# SGC-CK2-1: the first selective chemical probe for the pleiotropic kinase CK2

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**ABSTRACT:** Building upon a wealth of published knowledge surrounding the pyrazolopyrimidine scaffold, we designed a small library around the most selective small molecule CK2 inhibitors reported. Through extensive evaluation of this library we identified inhibitor **24** (SGC-CK2-1) as a potent, selective, and cell-active CK2 chemical probe. Remarkably, despite years of research pointing to CK2 as a key driver in cancer, our probe did not elicit an antiproliferative phenotype in cell lines tested. While many publications have attempted to characterize CK2 function, CK2 biology is complex and a high-quality chemical tool like SGC-CK2-1 will aid in connecting CK2 functions to phenotypes.

Protein phosphorylation is one of the most common and important post-translational modifications.[1] Protein kinases catalyze this reaction, phosphorylating a specific substrate, and causing a conformational change that affects protein function.[2] One protein kinase, CK2 (casein kinase 2), phosphorylates more than 300 proteins.[3] Via genetic and biochemical studies in a variety of experimental models, CK2 has been found to be both constitutively active and ubiquitously expressed, making it important in many biological processes (Figure 1, Table S1).[3-6] CK2 exists as a tetrameric complex made up of two catalytic subunits, CSNK2A1 (CK2α) and CSNK2A2 (CK2α'), and two regulatory subunits, CSNK2B (CK2β).[7] Being able to selectively inhibit CK2 would prove useful in understanding its role in a multitude of pathways ranging from cell survival and proliferation to inflammation.[6]

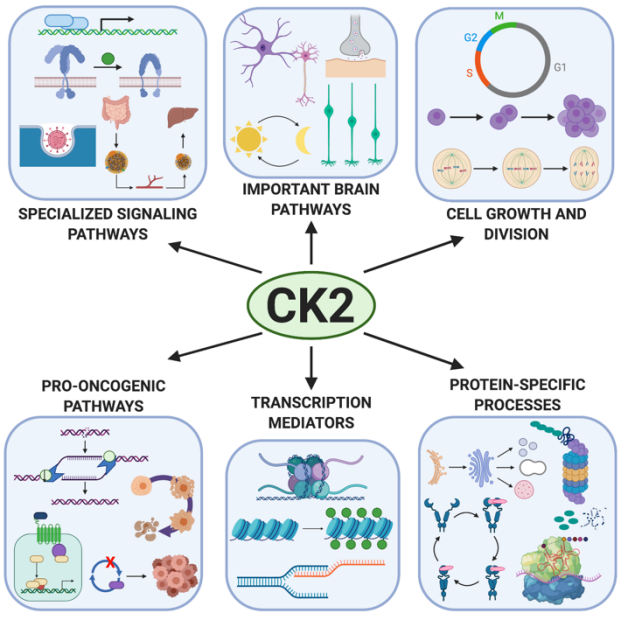



Figure 2. Summary of CK2 mediated pathways.

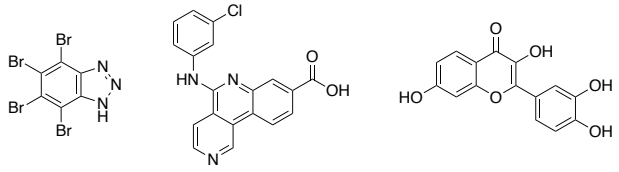



Figure 1. Literature CK2 inhibitors.

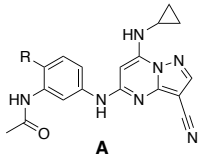
Although CK2 biology has been well-studied, with almost 750 references in PubMed, truly selective and potent inhibitors of CK2 are limited. The first reported compounds that bound to CK2 varied from benzotriazoles (e.g. – TBB) to natural product-like flavonoids (Figure 2). TBB was first published as a CK2 inhibitor in 1995 and demonstrated modest selectivity for CK2 over CK1 (casein kinase 1).[8] Later it was evaluated against a panel of 33[9] and 70[10] kinases and, while maintaining reasonable selectivity, it did inhibit several other kinases with >90% inhibition at a screening concentration of 10 μM (DYRK1A, DYRK2, DYRK3, HIPK2, and PIM1–3). In 2010, the compound CX-4945 was published, which has since become the most commonly employed CK2 inhibitor.[11] Given its antiproliferative activity in several different cancer cell lines, this inhibitor has been advanced and is currently being evaluated in clinical trials for

several oncology indications, basal cell carcinoma (NCT03897036), multiple myeloma (NCT01199718), cholangiocarcinoma (NCT02128282), breast cancer (NCT00891280) and medulloblastoma (NCT03904862).[12] Despite being fairly narrow spectrum, CX-4945 does inhibit other kinases below 100 nM (DAPK3, FLT3, TBK1, CLK3, HIPK3, PIM1, and CDK1).[12] The off-target activities of this inhibitor could provide confounding results when trying to attribute target to function to phenotype.

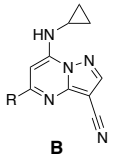
Given both the biological importance of this target and lack of selective inhibitors, we identified the need for a CK2 chemical probe to enable accurate elucidation of the biological effects of inhibition. Three recent publications from scientists at AstraZeneca disclose a series of pyrazolopyrimidines with nanomolar (nM) potency for CK2. They further evaluate this series for both mechanistic and phenotypic endpoints, including pAKT levels and antiproliferative activity.[13-16] While the compounds are potent, even one of their best compounds (**17**) also has off-targets (HIPK1–4, DAPK1–3, DYRK2, and BMPR1B) that may confound interpretation of biological results.[15] Due to its narrow kinome profile as well as potency, we started our CK2 probe project utilizing this promising scaffold.

Based on published pyrazolopyrimidines targeting CK2 and associated biochemical data, we opted to resynthesize a subset of the published molecules and expand into new chemical space by designing and preparing an additional 6 analogs not previously described in these papers. Table 1 shows the structures and corresponding CK2 enzymatic activity for our subset of previously exemplified molecules that we resynthesized for further characterization.

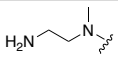
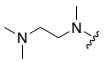
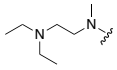
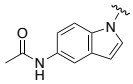
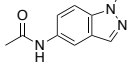
**Table 1. Reported CSNK2A inhibitors and biochemical activity.[13-16]**



A



B

| Cmpd | Scaffold | R | Reported CK2 $IC_{50}$ (nM) |
|---|---|---|---|
| **17** | **A** |  | **<3** |
| **15** | **A** |  | **<3** |
| **16** | **A** |  | **9** |
| **22** | **A** | **$CH_3$** | **<3** |
| **29** | **B** |  | **10** |
| **31** | **B** |  | **26** |

Three of these compounds (**17**, **22**, **29**) had been previously reported to have narrow selectivity profiles when screened at Ambit/DiscoverX in panels of 324 (**29**) or 402 (**17** and **22**) kinases. The remaining three compounds are close structural analogs with potent enzymatic activity, but for which limited selectivity data had been reported.[13-16] Figure 3 shows the compounds we designed based upon surveying the literature for structures and corresponding data. Structure–activity relationships were developed, and compounds designed that incorporated minor structural changes (**18**, **24**, **26**) and some slightly larger structural perturbations (**19**, **20**, **27**). While it was anticipated that the minor structural changes would result in compounds of broadly similar potency and selectivity to their progenitors, the others were designed to probe the pocket and determine which interactions were favorable and what changes might lead to enhanced selectivity.

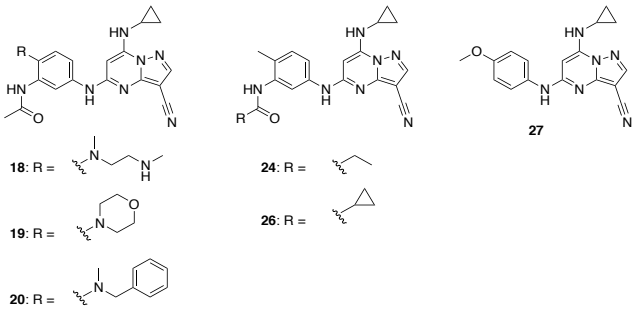



Figure 3. Synthetic CK2 targeting library.

All analogs were prepared using convergent chemistry and, where possible, utilizing previously reported synthetic routes.[13-15] Key transformations, including nucleophilic aromatic substitution and Buchwald-Hartwig amination, allowed preparation of all analogs. Detailed routes are included in the Supporting Information (Schemes S1–S3).

Upon preparation of the analogs shown in Table 1 and Figure 3, we assessed the cellular penetrance and target engagement of all compounds using the CSNK2A1 and CSNK2A2 nanoBRET assays in HEK-293 cells. Given the high sequence homology of the active sites of CSNK2A1 and CSNK2A2, we did not anticipate preferential binding to one versus the other.[17] The nanoBRET assay relies upon bioluminescence resonance energy transfer (BRET) between CSNK2A1–Nluc or CSNK2A2–Nluc and a tracer with a red-shifted fluorophore appended. Our analogs, which compete with the tracer for binding to the active site, were introduced in a dose-dependent manner and BRET was plotted versus concentration, allowing us to calculate a target engagement $IC_{50}$ value.[18] In parallel, the compound set was sent to Eurofins DiscoverX to be profiled against 403 wild type human kinases using their *scan*MAX platform to characterize the kinome-wide selectivity. Table 2 summarizes our findings related to the cellular potency and kinome-wide selectivity of the compounds we prepared.

From the entire set of compounds, 4 of our 6 novel pyrazolopyrimidines from Figure 3 (**20**, **24**, **26**, and **27**) exhibited the best kinome-wide selectivity (Table 2). Of note, our compounds are significantly more selective than the most frequently used CK2 inhibitor in the literature, CX-4945. CX-4945 has an $S_{10}(1\mu M) = 0.069$ with 28 kinases >90%I and compound **24**, for example, has an $S_{10}(1\mu M) = 0.007$ with only 3 kinases >90%I. The entire series proved to be active in the CSNK2A1 and CSNK2A2 cellular target engagement assays with several single digit nanomolar inhibitors (Figure S3). As expected, no difference in potency was observed between the two CSNK2A

**Table 2. Potency and selectivity of CK2-targeting pyrazolopyrimidines.**

| Cmpd | $S_{10}$(1μM) | # kinases >90%I at 1μM | CSNK2A1 NB $IC_{50}$ (nM) | CSNK2A2 NB $IC_{50}$ (nM) | Wild type kinases >90%I at 1μM (%I) | Enzymatic $IC_{50}$ values (nM) | Wild type kinases >35%I at 1μM (%I) | Off-target $IC_{50}$ or enzymatic assay values (nM) |
|---|---|---|---|---|---|---|---|---|
| 17 | 0.042 | 17 | 5.3 | 4.4 | Figure S2 | NT | | |
| 15 | 0.02 | 8 | 2.7 | 1.3 | Figure S2 | NT | | |
| 16 | 0.012 | 5 | 15 | 7.6 | Figure S2 | NT | | |
| 22 | 0.032 | 13 | 3.2 | 1.8 | Figure S2 | NT | | |
| 29 | 0.015 | 6 | 11 | 3.9 | Figure S2 | NT | | |
| 31 | 0.027 | 11 | 94 | 20 | Figure S2 | NT | | |
| 18 | 0.025 | 10 | 3.3 | 2.6 | Figure S2 | NT | | |
| 19 | 0.025 | 10 | 67 | 15 | Figure S2 | NT | | |
| 20 | 0.005 | 2 | 280 | 130 | CSNK2A2 (100)<br>CSNK2A1 (96.7) | CSNK2A2: 94<br>CSNK2A1: 91 | | |
| 24 | 0.007 | 3 | 36 | 16 | CSNK2A2 (100)<br>DRAK1 (100)<br>CSNK2A1 (99.5) | CSNK2A2: 2.3<br>DRAK1: >10,000<br>CSNK2A1: 4.2 | DYRK2 (86)<br>PLK4 (77)<br>HIPK2 (74)<br>MEK5 (72)<br>HIPK1 (68)<br>HIPK3 (66) | DYRK2: 440<br>PLK4: >10000<br>HIPK2: 3400<br>MEK5: 0%I at 1μM<br>HIPK1: 3700<br>HIPK3:8100 |
| 26 | 0.002 | 1 | 7700 | 2700 | CSNK2A2 (100) | CSNK2A2: 120 | CSNK2A1 (80)<br>SGK1 (68)<br>SGK3 (67) | CSNK2A1: 150<br>SGK1: >10000<br>SGK3: >10000 |
| 27 | 0.007 | 3 | 2200 | 1000 | CSNK2A2 (99.3)<br>CLK2 (97.6)<br>PHKG2 (95.6) | CSNK2A2: 240<br>CLK2: 2995 PHKG2: >10,000 | CAMK2A (84)<br>SGK1 (73)<br>CHEK2 (72)<br>BLK (67)<br>DAPK3 (66)<br>SGK3 (65)<br>DYRK2 (63) | CAMK2A: >10000<br>SGK1: 3600<br>CHEK2: >10000<br>BLK: >10000<br>DAPK3: >10000<br>SGK3: >10000<br>DYRK2: 720 |
| 32 | 0.00 | 0 | NT | >10000 | None >90%<br>CSNK2A2 (35)<br>CSNK2A1 (0) | CSNK2A1: >10000<br>CSNK2A2: >10000 | | |
| CX-4945 | 0.069 | 28 | NT | 45 | Figure S1 | NT | | |

**Table 3. Antiproliferative activity of pyrazolopyrimidines.**[13-16]

| Cmpd | Reported HCT-116 $IC_{50}$ (nM) | Experimental HCT-116 $IC_{50}$ (nM) |
|---|---|---|
| 17 | 10 | 33 ± 9 |
| 15 | 30 | 63 ± 23 |
| 18 | - | 31 ± 9 |
| 16 | - | 510 |
| 19 | - | 1800 ± 570 |
| 20 | - | >10000 |
| 22 | 81 | 150 ± 22 |
| 24 | - | >10000 |
| 26 | - | >10000 |
| 27 | - | >10000 |
| 29 | 530 | 1800 ± 250 |
| 31 | 3700 | >10000 |
| 32 | - | >10000 |
| CX-4945 | 2200 | >10000 |

subunits. Nine of the pyrazolopyrimidines demonstrated CSNK2A2 nanoBRET in cell target engagement $IC_{50}$ values less than or equal to 20 nM. CX-4945, for comparison, was shown to have a CSNK2A2 nanoBRET $IC_{50}$ = 45 nM. We followed up on the off-targets for the subset of very selective compounds that inhibited 3 or fewer kinases. Follow-up was carried out in dose-response using an enzymatic and/or nanoBRET assay corresponding to each of the wild type off-target kinases inhibited >60% in the DiscoverX *scan*MAX panel. Table 2 lists the kinases inhibited >60% for **20**, **24**, **26**, and **27**. While the selectivity of all four analogs fit within probe criteria,[19] the most potent compound in the CSNK2 nanoBRET assays (**24**) was chosen as the probe candidate. As shown in Table 2, DYRK2 was the only off-target kinase to demonstrate an $IC_{50}$ value < 1 μM. Of note, the potency of **24** in the CSNK2A1 and CSNK2A2 enzymatic assays was such that 100- fold selectivity for CK2 over DYRK2, its most potently inhibited off-target kinase, was observed. Since it seemed to be a common off-target of compounds in our library, all analogs in Table 2 were tested in the DYRK2 nanoBRET assay. Only **17** was found to be active at <1 μM against DYRK2 in cells (Figure S3, $IC_{50}$ = 160 nM). An $IC_{50}$ value of 3.7 μM was determined for **24** in the DYRK2 nanoBRET assay (Figure S3). The 100-fold selectivity for CK2 over DYRK2 in the respective enzymatic assays was maintained in the cell-based system.

Given its modest CK2 activity in the nanoBRET assay, narrow selectivity profile, and structural similarly to **24**, **26** was chosen as a chemical starting point from which to synthesize a negative control. As shown in Scheme S4, **26** was globally methylated using methyl iodide, the various products were separated,

and structural assignments made via spectroscopy. Compound **32** was profiled to determine both its kinome-wide selectivity and cellular target engagement of CSNK2A2. Based on no inhibition of any kinases >80% at 1 μM and no cellular activity in the CSNK2A2 nanoBRET assay up to 10 μM, **32** (SGC-CK2-1N) was chosen as the negative control: a structurally related compound that lacks CK2 affinity. In addition to a lack of cellular activity, **32** was also found to be devoid of CSNK2A1 and CSNK2A2 potency in the corresponding enzymatic assays.

Based upon reports that the previously exemplified pyrazolopyrimidines in our library inhibited the growth of HCT-116 cells and that **17** exhibited a high level of activity as a monotherapy in HCT-116 xenografts,[15] we evaluated the antiproliferative activity of our entire library in this colon cancer cell line. Table 3 shows the previously reported cytotoxicity data in HCT-116 cells alongside the data we collected following 72h treatment. Compounds that had previously been reported as cell growth inhibitors demonstrated antiproliferative activity in our hands as well. This was also true of the non-selective CK2 inhibitor CX-4945.[12] Our new analogs (**18**, **19**, **20**, **24**, **26**, and **27**) showed variable growth inhibition. Our negative control (**32**) did not demonstrate antiproliferative activity. Remarkably, the most selective compounds, **20**, **24**, **26**, and **27**, demonstrated no antiproliferative activity in this assay. Since these compounds were found to only inhibit CSNK2A1 and CSNK2A2, a working hypothesis is that the antiproliferative activity exhibited by less selective compounds was likely due to inhibition of an off-target kinase or combination of kinases and not due to inhibition of CK2.

Finally, we expanded our exploration of the antiproliferative activity of **24**. CK2 inhibition has been linked with suppressing glioblastoma invasiveness as well as pro-survival signaling pathways and growth.[20-21] As these studies employed U-87 MG cells, we tested the antiproliferative activity of **24** in this glioblastoma cell line after 72h compound treatment. As was observed in HCT-116 cells, **24** demonstrated no antiproliferative activity when tested up to 10 μM. We also investigated whether **24** could activate caspase 3/7 in U-87 MG cells.[22] At multiple time points, no caspase 3/7 activation was observed when tested up to 10 μM.

With so many putative substrates, CK2 is clearly a pleiotropic kinase. As such, CK2 inhibition is hypothesized to impact multiple pathways simultaneously, including several that drive cell growth and others that mediate apoptosis. Subcellular localization of CK2 has also been described as key to its function, adding another layer of complexity to CK2 biology.[17] Additional studies are underway that aim to deconvolute CK2 biology using this new probe and the negative control.

We have described the design, synthesis, and biological evaluation of a series of pyrazolopyrimidines as selective dual inhibitors of CSNK2A1 and CSNK2A2. Compound **24** (SGC-CK2-1) emerged as our best probe candidate. This probe outperforms all published inhibitors in terms of kinome-wide selectivity. When combined with its potent cellular activity, our probe represents the best available tool to interrogate CK2 biology. Its lack of antiproliferative activity in our preliminary studies have motivated study of **24** in a broader panel of cancer cell lines. Supported by the high expression of CK2 in the brain and its implication in the molecular pathology of neurodegenerative diseases, the non-toxic nature of selective CK2 inhibitors supports that CK2 inhibition could be a viable approach for treating disorders of the brain.

## ASSOCIATED CONTENT

### Supporting Information

Supplemental material is available free of charge via the Internet at http://pubs.acs.org.
NanoBRET and antiproliferative assay details, experimental methods, synthesis and characterization of target compounds are included (PDF).

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### Notes

The authors declare no competing financial interest.

### Author Contributions

⊥C.W. and D.H.D. contributed equally to this work. The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.


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## ABBREVIATIONS

nM, nanomolar; CK2, casein kinase 2; CSNK2A1/CK2α, casein kinase alpha 1; CSNK2A2/CK2α', casein kinase alpha 2; CK1, casein kinase 1; DYRK2, dual specificity tyrosine phosphorylation regulated kinase 2; TBB, 4,5,6,7-tetrabromobenzotriazole; BRET, bioluminescence resonance energy transfer; Nluc, Nanoluciferase; HEK-293, human embryonic kidney 293; HCT-116, human colon tumor 116; U-87 MG, Uppsala 87 malignant glioma

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