Supporting Information for:

Structure-Mechanism Insights and the Role of Nitric Oxide Donation Guide the Development of Oxadiazole-2-Oxides as Therapeutic Agents against Schistosomiasis.

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Supplemental Table 1. TGR inhibition and NO release profiles for selected analogues as judged by ABTS oxidation.

Analogue	**TGR**[a] *IC_{50} (μM)*	**% NO** *cmpd + TGR*	**% NO** *cmpd + TGR + NADPH*	**% NO** *cmpd + Cys*
2	6.3	1.5%	15%	61%
4	> 50	< 1%	3%	7%
5	> 50	< 1%	5%	10%
6	> 50	< 1%	< 1%	2%
7	11.2	1%	2%	6%
8	0.11	1%	7%	9%
9	0.63	2%	7%	10%
11	2.2	2%	16%	72%
12	2.5	2%	20%	78%
13	2.8	2%	19%	69%
14	2.8	2%	19%	70%
15	3.5	1%	12%	50%
16	3.5	4%	19%	68%
17	4.0	2%	16%	61%
18	7.1	2%	22%	80%
20	7.9	< 1%	13%	50%
22	8.9	< 1%	8%	20%
23	8.9	2%	9%	52%
24	10.0	2%	10%	55%
25	11.2	1%	15%	58%
28	2.8	2%	16%	60%
29	3.5	1%	2%	20%

TGR Biochemical Assay Protocol

Test compounds were dissolved in ACS grade DMSO to produce 10 mM starting stock solutions and arrayed as serial twofold dilutions in 1536-well plates as described previously (Yasgar A. et al. Compound Management for Quantitative High-Throughput Screening. *J. Assoc. Lab Automat.* **2008**, *13*, 79-89). The TGR assay was performed as described previously (see reference 16). Briefly, three μL of reagents (100 μM NADPH

and 15 nM TGR or 100 μM NADPH as no-enzyme control) were dispensed into 1536-well Greiner black clear-bottom assay plates. Compounds (23 nL) were transferred via a Kalypsys pin tool equipped with a 1536-pin array. The plate was incubated for 15 min at room temperature, and then a 1 μL aliquot of 500 μM NADPH was added, immediately followed by a 1 μL aliquot of 15 mM DTNB to start the reaction. The plate was transferred to a ViewLux high-throughput CCD imager (Perkin-Elmer, Wellesley, MA) where kinetic measurements (5 reads, one read every 2 minutes) of the TNB absorbance were acquired using a 405 nm excitation filter.

Ex vivo Worm Assay Protocol

Adult *S. mansoni* worms were obtained from mice 49 days post infection by perfusion with RPMI 1640 media as described [see reference 19 and Lewis, F. Schistosomiasis in Current Protocols in Immunology (ed. Coligan, J.E., Kruisbeek, A.M., Margulies, D.H., Shevach, E.M. & Strober, W.) Suppl. 28: 19.1.1–19.1.28 (John Wiley & Sons, New York, 1998)]. Compounds were dissolved in dimethylsulfoxide (DMSO) and added to freshly perfused worms in RPMI 1640 containing 25 mM Hepes, pH 7, 150 units/ml penicillin, 125 μg/ml streptomycin, and 10% fetal calf serum (Cell Grow, Fisher). Media were replaced every other day with fresh media with added compounds. Control worms were treated with equal volumes of DMSO alone. Worms were subsequently observed for motility and mortality. Mortality was scored as a complete lack of movement.

GR Assay Protocol

Recombinant human GR was provided by Pr. R. Heiner Schirmer, Biochemie-Zentrum der Universität Heidelberg. GR activity was determined as described (Carlberg, I. Glutathione Reductase *Methods Enzymol.* **1985**, *113*, 484-490.) after 15 min preincubation of the protein with compounds plus NADPH followed by addition of NADPH and glutathione disulfide to start the reaction. Reaction conditions for a 200 µL reaction were 100 µM NADPH, 0.1 U/ml hGR, 1mM GSSG, hGR assay buffer pH 6.9.

Biotin-Switch Assay Protocol

The biotin switch assay (see reference 50) was used to determine if S-nitrosylation is involved in the inhibition of TGR by compound **2**. Recombinant TGR was incubated +/- 10 µM NADPH, +/- compound **2** or +/- 10 µM glutathione (GSH). After incubation the reactants were removed from TGR on a spin column and by acetone precipitation of TGR. Free Cys-sulfhydryls in TGR were then alkylated by methyl methanethiosulfonate (MMTS) (Sigma). The S-nitrosothiols were then reduced by ascorbate generating free thiols, which then were reacted with the thiol-modifying reagent N-[6-(biotinamido)hexyl]-3'-(2'-pyridyldithio)propionamide (biotin-HPDP) (Pierce). After purification of the protein on a spin column (Micro Bio-Spin P6 column, BioRad) and acetone precipitation it was analyzed by native-12% acrylamide PAGE and Western blotting using an HRP-streptavidin conjugate (Sigma) to identify biotinylated proteins.

TrxR1 Assay Protocol (DNTB)

20 nM recombinant TrxR1 (40% specific activity, thus 8 nM fully active Sec-containing enzyme) in 250 µM NADPH, 1 mg/ml BSA, 50 mM Tris-HCl and 2 mM EDTA was

incubated with 5% DMSO containing compounds at various concentrations at room temperature. After 10 min, 1 μmol DTNB (dissolved in ethanol, final concentration of ethanol in the assay was 8.6 %) was added and the formation of TNB-anions absorbing at 412 nm was followed for 3 min at 30°C. A linear interval of 30 s was chosen to calculate the activity of TrxR1 (as percent of control) after treatment with compounds 1-31. 5% DMSO served as control.

Nitric Oxide Release Protocol:

Calibration of the Sievers Nitric Oxide Analyzer (NOA), model 280i (Instruments Business Group, Boulder, CO) was performed by injecting of various volumes of known concentrations of NO in helium (50 ppm, 500 ppm and 5%) certified standards into the reaction chamber and recording the peaks. Samples and reaction chambers were incubated at 37 °C. The contents of the reaction chamber were sparged with argon and swept into the chemiluminescence detector. Data were recorded using Agilent Chemstation software and processed using Microsoft Excel. Approximately 3 mL of 0.1 M pH 7.4 phosphate buffer containing cysteine (10 mM) and diethylenetriaminepentaacetic acid (DTPA, 50 μM) was placed into the reaction chamber of the NOA and then sparged for several minutes with argon. A DMSO solution (1 mM) of the prodrug was injected into the reaction chamber and nitric oxide release was recorded. Total amount of NO released was determined by measuring the area under the curve.

Reactivity of Oxadiazole-2-oxides with Cys (HPLC Assay):

The prodrug (0.1 mM) was exposed to cysteine (10 mM) in 0.1 M pH 7.4 phosphate buffer. The amount of prodrug unreacted was estimated by injecting aliquots into a Agilent 1100 series HPLC fitted with a Phenomenex Luna C18 column (250 × 4.6 mm) eluted with 75% acetonitrile and water with 0.1% HCOOH.

Characterization of Compounds.

The synthesis and characterization of compounds was previously reported (see Rai, G.; Thomas, C. J.; Leister, W.; Maloney, D. J. Synthesis of oxadiazole-2-oxide analogues as potential antischistosomal agents. *Tet. Lett.* **2009**, *50*, 1710-1713.). The lone exception was the bis-2,4-thiophene analogue (**35**). For convenience this data is presented here-in.

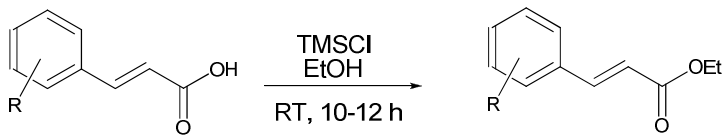
General Methods:

Unless otherwise stated, all reactions were carried out under an atmosphere of dry argon or nitrogen in dried glassware. Indicated reaction temperatures refer to those of the reaction bath, while room temperature (rt) is noted as 25 ^{o}C. All solvents were of anhydrous quality purchased from Aldrich Chemical Co. and used as received. Commercially available starting materials and reagents were purchased from Aldrich, TCI and Acros and were used as received.

Analytical thin layer chromatography (TLC) was performed with Sigma Aldrich TLC plates Aldrich TLC plates (5 x 20 cm, 60 Å, 250 μm). Visualization was accomplished by irradiation under a 254 nm UV lamp. Chromatography on silica gel was performed using forced flow (liquid) of the indicated solvent system on Biotage KP-Sil pre-packed cartridges and using the Biotage SP-1 automated chromatography system. ^{1}H- and ^{13}C NMR spectra were recorded on a Varian Inova 400 MHz spectrometer.

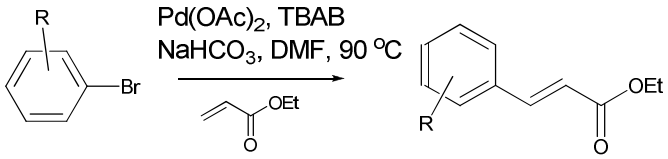
Chemical shifts are reported in ppm with the solvent resonance as the internal standard ($CDCl_3$ 7.26 ppm, 77.00 ppm, DMSO-d_6 2.5 ppm, 39.51 ppm for ^1H, ^{13}C respectively). Data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, dd = doublet of doublet, t = triplet, q = quartet, br = broad, m = multiplet), coupling constants, and number of protons. Low resolution mass spectra (electrospray ionization) were acquired on an Agilent Technologies 6130 quadrupole spectrometer coupled to an Agilent Technologies 1200 series HPLC. High resolution mass spectral data was collected in-house using and Agilent 6210 time-of-flight mass spectrometer, also coupled to an Agilent Technologies 1200 series HPLC system.

Experimental Procedures:



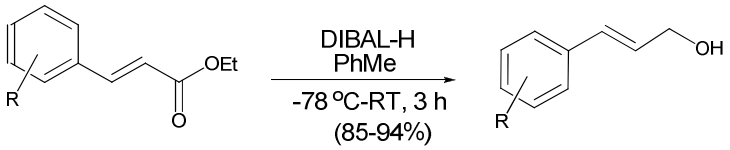
Esterification of Cinnamic acids.

To a solution containing the substituted cinnamic acid (10 mmol) in absolute ethanol (50 mL) was added TMSCl (22 mmol) and the reaction was stirred at RT for 12 h. After completion of the reaction, the solvent was removed under diminished pressure and the residue was re-dissolved in ethyl acetate. The ethyl acetate layer was washed successively with saturated $NaHCO_3$, water, brine then dried (Na_2SO_4) and concentrated under diminished pressure to give the pure product without need of further purification.



General Procedure for Heck Coupling.

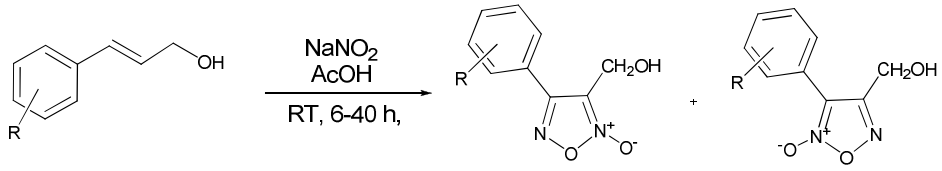
To a solution containing the aryl bromide (10 mmol) in DMF (20 mL) was added tetrabutylammonium bromide (10 mmol), palladium(II) acetate (5 mol %), sodium bicarbonate (40 mmol) and ethyl acrylate (20 mmol). The reaction mixture was stirred at 90 ^{o}C for 45 min, then cooled to room temperature, diluted with ethyl acetate and filtered through celite. The filtrate was poured into water and extracted with ethyl acetate. The organic layer was washed with water and brine then dried ($MgSO_4$), filtered and concentrated under diminished pressure. The crude solid was purified on a Biotage® silica gel column. Gradient elution with ethyl acetate (7→40%) in hexanes gave the product.



General Procedure for syntheses of substituted cinnamyl alcohols.

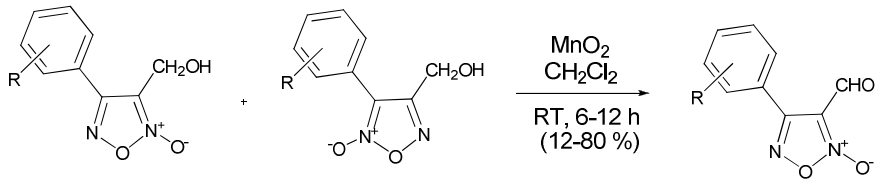
To a suspension of the corresponding ester (10 mmol) in toluene (25 mL) at –78 ^{o}C was added dropwise DIBAL (22 mmol, 1.0 M solution in toluene) over 45 min. The reaction mixture was allowed to warm to room temperature over 2 h and then stirred this temperature for an additional hour. The reaction mixture was quenched with ice containing dilute HCl. The organic layer was separated and the aqueous layer was extracted with ethyl acetate. The combined organic layer was washed with brine and water, dried ($MgSO_4$), filtered and concentrated under diminished pressure. The crude residue was purified on a Biotage® silica gel column. Gradient elution with ethyl acetate (12→80%) in hexanes gave the product. Analytical analysis was performed on an Agilent LC/MS (Agilent Technologies, Santa Clara, CA). Method 1: A 7 minute

gradient of 4% to 100% Acetonitrile (containing 0.025% trifluoroacetic acid) in water (containing 0.05% trifluoroacetic acid) was used with an 8 minute run time at a flow rate of 1 mL/min. A Phenomenex Luna C18 column (3 micron, 3 x 75 mm) was used at a temperature of 50°C. Method 2: A 3 minute gradient of 4% to 100% Acetonitrile (containing 0.025% trifluoroacetic acid) in water (containing 0.05% trifluoroacetic acid) was used with a 4.5 minute run time at a flow rate of 1 mL/min. A Phenomenex Gemini Phenyl column (3 micron, 3 x 100 mm) was used at a temperature of 50 °C. Purity determination was performed using an Agilent Diode Array Detector and all agents reported were found to have potencies ≥ 95%.



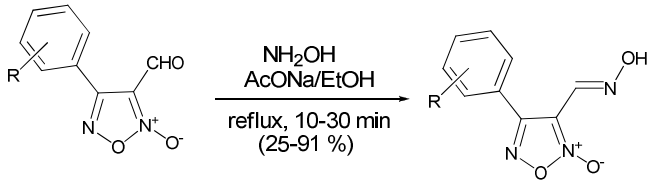
General Procedure for syntheses of 4-arylfuroxan-3-methanol.

To a solution of cinnamyl alcohol (10 mmol) in glacial acetic acid (50 mL) was added sodium nitrite (10-20 mmol for deactivated aryl groups/4mmol for activated aryl groups) portion wise over 45 min. The reaction mixture was stirred at RT for 6-48 h. After completion of the reaction, the reaction mixture was quenched with ice water and extracted with ethyl acetate. The ethyl acetate layer was washed successively with saturated $NaHCO_3$, water, brine then dried (Na_2SO_4) and concentrated under diminished pressure to give the crude product. The crude residue was purified on a Biotage® silica gel column. Gradient elution with ethyl acetate (7→80%) in hexanes gave the products as a mixture of isomers.



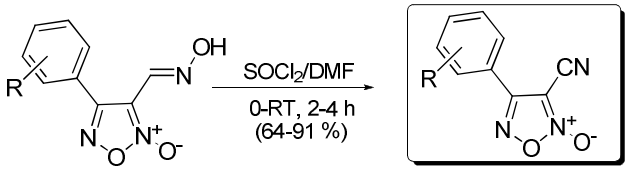
General Procedure for syntheses of 4-arylfuroxan-3-methanal:

4-Aryfuroxan-3-methanol (10 mmol) was dissolved in dichloromethane (50 mL) and treated with activated manganese dioxide (150 mmol). The reaction mixture was stirred at RT for 6-12 h then filtered through celite and concentrated under diminished pressure. The crude product was purified on a Biotage® silica gel column. Gradient elution with dichloromethane (12→100%) in hexanes gave the product.



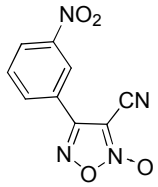
General Procedure for syntheses of 4-arylfuroxan-3-carbaldehyde oxime.

4-Aryfuroxan-3-methanal (10 mmol), hydroxylamine hydrochloride (15 mmol) and sodium acetate (10 mmol) in ethanol (50 mL) was refluxed for 10-20 min. After completion of the reaction, the solvent was removed under diminished pressure and the crude residue was purified on a Biotage® silica gel column. Gradient elution with ethyl acetate (7→60%) in hexanes gave the product.



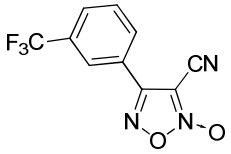
General Procedure for syntheses of 4-aryl-3-cyanofuroxan: To a solution of 4-arylfuroxan-3-carbaldehyde oxime (1 mmol) in DMF (4 mL) was added drop wise

thionyl chloride (4 mmol) at 0 °C. The reaction mixture was allowed to warm to room temperature with stirring over 3 h then stirred at this temperature for an additional hour. The reaction mixture was quenched with ice and extracted with dichloromethane. The combined organic layer was successively washed with saturated $NaHCO_3$, water, brine then dried (Na_2SO_4) and concentrated under diminished pressure to give the crude product. The crude product was purified on a Biotage® silica gel column/preparative HPLC®. Gradient elution with ethyl acetate (1→25%) in hexanes gave the product.



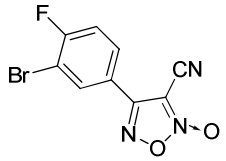
3-cyano-4-(3-nitrophenyl)-1,2,5-oxadiazole 2-oxide (11).

LC-MS: rt (min) = 5.82; ^{1}H NMR ($CDCl_3$) δ 7.85 (t, J = 8.0 Hz, 1H, Ar-H), 8.24-8.52 (m, 2H, Ar-H) and 8.84 (t, J = 1.8 Hz, 1H, Ar-H); ^{13}C NMR ($CDCl_3$) δ 94.2, 106.0, 122.0, 125.5, 127.1, 131.0, 132.2 149.0 and 152.3. HRMS (ESI) *m/z* 233.0314 $(M+H)^+$ ($C_9H_5N_4O_4$ requires 233.0311).



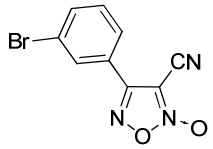
3-cyano-4-(3-(trifluoromethyl)phenyl)-1,2,5-oxadiazole 2-oxide (12).

LC-MS: rt (min) = 6.37; ^{1}H NMR ($CDCl_3$) δ 7.72-8.13 (m, 3H, Ar-H) and 8.21 (s, 1H, Ar-H); ^{13}C NMR ($CDCl_3$) δ 95.3, 106.0, 120.5, 125.8 (q, $J_{C\text{-}F}$ = 271.2 Hz), 123.7, 123.9 (q, $J_{C\text{-}F}$ = 4.1 Hz), 129.3, 129.4 (q, $J_{C\text{-}F}$ = 3.4 Hz), 130.0, 130.5, 132.4, 132.9 (q, $J_{C\text{-}F}$ = 33.5 Hz) and 153.1. HRMS (ESI) *m/z* 256.0326 $(M+H)^+$ ($C_{10}H_5F_3N_3O_2$ requires 256.0334).



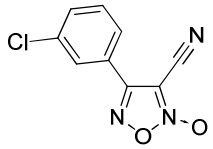
4-(3-bromo-4-fluorophenyl)-3-cyano-1,2,5-oxadiazole 2-oxide (13).

LC-MS: rt (min) = 6.37; ^{1}H NMR (DMSO-d_6) δ 7.73 (t, J = 8.6 Hz, 1H, Ar-H), 7.91-7.95 (m, 1H, Ar-H) and 8.18 (dd, J = 6.4 and 2.0 Hz, 1H, Ar-H); ^{13}C NMR (DMSO-d_6) δ 98.5, 107.1, 109.6 (d, J = 22.3 Hz), 118.3 (d, J = 23.1 Hz), 122.1, 129.1 (d, J =8.9 Hz), 132.3, 153.4 and 160.6 (d, J = 250.8 Hz). HRMS (ESI) m/z 285.9467 $(M+H)^+$ ($C_9H_4BrFN_3O_2$ requires 285.9463).



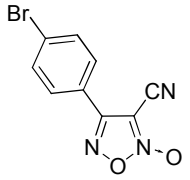
4-(3-bromophenyl)-3-cyano-1,2,5-oxadiazole 2-oxide (14).

LC-MS: rt (min) = 6.36; ^{1}H NMR ($CDCl_3$) δ 7.47 (t, J = 8.0 Hz, 1H, Ar-H), 7.76-7.86 (m, 2H, Ar-H) and 8.09 (t, J = 2 Hz, 1H, Ar-H); ^{13}C NMR ($CDCl_3$) δ 95.3, 106.1, 124.0, 125.5, 126.0, 130.0, 131.2, 136.0 and 153.0. HRMS (ESI) m/z 265.9565 $(M+H)^+$ ($C_9H_5BrN_3O_2$ requires 265.9566).



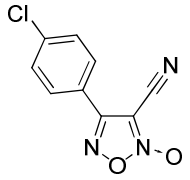
4-(3-chlorophenyl)-3-cyano-1,2,5-oxadiazole 2-oxide (15).

LC-MS: rt (min) = 6.27; ^{1}H NMR (DMSO-d_6) δ 7.34 (t, J = 7.6 Hz, 1H, Ar-H), 7.79-7.85 (m, 2H, Ar-H) and 7.89 (t, J = 1.6 Hz, 1H, Ar-H); ^{13}C NMR (DMSO-d_6) δ 98.5, 107.2, 125.8, 125.9, 124.6, 131.8, 132.5, 134.2 and 154.1. HRMS (ESI) m/z 222.0072 $(M+H)^+$ ($C_9H_5ClN_3O_2$ requires 222.0070).



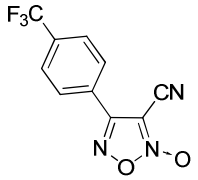
4-(4-bromophenyl)-3-cyano-1,2,5-oxadiazole 2-oxide (16).

LC-MS: rt (min) = 6.37; ^{1}H NMR (DMSO-d_6) δ 7.77 (d, J = 8.4 Hz, 2H, Ar-H) and 7.90 (d, J = 8.4 Hz, 2H, Ar-H); ^{13}C NMR (DMSO-d_6); δ 99.0, 108.0, 123.9, 127.0, 128.8, 130.5, 132.7, 134.4 and 155.0. HRMS (ESI) m/z 265.9572 $(M+H)^+$ ($C_9H_5BrN_3O_2$ requires 265.9566).



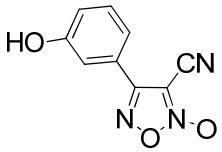
4-(4-chlorophenyl)-3-cyano-1,2,5-oxadiazole 2-oxide (17).

LC-MS: rt (min) = 6.29; ^{1}H NMR (DMSO-d_6) δ 7.75 (d, J = 8.4 Hz, 2H, Ar-H) and 7.86 (d, J = 8.8 Hz, 2H, Ar-H); ^{13}C NMR (DMSO-d_6); δ 99.1, 108.0, 123.5, 128.7, 129.8, 130.4, 131.5, 138.1 and 155.0. HRMS (ESI) m/z 222.0062 $(M+H)^+$ ($C_9H_5ClN_3O_2$ requires 222.0070).



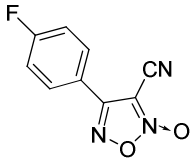
3-cyano-4-(4-(trifluoromethyl)phenyl)-1,2,5-oxadiazole 2-oxide (18).

LC-MS: rt (min) = 6.41; ^{1}H NMR ($CDCl_3$) δ 7.87 (d, J = 8.8 Hz, 2H, Ar-H) and 8.08 (d, J = 8.8 Hz, 2H, Ar-H); ^{13}C NMR ($CDCl_3$); δ 95.3, 106.1, 120.5, 125.9 (q, $J_{C\text{-}F}$ = 271.5 Hz), 126.7, 126.8 (q, $J_{C\text{-}F}$ = 3.7 Hz), 127.2, 127.4, 134.2, 134.8 (q, $J_{C\text{-}F}$ = 32.8 Hz) and 153.1. HRMS (ESI) m/z 256.0338 $(M+H)^+$ ($C_{10}H_5F_3N_3O_2$ requires 256.0334).



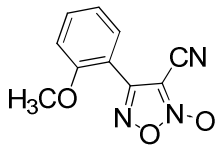
3-cyano-4-(3-hydroxyphenyl)-1,2,5-oxadiazole 2-oxide (19).

LC-MS: rt (min) = 5.23; ^{1}H NMR (DMSO-d_6) δ 7.06-7.30 (m, 3H, Ar-H), 7.70 (t, J = 8.0 Hz, 1H, Ar-H) and 10.15 (s, 1H, OH); ^{13}C-NMR (DMSO-d_6) δ 98.3, 107.5, 113.3, 117.5, 119.6, 124.9, 131.0, 155.0, 158.0. HRMS (ESI) m/z 204.0410 $(M+H)^+$ ($C_9H_6N_3O_3$ requires 204.0403).



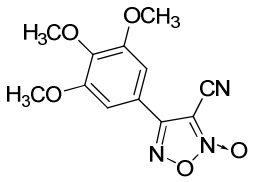
3-cyano-4-(4-fluorophenyl)-1,2,5-oxadiazole 2-oxide (20).

LC-MS: rt (min) = 5.94; ^{1}H NMR (DMSO-d_6) δ 7.53-7.58 (m, 2H, Ar-H) and 7.92-7.96 (m, 2H, Ar-H); ^{13}C-NMR (DMSO-d_6); δ 98.4, 107.3, 117.0 (d, J = 22.3 Hz), 120.5 (d, J = 3.0 Hz), 129.8 (d, J = 8.9 Hz), 154.4 and 164.4 (d, J = 250.7 Hz). HRMS (ESI) m/z 206.0361 $(M+H)^+$ ($C_9H_5FN_3O_2$ requires 206.0366).



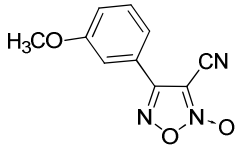
3-cyano-4-(2-methoxyphenyl)-1,2,5-oxadiazole 2-oxide (21).

LC-MS: rt (min) = 6.01; ^{1}H NMR ($CDCl_3$) δ 3.96 (s, 3H, OCH_3), 7.06-7.15 (m, 2H, Ar-H) and 7.58-7.72 (m, 2H, Ar-H); ^{13}C NMR ($CDCl_3$) δ 55.1, 98.4, 106.5, 111.6, 112.9, 121.4, 129.9, 134.3, 153.3 and 157.1. HRMS (ESI) m/z 218.0566 $(M+H)^+$ ($C_{10}H_8N_3O_3$ requires 218.0560).



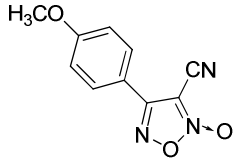
3-cyano-4-(3,4,5-trimethoxyphenyl)-1,2,5-oxadiazole 2-oxide (22).

LC-MS: rt (min) = 5.89; ^{1}H NMR (DMSO-d_6) δ 3.74 (s, 3H, OCH_3), 3.82 (s, 6H, OCH_3) and 7.11 (s, 2H, Ar-H); ^{13}C NMR (DMSO-d_6) δ 56.1, 58.6, 61.7, 99.1, 104.5, 106.1, 108.2, 119.6, 141.4, 141.5, 154.3 and 155.6. HRMS (ESI) *m/z* 278.0775 $(M+H)^+$ ($C_{12}H_{12}N_3O_5$ requires 278.0772).



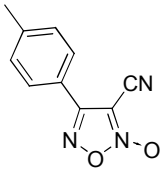
3-cyano-4-(3-methoxyphenyl)-1,2,5-oxadiazole 2-oxide (23).

LC-MS: rt (min) = 6.02; ^{1}H NMR ($CDCl_3$) δ 3.88 (s, 3H, OCH_3) and 7.14-7.50 (m, 4H, Ar-H); ^{13}C NMR ($CDCl_3$) δ 55.53, 96.0, 106.4, 111.6, 116.8, 119.2, 124.9, 130.9 154.2 and 160.34. HRMS (ESI) *m/z* 218.0562 $(M+H)^+$ ($C_{10}H_8N_3O_3$ requires 218.0560).



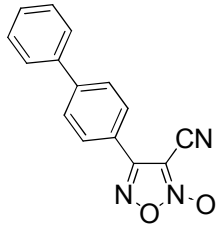
3-cyano-4-(4-methoxyphenyl)-1,2,5-oxadiazole 2-oxide (24).

LC-MS: rt (min) = 6.01; ^{1}H-NMR ($CDCl_3$) δ 3.91 (s, 3H, OCH_3), 7.06 (d, *J* = 8.8 Hz, 2H, Ar-H) and 7.87 (d, *J* = 9.2 Hz, 2H, Ar-H); ^{13}C-NMR ($CDCl_3$) δ 55.6, 95.5, 106.7, 115.1, 115.9, 128.2, 128.5, 153.9 and 163.0. HRMS (ESI) *m/z* 218.0564 $(M+H)^+$ ($C_{10}H_8N_3O_3$ requires 218.0560).



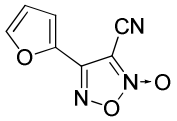
3-cyano-4-*p*-tolyl-1,2,5-oxadiazole 2-oxide (25).

LC-MS: rt (min) = 6.27; ^{1}H NMR ($CDCl_3$) δ 2.47 (s, 3H, CH_3), 7.38 (d, *J* = 8.0 Hz, 2H, Ar-H) and 7.81 (d, *J* = 8.4 Hz, 2H, Ar-H); ^{13}C NMR ($CDCl_3$) δ 22.0, 95.8, 106.8, 121.0, 127.0, 131.0, 146.0 and 154.8. HRMS (ESI) *m/z* 202.0610 $(M+H)^+$ ($C_{10}H_8N_3O_2$ requires 202.0610).



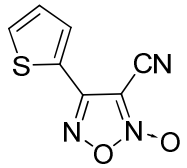
4-(biphenyl-4-yl)-3-cyano-1,2,5-oxadiazole 2-oxide (26).

LC-MS: rt (min) = 6.86; ^{1}H NMR ($CDCl_3$) δ 7.43-7.66 (m, 5H, Ar-H), 7.81 (d, *J* = 8.8 Hz, 2H, Ar-H) and 8.01 (d, *J* = 8.8 Hz, 2H, Ar-H); ^{13}C NMR ($CDCl_3$) δ 95.7, 106.4, 122.2, 127.1, 127.2, 128.1, 128.3, 129.1, 138.2, 146.0 and 154.0. HRMS (ESI) *m/z* 264.0779 $(M+H)^+$ ($C_{15}H_{10}N_3O_2$ requires 264.0773).



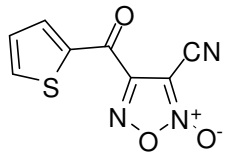
3-cyano-4-(furan-2-yl)-1,2,5-oxadiazole 2-oxide (28).

LC-MS: rt (min) = 5.36; ^{1}H NMR (DMSO-d_6) δ 6.88 (dd, *J* = 3.8 Hz and 1.8 Hz, 1H, Het-H), 7.37 (dd, *J* = 3.6 Hz and 0.8 Hz, 1H, Het-H), 8.18 (dd, *J* = 1.8 Hz and 0.6 Hz, 1H, Het-H); ^{13}C-NMR (DMSO-d_6) δ 96.8, 106.8, 113.0, 115.1, 138.2, 147.1 and 147.6. HRMS (ESI) *m/z* 178.0245 $(M+H)^+$ ($C_7H_4N_3O_3$ requires 178.0253).



3-cyano-4-(thiophen-2-yl)-1,2,5-oxadiazole 2-oxide (29).

LC-MS: rt (min) = 5.75; ^{1}H NMR ($CDCl_3$) δ 7.24-7.27 (m, 1H, Het-H), 7.67-7.69 (m, 1H, Het-H) and 7.82-7.83 (m, 1H, Het-H); ^{13}C NMR ($CDCl_3$) δ 94.9, 106.1, 124.6, 128.5, 130.5, 131.3 and 149.8. HRMS (ESI) *m/z* 194.0025 $(M+H)^+$ ($C_7H_4N_3O_2S$ requires 194.0024).



4-thienoyl-3-cyanofuroxan (30).

LC-MS: rt (min) = 5.66; ^{1}H NMR ($CDCl_3$) δ 7.30 (dd, *J* = 5.2 and 4.4 Hz, 1H, Het-H), 7.97 (dd, *J* = 4.8 and 1.2 Hz, 1H, Het-H) and 8.41 (dd, *J* = 4.0 Hz and 1.2 Hz, 1H, Het-H); ^{13}C-NMR ($CDCl_3$) δ 95.3, 104.5, 129.3, 137.4, 138.8, 138.9 152.6 and 171.4. HRMS (ESI) *m/z* 221.9981 $(M+H)^+$ ($C_8H_4N_3O_3S$ requires 221.9980).

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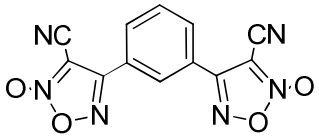
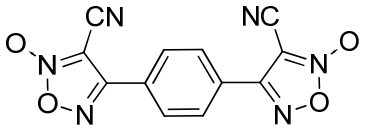


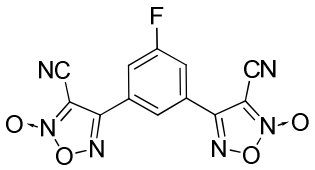
4,4'-(1,3-phenylene)bis(3-cyano-1,2,5-oxadiazole 2-oxide) (31).

LC-MS: rt (min) = 6.24; ^{1}H NMR ($CDCl_3$) δ 7.87 (t, *J* = 7.8 Hz, 1H, Ar-H), 8.19 (dd, *J* = 7.8 and 1.8 Hz, 2H, Ar-H), 8.53-8.54 (m, 1H, Ar-H); ^{13}C NMR ($CDCl_3$) δ 95.5, 106.0, 124.5, 125.8, 130.6, 131.2 and 153.0. HRMS (ESI) *m/z* 297.0382 $(M+H)^+$ ($C_{12}H_5N_6O_4$ requires 297.0386).



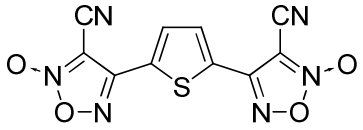
4**,4'-(1,4-phenylene)bis(3-cyano-1,2,5-oxadiazole 2-oxide) (32).**

LC-MS: rt (min) = 6.30; ^{1}H NMR ($CDCl_3$) δ 8.19 (s, 4H, Ar-H); ^{13}C NMR ($CDCl_3$) δ 95.2, 106.1, 127.8, 128.1 and 152.8. HRMS (ESI) *m/z* 297.0380 $(M+H)^+$ ($C_{12}H_5N_6O_4$ requires 297.0386).



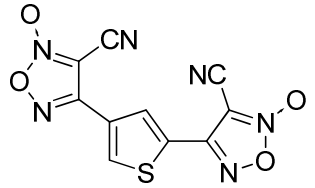
4,4'-(5-fluoro-1,3-phenylene)bis(3-cyano-1,2,5-oxadiazole 2-oxide) (33).

LC-MS: rt (min) = 6.30; ^{1}H NMR ($CDCl_3$) δ 7.92 (dd, J = 8 Hz and 1.6 Hz, 2H, Ar-H) and 8.31-8.32 (m, 1H, Ar-H); ^{13}C NMR ($CDCl_3$) δ 95.0, 105.7, 118.1 (d, $J_{C\text{-}F}$ = 23.8 Hz), 120.9 (d, $J_{C\text{-}F}$ = 3.7 Hz), 127.7 (d, $J_{C\text{-}F}$ = 8.2 Hz), 151.7 (d, $J_{C\text{-}F}$ = 2.2 Hz) and 163.3 (d, $J_{C\text{-}F}$ = 253 Hz). HRMS (ESI) m/z 315.0266 $(M+H)^+$ ($C_{12}H_4FN_6O_4$ requires 315.0265).



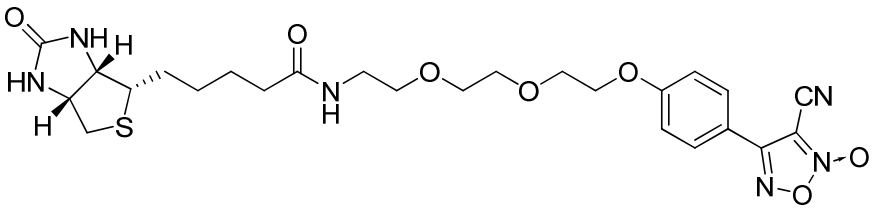
4,4'-(thiophene-2,5-diyl)bis(3-cyano-1,2,5-oxadiazole 2-oxide) (34).

LC-MS: rt (min) = 6.35; ^{1}H NMR ($CDCl_3$) δ 7.92 (s, 2H, Het-H); ^{13}C NMR ($CDCl_3$) δ 94.4, 105.6, 129.6, 130.8, and 148.5. HRMS (ESI) m/z 302.9934 $(M+H)^+$ ($C_{10}H_3N_6O_4S$ requires 302.9936).



4,4'-(thiophene-2,4-diyl)bis(3-cyano-1,2,5-oxadiazole 2-oxide) (35).

LC-MS: rt (min) = 6.12; ^{1}H NMR ($CDCl_3$) δ 8.30 (d, J = 1.6 Hz, 1H, Het-H) and 8.38 (d, J = 1.2 Hz, 1H, Het-H); ^{13}C NMR ($CDCl_3$) δ 94.8, 94.9, 105.7, 106.2, 126.3, 127.4, 128.1, 132.4, 148.6 and 148.9. HRMS (ESI) m/z 324.9754 $(M+Na)^+$ ($C_{10}H_3N_6O_4S$ requires 324.9753).



3-cyano-4-(4-(2-(2-(2-(5-((3aS,4S,6aR)-2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl)pentanamido)ethoxy)ethoxy)ethoxy)phenyl)-1,2,5-oxadiazole 2-oxide

LC-MS: rt (min) = 5.15; ^{1}H-NMR (DMSO-d_6) δ 1.26-1.61 (m, 6H), 2.05 (t, J = 7.6 Hz, 2H), 2.50-2.58 (m, 2H), 3.06-3.11 (m, 1H), 3.18 (q, J = 11.6 Hz and 6.0 Hz, 2H), 3.40 (t, J = 6.0 Hz, 2H), 3.52-3.55 (m, 2H), 3.59-3.62 (m, 2H), 3.77-3.79 (m, 2H), 4.10-4.13 (m, 1H), 4.21-4.22 (m, 2H), 4,28-4.31 (m, 1H), 6.35 (br, 1H), 6.40 (br, 1H), 7.24 (d, J = 9.2 Hz, 2H), and 7.81 (d, J = 9.2 Hz, 2H); ^{13}C-NMR (DMSO-d_6) δ 25.2, 28.0, 28.1, 35.1, 38.4, 55.3, 59.1, 60.9, 67.6, 68.7, 69.1, 69.5, 69.8, 107.6, 115.6, 115.7, 116.0, 128.6, 154.7, 161.6, 162.6 and 172.1 HRMS (ESI) m/z 561.2147 $(M+H)^+$ ($C_{25}H_{33}N_6O_7S$ requires 561.2131).