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Towards Covid-19 TMPRSS2 enzyme inhibitors and antimicrobial agents: Synthesis, antimicrobial potency, molecular docking, and drug-likeness prediction of thiadiazole-triazole hybrids

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ABSTRACT

1,3,4-Thiadiazole analogues 3 and 4 were synthesised via the reaction of 1-(5-methyl-1-(5-(methylthio)-1,3,4-thiadiazol-2-yl)-1H-1,2,3-triazol-4-yl)ethan-1-one 2 with vanillin or thiophene-2-carboxaldehyde, respectively through chalcone reaction. Compounds 3 and 4 were submitted to react with thiosemicarbazide affording 5-(4-hydroxy-3-methoxyphenyl)-3-(5-methyl-1-(5-(methylthio)-1,3,4-thiadiazol-2-yl)-1H-1,2,3-triazol-4-yl)-4,5-dihydro-1H-pyrazole-1-carbothioamide (5) give 3-(5-methyl-1-(5-(methylthio)-1,3,4-thiadiazol-2-yl)-1H-1,2,3-triazol-4-yl)-5-(thiophen-2-yl)-4,5-dihydro-1H-pyrazole-1-carbothioamide (6), respectively. The letters were reacted with N-(4-chlorophenyl)-2-oxopropanehydrazonoyl chloride to give compounds 7 and 8. The chemical compositions of the novel compounds were affirmed by spectral and microanalytical data. Meanwhile, all the newly synthesized compounds have been screened for their ability to prevent the proliferation of different pathogens named *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Staphylococcus aureus*, and *Candida albicans* in vitro. Additionally, the potency of the newly synthesized compounds to be anti-COVID-19 candidates was studied through a molecular docking study. The newly prepared molecules 2–8 were studied in silico against transmembrane serine protease 2 (TMPRSS2) to identify their potential therapeutic activity against Coronavirus. Moreover, the drug-likeness of the compounds was tested theoretically by ADMET studies. Compound 8 exhibited a better binding affinity (-9.1 kcal/mol) against the target enzyme TMPRSS2. Additionally, it respects Lipinski's rule of five and has acceptable ADMET properties, indicating that compound 8 could be interesting for the treatment of Covid-19.

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1. Introduction

Coronavirus 2019 (Covid-19) is the major cause of global public health problems [1,2]. Coronavirus is an infectious disease caused by SARS-Cov-2 virus [3]. The present need is to identify new antiviral therapeutic agents for Covid-19, which is why TMPRSS2 was selected as a drug target for Covid-19 as it plays a critical role in the propagation of disease. Heterocycles have always been regarded as the basic bedrock of the most modern biomedical and medicinal chemical research. Moreover, microbial infections are still among the major causes of death among the world's population due to increased bacterial antibiotic resistance phenomena and the development of resistance to chemotherapeutics. For these reasons, there is an urgent need to design and synthesize new antimicrobial agents.

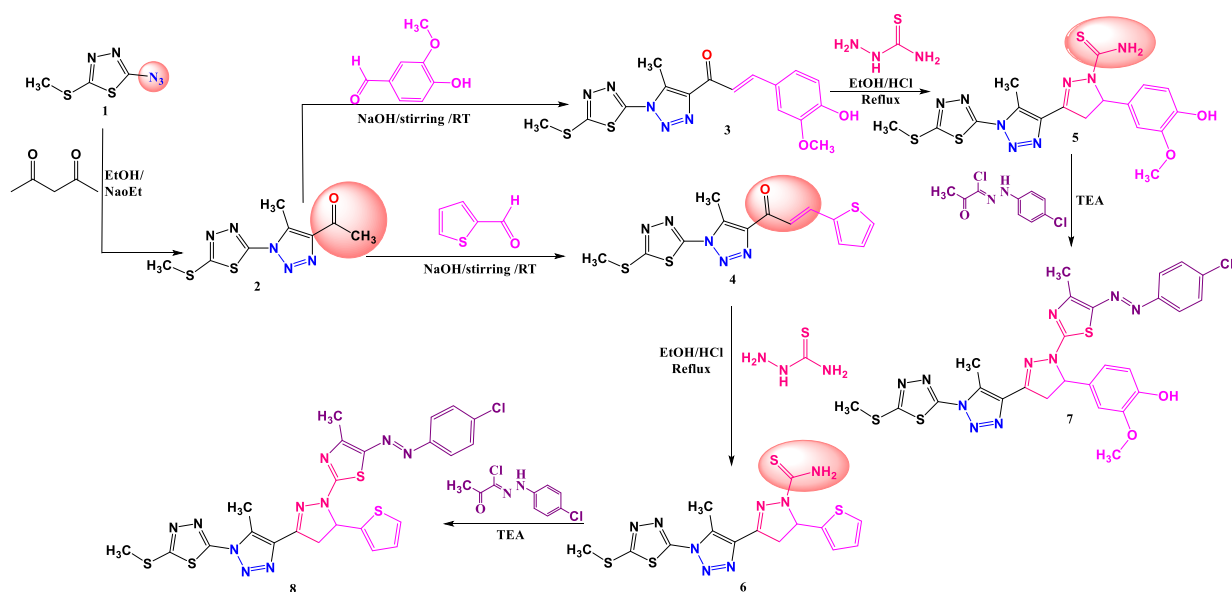
1,3,4-thiadiazoles and 1,2,3-triazoles as privileged ring systems. Considered among the most biologically promising nitrogenous five-membered heterocycles. They comprised a wide-ranging library of drug categories [4–15] like, anti-inflammatory, antidiabetic [16,17], antimicrobial [11,14], anticancer [6,15,18–21], antiviral [4], antileishmanial [22], etc.

Meanwhile, 1,3,4-thiadiazoles were found in several commercial drugs including antibiotics Cefazolin, sodium diuretics acetazolamide and methazolamide, anti-parasitic drug Megazol. Moreover, triazole moiety is present in many drugs, such as in aromatase inhibitor drugs Letrozole and Anastrozole.

Therefore, the present study conducts the synthesis of thiadiazole-triazole hybrid molecules 2–8 and antibacterial screening against different pathogens. Further, a molecular docking study of these compounds was performed against the target enzyme. Finally, ADMET analysis of the prepared molecules was calculated using free access tools such as Molinspiration, admetSAR, and SwissADME.

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Scheme 1. Synthesis of new 1,3,4-thiadiazole-based molecules 2-8.

2. Results and discussion

2.1. Chemistry

1-(5-methyl-1-(5-(methylthio)-1,3,4-thiadiazol-2-yl)-1H-1,2,3-triazol-4-yl) ethan-1-one (2) was allowed to react with vanillin or thiophene-2-carboxaldehyde in the presence of aqueous sodium hydroxide to produce the chalcones 3 and 4, respectively. Chemical structures of 3 and 4 were inferred from correct spectral and microanalytical data. As an authentic sample, ^1H NMR spectrum of compound 3 showed multiple signals 6.70–7.82 ppm for the aromatic hydrogen, and the proton of ($\text{CH}=\text{C}$) revealed doublet signal at 8.22 ppm for the proton of ($\text{CH}=\text{C}$) and showed a singlet signal at 9.52 ppm for the proton of OH group. Compounds 3 and 4 were submitted to react with thiosemicarbazide in boiling ethanolic sodium hydroxide to give 5-(4-hydroxy-3-methoxyphenyl)-3-(5-methyl-1-(5-(methylthio)-1,3,4-thiadiazol-2-yl)-1H-1,2,3-triazol-4-yl)-4,5-dihydro-1H-pyrazole-1-carbothioamide (5) (4-chlorophenyl diazenyl)-4-methylthiazol-2-yl)-3-(5-methyl-1-(5-(methylthio)-1,3,4-thiadiazol-2-yl)-1H-1,2,3-triazol-4-yl)-5-(thiophen-2-yl)-4,5-dihydro-1H-pyrazole-1-carbothioamide (6). The latter were reacted with N-(4-chlorophenyl)-2-oxopropanehydrazonoyl chloride in boiling ethanol containing catalytic amount of triethylamine gave 4-(1-(5-(4-chlorophenyl) diazenyl)-4-methylthiazol-2-yl)-3-(5-methyl-1-(5-(methylthio)-1,3,4-thiadiazol-2-yl)-1H-1,2,3-triazol-4-yl)-4,5-dihydro-1H-pyrazol-5-yl)-2-methoxyphenol (7) and 2-(4-(1-(5-(4-chlorophenyl) diazenyl)-4-methylthiazol-2-yl)-5-(thiophen-2-yl)-4,5-dihydro-1H-pyrazol-3-yl)-5-methyl-1H-1,2,3-triazol-1-yl)-5-(methylthio)-1,3,4-thiadiazole (8) (Scheme 1). For instance, ^1H NMR spectrum of compound 8 showed doublet of doublet signal at 3.65 ppm for CH_2 (pyraz), doublet of doublet signal at 4.01 ppm for CH_2 (pyraz), and doublet of doublet signal at 5.52 ppm for CH (pyraz). The structure of compound 8 was also confirmed by its mass spectrum (m/z (599)) [M^+] (see Experimental Part).

2.2. Antimicrobial activity

The up growing chemotherapeutic resistance of various microbial pathogens had been considered the most significant clinical problem globally. Consequently, investigation of new organic

compounds which could be derived from active groups to serve as wide-spectrum antimicrobials could contribute to compacting such challenges. In the current study, the antimicrobial potency of five newly synthesized compounds against some microbes was detected. The results revealed that compound 8 exhibited antimicrobial activity against all the tested microbes. The toxicity of the other tested compounds was noticed and there were different considerable activities observed against gram-negative bacteria and yeast. The MIC of the tested compound was also listed, as shown in Table 1.

It was worth noting that MIC is defined as the average of the lowest concentrations with no observable growth of microorganisms. Compound 8 revealed a considerable broad spectrum of antimicrobial activities against all strains of the tested pathogens with low concentrations which ranged from 5 to 10 $\mu\text{g}/\text{mL}$; Compound 8 exhibited significant antimicrobial activity against *Pseudomonas aeruginosa* at the concentration lower than the standard drug Ciprofloxacin. Likewise, Compound 2 provided MIC value at low concentration toward *Pseudomonas aeruginosa* and *Staphylococcus aureus* while it showed a relatively higher MIC concentration against *Escherichia coli*. Although compound 3 had a MIC value at low concentrations toward *Bacillus subtilis*, *Staphylococcus aureus*, and *Candida albicans*, it showed a relatively higher MIC concentration against *E. coli* and *Pseudomonas aeruginosa*. Compound 4 provided MIC value at low concentration toward *Candida albicans* and high MIC concentration against *Bacillus subtilis*, *Staphylococcus aureus*, and *Candida albicans*. Other tested organisms could be resistant to it even at high concentrations. Further, MIC was observed at high concentrations against *Pseudomonas aeruginosa*, and at low concentrations against *E. coli*, *Bacillus subtilis*, *Staphylococcus aureus*, and *Candida albicans* for compound 6. This study revealed that the antimicrobial activity of the tested new derivatives displayed potent antibacterial and antifungal activities.

2.3. In silico docking study and ADMET analysis

In the current study, the ability of the newly prepared molecules to inhibit Covid-19 TMPRSS2 was calculated by the molecular docking approach. The compounds have high binding affinities against TMPRSS2 enzyme ranging from -9.1 to -5.5 kcal/mol, which is better achieved using compound 8

Table 1
Antimicrobial activity and minimum inhibitory concentration (MIC) of the synthesized derivatives.

Sample No.	Minimum Inhibitory Concentration (MIC, µg/mL)				
	Escherichia coli	Pseudomonas aeruginosa	Bacillus subtilis	Staphylococcus aureus	Candida albicans
2	160	40	ND	40	ND
3	120	160	20	40	40
4	ND	ND	80	80	20
5	40	60	ND	ND	ND
6	40	160	40	20	20
7	30	40	120	ND	20
8	10	5	10	7	5
Ciprofloxacin	5	7	2.5	1.25	ND
Nystatin	ND	ND	ND	ND	5

Ciprofloxacin and Nystatin were used as standard drugs as control, ND: not determined.

Table 2
The binding energy values of the docked compounds 2–8 against TMPRSS2.

Binding Energy kcal/mol	Docked complex (amino acid–ligand) interactions	Distance (Å)
2	H-bonds TYR243:OH–compound 2	2.92
3	H-bonds TYR243:OH–compound 3 GLN350:NE2–compound 3 GLN331:OE1–compound 3 π - π interactions TRP377–compound 3 TRP377–compound 3 π -sigma interactions HIS203–compound 3 HIS203:CB–compound 3	2.82 2.91 2.15 5.13 4.36 3.65 3.73
4	H-bonds TYR243:OH–compound 4	2.94
5	H-bonds ARG208:NE–compound 5	2.86
6	H-bonds PRO245:O–compound 5	2.83
7	π -cation interactions LYS68:NZ–compound 6 ARG130:NH1–compound 6 ARG130:NH2–compound 6 π -sigma interactions GLN283:CG–compound 6	5.28 5.34 5.52 3.96
8	π - π interactions PHE66–compound 6 π -cation interactions LYS68:NZ–compound 6 π -sigma interactions GLN283:CG–compound 6	4.81 5.33 3.78

(−9.1 kcal/mol) (Fig. 1). The binding energies and intermolecular interactions with their distances are tabulated in Table 2. The 2D and 3D representations of the binding interactions of the docked compounds 2–8 against TMPRSS2 enzyme are shown in the (Supplementary materials). Compounds 2 and 4 docked with the target through one hydrogen bonding interaction with the residue TYR243 at 2.92 Å. Compound 3 exhibited binding energy (−6.8 kcal/mol) and showed three hydrogen bonding interactions with TYR243, GLN331, and GLN350. Additionally, it formed two π - π and π -sigma interactions with TRP377 and HIS203 at the distances 5.13, 4.36, 3.65, and 3.73 Å, respectively. Compound 6 docked with the residue PRO245 at 2.83 Å. Compound 5 showed binding energy (−7.4 kcal/mol), and formed one hydrogen bond with ARG208. Compound 7 docked with the residue LYS68, ARG130, and GLN283 through three π -cation and one π -sigma interactions, respectively. Finally, compound 8, with the highest

binding affinity (−9.1 kcal/mol), exhibited three π -stacked interactions with PHE66, LYS68, and GLN283.

The ADMET predictions were used to calculate the pharmacokinetics properties of the synthesized molecules. As observed in Table 3, all compounds respected the conditions mentioned in Lipinski's rule of five (Ro5) and had acceptable ADMET properties.

The acceptable ranges are as follows; Mol wt.: (130–725); % Human oral absorption: >80% high, <25% low; Donor HB: (0.0–6.0); Accept HB: (2.0–20.0); Predicted BBB permeability (−3 to 1.2); Predicted Caco cell permeability in nm/s (<25 is poor and >500 is great).

3. Experimental

3.1. Synthesis

3.1.1. Instruments

The reagents used in this work were used without purification. Melting points of the prepared compounds were determined using Griffin apparatus and are uncorrected. The completion of the reactions was mentioned by Thin Layer Chromatography (TLC) technique on aluminium plates coated with silica gel. IR spectra were recorded on Shimadzu 408 and Bruker Vect. 22. NMR spectra were run at 500 MHz using TMS as the internal standard. The chemical shifts were measured in ppm (δ) related to TMS (0.00 ppm). MS were recorded on an HP model, Mass 5988 Mass spectrometer at 70 eV. All new compounds were analysed for C, H, and N at Cairo University, Egypt.

3.1.2. 3-(4-hydroxy-3-methoxyphenyl)-1-(5-methyl-1-(5-(methylthio)-1, 3, 4-thiadiazol-2-yl)-1H-1, 2, 3-triazol-4-yl) prop-2-en-1-one (3)

Drop-wise addition of aqueous sodium hydroxide (10 mL, 10%) to a mixture of 1-(5-methyl-1-(5-(methylthio)-1,3,4-thiadiazol-2-yl)-1H-1,2,3-triazol-4-yl) ethan-1-one 2 (2.5 g, 10 mmol) and vanillin (1.5 g, 10 mmol) was conducted in ethanol while stirring at room temperature. The reaction mixture was left for 2 h, when the resulting solid was collected and recrystallized from ethanol to give 3 as white crystals; yield: 78%; m.p. 123–125 °C; FT-IR (KBr, ν , cm^{-1}): 3417 (OH), 1675 (C=O), 1600 (C=N), 1560 (C=C); ^1H NMR (DMSO d_6 , 500 MHz): δ (ppm) 2.47 (s, 3H, CH_3), 2.73 (s, 3H, CH_3), 3.83 (s, 3H, OCH_3), 6.70–7.82 (m, 4H, ArH, $\text{CH}=\text{C}$), 8.22 (d, 1H, $\text{CH}=\text{C}$), 9.52. ^{13}C NMR (DMSO d_6 , 100 MHz): δ 9.52 (CH_3), 16.21 (S-CH_3), 54.33 (OCH_3), 112.17 (Ar), 116.06 (Ar), 121.15 ($\text{CH}=\text{C}$), 122.27 (Ar), 127.18 (Ar), 133.92 (Ar), 138.51 (Ar), 145.14 ($\text{CH}=\text{C}$), 147.13 (Ar), 149.18 (Ar), 160.32 (Ar), 162.15 (Ar), 185.51 (C=O); MS: m/z [%]: 389 (M^+), 374 (75), 358 (12), 317 (30), 287 (28), 255 (86), 127 (48), 77 (100); Anal. Calcd for $\text{C}_{16}\text{H}_{15}\text{N}_5\text{O}_3\text{S}_2$ (389.45): C, 49.35; H, 3.88; N, 17.98%. Found C, 49.31; H, 3.82; N, 17.95%

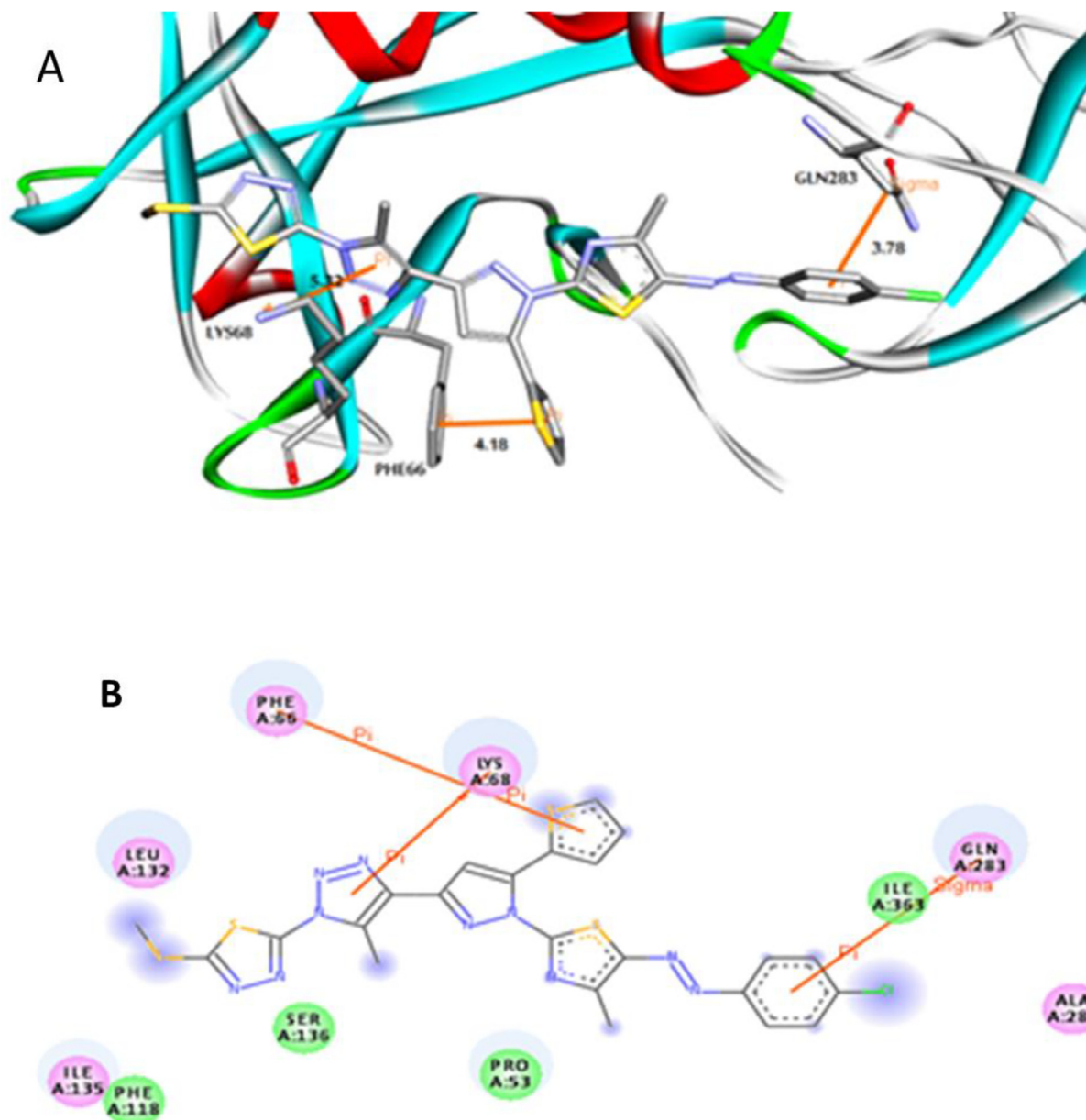


Fig. 1. A: (3D), and B: (2D) representations of the binding interactions of the docked compounds 8 against TMPRSS2 (PDB ID: 1z8g).

Table 3

ADMET profile and drug-likeness properties of the docked molecules 2–8.

	Molecular Weight (g/mol)	Caco-2 Permeability (Caco2+)	%Human Intestinal Absorption (HIA+)	logp	TPSA A ²	HBA	HBD	N rotatable	N violations	GI absorption	BBB permeant	Carcinogenicity	Bioavailability score
2	255.32	54.56	97.35	0.57	127.10	5	0	3	0	High	No		0.55
3	389.45	50.55	98.88	1.44	156.56	7	1	6	0	Low	No	Noncarcinogenic	0.55
4	349.45	54.36	98.81	1.76	155.34	5	0	5	0	Low	No	Noncarcinogenic	0.55
5	462.59	50.23	97.32	2.23	127.59	10	3	6	0	Low	No	Noncarcinogenic	0.55
6	422.57	50.84	87.91	1.65	211.98	5	1	5	0	Low	No	Noncarcinogenic	0.55
7	639.17	54.81	98.52	3.52	139.19	12	1	8	0	Low	No	Noncarcinogenic	0.17
8	599.18	54.04	92.75	3.92	219.78	8	0	7	0	Low	No	Noncarcinogenic	0.55

HBA, number of hydrogen bond acceptors; HBD, number of hydrogen bond donors; logp, logarithm of partition coefficient between n-octanol and water; N rotatable, number of rotatable bonds.

3.1.3. 1-(5-methyl-1-(5-(methylthio)-1,3,4-thiadiazol-2-yl)-1H-1,2,3-triazol-4-yl)-3-(thiophen-2-yl) prop-2-en-1-one (4)

Drop-wise addition of aqueous sodium hydroxide (10 mL, 10%) to a mixture of 1-(5-methyl-1-(5-(methylthio)-1,3,4-thiadiazol-2-yl)-1H-1,2,3-triazol-4-yl) ethan-1-one 2 (2.5 g, 10 mmol) and thiophene-2-carboxaldehyde (1.1 mL, 10 mmol) was carried out in ethanol while stirring at room temperature. The reaction mixture was left for 2 h, when the resulting solid was collected and recrystallized from ethanol to give 4 as white crystals; yield: 82%; m.p.142–144 °C; FT-IR (KBr, ν , cm^{-1}): 1682 (C = O), 1620 (C = N), 1595 (C = C); ^1H NMR (DMSO d_6 , 500 MHz): δ (ppm) 2.42 (s, 3H, CH₃), 2.76 (s, 3H, CH₃), 6.72–7.79 (m, 5H, ArH, CH=CH). ^{13}C NMR (DMSO d_6 , 100 MHz): δ 9.52(CH₃), 16.54 (CH₃), 127.12(CH=), 128.13(Ar), 129.14(Ar), 130.81(Ar), 133.27(Ar), 134.11(CH=), 138.33 (Ar), 140.13 (Ar), 160.61(Ar), 162.27(Ar), 182.15 (C=O); MS: m/z [%]: 350 (M+1), 335 (12), 292(10), 272(30), 265(15), 177(50), 65(100); Anal. Calcd for C₁₃H₁₁N₅O₃ (349.45): C, 44.68; H, 3.17; N, 20.04%. Found C, 44.62; H, 3.15; N, 19.98%

3.1.4. 5-(4-hydroxy-3-methoxyphenyl)-3-(5-methyl-1-(5-(methylthio)-1,3,4-thiadiazol-2-yl)-1H-1,2,3-triazol-4-yl)-4,5-dihydro-1H-pyrazole-1-carbothioamide (5)

A mixture of 3-(4-hydroxy-3-methoxyphenyl)-1-(5-methyl-1-(5-(methylthio)-1,3,4-thiadiazol-2-yl)-1H-1,2,3-triazol-4-yl) prop-2-en-1-one (3) (1.94 gm, 5 mmol) and thiosemicarbazide (0.46 g, 5 mmol) and sodium hydroxide (0.5 gm, 10 mmol) in ethanol (20 mL) was refluxed for 3–5 h. The resulting solid was collected and recrystallized from acetic acid to give 5 as orange solid; yield: 82%; m.p.180–182 °C; FT-IR (KBr, ν , cm^{-1}): 3491 (OH), 3382 (NH₂), 3065 (CH), 1620 (C = N), 1598 (C = C); ^1H NMR (DMSO d_6 , 500 MHz): δ (ppm) 2.47 (s, 3H, CH₃), 2.75 (s, 3H, CH₃), 3.56 (dd, 1H, CH₂ (pyraz)), 3.72(s, 3H, OCH₃), 3.82 (dd, 1H, CH₂ (pyraz)), 4.37 (dd, 1H, CH (pyraz)), 7.55–7.72 (m, 3H, ArH's), 8.15 (s, 1H, OH), 10.02 (s, 2H, NH₂).

^{13}C NMR (DMSO d_6 , 100 MHz): δ 10.11(CH₃), 16.58(CH₃), 41.82 (CH₂), 58.08 (OCH₃), 78.23(CH), 110.65(Ar), 115.80(Ar), 121.35(Ar), 133.17(Ar), 134.22(Ar), 138.30(Ar), 146.12(Ar), 148.12(Ar), 155.62(Ar), 161.02 (Ar), 162.33(Ar), 175.20 (C=S); MS: m/z [%]: 462 (M⁺); Anal. Calcd. for C₁₇H₁₈N₈O₂S₃ (462): C, 44.14; H, 3.92; N, 24.22; %. Found C, 44.19; H, 3.85; N, 24.18%

3.1.5. 3-(5-methyl-1-(5-(methylthio)-1,3,4-thiadiazol-2-yl)-1H-1,2,3-triazol-4-yl)-5-(thiophen-2-yl)-4,5-dihydro-1H-pyrazole-1-carbothioamide (6)

A mixture of 1-(5-methyl-1-(5-(methylthio)-1,3,4-thiadiazol-2-yl)-1H-1,2,3-triazol-4-yl)-3-(thiophen-2-yl) prop-2-en-1-one (4) (1.75 gm, 5 mmol) and thiosemicarbazide (0.46 gm, 5 mmol) and sodium hydroxide (0.5 gm, 10 mmol) in ethanol (20 mL) was refluxed for 3–5 h. The resulting solid was collected and recrystallized from acetic acid to give 6 as yellow crystals; yield: 76%; m.p.163–165 °C; FT-IR (KBr, ν , cm^{-1}): 3472, 3355 (NH₂), 3035 (CH), 1600 (C=N), 1562 (C=C); ^1H NMR (DMSO d_6 , 500 MHz): δ (ppm) 2.42 (s, 3H, CH₃), 2.72 (s, 3H, CH₃), 3.73 (dd, 1H, CH₂ (pyraz)), 3.84 (dd, 1H, J = 18, 12 Hz, CH₂ (pyraz)), 5.33 (dd, 1H, CH (pyraz)), 7.55–7.72 (m, 3H, ArH's), 9.11 (s. br., 2H, NH₂). ^{13}C NMR (DMSO d_6 , 100 MHz): δ 10.12 (CH₃), 16.52 (CH₃), 40.28(CH₂), 58.50(CH), 127.23(Ar), 127.52(Ar), 128.23(Ar), 129.3(Ar), 133.17(Ar), 138.37(Ar), 155.65(Ar), 160.14 (Ar), 162.85(Ar), 175.16 (C=S); MS: m/z [%]: 422 (M⁺), 382 (11), 370 (17), 342(14), 312(50), 272(10), 251 (30), 165(14), 77(100), 50(15); Anal. Calcd. for C₁₄H₁₄N₈S₄ (422.56): C, 39.79; H, 3.34; N, 26.52;%. Found C, 39.71; H, 3.32; N, 26.48%

3.1.6. 4-(1-(5-((4-chlorophenyl) diazenyl)-4-methylthiazol-2-yl)-3-(5-methyl-1-(5-(methylthio)-1,3,4-thiadiazol-2-yl)-1H-1,2,3-triazol-4-yl)-4,5-dihydro-1H-pyrazol-5-yl)-2-methoxyphenol (7)

A mixture of 5-(4-hydroxy-3-methoxyphenyl)-3-(5-methyl-1-(5-(methylthio)-1,3,4-thiadiazol-2-yl)-1H-1,2,3-triazol-4-yl)-4,5-dihydro-1H-pyrazole-1-carbothioamide (5) (2.31 g, 5 mmol) and triethylamine (0.75 mL, 0.5 g, 5 mmol) in ethanol (20 mL) was stirred under reflux for 4 h. The resulting solid was collected and recrystallized to give 7 as yellow solid; yield: 68%; m.p.222–224 °C; FT-IR (KBr, ν , cm^{-1}): 3487(OH), 3023 (CH), 1612 (C = N), 1592 (C = C); ^1H NMR (DMSO d_6 , 500 MHz): δ (ppm) 2.24 (s, 3H, CH₃), 2.47 (s, 3H, CH₃), 2.72 (s, 3H, CH₃), 3.65 (dd, 1H, CH₂(pyraz)), 3.81(s, 3H, OCH₃), 4.15 (dd, 1H, CH₂ (pyraz)), 5.21 (dd, 1H, CH(pyraz)), 7.52–7.88 (m, 7H, ArH's), 9.32(s, 1H, OH); ^{13}C NMR (DMSO d_6 , 100 MHz): δ 9.72(CH₃), 10.11(CH₃), 19.52(CH₃), 42.78(CH₂), 52.32(OCH₃), 60.32(CH), 110.23(Ar), 115.81(Ar), 119.33 (Ar), 125.15 (Ar), 126.17(Ar), 127.8(Ar), 128.12(Ar), 128.97(Ar), 130.02(Ar), 130.15(Ar), 131.21(Ar), 132.7(Ar), 134.19(Ar), 137.05(Ar), 146.12(Ar), 148.91(Ar), 156.16(Ar), 161.11(Ar), 165.8 (Ar), 168.11(Ar); MS: m/z [%]: 638 (M⁺, 18); Anal. Calcd. for C₂₆H₂₃ClN₁₀O₂S₃ (638): C, 48.86; H, 3.63; N, 21.91%. Found C, 48.68; H, 3.58; N, 21.87%

3.1.7. 2-(4-(1-(5-((4-chlorophenyl) diazenyl)-4-methylthiazol-2-yl)-5-(thiophen-2-yl)-4,5-dihydro-1H-pyrazol-3-yl)-5-methyl-1H-1,2,3-triazol-1-yl)-5-(methylthio)-1,3,4-thiadiazole (8)

A mixture of N-(4-chlorophenyl)-2-oxopropanehydrazonyl chloride (1.15 gm, 5 mmol) and 3-(5-methyl-1-(5-(methylthio)-1,3,4-thiadiazol-2-yl)-1H-1,2,3-triazol-4-yl)-5-(thiophen-2-yl)-4,5-dihydro-1H-pyrazole-1-carbothioamide (6) (2.11 gm, 5 mmol) and triethylamine (0.75 mL, 0.5 g, 5 mmol) in ethanol (20 mL) was stirred under reflux for 4 h. The resulting solid was collected and recrystallized to give 8 as yellow crystals; yield: 72%; m.p.222–224 °C; FT-IR (KBr, ν , cm^{-1}): 3023 (CH), 1612 (C=N), 1592 (C=C); ^1H NMR (DMSO d_6 , 500 MHz): δ (ppm) 2.42(s, 3H, CH₃), 2.52 (s, 3H, CH₃), 2.73 (s, 3H, CH₃), 3.65 (dd, 1H, CH₂(pyraz)), 4.01 (dd, 1H, CH₂ (pyraz)), 5.52 (dd, 1H, CH(pyraz)), 7.21–7.52 (m, 7H, ArH's). ^{13}C NMR (DMSO d_6 , 100 MHz): δ 9.77(CH₃), 10.12(CH₃), 16.52(CH₃), 40.72(CH₂), 52.35(OCH₃), 60.58(CH), 110.23(Ar), 115.22(Ar), 119.01(Ar), 126.15(Ar), 127.7(Ar), 128.61(Ar), 128.82(Ar) 130.21(Ar), 131.01(Ar), 133.71(Ar), 135.9 (Ar), 138.11(Ar), 147.19(Ar), 155.66 (Ar), 160.17(Ar), 162.18(Ar), 167.25(Ar); MS: m/z [%]: 601 (M+2, 10), 599 (M⁺, 12), 580 (15), 554 (18), 525 (16), 495 (17), 471 (32), 425 (21), 392 (17), 358 (24), 331(18), 314 (5), 167 (100), 77 (100), 65 (18); Anal. Calcd. for C₂₃H₁₉ClN₁₀S₄ (599.17): C, 46.11; H, 3.20; N, 23.38%. Found C, 46.08; H, 3.18; N, 23.35%

3.2. Antimicrobial activity

Antimicrobial susceptibility and minimum inhibitory concentration (MIC) of the synthesized Thiadiazoles were determined against two Gram-negative bacteria (*E. coli* ATCC 25955, *Pseudomonas aeruginosa* ATCC 10145), two Gram-positive bacteria (*Bacillus subtilis* ATCC 6633 and *Staphylococcus aureus* NRRL B-767), and unicellular fungi (*Candida albicans* ATCC 10231). The pathogens under study were provided by Al-Azhar University, Egypt. They were cultivated in Mueller Hinton broth at 35±2 °C for 24 h. The antimicrobial activity and MIC were carried out as described by Qader et al. (2021) [15]

3.3. In silico molecular docking, drug-likeness and toxicity predictions

PyRx—a virtual screening tool with the standard protocol [23] was used to perform the docking process between the target enzyme TMPRSS2 and the newly synthesized compounds 2–

8. The crystal structure of the target was retrieved from the RCSB Protein Data Bank web server (PDB ID: 1z8g) [24] and then prepared and energy minimized using CHARMM Force Field [25]. Additionally, the chemical structures (2D) of the compounds were drawn using ChemOffice tool (ChemDraw 16.0), then converted to three-dimensional (3D) using open Babel GUI [26]. Moreover, compounds were energetically minimized using UFF Force Field [27]. The energy-minimized ligand molecules were used as input for PyRx-tool to perform the docking simulation. Nine conformers were obtained for each docked compound, and the conformer with the highest binding affinity was selected for further study [4,15,28–30]. Finally, ADMET predictions of the molecules were calculated using free-access tools, such as Molinspiration, admetSAR, and SwissADME.

4. Conclusion

In search of anti-Covid-19 therapeutics, a new series of thiadiazole-triazole hybrids were synthesized. Meanwhile, the newly synthesized compounds showed potent antimicrobial activities against the tested pathogens, especially compound 8, which showed the highest activity against all the tested microbes at low MIC concentration. Additionally, the ability of these compounds to inhibit Covid-19 TMPRSS2 enzyme was investigated through a structure-based docking study to identify potent anti-Covid-19 drug candidates. It has been observed that compound 8 has the highest binding affinity toward the target enzyme. Therefore, it could be used as a potential therapeutic agent to combat Coronavirus.

Credit authors statement

All the authors designed the manuscript, synthesized the new compounds which were tested for their Covid-19 TMPRSS2 Enzyme Inhibition potency and their antimicrobial potency. Additionally, the authors revised the manuscript and designed it in its final form.

Declaration of Competing Interest

None.

Data for reference

Data will be made available on request.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.molstruc.2022.133659.

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