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Research Article

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Synthesis and studies of novel piperidine-substituted triazine derivatives as potential anti-inflammatory and antimicrobial agents

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ABSTRACT

A series of some novel 2,4-dimethoxy-6-(piperazin-1-yl)-1,3,5-triazine aryl ureido/aryl amido derivatives of biological interest were prepared and screened for their pro-inflammatory cytokines (TNF-α and IL-6) and antimicrobial activity. Among all the series of compounds screened, the compounds 5c, 5f, 6c, and 6f were found to have promising anti-inflammatory activity (up to 65–73% TNF-α and 73–85% IL-6 inhibitory activity) at concentration of 10 µM with reference to standard dexamethasone (75% TNF-α and 84% IL-6 inhibitory activities at 1µM) while the compounds 5a, 5d, 5e, 6a and 6e found to be potent antimicrobial agent showing even 2 to 2.5-fold more potency than that of standard ciprofloxacin and miconazole at the same MIC value of 10 µg/mL.

Keywords: 1,3,5-triazine, piperazine, aryl urea and amide, anti-inflammatory activity, antimicrobial activity.

INTRODUCTION

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] Non-steroidal anti-inflammatory drugs (NSADs) 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.[2]

Cytokines are intercellular messengers responsible for host defense mechanisms as inflammatory, immune and hematogenic responses. Although many of them are transient, they are produced by various cells acting as urgent response mediators in cases of invasive interventions. Disruption of this biological defense mechanism and continuous excessive cytokine production contributes to pathogenesis of inflammatory diseases. 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.[3]

One of the key pro-inflammatory cytokine Tumor necrosis factor- α (TNF- alpha) is a multitude of biological activities linked to pathology of autoimmune diseases such as rheumatoid arthritis (RA),[4] Crohn's disease,[5] systemic lupus erythematosus,[6] and multiple sclerosis,[7] septic shock,[8] and AIDS.[9] On the other hand, cytokine interleukin-6 (IL-6) (from the series of cytokine signaling pathway) contributes to the initiation and extension of the inflammatory

process and is considered as a central mediator in a range of inflammatory diseases but has not received the desired attention in drug discovery.[10]

TNF-α and IL-6 are thus pharmaceutically important molecular targets for the treatment of the abovementioned diseases. The available biopharmaceuticals (TNF soluble receptor (Enbrel ™) and TNF antibody (Remicade ™) are expensive, difficult to administer orally and have major side effects on prolonged clinical use. Therefore, there is an urgent medical need to discover small molecule agents to deal with higher level production of TNF-α.

Many compounds have been reported to have inhibitory activity against inflammation. Historically, natural products have provided the most consistent source of new scaffolds to design new therapeutic agents, and a number of naturally occurring phenolic compounds have been discovered as potent anti-inflammatory agents. On another side, 1,3,5-triazine ring has been often reported as an important scaffold in many biological targets. For example 1,3,5-triazine derivatives containing various amino groups on the position 2,4 or 6, such as tretamine, furazil and dioxadet, have been reported as anticancer agents.[11] Diaryl amino-triazines have been claimed as ALK kinase inhibitors,[11] Moreover, an anti-gastric ulcer agent that is commonly used in Japan,isogladine(2,4-diamino-6-(2,5-dichlorophenyl)-1,3,5triazine), was shown to possess antiangiogenic properties in connection with an anticancer effect.[12]

The numerous nitrogen containing structural classes (e.g., pyrroles, pyrimidines, pyridines, pyrimidones, indoles, oxyindoles and various fused bicyclic heterocycles) have been reported for their pro-inflammatory cytokine inhibitory activity.[13] Although the biological importance of heterocyclic derivatives of aryl ureas have been reported in the literature. For example, N-2,4- pyrimidine-N,N-phenyl/alkyl ureas were reported to be inhibitor of tumor necrosis factor alpha (TNF-α),[14] SA13353, substituted urea derivatives are reported as a potent inhibitor of TNF-α production,[15] pyrido-quinazolone analogues are reported as antifungal, antibacterial and anticancer agents.

On the basis of recent literature and our logical research to improve the anti-inflammatory and antimicrobial activity of the compound based on triazine core and related scaffold (Fig.1); we investigate that hybrid compound incorporating a piperazine core and urea/amide in single molecular frame could lead to the novel potent anti-inflammatory and antimicrobial agents. Thus as shown in scheme 1, the synthesis, anti-inflammatory and antimicrobial activity evaluation of novel 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-N-phenylpiperazine-1-carboxamide urea derivatives **(5a-h)** and (4-(4,6-dimethoxy-1,3,5-triazin-2-yl)piperazin-1-yl)(phenyl)methanone amide derivatives **(6a-h).**

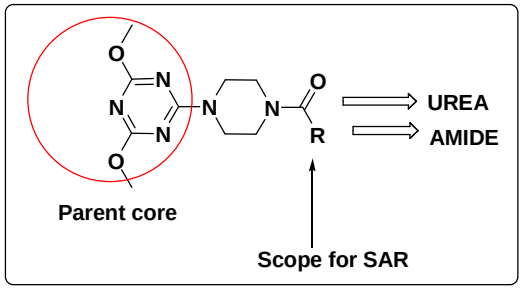
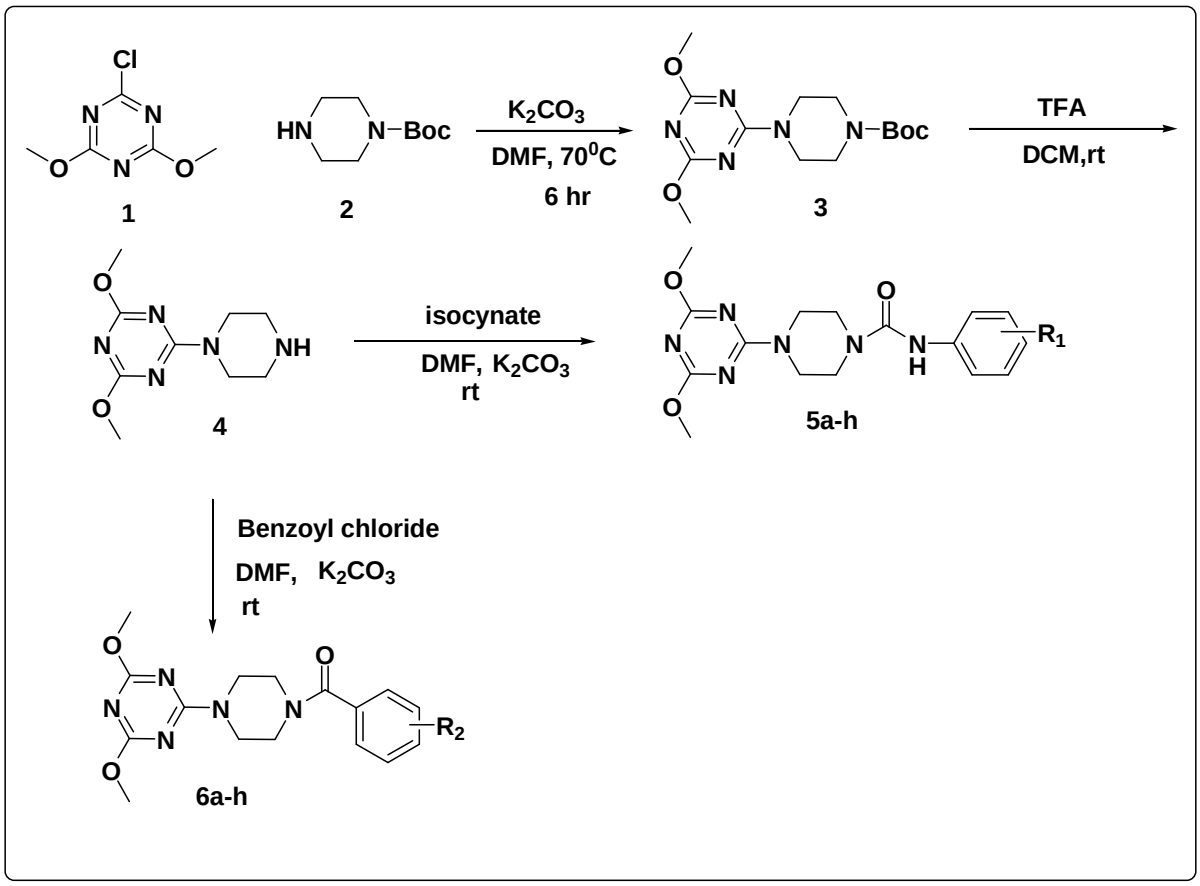


Fig.-1 Design Scaffold

Our synthetic strategy for novel compounds was outlined in Scheme 1. A commercially available 2-chloro-4,6-dimethoxy-1,3,5-triazine **(1)** treated with tert-butyl piperazine-1-carboxylate **(2)** under basic condition to gave tert-butyl 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)piperazine-1-carboxylate **(3).** On acidic deprotection of (3) gives common intermediate **(4)** which is treated with respective isocynate and benzoyl chloride to give 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-N-phenylpiperazine-1-carboxamide urea derivatives **(5a-h)** and (4-(4,6-dimethoxy-1,3,5-triazin-2-yl)piperazin-1-yl)(phenyl)methanone amide derivatives **(6a-h).**

Scheme 1: 2, 4-dimethoxy-6-(piperazin-1-yl)-1, 3, 5-triazine aryl ureido/aryl amido derivatives



	(5a-h)		(6a-h)
R_1 -	4-Methyl	R_2 -	4-Methyl
	4-Chloro		2-Chloro
	3-fluoro		3--fluoro
	4-NO_2		4-NO_2
	Phenyl		Phenyl
	2,4-difluoro		2,4-difluro
	4-Methoxy		4-Methoxy
	4-hydroxy		4-hydroxy

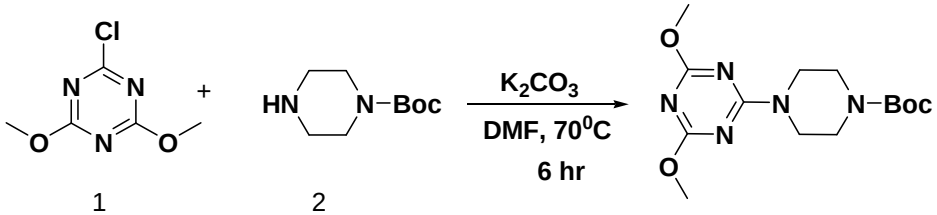
EXPERIMENTAL SECTION

All commercial chemicals and solvents used are of reagents grade and were used without further purification. The thin layer chromatography was performed on Merck pre-coated silica gel 60 F254 plates, with visualization under

UV light. ^{1}H NMR spectra were recorded with Bruker 400 MHz AVANCE instrument and *J* values are in Hertz and chemical shifts (δ) are reported in ppm relative to internal tetra methyl silane(TMS). The mass spectra were measured with Thermo Finnigan-TSQ Quarter Ultra (triple Quad).

Experimental Procedures

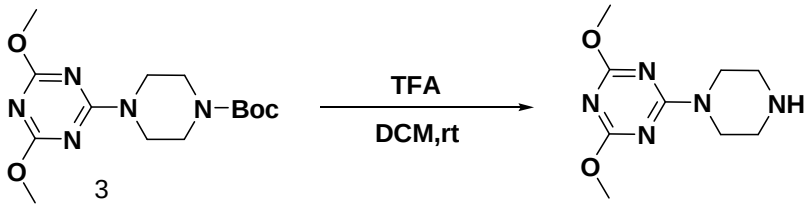
Synthesis of tert-butyl 4-(4, 6-dimethoxy-1, 3, 5-triazin-2-yl) piperazine-1-carboxylate (3)



1 2 3

Procedure- The mixture of 2-chloro-4, 6-dimethoxy-1, 3, 5-triazine [**1]** (1equi.), tert-butyl piperazine-1-carboxylate [**2]** (1equi.) and K_2CO_3 (2 equi.), in DMF (for 1gm, 25 ml) was heated at 70-80^{0}C for 6 hrs. The progress of reaction was monitored by TLC. (80% EtOAc/Hexane)/ (5% MeOH/DCM). After cooling the reaction mixture , water was added to give white precipitate which on filtration gives off a white solid as title compound. The solid compound obtained was dried under vacuum with 80% yield.

Synthesis of 2, 4-dimethoxy-6-(piperazin-1-yl)-1, 3, 5-triazine (4)

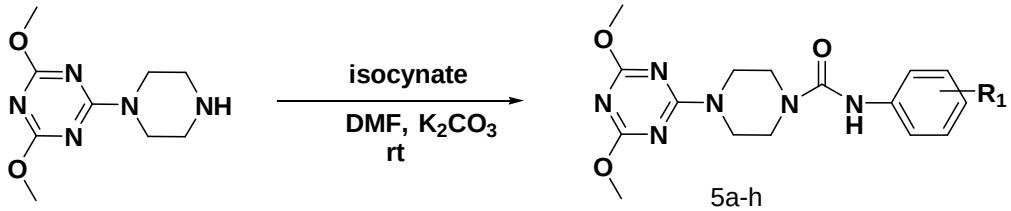


Procedure- To the solution of solution of tert-butyl 4-(4, 6-dimethoxy-1, 3, 5-triazin-2-yl) piperazine-1-carboxylate (1equi.) in DCM was added TFA (2 equi.) at 0^{0}C then stirred it at rt for 3-4 hrs. The reaction was monitored by TLC. (80% EtOAc/Hexane)/ (10% MeOH/DCM).

The reaction mixture was evaporated to give a sticky mass which was precipitated out in hexane. The precipitate on filtration gives off white solid as title compound. The solid compound obtained was dried under vacuum to give 70 % yield.

Derivatives

General procedure for the synthesis of 4-(4, 6-dimethoxy-1, 3, 5-triazin-2-yl)piperazine-1-carboxamide derivatives (5 a-h)



Procedure- To the solution of 2, 4-dimethoxy-6-(piperazin-1-yl)-1, 3, 5-triazine (1equi.), K_2CO_3 (1equi.) in DMF (for 1gm 25 ml) and substituted Isocynate (1equi.) was added at rt. The resulting mixture was stirred at rt for 2-3 hrs. and the progress of reaction was monitored by TLC. (70% EtOAc/Hexane) and (2% MeOH/DCM). On completion of reaction ,water was added to give white precipitate which on filtration gives solid . The resulting solid compound was washed with diethyl ether to give title compound which was dried under vacuum.

Experimental data

4-(4, 6-dimethoxy-1, 3, 5-triazin-2-yl)-N-p-tolylpiperazine-1-carboxamide (5a)

White solid: Yield 62%; ^{1}HNMR (DMSO-d6, 400MHz): δ 10.61 (s, 1H), 7.59 (m, 2H), 7.50 (m, 2H), 4.05 (m, 4H), 3.84 (m, 3H); 3.84 (m, 3H); 3.64 (m, 4H); 3.06 (s, 3H); MS (APCI); *m/z* 358.9 $[M+H]^+$. Anal. Calcd for $C_{17}H_{22}N_6O_3$: C, 56.97; H 6.19; N, 23.45. Found: C, 56.80; H, 6.08; N, 23.39 %.

N-(4-chlorophenyl)-4-(4,6-dimethoxy-1,3,5-triazin-2-yl)piperazine-1-carboxamide (5b)

White solid: Yield 65%; ^{1}HNMR (DMSO-d6, 400MHz): δ 10.31(s, 1H); 7.33 (d, *J* = 8 Hz, 2H), 6.92 (d, *J* = 8 Hz, 2H), 3.87(s, 3H), 3.87(s, 3H), 3.76 (m, 4H), 3.60 (m, 4H); MS (APCI); *m/z* 378.81 $[M+H]^+$. Anal. Calcd for $C_{16}H_{19}N_6O_3Cl$: C, 50.73; H 5.06; N, 22.19. Found: C, 50.63; H, 4.98; N, 22.09 %.

4-(4, 6-dimethoxy-1, 3, 5-triazin-2-yl)-N-(3-fluorophenyl) piperazine-1-carboxamide (5c)

White solid: Yield 66%; ^{1}HNMR (DMSO-d6, 400MHz): δ 9.91(s, 1H); 7.20 (d, *J* = 4 Hz, 2H), 7.08 (d, *J* = 8 Hz, 1H), 6.93 (d, *J* = 8 Hz, 1H), 4.10(m, 2H), 3.72 (m, 4H), 3.38(m, 3H); 3.38 (m, 3H); 3.25 (m, 2H); ^{13}C NMR (75 MHz, DMSO-d_6): δ =171.89, 168.82, 165.22, 155.20, 134.84,131.09, 131.05, 128.16, 110.10, 44.75, 43.19, 42.83, 40.83, 38.58 ppm; MS (APCI); *m/z* 362.9 $[M+H]^+$. Anal. Calcd for $C_{16}H_{19}N_6O_3F$: C, 53.03; H 5.29; N, 23.19. Found: C, 53.00; H, 5.18; N, 23.09 %.

4-(4, 6-dimethoxy-1, 3, 5-triazin-2-yl)-N-(4-nitrophenyl) piperazine-1-carboxamide (5d)

White solid: Yield 60%; ^{1}HNMR (DMSO-d6, 400MHz): δ 10.31(s, 1H); 7.50 (m, 2H), 7.29 (m, 2H), 4.46 (m, 2H), 4.05 (m, 2H); 3.88 (s, 6H); 3.64 (m, 4H); MS (APCI); *m/z* 389.3 $[M+H]^+$. Anal. Calcd for $C_{16}H_{19}N_7O_5$: C, 49.35; H 4.92; N, 25.18. Found: C, 49.23; H, 4.85; N, 25.09 %.

4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-N-phenylpiperazine-1-carboxamide (5e)

White solid: Yield 63%; ^{1}HNMR (DMSO-d6, 400MHz): δ 10.01(s, 1H); 7.75 (m, 3H), 7.59 (d, *J* = 8 Hz, 1H), 7.48 (m, 3H), 4.23(m, 4H), 3.71(s, 3H), 3.71 (s, 3H), 2.84 (m, 4H); ^{13}C NMR (75 MHz, DMSO-d_6): δ =172.89, 169.23, 166.25, 156.61, 135.63, 129.66, 128.43, 126.06, 57.54, 55.30, 39.22, 14.06 ppm; MS (APCI); *m/z* 344.9 $[M+H]^+$. Anal. Calcd for $C_{16}H_{20}N_6O_3$: C, 55.80; H 5.85; N, 24.40. Found: C, 55.73; H, 5.70; N, 24.31 %.

N-(2,4-difluorophenyl)-4-(4,6-dimethoxy-1,3,5-triazin-2-yl)piperazine-1-carboxamide (5f)

White solid: Yield 67%; ^{1}HNMR (DMSO-d6, 400MHz): δ 10.12(s, 1H); 7.14 (m, 1H), 6.84 (d, *J* = 8 Hz, 1H), 6.71 (d, *J* = 8 Hz, 1H), 3.55(s, 3H), 3.55(s, 3H), 3.19 (m, 2H), 3.05 (m, 4H); 2.97 (m, 2H); MS (APCI); *m/z* 380.6 $[M+H]^+$. Anal. Calcd for $C_{16}H_{18}N_6O_3F_2$: C, 50.52; H 4.77; N, 22.10. Found: C, 50.52; H, 4.77; N, 22.11 %.

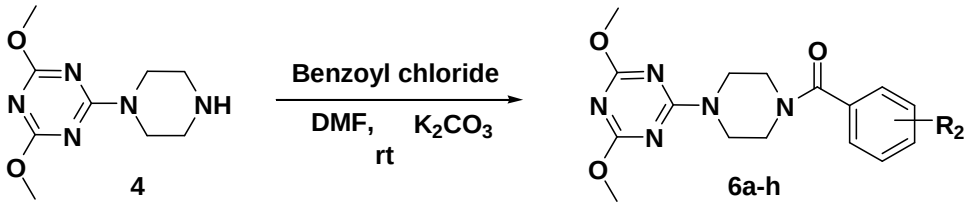
4-(4, 6-dimethoxy-1, 3, 5-triazin-2-yl)-N-(4-methoxyphenyl) piperazine-1-carboxamide (5g)

White solid: Yield 63%; ^{1}HNMR (DMSO-d6, 400MHz): δ 10.22(s, 1H); 7.54 (m,1H), 7.16 (d, *J* = 8 Hz, 2H), 7.11 (m, 1H), 4.08(m, 4H), 3.74(s, 3H), 3.74 (s, 3H), 3.45 (s, 3H), 2.88 (m, 2H), 2.61 (m, 2H); MS (APCI); *m/z* 375.3 $[M+H]^+$. Anal. Calcd for $C_{17}H_{22}N_6O_4$: C, 54.54; H 5.92; N, 22.45. Found: C, 54.44; H, 5.77; N, 22.31 %.

4-(4, 6-dimethoxy-1, 3, 5-triazin-2-yl)-N-(4-hydroxyphenyl) piperazine-1-carboxamide (5h)

White solid: Yield 62%; ^{1}HNMR (DMSO-d6, 400MHz): δ 10.25(s, 1H); 7.47 (m,1H), 7.18 (d, *J* = 8 Hz, 2H), 6.96 (m, 1H), 4.88(bs, 1H), 4.01(m, 4H), 3.85 (s, 3H), 3.85 (s, 3H), 2.88 (m, 2H); 2.72 (m, 2H); MS (APCI); *m/z* 360.9 $[M+H]^+$. Anal. Calcd for $C_{16}H_{20}N_6O_4$: C, 53.33; H 5.59; N, 23.32. Found: C, 53.24; H, 5.47; N, 23.31 %.

General procedure for the synthesis of (4-(4, 6-dimethoxy-1, 3, 5-triazin-2-yl) piperazin-1-yl)(phenyl) methanone derivatives (6 a-h)



Procedure- To the solution of 2, 4-dimethoxy-6-(piperazin-1-yl)-1, 3, 5-triazine (1equi.) , K_2CO_3 (1equi.) in DMF (for 1gm 30 ml) and a substituted benzoyl chloride was added at rt. The resulting mixture was stirred at rt for 12 hrs.

The progress of reaction was monitored by TLC. (70% EtOAc/Hexane) and (2% MeOH/DCM). On completion of reaction the sufficient water was added and the compound was extracted with ethyl acetate. The organic layer was washed with aq. $NaHCO_3$ solution and water separated out .The product was dried over sodium sulphate and filtered and then concentrated to give solid compound which is recrystalised with diethyl ether to give pure compound. It was dried under vacuum.

Experimental data

(4-(4, 6-dimethoxy-1, 3, 5-triazin-2-yl) piperazin-1-yl)(p-tolyl) methanone (6a):

White solid: Yield 66%; ^{1}HNMR (DMSO-d6, 400MHz): δ 6.86 (d, *J* = 8 Hz, 2H), 6.73 (d, *J* = 8 Hz, 2H), 3.55(s, 3H), 3.55(s, 3H), 3.19 (m, 2H), 2.98 (m, 4H); 2.96 (m, 2H); 0.94 (s, 3H); MS (APCI); *m/z* 343.8 $[M+H]^+$. Anal. Calcd for $C_{17}H_{21}N_5O_3$: C, 59.46; H 6.16; N, 20.40. Found: C, 59.39; H, 6.10; N, 20.35 %.

(2-chlorophenyl)(4-(4,6-dimethoxy-1,3,5-triazin-2-yl)piperazin-1-yl)methanone (6b)

Off white solid: Yield 66%; ^{1}HNMR (DMSO-d6, 400MHz): δ 7.29 (d, *J* = 8 Hz, 2H), 6.81 (d, *J* = 8 Hz, 2H), 3.83(s, 3H), 3.83(s, 3H), 3.79 (m, 4H), 3.54 (m, 4H); ^{13}C NMR (75 MHz, DMSO-d_6): *δ* =171.89, 168.82, 166.22, 134.84, 131.09, 131.05, 128.15, 54.20, 44.47, 43.18, 42.83, 40.83, 38.58 ppm; MS (APCI); *m/z* 364.9 $[M+H]^+$. Anal. Calcd for $C_{16}H_{18}N_5O_3Cl$: C, 52.82; H 4.99; N, 19.25. Found: C, 52.79; H, 4.87; N, 19.15 %.

(4-(4, 6-dimethoxy-1, 3, 5-triazin-2-yl) piperazin-1-yl)(3-fluorophenyl) methanone (6c)

White solid: Yield 66%; ^{1}HNMR (DMSO-d6, 400MHz): δ 6.69 (m, 2H), 6.31 (m, 2H), 3.84(s, 3H), 3.80(s, 3H), 3.24 (m, 4H), 3.04 (m, 4H); MS (APCI); *m/z* 348.1 $[M+H]^+$. Anal. Calcd for $C_{16}H_{18}N_5O_3F$: C, 55.33; H 5.22; N, 20.16. Found: C, 55.25; H, 5.18; N, 20.05

(4-(4,6-dimethoxy-1,3,5-triazin-2-yl)piperazin-1-yl)(4-nitrophenyl) methanone (6d)

White solid: Yield 66%; ^{1}HNMR (DMSO-d6, 400MHz): δ 8.10 (m, 2H), 7.90 (m, 2H), 4.02(m, 2H), 3.76(s, 6H), 3.73(m, 2H), 3.68(m, 2H), 3.65 (m, 2H); MS (APCI); *m/z* 374.8 $[M+H]^+$.Anal. Calcd for $C_{16}H_{18}N_6O_5$: C, 51.33; H 4.85; N, 22.45. Found: C, 51.25; H, 4.78; N, 22.35 %.

(4-(4,6-dimethoxy-1,3,5-triazin-2-yl)piperazin-1-yl)(Phenyl) methanone (6e)

Off white solid: Yield 71%; ^{1}HNMR (DMSO-d6, 400MHz): δ 7.22 (m, 2H), 6.99 (m, 2H), 6.68 (m, 1H), 4.46(m, 2H), 4.26(m, 2H), 3.60(s, 6H), 2.82 (m, 4H); ^{13}C NMR (75 MHz, DMSO-d_6): *δ* =171.92, 169.23, 166.25, 135.63, 129.66, 128.43, 127.03, 59.74, 54.20, 46.58, 43.17, 42.90, 41.22, 14.06 ppm; MS (APCI); *m/z* 330.2 $[M+H]^+$. Anal. Calcd for $C_{16}H_{19}N_5O_3$: C, 58.35; H, 5.81; N, 21.26. Found: C, 58.25; H, 5.78; N, 21.15 %.

(2,4-difluorophenyl)(4-(4,6-dimethoxy-1,3,5-triazin-2-yl)piperazin-1-yl)methanone (6f)

Off white solid: Yield 66%; ^{1}HNMR (DMSO-d6, 400MHz): δ 7.14 (m, 1H), 6.86 (d, *J* = 8 Hz, 1H), 6.71 (d, *J* = 8 Hz, 1H), 3.55 (s, 6H), 3.18 (m, 2H), 3.03(m, 4H), 2.96 (m, 2H); MS (APCI); *m/z* 365.7 $[M+H]^+$. Anal. Calcd for $C_{16}H_{17}N_5O_3F_2$: C, 52.60; H 4.69; N, 19.17. Found: C, 52.55; H, 4.58; N, 19.05 %.

(4-(4,6-dimethoxy-1,3,5-triazin-2-yl)piperazin-1-yl)(4-methoxyphenyl) methanone (6g)

White solid: Yield 66%; ^{1}HNMR (DMSO-d6, 400MHz): δ 7.27 (d, *J* = 8 Hz, 2H), 6.81 (d, *J* = 8 Hz, 2H), 3.83 (s, 9H), 3.79 (m, 4H), 3.54 (m, 4H); MS (APCI); *m/z* 360.0 $[M+H]^+$. Anal. Calcd for $C_{17}H_{21}N_5O_4$: C, 56.82; H 5.89; N, 19.49. Found: C, 56.75; H, 5.78; N, 19.39 %.

(4-(4,6-dimethoxy-1,3,5-triazin-2-yl)piperazin-1-yl)(4-hydroxyphenyl) methanone (6h)

White solid: Yield 66%; ^{1}HNMR (DMSO-d6, 400MHz): δ 6.69 (m, 2H), 6.32 (m, 2H), 3.84 (s, 6H), 3.01(m, 4H), 2.35 (m, 4H); MS (APCI); *m/z* 345.9 $[M+H]^+$. Anal. Calcd for $C_{16}H_{19}N_5O_4$: C, 55.64; H 5.55; N, 20.28. Found: C, 55.55; H, 5.48; N, 20.19 %.

Biological assay

Anti-inflammatory assay

Pro-inflammatory cytokine production by lip polysaccharide (LPS) in THP-1 cells was measured according to the method described in the reference (Hwang et al. 1993). During the 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 containing 10% fetal bovine serum (FBS, JRH). Cells were differentiated with phorbol myristate acetate (PMA, 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 ^{0}C. Finally, LPS (E. coli 0127:B8, Sigma Chemical Co., St. Louis, MO) was added, at a final concentration of 1 µg/mL in each well. Plates were further incubated at 37 ^{0}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).

Antibacterial assay

Newly synthesized compounds were screened for their antibacterial activity against selected Gram-positive organism's viz. *Staphylococcus aureus* (MTCC 96), *Bacillus subtilis* (MTCC 441) and Gram-negative organism's viz. *Escherichia coli* (MTCC 443), *Salmonella typhimurium* (MTCC 98) bacterial strains by agar well diffusion method with little modification. [2] Different concentrations (10-200 µg/ml) of test compounds were prepared in DMSO. The bacterial suspension was spread over nutrient agar plates and the well with 6 mm diameter was punched with sterile cork borer. The sample (50 µL) was added to the well and the plates were incubated at 37 °C for 24 h. Respective solvent control (DMSO) was kept and ciprofloxacin was used as standard antibacterial agent. The lowest concentration of compound which completely inhibits the bacterial growth was taken as minimum inhibitory concentration (MIC) and the minimum inhibitory concentrations (MIC) were noted for antibacterial assay.

Antifungal assay

Newly synthesized compounds were screened for their antifungal activity against *Candida albicans* (MTCC 227), *Aspergillus niger* (MTCC 281), *Fusarium solani* (MTCC 350) and *Aspergillus flavus* (MTCC 277) by agar well diffusion method with little modification(Sridhar et al. 2004). Normal saline was used to make a suspension of spores of fungal strain. The fungal suspension was spread over potato dextrose agar plates and the wells of 6 mm diameter were punched with sterile cork borer. The sample (50 µL) was added to the well and the plates were incubated at 37 °C for 2-3 days. Respective solvent control (DMSO) was kept and miconazole was used as standard antifungal agent. The lowest concentration of compound which completely inhibits the fungal growth was taken as minimum inhibitory concentration (MIC) and the minimum inhibitory concentrations (MIC) were noted for antifungal assay.

RESULTS AND DISCUSSION

Having secured the series of the novel 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-N-phenylpiperazine-1-carboxamide urea derivatives (5a-h) and (4-(4,6-dimethoxy-1,3,5-triazin-2-yl)piperazin-1-yl)(phenyl)methanone amide derivatives (6a-h), all compounds were evaluated for their ability to inhibit pro-inflammatory cytokines(TNF-α and IL-6) activity by TNF-α and IL-6 inhibition assay and anti-microbial activity against various gram-positive, gram-negative bacteria and fungal strains by using an agar well diffusion method. As presented in Tables 1 to 6, the compounds tested demonstrated a range of potencies, clearly showing the contributions of the urea/amide tri-azinic structure in terms of structure–activity relationships (SAR).

Thus, from the TNF-α and IL6 inhibitory activity data (Tables 1 and 4), it is observed that the compounds 5c, 5f, 6c and 6f found to be highly active as TNF-α and IL-6 inhibitor (up to 65–73% TNF-α and 73–85% IL-6 inhibitory activity) with compounds 5f (73% and 85%) and 6f (69% and 78%) exhibiting highest inhibition against TNF-α and IL-6 respectively at 10 µM. It is to be noted that almost all of these compound either found to be equally potent or more potent than that of the standard dexamethasone at MIC 1µM. Compounds 5b, 5g, 6g and 6h exhibited moderate activity (45–60% inhibition) while other compounds 5a, 5d, 5e and 6a, 6d, 6e exhibited low to very low or no activity all at same level of concentration. As shown in Table1 and 4, we firstly introduced urea and amide functionality on the triazine ring and a comparison of different substitutions at the position R_1 and R_2 for their activity has been made. The SAR with respect to the anti-inflammatory activity for the compounds **(5a-h)** and **(6a-h)** have shown an interesting trend as to the effect of substituent present on terminal ring of urea and amide moiety as well as the electronic nature of piperazine moiety in it has shown profound effect on the activity.

It is found from our results (Tables 1–3) that the nature and position of substituent on terminal benzene ring of urea and amide moiety has profound effect on the activity. When fluorine(F) atom is present on 2, 3 and 4 positions on the terminal benzene ring of urea and amide moiety , it is found that these position are the favorable sites for the higher potency. Evidently, the compound **5f** and **6f** with F at 2 and 4 positions on the terminal benzene rings of urea and amide series exhibiting highest TNF-α and IL-6 inhibitory activity. Also the presence of –F atom at position 3 of terminal benzene ring have shown the good activity(5c, 6c) ,while the presence of Cl at 4 and 2- position of terminal benzene ring **5b, and 6b** respectively exhibit moderate (47 to 59%) inhibitory activity. Interestingly, the

large bulky lipophilic group such as Methyl and NO_2 at 4-position of terminal benzene ring generated compound 5a, 5d ,6a and 6d from both series has no effect on TNF-α or IL-6 inhibitory activity. Unexpected result was shown in terms of –OH group in amide series with a moderate activity where as for urea series it shows less activity.

It reveals from our SAR studies that , the presence of lipophilic Cl and F tolerates the procytokine activity because it is found that Fluorine imparts the special characteristics that enhance therapeutic efficacy and improved pharmacological properties in bioactive molecules. There is no actual evidence in hand in support of the actual role of urea and amide functionality on the activity, at this time, so it is speculated that the H-bond donor ability of the urea and thiourea might be responsible for their high anti-inflammatory activity. Thus, the compound **5f** and **6f** were found to be most potential inflammatory agents amongst the series of compounds studied and could prove to be promising candidate for drug discovery.

Though there is no actual evidence in hand in support of the actual role of urea or amide moiety on the activity at this time, so it is speculated that the H-bond donor ability of the urea or amide along with the electronic effect of para substituent might be responsible for their high anti-inflammatory activity.

As a good response against anti-inflammatory activity shown by above mentioned *urea and amide derivatives of (piperazin-1-yl)-1, 3, 5-triazine*. It is found wise to go for the antimicrobial activity of these compounds to further assist for SAR study.

From antimicrobial activity data shown in Table 2, 3, 5 and 6, it is revealed that some analogues of this series have more potency than the standard drug Ciprofloxacin and Miconazole while some of them have comparable potency. Interestingly none of the compound with high anti-inflammatory activity found to be potent antibacterial or antifungal agents.

Thus, the compounds **5a, 5d, 5e** as well as **6a, 6d and 6e** bearing aryl 4-Me, 4-NO_2, and phenyl group respectively of both the series have higher potency against the tested antimicrobial strain. It is cleared from our results that the 4^{th} position of substituent on terminal benzene ring of urea and amide moiety is the favorable site for high antimicrobial activity. The high potency of these compounds may be attributed to the presence of H-bond acceptor type group's placement at 4-positions. While presence Cl, OH on terminal benzene ring of urea and amide series (**5b,5h 6b and 6h**) shows moderately potent antimicrobial activity with respect to standard drug. but interestingly the presence of NO_2 at 4^{th} position of terminal benzene ring in urea series **(5d)** shows potent antibacterial and antifungal activity while in amide series **(6d)** shows moderate antibacterial and antifungal activity. Any activity has not been observed in case of remaining compounds up to concentration of 200 µg/mL against same bacterial and fungal strains.

It is cleared from results i.e. Table 2, 3 for series of urea vs. Table 5, 6 for series of amide that , the SAR of antibacterial activity partially correlates with their SAR of antifungal activity as there is some divergence is observed. The same aryl 4^{th} position as observed already is favourable site of high activity for both series. The compounds **5d** and **6e** have been found to be 2 to 2.5-fold more potent than the standard drug Micanazole as similar to the antibacterial activity trend. Only dissimilarity is observed in case of compound **6d.** While the compounds (**6c, f and g)** have no major effect on the antifungal activity also. Explicitly the compound **5a, 5d** and **6d** bearing single substituent along with urea and amide functionality reflect 2.5 fold antimicrobial activity against the pathogenic bacteria and fungal strain.

Biological activity

Anti-inflammatory activity data of 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-N-phenylpiperazine-1-carboxamide urea derivatives (5a-h)(% inhibition at 10 µM)

Table-1

Comp. **(5a-h)**	**(R_1)**	% inhibition at 10 µM	
		TNF-α	**IL-6**
5a	4-Methyl	15	16
5b	4-Chloro	55	59
5c	3-fluro	**67**	**73**
5d	4-NO2	25	28
5e	Phenyl	10	13
5f	2,4-difluoro	**73**	**85**
5g	4-Methoxy	53	61
5h	4-Hydroxy	30	34
Ref.	Dexamethasone (1µM)	75	84

Antibacterial activity of 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-N-phenylpiperazine-1-carboxamide urea derivatives (5a-h).

Zone of inhibition [mm (MIC [a] values µg/mL)]

Table-2

Comp. (5a-h)	**Gram-positive** (MIC values µg/mL)		**Gram-negative** (MIC values µg/mL)	
	Staphylococcus aureus	*Bacillus subtilis*	*Escherichia coli*	*Salmonella typhimurium*
5a	**10**	**15**	**10**	**10**
5b	**40**	**30**	**45**	**50**
5c	80	65	80	80
5d	**15**	**10**	**10**	**10**
5e	**20**	**15**	**15**	**20**
5f	80	90	00	85
5g	70	55	65	75
5h	65	60	55	65
Ciprofloxacin(Ref.)	25	25	15	25

[a] *– Values are the average of three reading.*

Antifungal activity of 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-N-phenylpiperazine-1-carboxamide urea derivatives (5a-h).

Zone of inhibition [mm (MIC [a] values µg/mL)]

Table-3 MIC [a] values µg/mL

Comp. (5a-h)	*Candida albicans*	*Aspergillus niger*	*Fusarium – solani*	*Aspergillus flavus*
5a	**15**	**25**	**10**	**10**
5b	30	30	35	40
5c	75	65	95	80
5d	**10**	**20**	**10**	**10**
5e	45	30	40	35
5f	90	90	0	0
5g	95	75	85	90
5h	70	65	60	65
Miconazole(Ref.)	20	25	15	15

[a] *- Values are the average of three reading.*

***Anti-inflammatory activity data of (4-(4, 6-dimethoxy-1, 3, 5-triazin-2-yl) piperazin-1-yl)(phenyl)methanone amide derivatives* (6a-h)(% inhibition at 10 µM)**

Table-4

Comp. **(6a-h)**	**(R_2)**	% inhibition at 10 µM	
		TNF-α	**IL-6**
6a	4-Methyl	21	25
6b	2-Chloro	47	59
6c	3-fluro	**65**	**74**
6d	4-NO2	31	27
6e	Phenyl	0	10
6f	2,4-difluoro	**69**	**78**
6g	4-Methoxy	49	54
6h	4-hydroxy	43	47
Ref.	Dexamethasone (1µM)	75	84

***Antibacterial activity of (4-(4,6-dimethoxy-1,3,5-triazin-2-yl)piperazin-1-yl)(phenyl)methanone amide derivatives* (6a-h).**
Zone of inhibition [mm (MIC [a] values µg/mL)]

Table-5

Comp. (6a-h)	**Gram-positive** (MIC values µg/mL)		**Gram-negative** (MIC [a] values µg/mL)	
	Staphylococcus aureus	*Bacillus subtilis*	*Escherichia coli*	*Salmonella typhimurium*
6a	**20**	**15**	**15**	**20**
6b	60	65	70	65
6c	80	65	80	90
6d	**30**	**40**	**45**	**35**
6e	**25**	**15**	**20**	**10**
6f	75	95	00	85
6g	70	65	65	75
6h	55	50	60	75
Ciprofloxacin(Ref.)	20	25	20	15

[a] *- Values are the average of three reading.*

***Antifungal activity of (4-(4,6-dimethoxy-1,3,5-triazin-2-yl)piperazin-1-yl)(phenyl)methanone amide derivatives* (6a-h).**
Zone of inhibition [mm (MIC [a] values µg/mL)]

Table-6 (MIC [a] values µg/mL)

Comp. (6a-h)	*Candida* albicans	*Aspergillus niger*	*Fusarium - solani*	*Aspergillus flavus*
6a	**25**	**15**	**15**	**20**
6b	**65**	**75**	**80**	**65**
6c	80	65	0	80
6d	**35**	**40**	**45**	**45**
6e	**25**	**15**	**20**	**10**
6f	90	90	0	0
6g	95	80	85	90
6h	65	70	60	80
Miconazole(Ref.)	20	25	20	15

[a] *- Values are the average of three reading.*

CONCLUSION

we have synthesized the ***(piperazin-1-yl)-1, 3, 5-triazin*** based novel 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-N-phenylpiperazine-1-carboxamide urea derivatives **(5a-h)** and (4-(4,6-dimethoxy-1,3,5-triazin-2-yl)piperazin-1-yl)(phenyl)methanone amide derivatives **(6a-h)** and evaluated for their anti-inflammatory (against TNF-α and IL-6) as well as antimicrobial activities against different gram positive and gram negative bacterial and fungal strains. Among all the compounds screened (5a–h and 6a–h), the compounds **5c, 5f, 6c, and 6f** showed promising TNF-α

and IL-6 inhibitory activity with compounds **5f** and **6f** exhibiting highest activity while the compounds **5a, 5d, 5e, 6a, 6d and 6e** found to be the potent antimicrobial agent, showing even 2 to 2.5-fold more activity than that of standard ciprofloxacin and miconazole at the same MIC value of 10 µg/mL. Thus the presence of lipophilic Cl and F tolerates the procytokine activity as well as the H-bond donor ability of the urea and thiourea might be responsible for their high anti-inflammatory activity. While the presence of Methyl, NO_2, and phenyl group on 4^{th} position of terminal benzene ring of urea and amide functionality found to be effective potent antimicrobial agents.

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