Original Article

Synthesis, Characterization and Molecular Docking Study of New Coumarin β-Thio Carbonyl Derivatives against MCF-7 Breast Cancer Cell Line

Ali Mohammed Abdul-Hussain, Leaqaa Abd-ul-Redha Raheem and Maan Abdul Razzaq Nema

ABSTRACT

Objective: To prepare and compare a set of derivatives of 7-methoxy-3-(3-(4-R-phenyl)-3-(phenylthio) propanoyl)-2H-chromen-2-one to test their ability to induce anti-proliferative effects on MCF-7 human breast cancer cell line.
Study Design: Experimental study
Place and Duration of Study: This study was conducted at the College of Pharmacy, University of Basrah, Iraq from 15th March 2024 to 30th April 2025.
Methods: Five coumarin-chalcone derivatives were prepared using starting materials. The microculture tetrazolium assay was used to determine the IC50 values of these derivatives against the MCF-7 breast cancer cell line to determine its in vitro anticancer potential. The chemical structures of the synthesized derivatives were determined by determination of the melting point, mass spectrometry, infrared spectroscopy, and nuclear magnetic resonance spectroscopy (1H and 13C). The microculture tetrazolium assay was used to assess antiproliferative activity to evaluate cell viability and calculate IC50 values.
Results: Compound 11 demonstrated the best anti-proliferative activity with an IC50 of 6.25 ug/mL, compared to other derivatives. Compounds 8, 9 and 10 showed moderate cytotoxicity with the IC50 of 26.31, 29.29 and 33.49ug/mL respectively. Compound 7 on the other hand was less active with IC50 of 71.57 ug/mL. All the compounds that were synthesized showed a lower potency than the reference drug doxorubicin, the IC50 of which was 2.40 0 M.
Conclusion: The antiproliferative potency of the synthesized coumarin-chalcone derivatives was different and compound 11 was the strongest against the MCF-7 breast cancer cell line. Such results indicate that additional optimization of such compounds can result in the creation of more potent anticancer agents.
Key Words: Coumarin, β-thiocarbonyl, Michael addition, Microculture tetrazolium assay

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INTRODUCTION

Breast cancer is the most common among women worldwide and leading cause of cancer related deaths after that of lung cancer; epidemiological literature revealed a prevalence of 22-26 and a risk of breast cancer-related mortality of about 18%.[1-3] Multidrug resistance (MDR) are some of the significant obstacles to effective treatment of cancer.[4]

Department of Pharmaceutical Chemistry, College of Pharmacy, University of Basrah, Iraq.

Correspondence: Ali Mohammed Abdul Hussain, Department of Pharmaceutical Chemistry, College of Pharmacy, University of Basrah, Iraq.
Contact No: +9647717866860
Email: albandarali829@gmail.com

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In line with the (WHO) tumor data-base of 2021, over 2 million women are screened with breast cancer in the year.[3]

As the latest research indicates, coumarin has also been found to potentially treat cancer, potentially reducing the adverse impacts of radiation. Incorporation of coumarin into the hybridization structures is very effective in cancer treatment because the substance has the capability of destroying tumor cells as seen in various articles.[5,6]

It has vastly studied coumarin compounds in their anticancer effects against numerous cancers such as; melanoma, lymphoma, squamous cell carcinomas, prostate cancer progression, and against breast cancer rise. Toxic effects caused by radiation can be countered by using coumarins, hence, there is need to develop robust anti-tumor agents which are tissue-selective in addition to the distinct gamut of potency as a consequence of emergence of resistance to treatment, development of adverse effects and re-emergence of malignancies. Molecular hybrids have made them a

special agent especially to scientists and researchers because of the synergistic pharmacological effects. In the last 15 years or so, many MH based anticancer therapeutics are discovered.[7]

A key reaction in organic chemistry, the nucleophilic addition of thiols to form a carbon-sulfur bond, is the Michael addition reaction, which entails addition of carban-ions to unsaturated system in conjugation with an activating group (carbonyl group).[8] A wide range of reagents, have been reported in the literature to carry out the addition of thiols to conjugated alkenes.[9]

A very useful reaction in the asymmetric conversion is the catalytic asymmetric Michael addition, which has been developed to a very successful degree in new years in the organocatalytic form of the reaction.[10]

One of the many types of reactions is the sulfa-Michael addition that provides direct access to optically active sulfides, which can be used as a wide range of starting materials in the synthesis of biologically important chemicals.[11]

METHODS

The experimental research was done at the College of Pharmacy, University of Basrah, Iraq from the 15th March 2024 to 30th April 2025 by the letter No. 4545/QM/Approval/SJKDH379 dated March 9, 2024. Ethyl acetoacetate, thiophenol, 4- nitrobenzaldehyde, 4-bromobenzaldehyde, 4-methoxybenzaldehyde and piperidine were purchased off of MERCK. The sources of benzaldehyde, Ethanol and Methanol were purchased through Thomas Baker. Preparation of 3-Acetyl- 7-Methoxycoumarin: In a round bottom flask with 50 mL of ethyl acetoacetate (0.026 mol, 3 mL, 3.06 g), 2-methoxy- 4-hydroxy benzaldehyde (0.028 mol, 3 ml, 3.5 g) was added followed by the addition of dimethylamine (15 drop) as a catalyst, and the mixture was stirred A yellow precipitation formative, recrystallization using ethanol.[12]

Coumarin-Chalcone derivatives (2-6): 3-Acetyl-7-methoxy-2H -chromen-2-one (0.44 g, 2.0 mmol) was dissolved in 25 mL of DCM, and 0.5 mL of piperidine was added to it. The mixture was kept at reflux temperature of 10 hrs. The solution was cooled, filtered, dissolved in a small amount of aliquot of dichloromethane and further methanol was then added to cause precipitation.[13]

Preparation of new coumarin-2-thiocarbonyl derivatives (7-11): In a 250 mL round-bottom flask with magnetic stir bar dissolve coumarin-chalcone derivative (1.43mmol) in 20-25 mL of DCM. Add thiophenol (1.43 mmol) to the solution which is being stirred at room temperature. A base catalyst, triethylamine (0.715 mmol, 0.1 mL) is dropwise added to produce the thiolate species in the solution. The mixture is shaken at room temperature and the reaction is followed by (TLC) with nhexane/ethyl acetate (9:1) as a product. When it is ready, the mixture reaction is filtered into an ice-cold water to separate the product. DCM recrystallization provides the pure 2,14 -thiocarbonyl coumarin product.

Methods of characterization: Stuart SMP apparatus was used to determine the melting points of the synthesized compounds. A spectrophotometer was employed to get the infrared spectrum with a KBr disc (SHIMADZU, Japan). It used Bruker spectrometer (Switzerland) to record the 1H NMR and 1C NMR spectrums, using DMSO and CDCl3 as solvents, tetramethylsilane (TMS) as a reference and mass spectra of the synthesized coumarin beta thiocarbonyl molecule were recorded at the Faculty of Chemistry, Tehran University.

Preliminary cytotoxicity screening: The cells were inoculated on a (96) well plate at a concentration density of 2x104 cells per well. Each well was then filled with 150 0L of DMEM culture with 100 units/mL penicillin, 100 0L streptomycin, and 10 percent fetal bovine serum (FBS). After this, the plate was left at 37 ° C and exposed to 5% CO 2 and 95% relative humidity of a humid atmosphere in a 24 hour incubation. After the incubation period, fresh media having different doses of coumarin-2 compounds (1, 10, 25, 50, and 100 25g) and doxorubicin (1, 2.5, 5, 10, and 20 25g) were added to the media in each of the wells. The plate required 24-hour incubation. Each well was filled with 10 0L of MTT (4mg/mL) and incubated over 4 hours at 37C without light to dissolve formazan. 100 0L of dimethyl sulfoxide (DMSO) was added and allowed to dissolve. The optical density at 570 nm was measured after the full dissolution of the purple formazan using the ELISA microplate (BioRad, USA).15,16 The half maximum inhibitory concentration (IC50) of each cell line is calculated after triplicate treatments were done. Viability was determined by comparing the treated and untreated cells and measures were made thrice. Proliferative rate (PR) percentage =(A/B) 100, Equation (1). Inhibitor Rate % = 100 – PR Equation (2). A is the optical density of wells that are treated. B = Optical density of control (untreated) wells. The data was inputted and was analyzed using SPSS- 26.

RESULTS

The compounds of coumarin- 2 -thiocarbonyl derivatives (7-11) were elucidated with the help of multiple distinctive spectrum changes, which encompassed MS, IR, 1H NMR, and 13C NMR. Mass spectrometry was used to determine the molecular weight of the product synthesized and the majority of derivatives had molecular ion (M+.) peaks that indicated their respective molecular weight (Table 1).

FT-IR of coumarin- -thiocarbonyl Derivatives: FTIR spectral analysis of the synthetic coumarin -thiocarbonyl derivatives (compounds 7 -11) was used to confirm the key functional groups of these coumarin -

thiocarbonyl derivatives. Typical of the derivatives moiety, the high absorption band in the range of 1710-1735 cm -1 was attributed to the C=O vibration of coumarinmoiety. The bands at 650-800 cm -1 were additional proofs of the existence of correlated to aromatic C-S out-of-plane bending vibrations. A broad 3100-3000 cm-1 region of all compounds indicated the presence of aromatic C–H stretching whereas 1200-1250 cm-1 area of O-C of methoxy group. Table 2 shows the spectra that were obtained after correlation with reference to conventional infrared, thus establishing the structural integrity of the prepared compounds.

1H-NMR spectrums of coumarin -2-thiocarbonyl derivatives 7-11: the 1H-NMR spectrums of the five synthesized coumarin-2-thiocarbonyl derivatives as indicated in table 3 have characteristic pattern that is consistent with their speculated structure. The downfield area (6.9-8.2 ppm) will contain numerous aromatic proton peaks in each compound which will be a bearer of a substituted benzene ring of both the coumarin and 1-beta thiocarbonyl. Such signals are normally formed as multiplets or doublets through spin-spin coupling and they are also the outcome of the substituted pattern and symmetry of an aromatic system hence their successful synthesis and structural integrity.

13C-NMR spectra of coumarin -2 thiocarbonyl derivatives 711: The 13C nuclear magnetic resonance (NMR) spectra showed the appearance of the signals at approximately 41ppm and 49ppm, attributed to the evolution of the β-thiophenol (CHS) atom which is C11 and C10 respectively. Moreover, there are two peaks of 195.5 and 159.7 ppm. The signals are ketone carbonyl (C=O) of the acetyl (-COCH2) group and the lactone carbonyl (C=O), respectively. In all the synthesized compounds as well two signals in 165 ppm and 56 ppm are credited to the carbon attached with methoxy group on position C7 and methoxy C-7a respectively.

Lipinski Rule of Five (RO5): The generated products (7-11) were assessed based on Lipinski Rule of Five, which is usually used to assess the potential of a compound as an orally active pharmaceutical agent. The theoretical moles weight of the compounds were determined as 416.49 to 395.38 g/mol. The number of hydrogen bond donors was 0 and that of hydrogen bond acceptors was 4-6. The number of calculated rotatable bonds was between 7 and 8. The lipophilicity, as shown by the Log P mentioned was 3.27-3.80. Total polar surface area (T.P.S.A) of the compounds ranged between 81.81 and 127.63 A 2. The parameters fit the acceptable range of values that are set by Lipinski criteria which means that the compounds have favorable pharmacokinetic characteristics and can be orally bio-absorbed (Table 5).

Table No. 1: Physical characteristics and mass spectra of coumarin-β-thiocarbonyl derivatives 7-11

Compound	Melting Point (°C)	Yield %	Molecular weight (g/mole)	Mass (M.+) m/e	Appearance
7	138.5–140	45.89	416	416.3	Yellow
8	183–184	38.84	446	446.2	Pale Yellow
9	155–156.5	48.73	495	494.1	Chrome Yellow
10	168–169	39.66	461	461.1	Gold Yellow
11	243.5–245	26.09	460	459.2	Dark Red

Table No. 2: FT-IR characteristics of coumarin- β-thiocarbonyl derivatives 7-11

Absorption bond	Comp. 7	Comp. 8	Comp. 9	Comp. 10	Comp. 11
C=O (lactone – coumarin)	1728(s)	1730 (s)	1736(s)	1735 (s)	1728 (s)
C=O (thio-carbonyl)	1658 (m)	1672(m)	1672 (m)	1672 (m)	1643 (m)
C–S (stretching)	692 (m)	787 (m)	680 (m)	650 (m)	655 (m)
C–H (Aromatic)	3024–3074(w)	3040 (w)	3040 (w)	3040 (w)	3078 (w)
C–O (Methoxy)	–	1205-1255 (s)	–	–	–
$–CH_3$ (Methoxy, C–H stretch)	–	2841 (w)	–	–	–
C–Br (stretching)	–	–	574 (w)	–	–
N=O (asym. stretch)	–	–	–	1548 (s)	–
N=O (sym. stretch)	–	–	–	1365 (s)	–
$–CH_3$ (Dimethyl bending)	–	–	–	–	1381 (m)
Alkyl C–H stretching	–	–	–	–	2934 (s)

Table No. 3: ^{1}H-NMR spectra of coumarin -β-thiocarbonyl derivatives 7–11

Compound	Chemical shift					
	C4	C11	C10	C-7a	R	Aromatic protons
7	8.30 (s,1H)	4.80-4.84 J=14.4Hz (t,1H)	3.63-3.69 J1=18.2 J2=6.8 (dd,2H)	3.81 (s,3H)	-	6.72-7.6 (m,10H)
8	8.41 (s,1H)	4.33-4.37 J=14.4Hz (t,1H)	3.55-3.61 J1=18.2 J2=6.8 (dd,2H)	3.84 (s,3H)	3.94 (s,3H)	6.74-7.88 (m,9H)
9	8.40 (s,1H)	4.26-4.28 J=13.6Hz (t,1H)	3.69-3.76 J1=18.2 J2=6.8 (dd,2H)	3.83 (s,3H)	-	6.73-7.47 (m,9H)
10	8.51 (s,1H)	4.10-4.14 J=14.4Hz (t,1H)	3.50-3.56 J1=18.2 J2=6.8 (dd,2H)	3.93 (s,3H)	-	6.83-8.13 (m,9H)
11	8.51 (s,1H)	4.30-4.39 J=14.4Hz (t,1H)	3.61-3.66 J1=18.2 J2=6.8 (dd,2H)	3.90 (s,3H)	3.01 (s,3H) N-(CH3)2	6.78-7.88 (m,9H)

Table No. 4: ^{13}C NMR characteristics of coumarin–β-thio carbonyl derivatives (Compound 7–11)

Carbon position	Comp. 7	Comp. 8	Comp. 9	Comp. 10	Comp. 11
9	194.77	187.00	195.53	195.53	186.06
7	165.40	165.35	165.51	165.28	165.35
2	159.56	159.36	159.74	159.75	159.89
1b	157.74	157.47	157.75	157.79	157.75
1′	141.39	135.05	147.83	152.79	132.05
1``	134.97	140.01	136.94	135.31	140.90
4	134.32	143.70	140.62	138.82	147.84
3	133.01	132.43	133.18	131.51	131.68
2``	131.56	129.54	129.63	128.63	131.08
6``	131.56	129.54	129.63	128.63	131.08
5	130.69	131.21	130.64	129.83	131.19
3``	128.90	129.06	128.87	128.11	125.11
5``	128.90	129.06	128.87	128.11	125.11
3′	128.21	120.15	134.94	124.31	113.90
5′	128.21	120.15	134.94	124.31	113.90
2′	127.50	128.34	131.63	127.51	122.82
6′	127.59	128.34	131.63	127.51	122.82
4′	127.16	158.94	120.56	147.82	152.14
4``	124.17	125.18	127.12	125.60	120.59
6	113.91	113.99	113.89	113.90	111.71
1a	112.39	112.53	112.00	112.04	110.90
8	100.33	100.83	100.26	100.42	100.40
7a	57.07	56.77	56.07	56.06	56.00
10	48.42	50.75	48.17	50.36	49.96
11	40.54	43.48	40.47	40.03	40.68
R	-	56.68-OCH_3	-	-	44.58-$N(CH_3)_2$

Table No. 5: Lipinski's rules for coumarin-β-thio carbonyl compounds (7-11)

Cpd.	Mol. Wt. (g/mol)	NRB	NHA	NHD	TPSA (Å²)	Log P	Lipinski's Rule of Five Violation
7	416.49	7	4	0	81.81	3.27	0
8	446.51	8	5	0	91.04	3.53	0
9	495.38	7	4	0	81.81	3.76	1
10	461.49	8	6	0	127.63	3.11	0
11	459.56	8	4	0	85.05	3.80	0

DISCUSSION

The dose-response curve was obtained and used to determine the half maximal inhibitory concentration (IC50), which was expressed as the percentage of cell death at different drug concentrations. The production of all the produced compounds was cytotoxic with an IC50 range of 6.25 to 71.57 μM.[15,16]

Compound 11, which contains a dimethylamine group [-N(CH3)2], was the most active of the compounds produced, with an IC 50 of 6.25 0M. Compound 8 and 10that contained a methoxy, bromo and nitro group showed high activity with an IC of 26.31, 26.49 μM and 33.49 33.49 33.49 33.49 33.49 33.49 33.49 33.49 33.49 33.49 33.49 33.49 33.49 33.4 All the produced compounds exhibit less cytotoxic activity in comparison to doxorubicin, with a significantly lower IC50 of 2.40 0M. On the other hand, compounds 7 were less active. With no substituent on compound 7, the IC50 was 71.57mol/kg.[17,18]

CONCLUSION

The cytotoxic activities of the compounds produced were determined using the MCF-7 cell line. Compound 11 [N(CH3)2]substituted was found to have a superior cytotoxic activity with an IC50 value of 6.25 M compared to the other compounds obtained. However, their cytotoxic action was not as much powerful as the conventional doxorubicin.

Author's Contribution:

Concept & Design or acquisition of analysis or interpretation of data:	Ali Mohammed Abdul-Hussain, Leaqaa Abd-ul-Redha Raheem
Drafting or Revising Critically:	Ali Mohammed Abdul-Hussain, Maan Abdul Razzaq Nema
Final Approval of version:	All the above authors
Agreement to accountable for all aspects of work:	All the above authors

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REFERENCES

1. Chan JW, Hoyle CE, Lowe AB, Bowman M. Nucleophile-initiated thiol-Michael reactions: effect of organocatalyst, thiol, and ene. Macromolecules 2010; 43(15):6381–8.
2. Kenari F, Molnár S, Perjési P. Reaction of chalcones with cellular thiols. The effect of the 4-substitution of chalcones and protonation state of the thiols on the addition process. Diastereoselective thiol addition. Molecules 2021;26(14):4332.
3. Amslinger S, Al-Rifai N, Winter K, Wörmann K, Scholz R, Baumeister P, et al. Reactivity assessment of chalcones by a kinetic thiol assay. Org Biomol Chem 2013;11(4):549–54.
4. Movassagh B, Shaygan P. Michael addition of thiols to α, β-unsaturated carbonyl compounds under solvent-free conditions. Arkivoc 2006; 12:130-7.
5. Stiefel C, Schubert T, Morlock GE. Bioprofiling of cosmetics with focus on streamlined coumarin analysis. ACS Omega 2017;2(8):5242–50.
6. Rios R, Companyó X. Addition to alpha, beta-unsaturated aldehydes and ketones. In: Comprehensive enantioselective organocatalysis: catalysts, reactions and applications. Peter I. Dalko 2013; 977–1012.
7. Azimi S, Zonouzi A, Firuzi O, Iraji A, Saeedi M, Mahdavi M, et al. Discovery of imidazopyridines containing isoindoline-1, 3-dione framework as a new class of BACE1 inhibitors: design, synthesis and SAR analysis. Eur J Med Chem 2017;138: 729-37.
8. Jayasooriya RGPT, Molagoda IMN, Park C, Jeong JW, Choi YH, Moon DO, et al. Molecular chemotherapeutic potential of butein: A concise review. Food Chem Toxicol 2018;112:1-10.
9. Padmavathi G, Rathnakaram SR, Monisha J, Bordoloi D, Roy NK, Kunnumakkara AB. Potential of butein, a tetrahydroxychalcone to obliterate cancer. Phytomedicine 2015;22(13): 1163-71.
10. Wang KL, Yu YC, Hsia SM. Perspectives on the role of isoliquiritigenin in cancer. Cancers (Basel) 2021;13(1):115.
11. Peng F, Tang H, Liu P, Shen J, Guan X, Xie X, et al. Isoliquiritigenin modulates miR-

374a/PTEN/Akt axis to suppress breast cancer tumorigenesis and metastasis. Sci Rep 2017;7(1):9022.
12. Tchoupou IT, Manyeruke MH, Salami SA, Ezekiel CI, Ambassa P, Tembu J V, et al. An overview of the synthesis of coumarins via Knoevenagel condensation and their biological properties. Results Chem 2025;102181.
13. Moya-Alvarado G, Yañez O, Morales N, González-González A, Areche C, Núñez MT, et al. Coumarin-chalcone hybrids as inhibitors of MAO-B: Biological activity and in silico studies. Molecules 2021;26(9):2430.
14. Al-Jaber NA, Bougasim ASA, Karah MMS. Study of Michael addition on chalcones and or chalcone analogues. J Saudi Chem Soc 2012;16(1):45–53.
15. Jaafar ND, Al-Saffar AZ, Yousif EA. Genotoxic and cytotoxic activities of lantadene A-loaded gold nanoparticles (LA-AuNPS) in MCF-7 cell line: an in vitro assessment. Int J Toxicol 2020;39(5): 422–32.
16. Bashir MK. Effects of structural manipulation on the bioactivity of some coumarin-based products. Arch Razi Inst 2021;76(5):1297.
17. Fan D, Wang D, Zhang J, Fu X, Yan X, Wang D, et al. Cobalt-Catalyzed Cascade C–H Activation/ Annulation Polymerizations towards Diversified and Multifunctional Sulfur-Containing Fused Heterocyclic Polymers. J Am Chem Soc 2024; 146(25):17270-84.
18. Yang Y, Hu R, Tang BZ. Exploration of poly (thiazole-2-thione) structures from multicomponent polymerizations of elemental sulfur, dichalcones, and diisocyanides. Macromolecules 2025;13: 6577-89.