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Synthesis, in vitro urease inhibitory activity and molecular docking of 3,5-disubstituted thiadiazine-2-thiones

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Abstract

A series of 3,5-disubstituted-tetrahydro-thiadiazine-2-thione (**1**-**16**) have been synthesized, characterized by elemental analysis, infrared (IR), UV-visible, ^{1}H NMR, ^{13}C NMR, and MS spectroscopic techniques, and screened against jack bean urease. Among 16 compounds, compounds (**1**), (**2**), (**3**), (**4**), (**6**), (**7**), and (**9**) demonstrated excellent urease inhibitory activity with IC_{50} values (9.8 ± 0.5, 11.0 ± 0.6, 16.0 ± 1.5, 17.2 ± 0.5, 15.4 ± 0.5, 19.7 ± 0.4, and 15.8 ± 0.2μM), respectively, even better than the standard thiourea (IC_{50} = 21 ± 0.01μM). However, compound (**8**) shows an almost same level of inhibition (IC_{50} = 22.9 ± 0.3μM), as like standard. In this work, we reported for the first time urease inhibitory activity of thiadiazine thiones and its molecular docking studies.

1 | INTRODUCTION

Urease is a nickel metalloenzyme, isolated from the variety of bacteria, fungi, and plant.[1,2] Urease isolated from different sources have similar amino acid sequences and a common active site structure that contains two nickel ions; consequently, different ureases have the same mechanism of enzymatic activity.[3,4] It has been reported that urease has adverse effects on agriculture, human, and stockbreeding.[5,6] Urease is considered as a lethal factor in the infection of the urinary and gastrointestinal tract of human and animals and causes the development of kidney stones, chronic gastritis, duodenal ulcer, and gastric cancer.[7] Consequently, anti-ulcer drugs oriented around urease inhibitor remains the center of interest. Most recently, different types of compounds have been reported as urease inhibitor including quinines,[8] imidazoles,[9] hydroxamic acids,[10] barbiturates,[11] and thiobarbiturates.[12] Hence, in view of the above, there is high need to develop new urease inhibitors.

The thiadiazine thione nucleus and its derivatives have received particular attention due to its diverse range biological activities, most notably antibacterial,[13] antifungal,[14] antitubercular,[15] anthelmintic,[16] antiprotozoal,[17] leishmanicidal,[18] antimycobacterial,[19] and antiviral.[20]

In addition to its renowned biological potential, this versatile heterocyclic nucleus has been reported as a biolabile prodrug,[21] in the drug delivery system (DDS) because of its characteristic features like high liphophilicity and enzymatic hydrolysis. Subsequently, different types of amino acids,[22] peptides,[23] and drug based on primary amine have been reported to successfully attached to the THTT moiety,[24] which further enhance their cellular uptake by improving lipophilicity in the area where various physiological and enzyme activities causes the release of the drug molecule.

In spite of the tremendous progress over the last decades, in the synthesis and evaluation of the pharmaceutical and biological properties of the THTH moiety, there is still no example of the screening of these compounds against urease. Herein, we report for the first time the screening of thiadiazine thione derivatives for their urease inhibitory activities and their molecular docking studies.

2 | RESULTS AND DISCUSSION

2.1 | Chemistry

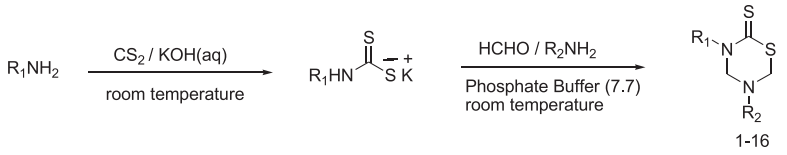
The desired 3,5-disubstituted-tetrahydro-thiadiazine-2-thiones (**1-16**) were synthesized in good to excellent yields (**65**%-**95**%), according to Scheme 1. The preparation of compounds (**1-16**) was achieved from the reaction of the primary amine including aniline, benzylamine, butylamine, *o*-toluidine, and 2,4,6-trimethylaniline with carbon disulfide, which leads to respective potassium salt of dithiocarbamate. Subsequently, its treatments with formaldehyde and different types of amino acid and amine including glycine, alanine, 6-amino-caproic acid, 2-aminopyrimidine, and ethanolamine in the presence of phosphate buffer (pH = 7.7) form respective 3,5-disubstituted thiadiazine-2-thiones (**1-16**).

Structures of all the synthesized compounds were confirmed by spectroscopic techniques and microanalysis. UV-visible analysis of the tetrahydrothiadiazine-2-thiones shows an absorption band at 205 to 290 nm. Infrared (IR) spectroscopy shows carboxylic and alcoholic O–H absorption band in the range of 3300 to 3450 cm^{-1}, while carbonyl (C═O) and carbon sulfide (C═S) in the range of 1760 to 1690 cm^{-1} and 1510 to 1450 cm^{-1}, respectively. Similarly, ^{1}H NMR spectra show two separate singlets for C-4 and C-6 methylene of tetrahydro-2H-1,3,5-thiadiazine-2-thione nucleus around 5.27 to 3.64 ppm.

In the same way, the ^{13}C NMR spectra of the compounds show the characteristics signals for C═S, C-4, and C-6 at δ:194.5, 69.7, 59.7, 195.0, 67.7, 57.1, 192.8, 68.2, 59.2, 194, 68.8, 59.7, 193.0, 67.6, 58.4, 192.8, 70.9, 59.4, 193.1, 71.5, 60.9, 198.3, 49.0, 47.7, 193.0, 71.0, 58.8, 193.4, 71.1, 58.8, 195.1, 72.2, 59.9, 194.0, 73.5, 58.5, 194.7, 73.9, 59.4, 194.3, 73.4, 60.8, 193.2, 72.0, 58.4, 194.2, 72.7, 57.7, in compounds **1**, **2**, **3**, **4**, **5**, **6**, **7**, **8**, **9**, **10**, **11**, **12**, **13**, **14**, **15**, and **16**, respectively. All the remaining data are mentioned in the experimental section.

2.2 | Urease inhibitory activity and molecular docking study

Here, our main focus was to screen all our synthesized compounds (**1-16**), for urease inhibitory activity against



SCHEME 1 Synthetic route of 3,5-disubstituted thiadiazine thiones

TABLE 1 In vitro activity of thiadiazine-2-thiones (**1-16**) against Jack bean

S. No	R^1	R^2	IC_{50} ± SEM, μM	Percent Inhibition	Yield, %
1	Benzyl–	$HOOCCH_2$–	9.8 ± 0.5	99.6	72
2	Benzyl–	$(CH_3)_2CH$–	11.0 ± 0.6	96.3	65
3	Benzyl–	$HOCH_2CH_2$–	16.0 ± 1.5	99.8	68
4	Benzyl–	$CH_3CH_2CH_2CH_2$–	17.2 ± 0.5	97	76
5	Benzyl–	$HOOCCH_2(CH_2)_4$–	25.08 ± 0.2	97	86
6	$CH_3CH_2CH_2CH_2$–	$HOOCCH_2$–	15.4 ± 0.5	97.5	80
7	$CH_3CH_2CH_2CH_2$–	$HOCH_2CH_2$–	19.7 ± 0.4	96.9	77
8	$CH_3CH_2CH_2CH_2$–	$C_4H_3N_2$–	22.9 ± 0.3	99.2	70
9	$CH_3CH_2CH_2CH_2$–	$HOOCCH_2(CH_2)_4$–	15.8 ± 0.2	98.3	82
10	$HOOCCH_2(CH_2)_4$–	$HOOCCH_2(CH_2)_4$–	35.08 ± 0.2	95	70
11	$HOOCCH_2$–	$HOOCCH_2$–	28.08 ± 0.2	96	75
12	C_6H_5–	$CH_3CH_2CH_2CH_2$–	46.3 ± 0.35	98.0	80
13	*o*-$CH_3C_6H_4$–	$HOOCCH_2$–	NI	—	70
14	2,4,6-$(CH_3)_3$-C_6H_3–	$HOCH_2CH_2$–	—	—	90
15	2,4,6-$(CH_3)_3$-C_6H_3–	$HOOCCH_2$–	—	—	95
16	2,4,6-$(CH_3)_3$-C_6H_3–	$HOOCCH_2(CH_2)_4$–	—	—	92
Standard urease inhibitor	Thiourea (standard urease inhibitor)	—	21 ± 0.01	—	—

Abbreviations: NI, no inhibitory activity. SEM, standard error of the mean.

Jack bean urease as shown in Table 1. Structure activity relationship of the synthesized compounds revealed that the thiadiazine thione nucleus having benzyl group at N-3 position and ethanoic acid group at N-5 position exhibited significant activity, for example, compound **1** (IC_{50} = 9.8 ± 0.5μM). The activities decreased when ethanoic group was replaced by isopropyl, butyl, and hexyl group at position N-5; for example, compounds **2** (IC_{50} = 11.0 ± 0.6μM), **4** (IC_{50} = 17.2 ± 0.5μM), and **5** **(**IC_{50} = 25.08 ± 0.2μM) show low activities as compare with compound **1**.

Similarly, decreased in activities was observed, when benzyl group at position N-3 was replaced by butyl group, for example, compounds **6** (IC_{50} = 15.4 ± 0.5μM), **7** (IC_{50} = 19.7 ± 0.4μM), **8** (IC_{50} = 22.9 ± 0.3μM), and **9** (IC_{50} = 15.8 ± 0.2μM). Surprisingly, low activity was found for compound **12** (IC_{50} = 46.3 ± 0.35μM), when the substituent at position N-3 was replaced by phenyl group, while activities completely abolished when monomethyl, dimethyl, and trimethyl substituted phenyl were used at N-3 position, such as compounds **10**, **11**, **12**, and **14** as shown in Table 1, which might be due to methyl group attached to the phenyl group.

To investigate the binding mode of the most active compound (compound **1**), the molecular docking was performed to determine the binding interaction between the active compound and urease enzyme. The docking result illustrated that the compound **1** was accommodated very well in the active site pocket. From the docking conformation of the most active compound **1**, it was observed that this compound formed three hydrogen bonds with the Glu 493, His 593, and Arg 609 residues of the enzyme (Figure 1).

The compound **1** has biological activity with the IC_{50} value of (9.8 ± 0.5μM) and the corresponding binding energy of −5.98 kcal/mol. His 492 was observed making an arene cation linkage with the benzene moiety of the compound. The Glu 493 and His 593 formed hydrogen bonding with the carboxyl moiety of the compound, while His 593 formed hydrogen bond with the nitrogen atom of the 3,5-dimethyl-1,3,5-thiadiazinane-2-thione moiety of the compound.

3 | CONCLUSION

In conclusion, a series of thiadiazine thione nucleus based compounds were prepared in good yield. The in vitro urease inhibition activity of these compounds revealed that the compounds (**1**, **2**, **3**, **4**, **6**, **7**, and **9**) were found more active than the reference compound thiourea, while the compounds **5** and **8** show same level of inhibitory activity as like standard drug. Among the series, the most active compound was investigated by docking study to explain their binding approach with target enzyme. Based on our above finding, we are optimistic that thiadiazine thione nucleus based moiety will be used to treat urease related problems in future.

4 | EXPERIMENTAL

4.1 | Materials

All solvents and chemical reagents were commercial grade quality and were used without further purification. All the reactions were monitored by thin layer chromatography, using precoated silica gel (aluminum sheets, layer thickness 0.2 mm and 60 HF-254). Melting points were determined in glass capillary tubes using a Gallen Kamp melting point apparatus and are uncorrected. Structures of all the synthesized compounds were determined with the help of spectroscopic techniques including: UV (Shimadzu), IR (Bio-Rad Win IR), ^{1}H NMR (Bruker), ^{13}C NMR (Bruker), MS (Jeol Mat 312), and elemental analysis (Carlo Erba 1106).

4.2 | General procedure for the synthesis of 3,5-disubstituted-tetrahydrothiadiazine-2-thiones (1-16)

To a magnetically stirred solution of primary amine/amino acid (20 mmol) and potassium hydroxide (20%, 20 mmol) in 30 mL of water, carbon disulfide (20 mmol) was added portion-wise. Further, the reaction materials were stirred for 4 hours, followed by addition of 37% formaldehyde (40 mmol) and stirred for 1 hour. Subsequently, the reaction material was filtered, and the filtrate was transferred dropwise to a suspension of amino acid/amine in 20 mL of phosphate buffer solution (pH 7.7) and stirs it for 1 hour. The reaction material was filtered, and the filtrate was extracted with dichloromethane. The aqueous solution was acidified with 15% HCl. The precipitate formed was filtered under suction and thoroughly washed with water and dried. At the end, the product was recrystallized from ethanol. The above general procedure represents a slightly modified version of already reported method.[19] The structures of the all the synthesized compounds were characterized by UV, IR, ^{1}H NMR, ^{13}C NMR, MS, and elemental analysis.

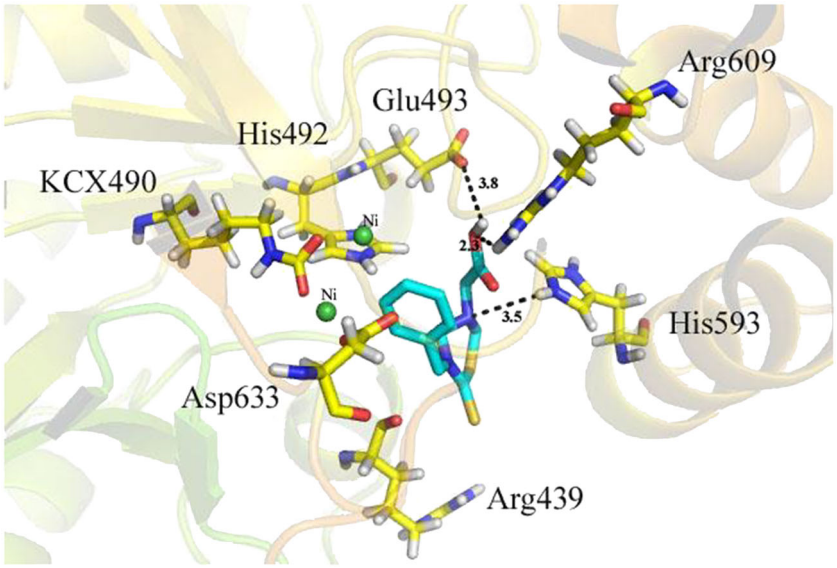


FIGURE 1 Solid ribbon representation of intermolecular interaction between compound **1** and urease crystal structure [Color figure can be viewed at wileyonlinelibrary.com]

4.2.1 | (5-Benzyl-6-thioxo-1,3,5-thiadiazinan-3-yl)acetic acid (1)

Benzylamine (2.18 mL, 20 mmol) and glycine (1.5 g, 20 mmol): recrystallized from ethanol, colourless solid,[21] Yield = 72%, m.p = 135-137°C; UV (methanol) λ_{max} nm: 205, 290; IR (KBr) υ_{max} cm^{-1}: 3430 (COOH), 3030 (Aromatic–C–H), 2883 (Aliphatic–C–H), 1724 (C═O), 1491 (C═S); ^{1}H NMR ($CDCl_3$ + CD_3OD) δ: 7.29-7.21 (m, 5H, Ar-H), 5.22 (s, 2H, $C_6H_5\underline{CH_2}$), 4.39 (s, 2H, H-4), 4.29 (s, 2H, H-6), 3.29 (s, 2H, $N\underline{CH_2}COOH$); ^{13}C NMR (CD_3OD) δ: 194.5 (C═S), 172.2 (C═O), 136.9 (Ar-C), 129.8, 129.5, and 129.1 (Ar–CH), 69.7 (C-4), 59.7 (C-6), 54.5 ($\underline{CH_2}C_6H_5$), 51.9 (NCH_2); EI-MS; m/z (rel. int. %): 281 (M^+), 149 (8), 133 (12), 119 (6), 101 (4), 91 (100), and 65 (12); *Anal.* calcd for $C_{12}H_{14}N_2O_2S_2$: C, 51.06; H, 4.96; N, 9.92. Found: C, 51.05; H, 4.92; N, 9.90.

4.2.2 | 2-(5-Benzyl-6-thioxo-1,3,5-thiadiazinan-3-yl)propanoic acid (2)

Benzylamine (2.18 mL, 20 mmol) and alanine (1.78 g, 20 mmol): recrystallized from ethanol, colourless solid, Yield = 65%, m.p = 143-144°C; UV (methanol) λ_{max} nm: 210, 290; IR (KBr) υ_{max} cm^{-1}: 3400 (COOH), 3010 (Aromatic–C–H), 2870 (Aliphatic–C–H), 1700 (C═O), 1456 (C═S); ^{1}H NMR (CD_3OD) δ: 7.32-7.03 (m, 5H, Ar–H), 5.22 (s, 2H, $C_6H_5\underline{CH_2}$), 4.28 (s, 2H, H-4), 4.22 (s, 2H, H-6), 3.57 (q, 1H, J = 7.0 Hz, $CH_3\underline{CH}$), 1.12 (d, 3H, J = 7.0 Hz, $CH\underline{CH_3}$); ^{13}C NMR (CD_3OD + $CDCl_3$) δ: 195.0 (C═S), 176.2 (C═O), 137.0 (Ar-C), 129.8, 129.7, and 129.1 (Ar–CH), 67.7 (C-4), 57.7 ($\underline{CH}CH_3$), 57.1 (C-6), 54.7 ($C_6H_5\underline{CH_2}$), 16.5 ($\underline{CH_3}CH$); EI-MS; m/z (rel. int. %): 296 (M^+), 281 (19), 148 (23), 133 (24), 119 (20), 90 (100), 76 (64), 65 (24), and 56 (26); *Anal.* calcd for $C_{13}H_{16}N_2O_2S_2$: C, 52.70; H, 5.40; N, 9.45. Found: C, 52.69; H, 5.40; N, 9.44.

4.2.3 | 3-Benzyl-5-(2-hydroxyethyl)-1,3,5-thiadiazinane-2-thione (3)

Benzylamine (2.18 mL, 20 mmol) and ethanolamine (1.20 mL, 20 mmol): recrystallized from ethanol, colourless solid, Yield = 68%, m.p = 99-101°C; UV (methanol) λ_{max} nm: 225, 290; IR (KBr) υ_{max} cm^{-1}: 3304 (OH), 3030 (Aromatic–C–H), 2958 (Aliphatic–C–H), 1491 (C═S); ^{1}H NMR (CD_3OD) δ: 7.40-7.28 (m, 5H, Ar–H), 5.33 (s, 2H, $\underline{CH_2}C_6H_5$), 4.41 (s, 2H, H-4), 4.32 (s, 2H, H-6), 3.43 (t, 2H, J = 5.4 Hz, $HO\underline{CH_2}CH_2$), 2.77 (t, 2H, J = 5.4 Hz, $N\underline{CH_2}CH_2$); ^{13}C NMR ($CDCl_3$) δ: 192.8 (C═S), 135.1 (Ar–C), 128.9, 128.6, and 128.3 (Ar–CH), 68.2 (C-4), 59.2 (C-6), 58.5 ($CH_2\underline{CH_2}OH$), 53.7 ($\underline{CH_2}C_6H_5$), 52.4 ($N\underline{CH_2}CH_2OH$); EI-MS; m/z (rel. int. %): 268 (M^+), 235 (18), 196 (8), 149 (11), 118 (13), 90 (100), 87 (31), 76 (16), 65 (13), and 56 (9); *Anal.* calcd for $C_{12}H_{16}N_2OS_2$: C, 53.73; H, 5.97; N, 10.44. Found: C, 54.72; H, 5.97; N, 10.43.

4.2.4 | 3-Benzyl-5-butyl-1,3,5-thiadiazinane-2-thione (4)

Benzylamine (2.18 mL, 20 mmol) and n-butylamine (1.97 mL, 20 mmol): recrystallized from ethanol, colourless solid,[25] Yield = 76%, m.*p* = 108-109°C; UV (methanol) λ_{max} nm: 210, 290; IR (KBr) υ_{max} cm^{-1}: 3025 (Aromatic–C–H), 2862 (Aliphatic–C–H), 1452 (C═S); ^{1}H NMR (CD_3OD) δ: 7.46-7.29 (m, 5H, Ar–H), 5.33 (s, 2H, $\underline{CH_2}C_6H_5$), 4.82 (s, 2H, H-4), 4.37 (s, 2H, H-6), 2.53 (t, 2H, J = 7.3 Hz, $N\underline{CH_2}CH_2$), 1.17-1.11 (m, 2H, $CH_2\underline{CH_2}CH_2$), 1.10-1.07 (m, 2H, $CH_2\underline{CH_2}CH_3$), 0.77 (t, J = 6.9 Hz, $\underline{CH_3}CH_2$); ^{13}C NMR (CD_3OD) δ: 194 (C═S), 137.3 (Ar–C),

129.8, 129.7, and 129.3 (Ar–CH), 68.8 (C-4), 59.7 (C-6), 54.29 ($\underline{CH_2}C_6H_5$), 50.9 ($N\underline{CH_2}CH_2$), 30.2 ($NCH_2\underline{CH_2}CH_2$), 21.03 ($CH_2\underline{CH_2}CH_3$), 14.0 ($CH_2\underline{CH_3}$); EI-MS; m/z (rel. int. %) 247 (M^+), 189 (2), 148 (2), 103 (7), 91 (100), 72 (12), 65 (27), and 51 (7); *Anal*. calcd for $C_{14}H_{20}N_2S_2$: C, 60.09; H, 7.14; N, 10.80. Found: C, 60.09; H, 7.12; N, 10.79.

4.2.5 | 6-(5-Benzyl-6-thioxo-1,3,5-thiadiazinan-3-yl)hexanoic acid (5)

Benzylamine (2.18 mL, 20 mmol) and 6-aminocaproic acid (2.62 g, 20 mmol): recrystallized from ethanol, colourless solid, Yield = 86%, m.p = 108-110°C; UV (methanol) λ_{max} nm: 240, 315; IR (KBr) υ_{max} cm^{-1}: 3400 (COOH), 3020 (Aromatic–C–H), 2850 (Aliphatic–C–H), 1729 (C═O), 1460 (C═S); ^{1}H NMR (CD_3OD) δ: 7.46-7.31 (m, 5H, Ar–H), 5.33 (s, 2H, $\underline{CH_2}C_6H_5$), 4.47 (s, 2H, H-4), 4.37 (s, 2H, H-6), 2.53 (t, 2H, J = 7.3 Hz, $CH_2\underline{CH_2}N$), 2.18 (t, 2H, J = 7.3 Hz, $CH_2\underline{CH_2}COOH$), 1.47-1.40 (m, 4H, $CH_2(\underline{CH_2})_2CH_2$), 1.18-1.10 (m, 2H, $CH_2\underline{CH_2}CH_2$); ^{13}C NMR (CD_3OD + $CDCl_3$) δ: 193.0 (C═S), 175.8 (C═O), 135.0 (Ar–C), 128.5, 128.2, and 127.9 (Ar–CH), 67.6 (C-4), 58.4 (C-6), 53.4 ($C_6H_5\underline{CH_2}$), 49.7 ($N\underline{CH_2}CH_2$), 33.5 ($\underline{CH_2}COOH$), 26.4 ($NCH_2\underline{CH_2}$), 25.9 ($NCH_2CH_2\underline{CH_2}$), 24.1 ($\underline{CH_2}CH_2COOH$); MS (FAB) m/z: 339; *Anal*. calcd for $C_{16}H_{22}N_2O_2S_2$: C, 56.77; H, 6.55; N, 8.28. Found: C, 56.76; H, 6.55; N,8.27.

4.2.6 | (5-Butyl-6-thioxo-1,3,5-thiadiazinan-3-yl)acetic acid (6)

n-Butylamine (1.97 mL, 20 mmol) and glycine (1.5 g, 20 mmol): recrystallized from ethanol, colourless solid,[21] Yield = 80%, m.p = 97-99°C; UV (methanol) λ_{max} nm: 210, 285: IR (KBr): 3420 (COOH), 2954 (Aliphatic–C–H), 1733 (C═O), 1508 (C═S); ^{1}H NMR (CD_3OD) δ: 4.53 (s, 2H, H-4), 4.52 (s, 2H, H-6), 3.97 (t, 2H, J = 7.8 Hz, $N\underline{CH_2}CH_2$), 3.30 (s, 2H, $\underline{CH_2}COOH$), 1.67-1.61 (m, 2H, $CH_2\underline{CH_2}CH_2$), 1.38-1.33 (m, 2H, $CH_2\underline{CH_2}CH_3$), 0.96 (t, J = 7.3 Hz, 3H, $CH_2\underline{CH_3}$); ^{13}C NMR (CD_3OD) δ: 192.8 (C═S), 172.8 (C═O), 70.9 (C-4), 59.4 (C-6), 52.9 ($\underline{CH_2}COOH$), 52.0 ($CH_2\underline{CH_2}N$), 29.5 ($CH_2\underline{CH_2}CH_2$), 20.9 ($CH_2\underline{CH_2}CH_3$), 14.1 ($\underline{CH_3}CH_2$); EI-MS; m/z (rel. int. %) 248 (M^+), 115 (22), 84 (86), 72 (96), 60 (58), and 57 (100); *Anal*. calcd for $C_9H_{16}N_2O_2S_2$: C, 43.54; H, 6.45; N, 11.29. Found: C, 43.52; H, 6.42; N, 11.28.

4.2.7 | 3-Butyl-5-(2-hydroxyethyl)-1,3,5-thiadiazinane-2-thione (7)

n-Butylamine (1.97 mL, 20 mmol) and ethanolamine (1.20 mL, 20 mmol): recrystallized from ethanol, colourless solid, Yield = 77%, m.p = 58-60°C; UV (methanol) λ_{max} nm: 245, 285; IR (KBr) υ_{max} cm^{-1}: 3300 (OH), 2860 (Aliphatic–C–H), 1480 (C═S); ^{1}H NMR (CD_3OD) δ: 4.84 (s, 2H, H-4), 4.50 (s, 2H, H-6), 3.90 (t, 2H, J = 7.9 Hz, $N\underline{CH_2}CH_2$), 3.72 (t, 2H, J = 5.4 Hz, $HO\underline{CH_2}CH_2$), 2.91 (t, 2H, J = 5.4 Hz, $N\underline{CH_2}CH_2$), 1.69-1.63 (m, 2H, $CH_2\underline{CH_2}CH_2$), 1.40-1.34 (m, 2H, $CH_2\underline{CH_2}CH_3$), 0.96 (t, 3H, J = 7.4 Hz, $\underline{CH_3}CH_2$); ^{13}C NMR (CD_3OD + $CDCl_3$) δ: 193.1(C═S), 71.5 (C-4), 60.9 (C-6), 59.5 (CH_2OH), 53.5 ($N\underline{CH_2}CH_2$), 53.0 ($N\underline{CH_2}CH_2OH$), 29.6 ($NCH_2\underline{CH_2}$), 20.9 ($\underline{CH_2}CH_3$), 14.1 ($\underline{CH_3}CH_2$); EI-MS; m/z (rel. Int. %): 234 (M^+), 201 (3), 161 (2), 110 (15), 84 (100), 72 (35), and 57 (15); *Anal*. calcd for $C_{14}H_{18}N_2O_2S_2$: C, 46.12; H, 7.74; N, 11.95. Found: C, 46.10; H, 7.72; N, 11.94.

4.2.8 | 3-Butyl-5-pyrimidin-2-yl-1,3,5-thiadiazinane-2-thione (8)

n-Butylamine (1.97 mL, 20 mmol) and 2-aminopyrimidine (1.90 g, 20 mmol): recrystallized from ethanol, colourless solid, Yield = 70%, m.*p* = 99-101°C; UV (methanol) λ_{max} nm: 275; IR (KBr) υ_{max} cm^{-1}: 2873 (Aliphatic–C–H), 1465 (C═S); ^{1}H NMR (CD_3OD) δ: 8.33 (d, 2H, J = 3.7 Hz, Ar–CH), 6.74 (t, 1H, J = 4.7 Hz, Ar–H), 5.27 (s, 2H, H-4), 4.84 (s, 2H, H-6), 3.65 (t, 2H, J = 7.2 Hz, $N\underline{CH_2}CH_2$), 1.63-1.56 (m, 2H, $CH_2\underline{CH_2}CH_2$), 1.38-1.31 (m, 2H, $CH_3\underline{CH_2}CH_2$), 0.92 (t, 3H, J = 7.3 Hz, $\underline{CH_3}CH2$);^{13}C NMR (CD_3OD) δ: 198.3 (C═S), 162.4 (Ar-C), 159.4 (Ar–C), 113.0 (Ar–CH), 49.0 (C-4), 48.3 ($N\underline{CH_2}CH_2$), 47.7 (C-6), 31.1 ($CH_2\underline{CH_2}CH_2$), 21.1 ($CH_3\underline{CH_2}CH_2$), 14.0 ($\underline{CH_3}CH_2$); MS (FAB) m/z: 286; *Anal*. Calcd for $C_{11}H_{16}N_4S_2$: C, 49.20; H, 5.97; N, 20.89. Found: C, 49.19; H, 5.95; N, 20.86.

4.2.9 | 6-(5-Butyl-6-thioxo-1,3,5-thiadiazinan-3-yl)hexanoic acid (9)

n-Butylamine (1.97 mL, 20 mmol) and 6-amino caproic acid (2.62 g, 20 mmol): recrystallized from ethanol, colourless solid, Yield = 82%, m.p = 88-90°C; UV (methanol) λ_{max} nm: 250, 285; IR (KBr) υ_{max} cm^{-1}: 3400 (COOH), 2865 (Aliphatic–C–H), 1695 (C═O), 1503 (C═S); ^{1}H NMR (CD_3OD) δ: 4.47 (s, 2H, H-4), 4.46 (s, 2H, H-6), 3.80 (t, 2H, J = 7.9 Hz, $N\underline{CH_2}CH_2$), 2.78 (t, 2H, J = 7.1 Hz, $N\underline{CH_2}CH_2$), 2.29 (t, 2H, J = 7.3 Hz, $CH_2\underline{CH_2}COOH$), 1.68-1.62 (m, 4H, $CH_2(\underline{CH_2})_2CH_2$), 1.61-1.55 (m, 2H, $CH_2\underline{CH_2}CH_2$), 1.44-1.40 (m, 2H, $CH_3\underline{CH_2}CH_2$), 1.39-1.34 (m, 2H, $CH_2\underline{CH_2}CH_2$), 0.96 (t, 3H, J = 7.3 Hz, $\underline{CH_3}CH_2$); ^{13}C NMR (CD_3OD) δ: 193.0 (C═S), 177.4 (C═O), 71.0 (C-4), 58.8 (C-6), 52.9 ($N\underline{CH_2}$), 50.9 ($N\underline{CH_2}$), 34.8 ($\underline{CH_2}COOH$), 29.5 ($CH_2\underline{CH_2}CH_2$), 28.1 ($CH_2\underline{CH_2}CH_2$), 27.5 ($\underline{CH_2}CH_2COOH$), 25.3 ($CH_2\underline{CH_2}CH_2$), 20.9 ($CH_2\underline{CH_2}CH_3$), 14.1 ($\underline{CH_3}CH_2$); EI-MS; m/z (rel. int. %):

246 (M^+), 156 (17), 115 (32), 98 (95), 84 (100), 76 (97), 57 (88), and 51 (7); *Anal*. calcd for $C_{13}H_{24}N_2O_2S_2$: C, 51.31; H, 7.89; N, 9.21. Found: C, 51.30; H, 7.87; N, 9.20.

4.2.10 | 6,6′-(2-Thioxo-1,3,5-thiadiazinane-3,5-diyl) dihexanoic acid (10)

6-Aminocaproic acid (2.62 g, 20 mmol) and 6-aminocaproic acid (2.62 g, 20 mmol): recrystallized from ethanol, colourless solid,[26] Yield = 70%, m.*p* = 143-144°C; UV (methanol) λ_{max} nm: 210, 285; IR (KBr) υ_{max} cm^{-1}: 3400 (COOH), 2862 (Aliphatic–C–H), 1704 (C═O), 1492 (C═S); ^{1}H NMR (CD_3OD) δ: 4.47 (s, 4H, H-4, H-6), 3.98 (t, 2H, (CH_2)-7, *J* = 7.8 Hz), 2.78 (t 2H, (CH_2)-7′, *J* = 7.1), 2.38 (t, 4H, (CH_2)-11 and (CH_2)-11′), 1.59-1.70 (m, 8H, (CH_2)-8 and 8′, (CH_2)-10 and 10′), 1.36-1.43 (m, 4H, (CH_2)-9 and 9′); ^{13}C NMR (CD_3OD + $CDCl_3$) δ: 193.4 (C═S), 177.5 (C═O), 71.1 (C-4), 58.8 (C-6), 52.95 (C-7), 50.95 (C-7′), 34.83 (C-11&11′), 28.10 (C-8), 27.57 (C-8′), 25.82 (C-9′), 25.6 (C-9), 27.2 (C-10), 27.2 (C-10′); EI-MS; m/z (rel. int. %): 362(M^+), 156 (20), 155 (18), 142 (13), 128(8), 111 (40), 98(18), and 55(66); *Anal*. Calcd for $C_{15}H_{28}N_2O_4S_2$: C, 49.72; H, 7.18; N, 7.73. Found: C, 49.70; H, 7.16; N, 7.71.

4.2.11 | 2,2′-(2-Thioxo-1,3,5-thiadiazinane-3,5-diyl) diacetic acid (11)

Glycine (1.5 g, 20 mmol) and glycine (1.5 g, 20 mmol): recrystallized from ethanol, colourless solid, Yield = 75%, m.p = 208-209°C; UV (methanol) λ_{max} nm: 250, 285; IR (KBr) υ_{max} cm^{-1}: 3430 (COOH), 2850 (Aliphatic–C–H), 1700 (C═O), 1475 (C═S); ^{1}H NMR (CD_3OD) δ: 4.74 (s, 2H, CH_2), 4.60 (s, 2H, H-4), 4.58 (s, 2H, H-6), 3.81(s, 2H, CH_2); ^{13}C NMR (CD_3OD) δ: 195.1 (C═S), 173.2 (C═O), 170.8 (C═O), 72.2 (C-4), 59.9 (C-6), 53.2 ($\underline{CH_2}$COOH), 52.4 ($\underline{CH_2}$COOH); Mass (FAB) m/z: 250; *Anal*. Calcd for $C_7H_{10}N_2O_4S_2$: C, 33.6; H, 4.0; N, 11.2. Found: C, 33.59; H, 3.97; N,10.99.

4.2.12 | 5-Butyl-3-phenyl-1,3,5-thiadiazinane-2-thione (12)

Aniline (2.18 mL, 20 mmol) and n-butyl amine (1.97 mL, 20 mmol): recrystallized from ethanol, colourless solid, Yield = 80%, m.p = 102-104°C; UV (methanol) λ_{max} nm: 215, 280; IR (KBr) υ_{max} cm^{-1}: 3030 (Aromatic–C–H), 2825 (Aliphatic–C–H), 1455 (C═S); ^{1}H NMR (CD_3OD) δ: 7.46-7.18 (m, 5H, Ar-H), 4.84 (s, 2H, H-4), 4.69 (s, 2H, H-6), 3.0 (t, 2H, *J* = 7.2 Hz, $N\underline{CH_2}CH_2$), 1.61-1.55 (m, 2H, $CH_2\underline{CH_2}CH_2$), 1.46-1.40 (m, 2H, $CH_3\underline{CH_2}CH_2$), 0.95 (t, 3H, *J* = 7.3 Hz, $\underline{CH_3}CH_2$);^{13}C NMR ($CDCl_3$) δ: 194.0 (C═), 144.5 (Ar–C), 129.8, 128.2, and 126.9 (Ar–CH), 73.5 (C-4), 58.5 (C-6), 50.1 ($N\underline{CH_2}$), 29.3 ($NCH_2\underline{CH_2}$), 20.0 ($NCH_2CH_2\underline{CH_2}$), 13.8 $CH_2\underline{CH_3}$); EI-MS; m/z (rel. int. %): 266 (M^+), 135 (32), 99 (67), 77 (99), 65(9), 57(30), and 51 (100); *Anal*. calcd for $C_{13}H_{18}N_2S_2$: C, 58.64; H, 6.76; N, 10.52. Found: C, 58.62; H, 6.74; N, 10.50.

4.2.13 | [5-(2-Methylphenyl)-6-thioxo-1,3,5-thiadiazinan-3-yl]acetic acid (13)

o-Toluidine (2.18 mL, 20 mmol) and glycine (1.5 g, 20 mmol): recrystallized from ethanol, colourless solid, Yield = 70%, m.*p* = 127-129°C; UV (methanol) λ_{max} nm: 215, 240; IR (KBr) υ_{max} cm^{-1}: 3400 (COOH), 3030 (Aromatic–C–H), 1716 (C═O), 1485 (C═S); ^{1}H NMR (CD_3OD) δ: 7.27-7.04 (m, Ar–H), 3.69 (s, 2H, H-4), 3.64 (s, 2H, H-6), 3.30 (s, 2H, $\underline{CH_2}$COOH), 2.22 (s, 3H, Ar–$\underline{CH_3}$);^{13}C NMR (CD_3OD) δ: 194.7 (C═S), 172.7 (C═O), 144, 136.6 (Ar–C), 132.3, 128, 129.7, and 127.7 (Ar–CH), 73.9 (C-4), 59.4 (C-6), 52.7 ($\underline{CH_2}$COOH), 17.8 (Ar–$\underline{CH_3}$); EI-MS; m/z (rel. int. %): 282 (M^+), 250 (15), 235 (12), 149 (62), 120 (100), 117 (53), 91 (94), 77 (58), 65 (66), and 51 (40); *Anal*. Calcd for $C_{12}H_{14}N_2O_2S_2$: C, 51.0; H, 4.96; N, 9.92. Found: C, 50.98; H, 4.94; N, 9.91.

4.2.14 | 5-(2-Hydroxyethyl)-3-mesityl-1,3,5-thiadiazinane-2-thione (14)

2,4,6-Trimethylaniline (2.18 mL, 20 mmol) and ethanolamine (1.20 mL, 20 mmol): recrystallized from ethanol, colourless solid, Yield = 90% m.p = 126-128°C; UV (methanol) λ_{max} nm: 240, 285; IR (KBr) υ_{max} cm^{-1}: 3315 (OH), 3020 (Aromatic–C–H), 2833 (Aliphatic–C–H), 1750 (C═O), 1475 (C═S); ^{1}H NMR (CD_3OD) δ: 6.90 (s, 2H, Ar–H), 4.65 (s, 2H, H-4), 4.51 (s, 2H, H-6), 3.75 (t, 2H, *J* = 6.9 Hz, $CH_2\underline{CH_2}OH$), 3.22 (t, 2H, *J* = 6.9 Hz, $N\underline{CH_2}CH_2OH$), 2.29 (s, 3H, Ar–CH_3), 2.14 (s, 6H, Ar–CH_3); ^{13}C NMR (CD_3OD + $CDCl_3$) δ: 194.3 (C═S), 139.5, 138.5 (Ar-C), 129.7 (Ar–CH), 73.4 (C-4), 60.8 (C-6), 58.4 ($\underline{CH_2}OH$), 54.6 ($N\underline{CH_2}$), 21.0, 18.4 (Ar–CH_3); EI-MS; m/z (rel. int. %): 296 (M^+), 250 (3), 177 (100), 119 (89), 104 (4), 91 (18), 87 (4), 76 (45), and 65 (6); *Anal*. calcd for $C_{14}H_{20}N_2OS_2$: C, 56.72; H, 6.80; N, 9.45. Found: C, 56.70; H, 6.79; N, 9.43.

4.2.15 | 5-Mesityl-6-thioxo-1,3,5-thiadiazinan-3-yl)acetic acid (15)

2,4,6-Trimethylaniline (2.18 mL, 20 mmol) and glycine (1.5 g, 20 mmol): recrystallized from ethanol, colourless

solid,[27] Yield = 95%, m.p = 148-150°C; UV (methanol) λ_{max} nm: 215, 245; IR (KBr) υ_{max} cm^{-1}: 3460 (COOH), 3010 (Aromatic–C–H), 2885 (Aliphatic–C–H), 1733 (C═O), 1471 (C═S); ^{1}H NMR (CD_3OD + $CDCl_3$) δ: 6.90 (s, 2H, Ar–H), 4.63 (s, 2H, H-4), 4.52 (s, 2H, H-6), 3.90 (s, $\underline{CH_2}COOH$), 2.24 (s, 3H, Ar–CH_3), 2.14 (s, 6H, Ar–CH_3); ^{13}C NMR (CD_3OD + $CDCl_3$) δ: 193.2 (C═S), 172.2 (C═O), 139.2, 138.5, and 134.9 (Ar–C), 130.5 (Ar–CH), 72.0 (C-4), 58.4 (C-6), 52.0 ($\underline{CH_2}COOH$), 21.1, 18.2 (Ar–CH_3); EI-MS; m/z (rel. int. %), 310 (M^+), 177 (100), 162 (40), 147 (39), 119 (37), 100 (13), 104 (7), and 91 (31), and 65 (12); *Anal*. calcd for $C_{14}H_{18}N_2O_2S_2$: C, 54.19; H, 5.80; N, 9.03. Found: C, 54.17; H, 5.78; N, 9.01.

4.2.16 | 6-(5-Mesityl-6-thioxo-1,3,5-thiadiazinan-3-yl)hexanoic acid (16)

2,4,6-Trimethylaniline and 6-aminocaproic acid (2.62 g, 20 mmol): recrystallized from ethanol, colourless solid, Yield = 95%, m.p = 122-124°C; UV (methanol) λ_{max} nm: 215, 285; IR (KBr) υ_{max} cm^{-1}: 3420 (COOH), 3030 (Aromatic–C–H), 2867 (Aliphatic–C–H), 1726 (C═O), 1490 (C═S); ^{1}H NMR (CD_3OD) δ: 6.91 (s, 2H, Ar–H), 4.63 (s, 2H, H-4), 4.50 (s, 2H, H-6), 3.09 (t, 2H, J = 8.9 Hz, $N\underline{CH_2}CH_2$), 2.29 (t, 2H, J = 9.1 Hz, $CH_2\underline{CH_2}COOH$), 2.26 (s, 3H, Ar–CH_3), 2.15 (s, 6H, Ar-CH_3), 1.66-1.61 (m, 4H, $CH_2(\underline{CH_2})_2CH_2$), 1.45-1.41 (m, 2H, $CH_2\underline{CH_2}CH_2$); ^{13}C NMR (CD_3OD + $CDCl_3$) δ: 194.2 (C═S), 176.0 (C═O), 139.4, 139.0, and 135.4 (Ar–C), 129.7 (Ar–CH), 72.7 (C-4), 57.7 (C-6), 51.9 ($N\underline{CH_2}CH_2$), 34.9 ($\underline{CH_2}COOH$), 27.3 ($NCH_2\underline{CH_2}$), 21.0 ($NCH_2CH_2\underline{CH_2}$), 17.9 ($\underline{CH_2}CH_2COOH$); MS (FAB) m/z: 366; *Anal*. calcd for $C_{18}H_{26}N_2O_2S_2$: C, 59.01; H, 7.10; N, 7.65. Found: C, 59.01; H, 7.08; N, 7.62.

5 | UREASE INHIBITION BIOASSAY (IN VITRO)

All urease inhibition assays were performed on 96-well microplate format using a 96-well microplate reader (Molecular devices, USA). The pure enzyme solution and urea were incubated at 30°C for 15 minutes. The buffer used in this assay was 10nM K_2HPO_4, 1mM $LiCl_2$, and 1mM EDTA buffer (pH 8.2). For ammonia determination using the indophenol method the absorbance at 630 nm was measured after every 2 minutes continuously for 1 hour.[28] Results were compared with thiourea; the standard inhibitor of urease. IC_{50} values were calculated as a mean of three individual readings.

6 | MOLECULAR MODELING METHOD

The crystallographic structure of Jack bean urease (PDB ID: 4H9M) was obtained from the RSCB Protein Data Bank.[29] The bound ligand acetohydroxamic acid and crystallographic water molecules were removed from the structure, and hypothetical hydrogen atoms were added to the X-ray structure in standard geometries with the Molecular Operating Environment (MOE) 2016.08. The point charge of the two catalytic nickel cations was set to +2. Gasteiger charges were calculated using AutoDock Tools, and nonpolar hydrogens were merged. Docking was performed with AutoDock[30] 4.2 with rigid protein structure and fully flexible ligands. The 3D structure of the compound of the series was saved as pdb files, which were then transformed to pdbqt files after the charges of the nonpolar hydrogen atoms were assigned with the aid of ADT. The grid calculation was performed using the Autogrid4 program; grid spacing of 0.375 Å parameters was set. A grid spacing based on the default setting was 0.375 Å. And the gpf file was generated to run AutoGrid for the calculation of the energetic map. After successful calculations, the docking parameter file (dpf file) was prepared to run AutoDock. Default settings were used with an initial population size of 150 randomly placed individuals, a maximum number of 2.5 × 106 (medium) energy evaluations, and a maximum number of 2.7 × 104 generations. A mutation rate of 0.02, a crossover rate of 0.8, and GA crossover mode of two points were chosen. One hundred runs were generated by using Lamarckian genetic algorithm searches. On successful completion of docking, the resultant complex structures were selected based on the most favorable free energy of binding.

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REFERENCES AND NOTES

[1] J. C. Polacco, M. A. Holland, *Int. Rev. Cytol.* **1993**, *145*, 65.

[2] H. L. Mobley, R. P. Hausinger, *Microbiol. Rev.* **1989**, *53*, 85.

[3] H. L. Mobley, M. D. Island, R. P. Hausinger, *Microbiol. Rev.* **1995**, *59*, 451.

[4] P. A. Karplus, M. A. Pearson, R. P. Hausinger, *Acc. Chem. Res.* **1997**, *30*, 330.

[5] Z. P. Xiao, T. W. Ma, W. C. Fu, X. C. Peng, A. H. Zhang, H. L. Zhu, *Eur. J. Med. Chem.* **2010**, *45*, 5064.

[6] G. B. Thalita, S. W. John, O. A. C. Antunes, M. F. M. Estela, *Lett. Drug. Des. Dis.* **2009**, *6*, 186.

[7] M. Houimel, J. P. Mach, I. Corthesy-Theulaz, B. Corthesy, I. Fisch, *Eur. J. Biochem.* **1999**, *262*, 774.

[8] T. C. Kuhler, J. Fryklund, N. A. Bergman, J. Weilitz, A. Lee, H. Larsson, *J. Med. Chem.* **1995**, *38*, 4906.

[9] N. E. Dixon, T. C. Gazzola, R. L. Blakeley, B. Zermer, *J. Am. Chem. Soc.* **1975**, *97*, 4131.

[10] K. Kobashi, S. Takebe, N. Terashima, J. Hase, *J. Biochem.* **1975**, *77*, 837.

[11] A. Rauf, F. Ahmed, A. Qureshi, A. Khan, M. Qadir, M. Choudhary, Z. Chohan, M. Youssoufid, T. B. Haddad, *J. Chin. Chem. Soc.* **2011**, *58*, 528.

[12] A. M. Qureshi, S. Mumtaz, A. Rauf, M. Ashraf, R. Nasar, Z. H. Chohan, *J. Enzyme Inhib. Med. Chem.* **2015**, *30*, 119.

[13] S. Saraç, M. Ertan, A. Balkan, N. Yulug, *Arch. Pharm.* **1991**, *324*, 449.

[14] M. A. Hussein, A. N. El-Shorbagi, A. R. Khallil, *Arch. Pharm.* **2001**, *334*, 305.

[15] S. A. A. El Bialy, A. M. Abdelal, A.-N. El-Shorbagi, S. M. M. Kheira, *Arch. Pharm.* **2005**, *338*, 38.

[16] M. Schorr, W. Dürckheimer, P. Klatt, G. Lämmler, G. Nesemann, E. Schrinner, *Arzmeim-Forsch/Drug Res* **1969**, *19*, 1807.

[17] J. Coro, R. Perez, H. Rodriguez, M. Suarez, C. Vega, M. Rolon, D. Montero, J. J. Nogal, A. Gomez-Barrio, *Bioorg. Med. Chem.* **2005**, *13*, 3413.

[18] L. Monzote, A. M. Montalvo, L. Fonseca, R. Perez, M. Suarez, H. Rodriguez, *Arzneimittelforschung* **2005**, *55*, 232.

[19] D. Katiyar, V. K. Tiwari, R. P. Tripathi, A. Srivastava, V. Chaturvedi, R. Srivastava, B. S. Srivastava, *Bioorg. Med. Chem.* **2003**, *11*, 4369.

[20] A. N. El-Shorbagi, *Eur. J. Med. Chem.* **1994**, *29*, 11.

[21] T. Aboul-Fadl, A. El-Shorbagi, *Eur. J. Med. Chem.* **1996**, *31*, 165.

[22] M. I. Abd-Elrahman, M. O. Ahmed, S. M. Ahmed, T. Aboul-Fadl, A. El-Shorbagi, *Biophys. Chem.* **2002**, *97*, 113.

[23] A. N. El-Shorbagi, *Arch Pharm (Weinheim)* **2000**, *333*, 281.

[24] M. Kot, W. Zaborska, *J. Enzyme Inhib. Med. Chem.* **2006**, *21*, 537.

[25] M. Arfan, M. N. Tahir, M. I. A. Shah, M. S. Iqbal, *Acta Crystallogr. Sect. E.* **2009**, *65*, o468.

[26] R. Pérez, O. Reyes, M. Suarez, H. E. Garay, L. J. Cruz, H. Rodríguez, M. A. D. Molero-Vilchez, C. Ochoa, *Tetrahedron Lett.* **2000**, *41*, 613.

[27] M. Arfan, M. N. Tahir, M. I. A. Shah, R. Khan, M. S. Iqbal, *Acta Crystallogr. Sect. E.* **2009**, *65*, o902.

[28] M. W. Weatherburn, *Anal. Chem.* **1967**, *39*, 971.

[29] H. M. Berman, J. Westbrook, Z. Feng, G. Gilliland, T. N. Bhat, H. Weissig, I. N. Shindyalov, P. E. Bourne, *Nucleic Acids Res.* **2000**, *28*(1), 235.

[30] G. M. Morris, R. Huey, W. Lindstrom, M. F. Sanner, R. K. Belew, D. S. Goodsell, A. J. Olson, *J. Comput. Chem.* **2009**, *30*, 2785.

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