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Review Article

Selective small-molecule EPAC activators

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The cellular signalling enzymes, EPAC1 and EPAC2, have emerged as key intracellular sensors of the secondary messenger cyclic 3′,5′-adenosine monophosphate (cyclic adenosine monophosphate) alongside protein kinase A. Interest has been galvanised in recent years thanks to the emergence of these species as potential targets for new cardiovascular disease therapies, including vascular inflammation and insulin resistance in vascular endothelial cells. We herein summarise the current state-of-the-art in small-molecule EPAC activity modulators, including cyclic nucleotides, sulphonylureas, and *N*-acylsulphonamides.

Introduction

Cyclic adenosine monophosphate (cAMP) is a prototypical secondary messenger involved in the regulation of many cellular processes in response to extracellular stimuli [1,2]. cAMP signalling is regulated by the relative expression and localisation of adenylyl cyclases (ACs) and phosphodiesterases (PDEs) within the cell [3–9]. cAMP mediates its effects mostly via protein kinase A (PKA) [10–13] and exchanges proteins activated by cAMP (EPACs) [14–16] in addition to Popeye domain-containing proteins (POPDCs) [17–20] and cyclic nucleotide-gated ion channels (CNGs) [21–24]. Controlling diverse physiological responses, many drugs have been developed to elevate intracellular cAMP levels, either through inhibition of PDEs or activation of ACs [7,25–34]. However, the indiscriminate nature of cAMP signalling points to the potential to concurrently activates all of the cAMP-sensor signalling pathways [35–38]. In this review, we summarise recent progress in the development of selective small-molecule activators of EPACs.

EPAC1 and EPAC2 are multi-domain proteins, encoded by different genes, which act as guanine nucleotide exchange factors for the Ras-like GTPases Rap1 and Rap2 [15,16,39]. They differ by function and tissue expression patterns, but share the same functional domain organisation and mode of activation. In each case, the structure consists of a regulatory *N*-terminal domain with a dishevelled-EGL pleckstrin homology domain and a cyclic nucleotide-binding domain (CNBD), and a *C*-terminal domain which includes a Ras exchange motif (REM), a Ras association domain and a CDC25 homology domain [40–44]. EPAC2 contains an additional *N*-terminal CNBD with a reduced affinity for cAMP, thought to be involved in subcellular localisation [15,16,45]. In the inactive state, the proteins exist in an auto-inhibited conformation in which the regulatory domain blocks the Rap-binding site [42,43,46]. Binding of cAMP to the CNBD induces a conformational shift, which unveils the Rap-binding site, allowing for signal transduction [42–44].

Many studies have suggested that EPAC signalling dysfunction plays a role in such diverse conditions as hypertension [47,48], diabetes [49], cancer [50,51], cardiac arrhythmia [52], and inflammatory pain [53]. EPAC1 has been shown to play a role in mitigating pro-inflammatory cytokine signalling in vascular endothelial cells (VECs), through induction of SOCS3 and subsequent inhibition of IL-6 signalling via the JAK/STAT3 pathway [54,55]. In the context of lung inflammation, EPAC1 and 2 appear to play disparate roles in the IL-8 signalling pathway associated with the chronic obstructive pulmonary disorder (COPD), in which EPAC1 suppresses airway remodelling, while EPAC2 is pro-inflammatory [56–60].

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With involvement in varied disease states [14], it is unsurprising that EPAC activity modulators have attracted attention as both tool molecules for further elucidating EPAC signalling roles and as drug development candidates. With differing and often opposing roles played by EPAC1 and 2 as well as other cAMP sensors, any EPAC1 or 2 agonist or antagonist would ideally be specific for a given EPAC isoform. In the case of EPAC antagonists, a recent review [14] has highlighted the use of selective inhibitors in various disease models; including the uncompetitive (CE3F4) and non-competitive EPAC1 (5225554 and 5376753) inhibitors and the EPAC2-selective inhibitor, ESI-05 (4-methylphenyl-2,4,6-trimethylphenylsulphone). For example, CE3F4 has been shown to inhibit autophagy in cardiomyocytes [61] and cardiac arrhythmia [62] and ESI-05 attenuates brain injury and neurological impairment [63,64] and inhibits pancreatic cancer cell migration [65]. ESI-09 (3-[5-(tert-butyl)isoxazol-3-yl]-2-[2-(3-chlorophenyl)hydrazono]-3-oxopropanenitrile) has also been defined as a non-selective inhibitor of EPAC1 and EPAC2 [66]. Herein, we review recent progress in the development of selective EPAC activators.

Cyclic nucleotide EPAC ligands

While cAMP activates both EPAC1 and EPAC2, many isoform-selective eight substituted cAMP analogues have been reported, the thioarylnucleotide 007 and its acetoxymethyl derivative 007-AM as well as the EPAC2-selective thiobenzyl thiophosphates S-220 and S-223 (Figure 1).

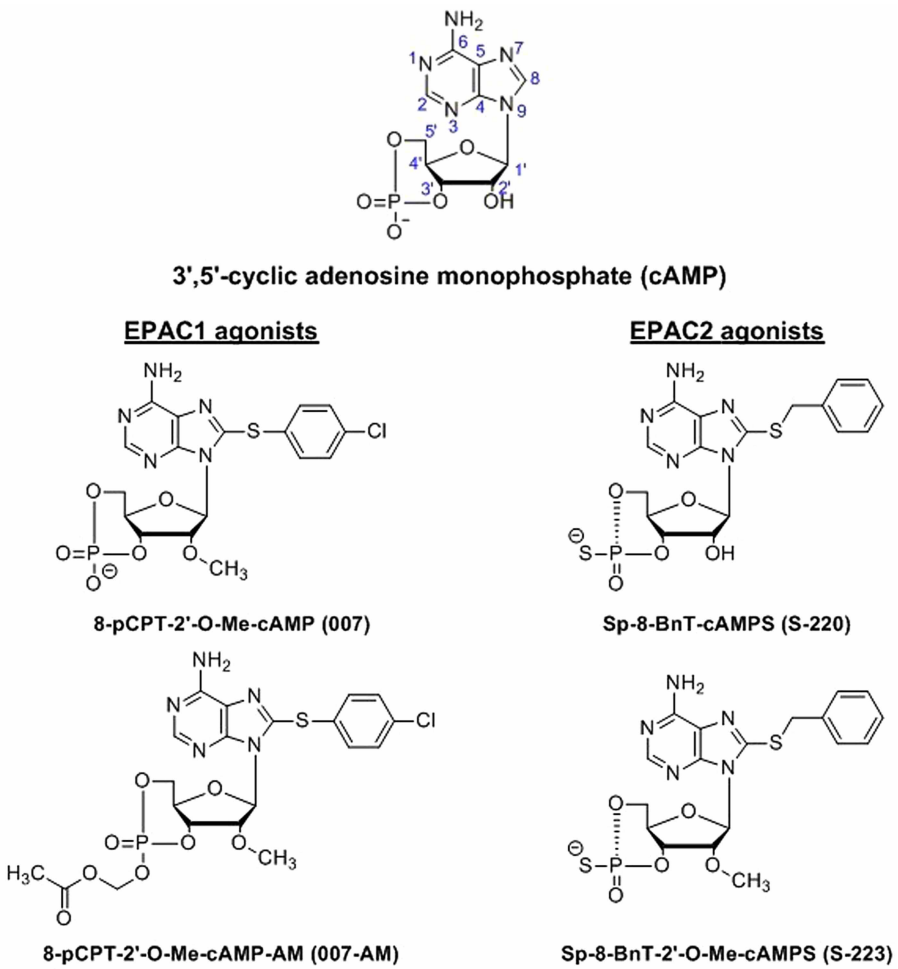

Figure 1. Extant cyclic nucleotide EPAC agonists.

Natural EPAC agonist cAMP compared to the EPAC1-selective chlorothiophenyl analogue 007 and its acetoxymethyl ester 007-AM which displays increased cell permeability. Also shown are the EPAC2-selective thiobenzyl thiophosphates S-220 and S-223.

Table 1 Comparison of activation constants for EPAC1 and EPAC2 obtained by *in vitro* biochemical Rap1 activation assays

	EPAC1		EPAC2	
	AC_{50} [μM]	k_{max}	AC_{50} [μM]	k_{max}
cAMP	45	1.0	1.8	1.0
007	1.8	3.3	3.5	0.8
S-220	13	0.3	0.1	7.7
S-223	30	0.2	1.5	4.7

Half-maximal concentration for activation (AC_{50}) describes the affinity of EPAC isoform for the cyclic nucleotide. Relative maximal activity (k_{max}) is the activity observed under saturating concentrations of the ligand and it is a measure of nucleotide's capability to shift the equilibrium towards the active state of EPAC.

007 and 007-AM

Enserink et al. first developed the EPAC-selective cAMP analogue, 8-(4-chlorophenylthio)-2′-*O*-methyladenosine-3′,5′-cyclic monophosphate (8-pCPT-2′-O-Me-cAMP, 007) as a tool to discriminate the respective roles of EPAC and PKA signalling pathways [67]. The CNBDs of PKA and CNGs have conserved glutamic acid residues that form a hydrogen bond with the 2′-hydroxy group of the cAMP molecule [67,68], while CNBDs of both EPAC1 and EPAC2 have different corresponding amino acids, glutamine (Q270 and lysine (K405), respectively [67,69]. It was discovered that the 2′-hydroxy group of cAMP is not necessary for the binding and activation of EPAC1, but 2′-*O*-alkyl substitutions, such as 2′-O-methyl, 2′-O-ethyl, 2′-O-propyl or 2′-*O*-butyl in cAMP analogues, greatly reduce their affinity for PKA [67,70]. 007 was shown to not only be an EPAC-selective agonist, but also a more potent EPAC1 activator than cAMP (see Table 1) [67,69]. Further study demonstrated that the *p*-chlorophenylthio (pCPT) substituent at the eight positions is responsible for high affinity, which Schwede et al. attributed to hydrophobic interactions in the binding pocket and the chlorophenyl motif shielding the binding pocket against solvent [69]. 2′-*O*-methylation provides not only discrimination against all four PKA isoforms in *in vitro* kinase assays (see Table 2), but also high maximal activity, presumably caused by the 2′-*O*-Me group pushing away Q270, which then interacts with the hinge region and changes its conformation to a more favourable one. While 007 activates both EPAC isoforms in *in vitro* Rap1 activation assays, importantly it activates EPAC1 to a greater degree ($k_{max} = 3.3$ for EPAC1 vs. $k_{max} = 0.8$ for EPAC2) as well as binding EPAC1 more strongly (with $AC_{50} = 1.8$ μM and for EPAC1 compared with $AC_{50} = 3.5$ μM for EPAC2, see: Table 1), due to the single amino acid difference between their cAMP-binding sites, as detailed above [69].

A disadvantage of 007 is its poor membrane permeability due to the presence of the extremely polar-charged phosphate [71–73]. Vliem et al. have developed an acetoxymethyl ester of 007 (007-AM) to overcome this problem via a simple protection protocol with bromomethyl acetate to give the product in ∼50:50

Table 2 Comparison of apparent activation constants (K_{act}) for four PKA isoforms obtained by *in vitro* biochemical kinase assay

	K_{act} [μM] PKA-Iα	PKA-Iβ	PKA-IIα	PKA-IIβ
cAMP	0.085	0.038	0.080	0.19
007	14	18	>70	50
S-220	0.29	0.29	0.27	0.21
S-223	>1000	>1000	>25	>1000

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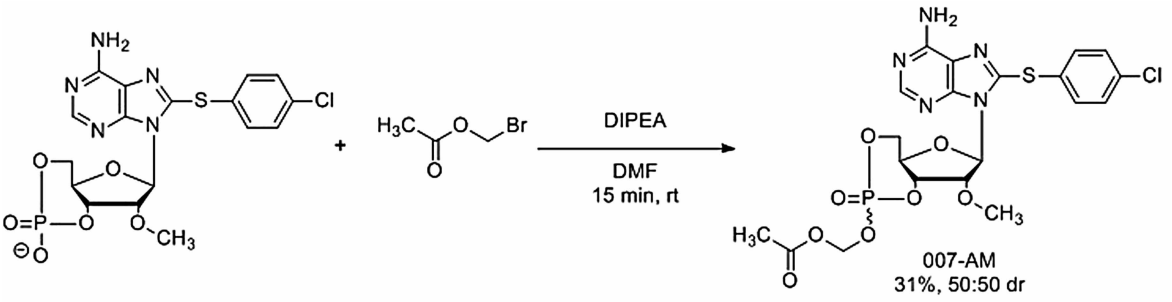
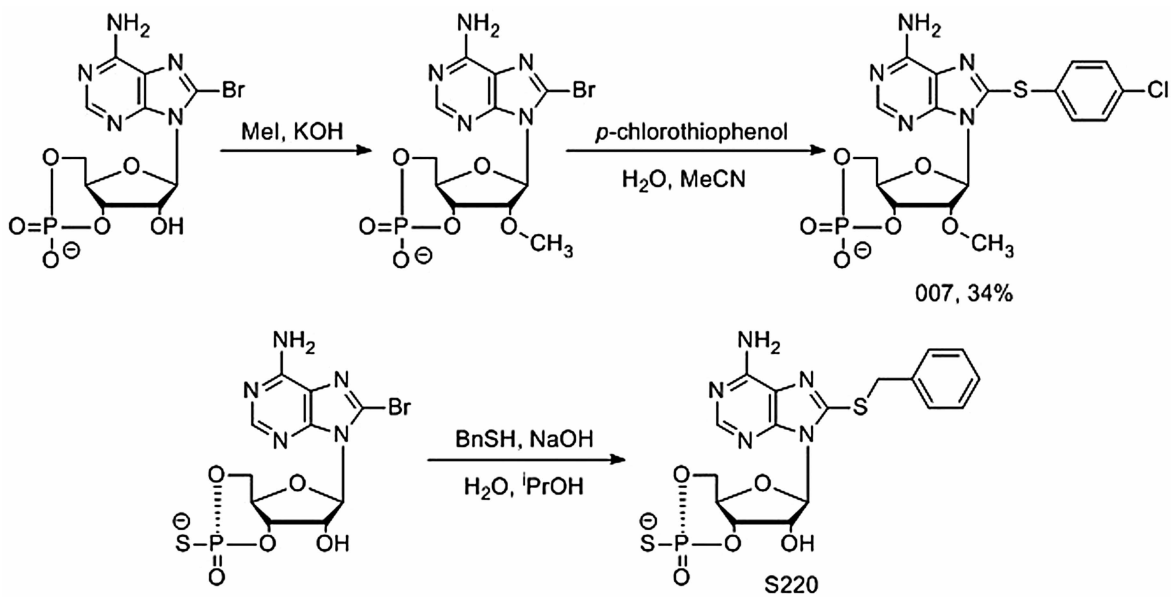


Scheme 1. Synthesis of 007-AM from 007.

007-AM may be prepared from 007 by the reaction of bromomethyl acetate with 007 in the presence of diisopropylethylamine in dimethylformamide to give 007-AM in 31% yield and 50:50 dr after 15 min at room temperature.

diastereomeric ratio (dr) and 31% yield (Scheme 1) [71]. While the diastereomers were separable, the pharmacokinetics of both were shown to be similar, so the mixture was used in bioactivity assays. Masking the phosphate group with a labile ester improves cell membrane penetration and subsequent hydrolysis of the ester by water or cellular esterases releases the active molecule, a strategy first employed by Schultz et al. to improve the permeability of dibutyryl cAMP [73]. Detection of active Rap1 in cell lysates and a FRET-based cellular assay demonstrated that 007-AM activates EPAC1 more readily than 007, and can be used at concentrations two to three orders of magnitude lower to achieve comparable effect [71], indicating that membrane permeability was indeed significantly improved over the parent 007.

007 and its esterified analogue have been widely used for investigating EPAC1 signalling pathways as well as for *in vivo* experiments [54,74–82] where the administration of 007 was reported to decrease renal failure [83] and oxidative stress [84] in ischaemia-reperfusion injury model mice. While both compounds are useful tool molecules, their utility as drug development candidates is lacking; plausible analogue syntheses are largely limited to sugar protections and substitution in the adenosine eight-position (see Scheme 2). Additionally,



Scheme 2. Synthesis of cyclic nucleotide EPAC activators.

007 is prepared from 8-bromo-cAMP *via* methylation with iodomethane followed by nucleophilic displacement of bromine using *p*-chlorothiophenol. In an analogous procedure, S-220 is prepared from 8-bromo-cAMPS *via* nucleophilic attack of benzyl thiol.

EPAC1-selective 007 is also a weak EPAC2 agonist (see Table 1), leading to off-target effects. Hothi et al. reported that activation of EPAC proteins in cardiomyocytes with 007 is associated with disturbed calcium homeostasis and arrhythmia [85]. Further studies on cardiomyocytes isolated from wild-type (WT), EPAC1 knockout (EPAC1-KO) and EPAC2 knockout (EPAC2-KO) mice have shown that the Ca^{2+} leak observed in both WT and EPAC1-KO after treatment with 007 did not occur in EPAC2-KO, pointing to the EPAC2 isoform as a mediator of this effect [86].

S-220 and S-223

The EPAC2-selective agonist 8-benzylthioadenosine-3′,5′-cyclic monophosphorothioate (Sp-8-BnT-cAMPS, S-220) was developed by Schwede et al. [69]. Improved maximal activity and affinity for EPAC2 were achieved by introducing a benzylthio (BnT) substituent at eight positions of adenine vs. the thioaryl present in the EPAC1-selective agonists 007 and 007-AM. Inspection of a crystal structure of S-220 bound to EPAC2 in the active conformation reveals that the BnT group is sandwiched between K450 (part of the lid, unique for EPAC2) and L379 (in the CNBD domain) and utilises hydrophobic interactions to promote the active conformation of CNBD. Replacing the axial oxygen atom of cyclic phosphate with sulfur provides an additional increase in maximal activity, while the crucial interaction with the phosphate-binding cassette (PBC) is retained [69]. Site-directed mutagenesis has shown that the single amino acid difference within cAMP-binding sites of EPAC1 and EPAC2 is responsible for S-220 isoform-selectivity (EPAC2 is activated with $AC_{50} = 0.1$ μM, while EPAC1 with $AC_{50} = 13$ μM, see Table 1). In *in vitro* kinase assays, S-220 was shown to activate all four PKA isoforms, though to a lesser extent than cAMP (see Table 2). In cell-based studies utilising the U2OS cell line stably transfected to overexpress EPAC2, however, only very low PKA activation was observed suggesting that this may not be a problem *in vivo* [69]. 2′-substitutions, which in the case of 007 resulted in highly improved selectivity for EPAC1 over PKA [67,69], considerably reduce the maximal activity and affinity for EPAC2, as in 2′-*O*-methylated S-220 analogue, S-223 ($k_{max} = 4.7$ and $AC_{50} = 1.5$ μM vs. $k_{max} = 7.7$ and $AC_{50} = 0.1$ for S-223 and parent compound, respectively). Although efficiently discriminating against PKA in *in vitro* kinase assays (see Table 2) and demonstrating reduced, but still significant potency for EPAC2 activation, S-223 failed to induce EPAC2 activity in cell-based tests [69]. S-220 was reported to enhance glucose-induced insulin release from isolated primary human islets [69]. It was also used for *in vivo* studies, where mice on high-fat diet treated with S-220 displayed reduced body weight gain [87].

Synthesis of cyclic nucleotide EPAC agonists

Both 007 and S-220 are accessed via bromonucleotide precursors (Scheme 2). For example, 8-bormo-cAMP may be efficiently methylated in the 2′-position using iodomethane, and subsequent treatment with 4-chlorothiophenol gives 007 in 34% yield [69]. Following an analogous procedure, S-220 may be synthesised via the treatment of 8-bromo-cAMPS with benzyl mercaptan (Scheme 2) — synthetic yields were not reported by the authors [69]. While both 8-bromo-cAMP and 8-bromo-cAMPS are commercially available, substitutions in other positions are synthetically challenging, limiting the potential of cyclic nucleotide EPAC ligands as drug development candidates.

Non-nucleotide EPAC ligands

Two classes of non-cyclic nucleotide small-molecule EPAC activators have emerged in recent years; sulphonylureas (SUs) are one such class. These compounds were initially of interest for use as anti-diabetic drugs, with clinically approved examples including Tolbutamide (TLB) and Glibenclamide (GLB) (Figure 2). SUs stimulates the secretion of insulin by closing pancreatic β cell K_{ATP} channels through binding to sulphonylurea receptor 1 (SUR1) [88].

Evidence for SU activity at EPAC1 and 2 has been controversial — Sunaga et al. have previously reported that SUs are selective activators of the EPAC2 isoform using in-cell FRET biosensor imaging to screen for EPAC2 activation, further noting that SU-induced insulin secretion was reduced (though not eliminated) in mice lacking EPAC2 [88]. Using the same technique, Zhang et al. later confirmed direct binding of SUs to EPAC2, and that this binding was at an allosteric site [89]. Further investigation of an L408W point mutant (known to bind cAMP but with reduced Rap1 activation activity in the L273W EPAC1 homologue) [44,90] suggested that EPAC2 activation by SUs occurred via the same molecular mechanism as cAMP activation [89].

Recent studies by Tsalkova et al. dispute these findings, however, showing that SUs were unable to bind to or activate EPAC2 *in vitro* [91]. In a fluorescence-based competition assay where Rap—1-bound fluorescent

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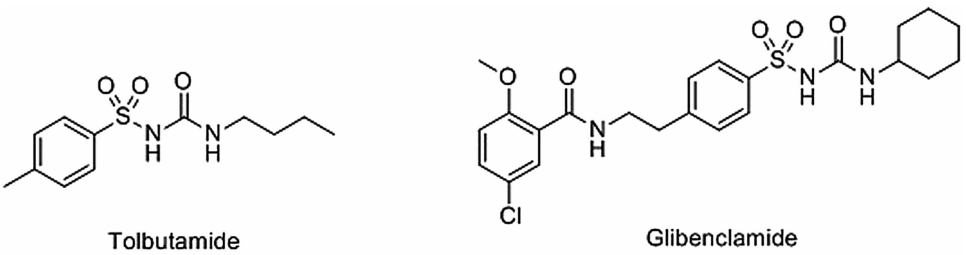


Figure 2. Two sulphonylurea drugs.

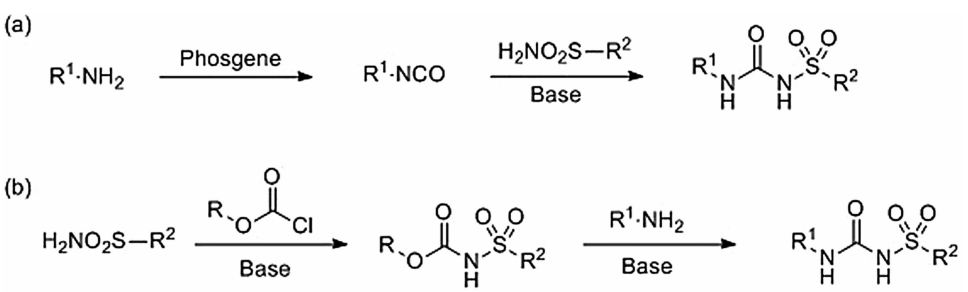
Approved sulphonylurea drugs include the anti-diabetics tolbutamide and gilbenclamide.

Mant-GDP is exchanged with GDP in the presence of activated EPAC2 leading to a decrease in fluorescence, it was observed that GLB failed to activate EPAC2 in concentrations from 0.001 to 100 μM, whilst 300 μM led to robust Mant-GDP dissociation. The authors proposed that the supposed activation of EPAC2 may be due to the previously observed increase in cellular cAMP levels in the presence of Sus [92,93]. This had, however, been ruled out by Sunaga et al. in their original report and the mechanism of EPAC2 activation by SUs remains ambiguous. However, SUs have found a role as probe molecules for studying EPAC2-mediated cellular processes, for example, insulin exocytosis as reported by Barg et al. [94].

The ease of synthesis and existence of multiple synthetic route to SUs is an attractive quality for the design of analogues [95]. The precursor amines, sulphonamides, and chloroformate esters are either inexpensive or easy to prepare. Commonly, SUs may be accessed by the treatment of a starting amine with phosgene followed by nucleophilic attack by a primary sulphonamide onto the resultant isocyanate (Scheme 3, route (a)) [96,97]. Alternatively, toxic phosgene may be avoided by the reaction of a primary sulphonamide with a chloroformate or anhydride to form the corresponding carbamate, followed by treatment with an amine to yield the sulphonylurea (Scheme 3, route (b)) [97,98].

N-acylsulphonamide EPAC agonists

Recently, Yarwood and co-workers reported the results of a screen of 5195 small molecules for binding at the EPAC1 CNBD, based on the competition for binding with the fluorescent cAMP analogue 8-NBD-cAMP [99]. The lead hit from the assay, I942, was found to have an IC_{50} of 35 μM compared with an IC_{50} of 4 μM for cAMP under the same conditions (Figure 3, alongside two other hit compounds from the same screen). Subsequent ligand-observed NMR studies confirmed the direct interaction of I942 with the CNBDs of both EPAC1 and EPAC2. The group then investigated EPAC activation by I942, based on activated EPAC-stimulated dissociation of fluorescent Mant-GDP from Rap1 in the presence of EPAC1/EPAC2 and I942, and observed partial agonist activity toward EPAC1, with very little concomitant activity at EPAC2. Notably, with I942 and cAMP binding the EPAC1 CNBD with roughly equal efficiency, the maximum activity induced by I942 is ∼10% that of cAMP. Rounding off the study, it was also shown that I942 had no effect *in vitro* on PKA activity,



Scheme 3. Sulphonyl urea synthesis.

Sulphonyl ureas are commonly synthesised *via* one of the two routes: (**a**) from the primary amines by the phosgene-mediated formation of an isocyanate followed by nucleophilic attack of a primary sulphonamide or (**b**) reaction of a primary sulphonamide with a chloroformate then nucleophilic substitution using a primary amine

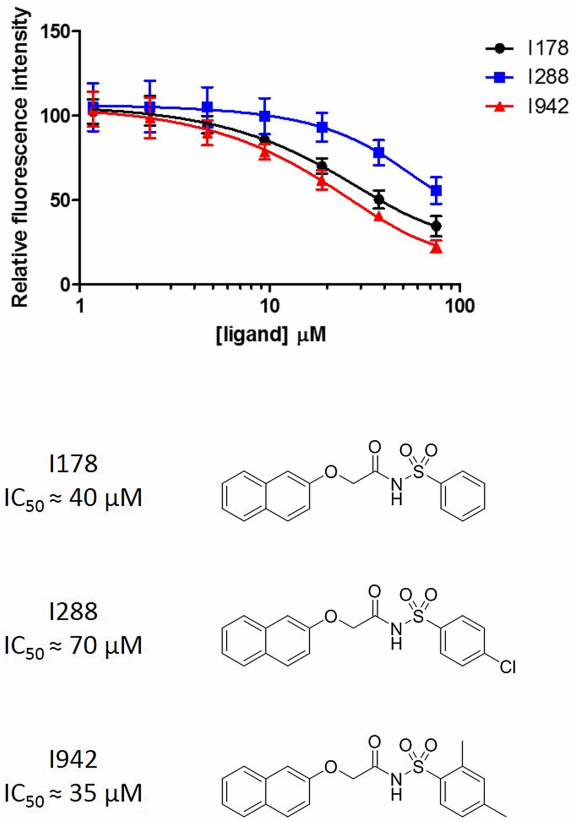


Figure 3. Dose–response curves for HTS hits against EPAC1-CNBD.

The three hit compounds I178, I288, and I942 were tested in a seven-point dose–response curve in the EPAC1-CNBD, 8-NBD-cAMP-binding assay.

as measured by phosphorylation of a PKA substrate peptide [99]. Importantly, recent work from the Yarwood laboratory has demonstrated that I942 can activate Rap1 in cells overexpressing EPAC1, but not EPAC2 (manuscript in preparation). This precludes any non-specific action of I942 through inhibition of endogenous PDEs or activation of ACs.

The same group subsequently investigated the in-cell activity of I942 and were able to demonstrate EPAC1 and Rap1 activation in HEK293 T cells as well as SOCS3 induction and suppression of IL6-stimulated JAK/STAT3 signalling in HuVECs [54]. SOCS3 induction was blocked by the EPAC1 antagonists ESI-09 and EPAC1 siRNA, but not the PKA inhibitor H89, demonstrating that SOCS3 induction by I942 does indeed proceed via EPAC1. RNA sequencing identified 425 genes regulated by I942 in HuVECs, the same regulated by the EPAC1-selective cyclic nucleotide agonist 007 as well as forskolin (a common cAMP-elevating tool molecule) [100] and rolipram (a cAMP-elevating PDE-4 inhibitor) [101]. Finally, I942 was shown to block the expression of the cell adhesion molecule VCAM1, known to play a role in the development of cardiovascular inflammation [102]. While the use of I942 as a probe molecule is in its infancy, a recent study has reported the use of I942 as an EPAC1-specific activator renders VECs more susceptible to infection by Ebola virus [103].

The mode of I942 binding at EPAC1 has yet to be fully elucidated, though we have advanced putative-binding models based on computational studies (Figure 4). In the absence of an EPAC1 structure, a homology model was constructed from an EPAC2 K405Q point mutant in the nucleotide-bound active conformation [55,69]. The findings suggest that the acidic *N*-acylsulphonamide moiety occupies the same volume as the cAMP phosphate, exploiting a key ionic interaction with R279 within the CNBD as well an engaging with charge-stabilised hydrogen bonds to A280 and A281; these residues are preserved in EPAC1. The model also suggests that the *m*-xylyl group of I942 occupies a similar space to that of the purine bicyclic ring of cAMP. However, I942 is unable to exploit the polar interactions available to cAMP through the adenine N1 and K353

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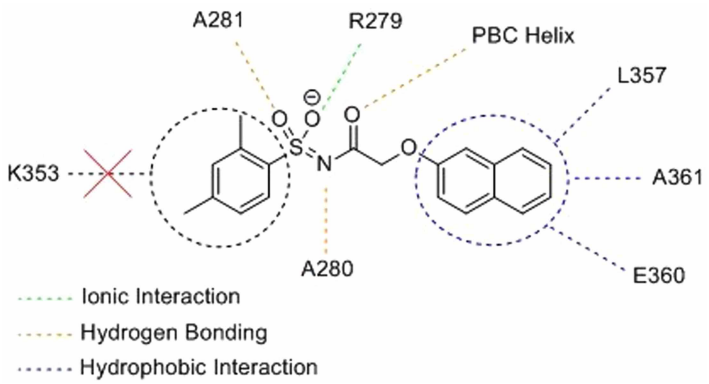


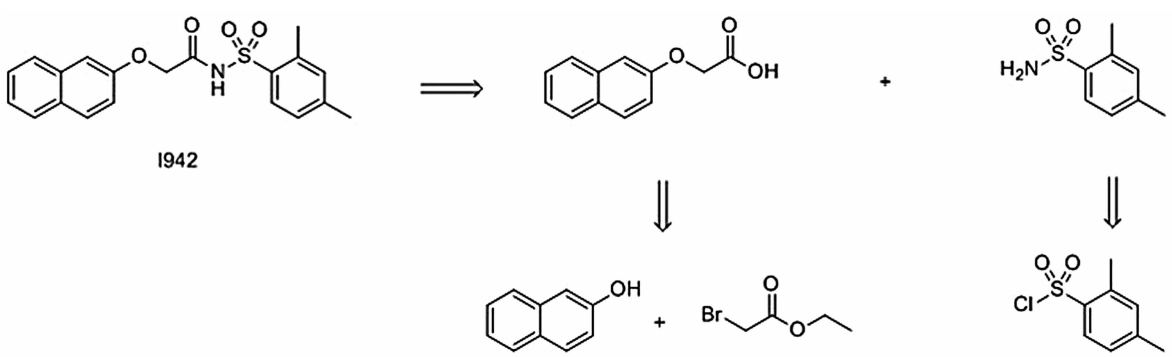
Figure 4. Proposed I942-binding interactions.

Specific ligand-residue interactions from a computed docking model for I942 at an EPAC1 homology model cAMP-binding site. Note that I942 does not exploit interaction with K353, a key cAMP-EPAC1-binding moiety.

on the REM domain α1 helix; this is proposed to be the key interaction, which stabilises the EPAC1 activation conformational reorganisation [42].

As an alternative EPAC1 activation model, we have proposed that, I942 may have access to some hydrophobic interactions through the naphthoxy group, which are not available to native cAMP. The model postulates that the oxymethylene motif threads a small passage, which leads to a hydrophobic channel that is occupied by the naphthoxy group of I942. Three residues L357, A361, and E360 on the REM α1 helix are theorised to stabilise the active state of EPAC1, and account for the agonistic properties of I942. The isoform-selectivity of I942 has been suggested to be due to the replacement of L357 and A361 by histidine and threonine, respectively, in EPAC2. The loss of L357 results in reduced surface contact with the naphthoxy group, whilst threonine induces steric hindrance. Furthermore, it was noted that packing of the naphthyl group against L357, A361, and E360 may stabilise the EPAC1 active state less effectively that cAMP, perhaps due to slightly altered seating of the CNBD against the core. Comparison of the three extant EPAC2 structures reveals a degree of plasticity in the positioning of the REM α1 helix and our docking models suggest a sterically crowded volume at the naphthyl–helix interface, accounting for the partial agonism of I942 due to this altered seating [55].

N-acylsulphonamides are common in modern medicinal chemistry; their synthesis and therapeutic potential have been recently reviewed [95]. In addition to phosphate mimetics, they have found use as carboxylate bioisosteres [104,105], with a pK_a in the range 3.5–4.5, similar to carboxylic acids [95]. An attractive feature of *N*-acylsulphonamides to the medicinal chemist is their ease of synthesis, with multiple synthetic routes starting from the readily obtained substrates [95]. For example, I942 may be disconnected (Scheme 4) back to a



Scheme 4. Retrosynthetic approach to I942.

I942 may be disconnected *via* an amide formation to a primary sulphonamide (itself readily obtained from the corresponding sulfonyl chloride) and a naphthoxy acid formed from 2-naphthol and ethyl bromoacetate.

sulphonyl chloride, of which a SciFinder search reveals 16 532 distinct commercially available examples and phenol (13 674commercially available examples). This ease of synthesis and plurality of substrates for analogue synthesis furnishes *N*-acylsulphonamides and SUs with a significant advantage over cyclic nucleotide EPAC ligands as drug development hit compounds.

Perspectives

- Exchange proteins directly activated by cAMP (EPACs) have been shown to play an important role in the development of many diseases. The naturally occurring EPAC activator, cAMP, indiscriminately activates EPAC1 and EPAC2 as well as other cAMP sensors such as PKA and POPDCs. With different and often opposing biological activity originating from these species, drugs or probe molecules targeting EPAC1 or 2 would ideally do so selectively.

- Extant small-molecule EPAC activators include cAMP-mimetic cyclic nucleotides such as 007 and its more cell-permeable acetoxymethyl ester 007-AM (EPAC1 selective) as well as S-220 and S-223 (EPAC2 selective). While these examples have found diverse uses as probe molecules, issues remain around debatable selectivity (cyclic nucleotides) and cell permeability (007). Moreover, EPAC-specific cAMP analogues, as well as their cellular metabolites, have been reported to exert off-target effects [106–110]. Other examples include sulphonylurea EPAC2 activators and *N*-acylsulphonamide EPAC1 activators. However, SUs have an ambiguous mode of action and may not target EPAC2 in cells. For example, SUs do not increase insulin secretion in islets from SUR1 knockout mice, indicating that they may not be working through EPAC2 directly [111] and may involve and alternative pathway through interactions with β-arrestin [112].

- Recently identified *N*-acylsulphonamides provide a selective EPAC1 activator with a confirmed mode of activity *in vitro* and have demonstrated activity in cell and tissue cultures. With greater scope for analogue synthesis than cyclic nucleotides, we anticipate that future research in EPAC1 activators will focus on this compound series. The partial agonism displayed by I942 remains problematic in terms of therapeutic use due to competition with endogenous cyclic AMP as previously discussed [99]; however, it should be noted that I942 exerts agonist properties *in cellulae* [54], even in the presence of maximal cyclic AMP levels, as stimulated by a combination of forskolin and rolipram. Moreover, it can be anticipated that future I942 analogues may be designed to exploit additional binding/activation inducing interactions (e.g. through K353 via carboxylates of heterocylces) or to pose less of a steric challenge at the CNBD-REM α1 helix interface. Increasing potency of this series to full agonism will address much of the concern surrounding this compound series in the context of drug development.

Abbreviations

AC, adenylyl cyclase; BnT, benzylthio; cAMP, cyclic adenosine monophosphate; CNBD, cyclic nucleotide-binding domain; CNGs, cyclic nucleotide-gated ion channels; COPD, chronic obstructive pulmonary disorder; dr, diastereomeric ratio; EPAC, exchange protein directly activated by cAMP; FRET, fluorescence resonance energy transfer; GDP, guanosine diphosphate; GLB, glibenamide; HTS, high-throughput screen; HuVECs, human vascular endothelial cells; IL-6, interleukin 6; KO, knockout; NMR, nuclear magnetic resonance; PBC, phosphate-binding cassette; *p*-CPT, *para*-chlorophenylthio; PDEs, phosphodiesterases; PKA, protein kinase A; POPDC, popeye domain-containing protein; REM, Ras exchange motif; SOCS3, suppressor of cytokine signalling 3; STAT3, signal transducer and activator of transcription 3; SU, sulphonylurea; SUR1, sulfonylurea receptor 1; TLB, tolbutamide; VCAM, vascular cell adhesion molecule; VEC, vascular endothelial cell; WT, wild type.

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Competing Interests

The Authors declare that there are no competing interests associated with the manuscript.

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