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ANTIMICROBIAL AND PHARMACOLOGICAL EVALUATION OF NEWER SUBSTITUTED COUMARIN DERIVATIVES

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ABSTRACT

A series of substituted Coumarin derivatives (2a-j) have been screened *in vitro* for antibacterial, antifungal and *in vivo*, for their anti-inflammatory activity. Most of the compounds were found to be most potent antimicrobial and anti-inflammatory as compared with the reference drugs used.

key words: Substituted Coumarin, Antimicrobial, Anti-inflammatory.

INTRODUCTION

Compounds comprising a coumarin (2-oxo-2*H*-1-benzopyran) backbone have a wide range of biological activities. Vast literature on the synthesis and biological activities with the coumarin moiety has taken the coumarin framework to a unique position in the drug designing. Various coumarin derivatives are known for several bioactivities such as fungicidal [1-3], bactericidal [4-8], pesticidal [9,10], activities. Some coumarins have been reported as antimicrobial [11], the anticoagulant [12], CNS depressant[13], antitumour [14,15], HIV proliferator [16], intermediate for the synthesis of cardiac drug [17], 'carbocromen' have been reported for coumarins. It is pertinent to mention here that potent antibiotics chartreusin [18] and coumermycine [19] are the coumarin derivatives. Another coumarin containing antibiotics such as novobiocin, clorobiocin and coumermycin A_1, produced by number of Streptomyces species, have been known for more than 40 years [20]. Later it has been discovered that these antibiotics are potent inhibitors of bacterial DNA gyrase [21] and topoisomerase IV [22]. Recently *invitro* evaluation of novel highly potent coumarin [23] inhibitors of gyraseB is reported. Two series of amino substituted coumarins [24] were evaluated *in vitro* as inhibitors of DNA gyrase and as potential antibacterials. The excellent antimicrobial activity of novel 3-arylazo-7-hydroxy-4-methyl coumarins [25] has been reported. Khan IA [20] has evaluated some coumarins as antimicrobial agents. Coumarins are important building blocks in the synthesis of the antibiotic novobiocin [27] and aflatoxin [28]. The coumarin derivative (Warfarin) is being used as anticoagulant for management of thromboembolic vascular diseases [29]. The anticoagulant, fungicidal, tuberculostatic and diuretic properties of coumarin derivatives are reported [30]. 4-Hydroxycoumarins are believed to be promising drug candidates as non-peptidic HIV protease inhibitors [31]. Spino C *et al* [32] have reported anti-HIV coumarins from *calophyllum* seed oil. Earlier certain other pyranocoumarins such as soulattrolide [33] isolated from *calophyllum* species were found to be active against HIV. Some coumarin [34] with sulfonamide moiety were reported as antitubercular. During last few years the attention has been focussed on simpler synthetic models due to the discovery of the antiviral [35] properties of coumarins. Among the natural and synthetic coumarin derivatives there are compound possessing anti-inflammatory [36], anthelmintic [37], hypnotic, analgesic [38] and anti-hepatotoxic [39] etc. activities.

Due to such wide range of activities shown by coumarin derivatives, it was thought to be fruitful to study their antimicrobial and anti-inflammatory activities.

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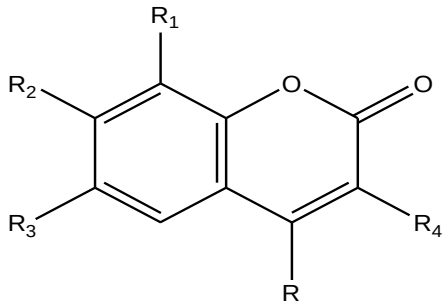
EXPERIMENTAL

Antimicrobial Activity

The antimicrobial screening of the synthesized compounds was determined by agar cup-plate method [40] at a concentration of 1000 µg/ml using DMF as solvent. All the compounds were screened *in vitro* for antibacterial for antibacterial activity against two gram-positive bacteria. *Staphylococcus aureus* and *Bacillus subtillis* and two gram-negative bacteria, *Escherichia coli* and *Salmonella species* and antifungal activity against *Aspargillus niger* and *Candida albicans*. The plates were incubated at 37^0 for 24 hour in case of antibacterial activity where as in case of antifungal activity for 48 hours. The control also maintained with 0.1 ml of DMF and zone of inhibition of the growth was measured in mm. The activity was compared with the standard drugs gentamicin (100 µg/ml) and griseofulvin (40 µg/ml) for antibacterial activity and antifungal activity respectively. Each experiment was conducted thrice and the averages of three were recorded. The results are presented in Table 1.



Coumarin (2a-2j)

	R	R_1	R_2	R_3	R_4
2a	CH_3	I	H	I	H
2b	CH_3	I	H	I	Ph
2c	CH_3	I	H	I	$NHCOCH_3$
2d	CH_3	I	H	I	NHCOPh
2e	CH_3	I	CH_3	Cl	H
2f	CH_3	I	CH_3	Cl	Ph
2g	CH_3	I	CH_3	Cl	$NHCOCH_3$
2h	CH_3	I	CH_3	Cl	NHCOPh
2i	H	I	OH	I	H
2j	H	I	OH	I	Ph

Anti-inflammatory Activity

Anti-inflammatory activity was measured using the formalin-induced paw odema test in rats [41]. Albino rats of either sex (150-200 g) were divided into control, standard and test groups, each consisting of six rats. A group of rats was treated with Tween-80 (1%) suspension I.P. (control). Another group was administered a dose of 10 mg/kg of suspension of phenylbutazone (standard) P.O. and the third group was treated with 100 mg/kg of the suspension of the test compounds. After 30 min the animals were injected with 0.1 ml of formalin (1% w/v) in the sub plantar region of left hind paw of the rats.

The volume of the paw was measured using the mercury displacement technique with the help of plethysmograph both in control as well as in standard animals including the test animals at 2 and 4 hour after injection. The initial volume of the paw was measured within 30 s of the injection. The percent inhibition of the inflammation after 2 and 4 h was calculated by using the following formula. % inhibition = $(1\text{-}V_t/V_c)$ 100, where V_c and V_t are the mean relative changes in the volume of paw odema in the control and test, respectively. The results are summarized in the table 2.

Table 1. *In vitro* antimicrobial activity of the compounds 2a-2j

Compound	Diameter of zone of inhibition in mm*					
	S.aureus	***B.subtilis***	***E.coli***	***S.species***	***A.niger***	***C.albicans***
2a	10	14	10	17	17	15
2b	14	17	12	19	15	19
2c	15	16	11	19	18	16
2d	17	19	13	20	16	14
2e	19	18	15	21	16	16
2f	21	22	15	25	22	18
2g	19	18	16	20	17	16
2h	21	20	14	19	20	22
2i	22	17	16	23	18	20
2j	23	16	14	24	21	24
Gentamicin	26	27	19	32	-	-
Griseofulvin	-	-	-	-	28	25

* Including diameter of the well-8 mm, Control (DMF)= No activity.

Table 2. Anti-inflammatory activity of the compounds 2a-2j

Compound	Dose(mg/kg)	Oedema volume (ml) different interval (mean±S.E.M.)		% Inhibition	
		2h	4h	2h	4h
2a	100	0.24 (±0.02)	0.17 (±0.02)	11.7	25.8
2b	100	0.24(±0.02)	0.17 (±0.02)	12.4	28.0
2c	100	0.22(±0.17)	0.15(±0.02)	18.2	34.5
2d	100	0.22(±0.01)	0.15(±0.02)	18.2	36.5
2e	100	0.20(±0.00)	0.11(±0.05)	26.6	52.5
2f	100	0.19(±0.01)	0.10(±0.00)	28.5	55.9
2g	100	0.19(±0.01)	0.10(±0.00)	28.8	55.1
2h	100	0.19(±0.01)	0.10(±0.00)	29.2	56.8
2i	100	0.20(±0.00)	0.11(±0.02)	24.0	51.7
2j	100	0.19(±0.01)	0.10(±0.02)	27.7	53.8
Standard (Phenyl butazone)	100	0.17(±0.02)	0.10(±0.00)	26.3	57.6
Control (Tween-80)	--	0.27(±0.02)	0.23(±0.02)	----	---

RESULT AND DISCUSSION

Antibacterial activity evaluation (table 1) reveals that, compounds 2e – 2j showed highest activity against *S. aureus*. Highest activity was also observed in the compounds 2d, 2f and 2h against *B.subtilis*. Compounds 2e to 2g and 2i displayed good activity against *S.species* and none of the compounds tested showed highest activity against *E.Coli*. Remaining compounds exhibited moderate to weak activity against all organisms. Antifungal activity evaluation (Table 1) displayed that compounds 2f, 2h and 2j showed very good activity against *A. niger and C.ablicans* respectively. Other compounds exhibited moderate to weak activity against both organisms. Amongst the compounds tested for antifungal activity, compounds possessing more halogen showed highest activity.

Amongst the compounds subjected to anti-inflammatory screening (table 2). Compounds, 2e(52.5%), 2f(55.9%), 2g(55.1%), 2h(56.8%), 2i(51.7%) and 2j(53.8)% were found to possess significant compared to that of the standard, phenylbutazone activity. The remaining compounds showed moderate activity (25.8-36.5%).

CONCLUSION

Hence, it can be concluded that, halogen subst. coumarins were evaluated for anti microbial and anti inflammatory potential using *in vitro* and *in vivo* methods respectively. It was found that certain compounds displayed strong activity compared to the standard compounds used and it suggested that these compounds could have great importance as therapeutic agents by exploring their further properties.

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REFERENCES

1. Reddy YD, Somayayula VV. *J Ind Chem Soc*, 58, 1981, 599.
2. Sangwan NK, Verma BS, Mallik OP, Dhindsa KS. *Ind J Chem*, 29B, 1990, 294.
3. Sardari S, Mori Y, Horita K, Micetich RG. *Bioorg Med Chem.* 7, 1999, 1933-1940, and Gursoy A, Ates O, Karali N, Cesur N, Kiraz M. *Eur J Med Chem*, 31, 1996, 643.
4. El-Naggar MA, Ahmed FSM, Abd El-Salam AM, Rade MA, Latif MSA. *J Het Chem*, 18, 1981, 1203.
5. Ueda Y, Chuang JM, Crast LB, Partyka RA. *J Antibiot*, 42, 1989, 1379.
6. Rao AK, Raju MS, Raju KM. *J Ind Chem Soc*, 58, 1981, 1021.
7. Shah S, Mehta RH. *J Ind Chem Soc*, 64, 1987, 708.
8. Vyas SR, Bapa ST, Mehta RH. *J Ind Chem Soc*, 67, 1990, 482-484 and Shah S, Desai D, Mehta RH, *J Ind Chem Soc*, 76, 1999, 507-508.
9. Firanz E, Klaubo E, Hammann I, *Ger Pat*-3012642/1981; *Chem Abstr*, 96, 1982, 14276.
10. Chavan VP, Mane AS, Shingare MS, *Ind J Chem*, 40B, 2001, 339-341.
11. Maxwell A. *Mol Microbiol*, 9, 1993, 681-688.
12. Bourgain RH, Oriessche RV, Smith P. *Arch Inst Pharmacodyn Ther*, 195, 1972, 240 and Manolov I, Danchen ND, *Eur J Med Chem*, 30, 1995, 531-535.
13. Moffett RB. *J Med Chem*, 7, 1964, 446.
14. Raev L, Voinov E, Ivanov I, Popov D. *Pharmazie*, 45, 1990, 696; *Chem Abstr*, 114, 1990, 74711b.

15. Dexeus FH, Logothetis CJ, Sella A, *J Clin Oncol*, 8, 1990, 325-329.
16. Kun E, Aurelian L, US Pat, 412, 1991, 783; *Chem Abstr*, 115, 1991, 97071t.
17. Bohdan S, Elzbieta B. *Pol PL*, 136, 1985, 525; *Chem Abstr*, 113, 1990, 40455k.
18. Simontish E, Eisenhuth W, Stanam OA, Schrnid H, *Helv Chem Acta*, 43, 1960, 58.
19. Childres S. *Topics Med Chem*, 1, 1967, 109.
20. Hinman JW, Hoeksema H, Caron EL, Jackson WG. *J American Chem Soc*, 78, 1956, 1072-1074.
21. Gellert M, O'dea MH, Itoh T, Tomizawa JI. *Proc Natl Acad Sci, USA*, 73, 1976, 4474.
22. Peng H, Marians KJ. *J Biol Chem*, 268, 1993, 24481-24490.
23. Laurin P, Ferroud D. *Bioorg Med Chem Lett*, 9, 1999, 2079-84.
24. Laurin P, Ferroud D, *Bioorg Med Chem Lett*, 9, 1999, 2875-2880.
25. Sharma P, Pritmani S. *Ind J Chem*, 38B, 1999, 1139-1142.
26. Khan IA, Kulkarni MV. *Ind J Chem*, 38B, 1999, 491-494.
27. Smissman EE. "Text Book of Org. Medicinal and Pharmaceutical Chemistry", 8th Edition, Lippincott Co., Toronto USA, 1982, 291-292.
28. Ueno Y. "Mycotoxins in Human and Animal Health" Park Forest South 4, Panthotox, 1977.
29. D'Melo AP, Venkataraman RV, Bates TR. *Drug Metabolism and Disposition*, 20, 1992, 572.
30. Sumiki Y, Ishi Y, Japan, 1955, 6697; *Chem Abstr*, 51, 1957, 170-199 and Kulkarni MV, Patil VD. *Arch Pharm*, (Weinheim) 314, 1981, 435-439.
31. Thaisrivongs S. *J Med Chem*, 39, 1996, 2400.
32. Spino C, Dodier M, Sotheeswaran S. *Bioorg Med Chem Lett*, 8, 1998, 3475-3478.
33. Fuller WR, Bokesch HR, Gustafsn KR, *Bioorg Med Chem Lett*, 4, 1994, 1961.
34. Gupta AS, Phull MS. *Ind J Chem*, 35B, 1996, 276.
35. Zembower DE, Liao SY, Flavin MT, Xu ZQ, Stup TL, Buckheit RW. *J Med Chem*, 40, 1997, 1007-1017.
36. Bylov IE, Vasylyev MV, Bilokin YV. *Eur J Med Chem*, 34, 1999, 997-1001.
37. Billeres D, Blondeau D, Sliwa H, *J Heterocyclic Chem*, 30, 1993, 671 and references cited therein.
38. Pachecho H, Gotto R. *Bull Soc Chem France*, 1960, 93.
39. Susan T and Saraswathy A. *Ind Drugs*, 38(3), 2001, 143-146.
40. Indian Pharmacopoeia, 3rd Edn, Apendix IV, Controller of Publications New Delhi, 1985, 90.
41. Kulkarni SK. In; Hand book of experimental pharmacology, 1st Edn. Vallabh Prakashan, New Delhi, 1987, 63.