Supplementary Information

Potent DNA gyrase inhibitors bind asymmetrically to their target using symmetrical bifurcated halogen bonds

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Supplementary Table S1. X-Ray Data Collection and Refinement Statistics.

| Inhibitor | 4 |
|-----------|---|
| **Data collection** | |
| Beamline | I04 Diamond Light Source, UK |
| Wavelength (Å) | 0.9795 |
| Detector | Eiger2 X 16M |
| Resolution range (Å) | 81.09 – 2.30 (2.34 – 2.30) |
| Space Group | P6₁ |
| Cell parameters (Å) | a = b = 92.6, c = 405.5 |
| Total no. of measured intensities | 1751993 (70928) |
| Unique reflections | 86845 (4546) |
| Multiplicity | 20.2 (15.6) |
| Mean I/σ(I) | 11.7 (1.4) |
| Completeness (%) | 100.0 (99.5) |
| Rmerge a | 0.193 (2.017) |
| Rmerge b | 0.198 (2.085) |
| CC½ c | 0.998 (0.557) |
| Wilson B value (Å²) | 41.2 |
| **Refinement** | |
| Resolution range (Å) | 80.32 – 2.30 (2.36 – 2.30) |
| Reflections: working/free d | 82229/4484 |
| Rw/Rfree e | 0.179/0.217 (0.278/0.314) |
| Ramachandran plot: |
| favoured/allowed/disallowed (% | 97.1/2.8/0.1 |
| R.m.s. bond distance deviation (Å) | 0.010 |
| R.m.s. bond angle deviation (°) | 1.636 |
| No. protein residues | B chain:672; D chain:670 |
| No. DNA bases | E/F chains:8; G/H chains:12 |
| No. of water/ligands/ions | 343/6/5 |
| Mean B factors: protein/DNA/water/ligands/ions/overall (Å²) | 49/52/44/75/62/49 |
| RSCC score f for 4 | 0.90 |
| Accession code | 6Z1A |

Values in parentheses are for the outer resolution shell.

a Rmerge = \( \frac{\sum| I(hkl) - \langle I(hkl) \rangle |}{\sum| I(hkl) |} \).

b Rmerge = \( \frac{\sum| I(hkl) - \langle I(hkl) \rangle |}{\sum| I(hkl) |} \) × \( \frac{\sum| I(hkl) |}{\sum| I(hkl) |} \), where I(hkl) is the rth observation of reflection hkl, \langle I(hkl) \rangle is the weighted average intensity for all observations i of reflection hkl and N is the number of observations of reflection hkl.

c CC½ is the correlation coefficient between symmetry equivalent intensities from random halves of the dataset.

d The data set was split into “working” and “free” sets consisting of 95 and 5% of the data respectively. The free set was not used for refinement.

e The R-factors Rw and Rfree are calculated as follows: R = \( \frac{\sum| F_{obs} - F_{calc} |}{\sum| F_{obs} |} \), where Fobs and Fcalc are the observed and calculated structure factor amplitudes, respectively.

f From MolProbity.27

27 From the PDB validation server.
Molecular Docking
Supplementary Figure 1: Predicted binding modes of compounds 2-6. Molecular docking results representing compounds (sticks representation colour-coded by element) with differently substituted phenyl RHSs, within the S. aureus DNA gyrase NBTI-binding site (PDB ID: 2XCS)\(^1\), revealing key intermolecular interactions. a compound 2 (unsubstituted phenyl); b compound 3 (p-fluoro phenyl, fluorine atom in cyan); c compound 4 (p-chloro phenyl, chlorine atom in green); d compound 5 (p-bromo phenyl, bromine atom in red); e compound 6 (p-iodo phenyl, iodine atom in violet). Hydrogen bonds are represented as blue dots, while predicted X···O halogen bonding interactions as yellow dots. GyrA, GyrB and DNA molecules are represented as cartoons in blue, yellow, and red, respectively. The amino acid residues are represented as sticks colour-coded by element.

Supplementary Table 2. Distance and angle parameters from predicted binding modes (Fig. S1), indicating the ability for the halogen bonds formation between p-halogenated inhibitors 4-6 (Cl, Br, and I) and Ala68 backbone carbonyl oxygen from one GyrA subunit.

| Compound | distance (Å) X···O | Θ\(_1\) angle (°) C-X···O | Θ\(_2\) angle (°) X···O=C |
|----------|-------------------|--------------------------|--------------------------|
| 4        | 2.79              | 162.9                    | 123.8                    |
| 5        | 2.96              | 169.2                    | 129.1                    |
| 6        | 2.90              | 157.5                    | 128.4                    |

Molecular dynamics simulations

All-atom molecular dynamics simulations on apo (ligand-free) and ligated DNA gyrase complexes (PDB ID: 2XCS)\(^1\) with the previously docked NBTI binding poses containing unsubstituted phenyl (2), i.e., p-halogenated phenyl RHSs (3-6) were conducted in duration of 500 ns. The stability, dynamics profile, and compactness of the simulated systems in first instance was assessed by inspecting the resulting MD trajectories in terms of analysing the plotted root-mean-square deviations (RMSD) and radius of gyration (Rg) for each system separately (Supplementary Fig. 2).

Taking into account the complexity of the studied systems as well as to obtain a clearer insight into their overall stability, RMSD plots over entire simulation time were calculated for each entity separately (e.g., backbone protein, DNA, and NBTI ligands; Supplementary Fig. 2). As depicted in Supplementary Fig. 2a, one can perceive well-equilibrated DNA gyrase systems (apo and ligated forms) as demonstrated by their calculated backbone protein RMSD values with no significant deviations (~2.01-2.78 Å). The apo form itself is typified by a good overall stability (~2.27-2.47 Å) that increases by complexation with our studied NBTI ligands, which is also evident by stabilization of the DNA (Supplementary Fig. 2b) in ligated NBTI complexes (~1.1-1.74 Å; intercalation of NBTIs
methoxy-naphthiridine LHS between central DNA base pairs) relative to the apo form (a significant fluctuation of ~2.00-3.24 Å). Among studied NBTIs (Supplementary Fig. 2c), p-bromophenyl derivative (5) can be assigned as the most stable one (~1.3-1.5 Å) followed by a similar stability pattern (~0.5-1.25 Å) for p-fluorophenyl (3) and p-chlorophenyl (4) derivatives (Supplementary Figs. 2a-b), while highest RMSD values (~0.36-1.50 Å) are noticeable for p-iodophenyl derivative (6), however with no dramatic overall fluctuations of around 1.0 Å on average.

These findings are congruent with the compactness assessments for apo and NBTIs ligated gyrase systems as depicted by the Rg plots (Supplementary Fig. 2d); although, one can observe similar Rg deviations of ~0.5-0.6 Å between all investigated systems, the system containing NBTI ligand with p-bromophenyl RHS (5) is the most compact one (~38.08-37.68 Å) followed by the complexes containing NBTIs with p-iodophenyl (6) and p-chlorophenyl (4) RHS (~38.23-37.73 Å and ~38.31-37.75 Å), while highest Rg fluctuations (~38.33-37.73 Å) could be attributed for the complex comprising an NBTI with p-fluorophenyl RHS (3) relative to the apo form (~38.1-37.56 Å). Such behaviour was expected to a certain extent, particularly in the case of (3), since despite the fluorine’s high electronegativity as well as lack of polarizability, it is not involved in halogen bonding, and thus forms significantly weaker interactions compared to chloride, bromide, and iodine containing compounds, as previously hypothesized (Supplementary Fig. 1 and Supplementary Table 2).
Supplementary Figure 2: Root-mean-square deviation (RMSD [Å]) and Radius of gyration (Rg [Å]) plots of 500 ns MD-simulated S. aureus apo (ligand-free) and DNA gyrase complexes (PDB ID: 2XCS) with the compounds (2-6). a backbone DNA gyrase protein RMSD; b DNA RMSD; c compounds 2-6 RMSD; d Rg plots. RMSD and Rg curves are coloured as follows: apo form (inset: light-blue), (2)-DNA gyrase complex (inset: gray), (3)-DNA gyrase complex (inset: yellow), (4)-DNA
 Supplementary Table 3. Average interatomic X···O distances [Å] and $\Theta_1$ [C-X···O], i.e., $\Theta_2$ [X···O=C] angles reflecting the halogen bonding propensities of compounds 3-6 to the backbone carbonyl oxygen of Ala68 from both GyrA subunits (PDB ID: 2XCS)\(^1\) calculated from their resulting MD trajectories.

| Comp. | $d_1$ [Å] | $d_2$ [Å] | $\Theta_1$ [C-X···O] | $\Theta_1$ [C-X···O]'' | $\Theta_2$ [X···O=C] | $\Theta_2$ [X···O=C]'' |
|-------|-----------|-----------|----------------------|---------------------|---------------------|---------------------|
| 3     | 3.45      | 3.82      | 147.20°              | 127.39°             | 125.80°             | 110.98°             |
| 4     | 3.69      | 3.74      | 132.31°              | 150.24°             | 117.33°             | 118.25°             |
| 5     | 3.99      | 3.39      | 150.48°              | 123.82°             | 118.27°             |                     |
| 6     | 3.75      | 3.47      | 140.24°              | 131.09°             | 123.10°             | 121.09°             |
Supplementary Figure 3: X···O distances [Å] and $\Theta_1$ [C-X···O], i.e., $\Theta_2$ [X···O=C] angles distribution plots accounting for the halogen bonding propensity between $p$-substituted halogens of investigated NBTTs (3-6) and the backbone carbonyl oxygen of GyrA Ala68 residues (PDB ID: 2XCS)$^1$ calculated over the entire MD simulations time of 500 ns. a compound 3; b compound 4; c compound 5; d compound 6. Ala68’ and Ala68” X···O distances are represented as green and olive bars, Ala68’ and Ala68” $\Theta_1$ [C-X···O] angles as light-blue and violet bars, while Ala68’ and Ala68” $\Theta_2$ [X···O=C] angles as dark-blue and red bars, respectively. The gray shaded areas represent the allowed halogen bonding interatomic distances (F···O < 2.99 Å, Cl···O < 3.27 Å, Br···O < 3.37 Å, I···O < 3.50 Å) and angles (140° $\leq \Theta_1$ [C-X···O] $\leq$ 180° and 90° $\leq \Theta_2$ [X···O=C] $\sim$ 120°)$^4,5$. 
Supplementary Figure 4: Representation of TOPRIM, WHD and Tower domains. The TOPRIM domain of one GyrB subunit is depicted in yellow and the WHD and tower domains of one GyrA subunit are depicted in blue and green, respectively. Other subunits and second GyrA and GyrB subunits are depicted in grey.1

Supplementary Figure 5: Crystallographic evidence for the occupancies of the two metal sites. Detail of the catalytic sites in the A-configuration, a and B-configuration, b (PDB ID: 6Z1A). Key residues in the vicinity of the active site are shown with white carbons, with the exception of the mutated catalytic residue, Phe1123, where the side-chain is shown with yellow carbons and thinner bonds (in the foreground relative to the rest of the figure). For the DNA fragment upstream of the break, the carbons are in green (in the background) and for the downstream fragment, the carbons are pale brown (in the foreground). Also shown in blue mesh is the final 2mF_{obs}-DF_{calc} electron density for the residues shown (with the exception of that for Phe1123; at 2.3 Å resolution and contoured at 1.1 σ), and the locations of the metal ions (grey spheres) are highlighted by peaks in an anomalous difference Fourier map shown in magenta mesh (contoured at 4.0 σ). The latter shows evidence for...
partial occupancy of the A-site by metal in the B-configuration, although this was modelled as a fully occupied water molecule.

Supplementary Figure 6: Crystallographic evidence supporting the placement, orientation and conformation of compound 4 in the complex with DNA gyrase and DNA (PDB ID: 6Z1A). a Omit mFobs-DFcalc difference electron density for the bound ligand calculated at 2.3-Å resolution and contoured at 1.6 σ (magenta mesh) and at 6.0 σ (cyan semi-transparent surface). Note that the latter highlights the confidence in the placement of the chlorine atom. The three orientations are chosen to emphasize the how the density associated with each ring is flattened, which aids in the assignment of their orientations. b The refined ligand has a overall B-factor that is somewhat elevated relative to the surrounding protein, but is comparable to that of the DNA. A breakdown shows there is a noticeably higher value for the aminopiperidine linker, which is in line with the lack of interactions with protein or DNA for this region of the ligand. For clarity, the ligand is shown in isolation (left) and in the context of the complex (right). In both panels, atoms are coloured according to their B-factors by interpolation between the colours shown in the key. So, for example an atom with a B-factor of 55 Å² would have a colour intermediate between green and yellow.
**Supplementary Figure 7: Predicted binding modes of compound 9.** Molecular docking results representing compound 9 (p-dimethylamino phenyl; sticks representation colour-coded by element) within the *S. aureus* DNA gyrase NBTI-binding site of our crystal structure (PDB ID: 6Z1A), revealing key intermolecular interactions. Hydrogen bonds are represented as blue dots, whereby, each methyl group forms pseudohydrogen bond with Ala68. GyrA, GyrB, and DNA are represented as cartoons in blue, yellow, and red, respectively. The amino acid residues are represented as sticks colour-coded by element.

**Supplementary Table 4. logP and logD of compounds 2-9 calculated with MarvinSketch 20.17.**

| Cmpd | logD | logP |
|------|------|------|
| 2    | 1.17 | 3.33 |
| 3    | 1.47 | 3.48 |
| 4    | 1.89 | 3.94 |
| 5    | 2.02 | 4.10 |
| 6    | 2.18 | 4.26 |
| 7    | 0.44 | 2.57 |
| 8    | 0.19 | 2.18 |
| 9    | 0.18 | 3.44 |
Supplementary Figure 8 A truncated system representing the complex between compound 4 and gyrase used for QM calculations of the interaction energies. Compound 4 (taken from PDB ID: 6Z1A) was truncated solely on the RHS part (chlorophenyl moiety; sticks representation colour-coded by element) that establishes direct, bifurcated halogen-bonding interactions (yellow dots) with the backbone carbonyl oxygens of Ala68 residues (sticks representation colour-coded by element) of each GyrA subunit (truncated and patched with methyl groups).

Supplementary Table 5. Interaction energies between the compound 4 (chlorophenyl RHS), 2 (unsubstituted phenyl RHS) and Ala68 residues obtained by QM calculations. The interaction energies, i.e., dispersion components on HF level were calculated by $\Delta E = E(\text{lig.}_B - \text{AlaBD complex}) - E(\text{lig.}_D) - E(\text{AlaBD})$.

| Comp. | $\Delta E_{\text{int}[\text{Ala68B}]}$ (kcal mol$^{-1}$) | $\Delta E_{\text{int}[\text{Ala68D}]}$ (kcal mol$^{-1}$) | $\Delta E_{\text{int}[\text{Ala68BD}]}$ (kcal mol$^{-1}$) | $\Delta E_{\text{disp}_\text{HF}[\text{Ala68BD}]}$ (kcal mol$^{-1}$) |
|-------|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|
| 4     | -2.00                                         | -2.02                                         | -4.23                                         | 1.38                                          |
| 2     | -1.59                                         | -1.60                                         | -3.18                                         | -0.58                                         |
Synthesis

Supplementary Figure 9: Synthesis of final compounds 2-9. Reagents and conditions: a) 1M HCl in CH₃COOH, 30 min, RT; b) RCHO, MeOH, CH₃COOH, 2 h, then NaCNBH₃, 0 °C, 10 min, then RT.

1-(2-(6-methoxy-1,5-naphthyridin-4-yl)ethyl)piperidin-4-amine (10)

Tert-butyl 1-(2-(6-methoxy-1,5-naphthyridin-4-yl)ethyl)piperidin-4-ylcarbamate (0.63 g, 1.63 mmol, 1 equiv) was dissolved in 12.5 ml of 1 M HCl in acetic acid and stirred for 30 min at room temperature. Solvent was evaporated, the residue dissolved in 30 mL of saturated NaHCO₃(aq) and pH adjusted to 12 with 1 M NaOH. Water layer was washed with DCM (3 × 30 mL), combined organic layers dried over Na₂SO₄ and concentrated in vacuum to afford compound 10 as dark-brown viscous liquid (0.33 g, 71%).

TLC (DCM:MeOH 4:1 + 1% Et₃N v/v): Rf = 0.10; ¹H NMR (400 MHz, 295 K, CDCl₃): δ 1.41–1.51 (m, 2H), 1.88 (d, J = 11.9 Hz, 2H), 2.20 (t, J = 11.0 Hz, 2H), 2.66–2.78 (m, 1H), 2.73–2.84 (m, 2H), 3.05 (d, J = 11.5 Hz, 2H), 3.30–3.46 (m, 2H), 4.08 (s, 3H), 7.11 (d, J = 9.0 Hz, 1H), 7.41 (d, J = 4.5 Hz, 1H), 8.19 (d, J = 9.0 Hz, 1H), 8.66 (d, J = 4.5 Hz, 1H) ppm; ¹³C NMR (100 MHz, 295 K, CDCl₃): δ 28.46, 35.83, 52.37, 53.72, 58.37, 77.23, 116.31, 124.24, 140.33, 140.97, 141.48, 146.64, 147.70, 161.43 ppm; IR (ATR): 3271, 2941, 2811, 1614, 1590, 1503, 1489, 1438, 1398, 1334, 1125, 1079, 1018, 932, 853, 807, 751, 713, 615, 584, 536 cm⁻¹; HRMS (m/z): [M + H]⁺ calcd. for C₁₆H₂₂N₄O₁₄ 287.1872; found 287.1875. *signal is overlapping with residual solvent peak.

General procedure for reductive amination: To a solution of amine 10 (1 equiv) in dry methanol the appropriate aldehyde (1.05-1.3 equiv) was added, together with catalytic amount of acetic acid. The reaction mixture was stirred for 2 h at room temperature under inert atmosphere. Next, the reaction mixture was cooled to 0 °C and NaCNBH₃ (3-5 equiv) dissolved in small amount of dry methanol was added drop-wise. After stirring overnight at room temperature under inert atmosphere, the solvent was evaporated in vacuum and the residue diluted in ethyl acetate. Organic phase was washed with saturated Na₂CO₃ three times, dried over Na₂SO₄, filtered and the solvent evaporated under reduced pressure. The residue was purified by flash column chromatography to afford appropriate substituted product.

N-benzyl-1-(2-(6-methoxy-1,5-naphthyridin-4-yl)ethyl)piperidin-4-amine (2)

According to the general procedure for reductive amination, compound 10 (0.2 g, 0.698 mmol, 1 equiv) was used together with benzaldehyde (0.092 mL, 0.908 mmol, 1.3 equiv). The residue was
purified by flash column chromatography using CHCl₃/MeOH (9:1) to afford compound 2 (0.064 g, 24%) as yellow solid.

mp: 47.7–50.1 °C; TLC (DCM:MeOH 4:1 v/v and CHCl₃:MeOH 9:1 v/v): Rₜ = 0.28 and 0.14;¹H NMR (400 MHz, 295 K, CDCl₃): δ 1.48–1.58 (m, 2H), 1.98–2.01 (m, 2H), 2.19–2.25 (m, 2H), 2.57–2.62 (m, 1H), 2.80–2.84 (m, 2H), 3.07–3.10 (m, 2H), 3.39–3.43 (m, 2H), 3.86 (s, 2H), 4.10 (s, 3H), 7.13 (d, J = 8.8 Hz, 1H), 7.28–7.30 (m, 1H)*, 7.36 (d, J = 4.4 Hz, 4H), 7.43 (d, J = 4.4 Hz, 1H), 8.21 (d, J = 9.2 Hz, 1H), 8.68 (d, J = 4.4 Hz, 1H) ppm;¹³C (100 MHz, 295 K, CDCl₃): δ 28.47, 32.80, 50.82, 52.35, 53.72, 54.09, 58.46, 77.24*, 116.27, 124.24, 126.90, 128.06, 128.44, 140.34, 140.75, 140.99, 141.49, 146.75, 147.71, 161.40 ppm; IR (ATR): 2942, 2817, 1611, 1590, 1489, 1452, 1397, 1361, 1334, 1301, 1256, 1183, 1107, 1075, 1018, 987, 849, 808, 747, 697, 649, 614, 537 cm⁻¹; HRMS (m/z) [M + H]+ calcd. for C₂₉H₂₈N₃O, 377.2336; found 377.2334; HPLC purity: 91.9%.

*signal is overlapping with residual solvent peak.

**N**-(4-fluorobenzyl)-1-(2-(6-methoxy-1,5-naphthyridin-4-yl)ethyl)piperidin-4-amine (3)

According to the general procedure for reductive amination, compound 10 (0.233 g, 0.814 mmol, 1 equiv) was used together with 4-fluorobenzaldehyde (0.09 mL, 0.853 mmol, 1.05 equiv). The residue was purified by flash column chromatography using CHCl₃/MeOH (9:1 + 1% Et₃N) to afford compound 3 (0.233 g, 69%) as light brown solid.

mp: 55–60 °C; TLC (DCM:MeOH 4:1 + 1 % Et₃N v/v): Rₜ = 0.2;¹H NMR (400 MHz, 295 K, CDCl₃): δ 1.46–1.55 (m, 2H)*, 1.97 (d, J = 11.6 Hz, 2H), 2.20 (t, J = 10.9 Hz, 2H), 2.52–2.61 (m, 1H), 2.77–2.85 (m, 2H), 3.07 (d, J = 11.8 Hz, 2H), 3.36–3.44 (m, 2H), 3.82 (s, 2H), 4.10 (s, 3H), 7.01–7.06 (m, 2H), 7.13 (d, J = 9.0 Hz, 1H), 7.30–7.35 (m, 3H), 7.43 (d, J = 4.5 Hz, 1H), 8.21 (d, J = 9.0 Hz, 1H), 8.68 (d, J = 4.5 Hz, 1H) ppm;¹³C NMR (100 MHz, 295 K, CDCl₃): δ 28.43, 32.71, 50.07, 52.32, 53.71, 58.41, 77.24*, 115.20 (d, J = 21.2 Hz), 116.29, 124.25, 129.54 (d, J = 8.0 Hz), 136.41, 140.35, 140.97, 141.50, 146.67, 147.71, 161.42, 161.88 (d, J = 244.6 Hz) ppm; IR (ATR): 3035, 2935, 2811, 1611, 1592, 1505, 1489, 1468, 1441, 1398, 1369, 1334, 1299, 1263, 1215, 1106, 1077, 1017, 932, 850, 812, 790, 751, 712, 696, 647, 607, 584, 564 cm⁻¹; HRMS (m/z) [M + H]+ calcd. for C₂₉H₂₇FN₃O, 395.2242; found 395.2238; HPLC purity: 94.9%.

*signal is overlapping with residual solvent peak.

**N**-(4-chlorobenzyl)-1-(2-(6-methoxy-1,5-naphthyridin-4-yl)ethyl)piperidin-4-amine (4)

According to the general procedure for reductive amination, compound 10 (0.2 g, 0.698 mmol, 1 equiv) was used together with 4-chlorobenzaldehyde (0.128 g, 0.908 mmol, 1.3 equiv). The residue was purified by flash column chromatography using CHCl₃/MeOH (9:1) to afford compound 4 (0.220 g, 77%) as orange-brown solid.

mp: 67.6–68.4 °C; TLC (DCM:MeOH 4:1 and CHCl₃:MeOH 4:1): Rₜ = 0.44 and 0.32;¹H NMR (400 MHz, 295 K, CDCl₃): δ 1.43–1.53 (m, 2H), 1.93–1.96 (m, 2H), 2.15–2.20 (m, 2H), 2.50–2.55 (m, 1H), 2.77–2.81 (m, 2H), 3.03–3.06 (m, 2H), 3.36–3.40 (m, 2H), 3.80 (s, 2H), 4.07 (s, 3H), 7.11 (d, J = 8.8 Hz, 1H), 7.26–7.31 (m, 4H)*, 7.41 (d, J = 4.4 Hz, 1H), 8.18 (d, J = 8.8 Hz, 1H), 8.65 (d, J = 4.4 Hz, 1H) ppm;¹³C (100 MHz, 295 K, CDCl₃): δ 28.44, 32.69, 50.06, 52.29, 53.71, 54.04, 58.40, 77.25*, 116.30, 124.74, 128.53, 129.39, 132.56, 139.19, 140.32, 140.96, 141.47, 146.65, 147.69, 161.42 ppm;
IR (ATR): 3223, 2945, 2827, 1490, 1262, 1092, 857, 810, 757, 641, 603 cm⁻¹; HRMS (m/z) [M + H]⁺ calcd. for C₂₃H₂₈ON₄Cl, 411.1946; found 411.1930; HPLC purity: 96.69%.

*signal is overlapping with residual solvent peak.

N-(4-bromobenzyl)-1-(2-(6-methoxy-1,5-naphthyridin-4-yl)ethyl)piperidin-4-amine (5)

According to the general procedure for reductive amination, compound 10 (0.2 g, 0.698 mmol, 1 equiv) was used together with 4-bromobenzaldehyde (0.168 g, 0.908 mmol, 1.3 equiv). The residue was purified by flash column chromatography using CHCl₃/MeOH (9:1) to afford compound 5 (0.293 g, 92%) as yellow-orange solid.

mp = 74.7–75.8 °C; TLC (DCM:MeOH 4:1 and CHCl₃:MeOH 9:1) R₅ = 0.55 and 0.29; ¹H NMR (400 MHz, 295 K, CDCl₃): δ 1.43–1.52 (m, 2H), 1.93–1.96 (m, 2H), 1.95–2.20 (m, 2H), 2.50–2.55 (m, 1H), 2.77–2.81 (m, 2H), 3.03–3.06 (m, 2H), 3.36–3.40 (m, 2H), 3.79 (s, 2H), 4.07 (s, 3H), 7.11 (d, J = 8.8 Hz, 1H), 7.22 (d, J = 6.32, 2H), 7.40–7.45 (m, 3H), 8.18 (d, J = 8.8 Hz, 1H), 8.65 (d, J = 4.4 Hz, 1H) ppm; ¹³C (100 MHz, 295 K, CDCl₃): δ 28.44, 32.69, 50.10, 52.29, 53.71, 54.03, 58.40, 77.25*, 116.30, 120.63, 124.74, 129.77, 131.48, 139.72, 140.32, 140.97, 141.46, 146.66, 147.68, 161.42 ppm; IR (ATR): 3224, 2942, 2826, 1589, 1489, 1402, 1335, 1263, 1092, 1006, 856, 810, 757, 656, 634, 598, 545, 521 cm⁻¹; HRMS (m/z) [M + H]⁺ calcd. for C₂₃H₂₈ON₄Br, 455.1441; found 455.1424; HPLC purity: 97.53%.

*signal is overlapping with residual solvent peak.

N-(4-iodobenzyl)-1-(2-(6-methoxy-1,5-naphthyridin-4-yl)ethyl)piperidin-4-amine (6)

According to the general procedure for reductive amination, compound 10 (0.2 g, 0.698 mmol, 1 equiv) was used together with 4-iodobenzaldehyde (0.210 g, 0.908 mmol, 1.3 equiv). The residue was purified by flash column chromatography using CHCl₃/MeOH (9:1) to afford compound 6 (0.285 g, 81%) as brown-yellow solid.

mp = 80.8–82.7 °C; TLC (DCM:MeOH 4:1 and CHCl₃:MeOH 9:1) R₅ = 0.4 and 0.17; ¹H NMR (400 MHz, 295 K, CDCl₃): δ 1.43–1.52 (m, 2H)*, 1.92–1.95 (m, 2H), 2.14–2.20 (m, 2H), 2.50–2.55 (m, 1H), 2.77–2.81 (m, 2H), 3.03–3.06 (m, 2H), 3.36–3.40 (m, 2H), 3.78 (s, 2H), 4.07 (s, 3H), 7.08–7.12 (m, 3H), 7.41 (d, J = 4.4 Hz, 1H), 7.41 (d, J = 4.4 Hz, 1H), 7.64 (d, J = 8.4 Hz, 2H), 8.18 (d, J = 8.8 Hz, 1H), 8.66 (d, J = 4.4 Hz, 1H) ppm; ¹³C (100 MHz, 295 K, DMSO-d₆): δ 28.42, 32.66, 50.17, 52.27, 53.72, 58.38, 77.24*, 92.10, 116.30, 124.26, 130.07, 137.46, 140.36, 140.44, 140.96, 141.50, 146.60, 147.72, 161.43 ppm; IR (ATR): 2921, 2791, 1612, 1586, 1502, 1487, 1398, 1333, 1260, 1142, 1120, 1095, 1076, 1007, 930, 886, 855, 800, 737, 706, 627, 583, 538 cm⁻¹; HRMS (m/z) [M + H]⁺ calcd. for C₂₃H₂₈ON₄, 503.1302; found 503.1298; HPLC purity: 99.62%.

*signal is overlapping with residual solvent peak.

(4-((1-(2-(6-methoxy-1,5-naphthyridin-4-yl)ethyl)piperidin-4-ylamino)methyl)phenyl)methanol (7)
According to the general procedure for reductive amination, compound 10 (0.2 g, 0.698 mmol, 1 equiv) was used together with 4-(hydroxymethyl)benzaldehyde (0.123 g, 0.908 mmol, 1.3 equiv). The residue was purified by flash column chromatography using CHCl₃/MeOH (9:1) to afford compound 7 (0.122 g, 43%) as light brown solid.

mp = 73.4–76.7 °C; TLC (DCM:MeOH 4:1 and CHCl₃:MeOH 9:1) R₇ = 0.07 and 0.03; ¹H NMR (400 MHz, 295 K, CDCl₃): δ 1.48–1.57 (m, 2H), 1.97–2.00 (m, 2H), 2.18–2.23 (m, 2H), 2.55–2.61 (m, 1H), 2.79–2.83 (m, 2H), 3.06–3.09 (m, 2H), 3.38–3.42 (m, 2H), 3.85 (s, 2H), 4.09 (s, 3H), 4.71 (s, 2H), 7.13 (d, J = 8.8 Hz, 1H), 7.43 (d, J = 4.8 Hz, 1H) ppm; ¹³C (100 MHz, 295 K, CDCl₃): δ 28.39, 32.48, 50.39, 52.24, 53.67, 53.79, 58.35, 65.04, 77.21*, 116.32, 124.25, 127.20, 128.33, 139.79, 140.32, 140.96, 141.46, 146.62, 147.70, 161.43 ppm; IR (ATR): 2938, 2821, 1611, 15920, 1503, 1487, 1446, 1397, 1333, 1257, 1174, 1104, 1073, 1050, 990, 849, 805, 753, 613, 585 cm⁻¹; HRMS (m/z) [M + H]^+ calcd. for C₂₄H₃₁O₂N₄, 407.2442; found 407.2440; HPLC purity: 98.50%.

*signal is overlapping with residual solvent peak.

4-((1-(2-(6-methoxy-1,5-naphthyridin-4-yl)ethyl)piperidin-4-ylamino)methyl)benzamide (8)

According to the general procedure for reductive amination, compound 10 (0.2 g, 0.698 mmol, 1 equiv) was used together with 4-formylbenzamide (0.135 g, 0.908 mmol, 1.3 equiv). The residue was purified by flash column chromatography using CHCl₃/MeOH (9:1) to afford compound 8 (0.033 g, 11%) as yellow solid.

mp = 116.1–122.1 °C; TLC (DCM:MeOH 4:1 and CHCl₃:MeOH 9:1) R₇ = 0.06 and 0.02; ¹H NMR (400 MHz, 295 K, CDCl₃): δ 1.44–1.54 (m, 2H), 1.94–1.96 (m, 2H), 2.14–2.20 (m, 2H), 2.51–2.56 (m, 1H), 2.77–2.81 (m, 2H), 3.04–3.07 (m, 2H), 3.36–3.40 (m, 2H), 3.90 (s, 2H), 4.07 (s, 3H), 5.59–6.17 (m, 2H), 7.11 (d, J = 8.8 Hz, 1H), 7.41–7.44 (m, 3H), 7.78 (d, J = 4.4 Hz, 1H) ppm; ¹³C (100 MHz, 295 K, DMSO): δ 27.49, 32.05, 49.33, 51.67, 53.27, 53.58, 57.85, 115.99, 124.46, 127.20, 127.41, 139.79, 140.32, 140.96, 141.46, 146.62, 147.70, 160.80, 167.10 ppm; IR (ATR): 3288, 3148, 2941, 2838, 1681, 1614, 1591, 1567, 1495, 1450, 1398, 1336, 1289, 1264, 1134, 1105, 1083, 1017, 990, 869, 851, 807, 782, 757, 691, 657, 631, 602, 586, 547 cm⁻¹; HRMS (m/z) [M + H]^+ calcd. for C₂₅H₃₀O₂N₅, 420.2394; found 420.2391; HPLC purity: 99.25%.

4-((1-(2-(6-methoxy-1,5-naphthyridin-4-yl)ethyl)piperidin-4-ylamino)methyl)benzamide (8)

According to the general procedure for reductive amination, compound 10 (0.2 g, 0.698 mmol, 1 equiv) was used together with 4-(dimethylamino)benzaldehyde (0.135 g, 0.908 mmol, 1.3 equiv). The residue was purified by flash column chromatography using CHCl₃/MeOH (9:1) to afford compound 8 (0.033 g, 11%) as yellow solid.

mp = 116.1–122.1 °C; TLC (DCM:MeOH 4:1 and CHCl₃:MeOH 9:1) R₇ = 0.06 and 0.02; ¹H NMR (400 MHz, 295 K, CDCl₃): δ 1.44–1.54 (m, 2H), 1.94–1.96 (m, 2H), 2.14–2.20 (m, 2H), 2.51–2.56 (m, 1H), 2.77–2.81 (m, 2H), 3.04–3.07 (m, 2H), 3.36–3.40 (m, 2H), 3.90 (s, 2H), 4.07 (s, 3H), 5.59–6.17 (m, 2H), 7.11 (d, J = 8.8 Hz, 1H), 7.41–7.44 (m, 3H), 7.78 (d, J = 4.4 Hz, 1H) ppm; ¹³C (100 MHz, 295 K, DMSO): δ 27.49, 32.05, 49.33, 51.67, 53.27, 53.58, 57.85, 115.99, 124.46, 127.20, 127.41, 139.79, 140.32, 140.96, 141.46, 146.62, 147.70, 160.80, 167.10 ppm; IR (ATR): 3288, 3148, 2941, 2838, 1681, 1614, 1591, 1567, 1495, 1450, 1398, 1336, 1289, 1264, 1134, 1105, 1083, 1017, 990, 869, 851, 807, 782, 757, 691, 657, 631, 602, 586, 547 cm⁻¹; HRMS (m/z) [M + H]^+ calcd. for C₂₅H₃₀O₂N₅, 420.2394; found 420.2391; HPLC purity: 99.25%.

N-(4-(dimethylamino)benzyl)-1-(2-(6-methoxy-1,5-naphthyridin-4-yl)ethyl)piperidin-4-amine (9)

According to the general procedure for reductive amination, compound 10 (0.2 g, 0.698 mmol, 1 equiv) was used together with 4-(dimethylamino)benzaldehyde (0.135 g, 0.908 mmol, 1.3 equiv). The
Residue was purified by flash column chromatography using CHCl₃/MeOH (9:1) to afford compound 9 (0.196 g, 67%) as yellow-orange solid. mp = 89.4–91.6 °C; TLC (DCM:MeOH 4:1 and CHCl₃:MeOH 9:1) Rᵣ = 0.07 and 0.05; ¹H NMR (400 MHz, 295 K, CDCl₃): δ 1.47–1.57 (m, 2H), 1.95–1.98 (m, 2H), 2.15–2.21 (m, 2H), 2.56–2.62 (m, 1H), 2.76–2.80 (m, 2H), 2.93 (s, 6H), 2.98–3.09 (m, 2H), 3.35–3.39 (m, 2H), 3.74 (s, 2H), 4.07 (s, 3H), 6.71 (d, J = 8.8 Hz, 2H), 7.11 (d, J = 8.8 Hz, 1H), 7.21 (d, J = 8.8 Hz, 2H), 7.41 (d, J = 4.4 Hz, 1H), 8.18 (d, J = 8.8 Hz, 1H), 8.66 (d, J = 4.4 Hz, 1H) ppm; ¹³C (100 MHz, 295 K, CDCl₃): δ 28.43, 32.33, 40.74, 49.97, 52.26, 53.72, 58.39, 77.23*, 112.71, 116.26, 124.24, 129.19, 140.31, 140.96, 141.46, 146.71, 147.67, 149.87, 161.39 ppm; IR (ATR): 2920, 2802, 1611, 1593, 1523, 1488, 1397, 2335, 1262, 1217, 1191, 1166, 1146, 1104, 1063, 1018, 958, 934, 856, 837, 809, 755, 712, 696, 647, 586, 566 cm⁻¹; HRMS (m/z) [M + H]⁺ calcd. for C₂₅H₃₄ON₅, 420.2758; found 420.2754; HPLC purity: 95.68%. *signal is overlapping with residual solvent peak.
NMR spectra

*N*-benzyl-1-(2-(6-methoxy-1,5-naphthyridin-4-yl)ethyl)piperidin-4-amine (2)

$^1$H NMR (400 MHz, CDCl$_3$)

$^{13}$C NMR (100 MHz, CDCl$_3$)
$N$-(4-fluorobenzyl)-1-(2-(6-methoxy-1,5-naphthyridin-4-yl)ethyl)piperidin-4-amine (3)

$^1$H NMR (400 MHz, CDCl$_3$)

$^{13}$C NMR (100 MHz, CDCl$_3$)
$N$-(4-chlorobenzyl)-1-(2-(6-methoxy-1,5-naphthridin-4-yl)ethyl)piperidin-4-amine (4)

$^1$H NMR (400 MHz, CDCl$_3$)

$^{13}$C NMR (100 MHz, CDCl$_3$)
$N$-(4-bromobenzyl)-1-(2-(6-methoxy-1,5-naphthyridin-4-yl)ethyl)piperidin-4-amine (5)

$^1$H NMR (400 MHz, CDCl$_3$)

$^{13}$C NMR (100 MHz, CDCl$_3$)
N-(4-iodobenzyl)-1-(2-(6-methoxy-1,5-naphthyridin-4-yl)ethyl)piperidin-4-amine (6)

$^1$H NMR (400 MHz, CDCl$_3$)

$^{13}$C NMR (100 MHz, CDCl$_3$)
(4-((1-(2-(6-methoxy-1,5-naphthyridin-4-yl)ethyl)piperidin-4-ylamino)methyl)phenyl)methanol (7)

$^1$H NMR (400 MHz, CDCl$_3$)

$^{13}$C NMR (100 MHz, CDCl$_3$)
4-((1-(2-(6-methoxy-1,5-naphthyridin-4-yl)ethyl)piperidin-4-ylamino)methyl)benzamide (8)

$^1$H NMR (400 MHz, CDCl$_3$)

$^{13}$C NMR (100 MHz, CDCl$_3$)
$N$-(4-(dimethylamino)benzyl)-1-(2-(6-methoxy-1,5-naphthyridin-4-yl)ethyl)piperidin-4-amine (9)

$^1$H NMR (400 MHz, CDCl$_3$)

$^{13}$C NMR (100 MHz, CDCl$_3$)
Determination of IC$_{50}$ values of compounds 5 and 6 by using *S. aureus* gyrase supercoiling assays

**Assay**
In all experiments, the activity of the enzyme was determined prior to the testing of the compounds and 1 U defined as the amount of enzyme required to fully supercoil the substrate. This amount of enzyme was used in all subsequent assays. The final DMSO concentration in all the reactions was 1% (v/v). Compounds were serially diluted in DMSO and added to the reaction before the addition of the enzyme. Control compound for all assays was ciprofloxacin.

**Staphylococcus aureus gyrase supercoiling assay**
1 U of DNA gyrase was incubated with 0.5 μg of relaxed pBR322 DNA in a 30 μl reaction at 37 °C for 30 min under the following conditions: 40 mM HEPES·KOH (pH 7.6), 10 mM magnesium acetate, 10 mM DTT, 2 mM ATP, 500 mM potassium glutamate and 0.05 mg/ml BSA. Each reaction was stopped by the addition of 30 μl chloroform/iso-amyl alcohol (24:1) and 20 μl Stop Dye (40% sucrose, 100 mM Tris.HCl (pH 7.5), 10 mM EDTA, 0.5 μg/ml bromophenol blue), before being loaded on a 1.0% TAE (Tris.acetate 0.04 mM, EDTA 0.002 mM). Gels run at 70V for 3 hours.

**Data acquisition and analysis**
Bands were visualised by ethidium staining for 10 min, destained for 10 min in water and bands were analysed by gel documentation equipment (Syngene, Cambridge, UK) and quantitated using Syngene GeneTools software. Raw gel data (fluorescent band volumes) collected from Syngene, GeneTools gel analysis software were converted to a % of the 100% control (the fully supercoiled or relaxed DNA band) and plotted against the inhibitor concentration using SigmaPlot Version 13 (2015). The global curve fit non-linear regression tool was used to calculate IC$_{50}$ data using the following equation:

Equation: Exponential Decay, Single, 2 Parameter $f = ae^{-bx}$

**Supplementary Table 6. IC$_{50}$ results of *S. aureus* DNA gyrase supercoiling.**

| Compound | S. aureus gyrase supercoiling IC$_{50}$ (μM) | Average |
|----------|------------------------------------------|---------|
|          | Assay 1 | Assay 2 |
| Ciprofloxacin | 27.49 | 25.18 | 26.34 |
| 5         | 0.007  | 0.007  | 0.007 |
| 6         | 0.013  | 0.009  | 0.011 |

| Assay 1 | S. aureus gyrase | Ciprofloxacin |
|---------|------------------|--------------|
| -       | +                |              |
| 0.001   | 0.005            | 0.01         |
| 0.05    | 0.1              | 0.5          |
| 1.0     | 5.0              | 25           |

| Assay 2 | S. aureus gyrase | Ciprofloxacin |
|---------|------------------|--------------|
| -       | +                |              |
| 0.001   | 0.005            | 0.01         |
| 0.05    | 0.1              | 0.5          |
| 1.0     | 5.0              | 25           |
Supplementary Fig. 10. Gel Image. Inhibition of *S. aureus* DNA gyrase supercoiling by Ciprofloxacin.

**Assay 1**

![Graph showing inhibition of DNA gyrase supercoiling by Ciprofloxacin](image1)

**Assay 2**

![Graph showing inhibition of DNA gyrase supercoiling by Ciprofloxacin](image2)

Supplementary Fig. 11. DNA gyrase supercoiling inhibition plot. The plot shows the results of the supercoiling inhibition with Ciprofloxacin. IC$_{50}$ calculated from assay 1 is 27.49 μM and 25.18 μM from assay 2.

**Assay 1**

*S. aureus* gyrase  | CFX  | 5
--- | --- | ---
- | + | 25 50 0.001 0.005 0.01 0.05 0.1 0.5 1.0 5.0 10 25 50 100 μM

**Assay 2**

*S. aureus* gyrase  | CFX  | 5
--- | --- | ---
- | + | 25 50 0.001 0.005 0.01 0.05 0.1 0.5 1.0 5.0 10 25 50 100 μM

Supplementary Fig. 12. Gel Image. Inhibition of *S. aureus* DNA gyrase supercoiling by compound 5. Ciprofloxacin (CFX) was used for comparison.

**Assay 1**

![Graph showing inhibition of DNA gyrase supercoiling by compound 5](image3)

**Assay 2**

![Graph showing inhibition of DNA gyrase supercoiling by compound 5](image4)
**Supplementary Fig. 13. DNA gyrase supercoiling inhibition plot.** The plot shows the results of the supercoiling inhibition with compound 5. IC$_{50}$ calculated from assay 1 is 0.007 µM and 0.007 µM from assay 2. Data were plotted up to 0.5 µM to improve the appearance of the plot.

**Supplementary Fig. 14. Gel Image.** Inhibition of *S. aureus* DNA gyrase supercoiling by compound 6. Ciprofloxacin (CFX) was used for comparison.

**Supplementary Fig. 15. DNA gyrase supercoiling inhibition plot.** The plot shows the results of the supercoiling inhibition with compound 6. IC$_{50}$ calculated from assay 1 is 0.013 µM and 0.009 µM from assay 2. Data were plotted up to 0.5 µM to improve the appearance of the plot.
Cleavage assay with E. coli DNA gyrase

The gels relate to the Fig. 3 in the main text.

Supplementary Figure 16: Stabilisation of single-strand cleavage by the E. coli DNA gyrase with compounds 4, 5 and 6. Cleavage complexes formed by 4, 5 and 6 were trapped with SDS (Materials and Methods) before treatment with Proteinase K. The plasmid substrate is mostly intact (top left) and thereby compacted by ethidium bromide, which is present in all the gels. Some nicking is observed. Addition of enzyme without any compound present induces a low amount of double-strand cleavage, which arise from the natural catalytic cycle of the enzyme. All three compound were tested without enzyme at a wide range of concentration (left column of gels) and no extra cleavage is observed (compared to the “no enzyme, no compound” lane). The right column of gels shows compounds tested with enzyme. The intensity of the nicked band increases with compound concentration, reflecting the stabilization of a singly cleaved cleavage complex by the compound. The level of cleavage remains relatively low when compared with the enzyme concentration (around 70 nM in all experiment). This is due to only a subset of enzyme-compound-DNA complexes being cleaved (see main text). The enzyme controls were reproduced twice with similar results and the cleavage assays (enzyme with compounds) were reproduced three times with similar results. Uncropped version of the gel image is available as Supplementary Figure 24.
Cytotoxicity studies.

Supplementary Table 7. Results of cytotoxicity studies.

| Cmpd | HUVEC IC₅₀ [µM] | HepG2 IC₅₀ [µM] | hERG IC₅₀ [µM] |
|------|-----------------|-----------------|----------------|
| 2    | 54.08 ± 3.45    | 17.67 ± 2.16    | 1.05           |
| 3    | >50             | 12.67 ± 0.21    | 0.59           |
| 4    | 26.96 ± 0.38    | 10.16 ± 0.10    | 0.27           |
| 5    | 25.42 ± 0.01    | 9.37 ± 0.07     | 0.29           |
| 6    | 18.98 ± 0.85    | 8.47 ± 0.09     | 0.18           |
| 7    | >50             | nd              | 3.24           |
| 8    | >50             | nd              | 13.45          |
| 9    | 23.85 ± 0.09    | 10.03 ± 0.06    | 2.11           |

nd: not determined

Metabolic activity assessment

Cells of the HepG2 line (ATCC® HB-8065™) and HUVEC (ATCC® CRL-1730™) were cultured in Dulbecco’s Modified Eagle Medium (DMEM; Sigma-Aldrich, St. Louis, MO, USA) supplemented with 10% heat-deactivated FBS (Gibco, Grand Island/NY, USA), 2 mM L-glutamine, 100 U/mL penicillin, 100 µg/mL streptomycin (all from Sigma-Aldrich) in a humidified chamber at 37 °C and 5% CO₂. The experiments were carried out on passages 7 - 9 for both cell lines. The tested compounds were dissolved in DMSO and further diluted in culture medium to a desired final concentration. Cells were seeded into 96-well plates at 8 × 10⁴ cells/mL (100 µL/well) and incubated for 24 h to attach onto the wells. Cells were treated with 1 µM and 50 µM of each compound of interest or corresponding vehicle as control. For IC₅₀ determination, cells were seeded into 96-well plates at 5 × 10⁴ cells/mL (100 µL/well) and after attachment treated with seven different concentration of the selected compounds or corresponding vehicle as control. The metabolic activity was assessed after 72 h treatment using the CellTiter 96 Aqueous One Solution Cell Proliferation Assay (Promega, Madison/WI, USA), in accordance with the manufacturer’s instructions. The absorbance was measured at 492 nm on an automated microplate reader BioTek Synergy HT (BioTek Instruments, Inc.). Data are presented as IC₅₀ value (mean ± SD) from two independent experiments, each conducted in duplicate or triplicate. IC₅₀ values were calculated by a non-linear regression model using Graph Pad Prism 7 software.

Cardiovascular hERG inhibition

Cardiovascular safety profile was entirely outsourced. hERG screening was performed at TCG Lifesciences Pvt. Ltd. India with FP-based hERG binding assay (Invitrogen kit).
Results of pdb database search

We have searched the RCSB Protein Data Bank for biological complexes in which a ligand is able to form bifurcated halogen bond with the protein. Recently, Shinada et al. performed a detailed RCSB PDB search, identifying bifurcated halogen bonds in five protein-ligand complexes (1GJD, 1UHI, 4CMJ, 4JYI, and 5A86)\(^8\). Our search was performed with advanced search of the Structure title, Primary Citation title, and PubMed abstract fields, utilizing keywords “bifurcated halogen bonds”, “symmetrical halogen bonds”, and “two halogen bonds”. Two complexes (5YC6 and 5YC7) arose from this search. Hits for all complexes are exemplified in Tables S7 and Figure S11, along with information about X···O distances, \(\Theta_1\), and \(\Theta_2\) angles.

Supplementary Table 8. PDB database search results of ligands forming bifurcated halogen bond with protein.

| PDB ID | Halogen involved | \(d_1\) [Å]\(^*\) | \(d_1\) [Å]\(^*\)' | \(\Theta_1\) [°]\(^*\) | \(\Theta_1\) [°]\(^*\)' | \(\Theta_2\) [°]\(^*\) | \(\Theta_2\) [°]\(^*\)' |
|--------|------------------|----------------|----------------|----------------|----------------|----------------|----------------|
| 1GJD   | I                | 3.4            | 3.7            | 163.9          | 143.3          | 156.3          | 133.6          |
| 1UHI   | I                | 4.5            | 3.1            | 147.4          | 174.2          | 136.1          | 127.4          |
| 4CMJ   | Br               | 4.4            | 3.8            | 143.8          | 142.4          | 78.0           | 142.2          |
| 4JYI   | Cl               | 3.9            | 3.3            | 141.8          | 150.5          | 97.8           | 140.0          |
| 5A86   | Cl               | 3.7            | 4.4            | 140.4          | 141.1          | 68.2           | 141.4          |
Supplementary Figure 17: Results of pdb databank search. a 1GJD (protein in cartoon representation in blue); b 1UHI (protein in cartoon representation in cyan); c 4CMJ (protein in cartoon representation in tan); d 4JYI (protein in cartoon representation in orange); e 5A86 (protein in cartoon representation in yellow). Amino acid residues are represented as sticks colour-coded by element. Halogen bonds are represented as yellow dots.
Results of small molecule database (Cambridge Structural Database (CSD)) search

The search of small molecules in Cambridge Structure Database\(^9\) (CSD version 2020.1), able to form bifurcated halogen bonds was performed using ConQuest (version 2020.1.1) program with the following criteria: halogen atom (Cl, Br or I) is bound to phenyl and interacts with two carbonyl oxygen atoms. All the obtained structures were visually inspected using Mercury (version 2020.1). Those structures with the distances between the halogen atom and both oxygen atoms lower than the sum of van der Waals radii were selected as hits. As reported by Shinada et al. a majority of chlorine atoms forms halogen bonds at a distance 0.3 Å longer than the sum of van der Waals radii.\(^8\) Therefore we applied this criteria for another selection of structures with a chloro atom. Although the search was based on halogen bonding with carbonyl oxygen atoms, structures in which halogen forms halogen bond with two non-carbonyl oxygens or one carbonyl and one non-carbonyl were also considered as hits. Hits for all halogen atoms are exemplified in Tables S8-11 along with information about X···O distances, \(\Theta_1\), and \(\Theta_2\) angles. Statistics on their geometric parameters are shown on Fig. S12-15.

Supplementary Table 9. Summary of hits from CSD search, forming bifurcated Cl···O bonds at the distance lower than the sum of van der Waals radii.

| No | Crystal structure identifier | \(d_1\) [Å]’ | \(d_2\) [Å]” | \(\Theta_1\)° [C-X···O]’ | \(\Theta_1\)° [C-X···O]” | \(\Theta_2\)° [X···O-R]’ | \(\Theta_2\)° [X···O-R]” |
|----|-----------------------------|-------------|-------------|----------------|----------------|---------------|---------------|
| 1  | KOVJEN                     | 2.951       | 3.229       | 166.01         | 149.41         | 74.3          |               |
| 2  | POZHOE                     | 2.917       | 2.623       | 150.1          | 114.11         | 101.54/135.64 | 124.82        |
| 3  | IJOCIT                     | 3.254       | 3.18        | 155.91         | 136.63         | 104.73/114.02 | 103.32/94.89  |
| 4  | MAVZER                     | 3.268       | 3.238       | 89.64          | 129.57         | 73.85         | 74.93/90.8    |
| 5  | SAGRIF                     | 3.124       | 3.259       | 160.82         | 96.31          | 125.07        | 154.29        |
Supplementary Figure 18: Statistics on geometrical properties of bifurcated Cl···O halogen bond based on sum of van der Waals radii. a Distribution plot of bifurcated Cl···O halogen distances. b Distribution plot of $\theta_1$ [C-Cl···O] angles. c Distribution plot of $\theta_2$ [C···O-R] angles. d Scatter plots (angle ($\theta_i$) versus distance) obtained from the halogen bond CSD search. Distance between halogen atom and first oxygen, and associated $\theta_1$, $\theta_2$ angles are represented as blue bars, while distance between halogen atom and second oxygen, and associated $\theta_1$, $\theta_2$ angles are represented as red bars.
Supplementary Table 10. Summary of hits from CSD search, forming bifurcated Cl⋯O bonds at the distance lower than the sum of van der Waals radii + 0.3 Å.

| No | Crystal structure identifier | $d_1$ [Å]$^*$ | $d_2$ [Å]$^*$ | $d_3$ [Å]$^{**}$ | $\Theta_{[\text{C-X}⋯\text{O}]}^\circ$ | $\Theta_{[\text{C-X}⋯\text{O}]}^\circ$ | $\Theta_{[\text{X}⋯\text{O-R}]}^\circ$ | $\Theta_{[\text{X}⋯\text{O-R}]}^\circ$ | $\Theta_{[\text{X}⋯\text{O-R}]}^\circ$ |
|----|-------------------------------|---------------|---------------|------------------|-------------------|-------------------|-------------------|-------------------|-------------------|
| 1  | GUMNOU                        | 3.429         | 3.451         | 3.179            | 112               | 143.4             | 143.5             | 112.7             | 120.9             |
| 2  | JUDPGE                        | 3.095         | 3.454         | /                | 154.52            | 92.04             | /                 | 116.7             | 166.5             |
| 3  | KOQCFI                        | 3.415         | 3.46          | /                | 93.1              | 136.9             | /                 | 90.2              | 87.3              |
| 4  | KOVJEN                        | 3.493         | 3.279         | /                | 109.73            | 175.14            | /                 | 103.24            | 145.67            |
| 5  | LOYHOZ                       | 2.951         | 3.229         | /                | 166.01            | 149.41            | /                 | 74.3              | 131               |
| 6  | NUBDEW                       | 3.485         | 3.437         | /                | 100.03            | 120.11            | /                 | 111.33/75.62      | 132.43            |
| 7  | POZHOE                        | 2.917         | 2.623         | /                | 150.1             | 114.11            | /                 | 101.54/135.64     | 124.82            |
| 8  | PUGQOA                        | 3.549         | 3.276         | /                | 92.12             | 174.64            | /                 | 91.36             | 101.74            |
| 9  | QOWWEH                        | 3.416         | 3.413         | /                | 99.51             | 92.89             | /                 | 107.98/128.01     | 110.52            |
| 10 | ZONTIO                        | 3.46          | 3.289         | /                | 64.03             | 132.62            | /                 | 87.27             | 90.93             |
| 11 | AJAKUR                        | 3.537         | 3.42          | /                | 70.66             | 85.1              | /                 | 119.59            | 77.31             |
| 12 | AKOZEF                        | 3.489         | 3.149         | /                | 75.02             | 160.52            | /                 | 111.47            | 131.3             |
| 13 | ANEQHU                        | 3.458         | 3.46          | /                | 152.8             | 124.72            | /                 | 100.71/106.33     | 101.33            |
| 14 | ANUQIL                        | 3.429         | 3.547         | /                | 150.08            | 113.98            | /                 | 115.79            | 105.51/135.83     |
| 15 | APUGEY                        | 3.492         | 3.37          | /                | 152.31            | 123.77            | /                 | 105.56/125.51     | 141               |
| 16 | ASOPEE                        | 3.44          | 3.257         | /                | 82.19             | 137.86            | /                 | 132               | 97.64             |
| 17 | ASUMUX                        | 3.247         | 3.332         | /                | 124.94            | 148.19            | /                 | 102.95/86.04      | 128.82            |
| 18 | AWEJIX                        | 3.428         | 3.41          | /                | 160.48            | 124.94            | /                 | 149.21            | 97.76             |
| 19 | AWITAB                        | 2.952         | 3.407         | /                | 165.9             | 71.27             | /                 | 129.76            | 149.66            |
| 20 | BACBUC                        | 3.559         | 3.558         | /                | 103.62            | 90.73             | /                 | 163.98            | 138.04            |
| 21 | BACDAK                        | 3.493         | 3.506         | /                | 104.47            | 91.85             | /                 | 163.27            | 136.13            |
| 22 | BANQUD                        | 3.404         | 3.36          | /                | 131.09            | 147.94            | /                 | 113.31            | 82.38/98.62       |
| 23 | BARTAP                        | 3.518         | 3.472         | 3.538            | 138.23            | 121.02            | 164.43            | 93.78             | 95.85             | 94.83/105.87      |
| 24 | BEBCAM                        | 3.445         | 3.461         | /                | 151.48            | 139.41            | /                 | 80.14/129.52/81.88 | 83.11             |
| 25 | BETXOQ                        | 3.238         | 3.258         | /                | 131.4             | 166.26            | /                 | 143.68            | 148.17            |
| 26 | BICWOÇ                        | 3.404         | 3.468         | /                | 109.7             | 139.51            | /                 | 75.42             | 72.22/89.64       |
| 27 | BIHXAIA                       | 3.207         | 3.365         | /                | 154.93            | 143.18            | /                 | 143.05            | 144.49            |
| 28 | BIPFUE                        | 3.515         | 3.315         | /                | 154.43            | 143.08            | /                 | 116.13/99.09      | 128.41/95.97      |
| 29 | BIPGAL                        | 3.305         | 3.494         | /                | 143.5             | 155.4             | /                 | 128.59/45.4       | 98.5/116.2        |
| 30 | BIVNUP                        | 3.541         | 3.559         | /                | 96.37             | 126               | /                 | 101.6/67.16       | 66.41             |
|   |   |   |   |   |   |   |   |   |   |   |   |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 31 | BIZFUM | 3.397 | 3.097 | / | 78.16 | 174.31 | / | 101.28 | 136.96 | / |
| 32 | BONPOM | 3.291 | 3.461 | / | 138.26 | 83.52 | / | 138.92 | 102.85 | / |
| 33 | BUIJLEZ | 3.568 | 3.462 | 3.532 | 92.11 | 134.37 | 147.05 | 154.47 | 156.59 | 130.63/76.39 |
| 34 | BUSQUUD | 3.44 | 3.522 | / | 91.88 | 128.63 | / | 73.15 | 69.39/97.97 | / |
| 35 | CAJSAK | 3.481 | 3.135 | / | 75.3 | 150.71 | / | 100.55 | 98.69 | / |
| 36 | CEDWAL | 3.404 | 3.558 | / | 71.36 | 100.71 | / | 96.67 | 81.13 | / |
| 37 | CIGWUK | 3.531 | 3.039 | / | 102.12 | 175.2 | / | 116.88 | 133.21/109.73 | / |
| 38 | CIJFEI | 3.562 | 3.518 | / | 154.33 | 159.17 | / | 83.79 | 85.59 | / |
| 39 | CIJWAV | 3.308 | 3.526 | / | 157.65 | 116.8 | / | 118.04 | 153.99 | / |
| 40 | CIMHIP01 | 3.142 | 3.543 | / | 150.82 | 133.18 | / | 147.08 | 106.18/115.6 | / |
| 41 | CIPZIL | 3.449 | 3.415 | / | 158.68 | 68.38 | / | 97.14 | 109.4 | / |
| 42 | CIVWAH | 3.526 | 3.143 | / | 89.84 | 168.1 | / | 115.59/90.23 | 92.1 | / |
| 43 | CNAPAN | 3.439 | 3.118 | / | 144.53 | 177.24 | / | 146.82/94.06 | 115.06 | / |
| 44 | COLNAT | 3.458 | 3.533 | / | 134.7 | 159.34 | / | 137.51 | 97.09 | / |
| 45 | COSWOY | 3.466 | 2.772 | / | 161.81 | 153.07 | / | 92.74/54.62 | 83.95 | / |
| 46 | CUQZAR | 3.294 | 2.855 | 3.387 | 107.8 | 170.95 | 138.9 | 64.59/91.98 | 108.05 | 84.59 |
| 47 | DAQZED | 3.516 | 3.515 | / | 116.87 | 142.73 | / | 81.71 | 82.11 | / |
| 48 | DEJKEL | 3.107 | 3.478 | / | 166.79 | 118.64 | / | 107.21/113.43 | 149.91/94.3 | / |
| 49 | DEJMAI | 3.435 | 3.44 | / | 125.92 | 125.6 | / | 104.5 | 137.34/89.56 | / |
| 50 | DINCIOU | 3.072 | 3.356 | / | 138.08 | 94.44 | / | 153.73 | 150.87 | / |
| 51 | DIPHUH01 | 3.459 | 3.429 | / | 78.41 | 81.35 | / | 92.69 | 134.25 | / |
| 52 | DISHOE | 3.344 | 3.514 | / | 120.28 | 104.84 | / | 84.86 | 77.31 | / |
| 53 | DIXFUO | 3.376 | 3.481 | / | 166.83 | 66.33 | / | 154.78 | 134.02 | / |
| 54 | DICYAS | 3.463 | 3.384 | / | 148.8 | 146.85 | / | 104.68 | 121.1 | / |
| 55 | DOGBUW | 3.415 | 3.513 | / | 131.53 | 77.36 | / | 78.29 | 104.56 | / |
| 56 | DOKJUL | 3.191 | 3.108 | / | 141.36 | 142.07 | / | 110.01/112.79 | 117.38/123.24 | / |
| 57 | DOMDAL | 3.395 | 3.547 | / | 69.4 | 66.77 | / | 136.79/101.48 | 105.69/53.04 | / |
| 58 | DOXOQ | 3.49 | 3.359 | / | 138.4 | 93.69 | / | 148.19 | 156.96 | / |
| 59 | DOZQOA | 3.536 | 3.293 | / | 101.52 | 164.99 | / | 70.54 | 86.52 | / |
| 60 | DUKDUI | 3.426 | 3.456 | / | 91.74 | 133.76 | / | 127.87 | 138.79 | / |
| 61 | DUKHAT | 2.976 | 3.439 | / | 166.43 | 102.04 | / | 119.27/115.02 | 119.27 | / |
| 62 | DUPVOB | 3.458 | 3.293 | / | 107.16 | 97.23 | / | 99.26/75.78 | 103.63 | / |
| 63 | EBITEQ | 3.266 | 3.397 | / | 144.09 | 168.41 | / | 90.44 | 87.45 | / |
| 64 | ECOLOY | 3.404 | 3.351 | / | 89.51 | 160.99 | / | 91.86 | 116.65/76.14 | / |
| 65 | EHUITEM | 3.276 | 3.072 | / | 110.83 | 147.86 | / | 159.85 | 111.83 | / |
| 66 | EJIZEE | 3.099 | 3.496 | / | 147.82 | 111.31 | / | 134.04 | 93.93 | / |
|   |   |   |   |   |   |   |   |   |   |   |
|---|---|---|---|---|---|---|---|---|---|---|
| 67 | ENOFAP | 3.297 | 3.411 | / | 150.14 | 88.11 | / | 101.17 | 114.98 | / |
| 68 | ERORAG | 3.405 | 3.558 | / | 98.72 | 84.56 | / | 149.04 | 109.71 | / |
| 69 | ESJEX | 3.112 | 3.545 | / | 165.43 | 93.55 | / | 128.65 | 153.43 | / |
| 70 | ESUGIK | 3.372 | 3.139 | / | 140.25 | 151.16 | / | 76.14 | 125.3 | / |
| 71 | ETJEW | 3.317 | 3.348 | / | 129.16 | 95.31 | / | 155.72 | 157.93 | / |
| 72 | ETOGAG | 3.299 | 3.537 | 3.447 | 92.97 | 94.81 | 115.59 | 124.03 | 62.04 | 69.59 |
| 73 | ETUBEC | 3.373 | 3.442 | / | 104.23 | 138.4 | / | 127.2 | 66.51 | / |
| 74 | FAXHIW | 3.03 | 3.559 | / | 171.05 | 82.28 | / | 119.62/112.11 | 138.61 | / |
| 75 | FAPHEJ | 3.496 | 3.376 | / | 140.13 | 154.02 | / | 132.85 | 131.32 | / |
| 76 | FAYZEM | 3.39 | 3.335 | / | 81.43 | 150.63 | / | 150.5 | 156.59 | / |
| 77 | FECDID | 3.517 | 3.517 | / | 156.28 | 156.28 | / | 111.37 | 111.37 | / |
| 78 | FERLIA | 3.565 | 3.209 | / | 101.86 | 146.7 | / | 168.88 | 116.7 | / |
| 79 | FIYFEZ | 3.487 | 3.334 | / | 125.52 | 155.99 | / | 123.19/106.77 | 110.72/103.69 | / |
| 80 | FOCDEH | 3.504 | 3.493 | / | 108.54 | 146.41 | / | 74.05 | 93.64 | / |
| 81 | FOLSEH | 3.406 | 3.48 | / | 168.41 | 124.03 | / | 160.09 | 142.49 | / |
| 82 | FUFNUR | 3.29 | 3.564 | 3.469 | 158.88 | 114.56 | 139.69 | 101 | 93.41 | 136.91 |
| 83 | FUFVEI | 3.276 | 3.455 | / | 166.41 | 132.04 | / | 86.63 | 124.97 | / |
| 84 | FUHVOU | 3.569 | 3.273 | / | 160.16 | 160.02 | / | 75.54 | 88.38 | / |
| 85 | FUJWAJ | 3.542 | 3.224 | / | 100.24 | 77.92 | / | 170.86 | 106.66 | / |
| 86 | GAHPAJ | 3.21 | 3.253 | 3.546 | 78.08 | 131.26 | 104.14 | 146.38 | 160.04 | 100.82 |
| 87 | GAQZEE | 3.358 | 3.391 | / | 159.77 | 150.68 | / | 152.2 | 104.13 | / |
| 88 | GEGCOL | 3.383 | 3.2 | / | 122.49 | 162.13 | / | 148.48 | 126.51 | / |
| 89 | GEJZEC | 3.534 | 3.463 | / | 84.06 | 140.07 | / | 103.26 | 108.14/93.99 | / |
| 90 | GENBIL | 3.161 | 3.446 | / | 169.72 | 136 | / | 126.29/114.4 | 73.66 | / |
| 91 | GEPJIW | 3.18 | 3.266 | / | 163.81 | 140.55 | / | 139.35 | 146.16 | / |
| 92 | GIDLUB | 3.556 | 3.277 | / | 78.02 | 145.77 | / | 124.96 | 143.96 | / |
| 93 | GILMAN | 3.398 | 3.55 | / | 93.84 | 73.77 | / | 98.71 | 75.93 | / |
| 94 | GIQGUL | 3.284 | 3.532 | / | 111.21 | 83.34 | / | 143.02/97.95 | 96.71 | / |
| 95 | GODJER | 3.4 | 3.36 | / | 132.29 | 109.54 | / | 102.39 | 165.72 | / |
| 96 | GRISFL | 3.403 | 3.473 | / | 146.74 | 127.66 | / | 150.58/82.49 | 111.09/74.12 | / |
| 97 | GUDJAS | 3.349 | 2.98 | / | 126.54 | 171.19 | / | 92.68 | 105.64 | / |
| 98 | GUGVEL | 3.529 | 3.512 | / | 83.51 | 140.24 | / | 155.67/86.62 | 93.04/144.35 | / |
| 99 | GUHXEM | 3.165 | 3.39 | / | 115.89 | 92.56 | / | 125.85 | 105.77 | / |
| 100 | GUHXOW | 3.558 | 3.438 | / | 127.9 | 108.33 | / | 100.85 | 132.84/108.67 | / |
| 101 | HEGGIL | 3.548 | 3.323 | / | 109.53 | 96.06 | / | 71.79/102.02 | 159.25 | / |
| 102 | HETWEJ | 3.368 | 3.158 | / | 105.5 | 150.74 | / | 110.04/132.43 | 107.8 | / |
| 103 | HIJZUV | 3.522 | 3.484 | / | 77.4 | 146.4 | / | 120.48 | 141.26 | / |
|   |   |   |   |   |   |
|---|---|---|---|---|---|
| 104 | HOFCAJ | 3.48 | 3.567 | / | 151.98 | 87.13 | / | 149.63 | 89.6 | / |
| 105 | HOSMEJ | 3.324 | 3.303 | / | 101.64 | 167.57 | / | 85.91 | 111.19 | / |
| 106 | HOWHOS | 3.499 | 3.548 | / | 87.3 | 80.28 | / | 80.21/109.74 | 108.13/93.72 | / |
| 107 | HUFDES | 3.08 | 3.37 | / | 133.53 | 90 | / | 173.07 | 166.97 | / |
| 108 | HUGDOD | 3.237 | 3.38 | 3.243 | 111.01 | 138.36 | 170.76 | 107.9 | 169.49 | 176 |
| 109 | HUGNAZ | 3.372 | 3.38 | / | 173.37 | 117.63 | / | 100.87 | 109.91 | / |
| 110 | HUJMAB | 3.449 | 2.922 | / | 122.83 | 155.53 | / | 135.71/92.44 | 102.4/103.07/104.16 | / |
| 111 | IGABEY | 3.236 | 3.542 | / | 113.05 | 153.76 | / | 128.39 | 95.41 | / |
| 112 | IJOCTF | 3.254 | 3.18 | / | 155.91 | 136.63 | / | 104.73/114.02 | 103.32/94.89 | / |
| 113 | IKEYED | 3.435 | 3.464 | 3.31 | 127.5 | 94.11 | 166.42 | 77.9 | 77.45 | 159.34 |
| 114 | IMAKEN | 3.434 | 3.44 | / | 144.33 | 106.62 | / | 72.54 | 71.94/100.79 | / |
| 115 | INAWEB | 3.441 | 3.563 | / | 103.43 | 77.99 | / | 111.1 | 90.92 | / |
| 116 | IROREM | 3.331 | 3.564 | / | 132.73 | 75.38 | / | 109.46 | 92.67 | / |
| 117 | JAWBIT | 3.334 | 3.324 | / | 127.2 | 162.7 | / | 94.71/124.16 | 98.11 | / |
| 118 | JAZBIY | 3.216 | 3.231 | / | 140 | 163.62 | / | 143.42/96.27 | 139.75 | / |
| 119 | JIPCAP | 3.554 | 3.04 | / | 132.81 | 163.69 | / | 100.93/72.24 | 115.73/96.36 | / |
| 120 | JIRDAS | 3.307 | 3.521 | / | 69.82 | 98.17 | / | 82.09 | 99.79 | / |
| 121 | JOXWEA | 3.548 | 3.548 | / | 155.29 | 87.91 | / | 94.39/73.05 | 94.25 | / |
| 122 | JUHHAV | 3.461 | 3.433 | / | 118.15 | 112.74 | / | 89.58 | 90.72 | / |
| 123 | KAXFOH | 3.476 | 3.339 | / | 113.72 | 97.31 | / | 73.49 | 165.35 | / |
| 124 | KEFTOF | 3.058 | 3.48 | / | 167.79 | 93.15 | / | 90.43 | 72.3 | / |
| 125 | KERWIQ | 3.048 | 3.551 | / | 73.14 | 159.79 | / | 136.03/88.04 | 144.57 | / |
| 126 | KILBEM | 3.526 | 3.519 | / | 163.26 | 102.22 | / | 90.16 | 140.31 | / |
| 127 | KIMSIK | 3.277 | 2.953 | / | 135.71 | 162.67 | / | 120.31/119.04 | 120.31 | / |
| 128 | KIPHEZ | 3.386 | 3.563 | / | 81.49 | 76.07 | / | 99.61 | 89.14 | / |
| 129 | KIRKON | 3.354 | 3.416 | / | 76.7 | 88.87 | / | 141.4 | 137.86 | / |
| 130 | LAHZIE | 3.065 | 3.568 | / | 173.77 | 91.6 | / | 170.14 | 146.5 | / |
| 131 | LATLID | 2.993 | 3.511 | / | 175.62 | 110.92 | / | 97.79/75.73 | 78.76 | / |
| 132 | LEJDOV | 3.127 | 3.325 | / | 163.42 | 118.64 | / | 113.94 | 102.84 | / |
| 133 | LEWKII | 3.419 | 3.439 | / | 84.74 | 118.31 | / | 82.84 | 94.8 | / |
| 134 | LEWKUU | 3.454 | 2.978 | / | 117.87 | 141.98 | / | 167.75 | 141.14 | / |
| 135 | LIPVAAH | 3.298 | 3.332 | / | 113.55 | 65.16 | / | 136.01 | 134.11 | / |
| 136 | LONVUI | 3.449 | 3.28 | / | 145.62 | 82.54 | / | 156.45 | 127.41 | / |
| 137 | LORWIZ | 3.426 | 3.327 | / | 85.98 | 161.24 | / | 102.3 | 102.5 | / |
| 138 | LUDJAW | 3.293 | 3.374 | / | 130.95 | 97.45 | / | 84.63 | 125.47 | / |
| 139 | LUDJAW | 3.504 | 3.222 | / | 80.02 | 160.99 | / | 98.98 | 150.52 | / |
| 140 | LUDJAW | 3.102 | 3.57 | / | 154.76 | 132.84 | / | 132.02 | 92.03 | / |
| Code   | 1 | 2 | 3 | 4 | 5 | 6 |
|--------|---|---|---|---|---|---|
| PICCIQ | 2.929 | 3.442 | 3.564 | 3.405 | 3.459 | 3.509 |
| PEXLUC | 3.094 | 3.525 | 3.548 | 3.589 | 3.564 | 3.509 |
| PINMUW | 3.348 | 3.06 | 153.8 | 141.57 | 129.74 | 146.89/94.04 |
| POCGEW | 3.553 | 3.265 | 94.76 | 148.05 | 84.31 | 139.69/100.21 |
| POFLOO | 3.283 | 3.315 | 154.85 | 106.99 | 86.8/89.35 | 95.71/82.17 |
| MCHTEP03 | 3.458 | 3.538 | 102.45 | 165.96 | 73.62/95.48 | 159.98/82.25 |
| MEKKEM | 3.276 | 3.435 | 106.14 | 144.8 | 73.32 | 145.14 |
| MOLLIJ | 3.21 | 3.47 | 167.2 | 69.87 | 137.43 | 103.95 |
| MOYPUN | 3.468 | 3.499 | 75.14 | 116.93 | 166.49 | 93.83 |
| MUHIK | 3.404 | 3.255 | 133.98 | 169.23 | 114.69 | 160.66 |
| MUPSUN | 3.569 | 3.337 | 119.13 | 168.27 | 100.47 | 123.98/81.84 |
| NAMRA | 3.48 | 3.047 | 85.02 | 152.23 | 144.42 | 127.45 |
| NARIJAU | 3.467 | 3.159 | 97.36 | 147.8 | 88.99 | 87.17 |
| NACUH | 3.565 | 3.347 | 90.28 | 161.43 | 105.27 | 128.37 |
| NEYSAN | 3.333 | 3.389 | 69.15 | 73.85 | 64.09/93.55 | 89.65 |
| NIHGOB | 3.192 | 3.401 | 107.46 | 89.33 | 167.4 | 106.54 |
| NIKFIY | 3.553 | 3.489 | 139.57 | 74.32 | 124.07 | 108.5 |
| NIPAG | 3.044 | 3.399 | 162.86 | 75.06 | 138.75 | 93.79 |
| NITMOS | 3.428 | 3.459 | 177.74 | 123.48 | 84.36 | 87.1 |
| NITNAS | 3.286 | 3.53 | 144.55 | 118.68 | 94.4 | 93.92 |
| NUVHOX | 3.515 | 3.509 | 93.29 | 118.63 | 83.5 | 119.94 |
| OCOKOY | 3.137 | 3.526 | 152.59 | 129.96 | 121.21/119.58 | 134.5 |
| OGUOKOS | 3.279 | 3.405 | 130.38 | 145.93 | 83.34/131.73 | 94.08 |
| OKUCAZ | 3.334 | 3.405 | 165.71 | 104.46 | 106.06 | 156.87/95.58 |
| OLECAL | 3.137 | 3.564 | 171.35 | 126.05 | 127.78 | 159.51 |
| OTMAH | 3.315 | 3.288 | 84.8 | 161.72 | 97.19/97.24 | 100.8/114.81 |
| OTOWOK | 3.443 | 3.277 | 151.1 | 157.25 | 153.74 | 109.74 |
| OWETAN | 3.242 | 3.068 | 159.6 | 155.12 | 95.01 | 102.6 |
| OZOMUM | 3.454 | 3.344 | 132.07 | 167.64 | 136.51/101.53 | 97.81 |
| PABHEF | 3.254 | 3.463 | 173.65 | 140.3 | 102.58 | 95.06 |
| PCMVALA01 | 3.121 | 3.458 | 155.62 | 111.4 | 172.94 | 168.79 |
| PEPPEH | 3.555 | 3.489 | 159.46 | 161.72 | 70.24 | 93.93/72.78 |
| PEXLUC | 3.094 | 3.525 | 156.33 | 141.8 | 146.58 | 101.59 |
| PICCIQ | 3.125 | 3.442 | 172.12 | 88.94 | 98.2 | 111.89/75.69 |
| PIDLAR | 2.929 | 3.487 | 172.04 | 83.94 | 166.94 | 76.76 |
| POFLOO | 3.283 | 3.315 | 154.85 | 106.99 | 86.8/89.35 | 95.71/82.17 |
| Code    | Value1 | Value2 | Value3 | Value4 | Value5 | Value6 |
|---------|--------|--------|--------|--------|--------|--------|
| TIKCOF  | 3.108  | 3.516  | /      | /      | 138.2  | 100.92 |
| TICMAT  | 3.024  | 3.555  | /      | /      | 174.87 | 92.68  |
| TIXXEN  | 3.485  | 2.927  | /      | /      | 69.98  | 172.77 |
| TAQSEL  | 3.372  | 3.477  | /      | /      | 108.87 | 150.69 |
| TCLPHA  | 3.236  | 3.39   | /      | /      | 160.47 | 79.47  |
| TEXCOR  | 3.374  | 3.465  | /      | /      | 131.18 | 160.68 |
| TICMAT  | 3.024  | 3.555  | /      | /      | 174.87 | 92.68  |
| TIXXEN  | 3.485  | 2.927  | /      | /      | 69.98  | 172.77 |
| TAQSEL  | 3.372  | 3.477  | /      | /      | 108.87 | 150.69 |
| TCLPHA  | 3.236  | 3.39   | /      | /      | 160.47 | 79.47  |
| TEXCOR  | 3.374  | 3.465  | /      | /      | 131.18 | 160.68 |
| TICMAT  | 3.024  | 3.555  | /      | /      | 174.87 | 92.68  |
| TIXXEN  | 3.485  | 2.927  | /      | /      | 69.98  | 172.77 |
| TAQSEL  | 3.372  | 3.477  | /      | /      | 108.87 | 150.69 |
| TCLPHA  | 3.236  | 3.39   | /      | /      | 160.47 | 79.47  |
| TEXCOR  | 3.374  | 3.465  | /      | /      | 131.18 | 160.68 |
| TICMAT  | 3.024  | 3.555  | /      | /      | 174.87 | 92.68  |
| TIXXEN  | 3.485  | 2.927  | /      | /      | 69.98  | 172.77 |
| TAQSEL  | 3.372  | 3.477  | /      | /      | 108.87 | 150.69 |
| TCLPHA  | 3.236  | 3.39   | /      | /      | 160.47 | 79.47  |
| TEXCOR  | 3.374  | 3.465  | /      | /      | 131.18 | 160.68 |
| TICMAT  | 3.024  | 3.555  | /      | /      | 174.87 | 92.68  |
| TIXXEN  | 3.485  | 2.927  | /      | /      | 69.98  | 172.77 |
| TAQSEL  | 3.372  | 3.477  | /      | /      | 108.87 | 150.69 |
| TCLPHA  | 3.236  | 3.39   | /      | /      | 160.47 | 79.47  |
| TEXCOR  | 3.374  | 3.465  | /      | /      | 131.18 | 160.68 |
| TICMAT  | 3.024  | 3.555  | /      | /      | 174.87 | 92.68  |
| TIXXEN  | 3.485  | 2.927  | /      | /      | 69.98  | 172.77 |
| TAQSEL  | 3.372  | 3.477  | /      | /      | 108.87 | 150.69 |
| TCLPHA  | 3.236  | 3.39   | /      | /      | 160.47 | 79.47  |
| TEXCOR  | 3.374  | 3.465  | /      | /      | 131.18 | 160.68 |
| TICMAT  | 3.024  | 3.555  | /      | /      | 174.87 | 92.68  |
| TIXXEN  | 3.485  | 2.927  | /      | /      | 69.98  | 172.77 |
| TAQSEL  | 3.372  | 3.477  | /      | /      | 108.87 | 150.69 |
| TCLPHA  | 3.236  | 3.39   | /      | /      | 160.47 | 79.47  |
| TEXCOR  | 3.374  | 3.465  | /      | /      | 131.18 | 160.68 |
| TICMAT  | 3.024  | 3.555  | /      | /      | 174.87 | 92.68  |
| TIXXEN  | 3.485  | 2.927  | /      | /      | 69.98  | 172.77 |
| TAQSEL  | 3.372  | 3.477  | /      | /      | 108.87 | 150.69 |
| TCLPHA  | 3.236  | 3.39   | /      | /      | 160.47 | 79.47  |
| TEXCOR  | 3.374  | 3.465  | /      | /      | 131.18 | 160.68 |
| TICMAT  | 3.024  | 3.555  | /      | /      | 174.87 | 92.68  |
| TIXXEN  | 3.485  | 2.927  | /      | /      | 69.98  | 172.77 |
| TAQSEL  | 3.372  | 3.477  | /      | /      | 108.87 | 150.69 |
| TCLPHA  | 3.236  | 3.39   | /      | /      | 160.47 | 79.47  |
| TEXCOR  | 3.374  | 3.465  | /      | /      | 131.18 | 160.68 |
| TICMAT  | 3.024  | 3.555  | /      | /      | 174.87 | 92.68  |
| TIXXEN  | 3.485  | 2.927  | /      | /      | 69.98  | 172.77 |
|     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| 212 | TITQOF | 3.343 | 3.489 | / | 125.08 | 165.4 | / | 112.03 | 107.08/90.85 | / |
| 213 | TUNVIH | 3.485 | 3.228 | / | 88.91 | 107.27 | / | 81.48 | 96.85 | / |
| 214 | UGUQY | 3.392 | 3.159 | / | 95.57 | 138.15 | / | 85.3 | 168.25 | / |
| 215 | UHUCQ | 3.472 | 3.182 | / | 177.26 | 126.47 | / | 120.21 | 145.5 | / |
| 216 | UPEVER | 3.229 | 3.309 | 3.456 | 152.5 | 140.04 | 83.21 | 119.64/87.77 | 85.36 | 71.88 |
| 217 | USAMIM | 3.521 | 3.524 | / | 130.11 | 88.21 | / | 84.85 | 129.79/98.18 | / |
| 218 | USUQEF | 3.403 | 3.409 | / | 99.7 | 129.07 | / | 111.61/126.64 | 105.67 | / |
| 219 | UWAJAF | 3.08 | 3.487 | / | 166.84 | 137.11 | / | 98.58 | 79.45 | / |
| 220 | UZUPEL | 3.417 | 3.489 | / | 68.37 | 67.79 | / | 89.21 | 55.24 | / |
| 221 | VADGUOC | 3.134 | 3.108 | / | 137.71 | 158.61 | / | 142.1/98.63 | 134.8 | / |
| 222 | VAFQAW | 3.401 | 3.17 | / | 155.4 | 127.57 | / | 91.82 | 97.23 | / |
| 223 | VECTOP | 3.544 | 3.463 | / | 123.37 | 102.12 | / | 133.47 | 134.06 | / |
| 224 | VECVEH | 3.498 | 3.55 | 3.431 | 88.48 | 162.74 | 72.68 | 156.23 | 81.4 | 171.62 |
| 225 | VEXDIO | 3.539 | 3.383 | / | 71.89 | 160.25 | / | 72.5/89.16 | 136.53 | / |
| 226 | VIFGIC | 3.388 | 3.47 | / | 166.51 | 94.37 | / | 106.65 | 84.42 | / |
| 227 | VIMHOQ | 3.492 | 3.36 | / | 127.1 | 171.12 | / | 111.62 | 81.19 | / |
| 228 | VIZNUP | 3.513 | 3.406 | / | 98.68 | 82.2 | / | 116.52 | 114.96 | / |
| 229 | VOJRUJ | 3.345 | 3.347 | 3.506 | 158.07 | 99.38 | 97.17 | 111.06 | 144.29 | 147.45 |
| 230 | WAHTUW | 3.53 | 3.29 | / | 134.27 | 150.31 | / | 71.02/104.69 | 149.92/87.53 | / |
| 231 | WEDBUE | 3.468 | 3.292 | / | 117.01 | 159.43 | / | 103.32 | 112.99 | / |
| 232 | WIHTEN | 3.564 | 3.489 | / | 125.47 | 177.8 | / | 102.8/80.45 | 103.14/70.75 | / |
| 233 | WIPGAF | 3.549 | 3.386 | / | 122.53 | 150.2 | / | 121.18 | 108.95/84.7 | / |
| 234 | WODLAF | 3.405 | 3.32 | / | 74.18 | 156.93 | / | 87.05 | 89.53 | / |
| 235 | WOGXUM | 3.503 | 3.48 | / | 87.78 | 98.23 | / | 75.53 | 85.61 | / |
| 236 | XAHGUL | 3.521 | 3.564 | / | 94.22 | 66.81 | / | 144.11 | 118.72/84.6 | / |
| 237 | XAPGUS | 3.36 | 3.462 | / | 79.46 | 144.08 | / | 145.37 | 90.48 | / |
| 238 | XAQGED | 3.37 | 3.143 | / | 140.16 | 151.14 | / | 76.1 | 125.12 | / |
| 239 | XETNES | 3.504 | 3.226 | 3.441 | 85.21 | 173.7 | 108.26 | 164.7 | 91.61/84.94 | 87.21/79.2 |
| 240 | XILDUT | 3.469 | 3.474 | 3.487 | 68.5 | 77.17 | 65.43 | 124.02/58.05 | 98.97/95.82 | 57.82/118.8 |
| 241 | XILFAB | 3.493 | 3.317 | / | 91.37 | 68.74 | / | 109.18/81.25 | 116.45/65.54 | / |
| 242 | XILYAT | 3.539 | 3.522 | / | 109.64 | 97.79 | / | 89.66 | 130.0/84.97 | / |
| 243 | XIMMUC | 2.864 | 3.437 | / | 171.58 | 88.17 | / | 125.67 | 93.32 | / |
| 244 | XOMIJUE | 3.141 | 3.403 | / | 165.2 | 83.04 | / | 132.29 | 101.01 | / |
| 245 | YASMOX | 3.465 | 3.477 | / | 154.83 | 124.58 | / | 97.66 | 127.03 | / |
| 246 | YASMOX | 3.264 | 3.293 | / | 165.05 | 133.27 | / | 94.87 | 106.57 | / |
| 247 | YEGVAI | 3.336 | 3.522 | / | 154.29 | 90.54 | / | 119.1 | 160.02 | / |
Supplementary Figure 19: Statistics on geometrical properties of bifurcated Cl···O halogen bond based on sum of van der Waals radii + 0.3 Å. a Distribution plot of bifurcated Cl···O halogen distances. b Distribution plot of $\Theta_1$ [C-Cl···O] angles. c Distribution plot of $\Theta_2$ [Cl···O-R] angles. d Scatter plots (angle ($\Theta_i$) versus distance) obtained from the halogen bond CSD search. Distance between halogen atom and first oxygen, and associated $\Theta_1$, $\Theta_2$ angles are represented as blue bars, while distance between halogen atom and second oxygen, and associated $\Theta_1$, $\Theta_2$ angles are represented as red bars. In some cases halogen forms bonds with three oxygens and their distance and associated $\Theta_1$, $\Theta_2$ angles are represented as green bars.
Supplementary Table 11. Summary of hits from CSD search, forming bifurcated Br⋯O bonds at the distance lower than the sum of van der Waals radii.

| No | Crystal structure identifier | d₁ [Å] | d₂ [Å] | Θ₁° [C-X⋯O] | Θ₁° [C-X⋯O] | Θ₂° [X⋯O-R] | Θ₂° [X⋯O-R] |
|----|-----------------------------|--------|--------|--------------|--------------|-------------|-------------|
| 1  | FOTFIF                      | 3.32   | 3.332  | 165.24       | 156.01       | 99.15       | 98.61       |
| 2  | HAMXAW                      | 3.227  | 3.341  | 164.06       | 142.97       | 122.94/101.95 | 126.33     |
| 3  | HOKBAN                      | 3.352  | 2.965  | 80.11        | 172.06       | 132.75      | 144.25      |
| 4  | JARHUG                      | 3.302  | 3.292  | 132.47       | 163.19       | 91.11/95.44 | 88.84       |
| 5  | JEMTIF                      | 3.271  | 3.37   | 138.42       | 134.8        | 127.87      | 112.12      |
| 6  | JIWCUO                      | 3.308  | 3.268  | 149.21       | 152.4        | 96.11/123.01 | 144.17     |
| 7  | MIJDOA                      | 3.145  | 3.369  | 150.35       | 158.04       | 128.72/119.93 | 119.85     |
| 8  | VESROC                      | 3.229  | 3.124  | 126.83       | 165.25       | 140.38      | 143.22      |
| 9  | XAKQOQ                      | 3.33   | 3.368  | 147.07       | 147.58       | 132.93/111.33 | 109.77/141.64 |
| 10 | FOTFIF                      | 3.32   | 3.332  | 165.24       | 156.01       | 99.15       | 98.61       |
Supplementary Figure 20: Statistics on geometrical properties of bifurcated Br···O halogen bond based on sum of van der Waals radii. 

**a** Distribution plot of bifurcated Br···O halogen distances. 

**b** Distribution plot of $\Theta_1$ [C-Br···O] angles. 

**c** Distribution plot of $\Theta_2$ [Br···O-R] angles. 

**d** Scatter plots (angle ($\Theta_1$) versus distance) obtained from the halogen bond CSD search. Distance between halogen atom and first oxygen, and associated $\Theta_1$, $\Theta_2$ angles are represented as blue bars, while distance between halogen atom and second oxygen, and associated $\Theta_1$, $\Theta_2$ angles are represented as red bars.
Supplementary Table 12. Summary of hits from CSD search, forming bifurcated I···O bonds at the distance lower than the sum of van der Waals radii.

| No | Crystal structure identifier | $d_1$ [Å]$^\dagger$ | $d_2$ [Å]$^\ddagger$ | $\Theta_1^\circ$ [C-X···O]$^\dagger$ | $\Theta_1^\circ$ [C-X···O]$^\ddagger$ | $\Theta_2^\circ$ [X···O-R]$^\dagger$ | $\Theta_2^\circ$ [X···O-R]$^\ddagger$ |
|----|-----------------------------|----------------------|----------------------|--------------------------------|--------------------------------|--------------------------------|--------------------------------|
| 1  | NORQOD                      | 3.443                | 2.886                | 146.05                          | 172.57                          | 79.47                          | 105.42                          |
| 2  | AGAHAT                      | 3.027                | 3.311                | 155.39                          | 148.89                          | 147.73                          | 142.85                          |
| 3  | COZGII                      | 3.473                | 3.134                | 154.32                          | 168.29                          | 90.74                          | 107.76                          |
| 4  | EXIFEX                      | 3.214                | 3.214                | 155.14                          | 155.14                          | 126.11                          | 126.11                          |
| 5  | EYUPUL01                    | 3.456                | 3.437                | 140.36                          | 141.57                          | 147.09                          | 147.09                          |
| 6  | FOTDUP                      | 3.308                | 3.457                | 168.77                          | 153.67                          | 104.08                          | 97.33                           |
| 7  | IWEVUC                      | 3.107                | 3.371                | 159.55                          | 160.42                          | 135.23/103.82                   | 91.21                           |
| 8  | KIJQUR                      | 3.138                | 3.138                | 127.71                          | 127.71                          | 130.22                          | 130.22                          |
| 9  | NERXUE                      | 3.414                | 3.448                | 142.76                          | 154.14                          | 124.58                          | 121.35                          |
| 10 | QOGFUO                      | 3.482                | 3.387                | 142.8                           | 162.57                          | 141.24/94.1                     | 93.89/104.97                    |
Supplementary Figure 21: Statistics on geometrical properties of bifurcated I⋯O halogen bond based on sum of van der Waals radii. a Distribution plot of bifurcated I⋯O halogen distances. b Distribution plot of $\Theta_1$ [C-I⋯O] angles. c Distribution plot of $\Theta_2$ [I⋯O-R] angles. d Scatter plots (angle ($\Theta_1$) versus distance) obtained from the halogen bond CSD search. Distance between halogen atom and first oxygen, and associated $\Theta_1$, $\Theta_2$ angles are represented as blue bars, while distance between halogen atom and second oxygen, and associated $\Theta_1$, $\Theta_2$ angles are represented as red bars.
**Supplementary Figure 22:** Uncropped gel of the Figure 3 in the main text (supplied also as the supplementary file Dataset 2).
**Supplementary Figure 23**: Uncropped gel of the Figure 3 in the main text (no enzyme/enzyme – control, supplied also as the supplementary file Dataset 3).
**Supplementary Figure 24:** Uncropped gel of the Supplementary Figure 16 (supplied also as the supplementary file Dataset 4).
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