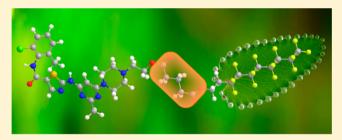


Improved Angiostatic Activity of Dasatinib by Modulation with **Hydrophobic Chains**

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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

asatinib, [N-(2-chloro-6-methylphenyl)-2-[[6-[4-(2-hy-6-methylphenyl)-2-[6-[4-(2-hy-6-(2-methylphenyl)-2-[6-[4-(2-hy-6-(2-methylphenyl)-2-[6-[4-(2-hy-6-(2-methylphenyl)-2-[6-[4-(2-hy-6-(2droxyethyl)-1-piperazinyl]-2-methyl-4-pyrimidinyl]amino]-5-thiazole carboxamide 1 (Figure 1), is a potent, orally

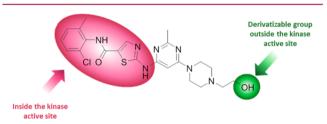


Figure 1. Structure of dasatinib 1 highlighting the part of the structure that interacts with kinases and the site that can be modified without impeding kinase inhibition.

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}

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 proangiogenic 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, 42-4 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

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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 ¹H, ¹³C, and ¹⁹F (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 (IC₅₀ = 10.6 and 12 μ M, respectively), inhibiting cell proliferation slightly less effectively than 1 (8.7 μ M). Compound 2a also showed some selectivity for A2780 and ECRF24, being less toxic to the HEK-293 cells (52 μ M). 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 (IC₅₀ > 500 μ M) on HEK-293 cells. An exception to this overall trend is 2e, which has an IC₅₀ value of $58 \mu M$ 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

Table 1. IC₅₀ Values (μ M) 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 \pm 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

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.

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 μ M, 100 μ L/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 μ M. 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

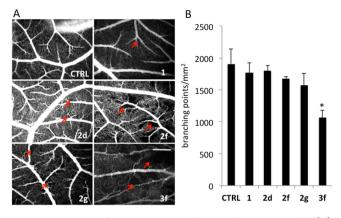


Figure 2. Activity of 1, 2d, 2f, 2g, and 3f in the CAM model. (A) Representative fluorescence angiographies taken on EDD14. Red arrows show examples of the avascular regions. Bar in lower right panel represents 400 μ m. (B) Branching points/mm² after application of 1, 2d, 2f, 2g, and 3f on the CAM administered intravenously at (20 μ M, 100 μ L/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 μ M, 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

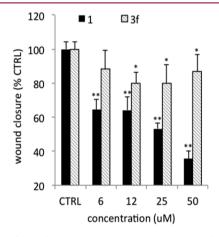


Figure 3. Effect of **1** and **3f** in the endothelial cell (ECRF24) migration assay. Concentration-dependent wound closure in ECRF24 cultures after 7 h of incubation with **1** or **3f**. Error bars represent the standard error of the mean. *p < 0.05 and **p < 0.01 are considered statistically significant.

 $50~\mu\text{M}$, approximately corresponding to the IC₅₀ 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 $_{50} > 500~\mu \rm M)$ 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.

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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

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