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SYNTHESIS OF ACETYLENIC AMINO DERIVATIVES OF 2-(2-((2,6-DICHLOROPHENYL) AMINO)PHENYL)ACETIC ACID

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Abstract: *After reviewing a number of researches that demonstrate the effectiveness of a number of compounds containing the acetylenic amine group as antimicrobials and antiproliferative activity, our goal is to prepare new acetylenic amine derivatives from the compound 2-(2-((2,6-dichlorophenyl)amino)phenyl)acetic acid using the Mannich reaction by the react salt of a carboxylic acid with propargyl bromide to produce the compound (prop-2-yn-1-yl 2-(2-((2,6-dichlorophenyl)amino)phenyl)acetate) (1), which was refluxed with a number of secondary amines and formaldehyde in presence of the copper chloride as a catalyst to obtain 7 compounds (4-(dialkylamino)but-2-yn-1-yl 2-(2-((2,6-dichlorophenyl)amino)phenyl)acetate)(2-8). The physical and spectral properties of the prepared compounds were measured, and the bioassay conducted for some substances by investigating their antibiotic activity. The two compounds (1,2) were also selected to test their anticancer activity against one of its types, a breast cancer cell line (MCF-7), using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay using multiple concentrations compared to the normal cell line (WRL-68). The previous results showed the effect of the two compounds on the cancer cell line more than its effect on the normal cell line, the results showed significant differences ($p \leq 0.0001$) by calculating the (IC_{50}) when treating compounds (1,2) for MCF-7 cancer cells at (250,300) µg/ml and for normal cells at (983,1292) µg/ml respectively. It is believed that the reason for its effectiveness is because contains acetylenic amine in compound (1) in addition to morpholine ring in compound (2), which research has proven to be effective in multiple medicines. Some pharmacokinetic activities of the prepared compounds have been predicted to support the discovery of new drugs using the Swiss ADME website*.

Keywords: *Propargyl bromide, Mannich base, MTT method, Cytotoxicity Assay, ADME.*

1. Introduction

Many derivatives containing an acetylene moiety over the years were studied and many of them had drug effectiveness in the clinical test phase. Compounds derived from acetylene have shown promising results as a treatment for various diseases. These derivatives have been found to possess anti-malarial [1], anti-tuberculosis, anti-cancer, and anti-microbial properties [2]. Acetylene compounds are also included in the composition of some plants, such as the mushrooms Hydnum repandum and Polyporus biformis, which have been used to enhance and strengthen immunity as antimicrobials and anticancers [3].

Not only are acetylene compounds essential in the preparation of various substances, but they can also be found in a plethora of marine sponges, corals, and plant species, as well as in fungal and bacterial cultures [4, 5]. These aforementioned compounds play a fundamental role in medicinal chemistry due to the presence of electronic effects mimicking the influences of aromatic rings, of which present in sedative-hypnotic drugs and synthetic retinoids; tazarotene, various contraceptives, and natural pesticides [4, 6]. These compounds have been found to have pharmacological activity, due to their advantages summarized in their activity and low toxicity [7], in addition to being rich in electrons, which facilitates their association with receptor proteins within living tissues [8]. Some

research has demonstrated their efficacy as anticancer agents [9, 10], hypertensive agents [11], and antiproliferative activity [7, 12, 13].

2. Materials and methods

Chemicals and reagents: All the reagents and solvents were purchased; [2-(2-((2,6-dichlorophenyl)amino)phenyl)acetic acid], Cu2Cl2, Dioxane, formaldehyde, secondary amines, glacial acetic acid and Propargyl bromide from Flulka Co. All solvents used were checked for purity before use by measuring their boiling points. Instrumentation: The products were characterized by FT-IR spectra recorded on Burker's Alpha-platinum ATR (Germany) FT-IR spectrophotometer, melting points were uncorrected and measured with a thermo-digital device IA 9300.

In the lab at the University of Ega-Oxford, England, a few tools were utilized to conduct our experiment. First, TMS was used as an internal reference to ensure measurements were accurate. Secondly, d6-DMSO was utilized as a solvent to dissolve the sample. To capture the data needed, the proton NMR spectra need to be recorded on a Bruker (AS 400 MHz). (1H-NMR) spectra provide insight into the various atoms in a molecule and how they are arranged. To better understand the data, signals assigned using the following abbreviations: *s* for singlet, *d* for doublet, *t* for triplet, and *m* for multiplet. These signals helped us analyze the molecular structure of tested sample.

2.1. Chemicals

2.1.1. General procedure for preparation of sodium 2-(2-((2,6-dichlorophenyl)amino)phenyl)acetate (I). The carboxylic acid was converted to its salt by mixing (0.01 mol, 3 g) of the starting material [2-(2-((2,6-dichlorophenyl)amino)phenyl)acetic acid] with an aqueous solution of sodium bicarbonate (0.01 mol, 0.84 g) dissolved in 15 ml of distilled water and stirred for half an hour until a clear solution was obtained. Filter and evaporate the water from the filtrate completely under vacuum pressure to obtain the acid salt in the form of a pure white powder.

1. Synthesis of prop-2-yn-1-yl 2-(2-((2,6-dichlorophenyl)amino)phenyl)acetate (1) [14].

In a 100 mL round flask, a mixture of (0.02 mol, 6.4 g) of acid salt (I), (10 mL) of propargyl bromide and (10 ml) of DMSO was refluxed for 3 h in a water bath at 80°C, and the solution was poured onto a beaker containing crushed ice (40 ml) with stirring to obtain an oily product which was treated by adding more crushed ice with continuous rubbing and stirring until it turned into a precipitate, then filtered , dried and recrystallized with benzene to obtain a precipitate with yellow color 76 % yield, and a melting point (87-89 °C). IR (υmax cm^{-1}): 3318 (N-H), 3279 (≡C-H), 2977-2898 (C-H), 2160 (C≡C), 1716 (C=O ester), 1206,1138 (C-O-C asy, sym) ; (^{1}H-NMR) (DMSO-d6 ppm): δ= 9.23 (s, 1H, NH), 7.53-6.75 m, 7H, Ar-H), 4.65 (m, 2H, $OCH_2C{\equiv}C$), 3.61 (t, 2H, CH_2CO_2), 3.42 (s,1H, C≡C-H).

2. A general method for the synthesis of acetylenic amine compounds (2-8) [14, 15]:

Mannich bases were prepared by mixing (0.002 mol, 0.67 g) of acetylene compound (1) with a secondary amine (0.003 mol) in a round bottom flask fitted with a reflux condenser and after cooling the mixture to (0 °C), adding (0.1 g) of Cu_2Cl_2 and paraformaldehyde (0.003 mol) with (15 mL) dioxane free peroxide at 0°C, then slowly added dropwise glacial acetic acid (0.5 mL) with stirring for five minutes, refluxing with continues stirring for 2 hours in dry condition. To obtain a pure product, the solution is acidified by adding dilute hydrochloric acid (1:1) to pH = 1 with continuous stirring, then the mixture is extracted three times by adding (10 ml) of diethyl ether and after collecting the aqueous layer, it is neutralized by adding sodium bicarbonate to form a precipitate that is filtered, dried and then recrystallized with a mixture of benzene and petroleum ether (80-100 °C) as shown in **Scheme 1** and **Table 1**.

4-morpholinobut-2-yn-1-yl-2-(2-((2,6-dichlorophenyl)amino)phenyl)acetate (2): Pale yellow, yield 68 %, m.p (139-141°C), IR (υmax cm^{-1}): 3312 (N-H), 2972-2909 (C-H), 2196 (C≡C), 1722 (C=O ester), 1211,1129 (C-O-C asy, sym) ; (^{1}H-NMR) (DMSO-d6 ppm): δ= 9.36 (s, 1H, NH),

7.64-6.86 (m, 7H, Ar-H), 4.77 (m, 2H, $OCH_2C{\equiv}C$), 3.67 (t, 2H, CH_2CO_2), 3.58 (t, 4H, CH_2O morpholine ring), 3.36 (t, 2H, $C{\equiv}C\text{-}CH_2N$), 2.52 (t, 4H, CH_2N morpholine ring).

4-(9H-carbazol-9-yl)but-2-yn-1-yl-2-(2-((2,6-dichlorophenyl)amino)phenyl)acetate (3): Pale orange, yield 59 %, m.p (209-211°C), IR (υmax cm^{-1}): 3317 (N-H), 2976-2911 (C-H), 2183 (C≡C), 1726 (C=O ester), 1208,1133 (C-O-C asy, sym) ; (^{1}H-NMR) (DMSO-d6 ppm): δ= 9.41 (s, 1H, NH), 8.22-6.63 (m, 15H, Ar-H), 4.69 (m, 2H, $OCH_2C{\equiv}C$), 4.51 (t, 2H, $C{\equiv}C\text{-}CH_2N$), 3.65 (t, 2H, CH_2CO_2).

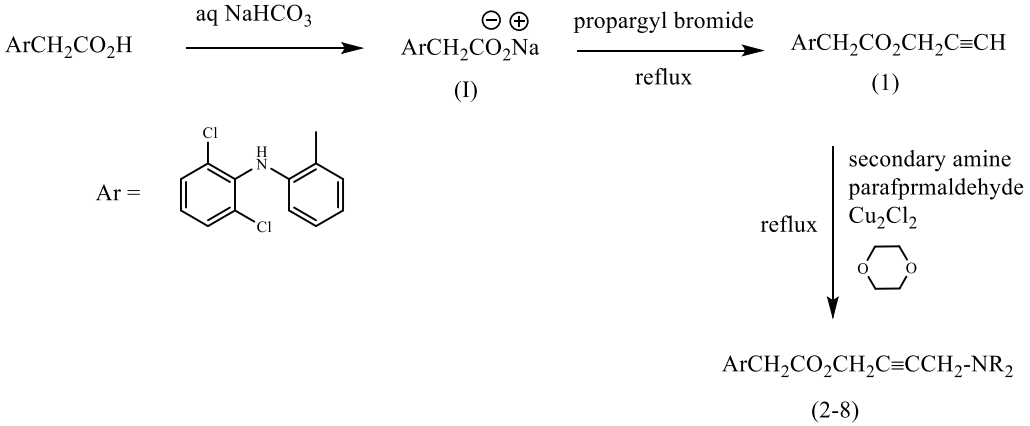
4-(diphenylamino)but-2-yn-1-yl-2-(2-((2,6-dichlorophenyl)amino)phenyl)acetate (4): Yellow, yield 53 %, m.p (101-104°C), IR (υmax cm^{-1}): 3310 (N-H), 2979-2915 (C-H), 2187 (C≡C), 1728 (C=O ester), 1210,1143 (C-O-C asy, sym). (^{1}H-NMR) (DMSO-d6 ppm): δ= 9.39 (s, 1H, NH), 7.62-6.57 (m, 15H, Ar-H), 4.66 (m, 2H, $OCH_2C{\equiv}C$), 4.54 (t, 2H, $C{\equiv}C\text{-}CH_2N$), 3.72 (t, 2H, CH_2CO_2).

4-(2-ethylpiperidin-1-yl)but-2-yn-1-yl-2-(2-((2,6-dichlorophenyl)amino)phenyl) acetate (5): Pale yellow, yield 62 %, m.p (153-155°C), IR (υmax cm^{-1}): 3289 (N-H), 2976-2857 (C-H), 2182 (C≡C), 1730 (C=O ester), 1211,1132 (C-O-C asy, sym) ; (^{1}H-NMR) (DMSO-d6 ppm): δ= 9.34 (s, 1H, NH), 7.52-6.58 (m, 7H, Ar-H), 4.63 (m, 2H, $OCH_2C{\equiv}C$), 3.67 (t, 2H, CH_2CO_2), 3.22 (t, 2H, $C{\equiv}C\text{-}CH_2N$), 2.46-1.28 (m, 10H, CH_2), 0.91 (m, 3H, CH_3) for secondary amine ring).

4-(dibutylamino)but-2-yn-1-yl-2-(2-((2,6-dichlorophenyl)amino)phenyl)acetate (6): Pale brown, yield 64 %, m.p (123-125°C), IR (υmax cm^{-1}): 3293 (N-H), 2975-2893 (C-H), 2189 (C≡C), 1729 (C=O ester), 1205,1144 (C-O-C asy, sym); (^{1}H-NMR) (DMSO-d6 ppm): δ= 9.42 (s, 1H, NH), 7.46-6.43 (m, 7H, Ar-H), 4.57 (m, 2H, $OCH_2C{\equiv}C$), 3.63 (t, 2H, CH_2CO_2), 3.34 (t, 2H, $C{\equiv}C\text{-}CH_2N$), 2.52-1.36 (m, 12H, CH_2) & 0.99 (t, 6H, CH_3) for secondary amine.

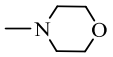
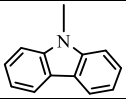
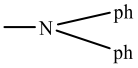
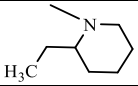
4-(dipropylamino)but-2-yn-1-yl-2-(2-((2,6-dichlorophenyl)amino)phenyl)acetate (7): Pale yellow, yield 58 %, m.p (108-110 °C), IR (υmax cm^{-1}): 3291 (N-H), 2973-2858 (C-H), 2185 (C≡C), 1724 (C=O ester), 1201,1146 (C-O-C asy, sym); (^{1}H-NMR) (DMSO-d6 ppm): δ= 9.37 (s, 1H, NH), 7.58-6.47 (m, 7H, Ar-H), 4.64 (m, 2H, $OCH_2C{\equiv}C$), 3.59 (t, 2H, CH_2CO_2), 3.28 (t, 2H, $C{\equiv}C\text{-}CH_2N$), 2.42-1.48 (m, 8H, CH_2) & 1.07 (t, 6H, CH_3) for secondary amine.

4-(diethylamino)but-2-yn-1-yl-2-(2-((2,6-dichlorophenyl)amino)phenyl)acetate (8): Pale yellow, yield 67 %, m.p (98-100 °C), IR (υmax cm^{-1}): 3302 (N-H), 2978-2867 (C-H), 2191 (C≡C), 1729 (C=O ester), 1209,1145 (C-O-C asy, sym) ; (^{1}H-NMR) (DMSO-d6 ppm): δ= 9.41 (s, 1H, NH), 7.43-6.52 (m, 7H, Ar-H), 4.68 (m, 2H, $OCH_2C{\equiv}C$), 3.64 (t, 2H, CH_2CO_2), 3.36 (t, 2H, $C{\equiv}C\text{-}CH_2N$), 2.51 (t, 4H, CH_2) & 1.1 (t, 6H, CH_3) for secondary amine).



Scheme 1. Route of the synthesis of acetylenic amine compounds (2-8)

Table 1. The derivatives of acetylenic amine compounds (2-8)

Compounds No.	-NR_2	Compounds No.	-NR_2
2		6	—$N(C_4H_9)_2$
3		7	—$N(C_3H_7)_2$
4		8	—$N(C_2H_5)_2$
5			

2.2. Antimicrobial activity:

To evaluate the antimicrobial activity of the compounds numbered (1, 2, 3, and 5); disk diffusion method (16) was performed (**Table 2**). Two G^+ and two G^- bacteria were submitted to the current study (*Streptococcus pneumonia*, *Staphyllococcus aureus, Klebsiella spp and E coli;* respectively), added to one pathogenic fungus, *Candida albicans*. The microbial isolates were obtained and authenticated in the Microbiology Laboratory, College of Science - University of Mosul. Each isolate was sub-cultured in a selective media, then the dilution method was applied to assume Minimum inhibitory concentration (MIC). After being inoculated, the Petridishes were placed in a warm incubator where they could grow and thrive. They were given plenty of fresh air and left to incubate for a full day at a toasty 37°C.

Table 2. Microorganisms used to test the antibiotic action of each substance (1, 2, 3, and 5).

Microorganisms used		Species
Bacteria species.	Gram $^+$	*Streptococcus pneumonia*
		Staphylococcus aureus
	Gram $^-$	*Klebsiella spp.*
		E. coli
Yeast	fungus	*Candida albicans*

Prepare ingredient concentrations: A stock solution was prepared from each of the synthesized compounds (1, 2, 3, and 5) in a concentration of (100 µg/mL), that used then to prepare ingredient concentrations (75, 50, 25 µg/mL) of each compound [16, 17].

Disc diffusion test: A suspension of 0.5 MacFarland was prepared from bacteria mixed with Muller-Hinton broth, and cultured on the Muller–Hinton agar plates exploiting a sterile cotton swab [16, 17].

Estimating antibacterial activity: The tested microbes were cultured on Müller-Hinton to perform a disc diffusion sensitivity test. In each Petridis, five wells were perforated in agar plates at 4 mm depth, and then 0.1 ml of each dilution of the studied compounds was put inside the well. Cultured (temperature 37 °C, duration 24 hours), then the inhibition zone (mm) was estimated to estimate the antibiotic actions of each substance at various dilutions [17].

2.3. Cytotoxicity assay [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay] [18, 19]:

At the Center for Natural Product Research and Drug Discovery, the team selected two compounds (1 and 2) that showed promise for fighting breast cancer. But they didn't stop there. To truly test their potential, the compounds underwent rigorous cytotoxicity testing against both cancerous and normal cell lines (MCF-7 and WRL-68, respectively). The team used the MTT colorimetric method to evaluate the efficacy of these compounds. Department of Pharmacology at the Faculty of Medicine, University of Malaya in Kuala Lumpur kindly provided the cell lines,

which were carefully preserved in liquid nitrogen. The cancer cell lines were propagated, purified, and put to the test at the Biotechnology Research Center at Al-Nahrain University. The results of this testing could have far-reaching implications for the future of breast cancer treatment.

2.4. Swiss ADME calculations:

The synthesized substances were tested for their ability to predict appropriate pharmacokinetic properties. Our combined analogs' ADME was taken into account by efficient and precise techniques that predicted the physical and pharmacological features of the produced drugs. Several parameters and their recommended values have been determined, and are listed in **Table 3**.

Table 3. Some Swiss ADME parameters of compounds (1-8)

Molecule No.	Formula	MW	TPSA	ESOL Class	GI absorption	BBB permeant	Lipinski violations	Bio availability Score	Synthetic Accessibility
1	$C_{17}H_{13}C_{l2}NO_2$	334.2	38.33	MS	High	Yes	1	0.55	2.74
2	$C_{22}H_{22}C_{l2}N_2O_3$	433.33	50.8	MS	High	Yes	0	0.55	3.62
3	$C_{30}H_{22}C_{l2}N_2O_2$	513.41	43.26	PS	Low	No	2	0.17	3.75
4	$C_{30}H_{24}C_{l2}N_2O_2$	515.43	41.57	PS	Low	No	2	0.17	3.96
5	$C_{25}H_{28}C_{l2}N_2O_2$	459.41	41.57	PS	High	Yes	1	0.55	4.25
6	$C_{26}H_{32}C_{l2}N_2O_2$	475.45	41.57	PS	Low	No	1	0.55	4.02
7	$C_{24}H_{28}C_{l2}N_2O_2$	447.4	41.57	PS	High	No	1	0.55	3.85
8	$C_{22}H_{24}C_{l2}N_2O_2$	419.34	41.57	MS	High	Yes	1	0.55	3.66
PS= Poorly soluble, MS= Moderately soluble									

3. Results and Discussion

3.1. The chemistry: The validity of the methods used in this research has been proven by measuring some of the physical and spectral properties of the prepared compounds. It was found that there is a difference in the melting points between compound (1) and compounds (2-8) by more than 10 degrees. It is noted that some of the products were in small percentages, and this may be due to the reaction requiring a longer time to complete and not because of the side products, because the Mannich method used was taken from reliable sources and is a well-known method, especially using the catalyst (Cu_2Cl_2) in the reaction. The products were also processed by purifying them as described above in the synthesis of compounds (2-8). The infrared spectroscopy of the compounds (1-8), where they gave stretching bands of the N-H bond with limits (3318-3289) in the form of medium-intensity bands and at the range (2196-2160) they gave weakly-stretched of the (C≡C) bond, while the ester bond stretching (C=O) appeared in the range (1730-1716) of strong intensity accompanied by two asymmetrical and symmetrical bands of strong (C-O-C) bonding at (1211-1201) and (1146-1129) respectively, noting the disappearance of the stretching absorption (C=O) of the carboxylic acid of the starting material and also the disappearance of the stretching band which belongs to hydroxyl group. What confirmed the validity of the method is the appearance of the (≡C-H) at (3279 cm^{-1}) in compound (1) and its disappearance in the compounds (2-8) with the appearance of additional bands belonging to the secondary amine represented by the C-H stretch bands of the groups of CH_2, CH_3 in the compounds (2, 5-8). As for the (^{1}H-NMR), several bands appeared, including: the N-H amine proton in all compounds at the region (9.42-9.23) in the form of a single band with the disappearance of the O-H band of carboxylic acid. Multiple bands also appeared at (8.22-6.43) belonging to the protons of the aromatic rings and multiple bands at (4.77-4.57) belonging to the CH_2 protons adjacent to the high electronegative oxygen atom ($OCH_2C{\equiv}C$), protons of CH_2CO_2 appeared at (3.72-3.59) in the form of triple beams with the appearance of triples also at (4.54-3.22) belonging to the CH_2 protons adjacent to the nitrogen atom $C{\equiv}CCH_2N$, which were entered by the Mannich reaction. The triple-terminal bond proton (≡C-H)

appeared only in compound (1) in the form of a single band at (3.42) and disappeared in compounds (2-8).

3.2. Biological Stady: To make the work more feasible, the biological activity of some of the synthesized compounds (1, 2, 3, 5) as antimicrobial compounds was tested. Two species of $Gram^{+}$ bacteria (*Streptococcus pneumonia and Staphylococcus aureus*) and two species of $Gram^{-}$ bacteria (*Klebsiella spp* and *E.coli*) , added to one species of fungus (*Candida albicans*) were chosen as targeted opportunistic microorganisms. According to disk diffusion test, it was found that the compounds 1, 3, and 5 had no considerable inhibitory effect against the four microbes submitted to the current study. On the other hands, compound (2) award inhibitory effect against *Streptococcus pneumoniae* and *Staphylococcus aureos* at concentrations (100, 75, 50, 25µg/ml) with inhibitory zones of (9.68, 8.53, 7.78, 7.32mm) and (14.92, 13.94, 8.77, 7.47 mm); respectively, as listed in **Table 4**.

Table 4. The antimicrobial effect of compounds (1,2,3, and 5) according disk diffusion test.

Micro organisms	1				2				3				5			
	100 µg/ml	75 µg/ml	50 µg/ml	25 µg/ml	100 µg/ml	75 µg/ml	50 µg/ml	25 µg/ml	100 µg/ml	75 µg/ml	50 µg/ml	25 µg/ml	100 µg/ml	75 µg/ml	50 µg/ml	25 µg/ml
Streptococcus pneumonia	R	R	R	R	9.68	8.53	7.78	7.32	R	R	R	R	R	R	R	R
Staphylococcus aureus	R	R	R	R	14.92	13.94	8.77	7.47	R	R	R	R	R	R	R	R
Klebsiella spp	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
E-coli	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Candida albicans	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
R = Resistance ≤ 7 value																

The activity of compound (2) may be attributed to the presence of a heterocyclic morpholine ring, which has been proven to have multiple bioactivities. M. Marinescu and *et.al*. Was referred to a number of Mannich bases those prepared from benzimidazole and had antibacterial activity against some G^{-} bacteria, like: (*S. aureus, B. flexus, C sporogenes and S. mutans*) [20]. Christina Zalaro and *et.al* were prepared a new series of hybrid Mannich bases of pyrazolo benzimidazole, they recorded antibacterial effects of the synthesized compounds against G+ bacteria (*Staphylococcus aureus* and *enterococcus faecalis) and G- bacteria* (*Pseudomonas aeruginosa and Escherichia coli)*. They concluded that the synthesized compounds illustrated a higher inhibitory effect against all bacterial isolates than the antibiotic of choices (Metronidazole, Nitrofurantoin) [21]. As for the researcher (S. Manap), he prepared number of derivatives that contain the morpholine ring; it was found that these compounds manifested a significant bioactivity against some pathogenic bacteria species [22].

3.3. Cytotoxicity Assay [23, 24]: The cytotoxicity assay (MTT assay) was performed to determine the toxic effect of the two compounds (1,2) at different concentrations on (MCF-7) breast cancer cells compared to the healthy cell line (WRL-68). Compound (2) has been carefully selected to contain the powerful morpholine ring. This ring has proven to be a highly effective pharmaceutical compound, thanks to its unique physio-chemical properties that boost both hydrophilic and lipophilic reactions. These properties work together to enhance the solubility of blood and improve the permeability of the brain's overall structure. What's truly remarkable about the morpholine ring is its similarity to neurotransmitters found in the active compounds of the central nervous system. For example, aryl-morpholines have been shown to play a key role in crossing the blood-brain barrier and improving brain function [23]. It's no wonder that this ring has been the subject of many studies and reviews. So, while the biological activity of morpholine derivatives has been covered in

other studies, the potential benefits of this powerful compound cannot be overstated. With compound (2), researchers have unlocked a new level of potential in the world of pharmaceuticals [23-27].

The cytotoxicity of the two selected compounds (1, 2) was tested by measuring their *invitro* anti-proliferative activity against one of the cancer cells lines (MCF-7). The assay is based on the conversion of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) yellow by the action of mitochondrial succinate dehydrogenase to insoluble formazan violet. The test results of compound (1) showed efficacy against MCF-7 breast cancer cells with inhibition rates (51.47, 36.58, 31.98, 9.95, 3.78, 3.36, 3.63) % treated with concentrations (1000, 500, 250, 125, 62.5, 31.25, 15.75) µg/ml, respectively, while it gave less cytotoxicity on healthy cells (WR-68), where the percentage of inhibition (21.87, 11.73, 6.41, 5.05, 4.94, 4.13, 4.06) % ranged at concentrations (1000, 500, 250, 125, 62.5, 31.25, 15.75) µg/ml as shown in **Table 5**.

Table 5. Effect of compound (1) on breast cancer cell line MCF-7 and normal cell line WRL-68 using the MTT assay for a period of 24 hours at a temperature of 37°C.

Cell line	Viability% (SD± mean)						
	The concentrations used (µg/ml)						
	1000	500	250	125	62.5	31.25	15.75
MCF-7	48.53±2.03	63.42±2.75	68.02±3.87	90.05±2.42	96.22±0.67	96.64±1.36	96.37±0.81
WRL68	78.13±1.44	88.27±1.45	93.59±2.10	94.95±0.92	95.06±0.63	95.87±1.12	95.94±0.20

In vitro cytotoxic activity results were determined by calculating the (IC_{50}) value, which represents the concentration of a compound required to halt the growth of 50% of tumor cells in comparison to untreated cells. We used Cisplatin as a positive control for our test. This allowed us to compare our results with known cytotoxic agents and determine the effectiveness of our compound. The results showed significant differences ($p \leq 0.0001$) by calculating the (IC_{50}) when treating compound (1) for MCF-7 cancer cells at (250) µg/ml and for normal cells at (983) µg/ml as shown in **Fig.1**.

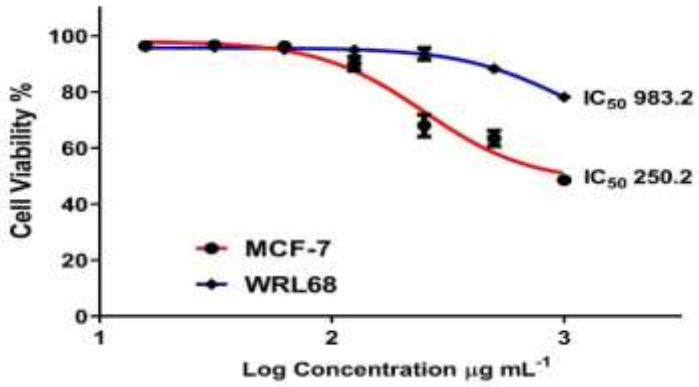


Fig. 1. Effect of compound (1) on MCF-7 and WRL-68 cell line using MTT assay

When investigating the effect of the new compound (2) on the MCF-7 cancer cell line, the results showed effectiveness, the inhibition rates reached (45.10, 36.30, 22.38, 9.41, 5.21, 4.59, 3.86) % tagged with concentrations (1000, 500, 250, 125, 62.5, 31.25, 15.75) µg/ml, respectively. But when testing the toxicity of compound (2) on the normal cell line WRL-68, it showed inhibition rates that ranged between (30.44, 13.97, 7.87, 3.82, 3.05, 5.09, 3.90) % at concentrations (1000, 500, 250, 125, 62.5, 31.25, 15.75) µg/ml, as shown in **Table 6**.

Table 6. Effect of compound (2) on the breast cancer cell line MCF-7 and the normal cell line WRL-68 using MTT assay for a period of 24 hours at a temperature of 37°C.

Cell line	Viability% (SD± mean)						
	The concentrations used (µg/ml)						
	1000	500	250	125	62.5	31.25	15.75
MCF-7	54.90±1.00	63.70±2.12	77.62±2.41	90.59±1.80	94.79±1.33	95.41±1.41	96.14±1.05
WRL68	69.56±3.20	86.03±0.85	92.13±1.56	96.18±1.25	96.95±1.14	94.91±2.20	96.10±0.48

The results showed significant differences ($p \leq 0.0001$) when calculating the (IC_{50}) upon compound (2) treatment for (MCF-7) cancer cells (300) µg/ml and for (WRL-68) normal cells (1292) µg/ml. The previous results showed the effect of the two compounds on the cancer cell line more than its effect on the normal cell line, as shown **in Fig. 2**.

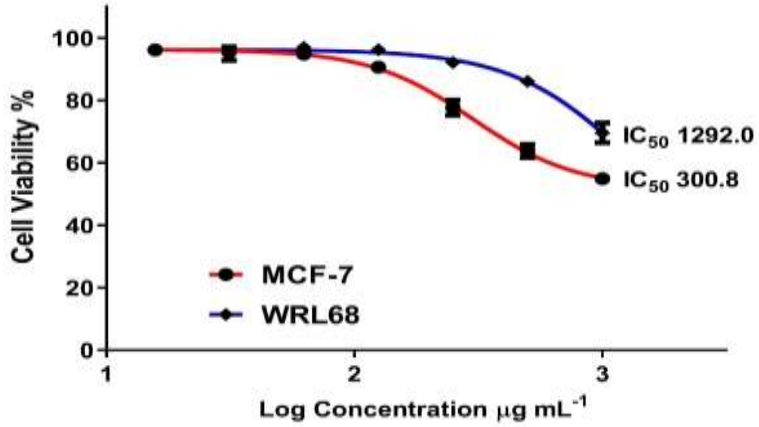


Fig. 2. Effect of compound (2) on MCF-7 and WRL-68 cell line using MTT assay

Upon conducting a thorough examination of the published research, it was discovered that Boryczka and their group had successfully synthesized a plethora of novel acetylene derivatives from betulin. These newly formulated compounds were then subjected to rigorous testing to determine their cytotoxic potential against a variety of human cancer cell lines including SW707 (colorectal adenocarcinoma), CCRF/CEM (leukemia), T47D (breast cancer), and P388 murine leukemia. Furthermore, the anti-proliferative activity of these compounds was evaluated using the MTT method in vitro, alongside normal Balb3T3 fibroblast cell lines. The results showed that there is a noticeable effect of the prepared compounds against the above-mentioned cancer cell lines in varying proportions [28]. Research has also proven that natural and synthetic naphthoquinones possess many anti-cancer efficacies for a number of derivatives prepared from Lawsone (2-hydroxy-1, 4-naphthoquinone) obtained from dried henna leaves (Lawsonia inermis). New Lawone-derived aminonaphthoquinones were synthesized by Mannich reaction and after testing their anticancer activity using the MTT method, they gave toxic effect against human hepatocellular carcinoma cell line HepG2 [29]. There are other researchers who tested the effectiveness of some morpholine derivatives and gave their results which showed that both N-acetyl and N-benzoyl derivatives give high effectiveness against some cancer lines [30].

3.4. Swiss ADME prediction: According to the recorded values in Table (3), it is clear that the compounds (1,2,3,6, and 9) penetrate the blood brain barrier and are active in the CNS, while compound (1,3) does not violate any of Lipinski's five principles. In terms of projected human gastrointestinal absorption, the bulk of the compounds had high GIT absorption, low solubility, and good bioavailability.

4. Conclusions

This study focuses on the preparation of acetylenic amine derivatives derived from 2-(2-((2,6-dichlorophenyl)amino)phenyl)acetic acid through the Mannich reaction using (Cu_2Cl_2) as catalyst, a series of compounds (1-8) were successfully prepared and characterized. The antimicrobial and antiproliferative activities of some compounds were evaluated, revealing promising results.

Testing the biological activity of some of the prepared compounds (1, 2, 3, 5) against five types of microbes at concentrations (25, 50, 75, 100 μg/ml), where the highest inhibitory activity was for compound 2 against Gram+ bacteria (*Streptococcus pneumonia*, *Staphylococcus aureus*) at a concentration of (100 μg/ml). A cytotoxicity test (MTT assay) was performed to determine the toxic effect of the two compounds (1,2) at different concentrations on breast cancer cells (MCF-7) compared to a healthy cell line (WRL-68). The compounds showed significant effectiveness in inhibiting the growth of breast cancer cells compared to normal cells, indicating their potential as anti-cancer agents. Compound (2) was carefully selected because it contains a morpholine ring. This ring has proven to be a highly effective pharmaceutical compound, thanks to its unique physicochemical properties that promote hydrophilic and lipophilic interactions. These properties work together to enhance the solubility of the blood and improve the permeability of the overall structure of the brain.

The efficacy achieved might be linked to acetylinic amine group and morphine ring in compound 1 and 2, respectively. With the help of ADME website, these produced compounds might be a template for new drug discovery opening the horizons for new pharmaceutical product, research, and design.

References

1. Maganti L.H.B., Ramesh D., Vijayakumar B.G., Khan M.I.K., Dhayalan A., Kamalraja J., Kannan T. Acetylene containing 2-(2-hydrazinyl) thiazole derivatives: design, synthesis, and in vitro and in silico evaluation of antimycobacterial activity against Mycobacterium tuberculosis // RSC advances, 2022, V. 12(14), p. 8771-8782. https://doi.org/10.1039/D2RA00928E
2. Ruddarraju R.R., Murugulla A.C., Kotla R., Tirumalasetty M.C.B., Wudayagiri R., Donthabakthuni S., Parasa L.S. Design, synthesis, anticancer, antimicrobial activities and molecular docking studies of theophylline containing acetylenes and theophylline containing 1, 2, 3-triazoles with variant nucleoside derivatives // European journal of medicinal chemistry, 2016, V. 123, p. 379-396. https://doi.org/10.1016/j.ejmech.2016.07.024
3. Kuklev D.V., Domb A.J., Dembitsky V.M. Bioactive acetylenic metabolites // Phytomedicine, 2013, V. 20(13), p. 1145-1159. https://doi.org/10.1016/j.phymed.2013.06.009
4. Almohaywi B., Yu T.T., Iskander G., Sabir S., Bhadbhade M., Black D.S., Kumar N. Synthesis of alkyne-substituted dihydropyrrolones as bacterial quorum-sensing inhibitors of Pseudomonas aeruginosa // Antibiotics, 2022, V. 11(2), p. 151. https://doi.org/10.3390/antibiotics11020151
5. Trofimov B.A., Gusarova N.K. Acetylene: New prospects of classical reactions // Russian Chemical Reviews, 2007,V. 76(6), p. 550-570. https://doi.org/10.1070/RC2007v076n06ABEH003712.
6. Camille G.W., Aldous D., Raboisson P., Rognan D. The Practice of Medicinal Chemistry- 4th Edition, 2015, eBook ISBN: 9780124172135
7. Kadela M., Jastrzębska M., Bębenek E., Chrobak E., Latocha M., Kusz J., Książek M., Boryczka S. Synthesis, structure and cytotoxic activity of mono-and dialkoxy derivatives of 5, 8-quinolinedione // Molecules, 2016, V. 21(2), 156. https://doi.org/10.3390/molecules21020156.
8. AL-Obaidi O.H.S. Preparation and Theoretical Study of some Transition Metal Complexes with Acetylenic Amine and Study of its Biological Activity // International Journal of Applied, 2011, V. 1(3), p. 41-46.

9. Siddiq A., Dembitsky V. Acetylenic anticancer agents // Anti-Cancer Agents in Medicinal Chemistry, 2008, V. 8(2), p. 132-170. https://doi.org/10.2174/187152008783497073
10. Sirazhetdinova N.S., Savelyev V.A., Baev D.S., Golubeva T.S., Klimenko L.S., Tolstikova T.G., Shults E.E. Synthesis, characterization and anticancer evaluation of nitrogen-substituted 1-(3-aminoprop-1-ynyl)-4-hydroxyanthraquinone derivatives // Medicinal Chemistry Research, 2021, V. 30(8), p. 1541-1556.https://doi.org/10.1007/s00044-021-02754-1
11. Voronin V.V., Ledovskaya M.S., Bogachenkov A.S., Rodygin K.S., Ananikov V.P. Acetylene in organic synthesis: Recent progress and new uses // Molecules, 2018, V. 23(10), 2442. https://doi.org/10.3390/molecules23102442
12. Kadela-Tomanek M., Jastrzębska M., Bębenek E., Chrobak E., Latocha M., Kusz J., Boryczka S. New acetylenic amine derivatives of 5, 8-quinolinediones: Synthesis, crystal structure and antiproliferative activity // Crystals, 2017, V. 7(1), 15. https://doi.org/10.3390/cryst7010015
13. Gayathri S., Viswanathamurthi P., Malecki J.G. Efficient multicomponent synthesis of propargylamines catalyzed by Cu (I) complexes encompassing hydrazone ligands under solvent-free condition // Inorganica Chimica Acta, 2022, V. 535, 120853. https://doi.org/10.1016/j.ica.2022.120853
14. Sheat M.A., Saeed Z.F. Synthesis and study of acetylenic amines derevitives containing sulfonamide, β-naphthoxy and hippurate groups // National J. Chemistry, 2006, V. 21, p. 53-62.
15. Saeed F.Z. Synthesis of some Acetylenic Amino Derivatives from Salicylic Acid // Journal of Education and Science, 2010, V. 23(3), p. 48-53.
16. Acs K., Aguilar C.N., Aguirre-Joya J., Amin A., Aumeeruddy-Elalfi Z., Ballal M., Sukhov B.G. Antibiotic Resistance. Mechanisms and New Antimicrobial Approaches, 2016, Academic Press. https://doi.org/10.1016/B978-0-12-803642-6.00012-5
17. Abdollahzadeh E., Nematollahi A., Hosseini H. Composition of antimicrobial edible films and methods for assessing their antimicrobial activity: A review // Trends in Food Science & Technology, 2021, V. 110, V. 291-303. https://doi.org/10.1016/j.tifs.2021.01.084
18. Arimah B.D., Ogunlowo O.P. Identification of lactic acid bacteria isolated from Nigerian foods: medical importance and comparison of their bacteriocins activities // J. Nat. Sci. Res, 2014, V. 23(4), p. 76-86.
19. Stefanowicz-Hajduk J., Ochocka J.R. Real-time cell analysis system in cytotoxicity applications: Usefulness and comparison with tetrazolium salt assays // Toxicology Reports, 2020, V. 7, p. 335-344. https://doi.org/10.1016/j.toxrep.2020.02.002
20. Marinescu M., Cinteză L.O., Marton G.I., Chifiriuc M.C., Popa M., Stănculescu I., Stavarache C.E. Synthesis, density functional theory study and in vitro antimicrobial evaluation of new benzimidazole Mannich bases // BMC chemistry, 2020, V. 14(1), p. 1-16. https://doi.org/10.1186/s13065-020-00697-z
21. Zalaru C., Dumitrascu F., Draghici C., Tarcomnicu I., Marinescu M., Nitulescu G.M., Chifiriuc M.C. New Pyrazolo-Benzimidazole Mannich Bases with Antimicrobial and Antibiofilm Activities // Antibiotics, 2022, V. 11(8), 1094. https://doi.org/10.3390/antibiotics11081094
22. Manap S. Synthesis and in vitro antioxidant and antimicrobial activities of novel 3-alkyl (aryl)-4-[3-methoxy-4-(2-furylcarbonyloxy)-benzylidenamino]-4, 5-dihydro-1 H-1, 2, 4-triazol-5-ones, and their N-acetyl, N-Mannich base derivatives // Journal of the Iranian Chemical Society, 2022, V. 19(4), p. 1347-1368. https://doi.org/10.1007/s13738-021-02386-7
23. Lenci E., Calugi L., Trabocchi A. Occurrence of morpholine in central nervous system drug discovery // ACS Chemical Neuroscience, 2021, V. 12(3), p. 378-390. https://doi.org/10.1021/acschemneuro.0c00729
24. Saeed B.B., Saeed Z.F., Dawood N.MZ. Synthesis of some novel heterocyclic compounds from chalcones // Przemyst Chemiczny, 2023, V. 102(9), p. 884-894. https://doi.org/10.15199/62.2023.9.2.
25. Kourounakis A.P., Xanthopoulos D., Tzara A. Morpholine as a privileged structure: A review on the medicinal chemistry and pharmacological activity of morpholine containing bioactive

molecules // Medicinal Research Reviews, 2020, V. 40(2), p. 709-752. https://doi.org/10.1002/med.21634.
26. Arshad F., Khan M.F., Akhtar W., Alam M.M., Nainwal L.M., Kaushik S.K., Shaquiquzzaman M. Revealing quinquennial anticancer journey of morpholine: A SAR based review // European journal of medicinal chemistry, 2019, V. 167, p. 324-356. https://doi.org/10.1016/j.ejmech.2019.02.015.
27. Kumari A., Singh R.K. Morpholine as ubiquitous pharmacophore in medicinal chemistry: Deep insight into the structure-activity relationship (SAR) // Bioorganic Chemistry, 2020, V. 96, https://doi.org/103578.10.1016/j.bioorg.2020.103578.
28. Boryczka S., Bębenek E., Wietrzyk J., Kempińska K., Jastrzębska M., Kusz J., Nowak M. Synthesis, structure and cytotoxic activity of new acetylenic derivatives of botulin // Molecules, 2013, V. 18(4), p. 4526-4543. https://doi.org/10.3390/molecules18044526
29. Nariya P., Shukla F., Vyas H., Devkar R., Thakore S. Synthesis and characterization of Mannich bases of lawsone and their anticancer activity // Synthetic Communications, 2020, V. 50(11), p. 1724-1735. https://doi.org/10.1080/00397911.2020.1755440
30. Bold C.P., Gut M., Schürmann J., Lucena-Agell D., Gertsch J., Diaz J.F., Altmann K.H. Synthesis of Morpholine-Based Analogues of (−)-Zampanolide and Their Biological Activity // Chemistry–A European Journal, 2021, V. 27(19), p. 5936-5943. https://doi.org/10.1002/chem.202003996.

2-(2-((2,6-DİXLORFENİL)AMİN)FENİL)SİRKƏ TURŞUSUNUN ASETİLEN-AMİN TÖRƏMƏLƏRİNİN SİNTEZİ

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Xülasə: Tərkibində asetilen-amin qrupu olan bəzi birləşmələrin antimikrob və antiproliferativ təsir effektivliyini təsdiq edən bir sıra tədqiqatları təhlil edərək, bu işdə məqsədimiz 2-(2-((2,6-diklorofenil)amin)fenil)sirkə turşusu əsasında yeni asetilen-amin törəmələrinin alınması olmuşdur. Karbon turşusu duzunun propargil bromidlə Mannix reaksiyası nəticəsində (prop-2-yn-1-yl 2-(2-((2,6-diklorofenil)amino)fenil)asetat) birləşməsi (1) alınmışdır. Alınmış birləşmə mis xlorid katalizatorunun iştirakı ilə bir sıra ikinci dərəcəli aminlər və formaldehidlə qaynadılmış və nəticədə 7 (4-(dialkilamino)but-2-yn-1-il-2-(2-((2,6-diklorofenil)amino)fenil)asetat) birləşmələri (2-8) əldə edilmişdir. Alınan birləşmələrin fiziki və spektral xassələri ölçülmüş, bəzi maddələrin antibiotik aktivliyinin öyrənilməsi üçün bioanalizi aparılmışdır. 3-(4,5-dimetiltiazol-2-il)-2 birləşməsindən istifadə edərək onun növlərindən birinə, döş xərçəngi hüceyrə xəttinə (MCF-7) qarşı antionkoloji fəaliyyətini yoxlamaq üçün iki birləşmə (1 və 2) də seçilmişdir. 5-difeniltetrazolium bromidin (MTT) analizi normal hüceyrə xətti (WRL-68) ilə müqayisədə bir neçə qatılıqdan istifadə etməklə aparılmışdır. Əvvəlki nəticələr bu iki birləşmənin xərçəng hüceyrə xəttinə təsirinin normal hüceyrə xəttinə nisbətən daha çox olduğunu göstərmişdir. Nəticələr göstərir ki, (250-300) μg/ml-də MCF-7 xərçəng hüceyrələri və 983 və 1292 μg/ml-də normal hüceyrələr üçün birləşmələr (1 və 2) ilə müalicə edildikdə (IC50) hesablandıqda əhəmiyyətli fərqlər ($p \leq 0.0001$) alınır: müvafiq olaraq 983 və 1292 μg/ml. Hesab olunur ki, effektivliyin səbəbi, (1) və (2) birləşmələrinin tərkibində morfolin halqasının və asetilen-aminin olmasıdır ki, bunların da bir çox dərmanlarda təsirli olduğu göstərilmişdir. Alınmış birləşmələrin bəzi farmakokinetik xassələri İsveçrə ADME veb saytından istifadə edilməklə dərmanların kəşfinə töhfə verəcəyi proqnozlaşdırılmışdır.
Açar sözləri: propargil bromid, Mannix əsasları, MTT metodu, sitotoksiklik analiz, ADME.

СИНТЕЗ АЦЕТИЛЕНОВЫХ АМИНОПРОИЗВОДНЫХ 2-(2-((2,6-ДИХЛОРФЕНИЛ)АМИНО)ФЕНИЛ)УКСУСНОЙ КИСЛОТЫ

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Резюме: Проанализировав ряд исследований, демонстрирующих эффективность некоторых соединений, содержащих ацетиленовую аминогруппу, в качестве антимикробного и антипролиферативного действия, нашей целью являлось получение новых производных ацетиленового амина на основе соединения 2-(2-((2,6-дихлорфенил))амино)фенил)уксусной кислоты реакцией Манниха взаимодействием соли карбоновой кислоты с пропаргилбромидом и получением соединения (проп-2-ин-1-ил 2-(2-((2,6-дихлорфенил)амино))фенил)ацетат) (1). Полученное соединение кипятили с рядом вторичных аминов и формальдегидом в присутствии хлорида меди в качестве катализатора с получением 7 соединений (4-(диалкиламино)бут-2-ин-1-ил-2-(2-((2,6-дихлорфенил)амино)фенил)ацетат) (2-8). Измерены физические и спектральные свойства полученных соединений, а также проведен биоанализ некоторых веществ путем исследования их антибиотической активности. Два соединения (1 и 2) были выбраны также для проверки их противораковой активности против одного из его типов, линии клеток рака молочной железы (MCF-7), с использованием 3-(4,5-диметилтиазол-2-ил)-2. Анализ 5-дифенилтетразолия бромида (MTT) проводили с использованием нескольких концентраций по сравнению с нормальной клеточной линией (WRL-68). Предыдущие результаты показали, что влияние этих двух соединений на линию раковых клеток больше, чем на нормальную клеточную линию. Результаты показали значительные различия ($p \leq 0{,}0001$) при расчете (IC50) при обработке соединениями (1,2) для раковых клеток MCF-7 при (250 300) мкг/мл и нормальных клеток при 983 и 1292 мкг/мл, соответственно. Считается, что причина его эффективности заключается в том, что в соединениях (1) и (2) содержится ацетиленовый амин в дополнение к морфолиновому кольцу, эффективность которого, как показали исследования, применяется во многих лекарствах. Было предсказано, что некоторая фармакокинетическая активность полученных соединений будет способствовать открытию новых лекарств с использованием швейцарского веб-сайта ADME.

Ключевые слова: пропаргил бромид, основание Манниха, метод MTT, анализ цитотоксичности, ADME.