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Novel piperazine based compounds as potential inhibitors for SARS-CoV-2 Protease Enzyme: Synthesis and molecular docking study

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Structurally diverse piperazine-based compounds hybrid with thiadiazole, isatin or with sulfur/nitrogen, functionalities were synthesized. The structures of the new compounds were established based on their spectral data and elemental analysis. The physicochemical, bioactivity scores and pharmacokinetic behavior of all the prepared ligands were evaluated using in silico computational tools. The new piperazine ligands have been screened for their inhibition activity against SARS-CoV-2 protease enzyme using molecular docking analysis. The docking studies showed that all the ligands have been docked with negative dock energy onto the target protease protein. Moreover, Molecular interaction studies revealed that SARS-CoV-2 protease enzyme had strong hydrogen bonding interactions with piperazine ligands. The present in silico study thus, provided some guidance to facilitate drug design targeting the SARS-CoV-2 main protease.

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1. Introduction

In December 2019, the seventh strain of Human coronaviruses has begun in the animal and seafood market in Wuhan, China, and rapidly identified as a novel beta coronavirus. Patients initially infected with this virus suffered severe respiratory tract infections, pneumonia illness, and fever [1-4]. WHO (World Health Organization) characterized the virus disease as COVID-19 (coronavirus disease-2019). Recently, WHO changed the name of the virus to be SARS-CoV-2 (severe acute respiratory syndrome-coronavirus-2) [5-7] instead of (2019-nCoV) that’s due to the high similarity of the nucleotide sequence with SARS-CoV and MERS-CoV. In March 2020, SARS-CoV-2 rapidly spread worldwide in more than 100 countries and WHO officially declared COVID-19 as pandemic or global infectious disease [8,9]. In 10th of September 2020 the number of COVID-19 infected people patients reached more than 28,021,800 people all over the world out of which ≈ 20,099,000 patients have been recovered and ≈908,000 death [10]. The number of infected people is still growing rapidly which make the finding of anti-SARS-CoV-2 drug become an essential and a challenge for scientists.

SARS-CoV-2 are positive sense single-stranded RNA virus, long about 30,000 bp, employs host cellular components to achieve its physiological activities such as protein synthesis and replication, subsequently accomplishes pathological damage to the host cells although some probable drugs are under investigation worldwide [11-15]. The severity of SARS-CoV-2 requires an urgency to develop a vaccine, which is an expensive and time-consuming process. However, nowadays different ligand-based computational techniques and structure-based modeling techniques could be fruitful approach to design novel inhibitors against SARS-CoV-2 [16,17].

Several significant targets have been documented to participate in the biological events critical to SARS-CoV replication, one of these targets is the main protease (Mpro)/chymotrypsin-like protease (3CLpro) [18-22]. It have been successfully crystallized from COVID-19, structured and repositioned in the Protein Data Bank (PDB) and is reachable by the public. This protease represents a potential target for the inhibition of CoV replication [12,23].

Piperazine scaffolds exist in several different biologically active compounds including some antiviral agents such as Delavirdine and Indinavir, Fig. 1, which are commonly used to treat human immunodeficiency virus (HIV) [24-27]. The previously reported antiviral activity of piperazine-based drugs encouraged us to design a variety of ligands containing piperazine moiety along with other functionalities or other heterocyclic rings in order to make screening of large library of piperazine based drugs to evaluate their inhibition against SARS-CoV-2 and to identify new active compounds.
using high throughput technique. Herein we report our results on synthesis of several compounds containing piperazine ring hybrid with other heterocyclic rings or with sulfur or nitrogen functionalities followed by finding the binding modes of synthesized ligands with SARS-CoV-2 main protease using docking studies. The aim of the present research is focused on the use of computational techniques and structure-based modeling techniques for some synthesized piperazine-based compounds as possible inhibitors against SARS-CoV-2. Thus, this study is an initiative to facilitate development of antiviral drug discovery model that could help to pave the way to the worldwide efforts to combat SARS-CoV-2.

2. Results and discussion

2.1. Chemistry

Multiple functionalization of piperazine at 1 and/or 4 positions were synthesized owing to the biological and pharmacological activities of piperazine derivatives. The nucleophilic character of piperazine at these positions promotes a variety of substitution reactions which allow the existence of hydrophobic and hydrophilic parts necessary to bind through different electrostatic and hydrogen bonding interactions. The synthetic route for the target piperazine-based compounds 2–11 is simple, straightforward and illustrated in Schemes 1–4. The structures of the synthesized compounds were confirmed by their IR, 1H NMR, 13C NMR and elemental analysis.

Piperazine bis-thiosemicarbazide 4 was prepared via multistep reaction involved nucleophilic addition of carbon disulfide to piperazine 1 in presence of triethyl amine, producing intermediate salt 2 [28]. That subjected to S-alkylation reaction using alkyl halide to afford bis(carbo-dithioate) 3 which on subsequent treatment with hydrazine hydrate afforded the desired target compound 4, Scheme 1.

It has been reported that 1,3,4-thiadiazole nucleus has the ability to form mesosonic systems associated with discrete regions of negative and positive charges [29,30]. Furthermore, it gave the associated compounds high lipophilicity which allow their effective cross of cellular membranes [31], leading to good oral absorption and strong interactions with biomolecules (e.g., proteins, nucleic acids, etc.) [32]. Accordingly, the bis thiosemicarbazide 4 has been used as a precursor for building up thia diazole-piperazine hybrid compounds 5–7.

The treatment of compound 4 with carbon disulfide (CS2) in boiling DMF led to the formation of 5,5-(piperazin-1,4-diy)bis(1,3,4-thiadiazole-2-thiol) 5 through additive cyclization process. Subsequent thiol-alkylation of product 5 using either methyl or ethyl iodide in the presence of ethanolic potassium hydroxide afforded S-methyl alkylated product 6a or S-ethyl alkylated product 6b, respectively. Further oxidation of 6a using ethanolic hydrogen peroxide produced the corresponding sulphone 7. Moreover, the Condensation of 4 with 4-nitrobenzaldehyde in presence of acetic acid has given 1,4-bis(4-nitrobenzylidene)piperazine-1,4-bis(carbothiohydrazide) 8, Scheme 2.

The FTIR analysis of the bis-dithiocarbamate 3a, 3b showed bands at 1458 and 1463 cm⁻¹ that can be ascribed to the vibration characteristic for dithiocarbamate group (NCSS). The peaks observed at 2913 cm⁻¹ and 2992 cm⁻¹ are respectively located to the symmetric and asymmetric stretching vibrational of C–H of alkyl groups. Moreover, proton NMR (DMSO–d₆) of 3a revealed two singlets at δ 4.16 and 2.67 ppm due to methylene groups of piperazine and methyl groups, respectively. Whereas, ethyl groups of 3b revealed a quartet and triplet signals in 1H NMR at 3.23 and 1.26 ppm, respectively. The infrared spectrum of 4 exhibited bands in the region 3267–3088 cm⁻¹ corresponding to the absorption characteristic for N–H stretching. Moreover, 1H NMR (DMSO–d₆) of 4 revealed broad D₂O-exchangeable singlets at δ 9.07 and 4.87 ppm attributed to the existence of hydrazino group. Furthermore, 13C NMR spectrum of 3a, 3b and 4 exhibited signals at δ 199, 195 and 182 ppm respective to their thiacarbonyl carbon, respectively. The 1H NMR (DMSO–d₆) spectrum of 5 exhibited a broad exchangeable-signal corresponding to protons of thiadiazole group. Moreover, methyl protons of 6a and 7 revealed signals in proton NMR at δ 2.68 and 3.04 ppm, respectively. The ethyl group of 6b was also displayed in 1H NMR as a quartet and a triplet at δ 3.17 and 1.41 ppm. Additionally, 13C NMR spectra of 5–7 showed two quaternary carbons in region 128.27–184.46 ppm corresponding to thia diazole ring. Compound 8 showed the characteristic infrared absorption bands for NO₂ stretching at 1519 and 1344 cm⁻¹. Furthermore, 1H NMR spectrum of 8 exhibited singlet at δ 8.86 ppm for azomethine proton (N–CH). 13C NMR spectrum of 8 showed two quaternary carbons corresponding to its phenyl rings.
The good antiviral activity of N-substituted isatin grabbed our attention to link isatin to piperazine [33-36]. Accordingly, bis-Mannich base 10, which represent piperazine-isatin hybrid compound was prepared by Aminomethylation of isatin via Mannich reaction using piperazine 1 as secondary amine and formaldehyde at room temperature. Furthermore, piperazine 1 has been transformed into diethyl piperazine-1,4-dicarboxylate 9 by stirring with ethyl chloroformate in dichloromethane at 0 °C followed by addition of triethylamine, Scheme 3. This because carbamates have been known as a peptide bond surrogate in medicinal chemistry [37], due to their ability to permeate cell membranes and chemical stability.

The infrared spectrum of the bis-dicarbamate 9 showed absorption band at 1708 cm⁻¹ corresponding to the stretching vibration of carbamate group. The ¹H NMR spectrum of 9 exhibited a quartet and triplet at 4.13 and 1.24 ppm for the ethyl protons. The carbonyl carbon of 9 was displayed also in ¹³C NMR at 155.37 ppm. The infrared spectrum of the bis-Mannich base 10 revealed two sharp absorption bands at 1738 and 1612 cm⁻¹ attributable to the vibration of ketonic and amidic carbonyls in isatin moiety, respectively. ¹H NMR spectrum of 10 showed a singlet at δ 4.38 ppm corresponding to the protons of the methylene’s spacer. Moreover, ¹³C NMR spectrum of 10 exhibited two signals at δ 183.20 and 158.99 can be ascribed to carbonyl carbons of isatin moiety and a signal at 61.53 for carbon of the methylene spacer.

As an extension to the present investigation, mono substituted piperazine has been synthesized either by direct replacement reaction of equimolar amounts of piperazine and active aryl halide or by amination: cyclization of bis[2-chloroethyl]amine. In this way, pyridyl piperazine 11 has been synthesized by reaction of piperazine 1 and 2-chloro-5-nitropyridine in presence of sodium hydride in 1,4-dioxane, Scheme 3. Moreover, phenyl piperazine 12 has been prepared by refluxing bis[2-chloroethyl]amine hydrochloride with p-bromoaniline in presence of n-propanol and potassium carbonate. The bis[2-chloroethyl]amine has been produced by chlorination of the commercially available diethanolamine using thionyl chloride [38], Scheme 4.

The infrared spectrum of 11 and 12 showed absorption characteristic for N–H stretching in the region 3404–3340 cm⁻¹. The asymmetric structure of 11 and 12 has been verified through NMR spectroscopic data. Accordingly, due to the molecular asymmetry of 11 and 12 their piperazine moiety showed two peaks in both proton and ¹³C NMR.

2.2. Physicochemical properties [39-41]

The physicochemical and biochemical properties of all the candidate ligands 3–12 were evaluated by using in silico computational tools. The oral bioavailability of a drug candidate can be predicted via Lipinski’s rule of five (RO5). This rule is based on physicochemical parameters of the tested ligands, including: Molecular weight (MW) not greater than 500 g/mol; A partition coefficient clogP less than or equal five; number of hydrogen bond donors (HBD) (NH and OH groups) not greater than five; and number of hydrogen bond acceptors (HBA) (O and N atoms) not exceeds ten. The synthesized ligands 3–12 were validated through descriptors of Lipinski’s rule of five (RO5 analysis). Results, Table 1, interestingly showed that nine of the tested ligands namely, 3a, 3b, 5, 6, 7, 9, 10, 11, and 12 were fully in agreement to Lipinski’s rule of five. Ligand 4 exhibited single violation regarding hydrogen donor as well as ligand 8 showed two violations regarding hydrogen acceptor and molecular weight. Another useful predication for oral bioavailability is computation of Veber descriptors namely, num-
Table 1
Predicted physicochemical properties for ligands 3–11.

| Lig. | Mwt | Log P | H-DON | H-ACC | Violation | TPSA | NROTBP | Molecular flexibility | Drug like test | Polar volume | volume | Drug Likeness |
|------|-----|-------|-------|-------|-----------|------|--------|----------------------|---------------|--------------|--------|----------------|
| 3a   | 266.00 | 2.48 | 0     | 2     | 0         | 6.22 | 4      | 8.17                 | 1             | 32.87        | 247.50 | −0.99         |
| 3b   | 294.03 | 3.25 | 0     | 2     | 0         | 6.22 | 6      | 9.93                 | 1             | 38.875       | 285.01 | −0.92         |
| 4    | 234.07 | −1.19 | 6     | 6     | 1         | 73.47 | 4      | 4.44                 | 1             | 242.75       | 228.27 | −0.24         |
| 5    | 317.98 | 0.90 | 0     | 6     | 0         | 51.18 | 2      | 4.71                 | 1             | 78.25        | 230.05 | −0.72         |
| 6a   | 346.01 | 2.98 | 0     | 6     | 0         | 52.31 | 4      | 5.89                 | 1             | 55.62        | 270.65 | −0.71         |
| 6b   | 374.04 | 3.70 | 0     | 6     | 0         | 52.31 | 6      | 7.16                 | 1             | 57.625       | 308.68 | −0.61         |
| 7    | 409.99 | −0.40 | 0 | 10 | 0         | 112.63 | 4      | 6.13                 | 1             | 70.75        | 310.26 | −0.34         |
| 8    | 500.10 | 1.91 | 2     | 12    | 2         | 122.81 | 10     | 6.82                 | 0             | 150.25       | 466.96 | −0.17         |
| 9    | 230.12 | 1.27 | 0     | 6     | 0         | 45.09 | 6      | 4.35                 | 1             | 18.05        | 247.05 | −0.96         |
| 10   | 404.14 | 1.14 | 0     | 8     | 0         | 68.12 | 4      | 3.34                 | 1             | 72.25        | 431.14 | 0.62          |
| 11   | 208.09 | 0.15 | 1     | 6     | 0         | 62.51 | 2      | 2.23                 | 1             | 70.5         | 186.72 | −0.90         |
| 12   | 240.02 | 2.31 | 1     | 2     | 0         | 15.65 | 1      | 3.01                 | 1             | 51.75        | 188.20 | −1.62         |

Table 2
Assessment of bioactivity scores for ligands 3–12 using Molinspiration software.

| Lig. | GPCR | ICM | KI | NRL | PI | EI |
|------|------|-----|----|-----|----|----|
| 3a   | −0.62 | −0.93 | −1.52 | −1.52 | −1.20 | −0.46 |
| 3b   | −0.67 | −0.92 | −1.41 | −1.33 | −1.05 | −0.45 |
| 4    | −1.06 | −0.9 | −1.00 | −1.62 | −1.10 | −0.45 |
| 5    | −0.86 | −0.73 | −0.57 | −1.04 | −0.54 | −0.16 |
| 6a   | −0.74 | −0.68 | −0.56 | −0.72 | −0.70 | −0.30 |
| 6b   | −0.65 | −0.63 | −0.65 | −0.67 | −0.58 | −0.35 |
| 7    | −0.54 | −0.69 | −0.39 | −0.48 | −0.36 | −0.20 |
| 8    | −0.47 | −0.44 | −0.48 | −0.58 | −0.50 | −0.29 |
| 9    | −0.19 | 0.04  | −0.44 | −0.38 | −0.20 | −0.12 |
| 10   | −0.18 | −0.45 | −0.14 | −0.58 | −0.02 | −0.03 |
| 11   | −0.34 | 0.12  | −0.25 | −0.90 | −0.62 | −0.20 |
| 12   | −0.64 | −0.20 | −0.61 | −1.14 | −0.96 | −0.51 |

2.3. Prediction of bioactivity scores [42-44]

Encouraged by the good physicochemical properties of the ligands under study we extend the calculation of drug likeness score towards G protein-coupled receptors (GPCR), ion channel modulators (ICM), kinase inhibitors (KI), nuclear receptor ligands (NRL), protease inhibitors (PI) and other enzyme (EI) targets. Calculations have been done using Molinspiration software, Table 2. As a rule, a compound having bioactivity score with positive value (>0.00) is most probable to possess considerable biological activities, while values 0.00 to −0.50 are expected to be moderately active and if score is less than −0.50 it is assumed to be inactive. The predicted bioactivity scores of all synthesized ligands showed moderate activity against EI which indicate efficient binding to enzymes. Moreover, the results of the present study demonstrated that ligands 9 and 11 showed good bioactivity score (0.04 and 0.12, respectively) towards ICM, while ligands 8, 10 and 12 exhibited moderate bioactivities to ICM. Furthermore, ligands 7–10 displayed moderate bioactivity towards PI. Similar behavior has been shown by ligands 7–11 and ligands 8–11 with respect to KI and GPCR respectively. Additionally, moderate bioactivity scores toward the target NRL has been predicted for ligands 7, 9 only.

2.4. Pharmacokinetics and toxicity

To assess the opportunity of future therapeutic application of the synthesized ligands, pharmacokinetic [45-47] behavior via ADME properties (absorption, distribution, metabolism and excretion) have been predicted in silico using pKCSM tool, Table 3. Regards to the predicted absorption, the obtained data revealed that all ligands 3–12 exhibited favorable human intestinal absorption (61–100%) where molecules with an absorbance less than 30% are poorly absorbed and not suitable for oral administration. Moreover, it was found that ligands displayed reasonable predicted water solubility except for ligands 7 and 9–12. Furthermore, the skin permeability predicted values indicate that all of the ligands are likely skin permeable except for ligands 3b and 12 which have skin permeability constant log kp greater than −2.5 cm/h. Con-
cerning blood brain permeability, the ability of the drug to cross into the brain is important to decrease toxicities and side effects. The predicted data revealed that ligands are lipophilic enough to cross the blood brain barrier except for ligand 7 which exhibited log BB less than −1. Additionally, most of the ligands having no permeation to central nervous system (CNS) with blood brain permeability-surface area product logPS less than −3. Regards to the predicted Metabolism properties, it was found that the ligands under study were predicted as inhibitors or non-inhibitors for some cytochrome P450 isoenzymes such as CYP1A2, CYP2C19 and CYP2C9. None of the assessed ligands was predicted as inhibitor for CYP2C19 or CYP2C9 except ligands 3b and 12, respectively. On the other hand, ligands 3b, 5, 6a, 6b and 12 were predicted as CYP1A2 inhibitor. This inhibitory effect on CYP isoenzyme activity may cause the probability of drug interactions. Moreover, excretion properties expressed in total clearance log(ClClot) have been predicted for all ligands under study which is important for determining dosing rates to reach steady state concentrations.

The parameters related to toxicity including maximum tolerated dose (MTD), AMES test, oral rat acute toxicity (ORAT), hepatotoxicity (HT), and skin sensitization (SS) have been predicted. AMES test is employed to assess whether the drug is mutagenic or not [48]. All the synthesized ligands except 8 and 11 exhibited negative AMES tests indicating that they are non-mutagenic. Moreover, ligands showed relatively high predicted lethal dose value L50 (2.53–3.33 mol/Kg) which is indicative of low acute toxicity.

The predicted hepatotoxicity showed that ligands 3a, 5, 6, 7, 8, 10, 11 and 12 would probably hepatotoxicity associated with disrupted normal function of liver. However, most of the assessed ligands don’t show skin sensitization.

### 2.5. Molecular docking

Considering the global threat posed by COVID-19, and with no proven antiviral agent available for immediate relief, the current *in silico* study provide structural insights about the protease of SARS-CoV-2 and its molecular interactions with synthesized ligands 3–12 as protease inhibitors. Protease enzyme is essential for viral replication because it catalyzes the proteolytic process for the polyproteins that are translated from the viral RNA [49]. Thus, inhibition of the protease activity would block viral replication and unlikely to be toxic for human, which make protease one of the best drug discovery targets in case of coronaviruses. The activity of protease enzyme is blocked by binding of inhibitor molecules to the active site of the enzyme. Moreover, protease is suitable for designing wide-spectrum inhibitors because it contains large number of amino acids. All the eleven protease inhibitors candidates got docked onto the predicted 3D model of protease of SARS-CoV-2 with a negative dock energy value as shown in Table 4. Molecular interaction studies, Fig. 2, showed that protease model of SARS-CoV-2 had

| Lig. | Absorption | Distribution | Metabolism | Excretion | Toxicity | SS |
|-----|------------|--------------|------------|-----------|----------|----|
| S   | IS         | SP           | BBP        | CNS       | CYP1A2   | CYP2C19 | CYP2C9 | Exc, TC | MTD | AMES | ORAT | HT | SS |
| 3a  | −3.45      | 91.88        | −2.59      | 0.39      | −3.36    | No       | No     | 0.53    | 0.57   | No     | 3.03    | Yes | Yes |
| 3b  | −4.18      | 91.04        | −2.42      | 0.57      | −3.06    | Yes      | Yes    | 0.51    | 0.44   | No     | 3.16    | Yes | Yes |
| 4   | −3.14      | 62.84        | −3.24      | −0.87     | −3.69    | No       | No     | 0.21    | 1.37   | No     | 3.33    | Yes | No  |
| 5   | −4.40      | 100.00       | −2.94      | −0.32     | −3.22    | Yes      | No     | 0.20    | 0.28   | No     | 2.64    | Yes | No  |
| 6a  | −4.50      | 95.77        | −2.90      | 0.05      | −3.13    | Yes      | No     | 0.29    | 0.17   | No     | 3.19    | Yes | Yes |
| 6b  | −4.88      | 94.74        | −2.96      | 0.01      | −3.10    | Yes      | No     | 0.33    | 0.19   | No     | 3.09    | Yes | No  |
| 7   | −2.86      | 54.77        | −2.73      | −2.08     | −3.40    | No       | No     | 0.24    | 0.65   | No     | 2.96    | Yes | No  |
| 8   | −4.72      | 66.53        | −2.74      | −1.05     | −2.67    | No       | No     | −0.09   | −0.46  | Yes    | 2.57    | Yes | No  |
| 9   | −1.97      | 90.46        | −4.07      | −0.23     | −3.08    | No       | No     | 0.67    | 0.72   | No     | 2.65    | No  | No  |
| 10  | −2.97      | 61.70        | −2.90      | −0.33     | −2.73    | No       | No     | 1.09    | −0.58  | No     | 2.65    | Yes | Yes |
| 11  | −1.93      | 90.71        | −2.93      | −0.45     | −2.97    | No       | No     | 0.48    | 0.23   | Yes    | 2.53    | Yes | No  |
| 12  | −1.96      | 92.70        | −1.86      | 0.627     | −2.27    | Yes      | Yes    | 0.82    | 0.13   | No     | 2.78    | Yes | Yes |

### 3. Experimental

#### 3.1. Instruments and apparatus

Melting points were determined by MEL-TEMP II melting point apparatus in open glass capillaries. The IR spectra were recorded as potassium bromide (KBr) discs on a Perkin-Elmer FT-IR (Fourier-Transform Infrared Spectroscopy), Faculty of Science, Alexandria University. The NMR spectra were carried out at ambient temperature (~25 °C) on a (JEOL) 500 MHz spectrophotometer using tetramethylsilane (TMS) as an internal standard, NMR Unit, Faculty of Science, Mansoura University. Elemental analyses were analyzed at the Regional Center for Mycology and Biotechnology, Al-Azhar University, Cairo, Egypt.

#### 3.2. Docking program

Molecular docking simulations were performed to achieve the mode of interaction of prepared piperazines with the binding pocket of SARS-CoV-2 protease. The newly released crystal structure of SARS-CoV-2 main protease as a receptor was retrieved from protein data bank (www.rcsb.org) with PDB ID: 6M03 [50]. Software version 2015.10 of Molecular Operating Environment (MOE)
was used to prepare the input files and analyzing the result. All water molecules, ligands and ions were removed from pdb file for the preparation of protein input file. The active site was selected utilizing ‘Site Finder’ MOE 2015.10 feature. Prior to docking, the piperazines structures were subjected to energy minimization and geometry optimization before docking. Docking simulations were conducted several times with various fitting protocols to observe the best molecular interactions and free binding energies. All docking results were sorted by scoring binding energy.

3.3. General method for synthesis of piperazines 2–12

3.3.1. Bis(triethylammonium) piperazine-1,4-bis(dithiocarbamate) 2

A mixture of piperazine (2 g, 0.023 mol) and triethylamine (12.8 mL) dissolved in THF (10 mL) in an ice bath was received carbon disulfide (4.25 mL) drop wise with constant stirring. After complete addition of carbon disulfide, the reaction was stirred at room temperature for 18 h. Pale yellow precipitate was formed filtered, washed 4 times with hexane:ethylacetate (5:95), 8.9 g (87%) yield; m.p.215–220 °C. [28]

3.3.2. General procedure of dialkylpiperazine-1,4-bis(carbothioylate) 3a,b

Alkal iodide [ethyl iodide (2.31 mL) or methyl iodide (1.23 mL)] was drop wisely added to a stirred solution of piperazine-1,4-bis(dithiocarbamate salt) 2 (4 g, 0.009 mol) in water (25 mL) under an ice cooled condition. The reaction mixture was stirred overnight at room temperature. The separated solid was filtered off then crystallized from ethanol.

3.3.3. Dimethyl piperazine-1,4-bis(carbothioylate) 3a [51]

Pale yellow crystals, 2.33 g (87%) yield; m.p. 180 °C. IR cm⁻¹ (KBr): 2992 (sp³C-H), 1458 (N–CSS, dithiocarbamate) and 1158 (–C-N) cm⁻¹. 1H NMR (400 MHz, CDCl₃): δ ppm: 4.16 (s, 8H, piperazine-4CH₂) and 2.67 (s, 6H, 2CH₃) ppm. 13CAPT NMR (101 MHz, CDCl₃): δ 199.22, 46.24 and 20.11 ppm. C₈H₁₈N₂S₄ requires: C: 36.05; H: 5.30; N: 10.51%, found: C: 36.47; H: 5.54; N: 10.27%.

3.3.4. Diethyl piperazine-1,4-bis-carbothioylate 3b [51,52]

White crystals, 2.26 g (97%) yield; m.p. 125 °C. IR cm⁻¹ (KBr): 2997 (sp³C-H), 1463 (N–CSS, dithiocarbamate) and 1159 (C–N) cm⁻¹. 1H NMR (500 MHz, CDCl₃) δ ppm: 3.34 (s, 8H, piperazine-4CH₂), 3.23 (g, J = 7.4 Hz, 4H, 25-CH₂) and 1.26 (t, J = 7.3 Hz, 6H, 2CH₃) ppm. 13CAPT NMR (126 MHz, CDCl₃): δ 195.70, 47.63, 30.51 and 13.54 ppm. C₁₀H₁₈N₂S₄ requires: C: 40.77; H: 6.17; N: 9.51%, found: C: 41.01; H: 6.25; N: 9.21%.

3.3.5. Piperazine-1,4-bis(carbothioylhydrazide) 4 [53]

A mixture of dimethyl piperazine-1,4-bis-carbothioylate 3a (4 gm, 0.013 mol) and hydrazide hydrate (98%) (19.8 ml) in ethanol (25 ml) was refluxed for 30 h. On cooling the reaction mixture, the solid which separated was filtered, washed, dried and no need to extra purification. gray powder, 2.23 g (70%) yield; m.p. 218–220 °C. IR cm⁻¹ (KBr): 3267 (NH₂), 3088 (NH), 2927 (sp³–C–H), 1159 (C–N), 1645 (–N=C=S, thiouamide) and 1159 (C–N). 1H NMR (500 MHz, DMSO-d₆) δ ppm: 9.07 (brs, 2H, NH₂, D₂O exchangeable), 4.87 (brs, 4H, 2NH₂, D₂O exchangeable) and 3.78 (s, 8H, piperazine-4CH₂) ppm. 13CAPT NMR (126 MHz, DMSO-d₆): 182.47 and 46.11 ppm. C₉H₁₈N₄S₄ requires: C: 30.74, H: 6.03, N, 35.86%, found: C: 31.45, H: 6.29, N, 35.46%.

3.3.6. 5,5’-(Piperazin-1,4-diyli)bis(1,3,4-thiadiazole-2-thiol) 5

The mixture of piperazine-1,4-bis(carbothioylhydrazide) 4 (5 gm, 0.021 mol), dimethylformamide (12 mL) and carbon disulphide (10 mL) were refluxed for 4 h. The mixture was poured into ice water and the precipitated product was filtered then crystallized from ethanol to yield yellow powder, 6.61 g (97%) yield; m.p. 290–300 °C. IR (KBr): 2909 (sp³-C-H), 2580 (SH), 1554 (C = N), 1172 (C–N) and 718 (C-S) cm⁻¹. 1H NMR (500 MHz,
DMSO–d$_6$): $\delta$ 13.65 (brs, 2H, D$_2$O exchangeable) and 3.39 (s, 8H, piperazine-4CH$_2$) ppm. $^{13}$CAPT NMR (126 MHz, DMSO–d$_6$): $\delta$ 181.57, 163.20 and 46.86 ppm. C$_8$H$_{10}$N$_6$S$_4$ requires: C: 30.16; H: 3.17; N: 26.39%, found: C: 30.43; H: 3.25; N: 26.57%.

### 3.3.7. General procedure of 1,4-bis(5-(alkylthio)-1,3,4-thiadiazol-2-yl)piperazine 6a,b

Alkyl iodide [methyl iodide (12.5 mL) or ethyl iodide (12.5 mL)] was drop wise added to stirring mixture of 5,5′-(piperazin-1,4-diyl)bis(1,3,4-thiadiazole-2-thiol) 8 (5 gm, 0.015 mol) and KOH (1.5 gm, 0.026 mol) in ethanol (35 mL). The mixture was further stirred for 4 h then poured into ice water and the precipitated formed washed with water, dried and crystallized from ethanol.

### 3.3.8. 1,4-Bis(5-(methylthio)-1,3,4-thiadiazol-2-yl)piperazine 6a

Pale white powder, 3.34 g (61%) yield; m.p. 215 ºC. IR cm$^{-1}$ (KBr): 2951 (sp$^3$ C-H), 1517 (C = N), 1107 (C-N), and 742 (C-S). $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ ppm: 3.64 (s, 8H, piperazine-4CH$_2$) and 2.68 (s, 6H, 2CH$_3$) ppm. $^{13}$CAPT NMR (101 MHz, CDCl$_3$): 171.95, 155.95, 159.95, 49.08 and 16.83 ppm. C$_{10}$H$_{14}$N$_6$S$_4$ requires: C: 34.65; H: 4.08; N: 24.25%, found: C: 34.35; H: 4.27; N: 24.64%.

### 3.3.9. 1,4-Bis(5-(ethylthio)-1,3,4-thiadiazol-2-yl)piperazine 6b

Pale white crystalline powder, 3.38 g (57%) yield; m.p. 220 ºC. IR cm$^{-1}$ (KBr): 2983 (sp$^3$ C-H), 1517 (C = N), 1105 (C-N), and 742 (C-S). $^1$H NMR (500 MHz, CDCl$_3$): $\delta$ ppm: 3.67 (s, 8H, piperazine-4CH$_2$), 3.17 (q, 4H, 2CH$_2$) and 1.41 (s, 6H, 2CH$_3$). $^{13}$CAPT NMR

Fig. 2. 2D and 3D binding modes of 3–12 (orange tube) in protease active sites (PDB: 6M03 [50]).
(126 MHz, CDCl₃): δ 172.23, 154.61, 49.06, 29.32 and 14.95 ppm. C₁₂H₁₈N₆S₄ requires: C: 38.47; H: 4.85; N: 22.43%, found: C: 38.53; H: 4.52; N: 22.87%.

3.3.10. 1,4-Bis(5-(methylsulfonyl)-1,3,4-thiadiazol-2-yl)piperazine 7

The treatment of 1,4-bis(5-(methylthio)-1,3,4-thiadiazol-2-yl)piperazine 187a (0.25 gm, 0.0007 mol) dissolved in ethanol (20 mL), with hydrogen peroxide (34%, 7 mL) was stirred at room temperature. After the completion of addition, the stirring was continuing overnight, then the excess of solvent was evaporated, cooled, filtered and then crystalizes from ethanol to give white powder, 0.14 g (48%) yield; m.p. 250 °C. IR cm⁻¹ (KBr): 2962 (sp³C-H), 1516 (C = N), 1103 (C-N), 767 (C-S) and 1308–1152 (SO₂, asymmetry and Symmetry). ¹H NMR (400 MHz, DMSO–d₆), δ ppm: 3.77 (s, 8H, piperazine-4CH₂) and 3.04 (s, 6H, 2CH₃) ppm. ¹³C APT NMR (125 MHz; DMSO–d₆): 184.46, 161.90, 48.85 and 42.84. C₁₀H₁₄N₆O₄S₄ requires: C: 29.25; H: 3.44; N: 20.47% found: C: 29.12; H: 3.27; N: 20.32%.
3.3.11. 1,4-Bis(4-nitrobenzylidene)piperazine-1,4-bis(carbothiohydrazide) 8 [54]

Piperazine-1,4-bis(carbothiohydrazide) 4 (0.5 gm, 0.00213 mol) dissolved in acetic acid (10 mL) was treated with p-nitrobenzaldehyde (0.64 gm, 0.00213 mol) and the mixture was refluxed for 25 h. The product precipitated while hot was filtered and dried to give dark red powder, 0.56 g (52%) yield; m.p. 175 °C. IR (KBr): 3440 (N–H), 2990 (sp$^3$-C-H), 1594 (S = C–N–, thioamide), 1106 (C–N) and 1519–1344 (NO$_2$, Asymmetry and Symmetry) cm$^{-1}$. $^1$H NMR (500 MHz, DMSO–d$_6$): δ 10.16 (br.s, 2H, D$_2$O exchangeable), 8.86 (s, 2H, 2N=CH), 8.25 (m, 8H, Ar-H) and 4.09 (s, 8H, piperazine-4CH$_2$) ppm. $^{13}$C APT NMR (126 MHz, DMSO–d$_6$): δ 192.40, 147.89, 140.08, 130.68, 129.62, 124.32 and 49.05 ppm. C$_{20}$H$_{20}$N$_8$O$_4$S$_4$ requires: C: 47.98; H: 4.03; N: 22.38%; found: C: 48.25; H: 4.12; N: 22.12%.

3.3.12. Diethyl piperazine-1,4-dicarboxylate 9

A solution of piperazine 1 (5 gm, 0.058 mol) in dichloromethane (75 mL) was cooled to 0 °C then triethylamine (24 mL) was added. The reaction was proceeded by drop wise addition of a solution of ethyl chloroformate (11 mL) in dichloromethane (35 mL). After stirring overnight at room temperature, the mixture was filtered and subject to extract with 3 M
HCl. The organic layer was dried over sodium sulfate then evaporated to give diethyl piperazin-1,4-dicarbamate. White solid, 7.23 g (54% yield; m.p. 45–47 °C; IR cm⁻¹ (KBr): 2994 (sp²-C=H), 1708 (N=O-CO carbamate) and 1109 (C-N). ¹H NMR (500 MHz, CDCl₃): δ ppm: 4.13 (q, 4H, 2CH₃), 3.42 (s, 8H, piperazine-4CH₂) and 1.24 (t, 6H, 2CH₃). ¹³C CPMG (101 MHz, CDCl₃): δ ppm: 151.37, 61.58, 43.02 and 14.68. C₁₂H₁₄N₂O₄ requires: C: 6.93; H: 5.21, N: 11.35%.

Credit author statement

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Declaration of Competing Interest

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

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.molstruc.2021.131020.

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