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Synthesizing Derivatives of 7-Hydroxy-4-{[(5-Methyl-1h- enzimidazol-2-Yl) Sulfanyl] Methyl}-2h-Chromen-2-One and Evaluating Their Potential Anti-Cancer Properties

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KEYWORDS

Pechmann condensation, Synthesis, Structural characterization, MCF cell lines, Trypan Blue assay, Therapeutic applications, Drug development

ABSTRACT:

Objective: The research aimed to synthesize novel chromen-2-one derivatives incorporating benzimidazolyl moieties and evaluate their anti-cancer properties.

Experimental: The compounds were synthesized using the Pechmann condensation method. Structural characterization was performed utilizing Fourier-transform infrared spectroscopy (FTIR), proton nuclear magnetic resonance (1H-NMR), and mass spectrometry (MS). The anti-cancer potential of the synthesized derivatives was assessed using the Trypan Blue assay on MCF cell lines.

Results: Several of the synthesized benzimidazolyl chromen-2-one derivatives demonstrated significant anti-cancer activity. The structural characterization confirmed the successful incorporation of benzimidazolyl moieties into the chromen-2-one framework.

Discussion: The promising anti-cancer activities observed in several derivatives suggest their potential as lead compounds for further development in anti-cancer drug research. The use of the Pechmann condensation method proved effective in synthesizing diverse chromen-2-one derivatives with therapeutic potential.

Conclusion: This study successfully synthesized and characterized novel benzimidazolyl chromen-2-one derivatives with noteworthy anti-cancer properties, validating their potential for future anti-cancer drug development. The methodology employed provides a valuable approach for the diversification of chromen-2-one derivatives with potential therapeutic applications.

1. Introduction

Given the substantial impact of cancer as a leading cause of mortality, it poses a significant global health concern. The International Agency for Research on Cancer estimates a staggering 19.3 million new cancer cases worldwide in 2020, with colorectal, lung, and female breast cancers each contributing to approximately 10% of the total cases.[1] Correspondingly, an estimated 10.0 million cancer-related fatalities are projected for the same year.[2] Despite notable progress in targeted therapies, including immunotherapy, gene therapy, and small molecule medicines, efficient treatment modalities are still lacking for many recurrent or refractory tumors.

Metastasis, a critical factor in poor prognosis, remains a challenge in cancer treatment. The underlying mechanisms of metastasis are not fully understood, further complicating efforts to prevent cancer

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progression.[3] Improving the effectiveness of therapeutic regimens is an ongoing challenge, emphasizing the urgent need for innovative treatment alternatives to halt the spread of cancer.[4]

Chemotherapy, while essential in cancer treatment, is often associated with harmful side effects. The primary goals of using popular anticancer medications involve slowing down cancer growth and ideally stopping it altogether.[5] Chemotherapeutic drugs, which target rapidly dividing cells, achieve this by preventing mitosis and causing cell damage, often referred to as cytotoxic effects. Strategies include disrupting cancer cells' DNA and hindering the formation of DNA strands necessary for cell division.[6]

Recent research in cancer treatment has explored immunotherapeutics and molecularly targeted therapies. However, the rapid development of drug resistance has become a global issue, necessitating the exploration of new compounds to address this challenge.[7] A promising approach involves the hybridization of two or more bioactive drug fragments with complementary functionalities or different mechanisms of action into a single molecule. Solomon et al. first reported on this isatin-based dual action/hybrid pharmacophore approach, demonstrating its potential for synergistic activity and increased drug efficacy.[8] Coumarin, the most prevalent naturally occurring secondary metabolite, is found in various plant families, essential oils, microorganisms, and some animal species. Beyond its application in cancer treatment, coumarin is beneficial for managing radiation side effects. The molecular characteristics of coumarin exhibit specific biological impacts on the cellular environment,[9] making it effective in melanoma maintenance therapy and demonstrating anti-viral, anti-tumor, anti-HIV, antimicrobial, and antioxidant activities. Coumarin derivatives, such as benzopyrones, isoflavones, furanocoumarin, and pyranocoumarin, have shown effectiveness in treating different cancer scenarios, including leukaemia, prostate cancer, and renal cell carcinoma.[10]

2. Objectives

Synthesis of Derivatives:

Design and synthesize a series of derivatives of 7-hydroxy-4-{[(5-methyl-1H-benzimidazol-2-yl)sulfanyl]methyl}-2H-chromen-2-one, incorporating various functional groups to explore structural modifications. Optimize synthetic routes for efficient production of the desired compounds. Characterize the synthesized derivatives using techniques such as nuclear magnetic resonance (NMR) spectroscopy, mass spectrometry (MS), and infrared (IR) spectroscopy to confirm their structures. In Vitro Anti-Cancer Evaluation: Conduct cytotoxicity assays to evaluate the anti- cancer activity of the synthesized derivatives against a panel of human cancer cell lines, including but not limited to breast cancer, lung cancer, colon cancer, and leukemia. Determine the half-maximal inhibitory concentration (IC50) values for each derivative to assess their potency. Structure-Activity Relationship (SAR) Analysis: Perform SAR analysis to correlate the structural features of the derivatives with their observed biological activities. Identify key structural modifications that enhance anti-cancer efficacy and selectivity.

3. Methods

EXPERIMENTAL

The melting points were determined without correction using the capillary method on a melting point apparatus. The infrared (IR) spectra of the synthesized compounds were acquired using a Bruker alpha-E Fourier-transform infrared attenuated total reflection (FTIR-ATR) spectrometer. The 1H nuclear magnetic resonance (NMR) spectra were obtained on a Bruker Avance II (400MHz) spectrometer using CDCl3 and DMSO as solvents, with TMS serving as the standard. Monitoring of the reaction progress was conducted through thin-layer chromatography (TLC) on pre-coated (Merck 60F254) and homemade silica gel-coated plates. The chromatogram was developed using a solvent system comprising chloroform and methanol in various ratios, and TLC spots were visualized using UV chambers.

SYNTHESIS OF 2-[(7- METHOXY -2-OXO-2H-CHROMEN-4-YL) METHOXY]-1H-IMIDAZOLE-5-CARBOXYLIC ACID (2A)

A reaction was conducted by heating 0.01 mole of 4-(bromomethyl)-7- methoxy -2H-chromen-2-one with 0.01 mole of 2-hydroxy-1H-imidazole-5-carboxylic acid in the presence of 5 ml of 10% potassium hydroxide solution for a duration of 2 hours. After completion of the reaction, the mixture was cooled to room temperature. The resulting precipitate, identified as the title compound, was obtained by adding dilute hydrochloric acid. The synthesis was followed by

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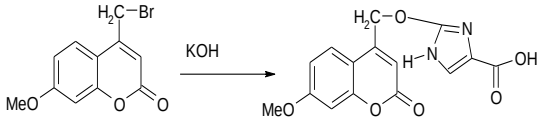
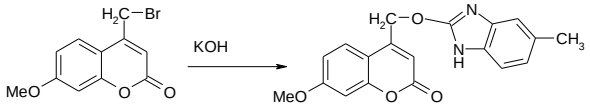
recording the melting point of the compound using the open capillary method.



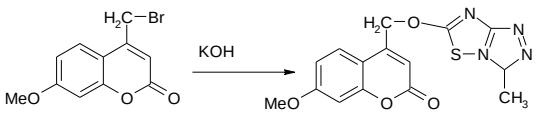
SYNTHESIS OF 7- METHOXY -4-{[(5-METHYL-1H-BENZIMIDAZOL-2-YL)OXY]METHYL}-2H-CHROMEN-2-ONE (2B)

In a synthetic procedure, 0.01 mole of 4-(bromomethyl)-7- methoxy -2H-chromen-2-one was subjected to heating with 0.01 mole of 5-methyl-1H-benzimidazol-2-ol in the presence of 5 ml of a 10% potassium hydroxide solution for a duration of 2 hours. Subsequently, the reaction mixture was cooled to room temperature, leading to the precipitation of the desired compound. The title compound was isolated by adding dilute hydrochloric acid. The synthesis was concluded by recording the melting point of the synthesized compound using the open capillary method.



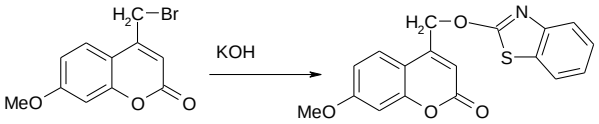
SYNTHESIS OF 7- METHOXY -4-{[(3-METHYL-3,7A-DIHYDRO[1,2,4]TRIAZOLO[4,3-B][1,2,4]THIADIAZOL-6-YL)OXY]METHYL}-2H-CHROMEN-2-ONE (2C)

0.01 mole of 4-(bromomethyl)-7- methoxy -2H-chromen-2-one underwent heating with 0.01 mole of 2-methoxy -1H-imidazole-5-carboxylic acid in the presence of 5 ml of a 10% potassium hydroxide solution for a duration of 2 hours. Following the reaction, the mixture was cooled to room temperature, resulting in the precipitation of the targeted compound. The title compound was then isolated by the addition of dilute hydrochloric acid. The synthesis was finalized by determining the melting point of the synthesized compound using the open capillary method.



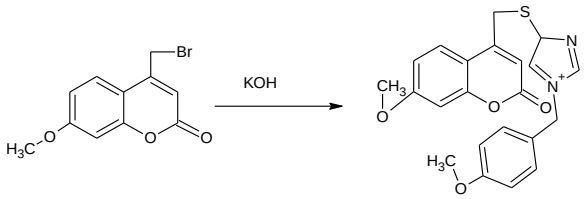
SYNTHESIS OF 4-[(1,3-BENZOTHIAZOL-2-YLOXY)METHYL]-7- METHOXY -2H-CHROMEN-2-ONE (2D)

0.01 mole of 4-(bromomethyl)-7- methoxy -2H-chromen-2-one was subjected to heating with 0.01 mole of 1,3-benzothiazol-2-ol in the presence of 5 ml of a 10% potassium hydroxide solution for a duration of 2 hours. Following the completion of the reaction, the mixture was cooled to room temperature, resulting in the precipitation of the intended compound. The title compound was then isolated by the addition of dilute hydrochloric acid. The synthesis was concluded by determining the melting point of the synthesized compound using the open capillary method.



Synthesis of 4-{[(7- methoxy -2-oxo-2H-chromen-4-yl)methyl]sulfanyl}-1-(4-methoxybenzyl)-4H-imidazol-1-ium (3a)

0.01 mole of 7- methoxy -4-(sulfanylmethyl)-2H-chromen-2-one was heated with 0.01 mole of 1-(4-methoxybenzyl)-4H-imidazol-1-ium in the presence of 5 ml of a 10% potassium hydroxide solution for a duration of 2 hours. After the completion of the reaction, the mixture was cooled to room temperature, leading to the precipitation of the desired compound. The title compound was then isolated by the addition of dilute hydrochloric acid. The synthesis was finalized by determining the melting point of the synthesized compound using the open capillary method.



Synthesis of 7- methoxy -4-{[(5-methyl-1H-benzimidazol-2-yl)sulfanyl]methyl}-2H-chromen-2-one (3b)

0.01 mole of 7- methoxy -4-(sulfanylmethyl)-2H-chromen-2-one was subjected to heating with 0.01 mole of 5-methyl-1H-benzimidazole-2-thiol in the presence of 5 ml of a 10% potassium hydroxide solution for a duration of 2 hours. Following the completion of the reaction, the mixture was cooled to room temperature, resulting in the precipitation of the intended compound. The title compound was then isolated by the addition of

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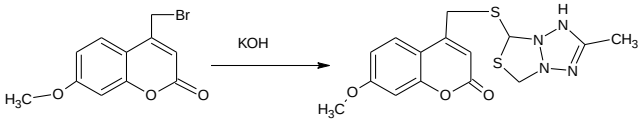
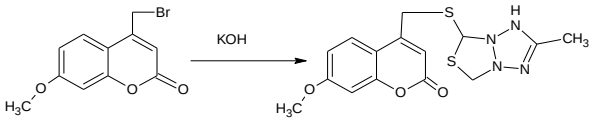
dilute hydrochloric acid. The synthesis was concluded by determining the melting point of the synthesized compound using the open capillary method.



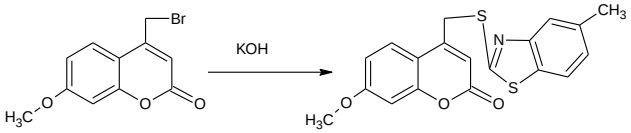
Synthesis Of 7- methoxy -4-{[(2-methyl-1H-[1,3,4]thiadiazolo[3,4-b]tetrazol-7-yl)sulfanyl]methyl}-2H-chromen-2-one (3c)

0.01 mole of 7- methoxy -4-(sulfanylmethyl)-2H-chromen-2-one was heated with 0.01 mole of 2-methyl-1H-[1,3,4]thiadiazolo[3,4-b]tetrazole-7-thiol in the presence of 5 ml of a 10% potassium hydroxide solution for a duration of 2 hours. After the completion of the reaction, the mixture was cooled to room temperature, leading to the precipitation of the desired compound. The title compound was then isolated by the addition of dilute hydrochloric acid. The synthesis was concluded by determining the melting point of the synthesized compound using the open capillary method.



Synthesis of 7- methoxy -4-{[(5-methyl-1,3-benzothiazol-2-yl)sulfanyl]methyl}-2H-chromen-2-one (3d)

0.01 mole of 7-methoxy-4-(sulfanylmethyl)-2H-chromen-2-one was heated with 0.01 mole of 5-methyl-1,3-benzothiazole-2-thiol in the presence of 5 ml of a 10% potassium hydroxide solution for a duration of 2 hours. Following the completion of the reaction, the mixture was cooled to room temperature, resulting in the precipitation of the intended compound. The title compound was then isolated by the addition of dilute hydrochloric acid. The synthesis was finalized by determining the melting point of the synthesized compound using the open capillary method.



In Vitro Anti-Cancer Activity

Cell lines:

MCF cell lines, representative of cervical cancer, were cultured in DMEM 10% PBS complete medium. This culture medium was enriched with 10% heat-inactivated fetal bovine serum and antibiotics. The cells were meticulously maintained at a temperature of 37°C in a 5% CO2 incubator, ensuring optimal conditions for cell growth and viability. To support the cell culture, the medium was consistently refreshed, contributing to a controlled and favorable environment for the propagation and experimentation involving MCF cervical cancer cell lines.

Trypan blue test/ dye exclusion test:

A cell suspension at a high concentration, approximately 10^6 cells/ml, was meticulously prepared. A clean haemocytometer slide was selected, and a cover slip was securely fixed in place. Subsequently, 100 µl of cell suspensions ranging from 0.5 to 2.0 × 10^5 cells/ml were carefully seeded into each well of a 96-well microtiter plate. The plated cells were then incubated at 37˚C, providing an optimal environment for cell attachment to occur. This controlled incubation period facilitated the establishment and adherence of cells within the wells, ensuring a consistent and reliable experimental setup.

4. Results

The title compounds were synthesized through a Pechmann condensation reaction, wherein Ethyl acetoacetate underwent a reaction with substituted aldehydes in the presence of sulfuric acid, resulting in the formation of Chromen derivatives. These Chromen derivatives were subsequently subjected to reactions with substituted Benzimidazole and Benzthiazole derivatives to yield the final compounds. After synthesis, the compounds were purified and underwent structural interpretation using FTIR, 1H-NMR, and MS analyses.

Following structural characterization, the derivatives were evaluated for their anti-cancer potential using MCF cell lines through the Trypan Blue assay. The in vitro anti-cancer activity was assessed by comparing the results with the standard drug, 5-Fluro-Uracil. Physicochemical data of the synthesized compounds were compiled in Table no. 01, providing essential information on the synthesized compounds.

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Additionally, Table no. 02 documented the in vitro anti-cancer activity data, shedding light on the efficacy of the compounds in comparison to the standard drug.

Table. No. 01: Physiochemical data of the synthesized compounds (2A-2D & 3a-3d)

Compd.code	Mole. Formula	Mole weight	M.P. (^{0}C)	Elemental analysis		
				C	H	N
3a	$C_{22}H_{21}N_2O_4S$	409.45	179-182	63.78	4.84	7.08
3b	$C_{19}H_{16}N_2O_3S$	352.38	168-172	63.89	4.17	8.28
3c	$C_{15}H_{16}N_4O_3S_2$	364.41	169-173	47.99	4.03	15.99
3d	$C_{19}H_{15}NO_3S_2$	369.43	176-179	60.83	3.69	3.94
2A	$C_{14}H_{11}N_3O_6$	317.23	168-172	51.49	2.99	13.86
2B	$C_{19}H_{16}N_2O_4$	336.32	165-167	67.08	4.38	8.69
2C	$C_{15}H_{14}N_4O_4S$	346.34	184-187	50.60	3.64	16.86
2D	$C_{18}H_{13}NO_4S$	339.35	176-17	62.76	3.41	4.31
			9			

SPECTRAL DATA

2A: FTIR (cm-1): 3245. 56 (-OH str.); 3210.23 (-NH str.); 3024.86 (Ar-CH str.); 2825.68 (-CH2 str.); 1768.74 (-C=O str.); 1556.79 (-C=N str.); 1234.89 (-C-N str.); 1024.56 (-C-O-C str.)**1H-NMR (ppm):** 11.5 (1H of –NH); 7.2 (-1H of chromen-4-one); 6.7-6.9 (3H of phenyl); 1.0-1.2 (2H of -CH2); 0.8-1.2 (3H of –CH3).

2B: FTIR (cm-1): 3235. 43 (-OH str.); 3226.45 (-NH str.); 3086.34 (Ar-CH str.); 2815.79 (-CH2 str.); 1785.35 (-C=O str.); 1548.69 (-C=N str.); 1245.95 (-C-N str.); 1034.85 (-C-O-C str.) : **1H-NMR (ppm):** 12.0 (1H of –NH); 7.4 (-1H of chromen-4-one); 6.8-7.0 (6H of phenyl); 1.0-1.2 (2H of -CH2) 0.8-1.2 (6H of –CH3)

2C: FTIR (cm-1): 3250. 43 (-OH str.); 3230.45 (-NH str.); 3045.34 (Ar-CH str.); 2830.79 (-CH2 str.);1787.35 (-C=O str.); 1558.69 (-C=N str.); 1265.95 (-C-N str.); 1039.85 (-C-O-C str.)**1H-NMR (ppm):** 12.0 (1H of –NH); 7.4 (-1H of chromen-4-one); 6.8-7.0 (3H of phenyl); 1.0-1.2 (2H of -CH2) ; 0.8-1.2 (6H of –CH3)

2D: FTIR (cm-1): 3235. 43 (-OH str.); 3226.45 (-NH str.); 3086.34 (Ar-CH str.); 2815.79 (-CH2 str.); 1785.35 (-C=O str.); 1548.69 (-C=N str.); 1245.95 (-C-N str.); 1034.85 (-C-O-C str.) **1H-NMR (ppm):** 12.0 (1H of –NH); 7.4 (1H of chromen-4-one); 6.8-7.0 (7H of phenyl); 1.0-1.2 (2H of -CH2);0.8-1.2 (3H of –CH3)

3a: FTIR (cm-1): 3245. 56 (-OH str.); 3210.23 (-NH str.); 3024.86 (Ar-CH str.); 2825.68 (-CH2 str.); 1768.74 (-C=O str.); 1556.79 (-C=N str.); 1234.89 (-C-N str.); 1024.56 (-C-O-C str.) 945.67 (-C-S-C str.) **1H-NMR (ppm):** 8.0-8.4 (3H of imidazole); 7.4 (-1H of chromen-4-one); 6.7-6.9 (7H of phenyl); 1.0-1.2 (4H of -CH2); 0.8-1.0 (6H of –CH3)

3b: FTIR (cm-1): 3235. 43 (-OH str.); 3226.45 (-NH str.); 3086.34 (Ar-CH str.); 2815.79 (-CH2 str.); 1785.35 (-C=O str.); 1548.69 (-C=N str.); 1245.95 (-C-N str.); 1034.85 (-C-O-C str.) 948.77 (-C-S-C str.) **1H-NMR (ppm):** 12.0 (1H of –NH); 7.4 (-1H of chromen-4-one); 6.8-7.0 (6H of phenyl); 1.0-1.2 (2H of -CH2)0.8-1.2 (6H of –CH3)

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3c: FTIR (cm-1): 3250. 43 (-OH str.); 3230.45 (-NH str.); 3045.34 (Ar-CH str.); 2830.79 (-CH2 str.); 1787.35 (-C=O str.); 1558.69 (-C=N str.); 1265.95 (-C-N str.); 1039.85 (-C-O-C str.) 953.57 (-C-S-C str.)**1H-NMR (ppm):** 12.0 (1H of –NH); 7.8 (1H of thiadiazolyl) 7.4 (-1H of chromen-4-one); 6.8-7.0 (3H of phenyl); 1.0-1.2 (2H of -CH2) 0.8-1.2 (6H of –CH3)

3d: FTIR (cm-1): 3235. 43 (-OH str.); 3226.45 (-NH str.); 3086.34 (Ar-CH str.); 2815.79 (-CH2 str.); 1785.35 (-C=O str.); 1548.69 (-C=N str.); 1245.95 (-C-N str.); 1034.85 (-C-O-C str.) 956.71 (-C-S-C str.) **1H-NMR (ppm):** 7.4 (1H of chromen-4-one); 6.8-7.0 (6H of phenyl); 1.0-1.2 (2H of -CH2); 0.8-1.0 (6H of –CH3)

Table no. 02: Anti-cancer activity of synthesized compounds on MCF cell lines (Cervical Cancer cells)

Compound code	% cytotoxicity	IC50 value
2A	23.48	13.96
2B	21.24	14.53
2C	27.69	16.20
2D	31.96	32.68
3a	21.87	17.75
3b	27.54	16.25
3c	31.65	14.95
3d	30.68	31.85
5FU	65.42	5.20

5. Discussion

Hydrophobic and hydrophilic scaffolds were individually synthesized and subsequently subjected to a reaction, resulting in the final compounds characterized as Coumarins. Given the diverse biological activities associated with coumarins, the synthesized compounds were further examined for their anti-cancer potential against cervical cancer cells. The assessment included a comparison with the standard drug, 5-Fluro-Uracil.

Remarkably, the synthesized compounds exhibited significant anti-cancer potential, surpassing the efficacy of the standard drug. This noteworthy finding suggests that, with some minor structural modifications, these agents could potentially be repurposed for the treatment of other types of cancers. The versatility of coumarins and their demonstrated anti-cancer properties underscore their potential as promising candidates for further exploration in cancer therapeutics.

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