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Synthesis and Biological Evaluation of Some Novel Aryl Substituted thiazolyl-4-thiazolidinone fused Pyrimidines as Potent Anti-Microbial and Anti-Inflammatory Agents

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A B S T R A C T

An innovative series of aryl substituted thiazolyl 4-thiazolidino pyrimidines has been synthesized by the condensation of 5-dimethylaminomethylene-2-(4-phenyl-thiazol-2-ylimino)-thiazolidin-4-one with urea, thiourea and guanidine and evaluated for their anti-microbial and anti-inflammatory activities. Three compounds were used for screening of anti-bacterial activity as well as anti-fungal activitity and one compound screened for anti-inflammatoty activity.

Keywords: 5-Dimethylaminomethylene-2-(4-phenyl-thiazol-2-ylimino)-thiazolidin-4-one, thiazolidino-pyrimidines, Anti-Inflammatory Activity, anti-microbial activity.

A R T I C L E I N F O

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1. Introduction

In the midst of the extensive range of heterocycles that have been explored for developing potential pharmacologically active compounds, 4-thiazolidinones fused with different heterocycles that are acknowledged to contribute to various

chemotherapeutic effects as anti-microbial, anti-inflammatory, anti-cancer and anti-convulsant agents. Additionally, some 4-thiazolidinone fused derivatives work as non nucleoside inhibitors of HIV Type 1 Reverse Transcriptase [5]. On the other hands, pyrimidines have a long and distinguished history extending from the days of their discovery as important constituents of nucleic acids to their current use in the chemotherapy of AIDS [6]. Pyrimidine derivatives have been come into view as anti-cancer, anti-microbial, anti-hypertensive, anti-malarial and anti-inflammatory active drugs [11].

The role of these molecules in the field of medicinal chemistry provoked us to imagine that these two energetic pharmacophores, if associated mutually, would create novel molecular templates which are expected to exhibit interesting biological properties. Combination of the thiazolidinone moiety with the pyrimidine nucleus may enhance these activities. In view of these previous findings and in continuation of our interest in the synthesis of thiazolidino fused pyrimidines, some new pyrimidine derivatives containing thiazolidin-4-one moiety have been synthesized and evaluated for some biological activities. To our knowledge, no attempt has been made in the literature to examine the feasibility of the preparation of the pyrimidines, addition on one side and substitution with thiazolyl moiety on the other side on 4-thiazolidinones. The structures of the newly synthesized compounds were confirmed with elementary microanalyses and substantiated with IR and ^{1}H NMR data. The target compounds have been subjected to the Pinnacle Biomedical Research Institute, PBRI, Bhopal to screen their anti-inflammatory activities. In addition, the *in-vitro* anti-bacterial and anti-fungal activities of the target compounds were also experienced.

2. Materials and Methods

Chemistry

Melting points were determined in open glass capillary and are uncorrected. Progress of reaction was monitored by using TLC on silica gel 'G' coated plates using benzene: methanol (9:1). IR spectra on KBr were recorded on FTIR-8400S (SHIMADZU) spectrometer. Mass spectra were taken on 3000 LC/MS system. ^{1}H NMR spectra were recorded on model AC-300F (Bruker) using $CDCl_3$/DMSO-d_6 as solvent. Chemical shift (δ) are given in ppm relative to signal for TMS as internal standard.

General procedure for synthesis of compounds 5-dimethylaminomethylene-2-(4-phenyl-thiazol-2-ylimino)-thiazolidin-4-one (2):

To a mixture of 2-(4-phenyl-thiazol-2-ylimino)-thiazolidin-4-one **(1)** (0.3g, 0.001mol) was taken in N, N-dimethyl formamide dimethyl acetal (15ml), and the solution was heated under reflux for 3hrs. After completion of reaction, the reaction mixture was cooled and poured on crushed ice. The residue was filtered and washed with hexane to give yellow color compound. Yield (72.4%) and m.p.130-135^{o}C.

General procedure for synthesis of compounds 2-(4-phenyl-thiazol-2-ylimino)-2, 3-dihydro-thiazolo [4, 5-d] pyrimidine-5-ol / thiol / ylamine (3/ 4/ 5):

To a mixture of 5-dimethylaminomethylene-2-(4-phenyl-thiazol-2-ylimino)-thiazolidin-4-one **(2)** (0.5g, 0.001mol), urea (1.2g, 0.02mol) and sodium ethoxide (1.36g, 0.02mol) in 20ml ethanol was refluxed for 7-8 hrs. The solvent was removed by distillation and residue was treated with glacial acetic acid (5ml) just to dissolve sodium salt of the pyrimidine and refluxed for 15min. The reaction mixture was then poured on crushed ice and solid obtained was recrystallized from ethanol to give compounds **3**. Compounds **4** and **5** were also prepared by adopting same method described for **3**.

Biological Activity

Anti-bacterial activity

To check the anti-bacterial activity, using disc diffusion method [12], 10mg of compounds were first dissolved in the ethanol to form the stock solution of the compounds to be tested followed making various dilutions at 100, 200 and 400µg/ml. The media, which was used for testing of anti-bacterial activity, was composed with peptone (5g), NaCl (5g), agar-agar (15g) in 1000ml distilled water and nutrient agar was developed for the microbes to grow the organisms by settling over Petri dishes. Ciprofloxacin (100, 200, and 400ppm) was used as reference drug. The sterilized nutrient agar solution was taken out by micro-pipettes and large number of Petri dishes was prepared by pouring agar solution to it then accurately cupped plates were placed in incubator at 37^{o}C for 6 hours and 28±2^{o}C at 48 hours for bacterial growth**.** *E. coli* and *Bacillus cereus* were used for anti-bacterial activity.

Anti-fungal activity

The media, which was used for testing of anti-fungal activity, had glucose (20g), peptone (10g) and agar-agar (15g) in one liter distilled water. The composition of potato dextrose (Hi-Media) was developed for the microbes to grow the organisms by settling over Petri dishes. The method described in the anti-bacterial activity was also applied for anti-fungal screening. *Fusarium oxysporium* and *Aspergilus nigar* were used for anti-fungal activity.

Anti-inflammatory Activity

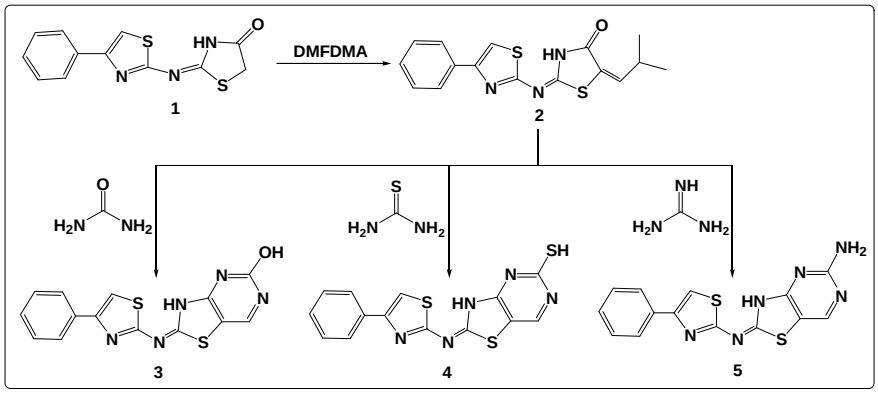
The anti-inflammatory activity of the standard drug Indomethacine and synthesized derivatives was determined using carrageenan induced paw oedema method [13] against carrageenan induced paw oedema in Sprague dawley rats (weighing 200±25 gm). Edema was induced by giving an intra-plantar injection of 1% carrageenan (Sigma Aldrich, Germany) in a normal saline of 0.1ml volume into the hind right paw using a 26 gauge needle. Paw thickness was measured with electronic digital calipers prior to 1, 3 and 5hrs following carrageenan administration. Peak effects occurred by 5h and carrageenan-induced paw edema returned to almost baseline levels by 24h, thus only the 5h data are reported. The standard drug (10mg/Kg) and synthesized derivatives was administered in animals by oral route. The differences in the paw diameter of each animal were calculated and compared with the changes in the paw diameter of control and the drug treated animals. The results were expressed as percentage decrease in paw diameter which can be calculated by using the formula:

Percent inhibition = [1 - paw diameter in treated (dt) / paw diameter in control (dc)] x 100.

3. Results and Discussion

Chemistry:

The aim in the present investigation is to examine the feasibility of the application of the intermediate dimethylamino methylenes and the synthesis of six-membered hetero-ring annulated compounds from their reaction with bidentate nucleophiles such as urea / thiourea / guanidine. Preparation of the objective compounds 3/4/5 was started through the reaction of compound **1** with DMF-DMA. The resulting compound **2** cyclised with urea / thiourea / guanidine. Compound **1** was synthesized by our previously described method [14]. The structure of pyrimidines was established by their physiochemical **(Table-1)** and spectral data **(Table-2).** The IR spectra of targeted pyrimidine derivatives show the absence of carbonyl stretching of the compound **2**. It is also revealed the characteristic absorption band at 1210cm^{-1} due to C=N stretching of pyrimidine ring. Their ^{1}H NMR spectrum (δ-ppm) have singlet at 3.00ppm assigned to one SH proton, a singlet at 7.80 ppm disperse the OH and at 4.05ppm due to NH_2 **(Scheme-1).**



Scheme 1: Synthesis of 4-Thiazolidinone fused pyrimidines

Biological Activities

The results of anti-microbial activity are given in **Table-3**. The zone of inhibition was measured in mm for each concentration. All of the tested compounds were found to exhibit good anti-microbial activity compared to standard drugs. The result of anti-inflammatory activity shows that compound **3** was found 58.14% inhibition as anti-inflammatory agent compared to standard drug idomethacine which show 82.29% inhibition, **Table-4**.

Table 1: Physicochemical characterization data for synthesized compounds 3, 4 and 5

S. No	Comp. Codes	Molecular Formula	M.W.	Yield (%)	M.P. (^{o}C)	Elemental Analysis Observed (Theoretical) percentage of C, H, N, & S			
						C	H	N	S
1.	3	$C_{20}H_{13}N_5OS_2$	453.99	60	255^{o}C-258^{o}C	59.78 (59.54)	3.46 (3.25)	17.67 (17.36)	15.85 (15.89)
2.	4	$C_{20}H_{13}N_5S_3$	498.44	64	278^{o}C-232^{o}C	57.21 (57.26)	3.42 (3.12)	16.57 (16.69)	22.95 (22.93)
3.	5	$C_{20}H_{14}N_6S_2$	449.57	61	284^{o}C-286^{o}C	59.34 (59.67)	3.53 (3.51)	20.89 (20.88)	15.62 (15.93)

Table 2: Spectral data of synthesized compounds 3, 4 and 5

S.No	Comp. Codes	IR (KBr) cm^{-1}	^{1}H NMR ($CDCl_3$ / DMSO) δ ppm
1.	3	3500 (O-H str.), 3355 (N-H str.), 3045 (C-H str.), 1586 (C=C str.), 1210 (C=N str.)	7.40-7.56 (5H, m, ArH), 7.00 (1H, s, CH), 7.06 (1H, s, ArH), 4.00 (1H, s, NH), 5.00 (1H, s, OH)
2.	4	3354 (N-H str.), 3038 (C-H str.), 2578 (S-H str.), 1210 (C=N str.), 2560 (S-H str.).	7.48-7.22 (4H, m, ArH), 7.77 (1H, s, ArH), 7.22 (1H, s, CH), 3.91 (1H, s, NH), 3.00 (1H, s, SH).
3.	5	3354 (N-H str.), 3037 (C-H str.), 1584 (C=C st.), 350, 1210 (C=N str.).	7.89-7.78 (5H, m, ArH), 7.56 (1H, s, ArH), 7.40 (1H, s, CH), 4.00 (2H, s, NH_2), 3.91 (1H, s, NH).

Table 4: Anti-inflammatory Activity: Paw Diameter (mm)

S. No	Treatment	Paw Diameter (mm)			% inhibition at 5hrs
		1hr	3hrs	5hrs	
1.	Control (Vehicle)	1.64±0.124	1.81±0.126	2.48±0.158	-
2.	Std. (Idomethacine)	0.76±0.113*	0.65±0.096*	0.44±0.104*	82.29
3.	5	1.12±0.106*	1.17±0.109*	1.04±0.112*	58.14

Values are expressed in MEAN±SD, n=6 animal in each group, One way Anova followed by Bonferroni test, *P<0.001 significant and NSP<0.001 non significant compared to the control.

Table 3: Anti-microbial activity of synthesized compounds 3, 4 and 5

S. No	Code of Comp.	Conc. (µg/ml)	*E. Coli*		*Bacillus cereus*		*Fusarium oxysporum*		*Aspergilus nigar*	
			Zone of inhibition (mm)	% activity compared to the standard	Zone of inhibition (mm)	% activity compared to the standard	Zone of Inhibition (mm)	% activity compared to the standard	Zone of Inhibition (mm)	% activity compared to the standard
1	**3**	400	19.0	94.44	13.0	72.22	18.0	60.00	20.5	75.92
		200	12.7	83.33	5.0	66.67	13.0	54.16	18.5	92.50
		100	7.0	74.50	35.0	65.50	8.5	47.22	12.1	80.66
2	**4**	400	20.0	83.33	17.0	94.44	25.0	83.33	26.3	97.40
		200	18.0	77.78	10.0	83.33	19.0	79.16	17.2	86.00
		100	6.0	66.67	6.0	74.50	14.0	77.78	12.3	82.00
3	**5**	400	17.0	79.16	12.6	70.00	21.0	70.00	23.1	85.55
		200	10.0	66.67	7.8	32.50	15.0	62.50	17.8	84.90
		100	6.0	58.33	4.8	60..00	9.0	50.00	13.6	80.22
Standard for Anti-bacterial activity	**Ciproflaxin**	400	24	100	18	100				
		200	18	100	12	100	-	-	-	-
		100	12	100	8	100				
Standard for Anti-fungal activity	**Fluconazole**	400					30.0	100	27.0	100
		200	-	-	-	-	24.0	100	20.0	100
		100					18.0	100	15.0	100

4. Conclusion

Compounds 2-(4-phenyl-thiazol-2-ylimino)-2, 3-dihydro-thiazolo [4, 5-d] pyrimidine-5-ol / thiol / yl-amine (3/ 4/ 5) have been synthesized and evaluated for anti-bacterial, anti-fungal and anti-inflammatory activities. All of the tested compounds were found to exhibit good anti-microbial activity compared to standard drugs. The result of anti-inflammatory activity shows that compound **3** was found 58.14% inhibition as anti-inflammatory agent.

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