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Development of quinone analogues as dynamin GTPase inhibitors

Kylie A. MacGregor [a, 2], Mohammed K. Abdel-Hamid [a, c, 2], Luke R. Odell [a, 1], Ngoc Chau [b], Ainslie Whiting [b], Phillip J. Robinson [b], Adam McCluskey [a, *]

[a] Centre for Chemical Biology, Chemistry, School of Environmental and Life Sciences, The University of Newcastle, Callaghan, NSW 2308, Australia
[b] Children's Medical Research Institute, University of Sydney, Westmead, NSW 2145, Australia
[c] Department of Medicinal Chemistry, Faculty of Pharmacy, Assiut University, Assiut 71526, Egypt

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ABSTRACT

Virtual screening of the ChemDiversity and ChemBridge compound databases against dynamin I (dynI) GTPase activity identified 2,5-*bis*-(benzylamino)-1,4-benzoquinone **1** as a 273 ± 106 μM inhibitor. In silico lead optimization and focused library-led synthesis resulted in the development of four discrete benzoquinone/naphthoquinone based compound libraries comprising 54 compounds in total. Sixteen analogues were more potent than lead **1**, with 2,5-*bis*-(4-hydroxyanilino)-1,4-benzoquinone (**45**) and 2,5-bis(4-carboxyanilino)-1,4-benzoquinone (**49**) the most active with IC_{50} values of 11.1 ± 3.6 and 10.6 ± 1.6 μM respectively. Molecular modelling suggested a number of hydrogen bonding and hydrophobic interactions were involved in stabilization of **49** within the dynI GTP binding site. Six of the most active inhibitors were evaluated for potential inhibition of clathrin-mediated endocytosis (CME). Quinone **45** was the most effective CME inhibitor with an $IC_{50(CME)}$ of 36 ± 16 μM.

1. Introduction

Dynamin GTPase belongs to the dynamin super-family of large GTPases which play crucial roles in the catalysis of membrane fission during clathrin mediated endocytosis (CME), regulation of cellular actin dynamics and are important for cytokinesis [1]. There are three classical dynamins (dynI, dynII and dynIII) which each have low affinity for guanine nucleotides (10–100 μM), high basal GTP turnover (0.4–1 min^{-1}) and the propensity for oligomerization into helical arrays around a template [2]. Each dynamin comprises five structural domains including an amino-terminal G domain that binds and hydrolyses GTP, a middle domain involved in self-assembly and oligomerization, a pleckstrin homology (PH) domain responsible for interactions with the plasma membrane and inducing the hemifission state, a GTPase effector domain (GED) which is also involved in self-assembly, and a proline- and arginine-rich (PRD) domain that interacts with SH3 domains in accessory proteins [3–5]. With the exception of the PRD, tertiary structures of all dynamin's individual domains have been reported [6–9]. In particular, two crystal structures for the near full length mammalian dynI (lacking the PRD domain) have been solved, providing valuable information regarding the complex dynamics involved in dynamins' GTPase cycle and its role as a fission protein in cells [10,11].

CME is a highly regulated cellular process in which growth factors and membrane-bound receptors are concentrated in invaginating clathrin-coated pits (CCPs). These CCPs pinch off to form vesicles which carry the cargo into the cell [10]. In the final stages of CME, dynamin GTPase can assemble into collars at the necks of the deeply invaginated CCPs to catalyse membrane fission apparently by neck constriction [3,11,12]. Synaptic vesicle endocytosis (SVE) in neural cells is based on a similar mechanism to CME and plays a role in the internalization of membrane to allow synaptic vesicle recycling thus sustaining synaptic transmission [11]. SVE and CME differ mainly in the dynamin genes that are utilized in each case and unique regulation of SVE by protein dephosphorylation. While dynII is found throughout the body and is the major scission protein for CME, dynI is mainly found in neuronal cells where it coexists with dynII and dynIII. In neural cells dynI protein is expressed at about 50-fold higher levels than the two other classical dynamins and is critical for neural cell functions [1].

We have previously reported the identification and development of the MiTMAB [13], Bis-T [14,15], RTIL [16], Iminiodyn™ [17], Pthaladyn™ [18], Dyngo™ [19,20], Rhodadyn™ [21] series and two generations of Dynole™ [22–24] compounds as dynamin

* Corresponding author.
E-mail address: Adam.McCluskey@newcastle.edu.au (A. McCluskey).
[1] Current address: Organic Pharmaceutical Chemistry, Department of Medicinal Chemistry, Uppsala Biomedical Centre, Uppsala University, Box 574, SE-751 23 Uppsala, Sweden.
[2] These authors contributed equally to this work.

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inhibitors [25]. We have also described the virtual screening of the ChemBridge and the ChemDiversity chemical databases against a dynI GTPase domain homology model (hDynI) using ICM flexible ligand-grid receptor algorithm to target the GTP binding site which led to the identification of the Pthaladyn™ compound series [17]. Herein we report on the development of a second series of compounds from our initial virtual screening hits were we identified 2,5-*bis*-(benzylamino)cyclohexa-2,5-diene-1,4-dione (**1**) as a 273 ± 106 μM potent dynI inhibitor (Fig. 1).

Quinones have been previously explored in a wide range of different drug development pathways with multiple analogues reported to be active in a myriad of different biological screens [26,27]. In our virtual screening approaches, quinones were retained due to their ability to link potential binding elements that would not otherwise be accessed [28,29]. A number of quinones have progressed to clinical trials, e.g. ubiquinone 10, diaziquone, mitoxantrone hydrochloride and monobenzone [29]; and in a related area (to this study) the natural product bolinaquinone has been shown to display high levels of specificity towards clathrin [30]. Herein we anticipated that our medicinal chemistry efforts with lead **1**, would add to the SAR of dynamin inhibition already established [13–25,31–33].

2. Results and discussion

Structural optimization of **1** commenced with molecular docking and investigation of its predicted orientation within dynI's GTP-binding site. Docking studies were conducted using AutoDock 4.2 and the dynI-GMPPCP co-crystal structure (PDB: 3ZYC) [3,34]. Examination of the GMMPCP binding pose revealed a number of strong hydrogen bonding interactions between the active site residues and GMPPCP (ESI). The hydrogen bonding interactions were similar to that observed with **1** (ESI). These included a strong hydrogen bond (2.4 Å) between one of the quinone carbonyl moieties and the side chain of the catalytically crucial Ser41 [2]. Other hydrogen bond interactions ranged between moderate and weak (2.8–3.2 Å) and involved the backbones and/or side chains of Ser45, Ser46 and Lys206. One of the terminal phenyl rings showed an arene–cation interaction with the side chain of Lys44, similar to that observed between Lys206 and the GMPPCP guanine core within the published co-crystal. However, compound **1** did not show any of the contacts with amino acids at the "dynamin-specific loop" (residues 236–246) which were present with GMPPCP [3]. The lipophilic/hydrophilic contact surface preference in the GTP active site showed a requirement to balance the hydrophilic/hydrophobic characters in subsequent modified quinone (Fig. 2). While the dynamin-specific loop favoured the presence of hydrophobic contacts (Fig. 2A) the P- and G4-loop region favoured hydrophilic interactions (Fig. 2B).

Our initial modelling studies suggested that 1,4-naphthoquinone analogues would retain the required interactions within the active site and consequently fuller engagement with the key active site residues. Thus, treatment of 1,4-naphthoquinone (**2**) and with a range of substituted amines we assembled a discrete library of amine substituted 1,4-naphthoquinones, which were screened for inhibition of dynI GTPase activity (Scheme 1 and Table 1) [35].

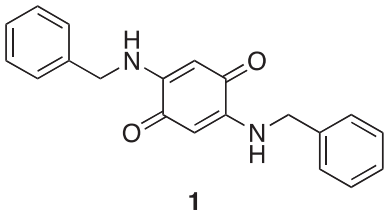

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Fig. 1. Chemical structure of virtual screening lead 2,5-*bis*-(benzylamino)cyclohexa-2,5-diene-1,4-dione (**1**).

Of the 13 analogues synthesized, only **9**, **10** and **12** showed significant improvement in activity compared to the lead **1** (IC_{50} = 273 ± 106 μM), with IC_{50} values of 30.3, 29.5 and 22.4 μM respectively. However, the activity profile for other derivatives was consistent, excepting **6**–**8**, with the contact preference maps and suggested the symmetry inherent in the parent compound was not absolutely required, but may have bestowed additional features advantageous for dynI inhibition.

The predicted binding poses for the **9**, **10** and **12** showed a high degree of similarity and revealed a number of hydrophobic and hydrogen bonding interactions with the binding site (Fig. 3). One of the quinone carbonyl groups was predicted to accept two hydrogen bonds from P-loop residues, specifically from the backbone and side chain protons of Ser45 and Lys44. The other carbonyl group formed a hydrogen bond with Ser41. The hydroxyl moiety donated a hydrogen bond to either the backbone or the side chain of Arg237 and accepted a Ser46 side chain hydrogen bond. The phenyl substituent made key hydrophobic interactions with the Ala42 and Ile242 side chains, whilst the naphthaquinone core participated in an arene–cation interaction with Lys44 in addition to making hydrophobic contacts with the Ile63, Leu137 and Pro138 side chains. The amine linker donated a moderate strength hydrogen bond to the backbone carbonyl of Gly60.

Given our findings with the naphthoquinone analogues (**3**–**16**), we next synthesized a focused library of symmetrically substituted *p*-benzoquinone analogues (**1, 17**–**33**) (Scheme 2). Treatment of *p*-benzoquinone (**16**) with two equivalents of 2-benzylamine in ethanol at reflux for 18 h [36]. These analogues were screened for their ability to inhibit dynI and these data are presented in Table 2.

Of the *p*-benzoquinone focused library analogues only **18**, **21**, **23** and **33** returned improved activity with dynI IC_{50} values of 29, 170, 112 and 58 μM, respectively. Only the oxygen bearing benzylamine analogues within this focused library returning IC_{50} values in the 29–170 μM range. The enhanced activity of **18** was attributed to interaction with most key residues at the active site as shown from its "Protein Ligand Interaction Fingerprints" (PLIF, Supplementary data). Although **23** bears a hydroxyl group suggesting a good hydrogen bonding network it showed a lower level of interactions compared to **18** (missing the interaction with Lys44, Gly139 and Asn208) suggesting that the C=O moiety in **18** plays an important role in favourable interactions within the GTP biding site. Methylation of the hydroxyl group giving the 4-methoxy substituted **23** further reduced potency with the loss of the interactions with Gly62 and Val64 in switch I region of the binding site (**21**, IC_{50} = 170 μM). This highlighted the potential importance of hydrogen bonding within the GTP-binding site. Other interesting results regarding the activities of the benzylamine derivatives were shown by the loss of the inhibitory activity in the case of **25**, directing attention to the importance of optimizing the length of the linker between the quinone and terminal aromatic moieties. In addition, the PLIF highlighted the binding site residues that were commonly involved in the interactions which include Ser41, Ser46 and Lys206.

Most of the alkyl or alkenyl substituted quinones (**26**–**33**) displayed no dynI inhibition, except **33** which was more active (IC_{50} = 58 ± 23 μM) than the lead **1**. This promoted us to further investigate the activity of aliphatic side chain derivatives through the introduction of heteroatom bearing substituents to target more hydrogen bond interactions at the binding site. Compounds **34**–**43** were synthesized essentially as described in Scheme 1, using a selected library of primary amines, and subsequently screened for dynI inhibition (Table 3) [35].