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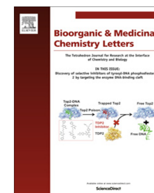

GSK114: A selective inhibitor for elucidating the biological role of TNNI3K

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ABSTRACT

A series of selective TNNI3K inhibitors were developed by modifying the hinge-binding heterocycle of a previously reported dual TNNI3K/B-Raf inhibitor. The resulting quinazoline-containing compounds exhibit a large preference (up to 250-fold) for binding to TNNI3K versus B-Raf, are useful probes for elucidating the biological pathways associated with TNNI3K, and are leads for discovering novel cardiac medicines. GSK114 emerged as a leading inhibitor, displaying significant bias (40-fold) for TNNI3K over B-Raf, exceptional broad spectrum kinase selectivity, and adequate oral exposure to enable its use in cellular and in vivo studies.

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Cardiac troponin I-interacting kinase (TNNI3K or CARK) is a member of the tyrosine-like kinase family that is selectively expressed in heart tissue. TNNI3K has been linked to the progression of dilated cardiomyopathy, cardiac hypertrophy, and ischemia/reperfusion injury using models that employ *Tnni3k* overexpressing or *Tnni3k* knockout animals.[1–5] Selective TNNI3K inhibitors are required to corroborate these findings in models that mimic clinical intervention and to elucidate the mechanisms that underlie the cardiac biology of TNNI3K.[1]

We recently reported the discovery of orally bioavailable 7-deazapurine TNNI3K inhibitors exemplified by **1** that show impressive broad spectrum kinase selectivity (Fig. 1).[6] However, **1** and its relatives exhibit potent activity at the structurally related B-Raf and c-Raf kinases, which share 67% sequence identity (82% similarity) with TNNI3K among residues comprising their ATP-binding sites. As Raf kinase inhibition has been linked to effects in heart failure models, the development of TNNI3K inhibitors that exhibit selectivity for TNNI3K over B-Raf and c-Raf is critical to enable efforts to characterize the function of TNNI3K.[7,8]

In the present report, we disclose efforts to introduce selectivity against B-Raf into analogs of **1** that culminate in the identification of GSK114 (**2**), which displays substantial preference (40-fold) for binding TNNI3K versus B-Raf (Fig. 1). Importantly, **2** maintains exquisite general kinase selectivity, is orally available, and has been employed, along with other TNNI3K inhibitors from our laboratories, in elucidating the pathways that lead to the cardioprotective effects of TNNI3K inhibition.[1]

Initial SAR studies on the 7-deazapurine template revealed that C7-substituents can modulate the TNNI3K-B-Raf selectivity of the series (Table 1).[6] Introducing halogens (**6**, **7**) or aryl groups (**8**, **9**) produced substantial increases in affinity for B-Raf (5–50 fold), with little change in TNNI3K binding (~2-fold). Among the simpler substituents, an electronic trend on the kinase selectivity is apparent (Me > H ~ F > Cl > Br) with electron withdrawing groups leading to compounds that favor B-Raf versus TNNI3K. This divergence in SAR suggests that the 7-deazapurine moiety participates in different binding interactions within TNNI3K and B-Raf. A comparison of crystal structures of substituted 7-deazapurines **10** bound to TNNI3K and **11** bound to B-Raf reveals that while the 7-deazapurine makes similar H-bonding interactions to the 'hinge' in both kinases, the residues that surround the 7-deazapurine heterocycle differ between the two kinases (Fig. 2).[9] Specifically, the 7-deazapurine is flanked by Tyr541 and Leu595 in TNNI3K but bounded by Trp530 and Phe582 in B-Raf, and presumably variable packing against these residues produces the non-identical SAR. These observations suggest that replacing the 7-deazapurine could modify selectivity and prompted an investigation of surrogates of the pyrrole ring of **1** in hopes of identifying an alternative core with preferential binding to TNNI3K.

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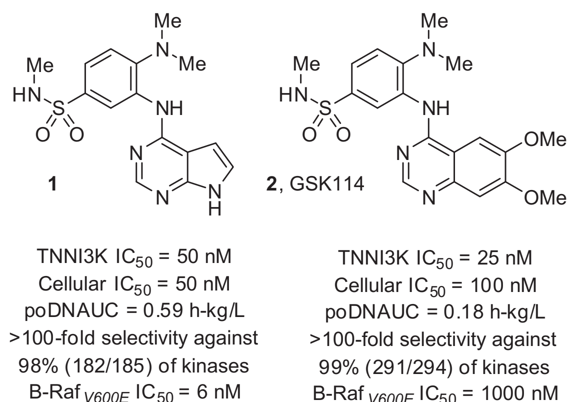


Figure 1. Comparison of **1** and **2**.

An assessment of several easily accessible replacements of the 7-deazapurine moiety of **1** including quinazoline (**12**), thienopyrimidine (**13**), and pyrrolopyrimidine (**14**, **15**) confirmed that both B-Raf and TNNI3K are sensitive to the heterocyclic hinge binding group (Table 2).[10] Each of these alternate heterocyclic cores exhibit improved preference for TNNI3K, showing up to 10-fold selectivity over B-Raf. Of interest, the affinity and partiality for TNNI3K increases as the ring systems of **12**–**15** become more electron rich (**12**–**13** vs **14**–**15**), providing additional evidence that the selectivity between TNNI3K and B-Raf is at least in part dictated by an electronic effect on the hinge binding heterocycle. Although the pyrrolopyrimidines (**14**, **15**) are the most potent and selective of the set, their poor pharmacokinetics (poDNAUC $\leqslant$ 0.02, Table 5) and limited sites of synthetic variation discouraged further evaluation. Instead, we focused on elaboration of the quinazoline under the premise that more electron-rich quinazolines should have significantly improved activity and selectivity compared to **12**.

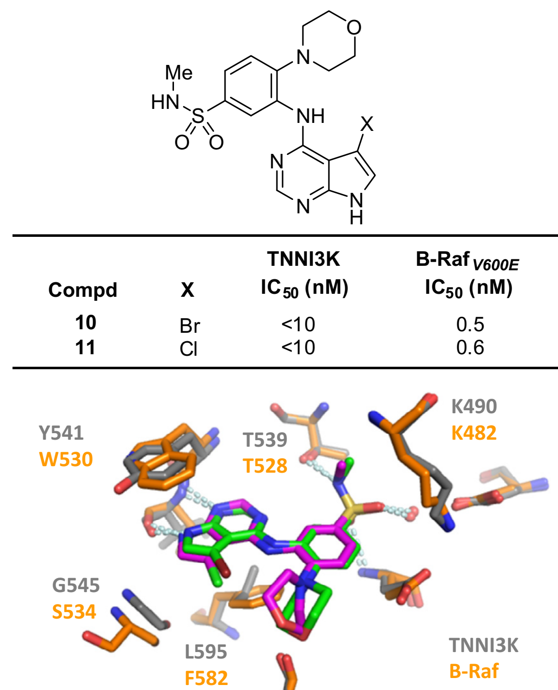
Introducing electron donating substituents onto either C6 (**16**–**18**) or C7 (**19**–**22**) of the quinazoline **12** improved both affinity for TNNI3K and selectivity over B-Raf (Table 3). As exemplified by a comparison of **18** (6-NHMe, IC_{50} = 50 nM)) and **20** (7-NHMe, IC_{50} = 40 nM), matched substituents produced similar effects on TNNI3K activity regardless of their exact position within the ring. This observation is consistent with the expectation that the substituents project away from the active site (Fig. 2) and serve as remote electronic modulators that may impact the quinazoline H-bonding interactions with Ile542 or its interaction with nearby hydrophobic residues, which are notably different for TNNI3K (Tyr541, Leu595) and B-Raf (Trp530, Phe582). However, **18** (6-NHMe, IC_{50} = 1000 nM) was substantially more active at B-Raf than its 7-substituted counterpart **20** (7-NHMe, IC_{50} = 10,000 nM) signaling another subtle area of distinction between the two kinases. Indeed, all 7-substituted quinazolines evaluated (**19**–**22**) showed greater selectivity than the 6-substituted analogs. B-Raf may display greater steric sensitivity to C7-substituents due to its narrower opening to the front pocket that results from its larger Trp530 versus Tyr541. Thus, we conclude that it is a combination of electronic and steric factors that ultimately give rise to the large preference for TNNI3K versus B-Raf exhibited by **20**, **21**, and **22**. 7-Substituents bearing elongated tails (**21**, **22**) impart elevated affinity for TNNI3K, which suggests they may introduce additional binding interactions with accessible residues (e.g. Ser549) on the edge of the kinase binding pocket. Notably, **22** has excellent potency (<10 nM) and specificity (>250-fold) for TNNI3K and is a suitable cellular probe (TNNI3K cellular IC_{50} = 25 nM, Table 5).

6,7-Di-MeO quinazoline **2** was more active at TNNI3K and more selective than its monosubstituted counterpart **16**, and this again highlights the enzyme's preference for electron rich hinge binders. The 5,7-di-MeO analog **23** further potentiated TNNI3K binding but also significantly increased the affinity for B-Raf.

Given that some of the highly selective quinazoline inhibitors (e.g. **22**) exhibit poor pharmacokinetics (Table 5), we evaluated selected substitution patterns in combination with an alternative 4-trifluoroethoxy bearing benzenesulfonamide headgroup (Table 4). We have previously observed that exchanging the 4-NMe_2 group for a 4-OCH_2CF_3 moiety improved clearance and oral bioavailability in a related series of TNNI3K inhibitors and we were pleased to find that this transformation was equally effective on the quinazoline scaffold, producing elevated oral exposure in each instance examined (Table 5). However, the 4-OCH_2CF_3 group displays inferior selectivity at TNNI3K versus the 4-NMe_2 functionality (Table 4), which partly limits its utility. A key observation in this regard is that the NMe_2 group is anticipated to project out of the plane of the benzenesulfonamide placing one methyl group into the area of the pocket occupied by Leu595 of TNNI3K and Phe582 of B-Raf.[6] This evidently creates a steric

Table 1
Effect of C7–7-deazapurine substituents[10]

Compd	R	TNNI3K IC_{50} (nM)	B-Raf_{V600E} IC_{50} (nM)	Selectivity (fold)
3	H	80	50	0.63
4	Me	40	63	1.58
5	F	130	79	0.63
6	Cl	32	13	0.41
7	Br	32	5	0.16
8	Ph	40	4	0.10
9	3-Cl-Ph	63	1	0.02



Compd	X	TNNI3K IC_{50} (nM)	B-Raf_{V600E} IC_{50} (nM)
10	Br	<10	0.5
11	Cl	<10	0.6

Figure 2. Structure of **10** (green) bound to TNNI3K (gray) overlaid with structure of **11** (magenta) bound to B-Raf (orange).[9]

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