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Research paper

Indole-like Trk receptor antagonists

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ABSTRACT

The virtual screening for new scaffolds for TrkA receptor antagonists resulted in potential low molecular weight drug candidates for the treatment of neuropathic pain and cancer. In particular, the compound (*Z*)-3-((5-methoxy-1*H*-indol-3-yl)methylene)-2-oxindole and its derivatives were assessed for their inhibitory activity against Trk receptors. The IC_{50} values were computationally predicted in combination of molecular and fragment-based QSAR. Thereafter, based on the structure-activity relationships (SAR), a series of new compounds were designed and synthesized. Among the final selection of 13 compounds, (*Z*)-3-((5-methoxy-1-methyl-1*H*-indol-3-yl)methylene)-*N*-methyl-2-oxindole-5-sulfonamide showed the best TrkA inhibitory activity using both biochemical and cellular assays and (*Z*)-3-((5-methoxy-1-methyl-1*H*-indol-3-yl)methylene)-2-oxindole-5-sulfonamide was the most potent inhibitor of TrkB and TrkC.

1. Introduction

The tropomyosin receptor kinase (Trk) family includes three homologous receptor tyrosine kinases: TrkA, TrkB, and TrkC, that specifically bind the neurotrophins nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), and neurotrophin 4 (NT4) and neurotrophin 3 (NT3), respectively. Activation of Trk receptors by the neurotrophins plays an important role in diverse biological responses, including differentiation, proliferation, survival, and other functional regulation of cells [1].

Neurotrophins are proteins that modulate the growth, maintenance, and survival of neurons [2]. In addition, NGF and BDNF function as key contributors in chronic neuropathic pain as well as hyperalgesia related to diverse pain states [3,4]. Hereditary sensory and autonomic neuropathy type V (HSANV) is caused by mutations in NGF gene, leading to the loss of ability to perceive deep pain [5]. Mutations in its receptor TrkA result in HSANIV, which is characterized by congenital insensitivity to pain, anhidrosis, and mental retardation [6].

Neurotrophin receptors have also been found to play an important role in the development and progression of tumor cells [7,8]. Alterations in Trk receptor expression, genomic rearrangements or mutations in the gene have been reported in different human cancers, e.g. pancreatic [9–12], prostate [13–15], breast [16–18], ovarian carcinoma [19,20], malignant melanomas [21], thyroid [22], and neuroblastoma [23,24]. Interestingly, TrkB receptor has been described to act as a suppressor of anoikis, a type of apoptosis important in prevention of metastasis [25], highlighting the importance of Trk receptor activity in tumor progression and formulating Trk receptors as potent targets of cancer therapy.

Changes in BDNF and its receptor expression are important in several central nervous system disorders, most notably the enhanced signaling in epilepsy and decreased or increased (depending on the brain region) levels in depression [26–28]. For this reason, inhibition of TrkB has been proposed as a candidate

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therapy for epilepsy. In a mouse model, inhibition of TrkB prevented recurrent seizures and alleviated anxiety-like behavior accompanied with a lower level of destructed hippocampal neurons [29].

Neurotrophins and their receptors have also been implicated to be important in age-related changes in cognition and Alzheimer's disease (AD). Although reports in this field are conflicting with some describing elevated levels of NGF in AD, the prevalent opinion seems to be that increasing the level of NGF or the activity of TrkA is therapeutic for AD [30,31]. Therefore, all inhibitors of Trk receptors might inflict unwanted side-effects if they are able to cross the blood-brain barrier.

In recent studies, several small molecule TrkA inhibitors have been shown to be effective in neuropathic and inflammatory pain models, able to attenuate cancer-induced pain as well as to block the development of some tumor cells [6,8,32]. Still, more potent and more Trk-selective inhibitors are searched for a therapeutic treatment of pain, cancer, and/or epilepsy.

The aim of the current study was to identify and characterize new low molecular weight antagonists of Trk receptors as drug candidates for the treatment of pain and cancer, using methods of computational modeling (molecular QSAR [33] and fragment-based QSAR [34]), chemical synthesis, and testing the compounds biochemically as well as in cellular assays. Several potent and highly Trk-selective inhibitors were identified. Future studies will determine if the compounds are able to penetrate the blood-brain barrier and if so, further drug development will be undertaken to render the inhibitors more specific for peripheral organs and less capable of inducing any unwanted side-effects due to Trk inhibition in the central nervous system.

This study was concentrated on indole-like Trk inhibitors. Some oxindoles and aza-oxindoles have been reported as selective TrkA inhibitors [35], a series of new 7-azaindole derivatives have demonstrated anticancer and antiangiogenic effects in human breast cancer cells [36], and oxindole amides and ureas have been investigated to elucidate the role of Trk receptors in cancer biology and other disease areas [37]. The indoles described in this study are targeting the ATP-binding pocket of the kinase domain, which is highly similar in the Trk family. Therefore, while TrkA was chosen as a representative for computational predictions and for the majority of the assays, the compounds are prevalently pan-Trk inhibitors.

2. Results and discussion

2.1. Model development

The data set obtained consisted of 47 indoles (Fig. 1 and Table S1 in Supplementary material). Using the QSARModel program [33], several multiple linear regression (MLR) models [38] were developed:

$$P = P_0 + \sum_{i=1}^{n} a_i D_i \quad (1)$$

Equation (1) correlates the studied property/activity P (P_0 - intercept) (in our case $\log IC_{50}$) with a certain number n of molecular descriptors D_i weighted by the regression coefficients a_i. Up to seven-parameters models were composed. As the compounds belong to three different structural classes and the corresponding biochemical assays differ from each other [35–37], the data set was investigated for outliers. Models based on four, five, and six descriptors with the best correlation were tested. The outliers mentioned below are rather related to the eventually selected model. At first 4 outliers (compounds 38, 40, 43, and 46 in Table S1), thereafter 3 outliers (compounds 7, 31, and 47 in Table S1) were identified by modified leverage analyses, i.e. compounds with large deviations from the model s^2 were removed:

$$\left(logIC_{50(predicted)} - logIC_{50(observed)}\right)^2 > s^2 \quad (2)$$

The final best MLR model of four descriptors possessed statistical characteristics is shown in Table 1. The coefficient of determination (Pearson's squared correlation coefficient) is $R^2 = 0.770$ for the data set of 40 compounds.

An ABC validation test [39] was applied to estimate the predictivity of Equation (1), taking into account the property data distribution. The ABC method consists of sorting the data in an ascending order according to the observed (experimental) values and three subsets (A, B, C) are formed: the 1st, 4th, 7th, etc. data points comprised the first subset (A), the 2nd, 5th, 8th, etc. comprised the second subset (B), and the 3rd, 6th, 9th, etc. comprised the third subset (C). Then three training sets were prepared as the combinations of any two subsets. Subsequently, the tested MLR model was rebuilt for each of the training sets, (AB, AC, and BC), with the same descriptors but with optimized regression coefficients. Further, these three models AB, AC, and BC were used to predict the property values for the C, B, and A subsets, respectively. The prediction was assessed based on the coefficient of determination R^2 between the predicted and observed property values. The final result was estimated by the averaged squared correlation coefficient by the three "external" sets C, B, and A. As regarding this ABC validation, the averaged R^2 is close to the R^2 of model, which is good for prediction purposes. In addition to the ABC validation, the standard leave-one-out cross-validation (R^2_{cv}) for the QSAR model resulted in $R^2_{cv} = 0.708$.

The descriptors appearing in the QSAR model (Table 1) are related to the stability, energy partition, and shape of the molecules [38]. The quantum-chemical descriptors, the lowest atomic state energy (AM1) for C atoms and the lowest atomic state energy (AM1) for H atoms are related to the ground states of these atoms in the molecule. The lower is the energy, the more stable is the atomic system and, thus, the more stable is the molecule with large C and H content. Besides, atomic state energies in QSAR models can be related to the change in the ligand electronic structure, steric hindrance, and the corresponding energetic effects in binding to the receptor. The quantum-chemical descriptor, the maximum electrophilic reactivity index (AM1) for C atoms comes from the LUMO coefficients and estimates the relative reactivity of the atoms within the molecule for a given series of compounds and is related to the activation energy of the corresponding chemical reaction. Since most atoms are the C atoms in the present investigation, this descriptor can be responsible for the reactivity of compounds. The quantum-chemical descriptors related to the frontier molecular orbitals such as various reactivity indices indirectly account also for the short-range intermolecular interactions, due to partial overlap of these orbitals. The molecular volume/XYZ box (AM1) is the geometrical descriptor that describes the bulk related properties and, by normalizing the descriptor with a unit box, shows how compact the molecule is.

2.2. Generation of a stable cell-line to monitor TrkA activity

A stably transfected cell line was generated in order to assess the capability of the compounds to inhibit the activation of TrkA receptor in the cellular context. Once the endogenous TrkA of the PC-12/luc/Elk1 cell line becomes activated, the downstream events will result in phosphorylation of Elk1, the GAL4-dbd fused to Elk1 binds then to GAL4 UAS and activates the transcription of luciferase