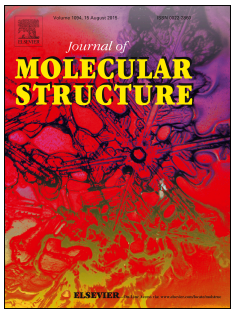

Design, synthesis, biological activity, molecular docking and computational studies on novel 1,4-disubstituted-1,2,3-Triazole-Thiosemicarbazone hybrid molecules

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Credit Author Statement

Naveen: Validation, Formal analysis, Investigation, Molinspiration Physicochemical Parameters Software **Ram Kumar Tittal:** Conceptualization, Methodology, Resources, Data Curation, Visualization, Writing -Original Draft, Writing- Review & Editing, Supervision **Ghule Vikas D.:** Software, Data Curation **Nikhil Kumar:** Load & solved X-ray Single Crystal data **Lokesh Kumar:** Formal analysis, Investigation **Kashmiri Lal:** Conceptualization, Methodology, Resources, Data Curation, **Ashwani Kumar:** Docking study.

Design, Synthesis, Biological Activity, Molecular Docking and Computational Studies on Novel 1,4-Disubstituted-1,2,3-Triazole-Thiosemicarbazone Hybrid Molecules

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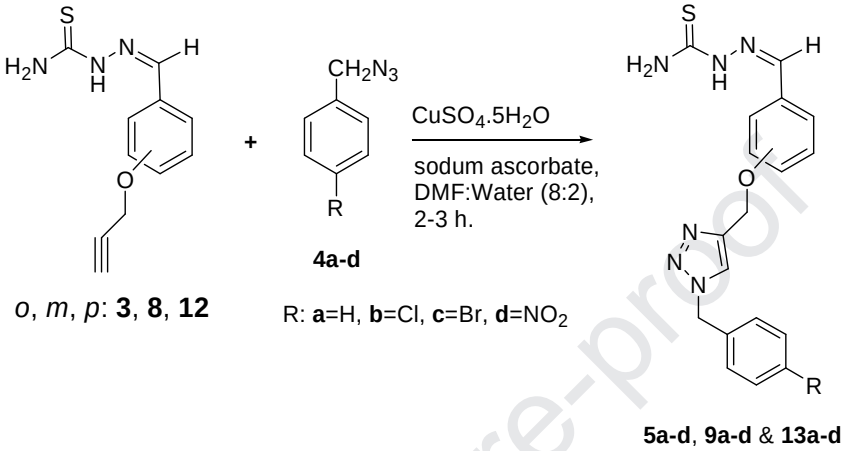

Biologically Significant 1,2,3-triazole-thiosemicarbazone hybrid molecules were synthesized through Cu(I)-catalysed cycloaddition reaction of thiosemicarbazone-linked alkynes and aromatic azides. The structures of all alkynes and 1,2,3-triazole-thiosemicarbazone hybrid molecules were characterized by IR, ^{1}H NMR, ^{13}C NMR & HRMS data structure of alkynes **3** & **12** were finally supported by X-ray crystallographic data. However, some alkynes were finally supported by single-crystal structures. The synthesized 1,2,3-triazole-TSC hybrid molecules were screened as active antimicrobials and activity results were supported by the molecular docking and DFT studies.

Design, Synthesis, Biological Activity, Molecular Docking and Computational Studies on Novel 1,4-Disubstituted-1,2,3-Triazole-Thiosemicarbazone Hybrid Molecules

Naveen,[a] Ram Kumar Tittal,*[,a] Ghule Vikas D.,[a] Nikhil Kumar,[a] Lokesh Kumar,[b] Kashmiri Lal,*[,b] and Ashwini Kumar[c]

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Abstract: A library of some novel 1,4-disubstituted-1,2,3-triazole-thiosemicarbazone hybrid molecules were designed and synthesized from (4-Prop-2-ynyloxy-benzylidene)-thiosemicarbazone and aryl azides under Cu(I)-catalyzed cycloaddition reaction. All newly synthesized [4-(1-Benzyl-1H-[1,2,3]triazol-4-ylmethoxy)-benzylidene] -thiosemicarbazone hybrid molecules were efficiently characterized by IR, ^{1}H NMR, ^{13}C NMR, HRMS and structure of alkynes **3** & **12** were finally supported by X-ray crystallographic data. Compounds **5c**, **5d**, **9c**, **9d 13c** and **13d** demonstrated excellent potency results for *B. Subtilis* and *P. Aeruginosa* bacterial strains with MIC values 0.0141, 0.0152, 0.0562, 0.0608, 0.0141, 0.0608, 0.0141, 0.0304, 0.0281, 0.0304, 0.0281, 0.0304, respectively as compared to reference drug Ciprofloxacin. Antibacterial activity results were supported by molecular docking and DFT studies**.**

Keywords: 1,2,3-triazole-thiosemicarbazone • CuAAC • Antibacterial activity • Molecular docking • DFT study.

Introduction:

In most of the cases, infections in human beings are non-invasive and temporarily silent. According to WHO, these slumbering infections lead to an active disease which may be cured by medicines. Sometimes the situation becomes intricate due to multidrug resistance (MDR) to bacterial and fungal diseases throughout the whole world.[1] This prominence MDR to pathogenic strains delays the proper remedy to the infections imposed by various strains.[2] Bacterial diseases many times become worse and even lead to death. In spite of substantial advancement in medicinal chemistry, it remains a challenge to the medicinal chemist to develop more efficient and safer antibacterial drugs. There is a desperate need of coherent approach for the development of novel and more potent drugs. The potency of drug mainly demonstrated by its prime efficacy, lesser virulence, and better selectivity. Now, medicinal chemists are mainly attracted towards the synthetic tactics of molecular hybridization. It involves the coupling of several pharmacophoric units via orthodox bonding to form a single paramount hybrid molecule. The resulted hybrid molecules generally show improved properties as compared to parent molecules.

Recently, authors have reported the synthesis, molecular docking and DFT studies of biologically active 1,4-disubstituted-1,2,3-triazolesemicarbazone hybrid molecules with better antibacterial activities as compared to commonly used antibiotic Ciprofloxacin.[3] In ancient times, elemental sulfur was known for pharmacophoric properties and was used for insecticides and fungicides. With these observations, we presumed to investigate, design and synthesize thiosemicarbazone linked triazole hybrid molecules. Thiosemicarbazone unit is a sulfur analogy of semicarbazone which may possess better biological activities. Thiosemicarbazide commonly abbreviated as TSC, also known as hydrazine carbothioamide, is a sulphur-containing thiocarbonyl organic compound that shares C=N-NH-(C=S)-NH moiety and known as thiosemicarbazone (TSC). The TSC and its derivatives usually prepared by the condensation reaction between aldehydes or ketones with thiosemicarbazide under Schiff's base condition.[4] Because of better chemical reactivity, physiological properties, versatile biological and pharmacological activities, TSC and its derivatives have unparalleled applications in the synthetic and

medicinal fields. The broad antimicrobial spectrum of thiosemicarbazone has made a suitable place in the pharmaceutical industry. Also, TSC is an excellent chelating ligand which remains a versatile synthon for the synthesis of important therapeutic compounds. Antiviral properties of thiosemicarbazone were first reported by Hamre et al in 1950.[5] In fact, thiosemicarbazone was the first representative to be used as an active molecule for the development of an antiviral drug. Thiosemicarbazone linked with isatin unit reportedly found active against smallpox or cowpox.[6] Sidwell and co-workers found purine-6-carboxyaldehyde linked with thiosemicarbazone effective against the herpes virus.[7] Brockman and co-workers have reported thiosemicarbazone as active against herpes simplex virus.[8] Klayman et al. tested the antimalarial activity of thiosemicarbazone linked with 2-acetyl pyridine.[9] Dobek lab found the activity of thiosemicarbazone substituted-2-acetyl pyridine activity against mycobacteria.[10] Hybrid compounds containing TSC moiety or metal complexes of TSC have been reported in the literature to possess more promising biological activities like antiviral,[11] antitumor,[12] antibacterial,[13] antitubercular,[14] antidiabetic,[15] antimicrobials,[16] anticonvulsant,[17] anticancer,[18] antimalarial,[19] anti-inflammatory and antiameobic activity, etc.[20]

Similarly, 5-member nitrogen-containing heterocycles like 1,2,3-triazoles are the most affluent constitutional ingredient to various drugs and biomaterials. This is due to stability towards acidic and basic hydrolysis even under extreme redox conditions.[21] Because of polar nature, triazoles behave as a hydrogen acceptor, hydrogen donor, can show dipole-dipole interaction and increases solubility in an aqueous medium. Thus, show several types of interactions with the target bioactive conjugates and protect it from metabolic degradation under extreme reaction conditions, chemical environments, and reagents. The 1,3-dipolar cycloaddition reaction between terminal alkyne and azide by Huisgen method is the most prevalent and robust strategy for the synthesis of 1,4-disubstituted-1,2,3-triazole but leads to the formation of an almost equivalent amount of 1,4 and 1,5-isomers. Recently, the modified Huisgen Cu(I)-catalyzed cycloaddition reaction between an azide and terminal alkyne under CuAAC reaction condition by Medal,[22] and Sharpless[23] has emerged as a premier example of click reaction. The term click reactions are versatile, highly selective, compatible with a wide variety of functional groups, efficient and accelerate the transformation of reactant into only one isomeric product. The removal of homogeneous Cu-catalyst from the reaction mixture always remains a big issue. Various types of procedures have emerged in literature for the regioselective synthesis of 1,4-disubstituted-1,2,3-triazole.[24] The ease in formation, high yield and stability of 1,4-disubstituted-1,2,3-triazoles are currently used as building blocks in fascinating biological activities like anticancer,[25-27] antioxidant,[28] antitubular,[29] antimalarial,[30] anti-HIV,[31] antimicrobial,[32-34] and anti-inflammatory etc.[35]

Considering medical importance of both TSC & 1,4-disubstituted-1,2,3-triazole units in the light of our attention for synthesizing pharmacologically active compounds, it was envisioned to synthesize a family of 1,2,3-triazole-thiosemicarbazone hybrids (**Figure 1**) via molecular hybridization approach with a view to assess their antimicrobial properties against the human pathogens like *E. coli*, *B. subtilis*, *S. aureus*, *P. Aeruginosa*, & *S. Enteric*.

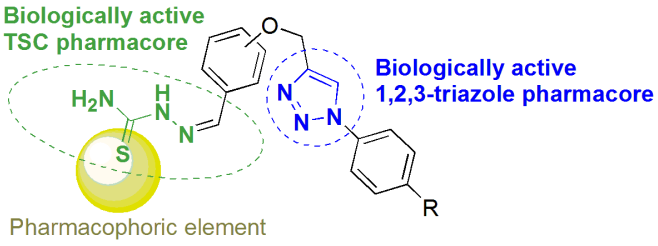
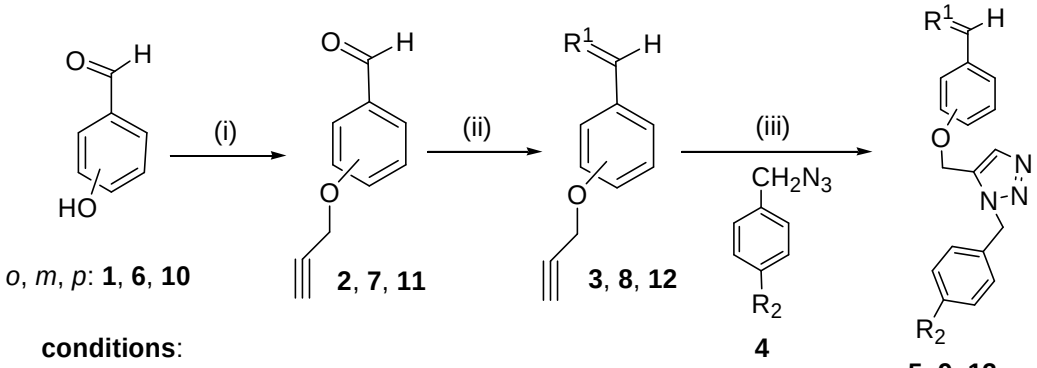


Figure 1. Designed 1,2,3-triazole-thiosemicarbazone hybrid molecule.

Results and Discussion

A series of novel 1,2,3-triazole-thiosemicarbazone hybrid molecules were synthesized in three steps using the reported procedures with slight modifications as described in **Scheme 1**.[3] The first step commenced with propargylation of commercially available *o*, *m* & *p*-hydroxybenzaldehyde by refluxing



conditions:
(i) Propargyl bromide, K_2CO_3, Acetone, refulx, 8h
(ii) $NH_2NHCSNH_2$.HCl, ethanol, reflux
(iii) **4a-d**, $CuSO_4.5H_2O$, sodum ascorbate, DMF:Water (8:2), 2-3 h.
R^1: $NNHCSNH_2$, R^2: **a**=H, **b**=Cl, **c**=Br, **d**=NO_2

Scheme 1. Synthesis of TSC-triazole hybrid molecules (**5a-d**, **9a-d**, **13a-d**).

with propargyl bromide in acetone using anhydrous K_2CO_3. In the second step, the prepared *o,m,p*-propargyloxy benzaldehyde was subjected for condensation reaction with thiosemicarbazide in dry C_2H_5OH for 4 hrs. The so obtained thiosemicarbazone linked alkynes (TSC-alkyne) were recrystallized with alcohol in 90-94% yields. The formation of TSC-alkynes (**3**, **8**, **12**) was efficiently characterized by IR, ^{1}H NMR, and ^{13}C NMR spectroscopic data. For reference, the Infra-Red spectroscopic data of compound **12** showed two major stretching peaks at ν_{max} 3282 & 1606 cm^{-1} due to terminal alkyne ≡CH and −C=N−, respectively for the formation of thiosemicarbazone of propargyl benzaldehyde. Also, the ^{1}H NMR spectrum of **12** showed a triplet peak at δ 3.60 ppm equivalent to one hydrogen and another peak at δ 11.34 ppm equivalent to another one hydrogen due to ≡CH(alkyne) group and H−C=N− group, respectively to confirm the formation of TSC-alkynes. The HRMS spectrum of alkyne **12** showed a major peak at m/z 234.077 corresponds to $[M+H]^+$. Finally, the structures of TSC-alkynes **3** and **12** were supported by the x-ray diffraction data.[36] The crystal structure diagram of alkynes **3** and **12** shown in **Figure 2** and **3**. The crystal data and crystal structure refinement parameters of **3** and **12** are given in **Table SI-1** (S.I. file).

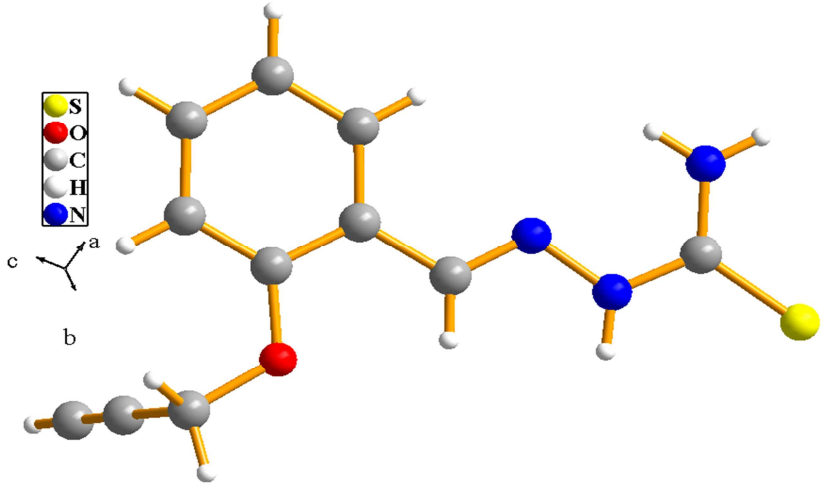


Figure 2. Crystal structure of compound **3**. Asymmetric unit contains two such molecular units.

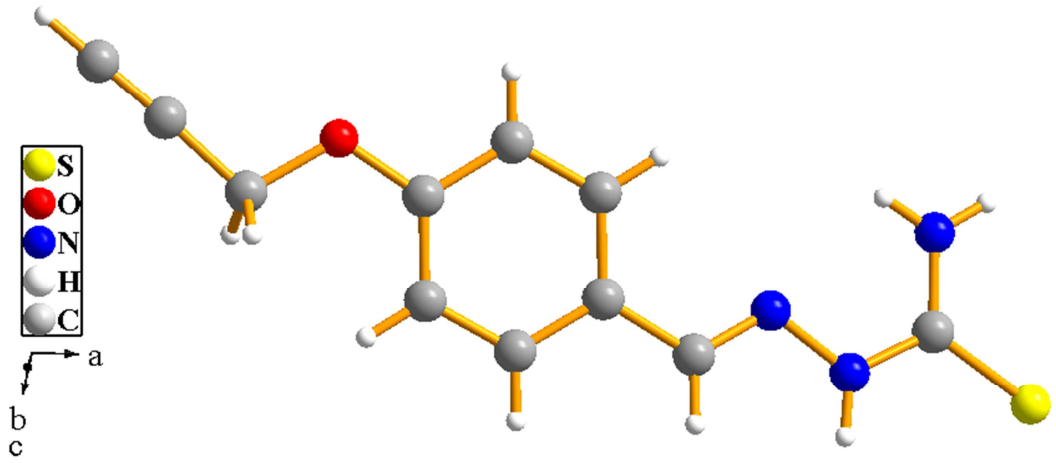


Figure 3. ORTEP diagram of **12** with 50% probability (H atoms are removed for clarity).

The starting material for the click reaction, i.e. TSC-alkyne (**3**, **8** & **12**) were subjected to react with in situ generated organic azides in the presence of $CuSO_4.5H_2O$ in DMF as a solvent to afford different 1,4-disubstituted-1,2,3-triazole-TSC hybrids (**5a-d**, **9a-d** & **13a-d**). The results with reaction details are summarized in **Table 1**. The structures of all the synthesized triazole hybrids were efficiently characterized with the help of IR, ^{1}H NMR, ^{13}C NMR and HRMS data. For instance, the appearance of a band at 3151 cm^{-1} in the IR spectrum of compound **13b** showed the formation of the triazole ring. The ^{1}H NMR spectrum of compound **13b** showed singlet peak at δ 7.93 ppm equivalent to one hydrogen due to -CH of triazole ring to confirm the formation of 1,2,3-triazole-TSC. The ^{13}C NMR spectrum of **13b** showed peaks at δ 143.29 and 125.25 ppm, which were assigned to C-4 and C-5 carbon atoms of the triazole rings. Two peaks appeared at δ 61.64 and 52.53 ppm were due to the presence of two methylene carbons attached to N-1 and C-4 of the triazole ring, respectively. The HRMS spectrum of **13b** showed the m/z peak at 401.1084 corresponds to $[M+H]^+$. All other compounds also exhibited a similar pattern in their spectra and the characterization details are presented in S.I. file.

Table 1. Synthesis of terminal alkyne (**3**, **8** & **12**) and 1,2,3-triazole-linked-thiosemicarbazone (**5a-d**, **9a-d**, **13a-d**)[a].

S. No.	Compounds	R^2	Time (h)	Yield (%)[b]
1	**3**	--	4	92
2	**5a**	H	3	85
3	**5b**	Cl	2.5	86
4	**5c**	Br	2.5	90
5	**5d**	NO_2	2	92
6	**8**	--	4	90
7	**9a**	H	3	90
8	**9b**	Cl	2.5	91
9	**9c**	Br	2.5	90
10	**9d**	NO_2	2	89
11	**12**	--	4	94
12	**13a**	H	2.5	88
13	**13b**	Cl	2.5	85
14	**13c**	Br	2	90
15	**13d**	NO_2	2	92

[a]Benzyl azide **4a-d** & alkynes **3**, **8**, & **12** (1 mmol); $CuSO_4.5H_2O$ (10 mol%) and Na-asc. (20 mol%), rt, 2-3 h. [b]Yield refers to pure compound obtained after recrystallization.

A library of newly synthesized TSC-1,2,3-triazole hybrid molecules was screened *in vitro* for antibacterial activity against gram-positive bacteria: *Staphylococcus Aureus* (MTCC 3160),

Staphylococcus Enteric and *Bacillus Subtilis* (MTCC441) and gram-negative bacteria: *Escherichia Coli* (MTCC 16521) and *Pseudomonas Aeruginosa* (MTCC 424) by a standard serial dilution method. A stock solution of 100 µg/ml concentration in DMSO solvent was prepared. A commonly used antibiotic drug Ciprofloxacin was selected as a reference drug. The stock solution of compounds and reference were diluted to 50 to 6.25 µg/mL concentration. Now each concentration lot was inoculated with 100 µg/mL suspension of respective bacteria in sterile saline and incubated at 37 °C for 24 hrs. To observe the effect of solvent on bacterial growth, a blank test was also performed at the experimental condition with the nutrient medium. The minimum inhibitory concentration (MIC) of the triazole was recorded in µmol/mL, as shown in **Table 2**.

Table 2. In vitro antibacterial data of compounds **3**, **5a-d**, **8**, **9a-d**, **12**, **13a-d** (MIC in µmol/mL).

Comp.	R	A	B	C	D	E
3	---	0.1073	0.1073	0.2145	0.1073	0.2146
5a	H	0.0683	0.1366	0.0683	0.1366	0.0683
5b	Cl	0.0312	0.0624	0.0312	0.0624	0.0624
5c	Br	0.0141	0.0562	0.0281	0.0141	0.0562
5d	NO_2	0.0152	0.0608	0.1217	0.0304	0.1217
8	----	0.1073	0.1073	0.2146	0.1073	0.2146
9a	H	0.0683	0.0683	0.0683	0.0683	0.0683
9b	Cl	0.0624	0.0312	0.0624	0.0312	0.1248
9c	Br	0.0562	0.0281	0.0562	0.0281	0.0281
9d	NO_2	0.0608	0.0304	0.0304	0.0304	0.0608
12	----	0.1073	0.1073	0.2146	0.1073	0.2146
13a	H	0.0683	0.0683	0.0683	0.1366	0.1366
13b	Cl	0.0624	0.0312	0.0312	0.0156	0.0624
13c	Br	0.0141	0.0562	0.0281	0.0281	0.1124
13d	NO_2	0.0608	0.0304	0.0608	0.0304	0.0304
Ciprofloxacin		**0.1465**	**0.0366**	**0.0732**	**0.0732**	**0.0732**

A: *B. Subtilis*; **B**: *S. Aureus*; **C**: *E. Coli*; **D**: *P. Aeruginosa*; **E**: *S. Enteric*.

The antibacterial screening results of alkynes and triazole hybrids (**3**, **5a-d**, **8**, **9a-d**, **12** & **13a-d**) were compared with each other and reference drug. Wherein, it was observed that the incorporation of triazole units on to the TSC-alkynes have increased the antibacterial potency of almost all 1,4-disubstituted-1,2,3-triazole-TSC hybrid molecules for all bacterial strains except few. Also, it was observed that on comparing antibacterial activity results of TSC-alkynes and triazole-TSC hybrid molecules with commonly used reference drug Ciprofloxacin, compounds **5c**, **5d**, **9c**, **9d 13c** & **13d** demonstrated excellent potency results for *B. Subtilis* and *P. Aeruginosa* bacterial strains with MIC values 0.0141, 0.0152, 0.0562, 0.0608, 0.0141, 0.0608, 0.0141, 0.0304, 0.0281, 0.0304, 0.0281, 0.0304, respectively. However, among other bacterial strains, *S. Aureus* showed excellent potency for **9c**, **9d**, & **13d**; *E. Coli* for **5c**, **9c**, **9d**, **13c** & **13d** and *S. Enteric* showed excellent activity for **5c**, **9c**, **9d** & **13d**. Similarly, for compound **5c** best potency results were observed for *B. Subtilis*, *E. Coli* and *P. Aeruginosa* with MIC 0.01405, 0.02809 & 0.01405 µmol/mL as compared to Ciprofloxacin with MIC 0.14648, 0.07324 & 0.07324 µmol/mL, respectively. Likewise, compound **9c** showed the better potency for both *S. Aureus*, and *S. Enteric* with MIC 0.0281 µmol/mL and compound **13c** showed best results for *B. Subtilis* and *E. Coli* with MIC 0.01405 & 0.02809 µmol/mL, respectively as compared to drug Ciprofloxacin (MIC 0.14648 & 0.07324 µmol/mL).

Also, the antibacterial activities of recently reported 1,2,3-triazole-semicarbazone hybrid molecules (**Figure 4a**) were compared with these newly synthesized 1,2,3-triazole-TSC molecules (**Figure 4b**) for some tested strains of bacteria, i.e. *E. Coli*, *B. Subtilis*, *S. Aureus*, *P. Aeruginosa* & *S. Enteric* as listed in **Table 3**. It was observed and revealed that TSC-derivatives are more potent or active as compared to

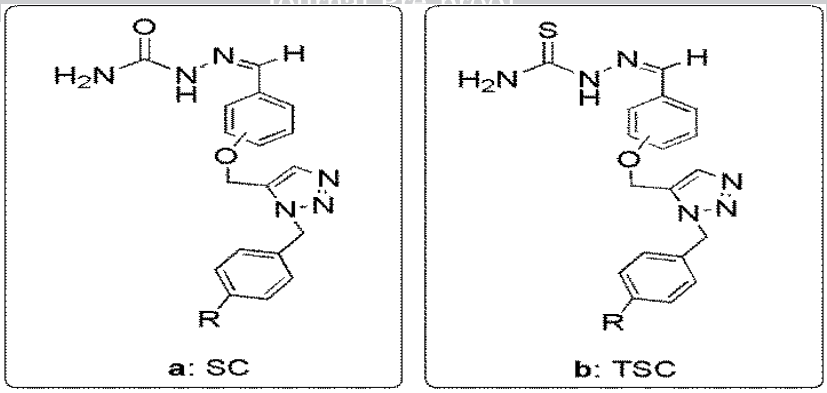


Figure 4. a: Semicarbazone hybrid & **b**: Thiosemicarbazone hybrids.

Table 3. Comparison of antimicrobial activity of SC & TSC hybrids.

Alkyne/R	***E. Coli***		***B. Subtilis***	
	SC	TSC	SC	TSC
o-Alkyne	0.11520	0.21459	0.11520	0.10730
H	0.07440	0.06831	0.07440	0.06831
Cl	0.06747	0.03121	0.06747	0.03121
Br	0.06024	0.02809	0.06024	0.01405
NO_2	0.13123	0.12166	0.06562	0.01521
p-Alkyne	0.23041	0.21459	0.11520	0.10730
H	0.07440	0.06831	0.07440	0.06831
Cl	0.13495	0.03121	0.06747	0.06242
Br	0.12048	0.02809	0.12048	0.01405
NO_2	0.06562	0.06083	0.06562	0.06083
Alkyne/R	***S. Aureus***		***P. Auroginosa***	
	SC	TSC	SC	TSC
o-Alkyne	0.11520	0.10730	0.11520	0.10730
H	0.03720	0.13661	0.07440	0.13661
Cl	0.06747	0.06242	0.06747	0.06242
Br	0.06024	0.05618	0.06024	0.01405
NO_2	0.06562	0.06083	0.06562	0.03041
p-Alkyne	0.05760	0.10730	0.11520	0.10730
H	0.03720	0.06831	0.07440	0.13661
Cl	0.03374	0.03121	0.06747	0.01561
Br	0.12048	0.05618	0.12048	0.02809
NO_2	0.03281	0.03041	0.06562	0.03041

o-/P-Alk: ortho/para-alkyne; SC: Semicarbazone; TSC: Thiosemicarbazone

the corresponding semicarbazone analogues except for **5a** and **13a** with unsubstituted benzyl (R=H), which showed little discouraging results for *S. Aureus* & *P. Auroginosa* bacterial strains. Also, TSC-lin--ked ortho-alkynes for *E. Coli* and para-alkyne for *S. Aureus* showed less potency then corresponding semicarbazone linked alkynes.

The structure-activity relationship (SAR) results showed that all triazole derivatives exhibited better efficacy and synergistic effect in the biological activity of TSC-triazole hybrids than the parent pharmacophoric units i.e. either TSC or 1,2,3-triazole. The **Table SI-1** (S.I. file) revealed that the substitution of benzyl moiety by electron-withdrawing groups (Br and NO_2) like **5c**, **5d**, **9c**, **9d**, **13c**, and **13d** exhibited broad-spectrum activity against studied bacterial strains. It was also observed that the compounds substituted with Br- on benzyl moiety (**5c**, **9c** and **13c**) showed more promising antibacterial activity than other compounds.

The physicochemical properties of 1,2,3-triazole-TSC hybrids were calculated by using Lipinski's rule of five, which acts as a filter for drug likeliness and to deduce their pharmacokinetic parameters. It includes absorption, distribution, metabolism and excretion from the human body. The value of milogP (octanol-water partition coefficient) of all synthesized compounds is less than 5.0 (2.1 to 4.09) indicating their high affinity and excellent permeability across the lipid bilayers of biological membranes. Also, the molecular weight less than 500 (366.45 to 445.35) showed easy diffusion, transportation and absorption. The sum of -OH and -NH groups showed that the hydrogen bond donor is less than 5.0 (3 for all) and hydrogen bond acceptor are less than 10 (i.e. 7-10). Therefore, the no. of violation to the Lipinski's rule of five is zero, confirming their easy binding to receptors. Also, the numbers of rotational bonds are less than 10 (i.e. 5-9), thus confirming their good flexibility. The value of total polar surface area (TPSA) was less than 160Å^2 (59.65 Å2 to 136.19 Å2) which presented it to be a good predictor of transport properties like intestinal absorption, blood barrier penetration, etc. The mathematical equation of %ABS is equal to 109-(0.345 x TPSA).[37] All the presented alkyne-TSC and triazole-TSC hybrid molecules exhibited good absorption (%ABS = 62.01 to 88.42). According to Lipinski's rule of five, any molecule violating more than one of these rule decreases the activity and selectivity of a drug-like compound to make unlikely orally-active for human beings. It is clear from the above discussion that parameters of all the tested compounds were within the limits of Lipinski's rule of five with violation number equal to zero and have good drug likeliness property. The bioactivity score of all the synthesized compounds summarized in **table SI-2** (S.I. file) by calculating the activity score of GPCR (protein-coupled receptor) ligand, ICM (ion channel modulator), KI (kinase inhibitor), NRL (nuclear receptor ligand), PI (protease inhibitor), and EI (enzyme inhibitor). The bioactivity score of all the compounds was >-0.5, which revealed that the synthesized compounds are active.

The reason for the higher activity of 1,2,3-triazole-TSC hybrid molecules as compared to TSC-alkynes could also be explained by the docking studies, the results of study represented in **Figure 5** & **6**.

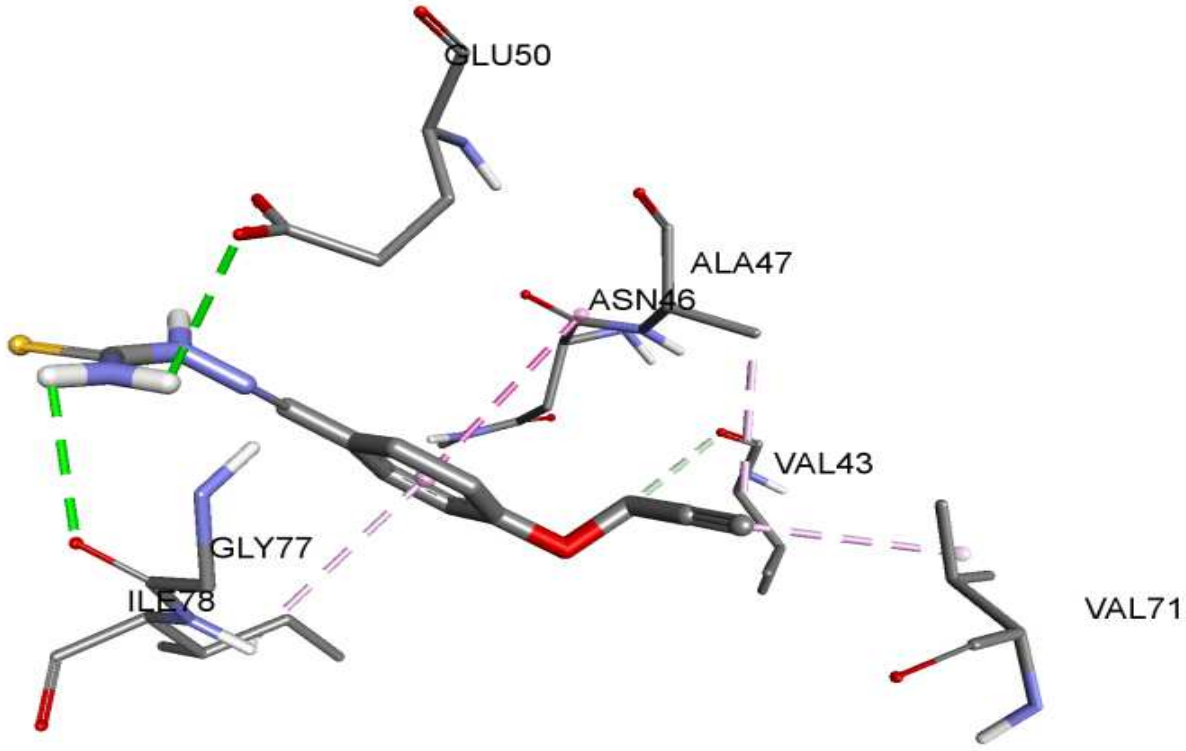


Figure 5. Interactions of alkyne **12** with active sites of DNA gyrase.

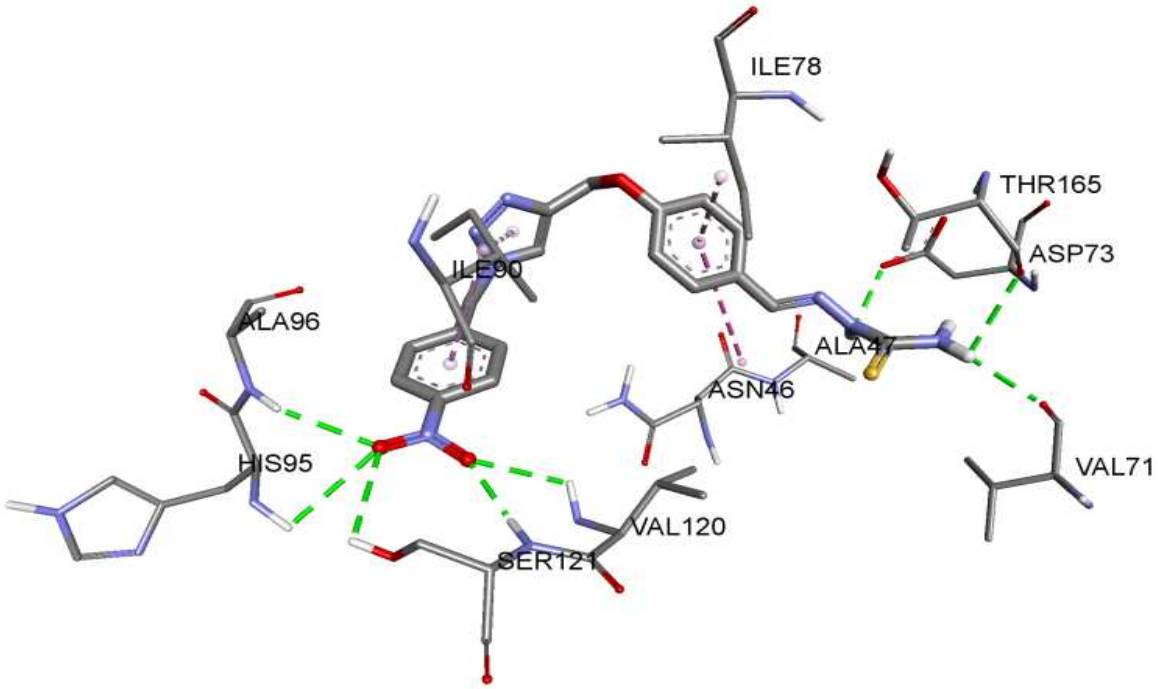


Figure 6. Interactions of a triazole **13d** with the active site residues of DNA gyrase.

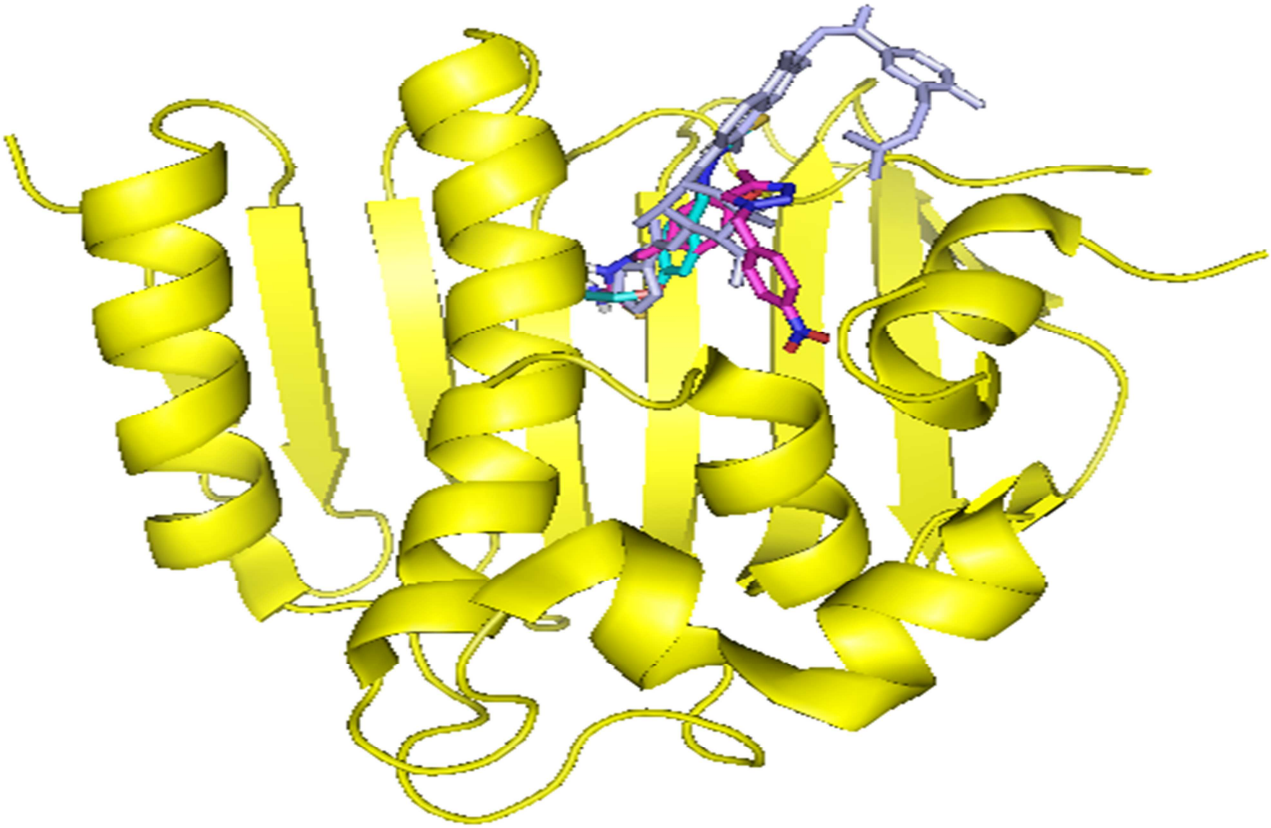


Figure 7. Cartoon diagram of DNA gyrase with co-crystallized ligand, alkyne **12**, and triazole **13d**.

Both molecules were docked in the active site of topoisomerase II of *E. Coli* (PDB ID: 1KZN).[38-40] This showed interactions of alkyne and triazole with the active sites of DNA gyrase. The two hydrogens of the terminal amino group were involved in hydrogen bonding (**Figure 5**) with oxygen atoms of Glu50 and Gly77. Amide-π interaction was observed between the aromatic ring of alkyne **12** and the peptide bond between Asn46 and Ala47. A one carbon-hydrogen bonding was seen between carbon attached to oxygen in alkyne and the carbonyl oxygen of Val43. In triazole **13d**, the NO_2 group has created a web of hydrogen bonding with His95, Ala96, Val120 and Ser121 (**Figure 6**). The terminal –

NH_2 group exhibited hydrogen bonding with Val71 and Thr165 while second NH group involved in hydrogen bonding with Asp73. The π-orbitals of the aromatic ring were involved in the amide-π stacking interactions with the peptide bond between Asn46 and Ala47. The hydrogen bonding was represented by the intense green colour dotted lines and the amide-π stacking represented by the pink dotted lines. In addition, the binding affinity of triazole was -8.2 kcal/mol in comparison to that of alkyne -6.6 kcal/mol. All these facts support the higher activity of triazole as compared to the alkyne. The cartoon diagram of DNA gyrase along with co-crystallized ligand, alkyne, and triazole is shown in **Figure 7**.

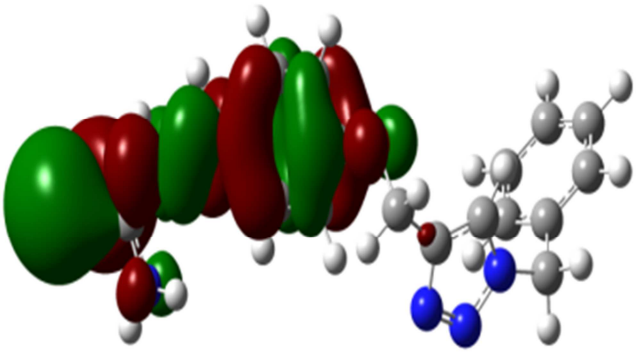
All the molecules included in the study were optimized at the B3LYP/6-311G(d,p) level using the Gaussian09 program package.[41] With the help of frontier molecular orbitals (FMOs) of any chemical compounds, one can determine important parameters such as chemical reactivity needed for drug design.[42-44] A molecule with a high value of highest occupied molecular orbitals (HOMOs) can donate electrons to the least unoccupied molecular orbitals (LUMOs). The value of HOMO and LUMO determines the electronic transitions and the reactivity of molecules. These values also help to predict the frontier orbital energies (FMOs), chemical potential (μ), chemical hardness (η), and electrophilicity index (ω) as presented in **Table 4**. The values of μ, η, and ω can be obtained by using Koopmans' theorem and Parr approximation,[45] as described in the SI (see, S. I. file). We have observed that **5a-5d** derivatives exhibited higher bandgap as compared to **9a-9d** and **13a-13d** derivatives. Replacement of Cl- of **5b** with –Br in **5c** didn't show any change in the bandgap.

Table 4. Calculated FMOs, energy gap, chemical potential, chemical hardness, and electrophilicity index in eV at the B3LYP/6-311G(d,p) level of theory.

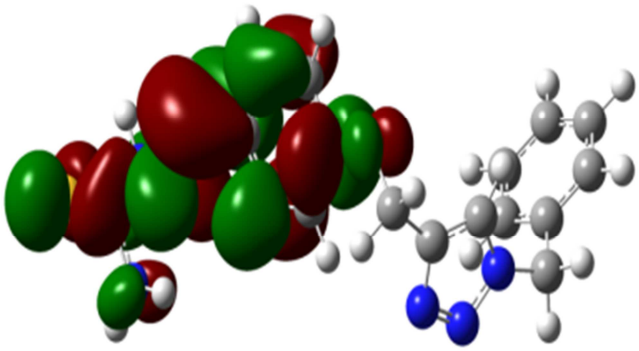
Compd.	E_{HOMO} (eV)	E_{LUMO} (eV)	$E_{LUMO-HOMO}$ (eV)	μ (eV)	η (eV)	ω (eV)
3	-5.60	-1.69	3.91	-3.64	1.96	3.39
5a	-5.64	-1.59	4.05	-3.62	2.03	3.23
5b	-5.91	-1.72	4.19	-3.81	2.10	3.47
5c	-5.91	-1.71	4.19	-3.81	2.10	3.46
5d	-6.01	-2.73	3.27	-4.37	1.64	5.84
8	-5.90	-1.84	4.05	-3.87	2.03	3.69
9a	-5.62	-1.93	3.69	-3.77	1.84	3.87
9b	-5.68	-1.60	4.08	-3.64	2.04	3.24
9c	-5.67	-1.59	4.08	-3.63	2.04	3.24
9d	-5.73	-2.78	2.95	-4.26	1.48	6.14
12	-5.71	-1.80	3.91	-3.75	1.96	3.60
13a	-5.58	-1.64	3.94	-3.61	1.97	3.31
13b	-5.75	-1.91	3.84	-3.83	1.92	3.81
13c	-5.75	-1.90	3.84	-3.82	1.92	3.80
13d	-5.86	-2.85	3.01	-4.35	1.50	6.30

However, the introduction of $–NO_2$ group in **5d** significantly reduced the bandgap and revealed more mobility of electrons. A similar trend was observed in meta and para derivatives. The predicted hardness values of $–NO_2$ derivatives of **5d**, **9d**, and **13d** were 1.64 eV, 1.48 eV, and 1.50 eV, respectively that showed higher reactivity. On the other hand, the corresponding electrophilicity index of these molecules revealed their fewer stabilization energies. The HOMO and LUMO of **13a-d** along with molecular electrostatic potential (MESP) maps were calculated as illustrated in **Figure 8**. In a comparison study of bandgap energy ($\Delta E_{LUMO-HOMO}$) of TSC-triazoles (**5a-d** & **13a-d**) with recently reported semicarbazone derivatives as shown in table 5 revealed that 1,2,3- triazole-TSC hybrids molecules are soft with less bandgap energy. Thus, 1,2,3-triazole-TSC hybrid molecules with lower bandgap energy were more reactive as compared to the 1,2,3-triazole-SC hybrid molecules. It was

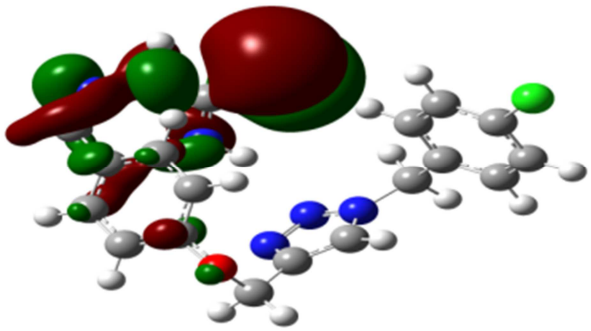
revealed that 1,2,3-triazole-thiosemicarbazone hybrid molecules are more reactive and are better antimicrobial substitutes to current drug Ciprofloxacin.



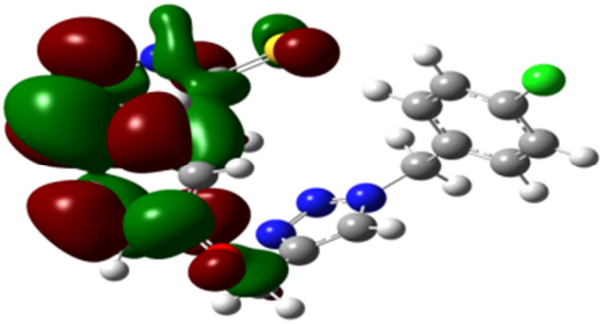
13a (HOMO)



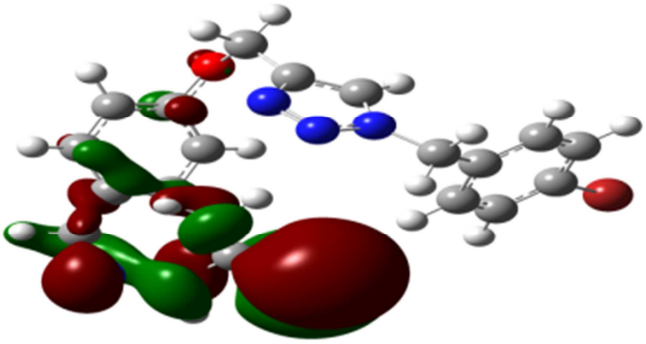
13a (LUMO)



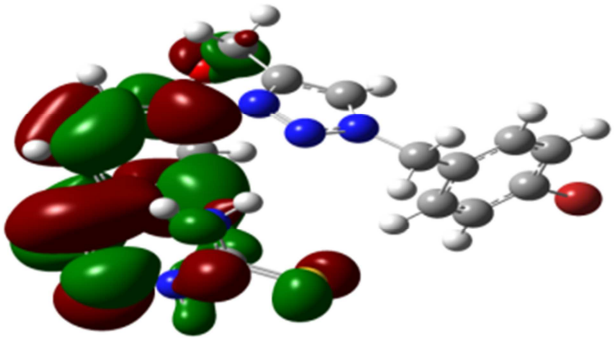
13b (HOMO)



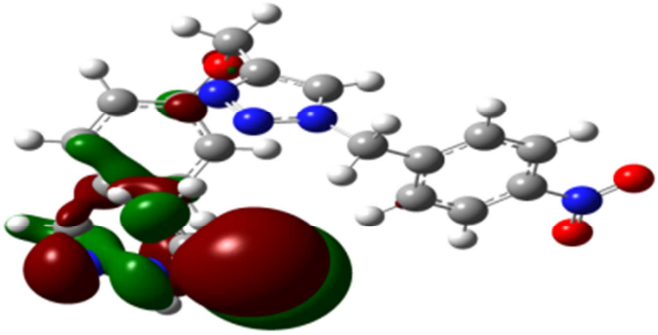
13b (LUMO)



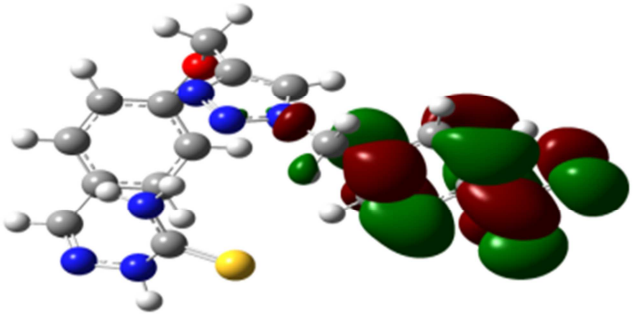
13c (HOMO)



13c (LUMO)



13d (HOMO)



13d (LUMO)

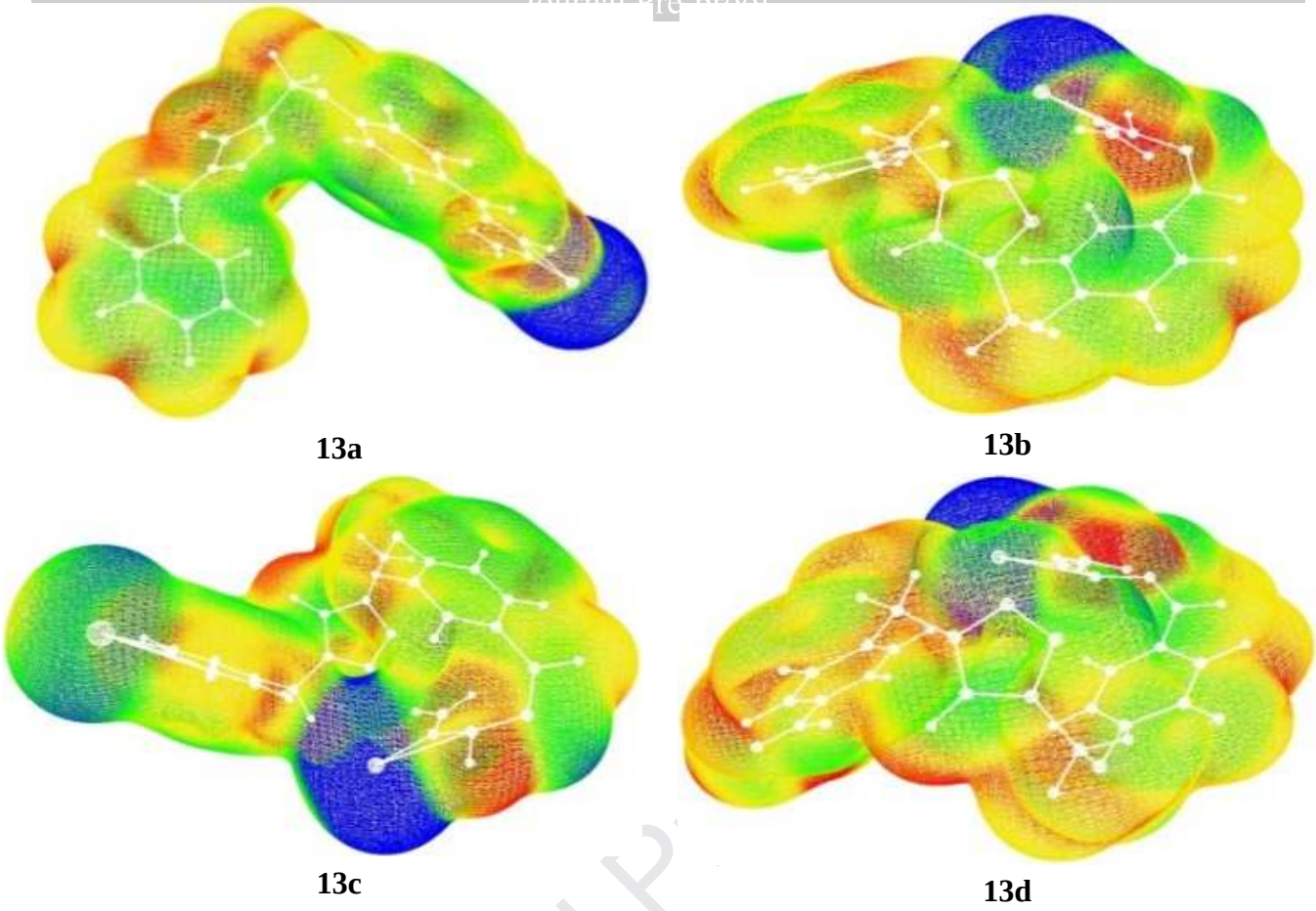


Figure 8. HOMO and LUMO distribution at the ground state of **13a-d** molecules and Molecular electrostatic potential (MESP) analysis of **13a-d** molecules.

Table 5. Comparison of bandgap energy ($E_{LUMO-HOMO}$) of 1,2,3-triazole-TSC hybrid and 1,2,3-triazole-SC hybrid molecules.

Compd.	TSC			SC		
	E_{HOMO} (eV)	E_{LUMO} (eV)	$E_{LUMO-HOMO}$ (eV)	E_{HOMO} (eV)	E_{LUMO} (eV)	$E_{LUMO-HOMO}$ (eV)
3	-5.60	-1.69	3.91	-5.91	-1.54	4.37
5a	-5.64	-1.59	4.05	-5.82	-1.56	4.26
5b	-5.91	-1.72	4.19	-5.99	-1.69	4.3
5c	-5.91	-1.71	4.19	-5.99	-1.68	4.31
5d	-6.01	-2.73	3.27	-6.53	-2.71	3.82
12	-5.71	-1.80	3.91	-5.69	-1.06	4.63
13a	-5.58	-1.64	3.94	-5.82	-1.31	4.51
13b	-5.75	-1.91	3.84	-6.22	-1.7	4.52
13c	-5.75	-1.90	3.84	-5.87	-1.57	4.3
13d	-5.86	-2.85	3.01	-6.31	-3.04	3.27

Conclusions

In precise, we have designed and synthesized a library of 1,4-disubstituted-1,2,3-triazole-thiosemicarbazone hybrid molecules via Cu(I)-catalyzed click reaction of thiosemicarbazone-linked alkynes with aromatic azides. Newly synthesized compounds were fully characterized by IR, ^{1}H NMR, ^{13}C NMR, HRMS and structures of alkynes **3** & **12** were finally supported by x-ray crystallographic data. Also, a synergistic effect in biological activity was exhibited by the incorporation of thiosemicarbazide unit with 1,2,3 triazoles. Compounds **5**, **9**, **13** (**c** & **d**) with MIC values 0.0141,

0.0152, 0.0562, 0.0608, 0.0141, 0.0608, 0.0141, 0.0304, 0.0281, 0.0304, 0.0281, 0.0304, respectively for *B. Subtilis* and *P. Aeruginosa* bacterial strains exhibited excellent potency as compared to classically used drug Ciprofloxacin. The FMOs revealed that incorporation of triazole moiety on to semicarbazone linked alkyne has improved the pharmacological activities of the resulted hybrid molecules. Antibacterial potency results of Triazole-TSC hybrid molecules were supported by computational docking and DFT studies**.**

Experimental Section

General Procedure for the synthesis of a terminal alkyne (2)

To a solution of *o*-/*m*-/*p*-substituted hydroxybenzaldehyde (1.0 mmol) in 20 mL acetone, anhydrous potassium carbonate (1.5 mmol) was added and the resulting suspension was refluxed for 30 min. Propargyl bromide (80% in toluene, 1.5 mmol) was added to it slowly and the reaction mixture was refluxed for 7-8 hrs. After completion of the reaction as monitored by TLC, the solvent was evaporated by a rotatory evaporator and the resulting solid residue was washed with ice-cold water then recrystallized from chloroform: hexane (7:3) to get pure terminal alkyne **2**.

General procedure for the synthesis of thiosemicarbazone linked alkyne (**3**, **8** & **12)**

Thiosemicarbazide hydrochloride (1 mmol dissolved in 10 mL water) was added to a stirred solution of ortho-propargyl-benzaldehyde (1 mmol) taken in ethanol at 70 °C. The reaction mixture was allowed to stir for 4 hours at this temperature. The progress and completion of the reaction were monitored by taking TLC at a frequent interval of time. After completion, the reaction mixture was cooled and light brown colour precipitates have appeared in the reaction mixture. Then, 20 mL of ice cool water was added to the reaction mixture. When the number of precipitates increased, filtered and dried the precipitates. Then, the precipitate was washed with ice-cold water followed by washing with ethanol to remove unreacted starting materials to produce the pure compound in 90-94% yield.

General procedure for the synthesis of thiosemicarbazone linked triazoles (**5a-d**, **9a-d** & **13a-d)**

A mixture of substituted benzyl bromide **4a-d** (1.0 mmol), sodium azide (3.0 mmol), alkyne **3**, **8** & **12** (1.0 mmol) in DMF/water (8:2), copper sulphate pentahydrate (10 mol%) and sodium ascorbate (20 mol%) was stirred for 2 to 3 hrs at room temperature. The progress and completion of the reaction was monitor by TLC at a frequent interval of time. After completion, the reaction mixture was diluted with ice-cold water (30 mL). The solid residues were filtered, washed with aqueous ammonium chloride: ammonia solution (9:1 v/v) followed by washing with water and recrystallized with ethyl acetate to get the desired 1,2,3-triazole derivatives (**5a-d**, **9a-d** & **13a-d**).

Conflict of Interest

The authors declare no conflict of interest.

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Highlights:

1) Newly designed 1,4-disubstituted-1,2,3-triazole-thiosemicarbazone hybrid molecules were synthesized in excellent yield.
2) All triazole hybrid molecules were fully characterized by IR, ^{1}H NMR, ^{13}C NMR, HRMS and alkynes **3** & **12** were supported by single-crystal structures.
3) Compounds **5**, **9**, **13** (**c** & **d**) exhibited excellent potency for *B. Subtilis* and *P. Aeruginosa* bacterial strains as compared to reference drug Ciprofloxacin.
4) Thiosemicarbazone hybrid molecules with lower bandgap energy were soft and more reactive than corresponding semicarbazone hybrid molecules and alkynes.
5) The antibacterial activity results were supported by the molecular docking and DFT studies**.**

Thank You,

Yours truly,
(Ram Kumar Tittal)

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

The authors declare no conflict of interest.