Fragment Based Drug Discovery: An Organic Synthesis Perspective

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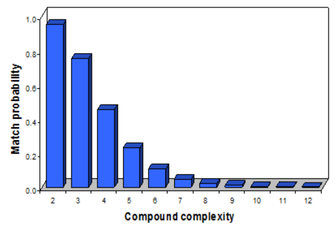
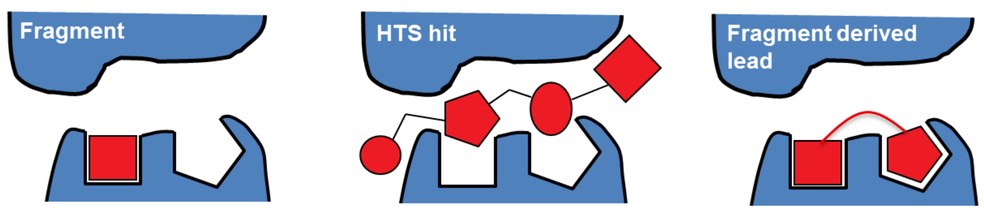
FRAGMENT BASED DRUG DISCOVERY (FBDD): KEY CONCEPTS

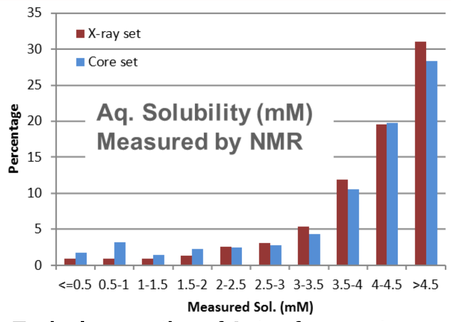
- Fragment hits bind with high energy interactions, so have high ligand efficiency (LE).
- Small libraries of fragments can sample chemical space more widely
  - There are estimated to be ~ 10^{50} compounds < 500 MW, cf. ~10^{6} fragments < 250 MW.
- Potencies in mM rather than µM range require sensitive biophysical techniques to detect interactions, e.g. X-Ray, NMR, SPR, ITC.
- Millimolar fragments can be converted to nanomolar leads with the support of structure based drug design.

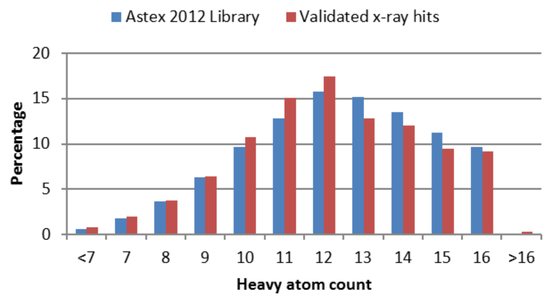


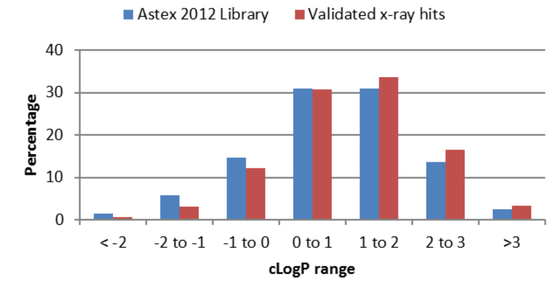
Simpler ligands are more likely to match receptors than complex ligands [1]



PROPERTIES OF ASTEX FRAGMENT LIBRARY







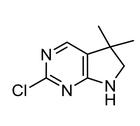
Typical properties of Astex fragments

- Molecular weight ~140-230 Da
- Non-hydrogen atoms 10-16
- Lipophilicity clogP ~0.0-2.0
- Aqueous solubility >5mM in 5% DMSO
- Stability >24 h in solution
- 3D Fragments are useful, but need to be kept small

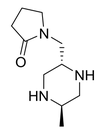
SOURCES OF FRAGMENTS AND SYNTHESIS OPPORTUNITIES IN FBDD

From Astex projects

- Synthetic intermediates can make good fragments
- Pd cyclization and piperazine synthesis generated multiple library members, e.g. Fragments 1, 2 (see AT-IAP example below)



HAC 12, cLogP 2.4
Fragment 1



HAC 14, cLogP -1.4
Fragment 2

Fragment Library Synthesis

- The Astex library contains many non-commercial, hand crafted molecules.
- Methods are needed to generate interesting pharmacophores with synthetically accessible growth vectors (Figure 1).
- Methods compatible with low logP substrates and 100 mg synthesis in ≤4 steps.
- Isolation of highly water soluble products is challenging!
- Precision synthesis guided by X-ray structural information requires bespoke transformations, e.g. incorporation of heteroatoms, stereo-control and regio-control
- CH activation example (Figure 2) [2,3] generates a bicycle with the possibility of substitution in a number of positions
- **Fragment synthesis represents an opportunity for the organic chemistry community**

Figure 1. Examples of fragment binding

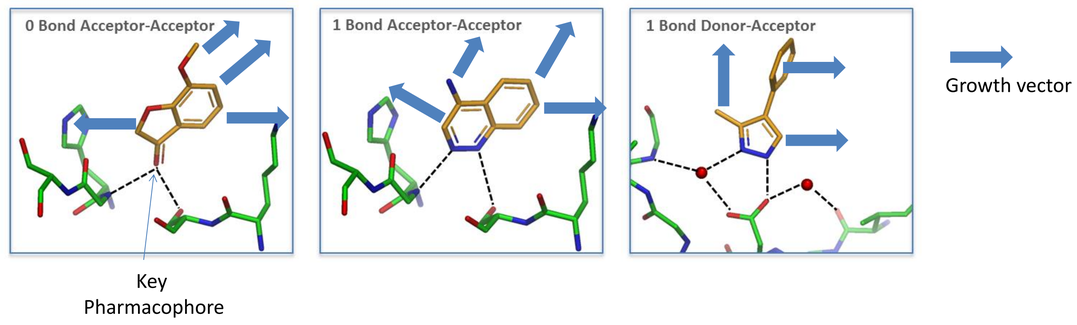
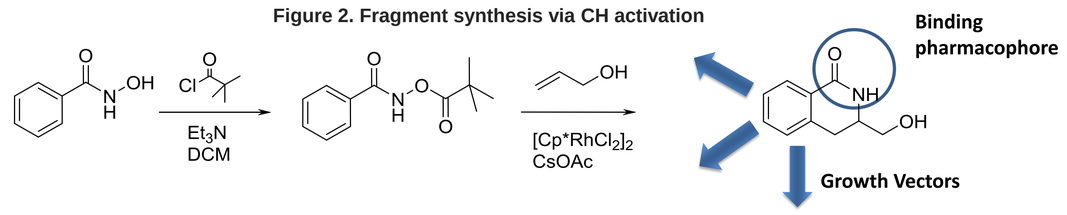
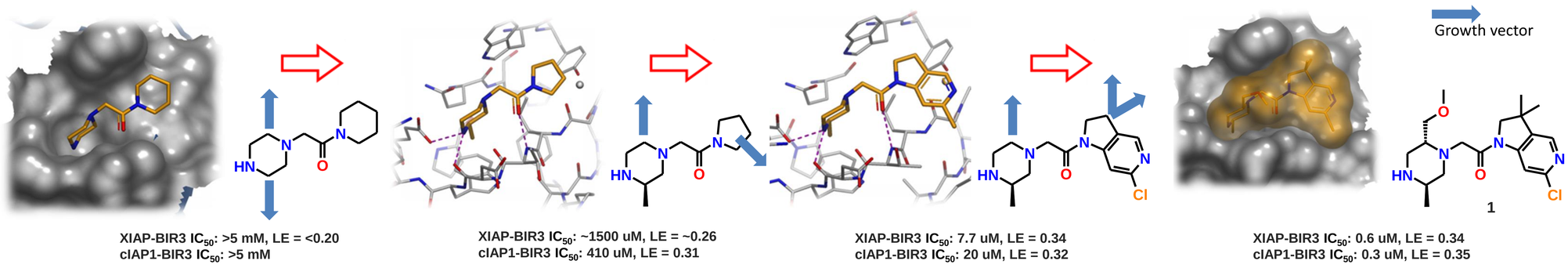


Figure 2. Fragment synthesis via CH activation



FRAGMENT TO LEAD OPTIMISATION EXAMPLE: IAP ANTAGONISTS

Inhibitor of apoptosis proteins (IAPs) are deregulated in many tumors and contribute to resistance to anticancer therapies. IAP antagonists can switch IAP-controlled pro-survival pathways towards apoptosis and cell death. Recent evidence suggests that a dual antagonist of cIAP1 and XIAP will promote a strong apoptotic response via caspase activation [4]. By applying the Astex Pyramid™ platform, we have identified fragments which bind to XIAP and cIAP1. Structure based drug design has been used to exploit the growth vectors of these fragments, generating potent lead molecules with balanced dual activity at both cIAP1 and XIAP.



XIAP-BIR3 IC_{50}: >5 mM, LE = <0.20
cIAP1-BIR3 IC_{50}: >5 mM

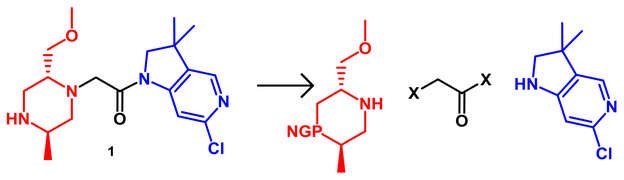
XIAP-BIR3 IC_{50}: ~1500 uM, LE = ~0.26
cIAP1-BIR3 IC_{50}: 410 uM, LE = 0.31

XIAP-BIR3 IC_{50}: 7.7 uM, LE = 0.34
cIAP1-BIR3 IC_{50}: 20 uM, LE = 0.32

XIAP-BIR3 IC_{50}: 0.6 uM, LE = 0.34
cIAP1-BIR3 IC_{50}: 0.3 uM, LE = 0.35

SYNTHESIS

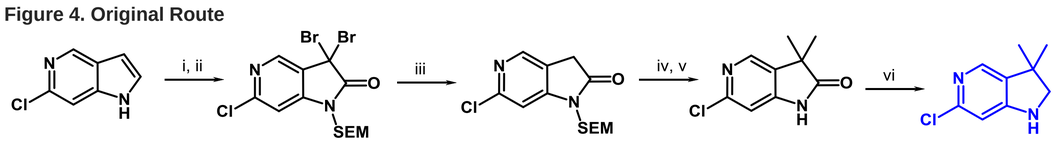
Figure 3 Retrosynthesis



- Retrosynthetic analysis of the target molecule **1** suggests a late-stage coupling (Figure3).
- The original route to the eastern half relied on the derivatisation of an azaindole (Figure 4). The route was low yielding and not suitable for large scale synthesis.
- Chemistry from the Larock group [5] makes use of an intramolecular, palladium catalysed cyclisation to synthesise indolines from aniline starting materials. We have now demonstrated that this chemistry is also applicable to heterocyclic amines (Figures 5,6). This gave us an improved route to **1** and also provided access to a series of analogous bicyclic groups.
- The western half contains a di-substituted piperazine with two chiral centres. The correct stereochemistry could be introduced from amino acid starting materials (Figure 7).
- Coupling of the two halves and deprotection provided the target molecule **1** (Figure 8).

SYNTHESIS OF AZAINDOLINE PORTION

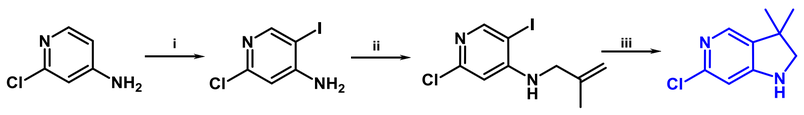
Figure 4. Original Route



- 6 Steps
- 20% Overall yield
- Unpredictable exotherm in step 3
- Not suitable for scale up

(i) NaH, SEMCl, DMF, 0 °C; (ii) $PyBr_3$, Dioxane, RT; (iii) Zn, $NH_4Cl_{(aq)}$, THF, RT; (iv) LHMDS, MeI, THF, -78 °C; (v) TFA, DCM, RT; (vi) BH_3-Me_2S, THF, 0 °C.

Figure 5. Improved Route

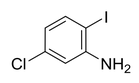
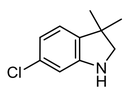
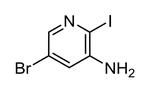
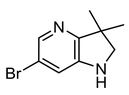
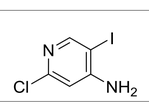
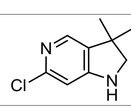
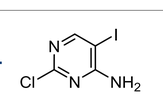
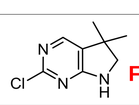
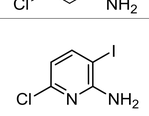
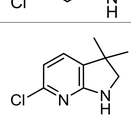
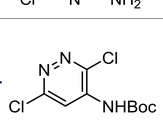
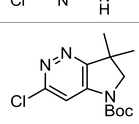


(i) NIS, MeCN, RT; (ii) KO^{t}Bu, 3-bromo-2-methylpropene, THF; (iii) $Pd(OAc)_2$, HCO_2Na, Bu_4NCl, Et_3N, PhMe, H_2O, 100 °C.

- 3 Steps
- Improved overall yield (28%)
- Performed on 100g scale
- Applicable to a range of heterocycles

APPLICATION TO OTHER BICYCLES [6]

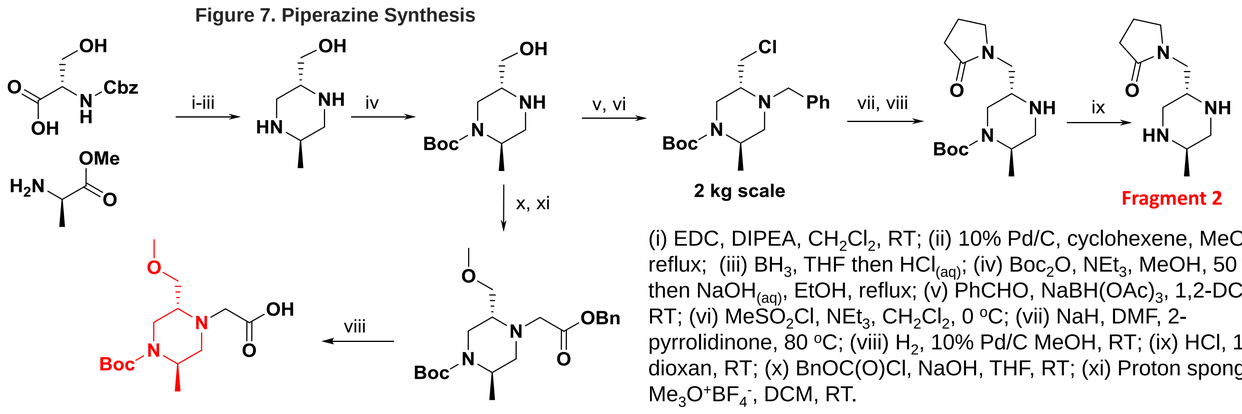
Figure 6. Other heterocycles

	Amine precursor	Product[a] (cyclization yield)		Amine precursor	Product[a] (cyclization yield)	
1.			(60%)	4.			(44%) **100 g**
2.			(89%) **100 g**	5.			(48%) **Fragment 1**
3.			(87%)	6.			(55%)[b]

a. (i) H_2C=C(Me)CH_2Br, KO^{t}Bu, THF, RT; (ii) $Pd(OAc)_2$, NEt_3, NaOCHO, Bu_4NCl, toluene, H_2O, 100 °C.
b. Cyclisation failed on unprotected substrate. 4,5-diazaindoline could be prepared from Boc protected material

SYNTHESIS OF PIPERAZINE PORTION

Figure 7. Piperazine Synthesis

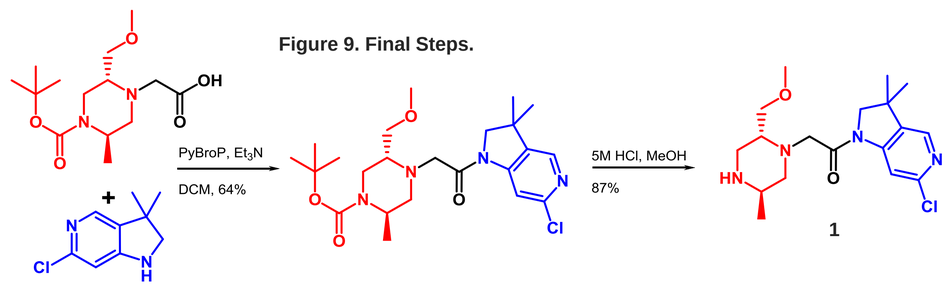


(i) EDC, DIPEA, CH_2Cl_2, RT; (ii) 10% Pd/C, cyclohexene, MeOH, reflux; (iii) BH_3, THF then $HCl_{(aq)}$; (iv) Boc_2O, NEt_3, MeOH, 50 °C then $NaOH_{(aq)}$, EtOH, reflux; (v) PhCHO, $NaBH(OAc)_3$, 1,2-DCE, RT; (vi) $MeSO_2Cl$, NEt_3, CH_2Cl_2, 0 °C; (vii) NaH, DMF, 2-pyrrolidinone, 80 °C; (viii) H_2, 10% Pd/C MeOH, RT; (ix) HCl, 1,4-dioxan, RT; (x) BnOC(O)Cl, NaOH, THF, RT; (xi) Proton sponge, $Me_3O^+BF_4^-$, DCM, RT.

- Scalable route developed to give trans disubstituted piperazines
- The chiral centres were introduced by employing amino acid starting materials

COUPLING AND DEPROTECTION

Figure 9. Final Steps.



SUMMARY

- **FBDD represents an opportunity for the organic synthesis community.**
  - Synthesis of fragments with diverse binding pharmacophores expressed in multiple scaffolds.
  - Chemistry methodology that allows multiple synthetically accessible vectors for fragment elaboration.
- Chemistry at Astex has been employed to prepare a series of azaindolines and diazaindolines as well as heavily substituted piperazines with defined stereochemistry

REFERENCES

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[2] Guimand, F. et al., JACS, 2011, 6449
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[5] Larock, R. C., Babu, S., Tet Lett, 1987, 28 (44), 5291
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