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Triazoloquinazolines: dual-target, a_{2a}r - pde 10a, anti-proliferative compounds

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Introduction Activation of the Adenosine A_{2A} receptor ($A_{2A}R$), and inhibition of phosphodiesterases, has been show to be anti-proliferative [1]. Using *in silico* docking, we identified a series of known PDE 10A inhibitors, triazoloquinazolines [2], which displayed promising docking scores to the orthosteric agonist-binding site of the $A_{2A}R$. Here we present data pertaining to the characterisation of these compounds with respect to activation of the $A_{2A}R$, identifying their selectivity profiles amongst adenosine receptors, as well as identifying their dual-target action to also be anti-proliferative.

Methods Test compounds were initially screened in yeast expressing either the A_1, A_{2A} or A_{2B} receptors [3]. cAMP accumulation/inhibition was measured using a LANCE cAMP kit (Perkin Elmer), for CHO-K1-$A_{2A}R$ and CHO-K1-A_3R cells, as well as C6 and HEK 293S cells. All cell types were stimulated with agonist (10 mM - 10 pM) for 30 minutes prior to determining intracellular cAMP concentrations. For proliferation assays, cells were seeded onto 96 well plates at a density of 2000 cells per well, and treated with agonists after 24 hours, for a period of 72 hours. Cell number was then quantified using CCK-8 (Sigma Aldrich).

Results Screening of test compounds (Cmpds) 1-5: in yeast (β-galactosidase), CHO-K1-$A_{2A}R$ and CHO-K1-A_3R cells (cAMP accumulation/inhibition), identified the ability of all compounds to act as agonists against the $A_{2A}R$, with Cmpds 1-3 being selective for the $A_{2A}R$ (Table 1). Further testing in cells endogenously expressing the $A_{2A}R$ and PDE 10A, C6 and HEK 293S cells, identified their abilities to elevate intracellular cAMP levels (Figure 1A-B), as well as inhibit proliferation (Figure 1C-D). This anti-proliferative action is specific to the triazoloquinazoline compounds, as CGS21680 (a selective $A_{2A}R$ agonist) had no effect upon proliferation in either cell type (Figure 1C-D). However, cAMP appears to be the key mediator of these compounds' actions as forskolin was also found to be anti-proliferative (Figure 1C-D).

Conclusions We have identified a series of dual action compounds, of the triazoloquinazoline chemical series, that are both $A_{2A}R$ agonists and PDE 10A inhibitors. Testing these compounds in cells endogenously expressing both of these targets identified that our compounds appear to have an anti-proliferative effect, which is cAMP dependent; this been particularly apparent in a rat model of glioma (C6 cells).

References

[**1**] Rickles *et al* (2010). *Blood.* **116**:593-602.
[**2**] Kehler *et al* (2011). *Bioorg. Med. Chem. Lett.* **21**:3738-42;
[**3**] Knight *et al* (2016). *J. Med. Chem.* **59**:947-64.

Table 1: Potency (pEC_{50}) and E_{max} values for Trizaoloquinazoline stimulation of adenosine A_1, A_{2A} and A_{2B} receptors in yeast expressing GPA1/$G\alpha_{i1/2}$ or GPA1/$G\alpha_s$ and CHO-K1-A_3R cells.

	A_1R - GPA1/$G\alpha_{i1/2}$		A_{2A}R - GPA1/$G\alpha_s$		A_{2B}R - GPA1/$G\alpha_s$		CHO-K1-A_3R	
	pEC_{50} [a]	E_{max} [b]	pEC_{50} [a]	E_{max} [b]	pEC_{50} [a]	E_{max} [b]	pEC_{50} [a]	Range [c]
NECA	5.87±0.1	100.40±3.2	5.50±0.2	92.84±5.1	4.14±0.1	99.76±13.2	9.75±0.1	-34.35±2.2
Cmpd 1	NR	NR	5.40±0.3*	63.68±6.5**	NR	NR	NR	NR
Cmpd 2	NR	NR	5.98±0.4	45.90±8.3***	NR	NR	NR	NR
Cmpd 3	NR	NR	5.71±0.4*	61.94±9.9**	NR	NR	NR	NR
Cmpd 4	5.44±0.3	60.88±9.0**	6.14±0.5	38.78±5.3**	4.79±0.2	17.62±2.5***	9.45±0.2	-16.59±1.1**
Cmpd 5	7.39±1.2**	12.94±3.9**	8.20±0.8***	11.09±3.9***	6.64±0.3	8.66±2.2***	NR	NR

Data ± SEM of 5 individual replicates

[a] Negative logarithm of agonist concentration producing half-maximal response

[b] Maximal response observed upon agonist stimulation, as a percentage of that observed upon stimulation with 100 μM NECA

[c] Range of response, as a percentage of that observed upon stimulation with 100 μM forskolin

NR – No response

Statistical difference between each agonist and NECA was calculated using a one-way ANOVA with Dunnet's post-test (*, $p < 0.05$, **, $p < 0.01$, ***, $p < 0.001$)

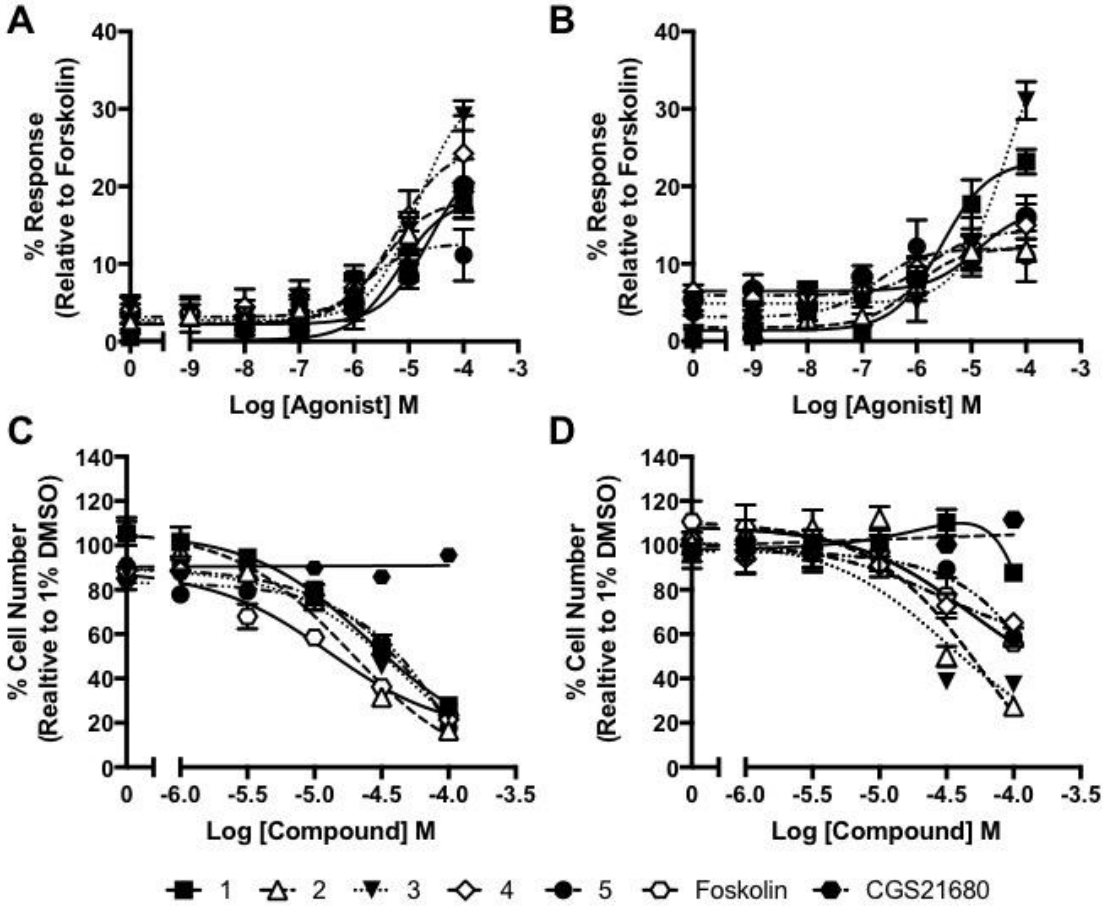

Figure 1. Triazoloquinazolines elevate intracellular cAMP and are anti-proliferative in both C6 and HEK 293S cells.

Cmpds 1-5 and CGS21680 elevate cAMP levels in C6 (A) and HEK 293S (B) cells. Additionally, both Cmpds 1-6 and forskolin inhibit proliferation in C6 cells (C), whilst only Cmpds 2-5 and forskolin act as anti-proliferative compounds upon HEK 293S cells (D). Data represented as the mean, ± SEM, of 3-6 replicates.