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# Halogen Bond: Its Role beyond Drug−Target Binding Affinity for Drug Discovery and Development

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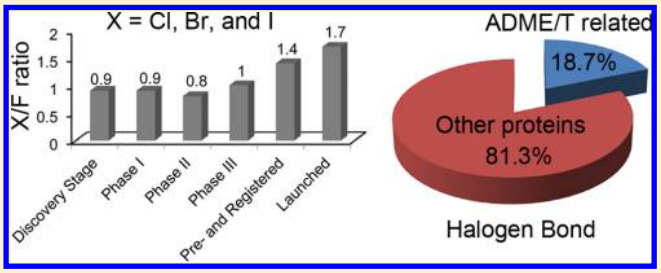
Supporting Information

**ABSTRACT:** Halogen bond has attracted a great deal of attention in the past years for hit-to-lead-to-candidate optimization aiming at improving drug-target binding affinity. In general, heavy organohalogens (i.e., organochlorines, organobromines, and organoiodines) are capable of forming halogen bonds while organofluorines are not. In order to explore the possible roles that halogen bonds could play beyond improving binding affinity, we performed a detailed database survey and quantum chemistry calculation with close attention paid to (1) the change of the ratio of heavy organohalogens to organofluorines along the drug discovery and development process and (2) the halogen bonds between organohalogens and nonbiopolymers or nontarget biopolymers. Our database survey revealed that (1) an obviously increasing trend of the ratio of heavy organohalogens to organofluorines was observed along the drug discovery and development process, illustrating that more organofluorines are worn and eliminated than heavy organohalogens during the process, suggesting that heavy halogens with the capability of forming halogen bonds should have priority for lead optimization; and (2) more than 16% of the halogen bonds in PDB are formed between organohalogens and water, and nearly 20% of the halogen bonds are formed with the proteins that are involved in the ADME/T process. Our QM/MM calculations validated the contribution of the halogen bond to the binding between organohalogens and plasma transport proteins. Thus, halogen bonds could play roles not only in improving drug−target binding affinity but also in tuning ADME/T property. Therefore, we suggest that albeit halogenation is a valuable approach for improving ligand bioactivity, more attention should be paid in the future to the application of the halogen bond for ligand ADME/T property optimization.




## 1. INTRODUCTION

Halogen bond (XB), a highly directional and specific interaction that acts in analogous to classical hydrogen bond (HB), is formed between a covalently bonded halogen atom (e.g., C−X, X = Cl, Br, I; XB donor) and a nucleophile (i.e., Lewis base; XB acceptor).[1−12] Due to the anisotropy of the charge distribution of halogen atoms,[13] a positively charged electrostatic region on the extension of C−X bonds, termed σ-hole, interacts attractively with the nucleophile.[6−8,14−22] Over the past few years, halogen bond has attracted more and more attention in drug discovery.[23−28] However, a detailed role of halogen bond is still difficult to elucidate.

It takes about 13 years to launch one new medicine (New Molecular Entity, NME) with the average cost of $1,778 million.[29] The drug discovery stage usually takes 5.5 years, while the drug development stage takes 8 years (Figure S1 in the Supporting Information). It is well recognized that halogen bond could play an important role during hit identification and lead optimization.[9,24,28,30−42] Some successful case studies have been reported. For example, a series of halogenated compounds targeting phosphodiesterase type 5 (PDE5) were designed and synthesized. The halogen bond between the phenolate oxygen atom of Y612 of PDE5 and the new halogenated inhibitors was validated by X-ray crystal structures (PDB ID: 3SIE).[24] Another series of halogenated compounds were synthesized as human Cathepsin L (hCatL) inhibitors.[9] In comparison with the unsubstituted phenyl derivative, the introduction of halogen bond between the 4-chlorophenyl moiety of the ligand and the backbone carbonyl oxygen of Gly61 in hCatL enhances the binding affinity by a factor of 13 (PDB ID: 2XU1).[9] A strong halogen bond was also observed between the backbone carbonyl oxygen of Leu112 in the α4β2 subtype of the nicotinic acetylcholine receptor (nAChR) and its halogenated antagonist. This specific halogen bond plays an essential role in establishing strong intersubunit anchoring that improves the drug efficacy (PDB ID: 3U8N).[42] Halogen bond could also be used for designing ligands to overcome drug resistance. A halogen bond between the iodine atom of an HIV-1 reverse transcriptase inhibitor and the backbone carbonyl oxygen of Tyr188 of the transcriptase not only enhances the affinity of the ligand but also avoids the arise of drug resistance (PDB ID:

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2BE2).[30] Nevertheless, all the examples shown above are aimed at improving the drug−target binding affinity in drug discovery stage. It remains unclear how important or prevalent the halogen bonds is to play other roles beyond drug−target binding affinity. Thus, it is of significance to perform a systematic study to explore its other potential roles in drug discovery and development process.

This study is intended to investigate the potential role of halogen bond beyond drug−target binding affinity through comprehensive database survey and quantum mechanics/molecular mechanics (QM/MM) calculations. The survey was performed with close attention paid to (1) the change of the ratio of heavy organohalogens to organofluorines along the drug discovery and development process and (2) the halogen bond between organohalogens and nonbiopolymers or non-target biopolymers. Our statistic and QM/MM calculation results revealed that more organofluorines are eliminated than heavy organohalogens during the drug development stage and that halogen bond involves the binding of organohalogens to the proteins that are involved in the ADME/T (absorption, distribution, metabolism, excretion, and toxicity) process. Accordingly, we suggest that more effort should be devoted in the future to address the potential role of halogen bond beyond the binding affinity, especially in optimizing ADME/T property.

## 2. MATERIALS AND METHODS

**Database Survey.** As most organofluorines (especially in pharmaceuticals) could not serve as halogen bond donors,[3,16,43,44] the ratio of heavy organohalogens (i.e., organochlorines, organobromines, and organoiodines) to organofluorines (called X/F ratio hereinafter) is calculated in the study, which could be used as an indicator for the possibility of halogen bond formed by organohalogens in each stage of drug discovery and development.

Three databases are used for the statistical analysis of organohalogens and halogen bonds in this article, viz., Thomson Reuters Pharma, ZINC (ZINC Is Not Commercial), and PDB (Protein Data Bank). Thomson Reuters Pharma collects comprehensive information on drug R&D, especially on drug development. The drug development stage in Thomson Reuters Pharma is divided into clinical phase I, clinical phase II, clinical phase III, preregistered, registered, and launched in chronological order. The statistical analysis of organohalogens in Thomson Reuters Pharma was performed by a substructure search for C−X (X = F, Cl, Br, and I). As small molecule drugs, especially the halogenated drugs, were our particular focus in this study, the search and the X/F ratio calculation were performed for the molecules with molecular weight less than 1,000.

ZINC is the most frequently used public resource for virtual screening, which contains over 21 million purchasable compounds over the world.[45,46] Standard subsets in ZINC are the approximations to the compounds appearing commonly in the literature, representing current interests in the drug discovery field.[45] These subsets include (1) 'Lead-like' subset, 4,628,981 unique molecules, with molecular weight between 250 and 350, xlogp ≤3.5, and the number of rotatable bonds no more than 7; (2) 'Fragment-like' subset, 275,053 unique molecules, with molecular weight less than or equal to 250, xlogp ≤3.5, and the number of rotatable bonds no more than 5; (3) 'Drug-like' subset, 9,658,014 unique molecules, with molecular weight between 150 and 500, xlogp ≤5, the number of rotatable bonds ≤7, no more than 5 hydrogen bond donors, and no more than 10 hydrogen bond acceptors; (4) 'All Purchasable' subset, 11,549,939 unique compounds purchasable for rapid testing of docking hypotheses (Table S1 in the Supporting Information).

The current PDB (April 2013 release) was used in the survey. As there are two types of halogen bond in PDB, i.e., C−X···Y (Y = O, N, or S) and C−X···$\pi$ (Figure 1).[47,48] The criteria

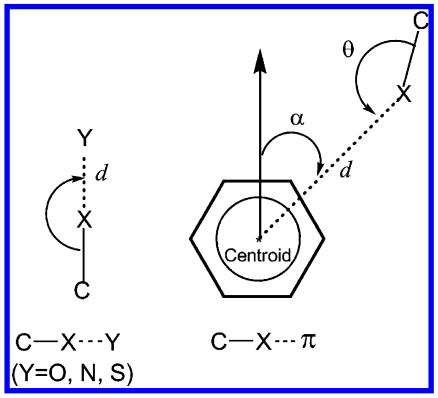


**Figure 1.** Two types of halogen bond, i.e., C−X···Y and C−X···$\pi$ in PDB.

were set as X···Y distances shorter than the sum of vdW radii[49] ($d$(Cl···O) < 3.27 Å, $d$(Br···O) < 3.37 Å, $d$(I···O) < 3.50 Å, $d$(Cl···N) < 3.30 Å, $d$(Br···N) < 3.40 Å, $d$(I···N) < 3.53 Å, $d$(Cl···S) < 3.55 Å, $d$(Br···S) < 3.65 Å, $d$(I···S) < 3.78 Å), and the C−X···Y angle is larger than 140°.[47] For C−X···$\pi$ halogen bond, $\pi$ systems from aromatic residues (Phe, Tyr, His, and Trp) are considered in this study with the following criteria: $d$(Cl···$\pi$) < 4.2 Å, $d$(Br···$\pi$) < 4.3 Å, $d$(I···$\pi$) < 4.5 Å, $\alpha$ < 60° and $\theta$ > 120°.[47] To achieve reliable results, only those high quality structures with resolution better than 2.5 Å or determined by solution NMR were chosen for further analysis. The protein structures with halogen bond may have three kinds of stoichiometry, i.e. monomer, homomer, and heteromer. Identical halogen bonds may exist in homomer and heteromer, as well as NMR structures, which would bias the results and thus were removed.

**QM/MM Calculations.** Two systems with crystal structures, viz., transthyretin (PDB ID: 1SN0[50]) and serum albumin (PDB ID: 1E7C[51]), were selected as examples for the QM/MM calculations of halogen bonds with the two-layer ONIOM (our own N-layered integrated molecular orbital and molecular mechanics) method.[52−54] Because the four subunits are almost identical in 1SN0, and the ligands lie between each two subunits (chain A, C and chain B, D), chains A and C were removed (Figure 2a). As for 1E7C, only the ligand involved in the halogen bond was kept, while the other small molecules were all removed (Figure 2b). The p$K_a$ values of ionizable residues in the proteins were calculated by the H++ Web site[55−57] at the pH values of the crystallization, and hydrogen atoms were added accordingly. The ligands and the corresponding protein backbones that form halogen bonds were included in the QM region which was described at the M06-2X/LanL2DZ level of density functional theory (DFT) for 1SN0 and M06-2X/6-311G++(d, p) for 1E7C. The M06-2X method[58] has been reported to give good performance on the optimization of the halogen bond complexes.[59−63] The MM


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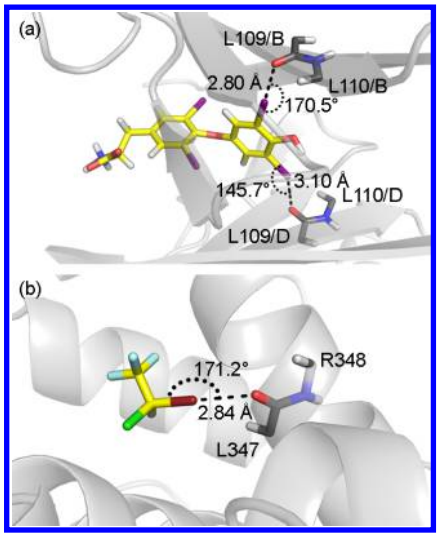


**Figure 2.** Optimized structures of full models at the ONIOM level for (a) transthyretin (PDB ID: 1SN0, M06-2X/LANL2DZ) and (b) serum albumin (PDB ID: 1E7C, M06-2X/6-311G++(d, p)). Atoms in the QM layer are presented as sticks with carbon atoms in yellow for ligands and gray for proteins, hydrogen in white, oxygen in red, nitrogen in blue, fluorine in cyan, chloride in green, bromine in brown, and iodine in purple. Atoms in the MM layer are shown as cartoon. The RMSD values between the crystal structures and the QM/MM optimized conformations of the heavy atoms in the QM layer are 1.32 Å and 1.07 Å for transthyretin and serum albumin, respectively, indicating that the optimized structures should be reliable.

layer of the system was described by the AMBER parm96 force field.[64]

The QM/MM optimization was carried out without any constrains. The QM layer of the model was then picked up for single-point energy calculation at the MP2/aug-cc-pVDZ level, while the SDD basis set was adapted for iodine atoms in the 1SN0 system (Figure 2a). The binding energies between the ligands and the protein backbones were then assessed from eq 1

$$\Delta E = E_{com} - (E_{lig} + E_{BB}) + BSSE \quad (1)$$

where $\Delta E$ is the binding energy, $E_{com}$ is the energy of the whole complex in the QM layer, $E_{lig}$ and $E_{BB}$ are the energies of the ligand and the backbone of the protein in the QM layer, respectively, and *BSSE* stands for the basis set superposition error corrections.[65] All these calculations were carried out with *Gaussian* 09 suite of programs.[66]

## 3. RESULTS AND DISCUSSION

**3.1. The Percentage of Organohalogens at Different Drug Development Stages.** Statistical analysis of organohalogens in different stages of drug discovery and development process may shed some light on the role of halogen bond in the process. The molecules in discovery stage represent the current opinions and interests in drug discovery, and the drug molecules in clinical phases represent the opinions and interests in drug discovery about a decade ago, while the launched phase collects the successful drugs over the past one hundred years. In Thomson Reuters Pharma, the organohalogens content are 34.6%, 36.4%, 29.8%, 36.4%, 33.1%, and 25.5% in drug discovery stage, clinical phase I, clinical phase II, clinical phase III, preregistered and registered, launched stages, respectively (Figure 3 and Table S2 in the Supporting Information). What is particularly worth mentioning is that more halogenated drugs (34%) are in clinic trials or at the stage of pre- and registered than that in the drugs launched (26%), implying that halogenations have been much appreciated nowadays. It is well-known that natural products contain little halogens in their structures. Therefore, halogenation should be an invaluable approach for the structural modification of natural products for drug development.

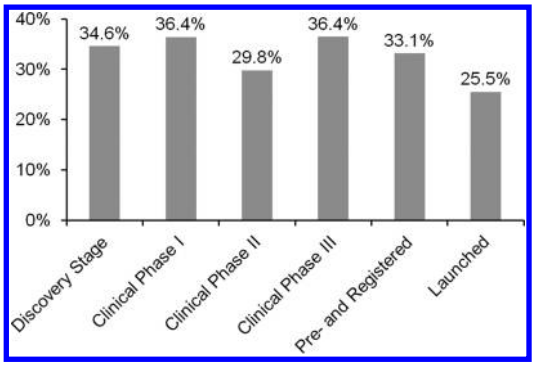


**Figure 3.** The percentage of organohalogens in the drug discovery (discovery stage) and development.

**3.2. The Ratios of Heavy Organohalogens to Organofluorines (X/F Ratios) at Different Drug Development Stages.** The X/F ratios in each stage were analyzed. In general, the larger the ratio is, the more prevalent the halogen bond is. As shown in Figure 4, the X/F ratios are 0.9, 0.9, 0.8, 1.0, 1.4, and 1.7 for drug discovery stage, clinical phase I, clinical phase II, clinical phase III, preregistered and registered, and launched stages, respectively. The content of heavy organohalogens increasing steadily from clinical phase II (44.0%) to the launched phase (63.3%) revealed that more organofluorines are worn and eliminated than heavy organohalogens during the drug development process (Figure 4 and Table S2 in the Supporting Information), suggesting that more attention should be paid to heavy organohalogens than organofluorine during drug discovery stage. The overall increasing trend for the X/F ratio in the drug discovery and development process implies a significant role for halogen bond in bringing a new drug to the market from hit identification.

**3.3. The Halogens Content in Different Chemical Libraries Derived from ZINC.** As for ZINC, the organohalogens occupy 27.0%, 16.8%, 33.1%, and 32.3% of the 'Lead-like' subset, 'Fragment-like' subset, 'Drug-like' subset, and 'All Purchasable' subset, respectively (Figure 5). Except for the 'Fragment-like' subset, the organohalogens content in ZINC is similar to that in Thomson Reuters Pharma, indicating that the value of organohalogens is highly appreciated nowadays in the drug discovery field. The relatively low content of organohalogen in 'Fragment-like' subset shows that more attention should be paid to the role of organohalogens in fragment-based drug design, which is in agreement with the opinion of Wilcken et al.[25,26]

The X/F ratios in ZINC are 0.6, 1.0, 0.8, and 0.9 for 'Lead-like', 'Fragment-like', 'Drug-like', and 'All Purchasable' subsets, respectively (Figure 6a-d), which are similar to the results of Thomson Reuters Pharma in the early stage of drug development. In comparison with the ratio for the compounds in clinical phases and launched phase, we may start with too many organofluorines during the drug discovery stage.


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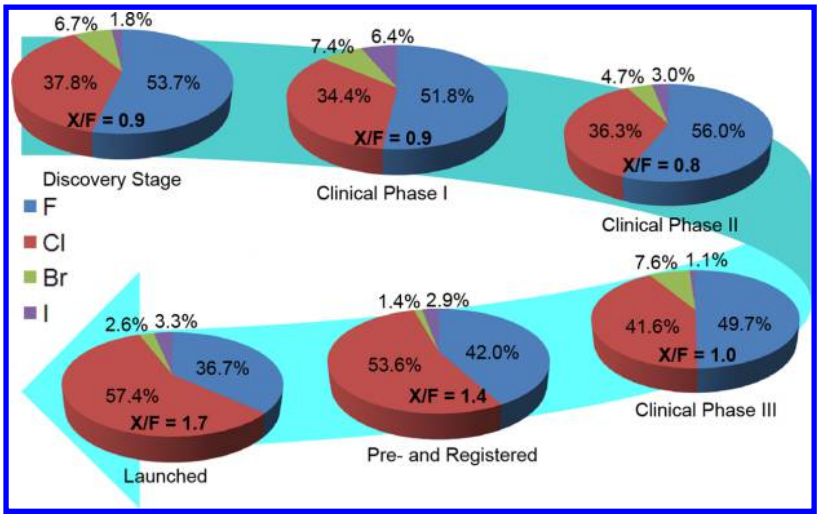


**Figure 4.** Composition of the organohalogens in different stages during drug discovery and development.

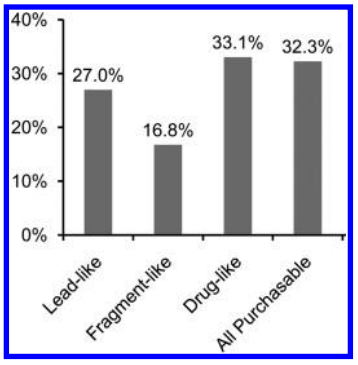


**Figure 5.** The percentage of organohalogens in the standard subset of ZINC ('Lead-like', 'Fragment-like', 'Drug-like', and 'All Purchasable').

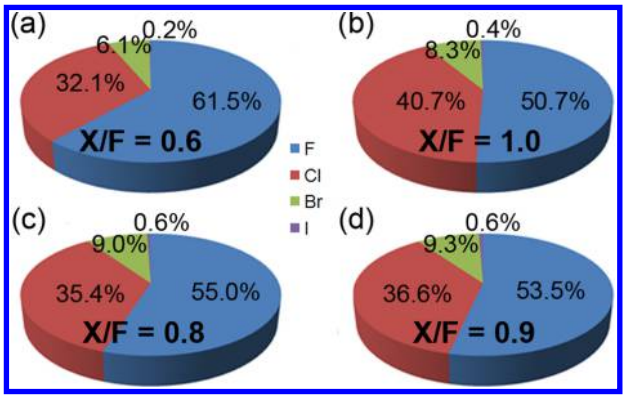


**Figure 6.** Composition of the organohalogens in the standard subset of ZINC: (a) Lead-like, (b) Fragment-like, (c) Drug-like, (d) All Purchasable.

Therefore, heavy-halogenation should be paid more attention during drug development process.

**3.4. Halogen Bond between Organohalogens and Water or ADME/T Related Proteins in PDB.** Among the 2,462 PDB structures that have heavy halogenated ligands, 791 structures were found to possess 1,295 halogen bonds. If using X···Y distances less than ΣvdW + 0.2 Å to account for uncertainties in the structures,[67,68] 1,894 halogen bonds were observed in 1,006 PDB structures. As shown in Figure 7a, 75.0% of the structures are determined by X-ray crystallography with a resolution better than 2.5 Å, while 0.8% are determined by solution NMR (high quality structures). After the redundancy were removed, 598 high quality structures containing 778 halogen bonds were yielded, which were used for further analysis. As shown in Figure 8a, 82.4% halogen bonds are formed between heavy organohalogens and biomacromolecules. To further investigate the role of halogen bond in drug discovery and development, the biopolymers that formed halogen bonds were analyzed from a variety of aspects. As shown in Figure 7b, more than one-half of the halogen bonds were found in human biomacromolecules. Based on SCOP (Structural Classification Of Proteins) classification, halogen bonds reside in various kinds of proteins i.e., all beta proteins (27.0%), alpha and beta proteins (a+b) (22.6%), alpha and beta proteins (a/b) (19.0%), all alpha proteins (16.1%), small proteins (12.5%), multidomain proteins (alpha and beta) (2.4%), and membrane and cell surface proteins and peptides (0.4%) (Figure 7d). With regard to enzyme classification, 38.1% halogen bonds reside in hydrolases, 34.0% in transferases, 21.7% in oxidoreductases, 2.7% in lyases, 1.9% in isomerases, and 1.7% in ligases (Figure 7c). The structural and functional diversity of the biopolymers implies other roles that halogen bond could play besides enhancing drug-target binding affinity. Indeed, based on the annotations (mostly from Uniprot and DrugBank), 112 of the 598 structures (18.7%) are found to be involved in the ADME/T process (Table S3 in the Supporting Information), e.g., cytochrome P450 proteins. Of the drugs cleared via metabolism, about 3/4 are metabolized by the cytochrome P450 superfamily.[69,70] Figure 8b is an example of typical halogen bonds in cytochrome P450, which is a C−Cl···$\pi$ type between a chloride and the phenyl ring of F365 in cytochrome P450 2B4 with $d$(Cl···$\pi$) = 3.83 Å, $\alpha$ = 24.7°, and $\theta$ = 132.1° (PDB ID: 3TMZ[71]).

There are 16.6% of the halogen bonds formed between heavy organohalogens and water molecules (Figure 8a). This kind of halogen bond should not be overlooked.[72] When a drug is administrated, it undergoes a very complicated process to exert therapeutic effect. Halogen bonds formed with water would play an important role since most of the process takes place in an aquatic environment. Quantum chemistry study has already demonstrated that halogen bonds formed with water are more thermodynamically stable than other water-involved interactions.[72] Figure 8c shows a typical C−Br···O halogen bond


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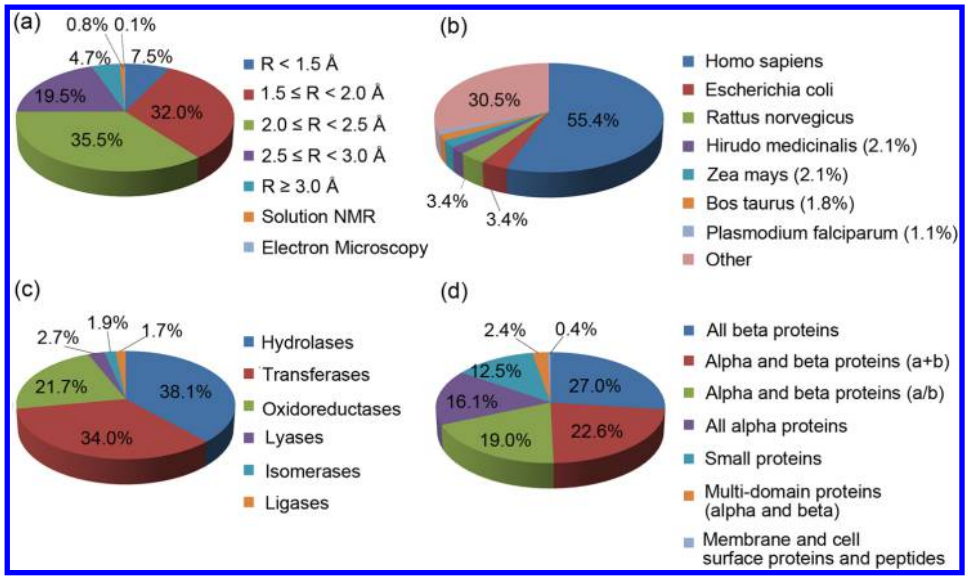


**Figure 7.** Halogen bonds in PDB. (a) Structures containing halogen bonds determined by different experimental methods. R stands for resolution. (b) Halogen bonds in different organisms. The proteins that could form a halogen bond are classified according to enzyme classification (c) and structural classification of proteins (SCOP) (d).

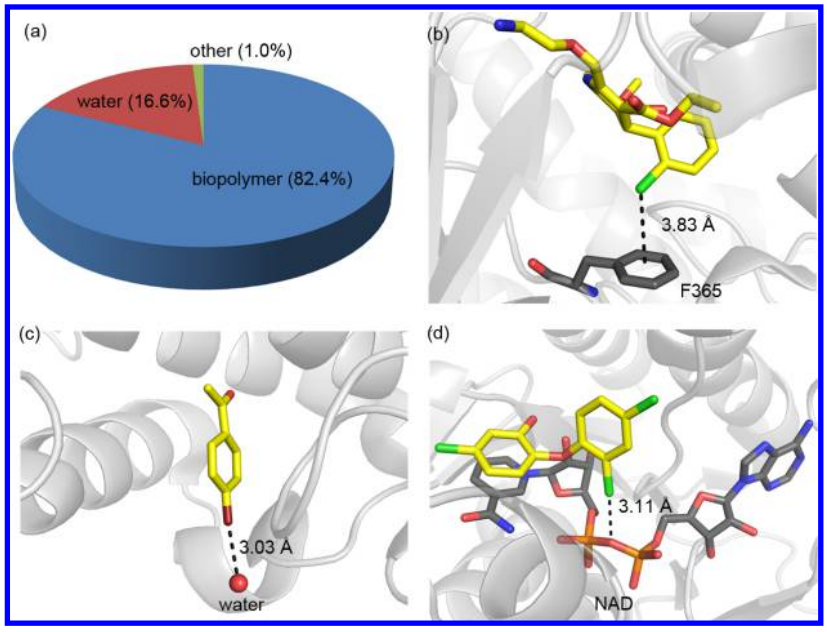


**Figure 8.** Halogen bonds formed between heavy organohalogens and biopolymer/water/other molecule. (a) The percentage composition of the three kinds of XB acceptors in PDB. Halogen bonds formed between heavy organohalogens and biopolymer (rabbit cytochrome P450 2B4) (b), water (c), and cofactor (d). Residues participating in halogen bonds are shown as sticks with carbon atoms in yellow for ligands and gray for protein/cofactor, oxygen in red, nitrogen in blue, phosphorus in orange, chloride in green, and bromine in brown. The rest of the proteins are shown in cartoon representation.

formed between a bromide and a water molecule in phospholipase A2 homologue bothropstoxin-1 (PDB ID: 3I03[73]). As many water molecules could not be resolved in a structure with a resolution better than 2.0 Å, the percentage of halogen bonds formed with water should be even higher in the real biological world.

Furthermore, halogen bonds could be formed between heavy organohalogens and other nonbiopolymers, e. g., cofactors, which account for 1% of all halogen bonds (Figure 8a). Figure 8d is an example of a C−Cl···O halogen bond with the cofactor NAD in Enoyl-[acyl-carrier-protein] reductase [NADH] with $d$(Cl···O) = 3.11 Å and ∠(C−Cl···O) = 168.1° (PDB ID: 3PJF[74]). Nevertheless, the statistic results demonstrated the high possibility and complicated roles of halogen bonds beyond improving drug-target binding affinity.

**3.5. The Geometrical and Energetic Parameters of Some Typical Halogen Bond with ADME/T Related Proteins.** Table 1 summarized some typical halogen bond systems from PDB with proteins related to the ADME/T property. For example, transthyretin, a plasma transport protein that was involved in the distribution of thyroid hormones and vitamin A, was found to transport many approved drugs, e.g., levothyroxine, liothyronine, liotrix, diflunisal, diclofenac, and diethylstilbestrol.[50,75−77] Among the halogenated compounds,


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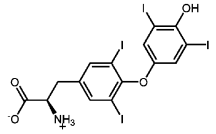
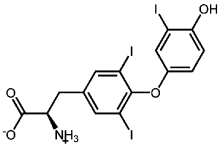
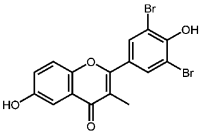
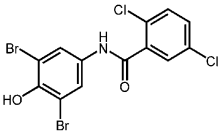
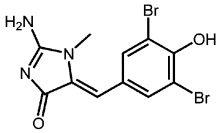
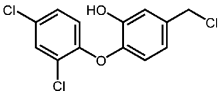
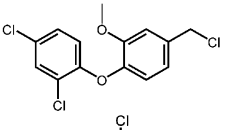
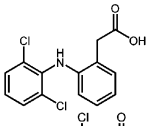
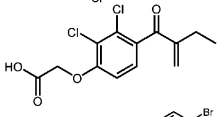
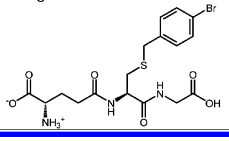
**Table 1. Summary of Typical Halogen Bonds in Selected ADME/T-Associated Protein-Ligand Complexes**

| Protein | Ligand name | Ligand structure [a] | PDB ID | XB acceptor | d(X···D), Å | ∠(C–X···D), deg |
|---|---|---|---|---|---|---|
| Transthyretin | T4 |  | 1ETA<br>1ETB<br>1SN0 | Ala109 O<br>Thr109 O<br>Leu109 O | 3.13<br>3.27<br>3.30 | 149.4<br>159.7<br>169.7 |
| Transthyretin | T3 |  | 1THA | Ser117 O | 3.22 | 144.8 |
| Transthyretin | FL8 |  | 1KGJ | Ser717 O | 3.35 | 150.1 |
| Transthyretin | DZ2 |  | 3ESO | Thr118 N | 3.15 | 165.3 |
| Transthyretin | 3M2 |  | 3P3S | Phe64 O | 3.27 | 169.3 |
| Transthyretin | FT2 |  | 4ABV | Thr119 OG1 | 2.69 | 159.1 |
| Transthyretin | 43F |  | 4AC2 | Thr119 OG1<br>HOH 2081 O | 2.63<br>3.15 | 151.8<br>161.8 |
| Serum albumin | HLT |  | 1E7B<br>1E7C | Leu347 O<br>Leu347 O | 3.22<br>3.18 | 150.9<br>149.1 |
| Cytochrome P450 2C5 | DIF |  | 1NR6 | HOH 853 O | 2.85 | 158.0 |
| Glutathione S-transferase P | EA |  | 2GSS<br>3KM6<br>3N9J | Tyr7 OH<br>HOH 414 O<br>Tyr7 OH | 3.10<br>2.96<br>2.89 | 162.3<br>163.2<br>159.6 |
| Glutathione S-transferase | 0HG |  | 3FRC | Asn111 ND2 | 3.32 | 146.6 |

[a]Halogen atoms that participate in halogen bond were depicted in red.

T4 and T3 are of great interest because they are both approved drugs targeting thyroid hormone receptors to execute their therapeutic effects. T4, thyroid hormone thyroxine, also levothyroxine or L-thyroxine, is used to treat hypothyroidism, chronic lymphocytic thyroiditis, goiter, myxedema coma, and stupor.[78] T3, L-triiodothyronine thyroid hormone, also liothyronine, is used to treat hypothyroidism.[78] The binding energy involved in halogen bond between T4 and transthyretin was calculated by QM/MM to be −3.32 kcal/mol (Figure 2a), manifesting that halogen bonds could enhance the binding between the drug and the transport protein, consequently, should play an import role in the distribution of drugs.

Another distribution related protein is serum albumin, the main plasma transport protein, which has a good binding capacity for many drugs. As shown in Table 1, HLT (halothane), a general inhalation anesthetic acting on multiple ion channels, is an approved drug which could form halogen bonds with serum albumin (PDB IDs: 1E7B and 1E7C). The calculated QM/MM binding energy correlating with halogen bond between halothane and serum albumin is −3.34 kcal/mol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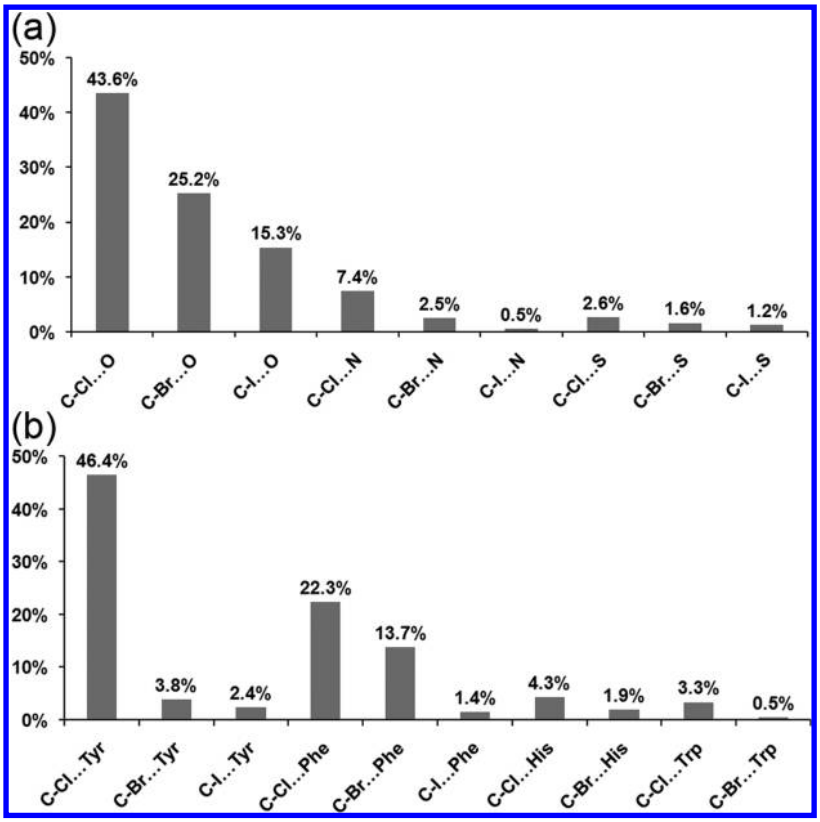


**Figure 9.** Halogen bonds in PDB. (a) C−X···Y halogen bonds are classified as C−Cl···O, C−Br···O, C−I···O, C−Cl···N, C−Br···N, C−I···N, C−Cl···S, C−Br···S, and C−I···S. (b) C−X···$\pi$ halogen bonds are classified as C−Cl···Tyr, C−Br···Tyr, C−I···Tyr, C−Cl···Phe, C−Br···Phe, C−I···Phe, C−Cl···His, C−Br···His, C−Cl···Trp, and C−Br···Trp.

(Figure 2b), manifesting again that halogen bond strengthens the binding of halothane to serum albumin, hence assisting the distribution of halothane.

Drug metabolism refers to the biotransformation of drug molecules, which is vital in defining the pharmacological and toxicological profile of drugs, attracting a particular interest in drug discovery and development process.[79] Figure 8b shows a C−Cl···$\pi$ halogen bond between cytochrome P450 and an antihypertensive drug amlodipine that targets calcium channel. Table 1 presents another halogen bond between cytochrome P450 and an anti-inflammatory drug diclofenac (DIF) that targets prostaglandin G/H synthase (PDB ID: 1NR6). It should be noted that this halogen bond is bridged by a water molecule, further demonstrating the role of halogen bonds formed with water molecules.

Toxicity is a key issue concerned during drug development. Glutathione S-transferases (GSTs) are well-known as their ability to conjugate the reduced glutathione to a large number of drugs for the purpose of detoxification. The halogen bonds could play a role in the detoxification process in aiding the binding of halogenated compounds to GSTs (Table 1). For example, EA (ethacrynic acid), an approved drug for the treatment of edema and high blood pressure that acts on sodium/potassium-transporting ATPase and solute carrier family, was found to form halogen bonds with GSTs (PDB IDs: 2GSS, 3KM6, and 3N9J). In addition, GSTs are phase II metabolic isozymes, implying a role of halogen bonds in drug metabolism process. Therefore, halogen bond could be used to optimize ligand ADME/T property.

**3.6. The Prevalent C−Cl···O Halogen Bond in PDB.** Among the 778 halogen bonds, 567 are C−X···Y contacts and 211 are C−X···$\pi$ contacts. For the C−X···$\pi$ contacts, some of them might be lone pair···$\pi$ interaction formed between the negative belt of the halogen atom and $\pi$ system.[80,81] So C−X···Y halogen bonds are more prevalent in biological systems, which is in agreement with the previous study.[47] C−X···O halogen bonds account for 84.1% of C−X···Y halogen bonds with C−Cl···O halogen bonds accounting for 43.6% (Figure 9a). In addition, there are 7.4% C−Cl···N, 2.5% C−Br···N, 0.5% C−I···N, 2.6% C−Cl···S, 1.6% C−Br···S, and 1.2% C−I···S halogen bonds (Figure 9a). Therefore, chlorination should be given the top priority during lead optimization for drug development.

**3.7. Halogen Bond with Backbone and Side Chain.** Among the 567 C−X···Y halogen bonds, 430 are formed between halogen atoms and protein residues. As shown in Figure 10, all of the twenty amino acids could form C−X···Y halogen bonds, with C−X···Leu accounting for 10.5%. 64.6% C−X···Y halogen bonds are formed with mainchain and 35.4% with side chain, manifesting that C−X···mainchain halogen bonds are dominant (Figure 10). However, for Tyr, Met, Cys, Thr, Asn, and Gln, C−X···side chain plays a more important role (Figure 10). For C−X···$\pi$ halogen bonds, nearly half are formed between chloride atom and tyrosine while 22.3% are C−Cl···Phe halogen bonds, and 13.7% are C−Br···Phe halogen bonds (Figure 9b). In addition, there are some C−Br···Tyr, C−I···Tyr, C−I···Phe, C−Cl···His, C−Br···His, C−Cl···Trp, and C−Br···Trp halogen bonds (Figure 9b).

## 4. CONCLUSIONS

About 13 years and $1,778 million on average are needed to launch one new medicine. The drug discovery stage usually


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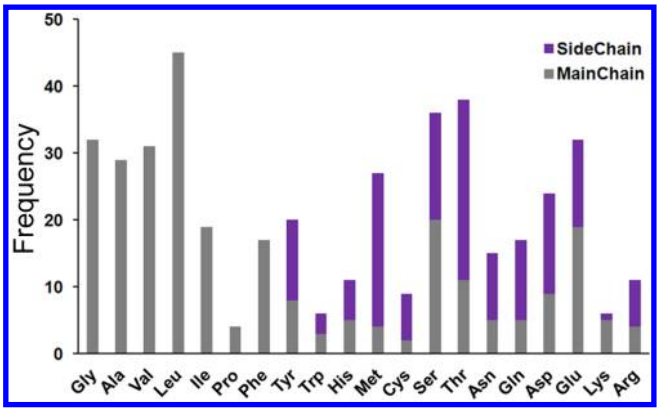


**Figure 10.** The frequency of the C–X···Y halogen bond between 20 amino acids and organohalogens.

takes 5.5 years, while the drug development stage takes 8 years. Halogen bond has gained widespread interest in the past years for hit-to-lead-to-candidate optimization aiming at improving drug–target binding affinity. In general, heavy organohalogens are able to form halogen bonds while organofluorines are not. Our database survey in this study revealed that halogenations have been highly appreciated nowadays. Meanwhile, the survey also showed an obviously increasing trend of the ratio of heavy organohalogens to organofluorine along drug discovery and development process, demonstrating that more fluorinated leads and candidates are eliminated than other organohalogens as drug discovery and development projects move on, revealing a more affirmative role for halogen bond in drug development stage. Impressively, apart from 82.4% halogen bonds formed between heavy organohalogens and biopolymer, 16.6% halogen bonds are formed between heavy organohalogens and water molecules. Considering that most biopolymers reside in an aquatic environment and a drug may interact with many kinds of biopolymers to exert therapeutic effect, these 16.6% halogen bonds would play even more complex physiological roles than we have recognized. Furthermore, halogen bonds were observed in the proteins with different functional characteristics, including hydrolases, transferases, oxidoreductases, lyases, isomerases, ligases, and transporters, nearly 20% of which are involved in the ADME/T process. QM/MM calculation results confirm the geometrical parameters of the halogen bonds between drugs to plasma transport proteins (transthyretin and serum albumin) and showed an attractive binding energy, which help the distribution of drugs. In addition, halogen bonds were found to participate in the metabolism and detoxification process.

In conclusion, halogen bond could play a positive role not only in improving drug–target binding affinity but also in tuning ADME/T property. Thus, more attention should be paid in the future to the application of halogen bond for ligand ADME/T property optimization.

## ■ ASSOCIATED CONTENT

### Ⓢ Supporting Information

An overview of the drug discovery and development process. Statistical analysis of organohalogens in Thomson Reuters Pharma and ZINC. 112 high quality structures that were involved in the ADME/T process. This material is available free of charge via the Internet at http://pubs.acs.org.

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### Author Contributions

§These authors contributed equally to the work.


### Notes

The authors declare no competing financial interest.

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