Application of virtual screening to the discovery of novel nicotinamide phosphoribosyltransferase (NAMPT) inhibitors with potential for the nn

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The structure-based virtual screen (SBVS) employed the publicly available X-ray crystal structures of NAMPT in complex with FK866 (PDB code: 2GVJ) and nicotinamide mononucleotide

TPSA $\leqslant$ 120 • ^{2}) and substructural criteria [14] to remove non-drug-like molecules. In the interests of generating the most diverse results possible in a timely manner, it was decided to run the following docking screens:

SBVS1 as the most conservative approach to Þnding close analogues of FK866, a 46,000-compound dataset of meta-pyridine-containing structures was docked against the 2GVJ structure including both Wat645 and Wat1339; the latter water helps compounds to achieve an FK866-like binding of the pyridine group;

SBVS2 as a more open-ended screen, a larger, more diverse dataset of 750,000 compounds was docked to the 2GVJ structure including only Wat645. Removal of Wat1339 enables more diverse chemotypes to bind to the pyridine subsite;

SBVS3 to encourage more diversity in ligand binding, the larger dataset was docked to the 2GVG structure containing no waters. Although 2GVG is very similar to 2GVJ, small changes in amino acid side chain positions can sometimes inßuence the docking acT*e W4(ch735.)J 0 -1

of the amide hydrogen bonds would not be very plausible ligands. For SBVS2 and SBVS3 the 750K-compound dataset was too large for docking with Glide SP in a reasonable timeframe. Therefore, the dataset was docked with the faster Glide HTVS protocol and the top-scoring 50,000 solutions were re-docked using Glide SP. Hydrogen bond constraints to Ser275 or Wat645 (if present) were added as before. Default Glide docking parameters were applied, with the post-docking geometry minimization stage (and incorporation of estimated strain energy into the docking score) included for the Glide SP jobs. One pose per input ligand was retained, and a maximum of 30,000 solutions per SP docking run retained for analysis.

The analysis of the docked compounds from each of the docking screens involved using the Maestro graphical interface to search for compounds of interest based on a combination of binding features and docking score, and manual visualization of sets of a few thousand docked ligands. Analysis was further facilitated by partitioning the ligands into molecular weight bins, so that a balanced selection could be made across different molecular weight ranges. The analysis resulted in a set of 630 compounds for consideration (221 from SBVS1, 276 from SBVS2 and 133 from SBVS3).

As a complement to the structure-based campaign described above, a ligand-based strategy was also pursued. An assessment of the known ligands for NAMPT suggested that the compounds fell into two broad classes: those with long aliphatic linkers and those with a phenyl ring in the linker. It was decided to carry out ligand-based virtual screening (LBVS) with a representative of each of these classes and so MPI-0479626[3] (1) and FK866 (2) (Table 1) were selected as query ligands.

Multiple 2D and 3D ligand-based searches were undertaken using these query compounds. The 2D searches utilized a database of 2.35 million commercially available compounds while the 3D searches were run against the 750,000-compound database mentioned earlier. In brief, the following searches were carried out:

2D substructure—given the presence of a terminal meta-pyridine in both query ligands plus a knowledge of this moiety's binding mode in the NAMPT active site, it was decided to search for compounds that possessed this group connected by a two-atom lin-

similarities to and differences from known NAMPT inhibitors, it was decided to order 102 compounds from the appropriate commercial vendors. 99 of the 102 compounds were received and of these, 42 showed 50% inhibition or greater when screened in the primary assay [21] at 100 μM in singlet. 40 compounds were confirmed to be hits when retested in duplicate at 100 μM and IC_{50}

nude mice using the same dosing regimen. Furthermore, the observed anti-tumor effects were achieved with minimal impact on the mouse body weights. [23] Coincidentally, the same companies also published a compound similar to our pyrazole-linked example in a slightly earlier (but still post-2012) paper [24] (15, Table 10) describing a structure-based design approach to NAMPT inhibitor discovery. Interestingly, the sulfone element in both compounds is reminiscent of the sulfonamide seen in 8 (Table 4).

In conclusion, we have described a structure- and ligand-based virtual screen that resulted in the rapid and cost-effective discovery of two series of NAMPT inhibitors that were novel in that context at the time of their discovery and had good potential for further optimization (although, for commercial reasons, this was never pursued). The subsequent publications from other groups reporting similar compounds bears out the effectiveness of the strategy employed. Encouragingly, one of the hits from the virtual screen showed a statistically signiÞcant protective effect when tested in a cellular model of axon degeneration adding weight to the hypothesis [6] that small molecule inhibition of NAMPT may be a fruitful approach to the treatment of neurodegenerative diseases.

Acknowledgments

References and notes

1. Garten, A.; Schuster, S.; Penke, M.; Gorski, T.; de Giorgis, T.; Kiess, W. Nat. Rev. Endocrinol. 2015, 11, 535.
2. Montecucco, F.; Cea, M.; Cagnetta, A.; Damonte, P.; Nahimana, A.; Ballestrero, A.; Del Rio, A.; Bruzzone, S.; Nencioni, A. Curr. Top. Med. Chem.RTravehie, C.; 420(Fakhf)-3e, G309(420(RDamoian,)-406(RA.;)4[(RTron,)-40 1G.;)4[(RC.;) [(CT*0 0Genazzane,)-306(A.)-