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ARC-TYPE HIGH AFFINITY FLUORESCENT PROBES FOR HIGH THROUGHPUT DRUG CANDIDATE SCREENING WITH FRET AND TR-FRET METHODS

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The estimation that more than 400 human diseases are linked to aberrant protein kinase (PK) signalling has made PKs important drug targets, especially for the different forms of cancer. Therefore the development of high throughput methods that allow drug candidate screening *in vitro* and *in vivo* is of high importance.

ARC-type bisubstrate analogue inhibitors (ARCs) are potent inhibitors of basophilic protein kinases of AGC family (Ki in low or sub nanomolar range) [1]. The bisubstrate character (simultaneous association with both binding sites of the kinase) allows ARC-based probes to be used for characterization of inhibitors targeted to either binding site of the kinase[2]. The unique structure of ARC-s allows the conjugation of fluorescent dyes without significant loss of binding affinity [3; 4]. Furthermore, fluorescently labelled ARCs can be efficiently combined with fluorescently labelled antibodies and PKs in Förster resonance energy transfer (FRET) based assays. Cell-permeable ARC-probes can be successfully exploited in combination with PKs expressed as fusions of fluorescent proteins in cell-based FRET assays for the characterisation of the ability of drug candidates to cross the plasma membrane and act on the expected target[5].

The application of lanthanide cryptates as fluorescence donors in time-resolved FRET assays overcomes both the excitation cross-talk and background fluorescence occurring in complicated biological samples such as live cells, cell or tissue extracts, and blood plasma Furthermore, even subnanomolar concentrations of lanthanide cryptate-labeled ARCs can be successfully used in complicated biological solutions without significant loss of sensitivity or disturbance by short-lived fluorecence characteristic for cellular components.

References

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