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ORIGINAL PAPER

Design, synthesis, and urease inhibition studies of a series of 4-amino-5-aryl-3*H*-1,2,4-triazole-3-thiones

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Abstract A series of 4-amino-5-aryl-3*H*-1,2,4-triazole-3-thiones was synthesized by reaction of aryl hydrazides with CS_2 and hydrazine hydrate. The synthesized compounds were characterized by spectroanalytical techniques, and their urease inhibition activity was evaluated using jack bean urease. All but one of the synthesized compounds were active, and two of them were found to be more potent than the standard, with 50% inhibition concentration (IC_{50}) values of 17.5 ± 0.52 and 4.3 ± 0.169 μM, respectively (standard IC_{50} = 21.0 ± 0.11 μM). Tentative statements regarding the role of different functional groups in binding to the enzyme active site are also presented.

Keywords Cyclizations · Enzyme inhibition · Heterocycles · Thiones · Urease activity

Introduction

The involvement of bacterial ureases in the formation of infectious stones [1] and development of peptic ulcers and stomach cancer [2] has been reported. Ureases are also involved in the development of urolithiasis, pyelonephritis, hepatic encephalopathy, hepatic coma, and urinary catheter encrustation [3]. The fertilizer urea is also hydrolyzed rapidly by the enzyme urease produced by fungi and bacteria on the soil surface [4, 5]. This reaction not only reduces the effective use of urea nitrogen by plants but also results in an increase of soil pH. In the past few years, a number of compounds have been proposed as urease inhibitors to tackle such problems [6–9]. The treatment of infections caused by urease-producing bacteria may also be possible by urease inhibition.

The important class of compounds formed by 1,2,4-triazole and its derivatives exhibits a broad spectrum of biological activities depending on the substitution pattern around the ring [10–15]. Recently, some triazoles have been reported as urease inhibitors [16]. Among triazoles, 1,2,4-triazole-3-thiones may behave as important pharmacophores for urease inhibition due to their structural similarity to the natural substrate of urease, i.e., urea. This aspect of 1,2,4-triazole-3-thiones has still not been explored fully, and only very few studies on the urease inhibition activities of these compounds have appeared in the literature [16, 17]. In addition, a number of such classes of compounds show other biological activities such as antifungal [18], anticonvulsant [13], anticancer [19], and antiviral [20]. In continuation of our work on the synthesis and biological evaluation of five-membered heterocycles [17, 21, 22], we report herein the synthesis, urease inhibition, and antimicrobial activities of some new 4-amino-5-aryl-2,4-dihydro-3*H*-1,2,4-triazole-3-thiones.

Results and discussion

The aryl hydrazides **3a**–**3l** were prepared from the corresponding benzoic acids **1a**–**1l** via esterification to **2a**–**2l**

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according to a reported procedure [23]. The structures of the aryl esters **2a–2l** and aryl hydrazides **3a–3l** were confirmed by comparison of their physical constants and infrared (IR) absorptions with reported values [23]. Treatment of the aryl hydrazides **3a–3l** with CS_2 in the presence of KOH, followed by cyclization with hydrazine hydrate [17], afforded the 4-amino-5-aryl-2,4-dihydro-3*H*-1,2,4-triazole-3-thiones **5a–5l**, as illustrated in Scheme 1.

The structures of 4-amino-5-aryl-2,4-dihydro-3*H*-1,2,4-triazole-3-thiones **5a–5l** were assigned by IR and nuclear magnetic resonance (NMR) spectra. In the IR spectra, the appearance of a comparatively weak band in the region 1,605–1,506 cm^{-1} for C=N, at the expense of strong absorption for the carbonyl of hydrazides, indicated the formation of triazoles. Furthermore, the appearance of a C=S absorption band in the region 1,352–1,309 cm^{-1} indicated that the triazoles are in their thione form. In ^{1}H NMR spectra, a broad downfield singlet in the region $\delta = 12.81$–14.03 ppm was assigned to the NH proton. A signal in the region 4.81–5.80 ppm, integrating to two protons, was assigned to NH_2 protons. The signals corresponding to the four aromatic protons were observed with different multiplicities, confirming the *o*-, *m*-, and *p*-substitutions on the ring. In ^{13}C NMR spectra, the signals in the regions $\delta = 177.6$–166.2 and 161.9–147.1 ppm were assigned to C-3 and C-5 of the triazole nucleus, respectively. Four or six signals for aromatic carbons were observed due to substitution on the benzene ring. In case of compounds **5d–5f** with a fluorine atom as a substituent, the doublets observed at 160.5 (**5d**), 162.4 (**5e**), and 163.7 (**5f**) ppm with large C–F coupling were assigned to C-2, C-3, and C-4 in the corresponding compounds. The structures were further confirmed by mass-spectral analysis. The molecular ion peak was observed for all the compounds.

Table 1 Urease inhibition activities of compounds **5a–5l**

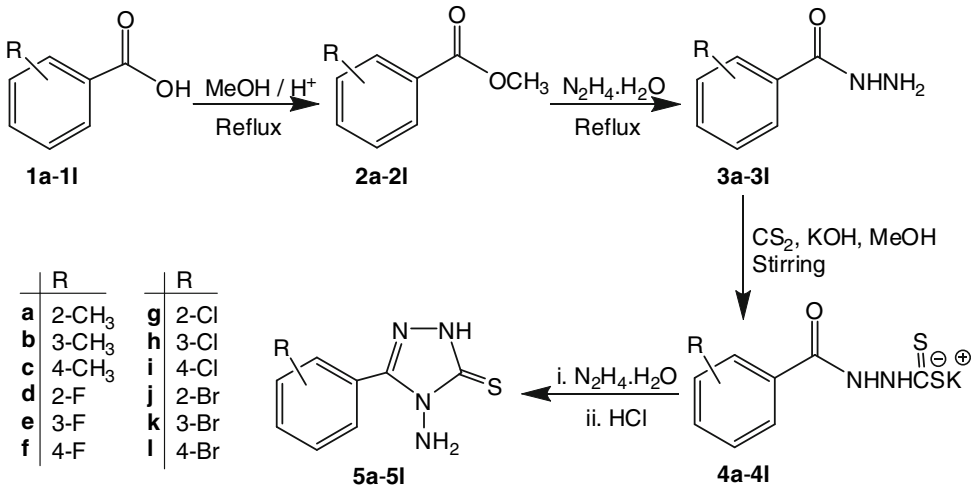
Compounds	Inhibition (%)	$IC_{50} \pm$ SEM (μM)
5a	70.9	321.1 ± 0.19
5b	88.4	44.96 ± 1.82
5c	99.0	25.9 ± 0.65
5d	99.0	44.9 ± 0.74
5e	96.9	69.9 ± 0.47
5f	77.3	41.7 ± 0.081
5g	96.5	73.3 ± 0.34
5h	98.2	26.7 ± 0.027
5i	95.1	89.7 ± 0.25
5j	97.4	17.5 ± 0.52
5k	93.6	29.1 ± 0.919
5l	98.8	4.3 ± 0.169

Urease inhibition activity

Compounds **5a–5l** were screened for urease inhibition activity against jack bean urease and exhibited remarkable inhibition (Table 1).

Compound **5l** was found to be the most potent ($IC_{50} = 4.3 \pm 0.16$ μM) of the series, with higher activity than the standard thiourea ($IC_{50} = 21.0 \pm 0.11$ μM). Such activity might be explained in term of the 4-bromophenyl residue at position 5 of the triazole nucleus. Similarly, compound **5j** with a 2-bromophenyl substituent at position 5 of the triazole nucleus exhibited more potency ($IC_{50} = 17.5 \pm 0.52$ μM) than the standard. The activities of compounds **5c** ($IC_{50} = 25.9 \pm 0.65$ μM), **5h** ($IC_{50} = 26.7 \pm 0.027$ μM), and **5k** ($IC_{50} = 29.1 \pm 0.919$ μM) were also comparable to that of the standard. Compounds **5b**, **5d**, and **5f** also exhibited activity against urease, with IC_{50} values of 44.96 ± 1.82, 44.9 ± 0.74, and 41.7 ± 0.081 μM,

Scheme 1



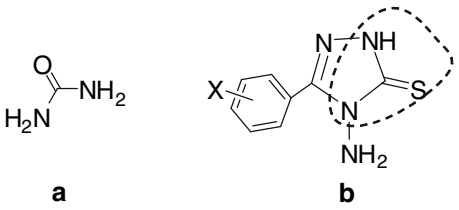


Fig. 1 Representation of the possible binding site to urease: (**a**) natural substrate of urease, i.e., urea, and (**b**) 4-amino-5-aryl-2,4-dihydro-3*H*-1,2,4-triazole-3-thiones

respectively. The activity of compounds **5e**, **5g**, and **5i** was moderate, with $IC_{50} = 69.9 \pm 0.47$, 73.3 ± 0.34, and 89.7 ± 0.25 μM, respectively, while **5a** showed poor activity ($IC_{50} = 321.1 \pm 0.196$ μM).

In general, it was observed that compounds **5j–5l** with bromophenyl substituents are more active than other analogues. Similarly, with the exception of the chlorophenyl-substituted triazoles (**5g–5i**), compounds with substitution at position 4 of the phenyl ring are more potent than their 2- and 3-analogues.

A part of the triazole nucleus has structural similarity with the natural substrate of urease, i.e., urea (Fig. 1) [24]. It appears that the aryl group, bearing electron-withdrawing or electron-donating groups, affects the binding of these molecules with the enzyme active site. This is also augmented by comparison of these results with the activity obtained for the 4-aminotriazoles with an alkyl substituent at position 5 of the nucleus [17].

It may be concluded on the basis of the observed results that the 4-amino group may not have any significant effect on urease inhibition. This may be the case as a 4-amino group is present in all compounds and the activity varied significantly with the change of aryl group. Probably, the aryl group is responsible for the enhancement or diminution of the activity, as the corresponding 5-alkyl compounds did not exhibit very good inhibition [17]. The planarity of the aryl group may increase or decrease the electron density on the triazole nucleus through conjugation and hence affect the binding of the molecule with the enzyme active site.

These preliminary investigations suggest that, among the investigated groups, only the bromo substituent has a significant effect on activity. It is also apparent that the major effect on the activity is that of the triazole nucleus, while the aryl group contributes in other ways, through either steric or electronic factors, as the corresponding hydrazides (**3a–3c** and **3g–3i**) [25] are inactive against jack bean urease. Only the hydrazides **3e**, **3f**, and **3j–3l** exhibited some activity. These results reveal that compounds **5j** and **5l** may serve as important and selective candidates for further investigations and structural modifications. These observations may also be applied to the development of antiulcer drugs.

Antimicrobial screenings

Compounds **5a–5l** were screened against different fungal and bacterial strains. Six fungal strains: *Trichphyton longifusus*, *Candida albicans*, *Aspergillus flavus*, *Microsporum canis*, *Fusarium solani*, and *Candida glabrata* were used. Only compound **5h** exhibited significant activity against *M. canis* [50% inhibition, 200 μg/cm^3 of dimethyl sulfoxide (DMSO), 7 days at 27 ± 1 °C]. All other compounds were found to be inactive at nontoxic concentrations. The compounds were also tested against six bacterial strains: *Escherichia coli*, *Bacillus subtillus*, *Shigella flexenari*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Salmonella typhi*, but none of the compounds was found to be active at nontoxic concentrations.

Experimental

The melting points of the compounds were determined using a Stuart SMP3 melting point apparatus. The infrared spectra (KBr discs) were recorded on a FTS 3000 MX, Bio-RAD Merlin (Excalibur model) spectrophotometer. The ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance 300-MHz NMR spectrophotometer, and signals were calibrated to the residual signal of the solvent. Coupling constants are reported in Hz. Electron ionization mass spectroscopy (EIMS) was carried out using Agilent Technologies 6890 N (GC) and an inert selective detector 5973 mass spectrometer.

General procedure for synthesis of 4-amino-5-aryl-2,4-dihydro-3H-1,2,4-triazole-3-thiones ***5a–5l***

The hydrazide (0.04 mol) and KOH (0.04 mol) in 50 cm^3 MeOH were treated with CS_2 (0.04 mol), and the mixture was stirred for 12–16 h at room temperature. Diethyl ether (50 cm^3) was added, and the precipitated solid was filtered, washed with ether, and vacuum-dried at 78 °C in a drying pestle. The potassium salts of substituted dithiocarbazinic acids were used for the next step without further purification.

The potassium salt of the substituted dithiocarbazinic acid (0.02 mol) and hydrazine hydrate (0.04 mol) in 2.0 cm^3 water were heated under reflux with stirring for 0.5–1.5 h. The color of the reaction mixture changed to green with the evolution of hydrogen sulfide, and a homogeneous solution was formed in half an hour. When evolution of hydrogen sulfide ceased (lead acetate test), the reaction mixture was diluted with 50 cm^3 cold water and

acidified with 6 N hydrochloric acid. The precipitated solid was filtered, washed with cold water, and recrystallized from aqueous EtOH.

4-Amino-2,4-dihydro-5-(2-methylphenyl)-3H-1,2,4-triazole-3-thione (**5a**, $C_9H_{10}N_4S$)
Yield 76%; m.p.: 205–207 °C; ^{1}H NMR ($CDCl_3$, 300 MHz): $\delta = 2.65$ (s, 3H, CH_3), 4.81 (s, 2H, NH_2), 7.33–7.38 (m, 2H, Ar–H), 7.47 (m, 1H, Ar–H), 7.93 (m, 1H, Ar–H), 12.93 (s, 1H, NH) ppm; ^{13}C NMR ($CDCl_3$, 75 MHz): $\delta = 22.0$ (CH_3), 121.1, 126.4, 128.7, 131.9, 132.1, 138.4 (6 × C-arom.), 161.9 (C-5), 177.6 (C-3) ppm; IR (KBr): $\bar{\nu} = 3{,}285, 3{,}118, 2{,}951, 1{,}506, 1{,}339, 1{,}194$ cm^{-1}; MS (EI): *m/z* (%) = 206 ($M^{+\cdot}$, 100), 191 (20), 135 (45), 118 (37), 102 (15), 91 (28), 65 (4), 60 (23).

4-Amino-2,4-dihydro-5-(3-methylphenyl)-3H-1,2,4-triazole-3-thione (**5b**, $C_9H_{10}N_4S$)
Yield 69%; m.p.: 206–208 °C; ^{1}H NMR ($CDCl_3$, 300 MHz): $\delta = 2.42$ (s, 3H, CH_3), 5.51 (s, 2H, NH_2), 7.36–7.45 (m, 2H, Ar–H), 7.96–7.98 (m, 2H, Ar–H), 12.84 (s, 1H, NH) ppm; ^{13}C NMR ($CDCl_3$, 75 MHz): $\delta = 20.5$ (CH_3), 125.3, 126.2, 128.3, 128.5, 131.1, 138.1 (6 × C-arom.), 149.7 (C-5), 166.2 (C-3) ppm; IR (KBr): $\bar{\nu} = 3{,}287, 3{,}108, 2{,}947, 1{,}532, 1{,}340, 1{,}179$ cm^{-1};; MS (EI): *m/z* (%) = 206 ($M^{+\cdot}$, 100), 191 (20), 175 (5), 135 (38), 118 (42), 102 (12), 91 (30), 65 (10), 60 (15).

4-Amino-2,4-dihydro-5-(4-methylphenyl)-3H-1,2,4-triazole-3-thione (**5c**, $C_9H_{10}N_4S$)
Yield 72%; m.p.: 201 °C; ^{1}H NMR ($(CD_3)_2CO$, 300 MHz): $\delta = 2.41$ (s, 3H, CH_3), 5.49 (s, 2H, NH_2), 7.83 (d, 2H, $J = 8.4$ Hz, Ar–H), 8.01 (d, 2H, $J = 8.4$ Hz, Ar–H), 12.81 (s, 1H, NH) ppm; ^{13}C NMR ($(CD_3)_2CO$, 75 MHz): $\delta = 20.7$ (CH_3), 120.2, 126.1, 129.9, 142.9 (4 × C-arom.), 161.1 (C-5), 178.2 (C-3) ppm; IR (KBr): $\bar{\nu} = 3{,}287, 3{,}116, 2{,}946, 1{,}515, 1{,}352, 1{,}176$cm^{-1}; MS (EI): *m/z* (%) = 206 ($M^{+\cdot}$, 100), 191 (2), 160 (3), 135 (42), 118 (40), 102 (3), 91 (18), 77 (3), 65(5), 60 (14).

4-Amino-5-(2-fluorophenyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione (**5d**, $C_8H_7FN_4S$)
Yield 64%; m.p.: 170–171 °C; ^{1}H NMR ($(CD_3)_2CO$, 300 MHz): $\delta = 5.35$ (s, 2H, NH_2), 7.34–7.43 (m, 2H, Ar–H), 7.67 (m, 1H, Ar–H), 7.77 (dt, 1H, $J = 7.5$, 1.5 Hz, Ar–H), 12.91 (s, 1H, NH) ppm; ^{13}C NMR ($(CD_3)_2CO$, 75 MHz): $\delta = 114.4$ (d, $J_{C,F} = 19.3$ Hz, C^3-arom.), 115.9 (d, $J_{C,F} = 20.2$ Hz, C^1-arom.), 124.4 (d, $J_{C,F} = 3.8$ Hz, C^5-arom.), 131.7 (d, $J_{C,F} = 2.2$ Hz, C^6-arom.), 133.0 (d, $J_{C,F} = 8.25$ Hz, C^4-arom.), 147.1 (C-5), 160.5 (d, $J_{C,F} = 249.7$ Hz, C^2-arom.), 168.2 (C-3) ppm; IR (KBr): $\bar{\nu} = 3{,}312, 3{,}171, 1{,}574, 1{,}316, 1{,}236$cm^{-1}; MS (EI): *m/z* (%) = 210 ($M^{+\cdot}$, 100), 190 (2), 139 (13), 122 (33), 102 (11), 95 (8), 75 (7), 60 (20).

4-Amino-5-(3-fluorophenyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione (**5e**, $C_8H_7FN_4S$)
Yield 72%; m.p.: 220 °C; ^{1}H NMR ($(CD_3)_2CO$, 300 MHz): $\delta = 5.53$ (s, 2H, NH_2), 7.34 (m, 1H, Ar–H), 7.79 (m, 1H, Ar–H), 8.01–8.04 (m, 2H, Ar–H), 12.94 (s, 1H, NH) ppm; ^{13}C NMR ($(CD_3)_2CO$, 75 MHz): $\delta = 114.7$ (d, $J_{C,F} = 24.7$ Hz, C^2-arom.), 117.2 (d, $J_{C,F} = 21.0$ Hz, C^4-arom.), 124.0 (d, $J_{C,F} = 3.0$ Hz, C^6-arom.), 128.2 (d, $J_{C,F} = 9.0$ Hz, C^5-arom.), 130.6 (d, $J_{C,F} = 8.3$ Hz, C^1-arom.), 148.4 (C-5), 162.4 (d, $J_{C,F} = 242.3$ Hz, C^3-arom.), 168.6 (C-3) ppm; IR (KBr): $\bar{\nu} = 3{,}288, 3{,}171, 1{,}536, 1{,}315, 1{,}192$cm^{-1}; MS (EI): *m/z* (%) = 210 ($M^{+\cdot}$, 100), 195 (2), 139 (25), 122 (34), 95 (23), 75 (8), 60 (12).

4-Amino-5-(4-fluorophenyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione (**5f**, $C_8H_7FN_4S$)
Yield 75%; m.p.: 208 °C; ^{1}H NMR (DMSO-d_6, 300 MHz): $\delta = 5.79$ (s, 2H, NH_2), 7.35–7.41 (m, 2H, Ar–H), 8.06–8.10 (m, 2H, Ar–H), 13.95 (s, 1H, NH) ppm; ^{13}C NMR (DMSO-d_6, 75 MHz): $\delta = 116.1$ (d, $J_{C,F} = 21.7$ Hz, C^3-arom., C^5-arom.), 122.7 (d, $J_{C,F} = 3.0$ Hz, C^1-arom.), 133.0 (d, $J_{C,F} = 9.0$ Hz, C^2-arom., C^6-arom.), 149.2 (C-5), 163.7 (d, $J_{C,F} = 246.8$ Hz, C^4-arom.), 167.4 (C-3) ppm; IR (KBr): $\bar{\nu} = 3{,}292, 3{,}113, 1{,}605, 1{,}309, 1{,}227$cm^{-1}; MS (EI): *m/z* (%) = 210 ($M^{+\cdot}$, 100), 195 (3), 139 (31), 122 (39), 95 (27), 75 (11), 60 (20).

4-Amino-5-(2-chlorophenyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione (**5g**, $C_8H_7ClN_4S$)
Yield 62%; m.p.: 170–172 °C; ^{1}H NMR (DMSO-d_6, 300 MHz): $\delta = 5.52$ (s, 2H, NH_2), 7.50 (dd, 1H, $J = 7.2$, 1.5 Hz, Ar–H), 7.57–7.66 (m, 3H, Ar–H), 13.99 (s, 1H, NH) ppm; ^{13}C NMR (DMSO-d_6, 75 MHz): $\delta = 125.7$, 127.6, 130.1, 132.8, 133.1, 133.9 (6 × C-arom.), 149.4 (C-5), 167.2 (C-3) ppm; IR (KBr): $\bar{\nu} = 3{,}285, 3{,}112, 2{,}946, 2{,}564, 1{,}539, 1{,}068$cm^{-1}; MS (EI): *m/z* (%) = 228 (M + 2, 23), 226 ($M^{+\cdot}$, 68), 191 (4), 157 (13), 155 (38), 140 (18), 138 (59), 113 (6), 111 (18), 102 (94), 89 (14), 75 (62), 60 (100).

4-Amino-5-(3-chlorophenyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione (**5h**, $C_8H_7ClN_4S$)
Yield 68%; m.p.: 215–217 °C; ^{1}H NMR (DMSO-d_6, 300 MHz): $\delta = 5.80$ (s, 2H, NH_2), 7.57 (dd, 1H, $J = 8.1$, 7.5 Hz, Ar–H), 7.62 (td, 1H, $J = 8.7$, 2.4 Hz, Ar–H), 7.97 (td, 1H, $J = 7.5$, 1.8 Hz, Ar–H), 8.14 (t, 1H, $J = 1.8$ Hz, Ar–H), 14.03 (s, 1H, NH) ppm; ^{13}C NMR (DMSO-d_6, 75 MHz): $\delta = 127.0$, 128.0, 128.1, 130.8, 131.0, 133.6 (6 × C-arom.), 148.6 (C-5), 167.7 (C-3) ppm; IR (KBr): $\bar{\nu} = 3{,}306, 3{,}104, 2{,}928, 1{,}535, 1{,}325, 1{,}080$cm^{-1}; MS (EI): *m/z* (%) = 228 (M + 2, 35), 226 ($M^{+\cdot}$, 100), 157 (8), 155 (25), 140 (11), 138 (34), 113 (10), 111 (25), 102 (24), 89 (8), 75 (25), 60 (43).

4-Amino-5-(4-chlorophenyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione (**5i**, $C_8H_7ClN_4S$)
Yield 64%; m.p.: 210–212 °C; ^{1}H NMR (DMSO-d_6, 300 MHz): $\delta = 5.79$ (s, 2H, NH_2), 7.56–7.63 (m, 2H, Ar–H), 8.04–8.08 (m, 2H, Ar–H), 13.98 (s, 1H, NH) ppm; ^{13}C NMR (DMSO-d_6, 75 MHz): $\delta = 125.1, 129.1, 130.2, 135.7$ (4 × C-arom.), 149.0 (C-5), 167.6 (C-3) ppm; IR (KBr): $\bar{\nu} = 3{,}246, 3{,}146, 2{,}932, 1{,}531, 1{,}314, 1{,}072\text{cm}^{-1}$; MS (EI): *m/z* (%) = 228 (M + 2, 36), 226 ($M^{+\cdot}$, 100), 157 (10), 155 (29), 140 (10), 138 (30), 113 (8), 111 (18), 102 (23), 75 (22), 60 (37).

4-Amino-5-(2-bromophenyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione (**5j**, $C_8H_7BrN_4S$)
Yield 69%; m.p.: 202 °C; ^{1}H NMR (DMSO-d_6, 300 MHz): $\delta = 5.50$ (s, 2H, NH_2), 7.47–7.60 (m, 3H, Ar–H), 7.79 (dd, 1H, $J = 7.8$, 1.8 Hz, Ar–H), 13.99 (s, 1H, NH) ppm; ^{13}C NMR (DMSO-d_6, 75 MHz): $\delta = 123.7, 127.8, 128.1, 132.9, 133.2, 135.4$ (6 × C-arom.), 150.5 (C-5), 167.1 (C-3) ppm; IR (KBr): $\bar{\nu} = 3{,}267, 3{,}125, 1{,}580, 1{,}326, 610\text{cm}^{-1}$; MS (EI): *m/z* (%) = 274 (M + 2, 98), 272 ($M^{+\cdot}$, 100), 241 (3), 239 (3), 201 (18), 199 (20), 184 (24), 182 (25), 158 (45), 102 (60), 75 (35), 60 (41).

4-Amino-5-(3-bromophenyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione (**5k**, $C_8H_7FN_4S$)
Yield 73%; m.p.: 212 °C; ^{1}H NMR ($(CD_3)_2CO$, 300 MHz): $\delta = 5.52$ (s, 2H, NH_2), 7.73–7.77 (m, 2H, Ar–H), 8.18 (td, 1H, $J = 7.8$, 1.8 Hz, Ar–H), 8.41 (dd, 1H, $J = 3.6$, 1.8 Hz, Ar–H), 12.94 (s, 1H, NH) ppm; ^{13}C NMR ($(CD_3)_2CO$, 75 MHz): $\delta = 121.8, 126.9, 128.3, 130.5, 130.6, 133.3$ (6 × C-arom.), 148.2 (C-5), 168.7 (C-3) ppm; IR (KBr): $\bar{\nu} = 3{,}271, 3{,}112, 1{,}577, 1{,}321, 596\text{cm}^{-1}$; MS (EI): *m/z* (%) = 274 (M + 2, 99), 272 ($M^{+\cdot}$, 100), 241 (2), 239 (3), 201 (21), 199 (23), 184 (17), 182 (18), 157 (7), 155 (7), 102 (37), 90 (8), 75 (17), 60 (39).

4-Amino-5-(4-bromophenyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione (**5l**, $C_8H_7BrN_4S$)
Yield 71%; m.p.: 205–206 °C; ^{1}H NMR ($(CD_3)_2CO$, 300 MHz): $\delta = 5.51$ (s, 2H, NH_2), 7.71–7.77 (m, 2H, Ar–H), 8.13–8.17 (m, 2H, Ar–H), 12.89 (s, 1H, NH) ppm; ^{13}C NMR ($(CD_3)_2CO$, 75 MHz): $\delta = 124.3, 125.4, 129.8, 131.6$ (4 × C-arom.), 148.8 (C-5), 168.6 (C-3) ppm; IR (KBr): $\bar{\nu} = 3{,}256, 3{,}104, 1{,}592, 1{,}317, 606\text{cm}^{-1}$; MS (EI): *m/z* (%) = 274 (M + 2, 98), 272 ($M^{+\cdot}$, 100), 241 (1), 239 (1), 201 (34), 199 (35), 184 (27), 182 (28), 157 (5), 155 (4), 102 (46), 90 (9), 75 (30), 60 (45).

Urease inhibition assay [17]

The reaction mixtures comprising 25 mm^3 jack bean urease solution, 55 mm^3 buffers, and 100 mM urea were incubated with 5 mm^3 (1 mM concentration) of the test compounds at 30 °C for 15 min in well plates. Measurement of ammonia production (indophenol method) [26] was used to determine urease activity. The phenol reagent (45 mm^3, 1% w/v phenol, and 0.005% w/v sodium nitroprusside) and alkali reagent (70 mm^3, 0.5% w/v sodium hydroxide, and 0.1% NaOCl) were added to each well, and the increasing absorbance at 630 nm was measured after 50 min, using a microplate reader (Molecular Device, USA). The change in absorbance per minute was noted, and results were processed using the SoftMax Pro software (Molecular Device, USA). All reactions were performed in triplicate. All assays were performed at pH 8.2 [0.01 M $K_2HPO_4{\cdot}3H_2O$, 1 mM ethylenediamine tetraacetic acid (EDTA), and 0.01 M $LiCl_2$]. Percentage inhibitions were calculated using the formula: $100 - (OD_{testwell}/OD_{control}) \times 100$. Thiourea was used as the standard inhibitor.

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