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A novel pyrimidine derivatives with aryl urea, thiourea and sulfonamide moieties: Synthesis, anti-inflammatory and antimicrobial evaluation

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ABSTRACT

A series of novel 4-(3-(trifluoromethyl)phenylamino-6-(4-(3-arylureiodo/arylthioureido/arylsulfonamido)-pyrimidine derivatives of biological interest were prepared by the sequential Suzuki cross coupling, acid amination, reduction followed by reaction of resulting amine with different arylisocyantes or arylisothiocyantes or arylsulfonyl chlorides. All the synthesized compounds (**1–25**) were screened for their pro-inflammatory cytokines (TNF-α and IL-6) and antimicrobial activity (antibacterial and antifungal). Biological data revealed that among all the compounds screened, compounds **5, 6, 11, 12, 16** and **20** were found to have moderate to potent anti-inflammatory activity (up to 48–78% TNF-α and 56–96% IL-6 inhibitory activity) with reference to standard dexamethasone at 10 μM. The compounds **10, 12, 13, 18, 20, 22, 24** and **25** found to have promising antimicrobial activity against all the selected pathogenic bacteria and fungi.

The treatment of bacterial infections remains a challenging therapeutic problem because of emerging infectious diseases and the increasing number of multidrug-resistant microbial pathogens. Despite the many antibiotics and chemotherapeutics available, the emergence of old and new antibiotic-resistant bacterial strains in the last decades leads to a substantial need for new classes of anti-bacterial agents.[1]

Pyrimidine and their derivatives are continuously attracting attention of the medicinal chemists in view of their profound range of biological activities, anti-HIV-1,[2] analgesic agents,[3] antiproliferative,[4] and antitumor.[5] The pyrimidine nucleus has increasingly attracted the attention of synthetic chemists. Though the antimicrobial activity of pyrimidine derivatives has been extensively studied and well documented in the literature,[6–8] however, relatively reports on the anti-inflammatory activity of the pyrimidine derivatives,[3,9–11] are scare. Moreover, the potential of pyrimidine derivatives bearing urea, thiourea and sulfonamide moieties as to their anti-inflammatory activity against the pro-inflammatory cytokines (TNF-α and IL-6) hitherto remained untested.

Non-steroidal anti-inflammatory drugs (NSAIDs) are therapeutically important in the treatment of rheumatoid 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.[12] Tumor necrosis factor alpha (TNF-α) and interleukin-6 (IL-6), the two important multifunctional pro-inflammatory cytokines are involved in the pathogenesis of autoimmune, inflammatory, cardiovascular, neurodegenerative and cancer diseases through a series of cytokine signaling pathways.[13,14] 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 it has not received the desired attention in drug discovery.[15] TNF-α and IL-6 are thus pharmaceutically important molecular targets for the treatment of the above-mentioned diseases.

In recent years we have been engaged in design, synthesis and anti-inflammatory and antimicrobial activity evaluation of novel urea, thiourea and sulfonamide derivatives of different heterocycles. We have previously reported the synthesis, anti-inflammatory and antimicrobial activity evaluation of novel 3,4-dihydropyrimidin-2(1*H*)-ones urea derivatives.[16] Encouraged by the results of our previous work, and in order to further expand the scope of pyrimidine derivatives as privileged medicinal scaffold, herein we disclose our results on the anti-inflammatory and antimicrobial activity evaluation of novel pyrimidine derivatives bearing urea, thiourea and sulfonamide moieties. Thus a large number 4-(3-(trifluoromethyl)phenylamino-6-(4-(3-arylureiodo/arylthioureido/

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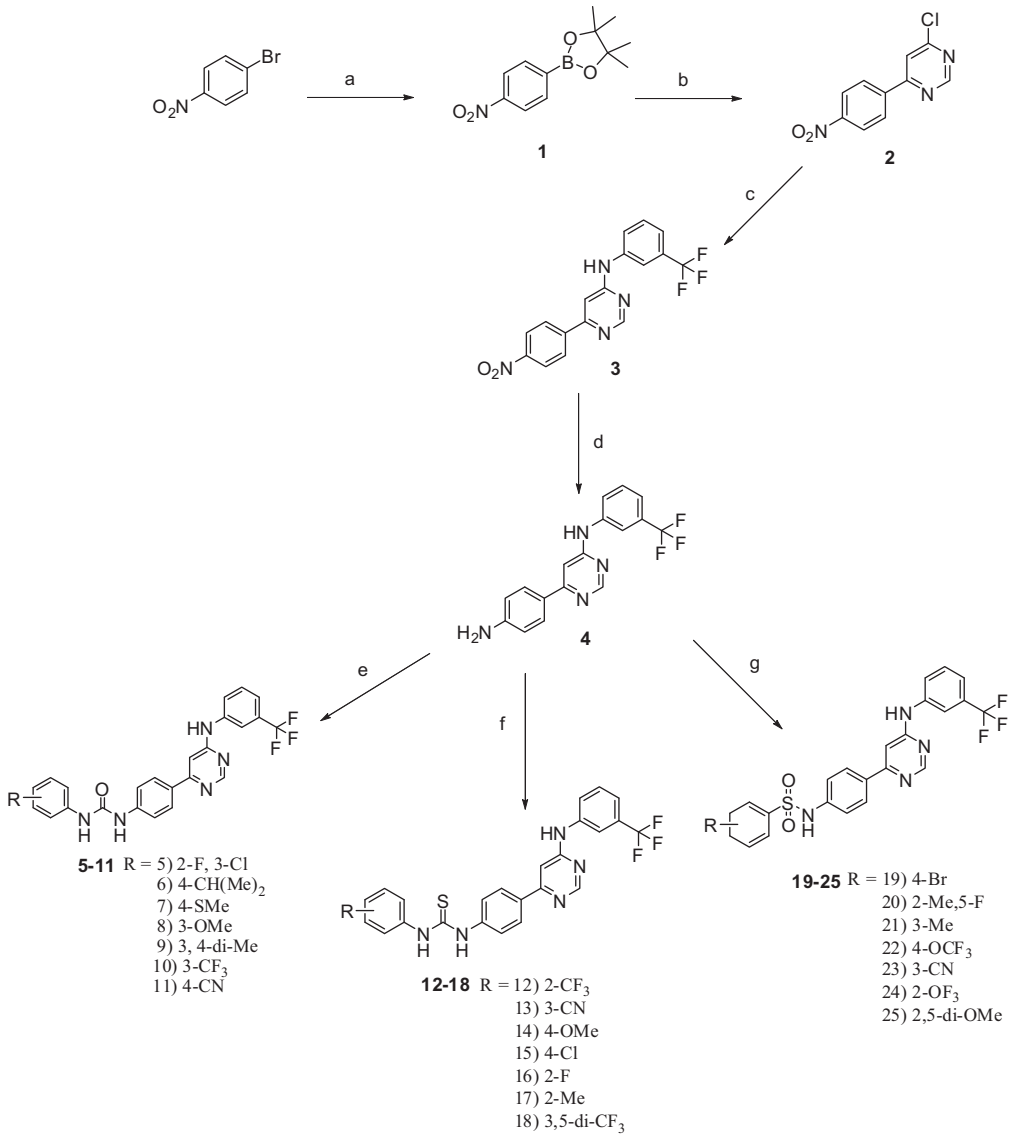
arylsulfonamido)-pyrimidine derivatives were conveniently synthesized and evaluated for their anti-inflammatory and antimicrobial activity.

Our synthetic strategy for the novel 4-(3-(trifluoromethyl) phenylamino-6-(4-(3-arylureiodo/arylthioureido/arylsulfonamido)-pyrimidine derivatives is illustrated in Scheme 1. The key intermediates 4-chloro-6-(4-nitrophenyl) pyrimidine (**2**) was synthesized by Suzuki cross coupling,[17] between 4,4,5,5-tetramethyl-2-(4-nitrophenyl)-1,3,2-dioxaborolane (**1**) and 2,4-dichloropyrimidine, in 53% yield. The microwave assisted amination of (**2**) with 3-(trifluoromethyl) aniline in 2-propanol, under acidic conditions afforded 4-(3-(trifluoromethyl) phenyl amino)-6-(4-nitrophenyl) pyrimidin (**3**) in 73% yield.[17] The desired amino analog (**4**) to be used in subsequent nucleophlic addition reaction, was obatined by reduction of (**3**) using $SnCl_2$ in ethyl acetate at room temperature in 67% yield. The purity of the compounds was checked by TLC and HPLC. Spectral data ^{1}H NMR, ^{13}C NMR, IR and MS of the newly synthesized compounds **1–25** was consistent with the proposed structures.

Having secured a series of structurally diverse 4-(3-(trifluoromethyl) phenyl amino-6-(4-(3-arylureiodo/arylthioureido/arylsulfonamido)-pyrimidine derivatives, next in-vitro anti-inflammatory and antimicrobial activity has been screened for the new compounds. The results of the anti-inflammatory, antibacterial and antifungal activity are collected in Tables 1–3 respectively. As shown in Table 1 six compounds, **5, 6, 11, 12, 16** and **20** found to be active against proactive kinas TNF-α and interleukin-6. Especially, compound **5**, found to be more active while **6** exhibited similar activity with respect to the standard dexamethasone at 10 μM concentration. The compounds **11, 12, 16** and **20**



Reagents andConditions :- a) Bis-pinacoloto diborane, Potassium acetate, $Pd(OAc)_2$, DMF, 80 °C, 2 h.
b) 2,4-dichloropyrimidine/Sodiumo carbonate, $Pd(PPh_3)_2Cl_2$, DME/EtOH/Water.
MW 120 C, 20 min.
c) 3-(trifluoromethyl)aniline/Iso-propanol, conc. HCl, MW 100 °C, 20 min.
d) $SnCl_2$/EtOAc, r.t. 12 h. e) Isocynate/THF, r.t. 1-2 h.
f) Isothiocynate/THF, r.t. 1-2 h. g) Solfonyl chlorides /DCM, Et_3N, r.t. 1-2 h.

Scheme 1. Synthesis of novel pyrimidine derivatives containing urea, thiourea, sulfonamide moieties.

Table 1
Anti-inflammatory activity of novel pyrimidine derivatives containing urea, thiourea, sulfonamide moieties

Compounds	% Inhibition at 10 μM NF-α	IL-6
1	0	0
2	0	0
3	0	0
4	0	6
5	78	96
6	71	90
7	0	0
8	11	18
9	16	27
10	28	40
11	61	80
12	68	82
13	11	16
14	3	8
15	23	28
16	50	62
17	0	0
18	0	0
19	6	11
20	48	56
21	14	25
22	0	8
23	0	12
24	0	0
25	8	26
Dexamethasone(1 μMl)	72	86

Table 2
Antibacterial activity of novel pyrimidine derivatives containing urea, thiourea, sulfonamide moieties (MIC[a] values μg/mL)

Compounds	Gram-positive		Gram-negative	
	S. aureus	*B. subtilis*	*E. coli*	*S. typhimurium*
1	—	—	—	—
2	—	—	—	—
3	—	—	—	—
4	—	—	—	—
5	90	—	—	—
6	25	40	20	40
7	75	90	90	90
8	35	30	30	30
9	90	90	—	—
10	25	40	35	80
11	—	—	—	—
12	—	—	—	—
13	90	—	90	90
14	—	—	—	—
15	35	35	30	30
16	90	90	90	—
17	—	—	—	—
18	20	25	25	25
19	80	—	—	—
20	15	10	15	15
21	90	—	90	90
22	15	20	15	10
23	40	65	25	45
24	10	10	20	10
25	45	30	25	35
Ciprofloxacin	20	15	15	20

No activity was observed up to 200 μg/ml.
[a] Values are the average of three reading.

Table 3
Antifungal activity of novel pyrimidine derivatives containing urea, thiourea, sulfonamide moieties (MIC[a] values μg/mL)

Compounds	*C. albicans*	*A. niger*	*F. solani*	*A. flavus*
1	90	—	—	—
2	90	90	—	90
3	—	—	—	—
4	80	80	90	80
5	90	—	—	—
6	—	—	—	—
7	90	—	90	90
8	55	35	20	20
9	40	75	60	30
10	25	35	20	20
11	80	90	75	80
12	15	25	15	15
13	20	40	20	20
14	80	—	90	90
15	—	—	90	—
16	90	—	90	90
17	—	—	—	—
18	90	—	75	—
19	80	95	0	95
20	90	—	90	90
21	—	—	—	—
22	—	—	—	—
23	55	20	20	35
24	30	60	20	20
25	25	15	15	40
Miconazole	20	15	15	20

No activity was observed up to 200 μg/ml.
[a] Values are the average of three reading.

were found to have moderate to good activity. TNF-α and interleukin-6 inhibitory activity of thses compounds was in the range of 48–78% and 56–96% respectively but at 10 μM and as compared to the standard (dexamethasone, at 1 μg/ml) they found to be moderately potent anti-inflammatory agents. Thus, the compound **5** found to be most potent anti-inflammatory agent amongst the series of compounds **1–25** and could prove to be promising candidate for drug discovery. The compounds **8, 9, 10, 13, 15, 21** exhibited low to very low anti-inflammatory activity and remaining compounds found to be ineffective as anti-inflammatory agents. It is to be noted that with only exception of compound **20** all of these active compounds viz., **5**, **6**, **11**, 12 and **16** from the newly synthesized series are either urea or thiourea derivatives and unfortunately almost no compounds from the corresponding sulfonamide series was found to be effective TNF-α or IL-6 inhibitor.

In order to serach for the potent compound from these newly synthesized compounds **1–25** were evaluated for in vitro antibacterial and antifungal activity against various Gram-positive, Gram-negative bacteria and fungal strains using agar well diffusion method. The antimicrobial evaluation data is represented in Tables 2 and 3. As can be seen from our results, many compounds from the newly synthesized series found to be potent antibacterial and antifungal agents. Thus the compounds **10, 12, 13, 18, 20, 22, 24** and **25** (Tables 2 and 3) exhibited comparable to or even higher antibacterial and antifungal activity than the standard Ciprofloxacin and Miconazole respectively almost against all the tested bacteria or fungi. Interestingly, the compounds **12**, **20**, **22**, **24** and **25** exhibited potent to same antibacterial and antifungal activity respectively as that of the standard Ciprofloxacin and Miconazole. Thus the compounds **20, 22** and **24** found to be most potent among the series and even more potent than standard ciprofloxacin as antibacterial agent. Moreover, compounds **22** and **24** were found to be more potent antibacterial agents than the standard drug against some bacteria Viz. *Staphylococcus aureus*, *Bacillus subtilis* and *Salmonella typhimurium*. Compounds **12** and **25** found to be good antifungal agents as compared to standard Miconazole. To our surprise, none of the most active anti-inflammatory agents **5**, **6**, **11**, **12**, **16**, and **20** was found to be active antibacterial or antifungal agents against all the bacteria or fungi screened. The remaining compounds of this series were found to have moderate or low activity or no activity.

The structural–activity relationship of these newly synthesized compounds is represented in Tables 1–3. It can be seen from our

result (Table 1) that anti-inflammatory activity can be attributed to the presence of urea or thiourea moiety in the pyrimidine scaffold. This is due to the fact that bearing one exception (compound **20**) all the active candidates, compound **5, 6, 11, 12** and **16** are the pyrimidine derivatives bearing either urea or thiourea moiety and none of the members from the corresponding sulfonamide series (compounds **19–25**) found to be a potent anti-inflammatory agent. This is further supported by the fact that compounds **1–3**, devoid of these functionalities found to be ineffective as the anti-inflammatory agents. The nature of the substituent on benzene ring of ureido or thioureido terminus found to have strong relevance to the biological activities. Thus the presence of functionalities such F, CF_3, Cl and isopropyl etc. were found to be suitable for high potency. Interestingly, in contrast to our previous results, here the presence of bulky lipophilic isopropyl group (compound **6**, 71% and 90% TNF-α and IL-6 inhibition respectively) found to have strong positive effect on the present activity. The position of F or CF_3 group on the ureido or thioureido terminal ring has dramatic effect on the said activity. Our anti-inflammatory activity data confirmed that the substitution of CF_3 at *o*-position is more relevant for increasing the biological activity than at *m* and *p* positions. Thus, while the compound **12** with *o*-CF_3 exhibited 68% and 82% TNF-α and IL-6 inhibitory activity respectively and proved to be a potent antiinflammatrory agent, the compound **18** with 3,5-di-CF_3 groups found to have no TNF-α or IL-6 inhibitory activity at all. Though we have not a concrete evidence in hand in support of the actual role of urea or thiourea moiety on the activity at this time, we can at least speculate that the H-bond donor ability of the urea or thiourea (not present in sulfonamide framework) along with the electronic effect of *ortho* or *para* substituent might be responsible for their high anti-inflammatory activity.

The structural–activity relationship of antibacterial and antifungal activity is represented in Tables 2 and 3 respectively. The compounds **6**, **8**, **10**, **12**, **13**, **18**, **20**, **22**, **24** and **25** exhibited moderate to potent antibacterial and antifungal activity against all the tested bacterial or fungal strains. No particular trend as to the effect of substituent on the antimicrobial activity has been observed. In contrast to the results of anti-inflammatory activity, majority of the active compounds **20, 22, 23, 24** and **25** except compound **6, 8, 10**, **12**, **13**, and **18** are from sulfonamide derivatives. Thus the pyrimidine derivatives bearing Isopropyl, CF_3, OCF_3, CN, and OMe, etc. at 2, 3 or 4-position of terminal ureido and thioureido ring (**10**, **12**, **13**, and **18**) or sulfonamide ring (**22–25**) found to be effective antimicrobial and antifungal agents. Remarkably, the compound **24** found to most potent antibacterial and antifungal agents among the series and exhibited well to same activity as compared to the standard Ciprofloxacin and Miconazole. The compound **6, 8, 10, 15, 23** and **25** exhibited moderate to low activity.

The remaining compounds found to have no antibacterial or antifungal activity. Again as similar to the anti-inflammatory activity, the position 2 and 4 on the terminal ring found to be the most favorable site for positive effect on these activities.

In conclusion, we have synthesized and evaluated the anti-inflammatory and antimicrobial activity of a novel series pyrimidine derivatives with aryl urea, aryl thiourea and aryl sulfonamide moieties. The compound **5**, **6**, 11 and **12** found to be promising anti-inflammatory agents while compounds **10**, 12 and **25** were found to active antibacterial and antifungal agents. With few exceptions, overall it has been observed that the urea or thiourea moiety found to be favorable structural feature for the aninflmmatory activity. Thus the presence of functionalities such as F, CF_3, Cl and isopropyl on benzene ring of ureido or thioureido terminus found to have strong relevance to the anti-inflammatory activity and Isopropyl, CF_3, OCF_3, CN, and OMe etc at 2, 3 or 4-position on benzene ring of ureido, thioureido and sulfonamide terminus found to be effective antimicrobial and antifungal agents.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2012.03.092.

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