Bioorganic & Medicinal Chemistry 23 (2015) 5303–5310

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

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

A new small molecule inhibitor of soluble guanylate cyclase

Filipa Mota [a], Paul Gane [a,†], Kathryn Hampden-Smith [a,‡], Charles K. Allerston [b,§], John Garthwaite [a], David L. Selwood [a,*]

[a] The Wolfson Institute for Biomedical Research, University College London, Gower Street, London WC1E 6BT, United Kingdom
[b] Structural Genomics Consortium, University of Oxford, Roosevelt Drive, Oxford OX3 7DQ, United Kingdom

ARTICLE INFO

Article history:
Received 12 May 2015
Revised 29 July 2015
Accepted 30 July 2015
Available online 4 August 2015

Keywords:
sGC (soluble guanylate cyclase)
Nitric oxide
ODQ
Surface plasmon resonance

ABSTRACT

Soluble guanylate cyclase (sGC) is a haem containing enzyme that regulates cardiovascular homeostasis and multiple mechanisms in the central and peripheral nervous system. Commonly used inhibitors of sGC activity act through oxidation of the haem moiety, however they also bind haemoglobin and this limits their bioavailability for in vivo studies. We have discovered a new class of small molecule inhibitors of sGC and have characterised a compound designated **D12** (compound **10**) which binds to the catalytic domain of the enzyme with a K_D of 11 μM in a SPR assay.

1. Introduction

The heterodimeric enzyme soluble guanylate cyclase (sGC) is an endogenous receptor for nitric oxide (NO). NO binds to a haem prosthetic group resulting in a conformational change which activates the enzyme. Upon activation, sGC converts guanosine triphosphate (GTP) into cyclic guanosine monophosphate (cGMP). NO-induced activation of sGC is key to maintaining cardiovascular homeostasis and in the brain, NO—sGC acts as a neurotransmitter—receptor system.[1–3]

NO-induced signalling has been implicated in the modulation of synaptic transmission and to act in long-term potentiation, one of the major cellular mechanisms that underlie the processes of learning and memory.[4] In rats, high cGMP levels promote neural stem cells differentiation to neurons whilst reduced cGMP levels promote differentiation to non-neuronal (mainly glial) cells, which consequently leads to impaired cognitive function.[5]

NO can mediate neurotoxicity and cause neuronal cell death. The rapid on–off-kinetics and desensitization profile of NO, combined with variations in the rate of cGMP breakdown, provide fundamental mechanisms for shaping cellular cGMP responses and is important in decoding NO signals under physiological and pathological conditions.[6] The neurotransmitter is associated with pathogenic mechanisms involved in multiple neurodegenerative diseases, including Parkinson's Disease (PD).[7] The NO—sGC system is also involved in the etiology of migraine.[8] Recent research has suggested the involvement of NO in PD is due to activation of sGC. Disruption of striatal NO-sGC-cGMP signalling cascades resulted in profound changes in behavioural, electrophysiological, and molecular responses to pharmacological manipulations of dopamine and glutamate transmission.[9] Studies performed in animal models of PD with a sGC inhibitor, ODQ **1** (Fig. 1), have shown that the enzyme could be a new drug target for restoring basal ganglia dysfunction and attenuating motor symptoms associated with PD.[9] ODQ **1** has been widely used to study the function of the NO-sGC-cGMP signal transduction pathway and it has been a valuable tool to distinguish signalling events mediated by sGC from those involving other nucleotide cyclases.[10] The small molecule binds to the ferrous haem in the β-subunit of the enzyme, yielding ferric haem which cannot bind NO.[11,12] Haem-binding compounds such as ODQ **1** and its 8-bromo-analogue NS2028, show activity against other haem containing proteins as such as haemoglobin, albeit at high concentrations.[13,14] ODQ may also not be able to block cGMP signalling in all circumstances.[15] Other known ways of inhibiting sGC activity include block of the catalytic site with ATP and GTP analogues[16–20] though these have weak inhibitory potency. Inhibitors such as LY-83583 act indirectly by generating superoxide which reacts rapidly with NO.[16] Previously we demonstrated that surface plasmon resonance (SPR) allied to biochemical screening was an effective way of

* Corresponding author. Tel.: +44 20 7679 2000.
E-mail address: d.selwood@ucl.ac.uk (D.L. Selwood).
† Current address: Chemical Computing Group, St John's Innovation Centre, Cambridge CB4 0WS, United Kingdom.
‡ Current address: University of Sussex, Brighton BN1 9RH, United Kingdom.
§ Current address: Dart NeuroScience, LLC, 12278 Scripps Summit Dr., San Diego, CA 92131, USA.

http://dx.doi.org/10.1016/j.bmc.2015.07.074
0968-0896/

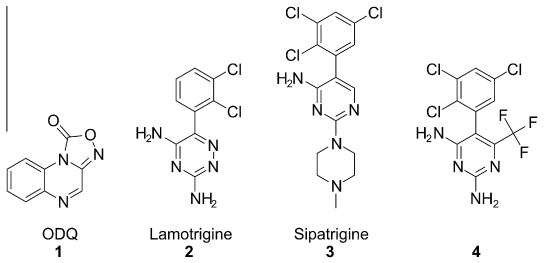

Figure 1. Chemical structures of sGC inhibitor ODQ **1**, Lamotrigine **2**, and analogues Sipatrigine **3** and **4**.

discovering new sGC ligands. In this study we identified a new small molecule inhibitor of sGC, which does not act through oxidation of the haem.

2. Results

2.1. In silico similarity searching and screening

In the course of studies in our laboratory we observed activation of sGC with the anti-epileptic drug lamotrigine **2** and inhibition with lamotrigine analogues sipatrigine **3** and **4**, all at low millimolar concentrations (data not shown) (Fig. 1). These compounds were used as the starting point for a screening study to find new inhibitors of sGC. The strategy was to conduct several rounds of similarity searching and screening to identify the best inhibitors. Some synthetic studies were conducted on the best hit to explore the structure–activity relationships.

Virtual screening was performed by similarity searches using MACCS fingerprints at 85% and 75% Tanimoto of commercial libraries and the selected compounds were screened at 100 μM against purified bovine lung sGC using diethylamine NONOate (30 nM) as the NO donor. Enzyme activity was determined by measuring cGMP production using a standard cGMP [^{3}H] radioimmunoassay.[21,22] The initial structure searched (Fig. 2, substructure A) resulted in circa 500 structures, out of which 16 compounds were selected based upon diversity, molecular size, and availability. Compounds **5** and **6** showed inhibition of enzyme activity by 51% at 100 μM. Subsequent searches of substructures B and C resulted in the identification of [1,2,5]oxadiazolo[3,4-*b*]pyrazines **7** and **8**, and *N*2,*N*3-diphenylquinoxaline-2,3-diamines **9** and **10** (designated **D12**) as inhibitors of sGC (Fig. 2).

2.2. Chemistry and structure activity studies

The hit compound **10** is formed of a quinoxaline scaffold with a nitro group in the 6-position of the heterocycle, and joined to two phenols via secondary amine linkers. A small set of analogues was synthesised to explore the binding role of the substituents, focusing on the nitro group and the phenols (Table 1).

Compound **10** and analogues **14**–**28** were synthesised via nucleophilic aromatic displacement using commercially available anilines and 2,3-dichloro-quinoxalines **12** when possible. In other cases 2,3-dichloroquinoxalines **12** were obtained via a known two-step synthesis, starting with the condensation of 1,2-diamines with oxalic acid.[23] The resulting 2,3-dihydroxyquinoxalines **11** were chlorinated with thionyl chloride and a catalytic amount of DMF. The carboxamide substituted quinoxaline **13** was obtained after amidation of the carbonyl chloride intermediate **12a** (Scheme 1).

Reduction of the nitro group in compound **10** was successfully achieved using tin(II) chloride in the presence of sodium borohydride to give compound **29** (Scheme 2).

We have previously described a surface plasmon resonance-based assay for the detection of binding of small molecules to full-length sGC and a smaller construct of the catalytic domain (sGCcat).[24] This assay was used to measure the binding of the compounds to sGCcat.[24] In general, compounds with hydroxyls on the anilino phenyl rings (**10**, **14**, **20**, **21**, **23**–**26**, **28**, **29**) showed higher binding to the enzyme, suggesting they might be involved in hydrogen bonding. The position of the hydroxyl group can be changed whilst retaining binding strength and activity. However, other groups such as acetamide **17**, **18**, methoxy **15**, or fluoro **19**, rendered the compound biochemically inactive and reduced its binding strength.

The electron-withdrawing nitro group commonly presents as a challenge in drug design. Nitro-aromatics are commonly associated with toxicity, but identifying suitable replacements has proven difficult.[25] A series of 6-substituted-2,3-dichloroquinoxalines was synthesised. Further modifications included the reduction of the nitro group to a primary amine, and the conversion of an acyl chloride into an amide (Schemes 1 and 2). Changes in the

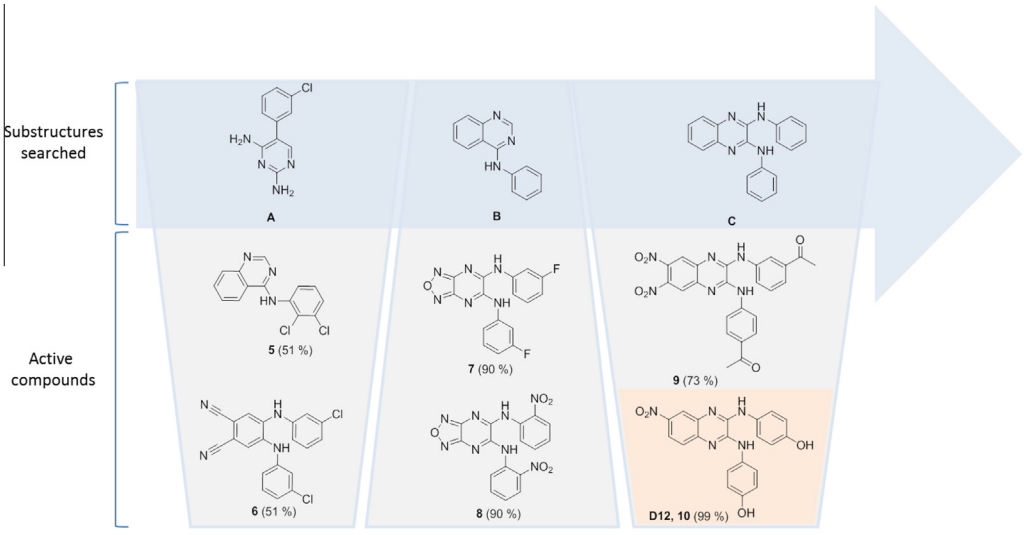

Figure 2. Virtual screening. Chemical structures of virtually searched substructures A–C and the most active compounds **5**–**10** of each series. The values in brackets correspond to the % inhibition of sGC activity at 100 μM compound.