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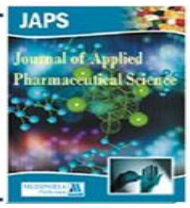

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Synthesis and antimicrobial activity of novel 3-Chloro- [1- (3,6-(Diphenyl) [1,2,4] Triazolo [3,4b] [1,3,4] Thiadiazole)] -4-(3,4-Diethoxy Phenyl-Azetidin-2-One and their derivatives

Kokila Parmar, Rekha Patel, Sarju Prajapati, Sejal Joshi and Rinku Patel

Kokila Parmar, Rekha Patel, Sarju Prajapati, Sejal Joshi and Rinku Patel
Department of Chemistry, Hemchandracharya North Gujarat University, Patan-384265, Gujarat, India

For Correspondence
Dr. Kokila A. Parmar
Department of Chemistry, Hemchandracharya North Gujarat University, Patan-384265, Gujarat.

ABSTRACT

A series of eight novel 2-Azetidinones **(8a-h)** have been synthesized by cyclocondensation of various Schiff bases based of ATT with chloroacetyl chloride in presences of triethylamine. Various Schiff bases were synthesized by condensation of ATT with various aryl aldehydes **(7a-h)**. The synthesized compounds **8a-h** were screened for their antibacterial activity against four microorganisms: Staphylococcus aureus (Gram positive), Bacillus subtilis (Gram positive), Psuedomonas aeruginosa (Gram negative) and Escherichia coli (Gram negative). They were found to exhibit good to moderate antibacterial activity. The structures were confirmed by elemental analysis, IR, ^{1}H-and ^{13}C-NMR spectral data.

Keywords: Synthesis, Antimicrobial activity, Triazolo and Azetidin-2-One.

INTRODUCTION

Azetidin-2-one, a four-membered cyclic lactam (β-lactam) skeleton has been recognized a useful building block for the synthesis of a large number of organic molecules by exploiting the strain energy associated with it. Efforts have been made in exploring such new aspects of β-lactam chemistry versatile intermediates for their synthesis of aromatic β-amino acid and their derivatives, peptides, polyamines, polyamino alcohols, amino sugars and polyamino ethers (Alcaide *et al.*, 2001) The cyclic 2-azetidinone skeleton has been extensively used as a template to build the heterocyclic structure fused to the four membered ring. This provides an access to diverse structural type synthetic target molecules lacking β-lactam ring structure. The β-lactam heterocycles are still the most prescribed antibiotics used in medicine. They are considered as an important contribution of science to humanity (Morin *et al.*, 1982). The most widely used antibiotics such as the penicillins, cephalosporins, carumonam, thienamycine, aztreonam and the norcardicins all contain β-lactum rings (Mata *et al.*, 2003). The long-term use of β-lactam antibiotics exerts selective pressure on bacteria and permits the proliferation of resistant organisms (Page *et al.*, 1992). Azetidinones are of great biological interest, especially as anti tubercular (Kagthara *et al.*, 2000), antibacterial (Rajasekaran *et al.*, 2010 and Ameya *et al.*, 2007), anti fungal (Pandey *et al.*, 2005) and as anti inflammatory (Mehta *et al.*, 2006), anti convulsant activity.

They also function as enzyme inhibitors and are effective on the central nervous system (Vashi *et al.,* 1995). 2-Aminobenzothiazoles constitute another class of heterocycles that posses antimicrobial and various other pharmacological activities like diuretic, antiulcer, antihistamine and anticancer properties (Russo et al.1994 , Katsura *et al.,* 1994, Kuuhle at el. 1998, and Baltork *et al.,* 2007). Some methods for the preparation of N-substituted-2-azetidinones, which are useful in the synthesis of taxol and taxol derivatives (Rey *et al.,* 1995). Singh *et al.,* have prepared some new 2-azetidinones from N-(salicylidene) amines and 2-diazo-1,2-diarylethanones(Singh et al.2007 and Singh *et al.,* 2004) . Wang *et al.,* synthesized fourteen derivatives of 2-azetidinones and reported for cholesterol absorption inhibitory action (Wang et al.2009). Hence, it was thought worthwhile to synthesize new congeners by incorporating ATT and azetidinone moieties in a single molecular frame work and to evaluate their antimicrobial activity.

RESULT AND DISCUSSION

The present protocol describes a simple and efficient method for the synthesis of azetidinones by different Schiff bases of ATT. It has been demonstrated that cyclocondensation of Schiff bases with chloroacetyl chloride in triethylamine revealed fairly high yields in a relatively short reaction time and easy work-up procedures. These conditions enable this method to be applicable for the synthesis of 2-azetidinone based heterocyclic. The purity of the synthesized compounds were confirmed by performing TLC. IR absorption band at 1697 cm^{-1} for stretching vibration of C=O of β-lactam the presences of one proton at 10.8 ppm (1H,s), confirms the condensation of reactants to form Schiff-base. Similarly IR, PMR and ^{13}CMR obtained were in correlation with synthesized azetidinones.

The synthesized compounds 7a-h was tested for their anti-bacterial activity proved to be effective particularly against *Staphylococcus aureus* (Gram positive), *Bacillus subtilis* (Gram positive), *Psuedomonas aeruginosa* (Gram negative) and *Escherichia coli* (Gram negative) Gram-positive. The compound 7b, 7d, 7f and 7h were shown significant activities and compound 7a, 7c, 7e, and 7g have shown moderate activity.

MATERIAL AND METHODS

I- Synthesis of methyl benzoate

Benzoic acid (0.01 mole) in 20 ml of methanol and 0.5 ml conc. Sulfuric acid was refluxed for 12 hrs. and poured into ice. The product was isolated and treated with standard sodium bicarbonate solution to give desired compounds. Yield: 96%.

II- Synthesis of benzoic acid hydrazide

A mixture methyl benzoate (0.01 mole) and hydrazine hydrate (0.5 g, 0.01 mole) was heated for 9 hrs. and poured into ice. The product was isolated and crystallized from ethanol. Yield: 85%.

III- Synthesis of potassium-benzoic acid hydrazide dithiocarbamate

A mixture of benzoic acid hydrazide (0.01 mole), KOH (0.84 g, 0.015 mole) and 1.5 ml CS_2 in absolute alcohol was stirred for 21 hrs. and product was isolated from diethyl ether. Yield: 87%.

IV- Synthesis of 4-amino-5-phenyl-4H-1,2,4-triazole-3-thiol

Potassium salt (0.01 mole) was taken in hydrazine hydrate and heated up to the evolution of H_2S gas cussed nearly 5 hrs. in oil bath. The reaction mixture was poured into crushed ice and treated with glacial Acetic acid .The product was filtered and purified by KOH treatment and crystallized from ethanol. Yield: 65%.

V- Synthesis of 3-(phenyl)-6-(4-N-acetylamino phenyl) [1,2,4]triazolo[3,4-b] [1,3,4]thiadiazole

A mixture of n-acetyl-p-amino benzoic acid (0.01 mole) and 4-amino-5-phenyl-4H-1,2,4-triazole-3-thiol (0.01 mole) in $POCl_3$ (25 ml) was refluxed for 10 hrs. The reaction mixture was poured into crushed ice and thus solid separated out was filtered, washed with water and crystallized from ethanol.

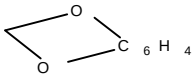
VI- Synthesis of 3-(phenyl)-6-(4-amino phenyl) [1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (ATT)

3-(phenyl)-6-(4-N-acetylamino phenyl) [1,2,4]triazolo [3,4-b][1,3,4]thiadiazole was hydrolysed by refluxing with 75 ml of ethanol containing 15 ml of concentrated HCl for 4-5 hrs. it was then poured into ice-cold water and finally made just alkaline with liquid ammonia. The resultant product 3-(phenyl)-6-(4-amino phenyl) [1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (ATT) is filtered off and washed with water and air dried. It was then recrystallised from ethanol to give product in near 60% yield. I.R. (KBr, cm^{-1}): 3362 (NH_2), 3030, 1500, 1600 (aromatic C-H), 1580 (C=N), 692, 1630 (NH-in and out plane), 1344 (C-S-C). PMR (δ ppm): 6.4-8.86 (m, aromatic CH). ^{13}CMR (δ ppm): 113, 130-150 (triazolo-thiadiazole), 120-129 (benzene)

VII- Synthesis Arylidine-[3,6-(diphenyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole] (7a-h)

A mixture of equimolar amount of 3-(phenyl)-6-(4-amino phenyl) [1,2,4] triazolo [3,4-b][1,3,4]thiadiazole (0.01 mole) and various aromatic aldehydes (0.01mole) in 50 ml acetic acid and refluxed for about 10-12 hrs. on oil bath. The reaction mixture was cooled and it was poured in to ice water and extracted with ethyl acetate and water and finally dried over anhydrous sodium sulfate. The solvent was evaporated to give the solid product. It was crystallized from ethyl acetate hexane using decolorizing charcoal to give various anils (a-h). Product in near 60% yield. I.R. (KBr, cm^{-1}): 3362 (NH_2), 30z30, 1500, 1600 (aromatic C-H), 1580 (C=N), 692, 1630 (NH-in and out plane), 1344 (C-S-C). PMR (δ ppm): 6.4-8.86 (m, aromatic CH). ^{13}CMR (δ ppm): 113, 130-150 (triazolo-thiadiazole), 120-129 (benzene).

Table. 1: Physical constant of 3-chloro-1-[3,6-(diphenyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole]-4-aryl-azetidin-2-ones (8a-h).

Sr. No	R	Molecular Formula	Molecular Weight	M.P. °C	Yield %	% of C, H, N, S, Cl Cal / Found				
						C	H	N	S	Cl
a	C_6H_6	$C_{24}H_{16}N_5OSCl$	457.5	190	60	62.9 62.7	3.5 3.4	15.3 15.1	7.0 7.0	7.7 7.3
b	4-OCH_3-C_6H_5	$C_{25}H_{18}N_5O_2SCl$	487.5	189	58	61.5 61.3	3.6 3.4	14.3 14.1	6.5 6.4	7.2 7.1
c	4-OH-C_6H_5	$C_{24}H_{16}N_5O_2SCl$	473.5	185	53	60.8 60.7	3.3 3.3	14.7 14.5	6.7 6.6	7.4 7.3
d	2-OH-C_6H_5	$C_{24}H_{16}N_5O_2SCl$	473.5	199	55	60.8 60.5	3.3 3.0	14.7 14.5	6.7 6.5	7.4 7.3
e	4-CH_3-C_6H_5	$C_{25}H_{18}N_5OSCl$	471.5	194	63	63.6 62.4	3.8 3.4	14.8 14.5	6.7 6.5	7.5 7.3
f		$C_{25}H_{16}N_5O_3SCl$	501.5	162	52	59.8 59.7	3.2 3.2	13.9 13.7	6.3 6.2	7.0 6.8
g	4-OH-3-OCH_3-C_6H_3-CHO	$C_{25}H_{18}N_2O_2SCl$	503.5	198	50	59.5 59.4	3.5 3.4	13.9 13.8	6.3 6.2	7.0 7.0
h	3-OC_2H_5-4-OC_2H_5-C_6H_3-CHO	$C_{28}H_{24}N_5O_3SCl$	545.5	180	47	61.5 61.5	4.4 4.4	12.8 12.7	5.8 5.7	6.5 6.3

VIII- Synthesis of 3-chloro-1-[3,6-(diphenyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole]-4-aryl-azetidin-2-ones (8a-h)

A mixture of Schiff base (1a-1h) (0.002 mole) and triethyl amine(TEA) (0.004 mole) was dissolved in 1,4-dioxane (50 ml), cooled and stirred. To this well-stirred cooled solution chloroacetyl chloride (0.004 mole) was added dropwise within a period of 30 minutes. The reaction mixture was then stirred for an additional 3 hours and left at room temperature for 48 hours. The resultant mixture was concentrated, cooled, poured into ice cold water, and then air-dried. The products thus obtained was purified by column chromatography over silica gel using 30% ethyl acetate; 70% benzene as eluent. Re-crystallization from ether/n-hexane gave 2-azetidinones (1a-1h), which were obtained in 55-70% yield.

Spectral Analysis of Synthesized 3-chloro-1-[3,6-(diphenyl)-[1,2,4]triazolo[3,4-b] [1,3,4]thiadiazole]-4-aryl-azetidin-2-ones

I- 3-chloro- [1-(3,6-(diphenyl) [1,2,4] triazolo [3,4-b] [1,3,4] thiadiazole)]-4-phenyl-azetidin-2-one (8a).

I.R. (KBr, cm^{-1}): 3030, 1500, 1600 (aromatic C-H, stretching), 1697 (C=O of β-lactam). PMR (δ ppm): 6.14-7.88 (m, aromatic C_4H), 10.8 (1H, CH_3). ^{13}CMR (δ ppm): 136-145 (triazole-thiadiazole), 114-130 (benzene), 48, 143, 156 (β-lactam), 169 (C=O), 136-145 (C_3).

II- 3-chloro- [1-(3,6-(diphenyl) [1,2,4] triazolo [3,4-b] [1,3,4] thiadiazole)]-4-(4-methoxy phenyl-azetidin-2-one (8b).

I.R. (KBr, cm^{-1}): 3400, 3030, 1500, 1600 (aromatic C-H, stretching), 1697 (C=O of β-lactam), 1200 (aryl-alkyl ether). PMR (δ ppm): 6.12-7.85 (m, aromatic C_4H of β-lactam), 10.4 (1H, CH_3 of β-lactam), 4.3 (3H, CH_3 of OCH_3). ^{13}CMR (δ ppm): 136-145 (triazole-thiadiazole), 114-130 (benzene), 48, 144, 156 (β-lactam), 46 (O-CH_3), 169 (C=O).

III- 3-chloro- [1-(3,6-(diphenyl) [1,2,4] triazolo [3,4-b] [1,3,4] thiadiazole)]-4-(4-hydroxy phenyl-azetidin-2-one (8c).

I.R. (KBr, cm^{-1}): 3370, 3030, 1500, 1600 (aromatic C-H, stretching), 1697 (C=O of β-lactam), 3200-2600 (-OH phenolic). PMR (δ ppm): 6.2-7.9 (m, aromatic C_4H of β-lactam), 10.8 (1H, CH_3 of β-lactam), 3.6 (H of OH). ^{13}CMR (δ ppm): 136-145 (triazole-thiadiazole), 119-130 (benzene), 48, 143, 156 (β-lactam), 119 (-C-O-H), 166 (C of CO).

IV-3-chloro- [1-(3,6-(diphenyl) [1,2,4] triazolo [3,4-b] [1,3,4] thiadiazole)]-4-(2-hydroxy phenyl-azetidin-2-one (8d).

I.R. (KBr, cm^{-1}): 3030, 1500, 1600 (aromatic C-H, stretching), 1690 (C=O of β-lactam), 3200-2600 (-OH phenolic). PMR (δ ppm): 6.2-7.9 (m, aromatic C_4H of β-lactam), 10.4 (1H, CH_3 of β-lactam), 3.9 (H of OH). ^{13}CMR (δ ppm): 136-145 (triazole-thiadiazole), 114-130 (benzene), 48, 143, 156 (β-lactam), 135 (C-OH), 165 (C of CO).

V- 3-chloro- [1-(3,6-(diphenyl) [1,2,4] triazolo [3,4-b] [1,3,4] thiadiazole)]-4-(4-methoxy phenyl-azetidin-2-one (8e).

I.R. (KBr, cm^{-1}): 3030, 1500, 1600 (aromatic C-H), 1690 (C=O of β-lactam), 2950, 1370 (-CH_3). PMR (δ ppm): 6.2-7.9 (m, aromatic C_4H of β-lactam), 10.4 (1H, CH_3 of β-lactam), 2.1 (3H, CH_3). ^{13}CMR (δ ppm): 136-148 (triazole-thiadiazole), 114-130 (benzene), 48, 144, 156 (β-lactam), 24.65 (CH_3).

VI-3-chloro- [1-(3,6-(diphenyl) [1,2,4] triazolo [3,4-b] [1,3,4] thiadiazole)]-4-(3,4-methlenedioxy phenyl-azetidin-2-one (8f).

I.R. (KBr, cm^{-1}): 3030, 1500, 1600 (aromatic C-H), 1690 (C=O of β-lactam), 1200 (Ar-O-alkyl). PMR (δ ppm): 6.2-7.9 (m, aromatic C_4H of β-lactam), 10.4 (1H, CH_3 of β-lactam), 5.8 (2H of O-CH_2-O). ^{13}CMR (δ ppm): 136-145 (triazole-thiadiazole), 114-130 (benzene), 48, 144, 156 (β-lactam), 135 (C-OH), 165 (C of CO), 91 (O-CH_2-O).

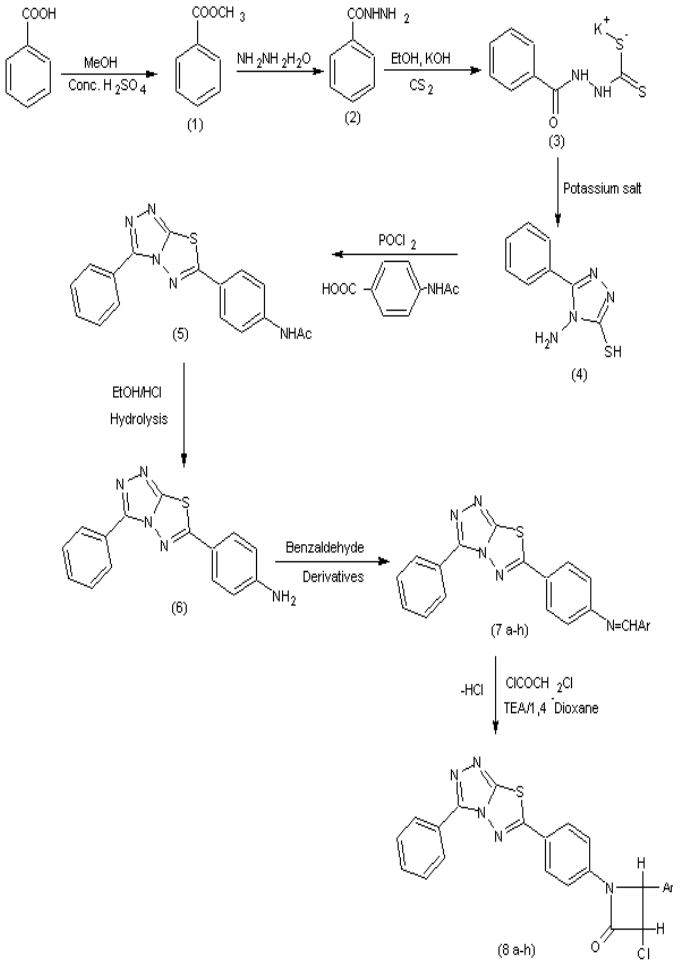
VII-3-chloro- [1-(3,6-(diphenyl) [1,2,4] triazolo [3,4-b] [1,3,4] thiadiazole)]-4-(4-hydroxy-3-methoxy phenyl-azetidin-2-one (8g).

I.R. (KBr, cm^{-1}): 3030, 1500, 1600 (aromatic C-H), 1690 (C=O of β-lactam), 3200-2600 (-OH phenolic), 1200 (aryl-alkyl ether). PMR (δ ppm): 6.1-7.9 (m, aromatic C_4H of β-lactam), 10.4 (1H, CH_3 of β-lactam), 3.36 (1H of OH), 4.3 (3H, s, -OCH_3).

^{13}CMR (δ ppm): 136-145 (triazole-thiadiazole), 114-130 (benzene), 48, 144, 156 (β-lactam), 135 (C-OH), 165 (C of CO), 56 (O-CH_3).

VIII-3-chloro- [1-(3,6-(diphenyl) [1,2,4] triazolo [3,4-b] [1,3,4] thiadiazole)]-4-(3,4-diethoxy phenyl-azetidin-2-one (8h).

I.R. (KBr, cm^{-1}): 3030, 1500, 1600 (aromatic C-H), 1690 (C=O of β-lactam), 1200 (aryl-alkyl ether), 2920, 2850,1450 (-CH_2-), 2950, 1370 (-CH_3-). PMR (δ ppm): 6.2-7.9 (m, aromatic C_4H of β-lactam), 10.4 (1H, CH_3 of β-lactam), 2.9-3.3 (4H of 2 CH_2), 2.5 (6H of 2 CH_3). ^{13}CMR (δ ppm): 136-145 (triazole-thiadiazole), 114-130 (benzene), 48, 144, 156 (β-lactam), 135 (C-OH), 165 (C of CO), 135 (C-O), 65 & 14 (CH_2 & CH_3).



Reaction Scheme

ANTIMICROBIAL ACTIVITY

Antimicrobial activity was carried out by cup-plate agar diffusion method which has been described as under. The purified products were screened for their antibacterial activity. Newly synthesized compound (8a-h) have been tested their antibacterial activity against *Staphylococcus aureus* (Gram positive), *Bacillus subtilis* (Gram positive), *Psuedomonas aeruginosa* (Gram negative) and *Escherichia coli* (Gram negative) by the help of borer in agar medium and filled with 0.04ml (40μg) solution of sample in DMF and Amoxicillin, Benzoylpenicillin, Ciprofloxacin, Erythromycin, Greseofulvin were used as a reference compound.Which are recorded in table-2. The compound 8b, 8d, 8f and 8h were shown significant activities and compound 8a, 8c, 8e, and 8g have shown moderate activity. The plates were incubated at 37^{o}C for 24 hours and the control was also maintained with 0.04 ml of DMF in a similar manner and the zones of inhibition of the bacterial growth were measured in millimeter and are recorded data in table-3.

Table. 2: Antibactarial activity of standards and solvent (DMF).

Compounds	Zone of Inhibition in (mm)			
	Gram Positive		Gram Negative	
	B. Subtillis	S. Aureus	E. Coli	Ps. Aeruginosa
DMF	06	05	05	05
Ampicillin	19	15	20	21
Tetracycline	21	20	15	18
Gentamycine	20	18	19	22
Chloramphenicol	20	23	18	24

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Tetracycline	21	20	15	18
Gentamycine	20	18	19	22
Chloramphenicol	20	23	18	24

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