Integrative identification of unexpected kinase–inhibitor interactions in the MAPK-mediated proliferation and differentiation of Mc3T3-E1 osteoblasts

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Abstract. Kinase-targeted therapy is a new and promising approach to disease treatment. However, some kinase inhibitors have been observed to cause an off-target adverse risk for skeletal system by influencing the growth of osteoblasts. It is known that the proliferation and differentiation of osteoblasts are essentially regulated by MAPK signaling pathway, and many off-target events are considered to influence this pathway. Here, the unexpected MAPK–inhibitor interactions in mouse MC3T3-E1 osteoblastic cells were investigated in detail using an integrative protocol. With bioinformatics analysis we successfully profiled a systematic noncognate interaction spectrum for off-target kinase inhibitors against mouse MAPK kinases, from which 13 potential MAPK–inhibitor interactions were identified. The inhibitors Nilotinib, Dasatinib and Bosutinib were suggested as promising candidates; their cytotoxicity on MC3T3-E1 and inhibitory activity against MAPK kinase were tested at cellular and molecular levels, respectively. We also tested two known MAPK inhibitors SP600125 and SB203580 as positive controls. Consequently, the Dasatinib was found to have high off-target risk for unexpectedly targeting osteoblast MAPK signaling pathway.

Key words: Mitogen-activated protein kinase — Kinase inhibitor — Off-target — Unexpected interaction — Molecular modeling — Osteoblast — MC3T3-E1

Introduction

Mitogen-activated protein kinase (MAPK) signaling pathway plays a critical role in bone development and maintenance (Thouverey and Caverzasio 2015). It has been reported that the MAPKs regulate bone mass through influencing osteoblast proliferation and differentiation (Ge et al. 2007; Greenblatt et al. 2010). This finding takes on particular relevance given that a majority of the secreted ligands that regulate osteoblast activity appear to signal in whole or in part through MAPK pathway (Greenblatt et al. 2013). Thus, shifting the kinase activity of MAPK members in osteoblasts can directly or indirectly influence bone formation and skeletal remodeling (Hu et al. 2003; Rodríguez-Carballo et al. 2016). For example, the natural product Resveratrol has been reported to reduce prostaglandin E1-stimulated osteoprotegerin synthesis in osteoblasts by suppressing MAPK family kinase JNK (Yamamoto et al. 2015), while the cytokine Irisin can promote osteoblast proliferation and differentiation via activating the MAPK pathway (Qiao et al. 2016).

Kinase-targeted therapy with small-molecule inhibitors has been widely used as therapeutic strategy to treat a variety of diseases such as cancer, inflammation and neurological disorder (Bhullar et al. 2018), which, however, were frequently observed to cause osteopenia and osteoporosis by unexpectedly disrupting and suppressing osteoblast growth (Aleman et al. 2014). A typical case is known as cancer treatment-induced bone loss (CTIBL), a long-term complication of anti-neoplastic treatments that leads to a high
fracture risk in oncologic patients (D’Oronzo et al. 2015). Previously, the untargeted kinase–inhibitor interactions in cancer signaling pathway have been successfully identified from kinase-targeted therapies (Meng et al. 2018). The ATP-binding site is highly conserved across the protein kinase family, and many ATP-completive inhibitors may not be able to well distinguish between the sites of different kinases, thus resulting in off-target side effects (Anastassiadis et al. 2011). The off-target effects on bone health have been found as the unexpected consequences of targeted therapies with kinase inhibition (Alemán et al. 2014).

Here, we hypothesize that one or more kinase members in the MAPK family could be targeted unexpectedly by certain noncognate kinase inhibitors to cause off-target skeletal effects on osteoblast-mediated bone formation. Although there probably is an association between the bone pathogenesis and unexpected kinase–inhibitor interactions in the MAPK signaling cascade of osteoblasts, the underlying molecular mechanism and biological implication still remain largely unexplored. In order to support our hypotheses and to identify the unexpected interactions that may cause off-target toxicity in osteogenic tissue, we herein profiled a systematic interaction spectrum of MAPK kinases expressed in mouse MC3T3-E1 osteoblastic cells with small-molecule inhibitors with reported off-target effects on bone formation, by integrating bioinformatics analysis, cell-based and cell-free assays, from which several MAPK–inhibitor interactions that are potentially associated with the osteopenia-related events were identified.

Materials and Methods

Kinase inhibitors with off-target effects on bone formation

A total of 18 small-molecule protein kinase inhibitors that have been reported to have off-target side effects on bone formation at cellular, animal and clinical levels were collected in Table 1. These inhibitors are diverse in terms of their chemical structures, including quinoline, indole, imidazole, and others.
| Inhibitor     | Chemical structure | Cognate target | Crystal complex | Off-target effect                                      |
|--------------|--------------------|----------------|-----------------|--------------------------------------------------------|
| Erlotinib    | ![Erlotinib](image1) | EGFR           | 1M17 cell       | promote osteoclast proliferation in low dose1          |
| Imatinib     | ![Imatinib](image2) | BCR-ABL        | 2HYY clinical   | alter bone and mineral metabolism2                     |
| Nilotinib    | ![Nilotinib](image3) | BCR-ABL        | 3CS9 cell       | decrease osteoclast development3                       |
| Dasatinib    | ![Dasatinib](image4) | Src | Abl | 3G5D animal | dysregulate bone remodeling4                           |
| Sunitinib    | ![Sunitinib](image5) | PDGFR | VEGFR | 3G0E clinical | amplify the inhibition of bone remodeling5             |
| Bosutinib    | ![Bosutinib](image6) | Src | Abl | 4MXO animal | exhibit a minor adverse effect on growing skeleton6    |
| Cabozantinib | ![Cabozantinib](image7) | MET | VEGFR | n.a. cell | have a dose-dependent biphasic effect on osteoblast activity7 |
| SYN1143      | ![SYN1143](image8) | c-Met          | n.a. animal     | potentiate the differentiation of precursor cells to osteoblasts8 |
| SGX523       | ![SGX523](image9) | c-Met          | 3DKF animal     | potentiate the differentiation of precursor cells to osteoblasts8 |
| Silmitasertib| ![Silmitasertib](image10) | CK2 | 3NGA cell | enhance the BMP2-induced osteoblast differentiation9    |
| Dovitinib    | ![Dovitinib](image11) | FGFR | c-Kit | 5A46 cell | enhance the BMP2-induced osteoblast differentiation10   |
| AG-1295      | ![AG-1295](image12) | PDGFR | n.a. cell | promote the osteogenic differentiation of pre-osteoblastic cells11 |

4 the crystal complex structure is formed by kinase inhibitor with its cognate target; 1 Bao et al. 2013; 2 Berman et al. 2016; 3 O’Sullivan et al. 2011; 4 Vandyke et al. 2010; 5 Brunello et al. 2009; 6 Tauer et al. 2013; 7 Dai et al. 2014; 8 Kim et al. 2017; 9 Son et al. 2015; 10 Lee et al. 2016; 11 Zhang et al. 2012; n.a., not available.
carboxamide, quinoxaline, etc; they are also diverse in terms of their cognate kinases, such as the sophisticated targets Src, EGFR, VEGFR and BCR-ABL. Some inhibitors (e.g. Imatinib) were found to have a long-term adverse effects on the mineral metabolism and bone mass of administrated patients (Berman et al. 2006), while some others (e.g. Dasatinib) were observed to dysregulate bone remodeling through inhibition of osteoclasts in vitro or in vivo (Vandyke et al. 2010). In particular, Bao and co-workers have performed a systematic kinase inhibitor screen against murine osteoblastic cell line and identified a number of candidates with potency to suppress cell viability in a dose- and time-dependent manner, although few compounds were observed to promote the cell proliferation at a very low concentration (Bao et al. 2013). These inhibitor compounds were also compiled here.

**Conventional mouse MAPK kinases**

The mouse MAPK family members can be classified into conventional and atypical kinases, which have distinct regulation and function. Only the conventional MAPKs are involved in the proliferation and differentiation of osteoblasts, which contains 10 kinases, namely extracellular signal-regulated kinase 1, 2 and 5 (Erk1/2/5), p38 isoforms (p38α/β/γ/δ) and c-Jun N-terminal kinases 1–3 (Jnk1/2/3) (Cargnello and Roux 2011), which are the central components of the MAPK signaling cascade and share a similar kinase domain at N-terminus but possess different additional portions at C-terminus.

In the 10 mouse MAPK kinases only p38α is currently available to its crystal structure in the protein data bank (PDB) database (Berman et al. 2000). Here, we performed BLAST sequence search (Altschul et al. 1990) against the PDB database to identify homologous protein crystal templates for other 9 kinases. As can be seen in Table 2, the identified homologous templates are all human or rat MAPK counterparts, which have a very high sequence identity with the mouse kinases. In particular, the two mouse Erk2 and Jnk1 kinase domains have fully identical sequences (100%) with that of human Erk2 and Jnk1 kinase domains, respectively; their structures would also be consistent. Considering the very high identity between other 6 mouse MAPK kinases and their homologous templates (>90%), we herein did not adopt the widely used homology modeling technique (Martí-Renom et al. 2000) to build the mouse kinase structures. Instead, virtual residue mutagenesis (VRM) and statistical modeling (Zhou et al. 2013ab) were carried out to computationally mutate homologous protein templates to the three-dimensional structures of mouse kinases (except p38α as well as Erk2 and Jnk1). Here, the VRM was performed using BetaSCPWeb server (Ryu et al. 2016).

**Building MAPK–inhibitor complex structures**

The complex structures of 18 off-target inhibitors with 10 MAPK kinases were computationally modeled by either inhibitor grafting or molecular docking. Here, a total of 13 kinase inhibitors in Table 1 are available to their cocrystallized complex structures with partner kinases. Therefore, we can graft their binding mode from the ATP-binding site of partner kinases to the ATP-binding site of MAPK kinases. This procedure can be seen in Figure 1. The MAPK kinase is superposed onto the crystal structure of partner kinase–inhibitor complex to obtain a superposed system of partner kinase-inhibitor-MAPK kinase and then the partner kinase was removed from the superposed system to generate the

### Table 2. The 10 conventional MAPK kinases expressed in mouse osteoblasts

| MAPK kinase | UniProt<sup>a</sup> | Specificity<sup>b</sup> | Kinase domain | Structure |
|-------------|---------------------|------------------------|---------------|-----------|
| MAPK3 Erk1  | Q63844              | Ser/Thr               | residues 43–331 | 2Z0Q      |
| MAPK1 Erk2  | P63085              | Ser/Thr               | residues 23–311 | 1ERK      |
| MAPK7 Erk5  | Q9WVS8              | Ser/Thr               | residues 55–347 | 5BYZ      |
| MAPK14 p38α | P47811              | Ser/Thr               | residues 24–308 | 5LAR      |
| MAPK11 p38β | Q9WU11              | Ser/Thr               | residues 24–308 | 3GP0      |
| MAPK12 p38γ | O08911              | Ser/Thr               | residues 27–311 | 1CM8      |
| MAPK13 p38δ | Q9Z1B7              | Ser/Thr               | residues 25–308 | 3COI      |
| MAPK8 Jnk1  | Q91Y6               | Ser/Thr               | residues 26–321 | 4QTD      |
| MAPK9 Jnk2  | Q9WTU6              | Ser/Thr               | residues 26–321 | 3E7O      |
| MAPK10 Jnk3 | Q61831              | Ser/Thr               | residues 64–359 | 1JNK      |

<sup>a</sup> the accession ID of kinase’s protein sequence in the UniProt database UniProt 2015;<sup>b</sup> kinase’s substrate specificity;<sup>c</sup> n.a., not available;<sup>d</sup> the crystal structure template of homologous protein used to model the mouse MAPK kinase domain structure.
modeled complex structure of MAPK kinase with the inhibitor. Previously, the grafting strategy has been successfully applied to structural modeling of the complex systems of mTOR and HER2 kinase with small-molecule compounds and phospholipase A2 with peptide ligands (Cui et al. 2015; Zhan et al. 2015; Shen et al. 2016). For other 5 kinase inhibitors in Table 1, molecular docking calculations were used to predict their binding mode to MAPK kinases. The atoms of kinases and inhibitors were assigned with Kollman and Gasteiger partial charges, respectively. In docking procedure, the AutoDock Tools was employed to set the center and size of grid boxes covering the kinase’s active site. The docking calculations were performed with AutoDock4 (Morris et al. 2009), which implemented Lamarckian genetic algorithm (LGA) to explore ligand conformational space in the active site of kinase receptor.

Dynamics simulation and energetics analysis

Through the inhibitor grafting and molecular docking a total of 180 MAPK–inhibitor complexes were obtained, which correspond to the systematic combination between 10 MAPK kinases and 18 off-target inhibitors. These complexes were subjected to molecular dynamics (MD) simulations implemented with AMBER14 software (Case et al. 2015). Inhibitor ligands were described with general AMBER force field (GAFF) (Wang et al. 2004), and their atomic charges were generated by using restrained electrostatic potential (RESP) fitting technique (Bayly et al. 1993). All the complex systems were immersed in a truncated octahedral box full of TIP3P water molecules and Na\(^+\) ions were added to keep the systems electroneutrality (Bai et al. 2017). Subsequently, the systems were heated from 0 to 300 K over 100 ps and 5-ns production simulations were carried in an isobaric-isothermal (NPT) ensemble with periodic boundary conditions (Zhou et al. 2016). The hydrogen atoms were constrained by performing the SHAKE method (Ryckaert et al. 1977) and time step was set to 2 fs. Long-range electrostatic forces were treated with particle-mesh Ewald (PME) (Darden et al. 1993) and nonbonded cutoff was set to 10 Å (Yang et al. 2015a, 2016).

During the production phase snapshots were saved every 50 ps, which were then employed to calculate the binding affinity score \(\Delta G\) by using molecular mechanics-Poisson Boltzmann/surface area (MM-PB/SA) method (Yang et al. 2015b):

\[
\Delta G = G_{\text{complex}} - (G_{\text{kinase}} + G_{\text{inhibitor}}) = \Delta E_{\text{interaction}} + \Delta D_{\text{desolvation}}
\]

where \(\Delta E_{\text{interaction}}\) is the intermolecular interaction energy between kinase and inhibitor which includes electrostatic and van der Waals potentials and was described using AMBER molecular force field, and \(\Delta D_{\text{desolvation}}\) is the desolvation effect upon kinase–inhibitor binding, which was computed by numerical solution of nonlinear Poisson-Boltzmann equation (for polar contribution) and surface area model (for nonpolar contribution) (Yu et al. 2014; Zhou et al. 2018).

Experimental part

The potency of compounds Nilotinib, Dasatinib, Bosutinib, SP600125 (pan-JNK inhibitor) and SB203580 (pan-p38 inhibitor) on murine osteoblasts was investigated using cell viability assays (Mackay et al. 1999; Karahashi et al. 2000). The mouse MC3T3-E1 osteoblastic cell line Subclone 4 were cultured and expanded in basal medium containing in DMEM with 10% fetal calf serum and 5% CO\(_2\) at 37°C. The cells were seeded in a 96 well plate at 1 × 10\(^4\) cells per well and treated with serial diluted compounds at different concentrations. Cell proliferation rate was measured using...
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The percentage of viable cells was calculated according to the following equation:

\[
\text{Viability} (\%) = \frac{(A_{\text{inhibitor}} - A_{\text{medium}})}{(A_{\text{control}} - A_{\text{medium}})} \times 100\% \quad (2)
\]

where the \( A_{\text{inhibitor}}, A_{\text{control}} \) and \( A_{\text{medium}} \) are the absorbance of cells treated with inhibitor, control and medium, respectively.

The activity of Dasatinib, SB203580 (positive control) and Bosutinib (negative control) against p38α kinase was determined using kinase inhibition assays (Lali et al. 2000; Hove et al. 2002). Kinase proteins were incubated in a buffer containing 50 mM Tris-HCl, pH 7.5, 1 mM EDTA, 10 mM NaF, 0.5 mM Na3VO4, 0.1% Triton X-100, 5 mM sodium pyrophosphate as well as 1 μCi [γ-32P]ATP and 0.5 mM ATP-2 as substrate. Compounds were mixed with the reaction buffer and incubated for 20 min at room temperature,

**Figure 2.** Multiple sequence alignment between the primary sequences of 10 mouse MAPK kinase domains (Erk1, Erk2, Erk5, p38α, p38β, p38γ, Jnk1, Jnk2 and Jnk3). The alignment was carried out using ESPript program (Gouet et al. 2003).
and then transferred onto a phosphocellulose piece. The piece was washed three times in phosphoric acid then in acetone at room temperature and $\gamma^{-32}$P incorporation was then measured by scintillation counting.

**Results and Discussion**

**Homology analysis and grafting testing**

The primary sequences of 10 mouse MAPK kinase domains (Erk1, Erk2, Erk5, p38α, p38β, p38γ, Jnk1, Jnk2 and Jnk3) were retrieved from the UniProt database (UniProt 2015), and their sequence homology was analyzed using multiple sequence alignment implemented with ESPript program (Gouet et al. 2003). As can be seen in Figure 2, the alignment revealed a high consensus between these kinases, with sequence identity > 30% – the lower threshold for homologous proteins (Geourjon et al. 2001). As compared to C-terminus, the N-terminus exhibits a larger conservation across the kinase family, as the conserved N-terminal section of kinases contains functional C-lobe and ATP-binding site. In addition, the 10 MAPK kinases can be divided into three subgroups, that is, Erk, p38 and Jnk; the kinases in the same subgroup are highly homologous to each other (identity > 60%). This is expected since a subgroup represents a panel of evolutionally conserved proteins that possess a very similar function. For example, the Erk subgroup consists of three MAPK kinases, i.e. Erk1, Erk2 and Erk5. An 88.9% sequence identity between Erk1 and Erk2 suggested that the two kinases are highly similar, displaying the same subcellular localization and sharing common activators and substrates (Lefloch et al. 2008). In contrast, the lower sequence identity (52.6%) of Erk5 indicates a different function of this kinase in cardiovascular development and neural differentiation (Nishimoto and Nishida 2006).

According to above analysis the MAPK kinases are homologous in sequence, structure and function. In particular, the ATP-binding site is highly conserved across the kinase family. Therefore, it is readily supposed that, if an ATP-completive inhibitor can target different kinases, the inhibitor would adopt similar binding mode to interact with the kinases’ ATP-binding sites. Previously, a systematic analysis of kinase–inhibitor crystal structure data deposited in the PDB database (Berman et al. 2000) also supported the supposition, although there are few exceptions (De Moliner et al. 2003; Vulpetti and Pevarello 2005).

Here, three off-target inhibitors, namely, Dasatinib, Imatinib and Nilotinib, were used to test the reliability of inhibitor grafting strategy. The Dasatinib has crystal complex structure with p38α (PDB: 3lfa), which was also cocryrstallized with its 11 partner kinases, including Abl (PDB: 2gag), Arg (PDB: 4xli), Bmx (PDB: 3sxr), EphA2 (PDB: 5i9y), EphA4 (PDB: 2y6o), Lyn (PDB: 2zva), Mst3 (PDB: 4qms), Mytl (PDB: 5vcv), Ptk6 (PDB: 5h2u), Src (PDB: 3g5d) and Stk10 (PDB: 5owr). We grafted the binding modes of Dasatinib ligand from these partner kinases to the ATP-binding site of p38α and then compared with its crystal binding mode in the site. Similarly, the cocryrstallized and grafted binding modes of Imatinib and Nilotinib were also compared in an analogous way. As can be seen in Figure 3, all

![Figure 3. Comparison between the cocryrstallized binding mode and grafted binding modes of inhibitors Dasatinib (A), Imatinib (B) and Nilotinib (C) to MAPK kinases. The multiple grafted binding modes were generated based on the crystal complexes of the inhibitors with their different partner kinases.](image-url)
the three inhibitor ligands exhibit an extended conformation in ATP-binding site, and they share a high consistence between the crystal and grafted binding modes in the site, with only a moderate displacement between core rings and a slight variation in side-chain moieties of different modes, indicating the rationality that same inhibitors bind similarly to different kinases, in particular those have high conservation.

A systematic interaction spectrum for off-target inhibitors against MAPK kinases

The complex structures of 18 off-target inhibitors with 10 mouse MAPK kinases were either solved by X-ray crystallography or modeled with inhibitor grafting and molecular docking, totally resulting in 180 inhibitor–kinase pairs, which were then subjected to MD simulations for conformational equilibrium and snapshot collection. Based on the collected snapshots the binding affinity score (DG) was calculated for each complex pair and, together, visualized as a heat map in Figure 4. The DG represents free energy change upon inhibitor binding to kinase (Tian et al. et al. 2011, 2014), and its negative and positive values indicate stabilization and destabilization of the binding, respectively (DG values are tabulated in Supplementary Material, Table S1).

As can be seen, a majority of inhibitor–kinase pairs exhibit a modest or low affinity (DG > −10 kcal/mol), albeit all of them seem to spontaneously interact with each other (DG < 0), suggesting that these off-target inhibitors are generally not the good binders of mouse MAPK kinases, and they are able to inhibit the kinases potently and should have a low adverse effect on bone system. However, several inhibitor–kinase pairs were predicted to have a high affinity (DG < −10 kcal/mol) that may cause the unexpected targeting of these inhibitors to MAPK kinases in mouse osteoblasts. In addition, the inhibitor binding profile displays a consistence within the same kinase subgroups. For example, the PF-04691502 can interact effectively with all the three kinases Jnk1, Jnk2 and Jnk3 in the Jnk subgroup (DG < −9 kcal/mol), while the Dasatinib exhibits high binding potency for three members p38α, p38β and p38γ of the p38 subgroup (DG < −10 kcal/mol). Interestingly, the Dasatinib has also been previously reported to influence the activation of p38 pathway (Dumka et al. 2009); this can be well reflected in the interaction spectrum. Here, totally 14 inhibitor–kinase pairs with high predicted affinity (DG < −10 kcal/mol) are derived from the interaction spectrum and listed in Table 3; they are considered as the potential unexpected interactions of off-target inhibitors with mouse MAPK kinases.

Cell-based and cell-free assays of unexpected inhibitor–kinase interactions

The unexpected targeting of MAPK pathway with off-target kinase inhibitors can address essential effects on the growth, proliferation and differentiation of osteoblasts (Greenblatt et al. 2013). The effects can be reflected at cellular level as dose-dependent cytotoxicity, which is the untargeted result of targeted therapies with off-target inhibitors. The mouse MC3T3-E1 osteoblastic cell line was treated separately with five compounds Nilotinib, Dasatinib, Bosutinib, SP600125

Figure 4. A systematic interaction spectrum for 18 off-target inhibitors against 10 mouse MAPK kinases. The plot is characterized by binding affinity score (ΔG).
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and SB203580; the former three were predicted to have potential unexpected interactions with Jnk, p38 and Erk subgroups, respectively, in terms of the inhibitor–kinase interaction spectrum, while the latter two are known MAPK inhibitors that were used as positive controls in this study.

As seen in Figure 5, the two positive controls SP600125 and SB203580 have high cytotoxic effect on MC3T3-E1, which can block the cell growth at a low concentration. Interestingly, the off-target inhibitor Dasatinib also exhibits a similar cytotoxicity profile with these positive controls, which can considerably reduce MC3T3-E1 viability with a low concentration, indicating that the Dasatinib is a potent inhibitor of MAPK pathway by targeting p38 subgroup, especially the p38α kinase (DG = –15.7 kcal/mol). The Nilotinib also exhibits a moderate cytotoxic effect on MC3T3-E1, which is roughly close to that of pan-Jnk inhibitor SP600125, albeit the former seems to be less effective than the latter. In addition, the Bosutinib was determined to have a weak potency that can influence the cell viability only at a high concentration (>100 μM).

Next, three compounds Dasatinib, SB203580 and Bosutinib were chosen to determine their inhibitory activity against mouse p38α kinase domain at molecular level (Table 4). The Dasatinib was supposed as a potential p38α inhibitor with a high binding affinity (DG = –15.7 kcal/mol), which has a similar cytotoxicity profile with the known p38α inhibitor SB203580 on mouse MC3T3-E1 cell line. In fact, the Dasatinib has been previously observed to dysregulate bone remodeling through disruption of osteoclasts in vivo, which increased trabecular bone volume by inhibiting osteoclast activity (Vandyke et al. 2010). In addition, although the Bosutinib was predicted as potent binder of Erk1/2 kinases, this inhibitor was estimated to have only a very low activity for p38α kinase (DG = –3.0 kcal/mol) and was measured to possess a moderate cytotoxicity for MC3T3-E1. Consequently, the positive control SB203580 was determined to have a high potency against mouse p38α (IC50 = 1.8 μM), which is basically in line with that against human p38α kinase (IC50 = 0.3–0.5 μM) (Lali et al. 2000). Analogous to the positive control, Dasatinib was also determined to have a strong potency, which can inhibit the mouse p38α kinase with IC50 value of 5.4 μM. In contract, no activity was found for the negative control Bosutinib. Overall, the experimental tests are well in line with computational findings.

Table 4. Binding score and inhibitory activity of SB203580, Bosutinib and Dasatinib against mouse p38α kinase

| Inhibitor   | Description          | IC50 (μM) | DG (kcal/mol) |
|-------------|----------------------|-----------|---------------|
| SB203580    | positive control     | 1.8 ± 0.3b| –16.2         |
| Bosutinib   | negative control     | no activity| –3.0         |
| Dasatinib   | predicted p38α inhibitor | 5.4 ± 1.6 | –15.7         |
|             | measured in triplicate. | IC50 = 0.3–0.5 μM for human p38α kinase (Lali et al. 2000). |
The complex structure of mouse p38α kinase with its potential inhibitor Dasatinib is shown in Figure 6. The inhibitor ligand adopts an extended conformation in the ATP-binding pocket of the mouse kinase, which exhibits a similar binding mode with that of human p38α kinase (PDB: 3lfa). Based on the structure the inhibitor molecule was identified to form three hydrogen bonds with the kinase residues Thr106 and Met109 by using PoseView program (Stierand et al. 2006), which should define the strong selectivity and specificity in inhibitor–kinase recognition, while a number of nonspecific chemical forces such as hydrophobic interactions and van der Waals contacts can also observed at the complex interface, which are considered to stabilize the inhibitor–kinase interaction (Luo et al. 2015). Overall, the Dasatinib is suggested as a good binder and a potent inhibitor of its noncognate p38α kinase, which can be either used to investigate its untargeted side effects on bone system or exploited as a new cognate p38α inhibitor for targeted therapy.

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Conflicts of interest. No potential conflicts of interest.

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