Halogen Bonding in Nucleic Acid Complexes

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Abstract

Halogen bonding (X-bonding) has attracted notable attention among noncovalent interactions. This highly directional attraction between a halogen atom and an electron donor has been exploited in knowledge-based drug design. A great deal of information has been gathered about X-bonds in protein-ligand complexes, as opposed to nucleic acid complexes. Here we provide a thorough analysis of nucleic acid complexes containing either halogenated building blocks or halogenated ligands. We analyzed close contacts between halogens and electron-rich moieties. The phosphate backbone oxygen is clearly the most common halogen acceptor. We identified 21 X-bonds within known structures of nucleic-acid complexes. A vast majority of the X-bonds is formed by halogenated nucleobases, such as bromouridine, and feature excellent geometries. Noncovalent ligands have been found to form only interactions with suboptimal interaction geometries. Hence, the first X-bonded nucleic-acid binder remains to be discovered.

Introduction

Bringing a new drug to the market consumes enormous intellectual and financial resources. It typically takes more than a decade from the discovery of an active compound (lead) to the final approval of a drug, which is based on it. The drug discovery and development declined from the trial-and-error approach when the number of recognized diseases steeply rose. Nowadays, the workflow stems from knowledge gained in a variety of studies believing that this can speed-up the whole drug development.

The number of known drug targets is slightly higher than 300. This amount is not extraordinarily high bearing in mind the number of recognized human genes (less than 20,000). Among the targets, there are mostly proteins and only a few nucleic acids (NAs). Given that NAs are ubiquitous biopolymers with a myriad of cellular functions though, they represent a clinically prominent class of targets.

Many strategies have appeared to optimize the lead compound into a therapeutic substance. The use of halogens is in this sense traditional. Xu et al. estimated that about 25% of approved drugs are halogenated, and the portion is similar in all stages of drug discovery and development. Halogen atoms modulate physicochemical properties of the molecular scaffolds; they affect the polarity and hydro/lipophilicity, which in turn changes membrane and blood brain barrier permeation of the molecule. Also, carbon-halogen covalent bond is difficult to metabolize, so the halogenation prolongs the lifetime of the active compound, but at the same time might increase its liver toxicity.
Apart from nonspecific effects, it was about a decade ago recognized that halogens might partake in a structurally specific and directional noncovalent interaction called a halogen bond (X-bond). The X-bond is an interaction between a halogen and a Lewis base or an electron-rich moiety. The electron density donors may be represented by electronegative atoms such as oxygen, nitrogen, sulfur, but also by aromatic rings or conjugated π-systems.

The X-bond has been found in many protein-ligand complexes including pharmaceutically relevant ones (for review see Refs. 6, 7, and 8). There have been only a few studies on X-bonds, where NAs played a role. The distinguished exceptions are the efforts of Shing Ho and co-workers. They focused on halogen bonding in so-called Holliday junction, a four-stranded (branched) complex of deoxyribonucleic acid (DNA). Using bromouridine as a building block, they directed a DNA into one of the several nearly isoenergetic conformers. The DNA model system also demonstrated that among the halogens it is the bromine that has the most favorable entropy/enthalpy compensation. Consequently, bromine was claimed an optimal element for X-bonding in DNA Holliday junctions.

To the best of our knowledge, no ligand has been reported so far to form an X-bond in any NA complex. The lack of information on X-bonding in NAs is somewhat surprising because NAs are naturally rich in electronegative atoms which make them (in theory) prospective X-bond acceptors. It seems that there is a missing relation between the worlds of NAs and X-bonds. This work aims at building such a relation. Hence, we continue with the introductions of the two worlds trying to find some overlap between them and highlight the pharmaceutical significance. Later, we analyze known structures of NAs and reveal main features of their complexes. Finally, we discuss all of the few examples of low-molecular compounds whose halogens are involved in interactions with electron donors.

### Halogen Bonding Features

The attraction of halogens with other electronegative atoms was observed as early as the 1950s in the crystallographic studies of Hassel et al. although the synthesis of X-bonded complexes dates back to 19th century. The puzzling nature of the attraction between two electron-rich chemical groups was attributed merely to charge transfer effects until Politzer et al. came up with a simple model explaining many of the X-bond features. Based on quantum chemical calculations of the molecular electrostatic potentials (MEPs) they proposed that the surface of halogens contains regions of both positive as well as negative electrostatic potential (ESP). The positive region was labeled a sigma-hole (σ-hole) (Fig. 1), and interestingly enough this label appeared only 15 years after its first evidence. The X-bond has been exploited in many areas of chemistry and material science (reviewed e.g. in Refs. 5 and 18) and a great amount of work has been done on the theoretical aspects of X-bonds too.

![Molecular structure of bromouracil](a), the intersection of a molecular surface (b), and full ESP in atomic units (au) projected onto the molecular surface (c). The blue disc of positive ESP in the forefront is the σ-hole.

X-bonds are similar in strength to the more common hydrogen bonds (H-bonds). The X-bond stabilization energy typically amounts to 5–25 kJ/mol and it increases with the increasing atomic number of the halogen involved. The reason for this is the increasing size and magnitude of the halogen σ-hole. Another factor is the halogen polarizability, which also increases with the halogen atomic number. Contrary to traditional view, modern theoretical studies suggest that the role of charge transfer in the X-bond stabilization is modest. The strongest X-bonds are found...
in complexes of iodinated molecules; brominated and chlorinated molecules form weaker X-bonds. Fluorine is the least polarizable and the most electronegative halogen. It possesses positive $\sigma$-holes in rare cases and mostly in inorganic molecules, so it is of lower importance for biological applications.

The structural trends of X-bonds in biomolecules have been inferred from several Protein Data Bank (PDB) surveys and theoretical analyses. All of the studies focused on protein-ligand X-bonds. The X-bonds are mostly established with the protein backbone. Its carbonyl oxygen is the most frequent electron donor. No preference for backbones of $\alpha$-helices, $\beta$-sheets or loop structures has been found. Lu et al. reported that about one-third of protein-ligand X-bonds involve an aromatic ring of the amino acid side chains. It was also reported that the X-bonds might not disrupt existing networks of H-bonds. Instead, an orthogonal pattern of X- and H-bonds is preferred involving the same electron donor atom. Another effect of ligand halogenation is a shortening of proximal H-bonds.

**Nucleic Acids as Drug Targets**

The deoxyribonucleic and ribonucleic acids (DNA and RNA) appear in living organisms in several forms. Whereas the DNA adopts only a few conformational classes, the structural diversity of RNA is much broader. DNA mostly occurs in the B-form helical conformation. A key RNA motif is the A-form double helix. However, loop regions, bulges, and other forms of mismatched nucleobases often disrupt the motif. Such a conformational variability is mirrored in the rapidly expanding variety of RNA functions recognized, especially in the last two decades. Apart from the classic roles of ribosomal, transfer and messenger RNAs, RNA was also shown to store genetic information, regulate gene expression, or act as enzyme.

Many NA-binders are halogenated. The DNA alkylating agents often contain halogen atoms because halogens facilitate the alkylating reactions on NAs. This way, anticancer cisplatin and its analogues covalently modify DNA. The reaction products block replication or transcription processes. The effect of alkylation is non-specific in terms of DNA sequence and also cell type. The modifications occur in both normal and cancer cells, but the higher proliferation rate of the cancer cells makes these drugs effective.

Alternatively to covalent modifications, various classes of compounds bind to double-stranded DNA (dsDNA) in a noncovalent manner. Examples include the anticancer anthracycline type antibiotics daunomycin and Adriamycin, and the polypeptide antibiotic dactylinmic, which works mainly by intercalating into DNA. The intercalation, described as an insertion of a planar molecule between consecutive base pairs, interferes with DNA-processing enzymes. Other small molecules bind to dsDNA interacting with base pair functional groups on the floor of the minor or major groove. Much of the research has concentrated on the DNA minor groove recognition leading to improved sequence selectivity of the compounds.

Important classes of drugs target ribosomal RNA (rRNA). In fact, most of the RNA-targeting drugs on the market act on ribosomes. The ribosome is a biomachine which synthesizes proteins. As such, it represents a critical center of cellular life. The differences between bacterial and eukaryotic ribosomes have allowed developing specific antibacterial agents that are often based on natural products. There is a variety of binding locations within the ribosome; the most frequent sites are the peptidyl-transferase center on the 50S subunit and the decoding center on the 30S subunit. Aminoglycosides and tetracyclines are the best-known classes of small molecules whose primary target is the 30S subunit. Oxazolidinones instead exert their antibacterial action by binding to the 23S rRNA present within the 50S subunit. Macrolides and related compounds (lincosamides and streptogramins), as well as chloramphenicol and clindamycin also target the 50S subunit. Several ribosome-binding molecules have been prepared contain-
ing a halogen atom stemming mostly from the pioneering case study of chloramphenicol.\(^46\)

In the past decade, other non-coding RNAs (ncRNAs) have emerged as prospective drug targets.\(^42,47\) Highly conserved ncRNAs provide new opportunities to expand the repertoire of drug targets to treat infections. Viral regulatory elements located in untranslated regions of mRNA often form folded structures that harbor potential binding sites for small molecules. The absence of homologous host cell RNAs makes them attractive for the development of innovative antiviral compounds.

There are a plethora of ncRNAs under intense pharmaceutical research due to their ability to interact with low-molecular ligands. For instance, human immunodeficiency virus type-1 (HIV-1) Trans-activation response (TAR) RNA plays an essential role in HIV-1 replication through its interaction with the viral trans-activator of transcription. Such interaction might be disrupted by ligands (reviewed in Refs.\(^48,49\)), where some of them contain halogen atoms.\(^50\)

Another example is an internal ribosome entry site (IRES) from Hepatitis C virus (HCV).\(^51\) The IRES RNA contains several independently folding domains that are potential targets for the development of selective viral translation inhibitors. A diverse set of ligands including oligonucleotides, peptides as well as small molecules have been reported to block IRES function by distinct mechanisms.\(^52\) Like in the case of other ncRNAs, the drug candidates occasionally contain halogens. Nevertheless, no drug targeting a ncRNA has been approved for the market.

Many lines of evidence are linking mutations and dysregulations of ncRNAs to neurodegenerative disorders.\(^53,54\) The presence of expanded CNG repeats in 5’ and 3’ untranslated regions is related to important diseases such as Myotonic dystrophy type 1, spinocerebellar ataxia type 3, and fragile X-associated tremor-ataxia syndrome. For example, the pathological expansion of CAG repeats (>35 consecutive CAG codons) in huntingtin exon 1 encodes a mutant protein whose abnormal function determines Huntington’s disease. Finding compounds able to bind pathogenic CNG repeats with high specificity may be a valuable strategy against these devastating diseases. Their design is still limited by the lack of structural information, although some small molecules have emerged through various strategies.\(^55,56\)

Both DNA and RNA can also adopt a non-canonical higher-order structure called G-quadruplexes (G4s) that are involved in regulating multiple biological pathways such as transcription, replication, translation and telomere structure.\(^57\) The building blocks of G4s are guanosine-rich quartets that self-associate into a square-planar platform through a cyclic Hoogsteen H-bonded arrangement. G4s are found in oncogene promoters, in telomeres, as well as in introns of mRNAs. These regions have been recognized as potential targets for anticancer drugs.\(^58\) A large number of small molecules are able to bind the quadruplex structures. They are characterized by polycyclic heteroaromatic scaffolds, or by cyclic/acyclic non-fused aromatic rings. Thus far, only a few molecules have been found to selectively bind the telomeric G4s, although their therapeutic potential appears high.\(^59\)

### Nucleic Acids as X-bond Acceptors

In the X-bond, the \(\sigma\)-hole on a halogen represents a Lewis acid that interacts with a Lewis base. NAs seem to offer an abundance of basic chemical groups. The backbone contains phosphate groups with two oxygens carrying a charge of \(-1\). Likewise, ribose and deoxyribose contain oxygens with lone electron pairs that could serve as \(\sigma\)-hole acceptors too. Further, the nucleobases form H-bonds that could be potentially replaced or augmented by X-bonds.

Nucleobases themselves show certain electrostatic diversity, where the negative sites may play a role of halogen acceptors. Fig.\(^2\) depicts MEPs of five most common nucleobases projected onto the plane of their aromatic systems. Cytosine and guanine contain areas of more negative ESP than the other nucleobases. Thymine and uracil resemble each other having
two negative sites on the oxygens separated by a small positive site. Perhaps the most heterogeneous ESP is around adenine which exhibits six areas of zero ESP near the molecular surface, as compared to four (G, T, U) and two (C). Apart from this, all of the nucleobases are aromatic, which allows them to act as electron donors via their π-electrons above and below their rings (not shown).

In nucleic acids, the situation is more complicated than in nucleobases: the three-dimensional structure of DNA and RNA is electrostatically diverse due to the presence of the phosphate backbone. For instance, the DNA minor groove was shown to be more electronegative than the major groove. Thanks to its plasticity, RNA may create folds with even more unusual electrostatic characteristics. Indeed, regions of strong negative electrostatic potentials were exploited in designing efficient TAR binders. Electrostatic interaction is also the main driving force of aminoglycoside binding to the ribosome. Overall, NAs seem to offer favorable electrostatics to attract positive σ-holes on halogens. It remains elusive, how is such ability employed in ligand recognition and NA self-assembly.

**Structural Survey Yields Two Data Sets**

To understand interaction preferences of halogens we analyzed known NA structures. We started with a broad set of X-ray structures from the PDB (September 2016). Apart from X-ray structures, the PDB also contains NA structures determined by nuclear magnetic resonance (NMR) techniques. For two reasons, we deliberately omitted those from our analyses. First, we wanted to be consistent with the strategies adopted by previous structural surveys of X-bonds in protein-ligand complexes. Second, there are indications that it may be difficult to assess the quality of the deposited NMR structural ensembles, which could complicate the geometric characterization.

Within the X-ray structures, we searched for complexes containing a nucleic acid and a halogen atom (Cl, Br, or I). Fluorine was excluded from the search due to its extremely low ability to form X-bonds in biological systems. The selected structures comprise nucleic acids and their complexes with other nucleic acids, low-molecular ligands, and/or proteins. To get reliable geometric characteristics, only data with the resolution better than 3.0 Å were considered. Note that the previous PDB surveys of X-bonds in protein complexes used the same resolution threshold.

From the PDB, we obtained 672 files which were subsequently filtered. We excluded structures containing halogens in the form of ions. Following the recommendation of the International Union of Pure and Applied Chemistry, we selected X-bonds as the contacts between halogens and electronegative atoms (N, O, P) shorter than or equal to the sum of van der Waals (vdW) radii (Tab. 1). The interactions involving at least one of the two interacting atoms with the crystal occupancy lower than 0.5 were omitted. Because of the X-bond directionality, we also required the angle of R–X···Y to be higher than 120°. Same or similar geometric criteria were used previously to define biological X-bonds with proteins, although the wider angular range was used in other studies as well.

|   | N  | O  | P  |
|---|----|----|----|
| Cl| 3.30| 3.27| 3.55|
| Br| 3.40| 3.37| 3.65|
| I | 3.53| 3.50| 3.78|

We also searched for the X-bonds that are formed with the aromatic systems of the nucleobases. To this aim, we considered only halogen contacts closer than 5 Å to the aromatic plane that make an angle between the plane normal vector and the X–C bond smaller than 60°.

In the end, we obtained a set of 21 X-bonds that satisfy the data quality, chemical and geometric criteria. This amount is a rather low. Scholfield et al. reported 760 protein complexes with an X-bond in 2012, which stands for about
1 % of the 80,000 protein structures deposited in the PDB at that time. The 21 X-bonds here represent about 0.2 % of ca 9,000 structures containing NA.

Hence to better capture possible geometric properties of halogen interactions, we collected a more extended set of complexes with a longer interaction distance. An arbitrary threshold of 4 Å was chosen such that it is higher than the X-bond length but still short enough to hint for an attractive interaction. Within the article, the interactions are referred to as linear contacts and they comprehend the X-bonds as well. We found 72 linear contacts. Table 2 summarizes various subsets of the PDB query.

**The X-Bonds Favor Nucleobase···Phosphate Pattern**

Within the X-bond set, the variety of interacting partners is low; 20 X-bonds involve a halogenated nucleobase, one X-bond is formed by cisplatin chlorine. The set contains 19 X-bonds with a phosphate oxygen as the electron donor; further, there is one X-bonds with an aromatic ring, and one with cytosine oxygen. Overall, only two X-bonds do not concur with the dominant nucleobase···phosphate interaction pattern.

The X-bond geometries are close to ideal. The X-bond lengths are shorter than the sum of the vdW radii by about 8 %, which conforms with the contractions reported for the protein X-bonds. About 90 % of the X-bonds are straighter than 160°.

The shortest X-bond in the set belongs to a structure of a Holliday junction; the X-bond is found between a bromodeoxyuridine and a phosphate oxygen of two neighboring residues (Fig. 3a) (PDB: 2org, resolution 2.0 Å). The X-bond was shown to stabilize particular DNA assembly in competition with H-bond. The straightest X-bond also involves a brominated uridine and phosphate oxygen (Fig. 3b) (PDB: 2bu1, resolution 2.2 Å) in a complex of RNA and phage MS2 coat protein and the overall geometry is remarkably similar to the one found in Holliday junction. Whereas the study on Holliday junction fully appreciated the role of X-bonding, in the latter case the specific interaction of the bromine remained unrecognized.

What is important, we have identified no non-covalent ligand involved in X-bonding. The only non-nucleobase residue that participates in an X-bond is cisplatin covalently bound to an adenine (PDB 5j4c, resolution 2.8 Å). It is also the only halogen donor that forms an X-bond with a π-system, although the interaction with one of the guanine nitrogens would alone classify the interaction as X-bond. The X-bond features superb geometric characteristics (Fig. 4). Unlike proteins, in nucleic acids,
Table 2: Analysis of the PDB. The contact is defined by the interatomic distance between a halogen and oxygen, nitrogen, or phosphorus shorter than 4 Å. The linear contact has the angle $R \cdots X \cdots O/P/N$ higher than $120^\circ$. X-bonds is a linear contact shorter than the sum of vdW radii.

|                          | Cl  | Br  | I   | sum |
|--------------------------|-----|-----|-----|-----|
| **Files**                |     |     |     |     |
| PDB search count         | 402 | 204 | 66  | 672 |
| Contains contact(s)      | 43  | 162 | 34  | 239 |
| Contains contact(s) with NA building block | 31  | 135 | 22  | 188 |
| Contains linear contacts(s) with NA building block | 22  | 29  | 2   | 53  |
| Contains X-bond(s) with NA building block | 2   | 17  | 2   | 21  |

| **Interactions**         |     |     |     |     |
|--------------------------|-----|-----|-----|-----|
| Number of contacts       | 611 | 1,319 | 205 | 2,135 |
| Contacts with NA building block | 43  | 315 | 41  | 399 |
| Linear contacts with NA building block | 22  | 48  | 2   | 72  |
| X-bonds with NA building block | 2   | 17  | 2   | 21  |

the halogen-$\pi$ interaction competes with $\pi-\pi$ interactions more often. Especially in dsDNA, it is hardly conceivable that there is a space for a halogen to attack the nucleobases from above or below of their aromatic planes. The situation in RNAs might be more favorable, but the single occurrence of such X-bond is hard to generalize.

In the NA complexes, the geometric quality of the halogen interactions increases in the order of Cl < Br < I. The interaction angles are more linear for heavier halogens. Especially the contacts of chlorine are rather bent with the median angle of $141^\circ$. The medians of the interaction lengths (Tab. 3) decrease with the increasing atomic number of the halogen. The surveys on protein-ligand X-bonds revealed the opposite trend, i.e. the increasing length of X-bonds with the increasing atomic number of the halogen involved. In this work (but also in Ref. [28]), the statistical sample might be insufficient to provide reliable statistics, which is true especially for the two iodine X-bonds.

Fig. 5 shows the histograms of the interaction lengths and angles. In the histogram of lengths, the minor peak near 3.0 Å, which is comparable with the sum of the vdW radii, stands for almost ideal X-bond length. There is a major peak near 3.5 Å too. Two peaks also appear in the histogram of interaction angles; around $140^\circ$ and $170^\circ$.

Each of the halogen subsets contributes to the histograms with a different weight. Chlorine- and bromine-containing complexes span the whole range of interaction lengths. The two iodine complexes feature short interactions. The situation with angles is different. Chlorine complexes appear only in the region of lower interaction angles. The highest angle in the chlorine

Figure 4: X-bond with an aromatic system between cisplatin (CPT) and a guanine (G). The distance stands for the perpendicular distance from the aromatic plane.

Interactions Longer Than X-Bonds

Table 3 summarizes counts of various types of interactions, and the statistics of the interaction geometries of the set of 72 halogen linear contacts.
Table 3: Medians (med) and interquartile ranges (iqr) of geometric characteristics of interactions involving various types of partners.

| Halogen          | Count | Length [Å] (med±iqr) | Angle [deg] (med±iqr) |
|------------------|-------|----------------------|-----------------------|
| chlorine         | 22    | 3.49±0.31            | 141±8                 |
| bromine          | 48    | 3.45±0.30            | 165±22                |
| iodine           | 2     | 3.07±0.06            | 173±3                 |
| Electron Donor   |       |                      |                       |
| N                | 21    | 3.47±0.28            | 140±11                |
| non-backbone O   | 9     | 3.69±0.42            | 135±25                |
| backbone O       | 42    | 3.41±0.45            | 166±8                 |
| Halogenated Residue |     |                      |                       |
| Halogenated nucleotide | 51  | 3.43±0.43            | 165±23                |
| Low-molecular ligand | 21  | 3.50±0.29            | 140±7                 |

Figure 5: Histograms of interaction lengths (a, c) and interaction angles represented by the R–X· · · Y angle (b, d); color-coded according to the halogen involved (a, b), or electron donating atom (c, d).

We analyzed the electron donors of halogen interactions found in the NA complexes. The backbone oxygen atom is the most common one. We identified 51 linear contacts of halogens with oxygen (71%), and 21 with nitrogen (29%). Although we included phosphorus as an electron donor in the search, no linear contact was found. The actual occurrence of the oxygen and nitrogen in NAs is roughly 2:1 (O:N), which likely contributes to the dominance of the linear contacts with oxygen. Most of the nitrogen interactions were found with chlorine, whereas subset is 154°, so chlorine interactions are likely weak. On the other hand, bromine complexes are scattered across the whole range of angles (Fig. 5b) with a cumulation around 170° (Fig. 6). The two iodine X-bonds are very straight (X-bond angle higher than 170°) suggesting a strong interaction.

Figure 6: Interaction geometries in polar coordinates color coded according to the halogen (a), or the electron donor atom (b).
bromine preferentially interacts with oxygen. 82% of the all interacting oxygens belong to the phosphate backbone. Unlike other oxygens and nitrogens in NAs, the phosphate oxygens carry a negative charge, which explains their higher propensity to halogen σ-holes.

According to the electron-donating atoms, the geometric quality of the interactions increases in the order of non-backbone oxygen < nitrogen < backbone oxygen (Tab. 3). The interactions which employ a backbone oxygen are typically shorter and straighter than the others.

We conclude that different interaction atoms likely occur in different interaction geometries. It is also apparent on a projection of the interaction geometries to the polar coordinates (Fig. 6). We observed no correlation between interaction lengths and the corresponding angles.

**Halogenated Ligands Show Sub-Optimal Interaction Geometries**

We did not find any X-bonded noncovalent ligands in NA complexes. Nevertheless, in the set of linear contacts we have found 15 unique ligands – 14 chlorinated and one brominated. The remaining interactions involve halogenated nucleobases as halogen donors. The halogen interactions of ligands are longer and less linear compared to the interactions of halogenated nucleobases (Tab. 3). The interaction angles deviate notably from linearity, which suggest that such interactions do not play a critical role in the NA-ligand recognition.

From the pharmaceutical point of view, the low-molecular ligands are of higher interest than the halogenated building blocks. Below we discuss all of the instances among the linear-contact data set which involve a low-molecular ligand. Structural formulas of the ligands discussed are shown in Fig. 7.

Several halogenated compounds bind to the bacterial ribosome. Chloramphenicol (1) is a classic antibiotic compound that targets the ribosomal A-site crevice in the 50S subunit (PDB 1nji, resolution 3.0 Å, 4v7w, resolution 3.0 Å). 1 contains two aliphatic chlorines that are activated by the nearby carbonyl group. Two distinct binding orientations in two different ribosomal system were proposed (T. thermophilus and H. marismortui) (Fig. 8). The lengths of both chlorine interactions are beyond the respective sums of the vdW radii (about 3.3 Å, Tab. 1), and quite bent. One of the chlorines approaches either the ribose inring oxygen (3.9 Å, 154°), or a guanine nitrogen (3.8 Å, 126°), respectively. The geometries suggest that the halogens interactions contribute weakly to the complex stability.

Clindamycin (2) is another halogenated ribosome binder (PDB 1yjn, resolution 3.0 Å) that binds into the 50S subunit (Fig. 9a) and forms linear contacts. 2 contains one chlorine that directs towards the sugar edge of guanosine. There is an interaction with guanine nitrogen (3.5 Å, 148°). At the same time, there is a shorter but more bent contact with sugar O2’ oxygen (3.1 Å, 112°).

7-chlorotetracycline (3) belongs to a class of tetracycline antibiotics that bind into the ribosomal 3OS subunit. This class acts by preventing correct processing of aminoacyl-tRNA. Although no complex of 3 with the ribosome
Figure 8: Chloramphenicol 1 (CLM) interactions in the 50S ribosomal subunit with guanosine (G) and uridine (U). Hydrogens were omitted for clarity. The overlay of the two binding poses found in *T. thermophilus* (PDB 1nji) and *H. marismortui* (PDB 4v7w) into a single reference frame is shown in red and black, respectively. Only residues within 7 Å from each of the halogens are shown.

satisfies the data-set criteria, a complex with an RNA aptamer does. The aptamer was designed to bind 3 with sub-nanomolar affinity (PDB 3egz, resolution 2.2 Å). It features an interaction between a chloride and a ribose oxygen of a cytidine (Fig. 9b) (3.7 Å, 151°). Due to the sub-optimal geometry, the role of halogen interaction in complex stabilization is likely marginal. Moreover, there are many other intermolecular interactions between the 3 and the aptamer, such as a stacking of a planar part of 3 with a guanine, and an oxygen-magnesium coordination (not shown). Those reduce the relative importance of the halogen contact even more.

Recently, crystal structures of *T. thermophilus* ribosomes with cisplatin (4 in Fig. 9c) were resolved (PDB 5j4b, resolution 2.6 Å; 5j4c, resolution 2.8 Å). Nine molecules of cisplatin are covalently bound to the ribosome in place of one of the chlorines. Two of the cisplatin residues were identified to interfere with the mRNA tunnel and the GTPase site – the places critical for the ribosome functions. One cisplatin out of the nine forms an X-bond with a guanine π-system (Fig. 4). Another cisplatin directs with its chlorine in between two stacked adenines with the shortest distance to a nitrogen of 4.0 Å and angle 151°. Another nitrogen is about 4.2 Å far away and forms the angle of 148° (Fig. 9c).

Our data set contains several ligands that bind into the prototype foamy virus (PFV) intasome, i.e. a complex of a viral integrase and DNA. Such a system serves as a model for HIV-1 integrase inhibition. Several inhibitors were developed with a halogenated phenyl ring (5 and 6) that form X-bonds with NAs. No difference in the interaction geometries was found between 5 and 6 analogs. 5-analog interactions show the best geometric characteristics among the PFV ligands (but generally still modest); the chlorine on 5 interacts with two adenine nitrogens (Fig. 9d). While the contact length of 3.3 Å is comparable with the sum of vdW radii, the angle of 142° is far from the ideal 180°. The other contact is 3.5 Å-long and even more bent (angle about 114°). We add that an aromatic fluorine activates the neighboring chlorine by making its σ-hole more positive. The effect of fluorination on the ligand binding affinity is not straightforward, however.

We found only one brominated ligand with a contacts shorter than 4 Å. A brominated imidazole analog (7) binds into a G-quadruplex formed within an RNA aptamer called Spinach (PDB 4q9q, resolution 2.45 Å). The Spinach module allows a green-fluorescence-protein-like functionality by selectively activating fluorescence of 7. 7 binds in a planar conformation with a strong stacking interaction with adenine. Besides this, it forms a contact with a phosphate oxygen of 3.5 Å under the angle of 137° (Fig. 9e).

**Summary and Outlook**

The role of X-bonding in drug development has been emphasized for a decade. Nonetheless, the knowledge-based design of halogenated drugs that feature an X-bond with their biomolecular targets is still a tedious task. Although there have been published several pioneering studies that involved protein targets, the nucleic acids still lack a successful application.

In this work, we focused on X-ray structures
of biomolecular complexes containing halogen interactions with nucleic acids. The general ability of nucleic acids to provide electron donating groups to X-bonding has been confirmed here. Using criteria recommended by IUPAC for X-bond definition, we found 21 NA complexes with the X-bond. We also analyzed interactions of the halogen atoms longer than X-bonds.

All but one of the X-bonds involved a halogenated nucleobase (preferably uridine). Halogenated nucleotides are often utilized in X-ray crystallography, which explains their high occurrence in the NA complexes. The incorporation of heavy atoms such as bromine into the structure helps to overcome the phase problem. Moreover, the halogenated nucleotides alter the physico-chemical properties of the NAs thus modulate the crystallization conditions; in the classical theory this is due to their stacking properties, but recently X-bonding has also been shown to play a role. What is more, halogenated nucleobases may be also used in radiation anti-cancer therapy as radiosensitizers.

Although, the exact mechanism of the increased radiosensitivity is not known, the changes in the ionization potential brought by the halogens or global structural changes associated with modified nucleobase pairing have been proposed.

An important feature of X-bonds in NA complexes is their preference towards backbone oxygens. This is true also for the longer halogen interactions. The phosphate oxygens tend to form straighter and shorter X-bonds than other electron donors, possibly due to their negative charge.

In NA····ligand noncovalent recognition, a variety of interactions plays a role. H-bonding and London dispersion-driven stacking interactions belong to the classic interactions, complemented by salt bridges, water-network disruptions, or metal-ion interactions. Strictly speaking, no noncovalent ligand has been found to employ X-bonding in the NA binding (according to the IUPAC recommendations). No X-bonded NA ligand found is an intriguing fact, which may be explained in two ways:

i) The X-bonding ligands may be inefficient in NA recognition. If so, the halogenated ligands that bind into NAs bind due to other kinds of interactions that are stronger than X-bonds. Our data support this hypothesis. All of the halogen interactions of ligands found in the survey here show sub-optimal geometries. Given
the high X-bond directionality, the stabilization energy of a complex reduces notably when deviating from the ideal geometry. Hence, the contribution to the stabilization of the NA complexes is likely not dominant. Of course, there are exceptions where X-bonding is the driving interaction. For instance, it was proven that a single X-bond/H-bond exchange might transform the DNA conformation completely.

ii) Medicinal chemists may under-appreciate the role of X-bonding in the drug-design strategies. Our data support also this hypothesis, because we found only a few halogenated ligands: 20 chlorinated, 8 brominated and one iodinated.

The current study on its own is unable to dissect which reason is more likely. The starting set of PDBs was biased towards the lighter halogens, which are, however, less suitable for strong X-bonds. For a better understanding of the role of X-bonding, structural and functional studies are required, especially of the less-frequent brominated and iodinated compounds.

The effects of halogens are not limited to the X-bonding, though. The halogenation affects the global electron distribution and consequently many of the molecular properties. For instance, Fanfrlík et al. demonstrated that solvation/desolvation may compensate for favorable σ-hole···lone pair interaction in halogen-to-hydrogen substitution in a protein-ligand complex. Also, a PDB survey of two protein families revealed shortening on H-bonds proximal to ligand halogens. This corresponds with the notion that halogenation increases the acidity of proximal H-bond donors. Still, these effects in NAs are difficult to inspect with the limited statistical sample of X-bonds here, and of halogenated NA ligands in general.

Nevertheless, the current analyses may help in designing novel X-bonded NA binders. Such binders should contain a bromine or iodine to form a strong X-bond. The ligand binding should be directed to the vicinity of sugar-phosphate backbone, for example to groove or bulge regions. Consequently, such a ligand should likely not contain extensive aromatic systems that are prone to intercalation into canonical helices. Systematic halogenation of known pharmacologically active compounds may be an optimal strategy to identify new agents with enhanced activity, presumably supported by the specific X-bonds.

Biographies

Michal H. Kolář received his Ph.D. in 2013 from Charles University in Prague, and from the Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic. With Pavel Hobza he focused on theoretical and computational description of noncovalent interactions. He is a recipient of the Humboldt Research Fellowship for Postdoctoral Researchers. From 2014 he worked with Paolo Carloni in Forschungszentrum Jülich, Germany, on RNA-ligand recognition. In 2016 he moved to Helmut Grubmüller’s group at the Max Planck Institute for Biophysical Chemistry, Göttingen, Germany, pursuing his interest in non-coding RNAs and computer simulations.

Oriana Tabarrini is a Professor of Medicinal Chemistry at the Department of Pharmaceutical Sciences (University of Perugia, Italy). After the degree in Pharmaceutical Chemistry and Technology she has worked for several years with research grants from pharmaceutical industries. In 2002 was promoted to Associate Professor. Her research has mainly aimed at developing small molecules as pharmacological tools and potential chemotherapeutics with particular focus on nucleic acid binders as antivirals and more recently for the treatment of neurodegenerative disorders. She has published over 85 research articles in leading peer-reviewed journals, including some invited reviews and patents. She has also received several project grants and is an ad-hoc reviewer for several top journals.

Abbreviations

BRU 5-bromouridine  
CLM chloramphenicol  
CLY clindamycine
CPT cisplatin
DNA deoxyribonucleic acid
dsDNA double-stranded DNA
G4 G-quadruplex
H-bond hydrogen bond
HCV Hepatitis C virus
HIV-1 human immunodeficiency virus type 1
IRES internal ribosome entry site
NA nucleic acid
ncRNA non-coding RNA
PDB Protein Data Bank
PFV prototype foamy virus
RNA ribonucleic acid
TAR trans-activation response RNA element
X-bond halogen bond

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