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# SYNTHESIS AND ANTIMICROBIALLY ACTIVITIES OF COUMARIN-3-CARBOXAMIDE DERIVATIVES**

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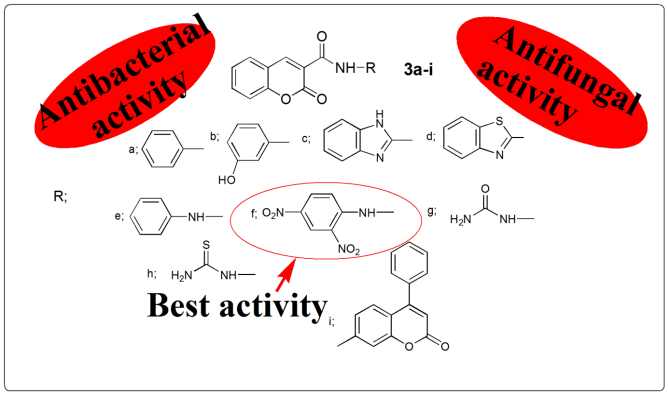
In this study nine coumarin-3-carboxamide derivatives 3a-3i were synthesized with coumarin-3-carboxylic acid chloride and different amines. Two of them, 3c and 3i, are original. The novel substances were purified by column chromatography, and their structures were identified by spectroscopic methods ($^1$H NMR, $^{13}$C NMR, IR) and elemental analysis. Coumarin-3-carboxamide derivatives were evaluated antimicrobially against ten different ATTC isolates. The compound 3f was found to be the most effective compound in terms of antibacterial and antifungal activities.




## INTRODUCTION

Coumarin compounds have a wide range of properties, especially pharmacological.[1, 2] Studies in the literature show that the activities vary according to the type and binding site of the attached substituent. The activities of coumarin 3-carboxamide compounds have been found in the literature as antitumor,[3-11] anti-inflammatory,[12, 13] anti-Alzheimer,[14-17] anti-coagulant, anti-Helicobacter pyroli agent,[19-21] and antimicrobial.[9, 22-25]

Carbendazim, which is a carboxylic acid amide, is one of the first fungicides to be mentioned in the literature as an excellent fungicide since the 1960s.[25] In later studies, the presence of the hydrazine group together with the amide group showed that the antifungal activity was increased.[25] Novobiocin and chlorobiocin are known antibiotics that are derivatives of carboxamide coumarin.[23]

In this study nine coumarin-3-carboxamide compounds synthesized. Two of these compounds are original 3c, 3i. The structures of these compounds were elucidated by spectroscopic measurements such as 1H NMR, 13C NMR, IR and elemental analysis. Antibacterial and antifungal activities of synthesized coumarin-3-carboxamides were investigated. No data on these activities related to these compounds were found in the literature.

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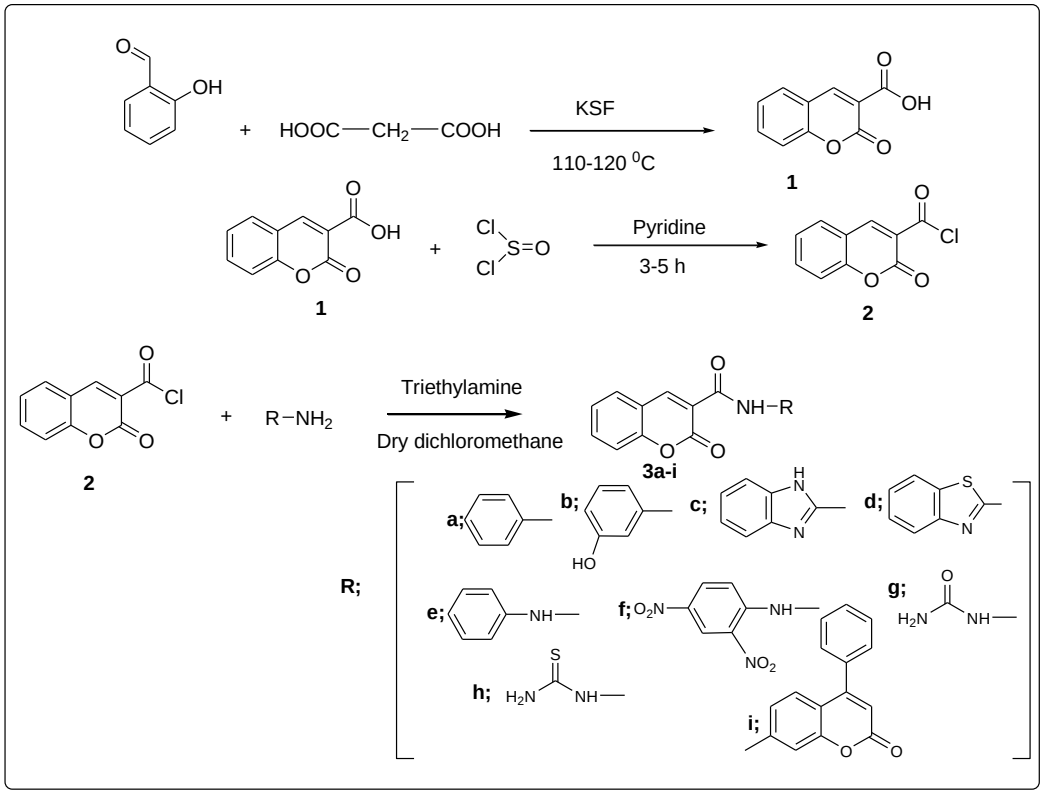


Scheme 1 – Synthesis of coumarin-3-carboxamide derivatives.

## RESULTS AND DISCUSSION

### Synthesis

Coumarin-3-carboxamides **3a-3i** were obtained in 60–90% yields by the method shown in Scheme 1.[26-28] **3c**, **3i** are original. IR, $^{1}$H NMR, $^{13}$C NMR and GC-MS spectras of new synthesized compounds are given in Supplementary material.

Characterization data of new coumarin-3-carboxamide derivatives are as follows:

***N-(1H-Benzimidazol-2-yl)-2-oxo-2H-chromene-3-carboxamide*** **(3c)**

Yellow solid. Purification: Column chromatography [Chloroform-Methanol (5:1)]. Yield: 73% IR (ATR, cm$^{-1}$): 3211 (NH), 1753 (C=O, lactone), 1668 (C=O, amid). $^{1}$H-NMR (DMSO-d$_6$, 500 MHz, δ / ppm): 7.08 (s, 2H, -NH, Arom. –CH), 7.27 (s, 2H, Arom.-CH), 7.40 (m, 2H, Arom.-CH), 7.69 (t, 1H, J=7.8 Hz, Arom.-CH), 7.85 (m, 3H, Arom.-CH, -CH), 8.60 (s, 1H,-NH). $^{13}$C-NMR (DMSO-d$_6$, 125 MHz, δ / ppm): 115.94, 118.13, 124.77, 125.30, 127.97, 129.13, 129.46, 130.29, 130.47, 134.44, 135.11, 141.21, 144.08, 147.40, 154.41 (14C, Arom.C and 1C Olefinic C), 157.87 (1C, C=O,lactone), 158.49 (1C, C=O, amide). MS (m/z): 51, 63, 75, 89, 101, 145, 173, 249, 265, 290, 305. Calculated C: 66.88, H: 3.63, N: 13.76, O: 15.72; Found C: 66.89, H:3.63, N: 13.75, O: 15.74.

***2-oxo-N-(2-oxo-4-phenyl-2H-chromen-7-yl)-2H-chromene-3-carboxamide*** **(3i)**

Brown solid. Purification: Column chromatography [Chloroform-Methanol (5:1)]. Yield: 72% IR (ATR, cm$^{-1}$): 3244 (NH), 1696 (C=O, lactone), 1599 (C=O, lactone), 1561 (C=O, amid). $^{1}$H-NMR (DMSO-d$_6$, 500 MHz, δ / ppm): 6.54 (dd, 1H, J=7.7 ve 2.8 Hz, Arom.-CH), 6.83 (t, 4H, J= 8.1 Hz, Arom.-CH), 7.03 (d, 1H, J=7.3 Hz, Arom.-CH), 7.16 (t, 1H, J=8.0 Hz, Arom.-CH), 7.32 (t, 1H, J=2.1 Hz, Arom.-CH), 7.42 (m, 1H, Arom. -CH), 7.48 (t, 1H, J=7.0 Hz, Arom.-CH), 7.55 (d, 1H, J=8.3 Hz, Arom.-CH), 8.01 (d, 1H, J=7.8 Hz, Arom.-CH), 8.59 (m, 1H,-CH), 8.90 (s, 1H,-CH), 10.57 (s, 1H, -NH). $^{13}$C-NMR (DMSO-d$_6$, 125 MHz, δ / ppm): 102.7, 105.66, 106.95, 107.07, 110.46, 111.43, 116.14, 118.31, 119.98, 123.93, 125.26, 129.6, 130.15, 134.21, 136.69, 138.79, 146.09, 147.21, 149.01, 153.8, 157.73, 158.09 (20C, Arom.-C and 2C Olefinic C), 159.68 (1C, -C=O, amide), 160.42 (1C, C=O; lactone), 161.61 (1C, C=O; lactone). Calculated C: 73.35, H: 3.69,

N: 3.42, O: 19.54; Found C: 73.36, H:3.67, N: 3.43, O: 19.52.

### In vitro antimicrobial activity

The in vitro antimicrobial activity of 9 coumarin-3-carboxamid derivatives against three Gram-positive bacteria, four Gram-negative bacteria, and three fungi by the broth microdilutions technique using the CLSI recommendations.[29,30] The well-known commercial antibiotics were used as the standard drugs and the minimal inhibitory concentrations (MIC) values compared with the standard drugs presented in Table 1 and 2.

Depending on the antibacterial results for all compounds, the test-culture *P. mirabilis ATCC 14153* appeared to be resistant to the all synthesized compounds. While **3b**, **3c** and **3d** did not show any antibacterial effect on gram-negative bacteria, they also showed moderate activity against gram-positive bacteria. **3f**, **3g** and **3h** showed moderate antibacterial activity against both gram (+) and gram (-) bacteria. **3a**, **3e** and **3i** showed no activity against any of the bacteria. **3b** and **3c** have the best antibacterial activity against to *S. epidermidis* ATCC 12228, **3f** against to *S. aureus* ATCC 29213 with an MIC value of 312.5 μg/mL.

The compound **3i** compound showed the best antifungal activity among the compounds studied with an MIC value of 312.5 μg/mL and 156.2 μg/mL. **3e** and **3f** showed activity against *C. Tropicalis* ATCC 750, while the other compounds showed no activity against any fungi.

*Table 1*

Minimal inhibitory concentrations (MICs) values of the tested coumarin derivatives for antibacterial activity

| Compound | *P. aeruginosa* ATCC 27853 | *E. coli* ATCC 25922 | *K. pneumoniae* ATCC 4352 | *P. mirabilis* ATCC 14153 | *E. faecalis* ATCC 29212 | *S. epidermidis* ATCC 12228 | *S. aureus* ATCC 29213 |
|---|---|---|---|---|---|---|---|
| 3a | - | - | - | - | - | - | - |
| 3b | - | - | - | - | 1250 | 312.5 | 625 |
| 3c | - | - | - | - | - | 312.5 | 625 |
| 3d | - | - | - | - | - | 1250 | - |
| 3e | - | - | - | - | - | - | - |
| 3f | - | 625 | - | - | 1250 | 625 | 312.5 |
| 3g | 625 | - | 625 | - | 625 | 1250 | - |
| 3h | - | - | - | - | 625 | - | - |
| 3i | - | - | - | - | - | - | - |
| Reference | 2.4[a] | 4.9[b] | 4.9[b] | 2.4[b] | 128[c] | 9.8[d] | 1.2[b] |

[a] Ceftazidime [b]Cefuroxime-Na [c]Amikacin [d] Cefuroxime

*Table 2*

Minimal inhibitory concentrations (MICs) values of the tested coumarin derivatives for antifungal activity

| Compound | *C. albicans* ATCC 10231 | *C. parapsilosis* ATCC 22019 | *C. tropicalis* ATCC 750 |
|---|---|---|---|
| 3a | - | - | - |
| 3b | - | - | - |
| 3c | - | - | - |
| 3d | - | - | - |
| 3e | - | - | 625 |
| 3f | - | - | 625 |
| 3g | - | - | - |
| 3h | - | - | - |
| 3i | 312.5 | 156.2 | - |
| Reference | 4.9[a] | 0.5[b] | 1[b] |

[a] Clotrimazole [b] Amphotericin B

## EXPERIMENTAL

### Materials and instrumentation

Chemicals used in this work were of high purity, they were analytical grade, and all of them were purchased from Sigma Aldrich and Fluka Company. The melting points of the synthesized compounds were determined with the Buchi Melting Point B-540 melting point instrument. FT-IR analysis: was done using the ATR technique with the Bruker Vertex 70 FT-IR Spectrophotometer in our department. $^{1}$H-NMR analysis with Bruker 500 MHz Gemini and $^{13}$C-NMR analysis with Varian 125 MHz Gemini were performed in Istanbul University-Cerrahpaşa Central laboratory and Yıldız Technical University Faculty of Arts and Sciences Molecular Biology and Genetics Department NMR laboratory, chemical shifts (δ) were expressed in parts per million (ppm) relative to tetramethylsilane (TMS) as a default (δ = 0.0 ppm). Mass (MS) spectra of the synthesized compounds were also taken from the device called GC-MS, Shimadzu QP2010Plus, located in the Department of Organic Chemistry, Department of Chemistry in Istanbul University-Cerrahpaşa. Elemental analyzes were carried out in the Thermo Finnigan Flash EA 1112 Series device at İstanbul University-Cerrahpaşa Central Laboratory.

### Synthesis of coumarin-3-carboxamides

Our starting compound coumarin-3-carboxylic acid 1 was obtained from the reaction of 2-hydroxybenzaldehyde with malonic acid.[26] Coumarin-3-carboxylic acid was converted to acid chloride 2 with thionylchloride.[27] Our final compounds, coumarin-3-carboxamides 3a-3i, were obtained in 60–90% yields by the reaction of coumarin carboxylic acid chloride with various amines or hydrazines.[28]

### Determination of Minimum Inhibitory Concentrations (MICs)

In vitro antimicrobial activities of 9 different coumarin-3-carboxamide molecules against ten different ATCC isolates (four Gram negative, three Gram positive and three fungi) were investigated. MICs of the compounds were identified by the broth microdilution technique as approved by the Clinical and Laboratory Standards Institute.[29,30] Molecules were dissolving in Dimethyl sulfoxide (DMSO, Sigma), and serial twofold dilutions (2500 to 1.22 μg/mL) were prepared in Mueller Hinton broth (MHB) for bacteria and RPMI-1640 medium for yeast. Bacterial inoculums were prepared with an approximately 5h MHB culture and a different microplate was used for each microorganism. Then, each individual bacterial inoculum was spectrophotometrically arranged with turbidity equivalent to a 0.5 McFarland and additionally diluted in MHB to obtain a final concentration of 5 × 105 CFU/mL in the test tray. Candida albicans ATCC 10231, C. parapsilosis ATCC 22019, and C. tropicalis ATCC 750 were prepared in RPMI-1640 medium to obtain a final concentration of 5 x 103 CFU/mL. Each test tray was stored in a plastic container to evade evaporation. Incubation: done for 18-24 hours at 37°C for trays containing MHB, and for 48 hours at 37°C for those existing RPMI-1640 medium. MIC was identified as the lowest concentrations of compounds that produced visible inhibition of apparent growth. The standard antimicrobials were also studied against the tested microorganisms.

## CONCLUSION

While the coumarin-3-carboxamide derivatives used in the study showed moderate antibacterial activity, they showed less antifungal activity. Compounds **3b**, **3c** and **3f** showed better antibacterial activity, while **3i**, **3e** and **3f** showed better antifungal activity. Pharmacologically active groups such as benzimidazole and benzthiazole used in addition to the amide group in the structure did not show the expected increase in microbial activity.

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## REFERENCES

1. N. Sakinah and J. Jumal, *Malays. J. Med. Health Sci.*, **2021**, *7*, 762–68. https://doi.org/10.33102/ mjosht.v7i1.145.
2. A. Carneiro, M. J. Matos, E. Uriarte and L. Santana, *Molecules*, **2021**, *26*, 501–514. https://doi.org/10.3390/ molecules26020501
3. P. S. Thacker, M. Alvala, M. Arifuddin, A. Angeli and C. T. Supuran, *Bioorg. Chem*., **2019**, *86*, 386–392. https://doi.org/10.1016/j.bioorg.2019.02.004.
4. T. Nasr, S. Bondock, H. M. Rashed, W. Fayad, M. Youns and T. M. Sakr, *Eur. J. Med. Chem*., **2018**, *151*, 723–739. https://doi.org/10.1016/j.ejmech.2018.04.014
5. H. Ji, Y. Tan, N. Gan, J. Zhang, V. Li, X. Zheng, Z. Wang and W. Yi, *Bioorg. Med. Chem*., **2021**, *29*, 115870–115881. https://doi.org/10.1016/j.bmc.2020.115870.
6. F. Comert Onder, S. Durdagi, K. Sahin, B. Ozpolat and M. Ay, *J. Chem. Inf. Model*., **2020**, *60*, 1766–1778. https://doi.org/10.1021/acs.jcim.9b01083.
7. N. S. Reddy, K. Gumireddy, M. R. Mallireddigari, S. C. Cosenza, P. Venkatapuram, S. C. Bell, E. P. Reddy and M. V. R. Reddy, *Bioorg. Med. Chem*., **2005**, *13*, 3141–3147. https://doi.org/10.1016/j.bmc.2005.02.051.
8. J. Shi, W. Lu, J. Chen, L. Sun, S. Yang, M. Zhou, L. Xu, Y. Ma and L. Yu, *Arch. Pharm*., **2021**, *354*, 2000236–2000245. https://doi.org/10.1002/ardp.202000236.
9. W. Phutdhawong, A. Chuenchid, T. Taechowisan, J. Sirirak and W. S. Phutdhawong, *Molecules*, **2021**, *26*, 1653–1664. https://doi.org/10.3390/molecules26061653.
10. E. M. Radwan, E. H. Elsayed, M. Abd El-Moneim and A. M. Youssef Moustafa, *Russ. J. Bioorg. Chem.*, **2021**, *47*, 149–157. https://doi.org/10.1134/S1068162021010180.
11. Y. Li, Q. Tang, Y. Xie, D. He, K. Yang and L. Zheng, *Bioorg. Med. Chem. Lett.*, 2021, 33, 127750–127757. https://doi.org/ 10.1016/j.bmcl.2020.127682.
12. G. Melagraki, A. Afantitis, O. Igglessi-Markopoulou, A. Detsi, M. Koufaki, C. Kontogiorgis and D. J. Hadjipavlou-Litina, *Eur. J. Med. Chem*., **2009**, *44*, 3020–3026. https://doi.org/ 10.1016/j.ejmech.2008.12.027
13. I. E. Bylov, M. V. Vasylyev and Y. V. Bilokin, *Eur. J. Med. Chem*., **1999**, *34*, 997–1001. https://doi.org/10.1016/ S0223–5234(99)00119–1.

14. A. Asadipour, M. Alipour, M. Jafari, M. Khoobi, S. Emami, H. Nadri, A. Sakhteman, A. Moradi, V. Sheibani, F. H. Moghadam, A. Shafiee and A. Foroumadi, *Eur. J. Med. Chem*., **2013**, *70*, 623–630. https://doi.org/10.1016/j.ejmech.2013.10.024.
15. X. He, Y. Y. Chen, J. B. Shi, W. J. Tang, Z. X. Pan, Z. Q. Dong, B. A. Song, J. Li and X. H. Liu, *Bioorg. Med. Chem*., **2014**, *22*, 3732–3738. https://doi.org/10.1016/j.bmc.2014.05.002.
16. M. B. Tehrani, Z. Rezaei, M. Asadi, H. Behnammanesh, H. Nadri, F. Afsharirad, A. Moradi, B. Larijani, M. Mohammadi-Khanaposhtani and M. Mahdavi, *Chem. Biodivers.*, **2019**, *16*, e1900144–e1900151. https://doi.org/10.1002/ cbdv.201900144.
17. F. Chimenti, D. Secci, A. Bolasco, P. Chimenti, B. Bizzarri, A. Granese, S. Carradori, M. Yanez, F. Orallo, F. Ortuso and S. Alcaro, *J. Med. Chem*., **2009**, *52*, 1935–1942. https://doi.org/10.1021/jm801496u.
18. S. Robert, C. Bertolla, B. Masereel, J. M. Dogne and L. Pochet, *J. Med. Chem.*, **2008**, *51*, 3077–3080. https://doi.org/10.1021/jm8002697.
19. F. Chimenti, B. Bizzarri, S. Carradori, A. Granese, D. Rivanera, D. Lilli, A. Zicari, M. M. Scaltrito and F. Sisto, *Bioorg. Med. Chem. Lett*., **2007**, *17*, 3065–3071. https://doi.org/10.1016/j.bmcl.2007.03.050.
20. F. Chimenti, B. Bizzarri, A. Bolasco, D. Secci, P. Chimenti, S. Carradori, A. Granese, D. Rivanera, D. Lilli, M. M. Scaltrito and M. I. Brenciaglia, *Eur. J. Med. Chem*., **2006**, *41*, 208–212. https://doi.org/10.1016/j.ejmech.2005.11.001.
21. F. Chimenti, B. Bizzarri, A. Bolasco, D. Secci, P. Chimenti, A. Granese, S. Carradori, D. Rivanera, A. Zicari, M. M. Scaltrito and F. Sisto, *Bioorg. Med Chem. Lett.*, **2010**, *20*, 4922–4926. https://doi.org/10.1016/j.bmcl.2010.06.048.
22. R. K. Sharma, V. Singh, N. Tiwari, R. J. Butcher and D. Katiyar, *Bioorg. Chem*., **2020**, *98*, 103700–103710. https://doi.org/10.1016/j.bioorg.2020.103700.
23. C. R. Sahoo, J. Sahoo, M. Mahapatra, D. Lenka, P. K. Sahu, B. Dehury, R. N. Padhy and S. K. Paidesetty, *Arab. J. Chem*., **2021**, *14*, 102922–102979. https://doi.org/10.1016/j.arabjc.2020.102922.
24. U. Salar, K. M. Khan, M. I. Fakhri, S. Hussain, S. Tauseef, S. Ameer, A. Wadood, H. Khan and S. Perveen, *Med. Chem*., **2018**, *14*, 86–101. https://doi.org/10.2174/ 1573406413666170623083116.
25. X. Yu, P. Teng, Y. L. Zhang, Z. J. Xu, M. Z. Zhang and W. H. Zhang, *Fitoterapia*, **2018**, *127*, 387–395. https://doi.org/10.1016/j.fitote.2018.03.013.
26. F. Bigi, L. Chesini, R. Maggi and G. Sartori, *J. Org. Chem.*, **1999**, *64*, 1033–1035.
27. L. L. Adreani and E. Lapi, *Boll. Chim. Farm*., **1960**, *99*, 583–586.
28. K. Wang, Z. Liu, R. Guan, D. Cao, H. Chen, Y. Shan and Q X. Y. Wu, *Spectrochim. Acta A Mol. Biomol. Spectrosc*., **2015**, *144*, 235–242. https://doi.org/10.1016/j.saa.2015.02.072.
29. Clinical and Laboratory Standards Institute (CLSI), Performance Standards for Antimicrobial Susceptibility Testing in Clinical and Laboratory Standards Institute, 950 West Valley Road, Suite 2500, Wayne, Pennsylvania 19087, USA, **2020**.
30. Clinical and Laboratory Standards Institute (CLSI), Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts; Approved Standard–Second Edition, in, Wayne, PA, USA, **1997**.