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Research Article

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Synthesis, characterization and biological activity of 1,6,7-triazaspiro-thiazolidine, tetrazoles and azetidines

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ABSTRACT

New novel derivatives of 3-chloro-6-(2,5-difluorobenzoyl)-8-(2-(4-substituted phenyl)-4-oxothiazolidin-3-yl)-1-((4-(trifluoromethyl)phenyl)amino)-1,6,7-triazaspiro[3.4]oct-7-ene-2,5-dione (10 a-f) were synthesized when 3-chloro-6-(2,5-difluorobenzoyl)-8-((4-substituted benzylidene) amino)-1-((4-(trifluoromethyl) phenyl)amino)-1,6,7-triazaspiro[3.4]oct-7-ene-2,5-dione (9 a-f) and marcapto acetic acid were refluxed in dioxane in presence of anhydrous Zinc Chloride. The synthesis of 3-chloro-6-(2,5-difluorobenzoyl)-8-(5-(4-substituted phenyl)-1H-tetrazol-1-yl)-1-((4-(trifluoromethyl) phenyl) amino)-1,6,7-triazaspiro[3.4]oct-7-ene-2,5-dione(11 a-f) were synthesized by refluxing a mixture of 3-chloro-6-(2,5-difluorobenzoyl)-8-((4-substituted benzylidene)amino)-1-((4-(trifluoromethyl)phenyl)amino)-1,6,7-triazaspiro[3.4]oct-7-ene-2,5-dione (9 a-f), sodium azide and PCl_5. The novel 3-chloro-8-(3-chloro-2-(4-substituted phenyl)-4-oxoazetidin-1-yl)-6-(2,5-difluoro benzoyl)-1((4(trifluoromethyl)phenyl)amino)-1,6,7-triazaspiro[3.4]oct-7-ene-2,5-dione(12a-f) were synthesized by refluxing a mixture of 3-chloro-6-(2,5-difluorobenzoyl)-8-((4-substituted benzylidene) amino)-1-((4-(trifluoromethyl) phenyl)amino)-1,6,7-triazaspiro [3.4]oct-7-ene-2,5-dione (9 a-f) and mono chloro acetyl chloride in di chloro methane. The newly synthesized compounds were characterized by IR, 1H-NMR, 13C-NMR, mass spectra & Elemental analysis. The newly synthesized compounds were screened for their Biological activity.

Key words: Thiazolidine, Tetrazole, Azitidine, antibacterial and anti-fungal activity.

INTRODUCTION

Hetero cyclic compounds represent an important class of biological active molecules specifically those containing the pyrazolone nucleus besides azaspiro, Thiazolidine and Tetrazole .rings have been shown to possess high biological activities [1-12] such as anti-tuberculosis, anti-neoplastic, anti-fertility and anti-hydro thyroid activity. The derivatives of pyrazolone-5-ones are important class of nitrogen hetero cycles, they found to possess tranquillizing, muscle relaxant, Psycho analeptic, anti convulsing, anti hypertensive, antidepressant, antipyretic and analgesic reactivates

2-Azetidinones and 4-thiazolidinones are the most common and important groups among the small ring hetero cyclic compounds.2-Azetidinones, commonly known as β-lactums, are the derivatives of azetidines with carbonyl group at 2^{nd}-position.The activity of the famous antibiotics such as penicillin, cephalosporin and carbapenems are attributed to the presence of 2- azetidinone ring in them. A large number of 3-chloromonocyclic β-lactum possesses powerful antibacterial [13], antifungal [14], anti-inflammatory [15], anti-tubercular [16], anticonvulsant [17], and analgesic [18], and cholesterol inhibitory activities [19]. 4-Thiazolidinones are the derivatives of Thiazolidines with carbonyl group at the 4^{th} – position and the compounds exhibited various biological activities such as antibacterial [20], antifungal[21], antioxidant[22], cytotoxic[23], analgesic[24], anti-inflammatory[25], anticonvulsant [26], anticancer [27], anti-HIV [28], ant tubercular [29], and anthelmintic activities [30].

In view of the importance of the above Hetero cycles we planned to synthesize System and Spiro-5on Thiazolidine-4-one(10 a-f) ,Spiro-pyrazolone Tetrazole(11 a-f) and Spiro-pyrazolone azitidine-2-one(12 a-f) ring System.

EXPERIMENTAL SECTION

All the chemicals used in the present investigation were purchased from Sigma-Aldrich Chemicals Company, Inc.USA. And used without further purification. TLC was performed on aluminum sheet of silica gel 60F254, E-Merk, Germany using iodine as visualizing agent. Melting point was determined in open capillary tubes on Mel-Tempapparatus and is uncorrected. Column chromatography was performed on silica gel with different solvent systems as eluents to afford the pure compound. The IR Spectra were recorded as KBr pellets on Perkin-Elmer 1000 units, instruments. All 1H and 13C-NMR spectra were recorded on a Varian XL-300 spectrometer operating at 400MHzfor 1H -NMR and 75 MHz for ^{13}C-NMR were recorded on a Varian XL-spectrometer operating at161.89MHz. The compounds were dissolved in DMSO-d6 and Chemical shifts were referenced to TMS (1H and^{13}C-NMR) .Mass spectral data was recorded on FAB-MS instrument at 70ev with direct inlet system. Elemental analysis were recorded on a Carlo Erba 1108 elemental Analyzer, Central Drug Research Institute, Lucknow, India.

RESULTS AND DISCUSSION

Synthesis of 3-chloro-6-(2,5-difluorobenzoyl)-8-(2-(4-substituted phenyl)-4-oxothiazolidin-3-yl)-1-((4-(trifluoro methyl)phenyl)amino)-1,6,7-triazaspiro[3.4]oct-7-ene-2,5-dione ***(10 a-f)***

A mixture of 3-chloro-6-(2,5-difluorobenzoyl)-8-((4-substituted benzylidene)amino)-1-((4-(trifluoromethyl) phenyl)amino)-1,6,7-triazaspiro[3.4]oct-7-ene-2,5-dione (9 a-f) (0.01 mol,) and Marcapto acetic acid (0.01 mol) dissolved in dioxane (20 ml), to the reaction mixture anhydrous zinc chloride (0.5 mg) was added and refluxed for 8 hrs.The progress of the reaction was monitored by TLC using cyclo hexane and ethyl acetate (7:3) solvent mixture. The reaction mixture was cooled and the resulting solid washed with sodium bi carbonate solution and recrystallized from absolute alcohol to affored3-chloro-6-(2,5-difluorobenzoyl)-8-(2-(4-substituted phenyl)-4-oxothiazolidin-3-yl)-1-((4-(trifluoromethyl)phenyl)amino)-1,6,7-triazaspiro[3.4]oct-7-ene-2,5-dione (10 a-f).The structure of 10 (a-f) was established by IR, ^{1}H-NMR, ^{13}C-NMR, Mass and Elemental analysis

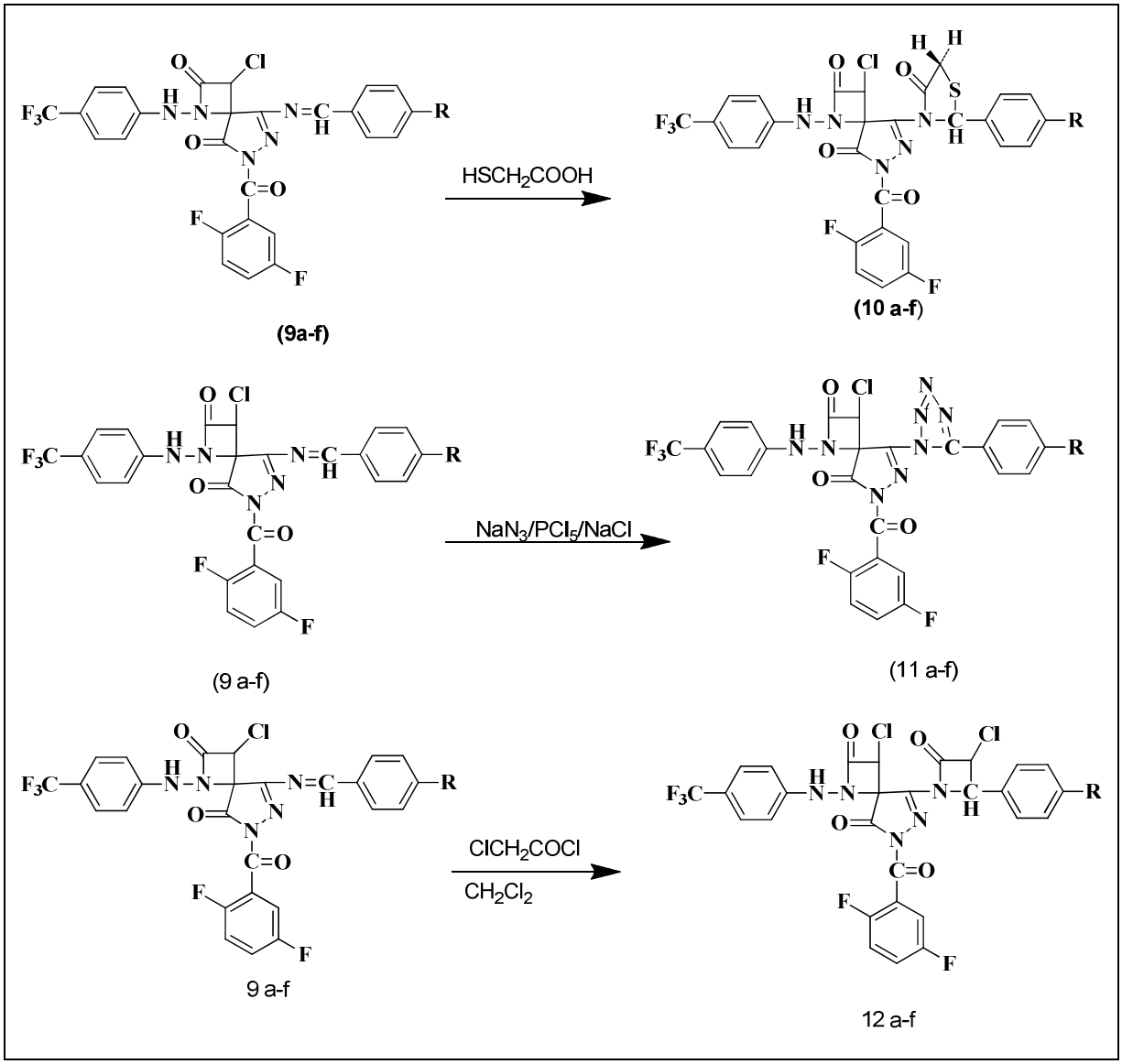
Synthesis of 3-chloro-6-(2,5-difluorobenzoyl)-8-(5-(4-substituted phenyl)-1H-tetrazol-1-yl)-1-((4-(trifluoromethyl) phenyl)amino)-1,6,7-triazaspiro[3.4]oct-7-ene-2,5-dione ***(11 a-f)***

A mixture of 3-chloro-6-(2,5-difluorobenzoyl)-8-((4-substituted benzylidene)amino)-1-((4-(trifluoromethyl) phenyl)amino)-1,6,7-triazaspiro[3.4]oct-7-ene-2,5-dione (9 a-f) (0.004 mol) and PCl_5 (0.004 mol) was heated at 100^0C for 1hour, when the evolution of fumes of HCl ceased, excess of PCl_3 was removed under reduced pressure. The residual imidoyl chloride was treated with an ice-cold solution of sodium azide (0.0075 mol) and excess of sodium acetate (0.008 mol)in water (25 ml) and acetone (30 ml). The reaction mixture was stirred for 20 hours. The progress of the reaction was monitored by TLC using cyclohexane and ethyl acetate solvent mixture (7:3).After completion of the reaction, the acetone was removed under reduced pressure. The remaining reaction mixture was extracted with chloroform (3x20 ml). The chloroform extract was dried under reduced pressure and the residue purified by column chromatography using silica (60-120 mesh) and cyclo hexane and ethyl acetate solvent mixture(7:3) as an elutent. The structure of 11 a-f was established by IR,1H-NMR, 13C-NMR, Mass spectra data and Elemental Analysis

*Synthesis of 3-chloro-8-(3-chloro-2-(4-substituted phenyl)-4-oxoazetidin-1-yl)-6-(2,5-difluorobenzoyl)-1((4(trifluoromethyl)phenyl)amino)-1,6,7-triazaspiro[3.4]oct-7-ene-2,5-dione****(12a-f)***

Monochloro acetyl chloride (0.01 mol)was added drop wise to 3-chloro-6-(2,5-difluorobenzoyl)-8-((4-substituted benzylidene)amino)-1-((4-(trifluoromethyl)phenyl)amino)-1,6,7-triazaspiro[3.4]oct-7-ene-2,5-dione (9 a-f), in di chloro methane the reaction mixture was stirred for 8 hours at room temperature. The progress of the reaction was monitored by TLC using cyclohexane and ethyl acetate (7:3) solvent mixture. The reaction mixture left for 3 days at room temperature. The excess of solvent was removed under vacuum by Rota evaporator. After solvent was removed the residue was purified by 60-120 mesh silica gel using cyclohexane-ethyl acetate solvent mixture as an elutent The reaction mixture was poured in crushed ice to yield3-chloro-8-(3-chloro-2-(4-substituted phenyl)-4-oxoazetidin-1-yl)-6-(2,5-difluorobenzoyl)-1((4(trifluoromethyl)phenyl)amino)-1,6,7-triazaspiro[3.4]oct-7-ene-2,5-dione(12a-f)

The structure of **(**12 a-f) was established by IR,1H-NMR, Mass spectra and Elemental analysis.



COMP NO	10a,11a,12a	10b,11b,12b	10c,11c,12c	10d,11d,12d	10e,11e,12e	10f,11f,12f
R	-H	$-CH_3$	$-OCH_3$	-Cl	-Br	$-NO_2$

Physical, analytical and spectral data for the compounds:

3-chloro-6-(2,5-difluorobenzoyl)-8-(4-oxo-2-phenylthiazolidin-3-yl)-1-((4-(trifluoromethyl)phenylamino)-1,6,7-triazaspiro[3.4]oct-7-ene-2,5-dione ***(10a)***

Yield:65%.m p: 141-143^{O}C. IR (KBr): 3225(stretching vibration of -NH), 1620(stretching vibration of >C=N), 1675(stretching vibration of cyclic carbonyl in pyrazoline-5-one ring), 1656(Exo cyclic >C=O group), 677(stretching vibration of C-Cl group), 1694(stretching vibration of >C=O group of azetidinone),1704(stretching vibration of C=O group of Thiazolidine).^{1}H-NMR (400 MH_Z DMSO-d6): 3.85(ud, 1H, Ha of Thiazolidine ring), 3.95(ud, 1H, Hb of Thiazolidine ring), 5.3(s, 1H, -CH of azetidinone group), 5.92(s, 1H, -CH- of Thiazolidine ring attached to phenyl group), 8.6(s, 1H, Ar-NH-, attached to azaspiro ring), 7.0-7.59(m, 12H, C_6H_5, C_6H_4 and C_6H_3 group).^{13}C-NMR(75MHz, DMSOd6): 154.3, 113.5, 125.6, 126.9, 163.5, 55.2, 75.9, 176.1, 157.0, 170.2, 126.7, 154.9, 118.7, 120.5, 158.6, 113.9, 172.1, 34.0, 56.4, 139.2, 126.9, 128.6, 127.1, 124.1 Corresponding to C_1, C_2 & C_6, C_3 & C_5, C_4, C_7, C_8, C_9, C_{10}, C_{11}, C_{12}, C_{13}, C_{14}, C_{15}, C_{16}, $C_{17,}$ C_{18}, C_{19}, C_{20}, C_{21}, C_{22}, C_{23} & C_{27}, C_{24} & C_{26}, C_{25}, C_{27}, C_{28}. Anal.Calcd. For: $C_{28}H_{17}ClF_5N_5O_4S$ C 53.75%, H 2.96% and N 10.81%. Found: C 53.43%, H 2.44% and N 10.51%.

3-chloro-6-(2,5-difluorobenzoyl)-8-(4-oxo-2-(p-tolyl)thiazolidin-3-yl)-1-((4-(trifluoromethyl)phenylamino)-1,6,7-triazaspiro[3.4]oct-7-ene-2,5-dione ***(10b)***

Yield 65%.m p: 128-130^{O}C. IR (KBr): 3230(stretching vibration of -NH), 1625(stretching vibration of >C=N), 1680(stretching vibration of cyclic carbonyl in pyrazoline-5-one ring), 1661(Exo cyclic >C=O group), 682(stretching vibration of C-Cl group), 1699(stretching vibration of >C=O group of azetidinone),1708(stretching vibration of C=O group of Thiazolidine).^{1}H-NMR (400 MH_Z DMSO-d6): 2.34(s, 3H, CH_3 group), 3.85(ud, 1H ,Ha of Thiazolidine ring), 3.95(ud, 1H, Hb of Thiazolidine ring), 5.3(s, 1H, -CH of azetidinone group), 5.92(s, 1H, -CH- of Thiazolidine ring attached to phenyl group), 8.6(s, 1H, Ar-NH-, attached to azaspiro ring), 7.0-7.59(m, 12H, C_6H_5, C_6H_4 and C_6H_3 group). ^{13}CNMR(75MHz, DMSOd6): 154.3, 113.5, 125.6, 126.9, 163.5, 55.2, 75.9, 176.1, 157.0, 170.2, 126.7, 154.9, 118.7, 120.5, 158.6, 113.9, 172.1, 34.0, 56.4, 136.2, 128.6, 128.9, 136.8, 124.1, 21.3 Corresponding to C_1, C_2 & C_6, C_3 & C_5, C_4, C_7, C_8, C_9, C_{10}, C_{11}, C_{12}, C_{13}, C_{14}, C_{15}, C_{16}, $C_{17,}$ C_{18}, C_{19}, C_{20}, $C_{2\ 1}$, C_{22}, C_{23} & C_{27}, C_{24} & C_{26}, C_{25}, C_{27}, C_{28}, C_{29} Anal. Calcd. For: $C_{29}H_{19}ClF_5N_5O_4S$ C 54.43%, H 3.20% and N 10.58%. Found: C 54.14%, H 3.00% and N 10.18%.

3-chloro-6-(2,5-difluoro benzoyl)-8-(2-(4-methoxy phenyl)-4-oxothiazolidin-3-yl)-1-((4-(trifluoro methyl) phenyl amino)-1,6,7-triazaspiro[3.4]oct-7-ene-2,5-dione ***(10c)***

Yield 65%.m p: 118-120^{O}C. IR (KBr): 3234(stretching vibration of -NH), 1627(stretching vibration of >C=N), 1682(stretching vibration of cyclic carbonyl in pyrazoline-5-one ring), 1668(Exo cyclic >C=O group), 684(stretching vibration of C-Cl group), 1701(stretching vibration of >C=O group of azetidinone),1702(stretching vibration of C=O group of Thiazolidine).^{1}H-NMR (400 MH_Z DMSO-d6): 3.6(s, 3H, -OCH_3 group), 3.85(ud, 1H ,Ha of Thiazolidine ring), 3.95(ud, 1H, Hb of Thiazolidine ring), 5.3(s, 1H, -CH of azetidinone group), 5.92(s, 1H, -CH- of Thiazolidine ring attached to phenyl group), 8.6(s, 1H, Ar-NH-, attached to azaspiro ring), 7.0-7.59(m, 12H, C_6H_5, C_6H_4 and C_6H_3 group). ^{13}CNMR(75MHz, DMSOd6): 154.3, 113.5, 125.6, 126.9, 163.5, 55.2, 75.9, 176.1, 157.0, 170.2, 126.7, 154.9, 118.7, 120.5, 158.6, 113.9, 172.1, 34.0, 56.4, 139.2, 131.5, 129.7, 114.2, 159.0, 124.1, 55.8 Corresponding to C_1,C_2 & C_6, C_3 & C_5, C_4, C_7, C_8, C_9, C_{10}, C_{11}, C_{12}, C_{13}, C_{14}, C_{15}, C_{16}, $C_{17,}$ C_{18}, C_{19}, C_{20}, C_{21}, C_{22}, C_{23} & C_{27}, C_{24} & C_{26}, C_{25}, $C_{27,}$ C_{28}, $C_{29.}$ Anal. Calcd For : $C_{29}H_{19}ClF_5N_5O_5S$ C 53.14% , H 3.12% and N10.33%. Found: C 53.01%, H 3.06% and N 10.03%.

3-chloro-8-(2-(4-chlorophenyl)-4-oxothiazolidin-3-yl)-6-(2,5-difluorobenzoyl)-1-((4-(trifluoromethyl)phenylamino)-1,6,7-triazaspiro[3.4]oct-7-ene-2,5-dione ***(10d)***

Yield 70%.m p: 136-138^{O}C. IR (KBr): 3220(stretching vibration of -NH), 1615(stretching vibration of >C=N), 1670(stretching vibration of cyclic carbonyl in pyrazoline-5-one ring), 1651(Exo cyclic >C=O group), 672(stretching vibration of C-Cl group), 1682(stretching vibration of >C=O group of azetidinone),1698(stretching vibration of C=O group of Thiazolidine). ^{1}H-NMR (400 MH_Z DMSO-d6): 3.85(ud, 1H ,Ha of Thiazolidine ring), 3.95(ud, 1H, Hb of Thiazolidine ring), 5.3(s, 1H, -CH of azetidinone group), 5.92(s, 1H.-CH- of Thiazolidine ring attached to phenyl group), 8.6(s, 1H, Ar-NH-, attached to azaspiro ring), 7.0-7.59(m, 12H, C_6H_5, C_6H_4 and C_6H_3 group). ^{13}CNMR(75MHz, DMSOd6): 154.3, 113.5, 125.6, 126.9, 163.5, 55.2, 75.9, 176.1, 157.0, 170.2, 126.7, 154.9, 118.7, 120.5, 158.6, 113.9, 172.134.0, 56.4, 131.5, 129.7, 128.7, 132.7, 124.1, Corresponding to C_1, C_2 & C_6, C_3 & C_5, C_4, C_7, C_8, C_9, C_{10}, C_{11}, C_{12}, C_{13}, C_{14}, C_{15}, C_{16}, $C_{17,}$ C_{18}, C_{19}, C_{20}, C_{21}, C_{22}, C_{23} & C_{27}, C_{24} & C_{26}, C_{25}, C_{27}, C_{28}. Anal. Calcd. For: $C_{28}H_{16}Cl_2F_5N_5O_4S$ C 51.04% , H 2.06% and N 10.24%. Found: C 50.94%, H 2.01% and N 10.04%.

8-(2-(4-bromophenyl)-4-oxothiazolidin-3-yl)-3-chloro-6-(2,5-difluorobenzoyl)-1-((4-(trifluoromethyl)phenylamino)-1,6,7-triazaspiro[3.4]oct-7-ene-2,5-dione ***(10e)***

Yield 70%.m p: 156-158^{O}C. IR (KBr): 3223(stretching vibration of -NH), 1616(stretching vibration of >C=N), 1671(stretching vibration of cyclic carbonyl in pyrazoline-5-one ring), 1652(Exo cyclic >C=O group), 672(stretching vibration of C-Cl group), 1671(stretching vibration of >C=O group of azetidinone),1700(stretching vibration of C=O group of Thiazolidine).^{1}H-NMR (400 MH_Z DMSO-d6): 3.85(ud, 1H ,Ha of Thiazolidine ring),3.95(ud,1H, Hb of Thiazolidine ring), 5.3(s,1H,-CH of azetidinone group), 5.92(s,1H.-CH- of Thiazolidine ring attached to phenyl group),8.6(s,1H,Ar-NH-,attached to azaspiro ring),7.0-7.59(m,12H,C_6H_5,C_6H_4 and C_6H_3 group).^{13}CNMR(75MHz,DMSOd6):154.3,113.5,125.6,126.9,163.5,55.2,75.9,176.1,157.0,170.2,126.7,154.9,118.7,120.5,158.6,113.9,172.1,34.0,56.4,138.2,130.9,131.6,121.1,124.1CorrespondingtoC$_1$,C$_2$&C$_6$,C$_3$&C$_5$,C$_4$,C$_7$,C$_8$,C$_9$,C$_{10}$,C$_{11}$,C$_{12}$,C$_{13}$,C$_{14}$,C$_{15}$,C$_{16}$,C$_{17,}$C$_{18}$,C$_{19}$,C$_{20}$,C$_{21}$,C$_{22}$,C$_{23}$&C$_{27}$,C$_{24}$&C$_{26}$,C$_{25}$,C$_{27}$C$_{28}$.,Anal.Calcd.For : $C_{28}H_{16}BrClF_5N_5O_4S$ C 47.92% , H 2.50% and N 9.63%. Found: C 47.56% , H 2.23% and N 9.33%.

3-chloro-6-(2,5-difluorobenzoyl)-8-(2-(4-nitrophenyl)-4-oxothiazolidin-3-yl)-1-((4-(trifluoromethyl)phenylamino)-1,6,7-triazaspiro[3.4]oct-7-ene-2,5-dione ***(10f)***

Yield 70%.m p: 172-174^{O}C. IR (KBr): 3215(stretching vibration of -NH), 1611(stretching vibration of >C=N), 1668(stretching vibration of cyclic carbonyl in pyrazoline-5-one ring), 1648(Exo cyclic >C=O group), 668(stretching vibration of C-Cl group), 1687(stretching vibration of >C=O group of azetidinone),1705(stretching

vibration of C=O group of Thiazolidine).^{1}H-NMR (400 MH_Z DMSO-d6): 3.85(ud, 1H ,Ha of Thiazolidine ring),3.95(ud,1H, Hb of Thiazolidine ring), 5.3(s,1H,-CH of azetidinone group), 5.92(s,1H.-CH- of Thiazolidine ring attached to phenyl group),8.6(s,1H,Ar-NH-,attached to azaspiro ring),7.0-7.59(m,12H,C_6H_5,C_6H_4 and C_6H_3 group).^{13}CNMR(75MHz,DMSO-d6): 154.3, 113.5, 125.6, 126.9, 163.5, 55.2, 75.9, 176.1, 157.0, 170.2, 126.7, 154.9,118.7,120.5,158.6,113.9,172.134.0,56.4,139.2,129.6,123.6,146.3,124.1CorrespondingtoC$_1$,C$_2$&C$_6$,C$_3$&C$_5$,C$_4$, C$_7$,C$_8$,C$_9$,C$_{10}$,C$_{11}$,C$_{12}$,C$_{13}$,C$_{14}$,C$_{15}$,C$_{16}$,C$_{17,}$C$_{18}$,C$_{19}$,C$_{20}$,C$_{21}$,C$_{22}$, C$_{23}$ & C$_{27}$, C$_{24}$ & C$_{26}$, C$_{25}$, C$_{27}$, C$_{28,}$ Anal.Calcd. For $C_{28}H_{16}ClF_5N_6O_6S$ C 58.26% , H 2.62% and N12.13%. Found: C 50.06% , H 2.0% and N 11.45%.

Mass Spectra:

The electron impact mass spectrum of 3-chloro-6-(2,5-difluorobenzoyl)-8-(4-oxo-2-phenylthiazolidin-3-yl)-1-((4-(trifluoromethyl)phenylamino)-1,6,7-triazaspiro[3.4]oct-7-ene-2,5-dione (10 a) was recorded and interpreted. The mass spectral data of compound (10a) showed the molecular ion [M^+] ion peak at m/z=649.06(100%) and M+2 peak at m/z=651.03(33%) the relative abundance of M^{7+} and M+2 were in there ratio of 3:1 indicate the presence of one chlorine atom. The odd m/z value of molecular ion (M^{7+}) indicates the presence of odd number of nitrogen atoms in molecular ion.

Table 1: Mass spectral data of primary fragmented ions for 3-chloro-6-(2,5-difluorobenzoyl)-8-(4-oxo-2-phenylthiazolidin-3-yl)-1-((4-(trifluoromethyl)phenylamino)-1,6,7-triazaspiro[3.4]oct-7-ene-2,5-dione (10a)

Molecular ion	Lost radical	Primary fragmented ion	m/z values	Relative abundance (R.A) (%)
$C_{28}H_{17}ClF_5N_5O_4S(M^+)$ m/z =649.06(100%) 651.06(33%)	$C_7H_3F_2O$•	$C_{21}H_{14}ClF_3N_5O_3S$+(II)	508.05 510.02	36.5
	$C_{21}H_{14}ClF_3N_5O_3S$•	$C_7H_3F_2O$+(III)	141.02	7.6
	$C_{22}H_{14}ClF_3N_5O_4S$•	$C_6H_3F_2$+(IV)	113.02	6.5
	$C_6H_3F_2$•	$C_{22}H_{14}ClF_3N_5O_4S$ +(V)	536.04 538.04	30.8
	$C_{22}H_{12}ClF_5N_5O_4S$•	C_6H_5+(VI)	77.04	6.5
	C_6H_5•	$C_{22}H_{12}ClF_5N_5O_4S$(VII)	572.04 574.04	36.9
	C_9H_8NOS•	$C_{19}H_9ClF_5N_4O_3$+(VIII)	471.03 473.03	32.0
	$C_{19}H_9ClF_5N_4O_3$•	C_9H_8NOS+(IX)	178.03	10.7
	$C_{21}H_{12}ClF_2N_4O_4S$•	$C_7H_5F_3N$+(X)	160.04	7.6
	$C_7H_5F_3N$•	$C_{21}H_{12}ClF_2N_4O_4S$+(XI)	489.02 491.02	36.8

The molecular ion signal was obeying nitrogen rule, while the primary fragmented ions derived from molecular ion signal may or may not obey nitrogen rule. The primary fragmented ions undergo fragmentation and forms secondary fragmented ions at different m/z values.

Table 2: Mass spectral data of secondary fragmented ions for 3-chloro-6-(2,5-difluorobenzoyl)-8-(4-oxo-2-phenylthiazolidin-3-yl)-1-((4-(trifluoromethyl)phenylamino)-1,6,7-triazaspiro[3.4]oct-7-ene-2,5-dione (10a)

Primary Fragmented ion	Lost free radical/neutral molecule	Secondary Fragmented ion	m/z values	Relative Abundance (R.A)
$C_{21}H_{14}ClF_3N_5O_3S$+(II)	$C_7H_5F_3N$•	$C_{14}H_9ClN_4O_3S$•+(XII)	348.01 350.01	32.9 9.9
	C_9H_8NOS•	$C_{12}H_6ClF_3N_4O_2$•+(XIII)	330.01 332.01	32.2 10.5
$C_{22}H_{14}ClF_3N_5O_4S$+(IV)	$C_7H_5F_3N$•	$C_{15}H_9ClN_4O_4S$•+(XIV)	376.00 378.00	36.8 12.2
	C_9H_8NOS•	$C_{13}H_6ClF_3N_4O_3$•+(XV)	358.01 360.01	33.7 10.9
$C_{22}H_{11}ClF_5N_5O_4S$+(VII)	$C_7H_3F_2O$•	$C_{15}H_8ClF_3N_5O_3S$•+(XVI)	410.98 412.98	33.0 10.8
	$C_7H_5F_3N$•	$C_{15}H_6ClF_2N_4O_4S$•+(XVII)	430.00 432.00	3.5 1.1
$C_{19}H_{10}ClF_5N_4O_3$ (IX)	$C_7H_5F_3N$•	$C_{12}H_4ClF_2N_3O_3$•+(XVIII)	310.99 312.99	32.1 9.4
	$C_7H_3F_2O$•	$C_{12}H_6ClF_3N_4O_2$•+(XIX)	330.01 332.01	32.2 9.9
$C_{21}H_{11}ClF_2N_4O_4S$+(XI)	$C_7H_3F_2O$•	$C_{14}H_9ClN_4O_3S$•+(XX)	348.01 350.01	32.9 10.2
	C_9H_8NOS•	$C_{12}H_4ClF_2N_3O_3$•+(XXI)	310.99 312.99	32.1 9.6

3-chloro-6-(2,5-difluorobenzoyl)-8-(5-phenyl-1H-tetrazol-1-yl)-1-((4-(trifluoromethyl)phenylamino)-1,6,7-triazaspiro[3.4]oct-7-ene-2,5-dione ***(11a)***

Yield 70%.m p: 132-134^{O}C. IR (KBr): 3225(stretching vibration of -NH), 1675(stretching vibration of cyclic carbonyl in pyrazoline-5-one ring), 1656(Exo cyclic >C=O group), 1694(stretching vibration of >C=O group of azetidinone),1157(stretching vibration of tetrazol),2012(stretching vibration of azide group).^{1}H-NMR (400 MH$_Z$ DMSO-d6):5.3(s,1H,-CH of azetidinone group), 8.6(s,1H,Ar-NH- attached to azaspiro ring), 6.81-7.81(m, 12H,C_6H_5,C_6H_4andC_6H_3group).^{13}CNMR(75MHz,DMSOd6):154.3,113.5,125.6,126.9,163.5,56.5,74.0,176.1,155.0,170.2,126.7,154.9,118.7,120.5,158.6,113.9,163.5,130.6,127.5,129.2,131.1,124.1corresponding to C_1,C_2&C_6,C_3 & C_5,C_4,C_7,C_8,C_9,C_{10},C_{11},C_{12},C_{13},C_{14},C_{15},C_{16},C_{17}, C_{18}, C_{19},C_{20} & C_{24}, C_{21} & C_{23},C_{22},C_{25},C_{26} Anal. Calcd.For: $C_{26}H_{15}ClF_5N_8O_3$ C 52.74% , H 2.62% and N18.22%. Found: C 53.23% , H 2.43% and N 18.02%.

3-chloro-6-(2,5-difluorobenzoyl)-8-(5-(p-tolyl)-1H-tetrazol-1-yl)-1-((4-(trifluoromethyl)phenylamino)-1,6,7-triazaspiro[3.4]oct-7-ene-2,5-dione ***(11b)***

Yield 65%.m p: 169-171^{O}C. IR (KBr): 3230(stretching vibration of -NH), 1680(stretching vibration of cyclic carbonyl in pyrazoline-5-one ring), 1661(Exo cyclic >C=O group), 1699(stretching vibration of >C=O group of azetidinone),1151(stretching vibration of tetrazol), 2094(stretching vibration of azide group).^{1}H-NMR (400 MH$_Z$ DMSO-d6): 2.34(s,3H,CH_3 group) 5.3(s,1H,-CH of azetidinone group), 8.6(s,1H,Ar-NH-,attached to azaspiro ring),6.81-7.81(m,11H,C_6H_4,C_6H_4 and C_6H_3 group). ^{13}CNMR(75MHz, DMSOd6): 154.3, 113.5, 125.6, 126.9, 163.5,56.5,74.0,176.1,155, 170.2,126.7,154.9,118.7, 120.5,158 ,113.9 ,163.5,127.6,125.7,129.5,131.7,124.1,21.3 corresponding to C_1,C_2&C_6,C_3& C_5,C_4,C_7,C_8,C_9,C_{10},C_{11},C_{12},C_{13},C_{14} ,C_{15},C_{16},C_{17},C_{18},C_{19}, C_{20}&C_{24},C_{21}&C_{23},C_{22},C_{25} ,C_{26},C_{27}Anal .C alcd. For: $C_{27}H_{16}ClF_5N_8O_3$ C 53.47% , H 2.88% and N17.82%. Found: C 53.12% , H 2.51% and N 17.22%.

3-chloro-6-(2,5-difluorobenzoyl)-8-(5-(4-methoxyphenyl)-1H-tetrazol-1-yl)-1-((4-(trifluoromethyl)phenylamino)-1,6,7-triazaspiro[3.4]oct-7-ene-2,5-dione ***(11c)***

Yield:65%.m p: 156-158^{O}C. IR (KBr): 3234(stretching vibration of -NH), 1682(stretching vibration of cyclic carbonyl in pyrazoline-5-one ring), 1668(Exo cyclic >C=O group), 1701 (stretching vibration of >C=O group of azetidinone),1149(stretching vibration of tetrazol), 2096(stretching vibration of azide group).^{1}H.^{1}H-NMR (400 MH$_Z$ DMSO-d6) : 3.6(s,3H,-OCH_3 group) 5.3(s,1H,-CH of azetidinone group), 8.6(s,1H,ArNH ,attached to azaspiro ring), 6.82-7.81(m,11H,C_6H_4,C_6H_4 and C_6H_3 group).^{13}CNMR(75MHz, DMSO-d6): 154.3 ,11 3.5, 125.6,126.9,163.5,56.5,74.0,176.1,155,170.2,126.7,154.9,118.7,120.5,158.,113.9,163.5,122.9,130.3,114.8,160.6,130 .3,124.1,55.8, corresponding to C_1,C_2&C_6,C_3&C_5,C_4,C_7, C_8,C_9,C_{10}, C_{11},C_{12},C_{13},C_{14} ,C_{15},C_{16},C_{17},C_{18},C_{19}, C_{20}&C_{24},C_{21}&C_{23},C_{22},C_{25},C_{26},C_{27},Anal.Calcd For :$C_{27}H_{16}ClF_5N_8O_4$ C52.18% , H 2.85% and N17.37%. Found: C 52.08% , H 2.31% and N 17.27%.

3-chloro-8-(5-(4-chlorophenyl)-1H-tetrazol-1-yl)-6-(2,5-difluorobenzoyl)-1-((4-(trifluoromethyl)phenylamino)-1,6,7-triazaspiro[3.4]oct-7-ene-2,5-dione ***(11d)***

Yield 65%.m p: 146-148^{O}C. IR (KBr): 3220(stretching vibration of -NH), 1670(stretching vibration of cyclic carbonyl in pyrazoline-5-one ring), 1651(Exo cyclic >C=O group), 1682(stretching vibration of >C=O group of azetidinone),1150(stretching vibration of tetrazol) ,2097(stretching vibration of azide group).^{1}H-NMR (400 MH$_Z$ DMSO-d6): 5.3(s,1H,-CH of azetidinone group), 8.6(s,1H,Ar-NH-,attached to azaspiro ring),6.90-7.85(m,11H,C_6H_4,C_6H_4 and C_6H_3 group)^{13}CNMR (75MHz, DMSO-d6): 154.3,113.5,125.6, 126.9, 163.5, 56.5, 74.0,176.1,155,170.2,126.7,154.9,118.7,120.5,158.,113.9,163.5,128.7,128.9,129.3,134.3,124.1 corresponding to C_1,C_2&C_6,C_3&C_5,C_4,C_7,C_8,C_9, C_{10}, C_{11},C_{12},C_{13},C_{14},C_{15},C_{16},C_{17},C_{18},C_{19}, C_{20} & C_{24}, C_{21} & C_{23}, C_{22}, C_{25}, C_{26}. Anal.Calcd.For : $C_{26}H_{13}Cl_2F_5N_8O_3$ C 49.94% , H 2.33% and N17.26%. Found: C 49.44% , H 2.01% and N 17.06%.

8-(5-(4-bromophenyl)-1H-tetrazol-1-yl)-3-chloro-6-(2,5-difluorobenzoyl)-1-((4-(trifluoromethyl)phenylamino)-1,6,7-triazaspiro[3.4]oct-7-ene-2,5-dione ***(11e)***

Yield 70%.m p: 139-141^{O}C. IR (KBr): 3223(stretching vibration of -NH), 1671(stretching vibration of cyclic carbonyl in pyrazoline-5-one ring), 1652(Exo cyclic >C=O group), 1671(stretching vibration of >C=O group of azetidinone),1154(stretching vibration of tetrazol) ,2098(stretching vibration of azide group).^{1}H-NMR (400 MH$_Z$ DMSO-d6): 5.3(s,1H,-CH of azetidinone group), 8.6(s,1H,Ar-NH-,attached to azaspiro ring),6.90-7.85(m,11H,C_6H_4,C_6H_4 and C_6H_3 group).^{13}CNMR(75MHz, DMSO-d6): 154.3, 113.5, 125.6, 126.9, 163.5, 56.5, 74.0, 176.1, 155,170.2,126.7,154.9,118.7,120.5,158.,113.9,163.5,129.6,131.1,132.1,123.1,124.1 corresponding to C_1,C_2&C_6,C_3&C_5,C_4,C_7,C_8, C_9, C_{10},C_{11}, C_{12},C_{13},C_{14} ,C_{15},C_{16},C_{17},C_{18},C_{19}, C_{20}&C_{24},C_{21}&C_{23},C_{22},C_{25},C_{26} . Anal. Calcd. For : $C_{26}H_{13}BrClF_5N_8O_3$ C 46.74% , H 2.25% and N16.39%. Found: C 46. 34% , H 1.93% and N 16.02%.

3-chloro-6-(2,5-difluorobenzoyl)-8-(5-(4-nitrophenyl)-1H-tetrazol-1-yl)-1-((4-(trifluoromethyl)phenylamino)-1,6,7-triazaspiro[3.4]oct-7-ene-2,5-dione ***(11f)***

Yield 70%.m p: 163-165^{O}C. IR (KBr): 3215(stretching vibration of -NH), 1668(stretching vibration of cyclic carbonyl in pyrazoline-5-one ring), 1648(Exo cyclic >C=O group), 1687(stretching vibration of >C=O group of azetidinone),1156(stretching vibration of tetrazol) ,2095(stretching vibration of azide group).^{1}H-NMR (400 MH_Z DMSO-d6): 5.3(s,1H,-CH of azetidinone group), 8.6(s,1H,Ar-NH-,attached to azaspiro ring),7.0-7.90(m,11H,C_6H_4,C_6H_4 and C_6H_3 group).^{13}CNMR(75MHz, DMSO-d6): 154.3, 113.5, 125.6, 126.9, 163.5, 56.5, 74.0, 176.1,155,170.2,126.7,154.9,118.7,120.5,158.,113.9,163.5,136.7,127.0,124.4,147.9,124.1 corresponding to C_1,C_2&C_6,C_3&C_5,C_4,C_7,C_8, C_9,C_{10} ,C_{11},C_{12},C_{13},C_{14},C_{15},C_{16},C_{17},C_{18},C_{19},C_{20}&C_{24},C_{21}&C_{23},C_{22},C_{25},C_{26}.Anal.Calcd.For $C_{26}H_{13}ClF_5N_9O_5$ C 49.74% , H 2.25% and N19.19%. Found: C 46.34% , H 1.93% and N 19.02%.

Mass Spectra:

The electron impact mass spectrum of 3-chloro-6-(2,5-difluorobenzoyl)-8-(5-phenyl-1H-tetrazol-1-yl)-1-((4-(trifluoromethyl)phenylamino)-1,6,7-triazaspiro[3.4]oct-7-ene-2,5-dione(11a) was recorded and interpreted The mass spectral data of compound (11a) showed the molecular ion [M^+] ion peak at m/z= 618.08(33.0%) and M+2 peak at m/z= 616.08(100%) the relative abundance of M^{7+} and M+2 were in there ratio of 3:1 indicate the presence of one chlorine atom. The odd m/z value of molecular ion (M^{7+}) indicates the presence of odd number of nitrogen atoms in molecular ion.

Table 3: Mass spectral data of primary fragmented ions for 3-chloro-6-(2,5-difluorobenzoyl)-8-(5-phenyl-1H-tetrazol-1-yl)-1-((4-(trifluoromethyl)phenylamino)-1,6,7-triazaspiro[3.4]oct-7-ene-2,5-dione (11a)

Molecular ion	Lost radical	Primary fragmented ion	m/z values	Relative abundance (R.A)(%)
$C_{26}H_{14}ClF_5N_8O_3$ (M^+) m/z: 616.08 (100%) 618.08(33.0%)	$C_7H_5F_3N$•	$C_{19}H_9ClF_2N_7O_3$+(II)	458.04 460.04	32.6 9.8
	$C_{19}H_9ClF_2N_7O_3$•	$C_7H_5F_3N$+(III)	161.04	7.6
	$C_7H_5N_4$•	$C_{19}H_9ClF_5N_4O_3$+(IV)	473.03 475.03	32.9 9.7
	$C_{19}H_9ClF_5N_4O_3$•	$C_7H_5N_4$+(V)	146.05	9.0
	$C_{20}H_9ClF_5N_8O_3$•	C_6H_5+(VI)	78.04	6.0
	C_6H_5•	$C_{20}H_9ClF_5N_8O_3$+(VII)	541.04 543.04	33.2 9.5
	$C_{20}H_{11}ClF_3N_8O_3$•	$C_6H_3F_2$+(VIII)	114.02	6.5
	$C_6H_3F_2$•	$C_{20}H_{11}ClF_3N_8O_3$+(IX)	505.06 507.06	33.2 9.4
	$C_{19}H_{11}ClF_3N_8O_2$•	$C_7H_3F_2O$+(X)	142.02	7.6
	$C_7H_3F_2O$•	$C_{19}H_{11}ClF_3N_8O_2$+(XI)	477.06 479.06	32.06 9.8

Table 4: Mass spectral data of secondary fragmented ions for 3-chloro-6-(2,5-difluorobenzoyl)-8-(5-phenyl-1H-tetrazol-1-yl)-1-((4-(trifluoromethyl)phenylamino)-1,6,7-triazaspiro[3.4]oct-7-ene-2,5-dione (11a)

Primary Fragmented ion	Lost radical	Secondary Fragmented ion	m/z values	Relative Abundance (R.A)
$C_{19}H_9ClF_2N_7O_3$+(II)	$C_7H_5F_3N$•	$C_{12}H_6ClN_7O_2$•+(12)	315.03 317.03	14.1 4.3
	$C_7H_5N_4$•	$C_{12}H_6ClF_3N_4O_2$•+(13)	310.99 312.99	13.1 4.2
$C_{19}H_9ClF_5N_4O_3$+(IV)	$C_7H_5F_3N$•	$C_{12}H_4ClF_2N_3O_3$•+(14)	310.99 312.99	14.2 4.5
	$C_7H_3F_2O$•	$C_{12}H_6ClF_3N_4O_2$•+(15)	330.01 332.01	13.1 4.3
$C_{20}H_9ClF_5N_8O_3$+(VII)	$C_7H_5F_3N$•	$C_{13}H_4ClF_2N_7O_3$•+(16)	379.00 381.00	14.2 4.2
	$C_7H_3F_2O$•	$C_{13}H_6ClF_3N_8O_2$•+(17)	398.03 400.03	14.2 4.5
$C_{20}H_{11}ClF_3N_8O_3$+(IX)	$C_7H_5F_3N$•	$C_{12}H_6ClN_7O_2$•+(18)	343.02 345.02	14.2 4.2
	$C_7H_5N_4$•	$C_{12}H_6ClF_3N_4O_2$•+(19)	358.01 360.01	15.7 4.7
$C_{19}H_{11}ClF_3N_8O_2$+(XI)	$C_7H_5F_3N$•	$C_{13}H_6ClN_7O_3$•+(20)	315.03 317.03	13.1 4.3
	$C_7H_5N_4$•	$C_{13}H_6ClF_3N_4O_3$•+(21)	330.01 332.01	13.1 4.3

3-chloro-8-(3-chloro-2-oxo-4-phenylazetidin-1-yl)-6-(2,5-difluorobenzoyl)-1-((4-(trifluoromethyl)phenylamino)-1,6,7-triazaspiro[3.4]oct-7-ene-2,5-dione ***(12a)***

Yield 70%.m p: 162-164^{O}C. IR (KBr): 3225(stretching vibration of -NH), 1620(stretching vibration of >C=N), 1675(stretching vibration of cyclic carbonyl in five membered hetero cyclic ring), 1656(Exo cyclic >C=O group), 677(stretching vibration of C-Cl group), 1694(stretching vibration of >C=O group of azetidinone).^{1}H-NMR (400 MH_Z DMSO-d6): 5.3(d,1H,-CH attached to phenyl group of azetidinone ring B), 5.46(d,1H,CH attached to chlorine of azetidinone ring-B) 5.38(s,1H,Azetidinone ring -A),6.81-7.81(m,12H,C_6H_5,C_6H_4 and C_6H_3 group), and 8.23(s,1H,Ar-NH- group attached to Azetidinone ring-A), ^{13}CNMR(75MHz, DMSOd6): 154.3, 113.5, 125.6, 126.9,163.5,55.4,78.1,176.1,171.2,170.2,126.7,154.9,118.7,120.5,158.6,113.9,163.7,133.7,129.2,128.8,131.0,124.1 Corresponding to C_1, C_2 & C_6, C_3 & C_5, C_4, C_7, C_8, C_9, C_{10}, C_{11}, C_{12}, C_{13}, C_{14}, C_{15}, C_{16}, $C_{17,}$ C_{18}, C_{19}, C_{20}, C_{21} & $C_{25,}$ C_{22} & C_{24},C_{23},C_{25},C_{26},C_{27},C_{28}, Anal. Calcd. For: $C_{28}H_{16}Cl_2F_5N_5O_3$ C 54.55% , H 2.45% and N10.74%. Found: C 51.05% , H 2.17% and N 10.24%.

3-chloro-8-(3-chloro-2-oxo-4-(p-tolyl) azetidin-1-yl)-6-(2, 5-difluorobenzoyl)-1-((4-(trifluoromethyl) phenylamino)-1, 6, 7-triazaspiro [3.4] oct-7-ene-2, 5-dione ***(12b)***

Yield 65%.m p: 114-116^{O}C. IR (KBr): 3230(stretching vibration of -NH), 1625(stretching vibration of >C=N), 1680(stretching vibration of cyclic carbonyl in pyrazoline-5-one ring), 1661(Exo cyclic >C=O group), 682(stretching vibration of C-Cl group), 1699(stretching vibration of >C=O group of azetidinone), ^{1}H-NMR (400 MH_Z DMSO-d6): 2.34 (s,3H, CH_3 group), 5.3(d,1H,-CH attached to phenyl group of azetidinone ring B), 5.46(d,1H,CH attached to chlorine of azetidinone ring-B) 5.38(s,1H,Azetidinone ring -A),6.81-7.81(m,11H,C_6H_4,C_6H_4 and C_6H_3 group), and 8.23(s,1H,Ar-NH- group attached to Azetidinone ring-A), ^{13}CNMR(75MHz,DMSOd6):154.3,113.5,125.6,126.9,163.5,55.4,78.1,176.1,171.2,170.2,126.7,154.9,118.7,120.5,158.6,113.9,163.7,130.7,129.1,129.1,140.7,124.1,21.3 Corresponding to C_1,C_2&C_6, C_3&C_5,C_4,C_7,C_8,C_9,C_{10},C_{11}, C_{12},C_{13},C_{14},C_{15},C_{16},$C_{17,}$$C_{18}$,$C_{19}$,$C_{20}$,$C_{21}$&$C_{25,}$ C_{22}& C_{24}, C_{23},C_{25},C_{26},C_{27},C_{28},C_{29}Anal .C alcd. For: $C_{27}H_{17}ClF_5N_5O$ C 46.74%, H 2.25% and N14.39%. Found: C 46. 34% , H 1.93% and N 14.02%.

3-chloro-8-(3-chloro-2-(4-methoxyphenyl)-4-oxoazetidin-1-yl)-6-(2,5-difluorobenzoyl)-1-((4-(trifluoromethyl) phenylamino)-1,6,7-triazaspiro[3.4]oct-7-ene-2,5-dione ***(12c)***

Yield 70%.m p: 148-150^{O}C. IR (KBr): 3230(stretching vibration of -NH), 1625(stretching vibration of >C=N), 1680(stretching vibration of cyclic carbonyl in pyrazoline-5-one ring), 1661(Exo cyclic >C=O group), 682(stretching vibration of C-Cl group), 1699(stretching vibration of >C=O group of azetidinone),).^{1}H-NMR (400 MH_Z DMSO-d63.6(s,3H,-OCH_3 group), 5.3(d,1H,-CH attached to phenyl group of azetidinone ring B), 5.46(d,1H,CH attached to chlorine of azetidinone ring-B), 5.38(s,1H,Azetidinone ring -A),6.81-7.81(m,11H,C_6H_4,C_6H_4 and C_6H_3 group), and 8.23(s,1H,Ar-NH- group attached to Azetidinone ring-A), ^{13}CNMR(75MHz,DMSOd6):154.3,113.5,125.6,126.9,163.5,55.4,78.1,176.1,171.2,170.2,126.7,154.9,118.7,120.5,158.6,113.9,163.7,126.0,130.2,114.4,162.9,124.1,55.8 Corresponding to C_1, C_2 & C_6, C_3 & C_5, C_4, C_7, C_8, C_9, C_{10}, C_{11}, C_{12}, C_{13},C_{14},C_{15}, C_{16},$C_{17,}$$C_{18}$,$C_{19}$,$C_{20}$,$C_{21}$ & $C_{25,}$ C_{22} & C_{24}, C_{23}, C_{25}, C_{26}, C_{27}, C_{28}, C_{29}., Anal. Calcd For : $C_{27}H_{17}ClF_5N_5O_4$ C 46.74%, H 2.25% and N14.39%. Found: C 46. 34%, H 1.93% and N 14.02%.

3-chloro-8-(3-chloro-2-(4-chlorophenyl)-4-oxoazetidin-1-yl)-6-(2,5-difluorobenzoyl)-1-((4-(trifluoromethyl) phenyl amino)-1,6,7-triazaspiro[3.4]oct-7-ene-2,5-dione ***(12d)***

Yield 75%.m p: 108-110^{O}C. IR (KBr): 3230(stretching vibration of -NH), 1625(stretching vibration of >C=N), 1680(stretching vibration of cyclic carbonyl in pyrazoline-5-one ring), 1661(Exo cyclic >C=O group), 682(stretching vibration of C-Cl group), 1699(stretching vibration of >C=O group of azetidinone).^{1}H-NMR (400 MH_Z DMSO-d6): 5.3(d,1H,-CH attached to phenyl group of azetidinone ring B), 5.46(d,1H,CH attached to chlorine of azetidinone ring-B) 5.38(s,1H,Azetidinone ring -A),6.81-7.81(m,11H,C_6H_4,C_6H_4 and C_6H_3 group), and 8.23(s,1H,Ar-NH- group attached to Azetidinone ring-A), ^{13}CNMR(75MHz,DMSO-d6): 154.3, 113.5, 125.6, 126.9,163.5,55.4,78.1,176.1,171.2,170.2,126.7,154.9,118.7,120.5,158.6,113.9,163.7,131.8,130.6,128.6,136.6,124.1 Corresponding to C_1, C_2 &C_6, C_3 & C_5, C_4, C_7, C_8, C_9, C_{10}, C_{11}, C_{12}, C_{13}, C_{14}, C_{15}, C_{16}, $C_{17,}$ C_{18}, C_{19}, C_{20}, C_{21} & $C_{25,}$ C_{22} & C_{24},C_{23},C_{25},C_{26}, C_{27}, C_{28}.,Anal.Calcd.For: $C_{26}H_{11}Cl_2F_5N_5O_3$ C 46.74% , H 2.25% and N14.39%. Found: C 46. 34% , H 1.93% and N 14.02%.

8-(2-(4-bromophenyl)-3-chloro-4-oxoazetidin-1-yl)-3-chloro-6-(2,5-difluorobenzoyl)-1-((4-(trifluoromethyl) phenyl amino)-1,6,7-triazaspiro[3.4]oct-7-ene-2,5-dione ***(12e)***

Yield 75%.m p: 135-137^{O}C. IR (KBr): 3230(stretching vibration of -NH), 1625(stretching vibration of >C=N), 1680(stretching vibration of cyclic carbonyl in pyrazoline-5-one ring), 1661(Exo cyclic >C=O group), 682(stretching vibration of C-Cl group), 1699(stretching vibration of >C=O group of azetidinone), ^{1}H-NMR (400 MH_Z DMSO-d6): 5.3(d,1H,-CH attached to phenyl group of azetidinone ring B), 5.46(d,1H,CH attached to chlorine of azetidinone ring-B) 5.38(s,1H,Azetidinone ring -A),6.90-7.85(m,11H,C_6H_4,C_6H_4 and C_6H_3 group), and 8.23(s , 1H,ArNHgroupattachedtoAzetidinoneringA),^{13}CNMR(75MHz,DMSOd6):154.3,113.5,125.6,126.9,163.5,55.4,78.1,

176.1,171.2,170.2,126.7,154.9,118.7,120.5,158.6,113.9,163.7,132.7,128.5,131.8,125.4,124.1 Corresponding to C_1, C_2 & C_6, C_3&C_5,C_4,C_7,C_8,C_9, C_{10}, C_{11},C_{12},C_{13},C_{14},C_{15},C_{16},$C_{17,}C_{18}$,C_{19},C_{20},C_{21} & $C_{25,}$ C_{22} & C_{24}, C_{23}, C_{25}, C_{26}, C_{27}, C_{28}, Anal.Calcd.For : $C_{26}H_{14}ClF_5N_5O_3$ C 46.74% , H 2.25% and N14.39%. Found: C 46. 34% , H 1.93% and N 14.02%.

3-chloro-8-(3-chloro-2-(4-nitrophenyl)-4-oxoazetidin-1-yl)-6-(2,5-difluorobenzoyl)-1-((4-(trifluoromethyl) phenyl amino)-1,6,7-triazaspiro[3.4]oct-7-ene-2,5-dione ***(12f)***

Yield 70%.m p: 124-126^{O}C. IR (KBr): 3230(stretching vibration of -NH), 1625(stretching vibration of >C=N), 1680(stretching vibration of cyclic carbonyl in pyrazoline-5-one ring), 1661(Exo cyclic >C=O group), 682(stretching vibration of C-Cl group), 1699(stretching vibration of >C=O group of azetidinone), ^{1}H-NMR (400 MH_Z DMSO-d6): 5.3(d,1H,-CH attached to phenyl group of azetidinone ring B), 5.46(d,1H,CH attached to chlorine of azetidinone ring-B) 5.38(s, 1H ,Azetidinone ring -A),7.0-7.90(m,11H,C_6H_4,C_6H_4 and C_6H_3 group), and 8.23 (s, 1H,Ar-NH- group attached to Azetidinone ring-A),^{13}CNMR(75MHz, DMSO-d6): 154.3,113.5,125.6,1 26.9,163.5,55.4,78.1,176.1,171.2,170.2,126.7,154.9,118.7,120.5,158.6,113.9,163.7,139.7,127.8,124.0,150.2,124.1 Corresponding to C_1,C_2 & C_6, C_3 & C_5,C_4,C_7,C_8,C_9,C_{10},C_{11},C_{12}, C_{13}, C_{14}, C_{15}, C_{16}, $C_{17,}$ C_{18}, C_{19}, C_{20}, C_{21} & $C_{25,}$ C_{22} & C_{24},C_{23},C_{25},C_{26}, C_{27}, C_{28},Anal.Calcd. For $C_{26}H_{14}ClF_5N_6O_5$ C 46.74% , H 2.25% and N14.39%. Found: C 46.34% , H 1.93% and N 14.02%.

Mass Spectra:

The electron impact mass spectrum 3-chloro-8-(3-chloro-2-oxo-4-phenylazetidin-1-yl)-6-(2,5-difluorobenzoyl)-1-((4-(trifluoromethyl)phenylamino)-1,6,7-triazaspiro[3.4]oct-7-ene-2,5-dione (12 a) was recorded and interpreted. The mass spectral data of compound (12a) showed the molecular ion [M^+] ion peak at m/z=651.05 and M+2 peak at m/z=653.05(33%) at m/z=655.03(100%) the relative abundances of M^{7+} and M+2 and M+4 peaks were in there ratio of 9:6:1 indicate the presence of two chlorine atoms. The odd m/z value of molecular ion (M^{7+}) indicates the presence of odd number of nitrogen atoms in molecular ion.

Table:5: Mass spectral data of primary fragmented ions for 3-chloro-8-(3-chloro-2-oxo-4-phenylazetidin-1-yl)-6-(2,5-difluorobenzoyl)-1-((4-(trifluoromethyl)phenylamino)-1,6,7-triazaspiro[3.4]oct-7-ene-2,5-dione (12a)

Molecular ion	Lost radical	Primary fragmented ionC	m/z values	Relative abundance (R.A)(%)
$C_{28}H_{16}Cl_2F_5N_5O_4(M^+)$ m/z: 651.05(100%) 653.05(33.3%) 655.03(11.1%)	$C_{21}H_{11}Cl_2F_2N_4O_4$•	$C_7H_5F_3N^+$ (II)	162.05	7.6
	$C_7H_5F_3N$•	$C_{21}H_{11}Cl_2F_2N_4O_4^+$(III)	493.01 495.01 497.01	27.9 18.6 3.1
	$C_{21}H_{13}Cl_2F_3N_5O_3$•	$C_7H_3F_2O^+$ (IV)	142.02	7.6
	$C_7H_3F_2O$•	$C_{21}H_{13}Cl_2F_3N_5O_3^+$ (V)	512.03 514.03 516.03	22.5 15 2.5
	$C_{22}H_{13}Cl_2F_3N_5O_4$•	$C_6H_3F_2^+$ (VI)	114.02	6.5
	$C_6H_3F_2$•	$C_{22}H_{13}Cl_2F_3N_5O_4^+$ (VII)	540.03 542.03 545.03	18.9 12.6 2.1
	$C_{22}H_{11}Cl_2F_5N_5O_4$•	$C_6H_5^+$ (VIII)	78.04	6.5
	C_6H_5•	$C_{22}H_{11}Cl_2F_5N_5O_4^+$ (IX)	575.00 577.00 579.00	24.3 16.2 2.7
	$C_{19}H_9ClF_5N_4O_3$•	$C_9H_7ClNO^+$ (X)	182.02 184.02	12.3 8.2 1.3
	C_9H_7ClNO•	$C_{19}H_9ClF_5N_4O_3^+$ (XI)	473.03 475.03 477.03	17.1 11.9 1.9

Table 6: Mass spectral data of secondary fragmented ions for 3-chloro-8-(3-chloro-2-oxo-4-phenylazetidin-1-yl)-6-(2,5-difluorobenzoyl)-1-((4-(trifluoromethyl)phenylamino)-1,6,7-triazaspiro[3.4]oct-7-ene-2,5-dione (12a)

Primary Fragmented ion	Lost radical	Secondary Fragmented ion	m/z values	Relative Abundance (R.A)
$C_{21}H_{11}Cl_2F_2N_4O_4$+(III)	C_9H_7ClNO•	$C_{12}H_4ClF_2N_3O_3$•+(12)	310.99 312.09 314.09	14.1 9.4 1.5
	$C_7H_3F_2O$•	$C_{14}H_8Cl_2N_4O_3$•+(13)	350.00 352.00 354.00	15.3 10.2 1.7
$C_{21}H_{13}Cl_2F_3N_5O_3$+ (IV)	$C_7H_5F_3N$•	$C_{14}H_8Cl_2N_4O_3$•+(14)	350.00 352.00 354.00	11.3 7.5 1.2
	C_9H_7ClNO•	$C_{12}H_6ClF_3N_4O_2$•+(15)	330.01 332.01 334.01	9.9 6.6 1.1
$C_{22}H_{13}Cl_2F_3N_5O_4$+ (VII)	$C_7H_5F_3N$•	$C_{15}H_8Cl_2N_4O_4$•+(16)	377.99 379.99 381.99	21.6 14.4 2.4
	C_9H_7ClNO•	$C_{13}H_6ClF_3N_4O_3$•+(17)	358.00 360.00	9.6 6.4 1.0
$C_{22}H_{10}Cl_2F_5N_5O_4$+ (IX)	$C_7H_5F_3N$•	$C_{15}H_5Cl_2F_2N_4O_4$•+(18)	412.97 414.97 416.99	16.2 10.8 1.8
	$C_7H_3F_2O$•	$C_{15}H_7Cl_2F_3N_5O_3$•+(19)	431.99 433.99 435.99	16.2 10.8 1.8
$C_{19}H_9ClF_5N_4O_3$+ (XI)	$C_7H_5F_3N$•	$C_{12}H_4ClF_2N_3O_3$•+(20)	310.99 312.99 314.99	7.2 4.8 0.8
	$C_7H_3F_2O$•	$C_{12}H_6ClF_3N_4O_2$•+(21)	330.01 312.01 314.01	15.3 10.2 1.7

Biological activity

The antimicrobial activity [31-33] of these newly synthesized compounds was performed according to disc diffusion method, as recommended by the National Committee for Clinical Laboratory [34]. The synthesized compounds Were used at the concentration of 250µg/ml DMF as a solvent [35].

Antibacterial activity

The antibacterial activity of 3-chloro-6-(2,5-difluorobenzoyl)-8-(2-(4-substituted phenyl)-4-oxothiazolidin-3-yl)-1-((4-(trifluoromethyl) phenyl)amino)-1,6,7-triazaspiro[3.4]oct-7-ene-2,5-dione (10 a-f), 3-chloro-6-(2,5-difluoro benzoyl)-8-(5-(4-substituted phenyl)-1H-tetrazol-1-yl)-1-((4-(trifluoromethyl) phenyl) amino)-1,6,7-triazaspiro[3.4]oct-7-ene-2,5-dione (11 a-f), 3-chloro-8-(3-chloro-2-(4-substituted phenyl)-4-oxoazetidin-1-yl)-6-(2,5-difluorobenzoyl)-1((4(trifluoromethyl)phenyl)amino)-1,6,7-triazaspiro[3.4]oct-7-ene-2,5-dione(12a-f) were screened against the Staphylococcus aureus (gram positive) and Escherichia coli (gram negative) organisms. Most of the compounds exhibited moderate antibacterial activity against both bacteria. The presence of chloro, bromo and nitro in the structure has shown increased effect on their antibacterial activity [36,37]

Table-7 Determination of zone of inhibition (in mm) of 3-chloro-6-(2,5-difluorobenzoyl)-8-(2-(4-substituted phenyl)-4-oxothiazolidin-3-yl)-1-((4-(trifluoromethyl)phenyl)amino)-1,6,7-triazaspiro[3.4]oct-7-ene-2,5-dione (10 a-f),) (µg/mL)

Entry	Bacteria						Fungi			
	Staphylococcus aureus **NCCS 2079**		***Bacillus cereus*** **NCCS 2106**		***Escherichia coli*** **NCCS 2065**		***Aspergillus niger*** **NCCS 1196**		***Candida albicans*** **NCCS 2106**	
	25	**50**	**25**	**50**	**25**	**50**	**25**	**50**	**25**	**50**
10a	-	**09**	-	**08**	-	**07**	-	**10**	-	**11**
10b	-	**08**	-	**10**	-	**09**	-	**09**	-	**12**
10c	-	**10**	-	**10**	-	**11**	-	**10**	-	**12**
10d	**10**	**14**	**10**	**15**	**07**	**13**	**08**	**13**	**07**	**12**
10e	**09**	**12**	**10**	**14**	**06**	**12**	**07**	**12**	**06**	**11**
10f	**13**	**16**	**13**	**17**	**10**	**15**	**11**	**16**	**12**	**15**
Chloromphenicol(5)	-	**25**	-	**26**	-	**22**	-	-	-	-
Ketocanazole(50)	-	-	-	-	-	-	-	**16**	-	**18**

Antifungal activity

Antifungal activity of(10 a-f, 11 a-f, 12 a-f,) was screened against Aspergillus Niger, Candida albicans [38]. The presence of chloro,bromo and nitro in the structure has shown increased effect on their anti-fungal activity.

Table-8 Determination of zone of inhibition (in mm) of 3-chloro-6-(2,5-difluorobenzoyl)-8-(5-(4-substituted phenyl)-1H-tetrazol-1-yl)-1-((4-(trifluoromethyl)phenyl)amino)-1,6,7-triazaspiro[3.4]oct-7-ene-2,5-dione (11 a-f), (µg/mL)

Entry	Bacteria						Fungi			
	Staphylococcus aureus NCCS 2079		*Bacillus cereus* NCCS 2106		*Escherichia coli* NCCS 2065		*Aspergillus niger* NCCS 1196		*Candida albicans* NCCS 2106	
	25	50	25	50	25	50	25	50	25	50
11a	-	12	-	11	-	10	-	13	-	14
11b	-	11	-	13	-	12	-	12	-	15
11c	-	17	-	13	-	14	-	13	-	15
11d	13	14	13	18	12	16	11	16	10	15
11e	12	15	13	12	11	15	10	15	09	14
11f	19	19	16	15	15	18	14	19	15	18
Chloromphenicol(5)	-	25	-	26	-	22	-	-	-	-
Ketocanazole(50)	-	-	-	-	-	-	-	16	-	18

Table-9 Determination of zone of inhibition (in mm) of 3-chloro-8-(3-chloro-2-(4-substituted phenyl)-4-oxoazetidin-1-yl)-6-(2,5-difluorobenzoyl)-1((4(trifluoromethyl)phenyl)amino)-1,6,7-triazaspiro[3.4]oct-7-ene-2,5-dione(12a-f) (µg/mL)

Entry	Bacteria						Fungi			
	Staphylococcus aureus NCCS 2079		*Bacillus cereus* NCCS 2106		*Escherichia coli* NCCS 2065		*Aspergillus niger* NCCS 1196		*Candida albicans* NCCS 2106	
	25	50	25	50	25	50	25	50	25	50
12a	-	11	-	10	-	09	-	12	-	13
12b	-	10	-	12	-	11	-	11	-	14
12c	-	12	-	12	-	13	-	12	-	14
12d	12	16	12	17	11	15	10	15	09	14
12e	11	14	12	16	10	14	09	14	08	13
12f	15	18	15	19	14	17	13	18	14	17
Chloromphenicol(5)	-	25	-	26	-	22	-	-	-	-
Ketocanazole(50)	-	-	-	-	-	-	-	16	-	18

Each well contains 25 and 50 µg of compounds ; Ch=Chloromphenicol 5 µg/mL, Ketocanazole 50 µg/mL

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