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Design, Synthesis, and Characterization of a Photoactivatable Caged Prodrug of Imatinib

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Imatinib is the first protein kinase inhibitor approved for clinical use and is a seminal drug for the concept of targeted therapy. Herein we report on the design, synthesis, photokinetic properties, and in vitro enzymatic evaluation of a photoactivatable caged prodrug of imatinib. This approach allows spatial and temporal control over the activation of imatinib triggered by ultraviolet light. The successful application of the photoactivation concept to this significant kinase inhibitor provides further evidence for the caging technique as a feasible approach in the kinase field. The presented photoactivatable imatinib prodrug will be highly useful as a pharmacological tool to study the impact of imatinib toward biological systems in greater detail.

Since the introduction of the tyrosine kinase inhibitor imatinib[1] (Gleevec/Glivec) into the market in 2001, protein kinase inhibitors have been successfully established mainly in targeted/personalized therapeutic approaches against cancer.[2] Originally, the prototypical type-II inhibitor[3] imatinib was approved for the therapy of Philadelphia chromosome-positive chronic myelogenous leukemia (Ph^+-CML).[4] In this way, at the molecular level the drug targets the Abelson murine leukemia viral oncogene homolog 1 (ABL1).[5] In addition to ABL1, it was discovered that imatinib also potently inhibits further tyrosine kinases involved in malignancies including c-KIT and platelet-derived growth factor receptor (PDGF-R).[6] Therefore, imatinib has become an option for the treatment of multiple cancers including gastrointestinal tumors (GIST).[7,8] Along with the fact that significant progress has been made in cancer therapy, imatinib has also been used in countless experimental in vitro and in vivo studies.[9–12] Considering the prominent role of this multi-kinase inhibitor in signal-transduction analysis, it would be beneficial to develop a photoactivatable imatinib prodrug.

Therefore, we aimed to provide a so-called caged compound that can be activated by irradiation with ultraviolet (UV) light.[13] In general, the implementation of a photoremovable protecting group (PPG)[14] provides spatial and temporal control over the release of a bioactive molecule.[15] As consequence, significant concentrations of the bioactive compound can be generated at a defined time point in an irradiated area of interest.[16,17] The caged prodrug concept is essentially based on covalently blocking a pharmacophore moiety by a PPG, rendering the compound biologically inactive.[18] For this purpose *o*-nitrobenzylic[19] and coumarylmethyl[20] derivatives have been widely used, among others, as PPGs in various biological applications.[21–23] There are currently only few reports on photoactivatable kinase inhibitors. These include a caged small-molecule Rho kinase inhibitor,[24] equivalents of Src kinase,[21] and peptidic PKA inhibitors.[25] Recently, a study about photo-switchable RET kinase inhibitors was published.[26]

In this study, we set out to design, synthesize, and characterize a photoactivatable derivative of imatinib. First, by using molecular modeling we sought to define a suitable pharmacophoric position in imatinib to covalently attach the PPG. For this purpose we docked the type-II binder imatinib into the active site of our in-house PDGF-Rβ homology model in the DFG-out conformation.[27] We also chose this receptor tyrosine kinase, as imatinib potently blocks PDGF-Rβ ($IC_{50} = 0.1$ μM),[28] and an enzymatic PDGF-Rβ assay has been established by our research group to test the compounds.[27,29]

The modeled ligand interaction diagram of imatinib in the ATP binding pocket of PDGF-Rβ revealed two prominent positions to covalently attach a PPG (Figure 1). Namely, the NH functions of the amide in the benzanilide and the *N*-arylpyrimidine-2-amine moiety both address key hydrogen bonds to PDGF-Rβ. Moreover, the tight ligand–protein complex suggested no steric tolerance at these positions within the binding pocket. In line with this notion, modeling of both *N*-*o*-nitrobenzyl-substituted imatinib derivatives in the active site of PDGF-Rβ did not result in plausible binding modes (not shown).

To determine if one of these NH positions in imatinib would be chemically suitable for subsequent PPG photocleavage, we first synthesized an imatinib fragment bound to 4,5-dimethoxy-2-nitrobenzyl (compound **1**, Figure 2; the *N*-[(2-nitrophenyl)methyl]-*N*-phenylpyrimidin-2-amine fragment will be investigated in due course of this project). Next, a 1 mM DMSO solution of **1** was subjected to irradiation (see the Supporting Information for UV spectrum and details). We found that photoinduced cleavage of compound **1** at $\lambda = 365$ nm (5.4 W) resulted in rapid and nearly quantitative cleavage under these conditions, yielding the parent benzanilide fragment (and the leaving group 4,5-dimethoxy-2-nitrosobenzaldehyde based on MS analysis, not shown) within 5 min (Figure 3). We therefore subsequently focused on the amide NH function in imatinib to attach the PPG.

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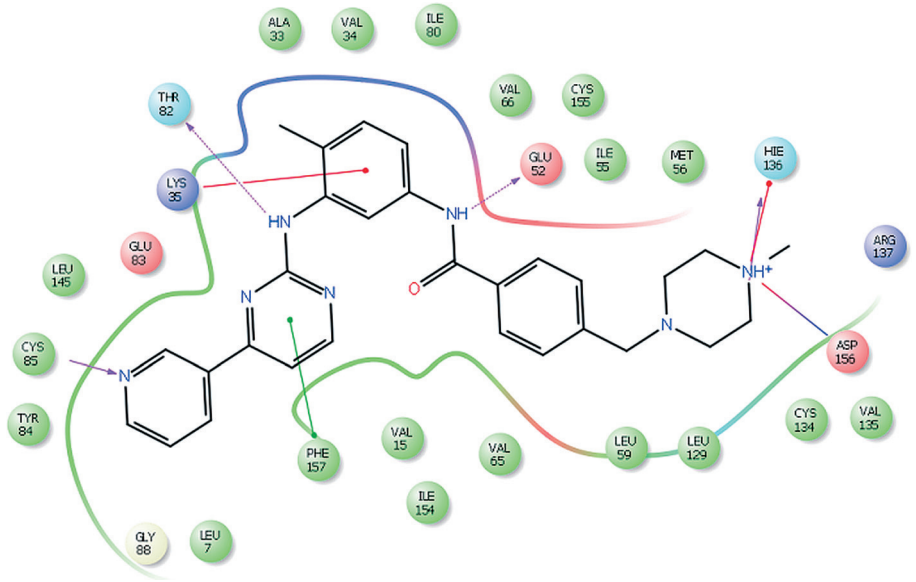


Figure 1. Modeled ligand interaction diagram of imatinib in the active site of a PDGF-Rβ homology model in the DFG-out conformation. Key ligand–protein interactions are shown. The binding mode of imatinib in the closely related kinase c-Src (PDB ID: 2OIQ)[30] is highly similar (not shown).

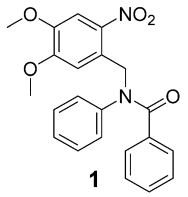


Figure 2. Photoactivatable imatinib fragment **1.** The compound contains the benzanilide scaffold of imatinib which is caged at the amide with the 4,5-dimethoxy-2-nitrobenzyl (DMNB) moiety.

Motivated by these results, we designed two differently PPG-caged prodrugs at the amide function of imatinib that bears the 4,5-dimethoxy-2-nitrobenzyl (DMNB, **2**) and coumarylmethyl moieties (**3**), respectively (Figure 4). Both PPGs have been reported to be suitable for caged prodrug applications in biological settings.[22]

We aimed to develop a straightforward synthetic strategy for the preparation of the planned caged prodrugs. Initially, our attempts toward base-catalyzed S_N reactions directly with imatinib and 1-(bromomethyl)-4,5-dimethoxy-2-nitrobenzene were unsuccessful, as only the imatinib–piperazine *N*-DMNB alkylated product could be isolated (which was stable under irradiation; see Supporting Information). However, an S_N reaction using the core scaffold of imatinib 4-methyl-*N*3-[4-(3-pyridyl)pyrimidin-2-yl]benzene-1,3-diamine **4** with the benzyl-brominated PPG reagents DMNB-Br or coumarylbromomethyl yielded compounds **5a** and **5b**, respectively (Scheme 1). In turn, introduction of the 4-[(4-methylpiperazin-1-yl)methyl]benzamide side chain in the final step yielded both PPG-protected compounds **2** and **3**, respectively (Scheme 1).

With these caged prodrugs of imatinib in hand, we next evaluated their photochemical properties. Based on the UV/Vis spectra of the compounds, we irradiated 1 mM solutions of **2** and **3** in DMSO for 12 min with light at a wavelength of 365 nm. Under these conditions, photocleavage of **3** was very poor, yielding only ~10% of imatinib (Supporting Information). In contrast, **2** was efficiently photocleaved within 10 min to produce imatinib (Figure 5) along with the leaving group 4,5-dimethoxy-2-nitrosobenzaldehyde as determined by MS analysis (not shown).

We next characterized the biological activity of imatinib and caged prodrug **2** in an enzymatic PDGF-Rβ assay without and with UV irradiation at $\lambda = 365$ nm. In this assay imatinib was found to potently inhibit PDGF-Rβ ($IC_{50} = 0.059$ μM); this is in good agreement with the reported value ($IC_{50} = 0.1$ μM).[28] In line with our modeling data, caged compound **2** was signifi-

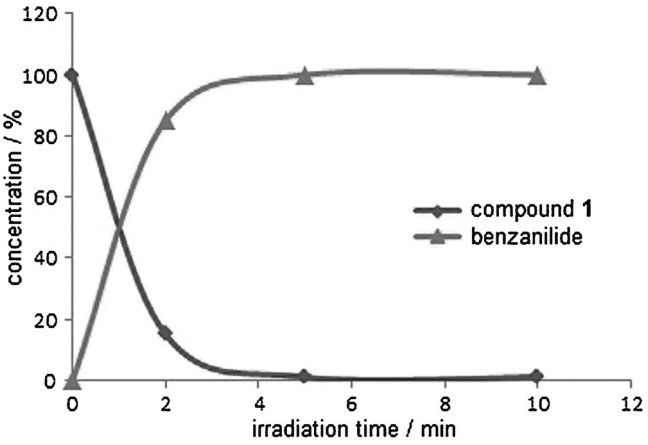


Figure 3. Time-dependent photoinduced cleavage of caged imatinib fragment **1** to yield benzanilide upon irradiation with UV light ($\lambda = 365$ nm, 5.4 W).

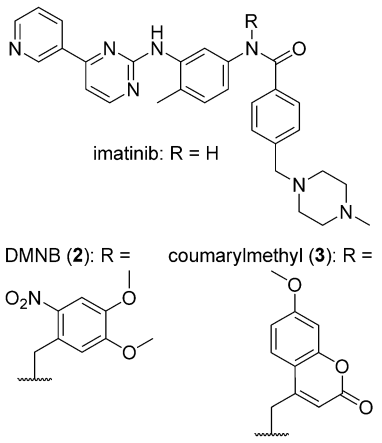
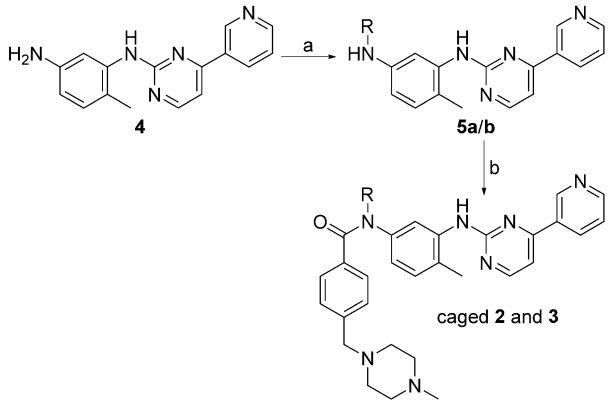


Figure 4. Chemical structures of imatinib and designed photoactivatable caged prodrugs **2** and **3**.



Scheme 1. Synthesis of caged imatinib derivatives **2** (R = DMNB) and **3** (R = coumarylmethyl). *Reagents and conditions*: a) DMNB-Br (for **5a**), 4-(bromomethyl)-7-methoxychromen-2-one (coumarylbromomethyl) (for **5b**), Li_2CO_3, DMF; b) 4-[(4-methylpiperazin-1-yl)methyl]benzoyl chloride, pyridine, 0 °C→RT. Full synthetic details are provided in the Supporting Information.

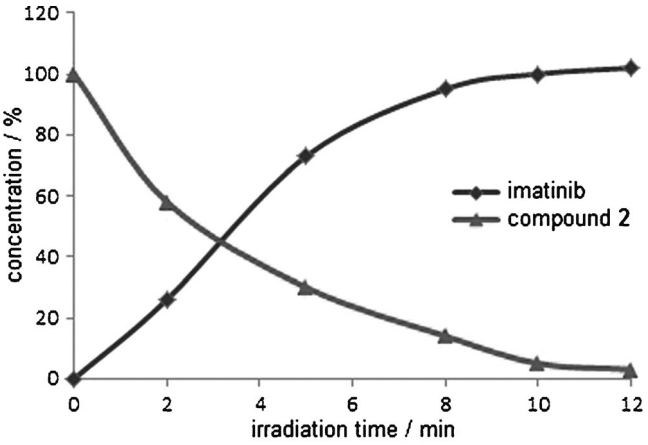


Figure 5. Time-dependent photoinduced cleavage of caged imatinib prodrug **2** upon irradiation with UV light ($\lambda = 365$ nm, 5.4 W). After 5 min irradiation, 70% of **3** was cleaved to produce imatinib.

cantly less biologically active without UV irradiation (**2** PDGF-Rβ $IC_{50} = 5.8$ μM). In contrast, testing **2** in the assay irradiated at $\lambda = 365$ nm (5.4 W) for 10 min revealed an inhibition of PDGF-Rβ ($IC_{50} = 0.089$ μM) similar to that of native imatinib (Figure 6). Notably, the residual biological activity of caged prodrug **2** results from minor impurities of uncaged imatinib in the sample (~1% based on HPLC analysis). Thus, at total compound concentrations >10 μM in the assay, the inhibitory effects on PDGF-Rβ cannot be differentiated. However, their difference regarding blockage of PDGF-Rβ is still significant in the range of typical in vitro assay concentrations (0.01–1 μM).

In conclusion, we have demonstrated that a caged prodrug of the kinase inhibitor imatinib could be successfully generated and characterized. Compound **2** was photoactivated by UV light at a wavelength of 365 nm within 10 min in an in vitro PDGF-Rβ assay to restore the biological activity of the parent imatinib. In contrast to imatinib, **1** can be almost quantitatively uncaged within 3 min. This indicates a significant influence of the leaving group (active inhibitor) toward the uncaging process. Thus, we will investigate further PPG systems in order to optimize the uncaging of imatinib. Our study further validates the powerful caging technique in the kinase field and provides a valuable pharmacological tool to study the biological effects of imatinib in novel settings and in greater detail. Finally, our approach holds great promise for future applications involving other kinase inhibitors as well.

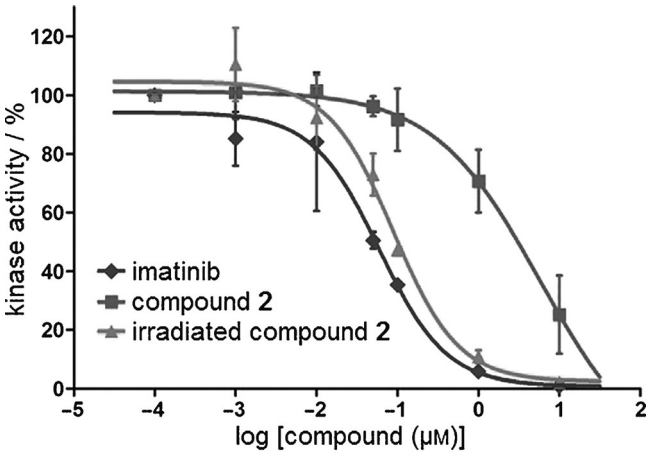


Figure 6. Biological activity of imatinib and caged prodrug **2** in an in vitro PDGF-Rβ assay without and with irradiation at $\lambda = 365$ nm (5.4 W) for 10 min (± SD, $n = 3$, see Supporting Information for details).

Experimental Section

All modeling was performed on a DELL 8 core system. For preparation, visualization, and building the 3D structures, Maestro (version 10.0.013, release 2014-4, Schrödinger LLC, New York, NY, USA) was used.

Synthesis of *N*-(4,5-dimethoxy-2-nitrobenzyl)-*N*-(4-methyl-3-((4-(pyridine-3-yl)pyrimidine-2-yl)amino)phenyl)-4-((4-methylpiperazin-1-yl)methyl)benzamide (2): Compound **5a** (330 mg, 700 μmol) was dissolved in dry pyridine (20 mL). The reaction mixture was cooled to 0 °C, and 4-[(4-methylpiperazin-1-yl)methyl]benzoyl chloride (288 mg, 1.00 mmol) was added in portions under a nitrogen atmosphere. The solution was stirred for 3 h at room temperature, and H_2O (100 mL) was added. The mixture was extracted with EtOAc (3×100 mL), and the combined organic layers were dried over anhydrous Na_2SO_4. The solvent was removed under vacuum, and the residue was purified by flash chromatography (SiO_2 reversed phase, MeOH/H_2O) to give compound **2** as a pale-yellow solid (40 mg, yield: 8%). ^{1}H NMR (300 MHz, [D_6]DMSO): $\delta = 2.09$ (s, 3H, pip-CH_3), 2.14 (s, 3H, CH_3), 2.21 (mc, 8H, pip-H-2,3,5,6), 3.33 (s, 2H, pip-CH_2), 3.74 (s, 3H, OCH_3), 3.82 (s, 3H, OCH_3), 5.38 (bs, 2H, CH_2), 6.79 (dd, 1H, $^3J = 8.1$ Hz, $^4J = 2.1$ Hz, ph-H-6), 7.03 (s, 1H, nitrobenz-H-6), 7.05 (d, 1H, $^3J = 8.1$ Hz, ph-H-5), 7.13 (d, 2H, $^3J = 8.2$ Hz, benz-H-3,5), 7.34 (d, 2H, $^3J = 8.2$ Hz, benz-H-2,6), 7.44 (d, 1H, $^3J = 5.2$ Hz, pyrim-H-5), 7.49 (ddd, 1H, $^3J = 8.0$ Hz, $^3J = 4.0$ Hz, pyr-H-5), 7.61 (s, 1H, nitrobenz-H-3), 7.62 (d, 1H, $^4J = 2.0$ Hz, ph-H-2), 8.36 (td, 1H, $^3J = 8.0$ Hz, $^4J = 2.0$ Hz, pyr-H-4), 8.44 (d, 1H, $^3J = 5.1$ Hz, pyrim-H-6), 8.69 (d, 1H, $^4J = 4.0$ Hz, pyr-H-6), 8.80 (s, 1H, pyrim-NH), 9.23 ppm (d, 1H, $^4J = 1.7$ Hz, pyr-H-2); ^{13}C NMR (75.5 MHz, [D_6]DMSO): $\delta = 17.5$ (q, CH_3), 45.6 (q, pip-CH_3), 50.6 (t, CH_2), 52.3, 54.6 (d, pip-C-2,3,5,6), 55.8, 55.9 (q, OCH_3), 61.4 (t, pip-CH_2), 107.9 (d, pyrim-C-5), 108.2 (d, nitrobenz-C-3), 110.5 (d, nitrobenz-C-6),

122.5 (d, ph-C-2), 122.9 (d, ph-C-6), 123.7 (d, pyr-C-5), 127.3 (s, nitrobenz-C-2), 128.1 (d, benz-C-3,5), 128.3 (d, benz-C-2,6), 129.5 (s, ph-C-1), 130.5 (d, ph-C-5), 131.9 (s, pyr-C-3), 134.1 (d, pyr-C-4), 134.3 (s, benz-C-4), 138.3 (s, ph-C-4), 139.9 (s, ph-C-3), 140.3 (s, benz-C-1), 140.7 (s, nitrobenz-C-1), 147.3 (s, nitrobenz-C-5), 148.0 (d, pyr-C-2), 151.4 (d, pyr-C-6), 152.9 (s, nitrobenz-C-4), 159.2 (d, pyrim-H-6), 160.6 (s, pyrim-C-4), 161.5 (s, pyrim-C-2), 169.9 ppm (s, C=O); IR (ATR): $\tilde{\nu}$ = 2836 (C–H), 1643 (C=O), 1574, 1330 (NO_2), 795 cm^{-1} (arom); MS (ESI, MeOH): *m/z* (%) = 689.1 (100) $[M+H]^+$. Further experimental details can be found in the Supporting Information.

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