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Synthesis, Characterization, Molecular docking, and Anticancer activity of New Formazan Derivatives

Abdulsattar Hashim A. Ghani[1], Moayed Nemma Mohammed[2] and Muqdad Irhaeem Kadhim[3]
[1]Al-Furat Al-Awsat Technical University, Iraq
[23]Al-Qadisiyah University, Iraq

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ABSTRACT
Objective: *This work includes novel formazan compounds containing 5-diazenyl-2-hydroxybenzoic acid with different Schiff bases. SB1 (N-(6-methoxybenzo[d]thiazol-2-yl)-1-(4-(pyridin-2-yl)phenyl)methanimine), SB2 (dimethylene(4-((4-(pyridin-2-yl)benzylidene)amino)phenyl)-l6-sulfanamine), SB3 4-(((4-methoxy-6-methyl-1,3,5-triazin-2-yl)imino)methyl)-N,N-dimethylaniline) and SB4 (4-(((4-methoxy-6-methyl-1,3,5-triazin-2-yl)imino)methyl)benzene-1,3-diol) leads to formation F1 (2-hydroxy-5-((((6-methoxybenzo[d]thiazol-2-yl)imino)(4-(pyridin-2-yl)phenyl)methyl)diazenyl)benzoic acid), F2 (5-((((4-(aminodimethylene-l6-sulfaneyl)phenyl)imino)(4-(pyridin-2-yl)phenyl)methyl)diazenyl)-2-hydroxybenzoic acid), F3 (5-(((4-(dimethylamino)phenyl)((4-methoxy-6-methyl-1,3,5-triazin-2-yl)imino)methyl)diazenyl)-2-hydroxybenzoic acid) and F4 (5-(((2,4-dihydroxyphenyl)((4-methoxy-6-methyl-1,3,5-triazin-2-yl)imino)methyl)diazenyl)-2-hydroxybenzoic acid) in order.* ***Method:*** *They have been synthesized by the addition reaction of the diazonium salts of 5-diazenyl-2-hydroxybenzoic acid with Schiff bases, and they were designed, synthesized, and confirmed by elemental analyses, FT-IR, 1H-NMR and 13C-NMR spectral data. It noticed the molecular docking results for the interaction of the tested ligands (F1, F2, F3, F4) with the 1T49 protein (PTP1B) reveal several important insights regarding their binding affinities, stability of poses, and interaction profiles within the active site of the enzyme.* ***Results:*** *This suggests that F1 is the best candidate for further development as a potential PTP1B inhibitor, especially in relation to treatment for the breast cancer. All these results are consistent with what this work has reached when conducting an anti-cancer test. This work has investigated the cytotoxic potential of F1 against cell line MCF-7 (breast cancer). Results showed that the compound exhibited significant cytotoxicity toward MCF-7 cells, with an IC_{50} of 147.6 µg/mL. These findings suggest a selective antiproliferative effect of the compound on cancerous cells compared to normal cells, highlighting its potential as a promising candidate for further anticancer drug development targeting breast cancer.* ***Novelty:*** *This work includes novel formazan compounds containing 5-diazenyl-2-hydroxybenzoic acid with different Schiff bases.*

INTRODUCTION

Formazan are organic compounds containing "N=N-C=N- or N=N-C=N-N", based on the kind and composition of the reactive (aromatic) amines [1]. There has been extensive research on formazans, including their synthesis, structure, evaluation, redox potentials, tautomer production, and photochromic transitions [2-6]. Formazans are compounds, depending on their structure, they can range in colour from red to orange to blue. Formazans and their complexes with transition metals are of interest in the fields of biochemistry, medicine, and dyes. Combining diazonium salts with substances that have an active (N=CH) group, such as cyanoacetic acid, nitromethane, malonic acid, and acetoacetic ester, is one of the different ways to produce formazans [7-10]. Formazans have been the focus of in-depth investigation because their easy availability, broad range of biological activity, varied chemical reactivity, and numerous uses [11 and 12]. These substances, along with heterocyclic hydrazones, are recognized for a variety biological processes, such as antiviral [13] anti-inflammatory [14], antimicrobial [15], antifungal [16]. They're used for sperm viability, tumour cell activity assessment, and anticancer drug screening [17-21], anti-HIV [22], etc. Several formazans have shown promising antifertility [23] and antiparkinsonian properties [24]. We synthesized formazan derivatives in this work by combining Schiff base that prepared from (**Comp.1** with **Comp.2 and 3**) and (**Comp.4** with **Comp.5 and 6**) in ethanol as a solvent (Scheme 1). These derivatives' structures were determined using spectral data ^{13}C-NMR, ^{1}H-NMR and FT-IR which are widely used for characterization of materials and chemicals [25–28]. Furthermore, Various heterocyclic systems are synthesized using formazans as starting materials [29].

Because of their significance in many different areas, researchers have studied formazan derivatives, concentrating mostly on their structures and spectra. Examples documented in the literature include X-ray diffraction data for formazan compounds, studies of (H-bonding) in formazan compounds, and description of the frequency at which they vibrate. Numerous theoretical and experimental investigations have been carried out to expand on the knowledge of potential proton transfer and tautomeric equilibrium mechanisms [30–32]. The structure and characteristics of formazans are greatly influenced by tautomerism, and certain formazans have been found to have intramolecular hydrogen bridges [33]. Penchmann [34] was the first to describe the tautomerism of formazans, although his findings were equivocal. Hunter and Roberts in 1941 [35].

RESEARCH METHOD

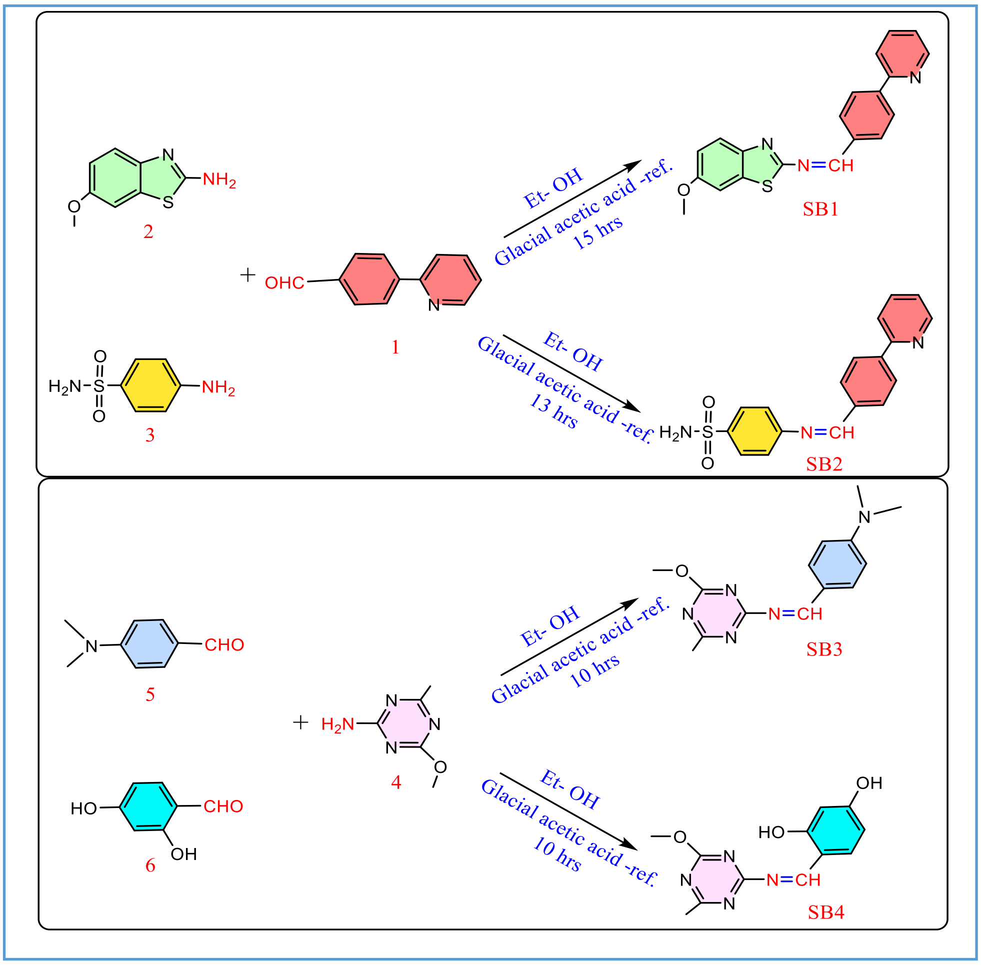
Experimental Part

1. Physical measurements and materrials

FT-IR was obtained on a Shimadzu (8400S) instrument at the BBC facility in Baghdad. The heat of fusion was modified by (Stewart, UK). Using Bruker Ultra Shield spectrophotometer, ^{13}C-NMR and ^{1}H-NMR spectra were measured @ 300 MHz in TMS as the internal average with (DMSO-d6) solution. Reference-grade reagents available from Fluka and Sigma-Aldrich were used in this study. The purity of the compounds was checked on Merck-TLC silica-coated chromatography plates using hexane, ethyl acetate, and ethanol as the mobile phase, in Iran.

2. General procedure of Schiff base [36]

Schiff bases were made using a refluxing process that involved an amine compound reacting with aromatic aldehyde in drops of glacial acetic acid in approximately 25 milliliters of 100% ethanol for many hours at 78 ^{o}C. TLC was used to determine the reaction's completion, and 100% ethanol was used to filter and re-crystallize the precipitate, see scheme 1.



Scheme 1. synthesis of Schiff base compounds (SB1- 4).

a. **This process leads to synthesize Schiff bases orderly:**

- **Synthesise of N-(6-methoxybenzo[d]thiazol-2-yl)-1-(4-(pyridin-2-yl)phenyl) methanimine (SB1)** yield; yellow precipitate, (0.290g, 38%), m.p. 178 ºC. It is from 2-Amino-6-methoxybenzothiazol (0.2g, 0.0011mol) (Comp.2) with 4-(2-pyridinylbenzaldehyde) (0.2g, 0.0011 mol) (Comp.1). FT-IR data in (cm^{-1}): 3051-3007 ʋ(C-H $_{arom.}$), 2862 ʋ(C-H $_{stre.}$ -OCH_3), 1645 ʋ(HC=N), 1462-1487 ʋ(C=C $_{arom.}$), 1433 ʋ(C-H from -CH_3 &-OCH_3), 1261 ʋ(C-N), 1224 ʋ(C-S stre.), 1111 ʋ(C-O from -OCH_3), 1051 ʋ(C-S stre.), 1020 ʋ(C-N). ^{1}H-NMR (300MHz, DMSO, δ in ppm) δ= 8.75 (d, 1H, HC=N $_{ring}$), 7.87 (s, 1H, HC=N), 7.98-7.11 (10H, Ar-H), and 3.86 (s, 3H, OCH3). ^{13}C NMR of (100 MHz, DMSO-d6) δ (ppm): 169.21 (N=C-N), 166.09 (CH=N), 157.88 (C-N and C-O), 150.29, 146.08, 143.28, 137.92, 136.05, 135.50, 130.84, 127.56, 123.85, 121.46 and 116.35 (Ar-H), 105.61 (C=C), and 56.22 (O-CH3).
- **Synthesise of dimethylene(4-((4-(pyridin-2-yl)benzylidene)amino)phenyl)-l6-sulfanamine (SB2)** yield; creamy white precipitate, (0.595g, 80%), m.p. 245 ºC. It is from 4-Aminobenzenesulfonamide (0.2g, 0.0011mol) (Comp.3) with compound (1) (0.21g, 0.0011 mol). FT-IR data in (cm^{-1}): 3282-3159 ʋ(NH), 2993 ʋ(C-H $_{stre.\ arom.}$), 1651 ʋ(HC=N), 1614- 1462 ʋ(C=C $_{stre.\ arom.}$), 1332-1153 ʋ(S=O) and 1012 ʋ(S-N). ^{1}H-NMR (300MHz, DMSO, δ in ppm) δ= 8.72 (s, 1H, HC=N) , 8.28(d, 1H, N=C$_{ring}$), 8.27- 7.86 (10H, Ar-H) and 7.42(s, 2H, NH_2). ^{13}C NMR of (100 MHz,

DMSO-d6) δ (ppm):162.72 (CH=N), 155.46 – 154.58 (C-N), 149.90 (C=N), 143.60 ($\underline{C}$-C-N), 140.35 (C-S), 136.86, 133.92, 132.94, 128.79, 123.55 and 122.72 (C-C_{ring}).

- **Synthesise of 4-(((4-methoxy-6-methyl-1,3,5-triazin-2-yl)imino)methyl)-N,N-dimethylaniline (SB3)** yield 83%; beige precipitate, (1.534g, 40%), m.p. (Decompose). It is from N,N-dimethylbenzaldehyde (1.06g, 0.0071mol) (Comp.5) with 2-Amino-4-methoxy-6-methyl-1,3,5-triazine (1g, 0.0071 mol) (Comp.4). FT-IR data in (cm^{-1}): 3051-3007 υ(C-H $_{arom.}$), 2920 υ(C-H $_{stre.}$ -OCH_3), 1658 υ(HC=N), 1566- 1512 υ(C=C $_{stre.\ arom.}$), 1462 υ(N=N), 1433 υ(C-H from -CH_3 &-OCH_3), 1261 υ(C-N), 1111 υ(C-O from -OCH_3), 1020 υ(C-N). ^{1}H-NMR (300MHz, DMSO, δ in ppm) δ=7.31 (s, 1H, HCN),7.50 -7.09 (4H, Ar-H), 3.80 (s, 3H, OCH3), 2.15 (s, 6H, $N(CH_3)_2$) and 1.65 (s, 3H, CH_3). ^{13}C NMR of (100 MHz, DMSO-d6) δ (ppm): 169.21 (N=C-N), 166.09 (CH=N), 157.88 (C-N and C-O), 150.29, 146.08, 143.28, 137.92, 136.05, 135.50, 130.84, 127.56, 123.85, 121.46 and 116.35 (Ar-H), 105.61 (C=C), and 56.22 (O-CH3).
- **Synthesise of 4-(((4-methoxy-6-methyl-1,3,5-triazin-2-yl)imino)methyl) benzene-1,3-diol (SB4)** yield; rosewood precipitate, (0.287g, 16%), m.p. (Decompose). It is from 2,4-dihydroxybenzaldehyde (0.49g, 0.0036mol) (Comp. 6) with compound (4) (0.5g, 0.0036 mol). FT-IR data in (cm^{-1}): 3327 and 3140 υ(OH), 3030 υ(C-H $_{arom.}$) 2953- 2814 υ(O-CH_3- C-H $_{stre.}$), 1658 υ(HC=N), 1566 υ(C=C $_{stre.\ arom.}$), 1462 υ(N=N), 1373 υ(C-C $_{arom.}$), 1344 υ(C-H from -CH_3 &-OCH_3), 1247 υ(C-N), 1103 υ(C-O from -OCH_3). ^{1}H-NMR (300MHz, DMSO, δ in ppm) δ= 7.32 (3H, Ar-H), 3.81 (s, 3H, OCH3) and 2.22 (s, 3H, CH3). ^{13}C NMR of (100 MHz, DMSO-d6) δ (ppm): 177.37 (HC=N), 171.21 (C-CH_3), 168.64 (C_{ring}), 164 (C-N and C-O), 160.07, 156.86, 143.18, 133.75, 123.75, 92.25, 54.20 (OCH_3) and 25.31 (-CH_3).

3. General procedure of Formazan derivatives (11-14)

Formazan derivatives F (1-4) were synthesized according to modified methods that described by Aqeel Abed Muhee [37 and 38]. The process includes mixing two solutions together:

Solution 1 dissolve (0.08 gm, 0.0005 mol) of 5-Amino-2-hydroxybenzoic acid (Mesalazine) each one separately in (2ml in 15ml) of HCL concentrated in distilled water kept between 0 and 5 °C in an ice bath while being constantly stirred. Following a dropwise addition of 15 ml (0.035 gm, 0.0005 mol) in the solution of sodium nitrite, the solution was cooled about fifteen minutes below 5°C in an ice bath.

Solution 2 dissolve (0.0005 mol) of compounds (F1-4) in (0.5 gm in 15ml) of NaOH with distilled water and (2ml) of absolute ethanol.

The result solution was added dropwise while stirring solution (1 to 2). After that, the contents were mixed about twenty minutes. The coloured solid (the pH was maintained at 6-8) [39]. After being separated, it is cleaned and filtered with distilled H_2O, then by ethanol, see scheme 2.

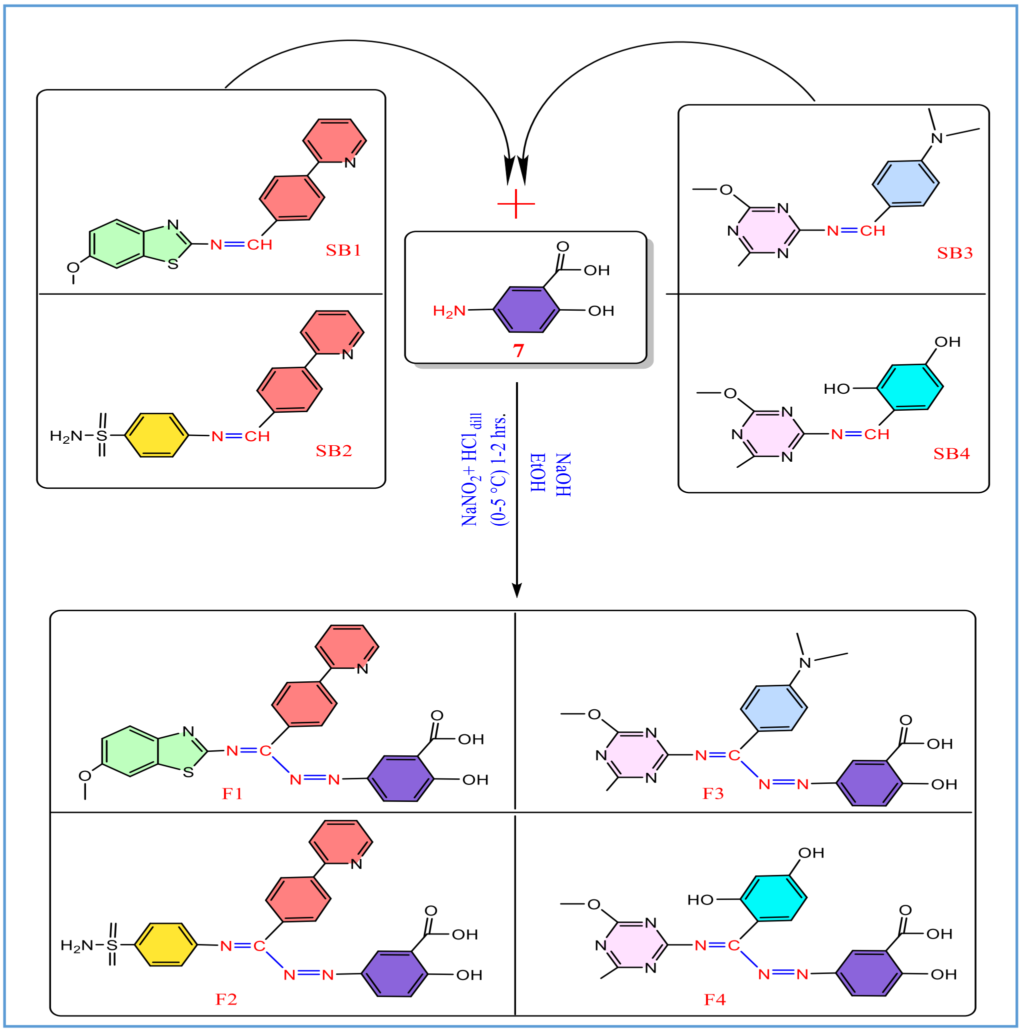
a. **Synthesise of 2-hydroxy-5-(((E)-((6-methoxybenzo[d]thiazol-2-yl)imino)(4-(pyridin-2-yl)phenyl)methyl)diazenyl)benzoic acid (F1)** yield; dark walnut gradient precipitate, (0.047g, 27%), m.p. 110 ºC. It is from SB1 (0.17g, 0.0005mol). FT-

IR data in (cm^{-1}): 3545 ʋ(COOH), 3475-3414 ʋ(OH_{phenol}), 3080 ʋ(C-H $_{stre.\ arom.}$), 2858 ʋ(C-H $_{alpha.}$), 1631 ʋ(HC=N), 1614- 1597 ʋ(C=N), 1487-1462 ʋ(C=C $_{stre.\ arom.}$), 1433 ʋ(C-H from -CH_3 &-OCH_3), 1257 ʋ(C-N), 1224 ʋ(C-S $_{stre.}$), 1153 ʋ(C-O from -OCH_3), and 1053 ʋ(C-S $_{stre.}$). ^{1}H-NMR (300MHz, DMSO, δ in ppm): δ= 10.09 (s, 1H, COOH), 9.21 (d, 1H, HC=N $_{ring}$ and OH), 7.75- 6.64 (10H, Ar-H), and 3.86 (s, 3H, OCH3). ^{13}C NMR of (100 MHz, DMSO-d6) δ (ppm): 169.21 (N=C-N), 166.09 (CH=N), 157.88 (C-N and C-O), 150.29, 146.08, 143.28, 137.92, 136.05, 135.50, 130.84, 127.56, 123.85, 121.46 and 116.35 (C=C_{phenyl}), 105.61 (C=C), and 56.22 (O-CH3).

b. **Synthesise of 2-hydroxy-5-(((4-(pyridin-2-yl)phenyl)((4sulfamoylphenyl) imino)methyl)diazenyl)benzoic acid (F2)** yield; creamy white precipitate, (0.595g, 83%), m.p. Dec. ºC. It is from SB 2 (0.17g, 0.0005mol). FT-IR data in (cm^{-1}): 3475 ʋ(COOH), 3414 ʋ(OH), 3344- 3248 ʋ(NH_2), 3080 ʋ(C-H $_{stre.\ arom,}$), 2179 ʋ(C=N $_{stre.}$), 1708 ʋ(C=O), 1627 ʋ(HC=N), 1585 ʋ(C=N_{ring}), 1483 ʋ(C=C $_{stre.\ arom.}$), 1365- 1334 ʋ(S=O $_{stre.\ asym.}$) and 1155 ʋ(S=O $_{stre.\ sym.}$). ^{1}H-NMR (300MHz, DMSO, δ in ppm): δ=10.09 (s, 1H, COOH), 8.90 (d, 1H, HC=N $_{ring}$), 7.74- 6.72 (14H, Ar-H), and 7.36 (s, 2H, NH_2). ^{13}C NMR of (100 MHz, DMSO-d6) δ (ppm):180.76 (COOH), 162.73 (N=C-N), 155.46 (C-N=N), 154.51, 150.22, 142.17, 141.78c, 139.45, 137.88, 135.36, 132.10, and 127.38 (C=C_{phenyl}), 123.70 (C=C-OH), and 121.25 (C-COOH).

c. **Synthesise of 5-(((4-(dimethylamino)phenyl)((4-methoxy-6-methyl-1,3,5-triazin-2-yl)imino)methyl)diazenyl)-2-hydroxybenzoic acid (F3)** yield; yellow precipitate, (0.19g, 72%), m.p. (>300) ºC. It is from SB 3 (0.14g, 0.0005mol). FT-IR data in (cm^{-1}): 3479- 3414 ʋ(COOH and OH), 3080- 3035 ʋ(C-H $_{stre.\ arom,}$), 2179 ʋ(C=N $_{stre.}$), 1710 ʋ(C=O), 1597- 1512 ʋ(N=N and C=N), 1475 ʋ(C=C $_{stre.\ arom.}$), 1417 ʋ(C-H from -CH_3 &-OCH_3), 1274 ʋ(C-N) and 1126 ʋ(C-O from -OCH_3). ^{1}H-NMR (300MHz, DMSO, δ in ppm) δ= 9.68 (s, 1H, Ph-OH), 8.90- 6.72 (7H, Ar-H), 3.05 (s, 3H, OCH_3), 2.09 (s, 3H, CH_3), and 1.24 (s, 6H, $N(CH_3)_2$). ^{13}C NMR of (100 MHz, DMSO-d6) δ (ppm) : 170.96 (COOH), 166.28 (N=C-N), 152.82 (C-N and C-O), 135.35, 126.59, 124.48, 121.39 and 110.22 (C=C $_{phenyl}$), 87.59 and 79.13 (C-OH), 45.38 (N-CH_3) and 26.86 (C-CH3).

d. **Synthesise of 5-(((2,4-dihydroxyphenyl)((4-methoxy-6-methyl-1,3,5-triazin-2-yl)imino)methyl)diazenyl)-2-hydroxybenzoic acid (F4)** yield; dark brown precipitate, (0.07g, 43%), m.p. (Decompose). It is from SB4 (0.13g, 0.0005mol). FT-IR data in (cm^{-1}): 3446 ʋ(COOH), 3400 ʋ(OH), 2981 ʋ(C-H $_{arom.}$), 1631- 1564 ʋ(C=O, N=N and C=N), 1541 ʋ(C=C $_{stre.\ arom.}$), 1487 ʋ(N=N), 1384- 1311 ʋ(C-N) and 1226- 1126 ʋ(C-O from -OCH_3). ^{1}H-NMR (300MHz, DMSO, δ in ppm) : δ=11.03 (s, 1H, COOH), 9.93 (s, 1H, OH), 8.20-6.41 (6H, Ar-H), 4.30- 4.23 (CH-N=N), 3.80 (s, 3H, OCH3), and 2.21 (s, 3H, CH_3). ^{13}C NMR of (100 MHz, DMSO-d6) δ (ppm): 172.44 (OH), 168.64- 162.95 (N=C-N), 145.04 (C-N=N), 132.99- 102.76 (C=C_{phenyl}), 62.37 (O-CH_3), 25.31 (C-CH_3) and 14.77 (N-CH_3).



Scheme 2. Synthesis of Formazan compounds (F1- 4).

4. Molecular docking

a. Molecules Library Preparation

The newly compounds **(F1, F2, F3, F4)** were synthesized and characterization to using in molecular docking process, Chemdraw Ultra 12.0 (https://chemdraw pro.software.informer.com/12.0/) software was utilized to generate the 3D structures of the compounds **(F1, F2, F3, F4)** and followed by energy minimization. Using Hyperchem 8.08 software, the semi-empirical AM1 investigate was used to pre-optimize the structures. To find the most stable conformation, the structures were optimized using the density functional theory (DFT) approach utilizing the B3LYP/6-31G basis set. Maximum force, root-mean-square (RMS) force, maximum displacement, and RMS displacement all converge to their default values "YES". Every principles are definite beyond shrewd vibrational repetitions for ligands; those results display that the ligands are stable. The increased structures were linked in one table on MOE program [40], in order to investigated ligands affinity; (**Comp.** F1-4). Also, Figure 1 illustrates the docking process's schematic illustration, examination of ligands, as well as ability to react.

5. Anticancer activity

After the result that it gets from molecular docking. It was taken **Comp.F1** to evaluate the cytotoxicity effect it's against the tumor cell lines (MCF-7) and the in vitro normal cell line HdFn. Human breast cancer cell line MCF-7 was created from a 69-year-old Caucasian woman who had metastatic breast adenocarcinoma. It's a widely used model in breast cancer research due to its characteristics, including estrogen receptor positivity and the ability to process estradiol. (MTT Assay) The sphere growth of MCF-7 cells in a 96-well ultra-low attachment plate with a circular bottom. We planted 2000 cells per well. From day 1 to day 21, a 100×microscope magnification was employed. A 40× microscope magnification was utilized after a 24-day period. Absorbance was calculated at 570 nm utilizing a microplate reader (Bio-Rad, USA). Cell viability was measured as a percentage relative to the un-treated control group. Every experiments were conducted in triplicate and at least three times repeated independently.

RESULTS AND DISCUSSION

1. Compounds Characterization

^{13}C-NMR, ^{1}H-NMR, and FT-IR spectroscopic techniques were used to determine the chemical structures of the synthesized products, these is provided under the section on experiments. FT-IR spectra of all compounds SB1-4 explained by stretching vibrations present, which In point with the (HC=N) group at 1645.28, 1651.07, 1658.78 and 1658.78 cm^{-1}, it acts as functional group in SB1-4; respectively while when synthesizing the compounds Formazan the FT-IR spectra data of theirs F1-4 are also characterized by appearing (3445 & 3414), (3475 & 3414), (3479 & 3414) and (3446 & 3400) for the bands (COOH & OH); respectively. Also shifting of frequencies for Imine group to (1631.78, 1627.92, 1597.06 band 1631.78) due to the stretching vibration of (N=N) at (1462.04, 1483.26, 1475.54 and 1460.11cm^{-1}); respectively). All compounds have been approved by ^{1}H-NMR spectra. The proton of (CH=N) is appear at (8.31, 8.72 and 7.31) ppm; respectively, but disappear in SB4 because of proton exchange between (OH) group in ortho position, while these signals didn't appear in the spectra of formazan compounds. The signals for (COOH & OH) appear in formazan compounds F1-4, it appears at (10.09 & 9.21), (10.09 & 9.21), (----, 9.68) and (11.03 & 9.93) ppm; respectively.

2. Molecular docking

It is used as a fundamental tool in the drug discovery pipeline. In the study, MOE program was applied to act all molecular docking calculations and predict the binding style of the prepared compounds **(F1, F2, F3, F4)** with the protein (**1T49**) (Figure 1). The expected binding affinities and characteristics of the products under investigation **(F1, F2, F3, F4)** towards (**1T49**) are listed in Table 1, and Table 2 shows the best binding poses of compounds **(F1, F2, F3, F4)** against target protein.

The 3D and 2D representations of contact of the inspected the key amino acid residues of (**1T49**) protein with compounds are illustrated in the next figures and tables. Compounds **(F1, F2, F3, F4)** showed good binding affinity values (Table 2, Table 3) with protein (PDB ID: **1T49**). The binding and mode of interactions of the compound **(F1, F2, F3, F4)** with the (PDB ID: **1T49**) protein are shown in 2D and 3D figures. From the interactions, it has been shown that primarily there are different types of interactions (hydrogen bonding and hydrophobic interactions). Contacted were further checked for

H bonds and bond lengths in the active site and were illustrated in the next figures. The products from these figures revealed that compounds (F1, F2, F3, F4) communicate accompanying various amino acid residues indifferent interplays: H-acceptor, H-donor, and H-pi, in addition to two pi-hydrogen and H-acceptor interplays accompanying the H_2O and various amino acids. The distance and energy binding of interaction are filed (Table 1). Hydrogen bonds and hydrophobic interactions significantly contributed to binding stability, suggesting a potential mechanism for inducing apoptosis and cell cycle arrest. These computational results are in line with the observed in vitro cytotoxicity, supporting the hypothesis that the compound may inhibit tumor progression by disrupting signalling pathways. Further in vivo validation is recommended to confirm these findings.

Table 1. Details of the best poses of ligands (**F1, F2, F3, F4**) with protein 1T49.

Compounds	Binding Affinity Kcal/mol	Rmsd (Å)	Atom of compound	Atom of Receptor	Involved receptor residues	Type of interaction bond	Distance (Å)	E (kcal/mol)
F1 -pose1	-7.91638	2.052064	N 35 5-ring 5-ring 6-ring 6-ring	ND2 5-ring 6-ring 5-ring 6-ring	ASN 193 892 301 892 301 892 301 892 301	(A) H-acceptor (A) pi-pi (A) pi-pi (A) pi-pi (A) pi-pi	3.57 3.32 1.59 3.48 1.34	-1.2 -0.1 -0.0 -0.2 -0.0
F2- pose 3	-7.13671	1.984281	C 35 6-ring 6-ring 6-ring 6-ring	CA 5-ring 6-ring 5-ring 6-ring	PHE 280 892 301 892 301 892 301 PHE 196	(A) H-acceptor (A) pi-pi (A) pi-pi (A) pi-pi (A) pi-pi	3.85 1.85 1.98 3.01 3.87	-3.3 -0.0 -0.0 -0.0 -0.0
F3- pose 2	-6.91622	2.053126	N 3 6-ring 6-ring 6-ring	ND2 CA 5-ring 6-ring	ASN 193 ASN 193 892 301 892 301	(A) H-acceptor (A) pi-H (A) pi-pi (A) pi-pi	3.60 4.28 2.05 3.74	-0.7 -0.7 -0.0 -0.0
F4- pose 1	-6.74426	2.132654	O 28 O 31 N 7 C 10	OE1 O ND2 6-ring	GLU 276 GLU 276 ASN 193 PHE 196	(A) H-donor (A) H-donor (A) H-acceptor (A) H-pi	2.96 2.82 2.92 4.19	-2.9 -1.8 -1.9 -0.6
Standard	-7.1244	2.126639	O20 4 O20 4 5-ring	NZ NZ 6-ring	LYS 197 LYS 197 PHE 280	(A) H-acceptor (A) ionic (A) pi-pi	2.95 2.95 3.47	-2.1 -4.8 -0.0

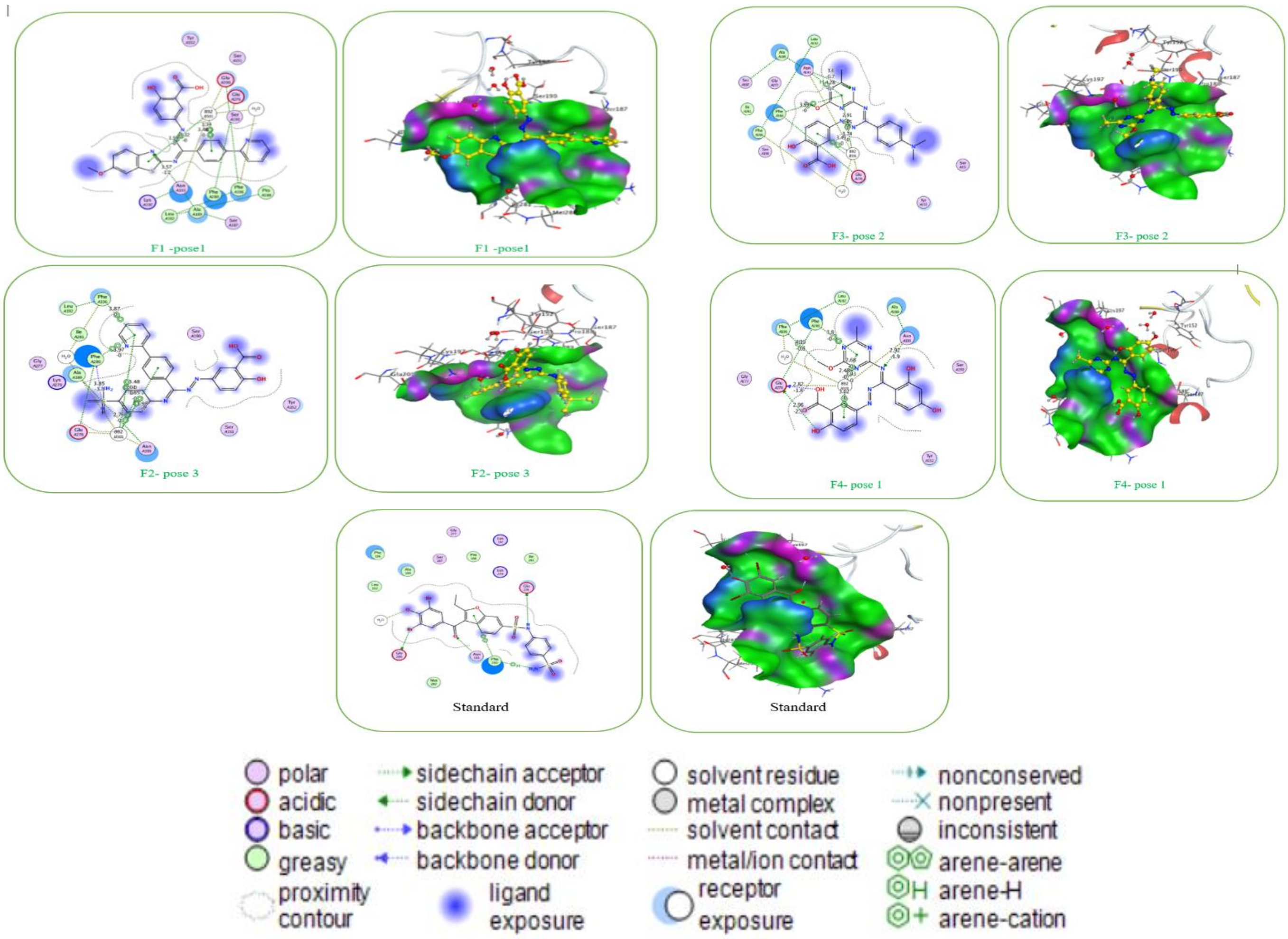


Figure 1. 2D and 3D of the best poses.

3. Anticancer activity

A human cell line was used to compare the synthesized formazan derivatives compound's anti-tumor efficiency [insert cell line, e.g., (breast cancer) MCF-7] using the MTT assay (Table 2). Cells were treated with the compound **(F1)** according to data that get it from molecular docking that is (F1) is a good activity. It uses at various concentrations (e.g., 1–100 μM) for 24/48 hours to assess their viability. Molecular docking analysis revealed strong binding interactions between the synthesized organic compound and key oncogenic proteins, including (**1T49)** The compound exhibited high binding affinity, and docking results indicated the formation of stable complexes at the active site. The IC50 values (Figure 2) of doxorubicin and F-1 were supposed at 25, 50, 100, 200, and 400 μg/ml, individually. The findings submitted that the exercise of F1 towards the breast cancer (MCF-7) cell line was more important. According to the MTT assay, 16 μg/ml doxorubicin, F-1 weakened container progress later 48 h. The qualified compounds showed an extreme answer in restricting and murder malignancy containers because of participation of the formazan group (-N=N-CH=N-NH-), that has existed justified in prior research to play ultimate main role in compound 's toxicity against malignancy containers [41]. Our results illustrated that F1 have the skill to restrict MCF-

7 container increase and have the potential to act as antagonistic-conscience tumour powers.

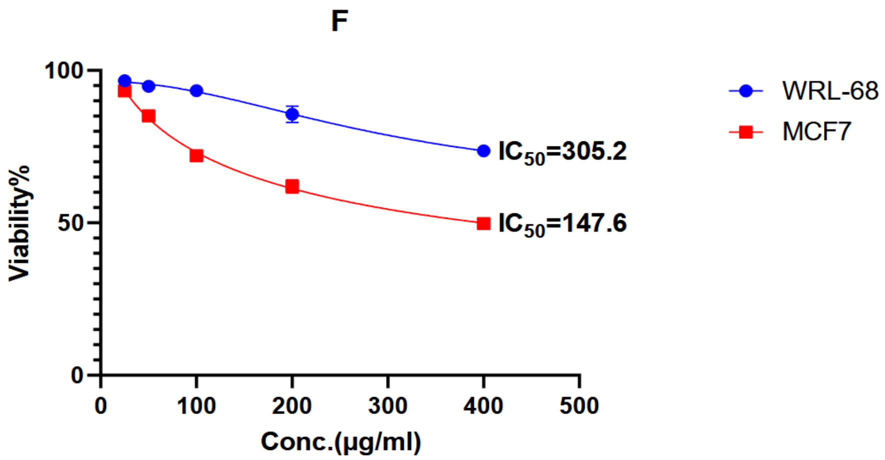


Figure 2. IC_{50} of F1 and control against MCF-7 cell line.

Table 2. Effect of F1 on the cell line MCF-7 (breast cancer) and compared with WRL-68 (normal cells) at the same concentration using MTT.

F	WRL-68		MCF-7	
Conc.	Mean	SD	Mean	SD
400	73.64967	0.998143	49.76867	1.557424
200	85.648	2.669311	61.99833	2.239138
100	93.36433	0.371955	72.029	1.043633
50	94.83	0.770839	85.14667	1.945085
25	96.605	0.65808	93.32567	0.522075

CONCLUSION

Fundamental Finding : We aimed to synthesis new derivatives of formazan compounds that is very important in the pharmaceutical, drugs, and different fields, so this work improved that compounds have that synthesized by the addition reaction of the diazonium salts of 5-diazenyl-2-hydroxybenzoic acid with Schiff bases, characterization by (the FT-IR, 1H-NMR and 13C-NMR techniques) and molecular docking results for the interaction of the tested ligands (F1, F2, F3, F4) with the 1T49 protein (PTP1B) reveal several important insights regarding their binding affinities, stability of poses, and interaction profiles within the active site of the enzyme. The results pointed to the cytotoxic potential of F1 against cell line MCF-7 (breast cancer). Results showed that the compound exhibited significant cytotoxicity toward MCF-7 cells, with an IC_{50} of 147.6 µg/mL. These findings suggest a selective antiproliferative effect of the compound on cancerous cells compared to normal cell. **Implication :** These findings suggest a selective antiproliferative effect of the compound on cancerous cells compared to normal cell, indicating its potential as a candidate for further anticancer drug development. The observed cytotoxic potential, coupled with the specific binding affinities and stability of poses within the active site of the enzyme, may contribute to the rational design of new therapeutic agents targeting cancer cells while minimizing effects on normal cells. **Limitation :** In this work, it tested F1 against MCF-7 cell line, and therefore the selectivity and activity of the other derivatives such as F2, F3, and F4 were not evaluated in the current study. Moreover, the pharmacological behaviour, bioavailability, and toxicity profiles were not explored in vivo, limiting the understanding of the compounds' potential clinical applications. **Future Research :** The authors recommended to use another method to synthesis these compounds by microwave irradiation that is more safety for researchers. Apply it to work that include the synthesis of metal–formazan complexes, particularly with transition metals such as Cu(II), Zn(II), or Co(II), as such complexes. It is advisable to evaluate the synthesized formazans against a wider spectrum of microbial strains and cancer cell lines (such as HeLa, HepG2, A549, and MDA-MB-231) to assess their selectivity, and another derivative of formazan such as F2, F3, and F4. It is advisable to study In vivo should be conducted to explore the pharmacological behaviour, bioavailability, and toxicity profiles of the most promising formazan compounds.

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***Abdulsattar Hashim A. Ghani (Corresponding Author)**
Al-Furat Al-Awsat Technical University, Iraq
Email: staarhashim@atu.edu.iq

Moayed Nemma Mohammed
Al-Qadisiyah University, Iraq
Email: moayad.alshbani@qu.edu.iq

Muqdad Irhaeem Kadhim
Al-Qadisiyah University, Iraq
Email: Muqdad.Khadhim@qu.edu.iq