Non-Classical Phenyl Bioisosteres as Effective Replacements in a Series of Novel Open Source Antimalarials

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ABSTRACT: The replacement of one chemical motif with another that is broadly similar is a common method in medicinal chemistry to modulate the physical and biological properties of a molecule (i.e. bioisosterism). In recent years, bioisosteres such as cubane and bicyclo[1.1.1]pentane (BCP) have been used as highly effective phenyl mimics. Herein we show the successful incorporation of a range of phenyl bioisosteres during the open source optimization of an antimalarial series. Cubane (**19**) and *closo*-carborane (**23**) analogues exhibited improved *in vitro* potency against *Plasmodium falciparum* when compared to the parent phenyl compound, however these changes resulted in a reduction in metabolic stability; unusually, enzyme-mediated oxidation was found to take place on the cubane core. A BCP analogue (**22)** was found to be equipotent to its parent phenyl compound and showed significantly improved metabolic properties. While these results demonstrate the utility of these atypical bioisosteres when used in a medicinal chemistry program, the search to find a suitable bioisostere may well require the preparation of many candidates, in our case, thirty-two compounds.

INTRODUCTION

Phenyl rings are ubiquitous in medicinal chemistry, appearing in all manner of biologically-relevant molecules. Yet phenyl groups may not always be the optimal motif: they are relatively non-polar, they may be involved in π-π stacking interactions that can contribute to low aqueous solubility, or have limited bioavailability and they can be a metabolic liability.[1,2] Fortunately, there are several well-established strategies for addressing solubility issues.[3,4] Perhaps the most common strategy is to append charged or polar groups such as alcohol or amine moieties to the parent compound. Such modifications will typically reduce the hydrophobicity of the compound, but can often dramatically worsen potency. Similar effects may be seen through the use of heterocyclic ring replacements, but again these may alter the potency of the desired compound.[5] Alternatively, modifications can be made to alter the crystal packing of a compound by either removing aromaticity *via* removing the phenyl rings or changing the molecular geometry or topology. For example, replacement of a phenyl ring by an alkyl group (linear, cyclic or caged) may help improve solubility by eliminating π-π stacking interactions, and have the added advantage of introducing further options for chemical derivatization that may not be accessible with a phenyl ring. Again, however, there is the caveat that these changes can heavily influence the binding interactions between a drug and its ultimate biological target.

Our recent experiences in dealing with such issues are related to an antimalarial medicinal chemistry program. Malaria is one of the most prevalent infectious diseases affecting low income countries, with 219 million cases reported worldwide in 2017, 2 million more cases than the previous year. This translates to around 1200 deaths per day, mostly involving young children.[6] With an increasing number of reports of resistance to current treatment and prevention strategies, new medicines must be discovered to combat the disease.[7,8] To help address this, the Open Source Malaria (OSM) consortium was created, with the aim of discovering new antimalarial medicines using an inclusive community operating on open science principles, where all data and experiments are shared in real time (for example every experiment involved in the current study is freely available to view online).[9] The most recent, fourth series examined by the consortium, the so-called Series 4 triazolopyrazine antimalarials, originated from a high-throughput screen performed at Pfizer in collaboration with the Medicines for Malaria Venture (MMV). The series was then passed to TCG Lifesciences for further optimization before being donated to the OSM consortium in 2013.[10] The series has produced many compounds with potent (<100 nM) activity against *Plasmodium falciparum*, promising physicochemical properties and low toxicity (Figure 1). Two compounds from the series were shown to have high potency in an *in vivo* mouse model (full results to be reported elsewhere).[11] While the potency of compounds in the series has been frequently high, issues still remain, particularly with regards to poor solubility and moderate clearance rates. As such, modification of the phenyl groups present in the structure was seen as an attractive strategy for mitigating these issues. Careful consideration of such modifications

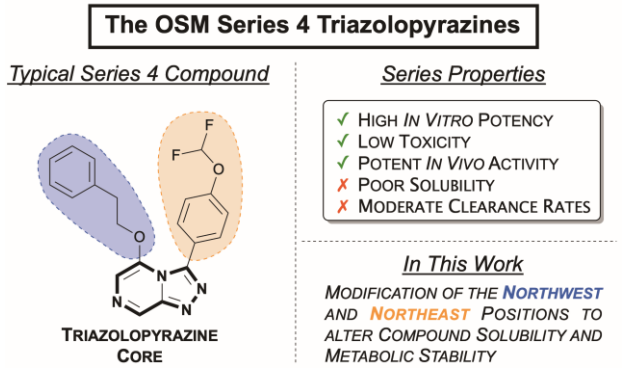


Figure 1. The Series 4 triazolopyrazines possess promising biological properties however further improvements to solubility and metabolic stability are required.

was required as the series is in the lead optimization stage of development and displays high sensitivity to functional group changes.

The approach adopted was based on bioisosteres, a concept first described by Harris Friedman in 1950 as "compounds or groups that possess near-equal molecular shapes and volumes, approximately the same distribution of electrons, and which exhibit similar physical properties".[12] The use of a bioisostere can be an extremely useful strategy in medicinal chemistry programs for altering the physical and biological properties of a compound.[13] Classical bioisosteres focus on the use of structurally simple atoms, groups and ring equivalents (e.g. replacement of a phenyl ring with a pyridine ring), while non-classical bioisosteres may differ quite dramatically from the original group.[14,15,16,17]

One of the more interesting non-classical phenyl bioisosteres is cubane, a motif that has been used in medicinal chemistry projects spanning a range of applications.[18,19,20] The size and shape of cubane mimics the rotational volume and shape of a phenyl ring.[21] Several biologically active molecules have been evaluated for their ability to tolerate a cubane as a replacement for an aromatic ring, examining changes in biological activity, solubility, metabolism, stability and synthetic tractability (A, Figure 2).[21] The five compounds evaluated included those with chemotherapeutic (Vorinostat), anesthetic (Benzocaine) and neotropic (Leteprinim) applications. It was found that four of the five showed equivalent or improved potency when the phenyl ring was replaced with a cubane. In these four cases, the slight increase in log*P* values with the cubane replacements (largest difference of log*P* ~0.5) did not have a significant impact on compound solubility. However, in the one case that led to decreased potency, a much larger increase in log*P* (~1.4) was seen for the cubane analogues that translated to a large reduction in solubility. A more recent evaluation of a further five pharmaceuticals revealed a mixed effect of the cubane replacement on both potency and solubility.[22]

In a similar manner to cubane, the bicyclo[1.1.1]pentane (BCP) motif has recently found use as a non-classical phenyl ring bioisostere.[23] While the size of the BCP motif does not match that of benzene as closely as cubane (*vide infra*), its use as an effective phenyl bioisostere has been demonstrated in a number of cases. For example, the phenyl ring in a γ-secretase inhibitor currently in development was replaced with the BCP motif resulting in a compound with not only equipotent enzyme inhibition, but also improved passive permeability, aqueous solubility and oral absorption characteristics.[24] In another example, a BCP replacement in a $LpPLA_2$ inhibitor resulted in maintenance of potency while improving the compound's physicochemical properties (B, Figure 2).[25] Increasing work has been done in recent years with the synthesis of BCP building blocks allowing for more possibilities for its use as a phenyl bioisostere.[26,27]

Perhaps less immediately obvious to medicinal chemists is the use of the boron-rich icosahedral clusters known as carboranes and their potential application as phenyl and adamantyl bioisosteres.[28] One prominent example is the *closo*-carboranyl derivative (closed form with all carbon and boron vertices) of tamoxifen.[29] The resulting carboranyl compounds showed high activity as anti-estrogen agents and were even found to be more stable to degradation than tamoxifen (C, Figure 2). Another example is that of asborin, a *closo*-1,2-carborane derivative of aspirin.[30] Interestingly in this case the pharmacological profile of the resulting carboranyl derivative was changed drastically. Instead of acting as a selective cyclooxygenase enzyme inhibitor (like aspirin), asborin was instead found to be a potent inhibitor to the unrelated aldo/keto 1A1 reductase family.[31] Other examples of carboranyl bioisosteres of polycycles such as adamantanes can be found in medicinal chemistry, including their *in vivo* application as CNS-modifying agents.[32]

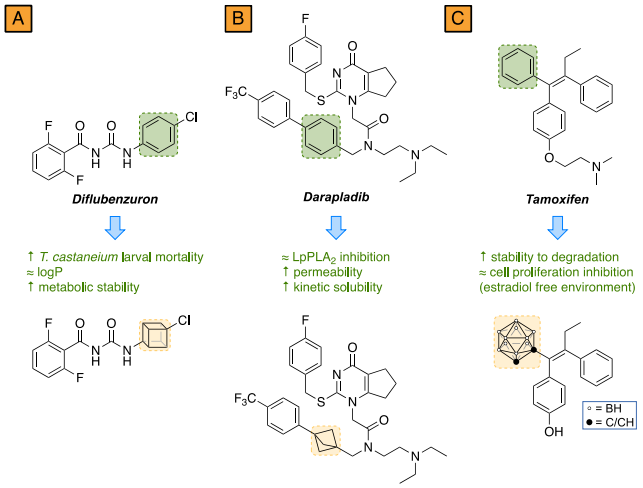


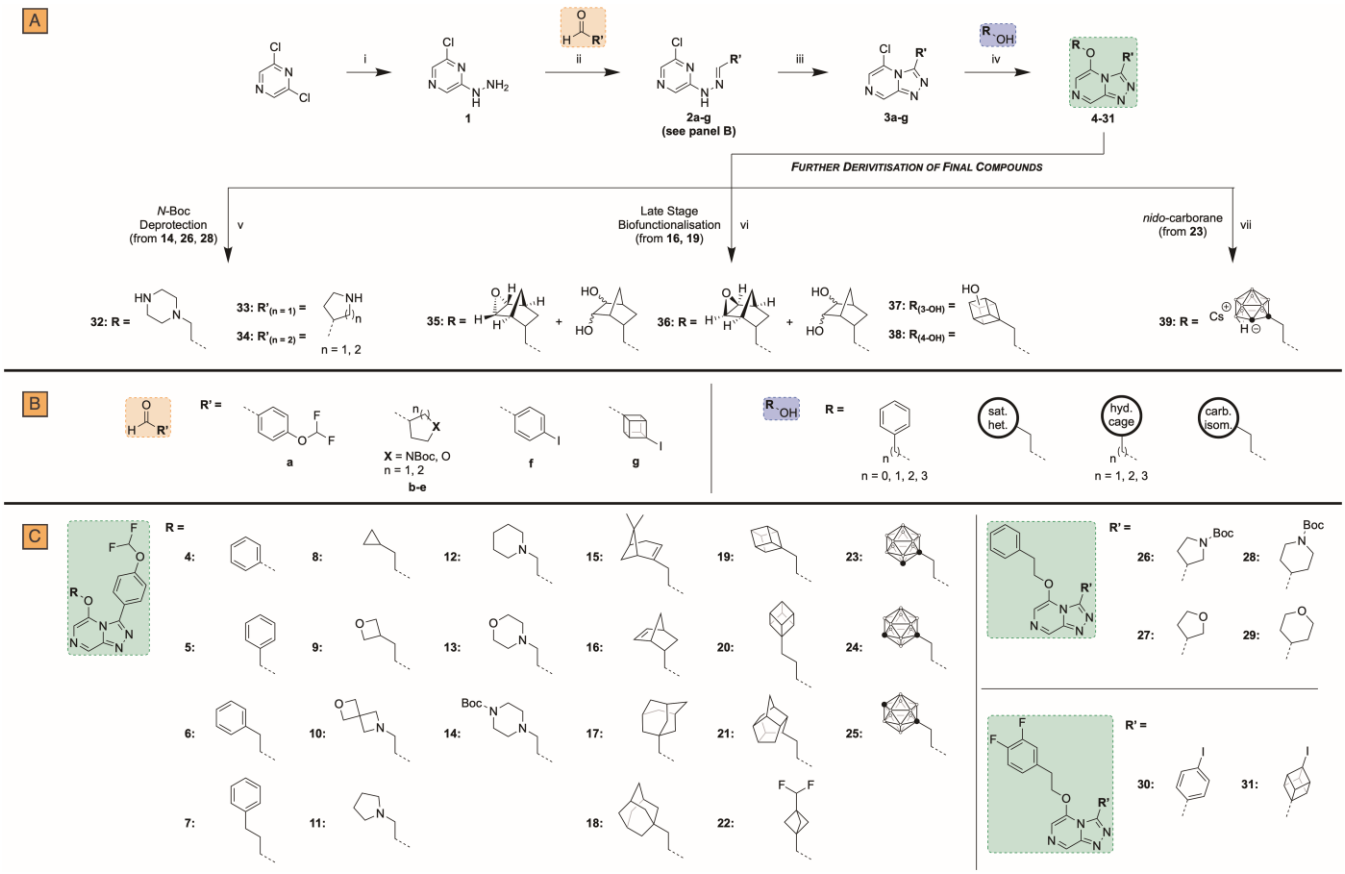
Figure 2. Examples of the use of phenyl bioisosteres. (A) Cubane in the insecticide, diflubenzuron. (B) BCP in the $LpPLA_2$ inhibitor, darapladib. (C) *closo*-1,2-carborane in the anti-estrogen agent, tamoxifen.

While it is not unusual for a medicinal chemistry campaign to explore select phenyl bioisosteres (typically cubane and BCP),[33,34] it is much less common to see the wider range of possible bioisosteres used in a single lead optimization program. In the case of the OSM Series 4 triazolopyrazines, the changes required to achieve our goal (improve the aqueous solubility and metabolic clearance parameters, but not significantly change the potency) were not immediately clear, in part because the project was a phenotypic drug discovery program with no confirmed biological target. While the calculation of cLog*P* values may give an indication of the potential aqueous solubility of a compound, these values can only be used as a general guideline and do not necessarily provide a good indication of bioavailability (note that in this study cLogP was prioritised over other possibly relevant parameters, such as molecular weight or total polar surface area; these values may be found in the Supporting Information, though they appear to show no correlation with experimental potency). This is particularly notable for some of the phenyl bioisosteres that are mentioned above, where the calculated log*P* values can be inconsistent across different software platforms,[35] particularly where the carborane motif is involved; in such cases it may be necessary to measure solubility experimentally.[22] Accordingly, we investigated a wide range of possible phenyl bioisosteres for Series 4, without relying heavily on the calculated cLog*P* values as a guide.

RESULTS AND DISCUSSION

Chemistry. The synthesis of the triazolopyrazine core was achieved in three steps from the commercially available 2,6-dichloropyrazine (Scheme 1). Initial displacement of a chlorine atom with hydrazine hydrate gave 2-chloro-6-hydrazinylpyrazine (**1**). Condensation with the appropriate aldehyde afforded the hydrazone intermediate (**2a**–**g**), which was subsequently cyclised to give the chlorinated triazolopyrazine core (**3a**–**g**). Final nucleophilic displacement

Scheme 1. Preparation of Series 4 target compounds[a]



[a]Synthetic route to target compounds and subsequent derivatisation where applicable. Reaction conditions: i) N_2H_4•H_2O, EtOH, 80 °C, overnight; ii) aldehyde, EtOH, rt, overnight; iii) $PhI(OAc)_2$, CH_2Cl_2, rt, overnight; iv) alcohol, 18-crown-6, PhMe, rt, overnight; v) TFA, CH_2Cl_2, rt, overnight; vi) see SI; vii) CsF, EtOH, 80 °C, 25 h. (B) Aldehyde and alcohol building blocks were either commercially available or synthesized; sat. het. = saturated heterocycle; hyd. cage = hydrocarbon cage; carb. isom. = *closo*-carborane isomer. (C) Target compounds.

of the chlorine atom with the appropriate alcohol produced the desired target compounds **4**–**31**. A number of additional transformations were performed on select target compounds. Deprotection of *N*-Boc compounds **14**, **26** and **28** was carried out using standard TFA conditions to give the corresponding free amine compounds **32**, **33** and **34**, respectively. Compounds **16** and **19** were used to generate late-stage biofunctionalized derivatives using dog or rabbit liver microsomes (see Experimental). In the case of the norbornene compound **16**, oxidation of the norbornene double bond led to a mixture of epoxides and *E* and/or *Z* diol compounds (**35** and **36**). In the case of the cubane compound **19**, two metabolites were isolated and were found to be hydroxylated on the cubane framework (**37** and **38**). The reaction of compound **23** with CsF in EtOH gave the hydrophilic *nido*-7,8-carboranyl derivative, as its cesium salt 39.

***In vitro* Activity.** All compounds were evaluated for *in vitro* antiplasmodial activity against the 3D7 strain of *P. falciparum*. The activities, indicated by their IC_{50} values, are summarized in the SI and shown graphically in Figure 3. While the inherited dataset for OSM Series 4 suggested an ether chain length of two methylene groups between the heterocyclic core and the northwest pendant phenyl ring was optimal, the specific compounds to prove this relationship were absent from the dataset. In order to validate this hypothesis, a set of compounds with an ether methylene chain length from zero to three were evaluated, clearly showing that this separation of the phenyl from the core was indeed important in order to maintain potency. Specifically, any chain length other than two carbon atoms (**6**) resulted in reduced potency with complete inactivity seen with a phenol side chain (**4**).

Surprisingly, when the saturated heterocyclic derivatives (**9**–**14**, **26**–**29** and **32**–**34**) were evaluated, it was found that they were all inactive with complete loss in potency compared to the parent phenyl compound. This was seen in both the northwest and northeast cases. The increased flexibility of these saturated heterocycles is presumably contributing to this loss of activity.

Encouragingly, the comparable two- and three-methylene linker cubane analogues **19** and **20** were only slightly less potent than the phenyl compounds but also followed the trend of decreased potency with longer chain length. The recovery of activity compared to the saturated heterocyclic derivatives provides strong evidence of the ability of cubane to mimic the rotational volume of a phenyl ring. It was also verified that the mechanism of action had not changed following these modifications: the potent bioisosteric compounds showed activity in an ion regulation assay which is linked to the malarial target *Pf*ATP4.[36,37]

However, on the contrary the northeast cubane derivative **31** was found to be completely inactive whereas the analogous phenyl compound **30** remained potent. This surprising result suggests that the ability of cubane to mimic a phenyl ring is reduced if the phenyl ring possesses restricted rotation (as is assumed to be the case here), in which case the volume occupied by the phenyl ring is not well mimicked by the bulk of the cubane. Alternatively, the change may have removed a key π-stacking interaction. Considering that all northeast phenyl replacements resulted in completely inactive compounds, the remaining investigation was focused on the further exploration of the northwest pendant phenyl ring.

The BCP derivative **22** was relatively well tolerated when compared to the analogous benzylic ether compound **3** with only a slight

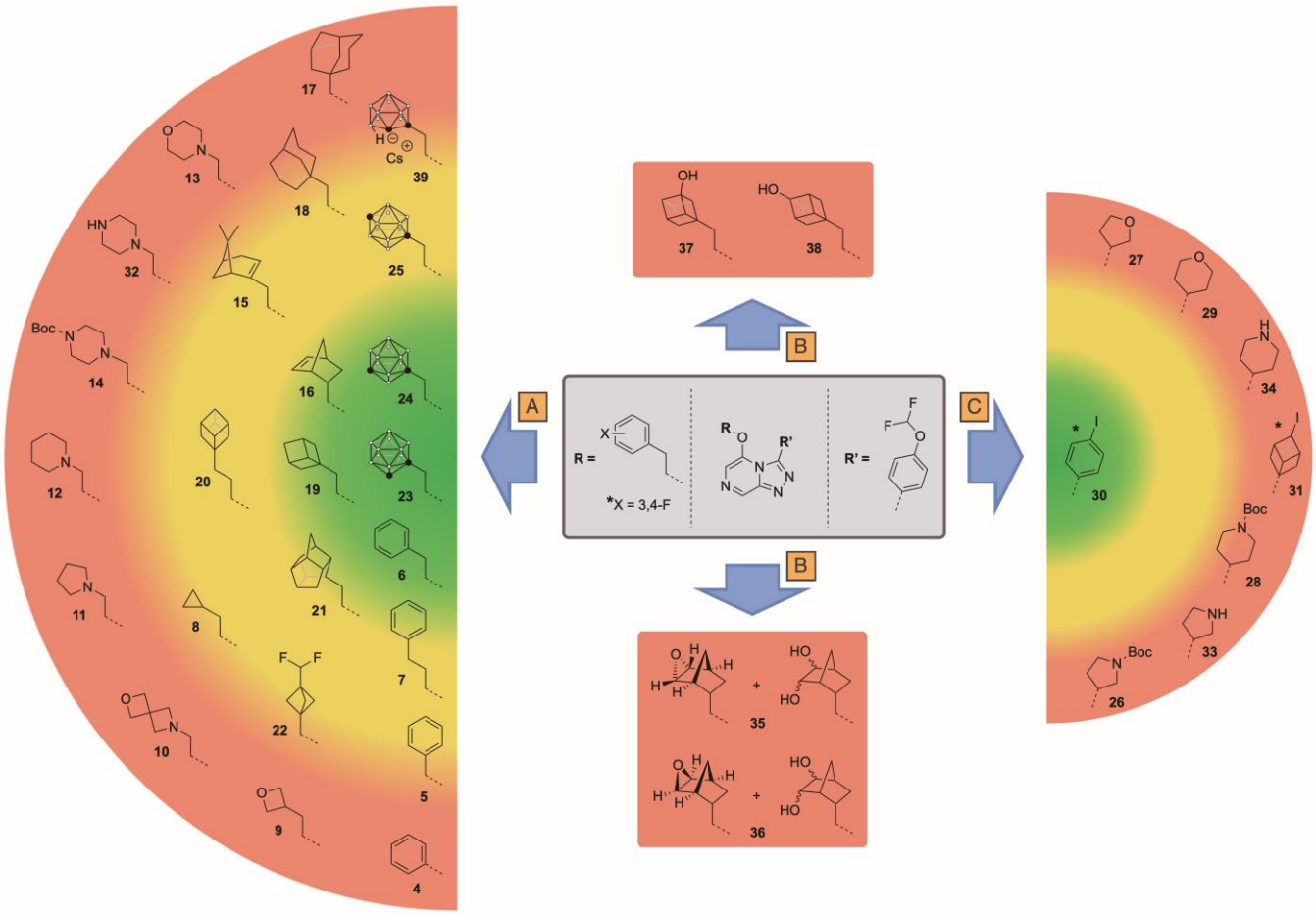


Figure 3. Heat map of *in vitro* potency. Color gradient: green = <1 μM; orange = 1–2.5 μM; red = >2.5 μM. (A) Variations in potency with northwest phenyl replacements. (B) Late-stage biofunctionalization compounds evaluated at lower concentrations than the other compounds. (C) Variations in potency with northeast phenyl replacements.

decrease in activity. This loss in potency may be due to the smaller size of the BCP motif, however it is noted that many examples of the use of BCP as a phenyl bioisostere tend to result in more significant changes to the metabolic and pharmacokinetic properties instead, as will be discussed below.

The larger adamantyl derivatives were less well tolerated with the one- and two-carbon chain compounds (**17** and **18**, respectively) showing potencies approximately 2 to 9 times lower than the corresponding phenyl compounds (**5** and **6**, respectively). This result may be attributed to the larger volume of adamantane (*vide infra*) compared to either a cubane or a rotating phenyl ring.[38] Other hydrocarbon-caged derivatives including nopol, norbornene and trishomocubane (**15**, **16** and **21**, respectively) were better tolerated, however these were still found to be less potent than the parent phenyl compounds.

As a final investigation point, the *closo*-1,2-, 1,7- and 1,12-carborane isomers were installed in place of the northwest phenyl position. Unexpectedly, the *closo*-1,2- and 1,7-carborane isomers (**23** and **24**) showed potencies greater than not only all the other previous derivatives but, more importantly, the parent phenyl compound. A significant drop in activity was seen between the *closo*-1,7- and 1,12-carborane isomers (**24** and **25**). The potency was observed to decrease from *closo*-1,2- < 1,7- < 1,12-carborane. This trend is likely related to the differences in polarity (and hydrophobicity) of the three carborane isomers.[39] The more hydrophobic and less polar the *closo*-carborane isomer is (as pertaining to the relative positions of the two carbon atoms in each carborane cage and the resulting dipole moments), the less potent the final compound was found to be.

In a similar manner to *closo*-1,2-carborane, the hydrophilic *nido*-7,8-carborane cage (open form with one boron vertex removed) has also shown potential as a phenyl bioisostere both in a carborane variant of tamoxifen[40] and trimethoprim,[41] for example. In the latter case, the *nido* form was found to be less toxic than the *closo* form, however it also performed less usefully as a boron neutron capture therapy (BNCT) agent, with poorer tumor retention and lower selectivity ratios for boron distribution in tumor versus normal tissues. In our case, the *nido*-7,8-carborane **39** was also found to be less active, with its potency decreasing significantly when compared to the *closo*-1,2-carborane **23**. Selective removal of a single BH group from the 3 (or 6) position of the *closo*-carborane cage affords a hydrophilic, anionic species which would likely show diminished binding to a hydrophobic receptor pocket.

With these encouraging results, select compounds were further evaluated for a number of other properties including toxicity, solubility and metabolic stability.

Toxicity. Having synthesized a number of highly potent compounds that possessed unusual motifs, it was important to check for any associated toxicity. While a number of examples in the literature show no significant increases in toxicity with cubane bioisosteres,[42,43] carborane associated toxicity has been far less studied. Encouragingly, when *closo*- and *nido*-carborane compounds **23** and **39** were evaluated for potential cytotoxicity in HepG2 cells, both compounds were found to be inactive, with IC_{50} values of >10 μM. Compounds **6**, **19** and **23** were additionally evaluated for their hERG activities with the phenyl compound **6** showing moderate activity (IC_{50} = 7.41 μM) and the cubane and carborane analogues showing slightly greater potencies (IC_{50} = 4.26 μM and 3.62 μM, respectively). While these values are not entirely desirable, further hERG optimiza-

tion in order to lower inhibition may be achieved through further derivatization.

Metabolic and Physicochemical Properties. The reported effects on metabolic and physicochemical properties of phenyl bioisosteric replacement by cubane have been mixed. While a number of cases have reported improvements in solubility following cubane substitutions,[34] the trend for metabolic stability is less consistent. There have been reports of both increased[21] and decreased[33] metabolic stability as a result of cubane substitution. In the present case, when compounds **6**, **19** and **23** were evaluated for their metabolic and physicochemical properties, they were found to perform poorly compared to the parent phenyl compound (Table 1). The solubility at pH 6.5 was low for **19** and **23** (<1.6 μg/mL) when compared to **6** (6.3 – 12.5 μg/mL). Both **19** and **23** also exhibited high clearance and short half-lives in human liver microsomes (HLM) and mouse liver microsomes (MLM). In rat cryopreserved hepatocytes (RCH), **19** and **23** showed intermediate and low degradation rates with low clearance rates and short half-lives. It is likely that clearance of the parent compound occurs *via* benzylic oxidation,[44] and that the removal of this phenyl ring should slow down the rate of clearance due to the absence of the benzylic position. Our results suggest that the incorporation of cubane in place of phenyl has led to the cubane becoming a metabolic liability. This appears to be in contrast to the notion that cubane derivatives are more metabolically stable to hydroxylation than the corresponding phenyl analogues (increased *s*-character from the strong and hindered tertiary C–H bonds).[45] In our case (*vide infra*), it appears that the metabolic 'hotspot' has shifted onto the cubane itself. Encouragingly, the BCP derivative **22** was found to possess significantly improved metabolic and physicochemical properties compared to the parent phenyl compound. While the solubilities were within the same range, **22** exhibited lower clearance and shorter half-life values across human, mouse and rat liver microsomes.

Table 1. Metabolic and physicochemical properties of select Series 4 phenyl bioisosteres compounds. The color coding of the values indicates the performance of the compound (green being more desirable).

	cLogP [a]	LogD (pH 7.4)	Solubility (pH 6.5)	HLM (CL_{int}/$T_{1/2}$)	MLM (CL_{int}/$T_{1/2}$)
6	3.2	3.7	6.3 – 12.5	66/26	262/7
19	2.5	4.3	<1.6	197/9	573/3
22	2.4	3.6	6.3 – 12.5	9/190	26/66
23	1.7[b]	4.4	<1.6	249/7	>866/<2

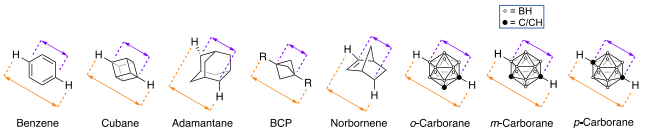
[a]cLogP values calculated using DataWarrior. [b]Carboranes are not currently handled well by prediction software: when this value is calculated with ChemDraw Professional the value is 4.0.

Late-Stage Biofunctionalization. Further light is shed on these results by the isolation of the four metabolites (**35**–**38**), which were found to be inactive. Similar metabolism of phenyl-substituted compounds leads to oxidation at the benzylic position, as expected, (these will be reported separately for this series) whereas for cubane the hydroxylation occurred on the hydrocarbon cage instead, i.e. we observe preferential hydroxylation of the benzylic position in the phenyl case but core hydroxylation for cubane. This is unexpected. Experiments on enzymatic oxidation of methylcubane have indicated that hydroxylation on the methyl group is favored over hydroxylation on the cubane framework with only small amounts of the latter products being identified.[46,47] If the cubane does become hydroxylated, it is thought that such products are unstable and lead to rearrangement to a ring-opened ketene product.[48] This possibly explains why there are a small number of reports of hydroxycubanes in the literature.[49,50] The present isolation of hydroxycubane derivatives is therefore unusual.

Comparison of Phenyl Bioisosteres. In literature reports where phenyl bioisosteres have been used, a size comparison between the bioisostere and the phenyl ring is often given. There is some variation between these values. For example, in one report the diagonal C–C atom distances for benzene and cubane have been quoted as the same (2.70 Å)[51], while another report has quoted them as different (2.82 Å and 2.72 Å for benzene and cubane, respectively).[34] Similar disagreements have been seen for BCP comparisons as well (*vide infra*). For meaningful comparisons, a unified approach was preferable. We have therefore examined deposited crystal structure files from The Cambridge Crystallographic Data Centre (CCDC), and calculated the dimensions of key phenyl bioisosteres with UCSF Chimera, allowing for a direct comparison (Table 2).

While the literature atom distances are, for the most part, consistent with those calculated with UCSF Chimera, there is a notable increase of about 10–15% between the calculated and

Table 2. Structural information of select phenyl bioisosteres calculated using UCSF Chimera. Where they exist, literature values are shown in parentheses. C–C and H–H distances are indicated in purple and orange respectively.



Structure (CCDC Identifier)	C–C Distance (Å)	H–H Distance (Å)	Surface Area (Å^2)[a]	Volume (Å^3)[a]
Benzene (ABELUO)	2.77 (2.70[51]; 2.79[21]; 2.82[34])	4.63 (5.90[52])	89.7	70.1 (79.0[38])
Cubane (CUBANE)	2.68 (2.70[51]; 2.72[21,34])	4.70 (4.88[53])	103	87.1
Adamantane (ADAMAN08)	3.53	4.92 (6.36[54]; 6.40[52])	126 (134[52])	119 (128[52]; 136[38])
BCP[b] (HEHRUJ)	1.88 (1.70[24]; 1.85[34]; 1.90[51])	4.78[c]	80.2[d]	61.4[d]
Norbornene (HOBBOP)	2.89	4.64	101	84.8
***closo*-1,2-Carborane** (TOKGIJ)	3.23[e]	5.26	135	127 (148[38])
***closo*-1,7-Carborane** (TOKGOP)	3.20[e]	5.12	134	126 (143[38])
***closo*-1,12-Carborane** (TOKGUV)	3.06	4.85 (5.35[55])	136	127 (141[38])

[a]Surface areas and volumes were calculated using a model of the solvent-excluded molecular surface with hydrogen atoms included. [b]R = terminal alkyne. [c]Alkyne C–C distance. [d]Surface area and volume calculated without alkyne substituents. [e]Carborane C–B distance.

literature values for surface area and volume. It remains largely unclear as to which methods provide calculated values closest to those measured from X-ray structures; accurate calculations would be useful for calculating the dimensions of novel bioisostere motifs. Most literature sources only provide a small selection of calculations and

specifically for volume calculations none take into account the free rotation of the bioisostere. However, as these comparisons are often made to show the relative dimensions, calculations performed using the same method or program would be the most reliable, such as in this case with UCSF Chimera. Nevertheless, it is interesting to note how different the dimensions can be for phenyl and an effective bioisostere. For example, the *closo*-carborane core was found experimentally to be an excellent mimic, yet its volume is significantly larger than that of a phenyl ring, meaning the functional significance of bioisosteric size comparisons must be treated with care.

CONCLUSION

While phenyl rings can be found in all manner of biologically-important molecules, they may not always be the ideal motif for drug-like molecules. By using classical and non-classical bioisostere replacements, the biological properties of a compound may be altered in dramatic ways. These changes may not always result in the desired improvements and accurate predictions can be challenging. In phenotypic projects like this one, which are now so common in early stage drug discovery, rational modifications, such as the substitution of a rotatable phenyl group with a cubane, may not lead to improvement in the overall properties of the molecule given how many other potential interactions may be involved before the molecule reaches its intended target. As seen in this work, equivalent substitutions for different phenyl rings in the same molecule can lead to widely different potencies. In any given case, it may well be necessary to explore a considerable range of possible bioisosteres before arriving at an effective solution. In our case, we have identified a series of novel *closo*-carborane containing compounds that possessed potencies greater than that of the parent phenyl compound. To get to this point required the synthesis of 30 variations of the initial hit. More rational substitutions may be possible in cases where the structures of the biological targets are known, particularly through consideration of the volumes occupied by the various isosteres.

EXPERIMENTAL SECTION

General Information. Reagents were purchased from either Sigma–Aldrich, Alfa Aesar, Acros, Merck, Fischer Scientific, Matrix Scientific, Ajax or Fluorochem. Unless otherwise specified, the reagents were used without further purification. Anhydrous conditions: glassware was dried at >130 ºC for >12 h, assembled hot and allowed to cool under a high vacuum where appropriate or purged with inert gas. Anhydrous solvents were obtained from the PureSolv system or by drying over activated 3 Å molecular sieves. Nitrogen gas was dried over silica and calcium chloride. Argon gas was used as acquired. The phrase *in vacuo* corresponds to ~1 mbar on a Schlenk line. Reduced pressure means under rotary evaporation at 40 ºC from 900–50 mbar. Flash chromatography was performed on Davisil Grace Davison 40–63 µm (230–400 mesh) silica gel or on a Biotage Isolera One. Analytical thin layer chromatography was performed on Merck Silica Gel 60 F_{254} precoated aluminium plates (0.2 mm) and visualised with UV irradiation (254 nm) and potassium permanganate, anisaldehyde or ninhydrin staining. High temperature reactions were carried out in silicone oil baths, controlled by temperature probe in the oil bath.

Melting points (mp) were recorded on a Stanford Research Systems OptiMelt at 1 ºC min^{-1} (capillaries ø = 1.5–1.6 mm, 90 mm) or a Stuart SMP10 at 2 ºC min^{-1} (capillaries ø = 1.8–1.9 mm, 100 mm). Infrared spectroscopy was carried out on a Bruker Alpha-E (attenuated total reflectance) without atmospheric compensation and processed using OPUS 7.0 software. Samples were analysed neat. Nuclear magnetic resonance spectroscopy was carried out at 300 K on Bruker spectrometers: either AVANCE 200 (^{1}H at 200 MHz), AVANCE 300 (^{1}H at 300 MHz, ^{13}C at 75 MHz), AVANCE III 400 (^{1}H at 400 MHz, ^{13}C at 101 MHz) or AVANCE III 500 (^{1}H at 500 MHz, ^{13}C at 126 MHz). Spectra were processed using Bruker Topspin or Mestrelab Research Mnova. Deuterated solvents ($CDCl_3$, DMSO-d_6, CD_3OD, acetone-d_6) obtained from the Cambridge Isotope Laboratories. ^{1}H and ^{13}C chemical shifts are reported in parts per million (ppm) with respect to TMS at 0.00 ppm. The chemical shifts of the spectra were calibrated to residual solvent peaks (^{1}H: $CHCl_3$ 7.26 ppm, DMSO 2.50 ppm, MeOH 3.31 ppm, $(CH_3)_2CO$ 2.05 ppm, TMS 0.00 ppm; ^{13}C: $CHCl_3$ 77.16 ppm, DMSO 39.52 ppm, MeOH 49.00 ppm, $(CH_3)_2CO$ 39.52 ppm, TMS 0.00 ppm). ^{1}H signal multiplicity is reported as: singlet (s), doublet (d), triplet (t), quartet (q), pentet (p) and combinations thereof, or multiplet (m). Broad signals are designated broad (br). Coupling constants (*J*) are reported in Hertz (Hz). Integrals are relative. $_{app}$ = apparent when the multiplicity was unexpected, e.g. coincidental or unresolved. Low resolution mass spectrometry (*m/z*) was carried out on a Finnigan quadrupole ion trap mass spectrometer using electrospray ionization (ESI) or atmospheric-pressure chemical ionization (APCI). High resolution mass spectrometry (HRMS) was performed on a Bruker 7T FT-ICR using ESI or APCI. Positive and negative detection is indicated by the charge of the ion, e.g. $[M+H]^+$ indicates positive ion detection.

Purity of all compounds was >95% as determined by NMR spectroscopy (provided for all compounds evaluated biologically).

2-Chloro-6-hydrazinylpyrazine (1) To a solution of 2,6-dichloropyrazine (22.3 g, 150 mmol, 1 equiv.) in EtOH (428 mL, 0.35 M) was added hydrazine monohydrate (14.7 mL, 299 mmol, 2 equiv.). The reaction was heated to 80 ºC until completion as indicated by TLC. The solution was allowed to cool to rt and the solvent was removed under reduced pressure. H_2O and EtOAc were added and the organic layer was separated. The aqueous layer was extracted with EtOAc (3 ×) and the combined organic layers washed with brine, dried (Na_2SO_4), filtered and concentrated under reduced pressure to give **1** as fine yellow needles (19.7 g, 93%); R_f 0.12 (30% EtOAc in hexanes); mp 133–135 ºC (lit.[56] 136–139 ºC); ^{1}H NMR (300 MHz; $CDCl_3$) δ: 8.13 (s, 1H), 7.89 (s, 1H), 6.38 (s, 1H), 3.86 (s, 2H); ^{13}C NMR (75 MHz; $CDCl_3$) δ: 156.4, 146.7, 132.4, 129.0. Spectroscopic data matched those in the literature.[56]

General Procedure for the Synthesis of 2a–g. Compound **1** (1 equiv.) was stirred into EtOH (112 mM). Aldehyde (1 equiv.) was added and the reaction stirred at rt until completion as indicated by TLC. The solvent was removed under reduced pressure to give compounds **2a–g** which were carried forward without further purification unless otherwise stated.

(*E*)-2-Chloro-6-(2-(4-(difluoromethoxy)benzylidene)hydrazinyl)pyrazine (2a). From 4-(difluoromethoxy)benzaldehyde (1.92 mL, 14.5 mmol) and **1** (2.10 g, 14.5 mmol) to give **2a** as light brown crystals (3.29 g, 76%); (3.29 g, 76%); Rf 0.63 (25% EtOAc in hexanes); mp 197–200 ºC; ^{1}H NMR (300 MHz, DMSO-d_6) δ: 11.55 (s, 1H), 8.56 (s, 1H), 8.05 (d, *J* = 5.2 Hz, 2H), 7.79 (d, *J* = 8.5 Hz, 2H), 7.29 (t, *J* = 73.9 Hz, 1H), 7.22 (d, *J* = 8.4 Hz, 2H); ^{13}C NMR (75 MHz, DMSO-d_6) δ: 152.3, 151.7, 145.6, 141.6, 132.4, 131.5, 128.8, 128.3, 118.8, 116.2 (t, *J* = 257.9 Hz).

***tert*-Butyl (*E*)-3-((2-(6-chloropyrazin-2-yl)hydrazinylidene)methyl)pyrrolidine-1-carboxylate (2b).** From 1-boc-3-pyrrolidine-carbaldehyde (200 mg, 1.00 mmol) and **1** (145 mg, 1.00 mmol); purified by automated flash chromatography on silica (Biotage Isolera, 6–50% EtOAc in hexanes) to give **2b** as an off-white powder (215 mg, 66%); R_f 0.27 (25% EtOAc in hexanes); mp 124–128 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.47 (s, 1H), 8.45 (s, 1H), 7.98 (s, 1H), 7.16 (d, *J* = 4.3 Hz, 1H), 3.61 (dt, *J* = 20.6 & 9.8 Hz, 1H), 3.54–3.44 (m, 1H), 3.44–3.32 (m, 2H), 3.09 (s, 1H), 2.18–2.06 (m, 1H), 2.06–1.90 (m, 1H), 1.46 (s, 9H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 154.6, 152.0, 146.2, 144.9, 134.0, 129.1, 79.6, 48.9, 45.2, 41.4, 40.5, 28.6; *m/z* (ESI+) 348 ($[M+Na]^+$, 100%); HRMS (ESI+) found 348.1194 ($[M+Na]^+$), $C_{14}H_{20}ClN_5O_2Na^+$ requires 348.1198.

(*E*)-2-Chloro-6-(2-((tetrahydrofuran-3-yl)methylene)hydrazinyl)pyrazine (2c). From tetrahydrofuran-3-carbaldehyde (50% in H_2O, 800 mg, 1.88 mmol) and **1** (578 mg, 1.88 mmol); purified by automated flash chromatography on silica (Biotage Isolera, 6–50% EtOAc in hexanes) to give **2c** as an off-white powder (662 mg, 73%); R_f 0.28 (25% EtOAc in hexanes); mp 135–138 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.46 (s, 1H), 8.40 (s, 1H), 7.98 (s, 1H), 7.14 (d, *J* = 5.9 Hz, 1H), 3.95 (ddd, *J* = 13.8, 8.4 & 6.6 Hz, 2H), 3.89–3.71 (m, 2H), 3.17 (dq, *J* = 13.8 & 6.3 Hz, 1H), 2.20

(dtd, *J* = 12.8, 7.8 & 5.4 Hz, 1H), 2.10–1.95 (m, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 152.0, 146.2, 146.0, 134.0, 129.1, 70.9, 68.2, 42.0, 30.6; *m/z* (ESI+) 249 ([M+Na]$^+$, 100%); HRMS (ESI+) found 227.0691 ([M+H]$^+$), $C_9H_{11}ClN_4OH^+$ requires 227.0694.

***tert*-Butyl (*E*)-4-((2-(6-chloropyrazin-2-yl)hydrazinylidene)methyl)piperidine-1-carboxylate (2d)**. From 1-boc-4-piperidine-carboxaldehyde (400 mg, 1.88 mmol) and **1** (271 mg, 1.88 mmol); filtered and washed with EtOH to give **2d** as a light brown powder (352 mg, 55%); R_f 0.52 (25% EtOAc in hexanes); mp 179–183 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.47 (s, 1H), 8.15 (s, 1H), 7.99 (s, 1H), 7.09 (s, 1H), 4.06 (br s, 1H), 3.85 (br s, 1H), 2.93 (q, *J* = 12.2 Hz, 2H), 2.47 (dq, *J* = 9.3 & 4.5 Hz, 1H), 1.99 (dd, *J* = 9.2 & 3.8 Hz, 1H), 1.74 (dd, *J* = 8.6 & 3.9 Hz, 1H), 1.66–1.44 (m, 2H), 1.46 (s, 9H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 154.9, 152.1, 146.2, 145.1, 134.0, 129.2, 79.9, 38.7, 28.6, 28.5, 24.4; *m/z* (ESI+) 362 ([M+Na]$^+$, 100%); HRMS (ESI+) found 362.1350 ([M+Na]$^+$), $C_{15}H_{22}ClN_5O_2Na^+$ requires 362.1354.

(*E*)-2-Chloro-6-(2-((tetrahydro-2*H*-pyran-4-yl)methylene)hydrazinyl)pyrazine (2e). From 4-formyltetrahydropyran (200 mg, 1.75 mmol) and **1** (253 mg, 1.75 mmol); filtered and washed with EtOH to give **2e** as a brown powder (284 mg, 67%); R_f 0.33 (25% EtOAc in hexanes); mp 160–168 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.47 (s, 1H), 8.40 (s, 1H), 7.98 (s, 1H), 7.09 (d, *J* = 4.7 Hz, 1H), 4.01 (dt, *J* = 11.1 & 3.5 Hz, 2H), 3.47 (td, *J* = 11.5 & 2.2 Hz, 2H), 2.72–2.40 (m, 1H), 1.85–1.73 (m, 2H), 1.73–1.57 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 152.2, 148.3, 146.1, 133.8, 129.2, 67.3, 37.9, 30.1; *m/z* (ESI+) 263 ([M+Na]$^+$, 100%); HRMS (ESI+) found 241.0848 ([M+H]$^+$), $C_{10}H_{13}ClN_4OH^+$ requires 241.0851.

(*E*)-2-Chloro-6-(2-(4-iodobenzylidene)hydrazinyl)pyrazine (2f). From **S13** (175 mg, 0.75 mmol) and **1** (109 mg, 0.75 mmol) to give **2f** as a light yellow powder (200 mg, 74%); carried forward without further purification or characterisation; R_f 0.26 (10% EtOAc in hexanes).

2-Chloro-6-(2-((*E*)-((2*r*,3*R*,4*r*,5*S*)-4-iodocuban-1-yl)methylene)hydrazinyl) pyrazine (2g). From **S15** (60.0 mg, 0.23 mmol) and **1** (33.6 mg, 0.23 mmol) to give **2g** as an off-white powder (90.1 mg, >100%); carried forward without further purification or characterisation; R_f 0.73 (2% MeOH in CH_2Cl_2).

General Procedure for the Synthesis of 3a–g. The product from General Procedure 1 (1 equiv.) was stirred into CH_2Cl_2 (112 mM). $PhI(OAc)_2$ (1 equiv.) was added and the reaction stirred at rt until completion as indication by TLC. The reaction was quenched with sat. aq. $NaHCO_3$, diluted with CH_2Cl_2 and the organic layer separated. The aqueous layer was extracted with CH_2Cl_2 (3 ×) and the combined organic layers were washed with brine, dried ($MgSO_4$), filtered and concentrated under reduced pressure to give the corresponding crude material which was purified by flash chromatography on silica to give the compounds **3a–g**.

5-Chloro-3-(4-(difluoromethoxy)phenyl)-[1,2,4]triazolo[4,3-*a*]pyrazine (3a). From **2a** (6.70 g, 22.4 mmol) to give the crude product, which was purified by automated flash chromatography on silica (Biotage Isolera, 12–100% EtOAc in hexanes) to give **3a** as a reddish-brown solid (4.91 g, 73%); R_f 0.42 (50% EtOAc in hexanes); mp 130–133 ºC; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 9.34 (s, 1H), 7.88 (s, 1H), 7.65 (d, *J* = 7.6 Hz, 2H), 7.27 (d, *J* = 7.7 Hz, 2H), 6.64 (t, *J* = 73.1 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 153.2, 147.4, 147.3, 143.2, 133.2, 130.0, 123.8, 122.0, 118.8, 115.6 (t, *J* = 261.4 Hz).

***tert*-Butyl 3-(5-chloro-[1,2,4]triazolo[4,3-*a*]pyrazin-3-yl)pyrrolidine-1-carboxylate (3b)**. From **2b** (160 mg, 0.49 mmol) to give the crude product, which was purified by automated flash chromatography on silica (Biotage Isolera, 12–100% EtOAc in hexanes) to give **3b** as a pale yellow powder (101 mg, 63%); R_f 0.07 (50% EtOAc in hexanes); mp 157–160 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.23 (s, 1H), 7.84 (s, 1H), 4.45–4.32 (m, 1H), 4.00–3.70 (m, 3H), 3.55 (dt, *J* = 10.7 & 7.3 Hz, 1H), 2.90–2.50 (m, 1H), 2.54–2.35 (m, 1H), 1.46 (s, 9H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 154.4, 149.3, 147.7, 143.3, 129.5, 121.3, 79.8, 51.4 (d, *J* = 57.4 Hz), 45.6, 36.7 (d, *J* = 85.9 Hz), 31.2 (d, *J* = 63.2 Hz), 28.6; *m/z* (ESI+) 346 ([M+Na]$^+$, 100%); HRMS (ESI+) found 346.1040 ([M+Na]$^+$), $C_{14}H_{18}ClN_5O_2Na^+$ requires 346.1041.

5-Chloro-3-(tetrahydrofuran-3-yl)-[1,2,4]triazolo[4,3-*a*]pyrazine (3c). From **2c** (600 mg, 2.65 mmol) to give the crude product, which was purified by automated flash chromatography on silica (Biotage Isolera, 12–100% EtOAc in hexanes) to give **3c** as a pale yellow powder (457 mg, 77%); R_f 0.06 (50% EtOAc in hexanes); mp 99–102 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.22 (s, 1H), 7.83 (s, 1H), 4.42 (dq, *J* = 8.7 & 6.3 Hz, 1H), 4.30 (dd, *J* = 8.5 & 7.3 Hz, 1H), 4.24–4.17 (m, 1H), 4.17–4.11 (m, 1H), 4.05 (td, *J* = 8.1 & 5.9 Hz, 1H), 2.77 (ddt, *J* = 12.2, 7.9 & 6.0 Hz, 1H), 2.48 (dddd, *J* = 12.5, 8.7, 7.8 & 6.5 Hz, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 149.8, 147.8, 143.4, 129.4, 121.3, 72.9, 68.6, 37.7, 32.4; *m/z* (ESI+) 247 ([M+Na]$^+$, 100%); HRMS (ESI+) found 225.0538 ([M+H]$^+$), $C_9H_9ClN_4OH^+$ requires 225.0538.

***tert*-Butyl 4-(5-chloro-[1,2,4]triazolo[4,3-*a*]pyrazin-3-yl)piperidine-1-carboxylate (3d).** From **2d** (300 mg, 0.88 mmol) to give the crude product, which was purified by automated flash chromatography on silica (Biotage Isolera, 12–100% EtOAc in hexanes) to give **3d** as a pale yellow powder (245 mg, 82%); R_f 0.14 (50% EtOAc in hexanes); mp 112–116 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.21 (s, 1H), 7.82 (s, 1H), 4.50 (d, *J* = 12.5 Hz, 1H), 4.16 (s, 1H), 3.81 (t, *J* = 10.8 Hz, 1H), 3.28 (t, *J* = 11.8 Hz, 1H), 2.88 (s, 1H), 2.31 (d, *J* = 13.2 Hz, 1H), 2.12 (qd, *J* = 12.8 & 3.6 Hz, 1H), 1.91 (dq, *J* = 9.7 & 3.0 Hz, 1H), 1.64 (q, *J* = 11.6 & 10.8 Hz, 1H), 1.44 (s, 9H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 154.5, 150.2, 147.3, 143.3, 129.4, 121.6, 80.1, 35.6, 31.0, 28.6, 25.0; *m/z* (ESI+) 360 ([M+Na]$^+$, 100%); HRMS (ESI+) found 360.1197 ([M+Na]$^+$), $C_{15}H_{20}ClN_5O_2Na^+$ requires 360.1198.

5-Chloro-3-(tetrahydro-2*H*-pyran-4-yl)-[1,2,4]triazolo[4,3-*a*]pyrazine (3e). From **2e** (230 mg, 0.96 mmol) to give the crude product, which was purified by automated flash chromatography on silica (Biotage Isolera, 12–100% EtOAc in hexanes) to give **3e** as pale yellow crystalline powder (145 mg, 63%); R_f 0.06 (50% EtOAc in hexanes); mp 185–193 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.23 (s, 1H), 7.82 (s, 1H), 4.27–4.09 (m, 2H), 3.95 (tt, *J* = 11.3 & 3.7 Hz, 1H), 3.61 (td, *J* = 11.7 & 2.1 Hz, 2H), 2.45–2.21 (m, 2H), 2.17–2.03 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 151.9, 147.4, 143.4, 129.5, 121.4, 67.5, 34.3, 32.4; *m/z* (ESI+) 261 ([M+Na]$^+$, 100%); HRMS (ESI+) found 239.0693 ([M+H]$^+$), $C_{10}H_{11}ClN_4OH^+$ requires 239.0694.

5-Chloro-3-(4-iodophenyl)-[1,2,4]triazolo[4,3-*a*]pyrazine (3f). From **2f** (200 mg, 0.56 mmol) to give the crude product, which was purified by automated flash chromatography on silica (Biotage Isolera, 12–100% EtOAc in hexanes) to give **3f** as a light brown powder (157 mg, 79%); carried forward without further characterisation; R_f 0.42 (50% EtOAc in hexanes); mp decomposed >200 ºC; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 9.34 (s, 1H), 7.88 (d_{app}, *J* = 5.8 Hz, 3H), 7.37 (d, *J* = 7.9 Hz, 2H).

5-Chloro-3-(4-iodocuban-1-yl)-[1,2,4]triazolo[4,3-*a*]pyrazine (3g). From **2g** (80.0 mg, 0.21 mmol) to give the crude product, which was purified by automated flash chromatography on silica (Biotage Isolera, 0–10% MeOH in CH_2Cl_2) to give **3g** as a brown powder (62.0 mg, 78%); R_f 0.37 (2% MeOH in CH_2Cl_2); mp decomposed >150 ºC; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 9.20 (s, 1H), 7.87 (s, 1H), 4.95–4.56 (m, 3H), 4.54–4.12 (m, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 147.7, 142.8, 129.1, 120.9, 54.7, 53.0, 51.7, 50.4, 34.5; *m/z* (ESI+) 405 ([M+Na]$^+$, 100%); HRMS (ESI+) found 404.9375 ([M+Na]$^+$), $C_{13}H_8ClIN_4Na^+$ requires 404.9374.

General Procedure for the Synthesis of 4–31. Nucleophile (1.0 equiv.) was added to PhMe (168 mM) along with the product from General Procedure 2 (1.0 equiv.), KOH (3.0 equiv.) and 18-crown-6 (0.1 equiv.). The reaction was stirred at rt until completion as indicated by TLC. The reaction was diluted with H_2O, then extracted with EtOAc. The organic layers were washed with H_2O until the aqueous layer became neutral, followed by brine, dried ($MgSO_4$), filtered and concentrated under reduced pressure to give the crude material, which was purified by flash chromatography on silica to give compounds **4–31**.

3-(4-(Difluoromethoxy)phenyl)-5-phenoxy-[1,2,4]triazolo[4,3-*a*]pyrazine (4). From phenol (31.7 mg, 0.34 mmol) and **3a** (100 mg, 0.34 mmol); the solvent was removed and the residue directly purified by automated flash chromatography on silica (Biotage Isolera, 25–100% EtOAc in hexanes) to give **4** as an off-white powder (44.9 mg, 38%); R_f 0.41 (100% EtOAc); mp 113–117 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.12 (s, 1H), 7.72 (d, *J* = 8.7 Hz, 2H), 7.36 (t, *J* = 7.9 Hz, 2H), 7.25–7.19 (m, 2H), 7.15 (d, *J* = 8.6 Hz, 2H), 6.93 (d, *J* = 8.0 Hz, 2H), 6.54 (t, *J* = 73.3 Hz, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 153.1, 152.6 (t, *J* = 2.8 Hz), 148.2, 146.5, 142.7, 138.4, 132.3, 130.6, 126.5, 124.4, 118.8, 115.7 (t, *J* = 261.0 Hz), 114.2; ^{19}F NMR (376 MHz, $CDCl_3$) δ: -81.36; *m/z* (ESI+) 377 ([M+Na]$^+$, 100%); HRMS (ESI+) found 355.0998 ([M+H]$^+$), $C_{18}H_{12}F_2N_4O_2H^+$ requires 355.1001.

5-(Benzyloxy)-3-(4-(difluoromethoxy)phenyl)-[1,2,4]triazolo[4,3-*a*]pyrazine (5). From benzyl alcohol (70.0 µL, 0.67 mmol) and **3a** (200 mg, 0.67 mmol) to give the crude product, which was purified by automated flash chromatography on silica (Biotage Isolera, 25–100% EtOAc in hexanes) to give **5** as a pale yellow powder (135 mg, 54%); R_f 0.08 (50% EtOAc in hexanes); mp 152–156 ºC; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 9.02 (s, 1H), 7.58 (d, *J* = 8.5 Hz, 2H), 7.42 (s, 1H), 7.38–7.22 (m, 3H), 7.07 (d, *J* = 7.3 Hz, 2H), 6.95 (d, *J* = 8.4 Hz, 2H), 6.46 (t, *J* = 73.4 Hz, 1H), 5.19 (s, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 152.4, 147.9, 146.5, 143.9, 136.7, 132.9, 132.5, 129.3, 128.8, 128.2, 124.5, 118.3, 115.7 (t, *J* = 260.4 Hz), 108.9, 72.9; *m/z* (ESI+) 391 ([M+Na]$^+$, 100%), 759 ([2M+Na]$^+$, 71%); HRMS (ESI+) 391.0975 ([M+Na]$^+$), $C_{19}H_{14}F_2N_4O_2Na^+$ requires 391.0977.

3-(4-(Difluoromethoxy)phenyl)-5-phenethoxy-[1,2,4]triazolo[4,3-*a*]pyrazine (6). From 2-phenylethanol (80.8 µL, 0.67 mmol) and **3a** (200 mg, 0.67 mmol) to give the crude product, which was purified by automated flash chromatography on silica (Biotage Isolera, 25–100% EtOAc in hexanes) to give **6** as a pale yellow powder (189 mg, 73%); R_f 0.10 (50% EtOAc in hexanes); mp 115–117 ºC; ^{1}H NMR (500 MHz, DMSO-d_6) δ: 9.03 (s, 1H), 7.77 (d, *J* = 8.6 Hz, 2H), 7.59 (s, 1H), 7.36 (t, *J* = 73.7 Hz, 1H), 7.30 (d, *J* = 8.6 Hz, 2H), 7.20 – 7.13 (m, 3H), 6.97 – 6.84 (m, 2H), 4.50 (t, *J* = 6.4 Hz, 2H), 2.88 (t, *J* = 6.4 Hz, 2H); ^{13}C NMR (126 MHz, DMSO-d_6) δ: 152.0, 147.4, 145.5, 143.8, 137.3, 135.0, 132.6, 128.6, 128.2, 126.4, 124.7, 117.6, 116.1 (t, *J* = 258.2 Hz), 108.8, 71.2, 33.8; *m/z* (ESI+) 405 ([M+Na]$^+$, 100%), 787 ([2M+Na]$^+$, 71%); HRMS (ESI+) 405.1131 ([M+Na]$^+$), $C_{20}H_{16}F_2N_4O_2Na^+$ requires 405.1133.

3-(4-(Difluoromethoxy)phenyl)-5-(3-phenylpropoxy)-[1,2,4]triazolo[4,3-*a*]pyrazine (7). From 3-phenylpropanol (45.9 µL, 0.34 mmol) and **3a** (100 mg, 0.34 mmol); the solvent was removed and the residue directly purified by automated flash chromatography on silica (Biotage Isolera, 25–100% EtOAc in hexanes) to give **7** as a dark yellow crystalline powder (111 mg, 83%); R_f 0.42 (100% EtOAc); mp 153–157 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.03 (s, 1H), 7.75 (d, *J* = 8.8 Hz, 2H), 7.32–7.16 (m, 6H), 6.96 (d, *J* = 7.0 Hz, 2H), 6.47 (t, *J* = 73.3 Hz, 1H), 4.19 (t, *J* = 5.9 Hz, 2H), 2.35 (t, *J* = 7.6 Hz, 2H), 1.96 (dt, *J* = 8.4 & 6.4 Hz, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 152.7, 147.9, 146.3, 144.2, 140.0, 136.5, 132.6, 128.8, 128.3, 126.6, 125.2, 118.6, 115.7 (t, *J* = 261.0 Hz), 108.4, 70.1, 31.6, 29.9; ^{19}F NMR (376 MHz, $CDCl_3$) δ: -81.33; *m/z* (ESI+) 419 ([M+Na]$^+$, 100%); HRMS (ESI+) found 397.1469 ([M+H]$^+$), $C_{21}H_{18}F_2N_4O_2H^+$ requires 397.1471.

5-(2-Cyclopropylethoxy)-3-(4-(difluoromethoxy)phenyl)-[1,2,4]triazolo[4,3-*a*]pyrazine (8). From 2-cyclopropylethanol (50.0 mg, 0.58 mmol) and **3a** (172 mg, 0.58 mmol) to give the crude product, which was purified by automated flash chromatography on silica (Biotage Isolera, 25–100% EtOAc in hexanes) to give an orange solid; repurifed by preparative TLC (75% EtOAc in hexanes) to give **8** as a yellow powder (42.3 mg, 21%); R_f 0.58 (100% EtOAc); mp 120–124 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.03 (s, 1H), 7.71 (d, *J* = 8.8 Hz, 2H), 7.32 (s, 1H), 7.23 (d, *J* = 8.7 Hz, 2H), 6.60 (t, *J* = 73.2 Hz, 1H), 4.25 (t, *J* = 6.3 Hz, 2H), 1.51 (q, *J* = 6.4 Hz, 2H), 0.54–0.19 (m, 3H), -0.01–-0.10 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 152.5, 148.0, 146.4, 144.4, 136.2, 132.6, 125.1, 118.8, 115.7 (t, *J* = 261.4 Hz), 108.3, 71.3, 33.5, 7.4, 4.3; ^{19}F NMR (376 MHz, $CDCl_3$) δ: -81.43; *m/z* (ESI+) 369 ([M+Na]$^+$, 100%), 715 ([2M+Na]$^+$, 35%); HRMS (ESI+) found 347.1313 ([M+H]$^+$), $C_{17}H_{16}F_2N_4O_2H^+$ requires 347.1314.

3-(4-(Difluoromethoxy)phenyl)-5-(2-(oxetan-3-yl)ethoxy)-[1,2,4]triazolo[4,3-*a*]pyrazine (9). From **S1** (35.0 mg, 0.34 mmol) and **3a** (102 mg, 0.34 mmol) to give the crude product, which was purified by automated flash chromatography on silica (Biotage Isolera, 1–10% MeOH in CH_2Cl_2) to give **9** as an orange powder (21.6 mg, 17%); R_f 0.06 (100% EtOAc); mp 110–114 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.04 (s, 1H), 7.68 (d, *J* = 8.7 Hz, 2H), 7.30 (s, 1H), 7.25 (d, *J* = 7.5 Hz, 2H), 6.62 (t, *J* = 73.1 Hz, 1H), 4.21–3.96 (m, 1H), 3.91–3.71 (m, 1H), 3.65 (q_{app}, *J* = 7.7 Hz, 1H), 3.48 (dd_{app}, *J* = 9.0 & 7.0 Hz, 1H), 3.34 (dd, *J* = 9.1 & 4.7 Hz, 1H), 2.47 (hept, *J* = 7.1 Hz, 1H), 1.86 (dtd, *J* = 12.9, 8.1 & 5.2 Hz, 1H), 1.42 (td, *J* = 13.0 & 7.6 Hz, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 152.4, 147.8, 146.2, 144.0, 136.8, 132.4, 125.2, 119.0, 115.6 (t, *J* = 262.0 Hz), 108.4, 72.3, 69.8, 67.5, 38.3, 28.4; *m/z* (ESI+) 385 ([M+Na]$^+$, 100%), 747 ([2M+Na]$^+$, 47%); HRMS (ESI+) found 385.1087 ([M+Na]$^+$), $C_{17}H_{16}F_2N_4O_3Na^+$ requires 385.1083.

6-(2-((3-(4-(Difluoromethoxy)phenyl)-[1,2,4]triazolo[4,3-*a*]pyrazin-5-yl)oxy)ethyl)-2-oxa-6-azaspiro[3.3]heptane (10). From 2-[2-oxa-6-azaspiro[3.3]heptan-6-yl]ethan-1-ol (100 mg, 0.70 mmol) and **3a** (207 mg, 0.70 mmol) to give the crude product, which was purified by automated flash chromatography on silica (Biotage Isolera, 1–10% MeOH in CH_2Cl_2) to give **10** as a light brown powder (145 mg, 52%); R_f 0.07 (5% MeOH in CH_2Cl_2); mp 136–141 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.03 (s, 1H), 7.75 (d, *J* = 8.8 Hz, 2H), 7.28 (s, 1H), 7.26 (d, *J* = 8.8 Hz, 2H), 6.64 (t, *J* = 73.0 Hz, 1H), 4.62 (s, 4H), 4.16 (t, *J* = 5.2 Hz, 2H), 3.09 (s, 4H), 2.58 (t, *J* = 5.2 Hz, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 152.2, 147.9, 146.3, 144.0, 136.7, 132.7, 125.1, 119.0, 115.5 (t, *J* = 262.4 Hz), 108.6, 81.0, 70.0, 64.2, 56.4, 39.5; *m/z* (ESI+) 426 ([M+Na]$^+$, 100%); HRMS (ESI+) found 426.1353 ([M+Na]$^+$), $C_{19}H_{19}F_2N_4O_3Na^+$ requires 426.1348.

3-(4-(Difluoromethoxy)phenyl)-5-(2-(pyrrolidin-1-yl)ethoxy)-[1,2,4]triazolo[4,3-*a*]pyrazine (11). From 1-(2-hydroxyethyl)pyrrolidine (59.1 µL, 0.51 mmol) and **3a** (150 mg, 0.51 mmol); the solvent was removed and the residue directly purified by automated flash chromatography on silica (Biotage Isolera, 1–10% MeOH in CH_2Cl_2) to give **11** as a brown powder (70.2 mg, 37%); R_f 0.12 (5% MeOH in CH_2Cl_2); mp 96–102 ºC; ^{1}H NMR (400 MHz, CD_3OD) δ: 8.98 (s, 1H), 7.80 (d, *J* = 8.8 Hz, 2H), 7.54 (s, 1H), 7.33 (d, *J* = 8.8 Hz, 2H), 7.00 (t, *J* = 73.5 Hz, 1H), 4.43 (t, *J* = 5.2 Hz, 2H), 2.77 (t, *J* = 5.2 Hz, 2H), 2.32 (t, *J* = 6.5 Hz, 4H), 1.87–1.59 (m, 4H); ^{13}C NMR (101 MHz, CD_3OD) δ: 151.9, 149.5, 149.1, 146.0, 136.3, 134.0, 125.9, 119.5, 117.4 (t, *J* = 259.5 Hz), 110.1, 71.3, 55.1, 54.6, 24.2; *m/z* (ESI+) 398 ([M+Na]$^+$, 100%); HRMS (ESI+) found 376.1582 ([M+H]$^+$), $C_{18}H_{19}F_2N_5O_2H^+$ requires 376.1580.

3-(4-(Difluoromethoxy)phenyl)-5-(2-(piperidin-1-yl)ethoxy)-[1,2,4]triazolo[4,3-*a*]pyrazine (12). From 1-(2-hydroxyethyl)piperidine (67.1 µL, 0.51 mmol) and **3a** (150 mg, 0.51 mmol); the solvent was removed and the residue directly purified by automated flash chromatography on silica (Biotage Isolera, 1–10% MeOH in CH_2Cl_2) to give **12** as a brown powder (70.2 mg, 36%); R_f 0.21 (5% MeOH in CH_2Cl_2); mp 123–129 ºC; ^{1}H NMR (400 MHz, CD_3OD) δ: 8.97 (s, 1H), 7.79 (d, *J* = 8.7 Hz, 2H), 7.53 (s, 1H), 7.33 (d, *J* = 8.7 Hz, 2H), 7.01 (t, *J* = 73.5 Hz, 1H), 4.43 (t, *J* = 5.1 Hz, 2H), 2.63 (t, *J* = 5.1 Hz, 2H), 2.21 (br s_{app}, 4H), 1.73–1.35 (m, 4H), 1.41–1.33 (m, 2H); ^{13}C NMR (101 MHz, CD_3OD) δ: 154.3, 149.0, 147.8, 146.0, 136.2, 133.9, 125.9, 119.4, 117.4 (t, *J* = 259.4 Hz), 110.1, 70.3, 57.7, 55.4, 26.5, 24.7; *m/z* (ESI+) 412 ([M+Na]$^+$, 100%); HRMS (ESI+) found 390.1737 ([M+H]$^+$), $C_{19}H_{21}F_2N_5O_2H^+$ requires 390.1736.

4-(2-((3-(4-(Difluoromethoxy)phenyl)-[1,2,4]triazolo[4,3-*a*]pyrazin-5-yl)oxy)ethyl)morpholine (13). From 4-(2-hydroxyethyl)morpholine (61.2 µL, 0.51 mmol) and **3a** (150 mg, 0.51 mmol); the solvent was removed and the residue directly purified by automated flash chromatography on silica (Biotage Isolera, 1–10% MeOH in CH_2Cl_2) to give **13** as a light brown powder (141 mg, 71%); R_f 0.26 (5% MeOH in CH_2Cl_2); mp 160–164 ºC; ^{1}H NMR (400 MHz,

CD_3OD) δ: 8.97 (s, 1H), 7.80 (d, *J* = 8.8 Hz, 2H), 7.53 (s, 1H), 7.33 (d, *J* = 8.7 Hz, 2H), 7.00 (t, *J* = 73.5 Hz, 1H), 4.42 (t, *J* = 5.1 Hz, 2H), 3.62–3.42 (m, 4H), 2.61 (t, *J* = 5.0 Hz, 2H), 2.37–2.04 (m, 4H); ^{13}C NMR (101 MHz, CD_3OD) δ: 154.3, 150.1, 149.0, 146.1, 136.2, 134.0, 125.9, 119.4, 117.5 (t, *J* = 259.3 Hz), 110.1, 70.2, 67.7, 57.5, 54.5; *m/z* (ESI+) 414 ([M+Na]$^+$, 100%); HRMS (ESI+) found 392.1530 ([M+H]$^+$), $C_{18}H_{19}F_2N_4O_3H^+$ requires 392.1529.

***tert*-Butyl 4-(2-((3-(4-(difluoromethoxy)phenyl)-[1,2,4]triazolo[4,3-*a*]pyrazin-5-yl)oxy)ethyl)piperazine-1-carboxylate (14)**. From **S2** (200 mg, 0.87 mmol) and **3a** (258 mg, 0.87 mmol) to give the crude product, which was purified by automated flash chromatography on silica (Biotage Isolera, 2–20% MeOH in CH_2Cl_2) to give **14** as a yellow powder with trace amounts of inseparable **S2** (205 mg, 48%); R_f 0.01 (100% EtOAc); mp 134–139 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.04 (s, 1H), 7.74 (d, *J* = 8.8 Hz, 2H), 7.32 (s, 1H), 7.24 (d, *J* = 8.8 Hz, 2H), 6.62 (t, *J* = 73.1 Hz, 1H), 4.30 (t, *J* = 5.2 Hz, 2H), 3.33–3.18 (m, 4H), 2.59 (t, *J* = 5.2 Hz, 2H), 2.25–2.05 (m, 4H), 1.45 (s, 9H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 154.7, 152.4, 147.9, 146.4, 144.0, 136.7, 132.6, 125.1, 118.9, 115.6 (t, *J* = 262.0 Hz), 108.6, 80.0, 69.0, 56.1, 53.1, 28.5; ^{19}F NMR (376 MHz, $CDCl_3$) δ: -81.50; *m/z* (ESI+) 513 ([M+Na]$^+$, 100%); HRMS (ESI+) found 491.2213 ([M+H]$^+$), $C_{23}H_{28}F_2N_6O_4H^+$ requires 491.2213.

3-(4-(Difluoromethoxy)phenyl)-5-(2-((1*R*,5*S*)-6,6-dimethylbicyclo[3.1.1]hept-2-en-2-yl)ethoxy)-[1,2,4]triazolo[4,3-*a*]pyrazine (15). From (1*R*)-(–)-nopol (57.6 µL, 0.34 mmol) and 3a (100 mg, 0.34 mmol); the solvent was removed and the residue directly purified by automated flash chromatography on silica (Biotage Isolera, 25–100% EtOAc in hexanes) to give 15 as a light red powder (103 mg, 71%); R_f 0.49 (100% EtOAc); mp 134–137 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.01 (s, 1H), 7.71 (d, *J* = 8.8 Hz, 2H), 7.29 (s, 1H), 7.24 (d, *J* = 8.8 Hz, 2H), 6.61 (t, *J* = 73.3 Hz, 1H), 4.97 (dt, *J* = 2.9 & 1.3 Hz, 1H), 4.29–4.11 (m, 2H), 2.39–2.22 (m, 3H), 2.13 (br s, 1H), 2.07 (br s, 1H), 2.05–1.98 (m, 1H), 1.83 (td, *J* = 5.7 & 1.5 Hz, 1H), 1.20 (s, 3H), 0.98 (d_{app}, *J* = 8.6 Hz, 1H), 0.63 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 152.5, 148.0, 146.4, 144.1, 142.4, 136.4, 132.6, 125.1, 120.0, 118.7, 115.7 (t, *J* = 261.3 Hz), 108.4, 69.3, 45.5, 40.7, 38.1, 35.5, 31.7, 31.4, 26.3, 21.1; *m/z* (ESI+) 449 ([M+Na]$^+$, 100%), 875 ([2M+Na]$^+$, 33%); HRMS (ESI+) found 449.1760 ([M+Na]$^+$), $C_{23}H_{24}F_2N_4O_2Na^+$ requires 449.1760.

5-(((1*S*,2*S*,4*S*)-Bicyclo[2.2.1]hept-5-en-2-yl)methoxy)-3-(4-(difluoromethoxy)phenyl)-[1,2,4]triazolo[4,3-*a*]pyrazine (16). From 5-norbornene-2-methanol (40.8 µL, 0.34 mmol) and **3a** (100 mg, 0.34 mmol); the solvent was removed and the residue directly purified by automated flash chromatography on silica (Biotage Isolera, 25–100% EtOAc in hexanes) to give **16** (present as a mixture of *endo* and *exo* isomers) as a yellow powder (80.0 mg, 62%); R_f 0.63 (100% EtOAc); mp 122–128 °C; ^{1}H NMR (400 MHz, $CDCl_3$, present as a mixture of *endo* and *exo* isomers, ~1:0.78 *maj*/*min*) δ: 9.01 (s, 1H$^{(min)}$), 9.00 (s, 1H$^{(maj)}$), 7.72 (d, *J* = 8.7 Hz, 4H$^{(comb)}$), 7.37–7.14 (m, 6H$^{(comb)}$), 6.61 (t, *J* = 73.2 Hz, 1H$^{(min)}$), 6.59 (t, *J* = 73.2 Hz, 1H$^{(maj)}$), 6.15 (dd, *J* = 5.7 & 3.0 Hz, 1H$^{(maj)}$), 6.06 (dd, *J* = 5.7 & 3.0 Hz, 1H$^{(min)}$), 5.90 (dd, *J* = 5.8 & 3.2 Hz, 1H$^{(min)}$), 5.74 (dd, *J* = 5.7 & 2.8 Hz, 1H$^{(maj)}$), 4.09 (ddd, *J* = 140.2, 8.8 & 6.2 Hz, 2H$^{(min)}$), 3.88 (dt, *J* = 90.9 & 9.1 Hz, 2H$^{(maj)}$), 2.89–2.65 (m, 2H$^{(comb)}$), 2.43–2.20 (m, 3H$^{(comb)}$), 1.71 (ddd, *J* = 12.4, 9.1 & 3.7 Hz, 1H$^{(maj)}$), 1.64 (dq, *J* = 9.5 & 4.8 Hz, 1H$^{(min)}$), 1.47–1.35 (m, 1H$^{(comb)}$), 1.34–1.23 (m, 1H$^{(comb)}$), 1.21–1.12 (m, 3H$^{(comb)}$), 1.10–0.99 (m, 1H$^{(min)}$), 0.47 (ddd, *J* = 11.7, 4.1 & 2.6 Hz, 1H$^{(maj)}$); ^{13}C NMR (101 MHz, $CDCl_3$, present as a mixture of *endo* and *exo* isomers) δ: 152.4, 152.3, 147.9, 146.3, 146.3, 144.24, 144.22, 138.6, 137.3, 136.3, 136.1, 135.7, 132.6, 132.5, 131.5, 129.1, 128.7, 125.3, 125.1, 119.1, 118.7, 115.7 (t, *J* = 261.6 Hz), 115.6 (t, *J* = 261.9 Hz), 108.2, 75.0, 74.4, 49.4, 44.8, 43.7, 43.4, 42.3, 41.6, 38.1, 37.9, 29.4, 28.9; *m/z* (ESI+) 407 ([M+Na]$^+$, 100%); HRMS (ESI+) found 407.1294 ([M+Na]$^+$), $C_{20}H_{18}F_2N_4O_2Na^+$ requires 407.1290.

5-(((3*r*,5*r*,7*r*)-Adamantan-1-yl)methoxy)-3-(4-(difluoromethoxy)phenyl)-[1,2,4]triazolo[4,3-*a*]pyrazine (17). From 1-adamantanemethanol (100 mg, 0.60 mmol) and **3a** (178 mg, 0.60 mmol) to give the crude product, which was purified by automated flash chromatography on silica (Biotage Isolera, 25–100% EtOAc in hexanes) to give **17** as a yellow powder (140 mg, 55%); R_f 0.53 (100% EtOAc); mp 210–219 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.01 (s, 1H), 7.67 (d, *J* = 8.7 Hz, 2H), 7.29 (s, 1H), 7.26 (d, *J* = 8.7 Hz, 2H), 6.59 (t, *J* = 73.2 Hz, 1H), 3.71 (s, 2H), 1.88 (br s, 3H), 1.67 (d, *J* = 12.3 Hz, 3H), 1.48 (d, *J* = 11.6 Hz, 3H), 1.26 (d_{app}, *J* = 2.5 Hz, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 152.6, 147.9, 146.0, 144.9, 136.2, 132.4, 125.5, 118.9, 115.7 (t, *J* = 261.2 Hz), 108.7, 81.6, 38.8, 36.6, 33.6, 27.8; *m/z* (ESI+) 449 ([M+Na]$^+$, 100%), 875 ([2M+Na]$^+$, 44%); HRMS (ESI+) found 449.1761 ([M+Na]$^+$), $C_{23}H_{24}F_2N_4O_2Na^+$ requires 449.1760.

5-(2-((1*s*,3*s*)-Adamantan-1-yl)ethoxy)-3-(4-(difluoromethoxy)phenyl)-[1,2,4]triazolo[4,3-*a*]pyrazine (18). From 1-adamantaneethanol (250 mg, 1.39 mmol) and **3a** (411 mg, 1.39 mmol) to give the crude product, which was purified by automated flash chromatography on silica (Biotage Isolera, 25–100% EtOAc in hexanes) to give **18** as a light brown powder (363 mg, 59%); R_f 0.63 (100% EtOAc); mp 182–185 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.00 (s, 1H), 7.73–7.64 (m, 2H), 7.29 (s, 1H), 7.25–7.20 (m, 2H), 6.61 (t, *J* = 73.2 Hz, 1H), 4.24 (t, *J* = 6.7 Hz, 2H), 1.87 (s, 3H), 1.66 (d, *J* = 12.3 Hz, 3H), 1.52 (d, *J* = 11.4 Hz, 3H), 1.39 (t, *J* = 6.7 Hz, 2H), 1.29 (d, *J* = 2.4 Hz, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 152.5, 147.9, 146.4, 144.2, 136.2, 132.7, 125.1, 118.7, 115.6 (t, *J* = 261.6 Hz), 108.3, 67.5, 42.3, 41.9, 36.8, 31.7, 28.5; *m/z* (ESI+) 463 ([M+Na]$^+$, 100%); HRMS (ESI+) found 463.1916 ([M+Na]$^+$), $C_{24}H_{26}F_2N_4O_2Na^+$ requires 463.1916.

5-(2-(Cuban-1-yl)ethoxy)-3-(4-(difluoromethoxy)phenyl)-[1,2,4]triazolo[4,3-*a*]pyrazine (19). From 2-cubylethan-1-ol[57] (19.0 mg, 0.12 mmol) and **3a** (37.0 mg, 0.12 mmol) to give the crude product, which was purified by automated flash chromatography on silica (Biotage Isolera, 18–100% EtOAc in hexanes) to give **19** as a pale yellow powder (28.0 mg, 55%); R_f 0.35 (75% EtOAc in hexanes); mp 146–149 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 9.05 (s, 1H), 7.73 (d, *J* = 8.7 Hz, 2H), 7.33 (s, 1H), 7.24 (d, *J* = 8.6 Hz, 2H), 6.61 (t, *J* = 73.2 Hz, 1H), 4.26 (t, *J* = 6.5 Hz, 2H), 3.98 (ddq, *J* = 7.2, 4.6 & 2.2 Hz, 1H), 3.78 (q, *J* = 5.0 Hz, 3H), 3.54 (ddd, *J* = 5.7, 4.4 & 2.3 Hz, 3H), 1.95 (t, *J* = 6.5 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 152.5, 147.9, 146.4, 144.2, 136.3, 132.7, 124.9, 119.1, 118.6, 115.7, 112.2, 108.4, 68.2, 55.7, 48.6, 48.3, 44.3, 31.8; *m/z* (ESI+) 431 ([M+Na]$^+$, 100%), 839 ([2M+Na]$^+$, 23%); HRMS (ESI+) found 431.1291 ([M+Na]$^+$), $C_{22}H_{18}F_2N_4O_2Na^+$ requires 431.1290.

5-(3-(Cuban-1-yl)propoxy)-3-(4-(difluoromethoxy)phenyl)-[1,2,4]triazolo[4,3-*a*]pyrazine (20). From **S3** (20.0 mg, 0.12 mmol) and **3a** (37.0 mg, 0.12 mmol) to give the crude product, which was purified by automated flash chromatography on silica (Biotage Isolera, 18–100% EtOAc in hexanes) to give **20** as a light brown powder (24.0 mg, 47%); R_f 0.33 (75% EtOAc in hexanes); mp 128–132 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 9.06 (s, 1H), 7.72 (d, *J* = 8.7 Hz, 2H), 7.32 (s, 1H), 7.23 (d, *J* = 8.5 Hz, 2H), 6.59 (t, *J* = 73.2 Hz, 1H), 4.22 (t, *J* = 6.2 Hz, 2H), 4.04 (qt, *J* = 4.5 & 2.2 Hz, 1H), 3.86 (q, *J* = 5.1 Hz, 3H), 3.63 (dt, *J* = 5.4 & 2.3 Hz, 3H), 1.75 (s, 4H); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 152.6, 147.9, 146.3, 144.3, 136.3, 132.5, 125.1, 119.2, 118.6, 115.7, 112.2, 108.4, 71.4, 58.2, 48.7, 48.2, 44.1, 29.1, 23.6; *m/z* (ESI+) 445 ([M+Na]$^+$, 100%), 867 ([2M+Na]$^+$, 13%); HRMS (ESI+) found 445.1448 ([M+Na]$^+$), $C_{23}H_{20}F_2N_4O_2Na^+$ requires 445.1446.

3-(4-(Difluoromethoxy)phenyl)-5-(2-(octahydro-1*H*-2,4,1-(epiethane[1,1,2]triyl)cyclobuta[*cd*]pentalen-7-yl)ethoxy)-[1,2,4]triazolo[4,3-*a*]pyrazine (21). From S7 (20.0 mg, 0.11 mmol) and 3a (31.2 mg, 0.11 mmol); the solvent was removed and the residue directly purified by automated flash chromatography on silica (Biotage Isolera, 25–100% EtOAc in hexanes) to give 21 as a light brown powder (14.5 mg, 31%); R_f 0.35 (100% EtOAc); mp 136–142 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.02 (s, 1H), 7.70 (d, *J* = 8.8 Hz, 2H), 7.29 (s, 1H), 7.23 (d, *J* = 8.7 Hz, 2H), 6.61 (t, *J* = 73.2 Hz, 1H), 4.18 (tq, *J* = 5.8 & 2.5 Hz, 2H), 2.60 (q, *J* = 6.5 Hz, 1H), 2.48 (dq, *J* = 27.4 & 6.8 Hz, 2H), 2.30 (dt, *J* = 7.8 & 4.0 Hz, 1H), 2.25–2.19 (m, 1H), 2.19–2.13 (m, 1H), 2.01–1.97 (m, 1H), 1.91 (q, *J* = 6.5 Hz, 2H), 1.68–1.49 (m, 4H), 1.18–1.05 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 148.0, 146.4, 145.8, 144.3, 136.3, 132.5, 125.1, 118.6, 115.7,

108.4, 71.2, 47.4, 47.0, 44.5, 42.3, 41.8, 41.5, 38.8, 38.4, 36.3, 34.2, 28.7, 28.6; *m/z* (ESI+) 473 ([M+Na]$^+$, 100%); HRMS (ESI+) found 451.1941 ([M+H]$^+$), $C_{25}H_{24}F_2N_4O_2H^+$ requires 451.1940.

3-(4-(Difluoromethoxy)phenyl)-5-((3-(difluoromethyl)bicyclo[1.1.1]pentan-1-yl)methoxy)-[1,2,4]triazolo[4,3-*a*]pyrazine (22). From **S8** (15.0 mg, 101 µmol) and **3a** (30.0 mg, 101 µmol); the solvent was removed and the residue directly purified by automated flash chromatography on silica (Biotage Isolera, 25–100% EtOAc in hexanes) to give **22** as a yellow powder (11.1 mg, 27%); R_f 0.40 (100% EtOAc); mp 129–134 ºC; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 9.05 (s, 1H), 7.69 (d, *J* = 8.8 Hz, 2H), 7.29 (d, *J* = 8.7 Hz, 2H), 7.28 (s, 1H), 6.61 (t, *J* = 73.0 Hz, 1H), 5.62 (t, *J* = 56.3 Hz, 1H), 4.20 (s, 2H), 1.63 (s, 6H); ^{13}C NMR (126 MHz, $CDCl_3$) δ: 152.2, 147.8, 146.1, 144.2, 136.8, 132.4, 125.5, 119.5, 115.6, 112.6, 108.5, 70.4, 47.6 (t, *J* = 3.9 Hz), 39.0, 37.2; *m/z* (ESI+) 431 ([M+Na]$^+$, 100%); HRMS (ESI+) found 409.1281 ([M+H]$^+$), $C_{19}H_{16}F_4N_4O_2H^+$ requires 409.1282.

5-((1,2-Dicarba-*closo*-decaborane-1-yl)ethoxy)-3-(4-(difluoromethoxy)phenyl)-[1,2,4]triazolo[4,3-*a*]pyrazine (23). From **S9** (50.0 mg, 0.27 mmol) and **3a** (78.8 mg, 0.27 mmol) to give the crude product, which was purified by automated flash chromatography on silica (Biotage Isolera, 25–100% EtOAc in hexanes) to give **23** as a light brown powder (43.6 mg, 37%); R_f 0.17 (100% EtOAc); mp 179–184 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.10 (s, 1H), 7.69 (d, *J* = 8.8 Hz, 2H), 7.33 (s, 1H), 7.29 (d, *J* = 8.7 Hz, 2H), 6.66 (t, *J* = 72.7 Hz, 1H), 4.32 (t, *J* = 5.6 Hz, 2H), 2.55 (t, *J* = 5.6 Hz, 2H), 3.11–1.14 (m, 11H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 152.6, 147.9, 145.8, 142.9, 137.8, 132.5, 124.8, 119.3, 115.4 (t, *J* = 263.3 Hz), 108.7, 71.0, 68.5, 60.1, 36.4; *m/z* (ESI+) 449 ([M+H]$^+$, 20%), 471 ([M+Na]$^+$, 100%); HRMS (ESI+) found 471.2610 ([M+Na]$^+$), $C_{16}H_{22}B_{10}F_2N_4O_2Na^+$ requires 471.2614.

5-((1,7-Dicarba-*closo*-decaborane-1-yl)ethoxy)-3-(4-(difluoromethoxy)phenyl)-[1,2,4]triazolo[4,3-*a*]pyrazine (24). From **S10** (20.0 mg, 106 µmol) and **3a** (31.5 mg, 106 µmol) the solvent was removed and the residue directly purified by automated flash chromatography on silica (Biotage Isolera, 25–100% EtOAc in hexanes) to give a yellow powder (11.1 mg, 23%); repurified by automated reversed-phase flash chromatography on silica (Biotage Isolera, 5–100% MeOH in H_2O) to give **24** as a light yellow powder (8.3 mg, 17%); R_f 0.36 (100% EtOAc); mp 158–162 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.07 (s, 1H), 7.69 (d, *J* = 8.7 Hz, 2H), 7.31–7.24 (m, 3H), 6.63 (t, *J* = 73.1 Hz, 1H), 4.16 (t, *J* = 6.5 Hz, 2H), 2.25 (t, *J* = 6.5 Hz, 2H), 3.28–0.98 (m, 11H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 152.6, 147.9, 146.3, 143.3, 137.4, 132.5, 125.0, 119.2, 115.6 (t, *J* = 262.0 Hz), 108.7, 71.2, 68.9, 55.5, 34.8; ^{19}F NMR (376 MHz, $CDCl_3$) δ: -81.47; *m/z* (ESI+) 471 ([M+Na]$^+$, 100%); HRMS (ESI+) found 449.2788 ([M+H]$^+$), $C_{16}H_{22}B_{10}F_2N_4O_2H^+$ requires 499.2787.

5-((1,12-Dicarba-*closo*-decaborane-1-yl)ethoxy)-3-(4-(difluoromethoxy)phenyl)-[1,2,4]triazolo[4,3-*a*]pyrazine (25). From **S12** (20.0 mg, 106 µmol) and **3a** (31.5 mg, 106 µmol); the solvent was removed and the residue directly purified by automated flash chromatography on silica (Biotage Isolera, 25–100% EtOAc in hexanes) to give **25** as a light brown powder (16.2 mg, 34%); R_f 0.43 (100% EtOAc); mp 137–143 ºC; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 9.05 (s, 1H), 7.65 (d, *J* = 8.7 Hz, 2H), 7.25 (d, *J* = 8.8 Hz, 1H), 7.23 (s, 1H), 6.63 (t, *J* = 73.1 Hz, 1H), 3.98 (t, *J* = 6.6 Hz, 2H), 1.91 (t, *J* = 6.6 Hz, 2H), 2.86–1.05 (m, 11H); ^{13}C NMR (126 MHz, $CDCl_3$) δ: 152.6, 147.9, 146.4, 143.3, 137.2, 132.5, 124.9, 119.0, 115.7 (t, *J* = 261.7 Hz), 108.6, 70.8, 68.6, 59.2, 36.4; *m/z* (ESI+) 449 ([M+H]$^+$, 100%); HRMS (ESI+) found 449.2806 ([M+H]$^+$), $C_{16}H_{22}B_{10}F_2N_4O_2H^+$ requires 449.2801.

***tert*-Butyl 3-(5-phenethoxy-[1,2,4]triazolo[4,3-*a*]pyrazin-3-yl)pyrrolidine-1-carboxylate (26)**. From 2-phenylethanol (25.9 µL, 0.22 mmol) and **3b** (70.0 mg, 0.22 mmol); the solvent was removed and the residue directly purified by automated flash chromatography on silica (Biotage Isolera, 25–100% EtOAc in hexanes) to give **26** as a pale yellow powder (38.7 mg, 44%); R_f 0.14 (100% EtOAc); mp 123–126 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.91 (s, 1H), 7.47–7.04 (m, 6H), 4.60 (d_{app}, *J* = 17.1 Hz, 2H), 3.79 (d_{app}, *J* = 7.0 Hz, 2H), 3.69 (s, 1H), 3.40 (br s, 1H), 3.27 (t, *J* = 7.0 Hz, 2H), 2.67 (br s, 1H), 2.38 (br s, 1H), 2.16 (br s, 1H), 1.49 (s, 9H); ^{13}C NMR (126 MHz, $CDCl_3$) δ: 154.6, 154.4, 148.1, 147.9, 147.8, 144.1, 136.8, 136.7, 136.2, 136.1, 129.2, 129.1, 128.6, 128.5, 127.6, 127.5, 108.0, 107.9, 79.7, 71.0, 51.5, 50.9, 45.7, 45.4, 37.0, 36.2, 35.12, 35.07, 30.9, 30.3, 28.7 (mixture of amide rotamers); *m/z* (ESI+) 432 ([M+Na]$^+$, 100%); HRMS (ESI+) found 432.2000 ([M+Na]$^+$), $C_{22}H_{27}N_5O_3Na^+$ requires 432.2006.

5-Phenethoxy-3-(tetrahydrofuran-3-yl)-[1,2,4]triazolo[4,3-*a*]pyrazine (27). From 2-phenylethanol (107 µL, 0.89 mmol) and **3c** (200 mg, 0.89 mmol); the solvent was removed and the residue directly purified by automated flash chromatography on silica (Biotage Isolera, 25–100% EtOAc in hexanes) to give **27** as a yellow powder (205 mg, 74%); R_f 0.11 (100% EtOAc); mp 127–133 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.91 (s, 1H), 7.41–7.33 (m, 2H), 7.30 (td, *J* = 8.3, 7.8 & 4.0 Hz, 3H), 7.25 (s, 1H), 4.89–4.48 (m, 2H), 4.37–3.73 (m, 5H), 3.27 (t, *J* = 6.6 Hz, 2H), 2.86–2.47 (m, 1H), 2.31–2.10 (m, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 148.5, 147.9, 144.1, 136.8, 136.2, 129.2, 128.7, 127.6, 107.8, 72.7, 71.1, 68.5, 37.6, 35.1, 31.8; *m/z* (ESI+) 333 ([M+Na]$^+$, 100%); HRMS (ESI+) found 311.1499 ([M+H]$^+$), $C_{17}H_{18}N_4O_2H^+$ requires 311.1503.

***tert*-Butyl 4-(5-phenethoxy-[1,2,4]triazolo[4,3-*a*]pyrazin-3-yl)piperidine-1-carboxylate (28).** From 2-phenylethanol (70.9 µL, 0.59 mmol) and **3d** (200 mg, 0.59 mmol); the solvent was removed and the residue directly purified by automated flash chromatography on silica (Biotage Isolera, 25–100% EtOAc in hexanes) to give **28** as a pale yellow powder (174 mg, 69%); R_f 0.26 (100% EtOAc); mp 145–149 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.89 (s, 1H), 7.56–7.10 (m, 6H), 4.80–4.41 (m, 2H), 4.26–4.04 (m, 1H), 3.56–3.40 (m, 1H), 3.37–3.15 (m, 2H), 2.87 (t, *J* = 12.2 Hz, 2H), 2.13 (d_{app}, *J* = 10.7 Hz, 2H), 1.86 (dq, *J* = 9.3 & 2.8 Hz, 1H), 1.59–1.39 (m, 2H), 1.46 (s, 9H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 154.7, 149.0, 147.5, 144.3, 136.5, 129.0, 128.9, 127.3, 107.8, 79.9, 71.4, 48.7, 44.7, 35.5, 34.7, 28.6, 25.2; *m/z* (ESI+) 446 ([M+Na]$^+$, 100%); HRMS (ESI+) found 424.2339 ([M+H]$^+$), $C_{23}H_{29}N_5O_3H^+$ requires 424.2343.

5-Phenethoxy-3-(tetrahydro-2*H*-pyran-4-yl)-[1,2,4]triazolo[4,3-*a*]pyrazine (29). From 2-phenylethanol (50.2 µL, 0.42 mmol) and **3e** (100 mg, 0.42 mmol); the solvent was removed and the residue directly purified by automated flash chromatography on silica (Biotage Isolera, 25–100% EtOAc in hexanes) to give **29** as a pale yellow powder (95.2 mg, 70%); R_f 0.10 (100% EtOAc); mp 146–149 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.92 (s, 1H), 7.42–7.35 (m, 2H), 7.33–7.26 (m, 4H), 4.62 (t, *J* = 6.7 Hz, 2H), 4.37–3.87 (m, 2H), 3.53 (tt, *J* = 11.4 & 3.8 Hz, 1H), 3.37 (td, *J* = 11.7 & 2.1 Hz, 2H), 3.29 (t, *J* = 6.6 Hz, 2H), 2.24–2.06 (m, 2H), 1.95–1.78 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 150.9, 147.6, 144.2, 136.9, 136.0, 129.2, 128.6, 127.6, 107.8, 70.6, 67.5, 34.8, 34.2, 32.0; *m/z* (ESI+) 347 ([M+Na]$^+$, 100%); HRMS (ESI+) found 325.1656 ([M+H]$^+$), $C_{18}H_{20}N_4O_2H^+$ requires 325.1659.

5-(3,4-Difluorophenethoxy)-3-(4-iodophenyl)-[1,2,4]triazolo[4,3-*a*]pyrazine (30). From 2-(3,4-difluorophenyl)ethanol (44.4 mg, 0.28 mmol) and **3f** (100 mg, 0.28 mmol) to give the crude product, which was purified by automated flash chromatography on silica (Biotage Isolera, 25–100% EtOAc in hexanes) to give **30** as a pale yellow powder (81.1 mg, 61%); R_f 0.50 (100% EtOAc); mp 155–157 ºC; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 9.02 (s, 1H), 7.83 (d, *J* = 7.7 Hz, 2H), 7.40 (d, *J* = 7.6 Hz, 2H), 7.31 (s, 1H), 7.00 (q, *J* = 8.7 Hz, 1H), 6.54 (t, *J* = 9.2 Hz, 2H), 4.42 (t, *J* = 5.9 Hz, 3H), 2.92 (t, *J* = 5.8 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 150.3 (dd, *J* = 249.0 & 12.7 Hz), 149.5 (dd, *J* = 248.0 & 12.5 Hz), 147.9, 146.3, 143.8, 137.1, 136.8, 133.3 (dd, *J* = 5.6 & 4.0 Hz), 132.3, 127.4, 124.5 (dd, *J* = 6.1 & 3.6 Hz), 117.5 (d, *J* = 17.4 Hz), 117.4 (d, *J* = 17.3 Hz), 108.5, 96.7, 70.9, 33.9; *m/z* (ESI+) 501 ([M+Na]$^+$, 100%); HRMS (ESI+) found 500.9986 ([M+Na]$^+$), $C_{19}H_{13}F_2IN_4ONa^+$ requires 500.9994.

5-(3,4-Difluorophenethoxy)-3-(4-iodocuban-1-yl)-[1,2,4]triazolo[4,3-*a*]pyrazine (31). From 2-(3,4-dilfuorophenyl)ethanol (20.7 mg, 0.13 mmol) and **3g** (50.0 mg, 0.13 mmol) to give the crude product, which was purified by automated

flash chromatography on silica (Biotage Isolera, 25–100% EtOAc in hexanes) to give **31** as a brown powder (19.9 mg, 30%); R_f 0.23 (100% EtOAc); mp 147–153 ºC; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.86 (s, 1H), 7.47–6.73 (m, 4H), 4.64–4.35 (m, 5H), 4.26 (s, 3H), 3.16 (t, *J* = 7.5 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 148.3, 146.3, 143.3, 136.7, 132.2, 125.0, 118.3, 118.0, 117.8, 108.1, 74.1, 71.4, 54.9, 52.2, 35.4, 34.2, 29.8; *m/z* (ESI+) 527 ([M+Na]$^+$, 100%); HRMS (ESI+) found 527.0150 ([M+Na]$^+$), $C_{21}H_{15}F_2IN_4ONa^+$ requires 527.0151.

3-(4-(Difluoromethoxy)phenyl)-5-(2-(piperazin-1-yl)ethoxy)-[1,2,4]triazolo[4,3-*a*]pyrazine (32). Compound **14** (150 mg, 0.31 mmol, 1.00 equiv.) was dissolved in CH_2Cl_2 (0.88 mL). TFA (0.26 mL, 3.42 mmol, 11.2 equiv.) was added and the reaction stirred at rt overnight. The solvent was removed and the residue directly purified by automated flash chromatography on silica (Biotage Isolera, 2–15% MeOH in CH_2Cl_2) to give **32** as a light yellow powder (106 mg, 89%); mp 140–148 ºC; ^{1}H NMR (400 MHz, CD_3OD) δ: 8.99 (s, 1H), 7.81 (d, *J* = 8.8 Hz, 2H), 7.53 (s, 1H), 7.35 (d, *J* = 8.7 Hz, 2H), 7.03 (t, *J* = 73.5 Hz, 1H), 4.42 (t, *J* = 5.0 Hz, 2H), 3.16–2.94 (m, 4H), 2.70 (t, *J* = 5.0 Hz, 2H), 2.58–2.35 (m, 4H) (amine NH signal not seen); ^{13}C NMR (101 MHz, CD_3OD) δ: 154.2, 149.0, 147.8, 146.0, 136.3, 134.0, 126.0, 119.4, 117.4 (t, *J* = 259.5 Hz), 110.1, 70.1, 56.6, 50.6, 44.8; *m/z* (ESI+) 413 ([M+Na]$^+$, 100%); HRMS (ESI+) found 319.1687 ([M+H]$^+$), $C_{18}H_{20}F_2N_6O_2H^+$ requires 391.1689.

5-Phenethoxy-3-(pyrrolidin-3-yl)-[1,2,4]triazolo[4,3-*a*]pyrazine (33). Compound **26** (20.0 mg, 48.8 µmol, 1.00 equiv.) was dissolved in CH_2Cl_2 (0.13 mL). TFA (42.1 µL, 0.55 mmol, 11.2 equiv.) was added and the reaction stirred at rt overnight. The solvent was removed and the residue directly purified by automated flash chromatography on silica (Biotage Isolera, 2–10% MeOH in CH_2Cl_2) to give a clear oil; repurified by automated reversed-phase flash chromatography on silica (Biotage Isolera, 5–100% MeOH in H_2O) to give **33** as a white powder (9.1 mg, 60%); mp 78–85 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.88 (s, 1H), 7.40–7.33 (m, 2H), 7.33–7.26 (m, 3H), 7.23 (s, 1H), 4.59 (t, *J* = 6.6 Hz, 2H), 3.86 (ddt, *J* = 8.7, 7.5 & 3.6 Hz, 1H), 3.27 (t, *J* = 6.6 Hz, 2H), 3.30–3.17 (m, 2H), 3.00 (dq, *J* = 41.7 & 7.5 Hz, 2H), 2.45 (br s, 1H), 2.34–2.18 (m, 1H), 2.16–1.94 (m, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 150.7, 147.8, 144.3, 136.7, 136.3, 129.1, 128.7, 127.5, 107.7, 70.9, 53.6, 47.4, 37.9, 35.1, 32.6; *m/z* (ESI+) 310 ([M+H]$^+$, 100%); HRMS (ESI+) found 310.1659 ([M+H]$^+$), $C_{17}H_{19}N_5OH^+$ requires 310.1662.

5-Phenethoxy-3-(piperidin-4-yl)-[1,2,4]triazolo[4,3-*a*]pyrazine (34). Compound **28** (100 mg, 0.24 mmol, 1.00 equiv.) was dissolved in CH_2Cl_2 (0.62 mL). TFA (0.2 mL, 2.64 mmol, 11.2 equiv.) was added and the reaction stirred at rt overnight. The solvent was removed and the residue directly purified by automated flash chromatography on silica (Biotage Isolera, 210% MeOH in CH_2Cl_2) to give a white solid; repurified by automated reversed-phase flash chromatography on silica (Biotage Isolera, 5–100% MeOH in H_2O) to give **34** as a white powder (61.7 mg, 81%); mp 175–180 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.90 (s, 1H), 7.48–6.67 (m, 6H), 4.77–4.40 (m, 2H), 4.04 (dt, *J* = 8.1 & 4.4 Hz, 1H), 3.68–3.51 (m, 2H), 3.39–3.23 (m, 3H), 3.09 (ddd, *J* = 12.5, 9.0 & 4.1 Hz, 1H), 2.33–1.90 (m, 1H), 1.90–1.64 (m, 3H) (amine NH signal not seen); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 147.5, 147.4, 143.9, 136.4, 136.0, 129.1, 128.7, 127.4, 108.5, 71.2, 46.9, 44.1, 34.5, 32.0, 29.2, 21.1; *m/z* (ESI+) 324 ([M+H]$^+$, 100%); HRMS (ESI+) found 324.1813 ([M+H]$^+$), $C_{18}H_{21}N_5OH^+$ requires 324.1819.

5-(((1*R*,2*R*,4*S*,5*S*,6*S*)-3-Oxatricyclo[3.2.1.0^{2,4}]octan-6-yl)methoxy)-3-(4-(difluoromethoxy)phenyl)-[1,2,4]triazolo[4,3-*a*]pyrazine (35). From the late-stage biofunctionalization of 16; ^{1}H NMR (600 MHz, DMSO-d_6) δ: 9.05 (s, 1H), 7.81 (d, *J* = 8.8 Hz, 2H), 7.57 (s, 1H), 7.35 (t, *J* = 73.1 Hz, 1H), 7.34 (d, *J* = 8.7 Hz, 2H), 4.31–4.13 (m, 2H), 3.13 (d, *J* = 3.5 Hz, 1H), 3.09 (d, *J* = 2.7 Hz, 1H), 2.35 (br d, *J* = 2.5 Hz, 1H), 2.16–2.09 (m, 1H), 1.89–1.78 (m, 1H), 1.46 (ddd, *J* = 12.6, 10.2, 4.3 Hz, 1H), 1.09–1.02 (m, 1H), 0.73 (ddd, *J* = 12.6, 4.8, 2.5 Hz, 1H), 0.62 (d, *J* = 9.5 Hz, 1H); HRMS (ESI+) found 401.1416 ([M+H]$^+$), $C_{20}H_{18}F_2N_4O_3H^+$ requires 401.1420.

5-(((1*R*,2*S*,4*R*,5*S*,6*S*)-3-Oxatricyclo[3.2.1.0^{2,4}]octan-6-yl)methoxy)-3-(4-(difluoromethoxy)phenyl)-[1,2,4]triazolo[4,3-*a*]pyrazine (36). From the late-stage biofunctionalization of 16; ^{1}H NMR (600 MHz, DMSO-d_6) δ: 9.04 (s, 1H), 7.81 (d, *J* = 8.7 Hz, 2H), 7.52 (s, 1H), 7.37 (d, *J* = 8.6 Hz, 2H), 7.37 (t, *J* = 73.6 Hz, 1H), 4.19–3.96 (m, 2H), 3.08 (d, *J* = 3.8 Hz, 1H), 2.84 (d, *J* = 3.5 Hz, 1H), 2.34–2.26 (m, 1H), 1.91–1.78 (m, 1H), 1.72–1.60 (m, 1H), 1.25 (ddd, *J* = 11.1, 8.5, 2.3 Hz, 1H), 0.98 (dt, *J* = 12.7, 4.2 Hz, 1H), 0.94 (d, *J* = 10.1 Hz, 1H), 0.71 (d, *J* = 10.1 Hz, 1H); HRMS (ESI+) found 401.1417 ([M+H]$^+$), $C_{20}H_{18}F_2N_4O_3H^+$ requires 401.1420.

3-(2-((3-(4-(Difluoromethoxy)phenyl)-[1,2,4]triazolo[4,3-a]pyrazin-5-yl)oxy)ethyl)cuban-1-ol (37). From the late-stage biofunctionalization of **19**; ^{1}H NMR (600 MHz, DMSO-d_6) δ: 9.05 (s, 1H), 7.82 (d, *J* = 8.6 Hz, 2H), 7.58 (s, 1H), 7.36 (t, *J* = 73.7 Hz, 1H), 7.31 (d, *J* = 8.5 Hz, 2H), 6.02 (s, 1H), 4.30 (t, *J* = 6.2 Hz, 2H), 3.81 (dq, *J* = 5.5, 2.7 Hz, 1H), 3.41 (dt, *J* = 5.1, 2.5 Hz, 2H), 3.37 (q, *J* = 5.1 Hz, 2H), 1.85 (t, *J* = 6.2 Hz, 2H). One cubane C–H at δ 3.33 obscured by solvent peak; HRMS (ESI+) found 425.1400 ([M+H]$^+$), $C_{22}H_{18}F_2N_4O_3H^+$ requires 425.1420.

4-(2-((3-(4-(Difluoromethoxy)phenyl)-[1,2,4]triazolo[4,3-*a*]pyrazin-5-yl)oxy)ethyl)cuban-1-ol (38). From the late-stage biofunctionalization of **19**; ^{1}H NMR (600 MHz, DMSO-d_6) δ: 9.04 (s, 1H), 7.82 (d, *J* = 8.6 Hz, 2H), 7.58 (s, 1H), 7.37 (t, *J* = 73.7 Hz, 1H), 7.31 (d, *J* = 8.6 Hz, 2H), 6.15 (s, 1H), 4.32 (t, *J* = 6.0 Hz, 2H), 3.58 (t, *J* = 5.2 Hz, 3H), 3.13 (t, *J* = 5.2 Hz, 3H), 1.89 (t, *J* = 6.0 Hz, 2H); HRMS (ESI+) found 425.1402 ([M+H]$^+$), $C_{22}H_{18}F_2N_4O_3H^+$ requires 425.1420.

5-((7,8-Dicarba-*nido*-undecaborane-7-yl)ethoxy)-3-(4-(difluoromethoxy)phenyl)-[1,2,4]triazolo[4,3-*a*]pyrazine (39). Adapted from the procedure of Yoo *et al.*[58]: Compound **23** (20.0 mg, 44.6 µmol, 1 equiv.) and CsF (20.3 mg, 134 µmol, 3 equiv.) were dissolved in EtOH (1 mL) and heated at reflux for 25 h. The solvent was removed. Acetone (2 mL) was added, the solution filtered through a fritted glass funnel, washed several times with acetone and the solvent removed to give the crude product as an off-white solid; purified by automated flash chromatography on silica (Biotage Isolera, 2–10% MeOH in CH_2Cl_2) to give **39** as a yellow powder (16.4 mg, 65%); R_f 0.13 (10% MeOH in CH_2Cl_2); mp 181–190 ºC; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.94 (s, 1H), 7.76 (d, *J* = 8.9 Hz, 2H), 7.50 (s, 1H), 7.28 (d, *J* = 8.9 Hz, 2H), 6.91 (t, *J* = 73.8 Hz, 1H), 4.56 (s, 2H), 4.46–4.29 (m, 2H), 1.94–1.65 (m, 2H) (carborane BH signals not seen); ^{13}C NMR (126 MHz, CD_3OD) δ: 154.6, 149.0, 148.0, 146.3, 135.6, 133.9, 125.6, 119.2, 117.7 (t, *J* = 257.9 Hz), 109.9, 74.2, 38.8 (carborane C signals not seen); *m/z* (ESI+) 484 ([M+2Na]$^+$, 100%); HRMS (ESI+) found 484.2381 ([M+2Na]$^+$), $C_{16}H_{22}B_9CsF_2N_4O_2Na_2^+$ requires 484.2375.

2-(Oxetan-3-yl)ethan-1-ol (S1). Lithium 2-(oxetan-3-yl)acetate (150 mg) was converted to the free acid by addition of conc. HCl and extraction with EtOAc to give a colorless oil (125 mg). The free acid (100 mg, 0.86 mmol, 1 equiv.) was dissolved in anhydrous THF (2 mL) and cooled to 0 ºC. $LiAlH_4$ (1 M in THF, 0.55 mL, 0.55 mmol, 0.64 equiv.) was added dropwise and the reaction mixture stirred for 10 min at 0 ºC, then at rt. The reaction was cooled in an ice bath and the LAH quenched with EtOAc dropwise. A sat. aq. solution of Rochelle's salt was added and the mixture stirred at 0 ºC, then at rt for 1.5 h. The organic layer was separated and the aqueous layer extracted with EtOAc (2 ×). The combined organic layers were dried ($MgSO_4$), filtered and concentrated under reduced pressure to give **S1** as a yellow oil (41.4 mg, 47%); carried forward without further purification or characterisation.

***tert*-Butyl 4-(2-hydroxyethyl)piperazine-1-carboxylate (S2)** Following the procedure of del Prado *et al.*[59]: A solution of Boc_2O (2.18 mL, 9.45 mmol, 1.23 equiv.) in CH_2Cl_2 (7 mL) was added dropwise to a solution of 1-(2-hydroxyethyl)piperazine (1.00 g, 7.68 mmol, 1.00 equiv.) in CH_2Cl_2 (10 mL) at 0 ºC. The reaction was warmed to rt and stirred for 2 h. Sat. aq. NH_4Cl (6 mL) was added and the organic layer separated. The aqueous layer was extracted with CH_2Cl_2 (2 × 8 mL) and the combined organic layers were dried (Na_2SO_4), filtered and concentrated under reduced pressure to give the crude product; purified by DCVC (25–100% EtOAc in hexanes) to give **S2** as a clear oil that crystallised on standing (674 mg, 38%); R_f 0.05 (100%

EtOAc, $KMnO_4$ stain); mp 45–52 °C; IR ν_{max} (film) /cm^{-1} 3426, 2974, 2933, 2868, 2812, 1695; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 3.61 (t, *J* = 5.4 Hz, 2H), 3.42 (t, *J* = 5.1 Hz, 4H), 2.54 (t, *J* = 5.4 Hz, 2H), 2.44 (t, *J* = 5.1 Hz, 4H), 1.45 (s, 9H) (alcohol OH signal not seen); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 154.8, 79.8, 59.5, 57.9, 52.9, 28.6 (1 obscured signal). Spectroscopic data matched those in the literature.[59]

3-Cubylpropan-1-ol (S3). Adapted from the procedure of Priefer *et al.*[60]: 3-Cubylacetic acid (58.0 mg, 0.36 mmol) was suspended in anhydrous THF (5 mL) under Ar. Borane dimethylsulfide complex (2.0 M in Et_2O, 0.51 mL, 1.02 mmol) was added and the solution was left to stir for 16 h. Sat. aq. NH_4Cl (1 mL) was cautiously added and the mixture was left to stir for 20 min. The THF was removed *in vacuo*, the residue was then diluted with H_2O (10 mL) and extracted with CH_2Cl_2 (3 × 10 mL). The combined organic layers were dried ($MgSO_4$), filtered and concentrated under reduced pressure. The crude product was purified by flash chromatography on silica (50% EtOAc in petroleum ether) to give **S3** as a yellow oil (55.0 mg, 94%); IR ν_{max} (film) /cm^{-1} 3307, 2968, 1444, 1214, 1054, 881, 841; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 4.07–4.01 (m, 1H), 3.87–3.84 (m, 3H), 3.74–3.71 (m, 3H), 3.65 (t, *J* = 6.6 Hz, 2H), 1.65–1.54 (m, 4H), 1.36 (br s, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 63.5, 59.0, 48.7, 48.5, 44.2, 29.4, 27.7; HRMS (ESI+) found 163.1115 ([M+H]$^+$), $C_{11}H_{14}OH^+$ requires 163.1117.

2,2-Dimethyl-5-(octahydro-1*H*-2,4,1-(epiethane[1,1,2]triyl)cyclobuta[*cd*]pentalen-7ylidene)-1,3-dioxane-4,6-dione (S4). Following the procedure of Aleksandrov *et al.*[61]: Octahydro-1*H*-2,4,1-(epiethane[1,1,2]triyl)cyclobuta[*cd*]pentalen-7-one (150 mg, 0.94 mmol, 1.0 equiv.) was dissolved in pyridine (1 mL) and isopropylidene malonate (162 mg, 1.12 mmol, 1.2 equiv.) was added. The mixture was agitated several times within the first hour to dissolve the acid, then left to stand for 5 days. The reaction mixture was poured over H_2O (20 mL) and the precipitate filtered to give the crude product as a white powder (153 mg, 57%); carried forward without further purification; the remaining crude material was purified by recrystallisation from 50% aq. acetone to give **S4** as white plates; mp 160–164 °C (lit.[61] 157–158 °C); IR ν_{max} (film) /cm^{-1} 1726, 1606; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 4.24 (ddd, *J* = 8.4, 5.9 & 2.4 Hz, 1H), 4.16–3.98 (m, 1H), 3.18 (td, *J* = 8.6 & 3.5 Hz, 1H), 2.94 (d, *J* = 10.1 Hz, 1H), 2.79 (q, *J* = 6.4 & 6.0 Hz, 1H), 2.66 (q, *J* = 7.0 & 6.4 Hz, 1H), 2.58–2.45 (m, 2H), 1.92 (d, *J* = 10.9 Hz, 1H), 1.71 (d, *J* = 5.3 Hz, 6H), 1.52 (d, *J* = 10.9 Hz, 1H), 1.40–1.15 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 194.6, 161.2, 161.0, 111.5, 103.9, 50.8, 50.6, 47.8, 47.5, 44.2, 43.0, 42.8, 39.8, 36.9, 30.9, 27.7, 27.1. Spectroscopic data matched those in the literature.[61]

2,2-Dimethyl-5-(octahydro-1*H*-2,4,1-(epiethane[1,1,2]triyl)cyclobuta[*cd*]pentalen-7yl)-1,3-dioxane-4,6-dione (S5). Following the procedure of Aleksandrov *et al.*[61]: Compound **S4** (120 mg, 0.42 mmol, 1.0 equiv.) was suspended in EtOH (8 mL) and $NaBH_4$ (17.4 mg, 0.42 mmol, 1.1 equiv.) was added portionwise keeping the temperature below 30 °C. The reaction was stirred for 1 h then filtered. The filtrate was reduced to half the volume and poured onto a H_2O (8 mL) and AcOH (0.13 mL) mixture and left to stand for 2 h. The solid was filtered, washed with H_2O and dried in vacuo to give the crude product as white crystals (91.8 mg, 76%); carried forward without further purification; the remaining crude material was purified by recrystallisation from 50% aq. acetone to give **S5** as white plates; mp 138–143 °C (lit.[61] 136–137 °C); IR ν_{max} (film) /cm^{-1} 1742; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 3.91 (d, *J* = 9.9 Hz, 1H), 3.05–2.73 (m, 2H), 2.59 (qd, *J* = 12.2, 10.8 & 4.8 Hz, 3H), 2.47 (d, *J* = 8.8 Hz, 1H), 2.24 (br d, *J* = 17.9 Hz, 2H), 1.92 (dt, *J* = 9.9 & 3.1 Hz, 1H), 1.81 (d, *J* = 13.2 Hz, 1H), 1.73 (d, *J* = 8.1 Hz, 6H), 1.70 (s_{app}, 1H), 1.30–1.08 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 166.0, 165.8, 105.4, 47.4, 46.4, 46.3, 44.3, 42.5, 42.1, 41.7, 41.5, 38.4, 36.4, 34.1, 29.2, 28.8, 28.3. Spectroscopic data matched those in the literature.[61]

2-(Octahydro-1*H*-2,4,1-(epiethane[1,1,2]triyl)cyclobuta[*cd*]pentalen-7-yl)acetic acid (S6). Following the procedure of Aleksandrov *et al.*[61]: Compound **S5** (65.0 mg, 0.23 mmol) was dissolved in a mixture of AcOH (0.5 mL) and conc. HCl (0.1 mL) and heated at reflux for 2.5 h. The reaction was cooled to rt and poured onto H_2O (3.75 mL), cooled in ice, filtered and washed with H_2O to give the crude product as an off-white powder (32.5 mg, 70%); carried forward without further purification; the remaining crude material was purified by recrystallisation from hexane to give **S6** as white crystals; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.82–2.49 (m, 6H), 2.30 (br s, 1H), 2.27–2.14 (m, 4H), 1.93 (tt, *J* = 6.8 & 2.8 Hz, 1H), 1.69 (s, 1H), 1.68–1.59 (m, 1H), 1.17 (d, *J* = 10.7 Hz, 1H), 1.03 (dt, *J* = 12.7 & 3.5 Hz, 1H). Spectroscopic data matched those in the literature.[61]

2-(Octahydro-1*H*-2,4,1-(epiethane[1,1,2]triyl)cyclobuta[*cd*]pentalen-7-yl)ethan-1ol (S7). Compound **S6** (25.0 mg, 0.12 mmol, 1 equiv.) was dissolved in anhydrous THF (1 mL) and cooled to 0 °C. $LiAlH_4$ (1 M in THF, 78.3 µL, 78.3 µmol, 0.64 equiv.) was added dropwise and the reaction mixture stirred for 10 min at 0 °C, then at rt. The reaction was cooled in an ice bath and the LAH quenched with EtOAc dropwise. A sat. aq. solution of Rochelle's salt was added and the mixture stirred at 0 °C, then at rt for 1.5 h. The organic layer was separated and the aqueous layer extracted with EtOAc (2 ×). The combined organic layers were dried ($MgSO_4$), filtered and concentrated under reduced pressure to give the crude product as a clear oil (25.6 mg, >100%); carried forward without further purification or characterisation.

(3-(Difluoromethyl)bicyclo[1.1.1]pentan-1-yl)methanol (S8). 3-(Difluoromethyl)bicyclo[1.1.1]pentane-1-carboxylic acid (25.8 mg, 159 µmol, 1.0 equiv.) was dissolved in THF (72.3 µL, 2.2 M) and cooled to 0 °C. $LiAlH_4$ (1 M in THF, 239 µL, 239 µmol, 1.5 equiv.) was added and the reaction allowed to warm to rt. The reaction was quenched with H_2O (18 µL) and anhydrous Na_2SO_4 (103 mg) and stirred for 20 min. The mixture was filtered, washed with THF and the filtrate concentrated under reduced pressure to give **S8** as a pale yellow oil (19.6 mg, 83%); carried forward without further purification or characterisation; R_f 0.34 (25% EtOAc in hexanes, $KMnO_4$ stain); ^{1}H NMR (200 MHz, $CDCl_3$) δ: 5.70 (t, *J* = 56.5 Hz, 1H), 3.66 (s, 2H), 1.81 (s, 6H) (alcohol OH signal not seen).

1,2-Dicarba-*closo*-dodecaborane-1-ethanol (S9). Following the procedure of Li *et al.*[62]: To a solution of *closo*-1,2-carborane (100 mg, 0.69 mmol, 1 equiv.) in anhydrous THF (2.25 mL) was added *n*-BuLi (1.6 M in hexanes, 0.43 mL, 0.69 mmol, 1 equiv.) at –78 °C under N_2. Stirring was continued for 30 min, then ethylene oxide (2.5 M in THF, 0.42 mL, 1.04 mmol, 1.5 equiv.) was added dropwise. Stirring was continued for 1 h at 0 °C, then the reaction was quenched with sat. aq. NH_4Cl (1 mL). The aqueous phase was extracted with EtOAc (3 × 1.5 mL) and the combined organic layers dried (Na_2SO_4), filtered and concentrated under reduced pressure to give the crude product, which was purified by automated flash chromatography on silica (Biotage Isolera, 5–40% EtOAc in hexanes) to give **S9** as a white solid (110 mg, 66%); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 3.98 (br s, 1H), 3.79 (t, *J* = 5.9 Hz, 2H), 2.49 (t, *J* = 5.9 Hz, 2H), 2.80–1.42 (m, 11H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 73.2, 60.8, 60.7, 39.9; *m/z* (ESI–) 189 ([M-H]$^-$, 100%); HRMS (ESI–) found 189.2058 ([M-H]$^-$), $C_4H_{15}B_{10}O^-$ requires 189.2059. Spectroscopic data matched those in the literature.[63]

1,7-Dicarba-*closo*-dodecaborane-1-ethanol (S10). Adapted from the procedure of Li *et al.*[62]: To a solution of *closo*-1,7-carborane (100 mg, 0.69 mmol, 1 equiv.) in anhydrous THF (2.25 mL) was added *n*-BuLi (1.6 M in hexanes, 0.43 mL, 0.69 mmol, 1 equiv.) at –78 °C under N_2. Stirring was continued for 30 min, then ethylene oxide (2.5 M in THF, 0.42 mL, 1.04 mmol, 1.5 equiv.) was added dropwise. Stirring was continued for 1 h at 0 °C, then the reaction was quenched with sat. aq. NH_4Cl (1 mL). The aqueous phase was extracted with EtOAc (3 × 1.5 mL) and the combined organic layers dried (Na_2SO_4), filtered and concentrated under reduced pressure to give the crude product, which was purified by automated flash chromatography on silica (Biotage Isolera, 6–50% EtOAc in hexanes) to give **S10** as a white solid (30.7 mg, 23%); R_f 0.47 (25% EtOAc in hexanes, $KMnO_4$ stain); ^{1}H NMR (200 MHz, $CDCl_3$) δ: 3.88–3.32 (m, 2H), 2.94 (s, 1H), 2.51–2.00 (m, 2H), 3.29–0.71 (m, 11H); *m/z* (APCI+) 191 ([M+H]$^+$, 100%); HRMS (ESI–) found 189.2058 ([M-

H]⁻), $C_4H_{15}B_{10}O^-$ requires 189.2059. Spectroscopic data matched those in the literature.[64]

1-(2-Benzyloxyethyl)-1,12-dicarba-*closo*-dodecaborane (S11). Following the procedure of Fujii *et al.*[65]: To a solution of *closo*-1,12-carborane (120 mg, 0.83 mmol, 1.0 equiv.) in anhydrous Et_2O (2.88 mL) was added *n*-BuLi (1.6 M in hexanes, 572 µL, 0.92 mmol, 1.1 equiv.) at 0 ºC under Ar. The reaction was stirred at rt for 1 h, then benzyl 2-bromoethyl ether (163 µL, 1.00 mmol, 1.2 equiv.) was added and the reaction stirred for 16 h. The reaction was cooled to 0 ºC, quenched with H_2O and diluted with EtOAc. The organic layer was washed with H_2O, brine, dried (Na_2SO_4), filtered and concentrated under reduced pressure to give the crude product, which was purified by automated flash chromatography on silica (Biotage Isolera, 6–50% EtOAc in hexanes) to give **S11** as a clear colorless oil (108 mg, 47%); R_f 0.83 (10% EtOAc in hexanes, $KMnO_4$ stain); ^{1}H NMR (200 MHz, $CDCl_3$) δ: 7.63–6.97 (m, 5H), 4.40 (s, 2H), 3.26 (t, *J* = 7.2 Hz, 2H), 2.64 (br s, 1H), 1.95 (t, *J* = 7.1 Hz, 2H), 3.72–0.63 (m, 10H). Spectroscopic data matched those in the literature.[65]

1,12-Dicarba-*closo*-dodecaborane-1-ethanol (S12) Adapted from the procedure of Mandal *et al.*[66]: Compound **S11** (90 mg, 0.32 mmol, 1.00 equiv.) and 5% Pd/C (34.4 mg, 0.02 mmol, 0.05 equiv.) were suspended in EtOH (2 mL). The reaction purged with Ar and Et_3SiH (0.52 mL, 3.23 mmol, 10.0 equiv.) was added dropwise and the reaction stirred at rt. The mixture was filtered through a pad of celite and concentrated under reduced pressure to give **S12** as a pale yellow solid (40.7 mg, 67%); carried forward without further purification; $\boldsymbol{R_f}$ 0.26 (10% EtOAc in hexanes, $KMnO_4$ stain); **^{1}H NMR** (400 MHz, $CDCl_3$) δ: 3.45 (t, *J* = 7.0 Hz, 2H), 2.66 (br s, 1H), 2.91–1.26 (m, 11H), 1.90 (t, *J* = 7.0 Hz, 2H). Spectroscopic data matched those in the literature.[63]

4-Iodobenzaldehyde (S13). Following the procedure of Yamashita *et al.*[67]: 1,3-Dimethyl-2-imidazolidinone (15 mL) was added to 4-bromobenzaldehyde (1.00 g, 5.40 mmol, 1.0 equiv.), KI (8.07 g, 48.6 mmol 9.0 equiv.) and CuI (3.19 g, 17.8 mmol, 3.1 equiv.). The mixture was purged with N_2 and heated with vigorous stirring at 200 ºC for 23 h. After cooling to rt, brine and ice were added and the reaction was placed in an ice bath for 3 h. The precipitated inorganic salts were removed by filtration and the filtrate extracted with Et_2O. The combined organic layers were washed with brine, dried (Na_2SO_4), filtered and concentrated under reduced pressure to give the crude product, which was purified by automated flash chromatography on silica (Biotage Isolera, 12–75% EtOAc in hexanes) to give **S13** as a yellow powder (263 mg, 21%); R_f 0.87 (50% EtOAc in hexanes); mp 72–79 ºC (lit.[68] 77–78 ºC); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.96 (s, 1H), 7.91 (d, *J* = 8.2 Hz, 2H), 7.59 (d, *J* = 8.5 Hz, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 191.5, 138.6, 135.7, 130.9, 103.0. Spectroscopic data matched those in the literature.[67]

1-Iodo-4-(hydroxymethyl)cubane (S14). Following the procedure of Griffiths *et al.*[69]: 4-Iodocubanecarboxylic acid[65] (200 mg, 0.73 mmol) was dissolved in anhydrous THF (7 mL) under Ar and cooled to 0 ºC. Borane dimethylsulfide complex (0.23 mL, 1.16 mmol) was added and the reaction stirred at 0 ºC for 20 min, then at rt for 4 h. The solution was quenched with H_2O and stirred overnight. After adding EtOAc, the solution was washed with H_2O, brine, dried ($MgSO_4$), filtered and concentrated under reduced pressure to give **S14** as a white solid (162 mg, 85%); carried forward without further purification; R_f 0.80 (100% EtOAc); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 4.21 (dd, *J* = 5.8 & 4.4 Hz, 3H), 4.04 (dd, *J* = 5.8 & 4.2 Hz, 3H), 3.77 (s, 2H) (alcohol OH signal not seen); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 63.2, 59.1, 54.8, 48.0, 39.0. Spectroscopic data matched those in the literature.[69]

1-Iodocubane-4-carboxaldehyde (S15). Following the procedure of Griffiths *et al.*[69]: A stirring solution of oxalyl chloride (63.8 µL, 0.75 mmol) in anhydrous CH_2Cl_2 (1 mL) was prepared under Ar at –78 ºC. Anhydrous DMSO (0.11 mL, 1.54 mmol) in anhydrous CH_2Cl_2 (1 mL) was added dropwise. After 20 min at –78 ºC, a solution of **S14** (162 mg, 0.62 mmol) in anhydrous CH_2Cl_2 (4.25 mL) was added dropwise under Ar. The mixture was maintained at –78 ºC for 1.5 h, then anhydrous Et_3N (0.39 mL, 2.80 mmol) was added. The mixture was warmed to rt and quenched with H_2O (4 mL). The aqueous layer was extracted with CH_2Cl_2 (2 × 4 mL) and the combined organic layers washed with H_2O (4 mL), brine (4 mL), dried ($MgSO_4$), filtered and concentrated under reduced pressure to give the crude product, which was purified by automated flash chromatography on silica (Biotage Isolera, 12–100% EtOAc in hexanes) to give **S15** as a white solid (130 mg, 81%); R_f 0.84 (50% EtOAc in hexanes); mp 124–130 ºC (lit.[69] 106–109 ºC); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 9.74 (s, 1H), 4.56–4.47 (m, 3H), 4.33–4.25 (m, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 197.1, 62.8, 54.8, 49.0, 35.6. Spectroscopic data matched those in the literature.[69]

***In Vitro* Antiplasmodial Activity (Drug Discovery Unit, University of Dundee).[70]** Cultures of the widely-used malaria reference strain of chloroquine-sensitive *P. falciparum* strain 3D7 were maintained in a 1.25% or 5% suspension of human red blood cells cultured in RPMI 1640 medium supplemented with 0.5% Albumax II (available from Gibco Life Technologies, San Diego, CA, cat. no. 11021-037), 12 mM sodium bicarbonate, 0.2 mM hypoxanthine, (pH 7.3), and 20 mg/L gentamicin at 37 ºC, in a humified atmosphere of 1% O_2, 3% CO_2 with a gas balance of nitrogen. Growth inhibition of the *P. falciparum* cultures was quantified in a 10-point dose response curve with a 1 in 3 dilution series. This 384 well plate based fluorescence assay utilises the binding of SYBRgreen I (Thermo Fisher Scientific/Invitrogen cat. no. S7585) to double stranded DNA, which greatly increases the fluorescent signal at 528 nm after excitation at 485 nm. Mefloquine was used as a drug control to monitor the quality of the assay (Z' = 0.6 to 0.8, where Z' is a measure of the discrimination between the positive and negative controls on a screen plate). Dose-response curves were determined from a minimum of 3 independent experiments. Compound bioactivity was expressed as IC_{50}, the concentration of compound causing 50% inhibition. IC_{50} values were determined from a minimum of 3 independent experiments. All data was processed using IDBS ActivityBase® raw data was converted into per cent inhibition through linear regression by setting the high inhibition control as 100% and the no inhibition control as 0%. Quality control criteria for passing plates were as follows: Z' >0.5, S:B >3, %CV(no inhibition control) <15. The formula used to calculate $Z' = 1 - \frac{3 \times (StDev_{high} + StDev_{low})}{ABS(Mean_{high} - Mean_{low})}$. All IC_{50} Curve fitting was undertaken using XLFit version 4.2 using Model 205 with the following 4 parametric equation: $y = A + \frac{B-A}{1+(C/x)^D}$, where A = % inhibition at bottom, B = % inhibition at top, C = IC_{50}, D = slope, x = inhibitor concentration and y = % inhibition. If curve did not reach 100% of inhibition, B was fixed to 100 only when at least 50% of inhibition was reached.

Cytotoxicity Studies (Drug Discovery Unit, University of Dundee).[70] *In vitro* cytotoxicity studies were carried out using HepG2 (Human Caucasian hepatocyte carcinoma, ECACC cat. no. 85011430) used as indicators for general mammalian cell toxicity. HepG2 *in vitro* cytotoxicity can be assessed using the assay procedure as described.[71]

Kinetic Solubility Estimation using Nephelometry (Centre for Drug Candidate Optimization, Monash Institute of Pharmaceutical Sciences). Compound in DMSO was spiked into either pH 6.5 phosphate buffer or 0.01 M HCl (approx. pH 2.0) at 7 concentrations with the final DMSO concentration of each being 1%. After 30 minutes had elapsed, samples were then analyzed for precipitation *via* Nephelometry to determine a solubility range.[72]

Distribution Coefficient Estimation using Chromatography (Centre for Drug Candidate Optimization, Monash Institute of Pharmaceutical Sciences). Partition coefficient values (LogD) of the test compounds were estimated at pH 7.4 by correlation of their chromatographic retention properties (mean of 2 injections) against the characteristics of a series of standard compounds with known partition coefficient values. The method employed is a gradient HPLC based derivation of the method developed by Lombardo.[73]

***In Vitro* Metabolic Stability (Centre for Drug Candidate Optimization, Monash Institute of Pharmaceutical Sciences).** The metabolic stability assay was performed by incubating each test compound with liver microsomes at 37 ºC and a protein concentration of

0.4 mg/mL. The metabolic reaction was initiated by the addition of an NADPH-regenerating system and quenched at 5 time points over a 60 minute incubation period by the addition of acetonitrile containing diazepam as internal standard. Control samples (containing no NADPH) were included (and quenched at 2, 30 and 60 minutes) to monitor for potential degradation in the absence of cofactor. All liver microsomes used in these experiments were purchased from XenoTech. Microsomal incubations were performed at a substrate concentration of 1 µM. The half-life and intrinsic clearance were determined by exponential regression of the concentration vs time data.

Late-Stage Biofunctionalization of Compounds 16 and 19 (Obach Lab, Pfizer). Compound **19** (40 µM) was incubated with dog liver microsomes (1 mg/mL) in a total volume of 40 mL potassium phosphate buffer (0.1 M; pH 7.4) containing $MgCl_2$ (3.3 mM) and NADPH (1.3 mM). The incubation was done in a 500 mL Erlenmeyer flask in a shaking water bath maintained at 37 ºC open to the air. After 1 h, the incubation was terminated by addition of MeCN (40 mL) and the precipitated protein was removed by spinning at 1700 × g for 5 min. The supernatant was partially evaporated in a vacuum centrifuge for 1.5 h and to the remaining liquid was added formic acid (0.5 mL), MeCN (0.5 mL) and H_2O to a final volume of 50 mL. This mixture was spun in a centrifuge at 40000 × g for 30 min. The clarified supernatant was applied to a Varian Polaris C18 column (4.6 × 250 mm; 5 µm) at 0.8 mL/min through a Jasco HPLC pump. After the entire solution was applied and another 5 mL of 0.1% formic acid in H_2O was washed through the system, the column was moved to an HPLC-UV-MS (Thermo Velos LTQ mass spectrometer, equipped with a Waters Acquity HPLC-UV system) in line with a fraction collector (Leap Analytics). A mobile phase gradient was applied at 0.8 mL/min beginning with 2% MeCN in 0.1% aqueous formic acid, raised to 20% MeCN at 1 min, held until 5 min, then increased linearly to 55% MeCN at 80 min, followed by a 10 min wash at 95% MeCN and 10 min re-equilibration to initial conditions. Fractions were collected every 20 sec. Fractions predicted to contain products of interest eluted around 39.5 and 42.0 min and were individually analysed on a Thermo Orbitrap Elite UHPLC-UV-HRMS system to ascertain identity and purity for pooling and qNMR analysis. Pooled fractions were evaporated in a vacuum centrifuge and the residue was taken up in DMSO-d_6 (0.05 mL). Compound **16** was subjected to a similar procedure except that rabbit liver microsomes were used as the source of enzyme, the substrate concentration was 25 µM, and the incubation time was 45 min. The fractionation was carried out similarly, except that the MeCN composition was raised to 70% instead of 55%.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website.

Table of molecular formula strings, *in vitro* potency values and calculated properties for target compounds (CSV)
^{1}H and ^{13}C NMR spectra for synthesized compounds (PDF)
Graphical table of *in vitro* potencies for target compounds (PDF)

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E.G.T. led the synthetic chemistry (laboratory-based and data interpretation), supervised by M.H.T., S.D.H., C.M.W., G.P.S. and L.M.R. provided reagents, protocols and experimental advice. I. H. developed the *Pf* assay, manually prepared and analyzed the compounds, collated all data and contributed to the paper (methods section and data table). M. A. carried out the routine *Pf* assay and analysis. R.S., G.S.W. and R.S.O. performed the biofunctionalization experiments and contributed data interpretation. E.G.T. and M.H.T. wrote the paper with inputs from all authors. M.H.T. conceived the study, secured the funding and founded Open Source Malaria where much of the work took place.

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Conflict of Interest

CSIRO (GPS) and UQ (CMW) have a formal relationship with Boron Molecular Pty Ltd. who supply cubane intermediates. RSO, GSW, and RS are employed by and shareholders in Pfizer Inc.

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ABBREVIATIONS

APCI, atmospheric-pressure chemical ionization; BCP, bicyclo[1.1.1]pentane; BNCT, boron neutron capture therapy; CCDC, Cambridge Crystallographic Data Centre; CNS, central nervous system; DCVC, dry column vacuum chromatography; hERG, human ether-à-go-go related gene; HLM, human liver microsomes; $LpPLA_2$, lipoprotein-associated phospholipase A_2; MLM, mouse liver microsomes; MMV, Medicines for Malaria Venture; OSM, Open Source Malaria; RCH, rat cryopreserved hepatocytes; UCSF, University of California San Francisco.

REFERENCES

[1] Ritchie, T. J.; Macdonald, S. J. F. The Impact of Aromatic Ring Count on Compound Developability – Are too many Aromatic Rings a Liability in Drug Design? *Drug Discov. Today* **2009**, *14*, 1011-1020.

[2] Ritchie, T. J.; Macdonald, S. J. F. Physicochemical Descriptors of Aromatic Character and Their Use in Drug Discovery. *J. Med. Chem.* **2014**, *57*, 7206-7215.

[3] Ishikawa, M.; Hashimoto, Y. Improvement in Aqueous Solubility in Small Molecule Drug Discovery Programs by Disruption of Molecular Planarity and Symmetry. *J. Med. Chem.* **2011**, *54*, 1539-1554.

[4] Walker, M. A. Novel Tactics for Designing Water-Soluble Molecules in Drug Discovery. *Expert Opin. Drug Discovery* **2014**, *9*, 1421-1433.

[5] Ritchie, T. J.; Macdonald, S. J. F. Heterocyclic Replacements for Benzene: Maximising ADME Benefits by Considering Individual Ring Isomers. *Eur. J. Med. Chem.* **2016**, *124*, 1057-1068.

[6] WHO World Malaria Report 2018. https://www.who.int/malaria/publications/world-malaria-report-2018/en/ (accessed Apr 4, 2018).

[7] Fight Against Malaria Stalling and Could Reverse, Warns 2017 World Malaria Report. https://www.devex.com/news/fight-against-malaria-stalling-and-could-reverse-warns-2017-world-malaria-report-91636 (accessed Mar 24, 2018).

[8] Global Malaria Report Reveals Africa's Hits and Missed: Here's What to Do. https://www.devex.com/news/fight-against-malaria-stalling-andcould-reverse-warns-2017-world-malaria-report-91636 (accessed Mar 24, 2018).

[9] a) Williamson, A. E.; Ylioja, P. M.; Robertson, M. N.; Antonova-Koch, Y.; Avery, V.; Baell, J. B.; Batchu, H.; Batra, S.; Burrows, J. N.; Bhattacharyya, S.; Calderon, F.; Charman, S. A.; Clark, J.; Crespo, B.; Dean, M.; Debbert, S. L.; Delves, M.; Dennis, A. S. M.; Deroose, F.; Duffy, S.; Fletcher, S.; Giaever, G.; Hallyburton, I.; Gamo, F.-J.; Gebbia, M.; Guy, R. K.; Hungerford, Z.; Kirk, K.; Lafuente-Monasterio, M. J.; Lee, A.; Meister, S.; Nislow, C.; Overington, J. P.; Papadatos, G.; Patiny, L.; Pham, J.; Ralph, S. A.; Ruecker, A.; Ryan, E.; Southan, C.; Srivastava, K.; Swain, C.; Tarnowski, M. J.; Thompson, P.; Turner, P.; Wallace, I. M.; Wells, T. N. C.; White, K.; White, L.; Willis, P.; Winzeler, E. A.; Wittlin, S.; Todd, M. H. Open Source Drug Discovery: Highly Potent Antimalarial Compounds Derived from the Tres Cantos Arylpyrroles. *ACS Cent. Sci.* **2016**, *2*, 687-701. b) Electronic Laboratory Notebook available at https://ses.library.usyd.edu.au/handle/2123/21118 (DOI: 10.25910/5d7af492b2c63).

[10] Potential new class of antimalarials now open source. https://www.mmv.org/newsroom/news/potential-new-class-antimalarials-now-open-source (accessed Nov 8, 2018).

[11] a) OSM Series 4 Wiki *In Vivo* Efficacy. https://github.com/OpenSourceMalaria/Series4/wiki/In-Vivo-Efficacy (accessed Nov 14, 2019). b) Compound **6**, containing a phenyl ring, was chosen as the comparison compound over either of the known *in vivo* actives because the latter contain difluorophenyl motifs; the comparison is valid because the potencies, solubilities and *in vitro* clearance values for the three compounds are similar (manuscript in preparation).

[12] Friedman, H. L. in *First Symposium on Chemical-Biological Correlation, May 26-27, 1950*, NAS-NRS: Washington, DC, **1951**, 295-358.

[13] Meanwell, N. A. Synopsis of some Recent Tactical Application of Bioisosteres in Drug Design. *J. Med. Chem.* **2011**, *54*, 2529-2591.

[14] Wanka, L.; Iqbal, K.; Schreiner, P. R. The Lipophilic Bullet Hits the Targets: Medicinal Chemistry of Adamantane Derivatives. *Chem Rev.* **2013**, *113*, 3516-3604.

[15] Carreira, E. M.; Fessard, T. C. Four-Membered Ring-Containing Spirocycles: Synthetic Strategies and Opportunities. *Chem. Rev.* **2014**, *114*, 8257-8322.

[16] Stockdale, T. P.; Williams, C. M. Pharmaceuticals that Contain Polycyclic Hydrocarbon Scaffolds. *Chem. Soc. Rev.* **2015**, *44*, 7737-7763.

[17] Locke, G. M.; Bernhard, S. S. R.; Senge, M. O. Nonconjugated Hydrocarbonds as Rigid-Linear Motifs: Isosteres for Material Sciences and Bioorganic and Medicinal Chemistry. *Chem. Eur. J.* **2018**, *25*, 4590-4647.

[18] Wlochal, J.; Davies, R. D. M.; Burton, J. Cubanes in Medicinal Chemistry: Synthesis of Functionalized Building Blocks. *Org. Lett.* **2014**, *16*, 4094-4097.

[19] Biegawiewicz, K. F.; Griffiths, J. R.; Savage, G. P.; Tsanaktsidis, J.; Priefer, R. Cubane: 50 Years Later. *Chem. Rev.* **2015**, *115*, 6719-6745.

[20] Reekie, T. A.; Williams, C. M.; Rendina, L. M.; Kassiou, M. Cubanes in Medicinal Chemistry. *J. Med. Chem.* **2019**, *62*, 1078-1095.

[21] Chalmers, B. A.; Xing, H.; Houston, S.; Clark, C.; Ghassabian, S.; Kuo, A.; Cao, B.; Reitsma, A.; Murray, C.-E. P.; Stok, J. E.; Boyle, G. M.; Pierce, C. J.; Littler, S. W.; Winkler, D. A.; Bernhardt, P. V.; Pasay, C.; De Voss, J. J.; McCarthy, J.; Parsons, P. G.; Walter, G. H.; Smith, M. T.; Cooper, H. M.; Nilsson, S. K.; Tsanaktsidis, J.; Savage, G. P.; Williams, C. M. Cubane as a Benzene Bioisostere. *Angew. Chem Int. Ed.* **2016**, *55*, 3580-3585; *Angew. Chem.* **2016**, *128*, 3644-3649.

[22] Houston, S. D.; Fahrenhorst-Jones, T.; Xing, H.; Chalmers, B. A.; Sykes, M. L.; Stok, J. E.; Soto, C. F.; Burns, J. M.; Bernhardt, P. V.; De Voss, J. J.; Boyle, G. M.; Smith, M. T.; Tsanaktsidis, J.; Savage, G. P.; Avery, V. M.; Williams, C. M. The Cubane Paradigm in Bioactive Molecule Discovery: Further Scope, Limitations and the Cyclooctatetraene Complement. *Org. Biomol. Chem.* **2019**, *17*, 6790-6798.

[23] Caputo, D. F. J.; Arroniz, C.; Dürr, A. B.; Mousseau, J. J.; Stepan, A. F.; Mansfield, S. J.; Anderson, E. A. Synthesis and Applications of Highly Functionalized 1-Halo-3-substituted Bicyclo[1.1.1]pentanes. *Chem. Sci.* **2018**, *9*, 5295-5300.

[24] Stepan, A. F.; Subramanyam, C.; Efremov, I. V.; Dutra, J. K.; O'Sullivan, T. J.; DiRico, K. J.; McDonald, W. S.; Won, A.; Dorff, P. H.; Nolan, C. E.; Becker, S. L.; Pustilnik, L. R.; Riddell, D. R.; Kauffman, G. W.; Kormos, B. L.; Zhang, L.; Lu, Y.; Capetta, S. H.; Green, M. E.; Karki, K.; Sibley, E.; Atchison, K. P.; Hallgren, A. J.; Oborski, C. E.; Robshaw, A. E.; Sneed, B.; O'Donnell, C. J. Application of the Bicyclo[1.1.1]pentane Motif as a Nonclassical Phenyl Ring Bioisostere in the Design of a Potent and Orally Active γ-Secretase Inhibitor. *J. Med. Chem.* **2012**, *55*, 3414-3424.

[25] Measom, N. D.; Down, K. D.; Hirst, D. J.; Jamieson, C.; Manas, E. S.; Patel, V. K.; Somers, D. O. Investigation of a Bicyclo[1.1.1]pentane as a Phenyl Replacement within an $LpPLA_2$ Inhibitor. *ACS Med. Chem. Lett.* **2017**, *8*, 43-48.

[26] Ma, X.; Pham, L. N. Selected Topics in the Syntheses of Bicyclo[1.1.1]pentane (BCP) Analogues. *Asian J. Org. Chem.* **2019**, *9*, 8-22.

[27] Nugent, J.; Arroniz, C.; Shire, B. R.; Sterling, A. J.; Pickford, H. D.; Wong, M. L. J.; Mansfield, S. J.; Caputo, D. F. J.; Owen, B.; Mousseau, J. J.; Duarte, F.; Anderson, E. A. A General Route to Bicyclo[1.1.1]pentanes through Photoredox Catalysis. *ACS Catal.* **2019**, *9*, 9568-9574.

[28] Issa, F.; Kassiou, M.; Rendina, L. M. Boron in Drug Discovery: Carboranes as Unique Pharmacophores in Biologically Active Compounds. *Chem. Rev.* **2011**, *111*, 5701-5722.

[29] Beer, M. L.; Lemon, J.; Valliant, J. F. Preparation and Evaluation of Carborane Analogues of Tamoxifen. *J. Med. Chem.* **2010**, *53*, 8012-8020.

[30] Scholz, M.; Bensdorf, K.; Gust, R.; Hey-Hawkins, E. Asborin: The Carbaborane Analogue of Aspirin. *ChemMedChem* **2009**, *4*, 746-748.

[31] Scholz, M.; Steinhagen, M.; Heiker, J. T.; Beck-Sickinger, A. G.; Hey-Hawkins, E. Asborin Inhibits Aldo/Keto Reductase 1A1. *ChemMedChem* **2011**, *6*, 89-93.

[32] Wilkinson, S. M.; Gunosewoyo, H.; Barron, M. L.; Boucher, A.; McDonnell, M.; Turner, P.; Morrison, D. E.; Bennett, M. R.; McGregor, I. S.; Rendina, L. M.; Kassiou, M. The First CNS-Active Carborane: A Novel $P2X_7$ Receptor Antagonist with Antidepressant Activity. *ACS Chem Neurosci.* **2014**, *5*, 335-339.

[33] Nicolaou, K. C.; Vourloumis, D.; Totokotsopoulos, S.; Papakyriakou, A.; Karsunky, H.; Fernando, H.; Gavrilyuk, J.; Webb, D.; Stepan, A. F. Synthesis and Biopharmaceutical Evaluation of Imatinib Analogues Featuring Unusual Structural Motifs. *ChemMedChem* **2016**, *11*, 31-37.

[34] Auberson, Y. P.; Brocklehurst, C.; Furegati, M.; Fessard, T. C.; Koch, G.; Decker, A.; La Vecchia, L.; Briard, E. Improving Nonspecific Binding and Solubility: Bicycloalkyl Groups and Cubanes as *para*-Phenyl Bioisosteres. *ChemMedChem* **2017**, *12*, 590-598.

[35] Shultz, M. D. Two Decades under the Influence of the Rule of Five and the Changing Properties of Approved Oral Drugs. *J. Med. Chem.* **2019**, *62*, 1701-1714.

[36] OSM Series 4 Wiki Mechanism of Action: Possible PfATP4 Activity Deduced from Parasite Ion Regulation Assays. https://github.com/OpenSourceMalaria/Series4/wiki/Mechanism-of-Action%3A-Possible-PfATP4-Activity-Deduced-from-Parasite-Ion-Regulation-Assays (accessed Apr 19, 2020).

[37] Lehane, A. M.; Ridgway, M. C.; Baker, E.; Kirk, K. Diverse Chemotypes Disrupt Ion Homeostasis in the Malaria Parasite. *Mol. Microbiol.* **2014**, *94*, 327-339.

[38] Scholz, M.; Hey-Hawkins, E. Carbaboranes as Pharmacophores: Properties, Synthesis, and Application Strategies. *Chem. Rev.* **2011**, *111*, 7035-7062.

[39] Lesnikowski, Z. J. Boron Units as Pharmacophores – New Applications and Opportunities of Boron Cluster Chemistry. *Collect. Czech. Chem. Commun.* **2007**, *72*, 1646-1658.

[40] Valliant, J. F.; Schaffer, P.; Stephenson, K. A.; Britten, J. F. Synthesis of Boroxifen, A *Nido*-Carborane Analogue of Tamoxifen. *J. Org. Chem.* **2002**, *67*, 383-387.

[41] Reynolds, R. C.; Campbell, S. R.; Fairchild, R. G.; Kisliuk, R. L.; Micca, P. L.; Queener, S. F.; Riordan, J. M.; Sedwick, W. D.; Waud, W. R.; Leung, A. K. W.; Dixon, R. W.; Suling, W. J.; Borhani, D. W. Novel Boron-Containing, Nonclassical Antifolates: Synthesis and Preliminary Biological and Structural Evaluation. *J. Med. Chem.* **2007**, *50*, 3283-3289.

[42] Eaton, P. E. Cubanes: Starting Materials for the Chemistry of the 1990s and the New Centure. *Angew. Chem. Int. Ed.* **1992**, *31*, 1421-1436.

[43] Bashir-Hashemi, A. Cubanes: Super explosives and potential pharmaceutical intermediates. *A. NASA Conf. Pub.* **1994**, 127-130.

[44] Uetrecht, J. P.; Trager, W. *Drug Metabolism: Chemical and Enzymatic Aspects*, CRC Press, **2007**, pp 71-75.

[45] Feng, Y.; Liu, L.; Wang, J.-T.; Zhao, S.-W.; Guo, Q.-X. Homolytic C–H and N–H Bond Dissociation Energies of Strained Organic Compounds. *J. Org. Chem.* **2004**, *69*, 3129-3138.

[46] Jin, Y.; Lipscomb, J. D. Probing the Mechanism of C–H Activation: Oxidation of Methylcubane by Soluble Methane Monooxygenase from *Methylosinus trichosporium* OB3b. *Biochemistry* **1999**, *38*, 6178-6186.

[47] Sarkar, M. R.; Houston, S. D.; Savage, G. P.; Williams, C. M.; Krenske, E. H.; Bell, S. G.; De Voss, J. J. Rearrangement-Free Hydroxylation of Methylcubanes by a Cytochrome P450: The Case for Dynamical Coupling of C–H Abstraction and Rebound. *J. Am. Chem. Soc.* **2019**, *141*, 19688-19699.

[48] Eaton, P. E.; Yip, Y. C. The Preparation and Fate of Cubylcarbinyl Radicals. *J. Am. Chem. Soc.* **1991**, *113*, 7692-7697.

[49] Annese, C.; D'Accolti, L.; Fusco, C.; Gandolfi, R.; Eaton, P. E.; Curci, R. Oxyfunctionalization of Non-Natural Targets by Dioxiranes. 6. On the Selective Hydroxylation of Cubane. *Org. Lett.* **2009**, *11*, 3574-3577.

[50] Collin, D. E.; Folgueiras-Amador, A. A.; Pletcher, D.; Light, M. E.; Linclau, B.; Brown, R. C. D. Cubane Electrochemistry: Direct Conversion of Cubane Carboxylic Acids to Alkoxy Cubanes Using the Hofer-Moest Reaction under Flow Conditions. *Chem. – Eur. J.* **2019**, *26*, 374-378.

[51] Bandak, D.; Babii, O.; Vasiuta, R.; Komarov, I. V.; Mykhailiuk, P. K. Design and Synthesis of Novel ^{19}F-Amino Acid: A Promising ^{19}F NMR Label for Peptide Studies. *Org. Lett.* **2015**, *17*, 226-229.

[52] Lamoureux, G.; Artavia, G. Use of the Adamantane Structure in Medicinal Chemistry. *Curr. Med. Chem.* **2010**, *17*, 2967-2978.

[53] Xing, H.; Houston, S. D.; Chen, X.; Ghassabian, S.; Fahrenhorst-Jones, T.; Kuo, A.; Murray, C.-E. P.; Conn, K.-A.; Jaeschke, K. N.; Jin, D.-Y.; Pasay, C.; Bernhardt, P. V.; Burns, J. M.; Tsanaktsidis, J.; Savage, G. P.; Boyle, G. M.; De Voss, G. J.; McCarthy, J.; Walter, G. H.; Burne, T. H. J.; Smith, M. T.; Tie, J.-K.; Williams, C. M. Cyclooctatetraene: A Bioactive Cubane Paradigm Complement. *Chem. Eur. J.* **2019**, *25*, 2729-2734.

[54] Morel-Desrosiers, N.; Morel, J.-P. Standard Molar Enthalpies, Volumes, and Heat Capacities of Adamantane in Cyclohexane, *n*-Hexane, and Carbon Tetrachloride. Interpretation using the Scaled-Particle Theory. *J. Solution Chem.* **1979**, *8*, 579-592.

[55] Bohn, R. K.; Bohn, M. D. The Molecular Structures of 1,2-, 1,7-, and 1,12-dicarboclosododecarborane (12), B10C2H12. *Inorg. Chem.* **1971**, *10*, 350-355.

[56] Bradac, J.; Furek, Z.; Janezic, D.; Molan, S.; Smerkolj, I.; Stanovnik, B.; Tisler, M.; Vercek, B. Heterocycles. 167. Telesubstitution and other Transformations of Imidazo[1,2-*a*]- and *s*-Triazolo[4,3-*a*]pyrazines. *J. Org. Chem.* **1977**, *42*, 4197-4201.

[57] Houston, S. D.; Xing, H.; Bernhardt, P. V.; Vanden Berg, T. J.; Tsanaktsidis, J.; Savage, G. P.; Williams, C. M. Cyclooctatetraenes through Valence Isomerization of Cubanes: Scope and Limitations. *Chem. Eur. J.* **2019**, *25*, 2735-2739.

[58] Yoo, J.; Hwang, J.-W.; Do, Y. Facile and Mild Deboronation of *o*-Carboranes Using Cesium Fluoride. *Inorg. Chem.* **2001**, *40*, 568-570.

[59] del Prado, A.; Navarro, R.; Levkin, P.; Gallardo, A.; Elvira, C.; Reinecke, H. Dual Stimuli-Responsive Polyamines Derived from Modified *N*-Vinylpyrrolidones through CuAAC Click Chemistry. *J. Polym. Sci., Part A: Polym. Chem.* **2016**, *54*, 1098-1108.

[60] Priefer, R.; Farrell, P. G.; Harpp, D. N. Effective Synthetic Routes to Cubylcarbinol Derivatives. *Synthesis* **2002**, *18*, 2671-2673.

[61] Aleksandrov, A. M.; Pehk, T. J.; Petrenko, A. E.; Watson, W. H. Accessible Route to 4-Substituted "Bird-Cage" Hydrocarbon Derivatives. *J. Org. Chem.* **1994**, *59*, 3709-3711.

[62] Li, K.; Wang, Y.; Yang, G.; Byun, S.; Rao, G.; Shoen, C.; Yang, H.; Gulati, A.; Crick, D. C.; Cynamon, M.; Huang, G.; Docampo, R.; No, J. H.; Oldfield, E. Oxa, Thia, Heterocycle, and Carborane Analogues of SQ109: Bacterial and Protozoal Cell Growth Inhibitors. *ACS Infect. Dis.* **2015**, *1*, 215-221.

[63] Nekvinda, J.; Grüner, B.; Gabel, D.; Nau, W. M.; Assaf, K. I. Host-Guest Chemistry of Carboranes: Synthesis of Carboxylate Derivatives and their Binding to Cyclodextrins. *Chem. Eur. J.* **2018**, *24*, 12970-12975.

[64] Agarwal, H. K.; Buszek, B.; Ricks, K. G.; Tjarks, W. Synthesis of *Closo*-1,7-Carboranyl Alkyl Amines. *Tetrahedron Lett.* **2011**, *52*, 5664-5667.

[65] Fujii, S.; Kano, A.; Songkram, C.; Masuno, H.; Taoda, Y.; Kawachi, E.; Hirano, T.; Tanatani, A.; Kagechika, H. Synthesis and Structure–Activity Relationship of *p*-Carborane-Based Non-Secosteroidal Vitamin D Analogs. *Bioorg. Med. Chem.* **2014**, *22*, 1227-1235.

[66] Mandal, P. K.; McMurray, J. S. Pd–C-Induced Catalytic Transfer Hydrogenation with Triethylsilane. *J. Org. Chem.* **2007**, *72*, 6599-6601.

[67] Yamashita, K.-I.; Tsuboi, M.; Asano, M. S.; Sugiura, K.-I. Facile Aromatic Finkelstein Iodination (AFI) Reaction in 1,3-Dimethyl-2-imidazolidinone (DMI). *Synth. Commun.* **2011**, *42*, 170-175.

[68] Yang, H.; Li, Y.; Jiang, M.; Wang, J.; Fu, H. Ceneral Copper-Catalyzed Transformations of Functional Groups from Arylboronic Acids in Water. *Chem. Eur. J.* **2011**, *17*, 5652-5660.

[69] Griffiths, J. R.; Tsanaktsidis, J.; Savage, G. P.; Priefer, R. Thermochemical Properties of Iodinated Cubane Derivatives. *Thermochim. Acta* **2010**, *499*, 15-20.

[70] Baragaña, B.; Forte, B.; Choi, R.; Hewitt, S. N.; Bueren-Calabuig, J. A.; Pisco, J. P.; Peet, C.; Dranow, D. M.; Robinson, D. A.; Jansen, C.; Norcross, N. R.; Vinayak, S.; Anderson, M.; Brooks, C. F.; Cooper, C. A.; Damerow, S.; Delves, M.; Dowers, K.; Duffy, J.; Edwards, T. E.; Hallyburton, I.; Horst, B. G.; Hulverson, M. A.; Ferguson, L.; Jiménez-Díaz, M. B.; Jumani, R. S.; Lorimer, D. D.; Love, M. S.; Maher, S.; Matthews, H.; McNamara, C. W.; Miller, P.; O'Neill, S.; Ojo, K. K.; Osuna-Cabello, M.; Pinto, E.; Post, J.; Riley, J.; Rottmann, M.; Sanz, L. M.; Scullion, P.; Sharma, A.; Shepherd, S. M.; Shishikura, Y.; Simeons, F. R. C.; Stebbins, E. E.; Stojanovski, L.; Straschil, U.; Tamaki, F. K.; Tamjar, J.; Torrie, L. S.; Vantaux, A.; Witkowski, B.; Wittlin, S.; Yogavel, M.; Zuccotto, F.; Angulo-Barturen, I.; Sinden, R.; Baum, J.; Gamo, F.-J.; Mäser, P.; Kyle, D. E.; Winzeler, E. A.; Myler, P. J.; Wyatt, P. G.; Floyd, D.; Matthews, D.; Sharma, A.; Striepen, B.; Huston, C. D.; Gray, D. W.; Fairlamb, A. H.; Pisliakov, A. V.; Walpole, C.; Read, K. D.; Van Voorhis, W. C.; Gilbert, I. H. Lysyl-tRNA Synthetase as a Drug Target in Malaria and Cryptosporidiosis. *Proc. Natl. Acad. Sci. U.S.A.* **2019**, *116*, 7015-7020.

[71] Mersch-Sundermann, V.; Knasmüller, S.; Wu, X.-J.; Darroudi, F.; Kassie, F. Use of a Human-Derive Liver Cell Line for the Detection of Cytoprotective, Antigenotoxic and Cogenotoxic Agents. *Toxicology* **2004**, *198*, 329-340.

[72] Bevan, C. D.; Lloyd, R. S. A High-Throughput Screening Method for the Determination of Aqueous Drug Solubility Using Laser Nephelometry in Microtiter Plates. *Anal. Chem.* **2000**, *72*, 1781-1787.

[73] Lombardo, F.; Shalaeva, M. Y.; Tupper, K. A.; Gao, F. Elog-D_{oct}: A Tool for Lipophilicity Determination in Drug Discovery. 2. Basic and Neutral Compounds. *J. Med. Chem.* **2001**, *44*, 2490-2497.

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