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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-4*H*-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)bromopropanamides. In the final step, *N*-substitution of 5-[*p*-(Cl-phenoxy)-methyl]-4- phenyl-4*H*-1,2,4-triazole-3-thiol was carried out with *N*-(substituted-phenyl)bromopropanamides to give desired library of 3-[5-[(*p*-Cl-phenoxy)-methyl]-4- phenyl-4*H*-1,2,4-triazole-3-ylthio]-*N*-(substituted-phenyl) propan-amides (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-4*H*-1,2,4-triazole-3-ylthio]-*N*-(3-methyl-phenyl)propanamide 8k, was found to be most prominent hit as urease inhibitor (IC_{50}= 42.57± 0.13 μM) using thiourea as standard (IC_{50}= 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 NH_3 and CO_2 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 pyelonephritis, 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), inflamation 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 (Kucukgüzel *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 $CDCl_3$ on Bruker-AM 600 MHz. EIMS were evaluated on Jeol MS 600H-1.

Synthesis

*5-[(4-*Cl-phenoxy)-methyl]-4- phenyl-4*H*-1,2,4-triazole -*3-thiol (4)*

2-(*p*-Cl-phenoxy) acetohydrazide (5 g; 0.024 mol; 1) was mixed in CH_3OH (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 CH_3OH 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 H_2O 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% Na_2CO_3 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-4*H*-1,2,4-triazole-*3-ylthio}-N-(substituted -phenyl) propanamides (8a-k)***

*5-[(4-*Cl-phenoxy)-methyl]-4- phenyl-4*H*-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 $CHCl_3$ 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 30°C, 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 Na_3PO_4 (3mM, pH 6.8) and phenol red (7μg per mL) as indicator. Thiourea acted as control.

$$\%\,\text{age inhibition} = 100 - \frac{\text{OD (Test well)}}{\text{OD (Control)}} \times 100$$

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 anti-proliferative 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.

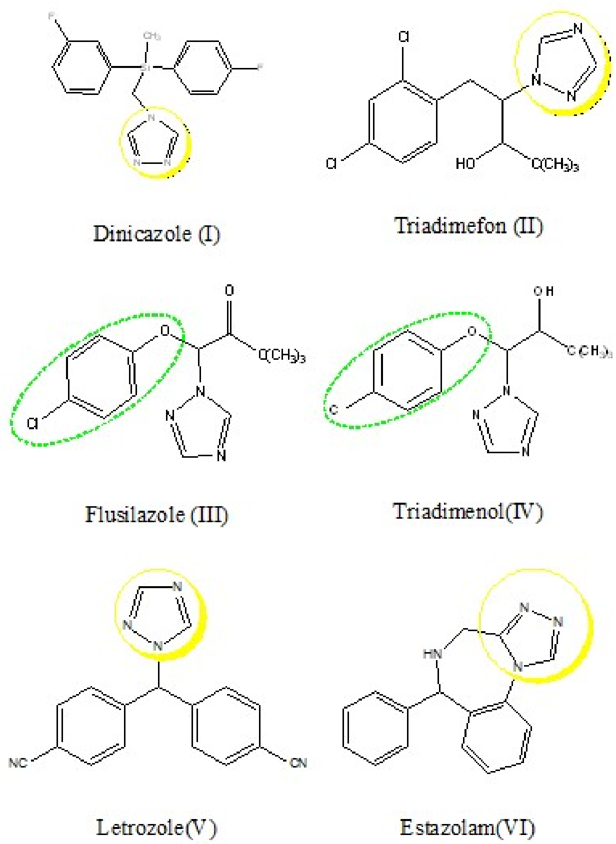


Fig. 1: 1,2,4-Triazoles as commercial fungicides

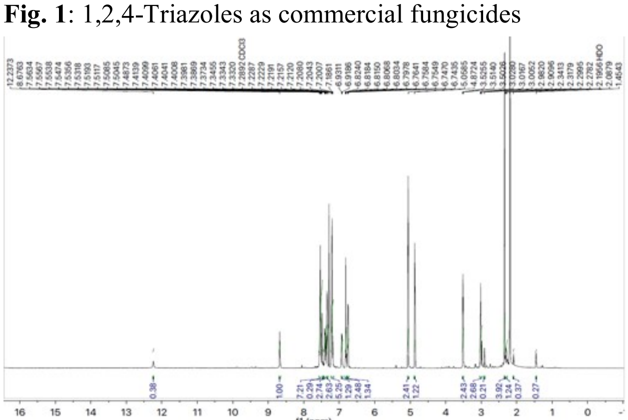


Fig. 2: ^{1}H-NMR spectrum of 8k

Chemistry

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.

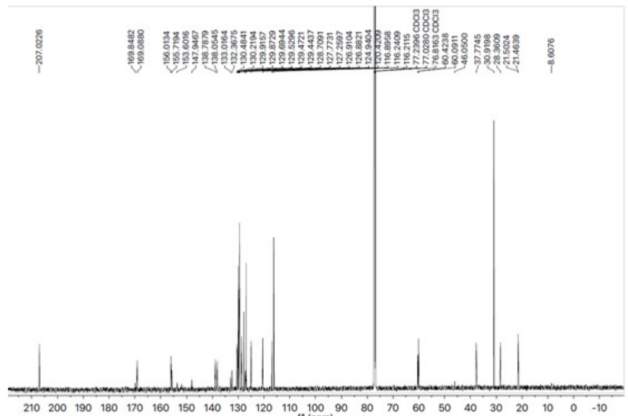


Fig. 3: ^{13}C-NMR spectrum of 8k

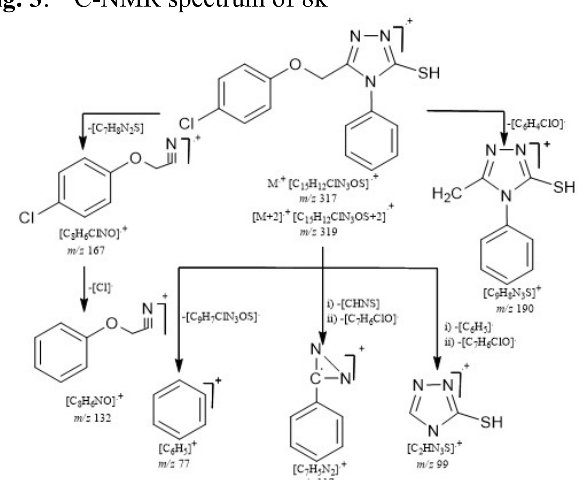


Fig. 4(a): EIMS proposed mass fragmentation pattern of 4

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)

Code	% Yield	M.W &M.F (g/mol)	Melting Point (°C)	(%) Inhibition 0.25 mM	IC_{50} (μM)
8a	92	493,$C_{26}H_{25}ClN_4O_2S$	138	12.43 ± 0.12	-
8b	97	478,$C_{25}H_{23}ClN_4O_2S$	115	76.53 ± 0.25	117.64 ± 0.18
8c	92	493,$C_{26}H_{25}ClN_4O_2S$	129	5.32 ± 0.11	-
8d	97	493, $C_{26}H_{25}ClN_4O_2S$	145	34.58 ± 0.14	-
8e	98	493, : $C_{26}H_{25}ClN_4O_2S$	124	4.76 ± 0.13	-
8f	95	493, : $C_{26}H_{25}ClN_4O_2S$	126	13.24 ± 0.16	-
8g	98	508, $C_{26}H_{25}ClN_4O_3S$	147	9.25 ± 0.14	-
8h	85	493, $C_{26}H_{25}ClN_4O_2S$	-	86.76 ± 0.17	85.43 ± 0.12
8i	84	493, $C_{26}H_{25}ClN_4O_2S$	-	87.35 ± 0.21	63.68 ± 0.16
8j	87	478, $C_{25}H_{23}ClN_4O_2S$	-	21.47 ± 0.15	-
8k	82	478, $C_{25}H_{23}ClN_4O_2S$	-	91.43 ± 0.19	42.57 ± 0.13
Control (Thiourea)				98.21 ± 0.18	21.25 ± 0.15

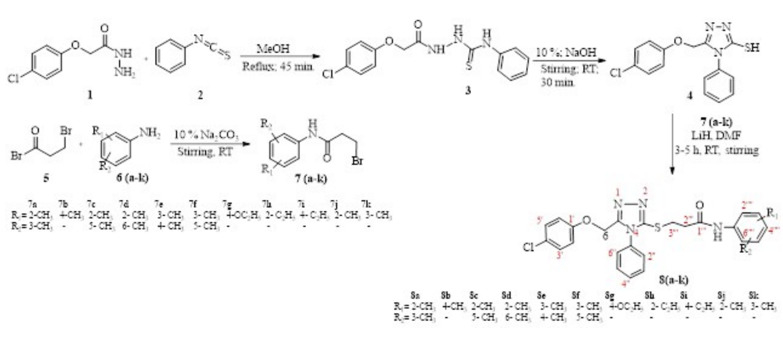
Table 2: SRB assay against HCT-116 cell lines (% age cell viability) at two concentration of (8a-k)

Code	Concentration	
	25 μM	50 μM
8a	24.44 ± 0.46	-
8b	44.84 ± 0.57	30.61 ± 4.88
8c	21.78 ± 0.63	0.16 ± 0.16
8d	31.14 ± 4.57	6.25 ± 0.53
8e	27.10 ± 1.16	22.25 ± 0.98
8f	46.83 ± 0.98	46.75 ± 2.48
8g	-	-
8h	20.68 ± 0.37	0.04 ± 0.04
8i	61.46 ± 4.69	53.64 ± 6.87
8j	-	-
8k	23.55 ± 0.92	0.75 ± 0.02
Control (DMSO)	100	

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.

Code	H-Bonding	Alkyl & л-Alkyl Interactions	Electrostatic Interactions (л-cation & anion)
8k	• ValA:744---NHCO • TyrA:32---C_6H_5 on triazole ring (л-donor)	• ValA: 744 & ValA: 36--C_6H_5 on triazole ring • AlaA: 16, ProA:743 & LeuA:13--CH_3 positioned at -NHC_6H_4 • AlaA: 16 & AlaA:37---$CONHC_6H_4$ • ValA: 744--O C_6H_4Cl & -Cl	• LysA:709 & GluA:718---OC_6H_4Cl • AspA:730 & LysA:716 --- Triazole ring • GluA:742----$CONHC_6H_4$



Scheme I: Scheme for the synthesis of (8a-k)

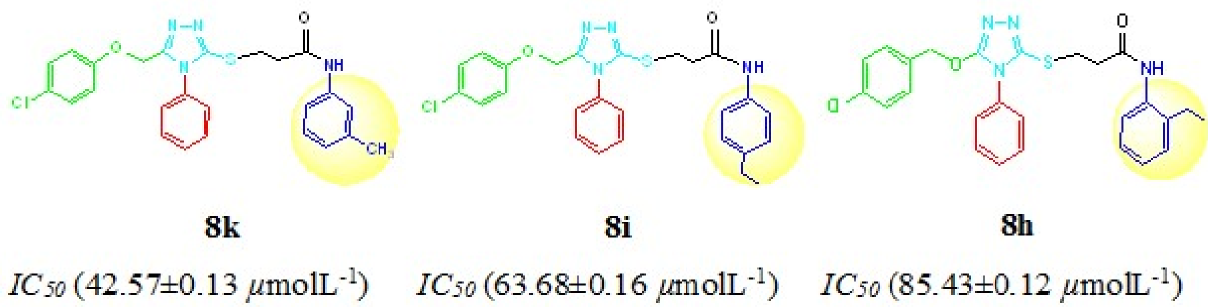


Fig. 5: Structure-activity relationship of compounds 8h, 8i & 8k

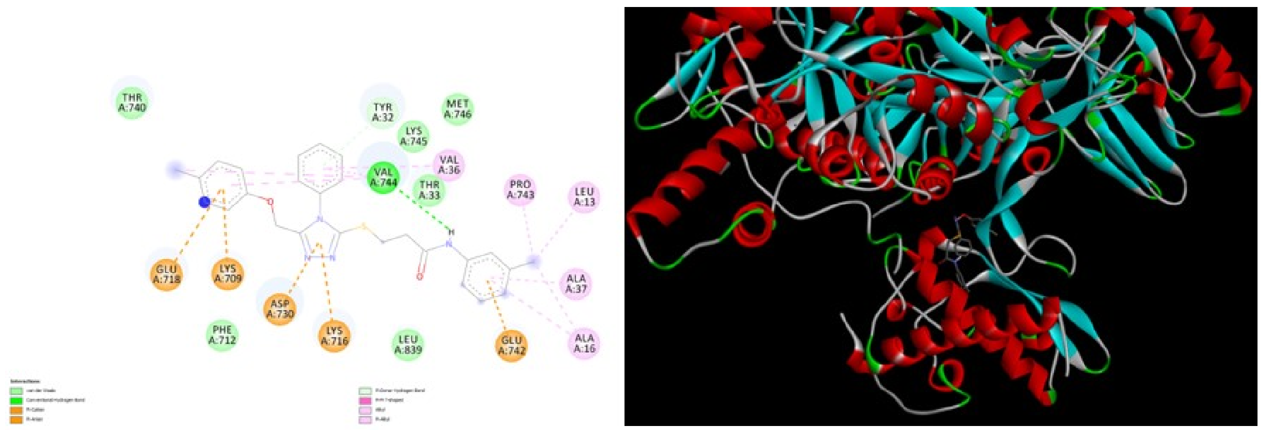


Fig. 6: Urease Binding 2D and 3D models of most potent synthetic inhibitor 8k

favored the expected structure. A broad singlet appeared downfield at δ 7.49 for H-b⁗ and a multiplet appeared at δ 7.54-7.51 for H-c″ - H-e″ confirming the presence of phenyl moiety. A_2B_2 spin arrangement was seen as *diortho* coupled doublets one at δ 7.21 and other at δ 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 δ 5.06 for -CH_2 group flanged between 4-chlorophenoxy and the triazole moieties. Two triplets resonated at δ 3.02 and 3.51 for 2 CH_2 groups at b‴ and c‴. Finally, a singlet at δ 2.32 confirmed the CH_3-a⁗ 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 δ 155.7. The remaining carbons exhibited peaks at δ 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 corelated to the MF of the synthesized derivative $C_{20}H_{23}N_3OS$.

Spectral Characterization

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

Solid; IR (KBr, cm^{-1}) 3023 s (C-H), 1591 s (C=N), 1590 s (C-N), 1487 s (C=C), 1164 s (C-Cl), 1123 s (C-O) and 771 s (C-S); ^{1}H-NMR ($CDCl_3$, 600), (*J*=Hz) δ_H 8.69 s (1H, NH), 7.57 t (*J* 7.0, 1H, H d‴), 7.56-7.49 m (2H, H c⁗ & e⁗), 7.27 br. s (2H, H b⁗ & H f⁗), 7.15 d (*J* 9.0, 2H, H c′ & H e′), 7.01 t (*J* 7.5, 1H, H e‴), 6.98 d (*J* 7.0, 2H, H d‴ & H f‴), 6.72 d (*J* 9.0, 2H, H b′ & H f′), 5.14 s (2H, -OCH_2 f), 3.70 br. s (2H, CH_2 c″), 3.67 br. s (2H, CH_3 b″), 2.24 s (3H, CH_3 b‴″), 2.13 s (3H, CH_3 a‴″); EIMS: *m/z (I, rel. %):* 285 $[M^{\cdot+}]$ $[(C_{15}H_{15}N_3OS)^{\cdot+}$;3], 258 $[(C_{14}H_{11}ClN_2O)^{\cdot+}$;3], 209 $[(C_9H_6N_3OCl+2)^+$;3], 207 $[(C_9H_6N_3OCl)^+$;3], 175 $[(C_8H_5N_3S)^+$;5], 121 $[(C_6H_5N_2O)^{\cdot+}$;100], 117 $[(C_7H_5N_2)^+$;40], 113$[(C_6H_5Cl+2)^+$;15], 77 $[(C_6H_5)^+$;40] & 57 $[(C_2H_3NO)^{\cdot+}$; 40].

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

Solid; IR (KBr, cm^{-1}) 3110 s (C-H), 1599 s (C=N), 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) δ_H 8.41 s (1H, NH), 7.56-7.51 m (3H, H c⁗ to 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, -OCH_2 f), 3.52 t (*J* 6.9, 2H, CH_2 c″), 3.00 t (*J* 6.9, 2H, CH_2 b″), 2.33 s (3H, CH_3 a⁗); EIMS: *m/z* 372 $[M^{\cdot+}]$ $[(C_{18}H_{15}ClN_3O_2S)^+$; 5] 285 $[(C_{15}H_{15}N_3OS)^{\cdot+}$; 3] 224 $[(C_{13}H_{16}N_3O)^+$; 4] 107 $[(C_6H_5NO)^+$; 100], 106 $[(C_7H_8N)^+$; 10]& 77 $[(C_6H_5)^+$; 40].

3-{5-[(4-Cl-phenoxy)-methyl]-4-*phenyl*-4H-1,2,4-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=C), 1383 b (-CH_2), 1122 s (C-O), 1052 s (C-Cl), 825 s (C-S); ^{1}H-NMR ($CDCl_3$, 600), (*J*=Hz) δ_H 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, -OCH_2 f), 4.72 t (*J* 6.8, 2H, CH_2 c′′), 3.11 t (*J* 6.8, 2H, CH_2 b′′), 2.32 s (3H, CH_3 b′′′′′), 2.26 s (3H, CH_3 a′′′′′); EIMS: *m/z (I, rel. %):* 285 [$M^{\cdot+}$] [$(C_{15}H_{15}N_3OS)^{\cdot+}$;3], 258 [$(C_{14}H_{11}ClN_2O)^{\cdot+}$;3], 209 [$(C_9H_6N_3OCl+2)^+$;3], 207 [$(C_9H_6N_3OCl)^+$;3], 175 [$(C_8H_5N_3S)^{\cdot+}$;5], 121 [$(C_6H_5N_2O)^{\cdot+}$;100], 117 [$(C_7H_5N_2)^{\cdot+}$;40], 113[$(C_6H_5Cl+2)^+$;15], 77 [$(C_6H_5)^+$;40] & 57 [$(C_2H_3NO)^{\cdot+}$; 40].

3-{5-[(4-Cl-phenoxy)-methyl]-4-*phenyl*-4H-1,2,4-triazole-3-ylthio}-N-(2,6-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); ^{1}H-NMR ($CDCl_3$, 600), (*J*=Hz) δ_H 7.80 s (1H,NH), 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, -OCH_2 f), 3.57 t (*J* 6.9, 2H, CH_2 c′′), 3.04 t (*J* 6.9, 2H, CH_2 b′′), 2.24 s (6H, CH_3 a′′′′′ & CH_3 b′′′′′); EIMS: *m/z (I, rel. %):* 285 [$M^{\cdot+}$] [$(C_{15}H_{15}N_3OS)^{\cdot+}$;3], 258 [$(C_{14}H_{11}ClN_2O)^{\cdot+}$;3], 209 [$(C_9H_6N_3OCl+2)^+$;3], 207 [$(C_9H_6N_3OCl)^+$;3], 175 [$(C_8H_5N_3S)^{\cdot+}$;5], 121 [$(C_6H_5N_2O)^{\cdot+}$;100], 117 [$(C_7H_5N_2)^{\cdot+}$;40], 113[$(C_6H_5Cl+2)^+$;15], 77 [$(C_6H_5)^+$;40] & 57 [$(C_2H_3NO)^{\cdot+}$;40].

3-{5-[(4-Cl-phenoxy)-methyl]-4-*phenyl*-4H-1,2,4-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=C), 1125 s (C-O), 1027 s (C-Cl), 780 s (C-S); ^{1}H-NMR ($CDCl_3$, 600), (*J*=Hz) δ_H 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* 7.0, 2H, H b′ & H f′), 5.05 s (2H, -OCH_2 f), 3.51 t (*J* 6.8, 2H, CH_2 c′′), 2.99 t (*J* 6.9, 2H, CH_2 b′′), 2.25 s (3H, CH_3 b′′′′′), 2.23 s (3H, CH_3 a′′′′′); EIMS: *m/z (I, rel. %):* 285 [$M^{\cdot+}$] [$(C_{15}H_{15}N_3OS)^{\cdot+}$; 47], 175 [$(C_8H_5N_3S)^{\cdot+}$; 50], 121 [$(C_6H_5N_2O)^{\cdot+}$; 100], 117 [$(C_7H_5N_2)^{\cdot+}$; 40] & 77 [$(C_6H_5)^+$; 30].

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

Solid; IR (KBr) 3056 s (C-H), 1598 s (C=N), 1591 s (C-N), 1491 s (C=C), 1162 s (C-Cl) 1129 s (C-O), 759 s (C-S); ^{1}H-NMR ($CDCl_3$, 600), (*J*=Hz) δ_H 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, -OCH_2 f), 3.51 t (*J* 6.7, 2H, CH_2 c′′), 2.98 t (*J* 6.7 2H, CH_2 b′′), 2.32 s (6H, CH_3 1′′′′′ & CH_3 b′′′′′); EIMS: *m/z* (I, rel. %): 387 [$M^{\cdot+}$] [$(C_{18}H_{16}ClN_4O_2S)^+$; 5], 285 [$(C_{15}H_{15}N_3OS)^{\cdot+}$; 40], 175 [$(C_8H_5N_3S)^+$; 40], 121 [$(C_6H_5N_2O)^+$; 100],91 [$(C_7H_7)^+$; 20] & 77 [$(C_6H_5)^+$; 100]

3-{5-[(4-Cl-phenoxy)-methyl]-4-*phenyl*-4H-1,2,4-triazole-3-ylthio}-N-(4-ethoxy-phenyl)propanamide (8g)

Solid; IR (KBr, cm^{-1}) 3470 s (C-H), 1589 s (C-N), 1586 s (C=N), 1486 s (C=C), 1150 s (C-Cl), 1130 s (C-O), 836 s (C-S); ^{1}H-NMR ($CDCl_3$, 600), (*J*=Hz) δ_H 7.56 s (1H, NH), 7.56-7.52 m (3H, H c′′′′ to H e′′′′), 7.51 d (*J* 8.9 , 2H, H b′′′ & H f′′), 7.33 d (*J* 7.6 2H, H c′ & H e′), 7.23 d (*J* 8.8, 2H, H b′′′ & H f′′), 6.87 d (*J* 8.8 , 2H, H c′′′ & H e′′′), 6.83 d (*J* 7.6 , 2H, H b′ & H f′), 5.06 s (2H, -OCH_2 f), 4.02 q (*J* 7.0 2H, CH_2 a′′′′′), 3.51 t (*J* 6.9 2H, CH_2 c′′), 2.98 t (*J* 6.9 2H, CH_2 b′′), 1.42 t (*J* 6.9 , 3H, CH_3 b′′′′′); EIMS: *m/z (I, rel. %)*: 285 [$M^{\cdot+}$] [$C_{15}H_{15}N_3OS$; 5]$^{\cdot+}$, 224 [$C_{11}H_{144}NO_2S$; 5]$^+$, 132 [C_8H_6NO; 50]$^{\cdot+}$, 117 [$C_6H_4N_3$; 40]$^+$, 108 [C_6H_6NO; 100]$^+$ & 77 [C_6H_5; 45]$^+$

3-{5-[(4-Cl-phenoxy)-methyl]-4-*phenyl*-4H-1,2,4-triazole-3-ylthio}-N-(2-ethyl-phenyl)propanamide (8h)

Semi-solid; IR (KBr, cm^{-1}) 3110 s (C-H), 1599 s (C=N), 1593 s (C-N), 1491 s (C=C), 1164 s (C-Cl), 1119 s (C-O), 652 s (C-S); ^{1}H-NMR ($CDCl_3$, 600), (*J*=Hz) δ_H 8.07 s (1H, NH), 7.75 d (*J* 7.9, 1H, H-f′′′), 7.53 d (*J* 7.2, 2H, H c′′′′ & H e′′′′), 7.38 br. d (*J* 7.5, 1H, H e′′′), 7.34 d (*J* 7.0, 2H, H b′′′′ & H f′′′′), 7.22 d (*J* 9.7, 2H, H c′ & H e′), 7.15 t (*J* 7.3, 1H, H d′′′), 6.82 d (*J* 9.7, 2H, H b′ & H f′), 6.74 d (*J* 8.2, 1H, H c′′′), 5.06 s (2H, -OCH_2 f), 3.54 t (*J* 6.7, 2H, CH_2 c′′), 3.06 t (*J* 6.7, 2H, CH_2 b′′), 2.68 q (*J* 7.5, 2H, CH_2 a′′′′′), 1.21 t (*J* 7.5, 3H, CH_3 b′′′′′); EIMS: *m/z (I, rel. %):* 492 [$M^{\cdot+}$] [$(C_{26}H_{25}ClN_4O_2S)^{\cdot+}$;5], 236 [$(C_{12}H_{14}N_2OS +2)^{\cdot+}$;5], 285 [$(C_{15}H_{15}N_3OS)^{\cdot+}$;5], 209 [$(C_9H_6ClN_3O +2)^+$; 5] & 77 [$(C_6H_5)^+$; 45].

3-{5-[(4-Cl-phenoxy)-methyl]-4-*phenyl*-4H-1,2,4-triazole-3-ylthio}-N-(4-ethyl-phenyl)propanamide (8i)

Semi-solid; IR (KBr, cm^{-1}) 3140 s (C-H), 1596 s (C=N), 1597 s (C-N), 1541 s (C=C), 1511 s (-NO_2), 1341 s (-NO_2), 1124 s (C-O), 695 s (C-S); ^{1}H-NMR ($CDCl_3$, 600), (*J*=Hz): δ_H 8.66 s (1H, NH), 7.55-7.51 m (3H, H-c′′′′ to H e′′′′), 7.34 d (*J* 7.0 ,2H, H b′′′′ & H f′′′′), 7.21 d (*J* 8.8, 2H, H b′′′ & H f′′), 7.13 d (*J* 8.0, 2H, H c′ & H e′), 6.81 d (*J* 8.0, 2H, H b′ & H f′), 6.75 d (*J* 8.8, 2H, H c′′′ & H e′′′), 5.05 s (2H, -OCH_2 f), 3.51 t (*J* 6.7, 2H, CH_2 c′′), 3.01 t (*J* 6.7 2H, CH_2 b′′), 2.62 q (*J* 7.5, 2H, CH_2 a′′′′′), 1.22 t (*J* 7.5 3H, CH_3 b′′′′′); EIMS: *m/z (I, rel. %):* 492 [$M^{\cdot+}$] [$(C_{26}H_{25}ClN_4O_2S)^{\cdot+}$;5], 236 [$(C_{12}H_{14}N_2OS +2)^{\cdot+}$;5], 285

[$(C_{15}H_{15}N_3OS)^{·+}$;5], 209 [$(C_9H_6ClN_3O +2)^+$;5] & 77 [$(C_6H_5)^+$; 45].

3-{5-[(4-Cl-phenoxy)-methyl]-4-*phenyl*-4H-1,2,4-triazole-3-ylthio}-N-(2-methyl-phenyl)propanamide (8j)

Semi-solid; IR (KBr, cm^{-1}) 3110 s (C-H), 1599 s (C=N), 1594 s (C-N), 1491 s (C=C), 1164 s (C-Cl), 1125 s (C-O), 652 s (C-S); ^{1}H-NMR ($CDCl_3$, 600), (*J*=Hz): δ_H 8.14 s (1H, NH), 7.78 d (*J* 7.9 , 1H, H f′′), 7.56-7.53 m (3H, H c′′′′ to H e′′′′), 7.35 d (*J* 6.9 , 2H, H b′′′′ & H f′′′′), merged-br. d (*J* = 9.0 , 3H, H c′ & H 5′ and H e′′′), 7.09 t (*J* 7.2 1H, H d′′′), 6.82 d (*J* 9.0 Hz, 2H, H 2′ & H f′),6.75 d (*J* 8.9 , 1H, H c′′′), 5.07 s (2H, -OCH$_2$ f), 3.55 t (*J* 6.6 , 2H, CH$_2$ c′′), 3.07 t (*J* 6.6 , 2H, CH$_2$ b′′), 2.28 s (3H, CH$_3$ a′′′′′); EIMS: *m/z* 465 {[$(M^{·+}+2)-CH_3^{·}]^+$ [$C_{24}H_{20}ClN_4O_2S+2]^+$;3}, 387 [$(C_{18}H_{16}N_4O_2S)^+$;3], 339 [$(C_{18}H_{17}N_4OS+2)^+$;3], 224 [$(C_{13}H_{16}N_3O)^+$;3], 134 [$(C_8H_8NO)^+$;3], 107 [$(C_6H_5NO)^+$;100], 113 [$(C_6H_4Cl+2)^+$;15] & 77 [$(C_6H_5)^+$;10].

3-{5-[(4-Cl-phenoxy)-methyl]-4-*phenyl*-4H-1,2,4-triazole-3-ylthio}-N-(3-methyl-phenyl)propanamide (8k)

Semi-solid; IR (KBr, cm^{-1}) 3110 s (C-H), 1599 s (C=N), 1590 s (C-N), 1491 s (C=C), 1164 s (C-Cl), 1122 s (C-O) and 652 s (C-S); ^{1}H-NMR ($CDCl_3$, 600), (*J*=Hz): δ_H 8.66 s (1H, NH), 7.54-7.51 m (3H, H c′′′′ to H e′′′′), 7.49 br. s (1H, H b′′′), 7.40 dis. dd-(merged in signal of H b′′′′ & f′′′′) (*J* 2.3 & 7.6 , 1H, H f′′), 7.34 d-(merged in dd of H f′′) (*J* 7.3 , 2H, H b′′′′ & H f′′′′), 7.21 br. d (*J* 8.6 2H, H c′ & H e′), 6.92 d (*J* 7.4, 1H, H e′′′), 6.80 br. d (*J* 8.6 , 2H, H b′ & H f′), 6.74 d (*J* 8.5 , 1H, H d′′′), 5.06 s (2H, -OCH$_2$ f), 3.51 t (*J* 6.7 2H, CH$_2$ c′′), 3.02 t (*J* 6.7 , 2H, CH$_2$ b′′), 2.32 s (3H, CH$_3$ a′′′′′); EIMS: *m/z(I, rel. %):* 285 [$M^{·+}$] [$(C_{15}H_{15}N_3OS)^{·+}$;3], 134 [$(C_8H_8NO)^+$;3], 107 [$(C_6H_5NO)^+$;100] & 77 [$(C_6H_5)^+$; 10]

The synthesized derivatives were screened against Urease which revealed that they exhibited moderate to good inhibitory potential as evident from their IC_{50} values. Thiourea was used as control having IC_{50} of 21.25±0.15 μmolL^{-1} tabulated in (table 1). The attachment of 8k with amino acid moieties of enzyme reaction sites all over the molecule supported the bioactivity results and was in accordance of the docking studies.

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 $C_{20}H_{23}N_3OS$ (8k), 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, 1590, 1491, 1164, 1122 and 652 for C-H, C=N 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. A_2B_2 spin system showed *diortho* coupled doublets one at δ 7.21 and other at δ 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 region a singlet was observed at δ 5.06 for -CH$_2$ group flanged between 4-Cl-phenoxy and 4-phenyl-1,2,4-triazole moieties. Two triplets resonated at δ 3.02 and 3.51 for 2 CH$_2$ groups at b′′′ and c′′′. Finally a singlet at δ 2.32 confirmed the CH$_3$-a′′′′′ group presence proving the projected structure[13]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, 129.4, 128.7, 126.8, 124.9, 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-a′′′′, 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-c′′′, C-a′′′′′ respectively. On basis of aforementioned collective substantiation, the structure was 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 50 μM concentration. 8c, 8k and 8d showed excellent activity with cell viability of 0.16±0.16 μM, 0.75±0.02 μM and 6.25±0.53 μM respectively. Compounds 8b, 8e, 8f & 8i showed moderate activity with cell viability in range of 22.25-53.64 μM. 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 8c, 8h and 8k could be considered as potent candidates.

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.

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