Synthesis, Characterization and Anticancer Evaluation of Some New Heteroatom Compounds

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Abstract. This work deals with the preparation of high yield compounds by simple methods through two parts: the first part involves the preparation of compounds RU1 and RU3 by the reaction of melamine with chloroacetic acid in existence of sodium acetate. The second part includes the preparation of compounds RU2 and RU4 by the reaction of benzidine with chloroacetic acid. The reactions were followed by TLC and the prepared compounds were characterized by FT-IR, ¹H-NMR and ¹³C-NMR techniques. The prepared compounds show a good biological activity against MCF-7.

Keywords. melamine, benzidine, Heteroatom, chloroacetic acid.

1. Introduction
Benzidine is a solid organic compound that contains the molecular formula C₆H₄(NH₂)₂ and is called aromatic amines. Its outward appearance is a yellow, reddish-gray crystalline powder, yellowish red or white which darkens when exposed to light or air [1, 2]. Benzidine has dangerous biological properties, and is considered to be carcinogenic, especially bladder and pancreatic cancer [3]. It is commonly used in the manufacture of paints, plastics, munitions and other synthetic chemicals. This is used in life sciences, biotechnology, medicine and practical molecular design [4]. It is integrated into the composition of pesticides and pharmaceutical industries [5], and azo compounds [6] such as red kango tincture [7]. In the past, in clinical laboratories, benzidine was also used to detect the presence of blood, as it was applied to the sample in the presence of hydrogen peroxide, providing the blue color for detection [2]. It is also used to make CHV compounds, widely used in drugs and liquid crystal [1]. Melamine is 1,3,5-Triazine-2,4,6- triamine, melamine has fire-resistant properties, and is considered to be carcinogenic, especially bladder and pancreatic cancer [3]. It is commonly used in the manufacture of paints, plastics, munitions and other synthetic chemicals. This is used in life sciences, biotechnology, medicine and practical molecular design [4]. It is integrated into the composition of pesticides and pharmaceutical industries [5], and azo compounds [6] such as red kango tincture [7]. In the past, in clinical laboratories, benzidine was also used to detect the presence of blood, as it was applied to the sample in the presence of hydrogen peroxide, providing the blue color for detection [2]. It is also used to make CHV compounds, widely used in drugs and liquid crystal [1]. Melamine is 1,3,5-Triazine-2,4,6- triamine, melamine has fire-resistant properties because it contains a high amount of nitrogen[8], melamine is part of the basic structure of a number of drugs, such as: Almitrine, Altreteramine, Cyromazine, Ethylhexyl Triazone, Iscotrimizol, Meladrazine, Melarsomine, Melarsoprol, Tretamine, Trinitrotiazine [9]. We note here a new compound of Heteroatom prepared from melamine and benzidine, they may have a biological activity as an anti-cancer.

2. Experimental
Chemicals: Sigma Fluka, MERCK, BDH and CDH developed all of the chemicals used: EtOH Absolute, glacial CH₃COOH, MeOH, HCl, aryl benzaldehyde, resorcinol, NaOH and H₂SO₄.
2.1. Instruments
The melting points of the prepared compounds were determined using the SMP30 melting point instrument, although the degrees of melting were not corrected. The "Testseon Shimadzu (FT-IR 8400 Series Japan)" using the KBr disk method (TLC) was once performed for TLC on silica gel G and spots were visualized by I₂ vapors. The ¹H-NMR spectra had been obtained using DMSO as solvent and TMS as an internal standard with "Bruker, Ultra Shield 400 MHz Switzerland."

2.2. Synthesis of compound (RU1)
Melamine (2 g, 0.016 mmol) was added to 10 mL of acetic acid in a 150 mL beaker, then add 1.5 g, 0.016 mole of ClCH₂COOH and 17 mL of saturated aqueous sodium acetate is stirred, then stirred, then added with continuous stirring, 40 ml of cold water is added to the sample. The product was then filtered, and then dried.

2.3. Synthesis of compound (RU2).
Benzidine (3 g, 0.016mol) was applied to 10ml of acetic acid in a 150ml flask. Added and stirred (1.5 g, 0.016mol) 2-chloroethanoic acid (ClCH₂COOH) and 17ml semi-saturated aqueous sodium acetate, then added 40ml to it. Gradually pouring cold water with continuous stirring, then filtering and drying the substance.

2.4. Synthesis of compound (RU3).
(2 g, 0.016mol) of Melamine are added to 30ml snowy acetic acid in a 150ml beaker, then (4.5g, 0.048mol) 2-chloroethanoic acid (ClCH₂COOH) and 50ml of semi-saturated aqueous sodium acetate were added. The result was stirred and added to it. 80ml gradually pour cold water with continuous stirring, then the product was filtered and dried.

2.5. Synthesis of compound (RU4).
In a 150ml flask, benzidine (3 g, 0.016mol) was added to 10ml of acetic acid, then 2-chloroethanoic acid (ClCH₂COOH) and 34ml of semi-saturated aqueous sodium acetate were added (3 g, 0.032mol). The resulting solution was stirred and then slowly applied 80ml to it. Pouring cold water with constant stirring, then filtering and drying. Table 1 displays physical properties of prepared compounds.

| Compound | Color  | Solubility      | Melting point °C | Yield % |
|----------|--------|-----------------|------------------|---------|
| RU1      | White  | Methanol        | Decompose > 235  | 88      |
| RU2      | Umber  | Methanol, ethanol, Acetone | 140 | 89      |
| RU3      | white  | Methanol, ethanol(Partial) | Decompose > 240 | 69      |
| RU4      | Umber  | Methanol        | 146              | 85      |

3. Results and Discussion
Amide derivatives prepared from melamine and benzidine reaction and usually occurs under acid catalysis, in semi-saturated aqueous sodium acetate solution (scheme1).
Scheme 1. Synthesis of amide derivatives (RU1-RU4)

The FT-IR spectra of the prepared compounds (RU1-RU4) showed disappearance, the band assigned to the $\nu$ (NH$_2$) of melamine and Benzidine derivatives at (3466-3345) cm$^{-1}$. The absorption bands of NH of amid at (3202-3194) cm$^{-1}$ were observed, (C = C, aromatic) two peaks at (1605-1432) cm$^{-1}$ were shown, two bands of absorption appeared at (3100-3032) and (2805-2970) cm$^{-1}$ belonging to (C-H, stretching) of the aromatic and aliphatic respectively. Also, (C-N) group appeared at stretching frequency (1378-1145) cm$^{-1}$. The results are showing in Figures (1-4). The $^1$H-NMR and $^{13}$C-NMR data for compounds (RU1, RU2) was recorded using DMSO as a solvent and a chemical shift in ppm. The $^1$H-NMR spectra shows NH signal (δ 8.02 -10.03), and appearance at 5.7 of protons (CH$_2$-NH), 3.8 for CH$_2$ signal at (4.53-4.41) as showing in Figures (5, 6). For Benzidine derivatives 4.4 for NH$_2$, 6.6 and 7.4 for CH-Ar. The $^{13}$C-NMR for compounds (RU1-RU3) show the appearance of (C=O) at (165-166), (C-NH) at (154.38) and (C-NH$_2$) at (172.85-171-18) as shown in figures (7, 8, 9).

Figure 1. FT-IR spectrum of compound (RU1).
Figure 2. FT-IR spectrum of compound (RU2).

Figure 3. FT-IR spectrum of compound (RU3).

Figure 4. FT-IR spectrum of compound (RU4).
Figure 5. $^1$H -NMR spectrum of compound (RU1).

Figure 6. $^1$H -NMR spectrum of compound (RU2).

Figure 7. $^{13}$C -NMR spectrum of compound (RU1).
4. ANTICANCER ACTIVITY

4.1. Maintenance of cell cultures
MCF-7 cells were maintained with 10 percent Fetal bovine serum, 100 units/mL penicillin, and 100 μg/mL streptomycin supplemented in RPMI-1640. Cells were reseeded at 80 percent confluence twice a week using Trypsin-EDTA and incubated at 37 °C [10].

5. Cytotoxicity Assays
The MTT assay was performed using 96-well plates [11] to assess the cytotoxic effect of (RU1, RU2, RU3, RU4). Cell lines were seeded at approximately 1 104 cells / well. Upon having invested 24 hours. The cells were loaded with compounds measured at various concentrations, or a confluent monolayer was obtained. Measuring cell viability after 72 hours. Treatment by removing the medium, add 28 μL of 2 mg / mL of MTT solution and incubate the cells at 37 °C for 2.5 h. After removing the MTT solution, the crystals remaining in the wells were solubilized by adding 130 μL of DMSO (Dimethyl Sulphoxide), followed by shaking for 15 minutes by 37 °C incubation [12]. The absorbency was measured at 492 nm on a microplate reader; the assay was conducted in triplicate. The rate of cell growth inhibition (the cytotoxicity percentage) was determined as the following equation [13]:-

\[
\text{Cytotoxicity} = \frac{A-B}{A} \times 100
\]

Where A and B are the optical density of control and the optical density of test.
6. Statistical analysis

The data obtained were evaluated statistically using an unpaired GraphPad Prism 6 t-test. The values have been reported as the triplicate measurements mean ± SEM [14].

Figure 10. Cytotoxic effect of RU1 in MCF-7 cells. IC50=32.18 μg/ml

Figure 11. Cytotoxic effect of RU2 in MCF-7 cells. IC50= 25.67 μg/ml

Figure 12. Cytotoxic effect of RU3 in MCF-7 cells. IC50=21.27 μg/ml
Figure 13. Cytotoxic effect of RU4 in MCF-7 cells. IC50= 42.18 μg/ml

7. Conclusion
New derivatives of the melamine and benzidine heteroatom were identified in this analysis. The compounds prepared were distinguished by techniques such as FT-IR, 1H-NMR and 13C-NMR. The cytotoxic effect was reported from (RU1, RU2, RU3, RU4). The prepared compounds showed strong biological activity against MCF-7, respectively with IC50 values 32.18, 25.67, 21.27, 42.18 μg/ml.

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