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# Synthesis, antibacterial activity, and docking studies of some novel *N*′-benzylidene-2-(2,4,5-trifluorophenyl)acetohydrazides

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**Abstract** An improved method was developed for synthesis of 2,4,5-trifluorobenzohydrazide derivatives through condensation of trifluorophenylacetohydrazide with different aryl aldehydes in ethanol with 70% perchloric acid as catalyst. The developed method is efficient in terms of shorter reaction time, easy workup, and purity of final products. We synthesized a total of 25 molecules and screened them for antibacterial activity. Among the synthesized compounds, **4q** showed good antibacterial activity against *Streptococcus mutans* MTCC 497 and *Staphylococcus aureus* MTCC 737 [50% inhibition concentration ($IC_{50}$): 30 and 35 μg/mL, respectively], followed by compound **4c** ($IC_{50}$: 45 μg/mL) against *Streptococcus mutans* MTCC 497 as well as *Salmonella enterica* MTCC 3858. Molecular docking study employing glucosamine 6-phosphate deaminase (NagB) enzyme (PDB: 2RI1) revealed that the Glide scores of the present ligands played a role. Compounds with 2-$NO_2$, 4-$(NCH_3)_2$, and 3,4-di-OH functional groups on the benzylidene ring (**4e**, **i**, **m**) exhibited significant Glide score of −8.2 kcal/mol.

**Keywords** Acetohydrazides · Antibacterial activity · NagB · Docking

**Electronic supplementary material** The online version of this article (doi:10.1007/s11164-017-2864-0) contains supplementary material, which is available to authorized users.

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## Introduction

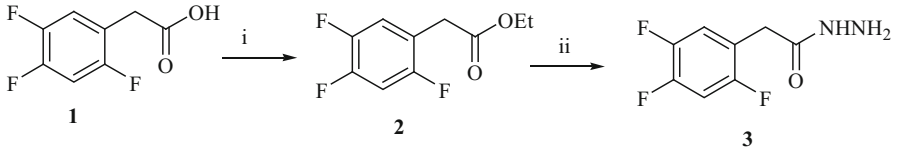
Benzylidenehydrazides are intermediates for numerous heterocyclic compounds [1, 2], containing –CH=NH–, i.e., imine group, usually called Schiff bases. Due to the presence of this imine connection, they show potent biological activities including anticancer, antibiotic, anti-human immunodeficiency virus (HIV), antifungal, anticonvulsant, antiinflammatory, anthelmintic, and antioxidant actions, etc. [3–26]. Because of this wide activity profile, these Schiff bases currently play a vital role in synthetic chemistry and have become versatile pharmacophores for design and synthesis of various drug molecules. In the present investigation, we attempted to synthesize a series of fluorinated benzylideneacetohydrazides, as most drugs and herbicides are found to contain trifluoromethyl and difluoromethyl groups [27–30] for enhanced activities [31].

Regarding experimental conditions for synthesis of these molecules, some established literature methods reported for synthesis of benzylidenehydrazides that use NaOH–ethanol [32], glacial acetic acid–ethanol [33], methanol–NaOH (pH 8) [34], $SnCl_2$ [35], glacial acetic acid–dry benzene [36], $TiCl_4$ [37], etc. are found to suffer from prolonged reaction time, dry conditions, vigorous heating, and low product yield, and for some methods, purification of products is also complicated. To address these issues, in the present investigation, the method shown in Schemes 1 and 2 was proposed to synthesize a series of *N*′-benzylidene-2-(2,4,5-trifluorophenyl)acetohydrazides.

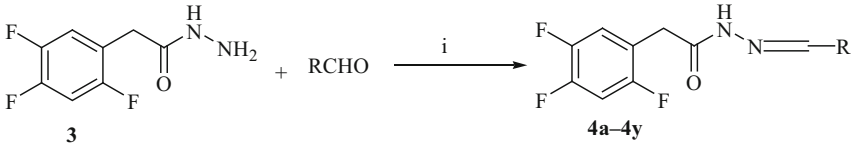
### Results and discussion

A series of 25 *N*′-benzylidene trifluorophenylacetohydrazides were successfully synthesized with enhanced yield and purity from trifluorophenylacetohydrazide intermediate **3** (Scheme 2). In the first stage, the trifluorophenylacetohydrazide **3** required for the title benzylideneacetohydrazides **4a–y** was obtained from trifluorophenyl acetic acid (**1**) via formation of respective ethyl ester by known approach [38] as shown in Scheme 1 with overall good yield.

To achieve the objectives of the present work, initially reagents such as sodium fluoride, mercuric chloride, and Celite were tested for condensation of hydrazide **3** with selected aldehydes, viz. phenyl, aryl, cinnamyl, and indolyl aldehydes. However, no considerable change in the reactants was observed with these reagents, even under different temperature and solvent conditions. However, use of catalytic quantity of 70% perchloric acid in methanol at room temperature indicated successful condensation of azide **3** and *p*-hydroxybenzaldehyde within 10 min.




**Scheme 1** Synthesis of trifluorophenylacetohydrazide intermediate **3**. Reagents and conditions: (i) EtOH, $H_2SO_4$, reflux, 4 h, yield 97%; (ii) $NH_2NH_2{\cdot}2H_2O$, EtOH, reflux, 4 h, yield 70%




R = Phenyl/ Aryl /Cinnamyl / indol

| Sl. No. | Solvent | Time (min.) | % Yield |
|---|---|---|---|
| 1 | Methanol | 40 | 80 |
| 2 | Ethanol | 25 | 96 |
| 3 | Dichloromethane | 60 | 72 |
| 4 | 1,2-dichloroethane | 65 | 76 |
| 5 | Tetrahydrofuran | 70 | 74 |
| 6 | 1,4-Dioxane | 75 | 70 |

**Scheme 2** Synthesis of *N*′-benzylidene-2-(2,4,5-trifluorophenyl)acetohydrazides. Reagents and conditions: (i) 70% $HClO_4$, EtOH, room temperature, 25–35 min, yield 93–96%

Moreover, the reaction was found to complete in 40 min with reasonably good yield of about 80%, indicating the suitability of $HClO_4$ as an efficient catalyst.

Furthermore, to improve the yield and reduce the reaction time, screening of the solvent for the perchloric acid catalyst was carried out using various solvents such as ethanol, dichloromethane (DCM), 1,2-dichloroethane (EDC), tetrahydrofuran (THF), and 1,4-dioxane for condensation of acetohydrazide **3** and *p*-hydroxybenzaldehyde. The results in the screened solvents are tabulated in Scheme 2. Based on these results, perchloric acid in ethanol was found to be a suitable catalyst–solvent system for the present condensation. Applying this improved method, a total of 25 new acetohydrazide derivatives (**4a–y**) were synthesized; the results are summarized in Table 1. All synthesized compounds were characterized by $^{1}H$ and $^{13}C$ nuclear magnetic resonance (NMR) and mass spectral data. The splitting pattern of proton and carbon signals for the respective atoms indicated presence of keto–enol tautomerism in the final products. Clearly separated peaks for NH, =CH, and $CH_2$ are indicated with an asterisk in the respective spectral data, with split peak values presented as the mean.

The yields and reaction times of the synthesized analogs indicated that compound **4a** with no substitution (R = Ph), compounds **4e**, **f** with electron-withdrawing groups (R = 2-$NO_2$Ph, 4-$NO_2$Ph), and compounds with electron-donating groups were obtained in 93–96% yield in 20–35 min at room temperature. Thus, the above results clearly indicate that aldehydes having electron-donating groups and electron-withdrawing groups were well tolerated with no considerable change in product yield.

As discussed above, the compounds were expected to possess antibacterial activity due to presence of CH=NH group, and the additional amide linkage formed from aceto group may also enhance the activity of the present compounds. So, the compounds were tested for their possible bacterial activity profile. Furthermore, to emphasize the antibacterial activities, the ligands were also subjected to protein

**Table 1** Reaction conditions using various aldehydes and corresponding products

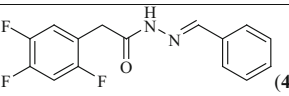
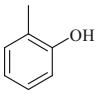
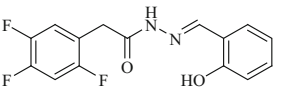
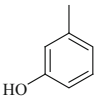
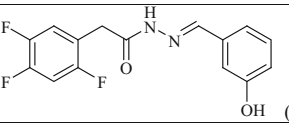
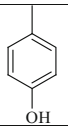
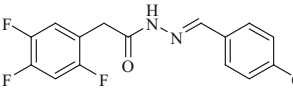
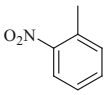
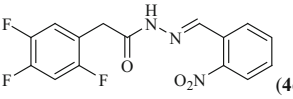
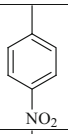
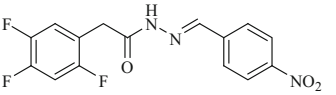
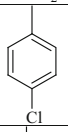
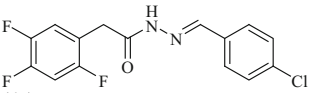
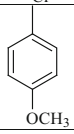
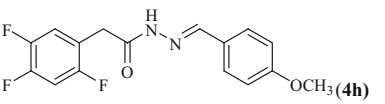
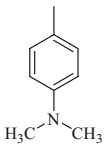
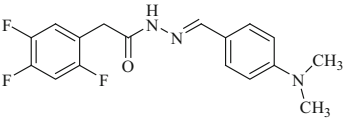
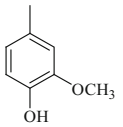
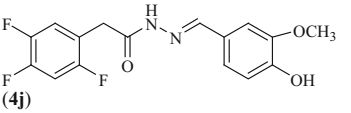
| Entry | (R) | Product (No.) | Time (min) | Yield (%) |
|---|---|---|---|---|
| 1 |  |  **(4a)** | 30 | 95 |
| 2 |  |  **(4b)** | 35 | 94 |
| 3 |  |  **(4c)** | 30 | 95 |
| 4 |  |  **(4d)** | 25 | 94 |
| 5 |  |  **(4e)** | 30 | 96 |
| 6 |  |  **(4f)** | 30 | 95 |
| 7 |  |  **(4g)** | 25 | 96 |
| 8 |  |  **(4h)** | 25 | 95 |
| 9 |  |  **(4i)** | 30 | 94 |
| 10 |  |  **(4j)** | 35 | 96 |

**Table 1** continued

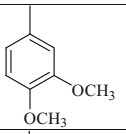
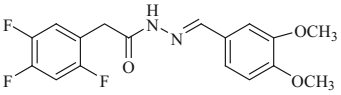
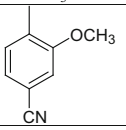
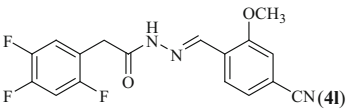
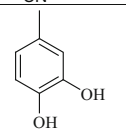
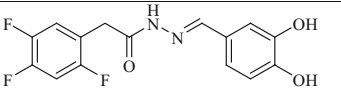
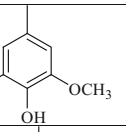
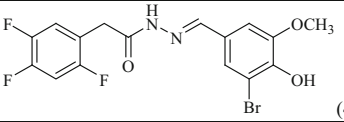
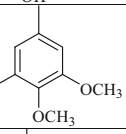
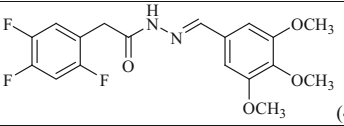
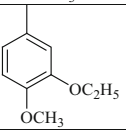
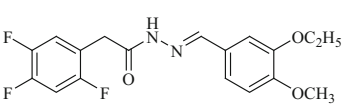
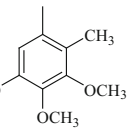
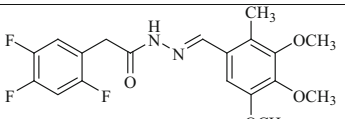
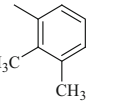
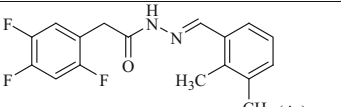
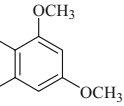
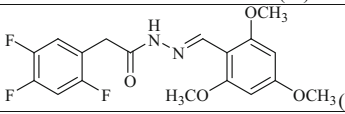
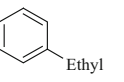
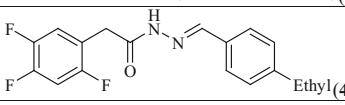
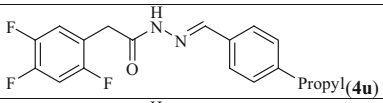
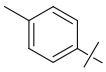
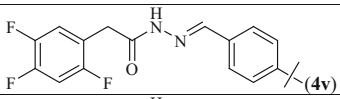
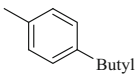
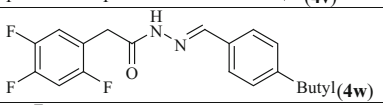
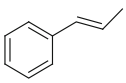
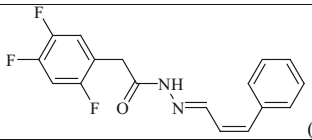
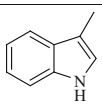
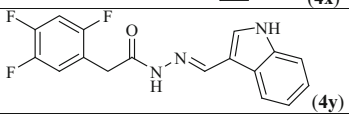
| Entry | (R) | Product (No.) | Time (min) | Yield (%) |
|---|---|---|---|---|
| 11 |  |  (**4k**) | 35 | 94 |
| 12 |  |  (**4l**) | 35 | 95 |
| 13 |  |  (**4m**) | 30 | 94 |
| 14 |  |  (**4n**) | 35 | 96 |
| 15 |  |  (**4o**) | 25 | 96 |
| 16 |  |  (**4p**) | 35 | 95 |
| 17 |  |  (**4q**) | 35 | 96 |
| 18 |  |  (**4r**) | 25 | 94 |
| 19 |  |  (**4s**) | 25 | 96 |
| 20 |  |  (**4t**) | 20 | 93 |

**Table 1** continued

| Entry | (R) | Product (No.) | Time (min) | Yield (%) |
|---|---|---|---|---|
| 21 |  |  | 30 | 94 |
| 22 |  |  | 30 | 93 |
| 23 |  |  | 25 | 94 |
| 24 |  |  | 35 | 93 |
| 25 |  |  | 30 | 94 |

binding in the protein binding pocket of NagB; the results are presented in the next section.

*Antibacterial activity*

All synthesized compounds **4a–y** were tested for their antibacterial activity against three Gram-positive (*Staphylococcus aureus* MTCC 737, *Enterococcus faecalis* MTCC 439, and *Streptococcus mutans* MTCC 497) and three Gram-negative (*Salmonella enterica* MTCC 3858, *Escherichia coli* MTCC 1687, and *Proteus vulgaris* MTCC 426) bacteria. The results obtained (Table 2) were compared with standard drug streptomycin. Among the tested compounds, **4q**, **c**, **i**, **h**, **f** showed considerably good antibacterial activity against at least one test bacteria. Compound **4q** exhibited $IC_{50}$ of 30 and 35 μg/mL against *Streptococcus mutans* MTCC 497 and *Staphylococcus aureus* MTCC 737, respectively. A similar $IC_{50}$ value of 45 μg/mL was shown by compound **4c** against both *Streptococcus mutans* MTCC 497 and *Salmonella enterica* MTCC 3858. Equipotent antibacterial activity with $IC_{50}$ value of 55 μg/mL was observed in the case of *Proteus vulgaris* MTCC 426 and *Enterococcus faecalis* MTCC 439 for compounds **4f** and **4h**, respectively.

*Molecular docking simulation studies*

Glucosamine 6-phosphate deaminase (NagB) enzyme (PDB: 2RI1) is believed to play an effective role in controlling the metabolic rate of *Streptococcus* species. The catalytic role of glucosamine 6-phosphate deaminase (NagB) enzyme in conversion

**Table 2** Antibacterial activity ($IC_{50}$ in μg/mL) of synthesized compounds **4a–y**

| Compound | Test organism (μg/mL) | | | | | |
|---|---|---|---|---|---|---|
| | *Staphylococcus aureus* MTCC 737 | *Enterococcus faecalis* MTCC 439 | *Streptococcus mutans* MTCC 497 | *Salmonella enterica* MTCC 3858 | *Escherichia coli* MTCC 1687 | *Proteus vulgaris* MTCC 426 |
| **4a** | 140 | 125 | 80 | 155 | 165 | 100 |
| **4b** | 100 | 187.5 | 140 | 165 | 230 | NA |
| **4c** | NA | 100 | 45 | 45 | 100 | 150 |
| **4d** | 145 | 145 | 160 | 200 | 140 | 135 |
| **4e** | 110 | 140 | 150 | NA | 187.5 | 90 |
| **4f** | 230 | 90 | 150 | 150 | NA | 55 |
| **4g** | NA | 135 | 90 | 145 | 95 | 145 |
| **4h** | 110 | 55 | 150 | 160 | 150 | 160 |
| **4i** | 50 | 95 | 80 | NA | 160 | 90 |
| **4j** | 145 | 145 | NA | 100 | 130 | NA |
| **4k** | 175 | 145 | 90 | 110 | 145 | 145 |
| **4l** | 85 | 135 | 155 | 160 | 150 | 100 |
| **4m** | 130 | 150 | 160 | 140 | NA | 145 |
| **4n** | 80 | 100 | 165 | 145 | 160 | 130 |
| **4o** | 135 | 90 | 145 | 155 | 128 | 100 |
| **4p** | NA | 187.5 | 175 | 150 | NA | 135 |
| **4q** | 35 | 110 | 30 | 145 | NA | 90 |
| **4r** | 135 | 187.5 | 145 | 140 | 150 | 140 |
| **4s** | 85 | 187.5 | 150 | 140 | 145 | 145 |
| **4t** | 130 | 90 | NA | 150 | 110 | NA |
| **4u** | 145 | NA | 155 | 155 | 120 | 125 |
| **4v** | 80 | 145 | NA | NA | NA | 90 |
| **4w** | NA | 100 | 70 | 150 | 145 | 135 |

**Table 2** continued

| Compound | Test organism (μg/mL) | | | | | |
|---|---|---|---|---|---|---|
| | *Staphylococcus aureus* MTCC 737 | *Enterococcus faecalis* MTCC 439 | *Streptococcus mutans* MTCC 497 | *Salmonella enterica* MTCC 3858 | *Escherichia coli* MTCC 1687 | *Proteus vulgaris* MTCC 426 |
| **4x** | 90 | 145 | 160 | 150 | 140 | 125 |
| **4y** | NA | 150 | 150 | 150 | NA | 140 |
| Streptomycin | 25 | 15 | 20 | 30 | 15 | 15 |

*NA* No Activity

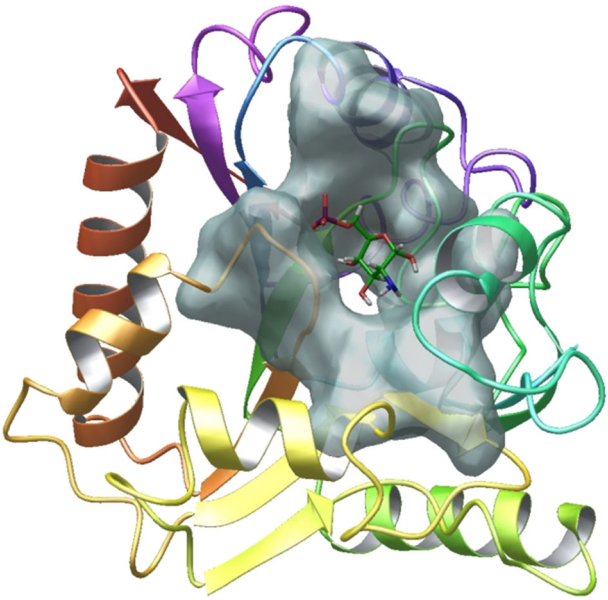


**Fig. 1** Glucosamine 6-phosphate deaminase (NagB) enzyme with reference ligand

of glucosamine to sugar and other byproducts provides crucial information about its catalytic mechanism [39] (Fig. 1). Hence, the X-ray crystalline protein glucosamine 6-phosphate deaminase (NagB) enzyme (PDB: 2RI1) was chosen for the present protein–ligand interpretation studies. The standard Glide docking protocol was followed for these studies, and the simulation results using glucosamine 6-phosphate deaminase (NagB) enzyme, a bacterial-related protein, are provided in Table 3. Binding mode analysis of the ligands clearly showed that compounds with 2-$NO_2$, 4-$(NCH_3)_2$, and 3,4-di-OH functional groups on the benzylidene ring (**4e**, **i**, **m**) had significant Glide score. Ligand **4e** was observed to be stabilized by hydrogen bonding at protein amino-acid residues Lys-196, Thr-40, and Ser-39 with bond length of 1.8, 2.1, and 2.2 Å respectively, and strongly surrounded by Gly-38, Thr-37, Ala-36, Gly-126, Gly-128, and Arg-129. Meanwhile, other ligands such as **4b**, **h**, **k**, **l**, **r**, **w** also showed satisfactory Glide scores ranging between −8.1 and −7.9 kcal/mol when compared with standard drug streptomycin. Interactions of ligand **4e** with active-site residues are depicted in Fig. 2.

## Experimental

### Materials and methods

Chemicals and solvents of laboratory grade (Merck) were used as such. Melting points were calculated on Remi melting point apparatus. All reactions were monitored by thin-layer chromatography (TLC), and yields refer to isolated

**Table 3** Molecular docking predicted results for compounds **4a–y** with NagB

| S. no. | Ligand code | Functional group (R) | Docking score (kcal/mol) | H bond (Å) | Active-site residues of glucosamine 6-phosphate deaminase (NagB) interacting with ligand |
|---|---|---|---|---|---|
| 1 | **4a** | Phenyl | −7.3 | 1.9, 2.4, 2.5 | Gly-126, Thr-40, Lys-196, Glu-195, Phe-192, Ser-39, Gly-38, Thr-37 |
| 2 | **4b** | *o*-OH phenyl | −8.1 | 2.2 | Thr-77, Phe-135, Gly-128, Gly-134, Ile-127, Gly-126, Thr-37, Gly-38 |
| 3 | **4c** | *m*-OH phenyl | −7.8 | 2.0 | Gln-75, Phe-80, Gly-38, Thr-37, Gly-126, Ile-127, Gly-134, Gly-128, Phe-135, Asn-159 |
| 4 | **4d** | *p*-OH phenyl | −7.7 | 2.2 | Gln-75, Phe-80, Tyr-77, Gly-38, Thr-37, Gly-126, Gly-134, Phe-135, Gly-128 |
| 5 | **4e** | *o*-$NO_2$ phenyl | −8.2 | 1.8, 2.1, 2.2 | Lys-196, Thr-40, Ser-39, Gly-38, Thr-37, Ala-36, Gly-134, Gly-126, Ile-127, Gly-128, Arg-129 |
| 6 | **4f** | *p*-$NO_2$ phenyl | −7.7 | 2.5, 2.8 | Thr-152, Thr-40, Ser-39, Gly-126, Gly-38, Thr-37, Gly-134, Phe-135, Glu-137, Thr-155 |
| 7 | **4g** | *p*-Cl phenyl | −7.6 | 2.3 | Gly-38, Thr-37, Ala-36, Ile-133, Gly-134, Phe-135, Thr-155 |
| 8 | **4h** | *p*-$OCH_3$ phenyl | −8.0 | 2.2, 2.5, 3.5 | Gly-38, Ile-135, Gly-126, Leu-125, Ala-36, Gly-38, Asn-159, Phe-135, Glu-134 |
| 9 | **4i** | 4-$N(CH_3)_2$ phenyl | −8.2 | 2.0 | Tyr-77, Ser-76, Gln-75, Asn-159, Gly-38, Thr-40, Thr-37, Gly-126, Gly-134 |
| 10 | **4j** | 4-OH,3-$OCH_3$ phenyl | −7.6 | 1.9, 2.0, 2.2, 2.5, 2.6, 2.7 | Ile-136, Thr-40, Ser-39, Gly-126, Gly-38, Ala-158, Thr-155, Gly-128, Thr-37 |
| 11 | **4k** | 3,4-Di-$OCH_3$ phenyl | −8.0 | 2.2, 2.5 | Gly-38, Gly-134, Thr-37, Ala-36, Asn-64, Phe-135, Thr-155 |
| 12 | **4l** | 3-$OCH_3$,4-CN phenyl | −7.9 | 2.5, 2.6, 3.4 | Thr-37, Tyr-77, Ile-127, Gly-128, Thr-155, Gln-75, Ser-76, Phe-80, Met-81 |
| 13 | **4m** | 3,4-Di-OH phenyl | −8.2 | 1.9, 2.2 | Gly-38, Thr-37, Ala-36, Gly-126, Ile-133, Gly-134, Phe-135, Thr-155, Asn-84 |
| 14 | **4n** | 3-Br,4-OH,5-$OCH_3$ phenyl | −7.8 | 2.5, 3.4, 3.5 | Thr-37, Gly-128, Ala-158, Ser-76, Phe-80, Tyr-77, Ala-36, Gly-126 |
| 15 | **4o** | 3,4,5-Tri-$OCH_3$ phenyl | −7.9 | 2.5, 3.5 | Thr-37, Ile-127, Gly-128, Arg-129, Phe-80, Tyr-77, Ala-36 |
| 16 | **4p** | 3-Ethoxy,4-$OCH_3$ phenyl | −7.8 | 2.3, 2.6, 2.7, 3.0 | Tyr-37, Ala-36, Gly-38, Tyr-77, Phe-80, Ser-76, Ala-158, Thr-155, Arg-129, Gly-128, Phe-135, Gly-126, Leu-125 |
| 17 | **4q** | 2-$CH_3$,3,4,5-tri-$OCH_3$ phenyl | −7.8 | 2.3, 2.9, 3.4, 3.5, 3.6 | Tyr-37, Ser-39, Gly-38, Thr-155, Gly-128, Phe-80 |

**Table 3** continued

| S. no. | Ligand code | Functional group (R) | Docking score (kcal/mol) | H bond (Å) | Active-site residues of glucosamine 6-phosphate deaminase (NagB) interacting with ligand |
|---|---|---|---|---|---|
| 18 | **4r** | 2,3-Di-$CH_3$ phenyl | −7.9 | 2.0 | Tyr-77, Phe-80, Ser-76, Thr-155, Gly-128, Phe-135, Glu-134, Ala-36, Thr-37 |
| 19 | **4s** | 2,4,6-Tri-$OCH_3$ phenyl | −7.6 | 2.0 | Tyr-77, Asn-64, Phe-135, Gly-134, Gly-128, Ala-36, Thr-37, Gly-38 |
| 20 | **4t** | 4-Ethyl phenyl | −7.5 | 3.2 | Gly-128, Thr-155, Ser-76, Phe-80, Gly-38, Thr-37, Ala-36, Gly-126 |
| 21 | **4u** | 4-Propyl phenyl | −7.6 | 2.3 | Tyr-77, Phe-80, Gly-38, Thr-37, Ala-36, Gly-134, Gly-128, Thr-155, Ala-158 |
| 22 | **4v** | 4-Tertiary butyl phenyl | −7.8 | 3.4 | Thr-155, Phe-135, Gly-134, Gly-126, Ala-36, Thr-37, Gly-38, Ser-39, Phe-80 |
| 23 | **4w** | 4-Butyl phenyl | −8.0 | 2.1 | Gly-38, Gln-75, Ser-76, Tyr-77, Asp-64, Leu-63, Phe-135, Gly-134, Ile-133, His-132, Gly-128 |
| 24 | **4x** | Cinnamyl methylene | −7.4 | 2.2, 2.6, 3.3 | Asn-159, Phe-80, Gln-75, Gln-83, Ser-76, Tyr-77, Thr-37, Ala-36, Thr-155, Ala-158 |
| 25 | **4y** | (1*H*-Indol-3-yl)methylene | −7.6 | 1.8, 2.2, 3.3 | Ala-36, Thr-37, Gly-134, Gly-128, Gly-38, Ser-39 |
| 26 | Standard | Streptomycin | 8.3 | 2.0, 2.2, 2.3, 2.4, 2.6, 2.8, 3.2, 3.3 | Ala-36, Gly-128, Thr-37, Gly-38, Arg-129, Phe-80, Phe-135 |

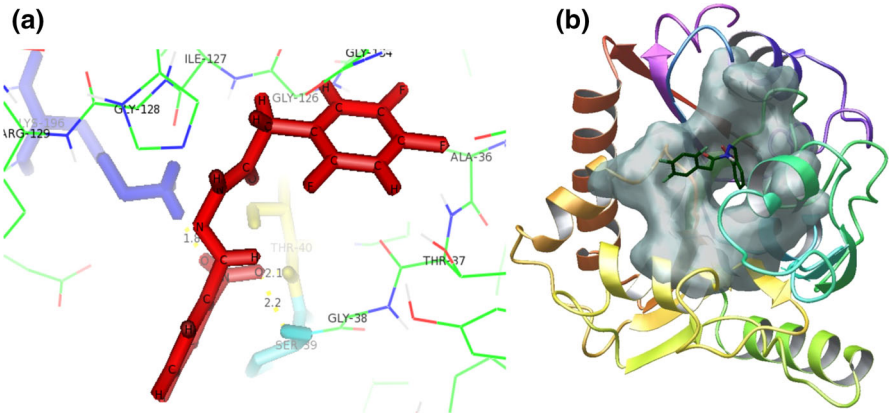



**Fig. 2** **a** NagB protein (*green lines*) active site complexed with **4e** (*thick red ball-and-stick model*). **b** Compound **4e** (*thick green ball-and-stick model*) at NagB active site. (Color figure online)

products. Proton NMR spectra were recorded in dimethyl sulfoxide (DMSO)-$d_6$ on Bruker 400 MHz apparatus at 400 MHz, and $^{13}C$ NMR spectra were recorded in DMSO-$d_6$ on a Bruker 400 MHz spectrometer at 100 MHz. Mass spectra were recorded on Elegant LC-1100 series instrument.

*Synthesis of ethyl-2-(2,4,5-trifluorophenyl)acetate (**2**)*

To solution of 2,4,5-trifluorophenyl acetic acid (**1**, 6 g, 31.57 mmol) in ethanol (50 mL) was added catalytic amount of $H_2SO_4$ at 0 °C followed by reflux for about 4 h. The reaction was monitored by TLC; after reaction completion, ethanol was distilled off. The reaction mass was poured into cold distilled water (100 mL) then extracted with EtOAc twice (2 × 50 mL). Later, the ethyl acetate layer was washed with distilled water twice (2 × 50 mL) to remove acid traces then dried over $Na_2SO_4$ (2 g, 17 mmoles). The total EtOAc layer was distilled off, and ethyl-2-(2,4,5-trifluorophenyl)acetate (**2**) finally obtained (6.67 g, 97% yield) as colorless oil. Analytical data: $^1H$ NMR (400 MHz, DMSO-$d_6$): $\delta$ 7.54–7.43 (m, 2H), 4.26 (s, 2H), 3.92 (m, 2H), 1.38 (t, 3H); LC–MS: *m/z* 219 $(M + H)^+$.

*Synthesis of 2-(2,4,5-trifluorophenyl)acetohydrazide (**3**)*

Solution of ethyl ester **2** (6 g, 27.52 mmoles) in ethanol (50 mL) and hydrazine hydrate (8.25 g, 8.0 mL, 165.12 mmoles) was refluxed for about 4 h. The reaction was monitored by using TLC; after reaction completion, the reaction mass was cooled to room temperature then poured into ice-cold water (100 mL). The precipitate was filtered off from the solid mass, washed with cold water, and dried at 100 °C for about 2–3 h to obtain 2-(2,4,5-trifluorophenyl)acetohydrazide (**3**, 3.93 g, 70% yield) as white solid. Analytical data: M.p.: 112–114 °C; $^1H$ NMR (400 MHz, DMSO-$d_6$): $\delta$ 11.59 (s, 1H), 7.53–7.42 (m, 2H), 4.25 (s, 2H), 3.40 (s, 2H); LC–MS: *m/z* 205 $(M + H)^+$.

*General procedure for preparation of 2-(2,4,5-trifluorophenyl)acetohydrazide derivatives **4a–y***

To mixture of 2-(2,4,5-trifluorophenyl)acetohydrazide (**3**, 1 mmol) and aromatic aldehyde (1 mmol) in ethanol (5 mL), catalytic amount of 70% perchloric acid was added. The reaction mass was stirred for about 20–35 min. Reaction was monitored by TLC; after reaction completion, the reaction mass was poured into ice-cold water (25 mL), and the product was filtered off then washed with cold water.

## Antibacterial activity investigation [40]

Test samples were prepared at different concentrations by dissolving in DMSO. Suspensions of tested bacterial cultures were prepared and inoculated into tubes containing freshly prepared nutrient broth medium. To these tubes, 1 mL of each concentration of test sample was added followed by incubation at 37 °C for 24 h. Control was maintained with bacterial inoculum but without compound sample.

After incubation, optical density (OD) values were taken at wavelength of 510 nm and the percentage bacterial inhibition calculated as

$$(A_C - A_S)/A_C \times 100,$$

where $A_c$ is the OD of control and $A_s$ is the OD of sample.

$IC_{50}$ values were calculated from plots of percentage inhibition on *Y*-axis versus sample concentration on *X*-axis.

*Protein and ligand preparation for glucosamine 6-phosphate deaminase (NagB) investigation*

NagB X-ray crystalline structure (PDB: 2RI1) was obtained from the Protein Data Bank (database site www.rcsb.org), and cocrystallized ligand was identified and removed. All water molecules and undesired ions were removed, and hydrogen atoms were added at suitable positions within the protein, followed by minimization and preparation using Protein Preparation Wizard (Schrödinger LLC). Entire ligands were drawn using ACD ChemSketch and converted into three dimensions (3D) using Maestro working panel and prepared by LigPrep. Glide 5.0 was chosen to investigate the binding modes of the current acetohydrazides, indicating glucosamine 6-phosphate deaminase (NagB) enzyme as target. The standard precision docking mode was employed in Glide 5.0 [39]. The binding mode analysis of glucosamine 6-phosphate deaminase (NagB) enzyme with the ligand complexes and their interactions along with bond distances were calculated and visualized using PyMOL. All obtained docking predictions are presented in Table 3.

## Spectral data

*N′-Benzylidene-2-(2,4,5-trifluorophenyl)acetohydrazide (**4a**)*

Compound obtained as white solid. M.p.: 158–160 °C; $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 11.59* (s, 1H, NH), 8.10* (s, 1H, $H_9$), 7.69 (s, 2H, $H_3$ and $H_6$), 7.50 (t, 2H, $J = 7.28$ Hz, $H_{12}$ and $H_{14}$), 7.42 (m, 3H, $H_{11}$, $H_{13}$, and $H_{15}$), 3.83 (s, 2H); $^{13}$C NMR (100 MHz, DMSO-$d_6$): $\delta$ 170.49* ($C_8$), 154.81 ($C_2$), 149.28 ($C_4$), 146.94 ($C_5$), 143.34 ($C_9$), 134.12 ($C_{10}$), 129.81 ($C_1$), 128.77 ($C_{11}$ and $C_{15}$), 127.02 ($C_{13}$), 126.75 ($C_{12}$ and $C_{14}$), 119.9 ($C_6$), 105.66 ($C_3$), 33.51* ($C_7$); MS: *m*/*z* 293 (M + 1) mass-292. M.F. $C_{15}H_{11}F_3N_2O$.

*N′-(2-Hydroxybenzylidene)-2-(2,4,5-trifluorophenyl)acetohydrazide (**4b**)*

Compound obtained as white solid. M.p.: 193–195 °C; $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 11.67* (s, 1H, NH), 10.5 (s, 1H, OH), 8.36* (s, 1H, $H_9$), 7.68 (brs, 1H, $H_{15}$), 7.52 (brs, 2H, $H_3$ and $H_6$), 7.25 (brs, 1H, $H_{13}$), 6.89 (brs, 2H, $H_{12}$ and $H_{14}$), 3.82* (s, 2H); $^{13}$C NMR (100 MHz, DMSO-$d_6$): $\delta$ 170.0 ($C_8$), 161.0 ($C_{11}$), 156.42 ($C_2$), 147.19 ($C_4$), 146.84 ($C_5$), 141.25 ($C_9$), 131.35 ($C_{13}$), 131.06 ($C_{15}$), 129.34 ($C_{14}$), 126.42 ($C_1$), 120.11 ($C_{10}$), 119.72 ($C_6$), 116.32 ($C_{12}$), 105.76 ($C_3$), 33.28 ($C_7$); MS: *m*/*z* 309 (M + 1) mass-308. M.F. $C_{15}H_{11}F_3N_2O_2$.

*N′-(3-Hydroxybenzylidene)-2-(2,4,5-trifluorophenyl)acetohydrazide (**4c**)*

Compound obtained as white solid. M.p.: 160–162 °C; $^{1}$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 11.54* (s, 1H, NH), 9.61 (s, 1H, OH), 8.01* (s, 1H, $H_9$), 7.52 (m, 2H, $H_3$ and $H_6$), 7.23 (t, 1H, $J = 7.6$ Hz, $H_{14}$), 7.09 (m, 2H, $H_{11}$ and $H_{15}$), 6.81 (d, 1H, $J = 7.6$ Hz, $H_{13}$), 3.81 (s, 2H, $H_7$); $^{13}$C NMR (100 MHz, DMSO-$d_6$): $\delta$ 170.39 ($C_8$), 164.50 ($C_{12}$), 157.63 ($C_2$), 147.0 ($C_4$), 146.80 ($C_5$), 143.53 ($C_9$), 135.35 ($C_{10}$), 129.81 ($C_{14}$), 127.32 ($C_1$), 119.91 ($C_6$), 118.75 ($C_{15}$), 117.38 ($C_{13}$), 112.63 ($C_{11}$), 105.17 ($C_3$), 32.02 ($C_7$); MS: *m/z* 309 (M + 1) mass-308. M.F. $C_{15}H_{11}F_3N_2O_2$.

*N′-(4-Hydroxybenzylidene)-2-(2,4,5-trifluorophenyl)acetohydrazide (**4d**)*

Compound obtained as white solid. M.p.: 186–188 °C; $^{1}$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 11.38* (s, 1H, NH), 9.90 (s, 1H, OH), 7.99* (s, 1H, $H_9$), 7.50 (m, 4H, $H_3$, $H_6$, $H_{11}$ and $H_{15}$), 6.81 (d, 2H, $J = 8.4$ Hz, $H_{12}$ and $H_{14}$), 3.79* (s, 2H, $H_7$); $^{13}$C NMR (100 MHz, DMSO-$d_6$): $\delta$ 170.10 ($C_8$), 159.18 ($C_{13}$), 159.36 ($C_2$), 147.08 ($C_4$), 146.8 ($C_5$), 143.63 ($C_9$), 128.76 ($C_1$), 128.45 ($C_{11}$ and $C_{15}$), 125.15 ($C_{10}$), 119.88 ($C_6$), 115.66 ($C_{12}$ and $C_{14}$), 105.63 ($C_3$), 32.08 ($C_7$); MS: *m/z* 309 (M + 1) mass-308. M.F. $C_{15}H_{11}F_3N_2O_2$.

*N′-(2-Nitrobenzylidene)-2-(2,4,5-trifluorophenyl)acetohydrazide (**4e**)*

Compound obtained as white solid. M.p.: 188–190 °C; $^{1}$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 11.67* (s, 1H, NH), 8.09* (s, 1H, $H_9$), 7.71 (m, 2H, $H_3$ and $H_6$), 7.51 (m, 4H, $H_{12}$, $H_{13}$, $H_{14}$, and $H_{15}$), 3.83 (s, 2H, $H_7$); $^{13}$C NMR (100 MHz, DMSO-$d_6$): $\delta$ 170.80 ($C_8$), 156.0 ($C_2$), 148.33 ($C_4$), 148.0 ($C_5$), 144.41 ($C_9$), 141.10 ($C_{11}$), 136.0 ($C_{14}$), 132.90 ($C_{12}$), 130.38 ($C_{10}$), 128.8 ($C_1$), 124.02 ($C_{15}$), 120.85 ($C_{13}$), 119.89 ($C_6$), 105.67 ($C_3$), 32.18 ($C_7$); MS: *m/z* 338 (M + 1) mass-337. M.F. $C_{15}H_{10}F_3N_3O_3$.

*N′-(4-Nitrobenzylidene)-2-(2,4,5-trifluorophenyl)acetohydrazide (**4f**)*

Compound obtained as white solid. M.p.: 205–207 °C; $^{1}$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 11.85 (s, 1H, NH), 8.29 (m, 2H, $H_3$ and $H_6$), 8.27 (s, 1H, $H_9$), 7.96 (t, 2H, $J = 8.4$ Hz, $H_{12}$ and $H_{14}$), 7.53 (m, 2H, $H_{11}$ and $H_{15}$), 3.88 (s, 2H, $H_7$); $^{13}$C NMR (100 MHz, DMSO-$d_6$): $\delta$ 170 ($C_8$), 154.81 ($C_2$), 147.9 ($C_4$), 147.73 ($C_5$), 141.01 ($C_{13}$), 140.41 ($C_9$), 134.50 ($C_{10}$), 127.97 ($C_1$), 127.71 ($C_{12}$ and $C_{14}$), 123.99 ($C_{11}$ and $C_{15}$), 119.90 ($C_6$), 105.50 ($C_3$), 32.12 ($C_7$); MS: *m/z* 338 (M + 1) mass-337. M.F. $C_{15}H_{10}F_3N_3O_3$.

*N′-(4-Chlorobenzylidene-2-(2,4,5-trifluorophenyl)acetohydrazide (**4g**)*

Compound obtained as white solid. M.p.: 180–182 °C; $^{1}$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 11.94* (s, 1H, NH), 8.11* (s, 1H, $H_9$), 7.72 (m, 2H, $H_3$ and $H_6$), 7.50 (m, 4H, $H_{11}$, $H_{12}$, $H_{14}$, and $H_{15}$), 3.84* (s, 2H, $H_7$); $^{13}$C NMR (100 MHz, DMSO-$d_6$): $\delta$ 170.56 ($C_8$), 156.96 ($C_2$), 146.82 ($C_4$), 145.48 ($C_5$), 142.06 ($C_9$), 134.27 ($C_{13}$), 133.08 ($C_{10}$), 128.85 ($C_{11}$ and $C_{15}$), 128.65 ($C_1$), 128.39 ($C_{12}$ and $C_{14}$), 119.89 ($C_6$),

105.44 ($C_3$), 32.07 ($C_7$); MS: *m/z* 325, 326, 327 (M, M + 2) mass-326. M.F. $C_{15}H_{10}ClF_3N_2O$.

*N′-(4-Methoxybenzylidene)-2-(2,4,5-trifluorophenyl)acetohydrazide (**4h**)*

Compound obtained as white solid. M.p.: 145–147 °C; $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 11.45* (s, 1H, NH), 8.05* (s, 1H, $H_9$), 7.64 (s, 1H, $H_3$), 7.62 (s, 1H, $H_6$), 7.51 (d, 2H, $J$ = 6.6 Hz, $H_{11}$ and $H_{15}$), 6.99 (d, 2H, $J$ = 7.52 Hz, $H_{12}$ and $H_{14}$), 3.9* (s, 2H, $H_7$), 3.79 (s, 3H, $OCH_3$); $^{13}$C NMR (100 MHz, DMSO-$d_6$): $\delta$ 170.24 ($C_8$), 160.65 ($C_{13}$), 154.82 ($C_2$), 146.89 ($C_4$), 146.66 ($C_5$), 143.21 ($C_9$), 128.62 ($C_1$), 128.32 ($C_{11}$ and $C_{15}$), 126.72 ($C_{10}$), 119.87 ($C_6$), 114.29 ($C_{12}$ and $C_{14}$), 105.64 ($C_3$), 55.26 ($OCH_3$), 32.09 ($C_7$); MS: *m/z* 323 (M + 1) mass-322. M.F. $C_{15}H_{13}F_3N_2O_2$.

*N′-(4-(Dimethylamino)benzylidene)-2-(2,4,5-trifluorophenyl)acetohydrazide (**4i**)*

Compound obtained as white solid. M.p.: 184–186 °C; $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 11.28* (s, 1H, NH), 7.96* (s, 1H, $H_9$), 7.50 (m, 4H, $H_3$, $H_6$, $H_{11}$, and $H_{15}$), 6.73 (d, 2H, $J$ = 8.48 Hz, $H_{12}$ and $H_{14}$), 3.78* (s, 2H, $H_7$), 2.96 (s, 6H, $N[CH_3]_2$); $^{13}$C NMR (100 MHz, DMSO-$d^6$): $\delta$ 169.87 ($C_8$), 154.69 ($C_2$), 151.36 ($C_{13}$), 147.61 ($C_4$), 146.87 ($C_5$), 144.16 ($C_9$), 128.34 ($C_1$), 128.01 ($C_{11}$ and $C_{15}$), 121.54 ($C_{10}$), 119.82 ($C_6$), 111.80 ($C_{12}$ and $C_{14}$), 105.76 ($C_3$), 39.71 ($N[CH_3]_2$), 32.07 ($C_7$); MS: *m/z* 336 (M + 1) mass-335. M.F. $C_{17}H_{16}F_3N_3O$.

*N′-(4-Hydroxy,3-methoxybenzylidene)-2-(2,4,5-trifluorophenyl)acetohydrazide (**4j**)*

Compound obtained as white solid. M.p.: 193–195 °C; $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 11.40* (s, 1H, NH), 9.52 (s, 1H, OH), 7.98* (s, 1H, $H_9$), 7.51 (m, 2H, $H_3$ and $H_6$), 7.26 (m, 1H, $H_{11}$), 7.5 (d, 1H, $J$ = 8.08 Hz, $H_{15}$), 6.81 (d, 1H, $J$ = 8.08 Hz, $H_{14}$), 3.80* (s, 2H, $H_7$), 3.80 (s, 3H, $OCH_3$); $^{13}$C NMR (100 MHz, DMSO-$d_6$): $\delta$ 170.17 ($C_8$), 157.07 ($C_2$), 148.97 ($C_4$), 148.74 ($C_5$), 147.99 ($C_{13}$), 147.33 ($C_{12}$), 143.74 ($C_9$), 125.58 ($C_1$), 121.93 ($C_{10}$), 121.30 ($C_{15}$), 119.71 ($C_6$), 115.51 ($C_{11}$), 109.37 ($C_{14}$), 105.44 ($C_3$), 55.58 ($OCH_3$), 32.24 ($C_7$); MS: *m/z* 339 (M + 1) mass-338. M.F. $C_{16}H_{13}F_3N_2O_3$.

*N′-(3,4-Dimethoxybenzylidene)-2-(2,4,5-trifluorophenyl)acetohydrazide (**4k**)*

Compound obtained as white solid. M.p.: 158–160 °C. $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 11.47* (s, 1H, NH), 8.02* (s, 1H, $H_9$), 7.51 (m, 2H, $H_3$ and $H_6$), 7.29 (m, 1H, $H_{11}$), 7.16 (m, 1H, $H_{15}$), 7.0 (m, 1H, $H_{14}$), 3.81* (s, 2H, $H_7$), 3.79 (s, 6H, $OCH_3$); $^{13}$C NMR (100 MHz, DMSO-$d_6$): $\delta$ 170.29 ($C_8$), 154.79 ($C_2$), 150.56 ($C_{13}$), 149.08 ($C_{12}$), 146.99 ($C_4$), 146.87 ($C_5$), 143.40 ($C_9$), 126.90 ($C_1$), 121.67 ($C_{10}$), 121.17 ($C_{15}$), 119.46 ($C_6$), 111.60 ($C_{14}$), 108.56 ($C_{11}$), 105.80 ($C_3$), 55.57 ($OCH_3$), 55.45 ($OCH_3$), 32.26 ($C_7$); MS: *m/z* 353 (M, M + 2) mass-352. M.F. $C_{17}H_{15}F_3N_2O_3$.

*N′-(2-Methoxy,4-nitrilebenzylidene)-2-(2,4,5-trifluorophenyl)acetohydrazide (**4l**)*

Compound obtained as white solid. M.p.: 223–225 °C; $^{1}$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 11.72* (s, 1H, NH), 8.43* (s, 1H, $H_9$), 7.99 (d, 1H, $J = 7.96$ Hz, $H_{15}$), 7.60 (s, 1H, $H_3$), 7.49 (m, 3H, $H_6$, $H_{12}$, and $H_{14}$), 3.91 (s, 3H, $OCH_3$), 3.84* (s, 2H, $H_7$); $^{13}$C NMR (100 MHz, DMSO-$d_6$): $\delta$ 174.44 ($C_8$), 157.40 ($C_2$), 157.29 ($C_{11}$), 146.98 ($C_4$), 146.75 ($C_5$), 140.39 ($C_9$), 137.10 ($C_{13}$), 127.10 ($C_1$), 126.18 ($C_{10}$), 124.63 ($C_{15}$), 119.85 ($C_6$), 118.58 (CN), 115.52 ($C_{14}$), 112.95 ($C_{12}$), 105.67 ($C_3$), 56.41 ($OCH_3$), 33.58 ($C_7$); MS: *m*/*z* 348 (M + 1) mass-347. M.F. $C_{17}H_{12}F_3N_3O_2$.

*N′-(3,4-Dihydroxybenzylidene)-2-(2,4,5-trifluorophenyl)acetohydrazide (**4m**)*

Compound obtained as white solid. M.p.: 182–184 °C; $^{1}$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 11.33* (s, 1H, NH), 9.37 (s, 1H, OH), 9.20 (s, 1H, OH), 7.92* (s, 1H, $H_9$), 7.51 (m, 2H, $H_3$ and $H_6$), 7.17 (m, 1H, $H_{11}$), 6.90 (m, 1H, $H_{15}$), 6.76 (m, 1H, $H_{14}$), 3.78* (s, 2H, $H_7$); $^{13}$C NMR (100 MHz, DMSO-$d_6$): $\delta$ 170.00 ($C_8$), 154.79 ($C_2$), 147.92 ($C_4$), 147.71 ($C_{13}$), 147.27 ($C_5$), 145.65 ($C_{12}$), 144.0 ($C_9$), 125.60 ($C_1$), 120.49 ($C_{10}$), 119.59 ($C_{15}$), 119.70 ($C_6$), 115.58 ($C_{14}$), 112.74 ($C_{11}$), 105.77 ($C_3$), 31.97 ($C_7$); MS: *m*/*z* 324 (M + 1) mass-324. M.F. $C_{15}H_{11}F_3N_2O_3$.

*N′-(3-Bromo,4-hydroxy,5-methoxybenzylidene)-2-(2,4,5-trifluorophenyl)acetohydrazide (**4n**)*

Compound obtained as white solid. M.p.: 222–224 °C; $^{1}$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 11.52* (s, 1H, NH), 9.0 (s, 1H, OH), 7.71* (s, 1H, $H_9$), 7.5 (m, 2H, $H_3$ and $H_6$), 7.40 (brs, 1H, $H_{11}$), 7.28 (m, 1H, $H_{15}$), 3.88 (s, 3H, $OCH_3$), 3.82* (s, 2H, $H_7$); $^{13}$C NMR (100 MHz, DMSO-$d_6$): $\delta$ 170.37 ($C_8$), 154.2 ($C_2$), 148.61 ($C_{14}$), 146.93 ($C_4$), 146.31 ($C_5$), 145.75 ($C_{12}$), 145.52 ($C_{13}$), 142.22 ($C_9$), 126.50 ($C_1$), 123.57 ($C_{10}$), 119.79 ($C_6$), 109.47 ($C_{11}$), 108.71 ($C_{15}$), 105.72 ($C_3$), 56.24 ($OCH_3$), 32.33 ($C_7$); MS: *m*/*z* 416, 418 (M, M + 2) mass-416. M.F. $C_{16}H_{12}BrF_3N_2O_3$.

*N′-(3,4,5-Trimethoxybenzylidene)-2-(2,4,5-trifluorophenyl)acetohydrazide (**4o**)*

Compound obtained as white solid. M.p.: 185–187 °C; $^{1}$H NMR (400 MHz, DMSO-$d_6$): $\delta$; 11.57* (s, 1H, NH), 8.02* (s, 1H, $H_9$), 7.52 (m, 2H, $H_3$ and $H_6$), 6.99 (s, 2H, $H_{11}$ and $H_{15}$), 3.83* (s, 2H, $H_7$), 3.82 (s, 6H, $[OCH_3]_2$), 3.69 (s, 3H, $OCH_3$); $^{13}$C NMR (100 MHz, DMSO-$d_6$): $\delta$ 170.51 ($C_8$), 153.17 ($C_2$, $C_{12}$, and $C_{14}$), 147.20 ($C_4$), 147.0 ($C_5$), 143.16 ($C_9$), 139.15 ($C_{13}$), 129.64 ($C_1$), 119.71 ($C_{10}$), 119.46 ($C_6$), 105.21 ($C_3$), 104.11 ($C_{11}$ and $C_{15}$), 60.09 ($C_{13}$-$OCH_3$), 55.98 ($C_{12}$, $C_{14}$-$OCH_3$), 33.51 ($C_7$); MS: *m*/*z* 383 (M + 1) mass-382. M.F. $C_{18}H_{17}F_3N_2O_4$.

*N′-(3-Ethoxy,4-methoxybenzylidene)-2-(2,4,5-trifluorophenyl)acetohydrazide (**4p**)*

Compound obtained as white solid. M.p.: 146–148 °C; $^{1}$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 11.44* (s, 1H, NH), 8.01* (s, 1H, $H_9$), 7.51 (m, 2H, $H_3$ and $H_6$), 7.27 (m, 1H, $H_{11}$), 7.16 (m, 1H, $H_{15}$), 7.0 (m, 1H, $H_{14}$), 4.05 (s, 3H, $OCH_3$), 3.831 (s, 2H,

$OCH_2$), 3.79* (s, 2H, $H_7$), 1.34 (t, 1H, $J = 6.8$ Hz, $CH_3$); $^{13}C$ NMR (100 MHz, DMSO-$d_6$): $\delta$ 170.26 ($C_8$), 157.18 ($C_2$), 150.74 ($C_{13}$), 149.33 ($C_{12}$), 148.28 ($C_5$), 147.02 ($C_4$), 143.44 ($C_9$), 128.3 ($C_1$), 121.60 ($C_{10}$), 121.19 ($C_{15}$), 119.76 ($C_6$), 111.76 ($C_{11}$), 109.82 ($C_{14}$), 105.66 ($C_3$), 63.81 ($OCH_2$), 55.57 ($OCH_3$), 33.50 ($C_7$), 14.64 ($CH_3$); MS: *m/z* 367 (M + 1) mass-366. M.F. $C_{18}H_{17}F_3N_2O_3$.

*N′-(3,4,5-Trimethoxy,2-methylbenzylidene)-2-(2,4,5-trifluorophenyl)acetohydrazide* ***(4q)***

Compound obtained as white solid. M.p.: 147–149 °C; $^1H$ NMR (400 MHz, DMSO-$d_6$): $\delta$ 11.43* (s, 1H, NH), 8.39* (s, 1H, $H_9$), 7.50 (m, 2H, $H_3$ and $H_6$), 6.76 (s, 1H, $H_{15}$), 3.78* (s, 2H, $H_7$), 3.79 (s, 3H, $C_{12}$-$OCH_3$), 3.78 (s, 3H, $C_{13}$-$OCH_3$), 3.73 (s, 3H, $C_{14}$-$OCH_3$), 2.49 (s, 3H, $CH_3$); $^{13}C$ NMR (100 MHz, DMSO-$d_6$): $\delta$ 170.0 ($C_8$), 153.46 ($C_2$), 153.19 ($C_{12}$), 146.70 ($C_4$), 146.2 ($C_5$), 144.30 ($C_{14}$), 141.35 ($C_9$), 139.63 ($C_{13}$), 133.24 ($C_1$), 119.90 ($C_6$), 118.30 ($C_{11}$), 115.38 ($C_{10}$), 111.23 ($C_{15}$), 105.66 ($C_3$), 61.52 ($C_{12}$-$OCH_3$), 60.42 ($C_{13}$-$OCH_3$), 55.84 ($C_{14}$-$OCH_3$), 32.0 ($C_7$), 22.50 ($CH_3$); MS: *m/z* 397 (M + 1) mass-396. M.F. $C_{19}H_{19}F_3N_2O_4$.

*N′-(2,3-Dimethylbenzylidene)-2-(2,4,5-trifluorophenyl)acetohydrazide* ***(4r)***

Compound obtained as white solid. M.p.: 164–166 °C; $^1H$ NMR (400 MHz, DMSO-$d_6$): $\delta$ 11.46* (s, 1H, NH), 8.33* (s, 1H, $H_9$), 7.65 (m, 1H, $H_{15}$), 7.50 (m, 2H, $H_3$ and $H_6$), 7.07 (m, 2H, $H_{13}$ and $H_{14}$), 3.81* (s, 2H, $H_7$), 2.38 (s, 3H, $C_{11}$-$CH_3$), 2.29 (s, 3H, $C_{12}$-$CH_3$); $^{13}C$ NMR (100 MHz, DMSO-$d_6$): $\delta$ 170.23 ($C_8$), 153.46 ($C_2$), 146.5 ($C_4$), 146.30 ($C_5$), 142.69 ($C_9$), 139.11 ($C_{11}$), 136.42 ($C_{12}$), 131.53 ($C_{10}$), 129.38 ($C_1$), 126.86 ($C_{13}$), 126.42 ($C_{15}$), 119.83 ($C_{14}$), 119.69 ($C_6$), 105.63 ($C_3$), 32.10 ($C_7$), 20.82 ($C_{12}$-$CH_3$), 19.30 ($C_{11}$-$CH_3$); MS: *m/z* 321 (M + 1) mass-320. M.F. $C_{17}H_{15}F_3N_2O$.

*N′-(2,4,6-Trimethoxybenzylidene)-2-(2,4,5-trifluorophenyl)acetohydrazide* ***(4s)***

Compound obtained as white solid. M.p.: 172–174 °C; $^1H$ NMR (400 MHz, DMSO-$d_6$): $\delta$ 11.20* (s, 1H, NH), 8.25* (s, 1H, $H_9$), 7.52 (m, 2H, $H_3$ and $H_6$), 6.28 (s, 2H, $H_{12}$ and $H_{14}$), 3.74* (s, 2H, $H_7$), 3.82 (s, 6H, $C_{11,\ 15}$-$OCH_3$), 3.80 (s, 3H, $C_{13}$-$OCH_3$); $^{13}C$ NMR (100 MHz, DMSO-$d_6$): $\delta$ 170.0 ($C_8$), 162.0 ($C_{13}$), 159.85 ($C_{11}$ and $C_{15}$), 153.46 ($C_2$), 146.9 ($C_4$), 146.2 ($C_5$), 138.72 ($C_9$), 127.85 ($C_1$), 119.65 ($C_6$), 105.62 ($C_3$), 104.01 ($C_{10}$), 91.28 ($C_{12}$ and $C_{14}$), 55.98 ($C_{11}$ and $C_{15}$-$OCH_3$), 55.93 ($C_{13}$-$OCH_3$), 31.87 ($C_7$); MS: *m/z* 383 (M + 1) mass-382. M.F. $C_{18}H_{17}F_3N_2O_4$.

*N′-(4-Ethylbenzylidene)-2-(2,4,5-trifluorophenyl)acetohydrazide* ***(4t)***

Compound obtained as white solid. M.p.: 148–150 °C; $^1H$ NMR (400 MHz, DMSO-$d_6$): $\delta$ 11.51* (s, 1H, NH), 8.08* (s, 1H, $H_9$), 7.60 (d, 1H, $J = 7.6$ Hz, $H_{11}$ and $H_{15}$), 7.52 (m, 2H, $H_3$ and $H_6$), 7.27 (d, 2H, $J = 7.6$ Hz, $H_{12}$ and $H_{14}$), 3.82* (s, 2H, $H_7$), 2.63 (q, 2H, $J = 7.2$ Hz, $C_{13}$-$CH_2$), 1.18 (t, 3H, $J = 7.2$ Hz, $C_{13}$-$CH_3$); $^{13}C$ NMR (100 MHz, DMSO-$d_6$): $\delta$ 170.37 ($C_8$), 157.2 ($C_2$), 146.82 ($C_4$), 145.86 ($C_5$),

143.42 ($C_9$), 131.70 ($C_{13}$), 128.19 ($C_{11}$ and $C_{15}$), 127.10 ($C_1$), 126.82 ($C_{12}$ and $C_{14}$), 125.31 ($C_{10}$), 119.63 ($C_6$), 105.65 ($C_3$), 32.11 ($C_7$), 28.03 ($CH_2$), 15.28 ($CH_3$); MS: *m/z* 321 (M + 1) mass-320. M.F. $C_{17}H_{15}F_3N_2O$.

*N′-(4-Propylbenzylidene)-2-(2,4,5-trifluorophenyl)acetohydrazide (**4u**)*

Compound obtained as white solid. M.p.: 146–148 °C; $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 11.51* (s, 1H, NH), 8.08* (s, 1H, $H_9$), 7.60 (d, 2H, $J$ = 8.4 Hz, $H_{11}$ and $H_{15}$), 7.5 (m, 2H, $H_3$ and $H_6$), 7.25 (d, 2H, $J$ = 7.6 Hz, $H_{12}$ and $H_{14}$), 3.82* (s, 2H, $C_7$), 2.58 (t, 2H, $J$ = 7.2 Hz, $CH_2$), 1.59 (m, 2H, $CH_2$), 0.89 (t, 3H, $J$ = 7.2 Hz, $CH_3$); $^{13}$C NMR (100 MHz, DMSO-$d_6$): $\delta$ 170.37 ($C_8$), 156.4 ($C_2$), 147.0 ($C_4$), 146.84 ($C_5$), 144.25 ($C_5$), 144.25 ($C_4$), 143.43 ($C_9$), 131.71 ($C_{13}$), 128.77 ($C_{11}$ and $C_{15}$), 127.01 ($C_1$), 126.74 ($C_{12}$ and $C_{14}$), 124.8 ($C_1$), 119.60 ($C_6$), 105.36 ($C_3$), 37.06 ($C_{13}$-$CH_2$), 32.10 ($C_7$), 23.80 ($CH_2$), 13.52 ($CH_3$); MS: *m/z* 333 (M, M + 2) mass-334. M.F. $C_{18}H_{16}F_3N_2O$.

*N′-(4-tert-Butylbenzylidene)-2-(2,4,5-trifluorophenyl)acetohydrazide (**4v**)*

Compound obtained as white solid. M.p.: 156–158 °C; $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 11.52* (s, 1H, NH), 8.08* (s, 1H, $H_9$), 7.61 (d, 2H, $J$ = 8.4 Hz, $H_{11}$ and $H_{15}$), 7.53 (m, 2H, $H_3$ and $H_6$), 7.45 (d, 2H, $J$ = 8.4 Hz, $H_{12}$ and $H_{14}$), 3.82* (s, 2H, $H_7$), 1.29 (s, 9H, $(CH_3)_3$); $^{13}$C NMR (100 MHz, DMSO-$d_6$): $\delta$ 170.37 ($C_8$), 152.66 ($C_2$), 149.25 ($C_4$), 146.76 ($C_5$), 143.32 ($C_9$), 131.43 ($C_{13}$), 126.86 ($C_1$), 126.58 ($C_{11}$ and $C_{15}$), 125.55 ($C_{12}$ and $C_{14}$), 124.67 ($C_{10}$), 119.80 ($C_6$), 105.80 ($C_3$), 34.52 (C13-C), 32.05 ($C_7$), 30.92 ($CH3)_3$; MS: *m/z* 349 (M + 1) mass-348. M.F. $C_{18}H_{16}F_3N_2O$.

*N′-(4-Butylbenzylidene)-2-(2,4,5-trifluorophenyl)acetohydrazide (**4w**)*

Compound obtained as white solid. M.p.: 144–146 °C; $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 11.51* (s, 1H, NH), 8.08* (s, 1H, $H_9$), 7.59 (d, 2H, $J$ = 8.0 Hz, $H_{11}$ and $H_{15}$), 7.52 (m, 2H, $H_3$ and $H_6$), 7.25 (d, 2H, $J$ = 7.6 Hz, $H_{12}$ and $H_{14}$), 3.82* (s, 2H, $H_7$), 2.60 (t, 2H, $J$ = 7.2 Hz, $C_{13}$-$CH_2$), 1.58 (m, 2H, $CH_2$), 1.30 (m, 2H, $CH_2$), 0.89 (t, 3H, $J$ = 7.2 Hz, $CH_3$); $^{13}$C NMR (100 MHz, DMSO-$d_6$): $\delta$ 170.36 ($C_8$), 154.9 ($C_2$), 146.85 ($C_4$), 144.47 ($C_5$), 143.43 ($C_9$), 131.69 ($C_{13}$), 128.70 ($C_{11}$ and $C_{15}$), 127.03 ($C_1$), 126.75 ($C_{12}$ and $C_{14}$), 125.37 ($C_{10}$), 119.68 ($C_6$), 105.64 ($C_3$), 34.66 ($C_{13}$-$CH_2$), 32.83 ($C_{13}$-$CH_2$), 32.09 ($C_7$), 21.67 ($C_{13}$-$CH_2$), 13.67 ($CH_3$); MS: *m/z* 349 (M + 1) mass-348. M.F. $C_{18}H_{16}F_3N_2O$.

*N′-(Cinnamylmethylene)-2-(2,4,5-trifluorophenyl)acetohydrazide (**4x**)*

Compound obtained as white solid. M.p.: 166–168 °C; $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 11.49* (s, 1H, NH), 7.91* (d, 1H, $J$ = 8.4 Hz, $H_9$), 7.60 (d, 1H, $J$ = 7.6 Hz, $H_{11}$), 7.49 (m, 2H, $H_2$ and $H_5$), 7.37 (m, 4H, $H_{10}$, $H_{13}$, $H_{15}$, and $H_{16}$), 6.98 (m, 2H, $H_{14}$ and $H_{16}$), 3.77 (s, 2H, $H_7$); NMR (100 MHz, DMSO-$d_6$): $\delta$ 170.16 ($C_8$), 157.0 ($C_2$), 148.66 ($C_4$), 145.87 ($C_5$), 138.97 ($C_{11}$), 138.70 ($C_9$), 135.86 ($C_{12}$),

128.78 ($C_{14}$, $C_{15}$, and $C_{16}$), 127.0 ($C_{13}$ and $C_{17}$), 126.3 ($C_1$) 125.08 ($C_{10}$), 119.82 ($C_6$), 105.80 ($C_3$), 31.89 ($C_7$); MS: *m/z* 319 (M + 1) mass-318. M.F. $C_{17}H_{13}F_3N_2O$.

*N′-(1H-Indol-3-yl)methylene)-2-(2,4,5-trifluorophenyl)acetohydrazide (**4y**)*

Compound obtained as white solid. M.p.: 165–167 °C; $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 11.54* (s, 1H, NH), 11.24 (s, 1H, NH), 8.29* (s, 1H, $H_9$), 8.15 (d, 1H, $J = 7.6$ Hz, $H_{12}$), 7.79 (s, 1H, $H_{10}$), 7.53 (m, 2H, $H_3$ and $H_6$), 7.43 (t, 1H, $J = 7.6$ Hz, $H_{14}$), 7.16 (m, 2H, $H_{13}$ and $H_{15}$), 3.84* (s, 2H, $H_7$); $^{13}$C NMR (100 MHz, DMSO-$d_6$): $\delta$ 169.61 ($C_8$), 154.73 ($C_2$), 146.32 ($C_4$), 143.97 ($C_5$), 140.82 ($C_9$), 137.07 ($C_{16}$), 130.27 ($C_{10}$), 127.4 ($C_1$), 124.07 ($C_{17}$), 122.55 ($C_{13}$), 121.50 ($C_{14}$), 120.48 ($C_{12}$), 119.94 ($C_6$), 111.85 ($C_{15}$), 111.49 ($C_{11}$), 105.77 ($C_3$), 32.06 ($C_7$); MS: *m/z* 332 (M + 1) mass-331. M.F. $C_{17}H_{12}F_3N_3O$.

## Conclusions

A simple and convenient method was developed for synthesis of *N′*-benzylidene-2,4,5-trifluoroacetohydrazide derivatives by condensation of trifluoroacetohydrazide with different arylaldehydes in ethanol with 70% perchloric acid as catalyst. Simple reaction conditions, easy workup, and good product purity without purification are advantages of the developed method. A total of 25 molecules were generated using the developed methodology. Antibacterial activity screening revealed that compound **4q** showed good antibacterial activity ($IC_{50}$) of 30 and 35 μg/mL against *Streptococcus mutans* MTCC 497 and *Staphylococcus aureus* MTCC 737, respectively, followed by compound **4c** ($IC_{50}$: 45 μg/mL) against *Streptococcus mutans* MTCC 497 as well as *Salmonella enterica* MTCC 3858. In silico simulation studies revealed that ligands with 2-$NO_2$, 4-$(NCH_3)_2$, and 3,4-di-OH functional groups on the benzylidene ring (**4e**, **i**, **m**) had greater affinity towards glucosamine 6-phosphate deaminase (NagB) enzyme.

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**Compliance with ethical standards**

**Conflict of interest** The authors confirm that this article has no conflict of interest.

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