

## Supporting Information: Triazolopyrimidines Identified as Reversible Myeloperoxidase Inhibitors

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### Experimental Methods

Abbreviations: acetate (Ac); acetonitrile (ACN); aminophenyl fluorescein (APF); amplex red (AR); apoA1 (apolipoprotein A1); argon (Ar); dimethoxyethane (DME); dimethylformamide (DMF); 4-dimethylaminopyridine (DMAP); dimethyl sulfoxide (DMSO); eosinophil peroxidase (EPX); equivalent (equiv.); ethyl (Et); excitation (ex); emission (em); high density lipoprotein (HDL); lactoperoxidase (LPO); methyl (Me); milligrams per kilogram (mpk); myeloperoxidase (MPO); room temperature (rt); tetrahydrofuran (THF); thyroid peroxidase (TPO); trifluoroacetic acid (TFA).

### Biological assay methods

#### *MPO Peroxidation Assay (Amplex Red Assay)*

MPO peroxidation activity was measured in 100 mM KPi (pH 7.4) by utilizing the non-fluorescent reagent Amplex Red (Invitrogen catalog # A12222) which can be oxidized to the highly fluorescent resorufin. Amplex Red is oxidized by the peroxidase action of MPO to resorufin. Reactions were carried out in 50  $\mu$ L total volume by adding a 25  $\mu$ L mixture of 200 pM myeloperoxidase (Calbiochem #475911) and 40 nM H<sub>2</sub>O<sub>2</sub> (Sigma #349887) to 100 nL inhibitor in 100% DMSO in a 384 well Perkin Elmer Optiplate. Enzyme and compound were preincubated for ten minutes at room temperature.

After the ten minute preincubation, 25  $\mu$ L of an Amplex Red mixture containing 200  $\mu$ M Amplex Red and 10mM H<sub>2</sub>O<sub>2</sub> was added to the plate. Kinetic determinations were carried out immediately on a Perkin Elmer Envision (15 minute kinetic read, Ex: 535 nm, Em: 590 nm).

IC<sub>50</sub> values were calculated by determining the slope of the linear portion of the kinetic trace (180-540 secs), and using that calculated slope to determine % inhibition occurring at each concentration of inhibitor using the following equation:

$$Y = A + \frac{B - A}{1 + (C/x)^D}$$

where A = minimal Y value (activity level of inhibited sample), B = maximal Y value (activity level of uninhibited sample), C = LogIC<sub>50</sub>, D = Hill Slope, x = concentration of inhibitor.

$$y = \frac{100\%}{1 + \left(\frac{x}{IC_{50}}\right)^s}$$

### ***MPO Chlorination Assay (APF Assay)***

MPO chlorination activity was measured in 100 mM KPi (pH 7.4) by utilizing the non-fluorescent reagent Aminophenyl fluorescein (APF-Invitrogen catalog # A36003). APF is cleaved by (-OCl) to yield the fluorescent compound fluorescein. Reactions were carried out in 50 μL total volume by adding a 25 μL mixture of 200 pM myeloperoxidase (Human MPO\_Calbiochem #475911; Mouse MPO\_R&D Systems#3667-MP) and 40 nM H<sub>2</sub>O<sub>2</sub> (Sigma #349887) to 100 nL inhibitor in 100% DMSO in a 384 well Perkin Elmer Optiplate. Enzyme and compound were preincubated for ten minutes at rt. After the ten minute preincubation, 25 μL of an APF mixture containing 10 mM APF, 240 mM NaCl and 10 μM H<sub>2</sub>O<sub>2</sub> was added to the plate. Kinetic determinations were carried out immediately on a Perkin Elmer Envision (15 minute kinetic read, ex: 485 nm, em: 535 nm). IC<sub>50</sub> values for inhibitors were calculated by taking the slope of the linear portion of the kinetic measurement (first ten minutes).

IC<sub>50</sub> values were determined by calculating % inhibition occurring at each concentration of inhibitor using the equation 100-(v<sub>i</sub>/v<sub>0</sub>\*100) where v<sub>i</sub> = the slope of the linear portion of an inhibited sample, v<sub>0</sub> = slope of the linear portion of the non-inhibited control sample and fitting data to:

where s is a slope factor, y is the percent activity remaining and x is the corresponding concentration for the inhibitor.

Activity was also measured without the 10 minute pre-incubation step using a similar procedure.

***High throughput screening assays:***

In order to enable the screening of BMS small molecule collection, the previously described assays were modified to a miniaturized 1536 well format.

**APF assay.** Briefly, the APF assay was conducted in a 4  $\mu$ L final volume in which 2 $\mu$ L of 1.75 nM MPO (Calbiochem #475911) with 20  $\mu$ M H<sub>2</sub>O<sub>2</sub> (final) were added to 20 nL of pre-plated compound, 10 $\mu$ M (final) and pre-incubated for 10 mins. Following pre-incubation, 2  $\mu$ L of the APF reagent was added and change in fluorescence (485nmEx/535nm Em) was measured kinetically at 30 second intervals for 5 minutes using a Viewlux™ (Perkin Elmer). The reaction rate was fit to linear equation and the determined slope used for further data analysis. A series of wells in columns 45 and 46 containing MPO and compound-equivalent concentration of DMSO were used to set 0% inhibition, and columns 47-48 in which MPO was absent serve as 100% inhibition. During the confirmation/retest phase of the experiment active compounds from the primary screening were examined for assay interference by measuring compound effect on the APF detection reaction, so called “NaOCl scavengers assay”. In these experiments, 50 $\mu$ M of NaOCl was substituted for MPO. Following 15 minutes of incubation using the fluorescence intensity measurement from the Viewlux™, compounds exhibiting 20% or greater inhibition of the fluorescence were deemed interfering and excluded from further progression.

**Amplex Red Assay.** Similarly, the Amplex Red assay was modified to a miniaturized 1536 well format. MPO was added in a 2  $\mu$ L volume (1.75 nM final) to 20 nL or pre-plated compounds. Following 10 minutes of pre-incubation, the reaction was initiated with a 2 $\mu$ L addition of mixture of 20  $\mu$ M H<sub>2</sub>O<sub>2</sub>, 200  $\mu$ M Amplex Red (final). The reaction was measured kinetically at 30 second intervals for 5 minutes using a Viewlux, with the rate of fluorescence change fit to a linear equation and the slope measurement further analyzed for percent inhibition determinations. To ensure compounds did not interfere with the detection method, compounds were evaluated for an effect on the conversion of the resazurin reagent to resorufin, so called “redox assay”. Test compounds (10  $\mu$ M final) were incubated with 5  $\mu$ M (final) of the resazurin reagent for 30 minutes.

Fluorescence intensity was subsequently measured on an Envision™ (Perkin Elmer) plate reader. Percent inhibition was determined by normalizing fluorescent values obtained from a known reactive standard, (E)-1,6-dimethyl-3-styrylpyrimido[5,4-e][1,2,4]triazine-5,7(1H,6H)-dione (10 μM) final, which served as 100% control (Lor *et al.*)<sup>1</sup>.

### ***MPO Spectral Scan Analysis***

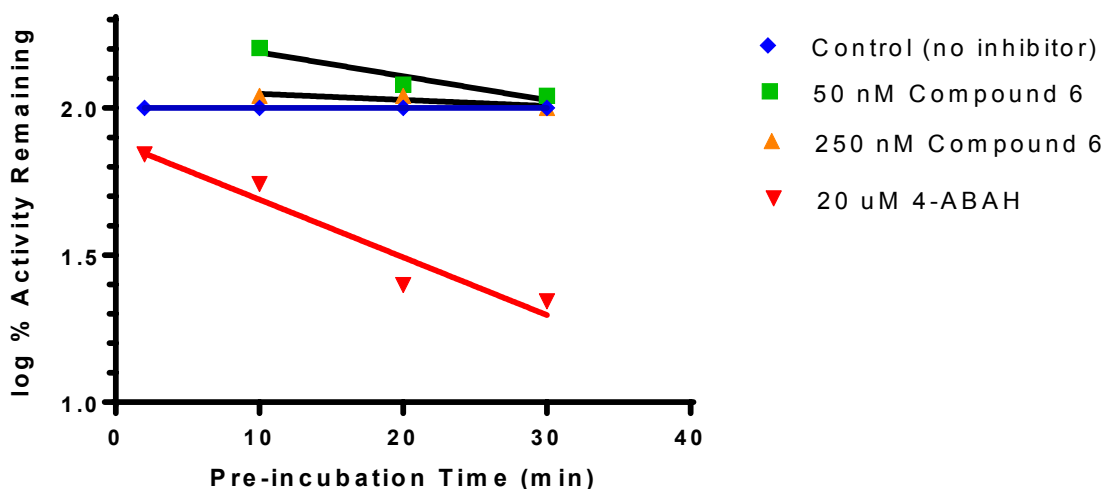
The formation and kinetics of the different MPO species in the presence of inhibitors was observed using an Applied Photophysics stopped flow spectrophotometer. For these studies, syringe 1 typically contained 2 μM MPO (Calbiochem #475911) and 5 μM H<sub>2</sub>O<sub>2</sub> in phosphate buffer. Syringe 2 contained the desired concentration of inhibitor and 5 μM H<sub>2</sub>O<sub>2</sub> in phosphate buffer. Equal volumes (40 μL) of each syringe was rapidly mixed at 25°C and data was acquired using PDA mode so that multiple MPO species could be monitored simultaneously. The amount of peroxide used was purposefully controlled to limit the number of turnovers which were observed in order to observe if any MPO II that was formed was converted back to resting MPO or if it remained in the MPO II form. Single wavelength data was collected under similar conditions but in separate experiments. Data shown is representative of numerous replicates.

### ***Compound 6 Reversibility Studies***

To determine whether the inhibitors were reversible or irreversible inhibitors of MPO, 20 nM MPO (Calbiochem #475911) in phosphate buffer was incubated with 5x - 10x the AR IC<sub>50</sub> for a given compound when that IC<sub>50</sub> is > 1 μM and 2 – 10x fold the enzyme concentration when the AR IC<sub>50</sub> is < 1 μM in the presence of 100 nM H<sub>2</sub>O<sub>2</sub> for various times along with control samples which contained peroxide 0.2% DMSO or the known irreversible inhibitor, 4-hydroxybenzoic acid hydrazide.<sup>2</sup> Typically after 2, 10, 20, and 30 mins pre-incubation, 80 μL of the reaction was removed and passed through a G50 spin column to remove any unbound inhibitor. A 5 μL aliquot of the flow-through was added to a 1x Amplex Red assay, and the rate of the reaction was measured in the usual way. Reversibility experiments were also conducted in the presence of 100 mM NaCl and 150 μM L-methionine. For the compound **6**, the addition of these components did not affect the results. The initial rate of the AR assay was used to calculate the percent activity remaining

compared to control. The figure below clearly shows that even after 30 mins of pre-incubation, the inhibition by compound **6** is reversible (SI Figure 1).

**SI. Figure 1:** Reversibility of Compound 6 compared to irreversible inhibitor 4-hydroxybenzoic acid hydrazide.



### ***Thyroid peroxidase (TPO) activity assay***

Thyroid peroxidase (TPO) (NCBI reference sequence NP\_000538.3; residues 15-839\_His tagged) was cloned with a C-terminal hexahistidine tag into a pFASTBAC donor plasmid, expressed in baculovirus-infected *Trichoplusia ni* insect cells, and purified using nickel affinity, anion exchange, and size exclusion chromatography. Protein was 97% pure by SDS-PAGE and 100% monomeric by dynamic light scattering. TPO activity was measured in 100 mM KPi (pH 7.4) by monitoring the H<sub>2</sub>O<sub>2</sub> catalyzed formation of 3, 5-iodo tyrosine from 3-iodotyrosine and potassium iodide. A 50  $\mu$ L mixture of 4.0 nM TPO (2.82 mg/ml) was added to 100 nL inhibitor in 100% DMSO in a 384 well REMP plate. Enzyme and compound were preincubated for ten minutes at room temperature. After preincubation, 25  $\mu$ L of a mixture containing 400  $\mu$ M potassium iodide and 240  $\mu$ M 3-iodo tyrosine was added to the plate containing enzyme and inhibitor, followed by the addition of 25  $\mu$ L of 20  $\mu$ M H<sub>2</sub>O<sub>2</sub>. The final concentrations of all components in the enzymatic reaction were 2.0 nM TPO, 100  $\mu$ M potassium iodide, 60  $\mu$ M 3-iodo tyrosine, 5  $\mu$ M H<sub>2</sub>O<sub>2</sub>, and 0.1% DMSO.

The reaction was allowed to proceed for 15 minutes, at which time it was quenched with 10  $\mu\text{L}$  of 20% TCA bringing the TCA concentration to 2.0%.  $\text{IC}_{50}$  values were determined by calculating the peak areas of 3-bromo-tyrosine present at the end of the 15 minute reaction and fitting the data to:

$$Y = A + \frac{B - A}{1 + (C/x)^D}$$

where A = minimal Y value (activity level of inhibited sample), B = maximal Y value (activity level of uninhibited sample), C =  $\log\text{IC}_{50}$ , D = Hill Slope, and x = concentration of inhibitor.

### ***Lactoperoxidase (LPO) Assay***

Lactoperoxidase (NCBI reference sequence NP\_006142.1-isoform 1; residues 26-712) was cloned into pFASTBAC donor plasmid, expressed in baculovirus-infected *Trichoplusia ni* insect cells and purified using cation exchange, hydrophobic interaction and size exclusion chromatography following procedures outlined in Shin *et al.*<sup>3</sup> Biochemical and Biophysical Research Communications 271,831-836 (2000). Protein was over 85% pure by SDS-PAGE and 99.9% monomeric by dynamic light scattering. LPO activity was measured in 100 mM KPi (pH 7.4) by monitoring the  $\text{H}_2\text{O}_2$  catalyzed formation of 3,5-iodo tyrosine from 3-iodotyrosine and potassium iodide. A 50  $\mu\text{L}$  mixture of 2.0 nM LPO (1.75 mg/ml) was added to 100 nL inhibitor in 100% DMSO in a 384 well REMP plate. Enzyme and compound were preincubated for ten minutes at room temperature. After preincubation, 25  $\mu\text{L}$  of a mixture containing 400  $\mu\text{M}$  potassium iodide and 240  $\mu\text{M}$  3-iodo tyrosine was added to the plate containing enzyme and inhibitor, followed by the addition of 25  $\mu\text{L}$  of 20  $\mu\text{M}$   $\text{H}_2\text{O}_2$ . The final concentrations of all components in the enzymatic reaction were 1.0 nM LPO, 100  $\mu\text{M}$  potassium iodide, 60  $\mu\text{M}$  3-iodo Tyrosine, 5  $\mu\text{M}$   $\text{H}_2\text{O}_2$ , and 0.1% DMSO.

The reaction was allowed to proceed for 15 minutes, at which time it was quenched with 10  $\mu\text{L}$  of 20% TCA bringing the TCA concentration to 2.0%.  $\text{IC}_{50}$  values were determined by calculating the peak areas of 3,5-diiodo-L-tyrosine present at the end of the 15 minute reaction and fitting the data to:

$$Y = A + \frac{B - A}{1 + (C/x)^D}$$

where A = minimal Y value (activity level of inhibited sample), B = maximal Y value (activity level of uninhibited sample), C =  $\log IC_{50}$ , D = Hill Slope, and x = concentration of inhibitor.

### ***Eosinophil peroxidase (EPX) Bromination Activity***

Eosinophil peroxidase (EPX) ) used in the assay was purchased from Lee Biosolutions (cat:342-60), with purity  $\geq 98\%$  (SDS-PAGE). EPX bromination activity was measured in 100 mM KPi (pH 7.4) by monitoring the  $H_2O_2$  catalyzed formation of 3-bromo tyrosine from tyrosine and potassium bromide. A 50  $\mu$ L mixture of 0.6  $\mu$ M EPX (Lee Biosolutions Cat# 342-60) was added to 100 nL inhibitor in 100% DMSO in a 384 well REMP plate. Enzyme and compound were preincubated for ten minutes at room temperature.

After the ten minute preincubation of enzyme and inhibitor, 25  $\mu$ L of a mixture containing 400  $\mu$ M tyrosine and 1200  $\mu$ M potassium bromide was added to the plate containing enzyme and inhibitor, followed by the addition of 25  $\mu$ L of 20  $\mu$ M  $H_2O_2$ . The reaction was allowed to proceed for 15 minutes, at which time it was quenched with 10  $\mu$ L of 20 % TCA. The final concentrations of all components were 0.3  $\mu$ M EPX, 100  $\mu$ M Tyrosine, 400  $\mu$ M potassium bromide, 5  $\mu$ M  $H_2O_2$ , 0.1% DMSO, and 2.0% TCA.

$IC_{50}$  values were determined by determining the peak areas of 3-bromo-tyrosine present at the end of the 15 minute reaction and fitting the data to:

$$Y = A + \frac{B - A}{1 + (C/x)^D}$$

where A = minimal Y value (activity level of inhibited sample), B = maximal Y value (activity level of uninhibited sample), C =  $\log IC_{50}$ , D = Hill Slope, x = concentration of inhibitor.

Reversed-phase analysis was performed on a Waters Acquity Ultra Performance LC system using an Acquity UPLC BEH  $C_{18}$  1.7  $\mu$ M, 2.1 x 50 mm analytical column. The column was maintained at 60 °C. Samples were eluted using a gradient of 0%-100% B over 2.5 minutes, followed by equilibration with 100% A for 1 minute where A consisted of 0.1 % TFA and B consisted of 90% MeOH/0.1 % TFA at a flow rate of 0.6 mL/min. The retention time of 3-bromo tyrosine was 1.22 min.

## ***APOA1 modification protocol - cholesterol efflux and ApoA1 peptide oxidation LCMS***

### ***assay:***

#### 1. Human ApoA1 MPO modification:

Human ApoA1 (Biodesign A95120H) was first dialyzed against PBS pH7.4, and then 200 ug/mL treated with a mixture containing 100  $\mu$ M diethylenetriamine penta-acetic acid (DTPA , Sigma D-6518), 250 $\mu$ M H<sub>2</sub>O<sub>2</sub> (J.T Baker 2180-01) with or without 50nM MPO (Calbiochem #475911), and small molecule inhibitors at 37 °C for 30 min. The reaction was stopped by adding 2 mM L-Methionine (Sigma M-9625).

#### 2. THP1 cholesterol efflux (adapted from Kritharides et al, 1998)<sup>4</sup>

THP1 cells (ATCC TIB-202) were cultured in RPMI-1640 plus 10% fetal bovine serum, L-glutamin (Gibco 25030-081), 2 mM, 25 mM hepes (Gibco 15630-080), 1% sodium pyruvate (Gibco 11360-070), 0.05 mM 2-mercaptoethanol( Sigma M-3148), and 1% antibiotic-antimycotic (Gibco 15240-096) in 5% CO<sub>2</sub>, differentiated with 100 nM PMA (Sigma P-8139) and plated in 96-well plates (1X 10<sup>5</sup> cells/well) for 4 days. Then 5 $\mu$ Ci/mL (1,2 <sup>3</sup>H)-cholesterol (Perkin Elmer NET-139) was used for labelling serum lipoprotein particles (100 $\mu$ L/well) in the presence with 5% lipid-deficient serum (LDS Intracel RP-056) and 50  $\mu$ g/mL ac-LDL (Biomedical Tech Inc, BT-906) for 24 hours. For cholesterol efflux measurement, 20  $\mu$ g/mL each apoA1 (treated with or without MPO and inhibitors) with 0.2% fatty acid-free BSA (Sigma A-6003) in RPMI-1640 with 1% sodium pyruvate, 1% antibiotic-antimycotic and 0.05 mM 2-mercaptoethanol were incubated with cells overnight. The apoA1 mediated cholesterol efflux in the THP1 cells media was determined by counting <sup>3</sup>H (Perkin Elmer Topcount-HTS, 12 DET, 96/384) present in cell media and in cell extracts and % cholesterol efflux calculated based on the formula: <sup>3</sup>H count in media/total <sup>3</sup>H (media+lysate) X 100.

#### 3. Method for ApoA1 peptide LCMS assay

ApoA1 peptides measured:

Y192cl - LAEYclHAK

Y192 - LAEYHAK



## W50- LLDNWDSVTSTFSK

Protein digestion: Protein samples were cleaned up with Zeba spin plate following vendor's protocol (Thermo Fisher Scientific). Trypsin was added to each well with 1 to 50 enzyme to protein ratio. Samples were incubated in 37 °C overnight.

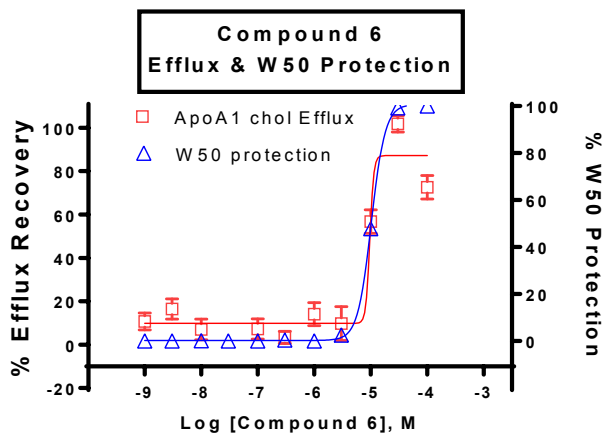
### SPE (C18 solid phase extraction):

To each sample, add 80 uL working internal standard (IS) solution with W50 IS (LLDNWDSVTSTFSK(13C, 15N)), Y192Cl IS (LAEYcIHAK (13C, 15N)), and Y192 IS (LAEYHAK(13C, 15N)). All heavy labeled internal standards were custom made by New England Peptide Co. Samples then were desalted using 3M Empore C18 plate. Briefly, the plate was conditioned with 300 uL 0.1% TFA in 90% acetonitrile followed by 300 uL 0.1% TFA in water. Samples were loaded, and the plate was washed with 300 uL 0.1% TFA in water. Finally bound peptides were eluted with 300 uL 0.1%TFA in 90% acetonitrile. Eluates were dried down by SpeedVac.

LCMS: Samples were reconstituted in 0.1% acetic acid in water and injected onto the column (Microm 0.3 x 50mm Halo C18 ) on Agilent 1200 CapLC system coupled with AB Sciex 5500 QTRAP.

Peak extraction was performed by AB Sciex Analysis software. Relative abundance of Y192cl, Y192 and W50 was estimated according to the ratio of AUCs (area under the curve) of the target peak and that of corresponding spiked in heavy labeled internal standard. Plots were generated by GraphPad Prism 5.0.

**SI Figure 2.** Dose response of inhibitor **6** on MPO oxidation of ApoA1 Trp (W) 50 (blue) and cholesterol efflux capacity (red). The EC<sub>50</sub> values were ~10 μM for W50 protection and ApoA1 cholesterol efflux capacity in THP-1 macrophages.



### ***Human Neutrophil PMA-induced MPO Activity Assay***

Neutrophils from healthy donors were isolated by the *Dextran Sedimentation Followed by Ficoll-Paque* method described by William M. Nauseef.<sup>5</sup> Blood was drawn in BD Vacutainer (BD Franklin Lakes 366480) and mixed with equal volumes of 3% dextran solution (Sigma D8906) by repeated inversion (10 times), and tubes were set upright for 18–20 min. Then the straw-colored, leukocyte-rich, erythrocyte-poor upper layer was aspirated with a sterile plastic pipet or a 10- to 15-mL sterile syringe and transfer to a sterile 50-mL conical tube. Leukocytes were pelleted by centrifugation at 500 g for 10 min at 4 °C. The cell pellets was resuspended with 35 mL sterile saline buffer. The leukocyte suspension was then carefully underlayered with 10 mL of Ficoll-Paque solution, and gradients centrifuged at 400 g for 40 min at rt. After centrifugation, the supernatant was discarded, and neutrophils were collected in sterile water supplemented with an equal volume of 1.8 % saline to promptly restore tonicity. Cells were centrifuged at 500 g for 5 min at 4 °C, and cells resuspended in HBSS (GIBCO # 14025) at about 30 million cells/mL.

The assay was adapted from protocol described by Gross *et al.*<sup>6</sup> 100 uL neutrophils at 1.106 Cells/mL were plated in Costar 96-well plate, and PMA ((Sigma # p8139) at 100 nM final concentration were added. Inhibitor (1 μL) in vehicle (10% Ethanol, 50% Peg, and 40% H<sub>2</sub>O) from a starting concentration of 22 μM down to 1 nM (1:3 dilution, total 10 doses) were incubated with cells for 3 min at rt. Luminol (100 μM final concentration) was added, and plate was read on LMaxII-384 luminometer (Molecular Devices) at 37 °C for 25 min for kinetics analysis. The rate of formation of luminescence due to oxidation of luminol by MPO-produced HOCl was measured

in the initial linear region of the reaction. No signal was detected using neutrophils from MPO knock-out mice used as negative control.

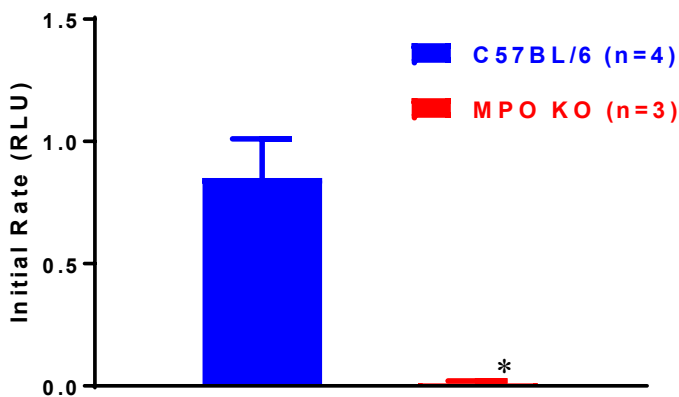
### ***Acute in Vivo PMA Model***

Mouse whole blood luminescence assay:

All animal studies were performed according to guidelines established by the American Association for Accreditation of Laboratory Animal Care and protocols were approved by the Bristol-Myers Squibb Hopewell Animal Care and Use Committee (ACUC). The protocol was modified from a similar method described by Gross *et al.*<sup>6</sup> C57Bl6 mice were orally dosed with 30 mpk **6** or vehicle 20 min prior to PMA (Sigma P-8139) retro-orbital injection at 0.62mg/kg. Two minutes post PMA injection, blood was drawn and kept on ice before measurement of MPO-produced HOCl quantification using luminol chemiluminescence as readout.

To measure mouse blood luminol activity, 100  $\mu$ L of mouse blood was plated in a 96-well plate (Costar 3925), combined with 2  $\mu$ L of 100 mM luminol (Sigma A4685, dissolved in H<sub>2</sub>O), and mixed. Luminol chemiluminescence was measured on LMaxII-384 luminometer (Molecular Devices) at 37 °C for 25 min for kinetics analysis and the HOCl rate of production was calculated. No luminol oxidation was detected in MPO knockout mice, which confirmed luminol chemiluminescence was dependent on MPO formation of HOCl (SI Figure 3).<sup>7</sup>

**SI. Figure 3.** Luminol chemiluminescence measured in wild type mice and MPO KO mice after PMA treatment.



### ***Protein crystallization***

Human MPO protein Isoform C was purchased from Lee Biosolutions Inc, St. Louis MO. The purchased protein was further purified by gel–filtration chromatography (Superdex 75) in a buffer containing 20 mM Na-Acetate pH 5.5, 100 mM NaCl. Crystals of MPO in complex with **6** were obtained at 22 °C by the hanging drop vapor diffusion method. The protein (25 mg/ml concentration) was incubated with **6** (protein:**6** molar ratio of 1:5) at 4 °C for 4 hours prior to crystallization setup. The reservoir solution contains 100 mM hepes (pH -7.5), 150 mM NaCl, and 20-25% (w/v) PEG3350. Crystals were cryoprotected by supplementing the mother liquor with 15% (v/v) ethylene glycol and harvested by flash-cooling in liquid nitrogen.

**Data Collection and Structure determination.** Data for the MPO/**6** complex were collected at beamline 08ID at the Canadian Light Source. The wavelength used was 1.0 Å and the detector was a Rayonix MX225. The diffraction images were indexed, integrated, and scaled with HKL-2000.<sup>8</sup> The structure was determined by molecular replacement using the program PHASER<sup>9</sup> with a previously reported structure of MPO (PDB entry 1CXP) used as the starting model in the refinement. The structure was refined with the program autoBUSTER (Global Phasing Ltd, Cambridge, England) and were examined and manually refitted with the program COOT.<sup>10</sup> The difference electron density clearly defined the position of the compound in the heme-binding pocket. Additional cycles of refinement were performed to complete the refinement and add solvent molecules. The crystallographic data-collection and reduction statistics are summarized in Table A.

**Table A.** Summary of crystallographic information

|                        |                                     |
|------------------------|-------------------------------------|
|                        | MPO<br>+ Compound 6                 |
| <b>PDB Code</b>        | 5WDJ                                |
| <b>Data collection</b> |                                     |
| Space group            | <i>P4<sub>3</sub>2<sub>1</sub>2</i> |
| Cell dimensions        |                                     |

|   |                     |
|---|---------------------|
| $a, b, c$ (Å)                           | 103.9, 103.9, 242.3 |
| $\alpha, \beta, \gamma$ (°)             | 90, 90, 90          |
| Resolution (Å)                          | 50-2.40             |
| $R_{\text{merge}}$ (%)                  | 11.3 (52.5)         |
| $I / \sigma I$                          | 15.11 ( 3.1)        |
| Completeness (%)                        | 100 (100)           |
| Redundancy                              | 4.8 (4.8)           |
| <b>Refinement</b>                       |                     |
| Resolution (Å)                          | 95.5 - 2.40         |
| Number of reflections                   | 47,290 (2676)       |
| $R_{\text{work}} / R_{\text{free}}$ (%) | 19.4 / 24.8         |
| No. atoms                               |                     |
| Protein                                 | 9048                |
| Ligand/ion                              | 326                 |
| Water                                   | 522                 |
| $B$ -factors (Å <sup>2</sup> )          |                     |
| Protein                                 | 24.3                |
| Ligand/ion                              | 30.1                |
| Water                                   | 28.9                |
| R.m.s. deviations                       |                     |
| Bond lengths (Å)                        | 0.006               |
| Bond angles (°)                         | 1.1                 |
| Ramachandran Plot Stats                 |                     |
| Most allowed (%)                        | 87.6                |
| Additional allowed (%)                  | 12.3                |
| Outliers (%)                            | 0.0                 |

<sup>a</sup>As defined by Laskowski *et al.*, 1993.<sup>11</sup>

The numbers in parentheses are for the highest-resolution shell. One crystal was used for each data collection.

### *Synthesis*

All reactions were carried out under a static atmosphere of argon or nitrogen and stirred magnetically unless otherwise stated. All reagents used were of commercial quality. <sup>1</sup>H NMR spectra were recorded on a Bruker 400 MHz system spectrometer unless otherwise noted. Chemical shifts are given in parts per million (ppm), and coupling constants (J values) are given in hertz (Hz). Selected data are reported in the following manner: chemical shift, multiplicity, coupling constants, proton number. All solvents were removed by rotary evaporation under vacuum using a standard rotary evaporator equipped with a dry ice condenser.

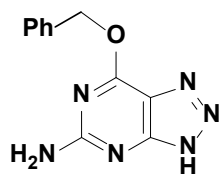
Final products were analyzed by reverse phase analytical HPLC: carried out on a Shimadzu Analytical HPLC: system running Discovery VP software. Reverse phase preparative HPLC of some final compounds was carried out using C18 columns with UV 220 nm or prep LCMS detection eluting with gradients of Solvent A (90% water, 10% MeOH, 0.1% TFA) and Solvent B (10% water, 90% MeOH, 0.1% TFA) or with gradients of Solvent A (90% water, 10% ACN, 0.1% TFA) and Solvent B (10% water, 90% ACN, 0.1% TFA) or with gradients of Solvent A (95% water, 2% ACN, 0.1% HCOOH) and Solvent B (98% ACN, 2% water, 0.1% HCOOH) or with gradients of Solvent A (95% water, 5% ACN, 10 mM NH<sub>4</sub>OAc) and Solvent B (98% ACN, 5% water, 10 mM NH<sub>4</sub>OAc) or with gradients of Solvent A (95% water, 2% ACN, 0.1% NH<sub>4</sub>OH) and Solvent B (98% ACN, 2% water, 0.1% NH<sub>4</sub>OH).

<sup>1</sup>H NMR spectra were obtained with Bruker or JEOL® Fourier transform spectrometers operating at frequencies as follows: <sup>1</sup>H NMR: 400 MHz (Bruker or JEOL®) or 500 MHz (Bruker or JEOL®). <sup>13</sup>C NMR: 100 MHz (Bruker or JEOL®). Spectra data are reported in the format: chemical shift (multiplicity, coupling constants, number of hydrogens). Chemical shifts are specified in ppm downfield of a tetramethylsilane internal standard ( $\delta$  units, tetramethylsilane = 0 ppm) and/or referenced to solvent peaks, which in <sup>1</sup>H NMR spectra appear at 2.49 ppm for CD<sub>2</sub>HSOCD<sub>3</sub>, 3.30 ppm for CD<sub>2</sub>HOD, 1.94 for CD<sub>3</sub>CN, and 7.24 ppm for CHCl<sub>3</sub>, and which in

$^{13}\text{C}$  NMR spectra appear at 39.7 ppm for  $\text{CD}_3\text{SOCD}_3$ , 49.0 ppm for  $\text{CD}_3\text{OD}$ , and 77.0 ppm for  $\text{CDCl}_3$ .

The pKa values were determined in the pH range between 2 and 11, and the data were obtained using a Sirius GLpKa instrument. The pKa values were measured using the spectrophotometric titration method, and the compound was dissolved in varying ratios of methanol/water with extrapolation to 0% cosolvent. The predicted pKa value was determined using the Jaguar pKa GUI method in the Maestro version 8.5.207 from Schroedinger, Inc.

Compounds reported in the paper were prepared by methods similar to those reported by Chae, M-Y. *et al.*<sup>12</sup> or by the methods below.



7-(benzyloxy)-3H-[1,2,3]triazolo[4,5-d]pyrimidin-5-amine (**6**).

A solution of 6N HCl (25 mL, 150 mmol) was added to 4-chloroaniline (5.0 g, 39 mmol) with vigorous stirring at 0 °C. A solution of  $\text{NaNO}_2$  (2.7 g, 39 mmol) in water (4.0 mL) was added to the reaction mixture, and the mixture was stirred at 0 °C for 30 min. Urea (0.47 g, 7.8 mmol) was added to destroy residual  $\text{NaNO}_2$ . This reaction mixture was added to a solution of 6-chloropyrimidine-2,4-diamine (5.6 g, 39 mmol) in water (30 mL). After 30 min, sodium acetate (14.5 g, 176 mmol) was added, and the reaction mixture was allowed to warm to rt overnight. The mixture was filtered, and the solids were washed thoroughly with water, and dried under vacuum overnight to obtain (*E*)-6-chloro-5-((4-chlorophenyl)diazenyl)pyrimidine-2,4-diamine (8.1 g, 28 mmol, 73 % yield). MS(ESI)  $m/z$  284.2 (M+H).

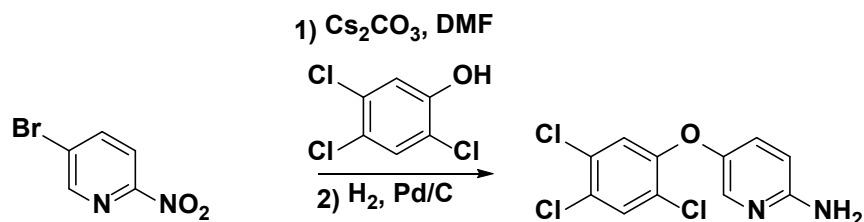
To a mixture of (*E*)-6-chloro-5-((4-chlorophenyl)diazenyl)pyrimidine-2,4-diamine (3.4 g, 12 mmol), and benzyl alcohol (2.5 mL, 24 mmol) in DMSO (25 mL) was added  $\text{Cs}_2\text{CO}_3$  (12 g, 36 mmol), and the resulting mixture was heated to 105 °C for 2h. The mixture was cooled to rt and diluted with water. The resulting slurry was filtered. The solid was washed with water, and triturated with ether to obtain a yellow solid (*E*)-6-(benzyloxy)-5-((4-

chlorophenyl)diazenyl)pyrimidine-2,4-diamine (4.2 g, 12 mmol), which was used without further manipulation. MS(ESI)  $m/z$  255.1 (M+H).

To a solution of (*E*)-6-(benzyloxy)-5-((4-chlorophenyl)diazenyl)pyrimidine-2,4-diamine (4.2 g, 12 mmol) in EtOH (10 mL) was added Zn powder (4.65 g, 71.0 mmol) followed by glacial AcOH (2.0 mL, 36 mmol). The resulting reaction mixture was heated at 70 °C for 2h. The mixture was filtered through a pad of celite, and the filtrate was concentrated to obtain an orange red product that was purified by silica gel chromatography (eluted with 0-20% MeOH in EtOAc) to furnish 6-(benzyloxy)pyrimidine-2,4,5-triamine (1.2 g, 5.2 mmol, 44 % yield).  $^1\text{H}$  NMR (500 MHz, MeOH- $d_4$ )  $\delta$  7.49 - 7.42 (m, 2H), 7.40 - 7.34 (m, 2H), 7.34 - 7.29 (m, 1H), 5.37 (s, 2H). MS(ESI)  $m/z$  232.2 (M+H).

To a solution of 6-(benzyloxy)pyrimidine-2,4,5-triamine (50 mg, 0.20 mmol) in DMSO (2 mL) was added a solution of isoamyl nitrite (0.03 mL, 0.2 mmol). The resulting solution was stirred at rt overnight. The reaction mixture was diluted with EtOAc, washed with saturated  $\text{NaHCO}_3$  and brine, dried over  $\text{MgSO}_4$ , and concentrated to obtain a reddish, sticky solid 7-(benzyloxy)-3H-[1,2,3]triazolo[4,5-d]pyrimidin-5-amine (45 mg, 0.19 mmol, 86 % yield), which was purified by silica gel chromatography (eluted with EtOAc/hex) and recrystallization from *i*PrOH.  $^1\text{H}$  NMR (500 MHz,  $\text{DMSO}-d_6$ )  $\delta$  ppm 15.34 (br. s., 1 H), 7.55 (d,  $J=6.88$  Hz, 2 H), 7.41 - 7.45 (m, 2 H), 7.35 - 7.40 (m, 1 H), 7.00 (br. s., 2 H), 5.57 (s, 2 H).  $^{13}\text{C}$  NMR (126 MHz,  $\text{DMSO}-d_6$ )  $\delta$  ppm 162.03, 160.43, 154.74, 135.82, 128.63, 128.38, 128.21, 119.65, 67.55. HRMS Observed: 243.09872; Calculated: 243.09899. HPLC Purity >96%.

*5-(2,4,5-trichlorophenoxy)pyridin-2-amine (7)*.

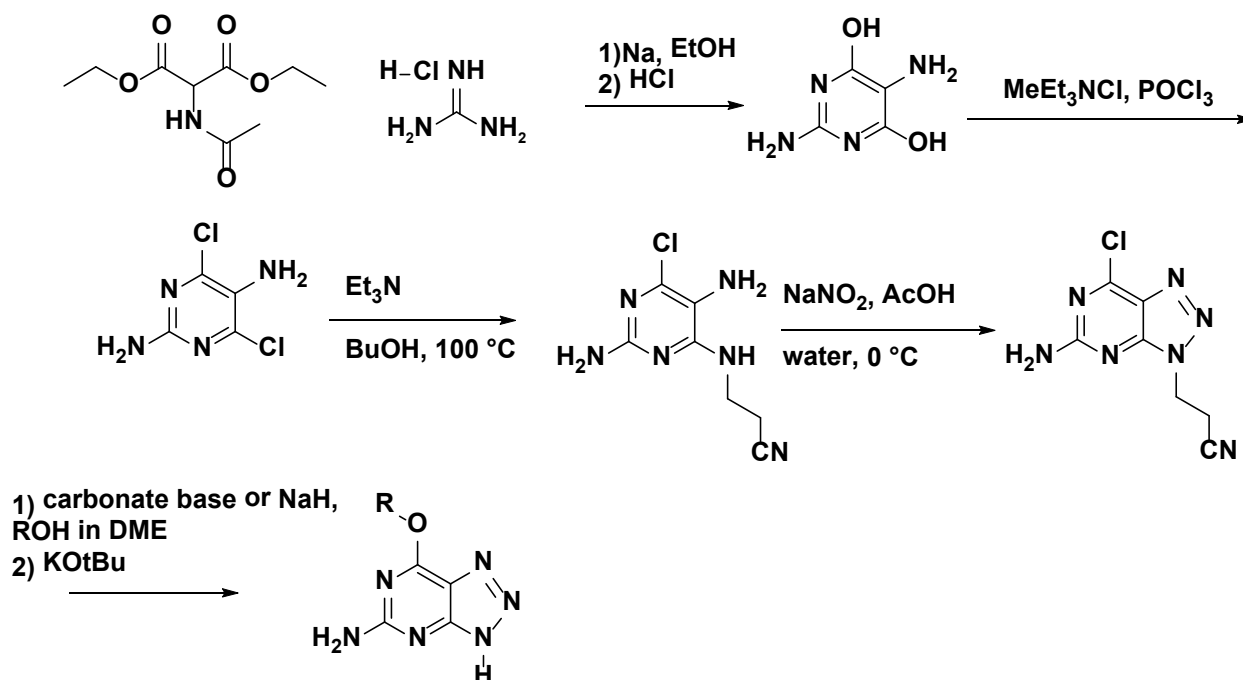


To a solution of 5-bromo-2-nitropyridine (0.47 g, 2.3 mmol) in DMF (7.7 mL) was added  $\text{Cs}_2\text{CO}_3$  (1.13 g, 3.47 mmol) at 0 °C followed by 2,4,5-trichlorophenol (0.50 g, 2.6 mmol) in DMF (7.7 mL). The solution was stirred at rt overnight. Water and EtOAc were added to the mixture and the organics were extracted from water with EtOAc. The organics were washed with water and



brine, then concentrated and purified by column chromatography (0-20% EtOAc/hexanes) to furnish 2-nitro-5-(2,4,5-trichlorophenoxy)pyridine (0.51 g, 1.60 mmol, 6) which was taken on directly. To a solution of 2-nitro-5-(2,4,5-trichlorophenoxy)pyridine (0.512 g, 1.60 mmol) in MeOH (16 mL) was added Pd/C (0.17 g, 0.16 mmol), and the mixture was blanketed under H<sub>2</sub> using a balloon. The reaction mixture was stirred overnight. The solution was filtered and concentrated under vacuum. The residue was purified by HPLC (10-90% ACN/water/0.1% TFA, Phenomenex AXIA 5u C18) to furnish 5-(2,4,5-trichlorophenoxy)pyridin-2-amine, TFA (0.047 g, 0.12 mmol, 5.2 % yield, two steps). <sup>1</sup>H NMR (400 MHz, CHLOROFORM-d) δ 7.91 (d, *J*=2.7 Hz, 1H), 7.53 (s, 1H), 7.19 (dd, *J*=8.8, 2.7 Hz, 1H), 6.86 (s, 1H), 6.55 (d, *J*=8.8 Hz, 1H), 4.45 (br s, 2H). MS(ESI) *m/z* 289.1 (M+H).

General Procedure to prepare triazolopyrimidine analogs.



To an ethanol (230 mL) solution was added sodium (2.65 g, 115 mmol) under Ar with stirring. After complete consumption of sodium, guanidine hydrochloride (11.0 g, 115 mmol) was added, and the solution was stirred for 10 min. This solution was heated to reflux and treated with diethyl acetamidomalonate (25.0 g, 115 mmol). After 2h, additional 460 mL of EtOH were added and heating to reflux was continued overnight. The mixture was cooled to rt. The mixture was filtered, and filtrate was washed with EtOH and  $\text{CHCl}_3$  to yield 21 g of intermediate acetamide. The intermediate solid was taken up in 60 mL of 6 N HCl and heated to 70 °C. After 2h the reaction mixture was cooled, and the solid was filtered followed by washing with 80 mL of 4N HCl. The resulting solid was dried in a vacuum oven at 80 °C overnight to furnish 2,5-diaminopyrimidine-4,6-diol, HCl (10 g, 56 mmol, 49 % yield). MS(ESI)  $m/z$  143.0 (M+H).

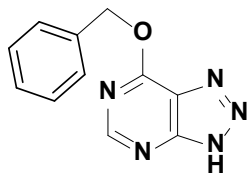
Triethylmethylammonium chloride (9.6 g, 63 mmol) and 2,5-diaminopyrimidine-4,6-diol, HCl (10 g, 56 mmol) were heated in  $\text{POCl}_3$  (13.6 mL, 146 mmol) to 105 °C overnight. The solution was concentrated (caution,  $\text{POCl}_3$  was neutralized in the collection bulb with 10 N NaOH solution), followed by addition of 200 mL water to the residue. The pH was adjusted to 4 (caution, EXOTHERM), and the solution was stirred for 1 h. The solution was adjusted to pH = 7, diluted with 1 L of EtOAc, and the solution was filtered through celite. The organic phase was separated,

passed through a silica plug and concentrated to furnish 4,6-dichloropyrimidine-2,5-diamine (2.55 g, 14.2 mmol, 25.4 % yield). <sup>1</sup>H NMR (500 MHz, DMSO-d<sub>6</sub>) δ 6.49 (s, 2H), 4.71 (s, 2H).

To a solution of 4,6-dichloropyrimidine-2,5-diamine (2.0 g, 11 mmol) and 3-aminopropionitrile (0.98 mL, 13 mmol) in butan-1-ol (45 mL) was added Et<sub>3</sub>N (1.6 mL, 11 mmol). The reaction mixture was heated to 100 °C and stirred overnight. The mixture was concentrated, and the volatiles were removed with MeOH azeotrope. The residue was carried forward as crude, 3-(2,5-diamino-6-chloropyrimidin-4-ylamino)propanenitrile (2.4 g, 11 mmol). MS(ESI) m/z 213.1 (M+H).

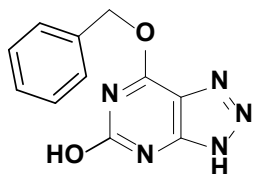
To a solution of 3-(2,5-diamino-6-chloropyrimidin-4-ylamino)propanenitrile (2.4 g, 11 mmol) in AcOH (9.3 mL)/water (28 mL) was added sodium nitrite (0.92 g, 13 mmol) in water (18 mL) at 0 °C. After 10 min, the reaction was complete by LCMS. The mixture was diluted with EtOAc and adjusted to pH = 6 using sat aq. NaHCO<sub>3</sub>. The organic layer was washed with brine, dried over Na<sub>2</sub>SO<sub>4</sub>, filtered and concentrated. The residue was purified by column chromatography (80-100% EtOAc/hexanes) to furnish 3-(5-amino-7-chloro-3H-[1,2,3]triazolo[4,5-d]pyrimidin-3-yl)propanenitrile (0.28 g, 1.2 mmol, 11 % yield). MS(ESI) m/z 239.1 (M+H).

To a solution of 3-(7-chloro-3H-[1,2,3]triazolo[4,5-d]pyrimidin-3-yl)propanenitrile (1 equiv.) and the benzyl alcohol starting material (1.5 equiv.) in DME (0.2 M reaction concentration) was added 3 equiv. of K<sub>2</sub>CO<sub>3</sub> or CsCO<sub>3</sub> or 2 equiv. of NaH. The reaction mixture was heated to 80 °C overnight. The reaction mixture was concentrated, and the residue purified by column chromatography (0-100% EtOAc/hexanes gradient) to furnish the appropriately substituted 3-(7-(benzyloxy)-3H-[1,2,3]triazolo[4,5-d]pyrimidin-3-yl)propanenitrile in 25-60 % yields. To a solution of the propane nitrile protected intermediate (1 equiv) in THF (1 mL) at 0 °C was added KOtBu (1 equiv). After 10 - 60 min, LCMS indicated formation of a single peak with product molecular ion mass. The reaction was quenched with AcOH (2 equiv), and then the mixture was concentrated. The residue was purified by prep HPLC to furnish the desired products in 5-70% yields.



7-(benzyloxy)-3H-[1,2,3]triazolo[4,5-d]pyrimidine (**8**).

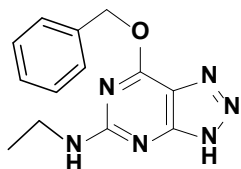
Prepared from 3-(7-chloro-3H-[1,2,3]triazolo[4,5-d]pyrimidin-3-yl)propanenitrile and benzyl alcohol by methods described in general procedure. <sup>1</sup>H NMR (500 MHz, DMSO-d<sub>6</sub>) δ 8.77 (s, 1H), 7.63 - 7.53 (m, 2H), 7.49 - 7.43 (m, 3H), 5.73 (s, 2H). MS(ESI) m/z 227.1 (M+H).



7-(benzyloxy)-3H-[1,2,3]triazolo[4,5-d]pyrimidin-5-ol (**9**)

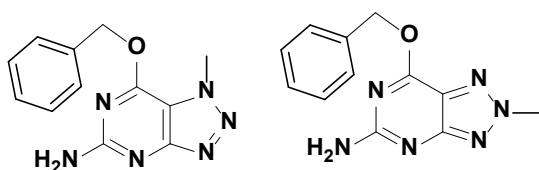
A suspension of 7-(benzyloxy)-3H-[1,2,3]triazolo[4,5-d]pyrimidin-5-amine, **6** (250 mg, 1.03 mmol) in acetone (4.5 mL) was poured into a solution of sodium nitrite (1.42 g, 20.6 mmol) in water (4.5 mL). Acetic acid (2.4 mL, 1.0 mmol) was added to the suspension with stirring. Minimum amounts of acetone were added as necessary to dissolve any suspended solid, and the solution was stirred for 5 h at rt. The acetone was removed by rotovap, and an orange precipitate formed in the aqueous solution. The precipitate was filtered from the solution, and washed with water. The light orange solid was dried on the high vacuum overnight to provide 7-(benzyloxy)-3H-[1,2,3]triazolo[4,5-d]pyrimidin-5-ol (139 mg, 0.557 mmol, 54% yield).

<sup>1</sup>H NMR (500 MHz, DMSO-d<sub>6</sub>) δ ppm 12.10 (1 H, br. s.), 7.52 (2 H, d, *J*=7.1 Hz), 7.32 - 7.47 (3 H, m), 5.56 (2 H, s). MS(ESI) m/z + 244.1 (M+H).



7-(benzyloxy)-N-ethyl-3H-[1,2,3]triazolo[4,5-d]pyrimidin-5-amine (**10**)

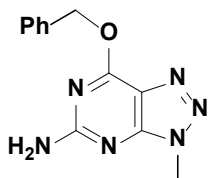
Dissolved excess acetaldehyde (40 mg) and 7-(benzyloxy)-3H-[1,2,3]triazolo[4,5-d]pyrimidin-5-amine (**6**, 0.05 g, 0.21 mmol) in MeOH (0.67 mL) with AcOH (0.067 mL), and a slightly opaque solution resulted. The mixture was heated to reflux and molecular sieves were added (~100 mg) followed by cooling to 40 °C. Sodium cyanoborohydride (0.052 g, 0.83 mmol) was added and the mixture was stirred overnight. The sieves were removed and washed with MeOH. The product was purified by reverse phase HPLC (10-90% MeOH in TFA buffered water, 30x75mm Phenomenex Axia Luna 5u column) to afford 5 mgs (0.018 mmol) of product. <sup>1</sup>H NMR (500 MHz, DMSO-*d*<sub>6</sub>) δ 7.55 (br d, *J*=7.2 Hz, 2H), 7.46 - 7.41 (m, 2H), 7.39 (d, *J*=7.2 Hz, 1H), 5.57 (br s, 2H), 3.34 (m, 2H), 1.15 (br t, *J*=6.7 Hz, 3H). MS(ESI) *m/z* + 271.1 (M+H).



Mixture of 7-(benzyloxy)-1-methyl-3H-[1,2,3]triazolo[4,5-d]pyrimidin-5-amine and 7-(benzyloxy)-2-methyl-2H-[1,2,3]triazolo[4,5-d]pyrimidin-5-amine (**11**)

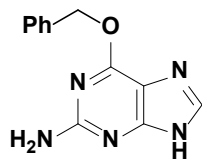
To a solution of 7-(benzyloxy)-3H-[1,2,3]triazolo[4,5-d]pyrimidin-5-amine (**6**, 0.050 g, 0.21 mmol) and potassium carbonate (0.031 g, 0.23 mmol) in DMF (2 mL) was added MeI (0.012 mL, 0.19 mmol) and the solution was allowed to stir overnight. The reaction was partitioned between EtOAc and 0.1M pH 7 phosphate buffer. The organic phase was washed with buffer, brine, and concentrated. The residue was dissolved in ACN and water, filtered and purified via preparative chromatography, 30x250 5u Phenomenex Luna column, 10-90% ACN / water (TFA buffer). The fraction were concentrated to furnish mixture of compounds **11** (20 mg, 0.027 mmol, 13% yield) and compound **12** (31 mg, 0.083 mmol, 40% yield).

Compound **11** <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) δ 7.56 - 7.37 (m, 10H), 5.67 (s, 2H), 5.63 (s, 2H), 4.38 (s, 3H), 4.32 (s, 3H). MS(ESI) *m/z* + 257.3 (M+H).



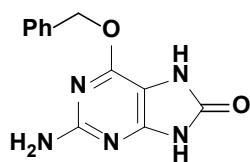
7-(benzyloxy)-3-methyl-3H-[1,2,3]triazolo[4,5-d]pyrimidin-5-amine (**12**)

$^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ )  $\delta$  7.51 (br d,  $J=7.2$  Hz, 2H), 7.43 - 7.30 (m, 3H), 5.69 – 5.52 (m, 4H), 4.10 (s, 3H). MS(ESI)  $m/z$  + 257.4 (M+H).



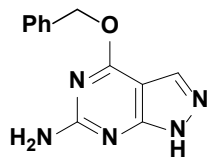
6-(benzyloxy)-9H-purin-2-amine (**13**).

Purchased from commercial sources.



2-amino-6-(benzyloxy)-7,9-dihydro-8H-purin-8-one (**14**).

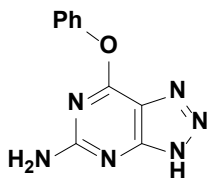
Phosgene in toluene (20% solution, 0.057 mL, 0.11 mmol) was added to a solution of 6-(benzyloxy)pyrimidine-2,4,5-triamine (25 mg, 0.11 mmol) in  $\text{CH}_2\text{Cl}_2$  (2 mL). The resulting mixture was stirred at rt for 2 h. The mixture was concentrated *in vacuo* and the residue was purified by reverse phase HPLC to yield Example 13 (0.68 mg, 1.69  $\mu\text{mol}$ , 1.6 % yield). MS (ESI)  $m/z$  258.4 (M+H) $^+$ .  $^1\text{H}$  NMR (500 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$  7.52 - 7.42 (m, 2H), 7.42 - 7.27 (m, 3H), 5.45 (s, 2H).



4-(benzyloxy)-1H-pyrazolo[3,4-d]pyrimidin-6-amine (**15**)

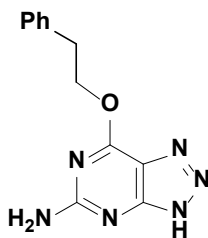
Cesium carbonate (120 mg, 0.35 mmol) was added to a mixture of 4-chloro-1H-pyrazolo[3,4-d]pyrimidin-6-amine (BIONET Supplier, 20 mg, 0.12 mmol) and phenylmethanol (0.061 mL, 0.59 mmol) in DMF (1 mL) and the mixture was heated in a microwave for 25 min at 200°C. The mixture was purified by reverse phase HPLC to yield Example 14 (0.96 mg, 2.7  $\mu\text{mol}$ , 2.3 %

yield). MS (ESI)  $m/z$  242.2 (M+H)<sup>+</sup>. <sup>1</sup>H NMR (500 MHz, DMSO- $d_6$ )  $\delta$  8.58 (s, 1H), 7.38 - 7.29 (m, 6H), 5.36 (s, 2H).



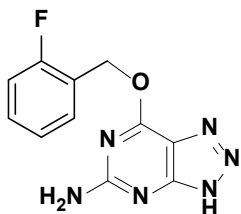
*7-phenoxy-3H-[1,2,3]triazolo[4,5-d]pyrimidin-5-amine (16)*

Prepared from 3-(5-amino-7-chloro-3H-[1,2,3]triazolo[4,5-d]pyrimidin-3-yl)propanenitrile and phenol as described in general procedure using NaH as base. The propanenitrile protecting group was removed under the reaction conditions. <sup>1</sup>H NMR (500 MHz, DMSO- $d_6$ )  $\delta$  7.55 - 7.44 (m, 2H), 7.38 - 7.27 (m, 3H), 6.94 (br d,  $J=2.2$  Hz, 2H). MS(ESI)  $m/z$  239.1 (M+H).



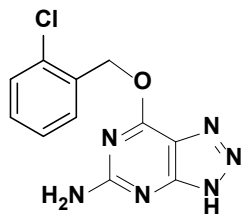
*7-phenethoxy-3H-[1,2,3]triazolo[4,5-d]pyrimidin-5-amine (17)*

Prepared from 3-(5-amino-7-chloro-3H-[1,2,3]triazolo[4,5-d]pyrimidin-3-yl)propanenitrile and 2-phenylethanol as described in general procedure using NaH as base. The propanenitrile protecting group was removed under the reaction conditions. <sup>1</sup>H NMR (500 MHz, DMSO- $d_6$ )  $\delta$  7.39 - 7.31 (m, 4H), 7.28 - 7.22 (m, 1H), 7.06 - 6.84 (m, 2H), 4.69 (t,  $J=7.2$  Hz, 2H), 3.15 (t,  $J=7.2$  Hz, 2H). MS(ESI)  $m/z$  257.0 (M+H).



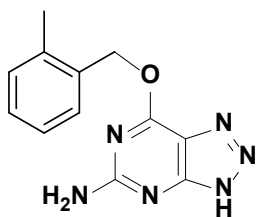
*7-((2-fluorobenzyl)oxy)-3H-[1,2,3]triazolo[4,5-d]pyrimidin-5-amine (18)*

Prepared from 3-(5-amino-7-chloro-3H-[1,2,3]triazolo[4,5-d]pyrimidin-3-yl)propanenitrile and (2-fluorophenyl)methanol as described in general procedure using  $K_2CO_3$ , followed by reaction with KOtBu in THF.  $^1H$  NMR (500 MHz, DMSO- $d_6$ )  $\delta$  7.69 (td,  $J=7.6, 1.7$  Hz, 1H), 7.52 - 7.42 (m, 1H), 7.34 - 7.22 (m, 2H), 7.04 (br d,  $J=3.3$  Hz, 2H), 5.62 (s, 2H). MS(ESI)  $m/z$  260.9 (M+H).



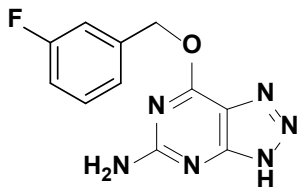
*7-((2-chlorobenzyl)oxy)-3H-[1,2,3]triazolo[4,5-d]pyrimidin-5-amine (19)*

Prepared from 3-(5-amino-7-chloro-3H-[1,2,3]triazolo[4,5-d]pyrimidin-3-yl)propanenitrile and (2-chlorophenyl)methanol as described in general procedure using  $K_2CO_3$ , followed by reaction with KOtBu in THF.  $^1H$  NMR (500 MHz, DMSO- $d_6$ )  $\delta$  7.76 - 7.66 (m, 1H), 7.56 (dd,  $J=7.4, 1.9$  Hz, 1H), 7.50 - 7.34 (m, 2H), 7.04 (br s, 2H), 5.72 - 5.57 (m, 2H). MS(ESI)  $m/z$  277.0 (M+H).



*7-((2-methylbenzyl)oxy)-3H-[1,2,3]triazolo[4,5-d]pyrimidin-5-amine (20)*

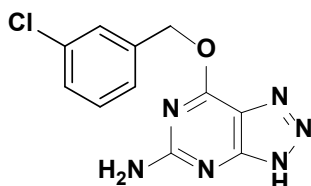
Prepared from 3-(5-amino-7-chloro-3H-[1,2,3]triazolo[4,5-d]pyrimidin-3-yl)propanenitrile and (2-methylphenyl)methanol as described in general procedure using  $K_2CO_3$ , followed by reaction with KOtBu in THF. MS(ESI)  $m/z$  257.1 (M+H).



*7-((3-fluorobenzyl)oxy)-3H-[1,2,3]triazolo[4,5-d]pyrimidin-5-amine (21)*

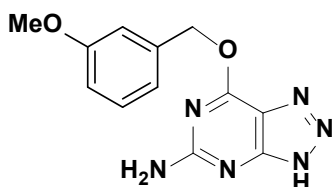


Prepared from 3-(5-amino-7-chloro-3H-[1,2,3]triazolo[4,5-d]pyrimidin-3-yl)propanenitrile and (3-fluorophenyl)methanol as described in general procedure using  $K_2CO_3$ , followed by reaction with KOtBu in THF.  $^1H$  NMR (500 MHz, DMSO- $d_6$ )  $\delta$  7.51 - 7.44 (m, 1H), 7.43 - 7.37 (m, 2H), 7.26 - 7.17 (m, 1H), 7.05 - 6.98 (m, 1H), 7.08 - 6.96 (m, 1H), 7.11 - 6.93 (m, 1H), 5.58 (s, 2H). MS(ESI) m/z 260.9 (M+H).



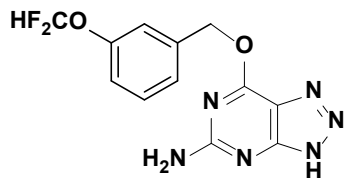
*7-((3-chlorobenzyl)oxy)-3H-[1,2,3]triazolo[4,5-d]pyrimidin-5-amine (22)*

Prepared from 3-(5-amino-7-chloro-3H-[1,2,3]triazolo[4,5-d]pyrimidin-3-yl)propanenitrile and (3-chlorophenyl)methanol as described in general procedure using  $K_2CO_3$ , followed by reaction with KOtBu in THF.  $^1H$  NMR (400 MHz, ACETONITRILE- $d_3$ )  $\delta$  7.54 (s, 1H), 7.48 - 7.30 (m, 3H), 6.95 - 6.33 (m, 2H), 5.56 (s, 2H). MS(ESI) m/z 275.3 (M+H).



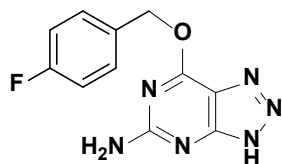
*7-((3-methoxybenzyl)oxy)-3H-[1,2,3]triazolo[4,5-d]pyrimidin-5-amine (23)*

Prepared from 3-(5-amino-7-chloro-3H-[1,2,3]triazolo[4,5-d]pyrimidin-3-yl)propanenitrile and (3-methoxyphenyl)methanol as described in general procedure using  $K_2CO_3$  followed by reaction with KOtBu in THF. MS(ESI) m/z 273.2 (M+H).



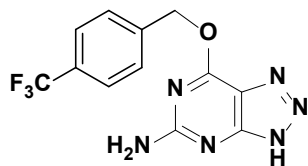
*7-((3-(difluoromethoxy)benzyl)oxy)-3H-[1,2,3]triazolo[4,5-d]pyrimidin-5-amine (24)*

Prepared from 3-(5-amino-7-chloro-3H-[1,2,3]triazolo[4,5-d]pyrimidin-3-yl)propanenitrile and (3-(difluoromethoxy)phenyl)methanol as described in general procedure using  $K_2CO_3$  followed by reaction with KOtBu in THF.  $^1H$  NMR (500 MHz, ACETONITRILE- $d_3$ )  $\delta$  7.56 - 7.43 (m, 2H), 7.38 (s, 1H), 7.19 (br d,  $J=7.7$  Hz, 1H), 7.02 - 6.64 (m, 1H), 5.70 - 5.60 (m, 2H). MS(ESI)  $m/z$  309.1 (M+H).



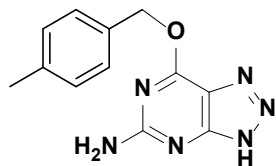
7-((4-fluorobenzyl)oxy)-3H-[1,2,3]triazolo[4,5-d]pyrimidin-5-amine (25)

Prepared from 3-(5-amino-7-chloro-3H-[1,2,3]triazolo[4,5-d]pyrimidin-3-yl)propanenitrile and (4-fluorophenyl)methanol as described in general procedure using  $K_2CO_3$  followed by reaction with KOtBu in THF.  $^1H$  NMR (500 MHz, DMSO- $d_6$ )  $\delta$  7.63 (dd,  $J=8.7, 5.6$  Hz, 2H), 7.25 (t,  $J=8.9$  Hz, 2H), 7.01 (br s, 2H), 5.54 (s, 2H). MS(ESI)  $m/z$  261.0 (M+H)



7-((4-(trifluoromethyl)benzyl)oxy)-3H-[1,2,3]triazolo[4,5-d]pyrimidin-5-amine (26)

Prepared from 3-(5-amino-7-chloro-3H-[1,2,3]triazolo[4,5-d]pyrimidin-3-yl)propanenitrile and (4-(trifluoromethyl)phenyl)methanol as described in general procedure using  $K_2CO_3$  followed by reaction with KOtBu in THF.  $^1H$  NMR (500 MHz, DMSO- $d_6$ )  $\delta$  8.01 - 7.67 (m, 4H), 7.24 - 6.85 (m, 2H), 5.67 (s, 2H). MS(ESI)  $m/z$  310.9 (M+H).



7-((4-methylbenzyl)oxy)-3H-[1,2,3]triazolo[4,5-d]pyrimidin-5-amine (27)

Prepared from 3-(5-amino-7-chloro-3H-[1,2,3]triazolo[4,5-d]pyrimidin-3-yl)propanenitrile and (4-methylphenyl)methanol as described in general procedure using  $K_2CO_3$  followed by reaction with KOtBu in THF.  $^1H$  NMR (500 MHz, DMSO- $d_6$ )  $\delta$  7.44 (d,  $J=8.0$  Hz, 2H), 7.23 (d,  $J=8.0$  Hz, 2H), 7.00 (br s, 2H), 5.52 (s, 2H), 2.33 (s, 3H). MS(ESI) m/z 257.0 (M+H)

## References

1. Lor, L. A.; Schneck, J.; McNulty, D. E.; Diaz, E.; Brandt, M.; Thrall, S. H.; Schwartz, B. *J. Biomolecular Screening* **2007**, *6*, 881.
2. Burner, U.; Obinger, C.; Paumann, M.; Furtmuller, P. G.; Kettle, A. J. *J Biol Chem* **1999**, *274*, 9494.
3. Shin, K.; Hayasawa, H.; Lonnerdal, B. *Biochem Biophys Res Commun* **2000**, *3*, 831.
4. Kritharides, L.; Christian, A.; Stoudt, G.; Morel, D.; Rothblat, G. H. *Arterioscler Thromb Vasc Biol* **1998**, *18*, 1589.
5. Nauseef, W. M. *Immunol Rev* **2007**, *219*, 88.
6. S. Gross, S. T. Gammon, B. L. Moss, D. Rauch, J. Harding, J. W. Heinecke, L. Ratner and D. Piwnica Worms, *Nat Med*, **2009**, *15*, 455.
7. Brennan, M. L.; Anderson, M. M.; Shih, D. M.; Qu, X. D.; Wang, X.; Mehta, A. C.; Lim, L. L.; Shi, W.; Hazen, S. L.; Jacob, J. S.; Crowley, J. R.; Heinecke, J. W.; Luscis, A. J. *J Clin Invest* **2001**, *107*, 419.
8. Otwinowski, Z.; Minor, W. *Methods Enzymol* **1997**, *276*, 307.
9. McCoy, A. J.; Grosse-Kunstleve, R. W.; Adams, P. D.; Winn, M. D.; Storoni, L. C.; Read, R. J. *J Appl Crystallogr* **2007**, *40*, 658.
10. Emsley, P.; Lohkamp, B.; Scott, W. G.; Cowtan, K. *Acta Crystallogr D Biol Crystallogr* **2010**, *66*, 486.
11. Laskowski, R. A.; Moss, D. S.; Thornton, J. M. *J Mol Biol* **1993**, *231*, 1049.
12. Chae, M. Y.; Swenn, K.; Kanugula, S.; Dolan, M. E.; Pegg, A. E.; Moschel, R. C. *J Med Chem* **1995**, *38*, 359.