4,5-dichloro-2-[(2-chlorophenyl)methyl]pyridazin-3-one

![Chemical structure](image)

**Category**

**Parameter**

**Description**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Additional names</th>
<th>4,5-dichloro-2-[(2-chlorophenyl)methyl]pyridazin-3-one</th>
</tr>
</thead>
</table>

**Citation**

Compounds targeting disulfide bond forming enzyme DsbB of Gram-negative bacteria

Cristina Landeta, Jessica L Blazyk, Feras Hatahet, Brian M Meehan, Markus Eser, Alissa Myrick, Ludmilla Bronstein, Shoko Minami, Holly Arnold, Na Ke, Eric J Rubin, Barbara C Furie, Bruce Furie, Jon Beckwith, Rachel Dutton & Dana Boyd

**Chemical descriptors**

SMILES: O=C1C(Cl)=C(Cl)C=NN1CC2=C(Cl)C=CC=C2

InChIKey: OCSOJUHXYHYEKJ-UHFFFAOYSA-N

**Chemical compound page**

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**Entries in chemical databases**

ST50189382 (PubChem)

**Availability**

Ambinter (France) Amb1343545

Enamine Ltd (Ukraine) Z19024333

**In vitro profiling**

<table>
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<tr>
<th>Target</th>
<th>DsbB enzyme</th>
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**Potency**

IC₅₀ of 18.85 nM against DsbB target in the *in vitro* assay; see Figure 2a.

**Selectivity**

Profiled against human PDI enzyme and found that up to 100 µM did not show significant inhibitory effect (Supplementary Figure 2b)

**Potential reactivities**

Not known

**SAR**

First, the presence of chlorine groups at positions 4 and 5 may be necessary for strong inhibitory activity. Second, the presence of a second aromatic ring at position 2, either phenyl or benzyl group increased the inhibitory activity, since the change from phenyl (compound 15) to methyl (compound 29) caused at least 100-fold decrease in activity. Third, the presence of benzyl (compound 12) caused a 5-fold increase of inhibitory activity compared to the phenyl substitution (compound 14), it may be possible that the carbon (CH₂) separating the two rings permits rotation of the o-chlorophenyl ring allowing the interaction with the protein. Fourth, the ortho substitution of a halogen group in the benzyl/phenyl ring improved inhibitory activity, while other small electronegative (such as nitro) or electron-donating (methyl) groups did not produce the same effect. Finally, substitutions of halogens at positions para or meta in the phenyl ring did not improve activity as the ortho substitution. These effects could be related to a rotation of the phenyl ring that influences interactions of the halogen with the protein. See Table 1.

**Mechanism of action**

Ion-trap mass spectrometry shows the appearance of an adduct of 253.6 Da of DsbB-DsbAC₃₃₃A dimer when treated with compound 12 (Supplementary Figure 4c). Since the theoretical Mw of compound 12 is 289.54 Da, the 35.9 Da mass loss is possibly representing a leaving chloride ion. This mass adduct was not observed when treating either DsbB (oxidized) or DsbAC₃₃₃A alone with the compound. The data indicated a covalent modification of Cys44 of DsbB by compound 12, which is occurring after the formation of the charge-transfer complex.
During ubiquinone reduction (Supplementary Figure 4d).

<table>
<thead>
<tr>
<th>Structure of target-probe complex</th>
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<tbody>
<tr>
<td>Additional comments</td>
<td></td>
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<tr>
<th>Cellular profiling</th>
<th>Validation of cellular target</th>
<th>Validation of cellular specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dose-dependently inhibited DsbA oxidation at 0.97 μM in <em>E. coli</em> cells as determined by <em>in vivo</em> chemical alkylation, see Figure 2b. DsbB re-oxidizes DsbA to start another catalytic cycle, thus the inhibition of DsbB causes the accumulation of DsbA in reduced state.</td>
<td>Profiled against <em>E. coli</em> expressing <em>Mycobacterium tuberculosis</em> VKOR enzyme and does not inhibit ≤ 100 μM in the β-galactosidase agar assay, see Figure 3. However when <em>E. coli</em> is expressing its DsbB enzyme the compound inhibits at 0.53 μM in the β-Galactosidase agar assay. In addition, the compound does not inhibit growth of <em>Mycobacterium smegmatis</em> when expressing VKOR enzyme at concentrations up to and including 100 μM, but it does at 400 nM when DsbB is expressed.</td>
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<td>Additional comments</td>
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| Additional comments              |                               |                                   |
