ACS Medicinal Chemistry Letters

Letter

pubs.acs.org/acsmedchemlett

Improved Angiostatic Activity of Dasatinib by Modulation with Hydrophobic Chains

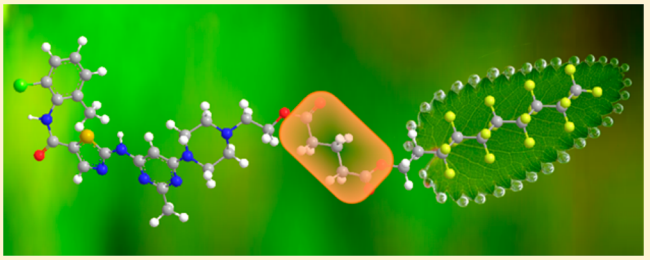
Emilia Păunescu,[†] Catherine M. Clavel,[†] Patrycja Nowak-Sliwinska,[†] Arjan W. Griffioen,[‡] and Paul J. Dyson*[,†]

[†]Institut des Sciences et Ingénierie Chimiques, Ecole Polytechnique Fédérale de Lausanne (EPFL), CH-1015 Lausanne, Switzerland

[‡]Angiogenesis Laboratory, Department of Medical Oncology, VUMC Cancer Center Amsterdam, 1081 HV Amsterdam, The Netherlands

Supporting Information

ABSTRACT: Dasatinib is an orally active nonselective tyrosine kinase inhibitor used to treat certain types of adult leukemia. By inhibiting PDGFR-β and SFKs in both tumor cells and tumor-associated endothelial cells, dasatinib inhibits tumor growth and angiogenesis. Herein, dasatinib derivatives modified with hydrophobic chains were prepared and evaluated for their *in vitro* antiproliferative selectivity and their *in vivo* antiangiogenic activity. For one of the derivatives, modified with a long perfluorinated chain, a significant enhancement in antiangiogenic activity was observed. Combined, these results suggest a possible generic route to modulate the angiostatic activity of drugs.



KEYWORDS: *Dasatinib, medicinal chemistry, angiogenesis, antiangiogenic drugs, kinase inhibitors*

Dasatinib, [*N*-(2-chloro-6-methylphenyl)-2-[[6-[4-(2-hydroxyethyl)-1-piperazinyl]-2-methyl-4-pyrimidinyl]-amino]-5-thiazole carboxamide **1** (Figure 1), is a potent, orally active, multitargeted inhibitor of several critical oncogenic kinases.[1−5] On the basis of the promising data obtained in clinical trials,[5,6] **1** was approved by regulatory agencies in 2006 for the treatment of adults with Bcr/Abl-dependent chronic myeloid leukemia or with Philadelphia chromosome-positive acute lymphoblastic leukemia with resistance or intolerance to imatinib therapy.[3,5,7−11] Several mechanisms are responsible for the **1**-induced suppression of leukemia including G1 arrest of the cell cycle,[12−14] apoptosis activation[12−16] and inhibition of cell migration, invasion, and metastasis,[12−19] associated most likely to kinase inhibition (binding to over 40 kinases).[20−23] The efficient inhibition of the Src family of kinases broadens the therapeutic potential of **1** toward solid tumors.[24,25] The proto-oncogene Src encodes a nonreceptor tyrosine kinase, whose expression and activity correlates with cancer progression, advanced malignancy, and poor prognosis in a variety of cancers.[26−30]

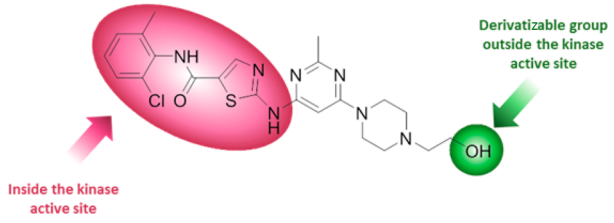


Figure 1. Structure of dasatinib **1** highlighting the part of the structure that interacts with kinases and the site that can be modified without impeding kinase inhibition.

Although most cancer therapies are directed against tumor cells, an emerging area of cancer therapeutics focuses on targeting the tumor microenvironment. As a Src inhibitor, **1** was shown to interfere with critical cell functions associated with angiogenesis and the metastatic cascade of human cancer cells such as motility and invasion.[31] Compound **1** reduces the ability of microvascular endothelial cells to form tubes *in vitro* and interferes with tumor-cell induced angiogenesis *in vivo*, by affecting endothelial cells directly and by inhibiting the pro-angiogenic signaling of certain cancer cells.[31−38]

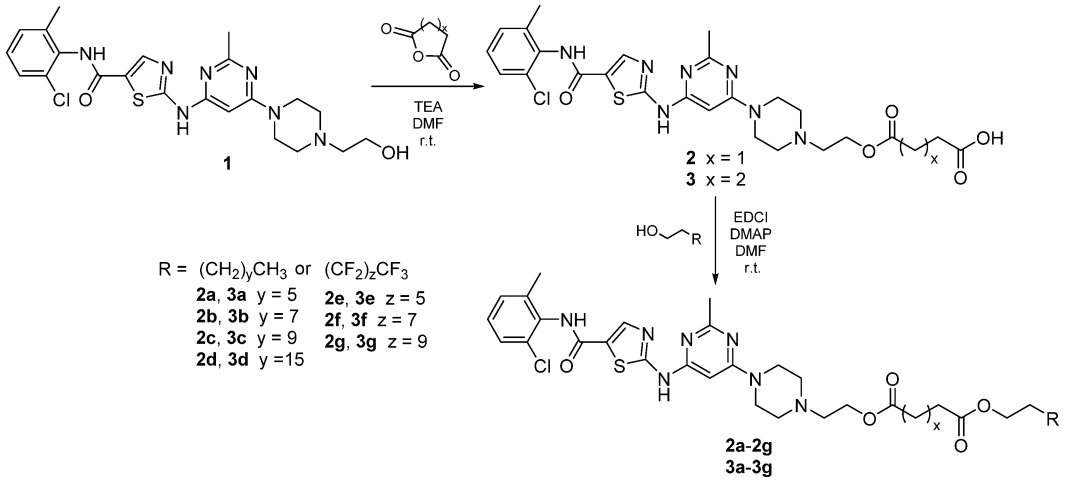
Previous studies showed the antiproliferative activity of **1** decreases when the part of the molecule that interacts with the kinase ATP binding site is modified.[1,2,39] Hence, the hydroxyl group is the preferred position for derivatization.[23,40,41] Various modifications of the hydroxyl group have been explored,[42−44] and it has been shown that derivatization of this position alters the selectivity of tyrosine kinase inhibition without necessarily reducing antiproliferative activity.[45]

Here, we describe the derivatization of **1** with a series of alkyl and perfluoroalkyl chains in order to increase the lipophilicity of the drug while maintaining the integrity of the region that directly interacts with the kinase active site (Figure 1).[46−48]

Received: December 3, 2014
Accepted: January 30, 2015
Published: January 30, 2015

DOI: 10.1021/ml500496u
ACS Med. Chem. Lett. 2015, 6, 313−317

Scheme 1. Synthesis of Alkyl (2a−2g) and Perfluoroalkyl (3a−3g) Derivatives of 1



The *in vitro* and *in vivo* properties of the new compounds were explored with improved selectivity observed *in vitro* that translates to superior angiostatic activity *in vivo*.

Dasatinib **1** was modified following a two-step route (Scheme 1) in which a hydrophobic chain is connected to the hydroxyl group of the drug via either a succinic group (**2a−2g**) or a glutaric group (**3a−3g**). Intermediates **2** and **3** were obtained by ring opening of succinic and glutaric anhydride under basic conditions. Coupling with the alkyl and perfluoroalkyl alcohols was performed in the presence of *N*-(3-(dimethylamino)propyl)-*N*′-ethylcarbodiimide hydrochloride (EDCI), used to activate the carboxylic acid group, and 4-(dimethylamino)pyridine (DMAP), employed as a catalyst. The compounds were obtained in yields of 49−72% for the succinic (**2a−2g**) series and only 28−65% for the glutaric (**2a−2g**) series, with the yield decreasing as the length of the hydrophobic chain increases. All compounds were fully characterized by ^{1}H, ^{13}C, and ^{19}F (where appropriate) NMR spectroscopy, ESI mass spectrometry, IR spectroscopy, and elemental analysis (see Supporting Information for details).

The cytotoxicity of **1**, **2a−2g**, and **3a−3g** was evaluated on human A2780 ovarian carcinoma cell line, ECRF24 immortalized endothelial cells, and noncancerous human HEK-293 embryonic kidney cells (Table 1). The inhibitory activity of the parent drug **1** to the three cells lines is similar, showing limited selectivity. Although all compounds with alkyl or perfluorinated chains exhibit a reduced inhibitory activity, significant changes in selectivity for the three cell lines were observed. Compounds **2a** and **3a**, with the shortest alkyl chains, have the largest effect on A2780 cancer cells (IC_{50} = 10.6 and 12 μM, respectively), inhibiting cell proliferation slightly less effectively than **1** (8.7 μM). Compound **2a** also showed some selectivity for A2780 and ECRF24, being less toxic to the HEK-293 cells (52 μM). Compound **2b**, with a longer alkyl chain than **2a**, is less cytotoxic for all cell lines, whereas **3b** lost activity against A2780 cells while maintaining a moderate inhibitory effect on ECRF24 and HEK-293 cells. As the length of the chains increase the compounds tend to become less inhibitory for A2780 cells and inactive (IC_{50} > 500 μM) on HEK-293 cells. An exception to this overall trend is **2e**, which has an IC_{50} value of 58 μM in the HEK-293 cell line. The compounds retained a reasonable activity toward ECRF24 cells, and there appears to be little difference between compounds with either an alkyl or perfluorinated chain. There is no clear correlation between lipophilicity (see Supporting Information for calculated log P values) and the IC_{50} values, suggesting that other physicochemical properties (e.g., the nature and rigidity of the chain) play an important role.

Table 1. IC_{50} Values (μM) Determined for Dasatinib (1) and Derivatives 2a−2g and 3a−3g Towards Human A2780 Ovarian Carcinoma, Human Immortalized ECRF24 Endothelial Cells, and Human HEK-293 Embryonic Kidney Cells; Values Are Given as Means ± SD

compd	A2780	ECRF24	HEK-293
1	8.7 ± 0.5	5.7 ± 0.3	14.3 ± 2.9
2a	10.6 ± 0.1	7.8 ± 2.4	52 ± 6
2b	22.5 ± 2.7	30 ± 5	46 ± 6
2c	45 ± 1	27 ± 3	>500
2d	108 ± 4	105 ± 12	>500
2e	130 ± 8	122 ± 0.5	58 ± 1
2f	118 ± 8	96 ± 7	>500
2g	90 ± 2	24 ± 2	>500
3a	12 ± 2.5	18 ± 2	18 ± 1
3b	119 ± 16	34 ± 5	29 ± 1.6
3c	53 ± 5	135 ± 4	>500
3d	125 ± 4	101 ± 11	>500
3e	28.3 ± 3.3	>500	>500
3f	121 ± 20	57 ± 2	>500
3g	>500	102 ± 2	>500

Compounds **2d**, **2f**, **2g**, and **3f** showed higher cytotoxicity to the ECRF24 cells while retaining tumor selectivity, i.e., they are noninhibitory for the noncancerous HEK-293 cells but are cytotoxic to A2780 cancer cells. Consequently, **2d**, **2f**, **2g**, and **3f** were tested for their effects on blood vessel development in the chorioallantoic membrane (CAM) assay of the chicken embryo to evaluate their antiangiogenic potential (Figure 2). The compounds were administered via a daily i.v. injection (20 μM, 100 μL/day for 3 consecutive days) between embryo development days (EDDs) 11 and 13, followed by imaging of the CAM vasculature on EDD 14 (Figure 2A).

The antiangiogenic effect of **1** was found to be moderate under the applied conditions, inhibiting the formation of vasculature by 8%, at a concentration of 20 μM. Interestingly, more pronounced efficacies on *in vivo* blood vessel formation were observed for some of the derivatives. Compound **2g** inhibited angiogenesis by 18%, and the angiostatic activity of **3f**

DOI: 10.1021/ml500496u
ACS Med. Chem. Lett. 2015, 6, 313−317

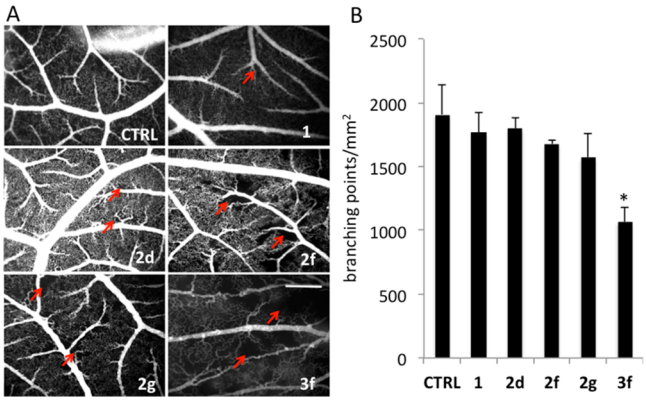


Figure 2. Activity of **1**, **2d**, **2f**, **2g**, and **3f** in the CAM model. (A) Representative fluorescence angiographies taken on EDD14. Red arrows show examples of the avascular regions. Bar in lower right panel represents 400 μm. (B) Branching points/mm^2 after application of **1**, **2d**, **2f**, **2g**, and **3f** on the CAM administered intravenously at (20 μM, 100 μL/day for three consecutive days). Error bars represent the standard error of the mean. *p < 0.05 is considered statistically significant.

was significantly higher with the number of capillaries decreasing by 45% (p < 0.05, Figure 2B). It should be emphasized that these effects were observed at a dose of 20 μM, which is at least 25-fold lower than the dose needed to inhibit the proliferation of noncancerous HEK-293 cells.

An important feature of angiogenesis is the motility of endothelial cells and, to confirm the angiostatic effect *in vivo*, this property was measured in an endothelial cells migration assay. Both **1** and **3f** diminished mobility of ECRF24 cells in a dose-dependent manner (Figure 3). Compound **3f** at a dose of

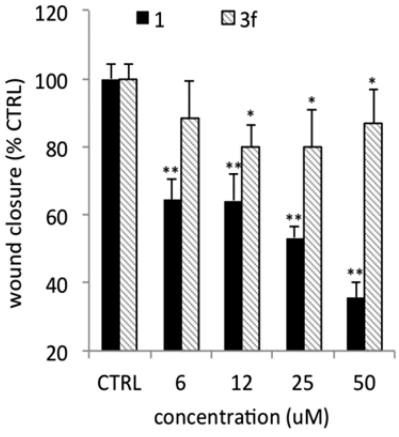


Figure 3. Effect of **1** and **3f** in the endothelial cell (ECRF24) migration assay. Concentration-dependent wound closure in ECRF24 cultures after 7 h of incubation with **1** or **3f**. Error bars represent the standard error of the mean. *p < 0.05 and **p < 0.01 are considered statistically significant.

50 μM, approximately corresponding to the IC_{50} in the ECRF24 proliferation inhibition assay (Table 1), induced cell migration inhibition by 15%, compared to 65% inhibition by **1** at the dame dose. This difference suggests that the angiostatic effect of **3f** corresponds to an antiproliferative mechanism rather than inhibition of mobility.

To conclude, the facile modulation of the hydroxyl group in **1** with a series of alkyl and perfluoroalkyl chains modifies the activity profile of the drug leading to a change in the selectivity profile (drug sensitivity is reduced, whereas selectivity is increased). As the size of the chain increases, the compounds become considerably less active against A2780 cancer cells and inactive (IC_{50} > 500 μM) on human embryonic kidney cells, while in some cases retaining the effect toward ECRF24 cells. The overall effect of these changes is to enhance the angiostatic properties of the compounds, especially in the case of **3f**, most likely through an antiproliferative mechanism.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Experimental procedures including synthesis and characterization of the compounds and *in vitro* and *in vivo* assays. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Phone: +41-21-6939854. Fax: +41-21-6939853. E-mail: paul.dyson@epfl.ch.

Author Contributions

All authors have given approval to the final version of the manuscript.

Funding

We thank the Swiss National Science Foundation and EPFL for financial support.

Notes

The authors declare no competing financial interest.

■ ABBREVIATIONS

A2780, human ovarian carcinoma cells; ATP, adenosine triphosphate; Bcr/Abl, breakpoint cluster region-Abelson oncogene; CAM, chorioallantoic membrane; DMF, *N*,*N*-dimethylformamide; DMAP, 4-(dimethylamino)pyridine; EDCI, *N*-(3-(dimethylamino)propyl)-*N*′-ethylcarbodiimide hydrochloride; ECRF24, immortalized human vascular endothelial cells; EDD, embryo development day; HEK-293, human embryonic kidney cells; Src, proto-oncogenes coding the SRC tyrosine kinases

■ REFERENCES

(1) Lombardo, L. J.; Lee, F. Y.; Chen, P.; Norris, D.; Barrish, J. C.; Behnia, K.; Castaneda, S.; Cornelius, L. A. M.; Das, J.; Doweyko, A. M.; Fairchild, C.; Hunt, J. T.; Inigo, I.; Johnston, K.; Kamath, A.; Kan, D.; Klei, H.; Marathe, P.; Pang, S. H.; Peterson, R.; Pitt, S.; Schieven, G. L.; Schmidt, R. J.; Tokarski, J.; Wen, M. L.; Wityak, J.; Borzilleri, R. M. Discovery of N-(2-chloro-6-methylphenyl)-2-(6-(4-(2-hydroxyethyl)-piperazin-1-yl)-2-methylpyrimidin-4-ylamino)thiazole-5-carboxamide (BMS-354825), a dual Src/Abl kinase inhibitor with potent antitumor activity in preclinical assays. *J. Med. Chem.* **2004**, *47*, 6658−6661.

(2) Das, J.; Chen, P.; Norris, D.; Padmanabha, R.; Lin, J.; Moquin, R. V.; Shen, Z. Q.; Cook, L. S.; Doweyko, A. M.; Pitt, S.; Pang, S. H.; Shen, D. R.; Fang, Q.; de Fex, H. F.; McIntyre, K. W.; Shuster, D. J.; Gillooly, K. M.; Behnia, K.; Schieven, G. L.; Wityak, J.; Barrish, J. C. 2-aminothiazole as a novel kinase inhibitor template. Structure-activity relationship studies toward the discovery of N-(2-chloro-6-methylphenyl)-2-[[6-[4-(2-hydroxyethyl)-1-piperazinyl)]-2-methyl-4-pyrimidinyl]amino)]-1,3-thiazole-5-carboxamide (Dasatinib, BMS-354825) as a potent pan-Src kinase inhibitor. *J. Med. Chem.* **2006**, *49*, 6819−6832.

DOI: 10.1021/ml500496u
ACS Med. Chem. Lett. 2015, 6, 313−317

(3) Shah, N. P.; Tran, C.; Lee, F. Y.; Chen, P.; Norris, D.; Sawyers, C. L. Overriding imatinib resistance with a novel ABL kinase inhibitor. *Science* **2004**, *305*, 399−401.
(4) Kantarjian, H.; Jabbour, E.; Grimley, J.; Kirkpatrick, P. Dasatinib. *Nat. Rev. Drug Discovery* **2006**, *5*, 717−718.
(5) Talpaz, M.; Shah, N. P.; Kantarjian, H.; Donato, N.; Nicoll, J.; Paquette, R.; Cortes, J.; O'Brien, S.; Nicaise, C.; Bleickardt, E.; Blackwood-Chirchir, M. A.; Iyer, V.; Chen, T. T.; Huang, F.; Decillis, A. P.; Sawyers, C. L. Dasatinib in imatinib-resistant Philadelphia chromosome-positive leukemias. *N. Engl. J. Med.* **2006**, *354*, 2531−2541.
(6) Cortes, J.; Rousselot, P.; Kim, D. W.; Ritchie, E.; Hamerschlak, N.; Coutre, S.; Hochhaus, A.; Guilhot, F.; Saglio, G.; Apperley, J.; Ottmann, O.; Shah, N.; Erben, P.; Branford, S.; Agarwal, P.; Gollerkeri, A.; Baccarani, M. Dasatinib induces complete hematologic and cytogenetic responses in patients with imatinib-resistant or -intolerant chronic myeloid leukemia in blast crisis. *Blood* **2007**, *109*, 3207−3213.
(7) Steinberg, M. Dasatinib: A tyrosine kinase inhibitor for the treatment of chronic myelogenous leukemia and Philadelphia chromosome-positive acute lymphoblastic leukemia. *Clin. Ther.* **2007**, *29*, 2289−2308.
(8) Hochhaus, A.; Kantarjian, H. The development of dasatinib as a treatment for chronic myeloid leukemia (CML): from initial studies to application in newly diagnosed patients. *J. Cancer. Res. Clin. Oncol.* **2013**, *139*, 1971−1984.
(9) Weisberg, E.; Manley, P. W.; Cowan-Jacob, S. W.; Hochhaus, A.; Griffin, J. D. Second generation inhibitors of BCR-ABL for the treatment of imatinib-resistant chronic myeloid leukaemia. *Nat. Rev. Cancer* **2007**, *7*, 345−356.
(10) Frame, M. C. Src in cancer: deregulation and consequences for cell behaviour. *Biochim. Biophys. Acta* **2002**, *1602*, 114−130.
(11) Haskell, M. D.; Slack, J. K.; Parsons, J. T.; Parsons, S. J. c-Src tyrosine phosphorylation of epidermal growth factor receptor, P190 RhoGAP, and focal adhesion kinase regulates diverse cellular processes. *Chem. Rev.* **2001**, *101*, 2425−2440.
(12) Eustace, A. J.; Crown, J.; Clynes, M.; O'Donovan, N. Preclinical evaluation of dasatinib, a potent Src kinase inhibitor, in melanoma cell lines. *J. Transl. Med.* **2008**, *6*, 53.
(13) Song, L. X.; Morris, M.; Bagui, T.; Lee, F. Y.; Jove, R.; Haura, E. B. Dasatinib (BMS-354825) selectively induces apoptosis in lung cancer cells dependent on epidermal growth factor receptor signaling for survival. *Cancer Res.* **2006**, *66*, 5542−5548.
(14) Johnson, F. M.; Saigal, B.; Talpaz, M.; Donato, N. J. Dasatinib (BMS-354825) tyrosine kinase inhibitor suppresses invasion and induces cell cycle arrest and apoptosis of head and neck squamous cell carcinoma and non-small cell lung cancer cells. *Clin. Cancer. Res.* **2005**, *11*, 6924−6932.
(15) Tsao, A. S.; He, D. D.; Saigal, B.; Liu, S. Y.; Lee, J. J.; Bakkannagari, S.; Ordonez, N. G.; Hong, W. K.; Wistuba, I.; Johnson, F. M. Inhibition of c-Src expression and activation in malignant pleural mesothelioma tissues leads to apoptosis, cell cycle arrest, and decreased migration and invasion. *Mol. Cancer Ther.* **2007**, *6*, 1962−1972.
(16) Shor, A. C.; Keschman, E. A.; Lee, F. Y.; Muro-Cacho, C.; Letson, G. D.; Trent, J. C.; Pledger, W. J.; Jove, R. Dasatinib inhibits migration and invasion in diverse human sarcoma cell lines and induces apoptosis in bone sarcoma cells dependent on Src kinase for survival. *Cancer Res.* **2007**, *67*, 2800−2808.
(17) Serrels, A.; Macpherson, I. R. J.; Evans, T. R. J.; Lee, F. Y.; Clark, E. A.; Sansom, O. J.; Ashton, G. H.; Frame, M. C.; Brunton, V. G. Identification of potential biomarkers for measuring inhibition of Src kinase activity in colon cancer cells following treatment with dasatinib. *Mol. Cancer Ther.* **2006**, *5*, 3014−3022.
(18) Nam, S.; Kim, D. W.; Cheng, J. Q.; Zhang, S. M.; Lee, J. H.; Buettner, R.; Mirosevich, J.; Lee, F. Y.; Jove, R. Action of the Src family kinase inhibitor, dasatinib (BMS-354825), on human prostate cancer cells. *Cancer Res.* **2005**, *65*, 9185−9189.
(19) Park, S. I.; Zhang, J.; Phihips, K. A.; Araujo, J. C.; Najjar, A. M.; Volgin, A. Y.; Gelovani, J. G.; Kim, S. J.; Wang, Z. X.; Gallick, G. E. Targeting src family kinases inhibits growth and lymph node metastases of prostate cancer in an orthotopic nude mouse model. *Cancer Res.* **2008**, *68*, 3323−3333.
(20) Bantscheff, M.; Eberhard, D.; Abraham, Y.; Bastuck, S.; Boesche, M.; Hobson, S.; Mathieson, T.; Perrin, J.; Raida, M.; Rau, C.; Reader, V.; Sweetman, G.; Bauer, A.; Bouwmeester, T.; Hopf, C.; Kruse, U.; Neubauer, G.; Ramsden, N.; Rick, J.; Kuster, B.; Drewes, G. Quantitative chemical proteomics reveals mechanisms of action of clinical ABL kinase inhibitors. *Nat. Biotechnol.* **2007**, *25*, 1035−1044.
(21) Karaman, M. W.; Herrgard, S.; Treiber, D. K.; Gallant, P.; Atteridge, C. E.; Campbell, B. T.; Chan, K. W.; Ciceri, P.; Davis, M. I.; Edeen, P. T.; Faraoni, R.; Floyd, M.; Hunt, J. P.; Lockhart, D. J.; Milanov, Z. V.; Morrison, M. J.; Pallares, G.; Patel, H. K.; Pritchard, S.; Wodicka, L. M.; Zarrinkar, P. P. A quantitative analysis of kinase inhibitor selectivity. *Nat. Biotechnol.* **2008**, *26*, 127−132.
(22) Hantschel, O.; Rix, U.; Superti-Furga, G. Target spectrum of the BCR-ABL inhibitors imatinib, nilotinib and dasatinib. *Leukemia Lymphoma* **2008**, *49*, 615−619.
(23) Li, J. N.; Rix, U.; Fang, B.; Bai, Y.; Edwards, A.; Colinge, J.; Bennett, K. L.; Gao, J. C.; Song, L. X.; Eschrich, S.; Superti-Furga, G.; Koomen, J.; Haura, E. B. A chemical and phosphoproteomic characterization of dasatinib action in lung cancer. *Nat. Chem. Biol.* **2010**, *6*, 291−299.
(24) Nam, S.; Kim, D.; Cheng, J. Q.; Zhang, S.; Lee, J. H.; Buettner, R.; Mirosevich, J.; Lee, F. Y.; Jove, R. Action of the Src family kinase inhibitor, dasatinib (BMS-354825), on human prostate cancer cells. *Cancer Res.* **2005**, *65*, 9185−9189.
(25) Araujo, J.; Logothetis, C. Dasatinib: a potent SRC inhibitor in clinical development for the treatment of solid tumors. *Cancer Treat. Rev.* **2010**, *36*, 492−500.
(26) Allgayer, H.; Boyd, D. D.; Heiss, M. M.; Abdalla, E. K.; Curley, S. A.; Gallick, G. E. Activation of Src kinase in primary colorectal carcinoma - An indicator of poor clinical prognosis. *Cancer* **2002**, *94*, 344−351.
(27) Chang, Y. M.; Bai, L.; Liu, S.; Yang, J. C.; Kung, H. J.; Evans, C. P. Src family kinase oncogenic potential and pathways in prostate cancer as revealed by AZD0530. *Oncogene* **2008**, *27*, 6365−6375.
(28) Duxbury, M. S.; Ito, H.; Zinner, M. J.; Ashley, S. W.; Whang, E. E. Inhibition of Src tyrosine kinase impairs inherent and acquired gemcitabine resistance in human pancreatic adenocarcinoma cells. *Clin. Cancer. Res.* **2004**, *10*, 2307−2318.
(29) Yezhelyev, M. V.; Koehl, G.; Guba, M.; Brabletz, T.; Jauch, K. W.; Ryan, A.; Barge, A.; Green, T.; Fennell, M.; Bruns, C. J. Inhibition of Src tyrosine kinase as treatment for human pancreatic cancer growing orthotopically in nude mice. *Clin. Cancer. Res.* **2004**, *10*, 8028−8036.
(30) Serrels, B.; Serrels, A.; Mason, S. M.; Baldeschi, C.; Ashton, G. H.; Canel, M.; Mackintosh, L. J.; Doyle, B.; Green, T. P.; Frame, M. C.; Sansom, O. J.; Brunton, V. G. A novel Src kinase inhibitor reduces tumour formation in a skin carcinogenesis model. *Carcinogenesis* **2009**, *30*, 249−257.
(31) Rice, L.; Lepler, S.; Pampo, C.; Siemann, D. W. Impact of the SRC inhibitor dasatinib on the metastatic phenotype of human prostate cancer cells. *Clin. Exp. Metastasis* **2012**, *29*, 133−142.
(32) Nautiyal, J.; Majumder, P.; Patel, B. B.; Lee, F. Y.; Majumdar, A. P. N. Src inhibitor dasatinib inhibits growth of breast cancer cells by modulating EGFR signaling. *Cancer Lett.* **2009**, *283*, 143−151.
(33) Coluccia, A. M.; Cirulli, T.; Neri, P.; Mangieri, D.; Colanardi, M. C.; Gnoni, A.; Di Renzo, N.; Dammacco, F.; Tassone, P.; Ribatti, D.; Gambacorti-Passerini, C.; Vacca, A. Validation of PDGFRbeta and c-Src tyrosine kinases as tumor/vessel targets in patients with multiple myeloma: preclinical efficacy of the novel, orally available inhibitor dasatinib. *Blood* **2008**, *112*, 1346−1356.
(34) Liang, W.; Kujawski, M.; Wu, J.; Lu, J. M.; Herrmann, A.; Loera, S.; Yen, Y.; Lee, F.; Yu, H.; Wen, W.; Jove, R. Antitumor Activity of Targeting Src Kinases in Endothelial and Myeloid Cell Compartments of the Tumor Microenvironment. *Clin. Cancer Res.* **2010**, *16*, 924−935.
(35) Mineo, M.; Garfield, S. H.; Taverna, S.; Flugy, A.; De Leo, G.; Alessandro, R.; Kohn, E. C. Exosomes released by K562 chronic

DOI: 10.1021/ml500496u
ACS Med. Chem. Lett. 2015, 6, 313−317

myeloid leukemia cells promote angiogenesis in a src-dependent fashion. *Angiogenesis* **2012**, *15*, 33−45.
(36) Heine, A.; Held, S. A. E.; Bringmann, A.; Holderried, T. A. W.; Brossart, P. Immunomodulatory effects of anti-angiogenic drugs. *Leukemia* **2011**, *25*, 899−905.
(37) Chung, A. S.; Ferrara, N. Targeting the tumor microenvironment with SRC kinase inhibition. *Clin. Cancer Res.* **2010**, *16*, 775−777.
(38) Saffran, V.; Siemann, D. Src Inhibitor dasatinib reduces the metastatic phenotype of breast cancer cells. *Mol. Cancer. Ther.* **2011**, *10*, B151.
(39) Liu, W.; Zhou, J. P.; Qi, F.; Bensdorf, K.; Li, Z. Y.; Zhang, H. B.; Qian, H.; Huang, W. L.; Cai, X. T.; Cao, P.; Wellner, A.; Gust, R. Synthesis and biological activities of 2-amino-thiazole-5-carboxylic acid phenylamide derivatives. *Arch. Pharm.* **2011**, *344*, 451−458.
(40) Shi, H. B.; Zhang, C. J.; Chen, G. Y. J.; Yao, S. Q. Cell-based proteome profiling of potential dasatinib targets by use of affinity-based probes. *J. Am. Chem. Soc.* **2012**, *134*, 3001−3014.
(41) Getlik, M.; Grutter, C.; Simard, J. R.; Kluter, S.; Rabiller, M.; Rode, H. B.; Robubi, A.; Rauh, D. Hybrid compound design to overcome the gatekeeper T338M mutation in cSrc. *J. Med. Chem.* **2009**, *52*, 3915−3926.
(42) Liu, F.; Lang, L. W.; Jiang, J.; Lu, H. J.; Wang, J. M.; Wang, S. C. Synthesis and biopharmaceutical studies of JLTN as potential dasatinib prodrug. *Chem. Pharm. Bull.* **2013**, *61*, 877−881.
(43) Kim, K. S.; Lee, F. Y.; Lombardo, L. J.; Luo, F. R.; Wityak, J. Prodrugs of Protein Tyrosine Kinase Inhibitors. U.S. Pat. Apl. 20060069101 A1, March 30, 2006..
(44) Riggs-Sauthier, J.; Zhang, W. Oligomer-Protein Tyrosine Kinase Inhibitor Conjugates As Antitumor Agents. U.S. Patent 8816077 B2, August 26, 2014.
(45) Tiwari, R. K.; Brown, A.; Shirazi, A. N.; Bolton, J.; Sun, G. Q.; Parang, K. Dasatinib-Fatty Acid Conjugates: Synthesis and Evaluation of Tyrosine Kinase Inhibitory and Anticancer Activities. In *The 244th American Chemical Society National Meeting & Exposition*, Philadelphia, PA, August 19−23, **2012**.
(46) Clavel, C. M.; Zava, O.; Schmitt, F.; Kenzaoui, B. H.; Nazarov, A. A.; Juillerat-Jeanneret, L.; Dyson, P. J. Thermoresponsive chlorambucil derivatives for tumour targeting. *Angew. Chem. Int. Ed.* **2011**, *50*, 7124−7127.
(47) Clavel, C. M.; Paunescu, E.; Nowak-Sliwinska, P.; Dyson, P. J. Thermoresponsive organometallic arene ruthenium complexes for tumour targeting. *Chem. Sci.* **2014**, *5*, 1097−1101.
(48) Clavel, C. M.; Paunescu, E.; Nowak-Sliwinska, P.; Griffioen, A. W.; Scopelliti, R.; Dyson, P. J. Discovery of a highly tumor-selective organometallic ruthenium(II)-arene complex. *J. Med. Chem.* **2014**, *57*, 3546−3558.

DOI: 10.1021/ml500496u
ACS Med. Chem. Lett. 2015, 6, 313−317