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Synthesis of novel derivatives of 7,8-dihydro-6*H*-imidazo[2,1-*b*][1,3]benzothiazol-5-one and their virus-inhibiting activity against influenza A virus

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Abstract

Influenza remains a highly pathogenic and hardly controlled human infection. The ability of selecting drug-resistant variants necessitates the search and development of novel anti-influenza drugs. Herein, we describe the synthesis and evaluation of a series of novel 2-substituted 7,8-dihydro-6*H*-imidazo[2,1-*b*][1,3]benzothiazol-5-ones **3a–k** for their virus-inhibiting activity against influenza A virus. The new analogues **3a–k** prepared in two steps from commercially available cyclohexane-1,3-diones were fully characterized by their NMR and mass spectral data. Among the new derivatives screened for cytotoxicity and *in vitro* antiviral activity against influenza virus A/Puerto Rico/8/34 (H1N1) in MDCK cells, three analogues **3i–k** containing a thiophene unit were found to exhibit high virus-inhibiting activity (high SI values) and a favorable toxicity profile. The compound **3j** (CC_{50}: >1000 μM, SI = 77) with higher potency is the best anti-influenza hit analogue for further structural optimization and drug development. The most active compounds did not inhibit viral neuraminidase and possess therefore other targets and mechanisms of activity than the currently used neuraminidase inhibitors.

KEYWORDS
1,3-dicarbonyl, antivirals, antiviral activity, benzothiazole, cytotoxicity, imidazole, influenza virus

1 | INTRODUCTION

Influenza is a highly contagious human disease. Every year, 500 million people suffer from the flu worldwide with about two million with fatal outcome.[1,2] The influenza virus causes annual epidemics and pandemics, the later are often associated with significant morbidity and mortality.[3] The pandemic of influenza A (H1N1) pdm09 caused by a swine-origin H1N1 virus was characterized by a wide coverage of the population of the world, severe clinical course and about 300000 lethal outcomes within 18 months.[4,5] Pregnant women, newborns, aged people, and persons with pre-existing pathologies (diabetes, kidney failure) are of high risk.[6] Moreover, in recent years, cases of human infection with avian influenza viruses H5N1, H7N2, H7N7, and H7N9

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have been reported.[7,8] Therefore, continuous monitoring and development of control measures to restrict influenza spreading is highly actual for medical science and health care.

Four classes of low-molecular compounds are currently used as etiotropic anti-influenza drugs (Figure 1).[9] Derivatives of adamantane, amantadine, and rimantadine inhibit the virus-specific protein M2.[10,11] Neuraminidase inhibitors (NAI), oseltamivir (Tamiflu®), zanamivir (Relenza®), laninamivir (Inavir®), and peramivir (Rapivab®) interfere with viral neuraminidase emzymatic activity and prevent the budding of progeny virions.[12,13] Nucleoside analogs ribavirin and favipiravir (T-705, Avigan®) induce lethal mutagenesis in the viral genome.[14] Finally, membrane fusion inhibitor arbidol (Umifenovir®) is approved in Russia and China for prevention and treatment of influenza.[15] Recently, structure-based *in silico* screening of library of compounds bearing a tetrazole moiety effectively suppressed the replication of influenza viruses.[16] Owing to error-prone activity and lack of correction mechanisms, viral polymerase provides high rate of mutations. This results in fast development and spreading of drug resistance. All influenza virus isolates are resistant to adamantanes, and almost 100% resistance to oseltamivir has been reported among H1N1 viruses.[17] There is therefore an urgent need for new drugs of high level and broad range of efficacy, high barrier for resistance, and targeting alternative viral proteins essential for viral life cycle.[18] Here we describe the synthesis and biological activity of novel derivatives of 7,8-dihydro-6*H*-imidazo[2,1-*b*][1,3]benzothiazol-5-one.

Recently fused heterocyclic systems with bridgehead nitrogen (Figure 2) have gained importance as pharmacophore exhibiting broad spectrum biological activities.[19] Imidazo[2,1-*b*]thiazole is one such fused ring system represented well in the literature.[20] Derivatives of this scaffold have been shown to possess a wide range of biological activity, from kinase inhibition,[21] antiviral,[22] anticancer,[20] and antioxidant activities to their use as potential antitubercular agents.[23,24] Interest in the biological activity of these compounds is also reflected in both the number of synthetic methods found to access them and the possibility for diverse functionalization.[19,20] Hence imidazo[2,1-*b*]thiazole is considered as platform of choice for designing new molecules for our ongoing medicinal chemistry pursuits.

The designed new scaffold (Figure 3) is based on recently reported antiviral agent **III**.[22] This compound together with relative ones were shown to inhibit non-structural protein of hepatitis C virus (HCV) NS4B. It interacts with other non-structural proteins of HCV, NS3, NS4A, NS5A and others that are involved in viral RNA synthesis.[25] In the current study, we describe synthesis and biological assessment of series of novel imidazo[2,1-*b*]thiazole-based compounds as potential inhibitors of another RNA-genome virus, influenza virus, *in vitro*. The designer scaffold has imidazo[2,1-*b*]thiazole core fused with cyclic ketone system. Variations in the proposed scaffold can be accomplished with substituted aromatic/heterocyclic ring units.

2 | RESULTS AND DISCUSSION

Initiating the synthesis (Scheme 1), 2-amino-5,6-dihydrobenzo[*d*]-thiazol-7(4*H*)-ones **1a**,**b** required was prepared from commercially available cyclic 1,3-dicarbonyls reacting with bromine followed by *in situ* condensation with thiourea. For example, 2-amino-5,6-dihydro-benzo[*d*]thiazol-7(4*H*)-one (**1a**) was prepared in 85% yield by the reaction of cyclohexane-1,3-dione with bromine in acetic acid in the presence of sodium acetate; *in situ* condensation with thiourea at 100°C. Similarly, 2-amino-5,5-dimethyl-5,6-dihydrobenzo[*d*]thiazol-7-(4*H*)-one (**1b**) was also prepared from 5,5-dimethylcyclohexane-1,3-dione in 82% yield. Both the compounds **1a** and **1b** were fully characterized by their NMR, IR, and ESI-mass spectral data.[23,24]

To build the desired fused heterocyclic analogues, 2-amino-5,6-dihydrobenzo[*d*]thiazol-7(4*H*)-ones **1a**,**b** were reacted with

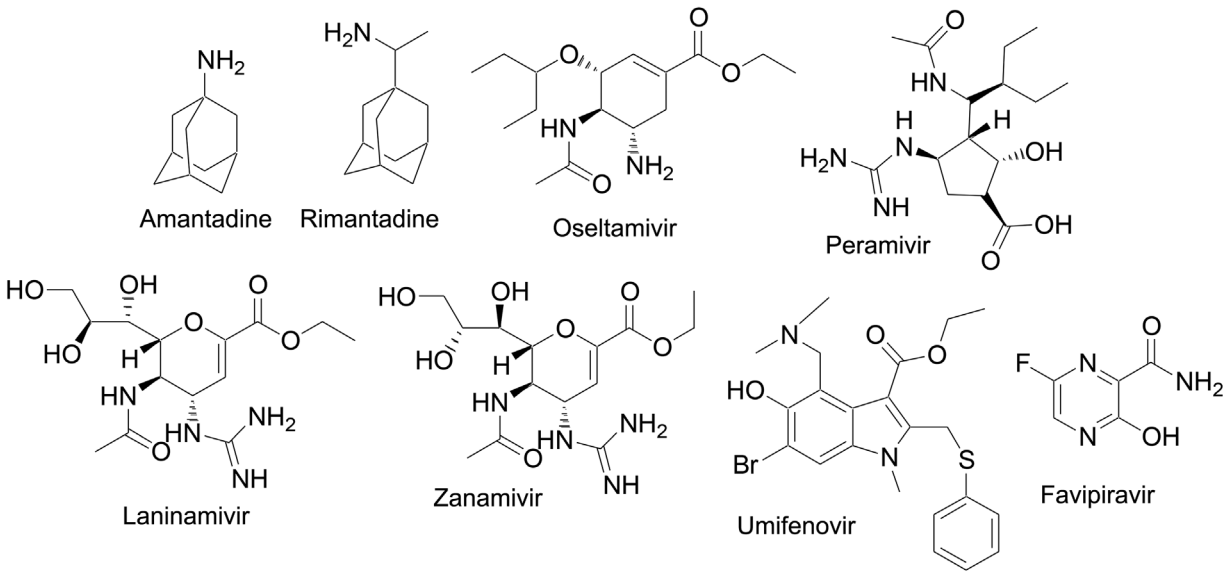


FIGURE 1 Anti-influenza drugs currently in use

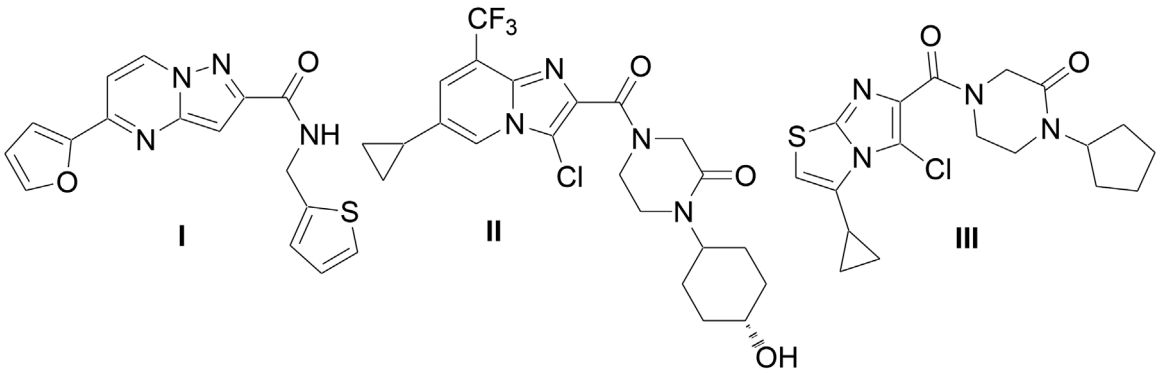


FIGURE 2 Antiviral chemical entities with heterocyclic fused ring system

substituted phenacyl bromides **2a–f** refluxing in 2-propanol (Scheme 1; Table 1). For example, 2-amino-5,6-dihydrobenzo[*d*]thiazol-7(4*H*)-one (**1a**) in 2-proponaol was reacted with *p*-bromophenacyl bromide (**2a**) at reflux to give 2-(4-bromophenyl)-6,7-dihydrobenzo[*d*]imidazo [2,1-*b*]-thiazol-8(5*H*)-one **3a** in 82% yield. Under similar conditions, all the compounds **3a–k** were synthesized and fully characterized by their ^{1}H and ^{13}C NMR, IR, and mass (ESI-MS and HRMS) spectral data. LogP and ClogP required to assess the lipophilic character of new analogs were calculated using ChemBioDraw 15.0 (Table 1).

The cytotoxicity of the compounds against MDCK cells and antiviral activity of the compounds **3a–k** against influenza virus A/Puerto Rico/8/34 (H1N1) in cell culture were studied by the technique described previously.[26,27] Based on the results obtained, 50% cytotoxic concentration (CC_{50}), 50% inhibiting concentration (IC_{50}) were calculated for each compound. The selectivity index was calculated for each compound as a ratio of CC_{50} to IC_{50}. The compounds with SI = 10 and higher were considered active entities. The test results are summarized in Table 1. Rimantadine and oseltamivir carboxylate were used as reference drugs.

In the group of 6,7-dihydrobenzo[*d*]imidazo[2,1-*b*]thiazol-8(5*H*)-one derivatives, eleven compounds **3a–k** analyzed had a wide range of cytotoxicity and their CC_{50} values varied from 49 μM to more than 1000 μM. All compounds carried phenyl or thiophene unit on the imidazole ring of imidazo[2,1-*b*]thiazole core. Structure–activity correlations of all new compounds **3a–k** revealed that in the presence of *para*-substituted benzene moieties appended on imidazole ring and simultaneous *gem*-dimethyl modification of the fused cyclohexane ring, the presence of bromo- or cyano-group in the *para* position led to an increase in toxicity, while the introduction of a methoxy group or a chlorine atom in this position led to its decrease. In some cases, the toxicity of *gem*-dimethyl derivatives was higher than that of hydrogen-bearing analogues. Indeed, introduction of methyl groups instead of hydrogen resulted in fivefold (compounds **3a** and **3b**), 1.5-fold (**3c** and **3d**) increase of toxicity. However, for methoxy- and chlorine-containing derivatives, the opposite relationship was observed. In these cases, *gem*-dimethyl-modification resulted in 7.5-fold and 20-fold decrease of toxicity, correspondingly.

All three thiophene-substituted analogues **3i–k** were of low toxicity (CC_{50}'s from 679 to >1000 μM). In this study, few such compounds were tested to conclude reliably the decreasing effect of thiophene group on toxicity of derivatives, although this trend should be taken into consideration in further studies.

Taken together, these results suggest that the toxicity of imidazo [2,1-*b*]thiazole-based compounds is complex, and more examples should be analyzed for reliable structure-toxicity dependence.

Activity against the influenza A virus showed that 3 out of 11 compounds (27%) possess selectivity indices higher than 10, which allows speaking in general of the moderate potential of this chemical library as an anti-influenza means. However, all compounds that had

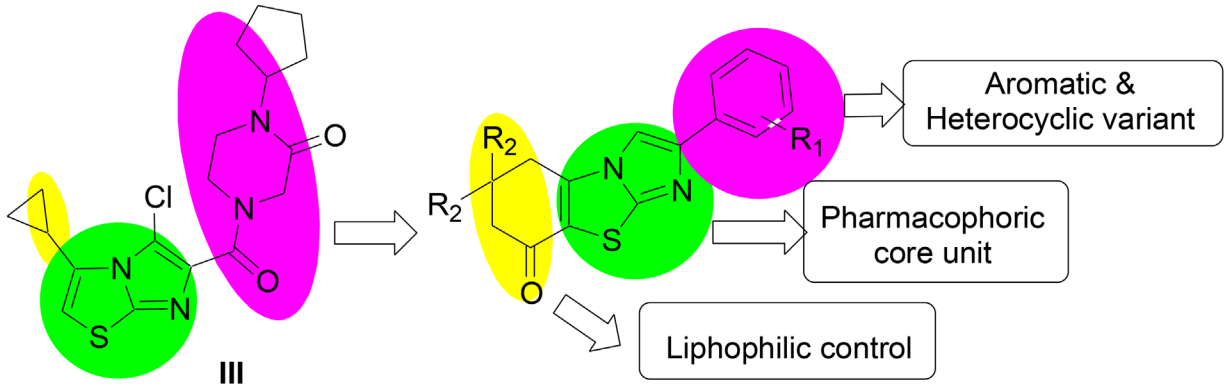
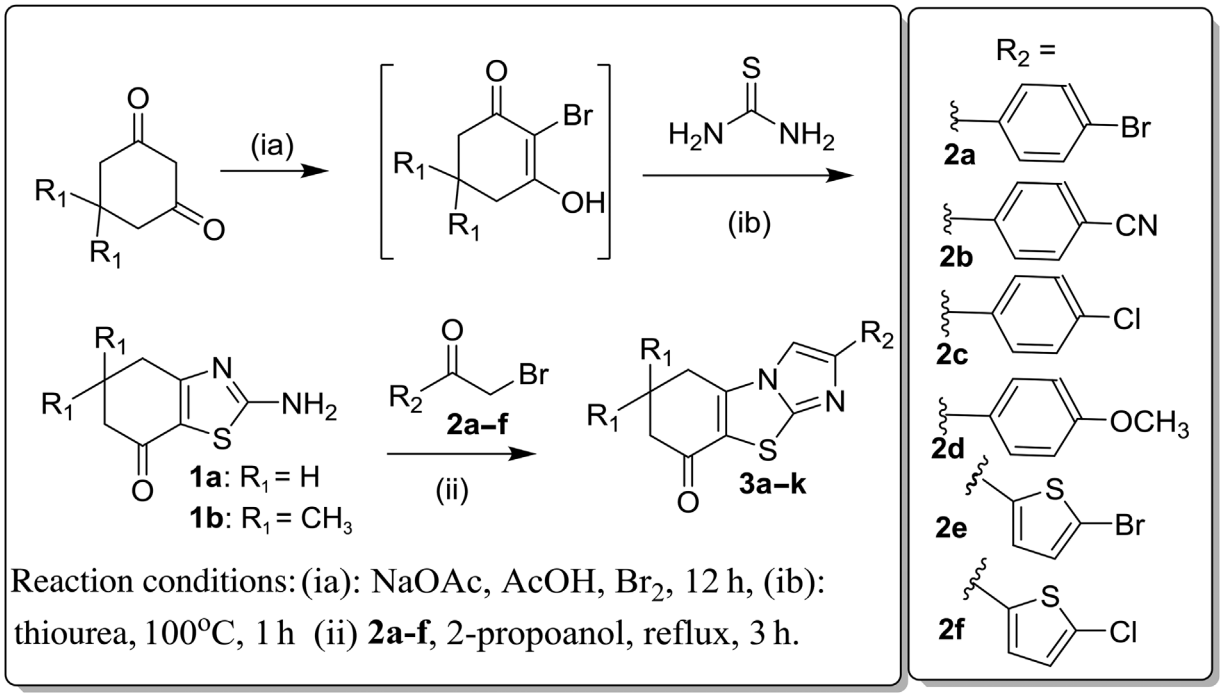


FIGURE 3 Design strategy for new derivatives



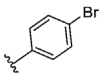
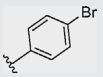
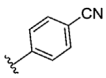
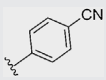
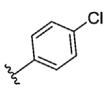
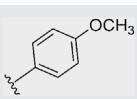
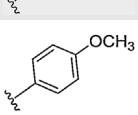
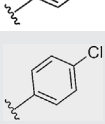
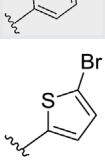
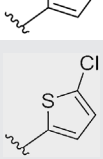
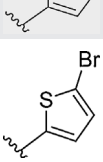
SCHEME 1 Synthesis of novel imidazo[2,1-*b*][1,3]benzothiazol-5-one derivatives **3a–k**

no inhibitory activity against influenza virus (**3a–h**) contained bulky phenyl substituents. Compared to reference drugs, all the phenyl substituted variants **3a–h** with or without *gem*-dimethyl group on fused cyclohexane ring were either more or less toxic but of no antiviral activity. A decrease in the volume of the substituent on the imidazole ring (replacement of the substituted benzene moiety by a less bulky substituted thiophene group) led to a sharp increase in antiviral activity in all three analyzed representatives of this group. The thiophene-substituted analogues **3i–k** has high activity in inhibiting influenza virus and lower toxicity than standard drug rimantadine. Compared to another standard drug oseltamivir (CC_{50} > 200 μM), thiophene analogues **3i–k** have favorable toxicity profile but exhibited lower antiviral activity. Importantly, the increase of virus-inhibiting activity (high SIs) after introducing of thiophene instead of benzene moiety was due to decrease of IC_{50}, and therefore increase of the affinity to specific target, rather than due to the decrease of toxicity. Thus, further optimization of the chemical structure in this direction can lead to the development of effective drugs against influenza. In particular, the range of thiophene-substituted compounds will be extended to identify more active compounds of described scaffold.

In attempt to decipher the mechanism of activity of this group of compounds, three lead compounds **3i–k** have been further tested for their ability to inhibit influenza virus neuraminidase in luminescent MUNANA-based assay.[28] Based on the data obtained, the values of IC_{50} were calculated that appeared 4.4, 121.9, and 6.9 mM for **3i–k** correspondingly (IC_{50} for reference compound oseltamivir carboxylate was 0.36 μM). Thus, no correlation was detected between anti-neuraminidase activity of compounds and their virus-inhibiting properties. We conclude therefore that despite relatively high anti-viral activity of novel compounds, they are not targeted against neuraminidase and have therefore other mechanism of action. Their further optimization may result in development of novel antivirals that are active against NAI-resistant variants of influenza virus.

The initial derivative of imidazo[2,1-*b*]thiazole was shown to suppress the activity of HCV-encoded protein NS4B. It was shown to interact with other non-structural proteins of HCV, NS3, NS4A, NS5A and others, that together provide correct synthesis of viral RNA.[25] NS4B also targets the replication complex components to specialized lipid raft compartments[29] and provides numerous regulating interactions with cellular proteins for benefit of viral replication.[30] As both HCV and influenza virus in their life cycles depend on specific membrane structures, and both contain RNA-dependent RNA-polymerase, we supposed that compounds able to inhibit NS4B, either its membrane-targeting or RNA-polymerase activity, could appear active against influenza virus as well. Indeed, three of 11 compounds demonstrated high suppressing activity against influenza virus. Importantly, all three thiophene-substituted derivatives demonstrated inhibiting activity against amantadine/rimantadine resistant virus that has been used in experiments. Adamantine derivatives, amantadine and rimantadine block the viral proton pump M2 thus preventing the acidification of the virion interior.[10] We conclude therefore that despite relatively high anti-viral activity of thiophene-containing imidazo[2,1-*b*]thiazole derivatives, they are targeted against neither neuraminidase nor M2 proton channel and have therefore other mechanism of action. This confirms indirectly our initial suggestion that their activity could be based on either inhibition of activity of influenza virus' polymerase complex or inhibition of membrane-linked processes in the viral cycle, like transport of RNPs to specific sites of plasma membrane or processes associated with membranes of Golgi complex.[31] Their further optimization may result

TABLE 1 Antiviral activity and cytotoxicity of **3a–k** against influenza virus A/Puerto Rico/8/34 (H1N1) in MDCK cells

Compound	R_1	R_2	Yield (%)	LogP/cLogP[a]	CC_{50}[b], μM	IC_{50}[c], μM	SI[d]
3a	H		82	4.15/4.35	>1000	>1000	1
3b	Me		79	4.95/5.38	210 ± 16	>111	2
3c	H		78	3.36/4.35	108 ± 8	86 ± 9	1
3d	Me		79	4.16/3.96	73 ± 4	37 ± 5	2
3e	H		72	3.88/4.19	49 ± 4	37 ± 4	1
3f	H		83	3.20/3.49	133 ± 9	>111	1
3g	Me		77	4.00/4.53	>1000	>1000	1
3h	Me		76	4.68/5.24	>1000	>1000	1
3i	H		73	4.82/5.29	679 ± 41	49 ± 6	14
3j	H		78	3.68/4.10	>1000	13 ± 3	77
3k	Me		72	4.02/4.26	>1000	46 ± 6	22
Rimantadine				N/A	319 ± 15	72 ± 8	4
Oseltamivir carboxylate				N/A	>200	0.2 ± 0.0	>1000

[a]LogP/cLogP was calculated using ChemBioDraw-2016 software.
[b]CC_{50} is the median cytotoxic concentration; i.e., the concentration causing 50% cell death.
[c]IC_{50} is the 50% inhibiting concentration; i.e., the concentration causing a 50% decrease in virus replication.
[d]SI is the selectivity index, the CC_{50}/IC_{50} ratio.

in development of novel antivirals that are active against drug-resistant variants of influenza virus.

3 | CONCLUSION

In the current study, we have designed and synthesized a series of novel 2-substituted 6,7-dihydrobenzo[*d*]imidazo[2,1-*b*]thiazol-8(5*H*)-ones **3a–k** through two-step reaction from readily available cyclohexane-1,3-diones. All the new analogues **3a–k** were fully characterized by their NMR and mass spectral data. Screening of all the 11 new derivatives against antiviral activity revealed that compounds **3i–k** are the most active agents inhibiting influenza A virus compared to the other evaluated compounds. The substituent on the imidazole ring was shown to have exceptional importance in anti-viral activity as the decrease of its volume (replacement of the substituted benzene moiety by a substituted thiophene group) led to a sharp increase in antiviral activity. Among all compounds, **3j** could be the best candidate to consider as drug-like hit analogue for further development of library compounds. The results described here demonstrate the potential utility of 6,7-dihydrobenzo[*d*]imidazo[2,1-*b*]thiazol-8(5*H*)-ones, tethered with thiophene unit as inhibitors of influenza A virus and data presented will help global efforts for identification of new chemical entities as potent drug-like novel antiviral candidates.

4 | EXPERIMENTAL

4.1 | Chemistry

4.1.1 | General

The original spectra of the investigated compounds as well as their InChI codes together with some biological activity data are provided as Supporting Information.

4.1.2 | General procedure for the preparation of 2-amino-5,6-dihydrobenzo[*d*]thiazol-7(4*H*)-ones 1a,b

To a mixture of cyclohexane-1,3-dione (5.0 g, 44.5 mmol) and NaOAc (3.65 g, 44.5 mmol) in acetic acid (50 mL) was added bromine (3.1 g, 44.5 mmol) dropwise at 15–20°C. After stirring the reaction mixture at room temperature for 12 h, thiourea (5.43 g, 71.3 mmol) was added in potions and heated at 100°C for 1 h. The reaction mixture was cooled to RT, acetic acid was removed under vacuum, the resulting crude product is treated with water (10 mL), neutralized with aqueous saturated $NaHCO_3$ solution, and extracted with ethylacetate (3 × 25 mL). The combined organic layer was dried over anhyd. Na_2SO_4, filtered and rotary evaporated under vacuum. The crude residue is triturated with water to give 2-amino-5,6-dihydrobenzo[*d*]thiazol-7(4*H*)-one (**1a**) as white solid in 85% yield. Mp: 185–187°C. ^{1}H NMR (DMSO, 300 MHz) δ 7.84 (s, 2H), 2.73 (t, *J* = 6.0 Hz, 2H), 2.42 (t, *J* = 6.6 Hz, 2H), 2.07 (qt, *J* = 6.32 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 189.40, 173.64, 167.73, 118.65, 36.63, 26.64, 22.29. IR (KBr) 3287, 2943, 1621, 1512, 1384, 1289, 1185, 1080, 835, 540 cm^{-1}. MS (ESI): *m/z*: 169 $[M+H]^+$; HR-MS (ESI) calcd. for $C_7H_9N_2OS$: 169.04301, found: 169.04326. Similarly, 2-amino-5,5-dimethyl-5,6-dihydrobenzo[*d*]thiazol-7(4*H*)-one (1**b**) was also prepared from dimedone in 82% yield. Mp: 205–207°C. ^{1}H NMR (DMSO, 300 MHz) δ 7.79 (s, 2H), 2.62 (s, 2H), 2.30 (s, 2H), 1.10 (s, 6H). ^{13}C NMR (75 MHz, $CDCl_3$ + DMSO) δ 188.97, 178.98, 166.12, 117.30, 50.67, 40.54, 34.03, 27.88. IR (KBr): 3375, 2924, 1628, 1507, 1366, 1053, 832, 524. MS (ESI) *m/z* 197 $[M+H]^+$: HR-MS (ESI) calcd. for $C_9H_{13}N_2OS$: 197.07505, found: 197.074.

4.1.3 | General procedure for the synthesis of 2-substituted 6,7-dihydrobenzo[*d*]imidazo[2,1-*b*]-thiazol-8(5*H*)-ones 3a–k

To a solution of **1a**,**b** (5.0 mmol) in 2-propanol (10 mL) was added bromides **2a–f** (5.0 mmol) and refluxed at 70°C for 3 h. After completion (TLC), the reaction mixture was cooled to RT, solvent was evaporated in vacuum. Crude residue was purified over silica gel column chromatography eluted with hexane/ethyl acetate (5:1) to give products 3**a–k** as pale yellow solids.

(2-(4-Bromophenyl)-6,7-dihydrobenzo[*d*]imidazo[2,1-*b*]thiazol-(5*H*)-one (3a)

Yield: 82%; mp: 257°C. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.78–7.63 (m, 2H), 7.66 (s, 1H), 7.56–7.52 (m, 2H), 3.02 (t, *J* = 6.23 Hz, 2H), 2.71 (t, *J* = 6.48 Hz, 2H), 2.37 (qt, *J* = 6.35 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 190.69, 143.48, 142.64, 140.76, 132.08, 131.18, 126.50, 122.78, 120.75, 107.70, 36.81, 22.61, 21.41. IR (KBr) 3422, 2929, 1658, 1476, 1353, 1320, 1128, 1002, 833, 725, 541 cm^{-1}. MS (ESI): *m/z*: 348.9287 $[M+H]^{+2}$: HRMS (ESI) calcd. for $C_{15}H_{11}BrN_2OS$: 345.97758, found: 345.97755.

2-(4-Bromophenyl)-6,6-dimethyl-6,7-dihydrobenzo[*d*]imidazo[2,1-*b*]thiazol-8(5*H*)-one (3b)

Yield: 79%; mp: 243–245°C. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.72–7.67 (m, 2H), 7.63 (s, 1H), 7.56–7.51 (m, 2H), 2.86 (s, 2H), 2.56 (s, 2H), 1.25 (s, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 190.75, 151.24, 148.41, 141.14, 132.14, 131.88, 126.91, 122.68, 121.93, 106.23, 51.38, 37.00, 35.00, 28.50. IR (KBr) 3417, 2953, 1663, 1586, 1477, 1355, 1323, 1123, 1002, 727, 543 cm^{-1}. MS (ESI) *m/z* 377 $[M+H]^{+2}$: HRMS (ESI) calcd. for $C_{17}H_{15}BrN_2OS$: 374.00859, found: 374.00885.

4-(8-Oxo-5,6,7,8-tetrahydrobenzo[*d*]imidazo[2,1-*b*]thiazol-2-yl)benzonitrile (3c)

Yield: 78%; mp: 203–206°C. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.73–7.69 (m, 2H), 7.66 (s, 1H), 7.56–7.57 (m, 2H), 3.02 (t, *J* = 6.23 Hz, 2H), 2.71 (t, *J* = 6.48 Hz, 2H), 2.37 (qt, *J* = 6.35 Hz, 6H). ^{13}C NMR (75 MHz, $CDCl_3$ + DMSO) δ 189.52, 173.32, 167.71, 167.25, 147.71, 138.09, 131.24, 125.29, 118.70, 104.10, 36.20, 26.22, 21.82. IR (KBr) 3377, 3140, 2929, 1639, 1536, 1339, 1127, 1039, 837, 700, 538 cm^{-1}. MS (ESI) *m/z* 294 $[M+H]^+$: HRMS (ESI) calcd. for $C_{16}H_{11}N_3OS$: 293.06227, found: 293.06228.

4-(6,6-Dimethyl-8-oxo-5,6,7,8-tetrahydrobenzo[*d*]imidazo[2,1-*b*]thiazol-2-yl)benzonitrile (3d)

Yield: 79%; mp: 228°C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.98–7.90 (m, 2H), 7.77 (s, 1H), 7.72–7.67 (m, 1H), 2.90 (s, 2H), 2.60 (s, 2H), 1.27 (s, 6H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 190.73, 151.56, 147.35, 141.08, 137.49, 132.56, 125.72, 123.21, 118.79, 111.09, 107.81, 51.34, 36.93, 35.00, 28.44. IR (KBr) 3294, 2942, 2225, 1622, 1512, 1380, 1287, 1184, 1080, 836, 663, 539 cm^{-1}. MS (ESI) *m/z* 322 $[M+H]^+$: HRMS (ESI) calcd. for $C_{18}H_{15}N_3OS$: 321.09634, found: 321.09653.

2-(4-Chlorophenyl)-6,7-dihydrobenzo[*d*]imidazo[2,1-*b*]thiazol-8(5*H*)-one (3e)

Yield: 72%; mp: 237–239°C. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.79–7.68 (m, 2H), 7.65 (s, 1H), 7.56–7.36 (m, 2H), 3.01 (t, *J* = 6.23 Hz, 2H), 2.71 (t, *J* = 6.48 Hz, 2H), 2.37 (qt, *J* = 6.35 Hz, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 191.06, 148.50, 142.58, 133.83, 132.07, 131.90, 128.96, 126.97, 126.68, 106.23, 37.33, 23.25, 22.00. IR (KBr) 3289, 2942, 1659, 1622, 1514, 1353, 1186, 1128, 1005, 837, 727, 539 cm^{-1}. MS (ESI) *m/z* 303 $[M+H]^+$: HRMS (ESI) calcd. for $C_{15}H_{11}ClN_2OS$: 302.02851, found: 302.02806.

2-(4-Methoxyphenyl)-6,7-dihydrobenzo[*d*]imidazo[2,1-*b*]-thiazol-8(5*H*)-one (3f)

Yield: 83%; mp: 230–231°C. ^{1}H NMR ($CDCl_3$ + DMSO, 300 MHz) δ 7.37–7.24 (m, 2H), 6.90–6.81 (m, 2H), 6.71 (s, 1H), 3.57 (s, 3H),

2.83 (t, J = 6.23 Hz, 2H), 2.62 (d, J = 6.48 Hz, 2H), 1.92 (qt, J = 6.35 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$ + DMSO) δ 189.37, 173.65, 168.25, 167.73, 148.02, 138.66, 131.77, 125.76, 118.47, 109.20, 104.58, 36.63, 26.65, 22.29. IR (KBr) 3379, 3113, 2944, 2226, 1640,1540, 1349, 1188, 1042, 838, 665 cm^{-1}. MS (ESI) *m/z* 299 $[M+H]^+$: HRMS (ESI) calcd. for $C_{16}H_{14}N_2O_2S$: 299.94520, found: 299.94518.

2-(4-Methoxyphenyl)-6,6-dimethyl-6,7-dihydrobenzo[*d*]-imidazo[2,1-*b*]thiazol-8(5*H*)-one (3g)

Yield: 77%; mp: 172–174°C. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.80–7.66 (m, 2H), 7.50 (s, 1H), 7.03–6.81 (m, 2H), 3.84 (s, 3H), 2.83 (s, 2H), 2.52 (s, 2H), 1.22 (s, 6H). ^{13}C NMR (75 MHz, $CDCl_3$ + DMSO) δ 178.15, 177.73, 168.28, 158.64, 154.22, 150.96, 146.50, 125.10, 120.87, 119.10, 32.63, 27.88, 21.67, 16.96, 14.42. IR (KBr): 3375, 3124, 2960, 1618, 1508, 1366, 1254, 1053, 951, 838, 526 cm^{-1}. MS (ESI) *m/z* 327.20 $[M+H]^+$: HRMS (ESI) calcd. for $C_{18}H_{19}N_2O_2S$: 327.11685, found: 327.11618.

2-(4-Chlorophenyl)-6,6-dimethyl-6,7-dihydrobenzo[*d*]-imidazo[2,1-*b*]thiazol-8(5*H*)-one (3h)

Yield: 76%; mp: 212–214°C. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.79–7.75 (M, 2H), 7.64 (s, 1H), 7.41–7.37 (m, 2H), 2.88 (s, 2H), 2.58 (s, 2H), 1.25 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 190.75, 151.24, 148.42, 141.15, 133.76, 131.71, 128.95, 126.63, 122.65, 106.18, 51.39, 37.01, 35.01, 28.50. IR (KBr): 3375, 3113, 2925, 1640, 1501, 1366, 1253, 1144, 1053, 949, 585, 522 cm^{-1}. MS (ESI) *m/z* 331 $[M+H]^+$: HRMS (ESI) calcd. for $C_{17}H_{15}ClN_2OS$ $(M+H)^+$: 330.05942, found: 330.05936.

2-(5-Bromothiophen-2-yl)-6,7-dihydrobenzo[*d*]imidazo[2,1-*b*]-thiazol-8(5*H*)-one (3i)

Yield: 73%; mp: 148–151°C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.52 (s, 1H), 7.13 (d, J = 3.91 Hz, 1H), 7.01 (d, J = 3.91 Hz, 1H), 2.99 (t, J = 6.23 Hz, 2H), 2.71 (t, J = 6.48 Hz, 2H), 2.36 (qt, J = 6.35 Hz, H). ^{13}C NMR ($CDCl_3$ + DMSO, 100 MHz): δ 194.43, 150.69, 139.14, 137.83, 136.60, 136.44, 136.27, 128.05, 127.25, 110.89, 35.24, 30.99, 27.49. IR (KBr): 3436, 3286, 3104, 2925, 1630, 1529, 1425, 1331, 1204, 1017, 791, 688 cm^{-1}. MS (ESI): *m/z* 355 $[M+H]^{+2}$; HRMS (ESI): calcd. for $C_{13}H_{14}BrN_2OS_2$ $(M+H)^{+2}$: 358.98898, found: 358.98819.

2-(5-Chlorothiophen-2-yl)-6,7-dihydrobenzo[*d*]imidazo[2,1-*b*]-thiazol-8(5*H*)-one (3j)

Yield: 78%; mp: 202–204°C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.50 (s, 1H), 7.13 (d, J = 3.79 Hz, 1H), 6.87 (d, J = 3.79 Hz, 1H), 2.99 (t, J = 6.23 Hz, 2H), 2.70 (t, J = 6.48 Hz, 2H), 2.36 (qt, J = 6.35 Hz, 2H). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 190.94, 150.86, 143.47, 142.47, 135.29, 129.53, 126.86, 124.01, 122.55, 105.32, 37.26, 23.17, 21.95. IR (KBr): 3134, 2931, 1661, 1577, 1478, 1354, 1325, 1119, 1026, 801, 710, 541 cm^{-1}. MS (ESI): *m/z* 309 $[M+H]^+$; HRMS (ESI) calcd. for $C_{13}H_9ClN_2OS_2$ $(M+H)^+$: 307.98484, found: 307.98448.

2-(5-Bromothiophen-2-yl)-6,6-dimethyl-6,7-dihydrobenzo[*d*] imidazo[2,1-*b*]thiazol-8(5*H*)-one (3k)

Yield: 72%; mp: 171–173°C. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.50 (s, 1H), 7.12 (d, J = 3.96 Hz, 1H), 7.01 (d, J = 3.96 Hz, 1H), 2.85 (s, 2H), 2.57 (s, 2H), 1.24 (s, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 190.24, 149.77, 142.15, 141.53, 138.01, 130.20, 122.86, 121.44, 110.65, 50.70, 36.01, 34.36, 27.76. IR (KBr): 3298, 2957, 1656, 1585, 1477, 1356, 1325, 1245, 1117, 78, 709, 535 cm^{-1}. MS (ESI) *m/z* 382 $[M+H]^{+2}$: HRMS (ESI) calcd. for $C_{15}H_{13}BrN_2OS_2$: 379.96538, found: 379.96538.

4.2 | Biological assays

4.2.1 | Virus inhibition assay

The compounds **3a–k** were dissolved in 0.1 mL DMSO to prepare stock solutions, and final solutions (1000.0–4.0 μM) were prepared by adding MEM with 1 μg/mL trypsin. Compounds were incubated with MDCK cells for 1 h at 36°C. Each concentration of the compounds was tested in triplicate. The cell culture was then infected with influenza virus A/Puerto Rico/8/34 (H1N1) (MOI 0.01) for 24 h at 36°C in the presence of 5% CO_2. A virus titer in the supernatant was determined by hemagglutination test after cultivating of the virus in MDCK cells for 48 h at 36°C in the presence of 5% CO_2. Rimantadine and oseltamivir carboxylate were used as reference drugs. For calculations, virus titer was expressed as per cent of the titer in control wells without compounds. The 50% inhibiting concentrations (IC_{50}) and the selectivity index (SI, the ratio of CC_{50} to IC_{50}) were calculated from the data obtained.

4.2.2 | Cytotoxicity assay

The microtetrazolium test (MTT)[26] was used to study the cytotoxicity of the compounds. Briefly, series of threefold dilutions of each compound in MEM were prepared. MDCK cells were incubated for 48 h at 36°C in 5% CO_2 in the presence of the dissolved substances. The cells were washed twice with phosphate-buffered saline (PBS), and a solution of 3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide (ICN Biochemicals Inc. Aurora, Ohio) (0.5 μg/mL) in PBS was added to the wells. After 1 h incubation, the wells were washed and the formazan residue was dissolved in DMSO (0.1 mL per well). The optical density in the wells was then measured on a Victor^2 1440 multifunctional reader (Perkin Elmer, Finland) at wavelength of 535 nm and plotted against the concentration of compounds. Each concentration was tested in three parallels. The 50% cytotoxic concentration (CC_{50}) of each compound was calculated from the data obtained.

4.2.3 | Neuraminidase activity assay

The study of virus susceptibility to **3j** was conducted by measurement of activity of neuraminidase in the reaction with fluorogenic substrate.[28] For this purpose, serial dilutions of the compounds in

the buffer (32.5 mM MES, pH 6.0, 4 mM $CaCl_2$) were mixed with fivefold dilutions of the virus in the wells of black 96-well plates (Corning). Plates were incubated 30 min at 37°C. Then 0.2 mM substrate solution (4-methylumbelliferyl-α-D-*N*-acetylneuraminic acid) was added to the wells and plates were further incubated for 30 min at room temperature. Reaction was stopped with the stop-solution (25% ethanol, 0.1 M glycine pH 10.7) following by measurement of luminescence on multifunctional plate reader Victor 1440 (Ex λ 365 nm, Em λ 450 nm). Oseltamivir carboxylate was used as reference compound. Based on the data obtained, 50% inhibiting concentrations of **3j** and oseltamivir were calculated.

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CONFLICT OF INTEREST

All authors declare no financial or commercial conflicts of interest.

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