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Synthesis, Breast Anticancer Activity and Theoretical Studies of Some New Barbituric Acid Derivatives

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ABSTRACT
A series ofnew barbituric acid derivatives containing heterogeneous rings such as pyrazolines (7-21), oxazolines (22-26) and oxiranes (27-31) have been prepared by reacting barbituric acid with some aldehydes to prepare chalcones (2-6) and then reacting the latter with aqueous hydrazine, phenylhydrazine, hydroxyamine hydrochloride and hydrogen peroxide. These derivatives were obtained by exploiting the presence of an active carbon atom (C5) in the structure of the starting material (barbituric acid) carrying an acidic hydrogen atom that provides the carbanion ion that enters into the aldol condensation to produce a number of □ □ □-unsaturated carbonyl compounds (2-6). After confirming the chemical formulas of the prepared compounds (2-31) by conducting some physical and spectral measurements, some theoretical studies were conducted on the starting material (1) and the prepared compounds (2-31) to predict their pharmacokinetic properties through (Swiss ADME evaluation) and to predict the extent of their toxicity to heart functions through (cardiotoxicity) test. Finally, in light of the previous results, the two compounds (25, 30) were selected to perform (cytotoxicity test), and the results were positive, i.e. the two selected compounds inhibited the growth of breast cancer cells (MCF-7) versus very slight inhibition of healthy cells of the type (HDFN) taken from neonatal dermal fibroblast cells.

Keywords: Barbituric acid, Breast Anticancer, Chalcones, Cardiotoxicity study, Cytotoxicity Assay, Oxazoline, Oxirane, Pyrazolines, Swiss ADME evaluation.

INTRODUCTION

Heterocyclic compounds are a basic building block for the preparation of many medicines. They are important and necessary compounds for the life system. There is a lot of research that aims to prepare new heterocyclic compounds that may have biological effectiveness for the development or discovery of new medicines [1]. Barbituric acid, chemically 2,4,6-trioxohexahydropyrimidine, a cyclic amide used as the parent compound to produce barbiturates that act as central nervous system depressants [2]. Barbituric acid itself does not give sedative and hypnotic effects but the substituted derivatives with alkyl or aryl group at position 5 provide effects. The derivatives of barbituric acid have especial place in pharmaceutical chemistry. Their biological activities range from classical applications in medical treatments as sedative, hypnotic, anticonvulsant, antiplasmodic and local anaesthetic drugs [3,4]. It has also more recent reports indicated that they have applications as anti-tumor, anti- cancer and anti- osteoporosis treatments [5,6].Researchers have been interested in preparing new derivatives of isoxazoles, as they are widely used in research related to the discovery of new drugs. This interest in isoxazole compounds came from the structural properties of this ring, as it has the ability to form hydrogen bonds with some proteins, which led to improving the pharmacokinetic properties of the compounds in which this ring was introduced into their structures. The compounds gave many biological activities, including antibacterial and antiviral, antituberculosis, anti-inflammatory, and anticancer [7,8]. Pyrazolo[3,4-d]pyrimidine compounds have also received wide attention from researchers in the field of new drug discovery. These compounds have been found to have many different biological activities such as antimicrobial and antiproliferative activities, as some compounds have shown strong cytotoxicity against some types of cancer cells [9-11].

MATERIAL AND METHODS

The compounds and solvents used were obtained in pure form from the German company Fluka. For the physical and spectral measurements, a digital melting point meter (IA 9300) and an infrared spectrometer (ATR Alpha-platinum from FT-IR Bruker) from Germany were used. For the nuclear magnetic resonance (^{1}H-NMR)

spectra, a Bruker device of type AS 400 MHz was used, using (DMSO-d_6) as a solvent and (TMS) as a reference.

Synthesis of 5-(arylmethylene)pyrimidine-2,4,6-trione (2-6) [12-16]

An equimolar of (0.05 mol) of barbituric acid, aromatic aldehyde, and potassium hydroxide were mixed. Then (15 ml) of ethanol was added and the mixture was stirred at laboratory temperature for (3 hours). The color of the solution changes within half an hour of stirring and a colored precipitate is formed. Stirring continues until the resulting color stabilizes. The precipitate was filtered, washed with ethanol several times, and air dried.

5-(3-Nitrobenzylidene)pyrimidine-2,4,6-trione (2): Pale brown, yield 82%, m.p= 270-272 °C. IR (υ cm^{-1}): 3153(N-H) ,3030(C-H arom.) , 1681(C=O) , 1594 (C=C) ,1515(assym.N02) 1350 (sym.NO2). ^{1}H-NMR(DMSO-d6,400MHz) δ(ppm): 10.15 [s,1H,2NH], 7.82-8.69 [m, 3H, Ar-H], 7.48 [s, H, =CH].

5-(Furan-2-ylmethylene)pyrimidine-2,4,6-trione (3): Dark gray,yield 87 %, m.p= 280°C. IR (υ cm^{-1}): 3190 (N-H) ,3040 (C-H arom.), 1644 (C=O), 1600 (C=C), 1266 (assym.C-O), 1097(sym.C-O)

5-(4-Methoxybenzylidene)pyrimidine-2,4,6-trione (4): Yellow, yield 80%, m.p= 309-311 °C. IR (υ cm^{-1}): 3109 (N-H),1669 (C=O), 1587 (C=C), 1364 (asym.C-O), 1066 (sym.C-O)

5-(Benzo[1,3]dioxol-5-ylmethylene)pyrimidine-2,4,6-trione (5): Pale brown, yield 77 %, m.p= 316-317 °C. IR (υ cm^{-1}): 3113 (N-H), 3052 (C-H aromatic), 1662 (C=O), 1600 (C=C). ^{1}H-NMR (DMSO-d6,400MHz) δ(ppm): 9.81 [s,2H,NH], [s,1H,CH], 6.17-7.54 [m,3H,Ar-H], 7.56 [s, 1H, =CH] , 5.89 [s,2H,CH_2].

5-(4-Dimethylaminobenzylidene)pyrimidine-2,4,6-trione (6): Orange dark, yield 70%, m.p= 345°C. IR(υcm^{-1}): 3180 (N-H), 1661 (C=O), 1600 (C=C).^{1}H-NMR(DMSO-d6,400MHz) δ(ppm): 10.02 [s,2H,2NH], 6.85 [s,1H,=CH], 6.57-6.83 [m,4H,Ar-H], 2.80 [s,6H,2CH_3].

Synthesis of 3-Aryl-2,3,3a,7-tetrahydro-pyrazolo[3,4-d]pyrimidine-4,6-dione (7-11) [17-20]

The α, β-unsaturated carbonyl compounds (2-6) (2.0 mmole) was mixed with (4.0 mmole) of $NH_2NH_2.H_2O$ in 10 mL of ethanol and the mixture was refluxed for four hours, then the reaction mixture was cooled and filtration of the precipitate and washed with ethanol.

3-(3-Nitro-phenyl)-2,3,3a,7-tetrahydro-pyrazolo[3,4-d]pyrimidine-4,6-dione (7): Yellow, 35%, m.p=199-202°C. IR (υ cm^{-1}): 3254 (N-H), 1645(C=O), 1502 (assym.NO2),1348 (sym.NO2)

3-(furan-2-yl)-2,3,3a,7-tetrahydro-pyrazolo[3,4-d]pyrimidine-4,6-dione (8) : Yellow, 70%,m.p= 294 °C. IR (υ cm-1): 3117(N-H), 1673 (C=O), 1245 (asym.C-O) 1108(sym.C-O)

3-(4-methoxyphenyl)-2,3,3a,7-tetrahydro-pyrazolo[3,4-d]pyrimidine-4,6-dione (9): Yellow, 74%, m.p=244°C. IR (υ,cm^{-1}): 3107 (N-H), 1657 (C=O),1590(C=N),1206 (assym.C-O), 1076 (sym.C-O). ^{1}H-NMR (DMSO-d6,400MHz) δ(ppm): 11.75 [s,1H,NH], 9.65 [s,1H,NH], 6.67 [d,1H,CH], 7.13-7.23 [m,4H,Ar-H], 8.31[s,1H, NH pyrazoline ring], 3.46 [s,3H,OCH_3], 2.52 [d,1H,CH].

3-(benzo[d][1,3]dioxol-5-yl)-2,3,3a,7-tetrahydro-pyrazolo[3,4-d]pyrimidine-4,6-dione (10): Yellow, 50%, m.p=292°C. IR (υ, cm^{-1}): 3311(N-H), 3090 (N-H amide), 1661 (C=O) ,1232(assym.C-O), 1014 (sym.C-O). ^{1}H-NMR(DMSO-d6,400MHz) δ(ppm): 9.53 [s,1H, NH], 8.88 [s,1H,NH], 2.01[d,1H,CH], 6.01 [d,1H, CH], 7.76-6.71[m,3H,Ar-H], 8.33 [s,1H, NH pyrazoline ring] 5.44 [s,2H,CH_2].

3-[4-(dimethylamino)phenyl]-2,3,3a,7-tetrahydro-pyrazolo[3,4-d]pyrimidine-4,6-dione (11): Yellow, 56%, m.p=280 °C. IR (υ,cm^{-1}): 3108(N-H), 1661 (C=O), 1290 (C-N), 2878 (C-H)

Synthesis of 2-Acetyl-3-(aryl)-2,3,3a,7-tetrahydro-pyrazolo[3,4-d]pyrimidine-4,6-dione (12-16) [17-20]

The compounds (2-6) (2.0 mmole) was mixed with (6.0 mmole) of $NH_2NH_2.H_2O$ in 10 mL of glacial acetic acid and the mixture was refluxed for four hours, then the reaction mixture was cooled and filtration of the precipitate and washed with ethanol.

2-Acetyl-3-(3-nitrophenyl)-2,3,3a,7-tetrahydro-pyrazolo[3,4-d]pyrimidine-4,6-dione (12):

Yield 44%, m.p= 313°C. IR (υ cm^{-1}): 3115(N-H), 1712 (C=O cyclic amide), 1668(C=O),1601 (C=N), 1545 (assym.NO2), 1342 (sym.NO2).

2-Acetyl-3-(furan-2-yl)-2,3,3a,7-tetrahydro-pyrazolo[3,4-d]pyrimidine-4,6-dione (13):

Yield 49 %, m.p= 87°C. IR (υ cm^{-1}): 3188 (N-H), 1696 (C=O cyclic amide), 1647 (C=O),1640 (C=N), 1271 (asym.C-O), 1045 (sym. C-O).

2-acetyl-3-(4-methoxyphenyl)-2,3,3a,7-tetrahydro-pyrazolo[3,4-d]pyrimidine-4,6-dione

(14): Yield 67%, m.p= 278°C. IR (υ cm^{-1}): 3212(N-H), 1698 (C=O cyclic amide), 1635(C=O), 1587 (C=N).

2-Acetyl-3-benzo[1,3]dioxol-5-yl-2,3,3a,7-tetrahydro-pyrazolo[3,4-d]pyrimidine-4,6-dione (15): Yield 50%, m.p= 267 °C. IR (υ cm^{-1}) : 3333 (N-H), 1736 (C=O),1674 (C=O), 1254 (assym.C-O), 1099 (sym. C-O). ^{1}H-NMR (DMSO-d6,400MHz), δ(ppm): 11.13 [s, 1H,NH],8.07 [s, 1H, NH], 6.93-7.25 [m, 3H, Ar-H],6.07 [s,2H,CH_2], 6.07 [d, 1H, CH], 2.18 [s, 3H, CH_3].

2-Acetyl-3-(4-(dimethylamino)phenyl)-2,3,3a,7-tetrahydro-pyrazolo[3,4-d]pyrimidine-4,6-dione (16): Yield 72 %, m.p= 220°C. IR (υ cm^{-1}): 3317 (N-H), 1743(C=O Cyclic amide), 1657 (C=O), 1590 (C=N). ^{1}H-NMR (DMSO-d6,400MHz), δ(ppm): 9.74 [s, 1H,NH], 9.1 [s,1H,NH], 6.76 [d.1H,CH], 6.66 -7.32 [d-d ,4H,Ar-H], 2.95 [d,1H,CH],2.89 [s,6H,2CH_3], 1.75 [s,3H,CH_3].

Synthesis of 3-aryl-2-phenyl-2,3,3a,7-tetrahydro-pyrazolo[3,4-d]pyrimidine-4,6-dione-pyrazolo[3,4-d] pyrimidine-4,6-dione (17-21) [17-20]

The compounds (2-6) (2.0 mmole) was mixed with (2.0 mmole) of phenyl hydrazine in 10 mL of glacial acetic acid and the mixture was refluxed for four hours. After the reaction was completed, it was cooled and poured onto crushed water. It was filtered immediately, washed with water and recrystallized with ethanol .

3-(3-Nitro-phenyl)-2-phenyl-2,3,3a,7-tetrahydro-pyrazolo[3,4-d]pyrimidine-4,6-dione (17): Yellow, 66 %, m.p=255°C. IR (υ cm^{-1}): 3183 (N-H), 1680 (C=O), 1523 (assym.NO2), 1347 (sym.NO2). ^{1}H-NMR (DMSO-d6,400MHz) δ(ppm): 10.72 [s, 1H, NH], 9.60 [s, 1H, NH], 6.91-8.10 [m, 9H, Ar-H], 6.66 [d, 1H, CH], 3.44 [d, 1H, CH].

3-(furan-2-yl)-2-phenyl-2,3,3a,7-tetrahydro-pyrazolo[3,4-d]pyrimidine-4,6-dione (18): Yellow, 27 %, m.p =291°C. IR (υ cm^{-1}): 3107 (N-H), 1610 (C=O), 1364 (asym.C-O), 1297 (sym.C-O). ^{1}H-NMR (DMSO-d6,400MHz) δ(ppm): 10.85 [s , 1H ,NH], 9.98 [s, 1H, ,NH], 6.6 [d, 1H, CH], 6.52-7.59 [m, 8H, Ar-H], 4.74 [d, 1H, CH].

3-(4-methoxyphenyl-2-phenyl-2,3,3a,7-tetrahydro-pyrazolo[3,4-d]pyrimidine-4,6-dione (19): Yellow, 54%, m.p=250°C. IR (υ cm^{-1}): 3079 (N-H), 1670 (C=O), 1220 (asym.C-O), 1030 (sym.C-O).

3-Benzo[1,3]dioxol-5-yl-2-phenyl-2,3,3a,7-tetrahydro-pyrazolo[3,4-d]pyrimidine-4,6-dione (20): Yellow, 30%, m.p = 240°C. IR (υ cm^{-1}): 3070 (N-H), 1666 (C=O), 1407 (N-C), 1247 (asym.C-O), 1045 (sym.C-O).

3-(4-(dimethylamino)phenyl)-2-phenyl-2,3,3a,7-tetrahydro-pyrazolo[3,4-d]pyrimidine-4,6-dione (21): Yellow, 67%, m.p=261°C. IR (υ cm^{-1}): 3190 (N-H), 1683 (C=O), 1246(C-N), 1400(C-C cyclic)

3-(aryl)-3,3a-dihydro-7H-isoxazolo[3,4-d]pyrimidine-4,6-dione(22-26) [17-20]

The compounds (2-6) (2.0 mmole) was mixed with(4.0 mmole) of NH2OH.HCl in 5 mL of pyridine and the mixture was refluxed for four hours, then the reaction mixture was cooled and Pour it over crushed ice and acidify the mixture with acetic acid. Leave the mixture until the next day at room temperature. Filter the precipitate and wash with water

3-(3-Nitro-phenyl)-3,3a-dihydro-7H-isoxazolo[3,4-d]pyrimidine-4,6-dione (22):

Yield 33%, m.p= 230°C. IR (υ cm^{-1}): 3010 (N-H), 1692 (C=O), 1580 (C=N), 1523 (asym.NO2), 1347 (sym.NO2).

3-(furan-2-yl)-3,3a-dihydroisoxazolo[3,4-d]pyrimidine-4,6(5H,7H)-dione (23):

Yield 40%, m.p= 224°C. IR (υ cm^{-1}): 3188 (N-H), 1720(C=O),1610(C=N), 1190 (asym. C-O), 1084 (sym.C-O).

3-(4-methoxyphenyl)-3,3a-dihydro-7H-isoxazolo[3,4-d]pyrimidine-4,6-dione (24):

Yield 77%, m.p= 290°C. IR (υ cm^{-1}): 3320 (N-H), 1698 (C=O), 1587 (C=N), 1210 (assym.C-O), 1089 (sym.C-O)

3-(benzo[d][1,3]dioxol-5-yl)-3,3a-dihydro-7H-isoxazolo[3,4-d]pyrimidine-4,6-dione (25): Yield 45 %, m.p= 214°C. IR (υ cm^{-1}): 3020 (N-H),1697(C=O), 1597 (C=N), 1220 (assym.C-O), 1150 (sym.C-O). ^{1}H-NMR (DMSO-d6,400MHz) δ(ppm): 10.83 [s, 1H,NH], 9.46 [s, 1H, NH], 7.81 [d, 1H, CH], 7.60-7.34 [m, 3H, Ar-H], 4.56 [s, 2H , CH_2], 2.95 [d, 1H,CH].

3-(4-(dimethylamino)phenyl)-3,3a-dihydro-7H-isoxazolo[3,4-d]pyrimidine-4,6-dione (26):

Yield 63% m.p=288°C. IR (υ cm^{-1}): 3138 (N-H), 1645 (C=O), 1598 (C=N), 2974 (C-H), 1215 (assym.C-O), 1059 (sym.C-O).

Synthesis 2-Aryl-1-oxa-5,7-diazaspiro[2.5]octane-4,6,8-trione (27-31) [17-20]

To a solution of α,β-unsaturated carbonyl compounds (2-6) (2.0 mmole) in (25 mL) of ethanol was heated until had completely dissolved, was added (0.5 g.) of a 30 % hydrogen peroxide and then slowly (0.5 mL) of 16 % sodium hydroxide . After (3) hours of stirring the mixture was left overnight at room temperature. The white powder was collected by filtration and washed with cold water to give epoxide derivatives.

2-(3-Nitro-phenyl)-1-oxa-5,7-diaza-spiro[2.5]octane-4,6,8-trione (27) : White, yield 83 % ,m.p= 70°C. IR (υ cm^{-1}): 3168 (N-H), 1641 (C=O), 1515 (assym. NO2), 1368 (sym.NO2), 1219 (assym.C-O), 1037 (sym.C-O)

2-Furan-2-yl-1-oxa-5,7-diaza-spiro[2.5]octane-4,6,8-trione (28): White, yield76 %, m.p= 58°C. IR (υ cm-1): 3154(N-H), 1648 (C=O), 1439 (C-N), 1220 (assym. C-O), 1014 (symC-O),

2-(4-Methoxy-phenyl)-1-oxa-5,7-diaza-spiro[2.5]octane-4,6,8-trione (29): White, yield 67 % , m.p= 86°C. IR (υ cm^{-1}): 3217(N-H) , 1651(C=O) , 1427(C-N)

2-Benzo[1,3]dioxol-5-yl-1-oxa-5,7-diaza-spiro[2.5]octane-4,6,8-trione (30) : White, yield 68%, m.p= 48°C. IR (υ cm^{-1}): 3201 (N-H),1675 (C=O), 1439 (N-C), 1215 (assym.C-O), 1039 (sym.C-O),

2-(4-Dimethylamino-phenyl)-1-oxa-5,7-diaza-spiro[2.5]octane-4,6,8-trione (31): White, yield 82 %, m.p= 74°C. IR (υ cm^{-1}): 3356 (N-H), 1596 (C=O), 1238 (assym. C-O), 1154 (sym. C-O).

Theoretical studies

Swiss ADME evaluation [21]

One of the important theoretical tests to predict the behavior of compounds inside the body is the Swiss ADME test, which provides information about some of the pharmacokinetic properties of the proposed drug compound in terms of (absorption, distribution, metabolism and excretion) in addition to some of its physical properties such as solubility and affinity with water and fats and whether the compound contains toxic and active parts that affect metabolism or not. All this information, in addition to laboratory and clinical studies, must be studied before using any chemical compound as a proposed drug, as it saves time, effort and costs. The results are listed in Table (1).

Cardiotoxicity study [22]

There are a large number of pharmaceutical and non-pharmaceutical compounds that affect heart functions directly and indirectly. Accordingly, the prepared compounds were examined using the link (https://biosig.lab.uq.edu.au/cardiotoxcsm/
prediction results/all_1672781700.53) to predict the effect of these compounds on five heart functions, which are (irregular heartbeat, heart failure, heart block, HERG toxicity, high blood pressure, and myocardial infarction). The results we obtained are shown in Table (2)

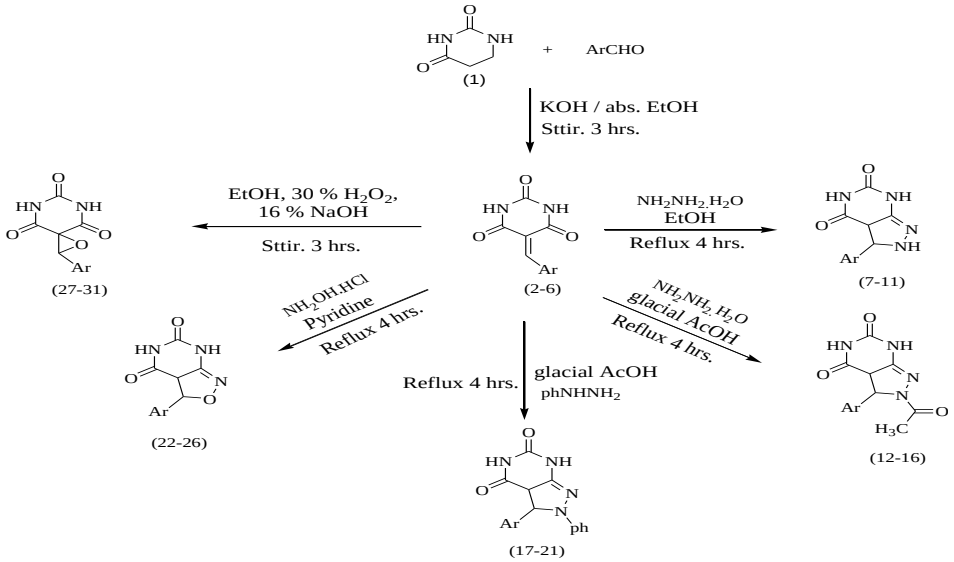
Cytotoxicity Assay [23]

Cancer is a disease that kills thousands of people annually, so researchers are interested in trying to discover drugs that may stop the growth of these cells or eliminate them. Most of the drugs currently used have several side effects that may harm both diseased and healthy cells. In this research, two compounds were selected from 30 compounds that were prepared for the purpose of examining their cytotoxicity. The two compounds (25, 30) were selected based on theoretical studies of them as they contain the piperinal ring and gave good results in the Swiss ADME test, in addition to the fact that they did not show toxicity to heart functions in the cardiotoxicity test. The MTT method was used to test the effect of the two compounds on breast cancer cells (MCF-7) and in comparison with healthy fibroblast cells type (HDFN). The cancerous and healthy cell lines were obtained from the University of Malaya in Kuala Lumpur \ Faculty of Medicine \ Department of Pharmacology. The cells were preserved in liquid nitrogen and the cells were purified, multiplied and tested at Al-Nahrain University \ Biotechnology Research Center.

RESULTS AND DISCUSSION

Chemical synthesis

The□ □ □ □-□unsaturated carbonyl compounds possess high reactivity due to the presence of the carbonyl group conjugated with the double bond. This instance suggests that the nucleophiles can react with chalcones at both the carbonyl group and the double bond. The reactions with binucleophiles leading to the broad range of cyclized compounds are of particular interest as shown in the Schemes (1).



Ar = 3-$NO_2C_6H_4$, Furfural, 4-$MeOC_6H_4$, Piperonal, 4-N,N-diMeC_6H_4

Scheme 1: Synthesis of a,b-Unsaturated Carbonyl Compounds, Pyrazoline, Oxazoline and Epoxide Derivatives (2-31)

A novel series of compounds (2-31) were synthesized and characterized. Five membered heterocyclic ring systems represented pyrazolines derivative (7-21) have been prepared through the reaction between hydrazine hydrate or phenyl hydrazine with barbituric acid-chalcone (2-6) in neutral or acidic medium. The reaction proceeded through the formation of the conjugate addition product which was cyclized to give the final compounds. Also, the formation of oxazolines derivatives (22-26) by the reaction of □ □ □ -unsaturated carbonyl compounds (2-6) with hydroxyl amine hydrochloride in basic medium to give the desired compounds. Finally, three membered heterocyclic ring systems represented oxirane derivative (27-31) via the reaction of □ □ □ -unsaturated carbonyl compounds (2-6) with peroxide hydrogen in basic medium.

Theoretical evaluations

Theoretical tests have been used through software that follows special standards in the past few years to evaluate some chemical and kinetic properties in addition to evaluating the toxicity of prepared chemical compounds. This is an important step before biological, industrial and clinical applications as proposed drugs. This step will save money and effort and also allow the composition to be modified to suit the required properties. There are many drugs that have been withdrawn from the market due to causing serious side effects, including chlorpheniramine and clonorex, which were withdrawn from the German market in 1969because they cause cardiovascular toxicity [24], as well as the drug valdecoxib, which was withdrawn from the US market in 2004 because it causes heart attacks and strokes [25], and also the drug norcaserin, which was withdrawn from the US market in 2020 because it increases the risk of cancer [26].

Accordingly, some of the physical and pharmacokinetic properties of the compounds prepared in this research were examined using the following applications:

Swiss ADME calculations

The pharmacokinetic properties test and some physical properties of compounds can give an expectation about the possibility of using the compound as a drug or not after completing the rest of the necessary biological and clinical tests. The SwissADME test provides a lot of information such as solubility, polarity, permeability, absorption and other information, which saves time to modify the structure of the compound to achieve a balance between the required properties or exclude the compound and not waste time testing it clinically and biologically. Table (1) provided a large amount of information about the prepared compounds (1-31) compared to the starting material (barbituric acid)(compound 1), the TPSA (Topological Polar Surface Area) values ranged between (136.69 - 75.27), where the high value indicates that the compound has a functional group that is subject to metabolic transformation. The lowest value was for the starting material, then for compounds (18, 26) the value was (83.03) and the highest values were (133.62, 136.69) for compounds (12, 27) respectively.It is believed that the reason for the high TPSA value for the two compounds (12, 27) is the presence of the nitro group in their structure, which makes the two compounds metabolize quickly, making them less permeable and less absorbable in the digestive system.

The prediction of the compound's ability to dissolve in fats was done by calculating the ilog p, and the values ranged between (1.94-0.08). The lowest value is for the initial material(1) and the highest for the compound (21).The reason is that compound (21) contains two hydrophobic aromatic rings in its structure.

As for evaluating the pharmacokinetics of the compounds, it is done by estimating the ability to absorb in the digestive system. From Table (1) it is clear that all compounds are absorbed except for compounds (7,12) noting that the two compounds share the presence of a benzene ring replaced by a nitro group. Regarding permeability through the blood-brain barrier, it was found that all compounds cannot cross this barrier, which indicates that they have pharmacokinetic properties that are not similar to narcotic compounds, as most narcotic drugs cross the blood-brain barrier to affect the central nervous system.

While, the values of Pgp substract (p-glycoprotien), it was shown that compounds (10, 11, 15, 17-21, 25, 30) have the ability to be secreted faster than the rest, to be excreted from the body before being well absorbed.

While Lipinski's rule applied to all the prepared compounds, indicating that all compounds may be orally active and do not have similar properties to narcotics in terms of pharmacokinetics. Also, the Ghose values ranged between (0,1), which gives another indication of the dissimilarity of the properties of these compounds to narcotics, meaning that they have somewhat suitable ADME properties, and the highest value was (4), which is for the starting material. Let us expect that the compound contains active groups that may give incorrect values in biological activity tests. The highest PAINs values were (2), which are for compounds (6, 11, 16, 21), noting that they all share the presence of an aniline ring substituted with two methyl groups on the nitrogen in their structure, so it is preferable to avoid these derivatives to prepare proposed drugs. As for the Leadikeness values, they indicate that the compound has good solubility, permeability and metabolic stability properties, as the values ranged between (0,1). From the above it is clear that all the prepared compounds can be used as drug candidates except some compounds containing nitrophenyl groups and tertiary aniline groups which have some poor ADME properties.

Table 1: Some physical and pharmacokinetic properties of compounds (1-31)

Molecule No.	Formula	MW	TPSA	Ilogp	GI absorption	BBB permeant	Pgp substrate	Lipinski #violations	Ghose #violations	PAINS #alerts	Leadlikeness #violations
1	C4H4N2O3	128.09	75.27	-0.08	High	No	No	0	4	0	1
2	C11H7N3O5	261.19	121.09	0.65	High	No	No	0	0	1	0
3	C9H6N2O4	206.15	88.41	0.72	High	No	No	0	1	1	1
4	C12H10N2O4	246.22	84.5	1.21	High	No	No	0	1	1	1
5	C12H8N2O5	260.2	93.73	1.19	High	No	No	0	1	1	0
6	C13H13N3O3	259.26	78.51	1.2	High	No	No	0	0	2	0
7	C11H9N5O4	275.22	128.41	0.5	Low	No	No	0	1	0	0
8	C9H8N4O3	220.18	95.73	0.75	High	No	No	0	1	0	1
9	C12H12N4O3	260.25	91.82	1.11	High	No	No	0	1	0	0
10	C12H10N4O4	274.23	101.05	0.85	High	No	Yes	0	1	0	0
11	C13H15N5O2	273.29	85.83	1.09	High	No	Yes	0	1	2	0
12	C13H11N5O5	317.26	136.69	0.85	Low	No	No	0	1	0	0
13	C11H10N4O4	262.22	104.01	1.11	High	No	No	0	1	0	0
14	C14H14N4O4	302.29	100.1	1.38	High	No	No	0	1	0	0
15	C14H12N4O5	316.27	109.33	1.43	High	No	Yes	0	1	0	0
16	C15H17N5O3	315.33	94.11	1.39	High	No	No	0	1	2	0
17	C17H13N5O4	351.32	119.62	1.27	High	No	Yes	0	0	0	1
18	C15H12N4O3	296.28	86.94	1.35	High	No	Yes	0	0	0	0
19	C18H16N4O3	336.34	83.03	1.79	High	No	Yes	0	0	0	0
20	C18H14N4O4	350.33	92.26	1.76	High	No	Yes	0	0	0	1
21	C19H19N5O2	349.39	77.04	1.94	High	No	Yes	0	0	2	0
22	C11H8N4O5	276.21	125.61	0.53	High	No	No	0	1	0	0
23	C9H7N3O4	221.17	92.93	0.66	High	No	No	0	1	0	1
24	C12H11N3O4	261.23	89.02	1.08	High	No	No	0	1	0	0
25	C12H9N3O5	275.22	98.25	1.17	High	No	Yes	0	1	0	0
26	C13H14N4O3	274.28	83.03	1.15	High	No	No	0	1	1	0
27	C11H7N3O6	277.19	133.62	0.57	High	No	No	0	1	0	0
28	C9H6N2O5	222.15	100.94	0.64	High	No	No	0	1	0	1
29	C12H10N2O5	262.22	97.03	0.98	High	No	No	0	1	0	0
30	C12H8N2O6	276.2	106.26	1.21	High	No	Yes	0	1	0	0
31	C13H13N3O4	275.26	91.04	0.95	High	No	No	0	1	0	0

Cardinal Toxicity study [27]

There are a large number of pharmaceutical compounds that were withdrawn from the local markets because they caused serious side effects that led to death, including their effect on the health of the heart and arteries(X). These symptoms did not appear through clinical tests before listing the compounds as drugs. Therefore, it is better to conduct some theoretical studies on the compounds before biological and clinical applications. One of the simple programs that provides information or predicts whether the compound affects heart functions or not is the cardiac toxicity test used in this research. The cardiac toxicity of the prepared compounds (1-31) was examined and the results are recorded in Table (2). It was found that compounds (6, 11, 14, 16, 26, 31) may cause disturbance and irregularity in the heartbeat (Arrhythmia), i.e. the number of beats may increase to more than 100 beats per minute or decrease to less than 60 beats per minute. All compounds do not cause Cardiac

failure nor do they cause a delay in the transmission of the electrical signal when it is transmitted from the atria to the ventricles (Heart Block). As for compound (21), we expect that it may disrupt the function of the potassium channel, which causes a disturbance in the electrical activity of the heart (HERG Toxicity). As for the effect of the compounds on blood pressure, Table (2) shows that all compounds are safe except (6, 11, 14, 16, 21, 26, 31), which may raise blood pressure (Hypertension). As for compounds (16, 19, 21, 27), it has been shown that they may cause myocardial infarction or a heart attack that may be caused by a blood clot or blockage of the coronary artery as a result of the transfer of a foreign substance to it.

Table 2: The Cardinal Toxicity Prediction of compounds (1-31)

Comp. No.	Smiles	Arrhythmia	Cardiac failure	Heart block	HERG toxicity	Hypertension	Myocardial infarction
1	O=C1CC(=O)NC(=O)N1	Safe	Safe	Safe	Safe	Safe	Safe
2	O=C1NC(=O)C(=Cc2cccc(c2)N(=O)=O)C(=O)N1	Safe	Safe	Safe	Safe	Safe	Safe
3	O=C1NC(=O)C(=Cc2ccco2)C(=O)N1	Safe	Safe	Safe	Safe	Safe	Safe
4	COc1ccc(cc1)C=C1C(=O)NC(=O)NC1=O	Safe	Safe	Safe	Safe	Safe	Safe
5	O=C1NC(=O)C(=Cc2ccc3c(c2)OCO3)C(=O)N1	Safe	Safe	Safe	Safe	Safe	Safe
6	CN(c1ccc(cc1)C=C1C(=O)NC(=O)NC1=O)C	Toxic	Safe	Safe	Safe	Toxic	Safe
7	O=C1NC(=O)C2C(=NNC2c2cccc(c2)N(=O)=O)N1	Safe	Safe	Safe	Safe	Safe	Safe
8	O=C1NC(=O)C2C(=NNC2c2ccco2)N1	Safe	Safe	Safe	Safe	Safe	Safe
9	COc1ccc(cc1)C1NN=C2C1C(=O)NC(=O)N2	Safe	Safe	Safe	Safe	Safe	Safe
10	O=C1NC(=O)C2C(=NNC2c2ccc3c(c2)OCO3)N1	Safe	Safe	Safe	Safe	Safe	Safe
11	O=C1NC(=O)C2C(=NNC2c2ccc(cc2)N(C)C)N1	Toxic	Safe	Safe	Safe	Toxic	Safe
12	O=C1NC(=O)C2C(=NN(C2c2cccc(c2)N(=O)=O)C(=O)C)N1	Safe	Safe	Safe	Safe	Safe	Safe
13	O=C1NC(=O)C2C(=NN(C2c2ccco2)C(=O)C)N1	Safe	Safe	Safe	Safe	Safe	Safe
14	COc1ccc(cc1)C1N(N=C2C1C(=O)NC(=O)N2)C(=O)C	Toxic	Safe	Safe	Safe	Toxic	Safe
15	O=C1NC(=O)C2C(=NN(C2c2ccc3c(c2)OCO3)C(=O)C)N1	Safe	Safe	Safe	Safe	Safe	Safe
16	O=C1NC(=O)C2C(=NN(C2c2ccc(cc2)N(C)C)C(=O)C)N1	Toxic	Safe	Safe	Safe	Toxic	Toxic
17	O=C1NC(=O)C2C(=NN(C2c2cccc(c2)N(=O)=O)c2ccccc2)N1	Safe	Safe	Safe	Safe	Safe	Safe
18	O=C1NC2=NN(C(C2C(=O)N1)c1ccco1)c1ccccc1	Safe	Safe	Safe	Safe	Safe	Safe
19	COc1ccc(cc1)C1C2C(=O)NC(=O)NC2=NN1c1ccccc1	Safe	Safe	Safe	Safe	Safe	Toxic
20	O=C1NC(=O)C2C(=NN(C2c2ccc3c(c2)OCO3)c2ccccc2)N1	Safe	Safe	Safe	Safe	Safe	Safe
21	O=C1NC(=O)C2C(=NN(C2c2ccc(cc2)N(C)C)c2ccccc2)N1	Safe	Safe	Safe	Toxic	Toxic	Toxic
22	O=C1NC(=O)C2C(=NOC2c2cccc(c2)N(=O)=O)N1	Safe	Safe	Safe	Safe	Safe	Safe
23	O=C1NC(=O)C2C(=NOC2c2ccco2)N1	Safe	Safe	Safe	Safe	Safe	Safe
24	COc1ccc(cc1)C1ON=C2C1C(=O)NC(=O)N2	Safe	Safe	Safe	Safe	Safe	Safe
25	O=C1NC(=O)C2C(=NOC2c2ccc3c(c2)OCO3)N1	Safe	Safe	Safe	Safe	Safe	Safe
26	O=C1NC(=O)C2C(=NOC2c2ccc(cc2)N(C)C)N1	Toxic	Safe	Safe	Safe	Toxic	Safe
27	O=C1NC(=O)C2(C(=O)N1)OC2c1cccc(c1)N(=O)=O	Safe	Safe	Safe	Safe	Safe	Toxic
28	O=C1NC(=O)C2(C(=O)N1)OC2c1ccco1	Safe	Safe	Safe	Safe	Safe	Safe
29	COc1ccc(cc1)C1OC21C(=O)NC(=O)NC2=O	Safe	Safe	Safe	Safe	Safe	Safe
30	O=C1NC(=O)C2(C(=O)N1)OC2c1ccc2c(c1)OCO2	Safe	Safe	Safe	Safe	Safe	Safe
31	CN(c1ccc(cc1)C1OC21C(=O)NC(=O)NC2=O)C	Toxic	Safe	Safe	Safe	Toxic	Safe

Cytotoxicity Assay [23, 28]

Compounds (25, 30) were selected for cytotoxicity testing using the MTT colorimetric assay method against the MCF-7 cancer cell line (breast cancer) and compared with a healthy cell line taken from the dermis of a newborn child, HDFN (Human dermal fibroblasts neonatal). Different concentrations of the two compounds were used, each test was performed four times and the average readings were taken. DMSO was used as a solvent.

The anti-proliferation activity of compounds (25, 30) was measured against the breast cancer cell line (MCF-7) and it was found that the yellow color of the compound MTT (3-(4,5-dimethylthiazol-2 yl)-2,5-diphenyl tetrazolium bromide) turned formazan purple with different color shades according to the concentrations used in the research. The inhibition percentages for compound (25) were (0, 0.54, 5.60, 16.05, 25.26, 36.24, 42.34) % at concentrations (0, 15.6, 31.25, 62.5, 125, 250, 500) micrograms per ml, respectively, as shown in Table (3). As

for compound (30), the inhibition percentages were (0, 4.45, 8.48, 15.12, 21.27, 24.15, 33.78) % at the same the previous concentrations, respectively, as shown in Table (4).

Table 3: The effect of compound (25) on the breast cancer cell line (MCF-7) at 37°C for 48 hours

Concentration (µg\ml)	**0**	**15.60**	**31.25**	**62.5**	**125**	**250**	**500**	**DMSO**
Test 1	661	660	621	551	500	423	388	628
Test 2	662	652	618	550	488	412	368	625
Test 3	624	648	608	548	481	408	380	631
Test 4	658	631	612	538	478	418	366	618
Mean	651.25	647.75	614.75	546.75	486.75	415.25	375.5	625.5
Viability %	100	99.4626	94.3954	83.9539	74.7409	63.762	57.6583	96.0461
Standard deviation (SD)	18.24600	12.23042	5.85234	5.96517	9.77667	6.60176	10.37625	5.56776

Table 4: The effect of compound (30) on the breast cancer cell line (MCF-7) at 37°C for 48 hours

Concentration (µg\ml)	**0**	**15.60**	**31.25**	**62.5**	**125**	**250**	**500**	**DMSO**
Test 1	661	620	588	555	512	501	428	628
Test 2	662	645	598	550	516	488	426	625
Test 3	624	613	598	548	518	498	440	631
Test 4	658	611	600	558	505	489	431	618
Mean	651.25	622.25	596	552.75	512.75	494	431.25	625.5
Viability %	100	95.547	91.5163	84.8752	78.7332	75.8541	66.2188	96.0461
Standard deviation (SD)	18.24600	15.64981	5.41602	4.57347	5.73730	6.48074	6.18465	5.56776

After calculating the (IC 50) value for compounds (25, 30), the values were (510.50, 713.75) µg/ml respectively as shown in Figure (1).

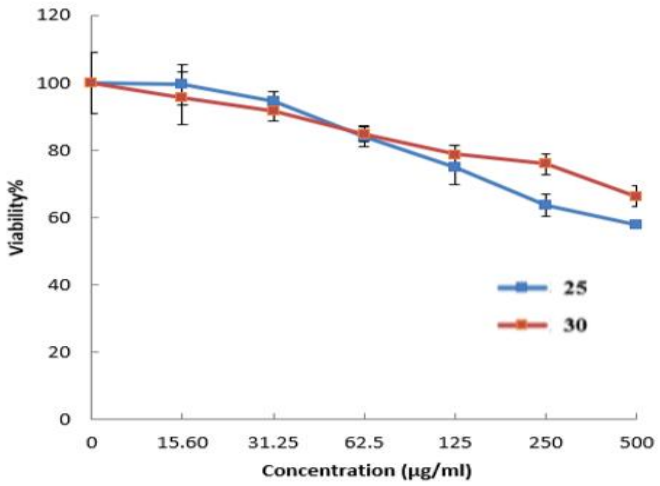


Fig 1: The effect of compounds (25, 30) on MCF-7 cell line using MTT assay

Electron microscopy showed morphological changes in monolayers of cultured MCF-7 breast cancer cells treated with different concentrations of the two compounds (25, 30) for 48 h, at 40× magnification by phase contrast microscopy as shown in Figure (2).

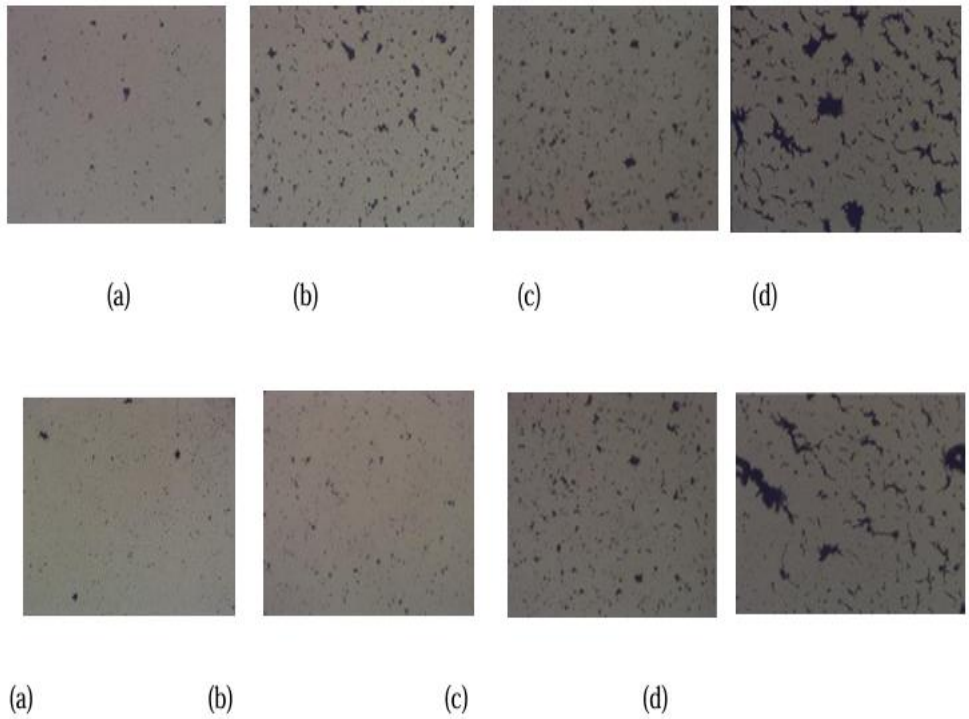


Figure 2: Microscopic images of the effect of compound (25, 30), respectively on MCF-7 cell line where a=62.5, b=125, c=250, d=500 µg/ml

The cytotoxicity recorded by the two compounds (25, 30) against the healthy cell line (HDFN) was (0, 4.25, 9.38, 10.14, 18.63, 19.63, 24.54) and (0, 3.27, 5.56, 9.71, 12.87, 17.45, 21.05) % at a concentration (0, 15.6, 31.25, 62.5, 125, 250, 500) microgram/ml, respectively, as shown in the tables (5,6) and the figure (3) below.

Table 5: The effect of compound (25) on the healthy cell line (HDFN) at 37◦C for 48 hours

Concentration (µg\ml)	**0**	**15.60**	**31.25**	**62.5**	**125**	**250**	**500**
Test 1	230	211	210	204	201	188	167
Test 2	219	231	211	209	200	182	181
Test 3	210	226	209	204	194	181	174
Test 4	258	210	201	207	197	186	170
Mean	229.25	219.5	207.75	206	198	184.25	173
Viability %	100	95.747	90.6216	89.8582	86.3686	80.3708	75.4635
Standard deviation (SD)	20.83866	10.59874	4.57347	2.44948	3.16227	3.30403	6.05530

Table 6: The effect of compound (30) on the healthy cell line (HDFN) at 37◦C for 48 hours

Concentration (µg\ml)	**0**	**15.60**	**31.25**	**62.5**	**125**	**250**	**500**
Test 1	230	222	218	215	200	188	181
Test 2	219	226	211	208	201	181	180
Test 3	210	218	217	204	195	198	185
Test 4	258	221	220	201	203	190	178
Mean	229.25	221.75	216.5	207	199.75	189.25	181
Viability %	100	96.7285	94.4384	90.2944	87.132	82.5518	78.9531
Standard deviation (SD)	20.83866	3.30403	3.87298	6.05530	3.40342	6.99404	2.94392

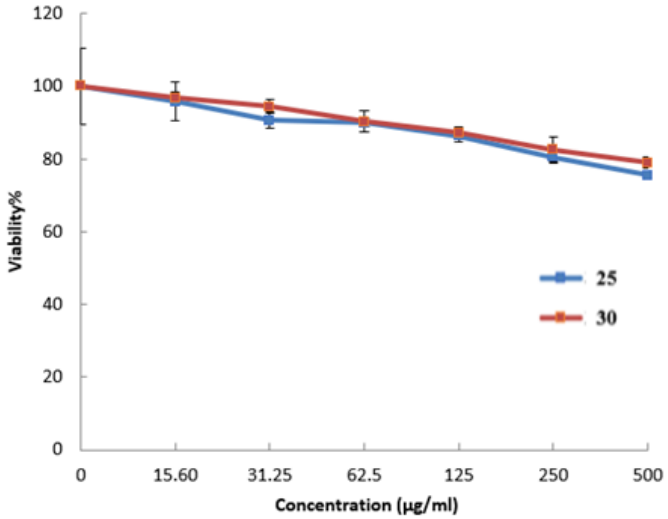


Figure 3: The effect of compounds (25, 30) on HDFN cell line using MTT assay

The previous results showed that both compounds (25, 30) had a clear inhibitory effect against the cancer cell line, especially compound (25), as the inhibitory concentration for the growth of half of the diseased cells was (510.5) compared to (713.75) micrograms/ml for compound (30). As for the effect of the two compounds on the healthy cell line, it was less, not exceeding a quarter of the number of healthy cells at the highest concentration used in the experiment.

CONCLUSION

The previous results showed that the compounds (6, 7, 11, 12, 16, 21,27, 31) gave poor ADME values due to the presence of the benzene ring substituted with nitro, methoxy or tertiary amine groups in their structures. These groups gave the compounds poor pharmacokinetic properties. As for the compounds (6, 11, 14, 16, 19, 21, 26, 27, 31), they recorded cardiac toxicity due to the presence of the same substituted groups on the benzene ring in the structures. Accordingly, to prepare drug candidates, it is necessary to avoid substituted benzene rings and focus on the heterogeneous five-membered rings. The two compounds (25, 30) were chosen for cytotoxicity testing because they contain the piperinal ring, which is known for its inhibitory effect on cancer cell growth [S,D]. In addition, the two tested compounds have good pharmacokinetic properties proven by the ADME prediction. They also did not cause cardiac toxicity, as shown in Table (2). Indeed, the two selected compounds were found to have anti-growth properties against cancer cells (MCF-7) and their effect on healthy cells (HDFN) was very low. All the previous results gave the expectation that some of the prepared compounds have good pharmacokinetic properties and do not have cardiotoxicity, so we suggest that they should be examined biologically and clinically to be excellent drugcandidates [29-31]**.**

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