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Synthesis and evaluation of pyrazolo[3,4-*b*]pyridines and its structural analogues as TNF-α and IL-6 inhibitors

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ABSTRACT

In the present article, we have synthesized three different series of pyrazolo[3,4-*b*]pyridines and their structural analogues using novel synthetic strategy involving one-pot condensation of 5,6-dihydro-4*H*-pyran-3-carbaldehyde/2-formyl-3,4,6-tri-*O*-methyl-D-glucal/chromone-3-carbaldehyde with heteroaromatic amines. All synthesized compounds were evaluated for their anti-inflammatory activity against TNF-α and IL-6. Out of 28 compounds screened, **40**, **51**, **52** and **56** exhibited promising activity against IL-6 with 60–65% inhibition at 10 μM concentration. Amongst these, **51**, **52** and **56** showed potent IL-6 inhibitory activity with IC_{50}'s of 0.2, 0.3 and 0.16 μM, respectively. Compound **56** was not cytotoxic in CCK-8 cells up to the concentration of >100 μM.

1. Introduction

Proinflammatory cytokines are involved in the pathogenesis of a variety of autoimmune and inflammatory diseases. Interleukin-6 (IL-6) and tumour necrosis factor-alpha (TNF-α) are two multifunctional proinflammatory cytokines involved in the pathogenesis of cardiovascular, neurodegenerational diseases and cancer through a series of cytokine signalling pathways.[1,2] Inhibition of release of cytokines has become a major focus of current drug discovery and development, and an important method for evaluating the bioactivity of drugs. The inhibition of cytokines, in particular TNF-α, has been successful in several clinical trials for the treatment of rheumatoid arthritis.[3]

The inflammatory process is characterized by the production of leukotrienes, histamine, bradykinin, and a variety of cytokines and chemokines by tissues and migrating cells. Amongst many cytokines responsible for regulating cellular physiology, TNF-α has been proven to play a dominant role both in normal immune function and in disturbances leading to autoimmune disease. Overexpression of TNF-α can lead to a variety of pathological conditions including rheumatoid arthritis (RA), multiple sclerosis, cachexia, sepsis, ulcerative colitis, congestive heart failure and Crohn's disease. To date, much research has been directed towards the inhibition of TNF-α production, the antagonism of TNF-α and the shedding of TNF-α from the cell surface. Thus, TNF-α has received a considerable amount of attention as a molecular target for the treatment of diseases mentioned above.[4]

Amongst proinflammatory cytokines, interleukin-6 (IL-6) is considered to contribute to the initiation and extension of the inflammatory process. Interleukin-6 is a multifunctional cytokine produced by wide range of cells, usually at sites of tissue inflammation, and it regulates hepatic acute-phase response, the immune response, inflammation and haematopoiesis. It appears to be the central mediator in a range of inflammatory diseases, including end-stage renal disease and rheumatoid arthritis.[5] Inhibition of IL-6 has not received desired attention in drug discovery.

Pyrazolo[3,4-*b*]pyridine class of compounds are reported to possess diverse range of biological activities. Misra et al. identified 4-substituted 1*H*-pyrazolo[3,4-*b*]pyridine derivatives (**1**, SQ-67563: IC_{50} 0.11 μM) as a new class of cdk2 inhibitors.[6] Lin et al. identified substituted pyrazolo[3,4-*b*]pyridine analogue **2** as a potent and selective cyclin-dependent kinase and cellular anti-proliferative inhibitor (IC_{50} 0.7 nM) against CDK1/cyclin B.[7] Witherington et al. discovered 6-heteroaryl-pyrazolo[3,4-*b*]pyridine **3** as a glycogen synthase kinase-3 (GSK-3) inhibitor (IC_{50} 0.8 nM).[8] Pyrazolo[3,4-*b*]pyridines are also reported to possess anti-microbial (**4**, *Escherichia coli*: IC_{50} 22 μM; *Candida albicans*: IC_{50} 18 μM),[9,10] anti-chagasic (**5**, *Trypanosoma cruzi*: IC_{50} 1.9 μg/mL)[11] and anti-leishmanial (**6**, *Leishmania amazonensis*: IC_{50} 0.12 μM)[12] activities. It has also been reported that pyrazolo[3,4-*b*]pyridine analogue, Y-25510 (**7**), stimulates production of IL-1α and IL-6 at the level of messenger RNA expression in cultured

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human monocytes as well as enhances in vivo production of IL-lα and IL-6 in mice.[13,14] Compound **8** showed potent p38α MAP kinase inhibitory activity (IC_{50} 0.7 nM) and potent in vivo TNF-α inhibition (97% inhibition of LPS-induced TNF-α release in mice at 20 mg/kg po.).[15]

Pyrazolo[3,4-*b*]pyridines, **9** [IC_{50} 15.7 nM (TNF-α), >1 μM (PDE-IV)] and **10** [IC_{50} 27 nM (TNF-α), >1 μM (PDE-IV)], are reported to exhibit anti-inflammatory activity by inhibition of TNF-α and PDE-IV.[16] Similarly biologically active pyrazolo[1,5-*a*]pyrimidines, **11** (non-steroidal anti-inflammatory drug)[17] and **12** (COX-2 inhibitor)[18], and isoxazolo[5,4-*b*]pyridine **13** (TACE inhibitor)[19] are also reported (Fig. 1).

There are several reports on synthesis of pyrazolo-pyridines and pyrazolo-pyrimidines using different synthetic routes. Lavecchia et al. synthesized pyrazolo[3,4-*b*]pyridines via indirect iodination of 2-chloro-nicotinonitrile to yield 2-chloro-5-iodonicotinonitrile, which was cyclized with methyl hydrazine leading to 3-amino-5-iodopyrazolo[3,4-*b*]pyridine.[20] Chebanov et al. synthesized pyrazolo[3,4-*b*]pyridines via refluxing 5-amino-3-methyl-1-phenylpyrazole with arylidenepyruvic acids.[21] Jachak et al. synthesized pyrazolo[3,4-*b*]pyridines and pyrazolo[4′,3′:5,6]pyrido[2,3-*d*]pyrimidines via Friedlander condensation of 5-amino-pyrazole-4-carbaldehyde with various active methylene compounds.[22,23] Goda et al. in 2004 devised a novel route for synthesis of pyrazolo-pyridines starting from 1,3-diaryl chalcones and ketones to get substituted pyrazolo-pyridines.[9] Zheng et al. reported one-pot conversion of 5-azidopyrazole-4-carboxaldehyde to pyrazolo[3,4-*b*]pyridines via diazo-transfer and subsequent Friedlander reaction.[24] Apart from these reports, several other researchers published synthesis of substituted 1*H*-pyrazolo[3,4-*b*] pyridines and related skeletons.[25–28]

Based on the interesting biological activity profile of 1*H*-pyrazolo[3,4-*b*]pyridines and as a part of our continuing efforts towards discovery of new class of compounds against different therapeutic areas, we have designed and synthesized different series of pyrazolo-pyridines and their structural analogues for evaluation of anti-inflammatory activity against TNF-α and IL-6.

Our dual interest in this work was to synthesize new series of hybrid structures containing heterocycle and a carbohydrate (D-glucal) moiety as recognition element, and to evaluate these against different therapeutic targets. Herein, we report synthesis of three different series of pyrazolo[3,4-*b*]pyridines and other hybrid heterocycles via novel short and efficient synthetic route.

2. Results and discussion

2.1 Chemistry

Our initial aim in this work was to devise a short and efficient synthetic strategy for synthesis of hybrid structures containing heterocycle and a carbohydrate component. Based on literature reports on construction of heterocyclic skeletons using 3-alkoxyacroleins,[29,30] we designed synthetic strategy involving condensation of 3-alkoxyacrolein analogues with heterocyclic amines to get pyrazolo[3,4-*b*]pyridines via imine intermediate as shown in Fig. 2.

Based on this strategy, we visualized 2-formyl glucal (2-formyl-3,4,6-tri-*O*-protected-D-glucal) as a key starting material, which has 3-alkoxyacrolein moiety. In order to synthesize a key starting material, we started with commercially available 3,4,6-tri-*O*-acetyl-D-glucal (**14**). For conversion of 3,4,6-tri-*O*-acetyl-D-glucal (**14**) to 3,4,6-tri-*O*-methyl-D-glucal (**15**), a number of reaction conditions were attempted: (a) NaOH/tetrabutylammonium iodide (TBAI), CH_3I, THF; (b) K_2CO_3/MeOH; CH_3I, NaH/DMF; (c) Na/MeOH; CH_3I, NaH/DMF. Desired product was obtained using all three methods but TBAI/NaOH method was found to be better in terms

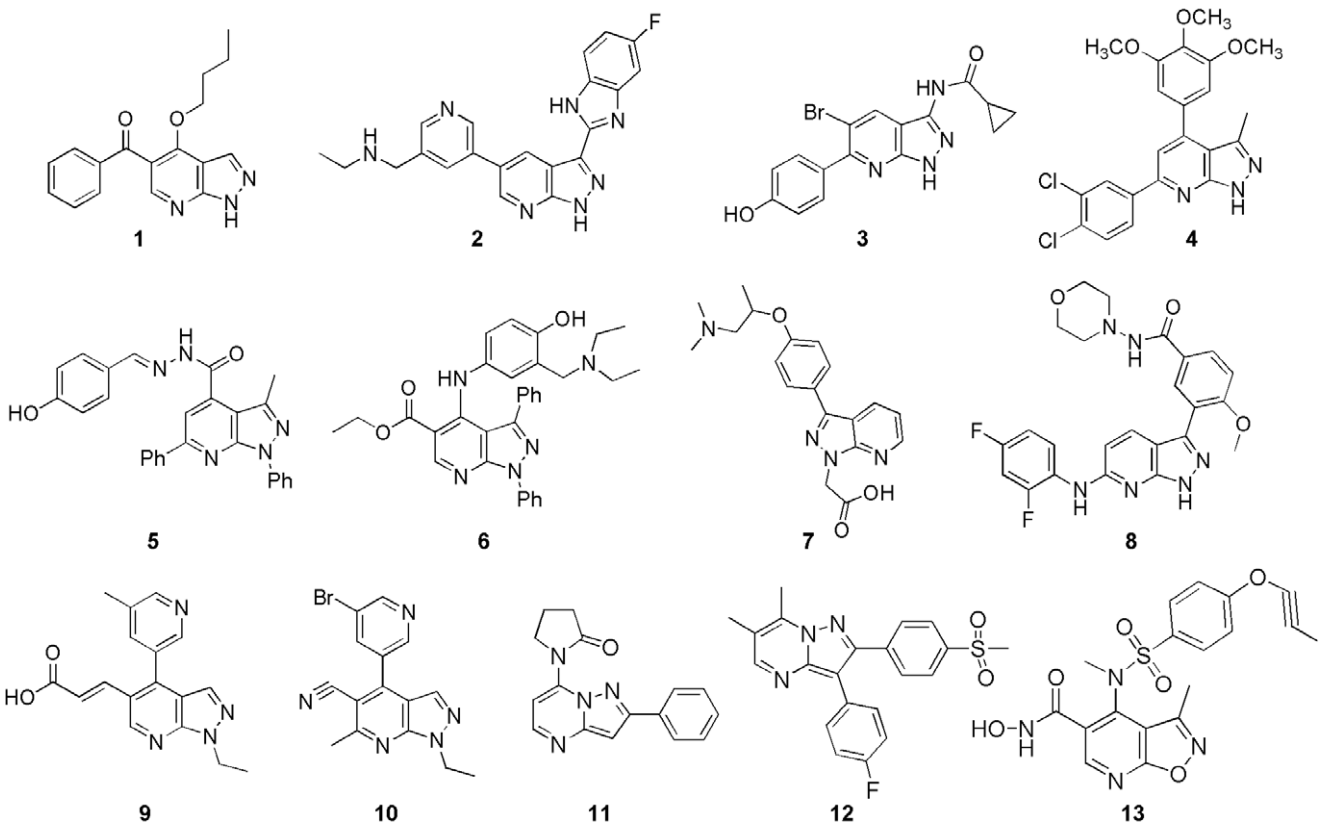


Figure 1. Some biologically active pyrazolo[3,4-*b*]pyridines, pyrazolo[1,5-*a*]pyrimidines and isoxazolo[5,4-*b*]pyridines.

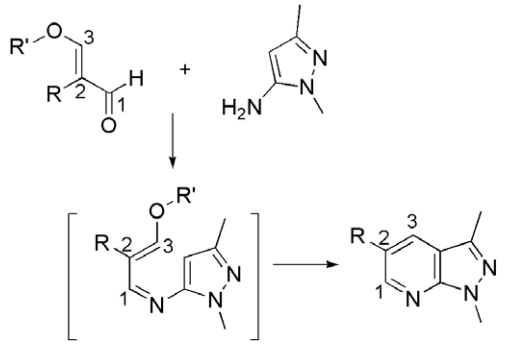


Figure 2. Synthetic approach for pyrazolo[3,4-*b*]pyridines.

of yield and also it is one-pot conversion compared with the first two methods. Treatment of 3,4,6-tri-*O*-acetyl-D-glucal (**14**) with solid NaOH and methyl iodide in THF in presence of TBAI resulted in formation of 3,4,6-tri-*O*-methyl-D-glucal (**15**) in 80% yield. Vilsmeier–Haack formylation[31] of **15** using $POCl_3$/DMF at room temperature led to formation of 2-formyl-3,4,6-tri-*O*-methyl-D-glucal (**16**) in 45% yield (Scheme 1).

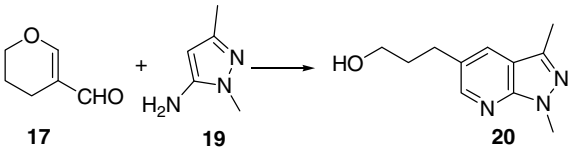
After synthesis of **16**, the key step involved in synthesis of target compounds was condensation of formyl precursor **16** with heterocyclic amine. As a model reaction, reaction of 5,6-dihydro-*4H*-pyran-3-carbaldehyde (**17**, synthesized from 3,4-dihydropyran **18** using Vilsmeier–Haack formylation) with 1,3-dimethyl-*1H*-pyrazol-5-amine (**19**) was attempted using different reaction conditions as depicted in Table 1.

As depicted in Table 1, desired hybrid compound **20** was obtained in 40–60% yield using ethanol as a solvent and acetic acid, HCl or *p*TSA as catalyst under reflux condition, while desired product was not formed when reaction was kept at room temperature even up to 10 h. The product **20** was characterized using ^{1}H, ^{13}C NMR, MS and IR data.

After optimization of reaction conditions for condensation, desired hybrid structures of glucal aldehyde **16** with different heterocyclic amines were synthesized (Scheme 2). Yields were not satisfactory (less than 15%) with the use of AcOH or HCl as a catalyst but comparatively better yields (20–24%) were obtained with *p*TSA, as well as very poor yields were obtained when only acetic acid was used (without ethanol). Treatment of 2-formyl-3,4,6-tri-*O*-methyl-D-glucal (**16**) with heterocyclic amines **19, 21–23** in presence of *p*TSA in EtOH under reflux for 2 h resulted in formation of hybrid compounds **24–27** in 20–24% yield (Scheme 2). Hybrid compounds **26–27** were further treated with BF_3–DMS, which resulted in formation of deprotected compounds **28–30** in 30% yield. All synthesized compounds were characterized by ^{1}H, ^{13}C NMR, MS and IR data.

Interestingly, it was noticed that during condensation of 2-formyl-3,4,6-tri-*O*-methyl-D-glucal (**16**) with 1-phenyl pyrazole amines (**21–22**), a side-chain methoxy located at α-position to pyridine ring gets replaced with ethoxy moiety, which appears to come from ethanol, a solvent used for reaction. But this ethyl exchange was not observed in the case of reaction of **16** with 1-methyl pyrazole amine (**19**). The probable mechanistic reason for

Table 1
Conditions for condensation of **17** with heterocyclic amine **19**



17 **19** **20**

Reagent/solvent	Condition	Yields (%)[a]
AcOH/EtOH	RT, 5–10 h	0
HCl/EtOH	RT, 5–10 h	0
*p*TSA/EtOH	RT, 5–10 h	0
AcOH	120 °C, 2 h	60
HCl/EtOH	90 °C, 2 h	40
*p*TSA/EtOH	90 °C, 2 h	45

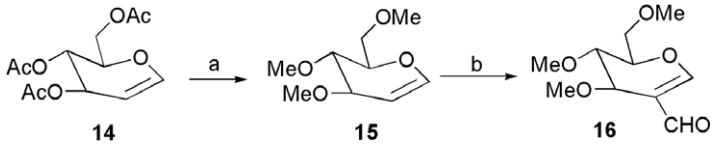
[a] Isolated yields.

this interesting observation can be justified as due to presence of phenyl moiety on N^1 of pyrazole ring, which in conjugation with pyridine ring makes the side chain α-methoxy a better leaving group and thus gets exchanged with comparatively stable ethoxy moiety. This observation was supported by ^{1}H, ^{13}C NMR and MS data.

In the case of reaction of 2-formyl-3,4,6-tri-*O*-methyl-D-glucal (**16**) with 3-(4-methoxyphenyl)-1-phenyl-*1H*-pyrazol-5-amine (**22**), hybrid structure **27b** was obtained instead of desired compound **27a** (Fig. 3). ^{1}H NMR spectrum of **27b** showed (a) only three methoxy signals (in region *δ3.15–4.00); (b)* two extra protons in region *δ 4.00–3.00* ppm; (c) triplet at δ 1.21 for three protons. This observation was also supported by ^{13}C NMR data where signal at δ 15.3 ppm ($-CH_2CH_3$) was observed. This observation along with mass spectral data (478, M+1 peak) led to conclusion that one of the methoxy groups of side chain gets replaced with ethoxy moiety. DEPT experiments further confirmed presence of ethyl functionality, as two CH_2's are seen in DEPT-135 spectrum of compound **27b**. Our assumption of ethanol (solvent used for reaction) as a source of ethoxy group was confirmed by changing the reaction solvent (methanol instead of ethanol). As expected, with the use of methanol as a solvent, no exchange of methoxy group was noticed, and compound **27a** was formed as predicted from ^{1}H NMR signals.

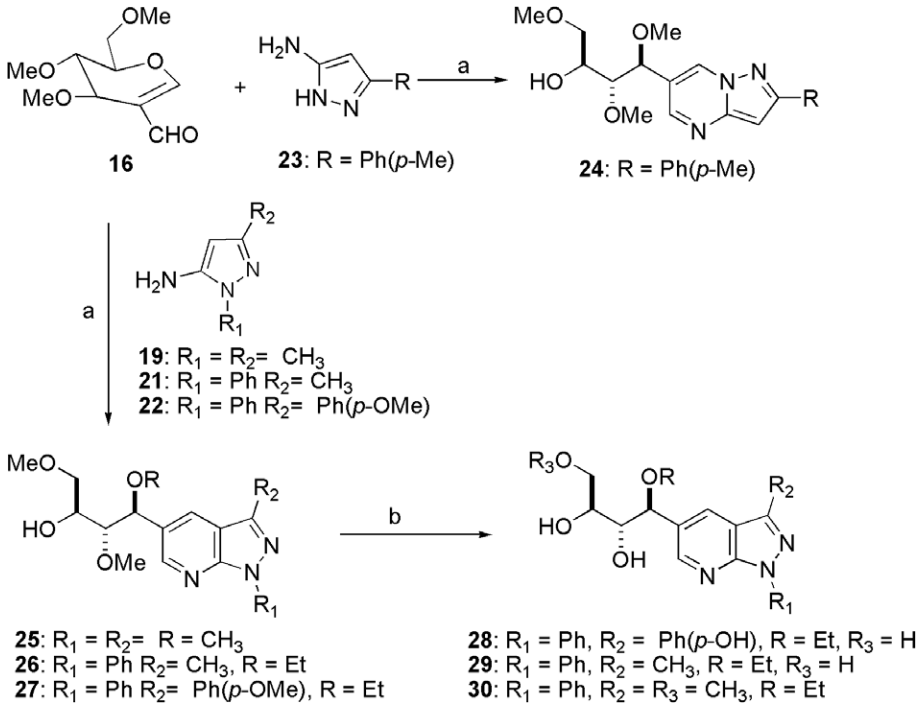
The location of ethyl functionality was further confirmed using extensive 2-D NMR experiments viz. HSQC and HMBC. The complete assignment of ^{1}H NMR and ^{13}C NMR signals (mentioned in experimental section; Section 4.4.5) for compound **27b** was done based on DEPT, HSQC and HMBC experiments.

Further, we synthesized several analogues of compound **20** by varying heterocycle component. Treatment of 5,6-dihydro-*4H*-pyran-3-carbaldehyde (**17**) with different heterocyclic amines **19, 21–23**, **31–35** in presence of *p*TSA or acetic acid resulted in formation of desired pyran-heterocycle hybrid structures **20**, **36–43** in 50–65% yield (Scheme 3). In the case of reaction of **17** with triazole amine (**31**), pyrazole amine (**32**) and 2-amino benzimidazole (**34**) using acetic acid, the terminal free hydroxyl of side chain underwent acetylation (acyl moiety from acetic acid), and acetylated products **38**, **40** and **41** were obtained. Acetylated products (**38**



14 **15** **16**

Scheme 1. Reagents and conditions: (a). CH_3I, solid NaOH, TBAI, THF, RT, 6 h, 80%; (b). $POCl_3$, DMF, rt, 4 h, 45%.



Scheme 2. Reagents and conditions: (a) pTSA, EtOH, 90 °C, 2 h, 20–24%; (b) BF_3–DMS, rt, 4 h, 25–30%.

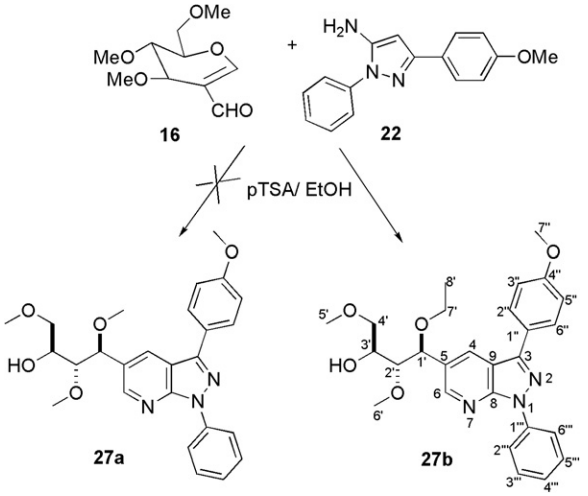


Figure 3. Condensation of 2-formyl-3,4,6-tri-*O*-methyl-D-glucal (**16**) with 1-phenyl-3-(4-methoxyphenyl)-5-pyrazole amine (**22**).

and **41**) further on treatment with catalytic amounts of potassium carbonate in methanol led to formation of desired products, **44–45,** in 85–90% yield. All synthesized compounds were characterized by ^{1}H, ^{13}C NMR, MS and IR spectroscopic data.

Based on structural similarity, we further selected 3-formyl chromones, **46–48,** as 3-alkoxyacrolein part and synthesized several hybrid compounds by varying heterocycle part. Treatment of 3-formyl chromones, **46–48,** with different heterocyclic amines **19, 21–23**, **34–35, 49–50** in presence of *p*TSA or acetic acid resulted in formation of desired chromone-heterocycle hybrid structures **51–60,** in 70–85% yield (Scheme 4). All synthesized compounds were characterized by ^{1}H, ^{13}C NMR, MS and IR spectroscopic data. The ^{1}H NMR spectrum of chromone-heterocycle hybrid structures, **51–60** showed presence of signals at δ *11.50–11.70* ppm for hydrogen bonded phenolic OH group. This was confirmed by deuterium exchange experiment.

2.2. Biological evaluation

All synthesized compounds were evaluated for anti-inflammatory activity against TNF-α and IL-6. Results are shown in Table 2. Dexamethasone was used as a reference standard for assay. Glucal-heterocycle hybrids**, 24–27,** exhibited 15–20% and 30–35% inhibition of TNF-α at 10 and 30 μM, respectively, while none of the compounds showed significant TNF-α inhibitory activity.

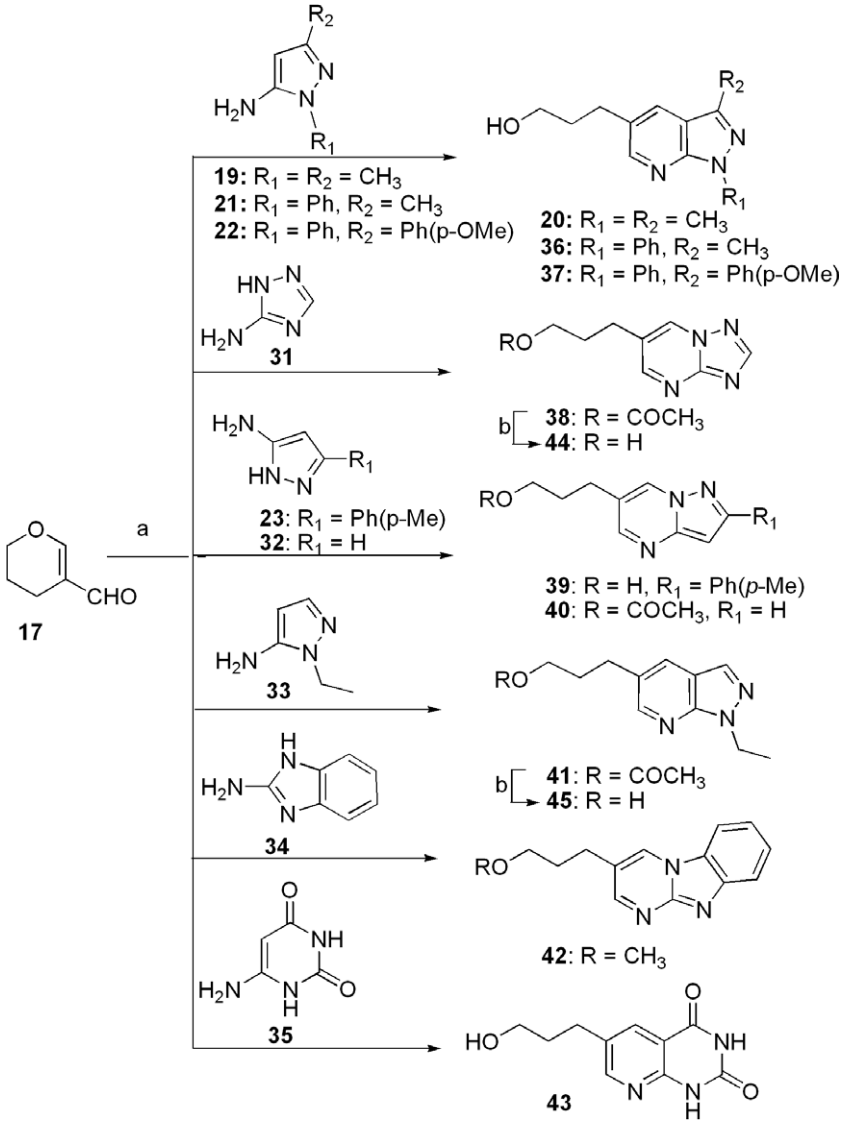
Several compounds showed promising activity against IL-6. Glucal-heterocycle hybrid structures, **26** and **27,** showed 44% and 35% inhibition of IL-6 at 10 μM while amongst pyran-heterocycle hybrid compounds pyrazolo-pyrimidine **40** showed promising activity against IL-6 with 60% inhibition at 10 μM. Compounds **37**, **43** and **44** showed 35–45% inhibition of IL-6 at 10 μM. From chromone-heterocycle hybrid structures, pyrazolo-pyridines, **51–52,** and isoxazolo-pyridines, **56–57,** showed 54–63% inhibition of IL-6 at 10 μM (Table 2). Most of the compounds did not show significant toxicity at 10 μM except compounds **51**, **52** and **58** (toxicity at 10 μM = 37–41% inhibition).

Most promising compounds **51, 52** and **56** were further tested, and IC_{50} was determined. These showed promising IC_{50} against IL-6 inhibition with 0.2, 0.3 and 0.16 μM, respectively (Table 3). A potent IL-6 inhibitory compound **56** did not show cytotoxicity (IC_{50} >100 μM).

From the present work, chromone-heterocycle hybrid compounds, isoxazolo-pyridine **56,** showed promising IL-6 inhibition with IC_{50} of 0.16 μM. Thus, isoxazolo-pyridine class of compounds have a potential to emerge as a new class of anti-inflammatory agents with specific IL-6 inhibitory activity.

3. Conclusion

In conclusion, three different series of pyrazolo[3,4-*b*]pyridines and other related structural analogues were synthesized using



Scheme 3. Reagents and conditions: (a) EtOH/pTSA, or AcOH, 90 °C, 2–4 h, 50-65%; (b) K_2CO_3, MeOH, 30 min, rt, 85–90%.

new, short and efficient synthetic strategy involving condensation of 3-alkoxyacroleins with different heterocyclic amines. From the activity results, we could conclude that only three analogues, **51, 52** and **56** (IC_{50} 0.2, 0.3 and 0.16 μM, respectively), are the most promising compounds. Moreover, since compound **56** did not show cytotoxicity even up to 100 μM concentration, we have selected it as a potential lead for further optimization as an IL-6 inhibitor.

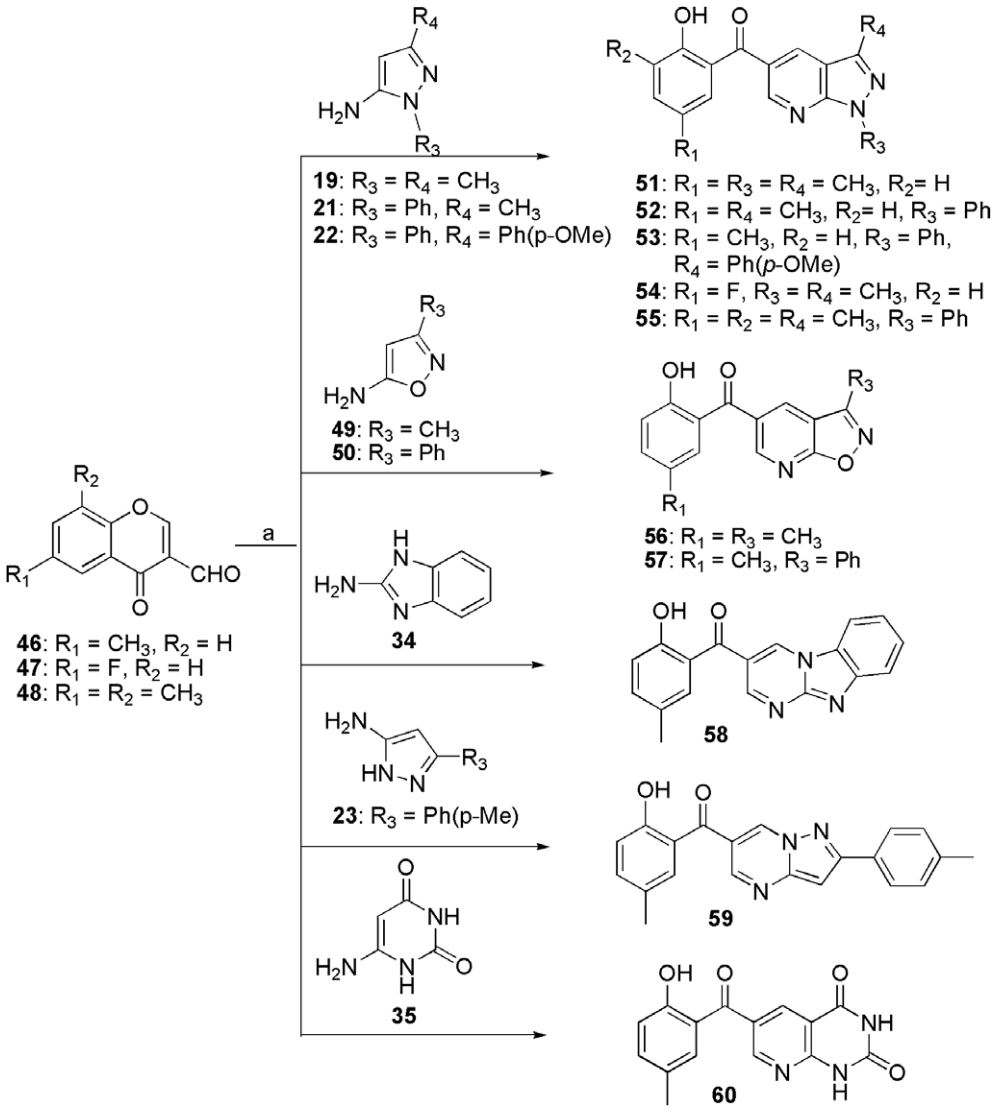
4. Experimental

4.1. General

Melting points were recorded on Labindia visual melting point apparatus. ^{1}H NMR spectra were recorded on 300 MHz Bruker FT-NMR (Avance DPX300) spectrometer using tetramethylsilane as internal standard, and chemical shifts are reported in δ units. Mass spectra were recorded on either GCMS (Focus GC with TSQ II mass analyzer and thermoelectron) with auto sampler/direct injection (EI/CI) or LCMS (APCI/ ESI; Bruker Daltonics MicroTOF Q). HPLC purity was checked using Waters Alliance or Dionex Ultima 3000 HPLC system. HPLC conditions used for checking purity were Kromasil C18 (150 × 4.6 mm, 3.5 μ) column; mobile phase: ACN (A), 0.01 M NH_4OAc + 0.5% TEA, pH 5.0, with AcOH (Time/A%: 0/10, 20/90, 22/10, 30/10); flow rate: 1 mL/min. Elemental analysis was carried out using elemental analyzer (Flash 1112 series EA). All chromatographic purifications were performed with silica gel (60–120 mesh), whereas all TLC (silica gel) developments were performed on silica gel coated (Merck Kiesel 60F_{254}, 0.2 mm thickness) sheets. All reagents and solvents were of commercial quality and were used as supplied unless otherwise stated. Yields reported are isolated yields of the materials.

4.2. Synthesis of 3,4,6-tri-*O*-methyl-D-glucal (15)

To a solution of 3,4,6-tri-*O*-acetyl-D-glucal (**14**, 1 mmol) in THF were added powdered NaOH (1 equiv), TBAI (0.5 equiv) and methyl iodide (5 equiv) successively. Reaction mixture was further allowed to stir for 6 h at room temperature. The reaction mixture was poured into water and extracted with EtOAc (3× 150 mL). The organic layer was washed with water, dried over sodium sulfate and concentrated to dryness. Crude reaction mixture was purified by column chromatography over silica gel (100–200 mesh) using 20% EtOAc/petroleum ether as eluent to get yellow viscous



Scheme 4. Reagents and conditions: (a) EtOH/pTSA, 90 °C, 2–4 h, 70–85%.

oil (yield: 60%). It was characterized by comparison of spectral data with literature values.[31]

4.3. Vilsmeier–Haack formylation: synthesis of 16–17

To a cooled solution of **15** or **18** (1 mmol) in *N*,*N*-dimethylformamide (1.1 equiv), phosphorus oxychloride (1.1 equiv) was added dropwise. Reaction mixture was allowed to stir for 4–6 h at room temperature. The reaction mixture was neutralized with saturated $NaHCO_3$ solution and extracted with EtOAc (3× 150 mL). Organic layer was washed 7–8 times with cold water followed by brine in order to remove DMF completely. After concentrating, crude reaction mixture was purified by column chromatography over silica gel (100–200 mesh) using 20% EtOAc/petroleum ether as eluent. Compounds **16** and **17** were characterized by comparison of spectral data with literature values.[31,32]

4.4. Synthesis of pyrazolo/triazolo[3,4-*b*]pyridines/pyrimidines

Method 1: To a mixture of equimolar amounts of appropriate aldehyde (1 mmol) and heterocyclic amine (1 mmol) in ethanol (20 mL), *p*TSA (catalytic amount) was added and resulting mixture was refluxed for 2–4 h. In the case of reactions of pyran aldehyde or glucal aldehyde with different amines, reaction mixture was concentrated under vacuum and purified by column chromatography over silica gel (100–200 mesh) using 50% EtOAc/petroleum ether as eluent. In the case of reaction of chromone aldehyde with different amines, the reaction mixture was filtered and residue was washed with cold ethanol and finally recrystallized from ethanol.

Method 2: The mixture of equimolar amounts of the appropriate aldehyde (1 mmol) and heterocyclic amine (1 mmol) in glacial acetic acid (20 mL) was refluxed for 2–4 h. The reaction mixture was neutralized with saturated $NaHCO_3$ solution and extracted with EtOAc (3× 150 mL). Organic layer was concentrated under reduced pressure and purified by column chromatography over silica gel (100–200 mesh) using 50% EtOAc/petroleum ether as eluent.

4.4.1. 5-Propyl-3-ol-1,3-dimethyl-*1H*-pyrazolo[3,4-*b*]pyridine (20)

Synthesized by method 1; brown sticky oil; yield: 71%; HPLC: 97.02%; IR (neat): 3326, 2926, 1624, 1508, 1053 cm^{-1}; ^1H NMR (300 MHz, CD_3OD): δ 8.41 (s, 1H), 8.01 (s, 1H), 4.00 (s, 3H), 3.59 (d, J = 6.3 *Hz*, 2H), 2.84 (d, J = 7.8 *Hz*, 2H), 2.52 (s, 3H), 1.95–1.84 (m, 2H); ^{13}C NMR (75 MHz, CD_3OD): δ 149.8, 149.6, 140.2, 129.9,

Table 2
Anti-inflammatory activity against TNF-α and IL-6

Entry	% Inhibition at 10 μM		
	TNF-α	IL-6	Toxicity
20	6	27	7
24	18	34	3
25	20	35	5
26	19	44	4
27	15	35	6
28	0	40	5
29	3	44	9
30	2	43	8
36	8	19	16
37	12	36	10
38	7	45	0
39	0	0	9
40	13	66	0
41	0	16	13
42	0	18	9
43	0	37	12
44	4	43	0
45	0	45	8
51	0	60	41
52	0	54	39
53	0	49	27
54	0	34	19
55	0	44	14
56	5	58	16
57	0	63	25
58	5	32	37
59	0	9	5
60	6	39	0
Dexamethasone (1 μM)	72	94	0

Table 3
IC_{50}'s of compounds **51**, **52** and **56**

Entry	IC_{50} (μM)[a]		
	TNF-α	IL-6	Toxicity
51	>100	0.2	2.6
52	>100	0.3	10
56	>100	0.16	>100
Dexamethasone	0.05	0.007	>100

[a] IC_{50}: the concentration that affords 50% inhibition of TNF-α or IL-6.

129.0, 115.0, 60.4, 34.3, 32.2, 28.5, 10.6; MS (APCI): *m/z* 206 $[M+1]^+$; analysis for $C_{11}H_{15}N_3O$ (205.1) calcd, C, 64.37; H, 7.37; N, 20.47; found, C, 64.07; H, 7.23; N, 20.13.

4.4.2 6-(1,2,4-Trimethoxybutyl-3-ol)-2-*p*-tolylpyrazolo[1,5-*a*]pyrimidine (24)

Synthesized by method 1; brown sticky solid; yield: 24%; HPLC: 96.06%; IR (Neat): 3323, 2924, 1717, 1622, 1521, 1447, 1322, 1123, 1003 cm^{-1}; ^1H NMR (300 MHz $CDCl_3$): δ 8.80 (s, 1H), 8.52 (d, *J* = 1.8 Hz, 1H), 7.90 (d, *J* = 9.0 *Hz,* 2H), 7.30 (d, *J* = 8.2 Hz, 2H), 6.97 (s, 1H), 4.65 (d, *J* = 3.0 *Hz* 1H), 4.02 (m, 1H), 3.62–3.56 (m, 2H), 3.39 (s, 3H), 3.32 (s, 3H), 3.32 (m, 1H), 3.18 (s, 3H), 2.42 (s, 3H); MS (APCI): *m/z* 372.2 $[M+1]^+$; 394.2 $[M+Na]^+$; analysis for $C_{20}H_{25}N_3O_4$ (371.2) calcd, C, 64.67; H, 6.78; N, 11.31; found, C, 64.38; H, 6.54; N, 11.10.

4.4.3. 5-(1,2,4-Trimethoxylbutyl-3-ol)-1,3-dimethyl-*1H*-pyrazolo[3,4-*b*]pyridine (25)

Synthesized by method 1; brown sticky solid; yield: 20%; HPLC: 98.65%; IR (Neat): 3020, 2855, 1610, 1516, 1216, 1093, 1032 cm^{-1}; ^1H NMR (300 MHz $CDCl_3$,): δ 8.56 (s, 1H), 8.07 (s, 1H), 4.74 (d, *J* = 3.2 *Hz,* 1H), 4.11 (s, 3H), 4.01–3.97 (m, 1H), 3.63-3.55 (m, 3H), 3.41 (s, 3H), 3.34 (s, 3H), 3.31-3.27 (m, 1H), 3.12 (s, 3H), 2.59 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 149.3, 146.9, 138.6, 126.6, 124.5, 113.1, 82.5, 78.7, 71.5, 67.9, 58.9, 57.2, 55.7, 31.8, 27.9; MS (APCI): *m/z* 310 $[M+1]^+$; 332 $[M+Na]^+$; analysis for $C_{15}H_{23}N_3O_4$ (309.2) calcd, C, 58.24; H, 7.49; N, 13.58; found, C, 58.12; H, 7.30; N, 13.42.

4.4.4. 5-(1-Ethoxy-2,4-dimethoxylbutyl-3-ol)-3-methyl-1-phenyl-*1H*-pyrazolo[3,4-*b*]pyridine (26)

Synthesized by method 1; brown sticky solid; yield: 22%; HPLC: 98.82%; IR (neat): 3433, 2867, 1532, 1548, 1052 cm^{-1}; ^1H NMR (300 MHz, CD_3OD): δ 8.68 (s, 1H), 8.26-8.18 (m, 3H), 7.56–7.52 (m, 2H), 7.35–7.31 (m, 1H), 4.62 (s, 1H), 3.91-3.85 (m, 1H), 3.54–3.46 (m, 4H), 3.40–3.38 (m, 1H), 3.36 (s, 3H), 3.08 (s, 3H), 2.66 (s, 3H), 1.22 (t, *J* = 7.5 Hz, 3H); MS (APCI): *m/z* 386.2 $[M+1]^+$, 408.2 $[M+Na]^+$; analysis for $C_{21}H_{27}N_3O_4$ (385.2) calcd, C, 65.44; H, 7.06; N, 10.90; found, C, 65.31; H, 6.96; N, 10.62.

4.4.5. 5-(1-Ethoxy-2,4-dimethoxylbutyl-3-ol)-3-(4-methoxyphenyl)-1-phenyl-1H-pyrazolo[3,4-*b*]pyridine (27)

For numbering see Fig. 3; synthesized by method 1; brown sticky solid; yield: 24%; HPLC: 97.04%; ^1H NMR (300 MHz, $CDCl_3$): δ 8.70 (d, *J* = 1.2 Hz, 1H, ArH-C_6), 8.39 (d, *J* = 1.2 Hz, 1H, ArCH-C_4), 8.35 (d, *J* = 8.1 Hz, 2H, ArH-$C_{2'''}$, $_{C6'''}$), 8.02 (d, *J* = 9 Hz, 2H, ArH-$C_{2''}$, $_{6''}$), 7.57-7.55 (m, 2H, ArH-$C_{3'''}$, $_{5'''}$), 7.32 (t, *J* = 6.0 *Hz,* 1H, ArH-$C_{4'''}$), 7.11 (d, *J* = 6 *Hz,* 2H, ArH-$C_{3''}$, $_{5''}$), 4.72 (d, *J* = 4.6 Hz, 1H, CH-$C_{1'}$), 3.91 (s, 3H, OCH_3-$C_{7''}$), 3.70–3.64 (m, 1H, CH_2-$C_{3'}$), 3.57–3.54 (m, 2H, CH_2-$C_{4'}$), 3.49 (t, *J* = 6.9 Hz, 1H, CH-$C_{2'}$), 3.44 (q, *J* = 3.2, 7.8 Hz, 2H, OCH_2-$C_{7'}$), 3.40 (s, 3H, OCH_3-$C_{5'}$), 3.25 (s, 3H, OCH_3-$C_{6'}$), 1.22 (t, *J* = 6.9 Hz, 3H, CH_3-$C_{8'}$); ^{13}C NMR (75 MHz, $CDCl_3$): δ 160.1 (C-$C_{4''}$), 151.3 (C-C_8), 149.5 (CH-C_6), 144.0 (C-C_3), 139.5 (C-$C_{1'''}$), 130.0 (CH-C_4), 129.1 (CH-$C_{3''',5'''}$), 128.8 (CH-C_5), 128.6 (CH-$C_{2''}$, $_{6''}$), 125.9 (CH-$C_{4'''}$), 125.4 (C-$C_{1''}$), 121.3 (CH-$C_{2'''}$, $_{6'''}$), 115.0 (C-C_9), 114.5 (CH-$C_{3''}$, $_{5''}$), 83.4 (CH-$C_{2'}$), 81.7 (CH-$C_{1'}$), 73.3 (CH_2-$C_{4'}$), 71.7 (CH-$C_{3'}$), 64.7 (CH_2-$C_{7'}$), 60.5 (OCH_3-$C_{6'}$), 59.1 (OCH_3-$C_{5'}$), 55.4 (OCH_3-$C_{7''}$), 15.3 (CH_3-$C_{8'}$); MS (APCI): *m/z* 478.2 $[M+1]^+$; analysis for $C_{27}H_{31}N_3O_5$ (477.2) calcd, C, 67.91; H, 6.54; N, 8.80; found, C, 67.43; H, 6.32; N, 8.44.

4.4.6. 5-Propyl-3-ol-3-methyl-1-phenyl-*1H*-pyrazolo[3,4-b]pyridine (36)

Synthesized by method 1; white amorphous solid; yield: 72%; mp 105–107 °C; HPLC: 97.02%; IR (KBr): 3403, 3018, 2862, 1593, 1508, 1063 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 8.43 (s, 1H), 8.19 (d, *J* = 8.1 Hz, 2H), 7.81 (s, 1H), 7.46 (t, *J* = 8.1 Hz, 2H), 7.22 (t, *J* = 7.2 Hz, 1H), 3.68 (t, *J* = 6 Hz, 2H), 2.84 (t, *J* = 7.8 Hz, 2H), 2.59 (s, 3H), 1.98-1.89 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 150.1, 149.9, 142.1, 139.6, 130.2, 129.1, 128.0, 125.4, 120.7, 117.0, 61.7, 34.5, 29.1, 12.4; MS (APCI): *m/z* 268 $[M+1]^+$; analysis for $C_{16}H_{17}N_3O_2$ (267.1) calcd, C 71.89; H 6.41; N 15.72; found, C 70.31, H 6.54, N 14.96.

4.4.7. 5-Propyl-3-ol-3-(4-methoxyphenyl)-1-phenyl-*1H*-pyrazolo[3,4-*b*]pyridine (37)

Synthesized by method 1; brown viscous oil; yield: 65%; HPLC: 99.01%; IR (KBr): 3373, 3040, 2927, 1598, 1500, 1032, 1249 cm^{-1}; ^1H NMR (300 MHz, CD_3OD + DMSO-d_6 - 4:1): δ 8.49 (d, *J* = 1.0 Hz, 1 H), 8.31 (d, *J* = 1.2 Hz, 1H), 8.27 (d, *J* = 4.8 Hz, 2H), 7.97 (d, *J* = 5.4 *Hz*, 2H), 7.50 (t, *J* = 4.8 *Hz*, 2H), 7.30 (t, *J* = 4.5 Hz, 1H), 7.08 (d, *J* = 5.1 Hz, 2H), 3.85 (s, 3 H), 3.59 (t, *J* = 3.6 Hz, 2 H), 2.88 (t, *J* = 4.8 *Hz*, 2H), 1.94-1.89 (m, 2H); ^{13}C NMR (75 MHz, CD_3OD): δ 158.5, 147.9, 141.9, 137.5, 130.1, 128.1, 126.4, 123.8, 123.1, 119.2, 113.2, 112.2, 58.6, 52.6, 32.4, 26.9; MS (APCI): *m/z* 360 $[M+1]^+$; analysis for $C_{22}H_{21}N_3O_2$ (359.2) calcd, C, 73.52; H, 5.89; N, 11.69; found, C, 73.29; H, 5.59; N, 11.72.

4.4.8. 6-(3-Acetoxypropyl)-[1,2,4]triazolo[1,5-*a*]pyrimidine (38)

Synthesized by method 2; brown sticky solid; yield: 62%; HPLC: 99.11%; IR (KBr): 3322, 2920, 1710, 1623, 1455, 1332, 1135 cm^{-1};

^{1}H NMR (300 MHz, $CDCl_3$): δ 8.74 (s, 1H), 8.70 (s, 1H), 8.48 (s, 1H), 4.16 (t, *J* = 6 *Hz*, 2H), 2.86 (t, *J* = 8.1 *Hz*, 2H), 2.11-2.04 (m, 2H), 2.08 (s, 3H); ^{13}C NMR (75 MHz, CD_3OD): δ 171.3, 157.4, 154.8, 153.8, 134.9, 125.2, 63.0, 29.1, 25.6, 19.3; MS (APCI): *m/z* 221 $[M+1]^+$; analysis for $C_{10}H_{12}N_4O_2$ (220.1) calcd, C, 54.54; H, 5.49; N, 25.44; found, C, 54.37; H, 5.41; N, 25.29.

4.4.9. 6-Propyl-3-ol-2-*p*-tolyl-pyrazolo[1,5-a]pyrimidine (39)

Synthesized by method 1; brown sticky solid; yield: 62%; HPLC: 98.08%; IR (KBr): 3326, 2926, 1711, 1624, 1528, 1457, 1342, 1140, 1053 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.47 (s, 1H), 8.39 (s, 1H), 6.87 (s, 1H), 7.82 (d, *J* = 8.1 Hz, 2H), 7.24 (d, *J* = 7.2 Hz, 2H), 3.70 (t, *J* = 6 Hz, 2H), 2.74 (t, *J* = 7.8 Hz, 2H), 2.36 (s, 3H), 1.94–1.85 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 154.2, 149.3, 146.8, 137.1, 130.8, 128.1, 127.7, 124.5, 119.5, 91.1, 59.3, 31.2, 24.3, 19.5; MS (APCI): *m/z* 268 $[M+1]^+$; analysis for $C_{16}H_{17}N_3O$ (267.1) calcd, C, 71.89; H, 6.41; N, 15.72; found, C, 71.65; H, 6.50; N, 15.40.

4.4.10. 6-(3-Acetoxy)propyl-pyrazolo[1,5-*a*]pyrimidine (40)

Synthesized by method 2; sticky brown solid; yield: 52%; HPLC: 95.42%; IR (KBr): 3131, 1736, 1629, 1559, 1239, 1123 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.51 (s, 1H), 8.39 (s, 1H), 8.07 (s, 1H), 6.68 (s, 1H), 4.14 (t, *J* = 6.3 Hz, 2H), 2.75 (t, *J* = 8.1 Hz, 2H), 2.07 (s, 3H), 2.07-2.01 (m, 2H,); ^{13}C NMR (75 MHz, CD_3OD): δ 169.6, 149.7, 145.2, 142.3, 126.7, 120.2, 93.7, 61.3, 27.3, 23.9, 17.5; MS (APCI): *m/z* 220 $[M+1]^+$; analysis for $C_{11}H_{13}N_3O_2$ (219.1) calcd, C, 60.26; H, 5.98; N, 19.17; found, C, 60.26; H, 5.98; N, 19.17.

4.4.11. 5-(3-Acetoxy)propyl-1-ethyl-1H-pyrazolo[3,4-*b*]pyridine (41)

Synthesized by method 2; brown sticky oil; yield: 85%; HPLC: 99.05%; IR (neat): 3020, 1734, 1612, 1567, 1513, 1508, 1368, 1216 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.41 (s, 1H), 7.96 (s, 1H), 7.87 (s, 1H), 4.60 (q, *J* = 7.2, 14.4 Hz, 2H), 4.36 (t, *J* = 6.6 Hz, 2H), 2.85 (t, *J* = 8.1 *Hz*, 2H), 2.08 (s, 3H), 2.05-1.98 (m, 2H), 1.55 (d, *J* = 7.2 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.6, 149.2, 148.4, 130.7, 128.8, 128.3, 115.2, 62.9, 41.7, 30.1, 28.9, 20.5, 14.5; MS (APCI): *m/z* 248 $[M+1]^+$; analysis for $C_{13}H_{17}N_3O_2$ (247.1) calcd, C, 63.14; H, 6.93; N, 16.99; found, C, 62.97; H, 7.03; N, 16.69.

4.4.12. 3-Propyl-3-ol-benzo[4,5]imidazo[1,2-*a*]pyrimidine (42)

Synthesized by method 1; green amorphous solid; yield: 87%; mp 120–121 °C; HPLC: 98.06%; IR (KBr): 3061, 2896, 1736, 1612, 1497, 1238, 1043 cm^{-1}; ^{1}H NMR (300 MHz, CD_3OD): δ 9.22 (s, 1H), 8.81 (s, 1H), 8.20 (d, *J* = 8.1 Hz, 1H), 7.86 (d, *J* = 8.1 Hz, 1H), 7.59 (t, *J* = 7.8 *Hz*, 1H), 7.49 (t, *J* = 7.8 Hz, 1H), 4.19 (t, *J* = 6.3 Hz, 2H), 2.89 (t, *J* = 8.1 *Hz*, 2H), 2.15–2.07 (m, 2H), 2.03 (s, 3H); ^{13}C NMR (75 MHz, CD_3OD): δ 169.6, 156.6, 148.0, 141.1, 130.8, 125.1, 120.0, 119.1, 116.7, 109.7, 61.3, 27.4, 24.0, 17.5; MS (ESI): *m/z* 255 $[M-1]^+$; analysis for $C_{15}H_{15}N_3O_2$ (269.1) calcd, C 66.90; H 5.61; N 15.60; found, C 67.28, H 5.85, N 15.30.

4.4.13. 6-(3-Hydroxypropyl)pyrido[2,3-*d*]pyrimidine-2,4-(*1H*,*3H*)-dione (43)

Synthesized by method 1; CH_2Cl_2: MeOH as mobile phase for column chromatography; greenish sticky semisolid; yield: 52%; HPLC: 98.09%; IR (KBr): 3754, 3438, 2856, 1701, 1438, 1388, 1290 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 11.57 (s, 1H), 11.39 (s, 1H), 8.45 (s, 1H), 8.06 (s, 1H), 4.50 (t, *J* = 4.8 Hz, 2H), 2.66 (t, *J* = 7.8 Hz, 2H), 1.74-1.65 (m, 2 H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 161.1, 153.3, 149.3, 149.0, 134.0, 131.3, 108.0, 58.2, 32.4, 26.4; MS (ESI): *m/z* 220 $[M-1]^+$; analysis for $C_{10}H_{11}N_3O_3$ (221.1) calcd, C, 54.29; H, 5.01; N, 19.00; found, C, 53.89; H, 5.27; N, 18.78.

4.4.14. 5-(2-Hydroxy-5-methyl-benzoyl)-1,3-dimethyl-*1H*-pyrazolo[3,4-*b*]pyridine (51)

Synthesized by method 1; yellow solid; yield: 70%; mp 173–175 °C; HPLC: 99.92%; IR (KBr): 3432, 2980, 2922, 2376, 1624, 1617, 1591, 1508, 1480, 1408, 1373, 1359, 1327, 1296, 1244, 1215, 1056 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 11.65 (s, 1H), 8.86 (d, *J* = 1.2 Hz, 1H), 8.39 (d, *J* = 1.2 Hz, 1H), 7.36–7.35 (m, 2 H), 7.02 (m, 1H), 4.14 (s, 3H), 2.62 (s, 3H), 2.27 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 197.3, 159.2, 149.8, 148.0, 140.7, 135.7, 130.9, 129.9, 126.3, 124.6, 117.3, 116.6, 112.6, 32.0, 18.7, 10.6; MS (APCI): *m/z* 282.2 $[M+1]^+$, 304.1 $[M+Na]^+$; analysis for $C_{16}H_{15}N_3O_2$ (281.1) calcd, C, 68.31; H, 5.37; N, 14.94; found, C, 68.20; H, 5.28; N, 14.88.

4.4.15. 5-(2-Hydroxy-5-methyl-benzoyl)-3-methyl-1-phenyl-*1H*-pyrazolo[3,4-*b*]pyridine (52)

Synthesized by method 1; yellow solid; yield: 73.23%; mp 128–130 °C; HPLC: 99.90%; IR (KBr): 3431, 3050, 2964, 2924, 1632, 1590, 1509, 1482, 1442 1338, 1299, 1226, 1143, 1116, 1017 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 11.69 (s, 1H), 8.95 (d, *J* = 2.1 *Hz*, 1H), 8.47 (d, *J* = 2.1 Hz, 1H), 8.26 (d, *J* = 7.8 Hz, 2H) 7.55 (t, *J* = 7.8 Hz, 2H), 7.39 (m, 3H), 7.05 (d, *J* = 8.7 Hz, 1H), 2.72 (s, 3H), 2.17 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 197.0, 159.3, 149.9, 148.2, 142.5, 137.1, 135.9, 130.9, 129.9, 127.4, 126.4, 125.7, 124.5, 119.5, 117.2, 116.7, 114.6, 18.7, 10.7; MS (APCI): *m/z* 344.1 $[M+1]^+$; analysis for $C_{21}H_{17}N_3O_2$ (343.1) calcd, C, 73.45; H, 4.99; N, 12.24; found, C, 73.34; H, 5.01; N, 12.32.

4.4.16. 5-(2-Hydroxy-5-methyl-benzoyl)-3-(4-methoxy phenyl)-1-phenyl-*1H*-pyrazolo[3,4-*b*]pyridine (53)

Synthesized by method 1; yellow solid; yield: 95.81%; mp 161–163 °C; HPLC: 99.70%; IR (KBr): 3029, 2967, 2930, 2837, 2558, 1938, 1685, 1632, 1608, 1587, 1479.4, 1438, 1278, 1256, 1185, 1137, 1022 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 11.71 (s, 1H), 8.97 (s, 1H), 8.76 (s, 1H), 8.37 (d, *J* = 8.1 *Hz*, 2H), 8.02 (d, *J* = 8.7 Hz, 2H), 7.59 (t, *J* = 7.5 Hz, 2H), 7.42-7.36 (m, 3H), 7.10-7.03 (m, 3H), 3.91 (s, 3H), 2.46 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 197.0, 159.3, 158.7, 149.8, 148.0, 143.8, 137.1, 136.0, 131.0, 130.9, 127.4, 126.9, 126.5, 126.4, 124.8, 122.7, 119.8, 117.2, 116.7, 113.0, 112.8, 53.6, 18.7; MS (ESI): *m/z* 434.2 $[M-1]^+$; analysis for $C_{27}H_{21}N_3O_3$ (435.1) calcd, C 74.74; H 4.86; N 9.65; found, C 74.37, H 4.70, N 9.91.

4.4.17. 5-(2-Hydroxy-5-fluoro-benzoyl)-1,3-dimethyl-*1H*-pyrazolo[3,4-b]pyridine (54)

Synthesized by method 1; yellow solid; yield: 54%; mp 158–160 °C; HPLC: 99.76%; IR (KBr): 3407, 3026, 2982, 2930, 2371, 1895, 1617, 1596, 1474, 1373, 1360, 1312, 1286, 1209, 1035, 1000 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$). δ 11.56 (s, 1H), 8.89 (s, 1H), 8.39 (s, 1H), 7.33–7.30 (m, 2H), 7.11-7.07 (m, 1H), 4.15 (s, 3H), 2.63 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 196.4, 167.9, 157.4, 149.9, 147.9, 140.9, 130.0, 123.9, 122.4, 122.1, 118.3, 116.1, 115.8, 32.0, 10.7; MS (ESI): *m/z* 284.2 $[M-1]^+$; analysis for $C_{15}H_{12}FN_3O_2$ (285.1) calcd, C, 63.15; H, 4.24; N, 14.73; found, C, 63.00; H, 4.17; N, 14.49.

4.4.18 5-(2-Hydroxy-3,5-dimethyl-benzoyl)-3-methyl-1-phenyl-*1H*-pyrazolo[3,4-*b*]pyridine (55)

Synthesized by method 1; yellow solid; yield: 72%, mp 162–164 °C; HPLC: 99.91%; IR (KBr): 3050, 2620, 1654, 1619, 1509, 1466, 1441, 1427 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 11.95 (s, 1H), 8.90 (s, 1H), 8.43 (s, 1H), 8.23 (d, *J* = 9 Hz, 2H), 7.52 (t, *J* = 9 Hz, 2H), 7.31 (t, *J* = 9 Hz, 1H), 7.12 (s, 1H), 2.68 (s, 3H), 2.29 (s, 3H), 2.21 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 197.3, 157.7, 149.4, 148.2, 142.4, 137.1, 136.9, 129.8, 128.4, 127.4, 125.9,

125.6, 124.5, 119.4, 116.5, 114.6, 18.7, 13.8, 10.7; MS (APCI): *m/z* 358.2 $[M+1]^+$; analysis for $C_{22}H_{14}N_3O_2$ (357.2) calcd, C 73.93; H 5.63; N 11.76; found, C 73.99, H 5.24, N 12.09.

4.4.19. 5-(2-Hydroxy-5-methyl-benzoyl)-3-methyl isoxazolo[5,4-*b*]pyridine (56)

Synthesized by method 1; yellow solid; yield: 69.12%; mp 175–177 °C; HPLC: 99.44%; IR (KBr): 3046, 2974, 2925, 2590, 2370, 1911, 1632, 1588, 1603, 1522, 1507, 1432, 1379, 1356, 1319, 1289, 1250, 1213, 1137, 1080 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 11.53 (s, 1H), 8.93 (s, 1H), 8.40 (s, 1H), 7.41 (d, J = 8.4 Hz, 1H), 7.28 (s, 1 H), 7.05 (d, J = 8.4 Hz, 1H), 2.67 (s, 3H), 2.27 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 196.1, 168.7, 159.4, 154.6, 149.7, 136.6, 131.1, 130.6, 128.6, 126.7, 116.9, 111.9, 18.7, 9.1; MS (APCI): *m/z* 268 $[M+1]^+$; analysis for $C_{15}H_{12}N_2O_3$ (268.2) calcd, C 67.16, H 4.51, N 10.44; found, C 67.37, H 3.93, N 10.39.

4.4.20. 5-(2-Hydroxy-5-methyl-benzoyl)-3-phenyl isoxazolo[5,4-*b*]pyridine (57)

Synthesized by method 1; yellow solid; yield: 70.6%; mp 179–182 °C; HPLC: 96.37%; IR (KBr): 3423, 3059, 2966, 2918, 1924, 1598, 1508, 1481.8, 1458, 1388, 1337, 1313, 1296, 1243, 1222, 1154, 1070, 1046, 1001 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 11.57 (s, 1H), 8.99 (d, J = 1.8 Hz, 1H), 8.69 (d, J = 1.8 Hz, 1H), 7.99 (m, 2H), 7.61 (m, 3H), 7.42 (d, J = 8.4 Hz, 1H), 7.30 (s, 1H), 7.06 (d, J = 8.7 Hz, 1H), 2.28 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 195.5, 158.4, 156.0, 149.7, 136.1, 132.2, 130.4, 129.1, 127.6, 127.4, 126.8, 125.8, 123.6, 117.4, 116.5, 95.9, 62.4, 18.5; MS (ESI): *m/z* 329 $[M-1]^+$; analysis for $C_{20}H_{14}N_2O_3$ (330.1) calcd, C 72.72; H 4.27; N 8.48; found, C 72.99, H 4.43, N 8.41.

4.4.21. 3-(2-Hydroxy5-methyl-benzoyl)-benzimidazo[1,2-*a*]pyrimidine (58)

Synthesized by method 1; yellow solid; yield: 51%; mp 260–262 °C; HPLC: 99.52%; IR (KBr): 3448, 3152, 3026, 2808, 2712, 1678.6, 1654, 1613, 1582, 1506, 1485, 1458, 1419, 1373, 1336, 1275, 1256, 1221, 1198, 1138, 1115, 1008 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 11.65 (s, 1H), 7.70-7.62 (m, 3H), 7.54-7.42 (m, 2H), 7.30 (s, 1H), 7.27-7.22 (m, 2H), 7.11 (d, J = 8.4 Hz, 1H), 2.32 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 178.1, 152.8, 142.6, 140.3, 135.3, 132.5, 130.5, 130.3, 125.0, 121.9, 121.7, 120.2, 116.8, 116.0, 109.7, 101.8, 79.6, 18.8; MS (ESI): *m/z* 304.1 $[M-1]^+$; analysis for $C_{18}H_{13}N_3O_2$ (305.1) calcd, C 71.28, H 4.32, N 13.85; found C 71.00, H 3.66, N 13.48.

4.4.22. 6-(2-Hydroxy-5-methyl-benzoyl)-2-*p*-tolyl pyrazolo[1,5-*a*]pyrimidine (59)

Synthesized by method 1; yellow solid; yield: 69.23%; mp 208–210 °C; HPLC: 99.96%; IR (KBr): 3422, 3134, 3078, 2917, 2370, 1631, 1597, 1476, 1460, 1372, 1337, 1308, 1295, 1248, 1200, 1165, 1064, 1001 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 11.37 (s, 1H), 9.07 (s, 1H), 8.82 (s, 1H), 7.92 (d, J = 7.8 Hz, 2H), 7.45 (s, 1H), 7.42 (d, J = 8.4 Hz, 1H), 7.33 (d, J = 7.8 Hz, 2H), 7.08 (s, 1H), 7.05 (d, J = 8.4 Hz, 1H), 2.43 (s, 3H), 2.32 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 193.0, 159.1, 157.8, 147.9, 147.4, 138.2, 136.4, 135.5, 129.7, 127.9, 127.2, 126.8, 124.8, 116.9, 116.7, 93.1, 19.6, 18.7; MS (ESI): *m/z* 344.1 $[M-1]^+$; analysis for $C_{21}H_{17}N_3O_2$ (343.4) calcd, C 73.45, H 4.99, N 12.24; found, C 73.54, H 5.26, N 12.12.

4.4.23. 6-(2-Hydroxy-5-methylbenzoyl) pyrido[2,3-*d*]pyrimidine-2,4-(1H,3H)-dione (60)

Synthesized by method 1; yellow solid; yield: 62%; mp 300–302 °C (degrad.); HPLC: 96.73%; IR (KBr): 3448, 3195, 3063, 2834, 1727, 1686, 1610, 1590, 1474, 1443, 1363, 1340, 1296, 1250, 1239, 1210, 1178, 1144, 1042, 1024 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 12.09 (s, 1H), 11.64 (s, 1H), 10.08 (s, 1H), 8.87 (d, J = 3 Hz, 1H), 8.35 (d, J = 3.2 Hz, 1H), 7.28 (d, J = 8.4 Hz, 1H), 7.19 (s, 1H), 6.90 (d, J = 8.1 Hz, 1H), 2.24 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 192.2, 160.6, 153.9, 153.2, 152.7, 148.9, 136.1, 132.9, 128.8, 126.9, 122.9, 115.3, 108.0, 18.5; MS (ESI): *m/z* 296.1 $[M-1]^+$; analysis for $C_{15}H_{11}N_3O_4$ (297.1) calcd, C 60.61; H 3.73; N 14.14; found, C 60.13, H 3.75, N 14.38.

4.5. Demethylation: synthesis of 28-30

To a stirred solution of **26** or **27** in dry CH_2Cl_2 at −48 °C, BF_3–DMS (3 equiv) was added dropwise and reaction mixture was stirred further at room temperature for 8–10 h. The reaction mixture was neutralized by $NaHCO_3$ and extracted with CH_2Cl_2. Solvent was evaporated on vacuo rotavapor and crude residue was purified by silica gel (100–200 mesh) column chromatography using CH_2Cl_2: MeOH (99:1).

4.5.1. 5-(1-Ethoxybutyl-2,3,4-triol)-3-(4-hydroxy phenyl)-1-phenyl-*1H*-pyrazolo[3,4-*b*]pyridine (28)

Yellowish brown viscous liquid; yield: 30%; HPLC: 98.53%; ^1H NMR (300 MHz, $CDCl_3$): δ 8.70 (d, J = 1.8 Hz, 1H), 8.53 (d, J = 1.8 Hz, 1H), 8.30 (d, J = 8.4 Hz, 2H), 7.94 (d, J = 6.9 Hz, 2H), 7.55 (t, J = 7.5 Hz, 2H), 7.34 (t, J = 7.2 Hz, 1H), 6.99 (d, J = 8.7 Hz, 2H), 4.84 (d, J = 4.2 Hz, 1H), 3.99 (dd, J = 4.5, 7.8 Hz, 1H), 3.73 (dd, J = 3.3, 11.4 Hz, 1H), 3.68–3.59 (m, 1H), 3.56–3.43 (m, 2H), 1.22 (t, J = 7.2 Hz, 3H); MS (APCI): *m/z* 336 $[M+1]^+$; analysis for $C_{24}H_{25}N_3O_5$ (435.12) calcd, C, 66.19; H, 5.79; N, 9.65; found, C, 66.12; H, 5.64; N, 9.71.

4.5.2. 5-(1-Ethoxybutyl-2,3,4-triol)-3-methyl-1-phenyl-*1H*-pyrazolo[3,4-*b*]pyridine (29)

Yellow oil; yield: 32%; HPLC: 97.03%; ^1H NMR (300 MHz, $CDCl_3$): δ 8.59 (d, J = 3 Hz, 1H), 8.24 (d, J = 8.9 Hz, 2H) 8.07 (d, J = 3 Hz, 1H), 7.51 (t, J = 6 Hz, 3H), 7.31-7.26 (m, 2H), 4.73 (d, J = 3 Hz, 1H), 3.81 (s, 1H), 3.52-3.45 (m, 3H), 2.67 (s, 3H), 1.22 (t, J = 7.2 Hz, 3H); MS (APCI): *m/z* 358.2 $[M+1]^+$; 380.1 $[M+Na]^+$; analysis for $C_{19}H_{23}N_3O_4$ (357.16) calcd, C, 63.85; H, 6.49; N, 11.76; found, C, 63.69; H, 6.38; N, 11.59.

4.5.3. 5-(4-Methoxy-1-ethoxybutyl-2,3-diol)-3-methyl-1-phenyl-*1H*-pyrazolo[3,4-*b*]pyridine (30)

Yellowish brown viscous liquid; yield: 30%; HPLC: 98.21%; ^1H NMR (300 MHz, $CDCl_3$,): δ 8.60 (s, 1H), 8.25 (d, J = 8.8 Hz, 2H), 8.09 (s, 1H), 7.52 (t, J = 6.2 Hz, 2H), 7.28 (t, J = 6.4 Hz, 1H), 4.88 (d, J = 3 Hz, 1H), 3.80–3.71 (m, 2H), 3.59-3.33 (m, 2H), 3.39 (s, 3H), 2.67 (s, 3H), 1.27 (t, J = 6 Hz, 3H); MS (APCI): *m/z* 372.2 $[M+1]^+$; 394.2 $[M+Na]^+$; analysis for $C_{20}H_{25}N_3O_4$ (371.18) calcd, C, 64.67; H, 6.78; N, 11.31; found, C, 64.54; H, 6.65; N, 11.19.

4.6. Deacetylation: synthesis of 44–45

To the solution of **38** or **41** in methanol, potassium carbonate (0.5 equiv) was added and reaction mixture was stirred at room temperature for 30 min. Water was added to reaction mixture and extracted with EtOAc (3× 150 mL). Solvent was evaporated under vacuum and purified by column chromatography over silica gel (100–200 mesh) using 50% EtOAc/petroleum ether as eluent.

4.6.1. 6-Propyl-3-ol-[1,2,4]triazolo[1,5-*a*]pyrimidine (44)

Brownish sticky solid; yield: 65%; HPLC: 97.05%; IR (KBr): 3366, 2929, 1628, 1450, 1322, 1102 cm^{-1}; ^1H NMR (300 MHz, CD_3OD): δ 9.09 (s, 1 H), 8.86 (s, 1H), 8.53 (s, 1H), 3.65 (t, J = 6.3 *Hz*, 2H), 2.91 (t, J = 8.1 Hz, 2H), 2.05–1.88 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$ + DMSO-d_6 = 4:1): δ 155.0, 153.9, 132.2,

123.0, 58.4, 31.2, 24.1; MS (APCI): *m/z* 179.2 $[M+1]^+$; analysis for $C_8H_{10}N_4O$ (178.1) calcd, C 53.92; H 5.66; N 31.44; found, C 52.02, H 5.38, N 33.05.

4.6.2. 5-Propyl-3-ol-1-ethyl-1H-pyrazolo[3,4-*b*] pyridine (45)

Greenish viscous liquid; yield: 60%; HPLC: 99.08%; IR (neat): 3584, 3384, 1612, 1569, 1508, 1457, 1414, 1299, 1260, 1057 cm^{-1}; ^1H NMR (300 MHz, CD_3OD): δ8.43 (d, *J* = 1.2 Hz, 1H), 8.01 (d, *J* = 1.2 Hz, 1H), 8.04 (s, 1H), 4.54 (q, *J* = 7.2, 14.4, 2H), 3.60 (t, *J* = 6.3 *Hz*, 2H), 2.85 (m, 2H), 1.93–1.86 (m, 2H), 1.47 (t, *J* = 7.5 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 147.9, 146.6, 129.3, 128.1, 127.0, 113.8, 59.8, 40.3, 32.7, 27.3, 13.2; MS (APCI): *m/z* 206 $[M+1]^+$; analysis for $C_{11}H_{15}N_3O$ (205.1) calcd, C, 64.37; H, 7.37; N, 20.47; found, C, 63.97; H, 7.27; N, 20.03.

4.7. Assay for TNF-α and IL-6 inhibition

Proinflammatory cytokine production by lipopolysaccharide (LPS) in THP-1 cells was measured according to the method described by Hwang et al., 1993.[33] Briefly, THP-1 cells were cultured in RPMI 1640 culture medium (Gibco BRL, Pasley, UK) containing 100 U/mL penicillin and 100 mg/mL streptomycin (100× solution, Sigma Chemical Co. St. Louis, MO) containing 10% fetal bovine serum (FBS, JRH). Cells were differentiated with phorbol myristate acetate (PMA, Sigma). Following cell plating, the test compounds or vehicle (0.5% DMSO) was added to each well and the plate was incubated for 30 min at 37 °C. Finally, LPS (*Escherichia coli* 0127:B8, Sigma Chemical Co., St. Louis, MO) was added, at a final concentration of 1 μg/mL. Plates were incubated at 37 °C for 24 h, 5% CO_2. Supernatants were harvested, and assayed for TNF-α and IL-6 by ELISA as described by the manufacturer (BD Biosciences). The cells were simultaneously evaluated for cytotoxicity using CCK-8 from Dojindo Laboratories. Percent inhibition of cytokine release compared to the control was calculated. The 50% inhibitory concentration (IC_{50}) values were calculated by a nonlinear regression method.[33]

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