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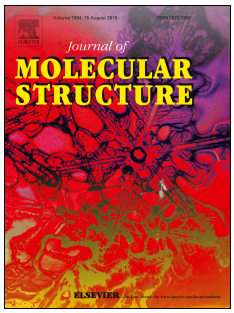

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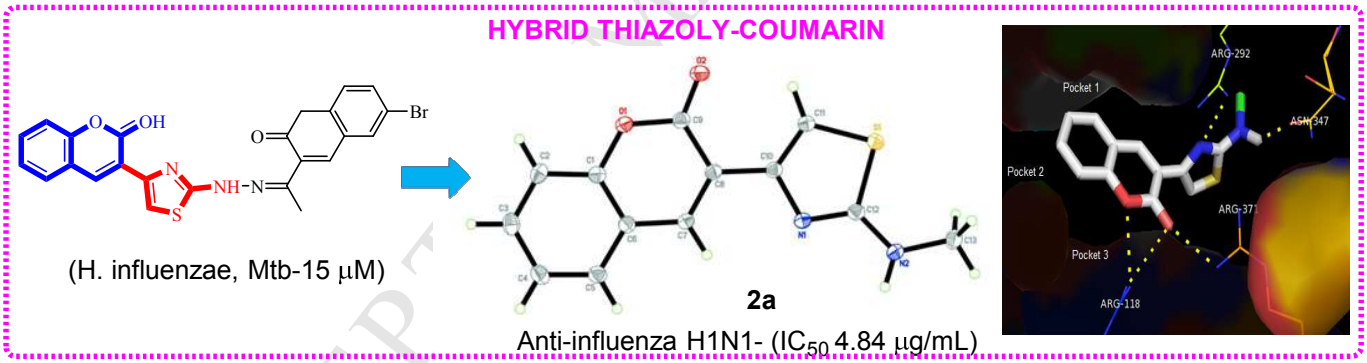

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ABSTRACT

A series of thiazole containing coumarin derivatives (**2a-o**) were efficiently synthesized as pharmacophore hybrids through Hantzsch cyclisation of 3-(2-bromoacetyl)-2*H*-chrome-2-ones with different *N*-substituted thiourea/*N*,*N*-di-substituted thiourea. All the synthesized compounds were evaluated for their potential as antibacterial, anti-tubercular and antiviral agents and some of the compounds displayed considerable potency against these strains. The presence of 2-bromophenylamino (**2h**) and 3,4-dichlorophenylamino (**2g**) group at thiazole moiety resulted the highest antibacterial (MIC 73 μM) and antitubercular (MIC 60 μM) activities, respectively. Antiviral screening displayed that methylamino derivative (**2a)** strongly inhibited the replication of H1N1 influenza A virus (IC_{50} value 4.84). Results of molecular docking studies also suggest that thiazolyl-coumarins could be an important scaffold for the design and development of novel antiviral agents.

Keywords: Thiazole, coumarin, antibacterial, antitubercular, anti-influenza.

1. Introduction

The discovery of antimicrobial agents changed the world of therapeutics by controlling the deadly infectious diseases which were the leading causes of morbidity and mortality [1]. But unfortunately, irrational use of antimicrobial agents and subsequent emergence of resistant microbes has resulted in treatment failures and increased mortality in epidemics like cholera, tuberculosis (TB), respiratory tract infections (RTI), sexually transmitted diseases (STD), and malaria [2]. Recent emergence of methicillin-resistant *Staphylococcus aureus* (MRSA) and carbapenem resistance mediated by New Delhi metallo *β*-lactamase (NDM-1 superbug) are also a serious epidemiological concern [3,4]. The rapid spread of drug resistance TB, *viz.* multiple drug-resistant (MDR) and extensively drug-resistant (XDR) TB is also becoming a foremost apprehension for TB control programs as there is no clinical resort available against these strains [5]. Therefore, development of new antimicrobials is required as newer resistance is always most likely to emerge. Moreover, Influenza or flu is a severe contagious respiratory infection caused by influenza viruses which sometimes results in hospitalization or death [6]. Although, several drugs are available for the treatment of a flu but each seasonal influenza epidemic claims approximately 0.5 million lives globally [7]. Consequently, the recent outbreaks of avian flu (H5N1), the persistent threat of human influenza A (H3N2, H1N1) and B, and rapid emergence of resistant viruses pose a new challenge emphasizing the importance of constant efforts in the development of novel anti-influenza agents [8].

Coumarin moiety is of great importance to chemists as well as biologists as it is found in a large variety of naturally occurring bioactive compounds [9]. A large number of coumarin derivatives were reported as antibacterial [10], antitubercular [11] and antiviral agents [12]. The biological activities of the resulting coumarin derivatives are effectively influenced by its substitution. Furthermore, thiazole moiety is a key pharmacophore for the synthesis of

various chemotherapeutic agents like antimicrobial agents [13]. The practice of incorporating two bioactive moieties into a single molecule through hybrid pharmacophore is a rational approach in a drug design. Thus in our previous studies, we introduced the thiazole moiety into coumarin skeleton, which has led to promising antibacterial and antiviral activities (Fig. 1) [14]. The molecular hybridization of thiazole and coumarin pharmacophores was chosen as a strategy to obtain more active compounds acting through multiple targets. Encouraged by these observations and in continuation of our research program [14,15], it was of interest to further synthesis and explore a novel series of hybrid thiazolyl-coumarin derivatives and preliminary evaluation of antibacterial, antitubercular and antiviral properties of the synthesized compounds.

2. Results and Discussion

2.1. Chemistry

The synthetic pathway leading to the title compounds **2(a-o)** is depicted in Scheme 1. In the initial step substituted-3-acetylcoumarins were brominated in ethanol free chloroform to get intermediates substituted-3-(2-bromoacetyl)-2*H*-chrome-2-ones **1(a-b).** The detailed procedure for the synthesis of compounds **1(a-b)** are presented in supportive information. In the subsequent step synthesis of title compound **2(a-o)** was accomplished by Hantzsch cyclization, which involves the cyclocondensation of **1(a-b)** with *N*-substituted thiourea and *N,N*-disubstituted thiourea. The structures of all the synthesized compounds **2(a-o**) were elucidated by IR, ^{1}H-NMR, ^{13}C-NMR, mass spectroscopy and elemental analysis. The IR spectra of prototype compound **2a** showed characteristic sharp bands at 1604, 1694 and 3362 cm^{-1} which could be assigned to C=N, lactone C=O and NH stretching, respectively. In the ^{1}H NMR spectra of compound **2a** showed an up-fielded singlet peak δ 2.90 due to methyl protons. The aromatic protons were observed as singlet at δ 7.61 and 8.65 due to thiazolyl H-10 and chromene H-4 protons. Two doublets were observed at δ 7.91 (J = 7.5 Hz) and δ 7.49

(*J* = 8.5 Hz) due to H-5 coupled with H-6 and H-8 coupled with H-6 proton, respectively. Two triplets at δ 7.66 (*J* = 8.0) and δ 7.43 (*J* = 8.0 Hz) can be assigned to H-7 proton coupled with H-6 and H-8 and H-6 proton coupled with H-5 and H-7 protons, respectively (otherwise the J4 coupling would be around 1.5 Hz). Moreover, the -NH proton resonated at δ 7.69 (*J* = 4.5 Hz) as doublet due to its coupling with methyl protons. It's worth to mention the absence of an up-field singlet at δ 4.75 due to methylene (-CH_2) protons of 3-(2-bromoacetyl)-2*H*-chrome-2-one (**1a**), also confirms the formation of thiazolyl ring. The ^{13}C NMR spectrum of **2a**, showed signals corresponded to all the 13 carbons in the compound. Signals at δ 143.55, 109.09 and 168.38 could be assigned to C-9, C-10 and C-11 of thiazolyl ring. The methyl carbon C-13 was found to resonate up-field at δ 30.92. Rest of the carbons of the coumarin nucleus were found to resonate in the expected chemical shift regions. The COSY spectrum of **2a** as shown in figure 2, revealed clear correlation between H-5 (δ 7.91) and H-6 (δ 7.43) and a cross correlation of H-6 (δ 7.43) with H-7 (δ 7.66). The proton H-7 (δ 7.66) was also found to correlate with H-8 (δ 7.49). Moreover, NH proton at δ 7.69 was found coupled with methyl protons (δ 2.90), which strongly suggested the formation of thiazolyl ring in the expected compound. The ^{1}H-^{13}C HMQC spectrum of **2a** (Fig. 3), confirmed the connectivity of the protons relative to their respective carbons showing that out of 13 carbons, six were quaternary, six were methines and the rest one was methyl carbons as determined by DEPT 135 and DEPT 90 experiments. The spectrum clearly showed the coupling of H-4 (δ_H 8.65) with C-4 (δ_C 138.22), H-5 (δ_H 7.91) with C-5 (δ_C 128.68), H-6 (δ_H 7.43) with C-6 (δ_C 124.66), H-7 (δ_H 7.66) with C-7 (δ_C 131.42) and H-8 (δ_H 7.49) with C-8 (δ_C 115.78). Direct correlation of H-10 (δ_H 7.61) with C-10 (δ_C 109.09) confirmed the presence of a thiazole ring in the structure. Besides this, correlation for methyl proton H-13 (δ_H 2.90) with C-13 (δ_C 30.92) was also observed. Structure elucidation of **2a** was further substantiated by ^{1}H-^{13}C HMBC spectrum (Fig. 4), in which long range correlations of C to H was observed. All these

possible correlations are shown in Fig. 5. It was clearly found that H-10 (δ_H 7.61) was correlated with C-9 (δ_C 143.55) and C-11 (δ_C 168.38), thus confirming the formation of a thiazole ring. H-4 (δ_H 8.65) was found to correlate with C-3 (δ_C 119.30) and C-9 (δ_C 143.55) which confirmed the attachment of thiazole ring to C-3 (δ_C 119.30) of coumarin core. The presence of coumarin nucleus was confirmed by observing the correlations of H-4 (δ_H 8.65) with C-8a (δ_C 152.20), C-5 (δ_C 128.68) and C-2 (δ_C 158.78). Moreover, H-5 (δ_H 7.91) was found to couple with C-7 (δ_C 131.42), C-4 (δ_C 38.22) and C-8a (δ_C 152.20). Correlation of H-7 (δ_H 7.66) with C-8 (δ_C 115.78) and C-5 (δ_C 128.68) was also observed. H-8 (δ_H 7.49) was found to couple with C-4a (δ_C 120.52), C-6 (δ_C 124.66) and C-8a (δ_C 152.20). Besides this, H-6 (δ_H 7.43) was found to couple with C-8 (δ_C 115.78) and C-4a (δ_C 120.52). In addition, correlation between methyl protons H-13 (δ_H 2.90) and C-11 (δ_C 138.38) was also observed. In addition, the structure and the stereochemistry of the thiazolyl-coumarins were confirmed by the single crystal X-ray crystallographic analysis of **2a**. Fig. 6 shows the ORTEP diagram and crystal packing respectively, for compound **2a** [16]. A computational study for prediction of Lipinski's parameters (≥ 1 violation) along with other physicochemical properties of all the molecules are presented in Table 1.

2.2. Biological Activities

2.2.1. Antibacterial Activity

Taking our previously reported compound hydrazinyl thiazolyl-coumarin (Minimum inhibitory concentration; MIC 15 μM) as the lead compound, we designed mono and di substituted thiazolyl-coumarin analogues as new antimicrobial agents. Substitution were focused mainly on the thiazole and coumarin nucleus in order to explore the structure activity relationship (SAR) of hybrid analogues (Fig. 1). All the synthesized compounds were evaluated for *in vitro* anti-bacterial activity against two Gram-positive bacteria (*S. pneumonia* and *S. aureus*) and three Gram negative bacteria (*E. coli*, *E. aerogenes* and *S.typhi*) using

broth microdilution method [17] and compared with reference drugs streptomycin, kanamycin, and vancomycin (Table 2). Intermediate, 3-(2-bromoacetyl)-2*H*-chrome-2-one (BAC) showed MIC of 560 μM against both Gram-positive and Gram-negative bacteria compared with standard drug streptomycin having MIC of in the range of 54-107 μM (Table 2). The results of antimicrobial activities showed that cyclisation of BAC into its thiazolyl derivatives resulted in enhanced antibacterial activities against the tested bacterial strains ranging in between 49-293 μM. Among the fifteen hybrid derivatives, four compounds **(2d, 2h, 2k,** and **2m)** showed promising antibacterial activity. The bromophenyl derivatives **2d** (MIC 79 μM) and **2h** (MIC 73 μM) displayed highly significant inhibition against all the pathogens with MIC values comparatively less than vancomycin, MIC 86-176 μM (except against Gram-positive bacteria). Further, di-substituted *N*,*N*-diphenyl derivative **2l** showed extremely low activity (MIC 158-316 μM) whereas, replacement with lesser sterically bulky alkyl groups led to compounds retaining the antimicrobial activity **2k** (MIC 115-230 μM) and **2m** (MIC 49-98 μM). From the results it is evident that mono-substituted thiazoles were comparatively more effective than the di-substituted thiazoles. Among the previous, compounds containing lipophilic electron withdrawing bromo group at phenyl ring showed promising antibacterial activity and substitution of electron releasing (-OCH_3) group at the coumarin nucleus slightly increases activity.

2.2.2. Anti-tubercular Activity

All the thiazolyl-coumarin derivatives **2(a-o)** were evaluated for their anti-TB potential against the well characterized virulent strain of *Mycobacterium tuberculosis*, H37Rv ATCC 25618 by BTG (BacTiter-Glo™) Microbial Cell Viability assay and compared with standard drug isoniazid (Table 2) [18,19]. Although, anti-tubercular activity of thiazolyl-coumarin compounds are not comparable with the standard drug isoniazid (MIC 1.3 μM) but among the fifteen compounds tested ten compounds showed moderate activity with MIC in the range of

60-194 μM. The highest activity was shown by compound **2g,** having 2,4-dichlorophenyl as substituent with MIC of 60 μM. Interestingly, the *N*,*N*-disubstituted derivatives (**2j, 2l, 2m, 2n** and **2o)** did not show any inhibition except compound **2k.** This might be as a consequence of a steric hindrance due to two bulky substituents and compound **2k** showed activity because of its less steric hindrance due to smaller methyl group.

2.2.3. Antiviral Activity

Anti-influenza activities of the thiazolyl-coumarins were evaluated using Madin−Darby canine kidney (MDCK) cell-based CPE (cytopathic effect protection) assays as described in supplementary material [20]. The antiviral activity data, as summarized in Table 3, revealed the moderate efficacies of thiazolyl-coumarins against the both strains (IC_{50} values 4.84 to >50 μg/mL) compared to the standard drug amantadine (2.76 and 50 μg/mL against the H3N2 and H1N1, respectively). The results of this study with regard to anti-influenza activity have been found to be remarkable against H1N1 strain. Compounds **2a**, **2c**, **2g** and **2k** seems to be promising agents having IC_{50} values 4.84, 19.72, 6.12 and 9.13 μg/mL, respectively against the H1N1 virus, of which **2a** being the most potent compound. Moreover, among these four compounds, only 2,4-dichlorophenyl derivative **2c** (IC_{50} 25.76 μg/mL) moderately inhibited the replication of H3N2 influenza A virus strains.

2.3. Molecular docking study

To gain further evidence regarding the antiviral effect of hybrid thiazolyl-coumarin analogues,

molecular docking study was carried out on neuraminidase (NA) enzyme (PDB ID: 3B7E) of H1N1 influenza A virus strain [21] using AutoDock program. The enzyme neuraminidase (NA) is an attractive target for antiviral strategy because of its essential role in the pathogenicity of many respiratory viruses. The binding energy, predicted inhibition constant, steric interactions along with hydrogen bonding with neuraminidase (NA) enzyme are used to

predict the binding affinities. The binding energy of compounds **2a-o** were in the range of -2.80 to -5.66 kcal/mol of which, the lowest docked energy was observed for compound **2a**. This study reveals that the presence of the aliphatic methyl group was important for neuraminidase inhibition. However, among the aromatic substituted ring, electron withdrawing groups (Cl, Br) enhanced inhibitory activity as compared to electron releasing group (OCH_3). Thus, the results of *in silico* studies were found in good agreement with the *in vitro* antiviral results (Table 3). The best binding modes of zanamivir, a neuraminidase inhibitor and compound **2a** in the active site of NA are presented in Figs. 7 and 8. The selectivity of zanamivir has been shown to result from the carboxylate group of sialic acid, which makes strong charge-charge interactions with a cluster of positively charged side chains of the Arg triad *viz*. Arg 118, 292 and 371 [22]. In contrast, compound **2a** lacks carboxylate group but forms four hydrogen bonds to the enzyme through its lactone group of coumarin and tertiary nitrogen of thiazole ring with the Arg triads *i.e.* at the same residue where the carboxylate moiety of the antiviral drug zanamivir binds [25]. Furthermore, the NH group of **2a** forms a hydrogen bond with ASN 347. Lipophilicity also appears to play crucial role in **2a** inhibitory activity, as chromene group is properly oriented to the more hydrophobic pocket formed by Ala-246, Ile-222 and an Arg-224. The binding energy of compound **2a** was found to be -5.66 and the inhibition constant (K_i) 70.4 µM. Therefore, the antiviral effect of compound **2a** might be due to inhibition of enzyme NA.

In summary, we have synthesized fifteen hybrid thiazolyl-coumarin analogs as antimicrobial agents. Most of the compounds had promising antibacterial, anti-tubercular and antiviral activities. In particular, mono-substituted thiazole derivatives containing phenyl ring with electron withdrawing groups were potent antimicrobial agents as compared to their corresponding di-substituted thiazole derivatives. Methoxy substitution at the coumarin nucleus led to slight enhance in antimicrobial potential. Molecular docking results also

suggested that anti-influenza activity might be due to neuraminidase inhibition. This study provides new thiazolyl-coumarin hybrids as potential lead molecules for further structural optimisation as anti-viral, anti-Tb and anti-bacterial agents.

3. EXPERIMENTAL

3.1. General procedure for the synthesis of thiazolyl coumarin derivatives (2a-o)

An equimolar mixture of 3-bromoacetyl coumarin (5.0 mmol) and various *N*-substituted as well as *N*,*N*-disubstituted thiourea (5.0 mmol) in $CHCl_3$/EtOH (2:1, v/v) was refluxed for 3 h. After completion of reaction, the clear solution formed was slowly evaporated under negative pressure to get a whitish crystalline solid. The obtained products were collected, dried and recrystallized from ethanol to afford pure compounds as whitish solid in good to moderate yields.

3.1.1. 3-(2-Methylamino-1, 3-thiazol-4-yl)-2H-chromen-2-one (2a)

White solid, (0.92 g, 71.3%), mp 192–194 °C. IR KBr (ν_{max}/cm^{-1}): 3362.50 (N-H), 2971.66 (C-H aliphatic, asymmetric, sp^3), 2881.90 (C-H aliphatic, symmetric, sp^3), 1694.40 (C=O lactone), 1604.95 (C=N); ^{1}H NMR (δ/ppm, 500 MHz, DMSO-d_6): 8.65 (1H, s, H-4), 7.91 (1H, d, *J* = 7.5 Hz, H-5), 7.69 (1H, d, *J* = 4.5, N-H), 7.66 (1H, t, *J* = 8.0 Hz , H-7), 7.61 (1H, s, H-10), 7.49 (1H, d, *J* = 8.5, H-8), 7.43 (1H, t, *J* = 8.0 Hz, H-6), 2.90 (3H, s, CH_3); ^{13}C NMR (δ/ppm, 125 MHz, DMSO-d_6): 168.38 (C-11), 158.78 (C-2), 152.20 (C-8a), 143.55 (C-9), 138.22 (C-4), 131.42 (C-7), 128.68 (C-5), 124.66 (C-6), 120.52 (C-4a), 119.30 (C-3), 115.78 (C-8), 109.09 (C-10), 30.92 (CH_3). Anal. Calcd. For $C_{13}H_{10}O_2N_2S$ (258.30 $gmol^{-1}$): C, 60.45; H, 3.90; N, 10.85 %. Found: C, 60.40; H, 3.86; N, 10.80 %. MS (+ESI) (m/z): 259.0532 (258.0463).

3.1.2. 3-(2-Ethylamino-1,3-thiazol-4-yl)-2H-chromen-2-one (2b)

White solid, (0.93 g, 68.3%), mp 210–221 °C. IR KBr (ν_{max}/cm^{-1}): 3362.50 (N-H), 1894.40 (C=O lactone), 1607.55 (C=N); ^{1}H NMR (δ/ppm, 500 MHz, $CDCl_3$): 8.54 (1H, s, H-4), 7.89

(1H, s, H-10), 7.58 (1H, dd, *J* = 8.0, 1.0 Hz, H-5), 7.50 (1H, ddd, *J* = 8.5, 1.0, 1.5 Hz , H-7), 7.34 (1H, d, *J* = 8.0, H-8), 7.28 (1H, t, *J* = 8.5 Hz, H-6), 5.29 (1H, s, N-H), 3.36-3.41 (2H, m, CH_2), 1.27 (3H, t, *J* = 7.0 Hz, CH_3); ^{13}C NMR (δ/ppm, 125 MHz, $CDCl_3$): 168.48 (C-11), 159.68 (C-2), 152.84 (C-8a), 143.78 (C-9), 138.59 (C-4), 131.16 (C-7), 128.23 (C-5), 124.50 (C-6), 120.90 (C-4a), 119.70 (C-3), 116.29 (C-8), 109.12 (C-10), 40.84 (CH_2), 14.75 (CH_3). Anal. Calcd. For $C_{14}H_{12}O_2N_2S$ (272.32 gmol^{-1}): C, 61.75; H, 4.44; N, 10.29 %. Found: C, 61.80; H, 4.40; N, 10.33 %. MS (+ESI) (m/z): 273.0685 (272.0162).

3.1.3. 3-(2-(3, 4-Dichlorophenylamino)-1, 3-thiazol-4-yl)-2H-chromen-2-one (2c)

White solid, (1.23 g, 63.0%), mp 226–228 °C. IR KBr (ν_{max}/cm^{-1}): 3285.23 (N-H), 1694.14 (C=O lactone), 1606.67 (C=N); ^{1}H NMR (δ/ppm, 500 MHz, DMSO-d_6): 10.64 (1H, s, N-H), 8.61 (1H, s, H-4), 8.01 (1H, d, *J* = 2.5, H-14), 7.84 (1H, dd, *J* = 8.0, 1.0 Hz, H-17), 7.82 (1H, s, H-10), 7.75 (1H, dd, *J* = 8.5, 3.0 Hz, H-5), 7.63 (1H, ddd, *J* = 9.5, 9.0, 2.0 Hz , H-7), 7.57 (1H, d, *J* = 9.0, H-18), 7.43 (1H, d, *J* = 8.0 Hz, H-8), 7.41 (1H, t, *J* = 7.0 Hz, H-6); ^{13}C NMR (δ/ppm, 125 MHz, DMSO-d_6): 161.85 (C-11), 158.73 (C-2), 152.33 (C-8a), 143.52 (C-4), 140.84 (C-9), 131.71 (C-13), 131.80 (C-14), 131.14 (C-16), 130.93 (C-18), 128.83 (C-7), 124.79 (C-15), 122.44 (C-3), 120.20 (C-17), 119.14 (C-5), 118.12 (C-6), 117.23 (C-4a), 115.90 (C-8), 110.81 (C-10). Anal. Calcd. For $C_{18}H_{10}O_2N_2Cl_2S$ (389.25 gmol^{-1}): C, 55.54; H, 2.59; N, 7.20 %. Found: C, 55.50; H, 2.36; N, 7.17 %. MS (+ESI) (m/z): 388.1041 (387.9840).

3.1.4. 3-(2-(2-Bromophenylamino)-1,3-thiazol-4-yl)-2H-chromen-2-one (2d)

White solid, (1.36 g, 68.0%), mp 119–201 °C. IR KBr (ν_{max}/cm^{-1}): 3381.85 (N-H), 1713.85 (C=O lactone), 1605.97 (C=N); ^{1}H NMR (δ/ppm, 500 MHz, DMSO-d_6): 10.60 (1H, s, N-H), 8.59 (1H, s, H-4), 8.25 (1H, d, *J* = 8.0 Hz, H-14), 7.89 (1H, dd, *J* = 7.5 Hz, H-5), 7.80 (1H, s, H-10), 7.69 (1H, dd, *J* = 7.5, 1.5 Hz, H-17), 7.63 (1H, ddd, *J* = 7.0, 7.0, 1.0 Hz, H-7), 7.49 (1H, d, *J* = 8.0, H-8), 7.46 (1H, t, *J* = 8.5 Hz, H-8), 7.39 (1H, t, *J* = 7.0 Hz, H-6), 7.09 (1H, td,

J = 8.0, 2.0 Hz, H-15); ^{13}C NMR (δ/ppm, 125 MHz, DMSO-d_6): 163.91 (C-11), 158.76 (C-2), 152.30 (C-8a), 143.25 (C-4), 138.80 (C-9), 138.51 (C-13), 133.02 (C-14), 131.64 (C-16), 128.89 (C-18), 128.61 (C-7), 124.98 (C-3), 124.67 (C-15), 123.00 (C-17), 120.31 (C-5), 119.19 (C-4a), 115.83 (C-6), 114.49 (C-8), 111.06 (C-10). Anal. Calcd. For $C_{18}H_{11}O_2N_2SBr$ (399.26 gmol^{-1}): C, 54.15; H, 2.78; N, 7.02 %. Found: C, 54.20; H, 2.83; N, 6.98 %. MS (+ESI) (m/z): 398.2685 (397.9724).

3.1.5. 8-Methoxy- 3-(2-methylamino-1, 3-thiazol-4-yl)-2H-chromen-2-one (2e)

White solid, (1.0 g, 69.4%), mp 234–236 °C. IR KBr (ν_{max}/cm^{-1}): 3159.97 (N-H), 1725.48 (C=O lactone), 1630.94 (C=N); ^{1}H NMR (δ/ppm, 500 MHz, DMSO-d_6): 8.56 (1H, s, H-4), 7.72 (1H, s, N-H), 7.57 (1H, s, H-10), 7.41 (1H, d, *J* = 7.5 Hz, H-5), 7.39 (1H, d, *J* = 8.0 Hz , H-7), 7.31 (1H, t, *J* = 8.0 Hz, H-6), 3.94 (3H, s, OCH_3), 2.94 (3H, s, CH_3); ^{13}C NMR (δ/ppm, 125 MHz, DMSO-d_6): 168.53 (C-11), 160.20 (C-2), 159.13 (C-8a), 146.15 (C-9), 143.11 (C-4), 141.36 (C-7), 138.59 (C-5), 124.75 (C-6), 120.37 (C-4a), 119.84 (C-3), 113.72 (C-8), 108.23 (C-10), 56.01 (OCH_3), 30.94 (CH_3). Anal. Calcd. For $C_{14}H_{12}O_3N_2S$ (288.32 gmol^{-1}): C, 58.32; H, 4.19; N, 9.72 %. Found: C, 58.36; H, 4.23; N, 9.77 %. MS (+ESI) (m/z): 289.0659 (288.0568).

3.1.6. 8-Methoxy- 3-(2-ethylamino-1, 3-thiazol-4-yl)-2H-chromen-2-one (2f)

White solid, (1.05 g, 69.5%), mp 231–233 °C. IR KBr (ν_{max}/cm^{-1}): 3245.10 (N-H), 1727.55 (C=O lactone), 1634.56 (C=N); ^{1}H NMR (δ/ppm, 500 MHz, $CDCl_3$): 9.23 (1H, s, N-H), 8.85 (1H, s, H-4), 7.83 (1H, s, H-10), 7.39 (1H, dd, *J* = 8.0, 1.5 Hz, H-5), 7.31 (1H, t, *J* = 8.0 Hz, H-6), 7.319 (1H, dd, *J* = 8.0, 1.0 Hz, H-7), 4.13 (3H, s, OCH_3), 3.44-3.49 (2H, m, CH_2), 1.49 (3H, t, J = 7.5 Hz, CH_3); ^{13}C NMR (δ/ppm, 125 MHz, $CDCl_3$): 169.83 (C-11), 157.58 (C-2), 146.86 (C-8a), 142.68 (C-9), 141.10 (C-4), 134.07 (C-7), 125.22 (C-5), 121.08 (C-6), 118.89 (C-4a), 115.15 (C-8), 114.49 (C-3), 106.05 (C-10), 56.36 (OCH_3), 42.98 (CH_2), 13.59 (CH_3).

Anal. Calcd. For $C_{15}H_{14}O_3N_2S$ (302.35 $gmol^{-1}$): C, 59.59; H, 4.67; N, 9.27 %. Found: C, 59.63; H, 4.71; N, 9.31 %. MS (+ESI) (m/z): 303.0793 (302.0752).

3.1.7. 8-Methoxy-3-(2-(3,4-dichlorophenylamino)-1,3-thiazol-4-yl)-2H-chromen-2-one (2g)

White solid, (1.42 g, 67.6%), mp 223–225 °C. IR KBr (ν_{max}/cm^{-1}): 3281.59 (N-H), 1708.76 (C=O lactone), 1604.74 (C=N); ^{1}H NMR (δ/ppm, 500 MHz, DMSO-d_6): 10.63 (1H, s, N-H), 8.54 (1H, s, H-4), 8.03 (1H, d, *J* = 2.5, H-14), 7.83 (1H, s, H-10), 7.73 (1H, dd, *J* = 9.0, 2.5 Hz, H-5), 7.57 (1H, d, *J* = 9.0 Hz, H-18), 7.37 (1H, dd, *J* = 7.5, 2.0 Hz , H-6), 7.34 (1H, d, *J* = 8.0, H-7), 7.31 (1H, dd, *J* = 8.0, 1.5 Hz, H-17), 3.93 (3H, s, OCH_3); ^{13}C NMR (δ/ppm, 125 MHz, DMSO-d_6): 161.79 (C-11), 158.40 (C-2), 146.26 (C-8a), 143.45 (C-4), 141.61 (C-9), 140.81 (C-13), 138.79 (C-14), 131.11 (C-16), 130.83 (C-18), 124.65 (C-7), 122.39 (C-15), 120.29 (C-3), 119.92 (C-17), 119.68 (C-5), 118.10 (C-6), 117.17 (C-8), 113.95 (C-4a), 110.79 (C-10), 56.05 (OCH_3). Anal. Calcd. For $C_{19}H_{12}O_3N_2Cl_2S$ (419.28 $gmol^{-1}$): C, 54.43; H, 2.88; N, 6.68 %. Found: C, 54.38; H, 2.83; N, 6.64 %. MS (+ESI) (m/z): 419.0029 (417.0045).

3.1.8. 8-Methoxy 3-(2-(2-bromophenylamino)-1,3-thiazol-4-yl)-2H-chromen-2-one (2h)

White solid, (1.51 g, 70.2%), mp 197–119 °C. IR KBr (ν_{max}/cm^{-1}): 3139.34 (N-H), 1727.50 (C=O lactone), 1591.67 (C=N); ^{1}H NMR (δ/ppm, 500 MHz, DMSO-d_6): 9.62 (1H, s, N-H), 8.54 (1H, s, H-4), 8.25 (1H, dd, *J* = 8.0, 1.5 Hz, H-14), 7.80 (1H, s, H-10), 7.69 (1H, dd, *J* = 8.0, 1.5 Hz, H-17), 7.48 (1H, td, *J* = 7.5, 1.5 Hz, H-16), 7.43 (1H, dd, *J* = 6.0, 3.5 Hz, H-6), 7.32 (1H, d, *J* = 7.5, H-7), 7.31 (1H, d, *J* = 7.5, 1.8 Hz, H-5), 7.08 (1H, td, *J* = 8.0, 1.5 Hz, H-15), 3.96 (3H, s, OCH_3); ^{13}C NMR (δ/ppm, 125 MHz, DMSO-d_6): 163.93 (C-11), 158.48 (C-2), 146.16 (C-8a), 143.17 (C-4), 141.58 (C-9), 138.78 (C-13), 138.68 (C-14), 131.01 (C-16), 128.62 (C-18), 124.99 (C-7), 124.60 (C-5), 122.98 (C-17), 120.41 (C-3), 120.07 (C-8), 119.75 (C-4a), 114.47 (C-5), 113.90 (C-6), 111.14 (C-10), 56.08 (OCH_3). Anal. Calcd. For

$C_{19}H_{13}O_3N_2SBr$ (427.29 $gmol^{-1}$): C, 53.16; H, 3.05; N, 6.53 %. Found: C, 53.20; H, 3.0; N, 6.51 %. MS (+ESI) (m/z): 428.9877 (427.9830).

3.1.9. 8-Methoxy 3-(2-(2-methoxyphenylamino)-1,3-thiazol-4-yl)-2H-chromen-2-one (2i)

White solid, (1.36 g, 71.6%), mp 247–249 °C. IR KBr (ν_{max}/cm^{-1}): 3381.63 (N-H), 1724.34 (C=O lactone), 1603.32 (C=N); ^{1}H NMR (δ/ppm, 500 MHz, DMSO-d_6): 9.67 (1H, s, N-H), 8.62 (1H, s, H-4), 8.50 (1H, d, *J* = 7.5 Hz, H-14), 7.77 (1H, s, H-10), 7.48 (1H, dd, *J* = 7.5, 2.0 Hz, H-17), 7.30-7.35 (2H, m, H-5 & H-7), 7.04-7.08 (3H, m, H-6, H-15 & H-14), 3.92 (3H, s, OCH_3) 3.89 (3H, s, Ar-OCH_3),; ^{13}C NMR (δ/ppm, 125 MHz, DMSO-d_6): 163.22 (C-11), 158.52 (C-2), 148.21 (C-8a), 146.24 (C-4), 143.14 (C-9), 141.55 (C-14), 138.47 (C-13), 129.97 (C-16), 124.56 (C-18), 122.27 (C-7), 120.87 (C-15), 120.51 (C-17), 120.09 (C-3), 119.84 (C-8), 118.56 (C-4a), 113.81 (C-5), 110.98 (C-6), 110.60 (C-10), 56.07 (OCH_3), 55.70 (Ar-OCH_3). Anal. Calcd. For $C_{20}H_{16}O_4N_2S$ (380.42 $gmol^{-1}$): C, 63.14; H, 4.24; N, 7.36 %. Found: C, 63.10; H, 4.20; N, 7.32 %. MS (+ESI) (m/z): 381.0901 (380.0830).

3.1.10. 3-(3-Ethyl-2-(ethylimino)-2,3-dihydrothiazol-4-yl)-2H-chromen-2-one (2j)

White solid, (1.02 g, 68.0%), mp 243–245 °C. IR KBr (ν_{max}/cm^{-1}): 1733.18 (C=O lactone), 1613.42 (C=N); ^{1}H NMR (δ/ppm, 500 MHz, DMSO-d_6): 8.45 (1H, s, H-4), 7.86 (1H, d, *J* = 7.5 Hz, H-5), 7.76 (1H, t, *J* = 8.5 Hz, H-7), 7.54 (1H, d, *J* = 8.0 Hz , H-8), 7.48 (1H, t, *J* = 8.5, H-6), 7.32 (1H, s, H-10), 4.05 (2H, q, *J* = 7.0 Hz, ,N$C\underline{H}_2$), 3.47 (2H, q, *J* = 7.0 Hz, =N$C\underline{H}_2$), 1.34 (3H, t, *J* = 7.0 Hz, $NCH_2C\underline{H}_3$), 1.18 (3H, t, *J* = 7.0 Hz, $=NCH_2C\underline{H}_3$); ^{13}C NMR (δ/ppm, 125 MHz, DMSO-d_6): 167.13 (C-11), 159.11 (C-2), 154.00 (C-8a), 147.84 (C-4), 135.99 (C-9), 133.59 (C-7), 129.45 (C-5), 125.04 (C-6), 118.43 (C-4a), 116.50 (C-3), 116.42 (C-8), 108.43 (C-10), 42.83 ($N\underline{C}H_2$), 42.19 ($=N\underline{C}H_2$), 13.00 ($=NCH_2\underline{C}H_3$), 12.71 ($=NCH_2\underline{C}H_3$). Anal. Calcd. For $C_{16}H_{16}O_2N_2S$ (300.37 $gmol^{-1}$): C, 63.98; H, 5.37; N, 9.33 %. Found: C, 64.03; H, 5.33; N, 9.30 %. MS (+ESI) (m/z): 301.1030 (300.0932).

3.1.11. 3-(3-Methyl-2-(methylimino)-2,3-dihydrothiazol-4-yl)-2H-chromen-2-one (2k)

White solid, (0.92 g, 67.6%), mp 196–198 °C. IR KBr (ν_{max}/cm^{-1}): 1712.83 (C=O lactone), 1615.21 (C=N); ^{1}H NMR (δ/ppm, 500 MHz, DMSO-d_6): 8.75 (1H, s, H-4), 8.00 (1H, dd, *J* = 8.0 2.0 Hz, H-5), 7.78 (1H, t, *J* = 9.0 Hz, H-7), 7.50 (1H, d, *J* = 8.5 Hz , H-8), 7.45 (1H, t, J = 7.5 Hz, H-6), 7.54 (1H, s, H-10), 3.55 (3H, s, NC$\underline{H}_3$), 1.17 (3H, s, =NC$\underline{H}_3$); ^{13}C NMR (δ/ppm, 125 MHz, DMSO-d_6): 164.28 (C-11), 158.31 (C-2), 154.47 (C-8a), 148.41 (C-9), 147.33 (C-4), 134.65 (C-7), 131.03 (C-5), 125.03 (C-6), 123.07 (C-4a), 118.08 (C-3), 116.14 (C-8), 108.10 (C-10), 65.95 (N$\underline{C}$H$_3$), 15.07 (=N$\underline{C}$H$_3$). Anal. Calcd. For $C_{14}H_{12}O_2N_2S$ (272.32): C, 61.75; H, 4.44; N, 10.29 %. Found: C, 61.70; H, 4.48; N, 10.25 %. MS (+ESI) (m/z): 273.0697 (272.0619).

3.1.12. 3-(3-Phenyl-2-(phenylimino)-2,3-dihydrothiazol-4-yl)-2H-chromen-2-one (2l)

White solid, (1.39 g, 70.2%), mp 216–218 °C. IR KBr (ν_{max}/cm^{-1}): 1718.40 (C=O lactone), 1605.97 (C=N); ^{1}H NMR (δ/ppm, 500 MHz, DMSO-d_6): 8.29 (1H, s, H-4), 7.76 (1H, dd, *J* = 8.0, 1.5 Hz, H-5), 7.66 (1H, ddd, *J* = 8.5, 7.5, 1.5 Hz, H-7), 7.59 (2H, dd, *J* = 7.5, 3.0 Hz, H-6 & H-8), 7.47-7.52 (5H, m, NC$_6$H$_5$), 7.34-7.41 (5H, m, =NC$_6$H$_5$), 7.23 (1H, s, H-10); ^{13}C NMR (δ/ppm, 125 MHz, DMSO-d_6): 157.82 (C-11), 157.28 (C-2), 156.14 (C-8a), 150.20 (C-9), 147.38 (C-4), 148.24 (C-13), 136.96 (C-12), 133.59 (C-15), 132.76 (C-17), 129.90 (C-16), 129.75 (C-19), 129.24 (C-14), 128.91 (C-23), 128.76 (C-20), 128.55 (C-21), 128.45 (C-22), 125.22 (C-18), 123.83 (C-3), 120.78 (C-7), 117.76 (C-8), 117.12 (C-4a), 116.40 (C-5), 116.22 (C-6), 109.21 (C-10). Anal. Calcd. For $C_{24}H_{16}O_2N_2S$ (396.46 gmol^{-1}): C, 72.71; H, 4.07; N, 7.07 %. Found: C, 72.77; H, 4.02; N, 7.03 %. MS (+ESI) (m/z): 397.1043 (396.0932).

3.1.13. 3-(3-Ethyl-2-(ethylimino)-2,3-dihydrothiazol-4-yl)-8-methoxy-2H-chromen-2-one (2m)

White solid, (1.05 g, 66.0%), mp 222–224 °C. IR KBr (ν_{max}/cm^{-1}): 1738.47 (C=O lactone), 1622.37 (C=N); ^{1}H NMR (δ/ppm, 500 MHz, DMSO-d_6): 8.45 (1H, s, H-4), 7.86 (1H, d, *J* =

7.5 Hz, H-5), 7.76 (1H, t, *J* = 8.5 Hz, H-7), 7.48 (1H, t, *J* = 8.5, H-6), 7.30 (1H, s, H-10), 4.05 (2H, q, *J* = 7.0 Hz, NC$\underline{H}_2$), 3.47 (2H, q, *J* = 7.0 Hz, =NC$\underline{H}_2$), 1.60 (3H, s, OCH_3), 1.34 (3H, t, *J* = 7.0 Hz, $NCH_2C\underline{H}_3$), 1.18 (3H, t, *J* = 7.0 Hz, $=NCH_2C\underline{H}_3$); ^{13}C NMR (δ/ppm, 125 MHz, DMSO-d_6): 165.91 (C-11), 159.11 (C-2), 154.00 (C-8a), 147.84 (C-4), 135.99 (C-9), 133.59 (C-7), 129.43 (C-5), 125.04 (C-6), 118.43 (C-4a), 116.50 (C-3), 116.42 (C-8), 110.28 (C-10), 42.83 ($N\underline{C}H_2$), 42.19 ($=N\underline{C}H_2$), 14.01 (OCH_3), 13.00 ($NCH_2\underline{C}H_3$), 12.71 ($=NCH_2\underline{C}H_3$). Anal. Calcd. For $C_{17}H_{18}O_3N_2S$ (318.39 gmol^{-1}): C, 60.36; H, 5.70; N, 8.80 %. Found: C, 60.46; H, 5.74; N, 8.85 %. MS (+ESI) (m/z): 311.1120 (330.1038).

3.1.14. 8-Methoxy-3-(3-methyl-2-(methylimino)-2,3-dihydrothiazol-4-yl-2H-chromen-2-one (2n)

White solid, (1.05 g, 69.5%), mp 195–197 °C. IR KBr (ν_{max}/cm^{-1}): 1698.76 (C=O lactone), 1623.19 (C=N); ^{1}H NMR (δ/ppm, 500 MHz, DMSO-d_6): 8.73 (1H, s, H-4), 8.01 (1H, dd, *J* = 8.0 2.0 Hz, H-5), 7.76 (1H, t, *J* = 9.0 Hz, H-7), 7.48 (1H, d, *J* = 8.5 Hz , H-8), 7.43 (1H, t, *J* = 7.5 Hz, H-6), 7.73 (1H, s, H-10), 3.71 (3H, s, OCH_3), 3.53 (3H, s, $NC\underline{H}_3$), 1.15 (3H, s, $=NC\underline{H}_3$); ^{13}C NMR (δ/ppm, 125 MHz, DMSO-d_6): 164.29 (C-11), 158.38 (C-2), 153.37 (C-8a), 148.40 (C-9), 146.03 (C-4), 134.65 (C-7), 131.22 (C-5), 125.23 (C-6), 123.80 (C-4a), 118.67 (C-3), 115.19 (C-8), 109.67 (C-10), 66.20 (C-14), 63.45 ($N\underline{C}H_3$), 14.30 (= $N\underline{C}H_3$). Anal. Calcd. For $C_{15}H_{14}O_3N_2S$ (302.35 gmol^{-1}): C, 59.59; H, 4.67; N, 9.27 %. Found: C, 60.03; H, 4.63; N, 9.23 %. MS (+ESI) (m/z): 303.0734 (302.0725).

3.1.15. 8-Methoxy-3-(3-phenyl-2-(phenylimino)-2,3-dihydrothiazol-4-yl)-2H-chromen-2-one (2o)

White solid, (1.46 g, 68.5%), mp 242–244 °C. IR KBr (ν_{max}/cm^{-1}): 1723.69 (C=O lactone), 1691.81 (C=N); ^{1}H NMR (δ/ppm, 500 MHz, DMSO-d_6): 8.29 (1H, s, H-4), 7.76 (1H, dd, *J* = 8.0, 1.5 Hz, H-5), 7.66 (1H, dd, *J* = 8.5, 1.5 Hz, H-7), 7.59 (1H, dd, *J* = 7.5, 3.0 Hz, H-6), 7.47-7.52 (5H, m, NC_6H_5), 7.34-7.41 (5H, m, $=NC_6\underline{H}_5$), 7.22 (1H, s, H-10), 1.60 (3H, s,

OCH_3); ^{13}C NMR (δ/ppm, 125 MHz, DMSO-d_6): 157.63 (C-11), 157.26 (C-2), 157.14 (C-8a), 146.38 (C-4), 150.22 (C-9), 148.25 (C-13), 136.36 (C-12), 135.55 (C-15), 134.67 (C-17), 129.90 (C-16), 129.67 (C-19), 129.42 (C-14), 129.24 (C-23), 129.10 (C-20), 129.07 (C-21), 129.03 (C-22), 127.22 (C-18), 125.03 (C-3), 120.87 (C-7), 119.700 (C-8), 119.12 (C-4a), 118.11 (C-5), 117.28 (C-6), 108.45 (C-10), 14.01 (OCH_3). Anal. Calcd. For $C_{25}H_{18}O_3N_2S$ (426.49): C, 70.40; H, 4.25; N, 6.57 %. Found: C, 70.44; H, 4.29; N, 6.62 %. MS (+ESI) (m/z): 427.1021 (426.1068).

3.2. Biological Activities

3.2.1. In vitro Evaluation of Antibacterial Activity

All the pure compounds were screened for *in vitro* anti-bacterial activity against two Gram-positive bacteria (*S. pneumoniae* and *S. aureus*) and three Gram-negative bacteria (*E. coli*, *E. aerogenes* and *S. typhi*) and the MIC was determined in μM by broth microdilution method [17]. The test organisms were freshly grown and incubated for 48 h at 37 °C. The 2-day old bacterial cultures were emulsified in small quantity of Muller Hinton broth (MHB) and incubated again at 37 °C overnight to attain log growth phase. The turbidity of each inoculum was then adjusted to McFarland standard no. 0.5 by further addition of MHB to give inoculum concentration 1.5 x 10^8 CFU/mL. A volume of 100 μL of MHB was added into all the wells of 96-well microtiter plates except the first column A. Then, 200 μL working solution of each tested compound was transferred to column A of each plate. Serial 2-fold dilution was made starting from column A, ranging 3.91-250 μg/mL and the final 100 μL of the working solution was dispensed off from the last well. Each well was then inoculated with 100 μL of each bacterial inoculum. Each plate was sealed with parafilm and incubated at 37 °C for 24 h. A volume of 50 μL of 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) (0.2 mg/mL dissolved in distilled water) was added into each

well and the plates were again incubated for 30 min at 37 °C. Streptomycin, kanamycin and vancomycin were used as positive controls, whereas dimethyl sulphoxide (DMSO) was used as the negative control. Each compound and drug was tested in triplicate twice. The color change of MTT after 30 min from yellow to purple indicated the presence of active bacterial cells. The MIC was calculated as the lowest concentration of compounds that prevented the color change.

3.2.2. *In Vitro* Evaluation of Anti-Tuberculosis Activity

The test samples were analyzed *in vitro* against *Mycobacterium tuberculosis* $H_{37}Rv$ (*Mtb* $H_{37}Rv$) in a high throughput screen using an assay adapted from the microdilution AlamarBlue (AB) broth assay as reported by Collins et al., 1997 [18]. All the operations of *Mtb* $H_{37}Rv$ were conducted in accordance with the Biosafety Level 3 restraint [19]. The media was added to 96 microtitre well plates with the drugs followed by the addition of culture (diluted x 1000) which were then serially diluted. Incubation was carried out at temperature of 37 °C for 48 h then AB dye was added to the plates and incubation was further extended for another 24 h. Controls was taken as a media and the solvent of same concentration used to dissolve the drugs. The plates were analyzed fluorometrically and the reduction of bacterial proliferation was calculated according to the manufacturer's formula.

3.2.3. MDCK Cell based Anti-Influenza Assay

Cell-based anti-influenza virus inhibitor screening was based on the principle of cytopathic effect (CPE) protection assay as described by Kao. In brief, Madin–Darby canine kidney (MDCK) cells cultured to approximately 90% confluence were detached with 0.25% Trypsin–EDTA (Invitrogen), washed and re-suspended in complete EMEM, 2.5x104 MDCK cells were plated in triplicate in a 96-well plate and incubated overnight at 37 ℃ in a humidified 5% CO_2 incubator. The confluent MDCK monolayers cells were rinsed twice with Hanks' solution devoid of serum, and then the cells were treated with 50μL medium

with 1 mg/mL TPCK and 0.3% BSA and infected by two influenza virus strains at a multiplicity of infection (MOI) of 0.01 PFU/cell. fter 2.0 hr. incubation, serially diluted compounds were added. After 3 days incubation, the medium was removed and 50μL medium containing 5μL CCK-8 reagent was added into each well followed by additional 2 hours incubation, the absorbance was measured at 450 nm using an UV star-Microplates Synergy HT plate reader [20]. The IC_{50} values were calculated by nonlinear regressions using Graph Pad Prism.

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References:

[1] J. Carlet, P. Collignon, D. Goldmann, H. Goosens, I.C. Gyssens, S. Harbarth, V. Jarlier, S.B. Levy, B. N'Doye, D. Pittet, R. Richtmann, W.H. Seto, J.W. van der Meer, A. Voss, Society's failure to protect a precious resource: antibiotics. Lancet, 378 (2011) 369-371.

[2] World Health Organization: The evolving threat of antimicrobial resistance – Options for action. 2012, Geneva: WHO. http://apps.who.int/iris/bitstream/10665/44812/1/9789241503181_eng.pdf (accessed 30.12.17)

[3] J. Walter, S. Haller, H.P. Blank, T. Eckmanns, M. Abu Sin, J. Hermes, Incidence of invasive meticillin-resistant Staphylococcus aureus infections in Germany, 2010 to 2014. Euro. Surveill, 20 (2015).

[4] M. Berrazeg, S. Diene, L. Medjahed, P. Parola, M. Drissi, D. Raoult, J. Rolain, Euro. Surveill. New Delhi Metallo-beta-lactamase around the world: an eReview using Google Maps. Euro. Surveill,19 (2014) 20809.

[5] R. Prasad and D.K Srivastava, Multi-drug and extensively drug-resistant TB (M/XDR-TB) management: Current issues. Clinical Epidemiology and Global Health. 1 (2013)124-128.

[6] E.D. Kilbourne, Influenza pandemics of the 20th century. Emerging Infectious Diseases, 12 (2006) 9-14.

[7] C. Ma, F. Li, R.G. Musharrafieh, J. Wang, Discovery of cyclosporine A and its analogs as broad-spectrum anti-influenza drugs with a high in vitro genetic barrier of drug resistance. Antiviral Research, 133 (2016) 62-72.

[8] E. De Clercq, Antiviral agents active against influenza A viruses. Nature Reviews Drug Discovery, 5 (2006) 1015-1025.

[9] A. Estevez-Braun, A.G. Gonzalez, Coumarins. Natural Product Reports, 14 (1997) 465-475.

[10] T.G. Kraljević, A. Harej, M. Sedić, S.K. Pavelić, V. Stepanić, D. Drenjančević, J., Talapko, S. Raić-Malić, Synthesis, in vitro anticancer and antibacterial activities and in silico studies of new 4-substituted 1,2,3-triazole-coumarin hybrids. European Journal Medicinal Chemistry, 124 (2016) 794-808.

[11] A. Anand, M.V. Kulkarni, S.D. Joshi, S.R. Dixit, One pot Click chemistry: A three component reaction for the synthesis of 2-mercaptobenzimidazole linked coumarinyl triazoles as anti-tubercular agents. Bioorganic Medicinal Chemistry Letter, 26 (2016) 4709-4713.

[12] M.Z. Hassan, H. Osman, M.A. Ali, M.J. Ahsan, Therapeutic potential of coumarins as antiviral agents. European Journal of Medicinal Chemistry. 123 (2016) 236-255.

[13] G.M. Reddy, J.R. Garcia, V.H. Reddy, A.M. de Andrade, A. Camilo Jr., R.A.P. Ribeiro, S.R. de Lazaro, Synthesis, antimicrobial activity and advances in structure-activity relationships (SARs) of novel tri-substituted thiazole derivatives. European Journal of Medicinal Chemistry. 123 (2016) 508-513.

[14] A. Arshad, H. Osman, M.C. Bagley, C.K. Lam, S. Mohamad, A.S.M. Zahariluddin, Synthesis and antimicrobial properties of some new thiazolyl coumarin derivatives, European Journal of Medicinal Chemistry. 46 (2011) 3788-3794.

[15] A.C. Wei, M.A. Ali, Y.K. Yoon, R. Ismail, T.S. Choon, R.S. Kumar, N. Arumugam, A.I. Almansour, H. Osman, Antimycobacterial activity: a facile three-component [3+2]-cycloaddition for the regioselective synthesis of highly functionalised dispiropyrrolidines, Bioorganic Medicinal Chemistry Letter, 22 (2012) 4930-4933.

[16] S.K. Yusufzai, H. Osman, A.S.A. Rahim, S. Arshad, I.A. Razak, 3-(2-Methyl-amino-1,3-thia-zol-4-yl)-2H-chromen-2-one. Acta Crystallogr Sect E, E68 (2012) o2416-o2417.

[17] J.D. MacLowry, M.J. Jaqua, S.T. Selepak, Detailed methodology and implementation of a semiautomated serial dilution microtechnique for antimicrobial susceptibility testing. Journal of Applied Microbiology, 20 (1970) 46-53.

[18] L. Collins, S.G. Franzblau, Microplate alamar blue assay versus BACTEC 460 system for high-throughput screening of compounds against Mycobacterium tuberculosis and Mycobacterium avium. Antimicro. Agent. Chemo. 41(1997) 1004-1009.

[19] Biosafety in Microbiological and Biomedical Laboratories (BMBL) (2009) 5th edn. US Department of Health and Human Services.

[20] G. Tang, X. Lin, Z. Qiu, W. Li, L. Zhu, L.Wang, S. Li, H. Li, W. Lin, M. Yang, T. Guo, L. Chen, D. Lee, J.Z. Wu, W. Yang, Design and synthesis of

benzenesulfonamide derivatives as potent anti-influenza hemagglutinin inhibitors. ACS Medicinal Chemistry Letter, 2 (2011) 603−607.

Table 1. Physicochemical and Lipinski's parameters and of synthesized compounds **2a-o.**

Compound	R	R_1	R_2	Volume	TPSA	NROTB	Log*P*	MW	HBA	HBD	Lipinski's violations
2a	H	Me	-	215.51	55.13	2	2.75	258.04	4	1	0
2b	H	Et	-	232.32	55.13	3	3.30	272.01	4	1	0
2c	H	3,4-Cl_2Ph	-	297.43	55.13	3	5.81	387.98	4	1	1
2d	H	2-BrPh	-	288.25	55.13	3	5.23	397.97	4	1	1
2e	OMe	Me	-	241.06	64.36	3	2.72	288.05	5	1	0
2f	OMe	Et	-	257.86	64.36	4	3.27	302.07	5	1	0
2g	OMe	3,4-Cl_2Ph	-	322.98	64.36	4	5.78	417.00	5	1	1
2h	OMe	2-BrPh	-	313.79	64.36	4	5.20	427.98	5	1	1
2i	OMe	2-OMePh	-	321.45	73.60	5	4.44	380.08	6	1	0
2j	H	Et	Et	266.06	47.51	3	3.31	300.09	4	0	0
2k	H	Me	Me	232.46	47.51	1	2.38	272.06	4	0	0
2l	H	Ph	Ph	342.15	47.51	3	5.16	396.09	4	0	1
2m	OMe	Et	Et	291.61	56.74	4	3.28	330.10	5	0	0
2n	OMe	Me	Me	258.00	56.74	2	2.35	302.07	5	0	0
2o	OMe	Ph	Ph	367.70	56.74	4	5.13	426.10	5	0	1
Rule				**-**	**-**	**-**	**≤ 5**	**≤ 500**	**≤ 10**	**≤ 5**	**≤ 1**

TPSA, topological polar surface area; NROTB, number of rotatable bonds; MW, molecular weight; Log *P*, logarithm of compound partition coefficient between *n*-octanol and water; HBA, number of hydrogen bond donors; HBD, number of hydrogen bond acceptors.

Table 2. *In vitro* bacterial activity (µM) of thiazolyl coumarin derivatives **2(a-o)** against different microbial species.

Compounds	MIC in µM					*M. tuberculosis*	Cytotoxicity (IC_{50} µg/mL)
	E. coli	*E. aerogenes*	*S. typhi*	*S. pneumoniae*	*S. aureus*		
2a	242	242	242	242	242	>194	>62.5
2b	230	230	230	115	230	184	>62.5
2c	161	161	161	81	161	129	>62.5
2d	79	79	79	79	79	126	>62.5
2e	434	217	217	217	217	174	>62.5
2f	414	414	414	414	207	83	>62.5
2g	150	150	150	75	150	60	>62.5
2h	73	73	73	73	73	>117	>62.5
2i	329	329	329	165	329	>132	>62.5
2j	208	208	208	104	208	-	ND
2k	115	115	115	115	230	184	>62.5
2l	316	158	158	316	316	-	ND
2m	98	98	98	98	49	-	ND
2n	103	207	207	103	207	-	ND
2o	293	293	293	293	293	-	ND
BAC	560	560	560	560	560	-	ND
Streptomycin*	54	107	107	54	54	-	ND
Kanamycin*	129	129	129	129	258	-	ND
Vancomycin*	173	86	86	22	22	-	ND
Isoniazid*	-	-	-	-	-	1.3	>62.5

Results are mean values of triplicates assays, (*****) indicates Positive control, (-) indicates no inhibition observed, ND indicates not done, BAC: 3-(2-bromoacetyl)-2*H*-chrome-2-one.

Table 3. *In-vitro* anti-influenza activity in MDCK cells

Compounds	Binding energy (Kcal/mol)	Antiviral activity (IC_{50}, µg/mL)	
		H1N1	H3N2
2a	-5.66	4.84	>50
2b	-2.80	>50	>50
2c	-5.02	19.72	25.76
2d	-4.60	>50	>50
2e	-4.90	>50	>50
2f	-3.14	>50	>50
2g	-5.40	6.12	>50
2h	-4.80	>50	>50
2i	-3.60	>50	>50
2j	-3.30	>50	>50
2k	-5.20	9.13	>50
2l	-3.03	>50	>50
2m	-3.20	-	-
2n	-3.12	-	-
2o	-2.96	-	-
Zanamivir	-6.90	0.8	ND
Amantadine*	ND	>50	2.76

IC_{50} values are mean values of triplicates assays, (*****) indicates Positive control, (-) indicates no inhibition observed and (ND) indicates not determined.

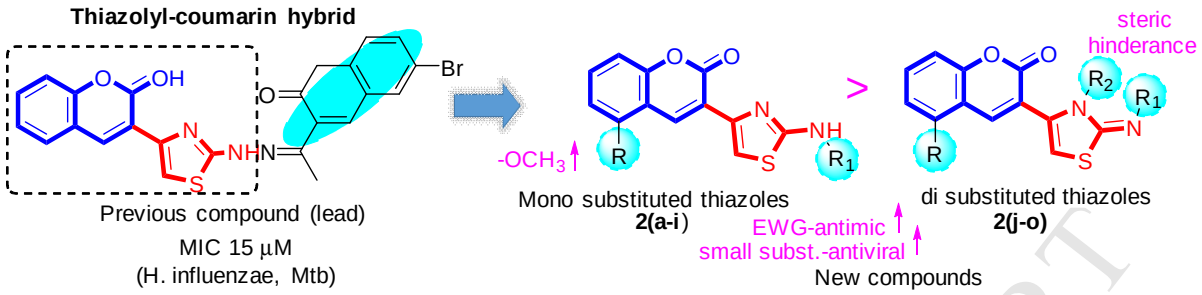


Fig. 1. Design of hybrid thiazolyl-coumarin analogues as antimicrobial agents

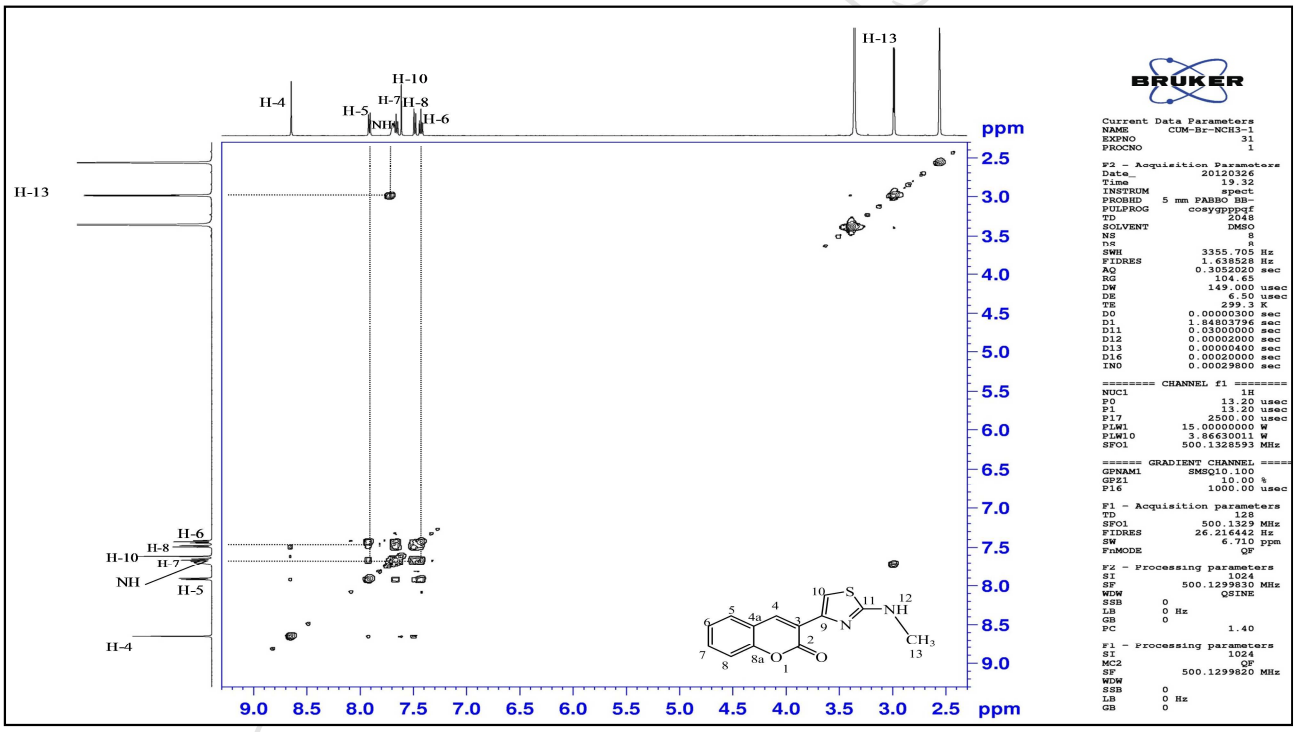


Fig.2. ^{1}H-^{1}H COSY spectrum of **2a** (500MHz) in DMSO-d_6

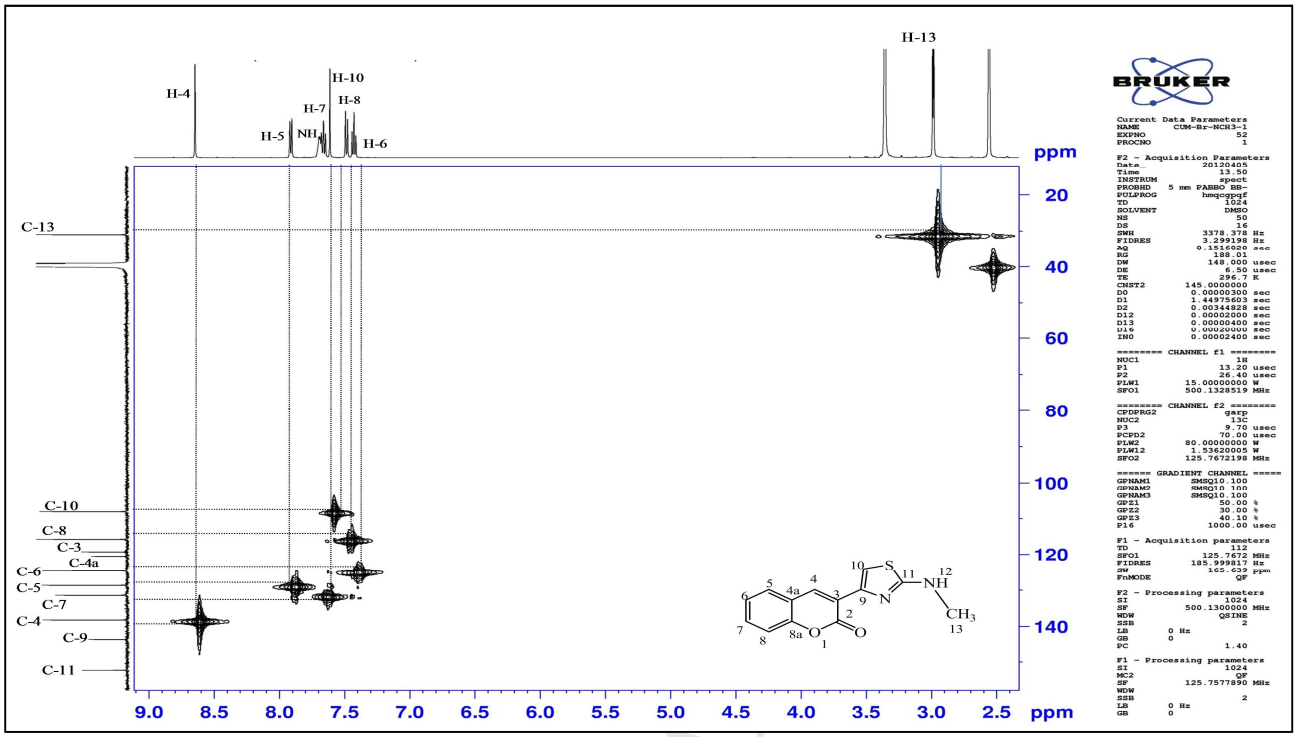


Fig.3. ^{1}H-^{13}C HMQC spectrum of **2a** (500MHz) in DMSO-d_6

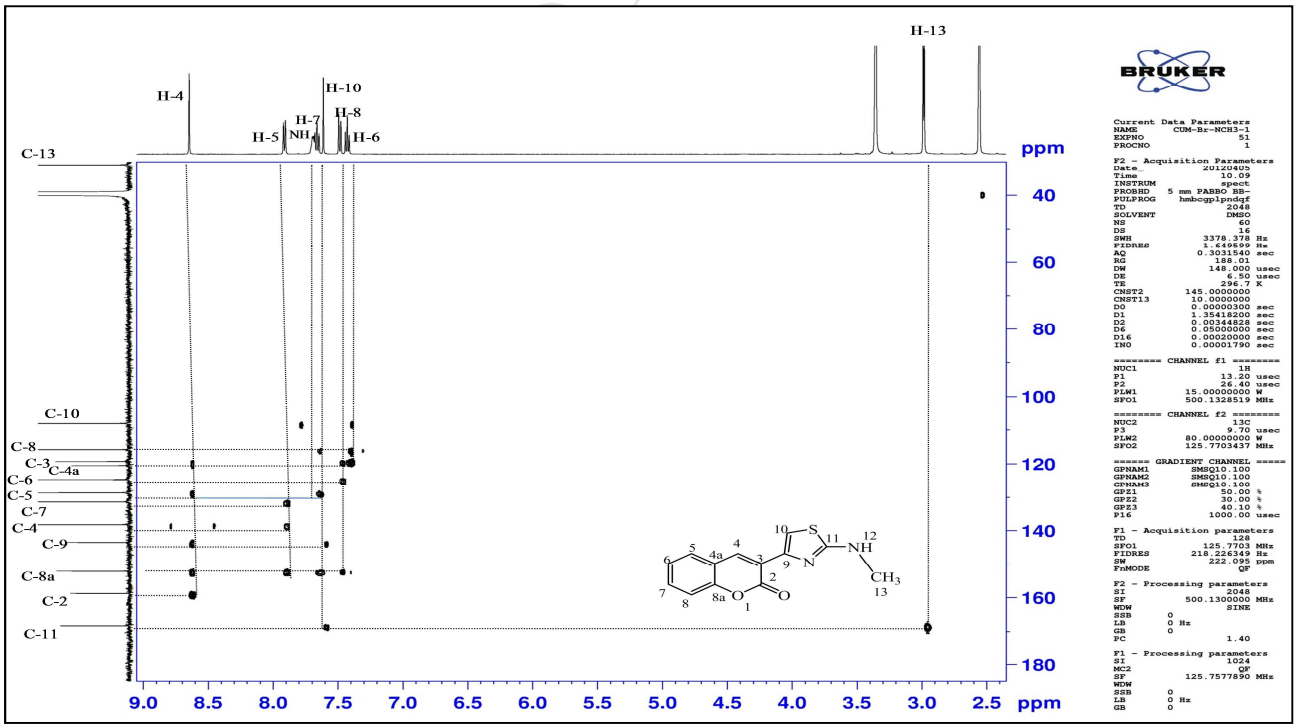


Fig.4. ^{1}H-^{13}C HMBC spectrum of **2a** (500MHz) in DMSO-d_6

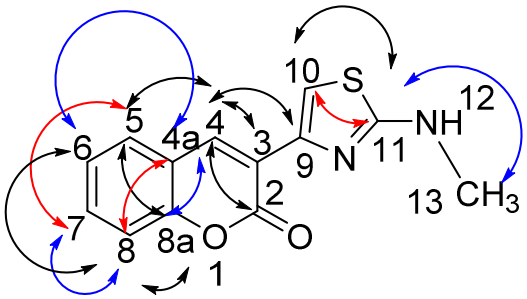


Fig. 5. ^{1}H-^{13}C HMBC correlations of **2a**

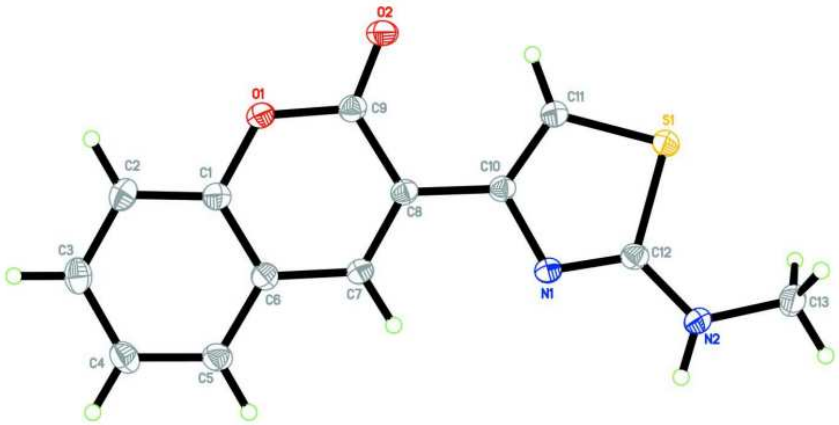


Fig. 6. Crystal structure with atom numbering scheme of **2a (**ORTEP view)

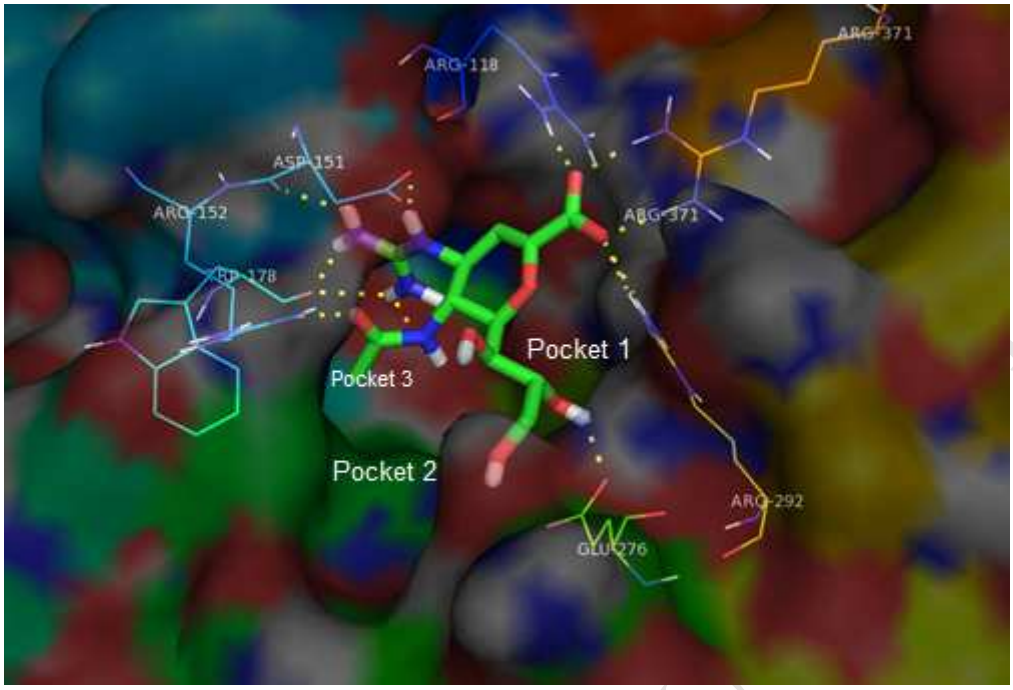


Fig. 7. Binding mode of zanamivir into NA pocket (PDB code: 3B7E) showing charge-charge interactions with a cluster of positively charged side chains of the Arg triad (Arg118, Arg292 & Arg371), Asp151, Arg152, Trp178 and Glu 276. Residues are colored by charge (positive is blue, negative is red, and neutral is white).

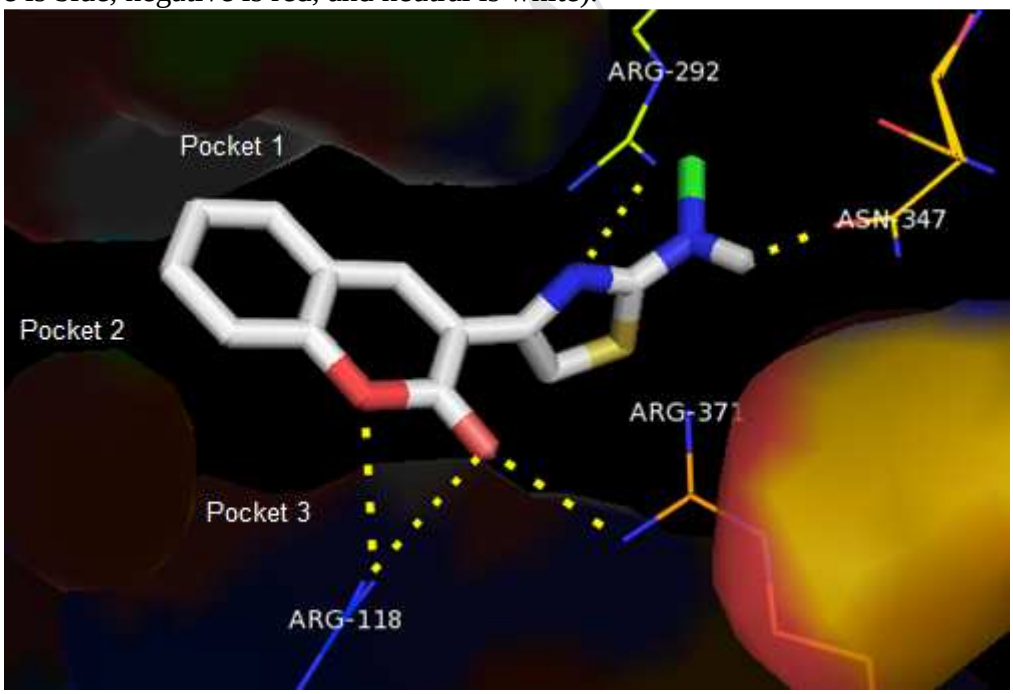
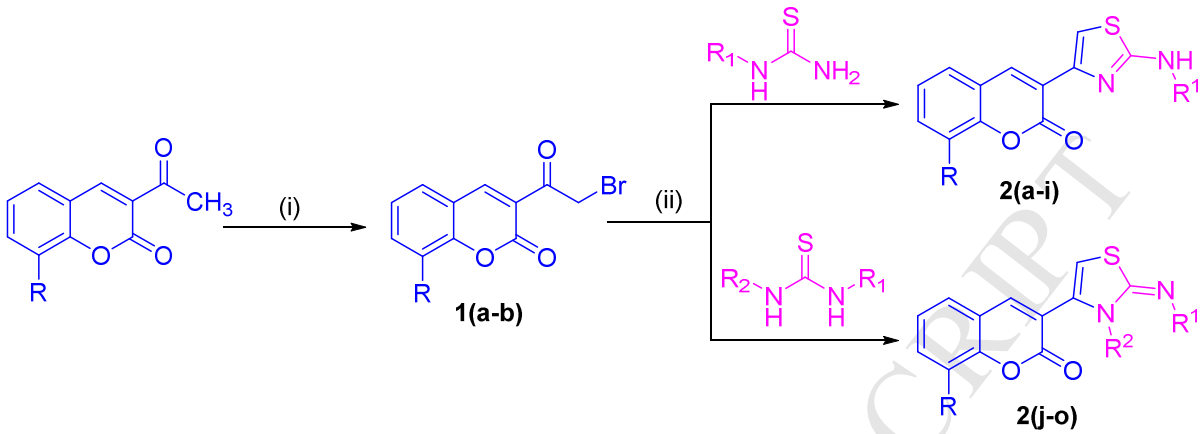


Fig. 8. Binding mode of compound **2a** at the binding site of neuraminidase (PDB code: 3B7E) showing hydrogen bond interactions (yellow dotted lines) with Arg triads: Arg118 (2.8 Aº), Arg292 (3.1 Aº), Arg371 (2.8 Aº), and Asn347 (1.8 Aº).



Scheme 1. Synthesis of thiazolyl coumarin derivatives **2(a-o)**. Reagents and reaction conditions: (i) Br_2, $CHCl_3$, 0-5 ºC stirring, 4-5 h; (ii) $CHCl_3$, EtOH (2:1), reflux, 3 h.

Highlights

- A series of novel hybrid thiazolyl-coumarin derivatives has been synthesized.
- Compound **2h** (MIC 73 μM) exhibited highest antibacterial activity.
- Compound **2g** emerged as the most effective anti-tubercular (MIC 60 μM) and anti-influenza agent (IC_{50} 6.12).
- Results of molecular docking provide a plausible binding interaction for the anti-influenza activity.