MOLEKUL
eISSN: 2503-0310

Articles
https://doi.org/10.20884/1.jm.2024.19.1.7799

# Synthesis of Some New Amino Carbonyl Compounds by Mannich Reaction

**Nagham M. Zaki Dawood[1*], Banan Borhan Saeed[1], Zainab Faiyq Saeed[2]**

[1]Department of Pharmaceutical Chemistry, College of Pharmacy, University of Mosul, Mosul, Iraq
[2]Department of Physiology, Biochemistry and Pharmacology, College of Veterinary Medicine, University of Mosul, Mosul, Iraq

*Corresponding author email: n3_m3_zmz@uomosul.edu.iq

**Received** January 19, 2023; **Accepted** January 11, 2024; **Available online** March 20, 2024

**ABSTRACT.** Beta-aminocarbonyl compounds are important intermediates produced from Mannich condensation. In this research, benzaldehyde, *para*-hydroxy acetophenone or *para*-nitro acetophenone were reacted with a various of primary aromatic amines to give compounds (1-7) and (8-14), respectively. The chemical formulas of the synthesized compounds were confirmed by conducting some physical and spectroscopic measurements, such as, melting points, IR and $H^1$-NMR spectrum. After testing the biological activity of some of the synthesized compounds (8,10,11,13) using Gram-positive (*Streptococcus,* and *Staphylococcus aureus*), Gram-negative (*Klebsiella spp.*, and *Escherichia coli*) bacteria, as well as yeast strains, were chosen for this investigation (*candidan albicans*). Isolated bacteria were identified using standard methods of isolation and identification, including direct assays and culture on various media. Some theoretical tests have also been carried out such as SwissADME and cardioToxCSM prediction on all prepared compounds to predict their activity in terms of pharmacokinetic properties (ADME) and also predict their cardiotoxicity on some heart functions such as arrhythmia, cardiac failure, heart block, hERG toxicity, Hypertension (HT), and Myocardial Infarction (MI).

**Keywords**: Secondary amines, aldehyde, Mannich condensation, antimicrobial activity.

## INTRODUCTION

The Mannich reaction is one of the essential reactions through which many useful compounds can be obtained in all fields, especially in the medical and pharmaceutical fields, as many drugs contain an amino part in their structure (Shi et al., 2021). The Mannich reaction provides a simple technology for creating amino carbonyl compounds because it combines three components into a three-component protocol (Guchhait et al., 2022). Many natural compounds and pharmaceuticals are heterocyclic amines (Cai et al., 2019). Amino carbonyl compounds play an important role both physiologically and medically (Raju et al., 2023a), especially the heterocyclic compounds in their structure that are remarkable compounds with a broad scope of medicinal properties, including antimicrobial (Jose et al., 2017; Wang et al., 2017; Marinescu et al., 2017; Zalaru et al., 2018; Liu et al., 2018; Marinescu et al., 2020), and anticancer (Zhang et al., 2014; Roman, 2022), anti-HIV (Zhang et al., 2014), and anticonvulsants (Zhang et al., 2014), analgesics (Sivakumar et al., 2015; Datar and Limaye, 2015; Hayun et al., 2019), anti-inflammatory analgesics (Al-Abdullah et al., 2014; Sivakumar et al., 2015; Datar and Limaye, 2015; Hayun et al., 2019), , anti-malarial (Raju et al., 2023b), antivirals (Raju et al., 2023b), anthelmintic (Raju et al., 2023b), anti-alzheimer's (Raju et al., 2023a), antioxidant (Raju et al., 2023a; Khan et al., 2023), also mannich bases are multifunctional drugs against Parkinson's disease (Tao et al, 2019). There are numerous prepared Mannich bases that have demonstrated biological activity against a variety of diseases. The compound 4-(5-(4-(N-phenyl-N-(R2-substituted)methylamino)phenyl)1-3-4-thiadiazole-2-yl)-1-R1-substituted-thiosemicarbazide was prepared and tested for its efficacy as an anti-inflammatory, antioxidant, and antimicrobial (Raoof and Sadiq, 2022). While in 2021, after testing the prepared two compounds 5-(3,4-dimethoxyphenyl)-3-{4-[(2-trifluorobenzyl)piperazine-1-yl]methyl}-1,3,4-oxadiazole-2(3H) -thione (Al-Wahaibi et al., 2021) and compound 2-(2-chlorophenyl)-5-(2-methoxyphenyl)-1,3,4-oxadiazole (Bajaj et al., 2021), it was found that these two compounds showed inhibitory activity on breast cancer cell line (MCF-7) . On the other hand, Cao et al. (2021), prepared 3-manish base derivatives of benzyldin/benzylphthalide as potential multifunctional agents for the treatment of Alzheimer's disease. As for the compound 1,2,4-triazole-adamantel N-Mannich, it was found that its bases have an effect in lowering blood sugar and antibacterial (Al-Abdullah et al., 2014). The purpose

of this research is to synthesize novel beta-minocarbonyl compounds by the Mannich reaction, and then to evaluate their biological activity against a variety of Gram-positive, Gram-negative, and fungus.

## EXPERIMENTAL SECTION

### Materials and Apparatus

Aldehydes, ketones, amines, sodium acetate were acquired and used as supplied without purification from Fluka (Germany). Melting points were measured in open capillaries with an electrothermal IA 9300 Digital-Series instrument, and data were used without adjustment. An Alpha platinum ATR (Germany) Bruker, FT-IR spectrophotometer has been used to determine the IR spectra. On 400 MHz Varian spectrometers, 1H NMR spectra were acquired with d6 DMSO as a solvent and tetramethylsilane (TMS) as an internal standard. Hereunder are the signals: - s stands for singlet, d for doublet, t for triplet, and m for multiplet.

**Synthesis of 1-(4-hydroxyphenyl)-3-phenyl-3-(arylamino) propan-1-one (1-7)** (Mousavi et al., 2013; Kumar & Choudhary, 2018; Taher-Maghsoodlou e al., 2018):

In a clean bottomed flask, equal moles (0.0025 mol) of benzaldehyde (0.265 g), aromatic primary amine derivatives, and p-hydroxy acetophenone (0.34 g) were stirred for 6 hours at room temperature with sodium acetate (0.0025 mole, 0.2 g) and ethanol as solvent (**Scheme 1**). The desired pure chemicals were extracted from the precipitate using simple filtration, ethanol washing, and air drying. Physical parameters and spectroscopic data (IR, 1H-NMR) were used to confirm the identity of the known products, as described in the paragraphs below.

1-(4-hydroxyphenyl)-3-phenyl-3-(pyrimidin-2-ylamino)propan-1-one (1). The color is pale- brown, The yield 71%, m.p. 220-222°C, IR (υ $cm^{-1}$), 3356 (phenolic O-H), 3222 (N-H secondary amine), 3046 (aromatic C-H), 1690 (C=O ketone), 1586 (conjugated C=N).

1-(4-hydroxyphenyl)-3-phenyl-3-(thiazol-2-ylamino)propan-1-one (2). The color is pale brown color, The yield 75%, m.p. 112-114 °C, IR (υ $cm^{-1}$), 3361 (phenolic O-H), 3164 (N-H amine), 3057 (aromatic C-H), 1642 (C=O ketone), 1601 (conjugated C=N): $^1$H-NMR (DMSO-d6 \ δ ppm), 9.71 (s, 1H, OH), 7.03 (s, 1H, NH), 7.87-6.73 (m,11H, Ar-H), 4.22 (t, 1H, CH), 3.28,2.38 (d, 2H, $CH_2$).

1-(4-hydroxyphenyl)-3-phenyl-3-(pyridin-2-ylamino) propan-1-one (3). Dark orange, the yield is 67%, m.p. 86-84 °C, IR (υ $cm^{-1}$), 3351 (phenolic O-H), 3268 (N-H amine), 3039 (C-H aromatic), 1661 (C=O ketone), 1593 (C=N).

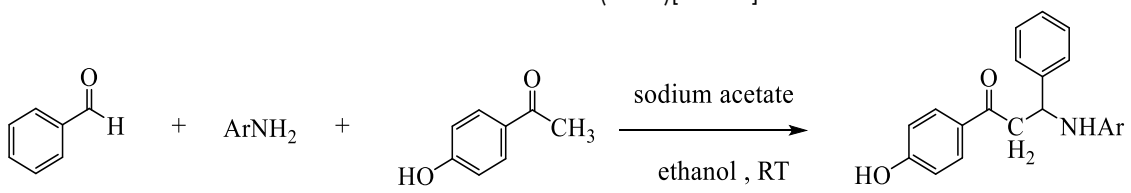
4-((3-(4-hydroxyphenyl)-3-oxo-1-phenylpropyl)amino)-1,5-dimethyl-2-phenyl-1,2-dihydro-3H-pyrazol-3-one (4). Pale brown, yield 88%, m.p 148-150°C, IR (υ $cm^{-1}$), 3396 (O-H phenol), 3268 (N-H amine), 3052 (C-H aromatic), 1672 (C=O ketone), 1636 (C=O aromatic amine), 1556 (C=C cycloalkene). $^1$H-NMR (DMSO-d6 / δ ppm), 9.63 (s, 1H, OH) , 8.63 (s, 1H, NH) , 7.69-6.81 (m,13H, Arom-H), 4.01 (t, 1H, CH), 3.29,2.63 (d, 2H, $CH_2$) , 3.24 (s, 3H, $NCH_3$), 2.35 (s, 3H, $=CCH_3$).

1-(4-hydroxyphenyl)-3-((4-methylpyridin-2-yl)amino)-3-phenylpropan-1-one (5). Brown, yield 82%, m.p 113-115°C, IR (υ $cm^{-1}$), 3386 (O-H phenol), 3191 (N-H amine), 3054 (C-H aromatic), 1676 (C=O ketone), 1600 (C=N).

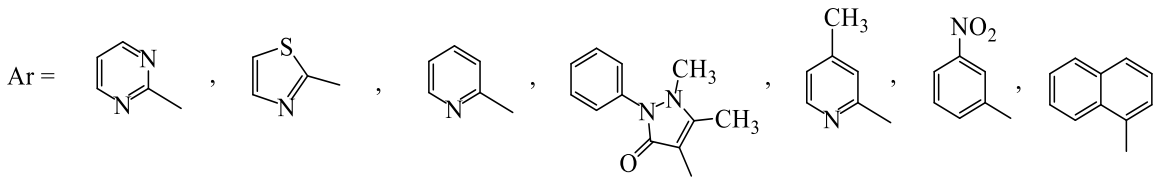
1-(4-hydroxyphenyl)-3-((3-nitrophenyl)amino)-3-phenylpropan-1-one (6). Yellowish-brown, yield 61%, m.p 145-147°C, IR (υ $cm^{-1}$), 3395 (O-H phenol), 3228 (N-H amine), 3051 (C-H aromatic), 1695 (C=O ketone), 1537,1263 ($NO_2$ asy, sym).

1-(4-hydroxyphenyl)-3-(naphthalen-1-ylamino)-3-phenylpropan-1-one (7). Brown, the yield is 69 %, m.p. 280-282 °C, IR (υ $cm^{-1}$), 3342 (phenolic O-H), 3284 (N-H amine), 3055 (aromatic C-H), 1673 (C=O ketone).

2.1.2. General procedure for the synthesis of 1-(4-nitrophenyl)-3-phenyl-3-(arylamino) propan-1-one (8-14)[25-27]:




(1-7)




**Scheme 1**. Synthesis of 1-(4-hydroxyphenyl)-3-phenyl-3-(arylamino) propan-1-one (1-7)

We followed the same above method (2.1.1), but only by replacing *p*-hydroxyacetophenone by *p*-nitroacetophenone (0.42 g) to form the compounds (8-14) as shown in scheme (2).

1-(4-nitrophenyl)-3-phenyl-3-(pyrimidin-2-ylamino)propan-1-one (8). Yellow, yield 83%, m.p 231-233 °C, IR (υ $cm^{-1}$), 3222 (N-H secondary amine), 3064 (aromatic C-H), 1686 (C=O ketone), 1580 (conjugated C=N), 1513,1248 ($NO_2$ asy, sym). $^1$H-NMR (DMSO-d6 / δ ppm), 6.88 (s, 1H, NH), 8.46-6.56 (m, 12H, Ar-H) , 4.19 (t , 1H, CH), 3.03,2.33 (d, 2H, $CH_2$).

1-(4-nittrophenyl)-3-phenyl-3-(thiazol-2-ylamino) propan-1-one (9). Pale orange, the yield is 72%, m.p. 123-125 °C; IR (υ $cm^{-1}$), 3230 (N-H amine), 3069,3106 (C-H aromatic), 1685 (C=O ketone), 1601 (C=N), 1516,1332 ($NO_2$ asy, sym). $^1$H-NMR (DMSO-d6 / δ ppm), 6.92 (s, 1H, NH), 7.90-7.32 (m ,11H , Ar-H), 4.18 (t , 1H, CH), 3.16, 2.47 (d, 2H, $CH_2$).

1-(4-nitrophenyl)-3-phenyl-3-(pyridin-2-ylamino) propan-1-one (10). Dark orang, yield 69%, m.p. 132-134 °C, IR (υ $cm^{-1}$), 3194 (N-H amine), 3055, 3015 (aromatic C-H), 1672 (C=O ketone), 1596 (conjugated C=N), 1515,1331 ($NO_2$ asy, sym). $^1$H-NMR (DMSO-d6 / δ ppm), 8.47 (s, 1H , NH), 8.01-6.39 (m,13H,Ar-H ) , 4.17 (t, 1H, CH), 3.33,2.47 (d, 2H, $CH_2$).

1,5-dimethyl-4-((3-(4-nitrophenyl)-3-oxo-1-phenylpropyl)amino)-2-phenyl-1,2-dihydro-3H-pyrazol-3-one (11). Yellow, the yield 91%, m.p. 158-159 °C, IR (υ $cm^{-1}$) , 3254 (N-H secondary amine), 3049 (aromatic C-H), 1688 (C=O ketone), 1637 (C=O amide), 1557 (C=C), 1518,1301 ($NO_2$ asy, sym) . $^1$H-NMR (DMSO-d6 / δ ppm) , 8.75 (s, 1H, NH), 8.11-7.05 (m , 14H, Ar-H), 3.57 (t , 1H, CH) , 3.41,2.88 (d , 2H, $CH_2$), 3.24 (s, 3H , $NCH_3$), 2.24 (s, 3H, $=CCH_3$) .

3-((4-methylpyridin-2-yl)amino)-1-(4-nitrophenyl)-3-phenylpropan-1-one (12). Yellowish-brown, the yield is 79% , m.p. 125-127 °C, IR (υ $cm^{-1}$), 3189 (N-H secondary amine), 3057 (aromatic C-H), 1682 (C=O ketone), 1601 (conjugated C=N), 1514,1335 ($NO_2$ asy, sym). $^1$H-NMR (DMSO-d6 /δ ppm) , 8.39 (s, 1H, NH), 8.35-6.56 (m, 12H , Ar-H), 4.29 (t, 1H, CH), 2.99,2.42 (d, 2H, $CH_2$), 2.37 (s, 3H, $=CCH_3$).

1-(4-nitrophenyl)-3-((3-nitrophenyl)amino)-3-phenylpropan-1-one (13). Bright yellow, the yield is 86%, m.p. 233-235 °C ; IR (υ $cm^{-1}$), 3329 (N-H secondary amine), 3100 (aromatic C-H) , 1689 (C=O ketone), 1516,1336 ($NO_2$ asy , sym) ; $^1$H-NMR (DMSO-d6 / δ ppm) , 8.47 (s, 1H, NH), 8.38-6.99 (m,13H, Ar-H), 4.23 (t , 1H, CH), 3.10,2.47 (d, 2H, $CH_2$).

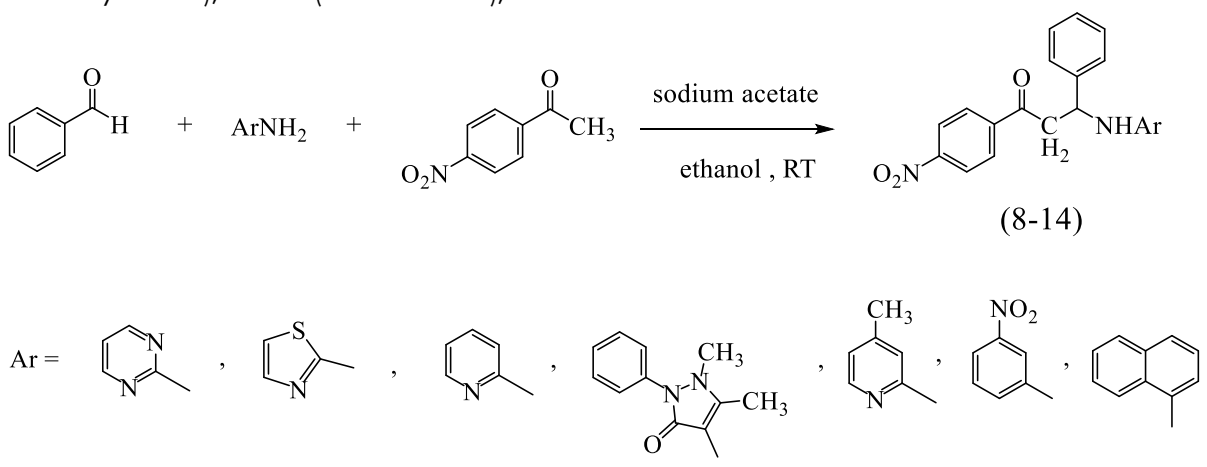
3-(naphthalen-1-ylamino)-1-(4-nitrophenyl)-3-phenylpropan-1-one (14). Bright yellow, yield 86%, m.p 233-235°C ; IR (υ $cm^{-1}$), 3279 (N-H amine), 3053 (C-H aromatic), 1690 (C=O ketone), 1514,1335 ($NO_2$ asy, sym) ; $^1$H-NMR (DMSO-d6 / δ ppm) , 8.37 (s, 1H , NH), 8.33-7.16 (m , 16H, Ar-H), 4.21 (t , 1H, CH), 3.28,2.43 (d, 2H, $CH_2$).

## Antimicrobial Activity

The newly prepared heterocyclic samples were evaluated *in vitro* against two species of G+ bacteria (*Streptococcus pneumoniae, Staphylococcus aureus*) as well as G- bacteria species (*Escherichia coli, Klebsiella* spp.) as well as the yeast strain (*Candida albicans*). Donation of the four microbes under study were done in Microbiology lab. In Al-Salam Hospital/Mosul province. The four microbes were subcultured at 37°C on Brain heart infusions agar (for bacteria) and dextrose agar (for fungus). Then the antimicrobial sensitivity test was applied using agar disc diffusion method (LaPierre et al., 2020).

## Preparation the discs:

Using the agar disc¬diffusion protocol, test samples are mixed with a known weight of the drug in dimethyl sulfoxide (DMSO) and after dilution to generate concentrations of 25, 50, 75 and 100 mg/ml. Soak sterile discs of filter paper (6 mm) in the solution and leave to dry at room temperature. Antibacterial activity was tested *in vitro* exploiting Mueller Hinton.




**Scheme 2.** Chemical route for the synthesis of 1-(4-nitrophenyl)-3-phenyl-3-(arylamino) propan-1-one (8-14)

3-5 colonies of bacterial isolates were twisted and transferred to a tube with (3 ml) of normal-saline solution with a vortex well. By the amount 100 microliters, of vaccine standard bacterial suspension (1.5108 CFU / ml) acquired from McFarland-turbidity standard (No. 0.5) were inoculated to each bacteria using a surface-glass sprayer. After incubation for 16–24 h on Mueller-Hinton Agar {MHA} plates, the discs were placed, and the plates were incubated, at 37° C for one day, however sensitivity test to control discs was done in this study for G+, G- bacteria, and *Candida albicans* (clindamycin 10, meropenem 10, azithromycin 18, and voriconazole 1) then, inhibition zone was measured as diameter in mm are given in the (**Table 1**) (Neli et al., 2011; Mahdi et al., 2015; Syal et al., 2017; Komenan, 2019).

### Theoretical Studies:

The use of early theoretical studies can reduce clinical failure of some drugs, among these studies are SwissADME prediction (absorption, distribution, metabolism, and excretion) and the cardioToxCSM test, which is concerned with studying the toxicity of the prepared compounds on heart functions such as Cardiac Failure, Heart Block, Arrhythmia, hERG toxicity, HT, and MI(Hassan et al., 2022).

### Swiss ADME Calculations:

The discovery of drugs goes through several important theoretical stages before applying them practically, including the absorption, distribution, metabolism and excretion test (ADME) in addition to some physiochemical properties of the proposed drug. This can be accessed theoretically using the Swiss ADME web tool by the link (http://www.swissadme.ch) which give the pharmacokinetics information of the proposed compound as a drug (Daina et al., 2017).

### Cardiotoxicity test:

This test studies the effect of prepared compounds on heart functions such as Arrhythmia, Cardiac Failure, Heart Block, HERG toxicity, Hypertension, and Myocardial Infarction by accessing the web server:
(https://biosig.lab.uq.edu.au/cardiotoxcsm/prediction_results/all_1672781700.53)
(cardioToxCSM: A web-server for predicting broad range cardiotoxicity of small molecules) (Iftkhar et al., 2022).

## RESULTS AND DISCUSSION

### Chemistry

Fourteen compounds of the class of Beta-amino carbonyl compounds were prepared in one step by simple Mannich reaction by mixing four components which are benzaldehyde, some selected aromatic primary amines, sodium acetate with either p-hydroxyacetophenone or p-nitroacetophenone with ethanol to obtain the target compounds (1-14). After drying the products, their melting points were measured, as there was a difference in melting points of more than 10°C between the reactants and the products of the Mannich reaction (1-14), and this gives preliminary evidence of the correctness of the method used.

As for spectroscopic measurements using IR, $^1$H-NMR, it confirmed the occurrence of a Mannich reaction, where the stretching absorption band of the aldehyde C-H and the carbonyl aldehyde stretch band supposed to appear at about (2730), (1720-1740) $cm^{-1}$ respectively disappeared, and one of the two (N-H) stretch bands for amine compounds disappeared and the bands appeared at frequencies less than the aromatic primary amines, new (O-H) stretching bands also appeared for compounds 1-7 and asymmetric, symmetric stretch bands for NO2 groups in compounds (8-14) , in addition to the appearance of stretching bands C=N for compounds (1,2,3,5, 8,9,10,12) in the range (1601-1586) of the amines containing heterocyclic rings included nitrogen. In general, IR absorbers in the regions (3396-3342), (3284-3164), (3057-3039), (1695-1661) $cm^{-1}$ for OH, NH, aromatic C-H, and C = O ketone alternately.

In the compounds, the chemical shift of the N-H protons (2,4,8-14) was resonated as a band-plug in the 1H-NMR (400 MHz, DMSO-d6) spectrum (8.63-6.88). The disappearance of the $CH_3$ group bands in compounds (2,8,9,10,13,14) gives conclusive evidence of the nucleophilic attack of the anion formed as a result of the withdrawal of the acidic proton of acetophenone compounds by the base (sodium acetate) and the emergence of new bands at the range (3.41-2.33) belonging to the new $CH_2$ group, as well as the disappearance of the benzaldehyde proton, which confirms the occurrence of the Mannich reaction.

### Antibacterial Activity

Beta-aminocarbonyl compounds are useful building blocks for the synthesis of biologically and medically important molecules. After testing the biological effectiveness of some of the compounds prepared in this research, which are listed in **Table 1** and **Figure 1**. The findings revealed that the compounds (8,10,11,13) had varying antimicrobial efficiency against the tested bacteria, with efficacy varying depending on the chemical concentration (25 mg/ml, 50 mg/ml, 75 mg/ml, 100 mg/ml). The chemicals' antibacterial action is dose-dependent, indicating that Gram + bacteria (*S. pneumonia*) resisted all of the compounds at varied concentrations and were not inhibited in their development, while all the tested compounds at all concentrations gave varying inhibition values against bacteria (*S. aureus*) and the highest values were for the two compounds (11,13). As for the valued positive bacteria (*E. coli*) compounds (8, 10, 11) only give growth inhibition values at concentrations (75 and 100) mg/ ml. Ultimately, with regard to the (*C. albicans*) yeast strain, the growth inhibition values for

the two compounds (8 and 10) were good and at all tested concentrations, while the compounds (11 and 13) resisted and did not give significant inhibition values.

We conclude from the foregoing that the compounds containing a hexagonal ring including a nitrogen atom gave good anti-bacterial activity and that all the tested compounds gave relatively high values as anti-bacterial (*S. aureus*).

**Table 1.** Antimicrobial activity in millimeters at various concentrations of substances in mg/ml (8,10,11,13) using disc diffusion method.

| Microorganisms | Concentration (mg/ml) | | | | compound No. |
|---|---|---|---|---|---|
| | 25 | 50 | 75 | 100 | |
| *Staphylococcus aureus* | 13.1 | 12.2 | 14.8 | 14.8 | 8 |
| | 20.5 | 20.7 | 29.4 | 30.4 | 10 |
| | 9.2 | 14.6 | 14.2 | 18.3 | 11 |
| | 17.4 | 25.5 | 27.4 | 30.7 | 13 |
| *Streptococcus pneumonia* | -ve | -ve | -ve | -ve | 8 |
| | -ve | -ve | -ve | -ve | 10 |
| | -ve | -ve | -ve | -ve | 11 |
| | -ve | -ve | -ve | -ve | 13 |
| *Escherichia coli* | -ve | -ve | 9.2 | 13.6 | 8 |
| | -ve | -ve | 9.8 | 10.2 | 10 |
| | -ve | -ve | -ve | -ve | 11 |
| | -ve | -ve | 10.6 | 11.2 | 13 |
| *Klebsiella spp.* | -ve | -ve | -ve | -ve | 8 |
| | -ve | -ve | 12.5 | 13.6 | 10 |
| | -ve | -ve | -ve | 11.3 | 11 |
| | 9.4 | 12.2 | 13.1 | 14.5 | 13 |
| *Candida albicans* | 12.8 | 13.4 | 13.5 | 17.3 | 8 |
| | 11.6 | 15.8 | 18 | 18.2 | 10 |
| | -ve | -ve | -ve | -ve | 11 |
| | -ve | -ve | -ve | -ve | 13 |
| | Inhibition zone (mm) | | | | |

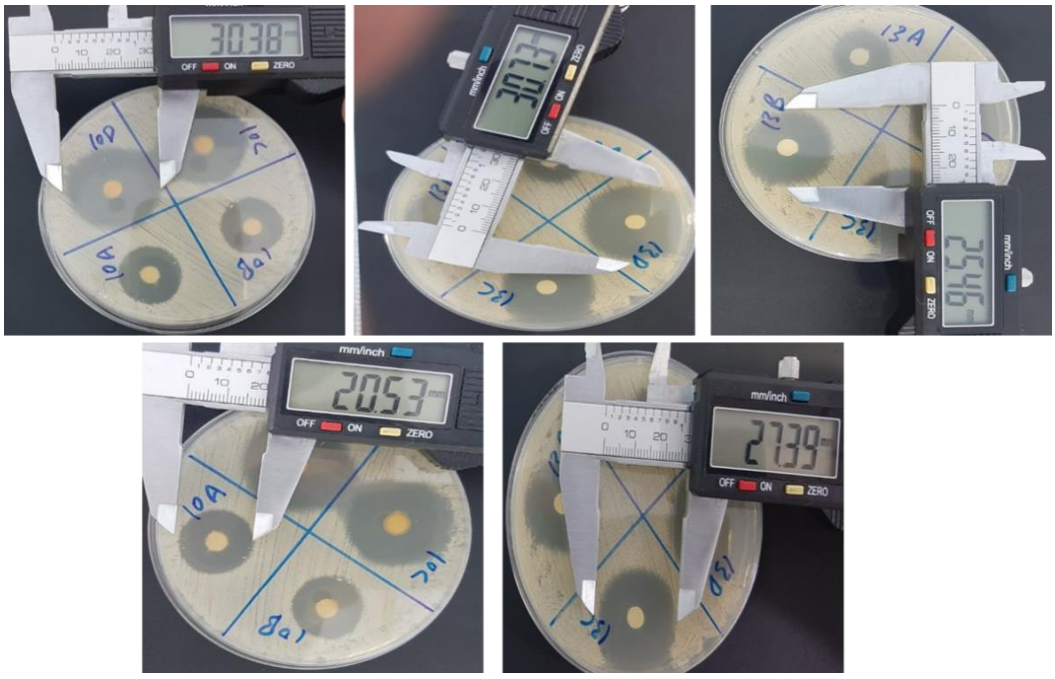


**Figure 1.** The inhibition zone of compounds (8,10,11,13) against *S. aureus*

To support the research findings, some previous research was reviewed, and it was found that Singh et al. (2016) discovered that derivatives of beta-aminocarbonyl molecules are essential building blocks for compounds such as amino acids, lactams, and amino alcohols, and are used in a variety of pharmaceutical and natural preparations. These derivatives have a wide range of medical applications such as antibacterial, anti-inflammatory, anticonvulsant, and anticancer drugs. In another study, a number of derivatives of Mannich bases were prepared, which gave inhibitory activity to the growth of a number of bacteria and fungi (Rambabu et al., 2022). A large number of studies prove the biological effectiveness of beta-aminocarbonyl compounds.

## Theoretical Study

The drug manufacturing process goes through complex stages and a long journey before being used on humans. In order to reduce costs and shorten time, some theoretical tests are conducted on compounds that give biological activity outside the body for the purpose of manufacturing a safe and effective drug at the same time.

## Swiss ADME Calculation

The use of early theoretical studies can reduce clinical failure of some drugs. Among these studies are ADME parameters (absorption, distribution, metabolism, and excretion). The prepared compounds (1-14) were examined using the SwissADME web tool to predict some pharmacokinetic and physicochemical properties of compounds to gather some information on their theoretical suitability in the body as chemical drugs (Daina & Zoete, 2016, Daina et al., 2017). The results are summarized in the **Table 2**.

Some theoretical measurements were made to anticipate the possibility of using compounds (1-14) to manufacture certain drugs according to specific principles (Daina & Zoete, 2016, Daina et al., 2017), where swissADME parameters gave the following information. The Lipinski Rule applies to all prepared compounds (1-14), where the value of violations = 0, and therefore it can be considered as one of the compounds that can be used orally after conducting all other tests. As for the solubility in water, it was found that all compounds have medium solubility except Compounds (7,11,14), its solubility was poor, the reason is that they contain a hydrophobic part. As for the Pharmacokinetics study, it indicates the possibility of compounds being absorbed through the gastrointestinal tract (GI absorption) into the bloodstream, in addition to the possibility of crossing compounds (1,3,5,7) through the blood-brain barrier (BBB permeant). The test (P-gp substrate) also gave an indication that the compounds (6, 7, 13, 14) do not travel with transport glycoproteins present in the cell wall, which works to push toxic substances out of the cell membrane. Alerts = 0 in the Pan Assay Interference Compounds (pains) test, gives an indication that all the prepared compounds do not have parts that can give false positives that lead to harmful interactions with proteins inside the body.

## Cardiotoxicity Predicting

Cardiac toxicity caused by some drugs is considered one of the most important reasons that led to the withdrawal of a number of drugs from the market and their failure, such as Fenpride, Propoxyphene, and Valdecoxib (Iftkhar et al., 2022), so it is necessary to study this aspect theoretically before starting to manufacture any compound suggested as a drug, and accordingly, a free and computational method was found on the Internet, CardioToxCSM, which gives information about the toxicity of compounds on the heart, including arrhythmia, cardiac failure, heart block, HERG toxicity, HT, and MI. Therefore, the prepared compounds (1-14) were studied to show their cardiotoxicity. The expected results are summarized in **Table 3**.

The above table shows that all compounds are expected to not cause Arrhythmias, and are also safe and do not cause Heart Block, and that only compound (7) can cause Cardiac failure. As for the test HERG toxicity, it gave an expectation that the compounds (1,2,3,5,6,12) do not bind to the cardiac potassium channel, and therefore do not cause sudden arrhythmias. While the compounds (4,11) are expected to cause high blood pressure, the study also showed that the compounds (1,2,3,4,5) are expected to obstruct blood flow to the heart muscle, perhaps resulting in a heart attack (MI). Accordingly, it was found that the two compounds (6, 12) do not have any cardiotoxicity, and compound (4) possesses the highest cardiac toxicity among the other compounds.

**Table 2**. Some parameters of Swiss ADME for compounds (1-14)

| Molecule No. | Formula | MW | H-bond acceptors | H-bond donors | iLOGP | XLOGP3 | WLOGP | MLOGP | Silicos-IT Log P | Consensus Log P | GI absorption | BBB permeant | Pgp substrate | Lipinski #violations | Bioavailability Score | PAINS #alerts |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | C19H17N3O2 | 319.4 | 4 | 2 | 2.09 | 3.56 | 3.09 | 1.72 | 3.04 | 2.7 | High | Yes | No | 0 | 0.55 | 0 |
| 2 | C18H16N2O25 | 324.4 | 3 | 2 | 2.32 | 4.31 | 3.76 | 1.95 | 4.23 | 3.31 | High | No | No | 0 | 0.55 | 0 |
| 3 | C20H18N2O2 | 318.4 | 3 | 2 | 2.44 | 4.21 | 3.7 | 2.37 | 3.6 | 3.26 | High | Yes | No | 0 | 0.55 | 0 |
| 4 | C26H25N3O3 | 427.5 | 3 | 2 | 3.42 | 5.22 | 4.1 | 3.25 | 3.8 | 3.96 | High | No | No | 0 | 0.55 | 0 |
| 5 | C21H20N2O2 | 332.4 | 3 | 2 | 2.67 | 4.57 | 4.01 | 2.59 | 4.12 | 3.59 | High | Yes | No | 0 | 0.55 | 0 |
| 6 | C21H18N2O4 | 362.4 | 4 | 2 | 2.29 | 4.77 | 4.21 | 2.42 | 2 | 3.14 | High | No | No | 0 | 0.55 | 0 |
| 7 | C25H21NO2 | 367.4 | 2 | 2 | 2.91 | 6.19 | 5.46 | 4.1 | 5.21 | 4.77 | High | Yes | Yes | 0 | 0.55 | 0 |
| 8 | C19H16N4O3 | 348.4 | 5 | 1 | 2.21 | 3.74 | 3.3 | 1.32 | 1.35 | 2.38 | High | No | Yes | 0 | 0.55 | 0 |
| 9 | C18H15N3O35 | 353.4 | 4 | 1 | 2.47 | 4.49 | 3.96 | 1.52 | 2.54 | 3 | High | No | No | 0 | 0.55 | 0 |
| 10 | C20H17N3O3 | 347.4 | 4 | 1 | 2.2 | 4.39 | 3.9 | 1.94 | 1.91 | 2.87 | High | No | No | 0 | 0.55 | 0 |
| 11 | C26H24N4O4 | 456.5 | 4 | 1 | 2.96 | 5.4 | 4.3 | 2.88 | 2.12 | 3.53 | High | No | No | 0 | 0.55 | 0 |
| 12 | C21H19N3O3 | 461.4 | 4 | 1 | 2.75 | 4.76 | 4.21 | 2.17 | 2.43 | 3.26 | High | No | No | 0 | 0.55 | 0 |
| 13 | C21H17N3O5 | 391.4 | 5 | 1 | 2.29 | 4.96 | 4.41 | 2.05 | 0.32 | 2.81 | High | No | Yes | 0 | 0.55 | 0 |
| 14 | C25H20N2O3 | 396.4 | 3 | 1 | 2.98 | 6.38 | 5.66 | 3.64 | 3.52 | 4.44 | High | No | Yes | 0 | 0.55 | 0 |

**Table 3.** Cardiotoxicity result of compounds (1-14)

| Comp. No. | Arrhythmia | Cardiac failure | Heart block | HERG toxicity | Hypertension | Myocardial infarction |
|---|---|---|---|---|---|---|
| 1 | Safe | Safe | Safe | Safe | Safe | Toxic |
| 2 | Safe | Safe | Safe | Safe | Safe | Toxic |
| 3 | Safe | Safe | Safe | Safe | Safe | Toxic |
| 4 | Safe | Safe | Safe | Toxic | Toxic | Toxic |
| 5 | Safe | Safe | Safe | Safe | Safe | Toxic |
| 6 | Safe | Safe | Safe | Safe | Safe | Safe |
| 7 | Safe | Toxic | Safe | Toxic | Safe | Safe |
| 8 | Safe | Safe | Safe | Toxic | Safe | Safe |
| 9 | Safe | Safe | Safe | Toxic | Safe | Safe |
| 10 | Safe | Safe | Safe | Toxic | Safe | Safe |
| 11 | Safe | Safe | Safe | Toxic | Toxic | Safe |
| 12 | Safe | Safe | Safe | Safe | Safe | Safe |
| 13 | Safe | Safe | Safe | Toxic | Safe | Safe |
| 14 | Safe | Safe | Safe | Toxic | Safe | Safe |

## CONCLUSIONS

The starting material for the research was chosen after reviewing several studies that reported that most nitro compounds have biological activity, which increases the possibility that the compounds produced by the study will be biologically beneficial. As for the compound *p*-hydroxyacetophenone, it was chosen on the basis that it contains an electron-donating hydroxyl group in order to compare the results. In addition to the availability of starting materials in our chemical stores. Two series of new aminocarbonyl compounds were prepared using the Mannich reaction, and after proving the chemical formulas physically and spectrally and based on practical and theoretical measurements, the results of the biological activity test for some of the prepared compounds (8,10,11,13) showed that the compound (13) gave the highest inhibition of *Staphylococcus bacteria aureus* at a concentration of 100 mg/ml and it was expected to give higher inhibition when tested at concentrations higher than 100 mg/ml. Theoretical measurements showed that all the prepared compounds were absorbed through the digestive system, and all of them applied Lipinski's rule (violation = 0) and also did not contain toxic parts that could be expelled by the cell wall to the outside (Alert = 0), but the cardiotoxicity tests gave an expectation that the two compounds ( 6, 12) The safest of them from the others, and the compound (4) had a higher effect than the rest of the compounds, as it is expected that it raises blood pressure and causes irregular heartbeat, which may cause a sudden heart attack.

## ACKNOWLEDGMENTS

Our thanks to the College of Pharmacy, University of Mosul.

## FUNDING

Self-Funded.

## APPROVAL BY THE ETHICS COMMITTEE

The research was authorized by the department of pharmaceutical chemistry's ethics committee at session number 6 on 20-2-2019.

## REFERENCES

Al-Abdullah, E. S., Al-Tuwaijri, H. M., Hassan, H. M., Haiba, M. E., Habib, E. E., & El-Emam, A. A. (2014). Antimicrobial and hypoglycemic activities of novel N-Mannich bases derived from 5-(1-adamantyl)-4-substituted-1, 2, 4-triazoline-3-thiones. *International journal of molecular sciences, 15*(12), 22995-23010.

Al-Wahaibi, L. H., Mohamed, A. A., Tawfik, S. S., Hassan, H. M., & El-Emam, A. A. (2021). 1, 3, 4-Oxadiazole N-Mannich bases: synthesis, antimicrobial, and anti-proliferative activities. *Molecules, 26*(8), 2110.

Bajaj, S., Kumar, M. S., Tinwala, H., & Mayur, Y. C. (2021). Design, synthesis, modelling studies and biological evaluation of 1, 3, 4-oxadiazole derivatives as potent anticancer agents targeting thymidine phosphorylase enzyme. *Bioorganic Chemistry, 111*, 104873.

Cai, L., Zhang, K., Chen, S., Lepage, R. J., Houk, K. N., Krenske, E. H., & Kwon, O. (2019). Catalytic asymmetric staudinger–aza-wittig reaction for the synthesis of heterocyclic amines. *Journal of the American Chemical Society, 141*(24), 9537-9542.

Cao, Z., Song, Q., Yu, G., Liu, Z., Cong, S., Tan, Z., & Deng,

Y. (2021). Novel 3-benzylidene/benzylphthalide mannich base derivatives as potential multifunctional agents for the treatment of

Alzheimer's disease. *Bioorganic & Medicinal Chemistry, 35*, 116074.

Daina, A. & Zoete, V. (2016). A Boiled-egg to predict gastrointestinal absorption and brain penetration of small molecules. *ChemMedChem, 11*,1117–1121.

Daina, A., Michielin, O., & Zoete, V. (2017). SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. *Scientific reports, 7*(1), 42717.

Datar, A.P., & Limaye, A.S. (2015). Design and synthesis of Mannich bases as benzimidazole derivatives as analgesic agents. *Anti-Inflammatory & Anti-Allergy Agents in Medicinal Chemistry,14*(1), 35-46.

Guchhait, T., Roy, S., & Jena, P. (2022). Mannich Reaction: An alternative synthetic approach for various pyrrole-based anion receptors and chelating ligands. *European Journal of Organic Chemistry, 2022*(34), e202200578.

Hassan, E. M., Mustafa, Y. F., & Merkhan, M. M. (2022). Computation in chemistry: representative software and resources, *International Journal of Pharmacy and Pharmaceutical Studies, 6*(4), 1-22.

Hayun, H. A. Y. U. N., Maggadani, B. P., Kurnia, A., Hanifah, A., Yuliandi, M., Fitriyani, I., & Hadrianti, S. P. (2019). Anti-inflammatory and antioxidant activity of synthesized mannich base derivatives of (2E, 6E)-2-[(4-Hydroxy-3-methoxyphenyl) methyl-idene]-6-(phenylmethylidene) cyclohexan-1-one. *Int J App Pharm, 11*(1), 246-50.

Iftkhar, S., de Sá, A. G., Velloso, J. P., Aljarf, R., Pires, D. E., & Ascher, D. B. (2022). CardioToxCSM: A web server for predicting cardiotoxicity of small molecules. *Journal of Chemical Information and Modeling, 62*(20), 4827-4836.

Jose, G., Kumara, T. H. S., Sowmya, H. B., Sriram, D., Row, T. N. G., Hosamani, A. A., ... & Ravikumar, Y. S. (2017). Synthesis, molecular docking, antimycobacterial and antimicrobial evaluation of new pyrrolo [3, 2-c] pyridine Mannich bases. *European Journal of Medicinal Chemistry, 131*, 275-288.

Khan, M. Q., Ullah, N., & Farooq, S. (2023). Mannich bases derivatives of 2-Phenyl-5-Benzimidazole sulfonic acid; Synthesis, Characterization, Computational studies and Biological evaluation. *Brazilian Journal of Pharmaceutical Sciences,* 59, e19544.

Kumar, A., & Choudhary, M. (2018). Modified series-fed dipole pair antenna using split-ring resonator directors for dual-band operation. *Journal of Taibah University for Science, 12*(2), 197-201.

LaPierre, L., Cornejo, J., Asun, A., Vergara, C., & Varela, D. (2020). M02–A12 Laboratory Guide: Methodologies for antimicrobial susceptibility testing, *APEC Sub-Committee on Standards and Conformance*.

Liu, Y., Xin, H., Yin, J., Yin, D., Yang, Z., & Li, J. (2018). Synthesis of novel ferrocenyl Mannich bases and their antibacterial activities. *Journal of Molecular Structure, 1157*, 482-485..

Mahdi, M. F., Al-Smaism, R. F., & Al-Khaliq, Z. M. A. (2015). Synthesis, characterization and antibacterial activity of new series of sulfamethoxazole derivatives. *World Journal of Pharmacy and Pharmaceutical Sciences, 4*(10), 284-293.

Marinescu, M., Cinteză, L. O., Marton, G. I., Chifiriuc, M. C., Popa, M., Stănculescu, I., ... & Stavarache, C. E. (2020). Synthesis, density functional theory study and in vitro antimicrobial evaluation of new benzimidazole Mannich bases. *BMC chemistry, 14*(1), 1-16.

Marinescu, M., Tudorache, D. G., Marton, G. I., Zalaru, C. M., Popa, M., Chifiriuc, M. C., ... & Constantinescu, C. (2017). Density functional theory molecular modeling, chemical synthesis, and antimicrobial behaviour of selected benzimidazole derivatives. *Journal of Molecular Structure, 1130*, 463-471.

Mousavi, M. R., Hazeri, N., Maghsoodlou, M. T., Salahi, S., & Habibi-Khorassani, S. M. (2013). Entirely green protocol for the synthesis of β-aminoketones using saccharose as a homogenous catalyst. *Chinese Chemical Letters, 24*(5), 411-414.

Neli, L.P., Yogendra, K., Berington, M., Ranjan, K.B. & Santa, R.J. (2011). In vitro antibacterial activity of alkaloid extract from stem bark of *Mahonia manipurensis* Takeda. *Journal of Medicinal Plants Research, 5*(5), pp.859-861.

Raju, S. K., Settu, A., Thiyagarajan, A., & Rama, D. (2023b). Synthetic applications of biologically important Mannich bases: An updated review. *Open Access Research Journal of Biology and Pharmacy, 07*(02), 001–015

Raju, S. K., Vengadhajalaphathy, P., Sundaram, R., Periyasamy, S., Chinnaraj, T., & Sekar, P. (2023a). Recent advances in biological applications of mannich bases—An overview. *International Journal of Pharmaceutical Chemistry and Analysis, 10*(1), 15-27

Rambabu, G., Kiran, Y. B. R., Tarakeswar, Y., Khalivulla, S. I., Barbosa, L. C. A., & Vijayakumar, V. (2022). Montmorillonite K10 clay catalyzed synthesis of novel β-aminocarbonyl compounds and their biological evaluation. *Chemistry & Biodiversity, 19*(10), e202200669.

Roman, G. (2022). Anticancer activity of Mannich bases: a review of recent literature. *ChemMedChem, 17*(16), e202200258.

Raoof, S. S., & Sadiq, A. S. (2022). Mannich bases: synthesis, pharmacological activity, and applications: A Review. *Iraqi Journal of Science*, 5086-5105.

Shi, S., Qiu, W., Miao, P., Li, R., Lin, X., & Sun, Z. (2021). Three-component radical homo Mannich reaction. *Nature Communications, 12*(1), 1006.

Singh, R., Bhasin, G., & Srivastava, R. (2016). β-aminocarbonyl compounds: chemistry and biological activities. *Mini-Reviews in Organic Chemistry, 13*(2), 143-153.

Sivakumar, K. K., Rajasekaran, A., Senthilkumar, P., & Wattamwar, P. P. (2014). Conventional and microwave assisted synthesis of pyrazolone Mannich bases possessing anti-inflammatory, analgesic, ulcerogenic effect and antimicrobial properties. *Bioorganic & Medicinal Chemistry Letters, 24*(13), 2940-2944.

Syal, K., Mo, M., Yu, H., Iriya, R., Jing, W., Guodong, S., & Tao, N. (2017). Current and emerging techniques for antibiotic susceptibility tests. *Theranostics, 7*(7), 1795.

Taher-Maghsoodlou, M., Rasul-Mousavi, S., Roygar, A., & Lashkari, M. (2018). Three-component carbon-carbon bond-forming reactions for the synthesis of β-amino carbonyl compounds using maleic acid-catalyzed one-pot mannich-type reaction in ethanol. *Kragujevac Journal of Science*, *40*, 71-81.

Tao, D., Wang, Y., Bao, X. Q., Yang, B. B., Gao, F., Wang, L., ... & Li, L. (2019). Discovery of coumarin Mannich base derivatives as multifunctional agents against monoamine oxidase B and neuroinflammation for the treatment of Parkinson's disease. *European Journal of Medicinal Chemistry*, *173*, 203-212.

Wang, B. L., Zhang, L. Y., Liu, X. H., Ma, Y., Zhang, Y., Li, Z. M., & Zhang, X. (2017). Synthesis, biological activities and SAR studies of new 3-substitutedpheny l-4-substitutedbenzylidene amino-1, 2, 4-triazole Mannich bases and bis-Mannich bases as ketol-acid reductoisomerase inhibitors. *Bioorganic & medicinal chemistry letters*, *27*(24), 5457-5462..

Zalaru, C., Dumitrascu, F., Draghici, C., Tarcomnicu, I., Tatia, R., Moldovan, L., ... & Ferbinteanu, M. (2018). Synthesis, spectroscopic characterization, DFT study and antimicrobial activity of novel alkylaminopyrazole derivatives. *Journal of Molecular Structure*, *1156*, 12-21..

Zhang, Z. Y., Zhu, Y. H., Zhou, C. H., Liu, Q., Lu, H. L., Ge, Y. J., & Wang, M. W. (2014). Development of β-amino-carbonyl compounds as androgen receptor antagonists. *Acta Pharmacologica Sinica*, *35*(5), 664-673.