Fig S.1 Sequence alignment between target protein TfdB-JLU and template protein 2-hydroxybiphenyl-3-monooxygenase. Sequence identity is 41.90%.
Fig S.2  A stereo view of the Cα backbone atoms of the homology model of TfdB (violet) superimposed with 5brt (cyan). Their root mean square deviation (RMSD) value was 1.85 Å. B The schematic diagram shows the detail of key residues: His47, Ile48, Trp222, Pro316, Phe424 of TfdB-JLU (magenta ball and stick) and His48, Ile49, Trp225, Pro320, Leu428 of 5brt (light cyan ball and stick). The values in parentheses stand for the RMSD value of key residues of TfdB-JLU versus matching residues of 5brt.
Fig S.3 FAD binding site of TfdB-JLU. A. Surface presentation of the FAD binding cavity. B. The extensive hydrogen bond interactions of FAD in the binding site. The hydrogen bonds are shown as green dashed lines.
Fig. S.4 The interaction of certain ligands with the active site of TfdB-JLU_WT (A, C, E)/ TfdB-JLU_P316Q (B, D, F). The ligands are 4-CP (in A, B), 2,5-DCP (in C, D) and 3,5-DCP (in E, F), respectively. The ligands are shown by magenta stick, FAD is shown by yellow line, and the residues in TfdB-JLU_WT/TfdB-JLU_P316Q are shown by gray line. The hydrogen bond interactions are shown by green dashed line, and the hydrophobic and halogen interactions are shown by pink dashed lines. The residues and FAD labeled have the hydrogen bond interaction with ligands.
Fig. S.5 The schematic diagram of the detail non-bond interaction formations of 2,4-DCP with the active site of TfdB-JLU_WT, TfdB-JLU-P316Q, TfdB-JLU-P316N and TfdB-JLU-P316I. The 2,4-DCP is shown by magenta stick, the 316 residue of TfdB-JLU_WT/TfdB-JLU-mutants is shown by yellow ball and stick, the residues of TfdB-JLU_WT/TfdB-JLU-mutants and FAD are shown by gray line. The hydrogen bond interactions are shown by green dashed line, and the hydrophobic and halogen interactions are shown by pink dashed lines.
Fig. S.6 Free energies of binding of CPs with TfdB-JLU_WT and TfdB-JLU_P316Q, and specific activities of TfdB-JLU_WT and TfdB-JLU_P316Q toward CPs.
**Supplementary Tables**

**Table S.1** Template search using SWISS-MODEL server.

| Template | Identity (%) | Coverage | Name | Ligands | Method | Oligo state |
|----------|--------------|----------|------|---------|--------|-------------|
| 4cy6     | 41.90        | 1-582    | 2-hydroxybiphenyl-3-monooxygenase | None | X-ray | homo-tetramer |
|          |              |          |      |         |        | 2.76Å       |
| 5btr     | 41.90        | 1-582    | 2-hydroxybiphenyl-3-monooxygenase | FAD, CH9 | X-ray | homo-tetramer |
|          |              |          |      |         |        | 2.30Å       |
| 3hg      | 37.28        | 1-582    | RdmE | FAD, VAK, SO4 | X-ray | monomer |
|          |              |          |      |         |        | 2.49Å       |
| 3ept     | 32.94        | 4-563    | RebC | FAD, NA | X-ray | monomer |
|          |              |          |      |         |        | 2.97Å       |
**Table S.2** Ramachandran Plot statistics for the target (TfdB-JLU) and template (5brt) models.

| Ramachandran Plot Statistics                          | TfdB-JLU | 5brt |
|--------------------------------------------------------|----------|------|
|                                                        | Nos.     | Nos. |
| Residues in the most favoured regions                  | 419      | 423  |
|                                                        | 85.0     | 89.6 |
| Residues in additional allowed regions                 | 72       | 46   |
|                                                        | 13.4     | 9.7  |
| Residues in generously allowed regions                 | 6        | 2    |
|                                                        | 1.2      | 0.4  |
| Residues in disallowed regions                         | 2        | 1    |
|                                                        | 0.4      | 0.2  |
| Number of non-glycine and non-proline residues         | 499      | 472  |
| Number of end residues (excl. Gly and Pro)             | 3        | 8    |
| Number of glycine residues (shown as triangles)        | 52       | 49   |
| Number of proline residues                             | 26       | 29   |
| Total number of residues                               | 580      | 558  |
|                                                        | 100      | 100  |
**Table S.3** Estimated free energy of binding of 2,4-DCP and TfdB-JLU_P316 and TfdB-JLU_W222 saturated mutants.

| Proteins       | Energy (kcal/mol) | Proteins       | Energy (kcal/mol) |
|----------------|-------------------|----------------|-------------------|
| TfdB-JLU       | -5.14             | TfdB-JLU       | -5.14             |
| TfdB-JLU_P316A | -4.80             | TfdB-JLU_W222A | -4.88             |
| TfdB-JLU_P316C | -5.00             | TfdB-JLU_W222C | -4.88             |
| TfdB-JLU_P316D | -5.13             | TfdB-JLU_W222D | -4.87             |
| TfdB-JLU_P316E | -5.04             | TfdB-JLU_W222E | -5.11             |
| TfdB-JLU_P316F | -4.64             | TfdB-JLU_W222F | -4.86             |
| TfdB-JLU_P316G | -4.77             | TfdB-JLU_W222G | -4.97             |
| TfdB-JLU_P316H | -4.63             | TfdB-JLU_W222H | -4.90             |
| TfdB-JLU_P316I | -4.51             | TfdB-JLU_W222I | -4.96             |
| TfdB-JLU_P316K | -4.66             | TfdB-JLU_W222K | -4.94             |
| TfdB-JLU_P316L | -4.64             | TfdB-JLU_W222L | -4.94             |
| TfdB-JLU_P316M | -4.73             | TfdB-JLU_W222M | -4.88             |
| TfdB-JLU_P316N | -5.13             | TfdB-JLU_W222N | -4.88             |
| TfdB-JLU_P316Q | -5.23             | TfdB-JLU_W222P | -4.88             |
| TfdB-JLU_P316R | -5.00             | TfdB-JLU_W222Q | -4.85             |
| TfdB-JLU_P316S | -4.67             | TfdB-JLU_W222R | -4.88             |
| TfdB-JLU_P316T | -4.57             | TfdB-JLU_W222S | -4.87             |
| TfdB-JLU_P316V | -4.72             | TfdB-JLU_W222T | -4.88             |
| TfdB-JLU_P316W | -4.81             | TfdB-JLU_W222V | -5.08             |
| TfdB-JLU_P316Y | -4.72             | TfdB-JLU_W222Y | -4.88             |
Table S.4 Halogen interaction parameters of 2,4-DCP with TfdB-JLU_P316Q.

| Types   | Form chemistry          | To chemistry   | Distances(Å)* |
|---------|-------------------------|----------------|---------------|
| Pi-Alkyl| TfdB_P316Q: Trp222      | 2,4-DCP: Cl12 | 4.95          |
| Pi-Alkyl| TfdB_P316Q: Phe424      | 2,4-DCP: Cl12 | 4.88          |

*The length of the hydrogen bonds.
Table S.5 Estimated free energy of binding of ligands and TfdB-JLU_WT/TfdB-JLU_P316Q.

| Ligands | With TfdB-JLU_WT Energy (kcal/mol) | With TfdB-JLU_P316Q Energy (kcal/mol) |
|---------|-----------------------------------|----------------------------------------|
| CP      | -3.99                             | -4.19                                  |
| 3-CP    | -4.55                             | -4.75                                  |
| 4-CP    | -4.49                             | -4.68                                  |
| 2,3-DCP | -4.92                             | -5.07                                  |
| 2,4-DCP | -5.14                             | -5.23                                  |
| 2,5-DCP | -5.10                             | -5.17                                  |
| 2,6-DCP | -4.94                             | -5.10                                  |
| 3,4-DCP | -4.92                             | -5.17                                  |
| 3,5-DCP | -5.19                             | -5.22                                  |
| 2,4,5-TCP | -5.25                         | -5.44                                  |
Table S.6 Hydrogen Bond parameters of ligands and TfdB-JLU_WT/TfdB-JLU_P316Q.

| Substrates | TfdB-JLU_WT | TfdB-JLU_P316Q |
|------------|-------------|----------------|
|            | Donors Atom | Receptor Atom  | Distances(Å)* | Donors Atom | Receptor Atom  | Distances(Å)* |
| 4-CP       | His47:HE2   | 4-CP: O        | 1.78          | Ser202: HG   | 4-CP: O        | 2.96          |
|            | 4-CP: H     | FAD: O4        | 2.10          | FAD: H5      | 4-CP: O        | 2.40          |
|            |             | 4-CP: H        |               |             | Arg45: O       | 1.98          |
| 2,5-DCP    | His47: HE2  | 2,5-DCP: O     | 1.84          | His47: HE2   | 2,5-DCP: O     | 1.74          |
|            | 2,5-DCP: H  | FAD: O4        | 2.50          | FAD: H3      | 2,5-DCP: O     | 2.19          |
|            |             | 2,5-DCP: H     |               |             | FAD: O4        | 2.89          |
| 3,5-DCP    | 3,5-DCP: H  | FAD: O4        | 2.12          | His47: HE2   | 3,5-DCP: O     | 1.98          |
|            |             |                 |               | FAD: H3      | 3,5-DCP: O     | 2.11          |
|            |             |                 |               | 3,5-DCP: H   | FAD: O4        | 2.90          |

*The length of the hydrogen bonds.
Table S.7  Hydropholic and halogen interaction parameters of 4-CP with TfdB-JLU_WT.

| Types   | Form chemistry          | To chemistry  | Distances(Å) |
|---------|-------------------------|---------------|--------------|
| Pi-Alkyl| TfdB-JLU_WT: Trp222     | 4-CP: Cl12    | 5.47         |
| Pi-Alkyl| TfdB-JLU_WT: Trp222     | 4-CP: Cl12    | 4.45         |
| Pi-Alkyl| TfdB-JLU_WT: Phe424     | 4-CP: Cl12    | 4.60         |
| Pi-Alkyl| 4-CP                    | TfdB-JLU_WT: Pro316 | 4.07       |
Table S.8 Hydrophobic and halogen interaction parameters of 4-CP with TfdB-JLU_P316Q.

| Types      | Form chemistry                  | To chemistry | Distances(Å) |
|------------|----------------------------------|--------------|--------------|
| Pi-Sigma   | TfdB-JLU_P316Q: Ile247:CG2       | 4-CP         | 3.90         |
| Pi-Sigma   | TfdB-JLU_P316Q: Leu249: CB       | 4-CP         | 3.70         |
| Alkyl      | 4-CP : Cl12                      | TfdB-JLU_P316Q: Val238 | 3.70         |
| Alkyl      | 4-CP: Cl12                       | TfdB-JLU_P316Q: Ile247 | 4.33         |
| Alkyl      | 4-CP: Cl12                       | TfdB-JLU_P316Q: Leu249 | 5.01         |
| Pi-Alkyl   | 4-CP                            | TfdB-JLU_P316Q: Val238 | 5.45         |
Table S.9 Hydrophobic and halogen interaction parameters of 2,5-DCP with TfdB-JLU_WT.

| Types   | Form chemistry | To chemistry      | Distances(Å) |
|---------|----------------|-------------------|--------------|
| Alkyl   | 2,5-DCP: Cl7   | TfdB-JLU_WT: Leu249 | 5.15         |
| Alkyl   | 2,5-DCP: Cl1   | TfdB-JLU_WT: Ile48  | 4.03         |
| Alkyl   | 2,5-DCP: Cl1   | TfdB-JLU_WT: Pro316 | 5.24         |
| Pi-Alkyl| TfdB-JLU_WT: His47 | 2,5-DCP: Cl1 | 3.93         |
| Pi-Alkyl| TfdB-JLU_WT: Trp222 | 2,5-DCP: Cl7 | 4.90         |
| Pi-Alkyl| 2,5-DCP        | TfdB-JLU_WT: Pro316 | 4.11         |
**Table S.10** Hydrophobic and halogen interaction parameters of 2,5-DCP with TfdB-JLU_P316Q.

| Types     | Form chemistry               | To chemistry  | Distances(Å) |
|-----------|-------------------------------|---------------|--------------|
| Pi-Sigma  | TfdB-JLU_P316Q: Gln316: CB    | 2,5-DCP       | 3.63         |
| Alkyl     | 2,5-DCP: Cl1                  | TfdB-JLU_P316Q: Ile48 | 3.96         |
| Pi-Alkyl  | TfdB-JLU_P316Q: His47         | 2,5-DCP: Cl1  | 3.94         |
| Pi-Alkyl  | TfdB-JLU_P316Q: Trp222        | 2,5-DCP: Cl7  | 5.18         |
| Pi-Alkyl  | TfdB-JLU_P316Q: Trp222        | 2,5-DCP: Cl7  | 4.47         |
| Pi-Alkyl  | TfdB-JLU_P316Q: FAD           | 2,5-DCP: Cl1  | 5.39         |
Table S.11 Hydrophobic and halogen interaction parameters of 3,5-DCP with TfdB-JLU_WT.

| Types    | Form chemistry                  | To chemistry   | Distances(Å) |
|----------|----------------------------------|----------------|--------------|
| Pi-Sigma | TfdB-JLU_WT: Pro316: CB          | 3,5-DCP        | 3.41         |
| Alkyl    | 3,5-DCP: Cl7                     | TfdB-JLU_WT: Ile48 | 4.12         |
| Pi-Alkyl | TfdB-JLU_WT: Trp96               | 3,5-DCP: Cl7   | 4.92         |
| Pi-Alkyl | TfdB-JLU_WT: Trp222              | 3,5-DCP: Cl13  | 4.69         |
| Pi-Alkyl | TfdB-JLU_WT: Phe424              | 3,5-DCP: Cl7   | 4.66         |
| Types  | Form chemistry       | To chemistry     | Distances(Å) |
|--------|----------------------|------------------|--------------|
| Alkyl  | 3,5-DCP: Cl7         | TfdB-JLU_P316Q:  | 5.28         |
|        |                      | Ile48            |              |
| Alkyl  | 3,5-DCP: Cl7         | TfdB-JLU_P316Q:  | 4.00         |
|        |                      | Leu220           |              |
| Pi-Alkyl| TfdB-JLU_P316Q: His47| 3,5-DCP: Cl7    | 5.05         |
| Pi-Alkyl| TfdB-JLU_P316Q: Trp96| 3,5-DCP: Cl7    | 4.99         |
| Pi-Alkyl| TfdB-JLU_P316Q: Trp96| 3,5-DCP: Cl7    | 5.13         |
| Pi-Alkyl| TfdB-JLU_P316Q: Trp222| 3,5-DCP: Cl13   | 4.91         |
| Pi-Alkyl| TfdB-JLU_P316Q: Trp222| 3,5-DCP: Cl13   | 4.17         |
| Pi-Alkyl| TfdB-JLU_P316Q: Phe424| 3,5-DCP: Cl7    | 4.26         |