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# Lead diversification. Application to existing drug molecules: Mifepristone 1 and antalarmin 8

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ABSTRACT

A series of C–H functionalisation plate-based chemical screens and other C–H activation protocols were developed for the chemical diversification of drug molecules. In this Letter, metalloporphyrin and other catalytic oxidation systems are described in addition to chlorination. Mifepristone and antalarmin are used as substrates. The products obtained and the biological data demonstrate the potential utility of this approach.



Structure–activity relationships (SAR) around a given chemical series is obviously limited to compounds that can actually be synthesised and tested in biological systems. Novel synthetic methodology which enables small subtle changes to complex lead molecules in a single step, for example, through the introduction of functional groups such as F, Cl, Me, OH, OMe, and CN would therefore be an extremely valuable tool. An arsenal of such chemical transformations could have advantages, such as beneficial tuning of physiochemical properties, blocking of metabolically vulnerable sites, or removal of toxicophores. As part of the drive within the pharmaceutical industry to find more efficient methods for optimising leads and reduce cycle times, the concept of lead diversification was explored in a drug discovery paradigm.

Lead diversification (LD) can be defined as the functionalisation of test compounds of around 450–500 MW using chemical or biochemical means to generate novel close-in analogues and expanded SAR. It is a different approach to chemical synthesis compared to the more traditional building block methodology where functionality is built in from the beginning. Typically transformations to introduce small functional groups such as OMe, OH, CN, F, Cl, Me, and deuterium via C–H activation were investigated. It was decided to apply this LD approach to late stage lead compounds for several reasons including the previously reported use of biomimetic oxidation[1] of drug molecules using metalloporphyrins to generate metabolites.

C–H activation is a rapidly expanding area of research and some of the novel chemistry described in the literature (e.g., Sanford's palladium-catalysed C–H activation[2] or iridium catalysed borylation[3]) had been reported using simple test systems such as *N*-phenylpyrazole. It was necessary to explore the breadth and limitations of these synthetic transformations in the context of real drug molecules and on novel substrates.

Our strategy was to find synthetic methodologies ideally for a plate based approach, but also for use as discrete reactions because inert atmosphere reactions are often not conducive to plate based methods. A survey of the literature was carried out to find methods for oxidation, fluorination, palladium and copper-catalysed C–H activation and other chemical methods for introduction of CN, Cl, Me, OMe either via metal-catalysed C–H activation or by electrophilic substitution.

The first step in the LD platform was to evaluate the structure of the test compound to determine which LD chemistries could be used based on synthetic experience and knowledge. For example, if there were alkyl groups, the BMO screen was used. For aromatic groups, the fluorination screen incorporating the chlorination conditions was used. If there was a Lewis basic $sp^2$ centre that could be used to coordinate for C–H activation, then copper or palladium-catalysed chemistry was attempted (Fig. 1).

Following structural analysis, the requisite chemistry was carried out. In the case of the BMO screen and other plate based screens, the data obtained indicated the conditions and catalyst for a scale-up

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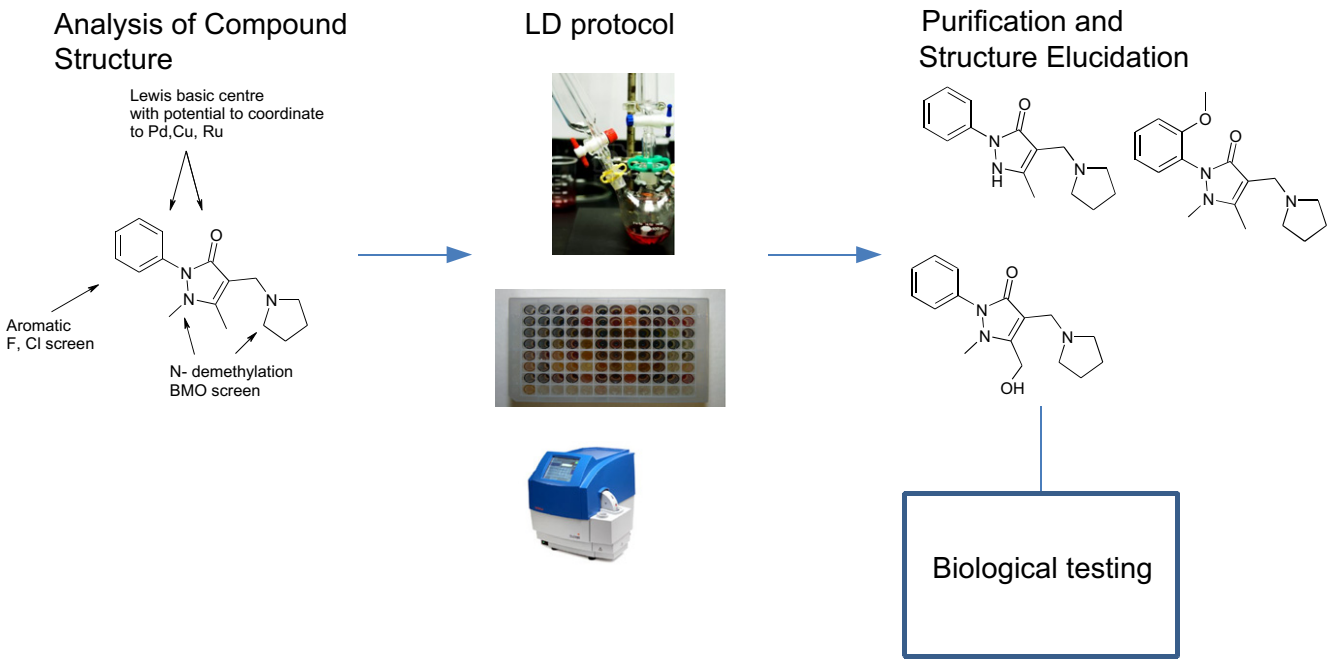



**Figure 1.** Process flow diagram for LD.

reaction. The reaction was then scaled up to ca. 100–150 mg scale of substrate. In the case of the metal-catalysed reactions, the crude reaction mixture was purified and then structure elucidation was carried out. The pure components were then submitted for biological testing. Chemical transformations that were not conducive to plate based chemistry including ultrasound mediated reaction benzylic acetylation and iridium catalysed borylation were also investigated.

While there are a plethora of oxidation catalysts that have been developed over the years, little work has been done to compare the reactivity of these oxidation catalysts over a diverse range of substrates to see if a selective catalyst/transformation can be found.

Metalloporphyrin-catalysed oxidations[4] have been extensively investigated over the past 40 years. They have been used for a variety of transformations such as alkane hydroxylation,[5] drug metabolite synthesis[6] and other reactions such as O-dealkylation, N-dealkylation, benzylic hydroxylation, sulphur and N-oxidation as well as epoxidation amongst other transformations.[7] Metalloporphyrins have been synthesized using every first row transition metal (Fig. 2) and the sheer number and complexity of these metalloporphyrins, as well as their different reactivities[8] meant that a combinatorial 96 well plate based chemical screen was necessary in order to cover a maximum number of conditions and reagents. There are over one hundred commercially available metalloporphyrins for oxidation reactions, however metal centres such as iron, manganese, ruthenium and chromium are the most widely reported.

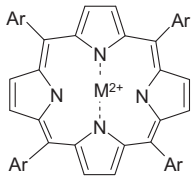



**Figure 2.** Structure of metalloporphyrins.

A review of the literature suggested that 9–10 commercial metalloporphyrins with the above transition metals would be a good starting point since these have been reported to be the most active catalysts displaying broad reactivity over a variety of substrates including drugs.[9] All the metalloporphyrins were purchased from commercial suppliers,[10] as were the oxidising agents.[11] A total of nine metalloporphyrins and thirteen oxidants were identified from literature searches. Initially, dimethyl aminoantipyrene **1** (Fig. 3) was used as a control substrate because metabolite studies had been reported[12] and it had previously been used as a substrate for metalloporphyrin based oxidations.[13]

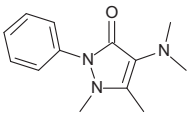
After several iterations using this substrate, an optimised plate consisting 36 wells and three metalloporphyrins: Fe(2-$NO_2$PP) (MP1), Mn(TDClPP) (MP2) and Fe(PFPP) (MP3) (Fig. 4) giving thirty six sets of conditions (2 solvents, 6 oxidants and 3 MP's) (Fig. 4). The screen was divided into two halves: one side using aprotic solvent and the other side using protic solvent and this gave results as informative as the whole 9 × 13 array. Often the products were detected in higher yields than the original screen (based on LCMS data).

The importance of identification and testing of drug metabolites at every stage of the drug discovery process, meant that oxidation methods to synthesise metabolites was a key area of work.

Our LD protocols were trialled on two internal Pfizer programs initially; the progesterone antagonist[14] and corticotrophin releasing factor-1 (CRF1)[15] antagonist programs.

Mifepristone[16] **2**, a known progesterone antagonist, is a very lipophilic steroid with a *c*log*P* of 4.65 (Log$D_{7.4}$ = 4.94) (Fig. 5).

Mifepristone **2** (1 mg) was reacted in the BMO screen[17] and analysis of the LCMS data showed several wells with multiple



Dimethylaminoantipyrene **1**

**Figure 3.** Dimethyl aminoantipyrene.

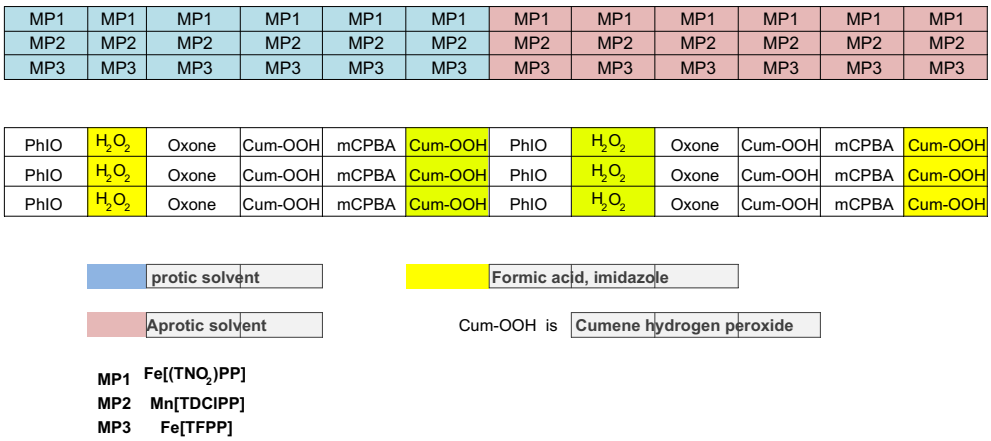
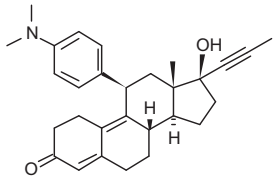



**Figure 4.** Plate map of biomimetic oxidation screen.




Mifepristone **2**
PR $IC_{50}$ : 10nM

**Figure 5.**

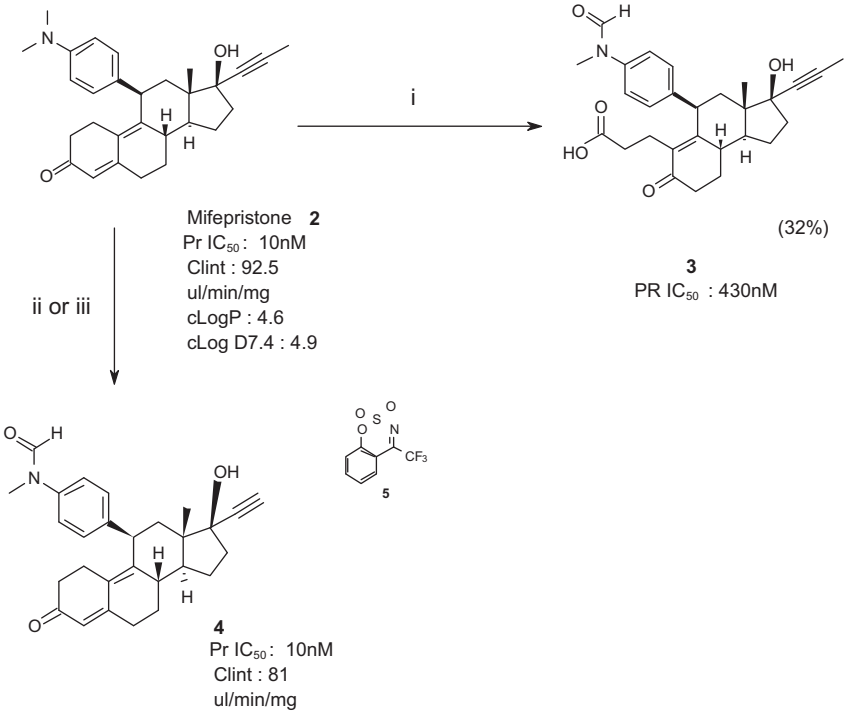
products; the wells which contained 1 major product were scaled up. The first well scaled up was Fe(PFPP), $H_2O_2$ in 1,2-dichloroethane, the major component was isolated, tested and structure elucidation carried out. It was found to be the ketocarboxylic acid **3** formed by oxidative cleavage of the steroid A ring (Scheme 1).

Acid **3** was found to be less potent than the parent mifepristone **2**. By cleaving the A-ring, the contribution to binding that the remainder of the molecule makes can be dissected.

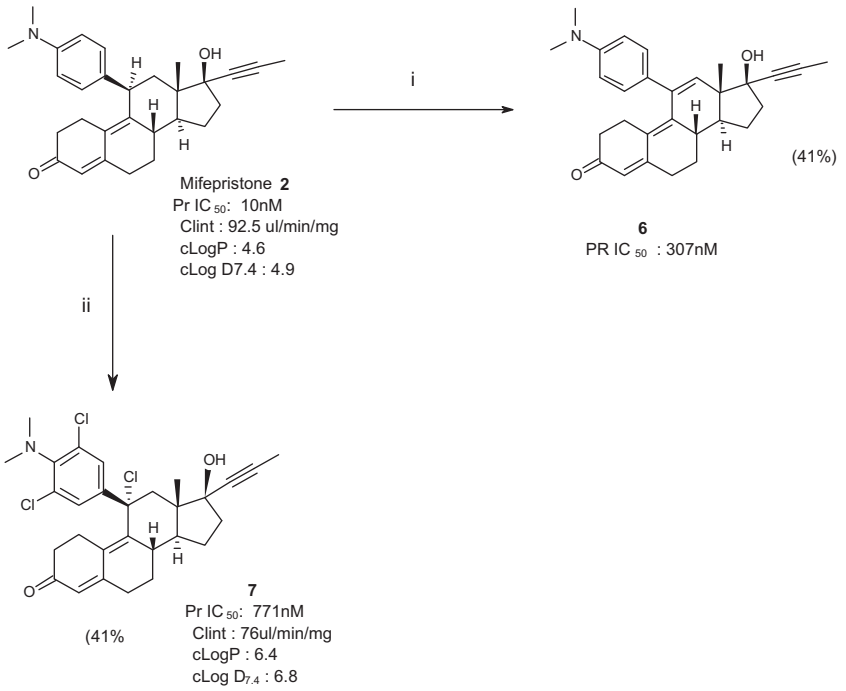
The second well scaled up was cumene hydrogen peroxide and Fe(2-$NO_2$)PP in 1,2-dichloroethane, the major product isolated in 19% yield was identified as the *N*-formyl adduct **4**. This was equipotent with mifepristone **2** and found to be metabolically more stable, representing a new piece of SAR. While the yield is low and the reaction gives access to milligram quantities, this is more than sufficient material to put into many high throughput screens and generate initial data.

Other oxidation catalysts were also explored such as DuBois' oxaziridine catalyst[18] **5** were found to be selective and showed comparable reactivity to the metalloporphyrin systems in some examples including mifepristone **2** (Scheme 3).

The BMO screen demonstrated that polar groups can be introduced to lipophilic molecules, quickly without the need to synthesize the molecule from suitable starting fragments. In several cases,




**Scheme 1.** BMO product. Reagents and conditions: (i) $H_2O_2$, Fe(PFPP) (10 mol %), dichloroethane, rt 4 h; (ii) Fe(2-$NO_2$)PP (10 mol %), cumene hydroperoxide, DCE, rt, 6 h; (iii) **5** (5 mol %), Urea·HOOH, PhSeSePh (3 mol %), Microwave (100 °C), 4 h DCE, rt, 6 h.




**Scheme 2.** Benzylic oxidation and elimination. Reagents and conditions: (i) DDQ, acetic acid, ultrasound, rt, 1 h; (ii) cyanuric chloride, $CH_3CN$, rt, 48 h.

this enabled the research team to explore new chemical space. Moving beyond neutral, lipophilic steroids, more polar compounds with basic centres were put through the BMO screen where the major product was often the N-oxide. In many cases these N-oxides were the drug metabolites. The BMO screen has proved to be a versatile and invaluable tool for LD.

A second robust oxidative transformation was benzylic oxidation by reaction with dichlorodicyanoquinone (DDQ) using ultrasound.[19] This was attempted on mifepristone **2** and gave clean transformation to the triene **6** in 41% isolated yield. Benzylic acetylation followed by elimination had occurred to give the double bond (Scheme 2).

Purification and structure elucidation were carried out. Biological testing showed a loss in potency and this is due to the change in conformation of mifepristone **2**. The dimethylaminobenzene group moves into the plane of conjugation following introduction of the double bond.

Later investigations focused on chlorination and fluorination. These atoms introduce small changes to drugs and have been widely used to moderate pharmacokinetic properties in drug molecules. Iodination and bromination was not explored as the changes in molecular weight were too large. A plate-based combinatorial chemical screen for fluorination was developed, which also incorporating chlorination conditions and this will be reported in later communications. Chlorination of mifepristone **2** using cyanuric chloride[20] in a mixture of tetrahydrofuran and acetonitrile (1:5) gave a mixture of products; the major product being identified as the trichlorinated steroid **7** in 41% yield.

The trichlorinated product **7** was seventy fold potent as a progesterone antagonist, but was more lipophilic and had greater stability in human liver microsomes.

Several other catalysts used in the screens that showed promising transformations[21] such as alkane hydroxylation included the DuBois catalyst **5** and $RuCl_3$.[22]

Attention then turned to antalarmin[23] **8**, which is a drug that acts as a CRF-1 antagonist. It is a very lipophilic compound with a $\log D_{7.4}$ of 8.6 and efforts were centered on making a more polar compound to improve aqueous solubility and pharmacokinetic properties.

Initially a computational analysis was carried out to determine if the selectivity of benzylic hydroxylation's could be predicted. An analysis of the regioisomeric radicals resulting from a hydrogen abstraction at all benzylic and nitrogen α-positions (x,y) was performed.[24] The observed regioselectivity was in exact agreement with the calculations as the experimental oxidation site was where the most stable radical had formed[25] (Fig. 6).

After running the BMO screen, analysis of the LCMS data showed three wells that had produced several new polar components. However, the well that had the cleanest profile was Fe(PFPP) using *m*-CPBA as the oxidant and methanol/acetonitrile (1:1) as the solvent (Scheme 3).

Scale-up of these best conditions on 200 mg of substrate gave a crude mixture of products with approximately 20% unreacted starting material. After work-up, purification and structure elucidation, it was found that the three components were all products of oxidation of the azaindole core on the 2-methyl position: the hydroxymethyl compound **9**, the aldehyde **10**, and the methoxy-substituted analogue **11**. The methoxy adduct **11** was formed by displacement of an activated benzylic intermediate by the methanol solvent. Testing of the compounds in a CRF-1 assay[26] showed that the methoxy **11** and hydroxy **9** derivatives were more potent. Evaluation of the physiochemical properties of **9**, **10** and **11** showed them to be more polar

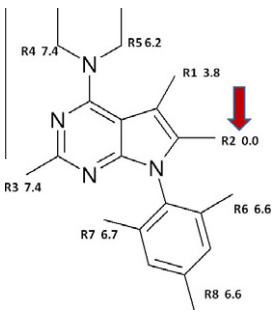
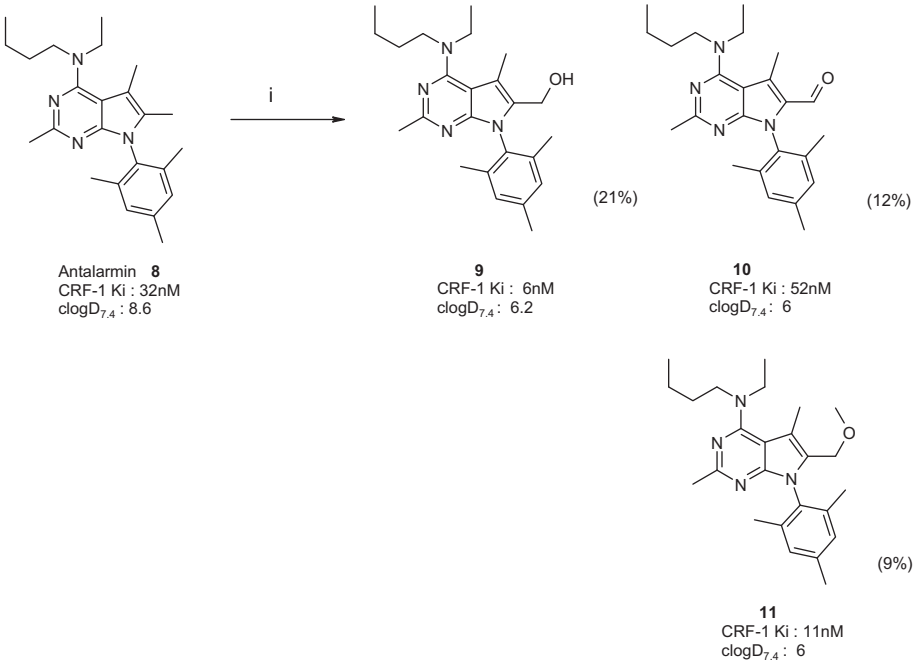



**Figure 6.** Relative energies (in kcal/mol) of the corresponding neutral radicals formed by C–H abstraction at the different benzylic positions.




**Scheme 3.** BMO scale-up of antalarmin to oxidation products. Reagents and Conditions: (i) mCPBA, Fe(PFPP) (10 mol %), MeOH/MeCN (1:1), 6 h, rt.

than the starting compound **8**. The carbonyl derivative **10** was slightly less potent, but was again a more polar compound. An analysis of the chemical literature showed that these oxidised compounds occupy new chemical space. CRF-1 has been targeted by several pharmaceutical companies over the past 10 years and it has been found to find new chemical space around some of the templates has been synthetically challenging. This example is a proof of concept for LD and it has been shown that by taking a lipophilic compound and adding small polar groups using a reaction catalyst screen, several compounds that were structurally close to the starting drug were made. These compounds were more potent, more polar (2 log units) and opened up new chemical space for this target.

$K_i$ values were determined from the $IC_{50}$ using the Cheng and Prussoff relationship and the $EC_{50}$ for the agonist dose–response curve which was determined on the same day.

Over the course of several screens and substrates it was found that in some wells, the same product was formed regardless of the metalloporphyrin used. This indicated that the oxidising agent was responsible for the reaction and the metalloporphyrin was not necessary in these cases.

Two case studies show the utility of LD. From a practical standpoint the main bottlenecks were: scaling-up of reactions, purification and structural elucidation. Metalloporphyrin-catalysed reactions have been reported over the past 30 years with some examples showing remarkable catalytic turnover and reactivity.[27]

It should be noted that the quantities made using LD were more than sufficient to test in multiple screens against many targets. However, in our hands scaleability was an issue; working on 10–90 mg gave adequate yields but scaling above this quantity seldom met with success, the reactions had low yields or no products were detected. There are many reasons for this including 'bleaching' of the metalloporphyrin by the oxidant rendering the catalyst inactive.[28]

Further work will address factors that will lead to more general applicability. Ease of purification was dependant on molecular weight and properties of the molecule. As a general rule of thumb: as the molecular weight increased, the products and starting material eluted closer on HPLC and so purification was difficult and sometimes impossible.

With macromolecules and peptides such as cyclosporin A[29] and FK506[30] the chemical yields were low (around 10–20%) and there were several products that proved too difficult to separate.

Structure elucidation was found to be the biggest bottleneck. As the molecular weight and hence complexity of the starting material and products increased, the more tedious and time consuming the purification and structure elucidation. However, this was balanced by the argument that mifepristone is synthesised in eleven chemical steps from (+) estrone.[31] Similarly antalarmin analogues generated by BMO versus direct synthesis from start show that LD is a viable approach. However, it is not a standalone tool and is dependant on the quality of the substrate. Other screens and transformations have been developed in collaboration with academic groups and will be published elsewhere.

## Conclusion

Chemical transformations to functionalise drug molecules in order to obtain SAR can circumvent a long synthesis to make the desired compound. C–H activation is a rapidly expanding area of research and so LD-type approaches are attractive as they can cut down the effort of a multistep synthesis of a target molecule. The examples shown in this report demonstrate proof of principle for LD and encouraged us to expand the types of transformations and screens for this platform technology.

In this report it has been shown that functionalization of late stage molecules is possible in a drug discovery environment and can potentially give results/SAR that are unexpected as well as leading project teams into new avenues and directions. The use of BMO to generate new close analogues has been discussed.

## Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.10.066.

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11. The metalloporphyrins were made up as stock solutions in 1,2-dichloroethane. The substrate was also dissolved in dichloroethane or acetonitrile (as these solvents are not susceptible to oxidation). The substrate was added to the metalloporphyrin and then further solvent added. Two solvent systems were used: protic (methanol/acetonitrile; 1:1) and aprotic (dichloroethane/acetonitrile; 1:1) as these solvents are not readily oxidised. The oxidants were added and in some wells a catalytic amount of formic acid and imidazole were also reaction variables.
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17. Substrate was dispensed into each well and agitated for 6 h at room temperature before quenching the reactions with sodium sulfite and finally analyzing by LCMS, using a 10 min run time.
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24. Note: the butyl group was shortened to the *N*-ethyl to help simplify the calculation.
25. All the calculations reported in this paper have been performed within density functional theory, using the hybrid three-parameter functional customarily denoted as B3LYP. In all cases the standard 6-31G** basis set, no molecular charge and duplet spin multiplicity (0,2) were used as implemented in Jaguar package (Schrodinger, LLC, Portland, Oregon). These intermediates were characterized by frequency calculations and have positive definite Hessian matrixes. In order to determine the minimum energy conformation(s) of the initial substrates conformational searches (Monte Carlo simulations) were previously performed using the AMBER* force field as implemented in the MacroModel package (Schrodinger, LLC, Portland, Oregon).All geometries are available on request to the authors.
26. CRF-1 potency is expressed as functional activity measured using CHO cells (Cell Sciences SNB0000377) expressing recombinant human CRF-1 receptor grown in DMEM:F12 (1:1) media containing 10% (V/V) Foetal Bovine Serum (PAA), 400 lg/ml Geneticin (GIBCO-BRL 10131-027) and 1% (V/V) Glutamax in a cell incubator at 37 °C, 5% $CO_2$ to 70% confluence. FAC hCRF (108 nM) was added with test compound to 10,000 cells/well in Phosphate Buffered Saline containing 500 lM FAC IBMX. The functional response was measured using DiscoveRx HitHunter cAMP II Assay kit (Amersham Biosciences—90-0034-03). Each compound was tested multiple times using a 0.5 log serial dilution dose–response with a top final assay concentration of 20 lM. The percent (%) response of the test compound at different test doses were then fitted to a 4-parameter logistic curve to determine the compound $IC_{50}$. $K_i$ values were determined from the $IC_{50}$ using the Cheng and Prussoff relationship and the $EC_{50}$ for the agonist dose–response curve which was determined on the same day.
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