“Magic Chloro”: Profound Effects of the Chlorine Atom in Drug Discovery

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Abstract

Chlorine is one of the most common atoms present in small-molecule drugs beyond carbon, hydrogen, nitrogen and oxygen. There are currently more than 250 FDA-approved chlorine-containing drugs, yet the beneficial effect of the chloro substituent has not yet been reviewed. The simple substitution of a hydrogen atom (R = H) with a chlorine atom (R = Cl) can result in incredible improvements in potency of up to 100,000-fold, and can lead to profound effects on pharmacokinetic parameters such as clearance, half-life, and drug exposure *in vivo*. Following the literature terminology of “magic methyl effect” in drugs, the term “magic chloro effect” has been coined herein. Reports of 500-fold or >1000-fold potency improvement are often serendipitous discoveries that can be considered “magic” rather than planned. However, hypotheses made to explain the magic chloro effect can lead to lessons that accelerate the cycle of drug discovery. With this in mind, we believe that medicinal chemists should place chlorine atoms into their lead scaffolds in judicious fashion, and organic chemists should invent more methods to place chlorine atoms selectively onto complex molecules.

Introduction

In preclinical studies of small-molecule drug discovery, the role of a medicinal chemist is to modify the structure of a chemical compound to improve its activity against the biological target. Understanding and optimizing this structure–activity relationship (SAR) is at the heart of any medicinal chemist's job. Throughout a medicinal chemistry campaign, it is critical to improve the compound's potency against the biological target, since potency is one of the deciding factors of what the patient's daily drug dose will be. Of course, potency against a target is one of many parameters that a medicinal chemist must simultaneously optimize.[1] Large changes in the chemical structure can result in large improvements in potency, but this can also cause drastic and unpredictable changes in the compound's aqueous solubility, cell membrane permeability, and pharmacokinetic parameters such as clearance, half-life, and so on. Medicinal chemists always wish to make a small change in the chemical structure and magically improve potency, while keeping the other parameters unchanged. In an ideal world, that would equate to modifying the C–H bond of a compound to a simple C–R group, where the R substituent would be chemically "small" and consist of a few atoms at most.

A notable example of such a substituent is the methyl group, in which R = CH_3 substitutes a hydrogen atom (R = H) in the molecule of interest. Although typical improvements in potency arising from an additional methyl group is on the order of 3.5- to 10-fold, a compilation of "magic methyl effects" of up to 590-fold improvement in potency (per methyl group) has been documented in a landmark review by Schönherr and Cernak in 2013.[2] First coined in 2009,[3] the term "magic methyl" has been used frequently since this review was published, both by an increasing number of organic chemists who develop C–H methylation strategies,[4–11] and by medicinal

chemists who attribute this term to SAR trends found in their drug discovery programs.[12–21] As such, the profound effect of this small methyl group in medicinal chemistry has been discussed at length.[2,22,23]

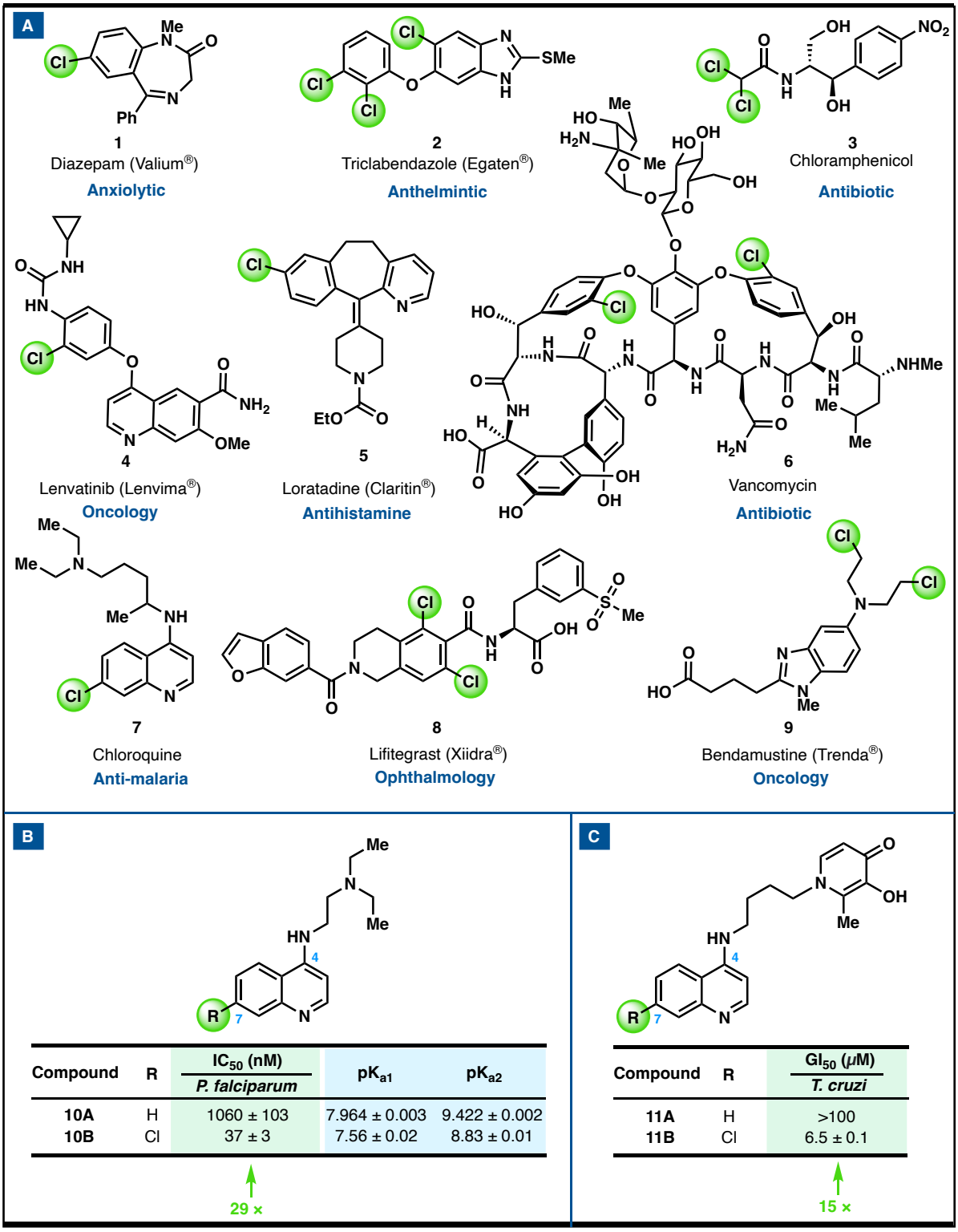
Are there other small substituents that could create a similar effect? The fluorine atom (R = F) has been known as a metabolically stable bioisostere of a hydrogen atom (R = H),[24–26] and the benefits of the fluoro substituent in medicinal chemistry such as for potency, physicochemical properties, and DMPK parameters have been extensively reviewed.[27–32] In a similar fashion, the trifluoromethyl group (R = CF_3) has been recognized as a metabolically stable bioisostere of a methyl group (R = CH_3) and,[26] although already prevalent in medicinal chemistry, the CF_3 group has been called "underexplored" in certain contexts.[33] Although potency improvements have been observed when replacing R = CH_3 with R = CF_3,[34,35] this phenomenon is not general and its benefit regarding bioactivity is still a topic of debate.[36]

A small substituent that has been used frequently in medicinal chemistry but not explicitly discussed is the chlorine atom (R = Cl). The chloro substituent can act as a bioisostere for many different functional groups, not only as a halide (replacing R = F, Br, and I with R = Cl) and as a monovalent substituent (replacing R = OH and SH with R = Cl), but also as a pseudo-halide (replacing R = CF_3 and R = CN with R = Cl) and even as methyl (replacing R = CH_3 with R = Cl).[24] The "methyl-chloro equivalence" had been identified in pesticides as early as 1953[37] but also in recent examples[38] displaying the isolipophilicity between methyl and chloro.[24] Other creative examples of a chloroalkene isosterically substituting an amide bond have also been designed.[39]

Introducing a chloro substituent on a compound, especially on an aromatic system, is typically easier than replacing a hydrogen atom with a fluoro, trifluoromethyl, or methyl group. Classic electrophilic aromatic substitution chemistry allows for a one-

step introduction of a chloro substituent, especially at the *para* position on a benzene ring. For this reason, the *para*-chloro analog is considered to be the best first choice when substituting phenyl groups in order to assess the logical next steps in what is now called the Topliss scheme.[40,41] In other words, the ability of the chlorine atom to mimic an electron-donating group (*e.g.*, OH, OCH_3), an electron-withdrawing group (*e.g.*, CF_3, CN), or a *quasi*-electron-neutral group (*e.g.*, CH_3) depending on the scaffold and context has been exploited in medicinal chemistry flowcharts.

The versatility of the chloro substituent highlighted above, as well as beneficial effects of halogen bonding[42] in protein–ligand interactions,[43–45] have ensured that the chlorine atom is prevalent in pharmaceutical agents. Chlorine atoms can often be found in Njardarson's "Top Pharmaceuticals Posters",[46] and in the chemical structures of well-known drugs and essential medicines (Figure 1A). In an analysis of the elemental composition of U.S. FDA-approved drugs, Njardarson observed that, after carbon, hydrogen, oxygen, and nitrogen, the most frequently encountered atoms are sulfur, chlorine, and fluorine, in this order.[47] The finding that the chlorine atom is more prevalent than the fluorine atom in drugs was rather unexpected.[47] A more recent review highlighting chlorinated drugs and synthetic approaches toward these scaffolds was also published.[48] Nevertheless, to the best of our knowledge, there is no review describing the profound effects of the chloro substituent in medicinal chemistry. Inspired by Schönherr and Cernak's 2013 review,[2] significant potency improvements of 10-fold or greater were searched in the literature and compiled. Herein, we describe the "magic chloro effect" in medicinal chemistry, with the objective of clearly establishing an informally known but poorly enunciated effect in drug discovery.



Compound	R	IC_{50} (nM) *P. falciparum*	pK_{a1}	pK_{a2}
10A	H	1060 ± 103	7.964 ± 0.003	9.422 ± 0.002
10B	Cl	37 ± 3	7.56 ± 0.02	8.83 ± 0.01

Compound	R	GI_{50} (μM) *T. cruzi*
11A	H	>100
11B	Cl	6.5 ± 0.1

Figure 1. A) The chloro substituent as an essential structural feature in drugs. **B) and C)** 7-Chloroquinoline, a privileged scaffold for anti-parasitic activity.

Methods of Literature Search

Medicinal chemistry journal articles were manually inspected and their SAR tables were examined to see whether potency values were present for matched molecular pairs[49] in which the only structural difference was R = H versus R = Cl. The years 2010–2022 (up to August 2022) of the following prominent medicinal chemistry journals were included in this study:

- *ACS Medicinal Chemistry Letters*
- *Bioorganic and Medicinal Chemistry*
- *Bioorganic and Medicinal Chemistry Letters*
- *ChemMedChem*
- *European Journal of Medicinal Chemistry*
- *Journal of Medicinal Chemistry*
- *Medicinal Chemistry Research*
- *MedChemComm* and *RSC Medicinal Chemistry*

2010 was chosen as the starting year because it was the first volume for *ACS Medicinal Chemistry Letters* and for *MedChemComm*. Furthermore, this 12.5-year span of journals conveniently delineated a total of 8 × 12.5 = 100 journal-years. This resulted in approximately 50,000 articles, whose matched molecular pair data were manually triaged. Out of the thousands of articles containing potency data comparing R = H and R = Cl, data were recorded only when the R = Cl compound presented a potency improvement of 10-fold (10 ×) or greater when compared to the corresponding R = H compound. This potency improvement cutoff resulted in >600 articles of at least 10 × potency improvement, >100 articles with >100 × potency improvement, and >20

articles with >1000 × potency improvement (see the Supplementary Information for spreadsheets containing these data).

This method of literature analysis ended up being a painstaking manual process despite databases of medicinal chemistry data being available in both commercial (*e.g.*, Reaxys Medicinal Chemistry) and public platforms (*e.g.*, PubMed, ChEMBL).[50] For example, matched molecular pair analysis for hERG affinity was conducted for R = H vs R = Cl using data that were extracted from ChEMBL.[51] However, Lipinski *et al.* stated that finding the necessary information from databases remains difficult, especially for bioactivity data, since there is no standardization for describing the activity metrics against a biological target (IC_{50}, pIC_{50}, IC_{80}, EC_{50}, GI_{50}, K_i, K_d, MIC, etc.).[52] Furthermore, a group from Roche mentioned that matched molecular pair analysis on large data sets is much more suited for ADMET and physicochemical properties than for biochemical activity.[53] Additional difficulties in sorting the nuances of on-target versus off-target activity in databases made it easier to read through each article and manually curating the desired matched molecular pairs of biological activity.

The potency improvement cutoff of 10-fold was determined as a corollary to Pfizer's lipophilic efficiency (LipE) metrics.[54] When substituting a molecule from R = H to R = Cl, the molecule most often increases in lipophilicity. Lipophilicity, as measured by partition coefficient (logP) or distribution coefficient (logD), is often predicted (as clogP or clogD) using additive effects. The lipophilic contributions of common functional groups have been compiled, and the change in logD (ΔlogD) caused by a chloro substituent was determined to have a median range of 0.60–0.70.[55] Of course, the ΔlogD for a given compound upon chloro substitution can be larger than 0.70, and is approximated herein as ΔlogD ~ 1.0 as the upper limit. If lipophilicity is the only driver of potency for a given biological target, then the expectation is that the logarithm

of the potency improvement should also be ~ 1.0, which equates to a 10-fold activity difference. Thus, in order to counteract a lipophilicity increase of ΔlogD ~ 1.0 (or 10-fold) when substituting R = H by R = Cl, the potency must at least improve by 10-fold to be considered significant. Using LipE terminology, the relevant equations are as follows:

(Eq. 1) $$LipE_{(R=H)} = -log\left(potency_{(R=H)}\right) - log\ D_{(R=H)}$$

(Eq. 2) $$LipE_{(R=Cl)} = -log\left(potency_{(R=Cl)}\right) - logD_{(R=Cl)}$$

(Eq.3 = Eq. 2 – Eq.1)

$$LipE_{(R=Cl)} - LipE_{(R=H)} = \left[-log\left(potency_{(R=Cl)}\right) - logD_{(R=Cl)}\right] - \left[-log\left(potency_{(R=H)}\right) - logD_{(R=H)}\right]$$

$$LipE_{(R=Cl)} - LipE_{(R=H)} = -\left[log\left(potency_{(R=Cl)}\right) - log\left(potency_{(R=H)}\right)\right] - \left[logD_{(R=Cl)} - logD_{(R=H)}\right]$$

$$\Delta LipE = -\Delta log(potency) - \Delta logD$$

In Eq.3, in order to at least maintain the LipE of the molecule when substituting R = H with R = Cl, $\Delta LipE = 0$. Then:

(Eq.4) $$0 = -\Delta log(potency) - \Delta logD$$

$$\Delta log(potency) = -\Delta logD$$

And if $\Delta logD \approx 1.0$,

(Eq.5) $$\Delta log(potency) = -1.0$$

(Eq.6) $$\frac{potency_{(R=Cl)}}{potency_{(R=H)}} = 10^{-1.0}$$

(Eq.7) $$potency_{(R=Cl)} = 0.1 \times potency_{(R=H)}$$

which means that the potency must decrease in numerical value by 10-fold (and therefore the activity must improve by 10-fold) when substituting R = H for R = Cl.

In some cases, serendipitous effects of >1000-fold have been achieved simply by making a one-atom change from H to Cl, which can only be attributed to “magic”. With this literature survey and analysis, the chloro substituent was found to be at least as important as “magic methyl”,[2] and therefore the same terminology was borrowed:

the magic chloro effect. Herein, we show rather unexpected, but dramatic results of R = H to R = Cl substitution in medicinal chemistry.

Data Analysis and Discussion

One of the most prevalent chlorinated motifs in the literature is a 4-amino-7-chloroquinoline core used for antiplasmodial activity against malaria. This motif is present in chloroquine (**7**; see Figure 1A), a molecule that is on the World Health Organization's list of essential medicines as an anti-malarial drug.[56] Since there is widespread chloroquine resistance due to *Plasmodium falciparum* malaria parasites acquiring *pfcrt* mutations,[57] there is still research interest in creating new anti-malarial drugs. Other anti-malarial drugs such as hydroxychloroquine and amodiaquine contain the same 4-amino-7-chloroquinoline core as well. A possible mechanism of action of chloroquine and related 4-amino-7-chloroquinoline molecules is that the drug in its uncharged form enters the digestive vacuole of the malaria parasite.[58] This digestive vacuole is a lysosome-like acidic compartment important for parasite metabolism and survival. Since 4-amino-7-chloroquinolines are weak bases, they increase the pH inside the digestive vacuole and disrupts the system, eventually leading to parasite death. To this end, the electron-withdrawing nature of the 7-chloro substituent decreasing the pK_a of the aminoquinoline (*i.e.*, rendering it more acidic) is critical in the pharmacophore of this drug. To study this effect, a series of 4-aminoquinolines with a substituent at the quinoline C7 position were synthesized and analyzed for both activity against *P. falciparum* and pK_a acidity values (Figure 1B).[59] The activity of the parent aminoquinoline **10A** (R = H) was improved 29 × by the introduction of the 7-chloro substituent (**10B**); this improvement in IC_{50} potency was likely caused by a difference in pK_{a1} of 0.4. Notably, when a 7-nitro group was introduced instead of the

7-chloro, the pK_{a1} difference was too large and the potency worsened. The 7-chloro substituent provided just the right amount of electron withdrawal to place the aminoquinoline pK_a in the optimal range for antiplasmodial activity.[59] A similar effect took place using this "privileged" 4-amino-7-chloroquinoline motif for anti-trypanosome activity.[60] When substituting the parent molecule from 7-H (**11A**) to 7-Cl (**11B**), the growth inhibitory activity against *T. cruzi* improved >15-fold (Figure 1C). Since potency improvements of 10~100 fold with the introduction of this 7-chloro substituent are common,[61] many medicinal chemistry programs have kept the 7-chloroquinoline as an obligatory element in the pharmacophore,[62,63] and organic chemistry methodology has even been developed specifically to facilitate functionalizations around the 7-chloroquinoline core.[64]

The Magic One-Chloro Effect in Pharmaceutical Lead Molecules

Unlike the privileged 7-chloroquinoline core, most of the scaffolds that show a drastic chloro effect in biological activity are isolated examples where a chlorine atom in the correct position of the molecule happens to create better binding to the target of interest. Partly due to Topliss' recommendation in 1972,[40] there is a bias among medicinal chemists to synthesize *para*-chlorophenyl compounds more frequently.[65] Although *para*-chlorophenyl compounds are not inherently privileged from a biological activity standpoint, this bias has led to increased numbers of examples where a *para*-chloro substituent on a benzene ring dramatically benefits the biological activity of a compound. Thus, several examples in Figure 2 and Figure 3 show these *para*-chloro effects, but these examples are not related in either the chemical scaffold or the disease of interest. Figure 2 specifically groups cases together because they were

supported by X-ray crystal structures or computational docking of the chemical compound onto the biological target of interest.

In the first example (Figure 2A), ribose nucleotide **12A** showed weak affinity for tumor-overexpressed eukaryotic initiation factor 4E (eIF4E), as measured in a scintillation proximity assay (SPA).[66] The *para*-chloro substituent in **12B** bestowed a 729 × potency improvement, allowing **12B** to have a good enough binding to eIF4E to allow for protein–ligand co-crystallization. The co-crystal structure of eIF4E complexed with **12B** showed that the *para*-chlorophenyl group fits the binding pocket almost perfectly, in both shape and size: the chlorophenyl moiety makes numerous favorable van der Waals interactions with residues including Phe48, Leu60, Asp90, Ser92, and Pro100 lining the pocket.[66] The chlorine atom specifically is in close contact with Phe48, Leu60, and Ser92. The lipophilicity of the chlorine atom is expected to fit well with lipophilic amino acid residues such as phenylalanine and leucine, but in this case, a halogen bond to the serine[43,44] also enhanced the protein–ligand interaction. The authors of this study noted that the chloro substituent prevents the phenyl ring from sampling other smaller pockets, further stabilizing the ligand binding.[66] Furthermore, this co-crystal structure revealed that the ribose ring only provides a conformationally restricted connection linking the terminal phosphate group with the chlorophenyl-guanine moiety. Since the ribose ring was in fact not necessary for binding, equipotent analogs were made without the ribose ring, successfully resulting in a scaffold truncation exercise.[66]

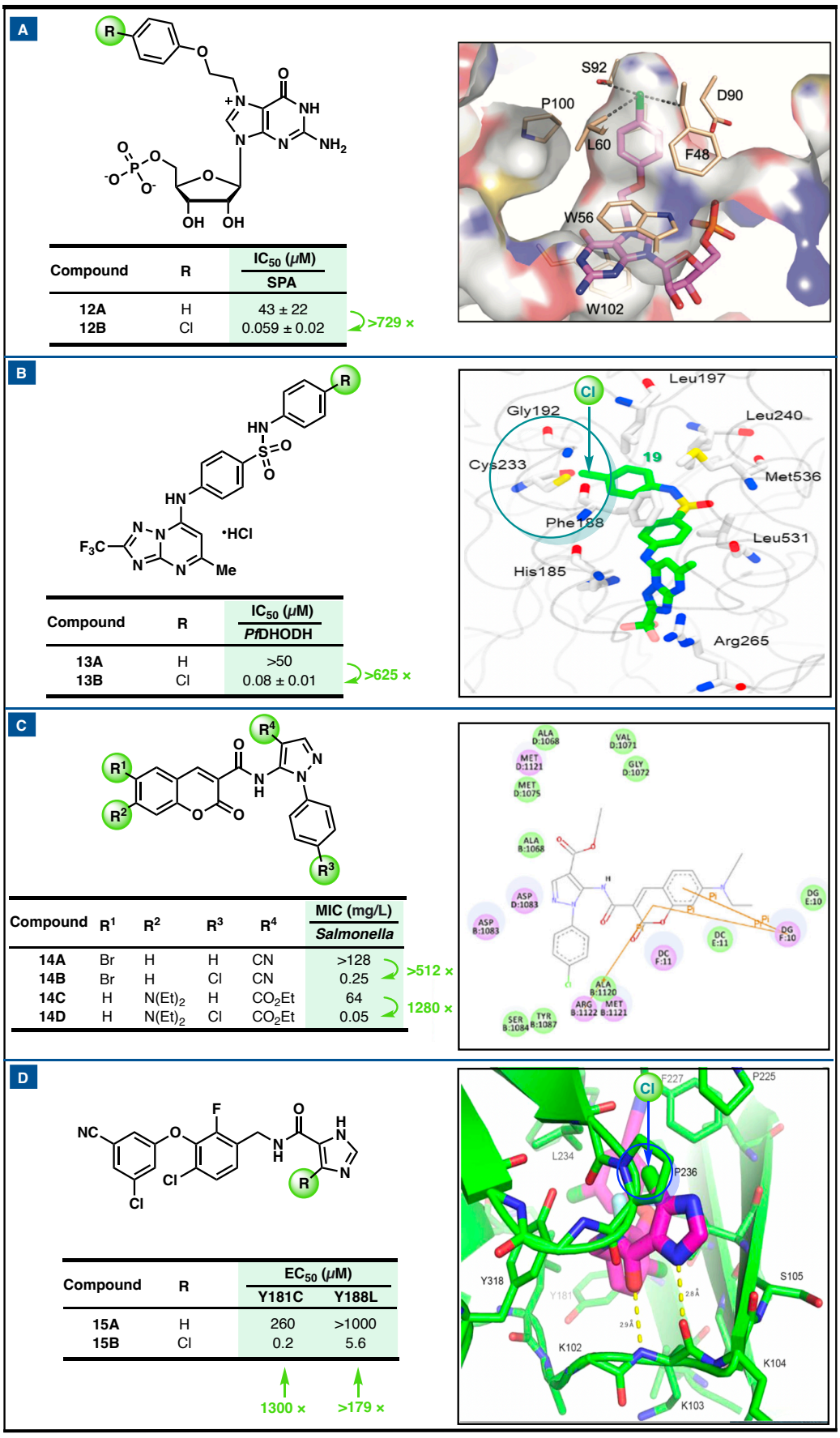


Figure 2. One-chloro effect on potency supported by pharmacophore models.

Combating *P. falciparum* as a way to treat malaria has steadily evolved, where researchers are now interested in exploiting dihydroorotate dehydrogenase enzyme (DHODH) inhibition as a strategy. An example of a *P. falciparum* dihydroorotate dehydrogenase enzyme (*Pf*DHODH) inhibitor is the class of triazolopyrimidines represented by compound **13A** (Figure 2B).[67] The corresponding *para*-chloro compound **13B** was the most active inhibitor in this study, representing a 625-fold IC_{50} improvement over the parent molecule **13A**. A co-crystal structure of the protein *Pf*DHODH bound to a small-molecule ligand, DSM265, is known because DSM265 is a potent inhibitor of *Pf*DHODH that is in clinical trials;[68,69] compound **13B** has many structural similarities to DSM265, and was computationally overlaid onto DSM265 inside the *Pf*DHODH binding pocket. In this binding model, the chlorophenyl group is surrounded by lipophilic amino acid residues such as Phe188 and Leu197, but the chlorine atom also engages with Cys233.[67]

Researchers around the world are trying to exploit different mechanisms of action to fight against antibiotic resistance. Bacterial DNA topoisomerase II inhibition is one way to develop new antibiotics that can target multidrug-resistant strains of bacteria such as methicillin-resistant *Staphylococcus aureus* (MRSA).[70] To this end, a class of topoisomerase II inhibitors containing a coumarin core and a phenylpyrazole unit was synthesized and tested against a Gram-negative bacterial strain such as *Salmonella* (Figure 2C).[71] Although very weak antibacterial activity was obtained with monosubstituted phenyl compounds **14A** and **14C** (where R^3 = H), a drastic improvement in minimal inhibitory concentration (MIC) was observed when *para*-chlorophenyl derivatives **14B** and **14D** (where R^3 = Cl) were tested. This study showed that the dramatic chloro effect takes place on more than one compound, and the

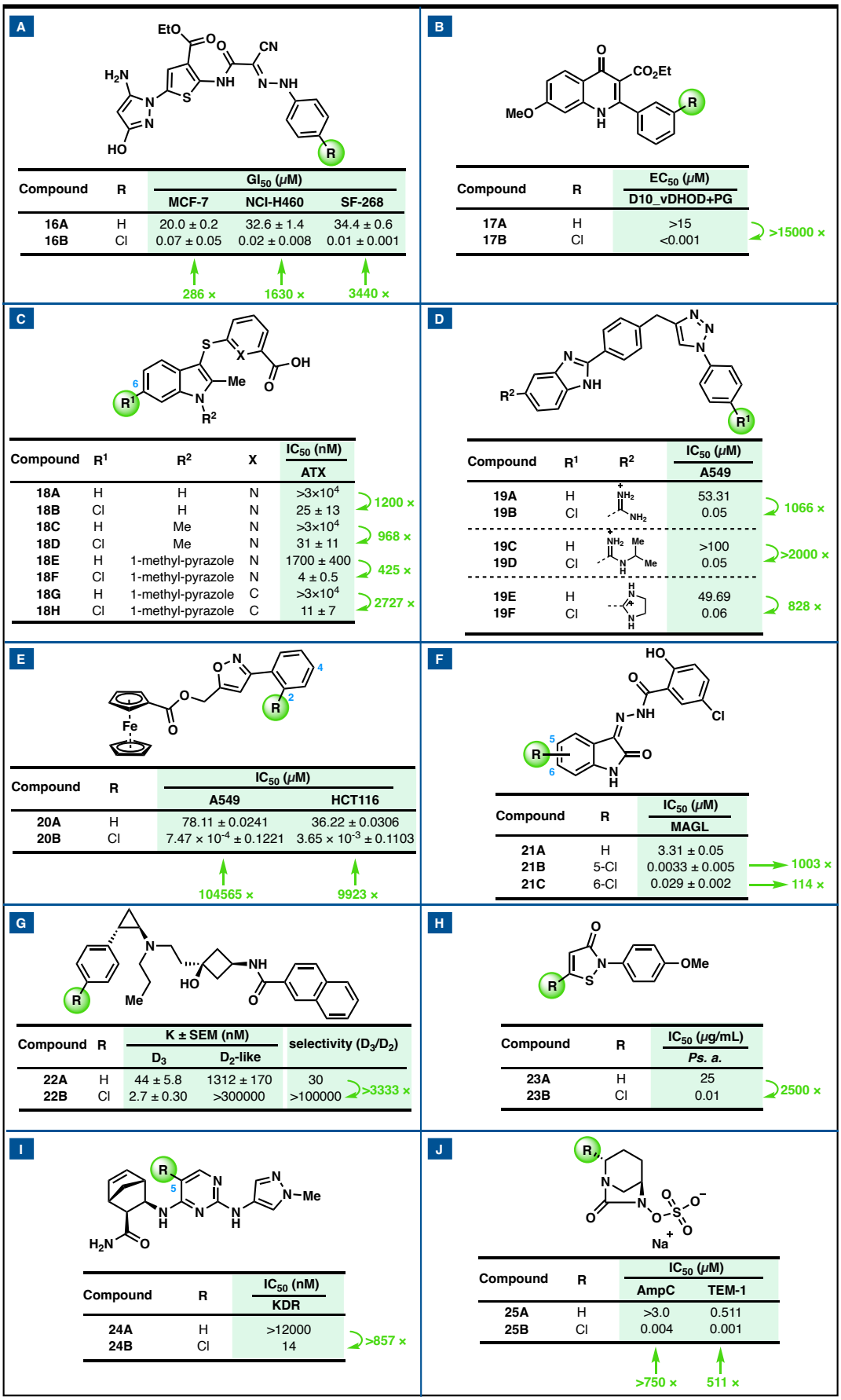
authors explained their finding by computationally docking their most active compound **14D** into the crystal structure of bacterial DNA topoisomerase II.[70]

HIV/AIDS therapy has greatly improved since the 1980s when contracting HIV was essentially a death sentence; today, it has become a rather manageable chronic disease, but there is still no cure to HIV. Although first-generation non-nucleoside reverse transcriptase inhibitors (NNRTIs) such as nevirapine or efavirenz have been effective, viral resistance to this mechanistic class of inhibitors continues to emerge.[72] In the quest toward discovering drugs against mutated HIV, binding of small molecules against NNRTI-resistant HIV reverse transcriptase proteins with Y181C or Y188L mutations was examined (Figure 2D).[73] In an example involving a chlorinated heteroarene, imidazole **15A** showed dramatic improvements in activity against Y181C and Y188L mutants of 1300 × and >179 ×, respectively, when a chlorine atom was introduced onto the imidazole ring to give **15B**. Analog **15B** was then bound to HIV-1 reverse transcriptase, and an X-ray crystal structure revealed the binding interactions around the chloroimidazole moiety. The researchers from GlaxoSmithKline noted that the beneficial chloro effect is caused by two main factors: 1) Small lipophilic groups like chloro in **15B** can occupy a small hydrophobic pocket created by Pro225, Phe227, and Pro236, thereby providing enhanced binding affinity over the parent compound **15A**; 2) the electron-withdrawing nature of the chloro substituent can lower the pK_a of the imidazole NH proton, facilitating hydrogen bonding and resulting in stronger binding of the imidazole NH to the amide carbonyl group on Lys103 in the protein backbone.[73]

Many other examples showing a magic chloro effect with >1000-fold potency enhancement were found in the literature (Figure 3). In the first example, three human tumor cell lines [breast adenocarcinoma (MCF-7), non-small cell lung cancer (NCI-

H460), and CNS cancer (SF-268)] were studied using a phenylhydrazone-containing thiophene lead compound (Figure 3A).[74] The parent phenylhydrazone **16A** only showed micromolar activity against these cancer cell lines, but a simple *para*-chloro substituent created the “magic” effect of drastically improving potencies against all three cancer cell lines, with 286 ×, 1630 ×, and 3440 × potency improvements against MCF-7, NCI-H460, and SF-268, respectively. The authors believed that these anti-cancer activities arise from the compound's affinity toward cyclin-dependent kinase 2 (CDK2), so they obtained a structure of CDK2 complexed with a related thiophenecarboxamide to conduct further studies on their system.[74]

In a rare *meta*-substituted chlorophenyl example, while examining antibacterial activity against chloroquine-sensitive *P. falciparum* strains (“D10” strains) with yeast dihydroorate dehydrogenase (thereby DHODH-inhibitor resistant), a multi-center research collaboration observed a magic chloro effect of more than 15,000 fold (Figure 3B).[75]



Compound	R	GI_{50} (μM) MCF-7	GI_{50} (μM) NCI-H460	GI_{50} (μM) SF-268
16A	H	20.0 ± 0.2	32.6 ± 1.4	34.4 ± 0.6
16B	Cl	0.07 ± 0.05	0.02 ± 0.008	0.01 ± 0.001

Compound	R	EC_{50} (μM) D10_vDHOD+PG
17A	H	>15
17B	Cl	<0.001

Compound	R^1	R^2	X	IC_{50} (nM) ATX
18A	H	H	N	$>3\times10^4$
18B	Cl	H	N	25 ± 13
18C	H	Me	N	$>3\times10^4$
18D	Cl	Me	N	31 ± 11
18E	H	1-methyl-pyrazole	N	1700 ± 400
18F	Cl	1-methyl-pyrazole	N	4 ± 0.5
18G	H	1-methyl-pyrazole	C	$>3\times10^4$
18H	Cl	1-methyl-pyrazole	C	11 ± 7

Compound	R^1	R^2	IC_{50} (μM) A549
19A	H		53.31
19B	Cl		0.05
19C	H		>100
19D	Cl		0.05
19E	H		49.69
19F	Cl		0.06

Compound	R	IC_{50} (μM) A549	IC_{50} (μM) HCT116
20A	H	78.11 ± 0.0241	36.22 ± 0.0306
20B	Cl	7.47×10^{-4} ± 0.1221	3.65×10^{-3} ± 0.1103

Compound	R	IC_{50} (μM) MAGL
21A	H	3.31 ± 0.05
21B	5-Cl	0.0033 ± 0.005
21C	6-Cl	0.029 ± 0.002

Compound	R	K ± SEM (nM) D_3	K ± SEM (nM) D_2-like	selectivity (D_3/D_2)
22A	H	44 ± 5.8	1312 ± 170	30
22B	Cl	2.7 ± 0.30	>300000	>100000

Compound	R	IC_{50} (μg/mL) *Ps. a.*
23A	H	25
23B	Cl	0.01

Compound	R	IC_{50} (nM) KDR
24A	H	>12000
24B	Cl	14

Compound	R	IC_{50} (μM) AmpC	IC_{50} (μM) TEM-1
25A	H	>3.0	0.511
25B	Cl	0.004	0.001

Figure 3. One-chloro effect on potency.

Having a series of consistent SAR results can solidify a medicinal chemist's understanding of the protein binding site and the minimally required ligand pharmacophore. When generating inhibitors against autotaxin (ATX), lysophosphatidylcholine (LPC) is used as a substrate in the assay because ATX hydrolyzes LPC; indole-based motifs such as **18A**–**18H** demonstrated the inhibition of ATX in a colorimetric LPC assay (Figure 3C).[76] In a series of examples, a chloro substituent at the indole C6 position was found to improve the IC_{50} potency against ATX by 400~2700 fold.[76] In another series of hydrogen-to-chlorine matched molecular pairs, anticancer activity against A549 lung cancer cells was demonstrated to be superior when a *para*-chloro substituent was present on the pendant phenyl moiety (Figure 3D).[77] This single chlorine atom was responsible for an 800~2000-fold improvement in potency for three related molecules **19A**, **19C**, and **19E**. This level of consistency achieved in a medicinal chemistry program's SAR can accurately define the pharmacophore of protein–ligand binding.

Another interesting example of the magic chloro effect was reported for compound **20B** containing an unusual ferrocenyl group and an isoxazole moiety (Figure 3E).[78] This compound was tested for its *in vitro* activity against the lung cancer cell line A549 and colorectal cancer cell line HCT116. Compound **20B**, which contains a chlorine atom at the *ortho* position on the benzene ring connected to the isoxazole, when compared to the parent compound **20A**, showed an exceptional potency improvement of 104565 × against A549 and 9923 × against HCT116. Other compounds containing a chlorine atom at the C4 position, or containing two chlorines at positions C2 and C4 were also evaluated, but these showed weaker potency.[78] Compound **20B**, with its potent anti-cancer activity against A549 and HCT116 cell lines (IC_{50} values of 0.747 and 3.65 nM, respectively), was identified as the most potent

compound in the study. It was also compared with the reference drug gefitinib (IC_{50} values of 17.90 and 21.55 mM, respectively, against A549 and HCT116), demonstrating a 23962-fold better activity than gefitinib against the A549 cell line, and a 5904-fold better activity than gefitinib against the HCT116 cell line.[78]

The endocannabinoid system (ECS) is a retrograde lipid signaling pathway that regulates a variety of physiological functions in the body. In the central nervous system (CNS), ECS is primarily involved in the regions of neuroprotection, nociception modulation, motor activity regulation, neurogenesis, synaptic plasticity, and regulation of certain memory processing phases. However, ECS has a significant role in immune and inflammatory responses,[79] and its functions are primarily mediated through its cannabinoid receptors CB1 and CB2, which are catabolized through serine hydrolase enzymes like monoacylglycerol lipase (MAGL) and fatty acid amide hydrolase (FAAH).[80] When studying MAGL inhibition, a notable chloro effect was observed with an isatin-derived hydrazone scaffold (Figure 3F).[81] Compared to the parent isatin compound **21A**, the 5-chloro analog **21B** was the most active against MAGL, with a 1003 × improvement in activity. Compound **21C**, with a Cl in position C6 of the isatin core, was found to be 114 × more active than compound **21A** as a MAGL inhibitor. It is important to note that **21C** also showed potent FAAH inhibition activity. The lead molecules **21B** and **21C** showed an acceptable pharmacokinetic profile and were deemed promising candidates for treating neurological and mood disorders.[81]

Another remarkable example of the magic chloro effect is demonstrated by compound **22B**, which is a potent and competitive antagonist of the human dopamine-3 (D_3) receptor (Figure 3G).[82] The D_3 receptor, one of the five subtypes of dopamine receptors, belonging to the subfamily of D_2-like receptors, is an important target for the treatment of a variety of neurological diseases, including schizophrenia, Parkinson's

disease, depression and substance use disorders. Many compounds that have targeted D_2-like receptors in non-selective fashion have failed because they cause undesirable side effects. Thus, there is an increased interest in identifying a compound that is selective for a specific D_2-like receptor subtype (such as D_3) to minimize these side effects and show therapeutic benefit.[83] In a study with the objective of enhancing D_3/D_2 selectivity, compound **22B**, containing a Cl at the *para* position of the benzene ring, was only 16 × more potent than the parent compound **22A**. However, unlike **22A**, **22B** did not bind to a D_2-like receptor, and therefore **22B** was 3333 × more selective towards the D_3 receptor over the D_2 receptor compared to **22A**. This chloro effect was attributed to the fact that the addition of a hydrophobic group such as chloro to the phenyl ring enhances the binding affinity of antagonists to the D_3 receptor without binding to the D_2-like receptors. [82]

Some magic chloro effects take place on rather unique heterocyclic architectures and not just on phenyl rings. For example, in an *N*-arylated isothiazolone scaffold, the chlorinated analog **23B** was 2500 × more potent than the parent compound **23A** against the bacteria *Pseudomonas aeruginosa (Ps. a.*) (Figure 3H).[84] In another example involving chlorinated heteroarenes, 5-chloropyrimidine **24B** was tested against kinase insert domain receptor (KDR) (Figure 3I).[85] The vascular endothelial growth factor (VEGF) receptor family of receptor tyrosine kinases (RTKs), most notably VEGFR2 or KDR, mediates the biological function of VEGF, which is a regulator of vascular permeability and an inducer of endothelial cell proliferation, migration and survival.[86] When optimizing for KDR activity, **24B** was found to be >857 fold more active than the parent compound **24A**.[85] The authors from Abbott Laboratories noted that a small, electron-withdrawing substituent was favored at this pyrimidine C5 position. In a closely related analog with the same 5-chloropyrimidine

with a bicyclic carboxamide, computational modeling showed that the chlorine atom is projected toward a small hydrophobic cavity in KDR kinase. Furthermore, although most magic chloro effects arise from chloro substituents at the periphery of a molecule, **24B** benefits from a chloro substituent in the central part of the molecule; this presumably forces a conformation in which the bicyclic moiety is pointing away from the pyrimidine C5 position and creates favorable binding.[85]

The chlorine atom can assume an important role not just in aromatic systems, but occasionally also in aliphatic ones, as exemplified by compound **25B** (Figure 3J).[87] This rather unusual structure containing a bicyclic urea was tested against a panel of serine β-lactamase (SBL) enzymes, in particular *Enterobacter cloacae* AmpC and TEM-1 for the treatment of Gram-negative bacteria. Compound **25B**, which contains a chlorine atom on an sp^3 carbon at the position α to the nitrogen atom of the urea, showed >750 × improved potency against AmpC and 511 × better activity against TEM1 when compared to the parent compound **25A**.[87]

The Magic Two-Chloro Effect in Pharmaceutical Lead Molecules

If one-chloro effects can be dramatic, in certain protein–ligand interactions, two-chloro effects can be even more drastic (Figure 4). When optimizing compounds against the fibroblast growth factor receptor 1 (FGFR1) kinase domain, bis-chloro compound **26B** was found to have a 12363 × potency improvement over the parent compound **26A** (Figure 4A).[88] The two *ortho*-chlorine atoms were found to render the phenyl ring perpendicular to the pyrazolopyridine core and engage in favorable hydrophobic interactions, thereby explaining this drastic effect.

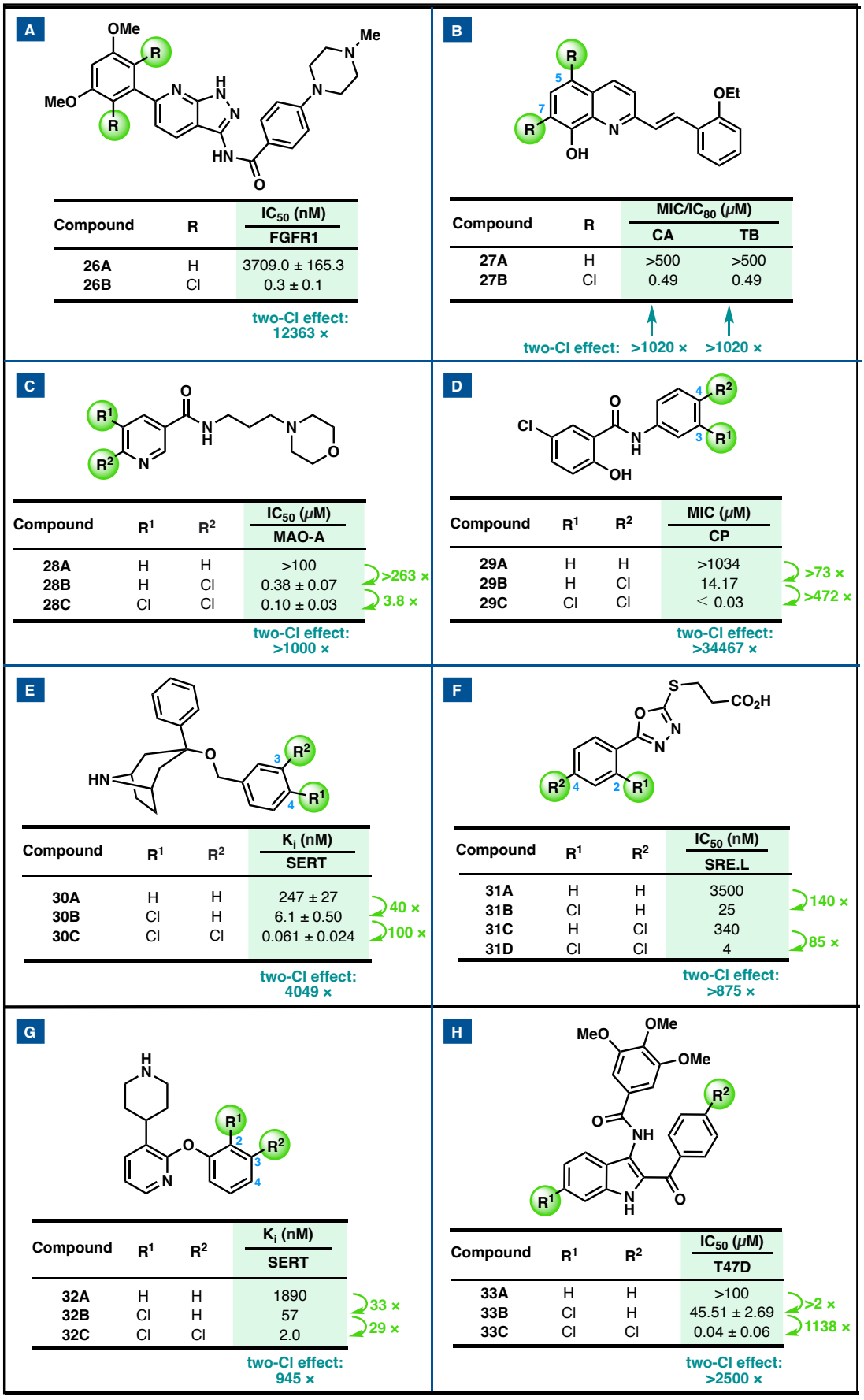


Figure 4. Two-chloro effect on potency.

Heteroaromatic motifs are prevalent in drugs, and fine-tuning their biological activity using chloro substituents is often conducted in medicinal chemistry. In an investigation of antimicrobial activity of quinolinols, the 5,7-dichloro motif was essential in all the tests conducted against Gram-positive bacteria (Figure 4B).[89] Specifically against *Candida albicans* (CA) and *Trichosporon beigelii* (TB), the two-chloro effect accounted for >1020 × improvements. The authors attributed this improved potency on the increased substrate lipophilicity, which is in general an important parameter for antimicrobial activity. The increased electrophilicity and acidity of the quinolinol core might also account for the potency improvement.[89] Next, researchers from Nanjing University found nicotinamides as potent and selective monoamine oxidase A (MAO-A) inhibitors.[90] The two-chloro effect from **28A** to **28C** accounted for a >1000 × potency improvement (Figure 4C). Based on the binding model of a related compound onto the MAO-A active site, π-π stacking of the pyridine core with one of the protein's tyrosine residues can be optimized when the pyridine ring is rendered more electron-deficient due to the two chlorine atoms.[90]

Dichlorophenyl compounds are synthesized rather often in the medicinal chemistry literature. This is partly due to the Topliss scheme that dictates that when a *para*-chlorophenyl compound is more active than the parent phenyl compound, the next logical compound to make is the 3,4-dichlorophenyl analog; 2,4-dichlorophenyl compounds can also be observed in Topliss' flowchart.[40] To this end, when testing antibacterial compounds, a 3,4-dichlorophenyl analog was synthesized, and it was found that the MIC against *Clostridium perfringens* (CP) improved >34467 × in a magic two-chloro effect (Figure 4D).[91] Compound **29C** benefited not only from the increased lipophilicity from the two chlorine atoms, but also from their electron-withdrawing properties. In order of worst to best potency, the substituents on the benzene ring of

29A–29C were classified as follows: 4-OCH_3 (least potent) < 4-CH_3 < 4-H < 4-Cl < 4-Br < 3-CF_3 < 3,4-Cl_2 (most potent). Rather impressively, Hammett's σ parameters followed the same trend: 4-OCH_3 (–0.27) < 4-CH_3 (–0.17) < 4-H (0) < 4-Cl (0.22) < 4-Br (0.23) < 3-CF_3 (0.51) < 3,4-Cl_2 (0.60). These matching trends supported the correlation between ring electron density and potency.[91] In another example with a 3,4-dichlorophenyl substrate, a high-affinity ligand for serotonin transporter (SERT) was obtained when benzyloxytropane **30A** was dichlorinated: the bis-chloro compound **30C** had a 4049 × stronger binding affinity than the parent compound **30A** (Figure 4E).[92] Next, for a 2,4-dichlorophenyl system such as **31D**, a serum responsive element promoter-driven luciferase (SRE.L) assay for scleroderma showed single-digit nanomolar activity (Figure 4F).[93] **31D** represents a >875 × activity improvement over the parent compound **31A**, which was only the beginning of a great potency improvement campaign. The three-carbon propionic acid in **31D** was elongated into a four-carbon butanoic acid chain for a 2.5 × activity improvement, and replacement of the 4-chloro atom (*i.e.*, R^2 = Cl) with a cyclopropyl group gave a further 840-fold IC_{50} improvement. This study achieved a 150,000-fold improvement in their hit-to-lead campaign, which is an exceptional feat that also resulted in a picomolar lead compound.[93]

The 2,3-dichloro-substituted phenyl group is a rare scaffold, partly because it does not feature in Topliss' recommended substitution pattern,[40] but also because it is more difficult to forge from a synthetic chemistry point of view. Nevertheless, scientists at Pfizer generated this substitution pattern on the benzene ring for a monoamine neurotransmission study because the C4 (*para*) position did not tolerate sterically larger groups (Figure 4G).[94] Even at the positions C2 and C3, only small substituents were tolerated, but the presence of an electronegative functional group in this aromatic

region was deemed favorable for SERT binding affinity. To this end, chlorine atoms were introduced onto phenyl compound **32A** at C2 and C3, and each chlorine atom was responsible for a ~30 × binding improvement, accounting for a two-chloro effect of 945 ×.[94]

Chloro effects from two different areas of the molecule could act cooperatively to result in a dramatic two-chloro effect. *In vitro* cytotoxicity studies against the human cancer cell line T47D have shown that the parent indole **33A**, which was essentially inactive, became the most active compound in the series when two hydrogen atoms on different rings were substituted with chlorine atoms (Figure 4H).[95] The lead compound **33C** showed a >2500 × improvement in potency, and was important enough to be tested in further studies, demonstrating cell cycle arrest and anti-tubulin activity.

Chloro Effects in Natural Products and Natural Product Analogs

Natural products and their derivatives have historically made a major contribution to drug discovery, especially against cancer and infectious diseases. Natural products have unique features when compared to non-natural compounds, which can be viewed as both an advantage and challenge for the drug discovery process.[96] Natural products showcase enormous scaffold diversity and structural complexity, and therefore cover a much wider portion of available chemical space compared to approved drugs.[97] The study of natural products can guide the exploration of biologically relevant chemical space, and can serve as an inspiration for the development of new drugs.[98] Natural products typically have a much larger number of sp^3 carbon atoms (characterized as "fraction sp^3", or Fsp^3). This "natural product likeness" is something that medicinal chemists strive for as a goal to "escape the

flatland" of sp^2-rich molecules that are often generated due to synthetic ease.[99,100] Other advantages include lower lipophilicity (lower logP/logD values) and greater molecular rigidity compared to synthetic compound libraries.[96] As a downside, natural products typically have a higher atom count and molecular weight, and their structural complexity often equates to lengthy and costly synthesis. Other unique features of natural products include a higher number of hydrogen bond acceptors and donors, as well as a higher oxygen atom count, but curiously with a lower nitrogen and halogen atom count than synthetic compounds.[96]

With these aspects in mind, it is occasionally beneficial to introduce halogen atoms, in this case a chlorine atom, onto natural products in order to get the best of both the natural and synthetic worlds and enhance their biological profile. Surprisingly, even though natural products are oftentimes characterized by a large and complex molecular structure, the effect of a simple chlorine atom can have a significant impact on its biological activity. Shown below is an analysis of three selected natural products and analogs: an artemisinin analog, kulokekahilide-2 and vancomycin aglycone (Figure 5).

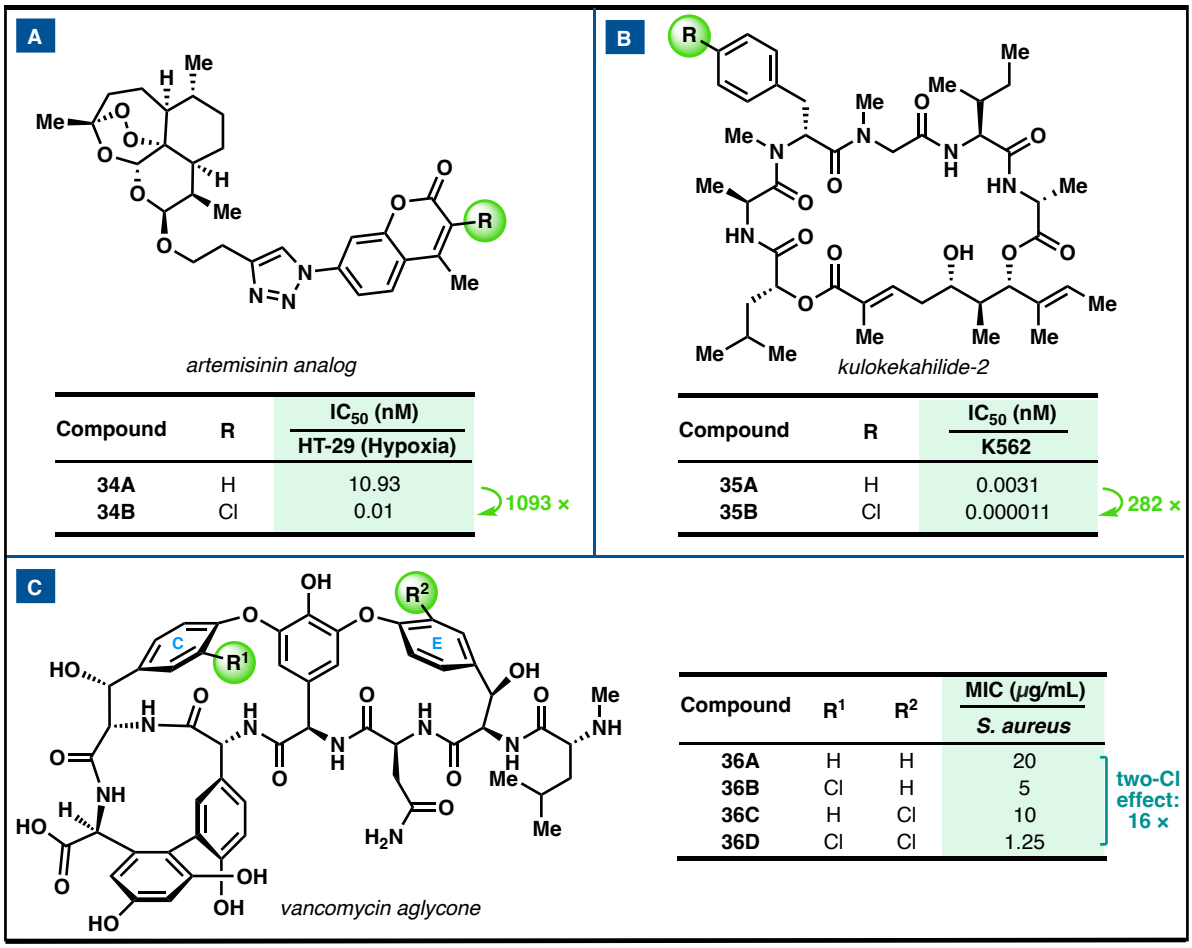


Figure 5. Chloro effect on natural products and natural product derivatives.

A dihydroartemisinin coumarin analog showed strong anti-cancer activity against the human colorectal adenocarcinoma cell line HT-29 (Figure 5A). In particular, compound **34B**, which contains a chlorine atom on the coumarin moiety, was 1093 × more potent than the parent compound **34A** against HT-29.[101] The importance of the chlorine atom in coumarin-based drugs was previously reported by Supuran and co-workers where they demonstrated that the incorporation of chloro- and/or chloromethyl groups in various positions of the heterocyclic ring significantly enhances inhibition against carbonic anhydrase.[102]

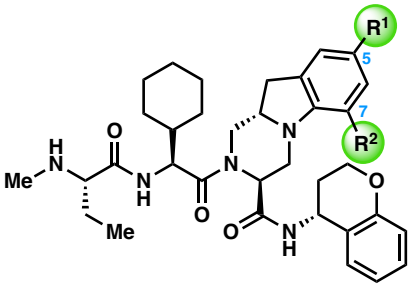
Kulokekahilide-2 (**35A**, Figure 5B), a 26-membered cyclodepsipeptide isolated from a Hawaiian marine mollusk, showed potent cytotoxicity in several mammalian tumor cells, and in particular against human cancer cell line K562 from chronic myelogenous leukemia.[103] After investigation of the SAR for kulokekahilide-2, it was found that its chlorinated derivative **35B** is 282 × more potent than the natural product itself (compound **35A**) against K562.[104] Presumably, the halogen-substituted natural product has enhanced potency because the chloro substituent enhances steric, electron-withdrawing, and hydrophobicity effects.

A highly recognizable molecule that benefits from the chloro effect is vancomycin, a glycopeptide antibiotic that is recommended intravenously and is employed for the treatment of complicated skin infections, bloodstream infections, endocarditis, bone and joint infections, and meningitis caused by methicillin-resistant *Staphylococcus aureus* (MRSA).[105] Vancomycin, and in particular the aglycone (*i.e.*, without the sugar moiety), contains two chlorine substituents on aryl rings C and E (compound **36D**, Figure 5C). In 2013, Boger and co-workers demonstrated that the lack of one or both of the chlorine atoms present in vancomycin strongly affects its antimicrobial activity due to the effect of the chlorine atoms on the binding affinity and selectivity for the D-Ala-D-Ala terminus.[106] This binding event is crucial because vancomycin functions by disrupting bacterial cell wall biosynthesis when binding to the peptide terminus D-Ala-D-Ala found in peptidoglycan precursors. It was found that vancomycin aglycone containing the two chlorine atoms in its structure (compound **36D**) is 16 × more potent against *Staphylococcus aureus* than the des-chloro compound **36A**, which is explained by a stronger binding to a D-Ala-D-Ala-containing peptide fragment.[106]

Chloro Effects on Pharmacokinetics

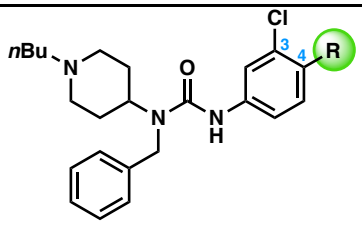
Although this review has mainly focused on the beneficial effect of the chloro substituent on potency, inhibitory activity, or binding affinity, over the course of this literature survey, pronounced chloro effects on pharmacokinetic parameters were also observed (Figure 6). Especially in the later stages of preclinical development (hit-to-lead and lead optimization), optimizing multiple parameters is essential for developing a drug with favorable absorption/distribution/metabolism/excretion/toxicology (ADMET) properties. For example, scientists from Takeda have been studying inhibitors of apoptosis proteins (IAPs), and realized that their most potent compound was very susceptible to MDR1-mediated efflux.[107] Using structure-based drug design, they succeeded in performing a scaffold hop to get to indoline compound **37A** (Figure 6A). Substitution at the indoline C5 position with a chlorine atom gave **37B**, which retained the strong inhibition of IAP binding (measured as X chromosome-linked IAP (XIAP) and cellular IAP (cIAP)), but simultaneously increased the apparent permeability (P_{app}) by 2.6 × and reduced the efflux ratio (ER) by 6 ×. The dichloro derivative **37C** exhibited an even better P_{app} and ER, but the IAP inhibitory activities were worse. Thus, **37B** was deemed to have the most balanced profile, and was chosen as the candidate for further evaluation.[107]

A



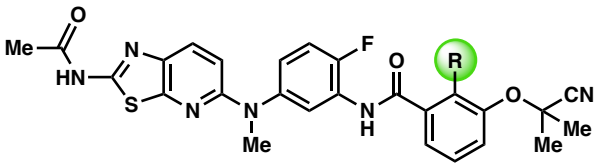
Compound	R^1	R^2	IC_{50} (μM) XIAP BIR3	IC_{50} (μM) cIAP BIR3	P_{app} (nm/s)		ER	
37A	H	H	19	1.4	25	2.6 ×	8.2	6 ×
37B	Cl	H	23	1.1	64	1.2 ×	1.4	2 ×
37C	Cl	Cl	62	1.4	75		0.69	

B



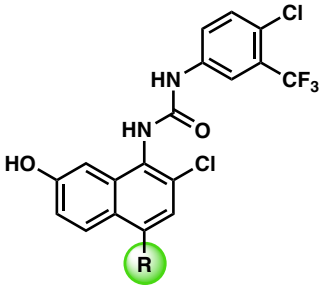
Compound	R	IC_{50} (μM) DCN1	$t_{1/2}$ (h)	CL_{int} (mL/min/kg)
38A	H	0.358	≤ 0.10	≥ 500
38B	Cl	0.045	2.11 ± 0.336	25.6 ± 4.08
			≥ 21 ×	≥ 20 ×

C



Compound	R	Kinase IC_{50} (nM) BRAF (V600E)	Kinase IC_{50} (nM) VEGFR2	Mouse PK AUC PO (μg·h/mL)	
39A	H	23	10	0.311	46 ×
39B	Cl	45	14	14.308	

D



Compound	R	IC_{50} (nM) TRPV1 (rat)	AUC (ng·h/mL)	
40A	H	4.5	3	28 ×
40B	Cl	2.1	84	

Figure 6. Chloro effect on pharmacokinetics.

Two of the key pharmacokinetic parameters that determines drug dosing intervals for a patient are half-life ($t_{½}$)[108] and intrinsic clearance (CL_{int}).[109] A group of researchers from St. Jude's Children Research Hospital have been studying "defective in cullin neddylation 1" (DCN1), which is an oncogenic driver gene that is common in squamous cell carcinoma.[110] After substituting the hydrogen atom in compound **38A** with a chlorine atom to give **38B**, not only did the IC_{50} potency value improve, but the $t_{½}$ and CL_{int} drastically improved by more than 20-fold (Figure 6B). This C4-chlorine atom effectively decreased the rate of microsomal oxidation, single-handedly fixing a key PK parameter. Compound **38B** showed the best combination of biochemical potency and intravenous PK values, and was therefore selected for further preclinical studies.[110]

Another PK parameter that is intrinsically related to the clearance is "area under the curve" (AUC). This is a parameter that is directly calculated from a concentration-time graph, and represents the total drug exposure in the living system of interest. In a rapidly accelerated fibrosarcoma (RAF) kinase study, B-RAF and vascular endothelial growth factor receptor 2 (VEGFR2) proteins were targeted, and medicinal chemistry studies led to compound **39A** with great potency but with poor AUC *per os* (AUC PO; AUC in oral administration) in mouse (Figure 6C).[111] The poor AUC value was attributed to the poor bioavailability of **39A**, and when R = H was substituted as R = Cl, the resulting compound **39B** showed a significant 46 × increase in AUC PO.

Finally, scientists from Bayer have been studying the transient receptor potential vanilloid 1 (TRPV1) ion channel for the treatment of urinary incontinence.[112] Although single-digit nanomolar levels of potency against TRPV1 in rat were achieved with their lead compound **40A**, the low AUC PO in rat made this compound non-advanceable (Figure 6D). The authors hypothesized that enhancing the naphthol's

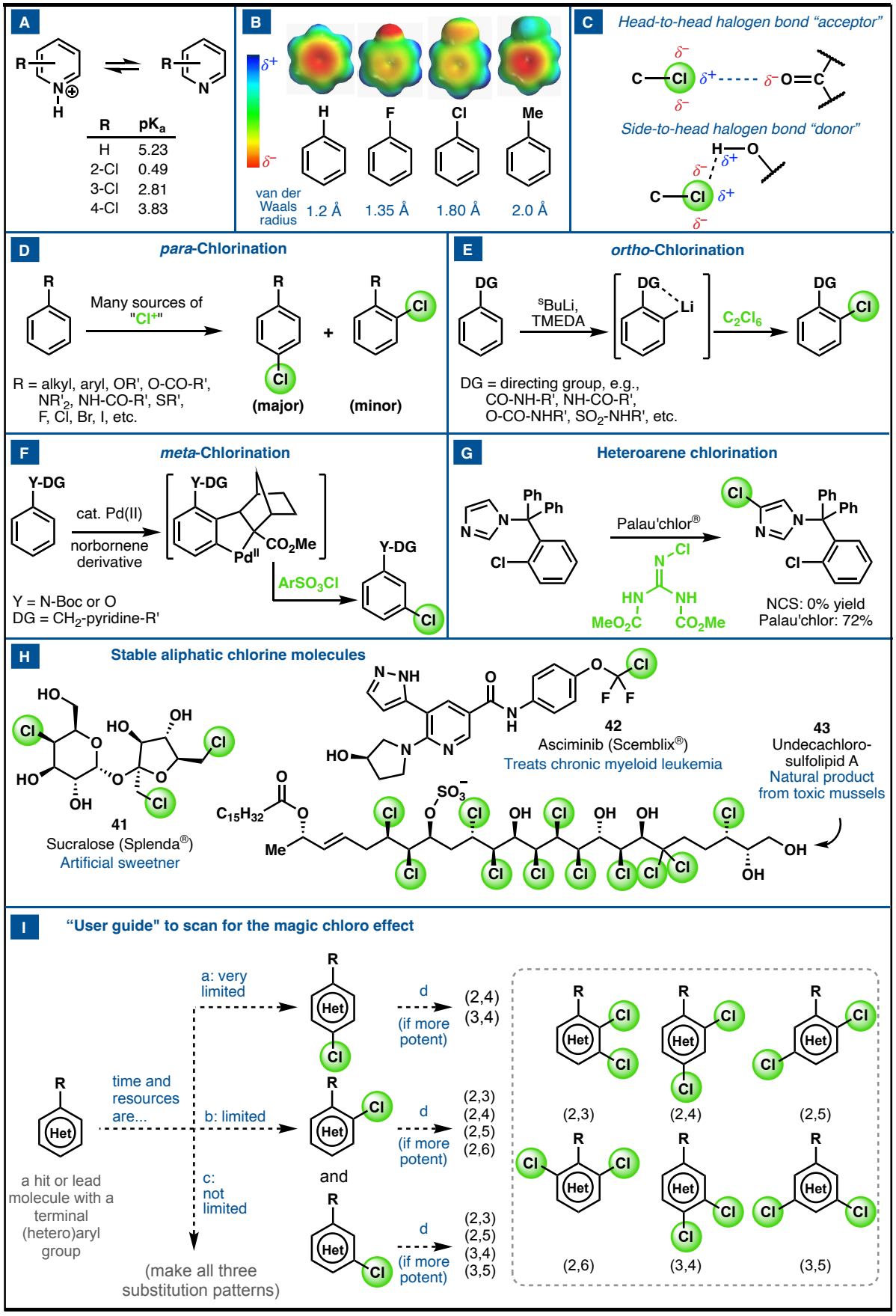
acidity by introducing electron-withdrawing substituents would reduce the clearance and enhance oral exposure. Indeed, introduction of an electron-withdrawing chloro substituent led to compound **40B**, which displayed a 28 × improvement in AUC; any single-atom change in a molecule that can produce this kind of dramatic PK effect is noteworthy.[112]

Unraveling the Magic of the "Magic Chloro Effect"

Fluorine is typically the go-to halogen atom for medicinal chemistry purposes and many reviews have been written on the topic.[24-32] Then, why should a chlorine atom be installed on a molecule, as opposed to a fluorine atom? Comparing the van der Waals radii of hydrogen (1.2 Å), fluoro (1.35 Å), chloro (1.80 Å), and methyl (2.0 Å) substituents (see Figure 7B),[113] a chloro substituent can be approximated as a methyl substituent, whereas fluoro is similar to hydrogen in size. It is therefore reasonable that chloro can follow in the footsteps of the "magic methyl effect",[2] whereas fluoro, despite its very frequent use in medicinal chemistry, is never considered "magic" when it comes to improving biochemical potency. This "magic" of the chloro effect can be further explained by the chlorine atom's ability to:

1) make (hetero)aromatic systems more acidic, as shown by the increased acidity (lower pK_a) of a pyridinium ion when it is substituted with chlorine (Figure 7A), as well as for other systems like phenols, anilines, and benzoic acids;[114]
2) render the benzene ring more electron-poor, as evidenced by electrostatic potential maps of substituted benzene where the center of the ring is depleted of electron density (to become less δ^-) when chloro is present (Figure 7B);[115,116,117]

3) change the scaffold's conformation, as demonstrated by examples in Figure 3I, Figure 4A, and Figure 5C;
4) enhance lipophilic binding, as graphically depicted by examples in Figure 2;
5) form halogen bonds (Figure 7C).[42–45] The chlorine-versus-fluorine difference is most pronounced in this effect, which takes into account the electron density surrounding these atoms. Although halogen atoms in general are electronegative and pull electron density toward themselves (and take on more δ^- charge), there is a positive electrostatic potential (the σ-hole)[42–44,117] at the extremity of the chlorine atom (see Figure 7B). This dual charge allows a chloro substituent to engage as a head-to-head halogen bond donor (*i.e.*, the Cl can interact with nucleophiles) as well as a side-to-head halogen bond acceptor (*i.e.*, the Cl can also interact with electrophiles; Figure 7C).[42–45] Because of its greater electronegativity, fluorine does not have a positively charged area and therefore cannot interact with nucleophiles.[43] Thus, chlorine-containing molecules have more possible binding modes than fluorine-containing molecules. A combination of all these factors can magnify the chloro effect on potency much more than for fluoro, and at least as much as methyl.


A
R
N
H
R
N
R | pKa
H 5.23
2-Cl 0.49
3-Cl 2.81
4-Cl 3.83
B
δ+
δ−
H
F
Cl
Me
van der Waals radius
1.2 Å
1.35 Å
1.80 Å
2.0 Å
C
Head-to-head halogen bond "acceptor"
C—Cl δ+ ----- δ− O=C
Side-to-head halogen bond "donor"
H—O
C—Cl
D
para-Chlorination
R
Many sources of "Cl+"
R
Cl
+
R
Cl
(major)
(minor)
R = alkyl, aryl, OR', O-CO-R', NR'2, NH-CO-R', SR', F, Cl, Br, I, etc.
E
ortho-Chlorination
DG
sBuLi, TMEDA
DG
Li
C2Cl6
DG
Cl
DG = directing group, e.g., CO-NH-R', NH-CO-R', O-CO-NHR', SO2-NHR', etc.
F
meta-Chlorination
Y-DG
cat. Pd(II)
norbornene derivative
Y-DG
Pd II
CO2Me
ArSO3Cl
Y-DG
Cl
Y = N-Boc or O
DG = CH2-pyridine-R'
G
Heteroarene chlorination
Ph
Ph
N
N
Cl
Palau'chlor®
N
Cl
HN
NH
MeO2C
CO2Me
Cl
Ph
Ph
N
N
Cl
NCS: 0% yield
Palau'chlor: 72%
H
Stable aliphatic chlorine molecules
HO
Cl
HO
OH
HO
Cl
O
O
O
HO
OH
Cl
41
Sucralose (Splenda®)
Artificial sweetner
N-NH
O
N
H
O
Cl
F
F
HO
N
N
42
Asciminib (Scemblix®)
Treats chronic myeloid leukemia
43
Undecachloro-sulfolipid A
Natural product from toxic mussels
O
C15H32
O
Me
SO3−
Cl
O
Cl
OH
Cl
OH
OH
Cl
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I
"User guide" to scan for the magic chloro effect
R
Het
a hit or lead molecule with a terminal (hetero)aryl group
time and resources are...
a: very limited
b: limited
c: not limited
(make all three substitution patterns)
R
Het
Cl
d
(if more potent)
(2,4)
(3,4)
(2,3)
(2,4)
(2,5)
(2,6)
and
(2,3)
(2,5)
(3,4)
(3,5)
(2,3)
(2,4)
(2,5)
(2,6)
(3,4)
(3,5)

Figure 7. A–C) Effects of chlorine on acidity, electron density, and halogen bonding. **D–G)** Chlorination methods in organic synthesis. **H)** Stability of aliphatic chlorine molecules. **I)** A "user guide" to help find the magic chloro effect.

Organic Chemistry Considerations

The ideal situation is to be able to take a lead compound of interest and subject it to one-step chlorination conditions to furnish a product with a chlorine atom at the desired position on the molecule. Although this might not be possible on molecules that have many (hetero)aromatic rings susceptible to chlorination, many monosubstituted benzene rings should be possible to simply chlorinate at the *para*-position by electrophilic aromatic substitution (S_EAr) using an electrophilic chlorinating agent (Figure 7A). *ortho*-Selective functionalization should be achievable by directed *ortho*-metalation then trapping with an electrophilic chlorinating agent (Figure 7E);[118] some Pd-catalyzed directed C–H chlorinations[119] and organocatalytic *ortho*-C–H chlorinations have also been developed.[120,121] Unless there is conveniently a strong electron-withdrawing group on the benzene ring forcing a *meta*-chlorination under S_EAr conditions, *meta*-selective chlorination is arguably the most difficult, and this has only been achieved recently by transition metal catalysis (Figure 7F).[122–124] For (hetero)aromatic systems where conventional chlorinating agents such as *N*-chlorosuccinimide is not sufficiently reactive, new chlorinating reagents have been invented in the past decade as well (Figure 7G).[125,126]

Although there are likely more ways to chlorinate a molecule than to methylate a molecule,[2] there is arguably still room for methodological development in chlorination, especially for *meta*-chlorination, and chlorination of biaryls and fused (hetero)aromatic systems. Computational methods that predict the most likely sites of

reactivity toward electrophilic halogenation are also useful[127] and should be expanded. Thus, we believe that the current state of organic chemistry still does not sufficiently accommodate the variety of medicinal chemistry scaffolds that need to be chlorinated selectively.

Conclusions and Outlook

Why install chlorine atoms in drug discovery?

The benefits of the chloro substituent can be extraordinary, with a reported effect of > 100000 × improvement in bioactivity bestowed by a single chlorine atom. The magic chloro effect can be caused by making heteroaromatic systems more acidic, rendering benzene rings more electron-poor, changing the scaffold's conformation, enhancing lipophilic binding, or forming halogen bonds—a combination of these factors can magnify the chloro effect even further. From an ADMET perspective, chlorine atoms can serve to block microsomal oxidation and reduce CL_{int}, or increase lipophilicity in order to increase P_{app}, %F, and AUC. If these are potential upsides that can be achieved by introducing one, if not two, chlorine atoms, then the chlorination effort is worth doing considering that making chlorinated analogs is often a simple, and sometimes even a one-step endeavor.

Despite all these possible advantages, there are some disadvantages as well. Depending on the mode of protein–ligand binding, an extra chloro substituent can be detrimental to the bioactivity, as the potency worsened by 100~550 fold in some of the surveyed cases.[128–130] Furthermore, chlorine atoms almost always make the parent molecule more lipophilic, which results in lower aqueous solubility. There is probably a lower “return on investment” as further chlorine atoms decorate the scaffold: the largest two-chloro effect was >34467-fold, amounting to ~200-fold potency

improvement per chloro substituent, whereas numerous one-chloro effects of >1000-fold have been observed. It is therefore unwise to continue to place more chlorine atoms (>4) on a molecule, considering that each chlorine atom can contribute up to ~1.0 in terms of logP value; the commonly recommended logP by “Lipinski's rule of 5” is 5.0,[131] therefore not leaving much left regarding “available lipophilicity”. In fact, random substitution of R = H by R = Cl is not recommended for every scaffold and context. A careful stepwise introduction of chlorine atoms and reassessment of bioactivity, akin to Topliss' flowchart,[40] should be done in systematic fashion for every aromatic ring in the molecule.

Notably, all but one example in this review have described chlorine atoms on (hetero)aromatic rings because drug discovery programs often avoid installing aliphatic chlorides into their lead candidates. This is mostly due to the false assumption that aliphatic chlorides are always susceptible to nucleophilic substitution reactions and present off-target toxicity. Although there are known covalent warheads such as a chloroacetamide ($ClCH_2CONR_2$)[132,133] or a nitrogen mustard ($[ClCH_2CH_2]_2NR$),[134,135] most aliphatic chlorides are surprisingly stable (Figure 7H), with a highly chlorinated substance such as sucralose (Splenda®; **41**) being excreted from the body unchanged after consumption.[136] Both the primary and secondary alkyl chloride functional groups in this structure are therefore stable in the body. A well-known class of natural products called polychlorinated sulfolipids (**43**) are toxic but bioactive, and this class of compounds can be chemically stable to ambient conditions (room temperature and moisture) for more than 30 years.[137] Furthermore, a seemingly reactive OCF_2Cl group is present in an approved drug called asciminib (Scemblix®; **42**); the role of the chlorine atom in **42** is to simply increase hydrophobic interactions with valine, leucine and isoleucine on the target protein, but not to act as a covalent warhead.[138] Even the

example of a secondary chloride in Figure 3J appears reactive toward various intracellular nucleophiles, but the chloro substituent simply enhances reversible binding potency.[87] Beneficially, tertiary alkyl chlorides can be used as stable bioisosteres for a *t*-butyl group.[139] These examples serve to demonstrate that most aliphatic chlorides are not covalently reactive, and that medicinal chemists should take advantage of the chlorine atom's unique properties even in aliphatic systems. However, as is the case for any drug candidate, metabolic stability and off-target toxicity must always be evaluated, since any substituent can potentially present issues in preclinical studies; even seemingly "safe" and oft-used substituents such as fluorine can present unwanted effects.[140]

When to install chlorine atoms in drug discovery?

An unwritten rule in drug discovery environments is to install methyl substituents in early preclinical work, when improving potency is of utmost importance and finding magic methyl effects can greatly accelerate the timelines of a program. In contrast, installing fluoro substituents is most effective in the later stages of preclinical studies, when improving PK parameters for ADMET optimization is crucial to prepare a lead compound for human clinical trials. The "magic chloro effect" has been demonstrated through Figures 2–5, and the benefit of the chlorine atom on PK parameters has been shown in Figure 6. Therefore, it can be argued that the chloro effect can be useful in all stages of preclinical work, but with a preference for early preclinical studies.

This review has attempted to bring to light a concept discussed informally among various medicinal chemistry groups but previously not summarized. The take-home messages are:

1) Potency improvements should exceed 10-fold per chloro substituent in order to counteract the effect of lipophilicity,[54,55] with an ideal contribution of 30-fold or greater. “Magic chloro effects” of 500 × or even >1000 × are often serendipitously obtained, and are to be cherished.
2) Considering the omnipresence of chlorine atoms in approved drugs,[46,47] medicinal chemists should make an effort to understand the exact benefit arising from each chlorine atom they place onto a molecule to avoid over-chlorinating their scaffold. If the guidance from 1) is not followed, the chloro substituent should be removed. This effort would be more clarifying if properly matched molecular pairs were always included in these discussions.[49]
3) Although the Topliss scheme can be beneficial,[40] and *para*-chlorinated phenyl building blocks are typically easier to purchase or to synthesize, medicinal chemists must try to avoid the “*para*-chlorophenyl bias”[65] and treat the *para*-chlorophenyl with no particular privileged status.
4) As a consequence of 3) above, organic chemists must in turn develop more *ortho*- and *meta*-chlorination methodology to help medicinal chemists create compounds that should be created from a protein–ligand binding point of view, rather than have them create compounds that are the easiest to create.
5) Aliphatic chlorides can be beneficial and should not be disregarded entirely due to preconceived notions of covalent warhead toxicity. The generated scientific data (*e.g.*, pharmacokinetic parameters, toxicology results) should always be

followed for guidance, just like for any other lead compound moving forward in preclinical studies.

6) Finally, the pharmacophore especially around the magic chloro substituent should be understood through protein–ligand X-ray crystal structures or careful analysis of computer models (*e.g.*, docking onto known structures or creating homology models).

Where to install chlorine atoms?

Along with the take-home messages above, here is a suggestion for a "user guide" of how to structurally modify the hit or lead compound in order to maximize the likelihood of encountering the magic chloro effect (Figure 7I):

a) Starting from a phenyl at the extremity of the molecule, when faced with very limited time/resources, make the *para*-chlorophenyl and follow the Topliss scheme. The caveat here is that a *para*-chlorophenyl is not more privileged than the *ortho*- or *meta*-chlorophenyl from a bioactivity standpoint.

b) Starting from a phenyl at the extremity of the molecule, if only two analogs could be made, the *ortho*-chloro and the *meta*-chloro should be made because the *ortho*-chloro changes the conformation of the phenyl linkage, and the *meta*-chloro is electronically different from *ortho*- and *para*-chloro substituents.

c) Ideally make all three *ortho*/*meta*/*para* substitution patterns because chlorophenyl building blocks are often readily available.

d) Only make dichlorophenyl systems if there is a dire need for potency, or if there is a good hypothesis/structural reason to make a dichloro system. Following the Topliss scheme and making only 3,4- and 2,4-dichlorophenyl systems is not recommended other than for synthetic ease.

e) Starting from a heteroarene at the extremity of the molecule, the above concepts a) ~ d) should be followed if applicable, but the pK_a of the heteroarene's conjugate acid should also be kept in mind for azines and azoles (for systems like imidazole, the NH of the parent compound could be acidic enough), and even the hydrogen bonding ability of oxazole-type systems could be considered.

f) For (hetero)aryl rings in the middle of a molecule (*i.e.*, already disubstituted), special care should be made when making chlorinated analogs because of conformational changes that could affect the entirety of the molecule both for biochemical potency and pharmacokinetic profiles.

g) Unless the chlorinated system is a chloroacetamide, a chloroethyl amine, or an allylic/benzylic chloride, chlorine atom incorporation on an aliphatic system should be attempted. The most likely area of success is where the R–H to R–Cl substitution is at the periphery of the molecule. A chloro substituent does not sterically influence an aliphatic system as much as a methyl substituent, and is perhaps less suited to restricting aliphatic conformation (*cf.* cyclohexane A-values of Cl vs CH_3 of 0.43 vs 1.7 kcal/mol, respectively);[141] however, the carefully positioned chlorine atoms in chlorosulfolipids are known to force the linear alkyl chain into staggered conformations.[137]

h) Chlorination should not be overused as a strategy if a compound's logP/logD lipophilicity is already too high, and aqueous solubility is already too low. In such a case, additional chlorine atoms might make the molecule too difficult to formulate.

There are so many medicinal chemistry lessons that can be learned from a simple substitution of a hydrogen atom to a chlorine atom on a hit or even a lead molecule. Although every medicinal chemist, every drug discovery program, and every corporation is prone to its own bias, attempting new chemistry, making novel substitution patterns on a scaffold, and simply following the data without bias could lead to unexpected and gratifying magic chloro effects. The resulting lessons can in turn generate new hypotheses, which triggers even more synthesis and data analysis, thereby accelerating the cycle of drug discovery.

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Both D. C. and Y. I. contributed to literature search, data analysis, and manuscript writing.

Competing Interests

The authors have no competing interests to declare.

Supplementary Information

The literature search and data collection were performed on a spreadsheet. These tables of data are organized in a Supplementary Information file.

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