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SYNTHESIS, CHARACTERIZATION AND EVALUATION OF ANTIMICROBIAL AND ANTIOXIDANT ACTIVITIES OF NEW SULFONAMIDE AND CARBAMATE DERIVATIVES OF 3-(TRIFLUOROMETHYL)-5,6,7,8-TETRA HYDRO-[1,2,4]-TRIAZOLO(4,3-A)-PYRAZINE (SITAGLIPTIN INTERMEDIATE)

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ABSTRACT
3-(Trifluoromethyl)-5,6,7,8-tetrahydro-[1,2,4]-triazolo(4,3-α)-pyrazine (Sitagliptin intermediate) **(1)** is a potent pharmacophore in sitagliptin and the drug is a selective dipeptidile peptidase-4 (DPP-4) inhibitor used for the treatment of type-2 diabetes mellitus (T2DM). The synthesis of a series of new sulphonamide **(7a-g)** and carbamate derivatives **(7h-j)** was accomplished in high yields by reacting equimolar quantities of substituted sulfonyl chlorides and chloroformates with compound **1** using 1,4-dimethyl piperazine as a base at 40-50 ^{o}C. The structures of the synthesized compounds were confirmed by IR, NMR (^{1}H,^{13}C), mass spectra and elemental analysis. The title compounds were screened for their antimicrobial antioxidant activities (DPPH, H_2O_2 and NO methods) and IC_{50} values were also determined. The compounds **7a, 7c** and **7f** exhibited potent activity while the rest showed moderate activity against bacteria, fungi and also antioxidant activity.

KEYWORDS:3-(trifluoromethyl)-5,6,7,8-tetrahydro-[1,2,4]-triazolo(4,3-α)-pyrazine, (Sitagliptin Intermediate), Dipeptidile Peptidase-4, Diabetes Mellitus-2, Antimicrobial activity, Antioxidant activity.

1. INTRODUCTION
Hetero cyclic compounds are the building blocks in organic synthesis and play an important role in the design and discovery of physiologically active compounds[1]. The fused aromatic heterocycles are very most important compound classes in drug discovery and play a vital role in living organisms. In particular, such scaffold is found as building blocks for DNA (guanine, adenine) and also in many approved drugs including slidenafil, zolpidem and trazodone, as well as in medicinal chemistry studies[2-5]. A series of novel β-amino amides incorporating in fused hetero cycles, i.e., triazolo pyrazines, were synthesized and evaluated their activity as inhibitors of dipeptidyl peptidase-IV (DPP-IV) and used for the treatment of type-2 diabetes[6]. The 3-(trifluoromethyl)-5,6,7,8-tetrahydro-[1,2,4]-triazolo(4,3-α)-pyrazine (Sitagliptin Intermediate) **(1)** is orally active DPP-IV inhibitor with excellent selectivity and oral bioavailability in preclinical species of the phosphate salt which was selected for development of new drug for the treatment of type-2 diabetes[7]. It was developed and marketed by Merk & Co. This drug inhibits the enzyme either alone or in combination with other oral anti hyper glycemic agents such as Metformin and Thiazolidenedione[8]. Type -2 diabetes mellitus (T2DM) is a global epidemic problem. The number of reported cases hasbeen doubled over the past 15 years[9]. Recently, dipeptidyl peptidase-IV (DPP -IV) inhibitors have emerged as a new class of anti-hyperglycemic agents for the treatment of T2DM[10] and offer several advantages over other existing anti diabetic agents such as lack of body weight gain and decreased incidence of hypoglycemia.

The (Sitagliptin intermediate) **(1)** is a selective, potent DPP-IV inhibitorand an active ingredient in JANUVIA and JANUMET (a fixed dose combination with the antidiabetic agent metformin) which have been approved for the treatment of type -2 diabetes by the FDA[11]. Triazolo pyrazine-based DPP-IV inhibitors , which led to the discovery of the phosphate salt of 3-(trifluoromethyl)-5,6,7,8-tetra hydro-[1,2,4]-triazolo(4,3-α)-pyrazine is currently in clinical development[12]. The sulfonamide derivatives occupy a unique position in the

drug industry with their potent antimicrobial and antioxidant properties[13]. The applications of sulfonamides have greatly extended from their primary functions as antitumor[14], hypoglycemic[15], antithyroid[16], carbonic anhydrase inhibitors[17], antiinflamma-tory[18], diuretic[19] and COX-inhibitors.

Sulfonamide–based medicines were the second antimicrobial agents, still widely used today for the treatment of various bacterial, protozoal and fungal infections[20] and the first effective chemotherapeutic agents to be available in safe therapeutic dosage in large ranges. They were the mainstay of therapy for bacterial infections in human beings before the introduction of penicillin in 1941[21]. The compounds containing sulfonyl groups have long been research focus as a result of their biological importance and chemical applications[22]. The carbamate moiety plays a pivot role in modern drug discovery in medicinal chemistry. It is found in drugs and also present in a more number of prodrugs as a means of achieving first-pass and systemic hydrolytic stability. Carbamates are playing a significant role in the human life and act as synthetic intermediates in medicinal, pharmaceutical industry and found in a variety of medicinally active compounds[23]. Carbamates also play an important role in the human life with different applications like the synthesis of pharmacological drugs and insecticides. They act as good antidiabetic[24], antioxidants[25], antimicrobial[26], antiviral, anticancer[27] and antitumor agents[28]. The organic carbamates are used in pharmaceutical industry, medicinal, agrochemical, polymer chemistry and also in peptide syntheses[29-31]. The carbamates serve as protecting groups for amines and amino acids in organic synthesis[32]. Valdagliptin[33] is used for increasing hormones in the body. Saxagliptin is an oral hyper glycemic agent and control blood glucose levels in diabetes-2[34]. The selective functionalization of 1,2,4-triazoles are one of the crucial biologically active class of heterocyclics. 1,2,4-Triazole derivatives are well known and found to exhibit antimicrobial[35-38], anticancer[39-41] and antitubercular activities and as therapeutically significant agents.

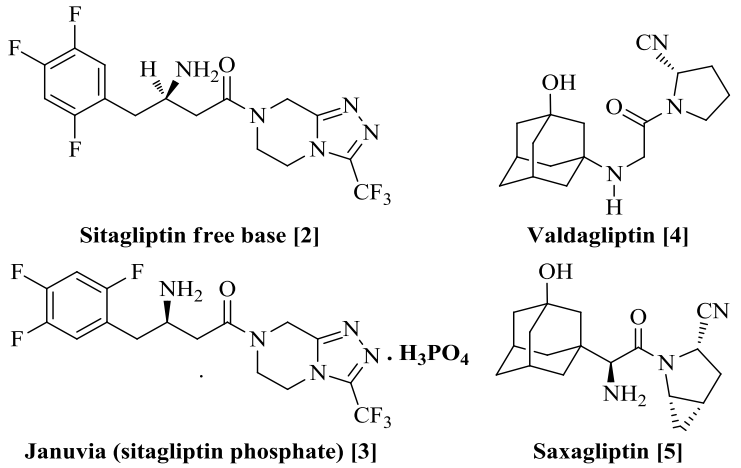


Fig-1: DPP-4 Inhibitors (2-5) used for the treatment of Type-2 diabetes.

A few drugs containing this heterocycle are Ribavirin (antiviral)[42], Rizatriptan (antimigraine)[43], Anastrozole and Letrazole (breast cancer)[44,45] which have showed in **Figure-2.**

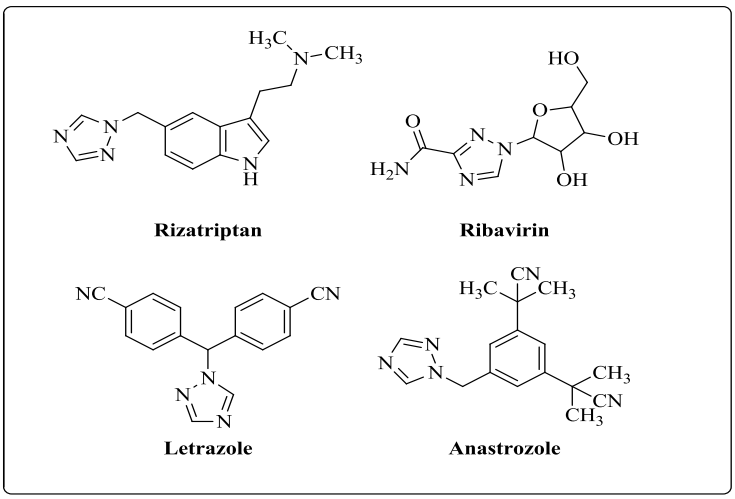


Figure-2: Biologically active drugs containing 1,2,4-triazole moiety.

Based on the review of the literature, medicinal and biological importance of triazolo pyrazine, sulfonamide and carbamate derivatives have led to the great challenge for chemists fordesigning new biologically active libraries which may be useful in treating the diseases. As a part of our research, we have synthesized a new series of sulphonamide and carbamate derivatives of 3-(trifluoromethyl)-5,6,7,8-tetrahydro-[1,2,4]-triazolo(4,3-α)-pyrazine (**1).** The title compounds were screened for their antimicrobial and antioxidant activities.

2. MATERIALS AND METHODS

2.1. Materials & Instruments

All the required chemicals and reagents were procured from Merck, Aldrich and S. D. Fine (India). Solvents were purified by distillation and drying using the appropriate drying agents. Melting points were determined in open capillaries on Guna melting point apparatus and are uncorrected. IR Spectra were recorded on JASCO-FT IR 5300 unit as KBr discs. The absorption peaks were expressed in cm^{-1}. ^1H and ^{13}C NMR Spectra were recorded on Bruker AV-400 Spectrometer operating at 400 MH_Z for 1HNMR, 125 MH_Z for ^{13}C NMR. The results are presented as chemical shift **i**n δ values/ ppm, multiplicity as 's' for singlet, 'd' for doublet, 't' for triplet, 'dd' for double doublet and 'm' for multiplet and **J** values in H_Z . Mass spectra were recorded on ESQUIRE 3000 mass spectrometer. The results are expressed in m/z values. The progress of the reactions was monitored by TLC on Merck Silica plates.

2.2. Methods

General procedure for the synthesis of Sulfonamide/Carbamate derivatives of sitagliptin drug Intermediate (1),(7a-j)

The 3-(trifluoromethyl)-5,6,7,8-tetrahydro-[1,2,4]-triazolo(4,3-α)-pyrazine **(1)** (1 m mol. 220 mg) was taken into 10 mL of THF contained in a 50 mL round bottom flask. Later 1,4-dimethyl piperazine (DMP) (1.2 m mol) was added. Then the reaction mixture was stirred for 2 hours at 65°C. Then DMP. HCl salt was removed from the reaction mixture by filtration. The filtrate was taken in a R.B. flask and 5 mL of THF and DMP (1.2 m mol) were added to the reaction mixture. Later 4-fluorophenyl sulfonyl chloride (**6a**) (1.0 m mol) contained in THF (10 mL) was added drop wise at 10°C to the reaction mixture. Later the reaction mixture was stirred for 4-5 hours by gradual raising of temperature up to 50°C. The progress of the reaction was monitored by TLC. After the completion of the reaction, the DMP. HCl precipitate was removed by filtration and the solvent was evaporated under vacuum to get the product, **7a**. The derivatives of the other products **7b-j** were synthesized by adopting the same procedure by reacting compound **1**with different sulfonyl chlorides **6b-g** and chloroformates **6h-j.** All the products were purified by simple workup followed by silica gel column chromatography using ethyl acetate and hexane (20-40%) as an eluent.

SPECTRAL DATA

7-(4-Flurophenylsulfonyl)-3-(trifluoromethyl)5,6,7,8tetrahydro[1,2,4]triazolo[4,3,] pyrazine (7a): Yield 83%**,** White solid, mp 201-203^0 C, IR (KBr): 1012 (-C-N), 1349 (-S=O_2, asymstr), 1439 (-N=N),1140(-SO_2, symstr), 1545 (-C=N) ; ^{1}H-NMR (500 MHz, DMSO-d_6, ppm): δ 7.99-7.47 (4H, m, Ar-H), 4.57 (2H, s, Pyrazine), 4.18 (2H, t, J=4.1 Hz, Pyrazine), 3.66 (2H, t, J=3.6 Hz, Pyrazine); ^{13}C-NMR (100 MHz, DMSO-d_6 ,ppm): δ 166.7 ($C_{4'}$), 132.4 ($C_{1'}$), 131.2 ($C_{2',6'}$), 122.7($C_{3',5'}$), (C-Aromatic), 117.1 (C_{10}, CF_3) ,164.2(C_5), 150.0 (C_7), 40.5(C_6) , 43.2 (C_2), 39.3 (C_3), (C-Pyrazine) : MS m/z;350.05 $(M+H)^+$. LCMS calculated for $C_{12}H_{10}F_4N_4O_2S$: C, 41.15; H, 2.88, N, 15.99, Found: C, 41.24; H, 2.86; N, 15.89.

7-(4-Iodophenylsulfonyl)-3-(trifluoromethyl)-5,6,7,8-tetrahydro[1,2,triazolo[4,3,α]pyrazine (7b): Yield 81%, White solid, mp 208-210^0 C; IR (KBr): 1026 (-C-N), 1347 (-SO_2asymstr), 2923 (-N-H), 1140 (-SO_2 symstr), 1506 (-C=N); ^{1}H-NMR (400 MHz, DMSO-d_6, ppm): δ 8.04 (2H, d, J=8.0 Hz, Ar-H), 7.65 (2H , d, J=7.6 Hz , Ar-H) 4.56 (2H, s, , Pyrazine), 4.17(2H, t, J=4.1 Hz, Pyrazine), 3.65 (2H, t, J=3.6 Hz, Pyrazine).

7-(4-Bromophenylsulfonyl)-3-(trifluoromethyl)-5,6,7,8-tetrahydro[1,2,4]triazolo[4,3,α] pyrazine (7c): Yield 80%, White solid, mp 205-207^0 C; IR (KBr): 1136 (-C-N), 1346 (-SO_2 asymstr), 1465 (-C=N), 1140 (-SO_2 symstr), 3410 (N- H) ; ^{1}H-NMR (400 MHz, DMSO-d_6, ppm) : δ (7.8-7.7 (4H, m, Ar-H), 4.53(2H, s, Pyrazine),4.1 (2H, t, J=4.1 Hz, Pyrazine), 3.6 (2H, t, J=3.6 Hz, Pyrazine).

7-(4-Nitrophenylsulfonyl)-3-(trifluoromethyl)-5,6,7,8-tetrahydro[1,2,4]triazolo[4,3,α] pyrazine (7d): Yield 79%, White solid , mp 220-222^0 C;. IR (KBr) : 115.1 (-S=O), 1514 (-NO_2), 1140 (-SO_2 symstr), ^{1}H-NMR 400 MHz , (DMSO-d_6, ppm); δ 8.45 (2H, d, J=8.4 Hz, Ar-H), 8.20 (2H, d, J=8.2 Hz, Ar-H), 4.65 (2H, s, Pyrazine), 4.23 (2H, t, J=4.2 Hz, Ar-H), 3.72 (2H, t, J=3.7 Hz, Ar-H).

7-(4-Chloro-3-nitrophenylsulfonyl)-3-(trifluoromethyl)-5,6,7,8-tetrahydro[1,2,4]triazolo [4,3,α] pyrazine (7e): Yield 75%, White solid, mp 215-217 ; ^{0}C.IR (KBr) : 1014 (-C-N), 1354 (-S=O), 1520 (-N=O), 1140 (-SO_2 symstr), 3847 (-N-H): ^{1}H-NMR (400MHz, DMSO-d_6, ppm) : δ 8.60 (1H, d, J=8.5 Hz, Ar-H), 8.20 (1H , m, Ar-H), 8.08 (1H, d, J=8.05 Hz, Ar-H), 4.66 (2H , s, Pyrazine), 4.25 (2H , t, J=4.2 Hz, Pyrazine), 3.73 (2H, s , Pyrazine).

7-(2-Nitrophenylsulfonyl)-3-(trifluoromethyl)-5,6,7,8-tetrahydro[1,2,4]triazolo[4,3,α] pyrazine (7f): Yield 76%, White solid, mp 150-152^0 C;. IR (KBr) : 1013 (-C-N), 1361 (-S=O^{2}asymstr), 1527 (-N=O), 1140 (-SO_2 symstr); ^{1}H-NMR (400 MHz, DMSO-d_6, ppm) : δ 8.28 (1H, d, J=8.2 H_Z, Ar-H), 8.11 (1H, d, J=8.10 H_Z, Ar-H), 8.01 (1H, d, J=8.0 H_Z, Ar-H), 7.98

(1H, d, J=7.9 H_Z,Ar-H), 4.65 (2H, s, Pyrazine), 4.3 (2H, t, J=4.3 H_Z,Ptrazine),3.91 (2H, t,J=3.9 H_Z, Ar-H).

7-(4-Chlorophenylsulfonyl)3--(trifluoromethyl)-5,6,7,8-tetrahydro[1,2,4]triazolo[4,3,α] pyrazine (7g): Yield 80%, White solid, Mol.wt.366.75, mp 198-200^0 C; .IR (KBr) :1005 (-C-N), 1133 (-SO_2asymstr),1497 (-C=N), 1140 (-SO_2 symstr), 3420 (-N-H);): ^{1}H-NMR (400 MHz , DMSO-d_6, ppm) : δ 7.92 (2H, d, J=7.9 Hz , Ar-H), 7.74 (2H, d, J=7.7 Hz, Ar-H), 4.58 (2H, s, Pyrazine), 4.19 (2H, t, J=4.1 Hz, Pyrazine), 3.67 (2H,t, J=3.6 Hz, Pyrazine).

7-(4-Nitrophenyl-3-(trifluoromethyl)-5,6-dihydro-[1,2,4]-triazolo[4,3,α]pyrazine-7(8H)-carboxylate (7h): Yield 81% , Brown solid, mp 175-177^0 C; IR (KBr) : 1005 (-C-N), 1418 (-N=N),1504 (-N=O), 1730 (-C=O), 3336 (-N-H) : ^{1}H-NMR (400 MHz, DMSO-d_6, ppm) : δ 8.32-8.30 (2H, m, Ar-H), 7.53-7.51 (2H, m, Ar-H), 5.12 (2H, s, Pyrazine), 4.34 (2H, s, Pyrazine), 3.35 (2H, s, Pyrazine).

Isobutyl-3-(trifluoromethyl)-5,6-dihydro-[1,2,4]-triazolo[4,3,α]pyrazine-7(8H)-carboxyl-ate (7i): Yield 85%, Ash coloured solid, mp183-185^0 C; IR (KBr) : 1135 (-C-N), 1506 (-C=N), 1718 (-C=O);^{1}H-NMR (400 MHz, DMSO-d_6, ppm) : δ 4.4-4.2 (5H , m, Pyrazine), 3.3 (1H, s, Pyrazine), 2.4 (2H, d, J=2.4 Hz, Aliphatic), 1.93 (1H, m ,Ali), 0.91 (6H, d, J=0.9 Hz, , Ali) ; ^{13}C-NMR (100 MHz, DMSO-d_6 ,ppm): δ 153.3 (-C=O), 148.5($C_{5,7}$), 44.0 (C_6), 43.0 (C_2), 39.6 (C_3), 115.3(CF_3), (Pyrazine); 73.5 (C_{12}), 39.4 (C_{13}), 27.8 (C_{14}), 19.2(C_{14}), ($C_{Aliphatic}$); MS m/z : 293.1 $(M+H)^+$. LCMS calculated For $C_{11}H_{15}$ F_3 N_4O_2: C, 45.21; H, 5.17; N, 19.17. Found C,45.33, H, 5.21, N, 19.07.

Ethyl-3-(trifluoromethyl)-5,6-dihydro-[1,2,4]-triazolo[4,3,α]pyrazine-7(8H)-carboxylate (7j) : Yield 83% , Ash coloured solid, Mol.wt.264.20, mp 153-155^0 C; IR (KBr) :1007 (-C-N), 1503 (-C=N), 1772 (-C=O):^{1}H-NMR (400 MHz, DMSO-d_6 , ppm) : δ 4.14 (2H, q, ,Ali-H), 1.29 (3H, t, J=1.2 Hz, CH_3), 4.48 (2H, s, Pyrazine), 4.30 (2H, s, Pyrazine), 3.34 (2H, s, Pyrazine).

Pharmacological Activity

Antibacterial activity

All the newly synthesized compounds **7(a-j)** have been assayed for their *in vitro* antibacterial activity by disc diffusion method[46] Gatifloxacin was used as a standard drug for antibacterial against pathogenic representative Gram-positive *Bacillus subtilis*(MTCC-441) *Basillus cereus* (MTCC-7190) and Gram-negative *Escherichia coli* (MTCC-40). The bacterial strains selected for this study are most common and easily available. They were grown individually in Luria Broth (LB) medium and the cell separation was spread over the surface of Muller-Hilton agar (MHA) plates with sterile spreaders. The plates were allowed to dry and a sterile well borer of 6 nm diameter was used to cut uniform wells in the agar. After incubation at 37 ^{0} C for 24 h, the plates were observed for a zone of inhibition (ZOI) in diameter around the well. The antibacterial activity was evaluated by measuring the diameter of clear Zone of inhibition. The inhibition zones of the test compounds were measured and compared with standard.

Antifungal activity

Antifungal activity was screened against two plant phathogenic fungi, viz, *Candida albicans* and *Aspergillus fumigates* (ATTC 9197)[47] and Amphotericin-B was used as a standard. Fungal stains *Candida albicans* and *Aspergillus fumigates*, Potato dextrose agar (PDA) was used as a medium to test fungi. Glass petri dishes were sterilized in an oven and melted PDA medium was poured into each petri dish. After solidification of the medium small portions of mycelium of each fungus were spread carefully over the centre of each PDA plate with the help of a sterilized needle. Then each fungus was transferred to a series of PDA plates. The PDA plates were then incubated at (25±2^0 C) and after five days of incubation they were ready for use Plates were inverted and incubated at 37 ºC for 72 hrs. The zone of inhibition was measured to assess for pathogenecity of test compound. The experiment was repeated thrice. After the incubation period, the diameter of inhibition zone was measured and documented as an indicator for the activity of the compounds.

Antioxidant activity

(DPPH) radical scavenging activity: The free radical scavenging activity by this method was determined by observing the bleaching of the purple colored methanol solution of 1,1-diphenyl-2-picrylhydrazyl (DPPH)[48]. The compounds which have hydrogen atom or electron donation ability only react with free radicals produced by the DPPH. 1 mL each compound four concentrations were prepared (25, 50, 75, and 100 µg/mL) in methanol and added to 4 mL of 0.004% w/v methanol solution of DPPH. After 30 min incubation period at room temperature, the absorbance was read against blank at 517 nm. Ascorbic acid was used as the standard. The per cent of inhibition of free radical production from DPPH was calculated by the following equation. Tests were carried out in triplicate. The % scavenging of DPPH radical was calculated by following equation.

$$\%\ Inhibition = \frac{(A\ Control - A\ sample)}{A\ Control} x100;$$ IC_{50} in µg/mL$= \frac{50x100}{\%\ Inhibition}$;

IC_{50} in µmol/mL$= \frac{\%\ of\ the\ IC_{50}}{MW\ of\ the\ Compound}$

Where $A_{control}$ is the absorbance of control (DPPH solution without the test compound) and A_{sample} is the absorbance of control (DPPH solution with the test compound solution).

Nitric oxide (NO) scavenging assay

Sodium nitroprusside[49] (5 µM) in phosphate buffer p^H 7.4 was incubated with different concentrations (25, 50,

75, and 100 µg / mL) of test compounds dissolved in methanol and tubes were incubated at 25°C for 2 h. Control experiment was conducted with equal amount of solvent in an identical manner. At intervals, 0.5 mL of incubation solution was taken and diluted with 0.5 mL of Griess reagent (1% Sulfanilamide, 0.1% N-naphthylethylenediaminedihydrochloride and 2% o-phosphoric acid dissolved in distilled water). The chromophore absorption was read at 546 nm which wasd after diazotization of nitrite with sulfonylamide and chlorides. The chromophore formed after diazotization of nitrite with sulfanilamide and subsequent N-naphthylethylenediaminedihydrochloride was read at 546 nm. The experiment was repeated in triplicate. Nitric oxide scavenging activity was calculated by the following equation is evaluated below.

% of scavenging = [(A control - A sample) / A control] X 100

Where (A control is the absorbance of the standard and - A sample is the absorbance in the presence of the sample and standard.

Hydrogen peroxide (H_2O_2) scavenging assay

The H_2O_2[50]scavenging ability of the title compounds was determined according to the method of solution of H_2O_2 (40 mm) was prepared in phosphate buffer (p^H 7.4). 25, 50, 75, 100 µg/mL concentrations of the test compounds in 3.4 mL of phosphate buffer were added to H_2O_2 solution of 0.6 mL, 40 mm). The absorbance value of the reaction mixture was recorded at 230 nm. Ascorbic acid was used as a standard. The per cent of scavenging of H_2O_2 was calculated using the following equation.

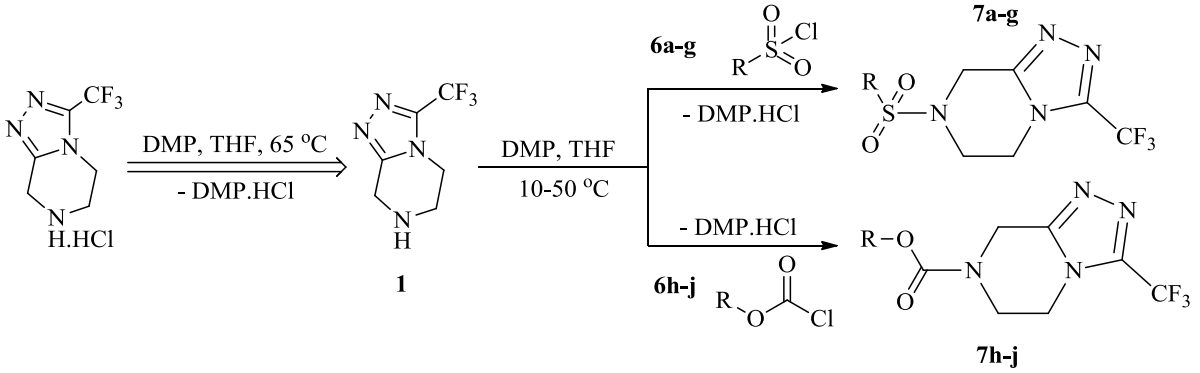
% of scavenging = [(A control - A sample) / A control] X 100

Where (A control is the absorbance of the standard and - A sample is the absorbance in the presence of the sample and standard.

RESULTS AND DISCUSSION

Chemistry

The synthetic strategy for the preparation of the title compounds, **7a-j** is depicted in **Scheme–1**. This is a two-step process. In the first step,3-(trifluoromethyl)-5,6,7,8-tetrahydro-[1,2,4]-triazolo(4,3-α)-pyrazine hydrochloride, the starting material was refluxed with N,N-dimethyl piperazine (DMP) in THF contained in a 50 mL RB flask at 60°Cto get the free base, **1**. In the second step, the N-H proton of **1** was substituted with sulfonamide and carbamate moieties using various sulfonylchlorides**6a-g** and chloroformates **6h-j**in the presence of DMP in THF contained in a 50 mL RB flask at 10-50°C to obtain the title compounds, **7a-j.** All the title compounds **7a-j** were isolated by simple workup and purified by silica gel column chromatography.



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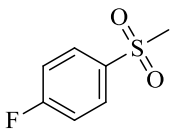
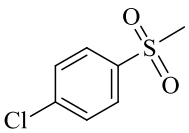
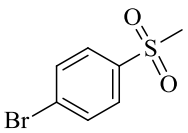
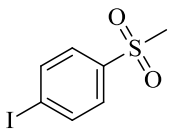
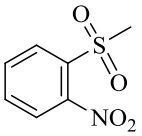
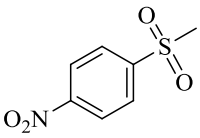
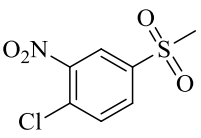
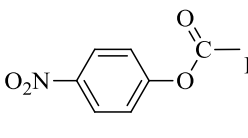
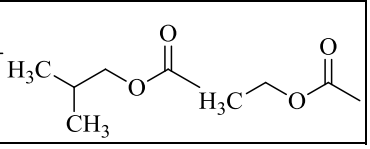
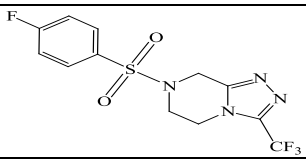
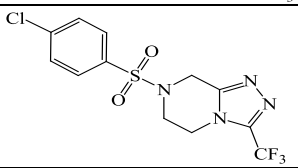
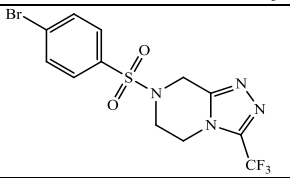
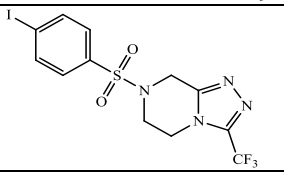
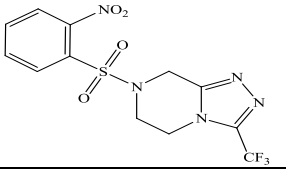
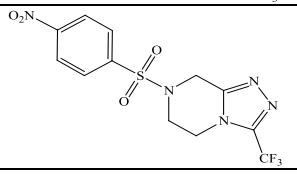
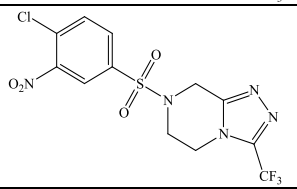
Structure of R					
Compound Code	**6f**	**6g**	**6h**	**6i**	**6j**
Structure of R					

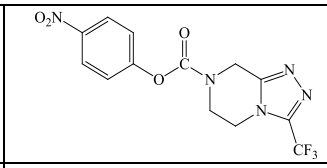
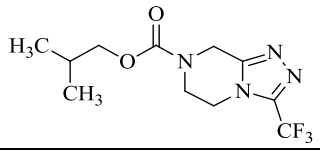
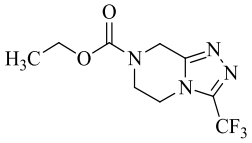
Scheme 1: Synthesis of sulfonamide/carbamate derivatives of 3-(trifluoromethyl)-5,6,7,8-tetrahydro-[1,2,4]-triazolo(4,3-α)pyrazine (7a-j).

All the newly synthesized title compounds were characterized by IR, ^1H and ^{13}C NMR and mass spectrometry and CHN analysis. In IR absorption bands for symmetric stretching of O=S=O were observed in the range of 1085-1140 cm^{-1} and asymmetric stretching bands in the range of 1310-1349 cm^{-1}.In ^1H NMR, a

multiplet signal at δ 7.99-7.47 indicates the aromatic protons; singlet and multiplet signals in the range of δ 4.57-3.66 are assigned to pyrazine moiety. In ^{13}C NMR spectra, appearance of signals in the range of δ 166.7-122.7 indicates the aromatic carbons of benzene moiety and peaks in the region of δ 40.5-39.3 are assigned to the pyrazine moiety. EIMS were recorded for representative compounds**7a** and **7i**. The **7a** gave $[M+H]^+$ion peak at m/z 351 and **7i** gave $[M+H]^+$ peak at m/z 293 corresponding to their molecular masses. The CHN analyses reports for **7a** and **7i**were in good agreement with their expected values. The title compounds **(7a-j)** were screened for antioxidant activity by DPPH, nitric oxide (NO) and hydrogen peroxide (H_2O_2) methods at 50, 100, 150 and 200 µg/mL concentrations. The structure-activity relationship of the tested compounds revealed that the compounds bearing fluorine, bromine, triazole and pyrazine moieties, showed higher radical scavenging activity when compared with those having other substituents in all three methods. Further, it was noticed that fluorine, bromine substituted compounds displayed higher antioxidant activity than that of chlorine, iodine, nitrogen substituted compounds. It was also observed that the compounds **7a** and **7c** exhibited good antioxidant activity, whereas other compounds displayed low activity. Further, the perusal of data presented in Tables 1, 2, 3 indicates that radical scavenging activity increases with increase in concentration in all the three methods. The compounds **7a** bearing 4-fluorophenylsulfonyl group**, 7c** bearing 4-bromophenylsulfonyl group exhibited high antioxidant activities. They exhibited promising antioxidant activity in the scavenging percentage range of 75-80% as compared with standard, Ascorbic acid (84-85%) at 100 µg/mL concentrations in all the three methods. The yield and physical data of the titled compounds are given in **Table-1**.

Physical data of the title compounds 7(a-j) compounds are given in Table -1.

Compound	Product	Reaction time (h)	Yield (%)	Mp (^{0}C)
7a		4	85	201-203
7b		4	80	198-200
7c		4	80	205-207
7d		4	81	208-210
7e		4.5	70	150-152
7f		4	79	220-222
7g		4	75	215-217

7h		4	82	175-177
7i		4	85	183-185
7j		4	78	153-155

Biological Activity

Anti microbial activity

The anti microbial activity of the newly synthesized title compounds, **(7a-j)** was screened against three bacterial and one fungal strains by disc diffusion method. Later their ZOI was measured.

The test solutions 100 µg/mL of the samples were prepared in dimethylformamide (DMF). The antibiotic Gatifloxacin was used as a standard for antibacterial activity.

Antibacterial activity

The newly synthesized sulfonamides **7(a-g)** and carbamates **7(h-j)** were screened against two Gram positive bacteria such as *Basillussubtilis MTCC 441*and *Bacillus cereus MTCC 7190*one Gram negative bacteria such as *Escherichia coli, MTCC 40* by the agar–well – diffusion method[46]. Four different concentrations (50, 100, 150 and 200 µg/mL) of the title compounds were prepared by dissolving in 1 mL of DMF. A 24 hour culture of the pathogenic strains were grown individually on Luria Broth (LB) medium and the cell separation was spread over the surface of Muller-Hilton agar (MHA) plates with sterile spreaders. The plates were allowed to dry and a sterile well borer of 6 nm diameter was used to cut uniform wells in the agar. After incubation at 37^{o}C for 24 hours, the plates were observed for a zone of inhibition (ZOI) in diameter around the well. Gatifloxacin was used as a standard drug for antibacterial assay. The zone of inhibition of the tested compounds was compared with standard. The bio-screening data revealed that two of the title compounds **7a** bearing 4-fluorophenylsulfonyl moiety and **7c** bonded with 4-bromophenylsulfonyl moiety exhibited good antibacterial activity against ***B. subtilis***, ***B. cereus*** and also ***E. coli***. The bacterial screening was performed in triplicate and their mean values were taken for SAR analysis which was depicted in **Table-S-1.** The title compounds showed their potential to serve as a good platform for further investigation in order to discover new derivatives having an improved overall biological profile.

Table -2: ***Inhibition zone (diameter) in mm of synthesized compounds (7a-j) tested bacterial strains by agar well diffusion method.***

Name of the Compounds	*E.coli* (µg/mL)				*Bacillus subtilis*(µg/mL)				*Basillus cereus* (µg/mL)			
	50	**100**	**150**	**200**	**50**	**100**	**150**	**200**	**50**	**100**	**150**	**200**
7a	1.4	2.4	3.7	8.7	0.2	2.7	3.5	7.5	-	1.4	2.8	6.9
7b	-	-	0.2	0.3	-	-	-	0.5	-	-	0.1	0.4
7c	1.1	3.2	6.4	10.2	0.2	2.2	3.4	7.3	1.4	2.8	4.6	7.9
7d	0.1	1.0	1.3	4.1	-	0.1	1.3	3.4	-	0.7	2.6	4.7
7e	-	-	0.2	0.3	-	-	0.1	0.3	-	-	0.3	0.4
7f	-	-	0.1	0.2	-	-	0.1	0.3	-	-	0.2	0.3
7g	-	-	0.1	0.2	-	-	0.1	0.2	-	-	0.1	0.2
7h	1.3	2.5	2.8	5.3	1.0	1.1	1.3	1.5	1.0	2.2	2.7	3.7
7i	0.1	1.0	1.3	4.1	-	0.2	1.3	3.4	-	0.7	2.6	4.7
7j	-	0.3	1.2	2.0	-	-	0.6	3.0	-	-	0.9	3.3
Std	2.2	3.9	6.6	12.3	5.6	9.1	13.0	18.0	4.0	8.0	12.5	17.0

Std: Gatifloxacin

* Antibacterial activity was carried out at 50,100, 150, 200 µg/mL

Anti fungal *activity*

The antifungal activity of the newly synthesized title compounds **7(a-j)** was screened against two pathogenic mold fungi viz, *Candida albicans* and *Aspergillus fumigates* (ATTC 9197)[47] and Amphotericin-B was used as the standard. The antifungal activity was

assessed by poisoned food technique with some modifications. Potato dextrose agar (PDA) was used as a medium to test fungi. Glass petri dishes were sterilized and melted PDA medium was poured into each petri dish. After the solidification of the medium, small portions of the mycelium of each fungus were spread carefully over the centre of each PDA plate with the help of a sterilized needle. Thus each fungus was transferred to a number of PDA plates. The PDA plates were then incubated at (25 ± 2^0 C) and after five days of incubation they were ready for use. The prepared discs of test samples were placed gently on the solidified agar plates and freshly seeded the test organisms with sterile forceps. The plates were then kept in a refrigerator at 4^0 C for 24 h in order that the materials have sufficient time to defuse to a considerable area of the plates. Afterwards the plates were incubated at 37^oC for 72 hours. Compounds **7a** bearing 4-fluoro phenyl sulfonyl group and **7c** bonded with 4- bromo phenyl sulfonyl moiety exhibited good antifungal activity shown in **Table-S-2.**

Table -3: *In vitro* evaluation of antifungal activity of the synthesized compounds 7(a-j)

Name of the compounds	*C. albicans* (µg/mL)				*A. fumigates* (µg/mL)			
	50	**100**	**150**	**200**	**50**	**100**	**150**	**200**
7a	0.5	2.0	3.3	8.0	0.5	1.5	2.9	6.4
7b	-	0.3	0.8	1.0	0.2	1.1	1.3	1.8
7c	1.0	3.3	6.2	10.1	1.0	2.2	4.1	7.5
7d	0.1	1.3	1.8	3.0	0.2	1.1	2.3	4.0
7e	-	-	0.1	0.3	-	0.1	0.3	0.8
7f	-	0.2	0.3	0.5	-	0.1	0.3	0.6
7g	-	-	0.1	0.2	-	-	0.2	0.4
7h	-	0.3	1.1	2.0	0.1	0.5	0.8	1.4
7i	-	0.5	1.3	2.2	0.1	0.8	1.3	3.5
7j	0.2	1.0	1.2	3.3	1.0	1.3	3.2	4.3
Std	2.5	3.8	6.0	12.1	1.5	2.8	5.6	11.8

Std: Amphotericin-B

*Antifungal activity was carried out at concentrations 50, 100, 150 and200µg/Ml

Antioxidant activity

The free radical scavenging activity by this method was determined by observing the bleaching of the purple colored methanol solution of 1,1-diphenyl-2-picrylhydrazyl (DPPH)[48]. The newly synthesized sulfonamide/carbamate derivatives of 3-(trifluoromethyl)-5,6,7,8-tetrahydro–[1,2,4]-triazolo(4,3-α)pyrazine **(7a-j)** derivatives were tested at different concentrations (25, 50, 75, 100 µg/mL) and showed potential to moderate activity. Among the compounds **7a** and **7c** exhibited good antioxidant activity, the reason might be the presence of electron withdrawing groups (fluoro and bromo groups) on phenyl ring of sulfonyl derivatives as shown in **Table S-3**. In NO method[49], compounds **7a** and **7c**exhibited good activity as shown in **Table-S-4.** In H_2O_2[50] method **7b,7e, 7g, 7h,7j** having good activity because the presence of electron withdrawing groups (chloro and nitro groups) on phenyl ring of sulfonyl and carbamate derivatives as shown in **Table-S-5.**The wide variations in free radical scavenging activities may be attributed to the various substituents on the phenyl ring.

Table -4: *The in vitro antioxidant activity of title compounds 7(a-j) by DPPH method*.

Compounds	Concentration (in µg/ mL)				
	25	**50**	**75**	**100**	**IC_{50} (µg/ mL)**
7a	37.71±0.12	44.21±0.14	59.34±0.11	72.54±0.19	56.54±0.05
7b	5±0.4	9.91±0.01	13.05±0.28	15.43±0.33	125.51±0.03
7c	35±0.19	45.22±0.34	63.24±0.21	72.29±0.39	55.28±0.23
7d	19±0.11	21.93±0.41	24.26±0.41	27.42±0.11	91.17±0.06
7e	5.5±0.2	8.1±1.02	12.5±0.31	15.2±0.23	20.3±0.19
7f	5.1±0.3	9.2±0.03	11.0±0.31	14.3±0.12	18.5±0.14
7g	4.8±0.4	8.12±0.15	13.53±0.65	16.8±0.16	21.8±0.033
7h	12±0.15	18.94±0.24	21.20±0.20	24.01±0.15	104.16±0.14
7i	15±0.32	19.22±0.25	24.16±0.08	25.82±0.16	96.82±0.12
7j	12±0.20	15.12±0.17	18.95±0.15	21.32±0.21	117.26±0.19
Ascorbic acid	72±0.15	76.18±0.17	78.29±0.15	80.14±0.10	34.72±0.12

Values were the means of three replicates ±SD

Table -5: The *in vitro* antioxidant activity of title compounds (7a-j) by NO method.

Compounds	Concentration (in µg/ mL)				
	25	50	75	100	IC_{50} (µg/ mL)
7a	35.71±0.12	40.21±0.14	56.34±0.11	62.54±0.19	44.37±0.05
7b	06±0.4	19.91±0.10	23.05±0.28	25.43±0.33	98.30±0.03
7c	45±0.19	55.22±0.34	60.24±0.21	72.29±0.39	55.55±0.23
7d	21±0.11	24.93±0.41	27.26±0.41	31.42±0.11	79.56±0.06
7e	6±0.2	9.1±1.02	13.5±0.31	15.9±0.23	20.5±0.19
7f	4.1±0.3	6.2±0.03	9.0±0.31	13.3±0.12	17.5±0.14
7g	4.8±0.4	8.12±0.15	13.53±0.65	16.8±0.16	21.8±0.03
7h	19±0.15	25.91±0.24	27.20±0.20	32.07±015	78.12±0.14
7i	17±0.32	20.22±0.25	23.16±0.08	30.82±0.16	81.82±0.12
7j	12±0.20	15.12±0.17	18.95±0.15	21.32±0.21	117.26±0.19
Ascorbic acid	75±015	80.18±0.17	83.29±0.15	86.14±0.10	33.33±0.12

Values were the means of three replicates ± SD

Table -6: The *in vitro* antioxidant activity of 7(a-j) compounds by H_2O_2method

Compounds	Concentration (in µg/ mL)				
	25	50	75	100	IC_{50} (µg/ mL)
7a	33.71±0.12	41.13±0.14	53.18±0.13	61.88±0.19	47.16±0.21
7b	05±0.11	16.72± 0.16	19.33±0.11	22.91±0.12	109.1±0.32
7c	49.39±0.18	59.06±0.14	67.03±0.14	77.71±0.13	50.61±0.11
7d	20.53±0.16	5.41±0.12	29.83±0.19	34.11±0.18	73.29±0.14
7e	5.6±0.23	6.1±0.13	6.6±0.22	9.3±0.29	268.33±0.33
7f	4.8±0.13	5.5±0.15	9.1±0.20	13.2±0.22	189.39±0.12
7g	5.1±0.10	5.8±0.13	6.5±0.19	10.2±0.11	245.09±0.22
7h	12.81±0.11	16.02±0.11	19.33±0.12	23.14±0.12	108.03±0.21
7i	18.23±0.19	21.04±0.17	24.61±0.13	26.09±0.11	95.82±0.20
7j	09.13±0.12	11.89±0.16	14.69±0.13	18.39±0.13	135.94±0.19
Ascorbic acid	71.19±0.18	76.18±0.17	78.29±0.15	80.14±0.10	35.17±0.22

Values were the means of three replicates ± SD

5. CONCLUSION

Finally it may be concluded, that a series of new sulfonamide/carbamate derivatives of 3-(trifluoromethyl)-5,6,7,8-tetrahydro-[1,2,4]-triazolo-(4,3-α)-pyrazine (sitagliptin intermediate) were designed and synthesized in high yields and their antimicrobial and antioxidant activities were evaluated. The compounds **7a** and **7c** exhibited potent antimicrobial and antioxidant activities. The results encouraged for the investigation of new potential lead compounds,**7a, 7c, 7e,7f** and**7g** to screen *in vivo* and also in the study of anti diabetic activity. Hence, the present investigation highlights the synthesized compounds **7(a-j)** will be the promising next generation anti-microbial drugs, which can be effectively used in the treatment of microbial infections. Further the title compounds may be screened for their antidiabetic activity as they contain sitaglyptin intermediate moiety in the near future.

ACKNOWLEDGEMENTS

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