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PIM kinase-responsive microsecond-lifetime photoluminescent probes based on selenium-containing heteroaromatic tricycle

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Abstract

A new structural fragment was synthesized for construction of protein binding-responsive photoluminescent probes. In complex with protein kinases of the PIM family, bisubstrate inhibitors containing benzo[4,5]seleno[3,2-d]pyrimidin-4-one moiety revealed microsecond-lifetime phosphorescence emission after pulse excitation with near-UV radiation. The phosphorescence signal was substantially (more than 50-fold) amplified by a covalently bound fluorescent dye (PromoFluor-555 or PromoFluor-647) whose absorption spectrum well overlapped with the phosphorescence emission spectrum of the selenium-containing heteroaromatic tricycle. The developed organic small-molecule long-lifetime photoluminescence probes possess subnanomolar affinity towards kinases of the PIM family and reveal especially strong emission signal with PIM-2 isozyme. The developed probes have potential to be used for monitoring of activity of PIM kinases for diagnosis of cancer.

1. Introduction

Binding-responsive small-molecule photoluminescent probes possess analyte-dependent photoluminescence properties that make them into simple and useful tools for analysis of chemical, biological and medical samples.[1] Such probes are in active use for determination of different types of analytes, specially ions and small molecules.[2] There is still a great need developing responsive probes for the analysis of several if not most pharmaceutically interesting proteins, thus such probes could be useful tools for both biological research and disease diagnostics.[3]

Different luminescence parameters of photoluminescent probes possess different sensitivity to binding of the probe to a protein. For example, fluorescence anisotropy (FA) of the fluorescent probe correlates with its rotation speed and thus it is dependent on the molecular weight of the fluorophore-containing complex. Therefore the association of a small molecule containing a fluorophore with a larger molecule (*e.g.*, a protein or a nucleic acid) inevitably leads to increase of the FA of the fluorophore. This change in FA can be measured with a common fluorescence spectrometer allowing the determination of binding affinity of the probe to the protein and application of the probe for characterization of unlabeled inhibitors in displacement assays.

The measurement of fluorescence intensity at a certain wavelength is the most straightforward and sensitive way to quantify the fluorophore, hence high sensitivity of an assay based on fluorescence intensity measurement can be achieved with simple equipment. Therefore just photoluminescent probes whose emission intensity is changed as a result of its binding to the target protein are defined as responsive probes. On the other side, fluorescence intensity of the probe may show only marginal change upon association of the target molecule with the probe, although a large change in fluorescence signal intensity upon specific binding of the probe to the protein has been achieved in some cases.[4] Additionally, biological samples often possess a high level of autofluorescence and scatter light leading to substantial background noise for the fluorescence intensity measurements thereby reducing the sensitivity of assays based on fluorescence intensity detection.

Compounds emitting luminescence with long decay time (luminescence lifetime in microsecond- or millisecond-scale) enable time-gated luminescence (TGL) measurement after radiation pulse excitation of the probe. This eliminates the noise caused by short lifetime fluorescence and light scattering.[5] Phosphorescence and other long-lifetime luminescence

phenomena are rare in water solution at room temperature.[5] Mostly lanthanide[6] and noble metal complexes (chelates, cryptates, *etc.*) containing organic antenna chromophores are emitting long-lifetime luminescence. Such probes have found wider application for bioanalytical measurements.[7] Luminescence intensity of such probes shows weak dependency on the specific association of the probes with target proteins. The responsiveness of the probes can be achieved by incorporation of additional fluorophores or quenchers or by labeling of target proteins which results in the dependency of luminescence intensity on binding of the probe to its labeled target protein because of change in the efficiency of energy transfer.[8]

The triplet excited-state of organic molecules is susceptible to nonradiative relaxation *via* thermal and collisional processes. Therefore its lifetime is sensitive to the presence of atmospheric triplet oxygen and other compounds in the measurement solution that can facilitate phosphorescence quenching processes. Organic phosphorescent probes have not found wider application for protein analysis in biological samples.[5,9]

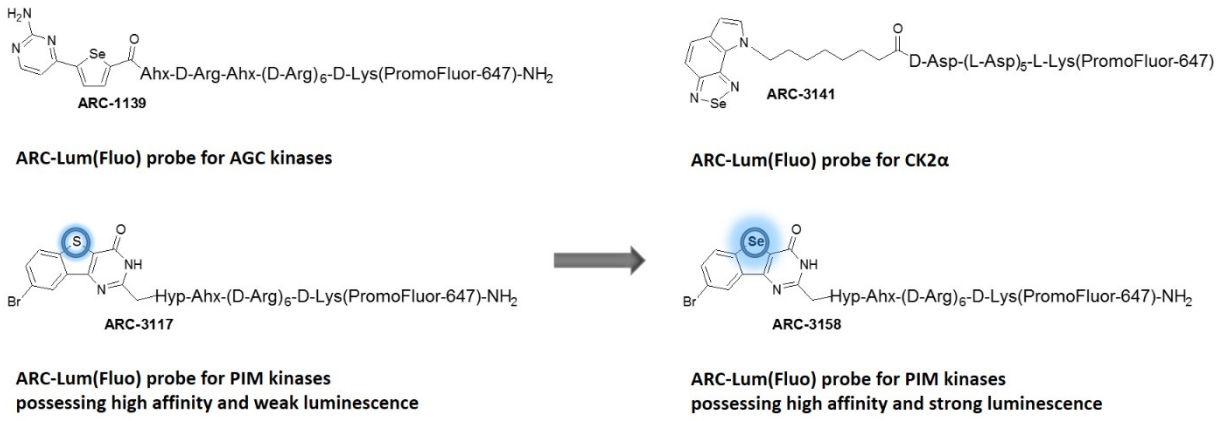


Fig. 1 Chemical structures of previously disclosed responsive microsecond-lifetime photoluminescent probes ARC-1139[10] and ARC-3141[12]. Generation of a PIM-selective selenium-comprising microsecond-lifetime photoluminescent probe ARC-3158, proceeding from its sulfur-comprising counterpart ARC-3117[10].

In recent publications we have reported on novel organic small-molecule protein binding-responsive photoluminescent ARC-Lum probes (Fig. 1).[10] After excitation with a flash of UV radiation these probes emit long-lifetime (luminescence lifetime $\tau = 20 – 250$ μs) luminescence in complex with a protein kinase (PK), while the free probes revealed very weak or no long-lifetime emission.[10] These probes have been derived from bisubstrate inhibitors (ARCs, adenosine analogue-peptide conjugates) of PKs[11] in which ATP-site binding heteroaromatic fragment contained a sulfur or a selenium atom.[10,12] The

heteroaromatic fragment of an ARC-Lum probe that targets the ATP-binding pocket of the PK possess a steady state fluorescence signal at 400 – 600 nm if excited with near-UV radiation (300 – 370 nm), both in free as well as in the bound state with a PK. Additionally, ARC-Lum probes possess a weak phosphorescence emission signal with microsecond-long decay time at wavelength range from 500 to 650 nm if associated with a PK (Fig. 2A).[10,12] Sulfur and selenium are heavy atoms that support intersystem crossing of electrons in the excited state leading to the triplet activated state that may emit light as phosphorescence. In contrast to the free (unbound) state of the probe, the hydrophobic and shielded environment in the ATP-binding pocket of the PK protects the exited triplet state from quenching by oxygen and restricts molecular movements, leading to increased phosphorescence and PK-binding responsiveness of ARC-Lum probes. Because of lack of a fluorescent dye these probes are designated as ARC-Lum(-) probes.

If an ARC-Lum probe additionally incorporates a conjugated fluorescence dye in close proximity to the phosphorescence donor [designated as ARC-Lum(Fluo) probes] immense sensitization (up to 2000-fold) of the luminescence signal takes place (Fig. 2B).[10] The delayed emission spectrum of ARC-Lum(Fluo) probes coincides with the fluorescence emission spectrum of the conjugated dye.[13] This phenomenon is probably induced by Förster-type resonant energy transfer (FRET) from the triplet electronic excited state of the sulfur- or selenium-containing aromatic system (luminescence donor) to the conjugated dye (acceptor fluorophore) leading to the singlet excitation state of the acceptor[14,15] and slow emission (τ = 20 – 250 μs) of light from the excited fluorophore.

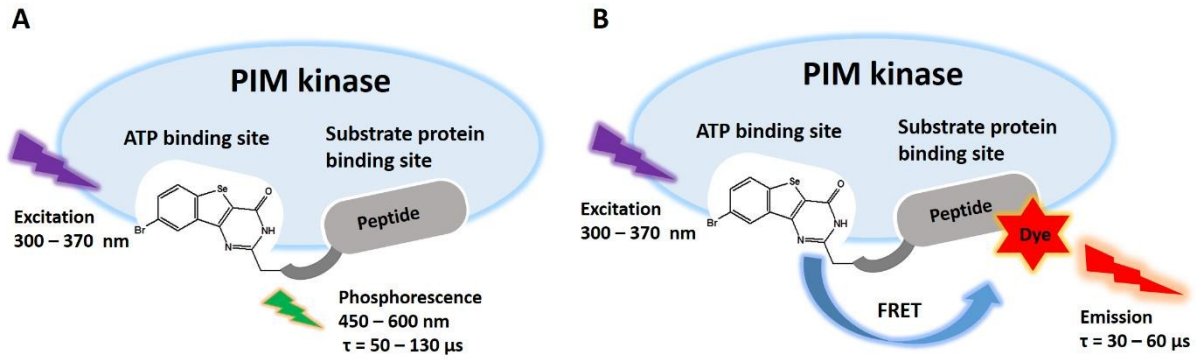


Fig. 2 Schematic representation of (A) ARC-Lum(-) and (B) ARC-Lum(Fluo) probe in complex with PIM kinase.

PIM family of PKs includes three constitutively active serine/threonine kinases, PIM-1, PIM-2 and PIM-3 that all regulate key biological processes, including cell survival, proliferation,

differentiation, and apoptosis.[16,17] Elevated expression levels of PIM-1 and PIM-2 have been observed in hematologic malignancies and prostate cancer, increased PIM-3 expression has been detected in some solid tumors. These findings suggest that PIM kinases are potential drug targets and biomarkers of human cancers.[16,17] Recent success with development of PIM-selective inhibitors with low picomolar inhibitory potency has activated clinical testing of these inhibitors for treating hematologic cancers.[18, 19]

We have described selective bisubstrate inhibitors with sub-nanomolar affinity for PK PIM-1 that contained benzo[4,5]thieno[3,2-d]pyrimidin-4-one fragment as the ATP pocket-binding moiety of the inhibitors.[20] Labelling of the inhibitors with a fluorescent dye led to high-affinity fluorescent probes for anisotropy measurement.[20,21] The benzothiophene moiety of these compounds incorporates a sulfur-containing heteroaromatic cycle, similarly to previous ARC-Lum probes, thus the long-lifetime signal of its complex with PIM-1 was tested. Only a negligible long-lifetime signal could be determined for the complex of the probe with PIM-1, therefore these probes were not studied further as ARC-Lum probes.

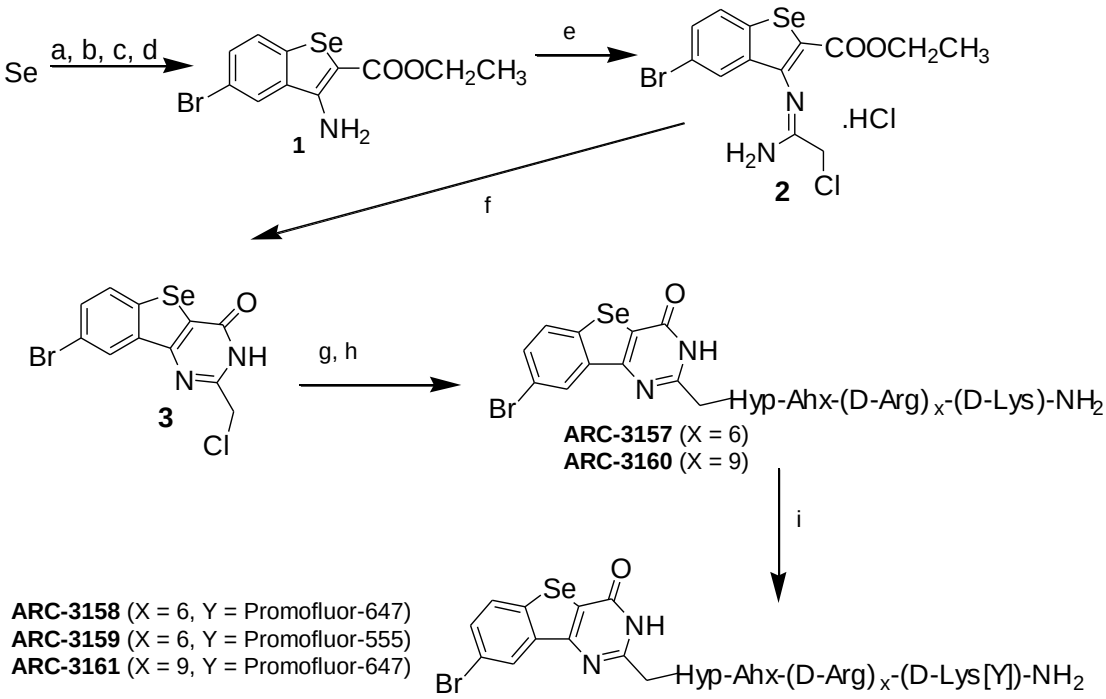
Here we report on selenium-containing counterparts of benzo[4,5]thieno[3,2-d]pyrimidin-4-one derived ARC-Lum(Fluo) probes. These new compounds possess strong microsecond-lifetime photoluminescence in complexes with PIM kinases. Especially strong binding-responsive luminescence signal was established for the PK PIM-2 that supports the application of the new probes in biomedical research.

2. Results and discussion

2.1. Synthesis of a selenium-containing aromatic tricycle and its fluorescent dye-labelled conjugates with peptides

Synthesis of benzo[4,5]seleno[3,2-d]pyrimidin-4-one fragment (Scheme 1) was generally similar to the preparation of its previously reported sulfur-containing counterpart.[20,22] Differently from the previous reaction set-up 5-bromo-2-fluorobenzonitrile was reacted with sodium selenide (that was produced from selenium and sodium borohydride just before the reaction), followed by treatment of the intermediate product with ethyl bromoacetate. The following cyclization reaction, performed in the presence of sodium hydroxide, led to the formation of compound **1.** Treatment of **1** with chloroacetonitrile in the presence of hydrochloric acid gave intermediate compound **2** that was cyclized by refluxing of the solution to yield benzo[4,5]seleno[3,2-d]pyrimidin-4-one derivative **3**. The peptidic structures

were synthesized on Rink-amide resin by using conventional Fmoc-chemistry procedures and thereafter the chloromethyl containing compound **3** was conjugated to the N-terminus of the peptides as described earlier.[20] Cleavage and deprotection of the resin-bound reaction products with trifluoroacetic acid (TFA) resulted in peptide conjugates [ARC-Lum(-) probes] ARC-3157 and ARC-3160. Fluorescent dyes PromoFluor-647 or PromoFluor-555 were connected to the conjugates *via* amino group of the side chain of lysine residue resulting in ARC-Lum(Fluo) probes ARC-3158, ARC-3159 and ARC-3161 (Scheme 1).



Scheme 1 Synthetic route to ARC-Lum(Fluo) probes: a) $NaBH_4/H_2O$, 0 °C; b) 5-Bromo-2-fluorobenzonitrile, DMF, 0-20 °C; c) $BrCH_2COOCH_2CH_3$, 0-20 °C; d) 4M NaOH, 0-60 °C; e) chloroacetonitrile, 4N HCl in dioxane; f) dioxane, reflux; g) peptide on resin, DIPEA, 60 °C, 8h; h) TFA, TIS, H_2O; i) NHS ester of PromoFluor dye, TEA, DMSO.

2.2. Affinity of ARC-Lum(Fluo) probes towards PIM kinases and PKAc

For the determination of affinity of probes their titration with PKs was performed using assays with FA or TGL intensity readouts. This study revealed very high affinity (K_D values close to 1 nM) for probes ARC-3158, ARC-3159 and ARC-3161 towards PIM kinases and approximately 100-fold lower affinity towards another basophilic PK, catalytic subunit of protein kinase A (PKAc) (Table 1). Selenium containing probes ARC-3158, ARC-3159, ARC-3161 and their sulfur containing counterpart ARC-3117 (Fig. 1, compound 17 in ref. 20) possessed similar affinity towards the tested PKs. This kinase affinity profile is substantially

different from that of the probe ARC-1139[10] containing 5-(2-aminopyrimidin-4-yl)selenophene-2-carboxylic acid moiety as the phosphorescent fragment; the latter probe possessed more than 100-fold higher affinity to PKAc than to PIM kinases.

Table 1 Dissociation constants (K_D,* nM) of complexes of ARC-Lum(Fluo) probes with PKs of the PIM family and PKAc

Compound	K_D*, nM			
	PIM-1	PIM-2	PIM-3	PKAc
ARC-3117[20]	0.6±0.2	0.7±0.2	1.2±0.4	276±26
ARC-3158	0.4±0.2	0.7±0.3	1.2±0.4	187±56
ARC-3159	0.5±0.2	0.9±0.4	1.8 ±0.5	95±27
ARC-3161	0.4±0.2	0.5±0.2	1.3±0.4	85±26
ARC-1139[10]	8±3	16±5	5±2	0.02

*K_D values (nM) were determined in a binding/displacement assay with FA or TGL intensity readout. Reported K_D values are the mean of 4 – 5 independent experiments with 95% confidence interval.

2.3. Spectral characterization of selenium-containing compounds

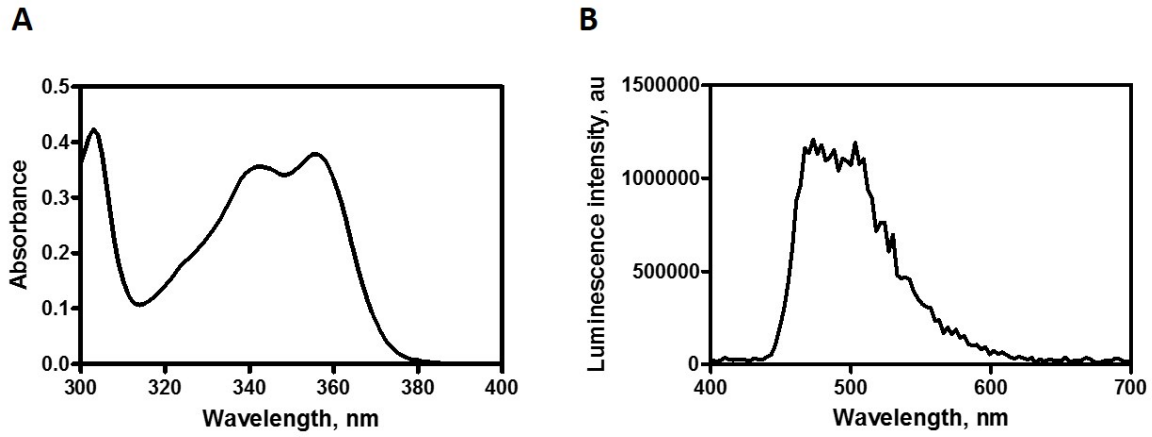


Fig. 3 Optical spectra of an ARC-Lum(-) probe ARC-3157. (A) UV absorption spectrum of ARC-3157 (670 μM) in water, pH 7.4 (path length 1 mm). (B) Phosphorescence emission spectrum of ARC-3157 (200 nM) in complex with PIM-2 (200 nM) in assay buffer (50 mM HEPES, 150 mM NaCl, 0.005% Tween 20, 5 mM DTT and 0.5 mg/ml BSA). Excitation at 360(5) nm, delay time 50 μs and gate 200 μs.

Benzo[4,5]seleno[3,2-d]pyrimidin-4-one fragment has an absorption maximum with molar absorption coefficient (ε) of 5700 $M^{-1}cm^{-1}$ at 356 nm (Fig. 3A). This value of ε is smaller than these values for previously reported ARC-Lum probes containing selenadiazole (ε = 10000 –

15000 $M^{-1}cm^{-1}$)[12] or thiophene and selenophene (ε = 15000 – 20000 $M^{-1}cm^{-1}$)[10] moieties but it is still sufficiently high for the application of the corresponding ARC-Lum probes in bioassays.

Table 2 Phosphorescence lifetimes (τ, μs) of ARC-Lum(-) probes ARC-3157 and ARC-3160 in complexes with PKs of the PIM family and PKAc

Compound	τ, μs			
	PIM-1	PIM-2	PIM-3	PKAc
ARC-3157	41 ± 4	84 ± 10	38 ± 6	132 ± 22
ARC-3160	53 ± 5	87 ± 15	42 ± 5	131 ± 20

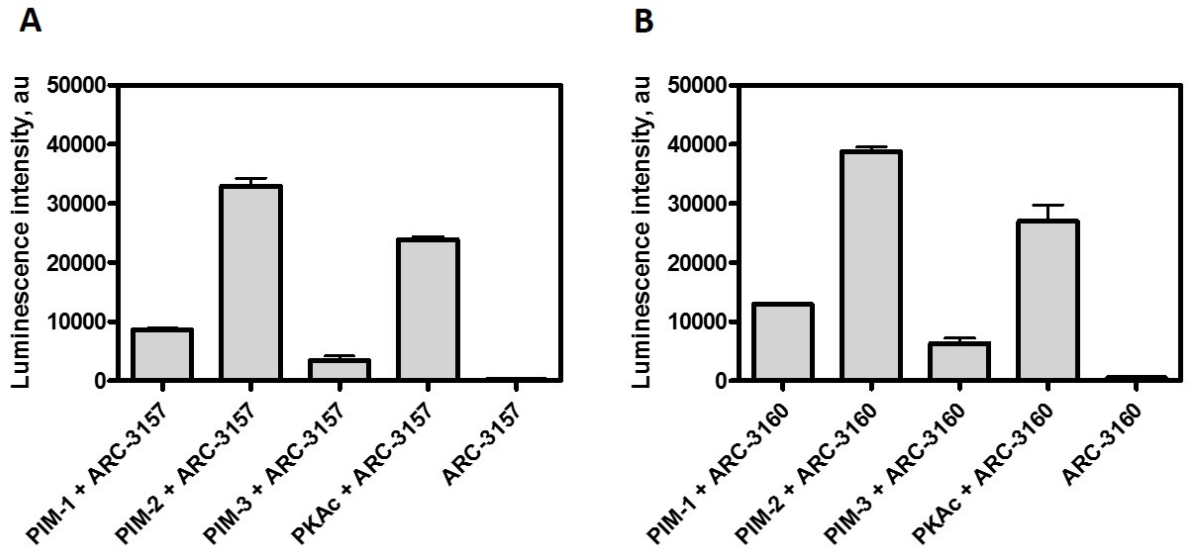


Fig. 4 Luminescence intensity of ARC-s with different PKs measured in TGL mode. (A) ARC-3157 (200 nM) and (B) ARC-3160 (200 nM) with PKs (500 nM). Excitation at 330(60) nm, emission at 520(14) nm, delay 50 μs and gate 150 μs.

Compound ARC-3157 is a selenium-containing counterpart of the previously reported sulfur-containing bisubstrate inhibitor (ARC-3104, compound **10** of ref. 20) of PIM kinases that showed about 100-fold selectivity for PIMs compared to another basophilic PK, PKAc.[20] Differently from sulfur-containing conjugates, binding of ARC-3157 and ARC-3160 to PKs resulted in complexes that emitted phosphorescence in wavelength range between 450 – 600 nm, after excitation at 360 nm (Fig. 3B). The phosphorescence intensity of the complexes of ARC-3157 (Fig. 4A) or ARC-3161 (Fig. 4B) with PIM-2 was 3 – 7-fold stronger than these with PKs PIM-1 and PIM-3 (Fig. 4, Table 2). This difference points to an unknown particularity of the three-dimensional structure of PIM-2 protein compared to other members

of the PIM kinase family, PIM-1 and PIM-3.[23] The signal intensity and lifetime of the probe in complex with PKAc was similar to that of the complex with PIM-2, but because of substantially lower (more than100-fold) affinity of PKAc high protein concentration was needed for complex formation and even at 500 nM concentration of PKAc the ARC-probes were still not fully bound to the PK. The phosphorescence decay times of the complexes of probes with PKs are listed in Table 2. ARC-3104 (Compound **10** of ref. 20), a sulfur-containing analogue of ARC-3157, gave a hardly detectable phosphorescence signal in complex with the studied PKs; the signal intensity of ARC-3104 was about 100-fold lower than the intensity of the corresponding signals of selenium-containing probes ARC-3157 and ARC-3160.

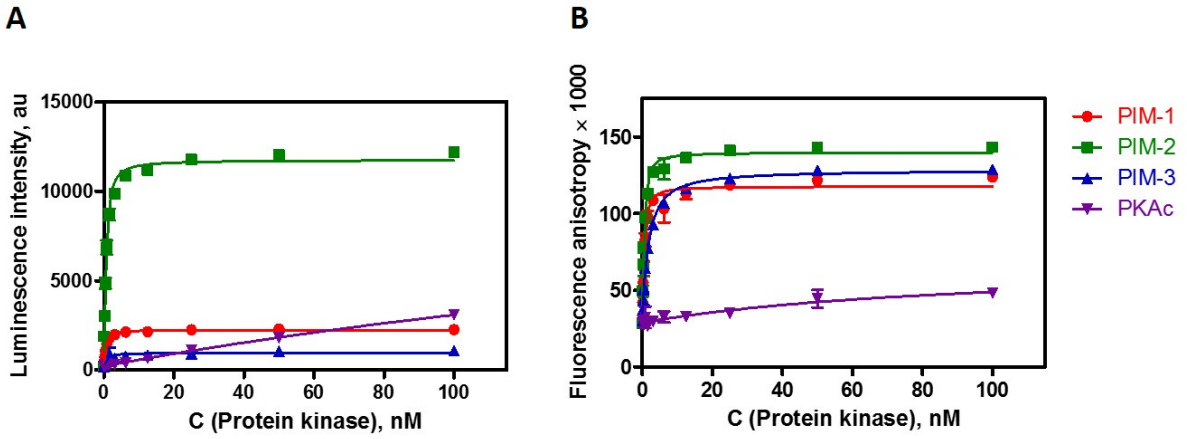


Fig. 5 Titration of ARC-3158 (1 nM) with PIM kinases and PKAc. (A) TGL intensity-based detection [excitation at 330(60) nm, emission at 675(50) nm, delay 50 μs and gate 150 μs,], (B) FA-based detection [excitation at 590(50) nm and emission at 675(50) nm].

Table 3 Luminescence lifetime τ (μs), relative zero-timepoint luminescence intensity A_0 (A_0 = 1 for ARC-3158 in complex with PIM-1) and efficiency of energy transfer from the donor to the acceptor luminophore E ($E = 1 - \tau_{DA}/\tau_D$) of microsecond lifetime photoluminescence for ARC-Lum(Fluo) probes in complex with PKs

Compound	PIM-1		PIM-2		PIM-3		PKAc	
	τ, μs (E)*	A_0	τ, μs (E)*	A_0	τ, μs (E)*	A_0	τ, μs (E)*	A_0
ARC-3158	28 ± 3 (0.32)	~1.00	67 ± 5 (0.20)	~0.93	22 ± 3 (0.42)	~0.84	57 ± 5 (0.57)	~0.57

ARC-3159	26 ± 2 (0.37)	~1.61	51 ± 4 (0.39)	~0.72	21 ± 3 (0.45)	~1.43	38 ± 3 (0.71)	~1.96
ARC-3161	42 ± 3 (0.21)	~0.35	67 ± 5 (0.23)	~0.50	30 ± 3 (0.29)	~0.44	68 ± 4 (0.48)	~0.43
ARC-3117	~27	~0.03	44 ± 5	~0.03	~29	~0.02	85± 13	~0.02

* $E = 1 - \tau_{DA}/\tau_D$, where τ_D is lifetime of the ARC-Lum probe without a dye [ARC-Lum(-) probes ARC-3157 or ARC-3160] and τ_{DA} is lifetime of the corresponding ARC-Lum(Fluo) probes containing an acceptor dye PromoFluor-555 or PromoFluor-647.

Compounds ARC-3158, ARC-3159 and ARC-3161 that incorporate a fluorescent dye gave much stronger (approximately 50 – 70-fold) microsecond-lifetime luminescence signals in the complexes with PKs (Fig. 5 and Table 3) than the compounds without a dye, ARC-3157 and ARC-3160 (Fig. 4 and Table 2). Such signal sensitization results from the efficient energy transfer from the excited triplet state of the selenium-containing heterocycle (donor) to the conjugated fluorescent dye (acceptor).[15,10] The amplification of the signal takes place as the rate of energy transfer between the luminophores is significantly higher than the rate of phosphorescence decay. Although triplet-singlet energy transfer is quantum mechanically forbidden (like phosphorescence), it can still slowly take place in case of strong spin-orbit coupling and this process can be very efficient if the luminescence decay of the donor is slow and the distance between donor and acceptor is short (shorter than the Förster distance of the luminophores).[15] Both of these conditions are fulfilled in case of PK-bound ARC-Lum(Fluo) probes. The efficiency of energy transfer can be estimated by comparing luminescence lifetimes of the compounds without a fluorescent dye (donor only) and compounds containing a dye (donor and acceptor) by using the equation $E = 1 - \tau_{DA}/\tau_D$, where τ_D is the luminescence lifetime of the ARC-Lum probe without a dye and τ_{DA} is lifetime of the compound containing an acceptor dye.[15] Lifetimes and normalized zero time-point intensity values A_0 together with efficiencies of energy transfer E are listed in Table 3. Zero time-point luminescence intensity (A_0) was established by extrapolation of luminescence decay curves to zero time-point. The illumination source used for luminescence measurements was xenon flash lamp that possesses long after-glow, therefore the application of shorter than 50 μs delay times was not possible. Thus, the extrapolated values of A_0 in Table 3 possessed larger uncertainty for complexes with shorter luminescence lifetime. The probe ARC-3159, incorporating a fluorescent dye PromoFluor-555 was measured with distinct optical filters than the probes that incorporated the fluorescent dye PromoFluor-647, hence A_0-values for ARC-3159 are not directly

comparable with the A_0-values measured for other probes. Generally, there was a good correlation between the luminescence lifetime and the A_0 values (better FRET efficiency leads to increased decay rate and probes with shorter lifetime possess higher A_0 values), but deviations from this rule are also known. ARC-3159 possessed the shortest lifetime with all tested PKs that points to efficient energy transfer between the luminophores in this probe. ARC-3159 also possessed high A_0-values. Absorption spectrum of PromoFluor-555 well overlaps with the phosphorescence emission spectrum of benzo[4,5]seleno[3,2-d]pyrimidin-4-one moiety (Fig. 2B) resulting in more efficient energy transfer from the donor luminophore to the acceptor fluorophore than in case of probes containing the dye PromoFluor-647 (Fig. 6A). Also the time-gated emission spectra of ARC-3158 and ARC-3159 well coincide with the fluorescence emission spectra of PromoFluor-647 and PromoFluor-555, respectively (Fig. 6B). ARC-3161 possesses the longest luminescence lifetime and the lowest energy transfer efficiency, caused by a longer peptide chain separating the interacting luminophores. ARC-3117 as a sulfur counterpart of the selenium-containing probe ARC-3158 possessed 50 – 100-fold weaker TGL emission than the latter compound (Fig. 1). The given values of luminescence lifetime of the probe ARC-3117 in complex with PIM-1 and PIM-3 (Table 2) are approximate estimations because of the weakness of the signals generated by the probe.

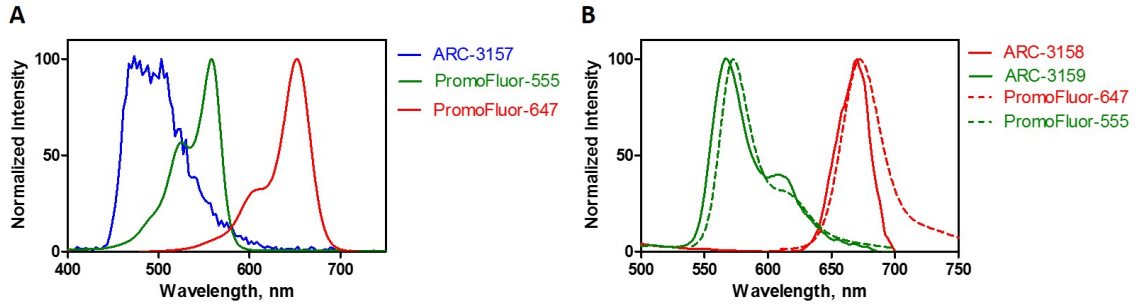


Fig. 6 TGL emission spectra of ARC-Lum probes as compared with fluorescence spectra of the attached fluorescent dyes PromoFluor-555 and PromoFluor-647. (A) The spectral overlap between the phosphorescence emission spectrum of ARC-3157 (donor) in complex with PIM-2 and the absorption spectra of free fluorescent dyes PromoFluor-555 and PromoFluor-647. (B) Coincidence of time-delayed emission spectra of probes ARC-3158 and ARC-3159 in complex with PIM-2 (continuous line) and the fluorescence emission spectra of the dyes incorporated in the probes, PromoFluor-647 and PromFluor-555, respectively (broken lines). TGL spectra of ARC-3158 (100 nM) and ARC-3159 (100 nM) in complex with of PIM-2 (500 nM) were recorded with excitation at 350(50) nm, using delay time of 50 μs and gate

time of 150 μs. The absorption and fluorescence emission spectra of non-conjugated PromoFluor dyes are taken from online sources.[24]

2.3. Comparison of photoluminescence properties of benzo[4,5]seleno[3,2-d]pyrimidin-4-one based probes and previously reported ARC-Lum probes based on other chemical scaffolds

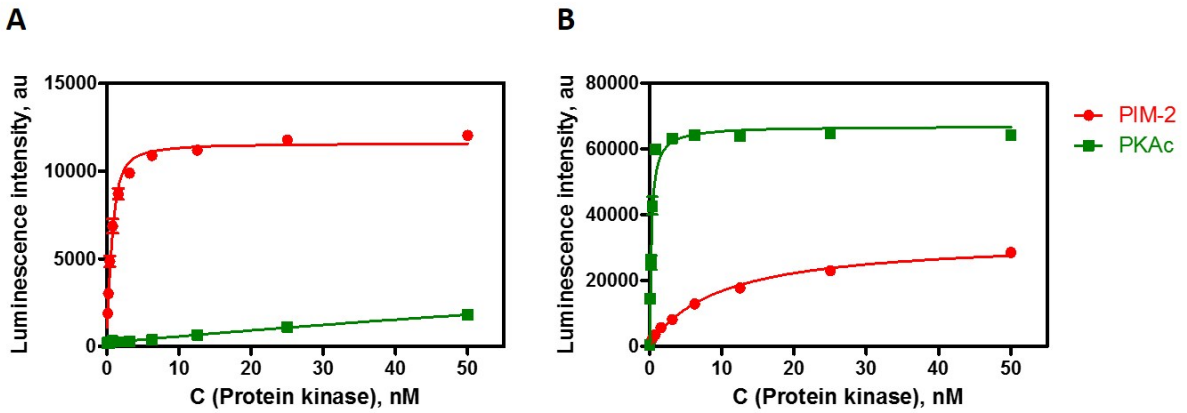


Fig. 7 Titration of (A) ARC-3158 (1 nM) and (B) ARC-1139 (1 nM) with PKs PIM-2 and PKAc [TGL detection: excitation at 330(60) nm, emission at 675(50) nm, delay 50 μs and gate 150 μs].

Compound ARC-1139[10] (Fig. 1) that comprises 5-(2-aminopyrimidin-4-yl)selenophene-2-carboxylic acid moiety as the phosphorescent fragment and PromoFluor-647 as the fluorescent acceptor dye is an ARC-Lum(Fluo) probe that has revealed high brightness in complex with AGC kinases. Now it was used as a reference probe to evaluate the optical properties of the new compounds. Both ARC-1139 and ARC-3158 are labelled with PromoFluor-647 dye and thus their luminescence can be directly compared using a 675(50) nm emission filter. ARC-1139 revealed about two-fold stronger signal in complex with PIM-2 than ARC-3158 although the affinity of the latter probe was higher (Fig. 7). The signal intensity difference was even larger in case of PKAc, where ARC-1139 revealed a 6-fold stronger signal. This difference may be caused by 3 – 4-fold smaller extinction coefficient of benzo[4,5]seleno[3,2-d]pyrimidin-4-one if compared to that of 5-(2-aminopyrimidin-4-yl)selenophene-2-carboxylic acid. Still ARC-3158 demonstrated more intense luminescence when bound to a kinase than most of the previously reported derivatives of thiophene[10] and selenadiazoles.[12] At low PK concentration ARC-3158 generates a PIM-2-selective signal, but

at higher (micromolar) concentration PKAc (and probably some other PKs of the AGC group) give rise to comparable signal intensity.

3. Conclusions

A new structural fragment was tested for construction of protein-responsive long-lifetime photoluminescent probes. Benzo[4,5]seleno[3,2-d]pyrimidin-4-one derived bisubstrate inhibitors with high affinity towards PKs of the PIM family revealed weak phosphorescence in complex with the kinases. Conjugation of a fluorescent dye PromoFluor-555 or PromoFluor-647 with the inhibitors led to ARC-Lum(Fluo) photoluminescent probes that in complex with PIM kinases after their excitation with a pulse of near-UV-radiation possessed intensive microsecond-lifetime emission at wavelengths corresponding to the fluorescence emission spectrum of the conjugated dye. In complex with PIM-2 new selenium-containing ARC-Lum(Fluo) probes revealed significantly (5 – 10-fold) stronger luminescence signal in the measurement window (50 μs delay, 150 μs gate) than in complex with other PKs of the PIM family, PIM-1 and PIM-3.

The results of the study also support our earlier assumption that numerous structurally diverse sulfur or selenium-containing heteroaromatic structures could be used for construction of binding-responsive long-lifetime photoluminescence probes for PKs and other proteins.

Supporting information

Electronic supplementary information (ESI) available:

Synthetic methods, structures of synthesized compounds, spectral characterization of compounds and experimental details of biochemical measurements are given in supporting information.

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