Research Paper

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Aryl Substituted Thiazolyl 4-Thiazolidinopyrazoles and Isoxazoles: Novel Anti-Microbial and Anti-Inflammatory Agents

Chemistry

KEYWORDS : Disc diffusion method, isoxazoles, paws oedema method, percent inhibition, pyrazoles, 4-thiazolidinone

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ABSTRACT

A new class of aryl substituted thiazolyl 4-thiazolidinopyrazoles and isoxazoles has been synthesized and evaluated for their anti-microbial and anti-inflammatory activities. Gram positive bacteria Bacillus cereus, and gram negative bacteria Escheria coli were used for estimation of anti-bacterial activity and Fusarium oxysporium and Aspergilus nigar fungi were used for evaluation of anti-fungal activities. In the present work, activities of synthesized compounds were evaluated by the "Disc Diffusion Method" using inhibition zone technique. The zone of inhibition was determined in comparison to the standard drugs i.e. Ciprofloxacin for bacteria and Fluconazole for fungi. The anti-inflammatory activity is expressed in percent inhibition by carrageenan induced paw oedema method using Idomethacine as standard drug. Results indicate that these compounds belong to a new class of highly potent anti-microbial and anti-inflammatory agents. Five compounds have used for the screening of anti-bacterial activity and as well as five for anti-fungal activity and three compounds evaluated as anti-inflammatory agents.

INTRODUCTION:

4-thiazolidinone template has emerged as a potent advantaged structural fragment in synthesis, caused by its extensive pharmacological spectrum and its affinity to various bio-targets [1,2]. 4-Thiazolidinone embraces the most important position among pharmaceutically significant natural products and synthetic compounds. 4-Thiazolidinones wok as anti-microbial [3], anti-tubercular [4], anti-inflammatory [5,6], anti-convulsant [7], anti-HIV [8], anti-cancer [9] and hypoglycemic active agents [10]. The role of 4-thiazolidinone nucleus in the field of medicinal chemistry provoked us to keep on working on synthesize novel derivatives having this moiety. The incredible ability of 4-thiazlidinones, to serve equally as biomimetics and reactive pharmacophore, makes up them an extremely considerable class of compounds.

Recent synthetic endeavors directed towards the development of biologically active materials have stressed the need to recognize certain building block set in synthesis and to explore their potential to provide access to the library of compounds of medicinal utility. Literature is full up with an extensive range of examples of one pot synthesis of heterocyclic systems having five-membered rings from the corresponding chalcones[11] and dimethylamino methylene ketones [12,13]. We envisioned that this concept could be applied in our synthetic work by using the potential of a novel building block developed from the thiazolyl substituted-4-thiazolidinones **(4a-b)** which could be synthesized according to scheme-**1** in three simple steps from phenacyl bromide **(1a-b)** involving first its cyclisation with thiourea, then reaction with chloroacetyl chloride to synthesize chloroacetamide **(3a-b)** followed by its cyclocondensation with NH_4SCN. 4-Thiazolidinones **(4a-b)** was further use to synthesize reactive intermediate like chalcones **(5a-f)** and dimethylamino methylene ketones **(6a-b)**. The five-membered heterocyclic ring systems *i.e.* isoxazole and pyrazole condensed thiazolidine moieties **(7-10)** prepared from **(5** and **6)** with the reaction of hydroxylamine hydrochloride and hydrazines respectively Scheme-**2**.

This study is to be expected to produce analogs with improved therapeutic profile. It is hoped that synthesis and biological evaluation of these compounds would provide a rationale approach to the study of structure activity relationship of these molecules. Based on these observations, it can be anticipated that incorporation of pyrazole, and isoxazole moieties on the one side of the 4-thiazolidinone nucleus and the pharmacophores like substituted thiazoles on the other side can produce interesting series of compounds with improved biological properties with minimum toxicity. As per to this prospect, compounds in scheme-**1** and scheme-**2** responded practically well in showing an outstandingly high level of anti-microbial and anti-inflammatory activity.

2. MATERIALS AND METHODS:

ANTI-BACTERIAL ACTIVITY:

To test the anti-bacterial activity through "Disc diffusion method" [14] 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, composed as 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 400 ppm) 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°C for 6 hours and 28±2°C at 48 hours for bacterial growth**.**

2.2 ANTI-FUNGAL ACTIVITY:

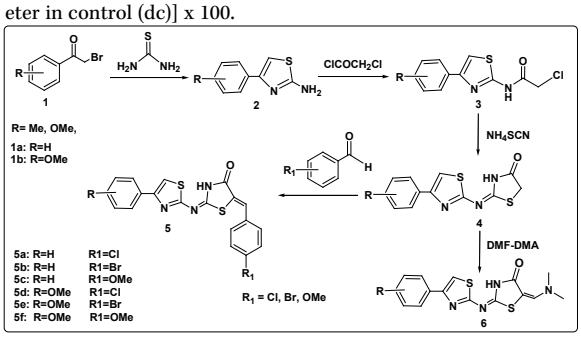
The media, which was used for testing of anti-fungal activity, had glucose (20g), peptone (10g) and agar-agar (15g) in one liter distilled water. The composition of potato dextrose (HI-Media) was developed for the microbes to grow the organisms by settling over Petri dishes. The method described in the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

2.3 ANTI-INFLAMMATORY ACTIVITY:

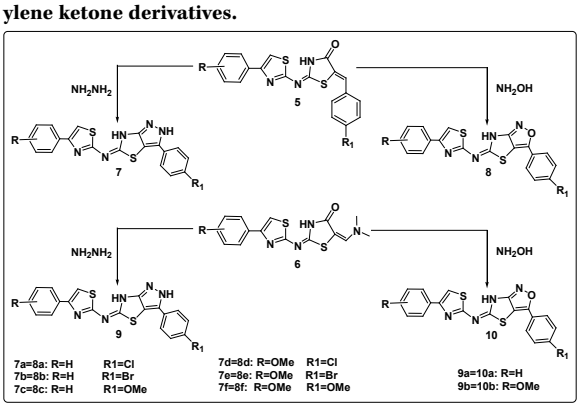
2.3.1 CARRAGEENAN INDUCED RAT PAW OEDEMA METHOD:

The anti-inflammatory activity of the standard drug Indomethacine and synthesized derivatives was determined using carrageenan induced paw oedema method [15] 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 5 hrs 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 reduction 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.



Scheme-1. Synthesis of Chalcone and Dimethyl aminomethylene ketone derivatives.



Scheme-2. Synthesis of pyrazole and isoxazole derivatives.

3. RESULT AND DISCUSSION:

Results originated from anti-microbial and anti-inflammatory studies of few selected compounds have been presented in Table-**1** and Table-**2** respectively. The results of anti-microbial and anti-inflammatory activities revealed that pyrazoles **(7a, 7d, 9a)** have good biological activities compared to the isoxazoles **(8b, 10b).** Insertion of arylidene moiety at position-**5** in five-membered ring may also increase the activities, when p-Cl aryl substitution has appeared it may more effectual for the compound. It was also observed that electron withdrawing group **(Cl)** improve the reactivity of drug and electron donating group **(OMe)** may reduced the effect of drug. Results show that among five of the tested compounds, compound **7d** shows highly remarkable anti-microbial and anti-inflammatory when compared with standard drugs. Compound **10b** was weakly active in biological activities as compared to the standard drug. It means these compounds possess good anti-microbial and anti-inflammatory activities.

Table:1 Anti-microbial activities of the synthesized compounds:

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
(A)	7a	400 200 100	19.0 12.7 7.0	94.44 83.33 74.50	13.0 5.0 35.0	72.22 66.67 65.50	21.0 15.0 9.0	70.00 62.50 50.00	24.3 17.1 12.6	90.00 85.50 84.00
(B)	7d	400 200 100	20.0 18.0 6.0	83.33 77.78 66.67	17.0 10.0 6.0	94.44 83.33 74.50	25.0 19.0 14.0	83.33 79.16 77.78	26.3 17.2 12.3	97.40 86.00 82.00
(C)	8b	400 200 100	17.0 10.0 6.0	79.16 66.67 58.33	12.6 7.8 4.8	70.00 32.50 60..00	18.0 13.0 8.5	60.00 54.16 47.22	23.1 17.8 13.6	85.55 84.90 80.22
(E)	9a	400 200 100	15.0 9.3 4.0	62.50 51.67 39.33	11.0 4.0 5.0	61.11 58.33 50.00	19.5 15.0 9.0	65.00 62.50 50.00	20.5 18.5 12.1	75.92 92.50 80.66
(F)	10b	400 200 100	14.0 9.0 5.1	58.33 50.00 42.50	12.0 7.5 4.6	66.67 62.50 57.50	15.0 11.0 8.0	50.33 48.00 44.44	18.3 17.2 12.3	67.77 86.00 82.00
Standard for Anti-bacterial activity	Ciproflaxin	400 200 100	24 18 12	100 100 100	18 12 8	100 100 100	-	-	-	-
Standard for Anti-fungal activity	Fluconazole	400 200 100	-	-	-	-	30.0 24.0 18.0	100 100 100	27.0 20.0 15.0	100 100 100

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Table-2: 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.	7d	0.93±0.103*	0.79±0.115*	0.66±0.088*	73.44
4.	8b	1.12±0.106*	1.17±0.109*	1.04±0.112*	58.14
5.	10b	1.53±0.132^{NS}	1.71±0.171^{NS}	2.08±0.064*	36.29

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

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REFERENCE

[1] Farhat, Mahmoud F.; Makhlouf, Mansour A.; El-Saghier, Ahmed M.; Mezoughi, Aysha B.A.; Awhida, Salma M.; El-mehdi, Amal A.M. Utilization of 2-ylidene-4-thiazolidinones in the synthesis of heterocyclic compounds. Part I: Synthesis of pyrazoles. Arabian J. Chem. 2011, 4(3), 307-311. | [2] Goel, B; Ram, T.; Tyagi, R.; Bansal, E.; Kumar, A.; Mukherjee, D.; Sinha, J. N. 2-Substituted-3-(4-bromo-2-carboxyphenyl)-5-methyl-4-thiazolidinones as potential anti-inflammatory agents. Eur. J. Med. Chem., 1999, 34(3), 265-269. | [3] Sattigeri, V. J.; Soni, A.; Singhal, S.; Khan, S.; Pandya, M.; Bhateja, P.; Mathur, T.; Rattan, A.; Khanna, J. M.; Mehta, A. Synthesis and antimicrobial activity of novel thiazolidinones. ARKIVOC, 2005, (ii), 46-59. | [4] Babaoglu, K.; Page, M. A.; Jones, V. C.; McNeil, M. R.; Dong, C.; Naismith, J. H.; Lee, R. E. Novel inhibitors of an emerging target in mycobacterium tuberculosis; substituted thiazolidinones as inhibitors of dTDP-rhamnose synthesis. Bioorg. Med. Chem. Lett., 2003, 13(19), 3227-3230. | [5] Vigorita, M. G.; Ottana, R.; Monforte, F.; Maccari, R.; Trovato, A.; Monforte, M. T.; Taviano, M. F. Synthesis and anti-inflammatory, analgesic activity of 3,3′ -(1,2-ethanediyl)-bis[2-aryl-4-thiazolidinone] chiral compounds. Part 10. Bioorg. Med. Chem. Lett., 2001, 11(21), 2791-2794. | [6] Deep, A.; Jain, S.; Sharma, P.C.; Phogat, P. Malhotra, M. Synthesis of 2-(aryl)-5-(arylidene)-4-thiazolidinone derivatives with potential analgesic and anti-inflammatory activity. Med. Chem. Res. 2012, 21(8), 1652-1659. | [7] Gaikwad, N. J.; Yunus, M.; Husain, H. A.; Meshram, D. B. Mannich reaction product of 5-arylidene-2-phenylimino-4-thiazolidinone as anti-convulsant. Indian J. Heterocyclic Chem., 2002, 12, 165-166. | [8] Rao, A.; Balzarini, J.; Carbone, A.; Chimirri, A.; Clercq, E. D.; Monforte, A. M.; Monforte, P.; Pannecouque, C.; Zappala, M. 2-(2,6-dihalophenyl)-3-(pyrimidine-2-yl)-1,3-thiazolidin-4-ones as non nucleoside HIV-1 reverse trascriptase inhibitors. Antiviral Res., 2004, 63(2), 79-84. | [9] Ottana, R.; Carotti, S.; Maccari, R.; Landini, I.; Chiricosta, G.; Caciagli, B.; Vigorita, M. G.; Mini, E. In vitro anti-proliferative activity against human colon cancer cell lines of representative 4-thiazolidinones Part I. Bioorg. Med. Chem. Lett., 2005, 15(17), 3930-3933. | [10] Bhat, B. A.; Ponnala, S. K.; Sahu, D. P.; Tiwari, P.; Tripathi, B. K. and Srivastava, A. K. Synthesis and anti-hyperglycemic activity profiles of novel thiazolidinedione derivatives. Bioorg. Med. Chem., 2004, 12(22), 5857-5864. | [11] An, H.; Eum, S-J.; Koh, M.; Lee, S. K.; Park, S. B. Diversity-oriented synthesis of privileged benzopyranyl heterocycles from s-cis-enones. J. Org. Chem., 2008, 73(5), 1752-1761. | [12] Martins, M. A. P. ; Fiss, G. F.; Frizzo, C. P.; Rosa, F. A.; Bonacorso, H. G.; Zanatta, N. Highly Regioselective Synthesis of Novel 1,4'-Bipyrazoles. J. Braz. Chem. Soc. 2010, 21, 240-247. | [13] Sharma, P.; Kumar, N.; Kaur, N.; Kishore, D. Synthesis of new derivatives of 2-Substituted 1,5-Benzodiazepine and Evaluation of their Anti-microbial activities. J. Appli. Chem. 2013, 2 (3): 426-432. | [14] Klancnik, A.; Piskernik, S.; Jersek, B.; Mozina, S.S. Evaluation of diffusion and dilution methods to determine the antibacterial activity of plant extracts. J. Microbiol. Methods. 2010, 81, 121-126. | [15] Wise, L. E.; Cannavacciulo, R.; Cravatt, B. F.; Martin, B. F.; Lichtman, A. H. Evaluation of fatty acid amides in the carrageenan-induced paw edema model, Neuropharmacology xx, 2007, 1-8. |