Supplementary Information

Functional and Structural Analysis of Phenazine *O*-Methyltransferase LaPhzM from *Lysobacter antibioticus* OH13 and One-Pot Enzymatic Synthesis of the Antibiotic Myxin

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Table S1. Sequence of primers used for the *LaPhzM* expression

LaPhzM-PF	**CCGGAATTC ATGACTGAGAATAACCGTGCC**
LaPhzM-PR	**CCCAAGCTT TCAGATCGGACGCGCTTC**

Table S2. Summary of the data processing statistics.

Space Group	$P2_1$
Cell Dimensions	
a, b, c (Å)	56.28, 81.02, 72.06
β	98.23°
Resolution (Å)[a]	40.0 — 1.42 (1.50 — 1.42)
R_{merge} (%)[a]	4.1 (44.1)
R_{pim} (%)[a]	2.6 (28.2)
Unique reflections[a]	395,285 (58,771)
Multiplicity[a]	3.4 (3.4)
Completeness (%)[a]	97.1 (98.0)
I/σ(I) [a]	15.3 (2.7)

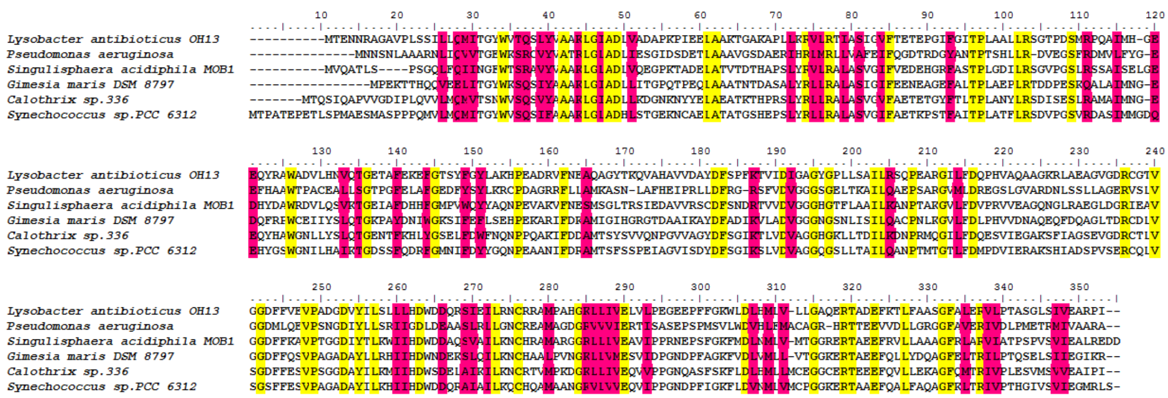
[a]The numbers in the parentheses are the values for the outer shells.

Table S3. Summary of the refinement statistics.

Resolution (Å)	40.0 — 1.42
R_{work} (%)	18.1
R_{free} (%)	20.2
Protein residues	664 (2 chains)
Ligands	2 SAH, 3 HCO_2^-, 3 Na^+, P33 (PEG fragment)
water	494
Average B-factor (Å^2)	
Protein and Ligands	18.5
Solvent	26.4
RMSD (bonds) (Å)	0.012
RMSD(Angles) (°)	1.537
Ramachandran Statistics	
Favored (%)	98.5
Allowed (%)	1.5
Outliers (%)	0.00
PDB ID	6C5B

Figure S1. Multiple alignment of the amino acid sequence of LaPhzM with homologous sequences (A) and a comparison between LaPhzM from *Lysobacter antibioticus* OH13 and PhzM from *Pseudomonas aeruginosa* (B).

A



B

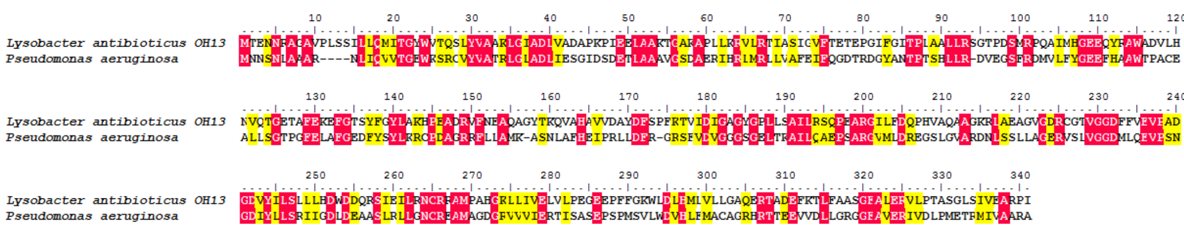


Figure S2. SDS-PAGE of LaPhzM produced in *E. coli*. The expected size of LaPhzM is 36.7 kDa.

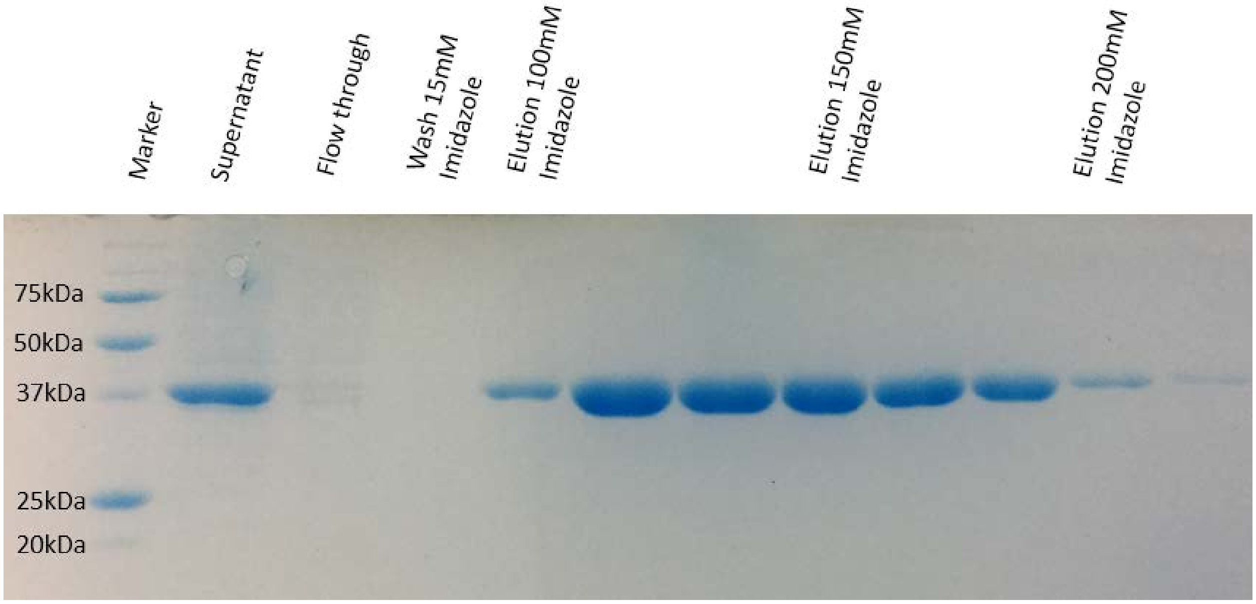


Figure S3. Enzyme kinetics of LaPhzM with 6-hydroxy-1-methoxyphenazine *N*5-oxide (**2**) as substrate to produce 1,6-dimethoxyphenazine *N*5-oxide (**4**). Lineweaver-Burk plot was used to determine K_M and V_{max}.

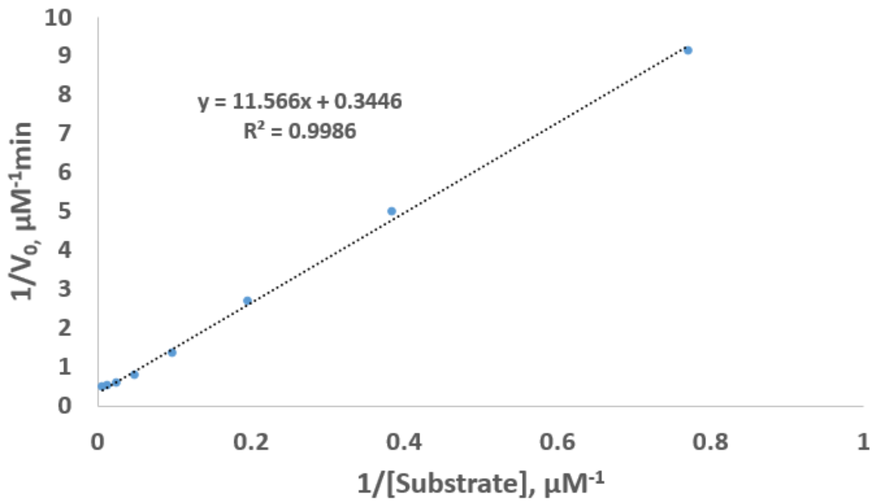
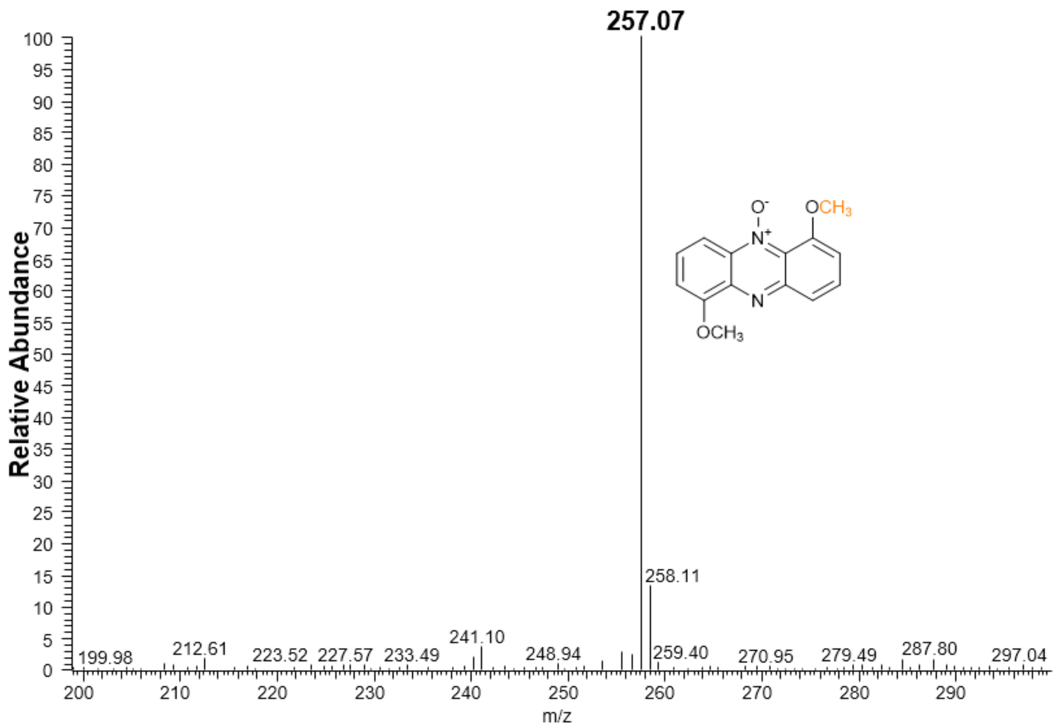
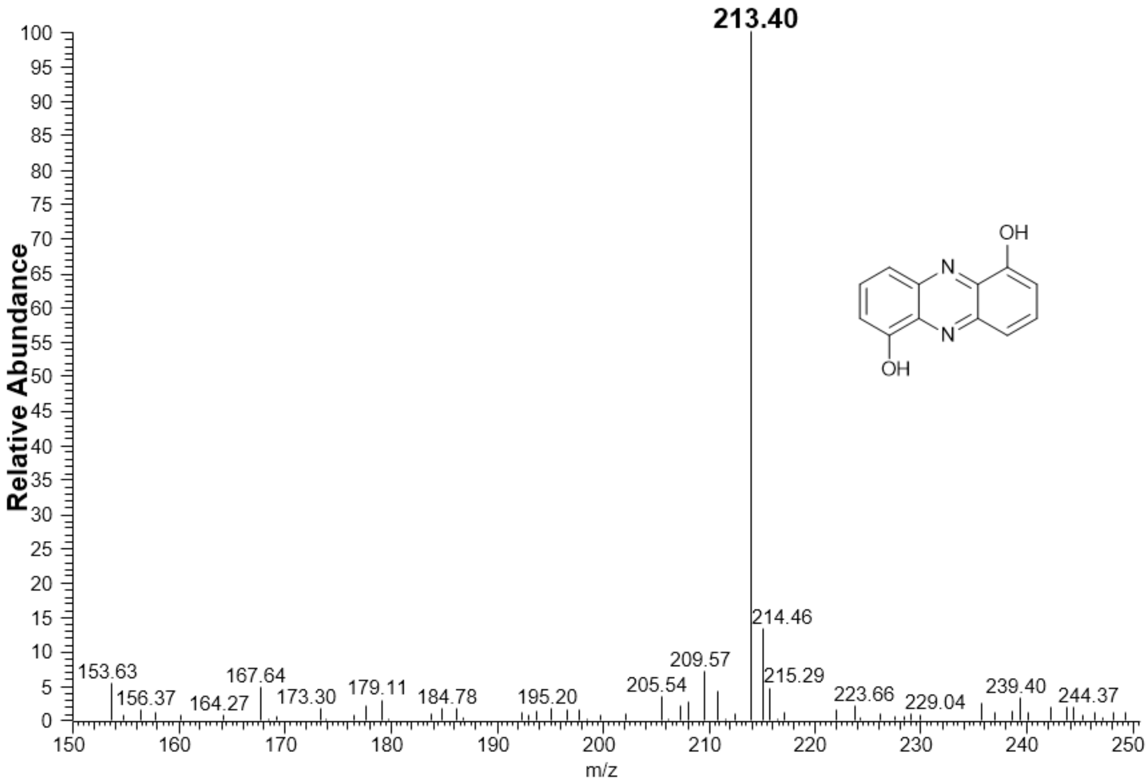


Figure S4. MS analysis of *in vitro* reaction products of LaPhzM. A. 1,6-dimethoxyphenazine *N*5-oxide (**4**); B. 1,6-dihydroxyphenazine (**7**); C. 1-hydroxy-6-methoxyphenazine (**6**); D. 1,6-dimethoxyphenazine (**5**); E. 1-hydroxyphenazine (**10**); F. 1-methoxyphenazine (**11**); G. pyocyanin; H. 1,6-dihydroxyphenazine *N*5-oxide (**8**); I. myxin (**1**); J. 1,6-dimethoxyphenazine *N*5,*N*10-dioxide (**9**).

A

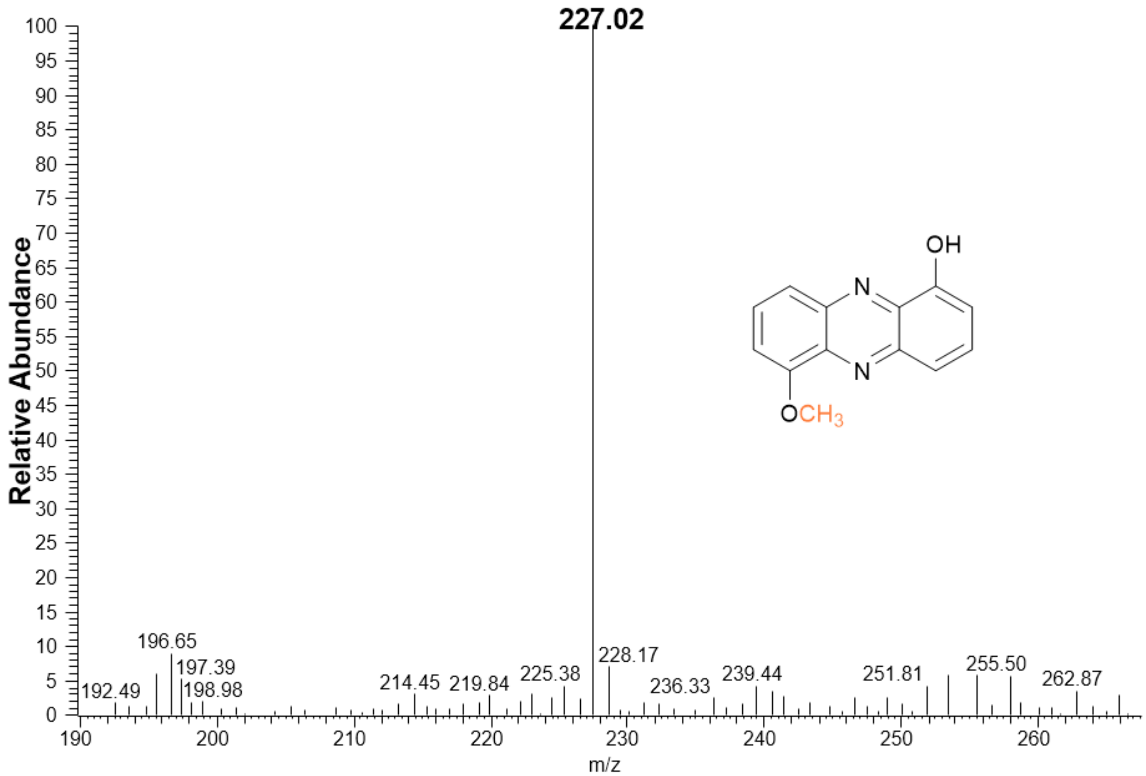


B

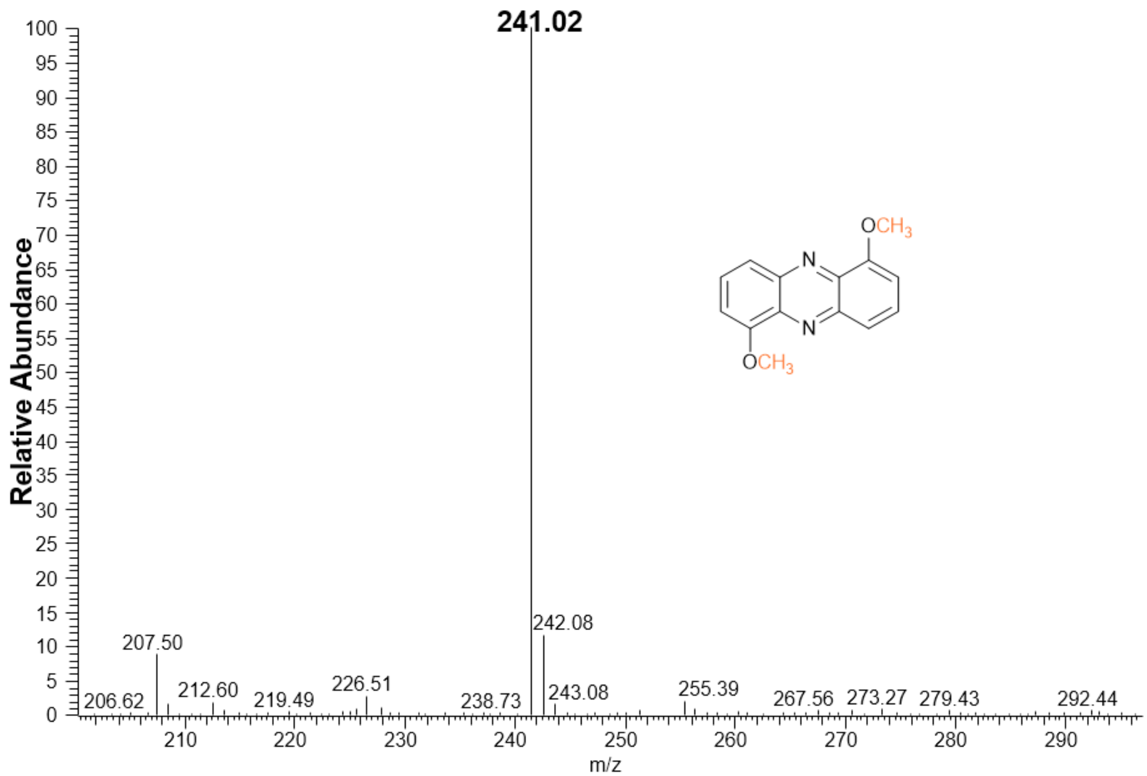


213.40
Relative Abundance
100
95
90
85
80
75
70
65
60
55
50
45
40
35
30
25
20
15
10
5
0
214.46
209.57
215.29
153.63
167.64
179.11
205.54
156.37
173.30
184.78
195.20
223.66
239.40
244.37
164.27
229.04
150
160
170
180
190
200
210
220
230
240
250
m/z
OH
N
N
OH

C

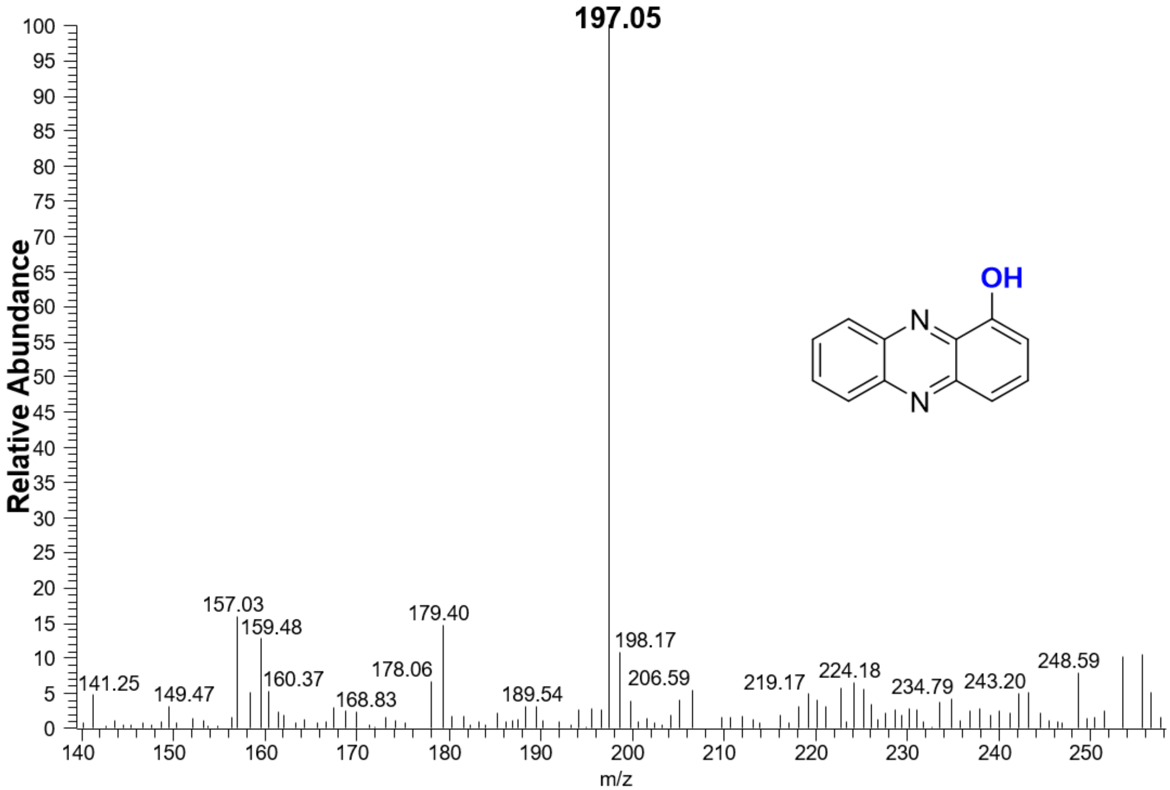

227.02
Relative Abundance
m/z
196.65
197.39
198.98
192.49
214.45
219.84
225.38
228.17
236.33
239.44
251.81
255.50
262.87
OH
N
N
OCH3

D



241.02
Relative Abundance
100
95
90
85
80
75
70
65
60
55
50
45
40
35
30
25
20
15
10
5
0
207.50
206.62
212.60
219.49
226.51
238.73
242.08
243.08
255.39
267.56
273.27
279.43
292.44
210
220
230
240
250
260
270
280
290
m/z
N
N
OCH3
OCH3

E



197.05
Relative Abundance
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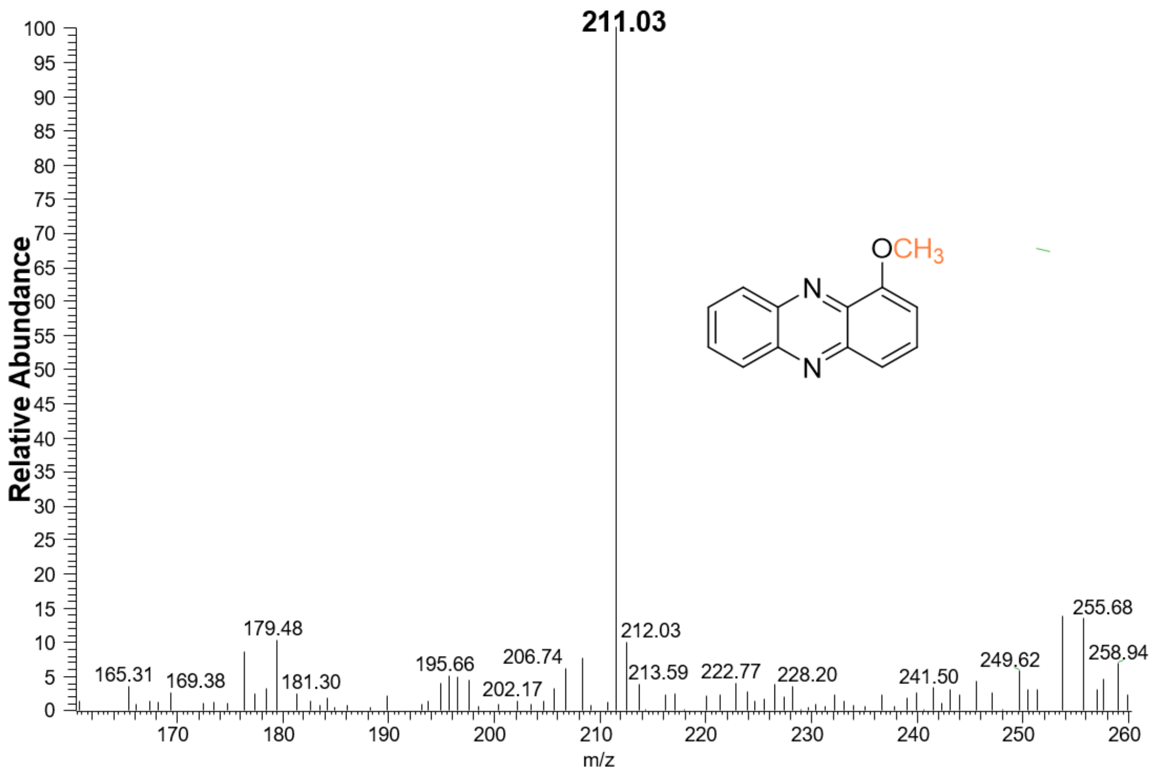
90
85
80
75
70
65
60
55
50
45
40
35
30
25
20
15
10
5
0
141.25
149.47
157.03
159.48
160.37
168.83
178.06
179.40
189.54
198.17
206.59
219.17
224.18
234.79
243.20
248.59
140
150
160
170
180
190
200
210
220
230
240
250
m/z
OH
N
N

F

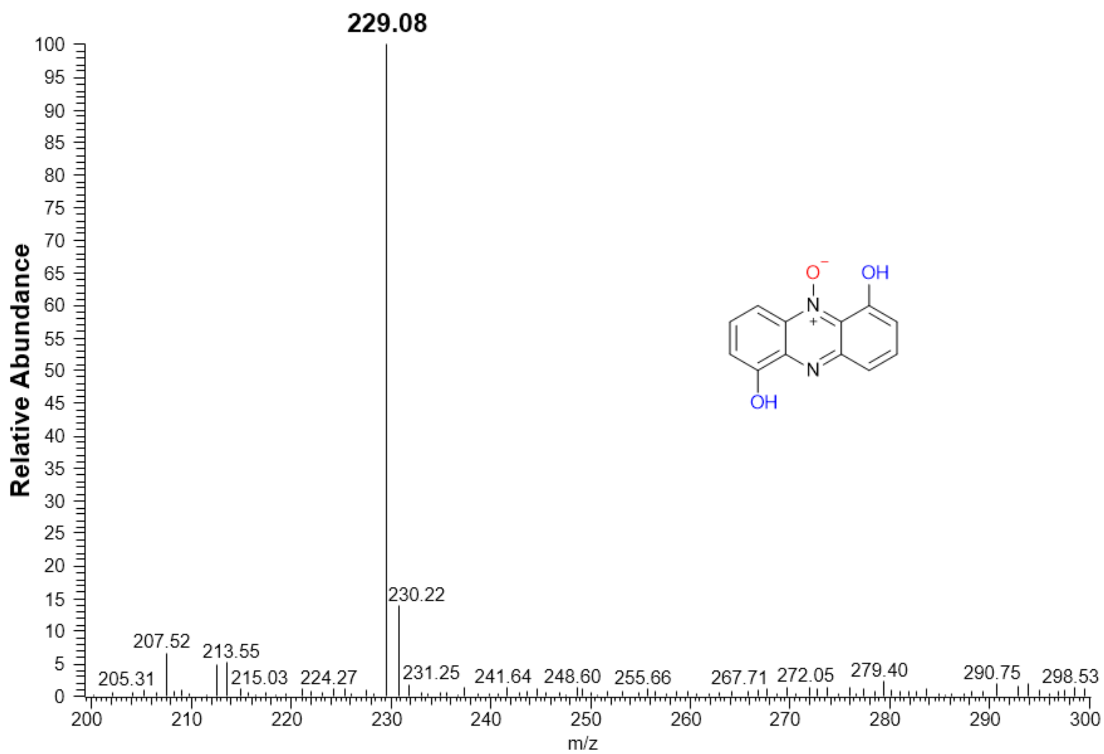


211.03
Relative Abundance
m/z
165.31
169.38
179.48
181.30
195.66
202.17
206.74
212.03
213.59
222.77
228.20
241.50
249.62
255.68
258.94
OCH3
N
N

G

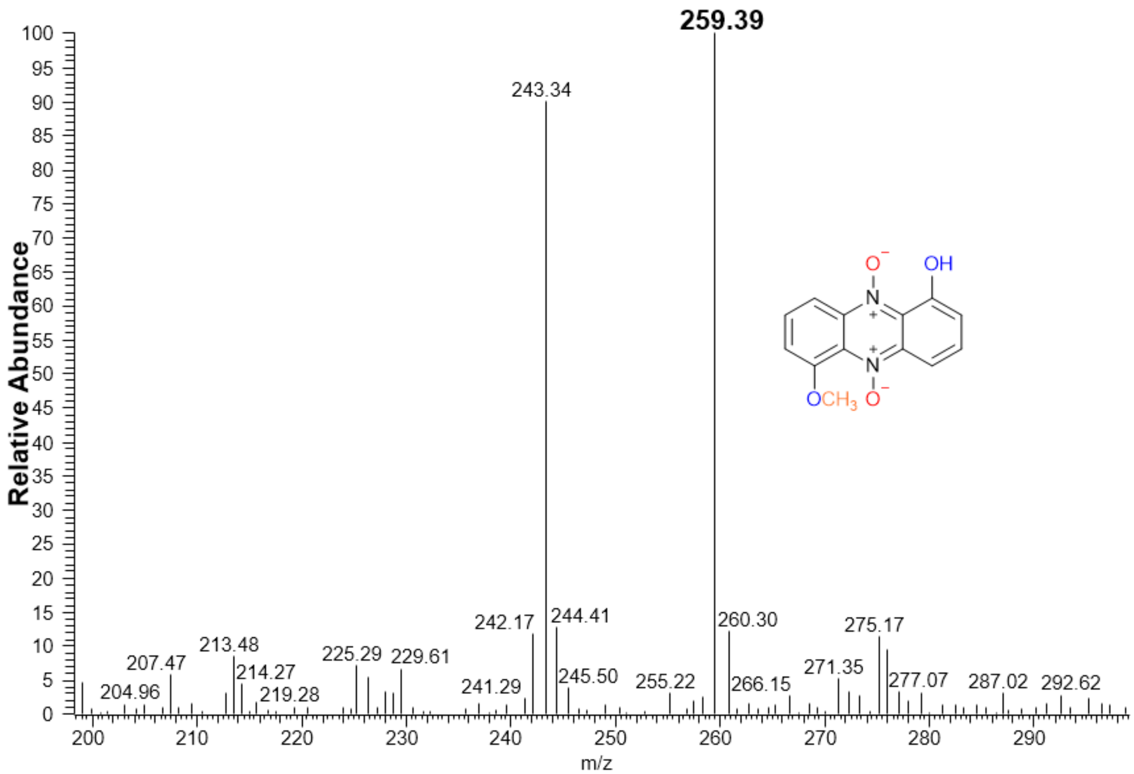

211.09
Relative Abundance
m/z
105.15
121.29
135.36
149.46
163.48
180.96
196.44
207.50
217.32
241.37
249.31
267.39
275.84
300.29
N
N
O

H



229.08
Relative Abundance
m/z
230.22
207.52
213.55
205.31
215.03
224.27
231.25
241.64
248.60
255.66
267.71
272.05
279.40
290.75
298.53
OH
OH
O
N
N

I



259.39
243.34
Relative Abundance
m/z
242.17
244.41
260.30
275.17
207.47
213.48
214.27
204.96
219.28
225.29
229.61
241.29
245.50
255.22
266.15
271.35
277.07
287.02
292.62
OH
OCH3

J

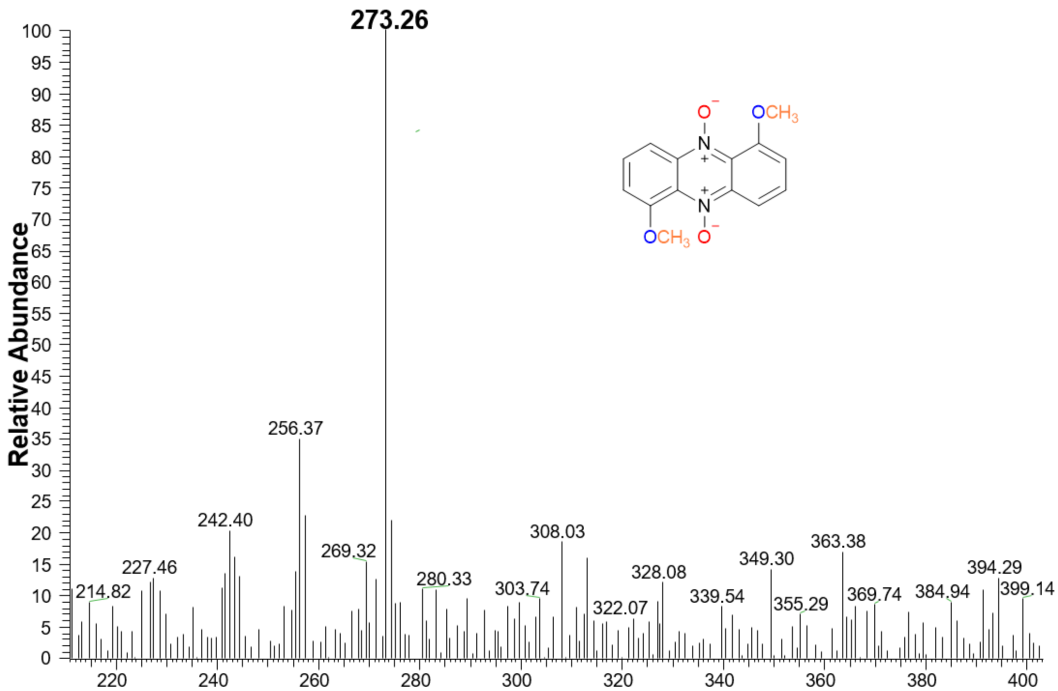
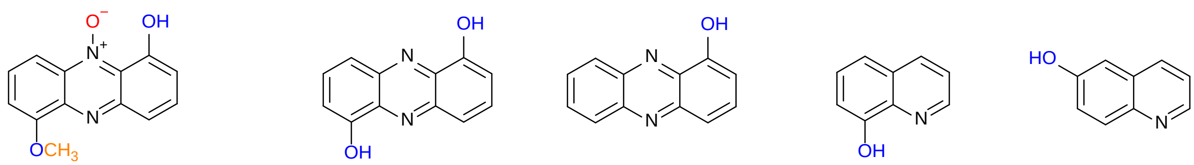

273.26
256.37
242.40
227.46
214.82
269.32
280.33
303.74
308.03
322.07
328.08
339.54
349.30
355.29
363.38
369.74
384.94
394.29
399.14
Relative Abundance
O^-
OCH_3
N^+
N^+
OCH_3
O^-

Figure S5. Relative reaction rate of LaPhzM toward different substrates. Note that when **7** was the substrate, the reaction condition was set to make **6** as the predominate product, with only a negligible amount of **5** produced.



6-Hydroxy-1-methoxy phenazine-*N*5-oxide (2) | **1,6-Dihydroxy phenazine (7)** | **1-Hydroxy phenazine (10)** | **8-Hydroxy quinoline** | **6-Hydroxy quinoline**

Substrate	Relative Rate
2	100 ± 2.8
7	264 ± 7.4
10	172 ± 3.5
8-hydroxyquinoline	9 ± 1.0
6-hydroxyquinoline	3 ± 0.6

Figure S6. Overview of the three-dimensional crystallographic structure of the LaPhzM-SAH complex. (A) The ribbon representation of the LaPhzM dimer, with the two protomers colored cyan and pale-cyan, respectively. The *S*-adenosyl-L-homocysteine (SAH) molecules are shown in sticks and colored by the atom type. (B) The secondary structure elements in a LaPhzM monomer with helices (α1-16) highlighted in cyan, strands (β1-9) in magenta and loops (not numbered) in salmon red.

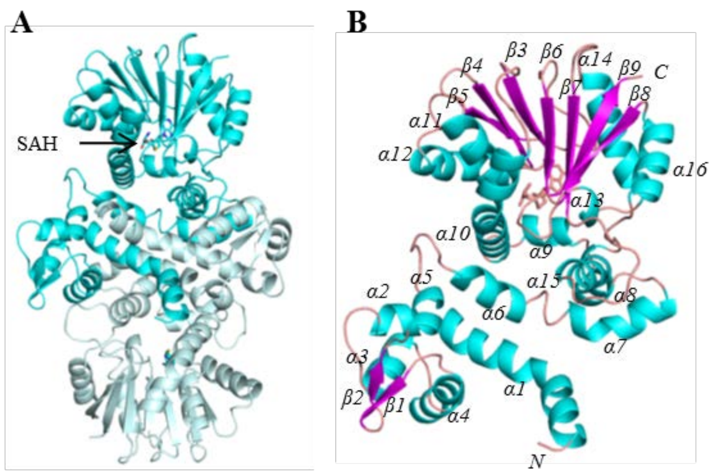


Figure S7. Comparison of the secondary structural elements between LaPhzM and (A) MmcR (PDB ID: 3GWZ, overall r.m.s.d. of Cα = 1.95 Å) and (B) PhzM (PDB ID: 2IP2, overall r.m.s.d. of Cα = 3.40 Å). LaPhzM is highlighted in cyan and MmcR/PhzM in magenta. The main differences between LaPhzM and MmcR lie in the loop region (residues 93 - 102) connecting the dimerization and catalytic domains. When the residues 10 – 93 and 94 – 341 of LaPhzM are overlaid with MmcR separately, it gives the core r.m.s.d of 0.98 Å and 1.42 Å, respectively.

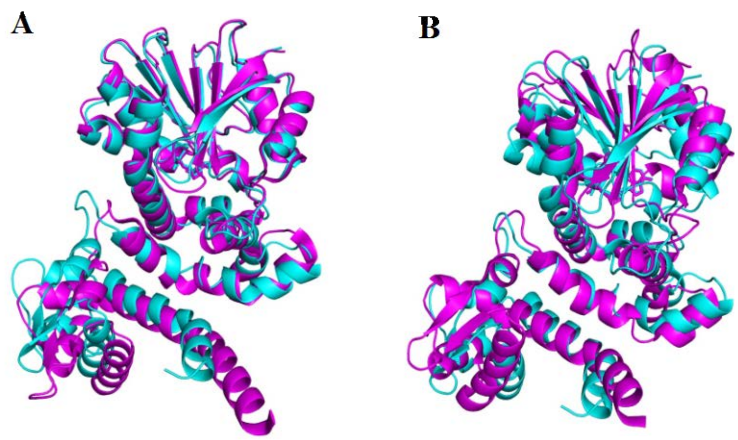


Figure S8. Stick representation of the SAH-binding environment in LaPhzM (carbon atoms in cyan) and MmcR (PDB ID: 3GXO, colored in gray). The residue labels are colored black for LaPhzM and gray for MmcR in the round bracket. For atoms in LaPhzM, the nitrogen atoms are highlighted in blue color, oxygen in red and sulfur in yellow.

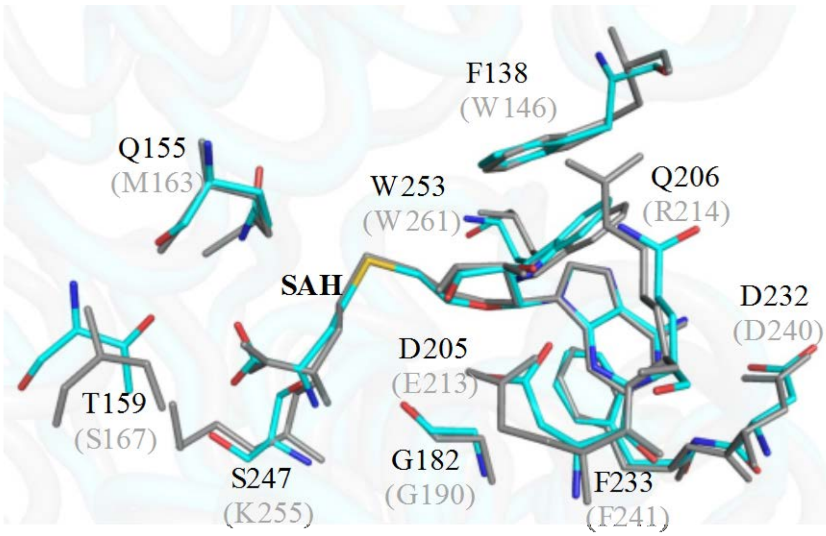


Figure S9. A model of LaPhzM in complex with 6-hydroxy-1-methoxyphenazine *N*5-oxide (**2**) at the catalytic site. **2** was fit into the LaPhzM structure by autodocking as described in the methods. The carbon atoms are highlighted in cyan, hydrogen in white, nitrogen in blue, oxygen in red and sulfur in yellow. The potential hydrogen bonds are highlighted by the dash lines.