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# Synthesis, docking and characterization of some novel 5-(S-alkyl)-1.3.4-thiadiazole-2-carboxamide derivatives as anti-inflammatory and antibacterial agents

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## Abstract

Because of the great pharmacological and industrial significance of 1,3,4-thiadiazole and its related compounds, researchers are still very interested in them. For this reason, in this study, we looked at ways to create new hybrid compounds containing carboxamide and 1,3,4-thiadiazole moieties. The thioxoacetamide derivatives used to make these compounds were reacted with various alkylated reagents to produce multiple *S*-alkyl groups. Additionally, these compounds were reacted with aldehydes to form novel derivatives known as 5-(substituent)-*N*-phenyl-1,3,4-thiadiazole-2-carboxamide. Here, we used the agar well diffusion method to examine the antibacterial activity of all the produced compounds against a few pathogenic bacteria that were resistant to multiple drugs. Additionally, look into their capacity to lower inflammation through the use of bovine serum albumin in the protein denaturation procedure. The substances were characterized by spectral analysis (IR, $^{1}$HNMR, $^{13}$CNMR and Elemental Analysis), and efficient as antibacterial agents against all the tested bacterial strains, except for *Escherichia coli*. Compounds **4a** and **8c** showed the highest level of inhibition zone against Gram-positive bacteria (*Staph. aureus, Bacillus subtilis*) at concentration 0.3, 0.4 and 0.5 mg/ml compared with ciprofloxacin at the same concentrations. The results demonstrated that every compound has significant anti-inflammatory activity. At a concentration of 250 µg/ml, compounds **3a**, **4c** and **8c** had the highest percentage inhibition of protein denaturation when (83.24%, 86.44% and 85.14%, respectively) compared to other compounds and diclofenac sodium as reference drug. Comparing compounds **4c** and **8c** to ciprofloxacin and diclofenac sodium, they showed powerful antibacterial and anti-inflammatory action. Furthermore, an investigation using molecular docking against DHPS from *S. aureus* (PDB ID: 6CLV) showed a strong connection with the intended protein and an elevated docking score, making it a prime candidate for antibiotics.

**Keywords** Carboxamide, Thioxoacetamide, 1,3,4-thiadiazole, Thiohydrazides, Antimicrobial, Anti-inflammatory agent

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## Background

Microbiological diseases are the most critical issue facing the economy and the world's health [1]. It has recently become more challenging to treat bacterial infections with conventional medicines [2]. Growing concern is being expressed throughout the world over the growth of bacterial resistance to well-known treatments and hospital-acquired illnesses [3]. In actuality, the development of microbial resistance to commercially accessible antibacterial medications is the main cause of illness and mortality [4]. Microbiological disorders that have recently caused a great deal of pain for humans include the epidemic of the plague, diphtheria, cholera, typhoid fever, a respiratory infection, and tuberculosis [5]. Additionally, some recent clinical studies mention the growth in enterococci that are resistant to vancomycin, *Staphylococcus epidermidis*, and methicillin-resistant *Staphylococcus aureus* (MRSA), which are the most prevalent bacterial infections that cause death in the majority wealthy countries [6, 7]. As per the World Health Organisation (WHO), traditional antibiotic therapy typically fails to treat diseases caused by resistant germs, which increases the risk of mortality and lengthens suffering [8]. Therefore, the development of novel antimicrobial drugs that differ from the widely used categories of antibacterial agents is still necessary [9]. Moreover, one potential solution to the problem of overloaded multidrug resistance (MDR) is the development of novel drugs with distinctive mechanisms of action to prevent cross-resistance with currently available therapies [10]. Because of their broad range of biological functions, heterocyclic ring structures in organic compounds continue to garner a lot of research. Numerous synthetic compounds that exhibit appealing biological effects such as antiviral [11], anticancer [12], cytotoxic [13], anticonvulsant [14], antihyperlipedemic [15], anti-inflammatory [16], analgesic [17], antidepressant [18], antioxidant [19], anti-pesticide [20], anti-COVID [21], antileishmanial [22], and antituberculosis [23] properties commonly use the scaffold 1,3,4-thiadiazole.

Many thiadiazole compounds have found extensive usage in chemotherapeutics as antimicrobial and antibacterial agents [24] that are effective against a wide range of pathogenic bacteria and resistant mycobacterium, such as compounds **A** and **B**. Moreover, mycobacterial activity has been observed to be significantly inhibited by compound **B** ($IC_{50}$ = 0.23 g/ml) [25]. Compound **C** was discovered to be superior to the industry standard (pyrimethanil) when the synthetic 1,3,4-thiadiazole scaffolds were tested using the mycelial growth rate method against a few fungus strains [26]. However, scaffolds **D** have anti-inflammatory activity and demonstrate COX-2 selectivity in the J774A.1 murine macrophage cell line [27]. (Fig. 1). The impressive anti-inflammatory properties of both heterocycles and carboxamide units have been demonstrated. As a result, a lot of research has focused on creating and studying oxicam derivatives as pharmacological agents. The success of the nonsteroidal anti-inflammatory medicines (NSAIDs) piroxicam (Feldene®), meloxicam (Mobic®), and tenoxicam stimulated research in this topic (Fig. 1). Additionally, Rimonabant exerted high activity via the inhibition of COX-2 (inducible) induced at sites of inflammation [28, 29].

As part of our ongoing efforts to produce anti-infective medicines [30–35]. In this study, we design and synthesize several new prototypes containing two pharmacophores, carboxamide and 1,3,4-thiadiazole inside one structural framework using environmentally friendly processes starting with 2-hydrazinyl-*N*-phenyl-2-thioxoacetamide derivatives [36]. We tested their anti-bacterial and anti-inflammatory activity for bioactive compounds.

## Result and discussion

### Chemistry

As a continuation of our strategy is to determine methods to utilize these molecules as the basis for the synthesis of many different five, six, and seven-membered rings [37–41]. Reaction of thioxoacetamide derivatives **1a–d** with carbon disulfide and potassium hydroxide in ethanol at room temperature considered an efficient method to synthesis potassium 5-(phenylcarbamoyl)-1,3,4-thiadiazole-2-thiolate derivatives (**2a–d)**, which treated with concentrated hydrochloric acid until pH 2–3 to afford novel moiety of 1,3,4-thiadiazole derivatives **3a–d** that can be used as a building block of some new 1,3,4-thiadiazole analogous (Scheme 1). The IR spectrum of compound **3a–d** revealed the disappearance of $NH_2$ group. $^1$HNMR for compound **3a** showed new singles at 15.06 for $NH_{thiadiazole}$ group, disappeared by $D_2O$, at the same time the peaks for amino group are disappeared. All the compounds show a new peak above 190 ppm in $^{13}$C NMR which come back to C=S of the formed 1,3,4-thiadiazole rings.

Moreover, compounds **2a–c** reacted with active halo compounds namely, methyl iodide ethyl iodide, 1-bromo-2-methylbutane and (bromomethyl)benzene at low temperature to give the corresponding S-alkyl derivatives with a substantial output, economical, gentle, straightforward, and environmentally friendly approach that produces suitable behaviors, see Scheme 2. Structures of the recently obtained compounds were verified based upon their IR, $^1$H-NMR, $^{13}$C-NMR, and elemental analyses. The IR spectra of compounds **4–7** exhibited the presence of broad band: at 3234–3537 $cm^{-1}$ corresponding to NH groups, at 1660–1680 $cm^{-1}$ corresponding to alkyl groups. The $^1$H-NMR spectrum, for example, of

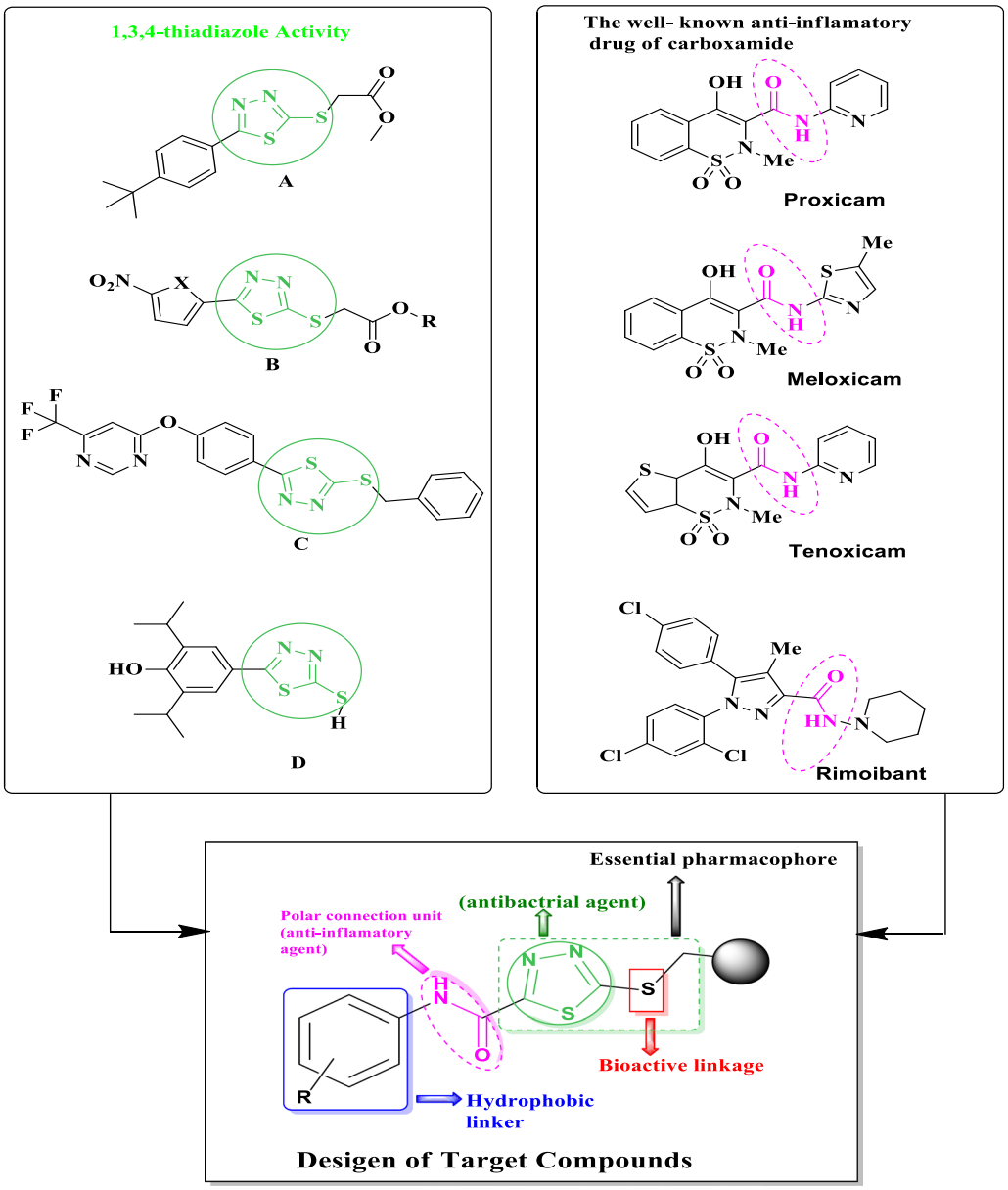
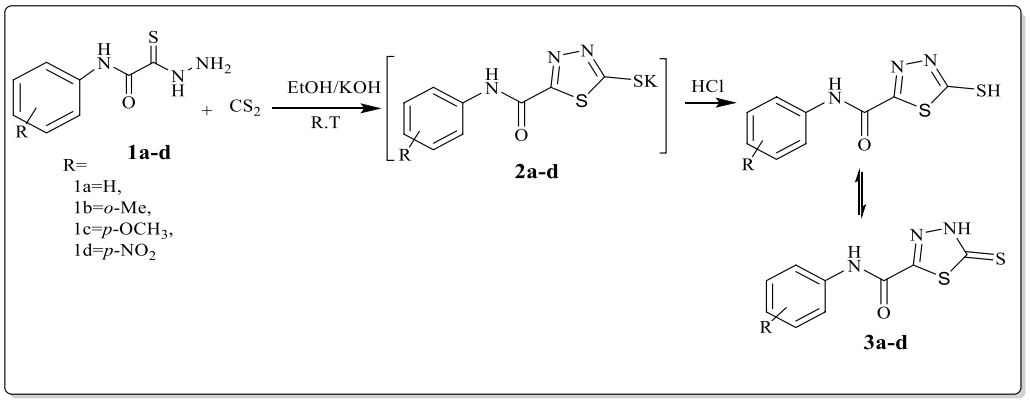


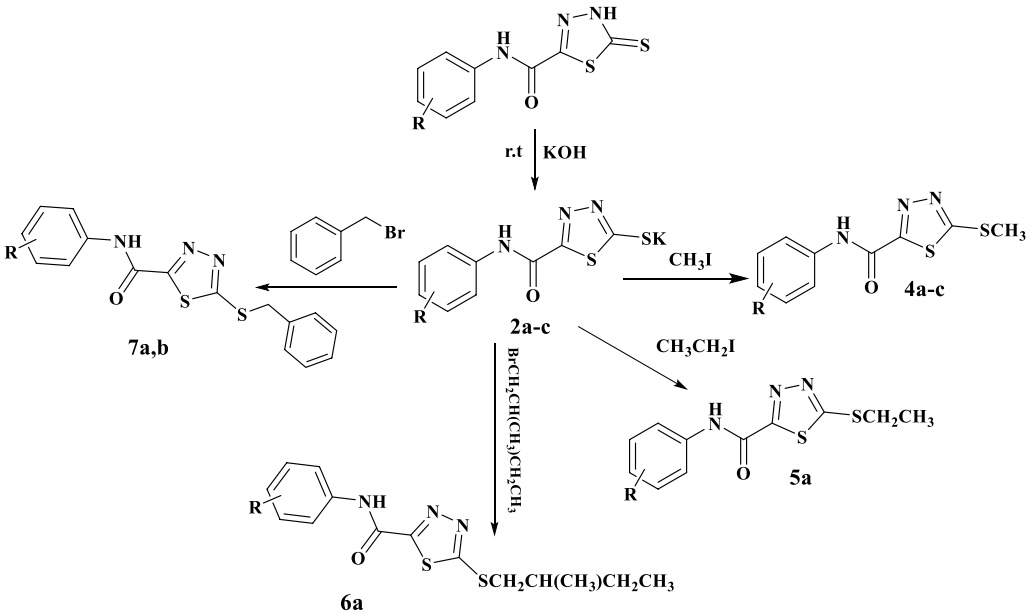

**Fig. 1** Strategy employed for designing 1.3.4-thiadiazole-2-carboxamide derivatives

compound **4a–c** revealed the presence of a broad band at 10.60–11.03 ppm characterized to NH group, a singlet signal at 2.24–2.84 ppm corresponding to S-alkyl group. $^{13}$CMR spectrum of compound **4a** revealed the following signals: 165.29, 156.32 (2C, thiadiazole), 173.51 (C=O), four signals at 138.12, 129.18, 125.11, 121.27 ppm for 5C of Aromatic group and singlet signal at 17.37 ppm of methylthiol group.

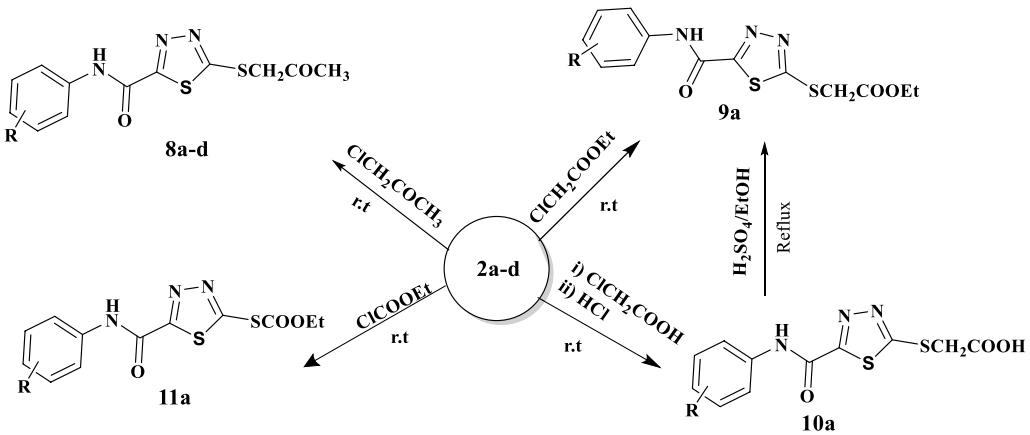
Nevertheless, alkylation reaction of compounds **2a–d** with chloroacetone, ethyl chloroacetate, chloroacetic acid and ethyl chloroformate, afforded the corresponding 5-(S-alkyl) sulfanyl-1,3,4-thiadiazole-2-carboxamide derivatives **8a–c**, **9a**, **10a** and **11a**, respectively. Additionally, it was easily to synthesis compound **9a** by another way through conversion of acidic group in compound **10a** into ester group in compound **9a**, see Scheme 3. The structures of the obtained 5-(S-alkyl)-1.3.4-thiadiazole-2-carboxamide derivatives **8–11** were distinguished by their spectral and elemental data. For instance, the IR spectrum of compound 9a had peak absorption for the NH group at 3537 cm$^{-1}$ and another distinctive band for the novel C=O group



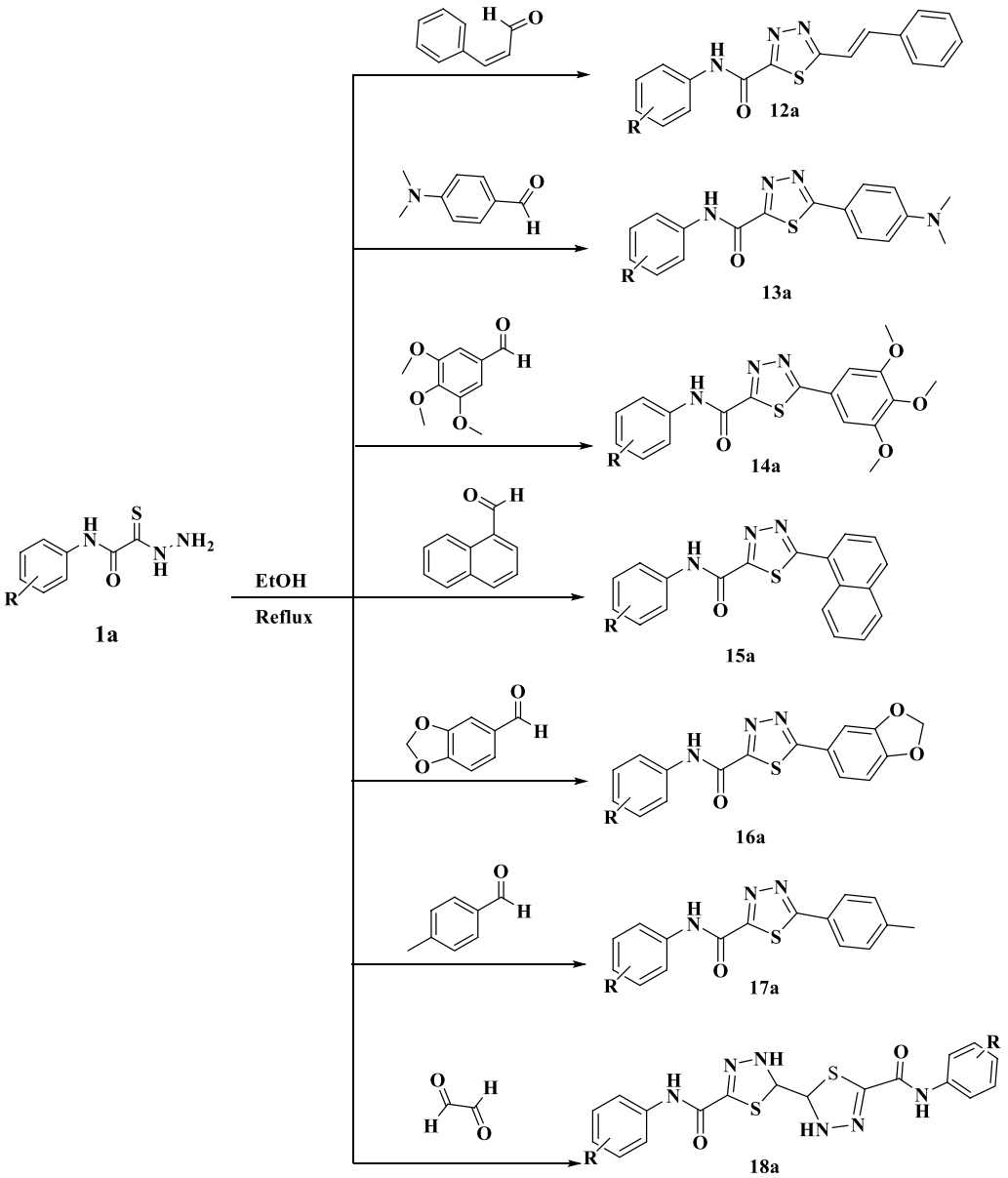

**Scheme 1** Synthesis of *N*-phenyl-5-thioxo-4,5-dihydro-1,3,4-thiadiazole-2-carboxamide derivatives




**Scheme 2** Synthesis of 5-(S-alkyl)-1.3.4-thiadiazole-2-carboxamide derivatives




**Scheme 3** Synthesis of 5-(S-alkyl)-1.3.4-thiadiazole-2-carboxamide derivatives




**Scheme 4** Synthesis of 5-(substituent)-*N*-phenyl-1,3,4-thiadiazole-2-carboxamide derivatives

at 1737 $cm^{-1}$. Where the $^{1}$H-NMR spectrum of this compound showed a singlet signal at 11.05 ppm for NH group which disappeared by $D_2O$, a multiples signals between 7.15 and 7.83 ppm for aromatic protons, singlet signal at 4.35 ppm for S–$CH_2$– group, quartet signal at 4.20–4.14 ppm and a triplet signal at 1.23–1.20 ppm with coupling constant equals to 7.08 Hz, which could be assigned for $CH_2CH_3$ groups. The signals of $^{13}$CNMR confirmed the expected structure by appearance of new carbonyl group at 168.08 ppm. Finally, the DEPT-135 obviously distinguished between the –$CH_2$– (62.12 ppm) and –$CH_3$ (14.56 ppm) of the ethyl chain where, it showed one $CH_3$ with a positive phase and two $CH_2$ with a negative one.

Reaction of compound **1a** with different aldehydes namely, cinnamaldehyde, *p-N, N* dimethylaminobenzaldehyde, 3,4,5-trimethoxy-benzaldehyde, 1-naphthaldehyde, pipreonal, *p* methylbenzaldehyde and glyoxal to afford; 5-(substituent)-*N*-phenyl-1,3,4-thiadiazole-2-carboxamide derivatives **(12a–18a)**, respectively, Scheme 4. IR spectrum of compounds **12a–18a** showed the disappearance of $NHNH_2$ group absorption bands. The $^{1}$H-NMR spectrum for compound **12a** showed signal at 10.70 ppm for NH group (disappeared by $D_2O$), between

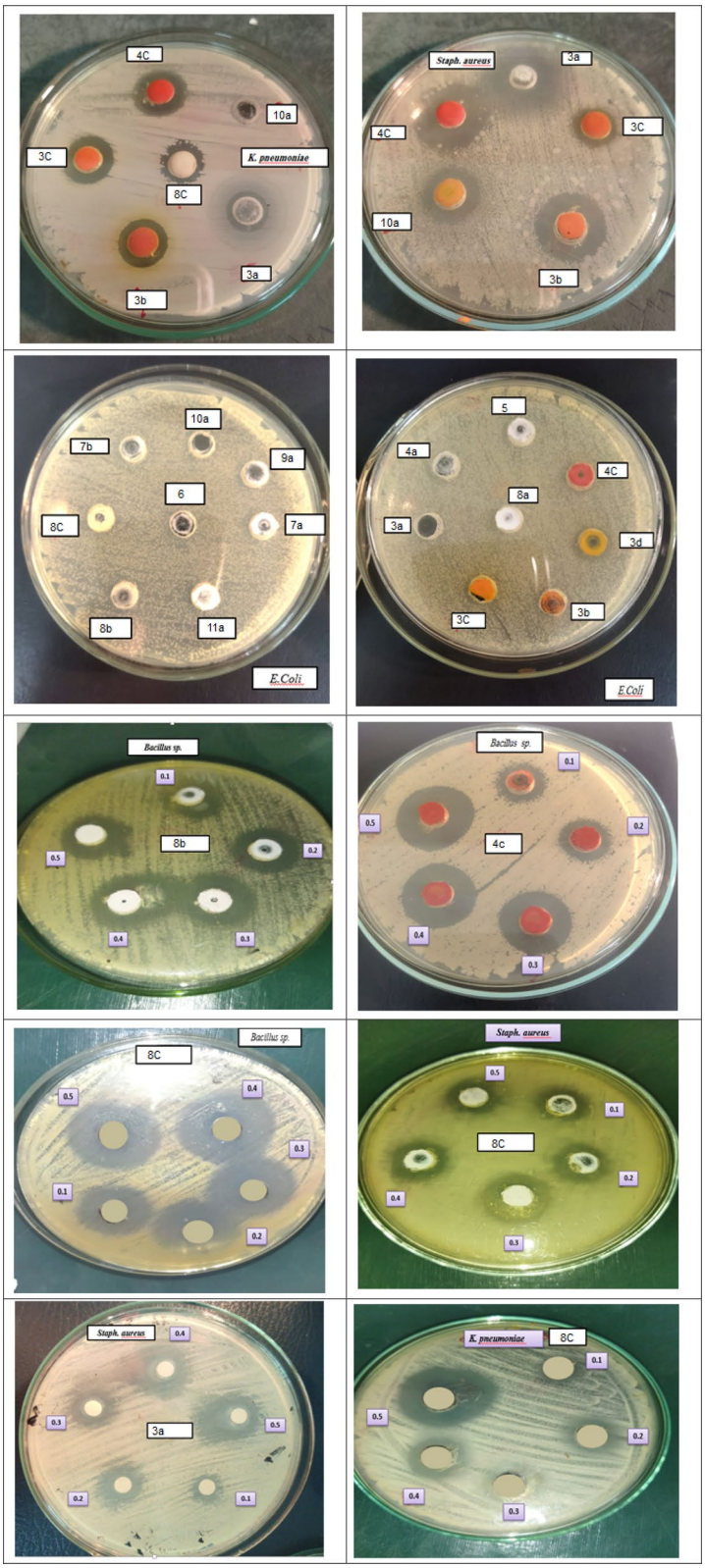



**Fig. 2** Antibacterial activity of the tested compounds

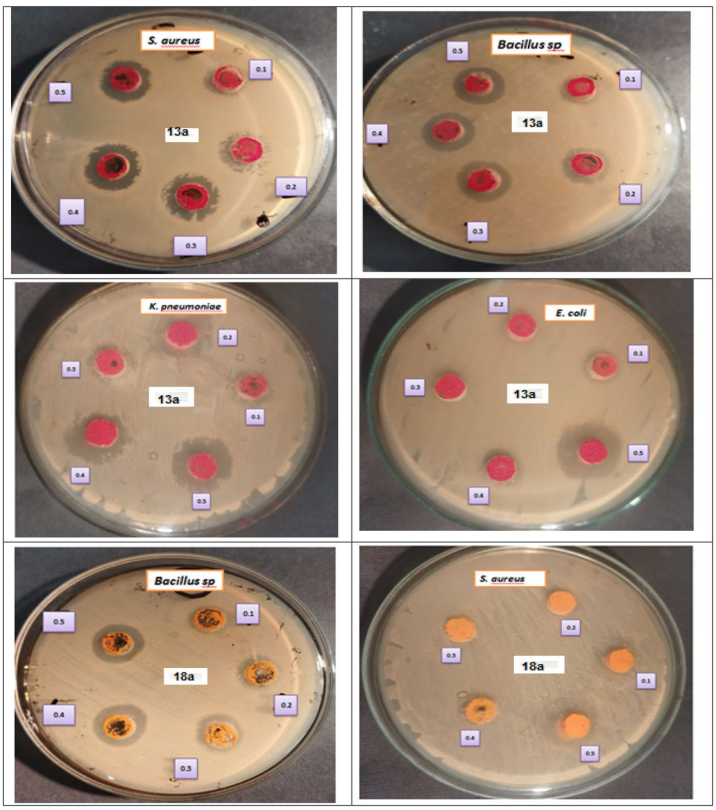



**Fig. 2** continued

7.85–7.08 ppm for aromatic protons and 6.68 ppm for the –CH=CH– group. The signals of $^{13}$C NMR spectrum confirmed the expected structure by appearance of new group signals at 163.20, 158.13 (2-S–C=N) and 120.74, 112.13 ppm for CH=CH– group. The $^{1}$H-NMR spectrum for compound **13a** showed signal at 11.31 ppm for NH group (disappeared by $D_2O$), between 8.23–7.09 ppm for aromatic protons and a new singlet signal at 2.84 ppm for the two methyl groups. The signals of $^{13}$CNMR spectrum confirmed the expected structure by appearance of new group signals at 171.33 (C=O), 166.60, 162.53 (2C, thiadiazole) and 36.42 ppm ($2CH_3$). In the case of compound **18a,** its $^{1}$H-NMR spectrum showed two signals at 10.19 and 9.04 ppm for NH groups (disappeared by $D_2O$), between 7.74–7.06 ppm for aromatic protons and new singlet signal at 5.53 for the two -CH groups. The signals of $^{13}$CNMR spectrum confirmed the expected structure by appearance of new signal at 159.13 (C=O), 139.04 (C, Thiadiazole), 138.66, 129.05, 124.29, 120.74 for aromatic ring and 76.13 ppm for quaternary carbon atom. Also, its Dept -135 spectrum showed signals at 129.09, 124.31, 120.74 ppm for aromatic ring and 75.98 ppm ($CH_{thiadiazole}$) in the positive phase.

## Biological evaluation

### *Antimicrobial screening*

Antimicrobial activity of the tested compounds was investigated against multidrug pathogenic bacteria. The tested compounds showed potential antibacterial effect against *Staph. aureus, Bacillus subtilis* and *K. pneumonia* and no inhibitory effect against *E. coli*. Ciprofloxacin is used in this investigation as a control. In clinic and hospital settings, ciprofloxacin is a widely used broad-spectrum antibiotic. The closest compounds to ciprofloxacin were **4c** and **8c**, which were more effective against Gram-positive bacteria (*Staph. aureus, Bacillus subtilis*) at concentration 0.3, 0.4 and 0.5 mg/ml. Furthermore, compounds **3a, 4a** and **6a** showed potential antibacterial effect against *Staph. Aureus* and *Bacillus subtilis*, respectively, as shown in (Table S1(supplementary file), Fig. 2).

### *Statistical results of antimicrobial screening*

Nineteen compounds studied with different concentrations on both Gram-positive and Gram-negative bacteria, formed four subsets in accordance with the zone of inhibition values. A one-way ANOVA was conducted to compare the effect of in-vitro antibacterial activity

**Table 1** In-vitro antibacterial activity of tested compounds

| Bacteria | Test compound | Mean of the size of the inhibition zone (mm) | Std. deviation Deviation | *p*-value* |
|---|---|---|---|---|
| *S. aureus* | 3a | 31.55 | 5.45 | 0.0001 |
| | 3b | 29.91 | 3.15 | |
| | 3c | 25.17 | 4.63 | |
| | 3d | 22.16 | 4.36 | |
| | 4a | 30.68 | 3.02 | |
| | 5a | 18.87 | 5.89 | |
| | 8a | 18.67 | 4.05 | |
| | 10a | 14.21 | 2.75 | |
| | 9a | 12.65 | 7.05 | |
| | 7a | 24.08 | 3.85 | |
| | 11a | 21.47 | 5.92 | |
| | 4c | 30.41 | 4.80 | |
| | 8b | 27.08 | 5.31 | |
| | 8c | 33.27 | 4.73 | |
| | 7b | 19.79 | 7.73 | |
| | 6a | 19.98 | 5.95 | |
| | 13a | 4.30 | 5.54 | |
| | 18a | 14.34 | 3.47 | |
| | Ciprofloxacin | 43.97 | 4.28 | |
| *Bacillus* sp. | 3a | 10.77 | 7.19 | 0.0001 |
| | 3b | 20.76 | 5.80 | |
| | 3c | 11.62 | 6.83 | |
| | 3d | 19.04 | 6.80 | |
| | 4a | 15.80 | 4.83 | |
| | 5a | 16.73 | 5.16 | |
| | 8a | 21.51 | 5.95 | |
| | 10a | 15.59 | 8.35 | |
| | 9a | 10.71 | 5.60 | |
| | 7a | 18.59 | 2.37 | |
| | 11a | 15.99 | 4.19 | |
| | 4c | 31.54 | 4.76 | |
| | 8b | 23.43 | 2.61 | |
| | 8c | 36.43 | 4.04 | |
| | 7b | 17.59 | 4.36 | |
| | 6a | 32.07 | 5.04 | |
| | 13a | 13.85 | 4.32 | |
| | 18a | 14.11 | 2.58 | |
| | Ciprofloxacin | 33.15 | 7.18 | |

**Table 1** (continued)

| Bacteria | Test compound | Mean of the size of the inhibition zone (mm) | Std. deviation Deviation | *p*-value* |
|---|---|---|---|---|
| *K. pnemoniae* | 3a | 15.17 | 2.43 | 0.0001 |
| | 3b | 17.47 | 3.37 | |
| | 3c | 14.84 | 2.29 | |
| | 3d | 19.89 | 5.96 | |
| | 4a | 19.26 | 6.11 | |
| | 5a | 20.32 | 6.38] | |
| | 8a | 15.44 | 8.25 | |
| | 10a | 0.00 | 0.00 | |
| | 9a | 6.53 | 5.61 | |
| | 7a | 20.23 | 2.55 | |
| | 11a | 11.42 | 6.05 | |
| | 4c | 19.24 | 3.10 | |
| | 8b | 13.55 | 8.43 | |
| | 8c | 26.32 | 7.10 | |
| | 7b | 15.40 | 4.35 | |
| | 6a | 0.00 | 0.00 | |
| | 13a | 0.00 | 0.00 | |
| | 18a | 15.24 | 10.91 | |
| | Ciprofloxacin | 38.16 | 9.46 | |
| *E. coli* | 3a | 0.00 | 0.00 | 0.0001 |
| | 3b | 0.00 | 0.00 | |
| | 3c | 0.60 | 2.32 | |
| | 3d | 0.00 | 0.00 | |
| | 4a | 0.00 | 0.00 | |
| | 5a | 0.00 | 0.00 | |
| | 8a | 0.00 | 0.00 | |
| | 10a | 0.00 | 0.00 | |
| | 9a | 0.00 | 0.00 | |
| | 7a | 0.00 | 0.00 | |
| | 11a | 0.00 | 0.00 | |
| | 4c | 4.25 | 5.49 | |
| | 8b | 0.00 | 0.00 | |
| | 8c | 6.02 | 5.27 | |
| | 7b | 0.00 | 0.00 | |
| | 6a | 0.00 | 0.00 | |
| | 13a | 0.00 | 0.00 | |
| | 18a | 0.00 | 0.00 | |
| | Ciprofloxacin | 45.02 | 11.25 | |

* $p < 0.05$ (significant), $p < 0.01$ (highly significant), $p < 0.001$ (very highly significant), NS: Non significant $p > 0.05$

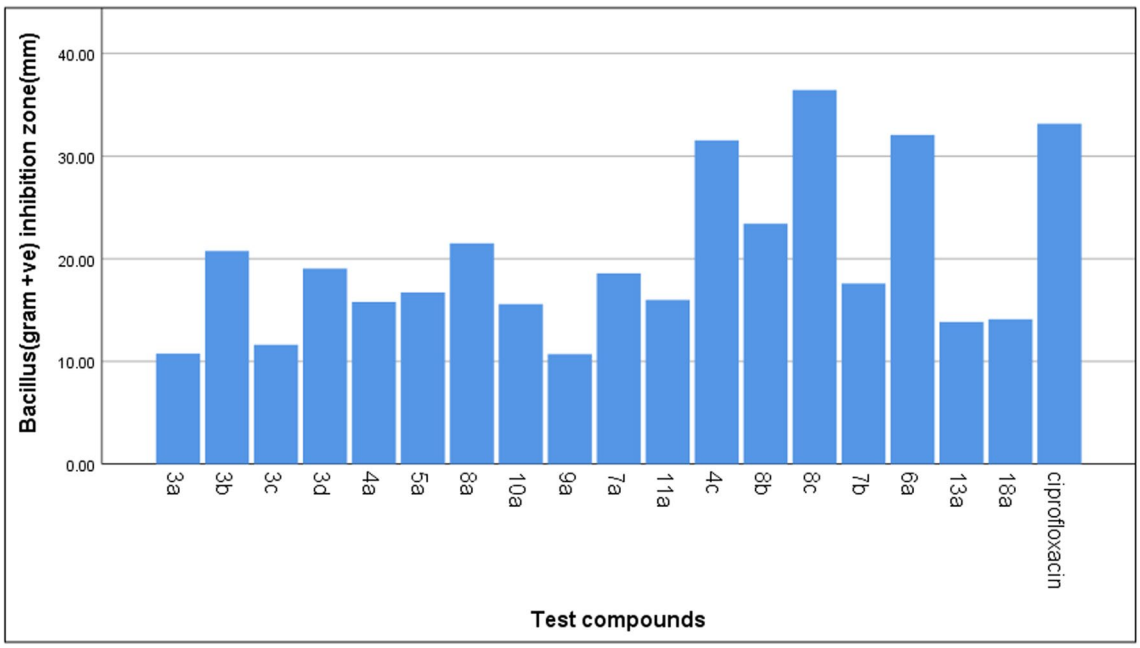



**Fig. 3** Shows a comparison between test compounds and ciprofloxacin with the size of the inhibition zone of *Bacillus* (gram +ve) strain of bacteria

of compounds (Table 1). From Table 1, we have found a statistically significant result. It is observed that the in-vitro antibacterial activity of compounds **19** (Ciprofloxacin), **8c, 6a**, and **4c** significantly different from all other compounds. But Ciprofloxacin is used as standard. It is evident from the ANOVA that the compounds (**8c, 6a**, and **4c**), exhibited significantly high antibacterial activity compare to the all other synthesized tested compounds and also with standard. As shown in Table 1, compound **8c** exhibit significantly high antibacterial activity against *S. aureus* (33.26 ± 4.73) and significantly excellent antibacterial effect against *Bacillus* strain (36.44 ± 4.05) (Fig. 3). Moreover, compound **6a, 4c** had exhibit significantly high antibacterial effect against *Bacillus* strain as mean = 32.66, 31.54 respectively.

#### *Anti-inflammatory activity of the tested compounds*

Proteins eliminate their tertiary and secondary structures when exposed to an external stressor or substance, such as a powerful base or acid, a highly concentrated inorganic salt, an organic solvent, or heating. This process is referred to as denaturation. The expected process of denaturation is a modification in electrostatic, hydrogen, hydrophobic, and disulphide coupling. There is a dose-dependent capacity of certain anti-inflammatory medications to avoid denaturation of proteins brought about by heating [42]. In this study all compounds were shown to have strong anti-inflammatory action by employing a protein denaturation inhibition technique at concentration of 50, 100, 150, 200 and 250 µg/ml in a concentration-dependent manner (Table 2). In comparison to other compounds, the compounds **3a**, **4c** and **8c** showed the highest levels of inhibition at concentrations of 250 µg/ml with percentage inhibition 83.24%, 86.44% and 85.14%, respectively. At the same concentrations, compounds **3b** and **8b** exhibited significant anti-inflammatory activity with percentage inhibition 81.99% and 80.99%. These substances could therefore be a viable substitute for agents that have anti-inflammatory properties. Hence, it could be a valuable medicinal ingredient for the treatment of bacterial infections and inflammation.

#### *Statistical results of anti-inflammatory activity*

All synthesized compounds were screened for in-vitro anti-inflammatory activity by inhibition of protein denaturation method using diclofenac as a standard drug. From Table 2, we have found a statistically significant result in all concentration (50, 100, 150, 250 µg /ml) in comparison to different test compounds. It is evident from the ANOVA that the compounds **3a**, **4c, 8c, 3b**

and **8b**, exhibited significantly high anti-inflammatory compare to the all-other synthesized tested compounds. Compound **8c** showed significant effect mean 65.23 compering with 70.85 for Diclofenac sodium.at concentrations of 50 µg/ml as shown in Fig. 4.

## Conclusion

Synthesis, characterization, and investigation of some 1,3,4-thiadiazole derivatives which prepared from thioxoacetamide derivatives were studied, their reactions with some alkyl halides to make alkylation reaction and with some aldehydes to form novel 5-(substituent)-*N*-phenyl-1,3,4-thiadiazole-2-carboxamide derivatives were investigated. Finally, we studied the possibility of 1,3,4-dihydrothiadiazole derivatives as antimicrobial potential on some multidrug-resistant pathogenic bacteria. Gram-positive and Gram-negative bacteria are both targets of antimicrobial action using the agar well diffusion method then screening data is subjected to statistical analysis using one way ANOVA technique. The compounds exhibited antibacterial efficacy against all tested bacterial strains except *Escherichia coli*. Also, the result revealed that all compounds possessed potent significant anti-inflammatory activity. In deep study, compounds **4c** and **8c** possess significant antimicrobial and anti-inflammatory activity as compared to ciprofloxacin and diclofenac sodium. Additionally, a study employing molecular docking against DHPS from *S. aureus* (PDB ID: 6CLV) found that it is a great option for antibiotics since it is used by nearly all bacterial strains to synthesize nucleic acids. The molecular docking study exhibited positive interaction with the target protein and a high docking score especially for compounds **3a**, **4c**, **8d** and **18a**. According to the study's findings, the substances in question have strong antibacterial and anti-inflammatory properties. The overall results of this study can be considered as very promising in the perspective of new antimicrobial drugs, especially when the medical importance of tested microorganisms is considered. However, pharmacological and toxicological studies, will be necessary to confirm this hypothesis.

## Methods/Experimental

### Chemistry

Thin layer chromatography (TLC) was employed to track all reactions utilizing percolated dishes of silica gel G/

**Table 2** Anti-denaturation activity of the tested compounds and positive control

| Concentrations µg/ml | Test compound | Mean of inhibition % | Std. deviation | *p*-value* |
|---|---|---|---|---|
| 50 | 3a | 63.19 | 0.00 | 0.0001 |
| | 3b | 59.25 | 0.00 | |
| | 3c | 58.44 | 0.00 | |
| | 3d | 58.63 | 0.00 | |
| | 4a | 30.60 | 0.00 | |
| | 5a | 2.76 | 0.00 | |
| | 8a | 1.39 | 0.00 | |
| | 10a | 2.12 | 0.00 | |
| | 9a | 4.76 | 0.00 | |
| | 7a | 35.22 | 0.00 | |
| | 11a | 34.72 | 0.00 | |
| | 4c | 64.13 | 0.00 | |
| | 8b | 59.75 | 0.00 | |
| | 8c | 65.23 | 0.00 | |
| | 7b | 32.72 | 0.00 | |
| | 6a | 30.60 | 0.0 | |
| | 13a | 35.22 | 0.0 | |
| | 18a | 6.76 | 0.0 | |
| | Diclofenac sodium | 70.85 | 0.0 | |
| 100 | 3a | 73.46 | 0.0 | 0.0001 |
| | 3b | 68.42 | 0.0 | |
| | 3c | 67.33 | 0.0 | |
| | 3d | 68.45 | 0.0 | |
| | 4a | 35.15 | 0.0 | |
| | 5a | 9.32 | 0.0 | |
| | 8a | 6.37 | 0.0 | |
| | 10a | 8.54 | 0.0 | |
| | 9a | 9.78 | 0.0 | |
| | 7a | 40.78 | 0.0 | |
| | 11a | 41.15 | 0.0 | |
| | 4c | 74.40 | 0.0 | |
| | 8b | 68.84 | 0.0 | |
| | 8c | 74.46 | 0.0 | |
| | 7b | 40.66 | 0.0 | |
| | 6a | 39.15 | 0.0 | |
| | 13a | 40.78 | 0.0 | |
| | 18a | 10.72 | 0.0 | |
| | Diclofenac sodium | 79.55 | 0.0 | |

**Table 2** (continued)

| Concentrations µg/ml | Test compound | Mean of inhibition % | Std. deviation | *p*-value* |
|---|---|---|---|---|
| 150 | 3a | 78.44 | 0.0 | 0.0001 |
| | 3b | 75.14 | 0.0 | |
| | 3c | 72.50 | 0.0 | |
| | 3d | 73.80 | 0.0 | |
| | 4a | 41.31 | 0.0 | |
| | 5a | 16.87 | 0.0 | |
| | 8a | 11.42 | 0.0 | |
| | 10a | 14.37 | 0.0 | |
| | 9a | 18.47 | 0.0 | |
| | 7a | 44.32 | 0.0 | |
| | 11a | 46.31 | 0.0 | |
| | 4c | 77.64 | 0.0 | |
| | 8b | 74.14 | 0.0 | |
| | 8c | 78.64 | 0.0 | |
| | 7b | 45.52 | 0.0 | |
| | 6a | 43.31 | 0.0 | |
| | 13a | 48.30 | 0.0 | |
| | 18a | 17.47 | 0.0 | |
| | Diclofenac sodium | 82.64 | 0.0 | |
| 200 | 3a | 81.72 | | 0.0001 |
| | 3b | 78.00 | 0.0 | |
| | 3c | 74.66 | 0.0 | |
| | 3d | 74.43 | 0.0 | |
| | 4a | 46.65 | 0.0 | |
| | 5a | 22.93 | 0.0 | |
| | 8a | 16.62 | 0.0 | |
| | 10a | 20.93 | 0.0 | |
| | 9a | 25.33 | 0.0 | |
| | 7a | 58.00 | 0.0 | |
| | 11a | 52.65 | 0.0 | |
| | 4c | 82.02 | 0.0 | |
| | 8b | 78.56 | 0.0 | |
| | 8c | 82.72 | 0.0 | |
| | 7b | 52.43 | 0.0 | |
| | 6a | 49.65 | 0.0 | |
| | 13a | 62.21 | 0.0 | |
| | 18a | 25.63 | 0.0 | |
| | Diclofenac sodium | 85.46 | 0.0 | |
| 250 | 3a | 83.24 | 0.0 | 0.0001 |
| | 3b | 81.99 | 0.0 | |
| | 3c | 79.65 | 0.0 | |
| | 3d | 78.00 | 0.0 | |
| | 4a | 51.43 | 0.0 | |
| | 5a | 29.12 | 0.0 | |
| | 8a | 21.86 | 0.0 | |
| | 10a | 23.12 | 0.0 | |
| | 9a | 29.76 | 0.0 | |
| | 7a | 64.62 | 0.0 | |
| | 11a | 60.43 | 0.0 | |
| | 4c | 85.14 | 0.0 | |
| | 8b | 80.99 | 0.0 | |
| | 8c | 86.44 | 0.0 | |
| | 7b | 60.77 | 0.0 | |
| | 6a | 55.43 | 0.0 | |
| | 13a | 70.62 | 0.0 | |
| | 18a | 33.76 | 0.0 | |
| | Diclofenac sodium | 97.85 | 0.0 | |

* $p < 0.05$ (significant), $p < 0.01$ (highly significant), $p < 0.001$ (very highly significant), NS: Non significant $p > 0.05$

UV-254 with a 0.25 mm thickness (Merck 60F254) and UV light (254 nm/365 nm) enable visualization. The uncorrected Kofeler melting point instrument was used to record all melting points. On an FT-IR spectrophotometer, KBr pellets were used to analyses IR spectra. At Sohag University, spectral characterizations of the compounds, Bruker Avance III at 400 MHz and 100 MHz for $^{1}H$ and $^{13}CNMR$ (DMSO-$d_6$, δ ppm), respectively were used. Tetramethylsilane (TMS) was selected as the standard for internal measurement and its chemical shifts (δ) were expressed in parts per million (ppm). TMS (=0 ppm) or DMSO (=39.51 ppm) were employed as internal standards for $^{13}C$ NMR. A Perkin-Elmer CHN analyzer model provided elemental analyses as shown in supplementary file.

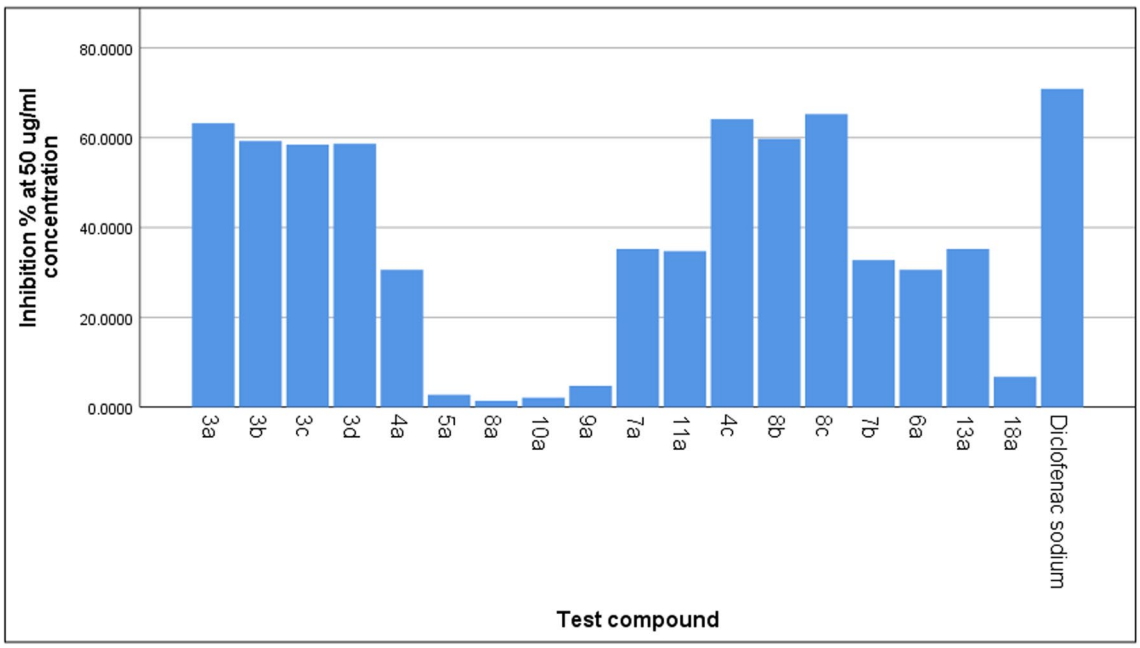



**Fig. 4** Shows a comparison between test compounds with 50 µg/ml concentration with % of inhibition

***General synthesis of N-phenyl-5-thioxo-4,5-dihydro-1,3,4-thiadiazole-2-carboxamide derivatives (3a–d)***

2-Hydrazino-*N*-Phenyl-2-thioxoacetamide **(1a–d)** (0.01 mmol), potassium hydroxide (0.03 mmol), carbon disulfide (0.03 mmol) was stirring in ethanol at room temperature for 6 h., then was poured in 20 ml distilled water. Concentrated hydrochloric acid was added until pH 2–3, precipitated formed crystallized with ethanol, see Figure (S1–S10).

*N*-phenyl-5-thioxo-4,5-dihydro-1,3,4-thiadiazole-2-carboxamide (**3a**): White crystals, yield 93%, mp. 180–182 °C; FT-IR (ATR) $\delta_{max}$: 3345, 3191 (2NH str.), 3104 ($CH_{arom}$ str.), 1678 (C=O str.), 1659 (C=N str.), 1236 (C=S str.); $^{1}$H NMR: $\delta$ 15.06 (s, H, $NH_{thiadiazole}$, exchangeable by $D_2O$), 10.79 (s, H, NH, exchangeable by $D_2O$), 7.77–7.14 (m, 5H, ArH) ppm; $^{13}$C NMR: $\delta$ 190.78 (C=S), 157.25 (C=O), 155.34, 137.91 (2C, Thiadiazole), 129.20, 125.23, 121.34 ppm (C of Arom.). Anal. Calcd. for $C_9H_7N_3OS_2$ (237.30): C, 45.55; H, 2.91; N, 17.71; S, 27.02% Found: C, 45.65; H, 2.81; N, 17.61; S, 27.12%.

5-Thioxo-*N*-(o-tolyl)-4,5-dihydro-1,3,4-thiadiazole-2-carboxamide (**3b**): Orange crystals, yield 91%, mp. 177–179 °C; FT-IR (ATR) $\delta_{max}$: 3342, 3311 (2NH str.), 3057 ($CH_{arom}$ str.), 1681 (C=O str.), 1648 (C=N str.), 1222 (C=S str.); $^{1}$H NMR: $\delta$ 15.06 (s, H, $NH_{thiadiazole}$), 10.79 (s, H, NH), 7.39–7.19 (m, 4H, ArH), 2.24 ppm (s, 3H, $CH_3$); $^{13}$C NMR: $\delta$ 190.55 (C=S), 157.25 (C=O), 155.15, 137.91 (2C, Thiadiazole), 134.62, 132.10, 129.20, 125.23, 121.34 (Arom.), 18.54 ppm ($CH_3$). Anal. Calcd. for $C_{10}H_9N_3OS_2$ (251.33): C, 47.79; H, 3.61; N, 16.72; S, 25.52% Found: C, 47.35; H, 3.95; N, 16.52; S, 25.31%.

*N*-(4-methoxyphenyl)-5-thioxo-4,5-dihydro-1,3,4-thiadiazole-2-carboxamide (**3c**): Orange crystals, yield 88%, mp. 193–195 °C; FT-IR (ATR) $\delta_{max}$: 3348, 3316 (2NH str.), 3132 ($CH_{arom}$ str.), 1675 (C=O str.), 1659 (C=N str.), 1236 (C=S str.); $^{1}$H NMR: $\delta$ 15.06 (s, H, $NH_{thiadiazole}$), 10.79 (s, H, NH), 7.77–7.35 (dd, 4H, ArH, $J$ = 8.08Hz), 4.18 (s, 3H, $OCH_3$) ppm; $^{13}$C NMR: $\delta$ 201.67 (C=S), 170.85 (C=O), 165.93 (C, Thiadiazole) 137.94, 129.25, 125.29, 121.31 (Arom.), 54.21 ($OCH_3$) ppm.Anal. Calcd. for $C_{10}H_9N_3O_2S_2$ (267.33): C, 44.93; H, 3.39; N, 15.72; S, 23.99% Found: C, 44.57; H, 3.75; N, 16.30; S, 23.58%.

*N*-(4-nitrophenyl)-5-thioxo-4,5-dihydro-1,3,4-thiadiazole-2-carboxamide (**3d**): Orange crystals, yield 70%, mp. 201–203 °C; FT-IR (ATR) $\delta_{max}$: 3351, 3327 (2NH str.), 3136 ($CH_{arom}$ str.), 1678 (C=O str.), 1666 (C=N str.), 1350, 1555 ($NO_2$ str.), 1225 (C=S str.); $^{1}$H NMR: $\delta$ 15.01 (s, H, $NH_{thiadiazole}$), 10.79 (s, H, NH), 7.77–7.35 ppm (dd, 4H, ArH, $J$ = 8.08Hz) ppm; $^{13}$C NM: $\delta$ 190.78 (C=S), 157.25 (C=O), 155.15 (C, Thiadiazole) 144.07, 129.25, 129.20, 125.23, 121.34 ppm (Arom.) Anal. Calcd. for $C_9H_6N_4O_3S_2$ (282.30): C, 38.29; H, 2.14; N, 19.85; S, 22.72% Found: C, 38.17; H, 2.24; N, 19.15; S, 22.52%.

### *General synthesis of 5-(S-alkyl)-1.3.4-thiadiazole-2-carboxamide derivatives*

A mixture of *N*-phenyl-5-thioxo-4,5-dihydro-1,3,4-thiadiazole-2-carboxamide derivatives **(2a–d)** (0.01 mmol), potassium hydroxide (0.03 mmol) and alkyl halide (0.015 mmol) were added and stirred in ethanol for 2 h. The formed precipitate was collected and crystallized from ethanol.

5-(Methylthio)-*N*-phenyl-1,3,4-thiadiazole-2-carboxamide (**4a**): White crystals, yield 97%, mp. 160–162 °C; FT-IR (ATR) $\delta_{max}$: 3381 (NH str.), 3156 ($CH_{arom}$ str.), 2923 ($CH_{3\ alip}$ str.), 1673 (C=O str.), 1659 (C=N str.); $^1$H NMR: $\delta$ 11.03 (s, H, NH), 7.85–7.14 (m, 5H, ArH), 2.83 ppm (s, 3H, $SCH_3$); $^{13}$C NMR: $\delta$ 173.51 (C=O), 165.29, 156.32 (2C, Thiadiazole), 138.12, 129.18, 125.11, 121.27 (Arom.), 17.37 ppm ($SCH_3$). Anal. Calcd. for $C_{10}H_9N_3OS_2$ (251.33): C, 47.79; H, 3.61; N, 16.72; S, 25.52% Found: C, 47.99; H, 3.41; N, 16.42; S, 25.80%.

5-(Methylthio)-*N*-(o-tolyl)-1,3,4-thiadiazole-2-carboxamide (**4b**): White crystals, yield 87%, mp. 155–157 °C; FT-IR (ATR) $\delta_{max}$: 3383 (NH str.), 3107 ($CH_{arom}$ str.), 2988, 2963 ($CH_3$ str.), 1679 (C=O str.), 1609 (C=N str.); $^1$H NMR: $\delta$ 10.60 (s, H, NH), 7.39–7.19 (m, 4H, ArH), 2.30 (s, 3H, $CH_3$ Arom), 2.24 (s, 3H, $SCH_3$) ppm; $^{13}$C NMR: $\delta$ 170.71 (C=O), 165.69, 156.44 (2C, Thiadiazole), 135.25, 134.08, 130.93, 127.11, 126.64, 125.23 (Arom.), 28.93 ($SCH_3$), 25.28**)** ppm ($CH_3$ Arom.). Anal. Calcd. for $C_{11}H_{11}N_3OS_2$ (265.35): C, 49.79; H, 4.18; N, 15.84; S, 24.17% Found: C, 49.63; H, 4.22; N, 15.76; S, 24.16%.

*N*-(4-methoxyphenyl)-5-(methylthio)-1,3,4-thiadiazole-2-carboxamide (**4c**): Orange crystals, yield 66%, mp. 198–200 °C; FT-IR (ATR) $\delta_{max}$: 3379 (NH str.), 3251 ($CH_{arom}$ str.), 2996, 2985 ($CH_{3alip}$ str.), 1675 (C=O str.), 1605 (C=N str.); $^1$H NMR: $\delta$ 11.07 (s, H, NH), 7.83–7.38 (dd, 4H, ArH, *J*=8.08Hz), 4.04 (s, 3H, $OCH_3$), 2.31 ppm (s, 3H, $SCH_3$); $^{13}$C NMR: $\delta$ 170.85, 165.93, 155.15, 137.94, 129.25, 125.29, 121.31 Arom, 54.21 **(**$OCH_3$**)**, 24.64 ppm **(**$SCH_3$**)**. Anal. Calcd. for $C_{11}H_{11}N_3O_2S_2$ (281.35): C, 46.96; H, 3.94; N, 14.93; S, 22.79% Found: C, 46.77; H, 3.89; N, 15.03; S, 22.64%**.**

5-(Ethylthio)-*N*-phenyl-1,3,4-thiadiazole-2-carboxamide (**5a**): White crystals, yield 73%, mp. 157–159 °C; FT-IR (ATR) $\delta_{max}$: 3336.85 (NH str.), 3061 ($CH_{arom}$ str.), 2979–2870 ($CH_2CH_{3alip}$ str.), 1670 ($C=O_{amidic}$ str.),1599 (C=N str.); $^1$H NMR: $\delta$ 11.04 (s, H, NH), 7.84–7.15 (m, 5H, ArH), 3.41, 3.40, 3.38, 3.36 (q, 2H, $SCH_2$, *J*=6.6 Hz), 1.45, 1.43, 1.41 ppm (t, 3H, $CH_3$, *J*=6.6 Hz); $^{13}$C NM: $\delta$ 171.47(C=O), 165.37, 156.31 (2C, Thiadiazole), 138.06, 131.87, 125.18, 121.28 (Arom.), 29.18 ($CH_2$), 14.64 ppm ($CH_3$). Dept -135 NMR; 129.35,124.90,121.31 Arom, 29.36, 14.57 ppm. Anal. Calcd. for $C_{11}H_{11}N_3OS_2$ (265.03): C, 49.79; H, 4.18; N, 15.84; S, 24.17% Found: C, 49.39; H, 4.18; N, 15.98; S, 24.03%.

5-((2-Methylbutyl) thio)-*N*-phenyl-1,3,4-thiadiazole-2-carboxamide (**6a**): White crystals, yield 84%, mp. 150–152 °C; FT-IR (ATR) $\delta_{max}$: 3340 ($NH_{amidic}$ str.), 3061 ($CH_{arom}$ str.), 2961–2869 ($CH_{aliphatic}$ str.), 1667 ($C=O_{amidic}$ str.), 1599 (C=N str.); $^1$H NMR: $\delta$ 11.04 (s, H, NH), 7.82–7.10 (m, 5H, ArH), 3.33, 3.31 (d, 2H,$SCH_2$), 1.62, 1.60, 1.59, 1.53, 1.50 (m, 3H, ($\underline{CH}(CH_3)\underline{CH_2}$)), 0.87, 0.86, 0.82 ppm (t, 6H, $\underline{CH_3}$)$CH_2\underline{CH_3}$); $^{13}$C NMR: $\delta$ 171.90 (C=O), 165.44, 156.25 (2C, Thiadiazole), 138.06, 129.15, 125.11, 121.21 (Arom.), 44.50, 37.88, 32.79, 27.40, 22.40 ppm. Anal. Calcd. for $C_{14}H_{17}N_3OS_2$ (307.43): C, 54.69; H, 5.57; N, 13.67; S, 20.86% Found: C, 54.46; H, 5.77; N, 13.58; S, 20.73%.

5-(Benzylthio)-*N*-phenyl-1,3,4-thiadiazole-2-carboxamide (**7a**): White crystals, yield 95%, mp. 237–239 °C; FT-IR (ATR) $\delta_{max}$: 3223 (NH str.), 3056 ($CH_{arom}$ str.), 2958 ($CH_{2alip}$ str.), 1667 ($C=O_{amidic}$ str.), 1625 (C=N str.); $^1$H NMR $\delta$ 11.05 (s, H, NH), 7.83–7.15(m, 10H, ArH), 4.67(s, 2H, $SCH_2$) ppm; $^{13}$C NMR $\delta$ 171.19 (C=O), 165.87,156.26 (2C, Thiadiazole), 138.03, 136.40, 129.66, 129.25, 129.11, 128.32, 125.23, 121.25 (Arom.), 38.109 ppm ($CH_2$). Dept-135 NMR; 129.61, 129.25, 129.11, 128.32, 125.28, 121.31 (Arom.), 38.20 ppm ($CH_2$). Anal. Calcd. for $C_{16}H_{13}N_3OS_2$ (327.42): C, 58.69; H, 4.00; N, 12.83; S, 19.59% Found: C, 58.66; H, 4.28; N, 12.19; S, 19.89%.

5-(Benzylthio)-*N*-(o-tolyl)-1,3,4-thiadiazole-2-carboxamide (**7b**): White crystals, yield 78%, mp. 211–213 °C; FT-IR (ATR) $\delta_{max}$: 3345 (NH str.), 3105 ($CH_{arom}$ str.), 2971 ($CH_3$ str.), 1662 (C=O str.), 1605 (C=N str.); $^1$H NMR: $\delta$ 11.05 (s, H, NH), 7.83–7.15 (m, 9H, ArH), 4.67(s, 2H, $SCH_2$), 1.21 ppm (s, 3H, CH3); $^{13}$C NMR: $\delta$ 171.19 (C=O), 165.87, 156.26 (2C, Thiadiazole), 140.98, 136.40, 133.74, 129.25, 129.11, 128.32, 125.23, 121.25 (Arom.), 38.10 ($CH_2$), 23.09 ppm ($CH_3$). Anal. Calcd. for $C_{17}H_{15}N_3OS_2$ (341.45): C, 59.80; H, 4.43; N, 12.31; S, 18.78% Found: C, 59.87; H, 4.33; N, 12.37; S, 18.54%.

5-((2-Oxopropyl) thio)-*N*-phenyl-1,3,4-thiadiazole-2-carboxamide (**8a**): White crystals, yield 93.5%, mp. 217–219 °C; FT-IR (ATR) $\delta_{max}$: 3332 (NH str.), 3060 ($CH_{arom}$ str.), 2917 ($CH_{3\ alip}$ str.), 1710 (C=O str.) 1661 ($C=O_{amidic}$ str.), 1622 (C=N str.); $^1$H NMR: $\delta$ 11.03 (s, H, NH), 7.83–7.17 (m, 5H, ArH), 4.52 (s, 2H, $SCH_2$), 2.31 ppm (s, 3H, $CH_3$); $^{13}$C NMR: $\delta$ 201.67 (C=O), 170.85($C=O_{amidic}$), 165.93, 1 56.31 (2C, Thiadiazole), 137.94, 129.25, 125.29, 121.06, 44.64 ($CH_2$), 29.18 ppm ($CH_3$), Dept-135; 129.34, 125.19, 121.39 (Arom.), 44.59 ($SCH_2$), 29.02 ppm ($CH_3$). Anal. Calcd. for $C_{12}H_{11}N3O_2S_2$ (293.36): C, 49.13; H, 3.78; N, 14.32; S, 21.86% Found: C, 49.53; H, 3.68; N, 14.39; S, 21.69%.

5-((2-Oxopropyl) thio)-*N*-(o-tolyl)-1,3,4-thiadiazole-2-carboxamide (**8b**): White powder, yield 81.5%, mp. 205–207 °C; FT-IR (ATR) $\delta_{max}$: 3325 (NH str.), 3060 ($CH_{arom}$ str.), 2957($CH_{3alip}$ str.), 1713 (C=O str.), 1667

($C{=}O_{amidic}$ str.), 1606 (C=N str.); $^1$H NMR: $\delta$ 10.61 (s, H, NH), 7.39–7.19 (m, 4H, ArH), 4.52 (s, 2H, $SCH_2$), 2.30 (s, 3H, $COCH_3$), 2.24 (s, 3H, $CH_3$ ArH) ppm; $^{13}$C NMR: $\delta$ 201.57 (C=O), 170.71 ($C{=}O_{amidic}$), 165.69, 156.44, (2C, Thiadiazole), 135.85, 130.93, 127.11, 126.66, 126.64 (Arom.), 44.64 ($SCH_2$), 28.93 ($CO\underline{CH_3}$), 18.10 ppm ($CH_3$ Arom). Anal. Calcd. for $C_{13}H_{13}N_3O_2S_2$ (307.39): C, 50.79; H, 4.26; N, 13.67; S, 20.86% Found: C, 50.33; H, 4.87; N, 13.47; S,20.71%.

*N*-(4-methoxyphenyl)-5-((2-oxopropyl) thio)-1,3,4-thiadiazole-2-carboxamide (**8c**): White crystals, yield 72%, mp. 267–269 °C; FT-IR (ATR) $\delta_{max}$: 3343 (NH), 3067 ($CH_{arom}$), 2949–2854 ($CH_2CH_{3alip}$), 1710 (C=O), 1661 ($C{=}O_{amidic}$), 1622 (C=N str.); $^1$H NMR: $\delta$ 10.07 (s, H, NH), 7.83–7.38 (dd, 4H, ArH, $J$ = 8.08 Hz), 4.52 (s, 2H, $S\underline{CH_2}$), 4.09 (s,3H, $COCH_3$)), 2.31 ppm (s, 3H, $OCH_3$); $^{13}$C NMR:$\delta$ 201.67 (C=O), 170.85 ($C{=}O_{amidic}$), 165.93, 156.31 (2C, Thiadiazole), 137.94, 129.25, 125.29, 121.31 (Arom.), 57.13 **(**$OCH_3$**)**, 44.64 ($SCH_2$), 29.35 ppm ($COCH_3$). Anal. Calcd. for $C_{13}H_{13}N_3O_3S_2$ (323.39): C, 48.28; H, 4.05; N, 12.99; S, 19.83% Found: C, 48.65; H, 4.35; N, 12.78; S, 19.48%.

*N*-(4-nitrophenyl)-5-((2-oxopropyl) thio)-1,3,4-thiadiazole-2-carboxamide (**8d**): White crystals, yield 66%, mp. 280–282 °C; FT-IR (ATR) $\delta_{max}$: 3345 (NH str.), 3191 ($CH_{arom}$ str.), 3104–2921 ($CH_2$, $CH_{3alip}$ str.), 1762 (C=O str.), 1678 ($C{=}O_{amidic}$ str.), 1659 (C=N str.), 1536,1341 ($NO_2$ str.); $^1$H NMR: $\delta$ 11.15 (s, H, NH), 7.89–7.44 (dd, 4H, ArH, $J$ = 8.08 Hz), 4.67 (s, 2H, $SCH_2$), 2.15 ppm (s, 3H, $CH_3$); $^{13}$C NMR:$\delta$ 202.02 (C=O), 171.89 ($C{=}O_{amidic}$), 166.54, 157.13 (2C, Thiadiazole), 138.11, 129.66, 125.51, 121.82 (Arom.), 44.69 ($SCH_2$), 29.67 ppm ($COCH_3$). Anal. Calcd. for $C_{12}H_{10}N_4O_4S_2$ (338.36): C, 42.60; H, 2.98; N, 16.56; S, 18.95% Found: C, 42.63; H, 2.95; N, 16.59; S, 18.94%.

Ethyl 2-((5-(phenylcarbamoyl)-1,3,4-thiadiazol-2-yl) thio) acetate (**9a**): White crystals, yield 64%, mp. 133–13; FT-IR (ATR) $\delta$ $_{max}$: 3537 ($NH_{amidic}$ str.), 3142 ($CH_{arom}$ str.), 2983–2905 ($CH_2$, $CH_{3alip}$ str.), 1737.94 (C=O str.), 1664.28 ($C{=}O_{amidic}$ str.), 1605 (C=N str.); $^1$H NMR: $\delta$ 11.05 (s, H, NH), 7.83–7.15 (m, 5H, ArH), 4.35 (s, 2H, $SCH_2$), 4.20, 4.18, 4.16, 4.14 (q, 2H, $CH_2$, $J$ = 7.08 Hz), 1.23, 1.22, 1.20 ppm (s, 3H, $CH_3$, $J$ = 7.08 Hz); $^{13}$C NMR: $\delta$ 170.42 (C=O), 168.08, ($C{=}O_{amidic}$), 166.38, 156.55 (2C, Thiadiazole), 137.87, 128.85, 125.61, 121.2 (Arom.), 3,62.19, 36.23, 14.34 ppm ($CH_3$). Dept-135; 129.31, 125.21, 121.40 (Arom.), 62.12, ($SCH_2$), 35.67 ($COO\underline{CH_2}$), 14.56 ppm ($CH_3$). Anal. Calcd. for $C_{13}H_{13}N_3O_3S_2$ (323.39): C, 48.28; H, 4.05; N, 12.99; S, 19.83% Found: C, 48.50; H, 4.17; N, 12.58; S, 19.48%.

2-((5-(Phenylcarbamoyl)-1,3,4-thiadiazol-2-yl) thio)acetic acid (**10a**): White crystals, yield 90%, mp. 199–201 °C; FT-IR (ATR) $\delta_{max}$: 3322 ($NH_{amidic}$ str.), 3104 (br OH str.), 3061 ($CH_{arom}$ str.), 2979–2926 ($CH_2CH_{3alip}$ str.), 1721 (C = O str.) 1665 ($C{=}O_{amidic}$ str.), 1599 (C=N str.); $^1$H NMR: $\delta$ 15.11 (s, H, COOH), 11.04 (s, H, NH), 7.81 -7.18, (m, 5H, ArH), 4.52 (s, 2H, $SCH_2$) ppm; $^{13}$C NMR: $\delta$ 170.63 (C=O), 169.39 ($C{=}O_{amidic}$), 165.95, 156.33 (2C, Thiadiazole), 137.86, 129.28, 125.36, 121.35 (Arom.), 36.36 ppm ($CH_2$). Dept-135; 129.32, 125.50, 121.40 (CH Arom), 36.27 ppm ($CH_2$). Anal. Calcd. for $C_{11}H_9N_3O_3S_2$ (295.34): C, 44.73; H, 3.07; N, 14.23; S, 21.71% Found: C, 44.33; H, 3.37; N, 14.43; S, 21.53%.

O-ethyl *S*-(5-(phenylcarbamoyl)-1,3,4-thiadiazol-2-yl) carbonothioate (**11a**): White powder, yield 53%, mp. 166–168 °C; FT-IR (ATR) $\delta_{max}$: 3349 ($NH_{amidic}$ str.), 3055 ($CH_{arom}$ str.), 2984–2870 ($CH_2CH_{3alip}$ str.), 1729.32 (C=O str.) 1682 ($C{=}O_{amidic}$ str.), 1641 (C=N str.); $^1$H NMR: $\delta$ 11.23 (s, H, NH), 7.82 -7.15 (m, 5H, ArH), 4.76–4.67 (q, 2H, $CH_2$, $J$ = 10.88 Hz), 1.05–1.00 ppm (t, 3H, $CH_3$, $J$ = 10.88 Hz); $^{13}$C NMR: $\delta$ 168.75 (C=O), 164.29 (C=O), 158.51, 155.86 (2C,Thiadiazole), 137.87, 129.20, 125.20, 121.35 (Arom.), 56.53 ($CH_2$), 18.86 ppm ($CH_3$). Anal. Calcd. for $C_{12}H_{11}N_3O_3S_2$ (309.36): C, 46.59; H, 3.58; N, 13.58; S, 20.73% Found: C, 46.44; H, 3.81; N, 13.78; S, 20.55%.

### *General synthesis of 5-(substituent)-N-phenyl-1,3,4-thiadiazole-2-carboxamide derivatives 12a–18a*

A mixture of 2-hydrazinyl-*N*-phenyl-2-thioxoacetamide **(1a)** (1.0 mmol) and an aldehyde namely; cinnamaldehyde, *p-N, N* dimethylaminobenzaldehyde, 3,4,5-trimethoxy-benzaldehyde, 1-naphthaldehyde, pipreonal, *p* methylbenzaldehyde and glyoxal (1.0 mmol) was refluxed for 3 h. in acetic acid. The solid product was filtrated and crystallized from ethanol, see Figure (S55–S71).

*N*-phenyl-5-styryl-1,3,4-thiadiazole-2-carboxamide (**12a**): Yellow crystals, yield 71%, mp. 226–228 °C FT-IR (ATR) $\delta_{max}$: 3328 (NH str.), 3108 ($CH_{arom}$ str.), 3057–2923 ($CH{=}CH_{alip}$ str.), 1685 (C=O str.), 1599 (C=N str.); $^1$H NMR: $\delta$ 10.70 (s, H, NH, exchangeable by $D_2O$), 7.85–7.08 (m, 10H, ArH), 6.68 (s, 2H, CH=CH) ppm; $^{13}$C NMR: $\delta$ 167.81 (C=O), 163.20, 158.13 (2C, Thiadiazole), 144.36, 141.98, 139.27, 139.04, 138.66, 133.38, 129.05, 124.05, 120.74, 112.13 (Arom.) ppm. Anal. Calcd. for $C_{17}H_{13}N_3OS$ (307.37): C, 66.43; H, 4.26; N, 13.67; S, 10.43% Found: C, 66.38; H, 4.31; N, 13.61; S, 10.45%.

5-[4-(Dimethylamino) phenyl]-*N*-phenyl-1,3,4-thiadiazole-2-carboxamide (**13a**): Red crystals, yield 91%, mp. 210–212 °C FT-IR (ATR) $\delta_{max}$: 3327 (NH str.), 3087 ($CH_{arom}$ str.), 2983–2874 ($CH_3$ str.), 1664 (C=O str.), 1625 (C=N str.); $^1$H NMR: $\delta$ 11.31 (s, H, $NH_{amidic}$, exchangeable

**Table 3** Docking data

| | Ligand | Receptor | Interaction | Distance | E (kcal/mol) | S (kcal/mol) |
|---|---|---|---|---|---|---|
| 5a | N 7 | MET 37 | H-donor | 3.880 | −1.090 | −5.783 |
| | S 15 | ALA 73 | H-donor | 3.300 | −1.190 | |
| | O 9 | ARG 176 | H-acceptor | 2.430 | −4.100 | |
| | 6-ring | ARG 204 | pi-H | 3.420 | −1.080 | |
| 8a | S 11 | MET 37 | H-donor | 3.730 | −1.130 | −5.809 |
| | S 11 | ASP 38 | H-donor | 3.990 | −1.160 | |
| | S 11 | ASP 38 | H-donor | 3.640 | −1.200 | |
| | S 15 | ASP 38 | H-donor | 3.410 | −1.290 | |
| | S 15 | ASP 38 | H-donor | 3.440 | −1.130 | |
| | O 18 | ARG 52 | H-acceptor | 2.750 | −1.340 | |
| 10a | S 11 | THR 214 | H-donor | 2.920 | −1.720 | −5.560 |
| | S 15 | THR 214 | H-donor | 3.230 | −1.530 | |
| | O 19 | ASP 42 | H-donor | 2.500 | −5.910 | |
| | 6-ring | LYS 203 | pi-H | 3.330 | −1.700 | |
| 9a | N 7 | MET 37 | H-donor | 3.210 | −2.220 | −5.616 |
| | S 15 | ARG 202 | H-donor | 3.530 | −1.510 | |
| | O 9 | ARG 176 | H-acceptor | 2.630 | −2.300 | |
| | 5-ring | ARG 204 | pi-H | 3.880 | −1.380 | |
| | 5-ring | ARG 204 | pi-H | 3.030 | −1.160 | |
| 7a | S 11 | MET 37 | H-donor | 2.610 | −1.960 | −6.098 |
| | S 11 | ALA 41 | H-donor | 3.160 | −1.710 | |
| | N 14 | THR 214 | H-acceptor | 2.700 | −2.800 | |
| 11a | S 11 | ASP 38 | H-donor | 3.640 | −1.140 | −6.368 |
| | S 15 | ASP 38 | H-donor | 2.730 | −1.390 | |
| | O 18 | LYS 248 | H-acceptor | 3.040 | −1.700 | |
| 8b | S 11 | ASP 38 | H-donor | 2.710 | −2.200 | −6.431 |
| | S 15 | ASP 38 | H-donor | 3.010 | −1.600 | |
| | O 19 | TYR 212 | H-acceptor | 2.920 | −1.620 | |
| 8d | S 11 | ASP 42 | H-donor | 2.750 | −2.540 | −6.814 |
| | O 21 | TYR 212 | H-acceptor | 2.540 | −1.810 | |
| | O 22 | LYS 248 | H-acceptor | 3.060 | −1.630 | |
| | 5-ring | ASP 213 | pi-H | 3.320 | −1.850 | |
| | 5-ring | THR 214 | pi-H | 3.990 | −1.140 | |
| 7b | S 11 | ASP 42 | H-donor | 2.750 | −1.830 | −6.315 |
| | S 15 | ASP 78 | H-donor | 3.950 | −1.510 | |
| | 6-ring | MET 37 | pi-H | 3.690 | −0.600 | |
| 4b | S 11 | ASP 38 | H-donor | 2.790 | −1.130 | −5.463 |
| | S 15 | ASP 38 | H-donor | 3.080 | −1.400 | |
| | 6-ring | TYR 212 | pi-H | 3.730 | −1.170 | |
| 6a | S 15 | MET 37 | H-donor | 2.320 | −1.140 | −5.817 |
| | O 9 | LYS 248 | H-acceptor | 2.840 | −1.600 | |
| 4a | N 7 | MET 37 | H-donor | 3.120 | −2.800 | −6.498 |
| | N 14 | MET 37 | H-donor | 3.160 | −1.130 | |
| | S 15 | ASP 38 | H-donor | 3.560 | −1.810 | |
| | S 15 | ASP 38 | H-donor | 3.570 | −0.740 | |
| | O 9 | ARG 176 | H-acceptor | 2.710 | −1.820 | |
| 3a | N 7 | MET 37 | H-donor | 3.860 | −1.350 | −6.821 |
| | S 15 | ALA 73 | H-donor | 3.160 | −1.300 | |
| | O 9 | ARG 176 | H-acceptor | 2.560 | −2.600 | |
| 4c | N 7 | THR 214 | H-donor | 2.740 | −1.560 | −6.809 |
| | S 11 | ASP 42 | H-donor | 2.760 | −2.430 | |
| | S 15 | ALA 41 | H-donor | 2.830 | −1.980 | |

**Table 3** (continued)

| | Ligand | Receptor | Interaction | Distance | E (kcal/mol) | S (kcal/mol) |
|---|---|---|---|---|---|---|
| 13a | S 14 | GLU39 | H-donor | 3.22 | −0.80 | −6.670 |
| | O 10 | LYS3 | H-acceptor | 3.12 | −1.40 | |
| | 6-ring | GLU39 | pi-H | 4.18 | −0.60 | |
| 18a | N 9 | ASP38 | H-donor | 2.91 | −1.60 | −7.380 |
| | O 22 | LYS248 | H-acceptor | 2.93 | −7.60 | |
| | 6-ring | THR214 | pi-H | 4.12 | −0.60 | |

by $D_2O$), 8.23–7.09 (m, 9H, ArH), 2.84 (s, 6H, $2CH_3$); $^{13}C$ NMR: $\delta$ 171.33 (C=O), 166.60, 162.53 (2C, Thiadiazole), 156.24, 137.00, 135.08, 133.21, 129.07, 125.90, 125.34, 121.20 (Arom.), 36.42 ppm ($2CH_3$). Anal. Calcd. for $C_{17}H_{16}N_4OS$ (324.40): C, 62.94; H, 4.97; N, 17.27; S, 9.88% Found: C, 62.85; H, 4.98; N, 17.29; S, 9.89%.

*N*-Phenyl-5-(3,4,5-trimethoxyphenyl)-1,3,4-thiadiazole-2-carboxamide (**14a**): White crystals, yield 76%, mp. 199–201 °C FT-IR (ATR) $\delta_{max}$3278 (NH str.), 3146 ($CH_{arom}$ str.), 2998–2874 ($OCH_3$ str.), 1671 (C=O str.), 1625 (C=N str.); $^1H$ NMR: $\delta$ 10.21 (s, H, NH, exchangeable by $D_2O$), 7.99–7.12 (m, 7H, ArH), 3.94 (s, 9H, $3OCH_3$) ppm; $^{13}C$ NMR: $\delta$ 173.54 (C=O), 167.53, 162.81 (2C, Thiadiazole), 151.80, 147.37, 135.70, 130.09, 126.28, 124.31, 122.13, 120.55 (Arom.), 56.56, 48.97 ($3OCH_3$) ppm. Anal. Calcd. for $C1_8H_{17}N_3O_4S$ (371.41): C, 58.21; H, 4.61; N, 11.31; S, 8.63% Found: C, 58.25; H, 4.62; N, 11.28; S, 8.58%.

5-(Naphthalen-1-yl)-*N*-phenyl-1,3,4-thiadiazole-2-carboxamide (**15a**): Pale yellow crystals, yield 67%, mp. 245–247 °C FT-IR (ATR) $\delta_{max}$: 3317 (NH str.), 3037–2985 ($CH_{arom}$ str.), 1673 (C=O str.), 1625 (C=N str.); $^1H$ NMR: $\delta$ 10.52 (s, H, $NH_{amidic}$, exchangeable by $D_2O$), 7.81–7.09 ppm (m, 12H, ArH) ppm; $^{13}C$ NMR: $\delta$ 171.96 (C=O), 164.76, 156.81 (2C, Thiadiazole), 135.95, 135.38, 133.68, 132.99, 129.43, 129.21, 129.25, 128.36, 128.21, 128.05, 121.29, 120.61, 119.76, 117.78 (Arom.) ppm. Anal. Calcd. For $C_{19}H_{13}N_3OS$ (331.39): C, 68.91; H, 3.93; N, 12.64; S, 9.68% Found: C, 68.86; H, 3.95; N, 12.68; S, 9.66%.

5-(Benzo[d][1,3]dioxol-5-yl)-*N*-phenyl-1,3,4-thiadiazole-2-carboxamide (**16a**): White crystals, yield 74%, 225–227 °C FT-IR (ATR) $\delta_{max}$: 3330 (NH str.), 3059 ($CH_{arom}$ str.), 2962 ($CH_{2alip}$ str.), 1690 (C=O str.), 1622 (C=N str.); $^1H$ NMR: $\delta$ 10.20 (s, H, NH, exchangeable by $D_2O$), 7.75–6.93 (m, 8H, ArH), 6.58 (s, 2H, $CH_2$ piprenal) ppm; $^{13}C$ NMR: $\delta$ 171.05 (C=O), 161.88, 158.45 (2C, Thiadiazole), 147.37, 146.15, 141.08, 139.21, 129.71, 127.85, 125.36, 121.57, 133.05, 111.04 (Arom.), 101.04 ($CH_2$ piprenal) ppm. Anal. Calcd. for $C_{16}H_{11}N_3O_3S$ (325.34): C, 59.07; H, 3.41; N, 12.92; S, 9.86% Found: C, 59.20; H, 3.38; N, 12.81; S, 9.84%.

*N*-phenyl-5-(p-tolyl)-1,3,4-thiadiazole-2-carboxamide (**17a**): Pale yellow, yield 77%, mp. 233-235°C FT-IR (ATR) $\delta_{max}$: 3322 (NH str.), 3125 ($CH_{arom}$ str.), 2976 ($CH_{3alip}$ str.), 1677 (C=O str.), 1625 (C=N str.); $^1H$ NMR: $\delta$ 10.20 (s, H, NH, exchangeable by $D_2O$), 7.89–7.13 (m, 9H, ArH), 3.41 (s, 3H, $CH_3$) ppm; $^{13}C$ NMR: $\delta$ 172.26 (C=O), 166.02, 156.66 (2C, Thiadiazole), 153.95, 141.17, 138.14, 129.24, 125.22, 124.85, 121.40, 105.40 (Arom.), 19.02 ppm ($CH_3$). Anal. Calcd. For $C_{16}H_{13}N_3OS$ (295.36): C, 65.06; H, 4.44; N, 14.23; S, 10.86% Found: C, 64.98; H, 4.46; N, 14.21; S, 10.88%.

*N*, *N*-diphenyl-2,2',3,3'-tetrahydro[2,2'-bi(1,3,4-thiadiazole)]-5,5'-dicarboxamide (18a): Orange crystals, yield 81%, mp. 187–189 °C FT-IR (ATR) $\delta_{max}$3321, 3255 (2NH str.), 3079 ($CH_{arom}$ str.), 2968 ($CH_{thiadiazole}$ str.), 1693 (C=O str.), 1625 (C=N str.); $^1H$ NMR: $\delta$ 10.19 (s, H, $NH_{amidic}$, exchangeable by $D_2O$), 9.04 (s, H, $NH_{thiadiazole}$, exchangeable by $D_2O$), 7.74–7.06 (m, 5H, ArH), 5.53 (s, 2H, $CH_{thiadiazole}$) ppm; $^{13}C$ NMR: $\delta$ 159.13 (C=O), 139.04 (C, Thiadiazole), 138.66, 129.05, 124.29, 120.74 (Arom.), 76.13 ppm ($CH_{thiadiazole}$); Dept-135 NMR; 129.09, 129.25, 124.31, 120.74 (Arom.), 75.98 ppm ($CH_{thiadiazole}$) Anal. Calcd. for $C_{18}H_{16}N_6O_2S_2$ (412.49): C, 52.41; H, 3.91; N, 20.37; S, 15.55% Found: C, 52.46; H, 3.90; N, 20.39; S, 15.52%.

## Biological evaluation

### *Antimicrobial screening*

According to the antibacterial activity of several compounds was screened using the agar well diffusion method [43]. Ciprofloxacin was utilized to compare the results as a positive control. Dimethylsulfoxide (DMSO) solution (10% v/v) was used as a negative control.

### *In-vitro anti-inflammatory activity (protein denaturation) of the tested compounds*

For the test compounds and the reference medication, diclofenac sodium, 0.05 mL of various concentrations (50, 100, 150, 200, and 250 µg/ml) were used, respectively. Then all tubes were combined with 0.45 ml (0.5% w/v) of BSA. The samples were heated for 3 min to maintain a temperature of 57 °C after being incubated at 37 °C for

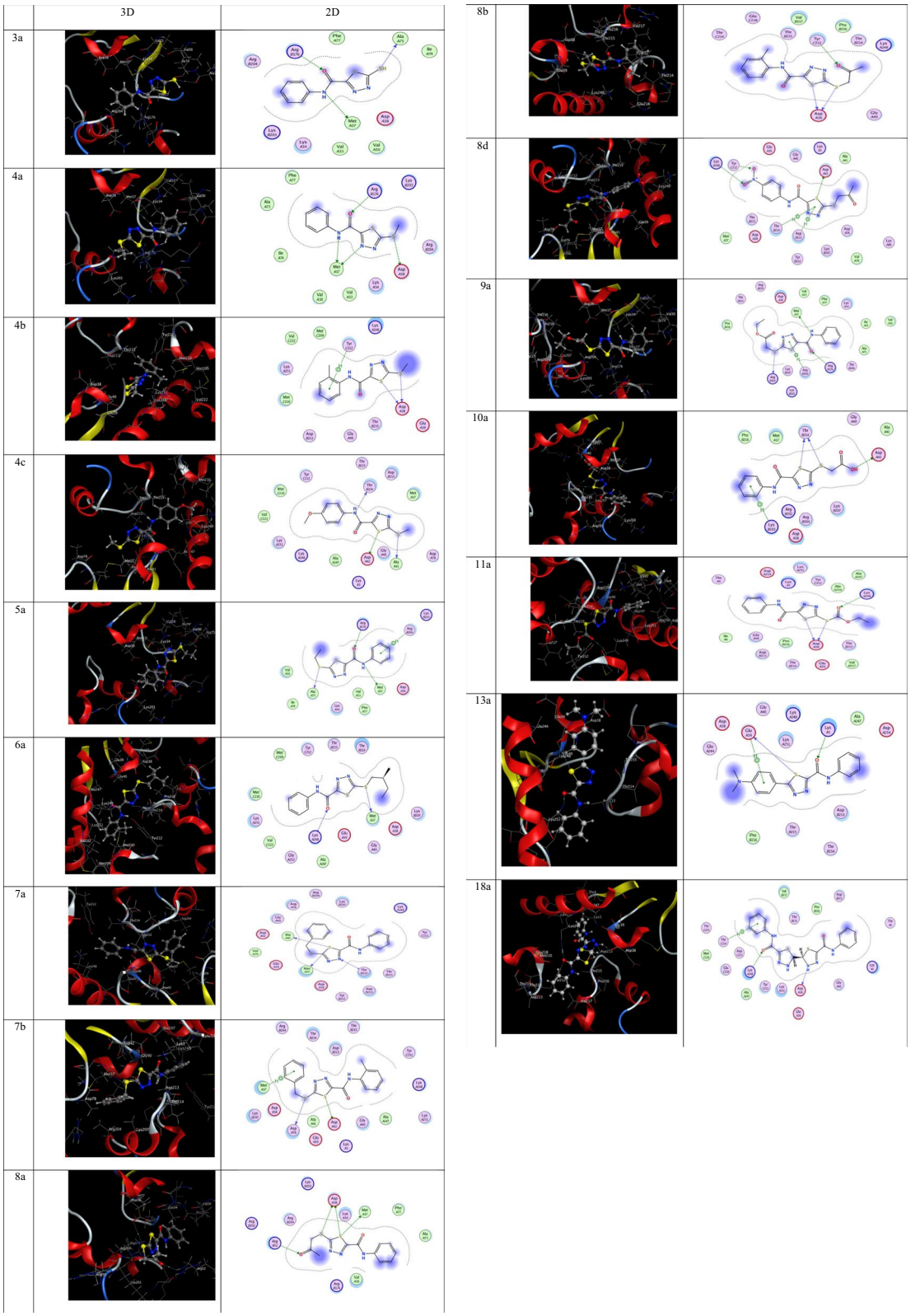



**Fig. 5** 2D and 3D views of the docked compounds with DHPS

20 min. Add 2.5 ml of phosphate buffer to the aforementioned solutions after cooling. At 660 nm, a UV–Visible spectrophotometer was used to detect the absorbance. Protein denaturation at 100% is represented by the control. A positive control drug called diclofenac sodium was used to compare the outcomes [44]. Calculations can be made to determine the degree of protein denaturation inhibition.

$$\% \text{ inhibition of denaturation} = 100 \times (1 - A2/A1)$$

A2 = Absorbance of the test sample, A1 = Absorbance of control.

### Statistical analysis

Analysis was performed using Statistical Program for Social Science (SPSS) version 26 (Armonk, NY: IBM Crop). The gathering of data was recorded and evaluated on an IBM-compatible computer. One-way ANOVA was used to determine if there was any statistically significant difference. P value $\leq 0.05$ was considered significant.

## Molecular docking

To predict the binding style and interactions of the aforementioned drugs with dihydropteroate synthase, molecular docking experiments were carried out to better understand their efficacy (DHPS). This last one is a crucial enzyme in the prokaryotic biosynthesis of folic acid and a crucial cofactor in the pathways that almost all bacterial strains use to synthesize nucleic acids, making it a prime candidate for antibiotics [45, 46]. Thus, crystal structure of DHPS in complex with pterin-sulfonamide conjugates [47]. PDB ID 6CLV, from *S. aureus* organism, was employed as a binding site in molecular docking simulations and downloaded from the RCSB protein data library. The docking results showed strong interactions with high docking scores (S) (more negative) of studied compounds to DHPS from *S. aureus*. The negative values of the calculated docking scores (S) for studied compounds, Table 3, demonstrates that the binding is spontaneous, and the chemicals are suitable for use as drugs [48, 49].

The subject compounds had a strong docking score from −6.821 **(3a)**, −6.814 **(8d),** −6.809 **(4c),** and −6.498 **(4a),** to −5.560 **(10a),** and −5.463 **(4b)** Kcal/mol toward the DHPS from *S. aureus* as can be shown from (Table 3). Due to their high docking score, **3a, 8d** and **4c** seem to be the most active. Compound **3a** revealed three hydrogen bonds interactions between N 7 with MET 37, S 15 with ALA 73, and O 9 with ARG 176; and **8d** revealed three hydrogen bonds interactions between S 11 with ASP 42, O 21 with TYR 212, and O 22 with LYS 248; furthermore, **4c** revealed three hydrogen bonds interactions between N 7 with THR 214, S 11 with ASP 42, S 15 with ALA 41. The docking results showed good interactions of the investigated **13a**, and **18a** compounds to DHPS from *S. aureus* (PDB ID: 6CLV). The subject **13a**, and **18a** compounds had good docking scores as −6.670 kcal/mol, and −7.380 kcal/mol, respectively. The **13a** revealed one H-donor, one H-acceptor, and one pi-H interactions between S14-GLU39, O10-LYS3, and 6-ring-GLU39, with distance of 3.22, 3.12, and 4.18 Angstrom, respectively, While, **18a** revealed one H-donor, one H-acceptor, and one pi-H interactions between N9-ASP38, O22-LYS248, and 6-ring-THR214, with distance of 2.91, 2.93, and 4.12 Angstrom, respectively (Fig. 5).

## Supplementary Information

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Supplementary Material 1.

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### Author contributions

A.M.E.: Formal analysis, data collection, funding procurement, first draught writing, writing reviews, and editing. A.A.: Writing the first draught, Writing the review and editing. O.M.E.: Writing (first draught), writing (review and editing), approach, resources, formal analysis, data curation, and writing (original draught). W.M.A. writing an initial draught, reviewing, and editing that draught. A.M.K. Original draughts of writing, reviewing and correcting written work, formal analysis, data collection and resources. The work's published form has been read by all authors and received their approval.

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### Data availability

The authors declare that the data supporting the findings of this study are available within the paper and its Supplementary Information files. Should any raw data files be needed in another format they are available from the corresponding author upon reasonable request.

## Declarations

### Ethics approval and consent to participate

Not applicable.

### Consent for publication

Not applicable.

### Competing interests

The authors declare that they have no competing interests.

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