International Journal for Pharmaceutical Research Scholars (IJPRS)

V-5, I-3, 2016 ISSN No: 2277 - 7873

REVIEW ARTICLE

Synthetic Approaches and Biological Activities with density and refractive index of 4-Hydroxycoumarin Derivatives

Dr. Govind Kher*[1], Dr. Haresh Ram[1]

[1] *Tolani College of Arts & Science, Adipur (Kutch), Gujarat, India*

Manuscript No: IJPRS/V5/I3/00129, Received On: 04/08/2016, Accepted On: 16/08/2016

ABSTRACT

An immaculate and disciplined method for synthesis of fresh Cumarin derivatives was skillful from different substituted-4-hydroxy-2H-chromen-2-one, acetic acid and POCl3 using with refluxed and no further purification requirement. The compounds were supported by FTIR, 1HMR and mass spectral data and biological activity completed.

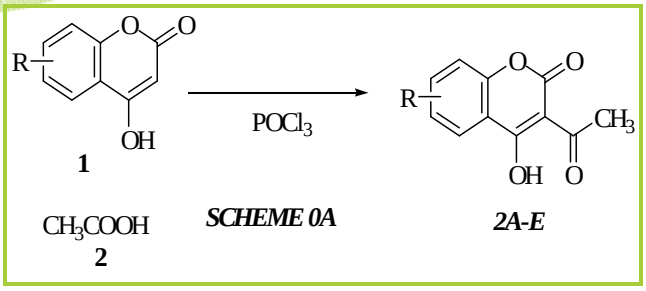
KEYWORDS

Substituted-4-hydroxy-2H-chromen-2-one; acetic acid and POCl3 only refluxed

INTRODUCTION

4-Hydroxycoumarins have evoked a immense deal of attention due to their biological properties and characteristic conjugated molecular structural design. Coumarins are present in natural and synthetic compounds possessing biological activity. They are acting at different stages of cancer formation. Some of them have cytostatic properties and the other have cytotoxic activity [1-3]. Two naturally occurring coumarins have been found to exhibit cytotoxicity against a panel of mammalian cancer cell lines [4]. In view of their importance as drugs, biologically active natural products and in other related applications, extensive studies have been carried out on the synthesis of coumarin compounds in recent years. It also represents the core structure of several molecules of pharmaceutical importance. Coumarin has been reported to serve as antibacterial [5-8], anti-oxidant [9,10], anti-inflammatory [11,12], anticoagulant8 and antitumour [13,14] agents. These pharmacological properties of coumarin aroused our interest in synthesizing some coumarin derivatives with the aim of testing their microbiological activity.

We have developed a new diffidence for the synthesis **3-acetyl-4-hydroxy-2H-chromen-2-one and its derivatives *(2A-E)*** with the advantage of fine yield and environmentally easiness (**Scheme-0A**).



SCHEME 0A

2A-E

METHOD

STEP 02

A 4-hydroxy coumarin derivative was mixed with glacial acetic acid, and phosphorous oxychloride was added slowly, the mixture was further reflux for 2-5 hrs. And then poured on

***Address for Correspondence:**
Dr. Govind Kher,
Tolani College of Arts & Science, Adipur (Kutch), Gujarat, India.
E-Mail Id: drgjkher@gmail.com

crushed ice with stirring. The solid separated out was filtered, washed with water and crystallize from methanol.

RESULTS & DISCUSSION

3-acetyl-6-chloro-4-hydroxy-2H-chromen-2-one (2A)

Yield: 60%; mp 149ºC; Anal. Calcd. for $C_{11}H_7ClO_4$: C, 55.37; H, 2.96; Cl, 14.86; O, 26.82; Found: C, 55.40; H, 2.93; Cl, 14.88; O, 26.80%; IR (cm^{-1}): 3313 (O-H stretching), 3101 (C-H stretching of aromatic ring), 2944 (C-H asymmetrical stretching of CH_3 group), 2841 (C-H symmetrical stretching of CH_3 group), 1658 (C=O stretching of ketone), 1602, 1535 & 1471 (C=C stretching of cyclic), 1404 (C-H asymmetrical deformation of CH_3 group), 1346 (C-H symmetrical deformation of CH_3 group), 1269 (C-O-C stretching), 922 (para-substituted), 772 (C-H in out plane deformation of aromatic ring), 680 (C-Cl stretching); ^{1}H NMR (DMSO) δ ppm: 2.50 (s, 3H, H), 7.93-7.97 (dd', 2H, H), 8.26-8.29 (dd', 1H, H), 13.09 (s, 1H, H), MS: *m/z* 239.

3-acetyl-7-chloro-4-hydroxy-2H-chromen-2-one (2B)

Yield: 68%; mp 144ºC; Anal. Calcd. for $C_{11}H_7ClO_4$: C, 55.37; H, 2.96; Cl, 14.86; O, 26.82; Found: C, 55.35; H, 2.94; Cl, 14.88; O, 26.84%; MS: *m/z* 239.

3-acetyl-4-hydroxy-6-methyl-2H-chromen-2-one (2C)

Yield: 54%; mp 147ºC; Anal. Calcd. for $C_{12}H_{10}O_4$: C, 66.05; H, 4.62; O, 29.38; Found: C, 66.00; H, 4.65; O, 29.30%; MS: *m/z* 218.

3-acetyl-6-fluoro-4-hydroxy-2H-chromen-2-one (2D)

Yield: 58%; mp 140ºC; Anal. Calcd. for $C_{11}H_7FO_4$: C, 59.47; H, 3.18; F, 8.55; O, 28.81; Found: C, 59.49; H, 3.25; F, 8.50; O, 28.80%; MS: *m/z* 222.

3-acetyl-7-fluoro-4-hydroxy-2H-chromen-2-one (2E)

Yield: 55%; mp 155ºC; Anal. Calcd. for $C_{11}H_7FO_4$: C, 59.47; H, 3.18; F, 8.55; O, 28.81; Found: C, 59.49; H, 3.20; F, 8.53; O, 28.79%; MS: *m/z* 222.

DENSITY AND REFRACTIVE INDEX

The solvents N. N-dimethyformamide (DMF) andtetrahydrofuran (THF) were of LR grade and are fractionally distilled by the reported method [15]. All the studied synthesized compounds were recrystalized from DMSO. For each compound, a series of solutions of different concentrations were prepared in DMF and THF solvents.

The density and refractive index of solutions were measured by using pyknometer and Abbe refractometer respectively. Study of refractive index and density was completed at constant temperature viz. 303.15 K, which is maintained by circulating water through jacket around the prisms of refractometer from an electronically controlled thermostatic water bath (NOVA NV-8550 E). The uncertainty of temperature was ±0.1º C.Mettler Toledo AB204-S, Switzerland electronic balance with uncertainty of ± 0.0001 g, was used for all the weights taken for density measurements.

The density of solution (ρ_{12}) is related to densities of the solvent, solute and their weight fractions g_1 and g_2 according to the equation:

$$\frac{1}{\rho_{12}} = \frac{g_1}{\rho_1} + \frac{g_2}{\rho_2}$$

Where ρ_{12} is the density of solution and ρ_1 and ρ_2 are the densities of solvent and solute respectively. The experimental values of densities and refractive index for synthesized compounds in different solutions.

The slope of the plot of $1/g_1\rho_{12}$ verses g_2/g_1 gives the density of these compounds. The plot of $1/g_1\rho_{12}$ verses g_2/g_1 is given in DMF and DMSO respectively. The densities of all the synthesized compounds were evaluated from the slope of such plots. The inverse of slope gives density of compound (ρ_2). These calculated

densities for all the compounds. Further, the density of compounds were calculated by using the following equation

$$\rho = KM / N_A \sum \Delta V_i$$

ρ indicates the density of the compound, K is packing fraction which is equal to 0.599, M is for molecular weight of the compound, N_A is the Avogadro's number and ΔV_i is the volume increment of the atoms and atomic groups present in the compound. The density of all the studied compounds have been evaluated and reported. The calculated volume increment ΔV_i for different atomic groups are given.

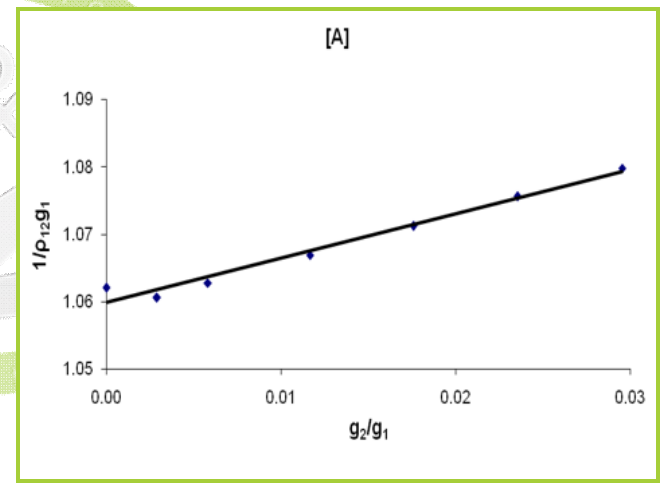
Comparison of densities evaluated from graphs and those calculated from equation showed that calculated values are different from those evaluated graphically. For the same compound, density in the two different solvents is different. This suggests that one has to consider the role of solvent in the measurement of the physical parameters of any solutions. It is because of the

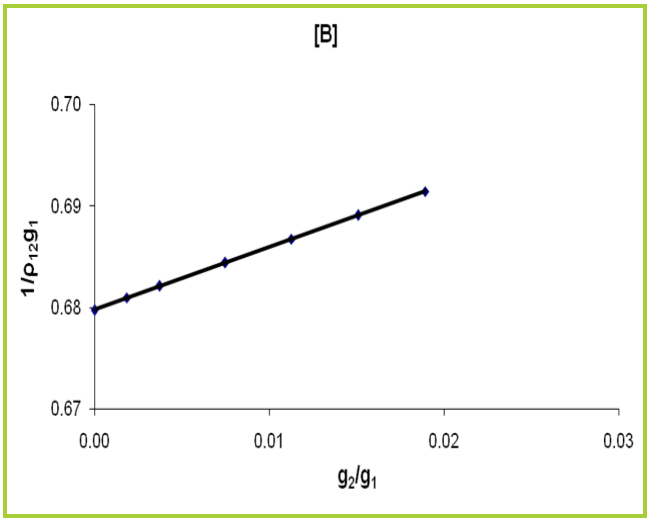
The density (ρ12) and refractive index (n) of in DMF & DMSO at 303.15 K.

Conc.(M)	DMF		THF	
	ρ12 (g.cm^{-3})	η	ρ12 (g.cm^{-3})	η
2A				
0.00	0.9303	1.4250	0.8777	1.4031
0.01	0.9321	1.4238	0.8775	1.4037
0.02	0.9325	1.4247	0.8791	1.4046
0.04	0.9330	1.4261	0.8793	1.4057
0.06	0.9337	1.4277	0.8801	1.4068
0.08	0.9341	1.4290	0.8815	1.4082
0.10	0.9352	1.4305	0.8847	1.4094
2B				
0.01	0.9303	1.4250	0.8777	1.4031
0.02	0.9344	1.4256	0.8768	1.4046
0.04	0.9352	1.4259	0.8784	1.4063
0.06	0.9355	1.4263	0.8804	1.4072
0.08	0.9357	1.4272	0.8823	1.4090
0.10	0.9358	1.4289	0.8832	1.4094

Conc.(M)	DMF		THF	
	ρ12 (g.cm^{-3})	η	ρ12 (g.cm^{-3})	η
2C				
0.01	0.9303	1.4250	0.8777	1.4031
0.02	0.9348	1.4258	0.8821	1.4059
0.04	0.9349	1.4268	0.8824	1.4063
0.06	0.9351	1.4282	0.8830	1.4071
0.08	0.9352	1.4292	0.8836	1.4079
0.10	0.9353	1.4302	0.8848	1.4092
2D				
0.01	0.9303	1.4250	0.8777	1.4031
0.02	0.9331	1.4238	0.8791	1.4052
0.04	0.9332	1.4249	0.8803	1.4071
0.06	0.9339	1.4260	0.8816	1.4083
0.08	0.9341	1.4272	0.8821	1.4090
0.10	0.9352	1.4292	0.8825	1.4103

The variation of 1/g1ρ12 with g2/g1 for 2A in [A] DMF and [B] DMSO at 303.15 K.





Experimental and calculated densities of compounds in DMF and DMSO solutions at 303.15 K.

Compounds	Density ($g.cm^{-3}$) calculated from slope of graph in two solvents		Density ($g.cm^{-3}$) calculated from Eqn. 3.5.2
	DMF	DMSO	
2A	1.5174	1.6235	1.2961
2B	1.2271	1.9381	1.2625
2C	1.2871	1.7824	1.3164
2D	1.7952	1.8693	1.3566

Facts that, in every solution molecular interactions exist which differ with different solvents. This is further confirmed by acoustical parameter which is already discussed. Generally, intermolecular interactions do not affect the density but due to the presence of different substituted groups in solutes, interactions differ in different solvents which may cause change in volume thereby affecting the density of solute in a particular solvent.

The molar refraction of a pure liquid $(MRD)_1$ can be calculated by the following equation:

$$(MRD)_1 = \left[\frac{n^2-1}{n^2+1}\right]\frac{M}{\rho}$$

wheren, M and ρ are refractive index , molecular weight and density of pure liquid respectively.

For solutions, the eq. was used to determining molar refraction.

$$(MRD)_{12} = \left[\frac{n_{12}^2-1}{n_{12}^2+1}\right]\left[\frac{X_1M_1+X_2M_2}{\rho_{12}}\right]$$

where$_{12}$ and ρ_{12} are refractive index and density of solution respectively. X_1 and X_2 are the mole fractions and M_1 and M_2 are the molecular weight of the solvent and solute respectively.

The plots of $(MRD)_{12}$ verses concentration for all the studied compounds in DMF and DMSO. It is evident from these figures that $(MRD)_{12}$ increases with the increase in concentration. From the values of the molar refraction of solution and pure solvent, molar refraction of solid compounds were determined by following equation:

$$(MRD)_{12} = X_1(MRD)_1 + X_2(MRD)_2$$

From the density and molar refraction data, the refractive indexes of all the compounds were calculated from equation. The molar refraction $(MRD)_2$ and refractive index of all the compounds are reported for 0.1 M solution.

Each solvent interacts differently with different functional groups, so that $(MRD)_2$ and refractive index of compounds is different in each solvent, as shown in below Table. As discussed above, in different solvents intermolecular interactions are different, which affect these parameters. In some solvents, aggregation or hydrogen bonding takes place whereas in others, breakage of bonds takes place. The refractive index and molar refraction depends not only upon atomic refraction but also upon single, double or triple bonds. However, it is reported that bond refraction is more effective than atomic refraction [16, 17]. Further, bond polarity also causes change in molar refraction. Thus, type of solvent affects the refractive Index and molar refraction of a solute.

Calculated molar refraction and refractive index of 0.1 M solution of Compounds in DMF and DMSO at 303.15 K.

Compounds	Solvents			
	DMF		DMSO	
	$(MRD)_2$	n	$(MRD)_2$	n
2a	121.6162	1.7681	76.5281	1.4704
2b	127.0075	1.6446	59.2673	1.4466
2c	114.3013	1.5262	64.0095	1.3923
2d	99.0074	1.7551	50.4576	1.3538

Antimicrobial evaluation

Total of the Prepared compounds ***(4A-E)*** were experienced for their antibacterial and antifungal activity (MIC) *in vitro* by broth dilution method [18-20] with two Gram-positive bacteria ***Staphylococcus aureus*** MTCC-96, ***Streptococcus pyogenes*** MTCC 443, two Gram-negative bacteria ***Escherichia coli*** MTCC 442, ***Pseudomonas aeruginosa*** MTCC 441 and three fungal strains ***Candida albicans*** MTCC 227, ***Aspergillus Niger*** MTCC 282, ***Aspergillus clavatus*** MTCC 1323 taking **gentamycin, ampicillin, chloramphenicol, ciprofloxacin, norfloxacin, nystatin and greseofulvin** as regular drugs.

The minimal inhibitory concentration (MIC) values for all the newly synthesized compounds, definited as the lowly concentration of the compound preventing the observable growth, were determined by using micro dilution broth method according to NCCLS standards .

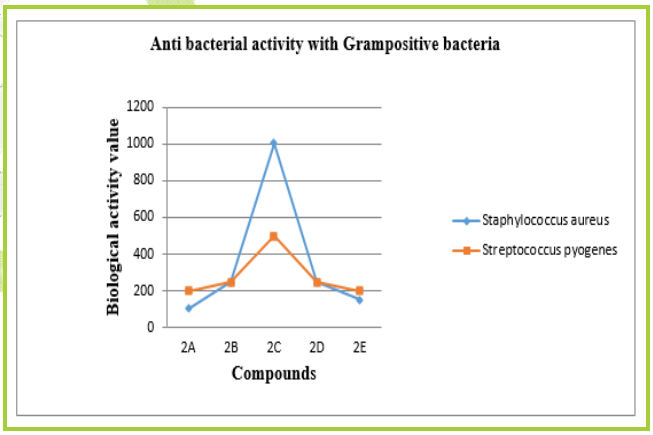
Minimal Inhibition Concentration [MIC]:-
The main advantage of the 'Broth Dilution Method' for MIC determination lies in the fact that it can readily be converted to determine the MIC as well.

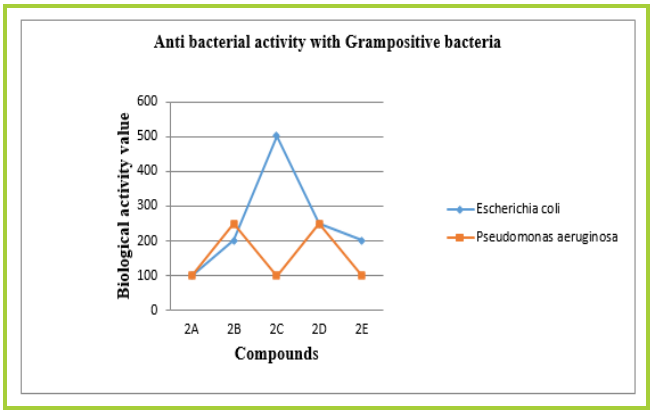
- Serial dilutions were prepared in primary and minor screening.
- The control tube containing no antibiotic is immediately subcultured by spreading a loopful evenly over a quarter of plate of medium suitable for the growth of the test organism and put for incubation at 37 ^{0}C overnight.
- The MIC of the control organism is read to check the accuracy of the drug concentrations.
- The lowest concentration inhibiting growth of the organism is recorded as the MIC.
- The amount of growth from the control tube before incubation (which represents the original inoculums) is compared.

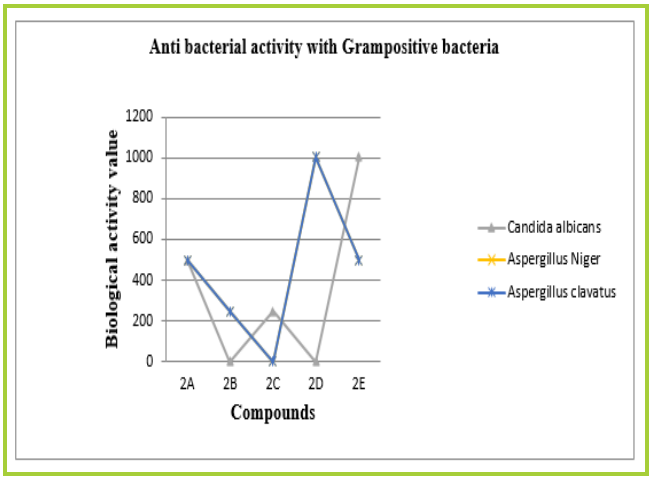
Antibacterial and antifungal activity of synthesized compounds (2a-e)

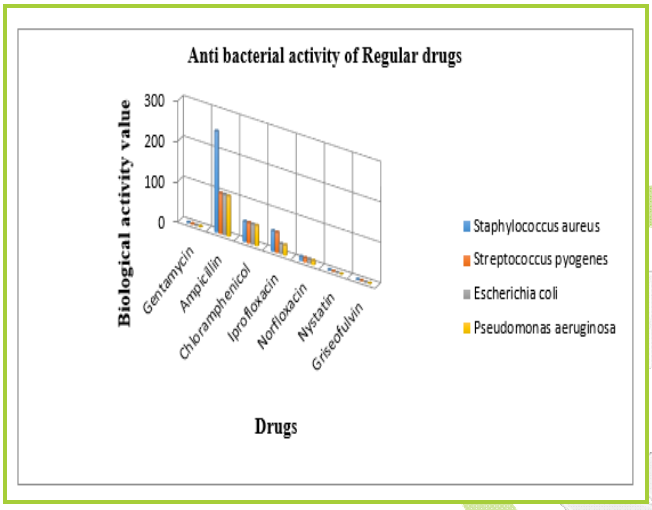
Code	Minimal inhibition concentration (µg mL^{-1})						
	Gram-positive		Gram-negative		Fungal species		
	S.a.	*S. p.*	*E.c.*	*P.a.*	*C. a.*	*A. n.*	*A.c.*
2A	100	200	100	100	500	500	500
2B	250	250	200	250	>1000	250	250
2C	1000	500	500	100	250	>1000	>1000
2D	250	250	250	250	>1000	1000	1000
2E	150	200	200	100	1000	500	500
Gentamycin	0.25	0.5	0.05	1	-	-	-
Ampicillin	250	100	100	100	-	-	-
Chloramphenicol	50	50	50	50	-	-	-
Iprofloxacin	50	50	25	25	-	-	-
Norfloxacin	10	10	10	10	-	-	-
Nystatin	-	-	-	-	100	100	100
Griseofulvin	-	-	-	-	500	100	100

Antibacterial and antifungal activities









CONCLUSION

In tallness, we include synthesized of fresh **3-acetyl-4-hydroxy-2H-chromen-2-one** derivatives using straightforward and appropriate method. This method produces these products in unmatched yields and difficulty-free workup. Product is isolated by smooth filtration. The isolated products are very pure and do not need any purification. This study opens up a new area of useful synthesis of potentially biologically active novel pyrimidine derivatives compounds.

ACKNOWLEDGEMENT

Authors are thankful to the department of chemistry, Tolani College of Arts & Science, Adipur (Kutch), India for providing laboratory facilities. One of us Dr Govind J. Kher is thankful to University Grants Commission, Bahadur Shah Zafar Marg, New Delhi for providing a research project [(**47-609/13(WRO)** dated **20 MAR 2014**].

REFERENCES

1. Wright, W. E., & Shay, J. W. (2001). Cellular senescence as a tumor-protection mechanism: the essential role of counting. *Current opinion in genetics & development*, *11*(1), 98-103.
2. Ag, B. (1998). Ouellette M. Frolkis M. Holt SE. Chiu CP. Morin GB. Harley CB. Shay JW. Lichtsteiner S. Wright WE. Extension of life-span by introduction of telomerase into normal human cells. *Science*, *279*, 349-352.
3. Gillis, A. J., Schuller, A. P., & Skordalakes, E. (2008). Structure of the Tribolium castaneum telomerase catalytic subunit TERT. *Nature*, *455*(7213), 633-637.
4. Marshall, M. E., Kervin, K., Benefield, C., Umerani, A., Albainy-Jenei, S., Zhao, Q., & Khazaeli, M. B. (1994). Growth-inhibitory effects of coumarin (1, 2-benzopyrone) and 7-hydroxycoumarin on human malignant cell lines in vitro. *Journal of cancer research and clinical oncology*, *120*, S3-S10.
5. Ukhov, S. V., Kon'shin, M. E., & Odegova, T. F. (2001). Synthesis and antimicrobial activity of 2-iminocoumarin-3-carboxylic acid amides. *Pharmaceutical Chemistry Journal*, *35*(7), 364-365.
6. Elhafez, O. M. A., Khrisy, E. E. D. A. M. E., Badria, F., & Fathy, A. E. D. M. (2003). Synthesis and biological investigations of new thiazolidinone and oxadiazoline coumarin derivatives. *Archives of pharmacal research*, *26*(9), 686-696.
7. Basanagouda, M., Kulkarni, M. V., Sharma, D., Gupta, V. K., Sandhyarani, P., & Rasal, V. P. (2009). Synthesis of some new 4-aryloxmethylcoumarins and examination of their antibacterial and antifungal activities. *Journal of chemical sciences*, *121*(4), 485-495.

8. Liu, X., Dong, M., Chen, X., Jiang, M., Lv, X., & Zhou, J. (2008). Antimicrobial activity of an endophytic Xylaria sp. YX-28 and identification of its antimicrobial compound 7-amino-4-methylcoumarin. *Applied Microbiology and Biotechnology*, *78*(2), 241-247.

9. Trapkov, V. A., Parfenov, E. A., & Smirnov, L. D. (1996). Synthesis and antiulcer activity of copperand zinc-containing coumarin antioxidants. *Pharmaceutical chemistry journal*, *30*(7), 445-447.

10. Vukovic, N., Sukdolak, S., Solujic, S., & Niciforovic, N. (2010). An efficient synthesis and antioxidant properties of novel imino and amino derivatives of 4-hydroxy coumarins. *Archives of pharmacal research*, *33*(1), 5-15.

11. Emmanuel-Giota, A. A., Fylaktakidou, K. C., Litinas, K. E., Nicolaides, D. N., & Hadjipavlou-Litina, D. J. (2001). Synthesis and biological evaluation of several 3-(coumarin-4-yl) tetrahydroisoxazole and 3-(coumarin-4-yl) dihydropyrazole derivatives. *Journal of Heterocyclic Chemistry*, *38*(3), 717-722.

12. Hamdi, N., & Dixneuf, P. (2007). Synthesis of triazole and coumarin compounds and their physiological activity. *Bioactive Heterocycles IV*, 123-153.

13. Wang, M., Wang, L. F., Li, Y. Z., Li, Q. X., Xu, Z. D., & Qu, D. M. (2001). Antitumour activity of transition metal complexes with the thiosemicarbazone derived from 3-acetylumbelliferone. *Transition Metal Chemistry*, *26*(3), 307-310.

14. Marchenko, M. M., Kopyl'chuk, G. P., Shmarakov, I. A., Ketsa, O. V., & Kushnir, V. N. (2006). Synthesis and antitumor activity of 5-(5′, 6′-benzocoumaro-3′-yl) methylaminouracil hydrobromide and its liposomal medicinal form. *Pharmaceutical Chemistry Journal*, *40*(6), 296-297.

15. Riddick, J. A., Bunger, W. B., & Sakano, T. K. (1986). Organic solvents: physical properties and methods of purification.

16. Vogel, A. I., Cresswell, W. T., Jeffery, G. J., & Leicester, J. (1950). Bond refractions and bond parachors. *CHEMISTRY & INDUSTRY*, (18), 358-358.

17. Aitken, A., & Taylor, H. F. W. (1960). Hydrothermal reactions in lime-quartz pastes. *Journal of Applied Chemistry*, *10*(1), 7-15.

18. National Committee for Clinical and Laboratory Standards. (1997). Method for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically Approved Standard, fourth ed. NCCLS, Villanova, Italy, Document M 100-S7. S100-S157.

19. Isenberg, D. H. (2001). Essential Procedure for Clinical Microbiology, American Society for Microbiology, Washington, 1998;(b) Zgoda JR and Porter JR. *Pharm Biol*, *39*, 221-225.

20. Zgoda, J. R., & Porter, J. R. (2001). A convenient microdilution method for screening natural products against bacteria and fungi. *Pharmaceutical Biology*, *39*(3), 221-225.

