European Journal of Medicinal Chemistry 69 (2013) 384–392

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Design, synthesis and efficacy of novel G protein-coupled receptor kinase 2 inhibitors

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ARTICLE INFO

Article history:
Received 8 April 2013
Received in revised form
19 July 2013
Accepted 22 August 2013
Available online 13 September 2013

Keywords:
GRK2 inhibitors
Cardiovascular system
Cyclic peptides
NMR conformational analysis

ABSTRACT

G protein-coupled receptor kinase 2 (GRK2) is a relevant signaling node of the cellular transduction network, playing major roles in the physiology of various organs/tissues including the heart and blood vessels. Emerging evidence suggests that GRK2 is up regulated in pathological situations such as heart failure, hypertrophy and hypertension, and its inhibition offers a potential therapeutic solution to these diseases. We explored the GRK2 inhibitory activity of a library of cyclic peptides derived from the HJ loop of G protein-coupled receptor kinases 2 (GRK2). The design of these cyclic compounds was based on the conformation of the HJ loop within the X-ray structure of GRK2. One of these compounds, the cyclic peptide **7**, inhibited potently and selectively the GRK2 activity, being more active than its linear precursor. In a cellular system, this peptide confirms the beneficial signaling properties of a potent GRK2 inhibitor. Preferred conformations of the most potent analog were investigated by NMR spectroscopy.

1. Introduction

The G protein-coupled receptor kinase family (GRKs) constitutes a group of seven protein kinases that specifically recognize and phosphorylate agonist-activated G protein coupled receptors (GPCRs) [1]. GRKs-mediated receptor phosphorylation triggers the binding of arrestin proteins that uncouple receptors from G proteins leading to rapid desensitization [2–4]. As a result of β-arrestin binding, phosphorylated receptors are also targeted for clathrin-mediated endocytosis, a process that classically serves to resensitize and recycle receptors back to the plasma membrane [5]. In addition, both arrestins and GRKs participate in signal propagation, cooperating in the assembly of macromolecular complexes in the receptor environment and interacting with different components of signal transduction [1,6]. The seven mammalian GRKs family can be divided into three subfamilies based on sequence and functional similarity: visual GRK subfamily (GRK1 and GRK7), the β-adrenergic receptor kinase (GRK2/GRK3), and the GRK4 subfamily (GRK4, GRK5 and GRK6) [1,7]. All GRKs share a common topological structure that includes an N-terminal regulator of G protein signaling homology domain (RH), a central kinase catalytic domain, and a C-terminal region containing a pleckstrin homology domain (PH) [1,8]. The best-characterized member of this family is the ubiquitously expressed GRK2, also known as β-adrenergic receptor kinase 1 (β-ARK1) [9]. Among others, GRK2 plays a major role in the agonist-specific desensitization of β-adrenergic

Abbreviations: DPC, dodecylphosphocholine; SAR, structure–activity relationship; DCM, dichloromethane; DIPEA, *N*,*N*-diisopropylethyl-amine; DMF, *N*,*N*-dimethylformamide; Pr_3SiH or TIS, triisopropylsilane; TFA, trifluoroacetic acid; Fmoc, 9-fluorenyl-methoxycarbonyl; HOBt, *N*-hydroxy-benzotriazole; HBTU, 2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluoro-phosphate; Trt, trityl; Pbf, 2,2,4,6,7–pentamethyldihydro benzofuran-5-sulfonyl; RP-HPLC, reversed-phase high performance liquid chromatography; ESI, electrospray ionization; LCQ, liquid chromatography quadrupole mass spectrometry; ATP, adenosine triphosphate; EDTA, Ethylene diamine tetraacetic acid; EGTA, ethylene glycol tetraacetic acid; cAMP, cyclic adenosine monophosphate.

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0223-5234/$ – see front matter © 2013 Published by Elsevier Masson SAS.
http://dx.doi.org/10.1016/j.ejmech.2013.08.039

Table 1
Different peptide fragments considered in this study.

Name		Sequences							
KRX-683_{107}	Myristyl	G	L	L	R	r	H	S	
KRX-683_{124}	Lauryl	G	L	L	R	r	H	S	I
$^{383-390}$HJ loop (**1**)		K	L	L	R	G	H	S	P
2		G	L	L	R	r	H	S	
3		G	L	L	R	r	H	S	I

receptors (βAR), and therefore in the signal transduction pathway of physiological relevance in the cardiovascular system [9–11]. Alterations in GRK2 levels and/or activity may have important effects in several cardiovascular pathologies, such as myocardiac ischemia, hypertrophy, and hypertension, in which it is up regulated [12–15]. In heart failure (HF), the relationship with increased cardiac GRK2 protein levels has been established in animal models and in patients [16–20]. These data underline the importance of GRK2 levels as a marker of predisposition to cardiac dysfunction [21,22] and support the idea that GRK2 offers a potential therapeutic target [9,23,24]. In fact, the inhibition of GRK2 activity by overexpression of GRK2ct (also termed β-ARKct), a construct that inhibits endogenous GRK2, has provided a successful approach for restoring cardiac function in mouse models with heart failure [25]. Nevertheless, β-ARKct failed to deliver to the clinical scenario due to its dimension (≈200 amino acids) and its need of genetic tools to express in the target tissue (adenovirus). Other inhibitors of GRK2 activity are currently available, even if they are characterized by low sensitivity and specificity [26–29]. Strategies to selectively inhibit the GRK2 activity have been attempted using shorter peptides [30–32] or RNA aptamers [33]. Myristyl or lauryl glycine derivatives of short peptides, such as KRX-683_{107} and KRX-683_{124} (Table 1), proved to be potent inhibitors of the kinase and possess hypoglycemic effect in animal models of Type 2 diabetes [32], and insulin resistance [34]. The peptide fragments of these compounds closely resemble the catalytic fragment 383–390 KLLRGHSP of GRK2 (**1**). This fragment is composed by the last part of the α-helix F (residues 383–386) and the first part of a strand (residues 387–390, Table 1) within the HJ loop. Several crystallographic and mutational studies, have pointed to HJ-αG residues as being involved in substrate binding and in binding to upstream activators [30,31]. We have recently found that peptides **2** and **3**, which are the not acylated derivatives of KRX-683_{107} and KRX-683_{124}, respectively (Table 1), selectively inhibit GRK2 *in vitro* [35]. Hence, these compounds are valuable starting points for the development of novel GRK2 inhibitors. Furthermore, conformational analysis of these peptides clearly indicated that their structures are very similar to the X-ray structure of the fragment encompassing the HJ loop of the GRK2 [36], indicating that the isolated peptide could keep the 3D structure of the protein segment.

Table 2
Structure, inhibition activities, and analytical data of peptides **2**–**9**.

Com.	Sequence	Inhibition[a]		HPLC[c]	ESI-MS (M + H)	
		GRK2[b]	GRK5[b]	*k′*	Calcd	Found
2[d]	GLLRrHS	47.6 ± 5.5	<5	1.70	836.97	837.66
3[d]	GLLRrHSI	49.6 ± 6.3	<5	1.72	950.13	950.70
4	[GLLRrHS]	47.8 ± 6.0	<5	1.70	819.96	820.53
5	[GLLRrHSI]	37.2 ± 10.7	<5	1.85	933.12	933.80
6	[KLLRrHD]	36.3 ± 4.4	<5	1.72	919.09	920.13
7	[KLLRrHD]I	55.3 ± 4.6	<5	1.75	1032.25	1033.11
8	[KLLRGHD]	47.2 ± 4.5	<5	1.76	819.47	820.51
9	[KLLRGHD]I	33.7 ± 7.8	<5	1.78	933.12	933.68

[a] Data represent mean values (±SD) of three independent determinations.
[b] GRK2 and GRK5 purified proteins activity (50 ng) were tested on rod outer segments (ROS) in presence or absence of 1 μM inhibitors.
[c] *k′* = [(peptide retention time solvent retention time)/solvent retention time].
[d] Already reported in Ref. [34].

Based on these results, we designed, synthesized, and evaluated the GRK2 inhibitor activities of small libraries of cyclic peptides. In addition, we discussed the biological effects of GRK2 inhibition on beta adrenergic receptor signaling for the most potent derivative characterized in this work.

2. Results

2.1. Design

We have recently found that peptides **2** and **3** selectively inhibit GRK2 *in vitro*. Their NMR solution conformations are very similar to the crystal structure of the fragment encompassing the HJ loop of the GRK2 (pdb entry 3CIK, Fig. 1) [35,36]. Hence, it can be hypothesized that the active conformation of the peptides resembles the HJ loop crystal structure. Accordingly, the stabilization of the peptide 3D structure by, for example, the cyclization of these linear compounds can be considered as a valid approach to the identification of more potent (stable and selective) compounds. As evident from Fig. 1, N- and C-terminal sides of the loop fragment are relatively close (~5 Å), hence we first carried out a head-to-tail cyclization of peptides **2** and **3** leading to peptides **4** and **5**, respectively (Table 2). The design of a second group of cyclic peptides is based on the consideration that, in the GRK2 crystal structure, the amino

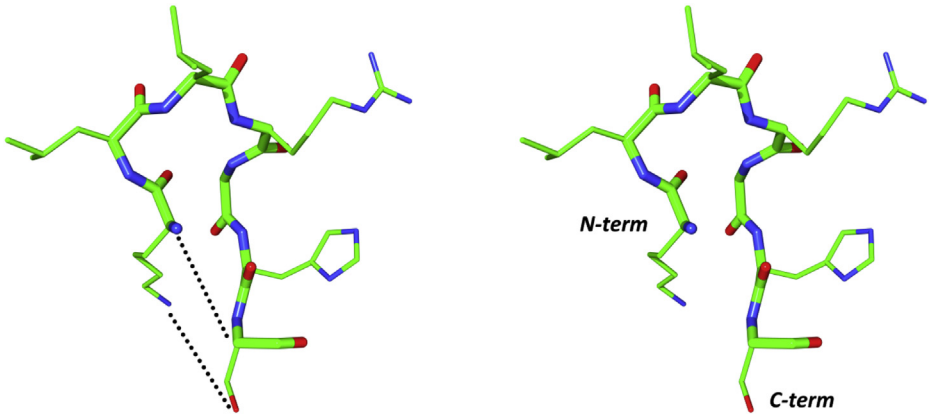

Fig. 1. Stereoview of the crystal structure of the fragment 383–390 of GRK2 (**1**, pdb entry 3CIK). Heavy atoms are shown with different colors (carbon, green; nitrogen, blue; oxygen, red). Hydrogen atoms are not shown for clarity. Cyclization strategies are shown as dotted lines. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)