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Original article

Quantitative structure activity relationship studies on thiourea analogues as influenza virus neuraminidase inhibitors

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Abstract

Influenza virus is a major global threat that impacts the world in one form or another as flu infections. Neuraminidase, one of the targets for these viruses, has recently been exploited in the treatment of these infections. Quantitative structure activity relationship studies were performed on thiourea analogues using spatial, topological, electronic, thermodynamic and E-state indices. Genetic algorithm based genetic function approximation method of variable selection was used to generate the model. Highly statistically significant model was obtained when number of descriptors in the equation was set to 5. The atom type log *P* and shadow indices descriptors showed enormous contributions to neuraminidase inhibition. The validation of the model was done by cross validation, randomization and external test set prediction. The model gives insight on structural requirements for designing more potent analogues against influenza virus neuraminidase.

Keywords: Neuraminidase; Thiourea; Influenza; QSAR; GFA; Descriptors

1. Introduction

The influenza virus is a pathogenic agent for humans and has been socializing in the human population since at least the sixteenth century leading to repeated febrile respiratory disease every year. One of the lethal outbreaks was the Spanish flu caused by influenza (H1N1) virus killing around 40–50 million people [1]. Taubenberger et al. sequenced nine fragments of viral RNA from the coding regions of hemagglutinin, neuraminidase, nucleoprotein, matrix protein 1 and matrix protein 2 isolated from the lung tissue sample of the victim [2]. The sequences were consistent with a novel H1N1 influenza A virus which belongs to the subgroup of strains that infect humans and swine but not the avian subgroup [2]. Even large-scale sequencing has been adopted to provide a more comprehensive picture of the evolution of influenza viruses and of their pattern of transmission through human and animal populations, but still there are many loopholes in understanding its mutational behavior which makes present therapy ineffective [3]. Some pandemics can be seen rapidly progressing to involve all parts of the world due to emergence of a unique virus to which the overall population holds no immunity. These pandemics show characteristics which include occurrence outside the usual season [4], extremely rapid transmission from person to person with concurrent outbreaks throughout the globe and high attack rates in all age groups [5].

Chemotherapy for influenza virus fails due to newly discovered drug resistance in mutant strains. Several probable molecular targets for influenza virus include M2 proteins, endonuclease, hemagglutin and neuraminidase [6–10]. Currently available classes of antivirals include M2 proton channel inhibitors (amantadine and rimantadine) and the neuraminidase inhibitors (zanamivir and oseltamivir) [9,11]. The genetic basis for resistance of M2 proton channel inhibitors is single nucleotide changes and corresponding single amino acid substitutions in the trans-membrane of the M2 ion channel

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protein. Clinical studies highlight that the resistant variants emerged rapidly in rimantadine treated patients and these resistant variants were able to spread widely in closed populations when the M2 inhibitors were used for treatment and prophylaxis [12]. The apparent choice remains to be the development of effective drugs which are not susceptible to such mutations.

Release of progeny virus from infected cells by cleaving sugars which bind the mature viral particles is facilitated by an enzyme called neuraminidase. Targeting influenza virus neuraminidase was one of the breakthroughs in developing agents against flu; developing such agents provides valuable perspectives applicable to the field of antiviral chemotherapy. Crystal structure of influenza A neuraminidase was solved in the year 1983 [13] and its complex with its natural substrate sialic acid was reported in 1992 [14]. Selective and potent neuraminidase inhibitors were achieved by structure-based drug design, a well known rational drug design approach [15]. Oseltamivir is the drug of choice for treating influenza in current scenario but it has been reported to cause vomiting and nausea. Tremendous antiviral activity is displayed by zanamivir when administered intranasally but it is less effective when delivered systemically. So, there is still an enormous need to design and identify new agents for the chemotherapy of influenza virus infection and formulate effective drugs for systemic administration.

Quantitative Structure Activity Relationships (QSAR) for different sets of compounds have been reported by Verma and Hansch [16]. Seventeen different QSAR equations to understand chemical–biological interactions governing their activities toward influenza neuraminidase were presented in their work. Out of 17, 8 contain a correlation between activity and hydrophobicity indicating that it is one of the most important properties to look upon while designing drug for flu virus [16]. Few of the equations also suggest bilinear relationship between hydrophobicity and activity i.e. activity is decreased if the hydrophobicity is increased after a certain limit [16]. We report here different QSAR models developed on newly synthesized thiourea analogues reported to be potent inhibitors of influenza virus neuraminidase. Genetic algorithm based genetic function approximation method of variable selection was used to generate these models. The best model was used for predicting the test molecules which were not included in the training set. Randomization test at various intervals of confidence levels was done to validate the final model. The model gave good insights for developing new analogues as influenza virus neuraminidase inhibitors.

2. Experimental

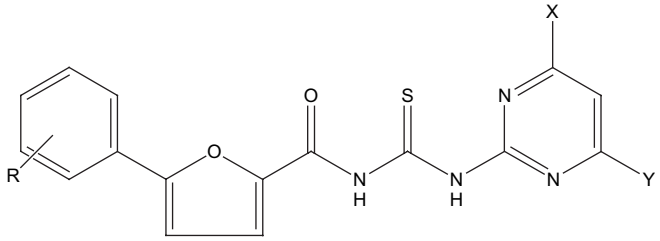
2.1. Dataset

In vitro inhibitory activity data (IC_{50} μM) of the thiourea analogues on influenza virus neuraminidase, reported by Sun et al. were taken for the study [17]. IC_{50} values were measured spectrofluorometrically using 2′-(4-methylumbelliferyl)-α-D-acetylneuraminic acid as substrate for neuraminidase to yield a fluorescent product which was quantified [17]. Out of 40 thiourea analogues reported, 30 molecules were selected for developing the model and 10 molecules for which the precise data were not available were discarded for the analysis. The IC_{50} (μM) values were taken in molar (M) range and converted to pIC_{50} according to the formula.

$$pIC_{50} = -\log IC_{50}$$

The dataset was randomly segregated into training and test sets comprising 24 and 6 molecules, respectively (Tables 1–3).

Table 1
Structure with actual and predicted activities of polysubstituted pyrimidinyl acyl(thio)urea analogues



Molecules	R	X	Y	IC_{50} (μM)	Actual pIC_{50} (M)	Predicted pIC_{50} (M)	Residual
1[a]	2-Cl	OEt	Me	1.65	5.78	6.69	−0.91
2	2-Cl	OEt	OEt	0.08	7.10	6.96	0.14
3	2-Cl	OH	Me	0.32	6.49	6.08	0.41
4	2-Cl	OMe	OMe	1.77	5.75	5.85	−0.10
5[a]	2-Cl	Cl	Cl	14.5	4.84	6.20	−1.36
6[b]	2-Cl	Cl	OEt	>20	–	–	–
7[b]	2-Cl	OMe	Me	>20	–	–	–
8	4-NO_2	Cl	Cl	1.66	5.78	5.85	−0.07
9[a]	4-NO_2	OEt	Me	2.30	5.64	6.41	−0.77
10	4-NO_2	OH	Me	0.36	6.44	5.87	0.57
11[b]	4-NO_2	OEt	OEt	>20	–	–	–

[a] Molecules used in test set.
[b] Molecules of discarded set.