents and conditions: (i) $LiOH.H_2O$, THF:MeOH:H_2O (3:1:1), rt, 16 h; (ii) Respective amine, EDCI.HCl, HOBt, Et_3N, DCM, 25 °C, 16 h; (iii) 4M HCl in dioxane, dioxane, rt, 5–10 h.

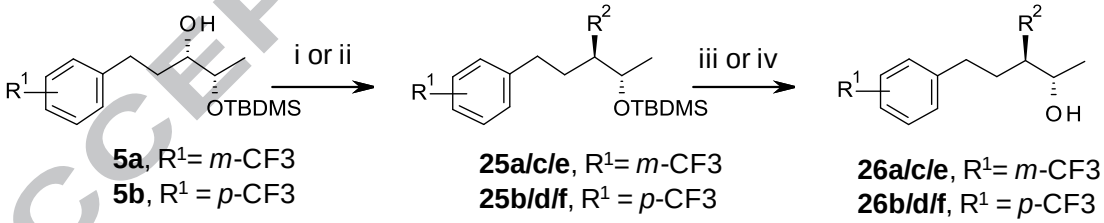


R = **a**, -C_4H_9; **b**, -Ph; **c**, -Ph-(*o*-CH_3); **d**, -Ph-(*m*-CH_3); **e**, -Ph-(*m*-CF_3);

Scheme 5. Reagents and conditions: (i) $NaBH_4$, THF, 0 ^{0}C, rt, overnight; (ii) tosyl choride, triethylamine, DCM, rt, 6 h; (iii) sodium salt of butanethiol/thiophenols, dioxane, rt, 5-8 h; (iv) methanolic HCl, 40 ^{0}C, 3 h; (v) 5-amino 4,6-dichloro purine, 1-butanol, triethylamine, reflux, 21 h; (vi) triethylorthoformate, conc. HCl 6 h; (vii) methanolic ammonia, 60 ^{0}C, 16 h; (viii) *p*-nitrobenzoic acid, triphenyl phosphine, DEAD, THF, rt, 16 h; (ix) NaOH, THF:MeOH:H_2O (3:1:1), rt, 5 h.

The synthesis of thio-derivatives of (+)-EHNA is outlined in **scheme 5**. Readily available boc protected L-threonine methyl ester **18** was subjected to ester reduction using $NaBH_4$ followed by tosylation gave compound **19**. Nucleophilic substitution of **19** with sodium salt of butane thiol and various thiophenols yielded aryl sulphides **20a-e** respectively. Deprotection of **20a-e** with methanolic HCl followed by condensation with 5-amino-4,6-dichloropyrimidine provided pyrimidine derivatives **21a-e**[26] in moderate yields. Treatment of **21a-e** with triethylorthoformate afforded cyclized chloropurine derivatives **22a-e**[26], subsequent amination with liquid ammonia provided thio- analogues of EHNA **23a-e** with 2′*R*, 3′*S threo* configuration. Finally inversion of C-2′ hydroxy with Mitsunobu reaction[27] followed by hydrolysis afforded desired thio-analogues of (+)-EHNA **24a-e** with 2′*S*, 3′*S erythro* configuration**.**

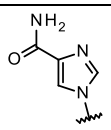
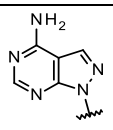
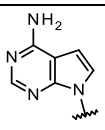
To understand the significance of adenine group towards potency contribution (P2 segment modification, Figure 2), we replaced adenine group by 4-carboxamide-imidazole (**26a-b**), 4-amino-pyrazolo[3,4-d]pyrimidine (**26c-d**) and 4-amino-pyrolo[3,4-d]pyrimidine (**26e-f**). The desired analogues were synthesized using intermediate **5a** and **5b** as described in **Scheme 6**. In two steps 3-hydroxy group of **5a/5b** was converted to mesylate and displacement by S_N2 reaction with 4-imidazolecarboxamide in the presence of NaH in DMF afforded **25a-b**.[28] Whereas **25c-f** were synthesized by reacting 4-Diboc-aminopyrazolo[3,4-d]pyrimidine[25a]/4-chloro-7H-pyrrolo[2,3-d]pyrimidine and **5a/5b** under Mitsunobu condition, Finally deprotection of **25a-f** afforded desired compounds **26a-f**.



5a, R^1= *m*-CF3
5b, R^1 = *p*-CF3

25a/c/e, R^1= *m*-CF3
25b/d/f, R^1 = *p*-CF3

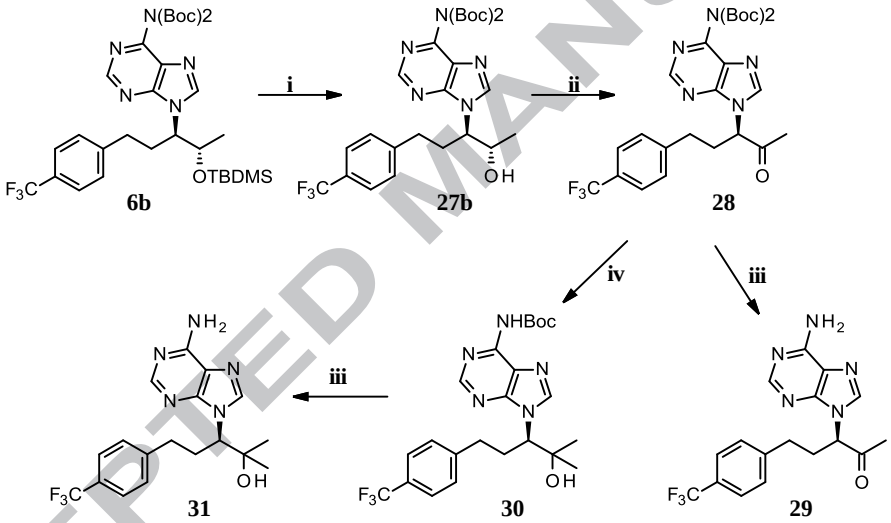
26a/c/e, R^1= *m*-CF3
26b/d/f, R^1 = *p*-CF3

	26a	26b	26c	26d	26e	26f
R^1	*m*-CF3	*p*-CF3	*m*-CF3	*p*-CF3	*m*-CF3	*p*-CF3
R^2						

Scheme 6. Reagents and conditions: (i) (a) MsCl, Et_3N, CH_2Cl_2, 0 °C, 1 h; (b) mesylate added to 4-imidazolecarboxamide, NaH, DMF, 80 °C, 36 - 48 h; (ii) 4-Diboc-aminopyrazolo[3,4-d]pyrimidine or 4-chloro-

7H-pyrrolo[2,3-d]pyrimidine, DEAD, PPh_3, THF, 0 °C to rt, 16 h; (iii) 4M HCl in Dioxane, Dioxane, rt, 5–10 h. (**iv**) NaN_3, PPh_3, DMSO, 110 °C, 1N HCl in water, 5 h.

We probed the role of C-2′ hydroxyl and chirality on potency (P3 segment modification, Figure 2) by converting secondary hydroxyl of **7b** to ketone **29** and tertiary hydroxyl group **31**. As described in **Scheme 7** selective deprotection of TDBMS group of intermediate **6b** yielded alcohol **27b** which was converted to ketone **28** by using pyridinium chlorochromate. Subsequent boc deprotection of **28** using 4M HCl in dioxane gave desired ketone derivative **29** in good yield. Intermediate **28** was treated with methyl magnesium bromide, which afforded the conversion of ketone to *tert*-hydroxy derivative along with deprotection of one boc group to get monoboc-adenine derivative **30**. The second boc group was deprotected using 4M HCl in dioxane yielded *tert*-alcohol derivative **31**.

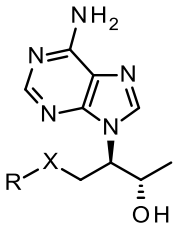


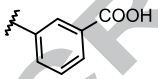
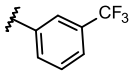
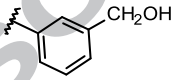
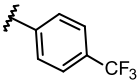
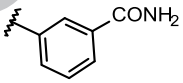
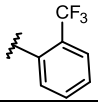
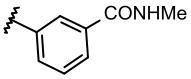
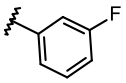
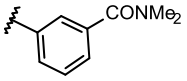
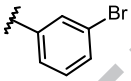
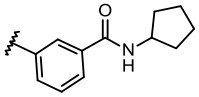
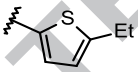
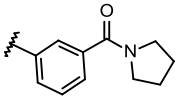
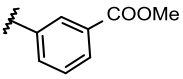
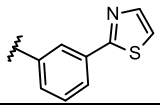
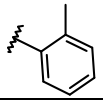
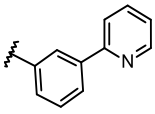
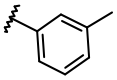
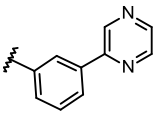
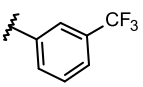
Scheme 7. Reagents and conditions: (i) TBAF, THF, 0 °C, 1 h.; (ii) PCC, DCM, rt, 4 h; (iii) 4M HCl in dioxane, dioxane, rt, 5–10 h; (iv) CH_3MgBr (3.0M in diethyl ether), THF, 0 °C to rt, 2 h.

2.2 Structure Activity Relationship

All analogues of (+)-EHNA were evaluated for their adenosine deaminase inhibitory potencies against bovine ADA using recombinant enzyme (**Table 1**).[29] Inhibitory potency of compounds was expressed as inhibitory constant (K_i). The (+)-EHNA showed potency with *Ki*: 2.2 nM which is comparable with reported data[30] and it is considered as a reference standard in this study.

Table 1. Inhibitory potency of (+)-EHNA analogues with bovine ADA



Compound Number	X	R	K_i (nM)	Compound No.	X	R	K_i (nM)
(+)-EHNA	-CH_2-	-C_4H_9	2.2	**11**	-CH_2-		53
7a	-CH_2-		1.1	**12**	-CH_2-		4.6
7b	-CH_2-		5.2	**13**	-CH_2-		2.9
7c	-CH_2-		6.2	**14**	-CH_2-		2.7
7d	-CH_2-		1.6	**17a**	-CH_2-		1.8
7e	-CH_2-		0.6	**17b**	-CH_2-		1.8
7f	-CH_2-		2.1	**17c**	-CH_2-		2.2
7g	-CH_2-		1.9	**24a**	-S-	-$(CH_2)_3CH_3$	54
7h	-CH_2-		2.2	**24b**	-S-		135
10a	-CH_2-		2.6	**24c**	-S-		66
10b	-CH_2-		2.3	**24d**	-S-		49
10c	-CH_2-		1.9	**24e**	-S-		25

Initially we focused our attention on the modification of segment P1 due to its involvement in potential metabolism of (+) EHNA.[18, 19] To avoid observed ω-oxidation of nonyl chain as well as to impart rigidity it was planned to replace C-6' to C-9' fragment of nonyl chain in EHNA by appropriately substituted phenyl, thiophene and cyclohexyl group. First we were interested to explore trifluoromethyl substitution on phenyl ring which may bring pharmacokinetic and pharmacodynamic advantages. Trifluoromethyl group enhances metabolic stability as well as increased lipophilicity may improve absorption, distribution or enhanced interaction with a hydrophobic binding region. We were pleased to find that *meta*-trifluoromethyl analogue (**7a**, *Ki*: 1.1 nM) appeared 2-fold more potent compared to (+)-EHNA (*Ki*: 2.2 nM) and ~5-fold better in potency compared to *para*- and *ortho*-trifluoromethyl analogues (**7b**, *Ki*: 5.2 nM & **7c**, *Ki*: 6.2 nM). These results suggested *meta*-trifluoromethyl is slightly better compared to *ortho*- and *para*- and moreover **7a** offered a good microsomal stability across species (refer **Table 3**).

Next, we explored various hydrophobic substituents by replacing *meta*-CF_3 of **7a**. Small hydrophobic substituent like fluorine **7d** (*Ki*: 1.6 nM) and bromine **7e (***Ki*: 0.6 nM) maintained good potency but had detrimental effect on metabolic stability (data not captured). Thiophene (**7f**, *Ki*: 1.1 nM), cyclohexyl (**7g**, *Ki*: 1.1 nM) and *meta*- methyl benzoate (**7h**, *Ki*: 2.2 nM) analogues showed comparable potency to (+)-EHNA but showed moderate to poor metabolic stability across species. Further analogues with bulkier substituent more specifically heterocycles like 2-thiazole **10a** (*Ki*: 2.6 nM), 2-pyridine **10b** (*Ki*: 2.3 nM), and 2-pyrazine **10c** (*Ki*: 1.9 nM) exhibited similar activity relative to **7a** and showed moderate to good metabolic stability.

Further, it was hypothesized that analogues appended with polar substitution on phenyl would lead an improvement in potency *via* additional hydrogen bond interaction with backbone residues. Also it was thought that the polar substitution may contribute towards improving drug like features. Encouragingly, most of the analogues synthesized in this series **12-14** and **17a-c** showed the potency in the range of 2–5 nM, exception of compound **11,** having carboxylic acid moiety showed a significant loss of the activity (*Ki*: 53 nM).

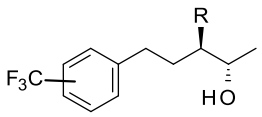
We were interested to explore *thio*-EHNA analogues, wherein C-5′ was replaced by sulfur. Previously described *oxo*-EHNA analogues (C-5′ replaced by oxygen) resulted in decreased potency, presumably due to loss of hydrophobic feature of alkyl chain. Hence it was rationalized the sulfur being less hydrophilic compare to oxygen *thio*-EHNA analogues may help to maintain the hydrophobicity of alkyl chain. Additionally, sulfur mimic C=C

bond, and will help to reduce the degree of freedom which in turn may improve pharmacokinetic properties. We examined focused *thio*-analogues **24a-e.** *Thio*-EHNA analogue **24a** (*Ki*: 54 nM) and constrained analogues **24c-d** showed 25-fold loss of potency compared to (+)-EHNA, whereas constrained analogue **24b** (*Ki*: 135 nM) without any substitution on phenyl showed significant loss in potency. *Thio*- analogue of **7a** (**24e,** *Ki*: 25 nM), exhibited 2-fold gain in the potency compared to *thio*-EHNA, highlighting the significance of *meta*- trifluoromethyl group.

Next, we examined the significance of adenine ring towards potency contribution by replacing adenine with appropriately substituted heterocyclic ring such as 4-carboxamide-imidazole (**26a-b**), 4-amino-pyrazolo[3,4-d]pyrimidine (**26c-d**) and 4-amino-pyrolo[3,4-d]pyrimidine (**26e-f**). Imidazole 4-carboxamide derivatives **26a** is equipotent to **7a** whereas **26b** appeared 5- fold less potent indicating the importance of *meta*-CF_3 substitution on phenyl. All other analogues **26c–f** led complete loss in the potency. Thus, it supports the earlier finding that N7 nitrogen is involved in hydrogen bonding with Asp-296[21] and is critical for the inhibitory activity of (+)-EHNA.[31]

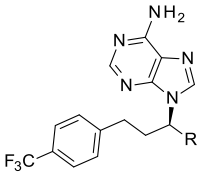
In order to validate the role of secondary hydroxyl in P3 segment, we thought of converting C-2′-hydroxy of **6b** to ketone and tertiary hydroxyl group and its influence on the potency. The affinity of keto compound **29** to the ADA was completely lost where as compound with *tert*-hydroxyl at C-2′ **31** (*Ki*: 69 nM) appeared less potent compared to (+)-EHNA and **7b.** This revealed that *sec*-hydroxyl group is necessary for the activity. The possible explanation for loss of activity in **31** may be due to extra methyl causing steric interaction as well as devoid of chirality at C-2′position

Table 2. Inhibitory potency of (+)-EHNA analogues with bovine ADA

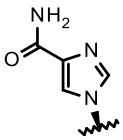
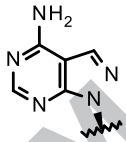
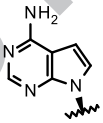


26a, 26c, 26e (*meta*-CF_3)

26b, 26d, 26f (*para*-CF_3)



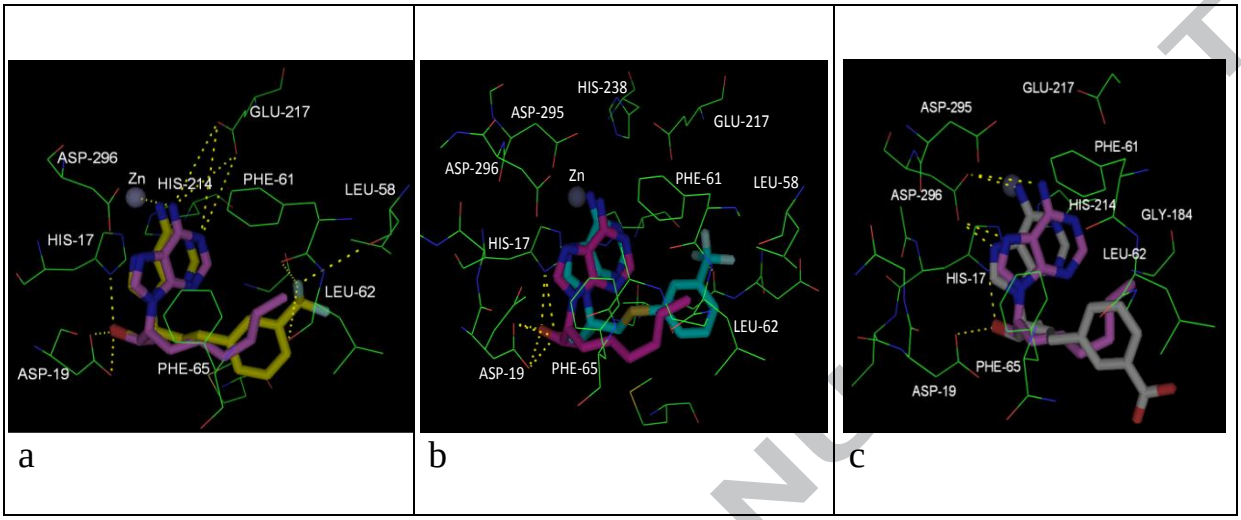
29, 31

Compound Number	R	K_i (nM)
26a		5.9
26b		29
26c		Inactive
26d		Inactive
26e		Inactive
26f		Inactive
29		Inactive
31		69

2.3 Docking studies in active site of ADA-(+)-EHNA co-crystal structure

To account for strong ADA inhibitory activity of **7a**, we build a docking model of ADA/**7a** complex based on X-ray crystal structure of ADA complex with (+)-EHNA (pdb id 2Z7G)[21] followed by energy minimization refinement using GOLD and MOE. Initially to confirm the reliability of docking procedure, (+)-EHNA was extracted from and re-docked to the crystal structure of ADA. As demonstrated in **Figure 3a** compound **7a** occupied an active site of ADA in a similar manner as observed in the complex of ADA/(+)-EHNA. The edited phenyl group of **7a** maintained most of the hydrophobic *van der* Walls interactions with Leu 58, Phe 61, Leu 62, Phe 65, Leu 106, Trp 117, Met 155 and Gly 184 amino acids of ADA. The appended trifluoromethyl group on phenyl appears to fit more favorably in ADA

active site pocket. The structural modification of **7a** does not disturb the hydrophobic interaction of C-2′ methyl and hydrogen bond interaction of adenine with Glu 217 and Asp 296 as well as C-2′-hydroxyl with His 17 and Asp 19.



a b c

Figure 3. (**a**) The docked pose of compound **7a** (Yellow) overlayed with cocrystal structure of (+)-EHNA (Magenta) from pdb id 2Z7G, (**b**) compound **24e** (cyans) overlayed with cocrystal structure of (+)-EHNA (Magenta) from pdb id 2Z7G and (**C**) compound **11** (White) overlayed with cocrystal structure of (+)-EHNA (Magenta) from pdb id 2Z7G. The oxygen (O), nitrogen (N), fluorine (F) and sulfur (S) atoms are colored in Red, Blue, Pale cyans and Olive respectively. Docking was done using program GOLD. The docked poses of compounds were further minimized using program MOE. The putative hydrogen bonds are shown by dotted line. The atomic numbering used here is from pdb id 2Z7G.

As shown in **Figure 3b** constrained *thio*-EHNA analogue **24e** oriented slightly in different way to that of terminal alkyl chain in (+)-EHNA. The possible explanation could be the smaller bond angle of C-S-C (~90 °) in **24e** as compared to bond angle of C-C-C (110 ^{o}) in (+)-EHNA**.** The change in orientation of terminal phenyl ring might have lost few hydrophobic interactions and thus affecting the potency of **24e**.

Another remarkable binding feature associated with compound **7a** and **24e** is that the trifluoromethyl group substituted on *meta*-position of terminal phenyl ring appears to fit more favorably and might allow *van der* Walls interaction between one of the fluorine and the carbonyl from backbone located in hydrophobic pocket. This additional interaction resulted in gaining two and five fold higher inhibitory activity for **24e** compared to **24d** and **24b** respectively.

Docking study of compound **11** exhibited that C-2′-hydroxy group maintaining its hydrogen bonding interactions with Asp-19 and His-17. On the other hand adenine ring of **11** showed

slight misalignment with respect to adenine ring of (+)-EHNA as shown in **Figure 3c**. This might have affected water mediated hydrogen bonding interactions of C6-amino group with Glu-217 as C6-amino group moving slightly away from Glu-217. Another interesting observation from docking pose of **11** clearly suggests the different orientation of terminal phenyl ring which results in loss of some hydrophobic interactions with amino acid residues like Phe-61, Phe-65, Leu-58 and Trp-117 leading the reduction in potency. The possible explanation is repulsive forces due to charge carboxylic group. This was further supported by molecular docking of compounds **7h** and **14** wherein carboxylic acid group is converted to methyl ester and N-methyl amide respectively. Both compounds docked well in ADA binding site. This modeling hypothesis was validated as compound **11** (Gold Score 17.01) has lost the potency whereas compounds **7h** (Gold Score 13.42) and **14** (Gold Score 13.14) showed good inhibitory potencies.

2.4 In vitro metabolic stability and intravenous pharmacokinetic in male Wistar rats

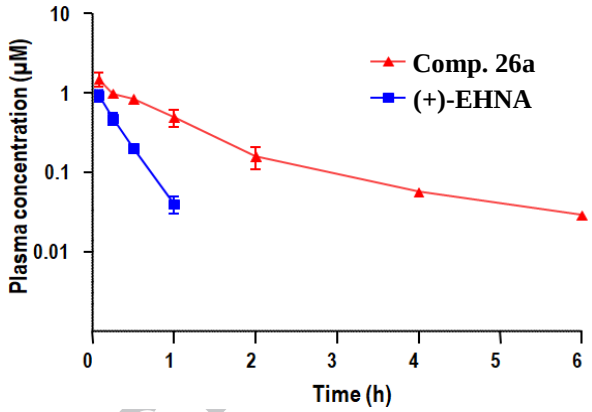
The metabolic stability results of all potent compounds from both series against mouse, rat and human liver microsomes are summarized in **Table 3**. In constrained carbon analogues of (+)-EHNA compounds **7a**, **7b**, **7h** and **14** were found to be stable whereas compounds **7f** and **7g** showed moderate stability. Compounds with bulkier hydrophobic substituent (**10a-c**) showed moderate metabolic stability across species. *Thio*-EHNA analogue **24e** showed good stability in rat and human but moderately stable in mouse.

Table 3. Metabolic Stability of selected compounds.

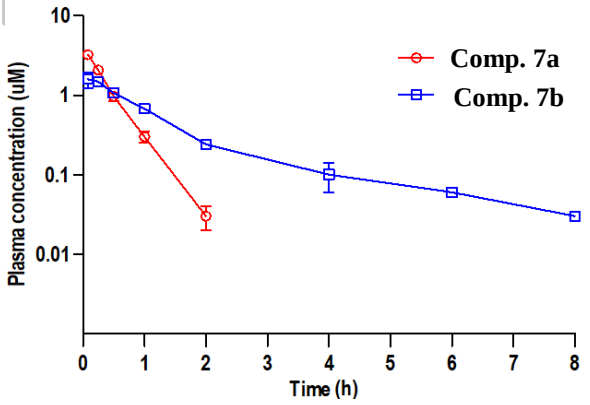
Compound Number	MR (nmol/min/mg) @0.125mg/ml protein	$t_{½}$ (min)	% R at 30min (+NADPH)	% R at 30min (-NADPH)	Matrix
7a	0.08	>30	74	89	MLM
	0.04	>30	86	91	RLM
	0.06	>30	80	95	HLM
7b	0.04	>30	85	94	MLM
	<0.04	>30	93	93	RLM
	<0.04	>30	99	95	HLM
7f	0.11	>30	64	90	MLM
	0.15	>30	58	95	RLM
	0.07	>30	76	95	HLM
7g	0.07	>30	77	92	MLM
	0.44	13	19	94	RLM
	0.11	>30	65	83	HLM
7h	0.04	>30	88	100	MLM
	<0.04	>30	96	99	RLM
	0.10	>30	68	65	HLM
14	<0.04	>30	100	96	MLM

	<0.04	>30	91	100	RLM
	<0.04	>30	90	92	HLM
10a	0.10	>30	65	92	MLM
	0.09	>30	76	91	RLM
	0.05	>30	84	100	HLM
10b	0.15	>30	58	100	MLM
	0.12	>30	64	100	RLM
	0.06	>30	82	91	HLM
10c	0.16	>30	57	94	MLM
	<0.04	>30	90	85	RLM
	0.09	>30	71	94	HLM
24a	<0.04	>30	100	94	MLM
	0.04	>30	86	97	RLM
	<0.04	>30	94	85	HLM
24e	0.22	25	43	100	MLM
	0.05	>100	83	100	RLM
	0.05	>100	83	89	HLM
26a	0.04	>30	89	93	MLM
	0.04	>30	87	92	RLM
	0.06	>30	79	94	HLM

Plasma Concentration *Vs* time profile of (+)-EHNA and **26a**



Plasma Concentration *Vs* time profile of **7a** and **7b**



Compound Number	IV Dose (mg/kg)	AUC_{0-t} (µM.h)	V_{ss} (L/Kg)	CL (mL/min/Kg)	$t_{1/2}$ (h)
(+)-EHNA	1	0.35	NR[#]	NR[#]	NR[#]
26a	1	1.42	2	33.3	0.94
7a	3	1.61	1.88	85	<0.5
7b	3	2.21	7.01	59	2.21

Formulation vehicle: NMP-10%, PEG-300-15%, 0.1M Ammonium acetate buffer q.s.(pH 3); [#]NR: not reportable due to very fast elimination. Route of

Figure 4. Intravenous pharmacokinetic profile of (+)-EHNA, **26a**, **7a** and **7b** in male *Wistar* rat

As the primary objective of this study was identification of ADA inhibitors with improved pharmacokinetics to that of (+)-EHNA, potent and stable compounds **26a**, **7a** and **7b** were evaluated for intravenous pharmacokinetic study in male *wistar* rat (**Figure 4**). (+)-EHNA showed very rapid plasma clearance and concentrations were found below quantification level beyond 1 h post dose administration. However, **26a, 7a** and **7b** demonstrated better pharmacokinetic properties than (+)-EHNA with respect clearance, half life and plasma exposures.

3. Conclusion

In summary, we explored SAR on three segments of (+)-EHNA by carrying out point modifications with aim to improve pharmacokinetic properties. All synthesized novel analogues were initially evaluated for their ability to inhibit ADA and subsequent profiling of potent compounds for microsomal stability and pharmacokinetics. Majority of modifications attempted on *n*-hexyl sidechain of (+)-EHNA were able to retain the potency with good microsomal stability. The constrained carbon analogues (**7a–h**, **10a–c**, **12**, **13, 14** and **17a–c**) exhibited low nanomolar potency whereas *thio*- analogues (**24a–e**) were found to be less potent. SAR supported by docking study also revealed that the hydrophobic binding pocket in the active site has much more tolerant of structural deviance and substitution on terminal phenyl ring allows polar as well as non-polar groups. Compound **7a**, **7b** and **26a** showed good balance of potency and microsomal stability and demonstrated better pharmacokinetic properties in rat compared to (+)-EHNA and therefore may have therapeutic potential for various inflammatory diseases, hypertension and cancer.

4. Experimental Section

4.1 General methods for chemistry.

All reagents, starting materials, and solvents (including dry solvents) were obtained from commercial suppliers and used as such without further purification. Reactions were carried out in oven-dried glassware under a positive pressure of argon/nitrogen unless otherwise mentioned. Reaction progress was determined by thin layer chromatography (TLC) analysis on Merck Kieselgel 60 F254 plates. Column chromatography was performed on silica gel (Rankem, 60-120 mesh). Deuterated solvents (Cambridge Isotope Laboratories) for NMR spectroscopic analysis were used as received. All NMR spectra were recorded on Varian 400 MHz spectrometer. Coupling constants are measured in Hertz. All chemical shifts are quoted in ppm, relative to tetramethylsilane, using the residual solvent peak as a reference standard.

Low-resolution mass spectra (MS) were determined on an Agilent 1200 series LC/MSD/VL. All MS experiments were performed using electrospray ionization (ESI) in positive ion mode. Analytical HPLC was performed on an Agilent 1200 series instrument equipped with Diode Array Detector Reagents and solvents were obtained from commercial sources and used without further purification. (Method: Column Agilent Eclipse XDB C-18, 5µM, 250mm X 4.6mm, at a flow of 1.5 ml/min, mobile phase: 0.05% formic acid in water and acetonitrile) Abbreviations of the solvents are used as follows: AcOEt, ethyl acetate; THF, tetrahydrofuran; EtOH, ethanol; DMF, N,N-dimethylformamide; Et_2O, diethyl ether; MeOH, methanol; CH_3CN, acetonitrile; K_2CO_3, potassium carbonate;

4.1.1. Ethyl (2S)-2-[tert-butyl(dimethyl)silyl]oxypropanoate (***1***)

To a stirred solution of ethyl-L-lactate (25 g, 211 mmol) in DMF (500 mL) was added *tert*-butyl dimethylsilyl chloride (33.52 g, 222 mmol), 4-dimethylamino pyridine (2.58 g, 21 mmol) followed by triethylamine (103 mL, 741mmol) at room temperature. The reaction mixture was stirred for 15 h (progress of reaction was monitored by TLC). Upon reaction completion, the mixture was diluted with water (300 mL) and the product was extracted with dichloromethane (250 mL x 3). Combined organic layer was washed with brine (200 mL), dried over anhydrous sodium sulfate and concentrated under reduced pressure to give the crude product. The crude product was purified by column chromatography using 4% EtOAc–Hexane as solvent system to afford pure **1** (39.8 g, 81%). ^{1}H NMR (400 MHz; $CDCl_3$) **:** δ 0.07 (s, 3H), 0.10 (s, 3H), 0.90 (s, 9H), 1.28 (t, *J* = 6.9 Hz, 3H), 1.39 (d, *J* = 6.9 Hz, 3H), 4.11-4.24 (m, 2H), 4.31 (q, *J* = 6.9 Hz, 1H); ^{13}C-NMR (100 MHz; $CDCl_3$) **:** δ −5.16, −4.83, 14.29, 18.42, 21.40, 25.83 (3C), 60.81, 68.58, 174.20; ESI-MS: m/z: 233.3 [M^+ + 1].

4.1.2. *(3S)-3-[tert-butyl(dimethyl)silyl]oxy-1-dimethoxyphosphoryl-butan-2-one* (***2***)

To a stirred solution of dimethylmethylphosphonate (32.03 g, 258 mmol) in THF (75 mL) maintained at −78 °C was added *n*-BuLi (103.3 mL, 258 mmol, 2.5 M soln. in Hexane) in dropwise manner under argon atmosphere. The reaction mixture was continued to stirr at −78 °C for 1 h followed by slow addition of solution of (*S*)-2(tert-butyl-methyl-silanyloxy)-propionic acid ethyl ester (**1**, 15g, 64.6 mmol) in THF (10 mL) and was stirred for additional 90 min.. After reaction completion, the mixture was quenched by the slow addition of aqueous ammonium chloride (250 mL). The aqueous layer was extracted with EtOAc (250 mL x 2), the combined organic layer was washed with water (200 mL), brine (100 mL), dried over anhydrous sodium sulfate and conc. under reduced pressure to give crude product as yellow oil. The crude product was purified by column chromatography using 35% EtOAc–Hexane as solvent system to afford pure **2** (14.3 g, 71%). ^{1}H NMR (400 MHz; $CDCl_3$)**:** δ

0.09 (s, 3H), 0.10 (s, 3H), 0.92 (s, 9H), 1.32 (d, J = 6.8 Hz, 3H), 3.26 (dd, J_{HH} = 14.7 Hz, $^2J_{HP}$ = 22 Hz, 1H), 3.38 (dd, J_{HH} = 15.2 Hz, $^2J_{HP}$ = 21 Hz, 1H), 3.78 (d, $^3J_{HP}$ = 3.4 Hz, 3H), 3.80 (d, $^3J_{HP}$ = 3.5 Hz, 3H), 4.24 (q, J = 6.4 Hz, 1H). ^{13}C-NMR (100 MHz; $(CD_3)_2SO$)**:** δ −5.00 ($SiCH_3$), −4.62 ($SiCH_3$), 18.05, 20.25, 25.76 (3C), 34.73 (d, $^1J_{PC}$ = 135.4 Hz), 52.88 (d, $^2J_{PC}$ = 6.2 Hz, OCH_3), 52.97 (d, $^2J_{PC}$ = 6.3 Hz, OCH_3), 74.83 (d, $^3J_{PC}$ = 3.1 Hz), 205.10 (d, $^2J_{PC}$ = 7.0 Hz); ESI-MS: m/z: 311.2 [M^+ + 1].

*4.1.3 General method for Wittig-Horner reaction to get **3a-d** and **3f-h***

To a stirred solution of 3-(*tert*-butyl-dimethyl-silanyloxy)-2-oxo-butyl]phosphonic acid dimethyl ester (**2**, 11.27 mmol) in THF (21 mL) maintained at −78 °C under argon atomosphere was added *n*-BuLi (11.27 mmol, 1.6 M in hexane) in a dropwise manner. After 10 minutes, to this reaction mixture corresponding aldehyde (11.27 mmol) in THF (28 mL) was added and the reaction temperature allowed to come to room temperature gradually. This reaction mixture was stirred for 18 h. The progress of reaction was monitored by TLC, upon completion the reaction was quenched with aq. ammonium chloride (20 mL). The reaction mixture was extracted with EtOAc (20 mL x 3). The combined organic layer was washed with brine, dried over anhy. sodium sulfate and concentrated under reduced pressure to obtain a crude product. The crude product was purified by column chromatography.

4.1.3.1. (E, 4S)-4-[tert-butyl(dimethyl)silyl]oxy-1-[3-(trifluoromethyl) phenyl] pent-1-en-3-one (***3a***). Yield: 82%; ^{1}H NMR (400 MHz; $CDCl_3$)**:** δ 0.01 (s, 3H), 0.11 (s, 3H), 0.95 (s, 9H), 1.38 (d, J = 6.9 Hz, 3H), 4.35 (q, J = 6.9 Hz, 1H), 7.36 (d, J = 16.1 Hz, 1H), 7.54 (t, J = 7.8 Hz, 1H), 7.65 (d, J = 7.8 Hz, 1H), 7.68-7.78 (m, 2H), 7.82 (s, 1H); ESI-MS *m/z***:** 359. [M^+ + 1].

4.1.3.2. (E, 4S)-4-[tert-butyl(dimethyl)silyl]oxy-1-[4-(trifluoromethyl) phenyl] pent-1-en-3-one (***3b***). Yield: 79%; ^{1}H NMR (400 MHz; $CDCl_3$)**:** δ 0.09 (s, 3H), 0.11 (s, 3H), 0.94 (s, 9H), 1.37 (d, J = 6.8 Hz, 3H), 4.34 (q, J = 6.8 Hz, 1H), 7.35 (d, J = 16.1 Hz, 1H), 7.65-7.68 (m, 4H), 7.71 (d, J = 16.2 Hz, 1H). ^{13}C-NMR (100 MHz; $CDCl_3$)**:** δ −4.82, –4.58, 18.30, 21.30, 25.90 (3C), 74.82, 122.59, 123.98 (q, $^1J_{FC}$ = 270.9 Hz), 126.04 (q, $^3J_{FC}$ = 3.9 Hz, 2C), 128.62 (2C), 132.02 (q, $^2J_{FC}$ = 32.5 Hz), 138.40, 141.95, 201.98; ESI-MS *m/z***:** 359.3 [M^+ + 1].

4.1.3.3. (E, 4S)-4-[tert-butyl(dimethyl)silyl]oxy-1-[2-(trifluoromethyl) phenyl] pent-1-en-3-one. (***3c***). Yield: 69%; ^{1}H NMR (400 MHz; $CDCl_3$)**:** δ 0.09 (s, 3H), 0.11 (s, 3H), 0.93 (s, 9H), 1.38 (d, J = 6.9 Hz, 3H), 4.37 (q, J = 6.9 Hz, 1H), 7.21 (d, J = 15.9 Hz, 1H), 7.49 (t, J = 7.9 Hz, 1H), 7.59 (t, J = 7.5 Hz, 1H), 7.74 (dd, J = 7.9 Hz, 17.4 Hz, 2H), 8.10 (dd, J = 2.0 Hz, 15.9 Hz, 1H); ^{13}C-NMR (100 MHz; $CDCl_3$)**:** δ −4.84, –4.64, 18.29, 21.28, 25.89(3C), 74.79,

124.10 (q, $^{1}J_{FC}$ = 274.1 Hz), 124.64, 126.37 (q, $^{3}J_{FC}$ = 5.4 Hz), 127.85, 129.49 (q, $^{2}J_{FC}$ = 30.2 Hz), 129.78, 132.21, 134.12, 139.22 (q, $^{4}J_{FC}$ = 1.5 Hz), 201.44; ESI-MS *m/z***:** 359.2 [M^{+} + 1].

4.1.3.4. (E, 4S)-4-[tert-butyl(dimethyl)silyl]oxy-1-(3-fluorophenyl) pent-1-en-3-one (***3d***). Yield: 75%; ^{1}H NMR (400 MHz; $CDCl_3$)**:** δ 0.09 (s, 3H), 0.11 (s, 3H), 0.94 (s, 9H), 1.37 (d, *J* = 6.8 Hz, 3H), 4.33 (q, *J* = 6.8 Hz, 1H), 7.06-7.13 (m, 1H), 7.23-7.31 (m, 2H), 7.32-7.41 (m, 2H), 7.66 (d, *J* = 16.2 Hz, 1H). ^{13}C-NMR (100 MHz; $CDCl_3$)**:** δ −4.84, −4.64, 18.27, 21.29, 25.87 (3C), 74.78, 114.58 (d $^{2}J_{FC}$ = 21.8 Hz), 117.42 (d, $^{2}J_{FC}$ = 21.0 Hz), 121.53, 124.57 (d, $^{4}J_{FC}$ = 3.1 Hz), 130.57 (d, $^{3}J_{FC}$ = 7.8 Hz), 137.27 (d, $^{3}J_{FC}$ = 7.7 Hz), 142.44 (d, $^{4}J_{FC}$ = 2.4 Hz), 163.14 (d, $^{1}J_{FC}$ = 246.8 Hz), 201.97; ESI-MS *m/z***:** 309.1 [M^{+} + 1].

4.1.3.5. (E, 4S)-4-[tert-butyl(dimethyl)silyl]oxy-1-(5-ethyl-2-thienyl)pent -1-en-3-one (***3f***). Yield: 71%; ^{1}H NMR (400 MHz; $CDCl_3$)**:** δ 0.09 (s, 6H), 0.94 (s, 9H), 1.30-1.36 (m, 6H), 2.85 (q, *J* = 7.6 Hz, 2H), 4.28 (q, *J* = 6.8 Hz, 1H), 6.75 (d, *J* = 3.6 Hz, 1H), 6.96 (d, *J* = 15.6 Hz, 1H), 7.13 (d, *J* = 4 Hz, 1H), 7.76 (d, *J* = 15.6 Hz, 1H); ESI-MS *m/z*: 325.2 [M + 1]$^{+}$

4.1.3.6. (E, 4S)-4-[tert-butyl(dimethyl)silyl]oxy-1-cyclohexyl-pent-1-en-3-one (**3g**). Yield: 61%; ^{1}H NMR (400 MHz; $CDCl_3$): δ 0.05 (s, 3H), 0.06 (s, 3H), 0.90 (s, 9H), 1.12-1.32 (m, 8H), 1.64-1.80 (m, 5H), 2.11-2.18 (m, 1H), 4.24 (q, *J* = 6.8 Hz, 1H), 6.54 (dd, *J* = 1.6 Hz, 16.4 Hz, 1H), 6.95 (dd, *J* = 6.8 Hz, 15.6Hz, 1H); ESI-MS *m/z*: 297.4 [M^{+} + 1].

4.1.3.7. 3-[(E, 4S)-4-[tert-butyl(dimethyl)silyl]oxy-3-oxo-pent-1-enyl] benzoate (***3h***). Yield: 76%; ^{1}H NMR (400 MHz; $CDCl_3$): δ 0.10 (s, 3H), 0.11 (s, 3H), 0.95 (s, 9H), 1.38 (d, *J* = 6.9 Hz, 3H), 3.95 (s, 3H), 4.35 (q, *J* = 6.9 Hz, 1H), 7.35 (d, *J* = 16.2 Hz, 1H), 7.49 (t, *J* = 7.9 Hz, 1H), 7.70-7.78 (m, 2H), 8.06 (d, *J* = 7.9 Hz, 1H), 8.27 (s, 1H). ^{13}C-NMR (100 MHz; ($CDCl_3$): δ −4.97, −4.75, 18.17, 21.16, 25.76, 52.32, 74.66, 121.36, 129.07, 129.21, 130.94, 131.22, 132.59, 135.20, 142.47, 166.45, 201.89. ESI-MS *m/z*: 349.3 [M^{+} + 1].

4.1.3.8. (E, 4S)-1-(3-bromophenyl)-4-[tert-butyl(dimethyl)silyl]oxy-pent-1-en-3-one (***3e***). To a stirred suspension of sodium hydride (1.13 g, 28.35 mmol, 60 % in oil) in THF (25 mL) maintained at 0 °C under argon atomosphere was slowly added solution of 3-(*tert*-butyl-dimethyl-silanyloxy)-2-oxo-butyl] phosphonic acid dimethyl ester (**2**, 8 g, 25.77 mmol) in THF (48 mL). The reaction mixture was stirred for 20 min. and then 3-bromobenzaldehyde (4.77 g, 25.77 mmol) in THF (64 mL) was added in a dropwise manner. The reaction temperature was allowed to come to room temperature gradually and then stirred for additional 4 h. The progress of reaction was monitored by TLC. Upon completion, the reaction mixture was slowly diluted with water (100 mL) and extracted with EtOAc (70 mL x 3). Combined organic layer was washed with brine, dried over anhydrous sodium sulfate and concentrated under reduced pressure to give a crude product which was purified by column

chromatography using 5% EtOAc–Hexane as solvent system to afford pure **3e** (6.4 g, 67%). ^{1}H NMR (400 MHz; $CDCl_3$): δ 0.09 (s, 3H), 0.11 (s, 3H), 0.94 (s, 9H), 1.37 (d, *J* = 6.8 Hz, 3H), 4.33 (q, *J* = 6.8 Hz, 1H), 7.25 (d, *J* = 15.6 Hz, 1H), 7.27 (t, *J* = 7.8 Hz, 1H), 7.47-7.54 (m, 2H), 7.62 (d, *J* = 16.1 Hz, 1H), 7.71 (t, *J* = 1.5 Hz, 1H). ESI-MS *m/z*: 369.2 [M^+ (^{79}Br) + 1], 371.1 [M^+ (^{81}Br) + 1].

*4.1.4. General method for stereoselective reduction of ketone to get **4a-f***

To a stirred solution of ketone (4.18 mmol) in methanol (50 mL) was added cerium chloride heptahydrate (4.60 mmol) and was cooled to −78 °C. To this suspension, sodium borohydride (4.60 mmol) in methanol (12 mL) was added dropwise manner. The reaction temperature was allowed to come to room temperature gradually and then stirred for additional 1 h. The reaction mixture was quenched by the addition of sat. aq. NH_4Cl (60 mL) and extracted with EtOAc (50 mL x 3). The combined organic layer was washed with water (50 mL), brine (50 mL), dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to afford the crude product. The crude product was purified by column chromatography.

4.1.4.1. (E, 3S, 4S)-4-(tert-butyl(dimethyl)silyl)oxy-1-[3-(trifluoro methyl) phenyl]pent-1-en-3-ol (***4a***). Yield: 78%; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.09 (s, 3H), 0.10 (s, 3H), 0.91 (s, 9H), 1.22 (d, *J* = 5.9 Hz, 3H), 2.73 (d, *J* = 2.9 Hz, 1H), 3.77 (quint. 1H), 3.98-4.05 (m, 1H), 6.25 (dd, *J* = 6.4 Hz, 16.1 Hz, 1H), 6.70 (d, *J* = 16.1 Hz, 1H), 7.39-7.57 (m, 3H), 7.61 (s, 1H); ESI-MS *m/z*: 229.2 [M – 131]$^+$ base peak, 383.1 [M^+ + Na].

4.1.4.2. Synthesize of (E, 3S, 4S)-4-[tert-butyl(dimethyl)silyl]oxy-1-[4-(trifluoromethyl) phenyl]pent-1-en-3-ol (***4b***). Yield: 81% yield; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.08 (s, 3H), 0.10 (s, 3H), 0.91 (s, 9H), 1.22 (d, *J* = 6.4 Hz, 3H), 2.76 (d, *J* = 4.9 Hz, 1H), 3.79 (quint. 1H), 4.03 (q, *J* = 5.2 Hz, 1H), 6.28 (dd, *J* = 6.1 Hz, 15.9 Hz, 1H), 6.70 (d, *J* = 15.8 Hz, 1H), 7.46 (d, *J* = 8.1 Hz, 2H), 7.56 (d, *J* = 8.3 Hz, 2H); ESI-MS *m/z* : 343.0 [M^+ – H_2O].

4.1.4.3. (E, 3S, 4S)-4-(tert-butyl(dimethyl)silyl)oxy-1-[2-(trifluoro methyl) phenyl]pent-1-en-3-ol (***4c***). Yield: 89%; ^{1}H NMR (400 MHz; $CDCl_3$): δ 0.09 (s, 3H), 0.11 (s, 3H), 0.91 (s, 9H), 1.21 (d, *J* = 6.2 Hz, 3H), 2.72 (d, *J* = 3.9 Hz, 1H, -OH), 3.78 (quint., 1H), 3.98-4.07 (m, 1H), 6.14 (dd, *J* = 6.6 Hz, 15.9 Hz, 1H), 7.02 (d, *J* = 15.7 Hz, 1H), 7.34 (t, *J* = 7.3 Hz, 1H), 7.49 (d, *J* = 7.3 Hz, 1H), 7.57-7.68 (m, 2H); ESI-MS *m/z*: 343.3 [M^+ – 17] base peak, 378.3 [M^+ + H_2O].

4.1.4.4. *(E, 3S, 4S)-4-(tert-butyl(dimethyl)silyl)oxy-1-(3-fluoro phenyl)pent-1-en-3-ol* (***4d***). Yield: 88%; ^{1}H NMR (400 MHz; $CDCl_3$): δ 0.09 (s, 3H), 0.10 (s, 3H), 0.91 (s, 9H), 1.21 (d, *J* = 6.2 Hz, 3H), 2.71 (d, *J* = 4.7 Hz, 1H), 3.76 (quint. 1H), 4.00 (app. q, *J* = 4.8 Hz, 1H), 6.18 (dd, *J* = 6.1 Hz, 15.9 Hz, 1H), 6.63 (d, *J* = 15.9 Hz, 1H), 6.92 (td, *J* = 2.4 Hz, 8.3 Hz, 1H), 7.07 (d, *J* = 10.3 Hz, 1H), 7.13 (d, *J* = 7.5 Hz, 1H), 7.23-7.30 (m, 1H); ^{13}C-NMR (100 MHz; $CDCl_3$): δ −4.69, −4.15, 18.13, 20.19, 25.90 (3C), 72.03, 76.80, 112.93 (d, $^{2}J_{FC}$ = 21.8 Hz), 114.44 (d, $^{2}J_{FC}$ = 21.0 Hz), 122.45 (d, $^{4}J_{FC}$ = 3.1 Hz), 130.07 (d, $^{3}J_{FC}$ = 7.8 Hz), 130.64 (d, $^{4}J_{FC}$ = 2.4 Hz), 130.70, 139.33 (d, $^{3}J_{FC}$ = 7.8 Hz), 163.19 (d, $^{1}J_{FC}$ = 244.5 Hz); ESI-MS *m/z*: 156.1 [½M + 1]$^{+}$ base peak, 293.1 [M^{+} – H_2O].

4.1.4.5. *(E, 3S, 4S)-1-(3-bromophenyl)-4-[tert-butyl(dimethyl)silyl] oxy-pent-1-en-3-ol* (***4e***). Yield: 71%; ^{1}H NMR (400 MHz; $CDCl_3$): δ 0.09 (s, 3H), 0.10 (s, 3H), 0.91 (s, 9H), 1.20 (d, *J* = 6.0 Hz, 3H), 2.71 (d, *J* = 4 Hz, 1H, -OH), 3.75 (quint., 1H), 3.76-4.03 (m, 1H), 6.17 (dd, *J* = 6.1 Hz, 16.0 Hz, 1H), 6.60 (d, *J* = 15.8 Hz, 1H), 7.18 (t, *J* = 7.7 Hz, 1H), 7.29 (d, *J* = 7.6 Hz, 1H), 7.36 (d, *J* = 7.7 Hz, 1H), 7.52 (s, 1H); ESI-MS *m/z*: 371.0 [M^{+} (^{79}Br) + 1] , 373.0 [M^{+} (^{81}Br) + 1].

4.1.4.6. *(E, 3S, 4S)-4-(tert-butyl(dimethyl)silyl)oxy-1-(5-ethyl-2-thienyl) pent-1-en-3-ol* (***4f***). Yield: 83%; ^{1}H NMR (400 MHz; $CDCl_3$): δ 0.09 (s, 6H), 0.91 (s, 9H), 1.19 (d, *J* = 5.8 Hz, 3H), 1.29 (t, *J* = 7.8 Hz, 3H), 2.65 (d, *J* = 4.4 Hz, 1H, -OH), 2.80 (q, *J* = 7.8 Hz, 2H), 3.72 (quint., 1H), 3.88-3.95 (m, 1H), 5.87 (dd, *J* = 6.8 Hz, 15.6 Hz, 1H), 6.62 (d, *J* = 3.4 Hz, 1H), 6.71 (d, *J* = 15.6 Hz, 1H), 7.74 (d, *J* = 3.4 Hz, 1H); ^{13}C-NMR (100 MHz; $CDCl_3$) : δ −4.71, −4.20, 15.82, 18.10, 20.18, 23.68, 25.88 (3C), 72.15, 76.91, 123.61, 125.51, 125.87, 127.39, 139.61, 146.79; ESI-MS *m/z*: 309.2 [M^{+} – 17].

4.1.4.7. *(E, 3S, 4S)-4-(tert-butyl(dimethyl)silyl)oxy-1-cyclohexyl-pent-1-en-3-ol* (***4g***). Yield: 94%; ^{1}H NMR (400 MHz; $CDCl_3$): δ 0.08 (s, 6H), 0.89 (s, 9H), 1.0-1.33 (m, 5H), 1.11 (d, *J* = 6.1 Hz, 3H), 1.60-1.77 (m, 5H), 1.90-2.0 (m, 1H), 2.59 (br. s, 1H), 3.62 (quint., 1H), 3.72 (t, *J* = 6.6 Hz, 1H), 5.33 (ddd, *J* = 1.2 Hz, 7.1 Hz, 15.4 Hz, 1H), 5.67 (dd, *J* = 6.3 Hz, 15.4 Hz, 1H); ^{13}C-NMR (100 MHz; $CDCl_3$) : δ −4.68, −4.09, 18.14, 20.10, 25.93 (3C), 26.13 (2C), 26.28, 32.79, 32.84, 40.53, 72.32, 77.59, 126.79, 139.94; ESI-MS *m/z*: 321.4 [M^{+} + Na] .

4.1.4.8. *Methyl 3-[(E, 3S, 4S)-4-(tert-butyl(dimethyl)silyl)oxy-3-hydroxy-pent-1-enyl] benzoate* (***4h***). Yield: 87%; ^{1}H NMR (400 MHz; $CDCl_3$): δ 0.09 (s, 3H), 0.11 (s, 3H), 0.91 (s, 9H), 1.21 (d, *J* = 6.0 Hz, 3H), 2.775 (d, *J* = 4 Hz, 1H, -OH), 3.78 (quint., 1H), 3.92 (s, 3H), 3.99-4.05 (m, 1H), 6.26 (dd, *J* = 6.2 Hz, 15.9 Hz, 1H), 6.70 (d, *J* = 15.9 Hz, 1H), 7.38 (t, *J* = 7.8 Hz, 1H), 7.55 (d, *J* = 7.7 Hz, 1H), 7.90 (d, *J* = 7.5 Hz, 1H), 8.06 (s, 1H); ^{13}C-NMR (100

MHz; ($CDCl_3$): δ −4.68, −4.12, 18.15, 20.24, 25.90 (3C), 52.28, 72.08, 76.97, 127.59, 128.68, 128.73, 130.54, 130.56, 130.87, 130.92, 137.24, 167.12. ESI-MS *m/z*: 368.3 [$M^+ + H_2O$] .

4.1.5. General method for double bond reduction of olefin (4a-d and 4f-h) to get ***5a-d*** *and* ***5f-h***

To a solution of olefin (***4a-d*** *and* ***4f-h***, 3.33 mmol) in EtOAc (72 mL) was added 10% palladium on carbon (20% w/w) and stirred at RT under hydrogen gas maintained at 1 atmospheric pressure for 30 min. The progress of reaction was monitored by LCMS. After completion of reaction, Pd–C was removed by filtration and the filtrate was evaporated to give the crude product. The crude product was purified by column chromatography.

4.1.5.1. (3S, 4S)-4-(tert-butyl(dimethyl)silyl)oxy-1-[3-(trifluoromethyl) phenyl] pentan-3-ol (***5a***). Yield: 92%; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.08 (s, 3H), 0.09 (s, 3H), 0.90 (s, 9H), 1.14 (d, *J* = 6.1 Hz, 3H), 1.68-1.75 (m, 2H), 2.45 (d, *J* = 5.1 Hz, 1H), 2.68-2.80 (m, 1H), 2.88-2.98 (m, 1H), 3.28 (quint., 1H), 3.64 (quint., 1H), 7.37-7.42 (m, 2H), 7.44 (d, *J* = 3.6 Hz, 1H), 7.46 (s, 1H). ^{13}C-NMR (100 MHz; $CDCl_3$): δ −4.79, −4.06, 18.09, 20.28, 25.89 (3C), 32.01, 35.10, 71.90, 74.85, 124.46 (q, $^1J_{FC}$ = 270.1 Hz), 125.34 (q, $^3J_{FC}$ = 3.1 Hz, 2C), 128.22 (q, $^2J_{FC}$ = 32.5 Hz), 128.88 (2C), 146.52; ESI-MS *m/z*: 363.3 [M^+ + 1].

4.1.5.2. (3S, 4S)-4-(tert-butyl(dimethyl)silyl)oxy-1-[4-(trifluoromethyl) phenyl] pentan-3-ol (***5b***). *Yield:* 94%; ^{1}H NMR (400 MHz; $CDCl_3$): δ 0.097 (s, 3H), 0.103 (s, 3H), 0.92 (s, 9H), 1.15 (d, *J* = 6.1 Hz, 3H), 1.72 (app. q, *J* = 7.4 Hz, 2H), 2.46 (br s, 1H), 2.71-2.82 (m, 1H), 2.94 (quint., 1H) 3.29 (d, *J* = 5.4 Hz, 1H), 3.65 (quint., 1H), 7.33 (d, *J* = 7.8 Hz, 2H), 7.55 (d, *J* = 8.1 Hz, 2H); ^{13}C-NMR (100 MHz; $CDCl_3$): δ −4.79, −4.06, 18.09, 20.28, 25.89 (3C), 32.01, 35.10, 71.90, 74.85, 124.46 (q, $^1J_{FC}$ = 270.1 Hz), 125.34 (q, $^3J_{FC}$ = 3.1 Hz, 2C), 128.22 (q, $^2J_{FC}$ = 32.5 Hz), 128.88 (2C), 146.52. ESI-MS *m/z*: 363.3 [M^+ + 1].

4.1.5.3. (3S, 4S)-4-(tert-butyl(dimethyl)silyl)oxy-1-[2-(trifluoro methyl) phenyl] pentan-3-ol (***5c***). Yield: 76% ; ^{1}H NMR (400 MHz; $CDCl_3$): δ 0.07 (s, 3H), 0.08 (s, 3H), 0.90 (s, 9H), 1.15 (d, *J* = 6.4 Hz, 3H), 1.66-1.75 (m, 2H), 2.47 (d, *J* = 3.4 Hz, 1H), 2.79-2.89 (m, 1H), 3.0-3.10 (m, 1H), 3.31-3.39 (m, 1H), 3.65 (quint., 1H), 7.29 (t, *J* = 7.6 Hz, 1H), 7.37 (d, *J* = 7.6 Hz, 1H), 7.46 (t, *J* = 7.6 Hz, 1H), 7.61 (d, *J* = 7.8 Hz, 1H); ^{13}C-NMR (100 MHz; $CDCl_3$): δ −4.79, −4.03, 18.11, 20.27, 25.91(3C), 29.23, 35.65, 71.83, 75.42, 124.75 (q, $^1J_{FC}$ = 274.0 Hz), 126.05 (q, $^3J_{FC}$ = 5.5 Hz, 2C), 128.56 (q, $^2J_{FC}$ = 29.6 Hz), 131.39, 131.84, 141.35 ESI-MS *m/z*: 345.1 [$M^+ - H_2O$] .

4.1.5.4. (3S, 4S)-4-(tert-butyl(dimethyl)silyl)oxy-1-(3-fluorophenyl) pentan-3-ol (***5d***). Yield: 83%; ^{1}H NMR (400 MHz; $CDCl_3$): δ 0.08 (s, 3H), 0.09 (s, 3H), 0.90 (s, 9H), 1.14 (d, *J* = 6.1 Hz, 3H), 1.65-1.74 (m, 2H), 2.43 (d, *J* = 3.7 Hz, 1H), 2.64-2.74 (m, 1H), 2.86 (quint.,

1H), 3.24-3.32 (m, 1H), 3.63 (quint., 1H), 6.84-6.94 (m, 2H), 6.98 (d, *J* = 7.6 Hz, 1H), 7.19-7.25 (m, 1H); ESI-MS *m/z*: 335.1 [M^+ + Na] .

4.1.5.5. (3S, 4S)-4-(tert-butyl(dimethyl)silyl)oxy-1-(5-ethyl-2-thienyl) pentan-3-ol (***5f***). Yield: 85%; ^{1}H NMR (400 MHz; $CDCl_3$): δ 0.09 (s, 6H), 0.90 (s, 9H), 1.15 (d, *J* = 6.1 Hz, 3H), 1.28 (t, *J* = 7.3 Hz, 3H), 1.70-1.78 (m, 2H), 2.39 (br s, 1H), 2.78 (q, *J* = 7.3 Hz, 2H), 2.82-2.91 (m, 1H), 2.99 (quint., 1H), 3.31-3.38 (m, 1H), 3.65 (quint., 1H), 6.57 (d, *J* = 3.4 Hz, 1H), 6.60 (d, *J* = 3.4 Hz, 1H); ^{13}C-NMR (100 MHz; $CDCl_3$): δ −4.71, −4.03, 16.04, 18.15, 20.34, 23.56, 25.96 (3C), 26.59, 35.73, 71.89, 74.92, 122.83, 123.82, 142.58, 145.15. ESI-MS *m/z*: 329.2 [M^+ + 1].

4.1.5.6. (3S, 4S)-4-(tert-butyl(dimethyl)silyl)oxy-1-cyclohexyl-pentan-3-ol (***5g***). Yield: 87%; ^{1}H NMR (400 MHz; $CDCl_3$): δ 0.08 (s, 3H), 0.08 (s, 3H), 0.89 (s, 9H), 1.14 (d, *J* = 6.1 Hz, 3H), 1.15-1.48 (m, 10H), 1.58-1.74 (m, 5H), 2.37 (br s, 1H), 3.20-3.27 (m, 1H), 3.62 (quint., 1H); ^{13}C-NMR (100 MHz; $CDCl_3$): δ −4.70, −3.99, 18.16, 20.44, 25.97 (3C), 26.53, 26.56, 26.86, 30.94, 33.34, 33.60, 33.69, 37.89, 71.83, 76.28. ESI-MS *m/z*: 301.3 [M^+ + 1].

4.1.5.7. Methyl 3-[(3S, 4S)-4-(tert-butyl(dimethyl)silyl)oxy-3-hydroxy-pentyl] benzoate (***5h***). Yield: 72%; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.09 (s, 6H), 0.90 (s, 9H), 1.14 (d, *J* = 5.9 Hz, 3H), 1.68-1.78 (m, 2H), 2.47 (d, *J* = 4.6 Hz, 1H), 2.69-2.81 (m, 1H), 2.87-2.97 (m, 1H), 3.28 (quint., 1H), 3.64 (quint., 1H), 3.91 (s, 3H), 7.35 (t, *J* = 7.4 Hz, 1H), 7.42 (d, *J* = 7.3 Hz, 1H), 7.87 (d, *J* = 7.8 Hz, 1H), 7.90 (s, 1H); ESI-MS *m/z*: 370.3 [M^+ + H_2O].

4.1.5.8. (3S, 4S)-1-(3-bromophenyl)-4-[tert-butyl(dimethyl)silyl]oxy-pentan-3-ol (***5e***). To a solution of (*E*, *3S*, *4S*)-1-(3-bromophenyl)-4-[*tert*-butyl(dimethyl)silyl]oxy-pent-1-en-3-ol (**4e**, 3.0 g, 8.07 mmol) in degassed solvent THF:*tert*-butyl alcohol (1: 1, 58 mL) Wilkinson's catalyst (0.45 g, 15 % w/w) was added. The solution was stirred at RT under hydrogen gas maintained at 1 atm. pressure for 16 h. After reaction completion the mixture was filtered through a short pad of alumina and washed thoroughly with ethyl acetate. The filtrate was evaporated to give crude product. The crude product was purified by column chromatography using 10–30% EtOAc–Hexane as solvent system to afford pure **5e** (2.5 g, 83% yield); ^{1}H NMR (400 MHz; $CDCl_3$): δ 0.08 (s, 6H), 0.90 (s, 9H), 1.13 (d, *J* = 6.3 Hz, 3H), 1.62-1.74 (m, 2H), 2.44 (d, *J* = 4.3 Hz, 1H -OH), 2.61-2.72 (m, 1H), 2.84 (quint., 1H) 3.27 (quint., 1H), 3.63 (quint., 1H), 7.11-7.19 (m, 2H), 7.28-7.35 (m, 1H), 7.36 (s, 1H); ^{13}C-NMR (100 MHz; $CDCl_3$): δ −4.71, −3.99, 18.15, 20.36, 25.95 (3C), 31.88, 35.21, 71.97, 74.92, 122.54, 127.33, 129.01, 130.04, 131.67, 144.80. ESI-MS *m/z*: 373.0 [M^+ (^{79}Br) + 1], 375.0 [M^+ (^{81}Br) + 1].

*4.1.6. General method for Mitsunobu reaction to get **6a-f***

To a solution of corresponding alcohol (0.55 mmol) in THF (15 mL) was added *tert*-butyl N-*tert*-butoxycarbonyl-*N*-(9H-purin-6-yl) carbamate (0.66 mmol) followed by triphenylphosphine (1.1 mmol). This reaction mixture was cooled to 0 °C and DEAD (1.1 mmol) was added dropwise manner. The reaction mixture was stirred at room temperature for 16 h. The reaction mixture was quenched by the addition of water (30 mL). Aqueous layer was extracted with EtOAc (35 mL x 3). The combined organic layer was washed with brine (30 mL), dried over anhydrous Na_2SO_4, and concentrated under reduced pressure to afford crude product which was purified by column chromatography

4.1.6.1. tert-butyl N-tert-butoxycarbonyl-N-[9-[(1R, 2S)-2-(tert-butyl (dimethyl)silyl)oxy-1-[2-[3-(trifluoromethyl)phenyl]ethyl] propyl]purin-6-yl]carbamate (***6a***). Yield: 52%; ^{1}H NMR (400 MHz; $CDCl_3$): δ −0.13 (s, 3H), 0.01 (s, 3H), 0.87 (s, 9H), 1.13 (d, *J* = 6.1 Hz, 3H), 1.43 (s, 18H), 2.36-2.64 (m, 4H), 4.10-4.16 (m, 1H), 4.43-4.50 (m, 1H), 7.18 (d, *J* = 7.6 Hz, 1H), 7.23 (s, 1H), 7.35 (t, *J* = 7.6 Hz, 1H), 7.43 (d, *J* = 7.5 Hz, 1H), 8.17 (s, 1H), 8.72 (s, 1H); ESI-MS *m/z*: 680.4 [M^+ + 1].

4.1.5.2. tert-butyl N-tert-butoxycarbonyl-N-[9-[(1R, 2S)-2-(tert-butyl (dimethyl)silyl)oxy-1-[2-[4-(trifluoromethyl)phenyl]ethyl]propyl]purin-6-yl]carbamate (***6b***). Yield: 66%; ^{1}H NMR (400 MHz; $CDCl_3$): δ −0.12 (s, 3H), 0.02 (s, 3H), 0.88 (s, 9H), 1.12 (d, *J* = 6.4 Hz, 3H), 1.44 (s, 18H), 2.35-2.64 (m, 4H), 4.20-4.30 (m, 1H), 4.43-4.51 (m, 1H), 7.13 (d, *J* = 8.0 Hz, 2H), 7.52 (d, *J* = 8.1 Hz, 2H), 8.14 (s, 1H), 8.86 (s, 1H). ESI-MS *m/z*: 680.5 [M^+ + 1].

*4.1.6.3. tert-butyl N-tert-butoxycarbonyl-N-[9-[(1R,2S)-2-(tert-butyl(dimethyl)silyl)oxy-1-[2-[2-(trifluoromethyl)phenyl]ethyl] propyl]purin-6-yl]carbamate (**6c**).* Yield: 58%; ^{1}H NMR (400 MHz; $CDCl_3$): δ −0.16 (s, 3H), 0.01 (s, 3H), 0.86 (s, 9H), 1.15 (d, *J* = 6.2 Hz, 3H), 1.41 (s, 18H), 2.32-2.45 (m, 1H), 2.47-2.59 (m, 2H), 2.62-2.74 (m, 1H), 4.15 (quint., 1H), 4.56-4.64 (m, 1H), 7.16 (d, *J* = 7.9 Hz, 1H), 7.31 (t, *J* = 7.5 Hz, 1H), 7.44 (t, *J* = 7.5 Hz, 1H), 7.60 (d, *J* = 8.0 Hz, 1H), 8.19 (s, 1H), 8.87 (s, 1H); ^{13}C-NMR (100 MHz; $CDCl_3$): δ −4.85, −4.20, 18.02, 20.62, 25.92 (3C), 27.91 (6C), 29.79, 30.03, 62.20, 70.25, 83.62 (2C), 124.65 (q, $^1J_{FC}$ = 274.1 Hz), 126.39 (q, $^3J_{FC}$ = 5.4 Hz), 126.69, 128.58 (q, $^2J_{FC}$ = 29.5 Hz), 129.32, 131.19, 132.14, 139.38 (q, $^4J_{FC}$ = 1.5 Hz), 144.39, 150.35, 150.67, 152.04, 153.84 (2C); ESI-MS *m/z*: 680.2 [M^+ + 1].

4.1.6.4. tert-butyl N-tert-butoxycarbonyl-N-[9-[(1R,2S)-2-(tert-butyl (di methyl)silyl)oxy-1-[2-(3-fluorophenyl)ethyl]propyl]purin-6-yl]carbamate (***6d***). Yield:

54%; ^{1}H NMR (400 MHz; $CDCl_3$): δ −0.14 (s, 3H), 0.01 (s, 3H), 0.87 (s, 9H), 1.11 (d, *J* = 6.2 Hz, 3H), 1.42 (s, 18H), 2.30-2.43 (m, 2H), 2.45-2.62 (m, 2H), 4.15 (quint., 1H), 4.42-4.48 (m, 1H), 6.73 (t, *J* = 8.4 Hz, 1H), 6.74 (d, *J* = 7.1 Hz, 1H), 6.88 (td, *J* = 2.2 Hz, 8.8 Hz, 1H), 7.19 (dd, *J* = 6.2 Hz, 8.0 Hz, 1H), 8.12 (s, 1H), 8.84 (s, 1H); ESI-MS *m/z*: 630.2 [M^+ + 1].

4.1.6.5. tert-butyl N-[9-[(1R,2S)-1-[2-(3-bromophenyl)ethyl]-2-[tert-butyl(dimethyl)silyl]oxy -propyl]purin-6-yl]-N-tert-butoxycarbonyl-carbamate (***6e***). Yield: 63%; ^{1}H NMR (400 MHz; $CDCl_3$): δ −0.14 (s, 3H), −0.01 (s, 3H), 0.87 (s, 9H), 1.11 (d, *J* = 6.2 Hz, 3H), 1.42 (s, 18H), 2.26-2.60 (m, 4H), 4.14 (quint., 1H), 4.41-4.48 (m, 1H), 6.91 (d, *J* = 7.9 Hz, 1H), 7.10 (t, *J* = 8.0 Hz, 1H), 7.13 (s, 1H), 7.34 (d, *J* = 7.9 Hz, 1H), 8.13 (s, 1H), 8.86 (s, 1H); ^{13}C-NMR (100 MHz; $CDCl_3$): δ −5.01, −4.38, 17.85, 20.47, 25.76 (3C), 27.74 (6C), 29.13, 31.71, 61.14, 69.94, 83.56 (2C), 122.61, 126.86, 128.98, 129.52, 130.11, 131.35, 142.39, 144.31, 150.22, 150.37, 151.91, 153.53 (C=O, 2C); ESI-MS *m/z*: 690.1 [M^+ (^{79}Br) + 1] $^+$, 692.1 [M^+ (^{81}Br) + 1].

4.1.6.6. tert-butyl N-tert-butoxycarbonyl-N-[9-[(1R,2S)-2-(tert-butyl (dimethyl)silyl)oxy-1-[2-(5-ethyl-2-thienyl)ethyl]propyl] purin-6-yl]carbamate (***6f***). Yield: 71; ^{1}H NMR (400 MHz, $CDCl_3$): δ −0.133 (s, 3H), −0.01 (s, 3H), 0.88 (s, 9H), 1.12 (d, *J* = 6.4 Hz, 3H), 1.31 (t, *J* = 7.1 Hz, 3H), 1.42 (s, 18H), 2.35-2.68 (m, 4H), 4.14-4.22 (m, 1H), 4.32 (q *J* = 7.1 Hz, 2H), 4.48-4.54 (m, 1H), 6.37 (d, *J* = 3.1 Hz, 1H), 6.53 (d, *J* = 3.4 Hz, 1H), 8.11 (s, 1H), 8.84 (s, 1H); ESI-MS *m/z*: 646.5 [M^+ + 1].

4.1.6.7. tert-butyl N-tert-butoxycarbonyl-N-[9-[(1R,2S)-2-(tert-butyl(dimethyl)silyl)oxy-1-(2-cyclohexylethyl)propyl]purin-6-yl]carbamate (***6g***). Yield: 63% ; ^{1}H NMR (400 MHz; $CDCl_3$): δ −0.15 (s, 3H), −0.01 (s, 3H), 0.70-0.85 (m, 3H), 0.88 (s, 9H), 0.98-1.11 (m, 3H), 1.16 (d, *J* = 6.4 Hz, 3H), 1.41 (s, 18H), 1.52-1.68 (m, 7H), 2.02-2.15 (m, 2H), 4.14 (quint., 1H), 4.47 (quint., 1H), 8.10 (s, 1H), 8.84 (s, 1H); ESI-MS *m/z*: 618.6 [M^+ + 1].

4.1.6.8. Methyl 3-[(3R,4S)-3-[6-[bis(tert-butoxycarbonyl)amino] purin-9-yl]-4-[tert-butyl (dimethyl)silyl]oxy-pentyl]benzoate (***6h***). Yield: 59%; ^{1}H NMR (400 MHz; $CDCl_3$): δ −0.13 (s, 3H), −0.01 (s, 3H), 0.87 (s, 9H), 1.12 (d, *J* = 6.3 Hz, 3H), 1.44 (s, 18H), 2.35-2.47 (m, 2H), 2.48-2.63 (m, 2H), 3.90 (s, 3H), 4.15 (quint., 1H), 4.43-4.51 (m, 1H), 7.17 (d, *J* = 7.6 Hz, 1H), 7.32 (t, *J* = 7.5 Hz, 1H), 7.70 (s, 1H), 7.88 (d, *J* = 7.9 Hz, 1H), 8.15 (s, 1H), 8.85 (s, 1H); ESI-MS *m/z*: 670.3 [M^+ + 1].

*4.1.7. General method for Diboc and TBDMS deprotection to get **7a-f***

To a solution of protected compound **6a-h** (0.17 mmol) in 1, 4-dioxane (2 mL) was added slowly 4M HCl in dioxane (1.76 mmol) at room temperature. The reaction mixture was

stirred for 16 h at RT. After completion of reaction, the volatiles were evaporated under reduced pressure and residue was netrualized with sat. aq. $NaHCO_3$ solution. Aquous layer was extracted with EtOAc (15 mL x 3). The combined organic layer was washed with water (20 mL), brine (20 mL), dried over anhydrous Na_2SO_4, and concentrated under reduced pressure to afford crude product. The crude product was purified by column chromatography.

4.1.7.1. *(2S, 3R)-3-(6-aminopurin-9-yl)-5-[3-(trifluoromethyl)phenyl] pentan-2-ol* (***7a***). Yield: 90%; ^{1}H NMR (400 MHz; CD_3OD): δ 1.07 (d, *J* = 6.4 Hz, 3H), 2.42-2.67 (m, 4H), 4.07 (quint., 1H), 4.33-4.42 (m, 1H), 7.24-7.42 (m, 4H), 8.17 (s, 1H), 8.19 (s, 1H); ^{13}C-NMR (100 MHz; (CD_3OD): δ 20.26, 31.06, 33.26, 62.42, 70.19, 119.99, 123.88 (q, $^3J_{FC}$ = 3.9 Hz), 125.64 (q, $^1J_{FC}$ = 271.7 Hz), 126.0 (q, $^3J_{FC}$ = 3.9 Hz), 130.92, 131.52 (q, $^2J_{FC}$ = 31.9 Hz), 133.17, 141.89, 143.27, 150.89, 153.54, 157.27; ESI-MS *m/z*: 366.2 [M^+ + 1].

4.1.7.2. *(2S, 3R)-3-(6-aminopurin-9-yl)-5-[4-(trifluoromethyl)phenyl] pentan-2-ol* (***7b***). Yield: 93 %; ^{1}H NMR (400 MHz; $(CD_3)_2SO$): δ 0.87 (d, *J* = 6.1 Hz, 3H), 2.32-2.48 (m, 4H), 4.00 (q, *J* = 6.2 Hz, 1H), 4.20-4.28 (m, 1H), 5.20 (d, *J* = 5.3 Hz, 1H), 7.22 (br s, 2H), 7.32 (d, *J* = 7.9 Hz, 2H), 7.58 (d, *J* = 8.2 Hz, 2H), 8.12 (s, 1H), 8.22 (s, 1H); ^{13}C-NMR (100 MHz; $(CD_3)_2SO$): δ 20.22, 30.34, 31.63, 60.52, 67.85, 118.80, 124.24 (q, $^1J_{Fc}$ = 271.7 Hz,), 125.03 (q, $^3J_{Fc}$ = 3.8 Hz, 2C), 126.68 (q, $^2J_{Fc}$ = 32.0 Hz), 129.08 (2C), 140.21, 146.05, 149.86, 152.28, 155.96; ESI-MS *m/z* : 366.2 [M^+ + 1]; HPLC purity: 98 %

4.1.7.3. *(2S, 3R)-3-(6-aminopurin-9-yl)-5-[2-(trifluoromethyl)phenyl] pentan-2-ol* (***7c***). Yield: 91%; ^{1}H NMR (400 MHz; CD_3OD): δ 1.11 (d, *J* = 6.4 Hz, 3H), 2.38-2.52 (m, 3H), 2.65-2.78 (m, 1H), 4.13 (quint., 1H), 4.48 (q, *J* = 6.4 Hz, 1H), 7.32 (t, *J* = 7.8 Hz, 1H), 7.33 (d, *J* = 7.8 Hz, 1H), 7.48 (t, *J* = 7.8 Hz, 1H), 7.57 (d, *J* = 8.1 Hz, 1H),8.21 (s, 1H), 8.25 (s, 1H); ^{13}C-NMR (100 MHz; CD_3OD): δ 20.39, 30.47, 32.08, 62.93, 70.03, 120.06, 125.98 (q, $^1J_{Fc}$ = 271.7 Hz, 1C), 126.88 (q, $^3J_{Fc}$ = 5.4 Hz, 1C), 127.57, 129.13 (q, $^2J_{Fc}$ = 28.7 Hz, 1C), 132.44, 133.29, 140.95, 141.91, 151.01, 153.59,157.34; ESI-MS *m/z*: 366.2 [M^+ + 1]; $[\alpha]_D^{23}$: +2.8 (*c* 0.25, CH_3OH); HPLC purity: 99 %

4.1.7.4. *(2S, 3R)-3-(6-aminopurin-9-yl)-5-(3-fluorophenyl)pentan-2-ol* (***7d***). Yield: 85 %; ^{1}H NMR (400 MHz; CD_3OD): δ 1.07 (d, *J* = 6.4 Hz, 3H), 2.40-2.58 (m, 4H), 4.09 (quint., 1H), 4.33-4.40 (m, 1H), 6.76 (dd, *J* =1.8 Hz, 10.0 Hz, 1H), 6.79-6.85 (m, 2H), 7.16 (q, *J* = 7.9 Hz, 1H), 8.18 (s, 1H), 8.19 (s, 1H). ^{13}C-NMR (100 MHz; CD_3OD): δ 20.26, 31.07, 33.15, 62.49, 70.17, 113.69 (d, $^2J_{FC}$ = 20.9 Hz), 115.97 (d, $^2J_{FC}$ = 21.7 Hz), 120.37, 125.17 (d, $^4J_{FC}$ = 2.3 Hz), 130.94 (d, $^3J_{FC}$ = 8.5 Hz), 141.96, 144.76 (d, $^3J_{FC}$ = 7.0 Hz), 150.93, 153.48,

157.26, 164.19 (d, $^{1}J_{FC}$ = 243.1 Hz); ESI-MS *m/z*: 316.3 [M^{+} + 1]; $[\alpha]_{D}^{23}$: +25.2 (*c* 0.25, CH_3OH); HPLC purity: 95 %.

4.1.7.5. *(2S, 3R)-3-(6-aminopurin-9-yl)-5-(3-bromophenyl)pentan-2-ol* (***7e***). Yield: 75 %; ^{1}H NMR (400 MHz, CD_3OD): δ 1.07 (d, *J* = 6.3 Hz, 3H), 2.40-2.58 (m, 4H), 4.07 (quint., 1H), 4.33-4.40 (m, 1H), 6.99 (d, *J* = 7.8 Hz, 1H), 7.07 (t, *J* = 7.8 Hz, 1H), 7.11 (s, 1H), 7.23 (d, *J* = 8.3 Hz, 1H), 8.16 (s, 1H), 8.19 (s, 1H); ^{13}C-NMR (100 MHz; CD_3OD): δ 20.22, 30.98, 33.13, 62.40, 70.20, 119.98, 123.23, 128.16, 130.10, 131.05, 132.38, 141.89, 144.48, 150.85, 153.52, 157.24; ESI-MS *m/z*: 376.2 [M^{+} (^{79}Br) + 1] , 378.1 [M^{+} (^{81}Br) + 1]; HPLC purity: 95 %.

4.1.7.6. *(2S, 3R)-3-(6-aminopurin-9-yl)-5-(5-ethyl-2-thienyl)pentan-2-ol* (***7f***). Yield: 87 %; ^{1}H NMR (400 MHz; CDCl3): δ 1.28 (t, *J* = 7.6 Hz, 3H), 1.30 (d, *J* = 4.8 Hz, 3H), 2.23-2.33 (m, 1H), 2.42-2.58 (m, 2H), 2.67-2.74 (m, 1H), 2.78 (q, *J* = 7.6 Hz, 2H), 4.21-4.32 (m, 2H), 5.79 (br s, 2H), 6.53 (d, *J* = 3.3 Hz, 1H), 6.58 (d, *J* = 3.3 Hz, 1H), 7.72 (s, 1H), 8.32 (s, 1H); ^{13}C-NMR (100 MHz; CDCl3): δ 16.17, 20.49, 23.69, 26.73, 29.08, 62.32, 69.71, 120.07, 123.14, 124.68, 140.39, 140.96, 146.13, 149.74, 152.58, 156.15; ESI-MS *m/z*: 332.2 [M^{+} + 1]; HPLC purity: 93 %

4.1.7.7. *(2S, 3R)-3-(6-aminopurin-9-yl)-5-cyclohexyl-pentan-2-ol* (***7g***). Yield: 86 %; ^{1}H NMR (400 MHz; CDCl3): δ 0.68-0.94 (m, 3H), 1.02-1.22 (m, 5H), 1.30 (d, *J* = 6.4 Hz, 3H), 1.56-1.70 (m, 2H), 1.86-2.25 (m, 5H), 4.15-4.32 (m, 2H), 5.79 (br s, 2H), 7.77 (s, 1H), 8.31 (s, 1H); ^{13}C-NMR (100 MHz; $CDCl_3$): δ 20.34, 25.09, 26.35, 26.42, 26.67, 30.09, 33.56, 34.06, 37.54, 63.36, 69.58, 119.75, 140.53, 149.88, 152.52, 156.08; ESI-MS *m/z*: 304.2 [M^{+} + 1]; HPLC purity: 98 %.

4.1.7.7.1. *Methyl 3-[(3R, 4S)-3-(6-aminopurin-9-yl)-4-hydroxy-pentyl] benzoate* (***7h***). Yield: 81%; ^{1}H NMR (400 MHz, CDCl3): δ 1.28 (d, *J* = 6.2 Hz, 3H), 2.26-2.40 (m, 1H), 2.45-2.62 (m, 3H), 3.91 (s, 3H), 4.19-4.31 (m, 2H), 4.95 (br s, 1H), 5.72 (br s, 2H), 7.23-7.27 (m, 1H), 7.33 (t, *J* = 7.5 Hz, 1H), 7.72-7.78 (m, 2H), 7.86 (d, *J* = 7.5 Hz, 1H), 8.33 (s, 1H); ^{13}C-NMR (100 MHz; $CDCl_3$): δ 20.29, 28.95, 32.19, 52.17, 62.19, 69.56, 119.78, 127.64, 128.63, 129.33, 130.45, 132.95, 140.35, 140.69, 149.84, 152.60, 155.97, 167.11; ESI-MS *m/z*: 356.3 [M^{+} + 1]. HPLC purity: 99 %.

4.1.8. *tert-butyl N-tert-butoxycarbonyl-N-[9-[(1R,2S)-2-[tert-butyl(dimethyl) silyl]oxy-1-[2-[3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl]ethyl]propyl]purin-6-yl] carbamate* (***8***).

A mixture of intermediate **6e** (1.9 g, 2.75 mmol), bis(pinacolato)diboron (1.04 g, 4.12 mmol) and potassium acetate (0.67 g, 6.87 mmol) in dioxane (35 mL) was degassed with Argon gas for 30 min. [1,1′-Bis(diphenylphosphino)ferrocene]dichloropalladium(II) complex with dichloromethane (0.22 g, 0.27 mmol) was added and the degassing continued again for further 15 min. Reaction mixture was refluxed for 3 h. Reaction progress was monitored by LCMS. After completion of the reaction, this mixture was passed through a short celite pad to remove insoluble material. The solvent was then removed under reduced pressure to give crude product. The crude product was purified by column chromatography using 20 % ethyl acetate–hexane as solvent system to afford pure **8** (1.8 g, 88 %). ^{1}H NMR (400 MHz; $CDCl_3$): δ –0.14 (s, 3H), –0.02 (s, 3H), 0.87 (s, 9H), 1.12 (d, *J* = 6.2 Hz, 3H), 1.34 (s, 12H), 1.42 (s, 18H), 2.30-2.61 (m, 4H), 4.16 (quint., 1H), 4.43-4.52 (m, 1H), 7.09 (d, *J* = 7.7 Hz, 1H), 7.27 (t, *J* = 7.3 Hz, 1H), 7.49 (s, 1H), 7.65 (d, *J* = 7.3 Hz, 1H), 8.15 (s, 1H), 8.85 (s, 1H); ^{13}C-NMR (100 MHz; $CDCl_3$): δ –5.02, –4.42, 17.86, 20.42, 24.84 (4C), 25.79 (3C), 27.75 (6C), 29.38, 32.04, 61.36, 69.92, 83.49 (2C), 83.79 (2C), 127.99, 128.57, 129.07, 131.17, 132.79, 134.57, 139.40, 144.53, 150.17, 150.29, 151.84, 153.63 (2C); ESI-MS *m/z*: 738.2 [M^+ + 1].

*4.1.9. General method for Suzuki reaction to get **9a-c***

A mixture of boronate ester **8** (0.33 mmol), heteroaryl halide (0.44 mmol) and potassium carbonate (0.84 mmol) in dioxane:water (4:1, 6.25 mL) was degassed with Argon gas for 10 min. [1,1′-Bis(diphenylphosphino)ferrocene]dichloro palladium(II), complex with dichloromethane (0.05 mmol) was added and the degassing continued again for further 5 min. Reaction mixture was heated at 90 °C for 3-5 h. Reaction progress was monitored by TLC. After reaction completion the mixture was passed through celite pad to remove insoluble material. The solvent was then removed under reduced pressure to give crude product. The crude product was purified by column chromatography.

4.1.9.1. tert-butyl N-tert-butoxycarbonyl-N-[9-[(1R,2S)-2-[tert-butyl(dimethyl)silyl]oxy-1-[2-(3-thiazol-2-ylphenyl)ethyl]propyl]purin-6-yl]carbamate (***9a***). Yield: 54%; ^{1}H NMR (400 MHz; $CDCl_3$): δ –0.13 (s, 3H), –0.02 (s, 3H), 0.87 (s, 9H), 1.12 (d, *J* = 6.4 Hz, 3H), 1.43 (s, 18H), 2.37-2.68 (m, 4H), 4.15 (quint., 1H), 4.48-4.56 (m, 1H), 7.05 (d, *J* = 7.3 Hz, 1H), 7.32 (t, *J* = 7.8 Hz, 1H), 7.33 (d, *J* = 3.4 Hz, 1H), 7.67 (s, 1H), 7.79 (d, *J* = 7.8 Hz, 1H), 7.86 (d, *J* = 3.0 Hz, 1H), 8.17 (s, 1H), 8.84 (s, 1H); ESI-MS *m/z*: 695.1 [M^+ + 1].

*4.1.9.2. tert-butyl N-tert-butoxycarbonyl-N-[9-[(1R, 2S)-2-[tert-butyl (dimethyl)silyl]oxy-1-[2-[3-(2-pyridyl)phenyl]ethyl]propyl] purin-6-yl]carbamate (**9b**)*. Yeld:

56%; ^{1}H NMR (400 MHz; $CDCl_3$): δ –0.14 (s, 3H), –0.02 (s, 3H), 0.86 (s, 9H), 1.12 (d, *J* = 6.2 Hz, 3H), 1.43 (s, 18H), 2.39-2.50 (m, 2H), 2.52-2.69 (m, 2H), 4.16 (quint., 1H), 4.49-4.56 (m, 1H), 7.06 (d, *J* = 7.9 Hz, 1H), 7.32 (ddd, *J* = 1.1 Hz, 4.9 Hz, 7.5 Hz, 1H), 7.36 (t, *J* = 7.7 Hz, 1H), 7.63-7.68 (m, 2H), 7.74 (td, *J* = 2.0 Hz, 8.0 Hz, 1H), 7.81 (dt, *J* = 1.6 Hz, 8.2 Hz, 1H), 8.18 (s, 1H), 8.66-8.69 (m, 1H), 8.82 (s, 1H); ^{13}C-NMR (100 MHz; $CDCl_3$): δ –5.02, –4.41, 17.87, 20.45, 25.77 (3C), 27.76 (6C), 29.67, 32.18, 61.26, 69.96, 83.55 (2C), 120.61, 122.19, 125.01, 126.98, 128.86, 129.01, 129.07, 136.78, 139.67, 140.65, 144.58, 149.60, 150.24, 150.31, 151.85, 153.63 (2C), 157.19; ESI-MS *m/z*: 689.2 [M^+ + 1].

4.1.9.3. tert-butyl N-tert-butoxycarbonyl-N-[9-[(1R, 2S)-2-[tert-butyl (dimethyl)silyl]oxy-1-[2-(3-pyrazin-2-ylphenyl)ethyl]propyl] purin-6-yl]carbamate (***9c***). Yield: 61%; ^{1}H NMR (400 MHz; $CDCl_3$): δ –0.13 (s, 3H), –0.01 (s, 3H), 0.87 (s, 9H), 1.12 (d, *J* = 6.2 Hz, 3H), 1.43 (s, 18H), 2.40-2.53 (m, 2H), 2.55-2.68 (m, 2H), 4.17 (quint., 1H), 4.48-4.56 (m, 1H), 7.12 (d, *J* = 7.8 Hz, 1H), 7.40 (t, *J* = 7.6 Hz, 1H), 7.72 (s, 1H), 7.83 (d, *J* = 7.8 Hz, 1H), 8.18 (s, 1H), 8.52 (d, *J* = 2.4 Hz, 1H), 8.62 (dd, *J* = 1.7 Hz, 2.4 Hz, 1H), 8.83 (s, 1H), 9.00 (d, *J* = 1.5 Hz, 1H); ^{13}C-NMR (100 MHz; $CDCl_3$): δ –4.99, –4.38, 17.87, 20.48, 25.77 (3C), 27.76 (6C), 29.67, 32.15, 61.26, 69.99, 83.59 (2C), 124.95, 126.99, 129.02, 129.30, 129.86, 136.64, 141.13, 142.21, 142.97, 144.14, 144.44, 150.27, 150.34, 151.89, 152.60, 153.60 (2C); ESI-MS *m/z*: 690.2 [M^+ + 1].

4.1.10. Synthesis of the biaryl derivatives ***10a-c***

Diboc and TBDMS deprotection of **9a-c** was carried employing general method described for synthesis of **7a-f**. All crude compounds were purified by column chromatography.

4.1.10.1. (2S, 3R)-3-(6-aminopurin-9-yl)-5-(3-thiazol-2-ylphenyl) pentan-2-ol (***10a***). Yield: 77 %; ^{1}H NMR (400 MHz; CD_3OD): δ 1.07 (d, *J* = 6.4 Hz, 3H), 2.46-2.66 (m, 4H), 4.07 (quint., 1H), 4.36-4.44 (m, 1H), 7.16 (d, *J* = 7.8 Hz, 1H), 7.28 (t, *J* = 7.8 Hz, 1H), 7.49 (s, 1H), 7.56 (d, *J* = 3.4 Hz, 1H), 7.67 (d, *J* = 7.8 Hz, 1H), 7.82 (d, *J* = 2.9 Hz, 1H), 8.11 (s, 1H), 8.18 (s, 1H); ^{13}C-NMR (100 MHz; CD_3OD): δ 20.16, 30.85, 33.36, 62.36, 70.27, 119.90, 120.80, 125.31, 127.48, 130.23, 131.37, 134.27, 141.91, 142.95, 144.19, 150.77, 153.43, 157.07, 170.12; ESI-MS *m/z* : 381.2 [M^+ + 1]; HPLC purity: 98 %.

*4.1.10.2. (2S, 3R)-3-(6-aminopurin-9-yl)-5-[3-(2-pyridyl)phenyl]pentan-2-ol (**10b**).* Yield: 75%; ^{1}H NMR (400 MHz; CD_3OD): δ 1.07 (d, *J* = 6.3 Hz, 3H), 2.48-2.65 (m, 4H), 4.07 (quint., 1H), 4.38-4.45 (m, 1H), 7.14 (d, *J* = 7.6 Hz, 1H), 7.31 (t, *J* = 7.8 Hz, 1H), 7.33-7.36 (m, 1H), 7.51 (s, 1H), 7.67 (d, *J* = 7.8 Hz, 1H), 7.73 (d, *J* = 7.8 Hz, 1H), 7.87 (td, *J* = 1.9 Hz,7.8 Hz, 1H), 8.09 (s, 1H), 8.19 (s, 1H), 8.55 (dd, *J* = 1.0 Hz, 5.9Hz, 1H); ^{13}C-NMR

(100 MHz; CD_3OD): δ 20.20, 31.10, 33.52, 62.38, 70.27, 120.01, 122.64, 123.69, 125.87, 128.26, 129.89, 130.21, 138.87, 140.36, 141.99, 142.54, 150.15, 150.93, 153.43, 151.19, 158.81; ESI-MS *m/z*: 375.3 [M^+ + 1]; HPLC purity: 99 %.

4.1.10.3. (2S, 3R)-3-(6-aminopurin-9-yl)-5-(3-pyrazin-2-ylphenyl) pentan-2-ol (***10c***). Yield: 81 %; ^{1}H NMR (400 MHz; CD_3OD): δ 1.07 (d, *J* = 6.4 Hz, 3H), 2.49-2.70 (m, 4H), 4.06 (quint., 1H), 4.37-4.45 (m, 1H), 7.19 (d, *J* = 7.9 Hz, 1H), 7.33 (t, *J* = 7.9 Hz, 1H), 7.61 (s, 1H), 7.79 (d, *J* = 7.8 Hz, 1H), 8.08 (s, 1H), 8.16 (s, 1H), 8.50 (d, *J* = 2.4 Hz, 1H), 8.62 (dd, *J* = 1.5 Hz, 2.4 Hz, 1H), 9.00 (d, *J* = 1.5 Hz, 1H); ^{13}C-NMR (100 MHz; CD_3OD): δ 20.22, 30.96, 33.57, 62.38, 70.29, 119.98, 125.70, 128.10, 130.11, 131.14, 137.32, 141.92, 142.84, 143.11, 143.87, 145.54, 150.89, 153.41, 154.16, 157.15; ESI-MS *m/z*: 376.3 [M^+ + 1]; HPLC purity: 99 %.

4.1.11. 3-[(3R, 4S)-3-(6-aminopurin-9-yl)-4-hydroxy-pentyl]benzoic acid.(***11***).

To a solution of ester **7h** (50 mg, 0.14 mmol) in THF:MeOH:H_2O (3:1:1, 2.5 mL) was added lithium hydroxide monohydrate (12 mg, 0.28 mmol) in one portion at RT. Reaction mixture was stirred for 16 h at RT. Solvent was removed under reduced pressure. The residue was diluted with cold water and acidified to pH ~5.0 with 1N hydrochloric acid. The aqueous layer was extracted with ethyl acetate (5 mL X 3) and combined organic layer was washed with brine, dried over anhydrous sodium sulfate and concentrated under reduced pressure to give a crude product. The crude product was purified by column chromatography to afford pure **11** (40 mg, 85% yield). ^{1}H NMR (400 MHz; CD_3OD): δ 1.07 (d, *J* = 6.3 Hz, 3H), 2.43-2.62 (m, 4H), 4.08 (quint., 1H), 4.32-4.41 (m, 1H), 7.24-7.29 (m, 2H), 7.64 (s, 1H), 7.74-7.78 (m, 1H), 8.17 (s, 2H); ^{13}C-NMR (100 MHz; $(CD_3)_2SO$): δ 20.25, 30.67, 31.55, 60.56, 67.94, 118.83, 126.91, 128.47, 129.00, 130.75, 132.83, 140.17, 141.49, 149.84, 152.19, 155.94, 167.33; ESI-MS *m/z*: 342.3 [M^+ + 1]; HPLC purity: 99 %.

4.1.12. (2S, 3R)-3-(6-aminopurin-9-yl)-5-[3-(hydroxymethyl)phenyl] pentan-2-ol (***12***).

An oven-dried, 25 mL 2 neck flask, fitted with a reflux condenser, was flushed with dry nitrogen. The flask was charged with a solution of lithium aluminum hydride (0.064 g, 1.68 mmol) in THF (10 mL). To the stirred solution at 0 °C, ester **7h** (0.1 g, 0.28 mmol) was added slowly. The reaction mixture was heated to reflux and stirred further for 16 h. Reaction mixture was slowly cooled to 0 °C and quenched with addition of EtOAc and sat. aqueous solution of Na_2SO_4. Reaction mixture was filtered through celite pad and filtrate was concentrated under reduced pressure to give crude product. The crude product was purified by column chromatography using 5% MeOH–DCM as solvent system to afford pure product **12** (75 mg, 81% yield); ^{1}H NMR (400 MHz, CD_3OD): δ 1.07 (d, *J* = 6.3 Hz, 3H), 2.4-2.58 (m,

4H), 4.08 (quint., 1H), 4.35-4.41 (m, 1H), 4.49 (s, 2H), 6.92 (d, *J* = 7.3 Hz, 1H), 6.98 (s, 1H), 7.10 (d, *J* = 7.3 Hz, 1H), 7.15 (t, *J* = 7.3 Hz, 1H), 8.16 (s, 1H), 8.18 (s, 1H); ^{13}C-NMR (100 MHz; CD_3OD): δ 20.25, 31.25, 33.39, 62.55, 65.15, 70.18, 120.05, 125.75, 128.00, 128.27, 129.38, 141.96, 142.06, 142.74, 150.95, 153.47, 157.27; ESI-MS *m/z*: 328.3 [M^+ + 1]; HPLC purity: 96 %.

4.1.13. 3-[(3R, 4S)-3-(6-aminopurin-9-yl)-4-hydroxy-pentyl]benzamide (***13***).

To a stirred solution of ester **7h** (0.11 g, 0.31 mmol) in methanol (5 mL) at 0 °C in a 15 mL seal tube was added magnesium nitrite (0.156 g, 1.54 mmol) in a single portion. The tube was sealed immediately and allowed to warm to room temperature. After 1 hour the reaction was heated at 80 °C for 48 h. The reaction was allowed to come to room temperature and diluted with chloroform (25 mL) and water (25 mL). The aqueous layer was neutralised with 3N HCl and the organic layer was separated. The aqueous layer was further extracted with chloroform (2 x 25 mL) and the combined organic layer washed with brine, and concentrated under reduced pressure to afford crude product. The crude product was purified by preparative TLC plate using 10 % MeOH–DCM as mobile phase to afford pure product **13** (52 mg, 50 % yield); ^{1}H NMR (400 MHz; CD_3OD): δ 1.07 (d, J = 6.2 Hz, 3H), 2.42-2.63 (m, 4H), 4.09 (quint., 1H), 4.36-4.44 (m, 1H), 7.21 (d, J = 7.5 Hz, 1H), 7.27 (t, J = 7.5 Hz, 1H), 7.56 (s, 1H), 7.63 (d, J = 7.5 Hz, 1H), 8.18 (s, 1H), 8.21 (s, 1H); ^{13}C-NMR (100 MHz; CD_3OD): δ 20.26, 31.28, 33.33, 62.56, 70.23, 120.11, 126.42, 128.59, 129.52, 132.90, 135.01, 141.96, 142.48, 151.03, 153.55, 157.31, 172.32; ESI-MS *m/z*: 341.1 [M^+ + 1]; HPLC purity: 91 %.

4.1.13. 3-[(3R, 4S)-3-(6-aminopurin-9-yl)-4-hydroxy-pentyl]-N-methyl-benzamide (***14***).

A solution of ester **7h** (150 mg, 0.42 mmol) in methylamine (40% in water, 8 mL) was placed in a 15 mL seal tube. The reaction mixture was heated at 80 °C for 10 h. The reaction mixture was allowed to come to room temperature and diluted with EtOAc (25 mL) and water (25 mL). The aqueous layer was neutralised with 1N HCl and the organic layer separated. The aqueous layer was further extracted with EtOAc (2 x 25mL) and the organic layers combined, washed with brine, and concentrated *in vacuo* to afford crude product. The crude product was purified by preparative TLC plate using 8% MeOH–DCM as mobile phase to afford pure product **14** (80 mg, 54% yield;. ^{1}H NMR (400 MHz; CD_3OD): δ 1.07 (d, *J* = 6.2 Hz, 3H), 2.43-2.62 (m, 4H), 2.89 (s, 3H), 4.08 (quint., 1H), 4.34-4.42 (m, 1H), 7.20 (d, *J* = 7.1 Hz, 1H), 7.26 (t, *J* = 7.5 Hz, 1H), 7.44 (s, 1H), 7.56 (d, *J* = 7.3 Hz, 1H), 8.17 (s, 1H), 8.19 (s, 1H); ESI-MS *m/z*: 355.1 [M^+ + 1]; HPLC purity: 99%.

4.1.15. 3-[(3R, 4S)-3-[6-(tert-butoxycarbonylamino)purin-9-yl]-4-[tert-butyl(dimethyl)silyl] oxy-pentyl]benzoic acid (***15***).

To a solution of ester **6h** (4.0 g, 5.97 mmol) in THF:MeOH:H_2O (3:1:1, 100 mL) was added lithium hydroxide monohydrate (0.50 g, 11.94 mmol) in one portion at RT. Reaction mixture was stirred for 16 h at RT. Solvent was removed under vacuum and diluted with cold water and acidified to pH ~ 5.0 with 1N hydrochloric acid. The aqueous layer was extracted with ethyl acetate (150 mL X 3) and combined organic layer was washed with brine, dried over anhydrous sodium sulfate and concentrated under reduced pressure to give a crude product. The crude product was purified by column chromatography using 5% MeOH–DCM as solvent system to afford pure **15** (2.38 g, 72% yield); ^{1}H NMR (400 MHz; $CDCl_3$): δ –0.12 (s, 3H), –0.01 (s, 3H), 0.88 (s, 9H), 1.11 (d, *J* = 6.3 Hz, 3H), 1.60 (s, 9H), 2.41-2.68 (m, 4H), 4.19 (quint., 1H), 4.35-4.45 (m, 1H), 7.20 (d, *J* = 7.3 Hz, 1H), 7.24 (t, *J* = 7.3 Hz, 1H), 7.69 (s, 1H), 7.88 (d, *J* = 7.4 Hz, 1H), 7.99 (s, 1H), 8.76 (s, 1H), 9.18 (br s, 1H). ESI-MS *m/z*: 556.2 $[M + 1]^+$ base peak.

*4.1.16. General method for Amide coupling to get **16a-c***

A 50 mL RB flask was charged with a magnetic spin bar, intermediate acid **15** (0.36 mmol), dimethyl amine (2.0 M in THF, 0.53 mmol), HoBt (0.53 mmol), DCM (12 mL), and triethylamine (1.08 mmol). To this reaction mixture maintained at 0 °C under stirring, was added EDCI.HCl (0.53 mmol). . The reaction was allowed to come to RT and stirred for 16 h. The reaction was then diluted with water and extracted with ethyl acetate (50 mL X 3). The combined organic layer was washed with brine, dried over anhy. sodium sulfate and concentrated under reduced pressure to give a crude product. The crude product was purified by column chromatography.

4.1.16.1. tert-butyl-N-[9-[(1R,2S)-2-[tert-butyl(dimethyl)silyl]oxy-1-[2-[3-(dimethylcarbamo yl)phenyl]ethyl]propyl]purin-6-yl]carbamate (***16a***). Yield: 51%; ^{1}H NMR (400 MHz; CD_3OD): δ –0.11 (s, 3H), –0.01 (s, 3H), 0.86 (s, 9H), 1.07 (d, *J* = 6.3 Hz, 3H), 1.59 (s, 9H), 2.42-2.69 (m, 4H), 2.95 (s, 3H), 3.08 (s, 3H), 4.23 (quint., 1H), 4.46-4.55 (m, 1H), 7.07 (s, 1H), 7.14 (d, *J* = 7.5 Hz, 1H), 7.19 (d, *J* = 7.8 Hz, 1H), 7.28 (t, *J* = 7.5 Hz, 1H), 8.35 (s, 1H), 8.54 (s, 1H); ^{13}C-NMR (100 MHz; CD_3OD): δ –4.94, –4.31, 18.75, 20.68, 26.28 (3C), 28.46 (3C), 30.54, 33.00, 35.61, 40.08, 62.61, 71.23, 82.79, 122.99, 123.01, 125.83, 128.01, 129.66, 130.97, 131.42, 142.49, 144.49, 151.25, 152.46, 153.01, 173.72; ESI-MS *m/z*: 583.3 $[M^+ + 1]$.

4.1.16.2. tert-butyl N-[9-[(1R,2S)-2-[tert-butyl(dimethyl)silyl]oxy-1-[2-[3-(cyclopentylcarba moyl)phenyl]ethyl]propyl]purin-6-yl] carbamate (***16b***). Yield: 75%; ^{1}H

NMR (400 MHz; $CDCl_3$): δ –0.16 (s, 3H), –0.01 (s, 3H), 0.87 (s, 9H), 1.13 (d, *J* = 6.4 Hz, 3H), 1.45-1.56 (m, 2H), 1.64 (s, 9H), 1.65-1.81 (m, 4H), 2.06-2.17 (m, 2H), 2.35-2.60 (m, 4H), 4.13 (quint., 1H), 4.40 (quint., 1H), 4.41-4.48 (m, 1H), 6.04 (d, *J* = 6.6 Hz, 1H), 7.08 (d, *J* = 8.0 Hz, 1H), 7.25 (t, *J* = 7.7 Hz, 1H), 7.43 (s, 1H), 7.48 (d, *J* = 7.7 Hz, 1H), 7.97 (s, 1H), 8.00 (s, 1H), 8.72 (s, 1H); ^{13}C-NMR (100 MHz; $CDCl_3$): δ –5.07, –4.36, 17.88, 20.60, 23.82 (2C), 25.79 (3C), 28.13 (3C), 28.98, 32.19, 33.21 (2C), 51.72, 61.23, 69.97, 82.17, 121.61, 124.55, 126.84, 128.59, 131.12, 135.24, 140.69, 141.99, 149.72, 149.76, 151.16, 152.69, 167.15; ESI-MS *m/z*: 623.2 [M^+ + 1].

4.1.16.3. tert-butyl N-[9-[(1R,2S)-2-[tert-butyl(dimethyl)silyl]oxy-1-[2-[3-(pyrrolidine-1-carbonyl)phenyl]ethyl]propyl]purin-6-yl]carbamate (***16c***). Yield: 69%; ^{1}H NMR (400 MHz; $CDCl_3$): δ –0.21 (s, 3H), –0.03 (s, 3H), 0.86 (s, 9H), 1.13 (d, *J* = 6.7 Hz, 3H), 1.56 (s, 9H), 1.85 (quint., 2H), 1.95 (quint., 2H), 2.31-2.58 (m, 4H), 3.35 (t, *J* = 6.7 Hz, 2H), 3.62 (t, *J* = 7.0 Hz, 2H), 4.12 (quint., 1H), 4.41-4.48 (m, 1H), 7.03 (d, *J* = 7.5 Hz, 1H), 7.20 (s, 1H), 7.25 (t, *J* = 7.9 Hz, 1H), 7.31 (d, *J* = 7.5 Hz, 1H), 7.98 (s, 1H), 8.07 (s, 1H), 8.72 (s, 1H); ^{13}C-NMR (100 MHz; $CDCl_3$): δ –4.89, –4.19, 18.06, 20.75, 24.59, 25.96 (3C), 26.53, 28.32 (3C), 29.21, 32.34, 46.29, 49.66, 61.47, 70.19, 82.29, 121.91, 125.18, 127.06, 128.58, 129.78, 137.84, 140.65, 142.16, 149.86, 149.99, 151.48, 152.88, 169.73; ESI-MS *m/z*: 609.3 [M^+ + 1].

*4.1.17. Synthesis of the Amide derivatives **17a**-c*

Diboc and TBDMS deprotection of **16a-c** was carried employing general method described for synthesis of **7a-f**. All crude compounds were purified by column chromatography.

4.1.17.1. 3-[(3R, 4S)-3-(6-aminopurin-9-yl)-4-hydroxy-pentyl]-N,N-dimethyl-benzamide (***17a***). Yield: 68 % ; ^{1}H NMR (400 MHz, CD_3OD): δ 1.06 (d, *J* = 6.3 Hz, 3H), 2.42-2.59 (m, 4H), 2.95 (s, 3H), 3.07 (s, 3H), 4.08 (quint., 1H), 4.35-4.43 (m, 1H), 7.09 (s, 1H), 7.12-7.21 (m, 2H), 7.27 (t, *J* = 7.6 Hz, 1H), 8.18 (s, 1H), 8.19 (s, 1H). ^{13}C-NMR (100 MHz; CD_3OD): δ 20.28, 31.22, 33.21, 35.61, 40.09, 62.47, 70.16, 120.07, 125.73, 128.01, 129.57, 130.97, 137.36, 142.03, 142.69, 151.03, 153.53, 157.27, 173.75; ESI-MS *m/z*: 369.1 [M^+ + 1]; HPLC purity: 96 %.

4.1.17.1. 3-[(3R, 4S)-3-(6-aminopurin-9-yl)-4-hydroxy-pentyl]-N-cyclopentyl-benzamide (***17b***). Yield: 59 %; ^{1}H NMR (400 MHz; CD_3OD): δ 1.08 (d, *J* = 6.4 Hz, 3H), 1.51-1.70 (m, 4H), 1.72-1.84 (m, 2H), 1.97-2.08 (m, 2H), 2.42-2.61 (m, 4H), 4.09 (quint., 1H), 4.29 (quint., 1H), 4.35-4.42 (m, 1H), 7.19 (d, *J* = 7.8 Hz, 1H), 7.26 (t, *J* = 7.8 Hz, 1H), 7.46 (s, 1H), 7.56 (d, *J* = 7.8 Hz, 1H), 8.17 (s, 1H), 8.19 (s, 1H);^{13}C-NMR (100 MHz; CD_3OD): δ 20.26, 24.95 (2C), 31.2 , 33.33 ,33.42 (2C), 53.03, 62.48, 70.19, 120.04, 126.14,

128.27, 129.48, 132.42, 136.02, 141.97, 142.36, 150.94, 153.53, 157.27, 170.01; ESI-MS *m/z*: 409.3 [M^+ + 1]; HPLC purity: 98 %.

4.1.17.1. [3-[(3R,4S)-3-(6-aminopurin-9-yl)-4-hydroxy-pentyl]phenyl]-pyrrolidin-1-yl-metha none (***17c***). Yield: 71 %; ^{1}H NMR (400 MHz; CD_3OD): δ 1.07 (d, *J* = 6.4 Hz, 3H), 1.89 (quint., 2H), 1.97 (quint., 2H), 2.44-2.60 (m, 4H), 3.34-3.42 (m, 2H), 3.54 (t, *J* = 6.9 Hz, 2H), 4.08 (quint., 1H), 4.35-4.42 (m, 1H), 7.14-7.18 (m, 2H), 7.24- 7.31 (m, 2H), 8.17 (s, 1H), 8.20 (s, 1H); ^{13}C-NMR (100 MHz; CD_3OD): δ 20.26, 25.33, 27.25, 31.16, 33.21, 47.44, 50.87, 62.41, 70.17, 120.07, 125.81, 128.06, 129.55, 131.28, 138.07, 142.03, 142.53, 151.02, 153.55, 157.29, 171.79; ESI-MS *m/z*: 395.3 [M^+ + 1]; HPLC purity: 98 %.

4.1.18. [(2R, 3R)-2-(tert-butoxycarbonylamino)-3-hydroxy-butyl] 4-methyl benzenesulfonate (***19***).

Procedure for reduction of ester using sodium borohydride: To a stirred solution of *N*-boc-L-theronine methyl ester (**18**, 20 g, 85.7 mmol) in dry THF (340 mL) at 0 °C under nitrogen was added sodium borohydride (3.24 g, 85.7 mmol) portion wise over the time period of 45 min. The reaction mixture was stirred at 0°C for an additional 1h and at room temperature for 16 h. The reaction mixture was cooled to 0 °C and diluted with distilled water (300 mL). The reaction mixture thus obtained was extracted with EtOAc (200 mL x 3). The combined organic layer was washed with brine and dried over anhydrous sodium sulfate. Volatiles were removed under reduced pressure to afford crude product as a viscous material. The crude product was purified by column chromatography using 40% ethyl acetate–hexane as solvent system to afford *the title compound* (17 g, 96% yield); ^{1}H NMR (400 MHz; CD_3OD): δ 1.14 (d, *J* = 6.4 Hz, 3H), 1.43 (s, 9H), 3.41-3.48 (m, 1H), 3.54 (dd, *J* = 5.9 Hz, 10.8 Hz, 1H), 3.60 (dd, *J* = 6.4 Hz, 10.8 Hz, 1H), 3.91-4.0 (m, 1H); ESI-MS *m/z*: 106.2 [M^+ – Boc].

Procedure for tosylation using tosyl chloride: To a stirred solution of above obtained alcohol (17 g, 82.8 mmol) in dry DCM (170 mL) at 0°C under nitrogen was added triethylamine (23.1 mL, 165.7 mmol). After the reaction mixture was stirred for 15 min *p*-toulene sulfonyl chloride (18.9 g, 99.4 mmol) in dry DCM (30 mL) was added slowly. The reaction mixture was stirred at 0°C for an additional 30 min and at room temperature for 5 h. After completion of reaction, volatiles were evaporated under reduced pressure and residue was poured into water (400 mL). Aqueous layer thus obtained was extracted with EtOAc (200 mL x 3). Combined organic layer was washed with water, brine, dried over anhydrous sodium sulfate and was evaporated under reduced pressure to afford crude product. The crude product was purified by column chromatography using 30% ethyl acetate–hexane as solvent

system to afford pure **19** (20.6 g, 69% yield); ^{1}H NMR (400 MHz; $CDCl_3$): δ 1.18 (d, *J* = 6.2 Hz, 3H), 1.40 (s, 9H), 2.18 (br s, 1H), 2.44 (s, 3H), 3.60-3.74 (m, 1H), 3.98-4.12 (m, 3H), 4.93 (d, *J* = 8.8 Hz, 1H), 7.35 (d, *J* = 7.9 Hz, 2H), 7.78 (d, *J* = 8.15 Hz, 2H); ESI-MS *m/z*: 260.0 [M^+ – Boc].

*4.1.19. General method for displacement of tosyl with to get **20a-e***

A mixture of sodium salt of thiol (9.3 mmol prepared from thiol and NaOH using stoichiometric ratio in methanol at room temperature, evaporation of methanol under reduced pressure furnished sodium thiolate) and [(*2R, 3R*)-2-(*tert*-butoxycarbonylamino)-3-hydroxy-butyl]4-methylbenzenesulfonate (**19**, 7.8 mmol) in 1,4-dioxane (20 mL) was stirred for 3-5 h at room temperature. The solvent was evaporated under reduced pressure to get the crude product which was purified by column chromatography.

4.1.19.1. tert-Butyl N-[(1S, 2R)-1-(butylsulfanylmethyl)-2-hydroxy-propyl] carbamate (***20a***). Yield: 84%; ^{1}H NMR (400 MHz; $CDCl_3$): δ 0.94 (t, *J* = 7.3 Hz, 3H), 1.25 (d, *J* = 6.36 Hz, 3H), 1.37-1.47 (m, 2H), 1.48 (s, 9H), 1.54-1.64 (m, 2H), 2.47 (br s, 1H), 2.58 (t, *J* = 7.3 Hz, 2H), 2.65-2.82 (m, 2H), 3.60 (br. s, 1H), 4.05-4.18 (m, 1H), 5.04 (br s, 1H); ESI-MS *m/z*: 178.1 [M^+ – Boc].

4.1.19.2. tert-butyl N-[(1S, 2R)-2-hydroxy-1-(phenylsulfanylmethyl) propyl]carbamate (***20b***). Yield: 94%; ^{1}H NMR (400 MHz; CD_3OD): δ 1.11 (d, *J* = 6.6 Hz, 3H), 1.44 (s, 9H), 3.01 (dd, *J* = 8.1, 13.6 Hz, 1H), 3.12 (dd, *J* = 6.2 Hz, 13.4 Hz, 1H), 3.51-3.62 (m, 1H), 3.93-4.02 (m, 1H), 7.16 (app. t, *J* = 7.27 Hz, 1H), 7.28 (t, *J* = 7.7 Hz, 2H), 7.38 (d, *J* = 7.7 Hz, 2H). ESI-MS *m/z*: 242.1 [M - 55] $^+$ base peak, 198.1 [M - Boc] $^+$, 320.1 [M + Na] $^+$

4.1.19.3. tert-Butyl N-[(1S, 2R)-2-hydroxy-1-(o-tolylsulfanylmethyl) propyl]carbamate (***20c***). Yield: 89%; ^{1}H NMR (400 MHz; $CDCl_3$): δ 1.21 (d, *J* = 6.3 Hz, 3H), 1.44 (s, 9H), 2.22 (d, *J* = 4.4 Hz, 1H), 2.38 (s, 3H), 3.06-3.17 (m, 2H), 3.60-3.69 (m, 1H), 4.09-4.18 (m, 1H), 4.98 (d, *J* = 8.3 Hz, 1H), 7.08-7.13 (m, 1H), 7.14-7.20 (m, 2H), 7.39 (d, *J* = 7.3 Hz, 1H); ^{13}C-NMR (100 MHz; $CDCl_3$): δ 20.54, 20.61, 28.46 (3C), 35.34, 54.96, 67.66, 79.74, 126.13, 126.74, 128.49, 130.34, 135.19, 137.81, 156.27; ESI-MS *m/z*: 212.1 [M^+ – Boc].

4.1.19.4. tert-Butyl N-[(1S, 2R)-2-hydroxy-1-(m-tolylsulfanylmethyl)propyl]carbamate (***20d***). Yield: 93%; ^{1}H NMR (400 MHz; $CDCl_3$): δ 1.20 (d, *J* = 6.4 Hz, 3H), 1.44 (s, 9H), 2.13 (br s, 1H), 2.33 (s, 3H), 3.06-3.20 (m, 2H), 3.58-3.67 (m, 1H), 4.08-4.17 (m, 1H), 4.96 (d, *J* = 7.8 Hz, 1H), 6.98-7.02 (m, 1H), 7.15-7.24 (m, 3H); ^{13}C-NMR (100 MHz; $CDCl_3$): δ 20.63, 21.44, 28.46 (3C), 36.14, 55.08, 67.59, 79.74, 126.43, 127.29, 129.03, 130.11, 135.68, 138.95, 156.26; ESI-MS *m/z*: 312.2 [M^+ + 1] $^+$.

4.1.19.5. *tert*-Butyl N-[(*1S, 2R*)-2-hydroxy-1-[[3-(trifluoromethyl) phenyl] sulfanylmethyl] propyl]carbamate (***20e***). Yield: 85%; ^{1}H NMR (400 MHz; $CDCl_3$): δ 1.22 (d, *J* = 6.4 Hz, 3H), 1.44 (s, 9H), 1.68 (br s, 1H), 3.10-3.26 (m, 2H), 3.58-3.67 (m, 1H), 4.13 (ddd, *J* = 2.4 Hz, 6.3 Hz, 12.7 Hz, 1H), 4.97 (d, *J* = 8.1 Hz, 1H), 7.38-7.44 (m, 2H), 7.55-7.60 (m, 1H), 7.61 (s, 1H); ^{13}C-NMR (100 MHz; $CDCl_3$): δ 20.77, 28.43 (3C), 35.59, 54.88, 67.35, 79.95, 122.66 (q, $^{3}J_{FC}$ = 3.9 Hz), 123.77 (q, $^{1}J_{FC}$ = 273.2 Hz), 125.23, 129.37, 131.19 (q, $^{2}J_{FC}$ = 31.9 Hz), 131.63, 137.75, 156.08; ESI-MS *m/z*: 266.0 [M^+ – Boc].

*4.1.20. General method for synthesis of **21a−e**.*

To a solution of compounds **20a−e** (5 mmol) in MeOH (10 mL) was added 1N HCl in MeOH (10 mL) at room temperature. Reaction mixture was stirred at room temperature for 3 h (progress of reaction was monitored on TLC). After completion, the reaction mixture was evaporated to dryness under reduced pressure to afford compounds **10a−f** as HCl salt which was used for next reaction without any purification. A solution of above obtained hydrochloride salt of beta amino compounds (3.98 mmol) in 40 mL of 1-butanol was added 4,6-dichloro-5-aminopyrimidine (7.96 mmol) and triethylamine (19.92 mmol). The reaction mixture was heated to reflux for 18-24 h. After completion of the reaction, the volatiles were removed under reduced pressure to obtain the oily residue which was dissolved in EtOAc (50 mL). Organic layer thus obtained was washed with water brinedried over anhydrous sodium sulfate and evapourated under reduced pressure to obtain crude product. The crude product was purified by column chromatography.

4.1.20.1. *(2R, 3S)-3-[(5-amino-6-chloro-pyrimidin-4-yl)amino]-4-butyl sulfanyl-butan-2-ol* (***21a***). Yield: 49%; ^{1}H NMR (400 MHz; $CDCl_3$): *δ* 0.89 (t, *J* = 7.3 Hz, 3H), 1.25 (d, *J* = 6.4 Hz, 3H), 1.32-1.46 (m, 2H), 1.56 (quint., 2H), 2.28 (br s, 1H), 2.49-2.63 (m, 2H), 2.75-2.90 (m, 2H), 3.54 (br. s, 2H), 4.17-4.32 (m, 2H), 5.43 (d, *J* = 8.3 Hz, 1H), 8.02 (s, 1H); ESI-MS *m/z*: 305.2 [M^+ (^{35}Cl) + 1].

4.1.20.2. *(2R, 3S)-3-[(5-amino-6-chloro-pyrimidin-4-yl)amino]-4-phenylsulfanyl-butan-2-ol* (***21b***). Yield: 59%; ^{1}H NMR (400 MHz; CD_3OD): δ 1.15 (d, *J* = 6.3 Hz, 3H), 3.19 (dd, *J* = 7.3 Hz, 13.7 Hz, 1H), 3.26 (dd, *J* = 6.4 Hz, 13.7 Hz, 1H), 4.18 (ddd, *J* = 2.9 Hz, 6.8 Hz, 13.2 Hz, 1H), 4.34-4.41 (m, 1H), 7.13-7.18 (m, 1H), 7.25 (t, *J* = 7.3 Hz, 2H), 7.37 (d, *J* = 7.8 Hz, 2H), 7.73 (s, 1H); ESI-MS *m/z*: 325.0 [M^+ (^{35}Cl) + 1].

4.1.20.3. *(2R, 3S)-3-[(5-amino-6-chloro-pyrimidin-4-yl)amino]-4-(o-tolylsulfanyl)butan-2-ol* (***21c***). Yield: 64%; ^{1}H NMR (400 MHz; $CDCl_3$): δ 1.23 (d, *J* = 6.4 Hz, 3H), 2.33 (s, 3H), 2.61 (br s, 1H), 3.22 (dd, *J* = 7.1 Hz, 13.7 Hz, 1H), 3.28 (dd, *J* = 5.9 Hz, 13.7 Hz, 1H), 3.38 (br. s, 2H), 4.23-4.37 (m, 2H), 5.37 (d, *J* = 8.3 Hz, 1H), 7.07-7.18 (m, 3H),

7.45 (d, *J* = 7.8 Hz, 1H), 8.0 (s, 1H); ^{13}C-NMR (100 MHz; $CDCl_3$): δ 20.55, 20.98, 34.69, 55.70, 67.80, 122.28, 126.27, 126.66, 128.59, 130.44, 134.95, 137.84, 143.08, 149.22, 154.62; ESI-MS *m/z*: 339.0 [M^+ (^{35}Cl) + 1].

4. *1.20.4.* *(2R, 3S)-3-[(5-amino-6-chloro-pyrimidin-4-yl)amino]-4-(m-tolylsulfanyl)butan-2-ol* (***21d***). Yield: 67%; ^{1}H NMR (400 MHz; CD_3OD): δ 1.14 (d, *J* = 6.4 Hz, 3H), 2.27 (s, 3H), 3.18 (dd, *J* = 7.6 Hz, 13.9 Hz, 1H), 3.25 (dd, *J* = 6.3 Hz, 13.9 Hz, 1H), 4.17 (ddd, *J* = 2.9 Hz, 6.3 Hz, 12.7 Hz, 1H), 4.36 (td, *J* = 2.9 Hz, 7.3 Hz, 1H), 6.97 (d, *J* = 7.1 Hz, 1H), 7.10-7.20 (m, 3H), 7.73 (s, 1H); ^{13}C-NMR (100 MHz; CD_3OD): δ 20.10, 21.38, 35.61, 56.73, 68.00, 124.97, 127.54, 127.89, 129.71, 130.91, 137.14, 139.02, 139.82, 147.46, 154.54; ESI-MS *m/z*: 339.2 [M^+ (^{35}Cl) + 1].

4.1.20.5. *(2R, 3S)-3-[(5-amino-6-chloro-pyrimidin-4-yl)amino]-4-[3-(trifluoromethyl)phenyl] sulfanyl-butan-2-ol* (***21e***). Yield: 64%; ^{1}H NMR (400 MHz; $CDCl_3$): δ 1.25 (d, *J* = 6.4 Hz, 3H), 2.65 (br s, 1H), 3.22 (dd, *J* = 8.3 Hz, 14.2 Hz, 1H), 3.34-3.45 (m, 3H), 4.19-4.28 (m, 1H), 4.38 (ddd, *J* = 2.4 Hz, 6.3 Hz, 12.7 Hz, 1H), 5.39 (d, *J* = 8.4 Hz, 1H), 7.37 (t, *J* = 7.8 Hz, 1H), 7.42 (d, *J* = 7.9 Hz, 1H), 7.57 (d, *J* = 7.8 Hz, 1H), 7.72 (s, 1H), 8.04 (s, 1H); ESI-MS *m/z*: 393.0 [$^+$ (^{35}Cl) + 1].

4.1.21. General method for cyclization of diamino pyrimidine intermediate 21a-e *to get chloropurine derivatives* ***22a−e.***

To a solution of diaminopyrimidine derivatives (0.99 mmol) in triethylorthoformate (18.88 mmol) was slowly added conc. HCl (0.05 mL). Reaction mixture was stirred for 2 h at room temperature. After completion the reaction was quenched with water (15 mL) and then aqueous layer was extracted with EtOAc (20 mL x 2). The combined organic layer was washed with brine dried over anhydrous sodium sulfate and the solvent was removed under reduced pressure to obtain the residue, which was stirred with 4M HCl in dioxane (5 mL) for 30 min. The solvent was evaporated under reduced pressure to obtain chloropurine derivative as a solid product.

4.1.21.1. ***(****2R, 3S)-4-butylsulfanyl-3-(6-chloropurin-9-yl)butan-2-ol* (***22a***). Yield: 82%; ^{1}H NMR (400 MHz; $CDCl_3$): δ 0.83 (t, *J* = 7.3 Hz, 3H), 1.07 (d, *J* = 6.4 Hz, 3H), 1.27-1.31 (m, 2H), 1.41-1.44 (m, 2H), 2.29-2.39 (m, 2H), 3.11-3.17 (m 2H), 4.34-4.41 (m, 1H), 4.43-4.49 (m, 1H), 4.60 (br s, 1H), 8.02 (s, 1H), 8.52 (s, 1H); ^{13}C-NMR (100 MHz; $CDCl_3$): δ 13.67, 20.93, 21.89, 31.60, 32.41, 34.15, 63.19, 67.95, 121.90, 143.51, 151.62 (2C), 161.49; ESI-MS *m/z*: 315.1 [M^+ (^{35}Cl) + 1].

4.1.21.2. *(2R, 3S)-3-(6-chloropurin-9-yl)-4-phenylsulfanyl-butan-2-ol* (***22b***). Yield: 81%; ^{1}H NMR (400 MHz; $CDCl_3$): *δ* 1.02 (d, *J* = 6.4 Hz, 3H), 3.53 (dd, *J* = 4.4 Hz, 14.7 Hz,

1H), 3.64 (dd, *J* = 9.9 Hz, 14.7 Hz, 1H), 4.25-4.40 (m, 2H), 4.60-4.69 (m, 1H), 7.08-7.22 (m, 5H), 7.79 (s, 1H), 8.41 (s, 1H); ESI-MS *m/z*: 335.0 [M^+ (^{35}Cl) + 1]. *4.1.22.3. (2R, 3S)-4.1.21.3. 3-(6-chloropurin-9-yl)-4-(o-tolylsulfanyl)butan-2-ol* (***22c***). Yield: 78%; ^{1}H NMR (400 MHz; CD_3OD): *δ* 1.07 (d, *J* = 6.3 Hz, 3H), 2.19 (s, 3H), 3.56 (dd, *J* = 4.4 Hz, 14.4 Hz, 1H), 3.69-3.76 (m, 1H), 4.30-4.40 (m, 1H), 4.68-4.76 (m, 1H), 6.98-7.12 (m, 3H), 7.25 (d, *J* = 6.8 Hz, 1H) 8.11 (s, 1H), 8.90 (s, 1H). ESI-MS *m/z*: 349.2 [M (^{35}Cl) + 1] $^+$ base peak. *4.1.22.4. (2R, 3S)-3-(6-chloropurin-9-yl)-4-(m-tolylsulfanyl)butan-2-ol* (***22d***). Yield: 69%; ^{1}H NMR (400 MHz; $CDCl_3$): δ 1.08 (d, *J* = 6.4 Hz, 3H), 2.23 (s, 3H), 3.48 (dd, *J* = 4.9 Hz, 14.6 Hz, 1H), 3.69 (dd, *J* = 9.8 Hz, 14.7 Hz, 1H), 3.96 (br s, 1H), 4.38-4.47 (m, 1H), 4.54 (quint., 1H), 6.89-6.98 (m, 3H), 7.03 (d, *J* = 7.3 Hz, 1H) 8.14 (s, 1H), 8.63 (s, 1H); ^{13}C-NMR (100 MHz; $CDCl_3$): δ 20.88, 21.23, 36.14, 63.51, 67.76, 127.58, 128.12, 128.81, 131.07, 131.62, 132.93, 139.0, 146.32, 151.19 (2C), 151.57; ESI-MS *m/z*: 349.2 [M^+ (^{35}Cl) + 1]. *4.1.21.5. (2R, 3S)-3-(6-chloropurin-9-yl)-4-[3-(trifluoromethyl)phenyl] sulfanyl-butan-2-ol* (***22e***). Yield: 75%; ^{1}H NMR (400 MHz; CD_3OD): *δ* 1.07 (d, *J* = 6.4 Hz, 3H), 3.66 (dd, *J* = 4.41 Hz, 14.77 Hz, 1H), 3.87 (dd, *J* = 10.58 Hz, 14.77 Hz, 1H), 4.38 (quint., 1H), 4.65-4.75 (m, 1H), 7.31-7.36 (m, 2H), 7.39 (d, *J* = 7.5 Hz, 1H), 7.44 (d, *J* = 7.7 Hz, 1H), 8.56 (s, 1H), 8.60 (s, 1H). (400 MHz; $CDCl_3$): *δ* 1.07 (d, *J* = 6.4 Hz, 3H), 3.56 (dd, *J* = 5.3 Hz, 14.7 Hz, 1H), 3.68-3.77 (m, 2H), 4.39-4.48 (m, 1H), 4.58 (quint., 1H), 7.27-7.42 (m, 2H), 7.37-7.42 (m, 2H), 8.19 (s, 1H), 8.65 (s, 1H); ESI-MS *m/z*: 403.1 [M^+ (^{35}Cl) + 1].

4.1.22. General method for amination of chloropurine 22a-e *to get adenine derivatives* ***23a−e.***

A mixture of chloropurine derivative (0.56 mmol) in liquid ammonia (1 g) was heated in seal tube at 65 °C for 16-20 h. After completion of the reaction, the ammonia gas was evaporated very carefully at 0 °C and the white residue was dried under *vacuo.* The solid mixture was triturated with 10 mL of boiling acetone and filter while hot to remove insoluble NH_4Cl the filtrate was evaporated to give pure product.

*4.1.22.1. (2R, 3S)-3-(6-aminopurin-9-yl)-4-butylsulfanyl-butan-2-ol (**23a**). Yield:* 85%; ^{1}H NMR (400 MHz; CD_3OD): δ 0.84 (t, *J* = 7.3 Hz, 3H), 1.04 (d, *J* = 6.3 Hz, 3H), 1.25-1.35 (m, 2H), 1.38-1.50 (m, 2H), 2.43 (t, *J* = 7.4 Hz, 2H), 3.15 (d, *J* = 2.0 Hz, 1H), 3.17 (d, *J* = 5.3 Hz, 1H), 4.27-4.35 (m, 1H), 4.59 (sept., 1H), 8.19 (s, 2H); ^{13}C-NMR (100 MHz, CD_3OD): δ 13.88, 20.86, 22.76, 32.46, 32.59, 34.67, 61.76, 68.29, 119.50, 142.61, 151.55, 153.49, 157.29; ESI-MS *m/z* : 296.1 [M^+ + 1]; HPLC purity: 94%.

4.1.22.2. *(2R, 3S)-3-(6-aminopurin-9-yl)-4-phenylsulfanyl-butan-2-ol* (***23b***). Yield: 83%; ^{1}H NMR (400 MHz; CD_3OD): δ 1.01 (d, *J* = 6.4 Hz, 3H), 3.53 (dd, *J* = 4.8 Hz, 14.5 Hz, 1H), 3.70 (dd, *J* = 10.3 Hz, 14.5 Hz, 1H), 4.28-4.35 (m, 1H), 4.55 (quint., 1H), 7.09-7.22 (m, 5H), 8.08 (s, 1H), 8.11 (s, 1H); ^{1}H NMR (400 MHz; $CDCl_3$): δ 1.03 (d, *J* = 6.4 Hz, 3H), 3.50 (dd, *J* = 3.7 Hz, 14.9 Hz, 1H), 3.67 (dd, *J* = 10.1 Hz, 14.9 Hz, 1H), 4.21-4.32 (m, 2H), 5.59 (br s, 2H), 5.68 (br s, 1H), 7.10-7.23 (m, 5H), 7.65 (s, 1H), 8.25 (s, 1H); ESI-MS *m/z*: 316.1 [M^+ + 1]; HPLC purity: 99%.

4.1.22.3. *(2R, 3S)-3-(6-aminopurin-9-yl)-4-(o-tolylsulfanyl)butan-2-ol* (***23c***). Yield: 81%; ^{1}H NMR (400 MHz; $CDCl_3$): δ 1.05 (d, *J* = 6.4 Hz, 3H), 2.27 (s, 3H), 3.45-3.57 (m, 1H), 3.62 (dd, *J* = 9.60 Hz, 14.4 Hz, 1H), 4.27-4.36 (m, 2H), 5.66 (br s, 2H), 5.97 (br s, 1H), 7.02-7.08 (m, 2H), 7.10-7.17 (m, 2H), 7.64 (s, 1H), 8.25 (s, 1H); ^{13}C-NMR (100 MHz; $CDCl_3$): δ 20.44, 20.91, 35.00, 63.29, 68.32, 120.05, 126.47, 126.69, 128.85, 130.58, 133.33, 138.42, 141.86, 149.11, 152.22, 155.77; ESI-MS *m/z*: 330.0 [M^+ + 1]; HPLC purity: 97%.

4.1.22.4. *(2R, 3S)-3-(6-aminopurin-9-yl)-4-(m-tolylsulfanyl)butan-2-ol* (***23d***). Yield: 87%; ^{1}H NMR (400 MHz; $CDCl_3$): δ 1.04 (d, *J* = 6.3 Hz, 3H), 2.24 (s, 3H), 3.47 (dd, *J* = 3.7 Hz, 14.4 Hz, 1H), 3.65 (dd, *J* = 10.1 Hz, 14.6 Hz, 1H), 4.20-4.38 (m, 2H), 5.72 (br s, 1H), 5.97 (br s, 2H), 6.93 (d, *J* = 7.2 Hz, 1H), 6.96-7.01 (m, 2H), 7.07 (t, *J* = 7.6 Hz, 1H), 7.67 (s, 1H), 8.23 (s, 1H); ^{13}C-NMR (100 MHz; $CDCl_3$): δ 20.91, 21.29, 36.08, 63.87, 68.29, 119.96, 127.12, 127.77, 128.84, 130.57, 133.73, 138.97, 141.99, 149.07, 152.09, 155.75; ESI-MS *m/z*: 330.0 [M^+ + 1]; HPLC purity: 99%.

4.1.22.5. *(2R, 3S)-3-(6-aminopurin-9-yl)-4-[3-(trifluoromethyl)phenyl] sulfanyl-butan-2-ol* (***23e***). Yield: 93%; ^{1}H NMR (400 MHz; CD_3OD): δ 1.03 (d, *J* = 6.4 Hz, 3H), 3.61 (dd, *J* = 4.4 Hz, 14.7 Hz, 1H), 3.81 (dd, *J* = 10.3 Hz, 14.5 Hz, 1H), 4.28-4.36 (m, 1H), 4.54-4.61 (m, 1H), 7.31-7.38 (m, 1H), 7.39-7.43 (m, 2H), 7.44-7.49 (m, 1H), 8.10 (s, 1H), 8.12 (s, 1H); ^{13}C-NMR (100 MHz; CD_3OD): δ 20.71, 36.82, 62.74, 68.42, 119.46, 124.42 (q, $^{3}J_{FC}$ = 3.9 Hz), 124.59(q, $^{3}J_{FC}$ = 3.9 Hz), 125.16 (q, $^{1}J_{FC}$ = 271.7 Hz), 130.58, 132.07 (q, $^{2}J_{FC}$ = 32.3 Hz), 134.71, 137.53, 142.49, 151.23, 153.35, 157.08; ESI-MS *m/z*: 384.1 [M^+ + 1]; HPLC purity: 99%.

4.1.23. General method for synthesis of ***24a−e****.*

Procedure for Mitsunobu reaction with 4-nitro benzoic acid: A 25 mL two neck RBF was equipped with a stirring bar, nitrogen inlet, and septum. To this flask secondary alcohol (0.29 mmol), 4-nitrobenzoic acid (1.19 mmol), triphenylphosphine (1.19 mmol) was added followed by 10 mL of THF. This reaction mixture was cooled to 0 °C in ice bath, and diethyl

azodicarboxylate (1.19 mmol) was added drop wise at a rate such that the temperature of reaction mixture was maintained below 10 °C. Upon completion of the addition the reaction mixture was warmed to room temperature and stirred 14-18 h at room temperature. After completion of reaction, aq. saturated solution of $NaHCO_3$ (10 mL) was added to reaction mixture and extracted with EtOAc (15 mL x 2). The combined organic layer was washed with water, brine, dried over anhydrous sodium sulfate and concentrated under reduced pressure to get crude product. The crude product was purified by column chromatography.

Procedure for hydrolysis of ester: To a solution of ester (0.145 mmol) in THF: H_2O (4:1, 10 mL) was added sodium hydroxide (0.29 mmol). The reaction mixture was stirred for 3-6 h at room temperature. After completion of reaction the reaction mixture was concentrated under reduced pressure. The residue obtained was dissolved in EtOAc (50 mL), washed with water, washed with $NaHCO_3$, with brine and dried over anhydrous sodium sulfate. The solvent was removed to obtain pure product.

4.1.23.1. *(2S, 3S)-3-(6-aminopurin-9-yl)-4-butylsulfanyl-butan-2-ol* ***(24a)***. Yield: 83%; ^{1}H NMR (400 MHz; CDCl3): δ 0.82 (t, *J* = 7.5 Hz, 3H), 1.21 (m, 2H), 1.36 (d, *J* = 6.7 Hz, 3H), 1.37-1.46 (m, 2H), 2.20-2.31 (m, 2H), 3.14 (d, *J* = 6.6 Hz, 2H), 4.25 (td, *J* = 1.8 Hz, 7.5 Hz, 1H), 4.41 (ddd, *J* = 1.8 Hz, 6.6 Hz, 8.4 Hz, 1H), 6.15 (br s, 3H), 7.84 (s, 1H), 8.29 (s, 1H); ^{13}C-NMR (100 MHz; $CDCl_3$): δ 13.61, 20.79, 21.89, 30.94, 31.63, 32.43, 64.81, 69.09, 120.32, 141.66, 149.39, 152.31, 155.99; ESI-MS *m/z*: 296.3 [M^+ + 1]; HPLC purity: 99%.

4.1.23.2. ***(****2S, 3S)-3-(6-aminopurin-9-yl)-4-phenylsulfanyl-butan-2-ol* (***24b***). Yield: 85%; ^{1}H NMR (400 MHz; CD_3OD): δ 1.06 (d, *J* = 6.2 Hz, 3H), 3.68-3.78 (m, 2H), 4.29 (quint., 1H), 4.36-4.45 (m, 1H), 7.05-7.20 (m, 5H), 8.05 (s, 1H), 8.10 (s, 1H); ^{13}C-NMR (100 MHz, CD_3OD): δ 19.29, 33.83, 63.29, 68.08, 118.95, 126.59, 128.56 (2C), 130.29 (2C), 134.34, 141.27, 149.52, 152.04, 155.90; ESI-MS *m/z*: 316.2 [M^+ + 1]. HPLC purity: 98%.

4.1.23.3. *(2S, 3S)-3-(6-aminopurin-9-yl)-4-(o-tolylsulfanyl)butan-2-ol* (***24c***). Yield: 85%; ^{1}H NMR (400 MHz; $CDCl_3$): δ 1.35 (d, *J* = 6.4 Hz, 3H), 2.27 (s, 3H), 3.56 (d, *J* = 6.4 Hz, 2H), 4.20-4.25 (m, 1H), 4.35-4.44 (m, 1H), 5.94 (br s, 2H), 6.18 (br s, 1H), 7.01-7.07 (m, 2H), 7.10-7.16 (m, 2H), 7.59 (s, 1H), 8.25 (s, 1H); ^{13}C-NMR (100 MHz, $CDCl_3$) : δ 20.42, 20.69, 31.65, 64.02, 69.17, 120.36, 126.41, 126.61, 128.67, 130.58, 133.28, 138.34, 141.59, 149.09, 152.10, 155.77; ESI-MS *m/z*: 329.9 [M^+ + 1]; HPLC purity: 96%.

4.1.23.4. *(2S, 3S)-3-(6-aminopurin-9-yl)-4-(m-tolylsulfanyl)butan-2-ol* (***24d***). Yield: 89%; ^{1}H NMR (400 MHz; $CDCl_3$): δ 1.33 (d, *J* = 6.4 Hz, 3H), 2.23 (s, 3H), 3.48-3.60 (m, 2H), 4.15-4.21 (m, 1H), 4.35-4.42 (m, 1H), 5.90 (br s, 2H), 6.24 (br s, 1H), 6.90-7.01 (m, 3H), 7.06-7.10 (m, 1H), 7.63 (s, 1H), 8.23 (s, 1H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 20.67, 21.28,

32.55, 64.57, 69.07, 120.10, 127.00, 127.69, 128.83, 130.38, 133.67, 138.95, 141.77, 149.0, 151.98, 155.73; ESI-MS *m/z*: 330.0 [M^+ + 1]; HPLC purity: 99%.

4.1.23.5. (2S, 3S)-3-(6-aminopurin-9-yl)-4-[3-(trifluoromethyl)phenyl] sulfanyl-butan-2-ol (***24e***). Yield: 91%; ^{1}H NMR (400 MHz; CD_3OD): δ 1.06 (d, *J* = 6.4 Hz, 3H), 3.79 (dd, *J* = 3.97 Hz, 14.99 Hz, 1H), 3.85 (dd, *J* = 10.36 Hz, 14.99 Hz, 1H), 4.31 (quint., 1H), 4.38-4.45 (m, 1H), 7.26-7.38 (m, 3H), 7.41 (d, *J* = 7.71 Hz, 1H), 8.07 (s, 1H), 8.08 (s, 1H); ESI-MS *m/z*: 384.0 [M^+ + 1]; HPLC purity: 95%.

4.1.24. *General method for synthesis of* ***25a*** *and* ***25b***

To a stirred solution of **5b** or **5c** (1.38 mmol) in CH_2Cl_2 (15 mL) was added MsCl (2.76 mmol). To this solution diisopropylethyl amine (2.76 mmol) was added dropwise at 0 °C. After 1 h, the reaction mixture was partitioned between CH_2Cl_2 and water. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated under reduced presure to give the methanesulfonate as an oil. This material was used for the next reaction without further purification.

To a solution of 4-imidazolecarboxamide (1.94 mmol) in DMF (3 mL) was added NaH (60% in mineral oil, 1.94 mmol) at room temperature and the reaction mixture was stirred for 20 min. The methanesulfonate (1.29 mmol) prepared above was added and the resulting mixture was stirred at 85 °C for 3 days. The reaction mixture was cooled, and the insoluble material was filtered and washed thoroughly with CH_2Cl_2. The filtrate and washings were combined and washed with brine. The organic layer was dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified by silica gel column chromatography.

4.1.24.1. 1-[(1R, 2S)-2-[tert-butyl(dimethyl)silyl]oxy-1-[2-[3-(trifluoro methyl)phenyl]ethyl] propyl]imidazole-4-carboxamide (***25a***). Yield: 41%; ^{1}H NMR (400 MHz, $CDCl_3$): δ −0.01 (s, 3H), 0.02 (s, 3H), 0.88 (s, 9H), 0.99 (d, *J* = 6.0 Hz, 3H), 2.06-2.19 (m, 1H), 2.28-2.40 (m, 1H), 2.41-2.52 (m, 1H), 2.58-2.69 (m, 1H), 2.72-2.80 (m, 1H), 3.90 (quint., 1H), 5.60 (br s, 1H), 7.01 (br s, 1H), 7.24 (d, *J* = 7.2 Hz, 1H), 7.35 (s, 1H), 7.39 (s, 1H), 7.41 (t, *J* = 8.0 Hz, 1H), 7.48 (d, *J* = 8.0 Hz, 1H), 7.63 (d, *J* = 0.8 Hz, 1H); ESI-MS *m/*: 456.2 [M^+ + 1]; HPLC purity: 87%.

4.1.24.2. 1-[(1R, 2S)-2-[tert-butyl(dimethyl)silyl]oxy-1-[2-[4-(trifluoromethyl)phenyl]ethyl] propyl]imidazole-4-carboxamide (***25b***). Yield: 36%; ^{1}H NMR (400 MHz, CD_3OD): δ −0.03 (s, 3H), 0.04 (s, 3H), 0.88 (s, 9H), 1.04 (d, *J* = 5.9 Hz, 3H), 2.20-2.40 (m, 2H), 2.50-2.67 (m, 2H), 3.96-4.05 (m, 2H), 7.31 (d, *J* = 8.1 Hz, 2H), 7.56 (d, *J* = 8.0 Hz, 2H), 7.71 (d, *J* = 1.2 Hz, 1H), 7.74 (d, *J* = 1 Hz, 1H); ESI-MS *m/z*: 456.3 [M^+ + 1].

4.1.25. General method for TBDMS deprotection using TBAF to get ***26a*** *and* ***26b***

To an ice cooled solution of intermediate **25a** or **25b** (0.438 mmol) in THF (10 mL) was added dropwise 1.0 M TBAF in THF (0.57 mmol). This reaction mixture was stirred at room temperature for 2-4 h. After completion of reaction 25% aq. $AcONH_4$ was added and the resulting mixture was stirred for 30 min. the reaction mixture was extracted with EtOAc (20 mL x 3). The combined organic layer was washed with brine (20 mL), dried over Na_2SO_4 and concentrated in *vacuo*. The residue was purified by silica gel column chromatography.

4.1.26.1. 1-[(1R, 2S)-2-hydroxy-1-[2-[3-(trifluoromethyl)phenyl]ethyl] propyl]imidazole-4-carboxamide (***26a***). Yield: 62%; ^{1}H NMR (400 MHz, CD_3OD): δ 1.04 (d, *J* = 5.9 Hz, 3H), 2.16-2.29 (m, 1H), 2.32-2.42 (m, 1H), 2.49-2.64 (m, 2H), 3.92 (quint., 1H), 3.97-4.04 (m, 1H), 7.37-7.50 (m, 4H), 7.72 (s, 1H), 7.79 (s, 1H); ^{13}C-NMR (100 MHz; CD_3OD): δ 19.56, 32.28, 32.96, 65.33, 70.47, 123.29, 124.02 (q, $^3J_{FC}$ = 3.1 Hz), 125.70 (q, $^1J_{FC}$ = 270.2 Hz), 125.98 (q, $^3J_{FC}$ = 3.9 Hz), 130.33, 131.80 (q, $^2J_{FC}$ = 31.7 Hz), 133.28, 137.12, 139.27, 143.47, 167.37; ESI-MS *m/z*: 342.3 [M^+ + 1]; HPLC purity: 96%.

4.1.25.2. 1-[(1R, 2S)-2-hydroxy-1-[2-[4-(trifluoromethyl) phenyl]ethyl]propyl] imidazole-4-carboxamide (***26b***). Yield: 56%; ^{1}H NMR (400 MHz, CD_3OD): δ 1.04 (d, *J* = 6.4 Hz, 3H), 2.16-2.29 (m, 1H), 2.32-2.44 (m, 1H), 2.56 (t, *J* = 7.4 Hz, 2H), 3.92 (quint., 1H), 3.96-4.05 (m, 1H), 7.32 (d, *J* = 8.1 Hz, 2H), 7.55 (d, *J* = 8.1 Hz, 2H), 7.73 (s, 1H), 7.78 (s, 1H); ^{13}C-NMR (100 MHz; CD_3OD): δ 19.56, 32.12, 32.94, 65.25, 70.46, 123.34, 125.81(q, $^1J_{FC}$ = 269.4 Hz), 126.40 (q, $^3J_{FC}$ = 3.9 Hz, 2C), 129.51 (q, $^2J_{FC}$ = 31.8 Hz), 130.08 (2C), 137.05, 139.29, 146.75,167.36; ESI-MS *m/z*: 342.1 [M^+ + 1]; HPLC purity: 99%.

4.1.26. Synthesis of intermediate ***25c-f***

4.1.26.1. tert-butyl N-tert-butoxycarbonyl-N-[1-[(1R,2S)-2-[tert-butyl (dimethyl)silyl]oxy-1-[2-[3-(trifluoromethyl)phenyl]ethyl]propyl]pyrazolo[3,4-d]pyrimidin-4-yl] carbamate (***25c***). Synthesis and purification was achieved according to the general method desribed *for Mitsunobu reaction to get 6a-f* by using of intermediate **5a** and 4-Diboc aminopyrazolo pyrimidine. Yield:78%; ^{1}H NMR (400 MHz; $CDCl_3$): δ 0.05 (s, 3H), 0.07 (s, 3H), 0.83 (d, *J* = 6.2 Hz, 3H), 0.87 (s, 9H), 1.56 (s, 18H), 2.29-2.57 (m, 4H), 4.23 (quint., 1H), 4.68-4.76 (m, 1H), 7.20 (d, *J* = 7.6 Hz, 1H), 7.25 (s, 1H), 7.31 (t, *J* = 7.6 Hz, 1H), 7.40 (d, *J* = 8.1 Hz, 1H), 8.21 (s, 1H), 8.69 (s, 1H); ESI-MS *m/z*: 680.2 [M^+ + 1]; HPLC purity: 98%.

4.1.26.2. tert-butyl N-tert-butoxycarbonyl-N-[1-[(1R,2S)-2-[tert-butyl(dimethyl)silyl]oxy-1-[2-[4-(trifluoromethyl)phenyl]ethyl]propyl]pyrazolo[3,4-d]pyrimidin-4-yl] carbamate (***25d***). Synthesis and purification was achieved according to the general method desribed *for Mitsunobu reaction to get 6a-f* by using of intermediate **5b** and

4-Diboc aminopyrazolo pyrimidine. Yield: 74%; ^{1}H NMR (400 MHz; $CDCl_3$): δ 0.05 (s, 3H), 0.06 (s, 3H), 0.84 (d, *J* = 6.1 Hz, 3H), 0.87 (s, 9H), 1.57 (s, 18H), 2.26-2.56 (m, 4H), 4.24 (quint., 1H), 4.70-4.79 (m, 1H), 7.11 (d, *J* = 8.1 Hz, 2H), 7.45 (d, *J* = 8.0 Hz, 2H), 8.19 (s, 1H), 8.70 (s, 1H); ESI-MS *m/z*: 680.1 [M^+ + 1]; HPLC purity: 85%.

4.1.26.3. tert-butyl-[(1S, 2R)-2-(4-chloropyrrolo[2,3-d]pyrimidin-7-yl)-1-methyl-4-[3-(trifluoromethyl)phenyl]butoxy]-dimethyl-silane (***25e***). Synthesis and purification was achieved according to the general method desribed *for Mitsunobu reaction to get* ***6a-f*** by using of intermediate **5a** and 4-chloro pyrrolo pyrimidine. Yield: 52%; ^{1}H NMR (400 MHz; $CDCl_3$): δ −0.09 (s, 3H), −0.01 (s, 3H), 0.88 (s, 9H), 1.04 (d, *J* = 6.2 Hz, 3H), 2.38-2.49 (m, 4H), 4.08 (quint., 1H), 4.57-4.65 (m, 1H), 6.63 (d, *J* = 3.5 Hz, 1H), 7.16 (d, *J* = 7.5 Hz, 1H), 7.22 (s, 1H), 7.27-7.35 (m, 3H), 8.61 (s, 1H); ESI-MS *m/z*: 498.3 [M^+ (^{35}Cl) + 1].

4.1.26.4. tert-butyl-[(1S,2R)-2-(4-chloropyrrolo[2,3-d]pyrimidin-7 -yl)-1-methyl-4-[4-(trifluoromethyl)phenyl]butoxy]-dimethyl-silane. (***25f***). Synthesis and purification was achieved according to the general method desribed *for Mitsunobu reaction to get 6a-f* by using of intermediate **5b** and 4-chloro pyrrolo pyrimidine. Yield: 58%; ^{1}H NMR (400 MHz; $CDCl_3$): δ −0.10 (s, 3H), −0.01 (s, 3H), 0.88 (s, 9H), 1.04 (d, *J* = 6.6 Hz, 3H), 2.30-2.48 (m, 4H), 4.09 (quint., 1H), 4.58-4.67 (m, 1H), 6.62 (d, *J* = 3.5 Hz, 1H), 7.09 (d, *J* = 8.0 Hz, 2H), 7.32 (d, *J* = 3.5 Hz, 1H), 7.53 (d, *J* = 8.0 Hz, 2H), 8.61 (s, 1H); ESI-MS *m/z*: 498.2 [M^+ (^{35}Cl) + 1].

4.1.27. Synthesis of intermediate ***26c*** *and* ***26d***

4.1.27.1. (2S, 3R)-3-(4-aminopyrazolo[3,4-d]pyrimidin-1-yl)-5-[3-(trifluoromethyl)phenyl] pentan-2-ol (***26c***). *Diboc and TBDMS deprotection to get* was achieved according to the general method desribed *for synthesis of* **7a-f** by employing intermediate ***25c***. Yield: 92%; ^{1}H NMR (400 MHz; CD_3OD): δ 0.87 (d, *J* = 6.3 Hz, 3H), 2.35-2.48 (m, 1H), 2.49-2.60 (m, 3H), 4.11 (quint., 1H), 4.52 (app.q, *J* = 7.8 Hz, 1H), 7.25 (s, 1H), 7.27-7.40 (m, 3H), 8.09 (s, 1H), 8.16 (s, 1H); ^{13}C-NMR (100 MHz; CD_3OD): δ 20.29, 32.71, 33.36, 64.04, 70.75, 101.61, 123.62 (q, $^3J_{FC}$ = 3.1 Hz), 125.69 (q, $^1J_{FC}$ = 269.4 Hz), 126.00 (q, $^3J_{FC}$ = 3.8 Hz), 129.88, 131.38 (q, $^2J_{FC}$ = 31.7 Hz), 133.19, 133.77, 143.62, 155.11, 156.67, 159.8;. ESI-MS *m/z*: 366.1 [M^+ + 1]. HPLC purity: 89%.

4.1.27.2. (2S,3R)-3-(4-aminopyrazolo [3,4-d]pyrimidin-1-yl)-5-[4-(trifluoromethy)phenyl] pentan-2-ol (***26d***). *Diboc and TBDMS deprotection to get* was achieved according to the general method desribed *for synthesis of* 7a-f by employing intermediate ***25d***. Yield: 90%; ^{1}H NMR (400 MHz; $CDCl_3$): δ 1.22 (d, *J* = 6.6 Hz, 3H), 2.21-2.32 (m, 1H), 2.34-2.45 (m, 1H), 2.50-2.66 (m, 2H), 4.18-4.27 (m, 1H), 4.70 (dt, *J* = 3.1 Hz,

10.7Hz, 1H), 5.85 (br. s, 2H), 7.16 (d, *J* = 8.0 Hz, 2H), 7.43 (d, *J* = 8.1 Hz, 2H), 7.94 (s, 1H), 8.36 (s, 1H); ^{13}C-NMR (100 MHz; $CDCl_3$): δ 19.72, 29.00, 32.19, 63.61, 70.16, 100.60, 124.26 (q, $^1J_{FC}$ = 271.7 Hz), 125.06 (q, $^3J_{FC}$ = 3.9 Hz, 2C), 128.21 (q, $^2J_{FC}$ = 31.9 Hz), 128.62 (2C), 130.79, 145.05, 153.49, 155.16, 157.26; ESI-MS *m/z*: 366.1 [M^+ + 1]; HPLC purity: 92%.

*4.1.28. General synthesis of **26e** and **26f***

To a stirred solution of **25e** or **25f** (0.60 mmol) in DMSO (4 mL) was added sodium azide (1.20 mmol) followed by triphenylphosphine (1.20 mmol) and the reaction mixture was stirred at 120 °C. Reaction progress was monitored by TLC. After 10-12h, 1N HCl (4 mL) was added to the reaction mixture and stirring was continued at 120 °C for an additional 2-3h. The reaction mixture was cooled to room temperature and further diluted with 1N HCl (2 mL). The resulting reaction mixture was poured into distilled water (10 mL) and the aqueous layer was extracted with EtOAc (10 mL x 2) to remove triphenylphosphine oxide (TPPO) and the majority of DMSO. The aqueous layer was slowly neutralized with saturated aqueous $NaHCO_3$ and thus obtained aqueous layer was extracted with EtOAc (10 mL x 3). The combined organic layer was washed with water, brine, dried over sodium sulfate and concentrated under reduced pressure to give crude product. The crude product was purified by column chromatography.

4.1.28.1. (2S, 3R)-3-(4-aminopyrrolo[2,3-d]pyrimidin-7-yl)-5-[3-(trifluoromethyl)phenyl] pentan-2-ol (***26e***). Yield: 45%; ^{1}H NMR (400 MHz; $CDCl_3$): δ 1.20 (d, *J* = 6.3 Hz, 3H), 2.24-2.38 (m, 1H), 2.40-2.57 (m, 3H), 4.18-4.32 (m, 2H), 5.44 (br s, 2H), 6.39 (d, *J* = 3.4 Hz, 1H), 6.95 (d, *J* = 3.7 Hz, 1H), 7.24 (d, *J* = 7.8 Hz, 1H), 7.28 (s, 1H), 7.33 (t, *J* = 7.6 Hz, 1H), 7.41 (d, *J* = 7.5 Hz, 1H), 8.25 (s, 1H); ^{13}C-NMR (100 MHz; CD_3OD): δ 20.54, 32.50, 33.19, 61.45, 71.18, 100.57, 104.24, 123.67, 123.74, 125.69 (q, $^1J_{FC}$ = 270.2 Hz), 126.03 (q, $^3J_{FC}$ = 3.1 Hz), 130.0, 131.48 (q, $^2J_{FC}$ = 31.8 Hz), 133.19, 143.76, 151.09, 151.86, 158.92; ESI-MS *m/z*: 365.2 [M^+ + 1]; HPLC purity: 94%.

4.1.28.2. (2S, 3R)-3-(4-aminopyrrolo[2,3-d]pyrimidin-7-yl)-5-[4-(trifluoromethyl)phenyl] pentan-2-ol (***26f***). Yield: 53%; ^{1}H NMR (400 MHz; $CDCl_3$): δ 1.22 (d, *J* = 6.3 Hz, 3H), 2.22-2.35 (m, 1H), 2.41-2.58 (m, 3H), 4.23 (d, *J* = 7.6 Hz, 2H), 5.41 (br s, 2H), 6.36 (d, *J* = 3.4 Hz, 1H), 6.93 (d, *J* = 3.7 Hz, 1H), 7.16 (d, *J* = 7.8 Hz, 2H), 7.48 (d, *J* = 8.1 Hz, 2H), 8.26 (s, 1H); ESI-MS *m/z*: 365.2 [M^+ + 1]; HPLC purity: 96%.

4.1.29. tert-butyl N-tert-butoxycarbonyl-N-[9-[(1R, 2S)-2-hydroxy-1-[2-[4-(trifluoromethyl) phenyl]ethyl]propyl]purin-6-yl]carbamate (***27***).

TBDMS deprotection using TBAF was achieved according to the general method described for synthesis of **26a** and **26b** by employing intermediate **6b**. Yield: 82%; ^{1}H NMR (400 MHz; $CDCl_3$): δ 1.25 (d, *J* = 6.3 Hz, 3H), 1.48 (s, 18H), 2.32-2.64 (m, 4H), 3.46 (br s, 1H), 4.20-4.29 (m, 1H), 4.41 (dt, *J* = 2.9 Hz, 11.2 Hz, 1H), 7.16 (d, *J* = 8.3 Hz, 2H), 7.52 (d, *J* = 7.9 Hz, 2H), 8.13 (s, 1H), 8.84 (s, 1H); ESI-MS *m/z*: 566.3 [M^+ + 1]; HPLC purity: 96%.

4.1.30. tert-butyl N-tert-butoxycarbonyl-N-[9-[(1R)-2-oxo-1-[2-[4-(trifluoromethyl) phenyl]ethyl]propyl]purin-6-yl]carbamate (***28***).

To a solution of intermediate **27** (0.1 g, 0.176 mmol) in DCM (4 mL) was added portionwise pyridinium chlorochromate (PCC) (0.076 g, 0.35 mmol) at RT. Reaction mixture slowly turns dark brown in color. This reaction mixture was stirred for 5 h at RT and monitored by TLC. After 5 h, solvent was evapourated under reduced pressure to afford crude product. The crude product was purified by preparative TLC plate using 50% EtOAc–hexanes as mobile phase to afford pure **28** (83 mg, 84% yield); ^{1}H NMR (400 MHz; $CDCl_3$): δ 1.48 (s, 18H), 2.23 (s, 3H), 2.40-2.70 (m, 4H), 5.37 (dd, *J* = 8.3 Hz, 3.9 Hz, 1H), 7.19 (d, *J* = 7.8 Hz, 2H), 7.55 (d, *J* = 8.3 Hz, 2H), 8.19 (s, 1H), 8.85 (s, 1H); ^{13}C-NMR (100 MHz; $CDCl_3$): δ 27.43, 27.75 (6C), 31.77, 31.96, 62.06, 83.89 (2C), 124.04 (q, $^1J_{FC}$ = 270.2 Hz), 125.72 (q, $^3J_{FC}$ = 3.8 Hz, 2C), 128.37, 128.65 (2C), 129.18 (q, $^2J_{FC}$ = 32.5 Hz), 143.14, 143.51, 150.38, 150.66, 152.22, 153.32 (2C), 202.02; ESI-MS *m/z*: 564.2 [M^+ + 1]; HPLC purity: 99%.

4.1.31. tert-butyl N-[9-[(1R)-2-hydroxy-2-methyl-1-[2-[4-(trifluoromethyl)phenyl]ethyl] propyl]purin-6-yl]carbamate (***30***).

To a solution of intermediate **28** (0.08 g, 0.142 mmol) in THF (5 mL) was added methyl magnesium bromide (0.14 mL, 0.42 mmol, 3M in diethyl ether) dropwise at 0 °C. The reaction mixture was stirred for 2 h at RT and progress of the reaction was monitored by TLC. After completion the reaction was quenched using aq. sat. NH_4Cl solution, and aqueous layer was extracted with EtOAc (10 mL x 2). The combined organic layer was washed with water, brine, dried over sodium sulfate and concentrated under reduced pressure to give crude product. The crude product was purified by preparative TLC Plate using 50% EtOAc–Hexanes as a mobile phase to afford pure **30** (57 mg, 70% yield); ^{1}H NMR (400 MHz; $CDCl_3$): δ 0.99 (s, 3H), 1.39 (s, 3H), 1.58 (s, 9H), 2.35-2.45 (m, 3H), 2.57-2.68 (m, 1H), 3.53 (br. s, 1H), 4.32 (d, *J* = 11.2 Hz, 1H), 7.11 (d, *J* = 8.3 Hz, 2H), 7.47 (d, *J* = 8.3 Hz, 2H), 7.98 (s, 1H), 8.02 (s, 1H), 8.72 (s, 1H); ^{13}C-NMR (100 MHz; $CDCl_3$): δ 27.65, 28.03, 28.09 (3C), 29.49, 30.89, 32.25, 72.37, 82.31, 124.07 (q, $^1J_{FC}$ = 268.5 Hz), 125.39 (q, $^3J_{FC}$ = 3.1 Hz, 2C),

128.47 (2C), 128.70, (q, $^{2}J_{FC}$ = 33.1 Hz), 142.63, 144.03, 149.70, 150.13, 151.31, 152.49, 206.98; ESI-MS *m/z*: 480.1 [M^{+} + 1]; HPLC purity: 98%.

4.1.32. (3R)-3-(6-aminopurin-9-yl)-5-[4-(trifluoromethyl)phenyl] pentan-2-one (***29***).

Diboc and TBDMS deprotection was achieved according to the general method desribed *for synthesis of* 7a-f by employing intermediate **28**. Yield: 88%; ^{1}H NMR (400 MHz; $(CD_3)_2SO$): δ 2.12 (s, 3H), 2.41-2.67 (m, 4H), 5.25-5.31 (m, 1H), 7.27 (br s, 2H), 7.35 (d, *J* = 7.8 Hz, 2H), 7.58 (d, *J* = 7.8 Hz, 2H), 8.09 (s, 1H), 8.21 (s, 1H); ^{13}C-NMR (100 MHz; $(CD_3)_2SO$): δ 26.79, 29.86, 31.52, 62.69, 118.62, 124.37 (q, $^{1}J_{FC}$ = 271.0 Hz), 125.09 (q, $^{3}J_{FC}$ = 3.9 Hz, 2C), 126.86 (q, $^{2}J_{FC}$ = 31.0 Hz), 129.15 (2C), 140.48, 145.28, 149.66, 152.45, 155.99, 203.81; ESI-MS *m/z*: 364.2 [M^{+} + 1]; HPLC purity: 98%.

5.1.33. (3R)-3-(6-aminopurin-9-yl)-2-methyl-5-[4-(trifluoromethyl)phenyl]pentan-2-ol (***31***).

Diboc and TBDMS deprotection was achieved according to the general method desribed *for synthesis of* 7a-f by employing intermediate **30**. Yield: 93%; ^{1}H NMR (400 MHz; CD_3OD): δ 0.95 (s, 3H), 1.32 (s, 3H), 2.39-2.62 (m, 4H), 4.44 (d, *J* = 11.2 Hz, 1H), 7.19 (d, *J* = 7.8 Hz, 2H), 7.44 (d, *J* = 8.3 Hz, 2H), 8.18 (s, 1H), 8.23 (s, 1H); ^{13}C-NMR (100 MHz; CD_3OD): δ 27.49, 27.54, 31.07, 33.49, 64.57, 72.99, 119.48, 125.79 (q, $^{1}J_{FC}$ = 270.2 Hz), 126.12 (q, $^{3}J_{FC}$ = 3.1 Hz, 2C), 129.37 (q, $^{2}J_{FC}$ = 32.5 Hz), 129.98 (2C), 142.04, 146.54, 151.56, 153.50, 157.30; ESI-MS *m/z*: 380.3 [$^{+}$ + 1]; HPLC purity: 96%.

4.2. Adenosine deaminase enzyme assay [29]

To different wells of a 96-well UV plate 80 µl of Enzyme solution (0.01 U/ml ADA in 50 mM potassium phosphate, pH 7.0) is added. Enzyme solution is incubated for 5 minutes with different concentrations of 10 µL of 10x final concentrations of test compounds to permit steady-state association of the compounds with ADA. The deamination reaction is initiated by the addition of 10 µl of 0.5 mM adenosine (in 50 mM potassium phosphate, pH 7.0), such that the final reaction mixture contains 50 µM adenosine. The reaction mixture is shaken at moderate speed for 5 seconds, after which the progress of the enzymatic reaction is monitored in a Tecan Safire plate reader by continuously measuring the loss of absorbance at 265 nm. Reaction rates are calculated from the slope of the line obtained at different inhibitor concentrations, plotted against the inhibitor concentration, and fit to the equation for a sigmoidal dose response to obtain the IC50 and Ki values. The enzymatic rate in the case of vehicle control is normalized to 100%. Data points represent the mean ± SEM of duplicate values.

4.3. Docking Studies

Enzyme and Ligand structure preparation: The Protein X-ray crystal structure of Adenosine Deaminase complex with an inhibitor EHNA is obtained from the RCSB Protein Data Bank (http://www.rcsb.org/pdb/home/home .do) in .pdb format (PDB ID: 2Z7G; Organism: Bos Taurus; Resolution 2.52 Å) for the purpose of molecular docking [21]. Among the several other crystal structures of ADA available in the Protein Data Bank (PDB), this structure was particularly selected due to its high resolution and structural similarity of co-crystallized ligand with the compounds to be docked. ADA enzyme structure was prepared by using the Gold Setup Wizard tool in GOLD Suite 5.2 (the Cambridge Crystallographic Data Center, UK) using the default parameters and standard protocol. The hydrogen atoms were added and ligand was extracted from protein structure. The 3D structures of the inhibitors were prepared and optimized by energy minimization using Molecular Operating Environment (MOE).

GOLD docking: The binding site was defined from the reference structure as bound ligand EHNA. The approximate radius is set to 10.0 Å. The Ligand Site is used to restrict the region of interest.The number of docking runs was set to 100 for each inhibitor. Default Genetic Algorithm (GA) settings were used for all calculations and a set of 10 solutions were saved for each ligand. Energy minimization for the protein in complex with the inhibitor was then conducted to remove energetically unfavorable contacts. The interaction energies between the enzyme and inhibitors were calculated with implicit distance-dependent dielectrics using Calculate Interaction Energy protocol. The best docking poses were selected based on the Gold fitness scores and the critical interactions reported in the literatures. The images were captured using PYMOL software.

4.4. Metabolic stability assay protocol:

A stock solution of the test compound(s) was prepared at 10 mM (active compound) in DMSO. The stock solution was diluted immediately before use using the phosphate buffer solution to create the working standard. All test compounds were completely soluble by visual inspection at rt. The assay was carried out by incubating test compound or positive control (i.e., Testosterone and verapamil), with species-specific liver microsomes (0.125 mg liver microsomal protein/mL), in 100 mM phosphate buffer (pH 7.4), and NADPH (1 mM) for up to 30 minutes at 37 ^{o}C (final volume 100 µL). All incubations were performed in

singlet. The mixtures containing all components except NADPH were pre-incubated at 37 ^{o}C for 5 minutes, and the reactions were started by the addition of NADPH. The final concentration of the organic solvent in the incubations was 0.1%. The reactions were terminated at 0, 3, 6, 9, 12, 15 and 30 min time-points with the addition of 100 µL of acetonitrile containing internal standard. Following protein precipitation and centrifugation, supernatant (100 µL) was transferred to a separate 96-well plate and analyzed by LC/MS/MS. Metabolic stability of a test compound, measured as the rate of disappearance of the parent (termed as metabolic turnover rate, MR), was calculated from the peak area ratios of the test compound at each time point as nmol/min/mg microsomal protein.

4.5. Intravenous pharmacokinetic experiment

Study Design: Male Wistar rats (weighing 230 ± 15 g) were obtained from inhouse breeding facility, Advinus Therapeutics Ltd., Pune, INDIA. The rats were grouped and housed in polycarbonate cages with not more than 3 rats per cage and maintained under standard laboratory conditions (temperature 25 ± 2 _C) with dark/light cycle (12 h). Rats were maintained on T.2014C Global 14% protein rodent maintenance diets (Harlan, Teklad diet, USA) and water ad libitum. All procedures described were reviewed and approved by the Institutional Animal Ethics committee (IAEC) constituted by Committee for the Purpose of Control and Supervision on Experiments (CPCSEA) on Animals, Govt. of India.

Pharmacokinetic experiments were carried-out in male Wistar rats following Intravenous (IV) administration. The animals were fasted overnight before the start of experimentation but had free access to water. For IV dosing, tested compounds were dissolved in different vehicles and solution formulation (dose volume: 5 mL/kg and formulation strength: 0.6 mg/mL) was administered to each rat at a dose of 3 mg/kg via tail vein.

Sample collection, processing and bioanalysis: Study used serial sampling design (n = 3/time point) with blood samples collected at 0.008 (IV only), 0.25, 0.5, 1, 2, 4, 6, 8 and 24 h

post dose into labeled micro-centrifuge tubes contained K2EDTA as an anticoagulant. Tissue samples were washed with phosphate buffer saline (pH 7.4), blotted dry and weighed. All samples were stored below 70 °C until bioanalysis. Blood samples were immediately centrifuged at 6000 RPM for 5 min to separate the plasma. Finally, supernatant (100 mL) was collected from each test sample microcentrifuge tube and transferred into HPLC vials for LC/MS/MS to determine the concentrations in plasma.

Pharmacokinetic parameters were calculated using the non-compartmental analysis tool of WinNonlin professional software (version 5.2.1).

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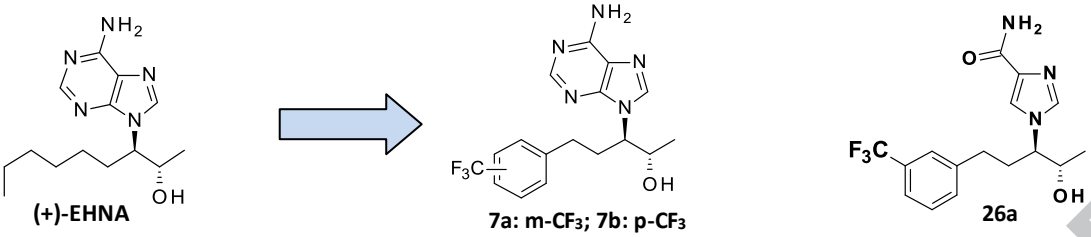
Graphical Abstract:



Compound	(+)-EHNA	7a	7b	26a
***Ki* (nM)**	2.2	1.0	5.2	5.9
RLM, MR (nmol/min/mg)	0.09	0.04	<0.04	0.04
***In vivo* Clearance (mL/min/Kg)**	Very rapid	85	59	33