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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

The synthesis, anti-inflammatory, and anti-microbial activity evaluation of new series of 4-(3-arylureido)phenyl-1,4-dihydropyridine urea derivatives

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Abstract A new series of 4-(3-arylureido)phenyl-1,4-dihydropyridine urea derivatives were synthesized using simple three component condensation, reduction, and nucleophilic addition sequence in moderate to good yields. All the synthesized compounds **6a–j** were evaluated for their anti-inflammatory [against the pro-inflammatory cytokines (tumor necrosis factor-alpha, TNF-α and interleukin-6, IL-6)] and anti-microbial activity (anti-bacterial and anti-fungal). Among all the compound screened, the compound **6b**, **6f**, and **6j** were found to have promising anti-inflammatory activity, 74–83 % TNF-α and 91–96 % IL-6 inhibitory activity, respectively as compared to the standard dexamethasone (71 and 86 % inhibition) but at the MIC of 10 μM/ml. The compounds **6d–e** and **6h** exhibited relatively lower TNF-α and IL-6 inhibitory activity and found to be moderately potent anti-inflammatory agents. The compounds **6c–e**, **6g**, and **6i** were found to be promising anti-bacterial and anti-fungal agents and remarkably some of the new compounds, viz. **6d** and **6i** were found be more potent than the standard ciprofloxacin or miconazole. It is to be noted that this is the first report on the anti-inflammatory activity evaluation of novel 1,4-dihydropyridine urea derivatives against the important molecular target, TNF-α, and IL-6.

Keywords 1,4-Dihyropyridine · Urea derivatives · Anti-inflammatory · Anti-bacterial · Anti-fungal

Introduction

1,4-Dihydropyridine (1,4-DHP) derivatives is an important class of bioactive molecules in the pharmaceutical field (Stout and Meyers, 1982). They possess anti-inflammatory, anti-microbial (Surendra Kumar *et al.*, 2011), anti-oxidant, and anti-ulcer activities (Swarnalatha *et al.*, 2011). DHPs are commercially used as calcium channel blocker for the treatment of cardiovascular diseases, including hypertension (Gaudio *et al.*, 1994). Recently, the synthesis of DHPs with respect to multidrug resistance reversal in tumor cell gave a new dimension to their application (Tanabe *et al.*, 1998; Tasaka *et al.*, 2001). In addition, 1,4-DHPs are excellent starting synthons for development of anti-tubercular agent (Eharkar *et al.*, 2002; Desai *et al.*, 2001). Oxidative aromatization reactions of DHPs are taking place in biological systems in the presence of certain enzymes. The nitrogen heterocycles thus prepared by Hantzsch method are of great importance because of their role in biological system. They have been served as model compounds for the NAD-NAPH biological redox systems (Vijesha *et al.*, 2011; Schellenberg and Weheimer, 1965; Kirschbaum, 1968; Norcross *et al.*, 1962).

Though, anti-microbial activity of 1,4-dihydropyridines derivatives has been studied and well established in the literature (Vijesha *et al.*, 2011; Sirisha *et al.*, 2011). However, there are no reports on the anti-inflammatory

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activity of the 1,4-dihydropyridines and most importantly, the potential of 1,4-dihydropyridines nucleus as to their anti-inflammatory activity against the pro-inflammatory cytokines (tumor necrosis factor-alpha, TNF-α and interleukin-6, IL-6) hitherto remained untested.

Non-steroidal anti-inflammatory drugs (NSAIDs) are therapeutically important in the treatment of rheumatic arthritis and in various types of inflammatory conditions, but their therapeutic utility has been limited due to their frequently observed gastrointestinal side effects. Thus, there is an urgent need for new targets that are required for the design and development of novel anti-inflammatory agents as an alternative to NSAIDs (Shishoo *et al.*, 1999). TNF-α and IL-6, the two important multifunctional pro-inflammatory cytokines that are involved in the pathogenesis of autoimmune, inflammatory, cardiovascular, neurodegenerative, and cancer diseases through a series of cytokine signaling pathways (Krishnamoorthy and Honn, 2006; Dominic and Raj, 2009). IL-6 contributes to the initiation and extension of the inflammatory process and considered as a central mediator in a range of inflammatory diseases but has not received the desired attention in drug discovery. TNF-α and IL-6 are thus pharmaceutically important molecular targets for the treatment of the above-mentioned diseases.

We have previously reported the anti-inflammatory and anti-microbial activity of novel 3,4-dihydropyrimidin-2(1H)-ones urea derivatives (Tale *et al.*, 2011). Encouraged by the results of our previous work, and to further expand the scope of 1,4-dihydropyridines derivatives as privileged medicinal scaffold, herein, we disclose our results on the synthesis, anti-inflammatory, and anti-microbial activity evaluation of novel 1,4-dihydropyridines urea derivatives.

Results and discussion

Chemistry

Our synthetic strategy for novel 4-(3-arylureido)phenyl-1,4-dihydropyridinurea derivatives is illustrated in Scheme 1. The key intermediate, 4-nitrophenyl-2,6-dimethyl-1,4-dihydropyridine-3,5-diethylcarboxylate **4** was synthesized by the three component condensation of 4-nitrobenzaldehyde **1**, ethyl acetoacetate **2,** and ammonium acetate **3** using PTSA as a catalyst in acetonitrile under reflux in high yield. The reduction of **4** with $SnCl_2$ in ethyl acetate at 40 °C readily afforded the corresponding amino derivative **5** in almost quantitative yield. Then, the treatment of the **5** with different arylisocyanate in THF at room temperature afforded the corresponding 4-(3-arylureido)phenyl-2,6-dimethyl-1,4-dihydropyridine-3,5-diethylcarboxylate derivatives **6a–j** in moderate to good yields. It is to be noted that the reactions of 4-amionophenyl-1,4-DHP **5** with different arylisocyanates were so rapid that the reactions were to be stopped shortly (within 2 min) failing which the formation of the side products and decrease in yield was observed. Thus, ten different 4-(3-arylureido)phenyl-1,4-dihydropyridinurea derivatives **6a–j** were obtained in moderate to good yields using simple three component condensation, reduction, and nucleophilic addition reaction sequence and straightforward starting material under mild conditions. The purity of the newly synthesized compounds was checked by TLC and HPLC. The ^{1}H NMR and mass spectral data was found to be consistent with structures of the newly synthesized 1,4-DHP analogs.

Anti-inflammatory activity

Having secured a series of structurally diverse 4-(3-arylureido)phenyl-1,4-dihydropyridine derivatives, next their anti-inflammatory and anti-microbial activity was evaluated. The results of the anti-inflammatory, anti-bacterial, and anti-fungal activity are collected in Tables 1, 2, and 3 respectively. As shown in Table 1, among all the compounds screened, compound **6b**, **6f**, **6j** exhibited promising TNF-α and IL-6 inhibitory activity ranging from 74–83 and 93–96 %, respectively at MIC of 10 μM. The other compounds, **6d–e** and **6h** exhibited comparable or little less anti-inflammatory activity to that of standard dexamethasone. It is noteworthy that the compounds **6b** (83 and 91 %) and **6j** (78 and 96 %) exhibited even higher TNF-α and IL-6 inhibitory activity than the standard dexamethasone (71 and 80 % TNF-α and IL-6 inhibition, respectively) but at MIC of 10 μM/ml. Thus, the two compounds **3b** and **3j** were found to be the potent anti-inflammatory agents among the series of compounds **6a–j**. The remaining compounds of this series found to have no activity at all or very low activity against TNF-α and IL-6.

Anti-microbial activity

Regarding anti-bacterial and anti-fungal activity (Tables 2, 3) compounds **6c–d** and **6i** exhibited broad spectrum anti-bacterial activity against all the bacteria, whereas the compounds **6c–e**, **6g**, and **6i** found to be the potent anti-fungal agents against all the fungi at the MIC ranging from 10 to 35 μM/ml. Remarkably, while the compounds **6e** and **6i** exhibited almost twofold more anti-bacterial activity that the standard ciprofloxacin almost against all the bacterial stain tested, the compounds **6d** and **6i** exhibited much higher (50 % higher) anti-fungal activity than the standard miconazole against the selected fungi. Thus, the compounds **6d–e** and **6i** found to be even more potent anti-bacterial or anti-fungal agents than standard drugs at the same concentration level.

Scheme 1 Reagents and conditions: (*a*) PTSA, acetonitrile, reflux, 3 h: (*b*) $SnCl_2$, EtOAc, 40 °C, 4 h: (*c*) different substituted isocyanates, THF, rt, 2 min

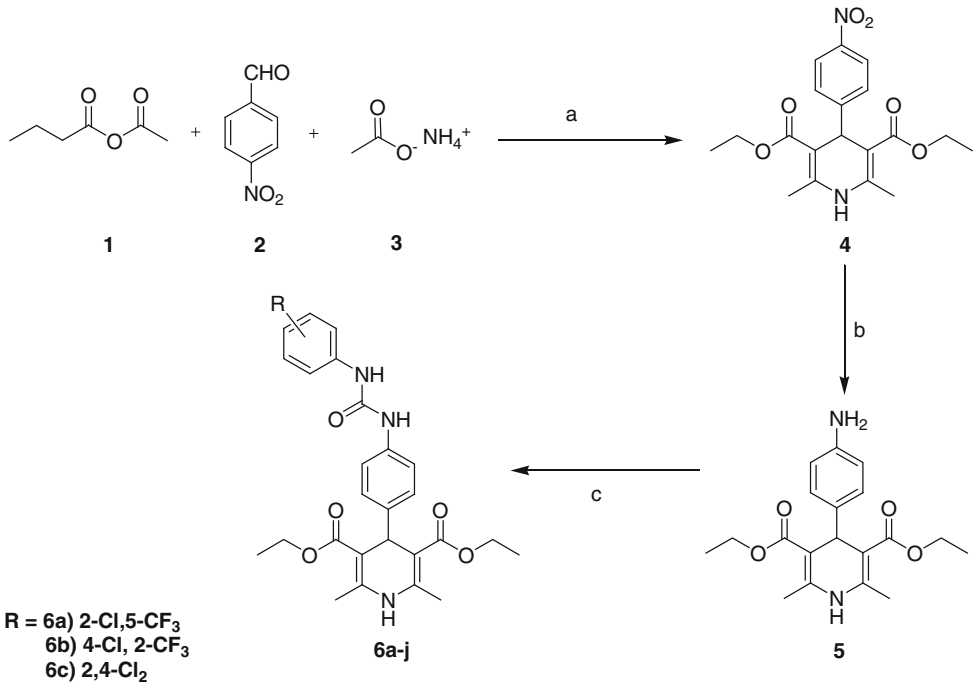

Table 1 Antibacterial activity of thioanalogs of 4-(3-Arylureido) Phenyl-1, 4-Dihydropyridine urea derivatives (minimum inhibitory concentration[a] values μg/ml)

Compounds	Gram-positive *Staphylococcus aureus*	*Bacillus subtilis*	Gram-negative *E. coli*	*Salmonella typhimurium*
4	90	–	90	90
5	60	85	40	45
6a	80	–	65	90
6b	55	70	45	60
6c	20	35	10	20
6d	15	25	15	15
6e	45	50	25	25
6f	60	85	90	90
6g	65	80	20	35
6h	40	80	65	65
6i	10	10	15	10
6j	–	–	70	90
Ciprofloxacin	20	25	20	25

No activity was observed up to 200 μg/ml

[a] Values are the average of three reading

Table 2 Antifungal activity of thioanalogs of 4-(3-Arylureido) Phenyl-1, 4-Dihydropyridine urea derivatives (minimum inhibitory concentration[a] values μg/ml)

Compounds	*Candida albicans*	*Aspergillus niger*	*Fusarium solani*	*Aspergillus flavus*
4	–	–	90	–
5	90	–	90	90
6a	65	45	90	90
6b	50	85	60	80
6c	15	10	25	20
6d	10	30	10	20
6e	35	20	20	35
6f	45	55	90	70
6g	30	20	20	20
6h	65	45	85	85
6i	20	10	25	20
6j	85	90	–	–
Miconazole	25	20	15	20

No activity was observed up to 200 μg/ml

[a] Values are the average of three reading

We believe that the incorporation of arylureido moiety onto the 4-substituted DHP core might have provided new chemicals space which resulted into favorable biological effect to the desired target. Thus, the prominent role of ureido moiety of this novel 1,4-DHP scaffold to impart the various activities evident by the fact that neither parent 4-nitrophenyl-1,4 DHP, **4** nor the corresponding amino

Table 3 Anti-inflammatory activity data

Compounds	% Inhibition at 10 μM	
	TNF-α	IL-6
4	0	0
5	0	0
6a	0	12
6b	83	91
6c	11	24
6d	36	51
6e	28	54
6f	74	86
6g	0	18
6h	42	68
6i	0	8
6j	78	96
Dexamethasone (1 μM)	71	80

analog 5 (Scheme 1) exhibited any TNF-α or IL-6 inhibitory activity nor the significant anti-microbial activity.

Regarding structure–activity relationship, biological activity data suggested the substituents on terminal benzene ring of ureido moiety has remarkable effect on the anti-inflammatory and anti-microbial activity. Thus, the compounds **6b**, bearing 2-CF_3, 4-Cl; and **6j**, bearing 2,5-F_2, the most potent anti-inflammatory agents from the series were found to be completely inactive as anti-bacterial or anti-fungal agents. However, the compounds with Cl, F, and CN etc. at one or more than one positions found to be suitable for high anti-bacterial and anti-fungal activity. The positions 2,4 or 2,5 were to found to be most suitable for the favorable effect on these activities but this seems not to be the general trend. This is evident by the fact that while compound **6d** with 2,5-Cl_2 found to be potent anti-microbial agent, **6e** bering 2,4-F_2 (Tables 2, 3, entry 7) exhibited moderate anti-bacterial activity and high anti-fungal activity, compound **6j** bearing 2,5-F_2 (Tables 2, 3, entry 12) on the other hand found to be completely ineffective as anti-bacterial and anti-fungal agent. Also, very high potency of the compound **6i** possessing 4-CN group, implicates that the presence of electron withdrawing substituent is favorable for high anti-microbial activity.

Conclusion

In conclusion, we have developed the novel approach for the synthesis of structurally diverse 4-(3-arylureido)phenyl-1,4-dihydropyridine urea derivatives using simple reaction sequence under mild conditions and their anti-inflammatory and anti-microbial activity was evaluated. The biological activity data of newly synthesized 1,4-DHP analogs suggested that the compound **6b**, **6f**, and **6j** exhibits the promising TNF-α and IL-6 inhibitory activity, while the compounds **6c–e**, **6g**, and **6i** found to be the potent anti-bacterial and anti-fungal agents.

Experimental

General techniques

All reagent used were of synthesis grade (Thomas Baker). ^{1}H NMR spectra were recorded on Bruker Advance spectrometer (300 or 500 MHz) using tetramethylsilane as internal standard. Chemical shifts are reported in ppm (δ) relative to the solvent peak, mass spectra were recorded on either GCMS (focus GC with TSQ II mass analyzer and thermoelectro) with auto sampler/direct injection (EI/CI) or LCMS (APCI/ESI; Bruker Daltanoics Micro TOFQ). HPLC purity was checked using Water Alliances or Dionex Ultima 3000 HPLC system. All purifications were done by Silica gel column chromatography (100–200#). Ethyl acetate and petroleum ether were used as mobile phase for TLC (Merck Kiesel 60 F254, 0.2 mm thickness sheet).

Chemistry

*Synthesis of diethyl 2,6-dimethyl-4-(4-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate (**4**)*

In a solution of ethyl acetoacetate (**1**) (6.62 mmol), 4-nitrobenzaldehyde (**2**) (6.62 mmol), and ammonium acetate (**3**) (6.62 mmol), in acetonitrile (30 ml), PTSA (0.66 mmol) was added then the resultant reaction mixture heated at 80 °C for a period of 12 h. After completion of reaction the reaction mixture was poured into ice cold water and extracted with ethyl acetate (50 × 3), organic layer concentrated under vacuum to get crude compound which was purified by silica gel column chromatography (100–200#). Yellow solid. Yield 68 %, ^{1}H NMR (DMSO, 300 MHz): 1.22 (t, $J = 7.6$ Hz, 6H), 2.28 (s, 6H), 4.12 (q, $J = 7.6$ Hz, 4H), 5.54 (s, 1H), 7.48–7.44 (m, 2H), 8.11–8.07 (m, 2H). MS (APCI); *m/z* 375.2 $[M+1]^+$.

*Synthesis of diethyl 4-(4-(3-(2-chloro-5-(trifluoromethyl)phenyl)ureido)phenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (**6a**)*

In a solution of diethyl 4-(4-aminophenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (**4**) (0.29 mmol) in THF (5 ml), 2-chloro-5-trifluoromethylphenylisocyanate (0.29 mmol) was added then the resultant reaction mixture was stirred at room temperature for a period of 2.5 min. After completion of reaction, the reaction mixture was

poured into ice cold water and extracted with ethyl acetate (20 × 3), organic layer concentrated under vacuum to get crude compound which was purified by silica gel column chromatography (100–200#). Pale yellow solid. Yield 19 %, mp 214–216; ^{1}H NMR (DMSO, 300 MHz): 1.12 (t, J = 7.2 Hz, 6H), 2.25 (s, 6H), 3.98 (q, J = 7.2 Hz, 4H), 4.80 (s, 1H), 7.08 (d, J = 8.8 Hz, 2H), 7.29 (d, J = 8.4 Hz, 2H), 7.35 (dd, J = 8.4, 2 Hz, 1H), 7.70 (d, J = 8.4 Hz, 1H), 8.57–8.63 (m, 2H), 8.79 (s, 1H), 9.44 (s, 1H). MS (APCI); m/z 566.3 $[M+1]^+$.

*Synthesis of diethyl 4-(4-(3-(4-chloro-3-(trifluoromethyl) phenyl) ureido) phenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (**6b**)*

The synthetic method followed the same as for **6a**. Pale yellow solid. Yield 33 %, mp 222–226; ^{1}H NMR (DMSO, 300 MHz): 1.12 (t, J = 6.8 Hz, 6H), 2.25 (s, 6H), 3.98 (q, J = 6.8 Hz, 4H), 4.79 (s, 1H), 7.06 (d, J = 8.8 Hz, 2H), 7.27 (d, J = 8.4 Hz, 2H), 7.71–7.69 (m, 2H), 7.99 (d, J = 8.8 Hz, 1H), 8.10 (s, 1H), 8.77 (s, 1H), 9.29 (s, 1H). MS (APCI); m/z 566.7 $[M+1]^+$.

*Synthesis of diethyl 4-(4-(3-(3,4-dichlorophenyl) ureido) phenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (**6c**)*

The synthetic method followed the same as for **6a**. Off white solid. Yield 29 %, mp 216–218; ^{1}H NMR (DMSO, 300 MHz): 1.14 (t, J = 7.2 Hz, 6H), 2.25 (s, 6H), 3.99 (q, J = 6.8 Hz, 4H), 4.80 (s, 1H), 7.06 (d, J = 8.4 Hz, 2H), 7.27 (d, J = 8.4 Hz, 2H), 7.36 (dd, J = 8.8, 2.4 Hz, 1H), 7.60 (d, J = 2.4 Hz, 1H), 8.19 (d, J = 9.2 Hz, 1H), 8.34 (s, 1H), 8.77 (s, 1H), 9.31 (s, 1H). MS (APCI); m/z 533.1 $[M+1]^+$.

*Synthesis of diethyl 4-(4-(3-(2,5-dichlorophenyl) ureido) phenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (**6d**)*

The synthetic method followed the same as for **6a**. Off white solid. Yield 41 %, mp 220–223; ^{1}H NMR (DMSO, 300 MHz): 1.12 (t, J = 7.2 Hz, 6H), 2.25 (s, 6H), 3.99 (q, J = 7.2 Hz, 4H), 4.80 (s, 1H), 7.08 (d, J = 8.8 Hz, 2H),7.29 (d, J = 8.4 Hz, 2H), 7.35 (dd, J = 8.4, 2 Hz, 1H), 7.70 (d, J = 8.4 Hz, 1H), 8.57–8.64 (m, 2H), 8.78 (s, 1H), 9.43 (s, 1H). MS (APCI); m/z 533.1 $[M+1]^+$.

*Synthesis of diethyl 4-(4-(3-(2,4-dichlorophenyl) ureido) phenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (**6e**)*

The synthetic method followed the same as for **6a**. Off white solid. Yield 32 %, mp 217–219; ^{1}H NMR (DMSO, 300 MHz): 1.14 (t, J = 7.2 Hz, 6H), 2.25 (s, 6H), 3.99 (q, J = 7.2 Hz, 4H), 4.80 (s, 1H), 7.06 (d, J = 8.4 Hz, 2H),7.27 (d, J = 8.4 Hz, 2H), 7.36 (dd, J = 8.8, 2.4 Hz, 1H), 7.60 (d, J = 2.4 Hz, 1H), 8.19 (d, J = 9.2 Hz, 2H), 8.34 (s, 1H), 8.77 (s, 1H), 9.31 (s, 1H). MS (APCI); m/z 533.1 $[M+1]^+$.

*Synthesis of diethyl 2,6-dimethyl-4-(4-(3-(4-(trifluoromethyl) phenyl) ureido) phenyl)-1,4-dihydropyridine-3,5-dicarboxylate (**6f**)*

The synthetic method followed the same as for **6a**. Pale yellow solid. Yield 21 %, mp 208–210; ^{1}H NMR (DMSO, 300 MHz): 1.12 (t, J = 7.2 Hz, 6H), 2.24 (s, 6H), 3.99 (q, J = 7.6 Hz, 4H), 4.78 (s, 1H), 7.04 (d, J = 8.4 Hz, 2H), 7.26–7.23 (m, 2H), 7.33–7.31 (m, 2H), 7.48–7.44 (m, 1H), 8.53 (s, 1H), 8.77–8.75 (m, 2H), 8.85 (s, 1H). MS (APCI); m/z 532.1 $[M+1]^+$.

*Synthesis of diethyl 4-(4-(3-(4-chlorophenyl) ureido) phenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (**6g**)*

The synthetic method followed the same as for **6a**. Pale yellow solid. Yield 18 %, mp 213–215; ^{1}H NMR (DMSO, 300 MHz): 1.12 (t, J = 7.2 Hz, 6H), 2.25 (s, 6H), 3.99 (q, J = 6.8 Hz, 4H), 4.79 (s, 1H), 7.04 (d, J = 8.4 Hz, 2H), 7.26–7.23 (m, 2H), 7.33–7.31 (m, 2H), 7.48–7.44 (m, 1H), 8.54 (s, 1H), 8.77–8.75 (m, 2H), 8.86 (s, 1H). MS (APCI); m/z 498.1 $[M+1]^+$.

*Synthesis of diethyl 4-(4-(3-(4-fluorophenyl) ureido) phenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (**6h**)*

The synthetic method followed the same as for **6a**. Off white solid. Yield 18 %, mp 228–223; ^{1}H NMR (DMSO, 300 MHz): 1.12 (t, J = 7.2 Hz, 6H), 2.25 (s, 6H), 3.99 (q, J = 7.2 Hz, 4H), 4.79 (s, 1H), 7.12–7.03 (m, 4H), 7.24 (d, J = 8.8 Hz, 2H), 7.45–7.41 (m, 2H), 8.52 (s, 1H), 8.75–8.65 (m, 2H). MS (APCI); m/z 482.1 $[M+1]^+$.

*Synthesis of diethyl 4-(4-(3-(4-cyanophenyl) ureido) phenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (**6i**)*

The synthetic method followed the same as for **6a**. Off white solid. Yield 18 %, mp 228–231; ^{1}H NMR (DMSO, 300 MHz): 1.12 (t, J = 7.2 Hz, 6H), 2.25 (s, 6H), 3.99 (q, J = 7.2 Hz, 4H), 4.75 (s, 1H), 7.04 (d, J = 8.4 Hz, 2H), 7.24 (d, J = 8.4 Hz, 2H), 8.51 (s, 1H), 8.63 (s, 1H), 8.74 (s, 1H). MS (APCI); m/z 489.1 $[M+1]^+$.

*Synthesis of diethyl 4-(4-(3-(2,5-difluorophenyl) ureido) phenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (**6j**)*

The synthetic method followed the same as for **6a**. Off white solid. Yield 18 %, mp 219–222; ^{1}H NMR (DMSO, 300 MHz): 1.12 (t, $J = 7.6$ Hz, 6H), 2.25 (s, 6H), 3.99 (q, $J = 7.6$ Hz, 4H), 4.80 (s, 1H), 7.08 (d, $J = 8.8$ Hz, 2H), 7.30 (d, $J = 8.4$ Hz, 2H), 7.35 (dd, $J = 8.4$, 2 Hz, 1H), 7.70 (d, $J = 8.4$ Hz, 1H), 8.57 (s, 1H), 8.64 (s, 1H), 8.78 (s, 1H), 9.43 (s, 1H). MS (APCI); *m/z* 500.1 $[M+1]^+$.

Biological assay

Anti-inflammatory assay

Pro-inflammatory cytokine production by lipopolysaccharide (LPS) in THP-1 cells was measured according to the method described by Hwang *et al*. (1993). During assay, THP-1 cells were cultured in RPMI 1640 culture medium (Gibco BRL, Pasley, UK) containing 100 U/ml penicillin and 100 mg/ml streptomycin, 10 % fetal bovine serum (JRH). Cells were differentiated with phorbol myristate acetate (Sigma). Following cell plating, the test compounds in 0.5 % DMSO were added to each well separately 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 1ug/ml in each well. Plates were further incubated at 37 °C for 24 h in 5 % CO_2. After incubation, supernatants were harvested, and assayed for TNF-α and IL-6 by ELISA as described by the manufacturer (BD Biosciences).

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