Supplementary Information

Small molecule microarray identifies inhibitors of tyrosyl-DNA phosphodiesterase 1 that simultaneously access the catalytic pocket and two substrate binding sites

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I. BIOLOGICAL EVALUATION

1. TDP1 Labelling using Alexa Fluor 647 (AF647) C2 Maleimide dye kit. Protein labelling was carried out using a similar procedure as previous previously described.[1] Briefly, Human TDP1 (148-608) (50 μL, 33 mg/mL)[2] in a buffer solution including Tris-HCl (pH 7.2, 25 mM), NaCl (150 mM), and tris(2-carboxyethyl)phosphine hydrochloride (TCEP, 2 mM) was diluted by adding phosphate-buffered saline (PBS) with 0.01% Tween-20 (PBST, 268 μL). To the diluted TDP1 (148-608) (318 μL, 0.1 mM), TCEP (3.2 μL, 100 mM in PBST buffer) and Alexa Fluor 647 C2 Maleimide dye kit (AF-647, ThermoFisher, Cat# A20347, 9.5 μL, 10 mM in DMSO) was added and incubated in the dark at 4°C overnight. Unreacted dye was quenched by the addition of DTT (32 μL, 100 mM in TBST buffer). The labelled protein was purified by gel filtration through a Sephadex PD-10 column (GE Life Sciences, Cat# 17-0851-01) which was pre-equilibrated with PBST buffer. The purified solution was dialyzed with PBST buffer (4 mL, 2×) using a 10 kDa molecular weight cut-off (MWCO) filter (Sigma-Aldrich, Cat# Z648027). The concentration of the final protein solution was determined by absorbance at 280 nm. Labeling and purity were assessed using absorbance measurements collected on a NanoDrop 2.0 Spectrophotometer (NanoDrop, 2000/2000C). AF-647 labelled TDP1 (148-608) (TDP1-AF647, 188 μL, 137.5 μM, labelling ratio: 0.87) was afforded. The AF647 labelled TDP1 solution (103 μL, 137.5 μM; Labelling ratio: 0.87) can be diluted in PBST buffer with 0.1% BSA and filtered through a sterilized syringe filter (Argos, Cat# FM32S) to afford a TDP1-AF647 solution (1 μM) for next step use.

2. Small Molecule Microarray Screening. Small molecule microarray screening was carried out as previously described.[3-5] Briefly, γ-aminopropyl silane (GAPS) microscope slides were functionalized with a short Fmoc-protected amino polyethylene glycol spacer. After

deprotection using piperidine, 1,6-diisocyanatohexane was coupled to the surface by urea bond formation to provide functionalized isocyanate-coated microarray slides that can react with primary alcohols and amines to form immobilized chemical screening libraries. A total of 20 000 unique small molecule stock solutions (10 mM in DMSO) purchased from ChemBridge and ChemDiv screening libraries, in addition to dyes and controls, were printed in duplicate onto four slides of 5000 compounds each and exposed to pyridine vapor to facilitate covalent attachment to the slide surface. After drying, slides were incubated with a polyethylene glycol solution to quench unreacted isocyanate surface. Microarray slides were pre-scanned using Fluorescence Scanner (InnoScan 1100AL) and their imagines were saved as TIFF files. Their barcodes were recorded. Microarray slides were ready to future use.

The microarray slides were incubated with blocking buffer 1% BSA/PBST (PBS with 0.01% Tween-20, 5 mL) in a Nunc 4-well Rectangular Tray (Thermo Scientific, Cat# 267061) and wrapped in aluminum foil with gentle shaking on a VWR Rocking Platform (3 rpm, 30 min, rt). The slides were washed by PBST buffer with 0.1% BSA by shaking (2 min, 3×) followed by TDP1-AF647 (1 μM, 3 mL/slide) was added to the slides. The slides were incubated by shaking on a VWR Rocking Platform (1.5 h, rt) and then washed by PBST (2 min, 3×) and deionized water followed by centrifugation (2600 rpm, 4 min, 25 ºC) to dry. Fluorescence of the small molecule screening slides were immediately measured (650 nm excitation, 670 nm emission) using a Microarray Fluorescence Scanner (MAPIX, InnoScan 1100 AL) and analyzed for hits by comparison to a slide incubated with buffer alone using software (SMM Analyzer). Hits were identified on the basis of signal-to-noise ratio (SNR),[6] defined as mean foreground − mean background/standard deviation of background, and Z-score, with the following criteria: (1) Raw SNR > 0, (2) SNR > 3, SD above negative control readings, (3) coefficient of variance (CV) of

replicate spots. Lead compounds (Z scores >3) are picked out. Totally 109 hits were found. 37 of them were tested by TDP1 *in vitro* assay (Table S1).

3. TDP1 Gel-based *in vitro* assay. TDP1 Gel-based *in vitro* assay was carried out as previously described.[2] 5′-Cy5-labeled DNA substrate (1 nM; N14Y; 5′-GATCTAAAAGACTT-pY-3′) was incubated with 10 pM recombinant TDP1 in the absence or presence of inhibitor (at concentrations ranging from 20 nM to 10 mM) for 15 min at room temperature in a buffer containing 50 mM Tris HCl, pH 7.5, 80 mM KCl, 2 mM EDTA, 1 mM DTT, 40 µg/ml BSA and 0.01% Tween-20. Reactions were terminated by addition of 1 volume of gel loading buffer [99.5% (v/v) formamide, 5 mM EDTA]. Samples were subjected to a 16% denaturing PAGE and gels were exposed after drying to a PhosphorImager screen (GE Healthcare). Gel images were scanned using a Typhoon FLA 9500 scanner (GE Healthcare) and densitometric analyses were performed using the ImageQuant software (GE Healthcare). Results for evaluation of analogues based imidazo[*1,2-a*]pyrazin-3-amine core are shown in Table S2 (page S51). Results for evaluation of imidazo[1,2-*a*]pyridin-3-amines are shown in Table 1.

4. TDP1 Lineweaver–Burk analysis (LB plot). To determine the kinetic parameters for the inhibition of TDP1 by **7b**, **8a**, **10a**, and **10b**, 400 nM of recombinant human TDP1 enzyme was incubated with 40 nM of labeled DNA substrate (CY5N14Y) with 0, 0.08, 0.2, 0.4, 0.8, or 1.2 µM of unlabeled DNA substrate (N14Y) in the presence or absence of the inhibitors in a final volume of 10 µL in 1 × LMP 1 reaction buffer (50 mM Tris-HCl, pH 7.5, 80 mM KCl, 2 mM EDTA, 1 mM DTT, 40 µg/mL BSA, 0.01% Tween 20). The reactions were carried out at room temperature for 0.25, 0.5, 1, 1.5, 3, 6, or 10 min and terminated by adding 1 volume of 2 × stop buffer (99.5% formamide, 10 mM EDTA). Samples were then analyzed with a 20% DNA sequencing gel and exposed to a PhosphorImager screen for further analysis by Typhoon FLA

9500 (GE Healthcare). The Lineweaver-Burk plots (Fig. S3) were generated based on the substrate concentrations and corresponding reaction velocities.

Table S1. SMM Hits and their Z scores. Selective hits (red) have been evaluated by gel-based TDP1 assay *in vitro.*

Commercial Resources and Catalogue Numbers	**Z score**
SMM Plate 1	
ChemDiv I (11 hits)	
4281-1757	5.75
5610-0620	4.03
6212-0075	4.18
3546-0609	3.11
4075-3330	3.37
4236-0434	3.56
4550-5935	5.08
5228-5358	3.08
5921-0208	3.49
6212-0056	6.27
6228-0205	4.52
SMM Plate 2	
ChemDiv II (3 hits)	
8012-8096	3.67
8014-8888	3.35
8451-16608	3.14
SMM Plate 3	

ChemDiv III (21 hits)	
C738-0015	8.98
C748-1353	7.73
C748-1355	4.45
C748-1397	3.16
C908-0409	3.82
C908-0431	5.49
C908-0490	3.24
C908-0491	3.96
C908-0520	4.53
C908-0547	4.35
C908-0549	6.68
D072-0526	3.05
D150-0324	3.41
D401-0782	7.84
D433-0001	3.94
E205-0046	4.51
C748-0155	4.69
C908-0545	5.09
D401-0754	3.07
D665-0492	3.78
D724-0943	4.40
SMM Plate 4	

ChemBridge I (22 hits)	
12918323	3.59
32314043	3.33
6682831	3.44
14734502	4.78
14850921	3.47
19469566	3.85
20313006	3.22
22611838	4.43
22827550	3.01
27185534	4.40
32635251	6.06
43033449	3.18
46417061	3.93
47504462	3.70
47913504	3.04
48264882	4.30
13949594	4.58
14655077	3.15
14744708	5.55
18402936	3.84
31891223	3.65
SMM Plate 5	

ChemBridge II (42 hits)	
13533883	3.72
17026190	3.56
33741474	4.18
14330381	3.58
14787340	3.11
15026584	3.82
15287123	3.06
16982874	3.38
18382016	4.01
18890687	3.36
19068090	4.22
32487380	4.37
12318921	3.23
13257468	3.89
15652090	7.19
17204632	3.35
18027827	3.32
33632022	3.50
41509658	4.21
46192145	4.07
13174251	4.53
16759064	5.02

17464509	5.84
18325829	3.19
19535702	3.27
22589337	3.01
26909894	6.56
31804073	3.06
42927908	4.48
58433631	3.06
13507134	3.32
14506204	6.37
14734744	3.75
15063357	3.32
21687324	4.02
27208483	4.48
33419523	3.36
40096953	3.45
42266387	4.99
12553104	4.36
14668002	3.38
60244866	3.44
SMM Plate 6	
Mipe FDA Clinical Drugs (3 + 6 hits)	

21725	3.66
36693	3.89
265372	3.60
NCGC00015482-05	3.65
NCGC00024631-01	3.14
NCGC00095838-03	3.18
NCGC00095899-04	3.12
NCGC00168108-02	3.54
NCGC00345022-02	3.60

Note: A total of 109 hits were found from SMM of a library of 21,000 “drug-like” compounds printed in 6 plates (0.5% hits to library). Based on the Z values of their SMM images and commercial availability, 37 hits were chosen (show red) for further analysis in gel-based TDP1 assay.

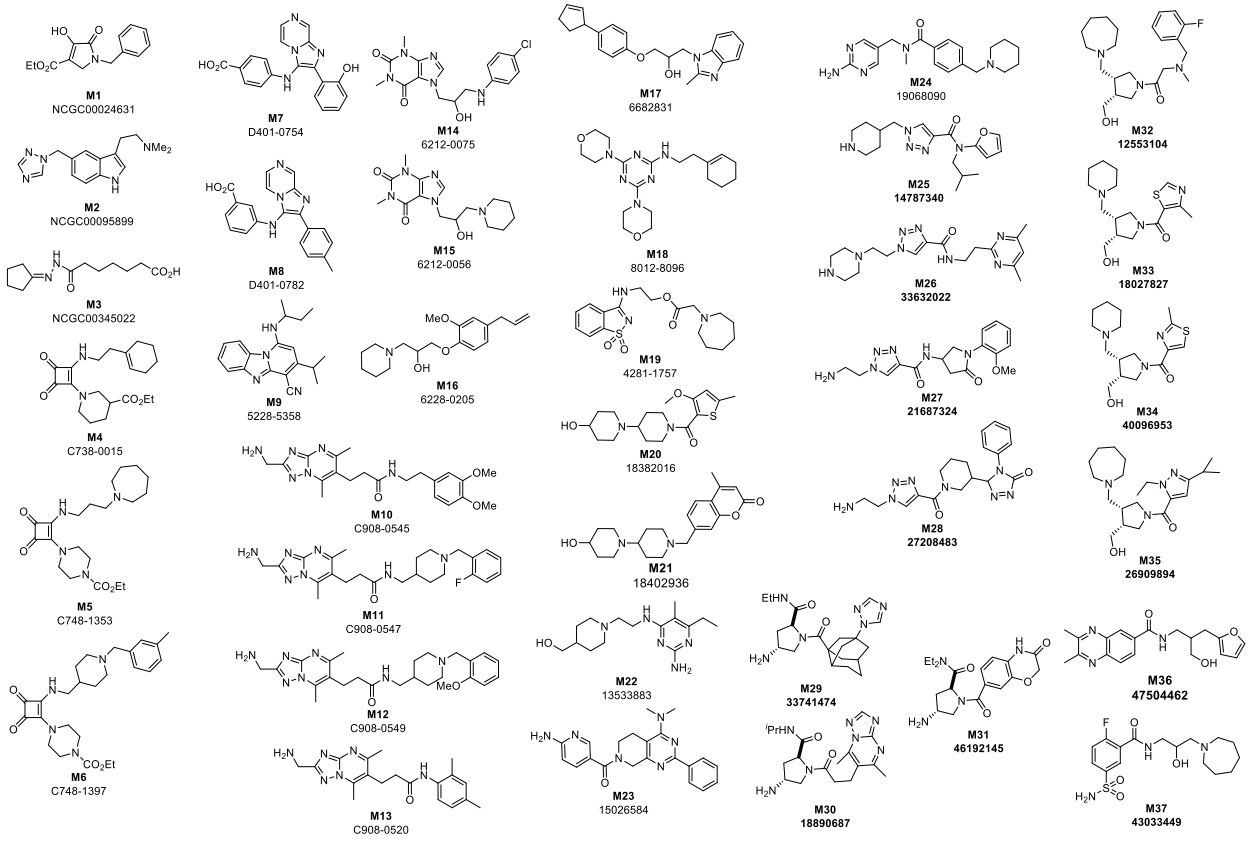


Figure S1. Structures of SMM lead compounds (37 compounds) which have been evaluated by gel-based TDP1 assays *in vitro*.

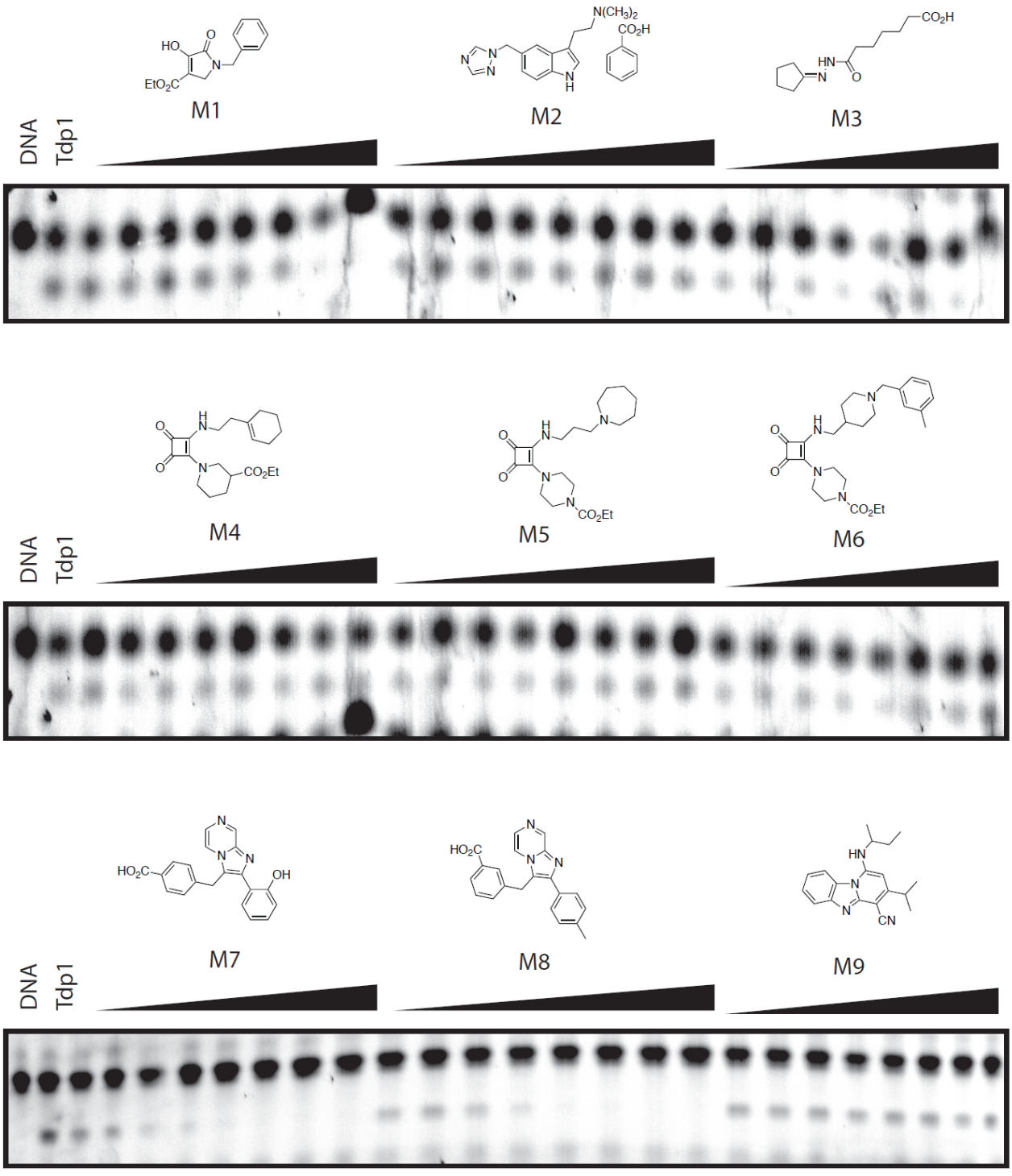


Figure S2. Gel images of the SMM lead compounds evaluated by TDP1 assays *in vitro*.

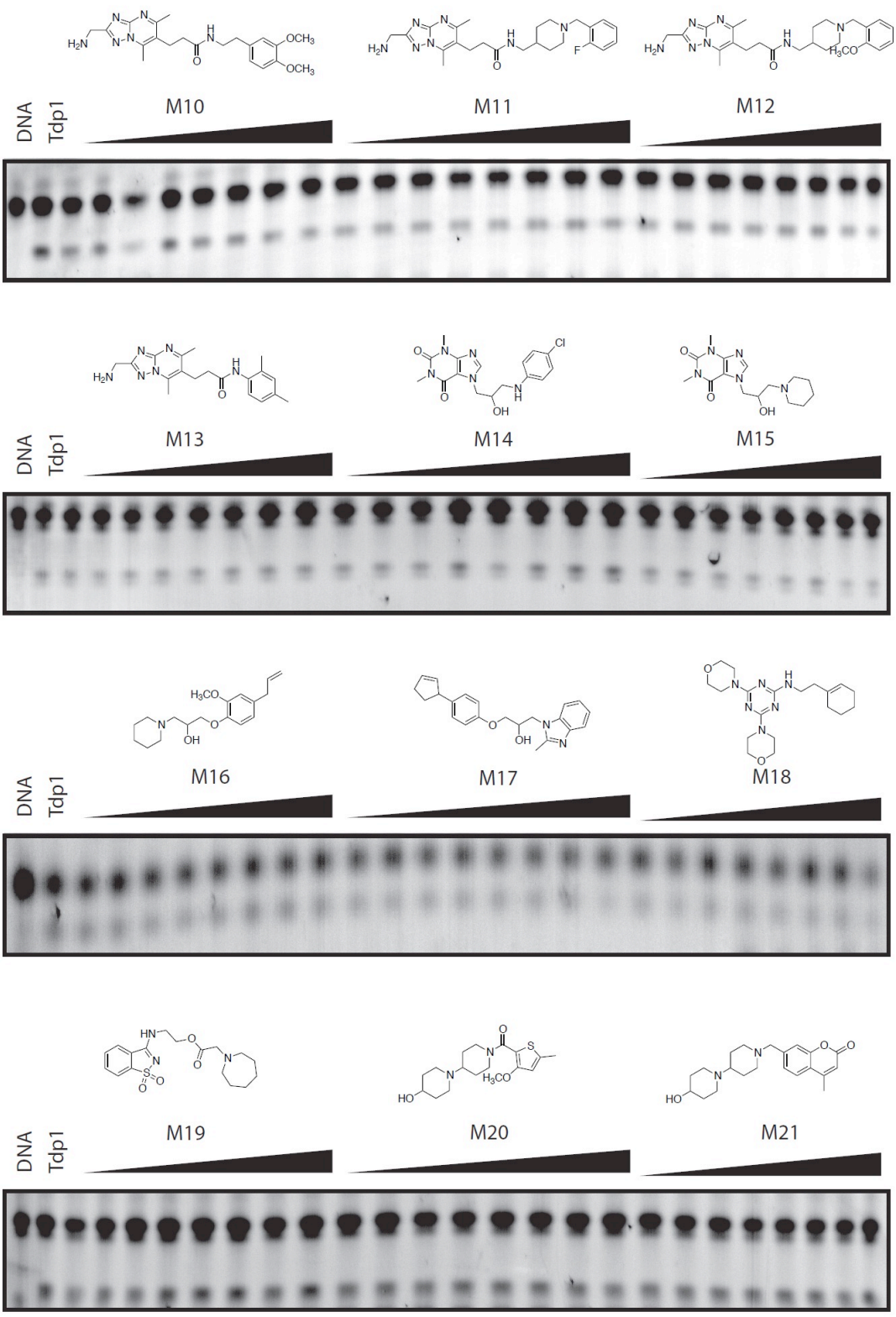


Figure S2 (cont.). Gel images of the SMM lead compounds evaluated by TDP1 assays *in vitro*.

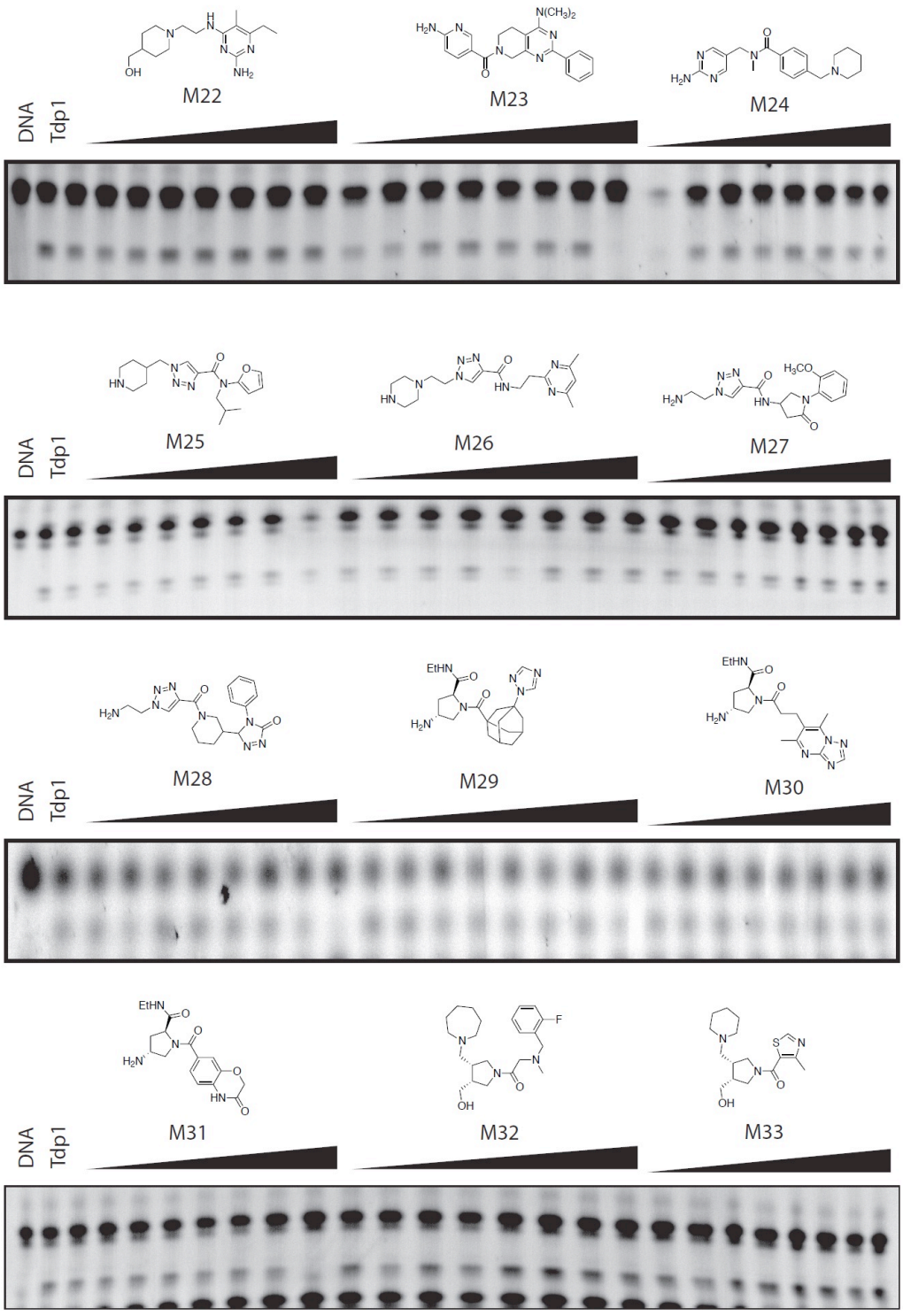


Figure S2 (cont.). Gel images of the SMM lead compounds evaluated by TDP1 assays *in vitro*.

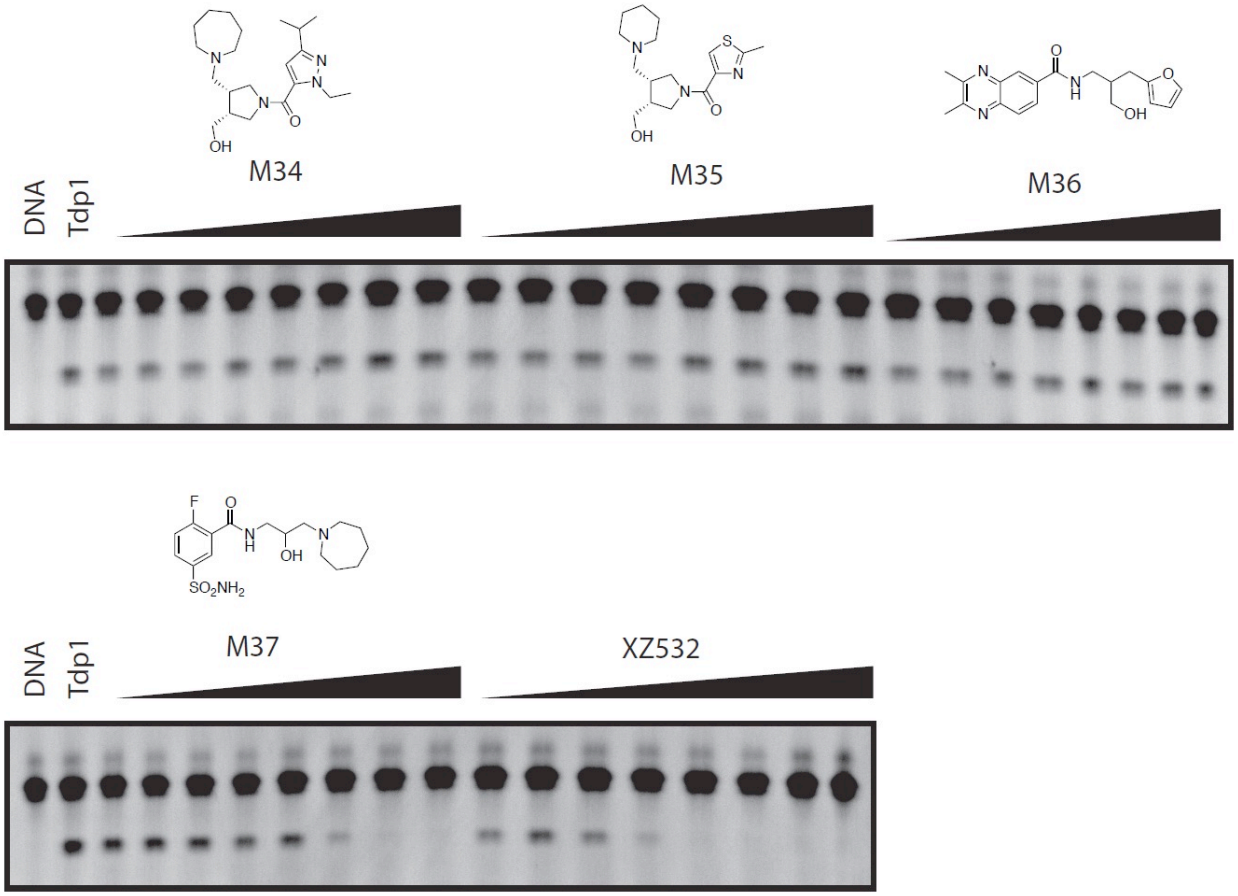


Figure S2 (cont.). Gel images of the SMM lead compounds evaluated by TDP1 assays *in vitro*.

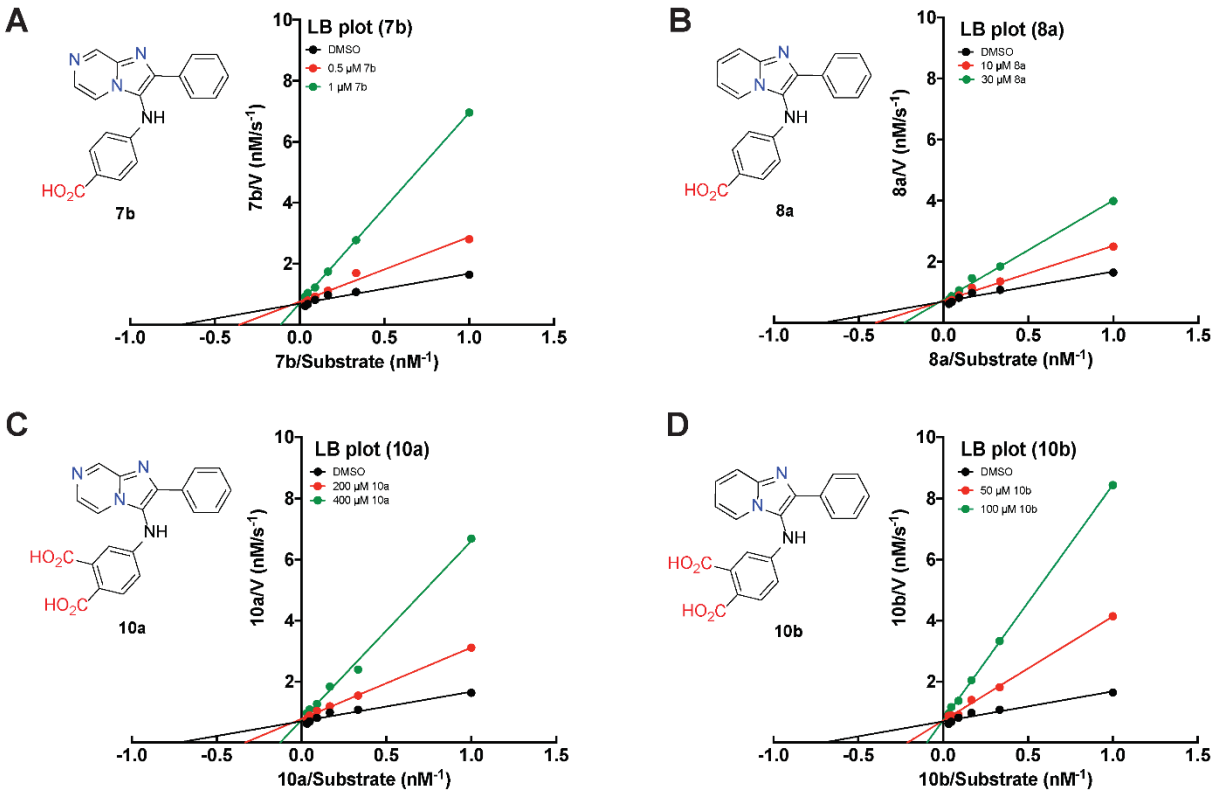
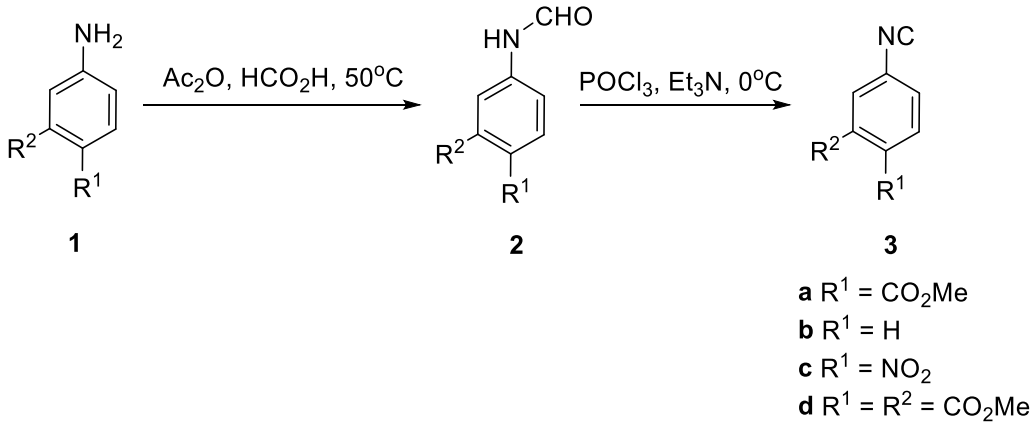


Figure S3. Lineweaver–Burk analysis (LB plot) of **7b, 8a, 10a, 10b** with TDP1. (A) Inhibitory constant of **7b**, K_i = 0.309 ± 0.122 μM. (B) Inhibitory constant of **8a**, K_i = 12.49 ± 0.49 μM. (C) Inhibitory constant of **10a**, K_i = 109.9 ± 30.9 μM. (D) Inhibitory constant of **10b**, K_i = 17.40 ± 2.80 μM.

II. SYNTHETIC PROCEDURES

1. General Procedures. Proton (^{1}H) and carbon (^{13}C) NMR spectra were recorded on a Varian 400 MHz spectrometer or a Varian 500 MHz spectrometer and are reported in ppm relative to TMS and referenced to the solvent in which the spectra were collected. Solvent was removed by rotary evaporation under reduced pressure, and anhydrous solvents were obtained commercially and used without further drying. Purification by silica gel chromatography was performed using Combiflash with EtOAc−hexanes solvent systems. Preparative high pressure liquid chromatography (HPLC) was conducted using a Waters Prep LC4000 system having

photodiode array detection and Phenomenex C18 columns (catalogue no. 00G4436-P0-AX, 250 mm × 21.2 mm 10 μm particle size, 110 Å pore) at a flow rate of 10 mL/min or 20 mL/min. Binary solvent systems consisting of A = 0.1% aqueous TFA and B = 0.1% TFA in acetonitrile were employed with gradients as indicated. Products were obtained as amorphous solids following lyophilization. Electrospray ionization-mass spectrometric (ESI-MS) were acquired with an Agilent LC/MSD system equipped with a multimode ion source. Purities of samples subjected to biological testing were assessed using this system and shown to be ≥95%. High resolution mass spectrometric (HRMS) were acquired by LC/MS-ESI using LTQ-Orbitrap-XL at 30K resolution.



Scheme S1. Synthesis of substituted isocyanobenzenes (**3a-d**).

2. General procedure A for the synthesis of substituted formamidobenzenes (2).[7, 8] A mixture of formic acid (15 mL, 407 mmol) and acetic anhydride (35 mL, 370 mmol) was stirred (55 °C, 2 h) and then cooled to rt to afford acetic formic anhydride (approximatley 3 equiv.) *in situ*. The mixture was added dropwise to a solution of substituted aminobenzene (**1**, 123 mmol) in THF (40 mL) at 0 °C and the reaction mixture was stirred (rt, 2 h). The solvent was evaporated and the solid was collected by filtrate and washed by hexanes to afford formamidobenzenes (**2**).

3. General procedure B for the synthesis of substituted isocyanobenzenes (3).[7, 8] To a solution of substituted formamidobenzene (**2**, 52 mmol) and triethylamine (21 mL, 157 mmol) in THF (50 mL) was added phosphoryl trichloride (5.9 mL, 63 mmol) dropwise at 0 °C over 1 h. The mixture was quenched by the addition of saturated aqueous Na_2CO_3 at 0 °C. The mixture was extracted by DCM and the organic phase was washed by brine, dried (Na_2SO_4), filtered and concentrated. The residue was purified by CombiFlash silica gel chromatography with solid loading. The final substituted isocyanobenzenes (**3**) were afforded.

3.1 Methyl 4-formamidobenzoate (2a). Treatment of methyl 4-aminobenzoate (**1a**) as outlined in general procedure A provided methyl 4-formamidobenzoate (**2a**) as a white solid (94 % yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.55 (s, 1H), 8.36 (d, J = 1.7 Hz, 1H), 7.94 (d, J = 8.7 Hz, 2H), 7.72 (d, J = 8.6 Hz, 1H), 3.83 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 166.20, 160.63, 142.97, 130.87 (2C), 124.82, 119.11 (2C), 52.39. DUIS-MS m/z: 180.0 (MH^+). ESI-MS m/z: 180.1 (MH^+).

3.2 N-Phenylformamide (2b). Treatment of aniline (**1b**) as outlined in general procedure A provided N-phenylformamide (**2b**) as a white solid (95 % yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.17 (s, 1H), 8.28 (d, J = 2.0 Hz, 1H), 7.60 (d, J = 7.2 Hz, 2H), 7.31 (t, J = 7.9 Hz, 2H), 7.07 (t, J = 7.4 Hz, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 160.02, 129.84, 129.30 (2C), 124.04, 119.59 (2C). ESI-MS m/z: 122.1 (MH^+), 144.0 (MNa^+), 243.1 (M_2H^+).

3.4 N-(4-Nitrophenyl)formamide (2c). Treatment of 4-nitroaniline (**1c**) as outlined in general procedure A provided N-(4-nitrophenyl)formamide (**2c**) as a yellow solid (98% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.70 (s, 1H), 8.32 (s, 1H), 8.17 (d, J = 8.9 Hz, 2H), 7.73 (d, J = 8.8 Hz, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 161.35, 144.08, 143.16, 125.42 (2C), 119.74 (2C). ESI-MS m/z: 167.0 (MH^+).

3.5 Dimethyl 4-formamidophthalate (2d). Treatment of dimethyl 4-aminophthalate (**1d**) as outlined in general procedure A provided dimethyl 4-formamidophthalate (**2d**) as white solid (94 % yield). ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.66 (s, 1H), 8.37 (s, 1H), 7.95 (s, 1H), 7.78 (s, 2H), 3.82 (s, 3H), 3.80 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 168.11, 166.76, 160.88, 141.69, 134.25, 130.93, 125.18, 121.06, 118.62, 53.14, 52.91. ESI-MS m/z: 238.1 (MH^+).

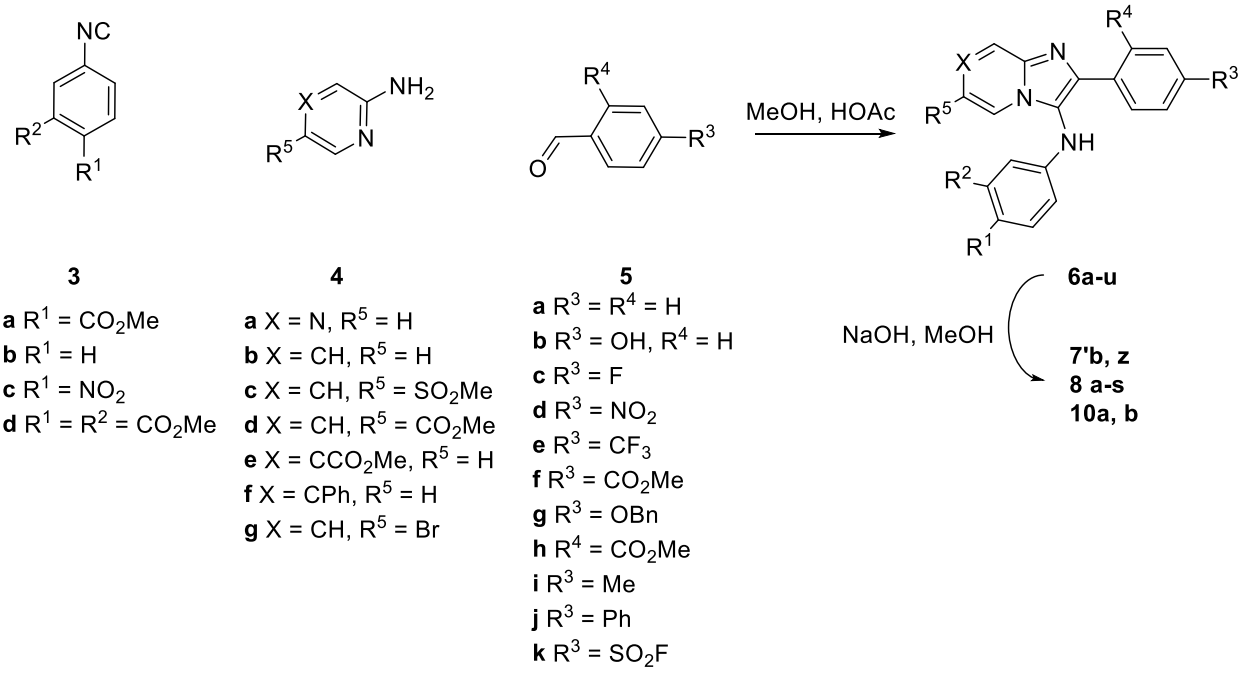
4. Preparation of substituted isocyanobenzenes (3).

4.1 Methyl 4-isocyanobenzoate (3a). Treatment of methyl 4-formamidobenzoate (**2a**) as outlined in general procedure B provided methyl 4-isocyanobenzoate (**3a**) as a white solid (88 % yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.05 (d, J = 8.6 Hz, 2H), 7.42 (d, J = 8.5 Hz, 2H), 3.91 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 167.19, 165.39, 130.90, 130.80 (2C), 126.43 (3C), 52.52.

4.2 Isocyanobenzene (3b). Treatment of N-phenylformamide (**2b**) as outlined in general procedure B (as reported in the literature[8, 9]) provided isocyanobenzene (**3b**) as a brown oil, which turned to green and dark green during pump drying (80 % yield).

4.3 1-Isocyano-4-nitrobenzene (3c). Treatment of N-(4-nitrophenyl)formamide (**2c**) as outlined in general procedure B provided 1-isocyano-4-nitrobenzene (**3c**) as a brown oil (98% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.24 (d, J = 8.9 Hz, 2H), 7.52 (d, J = 8.8 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.69, 147.52, 127.57 (2C), 125.08 (2C), 119.44.

4.4 Dimethyl 4-isocyanophthalate (3d). Treatment of dimethyl 4-formamidophthalate (**2d**) as outlined in general procedure B provided dimethyl 4-formamidophthalate (**3d**) as a brown oil (97 % yield). ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.95 (s, 1H), 7.87 (s, 2H), 3.85 (s, 3H), 3.84 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 167.55, 166.48, 166.14, 133.47, 132.42, 130.98, 129.93, 128.30, 127.17, 53.47, 53.42. ESI-MS m/z: 220.1 (MH^+).



Scheme S2. Synthesis of substituted imidazo[1,2-a]pyrazines **7'(b, z)**, **10a**, and imidazo[1,2-a]pyridines **8 (a-s), 10b**.

5. General procedure C for the synthesis of imidazo[1,2-a]pyrazines and imidazo[1,2-a]pyridines (6) by heating. Pyrizan-2-amines (**4**, 2 mmol), aldehydes (**5**, 2 mmol), and isonitrile (**3**, 2 mmol) (freshly prepared according to the literature[7-9]) were mixed in MeOH (2.0 mL), trimethyl orthoformate (TMOF, 2.0 mL) or dioxane (5.0 mL) and the mixture was heated and stirred. The final mixture was purified by HPLC to provide final imidazo[*1,2-a*]pyrazines (**6**).

6. General procedure D for the synthesis of imidazo[1,2-a]pyrazines and imidazo[1,2-a]pyridines (6). [10, 11] A solution of pyrizan-2-amines or pyridine-2-amines (**5**, 6 mmol), aldehydes (**4**, 6 mmol) and acetic acid (12 mmol) in MeOH (10 mL) was stirred (rt, 20 min) and then isonitrile (**3**, 6 mmol) was added and the rreaction mixture was stirred (rt, 24 h). The resulting suspension was collected by filteration and washed (hexanes and H_2O). The solid

product was collected and purified by HPLC to provide final imidazo[*1,2-a*]pyrazines or imidazo[*1,2-a*]pyridines (**6**).

7. General procedure E for the hydrolysis of esters (6) to prepare acids (7-10). Esters (**6**, 2 mmol) were mixed with sodium hydroxide (2 mL, 2N, 4 mmol) in MeOH (2 mL) and THF (2 mL) and the mixture was stirred (rt overnight). The reaction mixture was carefully adjusted to pH 3 using HCl (2N) to precipitate product out of solution. The resulting suspended solid was collected by filtration and the solid was washed (hexanes and H_2O) and dried. The final acids (**7-10**) were afforded by HPLC purification.

7.1 Methyl 4-((2-phenylimidazo[1,2-a]pyrazin-3-yl)amino)benzoate (6a). Treatment of pyrazin-2-amine (**4a**), benzaldehyde (**5a**), and methyl 4-isocyanobenzoate (**3a**) as outlined in general procedure D provided methyl 4-((2-phenylimidazo[1,2-a]pyrazin-3-yl)amino)benzoate (**6a**) as a grey solid (39% yield). ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.14 (d, J = 1.5 Hz, 1H), 9.05 (s, 1H), 8.07 (dd, J = 4.6, 1.5 Hz, 1H), 8.03 (dd, J = 8.3, 1.3 Hz, 2H), 7.92 (d, J = 4.5 Hz, 1H), 7.78 (d, J = 9.0 Hz, 2H), 7.44 (t, J = 7.6 Hz, 2H), 7.35 (t, J = 7.3 Hz, 1H), 6.61 (d, J = 8.0 Hz, 2H), 3.76 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 166.46, 149.87, 143.58, 140.12, 137.86, 133.02, 131.89 (2C), 129.94, 129.18 (2C), 128.93, 127.19 (2C), 120.34, 119.57, 116.93, 113.31 (2C), 52.03. ESI-MS m/z: 345.1 (MH^+).

7.2 Ethyl 4-((2-(4-fluorophenyl)imidazo[1,2-a]pyrazin-3-yl)amino)benzoate (6b). Treatment of pyrazin-2-amine (**4a**), 4-fluorobenzaldehyde (**5c**) and methyl 4-isocyanobenzoate (**3a**) as outline in general procedure C (dioxane, 50 °C, 3 h) and purification by HPLC (linear gradient of 20% B to 45% B over 20 min with a flow rate 20 mL/min; retention time = 14.1 min) provided methyl 4-((2-(4-fluorophenyl)imidazo[1,2-a]pyrazin-3-yl)amino)benzoate (**6b**) as a yellow solid (4.5% yield). ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.19 (d, J = 1.5 Hz, 1H), 9.09 (s,

1H), 8.10 (dd, *J* = 4.6, 1.5 Hz, 1H), 8.06 (dd, *J* = 8.7, 5.7 Hz, 2H), 7.94 (d, *J* = 4.6 Hz, 1H), 7.78 (d, *J* = 8.9 Hz, 2H), 7.30 (t, *J* = 8.9 Hz, 2H), 6.62 (d, *J* = 8.1 Hz, 2H), 3.76 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 166.44, 162.67 (d, *J* = 246.3 Hz), 149.59, 143.04, 139.59, 137.64, 131.88 (2C), 129.36 (d, *J* = 5.1 Hz), 129.34 (2C), 129.31 (2C, d, *J* = 8.1 Hz), 120.49, 119.74, 117.26, 116.24 (2C, d, *J* = 21.5 Hz), 113.40, 52.04. ESI-MS m/z: 363.1 (MH^+).

7.3 Methyl 4-((2-phenylimidazo[1,2-a]pyridin-3-yl)amino)benzoate (6c). Treatment of pyridin-2-amine (**4b**), benzaldehyde (**5a**), and methyl 4-isocyanobenzoate (**3a**) as outlined in general procedure D provided 4-((2-phenylimidazo[1,2-a]pyridin-3-yl)amino)benzoate (**6c**) as a white solid (41% yield). ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.90 (s, 1H), 8.01 (d, *J* = 6.9 Hz, 2H), 7.97 (d, *J* = 6.9 Hz, 1H), 7.77 (d, *J* = 8.5 Hz, 2H), 7.66 (d, *J* = 9.0 Hz, 1H), 7.40 (t, *J* = 7.7 Hz, 2H), 7.34 (ddd, *J* = 9.1, 6.7, 1.3 Hz, 1H), 7.29 (t, *J* = 7.3 Hz, 1H), 6.94 (td, *J* = 6.7, 1.2 Hz, 1H), 6.58 (brs, 2H), 3.76 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 166.50, 150.59, 142.48, 138.07, 133.87, 131.92 (2C), 129.00 (2C), 128.15, 126.88 (3C), 125.86, 123.49, 119.93, 117.97, 117.71, 113.02 (2C), 51.98. ESI-MS m/z: 344.1 (MH^+).

7.4 Methyl 4-((2-(p-tolyl)imidazo[1,2-a]pyridin-3-yl)amino)benzoate (6d). Treatment of pyridin-2-amine (**4b**), 4-methylbenzaldehyde (**5i**), and methyl 4-isocyanobenzoate (**3a**) as outlined in general procedure D provided methyl 4-((2-(p-tolyl)imidazo[1,2-a]pyridin-3-yl)amino)benzoate (**6d**) as a yellow solid (47% yield). ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.86 (s, 1H), 7.96 (d, *J* = 6.7 Hz, 1H), 7.90 (d, *J* = 8.2 Hz, 2H), 7.76 (d, *J* = 9.3 Hz, 2H), 7.63 (d, *J* = 9.1 Hz, 1H), 7.32 (ddd, *J* = 9.1, 6.7, 1.3 Hz, 1H), 7.20 (d, *J* = 7.9 Hz, 2H), 6.93 (td, *J* = 6.7, 1.1 Hz, 1H), 6.56 (brs, 2H), 3.75 (s, 3H), 2.29 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 166.50, 150.64, 142.40, 138.22, 137.48, 131.90 (2C), 131.07, 129.57 (2C), 126.81 (2C), 125.71, 123.41, 119.86, 117.58, 117.57, 112.99, 112.90 (2C), 51.97, 21.28. ESI-MS m/z: 358.2 (MH^+).

7.5 Methyl 4-((2-(4-(benzyloxy)phenyl)imidazo[1,2-a]pyridin-3-yl)amino)benzoate (6e). Treatment of pyridin-2-amine (**4b**), 4-(benzyloxy)benzaldehyde (**5g**), methyl 4-isocyanobenzoate (**3a**) as outlined in general procedure D provided methyl 4-((2-(4-(benzyloxy)phenyl)imidazo[1,2-a]pyridin-3-yl)amino)benzoate (**6e**) as a white solid (80% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.84 (s, 1H), 7.94 (dd, J = 7.5, 4.0 Hz, 3H), 7.78 (d, J = 8.5 Hz, 2H), 7.63 (d, J = 8.9 Hz, 1H), 7.45 (d, J = 7.4 Hz, 2H), 7.39 (t, J = 7.3 Hz, 2H), 7.34 – 7.30 (m, 2H), 7.05 (d, J = 8.2 Hz, 2H), 6.93 (t, J = 6.7 Hz, 1H), 6.57 (s, 2H), 5.12 (s, 2H), 3.77 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 166.52, 158.48, 150.69, 142.39, 138.13, 137.46, 131.93 (2C), 128.89 (2C), 128.30 (2C), 128.18 (3C), 126.60, 125.62, 123.36, 119.87, 117.47, 117.00, 115.35 (2C), 112.98 (2C), 112.82, 69.63, 51.98. ESI-MS m/z: 450.2 (MH^+).

7.6 Methyl 4-((2-(4-(trifluoromethyl)phenyl)imidazo[1,2-a]pyridin-3-yl)amino)benzoate (6f). Treatment of pyridin-2-amine (**4b**), 4-(trifluoromethyl)benzaldehyde (**5e**), and methyl 4-isocyanobenzoate (**3a**) as outlined in general procedure D provided methyl 4-((2-(4-(trifluoromethyl)phenyl)imidazo[1,2-a]pyridin-3-yl)amino)benzoate (**6f**) as a pale yellow solid (21 % yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.98 (s, 1H), 8.22 (d, J = 8.1 Hz, 2H), 8.01 (d, J = 6.8 Hz, 1H), 7.79 (d, J = 7.7 Hz, 4H), 7.70 (d, J = 9.1 Hz, 1H), 7.39 (ddd, J = 9.1, 6.8, 1.3 Hz, 1H), 6.98 (td, J = 6.8, 1.2 Hz, 1H), 6.61 (s, 2H), 3.76 (s, 3H).

7.7 Methyl 4-((2-(4-hydroxyphenyl)imidazo[1,2-a]pyridin-3-yl)amino)benzoate (6g). Treatment of pyridin-2-amine (**4b**), 4-hydroxybenzaldehyde (**5b**), and methyl 4-isocyanobenzoate (**3a**) as outlined in general procedure D provided methyl 4-((2-(4-hydroxyphenyl)imidazo[1,2-a]pyridin-3-yl)amino)benzoate (**6g**) as a white solid (50% yield). ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.56 (s, 1H), 8.81 (s, 1H), 7.91 (d, J = 6.8 Hz, 1H), 7.82 (d, J = 8.7 Hz, 2H), 7.77 (d, J = 8.7 Hz, 2H), 7.60 (d, J = 9.0 Hz, 1H), 7.31 – 7.28 (m, 1H), 6.90 (td, J =

6.7, 1.1 Hz, 1H), 6.77 (d, *J* = 8.7 Hz, 2H), 6.55 (s, 2H), 3.76 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 166.52, 157.67, 150.76, 142.29, 138.62, 131.90 (2C), 128.30 (2C), 125.40, 124.81, 123.26, 119.78, 117.34, 116.54, 115.77 (2C), 112.95 (2C), 112.66, 51.96. ESI-MS m/z: 360.2 (MH^+).

7.8 Methyl 4-(3-((4-(methoxycarbonyl)phenyl)amino)imidazo[1,2-a]pyridin-2-yl)benzoate (6h). Treatment of pyridin-2-amine (**4b**), methyl 4-formylbenzoate (**5f**) and methyl 4-isocyanobenzoate (**3a**) as outlined in general procedure D provided methyl 4-(3-((4-(methoxycarbonyl)phenyl)amino)imidazo[1,2-a]pyridin-2-yl)benzoate (**6h**) as a white solid (28% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.98 (s, 1H), 8.16 (d, *J* = 8.1 Hz, 2H), 7.99 (t, *J* = 6.5 Hz, 3H), 7.78 (d, *J* = 8.4 Hz, 2H), 7.69 (d, *J* = 9.1 Hz, 1H), 7.40 – 7.36 (m, 1H), 6.98 (t, *J* = 6.8 Hz, 1H), 6.61 – 6.59 (m, 2H), 3.85 (s, 3H), 3.76 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 166.47, 166.42, 150.21, 142.70, 138.46, 136.68, 131.92 (2C), 129.93 (2C), 128.82, 126.85 (2C), 126.40, 123.71, 120.15, 119.30, 117.92, 113.37, 113.15 (2C), 52.56, 52.00. ESI-MS m/z: 402.2 (MH^+).

7.9 Methyl 4-(3-(phenylamino)imidazo[1,2-a]pyridin-2-yl)benzoate (6i). Treatment of pyridin-2-amine (**4b**), methyl 4-formylbenzoate (**5f**), and isocyanobenzene (**3b**) as outlined in general procedure D provided methyl 4-(3-(phenylamino)imidazo[1,2-a]pyridin-2-yl)benzoate (**6i**) as a white solid (64% yield). ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.33 (s, 1H), 8.21 (d, *J* = 8.6 Hz, 2H), 7.98 – 7.96 (m, 3H), 7.66 (d, *J* = 9.0 Hz, 1H), 7.34 (ddd, *J* = 9.1, 6.7, 1.3 Hz, 1H), 7.15 (t, 2H, *J* = 9.0 Hz), 6.94 (td, *J* = 6.7, 1.1 Hz, 1H), 6.74 (t, *J* = 7.3 Hz, 1H), 6.52 (d, *J* = 7.3 Hz, 2H), 3.84 (s, 3H).^{13}C NMR (126 MHz, DMSO-d_6) δ 166.48, 145.67, 142.46, 138.79, 136.53, 130.03 (2C), 129.85 (2C), 128.62, 126.84 (2C), 126.09, 123.74, 120.76, 119.20, 117.84, 113.49, 113.04 (2C), 52.54. ESI-MS m/z: 344.2 (MH^+).

7.10 Methyl 6-(4-(methoxycarbonyl)phenyl)-5-oxo-5,6-dihydropyrido[2',1':2,3]imidazo[4,5-c]isoquinoline-9-carboxylate (6j).[12] Treatment of methyl 6-aminonicotinate (**4d**), methyl 2-formylbenzoate (**5h**) and methyl 4-isocyanobenzoate (**3a**) as outlined in general procedure C (MeOH, Biotage Initiator, microwave irradiation, 85 °C, 3 h) and provided methyl 6-(4-(methoxycarbonyl)phenyl)-5-oxo-5,6-dihydropyrido[2',1':2,3]imidazo[4,5-c]isoquinoline-9-carboxylate (**6j**) as a brown solid (5.0 % yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.56 (d, *J* = 7.4 Hz, 1H), 8.49 (d, *J* = 7.8 Hz, 1H), 8.41 (d, *J* = 8.5 Hz, 2H), 7.92 (ddd, *J* = 8.1, 7.2, 1.3 Hz, 1H), 7.71 – 7.62 (m, 5H), 7.40 (s, 1H), 4.05 (s, 3H), 3.75 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.84, 164.47, 161.34, 143.10, 139.40, 133.87, 131.97, 131.73, 131.64 (2C), 129.69, 129.30 (2C), 127.98, 127.03, 126.45, 124.70, 123.55, 123.26, 122.34, 117.64, 116.06, 52.64, 52.33. ESI-MS m/z: 428.1 (MH^+), 450.1 (MNa^+).

7.11 Methyl 2-phenyl-3-(phenylamino)imidazo[1,2-a]pyridine-6-carboxylate (6k). Treatment of methyl 6-aminonicotinate (**4d**), benzaldehyde (**5a**), and isocyanobenzene (**3b**) as outlined in general procedure D provided methyl 2-phenyl-3-(phenylamino)imidazo[1,2-a]pyridine-6-carboxylate (**6k**) as a pale yellow solid (54% yield). ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.33 (s, 1H), 8.21 (d, *J* = 8.6 Hz, 2H), 7.98 – 7.96 (m, 3H), 7.66 (d, *J* = 9.0 Hz, 1H), 7.34 (ddd, *J* = 8.9, 6.7, 1.1 Hz, 1H), 7.14 (t, *J* = 7.9 Hz, 2H), 6.99 – 6.92 (m, 1H), 6.74 (t, *J* = 7.3 Hz, 1H), 6.52 (d, *J* = 7.2 Hz, 2H), 3.84 (s, 3H).^{13}C NMR (126 MHz, DMSO-d_6) δ 165.22, 145.62, 142.66, 139.55, 133.39, 130.14 (2C), 129.04 (2C), 128.51, 127.06 (2C), 126.77, 124.50, 120.57, 119.43, 117.51, 115.88, 113.51 (2C), 52.90. ESI-MS m/z: 344.1 (MH^+).

7.12 Methyl 3-((4-nitrophenyl)amino)-2-phenylimidazo[1,2-a]pyridine-6-carboxylate (6l). Treatment of methyl 6-aminonicotinate (**4d**), benzaldehyde (**5a**), and 1-isocyano-4-nitrobenzene (**3c**) as outlined in general procedure D and purification by preparative HPLC

(linear gradient of 10% B to 40% B over 20 min with a flow rate 20 mL/min; retention time = 12.6 min) provided methyl 3-((4-nitrophenyl)amino)-2-phenylimidazo[1,2-a]pyridine-6-carboxylate (**6l**) as a pale brown solid (18% yield). ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.45 (s, 1H), 8.55 (s, 1H), 8.08 (d, J = 8.8 Hz, 2H), 8.00 (dd, J = 8.2, 1.2 Hz, 2H), 7.78 (t, J = 1.5 Hz, 2H), 7.43 (t, J = 7.7 Hz, 2H), 7.35 (t, J = 7.4 Hz, 1H), 6.73 (brs, 2H), 3.86 (s, 3H). ESI-MS m/z: 389.1 (MH^+).

7.13 Methyl 3-((4-(methoxycarbonyl)phenyl)amino)-2-phenylimidazo[1,2-a]pyridine-6-carboxylate (6m). Treatment of methyl 6-aminonicotinate (**4d**), benzaldehyde (**5a**), and methyl 4-isocyanobenzoate (**3a**) as outlined in general procedure D provided methyl 3-((4-(methoxycarbonyl)phenyl)amino)-2-phenylimidazo[1,2-a]pyridine-6-carboxylate (**6m**) as a green solid (47% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.98 (s, 1H), 8.51 (s, 1H), 8.02 – 8.00 (m, 2H), 7.79 – 7.7 (m, 4H), 7.41 (t, J = 7.6 Hz, 2H), 7.32 (t, J = 6.8 Hz, 1H), 6.53 (s, 2H), 3.84 (s, 3H), 3.76 (s, 3H). ESI-MS m/z: 402.2 (MH^+).

7.14 Methyl 4-((6-bromo-2-phenylimidazo[1,2-a]pyridin-3-yl)amino)benzoate (6n). treatment of 5-bromopyridin-2-amine (**4g**), benzaldehyde (**5a**), and methyl 4-isocyanobenzoate (**3a**) as outlined in general procedure D provided methyl 4-((6-bromo-2-phenylimidazo[1,2-a]pyridin-3-yl)amino)benzoate (**6n**) as a pale yellow solid (76 % yield). ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.88 (s, 1H), 8.25 – 8.21 (m, 1H), 7.98 (d, J = 7.0 Hz, 2H), 7.78 (d, J = 8.5 Hz, 2H), 7.66 (d, J = 9.5 Hz, 1H), 7.46 (dd, J = 9.5, 2.0 Hz, 1H), 7.40 (t, J = 7.7 Hz, 2H), 7.30 (t, J = 7.4 Hz, 1H), 6.60 (brs, 2H), 3.76 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 166.48, 150.19, 140.94, 138.81, 133.36, 131.86 (2C), 129.06 (2C), 128.80, 128.44, 126.92 (2C), 123.33, 120.16, 118.97, 118.60, 113.22 (2C), 107.10, 52.00. ESI-MS m/z: 422.1, 424.1 (MH^+).

7.15 Methyl 4-((2,7-diphenylimidazo[1,2-a]pyridin-3-yl)amino)benzoate (6o). Treatment of 4-phenylpyridin-2-amine (**4f**), benzaldehyde (**5a**), and methyl 4-isocyanobenzoate (**3a**) as outlined in general procedure D provided methyl 4-((2,7-diphenylimidazo[1,2-a]pyridin-3-yl)amino)benzoate (**6o**) as a white solid (49 % yield). ^{1}H NMR (500 MHz, C*DCl$_3$*) δ 8.01 – 7.97 (m, 2H), 7.95 (d, *J* = 9.0 Hz, 2H), 7.87 (s, 1H), 7.85 (d, *J* = 7.1 Hz, 1H), 7.67 (d, *J* = 7.0 Hz, 2H), 7.51 (t, *J* = 7.5 Hz, 2H), 7.44 (t, *J* = 7.4 Hz, 1H), 7.39 (t, *J* = 7.5 Hz, 2H), 7.34 – 7.31 (m, 1H), 7.10 (dd, *J* = 7.1, 1.8 Hz, 1H), 6.66 (d, *J* = 8.4 Hz, 2H), 6.08 (brs, 1H), 3.89 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 166.84, 148.88, 143.33, 138.48, 132.97, 132.04 (2C), 130.85, 129.16 (2C), 128.72 (2C), 128.43, 128.21, 127.02 (2C), 126.79 (2C), 126.47, 122.46, 121.83, 116.50, 114.49, 112.86 (2C), 112.53, 51.81. ESI-MS m/z: 420.2 (MH^+).

7.16 Methyl 4-((6-(methylsulfonyl)-2-phenylimidazo[1,2-a]pyridin-3-yl)amino)benzoate (6p). Treatment of 5-(methylsulfonyl)pyridin-2-amine (**4c**), benzaldehyde (**5a**), and methyl 4-isocyanobenzoate (**3a**) as outlined in general procedure D provided methyl 4-((6-(methylsulfonyl)-2-phenylimidazo[1,2-a]pyridin-3-yl)amino)benzoate (**6p**) as a green solid (10% yield). ^{1}H NMR (500 MHz, DMSO-*d$_6$*) δ 9.03 (s, 1H), 8.49 (d, *J* = 2.0 Hz, 1H), 8.02 (d, *J* = 7.3 Hz, 2H), 7.90 (d, *J* = 9.5 Hz, 1H), 7.79 (d, *J* = 8.5 Hz, 2H), 7.75 (dd, *J* = 9.5, 1.9 Hz, 1H), 7.43 (t, *J* = 7.6 Hz, 2H), 7.34 (t, *J* = 7.3 Hz, 1H), 6.67 (brs, 2H), 3.77 (s, 3H), 3.34 (s, 3H). ^{13}C NMR (126 MHz, DMSO-*d$_6$*) δ 166.46, 149.88, 142.29, 140.03, 132.94, 131.94 (2C), 129.17 (2C), 128.85, 127.54, 127.11 (2C), 125.50, 122.53, 120.46, 119.90, 118.44, 113.36 (2C), 52.04, 44.01.

7.17 Methyl 4-((2-([1,1'-biphenyl]-4-yl)-6-(methylsulfonyl)imidazo[1,2-a]pyridin-3-yl)amino)benzoate (6q). Treatment of 5-(methylsulfonyl)pyridin-2-amine (**4c**), (1,1'-biphenyl)-4-carbaldehyde (**5j**), and methyl 4-isocyanobenzoate (**3a**) as outlined in general procedure D provided methyl 4-((2-([1,1'-biphenyl]-4-yl)-6-(methylsulfonyl)imidazo[1,2-a]pyridin-3-

yl)amino)benzoate (**6q**) as a white solid (27 % yield). ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.08 (s, 1H), 8.50 (dd, J = 2.0, 0.9 Hz, 1H), 8.12 (d, J = 8.6 Hz, 2H), 7.91 (dd, J = 9.4, 0.9 Hz, 1H), 7.81 (d, J = 8.6 Hz, 2H), 7.78 – 7.74 (m, 3H), 7.71 (dd, J = 8.3, 1.3 Hz, 2H), 7.46 (t, J = 7.7 Hz, 2H), 7.39 – 7.34 (m, 1H), 6.70 (s, 2H), 3.76 (s, 3H), 3.34 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 166.46, 149.88, 142.38, 140.33, 139.85, 139.73, 132.01, 131.97 (2C), 129.43 (2C), 128.12, 127.63 (2C), 127.56, 127.35 (2C), 127.02 (2C), 125.50, 122.60, 120.52, 120.00, 118.42, 113.40 (2C), 52.04, 44.03. ESI-MS m/z: 498.1 (MH^+).

7.18 Methyl 3-((4-(methoxycarbonyl)phenyl)amino)-2-phenylimidazo[1,2-a]pyridine-7-carboxylate (6r). Treatment of methyl 2-aminoisonicotinate (**4e**), benzaldehyde (**5a**), and methyl 4-isocyanobenzoate (**3a**) as outlined in general procedure D provided methyl 3-((4-(methoxycarbonyl)phenyl)amino)-2-phenylimidazo[1,2-a]pyridine-7-carboxylate (**6r**) as a green solid (95% yield). ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.05 (s, 1H), 8.23 (s, 1H), 8.09 (d, J = 7.1 Hz, 1H), 8.02 (d, J = 7.1 Hz, 2H), 7.78 (d, J = 8.5 Hz, 2H), 7.43 (t, J = 7.6 Hz, 2H), 7.38 (dd, J = 7.0, 1.7 Hz, 1H), 7.34 (t, J = 7.2 Hz, 1H), 6.61 (s, 2H), 3.91 (s, 3H), 3.76 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 166.45, 165.59, 150.03, 141.23, 140.70, 133.22, 131.93 (2C), 129.13 (2C), 128.72, 127.07 (2C), 126.26, 123.77, 120.24, 119.86, 119.69, 113.21 (2C), 111.70, 53.07, 52.02. ESI-MS m/z: 402.2 (MH^+).

7.19 Dimethyl 4-((2-phenylimidazo[1,2-a]pyrazin-3-yl)amino)phthalate (6s). Treatment of pyrazin-2-amine (**4a**), benzaldehyde (**5a**), and dimethyl 4-isocyanophthalate (**3d**) as outlined in general procedure D provided dimethyl 4-((2-phenylimidazo[1,2-a]pyrazin-3-yl)amino) (**6s**) as a white solid (21% yield). ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.15 (s, 1H), 9.14 (d, J = 1.5 Hz, 1H), 8.11 (dd, J = 4.6, 1.5 Hz, 1H), 8.03 (d, J = 7.1 Hz, 2H), 7.92 (d, J = 4.6 Hz, 1H), 7.66 (d, J = 8.6 Hz, 1H), 7.45 (t, J = 7.6 Hz, 2H), 7.36 (t, J = 7.3 Hz, 1H), 6.78 (brs, 1H), 6.62 (brs, 1H), 3.74 (s,

3H), 3.74 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 168.86, 166.40, 148.98, 143.61, 140.22, 137.98, 136.44, 132.94, 132.23, 130.00, 129.23 (2C), 128.98, 127.16 (2C), 119.38, 118.98, 116.97, 114.40, 112.85, 52.89, 52.51. ESI-MS m/z: 403.1 (MH^+).

7.20 N,2-Diphenylimidazo[1,2-a]pyridin-3-amine (6t). Treatment of pyridin-2-amine (**4b**), benzaldehyde (**5a**), and isocyanobenzene (**3b**) as outlined in general procedure D and purification by preparative HPLC (linear gradient of 10% B to 50% B over 20 min with a flow rate 20 mL/min; retention time = 11.6 min) provided N,2-diphenylimidazo[1,2-a]pyridin-3-amine as a white solid (61 % yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.54 (s, 1H), 8.27 (d, J = 6.8 Hz, 1H), 7.97 – 7.94 (m, 2H), 7.91 (d, J = 9.0 Hz, 1H), 7.79 (t, J = 8.0 Hz, 1H), 7.52 (t, J = 7.5 Hz, 2H), 7.46 – 7.42 (m, 1H), 7.32 (t, J = 6.8 Hz, 1H), 7.18 (dd, J = 8.5, 7.3 Hz, 2H), 6.80 (t, J = 7.4 Hz, 1H), 6.66 (d, J = 7.4 Hz, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 146.05, 142.25, 137.95, 134.20, 130.00 (2C), 128.90 (2C), 127.94, 126.92 (2C), 125.54, 123.53, 119.40, 118.97, 117.63, 113.37 (2C), 112.69. ESI-MS m/z: 286.1 (MH^+). HRMS calcd. for $C_{19}H_{16}N_3$(MH^+), 286.1339; found, 286.1336.

7.21 6-(Methylsulfonyl)-N-(4-nitrophenyl)-2-phenylimidazo[1,2-a]pyridin-3-amine (6u). Treatment of 5-(methylsulfonyl)pyridin-2-amine (**4c**), benzaldehyde (**5a**), and 1-isocyano-4-nitrobenzene (**3c**) as outlined in general procedure D and purification by preparative HPLC (linear gradient of 10% B to 60% B over 20 min with a flow rate 20 mL/min; retention time = 13.7 min) provided 6-(methylsulfonyl)-N-(4-nitrophenyl)-2-phenylimidazo[1,2-a]pyridin-3-amine (**6u**) as a pale brown solid (18% yield). ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.48 (s, 1H), 8.54 (s, 1H), 8.09 (d, J = 8.9 Hz, 2H), 8.01 – 7.99 (m, 2H), 7.92 (dd, J = 9.5, 0.9 Hz, 1H), 7.77 (dd, J = 9.5, 1.9 Hz, 1H), 7.44 (t, J = 7.7 Hz, 2H), 7.36 (t, J = 7.4 Hz, 1H), 6.75 (brs, 2H), 3.34 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d6) δ 149.74, 140.34, 137.93, 137.64, 130.53, 127.14 (2C),

126.91, 125.66, 124.97 (2C), 124.69 (2C), 123.50, 120.74, 116.96, 116.28, 111.53 (2C), 41.90. ESI-MS m/z: 409.1 (MH^+). HRMS calcd. for $C_{20}H_{17}N_4O_4S(MH^+)$, 409.0965; found, 409.0966.

8. Preparation of imidazo[1,2-a]pyrazin-3-amines (7'b, s, t, z).

8.1 4-((2-Phenylimidazo[1,2-a]pyrazin-3-yl)amino)benzoic acid (7'b). Treatment of methyl 4-((2-phenylimidazo[1,2-a]pyrazin-3-yl)amino)benzoate (**6a**) as outlined in general procedure E (70 °C, 15 h) provided 4-((2-phenylimidazo[1,2-a]pyrazin-3-yl)amino)benzoic acid as a pale yellow solid (82% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.36 (s, 1H), 9.14 (d, J = 1.5 Hz, 1H), 8.98 (s, 1H), 8.07 – 8.04 (m, 3H), 7.92 (d, J = 4.6 Hz, 1H), 7.76 (d, J = 8.8 Hz, 2H), 7.44 (t, J = 7.5 Hz, 2H), 7.38 – 7.34 (m, 1H), 6.59 (d, J = 8.1 Hz, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 167.56, 149.51, 143.54, 140.13, 137.83, 133.05, 132.03 (2C), 129.90, 129.18 (2C), 128.91, 127.20 (2C), 121.49, 119.75, 116.95, 113.15 (2C). ESI-MS m/z: 331.1 (MH^+). HRMS calcd. for $C_{19}H_{15}N_4O_2$ (MH^+): 331.1190; found: 331.1180. Commercially obtained **7b**: ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.36 (brs, 1H), 9.13 (d, J = 1.5 Hz, 1H), 8.97 (s, 1H), 8.07 – 8.03 (m, 2H), 7.91 (d, J = 4.5 Hz, 1H), 7.75 (d, J = 9.0 Hz, 2H), 7.44 (t, J = 7.6 Hz, 2H), 7.37 - 7.34 (m, 1H), 6.58 (d, J = 8.2 Hz, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 167.67, 149.35, 143.55, 140.09, 137.82, 133.06, 131.99 (2C), 129.90, 129.18 (2C), 128.90, 127.19 (2C), 121.95, 119.80, 116.95, 113.10 (2C). ESI-MS m/z: 331.1 (MH^+).

8.2 2-((2-(2-Hydroxyphenyl)imidazo[1,2-a]pyrazin-3-yl)amino)benzoic acid (7's). Treatment of commercially available methyl 2-((2-(2-hydroxyphenyl)imidazo[1,2-a]pyrazin-3-yl)amino)benzoate as outline in general procedure E and purification by HPLC (linear gradient of 20% B to 70% B over 20 min with a flow rate 20 mL/min; retention time = 10.0 min) provided 2-((2-(2-hydroxyphenyl)imidazo[1,2-a]pyrazin-3-yl)amino)benzoic acid as a yellow solid (78% yield). ^{1}H NMR (500 MHz, DMSO-d_6) δ 13.19 (brs, 1H), 9.64 (s, 1H), 9.19 (d, J =

1.4 Hz, 1H), 8.07 (dd, *J* = 4.6, 1.5 Hz, 1H), 7.94 (d, *J* = 4.6 Hz, 1H), 7.91 (dd, *J* = 8.0, 1.7 Hz, 1H), 7.72 (dd, *J* = 7.9, 1.7 Hz, 1H), 7.19 - 7.15 (m, 2H), 6.91 (dd, *J* = 8.2, 1.2 Hz, 1H), 6.79 - 6.74 (m, 2H), 6.05 (d, *J* = 8.0 Hz, 1H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 170.06, 157.05, 147.26, 142.19, 139.16, 135.90, 135.22, 132.41, 130.79, 130.20, 127.92, 119.74, 119.66, 118.90, 117.36, 117.11, 116.68, 113.65 (2C). ESI-MS m/z: 347.1 (MH^+).

8.3 3-((2-(4-Carboxyphenyl)imidazo[1,2-a]pyrazin-3-yl)amino)benzoic acid (7't).

Treatment of commercially available 4-(3-((3-(ethoxycarbonyl)phenyl)amino)imidazo[1,2-a]pyrazin-2-yl)benzoic acid (**7r**) as outline in general procedure E and purification by HPLC (linear gradient of 20% B to 70% B over 20 min with a flow rate 20 mL/min; retention time = 6.3 min) provided 3-((2-(4-carboxyphenyl)imidazo[1,2-a]pyrazin-3-yl)amino)benzoic acid as a yellow solid (81% yield). ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.93 (brs, 2H), 9.18 (d, *J* = 1.5 Hz, 1H), 8.75 (s, 1H), 8.19 (d, *J* = 8.5 Hz, 2H), 8.10 (dd, *J* = 4.5, 1.5 Hz, 1H), 7.99 (d, *J* = 8.4 Hz, 2H), 7.93 (d, *J* = 4.6 Hz, 1H), 7.37 – 7.35 (m, 1H), 7.27 (t, *J* = 7.9 Hz, 1H), 7.16 (s, 1H), 6.73 (d, *J* = 7.4 Hz, 1H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 167.69, 167.41, 145.23, 143.76, 138.89, 137.77, 137.19, 132.51, 130.72, 130.34, 130.18 (2C), 129.77, 127.11 (2C), 121.52, 120.57, 117.91, 117.18, 114.41. ESI-MS m/z: 375.1 (MH^+).

8.4 4-((2-(4-Fluorophenyl)imidazo[1,2-a]pyrazin-3-yl)amino)benzoic acid (7'z).

Treatment of methyl 4-((2-(4-fluorophenyl)imidazo[1,2-a]pyrazin-3-yl)amino)benzoate (**6b**) as outline in general procedure E and purification by HPLC (linear gradient of 20% B to 40% B over 20 min with a flow rate 20 mL/min; retention time = 10.4 min) provided 4-((2-(4-fluorophenyl)imidazo[1,2-a]pyrazin-3-yl)amino)benzoic acid as a yellow solid (83% yield). ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.11 (d, *J* = 1.3 Hz, 1H), 8.94 (s, 1H), 8.03 – 7.98 (m, 3H), 7.87 (d, *J* = 4.6 Hz, 1H), 7.69 (d, *J* = 8.9 Hz, 2H), 7.23 (t, *J* = 8.9 Hz, 2H), 6.52 (d, *J* = 7.8 Hz, 2H).

^{13}C NMR (126 MHz, DMSO-d_6) δ 167.54, 162.65 (d, J = 246.1 Hz), 149.25, 143.09, 139.53, 137.65, 132.02 (2C), 129.43 (d, J = 3.6 Hz), 129.41, 129.30 (2C, d, J = 8.4 Hz), 121.64, 119.85, 116.23 (2C, d, J = 21.5 Hz), 117.23, 113.23 (2C). ESI-MS m/z: 349.1 (MH^+).

9. Preparation of imidazo[1,2-a]pyrazin-3-amines (8a-s).

9.1 4-((2-Phenylimidazo[1,2-a]pyridin-3-yl)amino)benzoic acid (8a). Treatment of methyl 4-((2-phenylimidazo[1,2-a]pyridin-3-yl)amino)benzoate (**6c**) as outlined in general procedure E (rt, 24 h) and purification by preparative HPLC (linear gradient of 10% B to 50% B over 20 min with a flow rate 20 mL/min; retention time = 9.0 min) provided 4-((2-phenylimidazo[1,2-a]pyridin-3-yl)amino)benzoic acid as a dark white solid (88% yield). ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.39 (s, 1H), 8.42 (d, J = 6.7 Hz, 1H), 8.06 (d, J = 9.0 Hz, 1H), 7.99 (t, J = 6.7 Hz, 3H), 7.77 (d, J = 8.7 Hz, 2H), 7.55 (t, J = 7.5 Hz, 2H), 7.48 (dt, J = 13.8, 7.0 Hz, 2H), 6.82 (d, J = 8.2 Hz, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 167.49, 148.97, 138.81, 133.72, 131.88 (2C), 130.52, 130.22, 129.70 (2C), 127.44 (2C), 127.16, 125.49, 122.25, 119.78, 117.51, 113.66 (2C), 113.45. ESI-MS m/z: 330.1 (MH^+). HRMS calcd. for $C_{20}H_{16}N_3O_2$(MH^+), 330.1237; found, 330.1229.

9.2 4-((2-(p-Tolyl)imidazo[1,2-a]pyridin-3-yl)amino)benzoic acid (8b). Treatment of methyl 4-((2-(p-tolyl)imidazo[1,2-a]pyridin-3-yl)amino)benzoate (**6d**) as outlined in general procedure E (rt, 24 h) and purification by preparative HPLC (linear gradient of 5% B to 30% B over 20 min with a flow rate 20 mL/min; retention time = 15.0 min) provided 4-((2-(p-tolyl)imidazo[1,2-a]pyridin-3-yl)amino)benzoic acid as a white solid (88% yield). ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.35 (brs, 1H), 8.94 (s, 1H), 8.02 (d, J = 6.5 Hz, 1H), 7.91 (d, J = 8.2 Hz, 2H), 7.75 (d, J = 9.1 Hz, 2H), 7.69 (d, J = 8.9 Hz, 1H), 7.42 – 7.38 (m, 1H), 7.22 (d, J = 8.0 Hz, 2H), 6.99 (t, J = 6.7 Hz, 1H), 6.59 (s, 2H), 2.30 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ

167.61, 150.15, 141.89, 137.82, 137.19, 132.00 (2C), 130.24, 129.65 (2C), 126.89 (2C), 126.67, 123.69, 121.15, 117.98, 117.01, 113.43, 112.93 (2C), 21.31. ESI-MS m/z: 344.2 (MH^+). HRMS calcd. for $C_{21}H_{18}N_3O_2(MH^+)$, 344.1394; found, 344.1387.

9.3 4-((2-(4-(Benzyloxy)phenyl)imidazo[1,2-a]pyridin-3-yl)amino)benzoic acid (8c). Treatment of methyl 4-((2-(4-(benzyloxy)phenyl)imidazo[1,2-a]pyridin-3-yl)amino)benzoate (**6e**) as outlined in general procedure E (70 °C, 24 h) and purification by preparative HPLC (linear gradient of 10% B to 40% B over 20 min with a flow rate 20 mL/min; retention time = 15.8 min) provided 4-((2-(4-(benzyloxy)phenyl)imidazo[1,2-a]pyridin-3-yl)amino)benzoic acid as a white solid (89% yield). ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.75 (s, 1H), 7.96 – 7.94 (m, 3H), 7.75 (d, J = 9.1 Hz, 2H), 7.62 (d, J = 9.0 Hz, 1H), 7.44 (d, J = 6.9 Hz, 2H), 7.39 (t, J = 7.4 Hz, 2H), 7.34 – 7.29 (m, 2H), 7.05 (d, J = 9.0 Hz, 2H), 6.92 (t, J = 6.7 Hz, 1H), 6.54 (s, 2H), 5.12 (s, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 167.88, 158.46, 150.01, 142.34, 138.11, 137.48, 131.98, 128.89 (4C), 128.29, 128.21 (2C), 128.18 (4C), 126.69, 125.55, 123.38, 117.44, 117.32, 115.33, 112.74 (2C), 69.63. HRMS calcd. for $C_{27}H_{22}N_3O_3(MH^+)$, 436.1656; found, 436.1646.

9.4 4-((2-(4-(Trifluoromethyl)phenyl)imidazo[1,2-a]pyridin-3-yl)amino)benzoic acid (8d). Treatment of methyl 4-((2-(4-(trifluoromethyl)phenyl)imidazo[1,2-a]pyridin-3-yl)amino)benzoate (**6f**) as outlined in general procedure E (rt, 24 h) and purification by preparative HPLC (linear gradient of 5% B to 30% B over 20 min with a flow rate 20 mL/min; retention time = 18.0 min) provided 4-((2-(4-(trifluoromethyl)phenyl)imidazo[1,2-a]pyridin-3-yl)amino)benzoic acid as a brown solid (25% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.38 (brs, 1H), 8.93 (s, 1H), 8.23 (d, J = 8.2 Hz, 2H), 8.02 (d, J = 6.8 Hz, 1H), 7.78 (dd, J = 11.4, 8.5 Hz, 4H), 7.71 (d, J = 9.0 Hz, 1H), 7.42 – 7.38 (m, 1H), 7.00 (t, J = 6.7 Hz, 1H), 6.59 (d, J = 8.3

Hz, 2H). ESI-MS m/z: 398.1 (MH^+). HRMS calcd. for $C_{21}H_{15}F_3N_3O_2(MH^+)$, 398.1111; found, 398.1109.

9.5 4-((2-(4-Nitrophenyl)imidazo[1,2-a]pyridin-3-yl)amino)benzoic acid (8e). Treatment of pyridin-2-amine (**4b**), 4-nitrobenzaldehyde (**5d**), and methyl 4-isocyanobenzoate (**3a**) as outlined in general procedure D provided methyl 4-((2-(4-nitrophenyl)imidazo[1,2-a]pyridin-3-yl)amino)benzoate as a yellow solid (34% yield). ESI-MS m/z: 389.1 (MH^+). Treatment of methyl 4-((2-(4-nitrophenyl)imidazo[1,2-a]pyridin-3-yl)amino)benzoate as outlined in general procedure E (rt, 24 h) and purification by preparative HPLC (linear gradient of 5% B to 30% B over 20 min with a flow rate 20 mL/min; retention time = 15.2 min) provided 4-((2-(4-nitrophenyl)imidazo[1,2-a]pyridin-3-yl)amino)benzoic acid as a brown solid (8.6% yield). ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.07 (s, 1H), 8.33 (d, J = 9.0 Hz, 2H), 8.25 (d, J = 8.9 Hz, 2H), 8.12 (d, J = 6.8 Hz, 1H), 7.80 (d, J = 9.0 Hz, 1H), 7.77 (d, J = 8.5 Hz, 2H), 7.55 (t, J = 7.9 Hz, 1H), 7.12 (t, J = 6.8 Hz, 1H), 6.65 (d, J = 8.3 Hz, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 167.53, 149.32, 147.15, 142.02, 138.75, 133.93, 132.04 (2C), 128.63, 127.72 (2C), 124.58 (2C), 124.33, 121.78, 120.64, 117.04, 114.61, 113.27 (2C). ESI-MS m/z: 375.1 (MH^+). HRMS calcd. for $C_{20}H_{15}N_4O_4(MH^+)$, 375.1088; found, 375.1082.

9.6 4-((2-(4-Hydroxyphenyl)imidazo[1,2-a]pyridin-3-yl)amino)benzoic acid (8f). Treatment of methyl 4-((2-(4-hydroxyphenyl)imidazo[1,2-a]pyridin-3-yl)amino)benzoate (**6g**) as outlined in general procedure E (65 °C, 24 h) and purification by preparative HPLC (linear gradient of 10% B to 50% B over 20 min with a flow rate 20 mL/min; retention time = 8.0 min) provided 4-((2-(4-hydroxyphenyl)imidazo[1,2-a]pyridin-3-yl)amino)benzoic acid as a pale pink solid (58% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.33 (s, 1H), 9.63 (s, 1H), 8.80 (s, 1H), 7.97 (d, J = 6.6 Hz, 1H), 7.83 (d, J = 8.7 Hz, 2H), 7.76 (d, J = 9.0 Hz, 2H), 7.64 (d, J = 9.0 Hz,

1H), 7.39 – 7.35 (m, 1H), 6.97 (t, J = 6.5 Hz, 1H), 6.80 (d, J = 8.7 Hz, 2H), 6.57 (d, J = 6.4 Hz, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 167.61, 157.89, 150.26, 141.82, 137.71, 132.01 (2C), 128.38 (2C), 126.25, 124.02, 123.50, 121.06, 116.88, 116.84, 115.85 (2C), 113.15, 112.87 (2C). ESI-MS m/z: 346.1 (MH^+). HRMS calcd. for $C_{20}H_{16}N_3O_3(MH^+)$, 346.1186; found, 346.1182.

9.7 4-((2-(4-Sulfophenyl)imidazo[1,2-a]pyridin-3-yl)amino)benzoic acid (8g). To a stirring solution of commercially available 4-formylbenzenesulfonyl chloride (1.1 g, 5.50 mmol) in acetonitrile (25 mL) at rt was added a potassium bifloride (KHF_2, 4.7 g, 61 mmol) in H_2O (25 mL). The resulting biphasic mixture was stirred vigorously (rt, 2 h). The reaction mixture was then diluted with H_2O (20 mL) and ethyl acetate (100 mL) the layers separated. The aqueous phase was extracted with 2 x100 mL portions of ethyl acetate, then the combined extracts were washed by brine (80 mL). The organic phase was dried over Na_2SO_4 and filtered. The filtrate was concentrated to afford the 4-formylbenzenesulfonyl fluoride[13] (**5k**, 780 mg) as brown solid (75 % yield). [^{1}H NMR (400 MHz, DMSO-d_6) δ 10.19 (s, 1H), 8.38 (d, J = 8.5 Hz, 2H), 8.26 (d, J = 7.7 Hz, 2H).^{19}F NMR (376 MHz, DMSO-d_6) δ 65.87. ^{13}C NMR (101 MHz, DMSO-d6) δ 192.81, 141.61, 136.17 (d, J = 24.0 Hz) 131.27 (2C), 129.74 (2C).] Treatment of pyridin-2-amine (**4b**), 4-formylbenzenesulfonyl fluoride (**5k**), and methyl 4-isocyanobenzoate (**3a**) as outlined in general procedure D provided methyl 4-((2-(4-(fluorosulfonyl)phenyl)imidazo[1,2-a]pyridin-3-yl)amino)benzoate as white solid (15 % yield). [^{1}H NMR (400 MHz, DMSO-d_6) δ 9.06 (s, 1H), 8.38 (d, J = 8.6 Hz, 2H), 8.18 (d, J = 8.8 Hz, 2H), 8.02 (d, J = 6.8 Hz, 1H), 7.79 (d, J = 9.1 Hz, 2H), 7.72 (d, J = 9.1 Hz, 1H), 7.41 (ddd, J = 9.1, 6.7, 1.3 Hz, 1H), 7.00 (td, J = 6.8, 1.1 Hz, 1H), 6.63 (d, J = 8.3 Hz, 2H), 3.76 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 166.46, 149.87, 142.95, 141.60, 135.36, 131.97 (2C), 130.13 (d, J = 23.4 Hz, 1C), 129.43 (2C), 127.92 (2C), 126.95, 123.92, 120.47 (2C), 118.16, 113.75, 113.29 (2C), 52.02. ^{19}F NMR (376 MHz, DMSO-d_6) δ

66.72. ESI-MS m/z: 426.1 (MH^+), 448.1 (MNa^+).] Treatment of methyl 4-((2-(4-(fluorosulfonyl)phenyl)imidazo[1,2-a]pyridin-3-yl)amino)benzoate as outlined in general procedure E (rt, 15 h) and purification by preparative HPLC (linear gradient of 5% B to 20% B over 20 min with a flow rate 20 mL/min; retention time = 11.4 min) provided 4-((2-(4-sulfophenyl)imidazo[*1,2-a*]pyridin-3-yl)amino)benzoic acid as white solid (52 % yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.14 (s, 1H), 8.36 (d, J = 6.8 Hz, 1H), 7.98 (d, J = 8.9 Hz, 1H), 7.92 (t, J = 8.0 Hz, 1H), 7.86 (d, J = 8.5 Hz, 2H), 7.77 (d, J = 8.9 Hz, 2H), 7.73 (d, J = 8.5 Hz, 2H), 7.42 (t, J = 6.8 Hz, 1H), 6.78 (d, J = 8.3 Hz, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 167.48, 150.01, 148.91, 139.12, 133.15, 131.91 (2C), 130.61, 127.59, 126.87 (2C), 126.75 (2C), 125.37, 122.26, 119.94, 117.15, 113.88, 113.63 (2C). ESI-MS m/z: 410.1 (MH^+). HRMS calcd. for $C_{20}H_{16}N_3O_5S(MH^+)$, 410.0805; found, 410.0803. HRMS calcd. for $C_{20}H_{15}N_3O_5SNa(MNa^+)$, 432.0625; found, 432.0622.

9.8 4-(3-((4-Carboxyphenyl)amino)imidazo[1,2-a]pyridin-2-yl)benzoic acid (8h).

Treatment of methyl 4-(3-((4-(methoxycarbonyl)phenyl)amino)imidazo[1,2-a]pyridin-2-yl)benzoate (**6h**) as outlined in general procedure E (65 °C, 24 h) and purification by preparative HPLC (linear gradient of 5% B to 20% B over 20 min with a flow rate 20 mL/min; retention time = 14.9 min) provided 4-(3-((4-carboxyphenyl)amino)imidazo[1,2-a]pyridin-2-yl)benzoic acid as a yellow solid (70% yield). ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.92 (s, 1H), 8.10 (d, J = 8.1 Hz, 2H), 7.98 (d, J = 6.8 Hz, 1H), 7.94 (d, J = 8.1 Hz, 2H), 7.75 (d, J = 8.4 Hz, 2H), 7.67 (d, J = 9.1 Hz, 1H), 7.37 – 7.34 (m, 1H), 6.96 (t, J = 6.7 Hz, 1H), 6.56 (s, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 168.05, 167.73, 149.87, 142.60, 137.22, 137.18, 132.26, 132.02 (2C), 129.95 (2C), 126.54 (2C), 126.16, 123.67, 121.65, 119.16, 117.83, 113.19, 112.92 (2C). ESI-MS m/z: 374.1 (MH^+). HRMS calcd. for $C_{21}H_{16}N_3O_4(MH^+)$, 374.1135; found, 374.1126.

9.9 4-(3-(Phenylamino)imidazo[1,2-a]pyridin-2-yl)benzoic acid (8i). Treatment of methyl 4-(3-(phenylamino)imidazo[1,2-a]pyridin-2-yl)benzoate (**6i**) as outlined in general procedure E (rt, 24 h) and purification by preparative HPLC (linear gradient of 5% B to 30% B over 20 min with a flow rate 20 mL/min; retention time = 15.9 min) provided 4-(3-(phenylamino)imidazo[1,2-a]pyridin-2-yl)benzoic acid as a white solid (95% yield). ^{1}H NMR (500 MHz, DMSO-d_6) δ 13.17 (brs, 1H), 8.82 (s, 1H), 8.33 (d, J = 6.8 Hz, 1H), 8.15 (d, J = 8.3 Hz, 2H), 8.04 (d, J = 8.3 Hz, 2H), 7.99 (d, J = 9.0 Hz, 1H), 7.88 (t, J = 7.9 Hz, 1H), 7.38 (t, J = 6.9 Hz, 1H), 7.18 (t, J = 7.7 Hz, 2H), 6.81 (t, J = 7.3 Hz, 1H), 6.72 (d, J = 7.9 Hz, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 167.12, 144.57, 139.45, 132.60, 131.68, 130.32 (3C), 130.05 (2C), 129.91, 127.35 (2C), 125.31, 121.75, 120.11, 116.72, 114.20, 114.12 (2C). ESI-MS m/z: 330.1 (MH^+). HRMS calcd. for $C_{20}H_{16}N_3O_2(MH^+)$, 330.1237; found, 330.1233.

9.10 2-(2-Carboxyphenyl)-3-((4-carboxyphenyl)amino)imidazo[1,2-a]pyridine-6-carboxylic acid (8j). Treatment of methyl 6-(4-(methoxycarbonyl)phenyl)-5-oxo-5,6-dihydropyrido[2',1':2,3]imidazo[4,5-c]isoquinoline-9-carboxylate (**6j**) as outlined in general procedure E (65 °C, 18 h) and purification by preparative HPLC (linear gradient of 5% B to 30% B over 20 min with a flow rate 20 mL/min; retention time = 14.7 min) provided 4-((2-(4-sulfophenyl)imidazo[1,2-a]pyridin-3-yl)amino)benzoic acid as white solid (52 % yield). ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.86 (s, 1H), 8.59 (s, 1H), 7.89 (d, J = 9.5 Hz, 1H), 7.82 (t, J = 7.9 Hz, 2H), 7.69 (d, J = 8.9 Hz, 2H), 7.60 – 7.48 (m, 3H), 6.68 (d, J = 8.3 Hz, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 169.07, 167.51, 165.79, 149.64, 141.18, 138.22, 133.21, 131.73(2C), 131.51, 131.11, 130.82, 130.13, 129.43, 127.20, 126.92, 121.53, 120.38, 118.30, 116.23, 113.26 (2C). HRMS calcd. for $C_{20}H_{16}N_3O_6(MH^+)$, 418.1034; found, 418.1033.

9.11 2-Phenyl-3-(phenylamino)imidazo[1,2-a]pyridine-6-carboxylic acid (8k).

Treatment of methyl 2-phenyl-3-(phenylamino)imidazo[1,2-a]pyridine-6-carboxylate (**6k**) as outlined in general procedure E (rt, 24 h) and purification by preparative HPLC provided 2-phenyl-3-(phenylamino)imidazo[1,2-a]pyridine-6-carboxylic acid as a pale green solid (83% yield). ^{1}H NMR (500 MHz, DMSO-d_6) δ 13.39 (brs, 1H), 8.52 (s, 1H), 8.38 (s, 1H), 8.06 (d, J = 7.3 Hz, 2H), 7.76 – 7.72 (m, 2H), 7.43 (t, J = 7.7 Hz, 2H), 7.33 (t, J = 7.3 Hz, 1H), 7.16 (t, J = 7.7 Hz, 2H), 6.77 (t, J = 7.3 Hz, 1H), 6.58 (d, J = 7.9 Hz, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 166.13, 145.54, 142.50, 138.82, 133.05, 130.12 (2C), 129.07 (2C), 128.59, 127.06 (2C), 126.86, 125.47, 120.51, 119.46, 117.17, 117.01, 113.55 (2C). ESI-MS m/z: 330.1 (MH^+). HRMS calcd. for $C_{20}H_{16}N_3O_2$(MH^+), 330.1237; found, 330.1231.

9.12 3-((4-Nitrophenyl)amino)-2-phenylimidazo[1,2-a]pyridine-6-carboxylic acid (8l).

Treatment of methyl 3-((4-nitrophenyl)amino)-2-phenylimidazo[1,2-a]pyridine-6-carboxylate (**6l**) as outlined in general procedure E (rt, 24 h) and purification by preparative HPLC (linear gradient of 10% B to 50% B over 20 min with a flow rate 20 mL/min; retention time = 12.1 min) provided 3-((4-nitrophenyl)amino)-2-phenylimidazo[1,2-a]pyridine-6-carboxylic acid as a brown solid (40% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.43 (brs, 1H), 9.44 (s, 1H), 8.51 (t, J = 1.4 Hz, 1H), 8.08 (d, J = 8.9 Hz, 2H), 8.01 – 7.98 (m, 2H), 7.76 (s, 2H), 7.43 (t, J = 7.5 Hz, 2H), 7.36 – 7.32 (m, 1H), 6.73 (brs, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 166.16, 152.15, 143.18, 139.66, 133.07, 129.21 (2C), 128.77, 127.02 (2C), 126.89 (2C), 126.87, 126.53, 125.55, 118.18, 117.78, 117.36, 112.83 (2C). ESI-MS m/z: 375.1 (MH^+). HRMS calcd. for $C_{20}H_{15}N_4O_4$(MH^+), 375.1088; found, 375.1083.

9.13 3-((4-Carboxyphenyl)amino)-2-phenylimidazo[1,2-a]pyridine-6-carboxylic acid (8m). Treatment of methyl 3-((4-(methoxycarbonyl)phenyl)amino)-2-phenylimidazo[1,2-

a]pyridine-6-carboxylate (**6m**) as outlined in general procedure E (65 °C, 24 h) and purification by preparative HPLC (linear gradient of 5% B to 50% B over 20 min with a flow rate 20 mL/min; retention time = 10.1 min) provided 3-((4-carboxyphenyl)amino)-2-phenylimidazo[1,2-a]pyridine-6-carboxylic acid as a dark brown solid (87% yield). ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 8.93 (s, 1H), 8.49 (t, *J* = 1.4 Hz, 1H), 8.02 – 8.00 (m, 2H), 7.79 – 7.73 (m, 4H), 7.43 (t, *J* = 7.6 Hz, 2H), 7.36 – 7.31 (m, 1H), 6.63 (d, *J* = 8.0 Hz, 2H). ^{13}C NMR (126 MHz, DMSO-*d*$_6$) δ 167.53, 166.05, 149.71, 142.71, 138.94, 132.78, 132.10 (2C), 129.17 (2C), 128.78, 127.05 (2C), 126.72, 125.75, 121.58, 119.33, 117.45, 117.10, 113.11 (2C). ESI-MS m/z: 374.2 (MH^+). HRMS calcd. for $C_{21}H_{16}N_3O_4$(MH^+), 374.1135; found, 374.1128.

9.14 2-([1,1'-Biphenyl]-4-yl)-3-((4-carboxyphenyl)amino)imidazo[1,2-a]pyridine-6-carboxylic acid (8n). Treatment of methyl 6-aminonicotinate (**4d**), (1,1'-biphenyl)-4-carbaldehyde (**5j**), and methyl 4-isocyanobenzoate (**3a**) as outlined in general procedure C (MeOH, Biotage Initiator, microwave irradiation, 85 °C, 3 h) provided methyl 2-([1,1'-biphenyl]-4-yl)-3-((4-(methoxycarbonyl)phenyl)amino)imidazo[1,2-a]pyridine-6-carboxylate as a yellow solid (40 % yield). ESI-MS m/z: 478.2 (MH^+). Treatment of methyl 2-([1,1'-biphenyl]-4-yl)-3-((4-(methoxycarbonyl)phenyl)amino)imidazo[1,2-a]pyridine-6-carboxylate as outlined in general procedure E (65 °C, 18 h) and purification by preparative HPLC (linear gradient of 10% B to 50% B over 20 min with a flow rate 20 mL/min; retention time = 17.1 min) provided 2-([1,1'-biphenyl]-4-yl)-3-((4-carboxyphenyl)amino)imidazo[1,2-a]pyridine-6-carboxylic acid as a brown solid (56 % yield). ESI-MS m/z: 450.2 (MH^+). HRMS calcd. for $C_{27}H_{20}N_3O_4$(MH^+), 450.1448; found, 450.1445.

9.15 4-((6-Bromo-2-phenylimidazo[1,2-a]pyridin-3-yl)amino)benzoic acid (8o). Treatment of methyl 4-((6-bromo-2-phenylimidazo[1,2-a]pyridin-3-yl)amino)benzoate (**6n**) as

outlined in general procedure E (65 °C, 18 h) and purification by preparative HPLC (linear gradient of 10% B to 60% B over 20 min with a flow rate 20 mL/min; retention time = 11.8 min) provided 4-((6-bromo-2-phenylimidazo[1,2-a]pyridin-3-yl)amino)benzoic acid as light brown solid (60 % yield). ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.86 (s, 1H), 8.30 (s, 1H), 8.01 – 7.94 (m, 2H), 7.75 (d, *J* = 9.1 Hz, 2H), 7.70 (d, *J* = 9.4 Hz, 1H), 7.54 (d, *J* = 9.3 Hz, 1H), 7.42 (t, *J* = 7.7 Hz, 2H), 7.33 (t, *J* = 7.4 Hz, 1H), 6.60 (d, *J* = 8.7 Hz, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 167.60, 149.72, 140.50, 137.75, 132.55, 131.98 (2C), 129.78, 129.16 (2C), 128.74, 127.00 (2C), 123.62, 121.46, 118.98, 118.43, 113.18 (2C), 107.66. HRMS calcd. for $C_{20}H_{15}BrN_3O_2$[M(^{79}Br)H^+], 408.0342; found, 408.0338.

9.16 4-((2,7-Diphenylimidazo[1,2-a]pyridin-3-yl)amino)benzoic acid (8p). Treatment of methyl 4-((2,7-diphenylimidazo[1,2-a]pyridin-3-yl)amino)benzoate (**6o**) as outlined in general procedure E (65 °C, 18 h) and purification by preparative HPLC (linear gradient of 10% B to 70% B over 20 min with a flow rate 20 mL/min; retention time = 13.4 min) provided 4-((2,7-diphenylimidazo[1,2-a]pyridin-3-yl)amino)benzoic acid as a white solid (65 % yield). ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.04 (s, 1H), 8.21 (d, *J* = 7.1 Hz, 1H), 8.04 (d, *J* = 1.6 Hz, 1H), 7.93 – 7.88 (m, 2H), 7.86 – 7.80 (m, 2H), 7.71 (d, *J* = 9.0 Hz, 2H), 7.57 – 7.53 (m, 1H), 7.51 (t, *J* = 7.5 Hz, 2H), 7.47 – 7.41 (m, 3H), 7.38 – 7.33 (m, 1H), 6.67 (d, *J* = 8.2 Hz, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 167.57, 149.54, 141.64, 140.83, 137.21, 134.24, 132.01 (2C), 130.06, 129.84 (2C), 129.81, 129.61, 129.47 (2C), 127.51 (2C), 127.21 (2C), 124.86, 121.85, 119.08, 114.73, 113.43, 113.40, 111.34. HRMS calcd. for $C_{26}H_{20}N_3O_2$(MH^+), 406.1550; found, 406.1541.

9.17 4-((6-(Methylsulfonyl)-2-phenylimidazo[1,2-a]pyridin-3-yl)amino)benzoic acid (8q). Treatment of methyl 4-((6-(methylsulfonyl)-2-phenylimidazo[1,2-a]pyridin-3-yl)amino)benzoate (**6p**) as outlined in general procedure E (80 °C, 24 h) and purification by

preparative HPLC (linear gradient of 10% B to 30% B over 20 min with a flow rate 20 mL/min; retention time = 15.7 min) provided 4-((6-(methylsulfonyl)-2-phenylimidazo[1,2-a]pyridin-3-yl)amino)benzoic acid as a brown solid (11% yield). ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.89 (s, 1H), 8.43 – 8.39 (m, 1H), 7.95 (d, J = 7.1 Hz, 2H), 7.82 (d, J = 9.4 Hz, 1H), 7.73 – 7.65 (m, 3H), 7.36 (t, J = 7.7 Hz, 2H), 7.27 (t, J = 7.4 Hz, 1H), 6.57 (d, J = 8.2 Hz, 2H), 3.26 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 167.56, 149.52, 142.21, 139.88, 132.86, 132.06 (2C), 129.18 (2C), 128.87, 127.59, 127.11 (2C), 125.51, 122.64, 121.63, 120.09, 118.36, 113.20 (2C), 44.01. ESI-MS m/z: 408.1 (MH^+). HRMS calcd. for $C_{21}H_{18}N_3O_4S(MH^+)$, 408.1013; found, 408.1015.

9.18 4-((2-([1,1'-Biphenyl]-4-yl)-6-(methylsulfonyl)imidazo[1,2-a]pyridin-3-yl)amino)benzoic acid (8r). Treatment of methyl 4-((2-([1,1'-biphenyl]-4-yl)-6-(methylsulfonyl)imidazo[1,2-a]pyridin-3-yl)amino)benzoate (**6q**) as outlined in general procedure E (65 °C, 18 h) and purification by preparative HPLC (linear gradient of 20% B to 60% B over 20 min with a flow rate 20 mL/min; retention time = 15.6 min) provided 4-((2-([1,1'-biphenyl]-4-yl)-6-(methylsulfonyl)imidazo[1,2-a]pyridin-3-yl)amino)benzoic acid as a brown solid (40 % yield). ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.94 (s, 1H), 8.46 – 8.40 (m, 1H), 8.05 (d, J = 8.5 Hz, 2H), 7.84 (d, J = 9.4 Hz, 1H), 7.75 – 7.66 (m, 4H), 7.64 (d, J = 7.3 Hz, 2H), 7.39 (t, J = 7.6 Hz, 2H), 7.30 (t, J = 7.3 Hz, 1H), 6.61 (brs, 2H), 3.27 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 167.54, 149.51, 142.30, 140.32, 139.84, 139.61, 132.09 (2C), 131.95, 129.42 (2C), 128.12, 127.62 (2C), 127.57, 127.35 (2C), 127.02 (2C), 125.49, 122.65, 121.67, 120.16, 118.36, 113.23 (2C), 44.02. ESI-MS m/z: 484.1 (MH^+). HRMS calcd. for $C_{27}H_{22}N_3O_4S(MH^+)$, 484.1326; found, 484.1321.

9.19 3-((4-Carboxyphenyl)amino)-2-phenylimidazo[1,2-a]pyridine-7-carboxylic acid (8s). Treatment of methyl 3-((4-(methoxycarbonyl)phenyl)amino)-2-phenylimidazo[1,2-

a]pyridine-7-carboxylate (**6r**) as outlined in general procedure E (rt, 24 h) and purification by preparative HPLC (linear gradient of 10% B to 50% B over 20 min with a flow rate 20 mL/min; retention time = 9.4 min) provided 3-((4-carboxyphenyl)amino)-2-phenylimidazo[1,2-a]pyridine-7-carboxylic acid as a dark pale yellow solid (95% yield). ^{1}H NMR (500 MHz, DMSO-*d*$_6$) δ 8.97 (s, 1H), 8.17 (s, 1H), 8.04 (t, *J* = 7.3 Hz, 3H), 7.75 (d, *J* = 8.5 Hz, 2H), 7.42 (t, *J* = 7.6 Hz, 2H), 7.37 (dd, *J* = 7.1, 1.6 Hz, 1H), 7.33 (t, *J* = 7.4 Hz, 1H), 6.58 (brs, 2H). ^{13}C NMR (126 MHz, DMSO-*d*$_6$) δ 167.58, 166.66, 149.77, 141.43, 140.38, 133.39, 132.06 (2C), 129.10 (2C), 128.59, 127.95, 127.05 (2C), 123.50, 121.34, 119.78, 119.50, 113.02 (2C), 112.12. ESI-MS m/z: 374.1 (MH^+). HRMS calcd. for $C_{21}H_{16}N_3O_4$(MH^+), 374.1135; found, 374.1125.

10. General procedure F preparation of methyl benzonates (12a, b).

Sodium hydride (15 mmol) was suspended in THF (15 mL). Commercial available 2-Phenyl-1H-benzo[d]imidazole (**11a**, 12 mmol) or 2-phenyl-1H-indole (**11b**, 12 mmol) and methyl 4-(bromomethyl)benzoate (12 mmol) were added at 0 °C. The reaction mixture was stirred (rt, 18 h). The mixture was purified by silica gel column and the methyl benzoates (**12a** or **12b**) were afforded.

10.1. Methyl 4-((2-phenyl-1H-benzo[d]imidazol-1-yl)methyl)benzoate (12a). Treatment of commercial available 2-phenyl-1H-benzo[d]imidazole (**11a**) and methyl 4-(bromomethyl)benzoate as outlined in general procedure F provided methyl 4-((2-phenyl-1H-benzo[d]imidazol-1-yl)methyl)benzoate (**12a**) as a white solid (77% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.02 (d, *J* = 8.4 Hz, 2H), 7.90 (d, *J* = 8.0 Hz, 1H), 7.66 (dd, *J* = 8.0, 1.7 Hz, 2H), 7.48 – 7.42 (m, 3H), 7.36 – 7.32 (m, 1H), 7.28 – 7.24 (m, 1H), 7.20 – 7.17 (m, 3H), 5.50 (s, 2H), 3.91 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.54, 154.16, 143.12, 141.44, 135.87, 130.39 (2C),

130.10, 129.86, 129.80, 129.18 (2C), 128.86 (2C), 126.01 (2C), 123.30, 122.96, 120.09, 110.33, 52.23, 48.22.

10.2. Methyl 4-((2-phenyl-1H-indol-1-yl)methyl)benzoate (12b). Treatment of commercial available 2-phenyl-1H-indole (**11b**) and methyl 4-(bromomethyl)benzoate as outlined in general procedure F provided methyl 4-((2-phenyl-1H-indol-1-yl)methyl)benzoate (**12b**) as a white solid (47% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.20 (s, 1H), 7.95 (d, *J* = 8.4 Hz, 2H), 7.51 (dd, *J* = 8.4, 1.4 Hz, 2H), 7.45 (t, *J* = 7.2 Hz, 3H), 7.42 – 7.37 (m, 2H), 7.32 (d, *J* = 8.6 Hz, 2H), 7.24 (ddd, *J* = 8.2, 7.1, 1.2 Hz, 1H), 7.10 (ddd, *J* = 8.0, 7.0, 1.0 Hz, 1H), 4.34 (s, 2H), 3.91 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 167.32, 147.22, 136.08, 135.76, 132.78, 129.83 (2C), 129.32, 128.96 (2C), 128.30 (2C), 127.92, 127.90 (2C), 127.86, 122.54, 119.94, 119.39, 110.98, 110.19, 52.03, 30.66.

10.3. 4-((2-Phenyl-1H-benzo[d]imidazol-1-yl)methyl)benzoic acid (9a). Treatment of methyl 4-((2-phenyl-1H-benzo[d]imidazol-1-yl)methyl)benzoate (**12a**) as outlined in general procedure E and purification by preparative HPLC (linear gradient of 20% B to 80% B over 20 min with a flow rate 20 mL/min; retention time = 6.5 min) provided 4-((2-phenyl-1H-benzo[d]imidazol-1-yl)methyl)benzoic acid (**9a**) as a white solid (97% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.91 – 7.85 (m, 3H), 7.83 – 7.78 (m, 2H), 7.70 – 7.59 (m, 4H), 7.52 – 7.42 (m, 2H), 7.23 (d, *J* = 8.3 Hz, 2H), 5.78 (s, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 167.32, 152.48, 141.01, 134.60, 132.02, 130.78, 130.30 (2C), 129.99 (2C), 129.63 (2C), 127.03 (2C), 126.55, 125.15, 117.90, 117.43, 114.98, 112.81, 48.36. ESI-MS m/z: 329.1 (MH^+). HRMS calcd. for $C_{21}H_{17}N_2O_2(MH^+)$, 329.1285; found, 329.1277.

10.4. 4-((2-Phenyl-1H-indol-1-yl)methyl)benzoic acid (9b). Treatment of methyl 4-((2-phenyl-1H-indol-1-yl)methyl)benzoate (**12b**) as outlined in general procedure E and purification

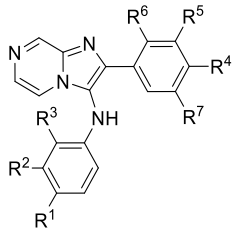
by preparative HPLC (linear gradient of 20% B to 80% B over 20 min with a flow rate 20 mL/min; retention time = 15.5 min) provided 4-((2-phenyl-1H-indol-1-yl)methyl)benzoic acid (**9b**) as a white solid (12% yield). ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.78 (brs, 1H), 11.37 (s, 1H), 7.83 (d, J = 9.1 Hz, 2H), 7.59 (d, J = 7.5 Hz, 2H), 7.48 (s, 2H), 7.42 – 7.31 (m, 3H), 7.28 (d, J = 8.7 Hz, 2H), 7.18 – 7.05 (m, 1H), 6.96 (t, J = 7.7 Hz, 1H), 4.31 (s, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 167.72, 147.43, 136.59, 135.63, 133.07, 129.95 (2C), 129.29 (2C), 129.22, 128.86, 128.57 (2C), 128.07 (2C), 128.00, 122.19, 119.44, 119.13, 111.75, 109.44, 30.48. ESI-MS m/z: 328.1 (MH^+). HRMS calcd. for $C_{22}H_{18}NO_2(MH^+)$, 328.1332; found, 328.1326.

10.5. 4-((2-Phenylimidazo[1,2-a]pyrazin-3-yl)amino)phthalic acid (10a). Treatment of dimethyl 4-((2-phenylimidazo[1,2-a]pyrazin-3-yl)amino)phthalate (**6s**) as outlined in general procedure E (70 °C, 24 h) and purification by preparative HPLC (linear gradient of 10% B to 20% B over 20 min with a flow rate 20 mL/min; retention time = 16.9 min) provided 4-((2-phenylimidazo[1,2-a]pyrazin-3-yl)amino)phthalic acid as a yellow solid (92% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.86 (brs, 2H), 9.15 (d, J = 1.5 Hz, 1H), 9.05 (s, 1H), 8.10 (dd, J = 4.5, 1.5 Hz, 1H), 8.05 (d, J = 7.3 Hz, 2H), 7.93 (d, J = 4.5 Hz, 1H), 7.62 (d, J = 8.5 Hz, 1H), 7.46 (t, J = 7.6 Hz, 2H), 7.37 (t, J = 7.3 Hz, 1H), 6.74 (brs, 1H), 6.52 (brs, 1H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 170.00, 167.65, 148.28, 143.59, 140.23, 137.94, 137.91, 132.99, 132.22, 129.95, 129.21 (2C), 128.95, 127.17 (2C), 120.90, 119.35, 116.94, 113.61, 112.75. ESI-MS m/z: 375.1 (MH^+). HRMS calcd. for $C_{20}H_{14}N_4O_4(MH^+)$, 375.1088; found, 375.1087.

10.6. 4-((2-Phenylimidazo[1,2-a]pyridin-3-yl)amino)phthalic acid (10b). Treatment of pyridin-2-amine (**4b**), benzaldehyde (**5a**), and dimethyl 4-isocyanophthalate (**3d**) as outlined in general procedure D provided dimethyl 4-((2-phenylimidazo[1,2-a]pyridin-3-yl)amino)phthalate as a green solid (3.1% yield). ESI-MS m/z: 402.2 (MH^+). Treatment of dimethyl 4-((2-

phenylimidazo[1,2-a]pyridin-3-yl)amino)phthalate as outlined in general procedure E (rt, 24 h) and purification by preparative HPLC (linear gradient of 5% B to 20% B over 20 min with a flow rate 20 mL/min; retention time = 16.5 min) provided 4-((2-phenylimidazo[1,2-a]pyridin-3-yl)amino)phthalic acid as a yellow solid (55% yield, two steps). ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.85 (s, 1H), 8.02 (d, J = 7.0 Hz, 2H), 7.99 (d, J = 6.8 Hz, 1H), 7.75 – 7.69 (m, 1H), 7.66 (d, J = 9.0 Hz, 1H), 7.42 (t, J = 7.7 Hz, 2H), 7.35 (ddd, J = 8.8, 6.7, 1.3 Hz, 1H), 7.30 (t, J = 7.4 Hz, 1H), 6.95 (t, J = 6.7 Hz, 1H), 6.67 (brs, 1H), 6.51 (brs, 1H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 193.73, 169.73, 167.84, 148.73, 142.46, 138.07, 138.05, 135.07, 133.81, 129.96, 129.64, 129.05 (2C), 128.21, 126.87 (2C), 126.00, 123.57, 117.95, 117.67, 113.10. ESI-MS m/z: 374.1 (MH^+). HRMS calcd. for $C_{21}H_{16}N_3O_4$(MH^+), 374.1135; found, 374.1133.

Table S2. Evaluation of analogues based imidazo[*1,2-a*]pyrazin-3-amine core using gel-based TDP1 assay *in vitro*.



Compd.[i]	M7	7a	7b	7c	7d	7e	7f	7g	7h
R^1	CO_2H	CO_2Et	CO_2H	$CONH_2$	CO_2H	CO_2H	CO_2H	H	$CONH_2$
R^2	H	H	H	H	H	H	H	H	H
R^3	H	H	H	H	H	H	OH	OMe	H
R^4	H	H	H	OMe	H	OH	H	CO_2H	Me
R^5	H	H	H	H	OH	H	H	H	H
R^6	OH	OH	H	H	H	H	H	H	H

IC_{50}(μM)[ii]	3.0	>1000	1.5	>1000	127	20	18	2300	>1000
Compd.[i]	**7i**	**7j**	**7k**	**7l**	**7m**	**7n**	**7o**	**7p**	**7q**
R^1	CONHMe	H	H	H	H	H	H	H	H
R^2	H	CO_2Me	H	CO_2H	CO_2H	CO_2H	CO_2H	CO_2H	CO_2H
R^3	H	H	CO_2Me	H	H	H	H	H	H
R^4	H	H	H	H	H	H	OH	$N(CH_2CH_2)_2O$	NMe_2
R^5	H	H	H	H	H	OH	H	H	H
R^6	H	OH	OH	H	OH	H	H	H	H
IC_{50}(μM)[ii]	>1000	>1000	>1000	>1000	370	2050	216	942	2900
Compd.[i]	**7r**	**7's**[iii]	**7't**[iii]	**7v**	**7w**	**7x**	**7y**	**7'z**[iii]	**M8**
R^1	H	H	H	CO_2H	CO_2H	CO_2H	CO_2H	CO_2H	H
R^2	CO_2Et	H	CO_2H	H	H	H	H	H	CO_2H
R^3	H	CO_2H	H	H	H	H	H	H	H
R^4	CO_2H	OH	CO_2H	NMe_2	Cl	H	H	F	Me
R^5	H	H	H	H	H	Cl	Br	H	H
R^6	H	H	H	H	H	H	H	H	H
IC_{50} (μM)[ii]	>1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000	233

Note: [i]Commercially available samples except **7's**, **7't**, **7'z**; [ii]The half maximal inhibitory concentration IC_{50} value are evaluated by TDP1 gel-based assay *in vitro*; [iii]Preparation method shown in experimental section.

III. X-RAY CRYSTALLOGRAPHY

1. Protein expression, purification, and crystallization. The catalytic domain of TDP1 (residues S148-S608) was expressed and purified as previously reported.[2] Crystals of TDP1 were grown by the hanging drop vapor diffusion method by mixing 2 μL of TDP1 (20 mg/mL in 25 mM Tris-HCl pH 7.2, 150 mM NaCl, 2 mM tris(2-carboxyethyl)phosphine) with 2 μL of well

solution composed of 0.1 M MOPS/HEPES pH 7.5, 10% (w/v) PEG 8000, 20% (v/v) ethylene glycol, 0.03 M sodium fluoride, 0.03 M sodium bromide, 0.03 M sodium iodide, 0.03 M sodium bromide and sealed over 500 μL well solution in a Nextal 15-well crystallization plate (Qiagen). To form the protein-inhibitor complex, crystals were transferred to a solution of mother liquor supplemented with 10 mM **10a** or **10b** (dissolved in DMSO, 10% (v/v) final DMSO concentration in drop) and soaked for 24 hours. Crystals for data collection were harvested with a LithoLoop (Mitegen) and flash-cooled by plunging into liquid N_2.

2. Data collection, structure determination, and refinement. X-ray diffraction data sets were collected remotely at the Advanced Photon Source, SER-CAT beamline 22-ID, Argonne National Laboratory. Data were collected using a wavelength of 1.0000 Å, a crystal to detector distance of 200 mm, exposure time of 0.5 seconds, and an oscillation range of 0.5° with an Eiger 16M detector. Diffraction images were processed using HKL3000.[14] The structures were determined by molecular replacement using the previously reported structure of TDP1 (PDB code: 6N19, chain A)[2] as a search model after removing all solvent and ligand molecules and searching for two molecules in the asymmetric unit with the program PHASER[15] in the PHENIX suite of programs.[16] Electron density maps were examined for difference electron density features (contoured at 3.0σ) to identify bound inhibitor. The coordinate files for the inhibitors were prepared using the molinspiration server (www.molinspiration.com) and .cif files for use during refinement were generated using eLBOW[17] in PHENIX. Iterative rounds of manual rebuilding of the structures were performed with COOT[19, 20] followed by refinement with phenix.refine.[18] Water molecules were identified with COOT, manually inspected, and refined with phenix.refine. Model quality and structure validation was performed using MolProbity.[19]

Table S3. Crystallographic data collection and refinement statistics.

Data collection statistics:		
	TDP1-10a	**TDP1-10b**
Program used:	HKL3000	HKL3000
Beamline	APS, SER-CAT, 22-ID	APS, SER-CAT, 22-ID
Wavelength (Å)	1.0000	1.0000
Space group	$P2_12_12_1$	$P2_12_12_1$
a, *b*, *c* (Å)	50.03, 105.82, 194.21	49.97, 105.03, 193.59
α, β, γ (°)	90, 90, 90	90, 90, 90
Resolution range*	50-1.86 (1.89-1.86)	50.0-1.70 (1.73-1.70)
Number of unique reflections	87892 (4308)	108780 (5192)
Completeness (%)	99.8 (100)	95.4 (91.4)
Redundancy	6.6 (6.8)	5.3 (5.1)
Mean I/σ (I)	38.4 (4.2)	26.5 (2.2)
R_{sym}	0.099 (0.750)	0.079 (0.889)
R_{pim}	0.047 (0.312)	0.036 (0.403)
CC ½ in highest resolution shell	0.812	0.714
Refinement statistics		
Program used:	phenix.refine	phenix.refine
Resolution range (Å)	48.55-1.86	48.40-1.70
Number of reflections used in refinement	87278	107820
R/R_{free}	0.182/0.219	0.189/0.219
No. of atoms		
Protein, chain A	3634	3618
Protein, chain B	3603	3584
10a	28 (A), 28 (B)	28 (A), 12 (B)
10b	-	
Water	537	593
Ethylene glycol	16	16
Average B factor ($Å^2$)		
Protein, chain A	34.2	22.8
Protein, chain B	41.3	29.2
10a	44.3 (A), 58.1 (B)	
10b		50.4 (A), 39.6 (B)
Water	46.0	35.3
Ethylene glycol	45.0	30.2
Root-mean-square-deviation from ideal		

Bond length (Å)	0.007	0.007
Bond angles (°)	0.88	0.83
Ramachandran plot		
Favored (%)	98.0	97.9
Allowed (%)	1.8	2.0
Outliers (%)	0.2	0.1
Molprobity analysis		
All atoms contact clash score	4.0 (98^{th} percentile)	3.9 (97^{th} percentile)
Molprobity score	1.2 (99^{th} percentile)	1.2 (99^{th} percentile)
PDB accession code	6W7L	6W7K
*Values in parenthesis are for the highest resolution shell		

IV Synergistic effect of TDP1 inhibitors with camptothecin (CPT) in human colon cancer cell line HCT116.

The synergistic effects of the TDP1 inhibitors with CPT were tested in human colon cancer cell line HCT116 based on cell viability (Table S4, Fig. S4-S7). Cells were first seeded in a 384-well black-clear plate until 30% confluency and then incubated with a serial dilution of CPT at the range of 0-100 nM in the present or the absence of TDP1 inhibitors for 72 h at 37^{o} C. Viable cell numbers were counted from the brightfield images taken by Biotek Cytation 5 (Fig. S4-S5). Synergistic scores of each TDP1 inhibitors were calculated and collected based on the data analysis from SynergyFinder (Fig. S6). [20] Fractional affect (Fa) and combination index (Ci) were analyzed with CompuSyn software (Fig. S7).

Table S4. The TDP1 selectivity of lead compounds over TDP2 using gel-based assays *in vitro*.

Compound	TDP1 IC_{50} (μM)[i]	TDP2 IC_{50} (μM)[ii]	TDP1 Selectivity[iii]	WCE hTDP1 IC_{50} (μM) [iv]	Ki (μM)[v]	Synergy Score [vii]
M7	19 ± 5.9 (3.0)[vi]	>100	5	>100	--	15.6
M8	>100 (233)[vi]	>100	--	--	--	--
7b	0.71 ± 0.03 (1.54)[vi]	>100	>140	28.7 ± 8.7	0.309 ± 0.122	3
8a	8.72 ± 1.81 (4.0) [vi]	>100	>11	>100	12.49 ± 0.49	-1.3
8n	2.94 ± 0.47 (1.5)[vi]	>100	>34	>100	--	11.3
8p	2.98 ± 0.24 (1.8)[vi]	>100	>33	50.2 ± 15.8	--	9.1
8q	3.03 ± 1.19 (1.8)[vi]	80	26	38.4 ± 9.95	--	9.5
8s	1.32 ± 0.22 (2.9)[vi]	>100	>75	>100	--	20.7
10a	>100 (45.5)[vi]	>100	--	--	109.9 ± 30.9	4
10b	15.9 ± 1.21 (11)[vi]	>100	>6	>100	17.40 ± 2.80	17.9

Note: [i]The half maximal inhibitory concentration (IC_{50}) based on gel based TDP1 fluorescence assay. [ii]The half maximal inhibitory concentration (IC_{50}) based on gel based TDP2 fluorescence assay. [iii]TDP1 selectivity based on the ratio of IC_{50} values of TDP1/TDP2. [iv]The half maximal inhibitory concentration (IC_{50}) based on gel based whole cell extract (WCE) human TDP1 fluorescence assay.[21] [v]Constant of inhibition Ki (Fig. S3). [vi] Separated data from previous experiments. [vii] Synergy Scores of TDP1 inhibitors with camptothecin (CPT) were caculated based on SynergyFinder 2.0 to reflect the synergistic effect (Fig. S6).[20]

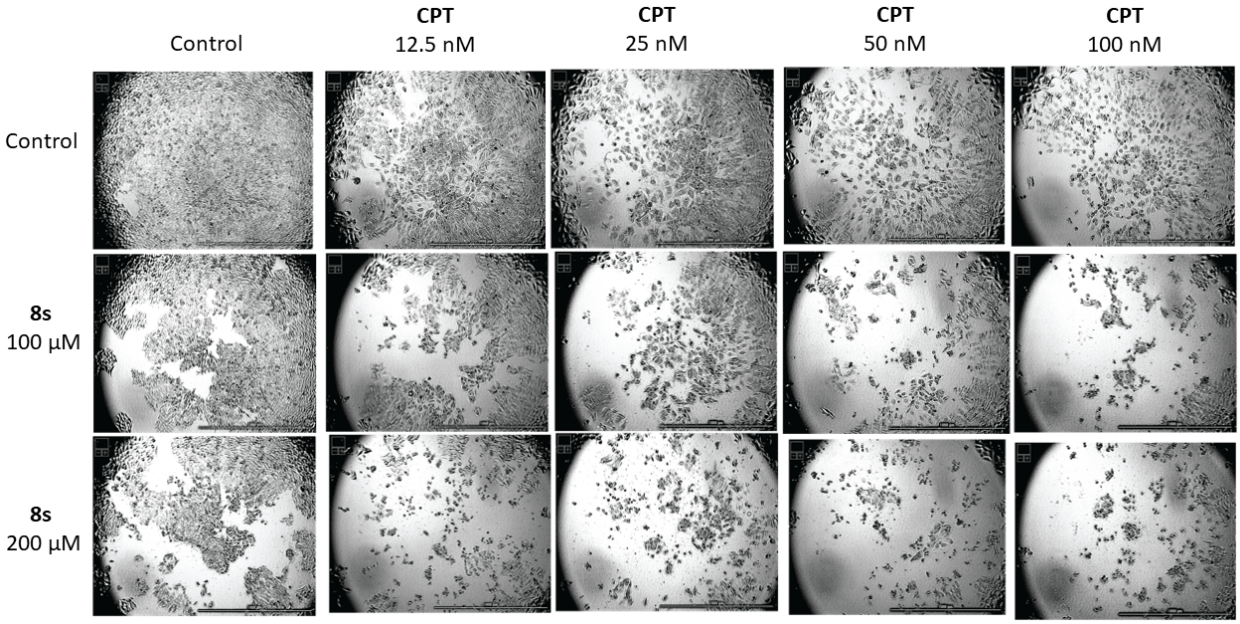


Figure S4. Synergistic effect of TDP1 inhibitor **8s** with camptothecin (CPT) in human colon cancer cell line HCT116. The brightfield images were taken by Biotek Cytation 5.

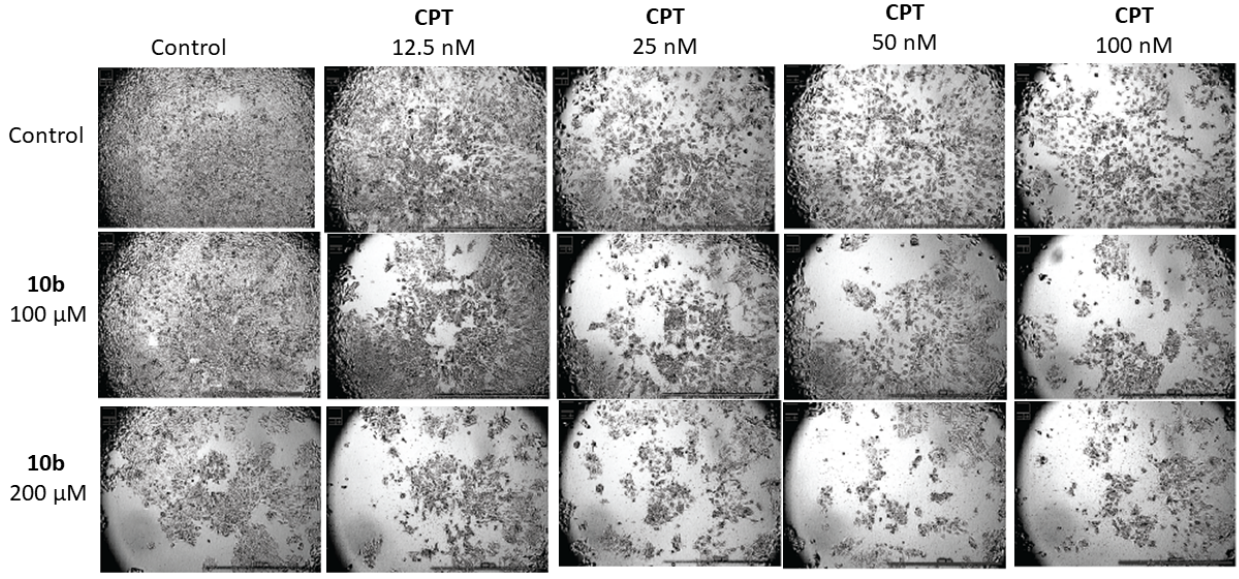


Figure S5. Synergistic effect of TDP1 inhibitor **10b** with camptothecin (CPT) in human colon cancer cell line HCT116. The brightfield images were taken by Biotek Cytation 5.

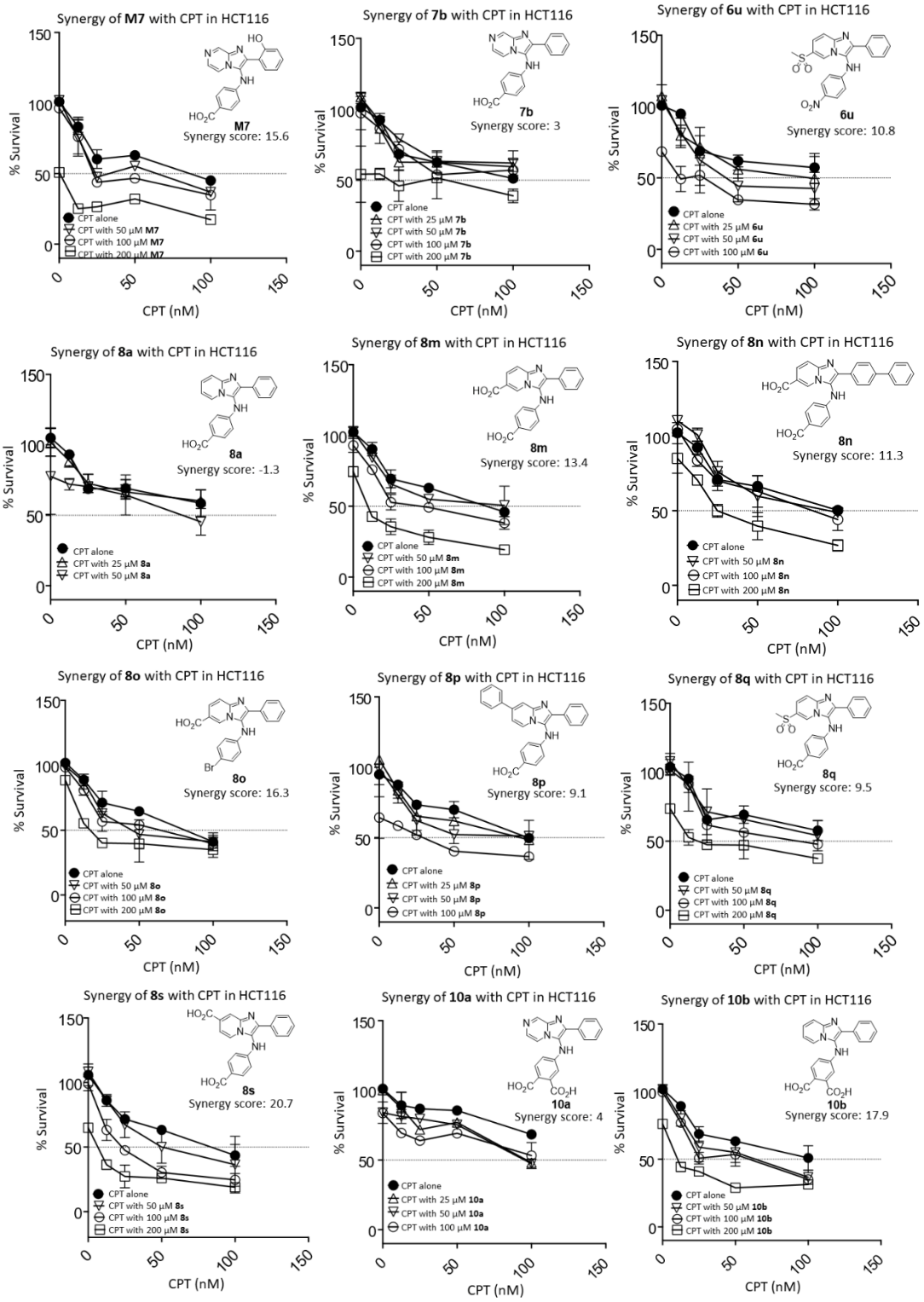


Figure S6. Synergistic effect of selective TDP1 inhibitors with camptothecin (CPT) in human colon cancer cell line HCT116 based on cell viability. Synergy Scores were calculated based on SynergyFinder 2.0.[20]

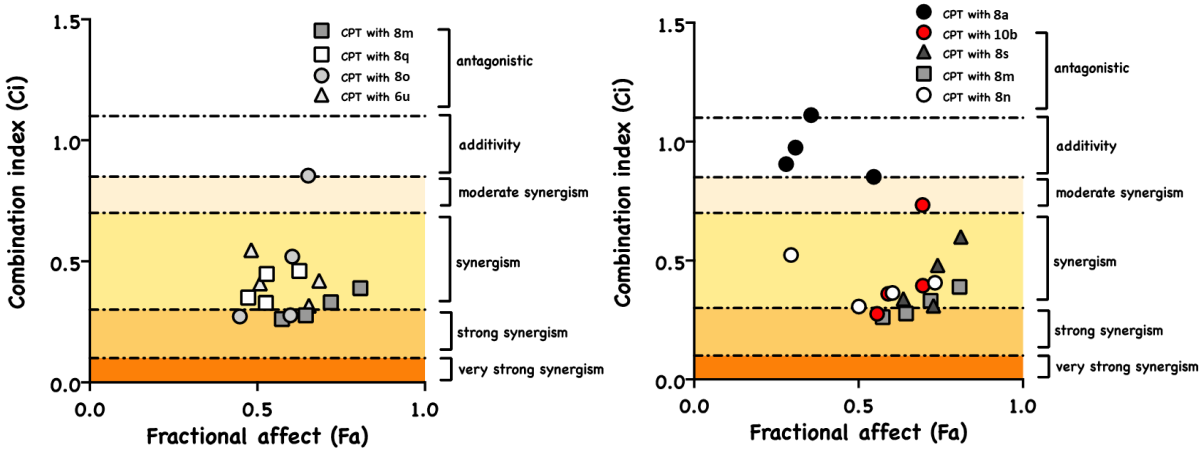


Figure S7. Fractional affect (Fa) and combination index (Ci) were analyzed with CompuSyn software.

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