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SYNTHESIS AND CHARACTERIZATION OF NOVEL SPIROAZETIDIN-2-ONES TETHERED WITH FURANS AS ANTIMICROBIAL AND ANTIINFLAMMATORY AGENTS

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ABSTRACT

Present investigation describes about the synthesis of some novel spiroazetidinones tethered with furans. These compounds were designed, based on the structure of β-lactam, which shows antimicrobial activity. Some new 1-(substituted phenyl)-3-chloro-5,9-bis(furan-2-ylmethylidene)-1-azaspiro[3.5]nonan-2-ones (3a - 3h) were synthesized by Staudinger's ketene–imine reaction. The structures of the newly synthesized compounds were confirmed on the basis of physical, IR, H^1 NMR, mass and elemental analysis data. All the synthesized compounds were screened for antibacterial, antifungal and invitro anti-inflammatory activities and the results of some of the derivatives exhibited promising activities. The zone of inhibition values of the compound 3h showed significant antibacterial activity, compounds 3f and 3g showed highest antifungal activity and remaining compounds had shown good antimicrobial activity. Highest invitro anti-inflammatory activity was observed with compounds 3c derivative possessing electron withdrawing substituents on phenyl ring.

KEYWORDS: Spiro, azetidinones, furan, antibacterial activity, antifungal activity, invitro anti-inflammatory activity.

INTRODUCTION

2-Azetidinones, commonly known as beta-lactams, are well-known heterocyclic compounds among the organic and medicinal chemists.[1] The activity of the famous antibiotics such as penicillin, cephalosporin, monobactams and carbapenems are attributed to

the presence of 2-azetidinone ring in them. Azetidin-2-ones can be prepared from Schiff's bases, which are the condensation products of aldehydes and amines. They are considered significant owing to their wide range of biological application. Recently, some other types of biological activity besides the antibacterial activity have been reported in compounds containing 2-azetidinone ring. Such biological activities include anti-fungal, anti-inflammatory, anti-tubercular, anti-convulsant, anthelmenthic, hypoglycemic, hypnotic, insecticidal, anti-parkinsonism etc.[2-7]

Furan belongs to the five membered heterocycles. Furan had proved to be the most useful framework for biological activities. Furans have attracted attention of medicinal chemists for both with regard to heterocyclic chemistry and the pharmacological activities associated with them. The pharmaceutical importance of these compounds lies in the fact that they can be effectively utilized as antimicrobial like antibacterial or antifungal or antiviral, anti-inflammatory, antidepressant, antianxiolytic, analgesic, antihypertensive, muscle relaxant, anti-arrhythmic, antiglaucoma activity, anti-ulcer, antidiuretic, anorectic, antiageing, antiparkinsonism, antihistaminic, anticholinergic, antineoplastic, insecticide, in the treatment of sickle cell aneamia etc.[8-10] Encouraged by these facts, we selected to work on Spiroazetidin-2-one containing furan with different substitutions on the phenyl ring.

In the present study we report the reaction of N-[2,6-bis(furan-2-ylmethylidene)cyclohexylidene]substituted aniline with chloroacetyl chloride to form spiroazetidinones tethered with furans (3a - 3h). The structures of the various synthesized compounds were assigned on the basis of IR, ^{1}H-NMR, mass spectral data and elemental analysis. These compounds were also screened for their antibacterial, antifungal and invitro anti-inflammatory activity.

MATERIALS AND METHODS

The structures of new compounds prepared during present investigation have been authentically established by their Melting point, TLC, IR, NMR, mass spectral studies and by Elemental analysis. Melting points (^{o}C) were determined on Theils tube by open capillary method and are triplicates. The IR spectra were recorded on Schimadzu FTIR 8400S spectrophotometer by using 1% KBr discs. 1H NMR spectra was recorded on AMX-400 MHz spectrometer using DMSO-d6 as solvent and TMS as an internal standard. Mass spectra on model LCMS-2010 A, Shimadzu, Japan and elemental analysis reports of compounds

were obtained from Instrument – FLASH EA 1112 Series Thermo finnigan. TLC was done using silica gel and visualization was accomplished with UV light (256 nm).

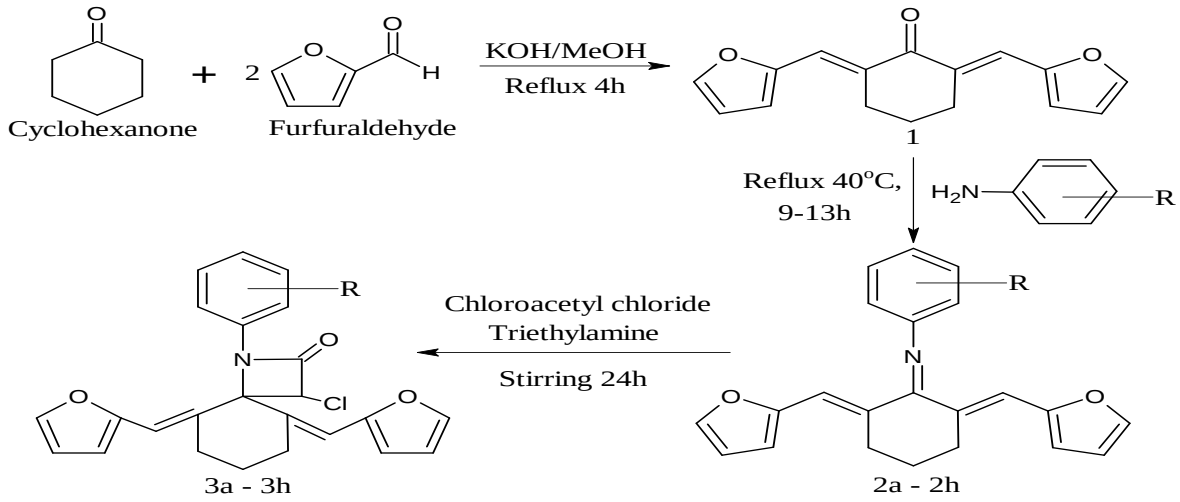

Fig. 1 Synthetic scheme for the preparation of spiroazetidin-2-one tethered with furans 3a - 3h

Procedure for synthesis of 2,6-bis(furan-2-ylmethylidene)cyclohexanone (1):

The mixture of cyclohexanone (0.1 mol, 9.8 g or 10.34 ml), fufuraldehyde (0.2 mol, 19.21 g or 16.58 ml) and potassium hydroxide (0.2 mol, 11.22 g) in 100ml of methanol was taken in a 500ml RBF and the reaction mixture was refluxed for 4hrs. The precipitate obtained was filtered, dried and recrystallized from aqueous ethanol.

General procedure for synthesis of N-[2,6-bis(furan-2-ylmethylidene)cyclohexylidene]substituted aniline (2a - 2h):

2,6-bis(furan-2-ylmethylidene)cyclohexanone (0.01 mol, 2.54 g) and 30 ml methanol were taken in a round bottom flask. Substituted anilines (0.01mol) was dissolved in 30 ml methanol and added slowly for about 20 min with vigorous stirring, maintaining the temperature at 40-50˚C. 1ml of glacial acetic acid was added and allowed to reflux for further 9-13 hrs (depending on the substituted aniline) at 40˚C. The mixture was poured into 250 ml ice-cold water and stirred. The precipitate obtained was filtered, dried and recrystallized from aqueous methanol.

General procedure for synthesis of 1-(substituted phenyl)-3-chloro-5,9-bis(furan-2-ylmethylidene)-1-azaspiro[3.5]nonan-2-one (3a - 3h):

A solution of Chloroacetyl chloride (0.01mol, 1.12 g or 0.79 ml) in 1,4-Dioxan was added drop wise to a well stirred solution of N-[2,6-bis(furan-2-

ylmethylidene)cyclohexylidene]substituted aniline (0.01mol) and Triethylamine (0.02mol, 2.02 g or 2.8 ml) in 1,4-Dioxan. The solution was then stirred for 24 hrs. The reaction mixture was added to ice cold water. The solid separated was filtered and purified from aqueous 1,4-Dioxane.

3-chloro-5,9-bis(furan-2-ylmethylidene)-1-phenyl-1-azaspiro[3.5]nonan-2-one (3a):

Dark yellow amorphous powder, m.p. 125°C, yield, 91.60 %. IR [KBr] cm^{-1} 565.10 (C-Cl),1741.60 (C=O of lactam), 2867.95 (Aliphatic CH), 3082.04 (Aromatic CH); 1HNMR (400 MHz, DMSO-d6):δ1.6-2.0 (6H, CH aliphatic), 4.8 (1H, CHCl β-lactam), 6.0 (2H, CH alkene), 6.6-7.6 (11H, CH aromatic); Mass: m/z:405 (M^+), 407 (M+2), 329, 76 and 67; Elemental analysis: C 70.08%, H 5.03%, N 3.57%.

1-(4-nitrophenyl)-3-chloro-5,9-bis(furan-2-yl methylidene)- 1-azaspiro[3.5]nonan-2-one (3b):

Light green colour crystals, m.p. 117°C, yield, 78.66%. IR [KBr] cm^{-1} 580.53 (C-Cl),1745.46 (C=O of lactam), 860.19 (Nitro C-N), 2867.96 (Aliphatic CH), 3051.18 (Aromatic CH); ^{1}HNMR (400 MHz, DMSO-d6):δ1.6-2.2(6H, CH aliphatic), 4.8 (1H, CHCl β-lactam), 6.0 (2H, CH alkene), 6.6-8.2 (10H, CH aromatic); Mass: m/z: 451 (M+1), 452 (M+2), 254, 241, 216 and 124; Elemental analysis: C 63.85%, H 4.29%, N 6.32%.

3-chloro-1-(4-chlorophenyl)-5,9-bis(furan-2-ylmethylidene) -1-azaspiro[3.5]nonan-2-one (3c):

Brown amorphous powder, m.p. 109°C, yield, 86.81%. IR [KBr] cm^{-1} 597.89 (C-Cl),1741.60 (C=O of lactam), 2925.81 (Aliphatic CH), 3055.03 (Aromatic CH); ^{1}HNMR (400 MHz, DMSO-d6):δ1.6-2.0 (6H, CH aliphatic), 4.8 (1H, CHCl β-lactam), 6.0 (2H, CH alkene), 6.6-7.8 (10H, CH aromatic); Mass: m/z: 440 (M^+), 442 (M+2), 444 (M+4), 366, 76 and 67; Elemental analysis: C 65.54%, H 4.39%, N 3.13%.

1-(4-bromophenyl)-3-chloro-5,9-bis(furan-2-ylmethylidene)-1-azaspiro[3.5]nonan-2-one (3d):

Dark yellow amorphous powder, m.p. 127°C, yield, 64.66%. IR [KBr] cm^{-1} 594.03 (C-Br), 750.26 (C-Cl),1737.74 (C=O of lactam), 2939.31 (Aliphatic CH), 3035.75 (Aromatic CH); ^{1}HNMR (400 MHz, DMSO-d6):δ1.6-2.0 (6H, CH aliphatic), 4.8 (1H, CHCl β-lactam), 6.0 (2H, CH alkene), 6.6-7.6 (10H, CH aromatic); Mass: m/z: 486 (M+2) & 484 (M^+), 488(M+4), 408, 410, 76 and 67; Elemental analysis: C 59.54%, H 3.92%, N 2.86%.

1-(2-nitrophenyl)-3-chloro-5,9-bis(furan-2-ylmethylidene)-1-azaspiro[3.5]nonan-2-one (3e):

Green amorphous powder, m.p. 119°C, yield, 93.11%. IR [KBr] cm^{-1} 594.03 (C-Cl),1747.39 (C=O of lactam), 881.41 (Nitro C-N), 2941.24 (Aliphatic CH), 3051.18 (Aromatic CH); ^{1}HNMR (400 MHz, DMSO-d6):δ1.6-2.0 (6H, CH aliphatic), 4.8 (1H, CHCl β-lactam), 6.0 (2H, CH alkene), 6.6-8.0 (10H, CH aromatic); Mass: m/z: 450 (M^{+}), 452 (M+2), 374, 76 and 67; Elemental analysis: C 63.97%, H 4.31%, N 6.18%.

3-chloro-1-(3,4-dichlorophenyl)-5,9-bis(furan-2-ylmethylidene) -1-azaspiro[3.5]nonan-2-one (3f):

Pale brown amorphous powder, m.p. 123°C, yield, 64.13%. IR [KBr] cm^{-1} 572.82 (C-Cl),1758.96 (C=O of lactam), 2867.96 (Aliphatic CH), 3043.46 (Aromatic CH); ^{1}HNMR (400 MHz, DMSO-d6):δ1.6-2.0 (6H, CH aliphatic), 4.8 (1H, CHCl β-lactam), 6.0 (2H, CH alkene), 6.6-7.8 (9H, CH aromatic); Mass: m/z: 474 (M^{+}), 476 (M+2), 478(M+4), 480 (M+6), 400, 402, 76 and 67; Elemental analysis: C 60.68%, H 3.86%, N 3.01%.

3-chloro-1-(4-fluorophenyl)-5,9-bis(furan-2-ylmethylidene) -1-azaspiro[3.5]nonan-2-one (3g):

Pale brown amorphous powder, m.p.111°C, yield, 71.39%. IR [KBr] cm^{-1} 597.89 (C-Cl),1745.46 (C=O of lactam), 1091.63 (C-F), 2918.10 (Aliphatic CH), 3041.53 (Aromatic CH); ^{1}HNMR (400 MHz, DMSO-d6):δ1.6-2.0 (6H, CH aliphatic), 4.8 (1H, CHCl β-lactam), 6.0 (2H, CH alkene), 6.6-7.6 (10H, CH aromatic); Mass: m/z: 423 (M^{+}), 425 (M+2), 347, 76 and 67; Elemental analysis: C 67.98%, H 4.63%, N 3.24%.

3-chloro-1-(2,6-dichlorophenyl)-5,9-bis(furan-2-ylmethylidene)-1-azaspiro[3.5]nonan-2-one (3h):

Green amorphous powder, m.p 119°C, yield, 78.27%. IR [KBr] cm^{-1} 597.89 (C-Cl),1747.39 (C=O of lactam), 2939.31 (Aliphatic CH), 3037.68 (Aromatic CH); ^{1}HNMR (400 MHz, DMSO-d6):δ1.6-2.0 (6H, CH aliphatic), 4.8 (1H, CHCl β-lactam), 6.0 (2H, CH alkene), 6.6-7.6 (9H, CH aromatic); Mass: m/z: 474 (M^{+}), 476 (M+2), 478(M+4), 480 (M+6), 400, 402, 76 and 67; Elemental analysis: C 60.64%, H 3.78%, N 2.74%.

RESULTS AND DISCUSSION

Chemistry

The synthetic methodology followed to obtain the target compounds is outlined in the figure 1. In the first step, Claisen-Schmidt condensation was performed by treating Cyclohexanone with Furfuraldehyde in the presence of potassium hydroxide in methanol for 4hrs to get 2,6-bis(furan-2-ylmethylidene)cyclohexanone **(1),** which on condensation with different aromatic anilines at 40^0C for 9-13 hrs furnished N-[2,6-bis(furan-2-ylmethylidene)cyclohexylidene]substituted aniline (2a - 2h)**.** β-lactam moiety was introduced in the compounds (2a - 2h), by addition of chloroacetyl chloride in 1,4-Dioxan was added drop wise to a well stirred solution of compounds (2a - 2h) and Triethylamine in 1,4-Dioxan, solution was then stirred for 24 hrs to yield 1-(substituted phenyl)-3-chloro-5,9-bis(furan-2-ylmethylidene)-1-azaspiro[3.5]nonan-2-one (3a - 3h) in reasonable yields**.**

The synthesized compounds were confirmed on the basis of spectral data. The 1H NMR spectrum of compound 3a showed a singlet at δ 4.9 due to the presence of CHCl proton due to the presence of lactam which was further confirmed by singlet at δ 6.1 indicates two methylidene protons attached to CH. A singlet at δ 1.6 and δ 2.1 due to presence of six aliphatic CH protons and 11 aromatic protons appeared in the range of δ 6.6-7.6, thus confirmed the structure of the compound 5a. Moreover, the mass spectrum of compound revealed a molecular ion peak at *m/z* 405(M^+).The structure was further supported by Elemental analysis data. In IR spectrum, the disappearance of imine absorption peak and the appearance of lactam stretching at 1741 cm^{-1} further confirmed the formation of the compound. Similarly the structures of other compounds were confirmed on the basis of IR, H^1 NMR, mass spectral and elemental analysis data.

Anti bacterial screening

All the synthesized compounds were screened for their antibacterial activity against two gram positive bacteria (*Bacillus subtilis, Staphylococcus aureus*) and two gram negative bacteria (*Escherichia coli, Pseudomonas aeruginosa*) with Amoxicillin and Ciprofloxacin as reference standards. The study was carried out by cup-plate method [11-13] to determine the zone of inhibition (mm) against four strains of bacteria. Antibacterial activity was carried out at a concentration 200 µg/ml. Further investigation of antibacterial data revealed that compounds 3h had showed highest activity and all the remaining compounds have shown good activity against both gram positive and gram negative strains as shown in table 1.

Table 1. Anti bacterial data of compounds 3a-3h.

Compound	Concentration (µg/ml)	Zone of inhibition in (mm)			
		S.aureus	***B.subtilis***	***P.aeruginosa***	***E.coli***
Control (DMF)	--	--	--	--	--
Amoxicillin	**20**	**24**	**26**	**25**	**32**
Ciprofloxacin	**20**	**33**	**36**	**32**	**38**
3a	200	20	16	17	16
3b	200	18	16	16	**17**
3c	200	**22**	14	16	13
3d	200	17	16	17	16
3e	200	18	14	14	15
3f	200	18	16	16	15
3g	200	17	17	17	**17**
3h	200	**22**	**18**	**35**	16

Anti fungal screening

All the synthesized compounds were screened for their antifungal activity against two fungal strains namely *Aspergillus niger* and *Candida albicans* with Fluconazole and Ketoconazole as reference standards. The study was carried out by cup-plate method to determine the zone of inhibition (mm) against two fungal strains. Antifungal activity was carried out at a concentration 200 µg/ml. Further investigation of antifungal data revealed that compounds 3f and 3g had showed highest activity against *C.albicans* and *A. niger* respectively and all the remaining compounds have shown good activity against both fungal strains as shown in the table 2.

Table 2. Anti fungal data of compounds 3a-3h.

Compound	Concentration (µg/ml)	Zone of inhibition (mm)	
		C. albicans	***A.niger***
Control	--	--	--
Fluconazole	**10**	**32**	**19**
Ketoconazole	**20**	**39**	**24**
3a	200	30	21
3b	200	35	25
3c	200	31	24
3d	200	31	24
3e	200	33	22
3f	200	**40**	24
3g	200	36	**27**
3h	200	37	30

Invitro Anti inflammatory activity (Inhibition of albumin denaturation)

All the synthesized compounds were screened for their anti-inflammatory activity by using inhibition of albumin denaturation technique which was studied according to Elias G et al.[14]

The reaction mixture was consists of test solution (1ml) containing different concentration of drug was mixed with 1ml of 1mg/ml albumin solution in phosphate buffer and incubated at $27^{o}\pm1^{o}C$ for 15 min. Denaturation was induced by keeping the reaction mixture at $60^{o}\pm1^{o}C$ in water bath for 10-20 min. After cooling, the turbidity was measured at 660 nm (UV -Visible Spectrophotometer Shimadzu Model 1601, Japan). The experiment was performed in triplicate.

The Percentage inhibition of protein denaturation was calculated as follows: Percentage inhibition =(Abs Control –Abs Sample) X 100/ Abs control.

Further investigation of anti-inflammatory data revealed that compounds 3c had showed highest activity and all the remaining compounds have shown good invitro anti-inflammatory activity as shown in the table 3.

Table 3. Anti - inflammatory data of compounds 3a-3h.

Compound	Inhibition of denaturation(%)	Concentration (mg/ml)					
		Blank	0.2	0.4	0.6	0.8	1.0
Ibuprofen		0	17.26	32.37	47.48	62.58	77.69
3a		0	17.26	28.77	35.25	46.47	52.51
3b		0	12.94	23.74	35.97	42.44	54.67
3c		0	20.14	25.89	50.35	50.35	**61.87**
3d		0	10.79	21.58	46.76	50.35	53.95
3e		0	3.59	8.63	41.72	42.44	44.60
3f		0	10.79	21.58	34.53	42.44	48.20
3g		0	5.03	13.66	23.74	35.97	47.48
3h		0	7.19	9.35	42.44	48.20	48.16

CONCLUSION

In this investigation, a new series of Spiroazetidin-2-ones (**3a - 3h**) have been synthesized by Straudinger ketene-imine reaction and the resulting compounds were characterized on the basis of spectral data. All the compounds were evaluated for antibacterial, antifungal and invitro anti-inflammatory activities. Some of them have exhibited potent anti-inflammatory activity, antifungal and anti - bacterial activity against both gram positive and gram negative organisms. This study suggests that Spiroazetidin-2-ones may serve as promising scaffolds for design of new antimicrobial and anti-inflammatory drugs.

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