Syntheses, Urease Inhibition, and Antimicrobial Studies of Some Chiral 3-Substituted-4-amino-5-thioxo-1*H*,4*H*-1,2,4-triazoles

Tashfeen Akhtar[1], Shahid Hameed[1,*], Khalid Mohammed Khan[2] and Muhammad Iqbal Choudhary[2]

[1]*Department of Chemistry, Quaid-e-Azam University, Islamabad, 45320 Pakistan;* [2]*HEJ Research Institute of Chemistry, International Center for Chemical and Biological Sciences, University of Karachi, Karachi-75270 Pakistan*

Abstract: Chiral 3-substituted-4-amino-5-thioxo-1*H*,4*H*-1,2,4-triazoles (**5a-i**) were synthesized. The target molecules were prepared by cyclization of the corresponding dithiocarbazinic acids, obtained from hydrazides, in the presence of hydrazine hydrate. The chiral hydrazides were in turn synthesized form L-amino acids. The structures of all the compounds were confirmed by modern spectroscopic techniques and purity ascertained by elemental analysis. The synthesized compounds **5a-i** were evaluated for urease inhibition and found to exhibit varying degrees of urease inhibition activity showing IC_{50} values ranging from 22.0 ± 0.5 to 43.8 ± 0.3 μM. Compound **5b** was found to be the most active, exhibiting IC_{50} = 22.0 ± 0.5 μM comparable to the standard, thiourea (IC_{50} = 21.0 ± 0.1 μM). Triazoles **5a-i** were also screened for their antimicrobial properties and promising antibacterial activities were observed against five pathogenic bacteria. However, all the compounds were devoid of any antifungal activity.

Key Words: Chiral triazoles, synthesis, antibacterial activity, urease inhibition.

INTRODUCTION

Urease inhibitors are considered new targets for antiulcer drugs [1]. The activity of bacterial ureases has been shown to be important virulence factor in the development of many harmful clinical conditions for human and animal health as well as agriculture [2]. Bacterial ureases have been reported to be involved in the formation of infectious stones [3] and development of peptic ulcers and stomach cancer [4]. Ureases also contribute to the development of urolithiasis, pyelonephritis, hepatic encephalopathy, hepatic coma, and urinary catheter encrustation [5]. In the near past, a number of compounds have been proposed as urease inhibitors to reduce environmental problems and enhance the uptake of urea nitrogen by plants [6-9]. The treatment of infections caused by urease producing bacteria may also be possible by urease inhibition.

Chiral compounds are highly selective biological agents because of the specificity associated with enzymatic structure. Synthesis of chiral heterocyclic compounds especially chiral triazoles is not well known and only few examples are found in the literature [10-12]. 1,2,4-Triazole and its derivatives show a broad spectrum of biological activities depending on the substitution pattern around the ring [13, 14]. Recently, some triazoles have been reported as urease inhibitors [15]. The 3-substituted-4-amino-1,2,4-triazole-5-thions/thiols have also been used as intermediates in the synthesis of various condensed heterocyclic systems [16,17]. In the present paper we wish to report the synthesis, urease inhibition and antimicrobial activities of some chiral 3-substituted-4-amino-5-thioxo-1*H*,4*H*-1,2,4-triazoles (**5a-i**). The structures of the synthesized compounds were established by modern spectroscopic techniques and their purity verified by elemental analysis.

*Address correspondence to this author at the Department of Chemistry, Quaid-e-Azam University, Islamabad, 45320 Pakistan; Tel: 0092-51-90642133; Fax: 0092-51-90642241; E-mail: shameed@qau.edu.pk

RESULTS AND DISCUSSION

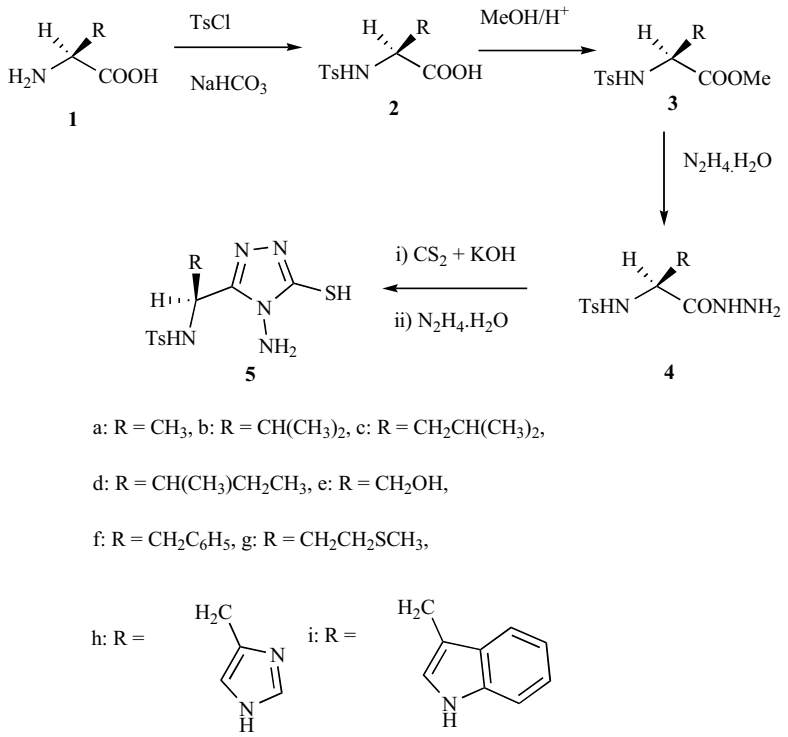
Chemistry

In order to synthesize the desired compounds, L-amino acids (**1a-i**) with different substitution pattern were selected. The acids were converted to their methyl esters (**3a-i**) after protection of the amino group [18]. The resulting esters were treated with 80% hydrazine hydrate to give the respective hydrazides (**4a-i**), which were then converted [19] to 3-substituted-4-amino-5-thioxo-1*H*,4*H*-1,2,4-triazoles (**5a-i**) by the reaction with CS_2 in presence of KOH followed by the addition of hydrazine hydrate as depicted in Scheme **1**.

The cyclization of the hydrazides (**4**) to triazoles (**5**) was indicated in the IR spectra by appearance of the absorption bands for C=N linkage in the region from 1597-1553 cm^{-1} at the expense of the strong carbonyl absorption in hydrazides. The cyclization was confirmed in the ^{1}H-NMR spectra by presence of the signals for three N-H protons (instead of four in hydrazides). Two signals for the iminium C-atoms were also observed in the ^{13}C-NMR spectra. In the mass spectra, the molecular ion peak was not observed for some of the synthesized compounds. The common fragment observed in all the compounds was at *m/z* 155 resulting by the cleavage of the sulfonamide linkage, followed by the loss of SO_2 to generate a fragment at *m/z* 91, observed as the base peak in all of the compounds except **5i**.

To check the possibility of racemization during the synthesis, the optical activity of all the compounds **5a-i** was measured. All the compounds were found to be optically active indicating that racemization, if occurred at all, was only partial. To ensure the optical purity of the compounds **5a-i**, the ^{1}H-NMR spectroscopy using lanthanide shift reagent Eu(hfc)$_3$ was used to determine the enantiomeric ratios. It was observed that, although enantiomerically pure amino acids have been employed as the starting materials, the enantiomeric ratios were 87-94 : 6-13 in the final products. It was

1573-4064/08 $55.00+.00



Scheme 1. Synthesis of 4-Aminotriazoles.

observed that these ratios were directly related to the reaction time. It is suggested that the reaction time must not be too long to avoid complete racemization.

BIOLOGY

Urease Inhibition Activity

The urease inhibition activity was carried out according to the literature protocol [20] using thiourea as the standard inhibitor having an IC_{50} value of 21.0 ± 0.1 μM. The results are presented in Table **1**.

Table 1. The Urease Inhibitory Activity of Triazoles

S. No.	Compound	IC_{50} ± S.E.M.
1	5a	35.9 ± 0.5
2	5b	22.0 ± 0.5
3	5c	33.5 ± 0.3
4	5e	33.6 ± 0.5
5	5g	43.8 ± 0.3
6	5i	29.7 ± 0.2
7	Thiourea	21.0 ± 0.1

All the scanned compounds exhibited good urease inhibition activity. Compound **5b** proved to be the most potent showing an enzyme inhibition activity with an IC_{50} = 22.0 ± 0.5 μM which is comparable to 21.0 ± 0.1μM of the standard. The compound **5i** also exhibited a good activity with an IC_{50} value of 29.7 ± 0.2 μM. The least active compound **5g** had an IC_{50} = 43.8 ± 0.3 μM, the activity of the rest of the compounds fall in the range 33.5-35.9 μM. From these results of urease inhibition activity, following tentative results can be drawn; it appears that there is no effect of the bulk of 'R' group (at chiral centre) on the activity of these compounds. Since all the synthesized chiral triazoles exhibited promising urease inhibitory activity, this may be due to their basic skeleton. It is suggested that some careful structural modifications like substitution either on free mercapto or amino group or on both simultaneously may escort to a potent lead molecule for future research in the field of urease inhibition. Of this series, the most active compound **5b** may act as a potential molecule for the structural modifications.

Antimicrobial Activity

The synthesized compounds **5a-5i** were tested for antibacterial activity at a sample concentration of 5 mg/mL of DMSO against five bacterial strains, *i.e. Escheria coli, Micrococcus luteus, Pseudomonas pickettii, Bordetella bronchseptica and Enterococcus avium*. The activity of the compounds **5e** and **5g-5i** was comparable to the standards (Roxy) against all the tested bacterial strains except *Pseudomonas pickettii* and *Bordetella bronchseptica*. The antibacterial activity data is tabulated in Table **2**.

The compounds **5d**, **5e** and **5g-5i** were also tested for their antifungal activity at sample concentration of 200 μg/mL of DMSO for 7 days at 27 °C against *Trichphyton longifusus, Candida albicans, Aspergillus flavus, Microsporum canis, Fusarium solani,* and *Candida glabrata* fungal strains but no significant activity was observed.

Table 2. Antibacterial Activity of Triazoles

Comp.	*Escheria Coli* (24/48h)	*Micrococcus Luteus* (24/48h)	*Pseudomonas Pickettii* (24/48h)	*Bordetella Bronchiseptica* (24/48h)	*Enterococcus Avium* (24/48h)
	(Zone of inhibition in mm)				
5a	9/Nil	Nil/Nil	Nil/Nil	Nil/ Nil	Nil/Nil
5c	Nil/Nil	Nil/Nil	Nil/Nil	Nil/Nil	Nil/Nil
5d	11/10.5	9/Nil	Nil/Nil	13/13	Nil/Nil
5e	14.5/13.5	13/14	10/12	18/17	12/12
5g	14.5/14.5	13/12.5	Nil/Nil	17/16	12/11.5
5h	14/14.5	13/13	9.5/10	16/16	10.5/11
5i	13.5/13	11/10	Nil/Nil	13/11	10/10
Roxy	13.5/17	10/11	22/27	24/22	12/12

CONCLUSION

All the synthesized compounds were scanned for urease inhibition and exhibited promising urease inhibitory activity which may be due to their basic skeleton. It was observed that the size of 'R' group had no effect on the enzyme inhibitory activity.

EXPERIMENTAL SECTION

Material and Instruments

The melting points were determined on Sanyo Gallenkamp melting point apparatus in open capillaries and are uncorrected. UV spectra were recorded on Lambda20, Perkin Elmer spectrophotometer. Specific rotation $[\alpha]_D$ was measured on ATAGO AP-100 automatic polarimeter. IR spectra were recorded on FTX 3000 MX BioRad Excalibar Series IR spectrophotometer using KBr pellets. The NMR spectra were recorded on Bruker 500 MHz and Bruker 300 MHz spectrometers. Mass spectra were measured on a MAT-112-S spectrometer at 70 eV. The elemental analysis was performed on Leco CHNS-932 Elemental Analyzer, Leco Corporation (USA). The reagents used were of analytical grade while the solvents were purified before use.

Urease Assay and Inhibition

The reaction mixtures comprising 25 μL of Jack bean Urease solution, 55 μL of buffers and 100 mM urea were incubated with 5 μL (1 mM conc.) of the test compounds at 30 $^{\circ}$C for 15 min in well plates. The measurement of ammonia production (indophenol method) [20] was used to determine the urease activity. The phenol reagent (45 μL, 1% w/v phenol and 0.005% w/v sodium nitroprusside) and alkali reagent (70 μL, 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 min was noted and the results processed using SoftMax Pro software (Molecular Device, USA). All the reactions were performed in triplicate. All the assays were performed at pH 8.2 (0.01 M $K_2HPO_4.3\ H_2O$, 1 mM EDTA and 0.01 M $LiCl_2$). The percentage inhibitions were calculated from the formula $100 - (OD_{testwell}/OD_{control}) \times 100$. Thiourea was used as the standard inhibitor.

General Procedure for the Syntheses of Hydrazides (4a-i)

The hydrazides (**4a-i**) were synthesized from the esters (**3a-i**). The esters (**3a-i**) in turn were synthesized from the selected amino acids (**1a-i**) after their conversion to *p*-toluenesulfonyl derivatrives [19]. The hydrazides were characterized by comparison of their physical constants and spectral data with literature values [21].

General Procedure for the Syntheses of 3-substituted-4-amino-5-thioxo-1H,4H-1,2,4-triazoles (5a-i)

A soln. of KOH (0.04 moles), MeOH (100 ml) and the respective hydrazide (0.04 moles) was treated with CS_2 (0.04 moles). The mixture was diluted with 150 ml of MeOH and stirred for 12-16 h at r.t. Diethyl ether (200 ml) was added and the precipitated solid was filtered, washed with Et_2O and vacuum dried at 78 $^{\circ}$C in drying pestle. The potassium salts of substituted dithiocarbazinic acids were obtained in nearly quantitative yields and used in the next step of cyclization without further purification.

A suspension of the potassium salt of substituted dithiocarbazinic acid (0.02 moles), hydrazine hydrate (0.04 moles) and 2.0 ml of H_2O was refluxed with stirring for 0.5-1.5 h. The color of the mixture changed to green with the evolution of H_2S and a homogeneous soln. was formed. When the evolution of H_2S ceased (lead acetate test), the reaction mixture was diluted with 100 ml of cold H_2O and acidified with conc. HCl resulting in the precipitation of a solid mass. The product was filtered, washed with cold H_2O and recrystallized from aq. EtOH.

4-Amino-3-[1-(4-methylbenzenesulfonamido)ethyl]-5-thioxo-1H,4H-1,2,4-triazole (5a)

Yield: 75%; mp 210-212 $^{\circ}$C; $[\alpha]_D^{25}$ = +35.38 (c 0.85, acetone). UV (λ_{max}) 227, 254. IR (KBr) ν_{max} 3387, 3289, 3041, 2925, 1597, 1317, 1155. ^{1}H-NMR (500 MHz, *methanol-d$_4$*) δ 1.45 (d, 3H, *J* = 7.1 Hz), 2.36 (s, 3H), 4.66 (q, 1H, *J*

= 7.3 Hz), 7.25 (d, 2H, *J* = 8.1 Hz), 7.59 (d, 2H, *J* = 8.2 Hz). ^{13}C-NMR (75 MHz, *acetone-d*$_6$) δ 167.89, 151.52, 143.47, 137.69, 129.24, 126.67, 45.35, 20.61, 19.27. EIMS (*m/z*) 313 (M^+, 17.1), 249 (2.1), 171 (3.2), 155 (16.6), 142 (24.8), 116 (5.0), 91 (100.0), 65 (38.3). Anal. calcd. for $C_{11}H_{15}N_5O_2S_2$: C 42.16; H 4.82; N 22.35; S 20.46; Found: C 42.26; H 4.73; N 22.23; S 20.06.

4-Amino-3-[1-(4-methylbenzenesulfonamido)-2-methylpropyl]-5-thioxo-1H,4H-1,2,4-triazole (5b)

Yield: 71%; mp 162-164 °C; $[\alpha]_D^{25}$ = +31.08 (c 0.50, MeOH). UV (λ_{max}) 228, 253. IR (KBr) ν_{max} 3415, 3267, 3041, 2956, 1553, 1369, 1153. ^{1}H-NMR (300 MHz, *methanol-d*$_4$) δ 0.82 (d, 3H, *J* = 6.6 Hz), 1.05 (d, 3H, *J* = 6.6 Hz), 2.20-2.08 (m, 1H), 2.39 (s, 3H), 4.25 (d, 1H, *J* = 8.4 Hz), 7.23 (d, 2H, *J* = 8.1 Hz), 7.56 (d, 2H, *J* = 8.1 Hz). ^{13}C-NMR (75 MHz, *methanol-d*$_4$) δ 166.98, 150.86, 143.72, 136.94, 128.96, 126.42, 55.0, 31.68, 20.25, 18.06, 17.89. EIMS (*m/z*) 341 (M^+, 28.2), 298 (47.8), 171 (6.2), 155 (40.1), 91 (100.0), 65 (18.7). Anal. calcd. for $C_{13}H_{19}N_5O_2S_2$: C 45.73; H 5.61; N 20.51; S 18.78; Found: C 45.66; H 5.68; N 20.60; S 18.90.

4-Amino-3-[1-(4-methylbenzenesulfonamido)-3-methylbutyl]-5-thioxo-1H,4H-1,2,4-triazole (5c)

Yield: 67%; mp 178-179 °C; $[\alpha]_D^{25}$ = +23.16 (c 0.50, MeOH). UV (λ_{max}) 228, 253. IR (KBr) ν_{max} 3345, 3279, 3057, 2965, 1567, 1372, 1158. ^{1}H-NMR (500 MHz, DMSO-*d*$_6$) δ 0.72 (d, 3H, *J* = 6.1 Hz), 0.79 (d, 3H, *J* = 6.1 Hz), 1.54-1.47 (m, 3H), 2.32 (s, 3H), 4.46 (dd, 1H, *J* = 7.5, 8.0 Hz), 5.30 (bs, 2H), 7.25 (d, 2H, *J* = 8.0 Hz), 7.54 (d, 2H, *J* = 8.1 Hz), 8.18 (s, 1H), 13.32 (s, 1H). ^{13}C-NMR (75 MHz, DMSO-*d*$_6$) δ 166.26, 151.73, 142.65, 137.46, 129.08, 126.46, 46.63, 41.82, 23.94, 22.47, 21.09, 20.98. EIMS (*m/z*) 355 (M^+, 14.1), 298 (5.9), 184 (8.5), 171 (11.9), 155 (30.3), 91 (100.0), 65 (25.0), 57 (3.9). Anal. calcd. for $C_{14}H_{21}N_5O_2S_2$: C 47.30; H 5.95; N 19.70; S 18.04; Found: C 47.17; H 6.01; N 19.53; S 17.91.

4-Amino-3-[1-(4-methylbenzenesulfonamido)-2-methylbutyl]-5-thioxo-1H,4H-1,2,4-triazole (5d)

Yield: 59%; mp 144-146 °C; $[\alpha]_D^{25}$ = +28057 (c 0.69, MeOH). UV (λ_{max}) 227, 254. IR (KBr) ν_{max} 3341, 3299, 3194, 2963, 1548, 1383, 1159. ^{1}H-NMR (500 MHz, DMSO-*d*6) δ 0.69 (dd, 3H, *J* = 8.0, 6.9 Hz), 0.71 (d, 3H, *J* = 7.4 Hz), 0.98 (m, 1H), 1.42 (m, 1H), 1.51 (m, 1H), 2.35 (s, 3H), 3.39 (t, 1H, *J* = 8.7 Hz), 5.25 (bs, 2H), 7.31 (d, 2H, *J* = 8.1 Hz), 7.61 (d, 2H, *J* = 8.1 Hz), 8.96 (bs, 1H), 12.68 (s, 1H). ^{13}C-NMR (75 MHz, DMSO-*d*$_6$) δ 169.32, 151.67, 142.26, 138.47, 128.96, 126.87, 59.09, 36.57, 24.57, 21.06, 15.60, 14.87. EIMS (*m/z*): 240 (42.1), 184 (7.4), 155 (43.2), 91 (100.0), 65 (21.0), 57 (9.6). Anal. calcd. for $C_{14}H_{21}N_5O_2S_2$: C 47.30; H 5.95; N 19.70; S 18.04; Found: C 47.23; H 5.87; N 19.59; S 17.86.

4-Amino-3-[1-(4-methylbenzenesulfonamido)-2-hydroxyethyl]-5-thioxo-1H,4H-1,2,4-triazole (5e)

Yield: 52%; mp 208 °C; $[\alpha]_D^{25}$ = +52.83 (c 1.27, MeOH). UV (λ_{max}) 228, 253. IR (KBr) ν_{max} 3426, 3262, 3037, 2930, 1560, 1327, 1161. ^{1}H-NMR (500 MHz, DMSO-*d*6) δ 2.33 (s, 3H), 3.54 (dd, 1H, *J* = 10.1, 6.4 Hz), 3.62 (dd, 1H, *J* = 10.0, 7.5 Hz), 4.47 (dd, 1H, *J* = 14.8, 7.2 Hz), 5.25 (s, 2H), 7.27 (d, 2H, *J* = 8.0 Hz), 7.59 (d, 2H, *J* = 8.1 Hz), 8.1 (bs, 1H), 13.40 (s, 1H). ^{13}C-NMR (75 MHz, DMSO-*d*$_6$) δ 166.17, 149.86, 142.71, 137.53, 129.14, 126.52, 61.64, 50.47, 21.11. EIMS (*m/z*): 329 (M^+, 4.8), 298 (8.7), 171 (9.9), 155 (23.6), 144 (14.2), 128 (2.7), 91 (100.0), 69 (2.8), 65 (36.9). Anal. calcd. for $C_{11}H_{15}N_6O_3S_2$: C 40.11; H 4.59; N 21.26; S 19.47; Found: C 39.90; H 4.63; N 21.13; S 19.83.

4-Amino-3-[1-(4-methylbenzenesulfonamido)-2-phenylethyl]-5-thioxo-1H,4H-1,2,4-triazole (5f)

Yield: 62%; mp 200-202 °C; $[\alpha]_D^{25}$ = +42.16 (c 1.01, acetone). UV (λ_{max}) 228, 254. IR (KBr) ν_{max} 3412, 3278, 3034, 2928, 1578, 1340, 1158. ^{1}H-NMR (300 MHz, *acetone-d*$_6$) δ 2.25 (s, 3H), 2.99-3.17 (m, 1H), 4.46-4.52 (m, 1H), 4.68-4.61 (m, 1H), 4.81 (bs, 2H), 6.97 (bs, 1H), 7.03-7.10 (m, 3H), 7.16 (d, 2H, *J* = 7.8 Hz), 7.42 (d, 2H, *J* = 8.1 Hz), 7.49 (d, 2H, *J* = 8.4 Hz). ^{13}C-NMR (75 MHz, *acetone-d*$_6$) δ 168.32, 149.26, 144.21, 137.10, 130.54, 130.31, 129.42, 128.14, 127.79, 127.28, 51.76, 32.34, 21.52. EIMS (*m/z*) 389 (M^+, 2.2), 298 (11.5), 274 (8.6), 155 (23.4), 91 (100.0), 77 (4.0), 65 (21.5). Anal. calcd. for $C_{17}H_{19}N_5O_2S_2$: C 52.43; H 4.92; N 17.99; S 17.05; Found: C 52.29; H 4.74; N 17.79; S 17.16.

4-Amino-3-[1-(4-methylbenzenesulfonamido)-3-(methylthio) propyl]-5-thioxo-1H,4H-1,2,4-triazole (5g)

Yield: 69%; mp 176-177 °C; $[\alpha]_D^{25}$ = +56.39 (c 1.35, MeOH). UV (λ_{max}) 228, 253. IR (KBr) ν_{max} 3320, 3253, 3045, 2928, 1567, 1383, 1163. ^{1}H-NMR (500 MHz, methanol-d$_4$) δ 2.02 (s, 3H), 2.37 (s, 3H), 2.04-2.10 (m, 2H), 2.44-2.54 (m, 2H), 4.75 (dd, 1H, J = 7.9, 6.9 Hz), 7.23 (d, 2H, J = 8.0 Hz), 7.57 (d, 2H, J = 8.2 Hz). ^{13}C-NMR (75 MHz, acetone-d$_6$) δ 168.15, 150.52, 143.51, 137.68, 129.21, 127.12, 48.46, 32.90, 20.70, 20.62, 14.17. EIMS (m/z) 218 (23.2), 171 (15.3), 155 (22.7), 102 (2.7), 91 (100.0), 75 (7.6), 65 (31.9), 61(38.4). Anal. calcd. for $C_{13}H_{19}N_5O_2S_3$: C 41.80; H 5.13; N 18.75; S 25.75; Found: C 41.89; H 5.11; N 18.79; S 25.69.

4-Amino-3-[1-(4-methylbenzenesulfonamido)-2-(1H-imidazol-4-yl)ethyl]-5-thioxo-1H,4H-1,2,4-triazole (5h)

Yield: 73%; mp 238 °C; $[\alpha]_D^{25}$ = +77.91 (c 1.87, acetone). UV (λ_{max}) 228, 266. IR (KBr) ν_{max} 3343, 3231, 3044, 2927, 1561, 1319, 1157. ^{1}H-NMR (500 MHz, DMSO-d6) δ 2.32 (s, 3H), 2.87 (dd, 1H, J = 7.3, 14.4 Hz), 3.23 (dd, 1H, J = 8.0, 14.4 Hz), 4.68 (dd, 1H, J = 6.6, 6.8 Hz), 5.25 (bs, 2H), 6.68 (s, 1H), 7.23 (d, 2H, J = 8.0 Hz), 7.43 (s, 1H), 7.49 (d, 2H, J = 8.0 Hz), 8.25 (bs, 1H), 11.72 (bs, 1H), 13.30 (bs, 1H). ^{13}C-NMR (75 MHz, acetone-d$_6$) δ 168.06, 151.36, 144.26, 138.32, 136.07, 135.70, 130.08, 128.03, 127.71, 50.71, 32.42, 21.54. EIMS (m/z): 246 (2.5), 171 (8.7), 155 (8.1), 107 (11.6), 91 (100.0), 89 (10.3), 81 (3.4), 65 (2.9). Anal. calcd. for $C_{14}H_{17}N_7O_2S_2$: C 44.31; H 4.52; N 25.84; S 16.90; Found: C 44.40; H 4.61; N 25.91;S 16.83.

4-Amino-3-[1-(4-methylbenzenesulfonamido)-2-1H-indolylethyl]-5-thioxo-1H,4H-1,2,4-triazole (5i)

Yield: 56%; mp 248-249 °C; $[\alpha]_D^{25}$ = +34.64 (c 0.82, acetone). UV (λ_{max}) 227, 253. IR (KBr) ν_{max} 3434, 3281, 3051, 2930, 1580, 1315, 1159. ^{1}H-NMR (500 MHz, methanol-d$_4$) δ 2.31 (s, 3H), 3.18-3.27 (m, 2H), 4.84 (dd, 1H, J =

7.6, 7.5 Hz), 6.90-6.96 (m, 2H), 7.04 (s, 1H), 7.06 (d, 1H, J = 8.2 Hz), 7.25 (d, 1H, J = 7.3 Hz), 7.32 (d, 2H, J = 8.0 Hz), 7.43 (d, 2H, J = 8.0 Hz). ^{13}C-NMR (75 MHz, DMSO-d_6) δ 166.96, 152.18, 142.77, 137.65, 136.49, 129.43, 127.19, 126.51, 124.59, 121.28, 118.77, 118.39, 111.82, 109.29, 49.90, 29.89, 21.52. EIMS (m/z): 273 (3.3), 155 (2.03), 131 (10.6), 130 (100.0), 91 (13.1), 77 (5.2), 65 (3.3). Anal. calcd. for $C_{19}H_{20}N_6O_2S_2$: C 53.25; H 4.70; N 19.63; S 14.97; Found: C 53.17; H 4.67; N 19.58; 15.03.

ACKNOELEDGEMENT

This work was financially supported by the Higher Education Commission (HEC) of Pakistan through project No. 20-674/R & D/06/1764 under National Research Program for Universities.

REFERENCES

[1] a) Onoda, Y.; Iwasaki, H.; Magaribuchi, T.; Tamaki, H. *Arzneimittelforschung Drug Res.,* **1991**, *41*, 546. (b) Onoda, Y.; Iwasaki, H.; Magaribuchi, T.; Tamaki, H. *Jpn. J. Pharmacol.,* **1990**, *52*, 631.

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

[3] a) Hausinger, R.P.; Karplus, P.A. in *Handbook of Metalloproteins;* (Messerschmidt, A.; Hubber, R.; Poulos, T.; Wieghardt, K. Ed.), John Wiley & Sons: Chichester, UK, **2001**, pp. 867; b) Rosenstein, I.J.M.; Hamilton-Miller, J.M.T. *CRC Crit. Rev. Microbiol*., **1984**, *11*, 1.

[4] Montecucco, C.; Rappuoli, R. *Nat. Rev. Mol. Cell Biol*., **2001**, *2*, 457.

[5] Mobley, H.L.T.; Hausinger, R. P. *Microbiol. Rev*., **1989**, *53*, 8.

[6] Hauck, R. D. in *Nitrogen in Crop Production*, (Hauck, R.D. Ed.), American Society of Agronomy: Madison, WI, **1984**, pp. 551.

[7] Andrews, R.K.; Dexter, A.; Blakeley, R.L.; Zerner, B. *J. Am. Chem. Soc*., **1986**, *108*, 7124.

[8] McCarty, G.W.; Bremner, J.M.; Lee, J.S. *Plant Soil,* **1990**, *127*, 269.

[9] Khan, K.M.; Iqbal, S.; Lodhi, M.A.; Maharvi, G.M.; Zia-Ullah; Choudhary, M.I.; Atta-ur-Rahman; Perveen, S. *Bioorg. Med. Chem*., **2004**, *12*, 1963.

[10] Elsharief, A. M. S.; Ghorab, M. M.; Al-Gaby, M. S. A.; Mohamed, S. I.; Ammar, Y. A. *Heteroatom Chem*., **2002**, *13*, 316.

[11] Akhtar, T.; Hameed, S., Al-Masoudi, N.A.; Khan K.M. *Heteroatom Chem*., **2007**, *18*, 316.

[12] Wang, Z.; Shi, H. *J. Heterocycl. Chem*., **2001**, *38*, 355.

[13] Yaseen, A.A.; Al-Masoudi, N.A.; Ferwanah, A.E.S.; *Bioorg. Med. Chem*., **2003**, *11*, 1701.

[14] Demirbas, N.; Ugurluoglu, R.; Demirbas, A. *Bioorg. Med. Chem*., **2002**, *10*, 3717; Zitouni, G.T.; Kaplancıkli, Z.A.; Yıldız, M.T.; Chevallet, P.; Kaya, D. *Eur. Med. Chem*., **2005**, *40*, 607; Almasirada, A.; Sayyed, A.; Tabatabai, Faizi, M.; Kebriaeezadeh, A.; Mehrabi, N.; Dalvandia, A.; Shafieea, A. *Bioorg. Med. Chem. Lett*., **2004**, *14*, 6057.

[15] Amtul, Z.; Rasheed, M.; Choudhary, M.I.; Supino, R.; Khan, K.M.; Atta-ur-Rahman, *Biochem Biophys. Res. Commun*., **2004**, *319*, 1053.

[16] Tozkoparan, B.; Gökhan, N.; Aktay, G.; Yesilada, E.; Ertana, M. *Eur. J. Med. Chem*., **2000**, *35*, 743.

[17] a) Karabasanagouda, T.; Adhikari, A.V.; Shetty, N.S. *Eur. J. Med. Chem.,* **2007**, *42*, 521. b) Chanda, M.S.; Barve, P.A.; Khanwelkar, R.R.; Athalye, S.S.; Venkataraman, D.S. *Can. J. Chem.*, **2007**, *85*, 21. c) El Ashry, E.S.H.; Awad, L.F.; Hamid, H.M.A.; Atta, A.I. *Nucleosides, Nucleutides, Nucleic Acids*, **2006**, *25*, 325 d) Holla, B.S.; Prasanna, C.S.; Poojary, B.; Rao, K. S.; Shridhara, K. *Ind. J. Chem*., **2006**, *45B*, 2071. e) Moustafa, A.H.; Haggam, R.A.; Younes, M.E.; El Ashry, E.S.H. *Nucleosides, Nucleutides, Nucleic Acids*, **2005**, *24*, 1885. f) Huia, X.; Zhanga, L.; Zhang, Z.; Wang, Q.; Wang, F. *J. Chin. Chem. Soc*., **2000**, *47*, 535.

[18] McChesney, E. W.; Kirkswamm, W.M. *J. Am. Chem. Soc.,* **1937**, *59*, 1116.

[19] Reid, J.R.; Heindel, N.D. *J. Heterocycl. Chem.,* **1976**, *13*, 925.

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

[21] Greenstein, J.P.; Winitz, M. *Chemistry of the Amino Acids*, Wiley: New York, **1961**, *Vol. 2*.

Received: 01 April, 2008 **Revised: 07 August, 2008** **Accepted: 22 August, 2008**