DOI Number: 10.37506/v14/i1/2020/ijfmt/192974

Study the Toxicity and Anticancer activity of Some New Amic Acid and Their Derivatives of Mefenamic acid

Ali A. Sabah[1], Muna S. Al-Rawi[1], Jumbad H. Tomma[1]

[1]Department of Chemistry, College of Education for Pure Science Ibn Al- Haitham, University of Baghdad, Baghdad, Iraq

Abstract

A series of amic acid derivatives of mefenamic acid were synthesized with the aim of inhibitting topical gastrointestinal toxicity of mefenamic acid**.** The key intermediate amic acid (III) was prepared from the reaction of acid hydrazid of mefenamic acid(II) with phthalic anhydrides in dry actone. The new type of imide compound (IV) was dehydrated the amic acid(III) with acetic anhydrous and sodium acetate. The esterification of hydroxyl groups of amic acid (III) produce corresponding ester(V), which was condensed with hydrazine hydrate to give acid hydrazide (VI), then the later compound reacted with syringaldehyde in dry benzene to yield new schiff base (VII). The new derivatives containing heterocyclic unit (VIII)-(X), four and five, member ring were successfully formed such as azetidin-2-one, thiazolidin-4-one, and,imidazolidin-4-one. The structures of the synthesized compounds were confirmed using FTIR, ^{1}HNMR, Mass and CHN-S. The antibacterial activities of some synthesized compounds were screened and showed a highest or low inhibition against *Staph.aureus (G+)*, *Bacillus subtilisa (G+)*, *Klebsiella pneumoniae (G-)*, and *E.coli (G-)*. Also, The cytotoxic effect of different concentrations of some the synthesized compounds was tested against MCF-7 cell line (human breast carcinoma cells) and positive results were obtained for some of them, which encouraged us to study the toxicity using living organisms (mice) to evaluate its acute toxicity and proved the resules of non-toxicity of the derivatives.

Keywords: *Mefenamic acid, imidazolidin-4-one, antibacterial activities, MCF-7 cell line, acute toxicity study.*

Introduction

Mefenamic acid is one of the anthranilic acid derivatives class of non-steroidal anti-inflammatory drugs (NSAID)) is thought to be due to their interference with prostaglandin biosynthesis, and is used to treat strong analgesic and antiinflammatory agents in the treatment of degeneration of joint cartilage, rheumatoid arthritis and musculosketal disease [1].

The general view in the literature shows that mefenamic acid undergoes a number of reactions, this has been used in the preparation of many compounds containing heterocyclic moiety of therapeutic molecules for different applications, including the presence of effective anti-cancer drugs, antimicrobial, inflammatory and cytotoxic activities[2].

Amic acid and its derivatives have been extremely used in drug industry, advanced materials and biologically active heterocycles. In fact, amic acid carrying suitable position of carboxylic group are prime precursors for synthesis of heterocyclic derivatives[3].

A large number of heterocyclic compounds have been explored for developing pharmaceutically molecules. Among this the derivatives: azetidin-2-one (β-lactam), thiazolidin-4-one, and imidazolidin-4-one, have been playing an important function in the pharmaceutical chemistry [4]. This study is aimed at synthesis new active molecules with fewer side effects that can be successfully used as drugs, the cytotoxic effect and non-toxicity proved the resules of this derivatives.

Corresponding Author:
Muna S. Al-rawi,
Assistant Professor,
E-mail: muna.samir67@gmail.com

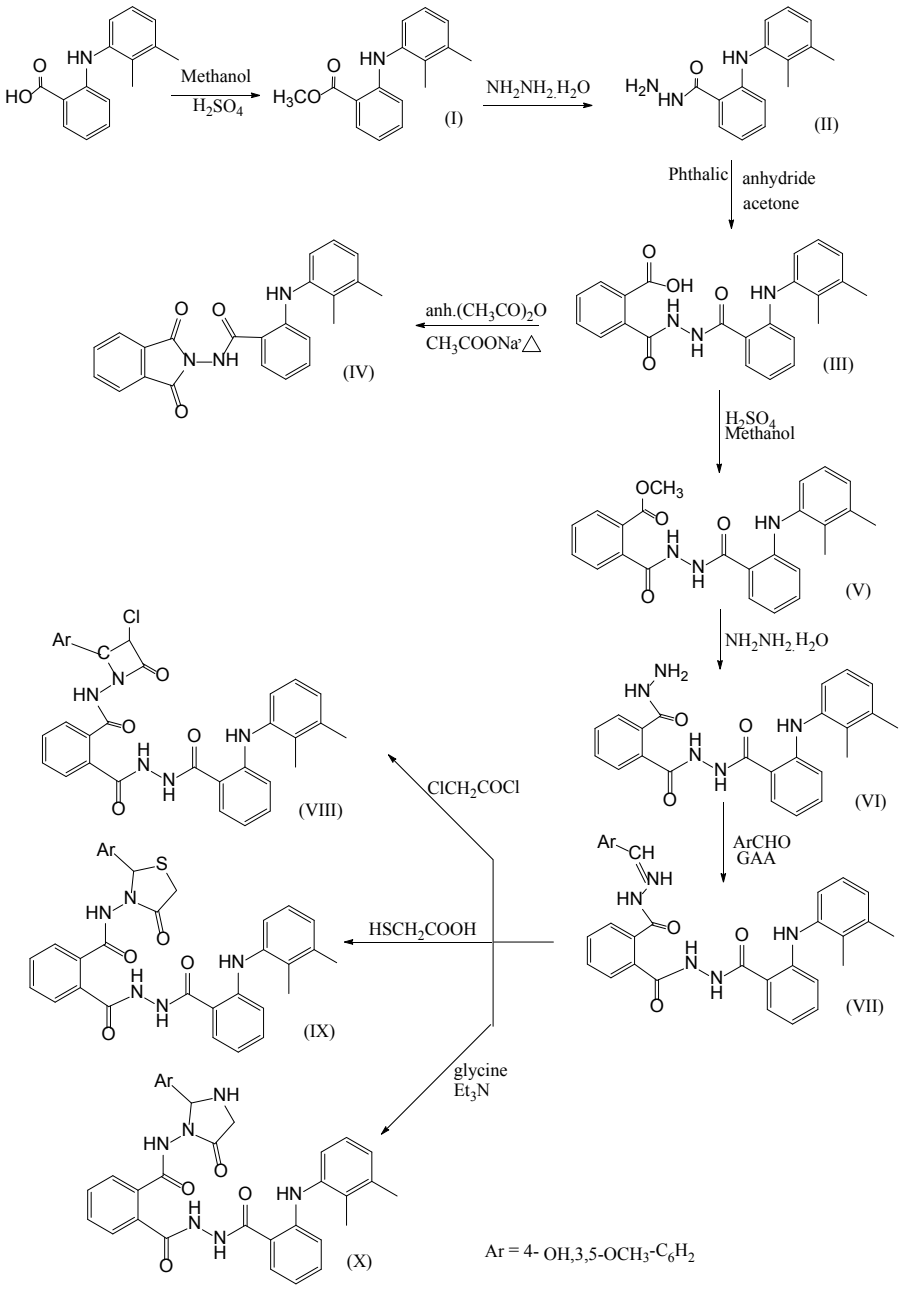
Experimental

Materials and instruments:

All solvents and chemicals were purchased from Sigma- Aldrich, and GCC Chemicals company. Melting points were recorded using electrothermal melting point apparatus and are uncorrected. The FTIR spectrums were registered using KBr discs on Shimadzo (Ir prestige-21) Fourier Transform Infrared Spectrometer. ^{1}HNMR spectra were recorded on Bruker, Ultra Shield (300)MHz, Switzerland and using DMSO-d_6, and $CDCl_3$ with reference to TMS as internal reference. Elemental micro-analyses were carried out using an EuroEA Analyzer. Mass spectra of compounds were measured by Electron Impact (EI) 70eVmass using a MS Model: 5973 spectrometer.

Synthetic Procedures

The routes for synthesis new derivatives as depending on to the Scheme (1):



Scheme (1): The synthetic route for target derivatives(I)-(X)

Preparation of methyl 2-(2,3-dimethylanilino) benzoate (I)

This compound (I)was prepared following the procedure described by Lit. [5] yield 80%; m.p(96-98)^{0}C.

Preparation of 2-(2,3-dimethylphenylamino) benzohydrazide (II)

This compound(II) was prepared following the procedure described by Lit. [5]. yield 78%,; m.p (118-120)ºC.

Synthesis of 2-(2-(2-((2,3-dimethylphenyl)amino) benzoyl)hydrazine-1-carbonyl) benzoic acid(III)

In a flask with magnetic stirrer, a solution of (2.55g, 0.01 mol) of acid hydrazide (II) in 20 mL of acetone was dripped into a solution of (1.48g, 0.01 mol) phthalic anhydride in 20 mL of acetone for 30 min at room temperature. Stirring was continued for 5 hrs. The brown solid precipitate was filtered, washed, dried and recrystallized from ethanol to give a new amic acid [III], yield 74% , m.p (167-169)º C; FTIR (ν ,cm^{-1}): 3392-2571(OH), 3340-3309 (NH), 3012 (C-H arom.), 2974-2912(C-H aliph.), 1712, 1653 (C=O), and 1255 (C-O); ^{1}H NMR (δ ppm): 2.09 (s, 3H, CH_3), 2.28 (s, 3H, CH_3), 6.66-8.08 (m,11H,Ar-H), 9.45, 7.91,and 7.58 for (s,1H,NH), 12.06(s, 1H, OH, broad); mass spectra (relative intensity%): m/z=403(5), 223 (75), 178(70), 75(100), 104(68), 51(25):Elemental analysis: Calcd. for $C_{23}H_{21}N_3O_4$: C, 68.48; H, 5.21; N, 10.42; Found: C, 68.18; H, 5.41; N, 10.73.

Synthesis of 2-((2,3-dimethylphenyl)amino) -N-(1,3-dioxoisoindolin-2-yl) benzamide (IV)

A mixture of 5 mL acetic anhydride and (0.82g , 0.01mol) anhydrous sodium acetate was allowed to dropping with stirring to a solution of amic acid(III) (4.03 g, 0.01 mol) in 10 mL ethanol for1hrs. The resulting mixture was refluxed over water path for 3hr. The reaction poured into ice water, the pale beige precipitated was collected filtered and washed with ice water and recrystallization from ethanol, yield 70% , mp. (188-190)ºC; FTIR (ν ,cm^{-1}): 3344 (NH), 2978 (C-H arom.), 2885-2735(C-H aliph.), 1747, 1645 (C=O), and 1238 (C-O); ^{1}H NMR (δ ppm): 2.09 (s, 3H, CH_3), 2.27 (s, 3H, CH_3), 6.66-8.06 (m,11H,Ar-H),9.57(s,1H,NH), 8.14(s,1H,NH); Elemental analysis: Calcd. for $C_{23}H_{19}N_3O_3$: C, 71.68; H, 4.93; N, 10.90; Found: C, 71.98; H, 5.11; N, 11.03.

Synthesis of methyl 2-(2-(2-((2,3-dimethylphenyl) amino) benzoyl) hydrazine -1-carbonyl) benzoate (V):

A mixture of compound (III) (4.03 g, 0.01mol), absolute methanol 30mL and H_2SO_4 5.4 mL was refluxed 6 hrs. After cooling washed the mixture with $NaHCO_3$ solution, The resulting of a white crystals solid was filtered off, washed with water, dried and recrystallized by ethanol to give compound (V), yield 71%; mp (137-139) ºC; FTIR (ν ,cm^{-1}): 3330-3309 (NH), 2987 (C-H arom.), 2939-2922(C-H aliph.), 1732, 1685, 1657 (C=O), and 1255 (C-O); ^{1}H NMR (δ ppm): 2.08 (s, 3H, CH_3), 2.27 (s, 3H, CH_3),3.86(s,3H,OCH_3), 6.66-8.09 (m,11H,Ar-H), 9.66,and 9.18 for (s,1H,NH); Elemental analysis for this compound : Calcd. for $C_{24}H_{23}N_3O_4$: C, 69.06; H, 5.51; N, 10.07; Found: C, 69.45; H, 5.80; N, 10.30.

Synthesis of N'-(2-((2,3-dimethylphenyl) amino) benzoyl) phthalohydrazide (VI):

A mixture of ester compound (V) (4.17gm,0.01mol), was dissolved in absolute ethanol 3mL. hydrazine hydrate(0.5gm,0.01 mol) was added slowly and the mixture was refluxed for 6hrs. The mixture was cooled and the pale yellow solid was filtered, then used ethanol to recrystallized, yield 70%, m.p (158-160)°C, FTIR (ν ,cm^{-1}): 3305-3145(NH_2,NH), 3001 (C-H arom.), 2941-2822(C-H aliph.), 1660,1639(C═O amide); ^{1}H NMR (δ ppm): 2.11 (s, 3H, CH_3), 2.28 (s, 3H, CH_3), 6.56-8.05 (m,11H,Ar-H),11.13, 9.18,and 8.13(s,1H,NH); Elemental analysis: Calcd. for $C_{23}H_{23}N_5O_3$: C, 66.18; H, 5.51; N, 16.78; Found: C, 66.70; H, 5.78; N, 17.01.

Synthesis of (Z)-N'1-(2-((2,3-dimethylphenyl) amino) benzoyl) - N'2-(4-hydroxy - 3,5-dimethoxybenzylidene) phthalohydrazide (VII)

A mixture of new compound (VI)(4.17g, 0.01 mol) , Syringaldehyde (1.82g, 0.01mol) with EtOH 15 mL and four drops of GAA glacial acetic acid was refluxed for 6hrs, cooled after that filtered the product and crystallized from ethanol,dark yellow powder, yield 70% , m.p. (150-152)°C, FTIR (ν ,cm^{-1}): 3548(OH), 3469-3344(NH), 3016 (C-H arom.), 2968-2935(C-H aliph.), 1651(C═O amide), 1626(C=N); ^{1}HNMR(δ,ppm,$CDCl_3$): 2.10 (s, 3H, CH_3), 2.29 (s, 3H, CH_3), 2.51(s, 1H,OH) , 3.84 (s,6H, OCH_3),6.68-8.58 (m,13H,Ar-H),7.32(s,1H,CH-N)

;11.98, 9.70,and 9.49 for groups (s,1H, NH); C.H.N-S: Calcd. for $C_{32}H_{31}N_5O_6$: C, 66.09; H, 5.33; N, 12.04; Found: C, 66.47; H, 5.58; N, 12.11.

Synthesis of N-((2R,3S)-3-chloro-2-(4-hydroxy-3,5-dimethoxyphenyl) -4-oxoazetidin-1-yl)-2-(2-((2,3-dimethylphenyl) amino) benzoyl) hydrazine-1-carbonyl) benzamide(VIII)

Chloroacetylchloride (0.01mol) in 10ml of dioxan calm down at (0-5) ^oC. Triethylamine (0.01mol) in(10mL) dioxane was added, then compound(VII) (5.81g, 0.01 mol) in 10mL of dioxane was added and heated 10hrs. The reaction poured into ice water to give red solid precipitate, filtered and dried. Yield 72%, m.p (142-144) ^0C; FTIR (ν ,cm^{-1}): 3514 (OH), 3438-3356(NH), 3080 (C-H arom.), 2974-2947(C-H aliph.), 1732 ,1651(C═O); ^1H NMR (δ ,ppm): 2.28 (s, 3H, CH_3), 3.02 (s, 3H, CH_3), 1.21(s, 1H,OH**),** 3.83 (s, 6H, OCH_3); 4.28 (d,1H, CH-Cl);4.16(d,1H, CH-N), 7.23-8.07 (m,13H,Ar-H), 10.53, 10.44, 8.70 for groups (s,1H,NH); Elemental analysis: Calcd. for $C_{34}H_{32}ClN_5O_7$: C, 62.05; H, 4.90; N, 10.64; Found: C62.49; H, 5.04; N, 10.92.

The mass fragment for (VIII)(%): m/z=657(5),293(100), 267 (50) , 163(30), 135(27), 63(10); Elemental analysis: Calcd. for $C_{34}H_{32}N_5O_7Cl$: C, 62.10; H, 4.87; N, 10.65; Found: C, 62.31; H, 4.98; N, 10.88.

Synthesis of S)-2-(2-(2-((2,3dimethylphenyl) amino)benzoyl) hydrazine -1- carbonyl) -N-(2-(4-hydroxy-3,5-dimethoxyphenyl) -4- oxothiazolidin-3-yl) benzamide (IX)

Compound of Schiff base (VII) (5.81g, 0.01 mol) and thioglycolic acid (0.01 mol) was heated in dry benzene 10 mL for 8hrs. The solvent was evaporated and the mixture was neutralized with $NaHCO_3$ solution, filtered and recrystallized from ethanol. The product was off white; yield : 66% m.p (223 -225)^0C, FTIR (ν,cm^{-1}): 3489(OH), 3344-3310(NH), 3068 (C-H arom.), 2941-2916(C-H aliph.), 1680 ,1653(C═O); ^1H NMR (δ ,ppm): 2.10 (s, 3H, CH_3), 2.29 (s, 3H, CH_3), 2.51(s, 1H,OH),3.33(s, 6H, OCH_3); 3.87 (s,2H, CH_2-S); 6.68-7.87 (m,13H,Ar-H), 7.37 (1H, CH-N), 12.98, 9.45, 9.18, for (s,1H,NH); CHN-S: Calcd. for $C_{34}H_{33}N_5O_7S$: C, 62.28; H, 5.07; N, 10.68; S,4.89; Found: C62.57; H, 5.38; N, 10.91; S,4.99.

Synthesis of R-2-(2-(2-((2,3-dimethylphenyl) amino)benzoyl) hydrazine-1-carbonyl)-N-(2-(4-hydroxy-3,5- dimethoxyphenyl)-5-oxoimidazolidin-1-yl) benzamide (X)

The Schiff base (VII) (5.81gm, 0.01 mol), glycine (0.75gm, 0.01mol) and triethylamine 1mL in ethanol 15mL was refluxed9hrs. The mixture was neutralized with diluted Hydrochloric Acid and then poured into ice- water. The peal yellow crystals were filtered off, washed with water and crystallized from ethanol, yield : 60% , m.p (198-200)^oC; FTIR (ν ,cm^{-1}): 3550 (OH), 3410-3380(NH), 3064 (C-H arom.), 2962-2933(C-H aliph.), 1736,1610(C=O); ^1H NMR (δ ,ppm): 2.09 (s, 3H, CH_3), 2.29 (s, 3H, CH_3), 1.85(s, 1H,OH), 3.75 (s, 6H, OCH_3); 3.87 (s,2H, CH_2-N); 6.41-7.91 (m,13H,Ar-H), 11.12(s,1H,NH), 9.18 (s,1H,NH), 8.13 (s,1H,NH); Elemental analysis: Calcd. for $C_{34}H_{34}N_6O_7$: C, 63.94; H, 5.37; N, 13.16; O, 17.54; Found: C63.57; H, 5.83; N, 13.82.

Results and Discussion

A number of new mefenamic acid derivatives were synthesized by the reaction between the carboxylic acid group in mefenamic acid and methanol in acidic medium (I), which was converted to acid hydrazide (II), then the later reacted with phthalic anhydride with heating in dry acetone to give compound (III) in good yield. FT-IR spectrum for (III) show the new absorption stretching bands due to O-H of COOH moiety in the region (3392-2571)cm^{-1}, a stretching band to C=O for COOH appeared at 1712 cm^{-1}, a stretching band asymmetry and symmetry of N-H group appeared at3340-3309 cm^{-1}. Imide compound (IV) was synthesized by cyclization of amic acid (III) using acetic anhydride and anhydrous sodium acetate. FT-IR spectrum for (IV) show characteristic absorption bands at 3344 cm^{-1} due to N-H imide, and absorption bands at (1747, 1645) to carbonyl imide groups[6].

The esterification of hydroxyl groups of amic acid (III) produce corresponding ester(V), the FTIR spectrum for (V) a display absence absorption stretching bands O-H and C═O groups together presents of new bands at 1732 $cm^{-1}$and 1255 cm^{-1}assigned to carbonyl and C-O groups of ester moiety, respectively. Ester(V) was condensed with NH_2NH_2 to give acid hydrazide (VI), the FTIR spectrum display stretching vibration asymmetry and symmetry of NH_2 and NH groups in the region(3305-3145)cm^{-1} as well as stretching absorption at 1651$cm^{-1}$for C═O amide. When the reacted compound(VI) reacted with syringaldehyde, new schiff base (VII) was formation. FTIR spectrum for

compound(VII) display absence of bands of NH_2, NH groups with a new stretching band C=N group at1626 cm^{-1}. Finally, three derivatives containing heterocyclic unit (VIII-X) four, and five member ring synthesized from reacted the shciff base(VII) with chloroacetylchloride, thioglycolic acid, and glycine in triethylamine, respectively. All compounds are identified by physical properties, and by spectral methods FT-IR, ^{1}HNMR and elemental analysis.

Biological Activity

Mefenamic acid has analgesic action three times more than that of aspirin. However, like all classical NSAIDs are associated with an increased risk of gastrointestinal ulcers. This study involves synthesis, evaluate antibacterial activity , cytotoxic effect, and toxicity *in vivo* of new amic acid and their derivatives of mefenamic acid, which are expected to have fewer side effects than the original compound.

Antibacterial activity

The antibacterial activity of the compounds were examined (*in vitro*) against *Staph.aureus (G+), Bacillus subtilisa (G+), Klebsiella pneumoniae (G-), and E.coli (G-)* using the agar diffusion method[7]. Most of the derivatives display high or low biological activity versus bacteria, the data are listed in table(1) . Compound (VIII) showed good inhibition against *Staphylococcus aureus*, this could be related to the presence of azetidin-2-one(β-lactam), while compound (X) showed slightly active against *Klebsiella pneumonia* and *E.coli*.

Anticancer screening

The cytotoxicity of new amic acid and their derivatives of mefenamic acid (III, IV, VII, VIII, IX) against a human breast cancer cell line[8] for 48 h. MCF-7 cells were seeded in (96-well)culture plates at 200 µl/ of cell suspension was filled to each well and the plates covered by plate and sealed at Para film and put in an incubator, then incubated for 24 h in humidified chamber at 37˚C with 5% Carbon dioxide gas and medium fill up with 10% bovine serum and 1% of penicillin / streptomycin mixture until the cells reached confluence. The plate was checked out for contamination, and cultured at different concentrations 10 - 500 µg/Ml, while 200µl of maintenance medium were added to each well of monitoring group, then plates were tight with Parra film and regressed to the incubator. Evaluation of cytotoxicity was carried out after 48 ,the supernatant was removed, 150 µL of DMSO was added to the solution then shaking, and absorbance values reeds at $\lambda = 450$ nm to calculated the rate of inhibition of cell growth. Cytotoxicity assay for derivatives (III, VII, and VIII) caused good inhibitory effect on the growth of cell line except compounds (IV, and IX), table (2). compound (III)showed more than 50% inhibition for MCF-7and compound (VII and IX) less than 50% inhibition for MCF-7cell line

Acute Toxicity Test

In this research, three groups of 45 albino mice (each consisting of 15 mice) were used to evaluate the acute toxicity of some synthesized compounds (III, IV, VII, VIII, IX), using the Lorke-written method[9]. Mice were fasted for 18 h with free arrival to water before experiment. The compounds were dissolved in distilled water and treated via the oral route (5 and 10 g/kg). The mice are fed, weighed and observation of general signs of toxicity symptoms, behavior, and mortality for 14 days. The study exhibited : no mortality with doses 5 and 10 g/kg body weight, no contrast in the weight of the mice (weighted every day) between the group control and the treated groups, no change in mice behaviors was in the weight of the mice daily measured between the group control and the treated groups, no modification in mice behaviors was recorded, and no toxicity symptoms were announced next 14 days. Moreover, some mice were sacrificed by cervical dislocation and kidneys, liver, and heart were weighed. Finally, visual estimate of organs of mice showed normal cases.

Conclusion

Amic acid represent a major class of organic compounds and used some of these derivatives in therapeutic purposes, therefore modification structure of the amic acid derived from mefenamic acid have allowed using multistep processes to the synthesized of new derivatives may be having a broad spectrum of biological activity. Some amic acid and their derivatives of mefenamic acid were screened for their ntibacterial, anticancer activity and acute toxicity test . We need to further examination to know mechanism by which the heterocyclic compounds act to give a potent cytotoxic effect that might get the mefenamic derivatives being blight anticancer product.

Acknowledgement : This work was supported by the Bio Technology Research Center in Al-Nahrain University.

Table (1) : antibacterial activity of some compounds

Comp.	Zone of inhibition in millimeter			
	Staphyl ococcus aureus	Bacillus sabtilis	Klebsiella pneumoniae	Escherichis coli
III	12	13	17	11
IV	11	5	12	11
VIII	26	13	15	11
IX	12	13	15	12
X	13	13	5	5

Table (2):The inhibition of cells growth of some compounds µl/well

Comp.	inhibition of cells growth forMCF-7
III	53.1%
IV	0%
VII	31.1%
VIII	10.1%
IX	0%

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

Funding: Self-funding

References

1- Cimolai N, The potential and promise of mefenamic acid, Expert Rev Clin Pharmacol. 2013;6(3):289-305.

2- Abbas A, synthesis and anti-inflammatory evaluation of novel thiadiazol derivatives of mefenamic acid, Bull. Chem. Soc. Ethiop, 2017, 31(1): 171-175.

3- Al-Azzawi A M, Raheem A A, Synthesis and Antibacterial Screening of New Schiff Bases Based on N-(4-acetophenyl) Succinimide, Journal of Science, 2017, 58(4A): 1790-1801.

4- AL-rawi M S, Hassan H A and Hassan D F, New Series of Substituted Heterocycles Derived from α , β-unsaturated Ketone and Their Cytotoxic Activity in Tumor Cell Lines, Orient. J. Chem., 2018,34(6), 2826-2831.

5- Lingam V R, Alishetty S,a Syed S B, Lakshmi N M, Khagga M,and Sarbani, Design and Synthesis of 1-Aroyl-2-ylidene Hydrazines under Conventional and Microwave Irradiation Conditions and their Cytotoxic Activities, J. Braz. Chem. Soc., 2010, 21(1): 98-104.

6- Tarek A F, Hatem A A, Adnan K, Ahmed B, Pervez A, Al-Samani T and Seik W, Microwave-Assisted One-Step Synthesis of Fenamic Acid Hydrazides from the CorrespondingAcids, Molecules 2011, 16:3544-3551.

7- Al-Rawi M S, Synthesis of some new heterocyclic compounds via chalcone derivatives, Ibn Al-Haitham J. for Pure & Appl. Sci., 2015,28 (1):88-99.

8- Qingqing H, Tiange C, Qianwen L, Yinghong

H, Qian L, Bingyue W, Preparation of psoralen polymer–lipid hybrid nanoparticles and their reversal of multidrug resistance in MCF-7/ADR cells ,Drug Deliv. 2018; 25(1): 1044-1054 .

9- Hanane M, Cytotoxicity of the Aqueous Extract and Organic Fractions from Origanum majorana on Human Breast Cell Line MDA-MB-231 and Human Colon Cell Line HT-29",Hindawi, Advance in pharmacological science, 2018 ID 3287193.