INTERACTION SCANNING LIBRARIES: A GENERAL STRATEGY FOR SCAFFOLD FUNCTIONALIZATION

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Introduction: Pharmacophoric interactions between small molecule ligand and its target protein rely both on the nature of the scaffold and some of its functional groups. It is then necessary to be able to vary rapidly and efficiently these small decorating substructures in order to optimize or add new interaction points between the ligand and its target. A general library synthesis strategy (**Interaction Scanning Libraries**) involving **8 sub-protocols** (**ISL-1** to **8**) and allowing the attachment of a diverse set of residues and linkers has been developed. A comprehensive set of **40 interaction groups** mapping all interaction types (hydrophobic, aromatic, acidic, basic, H-Bond Donor (HBD) and Acceptor (HBA)) through a wide range of distances (1-6 Å) has been carefully selected to provide a useful **tool for SAR generation**. The strategy is based on the use of a unique haloaromatic or heteroaromatic scaffold involved in several metallo-catalysed couplings. These 8 reactions (sub-protocols) are performed using **standardized library reaction conditions** (process, solvent, concentration) for maximum efficiency.

Concept & Strategies: The **ISL** concept relies on the fast preparation of analogs displaying a fundamental set of substituents on a unique scaffold. This fundamental set of 40 R-groups (Table 1) was chosen with an optimized balance of functional groups between aliphatic, aromatic, acidic, basic, H-Bond donor and acceptor features able to present additional interactions with the target protein. Such diverse set of decorative elements is attached to the scaffold with various linkers and has to be built with more than one chemistry involving deprotection steps in some cases. 8 metallo-catalysed processes are employed in a library format to cover such broad functionalization of an aryl-halide based scaffold (Figure 1).
The efficiency of the process relies on:

- The use of a unique aryl-halide based scaffold
- The constant availability of the derivatizing reagents and catalysts
- The late stage functionalization process in library format
- The only purification of the final compounds

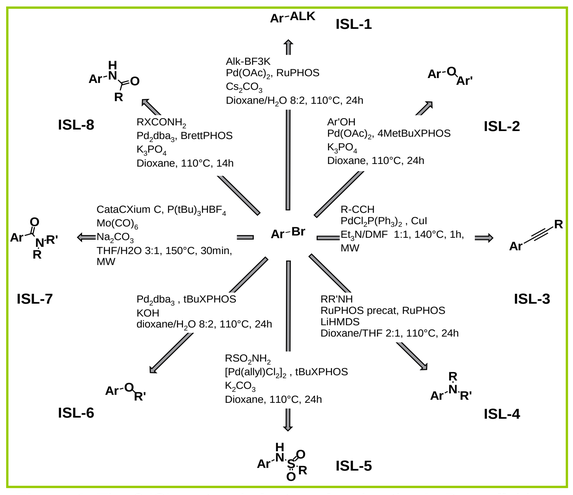

Figure 1: The 8 ISL protocols for transforming the Ar-Br scaffold

Hydrophobic Tails	Chemistry	Features (Distance Å)
	ISL1	HYDROPHOBIC (1.49)
	ISL1	HYDROPHOBIC (2.11)
	ISL1	HYDROPHOBIC (3.07; 3.89)
	ISL1	HYDROPHOBIC (1.93; 2.65; 4.37)
	ISL8	HYDROPHOBIC (4.46; 5.25; 5.65) ACEPTOR (3.01; 3.62) DONOR (1.42)

Aromatic Tails	Chemistry	Features (Distance Å)
	ISL1	AROMATIC (2.85)
	ISL1	AROMATIC (3.77)
	ISL2	AROMATIC (3.62) ACCEPTOR (1.36)
	ISL5	AROMATIC (4.16) ACCEPTOR (3.84; 3.15) ANION (1.35)
	ISL7	AROMATIC (5.16) ACCEPTOR (2.36) DONOR (2.42)
	ISL1	AROMATIC (5.21) HYDROPHOBIC(1.95)
	ISL4	AROMATIC (7.06) ACCEPTOR (4.27; 1.40)

HBD Tails	Chemistry	Features (Distance Å)
	ISL8*	DONOR (1.41)
	ISL8	DONOR (1.40) ACCEPTOR (3.01) HYDROPHOBIC (3.77)
	ISL6	DONOR (1.36) ACCEPTOR (1.36)
	ISL5	DONOR (1.43) ACCEPTOR (3.23; 3.86)
	ISL1*	DONOR (2.38) ACCEPTOR (2.38)
	ISL7	DONOR (2.44) ACCEPTOR (2.36)
	ISL1*	DONOR (2.97) CATION (2.97)
	ISL1*	DONOR (3.58) ACCEPTOR (3.69) AROMATIC (2.44)
	ISL1*	DONOR (4.55) ACCEPTOR (4.62) HYDROPHOBIC (1.92)
	ISL3	DONOR (4.77) ACCEPTOR (4.77)
	ISL3	DONOR (4.88) ACCEPTOR (4.88)
	ISL1*	DONOR (5.23) CATION (5.23; 2.46)

HBA Tails	Chemistry	Features (Distance Å)
	ISL6	ACCEPTOR (1.36) HYDROPHOBIC (2.38)
	ISL4	ACCEPTOR (1.41) *
	ISL1	ACCEPTOR (2.38)
	ISL7	ACCEPTOR (2.30)
	ISL8	ACCEPTOR (3.14) DONOR (1.42)
	ISL4	ACCEPTOR (4.22) DONOR (1.49)
	ISL3	ACCEPTOR (4.77)
	ISL6	ACCEPTOR (5.15; 1.37)
	ISL1	ACCEPTOR (6.71; 2.38) CATION (4.16)

Acidic Tails	Chemistry	Features (Distance Å)
	ISL7	ANION (2.09; 2.38)
	ISL1*	ANION (4.40; 4.42) HYDROPHOBIC (1.92)
	ISL3*	ANION (6.03; 5.18) HYDROPHOBIC (4.44)

Basic Tails	Chemistry	Features (Distance Å)
	ISL1	CATION (2.51) DONOR (2.51)
	ISL3	CATION (4.82)
	ISL1	CATION (5.11; 2.46)
	ISL1	CATION (5.73) DONOR (5.73) ACCEPTOR (2.38)

Table 1: The 40 functionalizing groups classified according to the 6 substructural features. *a subsequent deprotection step (TFA/H_2O/DCM 45:5:50, 4h rt) is used

Physico-Chemical Properties of ISL Members: Great care was taken in the choice of the substituents with a Focus on polarity and exposure of Hydrogen Bond Donating groups. 70% of residues are either HBD/HBA versus 30% aliphatic/aromatic. Basic and acidic groups are also included within the same library. Calculated LogP[1] for the 40 library members (Figure 2a) over 7 libraries (scaffold structures displayed in Table 3, R-groups displayed in Table 1) indicated that between 48% and 60% of library members have a LogP lower than the naked scaffold (Scaffold-H). In addition the LogP span over 4 units, (+/-2 units around the naked scaffold) showing a high level of variation of this parameter still keeping the scaffold unchanged. H Bond Acceptor coverage is also well represented with 11 chemical groups of various strength[2] (from carboxylic acid, sulfonamide, amide, amine, ether, alcohol, …) positioned at various distances (Table 1). **Control over MW** has been achieved with a moderate and well spread increase of the MW relative to the scaffold (between 20-70%) (Figure 2b). Typical example is 35% relative MW increase for a scaffold of MW=213 g/mol after addition of the 40 R-groups (Scaffold 2, Table 3). Obviously the maximum increase in MW is observed for the longer and bigger residues selected to potentially reaching the most remote interaction sites.

Figure 2a: Calculated ACDLogP for the 40 members of each of the 7 libraries (see Table 3 for scaffold structures)

Figure 2b: Calculated relative MW increase (ΔMW/MW) for the 40 members of each of the 7 libraries (see Table 3 for scaffold structures)

Spatial coverage is addressed with the use of several linker types of various flexibilities and directionalities (CH_2, O, N, SO_2, CO, NHCO, CC,..). Conformers of lower energies[3] have been aligned and distances of the different features[4] to the scaffold have been measured.[5] The 40 residues proposed more than one interaction type (feature) per residue due to their chemical complexity. Detailed distances of each member to the attachment point of the scaffold is depicted in Table 1. A 3D representation displays the explored target space (Figure 3a) with the 6 different interaction types (features). The distance to core frequency of each of the 6 features is well balanced between 1.5 and 6 Å as depicted in Figure 3b.

Figure 3a: 3D snapshot of aligned conformers of the 40 library members prepared with scaffold 1. 90 features are plotted for the 40 residues (see Table1)

Figure 3b: Distribution of the 6 features across the 1-7 Å distance range

Library Synthesis: Optimization of the reaction conditions focused on solvent type and concentration, reagent and catalyst equivalents and dispensing processes. Library is typically run on 0.25 mmol scale in 1 mL of dioxane at 110°C for 24h. Critical parameters such as base, catalyst and ligand could not be shared among all **ISL** protocols and were kept individually separated. Such issue was conveniently overcome by dispensing these reagents as stock solutions. Use of electron-rich bulky phosphine ligands was often crucial like with RuPhos for ISL-1 Molander type coupling,[6] 4MetBuXPhos for diphenylether ISL-2 synthesis,[7] or BrettPhos for ISL-8 primary urea or carbamate preparation.[8] Nature of the base is obviously important like KOH for alkoxyde formation in ISL-6[9] or Et_3N for Sonogashira coupling in ISL-3. The use of LiHMDS as strong base proved to be fairly general for coupling simple amines in ISL-4.[10] Other specific protocols such as ISL-5 for primary sulfonamide,[11] were found excellent. Only the Sonogashira type reaction of ISL-3 and Larhed carbonylation[12] used in ISL-7 needed a different solvent, higher temperature and microwave heating under pressure to achieve correct conversion. An example of scaffold functionalization is presented in Scheme 1 and Table 2 with the conversion results for the 40 prepared analogs derived from Scaffold 1.

Scaffold 1

ISL1	HPLC %	ISL1	HPLC %	ISL2/3	HPLC %	ISL4/5	HPLC %	ISL6/7	HPLC %	ISL8	HPLC %
ISL-1				ISL-2		ISL-4		ISL-6		ISL-8	
	79		75		74		88		78		64
	69		60				87	with Phenol Alkylation :			73
	26		80	ISL-3			89		88		
	62	with tBu or Boc deprotection :			29				83	with Boc deprotection :	
	90		32		75	ISL-5		ISL-7			67
	70		41		57		83		52	with Boc deprotection then Acylation :	
	84		69		67		79	with amide coupling :			65
	77		43	with tBu deprotection :					80		
	43	with tBu deprotection then NH4Cl coupling :			43				68		
	63		45						83		

Scheme 1, Table 2: Reactions were realized on 0.25mmol mmol scale. tBu or Boc deprotection were performed with TFA/H_2O/DCM 45:5:50, 4h r.t., Amide couplings were performed with EDCI/HOBt/DIEA in DCM 14h at rt. Phenol alkylations were performed with AlkX, Cs_2CO_3 in MeCN 14h at 80°C. Acylation was performed with AcCl/DIEA in DCM 14h at rt. Conversion were determined by LCMS/UV detection 210-260nm of the crude sample after aqueous workup.

The generalization of the **ISL** concept was confirmed over various scaffolds (Table 3) with good conversion results for the representative chemistries of the 8 ISL protocols. In some case, however, scaffolds (entry 2) or chemistries (ISL-2) provided lower overall results.

	Chemistries	ISL1	ISL2	ISL3	ISL4	ISL5	ISL6	ISL7	ISL8
Scaffold-X									
1		63	74	75	87	83	83	68	65
2		89	2	0	63	54	8	64	16
3		64	0	91	95	82	78	86	85
4		75	47	84	41	65	49	80	53
5		79	38	83	50	80	77	65	38
6		35	0	83	85	85	82	0	44
7		76	58	84	4	83	50	52	0

Table 3: Reactions were performed on 0.25mmol mmol scale. Conversion were determined by LCMS/UV detection 210-260nm of the crude sample after aqueous workup.

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4 Features were employed according to substructural definitions from Phase, verson 3.5, Schrödinger, LLC, New York, NY, 2012.
5 Coordinates and distances of features were obtained using Pipeline Pilot version 8.5; Accelrys Software Inc. San Diego, CA, USA.
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Applications:. This **ISL** library synthesis process can be efficiently applied to the functionalization of various aryl-halide based scaffolds for different goals:

- SAR generation in Hit to Lead program
- Fragment decoration in Fragment Based Drug Design
- Patent examplification
- 1st Generation library

Conclusion: A general library synthesis process (**Interaction Scanning Libraries**) involving **8 sub-protocols** (**ISL-1** to **8**) is described here for the rapid production of 40 analogs with selected residues offering a well balanced set of hydrophobic, aromatic, acidic, basic, HBD, HBA interactions through a wide range of distances (1-6 Å). This process is general and can be applied to many scaffold decoration strategies.