AJPS, 2013, Vol. 13, No.1

Synthesis, Characterization and Biological Activity of New Mefenamic acid - Oxoazetidine Derivatives

Ayad M.R. Raauf
Department of chemical pharmacy and clinical pharmacognacy,
Collage of pharmacy, University of Al-Musatansariya

Abstract:

Several new oxoazetidine derivatives based on mefenamic acid were synthesized via a four steps method in good yields.1st step was prepared 2-[2-chloro-N-(2,3-dimethylphenyl)acetamido]benzoic acid (2) by condensation of mefenamic acid with chloroacetyl chloride, which on amination with hydrazine hydrate in ethanol to give 2-[N-(2,3-dimethylphenyl)-2-hydrazinylacetamido] benzoic acid (3) in good yields(2nd step). Compound 3, on condensation with various aldehydes afforded a series of Schiff bases 2-[N-(2,3-dimethylphenyl)-2-(2-arylhydrazinyl)acetamido]benzoic acid (4a-f) (3rd step).

A series of oxoazetidine have been synthesized by cyclocondensation of various Schiff bases (4a-f) with chloroacetyl chloride or phenyl acetyl chloride in presence of triethyl amine to yield compounds (5a-f) and (6a-f), respectively (4th step). Purified compounds were characterized by mp, TLC, UV, FT-IR and elemental analysis.

The synthesized compounds (5a-f) were screened for their antibacterial activities against three species of bacteria. They were found to exhibit good antibacterial activity.

Key words: Synthesis,Mefenamic acid, Oxoazetidine, Biological Activity.

الخلاصة:

تحضير العديد من مشتقات الاوكسازتيدين الجديدة بنواتج عالية لحامض الميفيناميك باربع خطوات. الخطوة الاولى: تحضير المركب الجديد(2) بتكثيف حامض الميفانيميك مع كلورواسيتايل كلورايد والذي تم معاملته في الخطوة الثانية مع هيدرات الهيدرازين في الايثانول لاعطاء المركب الجديد(3). تحضير قواعد شيف مختلفة جديدة (4a–f) من خلال تكاثف المركب (3) مع عدد من الالديهيدات (الخطوة الثالثة).

تم اجراء الغلق الحلقي لتحضير مشتقات الاوكسازتيدين (5a–f) او (6a–f) بمعاملة (4a–f) مع كلورواسيتايل كلورايد او فنيل استيل كلورايد بوجود ثلاثي اثيل امين (الخطوة الرابعة) .

تم تشخيص المركبات الجديدة عن طريق تحليل العناصرواستخدام البيانات الطيفية .تمت دراسة الفعالية البايولوجية للمركبات (5a–f) ضد ثلاثة انواع من البكتريا وتبين ان لها فعالية بايولوجية جيدة .

Introduction:

Mefenamic acid has analgesis, non-steroidal anti-inflammatory and antipyretic actions drug and is used for the relief of less severe types of pains such as headache, toothache and muscular pains [1].

Azetidine, a four membered cyclic lactam (β-lactam) heterocycles, skeleton has been recognized as a useful building block for synthesis of large number of antibiotic used in medicine such as the penicillin, cephalosporin, aztreonam and thienamycine[2,3]. Azetidinones, which are part of the antibiotic structure, are known to exhibit interesting biological activeties[4,5].

A compendium on the generation of the β-lactam ring covering different methods has been reported [6]. The Staudinger reaction ([2+2] ketene-imine cycloaddition reaction) is regarded as one of the most fundamental and versatile methods for the synthesis of structurally diverse 2-azetidinone derivatives, although

many synthetic methods have been developed to date [7]. Azetidin-2-ones can also be synthesized by catalytic asymmetric reactions of ketenes and ketene enolates[8].

A β-lactam derivative has been synthesized as application of the catalytic asymmetric alkylation of α-cyanocarboxylates and acetoacetates with an alkyl halide under phase-transfer conditions [9]. A number of 2-azetidinones have been synthesized in good yields by a novel reaction between Schiff bases, substituted acetic acids and alkoxymethylene-*N*,*N*-dimethyliminium salts[10]

A series of novel azetedinones have been synthesized by cyclocondensation of various Schiff bases with chloroacetyl chloride in presence of triethylamine[4,11,12] or used phenyl acetyl chloride in presence of base[13].

Aim of the present work was to synthesis new mefenamic acid-oxoazetidine derivatives and studies their antibacterial activities.

Material and Methods:

Apparatus and Chemicals:

Melting points were determined by open capillary method on Stuart SMP10melting point apparatus and are uncorrected. IR spectra were recorded on Shimadzu FT-IR-8400S in KBr pellets. Elemental analysis was performed by Perkin-Elmer 240C analyzer series CHN analyzer. All reactions were monitored by TLC on silica gel plates. All solvents were distilled before used. Mefenamic acid, reagents and solvents used are commercially available.

Experimental:

Synthesis of 2-[2-chloro-N-(2,3-dimethylphenyl) acetamido]benzoic acid (2)[14]: A mixture of mefanemic acid (1, 0.001 mol) and chloroacetyl chloride (0.0035 mol) were heated with stirring to 90°C for 2h on a water bath. To destroy excess of the acid chloride, 2-propanol and water (2 ml each) was added and stirring was continued over night at room temperature. Toluene (10 ml) was added and the mixture extracted with 10% NaHCO3, organic phase was dried with magnesium sulfate and evaporated to dryness.

The compound obtained was recrystallized by ethanol to furnish a pale yellow solid. Yield 75%; m.p. 135-137°C; IR (KBr, cm^{-1}) 1633 (CONH). Anal. calcd. for $C_{17}H_{16}ClNO_3$: C 64.26, H 5.08, N 4.41%. %. Found: C 64.20, H 5.02, N 4.49%.

Synthesis of 2- [N- (2, 3-dimethylphenyl)-2-ydraziny-lacetamido] benzoic acid (3)[11]:

A mixture of 2-[2-chloro-N-(2,3-dimethylphenyl)acetamido]benzoic acid (2, 0.001 mol) and hydrazine hydrate (0.001 mol) in ethanol (30 ml) was refluxed for about 6 h.

After cooling the resulting solid was filtered, dried and recrystallized from methanol to give light yellow solid. Yield 80%; m.p. 155-158°C; IR (KBr, cm^{-1}) 3400 (NH), 3320 (NHNH2), 1650 (CONH). Anal. calcd. for $C_{17}H_{19}N_3O_3$: C 65.16, H 6.11, N 13.41%

%. Found: C 65.01, H 6.02, and N 13.47%.

Synthesis of 2- [N- (2, 3-dimethylphenyl)- 2- (2- arylhyd-razain) acet-amido]benzoic acid (4a-f) [15]:

A mixture of compound (3, 0.001 mol) and the corresponding aromatic aldehydes (0.001 mol) in 10 ml of absolute ethanol was stirred at room temperature for 4h, in the presence of two drops of glacial acetic acid as catalyst.

The end of the reaction was observed by TLC, and the hydrazones 4a-f was isolated by concentration of the reaction mixture under reduced pressure, followed by neutralization with a 10% $NaHCO_3$.

The resulting precipitate was filtered, washes with water and recrystallized ethanol. Melting points,

yields, CHN analysis and spectral characterization of the compounds (4a- f) were reported in Table 1, 2 and Scheme 1.

Synthesis of 2-[2-{(2-chloro-3-(4-aryl)-4-oxoazetidine-1-yl) amino}-N-(2,3-dimethylphenyl)acetamido]benzoic acid (5a-f) [4,11]:

A mixture of the compounds (4a-h, 0.001mol) in ethanol (10ml) and chloroacetyl chloride (0.003 mol) in presence of triethylamine (0.003 mol) were placed in round bottom flask and refluxed for 4 h.

After completion of the reaction was filtered to separate salt formed. The filtrate was concentrated to half its volume then poured on to crush ice.

The product was filtered wash with water and recrystallized from ethanol. Melting points, yields, CHN analysis and spectral characterization of the compounds (5a- f) were reported in Table 1, 2 and Scheme 1.

Synthesis 0f 2-[2-{(4-aryl-4-oxo-3-phenyl-azetidine-1-yl)amino}-N-(2,3-dimethylphenyl)acetamido]benzoic acid (6a-f)[16]:

To a solution of (4a-f) (0.003 mol) in dry benzene (10 ml) and (0.003 mol) triethylamine was added phenyl acetyl chloride (0.003 mol) and refluxed for 6 h.

The salt was filtered off. The filtrate was concentrated under reduced pressure. The product was filtered wash with water and recrystallized from ethanol. Melting points, yields, CHN analysis and spectral characterization of the compounds (6a- f) were reported in Table 1, 2 and Scheme 1.

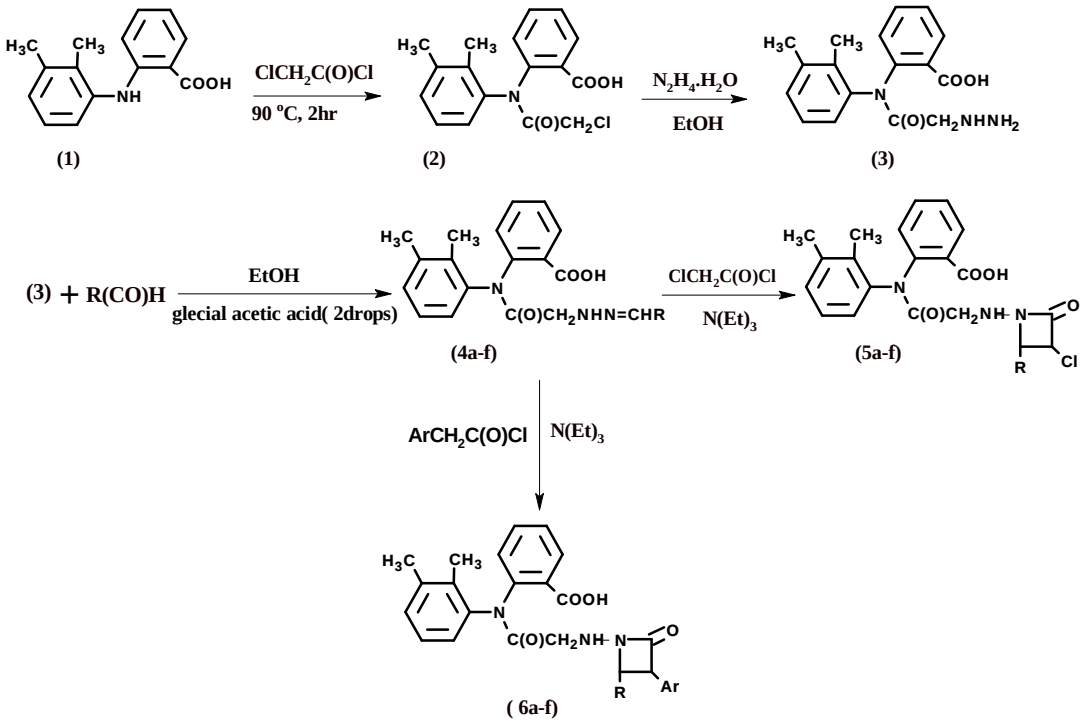
Biological Screening:

The compounds (5a-f) were screened for their antibacterial activity using *E. coli*. *Enterococcus sp.* and *Lactobacillus sp by* agar well-diffusion method [17, 18]. Uniform volume of different concentration 250 µg/ml of the test synthesized compounds was placed in each of the cavity, after overnight incubation at 37°C. The diameter of inhibition zone formed around the well was measured in mm.

DMSO was used as a solvent for all the compounds, which did not show any inhibition against test bacteria. Control experiment was carried out under similar condition by using ampicillin as a standard for comparison. The results of activity summarized in Table 3.

Result and Discussion:

This work is carried out in synthesizing new compounds of azitidinone (5a-f and 6a-f) have shown schematically below.



Compound	R
a	4- $NO_2C_6H_4$
b	4- $CH_3OC_6H_4$
c	4- ClC_6H_4
d	4- $(CH_3)_2NC_6H_4$
e	4-$CH_3C_6H_4$
f	4- OHC_6H_4

Scheme 1

All the new compounds prepared were initially characterized by analytical data Table 1 and IR spectra Table 2.

Mefenamic acid (1) on condensation with chloroacetyl chloride yield 2- [2- chloro- N- (2, 3-dimethylphenyl) acetamido] benzoic acid (2). The formation of compound (2) was evidenced by appearance band in IR spectra due to 1633 cm^{-1} (CONH) stretching. Compound (2) and hydrazine hydrate in ethanol as a reaction media afforded 2- [N-(2,3-dimethylphenyl)-2-ydrazinylacetamido] benzoic acid (3). In the IR spectra, the bands 3400 cm^{-1} (NH), 3320 cm^{-1} (NHNH2), 1650 cm^{-1} (CONH) confirms the formation of compound (3).

Compound (3) was then reacted with series of aromatic aldehydes (a-f) and 2 drops of glacial acetic acid in ethanol afforded compounds 2-[N-(2,3-dim - ethylphenyl)-2- (2-arylhydrazain) acet-amido]benzoic acid (4a-f).

Their IR spectrum was characterized by disappearance of the bands of (NHNH2) group and appearance of strong bands in the 3360-3377 cm^{-1} and 1548-1554 cm^{-1} due to stretching vibration of (NH) and (N=CH) groups respectively.

Compounds (4a-f), on reaction with chloroacetyl chloride in the presence of triethylamine afforded 2-[2-{(2-chloro-3-(4-aryl)-4-oxoazetidine-1-yl) amino}-N-(2,3-dimethylphenyl) acetamido] benzoic acid (5a-f). IR spectrum of compounds (5a-f) showed characteristic absorption band of (C=O β-lactum) in 1715-1730 cm^{-1} and band at 765-785 cm^{-1} attributed for (C-Cl).

Compounds (6a-f) were prepared from condensation of compounds (4a-f) with phenyl acetyl chloride in the presence of trietylamine.

The presence of (C=O β-lactum) group was indicated by the appearance of stretching frequencies in the range of 1725-1735 cm^{-1}.

Antibacterial Activity:

All the synthesized compounds (5a-f) were tested for antibacterial activity against bacterial species *E. coli. Enterococcus sp. a*nd *Lactobacillus sp.*the results from the Table 3 indicate that these compounds were active against all the bacteria used.

Table-1: Physical and analytical data of newly synthesized compounds (4a- f, 5a-f and 6a-f)

No.	Molecular Formula	MP °C	Yield (%)	Calc. (Found)%		
				C%	H%	N%
4a	$C_{24}H_{22}N_4O_5$	180-183	80	64.57 (64.60)	4.97 (4.95)	12.55 (12.57)
4b	$C_{25}H_{25}N_3O_4$	162-164	65	69.59 (69.55)	5.84 (5.81)	9.74 (9.79)
4c	$C_{24}H_{22}ClN_3O_3$	135-138	78	66.13 (66.15)	5.09 (5.12)	9.64 (9.62)
4d	$C_{26}H_{28}N_4O_3$	170-173	70	70.25 (70.22)	6.35 (6.30)	12.60 (12.57)
4e	$C_{25}H_{25}N_3O_3$	169-172	83	72.27 (72.23)	6.06 (6.26)	10.11 (10.15)
4f	$C_{24}H_{23}N_3O_4$	177-179	75	69.05 (69.02)	5.55 (5.53)	10.07 (10.11)
5a	$C_{26}H_{23}ClN_4O_6$	250-254	65	59.72 (59.67)	4.43 (4.41)	10.71 (10.74)
5b	$C_{27}H_{26}ClN_3O_5$	228-230	72	63.84 (63.88)	5.16 (5.13)	8.27 (8.31)
5c	$C_{26}H_{23}Cl_2N_3O_4$	248-250	67	60.95 (60.90)	4.52 (4.49)	8.20 (8.25)
5d	$C_{28}H_{29}ClN_4O_4$	220-223	65	64.55 (64.52)	5.61 (5.59)	10.75 (10.78)
5e	$C_{27}H_{26}ClN_3O_4$	235-238	70	65.92 (65.89)	5.33 (5.31)	8.54 (8.60)
5f	$C_{26}H_{24}ClN_3O_5$	227-229	69	63.22 (63.19)	4.90 (4.88)	8.51 (8.54)
6a	$C_{32}H_{28}N_4O_6$	270-273	70	68.07 (68.00)	5.00 (5.05)	9.92 (10.01)
6b	$C_{33}H_{31}N_3O_5$	250-252	72	72.11 (72.02)	5.69 (5.63)	7.65 (7.71)
6c	$C_{32}H_{28}ClN_3O_4$	255-257	63	69.37 (69.44)	5.09 (5.15)	7.58 (7.65)
6d	$C_{34}H_{34}N_4O_4$	239-241	65	72.58 (72.63)	6.09 (6.14)	9.96 (10.04)
6e	$C_{33}H_{31}N_3O_4$	244-246	70	74.28 (74.16)	5.86 (5.90)	7.87 (7.90)
6f	$C_{32}H_{29}ClN_3O_5$	250-252	75	71.76 (71.66)	5.46 (5.40)	7.85 (7.93)

Table-2: Spectral data of newly synthesized compounds (4a-f, 5a-f and 6a-f)

No.	IR: ν (cm^{-1})
4a	3367(NH), 1673(C=O), 1633(CONH), 1550(N=CH), 1340(Ar- NO_2)
4b	3360(NH), 2835(Ar-OCH_3), 1679(C=O), 1638(CONH), 1550(N=CH)
4c	3365(NH), 1705(C=O), 1640(CONH), 1548(N=CH), 825(Ar-Cl)
4d	3367(NH), 1668(C=O), 1633(CONH), 1550(N=CH)
4e	3377(NH), 1690(C=O), 1642(CONH), 1554(N=CH)
4f	3370(NH), 3450(ArOH), 1675(C=O), 1633(CONH), 1550(N=CH)
5a	3345(NH), 1655(CONH), 1715 (C=O β-lactum), 1345(Ar- NO_2),785(C-Cl)
5b	3343(NH), 1645(CONH),1720 (C=O β-lactum), 785 (C-Cl)
5c	3350(NH), 1653(CONH),1718(C=O β-lactum), 830(Ar-Cl) ,779(C-Cl)
5d	3340(NH), 1655(CONH),1720 (C=O β-lactum), 775 (C-Cl)
5e	3348(NH), 1650(CONH),1730 (C=O β-lactum), 765 (C-Cl)
5f	3350(NH), 3450(ArOH), 1649(CONH),1680 (C=O β-lactum), 768 (C-Cl)
6a	3349(NH), 1653(CONH),1735 (C=O β-lactum), 1343(Ar- NO_2)
6b	3351(NH), 1650(CONH),1725(C=O β-lactum)
6c	3355(NH), 1653(CONH),1728 (C=O β-lactum), 825(Ar-Cl)
6d	3345(NH), 1655(CONH),1735 (C=O β-lactum)
6e	3345(NH), 1657(CONH),1735 (C=O β-lactum)
6f	3348(NH), 3450(ArOH), 1648(CONH),1740(C=O β-lactum)

Table-3: Antibacterial activity of newly synthesized compounds (5a-f) (Zone of inhibition in mm).* 250㎎/ml.

Compound No.	*E.coli*	*Enterococcus sp.*	*Lactobacillus sp.*
5a*	15	16	15
(1982).	(1982) .	(1982).	(1982).
5c*	15	15	14
5d*	15	16	14
5e*	16	16	17
5f*	18	17	17
Ampicillin*	25	25	27

Referances:

1 - Brttain, H. G. Analytical profiles of drug substances and excipients.179-211, Academic Press, New York. 2002.

2 - Morin, R. B. and Gorman, M. Chemistry and biology of β-lactam antibiotics. Academic Press.New York. 1982.

3 - Southgate, R. The synthesis of natural β-lactam antibiotics. Contemp. Org. Synth. 1994. Vol . 1. Pp: 417-431.

4 - Ameya, A. Ch. and Nandini, Pai. R. Synthesis and Biological Activity of N- Substituted- 3- chloro- 2-azetidinones. Molecules, 2007. Vol. 12, Pp: 2467-2477.

5 - Mehta, P. D. ; Sengar, N. P. S. and Pathak, A. K. 2- Azetidinone- a new profile of various pharmacological activities. 2010. Vol. 45 (12) Pp: 5541-5560.

6 - Aranda, M.T.; Pérez-Faginas, P. and González-Muñiz, R. Periselectivity Switch of Acylketenesin

Cycloadditions with 1-Azadienes. Microwave-Assisted Diastereo selective Domino Three-Component Synthesis of r -Spiro-!–lactams *Curr. Org. Synth.* 2009 Vol. 6 (325).

7 - Jiaxi, X.u. Stereoselectivity in the synthesis of 2-azetidinones from ketenes and amines via Staudinger reaction. Arkivoc. 2009. Vol. 4. Pp: 21-44.

8 - Paull, D. H.; Weatherwax, A. and Lectka, T. Catalytic, asymmetric reactions of ketenes and ketene enolates. Tetrahedron, 2009. Vol. 65 (6771).

9 - Nagata, K.; Sano, D.; Shimizu, D.; Miyazaki, M.; Kanemitsu, T. and Itoh, T. Catalytic Asymmetric Alkylation of α-Cyanocarboxylates and Acetoacetate using a Phase-transfer Catalyst .Tetrahedron: Asymmetry, 2009. Vol. 20 Pp: 2530-2536.

10 - Jarrahpour, A. and Zarei, M. DMF-dimethyl sulfate as a new reagent for the synthesis of β -lactams. Tetrahedron Lett. 2009. Vol. 50. Pp: 1568-1570.

11 - Rajasekaran, A.; Periasamy, M. and Venkatesan, V. Synthesis, characterization and biological activity of some novel azetidinones. Journal of Developmental Biology and Tissue Engineering, 2010. Vol. 2 (1). Pp: 5-13.

12 - Raji, D. u. And Srivastava, S. K. Synthesis, characterization and antimicrobial activity of 2- (2′-substituted- benzylidine - hydrazino-acetyl)-mercapto-5-methyl-1,3,4-thiadi-azoles and 2-[2′-{4- substituted- aryl-3-chloro-2-oxo- azetidine}-acetyl- amino- mercapto]- 5- methyl-1,3,4-thiadiazoles. Inter. J. of Pharm. And Bio. Sci. 2010. Vol. 1 (2). Pp: 1-6.

13 - Endo, M. and Droghini, R. Synthesis of *trans*-1-*p*-methoxyphenyl-3-acetoxy- 4- phenylazetidin- 2- one. Bioorganic & Medicinal Chemistry Letters, 1993. Vol. 3. (11). Pp: 2483-2486.

14 - Acemoglu, M.; Allmendinger, Th.; Calienni, J.v.; Circus, J.; Sedelmeier, G. and Xu, D. Process for Phenylacetic acid derivatives. US. Pat. 2008. Vol. 249 (318). Pp: 548-486.

15 - Mobinikhaledi, A. K.; Foroughifar, N.; Khanpour, M. and Ebrahimi, S. Synthesis of some novel Schiff base containing 1,2,4–triazole ring. European Journal of Chemistry, 2010.Vol. 1 (1). Pp: 33-36.

16 - Ali, P.; Meshram, J. and Tiwari, V. Microwave Mediated Cyclo condensation of 2- aminothiazole into β-lactam Derivatives: Virtual Screening and In Vitro Antimicrobial Activity with Various Microorganisms. International Journal of ChemTech Research, 2010. Vol. 2 (2). Pp: 956-964.

17 - Wadher, S. J.; Karande, N. A.; Sonawane, S. D. and Yeole, P. G. Synthesis and Biological Evaluation of Schiff Base and 4- Thiazolidinoes of Amino salicylic Acid their derivatives as an Antimicrobial Agent. International Journal of ChemTech Research, 2009. Vol. 1 (2). Pp: 1303-1307.

18 - Ehta, P. D.; Sengar, N. P. S.; Subrahmanyam, E. V. S. and Satyanarayana, D. Synthesis and biological activity studies of some thiazolidinones and azetidinones. Indian journal of Pharmaceutical Science, 2006. Vol. 68 (1). Pp: 103-106.