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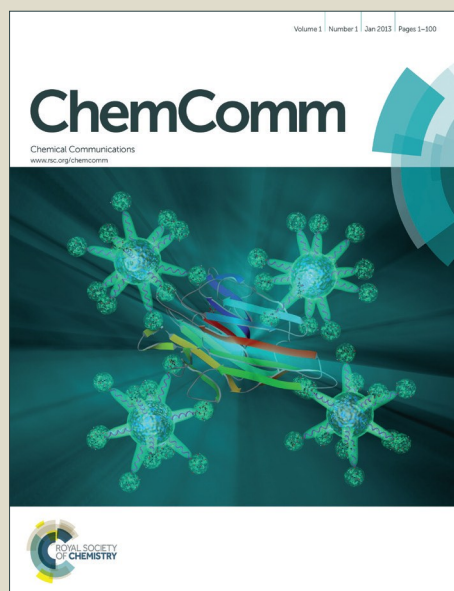
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## Highly solvatochromic and tunable fluorophores based on a 4,5-quinolimine scaffold: Novel CDK5 probes<sup>§,†</sup>

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**Novel 4,5-quinolimine-based fluorophores are more solvatochromic and red-shifted than known naphthalimide analogues. Conjugation of one of these fluorophores to a peptide derived from CDK5 kinase demonstrated its sensitivity for monitoring the interaction with its regulatory partner p25. Introduction of the quinolimine-labelled peptide into living glioblastoma cells probed the interaction with endogenous p25.**

Solvatochromic fluorophores have been used in the design of biosensors with applications ranging from the study of protein structural dynamics to the detection of protein-binding interactions.<sup>1</sup> Among these fluorophores, 1,8-naphthalimide derivatives have recently attracted considerable interest, due to their small size and their favorable photophysical properties.<sup>2</sup> These derivatives exhibit very weak fluorescence in water and polar solvents, compared to that in non-polar environments. This highly solvatochromic behavior confers advantage of high signal-to-noise ratio for detection of molecular interactions.<sup>1a, 1c</sup> 1,8-Naphthalimide-based fluorophores have found wide application in pH,<sup>3</sup> cation<sup>2c</sup> and anion<sup>2a</sup> chemosensors, DNA binders and anticancer agents,<sup>2b</sup> or as reporters of biomolecular interactions<sup>1a-c</sup> and cellular imaging agents.<sup>2b, 4</sup> One shortcoming of many of these sensors is their limited solubility in water.<sup>1a</sup> Taking into account that introduction of a protonatable nitrogen atom into the 1,8-naphthalimide scaffold could increase water solubility and red-shift the fluorescence emission,<sup>5</sup> along with the prevalence of the quinoline core in diverse fluorescence sensors,<sup>6</sup> we decided to explore the fluorescence properties of chimeric naphthalimide-quinoline fluorophores (Figure 1, B) and their potential for developing probes to monitor protein/protein

interactions. In this study we have investigated the utility of chimeric naphthalimide-quinoline fluorophores, to characterize the interaction between two peptides derived from the main interface between CDK5 kinase and its regulatory partner p25. Recent studies suggest that CDK5 expression and hyperactivation are involved in glioblastoma and correlate with the pathological grade of gliomas.<sup>7</sup> However, up to now, there are no available tools to monitor the CDK5/p25 interaction in its native environment. The crystal structure of this neurospecific kinase complex indeed shows that CDK5 binds p25 through the same interface as other CDKs, namely the C helix.<sup>8</sup> However, the affinity of this interface has not been characterized compared to that of other CDK/Cyclin complexes, such as CDK2/Cyclin A or CDK4/Cyclin D1. We show herein that one of the quinolimides herein described constitutes a much more sensitive probe for monitoring these peptide-peptide interactions than other standard dyes. Finally, we show that internalization of the quinolimine-conjugated peptide derived from CDK5 into cultured glioblastoma cells, provides means of localizing endogenous p25.

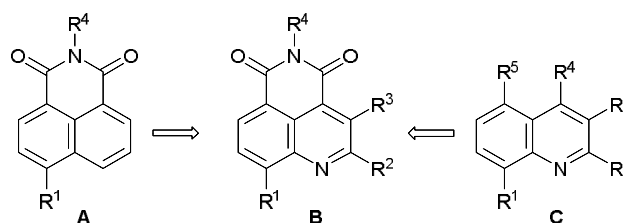


Fig. 1

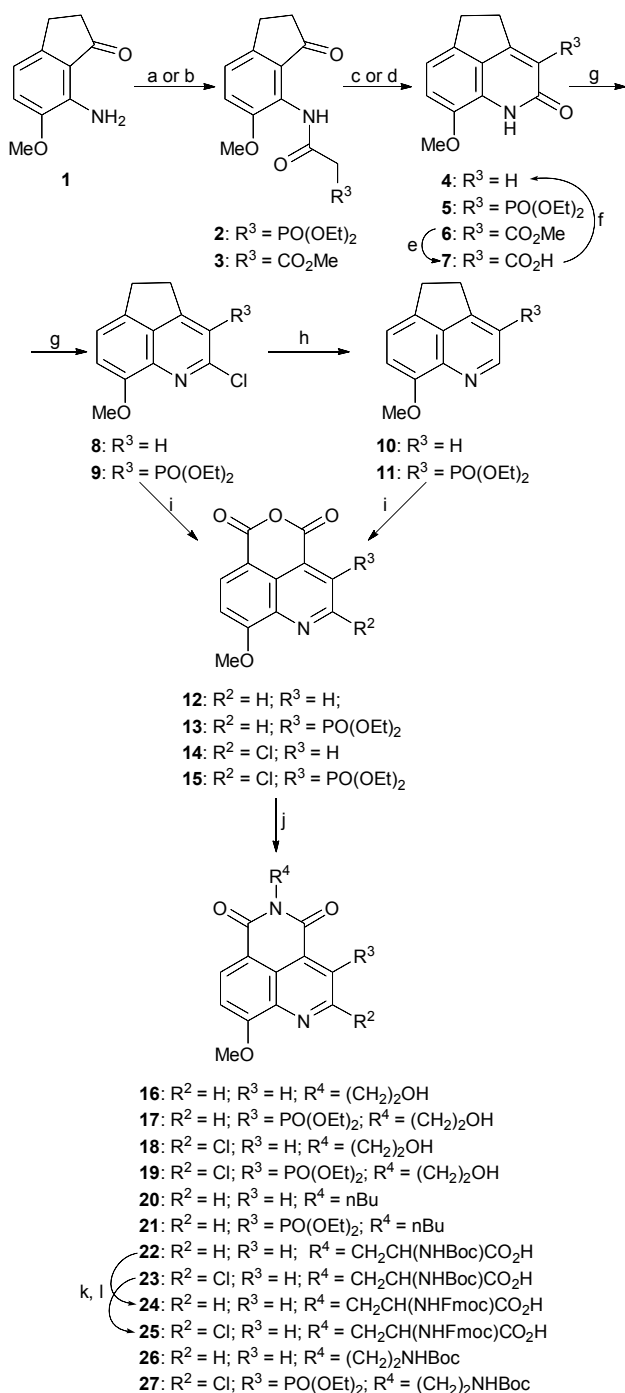
To the best of our knowledge only two articles<sup>9</sup> and a recent patent<sup>10</sup> have previously reported on the synthesis and biological activity of 4,5-quinolimine (4*H*-benzo[*de*][2,6]-naphthyridine-4,6(5*H*)-dione) derivatives. However, nothing about their fluorescence properties has been described yet and the synthetic methods used for the building of the quinolimine scaffold in these references are rather different from those herein described.

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<sup>§</sup>This manuscript is dedicated to Professor M. Teresa García-López on occasion of her retirement after a distinguished scientific career on Medicinal Chemistry.

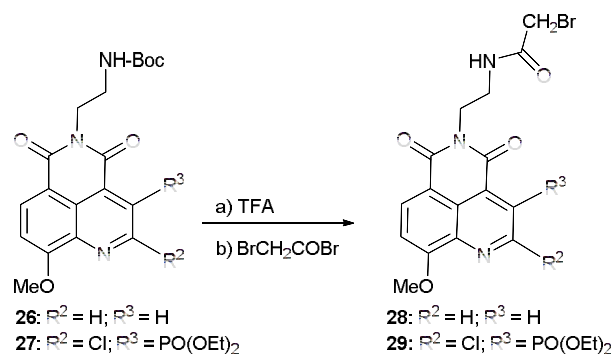
<sup>†</sup>Electronic Supplementary Information (ESI) available: Experimental procedures, photophysical and calculation data, figures of titration and cell imaging of a quinolimine labelled CDK5 peptide, as well as, NMR spectra. See DOI: 10.1039/x0xx00000x



**Scheme 1** Synthesis of quinolimide derivatives **16-27**. *Reagents and conditions:* (a) (EtO)<sub>2</sub>OPCH<sub>2</sub>CO<sub>2</sub>H, HATU, DIPEA, CH<sub>2</sub>Cl<sub>2</sub>, rt, 76 %; (b) ClCOCH<sub>2</sub>CO<sub>2</sub>Me, DIPEA, CH<sub>2</sub>Cl<sub>2</sub>, rt, 100 %; (c) LiCl, DBU, THF, rt, 100 %; (d) K<sub>2</sub>CO<sub>3</sub>, MeOH, 70 °C, 98%; (e) NaOH, H<sub>2</sub>O, 100 °C, 100%; (f) 180 °C, DMF, 86%; (g) POCl<sub>3</sub>, 110 °C, 96-100%; (h) H<sub>2</sub>, 10% Pd(C), MeOH, rt, 100%; (i) CrO<sub>3</sub>, (5:1) Ac<sub>2</sub>O/AcOH, 110 °C, 89-93%; (j) H<sub>2</sub>N-R<sup>4</sup>, EtOH, MW 120 °C, 29-81%; (k) TFA, dioxane, rt, 100%; (l) Fmoc-OSu, dioxane, rt, 71-78%.

As shown in Scheme 1, we devised a synthetic pathway starting for 4,5-quinolimides **B** starting from the 2,3-dihydro-1H-inden-1-one **1**<sup>11</sup>. Under reported conditions<sup>11</sup> (NaH, THF, 50 °C, 2h), the intramolecular Wittig-Horner reaction of amide **2** led to a mixture of the cyclized phosphonate **5** as major product (67%), along with the unsubstituted compound **4** (11%). However, when the cyclization was carried out with DBU/LiCl

in THF at room temperature the phosphonate **5** was obtained quantitatively. On the other hand, the unsubstituted quinolinone derivative **4** was obtained in 84% overall yield from the amide **3**, by intramolecular aldol reaction, followed by ester saponification and decarboxylation. Aromatization of the 4,5-dihydrocyclopenta[de]quinolin-2(1H)-ones **4** and **5**, by reaction with POCl<sub>3</sub>, led to the 2-chloro-quinoline derivatives **8** and **9**, whose catalytic hydrogenation produced the quinoline derivatives **10** and **11** in excellent yields. Then, oxidation of **8-11** by CrO<sub>3</sub> in (6:1) Ac<sub>2</sub>O/AcOH at 110 °C gave the corresponding anhydrides **12-15**. These anhydrides were not stable to silica gel of chromatography. Therefore, **12-15** were used for the quinolimide synthesis without further purification, after a thorough removal of chromium derivatives by filtration through Clarcel®. Reaction of anhydrides **12-15** with the corresponding amine under MW activated heating at 100 °C in EtOH yielded the desired quinolimide derivatives **16-27** in medium to good yields. Among these compounds, it is interesting to note the synthesis of the β-amino-alanine derivatives **22-25** as tools for the preparation of fluorophore-containing peptides. Furthermore, the ethylenediamines **26** and **27** were used for the preparation of thiol-reactive fluorophores, the bromomethyl acetamides **28** and **29** (Scheme 2).



**Scheme 2** Synthesis of the thiol-reactive bromomethyl acetamides **28** and **29**

The UV absorption and fluorescence properties of all synthesized quinolimide derivatives **16-26** were determined in solvents of varying polarity. The naphthalimide analogue **A1** [Figure 1, **A**, R<sup>4</sup> = (CH<sub>2</sub>)<sub>2</sub>OH] was also synthesized<sup>12</sup> for comparison purposes. The photophysical data of this reference compound and those of **16-19**, as quinolimide models, are summarized in Table 1. The UV studies show that replacement of the naphthalimide core by the quinolimide one (**A1** vs **16**) produced a small bathochromic shift (4-15 nm) in the λ<sub>max</sub><sup>abs</sup> and, except in water, a 30-35% decrease in the extinction coefficient. Introduction of the phosphonate group into the quinolimide scaffold (**17** vs **16** and **19** vs **18**), induced a significant bathochromic shift of 17-22 nm in the λ<sub>max</sub><sup>abs</sup>.

Regarding the fluorescence properties, the quinolimide derivatives showed a greater solvatochromism than the naphthalimide **A1**. A shift of 48 nm toward the red was observed in the λ<sub>max</sub><sup>em</sup> of **16** going from toluene to water (in **A1**

**Table 1.** Photophysical properties of the naphthalimide **A1** and quinolimide derivatives **16-19**

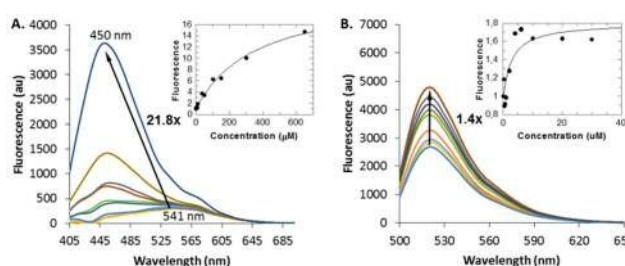
compd <sup>a</sup>	solvent	$\lambda_{\max}^{\text{abs}}$ (nm)	$\epsilon$ (M <sup>-1</sup> cm <sup>-1</sup> )	$\lambda_{\max}^{\text{em}}$ (nm)	$\Phi_F$ <sup>b</sup>
<b>A1</b>	toluene	362	12251	425	0.64
	dioxane	360	12085	425	0.61
	MeOH	366	12794	442	0.57
	DMSO	368	11898	442	0.47
	H <sub>2</sub> O	377	6770	457	0.61
	In vacuum <sup>c</sup>	346		390	
<b>16</b>	toluene	377	7873	455	0.90
	dioxane	372	8649	460	0.67
	MeOH	375	6760	490	0.28
	DMSO	377	6849	490	0.03
	H <sub>2</sub> O	381	8776	503	0.04
	In vacuum <sup>c</sup>	365		434	
<b>17</b>	toluene	395	4939	484	0.46
	dioxane	390	4252	487	0.54
	MeOH	397	5157	514	0.06
	DMSO	397	4325	510	0.01
	H <sub>2</sub> O	403	3752	525	0.01
	In vacuum <sup>c</sup>	382		463	
<b>18</b>	toluene	381	7555	467	0.83
	dioxane	376	7023	470	0.67
	MeOH	379	7562	500	0.12
	DMSO	382	4399	504	0.01
	H <sub>2</sub> O	385	2740	510	0.03
	In vacuum <sup>c</sup>	371		446	
<b>19</b>	toluene	401	4476	494	0.52
	dioxane	404	5280	503	0.31
	MeOH	411	3997	525	0.007
	DMSO	395	3725	527	0.0003
	H <sub>2</sub> O	402	3435	536	0.001
	In vacuum <sup>c</sup>	391		483	

<sup>a</sup>Measured in duplicate at a 12  $\mu\text{M}$  concentration, except for **A1** that was measured at a 7.5  $\mu\text{M}$ . <sup>b</sup>Quantum yields calculated with reference to quinine sulfate (in 0.1 M H<sub>2</sub>SO<sub>4</sub>), except for **17** and **19**, that were calculated with reference to coumarin 102 (in EtOH). <sup>c</sup>TD-DFT calculations in vacuum.

this shift was 32 nm), along with a 22-fold decrease in the quantum yield, which in **A1** was insignificant. On the other hand, introduction of a Cl at position 2 (**18** vs **16** and **19** vs **17**) produced an additional shift to the red of 7-12 nm, and a phosphonate group at position 3 (**16** vs **17** and **18** vs **19**) an additional shift of 22-29 nm. These substitutions also enhanced the sensitivity to the solvent polarity. For instance, the quantum yield of **19** decreases 520-fold from toluene ( $\Phi = 0.52$ ) to water ( $\Phi = 0.001$ ). However, no significant influence of the substituent at the imide nitrogen atom (for example, **16** vs **20** and **19** vs **21**) was observed in the photophysical properties. As a consequence of the red-shifts in the  $\lambda_{\max}^{\text{em}}$ , quinolimides **16-19** present larger Stokes shifts (30-45 nm larger in toluene and 46-58 nm in water) than the naphthalimide **A1**. Similarly to this reference compound, the fluorescence of the quinolimide **16** in dioxane did not decrease after 1h of irradiation at the  $\lambda_{\max}^{\text{abs}}$  (372 nm), demonstrating the high photostability of the fluorophore (Figure S1 of Supplementary Information). In addition, the quinolimide **16** displayed a 3.2-fold higher solubility in water<sup>13</sup> than the naphthalimide **A1**.

TD-DFT calculations at the PBE0/6-31+G(d,p) computational level<sup>14</sup> have been carried out within the Gaussian-09 program<sup>15</sup> to model the photophysical properties of compounds **A1** and **16-19**. The computational level used provided an adequate description of the UV and fluorescence spectra of quinolimide derivatives<sup>16</sup>. The calculated  $\lambda_{\max}^{\text{abs}}$  and  $\lambda_{\max}^{\text{em}}$  (Table 1) present excellent linear correlations with the experimental values in toluene ( $R^2 = 0.99$  and  $0.99$ , respectively, see Supplementary Information). Based on the calculations, both frontier orbitals HOMO and LUMO (Table S2 of Supplementary Information) are involved in absorption and fluorescence transitions, although the energetic value of the LUMO orbital is more affected by the structural differences of these compounds than the HOMO. The comparison of the geometrical parameters for both  $S_0$  and  $S_1$  states of compounds **A1** and **16-19** shows a similar behavior with alternating variations in bond lengths along the tricyclic structure (shown for **16** in Figure S3).

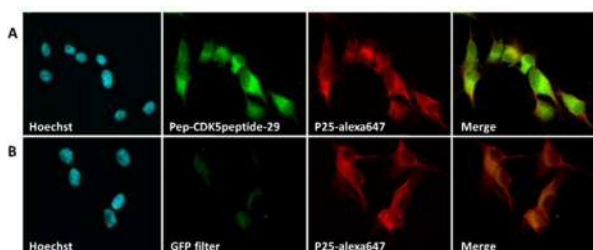
We next asked whether the bromomethyl acetamide **29** could serve to report on the interaction between the C helix-derived peptide of CDK5 (GVPSSALREICLLK, K5 peptide) and the interacting alpha helix in p25 (KEAFWDRCLSVINLM, p25 peptide). As shown in Figure 2A, titration of 5  $\mu\text{M}$  **29**-labelled-K5 peptide with the p25 peptide yielded a 14.7-fold increase in fluorescence emission intensity (21.8-fold at saturation) of this probe with a  $K_d$  value of  $355.0 \mu\text{M} \pm 57$ . Moreover, this interaction led to a shift in the emission maximum of probe **29** from 541 to 450 nm, whereas the same experiment performed with an irrelevant peptide derived from ubiquitin had no effect on fluorescence emission of **29**-labelled-K5 peptide (Figure S4), indicating that the probe does not contribute to promote a false interaction. In comparison, the fluorescein (FITC) labelled K5 peptide only yielded a 1.6-fold increase in fluorescence enhancement, with no associated spectral shift (Figure 2B). These results reveal that the quinolimide derivative **29** is a highly sensitive probe for monitoring interactions between peptides.



**Fig. 2** A) Titration of 5  $\mu\text{M}$  **29**-labelled CDK5 peptide with p25 peptide ( $\lambda_{\text{ex}} = 390$  nm) and curve fit. B) Titration of 200 nM FITC-labelled CDK5 peptide with p25 peptide ( $\lambda_{\text{ex}} = 495$  nm) and curve fit.

Finally, we asked whether the **29**-labelled-K5 peptide could serve as a probe to colocalize endogenous p25 in the U87 glioblastoma cell line when conjugated to the CDK5 peptide. As expected, the CDK5 **29**-labelled peptide did not enter cells alone. We therefore used a Pep-CDK5 peptide (KETWWETWWTEKK-GVPSSALREICLLK), which bears an N-terminal fusion of the Pep1 cell-penetrating peptide<sup>17</sup> linked to

the CDK5 peptide, and found that it penetrated readily into living U87 cells (Figure S5). To address whether the **29**-labelled Pep-CDK5 peptide indeed colocalized with endogenous p25, we performed indirect immunofluorescence of U87 cells overlaid with the **29**-labelled Pep-CDK5 peptide (Figure 3). These experiments revealed a complete overlay of the **29** signal observed through the GFP filter and of the signal revealed with Alexa647-labelled secondary/anti-p25 primary antibodies (Figure 3A). In contrast, cells which had not been overlaid with the **29**-labelled peptide did not reveal any signal through the GFP channel (Figure 3B).



**Fig. 3** Labelled CDK5 peptide colocalizes with intracellular p25 in U87 cells. A) **29**-labelled Pep-CDK5 peptide observed through the GFP filter was found to colocalize with p25 detected by immunofluorescence using Alexa647 secondary antibodies. B) Not overlaid with **29**-labelled peptide.

In conclusion, we have synthesized solvatochromic naphthalimide-quinoline hybrid fluorophores that show higher solvatochromic behavior and fluorescence emission red-shift than known naphthalimide analogues. Additionally, quinolimide derivatives display large Stokes shifts, photostability and 3.2-fold higher kinetic solubility in water than naphthalimides. The novel fluorophores have been incorporated into amino acid derivatives, as tools for the preparation of fluorophore-containing peptides, and into thiol-reactive compounds for protein labeling at cysteine. Conjugation of one of these quinolimide-based fluorophores to a CDK5 derived peptide has allowed to monitor the interaction between two peptides derived from the main interface between the catalytic kinase subunit CDK5 and its regulatory subunit p25, respectively. Finally, we have successfully employed the quinolimide-labelled peptide derived from the main interface of CDK5 with its regulatory partner p25 to probe endogenous p25 in living cells, revealing that this probe is sufficiently sensitive for detection of endogenous p25 protein, and further emphasizing the potential of quinolimides for cellular imaging applications.

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## Notes and references

- (a) G. S. Loving, M. Sainlos and B. Imperiali, *Trends Biotechnol.*, 2010, **28**, 73-83; (b) Z. Yang, J. Cao, Y. He, J. H. Yang, T. Kim, X. Peng and J. S. Kim, *Chem. Soc. Rev.*, 2014, **43**, 4563-4601; (c) A. S. Klymchenko and Y. Mely, *Prog. Mol. Biol. Transl. Sci.*, 2013, **113**, 35-58; (d) L. D. Lavis and R. T. Raines, *ACS Chem. Biol.*, 2008, **3**, 142-155.
- (a) R. M. Duke, E. B. Veale, F. M. Pfeffer, P. E. Kruger and T. Gunnlaugsson, *Chem. Soc. Rev.*, 2010, **39**, 3936-3953; (b) S. Banerjee, E. B. Veale, C. M. Phelan, S. A. Murphy, G. M. Tocci, L. J. Gillespie, D. O. Frimannsson, J. M. Kelly and T. Gunnlaugsson, *Chem. Soc. Rev.*, 2013, **42**, 1601-1618; (c) P. A. Panchenko, O. A. Fedorova and Y. V. Fedorov, *Russ. Chem. Rev.*, 2014, **83**, 155-182, 128 pp.
- (a) D. Cui, X. Qian, F. Liu and R. Zhang, *Org. Lett.*, 2004, **6**, 2757-2760; (b) V. B. Bojinov and T. N. Konstantinova, *Sens. Actuators, B*, 2007, **123**, 869-876; (c) N. I. Georgiev, V. B. Bojinov and P. S. Nikolov, *Dyes Pigm.*, 2010, **88**, 350-357.
- L. Yuan, W. Lin, K. Zheng, L. He and W. Huang, *Chem. Soc. Rev.*, 2013, **42**, 622-661.
- J. J. Bryant, B. D. Lindner and U. H. F. Bunz, *J. Org. Chem.*, 2013, **78**, 1038-1044.
- (a) X.-M. Meng, S.-X. Wang and M.-Z. Zhu, in *Molecular Photochemistry. Various Aspects*, ed. S. Saha, InTech, Shanghai, 2012, ch. 1, pp. 3-22; (b) J. A. González-Vera, E. Luković and B. Imperiali, *J. Org. Chem.*, 2009, **74**, 7309-7314; (c) D. Sarkar, A. Pramanik, S. Jana, P. Karmakar and T. K. Mondal, *Sens. Actuators, B*, 2015, **209**, 138-146; (d) B. Ojha and G. Das, *Photochem. Photobiol. Sci.*, 2011, **10**, 554-560; (e) C. Tran, T. Gallavardin, M. Petit, R. Slimi, H. Dhimane, M. Blanchard-Desce, F. C. Acher, D. Ogden and P. I. Dalko, *Org. Lett.*, 2015, **17**, 402-405; (f) S. Mukherjee, A. K. Paul, K. Krishna Rajak and H. Stoeckli-Evans, *Sens. Actuators, B*, 2014, **203**, 150-156; (g) C. Núñez, E. Oliveira, J. Garcia-Pardo, M. Diniz, J. Lorenzo, J. L. Capelo and C. Lodeiro, *J. Inorg. Biochem.*, 2014, **137**, 115-122; (h) X. Dong, C. H. Heo, S. Chen, H. M. Kim and Z. Liu, *Anal. Chem.*, 2014, **86**, 308-311.
- (a) R. Liu, B. Tian, M. Gearing, S. Hunter, K. Ye and Z. Mao, *Proc. Nat. Acad. Sci. U.S.A.*, 2008, **105**, 7570-7575; (b) Q. Xie, Q. Wu, C. M. Horbinski, W. A. Flavahan, K. Yang, W. Zhou, S. M. Dombrowski, Z. Huang, X. Fang, Y. Shi, A. N. Ferguson, D. F. Kashatus, S. Bao and J. N. Rich, *Nat. Neurosci.*, 2015, **18**, 501-510; (c) R. Yushan, G. Roodrajeetsing, C. Wenjie, D. Yiwu, Z. Tengfei, W. M. Madushi, L. Feifei, Z. Changwen, W. Xin, L. Zuyun, C. Gang and H. Suning, *World J. Surg. Oncol.*, 2015, **13**, 223.
- C. Tarricone, R. Dhavan, J. Peng, L. B. Areces, L.-H. Tsai and A. Musacchio, *Mol. Cell*, 2001, **8**, 657-669.
- (a) M. F. Braña, A. Gradillas, A. Gómez, N. Acero, F. Linares, D. Muñoz-Mingarro, C. Abradelo, F. Rey-Stolle, M. Yuste, J. Campos, M. A. Gallo and A. Espinosa, *J. Med. Chem.*, 2004, **47**, 2236-2242; (b) F. Castriconi, M. Paolino, G. Giuliani, M. Anzini, G. Campiani, L. Mennuni, C. Sabatini, M. Lanza, G. Caselli, F. De Rienzo, M. C. Menziani, M. Sbraccia, P. Molinari, T. Costa and A. Cappelli, *Eur. J. Med. Chem.*, 2014, **82**, 36-46.
- WO2014052906A1, 2014.
- WO2009062285A1, 2009.
- N. V. Marinova, N. I. Georgiev and V. B. Bojinov, *J. Photochem. Photobiol., A*, 2011, **222**, 132-140.
- (a) B. Bard, S. Martel and P.-A. Carrupt, *Eur. J. Pharm. Sci.*, 2008, **33**, 230-240; (b) J. Alsenz and M. Kansy, *Adv. Drug Delivery Rev.*, 2007, **59**, 546-567; (c) L. Di, P. V. Fish and T. Mano, *Drug Discovery Today*, 2012, **17**, 486-495.
- (a) C. Adamo and V. Barone, *J. Chem. Phys.*, 1999, **110**, 6158-6170; (b) P. C. Hariharan and J. A. Pople, *Theor. Chim. Acta*, 1973, **28**, 213-222.
- M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, G. Scalmani, V. Barone, B. Mennucci, G. A. Petersson, H. Nakatsuji, M. Caricato, X. Li, H. P. Hratchian, A. F. Izmaylov, J. Bloino, G. Zheng, J. L. Sonnenberg, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, T. Vreven, J. A. Montgomery Jr., J. E. Peralta, F. Ogliaro, M. J. Bearpark, J. Heyd, E. N. Brothers, K. N. Kudin, V. N. Staroverov, R. Kobayashi, J. Normand, K. Raghavachari, A. P. Rendell, J. C. Burant, S. S. Iyengar, J. Tomasi, M. Cossi, N. Rega, N. J. Millam, M. Klene, J. E. Knox, J. B. Cross, V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, R. E. Stratmann, O. Yazyev, A. J. Austin, R. Cammi, C. Pomelli, J. W. Ochterski, R. L. Martin, K. Morokuma, V. G. Zakrzewski, G. A. Voth, P. Salvador, J. J. Dannenberg, S. Dapprich, A. D. Daniels, Ö. Farkas, J. B. Foresman, J. V. Ortiz, J. Cioslowski and D. J. Fox, *Gaussian 09*, Gaussian, Inc., Wallingford, CT, USA, 2009.
- (a) D. Jacquemin, E. A. Perpete, G. Scalmani, I. Ciofini, C. Peltier and C. Adamo, *Chem. Phys.*, 2010, **372**, 61-66; (b) D. Jacquemin, E. A. Perpete, G. Scalmani, M. J. Frisch, I. Ciofini and C. Adamo, *Chem. Phys. Lett.*, 2007, **448**, 3-6.
- (a) M. C. Morris, J. Depollier, J. Mery, F. Heitz and G. Divita, *Nat. Biotech.*, 2001, **19**, 1173-1176; (b) L. Kurzawa, M. Pellerano and M. C. Morris, *Biochim. Biophys. Acta*, 2010, **1798**, 2274-2285.