International Journal of Chemical and Biochemical Sciences
(ISSN 2226-9614)

Journal Home page: www.iscientific.org/Journal.html



# Design, Synthesis and Evaluation of coumarin derivatives as antimicrobial activity

**B. Aparna[1], A. Harika[2], G. Surekha[3], S. Harshini[4], K. Divya Bharathi[5], G. Tulja Rani[6]**

[1,3,5] Assistant Professor, Department of Pharmaceutical Chemistry, Malla Reddy Pharmacy College, India.
2 University college of Engineering Science and Technology, Jntuh, India.
[4]Assistant Professor, Department of Pharmaceutical Analysis nd Quality Assurance, Malla Reddy Pharmacy College, India.
[6] Principal and Professor, Malla Reddy Pharmacy College, India.

**Abstract**

New substituted coumarins derivatives were synthesized by using nitration reaction to produce different nitro coumarin isomers which were separated from these isomers by using different solvent, and the reduction of nitro compounds was done to give corresponding amino coumarins. Temperature and reaction time of reaction were very important factors in determining the most productive nitro isotopes. A low temperature for three hours was sufficient to give a high product of a compound 6- nitro coumarin while increasing the temperature for a period of twenty-four hours that gave a high product of 8-nitro-coumarin. The synthesized compounds were confirmed by FT-IR, 1H-NMR, and 13C-NMR spectroscopy and all final compounds were tested for their antifungal and antibacterial activity. Some of them showed more biological activity than the standard drugs. Some synthesized compounds showed a variation in their anti-oxidant activities according to IC50 values. Some of these compounds had a very good antioxidant compared with ascorbic acid as a standard.

**Keywords:** Coumarin, Docking, Antimicrobial activity, Antioxidant activity.

**Full length article** **Corresponding Author*, e-mail: aparna.baliwada@gmail.com

## 1. Introduction

Heterocyclic compounds synthesis and their biological activities: Pharmacologically relevant heterocyclic compounds play a key role in the fight against diseases affecting both human and animal living organisms, as well as plants and they lead to new results about new molecules with a potential biological effect [1]. The shortage of novel antifungal drugs, the emergence of new infectious diseases, the resurgence of several infections, and the increased resistance of fungi to available chemotherapeutic agents are the essential issues in drug design and development, which prompted researchers to look for novel compounds that can combat with multidrug-resistant organisms [2]. Field of chemistry is forging ahead progressively, and hence newer molecules are synthesized in the laboratory to identify leads with target specific activity [3]. All synthesized compounds were tested for their biological activity as anti-fungal and antibacterial and also, they were tested for antioxidant. Bacterial and fungal diseases significantly influence on public health, and their resistance to antibiotics has prompted increased medical concerns, human contract bacterial infections from the air, food, water, or living vectors. Despite this, many bacterial organisms in human bodies do not cause disease [4]. Coumarins are classify under the benzopyrone family of heterocyclic compounds in which 6-membered α- pyrone ring fused with benzene ring and generally occurs in various natural products as a benzo derivative. Another efficient method for coumarin synthesis includes the interaction of resorcinol and ethyl acetoacetate by Pechmann condensation to provide pyrano coumarin derivatives with a good yield and atom-economy [5]. Several methods can be used to synthesize coumarins such as Pechmann condensation [6], Knoevenagel reaction [7], Claisen rearrangement [8], Baylis-Hill man reaction [9], and Wittig reaction [10].

Coumarins are heterocyclic compounds and have many medical properties [11] such as antifungal [12], antibacterial [13], antioxidants activity [14], and anti-inflammatory [15]. Coumarin and its derivatives possess anticancer activity against different types of cancers such as prostate, renal, breast, laryngeal, lung, colon, CNS, leukemia, and malignant melanoma [16]. Anti-HIV antiviral coumarin-based derivatives have the potential to inhibit different stages in the HIV replication cycle, inclusive of virus-host cell attachment, and cell membrane fusion [17].

Also, coumarins have been proven efficient pharmacophores in rest of coumarins studies as important biological activities, as depicted in Figure 1. We tried to synthesize new substituted coumarins as heterocyclic compounds having oxygen as a hetero atom, which has been reported as a common denominator of pharmacological and biochemical activities [18] so that we tested them as antibacterial, antifungal, and antioxidants.

## *2. Materials and methods*

1H-NMR spectra solvent DMSO-d6 was recorded on a 500 MHz spectrometer with TMS as an internal standard in Isfahan university, Iran and some in Jourdan. Melting points were determined on a Gallen-Kamp MFB-600 melting point apparatus and are uncorrected. Analytical thin layer chromatography (TLC) was performed on plates coated with silica gel (Merck 60 F254, 0.25 mm) and was visualized with ultraviolet light [19]. This process was conducted by using the preparative TLC method with preparative TLC plates and a solvent containing hexane: ethyl acetate (6:4). This experiment relied on visual observation with long-wave UV-light (365 nm) [19]. The biological activity test was performed at the University of Baghdad, College of Science.

### *Preparation of 6-nitro-4,7-dimethyl-chromen-2- one 2*

Preparation of compound 2 was carried out according to the same method in literature [20] by using 4,7-dimethyl coumarin, nitric acid, and sulfuric acid at 0-5 °C [21].

## *3. Result and discussion*

### *Synthesis of 8-nitro-4,7-dimethyl-chromen-2-one 3*

1 g from 4,7-dimethyl-chromen-2-on was dissolved in 15 mL of $H_2SO_4$ by stirring in an ice bath. The solution was added to a mixture of (0.4 ml) of $HNO_3$ and (1.2 mL) of $H_2SO_4$ dropwise at 0– 5 °C. The resulting solution was stirred for overnight at 0–5 °C**.** After the reaction was completed, the solution was added to the ice to produce the desired product. Then, the formed solid precipitate was filtered and washed from ethanol.

### *Synthesis of dinitro-4,7-dimethylcoumarin*

It was prepared with some modifications by using double amounts of nitrate mixture (nitric acid and sulfuric acid) at 0-5 °C. In initial (3 h), the reaction which was kept overnight, and then the temperature increased gradually to 15 °C. Two isomers, 3,6-dinitro-4,7-dimethylcoumarin 4, which were insoluble in dioxane, and 6,8- dinitro-4,7-dimethylcoumarin 5, which was soluble in dioxane, were produced.

### *Synthesis of 3,6,8 -tri nitro-4,7-dimethylcoumarin 6*

It was conducted by using thrice amount of nitrate mixture comprised of nitric acid and sulfuric acid under a gradual increase in temperature to 28 °C in which the reaction was kept overnight. Then, the solid product was poured in ice and after that, the precipitate was separated by filtration, washed with water, and dried. New nitro coumarin compounds were created by varying the concentration of nitric mixture and temperature of the reaction. Two nitro isomers of dinitro-4,7-dimethyl coumarin are 4,7-dimethyl-3,6-dinitro-2H-chromen-2-one 4 and 4,7- dimethyl-6,8-dinitro-2H-chromen-2-one 5 with trinitro-4,7-dimethyl coumarin that was 4,7- dimethyl-3,6,8-trinitro-2H-chromen-2-one 6 were confirmed by T.L.C, 1H-NMR, and 13C-NMR, as demonstrated in Table 1 and 2. FT-IR spectrum of compound 7 showed a decrease in absorption band at 1691 cm-1 due to carbonyl group for lactone ring that was due to the electrons releasing of the amino group instead of withdrawing nitro group which was disappereance of two absorption bands of the V NO2 and the appearance of absorption band at 3435 and 3360 cm-1 due to NH2 group.8 -amino-4,7-dimethyl-2H-chromen-2-one 8, 3,6- diamino-4,7-dimethyl-2H-chromen-2-one 9, and 6,8-diamino-4,7-dimethyl-2H-chromen-2-one 10 compounds: It was obtained by reducing compounds 3, 4, and 5, respectively. Likewise, compound 7 with double concentration of the reduction mixture was compared with the preparation of compounds 4 and 5. See Scheme 1.

### *Biological activity*

### *In in vitro antifungal studies*

synthesized compounds were tested for antifungal activity against candida albicans by using DMSO as a solvent by the agar well diffusion method (well diameter was 6 mm). Fluconazole was used as a standard drug for three days. Compounds 4 and 5 revealed a good antifungal activity and were better than the standard drug, as indicated in Table 3.

### *In vitro antibacterial studies*

The synthesized compounds 2-10 were tested for antibacterial activity against Escherichia coli and staphylococcus aureus by using cephalexin and amoxicillin as standard drugs by the agar well disc diffusion method, and the study showed that compound 6 was found to be more effective against E.coli than the standard drug.

### *Antioxidant activity*

### *DPPH radical scavenging activity [22]*

### *DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) [23]*

DPPH (1.434 mg) was dissolved in 100 mL of methanol, and by shielding the aluminum foil test tubes, various concentrations (100, 50, 25, 12.5, and 6.25 ppm) were prepared for seven compounds (3, 4, 5, 6, 7, 8, and 10) by using methanol to prepare the mentioned concentration above, ascorbic acid (vitamin C) was used as the reference compound. The absorbance of each solvent was measured with a spectrophotometer at 517 nm, after which incubation was done at 37 °C for 1 hour. Triple measurements were made by the following equation, which was able to determine the potential to scavenge DPPH (2, 2- diphenyl-1-picryl-hydrazyl-hydrate):

% inhibition = $(A0 – At) \times 100$

### *DPPH: Radical scavenging activity*

Some newly synthesized compounds showed antioxidant activity against DPPH (2, 2- diphenyl- 1-picryl-hydrazyl-hydrate) free radical and gave good scavenging percentage [24]. Hence, the compounds that were tested that showed antioxidant properties were selected for further testing (Figure 2).

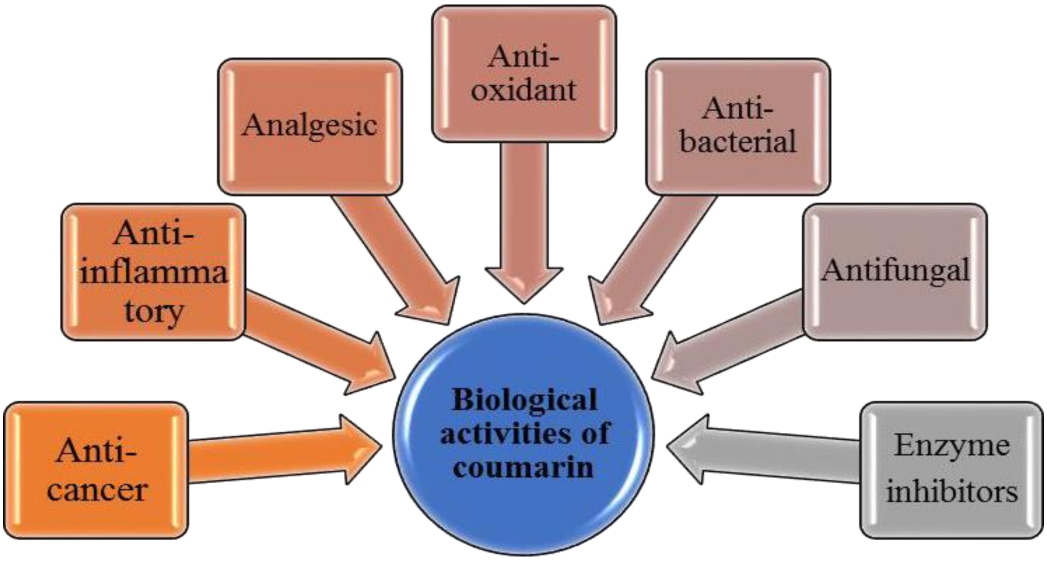



**Figure 1:** Biological activity of coumarin

**Scheme 1:** Synthesis of new amino coumarin from nitro coumari

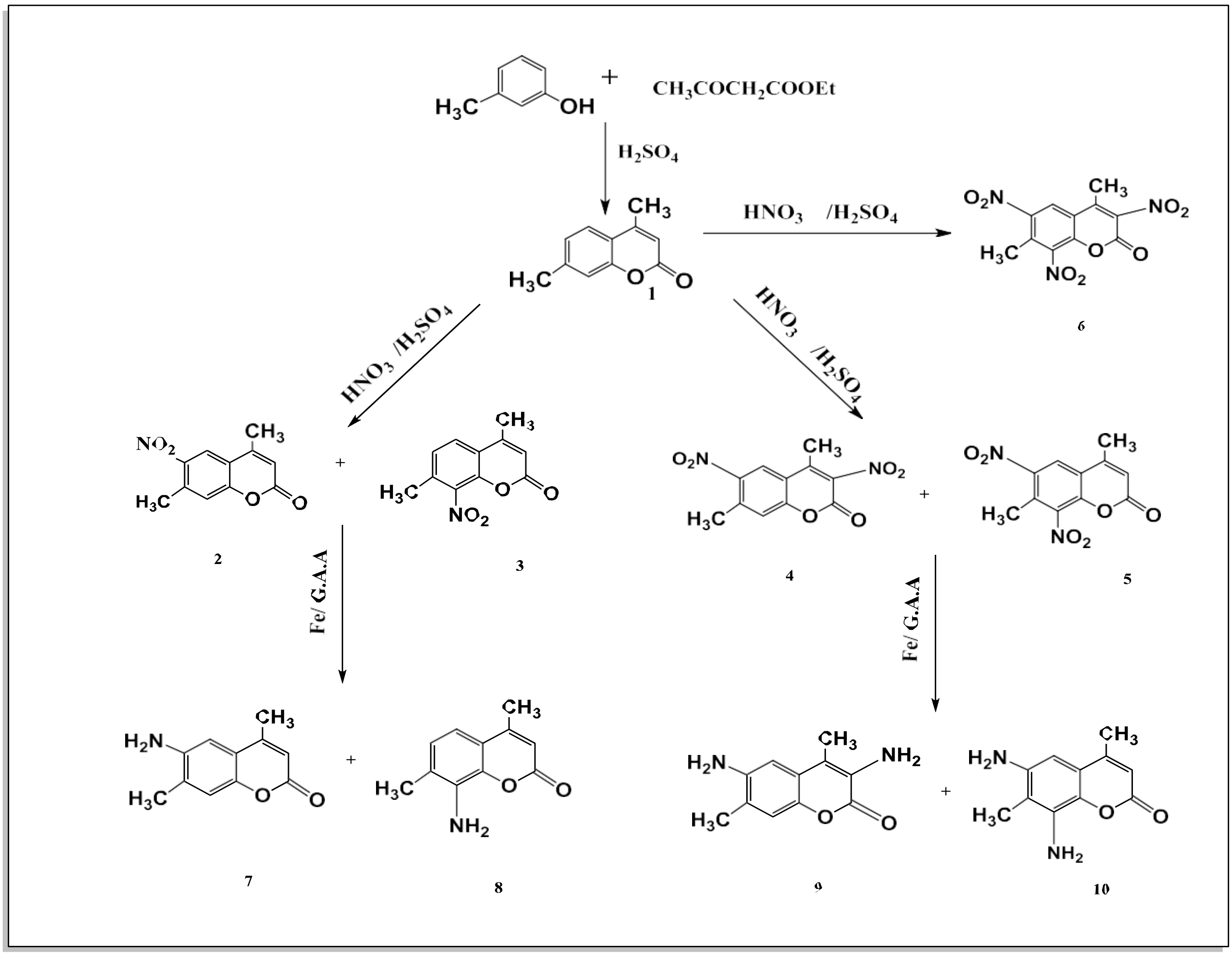


**Table 1:** The physical properties and FT-IR spectral data $cm^{-1}$ of synthesized compounds **2-10**

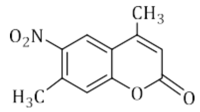
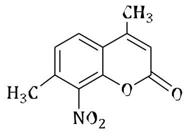
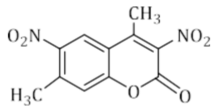
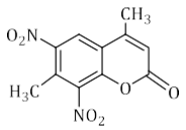
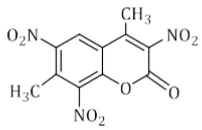
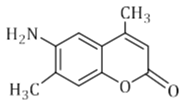
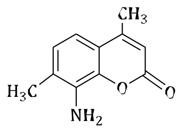
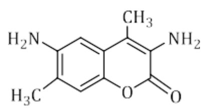
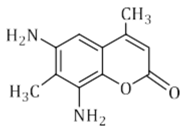
| Physical properties | | | | | Major FT-IR absorption $cm^{-1}$ | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| No. of compound | Structure of compounds | m.p. (°C) | RF | Yield% | υ (C-H) Aromatic | υ (C-H) Aliphatic as sy | Υ (C=O) lacton | υ C=C aromatic | υ (C-OC) | Other band as sy |
| 2 |  | 256-258 | 0.88 | 60 | 3095 | 2960 | 1734 | 1622 | 1251 | 1354 1527 $\upsilon NO_2$ |
| 3 |  | 189-190 | 0.73 | 40 | 3060 | 2945 as 2885sy | 1737 | 1625 1525 | 1255 | 1352 1525 $\upsilon NO_2$ |
| 4 |  | 156-158 | 0.74 | 35 | 3080 | 2993 | 1766 1739 | 1623 | 1253 | 1537 1350 $\upsilon NO_2$ |
| 5 |  | 164-166 | 0.76 | 65 | 3085 | 2360 | 1737 | 1623 | 1253 | 1527 1352 $\upsilon NO_2$ |
| 6 |  | 235 | 0.8 | 90 | 3001 | 2935 | 1764 | 1627 | 1226 | 1556 1342 $\upsilon NO_2$ |
| 7 |  | 210 | 0.14 | 70 | 3060 3035 | 2918 2948 | 1691 | 1556 1616 | 1220 1172 | 3434 3361 υ ($NH_2$) |
| 8 |  | 155-156 | 0.32 | 65 | 3056 | 2974 2918 | 1693 | 1558 1620 | 1176 | 3433 3359 υ ($NH_2$) |
| 9 |  | 188-189 | 0.23 | 55 | 3053 | 2974 | 1693 | 1556 | 1249 1222 | 3425 3361 υ ($NH_2$) |
| 10 |  | 204 dec | 0.21 | 65 | 3072 | 2968 2918 | 1681 | 1564 1620 | 1255 1234 | 3444 3425 υ ($NH_2$) |

**Table 2:** $^{1}$H-NMR and $^{13}$C-NMR spectral of some synthesized compounds

| No. of compund | $^{1}$H-NMR & $^{13}$C-NMR |
|---|---|
| 2 | $^{1}$H-NMR (500 MHz, DMSO): $\delta$ 2.4 (s, 3H, $CH_3$), 2.6 (s, 3H, $CH_3$), 6.5 (s, 1H, H-lactam ring), 7.5 (s, 1H, Ar-H), 8.3 (s, 1H, Ar-H). |
| 3 | $^{1}$H-NMR (500 MHz, DMSO): $\delta$ 2.49 (d, 3H, $CH_3$), 2.54 (d, 3H, $CH_3$), 6.53 (s, H, H-lactone ring), 7.73 (d, Ar-H5), 8.38 (d, Ar-H6). |
| 6 | $^{1}$H-NMR (500 MHz, DMSO): $\delta$ 8.8 (s, 1H, Ar-H). $^{13}$C-NMR (125 MHz, DMSO): $\delta$ 14.4, 14.7, 118, 126, 134, 138, 139, 145, 151. |
| 7 | $^{1}$H-NMR (500 MHz, DMSO): $\delta$ 2.34 (s, 3H, $CH_3$), 2.47 (s, 3H, $CH_3$), 6.23 (s, 1H, H-lactam ring), 8.19 (s, 2H, Ar-H), and 8.12 (s, 2H, Ar-H), 5.02 (s, 2H, $NH_2$). |
| 8 | $^{1}$H-NMR (500 MHz, DMSO): $\delta$ 2.12 (3, 3H, $CH_3$), 2.72 (s, 3H, $CH_3$), 5.1 (d, 2H, $NH_2$), 6.2 (s, 1H, H-lactam ring), 6.7 (d, *J*=8.4Hz, 1H, Ar-H), 6.9 (d, *J*=8.4Hz, 1H, Ar-H). $^{13}$C-NMR (125 MHz, DMSO): $\delta$ 17.7, 18.5, 106, 107, 140, 144, 145, 151, 153, 159, 160. |
| 9 | $^{1}$H-NMR (500 MHz, DMSO): $\delta$ 1.9 (s, 3H, $CH_3$), 2 (s, 3H, $CH_3$), 3.39 (d, 2H, $NH_2$), 5.0 (d, 2H, $NH_2$), 6.1 and 6.7 for (2 H, Ar-H). $^{13}$C-NMR (125 MHz, DMSO): $\delta$ 12.2, 17.3, 95, 129, 133, 143, 144, 155, 159. |

**Table 3:** Antifungal and antibacterial activities for synthesized compounds **1-10** by using agar well diffusion method.

The concentration of compounds is 10 mg/mL

| No.of sample | Escherichia coli | Staphylococcus aureus | Candida albicans |
|---|---|---|---|
| 1 | 16 | 17 | 16 |
| 2 | 17 | 19 | 16 |
| 3 | 18 | 19 | 16 |
| 4 | 17 | 22 | 16 |
| 5 | 16 | 20 | 18 |
| 6 | 30 | 23 | 25 |
| 7 | 16 | 21 | 17 |
| 8 | 16 | 21 | 15 |
| 9 | 20 | 20 | 14 |
| 10 | 22 | 20 | 19 |
| Amoxillin | 21 mm | 25 | - |
| Cephalexin | 21 mm | 20 | - |
| Fluconazole | - | - | 16 |

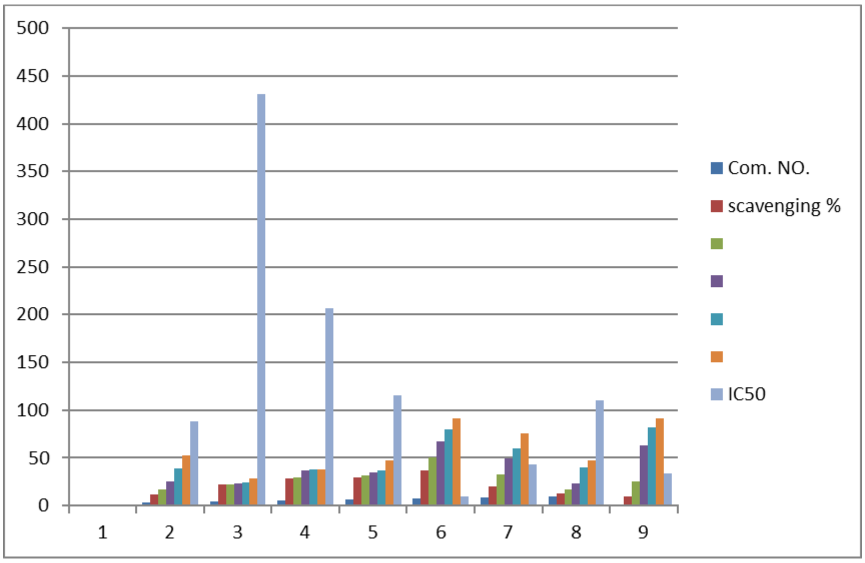



**Figure 2:** DPPH scavenging activity of all compounds.

**Table 4:** Anti-oxidant activities for synthesized compounds (**3**, **4**, **5**, **6**, **7**, **8**, and **10**)

| No of compound | Scavenging % | | | | | IC50 |
|---|---|---|---|---|---|---|
| | 6.25 µg\mL | 12.5 µg\mL | 25 µg\mL | 50 µg\mL | 100 µg\mL | |
| **3** | 12.1 | 16.9 | 24.8 | 39.1 | 52.1 | 88.5 |
| **4** | 21.76 | 22.45 | 23.29 | 24.2 | 28.24 | 431 |
| **5** | 28.66 | 29.29 | 37.03 | 37.38 | 38.21 | 206.1 |
| **6** | 28.94 | 31.1 | 35.08 | 37.03 | 47.28 | 115.2 |
| **7** | 36.8 | 50.3 | 67 | 80.1 | 91.2 | 10 |
| **8** | 20.1 | 33.1 | 49.3 | 60.3 | 75.9 | 42.9 |
| **10** | 12.2 | 16.9 | 22.9 | 39.9 | 46.9 | 110.2 |
| Ascorbic acid | 9.48 | 25.52 | 63.18 | 81.94 | 90.86 | 33.48 |

**Table 5:** Antioxidant activity according to Phongpaichit, 2017

| IC50 (µg/mL) | Mark |
|---|---|
| 10-50 | Strong Antioxidant Activity |
| 50-100 | Intermediate Antioxidant Activity |
| >100 | Weak Antioxidant Activity |

Accordingly, inhibitory concentrations (IC50) values were recorded and listed in Table 4. In this project, we applied the antioxidant activity classification, which was depended on IC50 range values published by Phongpaichit. For more details about these ranges, see Table 5. compounds showed a variation in their antioxidant activities according to IC50 values, which revealed a range of high, intermediate, and low activities, as can be seen in Table 2. The compound 7 has a very good antioxidant Compared with ascorbic acid as a standard, as represented in Table 2 and Table 3 (Activity according to Phongpaichit) [24].

## 4. Conclusions

In this work, different conditions were used to synthesize different nitro coumarin isomers. These compounds were reduced to corresponding amino coumarins by using Fe in dioxane in the presence of glacial acetic acid. All synthesized compounds were tested against different antibacterial, Escherichia coli, Staphylococcus aureus and tested against Candida albicans as anti-fungal. Compound 6 was found to be more effective against E. coli than the standard drug, Amoxicillin, which may be due to the presence of three nitro groups. The synthesized compounds were also tested for antioxidants by IC50 measurement by using (DPPH) and the result showed that compound 7 has a very good antioxidant compared with ascorbic acid as a standard.

## References


[1] B. Ramu, K. Kaushal, P. Chandrul, P. Shanmuga. Using 24 Factorial Designs optimization of Repaglinide Gastroretentive Drug Delivery System. Research J. Pharm. and Tech. 2021; 14(2):725-729.

[2] A.R. Tukur, J.D. Habila, R.G.O. Ayo, O.R.A. Lyun, Pharmacological Applications of Chalcones and Their Derivatives-A Mini Review, Journal of Chemical Reviews, 2022, 4

[3] S. Asirvatham, E. Thakor,H. Jain,T. Morpholine, Privileged Scaffold Possessing Diverse Bioactivity Profile, Journal of Chemical Reviews, 2021, 3

[4] A.J. Uttu, M.S. Sallau, O.R.A. Iyun, H. Ibrahim, Antimicrobial Efficacy of Selected Strychnos Species: A Mini Review, Journal of Chemical Reviews, 2022, 4:

[5] S. Sajjadifar, H. Hamidi, K. Pal, Revisiting of Boron Sulfonic Acid Applications in Organic Synthesis: Mini-Review, Journal of Chemical Reviews, 2019, 1:35

[6] A. Mirosanloo, D. Zareyee, M.A. Khalilzadeh, Recyclable cellulose nanocrystal supported Palladium nanoparticles as an efficient heterogeneous catalyst for the solvent-free synthesis of coumarin derivatives via von Pechmann condensation, Applied Organometallic Chemistry, 2018, 32:e4546

[7] M. M. Heravi, F. Janati, V. Zadsirjan. Applications of Knoevenagel condensation reaction in the total synthesis of natural products, Monatshefte für Chemie-Chemical Monthly, 2020, 151:439

[8] C. Schultze, B. Schmidt, Prenylcoumarins in One or Two Steps by a Microwave-Promoted Tandem Claisen Rearrangement/Wittig Olefination/Cyclization Sequence, The Journal of Organic Chemistry, 2018, 83:5210

[9] C. G. Lima, J.L. Monteiro, T. de Melo Lima, M. Weber Paixão, A.G. Corrêa, Angelica Lactones: From Biomass-Derived Platform Chemicals to Value-Added Products, ChemSusChem, 2018, 11:25

[10] M.M.Heravi, M. Ghanbarian, V. Zadsirjan, B. Alimadadi Jani, Recent advances in the applications of Wittig reaction in the total synthesis of natural products containing lactone, pyrone, and lactam as a scaffold, Monatshefte für Chemie-Chemical Monthly, 2019, 150:1365

[11] P. Govindaiah, N. Dumala, I. Mattan, P. Grover, M.J. Prakash, Design, synthesis, biological and in silico evaluation of coumarin-hydrazone derivatives as tubulin targeted antiproliferative agents, Bioorganic Chemistry, 2019, 91:103143

[12] Y.H. He, X.F. Shang, H.X. Li, A.P. Li, C. Tang, B. Q. hang, Z. J. Zhang, R. Wang, Y. Ma, S.S. Du, Y.M. Hu, T.L. Wu, W.B. Zhao, C.J. Yang, Y.Q. Liu, Antifungal activity and action mechanism study of coumarins from Cnidium monnieri fruit and structurally related compounds. Chemistry & Biodiversity, , 2021, 18:e2100633

[13] X.C. Yang, P.L. Zhang, K.V. Kumar, S. Li, R.X. Geng, C.H. Zhou, Discovery of unique thiazolidinone-conjugated coumarins as novel broad spectrum antibacterial agents, European Journal of Medicinal Chemistry, 2022, 232:114192

[14] V.P Koyiparambath., K.R. Prayaga, T.M. Rangarajan, A.G. Al-Sehemi, M. Pannipara, V. Bhaskar, A.S. Nair, S.T. Sudevan, S. Kumar, B. Mathew, Deciphering the detailed structure–activity relationship of coumarins as Monoamine oxidase enzyme inhibitors—An updated review, Chemical Biology & Drug Design, 2021, 98:655

[15] L. Tao, Y. T. Zhuo, Z. H. Qiao, J. Li, H. X. Tang, Q. M. Yu, Y. Y. Liu, Y. P. Liu, Prenylated coumarins from the fruits of Artocarpus heterophyllus with their potential anti-inflammatory and anti-HIV activities, Natural Product Research, 2022, 36:2526

[16] N. Bhattarai, A.A. Kumbhar, Y.R. Pokharel, P.N. Yadav, Anticancer potential of coumarin and its derivatives. Mini Reviews in MedicinalChemistry, 2021, 21:2996

[17] Z. Xu, Q. Chen, Y. Zhang, C. Liang, Coumarin-based derivatives with potential anti-HIV activity, Fitoterapia, 2021, 150:104863

[18] R. Hamid, I. Obaid, Synthesis and Characterization of Novel Subs.-1, 3, 4- Thiadiazolependant on Modified Poly (5-Vinyl Tetrazole-Co-MA), Iraqi Journal of Science, 2020, 472

[19] S.A. Abrahem, Analysing Aflatoxin Production Conditions in Feed Samples Using a Preparative Thin Layer Chromatography (TLC) Method, Iraqi Journal of Science, 2022, 9

[20] P. Chen, C. Shen, M. Qiu, J. Wu, Y. Bai, Y. Su, Synthesis of 5-fluoro-2-nitrobenzotrifluoride in a continuous-flow millireactor with a safe and

efficient protocol, Journal of Flow Chemistry, 2020, 10:207
[21] L.S. Ahamed, R.A. Ali, R.S. Ahmed, M.R. Ahamad, R.I. Al-Bayati, Synthesis of New 7-ethyl-4- methyl-2-Quinolone Derivatives, Al-Nahrain Journal of Science, 2019, 2:30
[22] A. Matuszewska, M. Jaszek, D. Stefaniuk, T. Ciszewski, Ł. Matuszewski, Anticancer, antioxidant, and antibacterial activities of low molecular weight bioactive subfractions isolated from cultures of wood degrading fungus Cerrena unicolor, PloS one, 2018, 13:e0197044
[23] A.H. Al-Mashhadani, Study of in vitro and in vivo free radical scavenging activity for radioprotection of cerium oxide nanoparticles, Iraqi Journal of Physics, 2017, 15:40