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Synthesis and biological evaluation of oxadiazole derivatives as inhibitors of soluble guanylyl cyclase

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ABSTRACT

Soluble guanylyl cyclase (sGC) is an ubiquitously expressed enzyme that generates the second messenger cGMP and hence, leads to a number of physiological responses including vasodilation, inhibition of platelet aggregation and neurotransmission. Whilst many activating and stimulating modulators of sGC were identified and studied in recent years, only two selective inhibitors are known: ODQ and NS 2028. Furthermore, a synthetic approach to these inhibitors has not been reported yet. Herein, we describe a novel and efficient synthesis of these inhibitors, as well as the preparation of three different classes of NS 2028 analogues. Biological evaluation of this library using rat aortic smooth muscle cells revealed four new compounds with good to moderate sGC inhibitory activity. Our experiments underline the major importance of the oxadiazole ring in ODQ and NS 2028 for the efficiency of this class of inhibitors.

1. Introduction

Endogenously produced nitric oxide participates in a number of cell communication and signal transduction cascades by activation of soluble guanylyl cyclases (sGC). These in turn, generate the second messenger cGMP which can influence physiological processes, as vasodilation or immune response. The NO–sGC–cGMP pathway plays an important role in cell life and has attracted much interest in medicinal research. Hence, several details concerning the mode of action of sGCs and their downstream effects have been collected. It is widely known that sGCs are heterodimeric proteins, consisting of a bigger α and a smaller β subunit. Two isoforms of each subunit have been discovered in different mammalian cells. The α1β1 type has been found to be the most abundant variant of sGCs.[1–5] The β subunit provides the histidine moiety His105, an essential element for the interaction with NO. By coordination of His105 to a prosthetic heme group a complex is formed that serves as NO receptor. It is assumed that NO binding induces the oxidation of ferrous heme to its ferric form, leading to the cleavage of its axial bond with His105 and ultimately, to heme pivoting. This in turn, causes a conformational change that is transmitted to the C-terminus of the enzyme harbouring the catalytic site to increase conversion of GTP to cGMP. The activation of sGC by NO increases the rate of cGMP formation several hundred-fold.[6–9] Once formed, cGMP promotes smooth muscle relaxation, inhibits platelet aggregation and leucocyte adhesion through the vessel wall by modulating the activity of phosphodiesterases, cGMP-dependent protein kinases and cGMP-gated ion channels.[10,11]

Dysfunction of the NO–sGC–cGMP pathway demonstrably induces an array of cardiovascular, immunological or neurodegenerative diseases. Several NO donors are currently in clinical use, although they are limited due to unspecific effects of NO with other biomolecules, lacking efficiency because of insufficient metabolism and long-term resistances development. Hence, the trend goes to novel sGC activators that do not release NO, and do not require the presence of heme. Such compounds are being tested in pulmonary hypertension and peripheral arterial occlusive disease.[12–19]

In contrast to sGC activators only two specific inhibitors are known. The first generation of sGC inhibitors included methylene blue and LY83583. However, later it was shown that these agents do not act as sGC inhibitors, but rather block NO-stimulated cGMP formation through the generation of superoxide anions that react with NO and inactivate it. In the mid-to-late 1990s, two chemically closely related sGC inhibitors became available, ODQ and NS 2028 (Fig. 1).

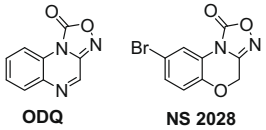

Figure 1. Chemical structures of selective inhibitors ODQ and NS 2028.

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These compounds have big advantages over the first generation of NO inhibitors, as they do not produce superoxide anions, and do not inhibit basal sGC activity. Moreover, they are selective for sGC, since they do not reduce particulate GC activity. Both ODQ and NS 2028 are thought to act as selective heme oxidants, rendering sGC insensitive to NO. These agents are valuable tools in unravelling the role of sGC in physiology and disease. Besides their utility in basic science studies, sGC inhibitors could become useful in treating hypotension, such as that seen during septic shock.[20–23]

Although ODQ and NS 2028 are known for approximately 15 years, their synthesis has not been reported yet. Moreover, the pharmacophore structure of these compounds has not been disclosed in detail. Some analogues of ODQ and NS 2028 have been prepared which were modified at the benzoxazine part. Thus, the molecules contained polar substituents at the phenyl ring or had a benzoxathine core. However, the oxadiazole element was not changed.[23] The aim of the present study is to establish a straightforward and efficient synthesis of ODQ and NS 2028 and furthermore to prepare analogues that provide information on the parts of the ODQ/NS 2028 scaffold that are crucial for sGC inhibitory activity.

2. Results and discussion

2.1. Chemistry

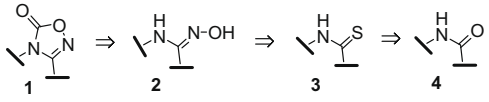
We first planned to establish a straightforward and efficient synthesis of the known inhibitors ODQ and NS 2028. In addition, we were interested in the preparation of a small library of inhibitors belonging to different substance classes in order to study structure–activity relationships. Hence, we intended to compare the inhibitory activity of compounds lacking the common feature in both ODQ/NS 2028 inhibitors with others that maintain this structure element but differ in their general scaffold.

A general retrosynthetic analysis of the oxadiazole ring is depicted in Scheme 1. Most of the desired compounds were synthesized in this manner. In this scheme all compounds are shown in a simplified manner for a better visualisation.

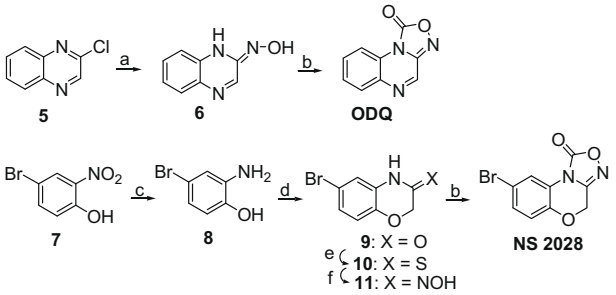
We planned to generate oxadiazole ring **1** from oxime **2** by insertion of CO and the following ring closure. The hydroxylimine functionality in **2** would arise from nucleophilic substitution of thiolactam **3** that in turn, would be generated by thionation of lactam species **4** with Lawesson reagent. The appropriate lactams would be bought from commercial sources or synthesized beforehand.

In the case of ODQ the synthesis of the oxadiazole ring proved to be easier, since oxime **6** was readily prepared in one step from commercially available 2-chloroquinoxaline **5** (Scheme 2). The nucleophilic substitution was performed with hydroxylamine hydrochloride under basic conditions and resulting oxime **6** was converted to ODQ by carbonylation with CDI[24] followed by cyclisation. Thus, ODQ was prepared in two steps with an overall yield of 32%.

The synthesis of NS 2028 started with the reduction of 4-bromo-2-nitrophenol **7** with tin(II)chloride dihydrate in concd hydrochloric acid.[25] Ring closure with chloroacetyl chloride under basic conditions gave rise to 6-bromo-2*H*-1,4-benzoxazin-3(4*H*)-one **9**.



Scheme 1. Retrosynthetic analysis of the oxadiazole element.



Scheme 2. Synthesis of ODQ and NS 2028. Reagents and conditions: (a) $H_2NOH{\cdot}HCl$, Na_2CO_3, DMSO, rt, 24 h (40%); (b) CDI, THF, reflux, 24 h (79% for ODQ; 95% for NS 2028); (c) $SnCl_2{\cdot}2H_2O$, concd HCl, MeOH, 0 °C→rt, 4.5 h (quant.); (d) i—$NaHCO_3$, H_2O, 4-methyl-2-pentanone; ii—chloroacetylchloride, 0 °C→reflux, 4 h (86%); (e) Lawesson reagent, THF, rt, 24 h (95%); (f) $H_2NOH{\cdot}HCl$, Et_3N, EtOH, rt, 24 h (78%).

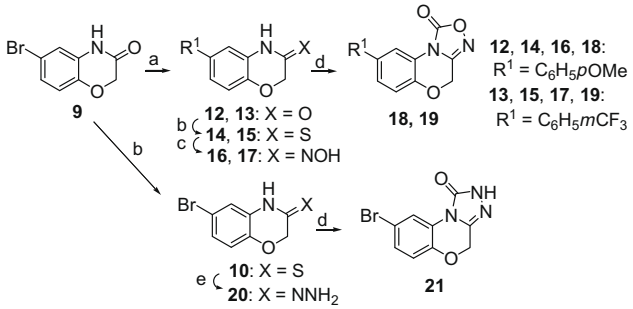
It is worth to point out that the use of 4-methyl-2-pentanone as solvent is important for a satisfactory yield.[26]

Lactam **9** was converted into NS 2028 in three steps according to the described retrosynthesis (Scheme 1). This procedure was performed in analogous way for all following oxadiazole compounds and is herein discussed using the example of NS 2028 (Scheme 2). At first, lactam **9** (respectively, **12** and **13** in Scheme 3, as well as **26** in Scheme 4) was thionated with Lawesson reagent by stirring for 24 h in THF at rt[27] yielding thiolactam **10** in 95% yield (respectively, 70–86%). For compound **23** (Scheme 4) a modified method was used, heating the reaction mixture in toluene at 70 °C increased the yield by 25% and decreased reaction time to 5 h.[28] The following nucleophilic substitution with hydroxylamine hydrochloride under basic conditions provided oxime **11** (respectively, **16**, **17** as well as **24**, **28**) without any problems.[24]

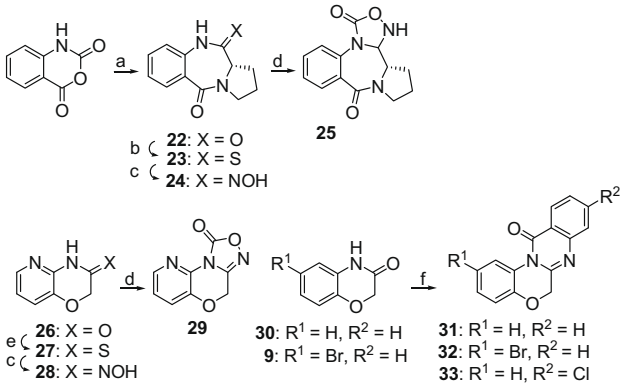
In case of the desired analogue **21** the oxadiazole ring is substituted by a triazole ring (Scheme 3). Therefore, thiolactam **10** was treated with 8 equiv hydrazine monohydrate for 12 h at rt.[29] In that way, hydrazone **20** was obtained in 58% yield.

The last step of the oxadiazole and triazole synthesis was the carbonylative cyclisation. For this purpose oxime **11** (respectively, **16**, **17** and **24**, **28** as well as hydrazone **20**) was treated with CDI in refluxing THF.[25] The oxadiazole compound NS 2028 (respectively, **18**, **19** and **25**, **29**) was prepared in five steps in an overall yield of 61%.

Analogues **18** and **19** were obtained by Suzuki coupling of lactam **9** with corresponding boronic acids (Scheme 3). We first intended to do a coupling as last step of the synthesis, which means with NS 2028, but that strategy was unsuccessful, since



Scheme 3. Synthesis of compounds **18**, **19** and **21**. Reagents and conditions: (a) approp. boronic acid, $Pd(OAc)_2$, PPh_3, Na_2CO_3, 1,4-dioxane/water, reflux, 12 h (96% for **12** and 99% for **13**); (b) Lawesson reagent, THF, rt, 24 h (70–86%); c) $H_2NOH{\cdot}HCl$, Et_3N, EtOH, rt, 24 h (78%); (d) CDI, THF, reflux, 24 h (57–69%); (e) $N_2H_4{\cdot}H_2O$, EtOH, rt, 12 h (58%).



Scheme 4. Synthesis of compounds **25**, **29** and **31**, **32** and **33**. Reagents and conditions: (a) L-proline, DMF, reflux, 4 h (quant.); (b) Lawesson reagent, toluene, 70 °C, 5 h (86%); (c) $H_2NOH{\cdot}HCl$, Et_3N, EtOH, rt, 24 h (76% for both oximes); (d) CDI, THF, reflux, 24 h (84% for **25** and 79% for **29**); (e) Lawesson reagent, THF, rt, 24 h (84%); (f) $POCl_3$, DCE, rt, 15 min, then approp. anthranilic acid, DCE, Et_3N, 0 °C→rt, 1 h, then reflux 1 h (88% for **31**, 67% for **32** and 48% for **33**).

the oxadiazole ring decomposed to oxime **9** by treatment with palladium. Therefore the coupling was done in an earlier step, with intermediate lactam **9**. Hence, we performed the Suzuki reaction in a two phase system (dioxane/water, 3:1, v/v) with palladium(II)acetate as catalytic species, triphenylphosphine as ligand and sodium carbonate as base. As a result we obtained lactams **12** and **13**, which were converted into the oxadiazoles **18** and **19** as described above in 27% and 29% overall yield.

Since benzodiazepines are privileged structures, we intended to investigate the influence of this scaffold on inhibitory activity. Hence, lactam **22** was synthesized by condensation of isatoic anhydride with L-proline[28], which was subsequently converted into oxadiazole **25** by above procedure in an overall yield of 55% (Scheme 4). Furthermore, in an analogous way commercially available 2*H*-pyrido[3,2-*b*][1,4]oxazin-3(4*H*)-one **26** led to compound **29** in 40% overall yield. This compound contains an additional H-bridge donor possibly leading to a stronger interaction with the enzyme. As last substance class, we prepared three differently substituted quinazolinbenzoxazine derivatives **31, 32** and **33**, lacking the oxadiazole structure element (Scheme 4). These compounds were prepared by one pot synthesis according to a procedure of Sastry[30] from either commercially available 2*H*-1,4-benzoxazin-3(4*H*)-one **30** or lactam **9** in an overall yield of 48–88%.

In subsequent biological assays all these compounds were tested on their inhibitory activity against sGC.

2.2. Biological evaluation

The inhibitory activity of the synthesized compounds was evaluated in vitro using rat aortic smooth muscle cells. cGMP levels in resting cultures were low and increased by more than 100-fold after stimulation with SNP. Pre-treatment of cells with ODQ dose-dependently inhibited SNP-stimulated cGMP formation, with the lower ODQ concentration reducing by 85%; the highest ODQ concentration almost abolished NO-stimulated cGMP formation.

From all experiments the most significant results are presented in Figure 2. The benzodiazepine **25**, quinazolinbenzoxazine derivatives **31** and **32** as well as triazole **21** do not exhibit an inhibitory effect on sGC, whilst oxadiazoles **18**, **19** and **29** as well as quinazolinbenzoxazine **33** are good to moderate inhibitors.

Although compound **19** features an extended scaffold, it is still as effective and potent as ODQ. In contrast, compound **18** has lower potency, as it only partially inhibits SNP-induced cGMP formation at 0.1 μM. Presumably, the trifluoromethane moiety of **19** is able to interact better with the enzyme than the methoxy group. The difference in activity could also be associated with the opposed electronic properties of these functional groups: the trifluoromethane residue has a strong electron-withdrawing effect, whereas1 the methoxy group is a good electron-donator. The additional H-bridge donor in compound **29** has also a positive effect on inhibitory activity, showing similar efficiency as ODQ. For compound **25** the expansion of the oxazine ring to a benzodiazepinone element results in a molecule that even at the highest concentration employed was a poor sGC inhibitor (although a trend to decrease cGMP is seen, this does not reach statistical significance at 10 μM). Probably, the additional pyrrolo element and the changed spatial configuration of the seven-membered ring lead to this result.

Substitution of the oxadiazole ring by a quinazoline element generally implicates loss of activity. An exception is compound **33** that is effective in blocking SNP-induced cGMP production when used at high concentrations. An explanation for this observation may be the chloride atom in position 9 of **33** which presumably interacts with the active site of the enzyme and thus blocks it. Surprisingly, compound **21** showed no inhibitory activity on sGCs. It differs from the inhibitor NS 2028 only by substitution of the oxygen atom in the oxadiazole ring by a nitrogen atom. The inactivity of triazole **21** may occur due to the inability of the NH moiety to serve as H-bridge acceptor. Alternatively, it is possible that the oxadiazole derivatives act by covalently binding to the enzyme and blocking its activity; a similar mode of action has been shown to occur in carbamates induced inhibition of acetylcholinesterase.[31] Consequently, the exchange of the oxadiazole structure by a less reactive triazole would lead to a loss activity. In order to obtain support for this hypothesis we incubated human recombinant sGC with NS 2028 and after, proteolytic processing of the sample we subjected it to mass spectrometric analysis. In these experiments, we could not detect any peptide fragment of sGC that is covalently bound to NS 2028 (data not shown); we were, thus, unable to prove our original hypothesis that NS 2028 inhibits sGC after covalent binding to the enzyme.

3. Conclusions

We describe a straightforward and efficient synthesis of the known inhibitors ODQ and NS 2028, as well as a series of different classes of analogues. The biological activity was evaluated in vitro with RASM cells. Thereby two novel inhibitors with good activity have been found, compounds **18** and **19**, as well as inhibitors **29** and **33** that showed to be more moderate. Furthermore, the results of our studies revealed new structure–activity relationships: the crucial role of the oxadiazole ring for inhibitory activity. In particular, compound **21** shows that the exchange of one atom leads to the loss of activity, as it comprises a triazole instead of the oxadiazole element. Considering the chemical reactivity of carbamides in comparison to carbamates this observation may be a reference to a different mode of inhibition.

4. Experimental

4.1. Chemistry

All reagents were commercially obtained from Acros, Sigma–Aldrich, Merck and Fluka and used without further purification unless otherwise stated. Anhydrous solvents were transferred via oven-dried syringe or cannula. Flasks were flame-dried under vacuum and cooled under a constant stream of argon. Melting points were measured with a NAGEMA K8-micro hot stage and are uncorrected. ^1H, ^{13}C and ^{19}F NMR spectra were recorded on Varian

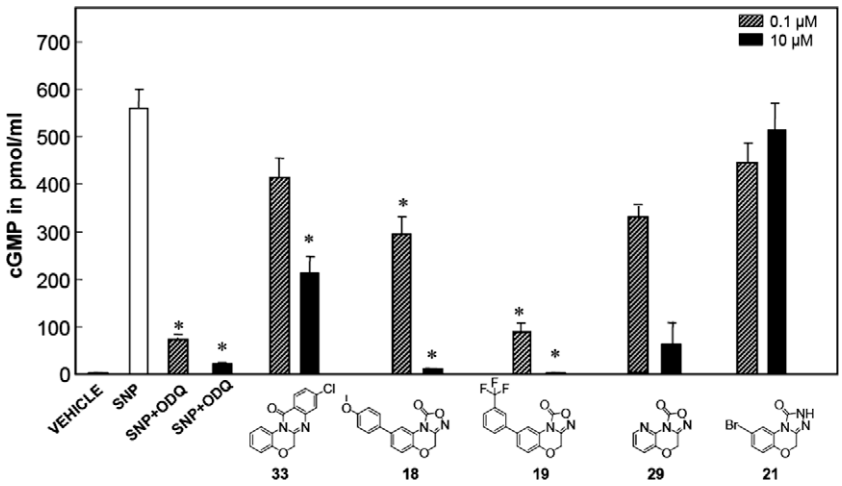


Figure 2. Effect of selected NS 2028/ODQ derivatives on cGMP accumulation. RASM cells were treated with ODQ or the newly synthesized compounds at the indicated concentration for 20 min. Cells were then stimulated with 10 µM SNP for 15 min, in the presence of the phosphodiesterase inhibitor isobutyl-methylxanthine (IBMX; 1 mM), prior to extraction of cGMP with HCl. Values are means ± SEM, $n = 11–12$ wells; $^*p < 0.05$ versus SNP.

Gemini 200 (200 MHz for ^{1}H NMR, 50 MHz for ^{13}C NMR, 188 MHz for ^{19}F NMR), 300 (300 MHz for ^{1}H NMR, 75 MHz for ^{13}C NMR, 228 MHz for ^{19}F NMR) and 400 (400 MHz for ^{1}H NMR, 100 MHz for ^{13}C NMR, 367 MHz for ^{19}F NMR). The chemical shifts are reported relative to the residual solvent peak, which was used as an internal reference (chemical shifts in δ values, J in Hz). HRMS were obtained on a Bruker Daltonics APEX II for ESI and on a Masslab Manchester VG 12-250 for EI. Optical rotations were measured with half automatical polarimeter Polartronic D (Schmidt + Haensch) and are uncorrected. Reactions involving moisture-sensitive reactants were performed in flame-dried glassware under an atmosphere of argon, reactants being added via syringe. Flash column chromatography was performed on silica gel (Acros 60 A, 0.035–0.070 mm) and analytical TLC on pre-coated silica gel plates (Merck 60 F_{254}, 0.25 mm).

4.1.1. 2-Amino-4-bromophenol (8)

A solution of tin(II)chloride dihydrate (41.4 g, 183.7 mmol) and 88 mL concd hydrochloric acid in 160 mL methanol was cooled to 0 °C and 4-bromo-2-nitrophenol **7** (8.0 g, 36.7 mmol) was added in one portion. The mixture was stirred for 4.5 h at room temperature, whereas the yellow solution turned colourless. Afterwards the solution was diluted with ethyl acetate and neutralized with satd $NaHCO_3$ solution (to pH 7). Thereby, a white solid precipitated, which was filtered and washed with ethyl acetate. The organic phase was separated and the water phase was extracted three times with ethyl acetate. The combined organic phases were dried over Na_2SO_4 and the solvent was removed in vacuo to yield pure **8** (6.9 g, 6.74 mmol; quantitative) as a fawn solid. Mp: 130–132 °C; ^{1}H NMR (300 MHz, DMSO-d_6): $\delta = 4.76$ (br, 2H, NH_2); 6.47 (dd, 1H, $^4J = 2.3$ Hz, $^3J = 8.2$ Hz, arom. 5-CH); 6.54 (d, 1H, $^3J = 8.2$ Hz, arom. 6-CH); 6.69 (d, 1H, $^4J = 2.3$ Hz, arom. 3-CH); 9.23 (br, 1H, OH). ^{13}C NMR (100 MHz, DMSO-d_6): $\delta = 110.6$ (arom. 4-CBr); 115.6 (arom. 3-CH); 116.0 (arom. 6-CH); 118.1 (arom. 5-C_q); 138.8 (arom. 2-CNH_2); 143.3 (arom. 1-COH). HRMS-ESI: m/z $[M+H]^-$ calcd for C_6H_5NOBr: 185.95600; found: 185.95563.

4.1.2. 6-Bromo-2*H*-1,4-benzoxazin-3(4*H*)-one (9)

To a suspension of 2-amino-4-bromophenol 8 (8.00 g, 42.55 mmol) in 25 mL 4-methyl-2-pentanone were added $NaHCO_3$ (8.54 g, 101.68 mmol) and 25 mL H_2O. The suspension was cooled to 0 °C and chloroacetylchloride (3.89 mL, 48.93 mmol) was added dropwise. After refluxing for 4 h the mixture was cooled to 0 °C. Thereby fawn crystals precipitated, which were filtered, washed with water and dried in vacuo to yield pure **9** (8.43 g, 36.96 mmol, 86%) as fawn crystals. Mp 221–223 °C. ^{1}H NMR (300 MHz, DMSO-d_6)[32]: $\delta = 4.57$ (s, 2H, 2-CH_2); 6.89 (d, 1H, $^3J = 8.8$ Hz, arom. 8-CH); 7.0 (d, 1H, $^4J = 2.2$ Hz, arom. 5-CH); 7.05 (dd, 1H, $^4J = 2.2$ Hz, $^3J = 8.8$ Hz, arom. 7-CH); 10.78 (br, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6): $\delta = 66.6$ (2-CH_2); 113.2 (arom. 6-CBr); 118.0 (arom. 8-CH and arom. 5-CH); 125.3 (arom. 7-CH); 129.0 (arom. 4a-CNH); 142.6 (arom. 8a-CO); 164.6 (3-C=O). HRMS-ESI: m/z $[M+H]^+$ calcd for $C_8H_6NO_2BrH$: 227.96547; found: 227.96546; $[2M+H]^+$ calcd for $C_{16}H_{12}N_2O_4Br_2H$: 454.92366; found: 454.92376.

4.2. General procedure for the preparation of 2*H*-1,4-benzoxazin-3(4*H*)-one derivatives (12) and (13) via Suzuki reaction

6-Bromo-2*H*-1,4-benzoxazin-3(4*H*)-one **9** and the appropriate boronic acid (1 equiv) were suspended in 1,4-dioxane (3 mL/100 mg) under argon atmosphere. Afterwards water (1 mL/100 mg), 2 M Na_2CO_3 solution (0.44 mL/100 mg), $Pd(OAc)_2$ (0.02 equiv) and triphenylphosphine (0.04 equiv) were added. The reaction mixture was refluxed for 12 h and afterwards extracted three times with ethyl acetate and the combined organic phases were washed with satd $NaHCO_3$ solution and brine. After drying over Na_2SO_4 the solvent was removed in vacuo and the crude product was purified by column chromatography.

4.2.1. 6-(4-Methoxyphenyl)-2*H*-1,4-benzoxazin-3(4*H*)-one (12)

According to the general procedure this compound was prepared from 6-bromo-2*H*-1,4-benzoxazin-3(4*H*)-one **9** (200 mg, 0.88 mmol) and 4-methoxy boronic acid (133 mg, 0.88 mmol) to give pure **12** as a colourless solid (216 mg, 0.85 mmol, 97%). Mp 205 °C. ^{1}H NMR (400 MHz, DMSO-d_6): $\delta = 3.77$ (s, 3H, OCH_3); 4.57 (s, 2H, 2-CH_2); 6.96–7.0 (m, 3H, 2× arom. 3′-CH and arom. 8-CH); 7.08 (d, 1H, $^4J = 2.0$ Hz, arom. 5-CH); 7.12 (dd, 1H, $^4J = 2.0$ Hz, $^3J = 8.3$ Hz, arom. 7-CH); 7.44–7.46 (dd, 2H, $^4J = 2.0$ Hz, $^3J = 6.8$ Hz, 2× arom. 2′-CH); 10.73 (br, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6): $\delta = 55.1$ (OCH_3); 66.8 (2-CH_2); 113.5 (arom. 8-CH); 114.4 (2C, 2× arom. 3′-CH); 116.5 (arom. 5-CH); 120.9 (arom. 7-CH); 127.3 (2C, 2× arom. 2′-CH); 127.6 (arom. 1′-C_q); 132.0 (arom. 4a-CNH); 134.4 (arom. 6-C_q); 142.4 (arom. 8a-CO); 158.7 (arom. 4′-C(OCH_3)); 164.9 (3-C=O). HRMS-ESI: m/z $[M+H]^+$ calcd for $C_{15}H_{13}NO_2H$: 256.09682; found:

256.09686; $[2M+H]^+$ calcd for $C_{30}H_{26}N_2O_4H$: 511.18636; found: 511.18675.

4.2.2. 6-[3-(Trifluoromethyl)phenyl]-2*H*-1,4-benzoxazin-3(4*H*)-one (13)

According to the general procedure this compound was prepared from 6-bromo-2*H*-1,4-benzoxazin-3(4*H*)-one **9** (300 mg, 1.32 mmol) and 3-trifluoromethylphenyl boronic acid (250 mg, 1.32 mmol) to yield pure **13** (381 mg, 1.3 mmol, 99%) as a colourless solid. Mp 227 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 4.61 (s, 2H, 2-CH_2); 7.04 (d, 1H, ^3J = 8.4 Hz, arom. 8-CH); 7.19 (d, 1H, ^4J = 2.2 Hz, arom. 5-CH); 7.28 (dd, 1H, ^4J = 2.2 Hz, ^3J = 8.4 Hz, arom. 7-CH); 7.67 (m, 2H, arom. 4′-CH and arom. 5′-CH); 7.81 (s, 1H, arom. 2′-CH); 7.85 (m, 1H, arom. 6′-CH); 10.74 (br, 1H, NH). APT (100 MHz, DMSO-d_6): δ = 66.8 (2-CH_2); 114.2 (arom. CH); 116.8 (arom. CH); 121.8 (arom. CH); 122.5 (q, ^3J = 4 Hz, arom. CH); 123.7 (q, ^3J = 4 Hz, arom. CH); 124.2 (q, ^1J = 273 Hz, CF_3); 127.8 (arom. C_q); 129.8 (q, ^2J = 32 Hz, arom. 3′-C_q); 130.1 (arom. CH); 130.3 (arom. CH); 132.9 (arom. C_q); 140.6 (arom. C_q); 143.5 (arom. C_q); 164.7 (3-C=O). ^{19}F NMR (282 MHz, DMSO-d_6): δ = −61.55 (CF_3). HRMS-ESI: *m/z* $[M+H]^+$ calcd for $C_{15}H_{10}NO_2F_3H$: 294.07364; found: 294.07373; $[2M+H]^+$ calcd for $C_{30}H_{20}N_2O_4F_6H$: 587.14000; found: 587.13979.

4.2.3. (11a*S*)-2,3-Dihydro-1*H*-pyrrolo[2,1-*c*][1,4]benzodiazepine-5,11(10*H*,11a*H*)-dione (22)

A suspension of isatoic anhydride (2.0 g, 12.26 mmol) and L-proline (1.41 g, 12.26 mmol) in 11 mL DMF was heated to 160 °C for 4 h. The solvent was removed in vacuo and the crude product was purified by column chromatography (dichloromethane/ethyl acetate 2:1 v/v). Benzodiazepine **22** (2.65 g, 12.26 mmol; quantitative) was gained as fawn crystals. Mp 215 °C. $[\alpha]_D^{23}$ +493 (*c* 1.03, MeOH). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.97–2.08 (m, 3H, 2-CH_2, 1-CH_aH_b); 2.72–2.80 (m, 1H, 1-CH_aH_b); 3.56–3.63 (m, 1H, 3-NCH_aH_b); 3.77–3.82 (m, 1H, 3-NCH_aH_b); 4.07 (d, 1H, ^3J = 6.2 Hz, 11a-NCH); 7.04 (d, 1H, ^3J = 8.1 Hz, arom. CH); 7.23–7.27 (m, 1H, arom. CH); 7.46 (m, 1H, arom. CH); 7.99 (dd, 1H, ^4J = 1.6 Hz, ^3J = 7.9 Hz, arom. 6-CH); 8.92 (br, 1H, NH). APT (100 MHz, $CDCl_3$): δ = 23.6 (2-CH_2); 26.4 (1-CH_2); 47.4 (3-NCH_2); 56.8 (11a-NCH); 121.2 (arom. CH); 125.2 (arom. CH); 127.3 (arom. 5a-C_q); 131.3 (arom. CH); 132.6 (arom. CH); 135.5 (arom. 9a-CNH); 165.6 (5-C=O); 171.52 (11-C=O). HRMS-ESI: *m/z* $[M+H]^+$ calcd for $C_{12}H_{12}N_2O_2H$: 217.09715; found: 217.09719; $[2M+H]^+$ calcd for $C_{24}H_{24}N_4O_4H$: 433.18703; found: 433.18697.

4.3. General procedure for the preparation of thiolactams (10), (14), (15), (23) and (27)

To a solution of the appropriate benzoxazine or benzodiazepine derivative in abs. THF (5 mL/mmol) was added Lawesson reagent (0.5 equiv). The yellow suspension was stirred for 12 h, in which a yellowish precipitate has formed. Subsequently, the solvent was removed in vacuo and the crude product was purified by column chromatography.

4.3.1. 6-Bromo-2*H*-1,4-benzoxazine-3(4*H*)-thione (10)

According to the general procedure this compound was prepared from 6-bromo-2*H*-1,4-benzoxazine-3(4*H*)-one **9** (8.40 g, 36.84 mmol) to yield pure **10** (8.56 g (35.07 mmol, 95%) as a yellowish solid. Mp 207 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 4.84 (s, 2H, 2-CH_2); 6.95 (d, 1H, ^3J = 7.8 Hz, arom. 8-CH); 7.16–7.2 (m, 2H, arom. 7-CH and arom. 5-CH); 12.77 (br, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ = 72.2 (2-CH_2); 113.2 (arom. 6-CBr); 118.3 (arom. 8-CH); 118.6 (arom. 5-CH); 127.4 (arom. 7-CH); 127.6 (arom. 4a-CNH); 144.3 (arom. 8a-CO); 191.2 (3-C=S). HRMS-ESI: *m/z* $[M+H]^+$ calcd for $C_8H_6NOBrSH$: 243.94262; found: 243.94257.

4.3.2. 6-(4-Methoxyphenyl)-2*H*-1,4-benzoxazine-3(4*H*)-thione (14)

According to the general procedure this compound was prepared from 6-(4-methoxyphenyl)-2*H*-1,4-benzoxazine-3(4*H*)-one **12** (200 mg, 0.78 mmol) to yield pure **14** (162 mg, 0.6 mmol; 77%) as a yellowish solid. Mp 175 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 4.84 (s, 3H, OCH_3); 4.84 (s, 2H, 2-CH_2); 7.0–7.04 (m, 3H, 2× arom. 3′-CH and arom. 8-CH); 7.24–7.27 (m, 2H, arom. 5-CH and arom. 7-CH); 7.46–7.48 (d, 2H, ^3J = 8.7 Hz, 2× arom. 2′-CH); 12.77 (br, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6): δ = 55.2 (OCH_3); 72.3 (2-CH_2); 114.0 (arom. 8-CH); 114.4 (2C, arom. 3′-CH); 116.7 (arom. 5-CH); 123.0 (arom. 7-CH); 126.6 (arom. 1′-C_q); 127.4 (2C, arom. 2′-CH); 131.7 (arom. 4a-CNH); 134.5 (arom. 6-C_q); 144.0 (arom. 8a-CO); 158.8 (arom. 4′-C(OCH_3)); 190.8 (3-C=S). HRMS-ESI: *m/z* $[M+H]^+$ calcd for $C_{15}H_{13}NO_2SH$: 272.07398; found: 272.07404.

4.3.3. 6-[3-(Trifluoromethyl)phenyl]-2*H*-1,4-benzoxazine-3(4*H*)-thione (15)

According to the general procedure this compound was prepared from 6-[3-(trifluoromethyl)phenyl]-2*H*-1,4-benzoxazine-3(4*H*)-one **13** (300 mg, 1.02 mmol) to yield pure **15** (222 mg, 0.72 mmol; 70%) as a yellowish solid. Mp 185–187 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 4.88 (s, 2H, 2-CH_2); 7.09 (d, 1H, ^3J = 8.3 Hz, arom. 8-CH); 7.38 (d, 1H, ^4J = 2.2 Hz, arom. 5-CH); 7.41 (dd, 1H, ^4J = 2.2 Hz, ^3J = 8.3 Hz, arom. 7-CH); 7.68–7.7 (m, 2H, arom. 4′-CH and arom. 5′-CH); 7.83 (s, 1H, arom. 2′-CH); 7.84–7.87 (m, 1H, arom. 6′-CH); 12.77 (br, 1H, NH). APT (100 MHz, DMSO-d_6): δ = 72.3 (2-CH_2); 114.8 (arom. CH); 117.0 (arom. CH); 122.7 (q, ^3J = 4 Hz, arom. CH); 123.9 (2C, 2× arom. CH); 124.1 (q, ^1J = 273 Hz, CF_3); 126.8 (arom. C_q); 129.8 (q, ^2J = 32 Hz, 3′-C_q); 130.2 (arom. CH); 130.4 (arom. CH); 133.0 (arom. C_q); 140.3 (arom. C_q); 145.2 (arom. C_q); 190.8 (3-C=S). ^{19}F NMR (188 MHz, DMSO-d_6): δ = −61.76 (CF_3). HRMS-ESI: *m/z* $[M+H]^+$ calcd for $C_{15}H_{10}NO_2F_3SH$: 310.05080; found: 310.05088.

4.3.4. (11a*S*)-11-Thioxo-1,2,3,10,11,11a-hexahydro-5*H*-pyrrolo[2,1-*c*][1,4]ben-zodiazepin-5-one (23)

To a suspension of 2,3-dihydro-1*H*-pyrrolo[2,1-*c*][1,4]-benzodiazepine-5,11(10*H*,11a*H*)-dione **22** (1.0 g, 4.62 mmol) in 100 mL toluene were added Lawesson reagent (0.94 g, 2.31 mmol). The yellow suspension was heated to 70 °C for 5 h. After this time a yellow solid has precipitated which was recrystallized from ethanol to yield pure **23** (923 mg, 3.97 mmol; 86%) as yellow crystals. Mp 253–254 °C. $[\alpha]_D^{23}$ +873 (*c* 1.026, DMSO). ^{1}H NMR (200 MHz, DMSO-d_6): δ = 1.84–2.07 (m, 3H, 2-CH_2, 1-CH_aH_b); 2.54 (m, 1H, 1-CH_aH_b); 3.4–3.5 (m, 2H, 3-NCH_2); 4.24 (d, 1H, ^3J = 6.0 Hz, 11a-NCH); 7.23–7.34 (m, 2H, 2× arom. CH); 7.50–7.58 (m, 1H, arom. CH); 7.8 (dd, 1H, ^4J = 1.7 Hz, ^3J = 7.7 Hz, arom. 6-CH); 12.43 (br, 1H, NH). APT (100 MHz, DMSO-d_6): δ = 22.7 (2-CH_2); 29.0 (1-CH_2); 46.8 (3-NCH_2); 59.8 (11a-NCH); 121.8 (arom. CH); 125.6 (arom. CH); 127.7 (arom. 5a-C_q); 130.2 (arom. CH); 132.2 (arom. CH); 136.4 (arom. 9a-CNH); 164.2 (5-C=O); 202.0 (11-C=S). HRMS-ESI: *m/z* $[M+Na]^+$ calcd for $C_{12}H_{12}N_2OSNa$: 255.05625; found: 255.05642; $[2M+Na]^+$ calcd for $C_{24}H_{24}N_4O_2S_2Na$: 487.12329; found: 487.12355.

4.3.5. 2*H*-Pyrido[3,2-*b*][1,4]oxazine-3(4*H*)-thione (27)

The preparation was done according to the general procedure starting from 2*H*-pyrido[3,2-*b*][1,4]oxazin-3(4*H*)-one 26 (1.0 g, 6.66 mmol). The yellow precipitate of **27** (933.7 mg, 5.62 mmol; 84%) was filtered, washed with cold *n*-hexane and dried in vacuo. Mp >239 °C (dec).[33] ^{1}H NMR (200 MHz, DMSO-d_6): δ = 4.9 (s, 2H,

2-CH_2); 7.08 (dd, 1H, ^3J = 4.9 Hz, ^3J = 7.9 Hz, arom. 7-CH); 7.4 (dd, 1H, ^4J = 1.2 Hz, ^3J = 7.9 Hz, arom. 8-CH); 7.97 (dd, 1H, ^4J = 1.2 Hz, ^3J = 4.9 Hz, arom. 6-CH); 13.13 (br, 1H, NH). ^{13}C NMR (50 MHz, DMSO-d_6): δ = 72.3 (2-CH_2); 120.9 (arom. 7-CH); 123.6 (arom. 8-CH); 140.1 (arom. 8a-CO); 141 (arom. 4a-C=N(NH)); 141.1 (arom. 6-CH); 193.6 (3-C=S). HRMS-EI: *m/z* $[M]^+$ calcd for $C_7H_6N_2OS$: 166.02009; found: 166.01802.

4.3.6. 6-Bromo-3-hydrazono-3,4-dihydro-2*H*-1,4-benzoxazine (20)

To a solution of 6-bromo-2*H*-1,4-benzoxazine-3(4*H*)-thione **10** (214.8 mg, 0.88 mmol) in 4 mL ethanol were added hydrazine monohydrate (352.4 mg, 7.04 mmol). The yellow suspension was stirred at room temperature for 12 h. After removal of the solvent in vacuo the crude product was washed with ethyl acetate to give 20 (123.6 mg, 0.51 mmol; 58%) as colourless solid. Mp 261 °C. ^{1}H NMR (200 MHz, DMSO-d_6): δ = 4.7 (s, 2H, 2-CH_2); 6.83 (d, 1H, ^3J = 8.1 Hz, arom. 8-CH); 6.95 (dd, 1H, ^4J = 2.2 Hz, ^3J = 8.1 Hz, arom. 7-CH); 7.39 (d, 1H, ^4J = 2.2 Hz, arom. 5-CH); 9.89 (br, 1H, NH). ^{13}C NMR (50 MHz, DMSO-d_6): δ = 65.5 (2-CH_2); 113.7 (arom. 6-CBr); 118.0 (arom. CH); 118.1 (arom. CH); 123.3 (arom. CH); 130.2 (arom. 4a-CNH); 143.6 (arom. 8a-CO); 145.7 (3-C=N). HRMS-ESI: *m/z* $[M+H]^+$ calcd for $C_8H_8N_3OBrH$: 241.99253; found: 241.98122.

4.4. General procedure for the preparation of oximes (11), (16), (17), (24) and (28)

The appropriate thiolactam was dissolved in ethanol (2 mL/mmol) and hydroxylamine hydrochloride (2 equiv) was added. After cooling to 0 °C triethyl amine (2 equiv) was added. The light yellow suspension was stirred at room temperature for 24 h, whereupon the mixture decolourised and H_2S was released. The solvent was removed in vacuo, the residue taken with dichloromethane and washed three times with water. The organic phase was dried with Na_2SO_4 and the solvent removed in vacuo. The crude product was purified by column chromatography.

4.4.1. 6-Bromo-2*H*-1,4-benzoxazin-3(4*H*)-one-oxime (11)

According to the general procedure this compound was prepared from 6-bromo-2*H*-1,4-benzoxazine-3(4*H*)-thione **10** (8.50 g, 34.82 mmol) to yield **11** (6.57 g, 27.02 mmol, 78%) as a colourless solid. Mp 196–198 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ = 4.47 (s, 2H, CH_2), 6.79 (d, 1H, ^3J = 9.0 Hz, arom. 8-CH), 6.86 (dd, 1H, ^3J = 9.6 Hz, ^4J = 2.4 Hz, arom. 7-CH), 7.22 (d, 1H, ^4J = 2.1 Hz, arom. 5-CH), 9.56 (s, 1H, OH), 10.01 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ = 63.3 (2-CH_2); 13.6 (arom. 6-CBr); 117.5 (arom. 8-CH); 118.1 (arom. 5-CH); 122.5 (arom. 7-CH); 130.6 (arom. 4a-CNH); 140.9 (arom. 8a-CO); 143.4 (3-C=N). HRMS-ESI: *m/z* $[M+H]^+$ calcd for $C_8H_7N_2OBrH$: 242.97691; found: 242.97633.

4.4.2. 6-(4-Methoxyphenyl)-2*H*-1,4-benzoxazin-3(4*H*)-one-oxime (16)

According to the general procedure this compound was prepared from 6-(4-methoxyphenyl)-2*H*-1,4-benzoxazine-3(4*H*)-thione 14 (100 mg, 0.37 mmol) to yield **16** (77 mg, 0.29 mmol, 77%) as colourless needles. Mp 207–208 °C. ^{1}H NMR (300 MHz, acetone-d_6): δ = 3.82 (s, 3H, OCH_3); 4.52 (s, 2H, 2-CH_2); 6.92 (d, 1H, ^3J = 8.3 Hz, arom. 8-CH); 6.99 (m, 2H, 2× arom. 3′-CH); 7.05 (dd, 1H, ^4J = 2.2 Hz, ^3J = 8.3 Hz, arom. 7-CH); 7.4 (d, 1H, ^4J = 2.2 Hz, arom. 5-CH); 7.50–7.53 (dd, ^4J = 1.9 Hz, ^3J = 6.8 Hz, 2H, 2× arom. 2′-CH); 8.57 (br, 1H, OH); 9.08 (br, 1H, NH). APT (100 MHz, acetone-d_6): δ = 55.6 (OCH_3); 64.9 (2-CH_2); 114.2 (arom. 3′-CH); 114.3 (arom. 3′-CH); 115.1 (arom. 8-CH); 117.6 (arom. 5-CH); 119.8 (arom. 7-CH); 128.4 (2C, 2× arom. 2′-CH); 130.0 (arom. 1′-C_q); 134.0 (arom. 4a-CNH); 136.3 (arom. 6-C_q); 143.2 (arom. 8a-CO); 144.8 (3-C=N); 160.1 (arom. 4′-C(OCH_3)). HRMS-ESI: *m/z* $[M+H]^+$ calcd for $C_{15}H_{14}N_2O_3H$: 271.10772; found: 271.10790.

4.4.3. 6-[3-(Trifluoromethyl)phenyl]-2*H*-1,4-benzoxazin-3(4*H*)-one-oxime (17)

According to the general procedure this compound was prepared from 6-[3-(trifluoromethyl)phenyl]-2*H*-1,4-benzoxazine-3(4*H*)-thione **15** (200 mg, 0.65 mmol) to yield **17** (148 mg, 0.48 mmol, 74%) as a colourless solid. Mp 121 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 4.52 (s, 2H, 2-CH_2); 6.96 (d, 1H, ^3J = 8.3 Hz, arom. 8-CH); 7.12 (dd, 1H, ^4J = 2.1 Hz, ^3J = 8.3 Hz arom. 7-CH); 7.48 (d, 1H, ^4J = 2.1 Hz, arom. 5-CH); 7.66 (m, 2H, arom. 4′-CH and arom. 5′-CH); 7.8 (s, 1H, arom. 2′-CH); 7.83–7.85 (m, 1H, arom. 6′-CH); 9.42 (br, 1H, OH); 9.92 (br, 1H, NH). APT (100 MHz, DMSO-d_6): δ = 63.7 (2-CH_2); 113.8 (arom. CH); 116.8 (arom. CH); 118.9 (arom. CH); 122.4 (q, ^3J = 4 Hz, arom. CH); 123.5 (q, ^3J = 4 Hz, arom. CH); 124.2 (q, ^1J = 273 Hz, CF_3); 129.4 (arom. C_q); 129.7 (q, ^2J = 33 Hz, arom. 3′-C_q); 130.0 (arom. CH); 130.2 (arom. CH); 132.9 (arom. C_q); 141.0 (arom. C_q); 141.4 (arom. C_q); 144.4 (arom. 3-C=N). ^{19}F NMR (376 MHz, DMSO-d_6): δ = −61.55 (CF_3). HRMS-ESI: *m/z* $[M+H]^+$ calcd for $C_{15}H_{11}N_2O_2F_3H$: 309.08454; found: 309.08452.

4.4.4. (11a*S*)-2,3-Dihydro-1*H*-pyrrolo[2,1-*c*][1,4]benzodiazepine-5,11(10*H*,11a*H*)-dione-11-oxime (24)

According to the general procedure this compound was prepared from (11a*S*)-11-thioxo-1,2,3,10,11,11a-hexahydro-5*H*-pyrrolo[2,1-*c*][1,4]benzodiazepin-5-one 23 (500 mg, 2.15 mmol) to yield **24** (380 mg, 1.64 mmol, 76%) as colourless crystals. Mp 132 °C. $[\alpha]_D^{23}$ +427 (*c*, 1.026 in MeOH). ^{1}H NMR (400 MHz, DMSO-d_6): δ = 1.84–2.0 (m, 3H, 2-CH_2, 1-CH_aH_b); 2.55–2.59 (m, 1H, 1-CH_aH_b); 3.47–3.6 (m, 2H, 3-NCH_2); 4.29–4.31 (dd, 1H, ^4J = 2.3 Hz, ^3J = 7.8 Hz, 11a-NCH); 7.0–7.08 (m, 1H, arom. CH); 7.23–7.25 (m, 1H, arom. CH); 7.34–7.39 (m, 1H, arom. CH); 7.66 (dd, 1H, ^4J = 1.6 Hz, ^3J = 7.8 Hz, arom. 6-CH); 8.69 (br, 1H, OH); 10.03 (br, 1H, NH). APT (100 MHz, DMSO-d_6): δ = 23.0 (2-CH_2); 25.5 (1-CH_2); 46.8 (3-NCH_2); 54.0 (11a-NCH); 121.2 (arom. CH); 121.7 (arom. CH); 125.1 (arom. 5a-C_q); 130.4 (arom. CH); 131.9 (arom. CH); 138.2 (arom. 9a-CNH); 149.0 (11-C=N); 165.2 (5-C=O). HRMS-ESI: *m/z* $[M+Na]^+$ calcd for $C_{12}H_{13}N_3O_2Na$: 254.09000; found: 254.09013; $[2M+Na]^+$ calcd for $C_{24}H_{26}N_6O_4Na$: 485.19077; found: 485.19087.

4.4.5. 2*H*-Pyrido[3,2-*b*][1,4]oxazin-3(4*H*)-one-oxime (28)

According to the general procedure this compound was prepared from 2*H*-Pyrido[3,2-*b*][1,4]oxazine-3(4*H*)-thione **27** (550 mg, 3.31 mmol) to yield **28** (400 mg, 2.42 mmol, 76%) as a colourless powder. Mp 253 °C. ^{1}H NMR (200 MHz, DMSO-d_6): δ = 4.56 (s, 2H, 2-CH_2); 6.83 (dd, 1H, ^3J = 4.9 Hz, ^3J = 7.9 Hz, arom. 7-CH); 7.24 (dd, 1H, ^4J = 1.4 Hz, ^3J = 7.9 Hz, arom. 8-CH); 7.83 (dd, 1H, ^4J = 1.4 Hz, ^3J = 4.9 Hz, arom. 6-CH); 9.59 (br, 1H, OH); 10.15 (br, 1H, NH). ^{13}C NMR (50 MHz, DMSO-d_6): δ = 63.5 (2-CH_2); 116.9 (arom. 7-CH); 122.9 (arom. 8-CH); 139.9 (arom. 8a-CO); 141.0 (arom. 6-CH); 141.1 (arom. 4a-C=N(NH)); 142.6 (3-C=N). HRMS-EI: *m/z* $[M]^+$ calcd for $C_7H_7N_3O_2$: 165.05386; found: 165.05375.

4.4.6. Quinoxalin-2(1*H*)-one oxime (6)

To a solution of 2-chloroquinoxaline **5** (1 g, 6.08 mmol) in 4 mL dimethyl sulfoxide were added hydroxylamine hydrochloride (0.84 g, 12.16 mmol) and anhydrous sodium carbonate (0.71 g, 6.69 mmol). The mixture was stirred at room temperature for 24 h. Afterwards the solution was diluted with water and extracted several times with dichloromethane. The combined organic phases were washed with brine and dried over $MgSO_4$. After concentration the solution was stored in the refrigerator over night. The yellow precipitate of **6** (388 mg, 2.4 mmol; 40%) was collected by filtration

and dried in vacuo. Mp 183 °C (dec). ^{1}H NMR (400 MHz, DMSO-d_6) δ = 6.88–6.92 (m, 1H, arom. 8-CH), 7.18–7.20 (m, 2H, arom. 6-CH, arom. 7-CH), 7.34 (d, 1H, ^3J = 7.2 Hz, arom. 5-CH), 7.80 (d, 1H, ^4J = 2.4 Hz, arom. 3-CH), 10.14 (br, 1H, OH), 10.41 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ = 114.4 (arom. CH); 120.9 (arom. CH); 128.0 (arom. CH); 129.4 (arom. CH); 132.5 (arom. C_q); 133.0 (arom. C_q); 142.4 (N=CH); 150.1 (C=N(NH)). HRMS-EI: *m/z* $[M]^+$ calcd for $C_8H_7N_3O$ 161. 05892; found: 161.05903.

4.5. General procedure for the preparation of triazole (21) and oxadiazoles ODQ, NS 2028, (18), (19), (25) and (29)

In an argon atmosphere the appropriate oxime or hydrazone was dissolved in abs. THF (4 mL/mmol) and subsequently treated with 1,1′-carbonyl diimidazole (1.1 equiv). The reaction mixture was refluxed for 24 h. Subsequently, the solvent was removed in vacuo, the residue taken with dichloromethane and washed three times with water. The organic phase was dried over Na_2SO_4 and the solvent was removed in vacuo. The crude product was purified by column chromatography.

4.5.1. 8-Bromo-2,4-dihydro-1*H*-[1,2,4]triazolo[3,4-*c*][1,4]-benzoxazin-1-one (21)

According to the general procedure this compound was prepared from 6-bromo-3-hydrazono-3,4-dihydro-2*H*-1,4-benzoxazine **20** (174 mg, 0.72 mmol) to yield **21** (86.1 mg, 0.32 mmol, 63%) as colourless needles. Mp 232 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ = 5.14 (s, 2H, 4-CH_2); 7.08 (d, 1H, ^3J = 8.2 Hz, arom. 6-CH); 7.34 (dd, 1H, ^4J = 2.3 Hz, ^3J = 8.2 Hz, arom. 7-CH); 8.2 (d, 1H, ^4J = 2.3 Hz, arom. 9-CH); 12.17 (br, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6): δ = 61.3 (4-CH_2); 113.6 (8-CBr); 118.3 (arom. 9-CH); 119.4 (arom. 6-CH); 124.3 (arom. 9a-CN); 128.7 (arom. 7-CH); 138.3 (3a-C=N(N)); 144.1 (arom. 5a-CO); 151.3 (1-C=O). IR (neat) 2923, 1647, 1462 cm^{-1}. HRMS-ESI: *m/z* $[M+H]^+$ calcd for $C_9H_6N_3O_2BrH$: 267.97162; found: 267.97179; $[2M+H]^+$ calcd for $C_{18}H_{12}N_6O_4BrH$: 534.93595; found: 534.93596.

4.5.2. 8-Bromo-4*H*-[1,2,4]oxadiazolo[3,4-*c*]benzoxazin-1-one (NS 2028)

According to the general procedure this compound was prepared from 6-bromo-2*H*-1,4-benzoxazin-3(4*H*)-one-oxime **11** (4.00 g, 16.45 mmol) to yield NS 2028 (4.21 g, 15.65 mmol, 95%) as a colourless solid. Mp 163–164 °C. ^{1}H NMR (200 MHz, DMSO-d_6): δ = 5.36 (s, 2H, 4-CH_2), 7.18 (d, 1H, ^3J = 8.8 Hz, arom. 6-CH), 7.47 (dd, 1H, ^3J = 8.8 Hz, ^4J = 2.2 Hz, arom. 7-CH), 8.02 (d, 1H, ^4J = 2.2 Hz, arom. 9-CH). ^{13}C NMR (75 MHz, DMSO-d_6): δ = 59.9 (4-CH_2), 113.8 (arom. 8-CBr), 117.9 (arom. 9-CH), 119.7 (arom. 6-CH), 122.5 (arom. 9a-C-N), 129.9 (arom. 7-CH), 144.0 (arom. 5a-C-O), 151.1 (3a-C=N(N)), 153 (1-C=O). IR (neat) 1782, 1627, 1493 cm^{-1}. HRMS-ESI: *m/z* $[M+Na]^+$ calcd for $C_9H_4N_2O_3Na$: 290.93758; found: 290.93772; $[2M+Na]^+$ calcd for $C_{18}H_8N_4O_6Na$: 558.88593; found: 558.88599.

4.5.3. 8-(4-Methoxyphenyl)-4*H*-[1,2,4]oxadiazolo[3,4-*c*][1,4]-benzoxazin-1-one (18)

According to the general procedure this compound was prepared from 6-(4-methoxyphenyl)-2*H*-1,4-benzoxazin-3(4*H*)-one-oxime **16** (70 mg, 0.26 mmol) to yield **18** (54 mg, 0.2 mmol, 57% concerning conversion) as colourless needles. Mp 209 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 3.86 (s, 3H, OCH_3); 5.12 (s, 2H, 4-CH_2); 6.98 (dd, 2H, ^4J = 1.9 Hz, ^3J = 6.8 Hz, 2× arom. 3′-CH); 7.16 (d, 1H, ^3J = 8.0 Hz, arom. 6-CH); 7.41 (dd, 1H, ^4J = 2.0 Hz, ^3J = 8.0 Hz, arom. 7-CH); 7.51 (dd, 2H, ^4J = 1.9 Hz, ^3J = 6.8 Hz, 2× arom. 2′-CH); 8.27 (d, 1H, ^4J = 2.0 Hz, arom. 9-CH). APT (100 MHz, $CDCl_3$): δ = 55.5 (OCH_3); 60.3 (4-CH_2); 114.5 (2C, 2× arom. 3′-CH); 114.6 (arom. 9-CH); 118.3 (arom. 6-CH); 121.5 (arom. 9a-CN); 126.0 (arom. 7-CH); 128.2 (2C, 2× arom. 2′-CH); 131.9 (arom. 1′-C_q); 137.3 (arom. 8-C_q); 143.6 (arom. 5a-CO); 151.0 (3a-C=N(N)); 154.3 (arom. 1-C=O); 159.7 (arom. 4′-C(OCH_3)). IR (neat) 1769, 1634, 1498 cm^{-1}. HRMS-ESI: *m/z* $[M+Na]^+$ calcd for $C_{16}H_{12}N_2O_4Na$: 319.06948; found: 319.06900; $[2M+Na]^+$ calcd for $C_{32}H_{24}N_4O_8Na$: 615.14918; found: 615.14858.

4.5.4 8-[3-(Trifluoromethyl)phenyl]-4*H*-[1,2,4]oxadiazolo[3,4-*c*]-benzoxazin-1-one (19)

According to the general procedure this compound was prepared from 6-[3-(trifluoromethyl)phenyl]-2*H*-1,4-benzoxazin-3(4*H*)-one-oxime **17** (100 mg, 0.32 mmol) to yield **19** (52 mg, 0.16 mmol, 69% concerning conversion) as colourless needles. Mp 163 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 5.16 (s, 2H, 4-CH_2); 7.22 (d, 1H, ^3J = 8.4 Hz, arom. 6-CH); 7.47 (dd, 1H, ^4J = 2.1 Hz, ^3J = 8.4 Hz, arom. 7-CH); 7.54–7.66 (m, 2H, arom. 4′-CH and arom. 5′-CH); 7.74–7.78 (m, 2H, arom. 2′-CH and arom. 6′-CH); 8.32 (d, 1H, ^4J = 2.1 Hz, arom. 9-CH). APT (75 MHz, $CDCl_3$): δ = 60.4 (4-CH_2); 115.2 (arom. 9-CH); 118.6 (arom. 6-CH); 121.7 (arom. 9a-CN); 123.9 (q, ^3J = 4 Hz, arom. 2′-CH); 124.2 (q, ^1J = 273 Hz, CF_3); 124.7 (q, ^3J = 4 Hz, arom. 4′-CH); 126.7 (arom. 7-CH); 129.7 (arom. 5′-CH); 130.5 (arom. 6′-CH); 131.6 (q, ^2J = 33 Hz, arom. 3′-C_q); 136.1 (arom. 8-C_q); 140.2 (arom. 1′-C_q); 144.6 (arom. 5a-CO); 150.7 (3a-C=N(N)); 154.2 (1-C=O). ^{19}F NMR (282 MHz, $CDCl_3$): δ = −63.03 (CF_3). IR (neat) 1777, 1634, 1488 cm^{-1}. HRMS-ESI: *m/z* $[M+Na]^+$ calcd for $C_{16}H_9N_2O_3F_3Na$: 357.04574; found: 357.04590; $[2M+Na]^+$ calcd for $C_{32}H_{18}N_4O_6F_6Na$: 691.10227; found: 691.10284.

4.5.5. (13a*S*)-11,12,13,13a-Tetrahydro-3*H*,9*H*-[1,2,4]oxadiazolo-[4,3-*a*]pyrrolo [2,1-*c*]-[1,4]-benzodiazepine-3,9-dione (25)

According to the general procedure this compound was prepared from (11a*S*)-2,3-dihydro-1*H*-pyrrolo[2,1-*c*][1,4]benzodiazepine-5,11(10*H*,11a*H*)-dione-11-oxime **24** (231 mg, 1 mmol) to yield **25** (164 mg, 0.64 mmol, 84%) as a colourless solid. Mp 176 °C. $[\alpha]_D^{23}$ +158 (*c* 1.098, $CHCl_3$). ^{1}H NMR (400 MHz, DMSO-d_6): δ = 2.04–2.13 (m, 2H, 12-CH_2); 2.27–2.32 (m, 1H, 13-CH_aH_b); 2.74–2.78 (m, 1H, 13-CH_aH_b); 3.63–3.68 (m, 1H, 11-NCH_aH_b); 3.80–3.86 (m, 1H, 11-NCH_aH_b); 4.54–4.57 (dd, 1H, ^4J = 3.0 Hz, ^3J = 8.4 Hz, 13a-NCH); 7.43–7.46 (m, 1H, arom. 7-CH); 7.56–7.61 (m, 1H, arom. 6-CH); 7.74 (d, 1H, ^3J = 8.0 Hz, arom. 5-CH); 7.94–7.96 (dd, 1H, ^4J = 1.3 Hz, ^3J = 7.7 Hz, arom. 8-CH). ^{13}C NMR (100 MHz, DMSO-d_6): δ = 23.5 (12-CH_2); 25.7 (13-CH_2); 47.9 (11-NCH_2); 51.3 (13a-NCH); 122.5 (arom. 5-CH); 128.7 (arom. 8a-C_q); 128.8 (arom. 7-CH); 128.8 (arom. 4a-CN); 132.2 (arom. 8-CH); 132.8 (arom. 6-CH); 156.4 (3-C=O); 158.2 (13b-C=N(N)); 164.2 (6-C=O). IR (neat) 1770, 1620 cm^{-1}. HRMS-ESI: *m/z* $[M+H]^+$ calcd for $C_{13}H_{11}N_3O_3H$: 258.08787; found: 258.08759; $[2M+H]^+$ calcd for $C_{26}H_{22}N_6O_6H$: 515.16791; found: 515.16709.

4.5.6. 6*H*-[1,2,4]Oxadiazolo[4,3-*d*]pyrido[3,2-*b*][1,4]oxazin-9-one (29)

According to the general procedure this compound was prepared from 2*H*-pyrido[3,2-*b*][1,4]oxazin-3(4*H*)-one-oxime **28** (310 mg, 1.88 mmol) to yield **29** (281 mg, 1.47 mmol, 79%) as colourless crystals. Mp 215 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ = 5.34 (s, 2H, 6-CH_2); 7.34 (dd, 1H, ^4J = 4.7 Hz, ^3J = 8.2 Hz, arom. 3-CH); 7.62 (dd, 1H, ^4J = 1.5 Hz, ^3J = 8.2 Hz, arom. 4-CH); 8.15 (dd, 1H, ^4J = 1.5 Hz, ^3J = 4.7 Hz, arom. 2-CH). APT (75 MHz, DMSO-d_6): δ = 60.7 (6-CH_2); 124.1 (arom. 3-CH); 126.3 (arom. 4-CH); 136.5 (arom. 10a-CN); 141.9 (arom. 4a-CO); 142.3 (arom. 2-CH); 152.5 (9-C=O); 153.0 (6a-C=N(N)). IR (neat) 1775, 1633, 1450 cm^{-1}. HRMS-ESI: *m/z* $[M+Na]^+$ calcd for $C_8H_5N_3O_3Na$: 214.02231; found: 214.02246; $[2M+Na]^+$ calcd for $C_{16}H_{10}N_6O_6Na$: 405.05540; found: 405.05579.

4.5.7. 1*H*-[1,2,4]Oxadiazolo[4,3-*a*]quinoxalin-1-one (ODQ)

According to the general procedure this compound was prepared from quinoxalin-2(1*H*)-one oxime **6** (40 mg, 0.25 mmol) to yield ODQ (45 mg, 0.24 mmol, 97%) as fawn crystals. Mp 163–164 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.55 (td, 1H, ^4J = 1.2 Hz, ^3J = 7.8 Hz, arom. 7-CH_2); 7.66 (td, 1H, ^4J = 1.5 Hz, ^3J = 7.8 Hz, arom. 8-CH_2); 7.92 (dd, 1H, ^4J = 1.2 Hz, ^3J = 7.8 Hz, arom. 6-CH); 8.54 (dd, 1H, ^4J = 1.2 Hz, ^3J = 8.4 Hz, arom. 9-CH); 8.68 (s, 1H, 4-CH). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 114.8 (arom. CH); 124.5 (arom. C_q); 127.8 (arom. CH); 130.6 (arom. CH); 131.9 (arom. CH); 134.8 (arom. C_q); 141.0 (4-CH); 146.7 (C=N(N)); 154.3 (C=O). IR (neat) 1780, 1769, 1573, 1462 cm^{-1}. HRMS-ESI: *m/z* $[M+Na]^+$ calcd for $C_9H_5N_3O_2Na$: 210.02740; found: 210.02732; $[2M+Na]^+$ calcd for $C_{18}H_{10}N_6O_4Na$: 397.06557; found: 397.06584.

4.6. General procedure for the preparation of 12-oxoquinazolino-benzoxazines (31), (32) and (33)[30]

A suspension of the appropriate 2*H*-1,4-benzoxazin-3(4*H*)-one derivative and phosphoryl chloride (1.5 eq) in dichloroethane (1 mL/mmol) was stirred for 15 min at room temperature. Subsequently triethyl amine (1.5 equiv) and anthranilic acid (1 equiv) were added dropwise at 0 °C. The resulting suspension was stirred for one hour at room temperature and then it was refluxed for another hour. After dilution of the mixture with dichloroethane and cold water the organic phase was separated and washed with 10% solution of sodium carbonate and water. The organic phase was dried over Na_2SO_4 and filtered, the solvent was removed in vacuo yielding the crude product which was recrystallized from ethanol.

4.6.1. 12-Oxo-6,12-dihydroquinazolino[2,3-*c*][1,4]benzoxazine (31)

According to the general procedure this compound was prepared from 2*H*-1,4-benzoxazin-3(4*H*)-one **30** (1.49 g, 10 mmol) and anthranilic acid (1.37 g, 10 mmol) to yield **31** (2.19 g, 8.75 mmol, 88%) as yellowish needles. Mp 123 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 4.98 (s, 2H, 6-CH_2); 7.13–7.18 (m, 2H, arom. 2-CH and arom. 4-CH); 7.23–7.27 (m, 1H, arom. 3-CH); 7.52 (m, 1H, arom. 10-CH); 7.66 (dd, ^4J = 0.8 Hz, ^3J = 8.0 Hz, 1H, arom. 8-CH); 7.77 (m, 1H, arom. 9-CH); 8.37 (dd, 1H, ^4J = 1.2 Hz, ^3J = 8.0 Hz, arom. 11-CH); 8.59 (dd, 1H, ^4J = 1.6 Hz, ^3J = 8.4 Hz, arom. 1-CH). APT (100 MHz, $CDCl_3$): δ = 69.5 (6-CH_2); 117.8 (arom. 4-CH); 122.5 (arom. 11a-C_q); 122.9 (arom. 2-CH); 123.5 (arom. 1-CH); 125.2 (arom. 13a-CN); 127.1 (arom. 8-CH); 127.6 (arom. 10-CH); 127.7 (arom. 11-CH); 128.0 (arom. 3-CH); 134.9 (arom. 9-CH); 146.1 (arom. 7a-C=N); 149.0 (arom. 6a-C=N(N)); 149.3 (arom. 4a-CO); 160.0 (12-C=O). IR (neat) 1689, 1625, 1490, 1352 cm^{-1}. HRMS-ESI: *m/z* $[M+Na]^+$ calcd for $C_{15}H_{10}N_2O_2Na$: 273.06345; found: 273.06368; $[2M+Na]^+$ calcd for $C_{30}H_{20}N_4O_4Na$: 523.13768; found: 523.13720.

4.6.2. 2-Bromo-12-oxo-6,12-dihydroquinazolino[2,3-*c*][1,4]-benzoxazine (32)

According to the general procedure this compound was prepared from 6-bromo-2*H*-1,4-benzoxazin-3(4*H*)-one **9** (228 mg, 1.0 mmol) and anthranilic acid (137 mg, 1.0 mmol) to yield **32** (221 mg, 0.67 mmol, 67%) as orange needles. Mp 149–151 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 4.97 (s, 2H, 6-CH_2); 7.02 (d, 1H, ^3J = 8.8 Hz, arom. 4-CH); 7.35 (dd, 1H, ^4J = 2.4 Hz, ^3J = 8.8 Hz, arom. 3-CH); 7.54 (m, 1H, arom. 10-CH); 7.66 (d, 1H, ^3J = 8.0 Hz arom. 8-CH); 7.77 (m, 1H, arom. 9-CH); 8.35 (dd, 1H, ^4J = 1.4 Hz, ^3J = 7.8 Hz, arom. 11-CH); 8.80 (d, 1H, ^4J = 2.4 Hz, arom. 1-CH). APT (100 MHz, $CDCl_3$): δ = 69.3 (6-CH_2); 115.0 (arom. 2-CBr); 119.1 (arom. 4-CH); 122.2 (arom. 11a-C_q); 126.1 (arom. 13a-CN); 126.3 (arom. 1-CH); 127.2 (arom. 8-CH); 127.7 (arom. 11-CH); 127.9 (arom. 10-CH); 130.8 (arom. 3-CH); 135.1 (arom. 9-CH); 145.8 (arom. 7a-CN); 148.1 (arom. 6a-C=N(N)); 148.2 (arom. 4a-CO); 159.8 (12-C=O). IR (neat) 1702, 1629, 1473, 1341 cm^{-1}. HRMS-ESI: *m/z* $[M+Na]^+$ calcd for $C_{15}H_9N_2O_2BrNa$: 350.97396; found: 350.97387; $[2M+Na]^+$ calcd for $C_{30}H_{18}N_4O_4Br_2Na$: 678.95870; found: 678.95885.

4.6.3. 9-Chloro-12-oxo-6,12-dihydroquinazolino[2,3-*c*][1,4]-benzoxazine (33)

According to the general procedure this compound was prepared from 2*H*-1,4-benzoxazin-3(4*H*)-one **30** (149.2 mg, 1.0 mmol) and 4-chloroanthranilic acid (171.6 mg, 1.0 mmol) to yield **33** (136 mg, 0.48 mmol, 48%) as orange needles. Mp 168–170 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 4.95 (s, 2H, 6-CH_2); 7.13–7.18 (m, 2H, arom. 2-CH and arom. 4-CH); 7.23–7.27 (m, 1H, arom. 3-CH); 7.46 (dd, 1H, ^4J = 2.4 Hz, ^3J = 8.6 Hz, arom. 10-CH); 7.65 (d, 1H, ^4J = 2.4 Hz, arom. 8-CH); 8.27 (d, 1H, ^3J = 8.6 Hz, arom. 11-CH); 8.54 (d, 1H, ^3J = 8.0 Hz, arom. 1-CH). APT (75 MHz, $CDCl_3$): δ = 69.3 (6-CH_2); 117.8 (arom. 4-CH); 120.9 (arom. 11a-C_q); 122.9 (arom. 2-CH); 123.4 (arom. 1-CH); 125.0 (arom. 13a-CN); 126.8 (arom. 8-CH); 128.2 (arom. 3-CH and arom. 10-CH); 129.2 (arom. 11-CH); 141.1 (arom. 9-C_q); 147.1 (arom. 7a-C=N); 149.2 (arom. 4a-CO); 150.3 (arom. 6a-C=N(N)); 159.3 (12-C=O). IR (neat) 1687, 1595, 1349 cm^{-1}. HRMS-ESI: *m/z* $[M+H]^+$ calcd for $C_{15}H_9N_2O_2ClH$: 285.04253; found: 285.04257; $[2M+H]^+$ calcd for $C_{30}H_{18}N_4O_4Cl_2H$: 569.07779; found: 569.07719.

4.7. Biology

Cell culture media and serum were obtained from Life Technologies Gibco–BRL (Paisley, UK). Penicillin and streptomycin from Applichem (Darmstadt, Germany). All cell culture plasticware was purchased from Corning-Costar Inc. (Corning, NY); L-NAME and isobutyl-methylxanthine (IBMX), were purchased from Sigma–Aldrich (St. Louis, MO). The cGMP EIA kit was purchased from Assay Designs (Ann Arbor, MI).

4.7.1. Rat aortic smooth cell culture

Rat aortic smooth muscle cells (RASMC) were grown on 100-mm dishes in high glucose DMEM supplemented with 10% foetal calf serum, 50 U/mL penicillin and 50 μg/mL streptomycin. RASMC between passages 3 and 4 were used for all experiments.

4.7.2. cGMP immunoassay

Confluent RASMC were initially treated with isobutyl-methylxanthine (IBMX; 1 mM, 5 min) to inhibit phosphodiesterase activity and were then exposed to the tested molecules (0, 1 or 10 μM) for 20 min in the presence of IBMX. At the end of the incubation period cells were treated with SNP (10 μM) for 15 min. Media were then aspirated, and 200 μl of 0.1 N HCl were added to each well to extract cGMP. After 30 min, HCl extracts were collected and centrifuged at 600*g* for 10 min to remove debris. The supernatants were directly analysed for cGMP by enzyme immunoassay.

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