Synthesis and evaluation of novel 1, 2, 4-substituted triazoles for urease and anti-proliferative activity

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Abstract: 1,2,4-triazoles are a major group of heterocyclic compounds. In the current work, a concise library of such triazoles synthesized through a multistep protocol. The synthesis involved hydrazinolysis of ethyl-2-(p-Cl-phenoxy) acetate followed by reflux with phenyl isothiocyanate to yield the intermediate 2-[2-(p-Cl-phenoxy)acetyl]-N-phenyl-hydrazinecarbothioamide. This intermediate was then cyclized to form 5-[p-(Cl-phenoxy)-methyl]-4-phenyl-4H-1,2,4-triazole-3-thiol (the parent moiety) at alkaline pH. In parallel, 3-bromopropionyl bromide was reacted with a series of phenylamines to yield N-(substituted-phenyl)bromopropamides. In the final step, N-substitution of 5-[p-(Cl-phenoxy)-methyl]-4-phenyl-4H-1,2,4-triazole-3-thiol was carried out with N-(substituted-phenyl)bromopropamides to give desired library of 3-[5-([p-(Cl-phenoxy)-methyl]-4-phenyl-4H-1,2,4-triazole-3-ylthio)]-N-(substituted-phenyl) propamides (8a-l). The prepared moieties were identified via IR, NMR, & EIMS and evaluated for urease and anti-proliferative activities. 3-[5-([p-(Cl-phenoxy)-methyl]-4-phenyl-4H-1,2,4-triazole-3-ylthio)]-N-(3-methyl-phenyl)propamidine 8k, was found to be most prominent hit as urease inhibitor (IC₅₀ = 42.57± 0.13 µM) using thiourea as standard (IC₅₀ = 21.25±0.15µM). The interaction of 8k with urease were studied using docking studies. Anti-proliferative activity results showed 8k as promising candidates and rest of the synthesized derivatives were found to be moderately anti-proliferative. Molecular docking results also displayed 8k, 8h, and 8c as potential hits for further study.

Keywords: 1, 2, 4-Triazole, phenyl isothiocyanate, N-(substitutedphenyl) bromopropionamides, urease inhibition, anti-proliferative.

INTRODUCTION

The urease enzyme is a nickel metal containing enzyme responsible for degradative processing of urea to NH₃ and CO₂ or carbamate and is found in many fungi and bacteria (Mobley et al., 1995, Karplus et al., 1997, Mobley et al., 1989 and Krajewska et al., 2007). It is. The enzyme is considered as an important virulence factor and is directly associated with the pylonephritis, peptic ulcer, urinary catheter encrustation, urolithiasis, hepatic coma etc. (Ragsdale et al., 2009). The enzyme particularly enables the survival of the pathogenic Helicobacter pylori in limited pH (4.0-8.2) range of human stomach. (Nakamura et al., 1998 and Clyne et al., 1996). Thus, H. pylori urease has been considered as a potential target for peptic ulcer and recurrent gastroduodenal inflammatory diseases and gastric adenocarcinoma are major risk factors associated with this bacteria (Hatzifoti et al., 2006).

1,2,4-Triazole is a major class of heterocyclic moieties that have been variously decorated to possess a variety of biological activities i.e, anticancer (Kaur et al., 2016), inflammation reductant (Shneine et al., 2016 and Palaska et al., 2002), antimicrobial (Kucukguzel et al., and Turan-Zitouni et al., 2005), antifungal (Bagihalli et al., 2008), antiviral (Sidwell et al., 1972), analgesic (Salgın-Gokşen et al., 2007), antioxidant (Khan et al.,2007) activities. Several 1,2,4-triazoles have been successfully developed into commercial fungicides, e.g. Diniconazole (I), Triadimefon (II), Flusilazole (III), Triadimenol (IV). Letrozole (V) is utilized for cancer treatment and contains aromatase inhibitor (Pragathi et al., 2021). Estazolam(VI) acts as anticonvulsant drug (Kucukguzel et al., 2015) (fig. 1).

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1,2,4-Triazole hybrids have multiple functionalities including potential to maximize efficacy, minimize side effects and exert multiple antitubercular mechanisms of action (Cao et al., 2021). Fluconazole has been widely used for antifungal activity because of its unique molecular structure, where nitrogen at 4 of 1,2,4-triazole ring has been coordinated to the heme Fe of CYP51 (Amin et al., 2021).

We have been interested in elaborating the chemistry and biology of the nitrogen containing heterocycles, in particular the 1,2,4-triazole ring system (Arfan et al., 2018, Saleem et al., 2010, Siddiqui et al., 2020, Butt et al., 2019 and Abbasi et al., 2018). In continuance of our interest, we have developed a concise library of the new bioactive compounds containing 1,2,4-triazole. The compounds have been evaluated for the ability to inhibit urease enzyme. Further, we have performed the docking to understand the binding of the hit with the enzyme. Lastly, we have evaluated the antiproliferative activity of the compounds. The work identifies a novel hit with potent antiurease and antiproliferative activity against HCT-116 cell lines and may be optimized further in the future investigation.

MATERIALS AND METHODS

Instrumentation and Materials
The chemicals and solvents utilized in the research work were highly pure and attained from Alfa Aesar. Griffin & George melting point instrument was utilized to monitor M.P are uncorrected. Silica gel coated plates (G-25-UV254) were used for TLC with various % of EtOAc and hexanes and spots were studied in UV light at 254 nm. The spectrophotometer used to observe IR spectra was Jasco-320-A. Proton & Carbon NMR spectra were documented in CDCl3 on Bruker-AM 600 MHz. EIMS were evaluated on Jeol MS 600H-1.

Synthesis
5-((4-Cl-phenoxy)-methyl)-4-phenyl-4H-1,2,4-triazole-3-thiol (4)
2-(p-Cl-phenoxy) acetohydrazide (5 g; 0.024 mol; 1) was mixed in CH3OH (40mL). The reaction was refluxed with continuous stirring for 45 min after adding phenyl isothiocyanate (3mL; 0.024 mol; 2). The contents of the flask were then cooled down to RT leading to the precipitation of 2-[2-(p-Cl-phenoxy) acetyl]-N-phenyl-hydrazinecarbothioamide (3). The crude compound was recrystallized from CH3OH and air-dried. Then it was poured to a flask followed by addition of 10% NaOH (30 mL). The mixture was agitated for 30mins at RT. Then the combination was quenched with ice & acidified till pH 4-5 by conc. HCl. The precipitates of 4 were collected, washed with distilled H2O and was air-dried to attain product.

N-(Substituted-phenyl) bromopropanamides (7a-k)
3-Bromopropanoylbromide (0.2 g; 0.1 mol; 5) was added to an RBF accompanied by addition of substituted phenyl amines (0.1 mol; 6a-k). The pH was balanced at 10 by 10% Na2CO3 and the compounds were run at room temperature. The obtained precipitates were dried to obtain pure N-(substituted-phenyl) bromopropanamides (7a-k).

{5-[(4-Cl-phenoxy)-methyl]-4- phenyl-4H-1,2,4-triazole-3-ylthio]-N-(substituted -phenyl) propanamides (8a-k)
5-[(4-Cl-phenoxy)-methyl]-4-phenyl-4H-1,2,4-triazole-3-thiol (0.2 g; 6 mmol; 4) was dissolved in DMF (7 mL) and LiH (0.0008g; 0.1 mmol) was poured to mixture. The solution was mixed for 30 mins. at RT. N-(substituted-phenyl) bromopropanamide (6 mmol; 7a-k) was dissolved in the mixture and it was run for 7-8 hours. The desired product was then obtained either through precipitation by quenching in ice or through solvent extraction with CHCl3 as organic medium to attain the desired compound (8a-k).

Biological Activity (In vitro)
Urease Inhibitory Activity
Urease inhibitory assay was performed as per reported method (Abbasi et al., 2004). At 30ºC, incubation of solutions comprising of enzyme (jack bean urease; 25µL) and buffer (55µL) having 50mM urea was performed with the test compd. (5µL) for 15 min in 96-well plates. Microplate reader was used to measure the increase in absorbance at 560nm after 10min. All the assays were run in triplicates having concentration 200 µL, buffer Na3PO4 (3mM, pH 6.8) and phenol red (7µg per mL) as indicator. Thiourea acted as control.

% age inhibition = 100 – (OD (Test well) / OD (Control)) × 100

Antiproliferative (SRB) activity
Effect of compounds on cell proliferation was determined by using Sulforhodamine B (SRB) assay as mentioned in reported method (Skehan et al., 1990). The antiproliferative activity was evaluated as follows:
Anti-proliferation (%) = [(B-S)/B] ×100
Whereas, B = Blank absorbance, S = Samples absorbance

Molecular docking
In order to understand the interaction of the hit, we performed molecular docking of compound 8k with urease enzyme using Auto Dock Vina (Trott O; 2010, Kryger et al., 2000, Petterson et al., 2004, Allouche; 2010 and William et al., 1994).

STATISTICAL ANALYSIS
All the measurements were carried out in triplicate and statistical analysis was performed by Microsoft Excel 2010. The results are presented as mean ± SEM.
RESULTS

New derivatives embodying 1,2,4-triazole nucleus were produced in current research work, the synthetic methodology & conditions are summarized in (Scheme 1). They were screened for enzyme inhibitory potential against urease and cytotoxic analysis (table 1 & table 2) for identification of the hit for subsequent detailed structure activity relationship studies to develop them into pharmacologically active candidates.

The multistep synthesis started with the reaction of ethyl-2-(p-Cl-phenoxy)acetate with hydrazine hydrate in methanol to afford 2-(p-Cl-phenoxy)acetohydrazide (1). The compound 1 was then refluxed with phenyl isothiocyanate (2) in methanol for 45 min. Reaction was cooled to obtain the precipitates of 2-[2-(4-Cl-phenoxy)acetyl]-N-phenyl-hydrazinecarbothioamide (3). Intermediate (3) was then cyclized under alkaline condition at RT. The acidification of the reaction mixture yielded key intermediate (4). At the same time, 3-bromopropionyl bromide (5) was reacted with a series of substituted phenyl-amines (6a-k) in basic media at room temperature to obtain (7a-k). In the last step, these propanamides (7a-k) were reacted with (4) in aprotic solvent at RT.

![Fig. 1: 1,2,4-Triazoles as commercial fungicides](image)

![Fig. 2: ^1H-NMR spectrum of 8k](image)

![Fig. 3: ^13C-NMR spectrum of 8k](image)

![Fig. 4(a): EIMS proposed mass fragmentation pattern of 4](image)

The desired products (8a-k) were obtained either through precipitation by quenching with ice or via solvent extraction using chloroform as the organic medium. The synthesized compounds, as shown in the experimental section, were characterized using IR, NMR, and EIMS. For example, for compound 8k, the IR spectrum showed absorption bands at 3110, 1599, 1590, 1491, 1164, 1122 and 652 cm$^{-1}$ for C-H, C=N imine, C-N, C=C, C-Cl, C-O, and C-S, respectively. The NMR spectral data also...
Table 1: Physical properties and urease inhibition activity of (8a-k)

<table>
<thead>
<tr>
<th>Code</th>
<th>% Yield</th>
<th>M.W &amp; M.F (g/mol)</th>
<th>Melting Point (°C)</th>
<th>(% Inhibition 0.25 mM)</th>
<th>IC₅₀ (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>8a</td>
<td>92</td>
<td>493, C₂₆H₂₅ClN₄O₂S</td>
<td>138</td>
<td>12.43 ± 0.12</td>
<td>-</td>
</tr>
<tr>
<td>8b</td>
<td>97</td>
<td>478, C₂₆H₂₅ClN₄O₂S</td>
<td>115</td>
<td>76.53 ± 0.25</td>
<td>117.64 ± 0.18</td>
</tr>
<tr>
<td>8c</td>
<td>92</td>
<td>493, C₂₆H₂₅ClN₄O₂S</td>
<td>129</td>
<td>5.32 ± 0.11</td>
<td>-</td>
</tr>
<tr>
<td>8d</td>
<td>97</td>
<td>493, C₂₆H₂₅ClN₄O₂S</td>
<td>145</td>
<td>34.58 ± 0.14</td>
<td>-</td>
</tr>
<tr>
<td>8e</td>
<td>98</td>
<td>493, C₂₆H₂₅ClN₄O₂S</td>
<td>124</td>
<td>4.76 ± 0.13</td>
<td>-</td>
</tr>
<tr>
<td>8f</td>
<td>95</td>
<td>493, C₂₆H₂₅ClN₄O₂S</td>
<td>126</td>
<td>13.24 ± 0.16</td>
<td>-</td>
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<td>8g</td>
<td>98</td>
<td>508, C₂₆H₂₅ClN₄O₂S</td>
<td>147</td>
<td>9.25 ± 0.14</td>
<td>-</td>
</tr>
<tr>
<td>8h</td>
<td>85</td>
<td>493, C₂₆H₂₅ClN₄O₂S</td>
<td>-</td>
<td>86.76 ± 0.17</td>
<td>85.43 ± 0.12</td>
</tr>
<tr>
<td>8i</td>
<td>84</td>
<td>493, C₂₆H₂₅ClN₄O₂S</td>
<td>-</td>
<td>87.35 ± 0.21</td>
<td>63.68 ± 0.16</td>
</tr>
<tr>
<td>8j</td>
<td>87</td>
<td>478, C₂₆H₂₅ClN₄O₂S</td>
<td>-</td>
<td>21.47 ± 0.15</td>
<td>-</td>
</tr>
<tr>
<td>8k</td>
<td>82</td>
<td>478, C₂₆H₂₅ClN₄O₂S</td>
<td>-</td>
<td>91.43 ± 0.19</td>
<td>42.57 ± 0.13</td>
</tr>
<tr>
<td>Control (Thiourea)</td>
<td>98.21 ± 0.18</td>
<td>21.25 ± 0.15</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2: SRB assay against HCT-116 cell lines (% age cell viability) at two concentration of (8a-k)

<table>
<thead>
<tr>
<th>Code</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>25 µM</td>
</tr>
<tr>
<td>8a</td>
<td>24.44 ± 0.46</td>
</tr>
<tr>
<td>8b</td>
<td>44.84 ± 0.57</td>
</tr>
<tr>
<td>8c</td>
<td>21.78 ± 0.63</td>
</tr>
<tr>
<td>8d</td>
<td>31.14 ± 4.57</td>
</tr>
<tr>
<td>8e</td>
<td>27.10 ± 1.16</td>
</tr>
<tr>
<td>8f</td>
<td>46.83 ± 0.98</td>
</tr>
<tr>
<td>8g</td>
<td>-</td>
</tr>
<tr>
<td>8h</td>
<td>20.68 ± 0.37</td>
</tr>
<tr>
<td>8i</td>
<td>61.46 ± 4.69</td>
</tr>
<tr>
<td>8j</td>
<td>-</td>
</tr>
<tr>
<td>8k</td>
<td>23.55 ± 0.92</td>
</tr>
<tr>
<td>Control (DMSO)</td>
<td>100</td>
</tr>
</tbody>
</table>

Experiments were replicated thrice (SEM, n = 3) and compounds were solubilized in methanol. The dash (-) showed no results.

Table 3: In-silico analysis of (8k) with active binding site interactions against Urease in 2D & 3D.

<table>
<thead>
<tr>
<th>Code</th>
<th>H-Bonding</th>
<th>Alkyl &amp; α-Alkyl Interactions</th>
<th>Electrostatic Interactions (α-cation &amp; anion)</th>
</tr>
</thead>
</table>
| 8k   | • ValA:744---NHCO  
• TyrA:32---C₆H₅ on triazole ring (α-donor)  
• AlaA: 16, ProA:743 & LeuA:13--CH₃ positioned at -NHC₆H₄  
• AlaA: 16 & AlaA:37---CONHC₆H₄  
• ValA:744--O C₆H₅Cl & -Cl | • ValA: 744 & ValA: 36--C₆H₅ on triazole ring  
• AlaA: 16, ProA:743 & LeuA:13--CH₃ positioned at -NHC₆H₄  
• AlaA: 16 & AlaA:37---CONHC₆H₄  
• ValA:744--O C₆H₅Cl & -Cl | • LysA:709 & GluA:718---OC₆H₅Cl  
• AspA:730 & LysA:716 --- Triazole ring  
• GluA:742----CONHC₆H₄ |

Scheme I: Scheme for the synthesis of (8a-k)
favored the expected structure. A broad singlet appeared downfield at $\delta$ 7.49 for H-b′′′′ and a multiplet appeared at $\delta$ 7.54-7.51 for H-c′′ - H-e′′ confirming the presence of phenyl moiety. A $^{2}B_{2}$ spin arrangement was seen as diortho coupled doublets one at $\delta$ 7.21 and other at $\delta$ 6.80 for 4 Hs positioned at H-c′ & H-e′ and H-b′ & H-f′, respectively, confirming the p-Cl-phenoxy group.

In aliphatic portion a singlet was observed at $\delta$ 5.06 for -CH₂ group flanged between 4-chlorophenoxy and the triazole moieties. Two triplets resonated at $\delta$ 3.02 and 3.51 for 2 CH₂ groups at b′′′ and cʹʹʹ. Finally, a singlet at $\delta$ 2.32 confirmed the CH₃-aʹʹʹ′ group presence proving the projected structure.

$^{13}$C-NMR spectrum further confirmed the successful reaction. The amide carbonyl gave peak at 169.0. The quaternary carbon (C-c) of the 1,2,4-triazole showed peak at $\delta$ 155.7. The remaining carbons exhibited peaks at $\delta$ 156.0, 147.9, 138.7, 138.1, 129.9, 129.6, 129.4, 128.7, 126.8, 124.9, 120.4, 116.8, 116.2, 60.1, 37.7, 28.3, and 21.5. The compound 8k showed MI peak at m/z 285 [M]+ in EIMS which correlated to the MF of the synthesized derivative C$_{20}$H$_{23}$N$_{3}$OS.

Spectral Characterization

3-{5-[(4-Cl-phenoxy)-methyl]-4-phenyl-4H-1,2,4-triazole-3-thiolato-N(2,3-dimethyl-phenyl)propanamide (8a)

Solid; IR (KBr, cm$^{-1}$) 3110 s (C-H), 1599 s (C=O), 1589 s (C-N), 1491 s (C=C), 1164 s (C-Cl), 1123 s (C-O) and 652 s (C-S); $^{1}$H-NMR (CDCl$_{3}$, 600), (J=Hz) $\delta$H 8.41 s (1H, NH), 7.56-7.51 m (3H, H cʹʹʹ to H eʹʹʹ), 7.48 d (J 8.3, 2H, H b′′′′ & H f′′′′), 7.33 br. d (J 7.6, 2H, H b′′ & H f′′′′), 7.22 d (J 5.6 Hz, 2H, H c′′ & H e′′), 7.13 br. d (J 7.6, 2H, H c''′ & H e''′), 6.82 d (J 5.6, 2H, H b′ & H f′), 5.06 s (2H, -OCH₂ f), 3.52 t (J 6.9, 2H, CH₂ c'), 3.00 t (J 6.9, 2H, CH₂ b′′), 2.33 s (3H, CH₃ a''''′'); EIMS: m/z 372 [M]+ [C$_{16}$H$_{13}$ClN$_{3}$O$_{2}$S]⁻; 285 [C$_{16}$H$_{13}$N$_{3}$OS]⁻; 224 [C$_{16}$H$_{13}$N$_{2}$O]⁻; 107 [C$_{8}$H$_{4}$N$_{2}$O]⁻; 106 [C$_{8}$H$_{4}$NO]⁻; 77 [(C$_{6}$H$_{5}$)⁻].

3-{5-[(4-Cl-phenoxy)-methyl]-4-phenyl-4H-1,2,4-triazole-3-thiolato-N(4-methyl-phenyl)propanamide (8b)

Solid; IR (KBr, cm$^{-1}$) 3110 s (C-H), 1599 s (C=O), 1589 s (C-N), 1491 s (C=C), 1164 s (C-Cl), 1123 s (C-O) and 652 s (C-S); $^{1}$H-NMR (CDCl$_{3}$, 600), (J=Hz) $\delta$H 8.41 s (1H, NH), 7.56-7.51 m (3H, H c''′ & H e''′), 7.48 d (J 8.3, 2H, H b′′′′ & H f′′′′), 7.33 br. d (J 7.6, 2H, H b′′ & H f′′′′), 7.22 d (J 5.6 Hz, 2H, H c′′ & H e′′), 7.13 br. d (J 7.6, 2H, H c''′ & H e''′), 6.82 d (J 5.6, 2H, H b′ & H f′), 5.06 s (2H, -OCH₂ f), 3.52 t (J 6.9, 2H, CH₂ c'), 3.00 t (J 6.9, 2H, CH₂ b′′), 2.33 s (3H, CH₃ a''''′'); EIMS: m/z 372 [M]+ [C$_{16}$H$_{13}$ClN$_{3}$O$_{2}$S]⁻; 285 [C$_{16}$H$_{13}$N$_{3}$OS]⁻; 224 [C$_{16}$H$_{13}$N$_{2}$O]⁻; 107 [C$_{8}$H$_{4}$N$_{2}$O]⁻; 106 [C$_{8}$H$_{4}$NO]⁻; 77 [(C$_{6}$H$_{5}$)⁻].

Fig. 5: Structure-activity relationship of compounds 8h, 8i & 8k

Fig. 6: Urease Binding 2D and 3D models of most potent synthetic inhibitor 8k

IC$_{50}$ (42.57±0.13 μmolL⁻¹) IC$_{50}$ (63.68±0.16 μmolL⁻¹) IC$_{50}$ (85.43±0.12 μmolL⁻¹)
3-[5-[(4-Cl-phenoxy)-methyl]-4-phenyl-1H,2,4-triazole-3-ylthio]-N-(2,5-dimethyl-phenyl)propanamide (8c)

Solid; IR (KBr, cm⁻¹) 3400 s (C-H), 1592 s (C=N), 1591 s (C-N), 1487 s (C=O), 1383 b (C=H), 1122 s (C-O), 1052 s (C=Cl), 825 s (C=S); H-NMR (CDCl₃, 600), (J=H) δ₁₆ 7.68 s (1H, NH), 7.55-7.54 m (3H, H c”’ to H e”’), 7.37 dd (J 2.5, 7.8, 1H, H d”), 7.33 br. s (1H, H f”), 7.19 d (J 8.7, 2H, H c’ & H e’), 7.08 d (J 7.6, 2H, H b”’ & H f”’), 6.93 d (J 7.5, 1H, H c”), 6.72 d (J 8.7 Hz, 2H, H b’ & H f’), 4.86 s (2H, -OCH₂), 4.72 t (J 6.8, 2H, CH₂c’), 3.11 t (J 6.8, 2H, CH₂b’), 2.32 s (3H, CH₃b”;”’); EIMS: m/z (I, rel. %): 285 [M⁺] [(C₉H₆N₅O₂S)⁺ ; 3], 258 [(C₉H₇NO₂⁺ ; 3), 209 [(C₈H₇NOCl⁺ ; 3), 207 [(C₈H₇NOCl⁺ ; 3), 175 [(C₇H₆N₂S)⁺ ; 5], 121 [(C₇H₆N₂S)⁺ ; 100], 117 [(C₇H₆N₂S)⁺ ; 40], 113[(C₇H₆Cl₂⁺ ; 15), 77 [(C₆H₅S)⁺ ; 40 & 57 [(C₆H₅NO₂⁺ ; 40].

3-[5-[(4-Cl-phenoxy)-methyl]-4-phenyl-1H,2,4-triazole-3-ylthio]-N-(6,6-dimethyl-phenyl)propanamide (8d)

Solid; IR (KBr, cm⁻¹) 2938 s (C-H), 1600 s (C=N), 1594 s (C-N), 1492 s (C=C), 1163 s (C-Cl), 1121 s (C-O), 792 s (C-S); H-NMR (CDCl₃, 600), (J=H) δ₁₆ 7.56-7.51 m (3H, H c”’ to H e”’), 7.33 dd (J 5.6, 9.6, 2H, H c”’ & H e”), 7.22 d (J 5.6, 2H, H c’ & H e’), 7.12-7.11 m (2H, H b”’ & H f”’), 7.08 t (J 9.3, 1H, H d”), 6.83 d (J 5.6, 2H, H b’ & H f’), 5.04 s (2H, -OCH₂), 3.57 t (J 6.9, 2H, CH₂c’), 3.04 t (J 6.9, 2H, CH₂b’), 2.24 s (6H, CH₃a”;”’ & CH₃b”;”’); EIMS: m/z (I, rel. %): 285 [M⁺] [(C₉H₆N₅O₂S)⁺ ; 3], 258 [(C₉H₇NO₂⁺ ; 3), 209 [(C₈H₇NOCl⁺ ; 3), 207 [(C₈H₇NOCl⁺ ; 3), 175 [(C₇H₆N₂S)⁺ ; 5], 121 [(C₇H₆N₂S)⁺ ; 100], 117 [(C₇H₆N₂S)⁺ ; 40], 113[(C₇H₆Cl₂⁺ ; 15), 77 [(C₆H₅S)⁺ ; 40 & 57 [(C₆H₅NO₂⁺ ; 40].

3-[5-[(4-Cl-phenoxy)-methyl]-4-phenyl-1H,2,4-triazole-3-ylthio]-N-(3,4-dimethyl-phenyl)propanamide (8e)

Solid; IR (KBr, cm⁻¹) 3062 s (C-H), 1596 s (C=N), 1592 s (C-N), 1487 s (C=O), 1125 s (C-O), 1027 s (C=Cl), 780 s (C-S); H-NMR (CDCl₃, 600), (J=H) δ₁₆ 8.34 s (1H, NH), 7.55-7.51 m (3H, H c”’ to H e”’), 7.40 br.d (J 6.0, 2H, H b”’ & H c”), 7.32 dd (J 1.3, 5.8 Hz, 1H, H f”), 7.30 br. d (J 1.9, 1H, H b”), 7.21 d (J 7.0, 2H, H c’ & H e’), 7.07 d (J 8.1, 1H, H e’), 6.83 d (J 8.0, 2H, H b’ & H f’), 5.05 s (2H, -OCH₂), 3.51 t (J 6.8, 2H, CH₂c’), 2.99 t (J 6.9, 2H, CH₃b’), 2.25 s (3H, CH₃b”;”’), 2.23 s (3H, CH₃a”;”’); EIMS: m/z (I, rel. %): 285 [M⁺] [(C₉H₆N₅O₂S)⁺ ; 47], 175 [(C₇H₆N₅S)⁺ ; 50], 121 [(C₇H₆N₂S)⁺ ; 100], 117 [(C₇H₆N₂S)⁺ ; 40 & 77 [(C₆H₅S)⁺ ; 30].

3-[5-[(4-Cl-phenoxy)-methyl]-4-phenyl-1H,2,4-triazole-3-ylthio]-N-(3,5-dimethyl-phenyl)propanamide (8f)

Solid; IR (KBr) 3056 s (C-H), 1598 s (C=N), 1591 s (C-S), 1491 s (C=C), 1162 s (C-Cl) 1129 s (C-O), 759 s (C-S); H-NMR (CDCl₃, 600), (J=H) δ₁₆ 8.18 s (1H, NH), 7.55-7.52 m (3H, H c”’ to H e”’), 7.32 dd (J 1.3 Hz, 1.8, 2H, H b”’ & H f”’), 7.22 merged-br. d (J 8.0, 4H, H c’ & H e’ and H b”’ & H f”’), 6.84 d (J 8.0, 2H, H b’ & H f’), 6.77 br. s (IH, H d”), 5.06 s (2H, -OCH₂), 3.51 t (J 6.7, 2H, CH₂c’), 2.98 t (J 6.7 Hz, CH₂b’), 2.32 s (6H, CH₃, 1”’ & CH₃b”’); EIMS: m/z (I, rel. %): 387 [M⁺] [(C₉H₆N₅O₂S)⁺ ; 5], 285 [(C₇H₆N₅O₂⁺ ; 40], 175 [(C₇H₆N₂S)⁺ ; 40], 121 [(C₇H₆N₂O)⁺ ; 100], 91 [(C₆H₅S)⁺ ; 20] & 77 [(C₆H₅O)⁺ ; 40].
The synthesized derivatives were screened against Urease enzyme. Compounds 8a, 8c-8g and 8j demonstrated no enzyme inhibitory potential and 8b showed weak enzyme inhibitory potential. Compounds 8a, 8g, & 8j were designated as 8k. Similarly other N-substituted derivative structures were also characterized.

**Biological Assays**

**Urease Assay**

The synthesized derivatives were analyzed against Urease enzyme. Compounds 8a, 8c-8g and 8j demonstrated no enzyme inhibitory potential and 8b showed weak inhibitory potential. Whereas, 8k, 8i and 8h showed promising inhibitory potential with respect to standard thiourea (fig. 5).

**In-silico analysis**

8k was attached to amino acid of the enzyme active site via different parts of the molecule making it competent for urease inhibition (table 1). The binding of synthesized analogue 8k with the amino acid residue of urease is explicated in table 3. 2D and 3D models of the most potent synthetic inhibitor 8k is shown in fig. 6.

**Anti-proliferative (SRB) assay**

All the synthesized derivatives showed good to excellent activities as tabulated in (table 2) as % age viability. 8h showed highest activity with cell viability of 0.04±0.04 at 21.25±0.15 µM concentration. Compounds 8a, 8g, & 8j were found to be inactive against HCT-116 cell lines. Dimethylsulfoxide utilized as control showed 100 value at both concentrations. This study suggested that compounds 8a, 8g and 8j could be considered as potent candidates.

DISCUSSION

Out of the synthesized compounds series, 8k was selected for further discussion on structural elucidation via various spectral methods. EIMS spectrum is used to deduce the molecular formula of this synthesized derivative [C94H124N18O8S] + , which exhibited molecular ion peak; m/z 285 [M] + and other distinctive mass fragments peaks emerged at m/z 134, 107 & 77. EIMS and suggested mass fragmentation pattern is shown in (fig. 4a; 4b). The IR spectrum showed absorption bands at 3110, 1599, 1500, 1491, 1164, 1122 and 652 for C-H, C=C imine, C=N, C=C, C-Cl, C=O and C-S respectively. NMR spectral data elucidated the structure by counting the no. of H via integration curves. A broad singlet appeared downfield at δ 7.49 for H-b‴, a multiplet appeared at δ 7.54-7.51 for H-c‴ - H-e‴ confirming the presence of phenyl moiety. A²B₂ spin system showed diortho coupled doublets one at δ 7.21 and another at δ 6.80 for 4 Hs positioned at H-c‴ & H-e‴ and H-b‴ & H-f‴ respectively confirming the p-CI-phenoxo group. In aliphatic region a singlet was observed at δ 5.06 for -CH₂ group flanged between 4-CI-phenoxo and 4-phenyl-1,2,4-triazole moieties. Two triplets resonated at δ 3.02 and 3.51 for 2 CH₃ groups at b‴″ and c‴″. Finally a singlet at δ 2.32 confirmed the CH₃-a‴‴ group presence proving the projected structure°C-NMR spectrum further confirmed the incorporation (fig. 2 & 3). The quaternary carbon (C-e) of the 1,2,4-triazole presented peak at δ 155.7, the other quaternary carbon (C-c) peak was not observed. The peaks of the remaining carbons were shown at δ 169.0, 156.0, 147.9, 138.7, 138.1, 129.9, 129.6, 128.7, 126.8, 124.0, 120.4, 116.8, 116.2, 60.1, 37.7, 28.3, 21.5 for carbons positioned at C-a‴‴, C-a‴, C-a‴‴‴, C-c‴‴‴, C-c‴‴ & C-e‴, C-b‴ & C-f‴, C-e‴‴, C-c‴ to C-e‴, C-d‴, C-d‴‴, C-b‴‴‴, C-f‴‴, C-b‴ & C-f‴, C-f‴, C-b‴‴, C-e‴‴‴, C-a‴‴‴ respectively. On basis of aforementioned collective substantiation, the structure was designated as 8k. Similarly other N-substituted derivative structures were also characterized.
CONCLUSION

Keeping the importance of the discovery of new urease inhibitors in mind, a concise library of the triazoles was prepared and characterized through contemporary spectroscopic data. In 8k, the presence of methyl group at meta position made this derivative excellent inhibitor against urease whereas, in 8h & 8i, the presence of ethyl group at ortho and para position of phenyl ring made these derivatives potent inhibitors. The biological evaluation of the library suggested compound 8k as the hit compound along with 8h, 8i showing prominent activities. Compound 8k was docked with the enzyme to understand the interaction of the molecule with the protein for further structure optimizations. The compounds were further investigated against HCT-116 cell line for the anti-proliferative activity and compound 8h showed the most promising results. This work identifies novel derivatives that can be optimized to further these derivatives into the drug discovery process.

REFERENCES


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