DOI: 10.1002/cmdc.201300378

The Fight against the Influenza A Virus H1N1: Synthesis, Molecular Modeling, and Biological Evaluation of Benzofurazan Derivatives as Viral RNA Polymerase Inhibitors

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The influenza RNA polymerase complex, which consists of the three subunits PA, PB1, and PB2, is a promising target for the development of new antiviral drugs. A large library of benzofurazan compounds was synthesized and assayed against influenza virus A/WSN/33 (H1N1). Most of the new derivatives were found to act by inhibiting the viral RNA polymerase complex through disruption of the complex formed between subunits PA and PB1. Docking studies were also performed to elucidate the binding mode of benzofurazans within the PB1 binding site in PA and to identify amino acids involved in their mechanism of action. The predicted binding pose is fully consistent with the biological data and lays the foundation for the rational development of more effective PA–PB1 inhibitors.

Introduction

Influenza is an infectious disease of birds and mammals caused by ribonucleic acid (RNA) viruses of the family *Orthomyxoviridae*. It is a contagious disease that occurs seasonally in epidemic and sometimes pandemic proportions. On the basis of serological subtyping, three distinct types of influenza—A, B, and C—are present, of which types A and B are of great concern as human pathogens.[1,2] The virus has two major antigenic glycoproteins: hemagglutinin (HA) and neuraminidase (NA). Three HA subtypes (H1, H2, and H3) and two NA subtypes (N1 and N2) have frequently been found in human influenza virus,[1,2] resulting in the possibility for distinct viral strains such as H1N1, H1N2, H2N1, H2N2, etc. Influenza epidemics and pandemics in the past century have had a serious impact on global morbidity, mortality, and economy.[3–5] Recent outbreaks of swine influenza (H1N1) in Mexico and other parts of the world have led to issuances of pandemic alerts by the World Health Organization (WHO).[6] Influenza virus continuously evolves to avoid host detection systems by changing its antigenicity through mutation of its surface glycoprotein (NA, HA) genes; in this way it maintains the capacity to reinfect the same individual in subsequent flu seasons. Another major factor for the emergence of extremely aggressive influenza viruses is the reassortment of genetic materials between different strains of the virus circulating in humans and avians.[7] Given this elevated rate of mutation, every season WHO experts identify the proper combination of antigenic strains for which vaccines are developed. However, the 9–12-month time gap between WHO recommendation and actual application of vaccine could result in a mismatch between the virus and vaccine, decreasing its effectiveness against the strains in circulation.[8] The development of an effective flu vaccine is hampered by these and other complicating factors. Therefore, additional weapons in the form of efficacious anti-influenza agents are a must in the fight against future seasonal or pandemic influenza outbreaks.[9,10]

A few antiviral drugs are effective in defending the body against infection, particularly in patients for whom it is not possible to administer an influenza vaccine or when a new type of virus enters circulation (i.e., swine H1N1 virus, 2009).[11] NA inhibitors, such as oseltamivir[12] and zanamivir, which inhibit virus budding,[13] are available for clinical use. Furthermore, M2 protein inhibitors, amantadine and rimantadine, historically the first drugs available for the treatment of influenza, have been used for over 50 years. Although both drugs can be effective against influenza virus A infection, they have been re-

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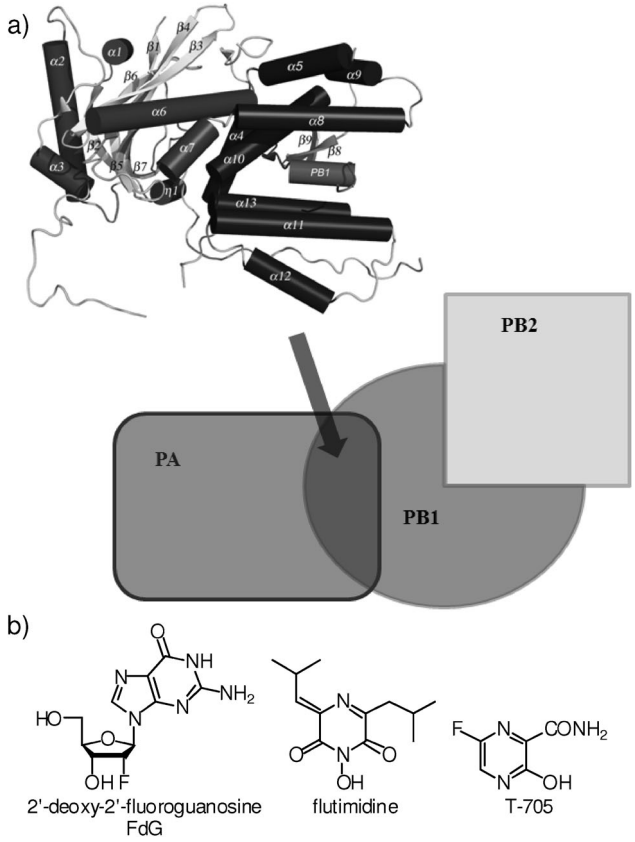


Figure 1. a) Structure of influenza RNA polymerase; b) influenza A RNA polymerase inhibitors.

ported to cause CNS side effects and have given rise to the rapid emergence of drug-resistant viral strains.[14–19]

In the last few years, influenza RNA polymerase has been identified as a new target for inhibition of the virus. This enzyme consists of a complex of three virus-encoded polypeptides (PB1, PB2, and PA; Figure 1a). In addition to RNA replicative activity, this polymerase also contains an endonuclease activity to ensure "cap snatching" to initiate the transcription and subsequent translation process. The polymerase complex genes contribute to the high virulence of the human H5N1 influenza virus isolate A/Vietnam/1203/04. This observation highlights the importance of novel antivirals that target the polymerase for further development of therapy and prophylaxis of human and avian influenza virus infections.[20] Few compounds have been reported to operate at the RNA polymerase. Like the inhibitors that have been found to act against reverse transcriptase (RNA-dependent DNA polymerase) of HIV or RNA replicase (RNA-dependent RNA polymerase) of HCV, influenza RNA polymerase inhibitors can be divided into two classes: nucleosides and non-nucleosides. Examples of the nucleoside-type inhibitors are 2′-deoxy-2′-fluoroguanosine (FdG)[21,22] and T-705[23–25] (Figure 1b). In addition, flutimide, a 2,6-diketopiperazine identified in extracts of the fungus *Delitschia confertaspora*, has been shown to specifically inhibit the cap-dependent endonuclease activity associated with influenza viral RNA polymerase and to inhibit the replication of influenza A and B virus in cell culture.[26] The discovery of molecules able to interact with the PA subunit is an extremely important goal in the synthesis of broad-spectrum antiviral drugs. In fact, because the N-terminal PA interaction domain of PB1 is highly conserved, a compound that can block the interaction between these two proteins can be expected to inhibit most, if not all, influenza strains. We recently reported the discovery of a novel class of influenza A (H1N1) inhibitors by taking advantage of a biochemical ELISA-based screening approach specifically designed to identify compounds that efficiently block the PA–PB1 interaction.[27,28] However, because only a limited amount of structure–activity relationship (SAR) information arose from our previous work, we decided to synthesize a larger library of benzofurazan derivatives to explore the chemical space around the heterocyclic nucleus. Both cell-based and enzyme bioassays were planned in order to test the antiviral activity and the ability of the benzofurazan compounds to interact with and inhibit the viral RNA polymerase complex. Furthermore, docking studies were performed to explore the structural features responsible for the biological activity of benzofurazan derivatives.

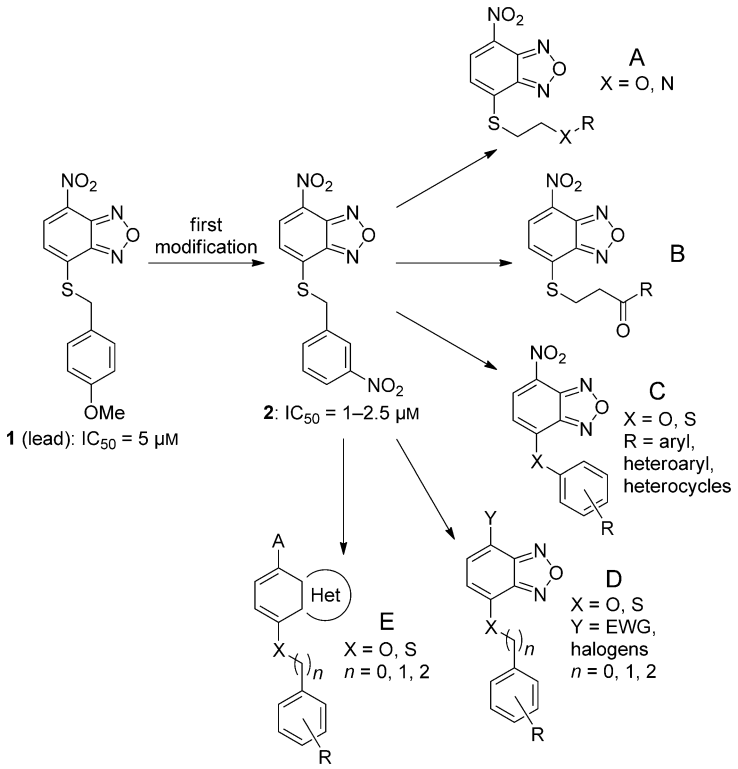


Figure 2. Second-generation benzofurazan derivatives.

Results and Discussion

Chemistry

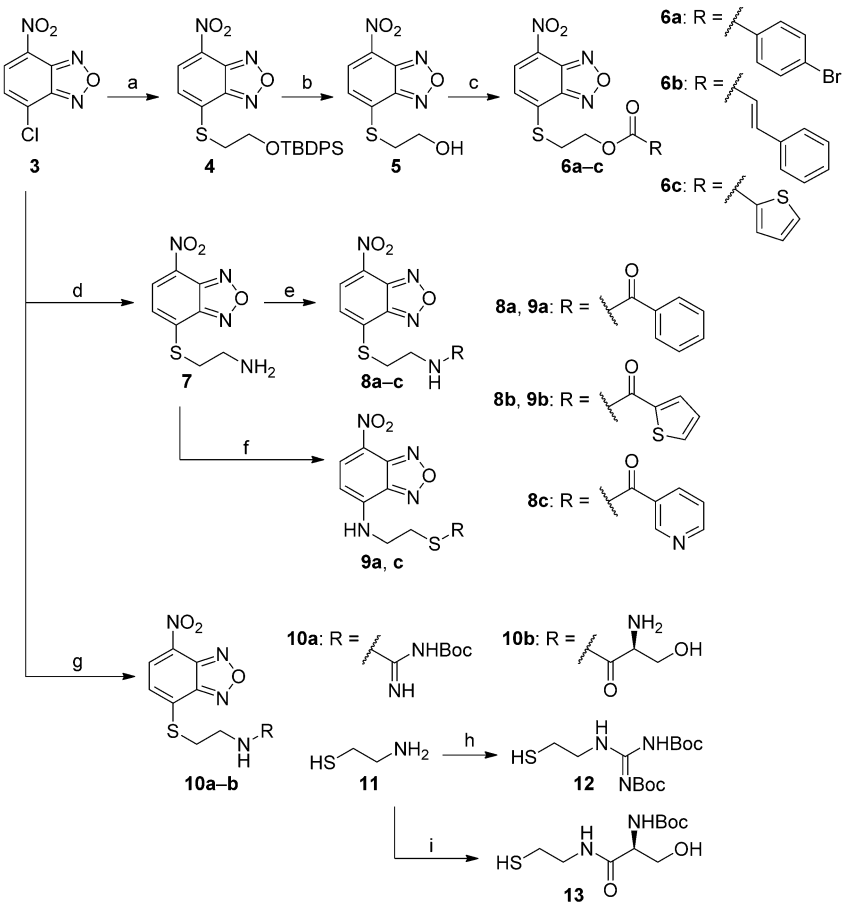
To identify novel benzofurazans as RNA polymerase inhibitors and to study the SARs, five series of analogues (**A**–**E**, Figure 2) of previously discovered compounds **1** and **2**[27] were designed and synthesized. Both series **A** and **B** bear an alkylthio substituent at C4 of the benzofurazan ring. In the first series of compounds with general structure **A** we planned to introduce a >1-carbon alkyl spacer between the sulfur and the aromatic ring. Moreover, enhancing the solubility of the compounds by replacing the aromatic moiety with hydrophilic groups was also investigated. Similarly, the second series of derivatives **B** was planned with the aim to introduce a hydrophilic/hydrogen-donor moiety at C4 and to evaluate their importance for activity. The **C** series is composed of benzofurazans bearing an *S*- or *O*-aryl moiety at C4. In particular, the introduction of heteroaryl moieties bound to the phenyl ring at C4 was planned in order to improve the solubility of the new compounds and thus to improve their activity in cellular assays. To determine the importance of the nitro group, the fourth series of compounds **D**, bearing various groups at C7 was also planned. Finally, the synthesis of compounds **E** with a non-benzofurazan core was accomplished. The benzofurazan core was replaced by (bio)isosteric (hetero)aromatic cores in order to investigate the importance of the oxadiazole ring for activity against influenza viruses.

Synthesis of derivatives with general structures A and B

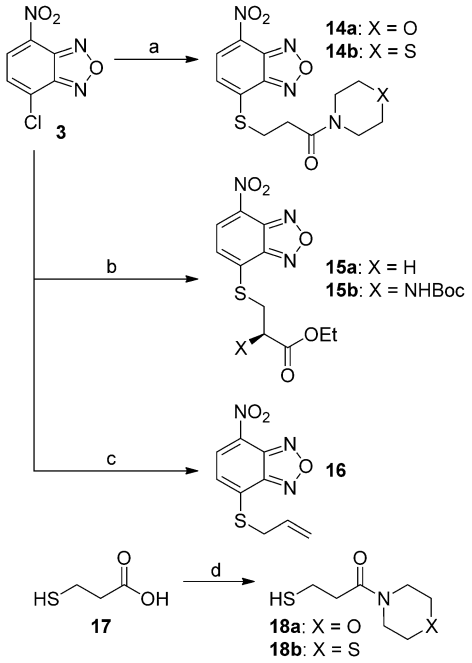
The synthesis of the first series of benzofurazan derivatives containing a cysteamine or thioethyl chain at C4 was carried out according to Scheme 1. Commercially available benzofurazan **3** was first treated with protected thioethanol to afford **4**, which, after deprotection with TBAF, led to intermediate **5**. Acylation of the latter afforded derivatives **6a**–**c** in excellent yields. Reaction of benzofurazan **3** with cysteamine gave intermediate **7**. Acylation of **7** with various acyl chlorides in the presence of pyridine as base selectively afforded compounds **8a**–**c**. If the same reaction was carried out in the presence of triethylamine, compounds **9a** and **9b** were also isolated. The formation of these latter compounds was due to an intramolecular Smiles rearrangement of the cysteamine chain catalyzed by triethylamine, as previously reported by our group.[29] Finally, reaction of **3** with **12** and **13**, in turn obtained through guanylation and amidation with cysteamine, and acid-mediated removal of the Boc protecting group led to the formation of derivatives **10a**,**b**. Compounds belonging to the **B** series of derivatives, bearing a thiopropionic acid side chain at C4, were then synthesized according to Scheme 2. Benzofurazan **3** was first allowed to react with amides **18a**,**b**, in turn obtained through the reaction of thiopropionic acid **14** with morpholine and thiomorpholine. Reaction of **3** with ethyl 3-thiopropionate or with *N*-Boc-cysteine ethyl ester led to derivatives **15a**,**b**. Finally, the allyl derivative **16** was obtained under the same reaction conditions.

Synthesis of derivatives with general structure C

A new series of benzofurazans bearing a thioaryl moiety at C4 was synthesized according to Scheme 3. The starting material



Scheme 1. *Reagents and conditions*: a) $HS(CH_2)_2OTBDPS$, K_2CO_3, CH_2Cl_2, reflux, 4 h; b) TBAF, THF, RT, 1 h; c) RCOCl, pyridine, DMAP, RT, 5 h; d) cysteamine, pyridine, KOAc, EtOH, reflux, 1 h; e) RCOCl, pyridine, DMAP, CH_2Cl_2, 6 h; f) RCOCl, Et_3N, CH_2Cl_2, DMAP, 30 min; g) **12** or **13**, pyridine, KOAc, EtOH, reflux, 1 h, then HCl, Et_2O, RT, 24 h; h) *N*,*N*′-di-Boc-*N*″-trifluoromethanesulfonylguanidine, Et_3N, CH_2Cl_2, RT, 6 h; i) *N*-Boc-serine, EDC, HOBt, DIPEA, DMF, RT, 10 h.

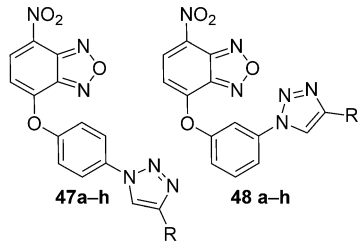


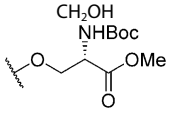
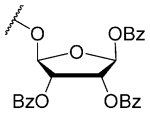
Scheme 2. *Reagents and conditions*: a) **18a** or **18b**, pyridine, KOAc, EtOH, reflux, 1 h; b) ethyl 3-thiopropionate or *N*-Boc-cysteine ethyl ester, pyridine, KOAc, EtOH, reflux, 1 h; c) allylthiol, pyridine, KOAc, EtOH, reflux, 1 h; d) morpholine or thiomorpholine, EDC, HOBt, DIPEA, DMF, RT, 10 h.

19, obtained from compound **3** by reaction with *m*-aminothiophenol in ethanol at reflux,[29] was then derivatized at the amino function. Imidazole derivative **20** was obtained through a microwave-accelerated one-pot reaction previously reported by our group,[30] leading to the formation of the heterocyclic ring in 10 min. Reaction of **19** with *p*-toluenesulfonyl isocyanate and cyanuric chloride led to derivatives **21** and **22**. This latter compound was also converted into its hydrochloride salt **23** in order to improve aqueous solubility. Finally, reductive amination of **19** with various aldehydes led to compounds **24a**–**d**. A second series of derivatives bearing an *O*-aryl moiety at C4 was then synthesized. With the purpose to avoid potential oxidation at the sulfur atom, which could reduce antiviral activity, several derivatives containing an ether moiety were synthesized. Moreover, the oxy-derivatives **27a**–**d** and **29** (Scheme 4) were synthesized with the aim to introduce an alkyl spacer between the two aromatic moieties and to obtain further SARs. Alkynes **26a**–**d** were obtained through Sonogashira coupling from 4-iodophenol **25** and then reacted with compound **3**. Hydrogenation of **26c** led to saturated phenol **28**, which was in turn converted into derivative **29**. Finally, derivative **33** was easily synthesized by starting from commercially available aldehyde **30**, which was converted into diene **31** in three steps. Ring-closing metathesis and deprotection led to phenol **32**, which, after reaction with 4-chloro-7-nitrobenzofurazan **3**, led to the desired compound **33**. We then turned our attention to the introduction of a heteroaliphatic group in order to improve the solubility of target compounds. Therefore, the synthesis of a series of phenol derivatives bearing a piperazine at the *para* position of the phenyl ring was planned (Scheme 5). Compound **34** was alkylated, acylated, and guanylated to give compounds **35**–**37**, which were in turn coupled with 4-chloro-7-nitrobenzofurazan **3** to give the desired products **38a**–**c**. Hydrochloride salts **39a**,**b** were also synthesized, and Boc deprotection of compound **38c** led to compound **40c**.

Finally, we planned to introduce a heteroaromatic moiety into the C4 chain. In particular, we focused our attention to the triazole ring, which is known to be a bioisostere of the amide bond. Azides **42a**,**b** were allowed to react with various alkynes through Huisgen-1,3-dipolar cycloaddition to afford triazoles **44a**–**f**, **45a**,**b**, and, after deprotection of **44d**, compound **46**. These were coupled with compound **3** to furnish the desired compounds **47**–**48a**–**h** (Scheme 6, Table 1). Both

Table 1. Triazole derivatives synthesized (series **C**).

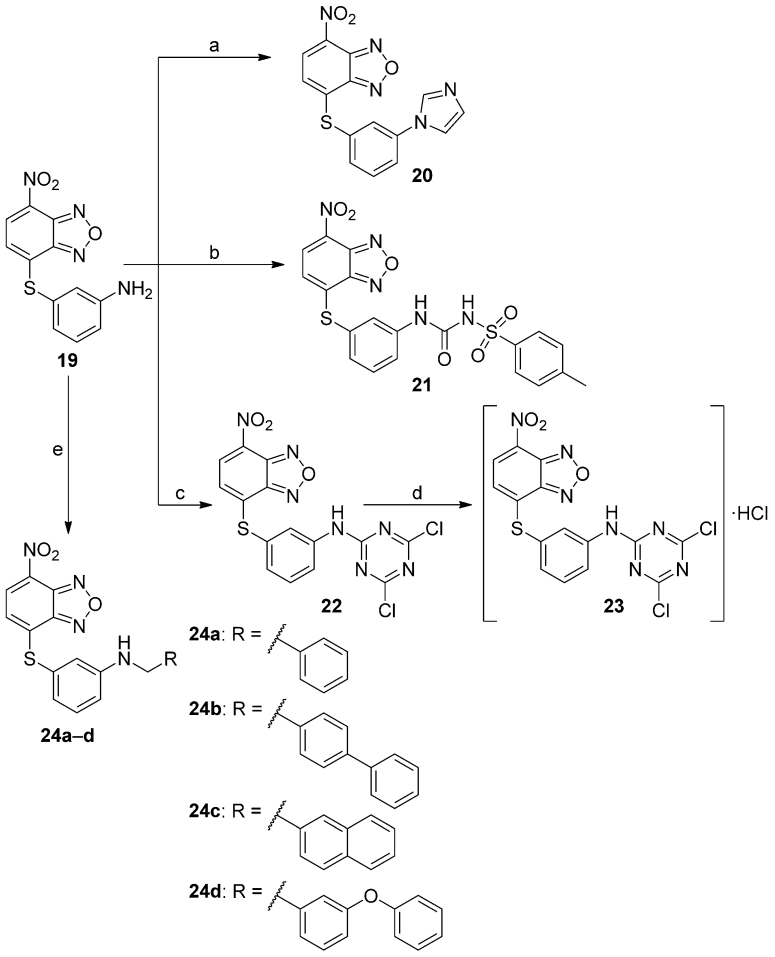


Entry	Azide	Alkyne	R	Triazole	Benzofurazan
1	**42a**	**43a**	Ph	**44a**	**47a**
2	**42a**	**43b**	*p*-MeOPh	**44b**	**47b**
3	**42a**	**43d**	*c*Hex	**44d**	**47d**
4	**42a**	**43e**	CH_2OH	**44e**	**47e**
5	**42a**	**43f**		**44f**	**47f**
6	**42a**	**43g**		**44g**	–
7	**42b**	**43a**	Ph	**45a**	**48a**
8	**42b**	**43b**	*p*-MeOPh	**45b**	**48b**
9	**42b**	**43c**	*p*-MeSPh	**45c**	**48c**
10				**46**	**47h**

meta- and *para*-substituted phenols were synthesized. The syntheses of benzofurazans **47f** and **47h**, respectively bearing an amino acid and a carbohydrate group, were planned in order to enhance the aqueous solubility of these derivatives.

Synthesis of derivatives with general structure D

A further series of compounds with general structure **D** was then planned with the aim of investigating the role of the substituents at C7. Compounds in which the nitro group was replaced by a halogen or an amino moiety were then designed and synthesized. Compounds **1**, **14a**, and **49**, which in our previous studies[27] showed high antiviral activity in cellular assays,



Scheme 3. *Reagents and conditions*: a) H_3PO_4, paraformaldehyde, glyoxal, NH_4Cl, H_2O/dioxane, MW (120 °C), 10 min; b) *p*-toluenesulfonyl isocyanate, CH_2Cl_2, RT, 24 h; c) cyanuric chloride, DME, −30 °C, 1 h; d) acetyl chloride, MeOH, CH_2Cl_2, RT, 2 min; e) RCHO, $NaBH_4$–PTSA, DCE, RT, 16 h.

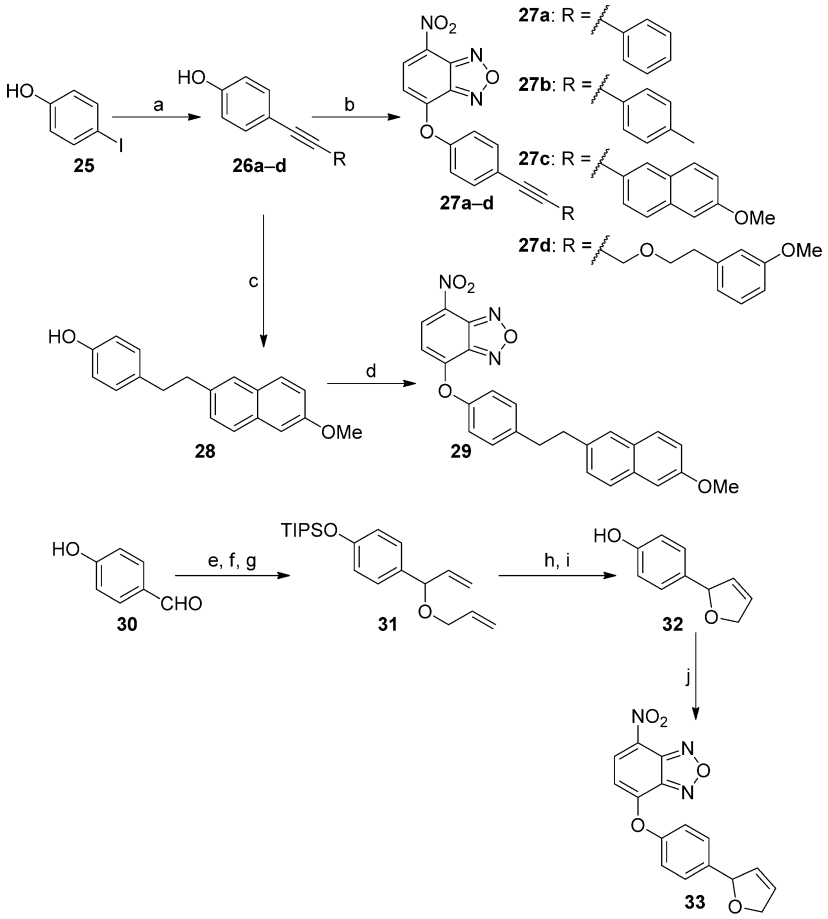
were chosen as substrates. Reduction of the nitro group was carried out with Fe^0 in acidic medium, to afford amines **50a**–**c**. Chlorination and iodination were performed with $CuCl_2$–*tert*-butylnitrile and $NaNO_2$–KI, respectively, to allow formation of **51b** and **52c**. Amines **53a** and **54c** were also synthesized by hydrochlorination and acylation reactions. Finally, sulfonamides **58a**–**d** were synthesized from amide **56**, in turn obtained from sulfonyl chloride **55** (Scheme 7).

Synthesis of the series of derivatives with general structure E: modifications of the heterocyclic core

The synthesis of a series of non-benzofurazan derivatives related to previous compounds was then planned in order to investigate the role and importance of the benzooxadiazole nucleus to anti-influenza activity. A series of tricyclic compounds strictly related to benzofurazans was obtained by Diels–Alder reaction of **49a**–**f** with the Danishefsky diene, leading to the formation of diastereomers **59a**–**f** and **60a**–**f** (Scheme 8). The benzo-2,1,3-thiadiazoles **63a**,**b** were then synthesized from commercially available **61**, which was converted into the bromo derivative **62** by holding it at reflux in concentrated nitric acid. Reaction of **62** with the appropriate nucleophile led to the desired products **63a**,**b**. The oxadiazole ring of the bezofurazans was then replaced with a pyridine ring. Hence, a series of quinoline and isoquinoline derivatives was synthesized. Skraup reaction between aniline **64** and acrolein **65** led to quinoline nucleus **66**, which, after coupling with the appropriate thiol or phenol, led to desired compounds **67a**–**d**. The synthesis of isoquinoline derivatives was accomplished by starting from **68**, which was converted into derivative **69** by bromination with *N*-bromosuccinimide (NBS) and nitration with potassium nitrate. Coupling with the appropriate thiol led to isoquinolines **70a**,**b** (Scheme 9). Finally, the oxadiazole ring was replaced with a 2-methylthiazole ring. Aniline **71** was acylated into **72** and cyclized into benzothiazole **73** by reaction with Lawesson's reagent. Nitration of **73** led to **74**, which was converted into desired compounds **75a**–**d** after reaction with the appropriate thiol/phenol. Finally, derivative **78** was obtained in a two-step sequence starting from aniline **76** in order to investigate the importance of the heterocyclic core for antiviral activity (Scheme 10).

Biological assays

The synthesized compounds were assayed for their inhibitory activity toward influenza virus strain A/WSN/33 (H1N1), and the results are listed in Table 2. With regard to derivatives with general structure **A**, compound **6a**, containing a thioethanol chain, showed good activity, with an IC_{50} value of 2.5 μM. However, **6a** proved to be slightly cytotoxic. On the other hand, related compounds **6b**,**c** were found to be inactive. The best activity data were obtained with cysteamine derivative **8**. The unsubstituted derivative **7** showed a good biological profile, with an IC_{50} value of 10 μM and low cytotoxicity. Acylated derivatives **8a**–**c** showed excellent antiviral activity (1–2.5 μM), but also proved to be highly cytotoxic. It seems clear that the presence of an aromatic moiety is fundamental for improvement of activity, yet it is also detrimental in terms of cytotoxicity. Compound **8c** proved to be a perfect compromise, bearing an aromatic moiety endowed with partial hydrophilic character, namely a pyridine. Compound **8d** was found to be active



Scheme 4. *Reagents and conditions*: a) alkyne, $(PPh_3)_2PdCl_2$, CuI, DMF/Et_3N, MW (120 °C), 5 min; b) **3**, cat. pyridine and KOAc, EtOH, reflux, 20 min; c) H_2, Pd/C, EtOAc, RT, 8 h; d) **3**, cat. pyridine and KOAc, EtOH, reflux, 20 min; e) TIPSCl, imidazole, DMF, 60 °C, 1 h; f) vinylmagnesium bromide, THF, RT, 1 h; g) allyl bromide, NaH, THF, RT, 5 h; h) 2nd-gen. Grubbs catalyst, CH_2Cl_2, RT, 1 h; i) TBAF, THF, RT, 1 h; j) **3**, cat. pyridine and KOAc, EtOH, reflux, 2 h.

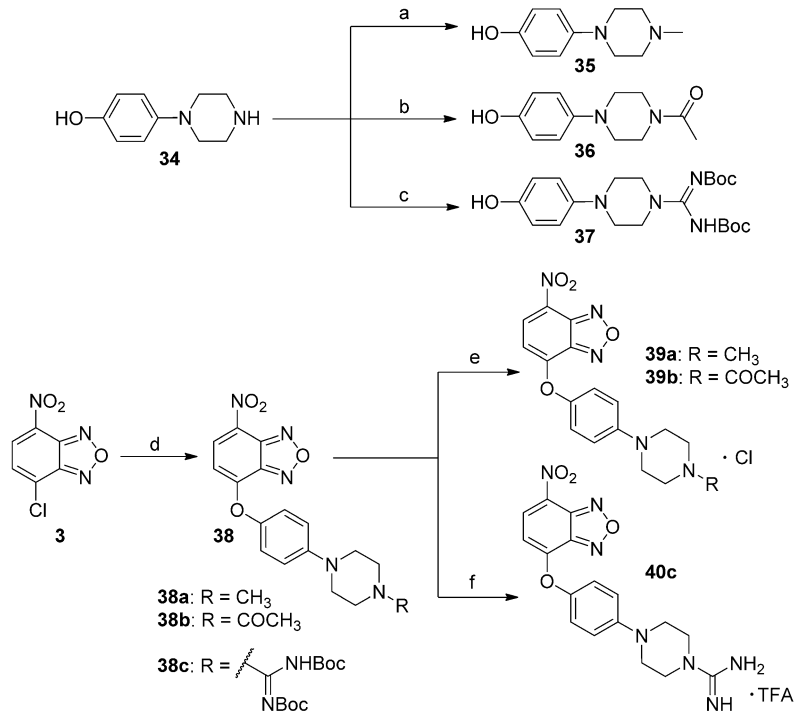
against influenza virus H1N1 and showed low cytotoxicity. Compounds with general structure **B** (namely **14**, **15**, and **16**) were all inactive against influenza H1N1 virus in cellular assays, with compound **16** as the only exception: IC_{50} 10 μM. In contrast, good results were obtained from compounds with general structure **C** (compounds **20**–**48**); all were active against influenza H1N1 virus, with IC_{50} values ranging from 1 to 60 μM. Overall, the introduction of aryl/benzyl ethers or thioethers or longer aliphatic/aromatic chains at C4 is favorable for activity against influenza H1N1. Notably, compound **47e** proved to be active against H1N1 virus, with an IC_{50} value of 1 μM and low cytotoxicity, thus proving to be the best benzofurazan derivative identified in this series.

Focusing on compounds of series **C** and **D** (compounds **49**–**78**), the biological data indicate that replacement of the nitro group or change of the benzofurazan core cause a loss of activity (data not shown). Similarly, the introduction of substituents at C6 is detrimental for antiviral activity. On the other hand, to study the mechanism of action of the new antiviral benzofurazan compounds, active derivatives were also tested in the novel biochemical ELISA previously described for influenza peptides and specifically designed to identify compounds that efficiently block the PA–PB1 interaction. Most of the new antiviral benzofurazan compounds (**8c**, **20**, **23**, **39b**, **40**, **47a**, **47e**, **48b**, **48c**) acted by disrupting the interaction between the PA and PB1 subunits of the viral RNA polymerase at the micromolar level, thus blocking an essential protein in the replication process of the virus. Notably, all the compounds observed to be active in the ELISA bear a heterocyclic or heteroaromatic ring in the benzofurazan chain. Imidazole derivative **20** showed a close correlation between the results of cell-based assays and ELISAs, confirming that its biological activity is due to interaction with the RNA polymerase complex. Close correlations between cellular and ELISAs were also observed for piperazine derivatives **39b** and **40**, and for triazoles **47a**,**e** and **48b**,**c**. On the other hand, the high ELISA IC_{50} values determined for some compounds (**16**, **21**, **24a**, and **33**), which were found active in cells at low micromolar levels, suggest that their mechanism of action could be different. Further studies are in progress to better elucidate this aspect.

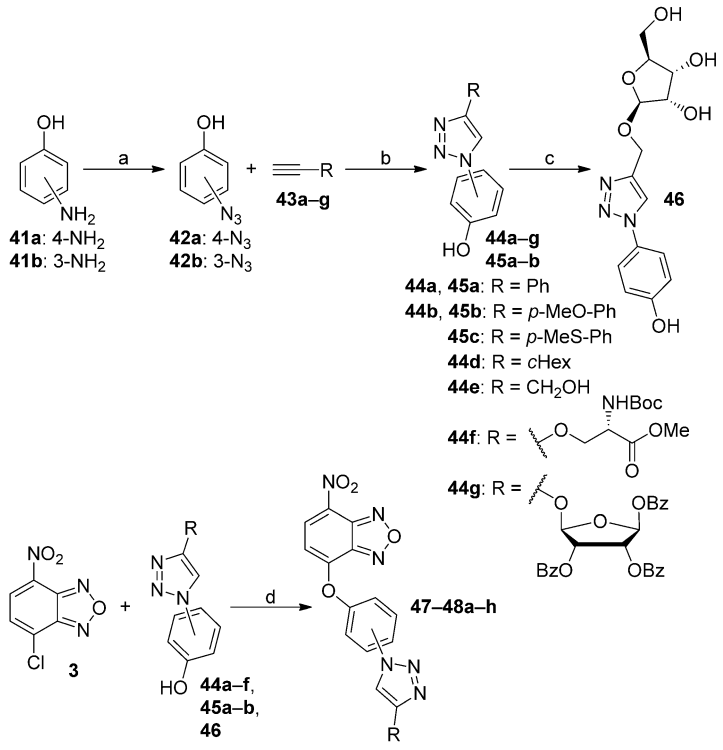
Molecular modeling

Docking studies were performed on the novel benzofurazans to explore the structural features responsible for their inhibitory activity toward influenza H1N1 virus. Because most of the derivatives were shown to act as PA–PB1 inhibitors, they were subjected to focused docking experiments within the PB1 binding site of PA.

Computational analysis was performed by using a protein assembly built with the aid of two recently deposited crystallographic structures of the PA–PB1 complex (PDB IDs 3CM8 and 2ZNL). The complete library of benzofurazans was docked by using both Glide and Gold programs. Docking results on derivatives reported herein did not converge to a single solution, but several binding modes were identified. The inability to recognize a unique pose was likely due to the large size of the pocket, adapted to the co-crystallized PB1 peptide. However, the most reliable binding mode identified for active inhibitors shows the benzofurazan scaffold involved in a π-stacking interaction with Trp706. Furthermore, a hydrogen bond between the oxygen atom of the furazan group and Gln408 and an



Scheme 5. *Reagents and conditions*: a) CH_2O, formic acid, MeOH, reflux, 12 h; b) Ac_2O, pyridine, RT, 1 h; c) *N*,*N*′-di-Boc-*N*″-trifluoromethanesulfonylguanidine, DIPEA, DMF, RT, 16 h; d) **35**, **36**, or **37**, cat. pyridine and/or KOAc, EtOH, reflux, 20 min; e) **38a** or **38b**, acetyl chloride, MeOH, CH_2Cl_2, RT, 2 min; f) **38c**, TFA, CH_2Cl_2, RT, 24 h.



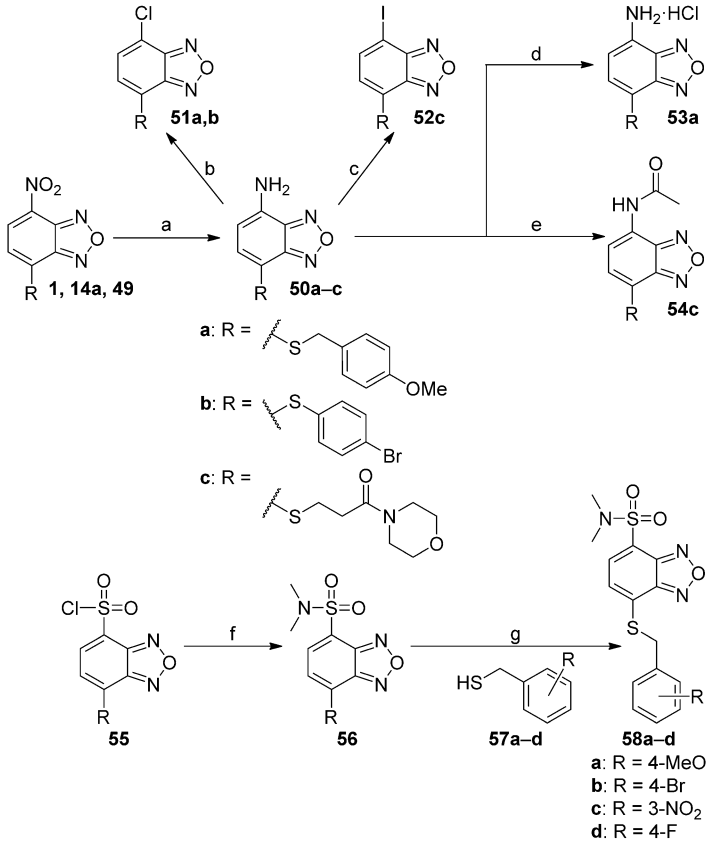
Scheme 6. *Reagents and conditions*: a) $NaNO_2$, NaN_3, $AcOH/H_2O$, 15 min; b) appropriate alkyne, sodium ascorbate/$Cu(SO)_4$, H_2O/*t*BuOH, MW (125 °C), 10 min; c) **44g**, MeOH/NH_4OH (4:1), RT, 18 h; d) **44**–**45a**–**f** or **46**, cat. pyridine and/or KOAc, EtOH, reflux, 3 h.

electrostatic interaction recurrent between the NH_3^+ of Lys643 and the nitro group were found. As an example, the binding mode of compound **39b** is shown in Figure 3. Similar interactions were previously described for other recently identified PA–PB1 inhibitors.[31,32] Accordingly, the lack of activity of compounds in which the nitro group is replaced by another group may depend on the loss of the electrostatic interaction with Lys643. Additionally, replacement of the benzofurazan core is detrimental to activity, probably from the loss of interactions with Trp706 and Gln408.

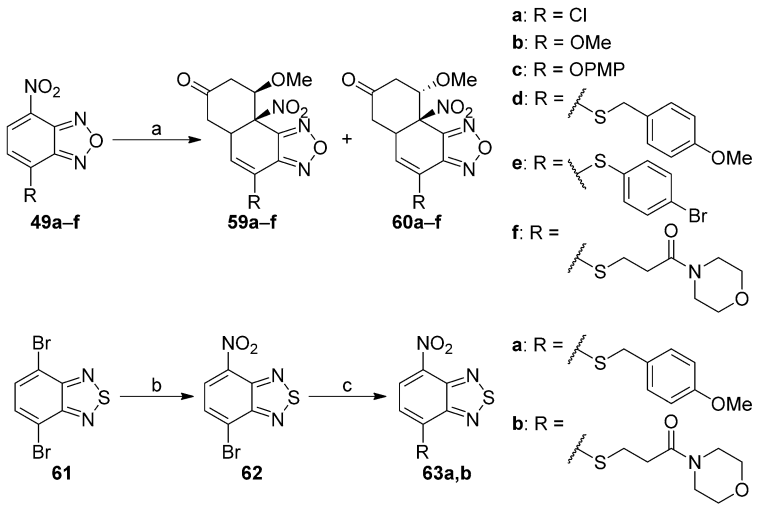
The ligand efficiency (LE)[33] of benzofurazan derivatives was calculated as the free energy of binding divided by the number of heavy atoms (non-hydrogen atoms, NHA) of the molecule (Table 2). Values of LE span from 0.16 to 0.34 kcal (mol·NHA)$^{-1}$, putting compounds **7**, **16**, and **20** in a promising range for further optimization, as an LE value of 0.24 kcal (mol·NHA)$^{-1}$ has been described as a limit over which small molecules are able to disrupt protein–protein interfaces.[34]

Conclusions

In summary, the present study demonstrates the potential of benzofurazan compounds as a new family of influenza virus inhibitors that act by targeting the interaction between the PA and PB1 subunits of the viral RNA polymerase. A large library of compounds was synthesized and biologically evaluated, leading to the identification of lead compounds (**8c** and **47e**) with high anti-H1N1 activity ($IC_{50} = 1$ μM). ELISA results confirmed the mechanism of action of benzofurazan derivatives being through inhibition of the viral RNA polymerase. These compounds are a starting point in the identification and development of novel therapeutic antiviral agents. Results of docking studies suggest that the residues Trp706, Gln408, and Lys643 are crucial for the binding of the benzofurazan derivatives to the PB1 binding pocket on PA, and therefore could be targeted for the design of novel PA–PB1 interaction inhibitors.



Scheme 7. *Reagents and conditions*: a) Fe^0, HCl, CH_2Cl_2/MeOH, RT, 25 min; b) $CuCl_2$, *tert*-butyl nitrite, CH_3CN, 65 °C, 3 h; c) 1. $NaNO_2$, H_2O, HCl/H_2SO_4, 0 °C, 40 min, 2. KI, H_2O, RT, 2 h; d) acetyl chloride, MeOH, CH_2Cl_2, RT, 2 min; e) Ac_2O, pyridine, cat. DMAP, RT, 4 h; f) Me_2NH, cat. Et_3N, CH_3CN, RT, 3 h; g) appropriate nucleophile, cat. pyridine and/or KOAc, EtOH, reflux, 2 h.



Scheme 8. *Reagents and conditions*: a) Danishefsky diene, CH_2Cl_2, RT, 6 h; b) conc HNO_3, reflux, 1 h; c) appropriate nucleophile, cat. pyridine and/or KOAc, EtOH, reflux, 3 h.

Experimental Section

Chemistry

Reagents were obtained from commercial suppliers and were used without further purification. ^1H and ^{13}C NMR spectra were measured on a Bruker Avance DPX400. Chemical shifts are reported relative to $CDCl_3$ at $\delta = 7.24$ ppm and tetramethylsilane at $\delta = 0.00$ ppm. Mass spectrometry (MS) data were obtained on an Agilent 1100 LC/MSD VL system (G1946C) at a flow rate of 0.4 mL min^{-1} using a binary solvent system of 95:5 MeOH/H_2O. UV detection was monitored at λ 254 nm. MS data were acquired in both positive and negative modes, scanning over the mass range of 50–1500. The following ion source parameters were used: drying gas flow: 9 mL min^{-1}; nebulizer pressure: 40 psig; drying gas temperature: 350 °C.

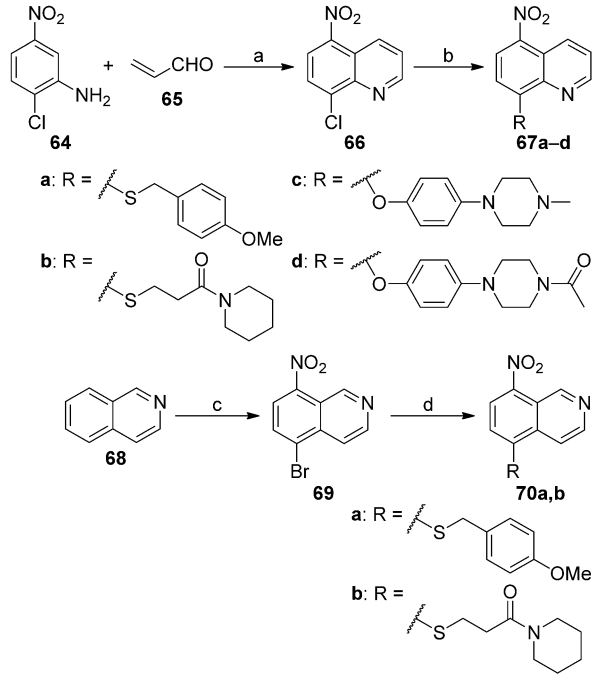
4-(2-(*tert*-Butyldiphenylsilyloxy)ethylthio)-7-nitrobenzo[*c*][1,2,5]oxadiazole (4): To a solution of 4-chloro-7-nitrobenzofurazan **3** (1 mmol) in 5 mL CH_2Cl_2, K_2CO_3 (1 mmol) and OTBDPS-thioethanol (1.1 mmol) were added and the reaction mixture was heated at reflux for 4 h. H_2O was added, and the organic layer was washed with brine, dried over Na_2SO_4, filtered off and the solvent was removed. The crude mixture was purified by flash chromatography (Hex/EtOAc 9:1) to give compound **5** as a yellow solid (0.71 mmol, 71%). ^1H NMR (400 MHz, CD_3OD): $\delta = 8.13$–8.11 (d, $J = 8$, 1H), 7.58–7.56 (m, 4H), 7.35–7.32 (m, 2H), 7.30–7.26 (m, 4H), 6.89–6.87 (d, $J = 8$, 1H), 4.00–3.96 (d, $J = 8$, 2H), 3.40–3.36 (d, $J = 8$, 2H), 0.97 ppm (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta =$ 143.78, 143.42, 135.66, 134.05, 130.07, 129.56, 126.23, 124.48, 119.08, 67.26, 43.39, 31.75, 26.88 ppm; MS (ESI) *m/z*: 502 $[M + Na]^+$.

2-(7-Nitrobenzo[*c*][1,2,5]oxadiazol-4-ylthio)ethanol (5): To a solution of **4** (134 mg, 0.28 mmol), in dry THF (5 mL) previously cooled to 0 °C, a 1 M TBAF solution in THF (280 μL, 0.28 mmol) was slowly added. The mixture was stirred at room temperature for 1 h, then the solvent was evaporated. The residue was purified by flash chromatography (EtOAc/Hex 4:1) giving the desired compound **5** as a colorless oil (0.28 mmol, quant). ^1H NMR (400 MHz, $CDCl_3$): $\delta = 8.38$–8.36 (d, $J = 8$, 1H), 7.37–7.35 (d, $J = 8$, 1H), 3.88–3.85 (t, $J = 4$, 2H), 3.42–3.38 ppm (t, $J = 4$, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 148.98$, 140.38, 135.78, 127.22, 124.53, 119.42, 57.13, 38.46 ppm; MS (ESI) *m/z*: 264 $[M + Na]^+$.

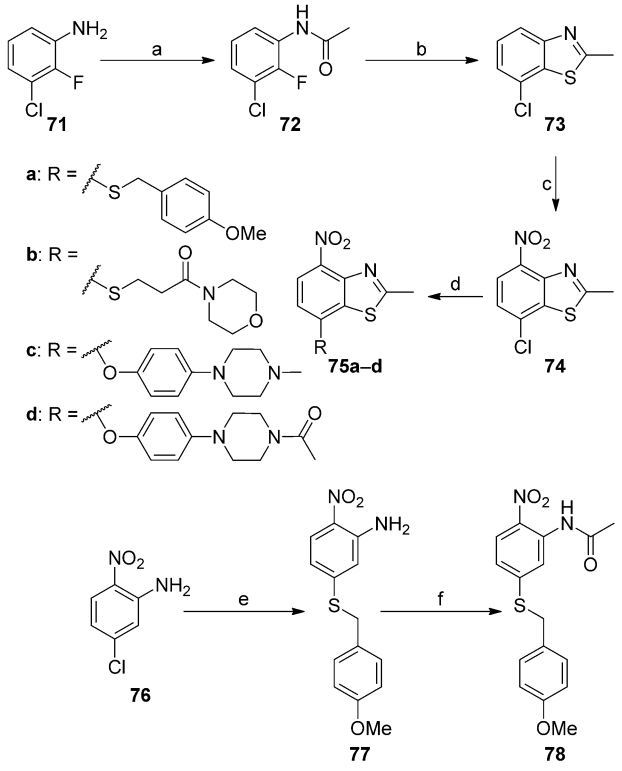
General procedure for esters 6a–c

To a suspension of **5** (35 mg, 0.14 mmol) in dry CH_2Cl_2 (5 mL), the appropriate acyl chloride (0.43 mmol), pyridine (50 μL, 0.58 mmol), and a catalytic amount of DMAP were added. The reaction mixture was stirred at room temperature for 6 h, after which time it was washed with $NaHCO_3$ and then with 1 N HCl. The combined organic layers were dried over Na_2SO_4, filtered off and the solvent removed under reduced pressure. The crude was purified by flash chromatography.

2-(7-Nitrobenzo[*c*][1,2,5]oxadiazol-4-ylthio)ethyl 4-bromobenzoate (6a): Yellow solid (0.13 mmol, 94%). ^1H NMR (400 MHz, $CDCl_3$): $\delta = 8.35$–8.33 (d, $J = 7.8$, 1H), 7.77–7.75 (d, $J = 8.3$, 2H), 7.52–7.50 (d, $J = 8.3$, 2H), 7.40–7.38 (d, $J = 7.8$, 1H), 4.62–4.59 (t, $J = 6.7$, 2H), 3.65–3.62 ppm (t, $J = 6.7$, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 165.46$, 149.22, 142.44, 139.31, 133.31, 131.87, 131.04, 130.40, 128.78, 127.96, 121.92, 62.00, 30.25 ppm; MS (ESI) *m/z*: 447 $[M + Na]^+$.

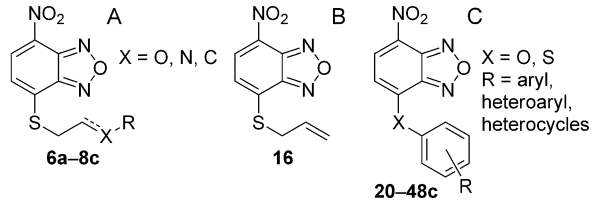


Scheme 9. *Reagents and conditions*: a) toluene, 100 °C, 2 h; b) appropriate nucleophile, K_2CO_3, DMF, MW (130 °C), 10 min; c) 1. NBS, conc H_2SO_4, −25 °C/−18 °C, 3 h, 2. conc H_2SO_4, KNO_3, −10 °C, 1 h; d) appropriate nucleophile, K_2CO_3, DMF, MW (130 °C), 10 min.



Scheme 10. *Reagents and conditions*: a) Ac_2O, reflux, 2 h; b) 1. Lawesson's reagent, xylene, 110 °C, 2 h. 2. Cs_2CO_3, reflux, 16 h; c) conc H_2SO_4, KNO_3, 1 h, −10 °C, then RT, 2 h; d) appropriate nucleophile, cat. pyridine and/or KOAc, EtOH, reflux, 3 h; e) 4-OMePhSH, K_2CO_3, EtOH, reflux, 3 h; f) Ac_2O, reflux, 2 h.

Table 2. In vitro inhibition of the PA–PB1 interaction (ELISA) and ligand efficiency (LE), along with activity (Act) and cytotoxicity (Cytox) of compounds **6–48** toward influenza virus strain A/WSN/33 (H1N1).



Compd	ELISA IC_{50} [μM][a]	LE [kcal (mol·NHA)$^{-1}$]	Act IC_{50} [μM][a]	Cytox [μM][a]
6a	100	0.22	2.5	15
6b	inactive	–	–	–
6c	inactive	–	–	–
7	100	0.34	10	40
8a	90	0.23	1	2.5
8b	50	0.26	2.5	10
8c	45	0.25	1	20
16	110	0.34	10	20
20	15	0.27	10	20
21	200	0.19	20	80
22	60	0.21	10	40
23	45	0.21	10	40
24a	75	0.21	15	40
24b	inactive	–	–	–
24c	inactive	–	–	–
24d	inactive	–	–	–
33	100	0.23	15	80
39a	40	0.23	10	20
39b	25	0.22	10	20
40	30	0.22	5	20
47a	25	0.21	5	40
47b	200	0.16	50	100
47d	250	0.16	60	60
47e	35	0.23	1	40
47f	inactive	–	–	–
47h	40	0.17	40	90
48a	inactive	–	–	–
48b	35	0.19	5	40
48c	20	0.20	5	40

[a] All compounds were tested twice; values are the average of two experiments.

2-(7-Nitrobenzo[*c*][1,2,5]oxadiazol-4-ylthio)ethyl cinnamate (6b): Yellow solid (0.12 mmol, 87%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.45–8.43 (d, J = 7.8, 1H), 7.71–7.65 (d, J = 16, 1H), 7.52–7.39 (m, 6H), 6.41–6.36 (d, J = 16, 1H), 4.58–4.54 (t, J = 7.2, 2H), 3.66–3.62 ppm (t, J = 7.2, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 166.83, 149.52, 146.46, 142.75, 139.97, 134.13, 131.03, 130.88, 129.25, 128.42, 121.92, 116.97, 61.64, 30.44 ppm; MS (ESI) *m/z*: 394 $[M+Na]^+$.

2-(7-Nitrobenzo[*c*][1,2,5]oxadiazol-4-ylthio)ethyl thiophene-2-carboxylate (6c): Yellow solid (0.116 mmol, 83%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.34–8.32 (d, J = 8, 1H), 7.69–7.68 (m, 1H), 7.52–7.51 (m, 1H), 7.43–7.41 (d, J = 8, 1H), 7.04–7.02 (m, 1H), 4.60–4.58 (t, J = 7.8, 2H), 3.65–3.62 ppm (t, J = 7.8, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 162.95, 143.86, 143.48, 136.72, 134.66, 133.82, 128.98, 126.78, 124.52, 119.13, 57.33, 36.67 ppm; MS (ESI) *m/z*: 260 $[M+Na]^+$.

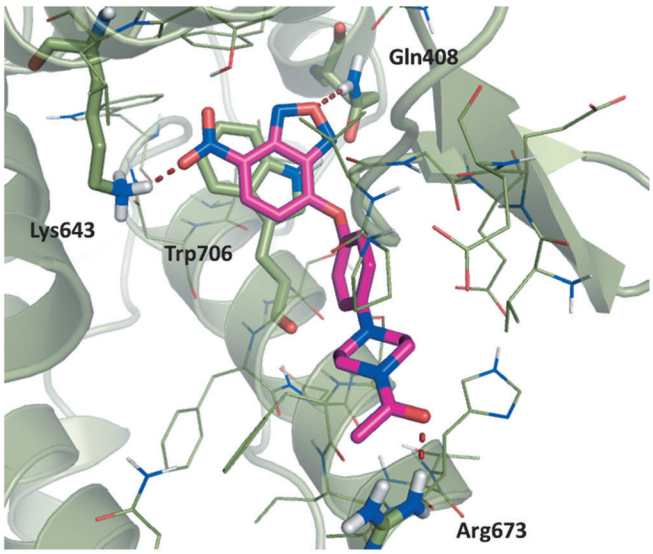


Figure 3. Binding mode of the benzofurazan derivative **39b**.

General procedure (A) for aromatic nucleophilic substitution

To a solution of 4-chloro-7-nitrobenzofuzan (50 mg, 0.25 mmol) in 5 mL EtOH, the appropriate nucleophile was added (0.27 mmol). The reaction mixture was heated at reflux for 5 min and, if the color had not changed, a catalytic amount of pyridine (0.05 mL) or of KOAc (0.01 mmol) was added, and reflux was continued for a further 20 min. If a strong red coloration did develop within 5 min, no catalyst was added, and reflux was continued for a further 15 min. The mixture was cooled to room temperature, and the precipitate formed was filtered off to give the desired product pure.

7-(2-Aminoethylthio)-4-nitrobenzofurazan (7): Compound **7** was synthesized according the general procedure for aromatic nucleophilic substitution. Yellow solid (0.217 mmol, 87%). ^{1}H NMR (400 MHz, CD_3OD): δ = 8.48–8.46 (d, J = 7.9, 1H), 7.55–7.53 (d, J = 7.9, 1H), 3.65–3.62, (m, 2H), 3.35–3.31 ppm (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 149.43, 142.68, 137.06, 133.52, 130.99, 122.86, 37.57, 27.78 ppm; MS (ESI) m/z: 241 $[M+H]^+$.

General procedure (B) for compounds 8a–c and 9a–c

To a solution of **7** (30 mg, 0.125 mmol) in dry CH_2Cl_2 (2 mL), pyridine (0.15 mmol), catalytic amount of DMAP (0.1) and the appropriate acyl chloride (0.15 mmol) were added. The reaction was stirred at room temperature for 6 h and then was quenched with $NaHCO_3$. The mixture was extracted with EtOAc and washed with 1 N HCl and brine. The organic layer was dried over Na_2SO_4, filtered off and the solvent was removed. The crude residue was purified by flash chromatography (Hex/EtOAc 1:1).

***N*-(2-(7-Nitrobenzo[*c*][1,2,5]oxadiazol-4-ylthio)ethyl)benzamide (8a)**: Yellow solid (0.09 mmol, 62%). ^{1}H NMR (400 MHz, [D_6]acetone): δ = 8.50–8.48 (d, J = 8, 1H), 8.19 (brs, 1H), 7.83–7.78 (m, 3H), 7.48–7.44, (m, 1H), 7.40–7.36 (m, 2H), 3.79–3.77 (m, 2H), 3.62–3.59 ppm (m, 2H); ^{13}C NMR (100 MHz, [D_6]acetone): δ = 167.07, 153.08, 149.97, 140.35, 134.76, 131.62, 131.40, 128.36, 127.12, 122.23, 38.24, 30.32 ppm; MS (ESI) m/z: 367 $[M+Na]^+$.

***N*-(2-(7-Nitrobenzo[*c*][1,2,5]oxadiazol-4-ylthio)ethyl)thiophene-2-carboxamide (8b)**: Yellow solid (0.072 mmol, 58%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.51–8.49 (d, J = 8, 1H), 7.77–7.75 (d, J = 8, 1H), 7.62–7.59 (m, 2H), 7.05–7.03 (m, 1H), 3.76–3.73 (m, 2H), 3.61–3.57 ppm (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 162.47, 143.78, 141.07, 136.53, 134.59, 133.44, 132.71, 131.71, 130.49, 128.17, 99.53, 44.62, 27.78 ppm; MS (ESI) m/z: 373 $[M+Na]^+$.

***N*-(2-(7-Nitrobenzo[*c*][1,2,5]oxadiazol-4-ylthio)ethyl)nicotinamide (8c)**: Yellow solid (0.072 mmol, 58%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.18 (s, 1H), 8.76 (brs, 1H), 8.75 (brs, 1H), 8.57 (brs, 1H), 8.51 (d, 1H, J = 8 Hz), 7.78 (d, 1H, J = 8 Hz), 3.90–3.85 (m, 2H), 3.68–3.64 ppm (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 165.92, 147.64, 146.73, 143.74, 143.05, 135.52, 131.46, 126.29, 122.27, 37.43, 30.17 ppm; MS: (ESI): m/z 345 $[M-H]^-$, 381 $[M+Cl]^-$

S-2-(7-Nitrobenzo[*c*][1,2,5]oxadiazol-4-ylamino)ethylbenzothioate (9a): Red solid (0.018 mmol, 15%). ^{1}H NMR (400 MHz, [D_6]DMSO): δ = 8.46 (d, 1H, J = 8.3 Hz), 7.84 (d, 2H, J = 8.5 Hz), 7.62 (t, 1H, J = 8.5 Hz), 7.48 (t, 2H, J = 8.5 Hz), 6.50 (d, 1H, J = 8.3 Hz), 3.68 (brs, 1H), 3.35 (t, 2H, J = 6.1 Hz), 3.23 ppm (t, 2H, J = 6.1 Hz); ^{13}C NMR (100 MHz, [D_6]DMSO): δ = 199.4, 149, 143.4, 141.4, 137.9, 134.5, 132.6, 129.7, 127, 123.8, 95.1, 68.5, 33.3 ppm.

S-2-(7-Nitrobenzo[*c*][1,2,5]oxadiazol-4-ylamino)ethylthiophene-2-carbothioate (9b): Red solid (0.027 mmol, 22%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.47–8.45 (d, J = 8, 1H), 7.79–7.77 (m, 1H), 7.65–7.63 (m, 1H), 7.10–7.08 (m, 1H), 6.34–6.32 (d, J = 8, 1H), 3.77–3.75(m, 2H), 3.39–3.36 ppm (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 192.33, 143.40, 140.87, 136.17, 133.95, 133.27, 132.11, 131.69, 129.99, 128.17, 99.02, 44.13, 27.49 ppm; MS (ESI) m/z: 373 $[M+Na]^+$.

General procedure for compounds 10a,b

Benzofurazans **10a,b** were prepared through the reaction of **3** with **12** or **13** according to general procedure (B).

***N*-Boc-(*N*-(2-(7-nitrobenzo[*c*][1,2,5]oxadiazol-4-ylthio)ethyl)guanidine (10a)**: Yellow solid (0.088 mmol, 71%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.60–8.58 (d, J = 8, 1H), 7.24–7.22 (d, J = 8, 1H), 3.84–3.82 (m, 2H), 3.73–3.70 (m, 2H), 1.35 ppm (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 163.12, 156.30, 153.17, 144.23, 143.73, 136.19, 124.12, 83.53, 79.47, 39.22, 36.69, 28.13 ppm; MS (ESI) m/z: 483 $[M+H]^+$.

(*S*)-2-Amino-3-hydroxy-*N*-(2-(7-nitrobenzo[*c*][1,2,5]oxadiazol-4-ylthio)ethyl)propanamide hydrochloride (10b): Yellow solid (0.125 mmol, quant). $[\alpha]_D$: −2.92° (c = 5.7, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.45 (d, 1H, J = 8 Hz, benzofurazan), 7.55 (d, 1H, J = 8 Hz), 3.88–3.85 (m, 2H), 3.78–3.73 (m, 1H), 3.64–3.59 (m, 4H), 3.47–3.44 ppm (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 167.20, 149.38, 143.53, 142.60, 139.23, 130.99, 121,81, 60.14, 54.79, 37.40, 29.90 ppm; MS (ESI) m/z: 483 $[M+H]^+$.

***tert*-Butyl-(*tert*-butoxycarbonylamino) (2- thioethylamino)methylenecarbamate (12)**: Cysteamine was reacted with protected guanidine according to the general procedure (B). The residue was purified by flash chromatography (Hex/EtOAc 4:1). White solid (0.098 mmol, 79%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.53 (brs, 1H), 3.67–3.69 (m, 2H), 2.80–2.77 (m, 2H), 1.40 ppm (s, 18H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 158.78, 157.83, 161.03, 84.92, 80.05, 40.23, 27.87 ppm; MS (ESI) m/z: 320 $[M+H]^+$.

(*S*)-*tert*-Butyl-3-hydroxy-1-(2-thioethylamino)-1-oxo-propan-2-ylcarbamate (13): Compound **13** was obtained using the same procedure as for **12**, but with cysteamine and Boc-serine as the starting materials (0.052 mmol, 42%). $[\alpha]_D$: −1.76° (c = 10, MeOH); ^{1}H NMR (400 MHz, [D_6]acetone): δ = 87.47 (brs, 1H), 5.96 (brs, 1H),

4.11–4.08 (m, 1H), 4.03 (brs, 1H), 3.76–3.72 (m, 1H), 3.68–3.63 (m, 1H), 3.36–3.29 (m, 2H), 2.59–2.53 (m, 2H), 1.78 (t, $J=8$ Hz, 1H), 1.34 ppm (s, 9H); ^{13}C NMR (100 MHz, [D$_6$]acetone): $\delta=170.6$, 155.9, 78.6, 62.4, 56.3, 42.3, 27.5, 23.6 ppm; MS (ESI) *m/z*: 287 $[M+H]^+$.

1-Morpholino-3-(7-nitrobenzo[*c*][1,2,5]oxadiazol-4-ylthio)propan-1-one (14a): Yellow solid (0.091 mmol, 73%). ^{1}H NMR (400 MHz, $CDCl_3$): $\delta=8.33$–8.31 (d, $J=8$, 1H), 7.21–7.19 (d, $J=8$, 1H), 3.62–3.52 (m, 8H), 3.41–3.38 (m, 2H), 2.81–2.77 ppm (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta=168.17$, 149.06, 142.42, 141.00, 132.64, 130.67, 120.74, 66.65, 66.33, 45.62, 42.14, 31.56, 26.86 ppm; MS (ESI) *m/z*: 361 $[M+Na]^+$.

3-(7-Nitrobenzo[*c*][1,2,5]oxadiazol-4-ylthio)-1-thiomorpholinopropan-1-one (14b): Yellow solid (0.096 mmol, 77%). ^{1}H NMR (400 MHz, $CDCl_3$): $\delta=8.28$–8.26 (d, $J=8$, 1H), 7.18–7.16 (d, $J=8$, 1H), 3.78–3.64 (m, 4H), 3.49–3.46 (m, 2H), 2.79–2.76 (m, 2H), 2.54–2.51 ppm (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta=167.96$, 149.00, 142.37, 141.17, 132.37, 130.94, 120.71, 47.96, 44.47, 31.76, 27.71, 27.22, 26.92 ppm; MS (ESI) *m/z*: 377 $[M+Na]^+$.

Ethyl 3-(7-nitrobenzo[*c*][1,2,5]oxadiazol-4-ylthio)propanoate (15a): Yellow solid (0.095 mmol, 76%). ^{1}H NMR (400 MHz, $CDCl_3$): $\delta=8.34$–8.32 (d, $J=8$, 1H), 7.20–7.18 (d, $J=8$, 1H), 4.11–4.06 (q, $J=8$, 2H), 3.50–3.46 (t, $J=8$, 2H), 2.78–2.75 (t, $J=8$, 2H), 1.19–1.16 ppm (t, $J=8$, 4H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta=170.56$, 149.08, 142.42, 140.41, 132.64, 120.91, 61.28, 32.88, 26.57, 14.12 ppm; MS (ESI) *m/z*: 320 $[M+Na]^+$.

(*R*)-Ethyl-2-(*tert*-butoxycarbonylamino)-3-(7-nitrobenzo[*c*][1,2,5]oxadiazol-4yl-thio)propanoate (15b): Yellow solid (0.086 mmol, 69%). ^{1}H NMR (400 MHz, CD_3OD): $\delta=8.53$–8.51 (d, $J=8$, 1H), 7.55–7.53 (d, $J=8$, 1H), 4.55–4.51 (m, 1H), 4.26–4.18 (m, 2H), 3.92–3.87 (m, 1H), 3.64–3.59 (m, 1H), 1.41 (s, 9H), 1.31–1.28 ppm (m, 3H); ^{13}C NMR (100 MHz, CD_3OD): $\delta=169.49$, 155.01, 149.23, 142.38, 139.67, 133.20, 130.55, 121.29, 80.83, 62.52, 52.85, 33.88, 28.15 14.06 ppm; MS (ESI) *m/z*: 413 $[M+H]^+$.

4-(Allylthio)-7-nitrobenzo[*c*][1,2,5]oxadiazole (16): Yellow solid (0.113 mmol, 91%). ^{1}H NMR (400 MHz, $CDCl_3$): $\delta=8.33$–8.31 (d, $J=7.9$, 1H), 7.15–7.13 (d, $J=7.9$, 2H), 5.94–5.84 (m, 1H), 5.40–5.25 (m, 2H), 3.90–3.88 ppm (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta=148.92$, 142.45, 137.41, 135.82, 133.25, 131.02, 130.76, 123.25, 122.68, 119.43, 37.66 ppm; MS (ESI) *m/z*: 260 $[M+Na]^+$.

3-Thio-1-morpholinopropan-1-one (18a): Morpholine (100 μL, 1.147 mmol) was dissolved in dry CH_2Cl_2 (10 mL) and cooled to 0 °C. Then 3-thiopropionic acid (100 μL, 1.147 mmol), EDC (200 mg, 1.043 mmol), HOBt (141 mg, 1.043 mmol) and DIPEA (218 μL, 1.251 mmol) were added and the mixture was stirred at room temperature for 16 h. The reaction mixture was quenched with saturated aqueous solution of $NaHCO_3$ (10 mL) and extracted with EtOAc (3×10 mL). The combined extract was dried over anhydrous Na_2SO_4 and evaporated to dryness. The crude product was purified by flash chromatography (EtOAc/Hex 9:1) to provide desired compound (0.48 mmol, 42%). ^{1}H NMR (400 MHz, $CDCl_3$): $\delta=3.45$ (brs, 4H), 3.39 (brs, 2H), 3.26 (brs, 2H), 2.61–2.56 (m, 2H), 2.44–2.41 (m, 2H), 1.55 ppm (t, $J=8$ Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta=$ 169.2, 66.6, 66.3, 45.5, 41.7, 36.7, 18.8 ppm.

3-Thio-1-thiomorpholinopropan-1-one (18b): To a solution of thiomorpholine (172 μL, 1.71 mmol) in dry DMF (5 mL) previously cooled to 0 °C, 3-thiopropionic acid 125 (164 μL, 1.88 mmol), EDC (328 mg, 1.71 mmol), HOBt (231 mg, 1.71 mmol), and DIPEA (338 mL, 2.05 mmol) were added. The reaction mixture was allowed to stir at room temperature for 10 h. After that time $NaHCO_3$ was added and the mixture was extracted with EtOAc. The organic layer was dried over Na_2SO_4, filtered off and the solvent removed under reduced pressure. The crude was purified by flash chromatography (Hex/EtOAc 20:80) to give the desired amide as a yellow oil (1.28 mmol, 75%). ^{1}H NMR (400 MHz, $CDCl_3$): $\delta=$ 3.69–3.67 (m, 4H), 3.55–3.53 (m, 4H), 2.64–2.59 (m, 2H), 2.47–2.44 (m, 2H), 1.60–1.56 ppm (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta=$ 169.00, 47.91, 44.19, 37.10, 27.68, 27.24, 19.94 ppm; MS (ESI) *m/z*: 214 $[M+Na]^+$.

4-(3-(1*H*-Imidazol-1-yl)phenylthio)-7-nitrobenzo[*c*][1,2,5]oxadiazole (20): Compound **19** (0.38 mmol) was dissolved into a solution of H_2O/dioxane (3:1 mL) and H_3PO_4 was added until pH 2 was reached. Solid paraformaldehyde (15 mg), glyoxal sol. 40% in H_2O (0.1 mL), and NH_4Cl saturated solution (0.5 mL) were then added and the resulting mixture was irradiated by microwave for 10 min at 120 °C. The mixture was then cooled to 0 °C, and NaOH was added to pH 12. The alkaline solution was extracted with EtOAc (2×10 mL). The combined organic layers were washed with brine, dried (Na_2SO_4), filtered and evaporated to give the crude **11**. The crude product was then purified by flash chromatography (EtOAc/MeOH 9:1) (0.17 mmol, 45%). ^{1}H NMR (400 MHz, $CDCl_3$): $\delta=8.21$ (d, $J=8$ Hz, 1H), 7.96, (s, 1H), 7.70 (s, 1H), 7.67–7.58 (m, 3H), 7.28, (s, 1H), 7.19 (s, 1H), 6.75 ppm (d, $J=8$ Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta=149.03$, 142.89, 138.84, 136.77, 136.28, 133.97, 132.52, 131.93, 130.03, 129.89, 125.74, 125.25, 122.51, 121.37, 116.71 ppm; MS (ESI) *m/z*: 340 $[M+H]^+$.

4-Methyl-*N*-(3-(7-nitrobenzo[*c*][1,2,5]oxadiazol-4-ylthio)phenylcarbamoyl)benzenesulfonamide (21): To a solution of compound **19** (0.2 mmol) in dry CH_2Cl_2 (4 mL) was added *p*-toluenesulfonyl isocyanate (1.5 equiv) and the solution was stirred at room temperature for 24 h. The mixture was then concentrated in vacuo, and the crude product was purified by flash chromatography (EtOAc/MeOH 9:1) (0.116 mmol, 58%). ^{1}H NMR (400 MHz, [D$_6$]DMSO): $\delta=$ 8.75 (brs, 1H), 8.41 (d, $J=8$ Hz, 1H), 7.86 (brs, 1H) 7.65–7.59 (m, 3H), 7.47 (d, $J=8$ Hz, 1H), 7.33–7.29 (m, 1H) 7.12 (d, $J=8$ Hz, 1H), 7.55 (d, 1H, $J=8$ Hz), 6.71 (d, $J=8$ Hz, 1H), 2.23 ppm (s, 1H); ^{13}C NMR (100 MHz, [D$_6$]DMSO): $\delta=157.95$, 151.23, 148.82, 144.44, 143.53, 143.19, 142.23, 141.12, 140.07, 133.09, 130.91, 129.19, 128.60, 126.94, 125.49, 123.64, 120.35 ppm; MS (ESI) *m/z*: 484 $[M-H]^-$.

4,6-Dichloro-*N*-(3-(7-nitrobenzo[*c*][1,2,5]oxadiazol-4-ylthio)phenyl)-1,3,5-triazin-2-amine (22): To a stirred solution of cyanuric chloride (51 mg, 0.8 equiv) in DME (10 mL) at −30 °C, compound **19** (100 mg, 1 equiv) was added and the resulting mixture was stirred at −30 °C for 1 h. The reaction mixture was then warmed to room temperature, diluted with EtOAc, washed with saturated aqueous solution of $NaHCO_3$ (10 mL), dried and concentrated in vacuo. The crude product was purified by flash chromatography (Hex/EtOAc 3:1) (0.263 mmol, 76%). ^{1}H NMR (400 MHz, $CDCl_3$): $\delta=$ 8.22 (d, $J=8$ Hz, 1H), 7.91 (s, 1H) 7.75 (brs, 1H) 7.67 (d, $J=8$ Hz, 1H), 7.57–7.53 (t, $J=8$ Hz, 1H), 7.46 (d, $J=8$ Hz, 1H), 6.79 ppm (d, $J=8$ Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta=178.48$, 165.48, 156.26, 155.05, 150.62, 144.85, 141.60, 141.35, 135.07, 132.22, 128.88, 125.69, 118.07, 117.65 ppm; MS (ESI) *m/z*: 434–438 $[M-H]^-$.

4,6-Dichloro-*N*-(3-(7-nitrobenzo[*c*][1,2,5]oxadiazol-4-ylthio)phenyl)-1,3,5-triazin-2-aminium chloride (23): To a solution of compound **22** (1 equiv) in CH_2Cl_2, a 1 M solution of HCl in MeOH (2 equiv) was added dropwise. The mixture was stirred at room temperature for 2 min, then the solvent was removed in vacuo to give the desired pure product (0.31 mmol, quant). ^{1}H NMR (400 MHz, [D$_6$]acetone): $\delta=10.09$ (s, 1H), 8.43 (d, $J=8$ Hz, 1H), 8.11 (s, 1H) 7.90 (d, $J=8$ Hz, 1H), 7.64–7.60 (t, $J=8$ Hz, 1H), 7.52 (d, $J=$

8 Hz, 1H), 7.03 (d, $J=8$ Hz, 1H), 2.88 ppm (br s, 1H); ^{13}C NMR (100 MHz, [D$_6$]acetone): $\delta=179.21$, 165.83, 156.31, 155.02, 150.82, 145.15, 141.77, 141.46, 135.12, 132.32, 129.02, 126.08, 118.23, 118.12 ppm; MS (ESI) *m/z*: 434–438 $[M-H]^-$.

General procedure (C) for reductive amination

To a solution of compound **19** (1.0 equiv) in DCE the appropriate aldehyde (1.0 equiv) was added and the mixture was stirred at room temperature for 10 min. Then a 1:1 mixture of $NaBH_4$/PTSA (1 equiv) was added and the suspension was stirred at room temperature for 16 h. The reaction mixture was quenched with saturated aqueous solution of $NaHCO_3$ (10 mL) and extracted with CH_2Cl_2 (3×10 mL). The combined extract was dried over anhydrous Na_2SO_4, filtered and concentrated. The crude products obtained were further purified by a flash column chromatography on silica gel (Hex/EtOAc 3:1).

***N*-Benzyl-3-(7-nitrobenzo[*c*][1,2,5]oxadiazol-4-ylthio)benzenamine (24a)**: (0.28 mmol, 91%). ^{1}H NMR (400 MHz, $CDCl_3$): $\delta=8.10$ (d, $J=8$ Hz, 1H), 7.28–7.23 (m, 5H), 6.91 (d, $J=8$ Hz, 1H), 6.78 (s, 1H), 6.59 (d, $J=8$ Hz, 1H), 4.30 ppm (s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta=152.82$, 146.40, 141.57, 141.44, 135.74, 134.64, 134.31, 134.14, 132.0, 130.79, 130.62, 130.03, 127.06, 124.46, 121.71, 119.07, 51.11 ppm; MS (ESI) *m/z*: 377 $[M-H]^-$, 413 $[M+Cl]^-$.

***N*-(Biphenyl-4-ylmethyl)-3-(7-nitrobenzo[*c*][1,2,5]oxa-diazol-4-ylthio)benzenamine (24b)**: (0.27 mmol, 87%). ^{1}H NMR (400 MHz, $CDCl_3$): $\delta=8.09$ (d, $J=8$ Hz, 1H), 7.52–7.48 (m, 4H), 7.39–7.34 (m, 4H), 7.31–7.26 (m, 2H), 6.91 (d, $J=8$ Hz, 1H), 6.81 (s, 1H), 6.61 (d, $J=8$ Hz, 1H), 4.35 ppm (s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta=$ 152.62, 151.45, 146.17, 145.50, 143.58, 143.41, 140.19, 134.31, 133.79, 132.40, 132.27, 132.19, 131.87, 130.83, 130.47, 129.93, 126.88, 124.18, 121.50, 118.82, 50.66 ppm; MS (ESI) *m/z*: 453 $[M-H]^-$.

***N*-(Naphthalen-2-ylmethyl)-3-(7-nitrobenzo[*c*][1,2,5]oxadiazol-4-ylthio)benzenamine (24c)**: (0.26 mmol, 85%). ^{1}H NMR (400 MHz, $CDCl_3$): $\delta=7.74$ (d, $J=8$ Hz, 1H), 7.69–7.64 (m, 4H), 7.43–7.36 (m, 3H), 7.28–7.24 (m, 1H), 6.88 (d, $J=8$ Hz, 1H), 6.82 (d, $J=8$ Hz, 1H), 6.75 (s, 1H), 6.35 (d, $J=8$ Hz, 1H), 4.48 ppm (s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta=144.30$, 143.92, 134.97, 134.81, 134.28, 133.89, 132.86, 132.16, 130.65, 130.17, 129.24, 129.01, 128.16, 127.72, 127.44, 126.81, 125.22, 122.53, 119.93, 117.82, 49.49 ppm; MS (ESI) *m/z*: 427 $[M-H]^-$, 463 $[M+Cl]^-$.

3-(7-Nitrobenzo[*c*][1,2,5]oxadiazol-4-ylthio)-*N*-(3-phenoxybenzyl)benzenamine (24d): (0.266 mmol, 86%). ^{1}H NMR (400 MHz, $CDCl_3$): $\delta=8.12$ (d, $J=8$ Hz, 1H), 7.28–7.21 (m, 4H), 7.05–7.00 (m, 2H), 6.94–6.88 (m, 4H), 6.84 (d, $J=8$ Hz, 1H), 6.75 (s, 1H), 6.59 (d, $J=8$ Hz, 1H), 4.29 ppm (s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta=$ 158.69, 158.61, 152.30, 150.49, 144.77, 143.97, 142.67, 134.53, 133.03, 131.97, 127.88, 125.33, 124.90, 124.29, 123.69, 123.29, 121.27, 120.64, 120.40, 119.64, 119.43, 118.95, 118.81, 117.09, 47.90 ppm; MS (ESI) *m/z*: 469 $[M-H]^-$.

General procedure for Sonogashira reaction

An oven-dried screw-cap tube was charged with 4-iodophenol (100 mg, 0.45 mmol), the appropriate alkyne (0.49 mmol), CuI (0.005 mmol), and $PdCl_2(PPh_3)_2$ (0.005 mmol). The reaction mixture was suspended in a DMF/Et_3N mixture (0.5:1 mL) and heated under microwave for 5 min at 120 °C. After that time the mixture was poured into 0.1 N HCl and extracted with Et_2O. The combined organic layers were washed with $NaHCO_3$, dried over Na_2SO_4 filtered off and the solvent was removed. The crude residue was purified by flash chromatography (Hex/EtOAc 4:1) to give the desired product.

4-(*p*-Phenylethynyl)phenol (26a): (0.35 mmol, 79%). ^{1}H NMR (400 MHz, CD_3OD): $\delta=7.61$–7.59 (m, 2H), 7.48–7.36 (m, 5H), 6.91–6.89 (m, 2H), 5.78 ppm (br s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta=$ 157.35, 133.82, 132.65, 128.47, 128.43, 128.37, 127.32, 115.78, 115.46, 89.88 ppm; MS (ESI) *m/z*: 195 $[M+H]^+$.

4-(*p*-Tolylethynyl)phenol (26b): (0.33 mmol, 75%). ^{1}H NMR (400 MHz, CD_3OD): $\delta=7.38$–7.36 (d, $J=8$, 4H), 7.10–7.08 (d, $J=8$, 2H), 6.75–6.73 (d, $J=8$, 2H), 2.31 ppm (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta=157.98$, 137.96, 133.56, 132.15, 130.89, 128.32, 119.83, 116.01, 115.65, 89.65, 22.87 ppm; MS (ESI) *m/z*: 209 $[M+H]^+$.

4-((6-Methoxynaphthalen)ethynyl)phenol (26c): (0.34 mmol, 78%). ^{1}H NMR (400 MHz, CD_3OD): $\delta=7.88$ (s, 1H), 7.69–7.61 (m, 2H), 7.47–7.45 (m, 1H), 7.10–7.08 (m, 1H), 7.05 (s, 1H), 6.77–6.75 ppm (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta=158.72$, 157.33, 135.02, 134.25, 133.78, 132.32, 129.97, 129.78, 128.92, 127.34, 119.01, 117.98, 115.67, 115.89, 94.75, 89.82, 55.98 ppm; MS (ESI) *m/z*: 275 $[M+H]+$.

4-(3-(4-Methoxybenzyloxy)prop-1-ynyl)phenol (26d): (0.27 mmol, 61%). ^{1}H NMR (400 MHz, CD_3OD): $\delta=7.27$–7.25 (d, $J=8$, 4H), 6.84–6.82 (d, $J=8$, 2H), 6.69–6.67 (d, $J=8$, 2H), 4.55 (s, 2H), 4.29 (s, 2H), 3.74 ppm (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta=158.35$, 157.98, 134.22, 130.01, 129.42, 127.89, 116.02, 115.56, 114.77, 97.01, 87.02, 57.53, 54.98 ppm; MS (ESI) *m/z*: 269 $[M+H]^+$.

4-Nitro-7-(4-(phenylethynyl)phenoxy)benzo[*c*][1,2,5]oxadiazole (27a): According to general procedure (A) for aromatic nucleophilic substitution. Compound was obtained as a yellow solid (0.21 mmol, 65%) ^{1}H NMR (400 MHz, $CDCl_3$): $\delta=8.37$–8.35 (d, $J=$ 7.8, 1H), 7.57–7.55 (d, $J=8$, 2H), 7.26–7.24 (d, $J=8$, 2H), 7.15–7.13 (d, $J=8$, 2H), 6.97–6.95 (d, $J=8$, 2H), 6.51–6.49 ppm (d, $J=7.8$, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta=159.67$, 148.77, 144.86, 141.03, 137.89, 135.84, 133.31, 133.07, 131.48, 120.53, 118.91, 118.73, 117.85, 117.58, 90.43, 90.41 ppm; MS (ESI) *m/z*: 380 $[M+Na]^+$.

4-Nitro-7-(4-(*p*-tolylethynyl)phenoxy)benzo[*c*][1,2,5]oxadiazole (27b): According to general procedure (A) for aromatic nucleophilic substitution. Compound was obtained as a yellow solid (0.2 mmol, 66%). ^{1}H NMR (400 MHz, CD_3OD): $\delta=8.36$–8.34 (d, $J=$ 7.8, 1H), 7.55–7.53 (d, $J=8$, 2H), 7.27–7.25 (d, $J=8$, 2H), 7.16–7.14 (d, $J=8$, 2H), 6.94–6.92 (d, $J=8$, 2H), 6.52–6.50 ppm (d, $J=7.8$, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta=158.43$, 147.61, 144.56, 140.28, 137.45, 135.21, 133.87, 133.52, 130.07, 120.35, 118.62, 118.03, 117.51, 117.36, 90.45, 90.41, 23.67 ppm; MS (ESI) *m/z*: 394 $[M+Na]^+$.

4-(4-((6-Methoxynaphthalen-2-yl)ethynyl)phenoxy)-7-nitrobenzo[*c*][1,2,5]oxadiazole (27c): According to general procedure (A) for aromatic nucleophilic substitution. Compound was obtained as a yellow solid (0.21 mmol, 68%). ^{1}H NMR (400 MHz, CD_3OD): $\delta=8.37$–8.35 (d, $J=8$, 1H), 7.93 (s, 1H), 7.68–7.64 (m, 4H), 7.50–7.48 (m, 1H), 7.20–7.18 (m, 2H), 7.13–7.11 (m, 1H), 7.07 (s, 1H), 6.55–6.53 (d, $J=8$, 1H), 3.88 ppm (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta=159.82$, 158.87, 145.02, 147.71, 136.75, 135.66, 134.82, 133.98, 132.75, 132.24, 131.69, 130.45, 129.99, 128.77, 121.37, 119.05, 118.27, 118.02, 117.48, 116.73, 116.52, 108.46, 106.81, 94.03, 88.72, 57.42 ppm; MS (ESI) *m/z*: 460 $[M+Na]^+$.

4-(4-(3-(3-Methoxyphenethoxy)prop-1-ynyl)phenoxy)-7-nitrobenzo[*c*][1,2,5]oxadiazole (27d): According to general procedure (A) for aromatic nucleophilic substitution. Compound was ob-

tained as a yellow solid (0.15 mmol, 61%). ^{1}H NMR (400 MHz, CD_3OD): δ = 8.37–8.35 (d, J = 8, 1H), 7.56–7.54 (d, J = 8, 2H), 7.27–7.25 (d, J = 8, 2H), 7.16–7.14 (d, J = 8, 2H), 6.52–6.50 (d, J = 8, 1H), 4.55 (s, 2H), 4.32 (s, 2H), 3.75 ppm (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 159.51, 153.75, 152.62, 145.12, 134.33, 134.13, 133.16, 129.82, 122.05, 120.99, 113.99, 113.86, 108.15, 107.96, 86.76, 84.73, 71.57, 56.94 ppm; MS (ESI) m/z: 454 $[M+Na]^+$.

4-(2-(6-Methoxynaphthalen-2-yl)ethyl)phenol (28): To a solution of **26c** (84 mg, 0.30 mmol) in EtOAc (4 mL) a catalytic amount of Pd/C (5%) was added. The reaction mixture was stirred under hydrogen atmosphere for 10 h. After that time the mixture was filtered on a Celite pad, the solvent was removed and the crude was purified by flash chromatography (Hex/EtOAc 4:1) to give the desired compound (0.24 mmol, 81%). ^{1}H NMR (400 MHz, CD_3OD): δ = 7.64–7.60 (m, 2H), 7.47 (s, 1H), 7.27–7.22 (m, 3H), 7.11–7.07 (m, 4H), 3.89 (s, 3H), 3.03–2.98 ppm (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 157.73, 156.34, 135.83, 134.28, 133.42, 131.72, 130.81, 129.78, 129.41, 128.64, 127.19, 119.93, 117.17, 115.85, 106.87, 57.26, 39.41, 38.93 ppm; MS (ESI) m/z: 279 $[M+H]^+$.

4-(4-(2-(6-Methoxynaphthalen-2-yl)ethyl)phenoxy)-7-nitrobenzo[*c*][1,2,5]oxadiazole (29): Compound **29** was obtained according to general procedure (B) for aromatic nucleophilic substitution. Compound was obtained as a yellow solid (0.16 mmol, 71%). ^{1}H NMR (400 MHz, CD_3OD): δ = 8.33–8.31 (d, J = 8, 1H), 7.62–7.58 (m, 2H), 7.44 (s, 1H), 7.25–7.20 (m, 3H), 7.09–7.05 (m, 4H), 6.40–6.38 (d, J = 8, 1H), 3.85 (s, 3H), 3.02 ppm (s, 4H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 157.35, 150.92, 141.01, 136.13, 133.38, 133.13, 130.77, 128.88, 127.68, 126.85, 126.49, 120.67, 118.84, 107.42, 105.71, 55.33, 37.70, 37.23 ppm; MS (ESI) m/z: 464 $[M+Na]^+$.

(4-(1-(Allyloxy)allyl)phenoxy)triisopropylsilane (31): a) To a solution of 4-hydroxybenzaldeyde (200 mg, 1.64 mmol) in dry DMF (4 mL), imidazole (267 mg, 3.93 mmol) was added. The mixture was cooled to 0 °C and TIPS-Cl (416 μL, 1.96 mmol) was slowly added. The mixture was stirred at 60 °C for 2 h. After that time H_2O and EtOAc were added. The mixture was extracted with EtOAc, the organic layer was dried over Na_2SO_4, filtered off and the solvent removed under reduced pressure. The crude was purified by flash chromatography (Hex/EtOAc 9:1) to give 4-(triisopropylsilyloxy)-benzaldehyde as a colorless oil (1.64 mmol, quant). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.72 (s, 1H), 7.64–7.62 (d, J = 8, 2H), 6.83–6.81 (d, J = 8, 2H), 1.17–1.09 (m, 3H), 0.96 ppm (m, 18H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 190.26, 161.62, 131.67, 130.16, 120.10, 17.64, 12.51 ppm; MS (ESI) m/z: 301 $[M+Na]^+$.

b) To a solution of 4-(triisopropylsilyloxy)benzaldehyde (459.2 mg, 1.64 mmol) in THF dry (20 mL) cooled to 0 °C was slowly added a solution 1 M of vinylmagnesium bromide in THF (1.96 mL, 1.96 mmol). The mixture was vigorously stirred for 1.5 h; NH_4Cl was then added and the mixture was extracted with EtOAc (2×5 mL). The organic layers were dried over Na_2SO_4, filtered and the solvent evaporated. The residue was purified by flash chromatography (Hex/EtOAc 4:1) to give 1-(4-(triisopropylsilyloxy)phenyl)prop-2-en-1-ol as a colorless oil (1.34 mmol, 82%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.13–7.11 (d, J = 8, 2H), 6.80–6.78 (d, J = 8, 2H), 5.97–5.89 (m, 1H), 5.22–5.17 (m, 1H), 5.07–5.04 (m, 1H), 5.00–4.98 (m, 1H), 1.25–1.15 (m, 3H), 1.05 ppm (s, 18H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 155.47, 140.49, 135.21, 127.56, 119.67, 114.41, 74.62, 17.85, 12.62 ppm; MS (ESI) m/z: 305 $[M-H]^-$.

c) To a solution of 1-(4-(triisopropylsilyloxy)phenyl)prop-2-en-1-ol (411 mg, 1.34 mmol) in dry THF (10 mL) under argon atmosphere previously cooled to 0 °C, NaH (32 mg, 1.34 mmol) was added. The mixture was stirred at room temperature for 15 min and allyl bromide (2 mmol) was added dropwise. The reaction mixture was allowed to stir at room temperature for a further 10 h. After that time H_2O and EtOAc were added. The mixture was extracted with EtOAc, the organic layer was dried over Na_2SO_4, filtered off and the solvent removed under reduced pressure. The crude was purified by flash chromatography (Hex/EtOAc 98:2) to give **31** as a colorless oil (0.71 mmol, 53%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.15–7.13 (d, J = 8, 2H), 6.77–6.75 (d, J = 8, 2H), 5.94–5.84 (m, 2H), 5.25–5.21 (m, 2H), 5.18–5.10 (m, 2H), 4.72–4.69 (m, 1H), 3.92–3.90 (m, 2H), 1.25–1.18 (m, 3H), 1.07 ppm (s, 18H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 155.52, 139.01, 134.89, 133.25, 128.13, 127.91, 127.66, 119.98, 119.70, 119.40, 117.46, 116.61, 115.83, 114.60, 81.55, 68.97, 17.85, 12.86 ppm; MS (ESI) m/z: 369 $[M+Na]^+$.

(4-(2,5-Dihydrofuran-2-yl)phenol (32): a) Compound **31** (103 mg, 0.29 mmol) was dissolved in CH_2Cl_2 dry (20 mL) under argon atmosphere, then second-generation Grubbs catalyst (2%) was added. The reaction mixture was allowed to stir at room temperature for 1 h, then the solvent was removed and the crude was purified by flash chromatography (Hex/Et_2O 96:4) to give the desired compound as a colorless oil (0.226 mmol, 78%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.10–7.08 (d, J = 8, 2H), 6.80–6.78 (d, J = 8, 2H), 5.97–5.95 (m, 1H), 5.81–5.79 (m, 1H), 5.67–5.66 (m, 1H), 4.81–4.76 (m, 1H), 4.69–4.65 (m, 1H), 1.23–1.14 (m, 3H), 1.05 ppm (s, 18H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 155.74, 134.26, 130.08, 127.71, 126.45, 119.67, 87.53, 75.41, 17.85, 12.61 ppm; MS (ESI) m/z: 341 $[M+Na]+$.

b) To a solution of the previous compound (67 mg, 0.21 mmol), in dry THF (3 mL) previously cooled to 0 °C was slowly added a solution 1 M of TBAF in THF (250 μL, 0.25 mmol). The mixture was stirred at room temperature for 1 h and then 1 N HCl and EtOAc were added. The mixture was extracted with EtOAc, the organic layer was dried over Na_2SO_4, filtered and the solvent evaporated. The residue was purified by flash chromatography Hex/Et_2O 3:2 to give desired compound as a colorless oil (0.17 mmol, 82%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.06–7.04 (d, J = 8, 2H), 6.64–6.62 (d, J = 8, 2H), 6.40 (br s, 1H), 5.99–5.97 (m, 1H), 5.79–5.78 (m, 1H), 5.70–5.69 (m, 1H), 4.81–4.77 (m, 1H), 4.69–4.66 ppm (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 155.85, 133.01, 129.70, 128.17, 126.45, 115.41, 87.70, 75.27 ppm; MS (ESI) m/z: 163 $[M+H]^+$.

4-(4-(2,5-Dihydrofuran-2-yl)phenoxy)-7-nitrobenzo[*c*]-[1,2,5]oxadiazole (33): Compound **33** was obtained according to general procedure (B) for aromatic nucleophilic substitution. Yellow solid (0.09 mmol, 59%). ^{1}H NMR (400 MHz, CD_3OD): δ = 8.35–8.33 (d, J = 8, 1H), 7.45–7.43 (d, J = 8, 2H), 7.18–7.16 (d, J = 8, 2H), 6.47–6.45 (d, J = 8, 1H), 6.05–6.04 (m, 1H), 5.87–5.86 (m, 1H), 5.79–5.78 (m, 1H), 4.87–4.82 (m, 1H), 4.76–4.73 ppm (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 154.35, 152.20, 145.09, 144.09, 141.34, 133.22, 129.34, 128.80, 127.32, 120.91, 107.53, 87.03, 75.03, 75.88, 29.58 ppm; MS (ESI) m/z: 348 $[M+Na]^+$.

4-(4-(4-Methylpiperazin-1-yl)phenoxy)-7-nitrobenzo[*c*]-[1,2,5]oxadiazole (38a): A mixture of 1-(4-hydroxyphenyl)piperazine (100 mg, 0.56 mmol), formic acid (16 equiv) and formaldehyde (1.1 equiv) in MeOH (6 mL) was held at reflux for 12 h. After evaporation of solvents in vacuo the residue was partitioned between CH_2Cl_2 and a saturated aqueous solution of $NaHCO_3$. The extract was dried over anhydrous Na_2SO_4, filtered and concentrated to give intermediate **35**. This was dissolved in EtOH and 4-chloro-7-nitrobenzofurazan (1 equiv) and a catalytic amount of KOAc and pyridine were added. The resulting mixture was heated at reflux for 3 h, then was concentrated in vacuo and the crude product was purified by flash chromatography (EtOAc/MeOH 7:3) to give de-

sired compound (0.44 mmol, 85%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.32 (d, J = 7.9 Hz, 1H), 7.05 (d, J = 8.2 Hz, 2H), 6.93 (d, J = 8.2 Hz, 2H), 6.45 (d, J = 7.9 Hz, 1H), 3.18 (4H, m), 2.54 (4H, m), 2.30 ppm (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 155.18, 150.05, 146.51, 145.22, 145.09, 133.46, 130.21, 121.36, 117.25, 107.09, 54.86, 48.85, 45.94 ppm; MS (ESI) m/z: 356 $[M+H]^+$, 378 $[M+Na]^+$.

1-(4-(4-(7-Nitrobenzo[*c*][1,2,5]oxadiazol-4-yloxy)phenyl)piperazin-1-yl)ethanone (38b): To a solution of 1-(4-hydroxyphenyl)piperazine (100 mg, 0.56 mmol) in pyridine (3 mL), Ac_2O (58 μL, 0.61 equiv) were added and the mixture was stirred at room temperature for 1 h. The reaction mixture was quenched with saturated aqueous solution of $NaHCO_3$ (10 mL) and extracted with EtOAc (3×10 mL). The combined extract was dried over anhydrous Na_2SO_4, filtered and concentrated to give intermediate **36**. This was dissolved in EtOH and 4-chloro-7-nitrobenzofurazan (1 equiv) and a catalytic amount of KOAc and pyridine were added. The resulting mixture was heated at reflux for 3 h. This was concentrated in vacuo and the crude product was purified by flash chromatography (EtOAc/Hex 3:1) to give desired compound (0.45 mmol, 87%). ^{1}H NMR (400 MHz, CD_3CN): δ = 8.41 (d, J = 7.9 Hz, 1H), 7.14 (d, J = 8.2 Hz, 2H), 7.03 (d, J = 8.2 Hz, 2H), 6.51 (d, J = 7.9 Hz, 1H), 3.61 (2H, m), 3.56 (2H, m), 3.16 (2H, m), 3.10 (2H, m), 2.00 ppm (3H, s); ^{13}C NMR (100 MHz, CD_3CN): δ = 170.29, 156.22, 151.42, 147.35, 147.14, 146.34, 137.68, 131.85, 123.36, 119.19, 110.78, 50.56, 50.19, 47.41, 42.64, 23.14 ppm; MS (ESI) m/z: 384 $[M+H]^+$, 406 $[M+Na]^+$.

***tert*-Butyl-(4-(4-hydroxyphenyl)piperazin-1-yl)methanediylidenedicarbamate (37)**: **Preparation of *N*,*N*′-di-Boc-*N*′′-trifluoromethanesulfonylguanidine**: To a solution of *N*,*N*′-di-Boc-guanidine (7.5 g, 29 mmol) in CH_2Cl_2 (100 mL) Et_3N (5.0 mL, 36 mmol) was added and the temperature of the mixture was equilibrated to −78 °C using a dry ice/*i*PrOH bath. Trifluoromethanesulfanyl anhydride (5.9 mL, 35 mmol) was added dropwise through the dropping funnel over a period of 20 min, and the resulting mixture was allowed to warm to −20 °C over 4 h. A 2 M aqueous $NaHSO_4$ solution was added to the mixture at −20 °C, such that the reaction temperature did not rise above −10 °C, and the resulting layers were stirred vigorously for 5 min. The layers were immediately separated, and the aqueous phase was extracted with CH_2Cl_2 (3×50 mL). The combined organic layers were washed with 2 M aqueous $NaHSO_4$ (80 mL), brine (50 mL), dried (Na_2SO_4), filtered, and concentrated under reduced pressure. The crude material was purified by flash column chromatography (CH_2Cl_2/Hex 4:1) to provide desired compound (24.6 mmol, 85%). ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 1.46 (s, 18H), 11.06 ppm (br s, 2H); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 27.5, 83.4, 119.1, 150.0, 152.3 ppm.

Preparation of 37: A mixture of *N*,*N*′-di-Boc-N′′-trifluoromethanesulfonylguanidine (218 mg, 0.56 equiv), 1-(4-hydroxyphenyl)piperazine (100 mg, 0.56 mmol), EDC (1.2 equiv) and DIPEA (1.2 equiv) in DMF (10 mL) was stirred for 16 h at room temperature. The mixture was partitioned between $NH_4Cl_{(aq)}$ (5 mL, 1 N) and EtOAc (25 mL). The organic layer was separated, dried (Na_2SO_4), filtered, and concentrated in vacuo. The residue was purified by column chromatography (EtOAc/Hex 3:2) to give desired compound (0.42 mmol, 76%). ^{1}H NMR (400 MHz, CD_3OD): δ = 6.80 (d, J = 8.3 Hz, 2H), 6.64 (d, J = 8.3 Hz, 2H), 3.65–3.63 (4H, m), 3.23–3.20 (4H, m), 1.42 ppm (18H, s); MS (ESI) m/z: 419 $[M-H]^-$.

di-*tert*-Butyl(4-(4-(7-nitrobenzo[*c*][1,2,5]oxadiazol-4-yloxy)phenyl)piperazin-1-yl)methanediylidenedicarbamate (38c): To a solution of 4-chloro-7-nitrobenzofurazan (1 equiv) in EtOH compound **20**, a catalytic amount of KOAc and pyridine were added. The resulting mixture was heated at reflux for 3 h, then concentrated in vacuo and the crude product was purified by flash chromatography (EtOAc/Hex 3:2) (0.23 mmol, 94%). ^{1}H NMR (400 MHz, CD_3OD): δ = 8.47 (d, J = 7.8 Hz, 1H), 7.16 (d, J = 8.2 Hz, 2H), 7.07 (d, J = 8.2 Hz, 2H), 6.55 (d, J = 7.8 Hz, 1H), 3.65–3.63 (4H, m), 3.23–3.20 (4H, m), 1.42 ppm (18H, s); ^{13}C NMR (100 MHz, CD_3OD): δ = 159.72, 154.14, 151.11, 149.15, 145.24, 144.24, 135.58, 129.74, 121.29, 118.24, 116.99, 115.43, 108.70, 80.09, 77.15, 47.82, 45.09, 27.87, 27.70 ppm; MS (ESI) m/z: 582 $[M-H]^-$.

1-Acetyl-4-(4-(7-nitrobenzo[*c*][1,2,5]oxadiazol-4-yloxy)phenyl)piperazin-1-ium chloride (38a) and 1-methyl-4-(4-(7-nitrobenzo[*c*]-[1,2,5]oxadiazol-4-yloxy)phenyl)piperazin-1-ium chloride (38b): To a solution of compounds **38a** or **38b** (1 equiv) in CH_2Cl_2 a solution of 1 M HCl in MeOH (2 equiv) was added dropwise. The mixture was stirred at room temperature for 2 min then the solvent was removed in vacuo to give the desired product pure. **38a**: (0.44 mmol, quant). ^{1}H NMR (400 MHz, CD_3OD): δ = 8.50 (d, J = 7.8 Hz, 1H), 7.69 (d, J = 8.3 Hz, 2H), 7.45 (d, J = 8.3 Hz, 2H), 6.74 (d, J = 7.8 Hz, 1H), 3.95 (4H, m), 3.63 (2H, m), 3.57 (2H, m), 2.15 ppm (3H, s); ^{13}C NMR (100 MHz, CD_3OD): δ = 170.63, 153.59, 149.62, 145.59, 145.35, 144.33, 133.76, 130.81, 121.65, 120.15, 108.77, 51.65, 51.35, 44.88, 40.13, 19.55 ppm; MS (ESI) m/z: 418 $[M-H]^-$. **38b**:(0.45 mmol, quant). ^{1}H NMR (400 MHz, CD_3OD): δ = 8.47 (d, J = 7.8 Hz, 1H), 7.21 (d, J = 8.1 Hz, 2H), 7.14 (d, J = 8.1 Hz, 2H), 6.56 (d, J = 7.8 Hz, 1H), 3.85–3.82 (2H, m), 3.60–3.57 (2H, m), 3.28–3.24 (2H, m), 3.12–3.09 (2H, m), 2.92 ppm (3H, s); ^{13}C NMR (100 MHz, CD_3OD): δ = 155.99, 150.09, 148.50, 146.94, 145.88, 135.61, 131.94, 122.97, 119.86, 109.39, 54.79, 48.30, 43.76 ppm; MS (ESI) m/z: 390 $[M-H]^-$.

4-(4-(7-Nitrobenzo[*c*][1,2,5]oxadiazol-4-yloxy)phenyl)piperazine-1-carboximidamide (40c): Compound **38c** (0.23 mmol) were dissolved in 5 mL dry CH_2Cl_2 and added with 0.5 mL of freshly distilled TFA. The mixtures were stirred at room temperature for 24 h. The solvent was removed under reduced pressure and the crude compound was washed several times with toluene to yield the desired product (0.23 mmol, quant). ^{1}H NMR (400 MHz, CD_3OD): δ = 8.47 (d, J = 7.8 Hz, 1H), 7.19 (d, J = 8.2 Hz, 2H), 7.09 (d, J = 8.2 Hz, 2H), 6.55 (d, J = 7.8 Hz, 1H), 3.81–3.79 (1H, dd), 3.63–3.60 (4H, m), 3.29–3.26 (4H, m), 3.16–3.14 ppm (1H, dd); ^{13}C NMR (100 MHz, CD_3OD): δ = 159.72, 149.15, 145.24, 144.24, 135.58, 129.74, 121.29, 118.24, 116.99, 115.43, 108.70, 47.82, 45.09 ppm; MS (ESI) m/z: 479 $[M-H]^-$.

4-Azidophenol (42a): To a stirred ice cooled solution of 4-hydroxylaniline (1.0 g, 18.3 mmol) dissolved in a (1:1) mixture of AcOH/H_2O (50 mL) was added $NaNO_2$ (2.1 g, 30.4 mmol) slowly. After 5 min were added NaN_3 (2 g, 30.76 mmol) and Et_2O (50 mL) and the reaction was stirred for further 10 min. The Et_2O layer was then separated, washed with H_2O, dried on Na_2SO_4, evaporated and the resultant black oil triturated with hexane (12.26 mmol, 67%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 6.8 ppm (s, 4H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 116, 120, 132, 152.5 ppm.

3-azidophenol (42b): 3-Azidophenol was obtained with the same procedure as for **42a** but starting from 3-hydroxylaniline. After being stirred for 20 min, the reaction mixture was filtered to remove black insoluble polymeric residues, extracted with CH_2Cl_2, dried on Na_2SO_4, and the solvent was removed in vacuo to give a yellow/brown oil (6.4 mmol, 35%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.15 (t, J = 8.0 Hz, 1H), 6.6 (d, J = 11.7 Hz, 2H), 6.5 ppm (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 106, 111, 112, 113, 130, 157 ppm.

(4-Ethynylphenyl)(methyl)sulfane (43c): To a solution of triisopropyl((4-(methylthio)phenyl)ethynyl)silane (0.46 mmol) in THF dry (4 mL) TBAF was added (0.56 mmol) and the mixture was stirred at

room temperature for 2 h. Then a NH_4Cl saturated solution (4 mL) was added and the aqueous phase was extracted with Et_2O (2× 10 mL). The organic layers were then collected, washed with saturated NaCl solution, dried with Na_2CO_3, and concentrated in vacuo to provide desired compound (0.45 mmol, 97%). ^1H NMR (400 MHz, $CDCl_3$): $\delta = 7.32$ (d, 2H, $J = 7.8$ Hz), 7.10 (d, 2H, $J = 7.8$ Hz), 3.0 (s, 1H), 2.40 ppm (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 139.98$, 132.29, 125.68, 118.26, 83.37, 77.31, 14.04 ppm; MS (ESI): m/z: 282 $[M-H]^-$.

***N*-*tert*-Butyloxycarbonyl-*O*-propargyl-L-serine methyl ester (43 f)**: To a stirred solution of *N*-tertbutyloxycarbonyl-L-serine (5.13 g, 25 mmol) in DMF (150 mL) at 0 °C was carefully added NaH (60% in mineral oil, 4.0 g, 100 mmol). The reaction mixture was stirred for 30 min until hydrogen evolution stopped. Propargyl bromide (4.46 mL, 50 mmol) was added, and the mixture was stirred at 0 °C for 30 min and then room temperature for 12 h; MeI (2.40 mL) was added. Four hours later the reaction mixture was poured into brine, and extracted with Et_2O (3×150 mL). The combined organic layers were washed with brine, dried (Na_2SO_4), and concentrated. The residue was purified by flash chromatography (Hex/Et_2O 4:1) to provide desired compound (9.75 mmol, 39%). $[\alpha]_D$: +17.8 ($c = 2.7$, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): $\delta = 5.38$ (d, $J = 8.4$ Hz, 1H), 4.46 (dt, $J = 8.4$, 3.3 Hz, 1H), 4.15 (d, $J = 2.4$ Hz, 2H), 3.96 (dd, $J = 9.3$, 3.3 Hz, 1H), 3.77 (s, 3H), 3.76 (dd, $J = 9.3$, 3.3 Hz, 1H), 2.46 (t, $J = 2.4$ Hz, 1H), 1.46 ppm (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 170.86$, 155.40, 79.98, 78.77, 75.03, 69.61, 58.49, 53.72, 52.49, 28.24 ppm.

1-Propynyl-2,3,4-tri-*O*-benzoyl-β-D-ribofuranose (43 g): To a solution of β-D-ribofuranose 1-acetate 2,3,5 tribenzoate (200 mg, 0.4 mmol) in CH_2Cl_2 (5 mL) was added propargyl alcohol (28 μL, 0.47 mmol) and $BF_3{\cdot}Et_2O$ (73 μL, 0.6 mmol) at 0 °C and the reaction mixture was stirred at room temperature for 1 h. After completion of the reaction anhydrous K_2CO_3 (100 mg) was added and stirring was continued for further 15 min. Then the reaction mixture was filtered and washed with CH_2Cl_2. The filtrate was washed with H_2O, the aqueous phase was separated and extracted with CH_2Cl_2 (2× 10 mL) and the combined organic phases were dried (Na_2SO_4) and concentrated to yield the desired compound as a crystalline solid (0.356 mmol, 89%). $[\alpha]_D$: +12.4° ($c = 10$, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): $\delta = 8.02$–7.96 (m, 4H), 7.81 (d, $J = 8.1$ Hz, 2H), 7.51–7.41 (m, 3H), 7.38–7.32 (m, 4H), 7.26–7.22 (m, 2H), 5.87–5.84 (m, 1H, H-2), 5.69 (d, $J = 4.2$ Hz, 1H, H-1), 5.44 (s, 1H, H-4), 4.70–4.65 (m, 2H), 4.50–4.46 (m, 1H, H-3), 4.20 (s, 2H), 2.39 ppm (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 166.10$, 165.26, 165.07, 133.44, 133.33, 133.10, 129.75, 129.67, 129.11, 128.84, 128.43, 128.34, 128.29, 103.30, 79.30, 78.25, 75.48, 75.24, 72.07, 64.42, 54.49 ppm; MS (ESI) m/z: 523 $[M+Na]^+$.

General procedure for the synthesis of triazoles 44–45 a–e

The appropriate alkyne (1 equiv) and the freshly synthesized phenylazide (1 equiv) were suspended in a 1:1 mixture of H_2O and *tert*-BuOH (1 mL each). To this, was added sodium ascorbate (0.1 equiv) and $CuSO_4{\cdot}5H_2O$ (0.01 equiv). The mixture was then irradiated for 10 min at 125 °C. The mixture was then partitioned between a saturated aqueous solution of NH_4Cl (10 mL + 2 gtt NH_4OH) and EtOAc (15 mL) and stirred for 15 min. The organic layer was separated, dried (Na_2SO_4), and solvent removed in vacuo to furnish the desired triazoles as pure compounds.

4-(4-Phenyl-1*H*-1,2,3-triazol-1-yl)phenol (44 a): (7.75 mmol, 79%). ^1H NMR (400 MHz, CD_3OD): $\delta = 8.65$ (s, 1H), 7.82 (d, $J = 8.1$ Hz, 2H), 7.61 (d, $J = 8.1$ Hz, 2H), 7.40–7.36 (m, 2H), 7.31–7.29 (m, 1H), 6.89 ppm (d, $J = 8.1$ Hz, 2H); ^{13}C NMR (100 MHz, CD_3OD): $\delta = 158.13$, 147.82, 130.05, 129.23, 128.50, 127.99, 125.28, 121.86, 118.90, 115.67 ppm; MS (ESI) m/z: 236 $[M-H]^-$.

4-(4-(4-Methoxyphenyl)-1*H*-1,2,3-triazol-1-yl)phenol (44 b): (2.72 mmol, 85%). ^1H NMR (400 MHz, CD_3OD): $\delta = 8.44$ (s, 1H), 7.68 (d, $J = 7.9$ Hz, 2H), 7.55 (d, $J = 7.9$ Hz, 2H), 6.89–6.85 (m, 4H), 3.72 ppm (s, 3H); ^{13}C NMR (100 MHz, CD_3OD): $\delta = 159.90$, 158.02, 147.74, 129.26, 126.62, 122.51, 121.78, 117.97, 115.65, 113.88, 54.28 ppm; MS (ESI) m/z: 268 $[M+H]^+$, 290 $[M+Na]^+$.

4-(4-Cyclohexenyl-1*H*-1,2,3-triazol-1-yl)phenol (44 d): (1.46 mmol, 73%). ^1H NMR (400 MHz, CD_3OD): $\delta = 8.19$ (s, 1H), 7.53 (d, $J = 8.1$ Hz, 2H), 6.86 (d, $J = 8.1$ Hz, 2H), 6.45 (s, 1H), 2.37 (brs, 2H), 2.15 (brs, 2H), 1.73 (brs, 2H), 1.64 ppm (brs, 2H); ^{13}C NMR (100 MHz, CD_3OD): $\delta = 157.95$, 149.41, 129.30, 126.83, 124.94, 121.71, 117.53, 115.61, 25.82, 24.80, 22.09, 21.79 ppm; MS (ESI) m/z: 240 $[M-H]^-$.

4-(4-(Hydroxymethyl)-1*H*-1,2,3-triazol-1-yl)phenol (44 e): (1.14 mmol, 57%). ^1H NMR (400 MHz, CD_3OD): $\delta = 8.21$ (s, 1H), 7.53 (d, $J = 8.2$ Hz, 2H), 6.87 (d, $J = 8$ Hz, 2H), 4.67 ppm (s, 2H); ^{13}C NMR (100 MHz, CD_3OD): $\delta = 158.09$, 148.11, 129.25, 121.92, 120.91, 115.64, 55 ppm; MS (ESI) m/z: 190 $[M-H]^-$.

(*S*)-Methyl-2-(*tert*-butoxycarbonylamino)-3-((1-(4-hydroxyphenyl)-1*H*-1,2,3-triazol-4-yl)methoxy)propanoate (44 f): (6.5 mmol, 67%). $[\alpha]_D$: −1.47° ($c = 10$, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): $\delta = 7.91$ (s, 1H), 7.85 (brs, 1H), 7.40 (d, $J = 7.8$ Hz, 1H), 6.89 (d, $J = 8$ Hz, 1H), 5.87 (brs, 1H), 5.65 (brs, 1H), 4.78 (brs, 2H), 3.70 (brs, 3H), 2.10 (brs, 2H), 1.36 ppm (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 165.30$, 157.16, 153.82, 144.74, 139.61, 129.75, 122.16, 121.38, 121.34, 118.48, 116.31, 82.02, 77.26, 76.94, 52.34, 45.16, 28.03 ppm; MS (ESI) m/z: 393 $[M+H]^+$.

1-(1-(4-Hydroxyphenyl)-1*H*-1,2,3-triazol-4-yl)-2,3,4-tri-*O*-benzoyl-β-D-ribofuranose (44 g): (0.256 mmol, 72%). $[\alpha]_D$: −4.25° ($c = 10$, $CHCl_3$); ^1H NMR (400 MHz, $CHCl_3$): $\delta = 8.77$ (brs, 1H), 7.97–7.91 (m, 4H), 7.80 (d, $J = 8.2$ Hz, 2H), 7.76 (s, 1H), 7.48–7.44 (m, 1H), 7.42–7.33 (m, 6H), 7.31–7.21 (m, 4H), 6.98 (d, $J = 8.2$ Hz, 2H), 5.88–5.85 (m, 1H, H-2), 5.69 (d, $J = 4.2$ Hz, 1H, H-1), 5.39 (s, 1H, H-4), 4.93–4.90 (m, 1H), 4.76–4.72 (m, 3H), 4.55–4.51 ppm (m, 1H, H-3); ^{13}C NMR (100 MHz, $CHCl_3$): $\delta = 166.34$, 165.48, 165.31, 157.66, 144.04, 133.57, 133.45, 133.22, 129.72, 129.67, 129.43, 129.36, 128.93, 128.72, 128.47, 128.34, 122.30, 121.52, 116.48, 104.78, 79.41, 75.59, 72.21, 64.56, 61.00 ppm; MS (ESI) m/z: 634 $[M-H]^-$.

3-(4-Phenyl-1*H*-1,2,3-triazol-1-yl)phenol (45 a): (4.72 mmol, 77%). ^1H NMR (400 MHz, CD_3OD): $\delta = 8.72$ (s, 1H), 7.83 (d, $J = 8.1$ Hz, 2H), 7.40–7.37 (m, 2H), 7.31–7.24 (m, 3H), 6.59 ppm (d, $J = 8.1$ Hz, 2H); ^{13}C NMR (100 MHz, CD_3OD): $\delta = 158.11$, 151.77, 130.31, 129.30, 128.55, 128.10, 125.33, 118.95, 115.54, 112.37 ppm; MS (ESI) m/z: 236 $[M-H]^-$.

3-(4-(4-Methoxyphenyl)-1*H*-1,2,3-triazol-1-yl)phenol (45 b): (2.24 mmol, 70%). ^1H NMR (400 MHz, CD_3OD): $\delta = 8.63$ (s, 1H), 7.75 (d, $J = 7.9$ Hz, 1H), 7.31–7.23 (m, 3H), 6.95 (d, $J = 7.9$ Hz, 1H), 6.84 ppm (d, $J = 8$ Hz, 1H); ^{13}C NMR (100 MHz, CD_3OD): $\delta = 160.04$, 158.56, 137.94, 130.27, 126.68, 122.45, 117.89, 115.43, 113.92, 110.63, 107.05, 54.30 ppm; MS (ESI) m/z: 266 $[M-H]^-$.

3-(4-(4-(Methylthio)phenyl)-1*H*-1,2,3-triazol-1-yl)phenol (45 c): (0.26 mmol, 58%). ^1H NMR (400 MHz, CD_3OD): $\delta = 8.72$ (s, 1H), 7.78 (s, 1H), 7.75 (s, 1H), 7.34–7.24 (m, 5H), 6.84 ppm (d, 1H, $J = 8.2$ Hz); ^{13}C NMR (100 MHz, CD_3OD): $\delta = 157.66$, 138.43, 129.82, 126.42, 125.85, 121.90, 118.18, 116, 14.42 ppm; MS (ESI) m/z: 282 $[M-H]^-$.

1-(1-(4-Hydroxyphenyl)-1*H*-1,2,3-triazol-4-yl)-2,3,4-trihydroxy-β-D-ribofuranose (46): Compound **44 g** (61 mg, 0.07 mmol) was dis-

solved in 4:1 MeOH/conc NH_4OH (5 mL) and stirred at room temperature for 18 h. The reaction mixture was concentrated in vacuo and azeotroped 3 times with EtOH. The crude product was dissolved in H_2O (5 mL), extracted with CH_2Cl_2 (3×50 mL) and the aqueous layer concentrated in vacuo to provide desired compound (0.069 mmol, 99%). $[\alpha]_D$: −1.28° (c=10, $CHCl_3$); ^{1}H NMR (400 MHz, CD_3OD): δ=8.27 (s, 1H), 7.52 (d, J=8.2 Hz, 2H), 6.86 (d, J=8.2 Hz, 2H), 4.95 (s, 1H, H-4), 4.81 (d, J=11.9 Hz, 1H), 4.62 (d, J=11.9 Hz, 1H), 4.08–4.05 (m, 1H, H-2), 3.95–3.88 (m, 2H), 3.72–3.69 (m, 1H, H-1), 3.56–3.51 ppm (m, 1H, H-3); MS (ESI) m/z: 322 $[M-H]^-$.

General procedure for the synthesis of compounds 47–48 a–h

To a solution of 4-chloro-7-nitrobenzofurazan (0.25 mmol, 1 equiv) in EtOH the appropriate triazole (1.1 equiv) and a catalytic amount of KOAc and pyridine were added. The resulting mixture was heated at reflux for 3 h. This was concentrated in vacuo and the crude product was purified by flash chromatography with the appropriate eluent.

4-Nitro-7-(4-(4-phenyl-1*H*-1,2,3-triazol-1-yl)phenoxy)benzo[*c*][1,2,5]oxadiazole (47 a): (0.23 mmol, 91%). ^{1}H NMR (400 MHz, [D$_6$]acetone): δ=9.00 (s, 1H), 8.64 (d, 1H, J=7.8 Hz), 8.13 (d, J=8.2 Hz, 2H) 7.94 (d, J=8.1 Hz, 2H), 7.64 (d, J=8.1 Hz, 2H), 7.45–7.41 (m, 2H), 7.32 (t, 1H, J=8.2 Hz), 6.94 ppm (d, 1H, J=7.8 Hz); ^{13}C NMR (100 MHz, [D$_6$]acetone): δ=153.23, 153.13, 148.03, 135.58, 134.27, 128.77, 128.14, 125.46, 122.39, 122.31, 118.73, 109.82 ppm; MS (ESI) m/z: 435 $[M+Cl]^-$.

4-(4-(4-(4-Methoxyphenyl)-1*H*-1,2,3-triazol-1-yl)phenoxy)-7-nitrobenzo[*c*][1,2,5]oxadiazole (47 b): (0.22 mmol, 88%). ^{1}H NMR (400 MHz, [D$_6$]acetone): δ=8.87 (s, 1H), 8.61 (d, J=7.8 Hz, 1H), 8.11 (d, 2H, J=8.2 Hz) 7.86 (d, 2H, J=8.1 Hz), 7.63 (d, J=8.1 Hz, 2H), 6.99 (d, J=8.2 Hz, 2H), 6.93 (d, J=7.8 Hz, 1H), 3.79 ppm (s, 3H); ^{13}C NMR (100 MHz, [D$_6$]acetone): δ=161.40, 154.76, 154.62, 149.44, 147.42, 146.41, 137.29, 136.94, 132.75, 128.76, 124.28, 123.79, 120.78, 118.12, 116.47, 112.59, 57.23 ppm; MS (ESI) m/z: 429 $[M-H]^-$.

4-(4-(4-Cyclohexenyl-1*H*-1,2,3-triazol-1-yl)phenoxy)-7-nitrobenzo[*c*][1,2,5]oxadiazole (47 d): (0.217 mmol, 87%). ^{1}H NMR (400 MHz, [D$_6$]acetone): δ=8.60 (d, J=7.9 Hz, 1H), 8.48 (s, 1H), 8.06 (d, J=8.0 Hz, 1H) 7.60 (d, J=8.0 Hz, 1H), 6.91 (d, J=7.9 Hz, 1H), 6.57–6.55 (m, 1H), 2.40–2.38 (m, 2H), 2.17–2.14 (m, 2H), 1.74–1.71 (m, 2H), 1.65–1.61 ppm (m, 2H); ^{13}C NMR (100 MHz, [D$_6$]acetone): δ=152.8, 145.5, 143.2, 135.6, 134.1, 127.3, 124.6, 122.25, 122.13, 117.11, 111.47, 109.73, 25.94, 24.85, 22.23, 22.0 ppm; MS (ESI) m/z: 403 $[M-H]^-$, 439 $[M+Cl]^-$.

(1-(4-(7-Nitrobenzo[*c*][1,2,5]oxadiazol-4-yloxy)phenyl)-1*H*-1,2,3-triazol-4-yl)methanol (47 e): 0.21 mmol, 85%. ^{1}H NMR (400 MHz, [D$_6$]acetone): δ=8.61 (d, J=7.8 Hz, 1H), 8.43 (s, 1H), 8.06 (d, J=8.2 Hz, 2H), 7.60 (d, J=8.2 Hz, 2H), 6.91 (d, J=7.8 Hz, 1H), 4.72 ppm (s, 2H); ^{13}C NMR (100 MHz, [D$_6$]acetone): δ=154.90, 154.55, 151.20, 147.19, 146.17, 137.27, 135.92, 132.81, 123.94, 123.88, 121.93, 111.36, 57.36 ppm; MS (ESI) m/z: 377 $[M+Cl]^-$, 389 $[M+Na]^+$.

(*R*)-Methyl-2-(*tert*-butoxycarbonylamino)-3-((1-(4-(7-nitrobenzo[*c*][1,2,5]oxadiazol-4-yloxy)phenyl)-1*H*-1,2,3-triazol-4-yl)methoxy)propanoate (47 f): (0.157 mmol, 63%). $[\alpha]_D$: +2.65° (c=10, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$): δ=8.37 (d, J=7.8 Hz, 1H), 8.10 (s, 1H), 7.83 (d, J=8.2 Hz, 1H), 7.37 (d, J=8.2 Hz, 1H), 6.59 (d, J=7.8 Hz, 1H), 5.82 (brs, 1H), 5.61 (brs, 1H), 4.78 (brs, 2H), 3.70 (brs, 4H), 1.34 ppm (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$): δ=165.24, 153.39, 152.58, 145.82, 144.98, 144.07, 139.82, 135.58, 133.06, 131.13, 122.64, 122.25, 117.61, 108.37,81.76, 52.26, 45.19, 27.99 ppm; MS (ESI) m/z: 556 $[M+H]^+$.

1-(1-(4-(7-Nitrobenzo[*c*][1,2,5]oxadiazol-4-yloxy)phenyl)-1*H*-1,2,3-triazol-4-yl)-2,3,4-tri-hydroxy-β-D-ribofuranose (47 h): (0.08 mmol, 32%). $[\alpha]_D$: −1.09° (c=10, MeOH); ^{1}H NMR (400 MHz, [D$_6$]acetone): δ=8.60 (d, J=8.0 Hz, 1H), 8.55 (s, 1H), 8.06 (d, J=8.2 Hz, 1H), 7.60 (d, J=8.2 Hz, 1H), 6.91 (d, J=8.0 Hz, 1H), 4.98 (s, 1H, H-4), 4.81 (d, J=11.8 Hz, 1H), 4.67 (d, J=11.8 Hz, 1H), 4.18–4.16 (m, 1H, H-2), 3.91–3.89 (m, 2H), 5.68 (d, J=11.9 Hz, 1H, H-1), 3.56–3.52 (m, 1H, H-3), 2.82 ppm (brs, 3H); ^{13}C NMR (100 MHz, [D$_6$]acetone): δ=153.24, 153.08, 145.73, 145.57, 144.56, 135.50, 134.32, 131.23, 122.48, 122.27, 121.71, 109.83, 106.83, 84.56, 75.26, 70.85, 62.92, 60.07 ppm; MS (ESI) m/z: 485 $[M-H]^-$.

4-Nitro-7-(3-(4-phenyl-1*H*-1,2,3-triazol-1-yl)phenoxy)benzo[*c*][1,2,5]oxadiazole (48 a): (0.225 mmol, 90%). ^{1}H NMR (400 MHz, [D$_6$]acetone): δ=8.99 (s, 1H), 8.60 (d, J=7.8 Hz, 1H), 8.02 (s, 1H), 8.00 (d, J=8.2 Hz, 1H), 7.94 (d, J=8.2 Hz, 1H), 7.79 (t, J=8.2 Hz, 1H), 7.51 (d, J=8.1 Hz, 1H), 7.42–7.40 (m, 2H), 7.32–7.29 (m, 1H), 6.99 ppm (d, J=7.8 Hz, 1H); ^{13}C NMR (100 MHz, [D$_6$]acetone): δ=155.94, 154.34, 149.50, 141.41, 146.40, 140.23, 137.26, 134.26, 132.88, 132.03, 131.01, 130.35, 127.36, 122.70, 121.77, 119.98, 114.61, 112.93 ppm; MS (ESI) m/z: 435 $[M+Cl]^-$.

4-(3-(4-(4-Methoxyphenyl)-1*H*-1,2,3-triazol-1-yl)phenoxy)-7-nitrobenzo[*c*][1,2,5]oxadiazole (48 b): (0.217 mmol, 87%). ^{1}H NMR (400 MHz, [D$_6$]acetone): δ=8.85 (s, 1H), 8.57 (d, J=7.8 Hz, 1H), 7.98 (s, 1H), 7.96 (d, J=8.2 Hz, 1H), 7.80–7.74 (m, 3H), 7.48 (d, J=8.2 Hz, 1H), 6.97–6.92 (m, 3H), 3.76 ppm (s, 1H); ^{13}C NMR (100 MHz, [D$_6$]acetone): δ=161.43, 155.91, 154.36, 149.46, 147.38, 146.37, 140.29, 137.23, 134.21, 132.84, 128.73, 124.55, 122.56, 120.67, 119.86, 116.45, 114.48, 112.84, 57.20 ppm; MS (ESI) m/z: 429 $[M-H]^-$.

4-(3-(4-(4-(Methylthio)phenyl)-1*H*-1,2,3-triazol-1-yl)phenoxy)-7-nitrobenzo[*c*][1,2,5]oxadiazole (48 c): (0.14 mmol, 57%). ^{1}H NMR (400 MHz, [D$_6$]acetone): δ=8.97 (s, 1H), 8.60 (d, J=7.8 Hz, 1H), 8.02 (s, 1H), 8.00 (d, J=8.2 Hz, 1H), 7.84 (d, J=8.1 Hz, 2H), 7.79 (t, J=8.2 Hz, 1H), 7.51 (d, J=8.2 Hz, 1H), 7.31 (d, J=8.1 Hz, 2H), 6.99 (d, J=8 Hz, 1H), 2.46 ppm (s, 1H); ^{13}C NMR (100 MHz, [D$_6$]acetone): δ=154.38, 153.02, 147.75, 145.59, 139.13, 138.90, 134.28, 131.37, 127.08, 126.37, 126.00, 125.75, 120.71, 120.48, 118.33, 112.97, 112.81, 110.12, 14.50 ppm; MS (ESI) m/z: 469 $[M+Na]^+$, 481 $[M+Cl]^-$.

General procedure for reduction of nitro to 7-amino derivatives (50 a–c)

The appropriate nitro compound (5.6 mmol) was dissolved in a mixture of CH_2Cl_2 (200 mL), conc HCl (12 mL) and MeOH (90 mL). After the addition of Fe^0 (2.13 g), the mixture was stirred for 25 min at room temperature. The reaction mixture was poured into H_2O (100 mL) and extracted with CH_2Cl_2 (3×150 mL). The organic layer was dried over anhydrous Na_2SO_4 and concentrated in vacuo. The crude product was then purified by flash chromatography (Hex/EtOAc 3:2).

7-(4-Bromobenzylthio)benzo[*c*][1,2,5]oxadiazol-4-amine (50 a): (4.87 mmol, 87%). ^{1}H NMR (400 MHz, $CDCl_3$): δ=7.25 (d, J=4.5 Hz, 2H), 7.01 (d, J=7.8 Hz, 1H), 6.94 (d, J=4 Hz, 2H), 6.13 (d, J=7.8 Hz, 1H), 4.57 (brs, 2H), 4.07 ppm (s, 2H); MS (ESI) m/z: 334–336 $[M-H]^-$.

7-(4-Bromophenylthio)benzo[*c*][1,2,5]oxadiazol-4-amine (50 b): (2.91 mmol, 52%). ^{1}H NMR (400 MHz, $CDCl_3$): δ=7.36–7.34 (d, J=

7.8, 1H), 7.29–7.27 (d, $J=8$, 2H), 7.09–7.07 (d, $J=8$, 2H), 6.29–6.27 (d, $J=7.8$, 1H), 4.73 ppm (br s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta=$ 147.99, 141.43, 136.89, 134.02, 131.96, 130.65, 129.72, 126.44, 125.05, 121.62 ppm; MS (ESI) *m/z*: 323 $[M+H]^+$.

3-(7-Aminobenzo[*c*][1,2,5]oxadiazol-4-ylthio)-1-morpholinopropan-1-one (50c): (5.15 mmol, 92%). ^{1}H NMR (400 MHz, $CDCl_3$): $\delta=$ 29 (d, $J=7.8$ Hz, 1H), 6.34 (d, $J=7.8$ Hz, 1H), 5.99 (br s, 2H), 3.49–3.44 (m, 4H), 3.38–3.35 (m, 4H), 3.13 (t, $J=8$ Hz, 2H), 2.59 ppm (t, $J=8$ Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta=169.40$, 151.13, 145.56, 139.05, 136.89, 106.84, 105.60, 66.58, 45.83, 41.81, 33.13 ppm; MS (ESI) *m/z*: 309 $[M+Na]^+$.

General procedure for 7-chloro derivatives 51a,b

To a solution of $CuCl_2$ (0.15 mmol) and *tert*-butyl nitrite (0.19 mmol) in dry CH_3CN at 65 °C was slowly added the appropriate amine previously solubilized in CH_3CN. During the addition the reaction solution turned black from the initial green color. The mixture was stirred at 65 °C for further 3 h, then was quenched with 3 N HCl and extracted with Et_2O. The organic layer was dried over Na_2SO_4 filtered off and concentrated and the residue was purified by flash chromatography (Hex/EtOAc 4:1).

4-Chloro-7-(4-methoxybenzylthio)benzo[*c*][1,2,5]oxadiazole (51a): Yellow solid (1.86 mmol, 65%). ^{1}H NMR (400 MHz, $CDCl_3$): $\delta=8.29$–8.27 (d, $J=8$, 1H), 7.29–7.27 (d, $J=8$, 2H), 7.13–7.11 (d, $J=8$, 1H), 6.82–6.80 (d, $J=8$, 2H), 4.41 (s, 2H), 3.73 ppm (s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta=159.76$, 146.32, 144.87, 130.48, 128.36, 127.51, 126.83, 117.53, 115.62, 113.56, 57.02, 36.45 ppm; MS (ESI) *m/z*: 329 $[M+Na]^+$.

4-(4-Bromophenylthio)-7-chlorobenzo[*c*][1,2,5]oxadiazole (51b): Yellow solid (1.51 mmol, 52%). ^{1}H NMR (400 MHz, $CDCl_3$): $\delta=7.51$–7.49 (d, $J=8.3$, 2H), 7.38–7.36 (d, $J=8.3$, 2H), 7.19–7.17 (d, $J=7.8$, 1H), 6.78–6.76 ppm (d, $J=7.8$, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta=$ 136.65, 135.69, 133.20, 130.41, 128.79, 128.28, 126.92, 124.27, 122.41, 119.73 ppm; MS (ESI) *m/z*: 342 $[M+H]^+$.

3-(7-Iodobenzo[*c*][1,2,5]oxadiazol-4-ylthio)-1-morpholinopropan-1-one (52c): To a cooled solution (0 °C) of compound **50c** (2.25 mmol) in concentrated HCl (0.56 mL) and 98% H_2SO_4 (2 mL) was added dropwise a solution of $NaNO_2$ (2.23 mmol) in 1 mL H_2O. The mixture was stirred vigorously at 0 °C for 40 min. Then, a solution of KI (4.5 mmol) in 0.9 mL of H_2O was added dropwise, and the mixture was stirred at room temperature for 2 h. The product was extracted with EtOAc, washed with a saturated Na_2CO_3 solution, H_2O and brine. After drying on anhydrous Na_2SO_4, the solution was concentrated in vacuo. The crude product was then purified by flash chromatography (Hex/EtOAc 3:1) to provide desired compound (2.07 mmol, 92%). ^{1}H NMR (400 MHz, $CDCl_3$): $\delta=7.66$ (d, $J=8$ Hz, 1H), 6.91 (d, $J=8$ Hz, 1H), 3.59–3.54 (m, 6H), 3.40 (t, $J=8$ Hz, 2H), 3.37–3.35 (m, 2H), 2.69 ppm (t, $J=8$ Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta=168.84$, 151.25, 147.77, 140.85, 128.12, 127.80, 66.69, 66.39, 45.69, 42.07, 32.42, 27.36 ppm.

7-(4-Methoxybenzylthio)benzo[*c*][1,2,5]oxadiazol-4-amine hydrochloride (53a): Compound **53a** was obtained using the same procedure as for **39a**,**b** (1.5 mmol, 75%). ^{1}H NMR (400 MHz, $CDCl_3$): $\delta=7.02$–7.01 (d, 3H), 6.68 (d, $J=8$ Hz, 2H), 6.12 (d, $J=8$ Hz, 1H), 4.55 (br s, 2H), 4.08 (s, 2H), 3.68 ppm (s, 3H); MS (ESI) *m/z*: 286 $[M-H]^-$.

***N*-(7-(3-Morpholino-3-oxopropylthio)benzo[*c*][1,2,5]oxadiazol-4-yl)acetamide (54c)**: To a solution of compound **50c** (0.56 mmol) in pyridine (3 mL), Ac_2O (0.61 mmol) was added and the mixture was stirred at room temperature for 1 h. The reaction mixture was quenched with saturated aqueous solution of $NaHCO_3$ (10 mL) and extracted with EtOAc (3×10 mL). The combined extract was dried over anhydrous Na_2SO_4, filtered and concentrated in vacuo and the crude product was purified by flash chromatography (EtOAc/Hex 3:1) to provide desired compound (0.509 mmol, 91%). ^{1}H NMR (400 MHz, $CDCl_3$): $\delta=8.12$ (d, $J=8$ Hz, 1H), 8.00 (br s, 1H), 7.25 (d, $J=8$ Hz, 1H), 3.59–3.54 (m, 6H), 3.39–3.34 (m, 4H), 2.65 (t, $J=8$ Hz, 2H), 2.24 ppm (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta=169.13$, 168.69, 139.88, 132.31, 124.42, 116.89, 111.18, 90.26, 66.73, 66.41, 45.69, 41.99, 32.99, 28.34, 24.55 ppm.

7-Chloro-*N*,*N*-dimethylbenzo[*c*][1,2,5]oxadiazole-4-sulfonamide (56): 4-Chlorosulfonyl-7-chloro-2,1,3-benzoxadiazole **55** (200 mg, 0.92 mmol) was dissolved in CH_3CN (6 mL). After the addition of Me_2NH (0.9 equiv) and Et_3N (cat.), the mixture was stirred at room temperature for 3 h. The reaction mixture was evaporated to dryness under reduced pressure, and the residue was separated by chromatography on silica gel using CH_2Cl_2. To give desired compound (0.84 mmol, 92%). ^{1}H NMR (400 MHz, $CDCl_3$): $\delta=7.89$ (d, $J=$ 7.8 Hz, 1H), 7.49 (d, $J=7.8$ Hz, 1H), 2.88 ppm (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta=150.40$, 147.20, 135.99, 130.68, 129.21, 127.58, 39.18 ppm; MS (ESI) *m/z*: 284–286 $[M+Na]^+$.

General procedure for the synthesis of compounds 58a–d

To a solution of compound **56** (65 mg, 0.25 mmol) in 5 mL of EtOH was added the appropriate nucleophile (0.27 mmol) and a catalytic amount of pyridine (0.05 mL) or of KOAc (0.01 mmol). The reaction mixture was heated at reflux for 2 h. The mixture was then concentrated in vacuo and the crude product was purified by flash chromatography (Hex/EtOAc 3:2).

7-(4-Methoxybenzylthio)-*N*,*N*-dimethylbenzo[*c*][1,2,5]oxadiazole-4-sulfonamide (58a): (0.23 mmol, 92%). ^{1}H NMR (400 MHz, $CDCl_3$): $\delta=7.77$ (d, $J=7.8$ Hz, 1H), 7.26 (d, $J=8.1$ Hz, 2H), 7.09 (d, $J=$ 7.8 Hz, 1H), 6.80 (d, $J=8.1$ Hz, 2H), 4.35 (s, 2H), 3.75 (s, 3H), 2.85 ppm (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta=160.96$, 150.32, 146.64, 137.24, 136.22, 131.56, 127.71, 124.61, 123.43, 115.90, 56.84, 39.22, 37.61 ppm; MS (ESI) *m/z*: 402 $[M+Na]^+$.

7-(4-Bromobenzylthio)-*N*,*N*-dimethylbenzo[*c*][1,2,5]oxadiazole-4-sulfonamide (58b): (0.227 mmol, 91%). ^{1}H NMR (400 MHz, $CDCl_3$): $\delta=7.76$ (d, $J=7.9$ Hz, 1H), 7.39 (d, $J=7.7$ Hz, 2H), 7.22 (d, $J=$ 7.7 Hz, 2H), 7.08 (d, $J=7.9$ Hz, 1H), 4.35 (s, 2H), 2.85 ppm (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta=150.32$, 146.65, 136.19, 136.08, 135.25, 133.65, 131.96, 125.30, 124.07, 123.64, 39.22, 37.43 ppm; MS (ESI) *m/z*: 450–452 $[M+Na]^+$.

***N*,*N*-Dimethyl-7-(3-nitrobenzylthio)benzo[*c*][1,2,5]oxadiazole-4-sulfonamide (58c)**: (0.23 mmol, 93%). ^{1}H NMR (400 MHz, $CDCl_3$): $\delta=8.21$ (s, 1H), 8.08 (d, $J=8.2$ Hz, 2H), 7.77 (d, $J=7.8$ Hz, 1H), 7.69 (d, $J=8.2$ Hz, 2H), 7.49–7.45 (m, 1H), 7.13 (d, $J=7.8$ Hz, 1H), 4.52 (s, 2H), 2.85 ppm (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta=148.47$, 137.19, 134.70, 134.33, 133.47, 129.96, 125.27, 124.71, 123.63, 123.26, 123.07, 117.43, 37.59, 35.73 ppm; MS (ESI) *m/z*: 393 $[M-H]^-$, 429 $[M+Cl]^-$.

7-(4-Fluorobenzylthio)-*N*,*N*-dimethylbenzo[*c*][1,2,5]oxadiazole-4-sulfonamide (58d): (0.23 mmol, 92%). ^{1}H NMR (400 MHz, $CDCl_3$): $\delta=7.77$ (d, $J=7.8$ Hz, 1H), 7.34–7.30 (m, 2H), 7.09 (d, $J=7.8$ Hz, 1H), 6.99–6.94 (m, 2H), 4.38 (s, 2H), 2.85 ppm (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta=163.58$–161.11 (C-F), 148.71, 145.04, 134.94, 134.52, 130.40, 123.48, 122.30, 115.85, 37.61, 35.73 ppm; MS (ESI) *m/z*: 366 $[M-H]^-$, 402 $[M+Cl]^-$.

General procedure for Diels–Alder with Danishefsky diene

In a sealed tube the appropriate starting material (**49 a–f**) (0.28 mmol) was dissolved in freshly distilled CH_2Cl_2 (2 mL) then *trans*-1-methoxy-3-trimethylsilyloxy-buta-1,3-diene was added. The reaction mixture was heated at 50 °C for 6–12 h. After that time 2 N HCl was added and the mixture was stirred for further 1 h then the mixture was extracted with CH_2Cl_2. The organic layer was dried over Na_2SO_4, filtered off and the solvent was removed under reduced pressure. The crude was then purified by flash chromatography to give two diastereomers **59 a–f** and **60 a–f**.

(9*R*,9a*R*)-4-Chloro-9-methoxy-9a-nitro-5a,6,9,9a-tetrahydronaphtho[2,1-*c*][1,2,5]oxadiazol-7(8*H*)-one (59 a): White solid (0.07 mmol, 25%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 6.72–6.70 (m, 1H), 5.11–5.10 (m, 1H), 4.45–4.39 (m, 1H), 3.42 (s, 3H), 2.98–2.94 (m, 1H), 2.83–2.77 (m, 1H), 2.71–2.67 (m, 1H), 1.91–1.84 ppm (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 200.09, 149.28, 145.75, 132.19, 121.02, 88.98, 79.70, 78.92, 58.73, 42.49, 42.17, 41.04 ppm.

(9*R*,9a*R*)-4,9-Dimethoxy-9a-nitro-5a,6,9,9a-tetrahydronaphtho-[2,1-*c*][1,2,5]oxadiazol-7(8*H*)-one (59 b): White solid (0.067 mmol, 24%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.48–5.46 (m, 1H), 5.01–5.00 (m, 1H), 4.31–4.24 (m, 1H), 3.74 (s, 3H), 3.35 (s, 3H), 2.89–2.83 (m, 1H), 2.73–2.67 (m, 1H), 2.64–2.59 (m, 1H), 1.80–1.73 ppm (m, 1H).

(9*R*,9a*R*)-9-Methoxy-4-(4-methoxyphenoxy)-9a-nitro-5a,6,9,9a-tetrahydronaphtho[2,1-*c*][1,2,5]oxadiazol-7(8*H*)-one (59 c): White solid (0.07 mmol, 25%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.19–7.17 (d, *J* = 8, 2H), 6.92–6.90 (d, *J* = 8, 2H), 5.58–5.56 (m, 1H), 5.11–5.09 (m, 1H), 4.28–4.22 (m, 1H), 3.87 (s, 3H), 3.41 (s, 3H), 2.96–2.91 (m, 1H), 2.73–2.68 (m, 2H), 1.90–1.83 ppm (m, 1H).

(9*R*,9a*R*)-9-Methoxy-4-(4-methoxybenzylthio)-9a-nitro-5a,6,9,9a-tetrahydronaphtho[2,1-*c*][1,2,5]oxadiazol-7(8*H*)-one (59 d): White solid (0.064 mmol, 23%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 6.98–6.96 (d, *J* = 8, 2H), 6.72–6.69 (d, *J* = 8, 2H), 6.37–6.36 (m, 1H), 4.98–4.97 (m, 1H), 4.18–4.12 (m, 1H), 4.06–3.96 (m, 2H), 3.71 (s, 3H), 3.35 (s, 3H), 2.85–2.80 (m, 1H), 2.59–2.55 (m, 1H), 2.53–2.47 (m, 1H), 1.60–1.49 ppm (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 201.11, 159.01, 148.65, 146.00, 138.40, 129.73, 129.20, 127.53, 122.11, 114.08, 87.05, 79.94, 57.77, 55.22, 42.35, 40.20, 37.72, 35.92 ppm.

(9*R*,9a*R*)-4-(4-Bromophenylthio)-9-methoxy-9a-nitro-5a,6,9,9a-tetrahydronaphtho[2,1-*c*][1,2,5]oxadiazol-7(8*H*)-one (59 e): White solid (0.064 mmol, 23%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.44–7.42 (d, *J* = 8, 2H), 7.23–7.21 (d, *J* = 8, 2H), 6.36–6.34 (m, 1H), 5.04–5.03 (m, 1H), 4.28–4.22 (m, 1H), 3.34 (s, 3H), 2.89–2.83 (m, 1H), 2.64–2.58 (m, 2H), 1.77–1.70 ppm (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 200.81, 146.15, 137.20, 134.43, 132.92, 128.65, 125.98, 123.66, 123.29, 113.73, 87.29, 79.75, 57.79, 42.22, 40.16, 37.96 ppm.

(9*R*,9a*R*)-9-Methoxy-4-(3-morpholino-3-oxopropylthio)-9a-nitro-5a,6,9,9a-tetrahydronaphtho[2,1-*c*][1,2,5]oxadiazol-7(8*H*)-one (59 f): White solid (0.07 mmol, 25%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 6.47–6.45 (m, 1H), 5.02–5.01 (m, 1H), 4.28–4.22 (m, 1H), 3.57–3.49 (m, 8H), 3.32 (s, 3H), 3.23–3.16 (m, 1H), 3.09–3.02 (m, 1H), 2.86–2.82 (m, 1H), 2.63–2.59 (m, 2H), 2.53–2.48 (m, 2H), 1.79–1.72 ppm (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 201.16, 168.61, 148.50, 146.11, 135.56, 122.65, 87.35, 79.83, 66.69, 66.38, 60.26, 57.76, 45.58, 42.36, 41.99, 40.18, 37.83, 32.38, 26.58, 20.92, 14.09 ppm.

(9*S*,9a*R*)-4-Chloro-9-methoxy-9a-nitro-5a,6,9,9a-tetrahydronaphtho[2,1-*c*][1,2,5]oxadiazol-7(8*H*)-one (60 a): White solid (0.117 mmol, 42%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 6.37–6.36 (m, 1H), 4.55–4.51 (m, 1H), 3.81–3.75 (m, 1H), 3.39 (s, 3H), 3.02–2.97 (m, 1H), 2.70–2.64 (m, 2H), 1.94–1.87 ppm (m, 1H).

(9*S*,9a*R*)-4,9-Dimethoxy-9a-nitro-5a,6,9,9a-tetrahydronaphtho-[2,1-*c*][1,2,5]oxadiazol-7(8*H*)-one (60 b): White solid (0.142 mmol, 51%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.22–5.21 (m, 1H), 4.52–4.47 (m, 1H), 3.73 (s, 3H), 3.69–3.62 (m, 1H), 3.40 (s, 3H), 3.02–2.97 (m, 1H), 2.66–2.61 (m, 2H), 1.81–1.74 ppm (m, 1H).

(9*S*,9a*R*)-9-Methoxy-4-(4-methoxyphenoxy)-9a-nitro-5a,6,9,9a-tetrahydronaphtho[2,1-*c*][1,2,5]oxadiazol-7(8*H*)-one (60 c): White solid (0.134 mmol, 48%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.18–7.16 (d, *J* = 8, 2H), 6.92–6.90 (d, *J* = 8, 2H), 6.52–6.50 (m, 1H), 5.30–5.28 (m, 1H), 4.60–4.56 (m, 1H), 3.87 (s, 3H), 3.66–3.59 129 (m, 1H), 3.49 (s, 3H), 3.10–3.05 (m, 1H), 2.75–2.66 (m, 2H), 2.64–2.62 (m, 1H), 1.91–1.87 ppm (m, 1H).

(9*S*,9a*R*)-9-Methoxy-4-(4-methoxybenzylthio)-9a-nitro-5a,6,9,9a-tetrahydronaphtho[2,1-*c*][1,2,5]oxadiazol-7(8*H*)-one (60 d): White solid (0.137 mmol, 49%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.07–7.05 (d, *J* = 8, 2H), 6.74–6.72 (d, *J* = 8, 2H), 6.05–6.03 (m, 1H), 4.47–4.43 (m, 1H), 4.03 (s, 2H), 3.69 (s, 3H), 3.61–3.57 (m, 1H), 3.37 (s, 3H), 2.96–2.91 (m, 1H), 2.64–2.57 (m, 1H), 2.46–2.41 (m, 1H), 1.69–1.61 ppm (m, 1H).

(9*S*,9a*R*)-4-(4-Bromophenylthio)-9-methoxy-9a-nitro-5a,6,9,9a-tetrahydronaphtho[2,1-*c*][1,2,5]oxadiazol-7(8*H*)-one (60 e): White solid (0.154 mmol, 55%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.45–7.43 (d, *J* = 8, 2H), 7.22–7.20 (d, *J* = 8, 2H), 6.02–6.00 (m, 1H), 4.53–4.49 (m, 1H), 3.67–3.61 (m, 1H), 3.40 (s, 3H), 3.02–2.97 (m, 1H), 2.67–2.61 (m, 1H), 2.58–2.53 (m, 1H), 1.81–1.74 ppm (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 200.25, 149.27, 145.51, 134.46, 132.90, 131.43, 128.39, 126.23, 123.71, 89.39, 79.88, 58.71, 42.95, 42.47, 41.02 ppm.

(9*S*,9a*R*)-9-Methoxy-4-(3-morpholino-3-oxopropylthio)-9a-nitro-5a,6,9,9a-tetrahydronaphtho[2,1-*c*][1,2,5]oxadiazol-7(8*H*)-one (60 f): White solid (0.131 mmol, 47%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 6.25–6.23 (m, 1H), 4.51–4.48 (m, 1H), 4.07–4.02 (m, 2H), 3.56–3.48 (m, 8H), 3.08–2.97 (m, 2H), 2.79–2.75 (m, 2H), 2.69–2.57 (m, 1H), 2.51–2.48 (m, 1H), 1.81–1.75 ppm (m, 1H).

4-Bromo-7-nitrobenzo[*c*][1,2,5]thiadiazole (62): A mixture of 4,7-dibromobenzo[*c*][1,2,5]thiadiazole (100 mg, 0.34 mmol) in conc HNO_3 (5 mL) was heated at reflux with stirring for 1 h. The resulting clear solution was poured into 10 mL of ice-H_2O and then extracted with EtOAc (2×10 mL). The combined organic phases were washed with brine, dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude product was separated by chromatography on silica gel (Hex/EtOAc 3:1) to afford the desired product (0.227 mmol, 67%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.41 (d, *J* = 7.89 Hz, 1H), 7.98 ppm (d, *J* = 7.89 Hz, 1H).

General procedure for the synthesis of compounds 63 a,b

To a solution of compound **62** (1 equiv) in EtOH the appropriate nucleophile and a catalytic amount of KOAc and pyridine were added. The resulting mixture was heated at reflux for 3 h. This was concentrated in vacuo and the crude product was purified by flash chromatography with the appropriate eluent.

4-(4-Methoxybenzylthio)-7-nitrobenzo[*c*][1,2,5]thiadiazole (63 a): (0.082 mmol, 72%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.42 (d, *J* = 8.1 Hz, 1H), 7.31–7.29 (m, 3H), 6.81 (d, *J* = 7.8 Hz, 2H), 4.35 (s, 2H), 3.73 ppm(s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 159.39, 153.24, 146.09, 143.64, 135.99, 129.94, 128.06, 125.90, 120.04, 114.34, 55.24, 35.84 ppm.

1-Morpholino-3-(7-nitrobenzo[*c*][1,2,5]thiadiazol-4-ylthio)propan-1-one (58 b): (0.078 mmol, 69%). ^{1}H NMR (400 MHz, $CDCl_3$):

$\delta = 8.46$ (d, $J = 8$ Hz, 1H), 7.34 (d, $J = 8$ Hz, 1H), 3.61–3.59 (m, 6H), 3.50 (t, $J = 8.2$ Hz, 2H), 3.40–3.38 (m, 2H), 2.77 ppm (t, $J = 8$ Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 168.46$, 153.25, 143.18, 135.96, 128.09, 119.52, 66.68, 66.36, 45.66, 42.14, 31.57, 26.46 ppm.

8-Chloro-5-nitroquinoline (66): To a solution of 2-chloro-5-nitroaniline (100 mg, 0.579 mmol) in 6 N HCl (15 mL), toluene (3 mL) and acrolein (76 μL, 1.158 mmol) were added and the mixture was heated at 100 °C for 2 h. The aqueous layer was separated and neutralized with aqueous NaOH to afford crude **66** as a crystalline solid. The crude product was purified by flash chromatography (Hex/EtOAc 3:1) to give desired compound (0.55 mmol, 95%). ^{1}H NMR (400 MHz, [D$_6$]acetone): $\delta = 9.09$ (d, $J = 4.2$ Hz, 1H), 8.92 (d, $J = 11.9$ Hz, 1H), 8.36 (d, $J = 7.8$ Hz, 1H), 8.07 (d, $J = 7.8$ Hz, 1H), 7.83 ppm (dd, $J = 4.2$ Hz and $J = 11.9$ Hz, 1H); ^{13}C NMR (100 MHz, [D$_6$]acetone): $\delta = 152.12$, 140.42, 132.12, 128.31, 127.99, 124.80, 124.71, 124.50, 121.97 ppm.

General procedure for aromatic nucleophilic substitution on quinoline 66

8-Chloro-5-nitroquinoline (0.25 mmol, 1 equiv) and the appropriate nucleophile (1.1 equiv) were suspended in the minimal amount of dry DMF (2 mL) in sealed vessels in the presence of K_2CO_3 (1 equiv), and the mixtures were irradiated at 130 °C for 10 min The reaction mixtures were then diluted with H_2O (2 mL) and extracted with EtOAc (3×10 mL). Finally, the organic phases were dried over anhydrous Na_2SO_4 and evaporated to dryness. The crude product was purified by flash chromatography with the appropriate eluent.

8-(4-Methoxybenzylthio)-5-nitroquinoline (67a): (0.22 mmol, 88%). ^{1}H NMR (400 MHz, [D$_6$]acetone): $\delta = 9.02$ (d, $J = 4.2$ Hz, 1H), 8.92 (d, $J = 11.9$ Hz, 1H), 8.35 (d, $J = 7.8$ Hz, 1H), 7.77 (dd, $J = 4.2$ Hz and $J = 11.9$ Hz, 1H), 7.71 (d, $J = 7.8$ Hz, 1H), 7.41 (d, $J = 8.2$ Hz, 1H), 6.86 (d, $J = 8.2$ Hz, 1H), 4.33 (s, 2H), 3.72 ppm (s, 3H).

1-Morpholino-3-(5-nitroquinolin-8-ylthio)propan-1-one (67b): (0.22 mmol, 88%). ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 9.04$ (d, $J = 4$ Hz, 1H), 8.88 (d, $J = 11.9$ Hz, 1H), 8.31 (d, $J = 7.8$ Hz, 1H), 7.60 (dd, $J = 4.2$ Hz and $J = 11.9$ Hz, 1H), 7.43 (d, $J = 7.8$ Hz, 1H), 3.58 (br s, 6H), 3.37 (br s, 2H), 3.36–3.34 (m, 2H), 2.77–2.73 ppm (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 168.87$, 149.94, 149.65, 144.39, 141.11, 132.82, 125.32, 124.61, 120.98, 120.14, 66.70, 66.38, 45.67, 42.09, 31.45, 25.83 ppm; MS (ESI) *m/z*: 348 $[M+H]^+$, 370 $[M+Na]^+$.

8-(4-(4-Methylpiperazin-1-yl)phenoxy)-5-nitroquinoline (67c): (0.205 mmol, 82%). ^{1}H NMR (400 MHz, [D$_6$]acetone): $\delta = 9.03$ (d, $J = 4.2$ Hz, 1H), 8.98 (d, $J = 11.9$ Hz, 1H), 8.36 (d, $J = 7.8$ Hz, 1H), 7.78 (dd, $J = 4.2$ Hz and $J = 11.9$ Hz, 1H), 7.08–6.90 (m, 4H), 6.73 (d, $J = 8.2$ Hz, 2H), 3.15–3.14 (4H, m), 2.47–2.46 (4H, m), 2.21 ppm (3H, s); ^{13}C NMR (100 MHz, [D$_6$]acetone): $\delta = 160.81$, 150.43, 149.41, 147.26, 139.42, 131.68, 126.78, 124.74, 122.78, 118.03, 115.43, 110.10, 54.86, 48.81, 45.31 ppm; MS (ESI) *m/z*: 365 $[M+H]^+$, 387 $[M+Na]^+$.

1-(4-(4-(5-Nitroquinolin-8-yloxy)phenyl)piperazin-1-yl)ethanone (67d): (0.207 mmol, 83%). ^{1}H NMR (400 MHz, [D$_6$]acetone): $\delta = 9.05$ (d, $J = 4.2$ Hz, 1H), 9.00 (d, $J = 11.9$ Hz, 1H), 8.38 (d, $J = 7.8$ Hz, 1H), 7.81 (dd, $J = 4.2$ Hz and $J = 11.9$ Hz, 1H), 7.09–7.06 (m, 4H), 6.95 (d, $J = 8.2$ Hz, 2H), 3.64–3.61 (4H, m), 3.20–3.18 (2H, m), 3.12–3.10 (2H, m), 2.01 ppm (3H, s); ^{13}C NMR (100 MHz, [D$_6$]acetone): $\delta = 150.48$, 149.19, 141.49, 139.13, 132.71, 131.88, 131.69, 126.72, 124.74, 121.28, 118.60, 117.88, 110.33, 49.57, 49.20, 45.76, 40.85, 20.35 ppm; MS (ESI) *m/z*: 393 $[M+H]^+$, 416 $[M+Na]^+$.

5-Bromo-8-nitroisoquinoline (69): Concentrated H_2SO_4 (96%, 20 mL) was cooled to 0 °C under nitrogen atmosphere and isoquinoline (500 μL, 4.21 mmol) was slowly added to the well-stirred acid at a rate such that the internal temperature was maintained < 30 °C. The solution was cooled to −25 °C in a dry ice–acetone bath and *N*-bromosuccinimide (976 mg, 5.48 mmol) was added to the vigorously stirred solution in portions such that the internal temperature was maintained between −22 and −26 °C. The suspension was stirred for 2 h at −22 °C and then for 3 h at −18 °C. KNO_3 (446 mg, 4.41 mmol) was then added at a rate such as to maintain the internal temperature below −10 °C and the mixture was then stirred at −10 °C for 1 h. The cooling bath was removed and the solution was stirred overnight. The resulting homogeneous reaction mixture was poured onto crushed ice in a 500 mL flask and the reaction flask was quickly washed with ice-cold H_2O, which was added to the 500 mL flask. The resulting mixture was stirred while the solution was adjusted to pH 8.0 using 25% aqueous NH_3 with the internal temperature maintained < 30 °C. The resulting suspension was stirred in an ice H_2O bath for 2 h and the precipitated solids were isolated by filtration. The solids were thoroughly washed three times with ice-cold H_2O and then air dried to constant weight. This material was suspended in 12 mL heptane and 3 mL toluene and heated at reflux for 1.5 h with stirring. The hot solution was then filtered through Celite using vacuum suction. The volume of the filtrate was decreased by distillation and the resulting orange solution was allowed to slowly cool with stirring overnight. The solids were isolated by filtration, washed with 100 mL ice-cold heptane, and dried to provide desired product (2.06 mmol, 49%). ^{1}H NMR (400 MHz, [D$_6$]acetone): $\delta = 9.77$ (s, 1H), 8.77 (d, $J = 6.2$ Hz, 1H), 8.26 (s, 2H), 8.10 ppm (d, $J = 8.1$ Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 148.20$, 145.81, 145.54, 135.12, 133.16, 127.80, 125.29, 120.49, 118.89 ppm; MS (ESI) *m/z*: 253–255 $[M+H]^+$.

General procedure for aromatic nucleophilic substitution on isoquinoline 69

Compounds **70a,b** were obtained by using the same procedure as for compounds **67a–d** but with 5-bromo-8-nitroisoquinoline as the starting material.

5-(4-Methoxybenzylthio)-8-nitroisoquinoline (70a): (0.207 mmol, 83%). ^{1}H NMR (400 MHz, [D$_6$]acetone): $\delta = 9.87$ (s, 1H), 8.65 (d, $J = 6.2$ Hz, 1H), 8.29 (d, $J = 8.1$ Hz, 1H), 8.05 (d, $J = 6.2$ Hz, 1H), 7.82 (d, $J = 8.1$ Hz, 1H), 7.36 (d, $J = 7.8$ Hz, 1H), 6.84 (d, $J = 7.8$ Hz, 1H), 4.44 (s, 2H), 3.71 ppm (s, 2H); ^{13}C NMR (100 MHz, [D$_6$]acetone): $\delta = 159.39$, 148.52, 148.39, 144.44, 142.98, 133.83, 130.27, 127.00, 125.11, 124.87, 119.72, 116.12, 114.01, 54.60, 35.83 ppm; MS (ESI) *m/z*: 327 $[M+H]^+$.

1-Morpholino-3-(8-nitroisoquinolin-5-ylthio)propan-1-one (70b): (0.217 mmol, 87%). ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 9.96$ (s, 1H), 8.58 (d, $J = 6.2$ Hz, 1H), 8.18 (d, $J = 8.1$ Hz, 1H), 7.94 (d, $J = 6.2$ Hz, 1H), 7.48 (d, $J = 8.1$ Hz, 1H), 3.58 (br s, 4H), 3.57 (br s, 2H), 3.42–3.49 (m, 2H), 3.36–3.34 (m, 2H), 2.73–2.69 ppm (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 168.52$, 148.81, 144.44, 142.77, 134.16, 125.04, 123.33, 119.98, 116.26, 66.67, 66.33, 45.61, 42.09, 31.65, 27.17 ppm; MS (ESI) *m/z*: 348 $[M+H]^+$, 370 $[M+Na]^+$.

***N*-(3-Chloro-2-fluorophenyl)acetamide (72)**: 3-Chloro-2-fluoroaniline (200 μL, 2.4 mmol) was mixed with acetic anhydride (10 mL) and heated at reflux for 2 h. The reaction was quenched with H_2O (3 mL), neutralized with a saturated solution of Na_2CO_3 and extracted with EtOAc (3×10 mL). The combined extract was dried over anhydrous Na_2SO_4 and evaporated to dryness to provide desired compound (2.4 mmol, quant). ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 8.18$–

8.16 (m, 1H), 7.31 (br s, 1H), 7.04–6.99 (m, 2H), 2.17 ppm (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 170.75, 154.36, 124.82, 124.65, 120.15, 119.90, 29.61 ppm; MS (ESI) *m/z*: 188 $[M+H]^+$.

7-Chloro-2-methylbenzo[*d*]thiazole (73): In a 50 mL flask were placed compound **72** (74 mg, 0.39 mmol), Lawesson's reagent (96 mg, 0.6 equiv), xylene (2 mL) and a magnetic stir bar. The mixture was stirred under argon at 105–115 °C for 2.5 h. Then Cs_2CO_3 (302.5 mg, 2 equiv) was added and the reaction mixture was stirred at reflux for 16 h. The solvent was evaporated to dryness and the crude product was separated by chromatography on silica gel (Hex/EtOAc 3:1) to afford the desired product (0.3 mmol, 77%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.76 (d, *J* = 8 Hz, 1H), 7.33–7.24 (m, 2H), 2.76 ppm (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 167.66, 153.97, 135.77, 126.77, 126.54, 124.33, 120.63, 20.09 ppm; MS (ESI) *m/z*: 185 $[M+H]^+$.

7-Chloro-2-methyl-4-nitrobenzo[*d*]thiazole (74): Concentrated H_2SO_4 (96%, 20 mL) was cooled to −10 °C under nitrogen atmosphere and compound **73** (370 mg, 2.02 mmol) was slowly added to the well-stirred acid at a rate such that the internal temperature was maintained < 30 °C. KNO_3 (213 mg, 2.1 mmol) was then added at a rate such as to maintain the internal temperature below −10 °C and the mixture was then stirred at −10 °C for 1 h. The cooling bath was removed and the solution was stirred at room temperature for 2 h. The resulting homogeneous reaction mixture was poured onto crushed ice and stirred while adjusting the solution to pH 8.0 using 25% aqueous NH_3 with the internal temperature maintained < 30 °C. This was extracted with EtOAc (3×25 mL) dried over anhydrous Na_2SO_4 and solvent removed in vacuo. The crude product was separated by chromatography on silica gel (Hex/EtOAc 3:1) to afford the desired product (1.35 mmol, 67%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.09 (d, *J* = 8 Hz, 1H), 7.40 (d, *J* = 8 Hz, 1H), 2.90 ppm (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 172.63, 145.67, 140.38, 138.96, 132.35, 123.81, 123.64, 20.75 ppm; MS (ESI) *m/z*: 230 $[M+H]^+$.

General procedure for the synthesis of compounds 75a–d

Compound **74** (0.25 mmol, 1 equiv) and the appropriate nucleophile (1 equiv) were suspended in anhydrous DMF (3 mL) in a 10 mL glass vial equipped with a small magnetic stir bar. K_2CO_3 (1 equiv) was then added to this solution and the mixture was irradiated under microwave for 10 min at 130 °C, using an irradiation power of 300 W. The mixture was then poured into H_2O (10 mL) and then extracted with EtOAc (2×10 mL). The combined organic phases were washed with brine, dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude products were purified by flash column chromatography using the appropriate eluent.

7-(4-Methoxybenzylthio)-2-methyl-4-nitrobenzo[*d*]thiazole (75a): (0.187 mmol, 75%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.00 (d, *J* = 8.2 Hz, 1H), 7.16 (m, 3H), 6.74 (d, *J* = 7.8 Hz, 2H), 4.21 (s, 2H), 3.69 (s, 3H), 2.83 ppm (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 171.79, 159.23, 144.86, 141.43, 139.40, 138.51, 129.92, 126.91, 123.22, 121.78, 114.16, 55.21, 37.52, 20.73 ppm; MS (ESI) *m/z*: 347 $[M+H]^+$, 369 $[M+Na]^+$.

3-(2-Methyl-4-nitrobenzo[*d*]thiazol-7-ylthio)-1-morpholinopropan-1-one (75b): (0.155 mmol, 62%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.03 (d, *J* = 8.2 Hz, 1H), 7.21 (d, *J* = 8 Hz, 1H), 3.54–3.52 (m, 6H), 3.38 (t, *J* = 8 Hz, 2H), 3.32–3.30 (m, 2H), 2.83 (s, 3H), 2.65 ppm (t, *J* = 8 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 171.80, 168.61, 144.95, 139.19, 138.45, 137.95, 123.37, 120.47, 66.65, 66.34, 45.59, 42.04, 32.32, 28.16, 20.75 ppm; MS (ESI) *m/z*: 368 $[M+H]^+$.

2-Methyl-7-(4-(4-methylpiperazin-1-yl)phenoxy)-4-nitrobenzo[*d*]-thiazole (75c): (0.14 mmol, 56%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.07 (d, *J* = 8.2 Hz, 1H), 6.97 (d, *J* = 7.9 Hz, 2H), 6.89 (d, *J* = 7.9 Hz, 2H), 6.59 (d, *J* = 8.2 Hz, 1H), 3.16–3.13 (4H, m), 2.88 (s, 3H), 2.53–2.51 (4H, m), 2.28 ppm (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 172.35, 157.59, 149.43, 148.09, 146.42, 136.36, 125.33, 121.48, 117.22, 108.20, 55.04, 54.95, 49.18, 46.04, 20.70 ppm; MS (ESI) *m/z*: 385 $[M+H]^+$.

1-(4-(4-(2-Methyl-4-nitrobenzo[*d*]thiazol-7-yloxy)phenyl)piperazin-1-yl)ethanone (75d): (0.135 mmol, 54%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.09 (d, J = 8.1 Hz, 1H), 7.01 (d, J = 7.8 Hz, 2H), 6.91 (d, J = 7.8 Hz, 2H), 6.61 (d, J = 8.1 Hz, 1H), 3.74–3.73 (m, 2H), 3.59–3.58 (m, 2H), 3.15–3.10 (m, 4H), 2.90 (s, 3H), 2.08 ppm (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 172.43, 157.38, 149.06, 147.47, 147.18, 136.52, 125.30, 121.62, 117.99, 108.29, 49.80, 49.50, 46.08, 41.21, 21.23, 20.70 ppm; MS (ESI) m/z: 413 $[M+H]^+$, 435 $[M+Na]^+$.

5-(4-Methoxybenzylthio)-2-nitroaniline (77): To a solution of 5-chloro-2-nitroaniline (100 mg, 0.58 mmol) in 10 mL of EtOH, 4-methoxytoluenethiol (80 μL, 0.58 mmol) and K_2CO_3 (1 equiv) were added. The reaction mixture was heated at reflux for 2 h. The mixture was then cooled to room temperature, the solvent was removed in vacuo. This was extracted with EtOAc (3×10 mL), washed with brine, dried over anhydrous Na_2SO_4 and the solvent was removed in vacuo. The crude product was separated by chromatography on silica gel (Hex/EtOAc 3:1) to afford the desired product (0.388 mmol, 67%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.06 (d, *J* = 8 Hz, 1H), 7.47 (s, 1H), 7.25 (d, *J* = 8 Hz, 1H), 6.82 (m, 4H), 4.30 (s, 2H), 3.70 ppm (s, 3H); MS (ESI) *m/z*: 291 $[M+H]^+$.

***N*-(5-(4-Methoxybenzylthio)-2-nitrophenyl)acetamide (78)**: To a solution of compound **77** (50 mg, 0.17 mmol) in CH_2Cl_2, Et_3N (45 μL, 0.34 mmol) and Ac_2O (24 μL, 0.26 mmol) were added, and the mixture was stirred at room temperature for 1 h. The reaction mixture was quenched with saturated aqueous $NaHCO_3$ (10 mL) and extracted with EtOAc (3×10 mL). The combined extracts were washed with brine, dried over anhydrous Na_2SO_4 and the solvent removed in vacuo. The crude product was separated by chromatography on silica gel (Hex/EtOAc 4:1) to afford the desired product (0.147 mmol, 87%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.47 (s, 1H), 8.22 (d, *J* = 8 Hz, 1H), 7.32 (d, *J* = 8 Hz, 1H), 6.82 (m, 4H), 4.30 (s, 2H), 3.70 (s, 3H), 2.07 ppm (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 160.2, 143.1, 138.8, 133.7, 129.4, 127.5, 120.1, 114.3, 55.4, 23.7 ppm; MS (ESI) *m/z*: 333 $[M+H]^+$.

Biology

Virus strains: Infection experiments were carried out using influenza A/WSN/33 (H1N1).[35]

Plasmid constructs: Plasmids pCA-Flag-GFP and pCA-PB_{11-25}A-GFP, pCAPB1-HA, the FluA minireplicon plasmids and the expression plasmids for the FluB minireplicon are described elsewhere.[36] The FluB minigenome expression plasmid, pPolI-lucRT_B, was obtained by cloning the firefly luciferase ORF (inverse orientation) flanked by the non-coding region of segment 8 of the B/Yamagata/73 into the SapI-digested plasmid pPolI-SapIRib according to Pleschka et al.[28] For the construction of pCA-PB_{11-25}B-GFP, a linker containing the first 25 codons of PB1 (B/Yamagata/73) was cloned into the EcoRI/NotI sites of pCA-Flag-GFP plasmid, replacing the Flag-coding sequence with PB_{11-25}B. Site-directed mutagenesis was carried out with pCA-PB_{11-25}A-GFP to create the plasmid pCAPB$_{11-}$

$_{25}A_{T61Y}$-GFP. The ORFs of PB1 (B/Yamagata/73) and PA (A/SC35M, A/Thailand/1(KAN-1)/04, A/Vietnam/1203/04, B/Yamagata/73, B/Lee/40) were PCR amplified with sense primers containing a NotI site (FluA strains) or a EcoRI site (FluB strains) upstream of the initiation codon and antisense primers with a deleted stop codon followed by an XmaI site, a coding sequence for an HA tag and a XhoI site. The PCR products were cloned into a modified pCAGGs-vector digested either with EcoRI/XhoI or NotI/XhoI, resulting in pCA-PB1-HA or pCA-PA-HA plasmids, coding for C-terminal-tagged versions of the polymerase subunits. To obtain the pCA-$PA_{A/SC35M}$-His plasmid, pCA-$PA_{A/SC35M}$-HA was digested with XmaI/XhoI, and the HA coding sequence was replaced by a His_6 linker. The A/B-chimeric expression plasmids were obtained by assembly PCR using the pCA-PB1-HA plasmids of SC35M and B/Yamagata/73 and by cloning the resulting PCR product in pCA-$PB1_{B/Yamagata/73}$-HA digested with EcoRI/EcoRV.

Reconstitution of viral polymerase activity: HEK293T cells were transiently transfected with a plasmid mixture containing either FluA- or FluB-derived PB1-, PB2-, PA- and NP-expression plasmids, polymerase I (Pol I)-driven plasmid transcribing an influenza A or influenza B virus-like RNA coding for the reporter protein firefly luciferase to monitor viral polymerase activity and with expression plasmids coding for the indicated GFP fusion proteins. Both minigenome RNAs were flanked by noncoding sequences of segment 8 of FluA and FluB, respectively. The transfection mixture also contained a plasmid constitutively expressing *Renilla* luciferase, which served to normalize variation in transfection efficiency. The reporter activity was determined 24 h post-transfection and normalized using the Dual-Glu Luciferase Assay System (Promega). The activity observed with transfection reactions containing Flag-GFP were set to 100%.

Immunoprecipitation experiments: HEK293T cells were transfected with the indicated plasmids in six-well plates using Metafectene (Biontex, Martinsried, Germany). Cells were incubated 24 h post-transfection with lysis buffer (20 mM Tris pH 7.5, 100 mM NaCl, 0.5 mM EDTA, 0.5% NP-40, 1% protease inhibitor Mix G, (Serva, Heidelberg, Germany), 1 mM DTT) for 15 min on ice. After centrifugation at 13000 rpm at 4 °C, the supernatant was incubated with anti-HA-specific antibodies coupled to agarose beads (Sigma) for 1 h at 4 °C. After three washes with 1 mL washing buffer (lysis buffer without protease inhibitor mix), bound material was eluted under denaturing conditions and separated by SDS-PAGE and transferred into PVDF membranes. Viral polymerase subunits and GFP fusion proteins were detected with antibodies directed against the HA tag (Covance, Berkeley, CA, USA) or His tag (Qiagen) or GFP tag (Santa Cruz Biotechnology).

Enzyme-linked immunosorbent assay (ELISA): Microwell plates (Pierce) were incubated at room temperature with saturating concentrations of small-molecule competitor compounds, washed, and subsequently incubated at room temperature with HA-tagged PA. To obtain PA-HA, HEK293T cells were seeded into 94-mm dishes, transfected with the respective plasmid, and treated with lysis buffer 24 h post-transfection as previously described.[36,37] After washing the microwell plates, the wells were incubated with an HA-specific primary antibody (Covance), followed by three washes and an incubation with a peroxidase-coupled secondary antibody (Jackson Immuno Research, Newmarket, UK) for a further 30 min. After the final wash step, ABTS substrate (Sigma, ready-to-use solution) was added, and the optical density was determined at λ 405 nm.

Plaque reduction assay: The experiments were carried out as previously described[37] with modifications. Confluent MDCK cells were infected with 100 PFU A/WSN/33, B/Yamagata/73, A/KAN-1, or VSV/Indiana in PBS containing BSA at room temperature. After removal of the inoculums, cells were overlaid with medium (DMEM with 20 mM HEPES, 0.01% DEAE Dextran, 0.001% $NaHCO_3$) containing 1% Oxoidagar and the benzofurazan compounds at the indicated concentrations. After incubation for 24 h (VSV), 48 h (A/WSN/33, A/KAN-1) at 37 °C with 5% CO_2, or 72 h at 33 °C with 5% CO_2 (B/Yamagata/73), cells were fixed with formaldehyde and stained with crystal violet. Plaques were counted, and mean plaque number of the water control was set at 100%.

Molecular modeling

Library preparation: All ligands were built with the Schrödinger Maestro 9.1 graphical interface.[38] Compounds were then processed with the Schrödinger LigPrep tool to generate separate files for all possible enantiomers and protonation states at physiological pH. OPLS_2005 was used as force field.

Protein preparation: The three-dimensional coordinates of H5N1 influenza A virus PA (C-terminal region, PAC) in complex with the PA binding region of PB1 (N-terminal region, PB1N, residues 1–25) were retrieved from the Protein Data Bank (PDB IDs 3CM8[39] and 2ZNL,[40] with resolutions of 2.90 and 2.30 Å, respectively). Because both structures contain gaps, a more complete assembly for PAC was obtained by applying a homology modeling procedure (Prime, Schrödinger). In detail, the FASTA sequence of the protein (influenza A virus, strain A/Wilson–Smith/1933 H1N1) was uploaded into the software, and the model was built using PDB IDs 2ZNL as the first template and 3CM8 as the second. Indeed, the gaps in the 2ZNL structure were partially filled with the corresponding residues solved in the 3CM8 structure, while the missing residues in both X-ray structures were completed by the program according to the FASTA sequence. The protein was then energy minimized to remove unfavorable contacts through the all-atom OPLS force field and Polak–Ribiere conjugate gradient method. A continuum solvation method, with water as the solvent, was also applied. Extended cutoffs were used, and convergence was set to 0.3 kJ mol^{-1}.

Docking studies: A consensus docking approach that takes advantage of two widely used docking programs (Glide[41] and Gold)[42] was used in this study. In detail, compounds were first docked using the Glide standard precision (SP) mode. A grid box of default size was centered on PB1-derived peptide of the X-ray crystal structure. No constraints were included during grid generation, while rotation of hydroxy groups was allowed. Default parameters were used for the docking runs. The top-five poses based on the Glide docking score were saved for each ligand. With regard to the docking with Gold, GoldScore and ChemScore were used as fitness functions. The GA parameter settings of Gold were used, with search efficiency set at 200%. Finally, results differing by <1.5 Å in ligand all-atom RMSD were clustered together. For each inhibitor, the first-ranked solution as well as the lowest-energy conformation of the most populated cluster were analyzed by comparing them with the best poses previously obtained with the Glide software. Poses were then selected by taking into account the overall match between the binding modes proposed by the two programs.

Ligand efficiency: Ligand efficiency (LE) was calculated by dividing the free energy of binding by the number of heavy (i.e., non-hydrogen) atoms. The activity value of each compound was converted into the free energy of binding at 300 K using the Gibbs equation ($\Delta G = -RT\ln K_d$), in which K_d is substituted by IC_{50} for the purpose of relative comparison, as previously reported.[33]

Acknowledgements

We gratefully acknowledge financial support provided by the FP7 FLUCURE (Ref. 259972) project.

Keywords: antiviral agents · benzofurazans · H1N1 · influenza A · RNA polymerase

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Received: September 19, 2013
Revised: November 6, 2013
Published online on November 27, 2013