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Lead Diversification 2: Application to P38, gMTP and lead compounds

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ABSTRACT

Lead Diversification is a new technology platform developed at Pfizer for the functionalization of drug molecules using C–H activation. We describe its application to some drug programs such as P38 and gMTP and the development of some new plate based screens including a fluorination screen.

In our previous paper the concept of Lead Diversification (LD) was outlined and some of the chemical screens developed such as the biomimetic oxidation[1] (BMO) screen were described. In this letter further transformations such as fluorination, chlorination, and methoxylation of drug molecules are discussed.

It should be noted that Wender et al. have also explored functionalization of natural products using a Lead Diversification (LD) approach[2]; however, our work explores a greater diversity of transformations on drug molecules.

As part of Pfizer's internal efforts to find a human gMTP inhibitor, close analogues of the aminoquinoline diamide **1** lead were synthesized using LD. The strategy was to make close chemical analogues to gain further structure–activity relationship (SAR) data and explore available LD chemistry options. The compound **1** is a good substrate to explore the chemoselectivity of such transformations.

Our initial LD efforts involved plate based screens for biomimetic oxidation (BMO) using metalloporphyrin catalyzed oxidations. Two polar compounds were observed in several wells. The more polar compound had a mass ion of M+16 amu and the less polar compound had a mass ion of M-41 amu. This led us to believe that the reaction had added polarity to substrate **1** or removed lipophilicity. Isolation and structure elucidation showed the products to be the *O*-dealkylated derivative **2** and the N-oxide **3** (Scheme 1). Both compounds were subsequently found to be inactive against gMTP. The *O*-dealkylated adduct **2** is the major human metabolite and the N-oxide **3** is a minor human metabolite. Using biomimetic oxidation, both metabolites were synthesized in a short time compared to a more time consuming conventional multistep synthesis.

Over the past five years there has been exponential growth in the field of C–H functionalisation and one of the earlier methodologies developed was by Sanford's group using a palladium II/IV system to catalyze C–H alkoxylation and O-acetoxylation onto aromatic groups.[3] Many of these new transformations had been developed using simple systems like *N*-phenylpyrazole[4] **4** (Scheme 2). Based on the published results, it was important to apply this to more complex systems such as drug-like compounds with a molecular weight of around 500 to see the scope of reactivity and chemoselectivity of these reactions. Sanford's work showed that C–H activation was directed by the palladium species co-ordinating to sp^2 nitrogen. It was reasoned that sp^2 oxygen atoms were also weakly Lewis basic such that a carbonyl could co-ordinate to palladium and enable Sanford-type directed C–H activation reactions to proceed.

Aminoquinoline diamide **1** was dissolved in acetic acid and palladium(II)acetate (10 mol %) and oxone were added then heated in a microwave reactor at 150 °C for 2 h.[5] LCMS analysis of the crude reaction mixture indicated the appearance of several close running peaks in addition to unreacted starting material (Fig. 1). The mass ions showed that OAc (M+44) had been added to the parent molecule.

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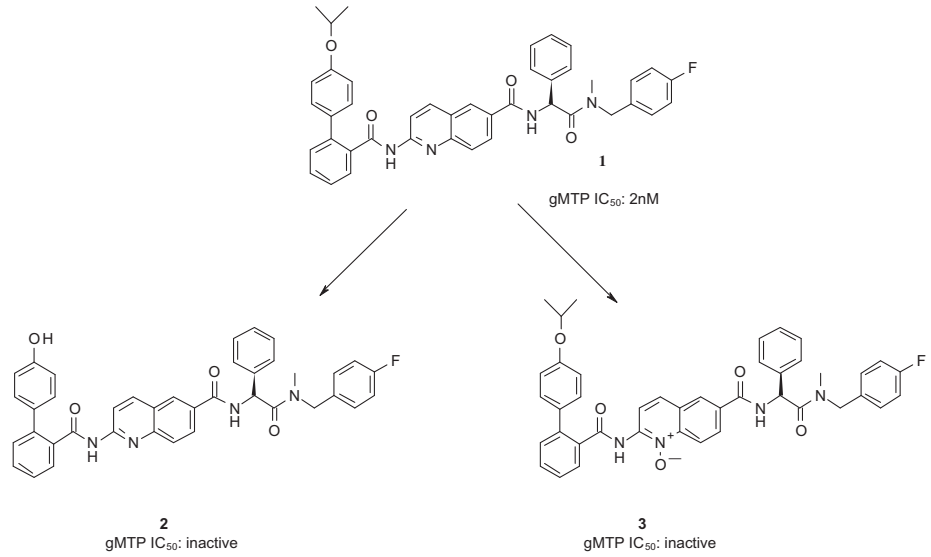
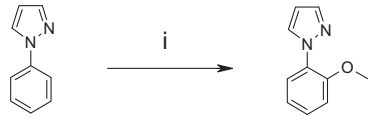


Scheme 1. Two products isolated from biomimetic oxidation scale-up reactions.



Scheme 2. Palladium(II) catalyzed methoxylation. Reagents and conditions: (i) Oxone, MeOH, $Pd(OAc)_2$ (10 mol %), 80° C, 4–6 h.

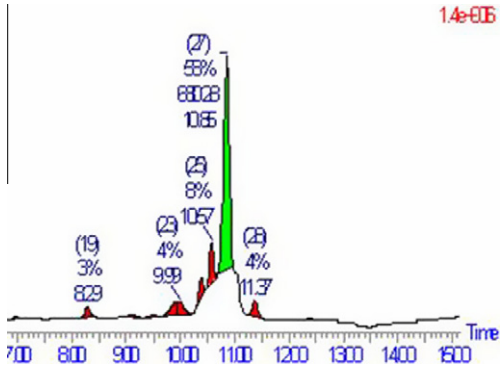


Figure 1. LCMS (ELSD) trace of Pd(II) acetate catalyzed methoxylation of **1** (crude reaction trace).

From the ELSD and UV (254) traces of the LCMS (Fig. 1), approximately 40% conversion of the starting compound **1** to products was seen. The reaction was repeated three times and found to be reproducible. Purification of the crude material was carried out by preparative HPLC with two compounds being isolated in high purity (>95%). Structure elucidation was carried out and the two derivatives were identified as the *O*-acetoxylated analogue **5** and the phenol **6** (Scheme 3).

Compound **5** is extremely interesting since to synthesize it from commercial starting materials would be lengthy and previous attempts at synthesis had failed because of a competing ring cyclization reaction.

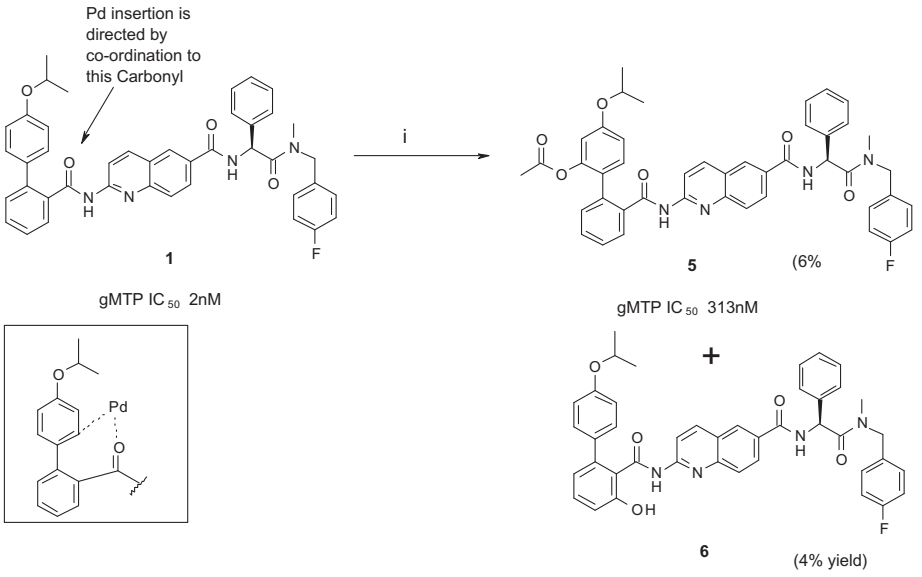
It was postulated that palladium coordinated to the carbonyl oxygen and inserted into the aryl C–H bond to give the observed regiochemistry. It should be noted that 6 products were observed, but only the products shown were isolated in sufficient purity and quantity for structure elucidation. Biological testing showed that the *O*-acetoxylated derivative **5** was less potent than the starting compound **1**.

The next chemical transformation attempted was Sanford's palladium(II)acetate catalyzed *O*-methoxylation on the aminoquinoline diamide lead compound **1** (Scheme 4). Again this resulted in a mixture of products. The methoxy derivative **7** was the only component isolated.

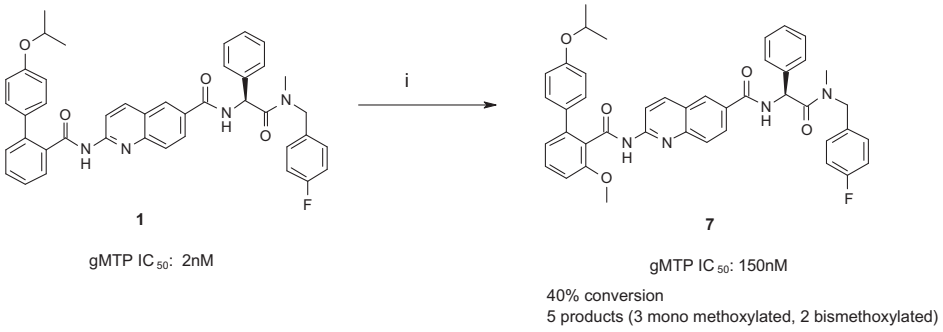
Methoxy derivative **7** was found to be a 100-fold less potent against human gMTP. Sanford reactions gave multiple products on drug molecules due to multiple palladium binding sites. As a result the effective palladium concentration is much lower and so the yield is lower. When the amount of catalyst was increased to 20 mol % the percent conversion based on ELSD and UV did increase, but adding more catalyst above this quantity did not significantly improve yield or conversion. It should be noted that synthesis of derivatives **5**, **6** and **7** from commercial fragments using conventional synthesis would have taken a long time as well as being very resource intensive. Using the LD approach sufficient material was isolated to obtain data that formed valuable SAR. While some of the chemistries developed were plate-based (oxidation screens, halogenation screens), other chemistries were found to work better as a single reaction using microwave heating.

The LD approach was applied to Pfizer's internal program to find an inhaled P38 inhibitor project lead, the aminopyrazole urea **8** was reasons why this compound was selected; one being submitted to the plate based BMO screen. There were several reasons; the desire to synthesize polar compounds to improve solubility, which had previously been a problem with this compound series. In addition, the compounds in this project were large complex molecules with heterocycles and functional groups that could react under the screening conditions. Palladium catalyzed C–H activation chemistry methods were attempted and met with some success, but purification proved impossible in all cases.

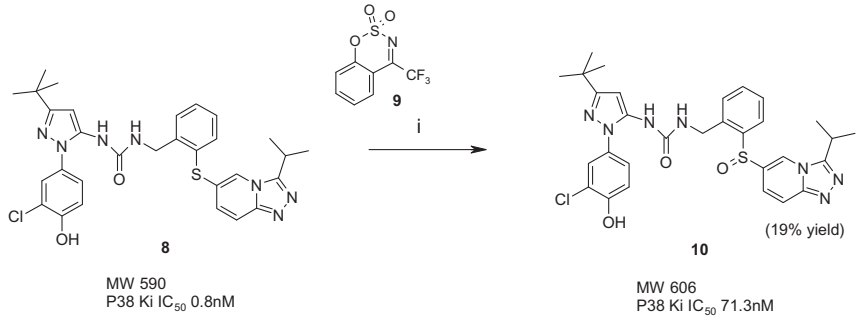
The aminopyrazole urea **8** was reacted in the BMO chemical screen and other oxidation screens. The BMO screen results showed two wells that contained more polar products and another oxidation screen showed one promising result with DuBois' catalyst[6] **9**, urea hydrogen peroxide and catalytic diphenydiselenide (Scheme 5). This reaction was scaled up and gave the sulfone **10** in 19% isolated yield.



Scheme 3. Pd(II) catalyzed O-acetoxylation of **1**. Reagents and conditions: (i) Oxone, acetic acid/acetic anhydride (1:1), $Pd(OAc)_2$ (20 mol %), microwave irradiation (120 °C), 4 h.



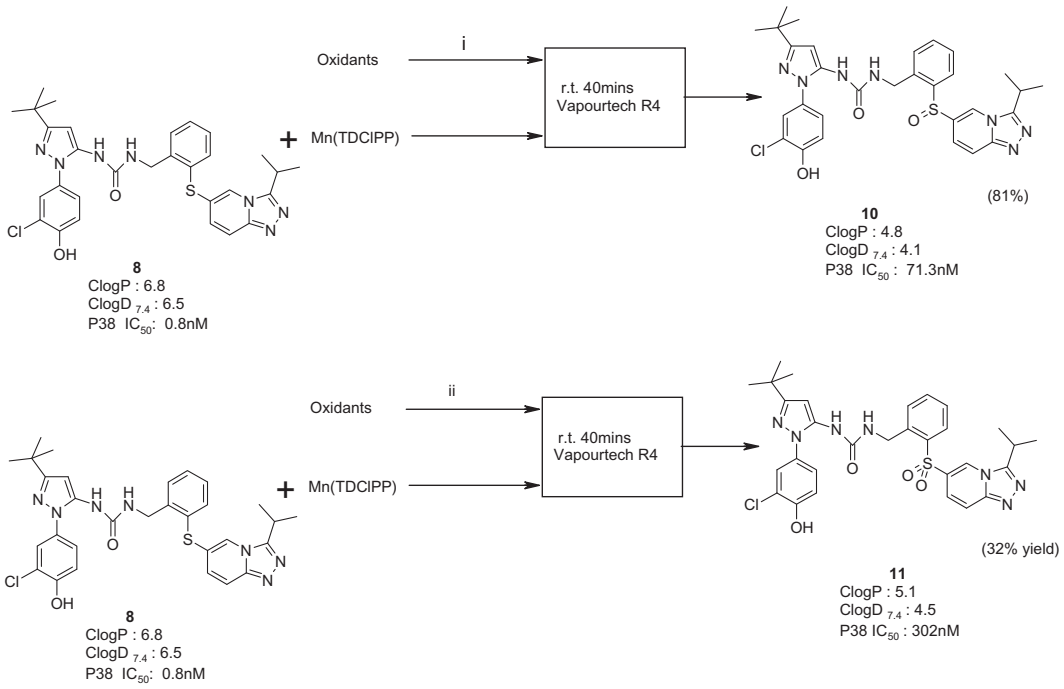
Scheme 4. Reagents and conditions: (i) Oxone, MeOH, $Pd(OAc)_2$ (20 mol %), microwave irradiation (120 °C), 4 h.



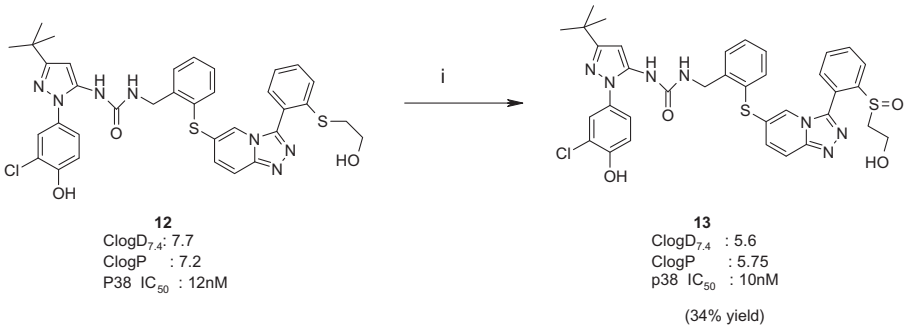
Scheme 5. Reagents and conditions: (i) **5** (5 mol %), Urea HOOH, dichloroethane, PhSeSePh (5 mol %), rt, 48 h.

The Mn(TDClPP),[7] H_2O_2, imidazole and formic acid (catalytic) wells were scaled up. Problems were encountered because we saw bleaching of the catalyst[8] and no reaction of the substrate. Bleaching is where degradation of the metalloporphyrin occurs by direct oxidation of the resting catalyst (M^{III}) rather than the reactive intermediates such as the high valent oxo-perferryl ($(TF_5PP^{+})M^{IV}=O$) and is known to be a particular problem with hydrogen peroxide. One way to circumvent metalloporphyrin bleaching is slow addition of the oxidant to the reaction. This reaction was attempted using flow chemistry with two reservoirs. One reservoir containing the substrate and metalloporphyrin catalyst and the second containing a mixture of the oxidant, catalytic formic acid and imidazole. The rationale for doing the reaction in flow was that the scale-up reaction was extremely exothermic and the risk of this could be minimized in flow. Furthermore, in flow, the amount of oxidant that the metalloporphyrin catalyst is exposed to at any one time can be limited and so in effect 'fresh' catalyst is exposed to oxidant over time and this would minimalise catalyst bleaching.[9]

The reaction was carried out on a Vapourtech R4 flow apparatus[10] using a flow rate of 200 μl/min and a 10 ml loop. The reaction time was 40 minutes and analysis of the crude product by LCMS



Scheme 6. Flow mediated biomimetic oxidation of P38 substrate. Reagents and conditions; (i) H_2O_2, imidazole (1 mol %) and formic acid (5 mol %), dichloroethane; (ii) mCPBA, CH_3OH/CH_3CN (1:1), rt.



Scheme 7. BMO scale-up reaction. Reagents and conditions: (i) Mn(TDClPP) (10 mol %), mCPBA, DCE, rt 8 h.

showed complete conversion of parent to the sulfoxide **10**. Synthesis of the sulfoxide **10** had been unsuccessfully attempted previously using conventional oxidation reagents. Scale-up of the second well using Mn(TDClPP) and a protic solvent gave the sulfone **11** in 32% yield (Scheme 6).

A closely related P38 compound **12** was reacted in the BMO chemical screen and one well showed clean conversion to a more polar product (Scheme 7).

The procedure was scaled up to 150 mmol of reactant using Mn(TDClPP), mCPBA in dichloroethane for 8 h at room temperature. The more polar product isolated was the sulfoxide **13** on the alkyl thioether portion in 34% isolated yield (Scheme 7). Biological testing against MAPK P38 showed that the alkyl sulfoxide **13** was equipotent to the starting dithioether.

Fluorination strategies are common in medicinal chemistry, serving to influence both pharmacological and metabolic processes whilst avoiding detrimental effects on favorable properties.[11] Fluorine is small, electronegative (4.0 on the Pauling scale) and can form hydrogen bonds.[12] In drug discovery, fluorine is most often used to block metabolically vulnerable positions on drug compounds.[13] However, introducing fluorine into a drug molecule at a late stage through synthesis is practically challenging.[14] Although there are many commercial fluorinating reagents, the chemistry can be problematic because fluorine is also an excellent oxidizing agent.[15] The bond dissociation energy of C–F is 452.5 $KJmol^{-1}$, C–H is 410 $KJmol^{-1}$ and that of N–F is approximately 318.6 $KJmol^{-1}$ (76.1 kcal/mol). The majority of electrophilic fluorinating agents have a nitrogen fluorine bond for example SelectFluor[16] and Accufluor, and so fluorination reactions can be extremely exothermic. The heat generated during fluorination is sufficient for fluorine radical formation which can destroy the substrate through uncontrolled reactions.[17] As a result methods for finding conditions and reagents to fluorinate organic molecules would be a useful tool for both the synthetic and medicinal chemist. A plate based combinatorial method was used to develop such a fluorination screen (Fig. 2) as a quick technique for finding optimum conditions for fluorinating drug molecules using a combinatorial array of reagents[18] and solvents. The fluorinating reagents were made up as stock

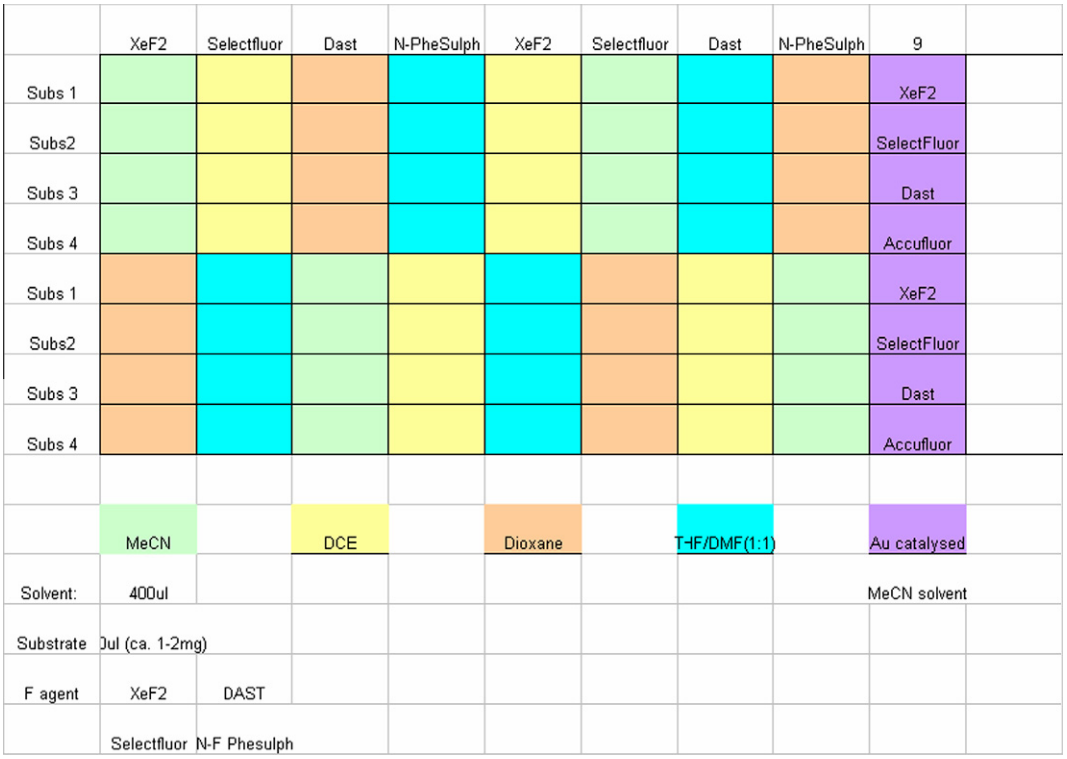
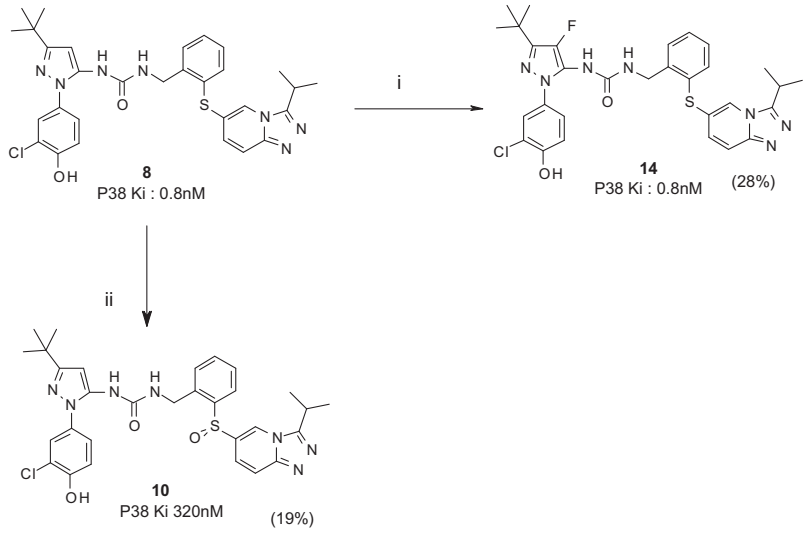


Figure 2. Plate map of fluorination screen.



Scheme 8. Fluorination screen scale-up reactions. Reagents and conditions: (i) Accufluor®, dichloroethane, rt, 50 h; (ii) Synfluor®, dichloroethane, rt, 30 h.

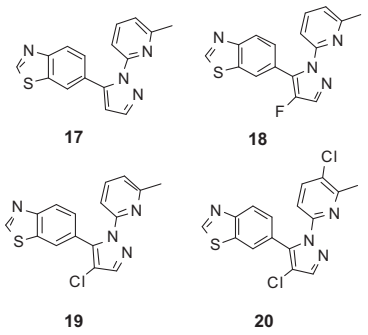
solutions in dichloroethane[19] or acetonitrile. Four solvents were used in the screen, based on literature searches[20] and Pfizer internal examples.

The reactions were easy to scale-up and were always carried out in plastic or Teflon vessels due to the reactivity of fluorinating reagents with glass.[14]

The pyrazole adduct **8** was subjected to the plate-based fluorination screen for 72 h.[21] After quenching and analysis by LCMS, two wells were found to have generated products. Scale-up, isolation of products and then structure elucidation, identified the fluoropyrazole derivative **14** and the sulfoxide **10** (Scheme 8). The fluoropyrazole derivative **14** was isolated in 28% yield and showed that selective fluorination of large drug molecules rich in heteroatoms and functional groups could be carried out. Biological testing against MAPK P38 showed that the fluoropyrazole derivative **14** was equipotent with the lead compound **8** and opened up new chemical space.

Using a conventional multistep synthetic approach to make the fluoropyrazole derivative **14** would have proved difficult at the urea forming step with the fluoro-aminopyrazole coupling being poor-yielding due to the low reactivity of the electron deficient aminopyrazole.

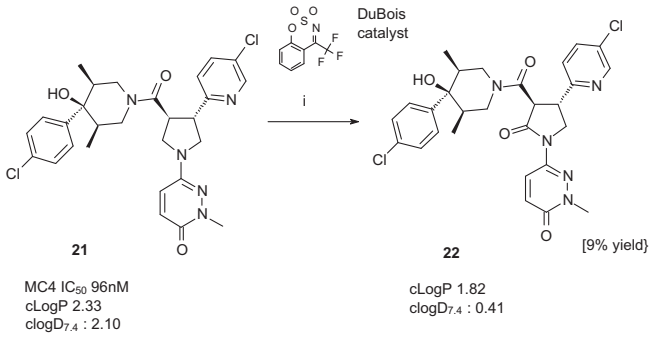
The sulfoxide **10** was an interesting result and showed the oxidizing power of fluorine. Sulphur has a great affinity for fluorine



R/ Compound	Alk5 IC_{50} (nM)	ActR2B : IC_{50} (nM)	cLogP	PLM[∞] Cl_{int} ul/min/mg	T1/2 min
17	1.19	541	3.65	122	11.4
18	1.36	1340	3.81	12.7	109
19	0.63	1060	4.13	<11.6	>120
20	77.8	>6000	4.81	NA	NA

[∞]Pig liver microsomes (PLM)

Figure 3.



Scheme 9. Reagents and conditions: (i) PhSeSePh (5 mol %), Urea.HOOH, dichloroethane, microwaves 100 °C, 2 h.

and had reacted to give the $ArSF_2Ar$[2] species,[22] which was hydrolyzed to the sulfoxide **10** on work-up.

Compound **1** was a lead compound for Pfizer's activin-like kinase 5 receptor (Alk5) antagonist program (for veterinary medicine). Pyrazole **17** had a short half life and one of the project objectives was to see if fluorination or chlorination could solve this issue. The pyrazole **17** was submitted to the fluorination screen. Several wells showed promising product profiles (Fig. 3). Taking the best, scale-up using accufluor® in acetonitrile gave the fluoropyrazole derivative **18** in 53% isolated yield. The half life in pig liver microsomes (PLM) increased tenfold as well as twofold increase in selectivity over ActR2B[23] as a result of this small change in molecular structure. Surprisingly the chlorinated adduct **19** was twofold more potent and had a longer half-life as well as having lower clearance in the in vivo pig model. The dichlorinated compound **20**, formed as an impurity in the chlorination reaction, was significantly less potent, but more selective than the mono-chloro adduct **19**.

In a similar plate based approach to chlorination, *N*-chlorosuccinimide (NCS) in acetonitrile was found to achieve chlorination generating two products as well as residual starting material. After scale-up, structure elucidation and testing it was found that the two products were the mono-chloro adduct **19** and the dichloro-adduct **20** where chlorination had occurred on both the pyrazole and the pyridine.

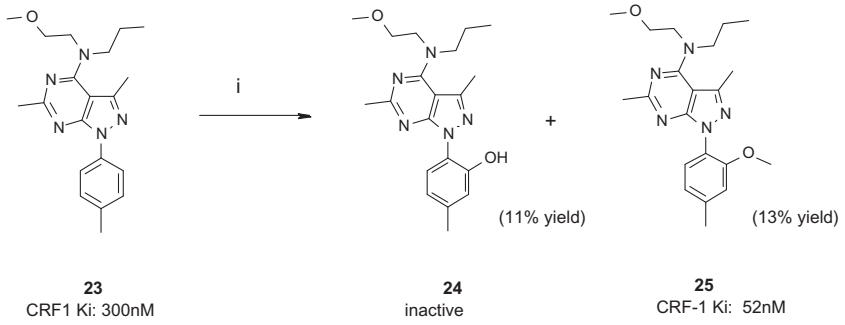
Through the screening approach, three close-in analogues had been synthesized and the problem of improving the half life without losing potency while keeping the *c*log *P* down had been resolved. These three halogenated derivatives were shown to have interesting pharmacological and metabolic properties. The impact on metabolism is most striking and suggests that LD has been successful in blocking metabolically vulnerable sites.

In a second example, compound **21** was the lead compound in the melanocortin-4 receptor antagonist (MC-4) program. Compound **21** is lipophilic and also required modification to improve pharmacological properties. Compound **21** was tested in the biomimetic oxidation screen (BMO) and while several wells showed promise, scale-up reactions were unsuccessful. In an extended oxidation screen, DuBois' catalyst[6] was found to give a clean reaction profile. The DuBois scale-up reaction on **21** was carried out by microwave heating of a solution of **21**, DuBois catalyst, diphenyl-diselenide and urea hydrogen peroxide in 1,2-dichloroethane at 100 °C for 2 h. The product was isolated in 9% yield and identified as the pyrrolidinone **22** (Scheme 9). Alternative synthetic strategies to this interesting target using conventional chemistry were very lengthy.

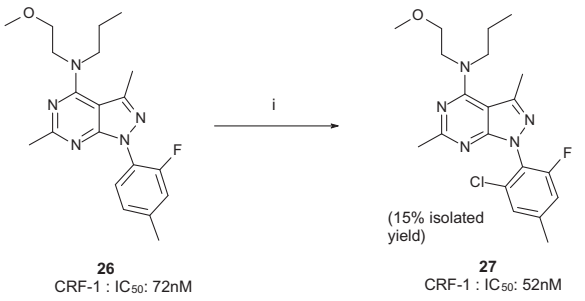
In our previous communication the biomimetic oxidation of the 3-methyl of an indazole core for the corticosteroid releasing factor (hCRF-1) project was described. The SAR generated helped to improve solubility for the azaindole series. The heterocyclic compound **23** was a key intermediate in a more polar series for Pfizer's CRF-1 program. The majority of the synthetic efforts were focused on adding polarity to the dialkylamine portion of the indazole and also onto the 2-aryl position as this takes the phenyl out of the plane. According to Miller et al. this conformational change is key for potency.[24] Also, it should be noted that no compound had been made where the phenyl had been in the plane (i.e., held in plane by an internal hydrogen bond) to confirm that the 'flat' molecule is inactive. At this time, Sanford's group had published the palladium catalyzed methoxylation of *N*-aryl pyrazoles using methanol via C–H activation.[25] It was felt that this methodology would prove invaluable to *ortho*-functionalize the phenyl group in **23** and generate structure–activity relationships (SAR). In addition, copper-catalyzed, directed aromatic hydroxylation had been reported by JinQuan Yu's group,[26] however in our hands these conditions were unsuccessful. The reaction was then attempted using a methanol/water mixture (2:1) as solvent with microwave heating (Scheme 10). This is a new method for aromatic hydroxylation, however the methoxy derivative **25** is also formed as the major adduct.

The reaction was heated at 100 °C and after 4 h, LCMS analysis showed two more polar products alongside the unreacted starting material. Purification using preparative HPLC followed by structure elucidation showed the products to be the 2-hydroxyphenyl **24** isolated in 11% yield and the 2-methoxyphenyl derivative **25**, which was isolated in 13% yield. Biological testing against CRF-1 showed the 2-methoxyphenyl compound **25** had an IC_{50} of 52 nM.[27] This is more potent than the starting compound **23**, one explanation for this is that the methoxy pushes the phenyl out of the plane and this change in conformation is important for activity. The 2-hydroxyphenol forms an internal hydrogen bond with the diazaindazole core and so the phenyl is held in the plane and this change in conformation results in the compound being inactive. The 2-hydroxyphenyl adduct **24** has an internal hydrogen bond that holds the phenyl ring in the plane and this change in conformation results in the compound being inactive. Another compound in the series was the 2-fluorophenyl derivative **26**; this compound had an IC_{50} of 72 nM.

As the molecule contained an *N*-phenylpyrazole, it was felt that the system was set up to attempt Sanford's palladium-catalyzed C–H functionalization. The transformation selected was a palla-



Scheme 10. Reagents and conditions: (i) Oxone, MeOH/H_2O (2:1), $Pd(OAc)_2$ (10 mol %), microwave heating 120 °C, 3 h.

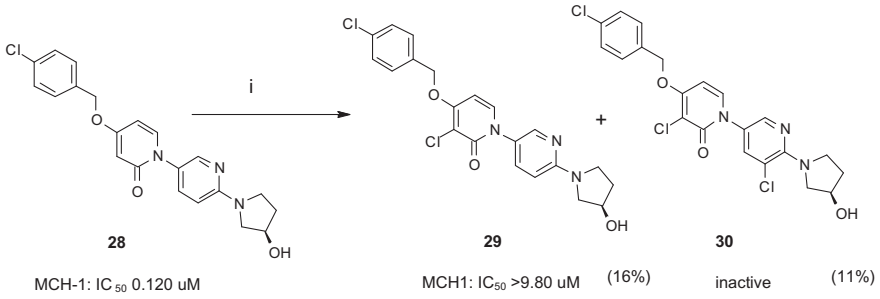


Scheme 11. Reagents and conditions: (i) $Pd(OAc)_2$ (20 mol %), CH_3CN, NCS, microwave heating 160 °C, 2 h.

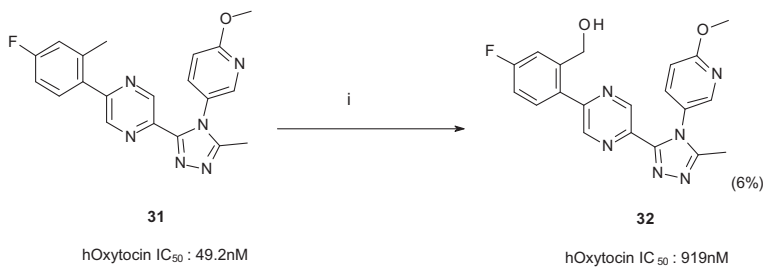
dium-catalyzed directed chlorination using *N*-chlorosuccinnimide (NCS) in acetonitrile.[27] The 2-chloro-6-fluoro analogue **27** was isolated in 15% yield (Scheme 11). As predicted, this chloro-derivative **27** was slightly more potent than the starting compound, primarily due to the slight change in the conformation of the phenyl; that is, the phenyl would be more perpendicular in the 2,6-disubstituted compound than the 2-substituted compound **26**. The activity of the products and 2-hydroxy adduct **24** demonstrated that conformation of the phenyl was key SAR.

In a different project, pyrimidone **28** was a lead compound in the melanin concentrating receptor 1 (MCHR-1) program. The pyrimidone **28** was tested in the BMO screen and found to be unreacted under all conditions. Pyrimidone **28** was then tested in the halogenation screen. Chlorination using NCS in acetonitrile/tetrahydrofuran (4:1) were optimum conditions and gave two close-running products which, after purification and structure elucidation, were found to be the monochloro- and dichloro-adducts, **29** and **30**, respectively (Scheme 12).

These reactions on **28** demonstrated that selective halogenation of heterocyclic groups in complex drug molecules was possible. Examples of compounds where the introduction of oxygen, fluorine and chlorine, have blocked metabolically vulnerable sites have been highlighted in this communication. However, biomimetic oxidation (BMO) has been previously used effectively to synthesize drug metabolites.[1] Metalloporphyrins are efficient catalysts for synthesizing drug metabolites because cytochrome p450's contain an iron metalloporphyrin in the active site of the enzyme and this is responsible for the oxidations.[1] As part of Pfizer's oxytocin antagonist program, a human specific metabolite **32** (Scheme 13) was identified and the synthesis of the metabolite **32** was needed for safety studies. The parent compound **31** was tested in the BMO screen and after scale-up; the hydroxymethyl metabolite **32** was isolated in 6% yield.



Scheme 12. Reagents and conditions: (i) MeCN/THF (4:1), NCS, rt, 19 h.



Scheme 13. Reagents and conditions: (i) Mn(TDClPP) (5 mol %), mCPBA, CH_3OH/CH_3CN (1:1), rt, 15 h.

Biological testing of the metabolite **32** showed it to be less potent than the starting compound **31**.

Transformations on drug molecules and the impact on some Pfizer medicinal chemistry projects have been elaborated. With the MCH lead compound, a simple but synthetically-difficult N-demethylation gave a more potent and more polar compound. Biomimetic oxidation has been reported as a method for metabolite synthesis, for example the oxytocin lead compound **31** was converted into the key metabolite **32** using this approach. LD is a viable and often practical approach for opening up chemical space and synthesizing key compounds. While the oxidation and BMO screens may not be successful on most substrates, other screens such as the halogenation screens can often work. As research into C–H activation increases and more transformations are discovered, LD will potentially have a more mainstream use in drug discovery.

Additional chemical transformations on drug molecules via C–H activation and oxidation have been described and their impact on drug discovery projects highlighted. The fluoropyrazole P38 compound **14** is another example of selective remote functionalization of a highly functionalized molecule. The conditions for fluorination were found using a combinatorial plate based screen that efficiently provided optimal methodology.

LD has demonstrated the difficulty in predicting selectivities and served to highlight purification as a significant hurdle, as the molecular weight of the drug increases.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.11.033.

References and notes

1. Chorghade, M. S.; Hill, D. R.; Lee, E. C.; Pariza, R. J.; Dolphin, D. H.; Hino, F.; Zhang, L.-Y. *Pure Appl. Chem.* **1996**, *68*, 753; Bernadou, J.; Bonnafous, M.; Labat, G.; Loiseau, P.; Meunier, B. *Drug Met. Dispos.* **1991**, *19*, 360; Segrestaa, J.; Verite, P.; Estour, F.; Menager, S.; Lafont, O. *Chem. Pharm. Bull* **2002**, *50*, 744; Mohajer, D.; Karimipour, G.; Bagherzadeh, M. *New J. Chem.* **2004**, *28*, 740.
2. Wender, P. A.; Hilinski, M. K.; Mayweg, A. V. W. *Org. Lett.* **2005**, *7*, 79.
3. Yoneyama, T.; Crabtree, R. H. *J. Mol. Catal. A* **1996**, *108*, 35; Sanford, M. S.; Deprez, N. R. *Inorg. Chem.* **2007**, *46*, 1924; Eberson, L.; Gomez-Gonzales, L. *J. Chem. Soc. Chem. Commun.* **1971**, 263; Zhang, R.; Nagraj, N.; Lansakara, D. S. P.; Lowell, P. H.; Newcombe, M. *Org. Lett.* **2006**, *8*, 2731.
4. Desai, L. V.; Stowers, K. J.; Sanford, M. S. *J. Am. Chem. Soc.* **2008**, *130*, 13285; Kalyani, D.; Deprez, N. R.; Desai, L. V.; Sanford, M. S. *J. Am. Chem. Soc.* **2005**, *127*, 7330.
5. *Palladium acetate catalyzed O-acetoxylation:*
Substrate (2.86 mmol) is suspended/dissolved in glacial acetic acid (2–3 ml) in a microwave vial. $PhI(OAc)_2$ (3.0 equiv) was added portion-wise followed by Palladium acetate (10–20 mg, excess). The vial is sealed by crimping on the lid. The vial was irradiated in the microwave reactor at 130 °C for 3600–7200 s (1–2 h). Typical yields on drug molecules were ca. 5–20%, it had not been shown conclusively whether adding more palladium catalyst and reheating improves the yield.
The solvent was evaporated under reduced pressure in the Genevac to give the crude reaction mixture, this was taken up in dichloromethane (40 ml) and washed with brine (saturated, 30 ml), water (30 ml), dried over magnesium sulphate, filtered and the solvent evaporated to give the crude material. This was then purified using preparative HPLC.
6. Litvinas, N. D.; Brodsky, B. H.; DuBois, J. *Angew. Chem., Int. Ed. Engl.* **2009**, *48*, 4513.
7. Doro, F. G.; Lindsay-Smith, J. R.; Ferreira, A. G.; Assis, M. D. *J. Mol. Catal. A: Chem.* **2000**, *164*, 97.
8. Cunningham, I. D.; Danks, T. N.; Hay, J. N.; Hamerton, I.; Gunathilagan, S.; Januczak, C. *J. Mol. Catal., A: Chem.* **2002**, *185*, 25.
9. Gonzalves, D'A. A. M. R. *J. Heterocycl. Chem.* **2002**, *34*, 499.
10. Ley, S. V.; Baxendale, I. R. *Chimia* **2008**, *62*, 162.
11. Park, B. K.; Kitteringham, N. R.; O'Neill, P. M. *Annu. Rev. Pharmacol. Toxicol.* **2001**, *41*, 443; Fisher, M. B.; Henne, K. R.; Boer, J. *Curr. Opin. Drug Discov. Devel.* **2006**, *9*, 101.
12. Bondi, A. *J. Chem. Phys.* **1964**, *68*, 441.
13. Ismail, M. D. *J. Fluorine Chem.* **2002**, *118*, 27.
14. Ramsden, C. A.; Smith, R. G. *J. Am. Chem. Soc.* **1998**, *120*, 6842.
15. *Organofluorine Chemistry. Principles and Commercial Applications*; Banks, R. E., Smart, B. E., Tatlow, J. C., Eds.; Plenum Press: New York, London, 1996; *Advances in Organic Synthesis Modern Organofluorine Chemistry—Synthetic Aspects*; Laali, K. K., Rahman, A.-U., Eds.; Bentham Science: Amsterdam, 2006; Vol. 2, Merrit, R. F.; Johnson, F. A. *J. Org. Chem.* **1966**, *31*, 1859; Purrington, S. T.; Kagen, B. S.; Patrick, T. B. *Chem. Rev.* **1986**, *86*, 997; Rozen, S. *Acc. Chem. Rev.* **1988**, *21*, 307; Chambers, R. D.; Parsons, M.; Sandford, G.; Thomas, E.; Trimcic, J.; Moilliet, J. S. *Tetrahedron* **2006**, *62*, 7162; Chambers, R. D.; Skinner, C. J.; Thomson, J.; Hutchinson, J. C. S. *Chem. Commun.* **1995**, 17; Chambers, R. D.; Holling, D.; Sanford, G.; Batsanov, A. S.; Howard, J. A. K. *J. Fluorine Chem.* **2004**, *125*, 661.
16. Banks, R. E. *J. Chem. Soc., Chem. Commun.* **1992**, 595; Singh, R. P.; Shreeve, J. M. *Acc. Chem. Res.* **2004**, *37*, 31.
17. Montgomery, J. A., Jr.; Petersson, G. A.; Al-Laham, M. A.; Mantzaris, J. *Chem. Phys. Lett.* **1990**, *169*, 497.
18. Lal, G. S.; Pez, G. P.; Syvret, R. G. *Chem. Rev.* **1996**, *96*, 1737; Gerstenberger, M. R. C.; Haas, A. *Angew. Chem., Int. Ed. Engl.* **1981**, *20*, 647; Welch, J. T. *Tetrahedron* **1987**, *43*, 3123; Borodkin, G. I.; Zaikina, P. A.; Shubin, V. G. *Tetrahedron Lett.* **2006**, *47*, 2639; Zhang, Y.; Shibatomi, K.; Yamamoto, H. *Synlett* **2005**, 2837.
19. Dichloroethane was used as an alternative to dichloromethane as it is less volatile and can be used with liquid handlers.
20. Chambers, R. D.; Spink, R. C. H. *Chem. Commun.* **1999**, 883; Chambers, R. D.; Fox, M. A.; Sanford, G. *Lab Chip* **2005**, *5*, 1132.
21. The plate was sealed up and agitated at room temperature for between 48 and 72 h as this was found to be the optimum reaction time. The reactions were quenched by the addition of aqueous methanol (1:1) and then analyzed by LCMS.
22. Marat, R. K.; Janzen, A. F. *Can. J. Chem.* **1977**, *55*, 3031.
23. Matsuzaki, T.; Hanai, S.; Kishi, H.; Liu, Z.-H.; Bao, Y.-L.; Kikuchi, A.; Tsuchida, K.; Sugino, H. *J. Biol. Chem.* **2002**, *277*, 19008.
24. Miller, D. C.; Klute, W.; Calabrese, A.; Brown, A. D. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 6144.
25. Chen, X.; Hao, X.-S.; Goodhue, C. E.; Yu, J.-Q. *J. Am. Chem. Soc.* **2006**, *128*, 6790.
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 (10 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 % 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.
27. Stowers, K. J.; Sanford, M. S. *Org. Lett.* **2009**, *11*, 4584; Kalyani, D.; Dick, A. R.; Anani, W. Q.; Sanford, M. S. *Org. Lett.* **2006**, *8*, 2523.