Synthesis, Characterization and Study of some of New Mefenamic Acid Derivatives as cytotoxic Agents

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Abstract

A new system drugs derivative was designed from Mefenamic Acid. These derivatives have been synthesized by reaction between mefenamic acid and different amino drugs via amide group. A number of compounds based on this new scaffold were prepared in good yields. These compounds have been purified and followed using TLC to determine purity with Rf value. All synthesized compounds structures were confirmed by ¹H-NMR, ¹³CNMR and FTIR techniques. All compounds were evaluated for their anticancer and antibacterial activities. Some of the compounds synthesized showed cytotoxic activities in vitro. Most of synthesized compounds induced significant reduction in the cytotoxic response as compared with controls.

Keywords: Mefenamic Acid, amino drugs, cytotoxic, Anti inflammatory
Introduction

Mefenamic acid [2-[N-(2,3-dimethylphenyl)amino]benzoic acid or ponstan] is one of a non-steroidal anti-inflammatory drug (NSAID) which exhibits anti-inflammatory, analgesic, and antipyretic activities [1,2]. It is extensively used as a therapeutic agent, moreover than 100 million treatments of NSAIDs throughout the world were made [3]. The dose allowed of drug is between 500 mg and 250 mg more than 7 days. The action mechanism of mefenamic acid is inhibition of COX (cyclooxygenase enzymes) which are required for prostaglandins production [4,5]. Mefenamic acid structure (figure 1) belongs to the aromatic amino acids and this make it having the biological activity [6]. This compound have very low solubility in gastric irritation, biological fluids and have a short biological half-life of two hours [7]. poorly water-soluble drugs and high permeability [8, 9].

![Mefenamic Acid Structure](image)

Figure 1. Mefenamic acid structure

It is still highly used to reduce pain, osteoarthritis, and a headache [10]. Pain is a problem not explained by medicine. According to the WHO, 90 % of diseases are related with pain [11]. Mefenamic acid (MA) is antirheumatic agent, furthermore, recent studies report on a therapeutic probable of the drug for cancer cell lines and Alzheimer’s disease [12, 13].

The expression ‘codrug’ or ( prodrug) pointing to two or more therapeutic compounds bonded via a covalent chemical linkage. In any case of being similar to prodrug it varies in having inactive group exchange by active group, which are combine indirectly or directly to the main moiety [14].

Here we are reported synthesized new prodrugs as mefenamic acid derivatives, some of them tested as anticancer and the Anti-inflammatory tested for all.
Experimental part

Materials and instruments

All used chemicals are in the highest available purity. All the starting materials used in this paper were purchased from Sigma Aldrich. The instruments used for the characterization of the prepared compounds were: Melting points were determined on a Gallenkamp MFB-600-Melting point Stuart apparatus, FT-IR spectra were recorded on a Bruker spectrometer. $^1$H-NMR were recorded on a Bruker AC 400 NMR spectrometer, operating at 400 MHz for $^1$H-NMR and 100 MHz for $^{13}$C-NMR. All chemical shifts ($\delta$) are reported in ppm relative to tetramethylsilane (TMS) as reference ($\delta$=0.0 ppm).

Biological activity:
The biological activity of the following compounds was studied against positive and negative bacteria, and cytotoxicity [15,16].

Maintenance of cell cultures

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

Synthesis of mefenamic acid derivatives:

5-(2-(2,3-dimethylphenylamino)benzamido)-2-hydroxybenzoic acid [RH6]
Mefenamic acid (0.24g, 1mmol), 10ml of DMSO (dimethylsulfoxide) and (0.102g, 1mmol) of SOCl$_2$ were mixed together and heating with stirring at (30-40) °C. Then, triethylamine (0.087 g, 3mmol) was added for (15min) at room temperature. After that, 5-aminosalicylic acid (5-ASA) (0.15g, 1mmol) was added and heating with stirring at (70°C) for (30 min.). The mixture were cooled by ice bath until the precipitate appeared, filtered and dried the product [17,18]. Colour: gray, m.p. 200°C Chemical Formula: C$_{22}$H$_{20}$N$_2$O$_4$ yield 87.6% . Elemental Analysis: C% 70.20 (71); H% 5.36(5.7); N% 7.44(7.22).

Synthesis of compounds R1-R5:
RH6 (0.5g, 1.3 mmol), 10ml of DMSO (dimethylsulfoxide) and (0.12g, 1.1mmol) of SOCl$_2$ were mixed together and heating with stirring at (30-40) °C. Then, triethylamine (0.09 g, 3.1mmol) was added for (15min) at room temperature. After that, amino drugs [(0.20g) 4-aminoantipyrin, (0.32g) cephalexin, (0.36g) amoxicillin, (0.33g) ciprofloxacin, (0.16)
pseudo-ephedrine] respectively, were added and heating with stirring at (70°C) for (30 min.). The mixture were cooled by ice bath until the precipitate appeared, filtered and dried the products.

5-(2-(2,3-dimethylphenylamino)benzamido)-2-hydroxy-N-(5-methyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)benzamide [R1]: (RH6 with 4-aminooantipyritin) Colour: black, m.p.145°C. Chemical Formula: C_{32}H_{29}N_{5}O_{4} yield 60%. Elemental Analysis: C% 70.19(69.9); H 5.34(5.1); N 12.79(12.5).

(2-(5-(2-(2,3-dimethylphenylamino)benzamido)-2-hydroxybenzamido)-2-phenylethylamino)-4-methyl-7-oxo-2-thiabi cyclo[4.2.0]oct-4-ene-5-carboxylic acid [R2] : (RH6 with cephalexin ) Colour: off white m.p. 110°C, chemical Formula: C_{39}H_{38}N_{4}O_{6}S yield 65%. Elemental Analysis: C% 67.81(66.4); H% 5.54(5.3); N% 8.11(7.9); S%, 4.64(4.1).

(5S)-6-((R)-2-(5-(2-(2,3-dimethylphenylamino) benzamido)-2-hydroxybenzamido)-2-(4-hydroxyphenyl)acetamido)-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo [3.2.0]heptane-2-carboxylic acid [R3]: (RH6 with amoxicillin ) Colour: light brown, m.p. 170°C Chemical Formula: C_{39}H_{37}N_{5}O_{8}S yield 70.5%. Elemental Analysis: C% 63.06(62.1); H% 5.15(5.01); N% 9.68(9.51); S%, 4.43(4.2).

1-cyclopropyl-6-(4-(5-(2-(2,3-dimethylphenylamino) benzamido)-2-hydroxybenzoyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid [R4] : (RH6 with ciprofloxacin ) Colour: dark brown, m.p 86°C Chemical Formula: C_{39}H_{37}N_{5}O_{6} yield 55.1% . Elemental Analysis: C% 69.73(68.5); H% 5.55(5.2); N% 10.43(10.1).

5-(2-(2,3-dimethylphenylamino)benzamido)-2-hydroxy-N-((1R,2R)-1-hydroxy-1-phenylpropan-2-yl)-N-methylbenzamide [R5] : (RH6 with pseudoephedrine) Colour: light pink , m.p 112°C Chemical Formula: C_{32}H_{33}N_{3}O_{4} yield 70% . Elemental Analysis: C% 73.40(72.1); H% 6.35(6.2); N% 8.02(7.8).

**Results and discussion**

New mefenamic acid derivatives were prepared starting by combined two drugs together mefenamic acid with Mesalazin to give new binary drug compound RH6 (scheme 1). This compound used as a starting material to design new tertiary drugs system. These derivatives synthesized via reacted RH6 with different amino drugs (4-aminooantipyritin, cephalexin, amoxicilllin, ciprofloxacin, pseudoephedrine respectively to give products R1-R5 (scheme 2). The structure of these compounds has been characterized by FT-IR, ^1^H-NMR and ^13^C-NMR spectra, and elemental analysis[19,20].

The FTIR(V_{max},cm^{-1}) spectrum for compound [RH6] show appearance 3307 (N-H str. ), 1795 (C=O carboxylic acid ), 1646 (C=O amid ) , 1561 –
2500 (OH carboxyl) , 2821 (O-H phenol) , 1487 and 1573 (C=C Ar.).

$^1$HNMR (400 MHz, DMSO-$d_6$, δ ppm) 15.0 (OH alcohol), 12.0 (OH carboxylic acid), 10.2, 9.90 (NH sec amine), 6.88-8.4 (CH-Ar), 2.1,2.3 CH3. $^{13}$CNMR (100 MHz, DMSO-$d_6$, δ ppm) 165.0, 148.0, 137.0,136.0, 133.3,132.2, 128.8,127.3,121.5,122.0, 119.5,118.2,117.1,20.2, 18.6

Scheme 1: synthesis of compound RH6
Scheme 2: Synthesis of compounds R1-R5
FTIR ($V_{\text{max}}, \text{cm}^{-1}$) of R1 shows: 2500 – 3069 (O-H carboxylic acid), 3343.6 (N-H str.), 1566.8 (NH bend.), 1648 (C=O amid), 1434 and 1492 (C=C Ar) 2943 (C-H bend. Sp$^3$). $^1$HNMR (400 MHz, DMSO-$d_6$, δ ppm) (OH, Ar) 10.16, (H,N-H amid) 9.47-9.75, (H,N-H amine) 8.45, (H-Ar) 6.68-8.16, (9H,CH$_3$) 2.25-3.63, (H,N-H)2.06. $^{13}$CNMR (100 MHz, DMSO-$d_6$, δ ppm):(C=O)170.67, (C-OH)149.22, (C-Ar)111.70-138.81, (C-CH$_3$) 39.80-14.15

FTIR ($V_{\text{max}}, \text{cm}^{-1}$) of R2 show up : 3307.05 (N-H str.), 1573 (N-H bend.), 1645 (C=O amid), 1739.7 (C=O carboxyl acid), 1328 and 1374 (C-N str.). $^1$HNMR (400 MHz, DMSO-$d_6$, δ ppm) (H-OH Ar) 12.99, (H, NH amid) 9.44-9.52, (H,NH amine) 9.48, (H, Ar) 6.64-7.92, (H,CH) 3.06-3.45,(H-CH$_3$) 3.01, (H-CH$_3$) 1.20-2. 59, (H,NH) 1.18. $^{13}$CNMR (100 MHz, DMSO-$d_6$, δ ppm) , (C=O) 170 971, (C,OH)149.25, (C-Ar) 111.72,(C,CH) 40.46-40.86 , (C,CH$_2$) 40.12-40.29, (C-CH$_3$) 14.10-20.65.

FTIR ($V_{\text{max}}, \text{cm}^{-1}$) spectrum of R3 show up : 3307.2 (N-H str.), 2570-3011.04 ( O-H alcohol), 1645.7 (C=O amid), 1469 and 1503 (C=C arom.), 1328 (C-N bend.). $^1$HNMR (400 MHz, DMSO-$d_6$, δ ppm) (H-OH carboxyl) 12.98, (H,NH amid) 8.29-9.51, (H,NH amine) 9.48, (H,OH, Ar) 9.44, (H,H-Ar) 6.68-8.29,(H,CH) 2.55-3.42, (H,CH$_3$) 1.44-2.51. $^{13}$CNMR (100 MHz, DMSO-$d_6$, δ ppm) , (C,C=O ring) 170.70, (C=C=O carboxyl) 149.24, (C,C=O amid) 134.62-138 82, (C,C=OH) 132.18, C,C=C Ar)111.17-131.70, (C,CH) 39.46-40.88, (C,CH$_3$) 14.11-20.67.

FTIR ($V_{\text{max}}, \text{cm}^{-1}$) spectrum of R4 shows: 3308 (N-H str.), 2648-3409 (O-H alcohol), 1645 (C=O amid), 1448 and 1504 (C=C arom.), 1329 (C-N bend.). $^1$HNMR (400 MHz, DMSO-$d_6$, δ ppm) (H, OH, Ar) 9.67, (H,NH amid) 9.45,(H,NH amine) 8.69, (H,H-Ar) 6.67-8.65, (H,CH) 3.85-3.86, (H,N-CH$_3$), (H,CH$_3$) 1.19-2.13. $^{13}$CNMR (100 MHz, DMSO-$d_6$, δ ppm) , (C,C=O ketone)176.74, (C,C=O amid)166.28-170.65, (C,C=O carboxyl) 152.31, (C,C=OH Ar)149.20 (C,N=C=O)148.48, (C,C=CAr) 107.22-144.53, (C,C=N) 40.87-46.78, (C,CH$_3$) 36.42-39.62, (C, C:cyclopropan) 8.09-20.68.

FTIR ($V_{\text{max}}, \text{cm}^{-1}$) spectrum of R5 show up : 3306 (N-H str.), 2639-3400.7 (O-H alcohol), 1645.5 (C=O amid), 1437 and 1499 (C=C arom.), 1326 (C-N bend.). $^1$HNMR (400 MHz, DMSO-$d_6$, δ ppm) (H,NH amid)12.91, (H,OH Ar) 9.50, (H,NH amine) 9.47, (H,H-Ar) 6.64-9.43, (H,OH alpha.) 4.54, (H,CH alpha.) 0.95-4.52. $^{13}$CNMR (100 MHz, DMSO-$d_6$, δ ppm) spectrum show up , (C,C=O amid)149.23-170.68, (C,OH Ar) 138.81, (C,C=C Ar)111.70-138.34, (C,CH$_3$) 40.89, (C,CH Alpha.) 14.12-40.49.
Physical properties of compounds Solubility:

Synthesized codrugs were highly soluble in water, ethanol, and acetone, DMSO, where as partial or soluble, ethyl acetate, diethyl ether, and chloroform indicating the presence of polar group.

Biological activity:

Cytotoxicity Assays

To determine the cytotoxic effect of (M6, M7), the MTT cell viability assay was done using 96-well plates. Cell lines were seeded at $1 \times 10^4$ cells/well. After 24 hrs. or a confluent monolayer was achieved, cells were treated with tested compounds at different concentration. Cell viability was measured after 72 hrs of treatment by removing the medium, adding 28 µL of 2 mg/mL solution of MTT and incubating the cells for 2.5 h at 37 °C. After removing the MTT solution, the crystals remaining in the wells were solubilized by the addition of 130 µL of DMSO (Dimethyl Sulphoxide) followed by 37 °C incubation for 15 min with shaking [19]. The absorbency was determined on a microplate reader at 492 nm; the assay was performed in triplicate. The inhibition rate of cell growth (the percentage of cytotoxicity) was calculated as the following equation [20]:

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

Where A and B are the optical density of control and the optical density of test

The obtained data were statically analyzed using an unpaired t-test with GraphPad Prism 6. The values were presented as the mean ± SEM of triplicate measurements[21].

MCF-7 is a breast cancer cell line evaluated the anti-proliferating effect of drug-loading RH6 and R1-R5 against the breast cancer cell lines. Based on cytotoxicity analyses, it concluded that prepared compounds RH6 and R1-R5 may be an appropriate and promising strategy for developing effective drug delivery system to clinical application against breast cancers. IC50 value was significantly decreased in these compounds (IC50=43.81, 37.24, 24.61, 22.69, 30.19 µM/ml respectively) in comparison with pure drugs and induced apoptotic cell death pathway. The results of this study suggest that RH6 and R1-R5 might be used for medical applications and offer a beneficial formulation for chemotherapy (figures 2-6).
The antibacterial activity

The antibacterial activity of prepared compounds RH6, R1-R5 was summarized in table 1. This activity was tested by the agar disc-diffusion method against negative gram bacteria Escherichia (E. coli.), and positive gram bacteria Staphylococcus aureus. Using different concentration of identical volume (250, 500, 100) µg/ml of the synthesized compounds, a solvent for all the compounds that used was DMSO, The results of these study shows that these compounds have a good anti-Bacterial activity.
Table 1: The antibacterial activities of compounds [RH6, R1-R5]
Antibacterial data in MIC [µg/ml]

| Compound | [gram +ve] | | | [gram -ve] | | |
|---|---|---|---|---|---|---|
| | 100mg | 50mg | 25mg | 100mg | 50mg | 25mg |
| RH6 | 23 | 18 | 15 | 20 | 14 | 6 |
| R1 | 20 | 15 | 7 | 20 | 16 | 8 |
| R2 | 22 | 18 | 8 | 19 | 10 | 9 |
| R3 | 30 | 20 | 10 | 30 | 22 | 15 |
| R4 | 30 | 25 | 25 | 30 | 25 | 23 |
| R5 | 20 | 12 | 10 | 18 | 15 | 10 |

Conclusion
A new system drugs were designed from mefenamic acid with new medicinal properties to expand the required drug. Where mefenamic acid is a major type of pharmaceutical organic compound, some of its derivatives may be used in the prepared of novel types of drugs that are produced via several steps. Mefenamic acid bounded with different amino drug all and prepared compounds were characterized by FTIR, $^1$H- NMR and $^{13}$C NMR spectroscopies. The biological activity for target molecules was studied, based on cytotoxicity analyses, and may be an appropriate and promising strategy for developing effective system to clinical application against breast cancers. The results of anti-Bacterial activity for these compounds was studied with good results. Physical properties have been investigated.

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