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Synthesis and antibacterial activity of some novel Piperazinophanes with intra annular ester functionality

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Abstract:

1:1 and 2:2 oligomeric piperazinophanes with intra annular ester functionality have been synthesized by Mannich reaction of various aromatic esters with piperazine by a one-pot reaction under benign condition through multicomponent reaction methodology. The newly synthesized piperazinophanes were characterized by spectral and analytical methods. The ester based 1:1 oligomeric piperazinophanes exhibit good target binding ability in molecular docking studies and also show better antibacterial activity against *Escherichia coli*, *Klebsiella pneumoniae, Staphylococcus aureus* and *Streptococcus pyogens* bacteria than the 2:2 oligomeric piperazinophanes.

Keywords: supramolecular, piperazine, host-guest, ester, Mannich reaction, antibacterial.

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Introduction:

Recently, cyclophanes containing piperazine bridge with intra annular ester functionality have attracted the researchers due to their biological activity and by the presence of multidentate coordinating sites. Mostly, ester derivatives such as aspirin, methyl salicylate are dispensed as prodrugs, which on hydrolysis get converted to the corresponding active form, because the drug molecules containing ester functionality undergo rapid hydrolysis when compared to other functional groups.[1] Ester

functionalized compounds are used in perfume industry because of their aroma nature.[2] In general ester derivatives are able to enhance solubility and crystallinity[3] and generate bioactive molecules or modify the properties of existing bioactive molecule. Ester derivatives are also used in electron transfer and electron exchange devices and also have been widely used in various fields such as textiles,[3] drug synthesis,[4] lubricant additives,[5] flavoring agents,[6] personal care,[7] plastics,[8] pharmaceuticals,[9] house hold products and for biological activities such as antibacterial,[10] antifungal[11] and so on.

Cyclophane with heterocyclic ring system possesses binding site for metal ions[12] and provide propitious properties as molecular hosts. Host Guest system observed in molecular recognition reveal molecular expletive in supramolecular chemistry.[13] Molecular recognition plays vital role in biological systems involving host guest interactions. Molecular recognition in supramolecular chemistry is regulated by non-covalent intermolecular interactions such as hydrogen bonding, metal coordination, hydrophobic π-π stacking, vanderwaal's and electrostatic interactions. Cyclophanes help in developing selective probes for various guest molecules by forming non-covalent interactions in the form of inclusion complexes inside the defined cavity with binding sites for metal ions providing propitious properties as molecular host.[14] However, the biological activity of piperazinophanes are rarely reported.[15] Piperazinophanes with ester functionality could furnish supramolecular sensing, recognition and self assembling properties.

Piperazines are new motif that can participate in multiple non-covalent interactions such as metal coordination, [16] anion recognition [17] self assembly [18] and also can exhibit wide applications in biology if used in the synthesis of rigid cavity

cyclophanes and hence can have ability to form complexes with selectivity that can sharply change the spectral properties. Piperazine moiety function as multiple target due to their auspicious properties such as solubility, bioavailability, hydrophilic and pharmocophoric nature. Further, it can enhance the hydrophilicity and binding with macromolecules as seen in drug molecules like sildenafil, imatinib.[19]

Considering the therapeutic importance of various aromatic esters and piperazine derivatives, our interest is to synthesize ester based piperazinophanes from readily available aromatic carboxylic acid and propargyl alcohol under Mannich reaction conditions. The present investigation focus on the synthesis, characterization and antibacterial activity of a new class of ester based piperazinophanes by a one pot Multi Component Reaction (MCR) of terminal alkyne with piperazine through Mannich reaction.

Result and discussions:

Ester based Piperazinophanes

Ester based piperazinophanes **1-10 (Figure 1)** can be synthesized from the corresponding bispropargyloxy biacyl precyclophane. The bisalkyne precyclophane can be obtained by the reaction of the various carboxylic acid chlorides with propargyl alcohol. Mannich reaction in the current study is efficiently utilized for the synthesize of macrocycles by a one pot process of coupling the terminal alkyne, secondary diamine, and formaldehyde. In order to synthesis ester functionalized piperazinophanes **1-10**, the required precyclophanes **16**, **17**, **18**, **19** and **20** are obtained by the reaction of various acid chlorides with propargyl alcohol.

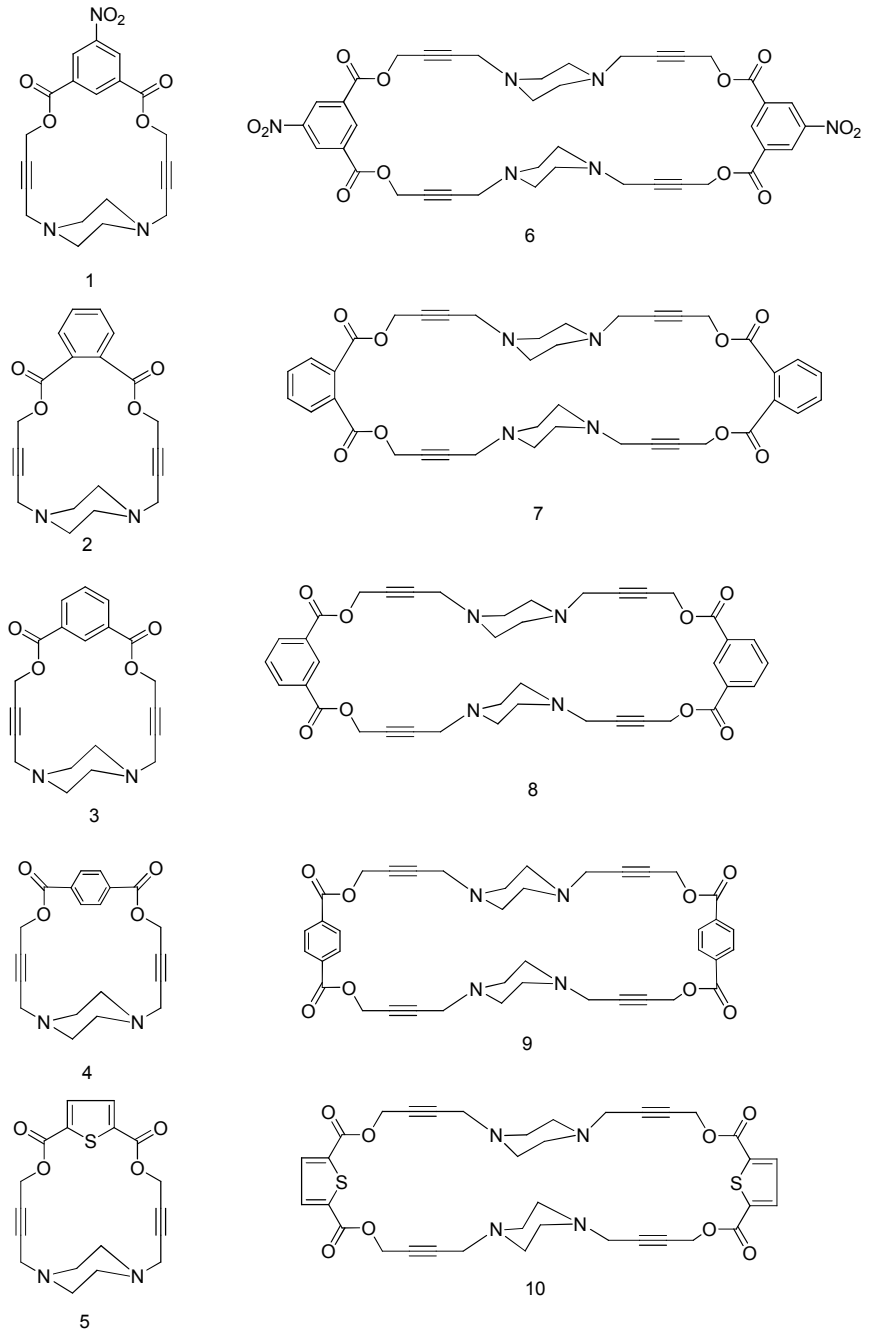


Figure 1: Molecular structure of ester based piperazinophane forms macrocycles **1–10**

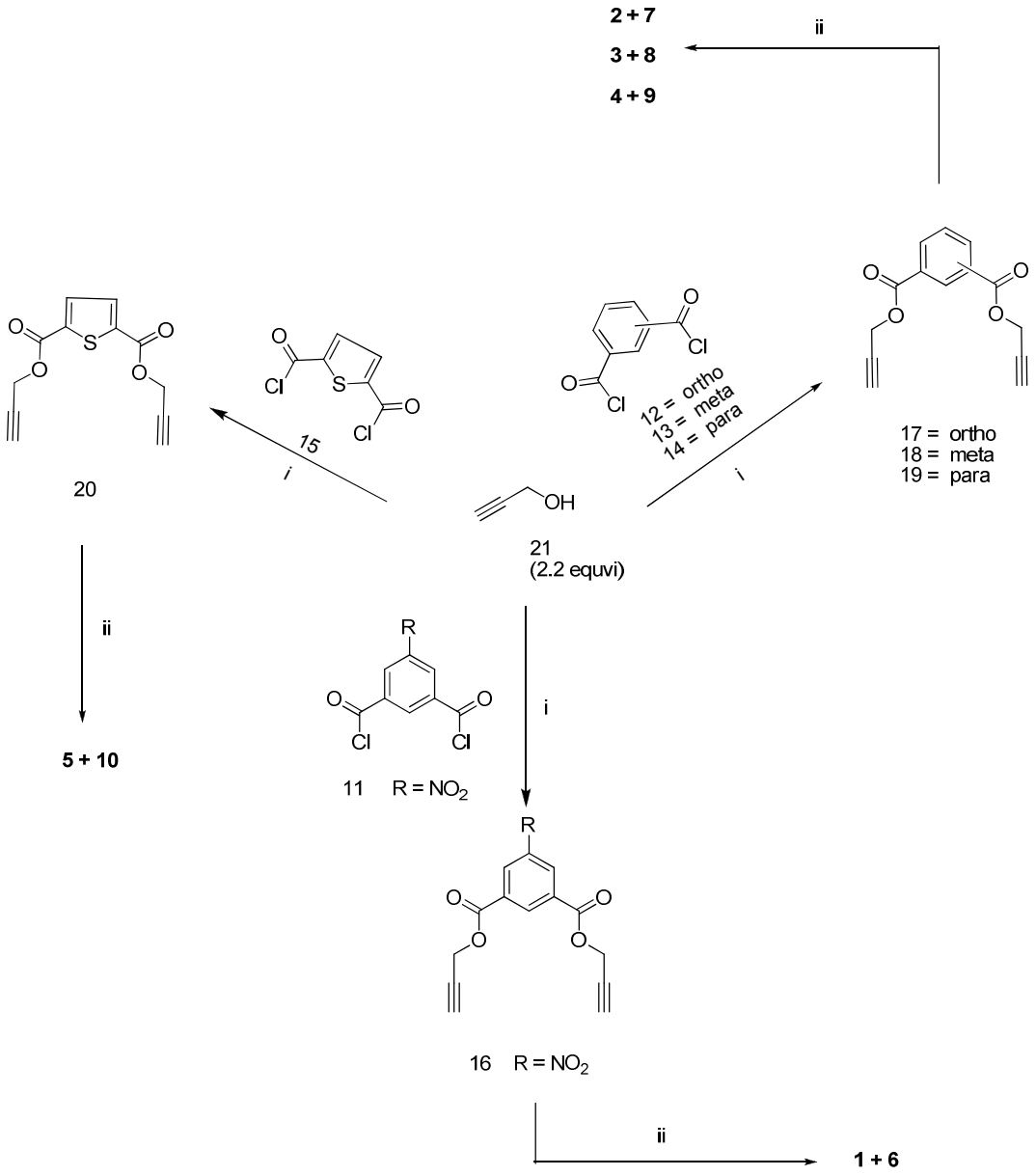
Synthesis and characterization of piperazinophanes

The synthesis of ester based 1:1 oligomeric cyclophanes **1, 2, 3, 4** and **5** and 2:2 oligomeric cyclophanes **6, 7, 8, 9** and **10** is shown in scheme 1. Reaction of 2.2 equiv. of propargyl alcohol, **21** with one equiv. of each 5-nitroisophthaloyl chloride **11,** phthaloyl

chloride **12**, isophthaloyl chloride **13**, terephthaloyl chloride **14** and thiophene-2,5-dicarbonyl dichloride **15** afforded the precyclophane ester **16**, **17**, **18**, **19** and **20**, in 76%, 80%, 82%, 78% and 70% yields, respectively (**Scheme 1**). The ^{1}H NMR spectrum of the precyclophane **16** showed the alkyne protons as a triplet at δ 2.59 and O-methylene protons as a doublet at δ 5.02 in addition to the signals for the aromatic protons. The ^{13}C NMR spectrum of the precyclophane **16** displayed signals for the O-methylene and the two acetylenic carbons at δ 53.5, and 76.0, 76.7, and ester carbonyl carbon at δ 162.8 in addition the signals for four aromatic carbons. In the mass spectrum the appearance of molecular ion peak at *m/z* 287 [M^{+}] also confirmed the structure of the precyclophane **16**. Further the elemental analysis also supported the composition of the precyclophane **16**. Similarly, the structure of the precyclophanes **17**, **18**, **19** and **20** was also confirmed from spectral and analytical data. Diacid chlorides **11, 12, 13, 14 and 15** were obtained by the reaction of the corresponding dicarboxylic acid and thionyl chloride by the usual procedure, as reported earlier.

Coupling of 1.0 equiv. of each of the precyclophane **16**, **17, 18, 19** and **20** with 1.0 equiv. of piperazine, and 2.0 equiv. of formaldehyde from 37-41% formalin solution in the presence of 1.0 equiv. CuCl in dioxane at 90 °C for 2 h afforded the 1:1 oligomeric ester functionalized piperazinophanes **1**, **2, 3, 4** and **5** in 32%, 26%, 33%, 35% and 30%, and 2:2 oligomeric piperazinophane esters **6**, **7, 8, 9** and **10** in 23%, 28% , 30%, 32% and 30% yields, respectively. **(Scheme 1)**

The ^{1}H NMR spectrum of 1:1 oligomeric macrocyclic ester **1** displayed three sharp singlets at δ 2.78, 3.37, 4.99 for eight protons of the piperazine, four protons of the *N*-methylene and four protons of *O*-methylene group in addition to the signals for the aromatic protons. The ^{13}C NMR spectrum of 1:1 oligomeric macrocyclic ester **1** showed five signals at δ 46.6, 51.4, 55.2 and 78.2, 84.7 for the piperazine, *N*-methylene,



Scheme 1: *Reagents and conditions:* i) DMAP, DCM (dry), 24 h, **16** (76%);. **17** (80%); **18** (82%); **19** (78%); **20** (70%). ii) piperazine (1.0 equiv.), formaldehyde (2.0 equiv.) from 37-41% formalin solution, CuCl (1.0 equiv.) 90 °C, 2 h. **1** (32%); **2** (26%); **3** (33%); **4** (35%); **5** (30%); **6** (23%); **7** (28%); **8** (30%); **9** (32%); **10** (30%)

O-methylene and acetylenic carbons and the ester carbonyl carbon at δ 163.4 in addition to the signals for four aromatic carbons. The appearance of molecular ion peak at *m/z* 397 and also the elemental analysis supported the structure of the macrocyclic ester **1.** Similarly, the structure of 1:1oligomeric ester macrocycles **2, 3, 4** and **5** was confirmed from spectral and analytical data as shown in SI.

The ^{1}H NMR spectrum of 2:2 oligomeric cyclophane ester **6** displayed a sharp singlet at δ 2.80 for sixteen protons of the piperazine and the *N*-CH_2 protons appeared as a singlet at δ 3.45, *O*-CH_2 protons as a singlet at δ 5.04 and the aromatic protons appeared in the region at δ 8.99 to 9.06. The ^{13}C NMR spectrum of the 2:2 oligomeric cyclophane ester **6** showed signals at δ 46.9, 51.6, 54.0 and 78.7; 82.7and for piperazine, N-methylene, *O*-CH_2 and alkyne carbons and the aromatic carbons appeared in the region at δ 128.6-148.5 the carbonyl carbon of the ester moiety appeared at δ 163.0. The mass spectrum of the 2:2 oligomeric cyclophane ester **6** showed the molecular ion peak at m/z 794 [M^+]. The structure of the 2:2 oligomeric cyclophane ester **6** was confirmed from analytical data (**Scheme 1**). Similarly the structure of the 2:2 oligomeric cyclophane ester **7**, **8**, **9** and **10** was characterized from spectral and analytical data as shown in SI.

Antibacterial activity:

There is always need to search for alternative antibacterial drugs for two reasons. The microorganism may develop immunity towards the existing drug and the drug itself can have side effects which will be seen only after long time usage. Antibacterial drugs could be of natural or semi synthetic or synthetic in origin. However, the synthetic drugs are more preferred because structural modification can be carried out from docking studies and structure activity relationship can be studied. Antibacterial activity of carbazolophane amides against *Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus* and *Staphylococcus pyogens* bacteria has been reported from our laboratory

earlier[20] and the reported carbazolophanes have shown remarkable antibacterial activity only against *Staphylococcus aureus*. Though, we have shown that imino cyclophane[21] exhibited good antibacterial activity against *Escherichia coli, Klebsiella pneumoniae* and *Staphylococcus aureus* bacteria, the synthesis and the yields are challenging. Hence, recently we have synthesized various sulphonophanes[22] and tested their antibacterial activity towards *Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus* bacteria and we have observed that their antibacterial activity is comparable to that of the piperazinophanes reported herein. In fact the antibacterial activity expressed as MIC values of some of the sulphonophanes reported earlier from our research group lies in the range of 10 μg/mL to 20 μg/mL and in the present study, the synthesized piperazinophanes shows the MIC values in the range of 12.5 μg/mL to 25 μg/mL for the three pathogens viz against *Escherichia coli, Klebsiella pneumoniae* and *Staphylococcus aureus*. The antibacterial activity of the compounds **S1 (piperazinophane 1)** to **S10 (piperazinophane 10)** is tested by resazurin reduction method. The four test bacteria chosen are *Escherichia coli, Klebsiella pneumoniae*, *Staphylococcus aureus* and *Staphylococcus pyogens* and streptomycin is employed as the standard. Table 1 shows the MIC values expressed in micromoles (μM) for all the synthesized compounds **1-10** against *Escherichia coli*, *Klebsiella pneumoniae, Staphylococcus aureus* and *Streptococcus pyogens* bacteria with streptomycin as the standard drug of choice. 1:1 Oligomeric cyclophane esters S**1** to S**5** show antibacterial activity against the human pathogens *Escherichia coli*, *Klebsiella pneumoniae, Staphylococcus aureus* and *Streptococcus pyogens* which is less than that of reference drug viz streptomycin. The 2:2 oligomeric cyclophane esters exhibit nil activity towards the two micro organism viz

Escherichia coli, *Klebsiella pneumoniae*, however they inhibit the growth of *Staphylococcus aureus* and *Streptococcus pyogens* to some extent. The ester based 2:2 oligomeric cyclophane **S10** is inactive towards all the tested pathogens. Piperazinophane **S1** show better activity in controlling the growth of *Escherichia coli* than cyclophanes S**3** and S**4** which are structurally similar. Surprisingly, the *ortho* isomer i.e. compound S**2** is less active than the piperazinophanes S**3** and S**4**, when tested with *Escherichia coli* bacteria. Cyclophanes S**2** and S**4** show similar activity towards the inhibition of the growth of *Klebsiella pneumoniae* and *Staphylococcus aureus*. Compounds S**2, S4** and S**8** show better activity than other piperazinophanes towards the inhibition of the growth of *Streptococcus pyogens*. In conclusion 2:2 oligomeric piperazinophanes **S7, S8, S9** and **S10** are inactive against gram negative bacteria and except piperazinophane **S10** all of the other piperazinophanes (**1-9)** show moderate activity against gram positive bacteria. Hence, based on the MIC values piperazinophanes **S2** and **S4** would be the better choice as antibacterial agent for the pathogen *Klebsiella pneumoniae, Staphylococcus aureus* and *Streptococcus pyogens* where as piperazinophane **S1** would be the better choice for the *Escherichia coli* bacteria when compared with all other synthesized cyclophanes. Dimethyl sulfoxide was used as the control and exhibits nil activity with reference to all the tested bacteria.

Table 1: Minimum Inhibitory Concentration of test compounds by resazurin reduction assay:

Samples / Cyclophanes	MIC (micromoles μM) Bacterial pathogens			
	Escherichia coli	*Klebsiella pneumoniae*	*Staphylococcus aureus*	*Streptococcus pyogens*
S1/1	0.031	0.126	0.126	0.126
S2/ 2	0.142	0.036	0.071	0.071
S3/3	0.071	0.071	0.142	0.142
S4/4	0.071	0.036	0.071	0.071
S5/5	0.279	0.279	0.139	0.279
S6/6	0.126	NA	0.126	0.126
S7/7	NA	NA	0.142	0.142
S8/8	NA	NA	0.142	0.071
S9/9	NA	NA	0.142	0.142
S10/10	NA	NA	NA	NA
Streptomycin	0.001	0.005	0.005	0.001
Control (DMSO)	NA	NA	NA	NA

NA= Nil activity

The antibacterial activity of the synthesized compounds were tested by resazurin reduction method as shown in Figure 2.

Figure 2: Antibacterial activity of the synthesized piperazinophanes by resazurin reduction method

Figure keys:

C4 -Control - dye + *Staphylococcus aureus*+ without compound

S10 – Compound + dye + *Escherichia coli*

S1b to S5b – Compound + dye + *Staphylococcus aureus*

P3 – Streptomycin + dye + *Staphylococcus aureus*

Structure activity relationship studies:

The *in vitro* antibacterial activity reveals that the ester and diyne functionality along with piperazine ring **(1-5)** show better antibacterial activity as compared to other piperazinophanes. Among the 1:1 oligomers the inhibitory activity depend on the nature of the substituents. Electron withdrawing group such as ester, when placed in the *p*-position of the intra annular cavity as in the piperazinophane **S4** show better activity than all other piperazinophanes. The 1:1 oligomer showed better antibacterial activity because of high solubility in polar solvents and hence can be easily applied to a substrate and also

has low molecular weight. Piperazinophane **1** shows better antibacterial activity against *Escherichia coli* may be due to the presence of the nitro functionality and rigid smaller cavity. Though cyclophane **6** also has nitro group, the cavity is bigger and hence shows nil activity against *Escherichia coli* bacteria. Similarly cyclophane **4** shows better antibacterial activity against *Klebsiella pneumoniae* however the corresponding 2:2 oligomer **8** shows nil activity due to larger cavity size. Further, with respect to the bacteria *Staphylococcus aureus* and *Streptococcus pyogens* both cyclophanes **2** and **4** show similar activity**.** Hence the cavity size and the presence of functional group at the intraanular position control the antibacterial activity. In conclusion, 1:1 oligomers provide better antibacterial activity against both gram-positive and gram-negative bacteria than high molecular weight 2:2 oligomeric piperazinophanes. Further, structure activity studies report that ester cyclophane **S5** having alkyl and thiophene ring show less inhibitory activity. Thus, the nature of the aromatic unit, the ester and diyne skeleton attached to piperazine has a strong influence on the extent of antibacterial activity.

Molecular docking studies:

The molecular docking study helps to find the binding of the tested compounds (ligand) with protein of interest (target). Molecular docking study has shown that in general compound binds well at the active site of protein biotin carboxylase (PDB ID: 2V59). The co-crystal ligand interacts with the residues GLU-201, LYS-202, LEU-204 and LYS-159 with the glide score of 11.742 and glide energy of -57.232 kcal/mol as evident from Fig 20 given in SI. Among the ten synthesized compounds, cyclophane **S1**, **S2** and **S4** showed binding with the target protein and the remaining compounds did not dock with the active pocket of the protein due to the incompatibility of their structures to the active site. Among **S1**, **S2** and **S4** cyclophane, **S4** showed the best binding affinity to the biotin carboxylase with a glide score and glide energy comparable to the co-crystal

ligand. **S2** also showed interaction with LYS-159, co-crystal active site. Compound **S1** showed hydrogen bond interaction with the HIS-236 with the glide score of 5.721 and glide energy of -49.417kcal/mol as evident from Fig 21 given in SI. Compound **S2** showed interactions with the TYR-203 and LyS-159 with the glide score of 8.212 and glide energy of 46.669 kcal/mol respectively as evident from Fig 22 given in SI. The compound **S4** showed hydrogen bond interaction with the residue LEU-204 with the glide score of 7.622 and glide energy of -40.772kcal/mol **(Figure 3)**.

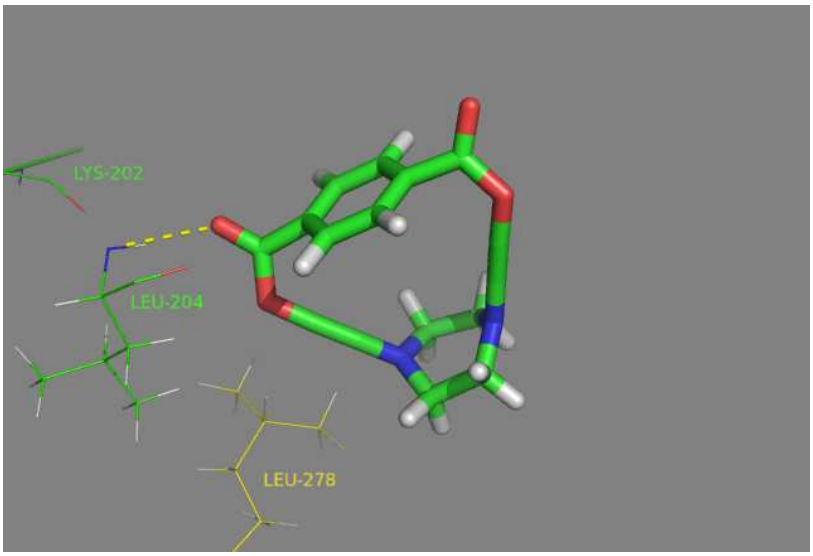


Figure 3: Molecular Docking of Compound **S4** in the active pocket of Protein (PDB ID-2V59)

Conclusions

In conclusion, efficient one-pot synthesis of the novel ester based 1:1 and 2:2 oligomeric piperazinophanes **1-10** has been successfully achieved under mild conditions and their *in vitro antibacterial activity* against four human pathogens Escherichia *coli*, *Klebsiella pneumoniae, Staphylococcus aureus* and *Streptococcus pyogens* revealed that piperazinophane **4** exhibited excellent antibacterial activity than all other synthesized piperazinophanes, which is also supported by molecular docking studies. All the 1:1

oligomeric piperazinophanes show better antibacterial activity against *Escherichia coli*, *Klebsiella pneumoniae, Staphylococcus aureus* and *Streptococcus pyogens* than 2:2 oligomeric piperazinophanes. The structure activity relationship also reveals that the ester functionality on the benzene ring at the *p*-position in the intra annular cavity and diyne skeleton attached to the piperazine molecule has a strong influence on the antibacterial activity.

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Synthesis and antibacterial activity of some novel Piperazinophanes with intra annular ester functionality

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Graphical Abstract

Ester based 1:1 and 2:2 oligomeric piperazinophanes were synthesized by multicomponent reactions (MCR) technique and assessed for their antibacterial activity and further supported by molecular docking studies.