Principles and Applications of CF₂X Moieties as Unconventional Halogen Bond Donors in Medicinal Chemistry, Chemical Biology, and Drug Discovery

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ABSTRACT: As an orthogonal principle to the established (hetero)aryl halides, we herein highlight the usefulness of CF₂X (X = Cl, Br, or I) moieties. Using tool compounds bearing CF₂X moieties, we study their chemical/metabolic stability and their logP/solubility, as well as the role of XB in their small molecular crystal structures. Employing QM techniques, we analyze the observed interactions, provide insights into the conformational flexibilities and preferences in the potential interaction space. For their application in molecular design, we characterize their XB donor capacities and its interaction strength dependent on geometric parameters. Implementation of CF₂X acetamides into our HEFLibs and biophysical evaluation (STD-NMR/ITC), followed by X-ray analysis, reveals a highly interesting binding mode for fragment 23 in JNK3, featuring an XB of CF₂Br toward the P-loop, as well as chalcogen bonds. We suggest that underexplored chemical space combined with unconventional binding modes provides excellent opportunities for patentable chemotypes for therapeutic intervention.

SIGNIFICANCE
Starting from asciminib, we thoroughly characterize the great potential of CF₂X moieties as strong halogen bond donors with a multitude of experimental and computational methods. While traditionally halogens in drug-like molecules are only attached to (hetero)aryl systems, these new moieties facilitate the escape from planarity into the third dimension. Hence, they provide completely new opportunities to harness the highly directional XB interactions in drug design and discovery.

INTRODUCTION
Halogen bonds (XB) are highly directional interactions between the electropositive region on the halogen (X = Cl, Br, I), the σ-hole, and a nucleophilic interaction partner (B), typically an atom containing one or more nonbonding electron pairs, a σ-system, or an anion.¹ Aryl halides with an sp²-hybridized carbon—halogen (C(sp²)−X) moiety or ethynyl halide species (C(sp)−X) form halogen bonds, which are well studied in organocatalysis,²–⁵ crystal engineering,⁶–⁸ biological systems,⁹–¹¹ and medicinal chemistry.¹²–¹⁴

In comparison, the use of alkyl halides with an sp³-hybridized carbon—halogen (C(sp³)−X) moiety is often limited by their reactivity, when X can serve as a leaving group for substitution, elimination, and radical reactions. It is also typically perceived that the smaller the s-part of the hybridization, the weaker will be the potential strength of the XB.¹⁵ As a consequence, strong electron-withdrawing groups (EWGs) are required as XB-tuning substituents, modulating the electron density at the halogen atom and, thus, improving the XB strength. Ideal EWGs will not only enhance the formation ofXBs but also substantially diminish the reactivity as a prerequisite to utilize this chemistry in different applications. From a design perspective, stabilized C(sp³)-X moieties help to overcome the limitations of in-plane XBs formed by (hetero)aryl halides and facilitate an orthogonal spatial behavior.

One embodiment of such an ideal EWG is fluorine. Halodifluoromethyl (C(sp³)F₂-X) groups are considered to be much less reactive than (C(sp³)H₂-X) groups based on the strong electron-withdrawing properties of both fluorine atoms and their shielding of the carbon atom. In addition, fluorine...
atoms are small in size and weight, while being able to engage in their own intermolecular contacts such as orthogonal multipolar interactions.

Numerous synthetic methods have been described in the literature as these CF$_2$X groups are suitable intermediates for the preparation of trifluoromethyl (CF$_3$),$^{17-19}$ difluoromethyl (CF$_2$H),$^{16}$ and difluoromethylene (CF$_2$)$^{18-21}$ building blocks or for $^{18}$F-labeling of trifluoromethylated compounds.$^{22-26}$

$\alpha_{\text{H}}$-Dihalo-perfluorinated alkanes or $\alpha$-halo-perfluorinated alkanes have been widely used as XB tool compounds for studies regarding anion recognition,$^{27-31}$ catalysis and synthesis,$^{32-35}$ crystal engineering,$^{36-39}$ host-guest complexes,$^{40-49}$ materials,$^{50-52}$ probes for molecular recognition,$^{53-57}$ self-assembly,$^{58-67}$ and supramolecular architecture.$^{68-71}$

Still, their use to target biological systems in medicinal chemistry, chemical biology, and drug discovery has been rather unexplored. Some halodifluoroacetamide (−NHCO−CF$_2$X) containing agents have been reported with respect to their antifungal activity,$^{72}$ as well as compounds targeting the calcium-activated chloride channel Transmembrane Protein 16A (TMEM16A).$^{73}$ We recently reported halodifluoroacetamide-containing peptides that bind to E3 ubiquitin-protein ligase Mdm2 and Mdm4, which are negative regulators of the p53 tumor suppressor.$^{74}$ The epilepsy drug candidate (phase III, 2019) padsevonil with 2-chloro-2,2-difluoroethyl (−CH$_2$CF$_2$Cl) moiety has selective affinity for both presynaptic synaptic vesicle 2 (SV2) proteins and postsynaptic central benzodiazepine receptor (CBZR) sites on the $\gamma$-aminobutyric acid (GABA$_\text{A}$) receptor.$^{75}$ Inhibitors of Vascular Endothelial Growth Factor (VEGF) receptors with CF$_2$Cl ether (−O−CF$_2$Cl) group have been published,$^{76-78}$ as well as ascinimib, an FDA-approved (2021) allosteric inhibitor of ABL1 kinase, targeting the fusion protein BRC-ABL in chronic myeloid leukemia (CML).$^{79,80}$ Except for the example of ascinimib, so far, no structural evidence for a CF$_2$X····B interaction is present in the PDB. Interestingly, the existence of this particular halogen bond was not even highlighted in the corresponding publication.$^{81}$

To the best of our knowledge, CF$_2$X groups are virtually absent from systematic methods for the exploration of therapeutics beyond these examples. We previously developed our Halogen-Enriched Fragment Library (HEFLib), which consists of about 200 halogenated fragments, to explore the potential of XB in fragment-based approaches.$^{82-85}$ So far, this library contains exclusively chlorine, bromine, or iodine bound to (hetero)aromatic systems. As a tool for a more systematic exploration of protein binding environments suitable for recognizing CF$_2$X moieties, we have started to integrate CF$_2$X-bearing fragments in our HEFLibs approach, as outlined herein.

■ RESULTS AND DISCUSSION

Study Design. The protein crystal structure of ascinimib with ABL1 kinase mutant T334I D382N (PDB ID: 5MO4)$^{86}$ solved by Wylie et al.,$^{87}$ revealed an XB interaction between the chlorine atom of the CF$_2$Cl ether group of ascinimib and the backbone carbonyl oxygen of L448 with an almost ideal C(sp$^3$)····Cl−O angle of 178.3° and a Cl····O distance of 3.3 Å in the allosteric myristate binding pocket (Figure 1). Based on this structure, we calculated the XB complex formation energies of ascinimib and other halogen-substituted derivatives of ascinimib at the MP2/TZVPP level of theory, as shown in Table 1. The structure of N-methylacetamide, as a shortened XB acceptor in the model system, was derived from the peptide bond of L448 in the protein crystal structure of the ABL1 kinase. All atoms of the CF$_2$X moiety, as well as all hydrogen atoms, were optimized for 13a−e, while the scaffold of ascinimib and the N-methylacetamide was fixed. The complex formation energy of ascinimib (13c) was −11.2 kJ mol$^{-1}$ (3.3 Å; 178.3°) by using the original coordinates of the CF$_2$Cl moiety. For reasons of comparability, the CF$_2$Cl group was relaxed like the modified CF$_2$X systems, yielding a very similar complex formation energy of −10.4 kJ mol$^{-1}$ at the same distance of 3.3 Å but with a slightly decreased σ-hole angle of 172.0°. The halogen is almost in plane with the peptide bond (dihedral angle $\sigma_{\text{Cl−O−C−Cl}}$ = 32.0°) at a bond angle $\alpha_{\text{C−O−Cl}}$ of 124.7°, which represents a reasonably good
interaction geometry (see the sphere in Figure 1). The best interaction geometries (about 15 kJ mol\(^{-1}\)) can only be formed perpendicular to the plane of the peptide bond. However, the hydrophobic residues A452, V487, and M491 prevent the formation of such an even more favorable interaction geometry.

The novel spatial interaction possibilities of CF\(_2\) with X = H, F, Br, I with N-Methylacetamide, Derived from the Peptide Bond of L448 in the Protein Crystal Structure of ABL1 (T3341 D382N) kinase (PDB ID: SMO4)

<table>
<thead>
<tr>
<th>Compound</th>
<th>X/H</th>
<th>(\Delta E (kJ \text{ mol}^{-1}))</th>
<th>X–O (Å)</th>
<th>C–X–O (deg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>13a</td>
<td>H</td>
<td>MP2/TZVPP</td>
<td>MP2/QZVPP</td>
<td>M06-2X-D3/TZVPP</td>
</tr>
<tr>
<td>13b</td>
<td>F</td>
<td>−2.7</td>
<td>−2.2</td>
<td>−2.7</td>
</tr>
<tr>
<td>Asciminib (SMO4)(^a)</td>
<td>Cl</td>
<td>−11.2</td>
<td>−11.2</td>
<td>−10.5</td>
</tr>
<tr>
<td>Asciminib (13c)</td>
<td>Cl</td>
<td>−10.4</td>
<td>−10.4</td>
<td>−9.7</td>
</tr>
<tr>
<td>13d</td>
<td>Br</td>
<td>−15.4</td>
<td>−15.3</td>
<td>−14.9</td>
</tr>
<tr>
<td>13e</td>
<td>I</td>
<td>−24.0</td>
<td>−24.9</td>
<td>−24.4</td>
</tr>
</tbody>
</table>

\(^a\)The CF\(_2\)Cl moiety of asciminib was not optimized in this reference calculation. \(^b\)Single point calculation based on MP2/TZVPP geometry.

Due to the smaller size of the hydrogen atom and the reduced single electron density, both Ph–Y–CF\(_2\)X units (X = H, F, Cl, Br, I) show less favorable interactions than any of the halogen bonds. Comparison with larger basis sets (MP2/QZVPP) or DFT-methods recommendable for halogen bonding (M06-2X-D3/TZVPP) was performed and is illustrated in Table 1. It shows rather good consistency of the reported complex formation energies.

We have shown that all of these compounds are synthetically accessible (for more information, see Supporting Information). The backbone oxygen of L448 can be addressed by C(sp\(^3\))–F, C(sp\(^3\))–X ethers with significant XB contributions, while it cannot be targeted by aromatic C(sp\(^3\))–X based on the asciminib scaffold. This finding strengthened our interest in investigating CF\(_2\)X groups with respect to the strength of their fluorine-tuned XB interactions and their unconventional interaction geometries.

Besides halodifluoromethoxy moieties (–O–CF\(_2\)X), we have also extended our studies of model systems to halodifluoroacetamide moieties (–NHCO–CF\(_2\)X). In contrast to aryl halides, both Ph–Y–CF\(_2\)X patterns (Y = O, NHCO) have two axes of rotation and consequently two dihedral angles (\(\phi\) and \(\psi\)), as illustrated in Figure 2. However, the amide is an easily synthetically accessible linker, providing an elongated distance between the CF\(_2\)X function and the attached scaffold. Based on the resonance effects of the amide bond, this linker behaves rigidly, not increasing the conformational degrees of freedom, while providing complementary, asymmetric capabilities for molecular interactions, e.g., HB donors or acceptors. The novel spatial interaction possibilities of CF\(_2\)X-bearing ether and amide molecules raise the question of their individual conformational isomerism and dynamics, which we investigated using in silico conformational analysis and comparative data analysis of small molecule crystal structures.

Using computational and experimental methods, we evaluate further properties of CF\(_2\)X moieties, related to their applicability in medicinal chemistry, such as stability, solubility, electrostatic properties (\(V_{\text{max}}\)), and their impact on XB strength. In the second pivotal part of this study, we implemented CF\(_2\)X-containing acetamides into a halogen-enriched fragment library (HEFLib), which we assessed by biophysical screening methods against c-Jun N-terminal kinases 1 and 3 (JNK1 and JNK3).

For our experimental studies using small molecule crystalization and conformational analysis, we have designed two series of ether- and amide-containing molecules (Figure 3) each with CF\(_2\)X units (X = H, F, Cl, Br, I), of which the hydrogen derivatives are enabled to form HBs and the chlorino, bromo, and iodine derivatives are enabled to form XBs. In the case of CF\(_2\)H, the fluorine atoms significantly increase the polarization of the C–H bond, leading to improved HBs. \(^b\) Herein, we do not focus on HBs, but we will use it for the purpose of comparing it to XBs. It was important to choose molecular patterns where all desired derivatives with X = H, F, Cl, Br, or I are synthetically accessible, solid compounds and capable of forming crystals. As shown in Figure 3, we synthesized CF\(_2\)H ether fragments (6a–e) derived from the structure of the BRC-ABL inhibitor asciminib. Depending on the starting compound, we obtained our desired urea derivatives in up to five synthesis steps. To obtain a similar series of acetamides (7a–e), we acetylated benzo[d][1,3]dioxol-5-amine with the appropriate reagents in one-step syntheses. Apart from CF\(_2\)X, both chemical patterns have comparable structural elements in different arrangements: a central benzene ring, at least one phenolic ether, and an
amidine substructure. None of the chemical functions is considered particularly reactive.

**Chemistry.** All relevant synthesis procedures are described in the Supporting Information. The syntheses of urea derivatives 6a–e also include the preparation of halogenated 4-methoxyanilines 5a–e, which are required for the synthesis of asciminib derivatives 13a–e. Synthesis methods of CF<sub>2</sub>Cl<sup>94,96</sup> and CF<sub>2</sub>Br<sup>25</sup> ethers were described in the literature. For CF<sub>2</sub>Br ether and its precursor syntheses, we adapted the methods of Khotavivattana et al. and Zhou et al.<sup>25,97</sup> Regarding CF<sub>2</sub>I ether, a new synthesis method had to be established, as described below. To our knowledge, only one synthesis method to obtain CF<sub>2</sub>I ether has been reported in the literature, but this method was not practical for our applications. Guo et al. described the reaction of nucleophiles such as alcohols with difluorodiodomethane (CF<sub>2</sub>I<sub>2</sub>) in DMF at room temperature to their corresponding carbamates as major products and the formation of CF<sub>2</sub>I ether moieties as a minor product with low yields of 0–15%.<sup>96</sup> Moreover, CF<sub>2</sub>I<sub>2</sub> is a relatively expensive reagent. For our iodination reaction, the commercially unavailable iodotrichloromethane (ICCl<sub>3</sub>) analogous to the bromination procedure of Khotavivattana et al. using BrCCl<sub>3</sub> was not an option. Instead, two equiv of iodoform (CHI<sub>3</sub>) in toluene were successfully established as an iodine source for CF<sub>2</sub>I ether synthesis with a yield of 27% for 4e. The benzo[d][1,3]dioxoles 7a–e were numbered lower than the asciminib derivatives due to their experimental affiliation with 6a–e and were prepared by using amide coupling methods. The preparation and two general procedures yielding the final test fragments (14–16, 19–22, 25, and 28–34) for our biophysical assessments by STD NMR and ITC are also described in the Supporting Information.

**Chemical Stability.** Since the chemical and metabolic stability of a ligand is of particular importance in drug discovery, we performed an initial evaluation of these unconventional CF<sub>2</sub>X-bearing moieties using a glutathione (GSH) and microsomal stability assay (MSA) under aqueous conditions. A graphical representation of the results of these assays can be found in the Supporting Information (Figure S1). As summarized in Table 2, the stabilities in the GSH assay vary considerably. With the exception of 6d,e, the half-life <i>t</i><sub>1/2</sub> of all compounds amounts to several days. The half-lives <i>t</i><sub>1/2</sub> derived from curve fits of the obtained data of 6d and 6e are 5.3 and 7.2 h, respectively. The resulting <i>t</i><sub>1/2</sub> values for amides 7b–e range from 130 to 290 h, while all other compounds (6a–c and 7a) are even more stable, exceeding 1200 h. In the MSA, amides 7a–e showed a metabolite with <i>m/z</i> = 138.1 in small amounts, corresponding to amide cleavage and a loss of the respective COCF<sub>2</sub>X group. Ethers 6a–c were stable in the MSA, whereas ethers 6d,e lost their CF<sub>2</sub>X group, which was accompanied by an increase in the corresponding phenolic metabolite (<i>m/z</i> 153.1). In particular, HB donors 6a and 7a as well as XB donors 6c and 7c–e are characterized by GSH half-lives of at least 10 days.

**LogP and Solubility.** As an indicator for the hydrophilicity/lipophilicity of a compound, the logP was calculated using Schrödinger QikProp (see Table 3).<sup>99,100</sup> This property is a key characteristic of drug molecules, affecting their absorption, distribution, metabolism, and excretion.<sup>101</sup> Although it can be an indicator of solubility, it is not the only criterion determining solubility.

For our compounds, the logP values of 6c–e range from 1.301 to 1.471, with the respective value increasing from Cl < Br < I. The logP values of 7c–e range from 2.169 to 2.315 following a similar trend for the different halogens than before. We virtually generated all different combinations of the benzo[d][1,3]dioxole scaffold (substituted in position 5) in the upper part of Table 3 or the phenylurea scaffold (substituted in position 4) in the lower part of Table 3 with the substituents being hydrogen or any of the halogens (F, Cl, Br, I) either directly attached to the scaffold (as a representation of the arylhalides) or attached to 2,2-difluoro-acetamide or difluoroacetonitrile as a linker. Independent of whether hydrogen or any halogen is attached, the amides always gave lower logP values by 1.18 ± 0.08 for the benzo[d][1,3]dioxoles and by 0.80 ± 0.03 for the phenylurea.

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**Table 2. GSH Stability Assay of Compounds 6a–e and 7a–e Measured at Concentrations of 250 μM with 5 mM GSH and 100 μM Internal Standard at 37 °C in PBS (pH 7.4) Containing 10% (v/v) ACN**

<table>
<thead>
<tr>
<th></th>
<th>ether 6a–e</th>
<th>amide 7a–e</th>
</tr>
</thead>
<tbody>
<tr>
<td>X</td>
<td>&lt;i&gt;t&lt;/i&gt;&lt;sub&gt;1/2&lt;/sub&gt; (h)</td>
<td>&lt;i&gt;t&lt;/i&gt;&lt;sub&gt;1/2&lt;/sub&gt; (h)</td>
</tr>
<tr>
<td>H</td>
<td>a</td>
<td>&gt;1200</td>
</tr>
<tr>
<td>F</td>
<td>b</td>
<td>&gt;1200</td>
</tr>
<tr>
<td>Cl</td>
<td>c</td>
<td>&gt;1200</td>
</tr>
<tr>
<td>Br</td>
<td>d</td>
<td>5.3</td>
</tr>
<tr>
<td>I</td>
<td>e</td>
<td>7.2</td>
</tr>
</tbody>
</table>

*Each measurement was performed in triplicate.*
derivatives. Based on the higher polarity of the amide linker and its capability to donate and accept hydrogen bonds, this systematic difference is unsurprising.

We also compared the arylhalides with each linker and found that the amides very often closely match the logP of the respective arylhalides (ΔlogP ≈ 0.08 ± 0.26) with 1-phenylurea (ΔlogP ≈ 0.64) and 1-(4-fluorophenyl)urea (ΔlogP ≈ 0.43) being outliers. The ether linker was always profoundly more lipophilic than the simple respective arylhalide. For the phenylurea scaffold the difference was ΔlogP ≈ 0.55 ± 0.27, for the benzo[ d ] [ 1,3 ]dioxole scaffold, the difference was even larger with ΔlogP ≈ 1.26 ± 0.05.

For the amides, the change from hydrogen to fluorine increased the logP by 0.24 ± 0.01, for the ether linker, by 0.15 ± 0.04. Halogen exchange from fluorine to chlorine produced a stronger increase (0.29 ± 0.01 for the amides and 0.33 ± 0.06 for the ether linkers) than exchange of chlorine to bromine (0.09 ± 0.04) or bromine to iodine (0.09 ± 0.02).

In summary, the lipophilicity of the compounds increases slightly from CF₃H to CF₃ to CF₂Cl to CF₂Br to CF₂I. It should be noted that the statistical fit of the

Table 3. LogP Values for Model Compounds, Including 6a−e and 7a−e, Calculated Using Schrödinger QikProp

<table>
<thead>
<tr>
<th></th>
<th>amide (7a-e)</th>
<th>ether</th>
<th>R₂ = I</th>
</tr>
</thead>
<tbody>
<tr>
<td>R₁ = H</td>
<td>1.631</td>
<td>1.500</td>
<td>2.315</td>
</tr>
<tr>
<td>R₁ = F</td>
<td>1.878</td>
<td>1.652</td>
<td>2.368</td>
</tr>
<tr>
<td>R₁ = Cl</td>
<td>2.169</td>
<td>2.107</td>
<td>3.027</td>
</tr>
<tr>
<td>R₁ = Br</td>
<td>2.239</td>
<td>2.263</td>
<td>3.582</td>
</tr>
<tr>
<td>R₁ = I</td>
<td>2.315</td>
<td>2.330</td>
<td>3.582</td>
</tr>
</tbody>
</table>

Figure 4. ESP isosurface depictions of model compounds (2,2-difluoro-2-halo-methoxybenzenes = “ether” or 2,2-difluoro-2-halo-N-phenylacetamides = “amide”) bearing different functional moieties (CF₃, CF₂Cl, CF₂Br, and CF₂I) calculated at the MP2/TZVPP level of theory. Negative ESP isosurfaces at an energy of −0.01 au are colored in dark blue and at an energy of −0.005 au in cyan. Positive ESP isosurfaces at +0.01 au are colored in red and at an energy of +0.005 au in orange. The isosurfaces at 0.000 au, indicating the boundaries for the transition between negative and positive ESPs, are shown as gray surfaces. Increasing positive potentials representing the σ-hole on the halogen opposite the C–X bond are visible from CF₃ to CF₂Cl to CF₂Br to CF₂I. They are highlighted by increasing green arrows, while red crosses mark the position where CF₃ lacks a similar σ-hole. Structures are shown as balls and sticks. All pictures were prepared with MOLCAD.²⁰,₁⁰³

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logP(octanol/water) model in QikProp was reported to have a coefficient of determination \( r^2 = 0.93 \) and an RMSD = 0.50. Thus, there are certain statistical limitations of the model, and the interpretation of smaller effects discussed before should be taken with caution.

In addition, it appears reasonable that the model cannot properly recognize the anisotropic nature of the electron distribution around chlorine, bromine, and iodine, as illustrated by the ESP plots in Figure 4. (Alternative mapping of positive and negative ESP onto the 0.001 au contour of the electronic density can be found in Figure S22 in the Supporting Information.) The size of the positive potential (orange/red surfaces, oriented toward above the aromatic ring in the ether model structures and oriented toward the right side away from the plane in the amide model structures) increases from \( \text{CF}_2\text{Cl} \) to \( \text{CF}_2\text{Br} \) to \( \text{CF}_2\text{I} \), while it is not visible in \( \text{CF}_6 \). This positive potential (highlighted by green arrows) is the “electrostatic embodiment” of the \( \sigma \)-hole, piercing though the negative electrostatic potential typically found equatorially around the halogen, chlorine, bromine, and iodine. The electron withdrawing effects of the geminal fluorine atoms foster the size and range of the positive potentials. From this electrostatic visualization it is difficult to estimate, how strong the influence of these electrostatic features will be on logP and solubility, however, it is plausible that they could favor iodine over bromine over chlorine over fluorine.

To investigate this more deeply, we measured the kinetic solubility of \( 6a-e \) and \( 7a-e \) with a turbidimetric assay in the concentration range of 0.43–5 mM in a buffer (50 mM HEPES, 100 mM NaCl, pH 7.4) containing 5% (v/v) DMSO to mimic typical assay conditions. The occurrence of precipitates was monitored in a time-dependent way over approximately 1 h by measuring light scattering between 600 and 800 nm in a 96 well plate, freshly shaken before each measurement cycle. The concentration-dependence absorption spectra for all compounds, measured at the beginning (3 min), an intermediate cycle (25 min), and at the end (53 min), can be found in the Supporting Information. The highest concentration up to which no scattering is observed is reported as “minimal instant solubility” (MIS) based on the first measurement cycle and as “minimal final solubility” (MFS) based on the last measurement cycle. These results were compared to logS predictions also done by QikProp.

For the phenylurea scaffold bearing an ether linker, MIS and MFS were identical for \( 6a, 6d, \) and \( 6c \) with values of 5, 5, and 4 mM, respectively. \( \text{CF}_3 \)-bearing \( 6b \) had an MIS of 3.2 mM and an MFS of 2.6 mM, while the \( \text{CF}_3\text{I} \)-bearing \( 6e \) had also an MIS of 3.2 mM, but an MFS of 2 mM. For \( 6a \) (\( \text{CF}_2\text{H} \)) and \( 6d \) (\( \text{CF}_2\text{Br} \)) no evidence of precipitation was found at the highest concentration tested. Thus, the actual solubility could be even significantly higher. Predictions by QikProp suggest solubilities of about 46 mM for \( 6a, 28 \) mM for \( 6b, 10 \) mM for \( 6c \) and \( 6d, \) and 8 mM for \( 6e \). Although the prediction is rather close to the measurement, the more limited solubility of \( \text{CF}_3 \) (\( 6b \)) and the rather good solubility of the bromine derivative (\( 6d \)) are somewhat surprising.

For the benzo[\( d \)][1,3]dioxole scaffold (\( 7a-e \)), for all compounds except \( 7e \) (\( \text{CF}_3\text{I} \)) an MIS value of 5 mM was determined. The same MIS of 5 mM was found for \( 7a, 7c, \) and \( 7d \). For \( \text{CF}_3 \) derivative \( 7b \), the MIS decreased to 4 mM. The \( \text{CF}_3\text{I} \) derivative \( 7e \) had a slightly more limited solubility of 3.2 mM (MIS) and 2 mM (MFS). QikProp predicted always a clearly lower solubility than that for the ether compounds (\( 6a-e \)). \( 7a \) was suggested to have a 7 mM solubility. As the determined concentration of 5 mM was the maximal concentration available, the real solubility could be actually 7 mM or higher. For \( 7b \), a slightly reduced solubility of 4 mM was predicted and confirmed by the experimental assay. While the solubility for \( 7c-e \) was predicted to be approximately the same (1.5 to 1.9 mM), the reduced solubility for chlorine (\( 7c \)) and bromine (\( 7d \)) cannot be confirmed by the experimental values (5 mM or higher). For the iodinated compound \( 7e \) an MIS of 3.2 mM and an MFS of 2 mM were found; thus, the prediction of 1.6 mM is quite good. Based on the published statistical parameters of the QikProp logS model (\( r^2 = 0.91 \) and RMSE = 0.63 log units), the prediction is reasonably well in line with the experiment. Still, it cannot be used to differentiate small but experimentally important differences. As a more general trend from the turbidimetric assay, we find slightly reduced solubility of the \( \text{CF}_3\text{I} \)-bearing compounds \( 6e \) and \( 7e \). The results for the \( \text{CF}_3 \) groups \( (6b/7b) \) were more heterogeneous. Overall, the tool compounds exhibited solubilities that still allow the usage of typical biophysical fragment-screening techniques. As a consequence, we provide QikProp logP(o/w) and logS predictions, as well as MIS and MFS values determined by experiment for all fragment-sized...
Figure 6. Overview of XB interactions between two single molecules. (a) Crystal structure of 6d (Br1···(C7=O1), 3.32 Å). (b) Crystal structure of 6e (I1···(C7=O1), 3.35 Å). (c) Crystal structure of 7c (molecule A, Cl1···O1, 3.01 Å). (d) Crystal structure of 7d (molecule A, Br1···O1, 3.01 Å). (e) Crystal structure of 7e (I1···(C1–6), 3.80 Å); view along the b axis.

Distances can be found at 3.524 and 4.281 Å, with σ-hole angles of 145.5° and 140.6°, respectively. 6d forms an asymmetric triclinic crystal system with space group P-1 and 6e constitutes a monoclinic space group P2₁/n. While in the crystals of 6a–c the polar urea and nonpolar halogenated methoxy groups have no spatial contact with each other, the lattice layers of 6d,e shift toward each other in an effort to form an XB, preserving the displaced π-stacking (Figure S12c,d). Thus, the bromine and iodine atoms are involved in XB interactions, in which the electron-rich π cloud of the urea carbonyl C=O bond interacts as an XB acceptor (Figure 6a,b: Br1···(C7=O1), 3.32 Å, 175.4°; I1···(C7=O1), 3.35 Å, 176.9°). Besides the modification change caused by XB, the differences in self-assembly between 6d and 6e can be explained by the different magnitudes of the van der Waals radii and C–X distances (C–Br, 1.95 Å; C–I, 2.15 Å). For 6d,e, we calculated the complex formation energies of the XB interactions between two single molecules of the crystals and performed distance scans by varying the distance along the C8–X1 axes (MP2/TZVPP), as shown in Figure 7. Previously we rotated the XB donor molecule of 6e 180° along its C8–I1 axis to reduce energy contributions of other molecular interactions. The XB of 6d contributes a −14.6 kJ mol⁻¹ value, and 6e gives a −22.8 kJ mol⁻¹ value (Table 4). Both deviate only slightly from the calculated energy minimum. It is important to note that we perform these calculations to support our analysis and interpretation of the interactions found in the crystal structures but not for purposes of systematically comparing the interaction strength of the CF₂X moieties.

Common characteristics of crystals 7a–e are parallel-displaced π–π stacking and HB between amides (O3···H1N, ~2.0 Å; Figures S7–S11 and S13). Various weaker CH side contacts on the halogens are additionally present. No solvent molecules are integrated in the crystal lattices. As shown in
with the triclinic space group P-1 and monoclinic Table S4.

Table 4. XB Distances X•••B, o-Hole Angles C•••X•••B, and Calculated Complex Formation Energies ΔE of 6d,e, 7c−e, and Asciminib (13c) (PDB ID: 5MO4)

<table>
<thead>
<tr>
<th>XB</th>
<th>XB acceptor</th>
<th>X•••B (Å)</th>
<th>C•••X•••B (°)</th>
<th>ΔE (kJ mol⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6d</td>
<td>Br1</td>
<td>(C7=O1)</td>
<td>3.321</td>
<td>175.4</td>
</tr>
<tr>
<td>6e</td>
<td>Cl</td>
<td>(C7=O1)</td>
<td>3.348</td>
<td>176.9</td>
</tr>
<tr>
<td>Asciminib</td>
<td>Cl</td>
<td>O(L448)</td>
<td>3.27</td>
<td>178.3</td>
</tr>
<tr>
<td>7c</td>
<td>Cl</td>
<td>O1</td>
<td>3.009(6)</td>
<td>170.8(3)</td>
</tr>
<tr>
<td>7d</td>
<td>Br1</td>
<td>O1</td>
<td>3.014(6)</td>
<td>171.9(3)</td>
</tr>
<tr>
<td>7e</td>
<td>H1</td>
<td>(C1−6)</td>
<td>3.797</td>
<td>175.0</td>
</tr>
</tbody>
</table>

*Based on the PDB ID: 5MO4. *Isomorphic crystal structures. *Represents the centroids of the XB acceptor. *Backbone oxygen of L448 in ABL1 (T334I_D382N). *ΔE of a pair of two single molecules with two mutual XB contacts.

Table S4, 7a and 7b assemble into asymmetric crystal systems with the triclinic space group P-1 and monoclinic C2/c, respectively. The cell of 7a contains two independent molecules (molecules A and B), each forming an HB between the CF3H group and the amide oxygen of the other type of molecule (Figure 7: O3B•••H9A, 2.89 Å, −16.9 kJ mol⁻¹; O3A•••H9B, 3.33 Å, −12.5 kJ mol⁻¹). It should be noted that these intermolecular interactions are likely dominated by the classical HBs (H1NA•••O3B, 1.99 Å; H2NB•••O3A, 2.96 Å). Thus, the CF3H•••O3A/B contact does not reach its full potential (see the curve optimum in Figure 7 for both contacts). In addition, neither the interaction partner of CF3Cl/CF3Br (O1 in the benzo[d][1,3]dioxole substructure) nor the interaction partner of CF3I (delocalized π-system of the aromatic ring) can be targeted by CF3H.

The symmetric orthorhombic crystals (Pna21) of compounds 7c and 7d are isomorphic. Interestingly, the amides are disordered, with two variants (molecules A and B) flipped 180° with respect to each other, each having an occupancy of 50% (Figures S9 and S10). The partitioned occupancy indicates that both nonsymmetric variants of the crystal molecules A and B are energetically equivalent. This also affects the respective locations of the fluorine atoms F1 and F2, while the heavier halogens Cl1 and Br1 remain largely fixed. The carbon atom C9, on the other hand, could not be meaningfully resolved into two positions. Hence, the C9–Cl1 and C9–Br1 bond vectors, which are important for forming the XB, have virtually the same orientation. Both structures are engaged in XB systems target O1 of the benzo[d][1,3]dioxole structure as an XB acceptor (Figure 6c,d, Cl1•••O1, 3.01 Å, 170.8°; Br1•••O1, 3.01 Å, 171.9°).

Acetamide 7e forms an asymmetric monoclinic crystal system with the space group P21/n and is the only crystal structure in which the heavier halogen is oriented toward the delocalized π-electrons of the aromatic ring (Figure 6e: I1−Cl−6(cenroid), 3.80 Å, 175.0°). Comparison with the trifluoracetamide 7b shows that enabling HB (7a) and XB (7c−e) leads to very specific changes in the crystal lattice. As shown in Figure S13, an HB network is maintained along the amides, and the individual molecules in 7a,c,d alternately adopt a position more orthogonal to that of each other (Figure S13c−e). In 7e, the lattice is rearranged so that two single molecules directly their iodine atom toward each other’s parallel-aligned aromatic rings (Figure S13b). We performed a distance scan analogous to that for 6d,e (Figure 7). The XB of 7c and 7d contribute −11.7 kJ mol⁻¹ and −14.1 kJ mol⁻¹, respectively (Table 4). The mutual iodine-π interactions of two single molecules of 7e yield a total energy ΔE of −51.3 kJ mol⁻¹. Artificially restricting this system to only one XB clarifies that slightly less than half of the interaction energy could be attributed to one XB contact. Unlike the XB-capable derivatives, 7a cannot fully exploit the potential of HB because the ΔE minimum requires a closer acceptor–donor contact, which is not realized in the crystal lattice. So far, the computational efforts were intended to illustrate the importance of certain contacts observed in our small molecule crystal structures. In subsequent paragraphs, we analyze the optimal conformations and interaction geometries that allow to harness the full potential of CF3-X moieties based on halogen bonding.

Conformational Analysis. In the next step, we used conformational analysis (gasphase) to investigate whether CF3X groups (X = H, Cl, Br, I) with ethereal or amide linker systems adopt preferred geometries and whether these are consistent with our experimental data. For this purpose, we
used geometry-optimized model molecules (Figure 2), which we rotated along their rotatable binding axes in increments of 10° steps to determine the relative energy changes ΔE in kJ mol⁻¹ from single point calculations. Because no optimizations were conducted after each rotation step with the dihedral angles (φ and ψ) being constrained, the resulting energies of geometries more distant to the starting point are typically higher than after constrained relaxation. This can slightly underestimate the number of good to excellent conformations and cause the plots to be not fully symmetric. As illustrated in Figure 9a,b, the potential energy surface profiles of the ethers are very different compared to the amide. The red mark: initial geometry-optimized conformation. Black marks: conformations at other ΔE minima. Orange marks: conformations found in the corresponding crystal structures of 6a−c−e and 7a,c−e. Green mark: conformation of asciminib (13c) found in ABL1 protein (T334I_D382N), PDB ID: 5MO4. (a) PES contour maps of ethers (−O−), from top to down: X = H, Cl, Br, or I. (b) PES contour maps of amides (−NHCO−), from top to down: X = H, Cl, Br, I. (c) Detailed PES contour map of Ph−O−CF₂Cl with geometries of 6c and asciminib (13c) found in PDB ID: 5MO4. (d) Corresponding Ph−O−CF₂Cl conformations of red and black marks in the PES contour map. (e) Corresponding Ph−NHCO−CF₂Cl conformations of red and black marks in the PES contour map. Structures of the energy minima C1−C3 in (d) and C4−C5 in (e) are shown as sticks. The flipped versions (ψ ± 180°) are depicted as transparent sticks.
While there are only few areas of very low conformational energies (dark blue in Figure 9a) for X = Cl, Br, I, these favored conformational areas are significantly increased for CF$_2$H. Thus, the XB donors will form good interactions with acceptors in the binding site more selectively than the HB donor. Likewise, the higher conformational restrictions of the XB donors are likely to give them an entropic advantage.

Among the amide molecules (Figure 9b), the iodine derivative shows the highest maximum of 67.2 kJ mol$^{-1}$ relative to its geometry-optimized structure. It is evident that the rotational barriers are significantly lower, and more conformational degrees of freedom of the CF$_2$X groups are possible, compared to the ether analogs (Figure 9a). The CF$_2$H and CF$_2$F groups show plots that are more similar for the amide linker. However, the preferred $\phi$ angle of CF$_2$H is located approximately between 120° and 180° (and between $-120^\circ$ and $-180^\circ$), whereas the preferred $\phi$ angle of CF$_2$I is roughly 60° to 120° (or $-60^\circ$ to $-120^\circ$).

To illustrate the agreement between this theoretical evaluation and our small molecular crystal structures, we annotated the plots with the experimentally determined conformations. Based on symmetry effects in these dihedral plots and alternatives to measure the $\psi$ angle due to the exact alignment of the 180° flipped phenyl ring, the same conformation found in a crystal structure could be annotated for positive or negative values of $\psi$. This is illustrated for ether derivatives in Figure 9d and for amide derivatives in Figure 9e.

The structure of the energy minima for OCF$_2$X is shown as C1, C2, and C3 with the flipped versions ($\psi \pm 180^\circ$) depicted as transparent sticks. Based on the coplanarity of the amide and the phenyl ring, for NHCOCF$_2$X only two $\psi$ angles, 0° and 180°, can occur. The C$_X$–X bonds in structures C4 and C5, representing the energy minima, are roughly orthogonal to the plane of the $\pi$-system ($\phi \approx \pm 90^\circ$). The flipped versions ($\psi \pm 180^\circ$) of C4 and C5 are again depicted as transparent sticks. For simplicity reasons, we provide all annotations in Figure 9a–c in one quadrant (ether: $\phi = 0^\circ$ to 180° and $\psi = -180^\circ$ to 0°; amide: $\phi = 0^\circ$ to 180° and $\psi = -90^\circ$ to 90°) representing several possible geometrically or energetically equivalent crystal conformers.

Figure 9a–c also shows the geometric data of the small molecule crystal structures (orange mark) and of asciminib in ABL1 protein crystal structure 5MO4 (green mark) as dihedral angles of the ether- or amide-linked CF$_2$X group. The torsion angles of the crystal data all coincide with the calculated data and are located in the dark blue areas, representing low energy values. Interestingly, two deviating features can be observed: Molecule F in the crystal lattice of 6c (single molecule with CI1F atom in Figure 5) is the only molecule showing an elongated conformation ($\phi = 179.3(9)^\circ$; $\psi = -100(1)^\circ$ corresponding to C3 in Figure 9d). This conformation is only a local minimum, compared to the global minimum represented by C1. Second, induced by the formation of a HB (Figure 8), the torsion angles of 7a (molecule A: $\phi = \pm 54(4)^\circ$; $\psi = \pm 11.0(8)^\circ$; molecule B: $\phi = \pm 48(4)^\circ$; $\psi = \pm 17.4(8)^\circ$) deviate significantly in their $\psi$ angle from the calculated energy minima. In summary, the crystal structures of molecules bearing chlorine, bromine, or iodine show excellent agreement with the PES minima (and their tolerances) obtained from QM calculations of the ether and amide model systems.

The hitherto discussed potential energy plots clearly highlight that despite preferential orientations of the halogens in CF$_2$X groups there is a significant degree of flexibility allowing these halogens to form XB to various acceptors in the binding site. In contrast to the typically considered (hetero)-arylated halide XB donors, CF$_2$X groups allow for an orthogonal binding vector strongly deviating from linearity and a far greater “allowed conformational space”. Thus, for CF$_2$X groups, the calculation of XB strength should always be adjusted by the relative conformational energy. As mentioned before, the distribution of conformational energies in this “allowed space” versus the strength of XB obtained from these conformations can play an important role in the enthalpy/entropy compensation of ligand binding.
Table 5. Calculated σ-Hole and XB Data of Geometry-Optimized Model Molecules with the General Formula Ph−Y−R (Y = N/A, O, NHCO; R = CF₃X) and X = H, Cl, Br, I

<table>
<thead>
<tr>
<th>Ph−Y−R</th>
<th>H</th>
<th>Cl</th>
<th>Br</th>
<th>I</th>
</tr>
</thead>
<tbody>
<tr>
<td>Y</td>
<td>R</td>
<td>QM-method</td>
<td>ΔE (kJ mol⁻¹)</td>
<td>X−O (Å)</td>
</tr>
<tr>
<td>−X</td>
<td>−X</td>
<td>MP2/TZVPP</td>
<td>−10.8 2.3</td>
<td>0.116</td>
</tr>
<tr>
<td>−X</td>
<td>−CF₃X</td>
<td>MP2/TZVPP</td>
<td>−20.3 2.1</td>
<td>0.135</td>
</tr>
<tr>
<td>−X</td>
<td>−CF₃X</td>
<td>MP2/TZVPP (BSSE corrected)</td>
<td>−16.0</td>
<td>−8.1</td>
</tr>
<tr>
<td>−X</td>
<td>−CF₃X</td>
<td>MP2/QZVPP</td>
<td>−19.8</td>
<td>−11.0</td>
</tr>
<tr>
<td>−X</td>
<td>−CF₃X</td>
<td>M06-2X-D3/TZVPP</td>
<td>−18.7</td>
<td>−10.1</td>
</tr>
<tr>
<td>−X</td>
<td>−CF₃X</td>
<td>MP2/TZVPP (BSSE corrected)</td>
<td>−14.9 2.1</td>
<td>0.141</td>
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<tr>
<td>−X</td>
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<td>−CF₃X</td>
<td>M06-2X-D3/TZVPP</td>
<td>−14.7</td>
<td>−14.4</td>
</tr>
</tbody>
</table>

The stated complex formation energies ΔE and the distance X−O correspond to the minima of distance scans between the HB/XB donor and the oxygen of N-methylacetamide as the HB/XB acceptor. The Cₓ−X−O angle is equal to 180°, and the X−O=C angle is equal to 120°. The V_max-values were calculated for an isodensity surface of 0.02 au. MP2/TZVPP calculations without BSSE are compared with BSSE-corrected MP2/TZVPP results as well as with MP2/QZVPP and M06-2X-D3/TZVPP. Additional weak secondary interactions between the aromatic ring and N-methylacetamide occur based on the ether geometry.

As guidelines for the molecular design of such moieties, we have tried to visualize the "XB interaction space" with close to optimal conformational energies. Figure 10 illustrates the conformation-dependent orientation of the Cₓ−X bond axes as elongated interaction vectors. Shown are the vectors of all determined geometries within Δϕ, Δψ = ± 90° (rotated in increments of 10°) and with ΔE equal to or less than 20 kJ mol⁻¹ in relation to the geometry-optimized minimum. From the graphics, it is evident that ethers (Figure 10a) and amides (Figure 10b) are capable of addressing different linker-specific spatial segments representing the locations of potential XB acceptors in a protein binding site. In case of the ether linker, the interaction vectors always lean toward the normal vector of the aromatic ring, in case of the amide linker, the interaction vectors always project away from the π-surface of the aromatic ring. Still, this "axial variation" is small for both linkers, leading to rather slim segments that can be targeted, displayed as a color-coded surface of pseudointeraction points at a distance of 3.0 Å to the halogen in Figure 10c,d. In comparison, the "equatorial variation" (with respect to the Cₓ−N or Cₓ−O bond) is much larger for both linkers, yielding a substantial segment of potential interaction partners. Within these boundaries, the size of the surface of potential interaction partners does not differ quite significantly between the different linkers and the different halogens.

Complex Formation Energies ofXBs and V_max. Since we have characterized the probable interaction space induced by suitable geometries of ether or amide linkers, we further investigated the XB strength dependent on the halogen, the type of linker, and the distance from the XB acceptor. We focus our theoretical view on this moiety only on the halogen bond. Hence, it should be noted that, obviously, interactions of the linker (HBs toward ether or amide), as well as interactions of the C−F bonds, such as orthogonal multipolar interactions, can strongly enhance the interaction energy of this moiety with particular amino acids in the binding site.⁶,¹⁰⁴−¹⁰⁶

As a model system for comparing XB and HB strength, we have used N-methylacetamide as a representative of the ubiquitously available backbone peptide bond.¹⁰⁷ We performed distance scans between the HB/XB donors of our model molecules (Figure 2) and the oxygen of N-methylacetamide as the HB/XB acceptor. The distance was changed in increments of 0.1 Å and the complex formation energies (ΔE) were calculated as single points for each increment. The Cₓ−X−O angle was fixed to 180°, and the X−O=C angle was fixed to 120°. As shown in Table 5, the results reveal tuned ΔE for ether and amide CFₓ moieties with Cl (ΔE = −10.5/−10.1 kJ mol⁻¹) < Br (ΔE = −13.7/−14.3 kJ mol⁻¹) < I (ΔE = −20.1/−21.6 kJ mol⁻¹). These results are quite similar to the computational analysis of the experimental small molecule crystal data already discussed (Table 4). Calculations were performed at the MP2/TZVPP level of theory as a standard. We often find for XB interactions that MP2-energies without correction of the basis set superposition error (BSSE) resemble benchmark calculations at the CCSD(T) level of theory with a complete basis set extrapolation (CBS) more closely than MP2-energies with BSSE. Still, MP2 is well-known to overestimate the strength of interactions. Thus, in Table 5, we additionally report MP2/TZVPP-energies with BSSE, MP2/QZVPP-energies, as well as M06-2X/TZVPP-energies with D3 dispersion correction. M06-2X has been shown to yield excellent geometries and energies for the XB18 and XB51 halogen bonding benchmark sets.¹⁰⁸

As expected, the BSSE correction reduced the adduct formation energies (on average by 21.6%). However, the consistency with the larger QZVPP basis set (+2.1% on average) and, particularly, the comparison with the established M06-2X hybrid functional (−2.8% on average) suggest that uncorrected MP2 values might only overestimate the adduct formation energy slightly and are not unreasonable to use for further discussions.

The respective average complex formation energies of the CFₓ-X amides and ethers are increased by a factor of 1.60 for chlorine, 1.36 for bromine, and 1.23 for iodine compared to MP2/TZVPP calculations without BSSE. This reflects the substantial polarization (tuning) effect by both fluorine
atoms attached to the same sp\(^3\) carbon atom onto the halogen X, as well as additional electron withdrawing effects from the linker oxygen or amide. Tuning of XB is very often represented by the increase of the σ-hole.\(^{109-116}\) As a useful descriptor of this improved anisotropic distribution of the electron density, the maximal positive electrostatic potential, V\(_{\text{max}}\) derived from plotting the ESP onto a specific electron isodensity surface, is provided. While most literature data is based on ESPs plotted onto an isodensity surface of 0.001 or 0.002 au, we have previously established reasons, why we use an isodensity surface of 0.02 au.\(^{117}\) From the data in Table 5, it can also be concluded that the V\(_{\text{max}}\) values of the CF\(_2\)X groups increase significantly by 7–22% compared to the corresponding halobenzenes. While the absolute changes in V\(_{\text{max}}\) and complex formation energies ΔE between Ph–X and Ph–O–CF\(_2\)X or Ph–NHCO–CF\(_2\)X, do not differ much for X = Cl, Br, or I, it should be noted that the strongest relative improvement is consistently found for chlorine.

In the case of HB, the complex formation energy is increased by a factor of 1.39 for the CF\(_2\)H moiety with an amide linker and by a factor of 1.89 with an ether linker in comparison with our library. The unmodified educt of \(16\) achieved, we also included 2-chloro-2,2-difluoro-1-(imidazo[2,1-b]pyridin-3-yl)ethan-1-one (\(7c\)), so that CF\(_{14-16}\) can be used to target more distant donors. This is even more pronounced based on the different C\(_x\)–X bond lengths, when considering the C\(_x\)–O distance (Figure 11b), instead of the X–O distance (Figure 11a).

**Biophysical Fragment Screening on JNK1 and JNK3.** To demonstrate the use of CF\(_2\)X in targeting binding sites, we have synthesized a series of fragments bearing R–NHCO–CF\(_2\)X with diversified scaffolds (R = organic scaffold; X = Cl, Br, I) as an addition to our HEFLibs concept.\(^{91,92,118-120}\) The advantage of this fragment-based strategy is its focus on only a few relevant key interactions. Using STD NMR and ITC as primary and secondary biophysical screening techniques, respectively, we applied this small library of CF\(_2\)X-fragments to the c-Jun N-terminal kinases 1 and 3 (JNK1 and JNK3).\(^{121,122}\) Here we focused on halogenated acetamides because their synthesis is much simpler, faster, and cheaper, which facilitates the rapid establishment of a fragment library enriched in CF\(_2\)X acetamides. In addition, the higher polarity of the amide linker in comparison to the ether linker, should be beneficial for the solubility of the resulting fragments. A small variety of diverse building blocks with few non-hydrogen atoms was purchased. Aside from aromatic amines with different aryl and heteroaryl scaffolds as starting materials (\(7c-e, 16-25, 28-29,\) and \(31-33\)), our test library includes amides prepared from acyclic primary (\(15\)) and cyclic secondary (\(30\)) alkyl amines. As acylation of imidazole[1,2-a]pyridine was easily achieved, we also included 2-chloro-2,2-difluoro-1-(imidazo[1,2-a]pyridin-3-yl)ethan-1-one (\(14\)) containing a carbonyl instead of an amide linker in our fragment library.

Table 6 summarizes the results of the STD NMR screening and ITC validation measurements. Initially, 18 fragments (\(7c-e, 14-16, 19-22, 25, 28-34\)) were tested for JNK3 binding by STD NMR. The CF\(_2\)Cl-containing 1,3,4-thiadiazole derivatives \(16\) and \(22\) were identified as hits and confirmed by ITC measurements as micromolar binders with affinities of 240 and 25 μM, respectively. To study the halogen influence on JNK3 binding, the matched molecular pairs of CF\(_2\)Br (\(17,\) 23) and CF\(_2\)I (\(18,\) 24) analogs were synthesized and added to our library. The unmodified educt of \(22-24,\) 5-(pyridin-4-yl)-1,3,4-thiadiazol-2-amine (\(35\)), was evaluated as a reference compound. For JNK1, all 23 listed fragments with the exception of \(35\) were screened by STD NMR, followed by individual ITC validation.

The smaller 1,3,4-thiadiazole fragments \(16-18\) (12 heavy atoms) bound to JNK1 at a K\(_D\) of 104–193 μM and on average slightly worse to JNK3 with a K\(_D\) of 186–248 μM. The N-(5-(pyridin-4-yl)-1,3,4-thiadiazol-2-yl)acetamide derivatives \(22-24\) (18 heavy atoms) bound with higher affinity in the low two-digit range to JNK1 (K\(_D\) = 10–20 μM) and JNK3 (K\(_D\) = 13–23 μM). Although the heavier fragments had a 10-fold higher affinity, they exhibited a lower LE due to the 50% higher number of heavy atoms (22–24: 0.36–0.39 versus 16–18: 0.42–0.46). In summary, the STD NMR signal of 16–18 indicated that the hydrogen atom of the thiazoil ring is involved in protein binding. However, substitution by a pyridine (22–24) increased the binding affinity by approximately 10-fold. Interestingly, loss of the HB acceptor by exchange of the N3 atom in the thiazoil ring of 17 into a carbon atom, yielding a thiazole ring (25), was accompanied by a significant reduction in affinity (K\(_D\) > 1000 μM). The comparison of 22–24 to their educt 35 (K\(_D\) = 162 μM) as a reference showed that halodifluoroacetylation of the hetero-
Table 6. Biophysical Measurements Using STD NMR and ITC against JNK1 and JNK3

<table>
<thead>
<tr>
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<th>STD</th>
<th>$K_D$ (μM)</th>
<th>LE</th>
<th>JNK1</th>
<th>STD</th>
<th>$K_D$ (μM)</th>
<th>LE</th>
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<tr>
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The results clearly show that halodifluoroacetylation contributes to affinity enhancement of JNK1 and JNK3, a moderate increase of affinity was measured for matched molecular pairs about XB interactions. For both JNK1 and JNK3, a moderate increase of affinity was measured for 16 (CF<sub>3</sub>-Cl, K<sub>D</sub><sup>JNK1</sup> = 186 μM; K<sub>D</sub><sup>JNK3</sup> = 243 μM) ≈ 17 (CF<sub>3</sub>-Br, K<sub>D</sub><sup>JNK1</sup> = 193 μM; K<sub>D</sub><sup>JNK3</sup> = 248 μM) toward 18 (CF<sub>2</sub>I, K<sub>D</sub><sup>JNK1</sup> = 104 μM; K<sub>D</sub><sup>JNK3</sup> = 186 μM).}

aromatic amine increased the affinity between 8-fold and 15-fold for JNK1.

Table 6. continued

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<th>STD</th>
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</table>

“Carboxyl functionality instead of an amide group. 5-(Pyridin-4-yl)-1,3,4-thiadiazol-2-amine: Educt of compounds 22–24. (+): no binding event or not detectable. (+): detected binding event. HA is the number of heavy atoms. ITC results are provided as K<sub>D</sub> in μM and transformed into ligand efficiencies by LE = −(ΔG/HA) = −log(K<sub>D</sub>/(1.4/HA)).”

For optimal XBs, these differences could be more pronounced, but steric restrictions in the binding site and increased conformational energies can diminish the size of the trend. To elucidate the structural basis for the observed SAR and to clarify the role of the involved interactions, we conducted crystallization experiments with JNK3.

Structure-Affinity Relationship and JNK3 Protein Crystallization.

The CF<sub>3</sub>-Br acetamide derivatives 17 and 23 were soaked in JNK3 crystals by using a sitting-drop procedure. Thus, far, soaking approaches of fragment 17 could not displace AMP-PCP, possibly due to the weaker affinity of 178 μM, but a JNK3 crystal was obtained with ligand 23 (K<sub>D</sub> = 17 μM) bound in the ATP binding pocket, as shown in Figure 12. The JNK3 crystal structure was determined in space group P2<sub>1</sub>2<sub>1</sub>2 (18) with two chains in the asymmetric unit and a resolution of 1.86 Å (Table S17). The chain B and its bound ligand 23 were insufficiently resolved, and therefore, the following analysis focuses on chain A.

For conformational energy differences of the CF<sub>3</sub>-Br moiety (23-A and 23-B) were built with 50:50 occupancy. The 4-pyridinyl-1,3,4-thiadiazole substructure is placed almost identically in both conformations, targeting the hinge region and gatekeeper M146 (Figure 12d). The N4 atom of pyridine forms a strong HB with the backbone N–H of M149 (d<sub>23-A,N–H</sub> = 2.9 Å; d<sub>23-B,N–H</sub> = 2.7 Å), whereas the C7–H and C8–H of pyridine form weaker HBs with the backbone oxygens of G147 (d<sub>23-A,C–O</sub> = 3.2 Å; d<sub>23-B,C–O</sub> = 3.1 Å) and M149 (d<sub>23-A,C–O</sub> = 3.9 Å; d<sub>23-B,C–O</sub> = 3.6 Å). Numerous C–H···π contacts are formed with the top and bottom side of the aromatic rings of the ligand. The gatekeeper M146 is engaged in a ChB with N3 of the thiadiazole ring system (d<sub>23,A,N3–S</sub> = 3.6 Å, α<sub>23,A,C–S–N</sub> = 156.4°; d<sub>23,B,N3–S</sub> = 3.5 Å, α<sub>23,B,C–S–N</sub> = 151.2°). A second ChB interaction is intramolecularly located between the sulfur atom of the thiadiazole and the oxygen atom of the amide (d<sub>23,AS–S</sub> = 3.0 Å, α<sub>23,AC–S–S</sub> = 153.8°; d<sub>23,B,AS–S</sub> = 3.0 Å, α<sub>23,B,C–S–S</sub> = 156.6°). Intramolecular ChB of this type have already been described in the literature. Nagao et al. calculated an ab initio geometry optimization at the HF/3-21G* level for a similar thiadiazole system. They obtained a relative energy difference of −36.6 kJ mol<sup>−1</sup> for the closest S···O contact of a 2,2,2-trifluoro-N-(1,3,4-thiadiazol-2-yl)acetamide derivative. This suggests that the rotatability of the thiadiazole-amide bond is significantly constrained by the ChB interaction and that a conformation with close S···O contact is typically preferred over the 180°-dipped conformation. This is consistent with the observed electron density in our crystal structure.

In conformation 23-B (ψ<sub>23-B</sub> = 107.4°), the bromine forms an XB with the backbone oxygen of G76 (d<sub>23-B,Br–O</sub> = 3.3 Å, α<sub>23-B,C–Br–O</sub> = 161.9°) located in the P-loop of JNK3 (Figure 12d). The side chain of K93 and the P-loop (G76 to S72) form a cavity around the bromine atom (distances of approximately 3.7–4.1 Å) and constrain the bromine in its effort to adopt a
more ideal σ-hole angle than 161.9°. Simultaneously, the protonated side chain amine of K93 is engaged in a fluorine interaction ($d_{23-B,N\cdots F} = 2.8$ Å). To elucidate the relevance of these interactions and compare both observed binding modes, we conducted model calculations at the MP2/TZVPP level of theory. The protein backbone between C$_\alpha$ of Q75 and C$_\alpha$ of I77 including the interaction partner G76 was represented as 2-acetamido-N-methylacetamide, as was the protein backbone of the beginning P-loop between the C$_\alpha$ of S72 and the C$_\alpha$ of A74. The side chain of lysine K93 was capped to 1-propylammonium. Hydrogen atoms were added and optimized, while all other atoms were fixed to the coordinates of the crystal structure. Despite the not ideal σ-hole angle, we observe a good interaction energy for the XB of $-12.3$ kJ mol$^{-1}$. The proximal P-loop (most likely G73) yields a complex formation energy of $-10.4$ kJ mol$^{-1}$ for the CF$_2$Br moiety. The charged HB between K93 and the fluorine atom exhibits the strongest complex formation energy ($-22.3$ kJ mol$^{-1}$) as expected.

The second conformation 23-A ($\psi_{23-A} = -64.7^\circ$) is based on a CF$_2$Br group, rotated approximately 170°, orienting one fluorine atom in the described cavity and the bromine atom facing toward the empty phosphate pocket (Figure 12c).

Possible key interactions stabilizing this binding mode could be HBs from the charged side chain of K93 to the electron-rich belts around the fluorine and bromine atoms ($d_{23-A,N\cdots F} = 3.4$ Å, $d_{23-A,N\cdots Br} = 3.6$ Å). From our model calculations, we have learned that the lysine contacts are slightly impaired with $-18.5$ kJ mol$^{-1}$ in comparison to conformation B. Likewise, the contacts to both stretches of backbone adjacent to the P-loop, S72–A74 and Q75–I77, give reduced interaction energies of $-5.1$ kJ mol$^{-1}$ and $-6.5$ kJ mol$^{-1}$, respectively. In addition, the conformational energy of B is more advantageous than that of A ($\Delta E = 11.4$ kJ mol$^{-1}$). Overall, conformation A shows surprisingly reasonable interaction energies; however, all calculations suggest that conformation B (Figure 12d) should be the stronger binder. It should be noted that the side chain of Q75 is not resolved in the crystal structure; however, it is possibly proximal to the CF$_2$X moiety in both conformations. Thus, an influence of this residue on the binding mode in solution cannot be ruled out.

The protein crystal structure of JNK3 in complex with ligand 23 provides plausible explanations for the SAR of 16–18, 22–24, 25, and 35 that were previously discussed. Compound 35 is not capable of forming the interactions of the halogenated acetamide group and binds substantially weaker to JNK1 ($K_d$...
The inability to form a ChB due to the absence of a suitable ChB donor atom (such as N3 in the thiadiazoles) most likely explains why thiazole (K_D > 1000 μM) was not detectable in the ITC experiments and emphasizes the importance of the ChB to the sulfur atom of gatekeeper M246 for the thiadiazole ligands. Compounds 16–18 showed only a minor trend of decreasing K_D values in the series Cl ≈ Br > I (K_D = 243 μM ≈ 248 μM > 186 μM), while 22–24 (K_D = 12–23 μM) bound better by a factor of approximately 10-fold to 15-fold. As stated before, the only significant difference is found for the exchange of chlorine (22) into iodine (24). The anchoring of fragments 22–24 in the hinge region by the pyridine ring as a hinge-binding motif likely plays an important role in the affinity boost compared to 16–18.

As presented herein (Table 5), the theoretically possible difference in interaction energy at an optimal XB geometry of each halogen shows that halogen exchange from chlorine to bromine could improve the ΔE by approximately −4.2 kJ mol⁻¹. Likewise, halogen exchange from bromine to iodine could strengthen the interaction by approximately 7.3 kJ mol⁻¹. Starting from a σ-hole angle α−br−C of 161.9° between bromine and the backbone oxygen of G76 located in the P-loop of JNK3, we astonishingly found that the bulkier iodine (24) can be accommodated with improved affinity in comparison to bromine (23). It remains speculative whether some small degree of adaptability of the P-loop is a necessary prerequisite for this observed structure-affinity relationship. This example highlights that the heavier halogens will not necessarily always be the sweet spot for forming XBs just based on their potential to exhibit enlarged σ-holes. For CF₂X moieties, the conformational strain, as well as strength and multitude of other interactions fixing the binding mode and limiting the formation of an optimal geometry, can be quite influential. In some binding sites and modes, an iodine will rise to the full potential of its possible XB strength. In other binding scenarios, an inadvertently sized bromine or smaller chlorine might be enabled to form optimal XB interactions.

**CONCLUSION**

We have demonstrated that molecules containing C(sp³)F₂X moieties (X = Cl, Br, I) attached by linker systems such as ethers or amides are synthetically accessible and that amide derivatives are particularly suitable for fragment-based drug discovery, providing the opportunity to identify highly interesting, unconventional XB-based binding modes in biological targets.

Starting from a PDB analysis fostering our interest in CF₂X derivatives based on the crystal structure of Ascininib in ABL1 kinase, we have created a series of matched molecular pairs for two different linker systems (6a–e and 7a–e). Using these tool compounds, we studied their chemical and metabolic stability, as well as their physicochemical properties, particularly with respect to solubility and logP. The polarization between fluorine and X = Cl, Br, or I and the role of the anisotropic electron distribution around X, as illustrated by ESP plots, certainly has an influence on stability and physicochemical properties. QikProp has been shown to provide reasonable predictions for quite a few solubilities. However, in some cases (15, 19, 21–24, 29, 33, 34) solubility issues were predicted that were fortunately not observed in the turbidimetric assay. In some cases, solubility issues were predicted correctly (31, 32). Thus, we recommend for compounds containing CF₂X moieties to always confirm predictions of solubility with experimental evidence and to avoid taking solubility warnings as the main criterion to decide for or against the synthesis (or procurement) of a compound.

In crystallization experiments with our tool compounds, we have shown that the self-assembly of the CF₂X derivatives in the crystalline solid-state is mainly driven by XBs and influenced by the size of the halogen. CF₂X ether and amide groups adopt preferred geometries, capable of targeting potential XB acceptors that are not readily addressable with C(sp³)−X moieties of (hetero)aryl halides, and they tend to strive in our crystal structures for the optimal XB angle and distance, unlike our analogous HB donor CF₂H (7a). Using QM methods, we characterized the conformational flexibility of such CF₂X ether and amide functions, finding good agreement with the experimental data from our small molecule crystals. We highlight clear conformational limitations, which are essential to consider, when using these moieties in molecular design. In addition, we find a reasonable degree of flexibility of these moieties to adapt to different XB acceptors in a binding site with low conformational strain, particularly in comparison with C(sp³)−X moieties. We further characterized the potential XB strength of these CF₂X donors in a distance-dependent manner. Compared to the respective halobenzenes, we find that the XB interactions are improved by approximately 3.5 to 4.5 kJ mol⁻¹, based on the tuning effects of the fluorine atoms and the linker functions.

The CF₂X structural motif is underrepresented in drug discovery and has hardly been applied so far. Organic amines are readily available and suitable starting materials for the facile preparation of a structurally diverse fragment library featuring CF₂X amides. We propose that unconventional or even rather unique binding modes could be explored based on such libraries, which are hardly accessible from conventional chemical space, allowing for the discovery of unclaimed, patentable chemotypes and the establishment of added therapeutic opportunities.

To demonstrate the usefulness and good applicability of this concept, we screened c-Jun N-terminal kinases 1 and 3 (JNK1 and JNK3), using STD NMR and validated hits by ITC, revealing several hits in the μM affinity range. Particularly the S-(pyridin-4-yl)-1,3,4-thiadiazole scaffold showed good binding to both kinases. The affinity of its bromine derivative (23) to JNK3 and JNK1 was 17 μM and 15 μM, respectively. We determined the crystal structure of 23 in complex with JNK3, elucidating that the CF₂Br moiety interacts with the P-loop in a unique way, which has not been reported so far. It can concomitantly donate an XB with a distance of 3.3 Å and a σ-hole angle of 161.9° toward the backbone oxygen of G76 located in the P-loop, while accepting a hydrogen bond from the charged side chain of K93 onto one of its fluorine atoms. Overall, the interactions in the binding site are shifted from sites that traditionally receive most attention, such as the hinge and the back pocket, toward the sugar/phosphate binding site of ATP. Only the pyridine substructure forms an HB with the hinge. Thus, fragment 23 has good optimization potential by elaborating the hinge-binding motif and performing fragment growth and merging approaches.

Future studies will focus on expanding the fragment library as well as developing further linker systems and other stable C(sp³)−X structures. The steadily expanding library will continue to be screened for additional binding fragments targeting therapeutically relevant proteins. Found hits are subject to lead optimization.
MATERIAL AND METHODS

Chemistry. Synthesis procedures of all synthesized compounds are described in the Supporting Information. Purity of all synthesized final compounds (6a–e, 7a–e, 13a–e, 14–25, 28–34) and 35 was >95% as determined by HPLC analysis (Table S1).

Glutathione Stability Assay. The glutathione stability assay was performed according to a modified procedure by Keeley et al.225 225 μL of PBS buffer, 12.5 μL of ACN, 250 μL of GSH solution (10 mM in PBS buffer containing 10% (v/v) ACN), and 6.25 μL of indoprofen or ketoprofen (8 mM in ACN) as internal standard were added into an HPLC vial. The assay was started by adding of fragment solution (20 μM in ACN) to give a final concentration of 250 μM fragment, 5 mM GSH, and 100 mM internal standard in 500 μL of PBS containing 10% (v/v) ACN. The final mixture was incubated at 37 °C and analyzed by injection of 5 μL into a analytical HPLC after different time intervals. Column: ReproSil-XR 120 C18, 5 μm, 150 mm × 4.6 mm; mobile phases: A: phosphate buffer pH 2.3, B: methanol; flow rate: 1.0 mL min⁻¹.

Microsomal Stability Assay. Samples were measured in triplicates and incubated at 37 °C. Each incubation had a total volume of 600 μL: 540 μL of phosphate buffer (100 mM, pH 7.4), 36 μL of NADPH regenerating system (1.3 mM NADP, 3.3 mM isocitrate, 0.4 U mL⁻¹ isocitrate dehydrogenase, 3.3 mM MgCl₂), 21 μL of microsomes pooled from human liver (10 mg mL⁻¹), and 3 μL of substance standard solution (1 mg mL⁻¹ for targets, 0.1 mg mL⁻¹ for positive controls). Testosterone, diclofenac, and propranolol were used as positive controls. Samples were taken at 0, 5, 15, 30, 45, 60, 120, and possibly 300 min. For this purpose, 50 μL of fragment solution was added to 50 μL of PBS buffer, 12.5 μL of ACN, 250 μL of GSH solution (10 mM in PBS buffer containing 10% (v/v) ACN), and 6.25 μL of indoprofen or ketoprofen (8 mM in ACN) as internal standard were added into an HPLC vial. The assay was started by adding of fragment solution (20 μM in ACN) to give a final concentration of 250 μM fragment, 5 mM GSH, and 100 mM internal standard in 500 μL of PBS containing 10% (v/v) ACN. The final mixture was incubated at 37 °C and analyzed by injection of 5 μL into a analytical HPLC after different time intervals. Column: ReproSil-XR 120 C18, 5 μm, 150 mm × 4.6 mm; mobile phases: A: phosphate buffer pH 2.3, B: methanol; flow rate: 1.0 mL min⁻¹.

Turbidimetric Solubility Assay. Turbidimetric solubility assay was performed in 50 mM HEPES at pH 7.4 and 100 mM NaCl at 25 °C. The 100 mM fragment stocks in DMSO were diluted by a factor 6a–e, and then a fragment solution (20 μM in ACN) was added to give a final concentration of 250 μM fragment, 5 μL of GSH solution (10 mM in ACN) as internal standard were added into an HPLC vial. The assay was started by adding of fragment solution (20 μM in ACN) to give a final concentration of 250 μM fragment, 5 mM GSH, and 100 mM internal standard in 500 μL of PBS containing 10% (v/v) ACN. The final mixture was incubated at 37 °C and analyzed by injection of 5 μL into a analytical HPLC after different time intervals. Column: ReproSil-XR 120 C18, 5 μm, 150 mm × 4.6 mm; mobile phases: A: phosphate buffer pH 2.3, B: methanol; flow rate: 1.0 mL min⁻¹.

Small Molecule Crystallization. Small molecule crystallization was performed overnight. Data were collected at the Swiss Light Source in Hemiksem with the instrument used, with the measuring cell set at 25 °C, while the cooling jacket was set at 15 °C. The measurement was performed by using a needle stirring speed of 1000 rpm and a reference heat rate of 10 μcal s⁻¹. Experiments were aborted when the measuring cell was unable to reach a heat rate greater than 9.0 μcal s⁻¹ during equilibration. After an initial delay of 120 s following a temperature equilibration, a first injection of 0.5 μL was done over 2 s. Nineteen injections of 2.0 μL were performed over 4 s every 120 s.

Cryostabilization and Data Acquisition. Cryostabilization was performed as previously described.211 Protein was concentrated to 7–9 mg mL⁻¹ and incubated with 1 mM AMP-PCP, 0.4 mM Zwittergent and 10% (v/v) ethylene glycol for 30–60 min. The cryostabilization condition consisted of 100 mM BisTris pH = 6, 27% PEG 3350 and 200 mM NaCl at 18 °C. Crystals grew within a few days.

For cryo protection, the previously described condition was supplemented with an additional 20% (v/v) glycerol and contained 5 mM fragment with no more than 5% (v/v) DMSO. Soaking was performed overnight. Data were collected at the Swiss Light Source in Switzerland at the Beaml ine Xo6SA (PXI).

Data Reduction and Refinement. Data reduction was performed using XDS.129 To obtain initial phases, PDB 4X21 was used as a search model for molecular replacement using Phaser.128 as part of the CCP4 suite.127 Multiple rounds of manual model building in COOT128 and refinement using PHENIX127 were performed. ACEDR123 was used to create restraints for the compound 23. The final model is deposited with the accession number 6ZBP (Table S1).

QLogP and QLogS Calculations. LogS and logP values (denoted QLogS and QLogP/w) were calculated using the QikProp module of Schrodinger suite version 2021–1.99 Molecules were protonated and preprocessed using Schrodinger’s LigPrep module with default parameters.109 All calculations were then carried out using the default parameters and the normal processing mode of QikProp.

MP2 Geometry Optimizations and Single Point Calculations. Geometry optimizations and single point calculations were carried out using TURBOMOLE 7.4.135 MP2 calculations were done in combination with the resolution of identity (RI) technique and the frozen core approximation.33–38 The frozen core orbitals were defined using default settings by which all orbitals possessing energies below −3.0 au were considered core orbitals. The SCF convergence criterion was increased to 10⁻⁸ hartree for all calculations. Throughout this study, we mainly used MP2-calculations combined with a triple-ζ basis set (def2-TZVPP).136 For comparison purposes, a quadruple-ζ basis set (def2-QZVPP)39 was used for the on-resonance frequency, which was determined from the 1H NMR spectra of the protein, by an offset between 0.5–0.6 ppm. Forty ppm was used as the off-resonance frequency. For an interleaved acquisition of the on- and off-resonance, a pseudo-2D scheme was applied. The saturation was done by Gaussian pulses with a length of 50 ms and 60 dB of attenuation, done with an interpulse delay of 1 ms leading to an excitation bandwidth of about 4 Hz. The screening was done with 16 scans of on- and off-resonance scans each, with a 3 s saturation time. A 1H NMR experiment was performed for each compound to act as a reference spectrum in the STD experiments. NMR experiments were carried out at 25 °C. Spectra were processed and analyzed with TopSpin v4.0.8 (Bruker) and reported as chemical shifts (δ) in parts per million (ppm) relative to the solvent peak.
selected interaction geometries. Furthermore, selected single points were counterpoise corrected using the procedure of Boys and Bernardi\textsuperscript{140} to correct for basis set superposition errors (BSSE). Additional single point calculations were performed using the hybrid functional MO06-2X\textsuperscript{141} in combination with Grimme’s dispersion correction (D3).\textsuperscript{142} When necessary, hydrogen atoms were added before starting the optimization using Protonate 3D in MOE 2018.0101.\textsuperscript{143,144}

Spherical Scan. The fragment (chlorodifluoromethoxy)benzene was derived from asciminib in crystal structure SM04. Hydrogen atoms were added using the Protonate3D feature of MOE at default settings. Next, the fragment was freely optimized using MP2/TZVPP. Using a custom Python/PyMOL script, 500 evenly distributed points were placed on a sphere with the radius of 3.27 Å (identical with the distance between the chlorine atom in asciminib and the backbone oxygen of L448 in the crystal structure) were generated around the oxygen of the MP2/TZVPP-optimized backbone model system N-methylacetamide. For each of these points, a complex of (chlorodifluoromethoxy)benzene and N-methylacetamide was generated using the following procedure: The fragment was translated by placing the chlorine atom at the coordinates of this data point. Next, the fragment was rotated around the coordinates of the chlorine atom to obtain an optimal σ-hole angle of 180° between the fragment and the oxygen of N-methylacetamide. In a final step, the fragment was rotated around its C−Cl bond in steps of 60° to obtain six geometries per data point. In total, 3000 complexes were generated, and single point calculations on the MP2/TZVPP-level of theory were performed. The six complex formation energies of each data point were averaged and colored according to the supplied color scheme by using PyMOL.

Potential Energy Surfaces (PES). Model molecules with the general formula Ph−Y−CF\textsubscript{2}X (X = H, Cl, Br, I) were geometry-optimized using the MP2/TZVPP-level of theory. Starting from these geometry-optimized molecules, further geometries were generated by rotation along their rotatable axes (φ, ψ) with an increment of 10° of each step. For each newly generated geometry, single point energies were calculated using MP2/TZVPP. Contour maps of potential energy surfaces (PESs) with ΔE in kJ mol\textsuperscript{-1} as a function of dihedral angles φ and ψ were generated for amide (Y = NHCO) and ether (Y = O) derivatives, each with 1296 geometries (36 × 36). All PES plots were generated using OriginPro 2020.\textsuperscript{144}

Electrostatic Potential and V\textsubscript{max} Calculations. Molecules were geometry-optimized using the MP2/TZVPP-level of theory. For conformers A and B of ligand 23, heavy atoms were kept frozen during optimization. Electrostatic potentials and electron densities for all geometry-optimized molecules were calculated using MP2/TZVPP. Contour maps of potential energy surfaces (PESs) with ΔE in kJ mol\textsuperscript{-1} as a function of dihedral angles φ and ψ were generated for amide (Y = NHCO) and ether (Y = O) derivatives, each with 1296 geometries (36 × 36). All PES plots were generated using OriginPro 2020.\textsuperscript{144}

Distance Scans. Molecules were optimized using the MP2/TZVPP-level of theory. For the evaluation of the small molecule crystals, heavy atoms of the molecules were kept frozen during optimization. Geometries shown in Figure 6 were altered in steps of 0.1 Å along the C\textsubscript{N}−X vectors of the respective halogen bond donating molecule. In the systematic approach, the freely geometry-optimized model molecules (Ph−X, Ph−O−CF\textsubscript{2}X, and Ph−NHCO−CF\textsubscript{2}X) and N-methylacetamide were oriented toward each other using the same protocol to avoid arbitrary differences: The C\textsubscript{N}−X−O angle is equal to 180°, the X−O=C angle is equal to 120° (with X = H, Cl, Br, I), and the dihedral angle N−C−O−X is equal to 90°. The distances were varied with an increment of 0.1 Å of each step. Distances X−O (X = H, Cl, Br, I) ranged from 1.5 to 5.0 Å.

Structural Depictions. Structural depictions of calculated molecules were prepared using PyMOL 2.3.3,\textsuperscript{145} The depictions of the crystal structures were prepared with Mercury (v.2020.2).\textsuperscript{146}

Interaction Vectors and Pseudo-Interaction Points. Data used for this visualization were taken from the potential energy surface scans described above. Interaction vectors and pseudointeraction points were visualized using a custom Python/PyMOL-script. Interaction vectors were generated using CYLINDER and CONE objects and colored according to the provided energy scale. The direction of the vector was taken from the respective C\textsubscript{N}−X bond. Vector length was set to 2 Å. Only vectors within Δφ,Δψ = ± 90° of the geometry-optimized molecule are shown. The energy threshold was set to 20 kJ mol\textsuperscript{-1} with respect to the geometry-optimized minimum. Pseudointeraction points were generated by elongation of the C\textsubscript{N}−X vector by 3 Å. Then, these points were combined into one PyMOL object. The “vdw radius” of each point was altered to 0.2 Å, and a surface was drawn. Surface quality was set to 3.

Conformational Energy Calculations. Hydrogen atoms were added to conformers A and B of compound 23 using the Protonate3D feature of MOE 2018.0101\textsuperscript{143} and, subsequently, optimized using MP2/TZVPP. Heavy atoms were kept frozen during optimization. The difference in the conformational energy was derived from these optimized structures.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jmedchem.3c00634.

Details on synthesis procedures, HPLC chromatograms, HPLC purity summary, NMR spectra, Solubility plots, NMR shifts, Stability assay, Small molecular X-ray data, Protein sequences, STD NMR spectra, ITC thermograms, Protein X-ray data, Ligand definition in PDB structure, Alternative ESP plots of tool compounds, List of compounds and properties (PDF)

SMILES (CSV)

Molecular formula strings (SMILES) and associated solubilities and biological data (XLSX)

3D-ESPs of CF\textsubscript{2}X ether (MP4)

3D-ESPs of amide moieties (MP4)

Accession Codes

Crystallographic data for the compounds have been deposited in the Cambridge Crystallographic Data Centre, CCDC, 12 Union Road, Cambridge CB21EZ, UK. Copies of the data can be obtained free of charge on quoting the depository numbers 2248877 (6a), 2232102 (6b), 2232103 (6c), 2232104 (6d), 2232105 (6e), 2232106 (7a), 2232107 (7b), 2232108 (7c), 2232109 (7d), and 2232110 (7e). The JNK3 protein structure in complex with 23 was deposited in the PDB under the accession code 8BZP. This material is available free of charge via the Internet at http://pubs.acs.org.

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M.E. calculated logP/logS values and distance scans of observed interactions in small molecular crystals, conforma-

Author Contributions
F.M.B. envisioned the research. S.V. and F.M.B. conceptualized the experiments. S.V. performed the chemistry, GSH assays, microsomal stability assays, small molecule crystallizations, and conducted the STD NMR and ITC experiments. J.R. and J.S. performed supplemental ITC measurements. J.R. conducted the turbidimetric solubility assay. S.V. and J.S. prepared the protein and conducted the protein crystallization experiments. J.S. performed protein data reduction and structure refinement. M.E. calculated logP/logS values and distance scans of 7a,c,e crystals. M.O.Z. performed QM calculations with respect to observed interactions in small molecular crystals, conforma-
tional analysis, potential interaction space, tuning, strength, and geometry dependence of XB interactions, electrostatic potentials of compounds and V_{max} values. D.S. performed X-ray measurements and data refinement of small molecule crystal structures. B.D., M.O., and M.L. conducted and analyzed the MS measurements of the microsomal stability assays. M.K. set up the STD NMR experiments. T.S. granted access to the SLS beamline. S.V. conducted data analysis and reprocessing. S.V. prepared the original draft. S.V., M.O.Z., and F.M.B. reviewed and edited the manuscript. All authors have approved the final version of the manuscript.

Notes
The authors declare no competing financial interest.

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Jason Stahlecker received his B.Sc. and M.Sc. in Biochemistry at the University of Tübingen. Since 2022, he is a PhD candidate in the lab of Prof. Dr. Frank Boeckler. His main focus lies on elucidating molecular interactions between small molecules and their targets using biophysical techniques.

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ABBREVIATIONS

ChB, chalcogen bond; FBDD, fragment-based drug discovery; GSH, glutathione; HA, heavy atom; HB, hydrogen bond; HEFLib, Halogen-Enriched Fragment Library; ITC, isothermal titration calorimetry; JNK1, c-Jun N-terminal kinase 1 (mitogen-activated protein kinase 8); JNK3, c-Jun N-terminal kinase 3 (mitogen-activated protein kinase 10); KDN, dissociation constant; MFS, minimal final solubility; MIS, minimal instant solubility; PDB, Protein Data Bank; PES, potential energy surface; SAR, structure–affinity relationship; STD, saturation transfer difference; TZVPP, valence triple-ζ with two sets of polarization functions; XB, halogen bond

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