

In this example, the set of compounds that were studied satisfied many of the criteria for "drug likeness". However, in this program, there were issues with potency, novelty, and metabolic stability. This program also focused on a known target which means that it is likely that other companies and research groups have been working on a similar program. This program will likely attract little or no commercial interest unless these issues associated with compounds can be overcome.

Drug Discovery Guide - Example 1					
Requested Information	Status of Experiment	Value Scale (1-5)	Summary of Results	Description of Requested Information	
Lead Investigator: Indiana Jones	results positive				
Target: NFkappaB	results unclear				
Indication: anti-inflammatory	results negative				
	experiment ongoing				
1	I. Final Product Profile			Characteristics of the product required for patient care	
2	Method of administration		oral	For small molecules, the goal is generally to develop an oral drug (unless it is targeted for a hospital setting where administration through IV is acceptable). One needs to consider bioavailability (intestinal absorption, metabolism, etc.) during development.	
3	Dose frequency		once daily	An oral drug should be taken several times over 24 hours. This means the drug should have activity of 1-10nM against the target in vitro. It is important to determine plasma stability and metabolic stability and other experiments outlined in Sections IV and V.	
4	Comparison to standard of care		does not have side effects like ulcers and safe for liver common in standard of care	Efficacy needs to be superior to standard of care with acceptable safety and side effect profiles. Experiments listed in Section V will help provide clarity on these issues.	
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6	II. Target			Assessing information on the target	
7	Is the target novel?		5		The target has not been reported in the scientific community.
8	Is the target known but it is associated with a new indication?		4		The target is known in the scientific community but research showed is related to a new indication.
9	Is the target known?		3	target known in literature	Target that has been studied for a particular indication and the drug candidates being developed is for the same indication.
10	Do animal model exists for this target?		4		Is there a gold standard animal model for target and the indication? Are there positive and negative controls using this model?
11	Is the target's relations to the indication supported by human genetics?		3		Are there correlations between genetic defect and outcome?
12	Is there a validated biomarker associated with the target?		3		Are there any clinically accepted biomarkers (e.g., HDL, LDL for heart disease) ?
13	Biochemistry				
14	Existence of in vitro assays with reliable readouts		5	assays have been published	Please identify the type of assay (e.g., binding, competition, selectivity, etc.). Are there negative and positive controls for each assay?
15	Existence of cell based models with reliable readouts		5	assays have been published	Are there negative and positive controls for each assay?
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17	Structural Biology - X ray				
18	Is there a X-ray crystal structure of the target?		1	apo structure known	Check in the Protein Data Bank (http://www.rcsb.org/). A crystal structure can simplify compound design efforts and can allow for virtual screening.
19	Is there a co-crystal structure(s) of the target complexed with a small molecule ligand?		1	co-crystal with small molecule ligand reported	A co-crystal with a small molecule can give insight on molecular interactions and can be used for virtual screening.
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21	III. Small Molecule Development - Hit Phase			Objective: To identify compounds with adequate potency and potential for structure-activity relationship (SAR) studies that can be further developed into 1-2 series (compound classes) for advancing to the Lead Stage. An "active compound" in the hit phase is one with IC50 or Ki < 10 µM in a majority of in vitro assays.
22				
23	Compound Activity			
24	IC50 or Ki < 10 µM (in majority of in vitro assays)		5	only one compound satisfied this criteria Please specify results and assay (e.g., binding, competition, selectivity, etc..)and specify the positive and negative controls.
25	IC50 or Ki < 10 µM (in majority of in vitro cell based assays)		5	Please give information on the cell based functional assays. Please specify the positive and negative controls.
26				
27	Compound Properties			
28	Are the active compounds novel (have compounds or class of compounds been reported)?		4	compounds tested were not novel Active compounds from a novel scaffold are of high commercial value. An "active compound" in the hit phase is one with IC50 or Ki < 10 µM in an in vitro assay. A medicinal chemist can perform searches on Scifinder (https://scifinder.cas.org/ - available via the Levy Library) and evaluate the patent landscape. It is also helpful to consult a patent attorney when a comprehensive patent analysis is needed.
29	Are the active compounds (IC50 or Ki < 10 µM in an in vitro assay) amenable to SAR?		5	depends on which scaffolds were chosen for further study Structure Activity Relationship (SAR) in a compound class is critical in drug development. A compound series needs to be amenable to chemical changes to increase potency and drug-like properties. A medicinal chemist will have knowledge of the synthesis and can assess synthetic viability.
30	Is there enough data to establish pharmacophore?		3	A pharmacophore is an abstract description of the molecular features required for molecular recognition. Examples of features include hydrophobic groups, aromatic groups, hydrogen bond acceptors and donors. This information helps in compound design and virtual screening.
31	Has there been more than 10 active compounds identified?		3	very few tested Having more than 10 active compounds is not an absolute requirement, but a larger sample of compounds gives the project more confidence that a drug candidate can be identified.
32	Do the active compounds have molecular weights (MW) < 500?		3	Molecular weight (MW) < 500 is an indicator of bioavailability; if the MW is > 500, the molecule would have to have the right balance of other physicochemical properties (e.g., high bioavailability, low toxicity).
33	Is the clogP < 5 for majority of compounds tested?		3	The partition coefficient (P) of un-ionized compound has correlation with solubility and bioavailability; its is the partition of a compound between octanol and water; clogP is a calculated parameter that can be obtained from ChemDraw or molinspiration (http://www.molinspiration.com/services/psa.html).

34	Do the most active 5 compounds follow Lipinski rules ?		2		Lipinski rules ((max 5-H bond donors; # N+O atoms < 10, clogP < 5; MW<500) are only guidelines to assess the "drug-likeness" of compounds. For a brief description please see (http://www.nature.com/nature/journal/v481/n7382/box/481455a_BX1.html)
35	Are the majority of the compounds tested soluble at pH 7.4?		4		The solubility > 1mg/ml at pH 7.4 is desirable and this parameter can be measured. At this stage, an exact measurement is not needed and can be done by observation of the compounds in solution.
36	Are there less than 10 rotatable bonds in the compounds tested?		3		Rotatable bond is defined as any single non-ring bond, bounded to nonterminal heavy (i.e., non-hydrogen) atom. Amide C-N bonds are not considered because of their high rotational energy barrier. As smaller number of rotatable bonds is an indicator for bioavailability but it is not absolute. The goal is <10.
37	Do the active compounds contain functional groups known to generate reactive metabolites?		3	compounds have functional groups that are known to cleave; will have metabolic stability issues	Certain functional groups on a compound are known to form reactive species in the body and may be lead to issues in metabolism. A medicinal chemist would know the list of functional groups to avoid. Also see SpotRM website (http://spotrm.com/index.php/home), which can do the analysis for you.
38	Is the polar surface area < 140 A2 in the majority of active compounds tested?		2	PSA < 120 A2	The Molecular Polar Surface Area (PSA), defined as the sum of surface contributions of polar atoms (usually oxygens, nitrogens and attached hydrogens) in a molecule, has been shown to correlate well with drug transport properties such as intestinal absorption or blood-brain barrier (BBB) penetration. For IV drugs, it may be more flexible. For compounds to penetrate BBB, the PSA should be < 90 A2. The PSA can be calculated from ChemDraw or molinspiration (http://www.molinspiration.com/services/psa.html).
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41	IV. Small Molecule Development - Lead Phase				Objective: To improve the potency of 1-2 series of compounds from the Hit Phase and examine at the pharmacokinetics (PK), including <u>a</u> bsorption, <u>d</u> istribution, <u>m</u> etabolism and <u>e</u> xcretion (ADME), and toxicity. The goal is to advance a small number of compounds for animal (beyond mouse or rat) studies. The minimum criteria for "active compound" in the Lead Phase is one with IC50 or Ki < 1 μ M in a majority of vitro assays. It is highly desirable to have compounds with IC50 or Ki < 0.1 μ M.
42					
43	Compound Activity				
44	In vitro assays				Please specify results and assay (i.e. binding, competition, selectivity, etc.); proper positive and negative controls used?
45	IC50/Ki < 1 μ M (in majority of assays)		5		
46	IC50/Ki < 0.1 μ M (in majority of assays)		4		
47	in vitro cell based assays				Please specify results and positive and negative controls used.
48	IC50/ Ki < 10 μ M (in majority of assays)		5		
49	IC50/ Ki < 1 μ M (in majority of assays)		4		
50					
51	Compound Properties				
52	Are the series of compounds novel?		4		Please see Line 28 for description on the importance of targeting novel chemical series.
53	Is there enough data from each series to confirm SAR?		5		There is enough data in each of the series tested to confirm SAR. Please see line 29 for description of SAR
54	Are key pharmacophores confirmed?		3		Please see Line 30 for description of pharmacophores.
55	Are the most active 5 compounds/series have MW < 500?		3		Please see Line 32 for description on importance of MW.
56	Is the clogP < 5 for the most active compounds?		3		Please see Line 33 for description importance of clogP.
57	Do the most active 5 compounds/series follow Lipinski rules?		2		Please see Line 34 for description of the Lipinski Rules of 5.
58	Are the most active compounds/series soluble at pH 7.4?		3		Please see Line 35 for description of solubility.
59	Is the number of rotatable bonds < 10 in the most active compounds/series?		3		Please see Line 36 for description of rotatable bonds.
60	Do the active compounds contain functional groups known to generate reactive metabolites?		4		Please see Line 37 for description of chemistries in metabolism.
61	Is the polar surface area < 140 A ² in the 5 most active compounds/series?		2		Please see Line 38 for description of polar surface area.
62					

63	ADME/Tox (tested for lead compounds)			Contract research organizations (CROs) should be used to perform the experiments to obtain data in this section. Examples of CROs include Eurofin (http://www.eurofins.com/); CEREP (www.cerep.fr/); Albany Molecular (ARMI - http://www.amrigoal.com/); Absorption Systems (http://www.absorption.com/); Cyprotex (http://www.cyprotex.com/); Drumetix (http://www.drumetix.com/php/dmpk-plasma-stability.php)
64	plasma protein binding assay		4	The test compound is incubated in plasma and supernatant and analyzed at different time points to see if compound is stable and whether is it bound to plasma proteins. (Estimated Cost: \$2000)
65	cytochrome P450 inhibition assay		4	This tests whether the compound interacts with the cytochrome P450 enzymes (CYP) in metabolism. Typical isoforms tested are CYP1A, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6 and CYP3A4 but others may be included. Showing there is no CYP liability is very positive. (Estimated Cost: \$3000)
66	PXR nuclear receptor assay		4	This assay test whether a compound activates the PXR nuclear receptor, which upregulates phase I oxidation enzymes and phase II conjugating enzymes in metabolism. Phase I reactions convert a parent drug to more polar (water soluble) active metabolites, and Phase II reactions convert a parent drug to more polar (water soluble) inactive metabolites by conjugation. See http://www.medbullets.com/step1-pharmacology/7006/phase-i-vs-phase-ii-metabolism . Compounds under development should not activate this receptor. (Estimated Cost \$700)
67	hERG assay		5	This assay tests the potential for a compound to cause arrhythmia called <i>Torsade de Pointes</i> . The human ether-a-go-go related gene (hERG) encodes the inward rectifying voltage gated potassium channel in the heart which is involved in cardiac repolarization. Compounds that inhibit this channel are likely to cause Torsade de Pointes. Cyprotex does this assay and tests other ion channels. (Estimated Cost: \$1000)
68	Animal Studies			These CROs have focus on animal studies: Sphaera (http://www.sphaerapharma.com/); Invivotek (http://www.invivotek.com/).
69	PK studies from animal models (rodent) - Single Dose (IV and/or oral)		4	A single dose study can give initial read on the following parameters: Tmax (time for max plasma concentration of compound), Cmax (max plasma concentration), t1/2 (half life, time for plasma concentration to drop by half of equilibrium concentration not the initial concentration.), AUC (area under curve, measurement of total drug exposure). See (http://www.thebody.com/content/art875.html). Having favorable stability and toxicity data early can significantly improve the value of the asset (Estimated Cost \$700-\$1200)
70	Results from animal models		5	It is important to confirm the animal model used is one that is endorsed by clinical experts for the indication. (Costs depend on model selection)
71				
72	PK studies from animal models (rodent)- ascending, multi-day dose (IV and/or oral)		3	This study gives more information on parameters outlined on Line 69 (Estimated Costs \$5000)
73				

74	V. Additional ADME/Tox			Items in this subsection are good, but are more likely to be important for IND enabling and IND Candidate selection. The CROs mentioned on Line 63 also provide these assays.
75				
76	Intestinal Absorption			
77	PAMPA (parallel membrane permeability assay)		3	This test measures a compound's ability to diffuse through a lipid-infused artificial membrane into an acceptor compartment. It avoids the complexities of active transport, allowing test compounds to be ranked based on a simple permeability property alone. (Estimated Costs \$450)
78	Caco-2 assay		3	Measures the amount of test compound that has crossed a Caco-2 cell monolayers grown on microporous membranes in multiwell insert systems (Estimated Costs \$600)
79	Metabolism			
80	Metabolic stability assay		3	This assay measures in vitro intrinsic clearance (ability of hepatic enzymes to metabolize a drug) and monitors disappearance of parent compound with time. It uses microsomes or hepatocytes. T _{1/2} can be used to estimate in vivo hepatic clearance. (Estimated Costs \$2100)
81	Metabolic profiling assay		3	Incubation of drug candidate with hepatocytes followed by identification of metabolites. Metabolites identification is used to optimize compound structure (decrease metabolic toxicity). It is also used to pick animal species in future tests. At times, hepatocytes from different animal species are used and data is used to help explain in vivo results. (Estimated Costs \$3000)
82				
83	Cytotoxicity			These assays test the compound for toxicity against different cell types (hepatocytes, endothelial cells, neuronal and glial cells, skeletal myocytes, cardiomyocytes)
84	Membrane integrity assay		2	This assay measures increase in cytoplasmic enzymes in culture after treatment. (Part of panel from Cyprotex of Estimated Cost \$1500-\$2500)
85	Cellular ATP assay		2	Dead or damaged cells contain little or no ATP. ATP levels are measured using a luciferin-luciferase assay. (Part of panel from Cyprotex of Estimated Cost \$1500-\$2500)
86	Mitochondrial function assay		2	This assay looks at the conversion of MTT from a yellow compound to a blue compound by NAD(P)H-dependent oxidoreductase enzymes in the cytosolic compartment of the cell which reflects cell metabolism. (Estimated Costs \$1300-\$1800)
87	Lysosomal function assay		2	This assay looks at Neutral Red uptake by cells due to lysosomal activities. Cell damage would have decrease in uptake. (Estimated Costs \$1200-\$2400)
88	Apoptosis induction assay		2	Measures caspase activity to reflect apoptosis. (Part of panel from Cyprotex of Estimated Cost \$1500-\$2500)
89				

90	Genotoxicity				
91	Micronucleus assay		3		This test screens for genotoxic carcinogens. It detects damage to the cell's chromosomes, or spindle apparatus. After exposure to a test substance, cells are allowed to divide. It is upon division that this type of damage can result in the formation of a smaller 'micronucleus', apart from the main nucleus. Litron Labs seem to specialize in this assay (http://litronlabs.com/default.htm) (Estimated Costs \$1600-\$3200)
92	AMES assay		3		This tests for whether a compound is a mutagen test. It uses a bacteria they require histidine for growth, but cannot produce it. The method tests the capability of the test compound to cause mutations that result in a return to a "prototrophic" state, so that the cells can grow on a histidine-free medium. There are kits for this assay (http://moltox.com/mutagenicity-assay-kits.php) but CROs can also do it. (Estimated Costs \$1800)
93					
94	Animal Studies				These CROs focus on animal studies. Sphaera (http://www.sphaerapharma.com/); Invivotek (http://www.invivotek.com/)
95	PK - non-rodent		2		Dog or monkey studies can be important pre-IND enabling dependent on the critical animal model species. It is critical to chose the species that is expected to show the most toxicity. (Costs depend on model selection)