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PREPARATION AND *IN VITRO* ANTICANCER ACTIVITY EVALUATION OF SOME COUMARIN DERIVATIVES

Mohd. Imran[1]*, Abdulhakim Bawadekji[2], Naira Nayeem[1]

1. ***Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Northern Border University, Rafha, Saudi Arabia.***
2. ***Department of Biological Sciences, College of Science, Northern Border University, Arar, Saudi Arabia.***

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ABSTRACT

Cancer has become the second main reason for worldwide disease-related deaths. There is a need to take remedial action to combat this condition and to develop new drugs and therapies for its treatment. Accordingly, it was decided to prepare and perform the anticancer activity of novel coumarin derivatives. The compounds 3a-3j were prepared from the reaction between 1a-1b and 2a-2e. The structures of 3a-3j were elucidated with the help of physical and spectral analysis. The sulforhodamine B (SRB) colorimetric method was adopted to assess the anticancer potential of 3a-3j with respect to HCT-116 and MCF-7 cell lines. The 3a derivative was the most promising compound that had IC_{50} of 1.93 µM and 1.25 µM concerning HCT-116 and MCF-7, respectively. However, its activity was almost 40% less than the doxorubicin. Accordingly, it was concluded that more effective anticancer agents can be developed by incorporating phenolic –OH group in the coumarin moiety and substituting a fluorine atom at an appropriate place of 3a-3j.

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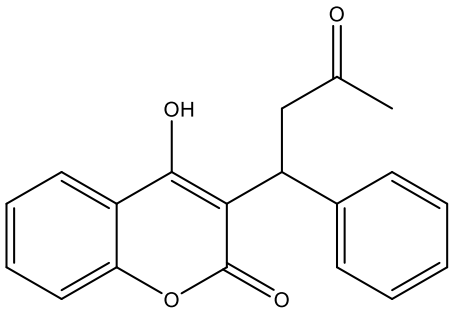
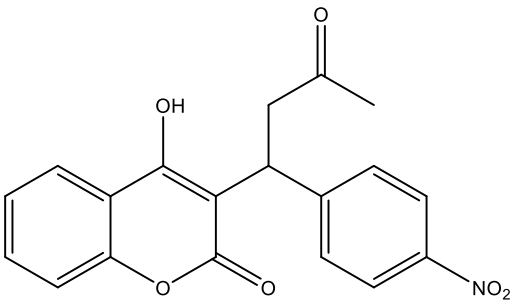
Introduction

Cancer has become the second main reason for worldwide disease-related deaths [1]. According to the key fact sheet of WHO (Accessed on May 1, 2019, https://www.who.int/en/news-room/fact-sheets/detail/cancer), cancer is responsible for about 9.6 million global demises and the cause of every sixth death in the year 2018. Although great progress has been made against cancer, it remains a large unmet need. A recent report in 2018 [2], about the Saudi Arabia cancer incidences, stated that about 4% of the Saudi population is suffering from cancer diseases. In view of the current situation regarding the cancer disease, there is a need to take remedial action to combat this disease and develop new drugs and therapies for cancer treatment. Coumarin derivatives are abundantly distributed in plants and many synthetic coumarins have also been prepared as medicinal agents, for example, warfarin [3], acenocoumarol [4], armillarisin A [5], hymecromone [6], carbochromen [7], phenprocoumon [8], and novobiocin [9].

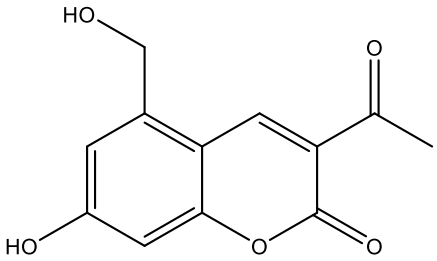


Warfarin

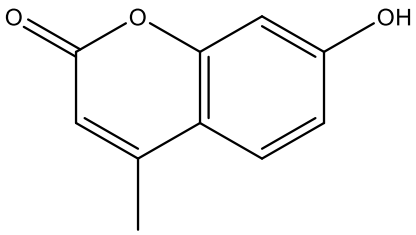


Acenocoumarol

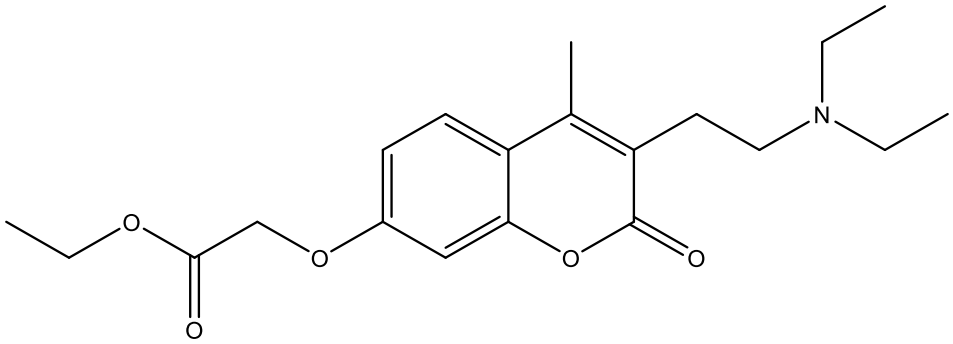
Corresponding Author: Mohd. Imran; Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Northern Border University, P.O. Box 840, Rafha 91911, Saudi Arabia. Email: imran_inderlok @ yahoo.co.in



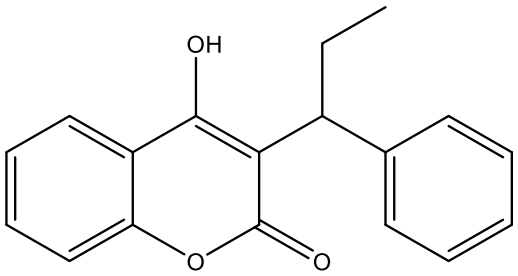
Armillarisin A



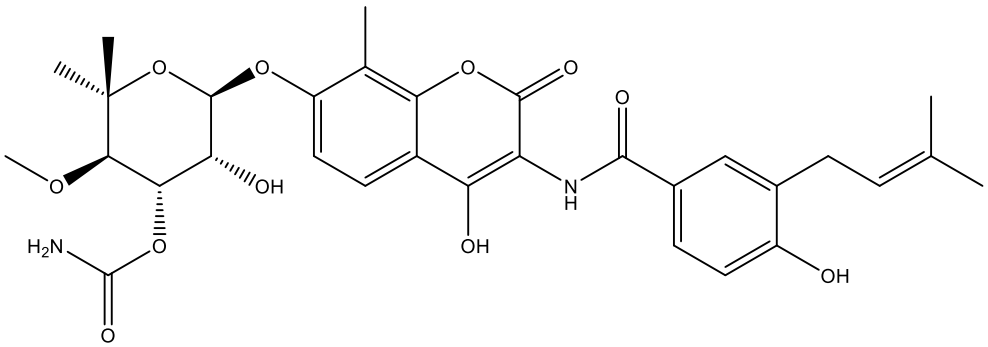
Hymecromone



Carbochromen

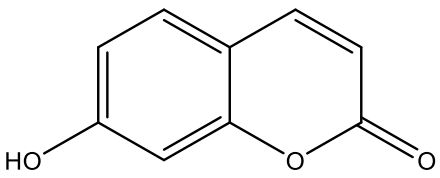


Phenprocoumon

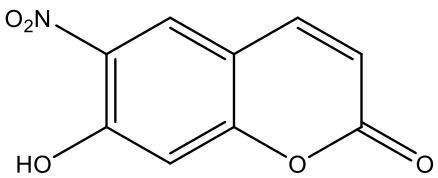


Novobiocin

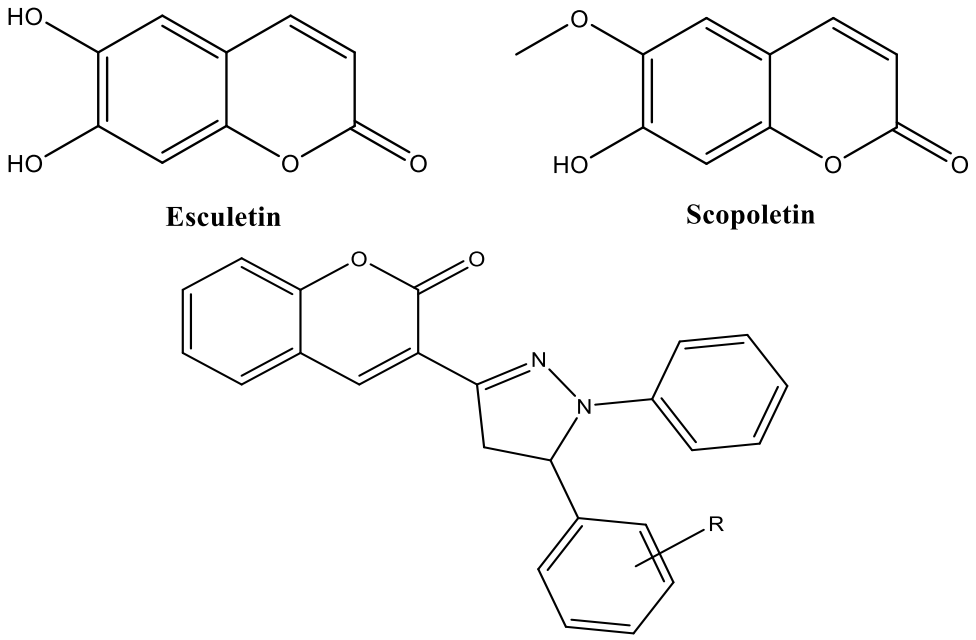
Recently, many reports have revealed coumarins as anticancer agents, which act by various mechanisms [10-15]. These reports have mentioned many coumarin derivatives as promising anticancer agents, including 7-Hydroxy-2*H*-chrome-2-one, 7-Hydroxy-6-nitrocoumarin, Esculetin, Scopoletin, Compound A, and Compound B.



7-hydroxy-2*H*-chromen-2-one



7-hydroxy-6-nitrocoumarin



In view of the above facts, it was decided to prepare and evaluate the anticancer activity of novel coumarin derivatives that could provide us a lead to develop new anticancer agents to treat the world's second largest mortality causing disease.

Materials and Methods

General

The Gallen Kamp apparatus was used to determine the melting point. The IR spectra (KBr; wave number in cm^{-1}), NMR analysis (DMSO-d_6; δ in ppm), mass analysis (M^+; m/z), and elemental investigation (C, H, N Anal.) were performed by Shimadzu spectrophotometer, Bruker DRX-300 spectrophotometer, Jeol-JMS-D-300 spectrometer, and VARIO El Elementary apparatus, respectively. The reaction monitoring and purity assessment were performed by TLC.

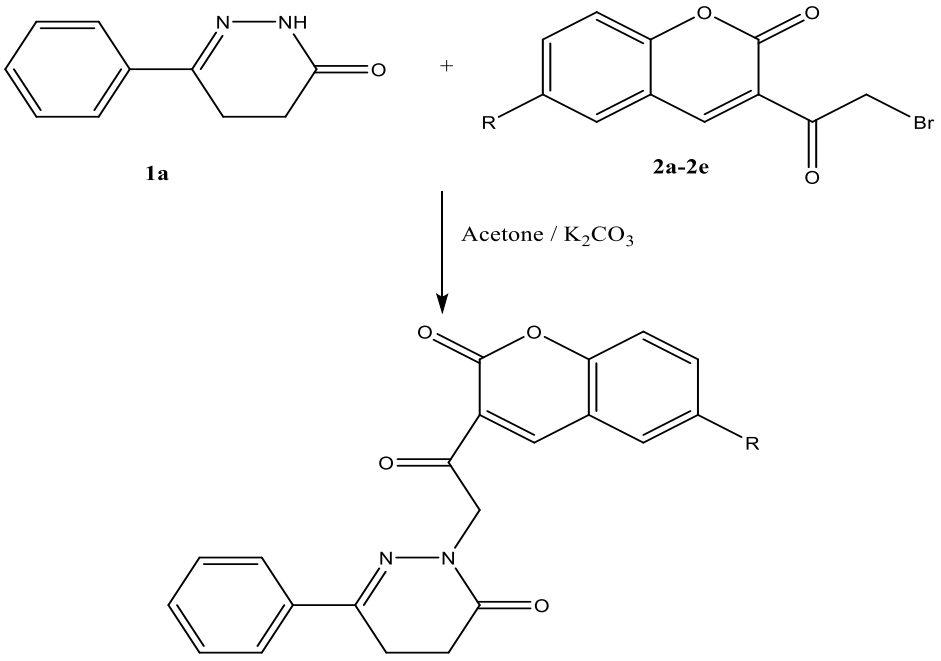
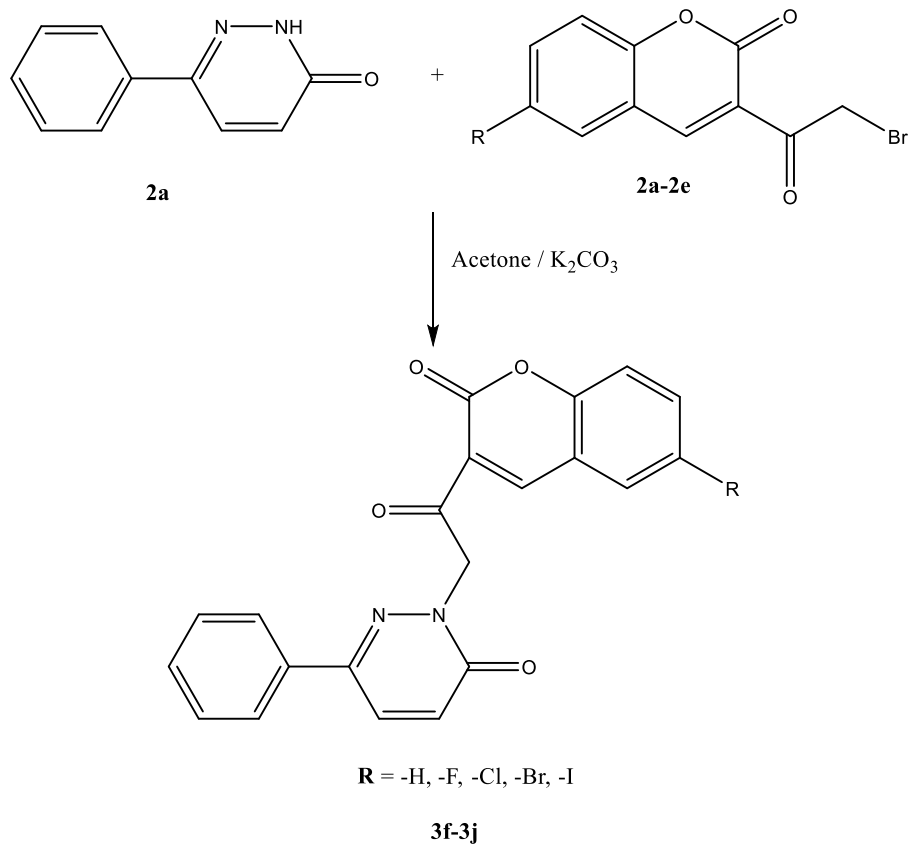


Figure 1: General procedure for the synthesis of the coumarin derivatives **3a-3e**



R = -H, -F, -Cl, -Br, -I

3f-3j

Figure 2: General procedure for the synthesis of the coumarin derivatives **3f-3j**

Preparation of the compound 1a and 1b
The intermediates 1a and 1b were obtained by the previous method [16].

Preparation of the compound 2a-2e
The compounds 2a and 2e were obtained by the previous method [17].

Preparation of 2-(2-oxo-2-(2-oxo-2*H*-chromen-3-yl)ethyl)-6-phenyl-4,5-dihydropyridazin-3(2*H*)-one (3a)
0.1 mol of each compound, 1a and 2a were stirred at 25ºC in acetone (30 ml) in the presence of K_2CO_3 for 20 hours. The reaction mass was concentrated to 15 ml and discharged in cold water (200 ml). The filtered residue was purified from ethanol.

Similar methods were applied to get the compounds 2-(2-oxo-2-(6-substituted-2-oxo-2*H*-chromen-3-yl)ethyl)-6-phenyl-4,5-dihydropyridazin-3(2*H*)-one (3b-3e) and 2-(2-oxo-2-(6-substituted-2-oxo-2*H*-chromen-3-yl)ethyl)-6-phenylpyridazin-3(2*H*)-one (3f-3j).

Anticancer activity
The sulforhodamine B (SRB) colorimetric method [18, 19] was adopted to assess the anticancer potential of 3a-3j with respect to breast cancer (MCF-7), and colon cancer (HCT-116) cell lines against doxorubicin as standard. In short, 100 µl of various dilutions of doxorubicin and 3a-3j, were mixed with cell lines at 37ºC. The unprocessed cells served as control. Each dilution was checked in six wells and the observations were made after 24 hours. Crystal violet was used to stain the cells and to identify the surviving cells [20, 21]. The lysing of the cells was done by 33% acetic acid. The absorbance of the control, standard and the test samples were read at 590 nm (SunRise ELISA reader).

$$\text{Percentage viability} = 1 - \frac{\text{The absorbance of the sample}}{\text{The absorbance of the control}} \times 100$$

The IC_{50} values were established by the regression equation using Microsoft Excel.

Statistical Analysis
The data (N =6, Mean, and Standard Error Mean) were analyzed by SPSS software, in which $p < 0.05$ specified the significant results.

Results

Figure 1 and Figure 2 depict the general process for the preparation of 3a-3e and 3f-3j, respectively. The structures of 3a-3j were characterized as per records of Table 1.

Table 1. The structure elucidation data of 3a-3j

Compound (Molecular Formula) (Melting Point)	**IR**	**^{1}H NMR**	**^{13}C NMR**	**Mass** (M^+)	**C, H, N Anal.,** [Found (Calculated)]
3a ($C_{21}H_{16}N_2O_4$) (155-157ºC)	1660, 1690, 1710, 1620, 1560, 1220	2.41 (t, 2H), 2.91 (t, 2H) 4.80 (s, 2H), 7.35-7.74 (m, 9H), 8.44 (s, 1H)	25.3, 33.4, 59.9, 117.0, 119.0, 126.3, 128.8, 129.1 (2C), 129.5, 130.0, 130.0, 132.0, 132.4, 137.3, 138.2, 147.4, 154.0, 159.9, 163.5, 195.4	360	C, 69.98 (69.99); H, 4.45 (4.48); N, 7.75 (7.77)
3b ($C_{21}H_{15}FN_2O_4$) (131-133ºC)	1670, 1700, 1720, 1630, 1570, 1230	2.44 (t, 2H), 2.93 (t, 2H) 4.83 (s, 2H), 7.35-7.73 (m, 8H), 8.46 (s, 1H)	25.3, 33.4, 59.9, 115.5, 116.0, 124.7, 126.0, 129.1 (2C), 129.7 (2C), 132.0, 132.3, 137.3, 138.3, 147.4, 149.5, 159.1, 159.9, 163.3, 195.5	378	C, 66.65 (66.66); H, 4.02 (4.00); N, 7.38 (7.40)
3c ($C_{21}H_{15}ClN_2O_4$) (164-166ºC)	1660, 1695, 1710, 1625, 1560, 1220	2.42 (t, 2H), 2.93 (t, 2H) 4.81 (s, 2H), 7.36-7.73 (m, 8H), 8.45 (s, 1H)	25.3, 33.4, 59.9, 119.0, 124.5, 127.7, 129.1 (2C), 129.8 (2C), 130.4, 132.0 (2C), 131.4, 137.3, 138.3, 147.4, 152.0, 159.9, 162.3, 195.4	394	C, 63.85 (63.89); H, 3.84 (3.83); N, 7.11 (7.10)
3d ($C_{21}H_{15}BrN_2O_4$) (159-161ºC)	1665, 1700, 1710, 1630, 1570, 1220	2.41 (t, 2H), 2.93 (t, 2H), 4.83 (s, 2H), 7.35-7.74 (m, 8H), 8.46 (s, 1H)	25.3, 33.4, 59.9, 119.1, 119.9, 125.3, 129.1 (2C), 129.7 (2C), 131.2, 132.0, 132.4, 135.1, 137.3, 138.3, 147.4, 153.1, 159.9, 163.3, 195.5	438	C, 57.40 (57.42); H, 3.45 (3.44); N, 6.38 (6.38)
3e ($C_{21}H_{15}IN_2O_4$) (178-180ºC)	1660, 1690, 1710, 1620, 1560, 1225	2.41 (t, 2H), 2.91 (t, 2H), 4.81 (s, 2H), 7.36-7.74 (m, 8H), 8.45 (s, 1H)	25.3, 33.4, 59.9, 93.7, 121.3, 124.7, 129.1 (2C), 129.7 (2C), 132.0, 132.4, 135.1, 137.3, 138.1, 138.3, 147.4, 152.8, 159.9, 163.3, 195.4	486	C, 51.85 (51.87); H, 3.10 (3.11); N, 5.75 (5.76)
3f ($C_{21}H_{14}N_2O_4$) (191-193ºC)	1665, 1700, 1715, 1625, 1565, 1225	4.60 (s, 2H), 6.48 (d, 1H), 6.70 (d, 1H), 7.35-7.72 (m, 9H), 8.44 (s, 1H)	59.9, 117.0, 119.0, 126.3, 128.8, 129.2, 129.7 (2C), 130.1 (2C), 131.0, 131.4, 132.0, 132.4, 135.2, 138.3, 145.2, 154.0, 159.4, 159.9, 195.5	358	C, 70.38 (70.39); H, 3.93 (3.94); N, 7.80 (7.82)
3g ($C_{21}H_{13}FN_2O_4$) (182-184ºC)	1665, 1695, 1720, 1630, 1570, 1230	4.63 (s, 2H), 6.50 (d, 1H), 6.73 (d, 1H), 7.35-7.74 (m, 8H), 8.46 (s, 1H)	59.9, 115.5, 116.0, 124.7, 126.0, 129.7 (2C), 130.1 (2C), 131.0, 131.4, 132.0, 132.3, 135.2, 138.3, 145.2, 149.5, 159.3, 159.5, 159.9, 195.5	376	C, 67.0 (67.02); H, 3.49 (3.48); N, 7.45 (7.44)
3h ($C_{21}H_{13}ClN_2O_4$) (210-212ºC)	1665, 1700, 1710, 1625, 1560, 1225	4.62 (s, 2H), 6.49 (d, 1H), 6.71 (d, 1H), 7.36-7.73 (m, 8H), 8.46 (s, 1H)	59.9, 119.0, 124.5, 127.7, 129.7 (2C), 130.2 (2C), 130.4, 131.0, 131.3, 132.0 (2C), 132.4, 135.2, 138.3, 145.2, 152.0, 159.4, 159.9, 195.5	392	C, 64.20 (64.21); H, 3.35 (3.34); N, 7.13 (7.13)
3i ($C_{21}H_{13}BrN_2O_4$) (222-224ºC)	1660, 1690, 1715, 1630, 1570, 1230	4.60 (s, 2H), 6.48 (d, 1H), 6.70 (d, 1H), 7.35-7.74 (m, 8H), 8.44 (s, 1H)	59.9, 119.1, 120.7, 125.3, 129.7 (2C), 130.2 (2C), 131.0, 131.4 (2C), 132.0, 132.3, 135.1, 135.2, 138.3, 145.2, 153.0, 159.4, 159.9, 195.5	436	C, 57.68 (57.69); H, 3.01 (3.00); N, 6.40 (6.41)
3j **(**$C_{21}H_{13}IN_2O_4$**)** **(**214-216ºC**)**	1665, 1700, 1710, 1625, 1570, 1225	4.63 (s, 2H), 6.50 (d, 1H), 6.73 (d, 1H), 7.35-7.74 (m, 8H), 8.46 (s, 1H)	59.9, 93.7, 121.3, 124.7, 129.7 (2C), 130.1 (2C), 131.0, 131.3, 132.0, 13.5, 135.1, 135.2, 138.1, 138.3, 145.2, 152.8, 159.4, 159.9, 195.5	483	C, 52.04 (52.09); H, 2.72 (2.71); N, 5.79 (5.79)

The sulforhodamine B (SRB) colorimetric method was adopted to assess the anticancer potential of 3a-3j with respect to HCT-116 / MCF-7 cell lines. Table 2 displays the anticancer activity data of 3a-3j.

Table 2. Anticancer activity of 3a-3j

Compound	**IC_{50} in µM (% inhibition)**	
	HCT-116	**MCF-7**
3a	1.93±0.22[a]	1.25±0.31[a]
3b	2.28±0.45[a]	2.09±0.12[a]
3c	3.88±0.38[a]	3.42±0.46[a]
3d	3.13±0.34[a]	4.12±0.36[a]
3e	5.55±0.51[a]	6.83±0.24[a]
3f	11.98±0.12[a]	13.55±0.33[a]

3g	9.98±0.28[a]	8.42±0.14[a]
3h	13.33±0.15[a]	15.12±0.29[a]
3i	9.55±0.15[a]	8.79±0.32[a]
3j	16.66±0.08[a]	18.23±0.23[a]
Doxorubicin	0.83±0.55[a]	0.74±0.41[a]

a = p < 0.5.

Discussion

The compounds 3a-3j were prepared according to Figure 1 and Figure 2. The compounds 1a & 1b [16], as well as 2a-2e, were obtained by the previous methods [17]. The facts of the comprehensive structural elucidations of 3a-3j are displayed in Table 1, which were in accordance with the assigned structure of the 3a-3j. Briefly, the IR spectra of 3a-3j displayed characteristic peaks for C=O at 1660-1720 cm^{-1}; C=N at 1620-1630 cm^{-1}; C=C at 1560-1570 cm^{-1}; and C-O-C at 1220-1230 cm^{-1}. The 1H NMR of 3a-3e displayed triplets of the pyridazine methylene groups of C-4 and C5 at δ 2.41-2.44 & 2.91-2.93 cm^{-1}, respectively; the C-4 and C5 hydrogens of 3f-3j appeared as doublets at δ 6.48-6.50 & 6.70-6.73 cm^{-1}, respectively; the methylene group of -CO-CH_2- moiety of 3a-3j appeared as singlet at δ 4.60-4.8 cm^{-1}; the C-4 hydrogen of coumarin appeared as singlet at δ 8.44-8.46 cm^{-1}; and other aromatic hydrogens appeared as multiplets at δ 7.35-7.74 cm^{-1}. The number of carbons as per 1C NMR, the mass analysis, and the elemental (C, H, and N) analysis data of 3a-3j were also in agreement with the allocated structures.

The anticancer activity record of Table 2 provided that the IC_{50} of doxorubicin was 0.83 µM (100%) and 0.74 µM (100%) relating to HCT-116 and MCF-7 cell lines, respectively. The compound 3a was the most promising compound that had IC_{50} values of 1.93 µM and 1.25 µM relating to HCT-116 and MCF-7, respectively. However, it was only 43.0% and 59.2% with respect to doxorubicin (100%) concerning HCT-116 and MCF-7, respectively. All other compounds showed less inhibition than 3a. It was also observed that the oxidized derivative of 3a, the compound 3f also displayed very good activity. As per the data of Table 2, it is evident that the compounds 3a-3e were more potent than their oxidized counterparts, 3f-3j. This finding is in accordance with the previous report [10] that stated easily oxidizable compounds act as better anticancer agents. It is also evident from the activity data that fluoro-substituted derivatives had better activity than the bromo-substituted derivatives, which in turn had better activity than the chloro-substituted derivatives. Furthermore, the iodine substituted compounds had the least potency. These data suggest that a modification in the light of these observations is possible to obtain better anticancer agents. For example, the coumarin ring may further be substituted with a phenolic –OH group that may increase its antioxidant potential, and ultimately its anticancer activity [12-14]. Accordingly, there is a high possibility that the fluoro- and the phenolic group containing coumarin moiety in these types of compounds may provide more efficient anticancer agents. Alternatively, the phenolic –OH group containing coumarin moiety and the fluoro-substituted benzene ring may also provide fruitful anticancer agents [13].

Conclusion

The compound **3a** had the highest anticancer activity among the tested compounds. However, its activity was almost 40% less than the doxorubicin. The chemical structure analysis showed that more worthy anticancer agents can be developed by incorporating phenolic –OH group in the coumarin moiety and substituting a fluorine atom at another place of the **3a-3j**. It is recommended to perform the suggested modifications in **3a-3j** to get the potent anticancer agents.

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Conflict of interest

No conflict of interest is associated with this work

References

1. Shin WJ, Pachynski RK. Chemerin modulation of tumor growth: Potential clinical applications in cancer. Discovery medicine. 2018 Aug 19;26(141):31-7.
2. Abuderman AA. Gastric cancer & prospects of cancer in Saudi Arabia peninsula. Saudi Journal of Biological Sciences. 2018 Feb 27. In press, https://doi.org/10.1016/j.sjbs.2018.02.016.
3. Pirmohamed M. Warfarin: the end or the end of one size fits all therapy?. Journal of personalized medicine. 2018 Sep;8(3):22.

4. Verhoef TI, Redekop WK, Daly AK, Van Schie RM, De Boer A, Maitland-van der Zee AH. Pharmacogenetic-guided dosing of coumarin anticoagulants: algorithms for warfarin, acenocoumarol, and phenprocoumon. British journal of clinical pharmacology. 2014 Apr;77(4):626-41.
5. Chen Q, Li P, Zhang J, Zhu J. Preclinical pharmacokinetic analysis of armillarisin succinate ester in mouse plasma and tissues by LC–MS/MS. Biomedical Chromatography. 2013 Jan;27(1):130-6.
6. Li X, Zeng H, Wang P, Lin L, Liu L, Zhen P, Fu Y, Lu P, Zhu H. Reactivation of latent HIV-1 in latently infected cells by coumarin compounds: Hymecromone and ScoparoneReactivation of Latent HIV-1 in Latently Infected Cells by Coumarin Compounds: Hymecromone and Scoparone. Current HIV research. 2016 Dec 1;14(6):484-90.
7. Opherk D, Schler G, Waas W, Dietz R, Kübler W. Intravenous carbochromen: A potent and effective drug for estimation of coronary dilatory capacity. European heart journal. 1990 Apr 1;11(4):342-7.
8. Ujeyl M, Köster I, Wille H, Stammschulte T, Hein R, Harder S, Gundert-Remy U, Bleek J, Ihle P, Schröder H, Schillinger G. Comparative risks of bleeding, ischemic stroke and mortality with direct oral anticoagulants versus phenprocoumon in patients with atrial fibrillation. European journal of clinical pharmacology. 2018 Oct 1;74(10):1317-25.
9. Dlugosz A, Janecka A. Novobiocin analogs as potential anticancer agents. Mini reviews in medicinal chemistry. 2017 Jun 1;17(9):728-33.
10. Thakur A, Singla R, Jaitak V. Coumarins as anticancer agents: A review on synthetic strategies, mechanism of action and SAR studies. European journal of medicinal chemistry. 2015 Aug 28;101:476-95.
11. Klenkar J, Molnar M. Natural and synthetic coumarins as potential anticancer agents. J. Chem. Pharm. Res. 2015 Jan 1;7:1223-38.
12. Lacy A, O'kennedy R. Studies on coumarins and coumarin-related compounds to determine their therapeutic role in the treatment of cancer. Current pharmaceutical design. 2004 Nov 1;10(30):3797-811.
13. Musa MA, Cooperwood JS, Khan MO. A review of coumarin derivatives in pharmacotherapy of breast cancer. Current medicinal chemistry. 2008 Nov 1;15(26):2664-79.
14. Dandriyal J, Singla R, Kumar M, Jaitak V. Recent developments of C-4 substituted coumarin derivatives as anticancer agents. European journal of medicinal chemistry. 2016 Aug 25;119:141-68.
15. Emami S, Dadashpour S. Current developments of coumarin-based anti-cancer agents in medicinal chemistry. European Journal of Medicinal Chemistry. 2015 Sep 18;102:611-30.
16. Sircar I, inventor; Warner-Lambert Co LLC, assignee. Substituted 6-phenyl-3 (2H)-pyridazinones useful as cardiotonic agents. United States patent US 4,404,203. 1983 Sep 13, available at https://patents.google.com/patent/US4404203.
17. Ansari MI, Khan SA. Synthesis and antimicrobial activity of some novel quinoline-pyrazoline-based coumarinyl thiazole derivatives. Medicinal Chemistry Research. 2017 Jul 1;26(7):1481-96.
18. Skehan P, Storeng R, Scudiero D, Monks A, McMahon J, Vistica D, Warren JT, Bokesch H, Kenney S, Boyd MR. New colorimetric cytotoxicity assay for anticancer-drug screening. JNCI: Journal of the National Cancer Institute. 1990 Jul 4;82(13):1107-12.
19. El-Feky SA, El-Fattah HA, Osman NA, Imran M, Zedan MN. Design, synthesis and in vitro antitumor activity of novel phthalazin-1, 4-dione/chalcone hybrids and phthalazin-1, 4-dione/pyrazoline hybrids. Journal of Chemical and Pharmaceutical Research. 2015;7(7):1154-66.
20. Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. Journal of immunological methods. 1983 Dec 16;65(1-2):55-63.
21. Gangadevi V, Muthumary J. Preliminary studies on cytotoxic effect of fungal taxol on cancer cell lines. African Journal of Biotechnology. 2007;6(12).