Available online at www.derpharmachemica.com

ISSN 0975-413X
CODEN (USA): PCHHAX

Der Pharma Chemica, 2017, 9(20):44-49
(http://www.derpharmachemica.com/archive.html)

Synthesis and Evaluation of New Diclofenac Acid having 2-Azetidinone

Yahya S Yaseen[1*], Muthanna S Farhan[2], Sabah J Salih[2]

[1]*Department of Pharmaceutical Chemistry, College of Pharmacy, University of Tikrit, Salah-Aldin, Iraq*
[2]*Department of Pharmaceutical Chemistry, College of Pharmacy, University of Baghdad, Baghdad, Iraq*

ABSTRACT

New series of 2-azetidinone (I-IV) were synthesized, the structure of these new derivatives were confirmed using spectral methods starting from diclofenac sodium. We prepared diclofenac acid by using hydrochloric acid, then converted to amide ester by using thionyl chloride, then converted to hydrazide by using hydrazine hydrate 99.5%, then a Schiff bases were synthesized using different aromatic aldehydes in ethanol, and the final compounds were obtained by cyclocondensation using chloroacetylchloride. The synthesis of the designed compounds has been successfully achieved. Purity and characterization were confirmed by determination of physical properties (melting points & Rf values), Fourier Transform Infra-Red (FTIR) spectroscopy and C, H, N analysis.

Keywords: Diclofenac, Schiff base, 2-Azetidinone, Antibacterial activity, Aldehyde

INTRODUCTION

The Non-steroidal Anti-inflammatory Drugs (NSAIDs) are among the most often prescribed drugs in the world. This heterogeneous class of drugs includes aspirin and several other selective or non-selective Cyclooxygenase (COX) inhibitors. The non-selective NSAIDs are the oldest ones and are called traditional or conventional NSAIDs. The selective NSAIDs are called COX-2 inhibitors [1]. The two main adverse drug reactions associated with NSAIDs relate to gastrointestinal effects and renal effects of the agents. These effects are dose-dependent, and in many cases severe enough to pose the risk of ulcer perforation, upper gastrointestinal bleeding, and death, limiting the use of NSAID therapy [2]. NSAIDs inhibit both the COX-1 and COX-2 enzymes. COX catalyses the formation of prostaglandins and thromboxane from Arachidonic Acid (AA) [3]. Diclofenac is a non-steroidal anti-inflammatory drug of the phenyl acetic acid class with anti-inflammatory, analgesic, and antipyretic properties. Contrary to the action of many traditional NSAIDs, diclofenac inhibits COX-2 enzyme with greater potency than it does COX-1. Similar to other NSAIDs, diclofenac is associated with serious dose-dependent gastrointestinal, cardiovascular, and renal adverse effects [4].

The cyclic 2-azetidinone skeleton has been extensively used as a template to build the heterocyclic structure fused to the four membered rings. The β-lactam heterocycles are still the most prescribed antibiotics used in medicine. They are considered as an important contribution of science to humanity [5].

The biological activity of β-lactam antibiotics such as penicillin and cephalosporin are attributed to the presence of 2-azetidinone ring in them [6]. Compounds carrying azetidin-2-one ring are reported to exhibit certain biological activities like antagonists [7], anti-inflammatory [8]. Cycloaddition of chloroacetylchloride with imine (Schiff base) result in formation of 2-azetidinone (β-lactam). The reaction involves direct acylation of imine with chloroacetylchloride. The reaction is carried out with base as triethylamine gives β-lactam [9].

New azetidinone bioactive agents have been synthesized with expected selectivity against COX-2 enzyme using naproxen and 2-azetidinone as pharmacophore (Figure 1). The Preliminary study of their anti-inflammatory activity showed that these synthesized compounds exhibited equivalent or better effect than naproxen. Also there antibacterial activity is more than Naproxen. Moreover the preliminary cytotoxic activity study of these compounds showed highly significant effect, and may represent an exploitable source of new anticancer agent more than naproxen [10].

Therefore, new derivatives of diclofenac containing azetidinone pharmacophore has been synthesized with expected activity against COX-2 and may have additional actions like the antibacterial effects.

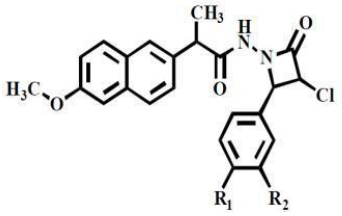


Figure 1: Series of naproxen derivatives containing 2-azetidinone pharmacophore [11]

MATERIALS AND METHODS

Materials and equipment's

Triethylamine, Thomas baker (India), Diclofenac working standard, hyperchemic (China), Thionyl chloride, BDH Chemicals Ltd. (England), Hydrazine Hydrate 99%, 99.5%, Provizer Pharma (India), Benzaldehyde, Scharlau (Spain), 4-Hydroxybenzaldehyde, Alfa Aesar (Germany), 3-Hydroxybenzaldehyde, Aldrich Chemistry (USA), 3-Nitrobenzaldehyde, Merk (Germany), Vanillin, Panreac Quimca (Spain), Chlorobenzaldahyde, Fluka AG; Buchssco (Switzerland), Chloro-acetyl chloride, Merk (Germany), Acetyl aldehyde, Thomas baker (India). The quality of all these chemicals together with the other ones used throughout the study and obtained from standard commercial sources were of the highest purity available and used without further purification. The melting points were determined by the open capillary method using Stuart SMP30 (USA) and were used uncorrected. Cooling of reactions when needed was done using a Julabo Chiller VC (F30) (GMBH, Germany). Infrared spectra were recorded in KBr disc on Shimadzu FTIR-8400 spectrophotometer (Japan), at the College of Pharmacy, University of Baghdad and on Shimadzu FTIR 8400-S spectrophotometer (Japan), at the College of Science, University of Al-Mustansiriyah. Elemental microanalysis was performed at the College of Pharmacy, University of Al-Mustansiriyah by using CHN Euro EA Elemental Analyzer (Italy).

The progress of the reaction was monitored by ascending thin layer chromatography which was run on Kieslgel G60 F254 pre-coated 0.2 mm thickness aluminium plates (E. Merck, Germany), and was used as well to check the purity of the product. The synthesized final products and their intermediates were revealed either by derivatization or reactivity toward iodine vapor or by irradiation with UV254 light. Chromatograms were eluted by using one or more of the following mobile phases: Solvent system (A): Ethyl acetate:n-Hexane (6:4 v/v), System (B): Chloroform:Methanol (3:7 v/v), System (C): Ethyl acetate:n-Hexane:Methanol (6:4:1 v/v) and System (D): Methanol:Ethyl acetate:n-Hexane (5:3:2 v/v).

General chemical tests such as the sodium fusion or other specific suitable tests were run to check the presence or absence of certain groups and the purity of the synthesized derivatives and intermediates (Vogel and Shriner). pH measurements of solutions was made using Alkacid™ pH Test Ribbons and Strips purchased from Fisher Scientific (USA).

EXPERIMENTAL SECTION

The synthetic procedures described below were adapted from those reported earlier in the literature and used with few minor alterations.

Chemical synthesis

Synthesis of diclofenac acid (intermediate A) Sodium diclofenac was dissolved in water at a concentrate ion of 7 mg/ml. When the sodium diclofenac was completely dissolved, it was titrated with an equal molar amount of hydrochloric acid. This solution was allowed to stir using a magnetic stir bar and plate for 10 min. Because the diclofenac free acid was not soluble in water, it immediately precipitated out of solution. The free acid of diclofenac was a suspension in water while the resulting sodium and chloride ions remained in solution. The mixture was filtered using 0.45 µm filter paper and a vacuum apparatus. The filtrate was washed with dilute HCl (0.001 N) and excess amounts of water to remove any excess sodium chloride and un-reacted sodium diclofenac. The powder was allowed to dry under a hood, collected and stored in a clear glass vial. The solubility of the free acid in methylene chloride was shown to be greater than 5 mg/ml.

Synthesis of diclofenac ethyl ester (intermediate B)

Suspension of diclofenac (0.01 mol, 2.96147 g) in 85 ml absolute ethanol was cooled down to -15°C, then thionyl chloride (0.01 mol, 0.734 ml, 1.1897 g) was added drop wise. The temperature was maintained below -10°C. The reaction mixture was kept at 40°C for 3 h. Followed by refluxing for three hours and left at room temperature overnight. The solvent was evaporated to dryness, re-dissolved in absolute ethanol and evaporated. The process was repeated several times to ensure complete removal of thionyl chloride excess.

Synthesis of diclofenac hydrazide (intermediate C)

Diclofenac ethyl ester (compound B) (0.00215 mol, 0.7 gm) and hydrazine hydrate 99.5% (an excess amount of 0. 0215, 0.06888 g, 0.0675 ml) were added to 50 ml of ethanol contained in a 100 ml round bottom flask and the mixture was first stirred overnight at Room Temperature (RT), after which the it was set to be refluxed at 80°C for 12 h. At the end of the reflux time, the mixture was left to be stirred overnight at RT. Later, the formed ppt was filtered off and washed several times with cold distilled water (4 × 15 ml), then the ppt was left to dry and the product was recrystallized from absolute ethanol to afford Compound C. Trials were made to synthesize Compound B using different percentages of hydrazine hydrate (99, 99.5%) and it was found that using the 99.5% one was found to be the best in running this step of the reaction compared to 99% ones.

Synthesis of diclofenac hydrazones (intermediate S1-S7)

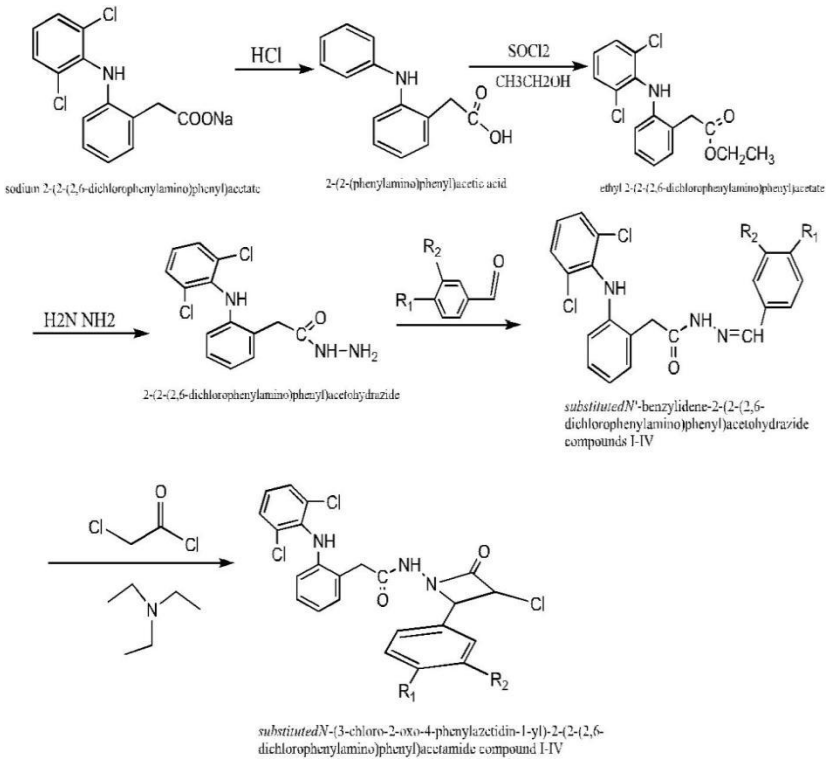
Five drops of glacial acetic acid was added to an ethanolic solution (5 ml) of one of the following an excess amount of aldehydes [(benzyl aldehydes: S1A (0.0016 mol, 0.16 ml, 0.1697 g), 4-hydroxybenzylaldehydes: S2A (00000644 mol, 000786 g), 3-hydroxy benzyl aldehydes: S3A (00000644 mol, 000786 g), 3-nitrobenzylaldehyde: SA4(0.000644 mol, 0.0973 g), Vanillin: S5A (0.000644 mol, 0.0979 g), 3-chlorobenzylaldehyde: SA6(0.000644 mol, 0.0905 g) and acetyl aldehyde: SA7 (0.0016 mol, 0.0704 g, 0.089 ml)] contained in a 100 ml round bottom flask equipped with a magnetic stirrer. Then (0.00032 mol, 0.1 g) of compound C dissolved in 20 ml of absolute ethanol was added with stirring to each of the above mentioned mixtures separately, after which, each reaction mixture was left to stir for (30 min) at RT and it was

noticed that the clear mixture has been converted into a suspended one, which was set to reflux at 80°C for 6 h. Later, the formed ppt was filtered and recrystallized from the following organic solvents to afford the corresponding intended hydra zones compound.

Synthesis of final target compounds (compound I-IV)

A mixture of one of compounds S1-S4 (0.002 mol) in Dimethylformamide (DMF) 15 ml and chloroacetyl chloride (0.006 mol, 0.67 g) in presence of triethylamine (0.006 mol, 0.60 g) was refluxed for 6 h. The high amounts of chloroacetyl chloride are to ensure the complete reaction of the reactants. The mixture was filtered to separate the precipitate that formed. The filtrate was concentrated to half its volume then poured onto crushed ice. Each final product (one of compounds II-IV) was filtered and washed with distilled water and recrystallized from absolute ethanol.

The general route illustrated in Scheme 1 was followed to synthesize the entire intermediate and final target compounds described earlier starting from diclofenac. The physical appearance, percent yield, Melting Point (m.p.°C) and Rf values of the synthesized compounds together with the elemental microanalysis (C, H, N analysis) of the final target compounds (I-IV) are given in Tables 1 and 2. The FTIR spectral data (KBr) υ cm^{-1} of the intermediate and final target compounds are listed below in Table 3.



Scheme 1: Series 1 general synthesis procedures of compounds A, B, C and S1-S7; where compound I has R1 and R2=H, compound II has R1=OH and R2=H, compound III has R1=H and R2=OH, compound IV has R1=NO_2 and R2

Table 1: Physicochemical characterization data of the synthesized compounds

Sym	Chemical name	Chemical formula	Physical appearance	% Yield	Melting point (°C)	R_f
A	2-(2-(phenyl amino)phenyl)acetic acid	$C_{14}H_{13}NO_2$	White powder	85	162	A=0.58 B=0.56
B	ethyl2-(2-(2,6-dichlorophenylamino)phenyl)acetate	$C_{16}H_{15}Cl_2NO_2$	Light Pale brown powder	95	61-63	A=0.75 B=0.87
C	2-(2-(2,6-dichlorophenylamino)phenyl acetohydrazide)	$C_{14}H_{13}Cl_2N_3O$	White powder	75	157-159	A=0.9 B=0.72
S1	N'-benzylidene-2-(2-(2,6 dichlorophenylamino)phenyl)acetohydrazide	$C_{21}H_{17}Cl_2N_3O$	White powder	94.5	255-260	A=0.33 B=0.88
S2	2-(2-(2,6-dichlorophenylamino)phenyl)-N'-(4-hydroxybenzylidene)acetohydrazide	$C_{21}H_{17}Cl_2N_3O_2$	Pale brown powder	96.3	230 dec	A=0.34 B=0.76
S3	2-(2-(2,6-dichlorophenylamino)phenyl)-N'-(3-hydroxybenzylidene)acetohydrazide	$C_{21}H_{17}Cl_2N_3O_2$	Off white powder	95.2	193 dec	A=0.37 B=0.77
S4	2-(2-(2,6-dichlorophenylamino)phenyl)-N'-(3-nitrobenzylidene)acetohydrazide	$C_{21}H_{16}Cl_2N_4O_3$	Yellow powder	97	56 dec	A=0.47 B=0.83
S5	2-(2-(2,6-dichlorophenylamino)phenyl)-N'-(4-hydroxy-methoxybenzylidene)acetohydrazide	$C_{22}H_{19}Cl_2N_3O_3$	Very light brown	85	210-212	A=0.44 B=0.73
S6	N'-(3-chlorobenzylidene)-2-(2-(2,6-dichlorophenylamino)phenyl)acetohydrazie	$C_{21}H_{16}Cl_3N_3O$	Off white powder	93.7	204	A=0.3 B=0.84
S7	2-(2-(2,6-dichlorophenylamino)phenyl)-N' ethylideneacetohydrazide	$C_{16}H_{15}Cl_2N_3O$	White powder	95	235-237	A=0.4 B=0.7
I	N-(3-chloro-2-oxo-4-phenylazetidin-1-yl)-2-(2-(2,6	$C_{23}H_{18}Cl_3N_3O_2$	Light brown powder	45.8	233-235	A=0.79 B=0.81

	dichlorophenylamino)phenyl)acetamide					
II	N-(3-chloro-2-(4-hydroxyphenyl)-4-oxoazetidin-1-yl)-2-(2-(2,6-dichlorophenylamino)phenyl)acetamide	$C_{23}H_{18}Cl_3N_3O_3$	Brown powder	38.1	196 dec	A=0.5 B=0.480.
III	N-(3-chloro-2-(3-hydroxyphenyl)-4-oxoazetidin-1-yl)-2-(2-(2,6-dichlorophenylamino)phenyl)acetamide	$C_{23}H_{18}Cl_3N_3O_3$	Brown powder	37.9	194 dec	A=0.77 B=0.75
IV	N-(3-chloro-2-(3-nitrophenyl)-4-oxoazetidin-1-yl)-2-(2-(2,6-dichlorophenylamino)phenyl)acetamide	$C_{23}H_{17}Cl_3N_4O_4$	Very brown powder	43.1	199-200	A=0.84 B=0.76

Table 2: The elemental microanalysis of the intended target compounds calculated/founded (I-IV)

Compound	Chemical formula	Mol. Wt.	Value type	C	H	N
I	$C_{23}H_{18}Cl_3N_3O_2$	474.76	calculated	58.19	3.82	8.85
			observed	56.459	3.65	8.62
II	$C_{23}H_{18}Cl_3N_3O_3$	490.76	calculated	56.29	3.70	8.56
			observed	54.675	3.58	8.24
III	$C_{23}H_{18}C_{l3}N_3O_3$	490.76	calculated	56.29	3.70	8.56
			observed	54.620	3.59	8.122
IV	$C_{23}H_{17}Cl_3N_4O_4$	519.76	calculated	53.15	3.30	10.78
			observed	51.919	3.113	10.330

Table 3: IR spectral data of synthesized compounds

Sym	Chemical name	Characteristics IR spectral bands (KBr) V cm^{-1} with its interpretation
A	2-(2-(phenyl amino)phenyl)acetic acid	3323 (O-H) stretching broad band of carboxylic acid, 1693 (C=O) stretching vibration of carboxylic acid 1568 (N-H) bending vibration of (amide II band)
B	ethyl2-(2-(2,6-dichlorophenylamino)phenyl)acetate	1712 (C=O) stretching vibration of ester, 1195 (C-O) stretching vibration of ester 767 (c-cl) stretching
C	2-(2-(2,6-dichlorophenylamino)phenyl)acetohydrazide	3263 (N-H) asymmetrical stretching vibration of primary amine 3202 (N-H) symmetrical stretching vibration of primary amine 1649 (C=O) stretching vibration of amide (amide I band) 163 (N-H) bending of amine
S1	N'-benzylidene-2-(2-(2,6-dichlorophenylamino)phenyl)acetohydrazide	3286 (N-H) stretching vibration of secondary amide 1647 (C=O) stretching vibration of amide (amide I band) overlapping with (C=N) group, 1267 (C-N) stretching vibration
S2	2-(2-(2,6-dichlorophenylamino)phenyl)-N'-(4-hydroxybenzylidene)acetohydrazide	3439 (o-H) stretching 3261 (N-H) stretching vibration of secondary amide 1651 (C=O) stretching vibration of amide (amide I band) overlapping with (C=N) group 1267 (C-N) stretching vibration
S3	2-(2-(2,6-dichlorophenylamino)phenyl)-N'-(3-hydroxybenzylidene)acetohydrazide	3385(o-H) stretching 3275 (N-H) stretching vibration of secondary amide 1651 (C=O) stretching vibration of amide (amide I band) overlapping with (C=N) group 1269 (C-N) stretching vibration
S4	2-(2-(2,6-dichlorophenylamino)phenyl)-N'-(3-nitrobenzylidene)acetohydrazide	3263 (N-H) stretching vibration of secondary amide,1707 (C=O) stretching of amide 1691 (C=N) stretching of imine 1535 (N–O) asymmetric stretching 1352 (N–O) symmetric stretching
S5	2-(2-(2,6-dichlorophenylamino)phenyl)-N'-(4-hydroxy-3-methoxybenzylidene)acetohydrazide	3385 (O-H) stretching broad band 1654 (C=O) stretching of amide, 1631 (C=N) stretching of imine
S6	N'-(3-chlorobenzylidene)-2-(2-(2,6-dichlorophenylamino)phenyl)acetohydrazide	3433 (N-H)Aromatic secondary amine 1656 (C=O) stretching of amide 1641 (C=N) stretching of imine 829 (Ar-cl) stretching
S7	2-(2-(2,6-dichlorophenylamino)phenyl)-N'-ethylideneacetohydrazide	3327 (N-H)Aromatic secondary amine, 1639 (C=O) stretching vibration of amide (amide I band) overlapping with (C=N) group 1589 (N-H) bending vibration of (amide II band)
I	N-(3-chloro-2-oxo-4-phenylazetidin-1-yl)-2-(2-(2,6-dichlorophenylamino)phenyl)acetamide	3439 Aromatic secondary amine NH stretch, 1726 (C=O) stretching vibration of B-lactam 1658 (C=O) stretching of amide
II	N-(3-chloro-2-(4-hydroxyphenyl)-4-oxoazetidin-1-yl)-2-(2-(2,6-dichlorophenylamino)phenyl)acetamide	(3435) Aromatic secondary amine, NH stretch overlapping with O-H, 1784 (C=O) stretching vibration of B-lactam,

		1658 (C=O) stretching of amide, 837 (Ar-cl) stretching
III	N-(3-chloro-2-(3-hydroxyphenyl)-4-oxoazetidin-1-yl)-2-(2-(2,6-dichlorophenylamino)phenyl)acetamide	(3223) Aromatic secondary amine, NH stretch, 1726 (C=O) stretching vibration of B-lactam, 1660 (C=O) stretching of amide, 837 (Ar-cl) stretching
IV	N-(3-chloro-2-(3-nitrophenyl)-4-oxoazetidin-1-yl)-2-(2-(2,6-dichlorophenylamino)phenyl)acetamide	(3458) Aromatic secondary amine NH stretch 1666 (C=O) stretching vibration of B-lactam overlapping with C=O stretching of amide 1531 (N–O) asymmetric stretching 1351 (N–O) symmetric stretching

Antimicrobial activity

The antimicrobial activity of the target compounds was done in Biology Department, College of Sciences/University of Almustansiriyah. A preliminary antibacterial activity has been carried out according to well diffusion method: The prepared compounds have been studied for their antimicrobial activity *in vitro* against four tested bacteria (*E. faecalis*, *Staphylococcus saprophyticus* as Gram-positive bacteria and, *Proteus mirabilis*, *E. coli* as Gram-negative bacteria), were clinical activated and maintained on nutrient agar medium for test in antibacterial activity. Amoxicillin was used as a reference drug for antibacterial activity [12] by using ANOVA two factor.

RESULTS AND DISCUSSION

The structure of the synthesized compounds was confirmed by using FTIR spectroscopy, C, H, N elemental microanalysis, and other physicochemical parameters (Tables 1-3). The synthesized diclofenac ethyl ester (Intermediate B) showed the appearance of the characteristic sharp band of the (C=O) stretching vibration of the formed ester around 1712 cm^{-1}, which is accompanied by the disappearance of characteristic broad band of the (OH) group of carboxylic acid of naproxen. The diclofenac hydrazide (intermediate C) showed the appearance of the characteristic sharp band around 1649 cm^{-1} which indicates the formation of the (C=O) group of the formed hydrazide (amide I band) and accompanied with the disappearance of the characteristic sharp band of the (C=O) stretching vibration of the ester at 1712 cm^{-1}.

The synthesized hydrazone derivatives (S1-S7) showed several characteristic sharp bands in the IR region, where the bands in the range between 1639-1691 cm^{-1} indicate the appearance of the (C=N) group stretching vibration of the imine, which was noticed that it appeared sometimes as separated band and sometime overlapped with the (C=O) stretching vibration of the amide I. The synthesized of the final target compound (I-IV) showed several characteristic sharp bands in the IR region, where the bands in the range between 1666-1784 cm^{-1} indicate the appearance of the B-lactam group stretching, which was noticed that it appeared sometimes as separated band and sometime overlapped with the (C=O) stretching vibration of the amide I (Table 3).

The elemental microanalysis revealed good agreement with the calculated percentages. The percent deviations of the observed/calculated values were found to be within the limits of accurate analysis (Table 2).

The results of the antibacterial activity are shown in (Table 4) and (Figure 2) all final target compound (I-IV) are more effective than amoxicillin which are used are standard. Compound IV got to the best result average of effects spots equal to 18.64 mm. *E. coli* is the most affected organism by all compounds with average of effects spots equal to 14.77 mm.

Table 4: The analyses of variance (P<0.01) of the antibacterial activity of compounds I-IV and propylene on *Enterococcus faecalis, Staphylococcus saprophyticus, Proteus mirabilis* and *Escheriea coli*

Compound name	***Enterococcus faecalis***	***Staphylococcus saprophyticus***	***Escheriea coli***	***Proteus mirabilis***	**Average**
Propelyne	0	0	0	0	0
Amoxyllin	13.66667	14.33333	18	15.66667	15.41667
Cpd. 1	15.33333	17.33333	17.6667	18.33333	17.16668
Cpd. 2	20.83333	13.66667	18.33333	16.5667	17.35001
Cpd. 3	16.66667	14	17	17.33333	16.25
Cpd. 4	21	16.66667	17.6667	19.23333	18.64168
average	14.58333	12.66667	14.77779	14.52223	
ANOVA (P ≤ 0.01)					
Source of variation	**SS**	**df**	**MS**	**F**	**F crit**
Rows	29.54351	4	7.385878	2.957029	4.77257799972321❶
Columns	21.02473	4	5.256182	2.104378	4.77257799972321❷
Error	39.96378	16	2.497736		
Total	90.53202	24			

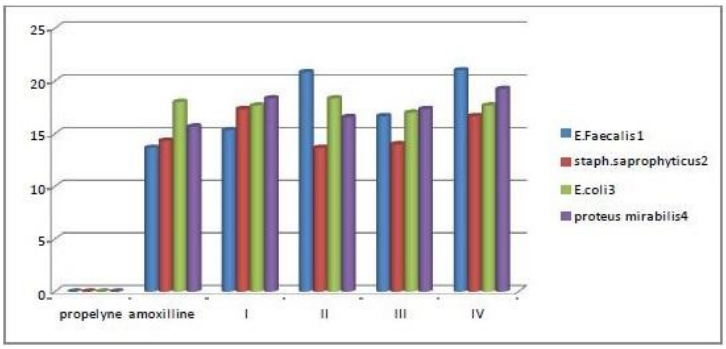


Figure 2: Biological activity of propylene glycol and compounds I-IV, on *Enterococcus faecalis, Staphylococcus saprophyticus, Proteus mirabilis* and *Escheriea coli*

CONCLUSION

The synthesized compounds were evaluated for antibacterial activity against (*E. faecalis*, *S. saprophyticus* as Gram-positive bacteria and, *P. mirabilis*, *E. coli* as Gram-negative bacteria) by using disc diffusion method. The antimicrobial activity of the newly synthesized compounds (I-IV) bearing a 2-azetidinone moiety revealed that all the tested compounds showed good antibacterial activities against the selected microbial strains.

ACKNOWLEDGEMENT

The data was abstracted from MSc thesis submitted to the Department of Pharmaceutical Chemistry, College of Pharmacy, University of Baghdad. The authors wish to thank both the University of Baghdad and the University of Tikrit for supporting this project.

REFERENCES

[1] A.H. Patricia, *J. Am. Coll. Cardiol*., **2004**, 43, 519-525.
[2] G. Ga, *Clin. Cornerstone.*, **2001**, 3(5), 50-60.
[3] H. Dai, K. Lamar Turepu Roos, N.K. Evanson, J. Tomsik, T.S. Elton, D.L. Simmons, *Proc. Natl. Acad. Sci*., **2002**, 99(21), 13926-13231.
[4] A. Roy, B. Bill, B. Kay, P. Paola, Y. Clarence, *Drugs.*, **2015**, 75(8), 859-877.
[5] N. Ramalakshmishmi, S. Deepa, A. Jenny, Gopi, S. Arunkumar, S.B. Junne, A.B. Kadam, S.B. Zangade, S.L. Shinde, Y.B. Vibhute, *IMRJ.*, **2012**, 2, 44-47.
[6] E.S. Rania, R. Parameshwara, V.H. Babu, Y.S. Ranganatha, B.N. Kumar, G.A. Kumar, *Int. J. Pharm. Pharm. Sci*., **2012**, 4, 424-427.
[7] P. Samadhiya, R. Sharma, S.K. Srivastava, S.D. Srivastava, *Quim. Nova*., **2012**, 35, 914-919.
[8] P. Sah, M. Seth, *JOCPR*., **2012**, 4, 146-153.
[9] P.J. Naik, D.V. Parekh, P.S. Desai, *Adv. Appl. Sci. Res*., **2013**, 4, 324-329.
[10] M. Ashutosh, V. Ravichandran, K.J. Prateek, K.D. Vinod, K.A. Ram, *J. Braz. Chem. Soc*., **2008**, 19(1).
[11] S.J. Salih, PhD Thesis, Baghdad University, Baghdad, Iraq, **2014**.
[12] L. Neli, K. Yogendra, M. Berington, *J. Med. Plants. Res*., **2011**, 5(5), 859-861.