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Design, Synthesis and Evaluation of New Diclofenac Derivative More Selective COX2 Inhibitor

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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 chloroacetylechloride.The synthesis of the designed compounds has been successfully achieved. Purity and characterization were confirmed by determination of physical properties (melting points & Rf values), FT-IR spectroscopy and CHN analysis.***

Keywords: Diclofenac, Schiff base, 2-Azetidinone, antibacterial activity, aldehyde

1. Introduction

The non-steroidal anti-inflammatory drugs (NSAIDs) are among the most common pain relief medicines 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 old 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 adverse 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 cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) isoenzymes. COX catalyzes 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 cyclooxygenase (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 is reported to exhibit certain biological activities like antagonists [7], anti-inflammatory [8].

Cycloaddition of chloroacetylechloride with imine (Schiff base) result in formation of 2-azetidinone (β-lactam).The reaction involves direct acylation of imine with chloroacetylechloride. 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 Pharmacophores (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].

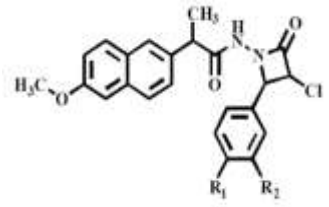


Figure 1: Series of naproxen derivatives containing 2-Azetidinone pharmacophore [11].

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

2. 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), Chlorobenzaldehyde, Fluka AG; buchssco (Switzerland), Chloro- acetylchloride, 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). Nuclear Magnetic Resonance (1H-NMR) Spectra were recorded on a Bruker-AV 400 instrument at 400.13 MHz.chemical shifts are in part per million (ppm)with reference to the chemical shift of the deuterated solvent or the internal standard tetramethylsilane. carried out at University of Nottingham in United Kingdom.

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 Aluminum 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 (6:4v/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).

3. Experimental Section

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

A.Chemical synthesis

1. Preparation of diclofenac acid from diclofenac salt (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 minutes. 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 0.45µm 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.

2. Synthesis of diclofenac ethyl ester (intermediate B).

Suspension of diclofenac (0.01mole, 2.96147gm) in 85ml absolute ethanol was cooled down to -15 ^{o}C, and then thionyl chloride (0.01mol, 0.734ml, 1.1897g) was added drop wise. The temperature was maintained below -10 ^{o}C. The reaction mixture was kept at 40 ^{o}C for 3 hrs. 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.

3. Synthesis of diclofenac hydrazide (intermediate C)

Diclofenac ethyl ester (compound B) (0.00215 mole, 0.7 gm.) and hydrazine hydrate 99.5% (an excess amount of 0. 0215, 0. 6888gm,0. 675ml) 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 ^{o}C for 12 hrs. 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 x 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.

4. Synthesis of diclofenac hydrazones (intermediate S1-S6).

Five drops of glacial acetic acid was added to an ethanolic solution (5 ml) of one of the following an excess amount of aldehydes [(benzylaldehydes: S1 (0.0016mole, 0.16ml, 0.1697gm);4-hydroxybenzylaldehydes: S2 (0.000644mole,0.0786gm.); 3-hydroxy benzyl aldehydes: S3 (0.000644 mole, 0.0786gm.); 3-nitrobenzylaldehyde: S4(0.000644mol,0.0973gm); Vanillin: S5 (0.000644 mole,0.0979 gm.)And; 3-chlorobenzylaldehyde: S6 (0.000644mole, 0.0905gm)] contained in a 100 ml round bottom flask equipped with a magnetic stirrer. Then (0.00032 mole, 0.1 gm.) 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 ^{o}C for 6 hrs. Later, the formed ppt was filtered and recrystallized from the following organic solvents to afford the corresponding intended hydra zones compound.

5. Synthesis of final Target compounds (compound I-IV).

A mixture of one of the compounds S1-S4 (0.002mole) in DMF (15 ml) and chloroacetyl chloride (0.006mole, 0.67gm) in the presence of triethylamine (0.006mole, 0.60gm) was refluxed for 6hr. The high amount of chloroacetyl chloride is 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 I-IV) was filtered and washed with distilled water and recrystallized from absolute ethanol.

Scheme (1): general synthesis procedures of compounds A,B,C and S1-S6; 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=NO2 and R2=H.

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. ^{o}C) and Rf values of the synthesized compounds together with the elemental microanalysis (CHN Analysis) of the final target compounds (I-IV) are given in (table 1) and (table2). The FTIR spectral data (KBr) υ cm-1of the intermediate and final Target compounds are listed below in (table 3).

Table 1: Physicochemical characterization data of the synthesized compounds

Sym	Chemical Name	Chemical Formula	Physical appearance	% Yield	Melting point ^{o}C	R_f
A	2-(2-(phenyl amino)phenyl)acetic acid	C14H13NO2	White powder	85	162	A=0.58 B=0.56
B	ethyl2-(2-(2,6-dichlorophenylamino)phenyl)acetate	C16H15Cl2NO2	Light Pale brown powder	95	61-63	A=0.75 B=0.87
C	2-(2-(2,6-dichlorophenylamino)phenyl) acetohydrazide	C14H13Cl2N3O	White powder	75	157-159	A=0.9 B=0.72
S1	N'-benzylidene-2-(2-(2,6-dichlorophenylamino)phenyl)acetohydrazide	C21H17Cl2N3O	White powder	94.5	255-260	A=0.33 B=0.88
S2	2-(2-(2,6-dichlorophenylamino)phenyl)-N'-(4-hydroxybenzylidene)acetohydrazide	C21H17Cl2N3O2	Pale brown powder	96.3	230 dec	A=0.34 B=0.76
S3	2-(2-(2,6-dichlorophenylamino)phenyl)-N'-(3-hydroxybenzylidene)acetohydrazide	C21H17Cl2N3O2	Off white powder	95.2	193 dec	A=0.37 B=0.77
S4	2-(2-(2,6-dichlorophenylamino)phenyl)-N'-(3-nitrobenzylidene)acetohydrazide	C21H16Cl2N4O3	Yellow powder	97	56 dec	A=0.47 B=0.83
S5	2-(2-(2,6-dichlorophenylamino)phenyl)-N'-(4-hydroxy-3-methoxybenzylidene)acetohydrazide	C22H19Cl2N3O3	Very light brown	85	210-212	A=0.44 B=0.73
S6	N'-(3-chlorobenzylidene)-2-(2-(2,6-dichlorophenylamino)phenyl)acetohydrazie	C21H16Cl3N3O	Off white powder	93.7	204	A=0.3 B=0.84
I	N-(3-chloro-2-oxo-4-phenylazetidin-1-yl)-2-(2-(2,6 dichlorophenylamino)phenyl)acetamide	C23H18Cl3N3O2	Light brown powder	45.8	233-235	A=0.79 B=0.81
II	N-(3-chloro-2-(4-hydroxyphenyl)-4-oxoazetidin-1-yl)-2-(2-(2,6-dichlorophenylamino)phenyl)acetamide	C23H18Cl3N3O3	Brown powder	38.1	196 dec	A=0.5 B=0.480.
III	N-(3-chloro-2-(3-hydroxyphenyl)-4-	C23H18Cl3N3O3	Brown powder	37.9	194 dec	A=0.77

	oxoazetidin-1-yl)-2-(2-(2,6-dichlorophenylamino)phenyl)acetamide					B=0.75
IV	N-(3-chloro-2-(3-nitrophenyl)-4-oxoazetidin-1-yl)-2-(2-(2,6-dichlorophenylamino)phenyl)acetamide	C23H17Cl3N4O4	Very brown powder	43.1	199-200	A=0.84 B=0.76

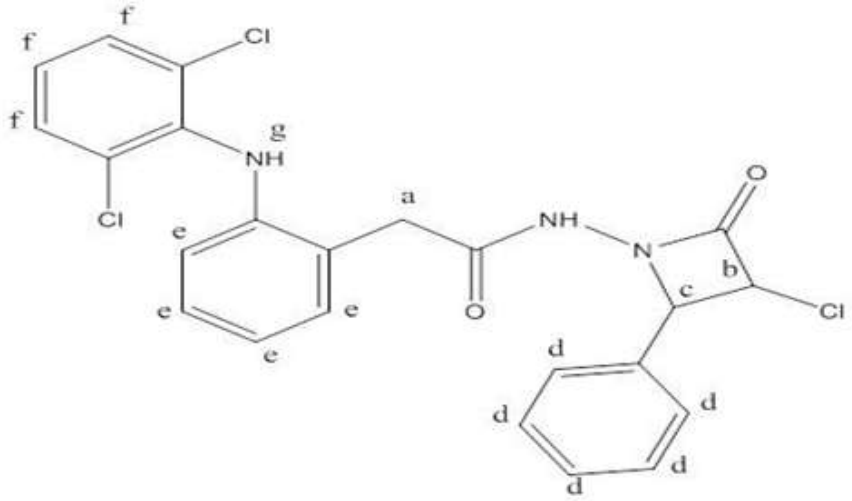
Table 2: The elemental microanalysis of the intended target compounds calculated / founded (I-IV)

Compound	Chemical Formula	Mol. wt.	Value type	C	H	N
I	C23H18Cl3N3O2	474.76	calculated observed	58.19 56.459	3.82 3.65	8.85 8.62
II	C23H18Cl3N3O3	490.76	calculated observed	56.29 54.675	3.70 3.58	8.56 8.24
III	C23H18Cl3N3O3	490.76	calculated observed	56.29 54.620	3.70 3.59	8.56 8.122
IV	C23H17Cl3N4O4	519.76	calculated observed	53.15 51.919	3.30 3.113	10.78 10.330

Table 3: IR spectral data of synthesized compounds

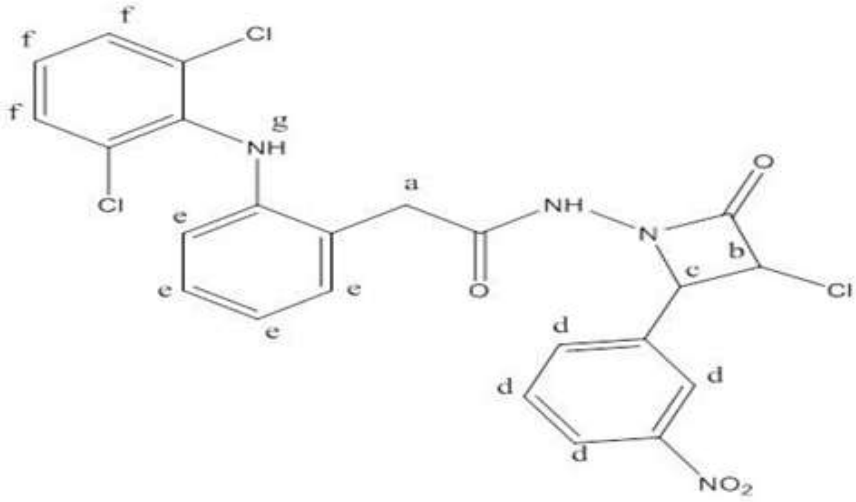
Sym	Chemical Name	Characteristics IR spectral bands (KBr) v cm-1with its Interpretation
A	2-(2-(phenyl amino)phenyl)acetic acid	3323(O-H) stretching broad band of carboxylic acid, 1693(C=O) stretching vibration of carboxylic acid,1568 (N-H) bending vibration of (amide II band)
B	ethyl2-(2-(2,6-dichlorophenylamino)phenyl)acetate	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) 1631(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
I	N-(3-chloro-2-oxo-4-phenylazetidin-1-yl)-2-(2-(2,6-dichlorophenylamino)phenyl)acetamide	3439Aromatic 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

Table 4: The 1HNMR data and their interpretation of compound II



Group	Chemical shift ppm	No. of H	Interpretation
A	3.33	2	(2H,s,CH2)
B	4.14	1	(1H, d, CH-Cl of Azetidinone ring)
C	3.71	1	(1H,d, Azetidinone proton)
D	7-7.5	5	(5H, m, Ar-H)
E	6-6.5	4	(4H, m, Ar-H)
F	7-7.5	3	(3H, m, Ar-H)
G	11.64	1	(1H,s,NH)
H	8.5	1	(1H, s, CONH)

Table 5: The 1HNMR data and their interpretation of compound IV



Group	Chemical shift ppm	No. of H	Interpretation
A	3.33	2	(2H,s,CH2)
B	4.17	1	(1H, d, CH-Cl of Azetidinone ring)
C	3.74	1	(1H, d, Azetidinone proton)
D	7.5-8.5	5	(4H, m, Ar-H)
E	6.5-7	4	(4H, m, Ar-H)
F	7-7.5	3	(3H, m, Ar-H)
G	11.86	1	(1H,s,NH)
H	8.5	1	(1H, s, CONH)

B. Anti-inflammatory Effect:

The anti-inflammatory activity of the target compounds was done in Department of Pharmaceutical Chemistry, College of Pharmacy, University of Baghdad. The evaluation of anti-inflammatory activity of newly synthesized compounds, is measured according to the method paw edema (the effects of testing compounds on egg-white albumine induced edema) which is an indicator of their anti-inflammatory activity. Diclofenac sodium was used as reference compounds of known profile of anti-inflammatory activity.

The anti-inflammatory activity of the tested compounds has been evaluated in comparison with their vehicle control group (DMSO) and reference drug. Westar rats of either sex weighing (210± 10 g) were supplied by the local bred of the animal house, department of pharmacology and toxicology, University of Baghdad and were housed in the same location

under standardized conditions. Animals were fed commercial chaw and tap water *ad libitum*.

The subcutaneous injection injection of 0.05ml fresh egg-white albumine into the sub planter side of the left hind paw produced the thickness in all animals designed as a control. Acute inflammation was produced by a (s.c) injection of undiluted fresh egg-white albumine 0.05 ml into the sub plantar side of the left hind paw of the rats; 30 min. after intraperitoneally injected administration of the drugs or their vehicle. The paw thickness was measured by digitalized vernier caliper at seven time intervals (0, 30, 60, 120, 180, min) after drug administration. Comparisons between different groups were made using ANOVA: Two factors without Replication. Probability (P) value of less than 0.05 was considered significant.

4. Results and Discussion

The structure of the synthesized compounds was confirmed by using FTIR spectroscopy, CHN elemental Microanalysis, 1H-NMR Spectroscopy, and other physicochemical parameters (tables 1,2,3,4 and 5). 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 the characteristic broad band of the (OH) group of carboxylic acid of Diclofenac. The diclofenac hydrazide (intermediate C) showed the appearance of the characteristic sharp band around1649 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-S6) 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 NMR spectrum of compound (II and IV) showed a doublets at (4.14, 4.17) respectively which integrated for one proton, identified as the CH-Cl of Azetidinone ring, supporting the formation of the Azetidinone ring.

The 1H-NMR Spectroscopy revealed good result indicated that final product appear from the data found in the (tab 4) and (tab 5) for compound II and 1Vrespectively and in the (fig 3) and (fig 4)

The results of anti inflammatory activity are shown in (table 4) and (figure 2). In the control group, paw edema was shown to be continually elevated reaching a maximum (5.741mm) after 30 minutes (1 hour after i.p. injection of vehicle or reference drug) of induction. For this reason, this time interval is used for the comparative analysis of anti-inflammatory effect of the reference drug and of the tested compounds. Paw thickness was reduced. back to lower value (5.265mm) after 180min.

intra-peritoneal injection of testing compounds produced varying degree of anti-inflammatory effect. Compound III exhibited potent effect than diclofenac (3mg/kg, i.p.); while compounds I,II and IV exhibited lower anti-inflammatory. The tested compounds and the reference drug produced significant reduction of paw edema with respect to the effect of propylene glycol 50%v/v (control group).

Table 6: The anti-inflammatory effect of control, diclofenac and compounds I-IV on egg-white induced paw edema in rats.

	Cpd	Time (min)					
		Baseline	0	30	60	120	180
Paw Thickness (mm) / n=6	Diclofenac	3.835	5.398	5.741667	5.365	5.21	5.043333
	Control	4.091	5.365	6.056667	5.913333	5.561667	5.265
	I	3.865	5.356	5.788333	5.475	5.133333	5.11
	II	3.705	5.18	5.771667	5.3	5.078333	5.126667
	III	4.098	5.3075	5.868333	5.13	5.121667	4.671667
	IV	3.648	4.873	5.925	5.401667	5.091667	5.108333

Source of Variation	*SS*	*df*	*MS*	*F*	*F crit*
Rows	0.014757	2	0.007379	0.219947	4.4589 ❶
Columns	1.455146	4	0.363787	10.84414	3.8378 ❷
Error	0.268375	8	0.033547		
Total	1.738278	14			

Data are expressed in mm paw thickness
n= number of animals.
Reference drugs were given 30 minutes before the injection of egg white
Time (0) is the time of injection of egg-white (induction of paw edema).

❶There are significant differences between compound's activity (rows) with P<0.05 with respect to each other.
❷There are no significant differences between time intervals (columns) with P<0.05 with respect to each other. This is due to lack of time to complete the experiments on rats until recovery.

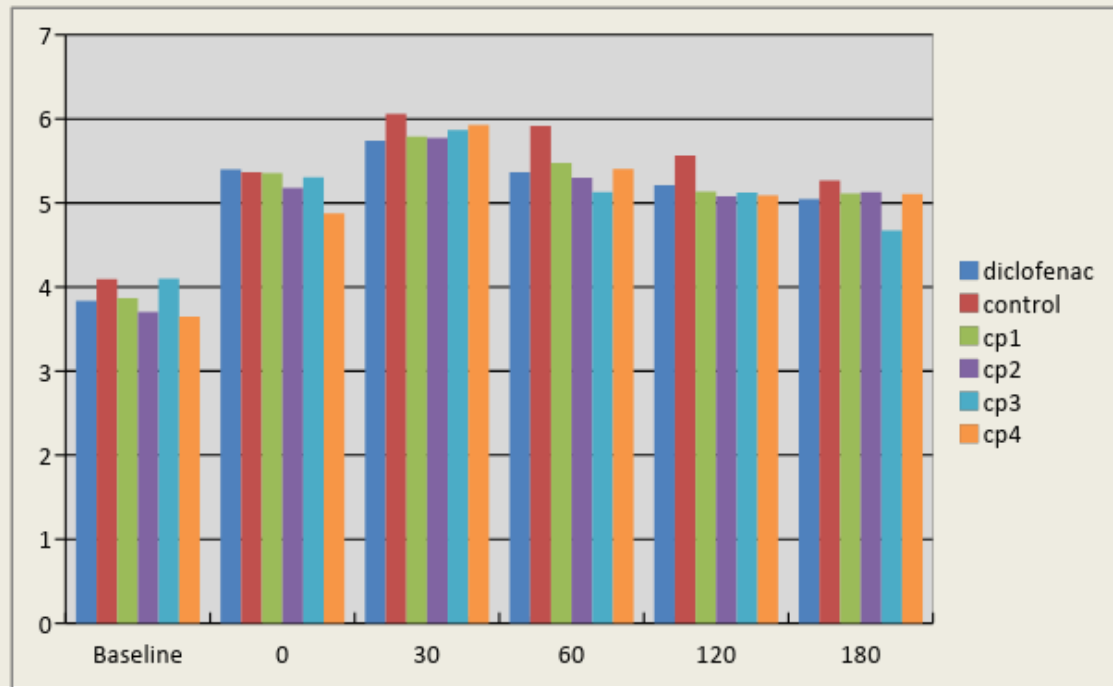


Figure 2: The effect of control, diclofenac and compounds I-IV on egg-white induced paw edema in rats

Figure 3: 1H-NMR spectrum of compound II

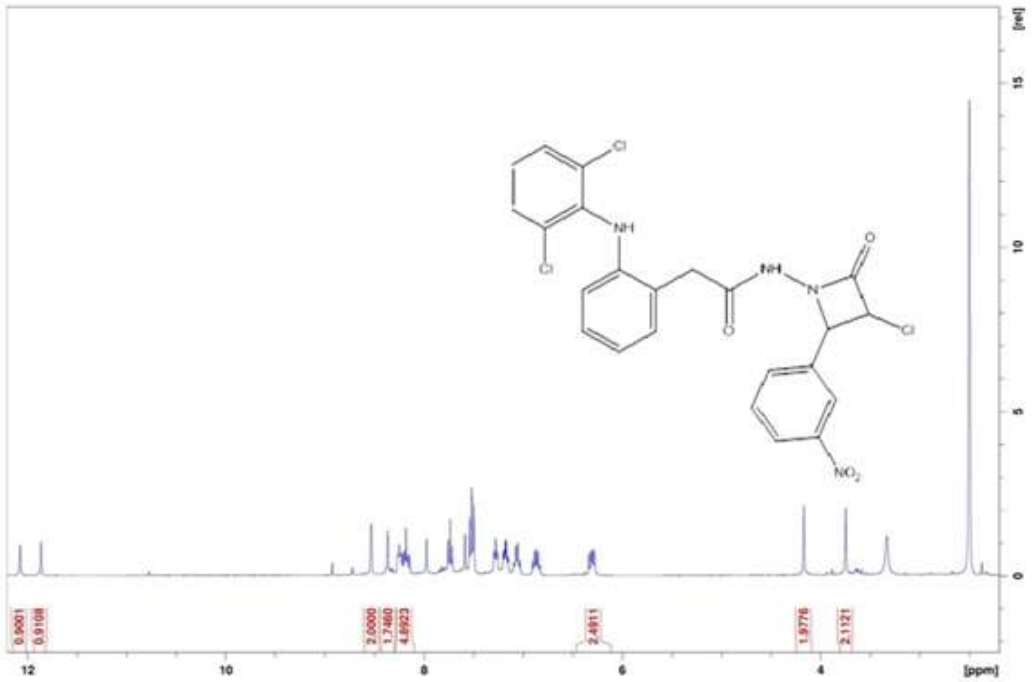


Figure 4: 1H-NMR spectrum of compound IV

5. Conclusion

The synthesized compounds were evaluated for anti-inflammatory activity by using paw method. The newly synthesized compounds I-IV produced varying degree of anti-inflammatory effect. Compound III exhibited potent effect than diclofenac (3mg/kg, i.p.); while compounds II, IV exhibited lower anti-inflammatory and I

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