Bioorganic & Medicinal Chemistry Letters xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Virtual screening and biophysical studies lead to HSP90 inhibitors

Renjie Huang, Daniel M. Ayine-Tora, M. Nasri Muhammad Rosdi, Yu Li, Jóhannes Reynisson *, Ivanhoe K.H. Leung *

School of Chemical Sciences, The University of Auckland, Private Bag 92019, Victoria Street West, Auckland 1142, New Zealand

ARTICLE INFO

Article history:
Received 12 November 2016
Revised 20 November 2016
Accepted 21 November 2016
Available online xxxx

Keywords:
HSP90
Virtual screening
Protein NMR
Thermal shift assay
Inhibitor

ABSTRACT

Heat shock protein 90 (HSP90) is a molecular chaperone that plays important functional roles in cells. The chaperone activity of HSP90 is regulated by the hydrolysis of ATP at the protein's N-terminal domain. HSP90, in particular the N-terminal domain, is a current inhibition target for therapeutic treatments of cancers. This paper describes an application of virtual screening, thermal shift assaying and protein NMR spectroscopy leading to the discovery of HSP90 inhibitors that contain the resorcinol structure. The resorcinol scaffold can be found in a class of HSP90 inhibitors that are currently undergoing clinical trials. The proved success of the resorcinol moiety in HSP90 inhibitors validates this combined virtual screen and biophysical technique approach, which may be applied for future inhibitor discovery work for HSP90 as well as other targets.

Heat Shock Protein 90 (HSP90) is a dimeric molecular chaperone that performs a range of essential housekeeping functions in cells.[1–5] These include the folding, maturation and stabilisation of client proteins, as well as repairing and degradation of damaged proteins. The HSP90 protomer consists of three domains: an N-terminal adenosine triphosphate (ATP)-binding domain, a middle protein-binding domain, and a C-terminal dimerisation domain where the two protomers are joined. This allows the two protomers to open and close in a pincer-like motion so that HSP90 can interact with its client proteins and other co-chaperones to perform its functions. The activity of HSP90 is regulated by the hydrolysis of ATP to adenosine diphosphate (ADP) at the protein's N-terminal domain.[6–8]

HSP90 has been linked to the progression of several types of cancers including melanoma, breast cancer, gastrointestinal stromal tumours and lung cancer.[9,10] In cancerous cells, the client proteins of HSP90 include those that are involved in oncogenic processes. The stabilisation and folding of oncogenic client proteins in cancerous cells by HSP90 enable the tumour cells to keep growing and proliferating, which eventually may lead to cancer.

Given the central role ATP hydrolysis plays in enabling and maintaining the function and activity of HSP90, the inhibition of ATP binding and hydrolysis by small molecules at the N-terminal domain of HSP90 (HSP90-ND) is potentially a useful strategy for therapeutic intervention against cancers.[11–15] First generation HSP90 inhibitors that target the ATP binding site were derived from natural products. These include geldanamycin, radicicol and their structural analogues. Whilst these compounds are effective HSP90 inhibitors, clinical trials have revealed dose and schedule-dependent toxicity.[16] Although second generation inhibitors, including CNF2024 and ganetespib, showed promising results in the early stages of clinical trials,[17–19] there is still a need to further develop and validate inhibitor scaffolds that target the ATP binding pocket of HSP90-ND.

Virtual screening is a cost-effective and efficient strategy in the identification of structural scaffolds and chemical moieties that are potentially important for binding to a target protein, provided a structural model of the protein target is available.[20–22] Biophysical techniques, including thermal shift assays[23–25] and protein nuclear magnetic resonance (NMR) spectroscopy,[26–28] are often applied as complementary methods to verify the hits obtained from virtual screens. Protein NMR spectroscopy is also useful in identifying ligand binding pocket on the protein, and for the quantification of their binding affinities (K_Ds).[29–31] Herein we describe our work in applying virtual screening, thermal shift assay and protein NMR spectroscopy in the identification and validation of HSP90-ND inhibitors.

Abbreviations: 17-AAG, 17-allylamino-17-demethoxygeldanamycin; 17-DMAG, 17-(dimethylaminoethylamino)-17-demethoxygeldanamycin (17-DMAG); ADP, adenosine diphosphate; AMPPCP, 5′-adenylylmethylenediphosphonate; ASP, Astex statistical potential; ATP, adenosine triphosphate; CS, ChemScore; GS, GoldScore; HB, hydrogen bonding; HSP90, heat shock protein 90; HSQC, heteronuclear single quantum correlation; K_D, binding affinity; ND, N-domain; NMR, nuclear magnetic resonance; NOE, nuclear Overhauser effect; T_m, protein denaturing temperature.

* Corresponding authors.
E-mail addresses: j.reynisson@auckland.ac.nz (J. Reynisson), i.leung@auckland.ac.nz (I.K.H. Leung).

http://dx.doi.org/10.1016/j.bmcl.2016.11.059
0960-894X/

High-throughput virtual screening was first conducted against the ATP binding pocket of HSP90-ND using a reported HSP90 structure that was bound with an analogue of the radicicol inhibitor (PDB id: 4CE1).[32] 9050 molecular entities were downloaded from the natural product InterBioScreen Ltd collection. The four scoring functions that are available in the GOLD software suite, including GoldScore (GS),[33] ChemScore (CS),[34,35] ChemPLP[36] and Astex Statistical Potential (ASP),[37] were used. In order to set a limit for these scoring functions, molecular modelling was conducted with nine known HSP90 inhibitors, which included 17-allylamino-17-demethoxygeldanamycin (17-AAG), 17-(dimethylaminoethylamino)-17-demethoxygeldanamycin (17-DMAG), CCT018159,

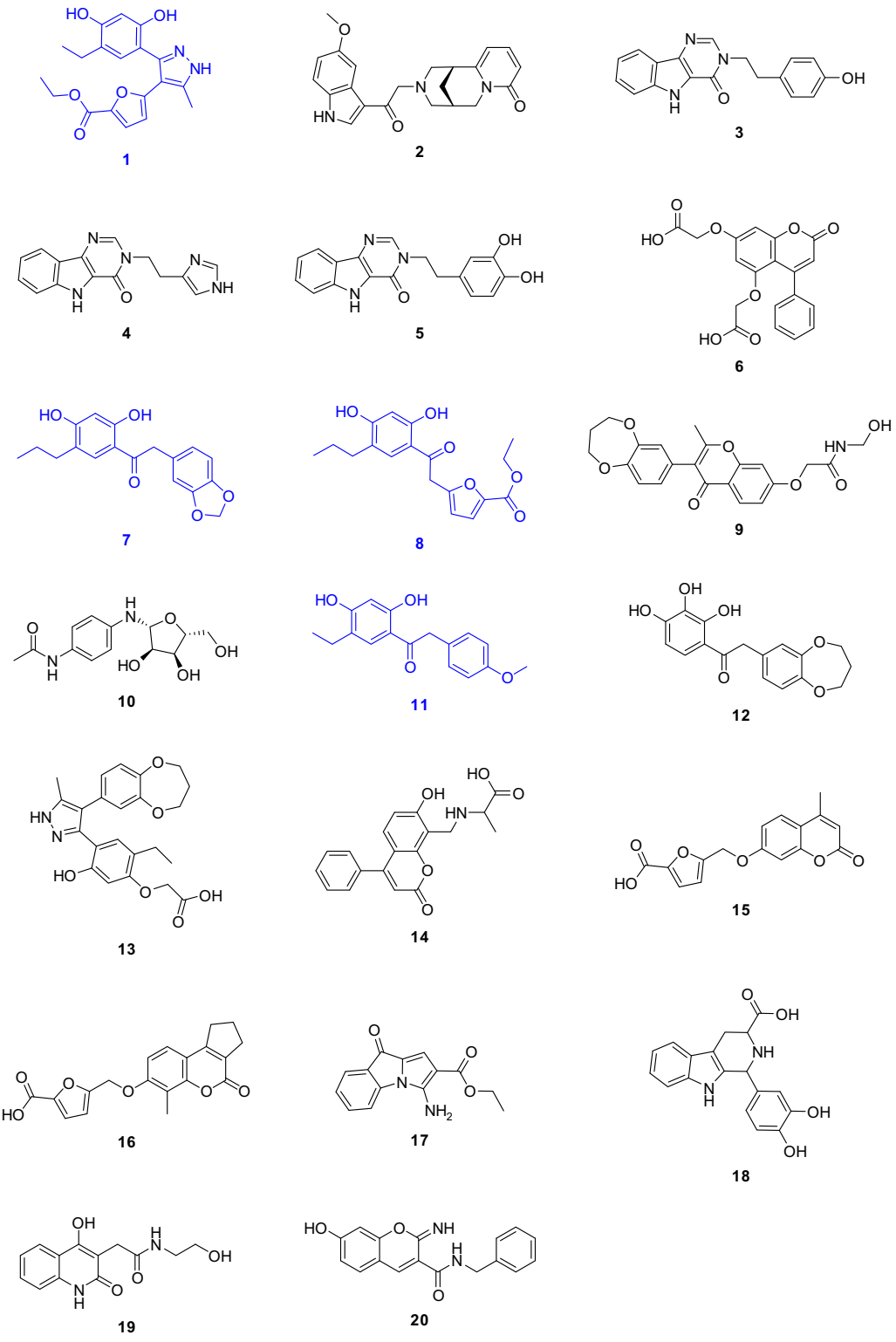

Fig. 1. Structures of the twenty compounds identified from the virtual screen. Blue indicates compounds that show binding to HSP90-ND through thermal shift assay and protein NMR spectroscopy. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Please cite this article in press as: Huang R., et al. Bioorg. Med. Chem. Lett. (2016), http://dx.doi.org/10.1016/j.bmcl.2016.11.059