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N-(thiazol-2-yl)-2-thiophene carboxamide derivatives as Abl inhibitors identified by a pharmacophore-based database screening of commercially available compounds

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ABSTRACT

Suggestions derived from a previous ligand-based ligand design approach and docking calculations aimed at finding compound with affinity toward Abl and molecular scaffolds previously untested as Abl inhibitors, led to the identification of commercially available *N*-(thiazol-2-yl)-2-thiophene carboxamide derivatives with affinity in a cell-free assay up to low nanomolar concentrations, significantly enhanced with respect to that of their parent compounds previously reported. In particular, among compounds of the Asinex database, molecular docking simulations guided the choice of high-affinity ligands, predicting their binding mode and their interaction pattern with the Abl catalytic binding site. Moreover, affinity of the new compounds was also rationalized in terms of their interactions with the enzyme.

In recent years, efforts made in the field of medicinal chemistry to find new Bcr-Abl tyrosine kinase inhibitors resulted in the design and synthesis of new generation compounds, some of which showed encouraging preliminary activity also in clinical trials.[1] In this context, the application of computational structure- and ligand-based drug design approaches could aid the identification of new classes of compounds previously untested as Abl inhibitors. As an example, a previous study based on a combination of docking/dynamics simulations and pharmacophoric modeling allowed us to find compounds having a chemical structure based on a central 1,3-thiazole or a 1,3,4-thiadiazole core bearing a substituted benzamido chain at position 2 and an aryl moiety at position 5 (the general structure is reported in Chart 1).[2,3] Such derivatives showed an inhibitory activity in the submicromolar range in a cell-free assay toward Abl. An analysis of the structure–affinity relationships suggested that the substituent at position 5 is important in influencing affinity. In fact, compounds with a phenyl ring directly bound to the C5 of the heterocyclic core resulted inactive toward Abl, while analogues with an alkyl spacer between the phenyl ring and the core showed good affinity. Moreover, docking simulations evidenced that the N3 of the thiadiazole or thiazole ring was engaged in a crucial hydrogen bond interaction with the NH group of Met318, while N4 of the thiadiazole derivatives was not involved in hydrogen bond contacts. Finally, a pharmacophore-based database search suggested that the phenyl ring of the benzamido moiety could be profitably replaced by different aromatic groups (among them, a thiophene ring led to the highest fit score

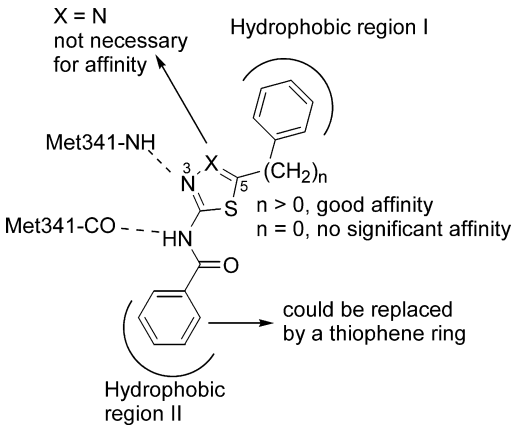

Chart 1. Schematic representation of Abi inhibitors based on a thiazole or a thiadiazole scaffold and their preliminary structure–affinity relationships.

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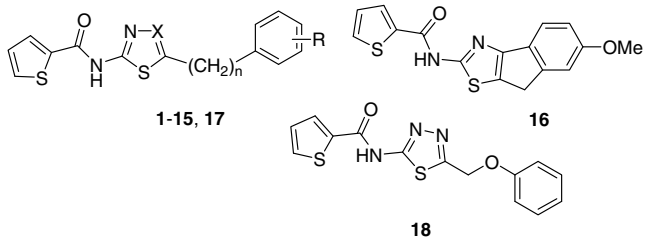
Table 1
Structure and inhibitory affinity of compounds **1–18** toward isolated Abl kinase



Compound	R	*n*	X	K_i (μM)[a]	Gold score[b]
1[c]	H	1	CH	0.090 ± 0.002	61.29
2[c]	4-F	1	CH	0.130 ± 0.005	61.13
3[d]	4-Cl	1	CH	0.624 ± 0.012	61.88
4[c]	3-Cl	1	CH	0.097 ± 0.024	62.64
5[c]	2-Cl	1	CH	0.245 ± 0.034	63.52
6[d]	2-F	1	CH	0.170 ± 0.011	61.05
7[c]	4-F	0	CH	1.646 ± 0.003	54.65
8[c]	4-Cl	0	CH	0.791 ± 0.007	58.37
9[c]	3-CH3	1	CH	0.059 ± 0.003	62.48
10[c]	3-CF3	1	CH	0.136 ± 0.005	62.67
11[d]	2-Cl,5-CF3	1	CH	0.845 ± 0.027	61.83
12[c]	2,5-diCl	1	CH	0.050 ± 0.006	67.36
13[d]	2,4-diCl	1	CH	3.474 ± 0.852	50.18
14[c]	4-OCH3	1	CH	0.016 ± 0.002	67.92
15[c]	4-CH3	1	CH	0.249 ± 0.018	62.39
16[c]				0.068 ± 0.002	50.58
17[c]	H	1	N	0.360 ± 0.004	59.01
18[c]				0.278 ± 0.006	60.68

[a] Values are means of three experiments and were calculated according to the following equation: $K_i = ID_{50}/\{E_0 + [E_0\ (K_m(ATP)/S_0)]\}/E_0$, where E_0 and S_0 are the enzyme and the ATP concentration (0.022 and 0.012 μM), respectively. Further details can be found in Ref. 2.

[b] Compound **16** was considered as an outlier and not reported in Fig. 1. Gold scores and K_i values were found to be highly correlated, with a correlation coefficient, excluding **16**, of 0.73).

[c] Compounds purchased from Asinex.[4]

[d] Compounds purchased from Chembridge Corporation.[5]

toward the pharmacophore model) which is equally able to fill the hydrophobic region II of the ATP binding site of Abl.

Taking into account all of these theoretical and experimental evidences, we have used the pharmacophoric model previously built to identify, within the Asinex and Chembridge databases,[4,5] a small set of compounds characterized by a *N*-(thiazol-2-yl)thiophene carboxamide scaffold bearing a benzyl moiety with different substituents and substitution pattern at position 5 of the thiazole nucleus (Table 1). Molecular docking simulations were also performed on such compounds to check their possibility to make profitable interactions with the ATP binding site of Abl and to further increase the probability of finding true positive (that is, compounds with significant affinity toward Abl). All the compounds, with the sole exception of **16**, were characterized by the same interaction pattern found for dasatinib in the complex with Abl, showing a hydrogen bond acceptor-donor motif involving the carbonyl oxygen and the NH group of the Met318 backbone.

The three-dimensional coordinates of the activated Abl kinase domain were extracted from its X-ray complex with dasatinib (entry code 2gqg of the Brookhaven protein data bank)[6] and used as the template for modeling studies. In particular, the portion of the complex constituted by the subunit A of Abl and by the inhibitor was used for calculations, upon an energy minimization performed to eliminate eventual steric clashes possibly affecting both protein and ligand. Structural optimization was performed by means of the software Macromodel,[7] using the Polak-Ribiere conjugate gradient algorithm and OPLS 2005 force field, and was terminated when the energy gradient root mean square fell below 0.01 kJ/Åmol. A 100 kJ/mol constraint was applied to the backbone atoms of the protein. The same optimization protocol was applied to complexes built during molecular docking calculations (see below).

The structure of inhibitors was sketched with Maestro[7] and submitted to the same minimization protocol, without any constraint.

Docking studies were performed using the Gold program,[8] by application of a genetic algorithm allowing for a partial flexibility of the protein. A 5 Å shell centered on dasatinib was chosen to dock thiazole and thiadiazole ligands into the Abl binding site. It is important to point out that docking programs are, in general, highly successful in generating the correct binding mode of ligands. However, when analyzing protein–ligand interactions, a poor correlation often does exist between the docking score and affinity. Correlations with binding affinity remain low even when scores are calculated directly from the experimentally determined protein–ligand structures. For this reason, we have applied both docking scoring functions available in Gold (namely, ChemScore and GoldScore), since the combination of multiple scoring functions could improve the enrichment of true positive and the probability to enhance correlation between docking scores and ligand affinity. As a result, both scoring functions found a good agreement between docking score/fitness results and experimental affinity data (in terms of K_i), although GoldScore was unable to handle compound **16**. As an example, GoldScore values and activity data showed a good correlation ($r^2 = 0.73$, excluding compound **16** which was considered as an outlier, Fig. 1 and Table 1), suggesting that the software was able to account for reliable binding mode and interactions of ligands with the protein and also supporting the hypothesis that GOLD docking calculations could be considered as a useful tool to drive the choice of new Abl ligand.

Selected compounds were then submitted to biological assays (following a protocol previously reported)[2] whose results showed that the benzyl derivative **1** was characterized by an affinity value of 0.090 μM, comparable to that of the corresponding *p*-F analogue **2** (0.130 μM). Docking calculations showed that the benzyl side chain of **1** was embedded into the hydrophobic region I (HRI), mainly interacting with the terminal methyl group of Thr315, Met290, Val299, Ile313, Ala380, Val256, and Ala253 (Fig. 2A). Most importantly, the nitrogen of the thiazole ring and the NH group of the amide moiety of **1** were the hydrogen bond acceptor and donor which had contacts with the backbone NH and CO groups of Met318, respectively, following a binding mode previously found

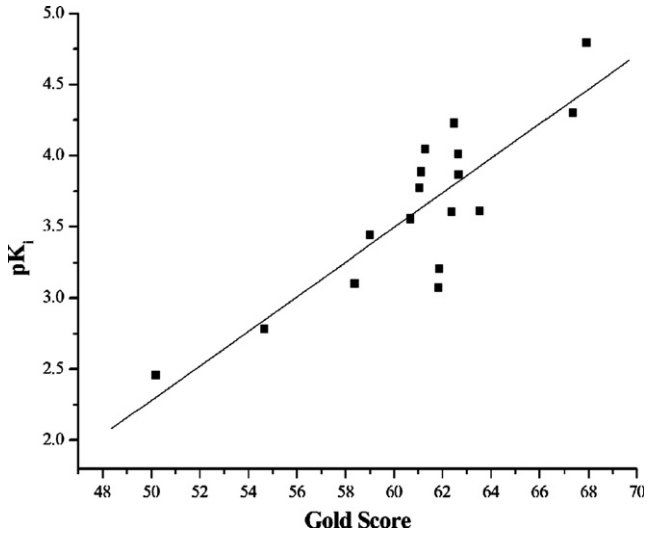


Figure 1. Graphical representation of the relationship between affinity data of compounds and Gold score fit values ($r^2 = 0.73$). Compound **16** was omitted.

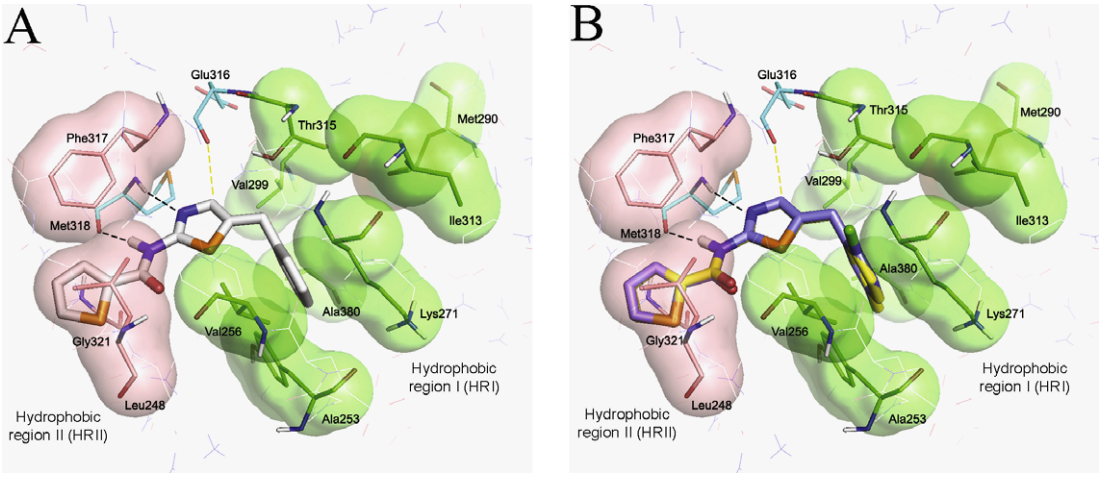


Figure 2. Graphical representation of the binding mode of the new compounds within the Abl binding pocket. The hydrogen bond donor/acceptor motif involving Met318 is represented by dashed black lines. The heteroaromatic CH···O=C interaction involving the thiazole ring and the backbone carbonyl oxygen of Glu316 is also depicted as a dashed orange line. Several of the amino acids forming the hydrophobic regions I and II are also shown in green and pink, respectively. A) Binding mode of **1**. B) Binding mode of **9** (orange) and **12** (cyan). The 3-methyl group of **9** is superposed to the 5-Cl substituent of **12** and both of them are located within HRII.

for 1,3,4-thiadiazole derivatives[2,3] and in the X-ray structure of the complex between BMS-354825 (dasatinib) and Abl (pdb entry 2gqg).[6] Moreover, the CH at the position 4 of the thiazole nucleus was also engaged in a heteroaromatic CH···O=C interaction with the backbone carbonyl oxygen of Glu316 at the hinge region. Similar interactions were found in other kinase-inhibitor complexes.[6] Finally, the terminal thiophene ring was accommodated within the hydrophobic region II (HRII) of the ATP binding site (mainly defined by the cleft created by Leu248, Gly321, and Phe317), while the carbonyl group of the amide moiety was not able to contact the receptor counterpart. Introduction of a 4-F substituent on the C5 phenyl ring (**2**) led to a partial reorientation of this aromatic moiety (with respect to that of **1**) and seemed to be an element disturbing the binding, instead to contribute to the stabilization of the complex. In fact, docking simulations found for the complex **2**-Abl a number of clusters significantly higher than that found for the **1**-Abl complex. A similar situation was found for the 4-chloro analogue (**3**) showing a slightly reduced affinity (0.624 μM) with respect to **2** (0.130 μM). Changing the substitution pattern to a 3-chloro derivative (**4**), affinity underwent a significant increase (0.097 μM), probably due to the favorable lipophilic interactions with HRI (see below). Moreover, both the 2-Cl and the 2-F analogues (**5** and **6**, respectively) showed comparable activity (0.245 and 0.170 μM, respectively).

The importance of the methylene spacer was supported by the fact that **7** showed an affinity (1.646 μM) more than one order of magnitude lower than that of the corresponding benzyl derivative **2** (0.130 μM). Similarly, **8**, the chloro analogue of **7**, was also characterized by a low affinity (0.791 μM). Although **7** and **8** had the same orientation of **1** and maintained the major contacts found between **1** and Met318, their halo substituent was located in a region of space mainly accommodating hydrophobic residues such as Ile313, Thr315, Val270, Val299, and Ala380. This finding was in agreement with the fact that the chloride (more lipophilic than the fluoride) was better accommodated within such a pocket, in comparison to the fluoride itself. In fact, affinity of **8** (0.791 μM) was twofold higher than that of **7** (1.646 μM). Moreover, further supporting this hypothesis, docking simulations found only one cluster for compound **8**, while many clusters were found for **7**, suggesting an uncertainty in the location of the fluorine substituent within the hydrophobic pocket.

Considering the usual orientation found for these compounds within the Abl binding site, in the attempt to better fill HRI of the binding pocket, a methyl group was inserted at the position 3 of the phenyl ring of the benzyl moiety, leading to **9** with an affinity of 0.059 μM, better than that of the unsubstituted benzyl derivative **1**. When the methyl group of **9** was transformed into a CF_3 as in **10**, affinity underwent a twofold decrease (0.136 μM). Similarly, the 2-Cl analogue of **10** (**11**) showed a decreased affinity (0.845 μM) with respect to **9**. On the other hand, affinity was restored to 0.050 μM with the 2,5-dichloro analogue **12**, due to the fact that the chlorine atom at position 5 was accommodated in the same region of the methyl group of **9** (Figure 2B), accounting for a very similar affinity. However, the 2,4-dichloro compound (**13**) had a remarkable lower affinity (3.474 μM), mainly due to the presence of the halogen at the para position not useful for profitable interactions with the target (as also found for **3**).

An analysis of the binding mode of **1** and **2** suggested that a hydrogen bond acceptor group at the para position of the phenyl ring could give profitable interaction with Lys271, possibly involving its terminal amino group as a hydrogen bond donor. Docking simulations showed that among Asinex entries, the *p*-methoxy derivative of **1** (compound **14**) was a putative ligand to meet such structural requirements. In fact, the orientation of **14** within the ATP binding site of Abl was very similar to that described for **1** and found for thiadiazole derivatives previously identified as Abl inhibitors, and the predicted hydrogen bond contact between the oxygen atom of the methoxy substituent and the amino group of Lys271 was found (2.5 Å distance). Accordingly, interactions of the *p*-methoxy substituent with the Abl binding site, in addition to the usual network of hydrogen bond contacts with Met318, led **14** to be the most active compound with an affinity of 0.016 μM. As expected, the 4-Me analogue (**15**) showed a reduced affinity (0.249 μM), further supporting the hypothesis that a lipophilic substituent at the para position of the phenyl ring does not profitably interact with HRI.

Transforming the thiazole nucleus of **1** into a 1,3,4-thiadiazole ring as in **17**, a fourfold decrease in affinity was found (0.090 vs 0.360 μM, respectively). Docking simulations showed for these two compounds a very similar binding mode and the same interactions with the binding pocket. The major difference was the lack of hydrophobic interactions between the nitrogen atom at the position 4 of the thiadiazole of **17** and the side chains of Leu370 and Ala269, on the contrary found between the CH group at the position 4 of the thiazole ring of **1** and the same residues. This differ-

ence in the interaction pattern could account for the lower affinity found for **17** in comparison to **1**.

Moreover, lengthening the benzyl chain of **17** by insertion of an oxygen atom led to **18**, without any significant variation of affinity (0.360 vs 0.278 μM, respectively).

Finally, to check the influence of flexibility on affinity toward Abl, the C5 benzyl side chain was rigidified by transforming the benzylthiazole system into a tricyclic core (**16**). An affinity fourfold lower than that of **14** was found (0.068 vs 0.016 μM), suggesting that a certain flexibility on the molecular portion filling HRI is required for better interactions. The binding mode of **16** is very different from that described for the remaining thiazole and thiadiazole derivatives. The donor-acceptor motif involving Met318 was replaced by hydrogen bond interactions between Leu322 and the carbonyl oxygen of **16** and between Thr315 and the oxygen atom of the methoxy group of the ligand.

In summary, in the attempt to optimize thiazole derivatives in terms of their affinity toward Abl tyrosine kinase, we have identified *N*-(thiazol-2-yl)-2-thiophene carboxamide compounds by application of a computer-aided drug design protocol based on a pharmacophoric model previously built and docking simulations of the interactions between the ligands and the target protein. The new compounds are characterized by a molecular scaffold previously untested in the field of Abl inhibitors, as well as by a high affinity toward Abl with IC_{50} values up to a 0.016 μM concentration. Docking results, in addition to allowing for a preliminary structure–activity relationship analysis, also provide details of the ligand-target interactions which could be taken into account in the next step of further ligand optimization. Additional assays are ongoing to assess for the ability of the new compounds to inhibit the mutated forms of Abl and results will be reported in due time.

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