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Synthesis, characterization and anticancer activity of new oxazepien derivative compounds

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Abstract

Colon cancer starts in the colon or rectum, usually as tiny, noncancerous cell groupings called polyps that form along the inner lining. There are several factors to consider that may influence the development, diagnosis, and treatment of the disease. Over time, some of these polyps can become cancerous. Compounds as Oxazepine derivatives of coumarin acid designed and synthesized in order to develop a lead molecule that demonstrates anti-cancer efficacy and the side effects of NSAIDs, including GI toxicity. Oxazepien derivative compounds diagnosed using FT-IR and 1HNMR. After that evaluate the cytotoxic effects of Oxazepine derivatives on investigated in vitro versus the human colon cell line CaCo-2 with normal human liver cell line WRL68. Effective of the test substances on cell growth was assessed by utilizing the MTT assay method after treating the cell line with various doses (1,5 ,10 ,15 ,20,25 ,50 and 75µM) for 24hours. The results showed that the most active compound against cancer cells 42.12±1.28 was 5b at a concentration of 75 µM and IC_{50} = 39.6 µM. It also has high selectivity and has a low toxicity rate when tested against normal cells WRL68 75.53±0.77. The current study showed the highly effective action of oxazepien derivative compounds and can be used in the management and treatment of cancer.

Keywords: Oxazepine, coumarin acid, FT-IR, 1HNMR anticancer activity, MTT assay.

Introduction

There is a class of phenolic compounds derived from cinnamic acid found in some types of plants, especially those suitable for food, medicine, and spices from different plant families. These compounds are known as benzopyrones or coumarins [1]. Coumarins are divided into four different sections: simple coumarins, furanocoumarins, pyranocumarins, and pyrone-substituted coumarins. Simple coumarin compounds are composed of molecules that have the ability to replace hydroxyl, alkoxyl, and alkyl in the basic structure, and glucosides are formed from them. There are many studies that have shown the

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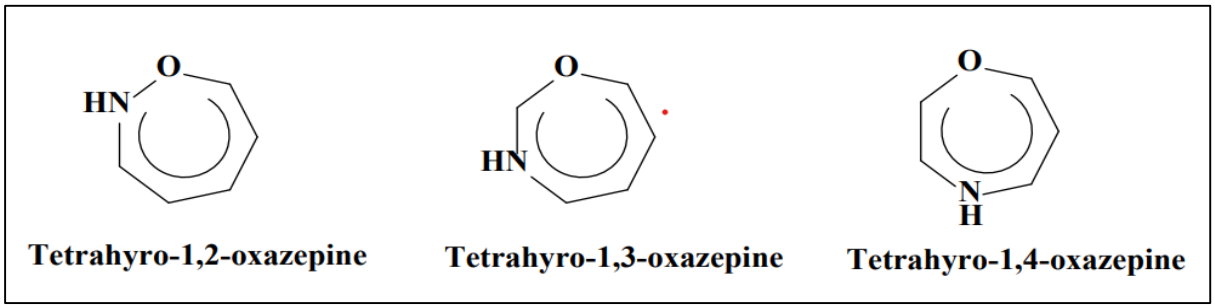
importance of coumarins in terms of their various biological activities in many fields due to their possession of antimicrobial, antioxidant, anticoagulant, and anticancer properties. [2].

Oxazepan is one of the heterogeneous cyclic compounds, as it consists of seven members linked to nitrogen and oxygen. Although it is less common, its importance has emerged due to its various pharmaceutical applications. Combining oxazepans and coumarins may lead to the development of new compounds with enhanced biological activities [3]. (Scheme 1).



Scheme1. Oxazepine compounds isomers,1,2-1,3, and1,4-oxazepine (Gomha et-al 2016), which are numbered based on where oxygen & nitrogen atoms are located on heptagonal ring.

Importance of 1,3-oxazine compounds attached to coumarins has been synthesized and explored due to their strong antimicrobial and antioxidant properties [4]. Modern 1,3-oxazine compounds attached to coumarins have contributed to the development of the therapeutic journey for many diseases. Using the MTT test technique on different 1,3-oxazine compounds attached to coumarin derivatives to evaluate their cytotoxicity and whether they have anticancer activity, their effective effect was demonstrated [5]. Coumarin derivatives have shown promising effects in the treatment of colon cancer. These compounds exhibit different mechanisms of action, including inducing apoptosis, inhibiting cell proliferation, and disrupting the cell cycle. Coumarin derivatives were found to inhibit the proliferation of colon cancer cells. For example, some coumarin-based compounds have shown anti-proliferation activity against colon cancer cell lines, inducing apoptosis, reducing tumor growth, and preventing angiogenesis, in which new blood vessels form to supply tumors with nutrients. [6] Through the process, tumor cells are effectively starved and their growth is inhibited by the synthesized compounds. These derivatives also possess potent antioxidant properties. By neutralizing free radicals and reducing oxidative stress, these compounds can protect cells from DNA damage and other harmful effects that contribute to the development of cancer.[7] Promising use in treating cancer, it may help mitigate the ad verse reactions of radiation [8]. The incorporation of coumarin into hybrid structures leads to a major treatment for cancer diseases due to the ability of the compound to destroy tumor cells, as shown by many studies. [9] Therefore, there is an urgent need to manufacture and study healthy, potent and highly selective antitumor drugs to suit tissues with a unique spectrum of activity, due to the emergence of resistance to treatment and the appearance of adverse reactions and the healing and recurrence of tumors. The similar action of the drug compound of molecular hybrids has made it particularly successful with scientists and researchers. In the last ten to fifteen years, many prepared anticancer drugs based on molecular hybridization have been identified [10].

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Materials and Methods

General Synthesis Procedure of 2-oxo 2H-chromene-3-carbohydrazid (2)[11]

The mixture of solution ethyl 2-oxo-2H-chromene-3-carboxylate (2.18 g, 10mmol) with hydrazine hydrate solution (purity 99%, 1.3 g, 25mmol) in 100% absolute ethanol (50 mL) under reflux for 6 hours at 80°C. Result precipitate that filtrate than washed with ethanol, and dried. Recrystallization from ethanol yields yellow color powder. precipitate, m.p. 206-207°C, 88 % yield.

General Synthesis Procedure of synthesis 3-(4-amino-5-mercapto-4H-1,2,4-triazol-3-yl)-2H-chromen-2-one (3)[12].

KOH (0.63gm, 100ml) was added in pure ethanol (0.200 L). Then, add 2-oxo-2H-chromene-3-carbohydrazide, CS_2 (0.15M) with slight amounts while stirring constantly, and hydrazine hydrate (15 ml, 0.3 M) and reflux for 7 hours in intermittent shaking. The product reaction was precipitate and filtered, extensively use cold water to wash it, then recrystallize it with ethanol. at 220-221°C, yielding 81%.

General Synthesis Procedure of 3-(4-bezylideneamino-5-mercapto-4H-1,2,4-triazol-3-yl)-2H-chromen-2-one (4)[13]

To a mixed of 4-subs. benzyl aldehyde (13.7g, 0.1mol) and 3-(4-amino-5-mercapto-4H-1,2,4-triazol-3-yl)-2H-chromen-2-one (11.2g, 0.1mol) in absolute ethanol (200mL), a few drops of glacial acetic acid were added at room temperature. There action mixture was stirred at 80°C for 5hrs. After cooling, the solid product was filtered and recrystallized from ethanol to yield colored compounds, m.p. 159-162°C, 90 % yield.

General Synthesis Procedure of 3,9-bis(4-hydroxyphenyl)-4,10-bis(3-mercapto-5-(2-oxo-2H-chromen-3-yl)-4H-1,2,4-triazol-4-yl)-9,10-dihydro-1H,3H-benzo[1,2-e:4,5-e'] bis ([1,3] oxazepine)-1,5,7,11(4H)-tetraone[14].

Oxazipen was synthesized from the reaction of Schiff base with phthalic anhydride. 2 mmole from prepared Schiff bases reacted with (1 mmoles, 0.0296 gm) of phthalic anhydride in 20 mL dry benzene was refluxed in a water bath at (85°C) for (7-9 hrs) by the reaction of cycloaddition, as shown in Scheme 1. After that, the product kept to cool at the room temperature than filtered to recrystallized.

MTT Assay:

The anticancer efficacy of CoCa-2cell line normal hepatic human cell line WRL 68 was evaluated. Initially, 200 µL/well of cells (106 cells/mL) were prepared and cultured in a 96-well tissue culture plate. Different concentrations of the test solution were prepared to evaluate the cytotoxic effect against the two cell lines examined (1-5-10-15-20-25-50-75 µM) using deionized water. Then, 100 µL of different concentrations were added to each well and incubated at 37 °C for 24 h. After the incubation period, 10 µL of MTT solution (5 mg/mL) was added to each well and incubated at 37 °C for 4 h. Finally, 50 µL of DMSO (dimethyl sulfoxide) was added to each well and incubated for 10 min only. The prepared WRL 68 and CoCa-2 cells were cultured in complete medium. Absorbance of each well was measured at 620 nm using

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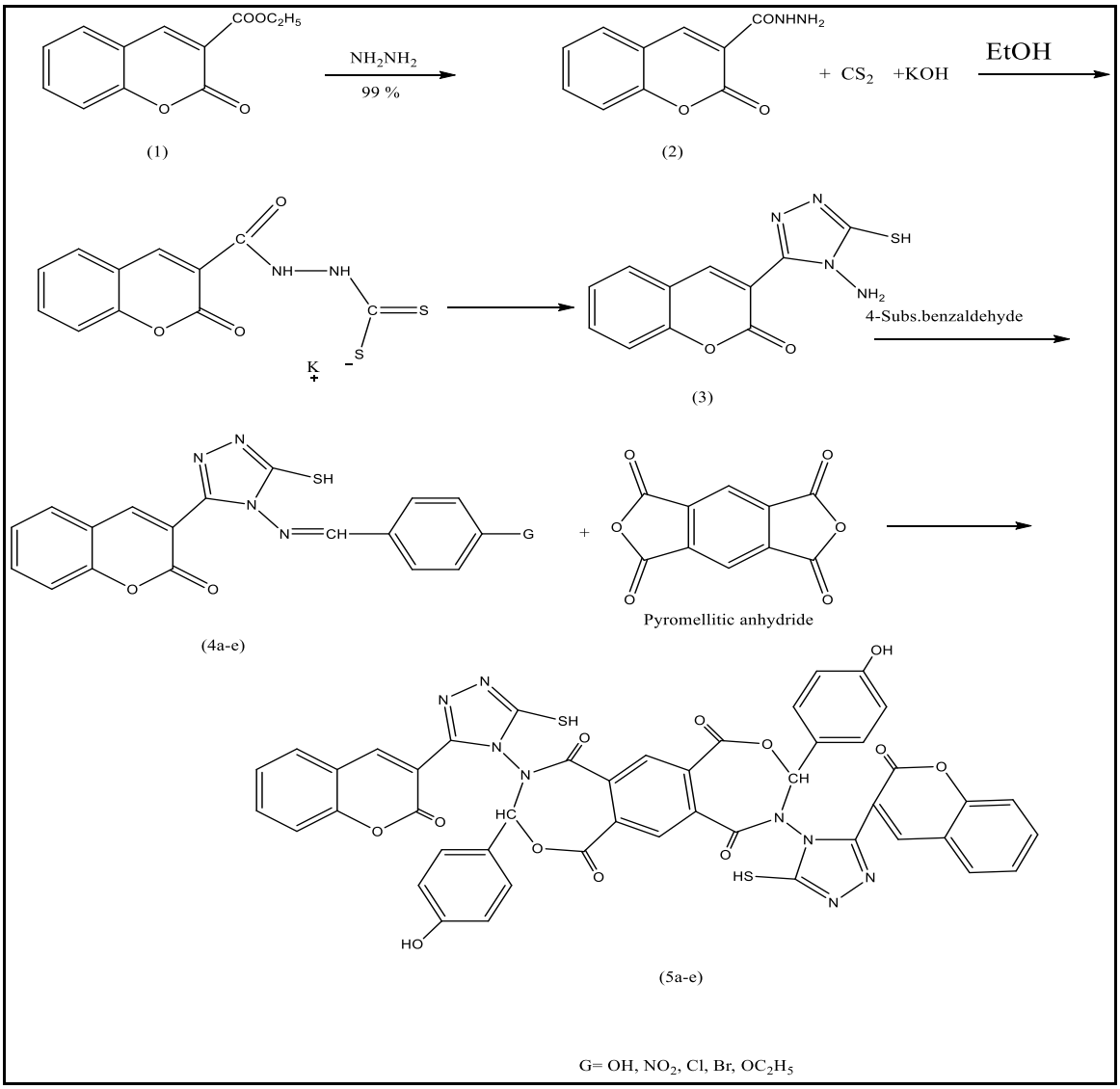
an ELISA reader. Only live cells were able to take up the dye while dead cells were not removed. IC_{50} values were calculated from the percent (%) of control. [15]

Statistical analysis:

The studies were completed in triplicate, and all data are presented as means ± standard deviation. The data was analyzed using one-way ANOVA in Prism Pad Graph version 16.0 software, and individual comparisons were made using Tukey's method. P-values < 0.05 were considered statistically significant.



Scheme.1: Synthetic steps for synthesis of 3,9-bis(4-hydroxyphenyl)-4,10-bis(3-mercapto-5-(2-oxo-2H-chromen-3-yl)-4H-1,2,4-triazol-4-yl)-9,10-dihydro-1H,3H-benzo[1,2-e:4,5-e']bis([1,3]oxazepine)-1,5,7,11(4H)-tetraone (5a-e)

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Results and Discussion

Spectral Data

The structure synthesized compounds were confirmed by FT-IR, ^{1}HNMR. FT-IR spectra showed the appearance of aliphatic C – H stretching at 2956 and 2874 cm^{-1} for asymmetrical and symmetrical stretching, respectively. Bands at 3054, 1632, 1705 and 834 cm-1 for aromatic C – H, azomethane CH = N [15], lactone carbonyl C = O stretching [16], and out of plane bending for *p*-disubstituted benzene ring. Many specific bands and their significance have been described in table (1).

Table (1).FT-IR spectral data for a series of compounds with different alkyl groups attached 5a-c.

Comp. No.	Alkyl Group	FT-IR Spectral information, cm^{-1}				
		νC=O Lactone	ν C-H (Aromatic)	ν C-H (Aliphatic)	ν C=C (Aromatic)	Others
5a	Cl	1706	3081	2936, 2886	1593	-
5b	Br	1696	3039	2939, 2832	1600	-
5c	OCH_3	1688	3055	2971, 2858	1599	1249 (C – O)

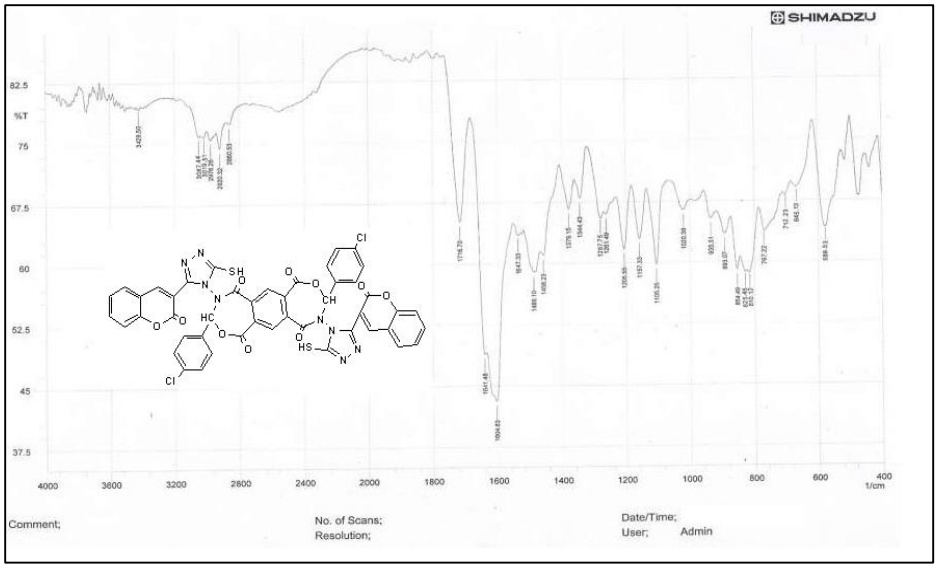

Figure (1). FT-IR spectrum of compound (5a).

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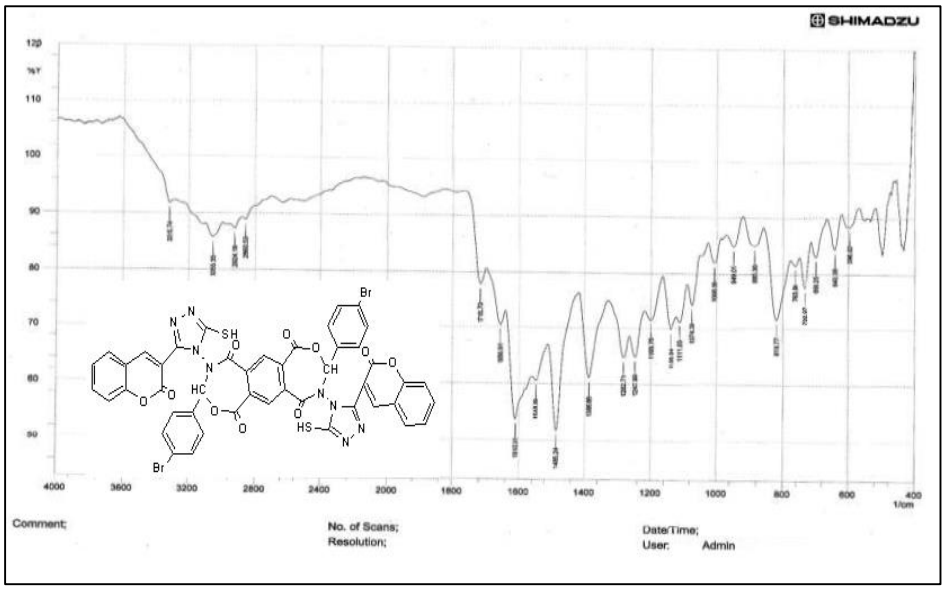

Figure (2). FT-IR spectrum of compound (5b)

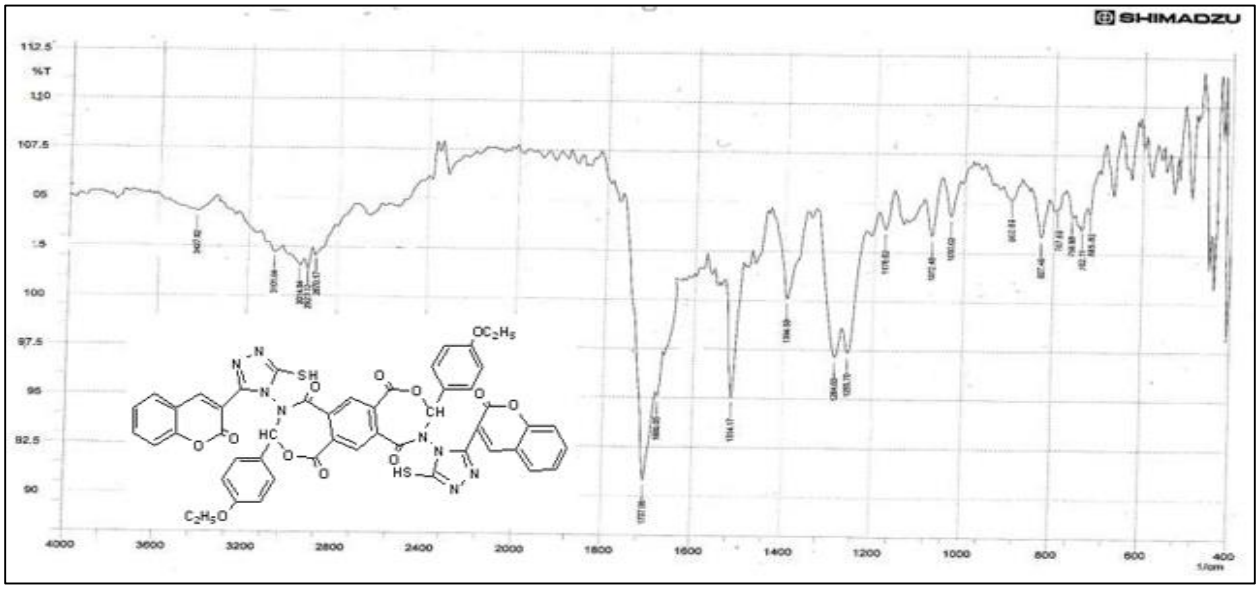

Figure (3). FT-IR spectrum of (5c).

The ^{1}H NMR spectra (μHz, Dimethyl sulfoxide **DMSO**-d6) of the 5c molecule revealed the following signals in δ (ppm): The signal at δ: (13.85) ppm corresponds to thio protons (s, SH, 2H) in triazole rings. The signal at (2.412-2.569) ppm is ascribed to the Dimethyl sulfoxide **DMSO** solvent. The singlet signal at 2.889 ppm corresponds to methyl group protons (6H, 2×CH3). The signal at (3.512-3.541) ppm is due to H_2O in dimethyl sulfoxide (**DMSO**). The signals of aromatic protons and protons of (C-H) groups inside oxazepine rings were examined in the range of (6.985 -8.541 ppm).

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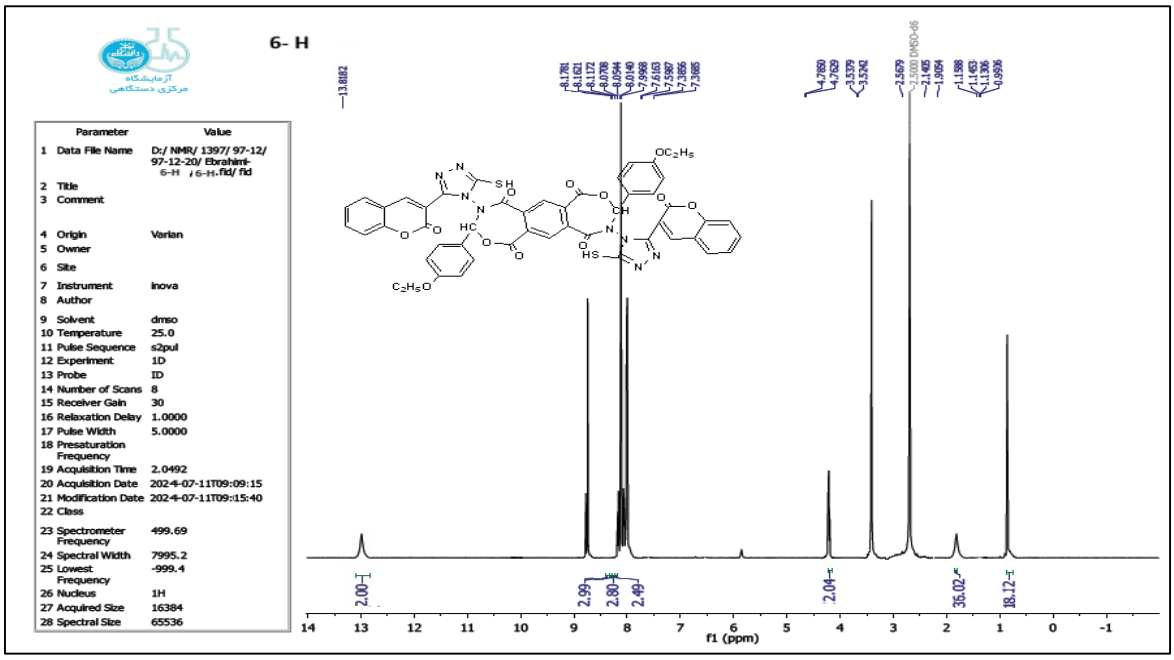

Figure (4). 1HNMR spectrum of (5c).

Cytotoxic effect of Compounds

The compounds were investigated in vitro compared between the human colon cell line CaCo-2 and the normal human liver cell line WRL68. Effective of compounds that tested on the cell line was treated with varying concentrations and the MTT assay method was employed to determine the results (1,5 ,10 ,15 ,20,25 ,50 and 75µM) for 24hours.

The cytotoxicity activity level of compound 5b was higher than compound 5a&5c. The selectivity of compound 5a was recorded as having the IC_{50} value against CaCo-2 cells (74.9 µM) and a relatively IC_{50} value against WRL68 cells (81.1µM), presented in table 2 and fig.5

Table (2). Effect of the compounds (5a) on CaCo-2 and WRL68 cell proliferation at various concentrations (µM/ml) cell viability data (Mean ± SEM)

Concentrations (µM/ml)	WRL68 (Mean±SEM)	CaCo-2 (Mean±SEM)
75	96.37±0.37	94.02±0.94
50	96.41±0.57	94.09±0.61
25	95.79±0.48	93.24±0.29
15	94.25±0.37	88.27±1.94
10	93.5±1.35	86.58±1.1
5	92.9±1.12	79.16±0.9
1	87.03±0.91	75.27±2.12

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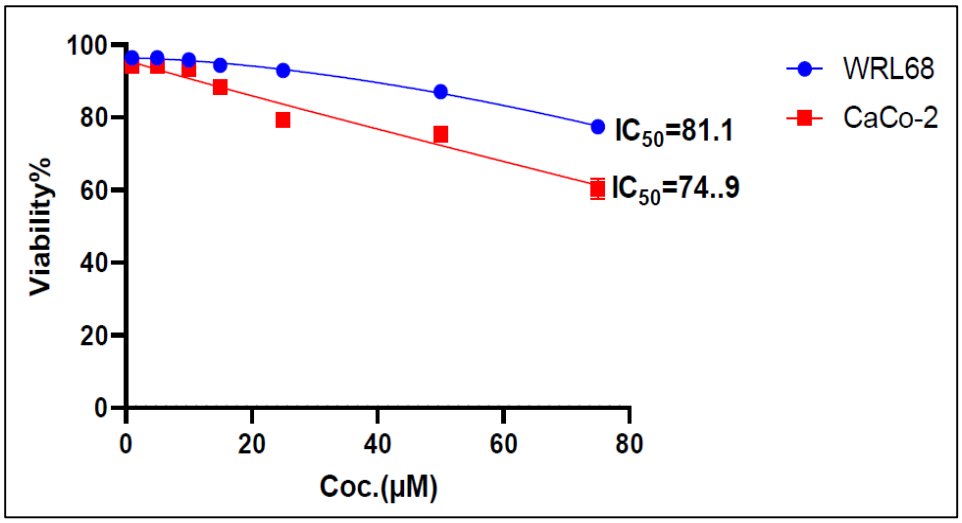

Figure (5). The distribution of IC_{50} values for compounds 5a against CaCo-2 and WRL68 cell lines

The compound 5b was recorded as having the IC_{50}value against CaCo-2 cells (24.53 µM) and a relatively IC_{50} value against WRL68 cells (39.6µM), indicating a good level of selectivity toward human cancer cells and making it a candidate anticancer agent presented in table 3 and Figure.6

Table (3). Effect of the compounds (5b) on CaCo-2 and WRL68 cell proliferation at various concentrations (µM/ml) cell viability data (Mean ± SEM)

Concentrations (µM/ml)	WRL68 (Mean±SEM)	CaCo-2 (Mean±SEM)
75	95.17±0.66	93.9±0.24
50	95.17±0.29	93.01±0.29
25	94.98±0.17	88.88±1.79
15	94.05±0.06	76.42±2.52
10	93.12±0.96	73.35±2.33
5	92.51±0.77	65.70±2.48
1	87.96±2.51	50.34±2.02

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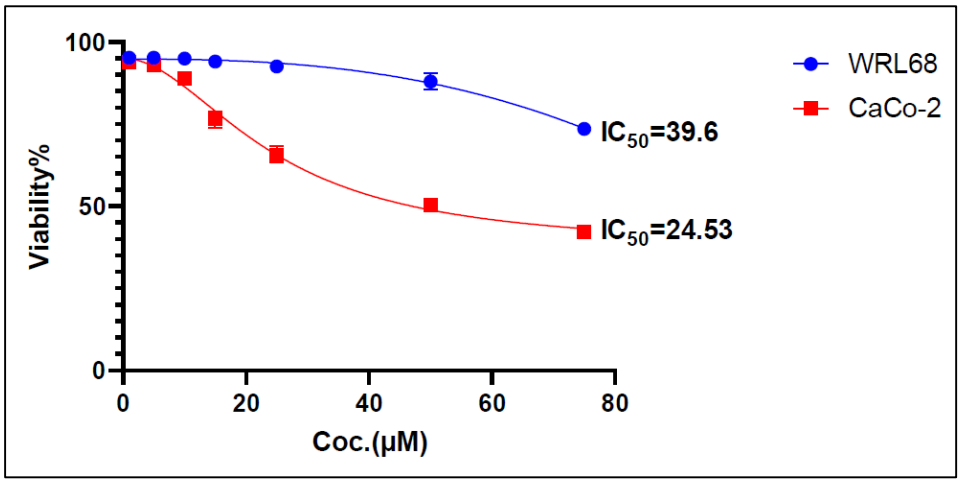

Figure (6). The distribution of IC_{50} values for compounds 5b against CaCo-2 and WRL68 cell lines

The compound 5c was recorded as having the IC_{50} value against CaCo-2 cells (40.66 µM) and a relatively IC_{50} value against WRL68 cells (50.48µM), it showed little effect against cancer cells, presented in table 4 and fig.7

Table (4). Effect of the compounds (5c) on CaCo-2 and WRL68 cell proliferation at various concentrations (µM/ml) cell viability data (Mean ± SEM)

Concentrations (µM/ml)	WRL68 (Mean±SEM)	CaCo-2 (Mean±SEM)
75	96.75±0.72	96.10±0.54
50	96.25±0.69	95.83±0.41
25	96.02±0.65	95.56±0.24
15	95.71±0.53	95.06±0.63
10	95.97±0.59	95.43±0.54
5	96.02±0.65	95.56±0.24
1	96.25±0.69	95.83±0.41

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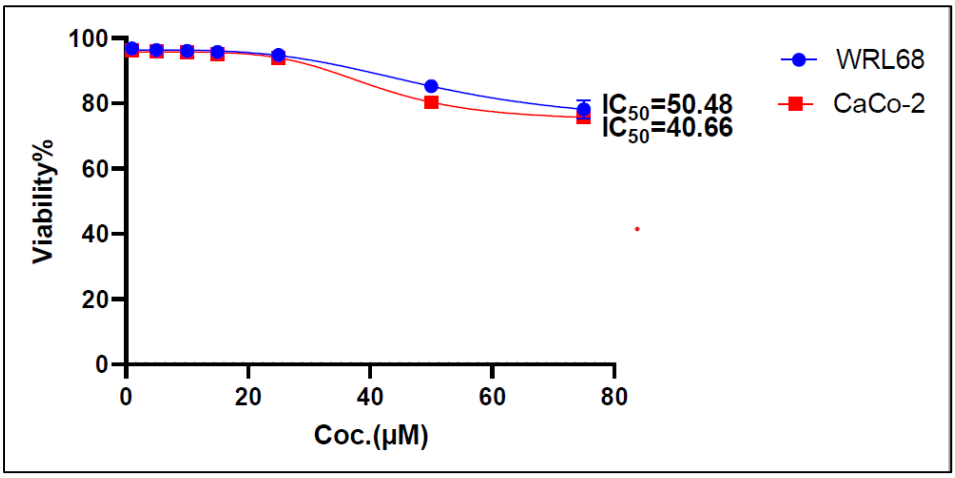

Figure (7). The distribution of IC_{50} values for compounds 5c against CaCo-2 and WRL68 cell lines

Many studies have focused on programmed as a key mechanism in tests of cell death in both cancerous and healthy cells. Even so, in cancer cells with a higher rate of mutation, malfunctioning signaling pathways may prevent planned cell death, which can occasionally result in therapy failure. According to a number of in vitro investigations, coumarin derivatives have potent anti-proliferation and cytotoxic actions on cancer cells and are highly selective for them [16,17]. Certain cancer cell types have been studied in vitro, such as human breast solid tumor lines, prostate cancer, pancreatic cancer, colon cancer, thyroid cancer, liver cancer, human lymphoma cell lines, lung cancer cell lines, and multidrug-resistant human thyroid cancer. [18,19]

Conclusions

The oxazepan derivatives synthesized according to the synthetic scheme under suitable experimental conditions from coumarin acid and characterized by FTIR & 1HNMR were sensitive to cancer cell lines, with IC_{50} values ranging from24.53 to 74.9 μM. Compounds 5b are highly selective for cancer cell inhibition at 75 μM compared to other compounds.

Acknowledgments

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Author's Declaration

- We hereby confirm that all the Figures and Tables in the manuscript are original and have been created by us.
- We have obtained ethical clearance for our study from the local ethical committee at [Al-Nahrain University/College of Science]. This approval underscores our commitment to ethical research practices and the well-being of our participants.

- Ethical Clearance: The project was approved by the local ethical committee at [Al-Nahrain University/College of Science], ensuring adherence to ethical standards and the protection of participants' rights and welfare.

Author's Contribution Statement

Hind S. Sdeeq: Contributed to the conception and design of the study, conducted some experiments, data rearrangement and drafted the initial manuscript.

Nasreen R. Jber: conducted some experiments, collection a part of literature review and conducted some characteristics of the products.

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