REVIEW PAPER

JMS Journal of Medical Science

Introducing bromine in the molecular structure as a good strategy to the drug design

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doi: https://doi.org/10.20883/medical.e1128

Keywords: bromine, halogen bond, radiopharmacy, drug resistance

Received 2024-08-29
Accepted 2024-09-24
Published 2024-09-30

How to Cite: Potapskyi E, Kustrzyńska K, Łażewski D, Skupin-Mrugalska P, Lesyk R, Wierzchowski M. Introducing bromine in the molecular structure as a good strategy to the drug design. Journal of Medical Science. 2024 September;93(3);e1128. doi:10.20883/medical.e1128

ABSTRACT

Nowadays, the search for new pharmaceuticals results in the development of thousands of new substances. One of the effective drug design strategies is to modify a previously obtained and studied substance. A very popular modification is the introduction of halogens into the structure of drugs, most often these are fluorine or chlorine atoms. However, the introduction of bromine into the structure of a potential drug also has a number of advantages. A good example would be natural substances extracted from marine organisms, which have been studied and proven to be effective in various diseases, including antibiotic therapy of resistant bacteria. Numerous studies justify the usage of bromine and its isotopes in therapy (both in diagnostic imaging and radiotherapy). To better explain the impact of "bromination," numerous researchers have described such a phenomenon as "halogen bond." Due to the presence of the so-called "sigma-hole" in the halogen atom of an organic molecule, it is possible to form these bonds, which results in a change in intermolecular and intramolecular interactions. Such changes can favorably affect drug-target interactions. The advantages of "bromination" include an increase in therapeutic activity, a beneficial effect on the metabolism of the drug and an increase in its duration of action. Besides, the phenomenon of heavy atom effect can be used to increase the effectiveness of photodynamic therapy and radiosensitization. Unfortunately, "bromination" is not without drawbacks, which we may include increased toxic effects and accumulation in the organism.

Introduction

Drug discovery strategies

From the beginning of pharmacotherapy to the present day, a huge number of drugs and substances with potential therapeutic effects have been developed. Since ancient times, products of natural origin, such as plants or animal products, have been used. With the advancement of science and the development of chemistry, the isolation of chemical compounds or their mixtures began. Even to this day, the searches for new drug-like substances often involve their discovery in natural sources. However, the main source of new potential drugs is their synthesis and development of previously unknown substances. Often these are modifications of already known drugs or molecules of natural origin to improve their therapeutic parameters, thus increasing their potential for treatment.

One of the strategies for finding new original drugs is to introduce new substituents into the molecule of an already known, studied drug. Substitution of new functional groups is always associated with a change in pharmacodynamic and pharmacokinetic properties. New moieties in the molecule often lead to an increase in therapeutic activity, reduction or complete elimination of side effects. Very often halogens are introduced into the structure of a drug. Excluding extended substituents and focusing on the percentage of individual atoms in the molecules of registered drugs, the FDA presented the following statistics [1].

It can be deduced from the diagram that halogens present the majority of structural modifications and are often chosen as a strategy for finding new therapeutics. Speaking of bromine, a relatively low level of interest among researchers is apparent compared to chlorine and fluorine. Nonetheless, the element appears among the top five heteroatoms in drugs used in disorders of the nervous, sensory and respiratory systems. This demonstrates its potential to pass through biological barriers including blood-brain (this subject will be expanded upon in later sections of the article). The history of bromine in medicine begins with ancient times.

History of the discovery of bromine

Molecular bromine was discovered by two scientists independently of each other. German chemist Carl Löwig isolated bromine from swamp water in 1825, and in 1826 French scientist Antoine Balard extracted bromine from marine algae remains [2,3]. Molecular bromine has strong disinfectant, irritant and oxidizing properties when in presence of organic molecules so it has no use in medicine. The simplest examples of bromine use are ammonium, sodium and potassium bromides. In the old days, these salts were used as sedatives. Due to a number of side effects, including so-called "bromism" and the existence of safer and more effec-

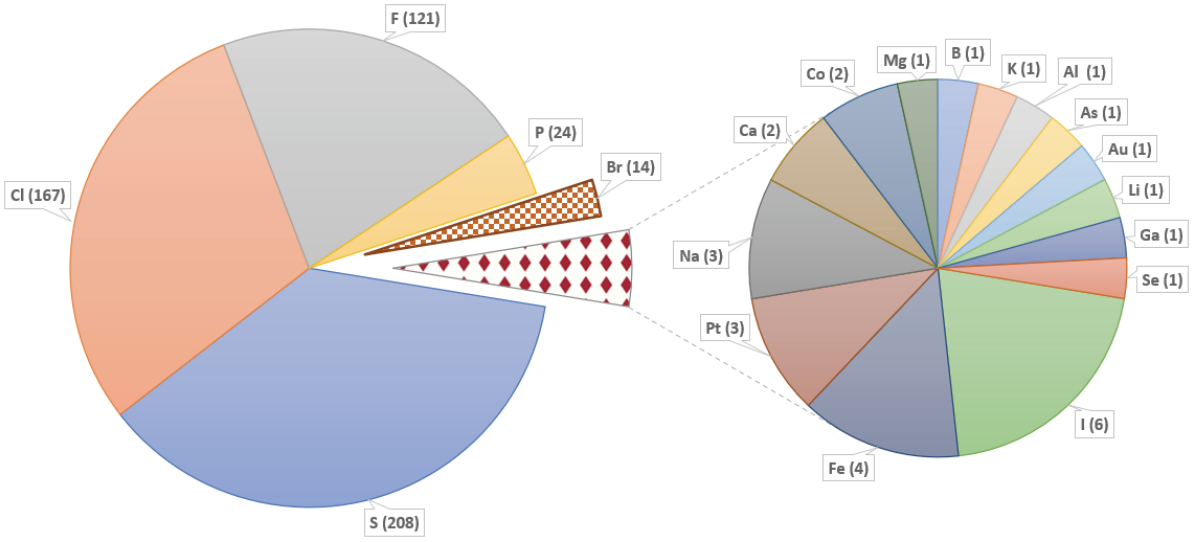

Figure 1. Occurrence of heteroatoms in medicinal substances (numbers in brackets mean the number of registered drugs reviewed in the paper) [1].

tive therapeutics, they fell out of use, although even today, they are still used in medicine in rare cases. Bromism is the consequence of bromide cumulation after long-term usage. The symptoms of this disease are anxiety, ataxia, psychosis, stupor, anorexia and rashes. But being incorporated into an organic molecule, it is devoid of these properties and can show a positive effect on the effectiveness in treating various diseases. The main sources of organic bromine derivatives are marine flora and fauna. Due to the high bromine content in seawater, these organisms produce metabolites, most of which contain a bromine atom in their structure. By 1999, more than 1600 compounds of this type had been isolated and identified. By 2020s another 12000 marine-derived compounds had been discovered, which not only expanded the list of potential drugs, but also served as inspiration for the design and synthesis of new derivatives. Moreover, it is estimated that every year the list of these compounds grows by several hundred more molecules [4].

Brominated indole derivatives – natural therapeutics with wide applications

A good example of potential therapeutics of natural origin are indole alkaloids. As mentioned above, due to the high halogen content of seawater, compounds from this source are often characterized by the presence of bromine in their structure. This group of metabolites is very varied and numerous studies indicate a potentially wide range of applications in medicine. For example, the following activities have been proven:

- Biocidal (including antibacterial, antifungal, antileishmanial, antiplasmodial, anti-HIV, larvicidal) [5–13];
- Opioid receptor agonist [14];
- Anti-inflammatory [15];
- Vasodilatory [16,17];
- Cholinesterase inhibitors [18];
- CB1 receptor inhibitors [19].

An interesting aspect of the therapeutic application of brominated indoles is their broad spectrum of activity against bacteria. As the problem of antibiotic resistance is increasing, the search for completely new structures seems to be crucial in solving this issue.

Weihong Wang et al. isolated from the tunicate species *Eudistoma sp.* 7 indole derivatives (**1–7**) (**Figure 2**) and tested their activity on selected bacterial strains (*Staphylococcus aureus* ATCC 6538, *Micrococcus lutes* ATCC 9341, *Staphylococcus epidermidis* ATCC12228, *Bacillus subtilis* ATCC 6633, *Escherichia coli* ATCC 11775, *Salmonella typhimurium* ATCC 14028, *Klebsiella pneumoniae* ATCC 4352). After conducting tests to determine MICs for the obtained compounds, the authors deduced that the most promising compound was **6**, which showed selectivity against *S. epidermidis* ATCC12228 (MIC = 12.5 µg/ml) and *Bacillus subtilis* ATCC 6633 (MIC = 25 µg/ml) strains. Derivatives **1** and **4** showed activity against the same microorganisms but with a much weaker effect – 50 µg/ml and 200 µg/ml for *S. epidermidis* ATCC12228 and *Bacillus subtilis* ATCC 6633 respectively. No bacteriostatic activity was found against the remaining bacteria. An MTT assay was also conducted for derivative **6**, which showed no cytotoxicity for a concentration of 100 µM [20].

Table 1. The results of the study by Finlayson Rhys et al.

	IC50 [µM]				
	T. b. rhod	*T. cruzi*	*L. don.*	*P. falc.* K1	L6
Didemnidine A	59	130	>180	41	24
Didemnidine B	44	82	>160	15	25
Precursor of Didemnidine A	34	88	>190	32	73
Precursor of Didemnidine B	9.9	28	>160	8.4	25
Melarsoprol	0.01				
Benznidazole		1.35			
Miltefosine			0.52		
Chloroquine				0.2	
Podophyllotoxin					0.01

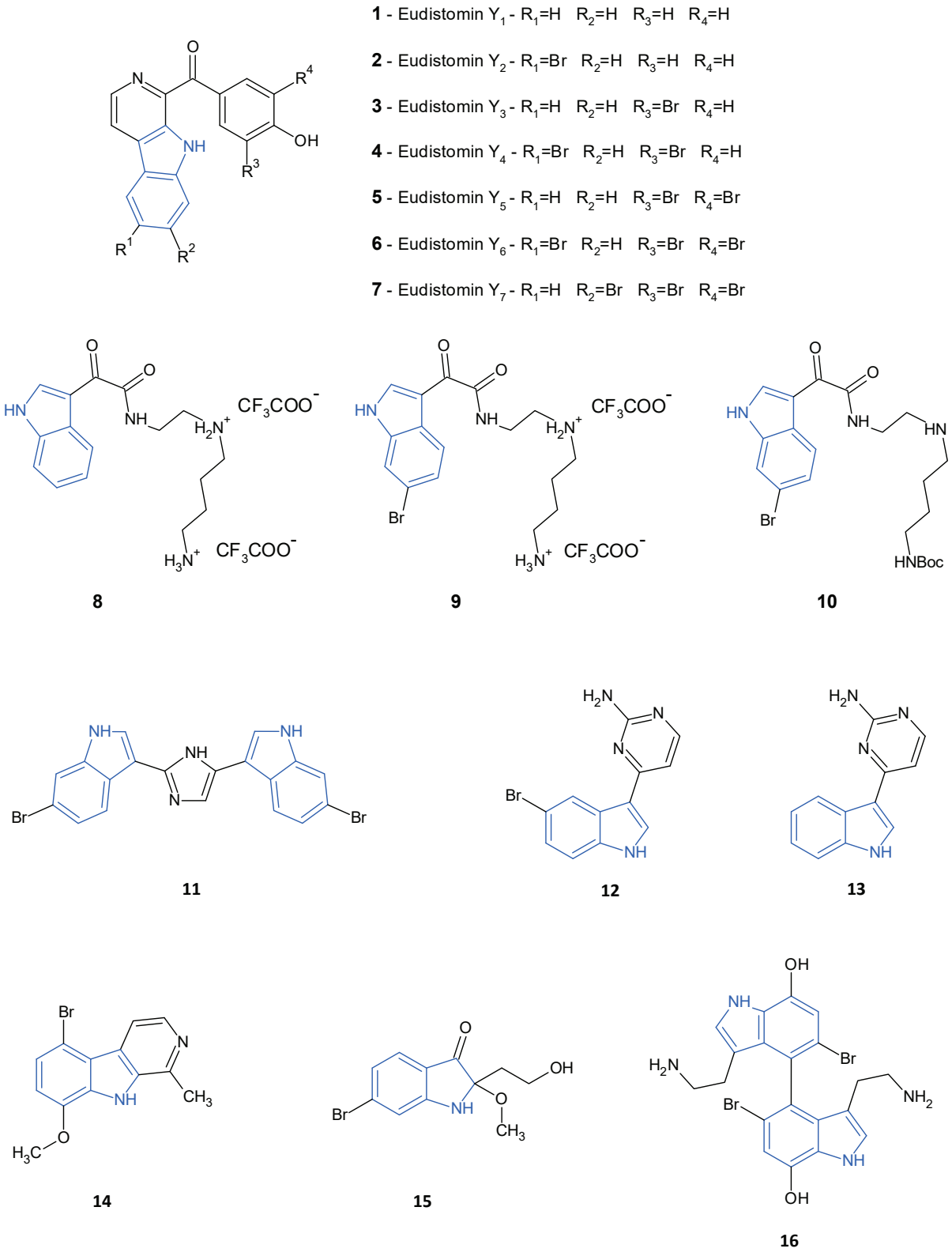

Figure 2. Structures of compounds 1–16 (1–7 – Eudistomin Y1–7; 8 – Didemnidine A; 9 – Didemnidine B; 10 – precursor of 9;11 – nortopsentin A; 12 – Meridianin C; 13 – Meridianin G; 14 – 5-bromo-8-methoxy-1-methyl-β-carboline; 15 – matemone; 16 – Dendridine A).

In their work, Finlayson Rhys et al. isolated certain metabolites of various types of sea squirts from the *Didemnidae* family. After extracting and confirming the structure of these compounds, they synthesized them and described two new derivatives, Didemnidine A (**8**) and Didemnidine B (**9**) (**Figure 2**). The obtained compounds and their precursors were then tested to determine the IC_{50} against selected parasites:

- *Trypanosoma brucei rhodesience*, STIB 900 strain (trypomastigotes stage);
- *Trypanosoma cruzi*, Tulahuen C4 strain (amastigotes stage);
- *Leishmania donovani*, MHOM-ET-67/L82 strain (amastigote/axenic stage);
- *Plasmodium falciparum*, K1 strain (IEF stage).

Drugs from the pharmaceutical market used to treat diseases caused by the above-mentioned organisms were used as controls. Cytotoxicity was determined against the L6 rat skeletal myoblast cell line. Noteworthy is the compound Didemnidine B and its precursor (**10**). With relatively low cytotoxicity, it shows medium growth inhibitory properties against *P. falciparum*. While its precursor is characterized by almost 2 times higher activity. The *T. brucei rhodesienc.* strain was also found to be susceptible to this compound. Although these therapeutic activities are not good enough, the authors' work may serve as inspiration for the development of new series of drugs, e.g. for malaria, which are modifications of Didemnidine B [21].

Two other studies have also described brominated indole derivatives that could be initial compounds for a new antimalarial drug. Alvarado Stephenie et al. tested nortopsentin A (**11**) (**Figure 2**), a compound derived from the sponge species *Spongosorites*, on chloroquine-resistant strains of *Plasmodium falciparum*. This derivative appears promising, as it shows an IC_{50} against this strain of 0.46 μM with a good selectivity index of 14.3 (against the NIH 3T3 fibroblast line). Bharate Sandip et al. in turn described compounds called Meridianins derived from the tunicates of the *Aplidium meridianum* species. Of the entire series, Meridianin C (**12**) and G (**13**) are the most promising (**Figure 2**), showing IC_{50} levels of 4.4–14.4 μM against other *P. falciparum* strains with selectivity coefficients of 25.1 and 24.1, respectively. Additionally, Meridianin C shows moderate activity against *Leishmania donovani* promastigotes (IC_{50} = 64 μM) [22,23].

Another example of potential pharmaceuticals is a group of compounds called β-carboline alkaloids. They have been isolated from animals in the family *Catenicellidae*. This family includes a species of invertebrate animals from New Zealand, *Pterocella Vesiculosa*, which was the subject of research by Till Marisa and Prinsep Michèle. In their work, the researchers isolated and described 5-bromo-8-methoxy-1-methyl-β-carboline (**14**) (**Figure 2**) and tested its inhibitory activity against the Gram-positive bacteria *Bacillus subtilis* (2–4 μg/ml), the fungi *Candida albicans* (4–5 μg/ml) and *Trichophyton mentagrophytes* (4–5 μg/ml) [24].

A compound that could serve as another initial substance for the development of new chemotherapeutics, called matemone (**15**), has been isolated from *Iotrochota purpurea*, a sponge that inhabits the Indian Ocean (**Figure 2**). The compound belongs to the oxindole group and was isolated by Carletti Isabelle et al. The authors tested the antimicrobial activity against *Staphylococcus aureus* by determining the zone of growth inhibition for discs containing 50, 100 and 200 μg/disc which were 7, 9 and 11 mm, respectively. Although the derivative showed bacteriostatic activity, it was not active against *Candida albicans* (at 200 μg/disc) [25].

Another species of sponges from which indole derivatives have been extracted is *Dictyodendrilla*, which resides in Okinawan waters. Tsuda Masashi et al. described the antimicrobial activity of a symmetrical dimer called Dendridine A (**16**) (**Figure 2**). According to the researchers, the compound shows promising results against both the bacteria *Bacillus subtilis* (MIC = 8.3 μg/ml) and *Micrococcus luteus* (MIC = 4.2 μg/ml), as well as the fungus *Cryptococcus neoformans* (MIC = 8.3 μg/ml) [26].

In the context of antibiotic resistance, an important discovery is the work of Zoraghi Roy et al. The researchers were interested in a series of alkaloids from the sponge species *Topsentia pachastrelloides*. The substances cis-3,4-dihydrohamacanthin B (**17**) and bromodeoxytopsentin (**18**) showed the best results (**Figure 3**). Against methicillin-resistant *Staphylococcus aureus* (MRSA) strains, the MIC values for these

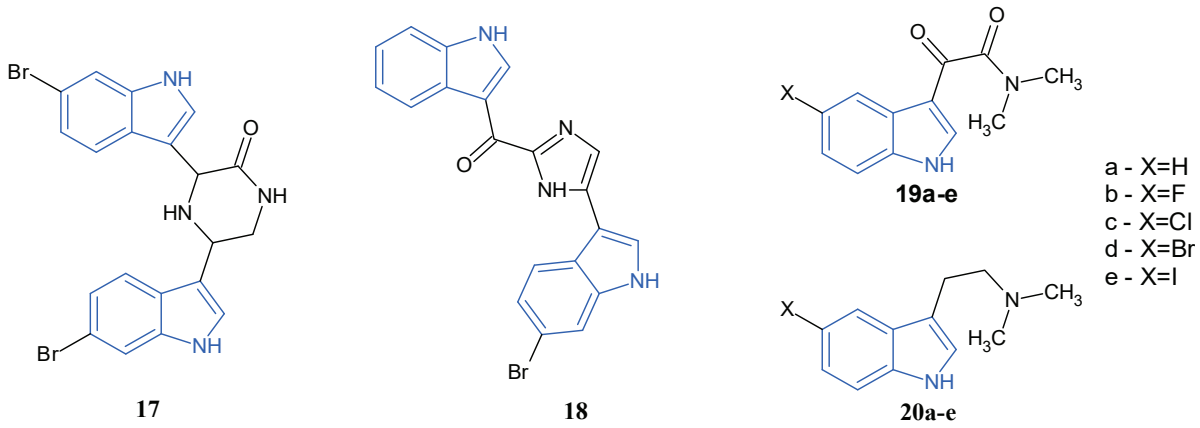

Figure 3. Structures of compounds 17–20a-e (17 – cis-3,4-dihydrohamacanthin B; 18 – bromodeoxytopsentin;19a-e – amide-derivatives of indole; 20a-e – amino-derivatives of indole).

compounds were 12.5 μg/ml and 6.25 μg/ml, respectively [27].

Moreover, brominated indole derivatives have applications beyond antimicrobial therapy. For example, for the above-mentioned matemone and 5-bromo-8-methoxy-1-methyl-β-carboline, anticancer activity has been proven in the same studies. The first one shows moderate anti-cancer activity against NSCLC-N6 L16 lung cancer cells (30 μg/ml), Mia PaCa-2 pancreatic cancer (24 μg/ml) and DU145 prostate cancer (27 μg/ml). The second one, on the other hand, has a relatively good IC_{50} = 5,089 μg/ml against the P388 murine leukemia cell line [24,25]. In addition, Mohamed A. Ibrahim et al. examined a series of halogenated amide- and amino-derivatives of indole (**19a-e** and **20a-e**) (**Figure 3**). In *in vitro* studies, compounds **20a**, **20c**, **20d**, **20e** were shown to have affinity for 5-HT1A and 5-HT7 receptors in the nanomole concentration range. Significant antidepressant-like effects for compounds **19a**, **20d**, **20a**, **20c**, **20e** were proven on mice *in vivo* (forced swim and locomotor activity tests). The authors explain such an increase in affinity for molecular targets in part by the presence of halogen bonding, which was observed during docking to the corresponding receptors [28].

Brominated pharmaceuticals that are approved

Bromine is not only occurring in experimental medicinal substances. Some drugs that have been tested and approved for pharmacological use also contain this element in their structure. Compared to other halogens, such as chlorine or fluorine, bromine is not as widely found. However, the range of pharmacological groups is quite wide. We can find drugs with this element in the following medications (**Figure 4**):

- Anesthetic drugs. The representative of this group is halothane (**21**). This drug was approved in 1956 for usage in general anesthesia. Halothane belongs to the group of inhaled pharmaceuticals, which also includes isoflurane and enflurane.
- Antihistamines. This group includes brompheniramine (**22**), dexbrompheniramine (**23**) and bromodiphenhydramine (**24**), which were registered in 1955s, 1970s and 1980s, respectively. These drugs belong to the first generation of antihistamines and in their days were used quite often alone or in combination with other drugs to treat allergy symptoms and the common cold. Dexbrompheniramine is the active enantiomer of brompheniramine, while bromodiphenhydramine has an additional ether group in its structure.
- Anticancer drugs. In this group we can note two medicines. Pipobroman (**25**) has an alkylating activity due to its two β-bromoamide moieties. Vandetanib (**26**), on the other hand, acts as a kinase inhibitor at various receptors, including VEGFR and EGFR. This pharmaceutical is used to treat thyroid cancer.
- Drugs used in CNS diseases. Necergoline (**27**) is an α_{1A}-adrenergic receptor antagonist and has found application in the treatment of CNS diseases of vascular origin, such as demen-

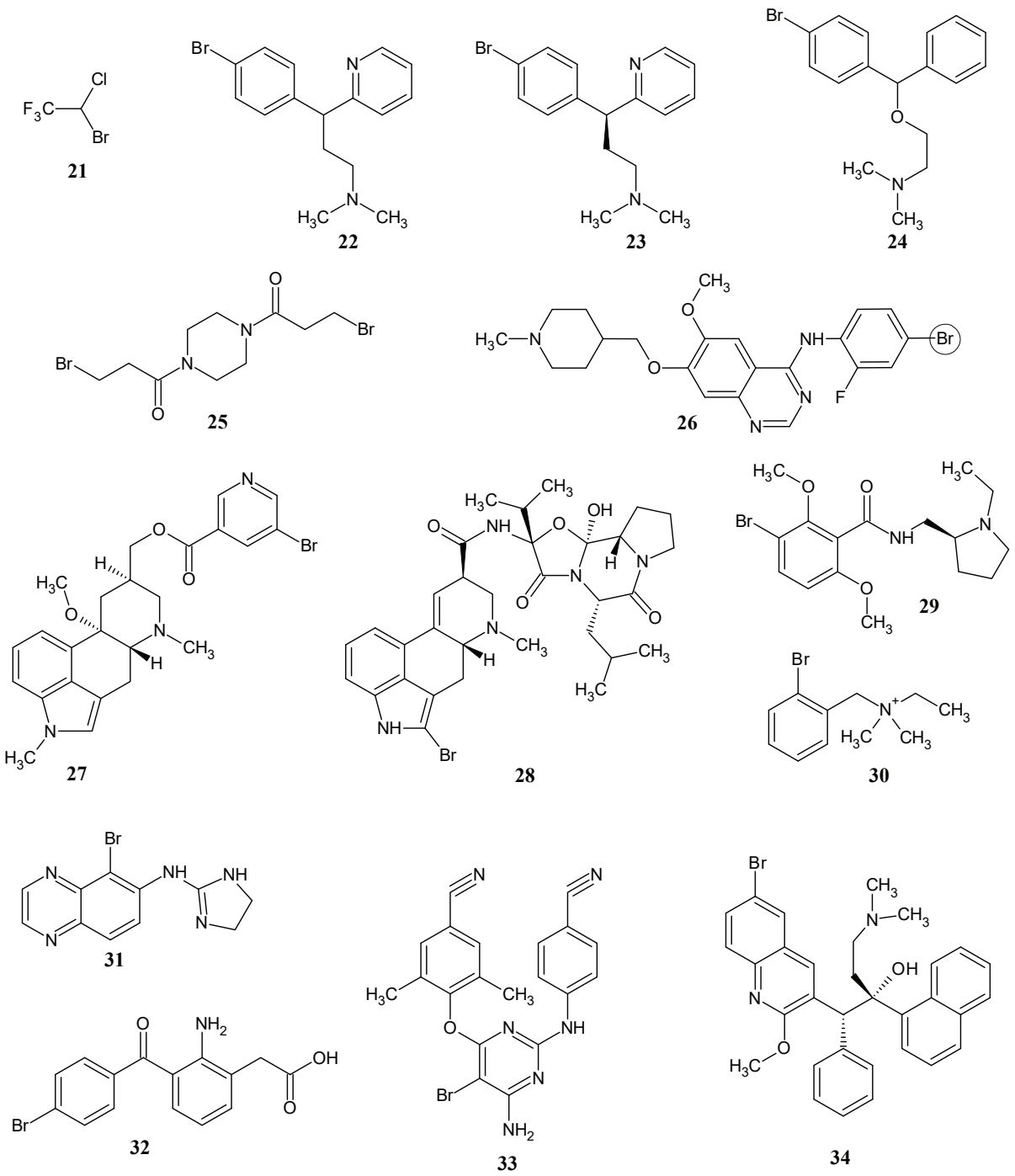

Figure 4. Approved drugs with bromine in their structure.

tia and migraine. Bromocriptine (**28**) is a dopaminergic derivative of ergoline and is used in the treatment of Parkinson's disease. Remoxipride (**29**) although it had success in treating drug-resistant schizophrenia, it was withdrawn quite quickly due to side effects.

› Cardiological drugs. This group includes bretylium (**30**), which was registered in 1959 for the treatment of hypertension. After time, however, it began to be used as an antiarrhythmic drug to treat tachycardia and fibrillation in emergency medicine.

› Ophthalmic drugs. Brimonidine (**31**) as an α-adrenergic medicine has found application in the treatment of open-angle glaucoma since 1996. Bromfenac (**32**) belongs to the NSAIDs and is a selective COX-2 antagonist. It is used to treat inflammation after ophthalmic

surgery since 2000. This drug has good pharmacokinetics and few side effects.

› Biocide drugs. In this category we can include two drugs, which are used in drug-resistant infections. Etravirine (**33**) is a non-nucleoside reverse transcriptase inhibitor (NNRTI) and there are no cases of resistance to this medication compared to other drugs in this group. It was approved in 2008 for the treatment of HIV. Bedaquiline (**34**) was registered in 2012 for the treatment of multi-drug-resistant tuberculosis in combination with other drugs.

Bromine in radiopharmacy

Production and labelling methods for bromine radionuclides

In addition to classical therapy, bromine and its isotopes have found applications in radiopharmacy. Radiopharmaceuticals are used in nuclear medicine as diagnostic tools and in therapy. The introduction of radionuclides allows for monitoring the cellular uptake of an active compound *in vivo*. Secondly, modification enables observing biochemical and pharmacological processes at the molecular level. Thirdly the radiation emitted by the radiopharmaceutical can kill and inhibit the proliferation of cancer cells. For mentioned applications, radiohalogens have potential use in this field. The leading halogen for imaging is the β^+ emitter – ^{18}F, used in positron emission tomography (PET). However, the isotope has a short half-life of 110 minutes, preventing monitoring metabolic pathways at later time points[29,30]. Iodine has a broader application range, presented by several isotopes with different decay characteristics. Nevertheless, the binding strength of iodine radionuclides to organic molecules sometimes does not allow the molecule to remain stable in the organism. On the contrary, bromines form stronger bonds in molecules, which increase resistance to unfavorable conditions in the body. Besides their purpose as imaging agents for PET with a longer half-life (^{76}Br), they are applicable as targeted therapeutic agents in treating tumors (^{77}Br, ^{80m}Br – Auger electron emitters. Considering these properties and new methods for producing isotopes and labeling molecules, bromine has potential in a broad spectrum of radiopharmacy [29,31]. Among well-known methods, the isotopes ^{75}Br and ^{76}Br manufacture proceeds through (p, 2n) nuclear reactions with selenium-76 and selenium-77 nuclides. Nevertheless, contamination occurs during the approach due to the competitive (p,n) reaction. Moreover, (p, α) ^{3}He and deuterons-induced reactions on ^{77,78,80}Se did not yield satisfactory results either [30]. The high vapor pressure and low boiling point of selenium result in the easy degradation of these isotopes, presenting another drawback. Degradation issues also arise with an α beam bombardment to arsenic. A newly proposed alternative for obtaining ^{76}Br involves heavy-ion fusion evaporation reactions. In the reaction, ^{28}Si transfers energy to natural chromium or ^{16}O to natural copper. McGuinness SR et al. successfully produce ^{76}Br and ^{77}Br isotopes using this method with advantages over the mentioned pathways. Radioisotopic impurities in the technique can be easily isolated using differential cold therapy or dry distillation. Equally significant aspects are characteristic of specific isotopic yield and renewable targets made from copper. High beam currents can also lead to better reaction yields [32]. An innovative approach for obtaining β^- -emitters with a half-life between 1 and 10 days is their extraction from molten salt reactors. For bromine radionuclides, the method applies to isotope ^{82}Br. The described manufacturing route for ^{82}Br is likely cheaper than other procedures. However, the production efficiency of the isotope in the experiment was below 0.02% [33].

Using a radioisotope itself does not enable it to image atypical cells or targeted therapy. The more precise action of radiation-emitting atoms is with a linked compound binding to specific cell receptors. The most commonly used reactions are electrophilic destannylation of the tributyltin precursor. This type of substitution without a carrier often results in lower efficiency due to the high activity of intermediates. Zhou Dong. et al. proposed a similar manufacturing process using a diaryliodonium salt precursor. The approach with microwave irradiation achieved high yields for 4-bromobenzoate and 4-bromobenzaldehyde. Furthermore, selecting a base-free method with subsequent HPLC purification treatment led to low contamination by other products. The particular two compounds are potential precursors for synthesizing radiopharmaceuticals, such as a radiolabeled poly-ADP-ribose polymerase-1 (PARP-1) in cancer imaging [34]. For the radiobro-

mination of specific membrane antigens of cancer cells, the copper-mediated reaction through an arylboronic precursor demonstrated satisfactory efficiency. At room temperature, selecting an appropriate boronic precursor and protecting its carboxyl groups with tert-butyl (t-Bu) groups achieved high labeling efficiency with ^{77}Br at 93%. T-Bu protection avoids the reduction in radiochemical conversion caused by the reaction with a carboxyl group. Other examples of reactions require oxidizing agents or organotin precursors. At a disadvantage, both reactions necessitate thorough product purification [35]. If a peptide or protein is brominated, a pre-conjugation using N-succinimidyl-2,6-dimethoxybenzoate allows the incorporation of bromine into the structure of any peptide with an α or ε-amino group. The presented solution enables a broader application than direct tyrosine residue labeling. Additionally, the presented approach's milder reaction conditions better preserve the peptide's biological activity [36].

Application of radiopharmaceuticals with ^{76}Br and ^{77}Br

Considering the similar properties of bromine and iodine, bromine could be a substitute radionuclide in nuclear medicine. Hashimoto T. et al. compared the isotope ^{77}Br with ^{125}I in a compound based on an inhibitor of p38α, which suppresses the inflammatory response. Bromine has a lower atomic number than iodine and probably will generate more Auger electrons than X-ray emission. In other words, it results in a more substantial destabilizing effect on cancer cells. Regrettably, in both cases, the inhibitory potential was similar. The radioactivity in the blood from the bromine nuclide persisted for at least 2 hours, indicating the stability of the compound. On the contrary to ^{77}Br, accumulation in the inflamed tissue was lower than with ^{125}I [37]. The magnified lipophilicity was the probable reason for low accumulation in the target tissue. In such cases, rapid uptake in the liver and transfer of the compound to the small intestine may occur. In this way, the result is minimal accumulation in the target tissue. Ogawa K. et al. improve the radiopharmaceutical biodistribution by introducing ethylene glycol into the molecule [38].

Another combination for studying the potential use of bromine-77 as a radiotherapeutic is with PARP-1 inhibitors. Incorporating ^{77}Br with rucaparib produced a more potent cytotoxic effect on prostate cancer cells. The Auger electron's high linear energy transfer is lethal but within a short range (10–100 nm). The nuclear localization of PARP molecules and their ability to bind DNA results in a beneficial response. The proximity of ^{77}Br nuclides to DNA significantly enhanced the destructive action in the tumor. Applying hybrid connection and radiation exposure caused cell cycle arrest at the G2-M phase by inducing double-strand DNA breaks. Even a single dose of the compound improved the survival of mice with prostate cancer [39]. *In vivo* studies on mice with ovarian cancer also observed favorable results. The ^{77}Br-rucaparib derivative (**35**) demonstrated specific cellular uptake and a thousand times lower median effective concentration than rucaparib alone. Compared to standalone PARP inhibitors, the hybrid compound showed higher cytotoxicity, independent of BRCA1 expression. Labeling with ^{76}Br in the same experiment did not show such good results. Significant uptake in the liver was observed after administration, followed by accumulation in the gallbladder in subsequent hours [40].

Tyrosine kinase inhibitors are an alternative class of targeting agents in cancer therapy with bromine radionuclides. These molecules can bind intracellularly to cancer cell lines with overexpressed mutated epidermal growth factor receptors (EGFR). Drugs used as tyrosine kinase inhibitors for cancer often do not effectively combat abnormal cells. During treatment, there is an increase in resistance mutations, including those occurring in EGFR. As a consequence, receptor variations create a problem in choosing a drug. On the other hand, immunohistochemical methods for determining EGFR mutations in tumors require tissue biopsies and have low specificity [41]. Radiobromine with rociletinib and osmitinib – drugs targeted at cells with the resistance mutation L858R/T790M of the EGFR receptor has the potential for usage. However, hybrid particles need improvements as therapeutic agents. Radiohalogen with rociletinib (**36**) was specific for double mutations L858R/T790M compared to wild-type EGFR and single mutation L858R. Unfortunately, the complex exhibited high hydrophobicity, which prevented effective tumor accumulation, and the marker lacked stability *in*

vivo. According to authors, there was undesirable accumulation in healthy lung cells, and the tested anticancer efficacy of the ^{77}Br complex was comparable to the non-halogenated compound [42,43].

A promising solution for using ^{77}Br radiotherapeutics in cancer treatment is the application of convection-enhanced delivery based on pressure-driven fluid flow. The procedure could prevent glioma cell growth recurrence after surgical resection. Raghavan R. et al. tested the effect of Auger electron emitters on a surgical resection model of a 2 cm tumor. By calculating the delivered radiation dose from radionuclides to cells, the toxicity to healthy cells would be very low for ^{77}Br. In the least promising calculations, it would be 0.51 Gy for healthy cells and 52 Gy for cancer cells. Most isotopes, including ^{123}I, ^{125}I, ^{99m}Tc, ^{195m}Pt, or ^{193m}Pt, would show higher toxicity to healthy cells [44].

The isotope ^{77}Br is frequently used in cancer research due to its relatively long half-life and dual capability as both an imaging agent (γ-emitter) and a targeted radiopharmaceutical. Instead, the positron-emitting isotopes ^{76}Br and ^{75}Br can have applications for PET imaging. However, ^{75}Br has a significantly shorter half-life than ^{76}Br (1.61 h vs. 16.2 h). Therefore, trials regarding ^{76}Br are more common [30,31]. For metabolic PET imaging of mouse DBT glioma cells, Bartels J.L. et al. tested the ^{76}Br complex with an amino acid. The (S)-amino-2-methyl-4-[^{76}Br]bromo-3-(E)-butenoic acid (**37**) undergoes mixed transport into the brain using amino acid transporters of systems L and A. The same compound, labeled with radionuclide ^{123}I, is transported by system A transporters. Effective transport to the brain via system A route is possible only with a damaged blood-brain barrier. Therefore, the described type of transport is very limited in terms of delivering imaging agents. System A transporters carry neutral and smaller amino acids, while system L transporters carry neutral and large ones. Despite mechanisms of transport, the brominated radiopharmaceutical was the one transported to a greater extent by the system L route. The results indicate that, in this case, the greater electronegativity of bromine and its impact on the molecule's charge were more significant than the larger substituent diameter of iodine. Differences in the compound transport routes resulted in higher uptake in subcutaneous glioma tumors of radiobrominated amino acids [30].

Combining ^{76}Br with meta-bromobenzylguanidine (**38**) presented promising out-turn in vitro for imaging carcinoma tissue expressing norepinephrine transporters. Imaging molecules allowed for detecting even small tumors in the PC-12 cell line. On the other hand, there was significant accumulation in the liver, intestines, stomach, and around the throat 3 hours after injection. Then, the radiopharmaceutical was washed out from non-targeted tissues [45]. The differences in biodistribution can appear when the site of atomic substitution changes. The bromine-76 atom's isomeric position in the aromatic structure influences the effectiveness of radionuclide delivery to the target site. Lang L. et al. demonstrated the relationship using the discussed isotope for potential corticotropin-releasing hormone receptor type 1 (CRF1) imaging. The [8-(4-bromo-2-,6-dimethoxyphenyl)-2,7-dimethylpyrazolo[1,5-a][1,3,5]triazin-4-yl]-N,N-bis-(2-methoxyethyl) amine (**39**) exhibited approximately 70 times greater affinity for the receptor than the 3-bromo isomer. Additionally, introducing methoxy groups into the phenolic ring reduced the compound's lipophilicity and subsequent adverse uptake by the liver. The modification resulted in better brain cellular uptake and reduced non-specific binding affinity [46]. Subsequent animal tests also indicated better properties of the 4-bromo derivative. The isomer had a higher affinity for CRF1 binding sites in monkeys' prefrontal cortex than the 3-bromo derivative and the corticotropin-releasing hormone. A missing element of the study is the analysis of *in vivo* biodistribution. The binding evaluation of the compound was conducted only through *ex vivo* autoradiographic studies on the brain [47].

Another evaluated combination of discussed radionuclide is with peptides containing sequence arginine-glycine-aspartic acid (RGD). Lang L et al. studied labeled RGD peptides to determine the expression of the integrin αvβ3 receptor in cancer cells. In a study on mice with glioma xenografts, the combination showed high accumulation in the tumor and comparable binding to the fluorine analog. However, the kidneys – excretory organs for integrin-binding peptides like RGD, also presented significant uptake for the studied molecule [36].

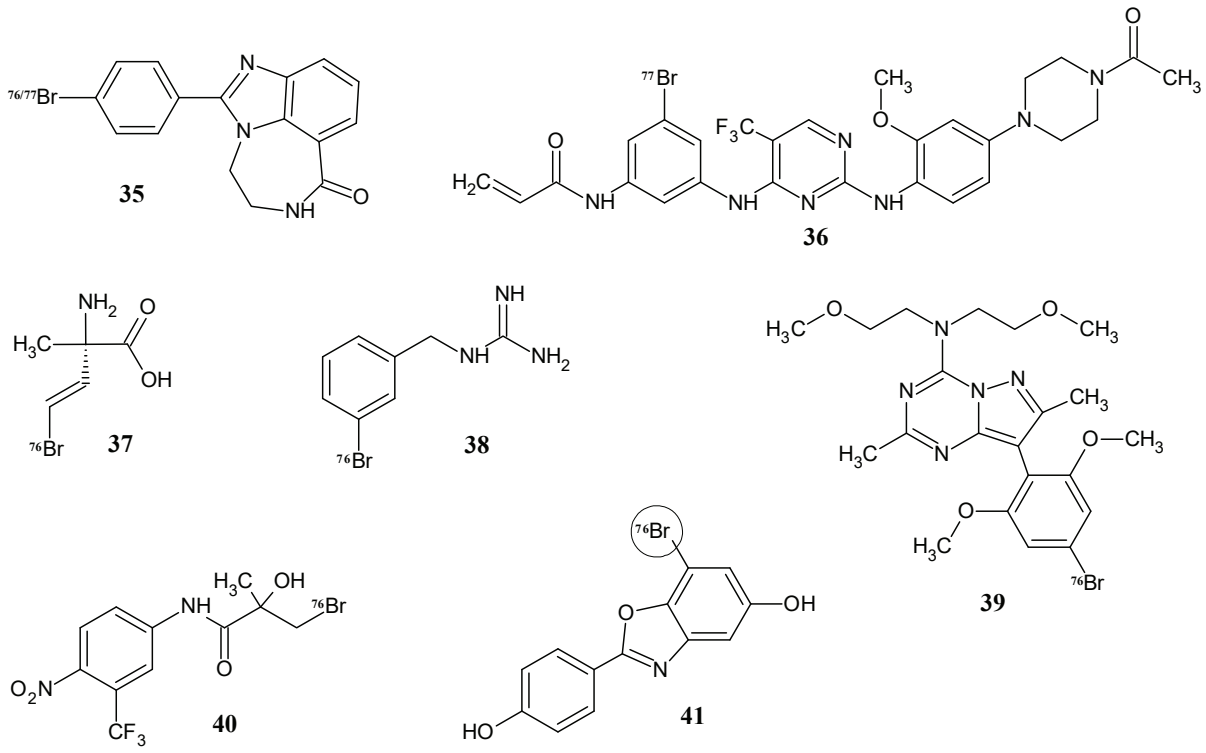

Figure 5 Radiofarmaceuticals with ^{76}Br and ^{77}Br.

In recent years, proposed ^{76}Br labeled compounds for imaging the occurrence of androgen, adenosine, and estrogen beta (ER-β) receptors were also ineffective [48–50]. The ligand for the androgen receptor, [^{76}Br]-hydroxyflutamide (**40**), had a higher binding affinity than the non-brominated compound. Still, its stability under physiological conditions was shallow, leading to rapid debromination of the complex [48]. Higher binding to the receptor in the halogenated version than the non-halogenated one appeared *in vitro* for the combination with the non-steroidal ER-β ligand (Br-041 (**41**)) – the ERB-041 analog, too. As well as some radiobrominated molecules, it did not have selective uptake by ER-β receptor cells *in vivo*. After administration to the body, there was more significant accumulation in the kidneys and liver than in the target tissue [50]. The same distribution was characteristic of the ^{76}Br – agonist – MRS3581 and the antagonist – MRS5147 of the adenosine receptor [49].

To summarise, studies focus on applying bromine nuclides as PET imaging agents or targeted cancer therapeutic. For the first implementation, research focuses on the radionuclide ^{76}Br. The β^+-emitter has a relatively long half-life, allowing for imaging of metabolic or pharmacokinetic processes at later time points compared to the commonly used ^{18}F. Conversely, for cancer therapy, the Auger electron emitter ^{77}Br is utilized. Its short range of action greatly minimizes damage to healthy cells caused by radiation emission [29–31]. On the other hand, a frequently encountered obstacle with brominated radiopharmaceuticals is their unfavorable biodistribution in tissues after introduction into the bloodstream. One of the reasons is the increased lipophilicity of the compound after introducing the bromine atom, leading to rapid hepatic uptake. Enriching compounds with hydrophilic groups can prevent observed mechanisms [38]. Besides introducing the bromine itself, the position of the halogen substitution also affects the efficiency of compound delivery in the body [46].

Isotopes such as ^{80m}Br, ^{75}Br, and ^{82}Br, despite their therapeutic or imaging purposes, have yet to be studied in recent years. The reason may be the better properties than commonly used radionuclides [31,33].

Influence of bromination on radiosensitization

The manufacture of radiopharmaceuticals requires more production stages than non-la-

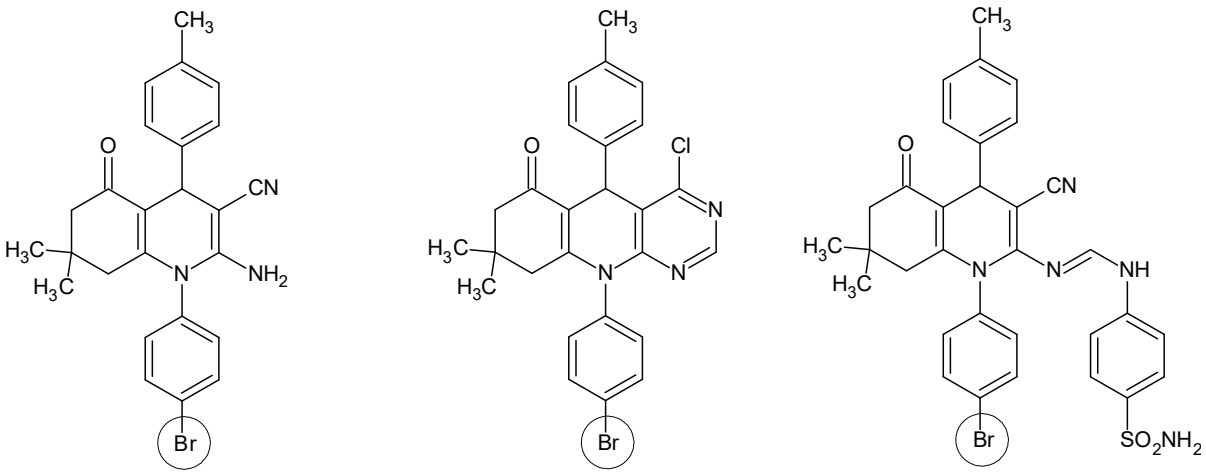

Figure 6. Quinoline derivatives with bromine in their structure.

beled chemotherapeutics. For this reason, the effectiveness of introducing natural bromine into the structure of a chemotherapeutic in combination with radiation is also being studied. Presence of bromine in nucleobases with exposition to low-energy electrons caused 2–3 times higher damage to oligonucleotides than in non-halogenated molecules. Their destabilizing potential ameliorates in the following order: 8-bromoadenine > 5-bromocytosine > 8-bromoguanine > 5-bromouracil. However, there are more interactions and bonds in cellular DNA, including hydrogen bonds between the bases of the two strands, which could alter the radiosensitizing effect on the nucleic acid [51]. In turn, Ghorab M. M. et al. studied the synergetic effect of combining a single radiation dose with quinoline derivatives. Of the synthesized compounds, the most active against the MCF7 breast cancer cell line were: 2-Amino-1-(4-bromophenyl)-7,7-dimethyl-5-oxo-4-p-tolyl-1,4,5,6,7,8-hexahydroquinoline-3-carbonitrile (**42**), 10-(4-Bromophenyl)-4-chloro-8,8-dimethyl-5-p-tolyl-7,8,9,10-tetrahydropyrimido[4,5-b]quinolin-6(5H)-one (**43**), and N'-(1-(4-bromophenyl)-3-cyano-7,7-dimethyl-5-oxo-4-p-tolyl-1,4,5,6,7,8-hexahydroquinolin-2-yl)-N-(4-sulfamoylphenyl)formimidamide (**44**). Each named hybrid molecule showed significantly higher anticancer activity than doxorubicin. After applying a single dose of γ radiation with the presented compounds, the IC_{50} value dropped by about 15 times for compounds **43** and **44** and 5 times for 08. The study results suggest that the mentioned modification can enhance the cytotoxic effect of radiation. Therefore, it is possible to reduce the chemotherapeutic or radiation intensity dose in therapy [52]. Picardi et al. chose porphyrins to study the aftermath of bromination. Enriched porphyrins demonstrated improved radiosensitization and photosensitization compared to their non-brominated versions. It is worth noticing that the authors consider that the strengthened effect is likely due to the higher cellular uptake rather than the enlarged production of cytotoxic species. In the case of photosensitizing, modification led to diminished singlet oxygen release while increasing the mean lethal dose. The same phenomenon could have occurred with enhanced radiosensitization [53].

In turn, modifying a compound with natural bromine can also improve the anticancer effect in radiotherapy. However, the synergistic effect is common to almost all chemotherapeutics. More studies with other molecules will determine the competitiveness of introducing such a modification into anticancer drugs compared to its absence [52].

Halogen bond

To better understand how bromine as a substituent in a potential drug molecule can affect pharmacokinetic and pharmacodynamic parameters, we need to look deeper into the physicochemical phenomena that occur between such a mol-

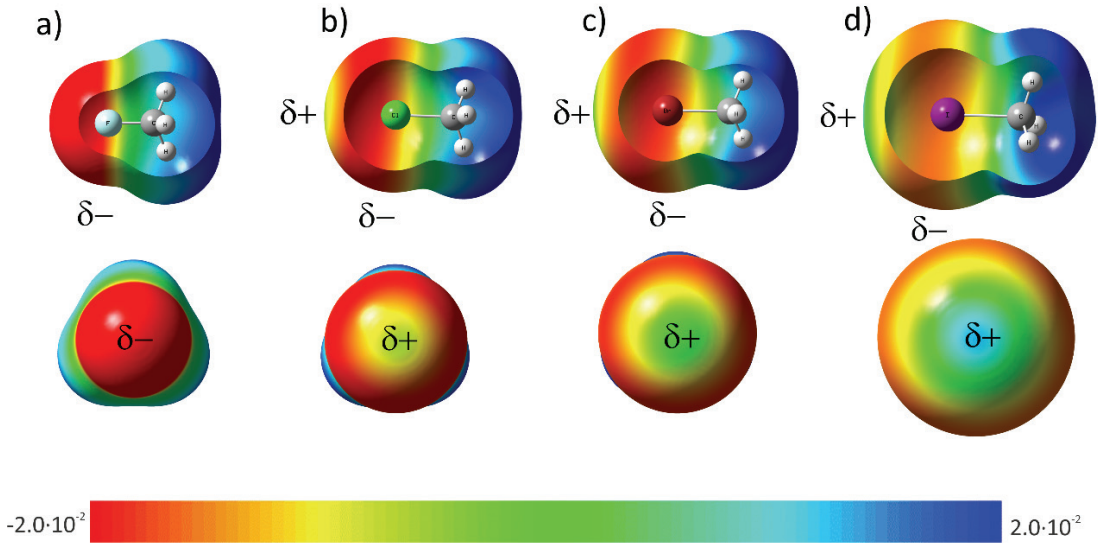

Figure 7. MEP of four halogenometanes: a) fluoromethane, b) chloromethane, c) bromomethane and d) iodomethane. Isosurfaces computed by Density Functional Theory method (functional M06–2X, basis function Def2TZVP).

ecule and a molecular target. The rational design of modern technologies in the fields of chemistry, biology, nanotechnology or medicine takes non-covalent interactions such as halogen bond into account.

Over the years, halogen substituents (-F, -Cl, -Br, -I) in organic molecules have been assumed to be nucleophilic substituents. Indeed, due to their higher electron density, halogens have a partial negative δ^- charge. Thus, they can be hydrogen bond acceptors, among other things. On the other hand, over the past few decades, numerous studies (e.g., molecular electrostatic potential (MEP) mapping, X-ray crystallographic studies) have been performed toward the possibility of halogens forming non-covalent bonds in organic compounds. Thus, the halogen bond (X-bond) was discovered. Through MEP mapping, the charge distribution of halogens in organic molecules was visualized, and it was found that halogen atoms (all except fluorine) do not have a homogeneous negative charge over the entire surface. On the halogen atom, opposite to the R—X bond and on its axis, a deficit in electron density occurs causing a region of positive electrostatic potential called the "sigma hole or σ-hole". Perpendicular to the R-X bond axis zone retains its negative electrostatic potential. The difference in electrostatic potential between the negative (equatorial) and positive (polar) zones of the atom is increasing in the Cl, Br, I order (**Figure 7**).

The σ-hole interacts with Lewis's base or any other electron-rich nucleophilic system to form a halogen bond. Since the observation of the first interactions in which the σ-hole plays a key role, several other types of bonds have been observed such as chalcogen, pnictogen and tetrel bonds [54]. Hence, the acceptor of such an X-bond in organic molecules can be atoms of oxygen, nitrogen, sulfur or the delocalized π orbital of the aromatic ring.

The first historically described compound in which a halogen bond occurs is the adduct of ammonia and the molecular iodine $NH_3 \cdots I_2$, described by M. Colin in 1814. However, a theoretical explanation of this phenomenon had to wait until the 20th century and the development of quantum chemistry. The explanation for this phenomenon remained unclear for a long time until the development of quantum chemistry. The first attempts to explain the formation of the halogen bond was the donor-acceptor model proposed by Mulliken. It involved the transfer of part of the electron density from the orbital occupied by the lone pair electron of the base to the orbitals of the halogen atom[55,56]. A theory describing the mechanism of halogen bond formation based on molecular electrostatic poten-

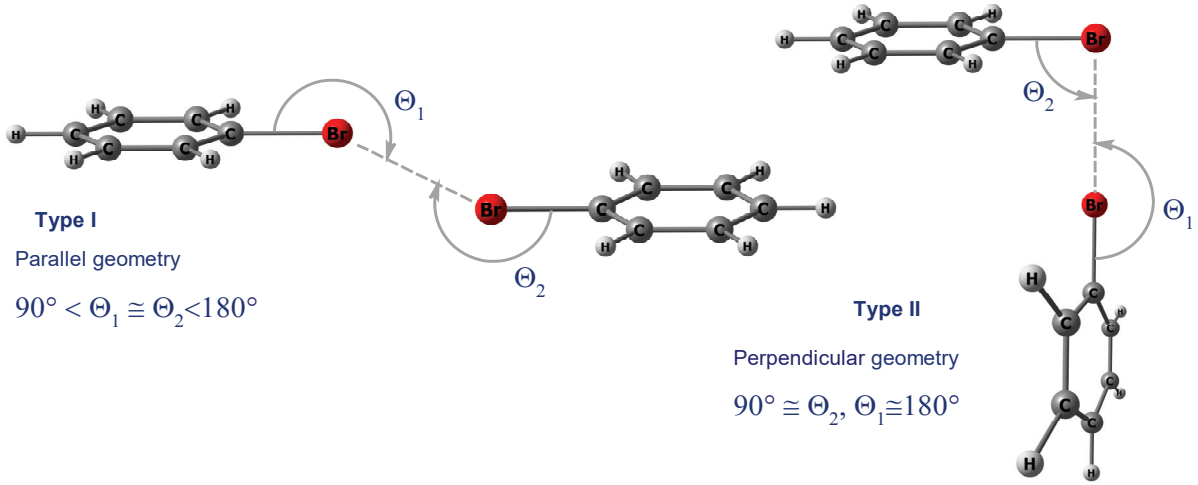

Figure 8. Two possible geometries of halogen-halogen bonds formed in bromobenzenes interactions: type I (parallel) and type II (perpendicular).

tial and sigma hole formation was presented by Polizer and co-workers[56]. Halogen bond energy depends on its type and ranges from 10 kJ/mol (N···Cl) to 150 kJ/mol (I_2···I^-) [57], whereas hydrogen bonds can vary in strength from weak 1 kJ/mol to strong (F—H···F^-) 161.5 kJ/mol [58,59].

According to the definition by IUPAC "a halogen bond occurs when there is evidence of a net attractive interaction between an electrophilic region associated with a halogen atom in a molecular entity and a nucleophilic region in another, or the same, molecular entity" [60]. Expanding on the definition, many data sources, such as protein and nucleic acid databases (PDB), crystallographic structures etc., show the angles between donor and acceptor of halogen bonds, where the angle C—X···Y is close to 180° and the bond length (**d**) X···Y is equal to or less than the sum of the van der Waals radii of these atoms. Consequently, two types of halogen bonds are distinguished – bond with a free electron pair where Y = O, N, S and bond with a delocalized π orbital [61]. In the first case, the angle **α** > 140°. While in the second, **α** > 120° and **θ** < 60° (**Figure 8**, **Figure 9**).

The strength of the halogen bond depends on many factors but mostly it is determined by the size of the σ-hole. Through MEP mapping, Ryan J. et al. discovered a relationship between σ-hole size and the radius of the halogen atom, which increases in the direction Cl < Br < I [62]. On the example of halogenated derivatives of benzodiazepines and their activity against the MDM2 protein, which is responsible for the down-regulation

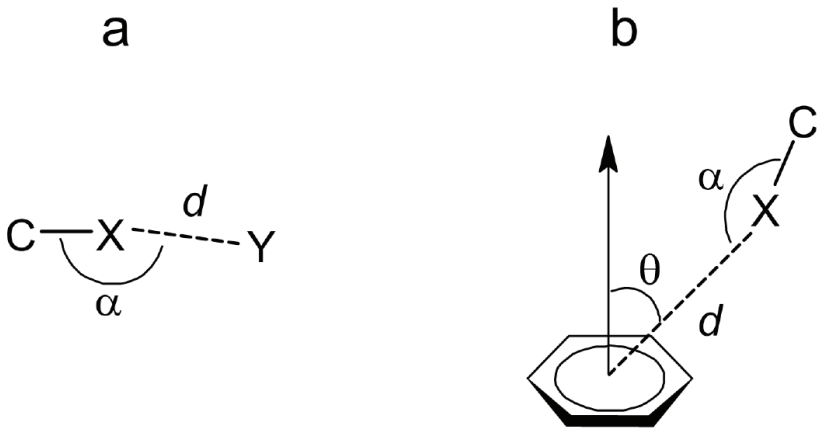

Figure 9. Types of halogen bonds: a – bond with nucleophile, where X = Cl, Br, I and Y = O, N, S; b – bond with aromatic ring.

of the p53 protein, Daniel J. Parks et al. demonstrated a twofold decrease in the affinity of the API for the molecular target after iodine replacement with bromine and a 3.6-fold decrease when substituted with chlorine. This is explained by a decrease in halogen bond strength [63].

The strength of the halogen bond can also be modified by substitution of electron-extracting groups (e.g. -F, -CF_3, -NO_2, -OCH_3, etc.). In their work, Riley K. et al. compared the electrostatic σ-hole potentials of various halobenzene derivatives. Comparing a series of compounds with different configurations of substituents in the ring (chloro-, bromo-, iodobenzene and their difluorinated and pentafluorinated derivatives), the authors concluded that the σ-hole exposure changes directly proportionally with the amount of fluorine in the ring. Namely, the electrostatic potentials of 1-chloro-2,6-difluorobenzene and 1-chloro-3,5-difluorobenzene are comparable to bromobenzene, while 1-bromo-2,6-difluorobenzene and 1-bromo-3,5-difluorobenzene are comparable to iodobenzene. In this way, it is possible to increase the strength of the halogen bond without changing the halogen in the molecule [64]. In a similar study, in addition to calculating the electrostatic potential, calculations were made for cocrystals with 21 X-bond acceptors and it was proven that the more the σ-hole is exposed, the stronger the above mentioned bond [65]. In another of their papers, Aakeröy Christer B. et al. proved the same dependence for aliphatic halo-derivatives [66]. The influence of other electron-withdrawing substituents was also studied by Forni A. et al. In their work, the researchers compared the gas-phase binding energy of formaldehyde with iodobenzene and its derivatives with different substituent configurations [67].

The strength of the halogen bond is also determined by external factors [68]. The medium in which the X-bond complex is formed is important. In the same study, Forni A. et al. compared halogen bond energies in complexes of iodobenzene and formaldehyde derivatives based on calculations in vacuum, diethyl ether and water. It turns out that as the dielectric constant of the solvent increases, the bond X length shortens and the angle of this bond increases. It would be worth expecting an increase in the strength of the bond, but at the same time with this another phenomenon occurs. Namely, the C—I bond gets extended, which plays a significant role in the formation of a halogen bond. Summing up, as the polarity of the solvent increases, the X-bond strength decreases [67].

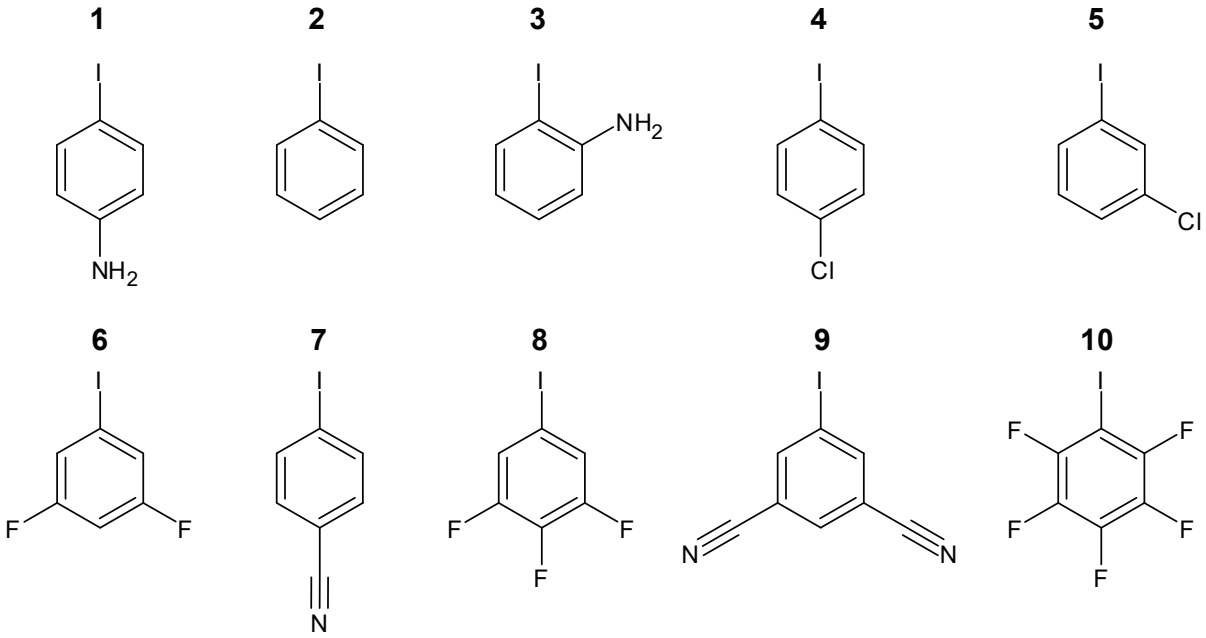

Figure 10. Iodobenzene derivatives for which halogen bond strength was calculated by Forni A [67]. et al. Compounds were placed in order of increase in bond strength (1 – p-aminoiodobenzene, 2 – iodobenzene, 3 – o-aminoiodobenzene, 4 – p-chloroiodobenzene, 5 – m-chloroiodobenzene, 6 – 3,5-difluoroiodobenzene, 7 – p-cyanoiodobenzene, 8 – 3,4,5-trifluoroiodobenzene, 9 – 3,5-dicyanoiodobenzene, 10 – pentafluoroiodobenzene).

So far, halogen substituents in API molecules have been considered as moieties that increase the lipophilicity of compounds. After the discovery of such a phenomenon as halogen bond, scientists began to consider the interactions of halogen-substituted bioactive substances with molecular targets in a slightly different way. It turns out that X-bonding has a significant impact on the interaction of drugs with biomolecules. As mentioned above, halogens form bonds with atoms such as oxygen, nitrogen or sulfur – atoms commonly found in proteins and protein structures of the cell (e.g., enzymes, receptors, etc.). If the molecular target of an API is such a structure, introducing a halogen into its molecule can increase the affinity and strength of binding to that target. At the same time, the X-bond is formed with both the backbone chain of the protein and the side chain [69]. One of the studies confirming the positive effect of halogens on biological activity is the work of Leo A. Hardegger et al. The authors examined a series of modified inhibitors of human cathepsin L (hCatL), an enzyme that, among other things, is responsible for the fusion of viruses into host cells. Through crystallographic analysis, it was proven that halogens form halogen bonds in the S3 pocket of cathepsin which contributes to an increase in the strength of the ligand's binding to the target, resulting in an increase in inhibitory activity. The affinity of such drugs increases 13-fold in some cases. The authors also compared the IC_{50} values of all derivatives. It turns out that chlorine substitution decreases the IC_{50} value by a dozen or even tens of times in some cases. It was also confirmed that substitution of larger halogens contributes to an even greater increase in inhibitory activity [70]. In another study, L. Rohde et al. compared the affinity of five agonists of the α4β2 nicotinic receptor, a therapeutic target for the treatment of certain psychiatric disorders and nicotine addiction. They proved that bromine-substituted APIs have 2–3 times higher affinity for the receptor compared to derivatives without a halogen in their structure [71]. D. Himmel et al. in turn examined the activity of three derivatives, two of which have an iodine atom in their structure, from the group of non-nucleoside reverse transcriptase inhibitors against HIV. The efficacy of these derivatives was compared on a dozen different strains, and it turns out that the halogenated derivatives have better activity against the above mentioned enzyme, making it possible to achieve nanomole or subnanomole IC_{50} values of these APIs. In some cases, the inhibitory effect is enhanced by several hundred times. More importantly, activity was tested against mutants that lack certain binding sites and are resistant to commercially available drugs. As the results show, by introducing a halogen into the molecule, the drug resistance of these mutants can be bypassed because halogen bonds can be formed with other transcriptase binding sites [72]. In addition to binding strength, halogens also affect drug residence time. The X-bonds formed stabilize the complex and significantly reduce the elimination rate of the API from the complex. This allows for the extension of the drug's duration of action, which has a direct impact on its efficacy [73].

Apart from improving the pharmacodynamic parameters of the drug, halogenation also has a positive effect on pharmacokinetic parameters. It is a well-known fact that the more lipophilic a compound is, the better it penetrates biological membranes, which also translates into the bioavailability of the drug. Since halogens in organic molecules were considered substituents that increase lipophilicity, such compounds are expected to have better permeability. This ability, on the other hand, depends on many factors, including the ability to form non-covalent bonds. Speaking in the context of halogen bonds, phospholipids, which are the main building block of biological membranes, have in their structure such acceptors as oxygen from the phosphate residue and carboxylic oxygen derived from fatty acids. In their work, R. Nunes et al. through simulations compared the ability of three benzene derivatives (pentafluoroiodobenzene, iodobenzene and p-iodophenol) to penetrate the phospholipid bilayer and the processes involved in their interaction. The crucial factor turned out to be the halogen bond that was formed during the penetration process. An interesting fact is that X-bonding took place in all stages of membrane penetration and even preceded this process, which allowed the authors to suggest that halogens in the molecule are promoters of passive transport inside the cell which is a property desirable in drug design [74]. Besides, the introduction of halogens into the structure of drugs is a good tool for modifying already existing drugs to improve their activ-

ity. Relatively lipophobic APIs targeting a protein structure have relatively weak properties to bind to the lipophilic part of the peptide (e.g., aromatic amino acids such as tryptophan, phenylalanine) and penetrate this so-called "hydrophobic pocket". Since the π orbital of the aromatic ring can serve as an acceptor for the halogen bond, in this way we can find new binding sites for the API and enhance its action several times or even find other therapeutic applications [75]. In conclusion, halogenation of drugs is a good tool to improve their bioavailability which has a significant impact on therapeutic efficacy.

As it turns out, the presence of halogens in the structure of a molecule also has a significant effect on its metabolism. In the case of prodrugs, their metabolism is a desirable phenomenon. However, it is often the case that the metabolites of a therapeutic substance show much lower activity or no activity at all. In extreme cases, the metabolites can even be toxic. In such cases, when designing a drug, we try to minimize its metabolism or eventually direct it in a different way. Enzymes of the cytochrome p450 family are mainly responsible for metabolism in the body. In itself, the C—X bond is very difficult to break [76]. This can be ascertained by the example of the tert-butyl group.

In their study, Hyeung-geun Park et al. compared a series of TRPV 1 receptor antagonists with analgesic effect. Looking at the two particular examples shown in Fig. 11 which differ only in the methyl group in the tert-butyl substituent, the researchers found similar activity for both derivatives. Prototype **a** was rapidly metabolized, which was associated not only with rapid elimination of the drug from the body, but also its metabolite lost selectivity to the target receptor. Replacing one methyl group with a perfluoromethyl group completely blocked this metabolic pathway at the cost of a slight increase in IC_{50} values, while lowering API clearance by 3.5-fold [77]. In addition to this fact, halogen bonds also affect the metabolism of xenobiotics in our body. In their study, S. Jiang et al. subjected chlorinated benzyl cyanides to the action of nitrilase, an enzyme that is responsible for the conversion of a nitrile group to a carboxyl group. In both cases, the aromatic ring was substituted with only one chlorine atom, while they differed in the position of the fluorine – in the first, the chlorine was in the meta position, in the second – the para. It turns out that the enzyme is selective towards the meta isomer, while it is not active towards the para isomer. It is known that in order for the enzyme to bind to the ligand it is necessary that the substrate is properly located in the active site. The enzymatic reaction occurs only when the substrate-enzyme complex settles into the correct space configuration. In both cases, the researchers observed an X-bond in the substrate-enzyme complex, but the acceptors of this bond were different amino

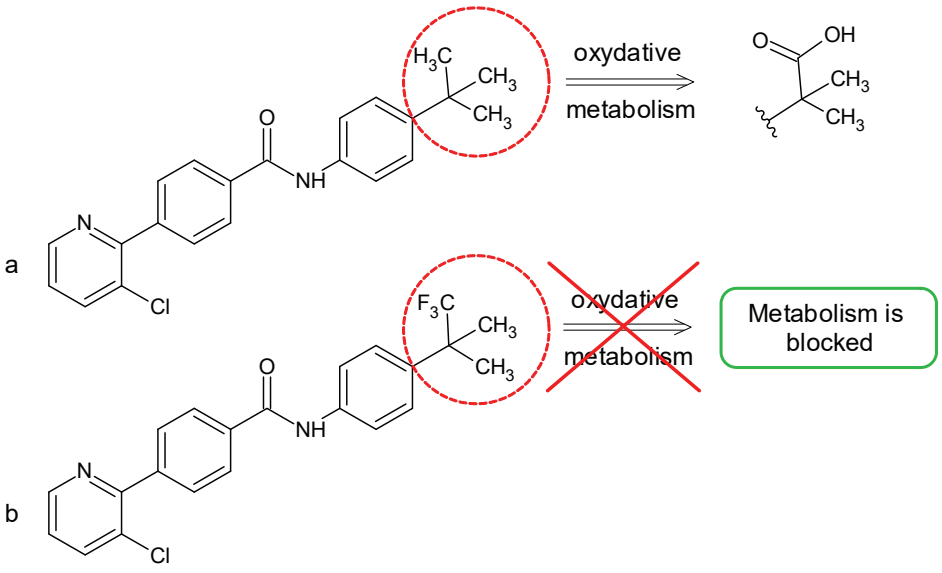

Figure 11. TRPV 1 receptor antagonists with analgesic effect and their metabolism. a – prototype with a tert-butyl group, b – prototype with one methyl group replaced by a perfluoromethyl group.

acids of the nitrilase – for the meta-derivative it was Gly195, while for the para-derivative it was Tyr173. Thanks to this difference, the compounds arranged in a completely different way. The nitrile group for the first derivative was exposed to the enzyme's active site and undergoes a reaction. For the second derivative, the nitrile group was unavailable [78].

Halogen bonds are found not only in xenobiotic-molecular target interactions. These bonds are also present in biomolecules of the human body. A very good example is the group of thyroid hormones (TH). These include thyroxine (T4) and triiodothyronine (T3), which is the more active form of T4. These hormones are very important for the human body, because they are responsible for fat and carbohydrate metabolism, body growth, brain development etc. Numerous studies by analyzing crystallographic structures confirm the presence of halogen bonds in every interaction of TH with other biomolecules of the body. Scientists also believe that halogen bonds play a key role in molecular recognition processes [79–81].

To begin with, it is worth mentioning the transport of THs. As the molecules are strongly lipophilic, certain transporters are required to transport them to their target site. Thyroxine binding globulin (TBG), transthyretin (TTR) and serum albumin (HSA) are responsible for this process in our body. The most important of these is TBG, as it has the highest affinity for T4. This protein has two cavities for binding T4 between helices H and A, and one of the bonds between T4 and TBG is actually the X-bond, which is formed between 3-iodine of thyroxine and oxygen from L269 of this protein. An interesting fact is that for TTR the key affinity factor is the number of iodine atoms in the molecule. T4 has 100% affinity, where T3 and T2 have only fractions of a percent. For this transporter, multiple halogen bonds can be observed at the same time in the hormone-protein complex. HSA z has four T4 binding sites (Tr1, Tr2, Tr3 and Tr4), where in each of them we can observe the formation of an X-bond [79].

In the human body, regulation of TH activity is very important. The iodothyronine deiodinases (Dio) family of enzymes is responsible for this process. These enzymes regulate THs activity by removing iodine atoms from THs molecules and their metabolites in a process called deiodination. The active site of this enzyme contains a peptide with selenium in its structure, which in this case is a halogen bond acceptor. The researchers say that this type of bond is responsible for regioselectivity in the deiodination process. In addition, the researchers hypothesize that the X-bond of I·····Se leads to the polarization of the C—X bond which allows the iodine to cleave from the TH molecule [80,81].

As THs are counted among the hormones that regulate gene expression, there is a family of appropriate nuclear receptors in cells. By analyzing ligand-receptor crystallographic structures, the researchers confirmed the presence of halogen bonds between 3-iodine of T3 and oxygen from F218 of binding pocket [79].

Taking into account the key factor, which is halogen bond, in the interaction between Dio and TH, it is possible to design suitable active substances to regulate the work of this enzyme which would lead to the regulation of TH activity. In several studies and computational research papers, scientists have analyzed the interactions between Dio and polybrominated diphenyl ethers (PBDEs). It turns out that halogen bonds can be observed in the PBDE-Dio complex. In some cases, the affinity of PBDEs is slightly weaker than that of THs, but this studies can serve as inspiration in designing drugs that can be used in diseases associated with TH activity [80].

As seen from the research presented up above, halogen bonds can be a useful tool for drug design. By introducing halogens, for example bromine, into a molecule, we can positively affect the parameters of a potential drug.

Conclusions

The objective of this review article is to explore the role of the bromine atom in drug molecules and its potential use in therapy. To summarize, both positive and negative aspects of it are presented below.

The arguments in favor of bromine are:

- There are natural compounds in nature containing bromine in their structure, which have proven therapeutic activities.
- The above-mentioned molecules can serve as inspiration in the design of new drugs.
- Derivatives from different chemical groups often show bacteriostatic and bactericidal

activity, even against drug-resistant strains. They also often show biocidal activity against other harmful organisms and viruses.

› Due to the occurrence of the halogen bond phenomenon, various parameters of potential drugs (e.g., drug elimination kinetics, drug metabolism, ability to penetrate biological membranes and bioavailability) can be improved compared to analogues without bromine.

› The above-mentioned binding also contributes to effects on drug potency and selectivity toward molecular targets.

› Compounds with various bromine isotopes may find application in radiotherapy. A positive effect has been proven in the treatment of various types of cancer.

› Bromine positively affects the effectiveness of photodynamic therapy as well as radiosensitization (heavy atom effect).

Disadvantages of using bromine in drug design:

› Although bromine contributes to prolonging the duration of action of therapeutics, it can also potentiate cumulative effects in the body, which in turn can lead to toxic effects.

Some bromine compounds including aromatics have proven harmful effects on the human body [82,83].

The presence of bromine in the API molecule and the formation of halogen bonds may not only contribute to improved pharmacokinetic parameters. In some cases, it can increase the affinity of the substance for enzymes, leading to faster metabolism.

Acknowledgements

Conflict of interest statement
The authors declare no conflict of interest.

Funding sources
There are no sources of funding to declare.

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