Supplementary information

Metabolic suppression identifies new antibacterial inhibitors under nutrient limitation

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SUPPLEMENTARY RESULTS

1. SUPPLEMENTARY TABLES

Supplementary Table 1. Small molecule screening data

Category	Parameter	Description
Assay	Type of assay	Cell-based
	Target	Whole organism *E. coli* K-12 MG1655
	Primary measurement	Measurement of optical density at 600 nm
	Assay protocol	Online methods section 'Primary screen in minimal media'
Library	Library size	29,569
	Library composition	Synthetic small molecules, off-patent FDA approved molecules, natural products and known bioactives
	Source	Maybridge, ChemBridge, BioMol, Prestwick, Sigma, MicroSource
	Additional comments	Library stock concentration: 250 μM in 25% DMSO
Screen	Format	96-well plates (Costar), 3 replicates
	Concentration(s) tested	10 μM, 0.2% DMSO
	Plate controls	High controls: 0.2% DMSO Low controls: 10 μg/ml of norfloxacin
	Reagent/ compound dispensing system	Biomek FX liquid handler (Beckman Coulter Inc., Fullerton, CA)
	Detection instrument and software	Envision (Perkin Elmer, Waltham, MA)
	Assay validation/QC	Average (n=3) Z' score: 0.8
	Correction factors	Optical density readings are background corrected
	Normalization	% Residual growth calculated based on the high and low controls
Post-HTS analysis	Hit criteria	80% Residual growth for the 3 replicate measurements
	Hit rate	1.7%
	Additional assay(s)	Dose-response and MIC determinations
	Confirmation of hit purity and structure	Compounds repurchased and retested, identity of key hits confirmed by LC/MS and NMR

Supplementary Table 2. Defined minimal media composition [a]

Supplement	Final conc. in M9 media (µg/ml)	Notes
Amino Acids		
DL-Alanine	100	
L-Arginine	22	
L-Asparagine	100	
L-Aspartic acid	100	
L-Cysteine	10	
Glycine	100	
L-Glutamic acid	100	
L-Glutamine	100	
L-Histidine	22	
L-Isoleucine	20	
L-Leucine	20	
L-Lysine	88	
L-Methionine	20	
L-Phenylalanine	20	Prepare in 0.001 N NaOH
L-Proline	30	
L-Serine	100	
L-Threonine	80	
L-Tryptophan	20	
L-Tyrosine	20	Prepare in 0.01 N NaOH
L-Valine	40	
Purines & Pyrimidines		
Adenine (A)	40	Prepare in 0.03 N HCl
Thymidine (T)	5	
Uracil (U)	40	
Guanosine (G)	40	
Vitamins		
Biotin (B7)	0.5	
Niacin (B3)	1	

Pantothenic acid (B5)	1	
Pyridoxine (B6)	1	
Thiamine (B1)	1	
Riboflavin (B2)	200	
PABA	2	
Cobalamin (B12)	10	
Other intermediates		
2,6- Diaminopimelate (DAP)	100	
L-Homoserine	20	
5-Aminolevulinic acid (5-ALA)	25	
3- Dehydroquinate (DHQ)	100	
Shikimate (SHIK)	100	
4-Hydroxybenzoate (4-HBA)	15	
2,3-Dihydroxybenzoate (2,3-DHBA)	2	
Citrulline	15	
Ornithine	15	
Putrescine	20	
Pools		
M9 ALL		All amino acids, vitamins and nucleobases
AA		All amino acids
VIT		All vitamins
NUC		All nucleobases
AA + VIT		All amino acids and vitamins
AA + NUC		All amino acids and nucleobases
VIT + NUC		All vitamins and nucleobases
PUR		Adenine and guanine
PYR		Thymine and uracil
ARO AA		Tyr, Phe,Trp
ARO		Tyr,PheTrp,PABA,2.3-DHBA,4-HBA

Pool 1		Adenine, His, Phe, Gln, Thymine
Pool 2		Guanine, Leu, Tyr, Asn, Ser
Pool 3		Cystine, Ile, Trp, Uracil, Glu
Pool 4		Met, Lys, Thr, Asp, DAP
Pool 5		B1, Val, Pro, Arg, Gly, Ile
Pool 6		Adenine, Guanine, Cystine, Met, B1
Pool 7		His, Leu, Ile, Lys, Val
Pool 8		Phe, Tyr, Trp, Thr, Pro
Pool 9		Gln, Asn, Uracil, Asp, Arg
Pool 10		Thymine, Ser, Glu, DAP, Gly
Trace metal solution	**Weight (g) in 100 ml solution**	
$FeSO_4 \cdot 7H_2O$	0.8	
$AlCl_3 \cdot 6H_2O$	0.1	
$ZnSO_4 \cdot 7H_2O$	0.02	
$CuCl_2 \cdot 2H_2O$	0.01	Add 0.1 ml of Trace metal solution in 1 L of M9 minimal media
$NaMoO_4 \cdot 2H_2O$	0.02	
$MnSO_4 \cdot H_2O$	0.1	
$CoCl_2$	0.04	
H_3BO_4	0.005	

[a] The metabolic suppression array was prepared in the format shown in **Fig. 3a** and in **Supplementary Fig. 4** as a 20× stock plate to be used for metabolic suppression profiling

Supplementary Table 3. Summary of EC_{50} and MIC values of prioritized actives

Priority Active	Supplier	Cat #	EC_{50} (μM) [a]		MIC (μg/ml) [b]	
			M9 [c]	M9 ALL [c]	M9	M9 ALL
MAC-0001907	MayBridge	BR 00067	0.5	17.9	4	16
MAC-0001908	MayBridge	BR 00068	0.6	18.7	8	32
MAC-0001958	MayBridge	BTB 00183	3.1	NI	16	> 256
MAC-0001961	MayBridge	BTB 00187	0.2	25.6	2	32
MAC-0003403	MayBridge	BTB 03305	0.7	NI	16	> 256
MAC-0005562	MayBridge	BTB 07373	1.5	NI	8	> 256
MAC-0006191	MayBridge	BTB 08617	0.6	NI	4	> 256
MAC-0007196	MayBridge	BTB 11383	0.8	NI	8	> 256
MAC-0007606	MayBridge	BTB 12380	5.1	NI	16	256
MAC-0008533	MayBridge	BTB 14373	0.7	13.4	2	64
MAC-0008972	MayBridge	BTB 15053	0.9	NI	8	256
MAC-0154388	MayBridge	CD 00793	1.1	63.3	2	128
MAC-0012416	MayBridge	CD 11546	0.3	13.4	1	16
MAC-0012616	MayBridge	DFP 00022	0.7	36.8	16	64
MAC-0013015	MayBridge	DP 00874	2.8	NI	8	> 256
MAC-0013528	MayBridge	DSHS 00086	0.1	6.8	0.5	64
MAC-0013532	MayBridge	DSHS 00100	2.1	NI	32	> 256
MAC-0013772	MayBridge	DSHS 00862	0.6	NI	4	> 256
MAC-0016505	MayBridge	HTS 00495	0.1	5.4	0.5	> 256
MAC-0018315	MayBridge	HTS 03738	0.2	NI	2	> 256
MAC-0018316	MayBridge	HTS 03741	1.5	NI	32	> 256
MAC-0158077	MayBridge	HTS 08964	0.4	73.6	32	256
MAC-0021596	MayBridge	HTS 09781	0.7	NI	16	> 256
MAC-0021835	MayBridge	JFD 00035	0.5	14.4	4	128
MAC-0023560	MayBridge	JFD 02936	5.5	NI	32	> 256
6-Azauracil	MayBridge	JFD 03556	6.0	NI	4	> 256
MAC-0024165	MayBridge	JFD 03885	0.4	NI	1	> 256
MAC-0024593	MayBridge	KM 00081	0.9	NI	4	> 256
MAC-0160523	MayBridge	KM 06089	4.1	NI	8	256
MAC-0161738	MayBridge	NRB 00886	1.0	74.7	4	256
MAC-0161992	MayBridge	NRB 04162	4.6	NI	32	> 256

MAC-0162065	MayBridge	NRB 05081	1.8	NI	4	> 256
MAC-0031448	MayBridge	PD 00549	0.4	16.2	4	128
MAC-0031946	MayBridge	RB 00158	0.4	35.3	8	64
MAC-0032479	MayBridge	RDR 01288	1.3	40.4	8	> 256
MAC-0032480	MayBridge	RDR 01289	2.4	71.2	16	> 256
MAC-0033880	MayBridge	RF 01653	0.9	NI	8	128
MAC-0033881	MayBridge	RF 01665	1.7	NI	32	256
MAC-0037439	MayBridge	RJC 02623	0.2	10.3	4	> 256
MAC-0037547	MayBridge	RJC 02756	3.4	NI	32	> 256
MAC-0164360	MayBridge	RJF 02182	0.7	NI	8	128
MAC-0039424	MayBridge	S 01335	1.0	24.5	8	128
MAC-0039804	MayBridge	S 04055	0.3	28.3	8	128
MAC-0039908	MayBridge	S 04548	0.0	44.7	0.5	256
MAC-0040158	MayBridge	S 07624	0.3	9.5	64	2
MAC-0041191	MayBridge	S 14125	1.6	32.3	8	128
MAC-0041942	MayBridge	SEW 00805	1.3	32.8	8	128
MAC-0165919	MayBridge	SEW 03166	0.7	47.6	16	64
MAC-0043268	MayBridge	SEW 03585	0.3	6.9	1	8
MAC-0000956	MayBridge	SPB 01986	0.5	15.6	8	128
MAC-0049900	MayBridge	SPB 07211	0.1	5.3	0.5	128
MAC-0168230	ChemBridge	5103499	0.7	NI	8	> 256
MAC-0168195	ChemBridge	5102267	0.2	47.3	2	64
MAC-0168120	ChemBridge	5100353	1.0	NI	32	> 256
MAC-0006508	ChemBridge	5110246	3.0	NI	32	> 256
MAC-0168425	ChemBridge	5107939	1.2	53.0	8	128
MAC-0168466	ChemBridge	5109197	3.5	NI	128	> 256
MAC-0168841	ChemBridge	5133775	0.7	NI	32	> 256
MAC-0168630	ChemBridge	5117127	0.5	NI	16	> 256
MAC-0170171	ChemBridge	5217926	0.3	11.0	4	32
MAC-0170172	ChemBridge	5217929	0.3	14.4	4	32
MAC-0170559	ChemBridge	5238776	1.7	NI	8	> 256
MAC-0170315	ChemBridge	5227194	0.6	47.1	32	> 256
MAC-0170316	ChemBridge	5227196	0.5	NI	32	> 256
MAC-0171530	ChemBridge	5283400	2.3	NI	16	> 256
MAC-0171823	ChemBridge	5309181	3.0	NI	8	128
MAC-0172113	ChemBridge	5326453	0.8	41.4	4	64

MAC-0173979	ChemBridge	5487829	0.4	NI	4	128
MAC-0176699	ChemBridge	5738215	9.9	NI	16	256
MAC-0088137	ChemBridge	5930551	0.3	13.5	4	64
MAC-0181370	MicroSource	01502210	0.5	NI	16	> 256
6-aminonicotinamide	MicroSource	01505315	0.8	39.3	4	> 256
5-fluorouracil	MicroSource	01505317	0.0	6.5	0.125	64
MAC-0182554	MicroSource	01505368	0.6	54.4	0.5	> 256

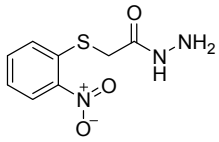
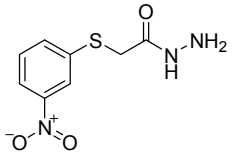
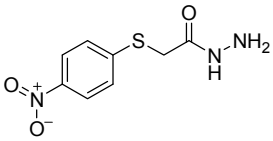
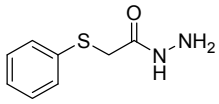
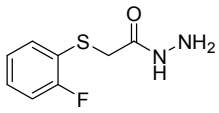
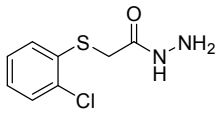
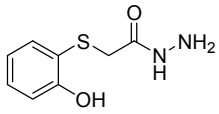
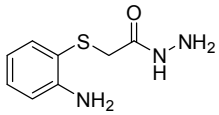
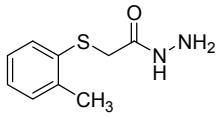
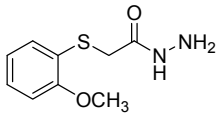
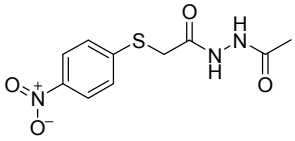
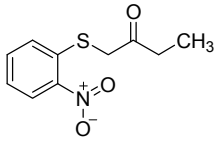
[a] The EC_{50} determinations were conducted within a concentration range of 1 nM- 80 µM, NI: no inhibition within the tested range (for details on experimental setup and data analysis, see **Online methods**).
[b] MIC determinations were conducted within a concentration range of 0.25- 256 µg/ml (for details on experimental setup and data analysis, see **Online methods**).
[c] M9= Minimal media; M9 ALL= Supplemented minimal media

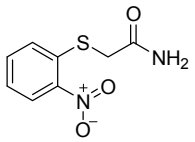
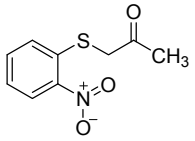
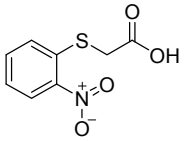
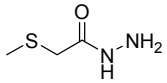
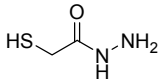
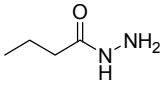
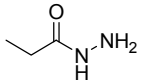
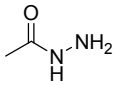
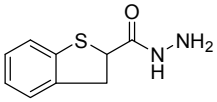
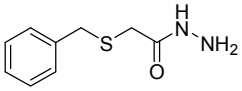
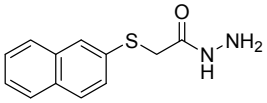
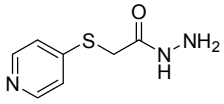
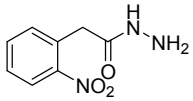
Supplementary Table 4: Antibacterial activity of MAC13772 against *E. coli* MG1655 in the presence of intermediates of biotin biosynthesis

Supplement [a]	MIC (µg/ml) [b]	Fold suppression [c]
None	8	-
AA+VIT+NUC	> 256	32
KAPA	8	1
DAPA	> 256	32
DTB	> 256	32
BIOTIN	> 256	32

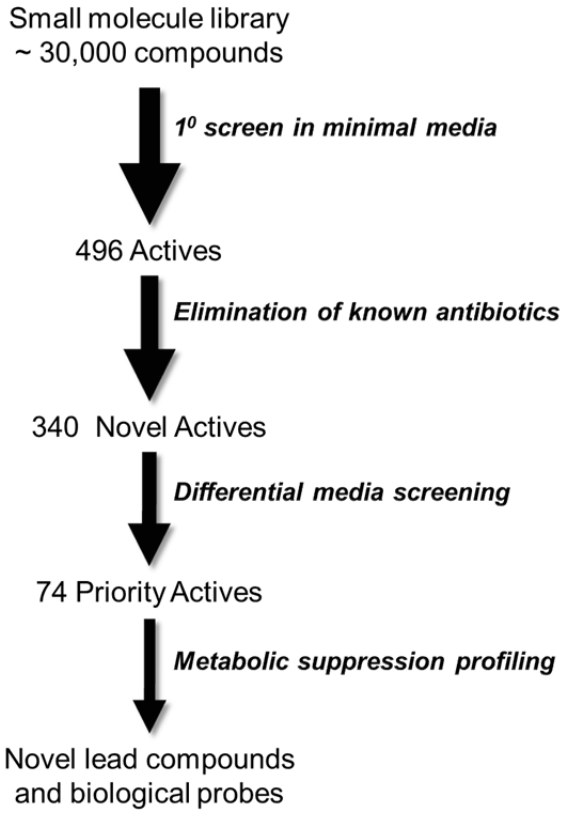
[a] AA: amino acids, VIT: vitamins, NUC: nucleobases, KAPA: 7-keto-8-aminopelargonate, DAPA: 7,8-diaminopelargonate and DTB: dethiobiotin
[b] MICs were determined as described in **Online methods**. Values are representative of three independent experiments
[c] Fold suppression is the ratio of the MIC in the presence of the supplement to the MIC without supplementation

Supplementary Table 5. Structures of MAC13772 analogs used in SAR studies

Compound	Structure	Supplier code
MAC13772		Ryan Scientific DSHS 00862SC
5		Ryan Scientific CAS36107-14-9
6		Ambinter (France) Amb 8998045
7		Ambinter (France) Amb 4065797
8		Ambinter (France) Amb 2336529
9		Ambinter (France) Amb 8858487
10		UORSY BBV-37826735
11		Ambinter (France) Amb 17305609
12		Ambinter (France) Amb 4074830
13		UORSY BBV-37662617
14		Ambinter (France) Amb 7951732
15		UORSY BBV-33761526

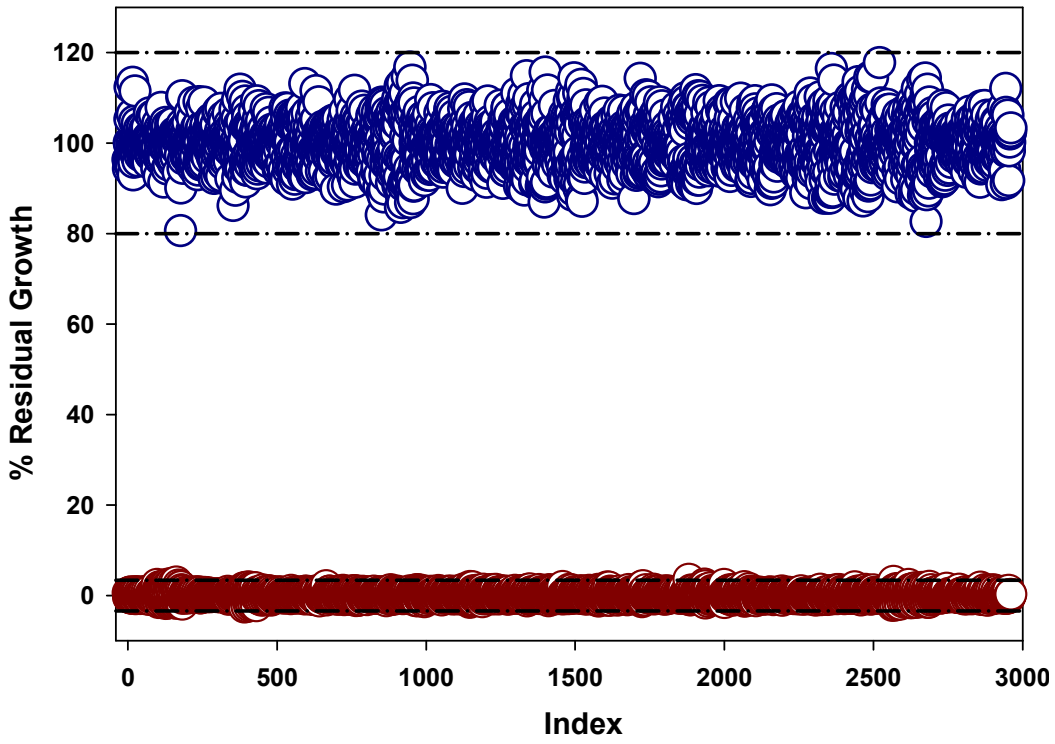
16		Ambinter (France) Amb 2795194
17		Ambinter (France) Amb 2624964
18		Ambinter (France) Amb 436644
19		UORSY BBV-39135451
20		UORSY BBV-37616329
21		Ambinter (France) Amb 6366352
22		Ambinter (France) Amb 1104708
23		Ambinter (France) Amb 1153969
24		Ambinter (France) Amb 8858703
25		Ambinter (France) Amb 6550464
26		Ambinter (France) Amb 4074831
27		Ambinter (France) Amb 8998355
28		Ambinter (France) Amb 6739870

2. SUPPLEMENTARY FIGURES



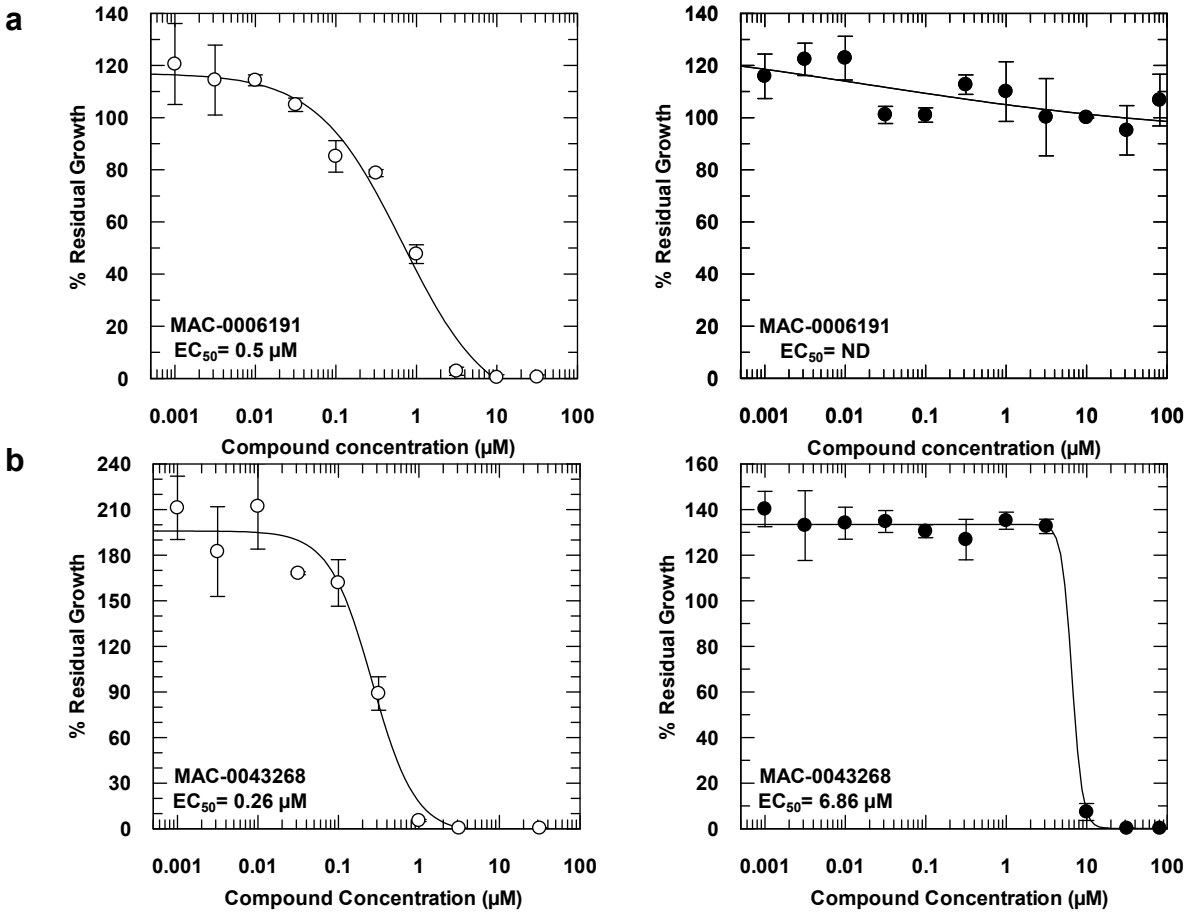
Supplementary Fig. 1. General approach to characterize inhibitors of bacterial physiology under nutrient-limited growth conditions.

A total of 496 compounds that inhibited the growth of *E. coli* MG1655 in minimal media were identified from a high-throughput screen of a library of ~ 30,000 diverse small molecules. After the removal of known antibiotics, a total of 340 novel actives were evaluated in dose-response studies in minimal and supplemented minimal media to identify potent inhibitors with a biological activity specific to bacterial growth under nutrient-limited conditions. From this evaluation, a total of 74 actives were prioritized for metabolic suppression profiling against a panel of primary metabolites to identify the potential target pathways of these inhibitors.



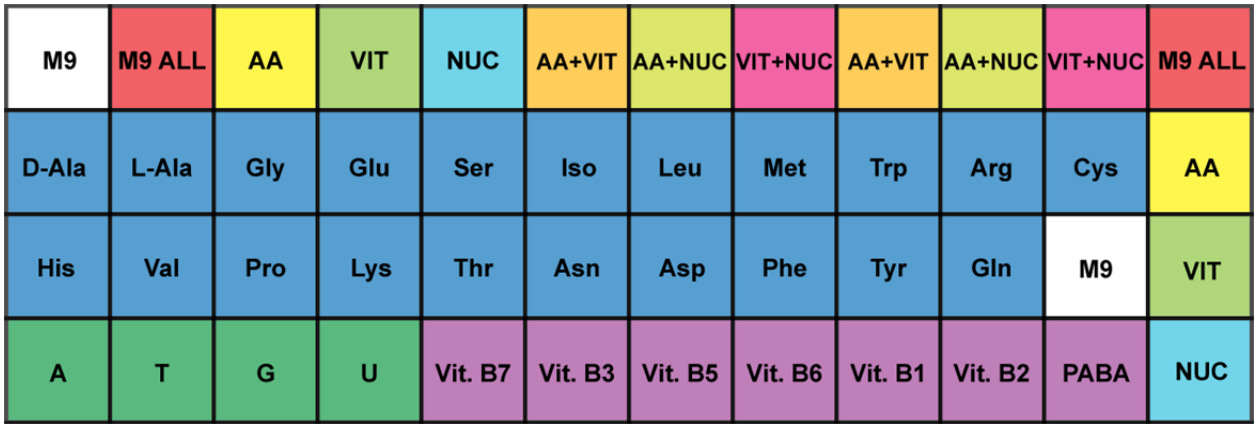
Supplementary Fig. 2. Primary screen in M9 minimal media- Control Data

The average high controls (100% residual growth; dark blue circles) and the average low controls (0% residual growth; maroon circles) containing 0.2% DMSO and 10 µg/ml of norfloxacin, respectively (n=3). The assay shows a large screening window between the high and the low controls and the calculated average Z' factor is 0.8.



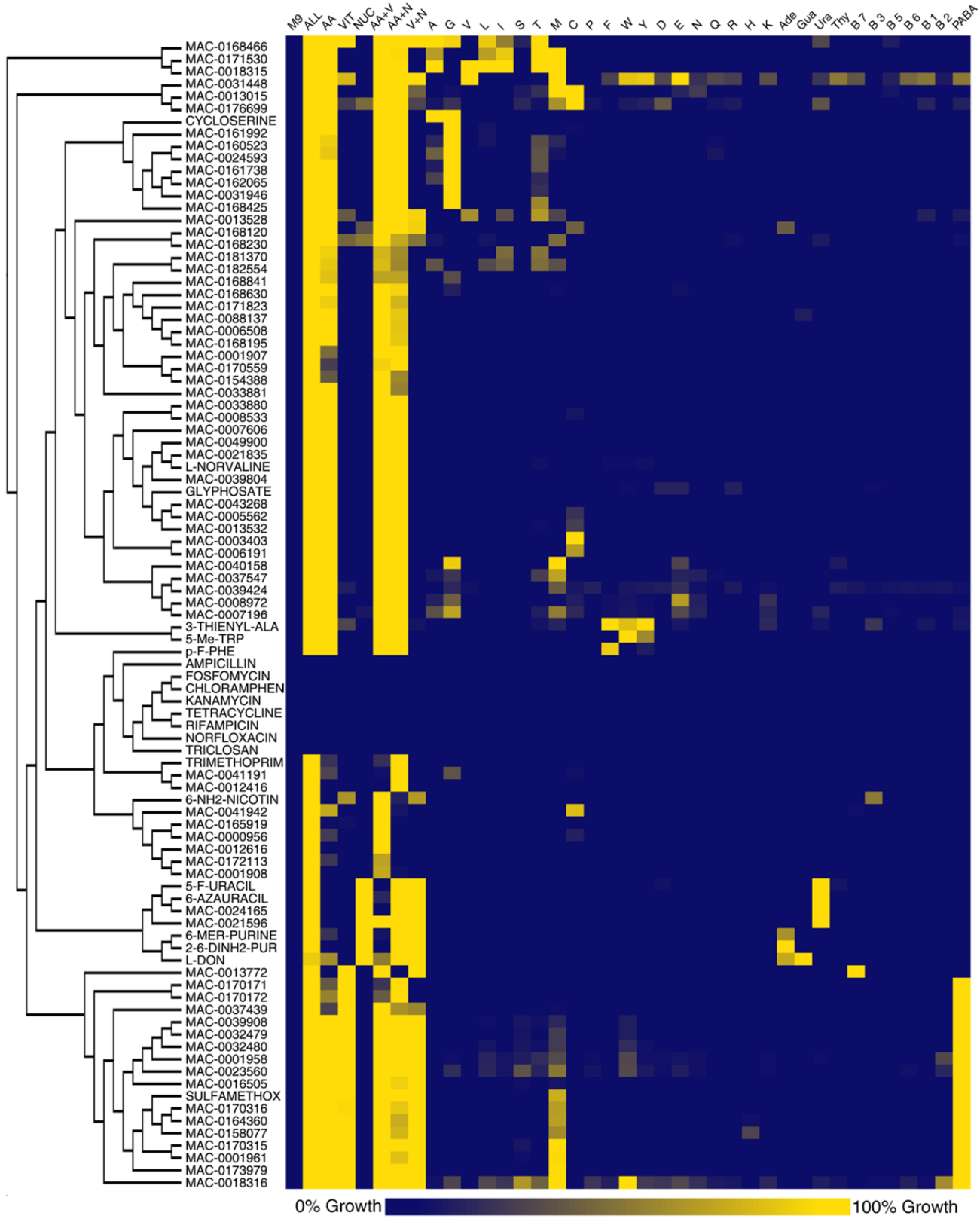
Supplementary Fig. 3. Effect of media composition on the EC_{50} of actives

Two representative examples of the EC_{50} analysis conducted on the actives prioritized from the primary screen. Dose response studies were carried out with each compound within a concentration range of 1 nM- 80 μM against *E. coli* MG1655 grown in either M9 minimal (open circles) or supplemented minimal media (closed circles). In panel **(a)**, MAC6191 has an EC_{50} of 0.5 μM in minimal media but has no growth inhibitory activity when tested in supplemented minimal media. In panel **(b)**, MAC43268 has EC_{50} values of 0.26 and 6.86 μM when tested in minimal and supplemented minimal media, respectively. This results into a ~ 26-fold suppression in its biological activity. Data with the error bars represent the mean % residual growth ± range of n=2 replicates.



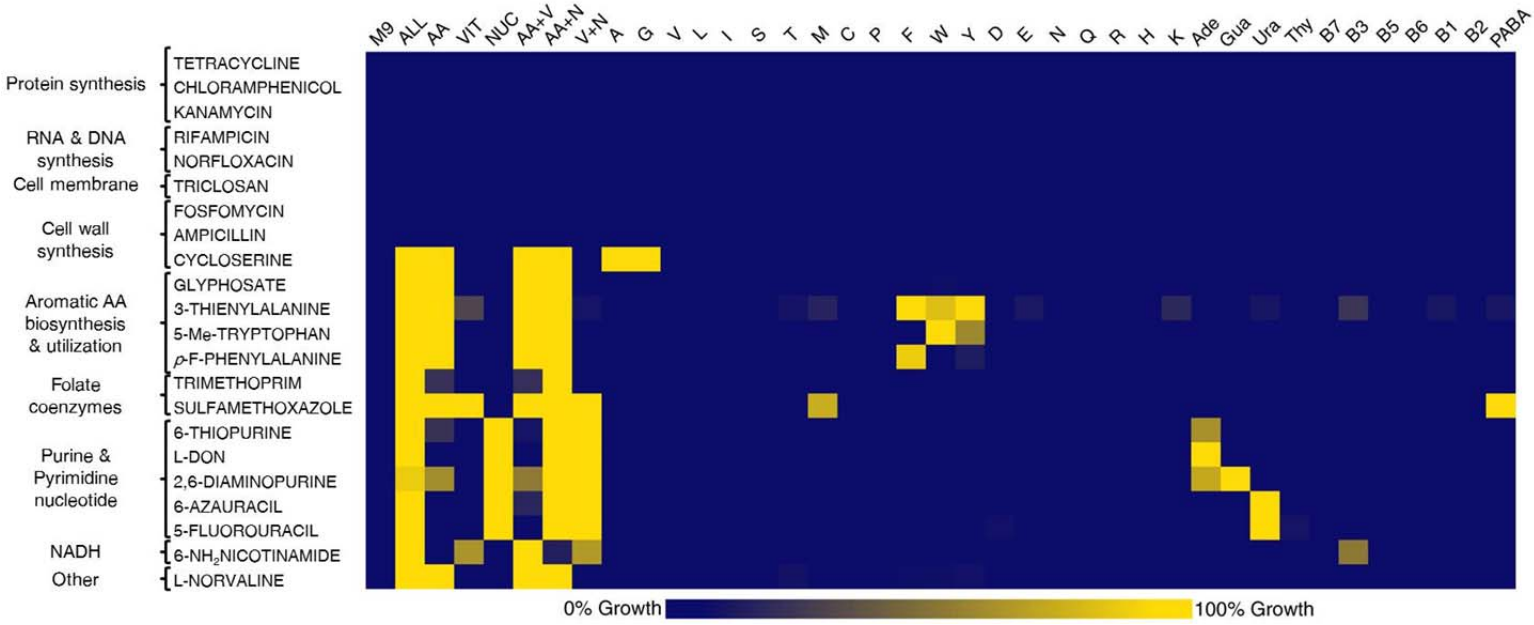
Supplementary Fig. 4. The Metabolic Suppression Array

For the secondary screen, key metabolites were systematically tested to look for suppression of the antibacterial activity of the prioritized actives. A 10^3-fold dilution of a mid-exponential culture of *E. coli* MG1655 was set up in M9 minimal media in 96-well plates containing 4× the MIC (minimum inhibitory concentration) of each and a 1/20 dilution of the metabolic suppression array stock plate. The arrays were incubated O/N at 37 ^{0}C then growth was determined turbidometrically by measuring the optical density of the plates at 600 nm. Amino acids are referred to by their 3-letter code, nucleotides by their 1-letter code, PABA: *p*-aminobenzoic acid. M9: no supplements; ALL: minimal media with all supplements; AA: minimal media with all amino acids; VIT: minimal media with all vitamins; NUC: minimal media with all nucleobases. For media recipe, pool compositions and concentrations of supplements refer to the **Online methods** and **Supplementary Table 2**.


M9
ALL
AA
VIT
NUC
AA+V
AA+N
V+N
A
G
V
L
I
S
T
M
C
P
F
W
Y
D
E
N
Q
R
H
K
Ade
Gua
Ura
Thy
B7
B3
B5
B6
B1
B2
PABA
MAC-0168466
MAC-0171530
MAC-0018315
MAC-0031448
MAC-0013015
MAC-0176699
CYCLOSERINE
MAC-0161992
MAC-0160523
MAC-0024593
MAC-0161738
MAC-0162065
MAC-0031946
MAC-0168425
MAC-0013528
MAC-0168120
MAC-0168230
MAC-0181370
MAC-0182554
MAC-0168841
MAC-0168630
MAC-0171823
MAC-0088137
MAC-0006508
MAC-0168195
MAC-0001907
MAC-0170559
MAC-0154388
MAC-0033881
MAC-0033880
MAC-0008533
MAC-0007606
MAC-0049900
MAC-0021835
L-NORVALINE
MAC-0039804
GLYPHOSATE
MAC-0043268
MAC-0005562
MAC-0013532
MAC-0003403
MAC-0006191
MAC-0040158
MAC-0037547
MAC-0039424
MAC-0008972
MAC-0007196
3-THIENYL-ALA
5-Me-TRP
p-F-PHE
AMPICILLIN
FOSFOMYCIN
CHLORAMPHEN
KANAMYCIN
TETRACYCLINE
RIFAMPICIN
NORFLOXACIN
TRICLOSAN
TRIMETHOPRIM
MAC-0041191
MAC-0012416
6-NH2-NICOTIN
MAC-0041942
MAC-0165919
MAC-0000956
MAC-0012616
MAC-0172113
MAC-0001908
5-F-URACIL
6-AZAURACIL
MAC-0024165
MAC-0021596
6-MER-PURINE
2-6-DINH2-PUR
L-DON
MAC-0013772
MAC-0170171
MAC-0170172
MAC-0037439
MAC-0039908
MAC-0032479
MAC-0032480
MAC-0001958
MAC-0023560
MAC-0016505
SULFAMETHOX
MAC-0170316
MAC-0164360
MAC-0158077
MAC-0170315
MAC-0001961
MAC-0173979
MAC-0018316
0% Growth
100% Growth

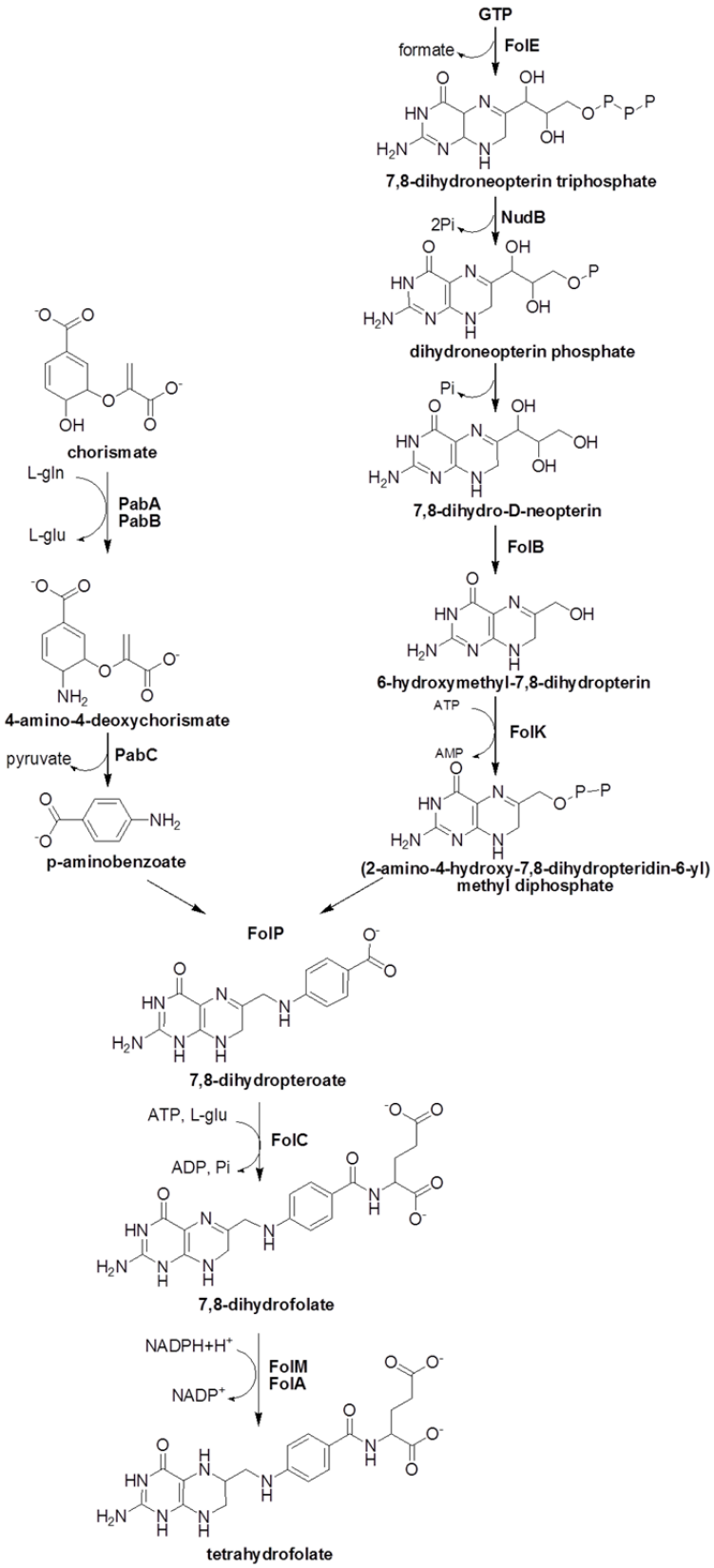
Supplementary Fig. 5. Heat map of the metabolic suppression profiles of priority actives.

Metabolic suppression profiles of 93 antibacterial inhibitors including 22 known antibiotics are hierarchically clustered based on the growth of *E. coli* MG1655 in the presence of each compound at 4× its MIC and with various primary metabolites or pools of metabolites in the metabolic suppression array (n= 2 replicates). Hierarchical cluster analysis of the percent residual growth was conducted as described in **Online methods**. Actives identified from the primary screen are designated by their MAC ID and known actives by their names. Amino acids are referred to by their 1-letter code, B vitamins by their respective numbers, PABA: *p*-aminobenzoic acid, Ade: adenine, Gua: guanine, Ura: uracil, Thy: thymine. M9: no supplements; ALL: minimal media with all supplements; AA: minimal media with all amino acids; VIT: minimal media with all vitamins; NUC: minimal media with all nucleobases; 3-THIENYL-ALA: L-3-thienylalanine, 5-Me-TRP: 5-methyltryptophan, p-F-PHE: p-fluorophenylalanine, CHLORAMPHEN: chloramphenicol , 6-NH2NICOTINAMIDE: 6-aminonicotinamide, 5-F-URACIL: 5-fluorouracil, 6-MER-PUR: 6-mercaptopurine (6-thiopurine), 2,6-DINH2-PUR: 2,6-diaminopurine, L-DON: 6-Diazo-5-oxo-L-norleucine, SULFAMETHOX: sulfamethoxazole.

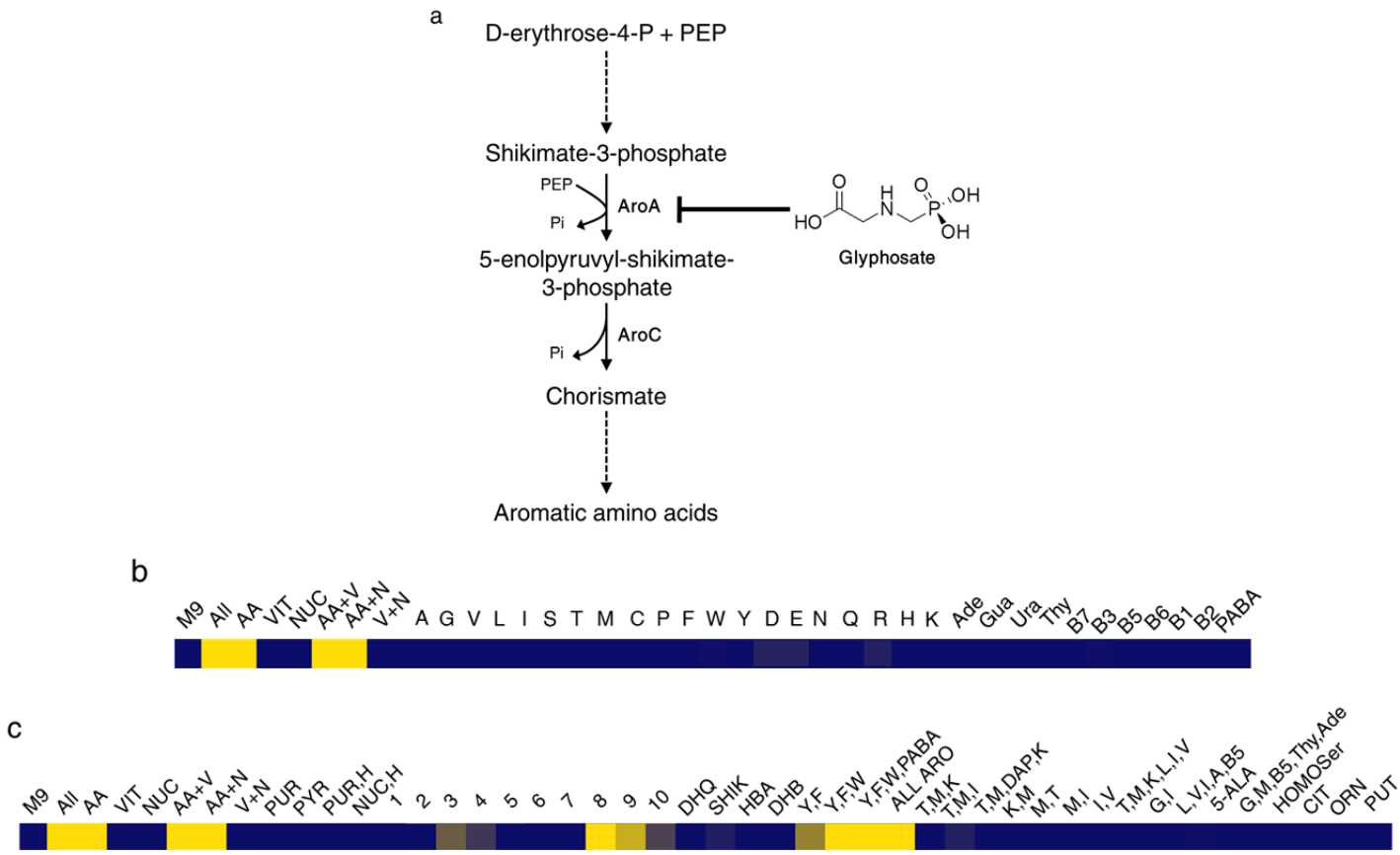


Supplementary Fig. 6. Metabolic suppression profiles of known antibiotics

The metabolic suppression profiles of 22 known antibiotics evaluated against a panel of primary metabolites and pools of metabolites through the metabolic suppression array (**Supplementary Fig. 4**) are derived from the heat map in **Supplementary Fig. 5**. These antibiotics are grouped based on their general MOA class. Note that only inhibitors that impair biosynthetic capabilities are suppressed by metabolites or pools of metabolites. Amino acids are referred to by their 1-letter code, B vitamins by their respective numbers, PABA: *p*-aminobenzoic acid, Ade: adenine, Gua: guanine, Ura: uracil, Thy: thymine. M9: no supplements; ALL: minimal media with all supplements; AA: minimal media with all amino acids; VIT: minimal media with all vitamins; NUC: minimal media with all nucleobases; 5-Me-Tryptophan: 5-methyltryptophan, *p*-F-Phenylalanine: *p*-fluorophenylalanine, 6-NH_2nicotinamide: 6- aminonicotinamide, L-DON: 6-Diazo-5-oxo-L-norleucine. Data represent the mean % residual growth of n=2 replicates.

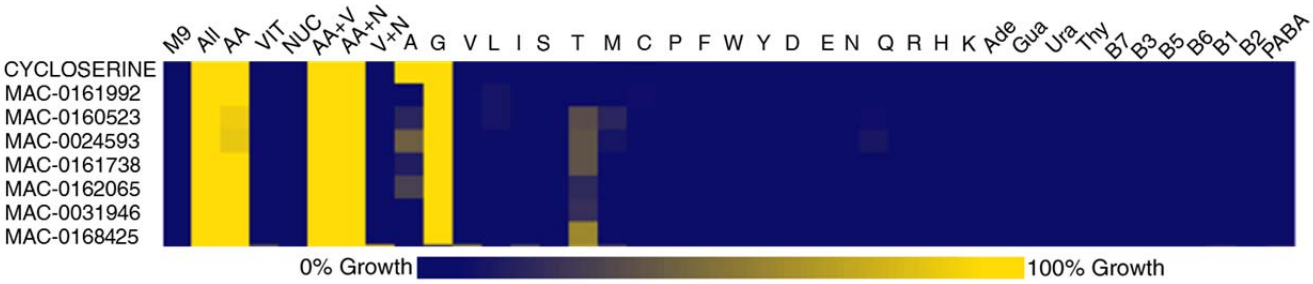


Supplementary Fig. 7. The tetrahydrofolate biosynthetic pathway in *E. coli*



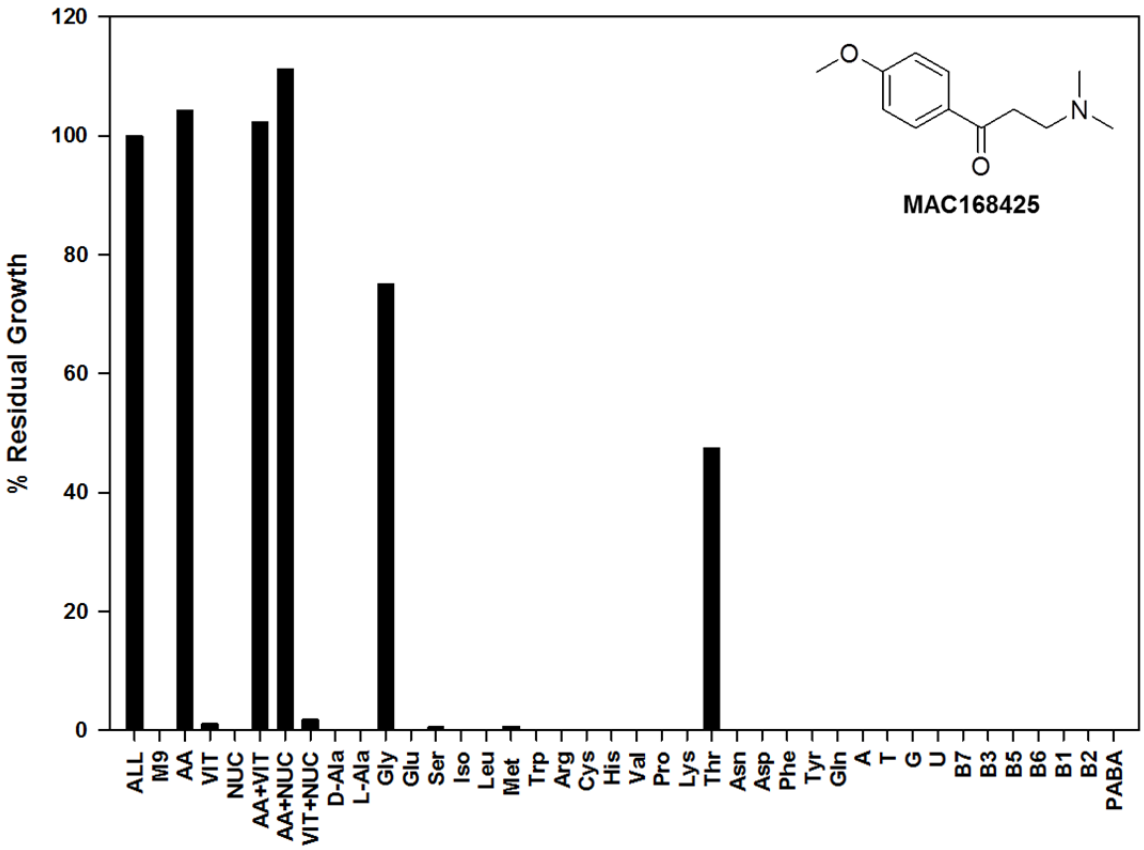
Supplementary Fig. 8. Metabolic suppression profile of glyphosate

(a) Schematic diagram of the biosynthetic pathway of chorismate in *E. coli*. Chorismate is a precursor of several metabolites including aromatic amino acids. Glyphosate inhibits the step catalyzed by AroA. **(b)** The metabolic suppression profile of glyphosate against a panel of primary metabolites in the metabolic suppression array (**Supplementary Fig. 4**) is extracted from the heat map in **Supplementary Fig. 5**. **(c)** The metabolic suppression profile of glyphosate against additional pools of metabolites in the expanded metabolic suppression array (**Fig. 3a**). The legend is the same as that for **Fig. 3**. Note the full suppression of the activity of glyphosate by pools containing the three aromatic amino acids.



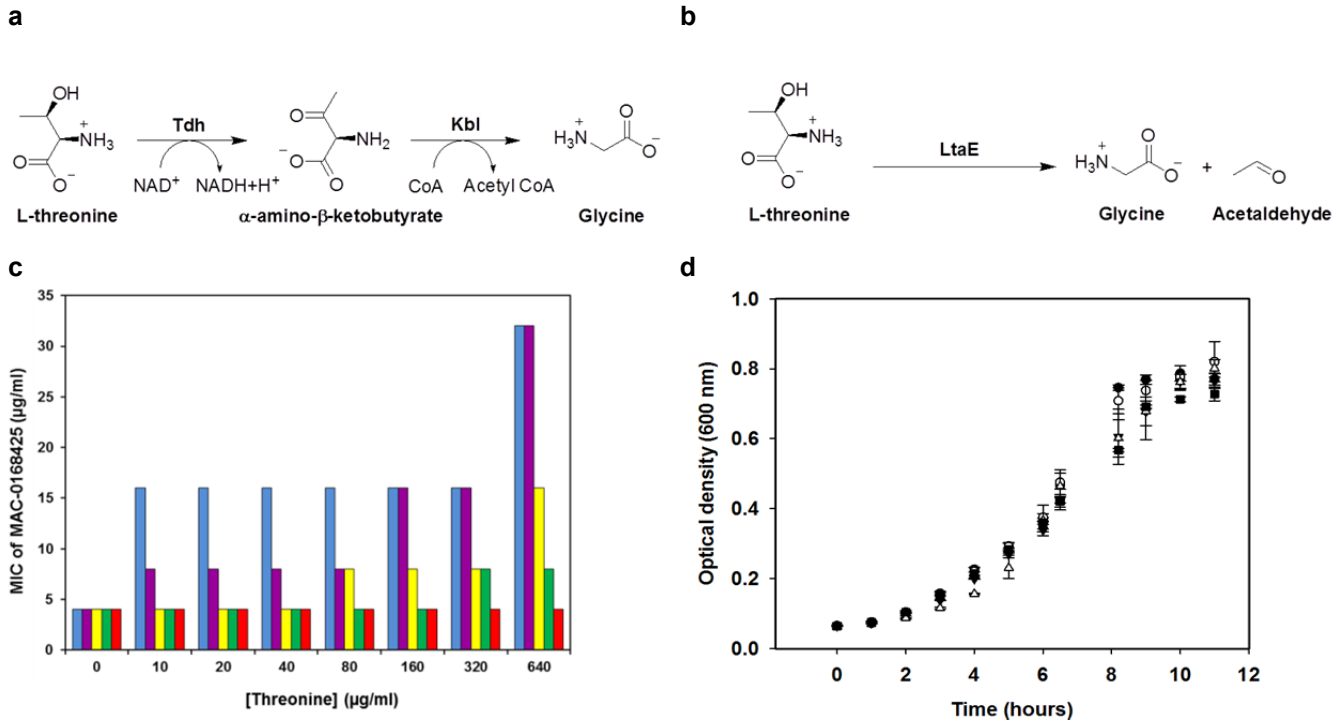
Supplementary Fig. 9. Cluster of actives suppressed by glycine

Cluster from the heat map in **Supplementary Fig. 5** showing the metabolic suppression profiles of 8 inhibitors (including the known antibiotic cycloserine) evaluated against a panel of primary metabolites and pools of metabolites through the metabolic suppression array (**Supplementary Fig. 4**). In this cluster, the activity of all of the compounds is fully suppressed by the amino acid glycine and to varying degrees by the amino acid, L-threonine. Amino acids are referred to by their 1-letter code, B vitamins by their respective numbers, PABA: *p*-aminobenzoic acid, Ade: adenine, Gua: guanine, Ura: uracil, Thy: thymine. M9: no supplements; ALL: minimal media with all supplements; AA: minimal media with all amino acids; VIT: minimal media with all vitamins; NUC: minimal media with all nucleobases. Data represent the mean % residual growth of n=2 replicates.



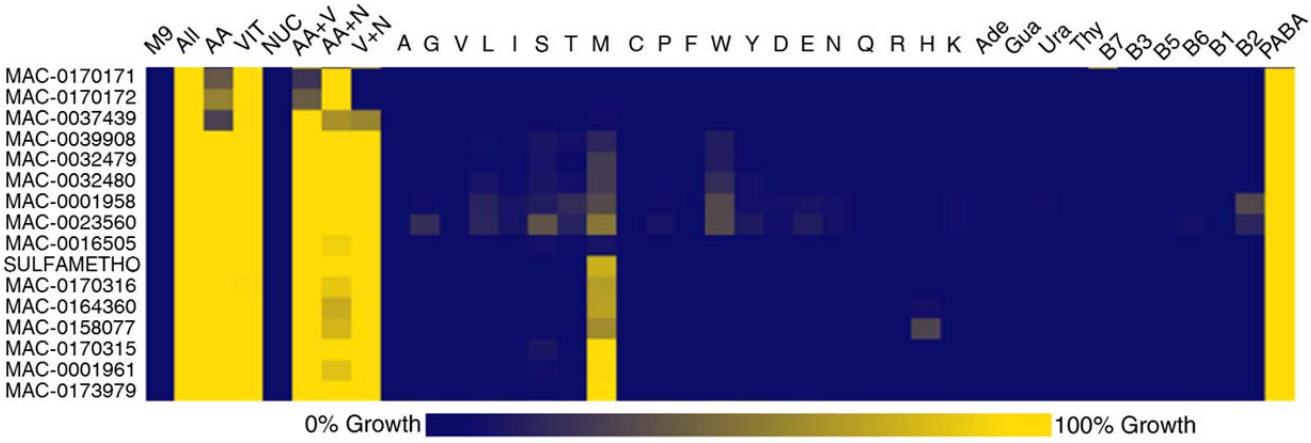
Supplementary Fig. 10. Metabolic suppression profile of MAC168425

A bar graph representation of the metabolic suppression of the activity of MAC168425 by a panel of primary metabolites and pools of metabolites in the metabolic suppression array (**Supplementary Fig. 4**). Note the high suppression of the inhibitory activity of this bioactive by glycine and to a lesser extent by L-threonine as well as pools containing a mixture of amino acids. Amino acids are referred to by their 3-letter code, nucleotides by their 1-letter code, PABA: *p*-aminobenzoic acid. M9: no supplements; ALL: minimal media with all supplements; AA: minimal media with all amino acids; VIT: minimal media with all vitamins; NUC: minimal media with all nucleobases. **Inset:** Chemical structure of MAC168425. Data are representative of at least two independent experiments.



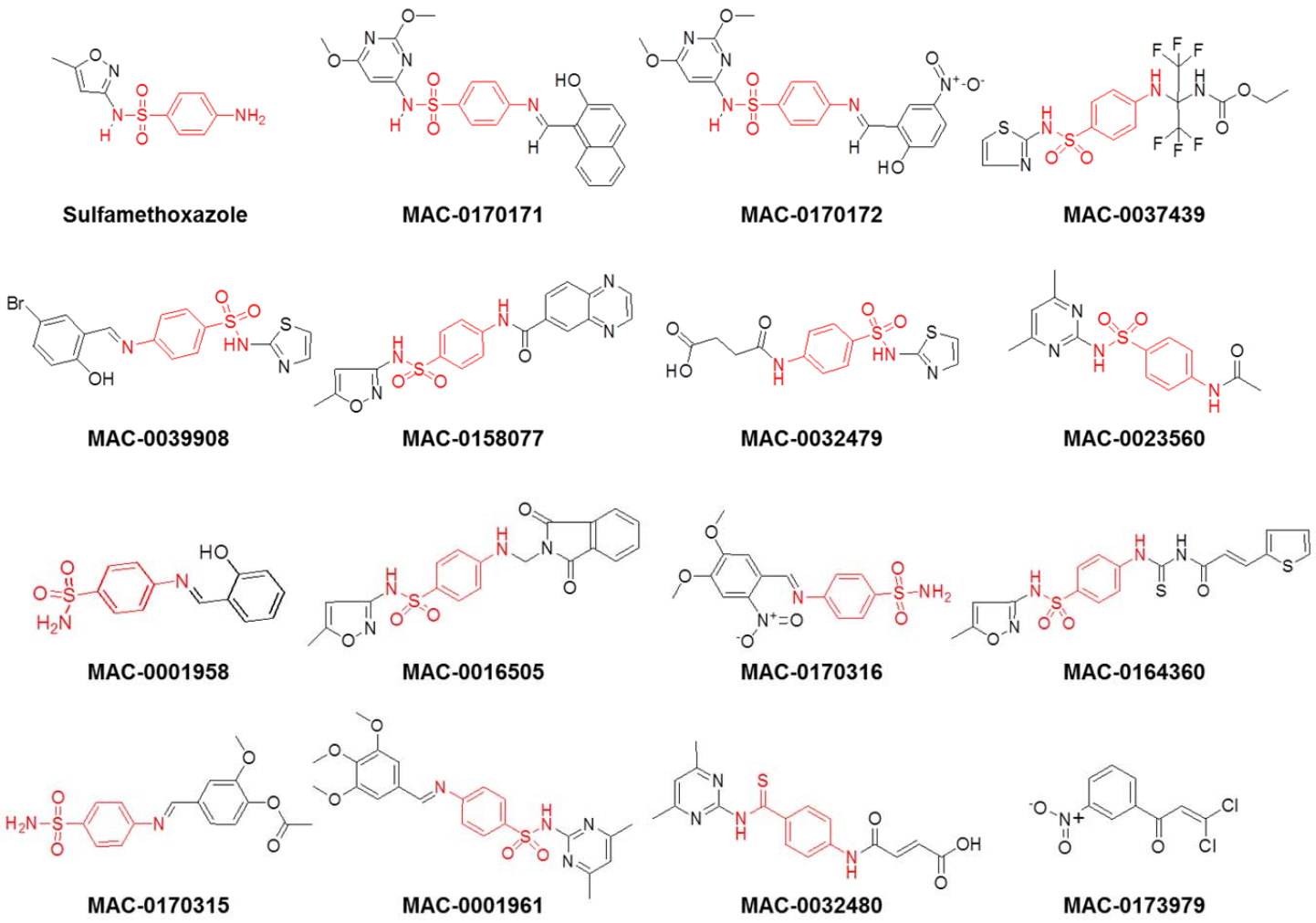
Supplementary Fig. 11. L-threonine suppresses MAC168425 through its conversion into glycine

(a) and **(b)** Pathways for the conversion of L-threonine into glycine in *E. coli*. In the major pathway **(a)** threonine dehydrogenase (Tdh) oxidizes L-threonine to α-amino-β-ketobutyrate which is cleaved by α-amino-β-ketobutyrate lyase (Kbl) to form glycine and acetyl CoA[3]. In the minor pathway **(b)** threonine is degraded by low-specificity threonine aldolase (LtaE) to form glycine and acetaldehyde[3]. **(c)** The MIC of MAC168425 increases in the presence of increasing concentrations of L-threonine. This effect is abolished in deletion mutants in the pathways that convert L-threonine to glycine. The parent strain *E. coli* BW25113 (blue bar), Δ*ltaE* (purple bar), Δ*tdh* (yellow bar), Δ*ltaE* Δ*tdh* (green bar), Δ*ltaE* Δ*tdh* Δ*kbl* (red bar). **(d)** Growth curves of the parent strain (closed circles), Δ*tdh* (open circles), Δ*ltaE* (closed triangles), Δ*ltaE* Δ*tdh* (open triangles), Δ*ltaE* Δ*tdh* Δ*kbl* (closed squares) in 96-well plates in glucose minimal media. Data and error bars represent mean values ± s.d of 4 biological replicates.



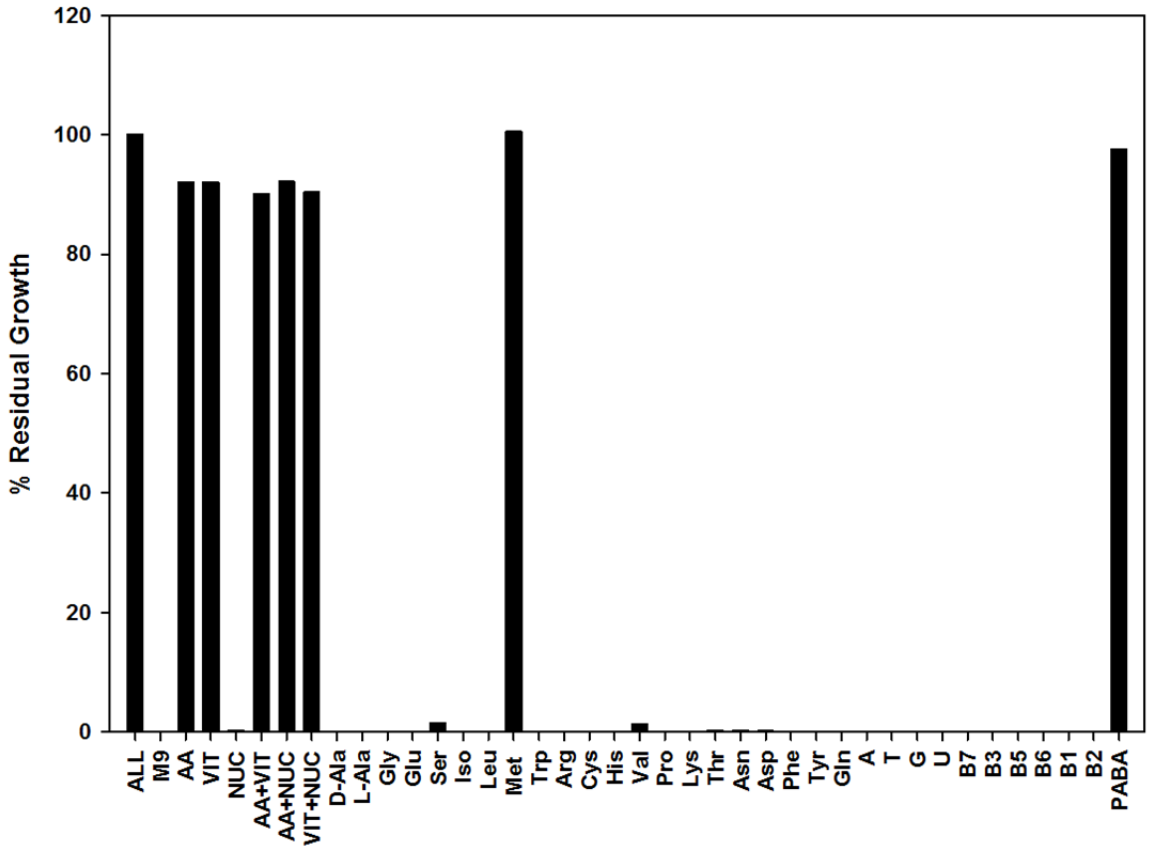
Supplementary Fig. 12. Cluster of actives suppressed by PABA

Cluster from the heat map in **Supplementary Fig. 5** showing the metabolic suppression profiles of 16 actives (including that of the known antibiotic sulfamethoxazole) evaluated against a panel of primary metabolites and pools of metabolites through the metabolic suppression array **(Supplementary Fig. 4)**. In this cluster, the activity of all of the compounds was fully suppressed by the folate biosynthesis intermediate, *p*-aminobenzoic acid (PABA) and to varying degrees by the amino acid, L-methionine. Amino acids are referred to by their 1-letter code, B vitamins by their respective numbers, PABA: *p*-aminobenzoic acid, Ade: adenine, Gua: guanine, Ura: uracil, Thy: thymine. M9: no supplements; ALL: minimal media with all supplements; AA: minimal media with all amino acids; VIT: minimal media with all vitamins; NUC: minimal media with all nucleobases. Data represent the mean % residual growth of n=2 replicates.



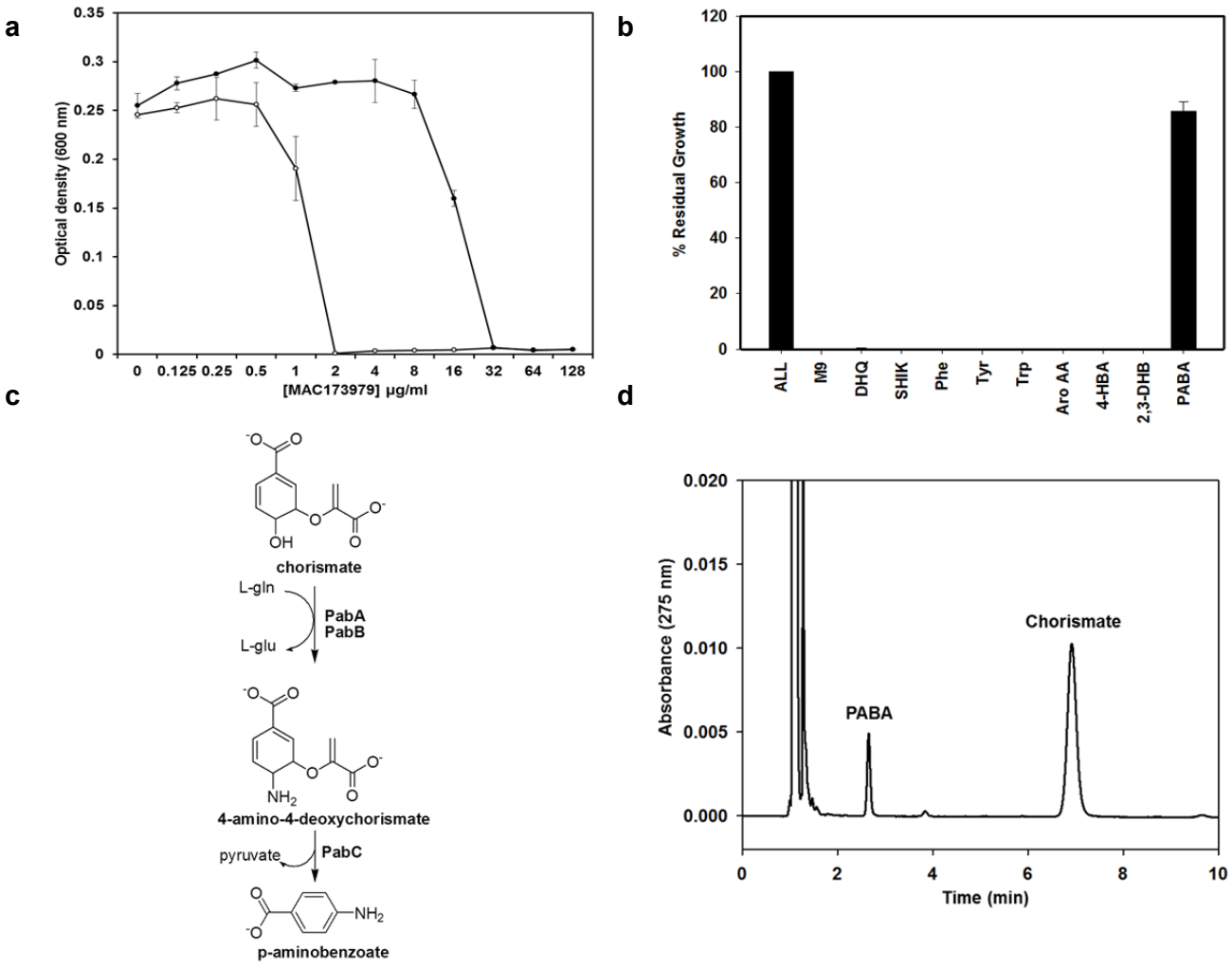
Supplementary Fig. 13. Structures of compounds suppressed by PABA

Chemical structures of the compounds in the cluster suppressed by PABA in the heat map in **Supplementary Fig. 5** and **Supplementary Fig. 12**. Note that the majority of the compounds in the cluster with the exception of MAC173979 contain the p-aminobenzenesulfonamide pharmacophore and are structural analogs of the known antibiotic sulfamethoxazole.



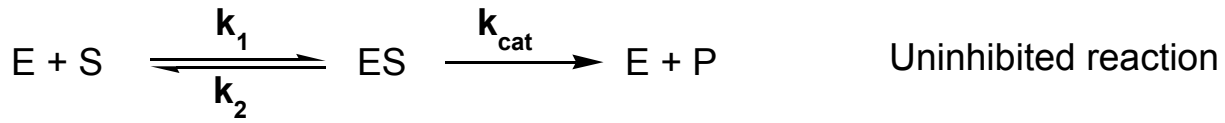
Supplementary Fig. 14. Metabolic suppression profile of MAC173979

A bar graph representation of the metabolic suppression of the activity of MAC173979 by a panel of primary metabolites and pools of metabolites in the metabolic suppression array (**Supplementary Fig. 4**). Note the complete suppression of the inhibitory activity of this bioactive by methionine and *p*-aminobenzoic acid as well as pools containing either or both metabolites. Amino acids are referred to by their 3-letter code, nucleotides by their 1-letter code, PABA: *p*-aminobenzoic acid. M9: no supplements; ALL: minimal media with all supplements; AA: minimal media with all amino acids; VIT: minimal media with all vitamins; NUC: minimal media with all nucleobases. Data are representative of at least two independent experiments.



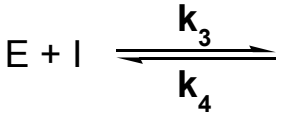
Supplementary Fig. 15. Suppression of MAC173979 and assay for PABA synthesis

(a) PABA suppresses up to 16-fold the activity of MAC173979 against *E. coli*. The MIC of the inhibitor was determined in minimal media in the absence (open circles) or presence (closed circles) of PABA (2 µg/ml). Data with the error bars represent the mean values ± range of n=2 biological replicates. **(b)** The activity of MAC173979 is not suppressed by precursors or products of chorismate other than PABA. Data with the error bars represent the mean % residual growth ± range of n=2 replicates. The legend is the same as that for **Fig. 3 (c)** PABA biosynthetic pathway in *E. coli*. **(d)** UV trace of a mixture containing standards of PABA and chorismate separated on a C_{18} reverse phase column and eluted isocratically with 5% acetic acid in double distilled H_2O[4].



a

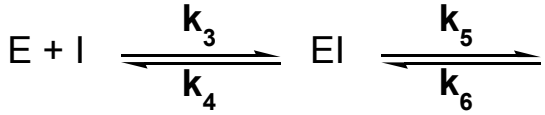
Simple reversible time dependent inhibition



$$Ki = \frac{k_4}{k_3} = \frac{[E][I]}{[EI]}$$

b

Two-step time dependent inhibition

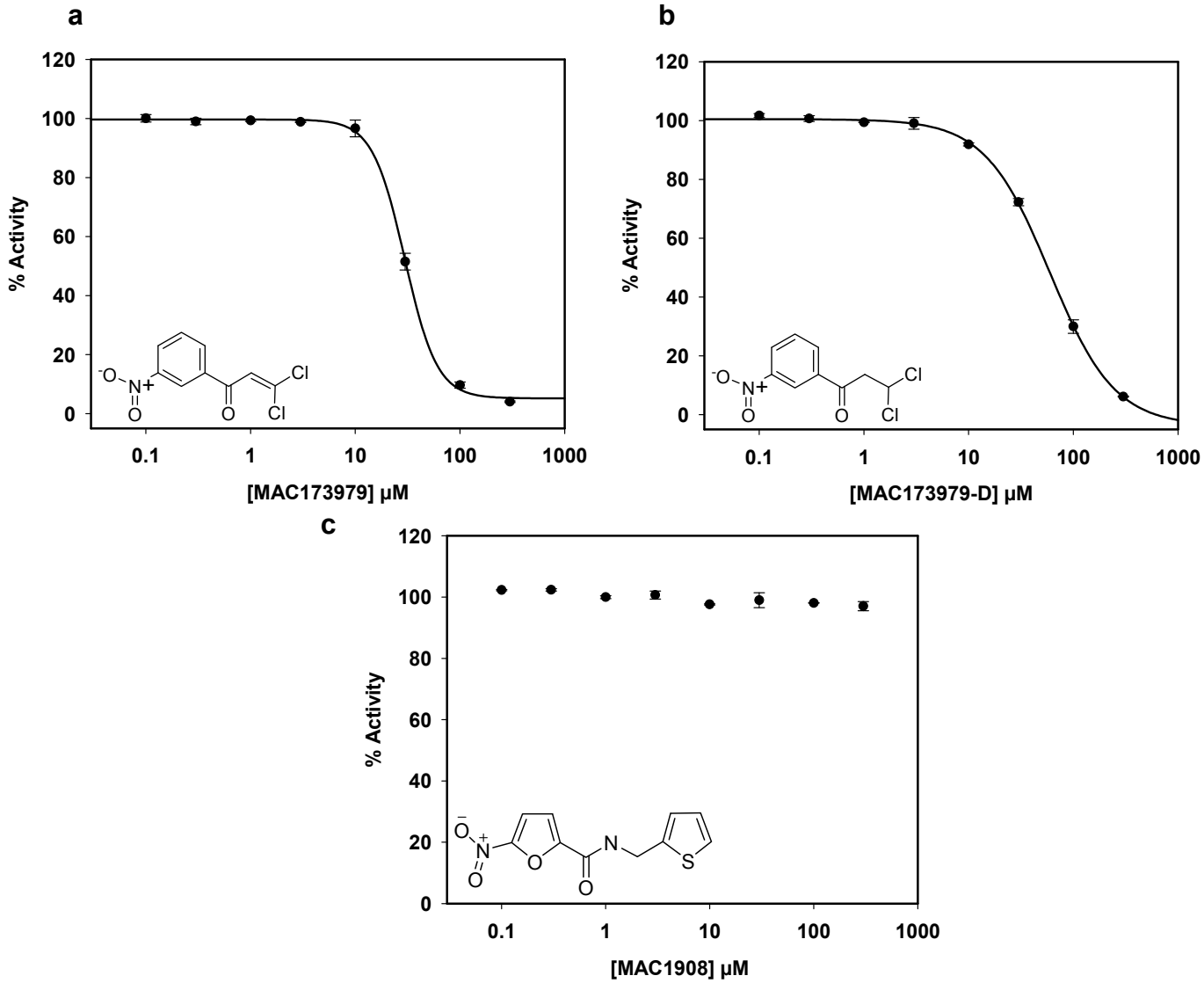


$$Ki = \frac{k_4}{k_3} = \frac{[E][I]}{[EI]}$$

$$Ki^{*} = \frac{Ki\,k_6}{k_5 + k_6} = \frac{[E][I]}{[EI] + [EI^{*}]}$$

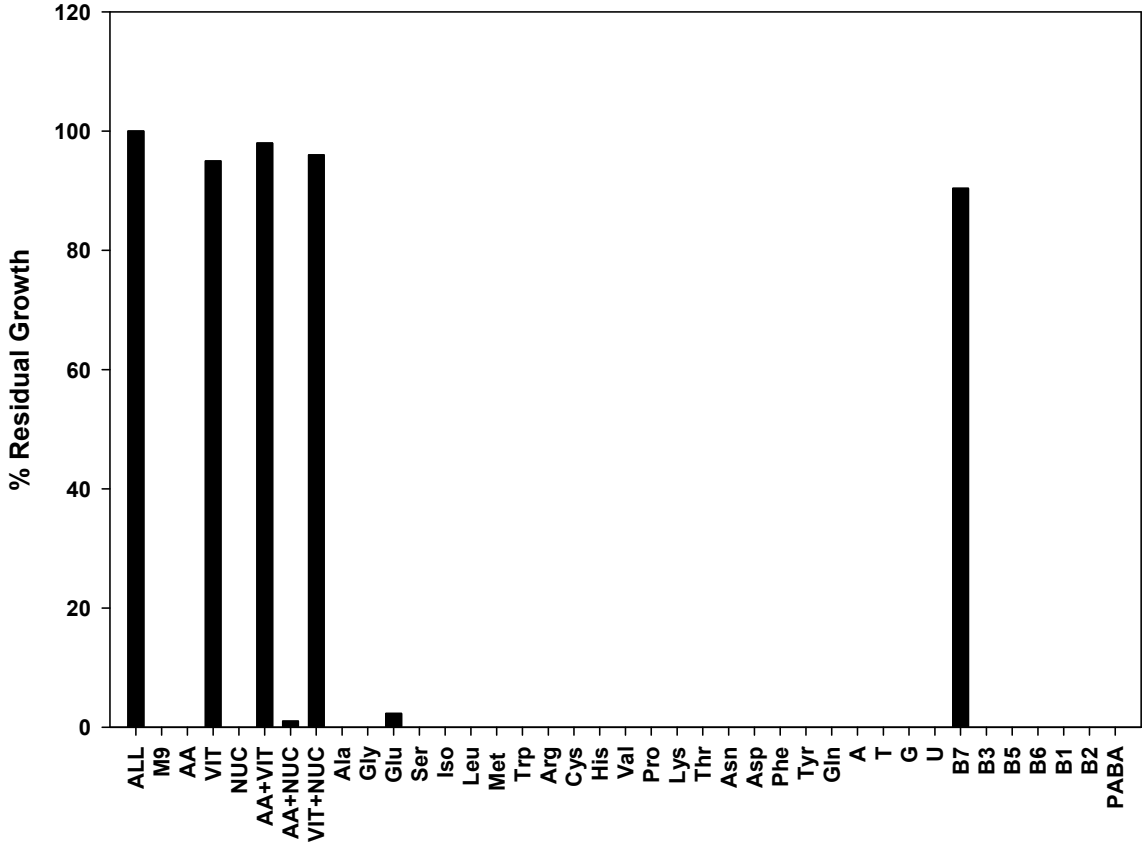
Supplementary Fig. 16. Schemes for time-dependent inhibition

Time-dependent inhibitors of enzyme activities can follow one of two kinetic schemes. In the case of simple reversible inhibition **(a)**, formation of the EI complex is a single step event that occurs on a slow time scale relative to the rate of catalysis and is in equilibrium with its dissociation. In a second scheme **(b)**, binding of the inhibitor to the enzyme occurs rapidly then through a second slower step the EI complex undergoes a conformational change to form the inactive EI* complex. In the case where k_6 is extremely low that it approaches zero, the inhibition is essentially irreversible[1,2].



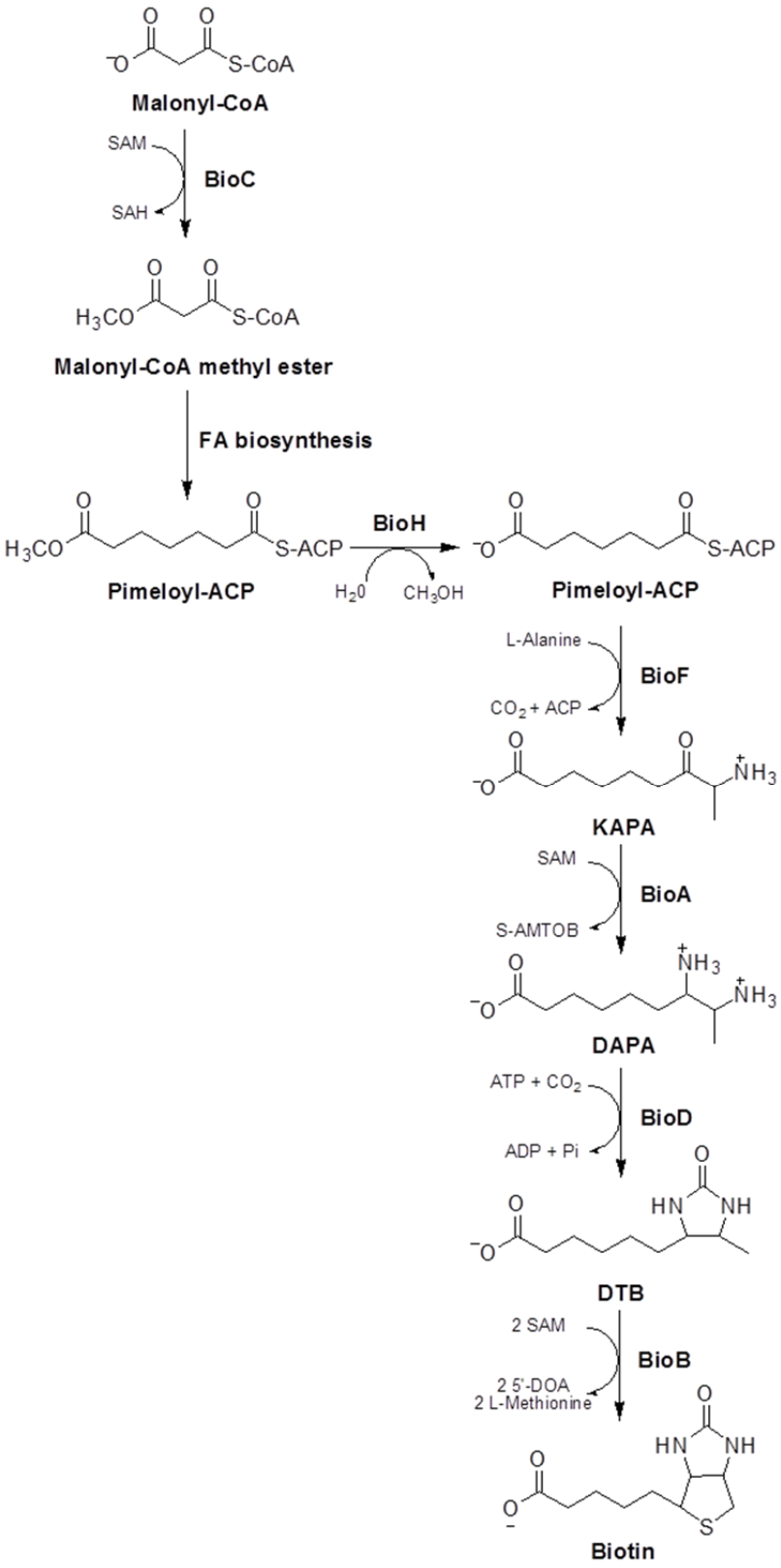
Supplementary Fig. 17. Dose-response determinations against PabA-B-C

Dose-response curves of MAC173979 **(a)** and an analog lacking the Michael acceptor, MAC173979-D **(b)**. As a negative control, an inhibitor not suppressed by PABA, MAC1908, was also tested **(c)**. Structures are shown as insets. Inhibitors (0-1000 µM) were incubated with the enzymes for 30 minutes prior to substrates addition. Reactions were quenched after 30 minutes with 8 M urea and analyzed by HPLC. Data with the error bars represent the mean % activity ± range of n=2 replicates and the dose response curves were fitted to the four parameter logistic nonlinear regression curve yielding IC_{50} values of 30 ± 2 µM **(a)**, 60 ± 7 µM **(b)** and no inhibition for **(c)**.

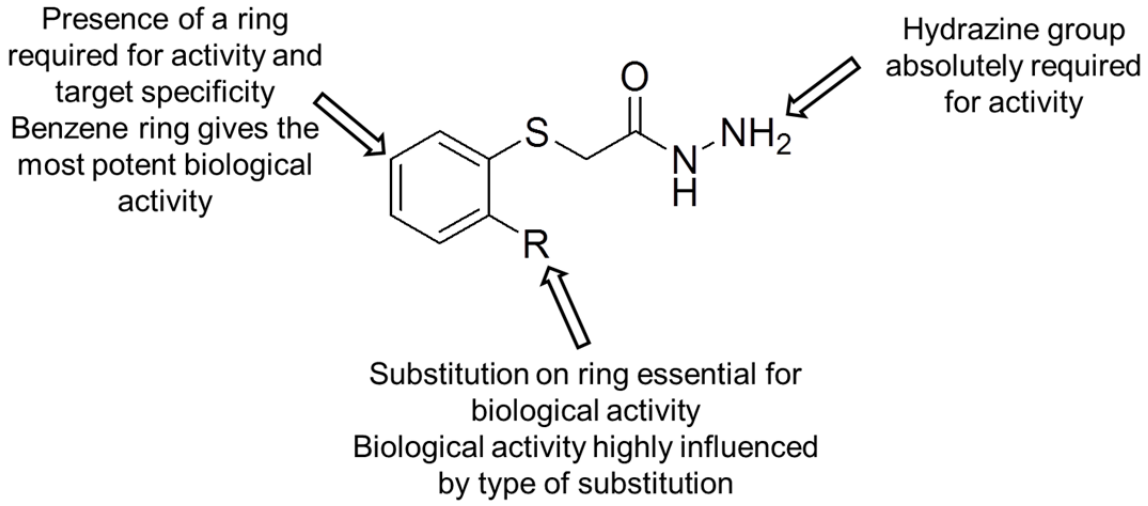


Supplementary Fig. 18. Metabolic suppression profile of MAC13772

A bar graph representation of the metabolic suppression of the activity of MAC13772 by a panel of primary metabolites and pools of metabolites in the metabolic suppression array (**Supplementary Fig. 4**). Note the complete suppression of the inhibitory activity of this bioactive by biotin (vit.B7) as well as pools containing vitamins. Amino acids are referred to by their 3-letter code, nucleotides by their 1-letter code, PABA: *p*-aminobenzoic acid. M9: no supplements; ALL: minimal media with all supplements; AA: minimal media with all amino acids; VIT: minimal media with all vitamins; NUC: minimal media with all nucleobases. Data are representative of at least two independent experiments.



Supplementary Fig. 19. Biotin biosynthesis in *E. coli*



Supplementary Fig. 20. Summary of structure-activity relationship investigation of MAC13772 and analogs

This schematic summarizes the main findings from the SAR study into the biological and biochemical activity of MAC13772 and 24 analogs (see **Table 1**, **Supplementary Table 5**).

3. SUPPLEMENTARY NOTES

CHEMICAL CHARACTERIZATION OF COMPOUNDS USED IN THIS STUDY

*3-(dimethylamino)-1-(4-methoxyphenyl)propan-1-one (**MAC168425**)*

^{1}H NMR (700 MHz, DMSO-d_6): δ ppm 8.01 (2 H, d, J=8.95 Hz), 7.13 (2 H, d, J=8.96 Hz), 3.90 (1 H, s), 3.60 (2 H, t, J=7.14, 0.95 Hz), 3.41 (2 H, t, J=7.56 Hz), 2.82 (6 H, s); ^{13}C NMR (176 MHz, DMSO-d_6): δ ppm 195.55, 163.99, 130.87, 129.36, 114.47, 56.11, 52.37, 42.66, 33.19. HRMS: for C12H18NO2 [M+1]+, calcd.: 208.1338, obs.: 208.1330.

*3,3-dichloro-1-(3-nitrophenyl)prop-2-en-1-one (**MAC173979**)*

^{1}H NMR (600 MHz, DMSO-d_6): δ ppm 8.70 (1 H, dd, J=2.3, 1.8 Hz), 8.51 (1 H, dd, J=8.2, 1.0 Hz), 8.44 (1 H, d, J=7.7 Hz), 8.04 (1 H, s), 7.86 (1 H, d, J=0.5); ^{13}C NMR (150.92 MHz, DMSO-d_6): δ ppm 184.68, 148.16, 137.60, 134.69, 134.64, 130.75, 128.02, 124.77, 122.91. HRMS: for C9H5Cl2NO3 [M], calcd.: 244.9668, obs.: 244.9668.

*3,3-dichloro-1-(3-nitrophenyl)propan-1-one: analog of MAC173979 without the Michael acceptor (**MAC173979-D**)* (purchased from Ambinter- France)

^{1}H NMR (700 MHz, DMSO-d_6): δ ppm 8.69 (1 H, t, J=1.83 Hz), 8.51 (1 H, ddd, J=8.14, 2.36, 1.06 Hz), 8.45 (1 H, dq, J=7.75, 0.95 Hz), 7.86 (1 H, t, J=8.09 Hz), 6.61 (1 H, t, J=6.07 Hz), 4.32 (2 H, d, J=6.17 Hz); ^{13}C NMR (176 MHz, DMSO-d_6): δ ppm 193.02, 148.09, 136.69, 134.42, 130.71, 128.00, 122.57, 69.18, 51.26. HRMS: for C9H7Cl2NO3 [M], calcd.: 246.9803, obs.: 246.9800.

*2-(2-nitrophenylthio)acetohydrazide (**Table 1**, Compound **MAC13772**)*

^{1}H-NMR (700 MHz, DMSO-d_6): δ ppm 9.40 (1 H, br. s.), 8.17 - 8.22 (1 H, m), 7.68 - 7.74 (2 H, m), 7.41 (1 H, ddd, *J*=8.30, 5.76, 2.69 Hz), 4.32 (2 H, s), 3.77 (2 H, s). ^{13}C-NMR

(176 MHz, DMSO-d_6): δ ppm 166.48, 145.40, 136.28, 134.23, 127.53, 125.81, 125.51, 34.06. HRMS: for C8H10N3O3S [M+1]+, calcd.: 228.0443, obs.: 228.0439.

*2-(3-nitrophenylthio)acetohydrazide (**Table 1**, Compound **5**)*

^{1}H-NMR (700 MHz, DMSO-d_6): δ ppm 9.20 (1 H, s), 7.36 - 7.39 (1 H, m), 7.32 - 7.34 (1 H, m), 6.98 (1 H, dd, *J*=8.08, 2.09 Hz), 6.67 (1 H, t, *J*=6.13 Hz), 4.26 (2 H, s), 3.73 (2 H, d, *J*=6.28 Hz); ^{13}C-NMR (176 MHz, DMSO-d_6): δ ppm 168.63, 149.53, 148.72, 129.83, 118.66, 110.33, 105.48, 44.67.

*2-(4-nitrophenylthio)acetohydrazide (**Table 1**, Compound **6**)*

^{1}H-NMR (700 MHz, DMSO-d_6): δ ppm 11.59 (1 H, br. s.), 8.15 (2 H, m, *J*=8.98 Hz), 7.60 (2 H, m, *J*=8.98 Hz), 4.08 (2 H, s), 3.56 (2 H, s); ^{13}C-NMR (176 MHz, DMSO-d_6): δ ppm 166.94, 126.66, 123.86, 66.35, 32.43; HRMS: for C8H10N3O3S [M+1]+, calcd.: 228.0443, obs.: 228.0453.

*2-(phenylthio)acetohydrazide (**Table 1**, Compound **7**)*

^{1}H-NMR (700 MHz, DMSO-d_6): δ ppm 9.24 (1 H, br. s.), 7.33 - 7.38 (2 H, m), 7.28 - 7.33 (2 H, m), 7.16 - 7.21 (1 H, m), 4.28 (2 H, br. s.), 3.59 (2 H, s); ^{13}C-NMR (176 MHz, DMSO-d_6): δ ppm 167.23, 136.16, 128.94, 127.79, 125.78, 34.59; HRMS: for C8H11N2OS [M+1]+, calcd.: 183.0592, obs.: 183.0589.

*2-(2-fluorophenylthio)acetohydrazide (**Table 1**, Compound **8**)*

^{1}H-NMR (700 MHz, DMSO-d_6): δ ppm 9.26 (1 H, br. s.), 7.48 (1 H, td, *J*=7.85, 1.65 Hz), 7.24 - 7.31 (1 H, m), 7.16 - 7.24 (2 H, m), 4.28 (2 H, s), 3.60 (2 H, s); ^{13}C-NMR (176 MHz, DMSO-d_6): δ ppm 166.85, 158.81, 130.16, 128.02, 125.03, 123.02, 115.29, 33.79; HRMS: for C8H10FN2OS [M+1]+, calcd.: 201.0498, obs.: 201.0506.

*2-(2-chlorophenylthio)acetohydrazide (**Table 1**, Compound **9**)*

^{1}H-NMR (700 MHz, DMSO-d_6): δ ppm 10.61 (1 H, br. s.), 7.47 (2 H, dt, *J*=7.78, 1.20 Hz), 7.33 (1 H, td, *J*=7.63, 1.50 Hz), 7.20 - 7.24 (1 H, m), 3.96 (2 H, s), 3.56 (2 H, s); ^{13}C-NMR (176 MHz, DMSO-d_6): δ ppm 167.13, 134.73, 130.74, 129.45, 127.82, 127.59, 126.91, 32.53; HRMS: for C8H10ClN2OS [M+1]+, calcd.: 217.0202, obs.: 217.0212.

*2-(2-hydroxyphenylthio)acetohydrazide (**Table 1**, Compound **10**)*

^{1}H-NMR (700 MHz, DMSO-d_6): δ ppm 9.24 (1 H, br. s.), 7.25 (1 H, dd, *J*=7.78, 1.80 Hz), 7.05 (1 H, td, *J*=7.63, 1.50 Hz), 6.81 (1 H, dd, *J*=8.08, 1.20 Hz), 6.76 (1 H, td, *J*=7.48, 1.20 Hz), 4.29 (2 H, br. s.), 3.49 (2 H, s); ^{13}C-NMR (176 MHz, DMSO-d_6): δ ppm 167.82, 155.40, 129.81, 127.52, 121.74, 119.53, 115.05, 34.22; HRMS: for C8H11N2O2S [M+1]+, calcd.: 199.0541, obs.: 199.0549.

*2-(2-aminophenylthio)acetohydrazide (**Table 1**, Compound **11**)*

^{1}H-NMR (700 MHz, DMSO-d_6): δ ppm 9.15 (1 H, br. s.), 7.24 (1 H, dd, *J*=7.78, 1.50 Hz), 6.99 - 7.05 (1 H, m), 6.69 (1 H, dd, *J*=8.08, 1.20 Hz), 6.49 (1 H, td, *J*=7.33, 1.20 Hz), 5.41 (2 H, s), 4.24 (2 H, br. s.), 3.31 (2 H, s); ^{13}C-NMR (176 MHz, DMSO-d_6): δ ppm 167.79, 149.45, 134.93, 129.53, 116.29, 115.92, 115.67, 114.39, 36.30; HRMS: for C8H12N3OS [M+1]+, calcd.: 198.0701, obs.: 198.0696.

*2-(o-tolylthio)acetohydrazide (**Table 1**, Compound **12**)*

^{1}H-NMR (700 MHz, DMSO-d_6): δ ppm 9.25 (1 H, s), 7.30 - 7.35 (1 H, m), 7.15 - 7.20 (2 H, m), 7.07 - 7.12 (1 H, m), 4.28 (2 H, s), 3.58 (2 H, s), 2.28 (3 H, s);^{13}C-NMR (176 MHz, DMSO-d_6): δ ppm 167.10, 135.80, 135.38, 129.85, 126.96, 126.57, 125.49, 34.11, 19.75. HRMS: for C9H13N2OS [M+1]+, calcd.: 197.0749, obs.: 197.0746.

*2-(2-methoxyphenylthio)acetohydrazide (**Table 1**, Compound **13**)*

^{1}H-NMR (700 MHz, DMSO-d_6): δ ppm 9.22 (1 H, br. s.), 7.27 (1 H, dd, *J*=7.78, 1.50 Hz), 7.14 - 7.20 (1 H, m), 6.97 (1 H, dd, *J*=8.08, 1.20 Hz), 6.92 (1 H, td, *J*=7.48, 1.20 Hz), 3.81 (3 H, s), 4.26 (2 H, s), 3.52 (2 H, s); ^{13}C-NMR (176 MHz, DMSO-d_6): δ ppm 167.31, 155.97, 127.42, 126.67, 124.42, 120.96, 110.73, 55.70, 33.09. HRMS: for C9H12N2O2S [M+1]+, calcd.: 213.0698, obs.: 213.0697.

*N'-{2-[(4-nitrophenyl)sulfanyl]acetyl}acetohydrazide (**Table 1**, Compound **14**)*

^{1}H-NMR (700 MHz, DMSO-d_6): δ ppm 10.20 (1 H, s), 9.94 (1 H, s), 8.10 - 8.15 (2 H, m), 7.56 - 7.61 (2 H, m), 3.92 (2 H, s), 1.85 (3 H, s);^{13}C-NMR (176 MHz, DMSO-d_6): δ ppm 167.88, 166.03, 146.76, 144.65, 126.45, 123.75, 32.99, 20.38. HRMS: for C10H12N3O4S [M+1]+, calcd.: 270.0549, obs.: 270.0557.

*1-(2-nitrophenylthio)butan-2-one (**Table 1**, Compound **15**)*

^{1}H-NMR (700 MHz, DMSO-d_6): δ ppm 8.19 (1 H, dd, *J*=8.23, 1.35 Hz), 7.68 (1 H, ddd, *J*=8.30, 7.11, 1.35 Hz), 7.47 (1 H, dd, *J*=8.23, 1.05 Hz), 7.39 (1 H, ddd, *J*=8.38, 7.18, 1.20 Hz), 4.24 (2 H, s), 2.63 (2 H, q, *J*=7.28 Hz), 0.92 - 0.98 (3 H, m); ^{13}C-NMR (176 MHz, DMSO-d_6): δ ppm 205.07, 145.58, 135.70, 134.14, 127.61, 125.85, 125.43, 41.59, 34.19, 7.61. HRMS: for C10H12NO3S [M+1]+, calcd.: 226.0538, obs.: 226.0542.

*2-(2-nitrophenylthio)acetamide (**Table 1**, Compound **16**)*

^{1}H-NMR (700 MHz, DMSO-d_6): δ ppm 8.20 (1 H, dd, *J*=8.23, 1.35 Hz), 7.72 (1 H, ddd, *J*=8.38, 7.18, 1.50 Hz), 7.68 (1 H, br. s.), 7.63 (1 H, dd, *J*=8.23, 1.05 Hz), 7.40 (1 H, ddd, *J*=8.15, 7.11, 1.20 Hz), 7.26 (1 H, br. s.), 3.78 (2 H, s); ^{13}C-NMR (176 MHz, DMSO-d_6): δ ppm 169.10, 145.34, 136.55, 134.22, 127.31, 125.86, 125.39, 35.68. HRMS: for C8H9N2O3S [M+1]+, calcd.: 213.0334, obs.: 213.0334.

*1-(2-nitrophenylthio)propan-2-one (**Table 1**, Compound **17**)*

^{1}H-NMR (700 MHz, DMSO-d_6): δ ppm 8.20 (1 H, dd, *J*=8.38, 1.50 Hz), 7.68 (1 H, td, *J*=7.78, 1.50 Hz), 7.47 (1 H, dd, *J*=8.08, 0.90 Hz), 7.36 - 7.42 (1 H, m), 4.25 (2 H, s), 2.26 (3 H, s); ^{13}C-NMR (176 MHz, DMSO-d_6): δ ppm 202.49, 145.56, 135.59, 134.17, 127.55, 125.89, 125.47, 42.51, 28.89. HRMS: for C9H10NO3S [M+1]+, calcd.: 212.0381, obs.: 212.0390.

*2-(2-nitrophenylthio)acetic acid (**Table 1**, Compound **18**)*

^{1}H-NMR (700 MHz, DMSO-d_6): δ ppm 10.55 (1 H, br. s.), 8.21 (1 H, dd, *J*=8.23, 1.35 Hz), 7.70 - 7.75 (1 H, m), 7.59 (1 H, dd, *J*=8.23, 1.05 Hz), 7.41 (1 H, ddd, *J*=8.15, 7.11, 1.20 Hz), 4.01 (2 H, s); ^{13}C-NMR (176 MHz, DMSO-d_6): δ ppm 169.98, 145.43, 135.83, 134.30, 127.32, 125.91, 125.55, 34.35. HRMS: for C9H10NO3S [M+NH4]+, calcd.: 231.0440, obs.: 231.0429.

*2-(methylthio)acetohydrazide (**Table 1**, Compound **19**)*

^{1}H-NMR (700 MHz, DMSO-d_6): δ ppm 9.10 (1 H, br. s.), 4.10 (2 H, br. s.), 3.00 (2 H, s), 2.09 (3 H, s); ^{13}C-NMR (176 MHz, DMSO-d_6): δ ppm 168.38, 34.59, 15.53. HRMS: for C3H9N2OS [M+1]+, calcd.: 121.0436, obs.: 121.0444.

*2-mercaptoacetohydrazide (**Table 1**, Compound **20**)*

^{1}H-NMR (700 MHz, DMSO-d_6): δ ppm 9.05 (1 H, br. s.), 4.36 (2 H, br. s.), 3.04 (1 H, br. s.), 1.74 (2 H, s); ^{13}C-NMR (176 MHz, DMSO-d_6): δ ppm 169.40, 33.49. HRMS: for C2H7N2OS [M+1]+, calcd.: 106.0201, obs.: 106.0213.

*butyrohydrazide (**Table 1**, Compound **21**)*

^{1}H-NMR (700 MHz, DMSO-d_6): δ ppm 8.90 (1 H, br. s.), 4.14 (2 H, br. s.), 1.97 (2 H, t, *J*=7.48 Hz), 1.48 - 1.52 (2 H, m), 0.84 (3 H, t, *J*=7.33 Hz); ^{13}C-NMR (176 MHz, DMSO-

d_6): δ ppm 170.90, 35.37, 18.62, 13.62. HRMS: for C4H10N2O [M+1]+, calcd.: 102.0793, obs.: 102.0804.

*propionohydrazide (**Table 1**, Compound **22**)*

^{1}H-NMR (700 MHz, DMSO-d_6): δ ppm 0.98 (3 H, t, *J*=7.63 Hz), 2.00 (2 H, q, *J*=7.48 Hz), 4.13 (2 H, br. s.), 8.89 (1 H, br. s.); ^{13}C-NMR (176 MHz, DMSO-d_6): δ ppm 172.46, 26.66, 9.98. HRMS: for C3H8N2O [M+1]+, calcd.: 88.0637, obs.: 88.0632.

*acetohydrazide (**Table 1**, Compound **23**)*

^{1}H-NMR (700 MHz, DMSO-d_6): δ ppm 8.92 (1 H, br. s.), 4.13 (2 H, br. s.), 1.74 (3 H, s); ^{13}C-NMR (176 MHz, DMSO-d_6): δ ppm 168.60, 20.50. HRMS: for C2H6N2O [M+1]+, calcd.: 74.048, obs.: 74.0466.

*2,3-dihydrobenzo[b]thiophene-2-carbohydrazide (**Table 1**, Compound **24**)*

^{1}H-NMR (700 MHz, DMSO-d_6): δ ppm 11.52 (1 H, br. s.), 7.23 (1 H, d, *J*=7.18 Hz), 7.18 (1 H, d, *J*=7.48 Hz), 7.12 (1 H, t, *J*=7.33 Hz), 7.03 (1 H, td, *J*=7.41, 1.05 Hz), 4.61 (1 H, dd, *J*=8.83, 4.94 Hz), 3.52 (1 H, dd, *J*=16.16, 4.79 Hz), 3.46 (1 H, dd, *J*=16.01, 8.83 Hz); ^{13}C-NMR (176 MHz, DMSO-d_6): δ ppm 170.43, 138.94, 138.88, 127.50, 124.69, 124.64, 121.27, 46.30, 37.38. HRMS: for C9H11N2OS [M+1]+, calcd.: 195.0592, obs.: 195.0597.

*2-(benzylthio)acetohydrazide (**Table 1**, Compound **25**)*

^{1}H-NMR (700 MHz, DMSO-d_6): δ ppm 9.12 (1 H, br. s.), 7.31 - 7.34 (4 H, m), 7.22 - 7.27 (1 H, m), 4.26 (2 H, s), 3.82 (2 H, s), 2.97 (2 H, s); ^{13}C-NMR (176 MHz, DMSO-d_6): δ ppm 168.26, 138.04, 128.94, 128.35, 126.87, 35.63, 32.09. HRMS: for C9H13N2OS [M+1]+, calcd.: 197.0749, obs.: 197.0756.

*2-(naphthalen-2-ylthio)acetohydrazide (**Table 1**, Compound **26**)*

^{1}H-NMR (700 MHz, DMSO-d_6): δ ppm 9.30 (1 H, s), 7.79 - 7.90 (4 H, m), 7.42 - 7.56 (3 H, m), 4.31 (2 H, br. s.), 3.71 (2 H, s); ^{13}C-NMR (176 MHz, DMSO-d_6): δ ppm 167.16, 133.77, 133.30, 131.13, 128.26, 127.59, 126.88, 126.68, 126.31, 125.65, 125.27, 34.57. HRMS: for C12H13N2OS [M+1]+, calcd.: 233.0749, obs.: 233.0746.

*2-(pyridin-4-ylthio)acetohydrazide (**Table 1**, Compound **27**)*

^{1}H-NMR (700 MHz, DMSO-d_6): δ ppm 11.90 (1 H, br. s.), 8.65 - 8.70 (2 H, m), 7.93 - 8.00 (2 H, m), 4.33 (2 H, s), 3.55 (2 H, s); ^{13}C-NMR (176 MHz, DMSO-d_6): δ ppm 166.01, 161.30, 139.81, 122.44, 66.35, 31.84. HRMS: for C7H10N3OS [M+1]+, calcd.: 184.0545, obs.: 184.0550.

*2-(2-nitrophenyl)acetohydrazide (**Table 1**, Compound **28**)*

^{1}H-NMR (700 MHz, DMSO-d_6): δ ppm 9.18 (1 H, br. s.), 7.98 (1 H, dd, *J*=8.23, 1.35 Hz), 7.66 (1 H, td, *J*=7.48, 1.20 Hz), 7.51 - 7.54 (1 H, m), 7.48 (1 H, dd, *J*=7.63, 1.05 Hz), 4.19 (2 H, s), 3.80 (2 H, s); ^{13}C-NMR (176 MHz, DMSO-d_6): δ ppm 168.12, 149.24, 133.26, 133.15, 130.55, 128.16, 124.44, 37.35. HRMS: for C8H10N3O3 [M+1]+, calcd.: 196.0722, obs.: 196.0732.

4. SUPPLEMENTARY REFERENCES

1. Morrison, J. F. & Walsh, C. T. The behavior and significance of slow-binding enzyme inhibitors. *Adv Enzymol Relat Areas Mol Biol* **61**, 201-301 (1988).
2. Copeland, R. A. *Enzymes: A Practical Introduction to Structure, Mechanism, and Data Analysis* (Wiley, New York, 2004).
3. Stauffer, G. V. in *Escherichia coli and Salmonella: Cellular and Molecular Biology* (eds. Böck, A. et al.) (ASM Press, Washington, D.C., 2004).
4. Walsh, C., Erion, M., Walts, A., Delany, J. & Berchtold, G. Chorismate aminations: partial purification of Escherichia coli PABA synthase and mechanistic comparison with anthranilate synthase. *Biochemistry* **26**, 4734-4779 (1987).