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Synthesis and Biological Evaluation of Novel Isopropyl 2-thiazolopyrimidine-6-carboxylate Derivatives

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ABSTRACT. In the present study, we have synthesized novel Isopropyl 2-(4-substitutedbenzylidene)-5-methyl-3-oxo-7-phenyl-3,7-dihydro-2*H*-thiazolo[3,2-a]-pyrimidine-6-carboxylate derivatives **(6a-j).** Elemental analysis, IR, ^{1}H NMR and mass spectral data elucidated structure of newly synthesized compounds. The newly synthesized compounds were screened for anti-inflammatory and anti microbial studies. Their biological activity data of the 10 compounds indicates that two compounds posses potent anti-inflammatory and five have antimicrobial activities.

Key words: Isopropyl 2-thiazolopyrimidine-6-carboxylate, Anti-inflammatory, Antibacterial, Antifungal activities

INTRODUCTION

Heterocycles are ubiquitous to among pharmaceutical compounds.[1] Pyrimidine moiety is an important class of *N*-containing heterocycles widely used as key building blocks for pharmaceutical agents. These compounds exhibit a wide spectrum of pharmacophore, as they act as bactericidal, fungicidal,[2] analgesic,[3] antioxidant,[4] antihypertensive,[5] antifilarial,[6] and anti-tumor agents.[7] Preclinical data from literature indicates the continuing research in polysubstituted pyrimidine as potential anti-tumor agents.[8-10] Among these, thiouracils in particular are used as anti-inflammatory and virucidal agents.[11] The biological and synthetic significance places this scaffold at a prestigious position in medicinal chemistry research.

The key role pyrimidines play in cellular processes has made them valuable leads for drug discovery. One important class of pyrimidines is 2-thiopyrimidine (2-TP) and its derivatives, which are also recognised as 2-mercaptopyrimidine compounds.[12] In 2-TP ring, sulfur atom serves as an interesting replacement for the existing oxygen atom bonded to C-2 in uridine base.[13,14] Base on this approach, 2-TPs have attracted significanat interest of synthetic-biochemists.[15,16] A patent[17] revealed the application of 2-TP derivatives in preparation of cardiotonic drugs. Pathak *et al*. have evaluated primary activity of 2-TP derivatives against Mycobacterium tuberculosis (Mtb).[18]

One-step synthesis of 3,4-dihydropyrimidin-2(1H)-one by three-component condensation of aldehydes, ethyl acetoacetate and urea in alcohol using strong mineral acid was first reported by Biginelli.[19] These substances, popularly known as Biginelli compounds possess several pharmaceutical properties like anti-bacterial, anti-viral, anti-inflammatory, anti-hypertensive and anti-tumor agents.[20] In continued quest of new antimicrobial, anti-inflammatory agents, we designed and synthesized novel Isopropyl 2-(4-substitutedbenzylidene)-5-methyl-3-oxo-7-phenyl-3,7-dihydro-2*H*-thiazolo[3,2-a]pyrimidine-6-carboxylate derivatives **(6a-j)** having substituted benzylthio groups. Structures of the products were characterized by IR, ^{1}H-NMR and LC-MS mass spectrometry and elemental analysis. Their biological activity data of the 10 compounds indicates that two compounds posses' potent anti-inflammatory and five have antimicrobial activities.

RESULTS AND DISCUSSION

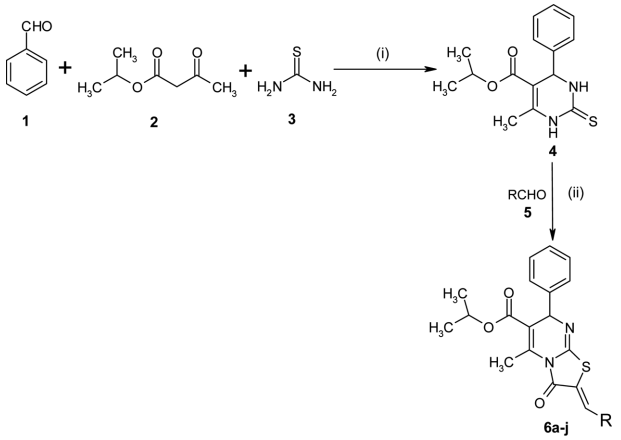
All the synthesized compounds were obtained in good to high yields. Products were purified and characterized by various spectroscopic techniques. The IR spectra of compounds (**6a-j**) showed characteristic absorption bands at 2981-2969 cm^{-1}, 1721-1706 cm^{-1}, 1632-1606, 1568-1525 and 652-647 cm^{-1} corresponding to the C-H$_{str}$, CO$_{str}$, CN$_{str}$, CC$_{str}$ and C-S$_{str}$ functions in the structures. Similarly the ^{1}H NMR spectra showed peaks due to in the range of δ 1.24-1.30 for OCH-($\underline{CH_3}$)$_2$, δ 2.28-2.39 for Ar-$\underline{CH_3}$, δ 4.20-4.28 for -CH, δ 4.89-4.92 for O$\underline{CH}$-(CH$_3$)$_2$, and δ 7.66-7.82 for -Ar-CH.

The mass spectrum of all the compounds showed molecular ion peak at M+H, at M+2H corresponding to its

molecular formula, which confirmed its chemical structure. The IR, ^{1}H NMR, mass spectra and elemental analysis showed the structure of various novel Isopropyl 2-(4-substitutedbenzylidene)-5-methyl-3-oxo-7-phenyl-3,7-dihydro-2*H*-thiazolo[3,2-a]pyrimidine-6-carboxylate derivatives **(6a-j)**.

CHEMISTRY

The synthesis of titled compounds (**6a-j**) was carried out according to *Scheme* 1. Benzaldehyde **1** and isopropyl acetoacetate **2** in ethanol was refluxed with thiourea **3** using ethanol as solvent in basic conditions to yield 5-isopropoxycarbonyl-6-methyl-4-phenyl-3,4-dihydropyrimidin-2(1*H*)-thione.[21] It was synthesized by the multicomponent Biginelli reaction. The Biginilli compound **4** was treated with substituted aromatic aldehydes **5** in presence of anhydrous sodium acetate to afford the titled compounds (**6a-j**). The reaction sequences are outlined in *Scheme* 1.



***Scheme* 1.** (i) $SrCl_2.6H_2O$, ethanol/reflux; (ii) $ClCH_2COOH$, AcONa, Ac_2O/AcOH.

Compound	R	Compound	R
6a	Ph	**6f**	4-N$(CH_3)_2$-Ph
6b	4-Br-Ph	**6g**	4-Cl-Ph
6c	4-C_2H_5-Ph	**6h**	4-CH$(CH_3)_2$-Ph
6d	4-CH_3-Ph	**6i**	4-C$(CH_3)_3$-Ph
6e	4-OCH_3-Ph	**6j**	4-NO_2-Ph

PHARMACOLOGICAL STUDIES

Anti-inflammatory activity

The results of tested compounds as well as reference standard were measured before administration of Carrageenan inflammation.[5] After the Carrageenan inflammation was administered on rats, the effect was measured in the intervals of 30, 60 and 120 min. The percent oedema inhibition was calculated reference to saline control group, as depicted in *Table* 1. All the newly obtained compounds **6a-j** were tested for anti-inflammatory activity. Compared to the standard, Nimesulide, bulk of the compounds exhibited moderate to good anti-inflammatory activity. The results revealed that, while **6b** and **6f** have shown potent anti-inflammatory activity, compounds **6a**,

***Table* 1.** The anti-inflammatory activity of Isopropyl 2-thiazolopyrimidine-6-carboxylate (**6a-j**)

Compound	Paw oedema thickness (mm)					
	30 m (X ± SE)	% oedema inhibition	60 m (X ± SE)	% oedema inhibition	120 m (X ± SE)	% oedema inhibition
Control	1.3 ± 0.05	–	1.5 ± 0.03	–	1.7 ± 0.03	–
6a	1.3 ± 0.05	7.6	1.2 ± 0.05**	20.0	1.3 ± 0.08**	23.5
6b	1.1 ± 0.03	**15.3**	1.1 ± 0.00**	**26.6**	1.1 ± 0.03**	**41.1**
6c	1.2 ± 0.05	7.6	1.3 ± 0.03**	13.3	1.3 ± 0.03**	23.5
6d	1.2 ± 0.06	7.6	1.1 ± 0.05**	26.6	1.2 ± 0.03**	29.4
6e	1.2 ± 0.03	7.6	1.1 ± 0.03**	26.6	1.2 ± 0.05**	29.4
6f	1.2 ± 0.03	**15.3**	1.1 ± 0.00**	**26.6**	1.1 ± 0.03**	**41.1**
6g	1.2 ± 0.03	7.6	1.1 ± 0.06**	26.6	1.4 ± 0.03**	17.6
6h	1.4 ± 0.00	7.6	1.2 ± 0.03**	20.0	1.3 ± 0.10**	23.5
6i	1.2 ± 0.05	7.6	1.2 ± 0.05**	20.0	1.3 ± 0.06**	23.5
6j	1.1 ± 0.03	15.3	1.2 ± 0.03**	20.0	1.3 ± 0.05**	23.5
Nimesulide	1.1 ± 0.05	15.3	1.1 ± 0.00**	26.6	1.0 ± 0.00**	41.1

Data represent mean values ±SE of six mice per group and the percent changes versus 30, 60 and 120 m post-carrageenan injection.
Data were analyzed using one-way ANOVA followed by Turkey-Krammer Multiple comparison test **p< 0.01.
Percent oedema inhibition was calculated as regards saline control group.
**Significant difference from the control value at p<0.01.
SE=standard error
The active compounds are marked in bold letters.

***Table* 2.** Antibacterial activity of Isopropyl 2-thiazolopyrimidine-6-carboxylate (**6a-j)**

Compound no	*Staphylococcus aureus*	*Eschrichia coli*	*Pseudomonas aeruginosa*	*Klebsiella pneumoniae*	*Streptococcus pyogenes*
6a	20 (6.25)	25 (6.25)	29 (6.25)	18 (6.25)	23 (6.25)
6b	23 (6.25)	28 (6.25)	30 (6.25)	18 (6.25)	21 (6.25)
6c	10 (12.5)	--	--	17 (6.25)	11 (6.25)
6d	8 (25)	23 (6.25)	9 (25)	--	8 (12.5)
6e	22 (6.25)	27 (6.25)	32 (6.25)	20 (6.25)	24 (6.25)
6f	21 (6.25)	29 (6.25)	32 (6.25)	20 (6.25)	23 (6.25)
6g	10 (12.5)	15 (25)	--	--	17 (12.5)
6h	12 (12.5)	--	21 (6.25)	--	8 (25)
6i	21 (6.25)	24 (6.25)	29 (6.25)	19 (6.25)	23 (6.25)
6j	21 (6.25)	26 (6.25)	32 (6.25)	21 (6.25)	24 (6.25)
Standard[a]	24 (6.25)	30 (6.25)	33 (6.25)	23 (6.25)	25 (6.25)

--Indicates bacteria is resistant to the compounds at >100 μg/ml, MIC values are given in brackets. MIC (μg/ml)=minimum inhibitory con centration, ie. Lowest concentration to completely inhibit bacterial growth. Zone of inhibition in mm.
[a]Ciprofloxacin was used as standard.

***Table* 3.** Antifungal activity of Isopropyl 2-thiazolopyrimidine-6-carboxylate (**6a-j**)

Compound no	*Aspergillus fumigatus*	*Aspergillus flavus*	*Trichophyton mentagrophytes*	*Penicillium marneffei*	*Candida albicans*
6a	22 (6.25)	22 (6.25)	25 (6.25)	22 (6.25)	20 (6.25)
6b	8 (25)	--	12 (12.5)	--	17 (6.25)
6c	22 (6.25)	20 (6.25)	22 (6.25)	25 (6.25)	17 (6.25)
6d	15 (6.25)	--	7 (25)	21 (6.25)	18 (6.25)
6e	5 (25)	18 (6.25)	--	12 (12.5)	17 (6.25)
6f	24 (6.25)	21 (6.25)	21 (6.25)	23 (6.25)	18 (6.25)
6g	11 (12.5)	12 (25)	--	--	14 (12.5)
6h	9 (25)	--	12 (12.5)	9 (25)	10 (12.5)
6i	22 (6.25)	19 (6.25)	20 (6.25)	23 (6.25)	19 (6.25)
6j	21 (6.25)	26 (6.25)	32 (6.25)	21 (6.25)	24 (6.25)
Standard[b]	25 (6.25)	21 (6.25)	23 (6.25)	25 (6.25)	19 (6.25)

--Indicates fungus is resistant to the compounds at >100 mg/ml, MIC values are given in brackets. MIC (mg/ml)=minimum inhibitory con centration, ie. Lowest concentration to completely inhibit fungal growth. Zone of Inhibition in mm.
[b]Amphotericin was used as standard.

6c, **6d**, **6e**, **6g**, **6h**, **6i** and **6j** exhibited good anti-inflammatory activities. Examining the structure-activity relationship (SAR), the bromosubstituted (**6b**) and diaminomethyl substituted (**6f**) thiopyrimidines have shown potent anti-inflammatory activity. This has revealed a new path in the synthesis of new class of Isopropyl 2-(4-substitutedbenzylidene)-5-methyl-3-oxo-7-phenyl-3,7-dihydro-2*H*-thiazolo[3,2-a]pyrimidine-6-carboxylate **(6a-j)** derivatives.

Antibacterial activity

The newly synthesized compounds were screened for their antibacterial activity against *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Streptococcus pyogenes* and *Klebsiella pneumoniae* (recultured) bacterial strains by disc diffusion method.[22,23] The investigation of antibacterial screening data revealed that all the tested compounds showed moderate to good bacterial inhibition. The compounds **6a**, **6b**, **6e**, **6f**, **6i** and **6j** showed potent activity against all the bacterial strains in *Table* 2.

Antifungal studies

All the newly prepared compounds were screened for their antifungal activity against *Aspergillus flavus, Aspergillus fumigatus, Candida albicans, Penicillium marneffei* and *Trichophyton mentagrophytes* (recultured) in DMSO by serial plate dilution method.[24,25] The antifungal screening data showed moderate to good activity. Compounds **6a**, **6c**, **6f**, **6i** and **6j** emerged as potent active against all the fungal strains in *Table* 3.

EXPERIMENTAL

All reagents and solvents (Aldrich or Merck) were purchased and used without further purification. Melting

points were determined on a Fisher-Johns melting point apparatus were uncorrected. Crude products were purified by column chromatography on silica gel of 60-120 mesh. IR spectra were obtained on a Perkin Elmer BX serried FT-IR 5000 spectrometer using KBr pellet. NMR spectra were recorded on a Varian 300 MHz spectrometer for ^{1}H NMR. The chemical shifts were reported as ppm down field using TMS as an internal standard. LC-MS Mass spectra were recorded on a MASPEC low resolution mass spectrometer operating at 70 eV.

5-Isopropoxycarbonyl-6-methyl-4-phenyl-3,4-dihydropyrimidin-2(1*H*)-thione(4)[21]

To a solution of isopropyl acetoacetate (1 mmol), aldehyde (1.1 mmol), thiourea (1.5 mmol), $SrCl_2.6H_2O$ (1 mmol, 10 mol%) and EtOH (20 ml). The mixture was heated at 40 ºC and the progress of the reaction was monitored by TLC. After completion of the reaction (about 3-5 h) the solution was cooled to room temperature and poured into crushed ice. The resultant solid product was collected and purified by column chromatography.

Isopropyl 2-(4-substitutedbenzylidene)-5-methyl-3-oxo-7-phenyl-3,7-dihydro-2*H*-thiazolo[3,2-a]pyrimidine-6-carboxylate (6 a-j)

A mixture of compound **4** (2 mmol), chloroacetic acid (2 mmol), sodium acetate anhydrous (2 g) in glacial acetic acid and acetic acid anhydride (40 mL, 3:1) was refluxed for 12 min., then equimolecular amount of the appropriate aromatic aldehydes were added. The reaction mixture was refluxed for 2 h, allowed to cool, poured onto cold water; the formed precipitate was filtered off, dried and crystallized from proper solvent to give the corresponding arylmethylene thiazolopyrimidine derivatives (**6a-j**) respectively.

Isopropyl 2-benzylidene-5-methyl-3-oxo-7-phenyl-3,7-dihydro-2*H*-thiazolo[3,2-a]pyrimidine-6-carboxylate (6a)

Pale yellow solid; Yield 69%; mp 196-198 ºC; IR (KBr, υ cm^{-1}): 2976 (C-H), 1709 (C=O), 1625 (C=N), 1549 (C=C), 641 (C-S); ^{1}H NMR (*δ*, DMSO-*d*$_6$): 1.24 (d, 6H, OCH-(C<u>H</u>$_3$)$_2$), 2.35 (s, 3H, Ar-C<u>H</u>$_3$), 4.21 (d, 1H, -CH), 4.91 (m, 1H, OC<u>H</u>-(CH$_3$)$_2$), 7.20-7.52 (m, 10H, Ar-H), 7.68 (s, 1H, Ar-CH); LCMS: (*m*/*z*) 419 [M+H]. *Anal*. Calcd. for $C_{24}H_{22}N_2O_3S$: C, 68.90; H, 5.30; N, 6.69. Found: C, 69.04; H, 5.24; N, 6.73.

Isopropyl 2-(4-bromobenzylidene)-5-methyl-3-oxo-7-phenyl-3,7-dihydro-2*H*-thiazolo[3,2-a]pyrimidine-6-carboxylate (6b)

Yellow solid; Yield 63%; mp 215-216 ºC; IR (KBr, υ cm^{-1}): 2973 (C-H), 1710 (C=O), 1613 (C=N), 1528 (C=C), 754 (C-Br), 638 (C-S); ^{1}H NMR (δ, DMSO-*d*$_6$): 1.30 (d, 6H, OCH-(C<u>H</u>$_3$)$_2$), 2.39 (s, 3H, Ar-C<u>H</u>$_3$), 4.27 (d, 1H, -CH), 4.93 (m, 1H, OC<u>H</u>-(CH$_3$)$_2$), 7.08-7.64 (m, 9H, Ar-H), 7.76 (s, 1H, Ar-CH); LCMS: (*m*/*z*) 496 [M$^+$] & 498 [M+2H]. *Anal*. Calcd. for $C_{24}H_{21}BrN_2O_3S$: C, 57.94; H, 4.26; N, 5.64. Found: C, 57.91; H, 4.32; N, 5.70.

Isopropyl 2-(4-ethylbenzylidene)-5-methyl-3-oxo-7-phenyl-3,7-dihydro-2*H*-thiazolo[3,2-a]pyrimidine-6-carboxylate (6c)

Yellow solid; Yield 75%; mp 174-175 ºC; IR (KBr, υ cm^{-1}): 2969 (C-H), 1711 (C=O), 1609 (C=N), 1546 (C=C), 654 (C-S); ^{1}H NMR (υ, DMSO-*d*$_6$): 1.18 (t, 3H, -ArCH$_2$-C<u>H</u>$_3$), 1.26 (d, 6H, OCH-(C<u>H</u>$_3$)$_2$), 2.34 (s, 3H, Ar-C<u>H</u>$_3$), 2.47 (q, 2H, -ArC<u>H</u>$_2$-CH$_3$), 4.23 (d, 1H, -CH), 4.90 (m, 1H, OC<u>H</u>-(CH$_3$)$_2$), 6.91-7.40 (m, 9H, Ar-H), 7.70 (s, 1H, Ar-CH); LCMS: (*m*/*z*) 447 [M+H]. *Anal*. Calcd. for $C_{26}H_{26}N_2O_3S$: C, 69.93; H, 5.87; N, 6.29. Found: C, 70.02; H, 5.95; N, 6.21.

Isopropyl 2-(4-methylbenzylidene)-5-methyl-3-oxo-7-phenyl-3,7-dihydro-2*H*-thiazolo[3,2-a]pyrimidine-6-carboxylate (6d)

Pale yellow solid; Yield 65%; mp 223-224 ºC; IR (KBr, υ cm^{-1}): 2976 (C-H), 1710 (C=O), 1617 (C=N), 1544 (C=C), 648 (C-S); ^{1}H NMR (δ, DMSO-*d*$_6$): 1.24 (d, 6H, OCH-(C<u>H</u>$_3$)$_2$), 2.28-2.33 (s, 6H, -C<u>H</u>$_3$), 4.21 (d, 1H, -CH), 4.89 (m, 1H, OC<u>H</u>-(CH$_3$)$_2$), 7.08-7.35 (m, 9H, Ar-H), 7.66 (s, 1H, Ar-CH); LCMS: (*m*/*z*) 433 [M+H]. *Anal*. Calcd. for $C_{25}H_{24}N_2O_3S$: C, 69.44; H, 5.59; N, 6.48. Found: C, 69.57; H, 5.53; N, 6.52.

Isopropyl 2-(4-methoxybenzylidene)-5-methyl-3-oxo-7-phenyl-3,7-dihydro-2*H*-thiazolo[3,2-a]pyrimidine-6-carboxylate (6e)

Yellow solid; Yield 76%; mp 170-171 ºC; IR (KBr, υ cm^{-1}): 2980 (C-H), 1706 (C=O), 1606 (C=N), 1568 (C=C), 645 (C-S); ^{1}H NMR (δ, DMSO-*d*$_6$): 1.30 (d, 6H, OCH-(C<u>H</u>$_3$)$_2$), 2.38 (s, 3H, Ar-C<u>H</u>$_3$), 3.76 (s, 3H, -ArOC<u>H</u>$_3$), 4.26 (d, 1H, -CH), 4.92 (m, 1H, OC<u>H</u>-(CH$_3$)$_2$), 6.84-7.36 (m, 9H, Ar-H), 7.80 (s, 1H, Ar-CH); LCMS: (*m*/*z*) 449 [M+H]. *Anal*. Calcd. for $C_{25}H_{24}N_2O_4S$: C, 66.95; H, 5.38; N, 6.26. Found: C, 67.04; H, 5.42; N, 6.33.

Isopropyl 2-(4-(dimethylamino)benzylidene)-5-methyl-3-oxo-7-phenyl-3,7-dihydro-2*H*-thiazolo[3,2-a]pyrimidine-6-carboxylate (6f)

Yellow solid; Yield 82%; mp 210-212 ºC; IR (KBr, υ cm^{-1}): 2973 (C-H), 1718 (C=O), 1610 (C=N), 1538 (C=C), 642 (C-S); ^{1}H NMR (δ, DMSO-*d*$_6$): 1.30 (d, 6H, OCH-(C<u>H</u>$_3$)$_2$), 2.39 (s, 3H, Ar-C<u>H</u>$_3$), 2.94 (s, 6H, -ArN(C<u>H</u>$_3$)$_2$), 4.28 (d, 1H, -CH), 4.92 (m, 1H, OC<u>H</u>-(CH$_3$)$_2$), 6.65-7.38 (m, 9H, Ar-H), 7.78 (s, 1H, Ar-CH); LCMS: (*m*/*z*) 462 [M+H]. *Anal*. Calcd. for $C_{26}H_{27}N_3O_3S$: C, 67.65; H, 5.90; N, 9.12. Found: C, 67.75; H, 5.97; N, 9.09.

Isopropyl 2-(4-chlorobenzylidene)-5-methyl-3-oxo-7-phenyl-3,7-dihydro-2*H*-thiazolo[3,2-a]pyrimidine-6-carboxylate (6g)

Yellow solid; Yield 80%; mp 252-253 °C; IR (KBr, υ cm^{-1}): 2981 (C-H), 1721 (C=O), 1628 (C=N), 1552 (C=C), 823 (C-Cl), 648 (C-S); ^{1}H NMR (υ, DMSO-d_6): 1.30 (d, 6H, OCH-($\underline{CH_3}$)$_2$), 2.38 (s, 3H, Ar-$\underline{CH_3}$), 4.27 (d, 1H, -CH), 4.91 (m, 1H, O$\underline{CH}$-(CH_3)$_2$), 7.18-7.42 (m, 9H, Ar-H), 7.74 (s, 1H, Ar-CH); LCMS: (*m*/*z*) 453 [M+H]. *Anal*. Calcd. for $C_{24}H_{21}ClN_2O_3S$: C, 63.65; H, 4.66; N, 6.18. Found: C, 63.78; H, 4.69; N, 6.23.

Isopropyl 2-(4-isopropylbenzylidene)-5-methyl-3-oxo-7-phenyl-3,7-dihydro-2*H*-thiazolo[3,2-a]pyrimidine-6-carboxylate (6h)

Yellow solid; Yield 78%; mp 185-187 °C; IR (KBr, υ cm^{-1}): 2972 (C-H), 1714 (C=O), 1618 (C=N), 1528 (C=C), 652 (C-S); ^{1}H NMR (δ, DMSO-d_6): 0.98 (d, 6H, -ArCH ($\underline{CH_3}$)$_2$), 1.26 (d, 6H, OCH-($\underline{CH_3}$)$_2$), 2.33 (s, 3H, Ar-$\underline{CH_3}$), 2.64 (m, 1H, -Ar$\underline{CH}$(CH_3)$_2$), 4.20 (d, 1H, -CH), 4.89 (m, 1H, O$\underline{CH}$-(CH_3)$_2$), 7.12-7.38 (m, 9H, Ar-H), 7.68 (s, 1H, Ar-CH); LCMS: (*m*/*z*) 461 [M+H]. *Anal*. Calcd. for $C_{27}H_{28}N_2O_3S$: C, 70.43; H, 6.13; N, 6.08. Found: C, 70.37; H, 6.18; N, 5.99.

Isopropyl 2-(4-*tert*-butylbenzylidene)-5-methyl-3-oxo-7-phenyl-3,7-dihydro-2*H*-thiazolo[3,2-a]pyrimidine-6-carboxylate (6i)

Pale yellow solid; Yield 71%; mp 201-202 °C; IR (KBr, υ cm^{-1}): 2974 (C-H), 1713 (C=O), 1616 (C=N), 1538 (C=C), 647 (C-S); ^{1}H NMR (δ, DMSO-d_6): 1.28 (d, 6H, OCH-($\underline{CH_3}$)$_2$), 1.36 (s, 9H, -ArC($\underline{CH_3}$)$_3$), 2.35 (s, 3H, Ar-$\underline{CH_3}$), 4.22 (d, 1H, -CH), 4.89 (m, 1H, O$\underline{CH}$-(CH_3)$_2$), 7.12-7.36 (m, 9H, Ar-H), 7.70 (s, 1H, Ar-CH); LCMS: (*m*/*z*) 475 [M+H]. *Anal*. Calcd. for $C_{28}H_{30}N_2O_3S$: C, 70.86; H, 6.37; N, 5.90. Found: C, 70.89; H, 6.40; N, 5.97.

Isopropyl 2-(4-nitrobenzylidene)-5-methyl-3-oxo-7-phenyl-3,7-dihydro-2*H*-thiazolo[3,2-a]pyrimidine-6-carboxylate (6j)

Yellow solid; Yield 69%; mp 221-223 °C; IR (KBr, υ cm^{-1}): 2980 (C-H), 1720 (C=O), 1632 (C=N), 1555 (C=C), 650 (C-S); ^{1}H NMR (δ, DMSO-d_6): 1.30 (d, 6H, OCH-($\underline{CH_3}$)$_2$), 2.38 (s, 3H, Ar-$\underline{CH_3}$), 4.28 (d, 1H, -CH), 4.93 (m, 1H, O$\underline{CH}$-(CH_3)$_2$), 7.10-7.43 (m, 5H, Ar-H), 7.82 (s, 1H, Ar-CH), 7.98-8.18 (m, 4H, Ar-H); LCMS: (*m*/*z*) 464 [M+H]. *Anal*. Calcd. for $C_{24}H_{21}N_3O_5S$: C, 62.18; H, 4.57; N, 9.07. Found: C, 62.23; H, 4.60; N, 9.12.

PHARMACOLOGICAL ASSAY

Anti-inflammatory activity

All the synthesized compounds were tested for their anti-inflammatory activity using Carrageenan induced rat hind paw oedema method of Winter *et al*..[5] The oedema hind paw was induced by injection of 0.1 mL of 1% Carrageenan solution into subplanter region of right hind paw. The volume of the paw was measured plethysmographically immediately and 120 m after the injection of the irritant. The difference in volume gave the amount of oedema developed. Percentage inhibition of the oedema between control group and the compound treated group was calculated and compared with the group receiving standard drug at 50 mg/kg b.w. The results are tabulated in *Table* 1.

Antibacterial assay

A standard inoculum (1-2×10^7 c.f.u/cm^3 0.5 McFarland standards) was introduced on to the surface of sterile agar plates, and a sterile glass spreader was used for even distribution of the inoculum. The discs measuring 6.25 mm in diameter were prepared from Whatman no.1 filter paper and sterilized by dry heat at 140 °C for 1 h. The sterile disc previously soaked in a known concentration of the test compounds were placed in nutrient agar medium. Solvent and growth controls were kept. The plates were inverted and incubated for 24 h at 37 °C. The inhibition zones were measured and compared with the controls. Minimum inhibitory concentration (MIC) was determined by broth dilution technique. The nutrient broth, which contained logarithmic serially two fold diluted amount of test compound and controls were inoculated with approximately 5×10^5 c.f.u of actively dividing bacteria cells. The cultures were incubated for 24 h at 37 °C and the growth was monitored visually and spectrophotometrically. The lowest concentration (highest dilution) required to arrest the growth of bacteria was regarded as minimum inhibitory concentrations (*MIC*). Ciprofloxacin was used as a standard drug. The diameter of the zone of inhibition and minimum inhibitory concentration values are given in *Table* 2.

Antifungal assay

Sabourauds agar media was prepared by dissolving 1 g peptone, 4 g D-glucose, and 2 g agar in 100 cm^3 distilled water, and adjusting pH to 5.7 using buffer. Normal saline was used to make a suspension of spore of fungal strain for lawning. A loop full of particular fungal strain was transferred to 3 cm^3 saline to get a suspension of corresponding species. 20 cm^3 of agar media was poured in to each Petri dish. Excess of suspension was decanted and the plates were dried by placing in a incubator at 37 °C for 1 h.

Using an agar punch, wells were made and each well was labeled. A control was also prepared in triplicate and maintained at 37 ºC for 3-4 d. The inhibition zones in diameter were measured and compared with the controls. The Nutrient Broth, which contained logarithmic serially two fold diluted amount of test compound and controls was inoculated with approximately 1.6×10^4-6×10^4 c.f.u cm^{-3}. The cultures were incubated for 48 h at 35 ºC and the growth was monitored. The lowest concentration (highest dilution) required to arrest the growth of fungus was regarded as minimum inhibitory concentrations (*MIC*). Amphotericin B was used as the standard drug. The diameter of zone of inhibition and minimum inhibitory concentration values are given in *Table* 3.

CONCLUSION

In conclusion, we have described simple and efficient protocol for the synthesis of novel Isopropyl 2-thiazolopyrimidine-6-carboxylate derivatives **(6a-j)** with good yields. All the synthesized compounds have been investigated for their anti-inflammatory, antibacterial and antifungal activities. With our newly synthesized compounds, it is evident that **6b** and **6f** have highest anti-inflammatory activity; **6a**, **6b**, **6e**, **6f**, **6i** and **6j** have antibacterial activity; and **6a**, **6c**, **6f**, **6i** and **6j** have antifungal activity. Accordingly, these novel class of Isopropyl 2-thiazolopyrimidine-6-carboxylate derivatives reported from our laboratory emerge as a valuable lead series with great potential to be used as anti-inflammatory, antibacterial and antifungal agents, and as promising candidates for further efficiency evaluation.

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