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In Vitro Antioxidant Activity Evaluation of 4-Methyl Coumarin Derivatives

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We have screened a number of heterocyclic compounds (4-methyl-coumarins) bearing different functionalities such as hydroxy, acetoxy and propoxy in combination with carboxylic acid group at different positions in the benzenoid ring of the coumarin nucleus for their effect on NADPH catalyzed liver microsomal lipid peroxidation and radical scavenging activity using diphenyl picryl hydrazyl (DPPH) with a view to make the structure activity relationship (SAR). Compounds **5**, **6**, **7** and **8** showed excellent *in vitro* inhibition of NADPH catalyzed lipid peroxidation and DPPH radical scavenging activity.

Key Words: Lipid peroxidation, Radical scavenging, Carboxylic acid group, 4-Methylcoumarin, Structure activity relationship.

INTRODUCTION

In recent years great efforts have been made to search for new antioxidants. This is mainly because man started paying attention to primary health care[1] and the main reason for this has been the increasing control of life threatening diseases by medicinal sciences. Reactive oxygen species (ROS) is mainly involved in the initiation of lipid peroxidation in healthy cells and causes adverse pathologies such as alzheimer arthrosclerosis, cancer, Parkinson's disease and retinal degradation[2]. In today's polluted environment natural antioxidant's defense is inadequate to neutralize the stray radicals in healthy individuals, therefore, scientists are trying to discover highly potent antioxidant candidate from synthetic chemicals. Among synthetic chemicals[3-5], coumarins are widely accepted for different pharmacological[6-8] and other biological activities[9]. Due to the wide spread applications, biological activity evaluation of coumarin derivatives has been a subject of intense investigations. Here in this report we have screened a number of coumarin derivatives possessing different functionalities like hydroxy, acetoxy and propoxy in combination with carboxylic acid group at different positions in the benzenoid ring of the coumarin

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(Fig. 1). The SAR studies showed that introduction of carboxylic acid group in the benzenoid ring of the coumarin nucleus enhanced the antioxidant and radical scavenging activity while monoacetoxy, monopropoxy and monobutoxy alone were totally ineffective for the same activity.

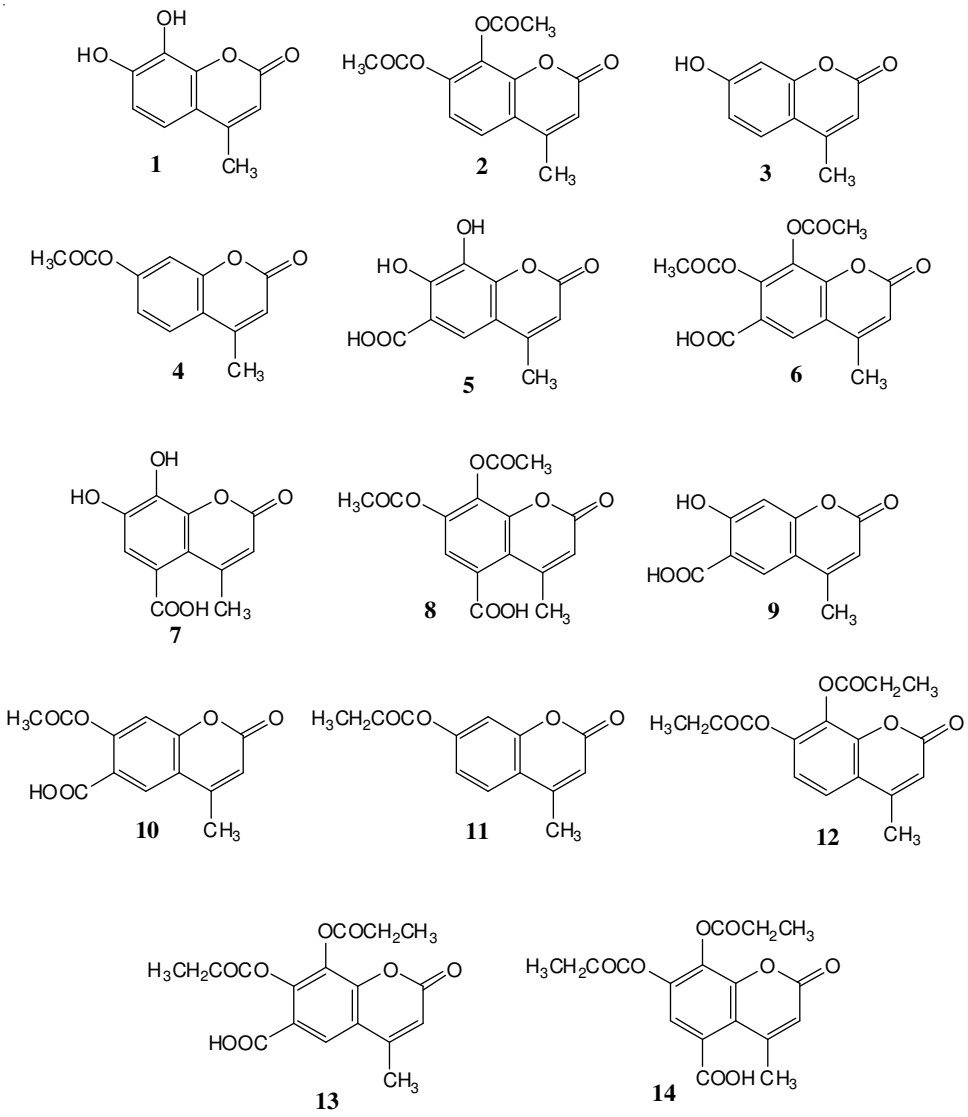


Fig. 1. Structure of the test compounds

EXPERIMENTAL

Adenosine diphosphate (ADP), nicotinamide adenine dinucleotide phosphate (NADPH), trichloroacetic acid (TCA) were purchased from Sisco Research Laboratory (Mumbai, India), Trizma (tris HCl), $FeCl_3$, thiobarbituric acid (TBA), dimethyl

sulfoxide (DMSO) of high purity grade were purchased from local suppliers. diphenyl picryl hydrazyl (DPPH) was obtained from Sigma Chemical Co. St Louis MO, USA.

Test compounds: The compounds **1**-**14** (Fig. 1) were synthesized in our laboratory by the well known Pechmann condensation[10] with little modifications followed by acetylation, propylation and butylation[11]. The model compounds **1**-**4**, **11** and **12** were confirmed by the spectral data reported in literature[12]. The physical and spectral data of compound **5**-**10**, **13** and **14** examined for *in vitro* antioxidant activity are already reported[13].

Animals: Male albino rats of wistar strain weighing around 180-200 g fed on rat chow supplied by Hindustan Lever Ltd., Mumbai (India) were used.

Preparation of rat liver microsomes: The method of Ernster and Nordenbrand[14] was used for the preparation of rat liver microsomes. Rats were killed by decapitation, liver removed and 30 % homogenate (w/v) was prepared in 0.25 M sucrose solution. The homogenate was centrifuged at 10,000 g for 0.5 h in a Sorvall refrigerated centrifuge. The supernatant was spun at 100,000 g for 1 h in the Beckman ultracentrifuge Model L-7 and the surface of microsomal pellets was washed twice with 0.15 M KCl and resuspended in 0.15 M KCl. The protein contents of microsomes were estimated by the method of Lowry *et al.*[15].

Assay for initiation of lipid peroxidation: Detailed assay procedures have been given in previous communication[16]. In short, rat liver microsomes (1 mg protein) were preincubated with tris HCl (0.025 M, pH 7.5) and test compound (100 µM, in DMSO) was added and incubated at 37 ºC for 10 min followed by the addition of ADP (3 mM) and $FeCl_3$ (0.15 mM). The initiation of enzymatic lipid peroxidation was started by the addition of NADPH (0.5 mM) and incubation of the reaction mixture continued for 10 min. The products of lipid peroxidation were quantified by estimation of thiobarbituric acid reactive substances (TBARS) thus formed as described earlier[16].

Assay for DPPH radical scavenging: A solution of test compounds in methanol (4 mL) at 100 µM concentration of the inhibitor was added to 1 mL of DPPH solution in methanol (0.15 mM). The contents were vigorously mixed, allowed to stand at 20 ºC for 0.5 h and the absorption was read at 517 nm.

IC_{50} values determination: Different concentrations of inhibitor ranging from 0.01-100 mM were incubated as described above to calculate the inhibitor concentration for 50 % inhibition (IC_{50}).

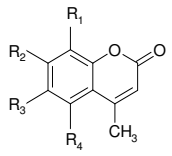
Statistical analysis: The final values are the mean of three observations and standard deviation was calculated with the help of following website: www.easycalculation.com/statistics/standard-deviation.

RESULTS AND DISCUSSION

In this communication, evaluation of inhibitory properties of 4-methylcoumarin derivatives on initiation of NADPH catalyzed lipid peroxidation *in vitro* in rat liver

microsomes was undertaken using earlier reported procedures[17]. The results given in Table-1 demonstrate the effect of 4-methylcoumarin derivatives on the initiation of lipid peroxidation enzymatically. The coumarin nucleus is derivatized by hydroxy, acetoxy and propoxy group(s) alone and in combination with carboxylic acid groups. The dihydroxy and diacetoxy coumarins in the combination with the carboxylic acid group at 6th position in benzenoid ring, compounds **5** and **6** were found to cause highest inhibition (91 and 88 %) for the initiation of lipid peroxidation which is slightly higher than model compound 7,8-dihydroxy-4-methylcoumarin (DHMC, **1**) and 7,8-diacetoxy-4-methylcoumarin (DAMC, **2**) (87 and 85 %, respectively). When carboxylic acid group was shifted from 6th to 5th positions in compound **7** and **8**, the inhibition of initiation of lipid peroxidation became absolutely equal to the model compounds **1** and **2**. The *ortho* dipropoxy coumarin derivative showed much less inhibition (44 %) as compared to model compounds but introduction of carboxylic acid group at the *ortho* position to propoxy group in the compound **13** increased the activity (55 %) moderately. The monohydroxy and monoacetoxy, **3** and **4** are totally ineffective for the same biological activity but contrary to the effect of above mentioned compounds, introduction of carboxylic acid group at the *ortho* position of hydroxyl and acetoxy group in the compounds **9** and **10** increased the activity.

TABLE-1
EFFECTS OF COUMARINS ON NADPH-DEPENDENT LIPID PEROXIDATION IN RAT LIVER MICROSOMES

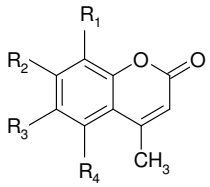


Comp. No.	R_1	R_2	R_3	R_4	Inhibition (%)	Standard deviation	Reference
1	OH	OH	H	H	87	0.2	17
2	$OCOCH_3$	$OCOCH_3$	H	H	85	0.2	17
3	H	OH	H	H	–	-	18
4	H	$OCOCH_3$	H	H	–	-	18
5	OH	OH	COOH	H	91	0.6	
6	$OCOCH_3$	$OCOCH_3$	COOH	H	88	0.4	
7	OH	OH	H	COOH	87	0.9	
8	$OCOCH_3$	$OCOCH_3$	H	COOH	85	0.6	
9	H	OH	COOH	H	35	1.5	
10	H	$OCOCH_3$	COOH	H	22	1.4	
11	OPr	OPr	H	H	44	0.4	
12	H	OPr	H	H	–	-	
13	OPr	OPr	COOH	H	55	0.2	
14	OPr	OPr	H	COOH	46	0.6	

The inhibitors concentration was 100 μM. The values represent mean of three separate experiments with variation of < 5 %.

As it is widely accepted that the coumarins play a key role in the antioxidant property[17] the relative antioxidant activities demonstrated by different functionalities on coumarins, screened in this report have been found to be dependant on the functionalities. The order for hydroxy-4-methylcoumarins in combination with carboxylic acid group was 7,8-dihydroxy-4-methylcoumarin-6-carboxylic acid (**5**) > 7,8-dihydroxy-4-methylcoumarin-5-carboxylic acid (**7**) = DHMC (**1**) > 7-hydroxy-4-methyl coumarin-6-carboxylic acid (**9**) and for acetoxy and propoxy 4-methylcoumarins in combination with carboxylic acid group was 7,8-diacetoxy-4-methyl coumarin-6-carboxylic acid (**6**) > 7,8-diacetoxy-4-methylcoumarin-5-carboxylic acid (**8**) = DAMC (**2**) > 7,8-dipropoxy-4-methylcoumarin-6-carboxylic acid (**13**) > 7,8-dipropoxy-4-methylcoumarin-5-carboxylic acid (**14**) > 7-acetoxy-4-methylcoumarin-6-carboxylic acid (**10**) (Table-1). The mechanism pathway for the inhibition of NADPH catalyzed micro-somal lipid peroxidation by coumarin derivatives are given in earlier publication[17]. Out of all the compounds examined, compounds **5**, **6**, **7** and **8** showed greater potency (IC_{50} 5.5, 6.2, 6.6 and 6.1 μM, respectively) in inhibiting the initiation of lipid peroxidation as compared to that of commercially available antioxidant compound α-tocopherol (IC_{50} 31.2 μM) (Table-2).

TABLE-2
COMPARATIVE INHIBITORY ACTION OF SOME 4-METHYLCOUMARINS



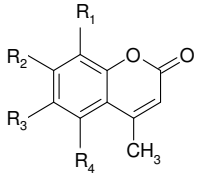
Comp. No.	R_1	R_2	R_3	R_4	IC_{50} (μM)	Reference
1	OH	OH	H	H	7.90	17
2	$OCOCH_3$	$OCOCH_3$	H	H	0.25	17
5	OH	OH	COOH	H	5.50	
6	$OCOCH_3$	$OCOCH_3$	COOH	H	6.20	
7	OH	OH	H	COOH	6.60	
8	$OCOCH_3$	$OCOCH_3$	H	COOH	6.10	
9	H	OH	COOH	H	61.8	
10	H	$OCOCH_3$	COOH	H	22.0	
11	OPr	OPr	H	H	52.6	
13	OPr	OPr	COOH	H	41.8	
α-tocopherol					31.2	18

The initiation of lipid peroxidation procedure is given in materials and methods. The effect of inhibitors concentration ranging from 0.01-100 μM on initial rate of lipid peroxidation was determined to calculate the inhibitor concentration for 50 % inhibition (IC_{50}). The values represent mean of three separate experiments with variation of < 5 %.

As a result of previous work[18], the monohydroxy and monoacetoxy derivatives of 4-methylcoumarin were totally ineffective in causing the radical scavenging activity. But 7-hydroxy-4-methylcoumarin-6-carboxylic acid (**9**) and 7-acetoxy-4-methyl-

coumarin-6-carboxylic acid (**10**) demonstrated good DPPH radical scavenging activity (Table-3). Almost same pattern of activity was observed between 7,8-dipropoxy-4-methylcoumarin and 7,8-dipropoxy-4-methyl coumarin-6-carboxylic acid. When carboxylic acid group was shifted from position 6th to 5th in the benzenoid ring of coumarin nucleus, radical scavenging activity was checked and found to be showing less activity than former. The deacetylation of acetoxy-4-methyl coumarin forming hydroxyl derivatives which performed the radical scavenging pathway as described in our previous publication[17]. It was also observed from the above mentioned data that depropylation of propoxy derivatives of 4-methyl coumarin forming hydroxyl derivatives with slower rate as compared to acetoxy derivatives which in turn showed less radical scavenging activity (Table-3).

TABLE-3
RADICAL SCAVENGING POTENTIAL OF SOME 4-METHYLCOUMARINS



Comp. No.	R_1	R_2	R_3	R_4	Inhibition (%)	Standard deviation	Reference
1	OH	OH	H	H	96	0.1	17
2	$OCOCH_3$	$OCOCH_3$	H	H	87	0.2	17
3	H	OH	H	H	–	–	18
4	H	$OCOCH_3$	H	H	–	–	18
5	OH	OH	COOH	H	98	0.2	
6	$OCOCH_3$	$OCOCH_3$	COOH	H	97	0.5	
7	OH	OH	H	COOH	97	0.2	
8	$OCOCH_3$	$OCOCH_3$	H	COOH	96	0.5	
9	H	OH	COOH	H	51	0.6	
10	H	$OCOCH_3$	COOH	H	51	0.6	
11	OPr	OPr	H	H	56	0.5	
12	H	OPr	H	H	–	–	
13	OPr	OPr	COOH	H	98	0.1	
14	OPr	OPr	H	COOH	96	0.2	

The inhibitors concentration was 100 μM. The values represent mean of three separate experiments with variation of < 5 %.

Conclusion

When tested *in vitro* on a panel of NADPH dependant microsomal lipid peroxidation and DPPH radical scavenging, some 4-methylcoumarins showed high and significant activities. In view of the *in vitro* results, compounds **5**, **6**, **7** and **8** showed high degree of inhibition for the initiation of lipid peroxidation and DPPH radical scavenging activities. The present results demonstrated that inclusion of the

carboxylic acid group at 5th and 6th position in the benzenoid ring of 4-methylcoumarin derivatives (**5**-**10**, **13**, **14**) imparted potent inhibition and the introduction of propoxy group in place of acetoxy group in 4-methylcoumarin decreased the inhibitory potency. All these results prompt us to examine more and more novel compounds with the ultimate aim of discovering new and highly active antioxidant compounds in future.

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REFERENCES

1. N.R. Farnsworth, O. Akerele, A.S. Bingel, D.D. Soejarto and Z. Guo, *Bull. World Health Org*., **63**, 965 (1985).
2. G.G. Duthie, *Eur. J. Clin. Nutr*., **47**, 759 (1993).
3. A.R. Liss, in eds.: V. Cody, E. Midleton and J.B. Harbone, Plant Flavonoids in Biology and Medicine, New York, Vol. 1, p. 15 (1986).
4. R.H. Midleton, *Trans. Pharmacol. Sci*., **5**, 335 (1985).
5. R. Edward, D.C. Sauter, T. Bruce, A.R. John and J.P. Andres, *Carcinogenesis*, **15**, 2269 (1994).
6. S. Takeda and M.J. Aburada, *J. Pharmacobio-Dyn*, **4**, 724 (1981).
7. D. Egan, R.O. Kennedy, E. Moran, D. Cox and E. Prosser, *Drug Metabol. Rev*., **22**, 503 (1990).
8. C.H. Yang, C. Chiang, K. Liu, S. Perg and R. Wang, *Yao Huseh Tung Pao*, **15**, 48 (1980).
9. M. Bhalla, P.K. Nathani, A. Kumar, T.N. Bhalla and K. Shanker, *Indian J. Chem*., **31B**, 183 (1992).
10. H.V. Pechmann and C. Duisberg, *Ber. Dtsch. Chem. Ges.*, **16**, 2119 (1883).
11. V.S. Parmar, K.S. Bisht, R. Jain, S. Singh, S.K. Sharma, O.D. Tyagi and H.N. Pati, *Indian J. Chem*., **35B**, 220 (1996).
12. H.G. Raj, S. Gupta, A.K. Prasad, P.M. Boll, J. Wengel, G. Biswas, S. Singh, S.K. Sharma, S.C. Jain and V.S. Parmar, *Bioorg. Med. Chem. Lett*., **5**, 1567 (1995).
13. Y.K. Tyagi, S. Tyagi, H.G. Raj and R.K. Gupta, *Sci. Pharm*., **76**, 395 (2008).
14. L. Ernster and K. Nordenbrand, *Methods Enzymol*., **10**, 574 (1967).
15. O.H. Lowry, N.K. Rosebrough, A.T. Farr and R.J. Randall, *J. Biol. Chem*., **193**, 265 (1951).
16. H.G. Raj, R.K. Sharma, B.S. Garg, V.S. Parmar, S.C. Jain, A. Singh, Y.K. Tyagi, H.N. Jha and C.E. Olsen, *Bioorg. Med. Chem*., **6**, 2205 (1998).
17. Y.K. Tyagi, A. Kumar, H.G. Raj, P. Vohra and R.K. Gupta, *Eur. J. Med. Chem*., **40**, 413 (2005).
18. H.G. Raj, V.S. Parmar, S.C. Jain, S. Goel, Poonam, Himanshu, S. Malhotra, A. Singh, C.E. Olsen and J. Wengel, *Bioorg. Med. Chem*., **6**, 833 (1998).

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