# FLUORINATED *N*-HETEROCYCLES AS CONFORMATIONALLY DIVERSE BIOACTIVES FOR DRUG DISCOVERY

DOI: http://dx.medra.org/10.17374/targets.2022.25.502

**Fei Liu,* Bilqees Sameem**

*Department of Molecular Sciences, Macquarie University, Sydney, NSW 2109, Australia*
*(e-mail: fei.liu@mq.edu.au)*

***Abstract.*** *N-heterocycles and their fluorinated analogues are prevalent in many fields of chemistry. In particular, they are an important class of compounds for bioactivity and drug discovery. The review here focuses on recent strategies in designing and accessing fluorinated and saturated N-heterocycles with conformational diversity for exploring new protein-ligand interactions. Some of the examples highlight the continuous investigation from the authors on fluorinated azepanes with complex conformational preferences.*

**Contents**

1. Introduction
2. Fluorine as a conformation tuning tool
3. Conformation-based bioactive drug discovery as new frontiers
4. Common fluorination methods for C–F bond formation
5. Conformational tuning by fluorine substitution on piperidines
6. Conformational tuning by fluorine substitution on azepanes
7. Conclusion
Acknowledgements
References

## 1. Introduction

Cyclic and nitrogen containing molecules are fundamental motifs of biological systems, most evidently as building blocks of genetic materials such as nucleic acids.[1-5] The electronic properties of the nitrogen in a heterocycle enable important acid/base chemistry for controlling vast and weak interactions, a feature that is essential for living systems. In addition, *N*-heterocycles confer diverse intermolecular interactions based on hydrogen-bonding, stereoelectronic and polarity effects, and van der Waals forces, embedded as key factors into aqueous biomolecular networks composed of large varieties of biopolymers. Not surprisingly, the prominent role of *N*-heterocycles in the finding and developing of pharmaceuticals is widely recognized.[6-9] *N*-heterocycles are found in a wide range of small molecule-based therapeutics for chronic diseases and neurological disorders, as well as anticancer, antiviral, antimicrobial, and antiparasitic agents (Figure 1). Analyses of FDA-approved small-molecule drugs indicated that over two-thirds contain at least one *N*-heterocycle.[7] The most-prevalent *N*-heterocycle is piperidine, followed by around two-dozens of nitrogen containing small to medium sized saturated rings, heteroaromatics, or bicycles. *N*-heterocycles have and will continue to occupy a unique area of chemistry with large impact on the daily human life.

In recent decades, fluorine substitution, typically on carbon, has become a focal point for developing bioactive *N*-heterocycles in pharmaceutical as well as agrochemical industries.[8] Fluorinated *N*-heterocycles with strong local dipole moments present unique effects on and interactions with the heteroatoms in the ring, resulting in very different electronic properties compared to their aromatic counterparts.[2] The presence of a C–F bond, often in rationally selected positions, significantly alter the pharmacokinetic or pharmacodynamic profiles of a therapeutic candidate.[10] Physical and chemical properties, such as polarity, acidity/basicity, lipophilicity, and metabolic stability, can be systematically tuned by using fluorine substitution. As *N*-heterocyclic research has been regularly discussed and summarized in nearly all fields of chemistry, this review will focus on more recent development of fluorination on saturated *N*-heterocyclic motifs, in particular six- and seven-membered rings with conformational diversity, with a special emphasis on our group's work on seven-membered saturated azepanes, in order to illustrate new intermolecular interactions based on conformational tuning for exploring new therapeutic opportunities by controlling dynamic protein-ligand interactions.

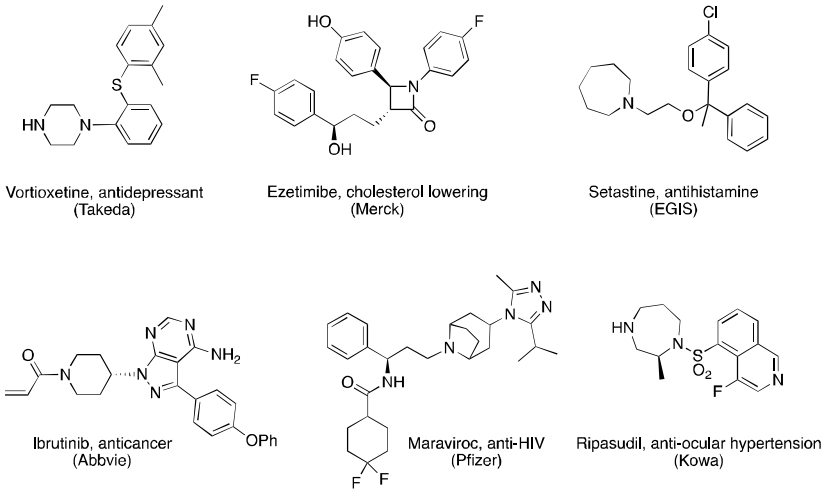



**Figure 1.** Representative examples of *N*-heterocycles (some with fluorination) as FDA-approved drugs.

## 2. Fluorine as a conformation tuning tool

The effects of C–F bonds, both electronic and stereoelectronic in a chemical and biological systems, are well recognized and extensively reviewed.[11-14] The polarity of the C–F bond, given the extremely highly electronegativity of fluorine, has a significant electrostatic character, creating a very strong C–F bond and relatively large dipole in fluorinated molecules for conferring dipole interactions with their environment (Figure 2a). These often include dipole-dipole, dipole-charge, H-bonding, and ion coordination interactions. The electron density concentrated on the small fluorine nucleus, with the carbon center significantly positive, also leads to geometric changes in the molecule and a preference for fluorine to bond to $sp^3$ carbon centers. While a poor leaving group in the $S_N2$ context, a fluoride can be reactive in driving addition-elimination reactions that involve cationic intermediates.

The highly polarized C–F bond also presents a low-lying σ antibonding orbital as an excellent acceptor for electron density from donors such as nitrogen or oxygen lone pairs.[12] The covalent character of the C–F bond is decreased by this secondary orbital overlap but the ionic character is increased by it, resulting in further bond stabilization. Constrained in a molecular context, this hyperconjugation, in conjunction with electronic effects, constitute the fluorine *gauche* effect underlying conformational preferences (Figure 2b). Compared to the dipole-based interactions up to 8 kcal/mol, this *gauche* effect is weaker in the range of 1-3 kcal/mol but can be applicable generally in many systems with electronegative substituents. The strength of the fluorine *gauche* effect is also dependent on solvent polarity for overall stabilization of the molecular dipole and tends to be more pronounced in polar solvents.

The C–F bond has been deployed, with positional control, in many cases for conformational tuning, including fluoroalkanes, fluorinated peptides and proteins, and fluorinated heterocycles.[13,15] The C–F bond, as shown in some of the representative examples, has significant impact on the conformational preferences, or overall shape, of the molecule (Figure 3). As a consequence, the biological activities, or physical properties, of these molecules, are dependent on the correct positioning of the C–F bond. When multiple C–F bonds are in use, the overall conformational effect can be difficult to predict, as many additional factors, such as electrostatic interactions, electronic repulsion, and solvent effects, add to the complexity of the conformational control. However, this also opens new opportunities for further investigation on new methods for the synthetic challenge of preparing these unusual molecules as well as processes for analyzing the multifactorial conformational effects by a combination of spectroscopic and computational techniques.

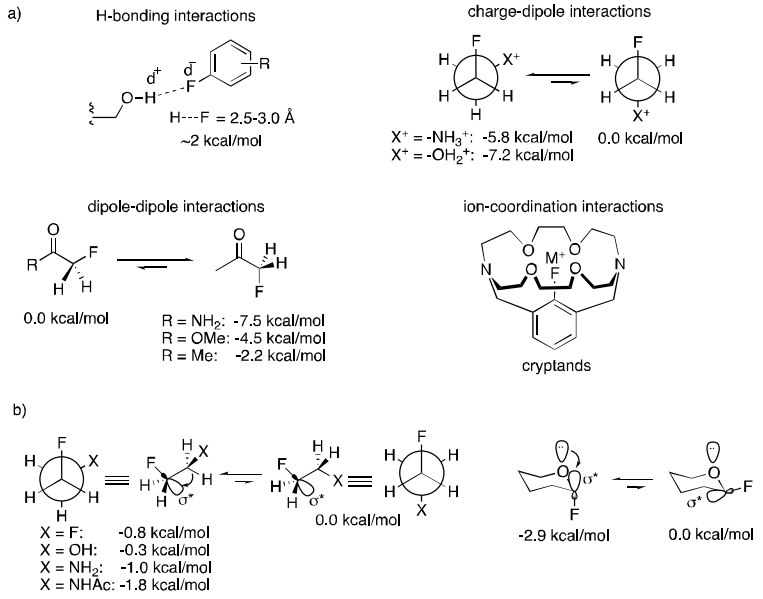



**Figure 2.** a) Main classes of polar interactions conferred by a C–F bond. b) Stereoelectronic C–F interactions.

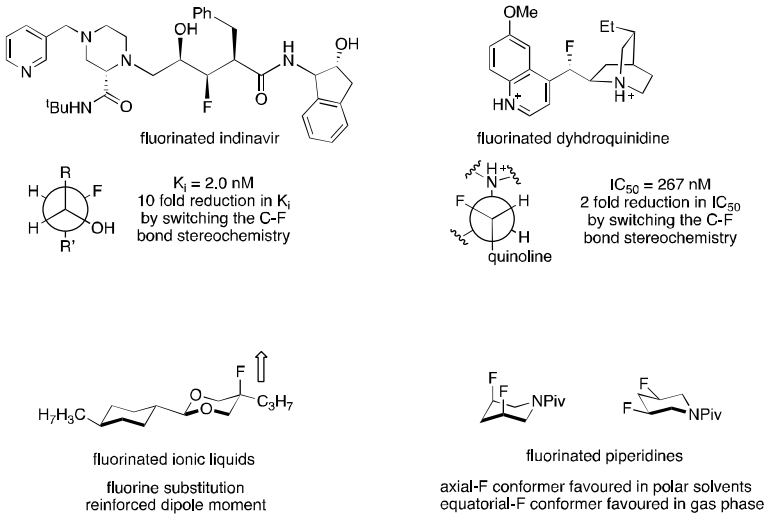



**Figure 3**. Examples of fluorine effects in heterocycles.

## 3. Conformation-based bioactive drug discovery as new frontiers

Modern drug discovery faces major challenges, foremost of which is to continuously find new modalities and address undruggable targets.[16,17] Improved understanding of target biology and genetic biomarkers help bridge some of the gap; however, the molecular design strategy also requires new principles in order to meet the demand of targeting selectively difficult disease-driving mechanisms and interactions. Proteins remain major drug targets, and their conformational flexibility is increasingly recognized as important factors to consider when developing drug leads with specificity.[18] As exemplified by Gleevec, the

first drug for chronic myelogenous leukemia (CML), a particular conformational state of its target Bcr-Abl kinase domain is the molecular entity through which Gleevec confers its efficacy with high selectivity.[19]

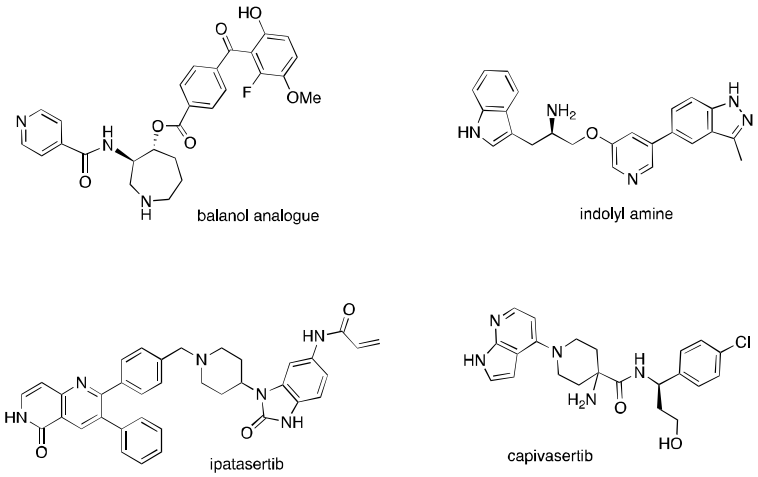



**Figure 4**. Examples of inhibitors investigated for achieving Akt isozyme specificity.

Protein flexibility however is difficult to predict in rational drug design. While computational techniques will continue to assist in providing initial insight into potential leads for exploration,[20] the slow step remains the physical discovery of molecular motifs that can be synthesized and analyzed with new modes of action on difficult targets. For example, the discovery of allostery-based inhibitors for Akt with isozyme specificity takes advantage of the selectivity of its pleckstrin homology (PH) domain.[21] The Akt protein kinase plays a key role in cell survival signalling, the dysregulation of which is directly involved in a variety of cancers. However, Akt isozymes differ in their specific regulatory roles, despite sharing a high level of primary sequence homology. Generations of Akt inhibitors have been explored with limited success in achieving Akt or Akt-isozyme specific activity (Figure 4). By combining PH-domain selective inhibitors with targeted covalent modifiers such as an acrylamide, highly potent and isozyme specific inhibitors for Akt1 and Akt2 based on the borussertib scaffold (Figure 5) have been reported to enable detailed biological and preclinical studies in order to elucidate isozyme-dependent disease etiology.[22-24]

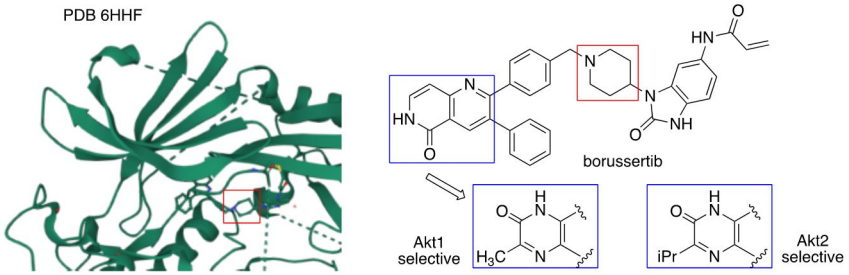



**Figure 5.** Derivatives of borussertib with Akt isozyme specificity found by structure-guided inhibitor design. The piperidine ring of the borussertib is highlighted in a red square in the PDB.

As more and more examples of biological signalling and regulatory events involving flexible motion either within the protein or in protein-protein interactions, new experimental approaches for conformational cooperativity will help tapping into an under-explored diversity space for drug lead discovery.[25] In the past two decades, fragment-based drug discovery (FBDD) has become an important strategy for drug dead

discovery, whereby a large number of fragments are screened as low-affinity modulator and then optimized.[26] The fragments may derive from synthetic libraries or natural products. For example, a chemoinformatic screen using more than 180000 natural products first identified a set of natural product fragments, rich in $sp^3$-centers, from Renieramycin P. The fragments were then further optimized to target p38α MAP kinase as well as other phosphatases.[27]

More recently, small molecules, in the form of Proteolysis Targeting Chimeras (PROTACs), regulate protein function by using the ubiquitin-proteasome system (UPS) to specifically eliminate targeted proteins.[28] One way of tuning permeability, lipophilicity and solubility of the PROTAC is to optimize the conformational flexibility for enhancing reversible and dynamic intramolecular hydrogen bonding in an environment-dependent manner. Conformational tuning is also vital to maximizing the binding specificity of the degrader to the targeted protein in order to reduce side effects.[29] For example, thalidomide, lenalidomide, and pomalidomide are able to recruit zinc-finger transcription factors and protein kinases to the cereblon-CRL4 ubiquitin ligase for degradation. Structural and biophysical investigations revealed that the phthalimide fragment of the small molecular glues binds at the protein interface with the glutarimide fragment embedded in a hydrophobic pocket of the cereblon ligase. The scaffolding of protein-protein interactions between cereblon and its protein substrates depends on the small molecule glue to conformationally induce-fit in the ternary complex.

In order to investigate new protein-ligand interactions in binary or ternary complexes with enhanced specificity, the FBDD approach can be coupled to conformational tuning to generate further chemical diversity as well as conformational diversity.[25] In this regard, the C–F bond from selective fluorination serves as a versatile tool for conferring both defined chemical and conformational effects. By combining conformational-coupled diversity-oriented synthesis (CDOS) with FBDD, new chemical and conformational space can be explored in an integrated manner for finding novel lead candidates.

## 4. Common fluorination methods for C–F bond formation

Stereogenic C–F bonds present additional challenges to fluorination methodology beyond just conversion.[30-32] Typically C–F bond formation can be achieved in two main avenues using common reagents (Figure 6): i) electrophilic fluorination with $F^+$ reagents; ii) nucleophilic fluorination involving a source of fluoride anion. The *N*-fluoropyridinium triflates and derivatives involving single-electron transfer processes were frequently used in fluorination of aromatic rings, carbanions, enol ethers and their derivatives. The *N*-fluorosulfonimides are neutral and easy to handle, and their chiral *N*-fluorosultam counterparts, as well as chiral Selectfluor® derivatives, have seen wide use in enantioselective fluorination reactions.

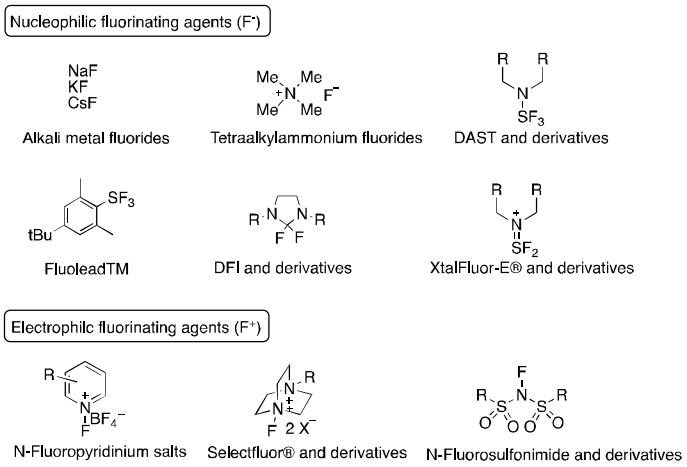
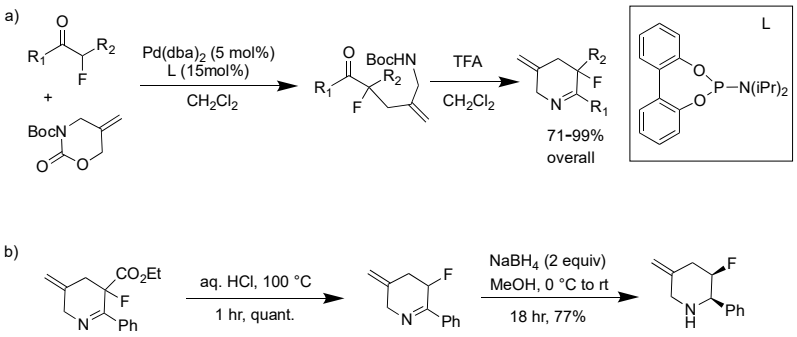



**Figure 6.** Common reagents for nucleophilic and electrophilic fluorination.

Nucleophilic fluorides are strongly solvated in protic solvents or form tight ion pairs in aprotic solvent, which can limit their use. The seminal work of the Middleton group on the first deoxyfluorinating reagent, (diethylamino)sulphur trifluoride (DAST) has prompted further development of other sulfur fluorides as efficient agents for mono- and difluorination of alcohols, ketones, aldehydes and carboxylic acids under mild conditions, with other similar reagents such as Deoxofluor®, Fluolead®, and XtalFluor-E®) as nonexplosive alternatives

Method development in transition-metal catalyzed ring annulation strategies has opened new ways of producing ring motifs with $sp^3C–F$ bonds. This serves as an alternative to deoxyfluorination in systems that may require extensive prefunctionalization. In particular, a recent report on 3-fluoro-piperidines involves a Pd-catalyzed [4+2] annulation approach to ring formation, using readily available α-fluoro-β-ketoesters and a cyclic carbamate (Scheme 1a).[33] This route led to facile preparation of 3-fluoropiperidines with orthogonal imine, ester, and alkene groups for further chemoselective and diastereoselective derivatization leading to ring C–F bond formation (Scheme 1b).




**Scheme 1**. a) Pd-catalyzed [4+2] annulation for ring cyclization from α-fluoro-β-ketoesters and a cyclic carbamate. b) Reduction for diastereoselective formation of a fluorinated piperidine.

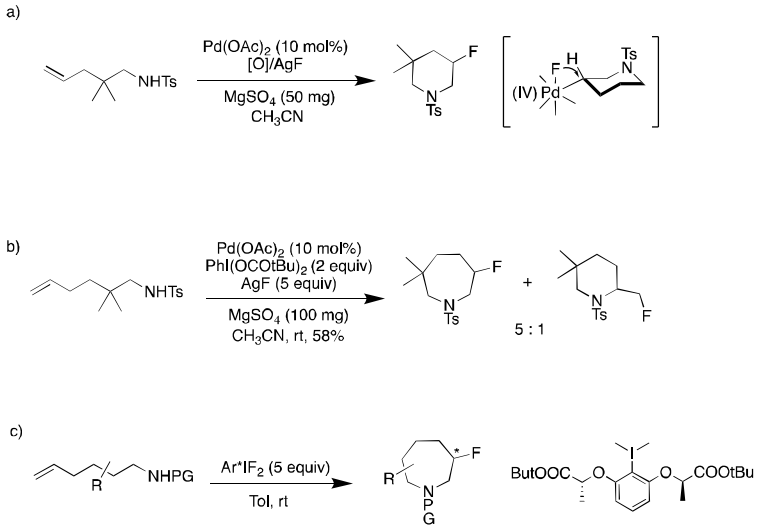
Other prevalent strategies based on Pd-catalyzed intramolecular aminofluorination of olefins require strong oxidants (Scheme 2a),[34] with the reaction likely proceeding through a fluoro-Pd(IV) intermediate in the reductive elimination step for C–F bond formation. The strongly oxidizing condition may be less tolerant of functional groups, in addition to regioselectivity issues for some substrates (Scheme 2b). Metal-free cyclization reactions for fluoro-azepane formation with good enantioselectivity (up to 77% *ee*) can be achieved by using chiral iodoarene difluorides (Scheme 2c), with the enantiopurity improved to 99% after crystalization.[35]

The use of I–F bond can be extended further from annulation or cyclization to C–C bond scission to provide fluorinated piperidines through a ring-expansion strategy.[36] A new fluoroiodane (III) reagent was prepared from silver difluoride and a chloro-hypervalent iodane precursor in excellent yield and at scale. This stable compound was used as a fluorine-transfer reagent after activation by a Lewis acid (Scheme 3a). The polarized I–F bond in turn activates the cyclopropane for C–C bond scission, followed by ring cyclization and elimination of an imide anion. The synthetic route was general and, when used with disubstituted cyclopropane, furnished fluoropiperidines with excellent diastereoselectivity (Scheme 3b). These novel ring formation strategies with concomitant fluoro-transfer steps will continue to emerge and produce novel fluorinated *N*-heterocycles with stereoselectivity and enantioselectivity, complementing the more established approach of C–F bond formation after prefunctionalization.

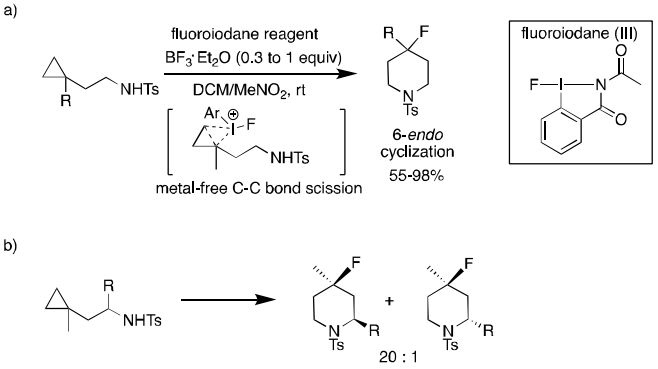
## 5. Conformational tuning by fluorine substitution on piperidines

Conformational analysis of six-membered ring systems has a remarkable history.[25] Compared to the smaller ring systems, six-membered rings exhibit more diverse conformational responses to substituent effects. As such, these rings serve as prototype model systems for understanding conformational tuning of

substituents such as fluorine. Comprehensive work on conformational properties of fluorinated cyclohexanes has produced semi-quantitative energetics of the fluorine gauche effect.[37]



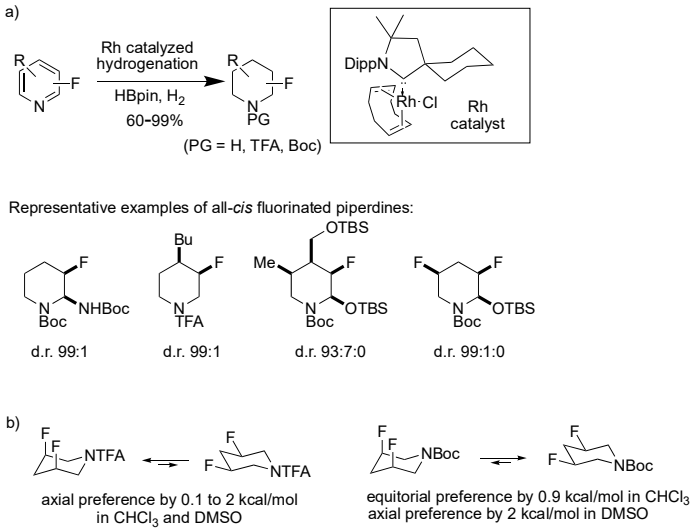

**Scheme 2**. a) Fluoro-Pd(IV) mediated ring cyclization. b) Fluoro-Pd(IV) mediated ring cyclization with competitive pathways to fluorinated azepane and piperidine ring formation. c) Metal-free oxidative ring cyclization to form fluorinated azepanes with enantioselectivity.




**Scheme 3**. a) A ring-expansion approach to fluorinated piperidine formation from substituted cyclopropanes. b) Diastereoselectivity in substituted fluoro-piperidine formation.

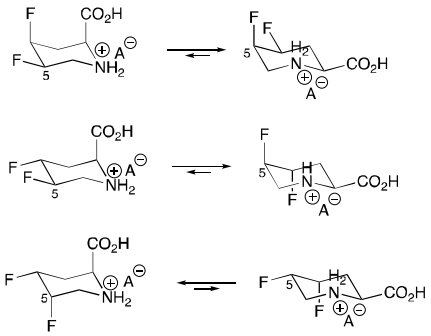
Conformational tuning of six-membered and saturated *N*-heterocycles present added complexity compared to their all-carbon counterparts, as demonstrated by work revealing the large axial fluorine effect independent of hydrogen-bonding.[38-40] Very recent work on conformational analysis of fluorinated piperidines has further refined the understanding of the axial-F preference, in part aided by more facile methods to access these compounds (Scheme 4).[41-44] With better access to fluorinated pyridines including metal-catalyzed fluorination of pyridines,[41] multi-substituted fluoro-pyridines can be directly hydrogenated using rhodium or palladium catalysts to furnish highly substituted fluoro-piperidine (Scheme 4a).[42,44] Conformational properties of these complex fluoro-piperidines depend not only on the overall substituent effect, but also on nitrogen protecting groups on the nitrogen and on the solvent (Scheme 4b).[43] The axial-F

preference can be reversed in the less polar solvent by changing the nitrogen-protecting group until the polarity of the solvent is high enough to prefer the more polar conformer.




**Scheme 4**. a) Rh-catalyzed hydrogenation of fluorinated pyridines for formation of fluorinated piperidines. b) Axial preferences of fluorinated piperidines.

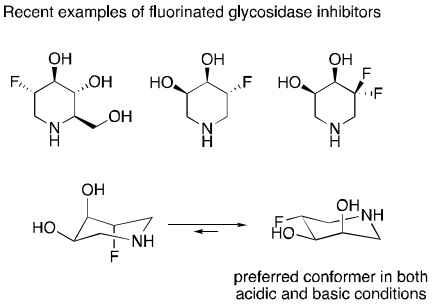
In the presence of highly polar substituents, the axial-fluorine effects are further complicated by neighbouring groups. In particular, difluorinated pipecolic acids, with a carboxylic acid substituent *ortho* to the basic nitrogen, provide unique conformational properties in this regard (Scheme 5).[45] As a prevalent motif in bioactive compounds, fluorinated pipecolic acids are valuable building blocks as they can be conformationally tuned to regulate binding. A combination of DFT studies and NMR analysis performed on a series of 4,5-difluoropipecolic acid suggests divergent conformational preferences. The all-*cis* difluoropipecolic acid (top row of Scheme 5) exhibits the highest conformational synergy to prefer the equatorial-acid conformer, while for the diastereomer in which both fluorine substituents are *trans* to the acid (bottom row of Scheme 5), the axial-acid conformer is preferred such that the C5 fluorine substituent is axial.



**Scheme 5**. Fluorine effects on conformation preferences of difluoropipecolic acids.

Fluorinated piperidine iminosugars present another important class of 6-membered and fluorinated *N*-heterocycles as bioactives mimicking traditional plant-based medicines based on piperidine triols.[46-48] The

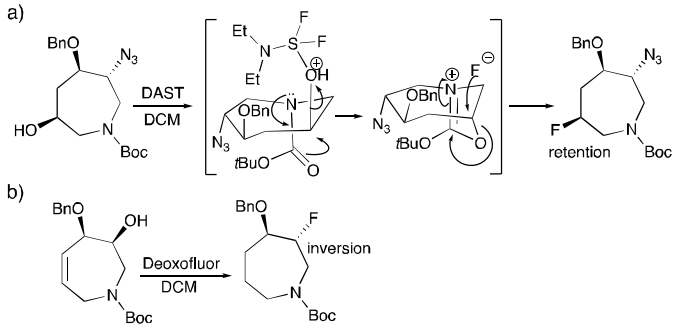
inclusion of fluorine is thought to improve liphophilicity, metabolic stability and bioavailability for enhanced immunomodulation. The conformational properties of these iminosugars as well as their bioactivity depend on the position of the fluorine substituent (Scheme 6). In the presence of multiple hydroxy groups, the equatorial or axial preference of the fluorine substituent is case-dependent and can be pH-independent.[47]




**Scheme 6**. Fluorine effects on conformation preferences of iminosugars.

## 6. Conformational tuning by fluorine substitution on azepanes

Seven-membered rings in general present more conformational freedom and therefore are significantly more difficult for conformational control. Conformational tuning of seven-membered *N*-heterocycles has received little attention until first examples of stereospecifically fluorinated azepanes were reported.[49] A practical approach of deoxyfluorination (Scheme 7) was investigated using a 1,2-*trans*-benzyloxyazido-disubstituted azepane framework, a motif frequently found in bioactive natural products. Monohydroxylation by hydroboration-oxidation was first performed to convert a ring-double bond into a secondary alcohol as a separable mixture of regioisomers, followed by stereospecific deoxyfluorination with readily accessible fluorinating agents such as DAST and Deoxofluor™. The retention of the secondary alcohol stereochemistry points to likely neighbouring group effect from the carbamate oxygen of the nitrogen-protecting Boc group given the seven-membered ring flexibility (Scheme 7a). The absence of this retention in an analogous deoxyfluorination (Scheme 7b) is supportive of the suggested role of the ring flexibility in affecting the selectivity of the fluorination. This again confirms the complexity associated with seven-membered ring systems not only in conformational properties but also in the stereochemical outcomes of their synthetic transformation.




**Scheme 7**. Diastereospecific fluorination of substituted azepanes by deoxyfluorination.

The conformational effects of the C6-monofluorinated azepane rings were investigated by $^{1}$H NMR spectroscopy and computational modelling.[50] The mutually *trans* benzyloxy and azido groups may prefer the diequatorial position in a twisted chair conformation, balanced with preference of the *gauche* effect of the azido group as well as the preference of the C–F bond to be *gauche* relative to the ring nitrogen. The

conformational complexity was difficult to predict, but a comparison of the C6-fluorinated diastereomers with the fluorine-less analogue (Figure 7) showed a clear trend of fluorine-based control of the conformational disorder. When the fluorine substitution gives the *R* configuration at the C6 position, the highly flexible ring settles into one major conformation, in which the fluorine *gauche* effect was clearly satisfied as well as that of the azido group, along with the C3/4 pseudo-diequatorial preference.

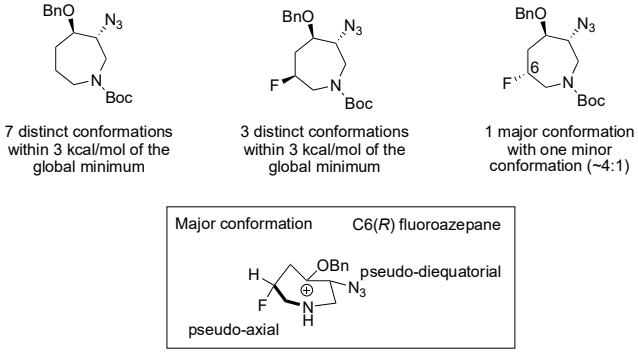
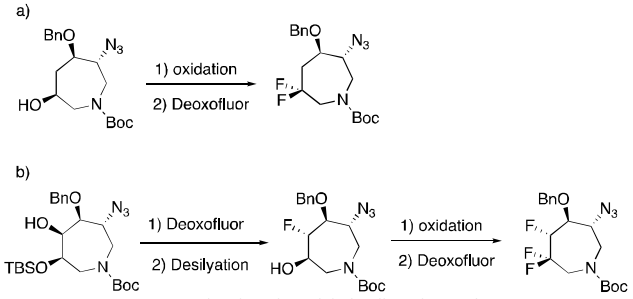



**Figure 7**. Complex conformational effects of fluorine on substituted azepanes.

As the azepane core becomes more substituted with additional polar substituents that are also highly influential on ring conformation, the fuller and more intricate conformational landscape was further explored by introducing additional fluorine and hydroxy groups (Scheme 8).[51] The preparation of the more substituted azepanes initiated with bis-hydroxylation, rather than monohydroxylation, and the deoxyfluorination protocol was effective as long as the appropriate protecting groups were in place. This produced geminally substituted difluoroazepanes as well as trifluoroazepanes. In general, fluorine was more effective than a hydroxy group in reducing conformational disorder. The geminally fluorinated or trifluorinated azepanes however did not provide stricter conformational control and in fact exhibited more complex conformational outcomes that were difficult to analyze by $^{1}$H NMR spectroscopy.




**Scheme 8**. Synthesis of multiply fluorinated azepanes.

The likely conflicting fluorine conformational control was investigated by X-ray analysis whereby the trifluorinated azepane was crystalized from dichloromethane.[52] The crystal formation was also highly problematic, producing extremely small crystals (0.015x0.01x0.01 mm) and requiring synchrotron radiation for diffraction. While weak with high residuals and a poor data-to-parameter ratio, the data was resolved and refined to provide a full structure. The asymmetric unit in fact contained two independent molecules corresponding to two different pucker-geometries in two conformers (Figure 8), both of which were part of the nine conformers found by computational analysis. The complexity arising from the solid-state analysis indirectly confirmed the conformational disorder from conflicting fluorine effects. The two conformers differed mainly in the puckering at C2 and C5 positions of ring that translated into significantly different

torsion angles around C3 and C4. Interesting, these two conformers form two C–H/F interactions in the dimeric unit, suggesting possible crystal-packing effects in selecting for these two conformers in particular. This X-ray structure not only validated the conformational analysis methodology but also was novel as the only crystal structure of a substituted azepanium salt at the time.

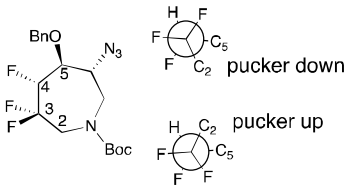
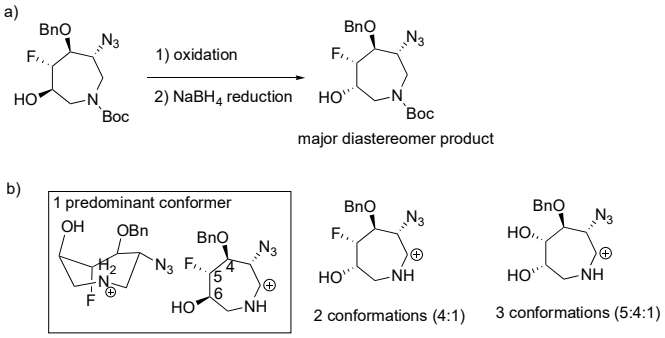



**Figure 8**. Conformational disorder revealed by X-ray analysis from conflicting fluorine effects driving different pucker geometries in two conformers in the same unit cell.

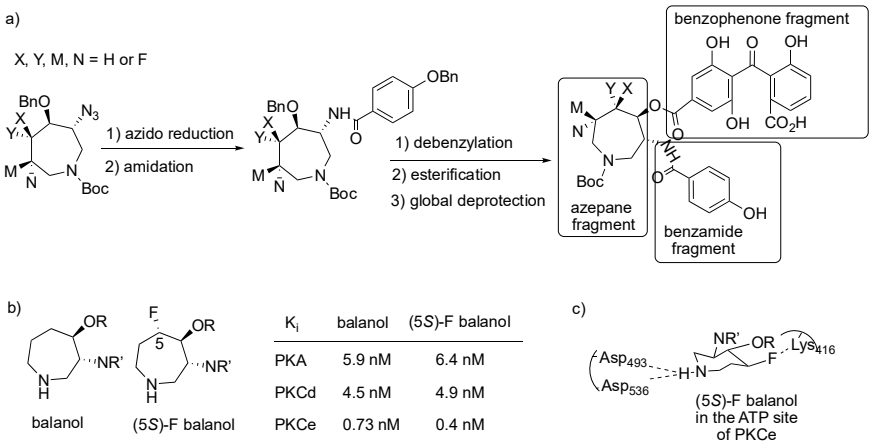
By replacing a fluorine with a hydroxy group in multiply fluorinated azepanes, enhanced conformational synergy leading to one predominant conformer was observed in some fluorohydrin azepanes. These fluorohydrins can be readily accessed from the same azepane precursors by red-ox based transformations followed by deoxyfluorination (Scheme 9a).[53] The fluorohydrin azepane with the C6 hydroxy group *trans* to the C5 fluorine showed clear conformational bias to one conformer, with fluorohydrin substituents on C5/6 in axial positions and the benzyloxy/azido groups in pseudo-diequatorial positions. In this azepane case, highest cooperativity in conformational control between the four substituents was permitted given their relative stereochemical configurations (Scheme 9b). Without the fluorohydrin in the *trans* configuration, a *cis* analogue showed more conformational diversity. The C5 fluorine appeared particularly effective in reducing conformational disorder as changing the C5 substituent from fluorine to a hydroxy group significantly altered the shape of the azepane to be more conformationally diverse. In this series, the C3/C4 pseudo-diequatorial preference and hydroxy gauche effect also tended to take priority over the azido gauche effect.




**Scheme 9**. a) Synthesis of highly substituted azepane fluorohydrins. b) Synergistic conformational tuning in azepane fluorohydrins.

To investigate how fluorine-based conformational tuning could provide new sources of physical/chemical perturbation and stereoelectronic effects for shape control in protein sites, some of the fluorinated azepanes were further converted into fluorinated natural products using the balanol framework (Scheme 10a).[54] Balanol is a potent antagonist ($IC_{50}$ 4-9 nM) for the ATP site of the AGC superfamily of protein kinases, in particular protein kinase C (PKC) isozymes, but with limited selectivity within the family. The PKC isozymes are highly homologous in the ATP site yet confer nonredundant and differentiated roles in regulating cell survival and motility. The dysregulation of the PKC isozyme activities is frequently implicated in cancer progression and metastasis.[55] However, the difficulty in isozyme-specific inhibition has

rendered the PKC isozymes one of the most elusive drug target classes.[56] As the azepane takes up the central position of the ATP-binding pocket, this balanol system is ideal for examining how shape-controlling fluorination impacts binding. Fluorination on the azepane ring of balanol should alter the binding of this high affinity ligand, and when coupled to structural modelling, may reveal new protein-ligand interactions conducive to binding and improved isozyme selectivity within the family. Indeed, one of the fluorinated balanol, with a single fluorine at C5 in the *S* configuration, did exhibit enhanced binding for only one isozyme PKCε, while its binding affinities for most other isozymes tested remained unchanged (Scheme 10b). In this fluorinated balanol analogue, the small F–O *gauche* preference was satisfied in the azepane ring, enabling H-bonding interactions from the protonated nitrogen to nearby aspartic residues while adding new H/F interactions to a nearby lysine (Scheme 10c). Furthermore, the fluorine substitution in this analogue did not perturb the binding interactions in the benzophenone and benzamide binding subsites, while for the other fluorinated balanol cases, the binding interactions in those subsites were adversely impacted in PKCε due to the cascade conformational change in the benzophenone and benzamide fragments due to fluorination. This also indicated the importance of considering the fluorine effect in other binding subsites further away. As the first demonstration of shape-controlling fluorination leading to additional selectivity in highly homologous kinase ATP sites, further structure-guided engineering or fragment-based lead design can be extended from this prototype to discover new selectivity-conferring protein-ligand interactions.




**Scheme 10**. a) Synthesis of fluorinated balanol analogues. b) Biological assays showing enhanced isozyme preference for PKCε from single fluorine substitution. c) Homology modelling showing new protein-ligand interactions conferred by stereospecific fluorine substitution.

To further understand remote fluorine effect on binding and level of congruency in local and remote fluorine effects, a more detailed computational analysis was performed on the acidity/basicity of functional groups on fluorinated balanol analogues. This further revealed the effect of the fluorine substituent on the nitrogen basicity depending on the fluorine position, whereby the C5(*S*)-F balanol analogue maintained the original basicity of the natural product the best, while the acidity of the phenols were relatively insensitive to the substitution.[57] The charged states of this balanol analogue thus resembled that of the natural product closest (Figure 9) while preserving the interactions with nearby acidic residues in the ATP-site.

Using homology modelling for PKA and PKCε, the global fluorine effect was investigated by molecular dynamics analysis in order to understand the differential binding of the C5(*S*)-F balanol to these highly homologous protein sites (Figure 10).[58,59] A structural homology model was built for PKCε using a known PKA/balanol binary X-ray structure (Figure 10a).[58] PKA in general showed much less sensitivity to ligand fluorination while PKCε was much more sensitive. As structurally equivalent residues in these two protein kinases, Thr184 in PKA and Ala549 in PKCε were found to have the "gating" effect driving the different binding responses (Figure 10b). The side chain of Thr184 is a key relay station for a

hydrogen-bond network between the oxygen of the amide linkage of the azepane and the ribose subsite of the protein, which secures the benzamide moiety in the adenine subsite and resists the fluorination effect in the azepane ring. The absence of this network in PKCε allows the fluorine effect to cascade significantly. Furthermore, the invariant and catalytic Lys, Lys73 in PKA and Lys437 in PKCε, is a major contributor to benzophenone binding in PKCε, including H-bonding, charge-charge, alkyl-π hydrophobic, and cation-π interactions. Azepane fluorination directed a phenol on the benzophenone to interact with Lys437 more extensively, providing additional charge-charge, alkyl-π hydrophobic, and cation-π interactions in PKCε. These protein-ligand interactions in PKA are much less extensive and weaker. Interestingly, the conformational dynamics of PKCε appeared unique amongst the PKC isozymes in its ability to lockdown its high level of flexibility in its bound form with the fluorinated balanol, showing conformational synergy with the ligand that is absent in other PKC isozymes.[59] This suggests that future design principles for improved inhibitor selectivity will need to take into account protein dynamics in the bound form for this class of enzymes.[60]

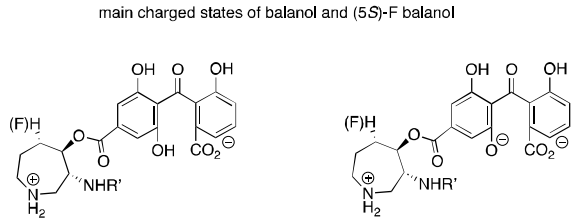



**Figure 9**. Charge states of balanol analogues in the ATP site of PKC isozymes.

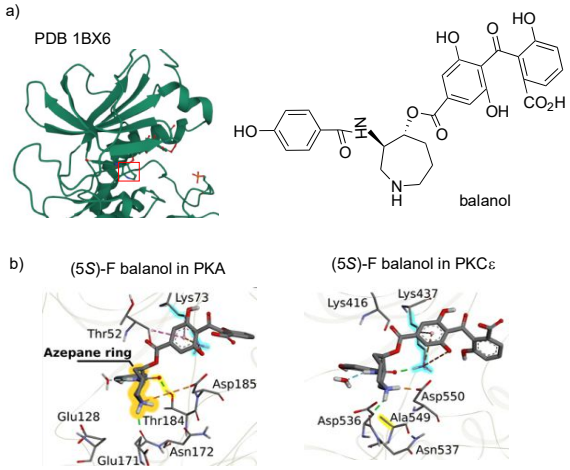



**Figure 10.** a) Binary X-ray structure of balanol in protein kinase A. b) Homology modelling based on the X-ray structure showing conformational synergy between the fluorinated balanol and PKCε that is absent in PKA.

## 7. Conclusion

*N*-Heterocyclic chemistry has continued to thrive as a major area of research due to the interesting physical and chemical properties of these molecules as well as their tremendous utility in pharmaceutical, agricultural, and materials industries. Fluorine, as a small but highly electronegative atom, has played a major role in the finding and developing of novel *N*-heterocyclic compounds with unique activities and applications. The emerging role of stereospecifically installed C–F bonds in conferring judiciously conformational control, particularly in protein-ligand interactions, has allowed new classes of *N*-heterocyclic

bioactives to become fruitful drivers in new fronts of modern drug discovery. In particular, using natural products as a source of bioactive motifs, novel fluorination chemistry has been developed to generate fluorinated fragments as building blocks for exploring conformationally regulated chemical space. By combining fluorine chemistry with fragment-based drug discovery platforms, new chemical diversity with conformational tuning will enable unconventional drug design strategies for addressing unprecedented challenges in finding more target-specific and mechanism-based therapeutics with clear mode-of-action to reduce side effects or combat drug resistance.

**Acknowledgements**

We thank Macquarie University for ongoing financial support as well as an iMQRES scholarship to B.S.

**References**

1. Petrov, V. A.: Fluorinate Heterocyclic Compounds: Synthesis, Chemistry, and Applications; John Wiley & Sons, INC., **2009**.
2. Gakh, A. A. a. K., Kennth L.: Fluorinated Heterocycles; American Chemical Society, **2009**.
3. Heravi, M. M.; Zadsirjan, V. *RSC Advances* **2020**, *10*, 44247-44311.
4. Kerru, N.; Gummidi, L.; Maddila, S.; Gangu, K. K.; Jonnalagadda, S. B. *Molecules* **2020**, 25.
5. Luzzio, F. A. *Adv. Heterocycl. Chem.* **2020**, *2020*, 1-84.
6. Leeson, P. D.; Springthorpe, B. *Nat. Rev. Drug Discov.* **2007**, *6*, 881-890.
7. Vitaku, E.; Smith, D. T.; Njardarson, J. T. *J. Med. Chem.* **2014**, *57*, 10257-10274.
8. Sedgwick, D. M.; Román, R.; Barrio, P.; Trabanco, A. A.; Fustero, S. In Fluorine in Life Sciences: Pharmaceuticals, Medicinal Diagnostics, and Agrochemicals **2019**, 575-606.
9 Zha, G. F.; Rakesh, K. P.; Manukumar, H. M.; Shantharam, C. S.; Long, S. *Eur. J. Med. Chem.* **2019**, *162*, 465-494.
10. Champagne, P. A.; Desroches, J.; Hamel, J. D.; Vandamme, M.; Paquin, J. F. *Chem. Rev.* **2015**, *115*, 9073-9174.
11. Kirk, K. L. *Org. Process Res. Dev.* **2008**, *12*, 305-321.
12. O'Hagan, D. *Chem. Soc. Rev.* **2008**, *37*, 308-319.
13. Hunter, L. *Beilstein J. Org. Chem.* **2010**, *6*, 38.
14. Hu, X. G.; Hunter, L. *Beilstein J. Org. Chem.* **2013**, *9*, 2696-2708.
15. Mondal, R.; Agbaria, M.; Nairoukh, Z. *Chemistry* **2021**, *27*, 7193-7213.
16. Lu, H.; Zhou, Q.; He, J.; Jiang, Z.; Peng, C.; Tong, R.; Shi, J. *Signal Transduct. Target Ther.* **2020**, *5*, 213.
17. Tautermann, C. S. *Meth. Mol. Biol.* **2020**, *2114*, 1-17.
18. Teague, S. J. *Nat. Rev. Drug. Discov.* **2003**, *2*, 527-541.
19. Agafonov, R. V.; Wilson, C.; Otten, R.; Buosi, V.; Kern, D. *Nat. Struct. Mol. Biol.* **2014**, *21*, 848-853.
20 Chatzigoulas, A.; Cournia, Z. *Wiley Interdiscip. Rev. Comput. Mol. Sci.* 2021, *11*, e1529.
21. Weisner, J.; Gontla, R.; van der Westhuizen, L.; Oeck, S.; Ketzer, J.; Janning, P.; Richters, A.; Muhlenberg, T.; Fang, Z.; Taher, A.; Jendrossek, V.; Pelly, S. C.; Bauer, S.; van Otterlo, W. A.; Rauh, D. *Angew. Chem. Int. Ed.* **2015**, *54*, 10313-10316.
22. Quambusch, L.; Landel, I.; Depta, L.; Weisner, J.; Uhlenbrock, N.; Muller, M. P.; Glanemann, F.; Althoff, K.; Siveke, J. T.; Rauh, D. *Angew. Chem. Int. Ed.* **2019**, *58*, 18823-18829.
23. Weisner, J.; Landel, I.; Reintjes, C.; Uhlenbrock, N.; Trajkovic-Arsic, M.; Dienstbier, N.; Hardick, J.; Ladigan, S.; Lindemann, M.; Smith, S.; Quambusch, L.; Scheinpflug, R.; Depta, L.; Gontla, R.; Unger, A.; Muller, H.; Baumann, M.; Schultz-Fademrecht, C.; Gunther, G.; Maghnouj, A.; Muller, M. P.; Pohl, M.; Teschendorf, C.; Wolters, H.; Viebahn, R.; Tannapfel, A.; Uhl, W.; Hengstler, J. G.; Hahn, S. A.; Siveke, J. T.; Rauh, D. *Cancer. Res.* **2019**, *79*, 2367-2378.
24. Uhlenbrock, N.; Smith, S.; Weisner, J.; Landel, I.; Lindemann, M.; Le, T. A.; Hardick, J.; Gontla, R.; Scheinpflug, R.; Czodrowski, P.; Janning, P.; Depta, L.; Quambusch, L.; Muller, M. P.; Engels, B.; Rauh, D. *Chem. Sci.* **2019**, *10*, 3573-3585.
25. Moore, A. F.; Newman, D. J.; Ranganathan, S.; Liu, F. *Austral. J. Chem.* **2018**, *71*, 917-930.

26. Zartler, E. R. *ACS Med. Chem. Lett.* **2014**, *5*, 952-953.
27. Over, B.; Wetzel, S.; Grutter, C.; Nakai, Y.; Renner, S.; Rauh, D.; Waldmann, H. *Nat. Chem.* **2013**, *5*, 21-28.
28. Cecchini, C.; Pannilunghi, S.; Tardy, S.; Scapozza, L. *Front. Chem.* **2021**, *9*, 672267.
29. Chamberlain, P. P.; D'Agostino, L. A.; Ellis, J. M.; Hansen, J. D.; Matyskiela, M. E.; McDonald, J. J.; Riggs, J. R.; Hamann, L. G. *ACS Med. Chem. Lett.* **2019**, *10*, 1592-1602.
30. Neumann, C. N.; Ritter, T. *Angew. Chem. Int. Ed.* **2015**, *54*, 3216-3221.
31. Tarantino, G.; Hammond, C. *Green Chem.* **2020**, *22*, 5195-5209.
32. Umemoto, T.; Yang, Y.; Hammond, G. B. *Beilstein J. Org. Chem.* **2021**, *17*, 1752-1813.
33. Garcia-Vazquez, V.; Hoteite, L.; Lakeland, C. P.; Watson, D. W.; Harrity, J. P. A. *Org. Lett.* **2021**, *23*, 2811-2815.
34. Wu, T. Y., G.; Liu, G. *J. Am. Chem. Soc.* **2009**, *131*, 16354-16355.
35. Kong, W.; Feige, P.; de Haro, T.; Nevado, C. *Angew. Chem. Int. Ed.* **2013**, *52*, 2469-2473.
36. Ren, J.; Du, F. H.; Jia, M. C.; Hu, Z. N.; Chen, Z.; Zhang, C. Angew. Chem. Int. Ed. **2021**, *60*, 24171-24178.
37. O'Hagan, D. *Chemistry* **2020**, *26*, 7981-7997.
38. Lankin, D. C. C., N. S.; Rao, S. N.; Spangler, D. P.; Snyder, J. P. *J. Am. Chem. Soc.* **1993**, *115*, 3356-3357.
39. Snyder, J. P. C., N. S.; Sato, H.; Lankin, D. C. *J. Am. Chem. Soc.* **2000**, *122*, 544-545.
40. Sun, A.; Lankin, D. C.; Hardcastle, K.; Snyder, J. P. *Chemistry* **2005**, *11*, 1579-1591.
41. Fier, P. S.; Hartwig, J. F. *Science* **2013**, *342*, 956-960.
42. Nairoukh, Z.; Wollenburg, M.; Schlepphorst, C.; Bergander, K.; Glorius, F. *Nat. Chem.* **2019**, *11*, 264-270.
43. Nairoukh, Z.; Strieth-Kalthoff, F.; Bergander, K.; Glorius, F. *Chemistry* **2020**, *26*, 6141-6146.
44. Wagener, T.; Heusler, A.; Nairoukh, Z.; Bergander, K.; Daniliuc, C. G.; Glorius, F. *ACS Catal.* **2020**, *10*, 12052-12057.
45. Chen, S.; Ruan, Y.; Lu, J. L.; Hunter, L.; Hu, X. G. *Org. Biomol. Chem.* **2020**, *18*, 8192-8198.
46. Le Guen, C.; Mena-Barragan, T.; Ortiz Mellet, C.; Gueyrard, D.; Pfund, E.; Lequeux, T. Org. Biomol. Chem. **2015**, *13*, 5983-5996.
47. Bhuma, N.; Burade, S. S.; Louat, T.; Herman, J.; Kawade, S.; Doshi, P. J.; Dhavale, D. D. *Tetrahedron* **2018**, *74*, 852-858.
48. Bilska-Markowska, M.; Szwajca, A.; Marciniak, B. *J. Fluor. Chem.* **2019**, *227*, 109364.
49. Patel, A. R.; Liu, F. *Tetrahedron* **2013**, *69*, 744-752.
50. Patel, A. R.; Ball, G.; Hunter, L.; Liu, F. *Org. Biomol. Chem.* **2013**, *11*, 3781-3785.
51. Patel, A. R.; Hunter, L.; Bhadbhade, M. M.; Liu, F. *Eur. J. Org. Chem.* **2014**, *2014*, 2584-2593.
52. Patel, A. R.; Bhadbhade, M. M.; Liu, F. *Acta Crystallogr. E: Crystallogr. Commun.* **2015**, *71*, 1361-1365.
53. Patel, A. R.; Liu, F. *Austral. J. Chem.* **2015**, *68*, 50-56.
54. Patel, A. R.; Hardianto, A.; Ranganathan, S.; Liu, F. *Org. Biomol. Chem.* **2017**, *15*, 1570-1574.
55. Parker, P. J.; Brown, S. J.; Calleja, V.; Chakravarty, P.; Cobbaut, M.; Linch, M.; Marshall, J. J. T.; Martini, S.; McDonald, N. Q.; Soliman, T.; Watson, L. *Nat. Rev. Cancer* **2021**, *21*, 51-63.
56. Mochly-Rosen, D.; Das, K.; Grimes, K. V. *Nat. Rev. Drug Discov.* **2012**, *11*, 937-957.
57. Hardianto, A.; Yusuf, M.; Liu, F.; Ranganathan, S. *BMC Bioinformatics* **2017**, *18 (Suppl. 16)*, 572.
58. Hardianto, A.; Liu, F.; Ranganathan, S. *J. Chem. Inf. Model* **2018**, *58*, 511-519.
59. Hardianto, A.; Khanna, V.; Liu, F.; Ranganathan, S. *BMC Bioinformatics* **2019**, *19 (Suppl. 13)*, 342.
60. Hardianto, A.; Yusuf, M.; Liu, F.; Ranganathan, S. Encyclopedia of Bioinformatics and Computational Biology **2019**, 273-282.