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ARTICLE

Synthesis, NMR Studies and Binding Interactions of (2*E*,2′*E*)-2,2′-(Propane-1,2-diylidene)*bis*(*N*-methylhydrazinecarbothioamide) with SARS-CoV Main Protease

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ABSTRACT

Chloroquine derivatives were one of the medications tested against the coronavirus pandemic in 2020 and they appeared to be effective. In this present work, (2*E*,2′*E*)-2,2′-(propane-1,2-diylidene)*bis*(*N*-methylhydrazinecarbothioamide) (PMTSC) has been postulated as a possible antiviral for the treatment of COVID-19 by using 1-Click docking. Compound PMTSC has been synthesized by the condensation reaction between pyruvaldehyde and *N*-methylthiosemicarbazide. The synthesized PMTSC was confirmed by elemental analysis and NMR spectral study. The binding interaction of PMTSC has been performed with SARS-CoV main protease (PDB code: 2GZ7 and 2GZ8). The docking results showed good binding energies and interactions.

KEYWORDS

Thiosemicarbazone, Schiff's base, SARS-CoV, Docking, Protein-Ligand binding interaction.

INTRODUCTION

With the spread of the pandemic corona virus, COVID-19 affecting the human population across the globe and the World Health Organization naming this pandemic virus as 'public enemy #1, the world has been in a hazardous condition since the beginning of 2020. As of May 21, 2020, the pandemic, which began on December 12, 2019, has infected over 4.68 million individuals in 216 nations and territories, leading to over 3 lakh deaths and the mortality rate because of COVID is still significantly high [1]. The causal pathogen of COVID-19 belongs to the beta-coronavirus (β-CoV) genera, according to its phylogenetic characteristics and genomic structure [2]. The 7th human coronavirus, 229E, NL63, OC43, HKU1, MERS-CoV and SARS-CoV are all pathogenic SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2) [3]. While the β-CoVs *i.e.* SARS-CoV and MERS-CoV are similar to SARS-CoV-2 triggered viral outbreaks in Nov'2002 and Sep'2012, respectively, the S-CoV-2 have shown rapid transmission across the world and has lead to the death of human lifes and the collapse of the international economy [4].

COVID-19 patients are being investigated for a various therapeutic remedies and drug discoveries for SARS-CoV,

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MERS-CoV and other viral infections, including present and potential antiviral medicines, potent steroids, plasma extracted from patients recovered from COVID-19 and also various other therapeutics like Chinese, Ayurvedic and Sidda medicine [5]. In American patient with COVID-19, intravenous administration of remdesivir, was found to be effective [6].

Ramdesivir is a nucleotide analogue and a antiviral medicine created by Gilead originally for treating patients affected by diseases caused by the Ebola, Marburg, MERS and SARS viruses, Interactions between baricitinib, interferon, lopinavir/ritonavir and ribavirin have already been identified as prospective therapy for patients with acute respiratory symptoms [7]. Despite the numerous experimental and computational investigations now underway in quest of a potential vaccine or medicine for this dreaded pandemic, there is currently no confirmed viable treatment for COVID-19. Many computational approaches have been explored to search for small molecular inhibitors against SARS-CoV-2 main protease and RdRp [8-11]. Analogous screening of potential drugs against the S protein of SARS-CoV-2 provided small molecular compounds with a high binding affinity.

Elfiky [12] and Domagk *et al.* [13] revealed the efficacy of thiosemicarbazones against tuberculosis, leading to the study of a large number of thiosemicarbazones for their anticancer [14], antibacterial [15-19], antifungal [16-20], antiprotozoal [21] and antiviral [22-25] properties. In present investigation, the synthesis and NMR characterization of (2*E*,2′*E*)-2,2′-(propane-1,2-diylidene)-*bis*(*N*-methylhydrazinecarbothioamide) (PMTSC) are reported. The binding affinity and binding interactions of PMTSC have been also investigated with SARS-CoV main protease (PDB code: 2GZ7 and 2GZ8).

EXPERIMENTAL

Pyruvaldehyde, *N*-methylthiosemicarbazide and other commercially available solvents were procured and utilized without further purification. The CHN analysis was performed by using Perkin-Elmer (USA) 2400 SERIES 2. The ^{1}H NMR and ^{13}C NMR spectrum were recorded by using Bruker (Germany) Avance III 400 in DMSO-d_6. The 3D structure of the targeted two SARS-CoV main protease (PDB code: 2GZ7 and 2GZ8, Fig. 1) were retrieved from RCSB PDB [26].

Synthesis of (2E,2′E)-2,2′-(propane-1,2-diylidene)-*bis*(*N*-methylhydrazinecarbothioamide) (PMTSC): The compound PMTSC has been synthesized by the reaction of pyruvaldehyde with *N*-methylthiosemicarbazide (MTSC) by the reported method (**Scheme-I**) [16-18]. Yield: 87%. Anal. calcd. calcd. (found) % for $C_7H_{14}N_6S_2$ (246.07 g mol^{-1}): C, 34.13 (34.37); H, 5.73 (5.69); N, 34.11 (34.23). ^{1}H NMR (400 MHz, DMSO-d_6, δ in ppm): 10.35 (s, 1H, CH=N, E_{Isomer}), 9.22 (s, 1H, CH=N, Z_{Isomer}), 8.57-8.51 (dd, 1H, NH-N), 8.43-8.39 (dd, 1H, NH-N), 7.96, 7.94 (d, 1H, NH), 7.65 (d, 1H, NH), 2.99, 2.88 (dd, 3H, -CH_3), 2.87, 2.86 (d, 3H, -CH_3), 2.16 (s, 3H, -CH_3). ^{13}C NMR (400 MHz, DMSO-d_6, δ in ppm): 178.26 (C=S), 177.74 (C=S), 147.10 (C=N), 141.87 (CH=N), 31.04 (N-CH_3), 30.93 (N-CH_3), 11.07 (C-CH_3).

Docking study: The 1-Click docking tool was used to simulate binding interactions of the synthesize compound

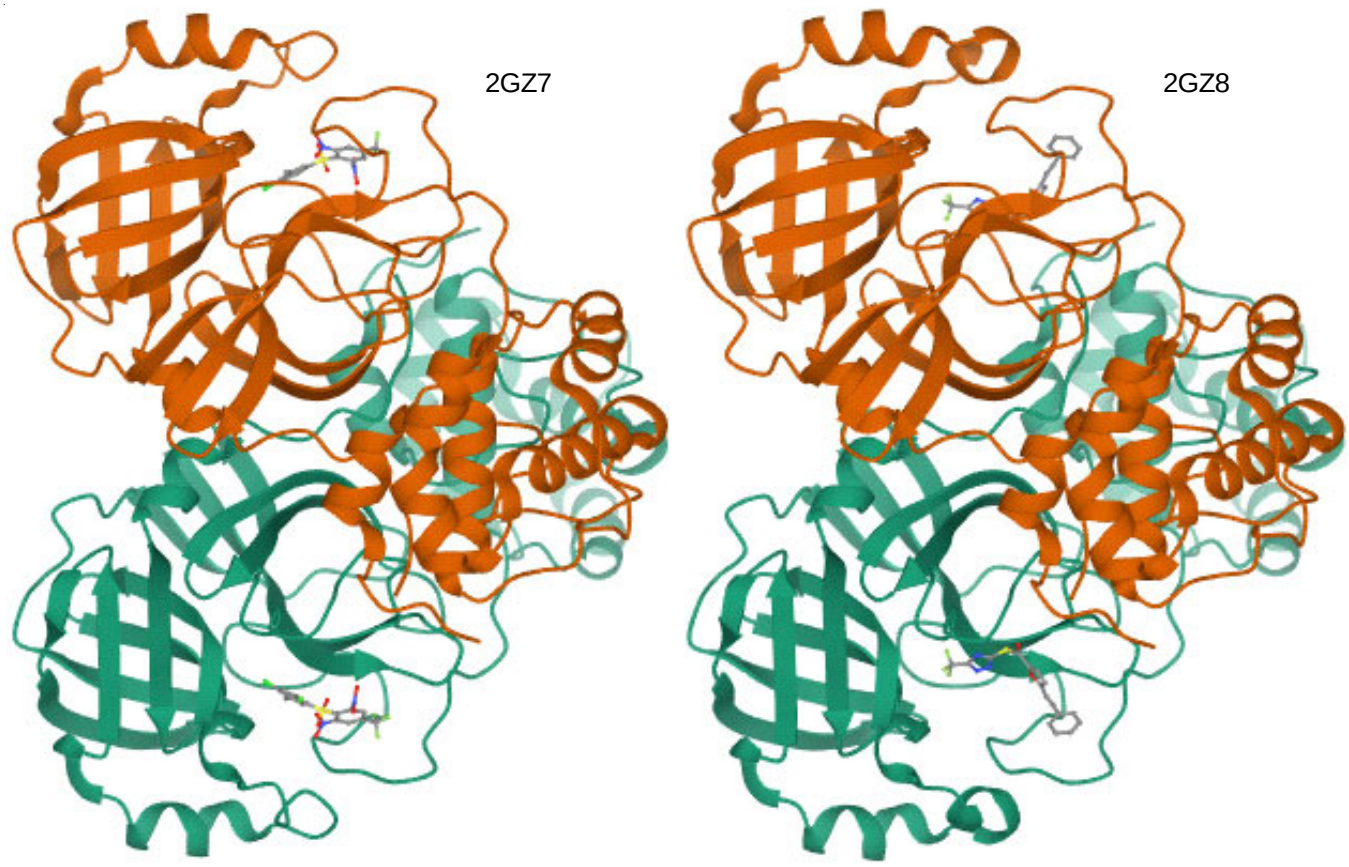
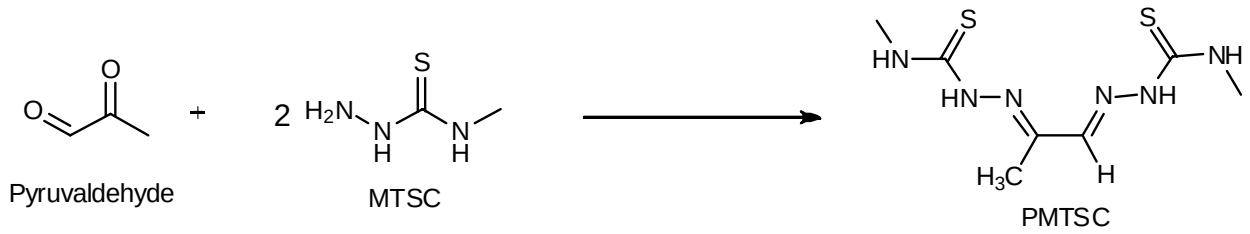


Fig. 1. Schematic representation of the structure of the targeted protease (2GZ7 and 2GZ8)



Scheme-I: Synthetic route of (2E,2′E)-2,2′-(propane-1,2-diylidene)-*bis*(*N*-methylhydrazinecarbothioamide) (PMTSC)

PMTSC and the docking poses and the modes of binding were displayed using the reported procedure [27].

RESULTS AND DISCUSSION

Compound PMTSC was successfully synthesized by the condensation of pyruvaldehyde with *N*-methylthiosemicarbazide with an admirable yield (**Scheme-I**, 87%). In ^{1}H NMR spectrum of PMTSC, one azomethine proton shown as singlet peak at 10.35 ppm and 9.22 ppm, which are attributed to the *E*-isomer (major) and *Z*-isomer (minor), respectively (Figs. 2 and 3). The two amino (hydrazine) groups are shown peaks in the range between 8.57-8.39 ppm. The two carbothioamidic protons are shown as doublet peak at 7.96 & 7.94 and 7.65 ppm [16-18]. The chemical shift of three methyl protons is observed at 2.99, 2.88 & 2.87, 2.86 ppm as doublets of doublet and doublet and 2.16 ppm as a singlet, which are assigned to two *N*-methyl and one C-methyl protons respectively [16-18]. In the neat ^{13}C NMR spectrum of PMTSC, the two thiocarbonyl groups are shown a peak at 178.26 and 177.74 ppm. The two distinctive azomethine carbons are shown a peak at 147.10 and 141.87 ppm, which are clearly indicated the azomethine group formed with the ketone and aldehyde environment respectively. The peaks at 31.04 and 30.93 are attributed to N-methyl carbons with two distinctive environments [16-18]. The C-CH_3 carbon shown a peak at 11.07 ppm. The CHN analysis of the synthesized PMTSC was also supported the formation of it.

Docking study: The synthesized PMTSC was simulated with SARS-CoV main protease (2GZ7 and 2GZ8) by using the 1-Click docking tool and its results are shown in Table-1 [27]. After that docking, compound PMTSC has been shown the tolerable binding affinities (Fig. 4). The binding affinity

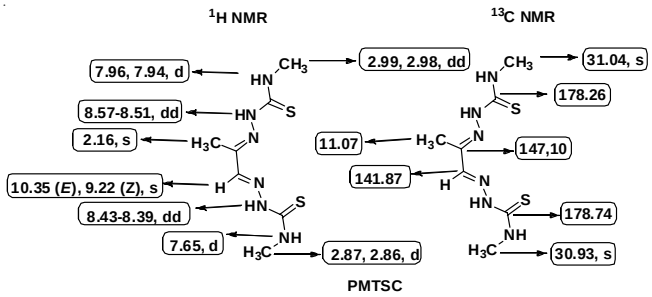


Fig. 2. Representation of ^{1}H NMR and ^{13}C NMR spectrum of PMTSC

values, -4.7 kcal/mol with 2GZ7 and -5.0 kcal/mol with 2GZ8, which are indicated the title compound shown a better and stable binding interaction with the 2GZ8 compared to 2GZ7. The compound PMTSC shown four hydrogen bonds with the docked protease 2GZ7. One H-bond interaction with the residue of ARG-188A in the distance with 2.02 Å. The second and third H-bond interaction with the residue of GLN-189A and GLN-192A with the distance of 2.43 and 3.27 Å, respectively. Compound PMTSC shown five H-bond interactions and two hydrophobic interactions with the docked protease 2GZ8. One H-bond interaction with the residue of THR-25A (1.96 Å), the three interactions with HIS-41A (2.57, 2.57 & 2.73) and one H-bond interaction with CYS-44A (2.23 Å) of SARS-CoV main protease. The observed two hydrophobic interactions of PMTSC with the residue of HIS-41A (3.80 Å) and MET-49A (3.86 Å) of the main protease (2GZ8).

Conclusion

In conclusion, compound (2*E*,2′*E*)-2,2′-(propane-1,2-diylidene)*bis*(*N*-methylhydrazinecarbothioamide) was synthesized as an excellent yield and characterized by CHN analysis, ^{1}H & ^{13}C NMR spectroscopic techniques. Then, the

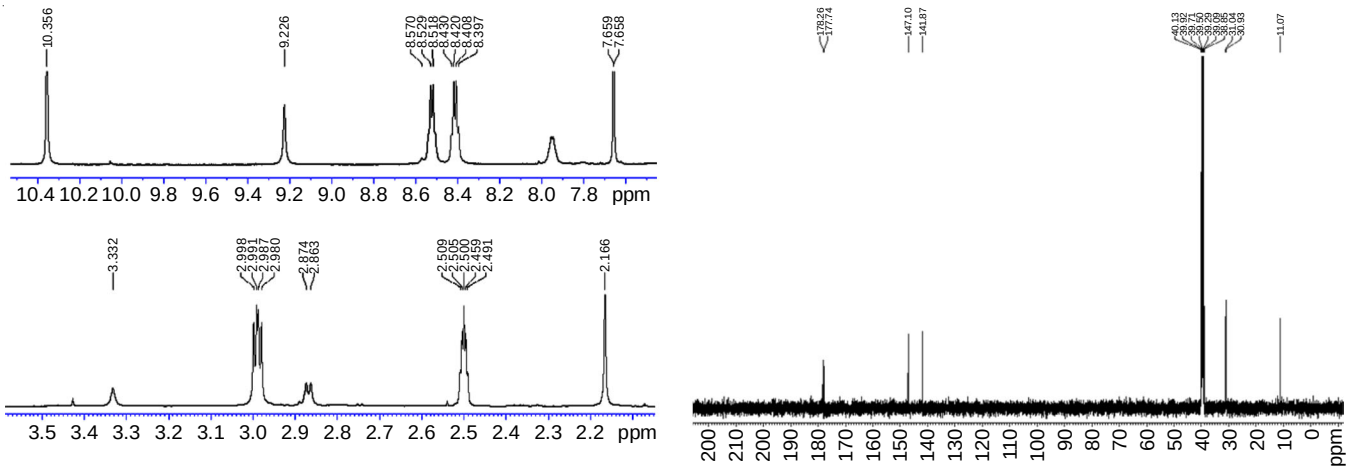


Fig. 3. ^{1}H and ^{13}C NMR spectrum of PMTSC

TABLE-1
PROTEIN-LIGAND INTERACTIONS OF PMTSC WITH SARS-CoV MAIN PROTEASE (2GZ7 AND 2GZ8)

Compound	PDB code							
	2GZ7			2GZ8				
	Binding affinity (kcal/mol)	H-bond interaction		Binding affinity (kcal/mol)	H-bond interaction		Hydrophobic interactions	
		Residue	Distance (Å)		Residue	Distance (Å)	Residue	Distance (Å)
PMTSC	-4.7	ARG-188A	2.02	-5.0	THR-25A	1.96	HIS-41A	3.80
		GLN-189A	2.43		HIS-41A	2.57	MET-49A	3.86
		GLN-192A	3.27			2.57	–	–
		–	–			2.73	–	–
		–	–		CYS-44A	2.23	–	–

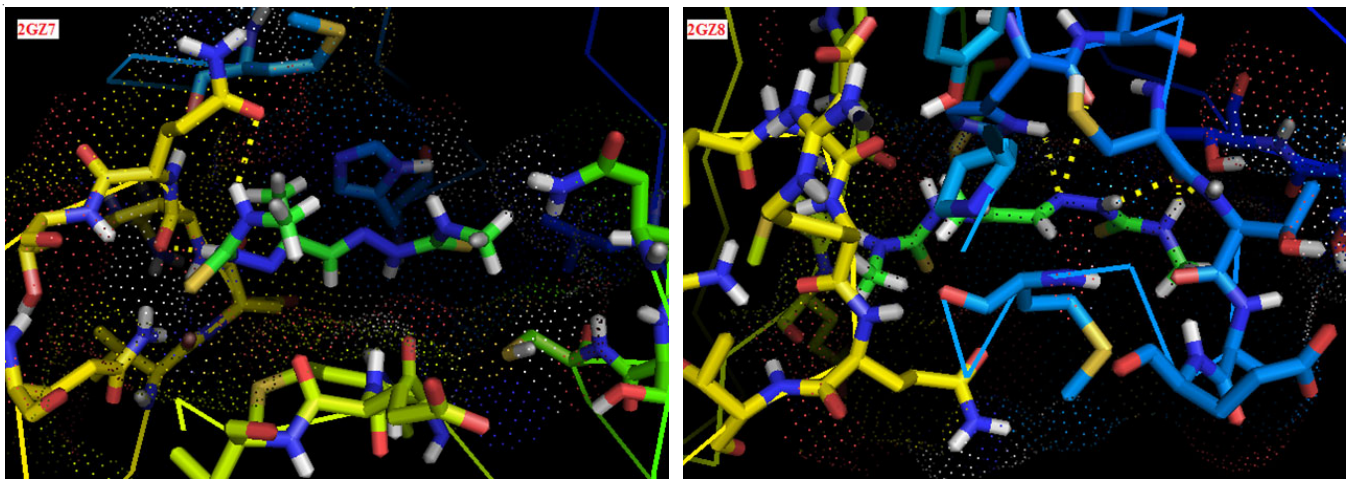


Fig. 4. Docking pose and binding interaction of PMTSC with 2GZ7 and 2GZ8

protein-ligand potential binding interactions of the synthesized PMTSC was also performed. A better binding affinity and binding interactions were obtained with the docked two main protease (2GZ7 and 2GZ8). Thus, the results of the present study warrant the need for the *in vitro* and *in vivo* testing of the related COVID-19 inhibitors as potential therapeutics against SARS-CoV-2 and related coronaviruses to check the alarming spread of the pandemic virus at present as well as in the future.

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