Soluble Guanylate Cyclase Stimulators and Activators

Peter Sandner, Daniel P. Zimmer, G. Todd Milne, Markus Follmann, Adrian Hobbs, and Johannes-Peter Stasch

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P. Sandner (✉)
Bayer AG, Pharmaceuticals R&D, Pharma Research Center, Wuppertal, Germany

Department of Pharmacology, Hannover Medical School, Hannover, Germany
e-mail: peter.sandner@bayer.com

D. P. Zimmer · G. T. Milne
Ironwood Pharmaceuticals, Cambridge, MA, USA

M. Follmann
Bayer AG, Pharmaceuticals R&D, Pharma Research Center, Wuppertal, Germany

A. Hobbs
Barts and the London School of Medicine and Dentistry QMUL, London, UK

J.-P. Stasch
Bayer AG, Pharmaceuticals R&D, Pharma Research Center, Wuppertal, Germany

Institute of Pharmacy, University Halle-Wittenberg, Halle, Germany

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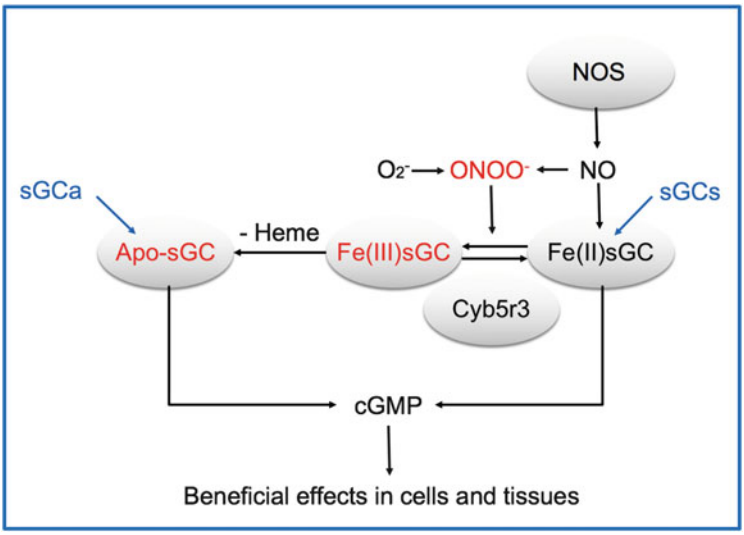
Abstract

When Furchgott, Murad, and Ignarro were honored with the Nobel prize for the identification of nitric oxide (NO) in 1998, the therapeutic implications of this discovery could not be fully anticipated. This was due to the fact that available therapeutics like NO donors did not allow a constant and long-lasting cyclic guanylyl monophosphate (cGMP) stimulation and had a narrow therapeutic window. Now, 20 years later, the stimulator of soluble guanylate cyclase (sGC), riociguat, is on the market and is the only drug approved for the treatment of two forms of pulmonary hypertension (PAH/CTEPH), and a variety of other sGC stimulators and sGC activators are in preclinical and clinical development for additional indications. The discovery of sGC stimulators and sGC activators is a milestone in the field of NO/sGC/cGMP pharmacology. The sGC stimulators and sGC activators bind directly to reduced, heme-containing and oxidized, heme-free sGC, respectively, which results in an increase in cGMP production. The action of sGC stimulators at the heme-containing enzyme is independent of NO but is enhanced in the presence of NO whereas the sGC activators interact with the heme-free form of sGC. These highly innovative pharmacological principles of sGC stimulation and activation seem to have a very broad therapeutic potential. Therefore, in both academia and industry, intensive research and development efforts have been undertaken to fully exploit the therapeutic benefit of these new compound classes. Here we summarize the discovery of sGC stimulators and sGC activators and the current developments in both compound classes, including the mode of action, the chemical structures, and the genesis of the terminology and nomenclature. In addition, preclinical studies exploring multiple aspects of their in vitro, ex vivo, and in vivo pharmacology are reviewed, providing an overview of multiple potential applications. Finally, the clinical developments, investigating the treatment potential of these compounds in various diseases like heart failure, diabetic kidney disease, fibrotic diseases, and hypertension, are reported. In summary, sGC stimulators and sGC activators have a unique mode of action with a broad treatment potential in cardiovascular diseases and beyond.

Graphical Abstract



Keywords

cGMP · Cyclic guanosine monophosphate · Nitric oxide · sGC · sGC activator · sGC stimulator · Soluble guanylyl cyclase

1 Heme-Containing and Heme-Free sGC: Structure, Function, and Regulation

The second messenger cyclic guanosine monophosphate (cGMP) is generated by the heterodimeric α/β-heme protein soluble guanylate cyclase (sGC) upon activation by its endogenous ligand nitric oxide (NO) (Derbyshire and Marletta 2012). The β sGC subunit carries the N-heme-nitric oxide binding domain (H-NOX). Since the H-NOX domain binds NO, this enzyme is also known as the NO-sensitive guanylyl cyclase. NO-dependent sGC stimulation triggers formation of cGMP and promotes vasodilation and inhibits smooth muscle proliferation, leukocyte recruitment, platelet aggregation, and vascular remodeling through a number of downstream targets such as protein kinases, cyclic nucleotide-gated channels, and phosphodiesterases, making the NO/sGC/cGMP signaling a central vasoprotective signaling pathway (Lucas et al. 2000; Feil et al. 2003; Lundberg et al. 2015).

sGC is a key signal-transduction enzyme in the cardiovascular system, and many cardiovascular diseases, such as hypertension, pulmonary hypertension, heart failure, chronic kidney disease, and erectile dysfunction, are associated with

dysfunction of the NO/sGC/cGMP-signaling pathway (Kemp-Harper and Feil 2008; Schulz et al. 2008; Stasch et al. 2011; Klinger and Kadowitz 2017). NO/sGC/cGMP signaling can be impaired in a variety of ways: increased ROS production by NADPH-oxidases and uncoupled NO-synthases, scavenging of NO via the reaction of NO and O^{2-} to form peroxynitrite, and oxidation of sGC to its NO-insensitive Fe^{3+} state and subsequent loss of the NO binding site on the prosthetic heme group (Stasch and Hobbs 2009; Stasch et al. 2011; Pan et al. 2016). Oxidative stress ultimately results in a reduced bioavailability of NO. The heme-free form of sGC is unresponsive to NO and prone to ubiquitin-mediated degradation (Stasch et al. 2006; Meurer et al. 2009; Hoffmann et al. 2009). In addition, sGC transcription and the stability of sGC mRNA are also affected by oxidative stress (Sharina and Martin 2017). Oxidative stress is associated with several cardiovascular diseases and is characterized by increased formation of reactive oxygen species (ROS) (Ritchie et al. 2017).

There is growing evidence supporting the relationship between genetic variants in the NO/sGC/cGMP pathway, and the prevalence and progression of cardiovascular, pulmonary, and renal diseases (Leineweber et al. 2017). Importantly, genetic alterations of the GUCY1A3 gene, which encodes the α1 subunit of the sGC, are associated with coronary artery disease as well as Moyamoya disease, achalasia, and hypertension (Erdmann et al. 2013; Kessler et al. 2017; Wallace et al. 2016). Moreover, associations with other mechanisms of sGC regulation have been described, such as membrane association and binding to the chaperone CCTη or heat shock protein 90 (HSP90) (Erdmann et al. 2013; Ghosh and Stuehr 2017).

Two distinct compound classes capable of activating sGC in an NO-independent manner were discovered at Bayer, the so-called sGC stimulators and sGC activators (Stasch and Hobbs 2009; Schmidt et al. 2009). Both classes of compounds directly bind to sGC and are allosteric modulators of guanylyl cyclase activity (Fig. 1).

sGC stimulators have a dual mode of action: they directly stimulate the native form of the enzyme independently of NO and they are also able to sensitize sGC to low levels of NO by stabilizing NO–sGC binding (Stasch et al. 2001; Stacy et al. 2018). The binding site of sGC stimulators has been a long-standing question that was recently addressed with a set of experiments incorporating photo-affinity crosslinking with LC-MS/MS and NMR approaches. Results from these experiments indicate that sGC stimulators likely bind near a previously identified tunnel of possible importance for NO escape from the heme pocket within the H-NOX domain of the β1 subunit. A potential mechanism of action of sGC stimulators involves the occlusion of a tunnel release by stimulator binding, thus leading to an observed higher affinity of NO to the ferrous heme-moiety (Wales et al. 2018; Winter et al. 2011). Maintaining sGC heme in the ferrous state is essential for sGC/cGMP signaling via NO and sGC stimulators. The ferrous heme group is non-covalently bound to the ß1 subunit of sGC via the proximal heme ligand H105 and the heme-binding motif Y-x-S-x-R, provided by ßTyr135, ßSer137, and ßArg139 (Schmidt et al. 2004).

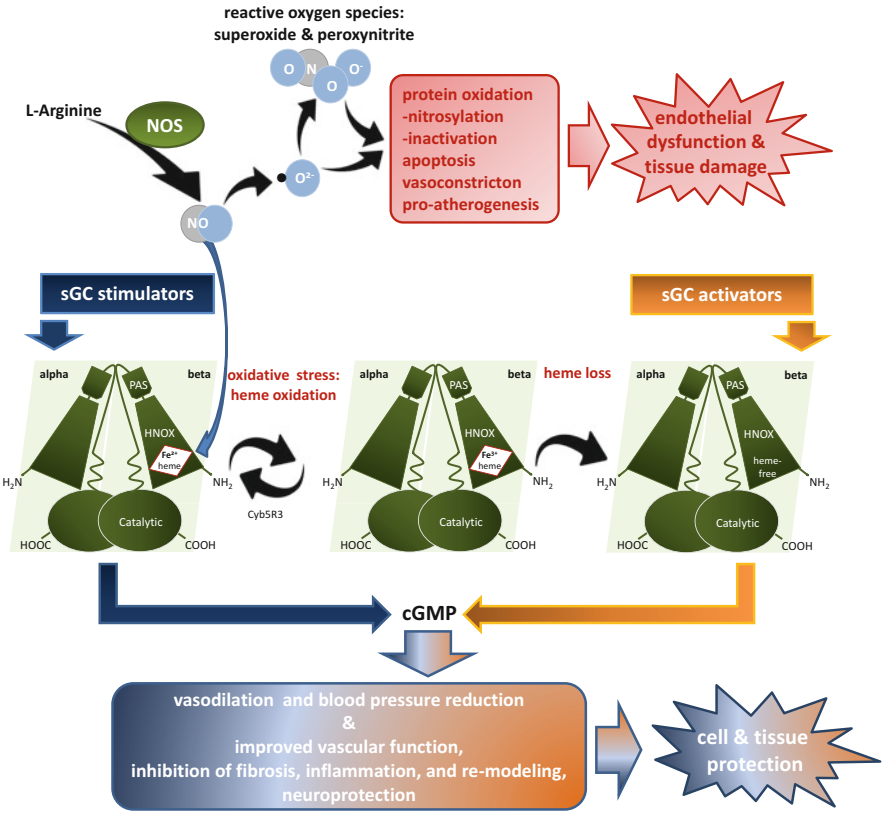


Fig. 1 Schematic representation of the sGC structure and the importance of heme-containing, native sGC and heme-free, dysfunctional form of sGC and its redox equilibrium. Oxidative stress is a risk factor for several cardiovascular diseases and is associated with increased formation of superoxide radicals, which react with NO to form the strong oxidant, peroxynitrite which is known to oxidize and inactivate many biomolecules, culminating in tissue damage. In particular, peroxynitrite oxidizes sGC, resulting in oxidized sGC and loss of the heme group which makes the enzyme unresponsive to NO. Balancing these effects, Cyb5R3, a heme iron reductase ubiquitously expressed in vascular smooth muscle cells, reduces the sGC heme iron and thereby resensitizes sGC to NO. The sGC stimulators and sGC activators are targeting the NO/sGC/cGMP pathway by stimulating the heme-containing sGC and the heme-free sGC, respectively, which is triggering the formation of cGMP which mediates the beneficial effects in cells and tissues

In contrast to sGC stimulators, sGC activators bind to the unoccupied heme-binding domain, thereby mimicking NO-bound heme, and activate the pathologically changed, heme-free, NO-unresponsive form of sGC (Stasch et al. 2015). Importantly, in isolated cells, in ex vivo blood vessels, and in vivo, sGC activators

such as cinaciguat had greater pharmacological activity under pathophysiological and oxidative stress conditions compared to sGC stimulators (Stasch et al. 2001; Thoonen et al. 2015). This therapeutic principle may preferentially dilate diseased versus normal blood vessels and therefore have far-reaching implications for the clinical use of sGC activators as unique diagnostic tools and innovative vascular therapy (Armitage et al. 2009; Stasch et al. 2006; Gladwin 2006).

Recently, heme-deficient sGC mice have been generated by a gene replacement approach; the codon for His105 is replaced by the codon for Phe105 (Thoonen et al. 2015). These mice represent a unique experimental platform to distinguish between heme-dependent and heme-independent effects of sGC as well as sGC stimulators and sGC activators. Furthermore, the in vivo relevance of heme-free, dysfunctional sGC could be investigated for the first time. The phenotype of these heme-deficient sGC mice is affected. Blood pressure was higher in these mice than in wild type (WT) mice. The heme-deficient sGC mice also showed gastrointestinal (GI) tract abnormalities, growth retardation, and a reduced life span. Importantly, the ability of aortic rings to relax in response to NO was completely abolished in aortas taken from heme-deficient sGC mice. In contrast, the sGC activator cinaciguat relaxed precontracted aortas from heme-deficient sGC mice at a lower concentration than required to relax those from WT mice. Consistent with the in vitro findings, in vivo NO effects were also abolished in heme-deficient sGC mice, and cinaciguat decreased blood pressure to a greater extent in heme-deficient sGC mice than in WT mice. This indicates the presence of a heme-free or dysfunctional sGC pool in vivo, and shows that it can be reactivated by sGC activators to overcome the pathophysiology of a disrupted NO/sGC/cGMP signaling pathway. Diseases associated with NO resistance would appear to be ideally suited for therapies directed at restoring redox homeostasis, sGC activity, and NO sensitivity.

There is a growing appreciation for the role of redox state in modulating NO/sGC/cGMP signaling. Data from research using stimulator and activator families of sGC agonists have provided support for the thesis that sGC bioactivity is redox regulated. Both agonism of native, heme-bound sGC by stimulators and of heme-free sGC by activators leads to increased formation of cGMP, which exerts multifaceted cellular and tissue effects (Stasch et al. 2011, 2015). However, oxidative stress shifts intracellular levels of native sGC toward the oxidized, dysfunctional, heme-free form that is unresponsive to both endogenous and exogenous NO (Evgenov et al. 2006; Munzel et al. 2007). This concept of NO resistance provides the rationale for sGC activators that bind to the unoccupied sGC heme binding site, thereby favoring the active enzyme state. In addition, assuming a sensitive balance between heme-free, oxidized, and heme-containing sGC in cells and tissues, it is proposed that sGC activators by virtue of very low Kd values are capable of shifting this equilibrium towards the heme-free sGC (Stasch et al. 2002; Schmidt et al. 2003; Kollau et al. 2018). While sGC undergoes proteasomal degradation once its heme is oxidized,

this process is prevented when agents such as sGC activators bind the sGC heme binding site (Meurer et al. 2009; Hoffmann et al. 2009).

In cardiovascular disease, the protective NO/sGC/cGMP signaling pathway is impaired due to a decreased pool of NO-sensitive heme-containing sGC accompanied by a concomitant increase in NO-insensitive heme-free sGC. However, no direct method exists to detect cellular heme-free sGC other than its activation by sGC activators (Stasch et al. 2006; Gladwin 2006). Fluorescence dequenching, based on the interaction of the optical active prosthetic heme group and the attached biarsenical fluorophor FlAsH, was used to detect changes in cellular sGC heme status (Hoffmann et al. 2011). Loss of the prosthetic group by oxidative stress was corroborated by an observed decrease in NO-induced sGC activity, reduced sGC protein levels, and an increased effect of sGC activators. The applicability of this approach based on the cellular expression of an engineered sGC variant is limited to recombinant expression systems. Nevertheless, it allows monitoring of sGC's redox regulation in living cells and future enhancements might be able to extend this approach to in vivo conditions.

While the oxidation of heme sGC under pathophysiological conditions and its association with enhanced sGC activation by sGC activators under these conditions are well documented, most of the hypothesized relationships between the function of ferrochelatase in heme biosynthesis and sGC regulation remain to be investigated (Patel et al. 2017). Mitochondrial heme biosynthesis is an important factor in controlling the expression and function of sGC and systems influencing superoxide generation and actions. Modulation of heme biosynthesis by ferrochelatase inhibition with *N*-methyl protoporphyrin IX promoted sGC depletion, superoxide elevation, and attenuation of relaxation to NO donors (Alruwaili et al. 2017). These studies suggest that disruption of heme biosynthesis resulting in a loss of cGMP production may serve as a contributing mechanism to the progression of cardiovascular disease.

Recently, a further important step in the enzymatic process that modulates sGC redox state and cGMP signaling has been discovered (Rahaman et al. 2017). Nicotinamide adenine dinucleotide (NADH) cytochrome b5 reductase 3 (Cyb5R3), a heme iron reductase ubiquitously expressed in vascular smooth muscle cells, sensitizes sGC to NO by reducing the sGC heme iron and thereby controls cGMP production and blood vessel dilation (Fig. 1). Consequently, Cyb5R3 expression and activity may also influence responses to therapeutics that activate and stimulate sGC (Rahaman et al. 2017).

2 NO Donors and Phosphodiesterase 5 (PDE5) Inhibitors as cGMP Increasing Drugs

Given the substantial disease relevance of impaired NO/sGC/cGMP signaling, it is no surprise that modulators that target the NO/sGC/cGMP signaling cascade other than sGC stimulators and activators have been successfully employed as pharmacological

interventions. Drugs acting on this pathway are useful for treating a variety of diseases. Although having distinct limitations, drugs have been successfully developed that act at the top of the NO/sGC/cGMP pathway to increase NO bioavailability (nitrates and NO donors) and that prolong signaling by stabilizing cGMP (PDE5 inhibitors).

2.1 NO Donors

In the nineteenth century, a long time before the discovery of NO and cGMP signaling, amylnitrate and nitroglycerine were known to be beneficial for the treatment of patients with angina pectoris (Brunton 1867; Murrell 1879). In fact, Alfred Nobel, who suffered from angina pectoris, was treated with nitroglycerine (glyceryltrinitrate GTN). Within the last almost 150 years of using NO donors, medicinal chemists synthesized a variety of NO-liberating drugs and organic nitrates that have been approved for angina pectoris: isosorbide mono and dinitrate (ISDN, ISMN), sodium nitroprusside (SNP), but also molsidomin in order to increase half-life. These NO donors liberate NO enzymatically or nonenzymatically and potently relax coronary blood vessels. Despite these intensive efforts and broad application, the main disadvantages of NO donors were only partly resolved. Nitrates still have a small therapeutic range and lead to tachyphylaxis. In addition, released NO reacts with ROS such as superoxide anions to produce peroxynitrite, which can cause tissue damage. Thus, stable and NO-independent stimulation of cGMP production could have major therapeutic advantages over NO donors.

2.2 PDE5 Inhibitors

Levels of cGMP can be increased by the use of phosphodiesterase type 5 inhibitors (PDE5i), which inhibit degradation of cGMP and were introduced into medical therapy for the treatment of erectile dysfunction (ED). The first compound approved for ED treatment was sildenafil (Viagra™) in 1998 followed by vardenafil (Levitra™) and tadalafil (Cialis™) in 2003. In 2007 and 2009, sildenafil and tadalafil were also approved for the treatment of pulmonary arterial hypertension (PAH) as Revatio™ and Adcirca™, respectively, followed by an additional approval of tadalafil for the treatment of symptomatic benign prostatic hyperplasia (BPH) in 2011. These different applications show the broad treatment potential of cGMP-enhancing drugs. Despite these advances, a substantial number of ED patients (estimated at 30–50% of all patients) do not sufficiently respond to PDE5i (Shabsigh 2004; Bruzziches et al. 2013). In addition, some pulmonary hypertension patients do not adequately respond to PDE5i therapy (Oudiz et al. 2011; Shapiro et al. 2012; Hoeper et al. 2017a, b). This nonresponse to PDE5i therapy could be mechanistically explained by the mode of action of PDE5i, which inhibit only cGMP degradation. Importantly, there are multiple phosphodiesterases that degrade cGMP and are differentially expressed with in cells and highly compartmentalized (Fischmeister et al. 2006). As a consequence, the pharmacology of PDE5i is limited to tissues that

express PDE5 and where PDE5 represents the primary mechanism of cGMP metabolism. Upon blockade of PDE5, other cGMP-metabolizing PDEs may compensate (Stasch et al. 2011). In addition, the efficacy of PDE5i may be substantially limited under conditions of very low endogenous NO production, resulting in low intracellular cGMP production. Decoupling of the NOS/NO/cGMP signaling cascade and low NO/cGMP production has been shown in ED patients (Bivalacqua et al. 2003) and also in patients with PAH and heart failure where endothelial dysfunction leads to impaired NO synthesis (Breitenstein et al. 2017). The low endogenous NO production could be due to aging (Garbán et al. 1995), but also metabolic syndrome, dyslipidemia (Mulhall et al. 2006), and obesity with and without hypogonadism (Gurbuz et al. 2008). Diabetes has also been associated with impaired NO production (Cartledge et al. 2001; Musicki and Burnett 2007).

The therapeutic success of NO-donors and PDE5 inhibitors validates the key pharmacological role of the NO/sGC/cGMP pathway and the broad therapeutic utility of targeting this pathway. However, distinct limitations with regard to tolerance, tissue expression, and robustness of pharmacological response underscore the opportunity for agents such as sGC stimulators and sGC activators that specifically target this pathway.

3 Nomenclature of sGC Stimulators and sGC Activators and INN Names

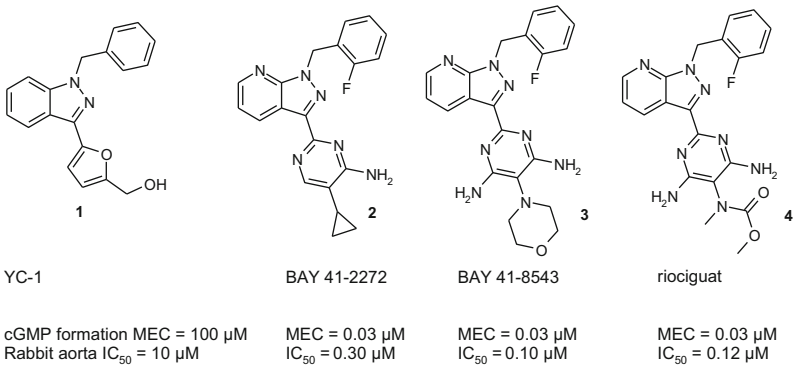
The evolution of terminology to define sGC stimulators and activators has followed closely the elucidation of, and distinction between, the mechanisms of action of these two series of sGC agonists. The name "sGC stimulator" was first coined by Bayer scientists who used a high-throughput approach to build upon earlier successes in the development of small molecules that directly triggered enzyme activity in a NO-independent fashion, yet also synergized with NO (e.g., YC-1, CFM-1571) (Ko et al. 1994; Selwood et al. 2001). Whereas compounds that did not promote enzyme turnover in synergy with NO, but rather triggered cGMP synthesis in oxidized or heme-deficient protein, were called "sGC activators" (e.g., BAY 58-2667, HMR1766). Indeed, the term "sGC activator" was arguably first described by Abbott researchers who reported a series of ortho-substituted sulfanyl-cinnamic acid (aminoalkyl) amides, highlighted by A-350619 (Miller et al. 2003), that were structurally dissimilar to YC-1, but utilized a similar mode of action (i.e., acting independently of NO and requiring the presence of a reduced heme moiety). Thus, despite identifying what are now termed "stimulators" they named the compounds GC "activators." In many ways, this exemplifies the flawed designation since stimulator and activator are used synonymously in scientific English to describe a mechanism of agonism at an enzyme, transcription factor, or receptor. Yet the differentiation between these two chemical classes is key to understanding not only the pharmacology but also novel (patho) physiological roles of the NO-sensitive cyclase. Specifically, it is believed that enzyme oxidation might occur during, and contribute to, cardiovascular disease by

leading to heme loss and NO insensitivity (Stasch et al. 2006); moreover, administration of "sGC activators" may proffer a means to target diseased vessels or organs. This slightly ambiguous terminology was in many respects improved upon by several groups, including scientists at Merck (Bittner et al. 2009), who created the labels "heme-dependent" and "heme-independent" sGC activators (HDA and HIA, respectively). This approach defined the disparate mechanisms of action more clearly, but muddied the waters by using the term "activator" for both sets of compounds, implying that each might work on oxidized/heme-free enzyme according to the original Bayer nomenclature.

The terminology to describe these novel classes of guanylyl cyclase ligands is further complicated by a recent revision of the enzyme nomenclature surrounding the target. Despite the ingrained use of sGC "stimulators" and "activators," and their formal approval as drug classes (i.e., name in approved drug labels or NDA/), the nomenclature surrounding their target protein, NO-sensitive guanylyl cyclase, has recently been updated (Alexander et al. 2015). Whilst these enzymes were originally termed soluble guanylyl cyclases, it has become clear that the term "soluble" is a misnomer, and these proteins are often associated with the cytoplasmic membrane, seemingly via interaction with chaperone proteins such as Hsp70 and Hsp90 (Balashova et al. 2005; Venema et al. 2003). The nomenclature of the NO-sensitive guanylyl cyclase isoforms have recently been modified to align with that of the homologous membrane-spanning proteins (e.g., GC-A, GC-B, GC-C; those that act as cognate receptors for the natriuretic peptide and guanylin family of hormones, or play a role in sensory perception (Kuhn 2016). Specifically, the ubiquitous NO-sensitive guanylyl cyclase comprising an α_1 and β_1 subunit is now referred to as GC-1, and the more tissue-specific (e.g., CNS, kidney, placenta) $\alpha_2\beta_1$ heterodimer is now termed GC-2 (Alexander et al. 2015). In addition, there has been considerable debate as to the correct chemical transformation catalyzed by the cGMP-synthesizing cyclase family; guanylate or guanylyl. Original discussions in the mid-1970s between scientists involved in the characterization of both cGMP- and cAMP-synthesizing enzymes resulted in the terms guanylate and adenylate begin adopted, if anything for ease of pronunciation rather than biochemical precision. However, from a chemical perspective the accurate nomenclature is unequivocally guanylyl, rather than guanylate (based on equivalent reactions of, for example, acetyl and acetate), since the α-oxygen of GTP leaves with the diphosphate group (Walseth et al. 1981) concomitant with reaction of the α-phosphorus with the ribose hydroxyl to cyclize GMP. Regardless of the new terminology, the identifiers "sGC stimulators" and "sGC activators" will persist to distinguish these family of molecules with distinct mechanisms of action, and their respective drug classes.

4 Discovery of sGC Stimulators

In 1994, scientists at Bayer started a screening campaign for substances that could induce an increase in NO synthesis and thereby stimulate sGC in porcine endothelial cells (Evgenov et al. 2006; Stasch and Hobbs 2009). These studies involved measurement of cGMP levels by radioimmunoassay, leading to the unexpected discovery of



Scheme 1 sGC stimulators YC-1 (**1**), BAY 41-2272 (**2**), BAY 41-8543 (**3**), and riociguat (**4**)

NO-independent sGC stimulators. At the same time, researchers at the National Taiwan University Taipei and Yung Shin Pharmaceuticals, Taiwan reported that a benzyl indazole compound named YC-1 (1) (Scheme 1) inhibited platelet aggregation via stimulation of cGMP synthesis. YC-1 (1) was subsequently characterized as a direct NO-independent, but heme-dependent, sGC stimulator. It stimulated isolated sGC by a factor of 30× to 40× at 100 μM, and showed a strong synergistic effect when combined with NO-releasing compounds and a loss of stimulation after oxidation or removal of the prosthetic heme moiety of sGC. YC-1 (1) exhibited a promising profile in various pharmacological studies. However, in addition to its relatively weak sGC stimulating potency, it revealed a poor pharmacokinetic profile and a lack of specificity as it was found to inhibit phosphodiesterases and to modulate many cGMP-independent effects. Therefore, further optimization of potency, pharmacokinetic properties, and specificity was required to realize the full therapeutic potential of this novel class of drugs.

Based on these initial results, extensive structure–activity relationship (SAR) studies were performed at Bayer to systematically optimize the structure of YC-1. The in vitro potency of the compounds was assessed by two different methods, a cGMP formation assay in sGC-overexpressing Chinese hamster ovary (CHO) cells and a functional assay based on the inhibition of phenylephrine-induced contraction of rabbit aortic rings. A first breakthrough in terms of improved potency resulted from the replacement of the benzyl indazole moiety of YC-1 by a (2-fluorobenzyl)pyrazolopyridine moiety and, even more importantly, the exchange of the (hydroxymethyl)furan portion for a 5-substituted 4-aminopyrimidine or 4,6-diaminopyrimidine group. Small molecule X-ray structures revealed a coplanar arrangement of this biaryl system, which is apparently important for achieving high potency. The 5-cyclopropyl-4-aminopyrimidine derivative BAY 41-2272 (2) (Scheme 1) showed a greatly improved sGC stimulating potency, with an IC_{50} of 0.3 μM for the contraction of rabbit aortic rings (YC-1, IC_{50} = 10 μM), and a minimum effective concentration (MEC) of 0.03 μM for cGMP formation in CHO cells (YC-1, MEC = 10 μM). In contrast to YC-1, BAY 41-2272 is a highly specific sGC stimulator and no relevant inhibition of phosphodiesterases was

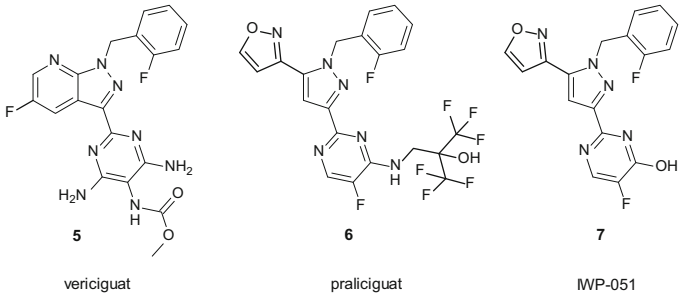
observed. Whereas the 1-(2-fluorobenzyl)-1H-pyrazolo[3,4-b]pyridine part of this new lead series turned out to be essential for potent sGC stimulating activity, the pyrimidine moiety allowed for broad variations. Further studies led to the 4,6-diamino-5-morpholino analogue BAY 41-8543 (3), displaying threefold higher potency in the phenylephrine-induced contraction of rabbit aorta ($IC_{50} = 0.10$ μM).

BAY 41-2272 and BAY 41-8543, however, displayed low metabolic stability and low oral bioavailability in rats, and BAY 41-2272 showed a strong inhibition as well as induction of metabolizing cytochrome P450 (CYP) enzymes. While these properties precluded further development, both compounds were used as tool compounds to study this novel class of drugs in numerous pharmacological experiments, resulting in more than 200 publications from various research groups around the world. Metabolite identification studies of BAY 41-2272 and BAY 41-8543 revealed an oxidative metabolism at the cyclopropyl and morpholino substituent, respectively. It was further demonstrated that, in contrast to compounds with small, lipophilic substituents at the pyrimidine C5-position (BAY 41-2272), derivatives with larger, more polar 5-substituents displayed no relevant CYP inhibition (BAY 41-8543). Continuous efforts to introduce other polar, potentially more stable substituents at the pyrimidine C5-position culminated in the identification of the *N*,*O*-dimethylcarbamate **4** (Scheme 1), BAY 63-2521 [International Nonproprietary Name (INN): riociguat]. Riociguat showed no relevant CYP interaction and a superior pharmacokinetic profile, including good oral bioavailability across different species.

In vitro, riociguat stimulated purified, recombinant sGC up to 73-fold, from 0.1 to 100 μM, and showed the typical profile of sGC stimulators: strong synergistic enzyme activation when combined with NO-releasing agents and crucial dependency on the presence of the reduced prosthetic heme moiety.

In conscious, spontaneously hypertensive rats, oral administration of riociguat resulted in a long-lasting and dose-dependent blood pressure decrease (Mittendorf et al. 2009). Importantly, and in contrast to nitrates, the effect is preserved over several weeks or when the rats are rendered nitrate tolerant. Riociguat was also investigated in different animal models of pulmonary hypertension (PH), including mice subjected to chronic hypoxia and rats injected subcutaneously with monocrotaline. In these experimental models, riociguat improved pulmonary hemodynamics and prevented, and even partially reversed, features of adverse structural remodeling such as right ventricular hypertrophy and muscularization of small pulmonary arteries (Stasch et al. 2011). Based on its combined profile of excellent potency, specificity, efficacy, and safety, riociguat was selected as a drug development candidate for the treatment of different forms of pulmonary hypertension (PH).

Riociguat was the first sGC stimulator to successfully transition from animal experiments to controlled clinical studies in patients. In randomized, double-blind, placebo-controlled Phase III trials in patients with the PH subforms, pulmonary arterial hypertension (PAH), and chronic thromboembolic PH (CTEPH), riociguat met the primary endpoint in exercise capacity (6-min-walking-distance, 6MWD). Riociguat showed a significant improvement in the 6MWD versus the placebo (+36 m, PAH; +46 m, CTEPH). Additionally, improvements were observed across



Scheme 2 sGC stimulators vericiguat (**5**), Praliciguat (**6**) and IWP-051 (**7**)

secondary endpoints, including pulmonary hemodynamics, functional class, and time to clinical worsening. Riociguat (Adempas^{TM}) is the first drug that has demonstrated efficacy in two life-threatening PH indications: CTEPH and PAH, and it is the only drug approved for CTEPH.

4.1 Activities Towards Next-Generation sGC Stimulators

Based on an increasing knowledge associated with this mode of action, the promising pharmacological effects of sGC stimulators and the clinical success of riociguat, several companies have pursued programs to further explore the structure–activity relationships (SAR) of the bis-heterocyclic pyrimidino-substituted pyrazolopyridines (Bayer, Pfizer, Merck) or to identify new lead series of sGC stimulators (Astellas, Bayer, Ironwood). From these efforts, three more sGC stimulators have made a successful transition to clinical studies: vericiguat (BAY 1021189) (**5**, Scheme 2) currently in phase 3 trials for heart failure with reduced ejection fraction (HFrEF), praliciguat (IW-1973; 6, scheme 2) currently in phase 2 trials for diabetic nephropathy and heart failure with preserved ejection fraction (HFpEF), and olinciguat (IW-1701) recently completed a phase 2a study in achalasia and currently in a phase 2 trial for sickle cell disease.

Vericiguat resulted from an optimization approach to identify orally bioavailable sGC stimulators with a longer duration of action than riociguat, in order to support a profile allowing for a once-daily oral dosing, and less oxidative metabolism in order to reduce drug interaction potential. Riociguat has a moderate half-life in different animal species and this pharmacokinetic profile translated into a three times daily dosing regimen in patients (Frey et al. 2017). The strategy was to further optimize the metabolic stability of riociguat mainly catalyzed by CYP1A1, and also by CYP3A4, CYP3A5, and CYP2J2 and hence reduce blood clearance to achieve a longer half-life. In these studies, vericiguat exhibited the best overall pharmacokinetic profile, with a low clearance and long half-life in rats and dogs after intravenous dosing, as well as high oral bioavailability (Follmann et al. 2017). In addition, vericiguat (**5**) had no inhibitory effects on major CYP isoforms (1A2, 2A6, 2B6, 2C8, 2C9, 2C19, 2D6, and

3A4), as indicated by IC_{50} values of >50 μM (Follmann et al. 2017). After thorough preclinical DMPK studies, vericiguat was selected as a clinical candidate and proved to have a pharmacokinetic profile in humans suitable for once-daily dosing.

Additional in vivo studies in animal models of hypertension, heart failure, and kidney disease have revealed dose-dependent antifibrotic and organ-protective properties in line with the sGC stimulator mode of action. Vericiguat is currently being investigated in a phase 3 clinical trial in patients with HFrEF (NCT02861534, Armstrong et al. 2017) and in a phase 2 clinical trial in patients with HFpEF (NCT03547583).

Researchers at Ironwood Pharmaceuticals have discovered several novel sGC stimulators and advanced three compounds into development (Buys et al. 2018). The medicinal chemistry effort that led to the bis-heteroaryl pyrazole IWP-051(7, scheme 2), a pharmacodynamically active compound with low clearance and a long half-life in rats, has been described (Nakai et al. 2016). Ironwood has advanced two other sGC stimulators, praliciguat and olinciguat, into clinical studies, and a third compound, IW-6463, that readily crosses the blood–brain barrier, is under preclinical evaluation for the potential treatment of CNS diseases.

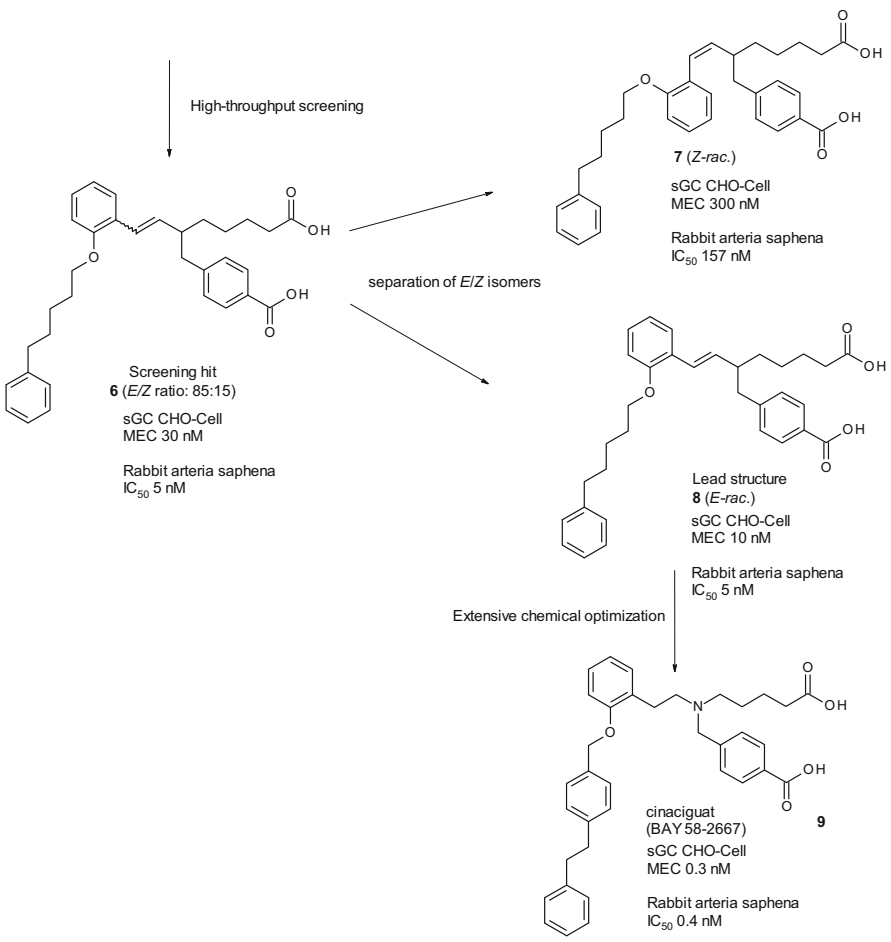
Praliciguat is an sGC stimulator with a long half-life in preclinical species, extensive tissue distribution, and mainly hepatic clearance (Tobin et al. 2018). Its pharmacokinetic half-life in humans is consistent with QD dosing (Hanrahan et al. 2018). Praliciguat has completed Phase 1 studies in healthy subjects and Phase 2a exploratory studies in patients with type 2 diabetes and a history of hypertension (NCT02906579, NCT03091920) Praliciguat is currently under investigation for treatment of diabetic nephropathy (NCT03217591) and heart failure with preserved ejection fraction (NCT03254485).

Olinciguat is an-sGC stimulator that has completed phase 1 studies as well as a Phase 2a study in patients with achalasia (NCT02931565). Olinciguat is also under investigation for the treatment of sickle cell disease (NCT03285178). In clinical studies in healthy adults, olinciguat demonstrated a long half-life and low peak-to-trough plasma ratio with QD dosing (Mittleman et al. 2017).

5 Discovery of sGC Activators

Following the discovery of the NO-independent, heme-dependent sGC stimulators, scientists at Bayer performed a high-throughput screen (HTS) in 1997 with the goal of identifying additional sGC stimulator leads. For this effort, the cGMP formation assay in sGC-overexpressing CHO cells was utilized. Surprisingly, a compound with an unprecedented and distinct dicarboxylic acid motif (6) was identified as a potent agonist of sGC (Scheme 3). After further mechanistic in vitro studies, it was established that this compound behaved in a completely different manner to the sGC stimulators, stimulating sGC in an NO- as well as heme-independent fashion. Thus, the novel pharmacological class was designated an sGC activator to clearly differentiate this new behavior and mode of action from sGC stimulators.

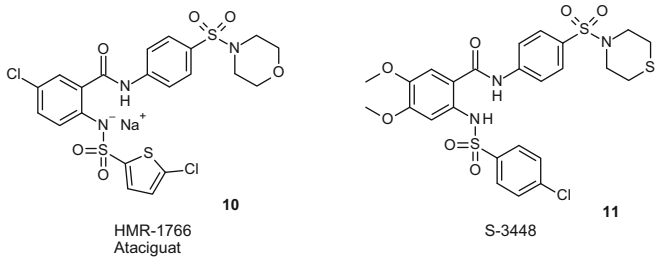
This serendipitous discovery provided a tool to further explore redox regulation of sGC and its role in the pathogenesis of several cardiovascular disorders. More specifically, this offered the prime opportunity to design drugs for selective binding



Scheme 3 Discovery of sGC activators by HTS and evolution towards cinaciguat (9)

to the oxidized, heme-free sGC generated by the influence of oxidative stress causally involved in many cardiovascular diseases.

Screening hit (6) presented as a racemic 85:15E/Z-mixture. After separation, the racemic E-isomer (8) turned out to be 30-fold more potent than the racemic Z-isomer (7). Subsequent separation of enantiomers revealed that the R,E-isomer of 8 is 70-fold more potent than the corresponding S-enantiomer. Moreover, lead structure 8 also showed promising in vitro potency on isolated recombinant sGC and relaxation of precontracted rabbit arteria saphena rings. Based on these initial results, an extensive lead optimization program was initiated with the goal of identifying a candidate suitable for intravenous dosing. The exchange of the central allylic moiety



Scheme 4 sGC activators reported by Hoechst Marion Roussel: ataciguat (10) and S-3448 (11)

for an ethylamino linkage and modification of the phenylpentyl side chain resulted in the discovery of clinical candidate BAY 58-2667 (9) (INN: cinaciguat).

The pharmacological efficacy profile of cinaciguat was explored in various in vivo models of myocardial infarction, chronic renal failure, arterial and pulmonary hypertension, and chronic heart failure. In a canine model of congestive heart failure (HF), intravenous administration of cinaciguat resulted in dose-dependent reductions in cardiac preload and afterload, and a concomitant increase in cardiac output and renal blood flow without further neurohumoral activation (Boerrigter et al. 2007).

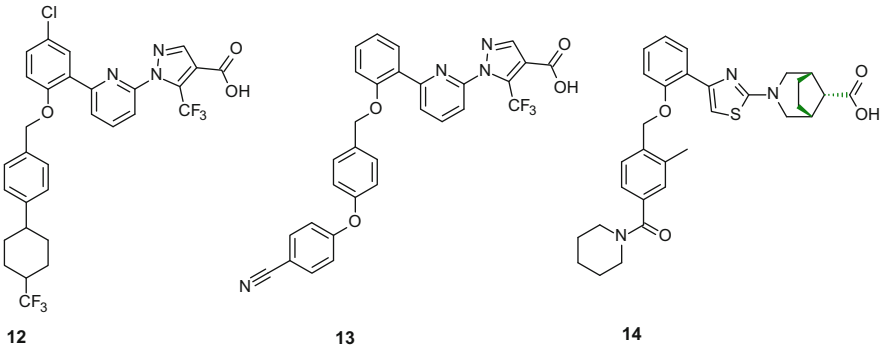
In 2001, researchers from Hoechst Marion Roussel disclosed anthranilic acid derivatives that represented a novel structural class of compounds also reported to activate the oxidized and/or heme-free form of sGC. The best-described examples are HMR 1766 (10) (INN: ataciguat) and S-3448 (11) (Scheme 4).

The inhibition of phenylephrine-induced contraction of rat aortic rings by both compounds is only moderate to weak; however, the pharmacological efficacy of ataciguat and S-3448 was demonstrated in various in vivo models of atherosclerosis and peripheral arterial occlusive disease. Chronic treatment of streptozotocin diabetic rats with ataciguat improved endothelial function and normalized platelet activation. Additionally, reduced atherosclerosis and improved endothelium-dependent vasorelaxation were observed in $ApoE^{-/-}$ mice treated with ataciguat. Stage II peripheral arterial occlusive disease is mainly characterized by exercise-induced muscle fatigue. Ataciguat improved ischemia-induced muscle fatigue in Zucker Diabetic Fatty (ZDF) rats with unilateral hind-limb ischemia as an experimental model of peripheral arterial occlusive disease.

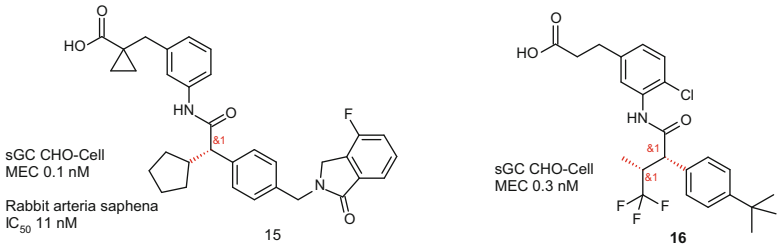
5.1 Activities Towards Second-Generation sGC Activators

The search for novel sGC activators has become an increasingly competitive field. Several approaches have been reported in the recent patent literature. Interestingly, all second-generation sGC activators contain a monocarboxylic acid moiety.

In 2009, Merck (12) and GlaxoSmithKline (GSK) (13) disclosed very similar sGC activators incorporating an identical 5-(trifluoromethyl)pyrazole-4-carboxylic acid moiety attached to a pyridine scaffold (Scheme 5). Even more recently,



Scheme 5 sGC activators described by Merck, GSK, and Boehringer Ingelheim



Scheme 6 3-Phenylpropionic acid sGC activators disclosed by Bayer

Boehringer Ingelheim disclosed BI 703704 (14) displaying in vivo activity in a ZSF1 rat model of type 2 diabetes mellitus (T2DM)–induced nephropathy.

Bayer has also disclosed monocarboxylic acids with novel structural features, highlighting branched 3-phenylpropionic acid derivatives, as exemplified by compound 15 (Scheme 6). With the aim of improving the DMPK profile of these compounds, lower molecular weight 3-phenylpropionic acid congeners have been prepared, as exemplified by compound 16 (Follmann et al. 2013). Bayer is currently developing three new generation sGC activators in phase 1 clinical development for pulmonary hypertension (PH), acute respiratory distress syndrome (ARDS) and chronic kidney disease (CKD).

6 Therapeutic Applications of sGC Stimulators and sGC Activators: A Preclinical Perspective

cGMP is a universal second messenger that regulates the function of many cell types, including smooth muscle cells, cardiomyocytes, fibroblasts, adipocytes, and neurons. Whereas the downstream signaling pathway remains to be fully elucidated, it is abundantly evident that cGMP is critical for the maintenance of cellular and organ homeostasis, and that NO/sGC/cGMP dysfunction is linked to the pathogenesis of numerous diseases. This ubiquitous signaling pathway is the pharmacologic target of

sGC stimulators and sGC activators. These pharmacologic agents have been intensively profiled in vitro, ex vivo, and in vivo in mechanistic and disease-relevant animal models to better understand their mode of action and to search for new therapeutic applications. Preclinical and clinical studies have revealed that sGC agonists affect contractility and proliferation of smooth muscle, reduce inflammation and fibrosis, positively impact metabolic risk factors (including weight gain, glucose, and cholesterol), and affect neuronal health and function.

The sGC stimulator riociguat is approved for the treatment of different forms of pulmonary hypertension. However, sGC stimulators and sGC activators have shown beneficial effects in animal models of a variety of other disease conditions. At the cGMP conference held in Bamberg, Germany in June 2017, world experts discussed not only novel targets for cGMP, but also new therapeutic applications of sGC modulators (Friebe et al. 2017). Although the details are beyond the scope of this chapter, some current and future lines of potential therapeutic applications are summarized below.

6.1 Cardiovascular Diseases: Pulmonary Hypertension, Arterial Hypertension, and Heart Failure

NO/sGC/cGMP signaling plays a central role in the cardiovascular system and thus is an obvious area of therapeutic interest, particularly with regard to pulmonary hypertension (PH), systemic hypertension, and heart failure. In 2013, riociguat (BAY 63-2521) was approved as first-in-class sGC stimulator for the treatment of pulmonary arterial hypertension (PAH) and chronic thromboembolic pulmonary hypertension (CTEPH) (Humbert and Ghofrani 2016; Hoeper 2015). In preclinical models of pulmonary hypertension, including hypoxia models and a monocrotaline model, the sGC stimulator BAY 41-2272 and the sGC activator cinaciguat reduced pulmonary hypertension, right ventricular hypertrophy, and lung vascular remodeling in a chronic hypoxia model of pulmonary hypertension, and both compounds reversed hemodynamic and structural changes in a rat monocrotaline model of severe pulmonary hypertension (Dumitrascu et al. 2006). In the Su5416/hypoxia model of pulmonary hypertension, riociguat decreased RV hypertrophy, increased cardiac output, and decreased total pulmonary resistance (Lang et al. 2012). In addition, riociguat reduced PH, pulmonary vascular remodeling and improved right ventricular function in a mouse TAC model of Group 2 PH, but did not improve left ventricular function or hypertrophy (Pradhan et al. 2016). Subsequently, there have been more than 30 preclinical publications demonstrating the effect of sGC stimulators including riociguat in cardiopulmonary diseases and pulmonary hypertension (Stasch and Evgenov 2013). These preclinical results anticipated clinical findings of reduction in PVR and NT-proBNP in PH patients, as well as improvement in exercise tolerance, which provided the basis for regulatory approvals of riociguat for treatment of PAH and CTEPH (Ghofrani et al. 2013a, b).

Activation of NO/sGC/cGMP signaling causes vascular smooth muscle relaxation and vasorelaxation. Consistent with a hypertensive phenotype in mouse sGC knockout models (Friebe et al. 2007; Buys et al. 2008), human genetic variants in the NO/sGC/cGMP pathway, including variants of sGC, have been associated with elevated blood pressure and increased cardiovascular disease risk (International Consortium for Blood Pressure Genome-Wide Association 2011). Although there are more than six classes of drugs that are used to treat hypertension, many patients do not achieve blood pressures below the guideline-recommended levels (Whelton et al. 2017; Pimenta and Calhoun 2016) of 130/80 mmHg. Of the drugs used to treat hypertension, only sodium nitroprusside targets the NO/sGC/cGMP pathway, but, due to tachyphylaxis, its use is limited to acute treatment of hypertensive crisis. It is expected that sGC stimulators and activators, which dose-dependently reduce blood pressure in animal models of hypertension (Mittendorf et al. 2009; Geschka et al. 2011; Tobin et al. 2018), would lower blood pressure in patients who are not at goal despite treatment with current standard of care. sGC stimulators in particular may provide potent blood pressure reduction in refractory patients with salt-sensitive hypertension, which is associated with endothelial dysfunction. Indeed, sGC stimulators have shown potent, dose-dependent blood pressure reduction in the Dahl salt-sensitive model of hypertension (Geschka et al. 2011; Tobin et al. 2018).

Chronic heart failure constitutes a major health problem worldwide. Pharmacological therapies, targeting the renin-angiotensin system or sympathetic nervous system, have limited efficacy (Lewis et al. 2017). Drugs targeting the cGMP pathway, including isosorbide dinitrate/hydralazine (BiDil) and the angiotensin receptor neprilysin inhibitor sacubitril combined with valsartan (Entresto), have proven effective in treatment of heart failure. In recent years, heart failure has been categorized into heart failure with reduced ejection fraction (HFrEF) and heart failure with preserved ejection fraction (HFpEF). The prevalence of HFpEF is now nearly equal to the prevalence of HFrEF. Although HFpEF has been characterized as a heterogenous condition and has been particularly refractory to pharmacologic treatment, an evolving understanding of the disease suggests that microvascular inflammation resulting in cardiac and systemic endothelial dysfunction may be a common underlying pathophysiology, highlighting the potential pharmacologic utility of sGC stimulators (Paulus and Tschope 2013). Nitrates have a long history of use in angina as well as acute and chronic heart failure, but their utility may be limited by development of tolerance and propensity to form highly damaging peroxynitrites. The rationale for treating both HFrEF and HFpEF with sGC agonists is strong and was recently reviewed (Breitenstein et al. 2017). sGC is expressed in critical cardiovascular tissues including vascular smooth muscle, the heart, and the kidney, and sGC $\alpha 1^{-/-}$ knockout mice have impaired ventricular relaxation and reduced cardiac output (Irvine et al. 2012). The sGC activator cinaciguat reduced cardiac hypertrophy and improved systolic and diastolic function in a diabetic cardiomyopathy model, and prevented cardiac hypertrophy in a pressure-overload model (Mátyás et al. 2015). Similarly, in Dahl salt-sensitive rats,

in the angiotensin II – pressure-overload models, or in post-myocardial infarction models of heart failure, treatment with sGC stimulators has consistently shown beneficial effects on cardiac function, remodeling, and fibrosis, as well as on levels of the ventricular stress hormone NT-proBNP (Masuyama et al. 2006; Masuyama et al. 2009; Methner et al. 2013; Geschka et al. 2011). Finally, NO/sGC/cGMP signaling plays an important role in regulating inflammation, which is believed to be a common underlying pathophysiology in HFpEF. In a double transgenic rat (dTGR) model of HFpEF, treatment with the sGC stimulator BAY 41-8543 dramatically improved survival rate, reduced cardiac fibrosis, macrophage infiltration, and gap junction remodeling. The expression of dysregulated cardiac genes associated with fibrosis, inflammation, apoptosis, oxidative stress, and ion channel function was restored in treated dTGR in the direction of healthy controls. Treatment reduced systemic blood pressure levels and improved endothelium-dependent vasorelaxation of resistance vessels. Further comprehensive in vivo phenotyping showed an improved diastolic cardiac function, improved hemodynamics, and also less susceptibility to ventricular arrhythmias. Thus, sGC stimulation was highly effective in improving several HFpEF facets in this animal model, underscoring its potential value for patients (Wilck et al. 2018). Moreover, in mouse studies, it has been shown that treatment with NO and sGC stimulators reduced P-selectin expression and leukocyte recruitment (Ahluwalia et al. 2004; Tchernychev et al. 2017), indicating potential to reduce microvascular inflammation.

6.2 Kidney Diseases

The potential benefits of cGMP increase in the kidney and of sGC stimulators and activators in renal disease were recently reviewed (Stasch et al. 2015; Krishnan et al. 2018). Chronic kidney disease, defined as reduced eGFR and/or increased urinary albumin, is associated with high morbidity and mortality, and treatment options are limited. NO/sGC/cGMP signaling is involved in vital renal functions including regulation of renal blood flow and glomerular hemodynamics as well as water and salt transport in the tubular system (Kone 1997). Indeed, advanced nephropathy is associated with progressive decline in levels of NO (Prabhakar 2004). sGC stimulators and activators have been tested in several models of kidney diseases including hypertensive nephropathy, unilateral ureteral obstruction, diabetic nephropathy, and acute glomerular nephritis. Treatment with both stimulators and activators has resulted in reduced proteinuria and/or albuminuria; decreased renal glomerulosclerosis, fibrosis and markers of fibrosis, and improved podocyte health (reviewed in Stasch et al. 2015). Data from in vitro studies in renal fibroblasts suggest cGMP-mediated suppression of TGFβ/P-smad3 signaling may contribute to antifibrotic effects in the kidney (Schinner et al. 2017). In human renal proximal tubular cells in vitro, treatment with sGC stimulators reduced TGFβ-induced apoptosis and TNFα-induced increases in MCP-1 (Liu et al. 2016). In addition, NO/sGC/cGMP stimulation mediates renal protection through systemic effects on the cardiovascular system, which, in health, may suppress tubular degeneration and subsequent renal fibrosis and hypertrophy.

6.3 Fibrotic Diseases (Lung Fibrosis and Systemic Sclerosis)

There is accumulating evidence that cGMP elevation can have an antifibrotic effect via directly targeting fibroblasts and myofibroblasts (Sandner et al. 2017; Sandner and Stasch 2017). Understanding these effects extends the mode of action of cGMP beyond vasodilation and may provide the basis for completely new applications of cGMP-enhancing drugs. As noted above, in models of hypertension, cardiomyopathy, and chronic kidney disease, treatment with sGC agonists has been associated with antifibrotic effects on target organs. In recent years, antifibrotic effects of sGC modulators have been explored in non-hypertensive models of fibrosis in organs outside of the cardiovascular system, including the lung and the skin. In a bleomycin mouse model of pulmonary fibrosis, riociguat treatment reduced pulmonary hypertension, right ventricular hypertrophy, inflammation, and pulmonary fibrosis (Evgenov et al. 2011). The sGC stimulator BAY 41-2272 halted development of skin fibrosis in bleomycin and Tsk-1 mouse models as demonstrated by reduced dermal thickening, myofibroblast number, and hydroxyproline content (Beyer et al. 2012). In many of these studies, sGC agonists reduced fibrosis at doses that did not affect blood pressure or heart rate, suggesting that the antifibrotic effects are independent from the hemodynamic effects and may be due to direct effects of cGMP on fibrotic processes. The antifibrotic mechanism has been explored in vitro in fibroblasts from lung, skin, kidney, liver, and heart (Vettel et al. 2014; Beyer et al. 2015; Hewitson et al. 2004; Hall et al. 2018; Lambers et al. 2014). Increasing cGMP signaling in these cellular studies suppressed TGFβ-mediated increases in collagen and ECM production, inhibited fibroblast-myofibroblast differentiation, and/or reduced fibroblast proliferation.

6.4 Liver Diseases

Chronic liver diseases such as hepatitis and alcoholic and nonalcoholic liver disease can lead to cirrhosis. Liver cirrhosis is characterized by extensive fibrotic scarring of the liver, which is associated with impaired hepatic function and leads to complications such as portal hypertension and esophageal varices. sGC agonists have shown promising effects in preclinical models of liver fibrosis (Knorr et al. 2008; Hall et al. 2017). The sGC stimulator riociguat was shown to reduce liver fibrosis and portal pressure in cirrhotic rats (Schwabl et al. 2018) and mechanistic studies suggest that sGC modulators may inhibit fibrotic differentiation of hepatic stellate cells (Xiao et al. 2015; Hall et al. 2017). Nonalcoholic steatohepatitis (NASH) is a liver disease with characteristics of steatosis, inflammation, and fibrosis, and is a growing health concern globally. Patients with NASH are at risk for developing cirrhosis, and also have elevated cardiovascular event risk. There are no approved treatments for NASH, and most of the treatments that are in clinical development address only one aspect of the NASH pathophysiology (i.e., steatosis, inflammation, or fibrosis). sGC agonists have the potential to impact all three aspects of NASH pathophysiology: inflammation [discussed above (Ahluwalia et al. 2004)],

fibrosis, and steatosis (see Sect. 6.5 below) (Hoffmann et al. 2015); indeed, in an experimental NASH model, the sGC stimulator praliciguat affected all three aspects (Flores-Costa et al. 2017).

6.5 Metabolic Disease

Elevated plasma glucose, excess visceral fat, abnormal cholesterol or triglyceride levels, and high blood pressure are components of the metabolic syndrome. When these factors occur together, they increase an individual's risk of developing heart disease, stroke, and diabetes. Treatment with the sGC stimulator BAY 41-8543 improved metabolic measures (weight gain, fat mass, diabetic phenotype) in a mouse diet-induced obesity (DIO) model (Hoffmann et al. 2015). In a similar DIO model, treatment with the sGC stimulator praliciguat improved glucose tolerance and insulin sensitivity and lower triglycerides (Schwartzkopf et al. 2018) Furthermore, olinciguat and praliciguat reduced fasting glucose in the ZSF1 model of diabetic nephropathy (Profy et al. 2017; Masferrer et al. 2016), and the sGC stimulator praliciguat reduced hepatic steatosis in an experimental NASH model (Flores-Costa et al. 2017). The promising metabolic effects suggest evaluation of sGC stimulators in individuals with metabolic diseases including obesity, diabetes, hyperlipidemia, and metabolic syndrome, and NASH may be warranted. One mechanism for the metabolic effects of sGC agonism may involve increased lipid uptake into brown adipose and increased whole-body energy expenditure (Hoffmann et al. 2015). This area of research warrants further exploration and may have broad relevance to treatment of metabolic disease and associated comorbidities.

6.6 Central and Peripheral Nervous System Disorders

The importance of cGMP in neuronal and sensory signaling, cognitive function, and brain health has gained greater appreciation in recent years. Both the ubiquitous NO-sensitive guanylyl cyclase comprising α1 and β1 subunits now referred to as GC-1, and the more tissue-specific (e.g., CNS, kidney, placenta) α2β1 heterodimer now termed GC-2 are expressed in the brain (Mergia et al. 2003; Ibarra et al. 2001), and cGMP has been shown to mediate memory formation and LTP (Bollen et al. 2014). In addition, NO and sGC regulate local blood flow in the CNS in response to neuronal activity through a process known as functional hyperemia (Faraco and Iadecola 2013). Vascular dysfunction may underlie forms of dementia and Alzheimer's disease as systemic hypertension is a leading risk factor for these diseases. There is also a growing interest in the role that neuroinflammation may play in the deterioration of brain health and cognitive function. Pharmacologic data with PDE5i suggest that cGMP signaling may suppress neuroinflammation (Agusti et al. 2017; Christina Alves et al. 2015; Raffaella et al. 2016). Drugs affecting the NO/sGC/cGMP signaling pathway may address multiple aspects of the pathophysiology of dementia. Inhibitors of PDE9, a cGMP-specific phosphodiesterase, have

shown promising results in preclinical models of learning and memory (van der Staay et al. 2008). However, a PDE9 inhibitor did not improve cognition in the clinic (Schwam et al. 2014). Relative to PDE9 inhibitors, sGC agonists, and particularly sGC stimulators, which enhance neuronal *and vascular* NO signaling, may have the potential to address the broader constellation of deficiencies in dementia. CNS-targeted sGC agonists have not been available for clinical investigation. IW-6463 is a novel sGC stimulator that penetrates the blood–brain barrier being evaluated for potential use in CNS diseases.

6.7 Gastrointestinal Motility Disorders

NO released from nitrergic neurons in the GI tract is an important regulator of GI smooth muscle relaxation and motility (Groneberg et al. 2016). Mice lacking sGC develop fatal GI obstruction (Friebe et al. 2007). There is strong evidence that dysfunctional nitrergic signaling is involved in GI motility disorders such as achalasia, gastroparesis, slow transit constipation, and Hirschsprung's disease. sGC is found in several cell types in the GI tract, including smooth muscle cells, interstitial cells of Cajal (ICC), and fibroblast-like cells; smooth muscle and ICC-specific sGC knockouts have increased understanding of the roles of sGC in each cell type in the regulation of intestinal peristalsis (Groneberg et al. 2016).

Achalasia is a swallowing disorder in which the lower esophageal sphincter (LES) remains in a contracted state, limiting passage of food from the esophagus into the stomach. Achalasia has been associated with loss of NO signaling neurons in the LES (Hoshino et al. 2013), mice deficient in neuronal nitric oxide synthase (nNOS) develop LES hypertension (Sivarao et al. 2001), and individuals with a rare homozygous loss of sGC mutation develop achalasia (Herve et al. 2014). NO donors and PDE5 inhibitors have reduced LES pressure in patients with achalasia (Patel et al. 2015; Bortolotti et al. 2000; Eherer et al. 2002). The sGC stimulator olinciguat was shown to relax human LES ex vivo (Zimmer et al. 2017), and a Phase 2a exploratory study was recently completed.

6.8 Hematologic (Sickle Cell Disease)

Sickle cell disease (SCD) is an inherited blood disorder resulting from an allele of the hemoglobin beta gene that results in sickling of red blood cells (Ingram 1956). Individuals with SCD can develop a number of complications including anemia, acute chest syndrome, pulmonary hypertension, fatigue, and vaso-occlusive crisis, which is characterized by extreme pain. Sickle cell disease is associated with endothelial and NO dysfunction (Nahavandi et al. 2002) resulting from increased circulating levels of free hemoglobin (an NO scavenger), arginase (which degrades the nitric oxide synthase substrate arginine), and ADMA (a nitric oxide synthase inhibitor). A main clinical feature of SCD is unpredictable and recurrent severe pain associated with sickle-cell-mediated small vessel vaso-occlusion, which may be

triggered or potentiated by vascular dysfunction and inflammation. Effective therapies targeting the SCD symptoms and quality of life including the cause of vaso-occlusive pain are needed.

Hydroxyurea is approved as a chronic use drug treatment for SCD. Although aspects of its mechanism of action are not completely understood, hydroxyurea may prevent red blood cell sickling by increasing fetal hemoglobin expression via NO release (Cokic et al. 2003). Red blood cells appear to have a functional NO/sGC/cGMP signaling pathway; furthermore, red blood cells from patients with endothelial dysfunction (associated with coronary artery disease) are responsive to NO as well as both sGC stimulators and sGC activators (Cortese-Krott et al. 2018). Stimulation of the NO/sGC/cGMP pathway has been shown to decrease vascular inflammation in vivo. As noted above, the sGC stimulator BAY 41-2272 and NO reduced leukocyte rolling and adhesion in an eNOS deficient mouse model (Ahluwalia et al. 2004), and the sGC stimulator olinciguat reduced makers of vascular inflammation and increased leukocyte rolling and velocity in a TNFα mouse model (Tchernychev et al. 2017). Chronic oral administration of the sGC activator cinaciguat improved endothelial function and reversed pulmonary hypertension and cardiac remodeling in a mouse model of SCD without affecting systemic blood pressure (Potoka et al. 2018). In a humanized SCD mouse model of TNFα-induced acute vaso-occlusion, BAY 73-6691, a PDE9 inhibitor, reduced leukocyte recruitment and red blood cell–leukocyte interactions, and improved leukocyte rolling and adhesion (Almeida et al. 2012). Finally, vasodilation mediated by sGC agonists is expected to increase blood flow in small vessels, preventing vaso-occlusion. In summary, sGC agonists could affect blood flow, vascular inflammation, and red blood cell sickling thereby preventing multiple complications of SCD.

6.9 Ocular Diseases

Glaucoma is a progressive optic neuropathy and a leading cause of blindness worldwide. Ocular pressure is a risk factor for development of primary open angle glaucoma, the most prevalent form of glaucoma. Mice deficient in sGC exhibit ophthalmic pathology resembling glaucoma, including increased intraocular pressure, optic neuropathy, and retinal vascular dysfunction. Additionally, human candidate gene studies revealed that a variant in the locus encoding genes for GC1 are associated with one form of primary open angle glaucoma (Buys et al. 2013). Furthermore, several studies have demonstrated that NO, cGMP, and sGC modulators may reduce intraocular pressure through regulation of aqueous humor outflow from the anterior chamber through the trabecular meshwork and Schlemm's canal (Kotikoski et al. 2003; Ge et al. 2016). Emerging data suggest that modulators of cGMP availability may also prevent optic nerve damage, independent of effects on ocular pressure. However, very recently a sGC activator from Novartis (MGV354) was profiled preclinically and clinically in Glaucoma. Despite promising preclinical results in animal models in which MGV354 significantly lowered intraocular pressure (Prasanna et al. 2018), MGV354 failed in the phase 1/2 clinical trial (Stacy et al. 2018).

6.10 Preclinical Summary

In summary, the multifaceted pharmacology of sGC modulators with effects on vascular function, inflammation, fibrosis, neuronal health and signaling, and metabolism affords the opportunity to positively impact a variety of pathologic conditions and organ systems. The availability of sGC stimulators and sGC activators enables the investigation of these unique mechanisms in both the preclinical and clinical settings.

7 Clinical Developments of sGC Stimulators and sGC Activators

Given the broad impact of the NO/sGC/cGMP pathway on regulation of cell, tissue, and body function, it is not surprising that there are many clinical trials, both completed and ongoing, investigating the treatment potential of sGC stimulators and sGC activators. Clinical trials with early sGC agonists that are no longer in clinical development are listed here for reference and completeness (Table 1).

Based on a broad preclinical profiling of riociguat in animal models of pulmonary hypertension in which a significant reduction of pulmonary artery pressure could be demonstrated, riociguat was developed for the treatment of pulmonary hypertension. Riociguat showed efficacy in two Phase 3 trials in pulmonary hypertension patients, namely, the PATENT trial in pulmonary hypertension patients, WHO Group 1 (pulmonary arterial hypertension – PAH) and the CHEST trial in WHO Group 4 (chronic thromboembolic pulmonary hypertension – CTEPH) (Ghofrani et al. 2013a, b; Frey et al. 2017). Treatment effects of riociguat were sustained for at least 2 years in the long-term Phase 3 extension studies PATENT-2 and CHEST-2 trials (Rubin et al. 2015; Simonneau et al. 2016; Ghofrani et al. 2016). A broad range of completed and ongoing Phase 3b, Phase 4, and investigator-initiated clinical trials with riociguat have also been performed and cannot be covered fully in the scope of this review. Importantly, riociguat was also tested in patients with pulmonary arterial hypertension with insufficient response to PDE5 inhibitors. This single-arm, open-label, uncontrolled study (RESPITE, NCT0200762) indicated that replacing PDE5i with riociguat may be a feasible and effective treatment strategy in these patients (Hoeper et al. 2017a, b). A randomized controlled, open-label multicenter international study is currently ongoing to confirm the results (REPLACE, NCT02891850). In addition, in the MOTION (NCT 02191137) study, an open-label Phase 4 program, treatment-naïve pulmonary arterial hypertension patients were studied for patient-reported outcome using three different quality of life instruments. The results showed a positive impact of riociguat treatment on patient-reported quality of life.

Beyond pulmonary hypertension there are also clinical trials ongoing to treat systemic, arterial hypertension with sGC stimulators. Vasorelaxation is a prominent effect of sGC stimulators at higher dose levels. Since sGC stimulators actively augment cGMP and downstream vasodilation rather than block vasoconstriction,

Table 1 Lists a selection of Phase 2 and 3 trials with sGC stimulators and sGC activators from the public ClinicalTrials.gov database (https://clinicaltrials.gov/)

sGC stimulators	Indication	Phase	NCT number	Study name	Status	
Riociguat (BAY 63-2521)	PAH	3	NCT00810693	PATENT	Completed	Approved for PAH in 2013
	CTEPH	3	NCT00855465	CHEST	Completed	Approved for CTEPH in 2013
	PAH	3	NCT00863681	PATENT-2	Completed	
	CTEPH	3	NCT00910429	CHEST_2	Completed	
	PAH children	3	NCT02562235	PATENT CHILD	Ongoing	
	PAH	3	NCT02007629	RESPITE	Completed	
	PH-LVD	2	NCT01065454	LEPHT	Completed	
	PH-IIPs	2	NCT02138825	RISE IIP	Terminated	
	dcSSc[a]	2	NCT02283762	RISE SSc	Completed	
	CF	2	NCT02170025		Terminated	
	SCD	2	NCT02633397		Ongoing	
Nelociguat (BAY 60-4552)	ED	2	NCT01168817		Completed	
Vericiguat (BAY 102-1189)	HFrEF	2	NCT01951625	SOCRATES-REDUCED	Completed	
	HFpEF	2	NCT01951638	SOCRATES-PRESERVED	Completed	
	HFrEF[a]	3	NCT02861534	VICTORIA	Ongoing	
	HFpEF[a]	2	NCT03547583	VITALITY-HFpEF	Ongoing	
Olinciguat (IW-1701)	Achalasia	2	NCT02931565		Completed	
	SCD	2	NCT03285178	STRONG SCD	Ongoing	
Praliciguat (IW-1973)	T2D and HTN	2	NCT03091920		Completed	
	T2D and HTN	2	NCT02906579		Completed	
	HFpEF	2	NCT03254485	CAPACITY-HFpEF	Ongoing	
	Diabetic Nephropathy	2	NCT03217591		Ongoing	

This table does not include Phase 1 clinical studies but does include development programs that are completed and terminated to reflect the full range of potential indications

[a]Bayer/MSD codevelopment; BAY 102-1189 = MK-1242

sGC stimulators might provide advantages over classical antihypertensive therapies. Bayer investigated a new chemical class of long-acting sGC stimulators in Phase 1 studies for the treatment of difficult to treat hypertension patients. Ironwood recently completed two Phase 2 studies with praliciguat in patients with T2DM and a history of hypertension on stable regimens of anti-glycemic and anti-hypertensive agents (Hanrahan et al. 2018a, b). Consistent with preclinical observations, treatment with praliciguat led to reductions in blood pressure and improvement in metabolic parameters including fasting plasma glucose and cholesterol levels in this patient population. These studies confirmed the pharmacokinetic profile of praliciguat supporting once-daily dosing and broad tissue distribution and set the stage for ongoing studies of this compound in patients with diabetic nephropathy and HFpEF discussed below.

The NO/sGC/cGMP signaling pathway plays a pivotal role in the regulation of the cardiovascular system, and sGC stimulators and sGC activators have the potential for broad impact on the treatment of cardiovascular diseases. cGMP increase by these compounds may result in systemic improvements driven by the vascular effects but could also have direct effects in cardiac or renal tissues improving heart and kidney function. In addition, there are hints from preclinical and clinical studies on metabolic effects and effects on adipose tissues. For these reasons, clinical trials, both completed and ongoing, have investigated the effects of sGC stimulators in chronic heart failure. Two Phase 2 studies, the so-called SOCRATES trials (SOluble guanylate Cyclase stimulatoR in heArT failure), were conducted in chronic heart failure patients with reduced and preserved ejection fraction, SOCRATES-REDUCED and SOCRATES-PRESERVED, respectively (Pieske et al. 2014). In the SOCRATES-REDUCED study (NCT01951625), the exploratory analysis suggested a dose-dependent reduction of NT-proBNP and a trend for reduction of CV deaths and HF hospitalizations (Gheorghiade et al. 2015). In the SOCRATES-PRESERVED study (NCT01951638), no significant effect on NT-proBNP was observed but there was an improvement in quality of life scores (Pieske et al. 2017; Filippatos et al. 2017). Currently a Phase 3 confirmatory trial with vericiguat in HFrEF, the so-called VICTORIA trial (VerICiguaT Global Study in Subjects With Heart Failure With Reduced Ejection Fraction, NCT02861534) is ongoing (Armstrong et al. 2017). More recently, Ironwood initiated the Phase 2 CAPACITY-HFpEF trial (NCT03254485) with praliciguat and Bayer together with MSD started a Phase 2 VITALITY-HFpEF trial (NCT03547583) with vericiguat. Both studies are evaluating the potential benefit of sGC stimulators in treating heart failure patients with preserved ejection fraction. Building on the preclinical data supporting positive renal effects of sGC stimulators (Stasch et al. 2015; Tobin et al. 2018), Praliciguat is also being studied in patients with diabetic nephropathy (NCT03217591).

In addition to the indications focusing on cardiopulmonary, cardiovascular, and heart and kidney diseases there are other indications under investigation in clinical trials. Based on preclinical profiling, several proof of concept and Phase 2 trials have been or are being conducted to explore the potential beneficial effects in patients. To explore the potential antifibrotic effects that have been observed in preclinical models of lung and skin fibrosis, Phase 2b trials were initiated. Riociguat was

investigated in patients with symptomatic PH, associated with idiopathic interstitial pneumonias including idiopathic pulmonary fibrosis (RISE-IIP, NCT02138825) (Nathan et al. 2017). Moreover, the effects of riociguat on skin fibrosis in SSc patients (RISE-SSc, NCT2283762) are currently studied. The RISE-IIP study was terminated prematurely due to an unfavorable risk versus benefit ratio in these patients (Nathan et al. 2017). The RISE-SSc study is ongoing with recruiting finished and data expected in 2018. Based on the various modes of actions and expression of sGC in different tissues and organs, smaller studies have been conducted or are underway exploring effects on rare diseases. The effects of the sGC stimulator olinciguat were evaluated in a recently completed exploratory study in patients with achalasia (NCT02931565). Olinciguat and riociguat are also in Phase 2 trials in sickle cell disease patients (NCT03285178 and NCT02633397, respectively). In addition to these more advanced clinical programs, there are still other sGC stimulators in preclinical development that might increase the number of compounds available for the benefit of patients (Friebe et al. 2017).

7.1 sGC Activators

Compared to sGC stimulators, the development pipeline of sGC activators is relatively limited and less advanced. There are no sGC activators in late stage development or approved to date. Multiple sGC activator projects have been terminated in Phase 2. sGC activators have not been explored in chronic heart failure; however, cinaciguat (BAY 58-2667) has been characterized in acute heart failure. Based on promising preclinical results, a Phase 2 study in patients with acute decompensated heart failure (ADHF) was initiated. Continuous intravenous infusion of cinaciguat was well tolerated and resulted in an improvement of cardiopulmonary hemodynamics. The subsequent clinical Phase 2b program studied the effects of cinaciguat in three randomized, double-blind, placebo-controlled studies in ADHF patients; however, the clinical development of cinaciguat was terminated prematurely because of hypotensive events without clear benefit (Breitenstein et al. 2017). The oral sGC activator ataciguat (HMR 1766) was investigated in Phase 2 studies for the treatment of peripheral arterial occlusive disease (PAD) and neuropathic pain. These projects were terminated due at least in part to the long-lasting blood pressure-lowering effects of these compounds in the absence of clear therapeutic benefit. In addition, the understanding of diseases with increased oxidative stress burden as the mode of action of sGC activators is still incompletely understood. Bayer recently reported three sGC activators in Phase 1 with the intention of potentially treating chronic kidney disease pulmonary hypertension and acute respiratory distress syndrome (ARDS). In addition, Boehringer-Ingelheim (BI) recently reported the early development of an sGC activator for chronic kidney disease (Friebe et al. 2017). It will become a very interesting topic in the future, how these compound class of sGC activators – acting on the heme-free sGC – could be differentiated from sGC stimulators. Especially in patients with diseases accompanied by increased oxidative stress burden, this could broaden the treatment potential or increase efficacy.

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