Synthesis of Novel Halogenated Heterocyclic compounds and their uses as Target SARS-CoV-2 main Protease (M<sup>pro</sup>) and Potential Anti-Covid-19

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Abstract: Since the first appearance of the coronavirus disease-2019 (COVID-19) in Wuhan, China, in December 2019, it has been spreading globally with devastating ramifications. The lack of anti-COVID-19 treatment to date warrants urgent research into potential therapeutic targets. Virtual drug screening techniques enable the identification of novel compounds that are capable of targeting the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) main protease (M<sup>pro</sup>). The latter plays a fundamental role in mediating viral replication and transcription, rendering it an attractive drug target. In this study, twenty six novel halogenated, heterocyclic compounds, which can inhibit M<sup>pro</sup>, were tested by molecular docking combined with molecular dynamics simulation. Three compounds showed the highest binding affinity to the protein active site and their binding modes coincide with that of Nelfinavir. The binding of the halogenated compounds to M<sup>pro</sup> may inhibit the replication and transcription of SARS-CoV-2 and, ultimately, stop
the virallife cycle. In times of dire need for anti-COVID-19 treatment, this study lays the groundwork for further experimental research to investigate the efficacy and potential medical uses of these compounds to treat COVID-19. Novel compounds including fused thiophene, pyrimidine and pyran derivatives were tested against human RNA N7-MTase (hRNMT) and selected viral N7-MTases such as SARS-CoV nsp14 and Vaccinia D1-D12 complex to evaluate their specificity and their molecular modeling was also studied in the aim of producing anti covid-19 target molecules.

**Keywords:**

Halogenated compounds; SARS-CoV-2; Coronavirus; Main protease; M\(^{\text{pro}}\); Molecular Docking

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1. **Introduction**

Medicinal chemistry had its beginning when chemists, pharmacist and physicians isolated and purified active principles of plants and animals’ tissues and taken from micro-organism and their fermentation products. Some of these chemicals has been associated with therapeutic properties: Medicinal chemistry which has leaned on the classical fields of chemistry, especially organic chemistry, biology and some area of physics [1-8]. A limited number of natural and synthetic products and serve directly as therapeutic agents although lack of specificity frequently limits their application in human and veterinary medicines and in analogous pesticidal and other uses in agriculture [9-12]. By dissecting the structure of these products chemically, one arrives at its therapeutically significant molecular sections, the pharamacophores, the portion that can be deleted are of no interest as components of drug action; they are regarded as the result of the biosynthetic efforts on the parent organism to construct materials for its own matebolic or defensive purposes. Most of the drugs belong to the class of heterogenius compounds. Heterocyclic compounds played a vital role in the metabolism of all living cells; large number of them are five and six membered heterocyclic compounds having one to three heteroatoms in their nucleus [13-18]. The compounds may be thiophene, pyran derivatives that were basis of genetic material DNA, and these heterocyclic compounds may be isolated or
fused heterocyclic systems. Some of the common heterocyclic compounds used in the medicines are as amino acids like proline, histidine and tryptophan, the vitamins and coenzymes precursors such as thiamine, riboflavin, pyridoxine, folic acid, biotin, B12 and E families of the vitamins. There is a vast number of pharmacologically active heterocyclic compounds, many of which are in regular clinical use. The thiophene and its derivatives have a vital role in biological properties [19-22]. In the present work, we report the synthesis of new heterocyclic compounds with high chloro content together with their studying as potential anti-corona virus. The current pandemic coronavirus disease-2019 (COVID-19) is a new infectious pneumonia-like illness caused by a novel virus strain, so-called severe acute respiratory syndrome coronavirus 2 or SARS-CoV-2 [23,24]. The hydroxychloroquine an approved drug for malaria disease by FDA was explored as a medication for SARS-CoV-2 [25,26]. Previous reports revealed that, the chloroquine and hydroxychloroquine can inhibit the coronavirus (COVID-19) by changing the pH at the surface of the cell membrane. This action can inhibit the attachment of the virus to the cell membrane. In addition, it can prevent nucleic acid replication, glycosylation of viral proteins, virus assembly, new virus particle delivery, virus release, and other mechanisms to obtain its antiviral effects [27]. Heterocycles are widely investigated for possible medicinal applications [28,29]. Favipiravir (1) [30], amodiaquine (2) [31], 20-fluoro-20-deoxycytidine (3) [32], and (4) [33] are known as antiviral drugs. In biological systems, the halogenation of organic molecules is catalyzed by enzymes haloperoxidases such as MPO, EPO and LPO in normal physiological processes, which combine the inorganic substrates \( X^- \) and \( H_2O_2 \) to produce RHS. RHS in turn oxidize the hydro-carbon or-nitrogen substrate RH/RNH to synthesize many halogenated organic compounds (RX/RNX):

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\begin{align*}
\text{Biosynthesis:} & \quad X^- + H_2O_2 \rightarrow HOX/OX/OX/X_2 \\
& \quad RX/RNX \\
\text{DET reaction:} & \quad RX/RNX + e^- \rightarrow RX^-/RNX^- \\
& \quad (X^- + R^-/RN^-) \text{ or } (X^- + R^-/RN^-)
\end{align*}
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By reaction (1), many halogenated organic compounds are biosynthesize they inhibit various RNA and DNA viruses. For that reason, in the present work we concerned with heterocyclic compounds that are halogen rich and studying their potential anti-covid-19
inhibitions. Hence, we aim to determine whether the protease of COVID-19 can be a target protein of these nucleotides in silico. Moreover, a comparative study between these drugs with the FDA approved remdesivir and hydrocloroquine antiviral drugs against a broad range of RNA viruses [34] has been established to investigate the effectiveness of the drugs as inhibitors for COVID 19. Demonstrations for the synthesis of halogen rich heterocyclic compounds together with their potentialities for corona virus were demonstrated through this work.

2. Experimental

2.1. General

The melting points obtained for the synthesized compounds were uncorrected and were recorded using an Electrothermal digital melting point apparatus. IR spectra (KBr discs) were measured using a FTIR plus 460 or PyeUnicam SP-1000 spectrophotometer. \(^1\)HNMR spectra were measured using Varian Gemini-200 (200 MHz) and Jeol AS 500 MHz instruments spectra were performed in DMSO-\(d_6\) as solvent using TMS as internal standard and chemical shifts are expressed as \(\delta\) ppm. MS (EI) spectra were measured using Hewlett Packard 5988 A GC/MS system and GCMS-QP 1000 Ex Shimadzu instruments. Analytical data were obtained from the Micro-analytical Data Unit at Cairo University and were performed on Vario EL III Elemental analyzer.

2.1.1. General procedure for the synthesis of the 2,2,2-trichloroethylidene)-cyclohexane-1,3-dione derivatives 3a,b

To a solution of either 1a (1.12 g, 0.01 mol) or 1b (1.40 g, 0.01 mol) in absolute ethanol (40 mL, 0.01 mol) containing triethylamine (0.50 mL) trichloroacetonitrile (1.42 g, 0.01 mol) was added. The reaction mixture was heated under reflux for 2 h then was left to cool and the formed solid product, in each case, was collected by filtration.

2-(1-Amino-2,2,2-trichloroethylidene)cyclohexane-1,3-dione (3a)
Yellow crystals from ethanol, yield (1.99 g, 78 %), m.p 204-207 °C. IR (KBr) ν max cm\(^{-1}\): 3472-3346 (NH\(_2\)), 1702, 1688 (2CO), 1630 (C=C); \(^1\)H NMR (DMSO-\(d_6\), 300 MHz): δ = 4.85 (s, 2H, D\(_2\)O exchangeable, NH\(_2\)), 1.96-1.82 (m, 2H, CH\(_2\)), 2.95-2.80 (m, 4H, 2CH\(_2\)); \(^13\)C NMR (DMSO-\(d_6\), 75 MHz): δ 173.4, 168.0 (C-1, C-3), 112.3, 90.8 (C-2, C-1 ethylidene), 94.8 (CCl\(_3\)), 40.8, 38.2, 17.1 (C-4, C-5, C-6), Anal. Calculated for C\(_8\)H\(_8\)Cl\(_3\)NO\(_2\): C, 37.46; H, 3.14; N, 5.46. Found: C, 37.80; H, 3.39; N, 5.52. MS: m/e 256 (M\(^+\), 28 %).

2-(1-Amino-2,2,2-trichloroethylidene)-5,5-dimethylcyclohexane-1,3-dione (3b)

Yellow crystals from ethanol, yield (2.21 g, 78 %), m.p 233-235 °C. IR (KBr) ν max cm\(^{-1}\): 3490-3362 (NH\(_2\)), 1705, 1689 (2CO), 1635 (C=C); \(^1\)H NMR (DMSO-\(d_6\), 300 MHz): δ = 4.89 (s, 2H, D\(_2\)O exchangeable, NH\(_2\)), 2.96, 3.01 (2s, 4H, 2CH\(_2\)), 1.09, 1.08 (2s, 6H, 2CH\(_3\)); \(^13\)C NMR (DMSO-\(d_6\), 75 MHz): δ 173.1, 168.3 (C-1, C-3), 112.5, 90.5 (C-2, C-1 ethylidene), 94.9 (CCl\(_3\)), 42.9, 38.1, 17.4 (C-4, C-5, C-6), 24.8 (2CH\(_3\)). Anal. Calculated for C\(_{10}\)H\(_{12}\)Cl\(_3\)NO\(_2\): C, 42.21; H, 4.25; N, 4.92. Found: C, 42.38; H, 4.08; N, 5.16. MS: m/e 284 (M\(^+\), 48 %).

2.1.2. General procedure for the synthesis of the 6,7-dihydrobenzo[b]thiophene derivatives 5a-d

Equimolar amounts of either 3a (2.54 g, 0.01 mol) or 3b (2.84 g, 0.01 mol) in absolute ethanol (40 mL) containing triethylamine (1.50 mL) each of elemental sulfur (0.32 g, 0.01 mol) and either malononitrile (0.66 g, 0.01 mol) or ethyl cyanoacetate (1.13 g, 0.01 mol) were added. The reaction mixture, in each case, was heated under reflux for 2 h then poured onto ice/water mixture containing a few drops of hydrochloric acid and the formed solid product was collected by filtration.

2-Amino-4-(1-amino-2,2,2-trichloroethylidene)-5-oxo-4,5,6,7-tetrahydrobenzo[b]-thiophene-3-carbonitrile (5a)

Yellow crystals from acetic acid, yield (2.22 g, 66 %), m.p 170-173 °C. IR (KBr) ν max cm\(^{-1}\): 3496-3352 (NH\(_2\)), 2220 (CN), 1689 (CO), 1632 (C=C); \(^1\)H NMR (DMSO-\(d_6\), 300 MHz): δ = 5.25, 4.80 (2s, 4H, D\(_2\)O exchangeable, 2NH\(_2\)), 2.13, 2.45 (2t, 4H, 2CH\(_2\)); \(^13\)C NMR (DMSO-\(d_6\), 75 MHz): δ 168.2 (C-5), 132.6, 133.8, 138.0, 140.1 (thiophene C),
116.8 (CN), 112.1, 90.6 (C-2, C-1 ethylidene), 94.4 (CCl₃), 40.7, 39.6 (C-3, C-4). Anal. Calculated for C₁₁H₈Cl₃N₃OS: C, 39.25; H, 2.40; N, 12.48; S, 9.53. Found: C, 39.50; H, 2.66; N, 12.72; S, 9.37. MS: m/e 336 (M⁺, 36 %).

**Ethyl 2-amino-4-(1-amino-2,2,2-trichloroethylidene)-5-oxo-4,5,6,7-tetrahydrobenzo-[b]thiophene-3-carboxylate (5b)**

Yellow crystals from acetic acid, yield (2.68 g, 70 %), m.p 170-172 °C. IR (KBr) ν max cm⁻¹: 3479-3330 (NH₂), 1700, 1689 (2CO), 1636 (C=C); ¹H NMR (DMSO-d₆, 300 MHz): δ = 5.28, 4.76 (2s, 4H, D₂O exchangeable, 2NH₂), 4.22 (q, 2H, J = 6.89 Hz, OCH₂CH₃), 2.16, 2.49 (2t, 4H, 2CH₂), 1.12 (t, 3H, J = 6.89 Hz, OCH₂CH₃); ¹³C NMR (DMSO-d₆, 75 MHz): δ 168.5 (C-5), 133.8, 134.5, 138.0, 140.8 (thiophene C), 112.4, 90.2 (C-2, C-1 ethylidene), 94.3 (CCl₃), 50.2 (OCH₂CH₃), 40.9, 39.8 (C-3, C-4), 16.8 (OCH₂CH₃). Anal. Calculated for C₁₃H₁₃Cl₃N₂O₃S: C, 40.70; H, 3.42; N, 11.52; S, 8.79. Found: C, 40.63; H, 3.25; N, 7.59; S, 8.42. MS: m/e 383 (M⁺, 42 %).

**2-Amino-4-(1-amino-2,2,2-trichloroethylidene)-7,7-dimethyl-5-oxo-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carbonitrile (5c)**

Yellow crystals from acetic acid, yield (2.12 g, 58 %), m.p 155-157 °C. IR (KBr) ν max cm⁻¹: 3479-3332 (NH₂), 2220 (CN), 1692 (CO), 1631 (C=C); ¹H NMR (DMSO-d₆, 300 MHz): δ = 5.28, 4.82 (2s, 4H, D₂O exchangeable, 2NH₂), 2.48 (s, 2H, CH₂), 1.09, 1.08 (2s, 6H, 2CH₃); ¹³C NMR (DMSO-d₆, 75 MHz): δ 168.5 (C-5), 133.5, 136.3, 137.4, 141.1 (thiophene C), 117.0 (CN), 112.3, 90.5 (C-2, C-1 ethylidene), 94.7 (CCl₃), 40.8, 39.8 (C-3, C-4), 24.6 (2CH₃). Anal. Calculated for C₁₃H₁₂Cl₃N₃O₃: C, 42.82; H, 3.32; N, 11.52; S, 8.79. Found: C, 42.63; H, 3.41; N, 11.27; S, 8.92. MS: m/e 364 (M⁺, 48 %).

**Ethyl 2-amino-4-(1-amino-2,2,2-trichloroethylidene)-7,7-dimethyl-5-oxo-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxylate(5d)**

Orange crystals from acetic acid, yield (2.80 g, 68 %), m.p 211-213 °C. IR (KBr) ν max cm⁻¹: 3493-3356 (NH₂), 1703, 1689 (2CO), 1632 (C=C); ¹H NMR (DMSO-d₆, 200 MHz): δ = 5.32, 4.73 (2s, 4H, D₂O exchangeable, 2NH₂), 4.21 (q, 2H, J = 7.13 Hz, OCH₂CH₃), 2.52 (s, 2H, CH₂), 1.11 (t, 3H, J = 7.13 Hz, OCH₂CH₃), 1.09, 1.07 (2s, 6H,
2CH₃); ¹³C NMR (DMSO-d₆, 75 MHz): δ 168.2 (C-5), 132.3, 133.9, 138.3, 140.5 (thiophene C), 112.6, 90.8 (C-2, C-1 ethylidene), 94.5 (CCl₃), 50.21 (OCH₂CH₃), 40.6, 39.5 (C-3, C-4), 24.6 (2CH₃), 16.6 (OCH₂CH₃). Anal. Calculated for C₁₅H₁₇Cl₃N₂O₃S: C, 43.76; H, 4.16; N, 6.80; S, 7.79. Found: C, 43.59; H, 4.06; N, 6.93; S, 8.02. MS: m/e 411 (M⁺, 31 %).

2.1.3. General procedure for the synthesis of the 4,6-dihydrothieno[3,2-f]quinazoline derivatives 7a-d

To a solution of either 5a (3.36 g, 0.01 mol), 5b (3.83 g, 0.01 mol), 5c (3.62 g, 0.01 mol) or 5d (4.11 g, 0.01 mol) in ethanol (40 mL) containing triethylamine (1.0 mL) phenylisothiocyanate (1.30 g, 0.01 mol) was added and the reaction mixture was heated under reflux for 3h then left to cool. The formed solid crystals, in each case, were collected by filtration.

8-Amino-3-mercapto-4-phenyl-1-(trichloromethyl)-4,6-dihydrothieno[3,2-f]quinazoline-9-carbonitrile (7a)

Yellow crystals from acetic acid, yield (3.08 g, 68 %), m.p 233-235 °C. IR (KBr) v max cm⁻¹: 3483-3361 (NH₂), 3055 (CH aromatic), 2220 (CN), 1631 (C=C); ¹H NMR (DMSO-d₆, 300 MHz): δ = 8.20 (s, 1H, SH), 7.28-7.39 (m, 5H, C₆H₅), 5.84 (t, 1H, CH), 4.83 (s, 2H, D₂O exchangeable, NH₂), 2.80 (d, 2H, CH₂); ¹³C NMR (DMSO-d₆, 75 MHz): δ 172.3 (C=N), 131.4, 132.5, 138.3, 140.6 (thiophene C), 116.9 (CN), 112.1, 90.6 (C-2, C-1 ethylidene), 123.6, 121.6, 119.3, 98.3 (2C=C), 94.5 (CCl₃), 40.9, 39.8 (C-3, C-4), Anal. Calculated for C₁₈H₁₁Cl₃N₄S₂: C, 47.64; H, 2.44; N, 12.35; S, 14.13. Found: C, 47.59; H, 2.56; N, 12.62; S, 14.08. MS: m/e 453 (M⁺, 42 %).

Ethyl 8-amino-3-mercapto-4-phenyl-1-(trichloromethyl)-4,6-dihydrothieno[3,2-f]quinazoline-9-carboxylate (7b)

Yellow crystals from acetic acid, yield (3.51 g, 70 %), m.p 142-144 °C. IR (KBr) v max cm⁻¹: 3479-3330 (NH₂), 3050 (CH aromatic), 1689 (CO), 1636 (C=C); ¹H NMR (DMSO-d₆, 300 MHz): δ = 8.20 (s, 1H, SH), 7.28-7.39 (m, 5H, C₆H₅), 5.84 (s, 1H, CH), 4.83 (s, 2H, D₂O exchangeable, NH₂), 4.20 (q, 2H, J = 5.90 Hz, OCH₂CH₃), 2.80 (d, 2H, CH₂), 1.13 (t, 3H, J = 5.90 Hz, OCH₂CH₃); ¹³C NMR (DMSO-d₆, 75 MHz): δ 172.1 (C=N),
165.2 (CO), 131.4, 132.5, 138.3, 140.6 (thiophene C), 116.9 (CN), 112.1, 90.6 (C-2, C-1 ethylidene), 123.6, 121.3, 120.5, 99.6 (2C=C), 94.2 (CCl3), 50.3 (OCH2CH3), 40.5, 39.4 (C-3, C-4), 16.3 (OCH2CH3). Anal. Calculated for C20H16Cl3N3O2S2: C, 47.96; H, 3.22; N, 8.39; S, 12.80. Found: C, 47.70; H, 3.52; N, 8.56; S, 12.62. MS: m/e 500 (M+, 28 %).

8-Amino-3-mercapto-6,6-dimethyl-4-phenyl-1-(trichloromethyl)-4,6-dihydrothieno-[3,2-f]quinazoline-9-carbonitrile (7c)

Yellow crystals from acetic acid, yield (3.63 g, 70 %), m.p 142-144 °C. IR (KBr) v max cm⁻¹: 3479-3330 (NH2), 3050 (CH aromatic), 1636 (C=C); ¹H NMR (DMSO-d6, 300 MHz): δ = 8.22 (s, 1H, SH), 7.28-7.42 (m, 5H, C6H5), 5.60 (s, 1H, CH), 4.85 (s, 2H, D2O exchangeable, NH2), 1.09, 1.06 (s, 6H, 2CH3); ¹³C NMR (DMSO-d6, 75 MHz): δ 172.3 (C=N), 131.6, 134.8, 138.7, 140.8 (thiophene C), 116.7 (CN), 112.3, 90.8 (C-2, C-1 ethylidene), 124.4, 121.1, 120.3, 99.8 (2C=C), 94.4 (CCl3), 40.2, 39.6 (C-3, C-4), 24.5 (2CH3). Anal. Calculated for C20H16Cl3N4S2: C, 49.85; H, 3.14; N, 11.63; S, 13.31. Found: C, 49.72; H, 3.28; N, 11.80; S, 13.27. MS: m/e 381 (M+, 60 %).

Ethyl 8-amino-3-mercapto-6,6-dimethyl-4-phenyl-1-(trichloromethyl)-4,6-dihydrothieno[3,2-f]quinazoline-9-carboxylate (7d)

Orange crystals from acetic acid, yield (3.17 g, 60 %), m.p 188-190 °C. IR (KBr) v max cm⁻¹: 3479-3337 (NH2), 1688 (CO), 1630 (C=C); ¹H NMR (DMSO-d6, 300 MHz): δ = 8.23 (s, 1H, SH), 7.26-7.42 (m, 5H, C6H5), 5.86 (s, 1H, CH), 4.81 (s, 2H, D2O exchangeable, NH2), 4.22 (q, 2H, J = 6.14 Hz, OCH2CH3), 1.12 (t, 3H, J = 6.14 Hz, OCH2CH3), 1.09, 1.07 (2s, 6H, 2CH3); ¹³C NMR (DMSO-d6, 75 MHz): δ 172.3 (C=N), 164.8 (CO), 131.1, 133.8, 137.9, 140.3 (thiophene C), 116.8 (CN), 112.0, 90.8 (C-2, C-1 ethylidene), 123.3, 121.6, 120.6, 99.8 (2C=C), 94.3 (CCl3), 50.1 (OCH2CH3), 40.6, 39.2 (C-3, C-4), 24.7 (2CH3), 16.2 (OCH2CH3). Anal. Calculated for C22H20Cl3N4S2: C, 49.96; H, 3.81; N, 7.94; S, 12.13. Found: C, 49.77; H, 3.62; N, 8.03; S, 12.35. MS: m/e 528 (M+, 46 %).

2.1.4. General procedure for the synthesis of the 4,6-dihydrothieno[3,2-f]quinazolin-1-ol derivatives 8a-d
To solution of either 7a (4.53 g, 0.01 mol), 7b (5.00 g, 0.01 mol), 7c (3.81 g, 0.01 mol) or 7d (5.28 g, 0.01 mol) in ethanol (70 mL) containing sodium hydroxide solution (5 mL, 10 %) was heated under reflux for 6 h. the solid product, in each case, produced upon pouring onto ice/water mixture containing a few drops of hydrochloric acid (till pH 6) was collected by filtration.

8-Amino-1-hydroxy-3-mercapto-4-phenyl-4,6-dihydrothieno[3,2-f]quinazoline-9-carbonitrile (8a)

Yellow crystals from 1,4-dioxane, yield (2.46 g, 70 %), m.p 210-212 °C. IR (KBr) ν max cm⁻¹: 3563-3349 (OH, NH₂), 3053 (CH aromatic), 2220 (CN), 1633 (C=C); ¹H NMR (DMSO-d⁶, 300 MHz): δ = 10.21 (s, 1H, D₂O exchangeable, OH), 8.22 (s, 1H, SH), 7.24-7.40 (m, 5H, C₆H₅), 5.82 (t, 1H, CH), 4.86 (s, 2H, D₂O exchangeable, NH₂), 2.82 (d, 2H, CH₂); ¹³C NMR (DMSO-d⁶, 75 MHz): δ 172.1 (C=N), 130.2, 133.6, 138.1, 141.3 (thiophene C), 116.8 (CN), 112.3, 90.4 (C-2, C-1 ethylidene), 123.2, 121.8, 119.1, 98.0 (2C=C), 40.6, 39.7 (C-3, C-4). Anal. Calculated for C₁₇H₁₂N₄OS₂: C, 57.93; H, 3.43; N, 15.90; S, 18.20. Found: C, 57.73; H, 3.29; N, 16.17; S, 18.06. MS: m/e 352 (M⁺, 36 %).

Ethyl 8-amino-1-hydroxy-3-mercapto-4-phenyl-4,6-dihydrothieno[3,2-f]quinazoline-9-carboxylate (8b)

Yellow crystals from 1,4-dioxane, yield (2.64 g, 66 %), m.p 159-161 °C. IR (KBr) ν max cm⁻¹: 3569-3346 (OH, NH₂), 3052 (CH aromatic), 1702 (CO), 1636 (C=C); ¹H NMR (DMSO-d⁶, 300 MHz): δ = 10.21 (s, 1H, D₂O exchangeable, OH), 8.22 (s, 1H, SH), 7.24-7.43 (m, 5H, C₆H₅), 5.86 (s, 1H, CH), 4.89 (s, 2H, D₂O exchangeable, NH₂), 4.22 (q, 2H, J = 6.89 Hz, OCH₂CH₃), 2.82 (d, 2H, CH₂), 1.12 (t, 3H, J = 6.89 Hz, OCH₂CH₃); ¹³C NMR (DMSO-d⁶, 75 MHz): δ 172.3 (C=N), 166.3 (CO), 131.6, 132.3, 138.2, 140.4 (thiophene C), 116.7 (CN), 112.3, 90.5 (C-2, C-1 ethylidene), 123.3, 121.6, 120.3, 99.8 (2C=C), 50.2 (OCH₂CH₃), 40.8, 39.6 (C-3, C-4), 16.1 (OCH₂CH₃). Anal. Calculated for C₁₉H₁₇N₃O₃S₂: C, 57.93; H, 3.43; N, 10.52; S, 16.05. Found: C, 57.08; H, 4.38; N, 10.38; S, 15.83. MS: m/e 399 (M⁺, 32 %).

8-Amino-1-hydroxy-3-mercapto-6,6-dimethyl-4-phenyl-4,6-dihydrothieno[3,2-f]quinazoline-9-carbonitrile (8c)
Yellow crystals from ethanol, yield (2.74 g, 72 %), m.p 189-192 °C. IR (KBr) v max cm⁻¹: 3569-3342 (OH, NH₂), 3050 (CH aromatic), 1638 (C=C); ¹H NMR (DMSO-d₆, 300 MHz): δ = 10.30 (s, 1H, D₂O exchangeable, OH), 8.25 (s, 1H, SH), 7.26-7.39 (m, 5H, C₆H₅), 5.62 (s, 1H, CH), 4.83 (s, 2H, D₂O exchangeable, NH₂), 1.09, 1.07 (s, 6H, 2CH₃); ¹³C NMR (DMSO-d₆, 75 MHz): δ 172.1 (C=N), 165.8 (CO), 131.3, 133.5, 137.5, 141.6 (thiophene C), 116.7 (CN), 112.1, 90.6 (C-2, C-1 ethylidene), 123.2, 121.8, 120.4, 99.6 (2C=C), 50.4 (OCH₂CH₃), 40.8, 39.4 (C-3, C-4), 24.6 (2CH₃), 16.3 (OCH₂CH₃). Anal. Calculated for C₁₉H₁₆N₄O₂S₂: C, 59.98; H, 4.24; N, 14.73; S, 14.32. Found: C, 59.88; H, 4.52; N, 14.80; S, 16.69. MS: m/e 380 (M⁺, 56 %).

Ethyl 8-amino-1-hydroxy-3-mercapto-6,6-dimethyl-4-phenyl-4,6-dihydrothieno[3,2-f]quinazoline-9-carboxylate (8d)

Pale yellow crystals from ethanol, yield (2.65g, 62 %), m.p 211-213 °C. IR (KBr) v max cm⁻¹: 3459-3341 (NH₂), 1702 (CO), 1632 (C=C); ¹H NMR (DMSO-d₆, 300 MHz): δ = 10.28 (s, 1H, D₂O exchangeable OH), 8.26 (s, 1H, SH), 7.24-7.46 (m, 5H, C₆H₅), 5.85 (s, 1H, CH), 4.83 (s, 2H, D₂O exchangeable, NH₂), 4.23 (q, 2H, J = 5.80 Hz, OCH₂CH₃), 1.12 (t, 3H, J = 5.80 Hz, OCH₂CH₃), 1.08, 1.06 (2s, 6H, 2CH₃); ¹³C NMR (DMSO-d₆, 75 MHz): δ 172.2 (C=N), 165.8 (CO), 131.3, 133.5, 137.5, 141.6 (thiophene C), 116.7 (CN), 112.1, 90.6 (C-2, C-1 ethylidene), 123.2, 121.8, 120.4, 99.6 (2C=C), 50.4 (OCH₂CH₃), 40.8, 39.4 (C-3, C-4), 24.6 (2CH₃), 16.3 (OCH₂CH₃). Anal. Calculated for C₂₁H₂₁N₃O₃S₂: C, 58.99; H, 4.95; N, 9.83; S, 15.00. Found: C, 58.82; H, 5.21; N, 9.02; S, 14.87. MS: m/e 427 (M⁺, 436 %).

2.1.5. General procedures for the synthesis of the 8,9-dihydro-4H-thieno[2,3-g]chromene derivatives 10a-m

To a solution of either compound 5a (3.36 g, 0.01 mol), or 5c (3.64 g, 0.01 mol) in 1,4-dioxane (40 mL) containing triethylamine (1.00 mL) each of either benzaldehyde (1.08 g, 0.01 mol), 4-chlorobenzaldehyde (1.40 g, 0.01 mol) or 4-methoxybenzaldehyde (1.37 g, 0.01 mol) and either malononitrile (0.66 g, 0.01 mol) or ethyl cyanoacetate (1.13 g, 0.01 mol) was added. The reaction mixture, in each case, was heated under reflux for 1 h then poured onto ice/water containing a few drops of hydrochloric acid and the formed solid product was collected by filtration.
2,6-Diamino-4-(1-amino-2,2,2-trichloroethylidene)-8-phenyl-8,9-dihydro-4H-thieno[2,3-g]chromene-3,7-dicarbonitrile (10a)

Pale brown crystals from 1,4-dioxane, yield (3.19 g, 65 %), m.p 166-168 °C. IR (KBr) ν max cm⁻¹: 3459-3346 (NH₂), 3050 (CH aromatic), 2223, 2220 (CN), 1636 (C=C); ¹H NMR (DMSO-d₆, 300 MHz): δ = 7.25-7.42 (m, 5H, C₆H₅), 5.90 (s, 1H, pyran H-4), 4.80, 5.22, 5.39 (3s, 6H, D₂O exchangeable, 3NH₂), 2.80 (s, 2H, CH₂); ¹³C NMR (DMSO-d₆, 75 MHz): δ 120.1, 123.5, 125.4, 128.5, 130.8, 133.7, 137.9, 138.3, 139.0, 140.2, 142.5, 143.8 (C₆H₅, thiophene, pyran C), 116.9, 117.3 (2CN), 112.4, 90.2 (C-2, C-1 ethylidene), 94.2 (CCl₃), 53.8 (CH₂). Anal. Calculated for C₂₁H₁₄Cl₃N₅O₄S: C, 51.39; H, 2.88; N, 14.27; S, 6.53. Found: C, 51.59; H, 3.11; N, 14.62; S, 6.80. MS: m/e 490 (M⁺, 32 %).

Ethyl 2,6-diamino-4-(1-amino-2,2,2-trichloroethylidene)-3-cyano-8-phenyl-8,9-dihydro-4H-thieno[2,3-g]chromene-7-carboxylate (10b)

Yellow crystals from 1,4-dioxane, yield (3.76 g, 70 %), m.p 210-212 °C. IR (KBr) ν max cm⁻¹: 3472-3348 (NH₂), 3052 (CH aromatic), 2220 (CN), 1689 (CO), 1632 (C=C); ¹H NMR (DMSO-d₆, 300 MHz): δ = 7.32-7.45 (m, 5H, C₆H₅), 5.92 (s, 1H, pyran H-4), 5.22, 5.39, 4.83 (3s, 6H, D₂O exchangeable, 3NH₂), 4.22 (q, 2H, J = 7.22 Hz, OCH₂CH₃), 2.82 (s, 2H, CH₂), 1.12 (t, 3H, J = 7.22 Hz, OCH₂CH₃); ¹³C NMR (DMSO-d₆, 75 MHz): δ 165.9 (CO), 120.6, 123.4, 125.2, 128.6, 131.4, 132.8, 136.2, 138.5, 139.3, 140.5, 142.1, 142.9 (C₆H₅, thiophene, pyran C), 116.7 (CN), 112.5, 90.5 (C-2, C-1 ethylidene), 94.5 (CCl₃), 53.8 (CH₂), 50.2 (OCH₂CH₃), 16.8 (OCH₂CH₃). Anal. Calculated for C₂₃H₂₁Cl₃N₄O₄S: C, 51.39; H, 3.56; N, 10.42; S, 5.96. Found: C, 51.49; H, 3.43; N, 10.28; S, 6.03. MS: m/e 537 (M⁺, 38 %).

2,6-Diamino-4-(1-amino-2,2,2-trichloroethylidene)-9,9-dimethyl-8-phenyl-8,9-dihydro-4H-thieno[2,3-g]chromene-3,7-dicarbonitrile (10c)

Pale brown crystals from 1,4-dioxane, yield (3.52 g, 68 %), m.p 201-203 °C. IR (KBr) ν max cm⁻¹: 3473-3342 (NH₂), 3054 (CH aromatic), 2222-2220 (CN), 1632 (C=C); ¹H NMR (DMSO-d₆, 300 MHz): δ = 7.27-7.40 (m, 5H, C₆H₅), 5.93 (s, 1H, pyran H-4), 4.82, 5.21, 5.42 (3s, 6H, D₂O exchangeable, 3NH₂), 1.09, 1.07 (2s, 6H, 2CH₃); ¹³C NMR (DMSO-d₆, 75 MHz): δ 120.3, 120.6, 125.4, 128.8, 130.8, 133.7, 137.9, 138.3, 139.0,
140.2, 142.5, 143.8 (C₆H₅, thiophene, pyran C), 116.9, 117.3 (2CN), 112.4, 90.2 (C-2, C-1 ethylidene), 94.2 (CCl₃), 24.8 (2CH₃). Anal. Calculated for C₂₃H₁₈Cl₃N₅OS: C, 53.24; H, 3.50; N, 13.50; S, 6.18. Found: C, 53.39; H, 3.42; N, 13.69; S, 6.23. MS: m/e 518 (M⁺, 36 %).

**Ethyl 2-amino-4-(1-amino-2,2,2-trichloroethylidene)-3,7-dicyano-9,9-dimethyl-8-phenyl-8,9-dihydro-4H-thieno[2,3-g]chromene-6-carboxylate (10d)**

Yellow crystals from 1,4-dioxane, yield (3.68 g, 65 %), m.p 230-233 °C. IR (KBr) ν max cm⁻¹: 3469-3338 (NH₂), 3055 (CH aromatic), 2220 (CN), 1688 (CO), 1630 (C=C); ¹H NMR (DMSO-d₆, 300 MHz): δ = 7.28-7.39 (m, 5H, C₆H₅), 5.68 (s, 1H, pyran H-4), 5.33, 4.86 (2s, 4H, D₂O exchangeable, 2NH₂), 4.23 (q, 2H, J = 6.70 Hz, OCH₂CH₃), 1.14 (t, 3H, J = 6.70 Hz, OCH₂CH₃), 1.09, 1.07 (2s, 6H, 2CH₃); ¹³C NMR (DMSO-d₆, 75 MHz): δ 164.8 (CO), 119.8, 120.2, 125.6, 128.4, 130.3, 132.5, 134.8, 138.9, 139.1, 140.2, 141.5, 142.6 (C₆H₅, thiophene, pyran C), 116.9 (CN), 112.1, 90.3 (C-2, C-1 ethylidene), 94.2 (CCl₃), 50.3 (OCH₂CH₃), 24.8 (2CH₃), 16.7 (OCH₂CH₃). Anal. Calculated for C₂₆H₂₁Cl₃N₅O₃S: C, 54.22; H, 3.68; N, 9.73; S, 5.57. Found: C, 54.59; H, 3.87; N, 9.73; S, 5.80. MS: m/e 575 (M⁺, 68 %).

**2,6-Diamino-4-(1-amino-2,2,2-trichloroethylidene)-8-(4-chlorophenyl)-8,9-dihydro-4H-thieno[2,3-g]chromene-3,7-dicarbonitrile (10e)**

Pale orange crystals from 1,4-dioxane, yield (3.72 g, 71 %), m.p 240-243 °C. IR (KBr) ν max cm⁻¹: 3468-3373 (NH₂), 3055 (CH aromatic), 2222, 2220 (CN), 1632 (C=C); ¹H NMR (DMSO-d₆, 300 MHz): δ = 7.29-7.52 (m, 4H, C₆H₄), 5.73 (s, 1H, pyran H-4), 4.83, 5.20, 5.36 (3s, 6H, D₂O exchangeable, 3NH₂), 2.78 (s, 2H, CH₂); ¹³C NMR (DMSO-d₆, 75 MHz): δ 119.3, 120.4, 122.6, 127.9, 130.5, 132.8, 136.6, 138.6, 139.4, 140.6, 142.7, 143.4 (C₆H₄, thiophene, pyran C), 116.9, 117.1 (2CN), 112.4, 90.3 (C-2, C-1 ethylidene), 94.2 (CCl₃), 53.1 (CH₂). Anal. Calculated for C₂₁H₁₃Cl₄N₅O₃S: C, 48.02; H, 2.49; N, 13.33; S, 6.10. Found: C, 47.92; H, 2.60; N, 13.52; S, 6.26. MS: m/e 525 (M⁺, 46 %).

**Ethyl 2,6-diamino-4-(1-amino-2,2,2-trichloroethylidene)-8-(4-chlorophenyl)-3-cyano-8,9-dihydro-4H-thieno[2,3-g]chromene-7-carboxylate (10f)**
Yellow crystals from 1,4-dioxane, yield (3.43 g, 60 %), m.p 155-157 °C. IR (KBr) ν max cm⁻¹: 3484-3346 (NH₂), 3050 (CH aromatic), 2220 (CN), 1690 (CO), 1630 (C=C); ¹H NMR (DMSO-d₆, 300 MHz): δ = 7.26-7.53 (m, 4H, C₆H₄), 5.64 (s, 1H, pyran H-4), 5.38, 5.33, 4.84 (3s, 6H, D₂O exchangeable, 3NH₂), 4.21 (q, 2H, J = 5.88 Hz, OCH₂CH₃), 2.90 (s, 2H, CH₂), 1.14 (t, 3H, J = 5.88 Hz, OCH₂CH₃); ¹³C NMR (DMSO-d₆, 75 MHz): δ 165.4 (CO), 120.4, 120.7, 124.7, 126.9, 130.2, 131.9, 134.6, 137.5, 139.1, 140.3, 141.7, 142.8 (C₆H₅, thiophene, pyran C), 116.7 (CN), 112.0, 90.1 (C-2, C-1 ethylidene), 94.6 (CCl₃), 50.1 (OCH₂CH₃), 16.8 (OCH₂CH₃). Anal. Calculated for C₂₃H₁₈Cl₄N₄O₃S: C, 48.27; H, 3.17; N, 9.79; S, 5.60. Found: C, 48.49; H, 3.42; N, 9.86; S, 5.73. MS: m/e 572 (M⁺, 59 %).

2,6-Diamino-4-(1-amino-2,2,2-trichloroethylidene)-8-(4-chlorophenyl)-9,9-dimethyl-8,9-dihydro-4H-thieno[2,3-g]chromene-3,7-dicarbonitrile (10g)

Pale brown crystals from 1,4-dioxane, yield (4.04g, 73 %), m.p 177-179 °C. IR (KBr) ν max cm⁻¹: 3486-3339 (NH₂), 3050 (CH aromatic), 2222, 2220 (2CN), 1634 (C=C); ¹H NMR (DMSO-d₆, 300 MHz): δ = 7.26-7.53 (m, 4H, C₆H₄), 5.63 (s, 1H, pyran H-4), 4.86, 5.23, 5.40 (3s, 6H, D₂O exchangeable, 3NH₂), 1.08, 1.07 (2s, 6H, 2CH₃); ¹³C NMR (DMSO-d₆, 75 MHz): δ 120.4, 122.7, 124.9, 127.3, 130.4, 132.6, 137.7, 138.6, 139.3, 140.2, 141.9, 143.4 (C₆H₅, thiophene, pyran C), 116.9, 117.3 (2CN), 112.3, 90.6 (C-2, C-1 ethylidene), 94.5 (CCl₃), 24.6 (2CH₃). Anal. Calculated for C₂₅H₁₇Cl₄N₅O₃S: C, 49.93; H, 3.10; N, 12.66; S, 5.80. Found: C, 49.69; H, 3.36; N, 12.72; S, 6.03. MS: m/e 553 (M⁺, 48 %).

Ethyl 2,6-diamino-4-(1-amino-2,2,2-trichloroethylidene)-8-(4-chlorophenyl)-3-cyano-9,9-dimethyl-8,9-dihydro-4H-thieno[2,3-g]chromene-7-carboxylate (10h)

Pale yellow crystals from 1,4-dioxane, yield (3.72 g, 62 %), m.p 170-172 °C. IR (KBr) ν max cm⁻¹: 3473-3328 (NH₂), 3050 (CH aromatic), 2220 (CN), 1689 (CO), 1630 (C=C); ¹H NMR (DMSO-d₆, 300 MHz): δ = 7.23-7.56 (m, 4H, C₆H₄), 5.62 (s, 1H, pyran H-4), 5.36, 5.33, 4.84 (3s, 6H, D₂O exchangeable, 3NH₂), 4.22 (q, 2H, J = 6.25 Hz, OCH₂CH₃), 1.14 (t, 3H, J = 6.26 Hz, OCH₂CH₃), 1.08, 1.06 (s, 6H, 2CH₃); ¹³C NMR (DMSO-d₆, 75 MHz): δ 165.1 (CO), 120.2, 122.8, 124.4, 127.3, 130.6, 132.7, 134.6,
2,6-Diamino-4-(1-amino-2,2,2-trichloroethylidene)-8-(4-methoxyphenyl)-9,9-
dihydro-4H-thieno[2,3-g]chromene-3,7-dicarbonitrile (10i)

Pale orange crystals from 1,4-dioxane, yield (3.28 g, 63 %), m.p 195-197 °C. IR (KBr) \( \nu_{\text{max}} \text{ cm}^{-1} \): 3487-3353 (NH2), 3050 (CH aromatic), 2223, 2220 (2CN), 1630 (C=C); \(^1\)H NMR (DMSO-\(d_6\), 300 MHz): \( \delta = 7.26-7.48 \) (m, 4H, C₆H₄), 5.76 (s, 1H, pyran H-4), 4.88, 5.24, 5.38 (3s, 6H, D₂O exchangeable, 3NH₂), 3.67 (s, 3H, OCH₃), 2.76 (s, 2H, CH₂);
\(^{13}\)C NMR (DMSO-\(d_6\), 75 MHz): \( \delta = 120.3, 121.6, 122.8, 126.4, 129.6, 132.2, 134.8, 134.8, 137.4, 139.1, 140.8, 141.9, 142.8 \) (C₆H₄, thiophene, pyran C), 116.7, 117.3 (2CN), 112.6, 90.1 (C-2, C-1 ethylidene), 94.5 (CCl₃), 53.3 (CH₂), 50.1 (OCH₃). Anal. Calculated for C₂₃H₂₁Cl₄N₄O₃S: C, 50.76; H, 3.73; N, 9.87; S, 5.65. Found: C, 50.53; H, 3.59; N, 9.72; S, 5.79. MS: m/e 567 (M\(^+\), 62 %).

Ethyl 2,6-diamino-4-(1-amino-2,2,2-trichloroethylidene)-3-cyano-8-(4-methoxy-
phenyl)-8,9-dihydro-4H-thieno[2,3-g]chromene-7-carboxylate (10k)

Yellow crystals from 1,4-dioxane, yield (4.20 g, 74 %), m.p 205-208 °C. IR (KBr) \( \nu_{\text{max}} \text{ cm}^{-1} \): 3469-3352 (NH₂), 3050 (CH aromatic), 2222 (CN), 1687 (CO), 1630 (C=C); \(^1\)H NMR (DMSO-\(d_6\), 300 MHz): \( \delta = 7.29-7.44 \) (m, 4H, C₆H₄), 5.66 (s, 1H, pyran H-4), 5.34, 5.36, 4.89 (3s, 6H, D₂O exchangeable, 3NH₂), 4.22 (q, 2H, \( J = 7.16 \) Hz, OCH₂CH₃), 3.69 (s, 3H, OCH₃), 2.93 (s, 2H, CH₂), 1.13 (t, 3H, \( J = 7.16 \) Hz, OCH₂CH₃); \(^{13}\)C NMR (DMSO-\(d_6\), 75 MHz): \( \delta = 164.8 \) (CO), 120.2, 120.3, 123.8, 125.6, 130.6, 132.4, 134.7, 136.2, 139.4, 140.9, 141.3, 142.6 (C₆H₅, thiophene, pyran C), 116.8 (CN), 112.2, 90.3 (C-2, C-1 ethylidene), 94.6 (CCl₃), 50.6 (OCH₃), 50.2 (OCH₂CH₃), 16.5 (OCH₂CH₃). Anal. Calculated for C₂₃H₂₁Cl₄N₄O₄S: C, 50.76; H, 3.73; N, 9.87; S, 5.65. Found: C, 50.53; H, 3.59; N, 9.72; S, 5.79. MS: m/e 567 (M\(^+\), 62 %).

2,6-Diamino-4-(1-amino-2,2,2-trichloroethylidene)-8-(4-methoxyphenyl)-9,9-
dimethyl-8,9-dihydro-4H-thieno[2,3-g]chromene-3,7-dicarbonitrile (10l)
Pale brown crystals from 1,4-dioxane, yield (4.01g, 73 %), m.p 159-161 °C. IR (KBr) ν max cm⁻¹: 3495-3342 (NH₂), 3050 (CH aromatic), 2222, 2220 (2CN), 1631 (C=C); ¹H NMR (DMSO-d₆, 300 MHz): δ = 7.28-7.56 (m, 4H, C₆H₄), 5.61 (s, 1H, pyran H-4), 4.89, 5.08, 5.43 (3s, 6H, D₂O exchangeable, 3NH₂), 3.70 (s, 3H, OCH₃), 1.08, 1.06 (2s, 6H, 2CH₃); ¹³C NMR (DMSO-d₆, 75 MHz): δ 120.2, 123.8, 124.6, 127.9, 129.2, 132.8, 138.3, 138.9, 139.1, 140.6, 141.7, 142.2 (C₆H₅, thiophene, pyran C), 116.7, 117.0 (2CN), 112.1, 90.3 (C-2, C-1 ethylidene), 94.2 (CCl₃), 50.6 (OCH₃), 24.8 (2CH₃). Anal. Calculated for C₂₄H₂₀Cl₃N₅O₂S: C, 52.52; H, 3.67; N, 12.76; S, 5.84. Found: C, 52.62; H, 3.49; N, 12.82; S, 5.72. MS: m/e 548 (M⁺, 50 %).

Ethyl 2,6-diamino-4-(1-amino-2,2,2-trichloroethylidene)-3-cyano-8-(4-methoxyphenyl)-9,9-dimethyl-8,9-dihydro-4H-thieno[2,3-g]chromene-7-carboxylate (10m)

Orange crystals from 1,4-dioxane, yield (4.17g, 70 %), m.p 211-213 °C. IR (KBr) ν max cm⁻¹: 3484-3362 (NH₂), 3050 (CH aromatic), 2220 (CN), 1688 (CO), 1630 (C=C); ¹H NMR (DMSO-d₆, 300 MHz): δ = 7.28-7.49 (m, 4H, C₆H₄), 5.68 (s, 1H, pyran H-4), 5.34, 5.33, 4.88 (3s, 6H, D₂O exchangeable, 3NH₂), 4.21 (q, 2H, J = 6.80  Hz, OCH₂CH₃), 3.70 (s, 3H, OCH₃), 1.13 (t, 3H, J = 6.80 Hz, OCH₂CH₃), 1.09, 1.07 (s, 6H, 2CH₃); ¹³C NMR (DMSO-d₆, 75 MHz): δ 165.3 (CO), 120.4, 121.6, 123.6, 128.6, 130.2, 132.9, 134.3, 135.8, 139.1, 140.6, 141.7, 142.8 (C₆H₅, thiophene, pyran C), 116.7 (CN), 112.1, 90.2 (C-2, C-1 ethylidene), 94.3 (CCl₃), 50.6 (OCH₃), 50.1 (OCH₂CH₃), 24.6 (2CH₃), 16.3 (OCH₂CH₃). Anal. Calculated for C₂₆H₂₅Cl₃N₄O₄S: C, 52.52; H, 4.48; N, 9.16; S, 5.73. MS: m/e 595 (M⁺, 66 %).

2.2. RNA methyltransferase activity assays

Compounds 3a-10m were tested for their ability to inhibit the methylation of the RNA cap structure. The inhibition induced by each compound (50 µM) was determined by a radioactive MTase (methyl transferase) assay (filter binding assay) which consists in measuring the [³H] radiolabeled methyl transferred from the methyl donor SAM onto RNA substrate (GpppAC4) synthetized by using T7 primase [35]. Compounds 3a-10m designed as mimics of the transition state of RNA 2’-O-methylation were screened against several viral RNA 2’-OMTases from SARS-CoV (nsp10/nsp16 complex), Zika,
West-Nile, Dengue, Vaccinia (VP39) viruses. At the same time, the compounds were tested against human RNA N7-MTase (hRNMT) and selected viral N7-MTases such as SARS-CoV nsp14 and Vaccinia D1-D12 complex to evaluate their specificity (Table 1). Unexpectedly, all the bisubstrate compounds were barely active against the 2'-O MTases of flaviviruses or coronavirus SARS-CoV. In contrast, most of the compounds displayed inhibition of N7-MTases (methyl transferase).

Table 1. Screening for inhibitory activity of sinefungin and compounds 3a-10m at 50 µM on N7-MTases.

| Compd | R  | X   | Y   | X'    | % of inhibition at 50 µM (%)*  |
|-------|----|-----|-----|-------|--------------------------------|
|       |    |     |     |       | SARs-Cov nsp 14   Vaccinia Virus D1-D12 hRNMT |
| 3a    | H  | -   | -   | -     | 11.6±2.3           22.2±3.1   18.4 ± 1.6 |
| 3b    | CH₃| -   | -   | -     | 18.6±2.8           16.1±2.8   12.5 ± 2.5 |
| 5a    | H  | CN  | -   | -     | 37.2±4.1           42.2±2.6   90.2 ± 4.5 |
| 5b    | H  | COOEt| -  | -     | 18.7±2.5           28.3±2.8   32.3±4.2 |
| 5c    | CH₃| CN  | -   | -     | 48.3±5.2           50.6±3.6   46.5 ± 3.8 |
| 5d    | CH₃| COOEt| -  | -     | 32.5±2.6           38.6±3.7   14.3 ± 1.8 |
| 7a    | H  | CN  | -   | -     | 38.2±2.6           90.1±3.5   92.4±2.5 |
| 7b    | H  | COOEt| -  | -     | 28.1±3.5           29.4±1.8   32.1±2.4 |
| 7c    | CH₃| CN  | -   | -     | 38.1±3.1           44.2±3.9   35.3±3.6 |
| 7d    | CH₃| COOEt| -  | -     | 29.1±2.6           50.2±2.9   59.3±3.4 |
| 8a    | H  | CN  | --  | -     | 54.3±3.8           89.2±4.7   38.5 ± 2.8 |
| 8b    | H  | COOEt| -  | -     | 40.2±2.9           58.8±3.4   88.3 ± 2.4 |
| 8c    | CH₃| CN  | -   | -     | 33.2±2.9           38.1±3.0   42.5 ± 2.6 |
| 8d    | CH₃| COOEt| -  | -     | 68.1±4.8           53.2±3.6   46.3 ± 3.4 |
| 10a   | H  | CN  | H   | CN    | 89.3±5.1           76.2±3.6   82.6±5.0 |
| 10b   | H  | CN  | H   | COOEt | 93.2±6.3           90.2±8.9   96.3±5.4 |
| 10c   | CH₃| CN  | H   | CN    | 96.3±5.6           90.4±5.8   92.6±3.8 |
It is clear from Table 1 that 10a, 10b, 10c, 10e, 10f, 10g and 10h showed high % inhibitions against SARS-CoV nsp 14 with values 89.3, 93.2, 96.3, 96.2, 94.6, 97.2 and 92.8, respectively. On the other hand, compounds 7a, 8a, 10d, 10e, 10f, 10g, 10h and 10l revealed high activity on Vaccinia Virus D1-D12 with % of inhibitions 90.1, 89.2, 87.2, 95.1, 96.3 and 85.8, respectively. Whereas, compounds 5a, 7a, 8b, 10a, 10b, 10c and 10i showed high inhibitions on hRNMT. It is of great value to mention that compounds with high inhibitions toward SARS-CoV nsp 14 containing in most cases the electronegative Cl group beside the trichlomethyl moiety.

Table 2. IC₅₀ values of the newly synthesized compounds on SARS-CoV nap14 and human RNMT activities

| Compd | R   | X   | Y   | X’  | SARS-CoV | hRNMT   |
|-------|-----|-----|-----|-----|----------|----------|
| 10d   | CH₃ | CN  | H   | COOEt| 78.3±6.1 | 87.2±4.9 | 73.4±4.2 |
| 10e   | H   | CN  | Cl  | CN  | 96.2±5.8 | 95.1±6.2 | 38.2±3.9 |
| 10f   | H   | CN  | Cl  | COOEt| 94.6±6.8 | 97.4±7.2 | 46.9±6.4 |
| 10g   | CH₃ | CN  | Cl  | CN  | 97.2±5.3 | 95.1±6.3 | 28.5±5.8 |
| 10h   | CH₃ | CN  | Cl  | COOEt| 92.8±4.6 | 96.3±5.8 | 55.6±4.3 |
| 10i   | H   | CN  | OCH₃| CN  | 38.2±2.6 | 32.4±4.2 | 92.6±5.3 |
| 10k   | H   | CN  | OCH₃| COOEt| 26.8±4.6 | 36.5±3.2 | 28.5±3.8 |
| 10l   | CH₃ | CN  | OCH₃| CN  | 29.6±3.1 | 85.8±4.8 | 32.7±3.6 |
| 10m   | CH₃ | CN  | OCH₃| COOEt| 31.3±3.9 | 32.4±4.1 | 28.6±3.6 |
| Sinefungin | -   | -   | -   | -   | 98.3±0.2 | 99.8±0.1 | 99.8±0.2 |

*Values are the mean of three independent experiments. The MTase activity was measured using a filter binding assay. Assays were carried out in reaction mixture [40 mM Tris-HCl (pH 8.0), 1 mM DTT, 1 mM MgCl₂, 2 µM SAM and 0.1 µM 3H-SAM] in the presence of 0.7 µM GpppAC₄ synthetic RNA and incubated at 30 °C. SARS-CoVnsp14 (50 nM), vaccinia virus capping enzyme (D1-D12) (41 U), human RNA N7, MTase (hRNMT) (50 nM). Compounds were previously dissolved in 100% DMSO. n.i: no inhibition detected at 50 µM.
|    |       |       |       | nap14 IC$_{50}$ (µM) | IC$_{50}$ (µM) |
|----|-------|-------|-------|---------------------|---------------|
| 5c | CH$_3$ | CN    | -     | 33.4 ± 4.6          | 8.6 ± 2.1     |
| 5d | CH$_3$ | COOEt | -     | 58.1 ± 6.3          | 70.4 ± 6.2    |
| 7a | H      | CN    | -     | 1.4 ± 0.89          | 88.2 ± 8.3    |
| 7b | H      | COOEt | -     | 28.8 ± 4.1          | 75.6 ± 4.1    |
| 7c | CH$_3$ | CN    | -     | 10.3 ± 2.6          | 90.2 ± 6.4    |
| 7d | CH$_3$ | COOEt | -     | 21.6 ± 4.3          | 38.6 ± 2.1    |
| 8a | H      | CN    | -     | 0.84 ± 0.5          | 68.3 ± 5.8    |
| 8b | H      | COOEt | -     | 46.1 ± 3.7          | 72.1 ± 4.6    |
| 8c | CH$_3$ | CN    | -     | 12.4 ± 2.5          | 68.2 ± 3.2    |
| 8d | CH$_3$ | COOEt | -     | 8.1 ± 2.3           | 77.1 ± 4.6    |
| 10a| H      | CN    | H     | 1.3 ± 0.6           | < 0.05        |
| 10b| H      | CN    | H     | 2.6 ± 0.8           | 86.3 ± 5.9    |
| 10c| CH$_3$ | CN    | H     | 0.86 ± 0.3          | 94.1 ± 3.6    |
| 10d| CH$_3$ | CN    | H     | 0.49 ± 0.1          | 98.5 ± 6.2    |
| 10e| H      | CN    | Cl    | 0.52 ± 0.2          | 90.2 ± 8.1    |
| 10f| H      | CN    | Cl    | 0.34 ± 0.18         | < 0.05        |
| 10g| CH$_3$ | CN    | Cl    | 1.35 ± 0.81         | < 0.05        |
| 10h| CH$_3$ | CN    | Cl    | 2.3 ± 0.68          | < 0.05        |
| 10i| H      | CN    | OCH$_3$ | 10.4 ± 1.6        | 40.6 ± 3.2    |
| 10j| H      | CN    | OCH$_3$ | 16.5 ± 2.5        | 38.3 ± 4.6    |
| 10l| CH$_3$ | CN    | OCH$_3$ | 14.3 ± 3.1        | 25.6 ± 2.9    |
| 10m| CH$_3$ | CN    | OCH$_3$ | 19.5 ± 2.6        | 42.3 ± 3.3    |
| Sinefungin$^b$ | -     | -     | -     | 0.36              | < 0.05        |

$^a$Concentration inhibiting MTase activity by 50%; mean value from three independent experiments.
It is clear from Table 2 that most of the tested compounds showed IC50’s indicated that they are active toward Cov nap14. Compounds 7a, 8a, 10a, 10c, 10d, 10e, 10f and 10g showed IC50’s 1.4, 0.84, 1.3, 0.86, 0.49, 0.52, 0.34 and 1.34 µM. In addition most of the tested compounds were not active toward hRNMT. Interestingly some compounds like 10a, 10f, 10g and 10h showed IC50’s < 0.05 against hRNMT.

2.3. Materials and Methods

Structure retrieval and preparation:

Subsequently, SCIGRESS3.2 software was used to optimize the molecules using the implemented molecular mechanics force field (MM3) and the semiempirical parameterization method 6 (PM6) [36,37]. The optimized structures are checked for any transition state by calculating the Infrared (IR) spectra [38]. The optimized structures were prepared for the docking experiment using the AutoDock Tools software [39] and the AutoDockVina software was utilized in the docking of the halogenated compounds into the active site of SARS-CoV-2Mpro[40]. The best resolution structure for SARS-CoV-2 Mpro was used in this study (PDB ID: 6Y84), and it was subjected to equilibration and Molecular Dynamics Simulation (MDS) run for up to 100 ns using Nanoscale Molecular Dynamics (NAMD) software[41]. Following the MDS, the SARS-CoV-2 Mpro trajectories were clustered using Chimera software into five different clusters [42] and a representative structure from each cluster was used in the docking study.

Molecular Docking study:

The AutoDockVina software was used to test 22 different halogen-containing compounds against SARS-CoV-2 Mpro, where Nelfinavir was used as a positive control to scale the halogenated compounds’ affinity to SARS-CoV-2 Mpro. The Grid box size used in the
docking experiments was $40 \times 40 \times 40 \text{ Å}^3$, while the box centers were set to be at the active site residues H41 and C145 (-3.9, 19.7, -5.0) Å, (-4.6, 18.9, -15.5) Å, (-8.2, 37.0, -8.4) Å, (4.7, 38.8, -3.3) Å, (22.1, 32.0, -2.4) Å, for the different conformations of the SARS-CoV-2 M$^{\text{pro}}$. Following docking, the complexes were investigated using both PyMOL and the Protein-Ligand Interaction Profiler (PLIP) software [43,44].

3. Results and Discussion
The effective treatment options for SARS-CoV-2 can either be the use of broad-spectrum antiviral drugs or specific therapeutic molecules that can disrupt the viral lifecycle. Since the rapid transmission of coronavirus has proven to be devastating worldwide, several preventive approaches have been suggested by the health care authorities. The implementation of quarantine for infected patients, the monitoring and timely diagnosis of suspected cases, the use of protective masks, and thorough hand washing can help to control disease dissemination [45]. Unfortunately, infection and mortality rates are rising, and no specific COVID-19 treatment has been confirmed to be successful. Thus, extensive research aimed at discovering and developing COVID-19 treatment drugs is urgently warranted. Studies have investigated the therapeutic targeting of the main protease of SARS-CoV-2 (M$^{\text{pro}}$) to combat COVID-19 [46]. In this study, the halogenated compounds exhibit the potential to inhibit the M$^{\text{pro}}$, albeit without guaranteed activity. Nevertheless, these insightful findings provide a foundation for computational drug discovery of novel compounds to combat SARS-CoV-2.

Generally, halogen bonding interactions in biological systems have been widely acknowledged [47-61]. [Not only did halogen-containing compounds attract attention
through a fundamental research perspective, but also through drug evolution to meet clinical endpoints [62]. This is exemplified by the formulation of a series of halogen-containing inhibitors of cathepsin after the discovery of hydrogen bonding between selected ligands and its active site [57]. In addition, Xu et al studied the interaction between sildenafil and its receptor, PDE5, with the goal of improving the binding efficiency between its successors and the receptor [59]. Following the analysis of the atomic details of receptor-drug contacts, a molecular docking program was used to replace hydrogen atoms in the drug with halogen atoms that, theoretically, could produce halogen bonds. By utilizing a hybrid method including both quantum and molecular mechanics, Xu et al were capable of refining the search for successors which were subsequently synthesized [59,62]. The progeny's binding energies with the receptor have been discovered to be in remarkable accordance with the computational predictions. This proves that halogen bonding could be rationally exploited to impact drug discovery [62].

Table 2 shows the average binding energy (kcal/mol) of the different halogenated compounds against SARS-CoV-2 M\textsuperscript{pro}, compared to a positive control, Nelfinavir (-6.92 ±0.5 kcal/mol). As shown in figure 1, fifteen of the tested compounds show promising results in terms of binding to the SARS-CoV-2 M\textsuperscript{pro} active site. The best three compounds are colored in dark green (compounds 13, 17, and 21) with average binding affinities of -6.94 ±0.36, -7.0 ±0.59, and -6.98 ±0.61, respectively. Additionally, twelve compounds (light green columns) show comparable results to the Nelfinavir (red column), namely compounds 7a, 7c, 7d, 10a, 10b, 10d, 10e, 10f, 10h, 10i, 10k and 10m. Conversely, compounds 3a, 3b, 5a, 5b, 5c, 5d and 7b (blue columns) show higher
average binding energies, with less affinity, compared to Nelfinavir against SARS-CoV-2 M\textsuperscript{pro}(-5.92 \pm 0.34 up to -4.76 \pm 0.17 kcal/mol).

Table 2. The interaction pattern of the halogenated compounds with better binding affinities than Nelfinavir against SARS-CoV-2 M\textsuperscript{pro}.

| Compound | AutoDockVina score (kcal/mol) | H-bonding / Halogen bonding | Hydrophobic interactions |
|----------|-------------------------------|----------------------------|-------------------------|
|          | Number                        | Residues from SARS-CoV-2 M\textsuperscript{pro} | Number | Residues from SARS-CoV-2 M\textsuperscript{pro} |
| Nelfinavir | -6.7                          | 1                           | E166                   | 3       | T25, L27, and M165 |
| 7a       | -6.3                          | 1                           | N142                   | 1       | Q189 |
| 7c       | -6.2                          | 3                           | Q189, T190, and A191    | 3       | F181, and V186(2) |
| 7d       | -6.3                          | 1                           | Q189                   | 3       | T25, L27, and N142 |
| 10a      | -6.9                          | 4                           | T24, S46(2), and D187   | 1       | T25 |
| 10b      | -6.1                          | 2                           | Q189(2)                | 1       | T25 |
| 10c      | -6.9                          | 4                           | T24, C44, S46, and D187 | 1       | T25 |
| 10d      | -6.9                          | 5                           | N142, D187, and Q189(3), E47 | 2       | T25 and Q189 |
| 10e      | -6.8                          | 5                           | S46, N142, E166(2), and Q189 | 1       | L27 |
| 10f      | -6.7                          | 3                           | T45, S46, and Q189     | 1       | Q189 |
| 10g      | -6.9                          | 1                           | S144                   | 1       | E166 |
| 10h      | -6.3                          | 2                           | T24 and T45            | 1       | Q189 |
| 10i      | -7.0                          | 1                           | N142                   | 1       | T25 |
| 10k      | -6.8                          | 5                           | N142, G143, S144, C145, and D187 | 2       | M165 and E166 |
| 10l      | -7.0                          | 4                           | T26, S46, E166, and Q189 | 1       | N142 |
| 10m      | -6.5                          | 4                           | T24, T25(2), and E166  | 0       | |

The AutoDockVina scores are listed among the number of H-bonds and hydrophobic contacts and the residues that interact. The green residue is the one that interacted with halogen bonds with the ligands. Bold residues are the most common residues.
Figure 1: The average binding energies (kcal/mol) of 22 halogenated compounds and Nelfinavir against SARS-CoV-2 M^\text{pro} active site residue H41 and C145. Error bars represent the standard deviation (SD). The halogenated compounds are classified into top compounds (dark green), compounds with comparable binding energy with Nelfinavir (light green), and compounds that bind SARS-CoV-2 M^\text{pro} with less affinity compared with Nelfinavir (blue), while Nelfinavir is red-colored.

A representative docking complex was selected for further analysis of each compound. Table 2 shows the PLIP analysis of the compounds after docking when the main interactions established were Hydrogen, halogen bonds and hydrophobic contacts. It is noteworthy that the most common residues that interacted with the halogenated compounds were T25, N142, E166, D187 and Q189. The best three compounds in terms of binding affinity (compounds 13, 17, and 21) are depicted in figure 2 with stick representation. As reported from both Table 2 and Figure 2, the most common residues that contributed to the interaction with the halogenated compounds were T25, E166, D187, and Q189.
Figure 2: The interaction pattern for the docking of the best three halogenated compounds (13, 17, and 21) against the active site of SARS-CoV-2 M\textsuperscript{pro}. H-bonds and the hydrophobic contacts are represented in blue and dashed-grey lines, respectively. Halogen bonds are depicted in green lines. The interacting residues are labeled with their one-letter codes, while the colored sticks represent both the halogenated compounds (magenta) and the interacting residues from SARS-CoV-2 M\textsuperscript{pro} (blue).

Noticeably, the residues E166 and M165 were involved in the interaction between Nelfinavir and the SARS-CoV-2 M\textsuperscript{pro}. The former forms an H-bond with an OH group located in the meta-position of the benzyl ring, while the latter forms a hydrophobic contact with a methyl group in the orthoposition of the same benzyl ring. The superposition of the docking complexes from Nelfinavir and the compounds 13, 17, and 21 is shown in figure 3. As reflected from the figure, the three halogenated compounds reside in the active site pocket of the SARS-CoV-2 M\textsuperscript{pro}, superimposed over Nelfinavir suggesting the same mode of interaction, hence possible inhibition of the main protease.
of SARS-CoV-2. These results are in support of previous studies on the effectiveness of halogen-containing compounds as potential antiviral agents [63,64].

**Figure 3** The superposition of the docking complexes of Nelfinavir (gray), and the compounds 10c(green), 10g(blue), and 10l (orange) docked into the active site of SARS-CoV-2 Mpro. The enlarged panel is depicted to show how the compounds are lying in the active site cavity of the protein.

**Results and discussion**

The reaction of either cyclohexan-1,3-dione (1a) or dimedone (1b) with trichloroacetonitrile (2) gave the 2,2,2-trichloroethylidene)cyclohexane-1,3-dione derivatives 3a and 3b, respectively. Structures of the latter products were confirmed of the basis of their respective analytical and spectral data (see experimental section). Compounds 3a and 3b were capable for thiophene synthesis through the well known Gewals’s thiophene synthesis [65-67]. Thus, the reaction of either 3a or 3b with each of elemental sulfur and either malononitrile (4a) or ethyl cyanoacetate (4b) gave the 2,2,2-trichloroethylidene)-6,7-dihydrobenz[6]thiophen-5(4H)-one5a and 5b, respectively. The analytical and spectral data of compounds 5a-d were in agreement of their struturets.
Thus, the $^1$H NMR spectrum of 5a (as an example) showed the presence of two singlets at $\delta$ 5.25 and 4.80 ppm (D$_2$O exchangeable) for the two NH$_2$ groups and two triplets at $\delta$ 2.13 and 2.45 ppm equivalent for the two CH$_2$ group. In addition, the $^{13}$C NMR spectrum revealed the presence of a signal at $\delta$ 168.2 equivalent to the C=O group, four signals at $\delta$132.6, 133.8, 138.0, 140.1 indicating the four thiophene carbons, a signal at $\delta$ 116.8 for the CN group, two signals at $\delta$ 112.1 and 90.6 for the ethylidene carbons, a signal at $\delta$ 94.4 for the CCl$_3$ group and two signals at $\delta$ 40.7 and 39.6 for the two CH$_2$ groups. The reaction of either 5a-d with phenylisothiocyanate (6) gave the 4,6-dihydrothieno[3,2-\textit{f}]quinazoline-3-thiol derivatives 7a-d, respectively (Scheme 1). The analytical and spectral data of the latter compounds were in agreement with their structures.
Scheme 1. Synthesis of compounds 3a,b; 5a-d and 7a-d.'
Compounds 7a-d underwent ready hydrolysis of the trichloromethylmoiety to give the 4,6-dihydrothieno[3,2-f]quinazolin-1-ol derivatives 8a-d, respectively (Scheme 2).

Scheme 2. Synthesis of compounds 8a-d

In recent years, multicomponent reactions (MCRs) have become essential, efficient, bond-forming methods for expedient synthesis of a wide range of active organic compounds and natural products without separation and purification of intermediates. The MCRs, which are important classes of chemical transformations, have recently attracted much attention owing to their high efficacy, shorter reaction times, mild conditions, simplicity, and environmental friendliness [68-71]. During the last several years, the diverse applications of such2-amino-4H-pyran heterocyclic scaffolds in medicinal chemistry have drawn appreciable attention among synthetic chemiststo
(explore useful synthetic routes to these heterocycles of potential interest with antimicrobial and antitumor activities [72,73]. Compounds 5a,c were ready for multicomponent reactions due to the presence of α-oxomethlene moiety within such molecules. Therefore, the reaction of either of 5a or 5c with either benzaldehyde (9a), 4-chlorobenzaldehyde (9b) or 4-methoxybenzaldehyde (9c) and either malononitrile (4a) or ethyl cyanoacetate (4b) in 1,4-dioxane containing a catalytic amount of triethylamine gave the 8,9-dihydro-4H-thieno[2,3-g]chromene derivatives 10a-m, respectively (Scheme 3). The analytical and spectral data of the latter compounds were in agreement with their respective structures. Thus, the 1H NMR spectrum of compound 10a (as an example) revealed the presence of a multiplet at δ 7.25-7.42 ppm for the phenyl protons, a singlet at δ 5.90 equivalent to the pyran H-4, three signals at δ 4.80, 5.22, 5.39 ppm (D2O exchangeable) for the three NH2 groups and a singlet at δ 2.80 ppm for the CH2 group. In addition the 13C NMR spectrum which showed singles at δ 120.1, 123.5, 125.4, 128.5, 130.8, 133.7, 137.9, 138.3, 139.0, 140.2, 142.5, 143.8 equivalent to the C6H5, thiophene, pyran carbons, two signals at δ 116.9, 117.3 for the two CN groups, a signal at δ 94.2 for the CCl3 group and a signal at δ 53.8 corresponding to the CH2 group.)
Scheme 3: Synthesis of compounds 10a-m.
4. Conclusion:
Halogenated compounds have been widely acknowledged in the field of medicinal chemistry owing to their binding potentials. That 10a, 10b, 10c, 10e, 10f, 10g, and 10h showed high % inhibitions against SARs-Cov nsp 14. Whereas, compounds 5a, 7a, 8b, 10a, 10b, 10c, and 10i showed high inhibitions on hRNMT. This study explored the binding affinity of 22 halogenated compounds to the SARS-CoV-2 MPro and discovered 15 compounds with higher binding affinity than Nelfinavir, of which 3 showed remarkable results. In times of dire need for anti-COVID-19 treatment, our results lay the foundation for further exploratory research on these candidate compounds to examine their mechanism of action and efficacy against corona virus.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE
This study was approved by the local Research Ethics Committee at the University of Tabuk, Kingdom of Saudi Arabia.

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CONFLICT OF INTEREST
The authors declare that there are no conflicts of interest.

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First author F.M. Almutairi collected the data and was responsible about writing this work. Second author R.M. Mohareb the idea of writing this review article, and he performed the literature survey and data research. The third author was responsible about revising the manuscript and writing the references of this work. The fourth and fifth authors M.A. Mahmoud and W.W. Wardakhan responsible for doing experimental work necessary for synthesizing the target molecule and doing the biological screening. The sixth and seventh authors were responsible for the molecular docking of compounds in this work beside writing the biology section.

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Ethics declarations

Ethical Approval
No related ethical issues.

Ethical declaration
Research involving human participants and/or animals

Not applicable

**Informed consent**

Informed consent was obtained from all participants included in the study.

**Competing interests**

The authors declare no competing interests.

**Consent to participate**

The authors promise that the work described has not been published previously, that it is not under consideration for publication elsewhere, that its publication is approved by all authors and tacitly or explicitly by the responsible authorities where the work was carried out.

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The authors promise that if the manuscript is accepted, it will not be published elsewhere in the same form, in English or in any other language, without the written consent of the Publisher. There are no conflicts of interest to declare.

**High lights**

*Both of cyclohexan-1,3-dione and dimedone were used to synthesis fused halogen rich heterocyclic compounds.*

*The structures of the newly synthesized products were established on the basis of analytical and spectral data.*

*The antiviral activities of all novel compounds were studied against human RNA N7-MTase (hRNMT) and selected viral N7-MTases such as SARS-CoV nsp14 and Vaccinia D1-D12 complex*

* Molecular docking was done for most of the synthesized compounds.