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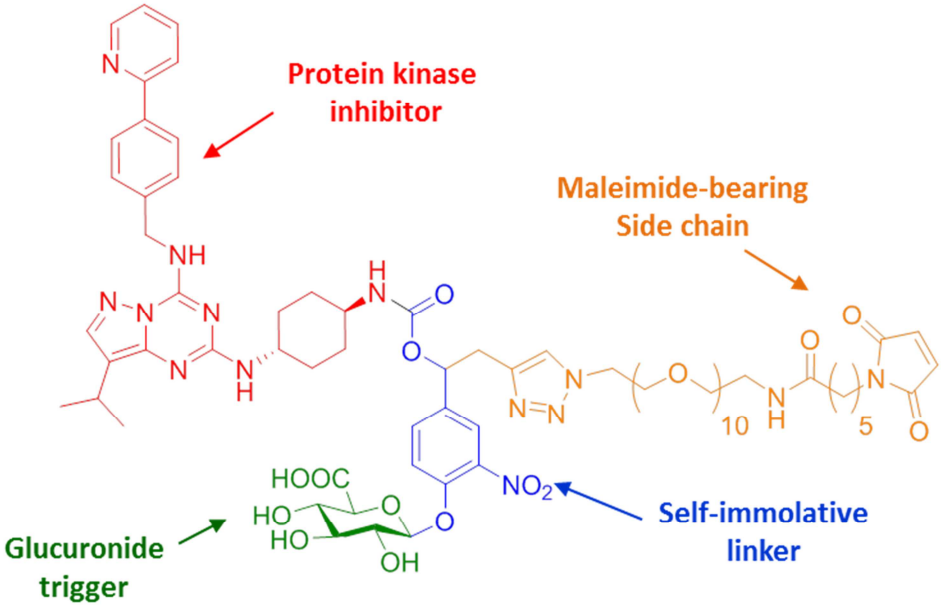

A β-glucuronidase-responsive albumin-binding prodrug for potential selective kinase inhibitor-based cancer chemotherapy

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Keywords: cancer, chemotherapy, drug delivery, self-immolative linker, β-glucuronidase-responsive albumin-binding prodrug, protein kinase inhibitor, tumour microenvironment

Abstract. We report on the synthesis and *in vitro* biological evaluations of a nanomolar protein kinase inhibitor (PKI) and its β-glucuronidase-responsive albumin-binding prodrug. The highly potent PKI is 400 TO 3400 times more cytotoxic than the well-known PKI Roscovitine. The prodrug is able to bind covalently to human serum albumin through Michael addition and release the cytotoxic PKI in the presence of β-glucuronidase, an enzyme over-expressed in the microenvironment of solid tumours.

Introduction

During the last two decades, protein kinase inhibitors have emerged as one of the most important class of anticancer agents and more than 28 PKIs already received Food and Drug Administration approval for applications in oncology [1–3]. However, almost all PKI developed so far induce severe adverse effects that hamper their therapeutic efficacy in clinic [4–7]. Therefore, the design of more selective PKIs is of great interest while remaining highly difficult since 518 genes encodes for kinase proteins [8]. An attractive alternative to skirt this challenging task relies on the development of molecular systems programmed for the selective delivery of PKI at the tumour site. Such a therapeutic approach is already validated in human with the clinical use of antibody-drug conjugates designed for the targeting of potent cytotoxics [9]. Within this framework, our research team developed recently the concept of β-glucuronidase-responsive albumin-binding prodrugs [10, 11]. These latter combine a glucuronide trigger [12, 13], a self-immolative linker [14, 15], a potent anticancer drug and a maleimide-bearing side chain in a single entity (Figure 1). Following intravenous administration, such prodrugs bind covalently to plasmatic albumin (step 1), thereby enabling their passive accumulation in tumours [16] where extracellular β-glucuronidase [17-20] launches the selective release of the targeted drug (step 2). This targeting strategy has led to outstanding therapeutic efficacy in mice, particularly for the treatment of triple-negative mammary and pancreatic cancers [11]. Thus, with the aim to enhance the selectivity of kinase inhibitor-based cancer chemotherapy, we now report on the study the β-glucuronidase-responsive albumin-binding prodrug **1** of the novel nanomolar PKI **2** (Figure 2).

Results and Discussion

In the course of our intensive efforts devoted to the discovery of new potent PKI, we focused on the synthesis of Roscovitine analogues (Figure 2) [21]. Roscovitine is indeed a well-known

cyclin dependant kinase inhibitor that showed promising results in various animal models, hence prompting its transfer to the clinic [22]. However, Roscovitine did not demonstrate significant anticancer therapeutic efficacy in human while inducing severe side effects due to its lack of selectivity. In the present study, compound **2** was selected from our screening since it possesses an amine functional group that is required for the attachment of the drug on the linker of our drug delivery system (Figures 1 and 2). PKI **2** was prepared in four steps from the 8-isopropyl-2-(methylthio)pyrazolo[1,5-a][1,3,5]triazin-4-ol **3** previously described in the literature (Scheme 1, see the supporting information). Thus, halogenation of the hydroxylated pyrazolotriazine **3** carried out in the presence of $POCl_3$ afforded the intermediate **4** (97%). The 4-(2-pyridyl)benzylamine moiety was incorporated by nucleophilic substitution to produce the derivative **5** in 98% yield. Oxidation of the thioether using 3-chloroperbenzoic acid provided the sulfoxide **6** (90%) which reacted with *trans*-1,4-diaminocyclohexane to give the expected PKI **2** (88%).

The inhibition profile of compound **2** was then evaluated on 14 different kinases and compared to that of Roscovitine (Table 1, see the supporting information). In each case, **2** inhibited kinase activity at lower concentrations than Roscovitine. With the exception of CLK3, PKI **2** was both efficient and versatile for kinase activity inhibition with IC_{50} values ranging from 0.049 to 0.12 µM. Since multiple oncogenic pathways are often involved in tumour growth, the high affinity of **2** for a wide range of kinases can be advantageous for cancer therapy.

We next pursued our investigations by measuring the antiproliferative activity of compound **2** on several cancer cell lines (Table 2). In these experiments, **2** was from 400- to 3400-fold more cytotoxic than Roscovitine used as a reference. Furthermore, with IC_{50} values in the low nanomolar (from 5 to 71 nM), the PKI **2** appeared as an excellent candidate for targeting strategies employing glucuronide prodrugs such as **1** [13, 23].

The synthesis of prodrug **1** was undertaken starting from the activated carbonate **7** [11] we recently reported in the literature (Scheme 2). Coupling between **7** and the primary amine of the drug **2** carried out in the presence of pyridine gave the alkyne **8** in 72% yield. The latter reacted in CH_2Cl_2 with commercially available *O*-(2-aminoethyl)-*O'*-(2-azidoethyl) nonaethylene glycol and $Cu(CH_3CN)_4PF_6$ in order to form the triazole **9** through the well-known copper(I)-catalyzed azide-alkyne 1,3-cycloaddition (CuAAC). After 1 hour under these conditions, the reaction mixture was washed with an aqueous solution of EDTA, the solvent was removed, and the crude product was engaged in the next step without any further purification. The full deprotection of the glucuronide moiety using LiOH produced the intermediate **10** which was directly coupled with the *N*-hydroxysuccinimide ester **11** to afford the prodrug **1** in 40% yield over three steps after purification by reverse phase column chromatography (purity > 95%).

Since β-glucuronidase-catalysed hydrolysis of the carbohydrate trigger is the key step in the process of drug release, our first aim was to confirmed that the glucuronide moiety was still substrate for the enzyme once bound to albumin. For this purpose, prodrug **1** was incubated with human serum albumin (HSA) at 37°C and the evolution of the mixture was monitored by HPLC-HRMS (see the supporting information). Under these conditions, prodrug **1** disappeared along the time as the result of its binding to HSA. In this experiment, no release of the PKI **2** was observed after 20 hours of incubation showing that our targeting device was highly stable in the absence of the activating enzyme. However, addition of β-glucuronidase in the medium triggered the release the drug indicating that the glucuronide was readily accessible for the enzyme even with the proximity of the bulky HSA (Figure 3).

Glucuronide **1** was then evaluated for its antiproliferative activity against A549 cancer cell line (Table 3). When incubated alone, prodrug **1** did not affect viability of cells at the highest tested dose of 200 nM whereas PKI **2** was highly toxic with an IC_{50} of 10.3 nM. As expected, derivatisation of drug **2** in the form of the β-glucuronidase-responsive albumin-binding prodrug **1** drastically reduced its toxicity**.** This decreased cytotoxicity can be explained by the hydrophilicity imparted by both the glucuronide and the polyethylene glycol-containing side chain that prevent passive penetration of the prodrug through cell membrane and subsequent intracellular activation by lysosomal β-glucuronidase. In contrast, addition of β-glucuronidase in the culture medium triggered the full release of the free drug thereby restoring its initial cytotoxicity (IC_{50} 9.4 nM). Overall, these results demonstrated that the biological activity of the PKI **2** can be turned off through its incorporation into an enzyme-responsive targeting assembly such as **1**. Moreover, kinases inhibition properties as well as the resulting cytotoxicity of **2** can be fully recovered in the presence of β-glucuronidase, an enzyme over-expressed in the microenvironment of almost all solid tumours.

Conclusion

In summary, we synthesised a novel PKI that exhibits a nanomolar activity for the inhibition of a wide range of protein kinase**.** The corresponding enzyme-responsive albumin-binding prodrug is far less toxic than the free drug and is readily activated in the presence of β-glucuronidase**.** Since the selective delivery of potent anticancer drugs by β-glucuronidase-responsive albumin-binding prodrugs was already demonstrated *in vivo*, our study can be of great interest to enhance the selectivity of kinase inhibitor-based cancer chemotherapy.

Experimental Section

General chemical procedure. All reactions were performed under a nitrogen atmosphere. Unless otherwise stated, solvents used were of HPLC quality. Chemicals were of analytical grade from commercial sources and were used without further purification. The reaction progress was monitored on precoated silica gel TLC plates MACHEREY-NAGEL ALUGRAM® SIL G/UV254 (0.2 mm silica gel 60). Spots were visualized under 254 nm UV light. Flash columns chromatography of compounds were performed using a Combi-flash Rf 200i apparatus using a silica phase column or a C_{18} reversed phase column.

^{1}H and ^{13}C NMR spectra were respectively recorded at 400 MHz and 100 MHz on a Bruker 400 Avance III instrument, equipped with an ultra-shielded magnet and a BBFO 5 mm broadband probe. The ^{13}C NMR of the compound **2** was recorded by the CRMPO (Centre Régional de Mesures Physiques de l'Ouest), at the University of Rennes 1, on a Bruker Avance III HD 500 MHz. Chemical shifts (δ) are reported in parts per million (ppm) from low to high field and referenced to residual solvent. Standard abbreviations indicating multiplicity are used as follows: br = broad, s = singlet, d = doublet, t = triplet, quin = quintet, m = multiplet. High resolution ESI mass spectrometry were carried out on a LC-QTOF MaXis Impact spectrometer (Bruker).

Analytical RP-HPLC was carried out on a Dionex Ultimate 3000 system equipped with a UV/Visible variable wavelength detector and with a reverse-phase column chromatography Acclaim(R) (C18, 250x4.6 mm, 5 µm, 120 Å, column A) or Nucleoshell (C18, 150x4.6 cm, 5 µm, column B) at 30°C and 1 $mL.min^{-1}$ with a linear gradient composed of A (0.2% TFA in water) and B (CH_3CN) beginning with A/B = 80/20 v/v and reaching A/B = 0/100 v/v within 30 min (method 1). All chromatograms were recorded at 254 nm.

HPLC/HRMS experiments were performed on an Accela UHPLC system coupled to a hybrid high resolution mass spectrometer (Q-ExactiveTM, Thermo Scientific). An Acclaim® C18 column (250x4.6 mm, 5 µm, 120 Å) at 30°C was used for chromatographic separation at a flow rate of 0.5 mL.min-1. The column effluent was introduced into the electrospray ionisation source (ESI) of the mass spectrometer. Analyses were performed in positive ion mode. The electrospray voltage was set at 4.0 kV. The capillary and heater temperatures were 275°C and 300°C respectively. The sheath, sweep and auxiliary gas (nitrogen) flow rates were set at 35, 10 and 20 (arbitrary units). Analysis of data was performed with XcaliburTM software. Enzymatic hydrolysis was monitored by HPLC/HRMS using a linear gradient composed of A (0.1% formic acid in water) and B (0.1% formic acid in CH_3CN) (method 2). After injection of 20 µL of sample, compounds were separated through a linear gradient starting from 20% of B and reaching 100% of B within 30 min.

Synthesis of compound 8: The carbonate **7** (0.0329 mmol, 22.6 mg) and the amine **2** (0.0329 mmol, 15 mg) were dissolved in 0.63 mL of dry DMF. 0.16 mL of pyridine was added followed by HOBt (0.0329 mmol, 4.4 mg). The mixture was stirred at room temperature for 19h. Then, the solvent was evaporated under high vacuum. The crude was purified by flash chromatography using combiflash® apparatus with a 4 gr gold column from redisep®. A progressive gradient of DCM/MeOH was used from 100/0 to 95/5 which afforded 24 mg of the desired compound **8** (72%). **^{1}H NMR** (400 MHz MHz, $CDCl_3$): δ 8.69 (d, 1H, *J* = 4.5 Hz), 7.96 (d, 2H, *J* = 8.3 Hz), 7.86 (dd, 1H, *J* = 2.2 Hz, *J* = 3.6 Hz), 7.74 (ddd, 1H, *J* = 15.5 Hz, *J* = 8.1 Hz, *J* = 2.0 Hz), 7.71 (td, 1H, *J* = 8.0 Hz, *J* = 1.2 Hz), 7.63 (s, 1H), 7.55 (dt, 1H, *J* = 8.6 Hz, *J* = 1.8 Hz), 7.44 (d, 1H, *J* = 8.2 Hz), 7.35 (dd, 1H, *J* = 8.6 Hz, *J* = 1.9 Hz), 7.24 (ddd, 2H, *J* = 7.1 Hz, *J* = 4.9 Hz, *J* = 1.3 Hz), 5.76 (t, 1H, *J* = 6.2 Hz), 5.39-5.28 (m, 4H), 5.20 (dd, 1H, *J* = 6.9 Hz, *J* = 3.8 Hz), 4.83 (br s, 1H), 4.76 (d, 3H, *J* = 5.8 Hz), 4.21 (d, 1H, *J* = 8.9

Hz), 3.77 (br s, 1H), 3.73 (d, 3H, *J* = 0.9 Hz), 3.45 (br s, 1H), 3.03 (td, 1H, *J* = 13.7 Hz, *J* = 6.9 Hz), 2.74 (m, 2H), 2.12 (d, 3H, *J* = 1.4 Hz), 2.06 (s, 3H), 2.05 (d, 3H, *J* = 0.4 Hz), 2.01 (t, 1H, *J* = 2.5 Hz), 1.93-2.24 (m, 6H), 1.28 (d, 6H, *J* = 6.9 Hz), 1.18-1.37 (m, 2H). **^{13}C NMR** (100 MHz, $CDCl_3$): δ 170.0, 169.3, 169.2, 166.7, 166.7, 156.8, 156.1, 154.1, 149.7, 149.0, 148.8, 143.6, 141.1, 141.0, 139.0, 138.1, 136.8, 135.9, 135.8, 132.2, 132.1, 127.9, 127.3, 123.3, 123.0, 122.2, 120.5, 119.8, 119.7, 99.7, 99.7, 78.6, 78.5, 72.6, 72.6, 72.1, 71.7, 71.1, 71.1, 70.2, 68.7, 68.7, 53.0, 49.7, 44.1, 31.8, 31.5, 26.5, 26.5, 23.4, 23.2, 20.6, 20.5, 20.5. Since the compound **8** was obtained as a mixture of two diastereoisomers, some ^{13}C NMR signals are split. **HMRS** (ESI+) for $C_{50}H_{55}N_9O_{14}$, calcd 1006.39467, found 1006.3948.

Synthesis of the compound 1: Under argon, the alkyne **8** (0.012 mmol, 12mg) was dissolved in DCM (0.61 mL). Then, a solution of the *O*-(2-aminoethyl)-*O'*-(2-azidoethyl) nonaethylene glycol in DCM (0.5 mL) was added, followed by $Cu(CH_3CN)_4PF_6$. The mixture was stirred at room temperature for 15h. A solution of EDTA (0.202 mmol, 75 mg) in water (1.1 mL) was added. After stirring at room temperature for 1h, the mixture was diluted with water and extracted four times with DCM. The organic layer was dried over $MgSO_4$ and the solvent was evaporated. The crude mixture was dissolved in 3 mL of MeOH and 0.2 mL of DCM and the solution was cooled down at 0°C. Then, a solution at 0°C of $LiOH.H_2O$ (0.106 mmol, 4.4 mg) in water (0.91 mL) was added. Two additional portions of $LiOH.H_2O$ (0.0.025 mmol, 1.1 mg) in water (0.23 mL) were added after stirring for 1h30 and 2h respectively. After 0.5h, Amberlyst acid resin was added and the mixture was stirred for 0.5h. The solution was filtered on celite which was washed with MeOH and drops of water. The solvent was evaporated under high vacuum. The crude mixture was dissolved in 0.29 mL of dry DMSO and NHS ester **11** (0.014 mmol, 4.4 mg) was added. The reaction mixture was stirred at room temperature overnight and DMSO was removed under high vaccum. The crude mixture was purified on reverse phase column using combiflash apparatus with a H_2O/MeCN gradient

(from 80/20 to 0/100 in 35 minutes). This procedure afforded the desired prodrug **1** (7.5 mg, 40% over 3 steps). **^{1}H NMR** (400 MHz, $CDCl_3$): δ 10.08 (bs, 1H), 8.66 (d, 1H, *J* = 4.5 Hz), 8.05 (d, 3H, *J* = 7.32 Hz), 7.94 (d, 2H, *J* = 8.2 Hz), 7.90 (m, 1H), 7.78 (m, 4H), 7.56 (d, 1H, *J* = 8.8 Hz), 7.52 (m, 2H), 7.37 (m, 2H), 7.30 (m, 1H), 7.00 (s, 2H), 5.85 (t, 1H, *J* = 6.5 Hz), 5.25 (d, 1H, *J* = 7.0 Hz), 4.69 (d, 2H, *J* = 5.1 Hz), 4.44 (t, 2H, *J* = 4.8 Hz), 3.97 (d, 1H, *J* = 9.7 Hz), 3.12-4.20 (m, 50H), 2.95 (m, 2H), 2.03 (t, 2H, *J* = 7.3 Hz), 1.82 (m, 3H), 1.73 (br s, 1H), 1.46 (quin, 4H, *J* = 7.3 Hz), 1.11-1.37 (m, 12H). **^{13}C NMR** (125 MHz, $CDCl_3$): δ 173.9, 172.1, 171.1, 169.9, 163.1, 157.9, 155.7, 154.5, 149.4, 148.6, 142.1, 139.8, 137.5, 134.9, 134.5, 128.2, 127.6, 126.6, 123.5, 122.7, 120.3, 116.6, 99.9, 75.9, 75.5, 72.8, 71.2, 69.8, 69.7, 69.6, 69.2, 68.9, 49.4, 38.5, 37.0, 35.1, 32.2, 30.8, 27.8, 26.7, 25.8, 24.8, 23.1. Since the compound **1** was obtained as a mixture of two diastereoisomers, some ^{13}C NMR signals are split. **HMRS** (ESI-): for $C_{75}H_{103}N_{14}O_{24}$, calcd 1583.72697, found 1583.7234.

Cell Culture. Human lung carcinoma A549 cells were grown in RPMI 1640-GlutaMax (Invitrogen) supplemented by 10% fetal bovine serum (Lonza), and 100u/ml Penicillin/Streptomycin (Life Technologies) in a humidified incubator at 37°C and 5% CO2. A549 cells were purchased from European Collection of Cell Cultures (ECACC). Cells were used at early passages (<10) and cultured as recommended by the manufacturer.

Cell Viability Assay. The Cell Proliferation Kit II (XTT, Roche) was used to assess cell viability. This assay is based on the cleavage of XTT by metabolic active cells resulting in the production of an orange formazan dye quantified by spectrophotometry. Assays were carried out essentially as described by the manufacturer. Briefly, 2.103 tumor cells/well were plated in a 96-well plate. Cells were cultured for 24h before adding the compound at the indicated concentration ± β-glucuronidase in the culture media. After 2 days of treatment, 25µl of the XTT labeling mixture were added per well. Cells were further incubated for additional 4h at

37°C before determination of the absorbance at 490nm on a 96-well microplate reader. IC_{50} values were determined using Graphpad software.

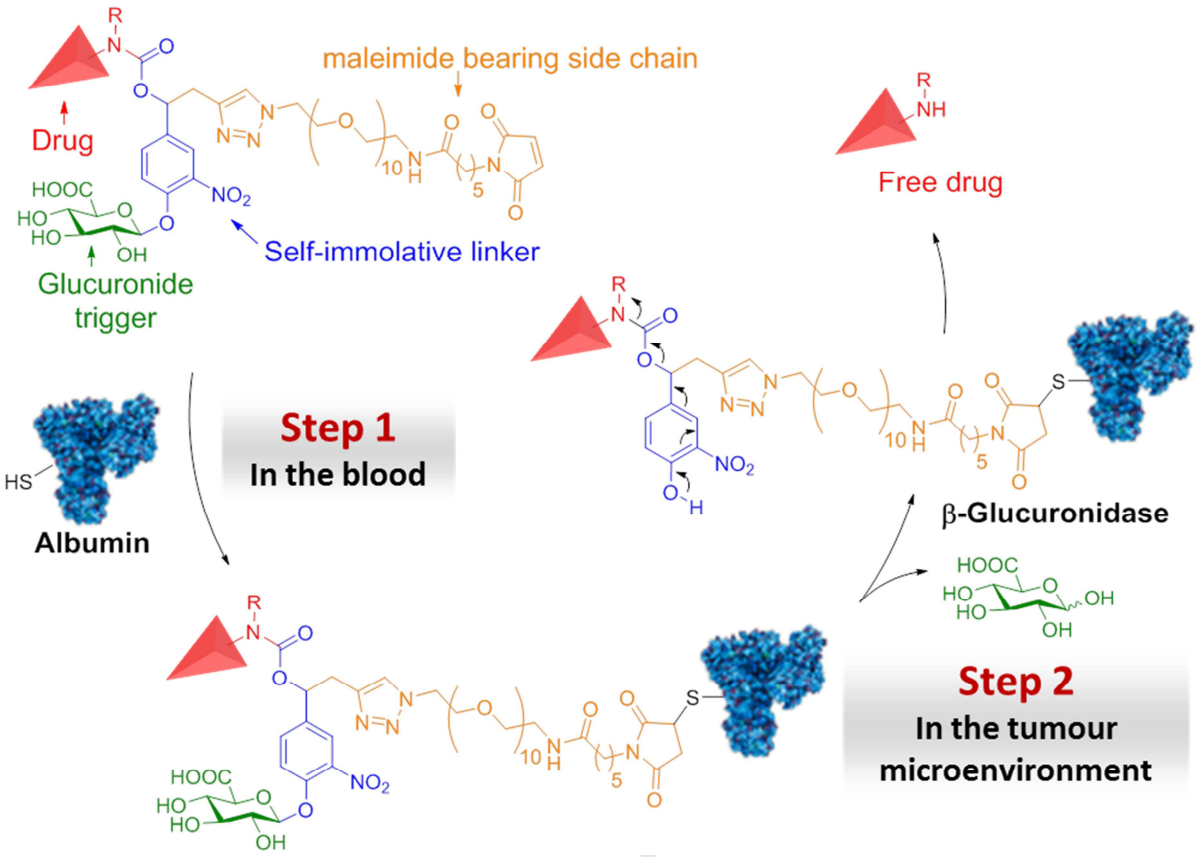


Figure 1 The principle of tumour targeting with the β-glucuronidase-responsive albumin-binding prodrugs: step 1: in the blood, the prodrug binds covalently to plasmatic albumin through Michael addition; step 2: in the tumour microenvironment, hydrolysis of the glycosidic bond triggered by extracellular β-glucuronidase initiates the self-decomposition of the linker leading to the release of the drug.

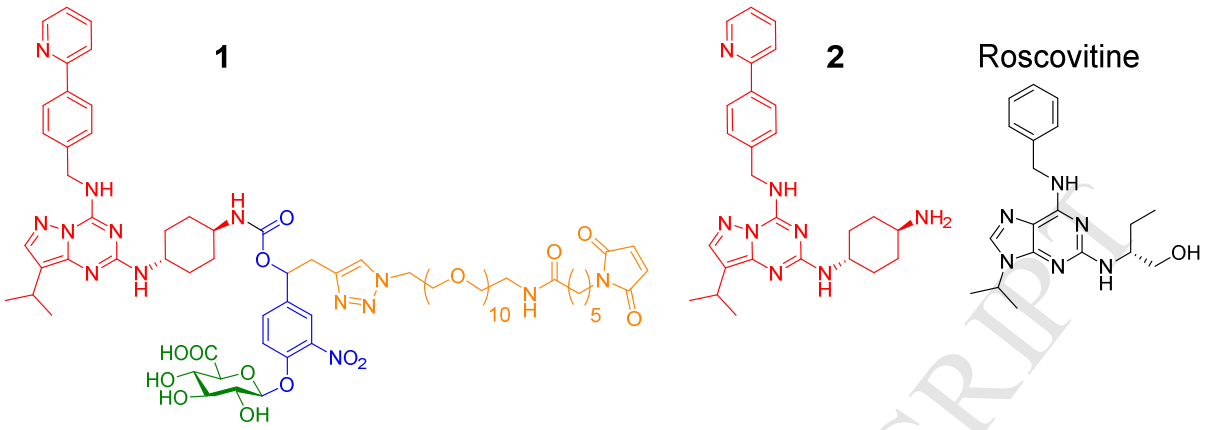
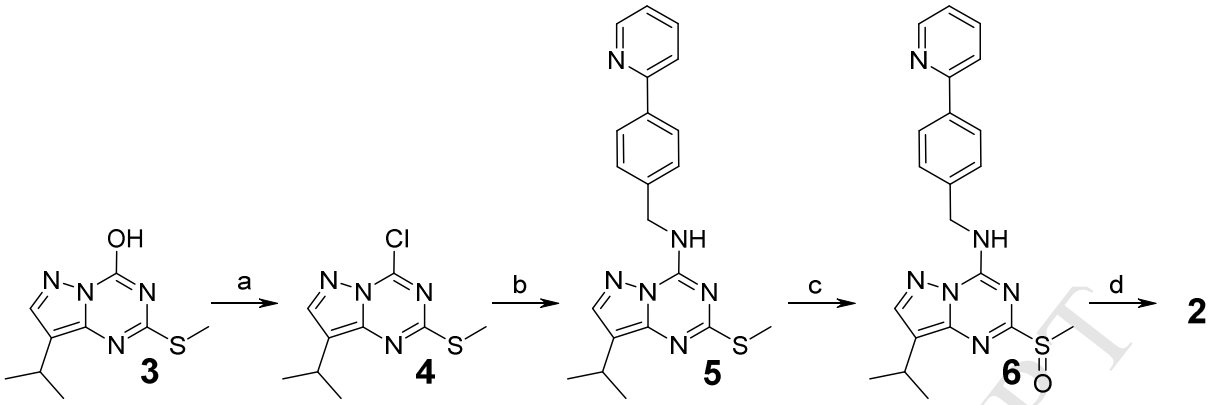


Figure 2 Structures of the β-glucuronidase-responsive albumin-binding prodrug **1**, the protein kinase inhibitor **2** and *(R)*-Roscovitine.



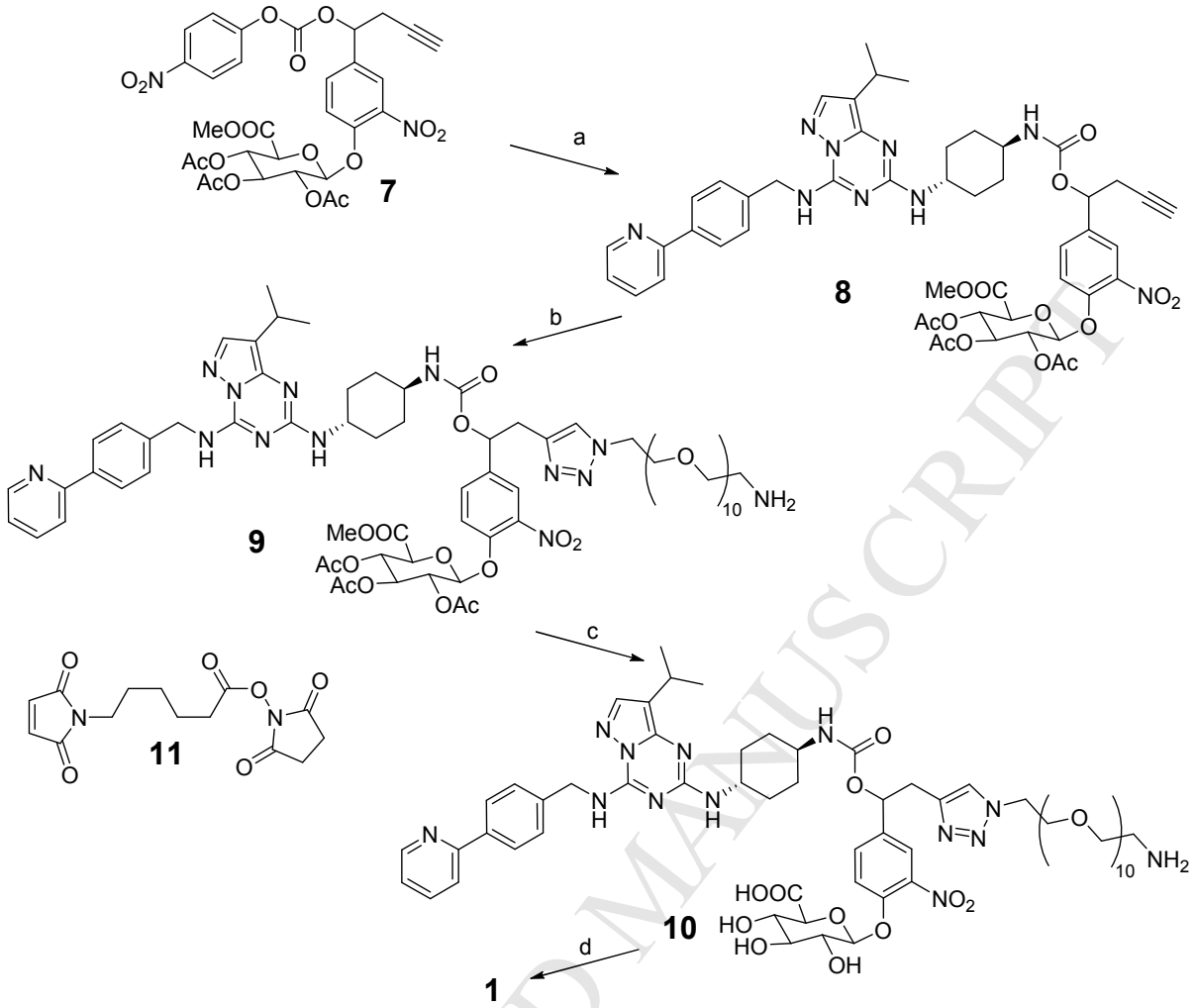
Scheme 1. Synthesis of PKI **2**. (a) $POCl_3$, NN-dimethylaniline 100°C, 4 h, 97%; (b) 2-(4-(ammoniomethyl)phenyl)pyridin-1-ium bis-trifluoroacetate salt, Et_3N, *n*BuOH, 80°C, 3 h, 98%; (c) 3-chloroperbenzoic acid, CH_2Cl_2, 0°C, 90%; (d) trans-1,4-diaminocyclohexane, 140°C, 4 h, 88%.

Table 1. Inhibition of kinase activity by **2** and *(R)*-Roscovitine (IC_{50} values are expressed in μM).

Kinase	Roscovitine	**2**
CDK1/ cyclin B	0.31	0.07
CDK2 / cyclin A	0.072	0.068
CDK5 / p25	0.2	0.05
CDK9 / cyclin T	0.22	0.049
CK1	0.73	0.071
CLK1	1.2	0.1
CLK2	0.71	0.13
CLK3	>10	2.9
CLK4	0.62	0.089
DYRK1A	1.2	0.12
DYRK1B	1.2	0.11
DYRK2	1.7	0.12
DYRK3	>10	0.38
GSK3	>10	0.32

Table 2. IC_{50} (µM) of *(R)*-Roscovitine and **2** on different cancer cell lines (SHSY-5Y: human neuroblastoma; IMR32: human neuroblastoma; Sc1: human B-lymphocyte, (Epstein-Barr virus transformed); Sc2: human embryonic stem cells; SCOV-3 and OVCAR-3: human ovarian adenocarcinoma)

Cell line	Roscovitine	**2**
SHSY-5Y	21	0.035
IMR32	17	0.005
Sc1	32	0.015
Sc2	21	0.013
SCOV-3	29	0.071
OVCAR-3	37	0.024



Scheme 2. Synthesis of the prodrug **1**. (a) dimethylformamide, HOBt, pyridine, H **2**, rt, 19h, 72%; (b) *O*-(2-aminoethyl)-*O'*-(2-azidoethyl) nonaethylene glycol, $Cu(MeCN)_4PF_6$, CH_2Cl_2, rt, 1h; (c) LiOH, H_2O/MeOH, 0°C, 4h; (d) **11**, DMSO, rt, overnight, 40% over 3 steps.

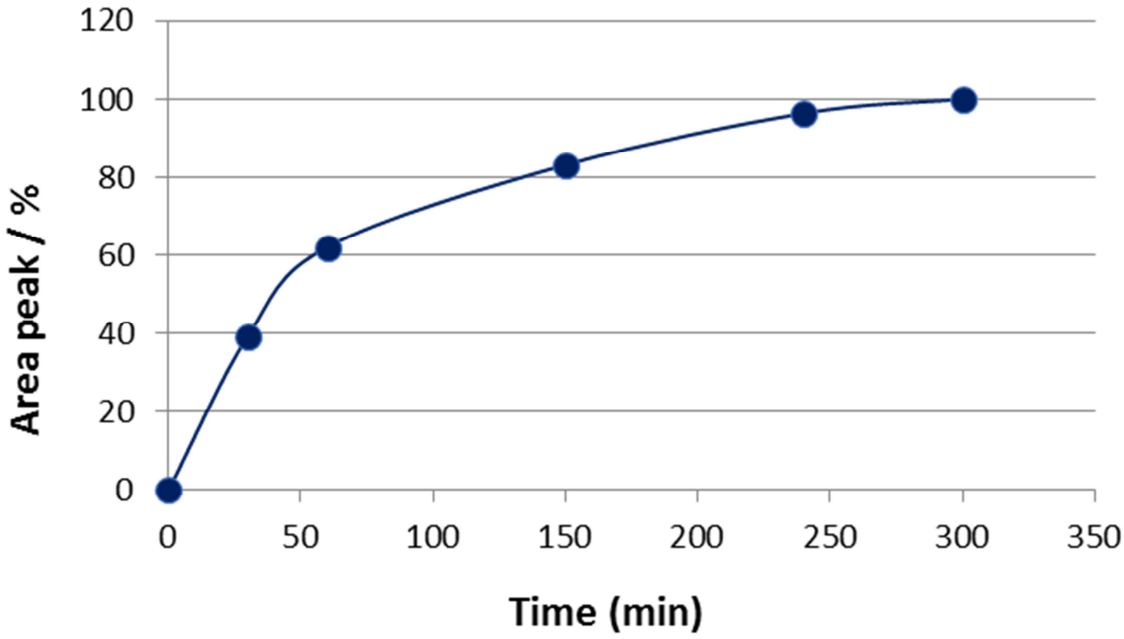


Figure 3 Kinetics of PKI **2** release from prodrug **1** in the presence of β-glucuronidase (133 $U.mL^{-1}$) at 37°C (0.02 M phosphate buffer, pH 7). The quantity of **2** released along the time was determined by HPLC-HRMS.

Table 3. Antiproliferative activities of PKI 2 and prodrug 1 (with or without β-glucuronidase) against human lung carcinoma A549 cell line after 3 days of treatment.

Compound	**IC_{50} (nM)**
1	>200
2	10.3±1.0
1 + β-glucuronidase	9.4±1.2

Appendix A. Supplementary data

Supplementary data related to this article can be found at

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References

[1] H.-Y. Zhao, H. Wei and X. Wang, The Reciprocal Interaction of Small Molecule Protein Kinase Inhibitors and ATP-Binding Cassette Transporters in Targeted Cancer Therapy, J. Cancer Res. Updat., 2 (2013) 68-86.

[2] O. Chahrour, D. Cairns and Z. Omran, Small Molecule Kinase Inhibitors as Anti-Cancer Therapeutics, Mini Rev. Med. Chem. 12 (2012) 399-411.

[3] P. Wu, T. E. Nielsen and M. H. Clausen, Small-molecule kinase inhibitors: an analysis of FDA-approved drugs, Drug Discov. Today 21 (2016) 5-10.

[4] S. K. Grant, Therapeutic Protein Kinase Inhibitors, Cell. Mol. Life Sci. 66 (2009) 1163-1177.

[5] D. R. Shah, R. R. Shah and J. Morganroth, Tyrosine Kinase Inhibitors: Their On-Target Toxicities as Potential Indicators of Efficacy, Drug Saf. 36 (2013) 413-426.

[6] S. Gross, R. Rahal, N. Stransky, C. Lengauer and K. P. Hoeflich, Targeting cancer with kinase inhibitors, J. Clin. Invest. 125 (2015) 1780-1789.

[7] R. R. Shah, Tyrosine Kinase Inhibitor-Induced Interstitial Lung Disease: Clinical Features, Diagnostic Challenges, and Therapeutic Dilemmas, Drug Saf. 39 (2016) 1073-1091.

[8] G. Manning, The protein kinase complement of the human genome, Science 298 (2002) 1912.

[9] A. Beck, L. Goetsch, C. Dumontet, N. Corvaïa, Strategies and challenges for the next generation of antibody drug conjugates, Nat. Rev. Drug Discov. 16 (2017) 315-337.

[10] T. Legigan, J. Clarhaut, B. Renoux, I. Tranoy-Opalinski, J.-M. Berjeaud, F. Guilhot and S. Papot, Synthesis and Antitumor Efficacy of a β-Glucuronidase-Responsive Albumin-Binding Prodrug of Doxorubicin, J. Med. Chem. 55 (2012) 4516-4520.

[11] B. Renoux, F. Raes, T. Legigan, E. Péraudeau, B. Eddhif, P. Poinot, I. Tranoy-Opalinski, J. Alsarraf, O. Koniev, S. Kolodych, S. Lerondel, A. Le Pape, J. Clarhaut and S. Papot, Targeting the Tumour Microenvironment with Enzyme-Responsive Drug Delivery System for Efficient Therapy of Breast and Pancreatic Cancers, Chem. Sci. 8 (2017) 3427-3433.

[12] M. de Graaf, E. Boven, H. W. Scheeren, H. J. Haisma and H. M. Pinedo, Beta-glucuronidase-mediated drug release, Curr. Pharm. Des. 8 (2002) 1391-1403.

[13] I. Tranoy-Opalinski, T. Legigan, R. Barat, J. Clarhaut, M. Thomas, B. Renoux and S. Papot, β-Glucuronidase-Responsive Prodrugs for Selective Cancer Chemotherapy: an Update, Eur. J. Med. Chem. 74 (2014) 302-313.

[14] I. Tranoy-Opalinski, A. Fernandes, M. Thomas, J.-P. Gesson and S. Papot, Design of Self-Immolative Linkers for Tumour-Activated Prodrug Therapy, *A*nti-Cancer Agents Med. Chem. 8 (2008) 618-637.

[15] A. Alouane, R. Labruère, T. Le Saux, F. Schmidt and L. Jullien, Self-Immolative Spacers: Kinetic Aspects, Structure-Property Relationships, and Applications, Angew. Chem. Int. Ed. 54 (2015) 7492-7509.

[16] B. Elsadek and F. Kratz, Impact of albumin on drug delivery - New applications on the horizon, J. Control. Release 157 (2012) 4-28.

[17] W. H. Fishman and A. J. Anlyan, Comparison of the beta-glucuronidase activity of normal, tumor, and lymph node tissues of surgical patients, *Science*, 106 (1947) 66-67.

[18] N. Albin, L. Massaad, C. Toussaint, M. C. Mathieu, J. Morizet, O. Parise, A. Gouyette and G. G. Chabot, Main drug-metabolizing enzyme-systems in human breast-tumors and peritumoral tissues, Cancer Res. 53 (1993) 3541-3546.

[19] K. Bosslet, J. Czech and D. Hoffmann, A novel one-step tumor-selective prodrug activation system Tumor Targeting, 1 (1995) 45-50.

[20] K. Bosslet, R. Straub, M. Blumrich, J. Czech, M. Gerken, B. Sperker, H. K. Kroemer, J.-P. Gesson, M. Koch and C. Monneret, Elucidation of the mechanism enabling tumor selective prodrug monotherapy Cancer Res., 58 (1998) 1195-1201.

[21] L. Meijer, H. Galons, B. Joseph, F. Popowycz, N. Oumata WO2010103486A1.

[22] J. Cicenas, K. Kalyan, A. Sorokinas, E. Stankunas, J. Levy, I. Meskinyte, V. Stankevicius, A. Kaupinis and M. Valius, Roscovitine in cancer and other diseases, Ann. Transl. Med. 3 (2015) 135.

[23] T. Legigan, J. Clarhaut, B. Renoux, I. Tranoy-Opalinski, A. Monvoisin, C. Jayle, J. Alsarraf, M. Thomas and S. Papot, Synthesis and biological evaluations of a

monomethylauristatin E glucuronide prodrug for selective cancer chemotherapy, Eur. J. Med. Chem. 67 (2013) 75-80.

Synthesis of a novel nanomolar protein kinase inhibitor.

Synthesis of the first β-glucuronidase-responsive albumin-binding prodrug for selective kinase inhibitor-based cancer chemotherapy.

Covalent binding to human serum albumin through Michael addition.

Efficient release of the drug upon enzymatic activation.