



**HAL**  
open science

## Light-Activatable Photocaged UNC2025 for Triggering TAM Kinase Inhibition in Bladder Cancer

Chloé Breton-Patient, Sébastien Billotte, Patricia Duchambon, Gaëlle Fontaine, Sophie Bombard, Sandrine Piguel

► **To cite this version:**

Chloé Breton-Patient, Sébastien Billotte, Patricia Duchambon, Gaëlle Fontaine, Sophie Bombard, et al.. Light-Activatable Photocaged UNC2025 for Triggering TAM Kinase Inhibition in Bladder Cancer. *ChemBioChem*, 2024, 25 (8), 10.1002/cbic.202300855 . hal-04783958

**HAL Id: hal-04783958**

**<https://hal.science/hal-04783958v1>**

Submitted on 18 Nov 2024

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

# Light-Activatable Photocaged UNC2025 for Triggering TAM Kinase Inhibition in Bladder Cancer

Chloé Breton-Patient,<sup>[a, b]</sup> Sébastien Billotte,<sup>[c]</sup> Patricia Duchambon,<sup>[a, b]</sup> Gaëlle Fontaine,<sup>[a, b]</sup>  
Sophie Bombard,<sup>[a, b]</sup> and Sandrine Piguel\*<sup>[c]</sup>

**Abstract:** Photopharmacology is an emerging field that utilizes photo-responsive molecules to enable control over the activity of a drug using light. The aim is to limit the therapeutic action of a drug at the level of diseased tissues and organs. Considering the well-known implications of protein kinases in cancer and the therapeutic issues associated with protein kinase inhibitors, the photopharmacology is seen as an innovative and alternative solution with great potential in oncology. In this context, we developed the first photocaged TAM kinase inhibitors based on **UNC2025**, a first-in-class small molecule kinase inhibitor. These prodrugs showed good stability in biologically relevant buffer and rapid photorelease of the photoremovable protecting group upon UV-light irradiation (<10 min.). These light-activatable prodrugs led to a 16-fold decrease to a complete loss of kinase inhibition, depending on the protein and the position at which the coumarin-type phototrigger was introduced. The most promising candidate was the *N,O*-dicaged compound, showing the superiority of having two photolabile protecting groups on **UNC2025** for being entirely inactive on TAM kinases. Under UV-light irradiation, the *N,O*-dicaged compound recovered its inhibitory potency in enzymatic assays and displayed excellent antiproliferative activity in RT112 cell lines.

## Introduction

Photopharmacology is an emerging field based on the spatial and temporal control of the biological activity of a therapeutic molecule activated by light.<sup>[1]</sup> The aim is to limit the therapeutic activity of a drug at the level of diseased tissues and organs. This is a very promising approach to overcome selectivity issues and off-target toxicities and then reduce side effects, the main pitfalls of current therapeutic treatments. Light is an attractive source of stimulation and has been recognized for decades for its therapeutic benefits. Indeed, light is considered non-invasive thanks to its high spatiotemporal precision and biorthogonality. Furthermore, the possibility of fine-tune the wavelength and the intensity of the light make this external stimulus as a valuable tool.<sup>[2]</sup>

The photopharmacology field relies on photochemical processes that are usually classified in two categories: molecular photoswitches<sup>[3]</sup> such as azobenzenes and photocleavable protecting groups<sup>[4]</sup> (PPG) including *o*-nitrobenzyl, coumarin or BODIPY derivatives. While the first strategy is based on a photochemically or thermally reversible geometrical change, the second one consists of an irreversible photochemical cleavage of a  $\sigma$ -bond between a PPG and the active molecule. Covalently attaching a photoremovable protecting group on key positions of the active drug leads to a temporary deactivation of the drug. Upon irradiation, the bond is broken and the again active drug is released with high spatiotemporal resolution. The choice between these two approaches depends on several factors including the synthetic facility of the photoactivatable drug, the significant difference in activity between the drug and its photoactivable version, the thermal and chemical stability in a biological environment, and structural considerations of the biological targets.<sup>[5]</sup> A tremendous amount of biological targets such as lipid membranes, ion channels, enzyme receptors and nucleic acids has been photoregulated.<sup>[6]</sup> Protein kinases have been recognized as valuable therapeutic targets. Almost 71 small-molecule kinase inhibitors have been approved by the Food and Drug Administration and reached the market for the treatment of malignancies and non-malignancies.<sup>[7]</sup> Despite this success, the poor drug selectivity

involving side-effects and resistance issues are still major drawbacks. Achieving selectivity proves to be challenging since most of the protein kinases are constitutively expressed in both healthy and diseased tissues leading to uncontrolled drug activity in time and space throughout the organism. It is therefore no surprise that the development of photoresponsive molecular tools targeting protein kinases has taken off over the past decade.<sup>[8]</sup> Indeed, combined with well-developed light-delivery devices and the target accessibility, they will deliver localized precise treatment.<sup>[9]</sup> In 1998, Wood *et al.* reported the first example of a photoactivatable kinase inhibitor directed against the c-AMP-dependant protein kinase.<sup>[10]</sup> It was only in 2012 that this area began to grow gently.<sup>[11]</sup> Since then, both photopharmacological approaches have been applied on a variety of protein kinase inhibitors targeting RET kinase<sup>[12]</sup>, protein kinase C<sup>[13]</sup>, MEK1<sup>[14]</sup>, VEGFR-2<sup>[15]</sup>, BRAF<sup>V600E</sup><sup>[16]</sup>, JNK3<sup>[17]</sup>, PKA<sup>[18]</sup>, Rho kinase<sup>[11]</sup>, CKI<sup>[19]</sup>, ERK1/2<sup>[20]</sup>, and PIK3<sup>[21]</sup>. Most interestingly, the group of Peifer developed a photocaged version of two marketed kinase inhibitors, imatinib<sup>[22]</sup> and vemurafenib.<sup>[23]</sup> Considering the 518 proteins of the human kinome, their implications in cancer and the large group of kinases inhibitors, the photopharmacology approach has just started to be explored.

The TAM family of transmembrane tyrosine kinases consists of three members: TYRO3, AXL and MERTK. These receptor tyrosine kinases are key regulators of cellular homeostasis, immune surveillance, and viral infection.<sup>[24]</sup> They are also important components of malignant cell survival in many cancers including myeloid leukemia, colon, liver, prostate, bladder and breast cancers.<sup>[25]</sup> Therefore, the TAM family is emerging as potential therapeutic targets in cancer treatment and other diseases leading to the recent growth of specific small molecule inhibitors. As part of an ongoing project aiming at implementing novel TAM kinase inhibitors, we recently reported the first photocaged version of TAM kinase inhibitors.<sup>[26]</sup>

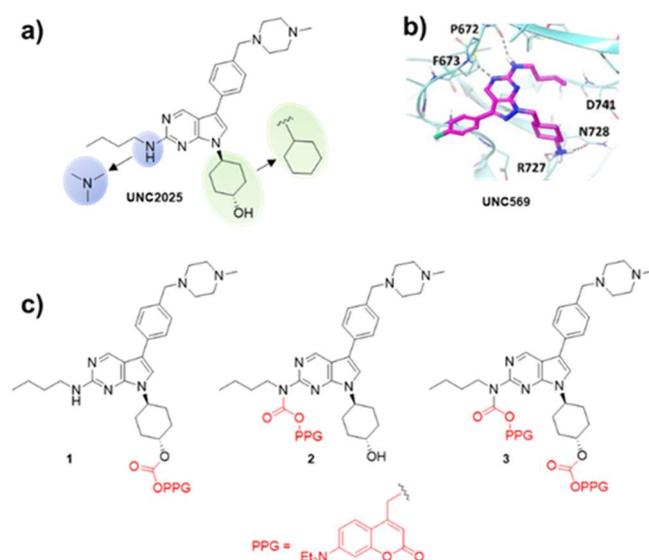
To go beyond this initial step and provide photopharmacological inhibitors targeting the TAM family that can be used on a pre-clinical level, we developed photocaged TAM kinase inhibitors based on **UNC2025**, also known as MRX-6313, a first-in-class small molecule kinase inhibitor identified as a MERTK/FLT3 dual inhibitor (Figure 1a).<sup>[27]</sup> **UNC2025** is characterized by excellent pharmacokinetic properties (low clearance, long half-life and high oral exposure) and high solubility. Furthermore, it is well tolerated in pre-clinical models such as human xenograft models of leukemia and solid cancers. In addition to MERTK (IC<sub>50</sub> = 0.74 nM), **UNC2025** targets the other two members of the TAM family with IC<sub>50</sub> values of 14 nM and 17 nM respectively for AXL, and TYRO3. These features make **UNC2025** an attractive choice to develop its photoactivatable version, which could be used as a photopharmacological tool in biomedical applications.

To determine the most suitable position(s) on **UNC2025** to graft the PPGs and to impair the binding to the active site, we based our hypothesis on reported structure-activity relationships (SAR) and docking experiments run on **UNC2025** and some analogs by Zhang *et al.*<sup>[27]</sup> The methylated version of **UNC2025** on the exocyclic amine had no significant effect on the inhibition of colony formation on A549 NSCLC and Molm-14 AML cell lines which are known to be dependent on MERTK and FLT3 respectively.<sup>[27]</sup> SAR studies on an analog of **UNC2025** show that

the replacement of the *trans*-4-hydroxycyclohexyl group by a cyclohexyl group resulted in a weaker inhibitor, up to 440-fold less active, for all members in the TAM family (Figure 1a).<sup>[28] [29]</sup> In a similar way, a X-ray structure of **UNC569**, an analog of **UNC2025**, in complex with MERTK revealed strong hydrogen bonding between two nitrogen atoms of the pyrazolopyrimidine core (N2, and exocyclic amine) and the methionine and proline residues from the hinge region (Figure 1b).<sup>[30]</sup> Another strong hydrogen bond takes place between the NH<sub>2</sub> group of the *trans*-aminocyclohexyl group and the carbonyl group of the Arg727 in the ATP sugar pocket. This result supported the importance of the hydroxyl substituent in **UNC2025** to maintain its potency towards the TAM family.

Taken all together, these analyses suggested that the hydroxyl and the exocyclic amine groups would be suitable positions for introducing one or two photocleavable protecting groups, further preventing the binding of the corresponding photocaged analogs within the TAM proteins. The commonly used phototrigger, [7-(diethylamino)coumarin-4-yl]methyl (7-DEACM), was chosen.<sup>[31]</sup>

Its interest relies on the rapidity of the photolytic reaction and on its photophysical properties that can be modulated by the presence of  $\pi$ -extended electron-donating or electron-withdrawing groups, permitting the photoactivation with light at wavelengths more compatible with the biological environment.<sup>[32]</sup> Therefore, we decided to develop three photoactivable versions of **UNC2025**: photocaged compound **1** bearing one 7-DEACM group on the hydroxyl function via a carbonate linkage, photocaged compound **2** bearing one 7-DEACM group on the exocyclic amine function via a carbamate linkage, and compound **3** being twice photoprotected on both the hydroxyl and exocyclic amine functions (Figure 1c).<sup>[33]</sup>



**Figure 1.** a) Structure of **UNC2025**; b) X-ray crystal structure of **UNC569** in complex with MERTK (kinase domain) (PDB ID: 3TCP); Reprinted with permission from X. Wang *et al.* *ACS Med. Chem. Lett.* **2016**, *7*, 1044-1049. Copyright © 2016, American Chemical Society; c) Structure of the three coumarin-caged **UNC2025** derivatives.

In this article, we describe the synthesis of three unprecedented photocaged **UNC2025** and their evaluation *in vitro* against the TAM family and in living cells upon irradiation with UV light. Our results highlight the promise of photocaged **UNC2025** to be a powerful light-controllable anticancer therapeutic agent.

## Results and Discussion

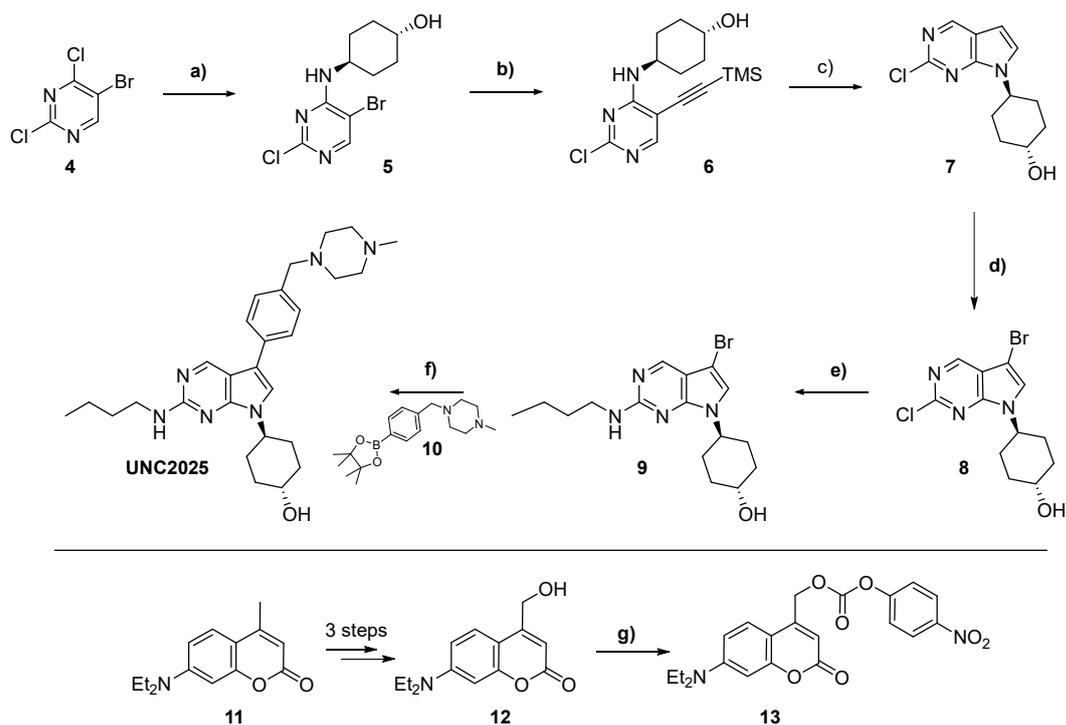
### Synthesis and photochemical characterisation

The previous synthetic path for **UNC2025**, described by Zhang *et al.*, was revisited and shortened from 8 to 6 steps with a comparable average yield (Scheme 1).<sup>[27]</sup> Starting from the commercially available 5-bromo-2,4-dichloropyrimidine **4**, nucleophilic aromatic substitution with *trans*-4-aminocyclohexanol afforded compound **5** with 92% yield. The trimethylsilylacetylene was introduced on the 5-position of the pyrimidine ring of **5** via a Sonogashira cross-coupling reaction cocatalysed by Pd(dppf)Cl<sub>2</sub>.DCM/CuI in THF to give the intermediate **6** with a moderate yield. The pyrrolo[2,3-*d*]pyrimidine ring **7** was formed after *in situ* deprotection of the trimethylsilyl (TMS) group with TBAF with 86% yield. Bromination of **7** with *N*-bromosuccinimide afforded compound **8** which subsequently reacted with butylamine through a nucleophilic aromatic substitution to yield compound **9** in 96%. Finally, a Suzuki cross-coupling between **9** and 4-(4-methylpiperazino)methylphenylboronic acid pinacol ester **10** as a coupling partner using Pd<sub>2</sub>(dba)<sub>3</sub> as catalyst and (*t*-Bu)<sub>3</sub>HPBF<sub>4</sub> as ligand in the presence of the base K<sub>3</sub>PO<sub>4</sub> in a mixture of dioxane/H<sub>2</sub>O, led to **UNC2025** in 80% yield.

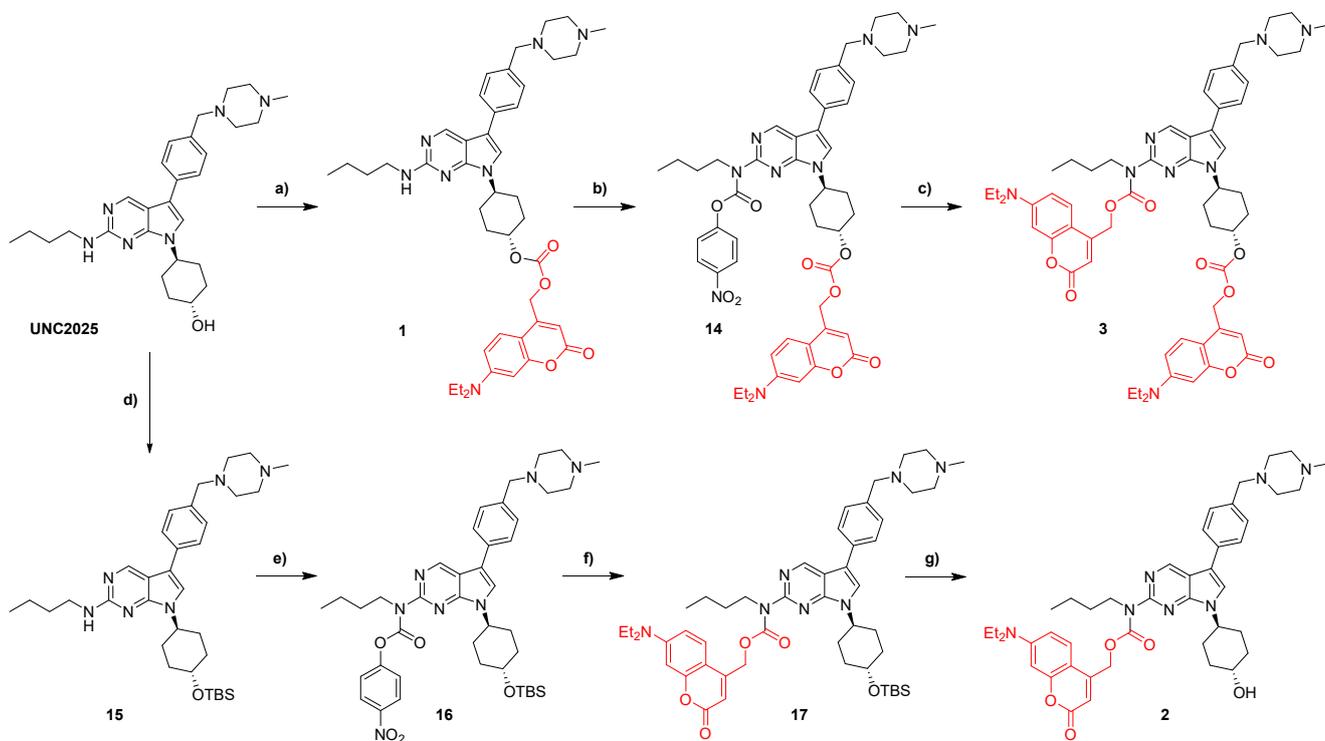
The 7-diethylamino-4-hydroxycoumarin **12** was prepared from an efficient three steps procedure, described by Weinrich *et al.*, starting from the 7-diethylamino-4-methylcoumarin **11**.<sup>[34]</sup> The subsequent reaction with 4-nitrobenzyl chloroformate led to the formation of carbonate **13** in good yield (Scheme 1).

The *O*-caged compound **1** was obtained, in 64% yield, by chemoselective reaction between the hydroxyl function of **UNC2025** and the carbonate **13** using NEt<sub>3</sub> and DMAP in THF (Scheme 2). Then, the secondary amino function was activated with the 4-nitrophenyl chloroformate to give the *N*-carbamate compound **14**. The displacement of the good leaving group, para-nitrophenol, by the alkoxide of coumarin **12** in the presence of NaH led to the *N,O*-dicaged compound **3** with an unoptimized yield of 29%.

Considering the chemoselective issues, another pathway for the synthesis of the *N*-caged compound **2** was set up. First, the primary alcohol function of **UNC2025** was protected with *tert*-butyldimethylsilyl group in 92% yield. The exocyclic amine was then activated using 4-nitrophenyl chloroformate to furnish the carbamate **16**, which subsequently reacted with the coumarin **12** in the presence of NaH to give the coumarin *N*-caged compound **17** in 60% yield. A final deprotection in acidic conditions afforded the expected *N*-caged compound **2** in 88% yield (Scheme 2).



**Scheme 1.** Synthesis of **UNC2025**. a) trans-aminocyclohexanol, DIPEA, *i*-PrOH, rt, 8 h, 92%, b) trimethylsilylacetylene, Pd(dppf)Cl<sub>2</sub>·DCM, CuI, Et<sub>3</sub>N, THF, 60°C, 24 h, 44%, c) TBAF, THF, 65°C, 5 h, 86%, d) NBS, DMF, rt, 1 h, 87%, e) BuNH<sub>2</sub>, DIPEA, *i*-PrOH, 95°C, 16 h, 96%, f) **10**, Pd<sub>2</sub>(dba)<sub>3</sub>, (*t*-Bu)<sub>3</sub>HPBF<sub>4</sub>, K<sub>3</sub>PO<sub>4</sub>, Dioxane/H<sub>2</sub>O, 80°C, 2 h, 80%. Synthesis of the coumarin cage derivatives **12** and **13**. g) 4-nitrophenylchloroformate, NEt<sub>3</sub>, THF, 77%.



**Scheme 2.** Synthesis of caged compounds **1**, and **2**, and dicaged compound **3**: a) **13**, DMAP, Et<sub>3</sub>N, THF, rt, 18 h, 64%, b) 4-nitrophenyl chloroformate, Et<sub>3</sub>N, THF, rt, 16 h, 38%, c) **12**, NaH, THF, rt, 16h, 29%, d) TBSCl, imidazole, DMF, rt, 16 h, 92%, e) 4-Nitrophenyl chloroformate, Et<sub>3</sub>N, THF, rt, 16 h, 86%, f) **13**, NaH, THF, rt, 16h, 60%, g) HCl 4N in dioxane, DCM, 0°C, 1 h, 88%.

The hydrolytic stability of the caged compounds in a 10% v/v DMSO/H<sub>2</sub>O mixture was also investigated to make sure that no active inhibitor was released before starting the irradiation. If compound **1** seems slightly unstable since a residual trace of **UNC2025** (~9% in HPLC, Figure S1) is present at t=0, both caged compounds **2** and **3** showed excellent stability at room temperature in the dark (Figures S1-S2). In contrast, 1h exposure to natural light led to a complete release of **UNC2025** (Figure S3) indicating that precautions should be taken when performing biological evaluations.

To evaluate the photoactivatable properties of the three caged **UNC2025** derivatives, we first recorded their absorption spectra. The maximum absorbance was reached at 379 nm for the *N*-caged and the *N,O*-caged compounds (Figure S4), while two absorption bands at 290 nm and 379 nm was measured for the *O*-caged compound **1**. Photolysis was carried out by irradiation of 20  $\mu$ M solutions of the three caged compounds in a 10% v/v DMSO/H<sub>2</sub>O mixture, using two LED (KESSIL, PR160L) at 370 nm, and the uncaging process was monitored by HPLC. Under these conditions, the DEACM groups of the *N,O*-dicaged compound **3** were rapidly photocleaved providing **UNC2025** in 5 minutes (Figures S5). During this process, we could also follow the formation of intermediate compounds **1** and **2** simultaneously with the release of **UNC2025** (Figure S6). The fast cleavage and the low amount of **1** during the photolysis of **3** suggest that the carbonate group is cleaved first. Indeed, in the case of compound **1**, the DEACM was photocleaved in 40 s only (Figure S7), while 5 minutes were necessary for compound **2** (Figure S8). In the decaging process of all three compounds, we also observed the release of the coumarin **12** (Figures S5, S7, S8, peak at 5.69 min)

### Enzymatic and *in cellulo* evaluations

Then, the enzymatic evaluation of all photocaged compounds and **UNC2025** on the three TAM kinases was performed with or without irradiation using a luminescence-based ADP-Glo™ Kinase Assay measuring the amount of ADP formed from ATP conversion by the kinase.

The residual kinase activity of the enzymes was first checked after two hours incubation in the presence of each compound, without irradiation. As expected, the caged molecules lost the inhibitory potency of **UNC2025** with various efficiency depending on the protein and the nature of the caged compound (Table 1, Figures S9-11). The *N,O*-dicaged compound **3** proved to be the most effective since a complete loss of inhibition was observed within the TAM family. On MERTK, the mono-caged compounds **1** and **2** were respectively 16- and 90-fold less potent than the original inhibitor (IC<sub>50</sub> = 1120 nM and 6430 nM respectively vs 71 nM for **UNC2025**), while a 704-fold less potency was obtained for the dicaged compound **3** (IC<sub>50</sub> = 50000 nM). On TYRO3, the same trend was observed for the mono-caged compounds **1** and **2**, being respectively 16- and 33-fold less active than **UNC2025** (IC<sub>50</sub> = 6100 nM and 12700 nM respectively vs 386 nM for **UNC2025**), whereas the dicaged compound **3** displayed no inhibitory effect (IC<sub>50</sub>>100  $\mu$ M). On AXL, the IC<sub>50</sub> of the mono-caged compounds **1** and **2** were significantly lower than those of **UNC2025**, respectively 19- and 200-fold (IC<sub>50</sub> = 148 nM and 1600 nM respectively vs 8 nM for **UNC2025**), while the dicaged compound **3** was totally inactive. Of the three photocaged compounds, the *O*-caged compound **1** lost the least activity. This minor residual activity can be partially explained by the contamination of free **UNC2025** (~9% in HPLC, figure S7). Compound **1**

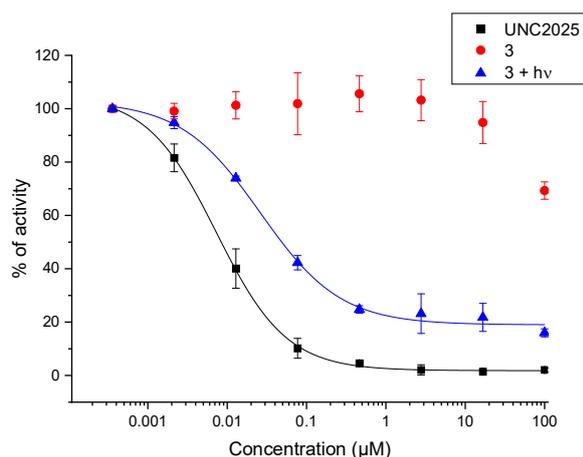
cleaves within 40 seconds, suggesting intrinsic instability at ambient light and, therefore, needing to be handled in the dark. Overall, these results validated our hypothesis that masking the hydroxyl group and/or the exocyclic amine with a photoremovable protecting group was deleterious to the TAM kinase inhibition, with a remarkable effect while masking both functional groups. In the meantime, we proved that the 7-DEACM **12** was totally inactive on the three kinases (Figure S12).

**Table 1.** IC<sub>50</sub> values of **UNC2025** and caged compounds **1**, **2**, and **3** on MERTK, TYRO3, and AXL without and with irradiation at 370 nm for 10 minutes (light intensity: 23.8 mW/cm<sup>2</sup>) using an ADP-Glo Kinase Assay. The ratios between IC<sub>50</sub> of the irradiated caged compounds and **UNC2025** are shown in brackets. The data represent means from duplicate experiments.

Enzymes	MERTK		TYRO3		AXL	
	IC <sub>50</sub>	IC <sub>50</sub> + UV	IC <sub>50</sub>	IC <sub>50</sub> +UV	IC <sub>50</sub>	IC <sub>50</sub> +UV
<b>UNC2025</b>	71 nM	81 nM	386 nM	360 nM	8 nM	8 nM
<b>1</b>	1120 nM (16)	246 nM (3)	6100 nM	810 nM	148 nM	25 nM (3)
<b>2</b>	6430 nM (90)	890 nM	12700 nM	2550 nM (7)	1600 nM	135 nM
<b>3</b>	50000 nM	359 nM (4)	>100 $\mu$ M	1680 nM (4)	>100 $\mu$ M	49 nM (6)

We then irradiated the photocaged compounds to demonstrate the feasibility of the desired uncaging process in *in vitro* enzymatic assays. First, we proved that the kinase activity of the three TAM kinases was not altered by irradiation within 1 hour. As a control, **UNC2025** was also tested under the same conditions, and its inhibitory activity remain unchanged regardless of the kinase (Table 1). The caged molecules were incubated with the enzymes and irradiated for 10 minutes at 370 nm. The residual kinase activity was then checked after two additional hours incubation under natural light. As expected, after irradiation, the TAM kinase activity was significantly inhibited.

On the three kinases (Table 1, figures S9-11), the three irradiated caged compounds **1**, **2**, and **3** were extremely more potent than the non-irradiated ones, with IC<sub>50</sub> of the same order of magnitude than **UNC2025**. The best recovery of the inhibitory activity was found for compounds **1** and **3** (3 and 4-6-fold increase IC<sub>50</sub> of **UNC2025** respectively, figure 2), whereas it was less efficient for **2** (7 to 16-fold increase IC<sub>50</sub> of **UNC2025**). The inhibitory efficacy of the three decaged compounds is slightly lower than that of **UNC2025**, probably because the drug is progressively released during the irradiation time (Figure S6), allowing competition between the newly decaged **UNC2025** with the kinase substrates already present in the medium (ATP, peptide). Since the difference between IC<sub>50</sub> with and without irradiation was the most significant for compound **3** (no or weak inhibitory effect), we selected it for cellular viability assays using two human-bladder cancer-derived cell lines: RT112 and SCaBER. While SCaBER strongly expresses AXL and, more weakly, TYRO3, the ratio is reversed for RT112. [35] MERTK is not expressed in both cell lines.



**Figure 2.** Kinase assay on AXL. Dose-response curve of **3** without irradiation (red line), **3** with irradiation at 370 nm (Light intensity: 23.8 mW/cm<sup>2</sup>, blue line), **UNC2025** as the positive control (black line). All enzymatic experiments were duplicated, error bars represent standard deviation

First, we showed that the cellular viability of both cell lines was not altered under 5 minutes irradiation at 370 nm (Figure S13). In the meantime, we proved that the 7-DEACM **12** was totally inactive on both cell lines with or without irradiation (Figure S14).

Without irradiation, compound **3** was 70-fold less active (IC<sub>50</sub> = 42.4 µM) than **UNC2025** (IC<sub>50</sub> = 0.6 µM) in the RT112 cell line, while a great loss of potency was observed in SCaBER (IC<sub>50</sub> = 125 µM, Table 2). Then, the antiproliferative activity of **3** was almost completely restored upon 5 minutes UV irradiation with 2.1 µM in RT112 and 39.8 µM in SCaBER as compared to 1 and 7.9 µM for **UNC2025**, highlighting the benefits of the UV uncaging process (Figure 3).

## Conclusion

We have developed the first photoactivatable versions of **UNC2025**, a first-in-class small molecule TAM kinase inhibitor, based on a rational molecular design. By grafting one coumarin-photocleavable protecting group on the hydroxyl or the exocyclic amine groups, the photocaged compounds showed from 16-fold to 200-fold less potent activity against all the three TAM kinases than **UNC2025**. We also introduced two coumarin groups respectively on the hydroxyl and amine groups. As a result, the corresponding *N,O*-dicaged compound **3** proved to be the most striking with a complete loss of activity in the enzymatic assays. As predicted from the photochemical experiments, the **UNC2025** activities of the caged compounds were restored upon UV irradiation with slightly lower efficacy than that of the native drug, and with a better efficiency for the monocaged compound **1** and the dicaged **3**. Thanks to its ineffective activity and recovered potent activity upon irradiation, analog to **UNC2025**, **3** fulfills the expected characteristics for a caged compound. Satisfyingly, the enzymatic inhibition assay could be translated to cellular viability assays in two human-bladder cancer cell lines. Indeed, the antiproliferative activity of **3** under UV irradiation was comparable to that of **UNC2025** in RT112 cell lines.

The successful application of the photopharmacology concept on the TAM kinase family in *in vitro* and *in cellulo* assays opens the way for further *in vivo* investigations, e.g. in zebrafish or mouse models. In addition, although light is very powerful, exposure to UV light associated with low penetration is still a major concern. Therefore, these results emphasize the possibility of incorporating red-shifted photoactivatable protecting groups in order to adjust the phototherapeutic window compatible to tissue

irradiation. Furthermore, they may also allow the use of two-photon sensitive photolabile groups that would enable lower energy and deeper tissue penetrating light.

## Supporting Information

The authors have cited additional references within the Supporting Information.<sup>[27, 34]</sup>

## Acknowledgements

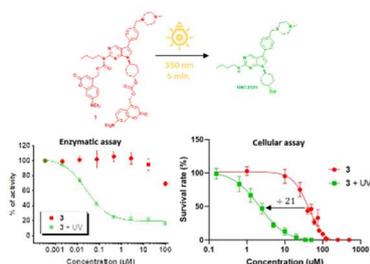
This project has received financial support from the CNRS through the MITI interdisciplinary programs, the Ministère de l'Enseignement Supérieur et de la Recherche for a doctoral fellowship for C.B.-P., the "Fondation pour la Recherche Médicale" for a doctoral fellowship for S.B. (ECO202206015545). This work has also benefited from the facilities and expertise of the Small Molecule Mass Spectrometry platform of IMAGIF (Centre de Recherche de Gif - [www.imagif.cnrs.fr](http://www.imagif.cnrs.fr)), the "service d'analyses Chromato-Masse", and the NMR service from BioCIS for LC-HRMS and NMR analysis respectively.

**Keywords:** photoactivatable • photopharmacology • prodrug • protein kinases • uncaging

- [1] a) I. M. Welleman, H. H. Boersma, M. W. H. Hoorens, B. L. Feringa, W. Szymanski, *Chem. Sci.* **2020**, *11*, 11672-11691; b) J. Morstein, D. Trauner, *Curr. Opin. Chem. Biol.* **2019**, *50*, 145-151; c) M. Michael Dcona, Koushambi Mitra, M. C. T. Hartman, *RSC Med. Chem.* **2020**, *11*, 982-1002.
- [2] M. Sharma, S. H. Friedman, *ChemPhotoChem* **2021**, *5*, 611-618.
- [3] P. Kobauri, F. J. Dekker, W. Szymanski, B. L. Feringa, *Angew. Chem. Int. Ed. Engl.* **2023**, *62*, e202300681.
- [4] a) R. Weinstain, T. Slanina, D. Sland, P. Klan, *Chem. Rev.* **2020**, *120*, 13135-13272; b) J. M. Silva, E. Silva, R. L. Reis, *J. Control Release* **2019**, *298*, 154-176; c) P. Klan, T. Solomek, C. G. Bochet, A. Blanc, R. Givens, M. Rubina, V. Popik, A. Kostikov, J. Wirz, *Chem. Rev.* **2013**, *113*, 119-191; d) L. Josa - Culleré, A. Llebaria, *ChemPhotoChem* **2020**, *5*, 296-314.
- [5] E. R. Thapaliy, L. Mony, R. Sanchez, B. Serraz, P. Paoletti, G. C. R. Ellis-Davies, *ChemPhotoChem* **2021**, *5*, 445-454.
- [6] a) W. Szymanski, J. M. Beierle, H. A. Kistemaker, W. A. Velema, B. L. Feringa, *Chem. Rev.* **2013**, *113*, 6114-6178; b) J. Broichhagen, J. A. Frank, D. Trauner, *Acc. Chem. Res.* **2015**, *48*, 1947-1960; c) N. Ankenbruck, T. Courtney, Y. Naro, A. Deiters, *Angew. Chem. Int. Ed. Engl.* **2018**, *57*, 2768-2798; d) Katharina Hüll, Johannes Morstein, D. Trauner, *Chem. Rev.* **2018**, *118*, 10710-10747.
- [7] a) R. Roskoski, Jr., *Pharmacol. Res.* **2019**, *144*, 19-50; b) F. Carles, S. Bourg, C. Meyer, P. Bonnet, *Molecules* **2018**, *23*, 1-18; c) M. M. Attwood, D. Fabbro, A. V. Sokolov, S. Knapp, H. B. Schioth, *Nat. Rev. Drug Discov.* **2021**, *20*, 839-861.
- [8] a) C. L. Fleming, M. Grötli, J. Andréasson, *ChemPhotoChem* **2019**, *3*, 318-326; b) D. Laczi, M. D. Johnstone, C. L. Fleming, *Chem. Asian J.* **2022**, *17*, e202200200.
- [9] a) J. F. Algorri, M. Ochoa, P. Roldan-Varona, L. Rodriguez-Cobo, J. M. Lopez-Higuera, *Cancers* **2021**, *13*, 3484; b) M. M. Lerch, M. J. Hansen, G. M. van Dam, W. Szymanski, B. L. Feringa, *Angew. Chem. Int. Ed. Engl.* **2016**, *55*, 10978-10999.
- [10] J. S. Wood, M. Koszelak, J. Liu, D. S. Lawrence, *J. Am. Chem. Soc.* **1998**, *120*, 7145-7146.
- [11] A. R. Morckel, H. Lusic, L. Farzana, J. A. Yoder, A. Deiters, N. M. Nascone-Yoder, *Development* **2012**, *139*, 437-442.
- [12] a) R. Ferreira, J. R. Nilsson, C. Solano, J. Andreasson, M. Grotli, *Sci. Rep.* **2015**, *5*, 9769; b) Y. Xu, C. Gao, M. Andreasson, L. Häversen, M. P. Carrasco, C. Fleming, T. Lundbäck, J. Andréasson, M. Grötli, *Eur. J. Med. Chem.* **2022**, *234*, 114226; c) D. Bliman, J. R. Nilsson, P. Kettunen, J. Andreasson, M. Grotli, *Sci. Rep.* **2015**, *5*, 13109.
- [13] D. Wilson, J. W. Li, N. R. Branda, *ChemMedChem* **2017**, *12*, 284-287.
- [14] Y. H. Tsai, S. Essig, J. R. James, K. Lang, J. W. Chin, *Nature Chem.* **2015**, *7*, 554-561.
- [15] a) D. Schmidt, T. Rodat, L. Heintze, J. Weber, R. Horbert, U. Girreser, T. Raeker, L. Bußmann, M. Kriegs, B. Hartke, C. Peifer, *ChemMedChem* **2018**, *13*, 2415-2426; b) B. Pinchuk, R. Horbert, A. Dobber, L. Kuhl, C. Peifer, *Molecules* **2016**, *21*.
- [16] a) M. W. H. Hoorens, M. E. Ourailidou, T. Rodat, P. E. van der Wouden, P. Kobauri, M. Kriegs, C. Peifer, B. L. Feringa, F. J. Dekker, W. Szymanski, *Eur. J. Med. Chem.* **2019**, *179*, 133-146; b) Zhiyuan Chen, Ran Ke, Zhiqiang Song, Yang Zhou, Xiaomei Ren, Weixue Huang, Zhen Wang, K. Ding, *Bioorg. Med. Chem. Lett.* **2022**, *64*, 128683.
- [17] a) M. Reynders, A. Chaikwad, B. T. Berger, K. Bauer, P. Koch, S. Laufer, S. Knapp, D. Trauner, *Angew. Chem. Int. Ed. Engl.* **2021**, *60*, 20178-20183; b) B. S. Hoffelner, S. Andreev, N. Plank, P. Koch, *Pharmaceuticals* **2023**, *16*, 264.
- [18] a) J. S. Wood, Koszelak, M., Liu, J., Lawrence, D. S., *J. Am. Chem. Soc.* **1998**, *120*, 7145-7146; b) T. Sommus, D. Lavogina, E. Enkvist, A. Uri, K. Viht, *Chem. Commun.* **2019**, *55*, 11147-11150.
- [19] D. Kolarski, A. Sugiyama, G. Breton, C. Rakers, D. Ono, A. Schulte, F. Tama, K. Itami, W. Szymanski, T. Hirota, B. L. Feringa, *J. Am. Chem. Soc.* **2019**, *141*, 15784-15791.
- [20] R. Chen, Z. Wang, L. Liu, Z. Pan, *Chem. Commun.* **2022**, *58*, 4901-4904.
- [21] K. Zhang, M. Ji, S. Lin, S. Peng, Z. Zhang, M. Zhang, J. Zhang, Y. Zhang, D. Wu, H. Tian, X. Chen, H. Xu, *J. Med. Chem.* **2021**, *64*, 7331-7340.

- [22] M. Zindler, B. Pinchuk, C. Renn, R. Horbert, A. Dobber, C. Peifer, *ChemMedChem* **2015**, *10*, 1335-1338.
- [23] R. Horbert, B. Pinchuk, P. Davies, D. Alessi, C. Peifer, *ACS Chem. Biol.* **2015**, *10*, 2099-2107.
- [24] a) R. M. Linger, A. K. Keating, H. S. Earp, D. K. Graham, *Adv Cancer Res* **2008**, *100*, 35-83; b) S. M. Post, M. Andreeff, C. DiNardo, J. D. Khoury, P. P. Ruvolo, *Biochim. Biophys. Acta Mol. Cell. Res.* **2021**, *1868*, 118992.
- [25] a) A. Mikolajczyk, F. Mitula, D. Popiel, B. Kaminska, M. Wieczorek, J. Pieczykolan, *Cancers* **2022**, *14*, 2488; b) S. K. Smart, E. Vasileiadi, X. Wang, D. DeRyckere, D. K. Graham, *Cancers* **2018**, *10*, 474; c) D. K. Graham, D. DeRyckere, K. D. Davies, H. S. Earp, *Nat. Rev. Cancer* **2014**, *14*, 769-785.
- [26] J. Le Bescont, L. Mouawad, T. Boddaert, S. Bombard, S. Piguel, *ChemPhotoChem* **2021**, *5*, 989-994.
- [27] W. Zhang, D. DeRyckere, D. Hunter, J. Liu, M. A. Stashko, K. A. Minson, C. T. Cummings, M. Lee, T. G. Glaros, D. L. Newton, S. Sather, D. Zhang, D. Kireev, W. P. Janzen, H. S. Earp, D. K. Graham, S. V. Frye, X. Wang, *J. Med. Chem.* **2014**, *57*, 7031-7041.
- [28] J. Liu, W. Zhang, M. A. Stashko, D. Deryckere, C. T. Cummings, D. Hunter, C. Yang, C. N. Jayakody, N. Cheng, C. Simpson, J. Norris-Drouin, S. Sather, D. Kireev, W. P. Janzen, H. S. Earp, D. K. Graham, S. V. Frye, X. Wang, *Eur. J. Med. Chem.* **2013**, *65*, 83-93.
- [29] J. Liu, C. Yang, C. Simpson, D. Deryckere, A. Van Deusen, M. J. Miley, D. Kireev, J. Norris-Drouin, S. Sather, D. Hunter, V. K. Korboukh, H. S. Patel, W. P. Janzen, M. Machius, G. L. Johnson, H. S. Earp, D. K. Graham, S. V. Frye, X. Wang, *ACS Med. Chem. Lett.* **2012**, *3*, 129-134.
- [30] X. Wang, J. Liu, W. Zhang, M. A. Stashko, J. Nichols, M. J. Miley, J. Norris-Drouin, Z. Chen, M. Machius, D. DeRyckere, E. Wood, D. K. Graham, H. S. Earp, D. Kireev, S. V. Frye, *ACS Med. Chem. Lett.* **2016**, *7*, 1044-1049.
- [31] M. J. Hansen, W. A. Velema, M. M. Lerch, W. Szymanski, B. L. Feringa, *Chem. Soc. Rev.* **2015**, *44*, 3358-3377.
- [32] a) J. P. Olson, M. R. Banghart, B. L. Sabatini, G. C. Ellis-Davies, *J. Am. Chem. Soc.* **2013**, *135*, 15948-15954; b) Q. Lin, L. Yang, Z. Wang, Y. Hua, D. Zhang, B. Bao, C. Bao, X. Gong, L. Zhu, *Angew. Chem. Int. Ed. Engl.* **2018**, *57*, 3722-3726; c) M. Klausen, V. Dubois, G. Clermont, C. Tonnele, F. Castet, M. Blanchard-Desce, *Chem. Sci.* **2019**, *10*, 4209-4219; d) M. Bojtar, A. Kormos, K. Kis-Petik, M. Kellermayer, P. Kele, *Org. Lett.* **2019**, *21*, 9410-9414.
- [33] a) P. Bourbon, Q. Peng, G. Ferraudi, C. Stauffacher, O. Wiest, P. Helquist, *Bioorg. Med. Chem. Lett.* **2013**, *23*, 6321-6324; b) P. Bourbon, Q. Peng, G. Ferraudi, C. Stauffacher, O. Wiest, P. Helquist, *Bioorg. Med. Chem. Lett.* **2013**, *23*, 2162-2165.
- [34] T. Weinrich, M. Gränz, C. Grünewald, T. F. Prisner, M. W. Göbel, *Eur. J. Org. Chem.* **2017**, 491-496.
- [35] F. Dufour, L. Silina, H. Neyret-Kahn, A. Moreno-Vega, C. Krucker, N. Karboul, M. arion Dorland-Galliot, P. Maillé, E. Chapeaublanc, Y. Allory, N. Stransky, H. Haegel, T. Menguy, V. Duong, F. Radvanyi, I. Bernard-Pierrot, *Br. J. Cancer* **2019**, *120*, 555-564

## Entry for the Table of Contents



Photopharmacology: three photoactivatable small molecules based on the **UNC2025** scaffold were developed for controlling TAM kinase activity *in vitro* and *in cellulo* assays. The dicaged compound **3** proved to be the most striking with a complete loss of inhibition while recovering full activity upon light irradiation.

Institute and/or researcher Twitter usernames: @SandrinePiguel, @BioCIS