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Novel Butan-2-ylidene Benzohydrazides; Synthesis, Antimicrobial Evaluation and Molecular Docking Study

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Abstract: A variety of butan-2-ylidene benzohydrazides were synthesized via the reaction of benzohydrazides with 4,4,4-triflouro-1-phenyl-1,-3-butanedione/4,4,4 – triflouro -1, 2, - (2 – napthyl) -1, 3 – butanedione. Acetylation and benzoylation of butan-2-ylidene benzohydrazides gave the derivatives previously unknown. The compounds were characterized by spectral analyses (Mass spec., IR, and NMR), and were evaluated for their *in vitro* antimicrobial activity against some clinical isolates of interest. The *in vitro* screening revealed that some of the compounds possessed strong inhibitory potentials against the isolates with IZD in the range of 7-14 mm. Predicted ADMET ensured the druggability properties of the synthesized compounds. The molecular docking study indicated compounds**7a** and **9a** to possess the highest docking store and established significant interactions in the active site of the target protein.

Keywords: Butan-2-ylidene benzohydrazides, Acetylation, Benzoylation, Clinical isolat*es*, *In vitro*, Drug-likeness

1.0 Introduction

Human exposure to pathogens has led to bacterial and fungal infections which have been one of the pivotal global health issues in recent memory. The treatment of these broad ranges of microbial infections is complicated because lots of microbes including bacteria, viruses, and fungi are becoming resistant due to inordinate and extended use of standard marketed antimicrobial and antibiotics drugs (Cassir *et al*. 2014). Antimicrobial resistance (AMR) and the tampering off of potent antimicrobial drugs are considered a major terror to human health. Multi-drug resistance of bacteria and fungi such as Methicillin-resistant *Staphylococcus aureus* (MRSA), Vancomycin-resistant *Staphylococcus aureus* (VRSA), Vancomycin-resistant *enterococcus* (VRE) and Fluconazole-resistant *Candida* have attained horrifying level becoming a global life-threatening medical issue (Mishra *et al*. 2013, Cetinkaya *et al*. 2000, Gulshan *et al*. 2007). The fight against drug-resistant microorganisms involves the strategic development of new, cheap, and more powerful innovative antimicrobial agents with minimal adverse effects (Khan *et al*. 2005, Klenke *et al*. 2003).

Therefore, the need to develop novel broad-spectrum antibacterial motifs against both Gram-positive (G+) and Gram-negative (G-) bacteria for the treatment of bacterial infections, especially drug-resistant strains remains a practicable objective of most chemists.

Benzohydrazide serves as one of the lead compounds in drug discovery. Published papers revealed that both natural and synthetic benzohydrazides possess a wide spectrum of pharmacological properties including antimalarial (Li *et al.* 1995), anti-inflammatory (Bayrak *et al.* 2009), antioxidant (Anto *et al.* 1995, Mukherjee *et al.* 2001), antileishmanial (Nielsen *et al*. 1998), antifungal (Rollas *et al.* 2000), antibacterial (Osório *et al*. 2012), antimycobacterial (Koçyiğit-Kaymakçıoğlu *et al*. 2012), anticancer (Veeramanikandan *et al*. 2015), antitumor (Galal *et al*. 2009), antimicrobial (Kirilmis *et al*. 2008), antiarrhythmic (Bourgery *et al.* 1981).

Furthermore, reports in recent years showed that hydrazide analogs having azomethine (–CONHN=CH-) group possess significant biological properties such as analgesic, anticonvulsant,

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antagonistic, anti-inflammatory (Sriram *et al.* 2006, Narasimhan *et al.* 2007, Duarte *et al.* 2007).

Moreover, *in vitro* metabolism analyses of hydrazide biological functionalities indicate that they can readily experience hydrolytic reactions, which is an advantage to treat several infectious diseases (Kömürcü *et al.* 1995, Ulgen *et al.* 1997).

Inspired by these findings, we decided to design some new libraries of benzohydrazides for their antimicrobial investigation. We had earlier reported other biological motifs as likely antimicrobial agents (Ezeokonkwo *et al*. 2018, 2019). In continuation of our effort to construct potent antimicrobial agents, we herein report the synthesis of (Z)-(substituted)-N'-(1,1,1-trifluoro-4-oxo-4-phenylbutan-2-ylidene)benzohydrazides (**3a-c**), (Z)-(substituted)-N'-(1,1,1-trifluoro-4-(naphthalen-2-yl)-4-oxobutan-2-ylidene)benzohydrazides (**5a-b**), (Z)-(substituted)-N-acetyl-N'-(1,1,1-trifluoro-4-oxo-4-phenylbutan-2-ylidene)benzohydrazides (**7a-b**), and (Z)-(substituted)-N-benzoyl-N'-(1,1,1-trifluoro-4-oxo-4-phenylbutan-2-ylidene)benzohydrazides (**9a-c**). The structural characterization of the synthesized compounds was done using UV, FTIR, NMR, and mass spectroscopy. The antimicrobial potentials of the synthesized compounds were evaluated *in vitro* via agar-well dilution method. The compounds were also assessed for their drug-likeness, and molecular docking was carried out to find out the binding affinities of the compounds and their bonding interactions with the target protein.

2.0 Results and Discussion

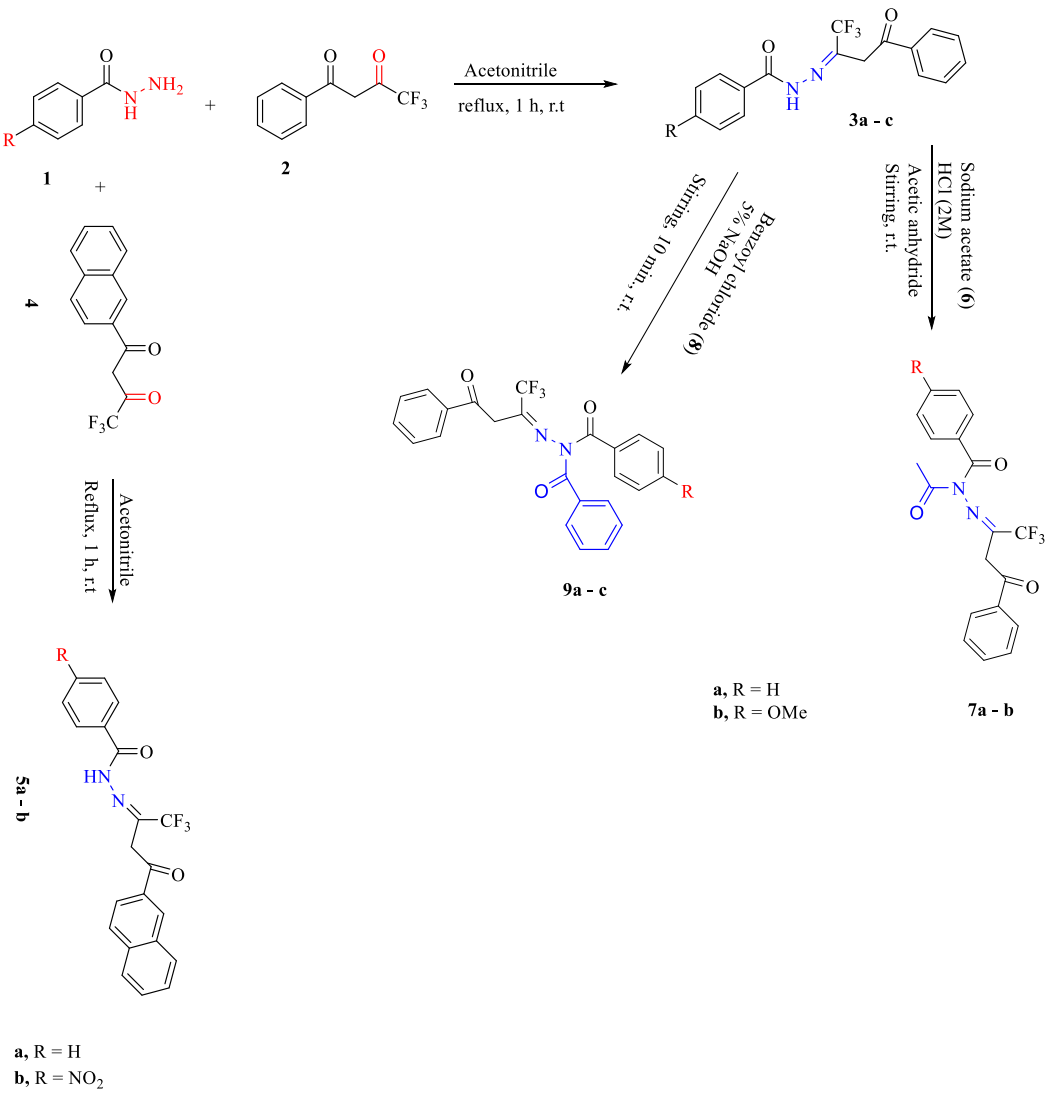
2.1 Chemistry

The synthetic strategies to the target compounds bearing the benzohydrazide moiety are illustrated in **scheme 1**. The room temperature refluxing of benzohydrazides (**1a-c**) and 4,4,4 – triflouro- 1-phenyl-1,-3-butanedione (**2**) in acetonitrile afforded the (Z)-(substituted)-N'-(1,1,1-trifluoro-4-oxo-4-phenylbutan-2-ylidene)benzohydrazides ***(3a-c)***. In the FTIR spectrum of compound **3c** for example, the band at 3490 cm^{-1} is due to N-H stretching, while the bands at 1653, 1498, and 1254 cm^{-1} are due to C=O, C=N, and N=O stretching respectively. In the ^{1}H NMR spectrum of compound **3c**, it was possible to observe peaks at 11.83 ppm due to N-H proton (H-9), 3.60 ppm due to methylidyne proton (H-12), while aromatic protons were observed in the range of 7.91-7.46 ppm. ^{13}C NMR spectrum of compound **3c** showed the characteristic carbon peaks in the compound such as 168.29 ppm for C=O (C-13), 166.47 ppm for C=O (C-7), 165.41 ppm for Ar-C-N=O (C-2), 158.41 ppm for C=N (C-11), 116.33 ppm (C-15), 48.81 ppm (C-12), while aromatic carbons were observed in the range of 149.96-127.40 ppm. The mass spectrum is consistent with the theoretical value.

Compounds (**3a-c**) were converted to their corresponding acetyl derivatives (**7a-b**) by acetylation using hydrated sodium acetate (**6**), and benzoyl derivatives (**9a-c**) via benzoylation using benzoylchloride (**8**) at room temperature. In the representative compound **7a**, the absorption bands at 1649 and 1495 cm^{-1} in the FTIR spectrum are due to C=O and C=N stretching respectively. The ^{1}H NMR spectrum of compound **7a** showed peaks at 3.70 ppm assigned to the methyl protons (H-24), 2.33 ppm assigned to methylidyne proton (H-12), while aromatic protons were observed in the range of 7.99-7.08 ppm. The ^{13}C NMR spectrum of compound **7a** showed peaks at 195.54, 179.33 and 170.67 ppm for C=O (C-13, C-7, and C-22 respectively), 154.08 ppm for C=N (C-11), 113.02 (C-15), while aromatic protons were observed at peaks in the range of 150.01-124.74 ppm.

In the representative compound **9b**, the stretching for C=O, C=N, and C-O in the FTIR spectrum was observed at 1651, 1498, and 1094 cm^{-1} respectively. In the ^{1}H NMR spectrum of compound **9b**, the peaks in the range of 8.14-7.07 ppm are due to aromatic protons, while the peaks at 3.92 and 3.31 are due to methyl protons (H-22) and methylidyne proton (H-12). The ^{13}C NMR spectrum of compound **9b** showed signals at 172.67 pmm due to C=O (C-13), 167.84 ppm due to C=O (C-7 and C-23). Other signals shown are in agreement with the proposed structure.

Furthermore, the reaction of benzohydrazides (**1a-b**) and 4,4,4-trifluoro-1-(naphthalen-2-yl)butane-1,3-dione (**4**) in acetonitrile at room temperature produced the ***(***Z)-(substituted)-N'-(1,1,1-trifluoro-4-(naphthalen-2-yl)-4-oxobutan-2-ylidene)benzohydrazides ***(5a-b)***. The compounds were obtained in good to excellent yields. The FTIR spectrum of compound **5a** for example showed absorption bands at 3445 cm^{-1} assigned to N-H, 1651 cm^{-1} assigned to C=O, and 1497 assigned to C=N stretching. The signals observed in the ^{1}H NMR and ^{1}C NMR spectra of compound **5b** also support the proposed structure.



Scheme 1. Synthetic rout to the target compounds

2.2 Antimicrobial activity

2.2.1 Antifungal

The synthesized compounds were evaluated for their antifungal activity against *C. albican* and *A. niger*. The result of the sensitivity test is presented in **Table 1**. Compounds **3c, 7a-b, 9a** showed marked antifungal activity against *C. albican*, with compound **7a** being the most active with the IZD of 14 mm. All the compounds were active against *A. niger* except compounds **3b, 7a** and **9c**, with compound **5a** showing the strongest activity with the IZD of 11mm.

2.2.2 Antibacterial

The synthesized compounds were screened for their antibacterial potential against gram +ve and gram –ve bacterial strains. All the compounds except compound **7b** were active against *S. typhi* with the IZD in the range of 7-11 mm. Compound **5b** showed marked activity against *E. coli* with the IZD of 11.5 mm. Compounds **5a** and **7a** revealed the highest inhibition against *S. aureus* and *B. subtilis* with the IZD of 10.5 and 10 mm respectively, while compound **5b** (IZD: 9 mm) had a comparable antibacterial inhibitory potential to Gentamicin standard (IZD: 10 mm) against *Shingella. sp*.

Table 1. Inhibition Zone Diameter (IZD) (mm) of the synthesized compounds

Comp.	R	*B. subtilis*	*S. aureus*	*Listeria sp.*	*E. coli*	*S. typhi*	*Klebsiella sp.*	*Pseudomonas sp.*	*Shigellia sp.*	*C. albican*	*A. niger*
3a	H	8	9	-	-	8	-	-	-	-	8
3b	OMe	-	-	-	-	9.5	8	11.5	-	-	-
3c	NO_2	-	-	-	-	9	-	-	-	11	8
5a	H	-	10.5	-	-	7	-	-	-	-	11
5b	NO_2	-	-	-	11.5	7.5	-	8	9	-	9
7a	H	10	-	7	-	11	-	-	-	14	-
7b	OMe	-	-	-	-	-	-	-	-	10.5	7
9a	H	9.5	-	-	-	10	-	8	-	10.5	-
9b	OMe	-	-	10.5	-	8	-	10	-	-	8.5
9c	NO_2	8	-	-	-	7.5	-	-	-	-	-
St. (A)		22	20	20	20	20	20	18	10	28	28
St. (B)		23	20	25	23	22	28	22	18	25	20

St. (A) = Gentamicin, St. (B) = Cyprofloxacin

2.3 In silico ADMET properties

The drug-likeness of the synthesized compounds was evaluated using SwissADME free online tool (http://www.swissadme.ch/). Lipinski's rule of five is a vital assessment of a drug-like molecule. According to the rule (MW ≤500, HBD ≤5, HBA ≤10, Log p ≤5, RBC ≤10) (Lipinski 2000), the synthesized compounds possessed good drug-like properties with zero violation of the rule, **Table 2**. Also, the compounds possessed tPSA in the range of 58.53-112.63 Å^2 (Veber rule: tPSA ≤140 Å^2) (Veber *et al.* 2002), which further validates their good oral bioavailability and cell permeability.

Table 2. *In-silico* ADMET screening of the synthesized compounds

Compound	MW	HBD	HBA	Log P	RBC	Lipinski's Violation	tPSA [Å^2]	Bioavailability score
Rule	≤500	≤5	≤10	≤5	≤10		-	-
3a	334.29	1	6	3.69	7	0	58.53	0.55
3b	364.32	1	7	3.74	8	0	89.14	0.55
3c	379.29	1	8	3.07	8	0	104.35	0.55
5a	384.35	1	6	4.58	7	0	58.53	0.55
5b	429.35	1	8	3.97	8	0	104.35	0.55
7a	376.33	0	7	3.79	8	0	66.81	0.55
7b	406.35	0	8	3.80	9	0	76.04	0.55
9a	438.40	0	7	4.89	8	0	66.81	0.55
9b	468.42	0	8	4.84	10	0	76.04	0.55
9c	483.40	0	9	4.43	10	0	112.63	0.55

2.4 Molecular Docking Analysis

Molecular docking is important in corroborating the binding reliability and interaction poses of ligands in the binding site of kinases (Tanoli *et al.* 2014). Docking study was performed by Autodock tools to predict the interaction mode of the benzohydrazides in the active site of the target protein. The Crystal structure of CDP-D-glucose 4,6-dehydratase from *Salmonella typhi* Ct18 (PDB code: 1WVG) containing the co-crystallized ligand (Cytidine-5'-diphospho-beta-d-xylose) (**Figure 3**) is available in the RCSB protein data bank (https://www.rcsb.org/structure/1WVG).

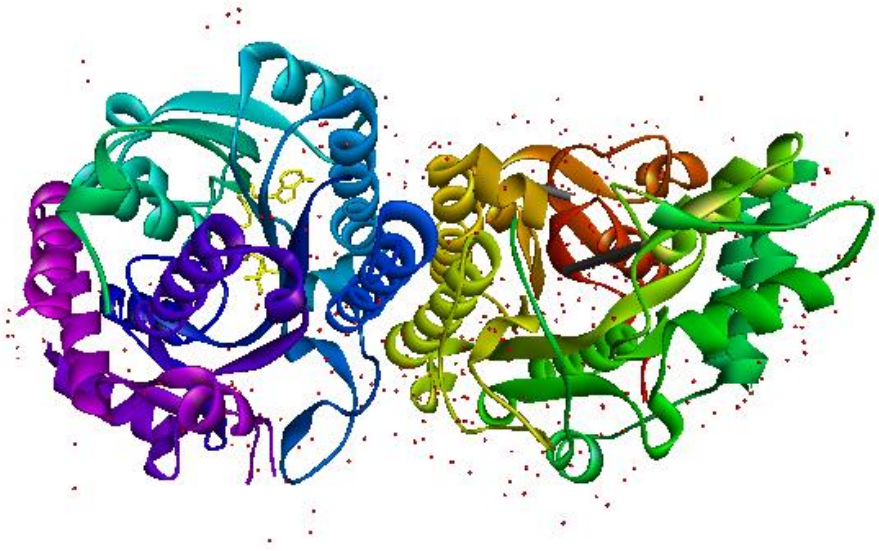


Figure 1: 2D Docking pose of target protein with the co-crystallized ligand

All the synthesized compounds except **9c** revealed stronger binding affinity with the protein, exhibiting better binding energy than ciprofloxacin standard and the native ligand, **Table 3**. Compounds **7a** and **9a** had the highest binding energy with -10.10 and -9.80 Kcal/mol respectively. The binding interactions of the most active compounds **7a** and **9a** against S. *typhi* have been shown in **Figures 4** and **5**. Compounds **7a** and **9a** are well stabilized in the active pocket of the target protein forming significant hydrogen bonding and other hydrophobic and electrostatic interactions. Compound **7a** established hydrogen bonding with VAL 1198, ARG 1208 and LYS 1023 residues, while compound **9a** formed hydrogen bonding with ARG 1232, ARG 1208, TRY 1159 and LYS 1023 residues. These strong chemical interactions may explain the relatively high inhibitory potentials of these compounds against S. *typhi*.

Table 3: Free binding energy of the synthesized compounds and the native ligand with the target protein (1WVG)

Compound	Binding energy ΔG (Kcal/mol)
3a	-9.60
3b	-8.90
3c	-9.40
5a	-9.60
5b	-8.80
7a	-10.10
7b	-9.60
7c	-9.70
9a	-9.80
9b	-9.00
9c	-7.90
Native ligand	-8.50
Ciprofloxacin	-8.80

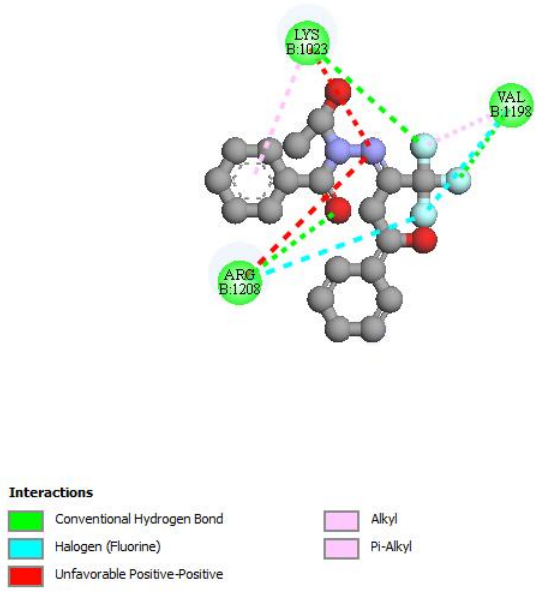


Figure 2: 2D bonding interaction of 7a with the protein (1WVG)

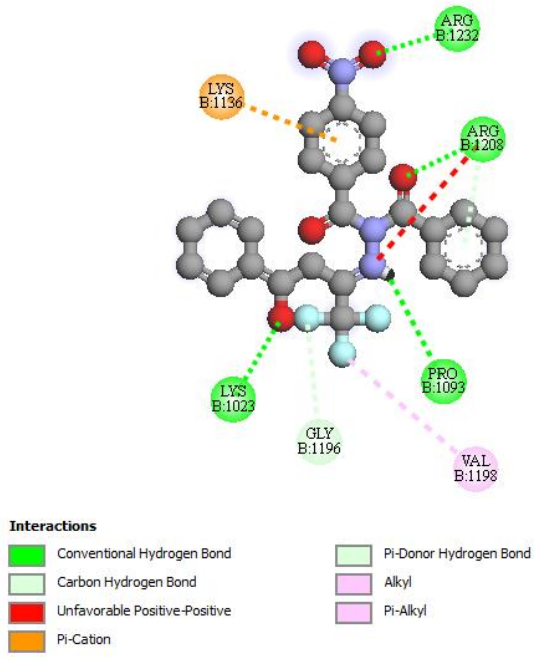


Figure 3: 2D interactions of 9a with the protein (1WVG)

Conclusions

We have synthesized novel benzohydrazides and evaluated their antimicrobial potentials against clinical isolates of interest. Some of the target compounds demonstrated weak to excellent inhibition against the tested microorganisms. All the compounds synthesized were active against *S. typhi*, with compounds **7a** and **9a** showing the highest sensitivity. *In silico* drug-like prediction favoured the oral bioavailability of the synthesized compounds. Molecular docking results revealed that the compounds had better binding affinity than ciprofloxacin thereby depicting good inhibition potential. Though the target compounds demonstrated lower *in vitro* antimicrobial inhibition compared to Gentamicin and Ciprofloxacin standard, the results suggest that these compounds are a good starting point for the rational development of novel antimicrobial agents.

3.0 Materials and methods

Chemistry

All commercial chemicals were used as received from Sigma-Aldrich and all solvents were purified using standard procedures prior to use. For thin-layer chromatography (TLC) analysis, Merck pre-coated plates (silica gel 60 F254) were used and spots were visualized under UV light. Melting points (m.p) determination was performed using Guoming Melting point tester and are uncorrected. Ultraviolet and visible spectra were recorded on UV-VIS G6860A spectrophotometer using matched 1 cm quart cell; absorption maxima were given in nanometers (nm). The IR spectra were recorded on 630 FT-IR spectrometer. ^{1}H NMR spectra were recorded on a Bruker Avance II 400 MHz and ^{13}C NMR spectra on Varian Mercury-400 100 MHz in DMSO as a solvent and tetramethylsilane (TMS) as an internal standard. Mass spectra were scanned on a Bruker Daltonics micrOTOF spectrometer at School of Science and Engineering Laboratory, University of Wakaito, Hamilton, New Zealand. Chemical shifts were reported in ppm units with the use of δ scale. The antimicrobial screening was done at the Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka. All the products were purified through repeated recrystallization using suitable solvent(s).

3.1 *General procedure for the preparation of (Z)-(substituted)-N'-(1,1,1-trifluoro-4-oxo-4-phenylbutan-2-ylidene)benzohydrazides (3a-c).*

A mixture of benzohydrazides (**1a-c)** (6.94 mmol), 4,4,4 – triflouro- 1-phenyl-1,-3-butanedione (**2)** (6.94 mmol), acetonitrile (100 ml) was refluxed for 1 h at room temperature. At the completion of the reaction as monitored using TLC, the mixture was filtered via suction, and the crude product obtained was washed well with distilled water, air dried, and recrystallized from methanol or ethanol.

3.1.1 *(Z)-N'-(1,1,1-trifluoro-4-oxo-4-phenylbutan-2-ylidene)benzohydrazide (3a)*

White crystals; yield: 81%; m.p: 175-177 ◦C; Uv-Vis (MeOH) (nm): 300 (1.5136), 410 (1.2023), 550 (1.0965); IR (KBr) (cm^{-1}): 3427 (N-H), 2931 (C-H aromatic), 1649 (C=O), 1496 (C=N); ^{1}H NMR (400 MHz, DMSO:d_6) δ ppm: 7.91 (s, 1H, H-N), 7.89-7.45 (m, 10H, Ar-H), 3.53 (s, 1H, CH); ^{13}C NMR (100 MHz, DMSO:d_6) δ ppm: 174.71 (C=O), 167.84 (C=O), 154.08 (C=N), 151.06-129.09 (Ar-C), 119.37

(CF_3), 59.00; MS: *m/z* 263.0284, 333.0188 (M-H). Anal. Calcd for $C_{17}H_{13}F_3N_2O_2$ (334.32).

3.1.2 ***(Z)-4-methoxy-N'-(1,1,1-trifluoro-4-oxo-4-phenylbutan-2-ylidene)benzohydrazide* (*3b*)**
Creamy white crystals; yield: 67%; m.p: 191-193 ◦C; Uv-Vis (MeOH) (nm): 300 (1.5136), 410 (1.1482), 550 (1.0232); IR (KBr) (cm^{-1}): 3401 (N-H), 2935 (C-H aromatic), 1647 (C=O), 1496 (C=N), 1062 (C-O); ^{1}H NMR (400 MHz, DMSO:d_6) δ ppm: 7.89 (s, 1H, N-H), 7.68-7.28 (m, 9H, Ar-H), 3.59 (s, 3H, O-CH_3), 3.39 (s, 1H, CH); ^{13}C NMR (100 MHz, DMSO:d_6) δ ppm: 171.29 (C=O), 170.74 (C=O), 168.43 (Ar-OCH_3), 158.45 (C=N), 142.50-127.06 (Ar-C), 105.86 (CF_3), 48.82 (O-CH_3), 40.02; MS: *m/z*: 211.0246, 293.0604, 363.0567 (M-H). Anal. Calcd for $C_{18}H_{15}F_3N_2O_3$ (364.10).

3.1.3 ***(Z)-4-nitro-N'-(1,1,1-trifluoro-4-oxo-4-phenylbutan-2-ylidene)benzohydrazide (3c)***
White powder; yield: 85%; m.p: 212-214 ◦C; Uv-Vis (MeOH) (nm): 300 (2.6303), 410 (1.7380), 550 (1.5136), 750 (1.2590); IR (KBr) (cm^{-1}): 3490 (N-H), 2931 (C-H aromatic), 1653 (C=O), 1498 (C=N), 1254 (N=O); ^{1}H NMR (400 MHz, DMSO:d_6) δ ppm: 11.83 (s, 1H, N-H), 7.91-7.46 (m, 9H, Ar-H), 3.60, (s, 1H, CH); ^{13}C-NMR (100 MHz, DMSO:d_6) δ ppm: 168.29 (C=O), 166.47 (C=O), 165.41 (Ar-C-NO_2), 158.41 (C=N), 149.96-127.40 (Ar-C), 116.33 (CF_3), 48.8; MS: *m/z* 308.0387, 378.0340 (M-H). Anal. Calcd for $C_{17}H_{11}F_3N_3O_4$ (379.08).

3.4 ***General procedure for the preparation of (Z)-(substituted)-N'-(1,1,1-trifluoro-4-(naphthalen-2-yl)-4-oxobutan-2-ylidene)benzohydrazide (5a-b).***
A mixture of benzohydrazides (**1a-b)** (5.63 mmol), 4,4,4-trifluoro-1-(naphthalen-2-yl)butane-1,3-dione(**4)** (5.63 mmol), acetonitrile (100 ml) was refluxed for 1 h at room temperature. At the completion of the reaction as monitored using TLC, the mixtrure was filtered via suction, and the crude product obtained was washed well with distilled water, air dried, and recrystallized from methanol.

3.4.1 ***(Z)-N'-(1,1,1-trifluoro-4-(naphthalen-2-yl)-4-oxobutan-2-ylidene)benzohydrazide (5a)***
White crystals; yield: 61%; m.p: 226-228 ◦C; Uv-Vis (MeOH) (nm): 300 (1.3804), 410 (1.5136), 550 (1.4261), 750 (1.2589); IR (KBr) (cm^{-1}): 3445 (N-H), 2931 (C-H aromatic), 1651 (C=O), 1496 (C=N); ^{1}H NMR (400 MHz, DMSO:d_6) δ ppm: 7.92 (s, 1H, N-H), 7.90-6.56 (m, 12H, Ar-H), 3.72 (s, 1H, CH); ^{13}C NMR (100 MHz, DMSO:d_6) δ ppm: 168.29 (C=O), 166.47 (C=O), 158.41 (C=N), 149.96-127.40 (Ar-C), 116.53 (CF_3), 48.81; MS: *m/z*: 369.0282, 370.0252, 371.0362, 383.0441, 384.0453 (M^+). Anal. Calcd for $C_{21}H_{15}F_3N_2O_2$ (384.36).

3.4.2 ***(Z)-4-nitro-N'-(1,1,1-trifluoro-4-(naphthalen-2-yl)-4-oxobutan-2-ylidene)benzohydrazide (5b)***
White powder; yield: 57%; m.p: >300 ◦C; Uv-Vis (MeOH) (nm): 300 (1.2589), 550 (1.0233); IR (KBr) (cm^{-1}): 3429 (N-H), 2933 (C-H aromatic), 1649 (C=O), 1496 (C=N), 1254 (N=O); ^{1}H NMR (400 MHz, DMSO:d_6) δ ppm: 8.36 (s, 1H, N-H), 8.10-7.45 (m, 11H, Ar-H), 3.66 (s, 1H, CH); ^{13}C NMR (100 MHz, DMSO:d_6) δ ppm: 170.18 (C=O), 167.84 (C=O), 150.05 (C=N), 146.53-128.65 (Ar-C), 124.91 (CF_3), 44.27; MS: *m/z*: 315.9474, 341.2036, 368.0946, 308.1068, 418.9731. Anal. Calcd for $C_{21}H_{14}F_3N_3O_4$ (429.36).

3.5 ***General procedure for the preparation of (Z)-N-acetyl-(substituted)-N'-(1,1,1-trifluoro-4-oxo-4-phenylbutan-2-ylidene)benzohydrazides (7a-b)***
(Z)-(substituted)-N'-(1,1,1-trifluoro-4-oxo-4-phenylbutan-2-ylidene)benzohydrazides (**3a-c**). (1.33 mmol) was dissolved in 2M HCl (10 ml) and crushed ice (10 g) was added. To this, a solution of hydrated sodium acetate (**6**) (5 g) in water (25 ml) was added, followed by acetic anhydride (5 ml) in drops with constant stirring at room temperature until the smell of the acetic anhydride vanished. The mixture was filtered via suction, and the crude product obtained was washed well with distilled water, air dried, and recrystallized from ethanol.

3.5.1 ***(Z)-N-acetyl-N'-(1,1,1-trifluoro-4-oxo-4-phenylbutan-2-ylidene)benzohydrazide (7a)***
White crystals; yield: 54%; m.p: 101-103 ◦C; Uv-Vis (MeOH) (nm): 300 (1.5136), 410 (1.2589), 550 (1.0233); IR (KBr) (cm^{-1}): 2933 (C-H aromatic), 1649 (C=O), 1495 (C=N); ^{1}H NMR (400 MHz, DMSO:d_6) δ ppm: 7.99-7.08 (m, 10H, Ar-H), 3.70 (s, 3H, CH_3), 2.47 (s, 1H, CH); ^{13}C NMR (100 MHz, DMSO:d_6) δ ppm: 195.54 (C=O), 179.33 (C=O), 170.67 (C=O), 154.08 (N=C), 150.01-124.74 (Ar-C), 113.02 (CF_3), 44.24 (O-CH_3), 40.38; MS: *m/z* 261.0606, 357.0315. Anal. Calcd for $C_{19}H_{15}F_3N_2O_3$ (376.34).

3.5.2 ***(Z)-N-acetyl-4-methoxy-N'-(1,1,1-trifluoro-4-oxo-4-phenylbutan-2-ylidene)benzohydrazide (7b)***
White crystals; yield: 54%; m.p: 98-100 ◦C; Uv-Vis (MeOH) (nm): 300 (1.5136), 410 (1.2589), 550 (1.0233); IR (KBr) (cm^{-1}): 2931 (C-H aromatic), 1649 (C=O), 1496 (C=N), 1095 (C-O); ^{1}H NMR (400 MHz, DMSO:d_6) δ ppm: 7.92-7.13 (m, 9H, Ar-H), 4.42 (s, 3H, OCH_3), 3.53 (s, 1H, CH); ^{13}C NMR (100 MHz, DMSO:d_6) δ ppm: 173.07 (C=O), 167.85 (C=O), 141.44-118.36 (Ar-C), 111.94 (CF_3), 57.19 (O-CH_3), 39.48; MS: *m/z* 363.0914, 399.0686. Anal. Calcd for $C_{20}H_{17}F_3N_2O_4$ (406.36).

3.6 ***General procedure for the preparation of (Z)-N-benzoyl-(substituted)-N'-(1,1,1-trifluoro-4-oxo-4-phenylbutan-2-ylidene)benzohydrazides (9a-c)***

N' – (1,1,1 – triflouro-4-oxo-4-phenylbutan-2-ylidene)benzohydrazides (**3a-c)** (1.33 mmol) was added to a 5% NaOH solution (10 ml) in a conical flask. To this, benzoylchloride (**8**) (1 ml) was added in drops with constant shaking in an ice bath. The resulting mixture was stirred vigorously for 10 minutes at room temperature until the smell of benzoylchloride vanished. The mixture was filtered via suction, and the crude product obtained was washed well with distilled water, air dried, and recrystallized from ethanol.

3.6.1 ***(Z)-N-benzoyl-N'-(1,1,1-trifluoro-4-(naphthalen-2-yl)-4-oxobutan-2-ylidene)benzohydrazide (9a)***

White crystals; yield: 43%; m.p: 144-146 ◦C; Uv-Vis (MeOH) (nm): 300 (1.5136), 410 (1.2589), 550 (1.0233); IR (KBr) (cm^{-1}): 2933 (C-H aromatic), 1649 (C=O), 1496 (C=N); ^{1}H NMR (400 MHz, DMSO:d_6) δ ppm: 7.92-7.15 (m, 15H, Ar-H), 3.30 (s, 1H, CH); ^{13}C NMR (100 MHz, DMSO:d_6) δ ppm: 172.80 (C=O), 167.84 (C=O), 133.38-131.29 (Ar-C), 39.54; MS: *m/z* 211.0379, 247.0172, 301.0802, 319.1616, 333.0822, 393.2776, 403.3044. Anal. Calcd for $C_{24}H_{17}F_3N_2O_3$ (438.41).

3.6.2 ***(Z)-N-benzoyl-4-methoxy-N'-(1,1,1-trifluoro-4-(naphthalen-2-yl)-4-oxobutan-2-ylidene)benzohydrazide (9b)***

White crystals; yield: 54%; m.p: 119-121 ◦C; Uv-Vis (MeOH) (nm): 300 (1.5136), 550 (1.0233); IR (KBr) (cm^{-1}): 2931 (C-H aromatic), 1651 (C=O), 1498 (C=N), 1094 (C-O); ^{1}H-NMR (400 MHz, DMSO:d_6) δ ppm: 8.14-7.07 (m, 14H, Ar-H), 3.92 (s, 3H, O-CH_3), 3.31 (s, 1H, CH); ^{13}C NMR (100 MHz, DMSO:d_6) δ ppm: 172.67 (C=O), 167.84 (C=O), 133.39-126.90 (Ar-C), 124.67 (CF_3), 58.16 (O-CH_3), 38.06; MS: *m/z* 207.0879, 221.1013, 301.1056, 339.0375, 381.2575, 387.0540, 413.2304, 437.1474, 461.0642. Anal. Calcd for $C_{25}H_{19}F_3N_2O_4$ (468.43).

3.6.3 ***(Z)-N-benzoyl-4-nitro-N'-(1,1,1-trifluoro-4-(naphthalen-2-yl)-4-oxobutan-2-ylidene)benzohydrazide (9c)***

White powder; yield: 42%; m.p: 120-122 ◦C; Uv-Vis (MeOH) (nm): 300 (1.5136), 550 (1.0233); IR (KBr) (cm^{-1}): 2931 (C-H aromatic), 1651 (C=O), 1498 (C=N), 1254 (N=O); ^{1}H NMR (400 MHz, DMSO:d_6) δ ppm: 7.91-6.55 (m, 14H, Ar-H), 3.48 (s, 1H, CH); ^{13}C NMR (100 MHz, DMSO:d_6) δ ppm: 170.34 (C=O), 166.07 (C=O), 157.91 (Ar-C-NO_2), 145.52-125.48 (Ar-C), 112.90 (CF_3), 44.44.

3.7 ***Antimicrobial assay***

Antimicrobial activity potentials of the synthesized benzohydrazides were determined by agar-well dilution method (Sridhar *et al.* 2016). The compounds were prepared at a concentration of 50 mg/ml and evaluated against the Gram-positive bacteria; *Staphylococcus aureus*, *Bacillus subtilis*, *Listeria monocytogenes*, Gram-negative bacteria; *Escherichia coli*, *Salmonella typhimurium*, *Klebsiella pneumoniae*, *Shigellia sp*, *Pseudomonas aeruginosa*, and fungi strains; *Aspergillus niger*, and *Candida albican*. Commercially available antibiotics Ciprofloxacin and Gentamicin were used as reference drugs.

3.8 ***In Silico Molecular Prediction***

SwissADME**,** a free online tool (http://www.swissadme.ch/) developed and maintained by the molecular modeling group of the Swiss Institute of Bioinformatics (SIB) was used to evaluate the drug-likeness of the synthesized compounds (Daina *et al.* 2017). The molecular descriptors calculated include molecular weight (MW), partition coefficient (log P), hydrogen bond acceptor (HBA), hydrogen bond donor (HBD), topological polar surface area (tPSA), number of rotatable bond (RBC).

3.9 ***Molecular Docking Study***

Auto Dock permits the understanding of the molecular interactions between a ligand and a receptor in terms of free binding energy and bonding interactions. Molecular docking was carried out to determine the binding energy and bonding interactions of the synthesized benzohydrazide against a *S. typhi* receptor. 3D crystal structure of the target receptor (PDB code: 1WVG**)** was retrieved from the protein data bank with resolution 3.46 Å and prepared using BIOVIA discovery studio 2017 R2 version 17.2.0.16349. The preparations included deleting of multiple chains, water of crystallization, energy minimization and defining of binding site. The grid box size of the binding site was determined by checking the binding site attributes (X= 30.055917, Y= 26.457861, Z= 39.084694, radius = 9.163073). The structures of synthesized compounds were drawn using ChemDraw and converted to their 3D form using Discovery studio. The synthesized compound was docked into the active site of the target receptor using Autodock/Autodock vina (Trott and Olson 2010). The docking results were analyzed using BIOVIA discovery studio. The binding modes with significant binding scores were selected (Lengaur and Rarey 1996, Reetu 2012).

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