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Biological evaluation of 4,5,7-trisubstituted Indeno[1,2-*b*]indoles reveals a potent inhibitor of Protein Kinase CK2 in tumor cells with diverse anti-cancer effects and preferential cytoplasmic localization

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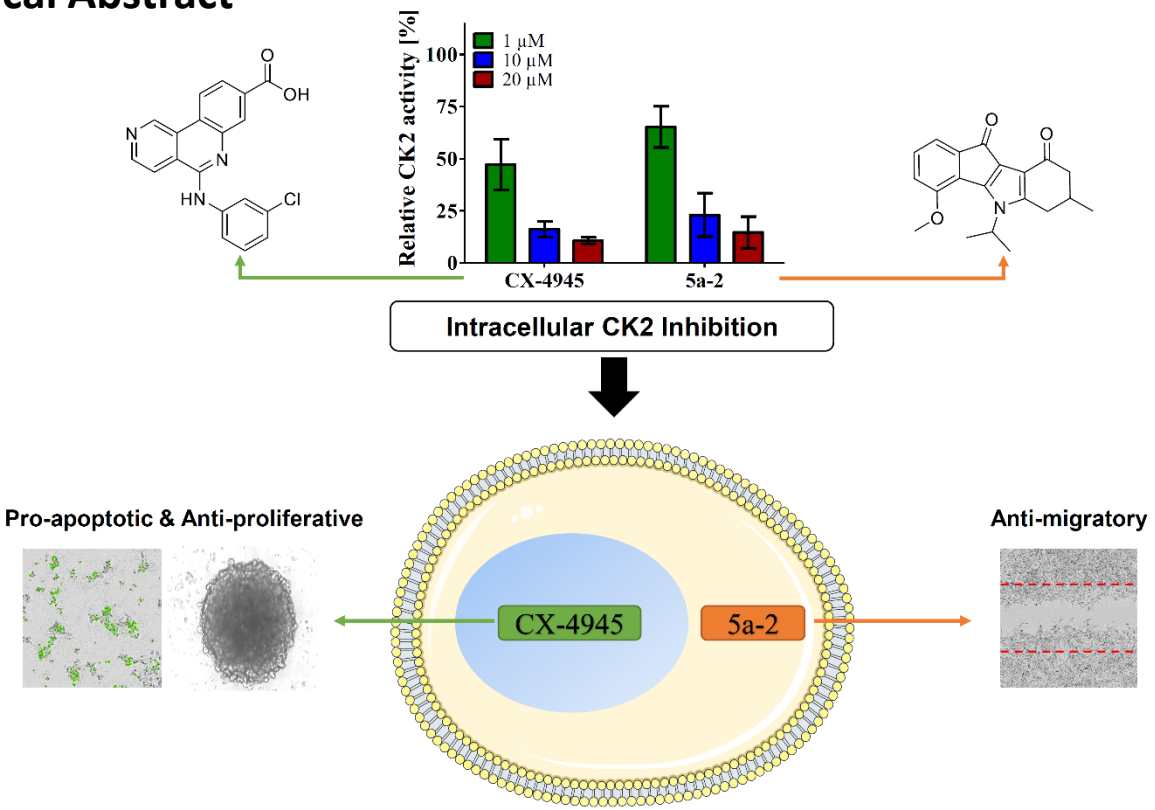
Graphical Abstract



Abstract:

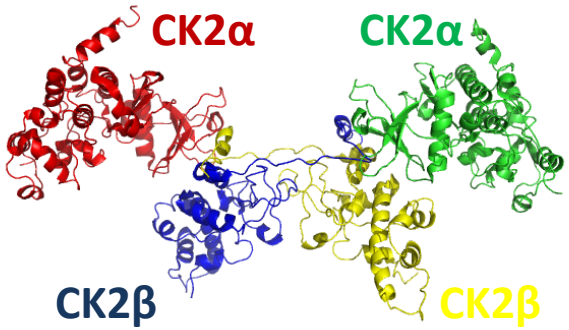
The highly pleiotropic and constitutively active serine/threonine protein kinase CK2 is considered a key target in cancer. The indeno[1,2-*b*]indole scaffold was previously shown to provide derivatives exhibiting strong CK2 inhibition and satisfactory drug-like characteristics. In this work, we evaluated one 4,5,7-trisubstituted indeno[1,2-*b*]indole derivative for its intracellular inhibition of CK2 activity and the accompanying effects on proliferation, migration and apoptosis in cancer cells. The compound 5-isopropyl-4-methoxy-7-methyl-5,6,7,8-tetrahydro-indeno[1,2-*b*]indole-9,10-dione **(5a-2)** strongly inhibited CK2 activity *in vitro* with IC_{50} value of 25 nM and in cultured A431, A549 and LNCaP cell lines (> 75% inhibition at 20 µM). The intracellular inhibition of CK2 by **5a-2** was comparable to that induced by the reference CK2 inhibitor CX-4945, though the latter exhibited > 6-fold higher inhibitory potency toward CK2 *in vitro* (IC_{50} = 3.7 nM). A possible explanation for this discrepancy is the significantly higher intracellular concentrations of **5a-2** compared to CX-4945 following their cellular uptake. Compared to CX-4945, **5a-2** induced similar anti-proliferative, weaker pro-apoptotic but stronger anti-migratory effects on cancer cells. These variations can be partly attributed to the observed differences in the subcellular localization of both compounds whereby 71% of the uptaken **5a-2** molecules were found in the cytoplasm while 49% of intracellular CX-4945 was detectable in the nuclear fraction.

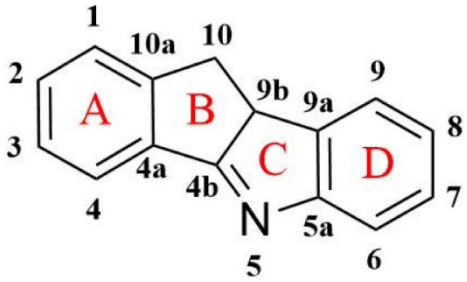
Our study emphasizes the potential of indeno[1,2-*b*]indole as an interesting framework for developing potent CK2 inhibitors and highlights the significance of subcellular distribution in dictating preferential cellular effects of CK2 inhibitors.

Keywords: Anti-cancer; CK2; Indeno[1,2-b]indole; Live cell imaging; Subcellular distribution

Introduction

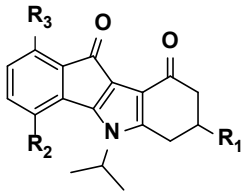
- Human CK2 is a constitutively active serine/threonine kinase.
- Shows ubiquitous expression and high pleiotropy (> 500 substrates) with a tightly regulated subcellular localization.
- Well-established therapeutic target in cancer.



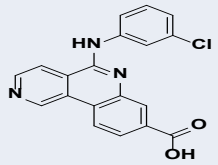


- indeno[1,2-*b*]indole scaffold is a flat tetracyclic structure allowing diverse derivatization of the ring system.
- Several derivatives were identified as potent CK2 inhibitors addressing the ATP-binding pocket of the kinase.

Results and discussion - Inhibition of human CK2 by trisubstituted indeno[1,2-*b*]indoles and the reference compound CX-4945 *in vitro*



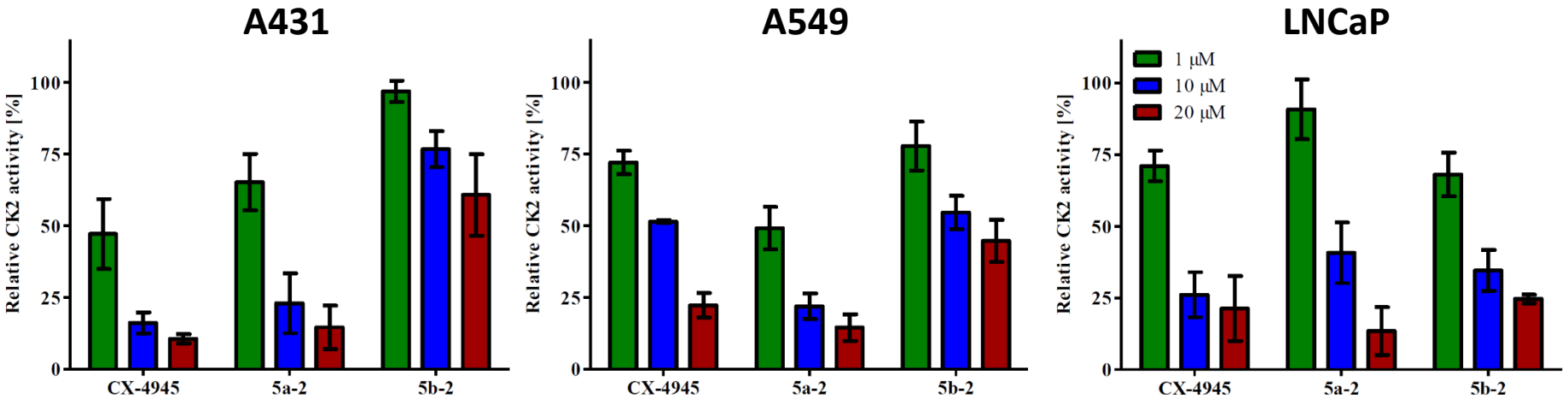
- Compounds **5a-2** and **5b-2** are potent CK2 inhibitors while their regioisomers (**5a-1** and **5b-1**) are not.
- A methoxy group in position 4 of the indeno[1,2-*b*]indole scaffold is more favorable for CK2 inhibition than in position 1.

Compound	R_1	R_2	R_3	Inhibition (%) 10 µM	IC_{50} (µM) †
5a-1	$-CH_3$	-H	$-OCH_3$	55	8.170
5a-2	$-CH_3$	$-OCH_3$	-H	100	0.025
5b-1	$-CH_2CH_3$	-H	$-OCH_3$	50	9.910
5b-2	$-CH_2CH_3$	$-OCH_3$	-H	100	0.047
CX-4945				100	0.0037‡

† Values were derived from dose-response curves with nine different inhibitor concentrations determined in a capillary electrophoresis (CE)-based kinase activity assay.

‡ Previously reported by Gozzi *et al. J. Med. Chem,* 58: 265–277 (2015).

Results and discussion - Intracellular inhibition of CK2 activity in three different cancer cell lines

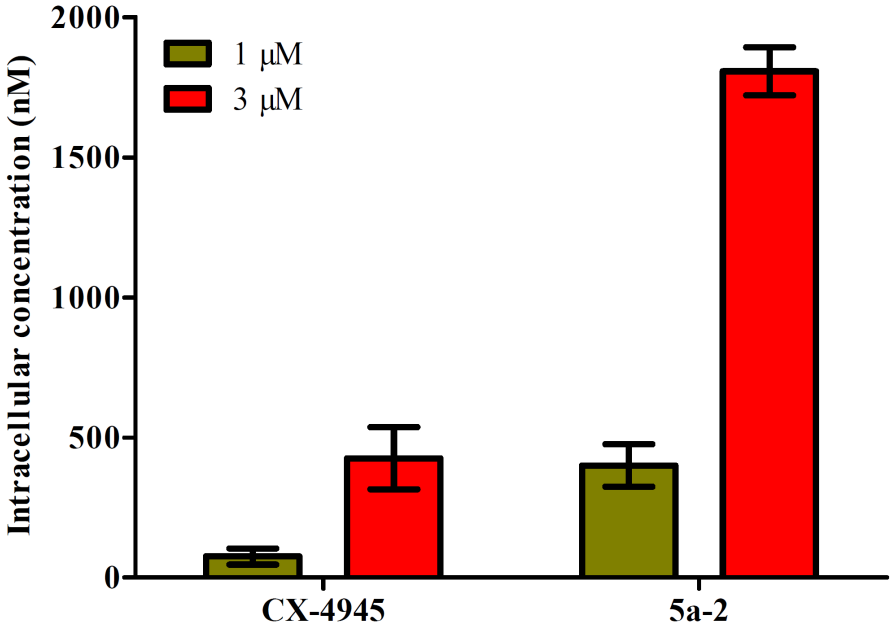


CK2 activity was determined in the soluble fraction of lysates from cultured cells treated for 24 h with 5a-2, 5b-2 or CX-4945 at the given concentrations using the previously developed CE-based kinase activity assay.

➡ Compound **5a-2** interferes strongly with intracellular CK2 activity to the same extent as **CX-4945** despite their different IC_{50} values.

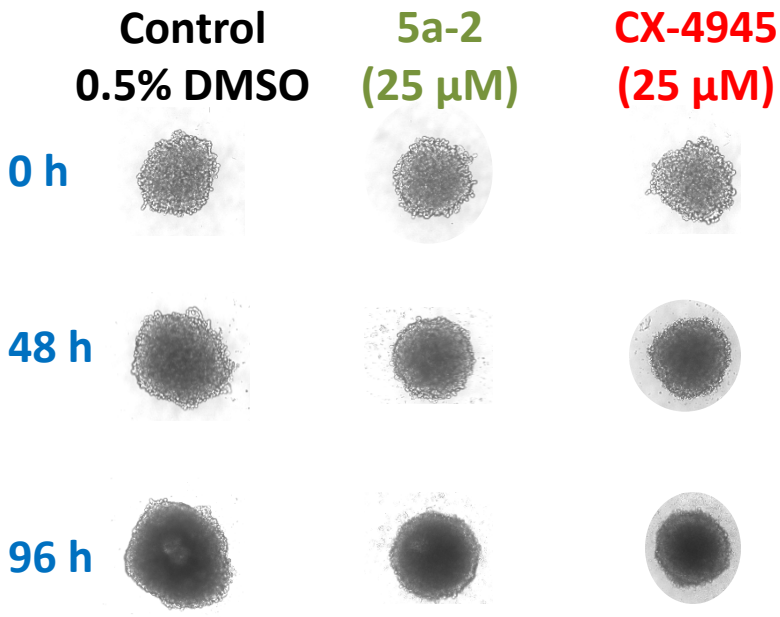
Results and discussion - Investigation of the cellular uptake of compounds 5a-2 and CX-4945

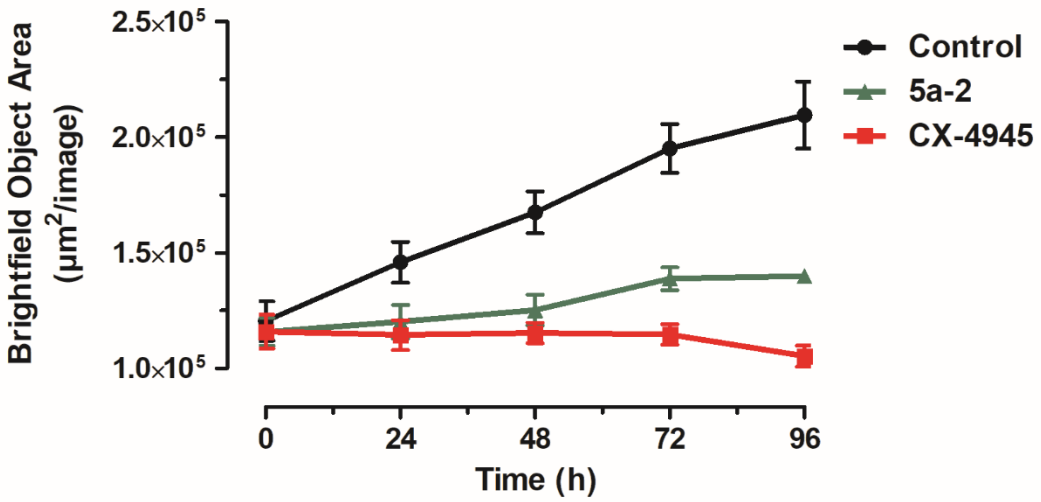
- Intracellular concentrations of **5a-2** are **> 3-fold** higher than those of **CX-4945**.
- Higher intracellular concentration of **5a-2** could provide an explanation for the comparable inhibitory effects of **5a-2** and **CX-4945** on cellular CK2 activity, despite the higher IC_{50} value of **5a-2**.



- Cultured A431 cells were treated with the inhibitors at the given concentrations for 5 h.
- Inhibitor concentrations in the lysates of treated cells were quantified using HPLC-MS/MS.

Results and discussion - Evaluation of anti-cancer activity (Effect on cell proliferation)

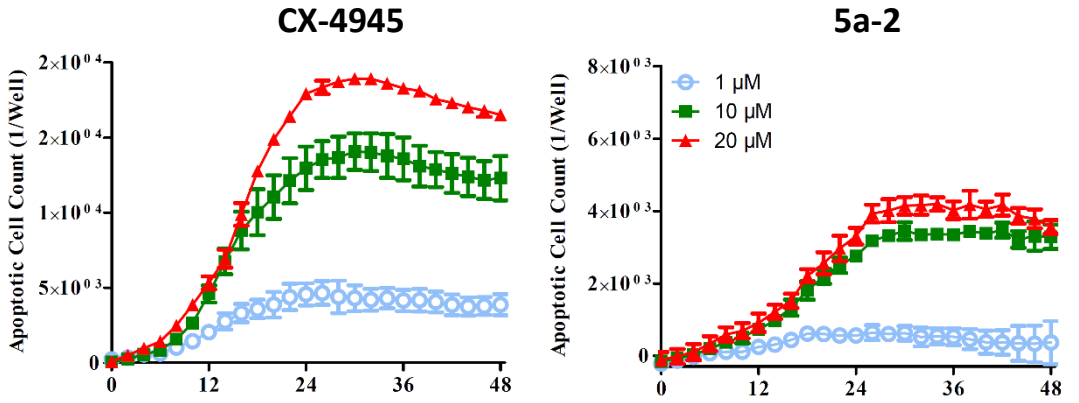




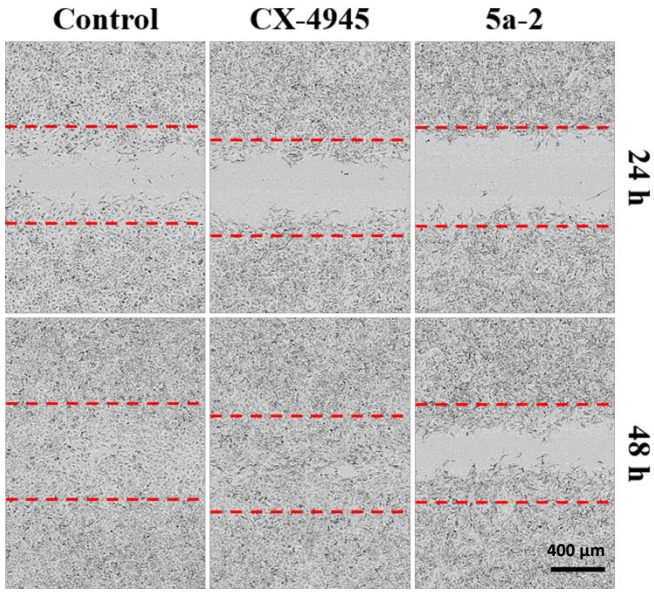
- Multicellular spheroids of A549 cells were incubated with 5a-2 or CX-4945 for 96 h and monitored for their growth in IncuCyte® S3 Live Cell Imaging system (Sartorius).

➡ **5a-2** inhibits the growth of 3D tumor spheroids similar to **CX-4945** up to 48 h post-treatment.

Results and discussion - Evaluation of anti-cancer activity (Effects on cell migration and apoptosis)



- A431 cells were cultured in 96-well plates to a confluence of 30%.
- Cells were treated with the indicated concentrations of each compound while control wells received 1% DMSO.
- IncuCyte® caspase 3/7 green reagent (5 μM) was added and the apoptotic cell count depicted from the green fluorescent signals was monitored for 48 h using IncuCyte® S3 live cell imaging system.
- The apoptotic cell counts in control wells were subtracted as background from those in treated wells.

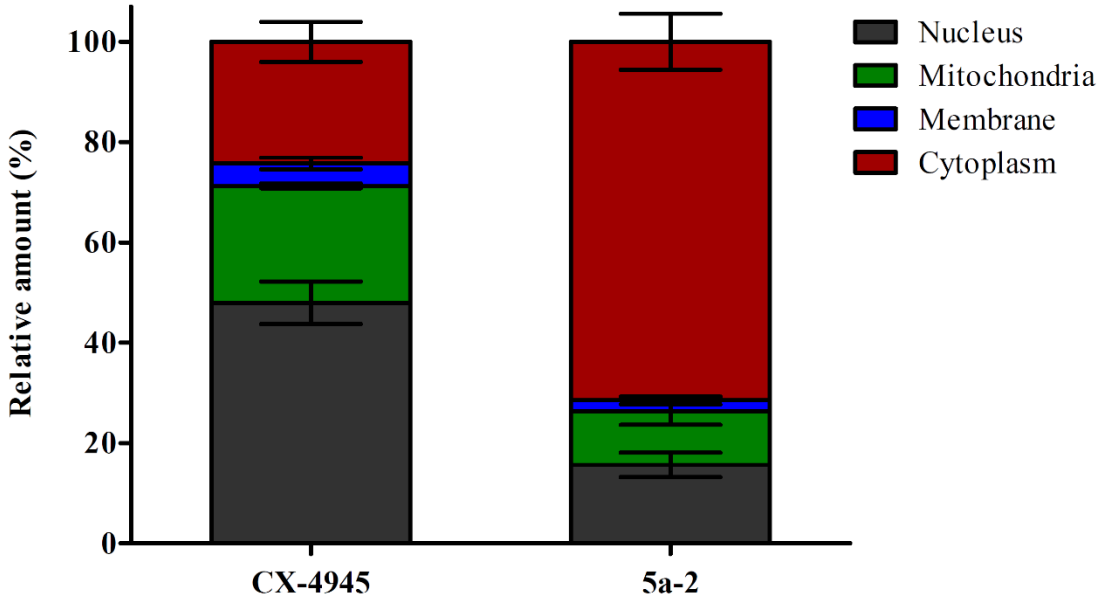


- A549 cells were cultured in 96-well Imagelock plates to a confluence of 100% and a scratch wound was created in each well using an IncuCyte® WoundMaker tool (Sartorius).
- Cells were treated with 10 μM 0f either CX-4945 or 5a-2 while control wells received 1% DMSO.
- Cell migration into the "wound" was monitored for 48 h using an IncuCyte® S3 Live Cell Imaging system (Sartorius).

5a-2 exhibits remarkably stronger anti-migratory but weaker pro-apoptotic effects compared to **CX-4945**.

Results and discussion - Investigation of the subcellular distribution of compounds 5a-2 and CX-4945

Following uptake, **5a-2** exhibits a preferential subcellular distribution in the cytoplasm (approx. 71 %) while **CX-4945** is mainly localized in the nuclear fraction of the treated cells (approx. 49 %).



- Cellular fractions of LNCaP cell lysates were separated and collected by differential centrifugation after 5 h of treatment of cultured cells with 1 µM of 5a-2 or CX-4945.
- The inhibitors were detected in each fraction using HPLC-MS/MS.

Conclusions

- The trisubstituted indeno[1,2-*b*]indole derivative **5a-2** demonstrates a strong inhibition of protein kinase CK2 activity in different cancer cell lines.
- Cellular uptake of **5a-2** is more efficient than that of **CX-4945**, probably due to more favorable physicochemical properties and/or different uptake mechanism(s).
- **5a-2** shows prominent anti-migratory and anti-proliferative effects but induces weaker pro-apoptotic effect as compared to **CX-4945**.
- For a highly pleiotropic and ubiquitously expressed target, like CK2, the subcellular distribution of the inhibitors can be determinant for their preferential cellular effects in cancer cells.