ACS Medicinal Chemistry Letters

Letter

pubs.acs.org/acsmedchemlett

Pyrazolo[3,4-*d*]pyrimidine Prodrugs: Strategic Optimization of the Aqueous Solubility of Dual Src/Abl Inhibitors

Giulia Vignaroli,[†] Claudio Zamperini,[†] Elena Dreassi,[†] Marco Radi,[†,‡] Adriano Angelucci,[§] Patrizia Sanità,[§] Emmanuele Crespan,[∥] Miroslava Kissova,[∥] Giovanni Maga,[∥] Silvia Schenone,[⊥] Francesca Musumeci,[⊥] and Maurizio Botta*[,†,#]

[†]Dipartimentodi Biotecnologie, Chimica e Farmacia, Università degli Studi di Siena, Via Aldo Moro 2, 53100 Siena, Italy

[‡]Dipartimento di Farmacia, Università degli Studi di Parma, Viale delle Scienze 27/A, 43124 Parma, Italy

[§]Dipartimento di Scienze Cliniche Applicate e Biotecnologiche, Università dell'Aquila, Via Vetoio, 67100 Coppito, L'Aquila, Italy

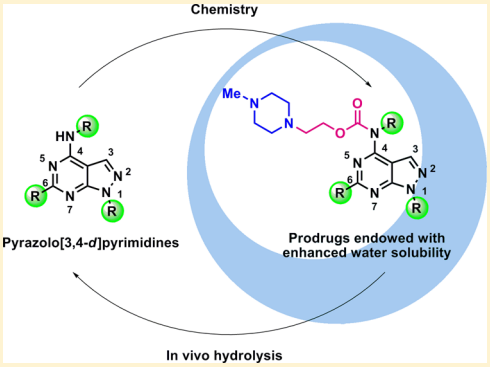
[∥]Istituto di Genetica Molecolare, IGM-CNR, Via Abbiategrasso 207, 27100 Pavia, Italy

[⊥]Dipartimento di Scienza Farmaceutiche, Università degli Studi di Genova, Viale benedetto XV 3, 16132 Genova, Italy

[#]Sbarro Institute for Cancer Research and Molecular Medicine, Center for Biotechnology, College of Science and Technology, Temple University, BioLife Science Building, Suite 333, 1900 North 12th Street, Philadelphia, Pennsylvania 19122, United States

S *Supporting Information*

ABSTRACT: Design and synthesis of prodrugs of promising drug candidates represents a valid strategy to overcome the lack of favorable ADME properties, in particular aqueous solubility and bioavailability. We report herein the successful application of this strategy with two representative pyrazolo[3,4-*d*]pyrimidine derivatives (**1** and **2**), which led to the development of the corresponding and highly water-soluble antitumor prodrugs (**7** and **8**). In vitro studies confirmed a significant improvement of aqueous solubility and, for compound **8**, good plasma stability, suggesting superior in vivo bioavailability. As expected, the uncleaved water-soluble prodrugs **7** and **8** showed no activity toward the enzymatic targets (c-Src and c-Abl) but revealed promising antiproliferative activity in myeloid cell lines, as a consequence of the in vitro hydrolysis of the selected solubilizing moiety, followed by the release of the active compounds (**1** and **2**).



KEYWORDS: *Prodrug, pyrazolo[3,4-d]pyrimidine, aqueous solubility, dual c-Src and c-Abl inhibitor*

Pyrazolo[3,4-*d*]pyrimidines represent a promising class of compounds capable of inhibiting several oncogenic tyrosine kinases, which represent an attractive target for the development of new therapeutic agents against cancer.[1] In this context, 4-amino-substituted pyrazolo[3,4-*d*]pyrimidines, extensively studied and regularly synthesized in our laboratories (general structure **A**, Figure 1), were found to inhibit c-Src and c-Abl activities in cell-free assays, some of them with nanomolar activity.[2,3] Furthermore, several members of this family were able to induce apoptosis and reduce cellular proliferation in different solid tumor cell lines (A431, 8701-BC, SaOS-2, and PC3).[4−7]

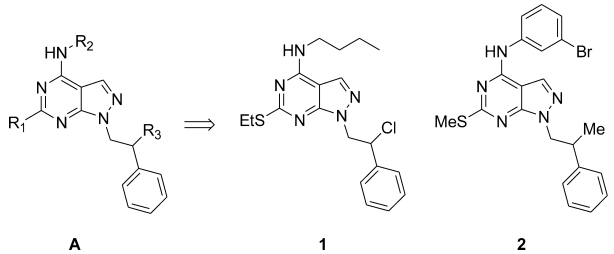


Figure 1. Pyrazolo[3,4-*d*]pyrimidines: general structure **A** and selected compounds **1** and **2**.

Other 4-amino-substituted pyrazolo[3,4-*d*]pyrimidines are known to inhibit the proliferation of Bcr-Abl-positive human leukemia cell lines (K-562, KU-812, and MEG-01), to reduce Bcr-Abl tyrosine phosphorylation and to promote apoptosis of Bcr-Abl expressing cells.[8]

The biological activity exhibited by this family of compounds, as for other tyrosine kinases inhibitors, is associated with low aqueous solubility; this could influence the pharmacokinetic parameters and cause issues in the future development of these putative drug candidates.[9,10]

Early assessment of pharmaceutical properties such as solubility, metabolic stability, and permeability has become a key step in the drug discovery process[11] since it is estimated

Received: February 25, 2013
Accepted: May 20, 2013

   dx.doi.org/10.1021/ml4000782 | *ACS Med. Chem. Lett.* XXXX, XXX, XXX−XXX

that 40% of potential drug candidates fail to reach the market due to poor physicochemical properties.[12,13]

In this context, the determination of solubility represents an important and crucial aspect, indeed a low aqueous solubility could possibly be responsible for irreproducible and inaccurate in vitro results.[14,15] This is why a coordinated and in-parallel optimization of biological activity and pharmaceutical properties, such as water solubility, likely represents a valid tactic to develop a new potential drug candidate. Herein, the use of prodrugs, chemically modified versions of the pharmaceutically active drug, which after undergoing in vivo transformations release the active drug, is reported. This approach represents a well established strategy to improve the physicochemical, biopharmaceutical, or pharmacokinetic properties of potential drug candidates.[16,17]

In the present study, a prodrug strategy was planned with the aim of validating a novel and useful approach to overcome the lack of aqueous solubility that characterizes the pyrazolo[3,4-*d*]pyrimidine class of compounds. Suitable prodrugs of pyrazolo[3,4-*d*]pyrimidine compounds, should display improved aqueous solubility in order to enhance pharmacokinetic properties, avoid problems faced during in vitro assays,[9,10] and facilitate in vivo distribution. Furthermore, the development of a rapid and versatile synthesis, applicable to a wide range of previously synthetized final compounds, was an appealing goal. To fulfill these aims, we first selected the most suitable position on the pyrazolo[3,4-*d*]pyrimidine core, on which to affix the prodrug functionality, and second chose the appropriate enzymatically cleavable linker and solubilizing moiety. Sticking to the plan to design the most versatile protocol, the secondary amine in the C-4 position was selected, as a consequence of being the moiety that most of the previously synthetized pyrazolo[3,4-*d*]pyrimidines shared. Indeed, the NH group on C-4 position represents an essential feature, able to create favorable interactions within the ATP-binding site of tyrosine kinases, as previously demonstrated by structure−activity relationships (SAR) and computational studies.[18] Furthermore, the high variability of the secondary amino moieties introduced in C-4 position could allow for the validation of a highly adaptable synthetic protocol.

A thorough literature search suggested the *O*-alkyl carbamate moiety, easily cleavable in vivo by hydrolases, as a viable linker to connect the secondary amino group at C-4 with the solubilizing group.[19,20]

Taken the water solubility issue into account, a *N*-methylpiperazino moiety, protonated at physiological pH (pK_a 9.27 ± 0.1)[21] and characterized by a high water solubility (molar solubility 9.98 mol L^{-1}, pH 7), was chosen in order to increase the water affinity of the resulting prodrugs (**7** and **8**) in comparison with the starting drugs (**1** and **2**). Figure 2 shows the three final components assembled to give the prodrug: (I) pyrazolo[3,4-*d*]pyrimidine core, (II) *O*-alkyl carbamate linker, and (III) the solubilizing moiety.

Two representative derivatives (**1** and **2**), previously synthetized in our laboratories, were chosen on the basis of their structural frameworks and biological activities. First, the different alkylamino or arylamino moieties on C-4 position (compound **1** and **2**, respectively) allowed for good structural variability; this was essential and required to initiate a highly adaptable synthesis. Second, they are associated with a remarkable ability to inhibit c-Abl and c-Src tyrosine kinases (Table 1).[22−25]

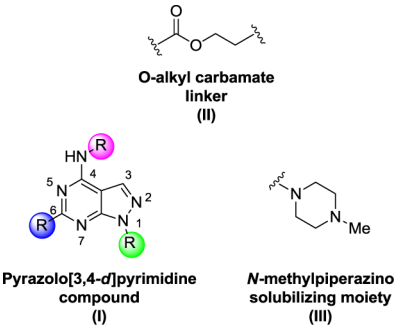


Figure 2. Prodrug's components.

Starting from the final pyrazolo[3,4-*d*]pyrimidine compounds (**1** and **2**), a classical carbamate synthesis was performed using triphosgene and sodium bicarbonate in CH_2Cl_2.[26] The resulting mixture, after filtration and evaporation under reduced pressure, gave the chlorocarbonate intermediates (**3** and **4**), which were immediately used in the next step without further purification. The subsequent addition of 2-*N*-methylpiperazinethanol (**6**), activated by sodium hydride, resulted in the formation of the desired prodrugs (**7** and **8**) (Scheme 1). In our laboratories, several in vitro experiments were initially conducted in order to define some ADME properties of the newly synthesized prodrugs (**7** and **8**) and compare them to those shown by parent drugs (**1** and **2**). Furthermore, these studies allowed us to establish the stability of the *O*-alkyl carbamate linker introduced into the parent drugs.

Aqueous solubility, stability in water, methanol, phosphate buffer (PBS, pH 7.4), and plasma were investigated, in addition to metabolic stability in presence of human liver microsomes (HLM) and permeability in parallel artificial membrane permeability assay (PAMPA). Solubility, stability, and permeability data obtained for the synthetized prodrugs (**7** and **8**) and their parent drugs (**1** and **2**) are shown in Table 1. Thermodynamic solubility was tested in water by adding 1 mL of H_2O to 1 mg of each compound; the resulting mixture was then shaken at ambient temperature for 24 h. The concentration of compounds (**1**, **2**, and **8**) was determined by UV/LC−MS, following filtration through a 0.45 μm nylon filter.[27] Prodrug **8**, when compared to its parent drug **2**, showed an encouraging enhancement of aqueous solubility. In fact, the solubility increased from less than 0.01 to 6 μg mL^{-1}, thus demonstrating that the prodrug approach could be the correct choice for overcoming the lack of aqueous solubility of these types of compounds. Solubility of compound **7** could not be tested by the in vitro assay, due to the fast rate of hydrolysis shown in aqueous medium; the value reported is calculated using Discovery Studio 3.0 software.[28] Nevertheless, the predicted solubility is 17.7 μg mL^{-1}, which represents an improvement compared to the solubility of the parent compound **2** (0.25 or 0.05 μg mL^{-1}, predicted and experimental values, respectively). The stability of prodrugs (**7** and **8**) was determined by UV/LC−MS in three polar media in order to evaluate if the contribution of chemical hydrolysis induced by water, methanol, and PBS to the overall rate of hydrolysis in subsequent plasma stability test is minimal. A 500 μM solution was prepared by dissolving the compounds (**7** and **8**) in PBS (12.5 μM, pH 7.4), water, and methanol, respectively. Twenty microliter aliquots were taken during 48 h (see Supporting Information for details with regards to

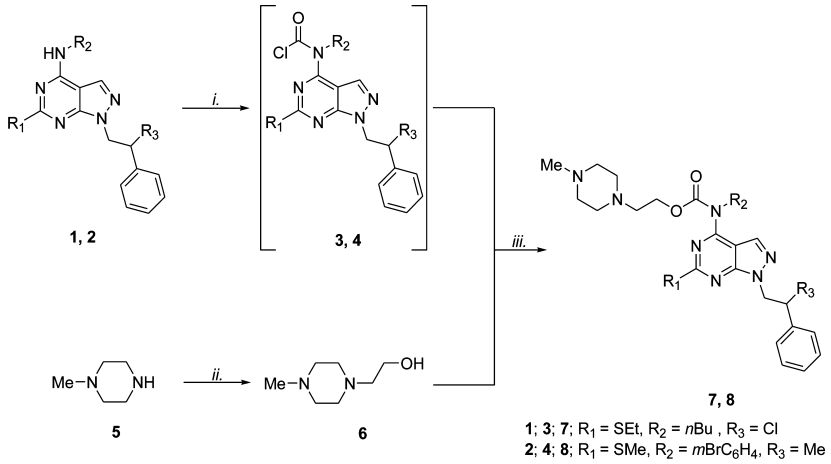
dx.doi.org/10.1021/ml4000782 | ACS Med. Chem. Lett. XXXX, XXX, XXX−XXX

Table 1. Characterization of Compounds 1, 2, 7, and 8: Solubility, Stability, and Membrane Permeability

		Stability[a]					
compd	H_2O solubility[a] $\mu g\ mL^{-1}$	H_2O $T_{1/2}$	PBS pH 7.4 $T_{1/2}$	MeOH $T_{1/2}$	human plasma $T_{1/2}$	metabolic stability[a,d] %	PAMPA[a,e] P_{app} 10^{-6} cm sec^{-1} (% MR)[a,f]
1	0.05 (0.25)[b]	ND[c]	ND[c]	ND[c]	ND[c]	91.5	5.99 (44.9)[f]
2	0.01 (0.02)[b]	ND[c]	ND[c]	ND[c]	ND[c]	95.1	0.01 (80.4)[f]
7	(17.7)[b]	30 min	63 min	125 min	28 min	ND[c]	ND[c]
8	6.47 (1.97)[b]	>48 h	>48 h	>48 h	193 min	99.9	2.11 (67.3)[f]

[a]Determined by UV/LC−MS. Data represent mean values of at least two experiments. [b]Calculated by Discovery Studio 3.0. [c]Not determined. [d]Expressed as percentage of unmodified drug. [e]PAMPA; see Supporting Information for experimental details. [f]Membrane retention (MR) expressed as percentage of compound unable to reach the acceptor compartment.

Scheme 1. Synthesis of Prodrugs 7 and 8[a]



[a]Reagents and conditions: (i) triphosgene, $NaHCO_3$, CH_2Cl_2; 3 h, 0 °C to r.t.; (ii) 2-bromoethanol, toluene, r.t., overnight then 80 °C, 2 h; (iii) NaH, CH_2Cl_2.

sampling) and analyzed by HPLC. Prodrug **8** displayed a good stability profile in all polar solutions tested and is thus regarded as chemically stable for the subsequent evaluation of its susceptibility to enzymatic hydrolysis. However, the stability of prodrug **7** was low in all the polar solutions tested (especially in water, with a half-life of 30 min); this high rate of hydrolysis is the reason why this compound could not be tested with regards to its aqueous solubility, metabolic stability, and permeability (these evaluations require higher water stability, for at least 1 h; see Supporting Information for experimental details). As already highlighted, plasma stability plays an important role in drug discovery and development, especially for groups susceptible to plasma enzyme hydrolysis, that could give rise to unstable fragments, which tend to have rapid clearance and a short half-life, resulting in poor in vivo performance. In order to understand the behavior of the *O*-alkyl carbamate linker toward plasma hydrolysis, an in vitro experiment using pooled human plasma was designed. The prodrugs **7** and **8** were incubated at 37 °C in human plasma, the disappearance of the substrate and the consequent formation of **1** and **2**, respectively, was monitored by HPLC analyses (Table 1). In the case of compound **7**, the plasma half-life confirmed the data obtained by water stability assay (half-life of 28 min) highlighting that the contribution of solvent-mediated hydrolysis in the cleavage of prodrug **7** can not be ignored. Compound **8** showed a half-life in plasma of about three hours, which is a good and encouraging value to guarantee an appropriate in vivo distribution.[11,29,30] Furthermore, for produg **8** passive membrane permeability could be evaluated performing a PAMPA assay.[31,32] The obtained passive permeability value (2.11×10^{-6} cm s^{-1}) showed a good enhancement with respect to the value obtained for parent drug **2** (0.01×10^{-6} cm s^{-1}), indicating that applying the prodrug approach could represent an interesting way to increase passive membrane permeability. Moreover, the prodrug strategy permitted to reduce the percentage of membrane-trapped compound; in fact, prodrug **8** showed a membrane retention (MR) percentage of 67.3% lower than the value obtained for parent drug **2** (80.4%).[33] In order to simulate phase I metabolism, compounds **1**, **2**, and **8** were incubated at 37 °C for one hour, with 5 μL of man-pooled HLM. After this time the percentage of drugs and metabolites was determined by LC−MS analysis: all tested compounds showed good metabolic stability (higher than 90%). Parent drug **1**, in a previously performed cell-free assay, showed K_i values of 0.60 and 0.32 μM toward c-Src and c-Abl, respectively, whereas **2** exhibited a high affinity toward c-Src (K_i value of 0.02 μM) and micromolar activity against c-Abl (1.07 μM) (Table 2). However, both parent drugs **1** and **2** showed activity as dual c-Src and c-Abl inhibitors. The newly synthetized prodrugs **7** and **8** were inactive when tested against both enzymes since the prodrug concept involves the chemical modification of a parent drug into a structurally different molecule with an altered set of pharmaceutical and pharmacokinetic properties.[34] In our example, the introduction of the highly aqueous soluble *N*-methylpiperazino group masks the C-4 secondary amino group needed to create favorable interactions within the ATP-binding site.[18] This is the reason why the modifications used to design our prodrugs (**7** and **8**)

dx.doi.org/10.1021/ml4000782 | ACS Med. Chem. Lett. XXXX, XXX, XXX−XXX

Table 2. Biological Evaluation of Compounds 1, 2, 7, and 8

	$K_i^{a,b}$ (μM)		$IC_{50}^{a,d,e}$ (SD) (μM)	
compd	c-Src	c-Ablwt	32D-p210	32D-T315I
1	0.60	0.32	3.5 (0.8)	6.7 (1.2)
2	0.02	1.07	6.2 (0.8)	5.8 (0.9)
7	NA[c]	NA[c]	1.2 (0.1)	2.4 (0.1)
8	NA[c]	NA[c]	2.8 (1.6)	2.6 (0.2)

[a]Data represent mean values of at least two experiments. [b]K_i values toward isolated kinases calculated according to eq 2 (see Supporting Information). [c]NA: not active at 100 μM (the highest concentration tested). [d]In vitro experiments conducted with 32D-T315I and 32D-p210 cells. [e]IC_{50}: the half maximal inhibitory concentration of the effectiveness in reducing the number of viable cells with respect to untreated cells. SD: standard deviation.

employed an *O*-alkyl carbamate linker, easily cleavable by hydrolysis and able to release the parent active compound in vivo.

The in vitro cytotoxic effects of the prodrugs **7** and **8** was then evaluated with a murine myeloid cell line (32D), expressing the nonmutated Bcr-Abl fusion protein (p210) or the mutated form (T315I). Cells were treated for 48 h with increasing concentrations of the inhibitors (0.01−50 μM), and IC_{50} values were calculated counting viable cells in comparison with untreated cells. When compared to the activities of the parent drugs (**1** and **2**), both prodrugs (**7** and **8**) showed an enhancement of their cytotoxic effects (Table 2).

As demonstrated by the enzymatic assays, prodrugs **7** and **8** were not able to interact directly with c-Src and c-Abl, so the molecular mechanism of action of these compounds in cells was still to be elucidated. In order to define whether the activities of **7** and **8** were dependent on the interaction between the released parent drugs **1** and **2** and tyrosine kinase enzyme c-Src, Western blot analysis was performed (Figure 3). The treatment

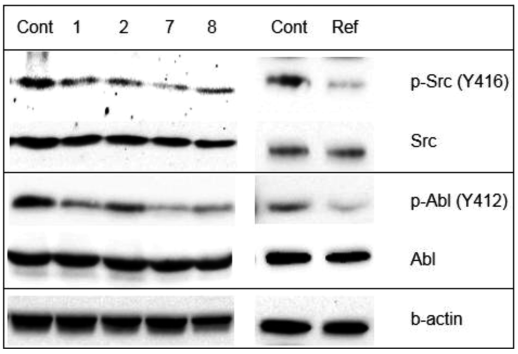


Figure 3. Western blot analysis of Src, Abl, and their respective phosphorylated active forms in murine myeloid cell line 32D-p210. Cells were treated with 1 μM parental compounds (**1** and **2**) and prodrugs (**7** and **8**). Dasatinib was added asa reference dual inhibitor compound (ref) and Beta-actin (b-actin) as loading control.

of myeloid cells with prodrugs **7** and **8** caused an effective reduction of the active phosphorylated form of c-Src and c-Abl, suggesting that parent compounds were readily available in the cell cytoplasm. However, in order to clarify the potential of this therapeutic strategy, further studies are needed, mainly to verify if the inhibition of target tyrosine kinases is obtained by a direct or indirect mechanism.

In conclusion, in the present work we employed a prodrug approach on previously synthetized pyrazolo[3,4-*d*]pyrimidines (**1** and **2**) to overcome the lack of good aqueous solubility shown by this promising class of compounds, which are able to inhibit c-Src and c-Abl in myeloid cell lines. We developed a versatile synthesis, which allowed the introduction of a *N*-methylpiperazino group linked by an *O*-alkyl carbamate chain. The increase of aqueous solubility was demonstrated by in vitro studies, as well as by the plasma stability and half-life. The newly synthesized prodrug **8** showed an improved passive membrane permeability and a lower membrane retention when compared to parent drug **2**. Enzymatic assays confirmed the expected inactivity of compounds **7** and **8**; meanwhile, the subsequent cellular assay showed a micromolar cytotoxic effect above the one of the corresponding parent compounds **1** and **2**. The Western blot analysis of produgs (**7** and **8**), conducted on murine myeloid cells, showed a reduction of phosphorylated (active) form of both c-Src and c-Abl, moderately higher than those associated with parent drugs (**1** and **2**).

The developed prodrug strategy allowed us to obtain pyrazolo[3,4-*d*]pyrimidines, endowed with an improved aqueous solubility profile. This method appears to be a powerful strategy to overcome the suboptimal pharmacokinetic properties of this class of compounds and, as consequence, makes their properties suitable for preclinical studies.

■ ASSOCIATED CONTENT

Supporting Information

Experimental details for the synthesis, ADME studies (solubility, stability, and PAMPA), enzymatic and cellular assays, and instrument details. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*(M.B.) E-mail: botta.maurizio@gmail.com.

Funding

We are grateful to Lead Discovery Siena (LDS), Associazione Italiana per la Ricerca sul Cancro (AIRC grant IG12084 to G.M.), Progetti di Ricerca di Interesse Nazionale (PRIN 2010 5YY2HL to S.S. and F.M.), Chemical Approaches to Targeting Drug Resistance in Cancer Stem Cells (CM1106), and Istituto Toscano Tumori (ITT grant proposal 2010).

Notes

The authors declare no competing financial interest.

■ REFERENCES

(1) Arora, A.; Scholar, E. M. Role of tyrosine kinase inhibitors in cancer therapy. *J. Pharm. Exp. Ther.* **2005**, *315*, 971−979.

(2) Crespan, E.; Radi, M.; Zanoli, S.; Schenone, S.; Botta, M.; Maga, G. Dual Src and Abl inhibitors target wild type Abl and the Abl T315I Imatinib-resistant mutant with different mechanisms. *Bioorg. Med. Chem.* **2010**, *18*, 3999−4008.

(3) Radi, M.; Dreassi, E.; Brullo, C.; Crespan, E.; Tintori, C.; Bernardo, V.; Valoti, M.; Zamperini, C.; Daigl, H.; Musumeci, F.; Carraro, F.; Naldini, A.; Filippi, I.; Maga, G.; Schenone, S.; Botta, M. Design, Synthesis, Biological Activity, and ADME Properties of Pyrazolo[3,4-*d*]pyrimidines Active in Hypoxic Human Leukemia Cells: A Lead Optimization Study. *J. Med. Chem.* **2011**, *54*, 2610−2626.

(4) Carraro, F.; Pucci, A.; Naldini, A.; Schenone, S.; Bruno, O.; Ranise, A.; Bondavalli, F.; Brullo, C.; Fossa, P.; Menozzi, G.; Mosti, L.; Manetti, F.; Botta, M. Pyrazolo[3,4-*d*]pyrimidines endowed with antiproliferative activity on ductal infiltrating carcinoma cells. *J. Med. Chem.* **2004**, *47*, 1595−1598.

(5) Carraro, F.; Naldini, A.; Pucci, A.; Locatelli, G. A.; Maga, G.; Schenone, S.; Bruno, O.; Ranise, A.; Bondavalli, F.; Brullo, C.; Fossa, P.; Menozzi, G.; Mosti, L.; Modugno, M.; Tintori, C.; Manetti, F.; Botta, M. Pyrazolo[3,4-*d*]pyrimidines as potent antiproliferative and proapoptotic agents toward A431 and 8701-BC cells in culture via inhibition of c-Src phosphorylation. *J. Med. Chem.* **2006**, *49*, 1549−1561.
(6) Manetti, F.; Santucci, A.; Locatelli, G. A.; Maga, G.; Spreafico, A.; Serchi, T.; Orlandini, M.; Bernardini, G.; Caradonna, N. P.; Spallarossa, A.; Brullo, C.; Schenone, S.; Bruno, O.; Ranise, A.; Bondavalli, F.; Hoffmann, O.; Bologna, M.; Angelucci, A.; Botta, M. Identification of a novel pyrazolo[3,4-*d*]pyrimidine able to inhibit cell proliferation of a human osteogenic sarcoma in vitro and in a xenograft model in mice. *J. Med. Chem.* **2007**, *50*, 5579−5588.
(7) Angelucci, A.; Schenone, S.; Gravina, G. L.; Muzi, P.; Festuccia, C.; Vicentini, C.; Botta, M.; Bologna, M. Pyrazolo[3,4-*d*]pyrimidines c-Src inhibitors reduce epidermal growth factor-induced migration in prostate cancer cells. *Eur. J. Cancer* **2006**, *42*, 2838−2845.
(8) Manetti, F.; Pucci, A.; Magnani, M.; Locatelli, G. A.; Brullo, C.; Naldini, A.; Schenone, S.; Maga, G.; Carraro, F.; Botta, M. Inhibition of Bcr-Abl phosphorylation and induction of apoptosis by pyrazolopyrimidine in human leukemia cells. *ChemMedChem* **2007**, *2*, 343−353.
(9) Dreassi, E.; Zizzari, A. T.; Mori, M.; Filippi, I.; Belfiore, A.; Naldini, A.; Carraro, F.; Santucci, A.; Schenone, S.; Botta, M. 2-Hydroxypropyl-*β*-cyclodextrin strongly improves water solubility and anti-proliferative activity of pyrazolo[3,4-*d*]pyrimidines Src-Abl dual inhibitors. *Eur. J. Med. Chem.* **2010**, *45*, 5958−5964.
(10) Radi, M.; Evensen, L.; Dreassi, E.; Zamperini, C.; Caporicci, M.; Falchi, F.; Musumeci, F.; Schenone, S.; Lorens, J. B.; Botta, M. A combined targeted/phenotypic approach for the identification of new antiangiogenics agents active on a zebrafish model: from in silico screening to cyclodextrin formulation. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 5579−5583.
(11) Kerns, E. H.; Di, L. Pharmaceutical profiling in drug discovery. *Drug Discovery Today* **2003**, *8*, 316−323.
(12) Di, L.; Kerns, E. H. Profiling drug-like properties in discovery research. *Curr. Opin. Chem. Biol.* **2003**, *7*, 402−408.
(13) Lipinski, C. A. Drug-like properties and the causes of poor solubility and poor permeability. *J. Pharmacol. Toxicol. Methods* **2000**, *44*, 235−249.
(14) Alsenz, J.; Kansy, M. High throughput solubility measurement in drug discovery and development. *Adv. Drug Delivery Rev.* **2007**, *59*, 546−567.
(15) Popa-Burke, I. G.; Issakova, O.; Arroway, J. D.; Bernasconi, P.; Chen, M.; Coudurier, L.; Galasinski, S.; Jadhav, A. P.; Janzen, W. P.; Lagasca, D.; Liu, D.; Lewis, R. S.; Mohney, R. P.; Sepetov, N.; Sparkman, D. A.; Hodge, C. N. Streamlined system for purifying and quantifying a diverse library of compounds and the effect of compound concentration measurements on the accurate interpretation of biological assay results. *Anal. Chem.* **2004**, *76*, 7278−7287.
(16) Rautio, J.; Kumpulainen, H.; Heimbach, T.; Oliyai, R.; Oh, D.; Järvinen, T.; Savolainen, J. Prodrugs: design and clinical applications. *Nat. Rev.* **2008**, *7*, 255−270.
(17) Huttunen, K. M.; Raunio, H.; Rautio, J. Prodrugs-from Serendipity to Rational Design. *Pharmacol. Rev.* **2011**, *63*, 750−771.
(18) Tintori, C.; Magnani, M.; Schenone, S.; Botta, M. Docking, 3D QSAR studies and in silico ADME prediction on c-Src tyrosine kinase inhibitors. *Eur. J. Med. Chem.* **2009**, *44*, 990−1000.
(19) Persson, G.; Pahlm, O.; Gnosspelius, Y. Oral bambuterol versus terbutaline in patients with asthma. *Curr. Ther. Res.* **1995**, *56*, 457−465.
(20) Slatter, J. G.; Schaaf, L. J.; Sams, J. P.; Feenstra, K. L.; Johnson, M. G.; Bombart, P. A.; Cathcart, K. S.; Verbug, M. T.; Pearson, L. K.; Compton, L. D.; Miller, L. L.; Baker, D. S.; Pesheck, C. V.; Lord, R. S. Pharmacokinetics, metabolism, and excretion of irinotecan (CPT-11) following I.V. infusion of [^{14}C]CPT-11 in cancer patients. *Drug Metab. Dispos.* **2000**, *28*, 423−433.
(21) Bjerrum, J.; Schwarzenbach, G.; Sillen, L. G. *Stability Constants. Part I: Organic Ligands*; Chemical Society Special Publication No. 6; The Chemical Society: London, U.K., 1957.
(22) Falchi, F.; Manetti, F.; Carraro, F.; Naldini, A.; Maga, G.; Crespan, E.; Schenone, S.; Bruno, O.; Brullo, C.; Botta, M. 3D QSAR models built on structure-based alignments of Abl tyrosine kinase inhibitors. *ChemMedChem* **2009**, *4*, 976−987.
(23) Schenone, S.; Brullo, C.; Bruno, O.; Bondavalli, F.; Mosti, L.; Maga, G.; Crespan, E.; Carraro, F.; Manetti, F.; Tintori, C.; Botta, M. Synthesis, biological evaluation and docking studies of 4-amino substituted 1*H*-pyrazolo[3,4-*d*]pyrimidines. *Eur. J. Med. Chem.* **2008**, *43*, 2665−2676.
(24) Santucci, M. A.; Corradi, V.; Mancini, M.; Manetti, F.; Radi, M.; Schenone, S.; Botta, M. C6-unsubstituted pyrazolo[3,4-*d*]pyrimidines are dual Src/Abl inhibitors effective against imatinib mesylate resistant chronic myeloid leukemia cell lines. *ChemMedChem* **2009**, *4*, 118−126.
(25) Radi, M.; Brullo, C.; Crespan, E.; Tintori, C.; Musumeci, F.; Biava, M.; Schenone, S.; Dreassi, E.; Zamperini, C.; Maga, G.; Pagano, D.; Angelucci, A.; Bologna, M.; Botta, M. Identification of potent c-Src inhibitors strongly affecting the proliferation of human neuroblastoma cells. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 5928−5933.
(26) Mangas-Sánchez, J.; Rodríguez-Mata, M.; Busto, E.; Gotor-Fernández, V.; Gotor, V. Chemoenzymatic synthesis of rivastigmine based on lipase-catalyzed processes. *J. Org. Chem.* **2009**, *74*, 5304−5310.
(27) Avdeef, A. High-throughput Measurements of Solubility Profiles. In *Pharmacokinetic Optimization in Drug Research: Biological, Physicochemical, and Computational Strategies*; Wiley: Lausanne, Switzerland, 2001; pp 305−325.
(28) Accelerys Software Inc. *Discovery Studio Modeling Environment Release 2.5*; Accelerys: San Diego, CA.
(29) Simoes, M. F.; Valente, E.; Gòmez, M. J. R.; Anes, E.; Constantino, L. Lipophilic pyrazinoic acid amide and ester prodrugs: Stability, activation and activity against *M. tuberculosis*. *Eur. J. Pharm. Sci.* **2009**, *37*, 257−263.
(30) Wang, Y. J.; Pan, M. H.; Cheng, A. L.; Lin, L. I.; Ho, Y. S.; Hsieh, C. Y.; Lin, J. K. Stability of curcumin in buffer solutions and characterization of its degradation products. *J. Pharm. Biomed. Anal.* **1997**, *15*, 1867−1876.
(31) Wohnsland, F.; Faller, B. High-throughput permeability pH profile and high-throughput alkane/water log P with artificial membranes. *J. Med. Chem.* **2001**, *44*, 923−930.
(32) Sugano, K.; Hamada, H.; Machida, M.; Ushio, H. High throughput prediction of oral absorption: improvement of the composition of the lipid solution used in parallel artificial membrane permeation assay. *J. Biomol. Screening* **2001**, *6*, 189−196.
(33) Avdeef, A.; Strafford, M.; Block, E.; Balogh, M. P.; Chambliss, W.; Khan, I. Drug absorption in vitro model: filter-immobilized artificial membranes. 2. Studies of the permeability properties of lactones in Piper methysticum Forst. *Eur. J. Pharm. Sci.* **2001**, *14*, 271−280.
(34) Rautio, J., Ed. *Prodrugs and Targeted Delivery: Towards Better ADME Properties*; Wiley-VCH: Berlin, Germany, 2011.