Cancer Research

Iraqi Journal of Cancer and Medical Genetics

Synthesis, characterization and cytotoxic effect of 2-((E)-((E)-2-(5-chloro-3,3-dimethylindolin-2-ylidene)-3-oxopropylidene) amino) benzoic acid on the MCF7 cancer cell line

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Keywords:

Aldehyde,
Aniline substituted,
Cytotoxic activity,
Heterocyclic compounds and
Schiff bases

Abstract

Indole Schiff base derivatives have attracted considerable interest in the field of medicinal chemistry because of their broad range of biological activities. This study aimed to synthesize a new indole Schiff base derivative, and its cytotoxic activity was subsequently tested against the MCF7 breast cancer cell line and the REF normal cell line. The new compound 2-((E)-((E)-2-(5-chloro-3,3-dimethylindolin-2-ylidene)-3-oxopropylidene) amino) benzoic acid was synthesized via the condensation reaction of malonaldehyde with 2-aminobenzoic acid in methanol under acidic conditions, as shown in Figure (1). The chemical structure of the synthesized compound was characterized by TLC, FT-IR, 1H NMR and 13C NMR, and the absorption bands confirmed the chemical structure of this new compound.

The cytotoxic activity of the new synthetic Schiff base compound was studied by using a breast cancer cell line (MCF7) and a normal cell line (REF). The results revealed a low inhibition rate at low concentration on normal cells with 11% and 12% for 48 hrs. and with (40, 60) µg/ml concentration showed 16% and 22% for the same time. The cytotoxic activity of this compound toward MCF7 showed a higher inhibition rate with 64% for the concentration (60 µg/ml), 63% for (40 µg/ml), 60% for (20 µg/ml), and 56% for (10 µg/ml) after 48 hrs. Therefore, this new compound has high potential to inhibit MCF7 breast cancer cell line.

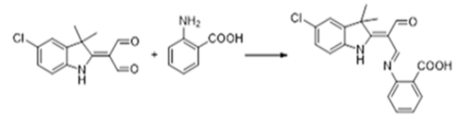

Figure (1): Synthetic pathway of 2-((E)-((E)-2-(5-chloro-3,3-dimethylindolin-2-ylidene)-3-oxopropylidene) amino) benzoic acid

Key Dates

Received:
2024-09-18
Revised:
2024-11-22
Accepted:
2025-04-07
Published:
2025-06-04

URL: https://ijcmg.uomustansiriyah.edu.iq/index.php/ijcmg/article/view/374/version/377
DOI: https://doi.org/10.29409/cfswjb94

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Introduction

Heterocyclic compounds constitute the largest family of organic compounds; these compounds have at least one heteroatom in their structure and are very important chemicals because of their many applications in life, such as drugs, dyes, and conductive materials (1). According to the literature data published in recent years, new anticancer drugs for heterocyclic compound families have undergone significant development. Anticancer research has focused on the versatility and dynamic structure of these molecules(2). Owing to their structural variety and biological importance, nitrogen-containing heterocycles are gaining much attention from scientists(3).

Indoles are a class of heterocyclic compounds that have a bicyclic structure containing a six-membered benzene ring fused to a five-membered nitrogen containing a pyrrole ring(4). Indole has been found in many natural products, and synthetic compounds have increased in popularity because of their exceptional pharmacological properties, such as anticancer, antiproliferative, anti-HIV, and antioxidant activities(5). Schiff bases contain a double bond between carbon and nitrogen atoms called the imine group (-C=N), and the imine is formed by the condensation of primary amines with carbonyl compounds by the removal of water under specific conditions(6). Schiff bases are an essential class of the most commonly used organic compounds and have a wide range of applications, including analytical, biological and medicinal drug (7).

Cancer is a large group of diseases that can start in any organ of the body when cells grow uncontrollably with damaged DNA expression and then spread to other parts of the body(8). Cancer has become the second leading cause of death over the years, with 9.6 million deaths in 2018, and will be the most frequently diagnosed cancer among women worldwide, with more than 2.2 million new cases in 2020(9). The development of treatments that inhibit the growth of cancer cells is challenging for medical chemists.

Indole Schiff bases represent a promising class of compounds for cancer therapy because of their ability to induce apoptosis(10), inhibit key enzymes(11), interact with DNA(12), and potentially modulate oxidative stress(13). Compared with traditional chemical agents, indole Schiff bases may exhibit lower toxicity, which often causes significant damage to healthy tissues(14).

Youssef et al. (2022) synthesized novel indolyl-1,2,4-triazole as an inhibitor of breast and liver cancer. Among the tested compounds, the most promising activity was inducing apoptosis, causing cell cycle arrest, and strongly inhibiting EGFR and PARP-1. In vivo studies confirmed its efficacy in reducing tumor proliferation. These results highlight the potential of indolyl-triazole hybrids in targeted cancer therapy(15).

Khanam et al. (2024) explored the anticancer potential of thiazole Schiff base derivatives. Their study evaluated novel compounds against breast cancer targets compared with FDA-approved drugs. Molecular docking revealed that the compounds exhibited strong binding affinities for the 4FX3 protein, suggesting significant inhibitory potential. These findings highlight the potential of thiazole Schiff bases as promising candidates for breast cancer therapy(16).

Aseel et al. (2022) synthesized a new Indole Schiff base compound [2-(5-chloro-3,3-dimethyl-1,3-dihydro-indol-2-ylidene)-3-(4-methoxy-phenylimino)-propionaldehyde]. The data revealed that the compounds have promising anticancer activity against AMJ13 cells and are safe for the normal growth of the REF cell line(17).

This study aimed to evaluate the cytotoxic effect of a newly synthesized indole-Schiff base compound, 2-((E)-((E)-2-(5-chloro-3,3-dimethylindolin-2-ylidene)-3-oxopropylidene) amino) benzoic acid, against the breast cancer cell line MCF-7 and compare its effects on (REF) normal cells. To expand the knowledge about the therapeutic properties of these compounds, creative frontiers for the development of drugs that contribute to combating cancer diseases are needed.

Materials and methods

Two types of cell lines were used in this research:

(MCF-7): Michigan Cancer Foundation as a breast cancer cell line and (REF): fibroblastic and epithelial cells as a normal murine cell line. were locally established at the Iraqi Center for Cancer and Medical Genetics Research/Mustansiriyah University. The MCF7 cell line was maintained in RPMI-1640 media, which contains glutamine (2 mmol/L), 10% fetal bovine serum (FBS), penicillin (100 U/ml) and streptomycin (100 U/ml), and the REF cell line was maintained in MEM, which contains glutamine (2 mmol/L), 10% fetal bovine serum (FBS), penicillin (100 U/ml) and streptomycin (100 U/ml)(18) . All the cells were maintained at 37°C in a 5% CO2 atmosphere.

The new Schiff base compound was dissolved in 0.5% (DMSO) dimethyl sulfoxide and then diluted with free serum nutrition medium (RPMI-1640 and MEM) to various concentrations (10 µg/ml, 20 µg/ml, 40 µg/ml and 60 µg/ml)(19). The melting point for the new synthetic compound was measured, and the sample was purified via thin layer chromatography via silica gel sheets. The spot was detected via a florescence analysis cabinet model UVT-2000. IR spectra were recorded on a Perkin-Elmer spectrometer, and 1H and 13C NMR spectra were recorded in DMSO on an Agilent Technologies 400 MHz spectrometer at 499.19 MHz for 1H NMR and at 125.54 MHz for 13C NMR.

Chemicals and solvents

Chemicals and solvents were obtained from different companies and used as received without purification. Malonaldehyde was synthesized as described by Baradarani and colleagues(20).

Synthetic methods:

The synthesis of 2-((E)-((E)-2-(5-chloro-3,3-dimethylindolin-2-ylidene)-3-oxopropylidene)amino) benzoic acid is shown in Figure (2). A solution of 0.5 g (2 mmol) of malonaldehyde was dissolved in 5 ml of methanol, and 0.27 g (2 mmol) of 2-aminobenzoic acid was dissolved in 10 ml of methanol to mix the mixture, after which 1 ml of glacial acetic acid was added. The mixture was refluxed in a water bath at 78°C for 7 hrs. A yellow precipitate was formed, filtered, washed with ethanol and dried at 78°C. TLC was used to measure the purity of this new compound, which yielded one spot.

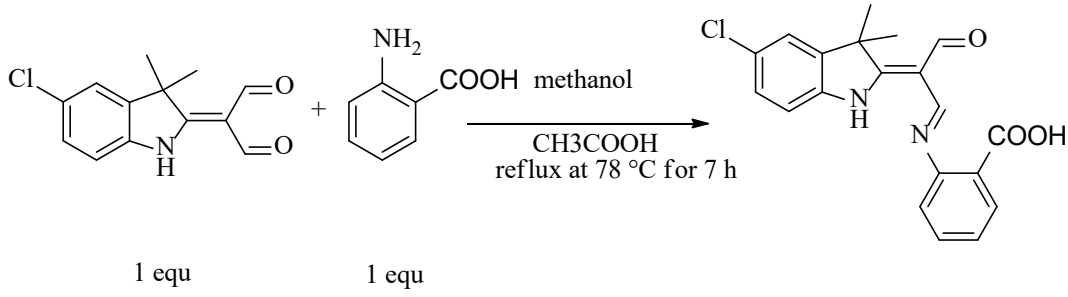

Figure 2: Synthesis of 2-((E)-((E)-2-(5-chloro-3,3-dimethylindolin-2-ylidene)-3-oxopropylidene) amino) benzoic acid

Yield (0.53 g 72%), m. p (1700 (172-C, M. wt. 368.81, IR data in (cm-1): 3131υ(O-H) acid, 2965υ (CH aromatic), 2848υ(CH aldehyde), 1662υ (C=O), 1573υ (CHN), 1506υ(C=C), 1402 (OH bend), 1268υ(C-O), 964 (out of plane O-H bend). 1HNMR (499.19, DMSO, δppm): 13.15(s, 1H, OH acid), 9.80 (s, 1H, HCO), 7.69 (1H, HCN), 7.656.36-7)H, Ar-H) and 1.65 (6H, s, CH3); 13C NMR (125.54 MHz, DMSO, δ ppm): 177.77 (Ar-N=C), 142.94 (CH=N), (139.50-115.18) Ar-CH, 109.60 (O=C=C), 51.25 (CH3CCH3) and 23.85 (2x CH3)

Cell line seeding

Approximately 200 µl (104 cells/well) of the suspension cells (MCF7 and REF) were seeded in two sterile 96-well plates and then cultured for 24 hrs. The media was replaced with 200 µl of 2-((E)-((E)-2-(5-chloro-3,3-dimethylindolin-2-ylidene)-3-oxopropylidene) amino) benzoic acid dilutions (10 µg/ml, 20 µg/ml, 40 µg/ml and 60 µg/ml), and the plates were subsequently incubated at 37°C for 48 hr. The protocol for treating cells was prepared as described by Evans and colleagues(21). Crystal violet dye was used to highlight the DNA by removing the culture medium, and the wells were stained with 100 µl of 0.05% crystal violet solution. The mixture was incubated for 20 minutes at 37°C, distilled water was used to wash the plates, and the mixture was left at room temperature to air dry(22). The plates were read with a microplate reader at 495 nm. The inhibition rate was measured by using the following equation(23)

% growth inhibition = (absorbance of control – absorbance of sample)/absorbance of control×100%.

Statistical analysis

A t test was used to determine the differences between the concentrations in each cell line and to determine the differences between two cells at each exposure time. GraphPad Prism V6 was used for statistical analysis. A Excel 2010 sheet was used to draw the curves.

Results

The newly synthesized compounds were subjected to thin layer chromatography (TLC) and spectral studies (HNMR, 13CNMR, and FTIR). The physical properties are listed in Table 1.

Table 1: Physical properties of the synthesized compounds

Molecular formula	Molecular weight	Percentage Yield	Melting Point °C
$C_{20}H_{17}CLN_2O_3$	368.81	72	170-172

IR Study

The infrared spectrum (4,000400- cm-1 range) of the 2-((E)-((E)-2-(5-chloro-3,3-dimethylindolin-2-ylidene)-3-oxopropylidene) amino) benzoic acid is shown in Fig. 3. The prominent band at 3131 cm-1 for (O-H) acid and 2965 cm-1 was attributed to CH aromatic stretching(24), and the benzoic acid band (C=O) was located at 1662 cm-1. The peak at 1402 and 964 cm-1 was attributed to OH bending and out-of-plane O-H bending, respectively(25). While the absorption band at 1537cm-1 was belonged to (CH=N)(26). The (C=C) group was identified by the frequency at 1506 cm-1 and 1268cm-1 was belonged to (C-O) group(27). All these foremost absorption bands are approved the chemical structure of the new compound.

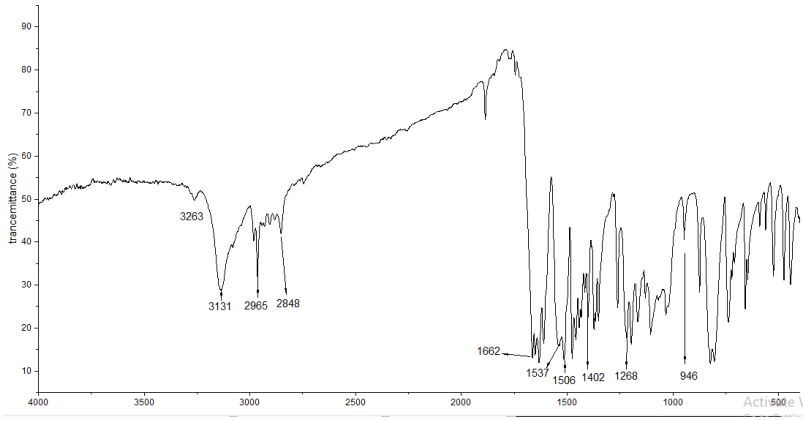

Figure 3: FTIR spectrum of 2-((E)-((E)-2-(5-chloro-3,3-dimethylindolin-2-ylidene)-3-oxopropylidene)

NMR results

HNMR and 13C NMR spectra were proved by Agilent Technologies (inova) spectrometer, at 499.19MHz for1HNMR and at 125.54 MHz for13CNMR. The chemical shifts are given in δ values (ppm) using TMS as the internal standard, (DMSO) and HDO were used as solvents.

The HNMR spectrum Fig. 4. Displayed a singlet signal appeared in the region at 7.69 ppm was attributed to proton atom of (H-C=N)(28), singlet signal at 13.15 ppm which was assigned to the Proton of the hydroxyl group (O-H)(29). A singlet peak also observed at 9.80 ppm gone to proton atom of C=O group(30), the aromatic protons appear in the region (7.656.36-) ppm. and 1.65 (6H, s, CH3); six protons atoms of two methyl groups(31).

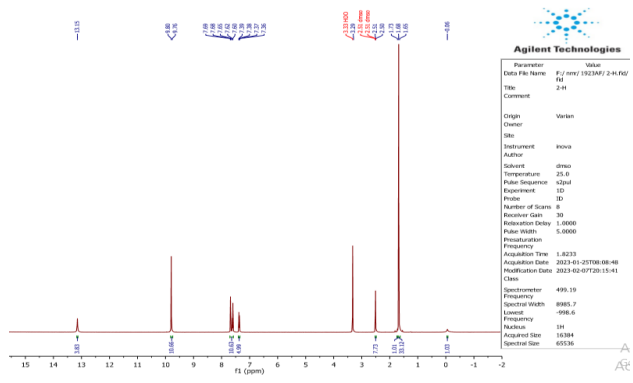

Figure 4: HNMR of 2-((E)-((E)-2-(5-chloro-3,3-dimethylindolin-2-ylidene)-3-oxopropylidene) amino) benzoic acid

13C NMR

C13 NMR data Fig.5. supported the 1H NMR results. A singlet signal appeared at 177.77 which was attributed to the carbon atom of (Ar-N=C)(32), while a new singlet signal observed at 142.94 ppm belonged to the carbon atom of the azomethine group(33) , peaks observed between (139.50-115.18) ppm were assigned to carbon atoms of the aromatic rings(34). Three peaks appeared at 109.60 ppm, 51.25 ppm and 23.85ppm belong to carbon atoms of C=C-CH=O, CH3CCH3 and (2x CH3). Respectively (35) .

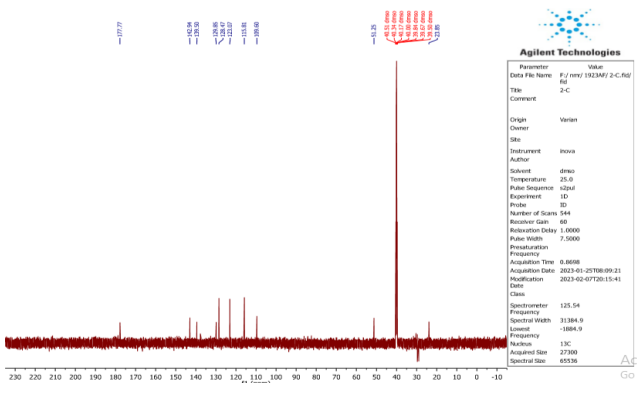

Figure 5: 13CNMR spectrum of 2-((E)-((E)-2-(5-chloro-3,3-dimethylindolin-2-ylidene)-3-oxopropylidene) amino) benzoic acid

The in vitro cytotoxic effects of 2-((E)-((E)-2-(5-chloro-3,3-dimethylindolin-2-ylidene)-3-oxopropylidene) amino acid) benzoic acid on the MCF7 breast cancer cell line were time dependent. The cytotoxic activity of the new compound (Fig. 6) reached a high concentration of 60 µg/ml after 48 hrs. The exposure time resulted in greater cytotoxicity against the MCF7 cell line, with 64% inhibition, and the 40 µg/ml concentration resulted in an inhibition rate of 63%. The cytotoxic activity at low concentrations (20 and 10 µg/ml) was 60% and 56%, respectively.

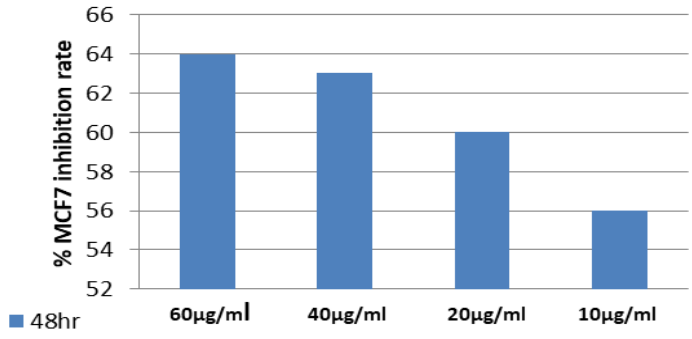

Figure 6: Cytotoxic activity of 2-((E)-((E)-2-(5-chloro-3,3-dimethylindolin-2-ylidene)-3-oxopropylidene) amino acid) benzoic acid against the MCF7 cancer cell line at different concentrations for 48 hr.

When the new compound was tested against the REF normal cell line (Fig. 7), the inhibition rates at the different concentrations were 12% and 11% after 48 hr. At a concentration of 40 µg/ml, 16% inhibition was detected. The cytotoxicity of the higher concentration (60 µg/ml) was 22%.

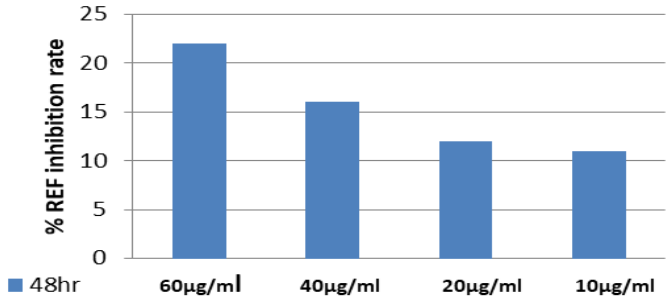

Figure 7: Cytotoxic activity of 2-((E)-((E)-2-(5-chloro-3,3-dimethylindolin-2-ylidene)-3-oxopropylidene) amino acid) benzoic acid against the MCF7 cancer cell line at different concentrations for 48 hr.

Dissection

A 2- ((E)-((E) - 2 - (5-chloro-3,3-dimethylindolin-2-ylidene)-3-oxopropylidene) amino) benzoic acid was synthesized by the reaction of malonaldehyde with one equivalent of anthranilic acid in refluxing methanol, and the resulting yellow precipitate of the new indole Schiff base compound (Fig. 1.) The product was then isolated by recrystallization and characterized by IR and NMR spectroscopy. The new compound was stable, soluble in organic solvents and very soluble in DMSO at room temperature.

Infra-red results of the new compound showed a disappearance the absorption bands of (NH2) group; while new absorption bands of schiff base group is found at 1537 was belonged to (CH=N) cm-1 this a new functional group was specified and approved to Schiff base formation(36).

H-NMR showed disappearance the signal of NH2 group on the spectrum of substituted anilines and shown a singlet signal in the region at 7.69 ppm was attributed to proton atom of (H-C=N)(37), 13C NMR results maintained the 1H NMR results, a signal of (CH=N) group was identified at 142.94 ppm(38). These results support the formation of this new compounds.

The in vitro anticancer activity of the new Schiff base compound against the MCF7 cancer cell line was promising after 48 hr. At concentrations of 60 and 40 µg/ml, the new compound had greater cytotoxicity (64% and 63%, respectively), whereas at 20 and 10 µg/ml, the new compound had inhibition rates of 60% and 56%, respectively. However, the cytotoxic activity is safe toward normal REF cell viability, with inhibition rates of 22%, 16%, 12% and 11% at concentrations of 60, 40, 20 and 10 µg/ml, respectively, so the ideal concentrations could be 40 µg/ml and 20 µg/ml because they inhibit the growth of the MCF7 cancer cell line and are safe for normal REF cell viability.

Conclusion

The new synthetic yellow compound 2-((E)-((E)-2-(5-chloro-3,3-dimethylindolin-2-ylidene)-3-oxopropylidene) amino) benzoic acid compound was characterized by TLC, IR and

NMR spectroscopy, this compound with concentration 40µg/ml and 20µg/ml has the ability to inhibit (MCF7) cancer cells and it will be safe toward (REF) normal cell growth. Let us to put a big focus for further research on these derivatives in cancer therapy.

Acknowledgment

The authors thank the Iraqi Center for Cancer and Medical Genetic Research, Mustansiriyah University, and the Department of Chemistry, Faculty of Science, University of Diyala, Iraq, for supporting this work.

Conflict of interest

All the authors declare that they have no conflicts of interest.

Contribution of authors

Author 1: Aseel Faeq Ghaidan

new compound by the condensation reaction of malonaldehyde with 4-aminobenzoic acid in methanol under acidic condition. Then the chemical structure of the synthesized compound was characterized by TLC, FT-IR, 1H NMR and 13C NMR .

Author 2: Khansaa Raed Dawood Al-Saadi :

Cytotoxic activity of the new synthetic Schiff base compound was studied by using cancer cell line (MCF7) and normal cell line (REF)

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