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Research article

In silico investigation of new binding pocket for mitogen activated kinase kinase (MEK): Development of new promising inhibitors

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ABSTRACT

It has been previously shown that the inhibition of mitogen activated protein kinase kinase (MEK) contributes to apoptosis and suppression of different cancer cells. Correspondingly, a number of MEK1/2 inhibitors have been designed and evaluated since 2001. However, they did not satisfy essential pharmacokinetic (PK) and pharmacodynamic (PD) properties thus, almost most of them were terminated in pre-clinical or clinical studies. This study aims to design new specific MEK1/2 inhibitors with improved PK/PD profiles to be used as alternative cancer medications. In first part of this study, a comprehensive screening, for the first time, was done on well-known MEK1/2 inhibitors using a number of computational programs such as AutoDock Tools 4.2 (ADT) and AutoDock Vina. Therefore a valuable training dataset as well as a reliable pharmacophore model were provided which were then used to design new inhibitors. According to the results of training dataset, Trametinib was determined as the best inhibitor provided, so far. So, Trametinib was used as the lead structure to design new inhibitors in this study. In second part of this investigation, a set of new allosteric MEK1/2 inhibitors were designed significantly improving the binding energy as well as the ADMET properties, suggesting more specific and stable ligand-receptor complexes. Consequently, the structures 14 and 15 of our inhibitors, as the most potent structures, are great substituents for Trametinib to be used and evaluated in clinical trials as alternative cancer drugs.

1. Introduction

The Ras/Raf/MEK/ERK pathway is one of the most important pathways of mitogen-activated protein kinase (MAPK) cascade. MAPK pathways control cellular processes including proliferation, differentiation and apoptosis (Wallace et al., 2010; Cargnello and Roux, 2011) and also their aberrant activation is frequently observed in tumor cells specifically those of K-Ras and B-Raf mutations (Davies et al., 2002; Cohen et al., 2003). Mitogen activated protein kinase kinase (MEK), also known as MKK, MAPKK and MAP kinase kinase (EC = 2.7.12.2), is activated being phosphorylated by Raf enzyme and has two known substrates; ERK1 and ERK2 (Isshiki et al., 2011). Although seven different MEK enzymes have been identified in Homo sapiens, a similar structure can be seen in all of them which includes a catalytic domain (also kinase domain), an N-terminal domain and a C-terminal domain. Of seven MEK enzymes, MEK1 (393 a.a.) and MEK2 (400 a.a.), as the key mediators of the Ras/Raf/MEK/ERK cascade, have similar structures and functions which include a docking site for ERK substrate placed in the N-terminal sequence (Anderson et al., 1990), an allosteric inhibitory fragment and also a unique nuclear export sequence (Meier et al., 2012). Moreover, the N-terminal domain embraces the main catalytic site located in a highly conserved kinase domain as well as the ATP binding site located adjacent to the allosteric inhibitory fragment (Anderson et al., 1990; Meier et al., 2012). However, there are some differences which distinguish each individual MEK enzyme, these various features can be found in the terminal sequences (Fischmann et al., 2009). Overexpression and uncontrolled activation of MEK/ERK pathway is shown to be associated with different types of human cancer (Fujii et al., 2011; Steelman et al., 2011; Luca et al., 2012). Therefore, the inhibition of MEK has the potential to suppress various cancer cells.

As the first MEK inhibitor tested clinically, CI-1040 (PD-184352) entered phase I clinical study but the development of this inhibitor was terminated because of low efficacy and poor pharmacokinetic profile. The main problems of CI-1040 were low water solubility,

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metabolic instability (Allen et al., 2003; Lorusso et al., 2005a) diarrhea, fatigue, nausea and skin rash (Ramnath and Adjei, 2007). As the second MEK inhibitor, PD0325901 was developed and tested in human clinical trials, and although some properties such as water solubility and stability were improved in comparison with CI-1040, sufficient efficacy was not exhibited. Besides, several side effects such as visual disturbance, syncope and negative effect on CNS caused this compound to fail in phase I clinical study (Lorusso et al., 2005b; Haura et al., 2010). Selumetinib (AZD6244, ARRY142886) was designed and used in phase I and II clinical studies in 2008 (Adjei et al., 2008) and had some progresses in comparison with previous MEK inhibitors such as having effect on various types of cancers (Jain et al., 2014). In 2010, (Kim et al. (2010) reported a novel MEK inhibitor, AS703026. Investigations on this inhibitor showed that it has good potency only in human multiple myeloma (MM), but other cancer types cannot be successfully treated by AS703026. Later in 2011, Dong et al., (2011) set out a new MEK inhibitor called TAK-733 which showed potent anticancer activities against melanoma, colorectal, non-small cell lung cancer (NSCLC), pancreatic and breast cancer. As these mentioned MEK inhibitors showed little benefits for cancer patients (Gilmartin et al., 2011), scientists came up with another MEK inhibitor namely Trametinib (also known as GSK1120212 and JTP-74057) in order to avoid previous MEK inhibitors' problems such as negative side effects, poor solubility, insufficient efficacy, low selectivity and *etc*. (Gilmartin et al., 2011). Trametinib was considered as a potent MEK inhibitor having long half-life, good selectivity and broad effect on various cancers. However, another study illustrated that there are still some adverse events in patients treated by Trametinib such as rash or dermatitis acneiform (82%) and diarrhoea (45%), most of which were grade II or lower, and also Trametinib had time-limited effect on patients in that investigation (Jeffrey et al., 2013). Trametinib was shown to have insufficient antitumor activity considering different cancers, and is advised to be used in combination with other drugs (Walters et al., 2013). Therefore, there is still a critical need for new MEK inhibitors which can effectively target MEK1 and MEK2 enzymes with high binding energy, low acute oral toxicity, reliable solubility and high intestinal absorption which may guaranty specific selectivity, less side effects and long term tumor suppression.

Computational studies have had great impacts on designing and producing new drugs during last decade and there is an upwards trend on using computational methods to predict the 3D structures and conformational factors of drugs as well as to calculate the ligand-receptor binding parameters (Asthagiri and Lauffenburger, 2001; Orton et al., 2005; Legewie et al., 2007) in order to prevent time- and money-consuming laboratory efforts. Lead structures are very helpful to develop new datasets and eventually new drugs, moreover, it is essential to predict the pharmacokinetic and pharmacodynmic properties of these lead structures as well as newly designed drugs prior to experimental procedures (Winkler, 2002). 3D-quantitative structure activity relationship (QSAR) is one of the most used regression models which uses force field calculations and requires 3D-structures. 3D-QSAR has been used frequently in order to design and analyze different inhibitors of Ras/Raf/MEK/ERK pathway (Thaimattam et al., 2004; Kim et al., 2011; Yang et al., 2012; Larifa et al., 2014).

In this study, we have done a comprehensive computational investigation on previously reported MEK1/2 inhibitors and classified them based on their pharmacokinetic and pharmacodynamic properties in order to determine the best designed inhibitors, and subsequently to use them as the lead structures to design new MEK1/2 inhibitors. Based on the results, Trametinib is the best inhibitor designed, so far, due to the highest binding energy and lowest inhibition constant (K_i) among others (Table 2). However, Trametinib did not show satisfactory results in clinical trials, as mentioned above. Therefore, we set out to design novel MEK allosteric site inhibitors using computational methods. Based on the results of the 3D-QSAR analysis in this research, new inhibitors were designed to complementary fit the inner unoccupied space of MEK enzyme. Two potential pockets were considered within the inner empty space of this enzyme by accurate analysis of the active and binding sites. Therefore, we have designed a set of novel MEK1/2 inhibitors with improved binding energy, Human Intestinal Absorption (HIA), blood brain barrier (BBB) penetration, solubility ($\log S$), acute oral toxicity (AOT), Ames toxicity, rat acute toxicity (LD_{50}), and K_i in comparison with Trametinib. Consequently, we provided a set of 15 compounds which can be used as potent, novel, selective and ATP-noncompetitive MEK1/2 allosteric site inhibitors.

2. Materials and methods

2.1. Structural investigation of MEK

In order to fully understand the binding and inhibitory mechanism of the MEK1/2 inhibitors, several structural and functional properties of the MEK active site have been investigated by molecular graphic software such as Swiss PDB Viewer (spdbv) 4.1 (Johansson et al., 2012) and Pymol 1.7 (Seeliger and Groot, 2010). To achieve the 3D-QSAR parameters of the MEK enzyme that is essentially important for novel inhibitor design, 43 MEK1/2 inhibitors which were previously reported and clinically tested, have been used in the current study. This helps us to obtain reliable pharmacophore models that could properly navigate our designing strategies and validate further results. Accordingly, we used the

Table 1
Previously provided MEK1/2 inhibitors which were screened in this study.

Inhibitor	Reference	Inhibitor	Reference	Inhibitor	Reference
CI-1040	Allen et al. (2003)	Com.12a	Wallace et al. (2010)	Trametinib	Gilmartin et al. (2011)
PD0325901	Lorusso et al. (2005a)	Com.13	Wallace et al. (2010)	RO5126766	Bahleda et al. (2012)
CH4987655	Isshiki et al. (2011)	Com.14	Wallace et al. (2010)	RO4987655	Maria et al. (2012)
PD318088	Shulin et al. (2005)	Com.17	Wallace et al. (2010)	Com.1	Adams et al. (2012)
Com.6	Isshiki et al. (2011)	Com.18	Wallace et al. (2010)	Com.27	Adams et al. (2012)
Com.22	Isshiki et al. (2011)	Com.19	Wallace et al. (2010)	Com.9	Hartung et al. (2013)
Com.25	Isshiki et al. (2011)	AS703026	Kim et al. (2010)	Com.13	Hartung et al. (2013)
Com.26	Isshiki et al. (2011)	MEK162	Shannon et al. (2010)	Com.16	Hartung et al. (2013)
Com.27	Isshiki et al. (2011)	Com.10	Dong et al. (2011)	Refametinib	Schmieder et al. (2013)
AZD8330	Wallace et al. (2009)	Com.11	Dong et al. (2011)	GDC0973	Aslan et al. (2013)
Com.3	Wallace et al. (2010)	Com.15	Dong et al. (2011)	Com.1a	Lu et al. (2014)
Com.7a	Wallace et al. (2010)	Com.16	Dong et al. (2011)	Com.10a	Lu et al. (2014)
Com.7b	Wallace et al. (2010)	Com.17	Dong et al. (2011)	Selumetinib	Jain et al. (2014)
Com.8a	Wallace et al. (2010)	Com.18	Dong et al. (2011)	–	–
Com.8b	Wallace et al. (2010)	TAK733	Dong et al. (2011)	–	–