Supporting Information

A PARP inhibitor with selectivity toward ADP-ribosyltransferase ARTD3/PARP3

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Supplementary Tables

Supplementary Table 1. STO1131 stabilizes ARTD3/PARP3 and other ADP-ribose transferases in thermal shift assays. [a]

Name	Alt. Name	T_m shift (°C)
ARTD1	PARP1	2.0±0.1
ARTD2	PARP2	1.4±0.3
ARTD3	PARP3	8.5±0.2
ARTD4	PARP4	0.3±0.4
ARTD5	TNKS1	0.0±0.04
ARTD6	TNKS2	0.2±0.1
ARTD7	PARP15	0.2±0. 5
ARTD8	PARP14	-0.3±0.5
ARTD9	PARP9	-0.1±0.6
ARTD10	PARP10	0.5±0.5
ARTD11	PARP11	n.d.
ARTD12	PARP12	0.1±0.1
ARTD13	PARP13	0.2±0.1
ARTD14	PARP7	n.d.
ARTD15	PARP16	0.5±0.2
ARTD16	PARP8	n.d.
ARTD17	PARP6	n.d.

[a] Determined by differential scanning fluorimetry. Values represent means ± S.D. of two to four independent experiments. n.d., not determined.

Supplementary Table 2. Crystallographic data collection and refinement statistics. [a]

Data collection	ARTD3•ME0355	ARTD3•ME0354	ARTD3•ME0328	ARTD1•ME0328
Synchrotron	ESRF	Diamond	Diamond	Bessy
Beam line	ID14-1	I04	I04	BL14.1
Wavelength (Å)	0.93340	0.99190	0.99190	0.91841
Space group	$P2_1$	$P2_1$	$P2_1$	$P2_1$
Unit cell dimensions				
a, b, c (Å)	54.8, 56.8, 56.9	55.2, 56.8, 56.6	54.7, 56.7, 57.0	87.1, 49.4, 183.5
α, β, γ (°)	90, 112.77, 90	90, 112.66, 90	90, 112.74, 90	90, 101.82, 90
Resolution (Å)	52.5 – 1.86 (1.96 – 1.86)	30.0 – 1.80 (1.85 – 1.80)	30.0 – 1.80 (1.85 – 1.80)	30.0 - 2.89 (2.90 -2.89)
Unique reflections	25790 (2775)	29876 (2165)	29828 (2148)	34835 (376)
R-merge (%)[b]	4.8 (19.8)	9.1 (48.0)	8.0 (44.1)	10.0 (56.4)
Completeness (%)	99.0 (94.5)	99.2 (98.2)	99.4 (97.6)	99.9 (99.7)
Redundancy	7.4 (6.4)	7.5 (7.2)	7.4 (7.1)	6.1 (6.1)
$\langle I/\sigma I \rangle$	30.4 (9.1)	14.5 (3.4)	21.5 (3.9)	15.5 (3.1)
Refinement				
Resolution (Å)	52.5 - 1.90 (2.00 - 1.90)	28.4 - 1.80 (1.85 - 1.80)	28.4 - 1.80 (1.85 - 1.80)	27.2 - 2.89 (2.98 - 2.89)
R-all (%)[c]	17.2 (27.1)	16.3 (22.0)	16.4 (21.5)	21.5 (25.6)
R-free (%)[c]	21.9 (37.7)	19.4 (24.8)	20.3 (25.8)	28.5 (37.4)
r.m.s.d. bond length (Å)	0.019	0.018	0.018	0.010
r.m.s.d. bond angle (°)	1.7	1.6	1.5	1.2
B-factors ($Å^2$)				
Protein	18.3	15.9	15.1	61.9
Ligand	15.0	18.2	14.2	35.2
Water	23.0	23.0	21.8	-
Ramachandran plot [d]				
Most favoured (%)	98.9	99.1	98.6	94.0
Allowed (%)	100	100	99.7	99.6

[a] Values in parentheses are for the outermost resolution shell.

[b] $R_{merge} = \sum| I - \langle I \rangle | / \sum I$, where I is the intensity measurement for a given reflection and $\langle I \rangle$ is the average intensity for multiple measurements of this reflection.

[c] $R = \sum|| F_{obs} | - | F_{calc} || / \sum| F_{obs} |$, where R_{free} is calculated for a randomly chosen 5-10% of reflections, which were not used for structure refinement, and R_{all} is calculated for all reflections.

[d] The Ramachandran plot was calculated using the Molprobity server.[1]

Supplementary Table 3. ME0328 and ME0355 specifically inhibit the enzymatic activity of ARTD3/PARP3 *in vitro*.[a]

			ME0328		ME0355	
Name	Alt. Name	K_m (µM)	T_m shift (°C)	IC_{50} (µM)	T_m shift (°C)	IC_{50} (µM)
ARTD1	PARP1	10±1.9	1.8±0.1	6.3±0.62	2.0±0.2	9.1±2.6
ARTD2	PARP2	159±5	1.2±0.1	10.8±4.0	0.7±0.6	
ARTD3	PARP3	230±47	8.5±0.6	0.89±0.28	9.7±0.4	1.3±0.2
ARTD4	PARP4	n.d.		n.d.[b]		n.d.[b]
ARTD5	TNKS1	41±11	0.1±0.1	47.3±29.0	0.1±0.1	n.d.
ARTD6	TNKS2	174±43		34.3±17.0		n.d.
ARTD7	PARP15	6.3±0.6		>100		n.d.
ARTD8	PARP14	164±17	-0.2±0.1	>100	0.3±0.3	n.d.
ARTD9	PARP9	n.d.[c]		n.d.[c]		n.d.[c]
ARTD10	PARP10	95±34		71.3±22.0		>50
ARTD11	PARP11	n.d.[d]		n.d.[d]		n.d.[d]
ARTD12	PARP12	n.d.		>100		>50
ARTD13	PARP13	n.d.[c]		n.d.[c]		n.d.[c]
ARTD14	PARP7	n.d.[d]		n.d.[d]		n.d.[d]
ARTD15	PARP16	n.d		n.d.[b]		n.d.[b]
ARTD16	PARP8	n.d.[d]		n.d.[d]		n.d.[d]
ARTD17	PARP6	n.d.[d]		n.d.[d]		n.d.[d]

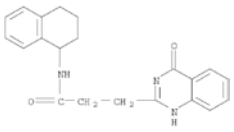
[a] Values represent means ± S.D. of three independent experiments (T_m shift data) or means ± S.E. of the fitted parameter (IC_{50} data; based on triplicates).
[b] Not determined – no transferase activity in presence of DMSO.
[c] Not determined – protein reportedly lacks ADP-ribose transferase activity.[2]
[d] Not determined – transferase domain could not be produced.

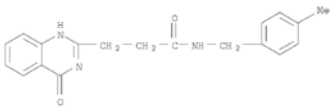
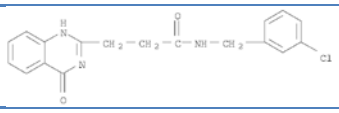
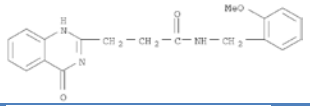
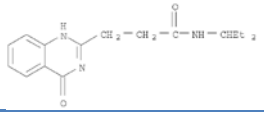
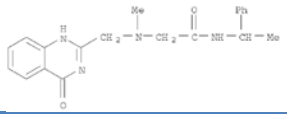
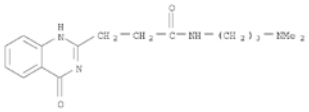
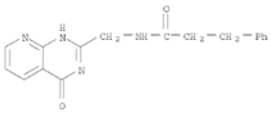
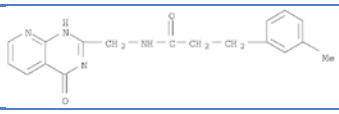
Supplementary Table 4. Physicochemical and metabolic stability profiling of ME0328.

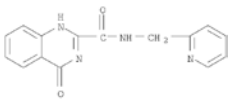
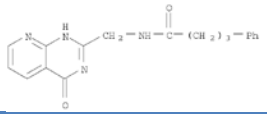
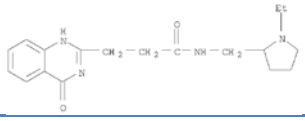
Property/mol. descriptor	ME0328	Range [a]	Comment
Formula molecular weight	321.27	Not available	
QPlogPo/w [b]	2.5	-2.0 – 6.5	Predicted octanol/water partition coefficient
QPPCaco [b]	559	<25 poor, >500 great	Predicted apparent Caco-2 cell permeability in nm/s
QPPMDCK [b]	371	<25 poor, >500 great	Predicted apparent MDCK cell permeability in nm/s
QPlogBB [b]	-1.0	-3.0 – 1.2	Predicted brain/blood partition coefficient
QPlogKhsa [b]	-0.1	-1.5 – 1.5	Prediction of binding to human serum albumin
Mean solubility (µM) [c]	25.4		
HLM CL_{int} (µl/min/mg) [c]	16.28		Internal clearance in human liver microsomes
HW rat hep CL_{int} (µl/min/1E6 cells) [c]	36.98		
HuCaco2 pH 6.5 A-to-B P_{app} (1E6 x cm/s) [c]	43.54		Apparent permeability coefficient in Caco-2 cells

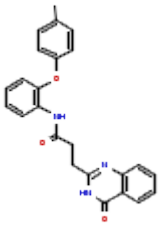
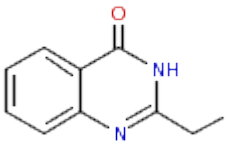
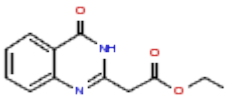
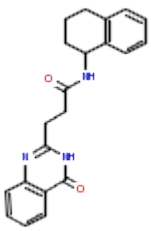
[a] For 95% of known drugs.
[b] Calculated using Qikprop 3.3 (Schrödinger Inc.).
[c] Determined by AstraZeneca R&D (Mölndal, Sweden).[3]

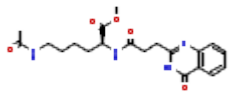
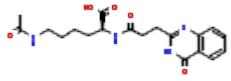
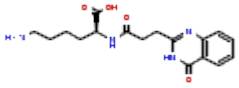
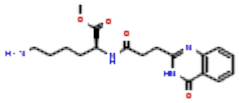
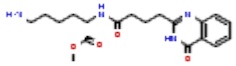
Supplementary Table 5. Database search results for similarity of ME0328/ME0355 among known bioactive compounds.

Substructure search using ME0328/ME0355 [a]		
CAS	**Structure**	**Reference**
870768-51-7		Variety of antiprion compounds discovered through an in silico screen based on cellular-form prion protein structure: correlation between antiprion activity and binding affinity. Antimicrobial Agents and Chemotherapy (2009), 53(2), 765-771.

Similarity search of compounds with ≥ 80 % similarity to ME0328/ME0355 [a]		
CAS	**Structure**	**Reference**
743451-47-0		Method using lifespan-altering compounds for altering the lifespan of eukaryotic organisms, and screening for such compounds. U.S. Pat. Appl. Publ. (2009), US 20090163545 A1 20090625.
851105-00-5		Same as 743451-47-0
785702-46-7		Same as 743451-47-0
731817-17-7		Same as 743451-47-0
794562-86-0		Same as 743451-47-0
749901-14-2		1. Identification of Non-Peptide Malignant Brain Tumor (MBT) Repeat Antagonists by Virtual Screening of Commercially Available Compounds. Journal of Medicinal Chemistry (2010), 53(21), 7625-7631 2. Same as 743451-47-0
958356-12-2		1. Pyrido pyrimidinones as selective agonists of the high affinity niacin receptor GPR109A: Optimization of in vitro activity. Bioorganic & Medicinal Chemistry Letters (2010), 20(18), 5426-5430. 2. Preparation of pyrido[2,3-d]pyrimidin-4-one derivatives as HM74A agonists. PCT Int. Appl. (2007), WO 2007134986 A1 20071129
958361-10-9		Preparation of pyrido[2,3-d]pyrimidin-4-one derivatives as HM74A agonists. PCT Int. Appl. (2007), WO 2007134986 A1 20071129

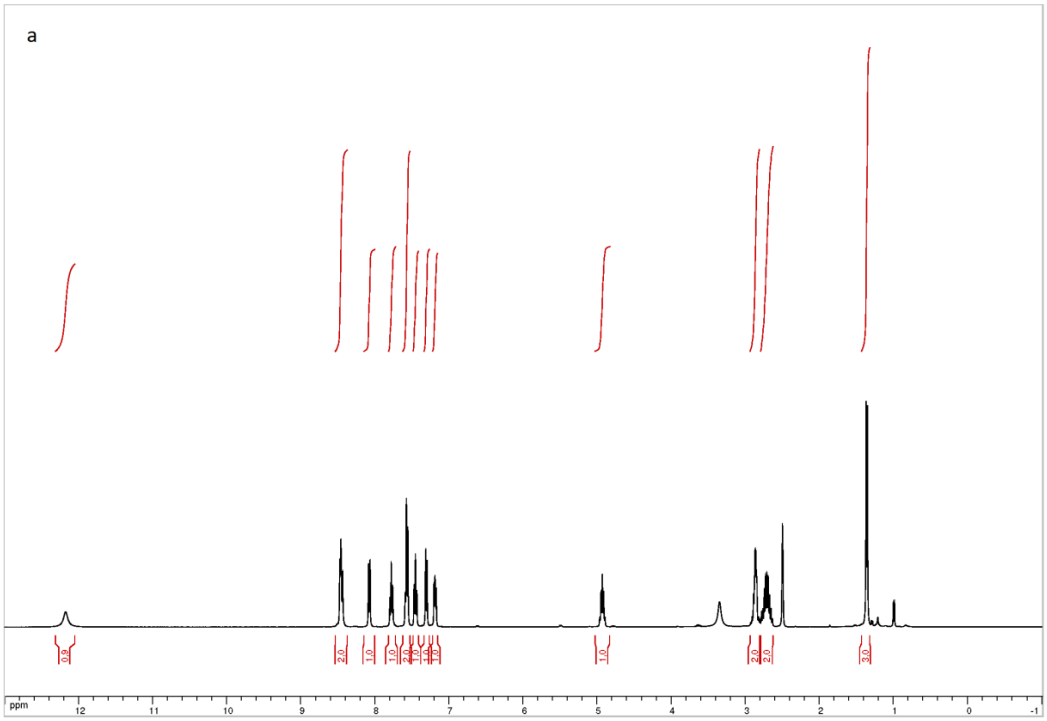
869298-87-3		Preparation of heterocyclic amides as MMP-13 inhibitors for treating osteoarthritis and rheumatoid arthritis. PCT Int. Appl. (2005), WO 2005105760 A1 20051110
958356-17-7		Same as 958361-10-9
930065-89-7		Same as 749901-14-2

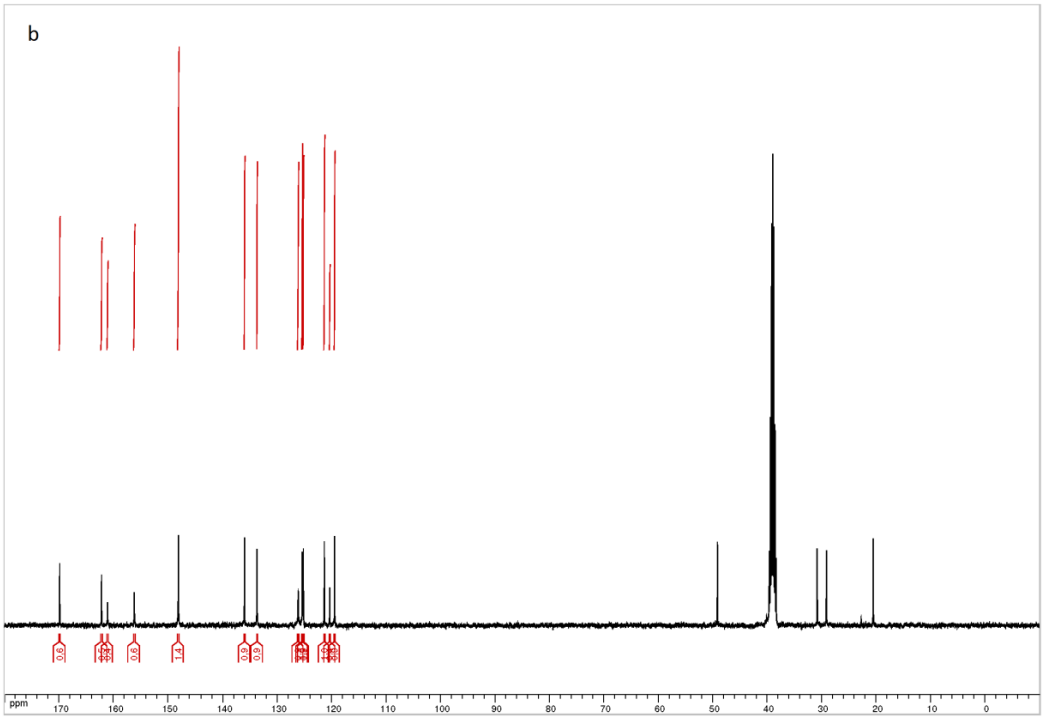
Similarity search of compounds with ≥ 80 % similarity to ME0328/ME0355 [b]		
Name	**Structure**	**Biological activity**
CHEMBL581442		TCMDC-125459 GSK Malaria Screening: Plasmodium falciparum GSK Malaria Screening: Homo sapiens
CHEMBL1164161		Inhibition of Bacillus thermoproteolyticus thermolysin
CHEMBL1164448		Inhibition of Bacillus thermoproteolyticus thermolysin
CHEMBL1631209		Antiprion activity against mouse recombinant PrP

CHEMBL1783023		Antibacterial activity against Xanthomonas oryzae. Antibacterial activity against Xanthomonas campestris pvs. Antibacterial activity against Escherichia coli. Antibacterial activity against Bacillus subtilis. Antibacterial activity against Pseudomonas fluorescens.
CHEMBL1783025		Same as CHEMBL1783023
CHEMBL1789526		Same as CHEMBL1783023
CHEMBL1789619		Same as CHEMBL1783023
CHEMBL1789671		Same as CHEMBL1783023

[a] Using SciFinder (https://www.cas.org/products/scifinder).
[b] Using ChEMBL (https://www.ebi.ac.uk/chembl).

Supplementary Figures





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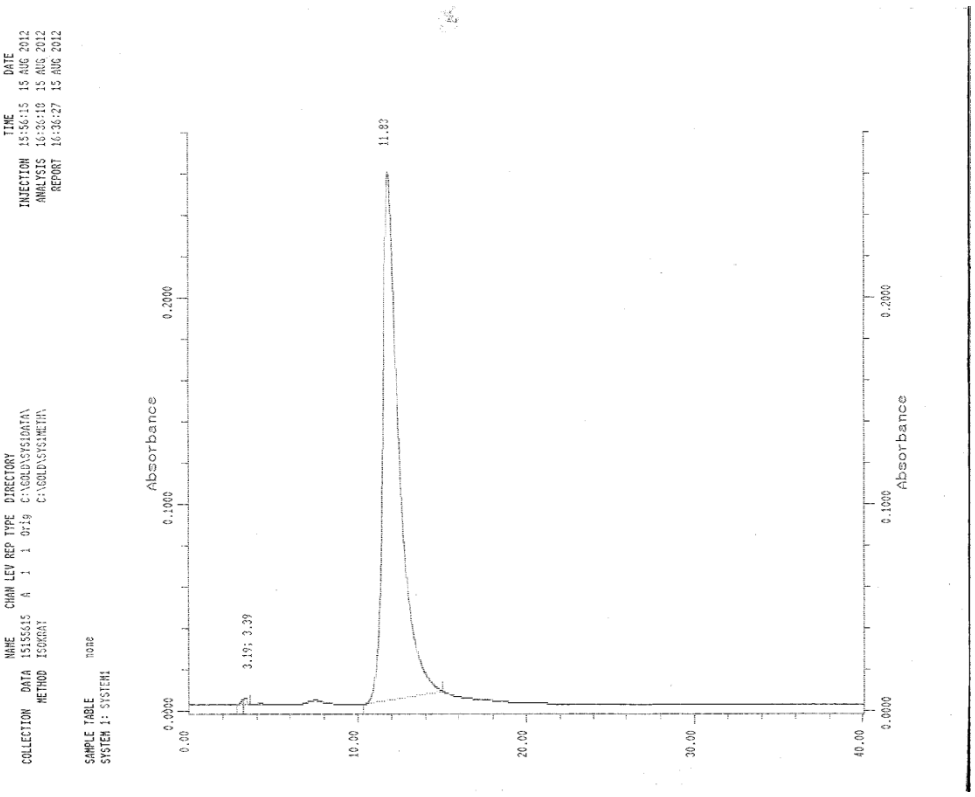
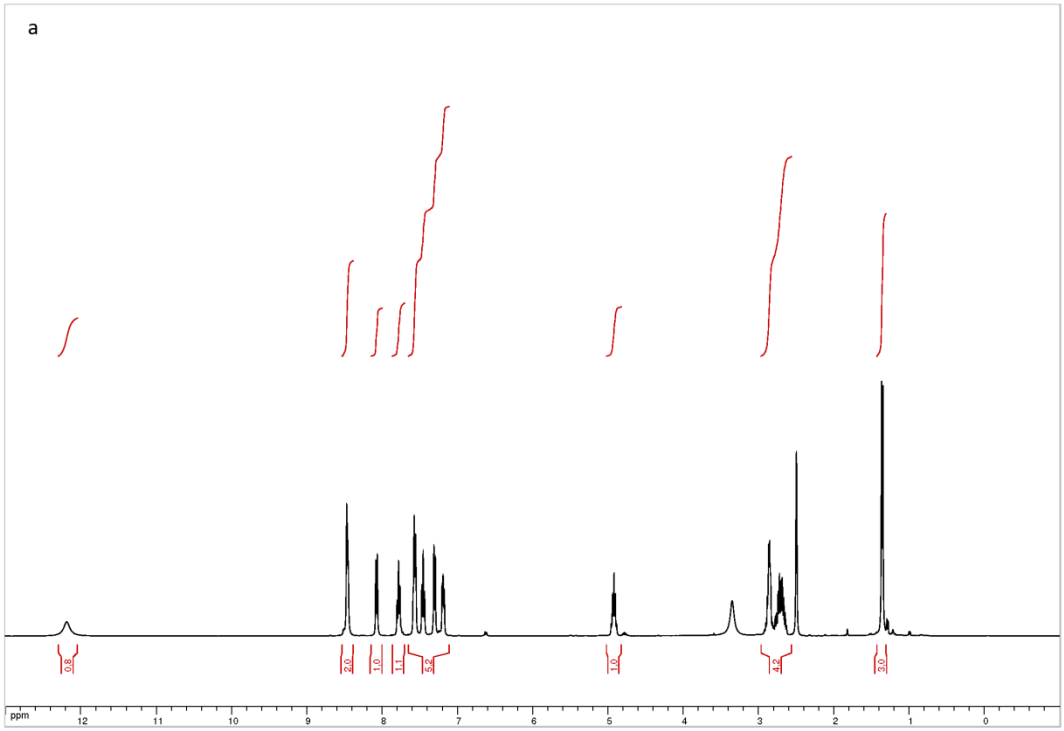


Supplementary Figure 1. Characterization of ME0354. (a) ^{1}H-NMR spectrum. **(b)** ^{13}C-NMR spectrum. **(c)** Chiral HPLC UV trace.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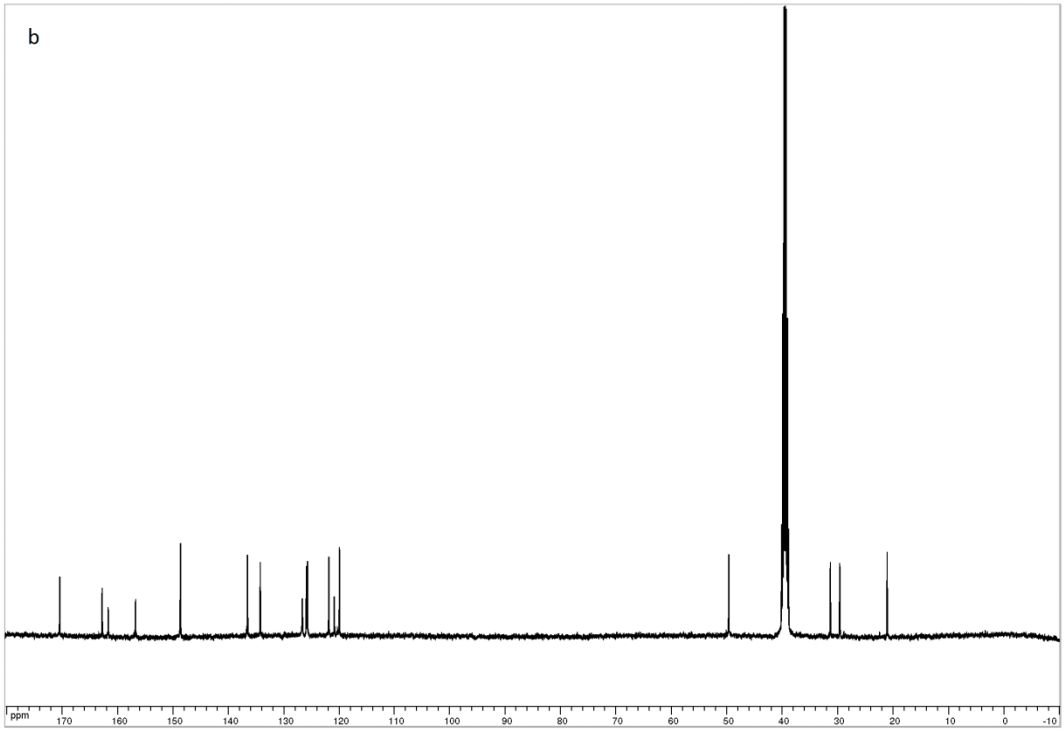
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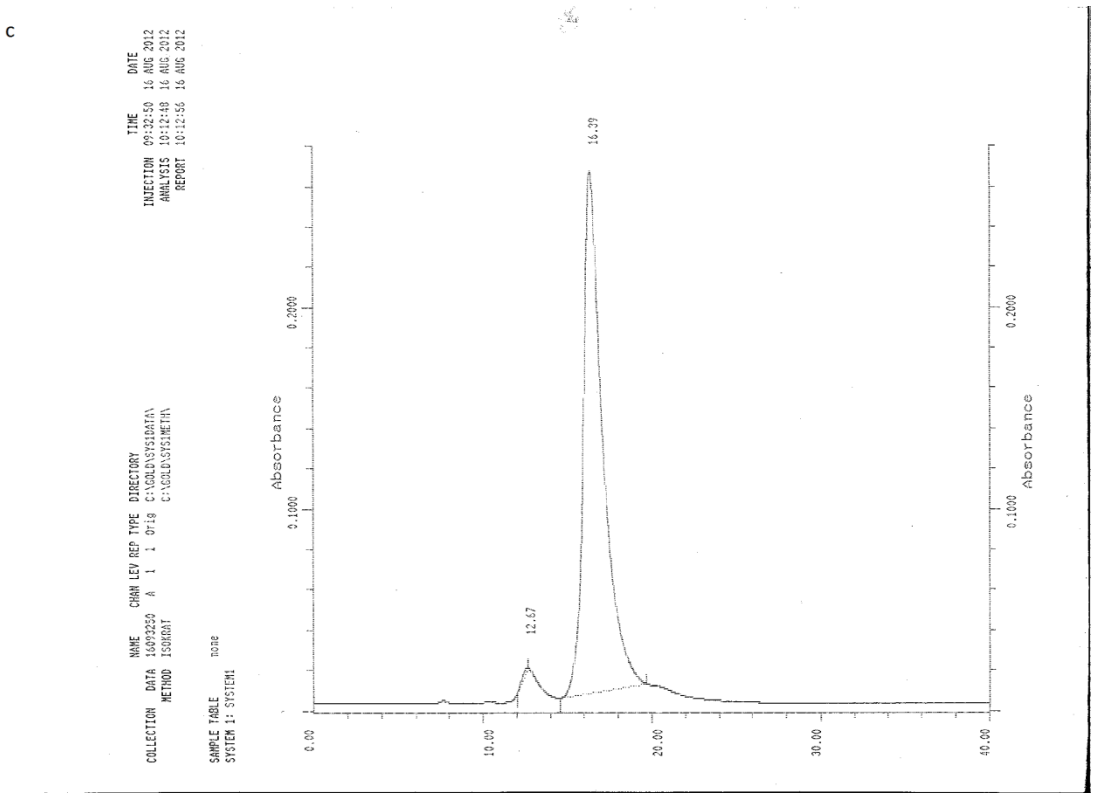
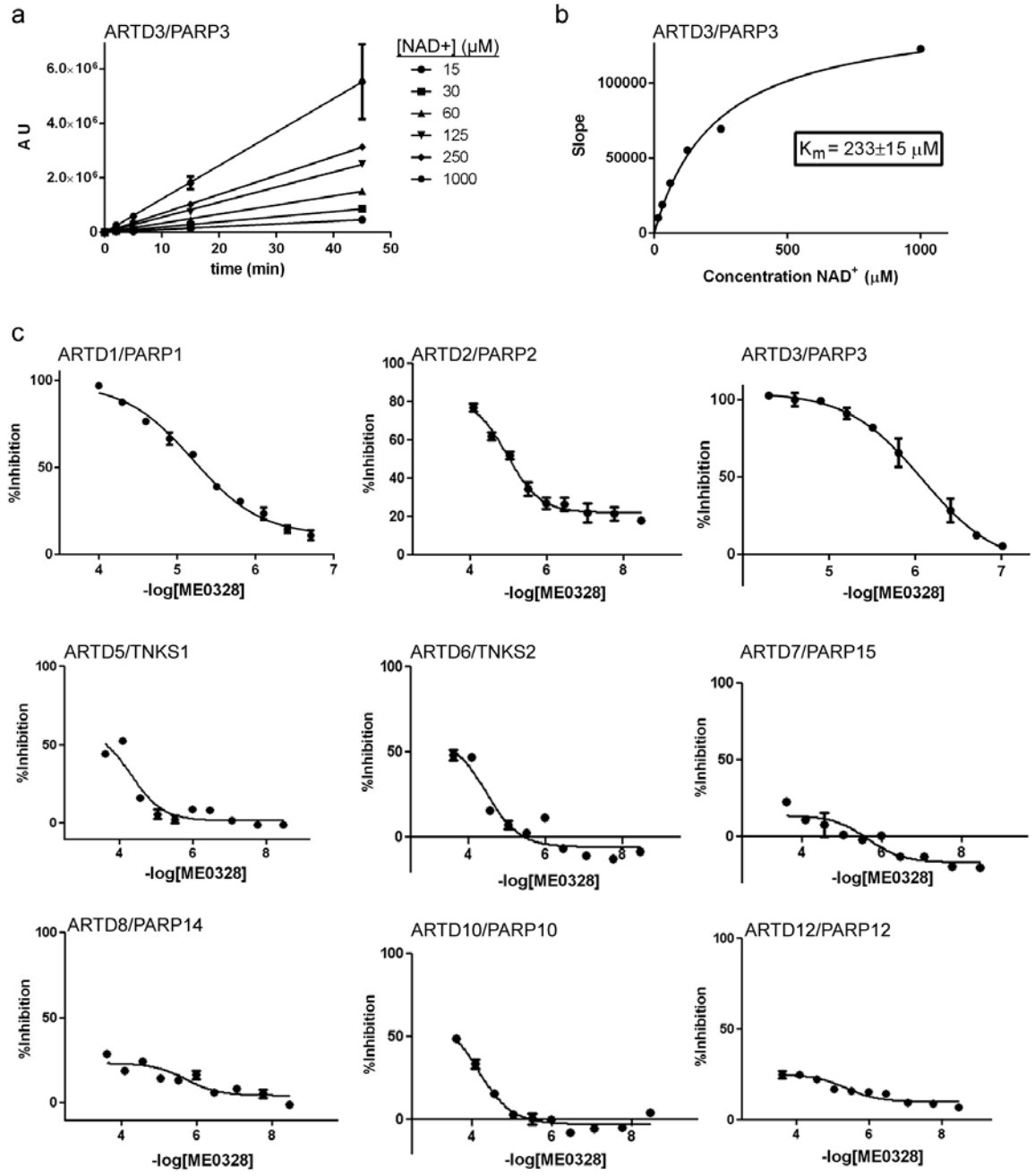
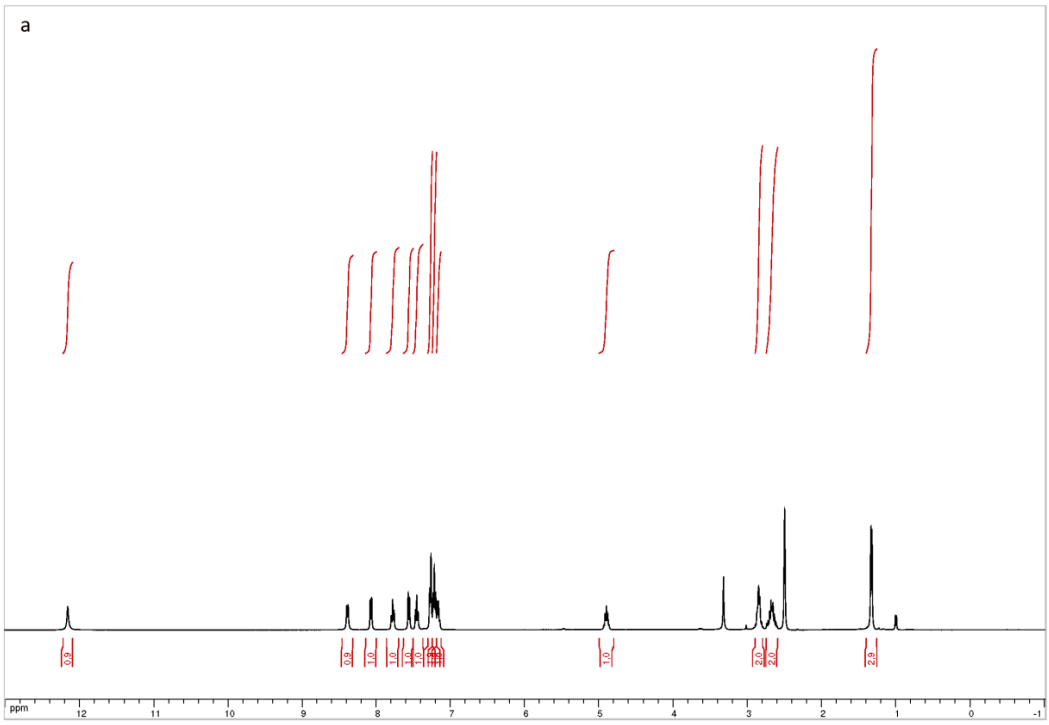


Supplementary Figure 2. Characterization of ME0355. (a) ^{1}H-NMR spectrum. **(b)** ^{13}C-NMR spectrum. **(c)** Chiral HPLC UV trace.



Supplementary Figure 3. Examples of enzymatic assay data. (**a**) Representative raw data of a K_m determination for ARTD3. All measurements were performed within the linear range of the assay. (**b**) K_m determination for ARTD3. Values listed in Supplementary Table 3 were determined accordingly. (**c**) IC50 determinations for ME0328 and 9 ARTD family members. Representative curves are shown. Values listed in Supplementary Table 3 represent means ± S.E. of the fitted parameter based on triplicates of such measurements.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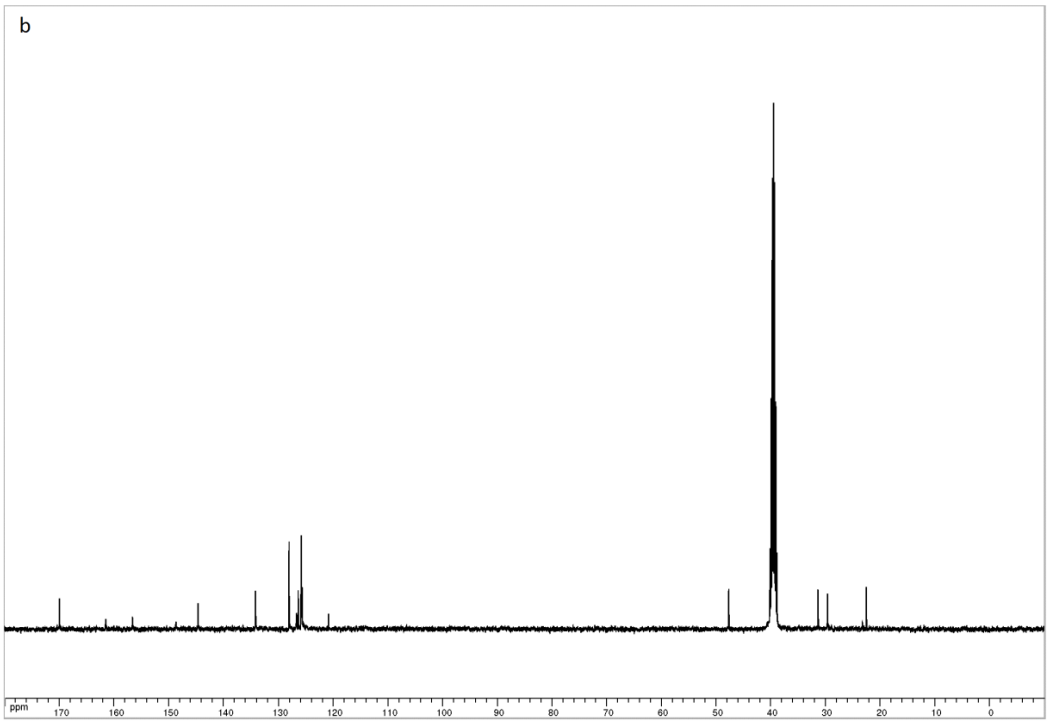
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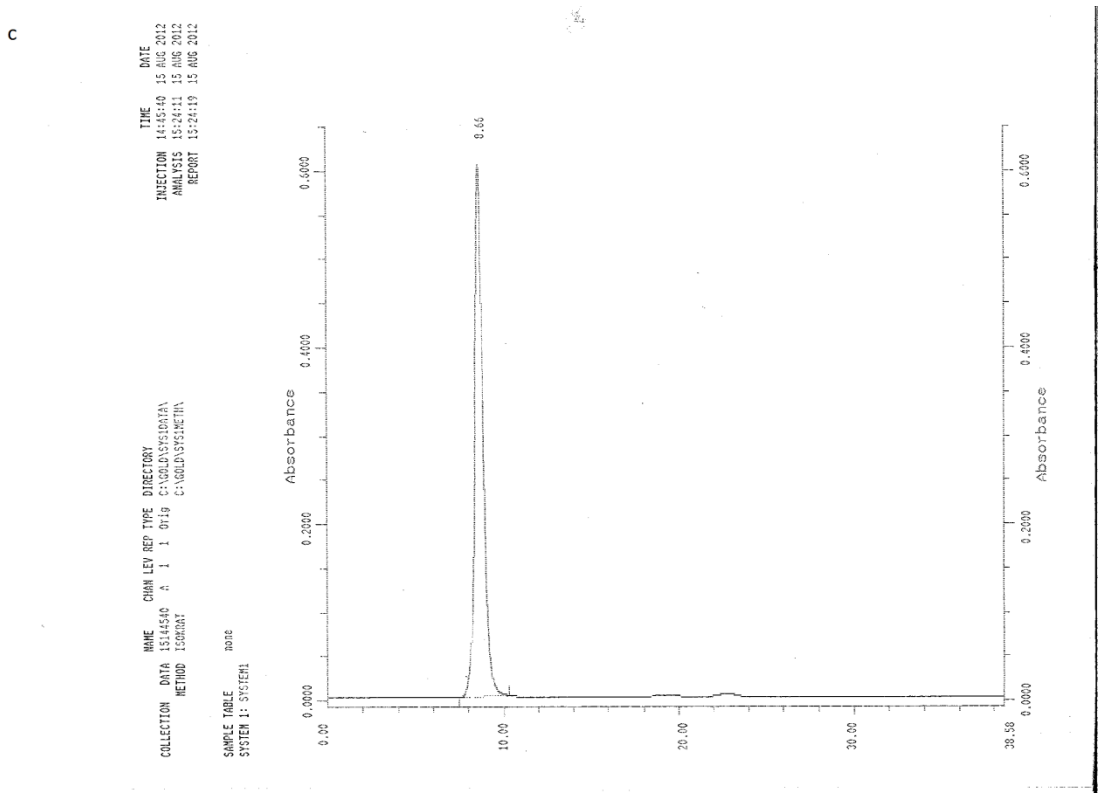
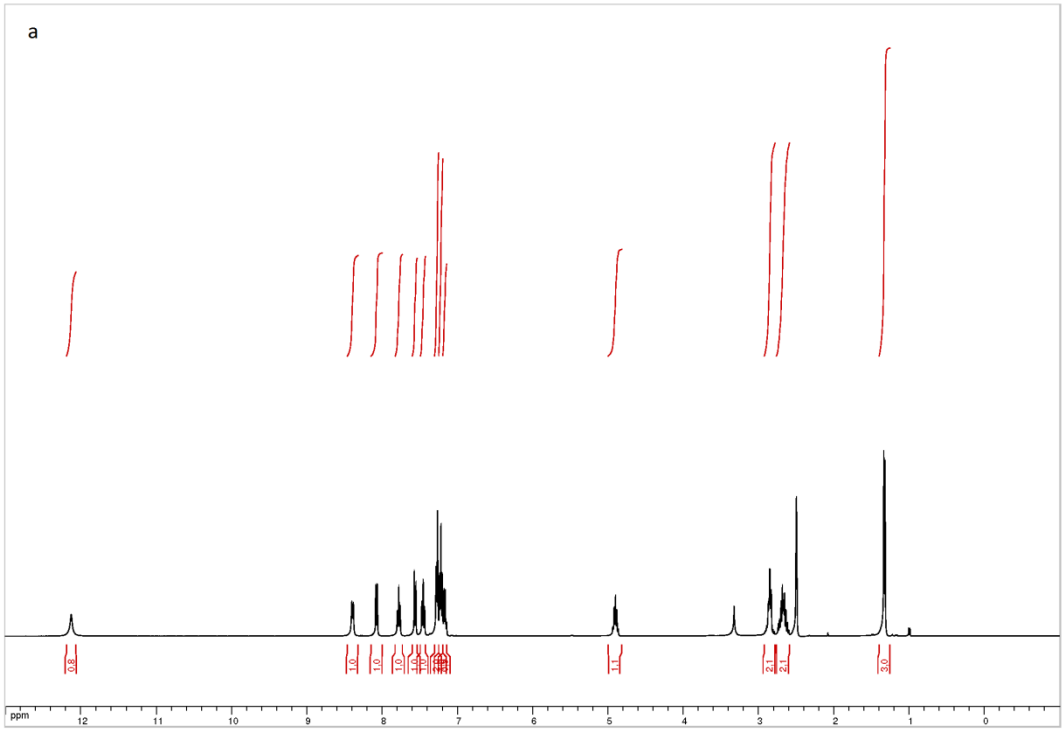


Supplementary Figure 4. Characterization of ME0327. (a) ^{1}H-NMR spectrum. **(b)** ^{13}C-NMR spectrum. **(c)** Chiral HPLC UV trace.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1.0
1.1
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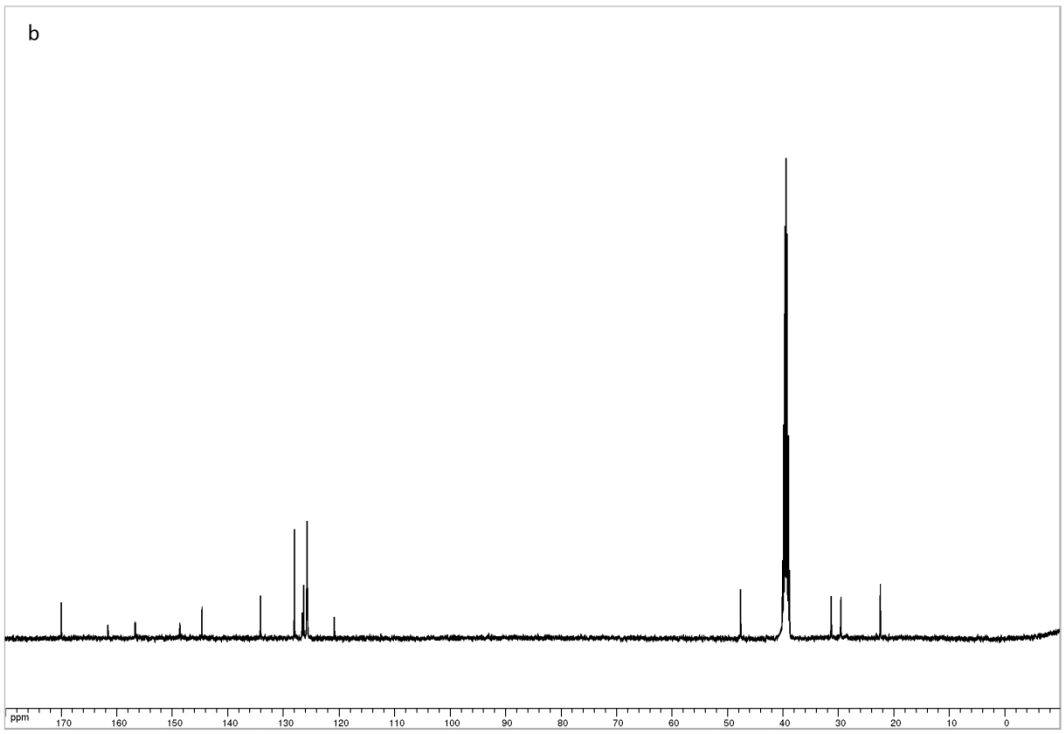
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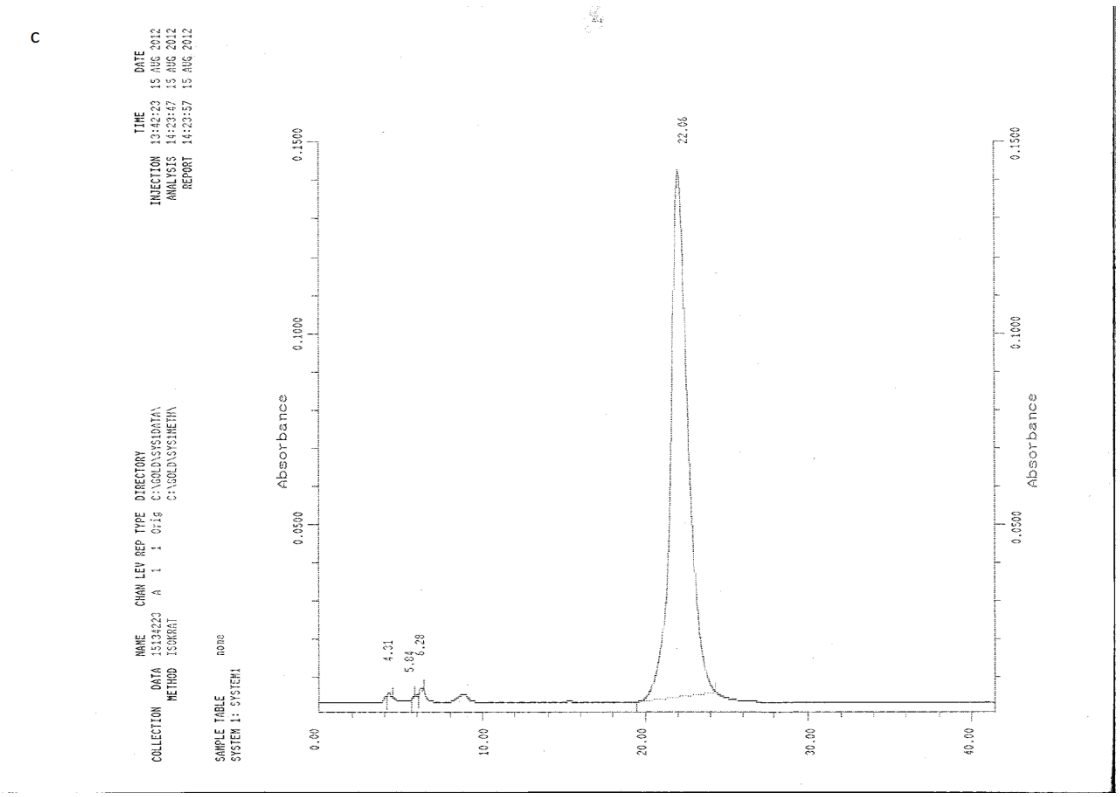
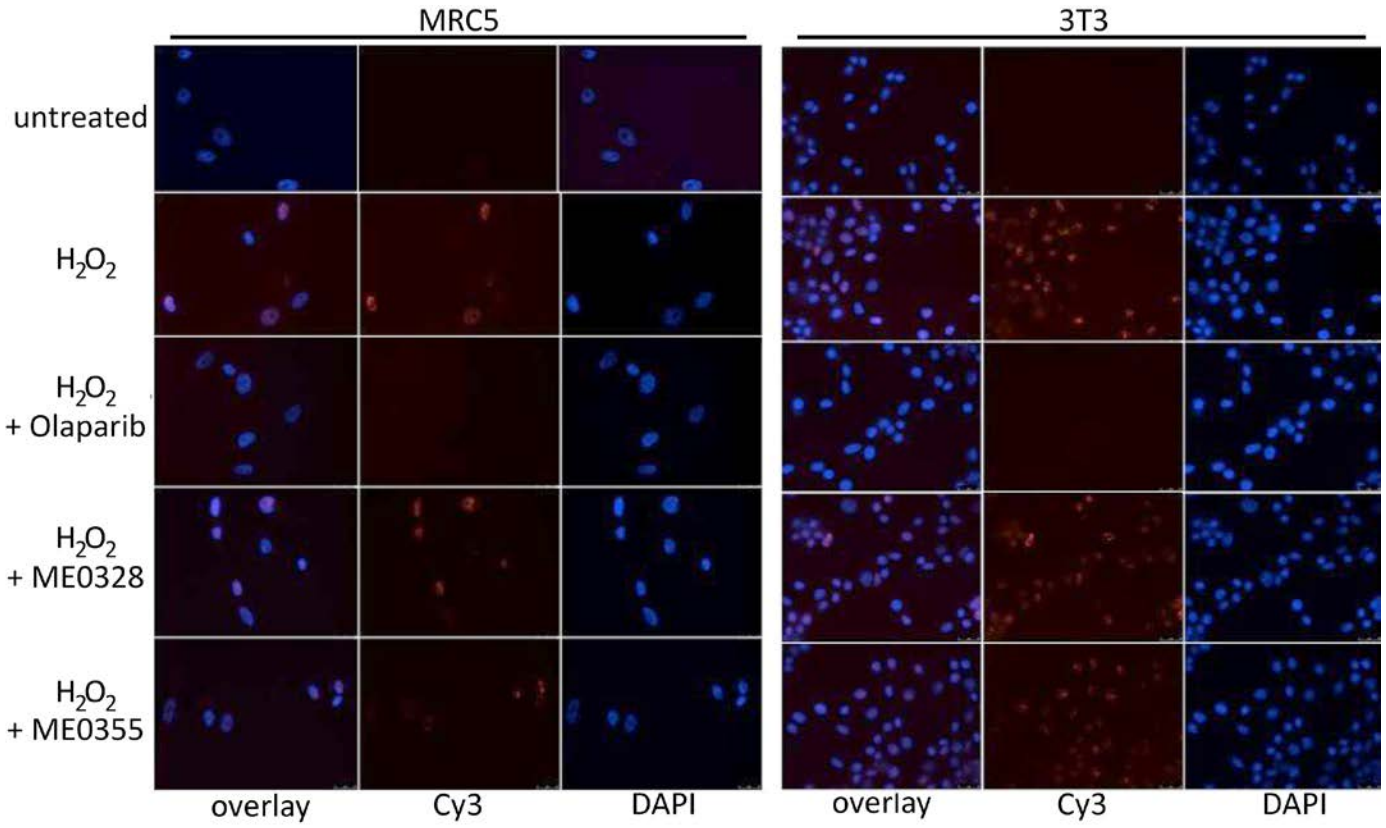


Supplementary Figure 5. Characterization of ME0328. (a) ^{1}H-NMR spectrum. **(b)** ^{13}C-NMR spectrum. **(c)** Chiral HPLC UV trace.



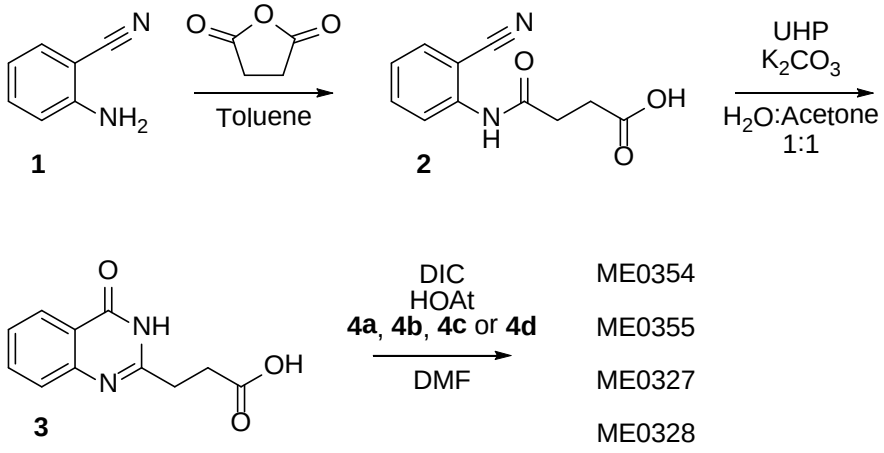
Supplementary Figure 6. **ME0328 and ME0355 have no effect on ARTD1/PARP1 activity in cells after H_2O_2-induced DNA damage.** MRC5 or 3T3 cells were pretreated for 1 hour with either Olaparib or ME0328 or ME0355 (20 μM) prior to H_2O_2 treatment (1 mM for 10 minutes). Cells were fixed (methanol/acetic acid) and stained with anti-PAR 10H antibody followed by Cy3-coupled anti-mouse antibody. Cells were co-stained with DAPI to visualize nuclei.

Supplementary Methods

General Chemical Procedures. LCMS analysis was carried out using a Waters LC system equipped with an Xterra MS C18 18.5 µm 4.6*50 mm column and an eluent system consisting of MeCN in water, both of which contained 0.2% formic acid. Detection was performed at 214 and 254 nm. Mass spectra were obtained using a Waters Micromass ZG 2000 using both positive and negative electrospray ionization. ^{1}H NMR and ^{13}C NMR spectra were recorded using a Bruker DRX-400 spectrometer in $CDCl_3$ solution (residual $CHCl_3$ (δ_H 7.26 ppm, δ_C 77.16 ppm) as internal standard) or in $(CD_3)_2SO$ solution (residual $(CH_3)_2SO$ (δ_H 2.50 ppm, δ_C 39.52 ppm) as internal standard). Optical rotations were measured on a Perkin Elmer Polarimeter 343 at 20 °C. All target compounds were ≥95% pure according to LCMS UV-traces. Chiral HPLC was performed using a Beckman system equipped with a Pirkle Covalent (S,S) Whelk-O 1 10/100 Krom FEC 25 cm x 4.6 mm column and an eluent system consisting of isopropanol and hexane in a one to one ratio. Detection was performed at 254 nm using a System Gold 166 Detector.

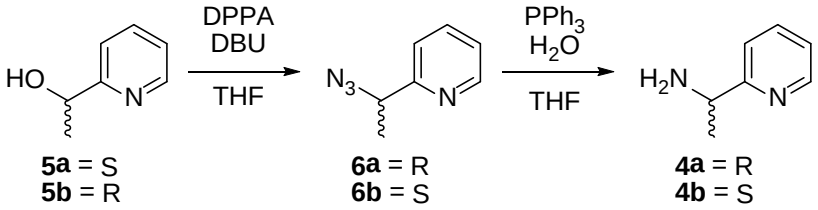
Synthetic strategy. The synthetic route to compounds ME0327, ME0328, ME0354 and ME0355 is presented in scheme 1. Reacting 2-aminobenzonitrile (**1**) with succinic anhydride in toluene gave the resulting anilide (**2**) in 96% yield. Compound **2** was then ring closed to the quinazolinone main fragment (**3**) in 68% yield using UHP and K_2CO_3 in a H_2O:acetone 1:1 mixture.[4] Finally, ME0327, ME0328, ME0354 and ME0355 were obtained from **3** in 50-78% yield using standard amide coupling reagents DIC and HOAt together with the appropriate amine in DMF.

Scheme 1. Synthesis of compounds ME0327, ME0328, ME0354 and ME0355.

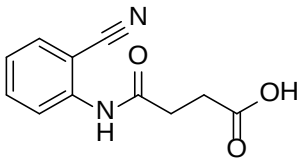


The two amines (**4a** and **4b**) corresponding to ME0354 and ME0355 were not commercially available and thus had to be synthesized from their respective alcohols (scheme 2). This was done by reacting **5a** or **5b** with DPPA and DBU in THF to produce the azides, **6a** and **6b**, in 53-64% yield with inversion of absolute configuration. The azides were then reduced to the desired amines in 79-97% yield using PPh_3 and H_2O in THF.

Scheme 2. Synthesis and absolute configuration of amine building blocks **4a** and **4b**.

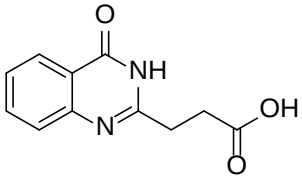


4-(2-cyanophenylamino)-4-oxobutanoic acid (2)



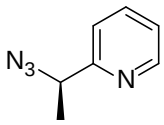
2-aminobenzonitrile (**1**) (1500 mg, 12.7 mmol) and succinic anhydride (1525 mg, 15.24 mmol) in toluene (15 ml) were heated to 90 °C for 3 hours. The solid material was filtered off, washed with Et_2O and dried *in vacuo* to give 4-(2-cyanophenylamino)-4-oxobutanoic acid (**2**) (2670 mg, 96%). ^{1}H NMR (400 MHz, $(CD_3)_2SO$) δ 12.16 (s, 1H), 10.18 (s, 1H), 7.79 (dd, J = 7.8, 1.6 Hz, 1H), 7.67 (ddd, J = 8.6, 7.4, 1.2 Hz, 1H), 7.59 (d, 7.7 Hz, 1H), 7.33 (t, J = 7.6 Hz, 1H), 2.63 (t, J = 6.8 Hz, 2H), 2.53 (t, 6.8 Hz, 2H); ^{13}C NMR (100 MHz, $(CD_3)_2SO$) δ 173.6, 170.6, 140.2, 133.7, 133.2, 125.4, 125.2, 116.8, 106.9, 30.5, 28.8; ESIMS *m/z* calcd $[M+H^+]^+$, 219.08; found 219.13.

3-(4-oxo-3H-quinazolin-2-yl)propanoic acid (3)



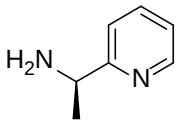
4-(2-cyanophenylamino)-4-oxobutanoic acid (**2**) (2100 mg, 9.62 mmol) was dissolved in Acetone:H_2O 1:1 (120 ml) and K_2CO_3 (2660 mg, 19.24 mmol) followed by UHP (2715 mg, 28.8 mmol) were added. The reaction mixture was heated to 82 °C for 40 hours. K_2CO_3 (2660 mg, 19.24 mmol) and UHP (2715 mg, 28.8 mmol) were added and the mixture was heated to 82 °C for another 48 hours. The pH of the water phase was adjusted to 4 using 6 M HCl. The solid material was filtered off and washed with MeOH and DCM to give 3-(4-oxo-3H-quinazolin-2-yl)propanoic acid (**3**) (1422 mg, 58%). ^{1}H NMR (400 MHz, $(CD_3)_2SO$) δ 12.27-12.15 (m, 2H), 8.07 (d, J = 7.7 Hz, 1H), 7.76 (ddd, J = 8.2, 7.2, 1.0 Hz, 1H), 7.57 (d, J = 8.3 Hz, 1H), 7.45 (t, J = 7.5 Hz, 1H), 2.85 (t, 6.9 Hz, 2H), 2.75 (t, 6.8 Hz, 2H); ^{13}C NMR (100 MHz, $(CD_3)_2SO$) δ 173.5, 161.6, 156.3, 148.6, 134.3, 126.7, 126.0, 125.7, 120.9, 29.8, 29.1; ESIMS *m/z* calcd $[M+H^+]^+$, 219.08; found 219.26.

(R)-2-(1-azidoethyl)pyridine (6a)



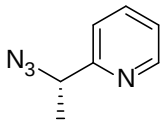
DBU (0.18 ml, 1.22 mmol) followed by DPPA (0.26 ml, 1.22 mmol) were added dropwise to a stirred solution of (S)-α-methyl-2-pyridinemethanol (**5a**) (100 mg, 0.1 mmol) in THF (3.5 ml). The reaction mixture was stirred at room temperature for 20 hours, concentrated and purified using column chromatography on silica gel (EtOAc:Heptane 25:75) to give (R)-2-(1-azidoethyl)pyridine (**6a**) (64 mg, 53%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.57 (dd, J = 4.9, 1.8 Hz, 1H), 7.69 (td, J = 7.7, 1.9 Hz, 1H), 7.33 (d, J = 7.8 Hz, 1H), 7.20 (dd, J = 7.6, 4.9 Hz, 1H), 4.65 (q, 6.9 Hz, 1H), 1.59 (d, J = 6.9 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 160,1, 149.5, 137.1, 122.9, 120.6, 61.7, 20.2; ESIMS *m/z* calcd $[M-N_2+H^+]^+$, 121.08; found 121.23.

(R)-1-(pyridin-2-yl)ethanamine (4a)



(R)-2-(1-azidoethyl)pyridine (**6a**) (64 mg (0.43 mmol) and triphenylphosphine (135 mg, 0.51 mmol) were dissolved in THF (2.5 ml) and H_2O (0.12 ml, 6.4 mmol) was added. The reaction mixture was stirred at room temperature for 26 hours, diluted with DCM and extracted with 1 M HCl. The pH of the water phase was adjusted to 12 and the water phase was extracted five times with DCM. The combined organic phases were dried (Na_2SO_4), filtered and concentrated to give (R)-1-(pyridin-2-yl)ethanamine (**4a**) (51 mg, 97%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.53 (d, 4.8 Hz, 1H), 7.62 (td, J = 7.7, 1.8 Hz, 1H), 7.28 (d, J = 7.8 Hz, 1H), 7.12 (dd, 7.5, 4.9 Hz, 1H), 4.13 (q, J = 6.7 Hz, 1H), 1.90 (s, 2H), 1.41 (d, 6.7 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.8, 149.2, 136.6, 121.9, 120.1, 52.5, 24.5; ESIMS *m/z* calcd $[M+H^+]^+$, 123.09; found 123.27.

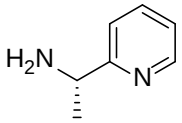
(S)-2-(1-azidoethyl)pyridine (6b)



DBU (0.18 ml, 1.22 mmol) followed by DPPA (0.26 ml, 1.22 mmol) were added dropwise to a stirred solution of (R)-α-methyl-2-pyridinemethanol (**5b**) (100 mg, 0.1 mmol) in THF (3.5 ml). The reaction mixture was stirred at room temperature for 20 hours, concentrated and purified using column chromatography on silica gel (EtOAc:Heptane 25:75) to give (S)-2-(1-azidoethyl)pyridine (**6b**) (77 mg, 64%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.57 (dd, J = 4.9, 1.8 Hz,

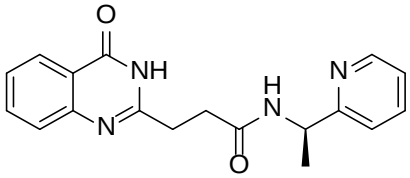
1H), 7.69 (td, J = 7.7, 1.9 Hz, 1H), 7.33 (d, J = 7.8 Hz, 1H), 7.20 (dd, J = 7.6, 4.9 Hz, 1H), 4.65 (q, 6.9 Hz, 1H), 1.59 (d, J = 6.9 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 160,1, 149.5, 137.1, 122.9, 120.6, 61.7, 20.2.

(S)-1-(pyridin-2-yl)ethanamine (4b)



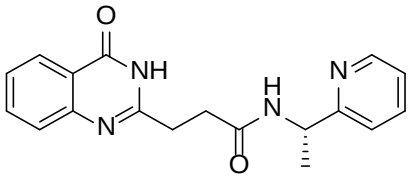
(S)-2-(1-azidoethyl)pyridine (**6b**) (60 mg (0.40 mmol) and triphenylphosphine (127 mg, 0.48 mmol) were dissolved in THF (2.5 ml) and H_2O (0.11 ml, 6.0 mmol) was added. The reaction mixture was stirred at room temperature for 26 hours, diluted with DCM and extracted with 1 M HCl. The pH of the water phase was adjusted to 12 and the water phase was extracted five times with DCM. The combined organic phases were dried (Na_2SO_4), filtered and concentrated to give (S)-1-(pyridin-2-yl)ethanamine (**4b**) (39 mg, 79%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.53 (d, 4.8 Hz, 1H), 7.62 (td, J = 7.7, 1.8 Hz, 1H), 7.28 (d, J = 7.8 Hz, 1H), 7.12 (dd, 7.5, 4.9 Hz, 1H), 4.13 (q, J = 6.7 Hz, 1H), 2.39 (s, 2H), 1.41 (d, 6.7 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.8, 149.2, 136.6, 121.9, 120.1, 52.5, 24.5.

(R)-3-(4-oxo-3H-quinazolin-2-yl)-N-(1-(pyridin-2-yl)ethyl)propanamide (ME0354)



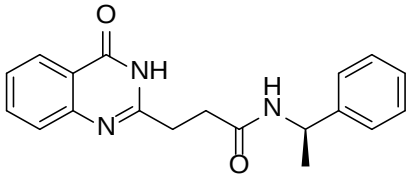
3-(4-oxo-3H-quinazolin-2-yl)propanoic acid (**3**) (40 mg, 0.183 mmol), (R)-1-(pyridin-2-yl)ethanamine (**4a**) (26.9 mg, 0.22 mmol) and HOAt (29.9 mg, 0.22 mmol) were dissolved in DMF (1.5 ml) and DIC (34.3 µl, 0.22 mmol) was added. The reaction mixture was stirred at room temperature for 23 hours, diluted with EtOAc and washed twice with saturated aqueous $NaHCO_3$. The organic phase was dried (Na_2SO_4), filtered and concentrated. The residue was purified using column chromatography on silica gel (DCM:MeOH 95:5) to give (R)-3-(4-oxo-3H-quinazolin-2-yl)-N-(1-(pyridin-2-yl)ethyl)propanamide (ME0354) (41 mg, 69%). $[\alpha]_D$ 27° (*c* 0.57, DMSO); 99% enantiomeric excess; ^{1}H NMR (400 MHz, $(CD_3)_2SO$) δ 12.17 (s, 1H), 8.49-8.42 (m, 2H), 8.08 (dd, J = 7.9, 1.6 Hz, 1H), 7.78 (ddd, J = 8.2, 7.2, 1.3 Hz, 1H), 7.60-7.53 (m, 2H), 7.45 (t, J = 7.4 Hz, 1H), 7.31 (d, J = 8.0 Hz, 1H), 7.19 (dd, J = 7.5, 4.8 Hz, 1H), 4.93 (quin, J = 7.3 Hz, 1H), 2.94-2.81 (m, 2H), 2.80-2.63 (m, 2H), 1.36 (d, J = 7.1 Hz, 3H); ^{13}C NMR (100 MHz, $(CD_3)_2SO$) δ 169.9, 162.2, 161.1, 156.2, 148.1 (2C), 136.0, 133.7, 126.1, 125.4, 125.2, 121.3, 120.4, 119.5, 49.2, 30.8, 29.1, 20.6; HRMS (ES+) calcd [M+Na+]+, 345.1322; found 345.1336.

(S)-3-(4-oxo-3H-quinazolin-2-yl)-N-(1-(pyridin-2-yl)ethyl)propanamide (ME0355**)**



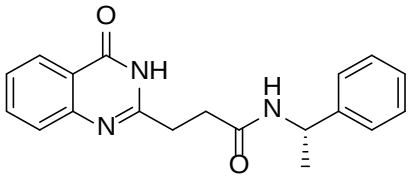
3-(4-oxo-3H-quinazolin-2-yl)propanoic acid (**3**) (40 mg, 0.183 mmol), (S)-1-(pyridin-2-yl)ethanamine (**4b**) (26.9 mg, 0.22 mmol) and HOAt (29.9 mg, 0.22 mmol) were dissolved in DMF (1.5 ml) and DIC (34.3 µl, 0.22 mmol) was added. The reaction mixture was stirred at room temperature for 23 hours, diluted with EtOAc and washed twice with saturated aqueous $NaHCO_3$. The organic phase was dried (Na_2SO_4), filtered and concentrated. The residue was purified using column chromatography on silica gel (DCM:MeOH 95:5) to give (R)-3-(4-oxo-3H-quinazolin-2-yl)-N-(1-(pyridin-2-yl)ethyl)propanamide (ME0355) (31 mg, 53%). $[\alpha]_D$ -24° (*c* 0.65, DMSO); 90% enantiomeric excess; ^{1}H NMR (400 MHz, $(CD_3)_2SO$) δ 12.17 (s, 1H), 8.49-8.42 (m, 2H), 8.08 (dd, J = 7.9, 1.6 Hz, 1H), 7.78 (ddd, J = 8.2, 7.2, 1.3 Hz, 1H), 7.60-7.53 (m, 2H), 7.45 (t, J = 7.4 Hz, 1H), 7.31 (d, J = 8.0 Hz, 1H), 7.19 (dd, J = 7.5, 4.8 Hz, 1H), 4.93 (quin, J = 7.3 Hz, 1H), 2.94-2.81 (m, 2H), 2.80-2.63 (m, 2H), 1.36 (d, J = 7.1 Hz, 3H); ^{13}C NMR (100 MHz, $(CD_3)_2SO$) δ 169.9, 162.2, 161.1, 156.2, 148.1 (2C), 136.0, 133.7, 126.1, 125.4, 125.2, 121.3, 120.4, 119.5, 49.2, 30.8, 29.1, 20.6; HRMS (ES+) calcd [M+Na+]+, 345.1322; found 345.1321.

(R)-3-(4-oxo-3H-quinazolin-2-yl)-N-(1-phenylethyl)propanamide (ME0327)



3-(4-oxo-3H-quinazolin-2-yl)propanoic acid (**3**) (40 mg, 0.183 mmol), (R)-(+)-1-phenylethylamine (**4c**) (28.1 µl, 0.22 mmol) and HOAt (29.9 mg, 0.22 mmol) were dissolved in DMF (1.5 ml) and DIC (34.3 µl, 0.22 mmol) was added. The reaction mixture was stirred at room temperature for 19 hours, diluted with EtOAc and washed twice with saturated aqueous $NaHCO_3$. The organic phase was dried (Na_2SO_4), filtered and concentrated. The residue was purified using column chromatography on silica gel (DCM:MeOH 96:4) to give (S)-3-(4-oxo-3H-quinazolin-2-yl)-N-(1-phenylethyl)propanamide (ME0327) (46 mg, 78%). $[\alpha]_D$ 21° (*c* 0.63, DMSO); 98% enantiomeric excess; ^{1}H NMR (400MHz, $(CD_3)_2SO$) δ 12.13 (s, 1H), 8.39 (d, J = 8.0 Hz, 1H), 8.08 (dd, J = 7.9, 1.6 Hz, 1H), 7.78 (ddd, J = 9.0, 7.1, 1.8 Hz, 1H), 7.56 (d, 8.3 Hz, 1H), 7.46 (t, J = 7.7 Hz, 1H), 7.30-7.25 (m, 2H), 7.25-7.20 (m, 2H), 7.20-7.15 (m, 1H), 4.90 (quin, J = 7.3 Hz, 1H), 2.93-2.79 (m, 2H), 2.76-2.60 (m, 2H), 1.33 (d, J = 7.0 Hz, 1H); ^{13}C NMR (100 MHz, $(CD_3)_2SO$) δ 170.1, 161.7, 156.8, 148.7, 144.7, 134.2, 128.1 (2C), 126.7, 126.4, 125.8 (2C), 125.8, 125.7, 120.9, 47.7, 31.4, 29.7, 22.5; HRMS (ES+) calcd [M+Na+]+, 344.1369; found 344.1370.

(S)-3-(4-oxo-3H-quinazolin-2-yl)-N-(1-phenylethyl)propanamide (ME0328**)**



3-(4-oxo-3H-quinazolin-2-yl)propanoic acid (**3**) (40 mg, 0.183 mmol), (S)-(-)-1-phenylethylamine (**4d**) (28.1 µl, 0.22 mmol) and HOAt (29.9 mg, 0.22 mmol) were dissolved in DMF (1.5 ml) and DIC (34.3 µl, 0.22 mmol) was added. The reaction mixture was stirred at room temperature for 19 hours, diluted with EtOAc and washed twice with saturated $NaHCO_3$. The organic phase was dried (Na_2SO_4), filtered and concentrated. The residue was purified using column chromatography on silica gel (DCM:MeOH 96:4) to give (S)-3-(4-oxo-3H-quinazolin-2-yl)-N-(1-phenylethyl)propanamide (ME0328) (46 mg, 78%). $[\alpha]_D$ -22° (*c* 0.65, DMSO); 98% enantiomeric excess; ^{1}H NMR (400 MHz, $(CD_3)_2SO$) δ 12.13 (s, 1H), 8.39 (d, J = 8.0 Hz, 1H), 8.08 (dd, J = 7.9, 1.6 Hz, 1H), 7.78 (ddd, J = 9.0, 7.1, 1.8 Hz, 1H), 7.56 (d, 8.3 Hz, 1H), 7.46 (t, J = 7.7 Hz, 1H), 7.30-7.25 (m, 2H), 7.25-7.20 (m, 2H), 7.20-7.15 (m, 1H), 4.90 (quin, J = 7.3 Hz, 1H), 2.93-2.79 (m, 2H), 2.76-2.60 (m, 2H), 1.33 (d, J = 7.0 Hz, 1H); ^{13}C NMR (100 MHz, $(CD_3)_2SO$) δ 170.1, 161.7, 156.8, 148.7, 144.7, 134.2, 128.1 (2C), 126.7, 126.4, 125.8 (2C), 125.8, 125.7, 120.9, 47.7, 31.4, 29.7, 22.5; HRMS (ES+) calcd [M+Na+]+, 344.1369; found 344.1374.

Abbreviations

Ac	Acetate
DBU	1,8-Diazabicyclo[5.4.0]undec-7-ene
DCM	Dichloromethane
DIC	*N,N′*-Diisopropylcarbodiimide
DMF	N,N-Dimethylformamide
DMSO	Dimethylsulfoxide
DPPA	Diphenyl phosphoryl azide
Et	Ethyl
FCS	Fetal calf serum
HOAt	1-Hydroxybenzotriazole
HRMS	High-resolution Mass Spectrometry
LCMS	Liquid Chromatography Mass Spectrometry
MDCK	Madin-Darby Canine Kidney
Me	Methyl
NMR	Nuclear Magnetic Resonance
Ph	Phenyl
THF	Tetrahydrofuran
UHP	Urea Hydrogen Peroxide
UV	Ultra Violet

Supplementary References

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