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# Synthesis, characterization and antimicrobial evaluation of novel compounds 8-(benzylideneamino)-3-chloro-6-(2,5-difluorobenzoyl)-1-((4-(trifluoromethyl) phenyl)amino)-1,6,7-triazaspiro[3,4]-oct-7-ene-2,5-dione

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**ABSTRACT**

*New novel derivatives of 8-(4-Substituted benzylideneamino)-3-Chloro-6-(2,5-difluorobenzoyl) -1-((4-(trifluoromethyl)phenylamino)-1,6,7-triazaspiro[3,4]-oct-7-ene-2,5-dione. (9a-f) were prepared by condensation of 4-substituted benzaldehyde(8a-f) with 8-amino-3-Chloro-6-(2,5-difluorobenzoyl)-1-((4-(trifluoromethyl)phenylamino)-1,6,7-triazaspiro[3,4]-oct-7-ene-2,5dione (7). The synthon (7) was obtained by deprotection of Tert-butyl(3-Chloro-7-(2,5-difluorobenzoyl)-2,8-dioxo-1-((4-(trifluoro methyl) phenyl)amino)-1,6,7-triazaspiro[3,4]-oct-5-en-5-yl)Carbamate(6). The synthons (6) was obtained by the reaction of chloro acetyl chloride with Tart-butyl (1-(2,5-difluorobnzoyl)-5-oxo-4-(2-(4-trifluoromethyl)phenylhydrazono)-4,5-dihydro-1H-pyrazole-3-yl)carbamate(5). The synthon (5) was obtained by the reaction between BOC (tert butyloxy carbamate) and 3-amino-1-(2, 5-di fluoro phenyl carbonoyl)-4-(2-(4-Substituted) phenyl) hydrazono)-1H-pyrazole-5(4H) -one (3). The synthon (3) was obtained by the condensation of 1,4- di fluoro benzoyl hydrazide(2) with Ethyl 2-(2-(4-(tri fluoromethyl) phenyl ) hydrazono)-2-iso cyano acetate(1).The synthon(1) was obtained by the condensation of 1-chloro-2-(4-(trifluoro methyl) phenyl)diazene(A) with ethyl-2-iso cyano acetate (B).*

**Key words**: 1, 6, 7-triazaspiro, chloro acetyl chloride, tert-butyloxy carbamate, Deprotection, Antibacterial and Antifungal activity.

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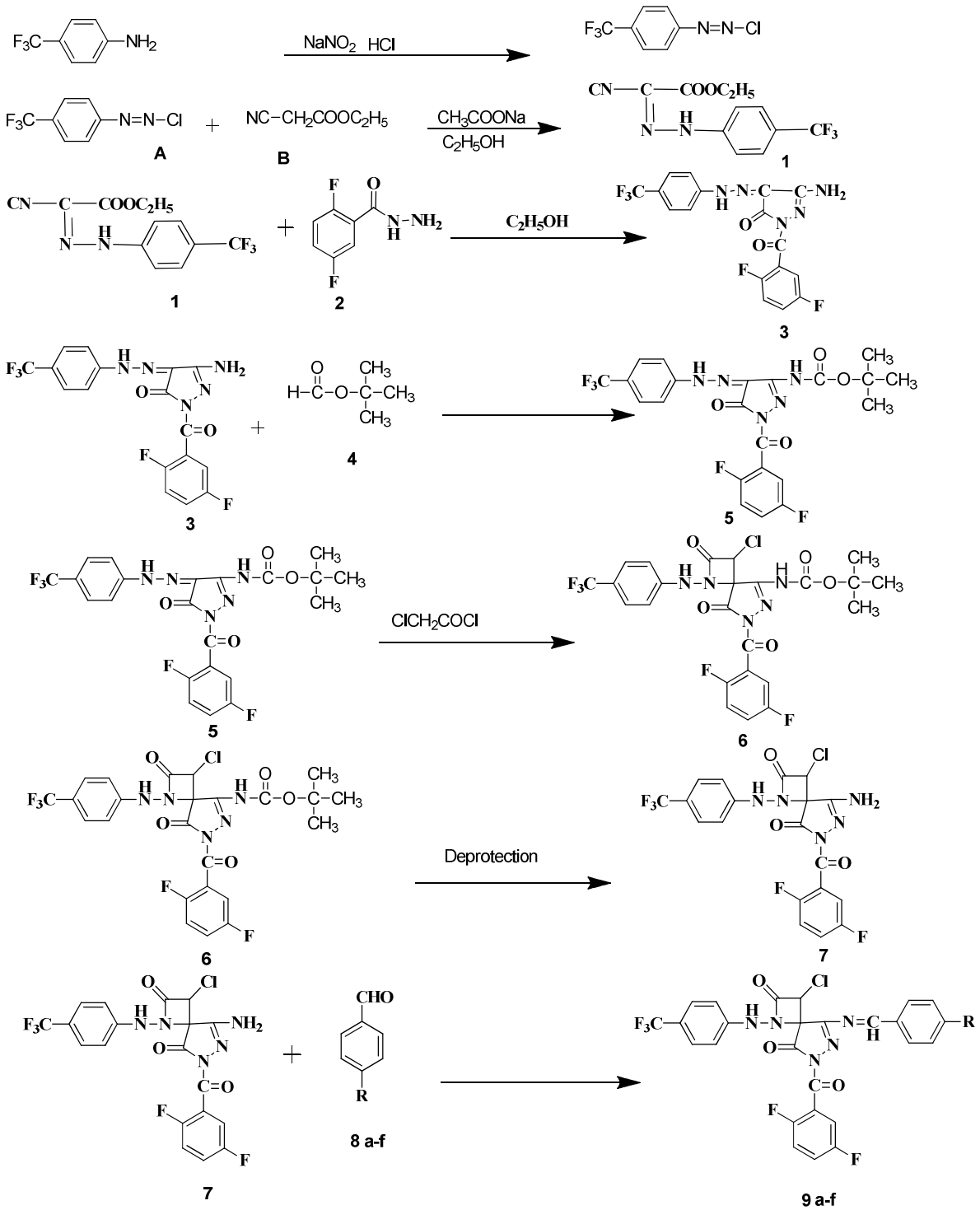
## INTRODUCTION

Hetero cyclic compounds represent an important class of biological active molecules specifically those containing the pyrazolone nucleus besides triazaspiro ring 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.

Triazaspiro compounds are the most common and important groups among the small ring hetero cyclic compounds. triazaspiro, 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, anticonvulsant [16], and analgesic [17], and cholesterol inhibitory activities [18].

In view of the importance of the above Hetero cycles we planned to synthesize Triazaspiro derivatives.



**Scheme II.1:** ***Synthesis of 8 - (4 - Substituted benzylideneamino) – 3 – Chloro – 6 - (2, 5 -difluorobenzoyl) – 1 - ((4 - (trifluoromethyl)phenylamino) - 1, 6, 7 – triazaspiro [3, 4] – oct – 7 – ene - 2, 5 - dione (9a-f)***

| COMP NO | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| R | H | $CH_3$ | $OCH_3$ | Cl | Br | $NO_2$ |

## MATERIALS AND METHODS

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-Temp. apparatus and are 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**-amino-1-(2, 5-difluorobenzoyl)-4-(2-(4-(trifluoromethyl)phenyl)hydrazono)-1H-pyrazol-5(4H)-one (3)**

The required primary amine is diazotized with sodium nitrite and HCl mixture at 0-5$^0$ c and it is coupled with cyano acetic ester to afford ethyl-2-2-(4-(tri fluoro methyl) phenyl hydrazono)-2- is cyano acetate (1).The compound (1) was prepared by the procedure described by H.M.Walborsky,M.E. Baum [19]

A mixture of ethyl-2-2-(4-(tri flouro methyl) phenyl hydrazono)-2- iso cyano acetate(1) 1,4-difluoro benzoyl hydrazide(2) and dimethyl formamide(1 ml) was subjected to microwave irradiation at 150 W intermittently at 30 seconds intervals for 2 min.After complete conversion as indicated by TLC,the reaction mixture was cooled and treated with cold water. The presipitate3-amino-1-(2,5-difluorobenzoyl)-4-(2-(4-(trifluoromethyl)phenyl) hydrazono)-1H-pyrazol-5(4H)-one(3) was filtered and recrystallized from ethyl alcohol. The structure of (3) was established by IR,1H-NMR and Elemental analysis

**Synthesis of Tert-butyl (1-(2, 5-difluorobnzoyl)-5-oxo-4-(2-(4-trifluoromethyl) phenylhydrazono)-4, 5-dihydro-1H-pyrazole-3-yl) carbamate (5)**

The protection of amino group was carried out by BOC anhydride using the procedure as reported in the literature [31] 3-amino - 1 - (2, 5 - difluorobenzoyl) – 4 - (2 - (4 -(trifluoromethyl)phenyl)hydrazono)-1H-pyrazol-5(4H)-one(3) was dissolved in dimethyl formamide and water mixture. To this to equivalents of sodium bi carbonate was added with stirring the resulting solution was cooled to 5$^o$C and (*t*-butyloxy) carbamate (BOC) anhydride (1.5 eq) was added slowly as a solution *Para*- dioxane. The resulting reaction mixture was stirred at 0$^o$C for 1hr and allowed to warm to room temperature over night. The progress of the reaction was monitored by TLC using cyclohexane-ethyl acetate (7:3) as a solvent mixture as an elutent. After completion of the reaction water was added to the reaction mixture. The aqueous layer was extracted with ethyl acetate(2x20 ml).The organic layer was back extracted twice with 20 ml of saturated sodium bi carbonate solution .The combined aqueous layers were acidified to a P$^H$ of 1 with 10% HCl.The combined organic layers were dried on anhydrous sodium sulphate. The solvent was evaporated with Rota evaporator. The resulting residue was purified with 60-120 silica mesh. The cyclohexane and ethyl acetate (7:3) solvent mixture was used as an elutent. The product tert-butyl(1-(2,5-difluorobnzoyl)-5-oxo-4-(2-(4-trifluoromethyl)phenylhydrazono)-4,5-dihydro-1H-pyrazole-3-yl)carbamate (5) was obtained .The structure of tert-butyl (1-(2,5-difluorobnzoyl)-5-oxo-4-(2-(4-trifluoromethyl)phenylhydrazono)-4,5-dihydro-1H-pyrazole-3-yl)carbamate (5) was established by IR, $^1$H-NMR and Elemental analysis

**Synthesis of Tert-butyl (3-Chloro – 7 - (2, 5 - difluorobenzoyl) - 2, 8 – dioxo – 1 - ((4 -(trifluoromethyl) phenyl) amino) - 1, 6, 7 – triazaspiro [3, 4] – oct – 5 – en – 5 - yl) Carbamate (6)**

Monochloro acetyl chloride (0.01 mol)was added drop wise to tert-butyl(1-(2,5-difluorobnzoyl)-5-oxo-4-(2-(4-trifluoromethyl)phenylhydrazono)-4,5-dihydro-1H-pyrazole-3-yl)carbamate .The reaction mixture was stirred for 8 hours. 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 a room temperature. The reaction mixture was poured in crushed ice. The product thus formed was filtered and washed with sodium bi carbonate solution. The dried product was recrystallized from absolute alcohol. The structure of Tert-butyl(3-Chloro-7-(2,5-difluorobenzoyl)-2,8-dioxo-1-((4-

(trifluoromethyl)phenyl)amino)-1,6,7-triazaspiro[3,4]-oct-5-en-5-yl)Carbamate(6 ) was established by IR,1H-NMR and Elemental analysis.

**Deprotection of BOC 8 – amino – 3 – Chloro – 6 - (2, 5 - difluorobenzoyl) – 1 - ((4-(trifluoromethyl)phenylamino) - 1, 6, 7-triazaspiro [3, 4] - oct-7-ene-2, 5dione (7)**

Tert-butyl(3-Chloro-7-(2,5-difluorobenzoyl)-2,8-dioxo-1-((4-(trifluoromethyl)phenyl)amino)-1,6,7-triazaspiro[3,4]-oct-5-en-5-yl)Carbamate(6 ) (0.01 mol) was dissolved in 1:1 tri fluoro acetic acid and water mixture. The reaction mixture was stirred at room temperature for 2 hr.The progress of the reaction was monitored by TLC using cyclohxane and ethyl acetate (7:3) solvent mixture after the reaction is completed .The solvent is evaporated in Rota evoparetar.The residue was purified by 60-120 silica mesh using cyclo hexane and ethyl acetate (7:3) solvent mixture as an elutent. The structure of (7) was established by IR, $^{1}$H-NMR and Elemental analysis

**Synthesis of 8-(4-subtituted benzylideneamino)-3-Chloro-6-(2, 5-difluorobenzoyl)-1-((4-(trifluoromethyl)phenylamino) - 1, 6, 7-triazaspiro [3, 4] - oct-7-ene-2, 5-dione (9a-f)**

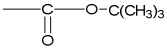
Equimolar quantity of 8-amino-3-Chloro-6-(2,5-difluorobenzoyl)-1-((4-(trifluoromethyl)phenylamino)-1,6,7-triazaspiro[3,4]-oct-7-ene-2,5dione(7) and benzaldehyde were dissolved in absolute alcohol. To this reaction mixture was added. The reaction mixture was heated on a steam bath for 2 hr at 100$^{0}$ C.The progress of the reaction was monitored by TLC using cyclohexane and ethyl acetate(7:3) solvent mixture. The product was dried and recrystallized from warm absolute alcohol. The reaction mixture kept overnight at room temperature. The solvent was evaporated by Rota evaporator. The residue was filtered and purified by 60-120 silica mesh using cyclohexane and ethyl acetate (7:3) as an elutent. The similar procedure was adopted to synthesize 8b-f from 8-amino-3-Chloro-6-(2,5-difluorobenzoyl)-1-((4-(trifluoromethyl)phenylamino) - 1, 6, 7-triazaspiro[3, 4] – oct – 7 – ene -2,5dione (7) with 4-methyl benzaldehyde, 4-methoxy benzaldehyde, 4-chloro benzaldehyde, 4-bromo benzaldehyde, 4-nitro benzaldehyde. The structure of 9 (a-f) was established by IR, $^{1}$H-NMR, $^{13}$C-NMR, Mass and Elemental analysis.

**Physical, analytical and spectral data for the compounds:**

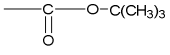
*3-amino-1-(2, 5-difluorophenylcarbonoyl)-4-(2-(4-Substituted) phenyl) hydrazono)-1H-pyrazole-5(4H) - one* ***(3)***

Yield 85%.m p: 184$^{O}$C. IR (KBr):3385,3405(two bands stretching vibration of $NH_2$),3225(stretching vibration of -NH),1620(stretching vibration of >C=N),1675(stretching vibration of cyclic carbonyl five membered hetero cyclic ring),1656(exo cyclic >C=O group), $^{1}$H-NMR (400 $MH_Z$ DMSO-d6): 2.15(s,2H,-$NH_2$ group),6.81-8.37(m,7H,$C_6H_4$ and $C_6H_3$), 10.15(s,1H,Ar –NH-N= group). Anal.Calcd.For $C_{17}H_{10}F_5N_5O_2$ C 49.64% , H 2.45% and N 17.03%. Found: C 49.53%, H 2.23% and N 16.80%.

*Tart-butyl (1-(2,5-difluorobnzoyl)-5-oxo-4-(2-(4-trifluoromethyl)phenylhydrazono)-4,5-dihydro-1H-pyrazole-3-yl)carbamate* ***(5)***

Yield 75%.m p: 156-158$^{O}$C. IR (KBr):3385,3405(two bands stretching vibration of $NH_2$),3225(stretching vibration of -NH),1620(stretching vibration of >C=N),1675(stretching vibration of cyclic carbonyl five membered hetero cyclic ring),1656(exo cyclic >C=O group) 1720(stretching vibration of >C=O group of an ester)1380(bending vibration of $C(CH_3)_3$), $^{1}$H-NMR (400 $MH_Z$ DMSO-d6): 1.32(s,9H,t-butyl group),8.2(s,1H,-NH attached to  ) 2.15(s,2H,-$NH_2$ group),6.81-8.37(m,7H,$C_6H_4$ and $C_6H_3$), 10.15(s,1H,Ar –NH-N= group). Anal.Calcd.For $C_{22}H_{18}F_5N_5O4$ C 51.64% , H 3.55% and N13.69%. Found: C 51. 34% , H 3.33% and N 13.42%.

*Tert-butyl (3-Chloro – 7 - (2,5-difluorobenzoyl) - 2, 8 – dioxo – 1 - ( (4 -(trifluoromethyl)phenyl) amino) -1,6,7-triazaspiro [3, 4] - oct-5-en-5-yl) Carbamate* **(6)**

Yield 70%.m p: 137-139$^{O}$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), 1720(stretching vibration of  group of an ester), 1250(C-O-C, stretching vibration of an ester),1380 (Bending vibration of $C(CH_3)_3$), 677(stretching vibration of C-Cl group), 1694(stretching vibration of >C=O group of azetidinone). $^{1}$H-NMR (400 $MH_Z$ DMSO-d6): ) 1.32(δ,9H,t-butyl group), 5.3(δ,1H attached to –CH of azetidinone group) 6.81-8.37(m,7H, $C_6H_4$ and $C_6H_3$), 8.2(δ,1H, -NH attached to  ).10.15(δ,1H Ar-NH-N= group),. Anal.Calcd.For $C_{24}H_{19}ClF_5N_5O5$ C 49.74% , H 3.25% and N11.99%. Found: C 48. 34% , H 3.07% and N 11.02%.

*8-amino-3-Chloro-6-(2,5-difluorobenzoyl)-1-((4-(trifluoromethyl)phenylamino)-1,6,7-triazaspiro[3,4]-oct-7-ene-2,5dione* ***(7)***
Yield 70%.m p: 136-138$^{O}$C. IR (KBr): 3385 ,3405 (two bands stretching vibration of $–NH_2$) , 3225(stretching vibration of -NH), 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): ) 2.15(s,2H,-$NH_2$ group),5.3(δ,1H attached to –CH of azetidinone group) 6.81-8.37(m,7H, $C_6H_4$ and $C_6H_3$), 10.15(δ,1H Ar-NH-N= group). . Anal.Calcd.For $C_{24}H_{11}ClF_5N_5O3$ C 46.74% , H 2.25% and N14.39%. Found: C 46. 34% , H 1.93% and N 14.02%.

*8-(4-benzylideneamino)-3-Chloro-6-(2,5-difluorobenzoyl)-1-((4-(trifluoromethyl)phenyl)amino)-1,6,7-triazaspiro [3,4]-oct-7-ene-2,5-dione* ***(9a)***
Yield 70%.m p: 160-162$^{O}$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(s,1H,-CH of azetidinone group),6.81-8.37(m,12H,$C_6H_5$,$C_6H_4$ and $C_6H_3$ group),8.48(s,1H,-N=CH- group), 10.15(s, 1H Ar-NH-N= group). $^{13}$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, 133.7, 129.2, 128.8, 131.0, 124. Corresponding to $C_1$, $C_2$ & $C_6$, $C_3$ & $C_5$, $C_4$, $C_7$, $C_8$, $C_9$, $C_{10}$, $C_{11}$, $C_{12}$, $C_{13}$, $C_{14}$, $C_{15}$, $C_{16}$, $C_{17,}$ $C_{18}$, $C_{19}$, $C_{20}$,$C_{21}$ & $C_{25,}$ $C_{22}$ & $C_{24}$, $C_{23}$, $C_{25}$, $C_{26}$, Anal. Calcd. For: $C_{23}H_{15}ClF_5N_5O_3$ C 54.24% , H 2.65% and N12.16%. Found: C 46. 34%, H 1.93% and N 14.02%.

*8- (4-methylbenzylideneamino) – 3 – Chloro – 6 - (2, 5-difluorobenzoyl) – 1 - ((4 -(trifluoromethyl)phenyl)amino) - 1, 6, 7-triazaspiro [3, 4] - oct – 7 – ene - 2, 5 - dione* ***(9b)***
Yield 65%.m p: 114-116$^{O}$C. IR (KBr): 3230(stretching vibration of -NH), 1699(stretching vibration of >C=N), 1680(stretching vibration of cyclic carbonyl in five membered hetero cyclic ring), 1661(Exo cyclic >C=O group), 682(stretching vibration of C-Cl group), 1625(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(s, 1H, -CH of azetidinone group), 6.76-7.71(m, 12H, $C_6H_5$, $C_6H_4$ and $C_6H_3$ group), 8.48(s, 1H, -N=CH- group), 10.15(s, 1H, Ar-NH-N= group). $^{13}$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, 130.7, 129.1, 129.1, 140.7, 124.1, 21.3 Corresponding to $C_1$, $C_2$ & $C_6$, $C_3$ & $C_5$, $C_4$, $C_7$, $C_8$, $C_9$, $C_{10}$, $C_{11}$, $C_{12}$, $C_{13}$ , $C_{14}$, $C_{15}$, $C_{16}$, $C_{17,}$ $C_{18}$, $C_{19}$, $C_{20}$, $C_{21}$ & $C_{25,}$ $C_{22}$ & $C_{24}$, $C_{23}$, $C_{25}$, $C_{26}$, $C_{27,}$ 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%.

*8 - (4 - methoxybenzylideneamino) – 3 – Chloro – 6 - (2, 5-difluorobenzoyl) – 1 - ((4-(trifluoromethyl) phenyl) amino) - 1, 6, 7-triazaspiro [3, 4]-oct-7-ene-2, 5-dione* ***(9c)***
Yield 70%.m p: 148-150$^{O}$C. IR (KBr): 3234(stretching vibration of -NH), 1701(stretching vibration of >C=N), 1682(stretching vibration of cyclic carbonyl in five membered hetero cyclic ring), 1668(Exo cyclic >C=O group), 684(stretching vibration of C-Cl group), 1627(stretching vibration of >C=O group of azetidinone). $^{1}$H-NMR (400 $MH_Z$ DMSO-d6): 3.6(s, 3H, -$OCH_3$ group), 5.3(s, 1H, -CH of azetidinone group), 6.76-7.84(m, 12H, $C_6H_5$, $C_6H_4$ and $C_6H_3$ group), 8.48(s, 1H, -N=CH- group), 10.15(s, 1H, Ar-NH-N= group). $^{13}$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, 126.0, 130.2, 114.4, 162.9, 124.1, 55.8 Corresponding to $C_1$, $C_2$ & $C_6$, $C_3$ & $C_5$, $C_4$, $C_7$, $C_8$, $C_9$, $C_{10}$, $C_{11}$, $C_{12}$, $C_{13}$, $C_{14}$, $C_{15}$, $C_{16}$, $C_{17,}$ $C_{18}$, $C_{19}$, $C_{20}$, $C_{21}$ & $C_{25,}$ $C_{22}$ & $C_{24}$, $C_{23}$, $C_{25}$, $C_{26}$, $C_{27}$, 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%.

*8-(4-chlorobenzylideneamino)-3-Chloro-6-(2, 5-difluorobenzoyl) - 1 - ((4 - (trifluoromethyl) phenyl) amino) - 1, 6, 7-triazaspiro[3, 4] - oct - 7 - ene - 2, 5-dione* ***(9d)***
Yield 75%.m p: 108-110$^{O}$C. IR (KBr): 3220(stretching vibration of -NH), 1689(stretching vibration of >C=N), 1670(stretching vibration of cyclic carbonyl in five membered hetero cyclic ring), 1651(Exo cyclic >C=O group), 672(stretching vibration of C-Cl group), 1615(stretching vibration of >C=O group of azetidinone). $^{1}$H-NMR (400 $MH_Z$ DMSO-d6): 5.3(s, 1H, -CH of azetidinone group), 6.76-7.77(m, 12H, $C_6H_5$, $C_6H_4$ and $C_6H_3$ group), 8.48(s, 1H, -N=CH- group), 10.15 (s, 1H Ar-NH-N= group). $^{13}$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 $C_1$, $C_2$ & $C_6$, $C_3$ & $C_5$, $C_4$, $C_7$, $C_8$, $C_9$, $C_{10}$, $C_{11}$, $C_{12}$, $C_{13}$, $C_{14}$, $C_{15}$, $C_{16}$, $C_{17}$, $C_{18}$, $C_{19}$, $C_{20}$, $C_{21}$ & $C_{25}$, $C_{22}$ & $C_{24}$, $C_{23}$, $C_{25}$, $C_{26}$, 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 - (4-bromobenzylideneamino) – 3 – Chloro – 6 - (2, 5 - difluorobenzoyl) – 1 - ((4 -(trifluoromethyl )phenyl) amino)-1, 6, 7- triazaspiro[3, 4] - oct – 7 - ene-2, 5-dione* ***(9e)***

Yield 75%.m p: 135-137$^O$C. IR (KBr): 3223(stretching vibration of -NH), 1690(stretching vibration of >C=N), 1671(stretching vibration of cyclic carbonyl in five membered hetero cyclic ring), 1652(Exo cyclic >C=O group), 672(stretching vibration of C-Cl group), 1616(stretching vibration of >C=O group of azetidinone). $^1$H-NMR (400 $MH_Z$ DMSO-d6): 5.3(s, 1H, -CH of azetidinone group), 6.76-7.72(m, 12H, $C_6H_5$, $C_6H_4$ and $C_6H_3$ group), 8.48(s, 1H, -N=CH- group), 10.15(s, 1H Ar-NH-N= group). $^{13}$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, 132.7, 128.5, 131.8, 125.4, 124.1 Corresponding to $C_1$, $C_2$ & $C_6$, $C_3$ & $C_5$, $C_4$, $C_7$, $C_8$, $C_9$, $C_{10}$, $C_{11}$, $C_{12}$, $C_{13}$, $C_{14}$, $C_{15}$, $C_{16}$, $C_{17}$, $C_{18}$, $C_{19}$, $C_{20}$, $C_{21}$ & $C_{25}$, $C_{22}$ & $C_{24}$, $C_{23}$, $C_{25}$, $C_{26}$, 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%.

*8-(4-nitrobenzylideneamino)-3-Chloro - 6 - (2, 5 - difluorobenzoyl) - 1 - ((4 - (trifluoromethyl) phenyl) amino) - 1, 6, 7-triazaspiro [3, 4]- oct-7-ene-2, 5-dione* ***(9f)***

Yield 70%.m p: 124-126$^O$C. IR (KBr): 3215(stretching vibration of -NH), 1687(stretching vibration of >C=N), 1668(stretching vibration of cyclic carbonyl in five membered hetero cyclic ring), 1648(Exo cyclic >C=O group), 668(stretching vibration of C-Cl group), 1611(stretching vibration of >C=O group of azetidinone). $^1$H-NMR (400 $MH_Z$ DMSO-d6): 5.3(s, 1H, -CH of azetidinone group), 6.81-8.33(m, 12H, $C_6H_5$, $C_6H_4$ and $C_6H_3$ group), 8.48(s, 1H, -N=CH- group), 10.15(s, 1H Ar-NH-N= group). $^{13}$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, 139.7, 127.8, 124.0, 150.2, 124.1 Corresponding to $C_1$, $C_2$ & $C_6$, $C_3$ & $C_5$, $C_4$, $C_7$, $C_8$, $C_9$, $C_{10}$, $C_{11}$, $C_{12}$, $C_{13}$, $C_{14}$, $C_{15}$, $C_{16}$, $C_{17}$, $C_{18}$, $C_{19}$, $C_{20}$, $C_{21}$ & $C_{25}$, $C_{22}$ & $C_{24}$, $C_{23}$, $C_{25}$, $C_{26}$, 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 mass spectral fragmentation process of (9a) was presented in chart-II.I.II. The molecular ion peak was observed at m/z=577.08(33% ) and the base peak was noticed at m/z 575.08(100% ) peaks appeared at different m/z values and these were shown in the **Table.I**

**Table:I: Mass spectral data of primary fragmented ions for 8-(4-benzylideneamino)-3-Chloro-6-(2,5-difluorobenzoyl)-1-((4-(trifluoromethyl)phenylamino)-1,6,7-triazaspiro[3,4]-oct-7-ene-2,5-dione (9a)**

| Molecular ion | Lost radical | Primary fragmented ion | m/z values | Relative abundance (R.A) (%) |
|---|---|---|---|---|
| $C_{26}H_{15}ClF_5N_5O_3(M^+)$ m/z: 575.08(100%) 577.08(33%) | $C_6H_3F_2$• | $C_{20}H_{12}ClF_3N_5O_3^+$ | 462.06<br>464.06 | 35.2<br>10.9 |
| | $C_{20}H_{12}ClF_3N_5O_3$• | $C_6H_3F_2^+$ | 113.02 | 6.6 |
| | $C_7H_6N$• | $C_{19}H_{10}ClF_2N_4O_3^+$ | 471.03<br>473.03 | 22.5<br>9.5 |
| | $C_{19}H_{10}ClF_2N_4O_3$• | $C_7H_6N^+$ | 104.05 | 8.2 |
| | $C_7H_3F_2O$• | $C_{19}H_9ClF_5N_4O_3^+$ | 401.04<br>403.04 | 18.4<br>6.2 |
| | $C_{19}H_9ClF_5N_4O_3$• | $C_7H_3F_2O^+$ | 141.02 | 7.2 |
| | $C_7H_5F_3N$• | $C_{19}H_{12}ClF_3N_5O_2^+$ | 434.06<br>436.06 | 21.4<br>6.9 |
| | $C_{19}H_{12}ClF_3N_5O_2$• | $C_7H_5F_3N^+$ | 160.04 | 7.9 |

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. The fragmentation processes of primary fragmented ions to afford secondary fragmented ions were shown in the **Table II**

The fragmented ions containing one chlorine atom showed two m/z values with difference of two units and the corresponding relative abundances were in the ratio of 3:1

**Table:II:Mass spectral data of secondary fragmented ions for 8-(4-benzylideneamino)-3-Chloro-6-(2,5-difluorobenzoyl)-1-((4-(trifluoromethyl)phenylamino)-1,6,7-triazaspiro[3,4]-oct-7-ene-2,5-dione(9a)**

| Primary Fragmented ion | Lost radical | Secondary Fragmented ion | m/z values | Relative Abundance (R.A) |
|---|---|---|---|---|
| $C_{20}H_{12}ClF_3N_5O_3$+(B) | $CO,NC\dot{O}$ $C_7H_6\dot{N}$ | $C_{11}H_6ClF_3N_3O$+(J) | 288.02<br>290.02 | 12.0<br>3.7 |
| $C_{19}H_9ClF_5N_4O_3$+(E) | $C_6H_3\dot{F}_2,NC\dot{O}$ | $C_5HClN_2O_2$.+(K) | 155.97<br>157.97 | 5.5<br>1.6 |
| $C_{19}H_{10}ClF_2N_4O_3$+(F) | $C_6H_3F_2NCO$ | $C_{12}H_7ClN_3O_2$+(L) | 260.02<br>262.02 | 13.1<br>3.9 |
| $C_9H_3F_2O$+(H) | CO | $C_6H_3F_2$+(M) | 113.02 | 3.5 |
| $C_{19}H_{12}ClF_3N_5O_2$+(I) | $N_2$ | $C_{19}H_{12}ClF_3N_3O_2$+(N) | 406.0<br>408.0 | 20.8<br>6.9 |

***Biological activity***

The antimicrobial activity [20-22] of these newly synthesized compounds was performed according to disc diffusion method, as recommended by the National Committee for Clinical Laboratory [23]. The synthesized compounds Were used at the concentration of 250µg/ml DMF as a solvent [24].

***Antibacterial activity***

The antibacterial activity of 8-(4-benzylideneamino)-3-Chloro-6-(2,5-difluorobenzoyl)-1-((4-(trifluoromethyl) phenyl)amino)-1,6,7-triazaspiro[3,4]-oct-7-ene-2,5-dione (9a-f) were screened against the Staphylococcus aureus (gram positive) and Escherichiacoli (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 [25, 26] in the following Table III

***Antifungal activity***

Antifungal activity of 8-(4-benzylideneamino)-3-Chloro-6-(2,5-difluorobenzoyl)-1-((4-(trifluoromethyl) phenyl) amino)-1,6,7-triazaspiro[3,4]-oct-7-ene-2,5-dione (9a-f) were screened against Aspergillus niger, Candida albicans [27]. The presence of chloro,bromo and nitro in the structure has shown increased effect on their antibacterial activity in the following Table III

| Entry | Bacteria | | | | | | fungi | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | *Staphylococcus aureus NCCS2079* | | *Bacillus cereus NCCS 2106* | | *Escherichia coli NCCS2065* | | *Aspergillus niger NCCS 1196* | | *Candida albicans NCCS 2106* | |
| | 25 | 50 | 25 | 50 | 25 | 50 | 25 | 50 | 25 | 50 |
| 9a | - | 07 | - | 06 | - | 05 | - | 08 | - | 09 |
| 9b | - | 06 | - | 08 | - | 07 | - | 07 | - | 10 |
| 9c | - | 08 | - | 08 | - | 09 | - | 08 | - | 10 |
| 9d | 08 | 12 | 08 | 13 | 07 | 11 | 06 | 11 | 05 | 10 |
| 9e | 07 | 10 | 08 | 12 | 06 | 10 | 05 | 10 | 04 | 09 |
| 9f | 11 | 14 | 11 | 15 | 10 | 13 | 09 | 14 | 10 | 13 |
| Chloromphenicol (5) | - | 25 | - | 26 | - | 22 | - | - | - | - |
| Ketocanazole (50) | - | - | - | - | - | - | - | 16 | - | 18 |

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