SYNTHESIS AND ANTI-CANER EVALUATION OF 7-HYDROXY-4-{[(5-METHYL-1H-BENZIMIDAZOL-2-YL) SULFANYL] METHYL}-2H-CHROMEN-2-ONE DERIVATIVES

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Abstract

The plethora subscribed in this research has been directed towards the synthesis and anti-cancer evaluation of the novel substituted chromen derivatives. The title compounds were synthesized by using conventional synthesis. The structure of the synthesized compounds was established by using FTIR, 1H-NMR, and MS. The synthesized compounds were then evaluated for their anti-cancer potential by using MCF cell lines using Trypan Blue assay. The few compounds show promising anti-cancer activities as compared to the standard drug.

Keywords: Anti-cancer, Chromen-2-one, Benzimidazolyl, Derivatives, Pechmann condensation

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1.0 INTRODUCTION

Given that cancer is one of the leading causes of mortality, it poses a serious threat to public health. According to the International Agency for Research on Cancer, there will be 19.3 million new cases of cancer worldwide in 2020, with colorectal, lung, and female breast cancer accounting for roughly 10% of the total cases each. [1] Additionally, according to the same analysis, 10.0 million fatalities related to cancer will occur worldwide in 2020. [2] Despite significant advancements in targeted therapies for recurrent or refractory tumours, such as immunotherapy, gene therapy, and small molecule medicines, many of them still lack efficient treatment modalities. Metastasis, a significant factor in poor prognosis, also poses a challenge to current treatments and prevents the prevention of cancer progression, despite the fact that the underlying mechanisms are not fully understood. [3] Therefore, it continues to be difficult to increase the effectiveness of therapeutic regimens, and it is urgently necessary to create new treatment alternatives to stop the spread of cancer.[4]

Because of their harmful side effects, chemo-therapy medications are occasionally dreaded. Their job is to slow down a cancer's growth and, ideally, stop it completely. The usage of the most popular anticancer medications has three objectives [5]. Chemotherapeutic medications (which target rapidly dividing cells) function by preventing mitosis. as these medications They are referred to as cytotoxic when they cause cell damage. They halt mitosis through a variety of strategies, such as disrupting the cancer cells' DNA and preventing the creation of DNA strands necessary for cell division [6]. Research on numerous types of cancer has recently included immunotherapeutics and molecularly targeted therapies. Resistance to medications is quickly growing to be a significant global issue. One of the most crucial areas of study today is the requirement to create new chemicals to deal with resistance [7]. A unique strategy that frequently produces synergistic activity and increased drug efficacy is the hybridization of two or more bioactive drug fragments with complimentary functionalities or different mechanisms of action into a single molecule. This isatin-based dual action/hybrid pharmacophore approach was first reported by Solomon et al. [8].

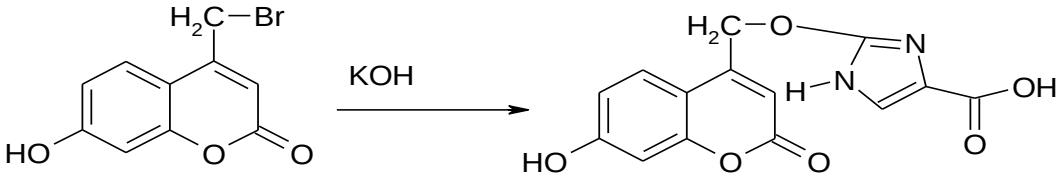
The most prevalent naturally occurring secondary metabolite is coumarin , which may be found in a variety of plant families, essential oils, microorganisms, and a few animal species. In addition to treating cancer, coumarin is also useful for treating radiation side effects. The molecular characteristics of coumarin have been shown to have specific biological impacts on the cellular environment [9]. In the instance of melanoma, coumarin has been proven to be an effective maintenance therapy. It has also been discovered to prevent tumour growth and to have a wide range of biological activities, including those that are anti-viral, anti-tumor, anti-HIV, antimicrobial, and antioxidant. The use of coumarin in the treatment of leukaemia, prostate cancer, and renal cell carcinoma is crucial [10]. Different cancer situations can be effectively treated with coumarin derivatives such benzopyrones, isoflavones, furanocoumarin, and pyranocoumarin.

2.0 EXPERIMENTAL

Using the capillary approach, melting points were uncorrectedly recorded on melting point apparatus. On a Bruker alpha-E FTIR-ATR, all of the IR spectra of the synthetic compounds were captured. 1HNMR spectra were captured using a Bruker Avance II (400MHz) spectrometer with CDCl3 and DMSO as solvents. TMS was used as the standard. Thin layer chromatography is used to monitor the reaction on pre-coated (Merck 60F254) and home-made silica gel coated plates. Chloroform and methanol were utilised as the solvent system for creating the chromatogram in various ratios. TLC spots were observed using UV chambers.

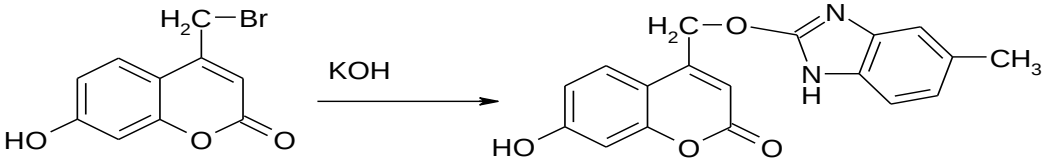
SYNTHESIS OF 2-[(7-HYDROXY-2-OXO-2H-CHROMEN-4-YL) METHOXY]-1H-IMIDA ZOLE-5-CARBOXYLIC ACID (2A)

0.01 mole of 4-(bromomethyl)-7-hydroxy-2H-chromen-2-one is heated with 0.01 mole of 2-hydroxy-1H-imidazole-5-carboxylic acid in presence of 5 ml of 10% potassium hydroxide solution for 2 hours. Cooled to room temperature. Precipitate out title compound with addition of dilute hydrochloric acid. Melting point of the synthesized compound was recorded with open capillary method.



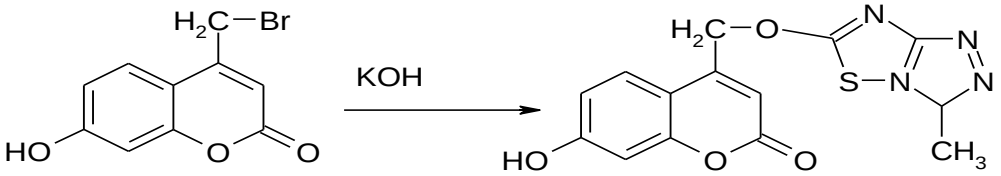
SYNTHESIS OF 7-HYDROXY-4-{[(5-METHYL-1H-BENZIMIDAZOL-2-YL)OXY] METHYL}-2H-CHROMEN-2-ONE (2B)

0.01 mole of 4-(bromomethyl)-7-hydroxy-2H-chromen-2-one is heated with 0.01 mole of 5-methyl-1H-benzimidazol-2-ol in presence of 5 ml of 10% potassium hydroxide solution for 2 hours. Cooled to room temperature. Precipitate out title compound with addition of dilute hydrochloric acid. Melting point of the synthesized compound was recorded with open capillary method.



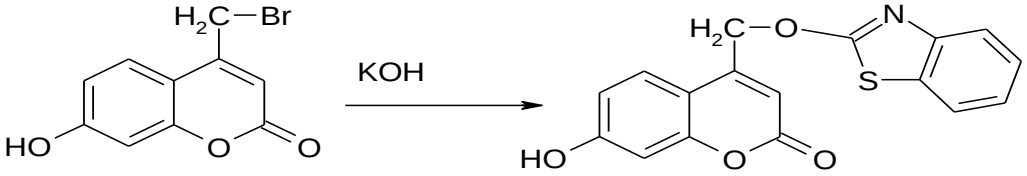
SYNTHESIS OF 7-HYDROXY-4-{[(3METHYL-3,7A-DIHYDRO[1,2,4]TRIAZOLO[4,3-B][1,2,4]THIADIAZOL-6-YL)OXY]METHYL}-2H-CHROMEN-2-ONE (2C)

0.01 mole of 4-(bromomethyl)-7-hydroxy-2H-chromen-2-one is heated with 0.01 mole of 2-hydroxy-1H-imidazole-5-carboxylic acid in presence of 5 ml of 10% potassium hydroxide solution for 2 hours. Cooled to room temperature. Precipitate out title compound with addition of dilute hydrochloric acid. Melting point of the synthesized compound was recorded with open capillary method.



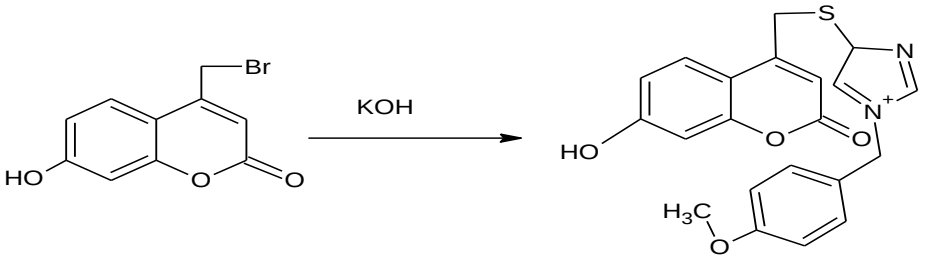
SYNTHESIS OF 4-[(1,3-BENZOTHIAZOL-2-YLOXY)METHYL]-7-HYDROXY-2H-CHROMEN-2-ONE (2D)

0.01 mole of 4-(bromomethyl)-7-hydroxy-2H-chromen-2-one is heated with 0.01 mole of 1,3-benzothiazol-2-ol in presence of 5 ml of 10% potassium hydroxide solution for 2 hours. Cooled to room temperature. Precipitate out title compound with addition of dilute hydrochloric acid. Melting point of the synthesized compound was recorded with open capillary method.



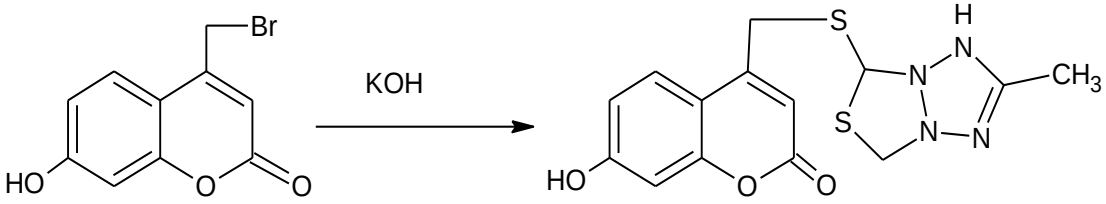
SYNTHESIS OF 4-{[(7-hydroxy-2-oxo-2H chromen-4-yl)methyl]sulfanyl}-1-(4-methoxybenzyl)-4H-imidazol-1-ium (3a)

0.01 mole 7-hydroxy-4-(sulfanylmethyl)-2H-chromen-2-one is heated with 0.01 mole of 1-(4-methoxybenzyl)-4H-imidazol-1-ium in presence of 5 ml of 10% potassium hydroxide solution for 2 hours. Cooled to room temperature. Precipitate out title compound with addition of dilute hydrochloric acid. Melting point of the synthesized compound was recorded with open capillary method.



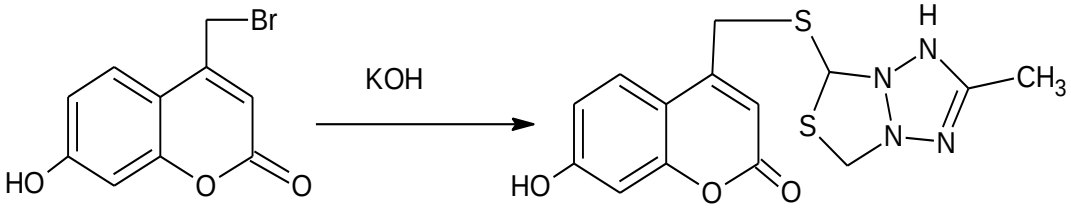
SYNTHESIS OF 7-hydroxy-4-{[(5-methyl-1H-benzimidazol-2-yl)sulfanyl]methyl}-2H-chromen-2-one (3b)

0.01 mole of 7-hydroxy-4-(sulfanylmethyl)-2H-chromen-2-one is heated with 0.01 mole of 5-methyl-1H-benzimidazole-2-thiol in presence of 5 ml of 10% potassium hydroxide solution for 2 hours. Cooled to room temperature. Precipitate out title compound with addition of dilute hydrochloric acid. Melting point of the synthesized compound was recorded with open capillary method.



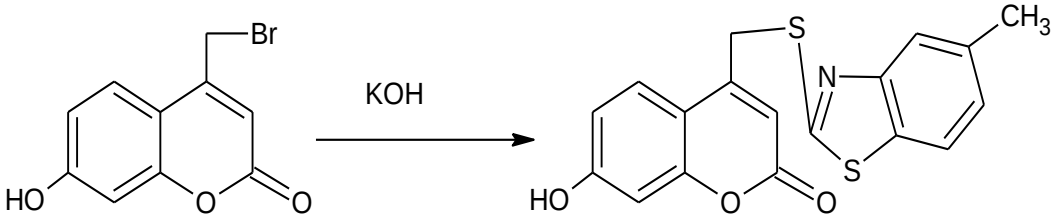
SYNTHESIS OF 7-hydroxy-4-{[(2-methyl-1H-[1,3,4]thiadiazolo[3,4-b]tetrazol-7-yl)sulfanyl]methyl}-2H-chromen-2-one (3c)

0.01 mole of 7-hydroxy-4-(sulfanylmethyl)-2H-chromen-2-one is heated with 0.01 mole of 2-methyl-1H-[1,3,4]thiadiazolo[3,4-b]tetrazole-7-thiol in presence of 5 ml of 10% potassium hydroxide solution for 2 hours. Cooled to room temperature. Precipitate out title compound with addition of dilute hydrochloric acid. Melting point of the synthesized compound was recorded with open capillary method.



SYNTHESIS OF 7-hydroxy-4-{[(5-methyl-1,3-benzothiazol-2-yl)sulfanyl]methyl}-2H-chromen-2-one (3d)

0.01 mole of 7-hydroxy-4-(sulfanylmethyl)-2H-chromen-2-one is heated with 0.01 mole of 5-methyl-1,3-benzothiazole-2-thiol in presence of 5 ml of 10% potassium hydroxide solution for 2 hours. Cooled to room temperature. Precipitate out title compound with addition of dilute hydrochloric acid. Melting point of the synthesized compound was recorded with open capillary method.



3.0 IN VITRO ANTI-CANCER ACTIVITY CELL LINES:

MCF (cervical cancer) Cell lines .The MCF cells were cultured in DMEM 10% PBS complete medium. The medium was supplemented with 10% heat inactivated fetal bovine serum, antibiotics. The cell lines were maintained at 37° C in a 5% CO2 incubator and the media were changed frequently.

TRYPAN BLUE TEST/ DYE EXCLUSION TEST:

Cell suspension at a high concentration (approx106 cells/ml) was prepared. Clean haemocytometer slide was taken and cover slip was fixed in place. 100 µl /well of cell suspensions (0.5-2.0×105 cells/ml) were seeded in 96 well micro titer plates and incubated at 37˚C to allow for cell attachment

4.0 RESULT AND DISCUSSION

The title compounds were synthesised by using Pechmann condensation reaction In which Ethyl acetoacetate reacts with substituted aldehydes in presence of sulfuric acid to offer Chromen derivatives. These chromen derivatives are then reacted with substituted Benzimidazole, Benzthia zole derivatives to obtain final compounds. The synthesized compounds were then purified and subjected for structural interpretation by using FTIR,1H-NMR and MS analysis.

Theses derivatives are then subjected for their anti-cancer potential using MCF ell lines using Trypan Blue assay. The in vitro anti cancer potential is evaluated by using the standard drug 5-Fluro-Uracil.The physicochemical data of the synthesized compounds was reported in Table no. 01 and In vitro anti-cancer activity data was reported in Table no. 02.

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	$C_{21}H_{19}N_2O_4S$	395.45	179-182	63.78	4.84	7.08
3b	C_{18}H14N2O3S	338.38	168-172	63.89	4.17	8.28
3c	C14H14N4O3S2	350.41	169-173	47.99	4.03	15.99
3d	C18H13NO3S2	355.43	176-179	60.83	3.69	3.94
2A	C13H9N3O6	303.23	168-172	51.49	2.99	13.86
2B	C18H14N2O4	322.32	165-167	67.08	4.38	8.69
2C	C14H12N4O4S	332.34	184-187	50.60	3.64	16.86
2D	C17H11NO4S	325.35	176-179	62.76	3.41	4.31

5.0 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); 5.0 (2H of –OH); 1.0-1.2 (2H of -CH2)

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); 5.0 (1H of –OH); 1.0-1.2 (2H of -CH2) 0.8-1.2 (3H 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); 5.0 (1H of –OH); 1.0-1.2 (2H of -CH2) ; 0.8-1.2 (3H 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); 5.0 (1H of –OH); 1.0-1.2 (2H of -CH2)

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); 5.0 (1H of –OH); 1.0-1.2 (4H of -CH2); 0.8-1.0 (3H 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); 5.0 (1H of –OH); 1.0-1.2 (2H of -CH2)0.8-1.2 (3H of –CH3)

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); 5.0 (1H of –OH); 1.0-1.2 (2H of -CH2) 0.8-1.2 (3H 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); 5.0 (1H of –OH); 1.0-1.2 (2H of -CH2); 0.8-1.0 (3H of –CH3)

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

Compound code	% cytotoxicity	IC50 value
2A	21.45	14.68
2B	24.58	13.57
2C	28.73	15.79
2D	32.43	33.75
3a	20.55	16.68
3b	26.28	14.42
3c	30.08	15.73
3d	33.64	32.86
5FU	66.54	4.76

6.0 CONCLUSION

The hydrophobic and hydrophilic scaffolds were synthesized and then reacted with each other to obtain the final compounds of Coumarins. As coumarins have wide variety of biological activities. Thus the synthesized compounds were then tested for their anti-cancer potential against cervical cancer cells using 5-Fluro-Uracil as a standard drug. The compounds show significant anti-cancer potential as compared to that of the standard drug. These agents with some minor structural modifications can be utilized for treatment of other types of cancers.

7.0 REFERENCES

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