

Electrostatic Complementarity in Structure-Based Drug Design

Miniperspective

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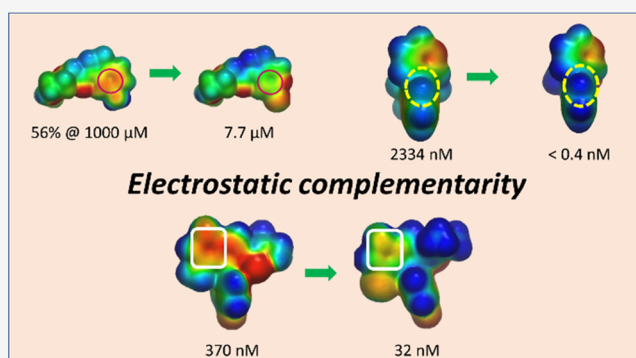
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ABSTRACT: Optimization of electrostatic complementarity is an important strategy in structure-based drug discovery for improving the affinity of molecules against a specific protein target. In this Miniperspective we identify examples where deliberate optimization of protein–ligand electrostatic complementarity or intramolecular electrostatic interactions gave improvements in target affinity (up to 250-fold), physicochemical properties, *in vitro* properties, and off-target selectivity. We also look retrospectively at a series of factor Xa inhibitors that show an almost 8000-fold range in potency that can be correlated with the calculated electrostatic potential (ESP) surfaces. Recent developments using a graph-convolutional deep neural network to rapidly generate high quality ESP surfaces have the potential to make this useful tool more accessible for a wider audience within the field of medicinal chemistry.



■ INTRODUCTION

Electrostatic interactions play a key role in molecular recognition, and their characterization and modulation are at the heart of structure-based drug design (SBDD). In SBDD, maximizing shape and electrostatic complementarity between ligand and protein is a well-established strategy to improve binding affinity and selectivity against antitargets. However, electrostatic complementarity is a more complex concept than shape complementarity.

The energy and preferred directionality of electrostatic interactions are not easy to assess, even for trained medicinal and computational chemists, and desolvation penalties make this task even more complex.

The calculation of electrostatic potential (ESP) surfaces can aid this endeavor both by identifying areas of electrostatic match or mismatch and by readily facilitating the comparison of multiple ligands bearing different substituents or core scaffolds. For example, the impact on ESP of substituents in aromatic systems has been extensively studied and attributed variously to π -resonance, σ -induction, and electrostatic field effects,^{1–4} with only a minor influence from the change in overall electron density of the aryl π -system.^{4,5} The use of ESP surfaces takes these factors into account to provide a rapid understanding of protein–ligand complementarity.

In this Miniperspective we describe a number of SBDD case studies where ESP analysis and subsequent modulation have been used to optimize protein–ligand electrostatic complementarity in order to improve potency and selectivity. Following

this we also discuss how ESP methods have been applied to modulate intramolecular interactions in order to stabilize the ligand bioactive conformation.

■ CALCULATION OF ELECTROSTATIC POTENTIALS

Molecular electrostatics are most widely studied by visualizing and analyzing the ESP surface of molecules.⁶ The ESP energy at any given point on the molecular surface is the energy of the interaction between the molecular charge distribution and a unit positive charge positioned at that surface point. The ESP can be most accurately computed by quantum mechanical (QM) *ab initio* methods, a technique that is mostly limited to small molecules due to the high computational time expense required for more complex species. For example, Figure 1B illustrates the ESP surface of chlorobenzene calculated using the *ab initio* density functional theory (DFT) method. Additionally, continuum solvent models have been developed and employed for QM methods to mimic the influence of solvents on the electrostatic interactions.^{7–9} To improve the computation speed, atom-centered charges can be parametrized to reproduce ESP surfaces obtained from QM methods.^{10–16} These charges

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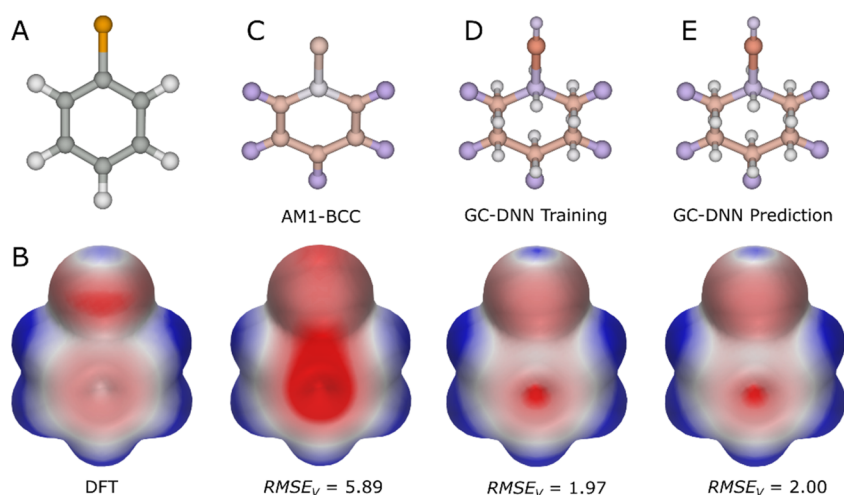


Figure 1. ESP of chlorobenzene obtained using various methods. (A) Structural model of chlorobenzene with carbon (gray spheres), chlorine (orange) and hydrogen atoms (white). (B) ESP surface (red-white-blue color gradient representing ESP values of -15 to 15 kcal/mol) was calculated using the *ab initio* DFT B3LYP method, and this surface is used as the standard for the comparison. (C) Atoms are colored (red-white-blue color gradient representing -0.5 to $+0.5$ charge) by AM1-BCC partial charge values (top), and ESP calculated from these charges (bottom). Root mean square error value ($RMSE_v$) shows the deviation in ESP energy with respect to the *ab initio* DFT ESP energy. (D) Additional off-atom charge centers representing p-orbitals and σ hole are included and represented by smaller spheres connected to the carbon and chlorine atoms, respectively. The atoms/features are colored by the partial charge values that were optimized to reproduce the *ab initio* DFT ESP surface. The ESP surface calculated using these fitted charges is shown at bottom. The low $RMSE_v$ value shows that the off-atom charges improve the accuracy of ESP surface. These charges were used to train graph-convolutional deep neural network (GC DNN) model in this study.²⁴ (E) Predicted charges from the GC DNN model (top) and calculated ESP surface using these charges. This method provides the ESP surface with almost the same accuracy as obtained by DFT fitted atoms/features charges without requiring time-expensive *ab initio* DFT calculations.

are used in molecular mechanics force fields and have been employed to calculate ESP surfaces;⁶ for example, Figure 1C shows the ESP surface of chlorobenzene generated using AM1-BCC atom-centered charges.¹⁷ This use of atomic charges enables fast ESP calculation for macromolecules including proteins and DNA because the same atomic charges can be used for different species based on the predefined atom types. To model the continuum solvent with atom-centered charges, methods have been developed to rapidly solve the Poisson–Boltzmann equation and its application has been demonstrated for complex macromolecules such as microtubules and ribosomes.^{18–23}

While ESP surfaces can be calculated more rapidly using atomic charges, they are less accurate when compared to those derived from QM methods, as shown by the root-mean-square error value ($RMSE_v$) in Figure 1C. One major limitation for using atomic charges is the absence of features such as σ holes, π orbitals, and lone pairs that are either merged on the atom center or not considered during the parametrization process.^{25–28} Figure 1D shows the π orbitals and σ hole of chlorobenzene as the off-atom-centered feature points (represented by smaller spheres connected to carbon and chlorine atoms). Some force fields, such as XED, explicitly include these off-atom sites as additional charge centers to improve accuracy and provide a better description of systems involving electron anisotropy.²⁹ ESPs calculated with the XED force field have been used to develop a SBDD tool to estimate electrostatic complementarity of protein–ligand complexes.³⁰

Another major limitation, particularly applicable to small molecules, is incorrect assignment of atomic charges due to the limited number of molecules used during the charge parametrizations. For many practical purposes, QM methods can be used to obtain ESPs for small molecules while atomic charges can be used for the proteins. However, QM methods still present

a bottleneck for interactive drug-design applications due to the high computational time cost (the calculation of a QM ESP for a typical drug-like molecule with molecular weight 400 takes approximately 30 min).

To overcome the issues in calculating charges by empirical models, various machine/deep learning methods have been developed to build models for prediction of the charges;^{31–37} these can be employed for molecular mechanics calculations and molecular dynamics simulations. When additional features such as σ holes, π orbitals, and lone pairs are included as off-atom sites and charges are derived (fitted by least-squares method) to reproduce QM ESPs, the calculated ESP surface is very similar to the *ab initio* ESP surface (Figure 1B,D).

Recently, Rathi et al. used atomic and off-atom site charges, obtained by fitting of QM ESPs for a diverse set of over 100 000 molecules, to train and validate a graph-convolutional deep neural network (GC-DNN) model.²⁴ The predicted charges for a set of test compounds were further used to calculate their ESP surfaces, which deviated on average by 3.27 ± 0.62 kcal/mol ($RMSE_v$) from the corresponding QM ESPs, a significant improvement on the average deviation of 7.86 ± 1.04 kcal/mol ($RMSE_v$) obtained for the AM1-BCC model. This difference is exemplified for chlorobenzene in Figure 1C,E. Additionally, the authors implemented an empirical model that scales down the partial charges of charged atoms in formally charged molecules to further improve the visualization of predicted ESP surfaces. While this scaling limits the direct comparison between the ESPs of uncharged and formally charged species, it still allows for the relative comparison within a series of formally charged molecules for the purpose of protein–ligand interaction optimization in SBDD. Overall, this model enables the computation of highly accurate ligand and protein ESP surfaces on a time scale suitable for interactive SBDD (the calculation of a GC-DNN ESP for a typical drug-like molecule with molecular

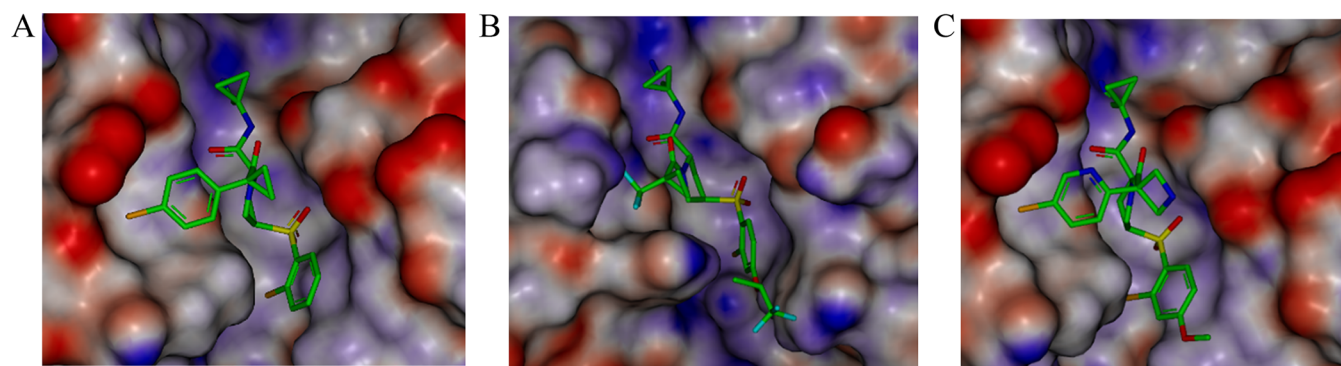


Figure 2. (A) Electrostatic potentials mapped onto the binding sites of human cathepsin L (left, PDB code 2ycj) and (B) mouse cathepsin S (middle, PDB code 4bpv). The following charged amino acids are found within 7 Å around **1**: human CatL (2 Glu, 4 Asp), human CatS (1 Asp, 1 Lys), mouse CatS (1 Glu, 1 Asp, 1 Lys). (C) X-ray cocrystal structure of **2** with human CatL (PDB code 5f02). Hydrogen atoms in protein and ligands were added and optimized using an internally developed code base (https://github.com/AstexUK/ESP_DNN/tree/master/esp_dnn/ext/pli). Electrostatic maps were generated using a GC DNN model and displayed as a solid Connolly surface with a color gradient of −50 (red) to 0 (white) to 50 (blue) kcal/mol.²⁴

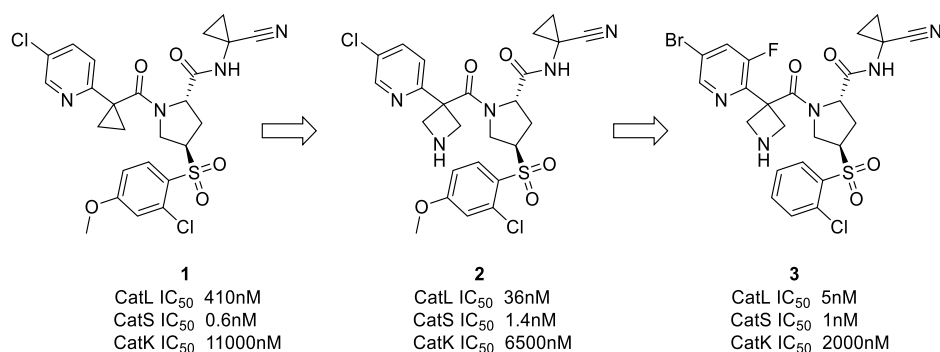


Figure 3. Optimization of CatL inhibitor **1** into dual CatL/CatS inhibitor **3**.

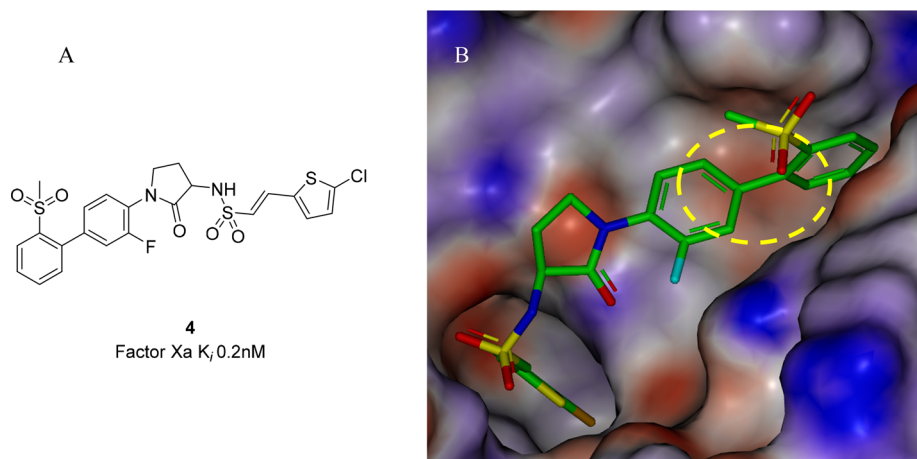


Figure 4. (A) Structure of factor Xa inhibitor **4**. (B) **4** in complex with factor Xa (PDB code 2VH6). Hydrogen atoms in protein and ligands were added and optimized as written in Figure 2. Electrostatic potential surface of the protein is calculated using GC-DNN method and is displayed on a solid Connolly surface with a color gradient of −50 (red) to 0 (white) to 50 (blue) kcal/mol.²⁴ Electron rich area is indicated by the yellow dashed oval.

weight 400 takes approximately 0.3 s) and is now being used by medicinal chemists at Astex.

■ OPTIMIZATION OF PROTEIN–LIGAND ELECTROSTATIC COMPLEMENTARITY

Dual Inhibition of Cathepsin S (CatS) and Cathepsin L (CatL). As a part of a wider review of computational methods used at Roche for small molecular drug discovery,³⁸ Stahl et al. report an example of using protein ESPs to develop a dual CatS/

CatL inhibitor. A series of selective CatS compounds had been previously reported by Roche which formed the starting point for the dual CatS/CatL inhibitors.³⁹ Analysis of the ESP surfaces of the two proteins revealed that the CatL binding site has a predominantly negative surface potential (six acidic residues (Asp and Glu) with no basic residues (Lys and Arg) within a 7 Å radius of the binding site), whereas CatS contains one Asp and one Lys within the same radius (Figure 2).

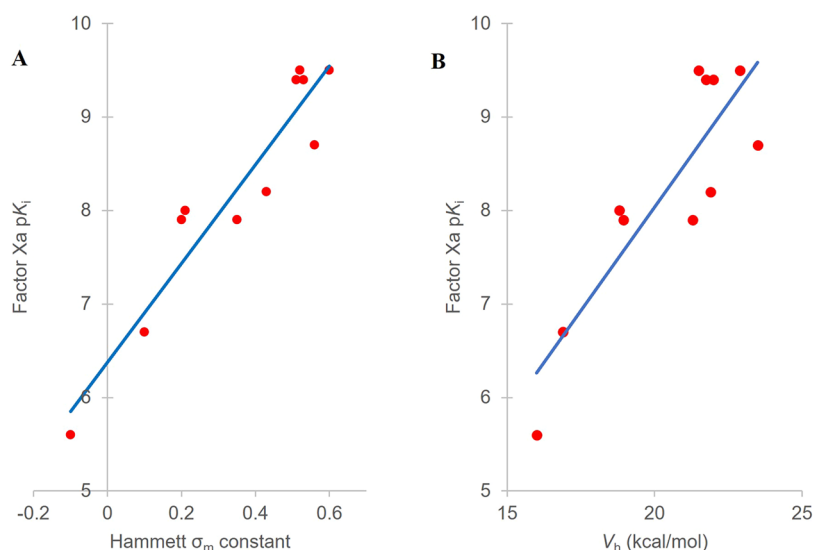


Figure 5. (A) Correlation of pK_i and Hammett σ_m constants for 5–15. pK_i values for 5–7 were based on their maximum possible affinity (0.4 nM (for 5) and 0.3 nM (for 6 and 7)). (B) Correlation between factor Xa pK_i and the calculated charge (V_h) on the ortho hydrogen of the terminal aromatic ring of 5–15. Charge was calculated using the GC-DNN model.

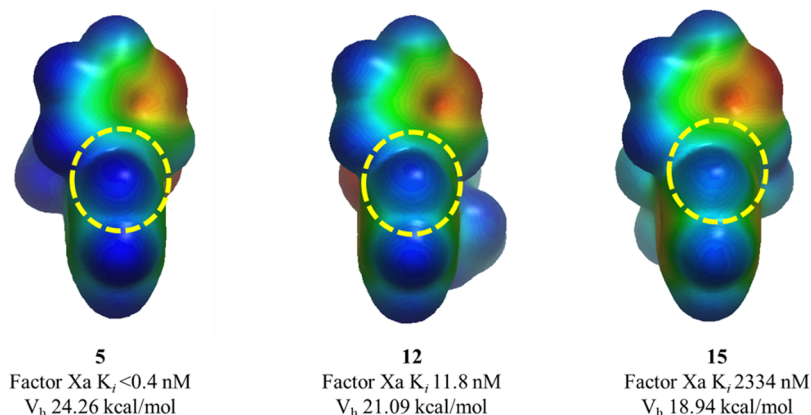


Figure 6. ESP surfaces of the biaryl S4 pocket substituent for 5, 12, and 15. Ortho hydrogen involved in interaction with Trp215 is highlighted in dashed yellow circle. V_h is the point charge on the ortho hydrogen in the dashed circle. ESP and point charges were calculated using GC-DNN and displayed on surface with a color gradient of -50 (red) to 0 (green) to 50 (blue) kcal/mol.²⁴

To improve the electrostatic complementarity with CatL, one of the cyclopropyl linkers in **1** was exchanged for a basic azetidine group (Figure 2). This resulted in an 11-fold improvement in CatL binding affinity while only reducing the affinity for CatS by 2-fold. **2** also maintained a good selectivity window over cathepsin K (CatK). The X-ray crystal structure of **2** bound to CatL (Figure 2c) shows that the azetidine makes no direct interactions with the acidic residues, but due to the long-range nature of electrostatic interactions, the benefit is seen in the improvement in potency. Further optimization of the 5-chloropyridine based on a previously reported⁴⁰ exploration of halogen bonding in the S3 pocket of CatL gave **3** which is a single digit nanomolar inhibitor of both CatL and CatS which maintains selectivity over CatK (Figure 3).

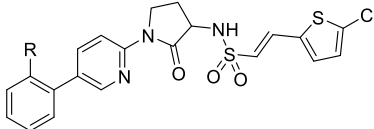
Factor Xa. In 2008, the optimization of a series of factor Xa inhibitors was disclosed by GSK.⁴¹ The X-ray structure of lead compound **4** complexed with factor Xa indicated that the aryl sulfone binds in the S4 pocket where it forms an edge-to-face aromatic interaction with Trp215. The ortho-hydrogen on the aryl sulfone is situated close to the electron-rich indole of Trp215 (yellow oval in Figure 4), and so the authors

hypothesized that this interaction, and therefore the affinity of the inhibitors, could be modulated by the σ -electron withdrawing effect of the substituent on the other ortho position (the methyl sulfone in **4**).⁴²

While looking to optimize the S4 pocket interactions, there was also a desire to reduce the lipophilicity of the compounds, and so the ortho-fluorophenyl in **4** was replaced with a pyridine and a range of substituents were installed in the ortho position of the terminal aromatic ring to give 5–15 (Table 1). The affinities of these compounds were determined and plotted against the Hammett σ_m coefficients⁴³ (Figure 5A) which confirmed that there was a clear relationship between the Hammett coefficient and the affinity for factor Xa. The calculated trend line showed that based on Hammett coefficients, it would be possible to predict the affinity of a prospective compound ($pK_i = 5.28\sigma_m + 6.39$; R^2 value of 0.897).

While the optimization work described here required the synthesis of all the compounds listed, it would have been possible to use ESP calculations to predict the most potent molecules and thus reduce the number of compounds needing to be synthesized. To illustrate this, we calculated the ESP

Table 1. Factor Xa Binding Affinities for 5–15



compd	R	factor Xa K_i (nM)	Hammett σ_m
5	SO ₂ NH ₂	<0.4	0.53
6	SO ₂ NHMe	<0.3	0.52
7	SO ₂ Me	<0.3	0.6
8	SO ₂ NMe ₂	0.4	0.51
9	CN	1.8	0.56
10	CF ₃	6	0.43
11	NMeSO ₂ Me	9.1	0.21
12	CONMe ₂	11.8	0.35
13	NHSO ₂ Me	14	0.2
14	O ^t Pr	180	0.1
15	^t Bu	2334	−0.1

surfaces of the biaryl S4 pocket motifs using the GC-DNN ESP model (examples 5, 12, and 15, Figure 6),²⁴ and it was found that the charge on the ortho-hydrogen (V_h) could be used to correctly rank the compounds' affinities (Figure 5B; $R^2 = 0.757$). This example demonstrates the power of ESP calculations to accurately predict compound potency in cases where an initial SAR is established, and similar approaches could be applied to a wide variety of target proteins.

Inhibitors of Apoptosis Proteins (IAP). In 2015, scientists from Astex reported the discovery and optimization of fragments against cellular inhibitor of apoptosis protein 1 and 2 (cIAP1/2) and X-linked inhibitor of apoptosis protein (XIAP).⁴⁴ From among the fragment hits against baculovirus IAP repeat 3 (BIR3) domain of XIAP, fragment 16 was prioritized for follow-up. By use of SBDD and a small, focused library of compounds, pyrrolidine 17 was optimized to indoline 18 (Figure 7).

While this change gave only a modest improvement in potency, the X-ray crystal structure showed good shape complementarity with the protein surface and promising vectors into an adjacent subpocket, warranting further optimization. To improve the binding affinity of 18, the electrostatic environment of the binding site was examined (Figure 8). The ESP map showed a negative electrostatic patch on the proximal protein surface due to a backbone carbonyl and the phenolic oxygen of tyrosine 324. The X-ray crystal structure indicated that the aromatic portion of the indoline stacks on top of this negative patch.

The authors hypothesized that the weak affinity of 18 for the target was caused by an electrostatic repulsion of the negative electrostatic π -cloud of the indoline and the negative electrostatic surface of the protein. To test this hypothesis, analogues of 18 with a range of electronegativities were synthesized, installing a selection of electron-donating or electron-withdrawing substituents in the C-6 position of the indoline based on the

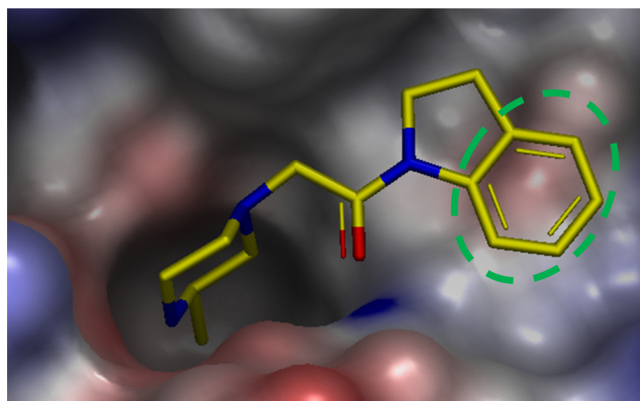
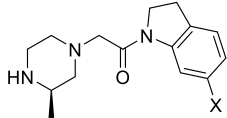


Figure 8. ESP surface of XIAP superimposed on the X-ray crystal structure of XIAP-BIR3 with 18. Green dashed oval indicates the negative electrostatic surface patch. The Connolly surface of the protein is colored by electrostatic potential with a color gradient of −50 (red) to 0 (white) to 50 (blue) kcal/mol. Hydrogen atoms in protein and ligands were added and optimized as described in Figure 2.

Table 2. Affinities of Substituted Indolines 18–27 for XIAP-BIR3 Domain and cIAP-BIR3 Domain



compd	X	Hammett σ_p	XIAP-BIR3 IC ₅₀ (μ M) or % I	cIAP-BIR3 IC ₅₀ (μ M) or % I
18	-H	0.00	52% @ 495 μ M	42% @ 495 μ M
19	-NH ₂	−0.66	56% @ 1000 μ M	8% @ 1000 μ M
20	-OMe	−0.27	49% @ 155 μ M	78
21	-Me	−0.17	46	95
22	- ⁱ Pr	−0.15	59	63
23	-F	0.06	51	51% @ 155 μ M
24	-Cl	0.23	13	29
25	-Br	0.23	9.8	40
26	-CF ₃	0.54	5.9	36
27	-SO ₂ Me	0.72	4.1	60% @ 99 μ M

Hammett σ_p values of the substituents (Table 2).⁴⁵ Figure 9A shows that binding affinities for XIAP-BIR3 are highly sensitive to the electronic properties of the indoline π -system: electron-poor indolines (26 and 27) are approximately 2 orders of magnitude more potent than electron-rich indolines (19 and 20).

In the related protein cIAP1, tyrosine 324 is replaced with a phenylalanine. This results in a reduction of the electronegative patch seen in XIAP, and when the affinities of 18–27 were measured against cIAP1 (Table 2), the affinities showed no correlation with the Hammett σ_p values (Figure 9B), further

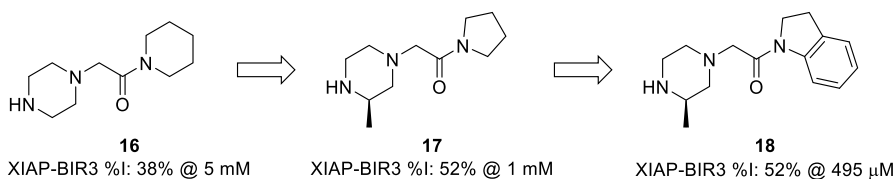


Figure 7. Optimization of fragment 16–18.

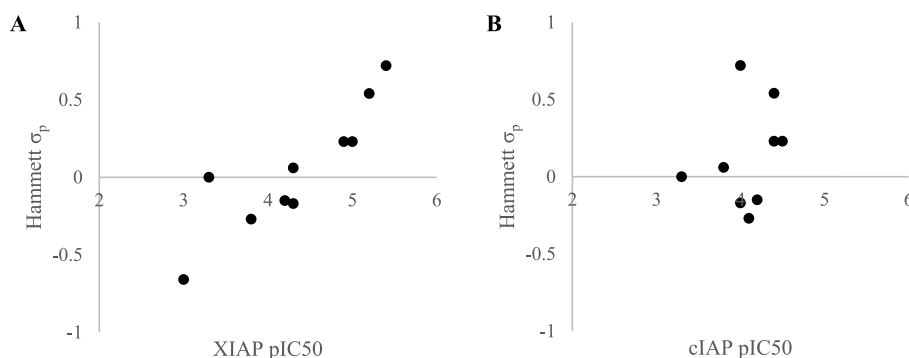


Figure 9. Plot of Hammett σ_p coefficients and affinities for indolines **18**–**27** against (A) XIAP-BIR3 domain and (B) cIAP-BIR3 domain (compound **19** not shown in plot B).

confirming that the optimization of the electrostatic complementarity between the indoline ring and the protein surface was responsible for the gain in affinity against XIAP.

To further dissipate the electropositivity of the indoline ring, the ESP surface of putative azaindoline target **28** was calculated using *ab initio* methods and compared to the ESP surface of indoline **19** (Figure 10). This analysis showed a significant

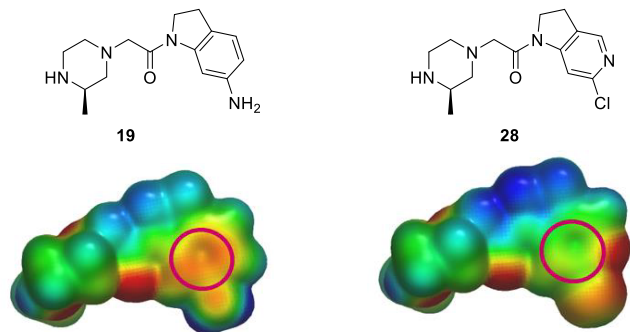


Figure 10. Comparison of electrostatic potential surfaces of **19** and **28** shown as color gradient of -50 (red) to 0 (green) to 50 (blue) kcal/mol. Red circle indicates aromatic surface in contact with the electronegative patch in the P3 pocket of XIAP.

reduction in the electropositivity of the aromatic ring. Compound **28** was synthesized and was shown to have an approximately 50-fold improvement in affinity for XIAP-BIR3 ($7.7 \mu\text{M}$) compared with **19** while also providing a useful synthetic handle to elaborate these compounds further.

Centromere-Associated Protein E (CENP-E). In the examples discussed to this point, X-ray crystal structures of the protein–ligand complex have been used to visualize the ESP surfaces; however Hirayama and colleagues at Takeda have published an example on the optimization of inhibitors of

centromere-associated protein E (CENP-E) where a homology model based on the motor domain of kinesin spindle protein (KSP) was used to generate the ESP data instead.^{46,47} Starting from thienopyridone **29**, which was identified from an HTS hit, optimization of the amide led to **30** which showed a significant improvement in biochemical affinity (Figure 11). Analysis of **29** in the homology model suggested that the pyridone carbonyl was not making any interactions with the protein; this led the authors to redesign the core scaffold to give thienopyrrole **31** which had 32 nM inhibition in a biochemical assay.

To better understand the SAR seen in the substitution at the C-3 position of the thieno[3,2-*b*]pyrrole, the ESP surfaces of a series of analogues were calculated (Figure 13). To simplify the calculations, the amide was replaced with a simple dimethylamide. Potent inhibitors, such as those bearing 3-Br (**32**) or 3-Cl (**33**), showed a neutral electrostatic potential on the thiophene ring (Figure 12). In contrast, 3-methyl derivative **34** showed a negative ESP and 3-nitrile analogue **35** showed a positive ESP on the thiophene ring. The homology model suggested that the scaffold was in a hydrophobic cavity that contained isoleucine, tyrosine, phenylalanine, and methionine residues; as a result of this, the authors hypothesized that a neutral scaffold was required for binding to CENP-E which is confirmed by the biochemical assay data.

While **31** showed good biochemical affinity the compound showed poor cellular activity (p-HHE $\text{EC}_{50} = 3.0 \mu\text{M}$). To improve the cellular activity, the authors attempted to investigate a second scaffold hop from the thienopyrrole. On the basis of the results of the previous ESP analysis, the ESP surfaces of potential scaffolds were generated, and as the bromoindoles (**36** and **38**) were predicted to have a negative ESP on the thiophene ring, these were not synthesized (Figure 13). Imidazopyridine **40** was predicted to have a neutral ESP and, as such, was selected for synthesis. Pleasingly, **41** showed a similar biochemical affinity to **31** (50 nM vs 32 nM) and saw a 2-

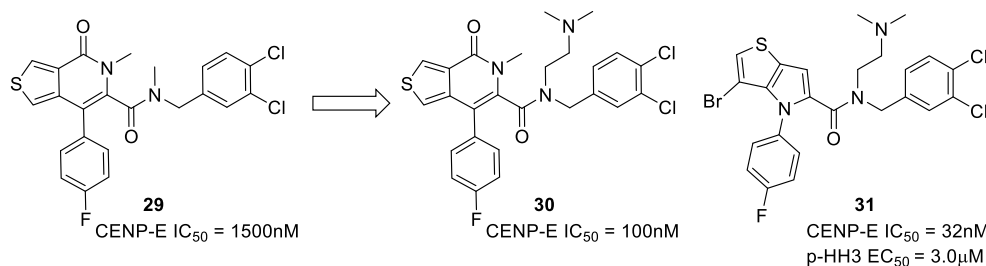


Figure 11. Optimization of HTS hit **29**.

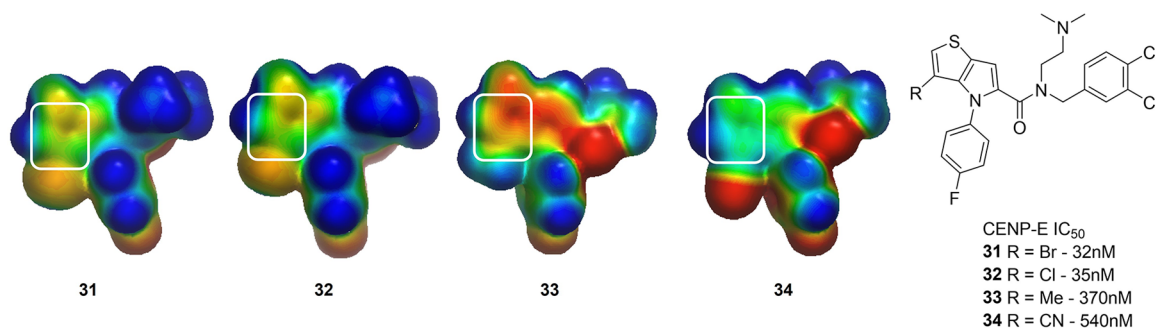


Figure 12. Using ESP to understand SAR around 31. Electrostatic potential of dimethylamide analogues were calculated to reduce computational burden. Area of interest is highlighted with a white rounded rectangle. ESPs were generated using a GC DNN model and displayed as Connolly surface with a color gradient of -50 (red) to 0 (green) to 50 (blue) kcal/mol.²⁴

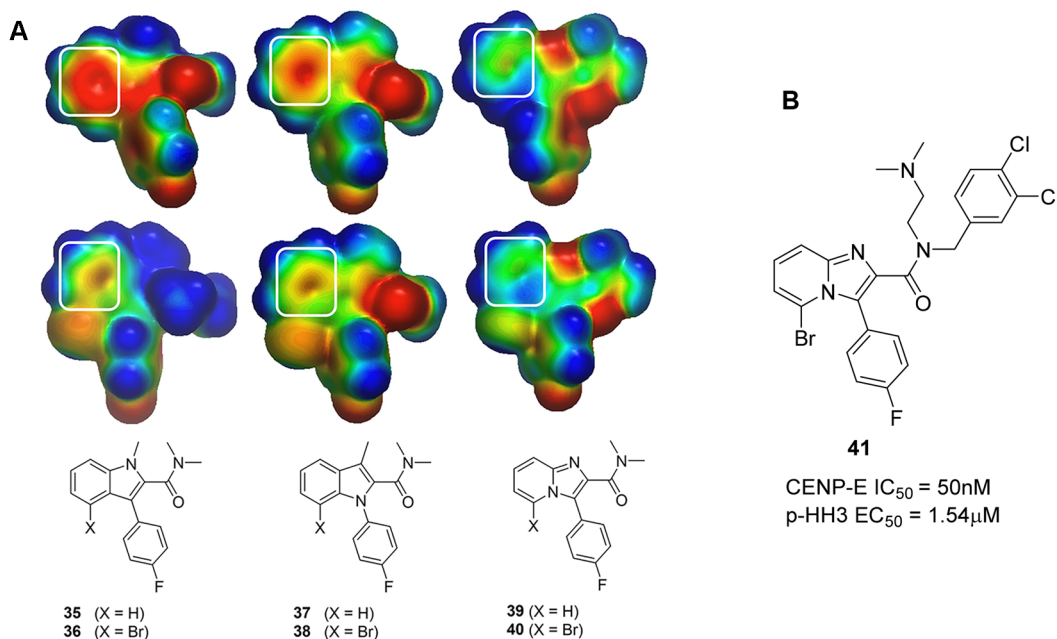


Figure 13. (A) Use of electrostatic potential surfaces to predict a heterocyclic scaffold hop to improve cellular affinity. Positive, neutral, and negative areas are colored blue, green, and red, respectively, as Connolly surfaces with a solid display style. ESPs generated using a GC DNN model and shown as color gradient of -50 (red) to 0 (green) to 50 (blue) kcal/mol.²⁴ Area of interest is highlighted with a white rounded rectangle. (B) Scaffold hop product 41.

fold improvement in cellular affinity ($1.54 \mu\text{M}$ vs $3.0 \mu\text{M}$) along with a notable improvement in permeability as measured in a PAMPA (pH7.4) assay (31 41 nm/s vs 41 353 nm/s). Although the change in cellular affinity is modest, these results provided the confidence to develop this lead molecule further.

OPTIMIZATION OF INTRAMOLECULAR ELECTROSTATIC INTERACTIONS

The use of ESP in SBDD extends beyond the optimization of protein–ligand interactions, discussed above, to also encompass the study of intramolecular interactions. Analysis of the electrostatic profile of a small molecule ligand in isolation can yield information regarding both beneficial and detrimental intramolecular interactions, leading to rationalization of existing SAR and providing the opportunity to prospectively design target molecules of increased binding affinity.

In the context of SBDD, analysis of intramolecular interactions in the bound, or bioactive, conformation of the ligand is of particular significance when compared with the available conformations of the ligand when unbound. Most

small molecules employed in drug discovery campaigns can adopt multiple conformations when free in solution, dictated principally by the number of rotatable bonds. Close correspondence between the dominant solution conformation and the observed bound conformation helps to minimize entropic penalties upon ligand binding, and hence stabilization of the bioactive conformation when in solution offers a secure strategy to increase binding affinity.^{48,49} Experimental determination of the lowest energy free ligand conformation is typically performed with 1D and 2D NMR spectroscopy, where proton chemical shift signatures and through-space interactions can be used to assess the degree of ligand flexibility and assign the major conformation.^{50,51}

Preorganization in solution of otherwise flexible ligand structures can be achieved via a range of methods, such as functional group changes, macrocyclization or the introduction of intramolecular interactions.^{38,52–54} In our experience, ESPs have proven particularly informative in the latter case, where careful analysis of an intramolecular interaction observed in the protein–ligand X-ray crystal structure can lead to rational

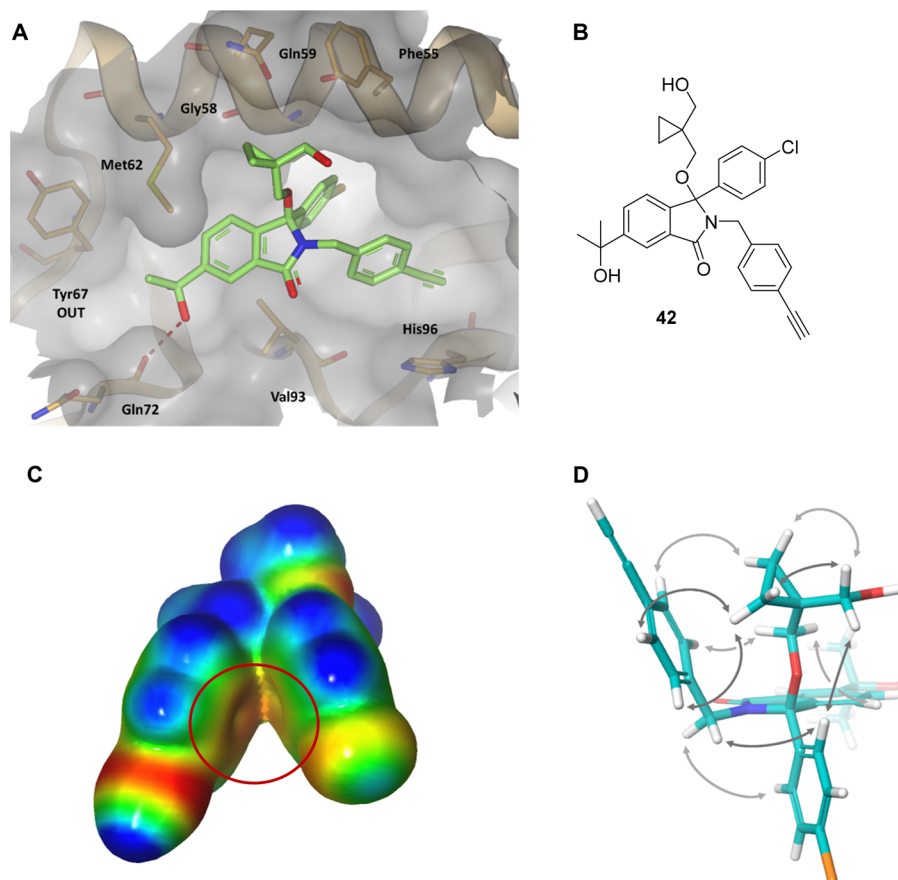


Figure 14. (A) X-ray crystal structure of compound **42** bound to MDM2 (PDB code 7BMG). (B) Structure of **42**. (C) ESP surface of **42** in the bound conformation. Negative areas are colored red, and positive areas are colored blue. The red circle indicates a potential electrostatic clash between the π systems of the aromatic rings. (D) Solution conformation of **42** as determined by NMR. Through-space ROESY correlations from the NMR spectrum are indicated with arrows. Reproduced with permission from Chessari et al. *J. Med. Chem.* **2020**, *64* (7), 4071–4088. Copyright 2021 American Chemical Society.

designs that attempt to recapitulate this in the major solution conformation. The examples described below feature ESP-driven optimization of π – π stacking and dipole–dipole interactions, but it is feasible that the approach could be applied to other intramolecular contacts such as cation– π , hydrophobic, or H-bonding interactions.

Murine Double Minute 2 (MDM2). Intramolecular ESP analysis was employed in the structure-guided design of novel isoindolinone-based inhibitors of MDM2.⁵⁵ Early protein–ligand X-ray crystal structures, such as that of compound **42** (Figure 14A), showed a folded conformation whereby the 4-chlorophenyl and 4-ethynylbenzyl substituents stack against each other. Having identified a putative intramolecular interaction, the ESP surface of the bound conformation of **42** was calculated. This analysis suggested an electrostatic repulsion between the two aromatic rings, as denoted by the red coloration of the ESP surface of both π systems in close proximity (Figure 14C). This theory was supported by solution NMR studies, which showed the dominant conformation of **42** to be in an open form, with the two aromatic rings directed away from each other to avoid the unfavorable electrostatic clash (Figure 14D).

Having established a discrepancy between the bioactive and solution conformations, further ESP calculations, alongside a computational “thermodynamic cycle” method,⁵⁶ were used to model potential alternatives to the 4-ethynylbenzyl group which would favor the folded conformation seen in the bound structure

(Figure 15). Both the quantitative analysis of the interaction energy between the two rings ($E_{\pi-\pi}$) and the qualitative inspection of the ESP surfaces showed a clear advantage to exploring more electron-deficient ring systems such as the chloropyridine example **43** shown in Figure 15. Gratifyingly, this theory was borne out as several synthesized analogues bearing more electron-deficient benzyl-type substituents showed improvements in both the primary binding assay and a SJSA-1 TP53^{WT} cell assay, with a concomitant decrease in lipophilicity to help control physicochemical properties. For example, compound **43** showed a 3-fold gain in potency in both assays and a decrease in cLogP of 1 log unit compared to compound **42**. While this improvement in potency is smaller than some of the examples seen above, it is what would be expected from the calculated differences in energies. This approach also allowed the installation of a polar heterocycle into a pocket which historically required a more lipophilic group resulting in an increase in lipophilic ligand efficiency (LLE or LipE) from 2.9 to 4.4.⁵⁷ Protein–ligand X-ray crystallography and a separate small molecule X-ray structure of **43** confirmed the bioactive folded conformation is retained for more polar aromatic groups, as predicted by the ESP modeling. This example shows the ability of ESP to identify possible intramolecular clashes which can then aid the rational design of new targets that favor the desired interaction in the solution phase, thus delivering increased binding potency.

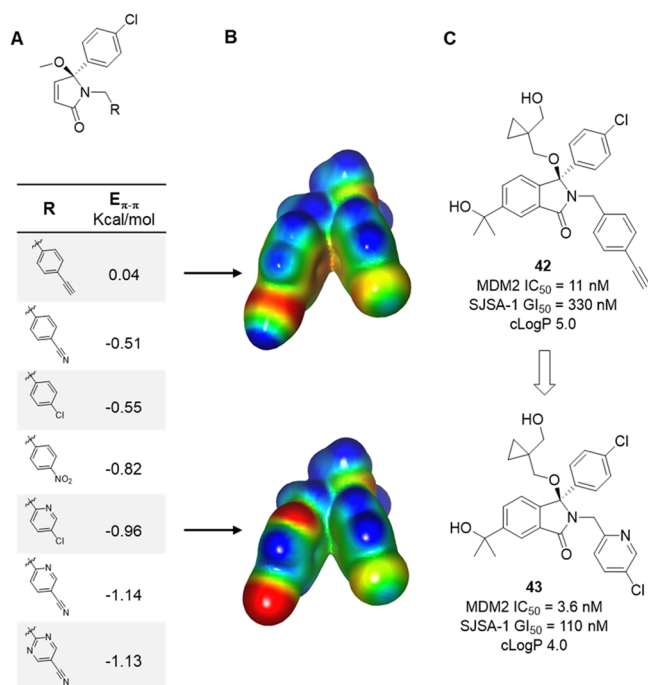


Figure 15. (A) Calculated values of the aromatic interaction ($E_{\pi-\pi}$). (B) ESP surfaces of two representative examples from (A). Negative areas are colored red, and positive areas are colored blue. (C) Improvement in binding and cellular potency and decrease in calculated lipophilicity from compound 42 to 43. Reproduced with permission from Chessari et al. *J. Med. Chem.* 2020, 64 (7), 4071–4088. Copyright 2021 American Chemical Society.

Inhibitors of Apoptosis Proteins (IAP). A second example of intramolecular ESP analysis can be found in the further optimization of IAP antagonists, discussed previously in the context of protein–ligand interactions.⁵⁸ Starting from compound 44, bearing a methoxymethyl group (Table 3), SAR for the piperazine C-5 substituent was explored. During this round of optimization it was noted that the protein–ligand X-ray structures of several analogues showed that the new substituent

Table 3. Affinities of cCompounds 44, 46, 47 Bearing Various Substituents

Compound	R	XIAP IC_{50} (nM)
44		220
46		110
47		44

preferentially adopted a folded conformation, packed on top of the central amide bond, for example, compound 45 from a subseries featuring an aza-isomeric core (Figure 16A). As in the previous example, this identification of an intramolecular stacking interaction was followed by solution NMR studies that, in this case, showed the lowest energy free conformation of compound 45 was consistent with the bioactive pose, with the folded arrangement observed (Figure 16B).

In order to gain a better understanding of this interaction and to facilitate the design of new compounds that maximize the observed stacking orientation, ESP surfaces of the piperazine-azaindoline core and of potential P2 substituents were calculated (Figure 17). This analysis suggested a number of groups whose electrostatic profiles would best complement that of the core amide bond, enhancing the attractive dipole–dipole interaction and favoring the desired folded geometry. Figure 17 shows two such examples: an aromatic and a saturated heterocycle.

Synthesis and testing of fluoropyrazole analogue 46 and pyrrolidinone species 47 gave 2-fold and 5-fold increases in activity, respectively, compared to parent compound 44 (Table 3), with 47 providing the most ligand efficient compound in the first round of SAR exploration (LE = 0.29). As in the previous example, this modest gain in affinity was accompanied by an improvement in LLE from 3.1 (compound 44) to 4.3 (compound 47), suggesting that this type of careful ESP analysis can be applied to improve broader physicochemical properties. The crystal structure of compound 47 bound to XIAP-BIR3 confirmed the persistence of the folded conformation, as anticipated from the ESP modeling (Figure 17). This example demonstrates that even in cases where the bound and free conformations are initially consistent with each other, ESP analysis can be used to profile intramolecular interactions (in this case, a dipole–dipole stack) and help to design new targets that favor the bioactive pose to the greatest extent.

CONCLUSIONS

As can be seen from the examples above, ESP can be used to benefit and accelerate structurally enabled drug discovery programs. Improving electrostatic complementarity has been shown not only to improve the affinity of compounds for the protein target but also, as illustrated by the CENP-E inhibitors, to identify alternative scaffolds that have improved physicochemical properties leading to improved cellular activity and potentially improved *in vivo* profiles. The example of the factor Xa inhibitors clearly illustrates that within a specific series, the ESP characteristics need to be considered, and careful optimization can yield significant gains in potency. This optimization of electronic properties of aromatic groups is reminiscent of the decision tree proposed by Topliss for optimizing substituents on aromatic groups; however as ESP can be calculated prospectively, this will reduce the number of compounds needing to be synthesized to arrive at the most efficient arrangement of substituents for a given protein target.⁵⁹

ESP calculations also give the opportunity to identify unconventional interactions that can be made between ligand and protein as shown by the IAP inhibitors. Stacking an aromatic ring on top of a backbone carbonyl may not be an obvious choice for developing a beneficial interaction, but by understanding the ESP of the protein and ligand, this interaction can be successfully optimized. The use of ESP to take a CatS specific inhibitor and convert it to a dual CatS/CatL inhibitor shows that by elegant use of surface potentials it is possible to optimize potency for one target without noticeably reducing the potency against another.

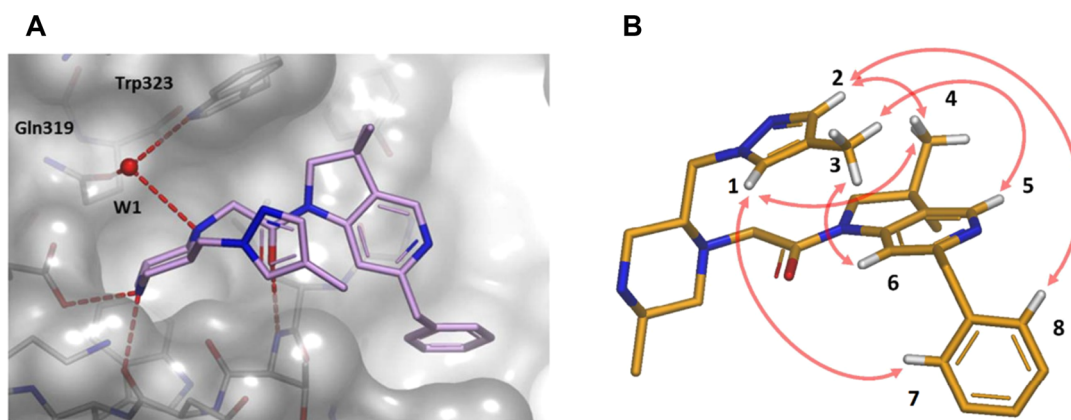


Figure 16. (A) X-ray crystal structure of compound **45** bound to XIAP-BIR3. Hydrogen bonds are shown as dashed lines. Structured water molecule is highlighted as W1. (B) Solution conformation of **45** as observed by NMR. Arrows indicate through-space ROESY correlations observed in the NMR spectrum. Reproduced with permission from Taminini et al. *J. Med. Chem.* **2017**, *60* (11), 4611–4625. Copyright 2017 American Chemical Society.

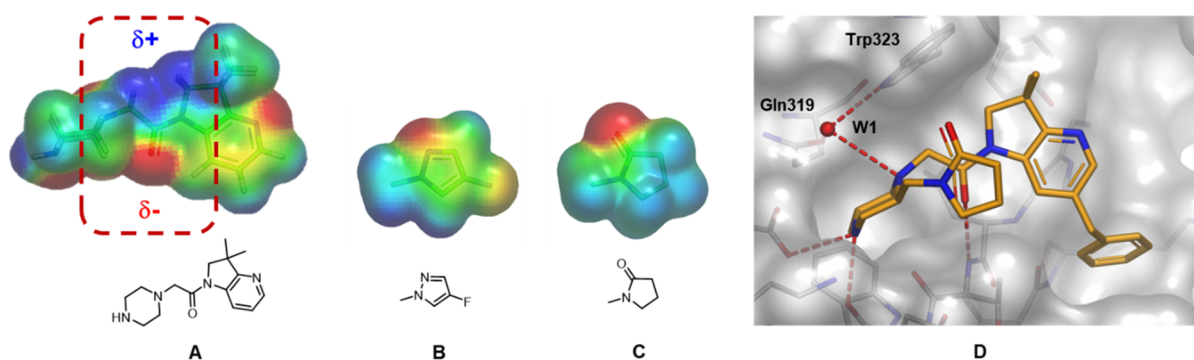


Figure 17. ESP surfaces of the core piperazine-azaindoline scaffold (A) and two examples of piperazine substituents that were selected for synthesis (B, C). Negative areas are colored red, and positive areas are colored blue. The key region surrounding the amide linkage of A is highlighted by the red dashed line. (D) X-ray crystal structure of **47** bound to XIAP-BIR3. Hydrogen bonds are shown as dashed lines. Structured water molecule is highlighted as W1. Reproduced with permission from Taminini et al. *J. Med. Chem.* **2017**, *60* (11), 4611–4625. Copyright 2017 American Chemical Society.

Similarly, while there are no examples here, it is also possible to imagine a situation where ESP could be used to engineer selectivity against a given antitarget.

In the two examples of ligand ESP modeling (MDM2 and IAP inhibitors), solution conformational studies were also performed to give further insight into the nature of the intramolecular interactions and their influence on the energetics of binding to the protein. However, it is conceivable that, even in the absence of this experimental information, ESP analysis alone could still have assisted in compound design and given similar conclusions. In the case of MDM2 antagonists, especially, the identification via ESP calculations of an electrostatic clash at the interface of the two aromatic rings would likely have warranted further exploration of SAR at this position of the molecule in any case, and the subsequent design of more favorable analogues did not rely specifically on knowledge of the solution conformation.

The ability to identify or confirm both unfavorable clashes and desirable interactions from structural information, in conjunction with either solution NMR experiments or other modeling techniques, makes intramolecular ESP analysis a valuable technique in SBDD.

It is also clear from the examples discussed that, while the benefits of optimizing electrostatic complementarity are clear, there is a limited uptake of this technology in medicinal chemistry groups. One reason for this limited uptake could be that, historically, generating ESP, even for small molecules, is a

relatively computationally expensive technology that requires expertise in performing the calculations. This computational expense increases exponentially when considering the complexity of generating ESP for macromolecules such as proteins. Moreover, ESP surfaces for formally charged molecules computed in vacuum are not practically useful, and it could be further improved by including an implicit solvation model; however, this further complicates the procedure and increases computation time by manyfold. The rise of graphical processing units (GPUs) for computational processing may have reduced the barrier to these calculations,^{60,61} but they are likely to still be out of reach of all but the best equipped laboratories. The recent development of a GC-DNN model for generation of near-DFT quality ESP surfaces many orders of magnitude faster than conventional QM methods (for example, a typical drug-like molecule of molecular weight 400 could take around 30 min to calculate a DFT ESP; this calculation time can be cut to ~0.3 s using the recently developed GC-DNN model), and using routinely available computer hardware, could open ESP generation to laboratories that had historically been unable to access conventionally generated QM ESP surfaces. We hope that lowering the activation barrier to accessing ESP data will act as a catalyst for an increased uptake of the use of ESP surfaces in medicinal chemistry.

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Notes

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Gianni Chessari is Vice President and Head of Medicinal Chemistry at Astex Pharmaceuticals where he has also served as Head of Modelling. He has extensive experience of fragment-based and structure-based drug design and has contributed to the discovery of a number of small molecule drug candidates that have progressed into human clinical trials. During his career he has worked on experimental and computational methods to evaluate electrostatic interactions in molecular recognition of small molecule–protein binding. He obtained his degree in Chemistry from the University of Catania (Italy). His Ph.D. studies were carried out at the University of Sheffield (U.K.), supervised by Professor Christopher A. Hunter. Gianni has authored more than 60 publications and patents.

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ABBREVIATIONS USED

BCC, bond charge correction; BIR, baculovirus IAP repeat; ESP, electrostatic potential; CatL, cathepsin L; CatS, cathepsin S; CENP-E, centromere-associated protein E; cIAP, cellular inhibitor of apoptosis protein; GC-DNN, graph-convolutional deep neural network; GPU, graphical processing unit; IAP, inhibitors of apoptosis proteins; KSP, kinesin spindle protein; LLE, lipophilic ligand efficiency; MDM2, murine double minute 2; p-HH3, phosphohistone H3; RMSE_√, root-mean-square error value; QM, quantum mechanical; XED, extended electron distribution; XIAP, X-linked inhibitor of apoptosis protein

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NOTE ADDED AFTER ASAP PUBLICATION

This paper originally published ASAP on May 5, 2022. Due to a production error, panel B of Figure 13 was previously missing and has now been updated. A change on page G was made, correcting compound 42 to compound 41. An updated version was reposted on May 18, 2022.

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