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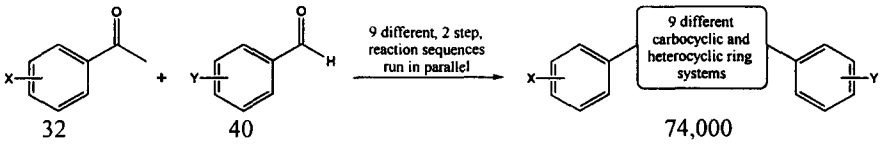
TETRAHEDRON

Automated Parallel Synthesis of Chalcone-Based Screening Libraries

David G. Powers*, David S. Casebier, Demosthenes Fokas, William J. Ryan, Jonah R. Troth, and David L. Coffen

ArQule, Inc. 200 Boston Ave., Medford, MA 02155

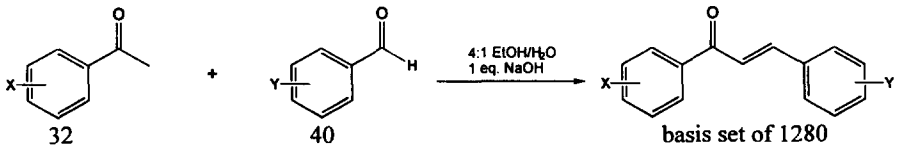
Abstract: Using a variety of condensation and cyclization reactions, nine combinatorial arrays of individual chalcone derivatives, over 74,000 in all, were produced in 50 μM quantities. The ease with which a broad range of diversity can be fabricated around a single molecular motif using automated parallel solution phase synthesis has been demonstrated.



The effort to prepare large numbers of novel, patentable, structures for lead discovery has historically relied on scaffolds and reagents that were commercially available. The need to expand the scope of both the chemistries available to prepare libraries, and the structural diversity present in those libraries, created a new direction in the area of parallel synthesis; the development of new sets of specialty materials that have been prepared for use as proprietary building blocks. This type of modular library design using sequential reactions in overlay on existing arrays allows for the very rapid generation of large numbers of compounds. In this example, chalcones were chosen as the featured building block because of their chemical flexibility toward further elaboration, ease of preparation, and by virtue of their already having incorporated two elements of diversity. Variation in library motif was then introduced by changing the chemistry to which the building block set was exposed.

The first step in this convergent strategy was the development of a chalcone library that would become a basis set for further elaboration. As can be seen in figure 1, chalcones are readily prepared by aldol condensation of an aryl aldehyde with an acetophenone.[1] The constituents that were chosen for the chalcone array included substituted aryl, including both 5-, and 6-membered ring heteroaryl, aldehydes and acetophenones. Initial investigational libraries were prepared to check whether the reagent sets that were chosen reliably afforded arrays of high purity. It was found that the chalcone array of 1280 compounds, prepared by the cross of 32 acetophenones by 40 aldehydes, routinely had an average purity of 96% as determined by mass spectral analysis and reverse phase HPLC (light scattering detection). The constituent aldehydes and acetophenones used in the formation of the basis set are listed in table 1.

Figure 1.



PII: S0040-4020(98)00137-9

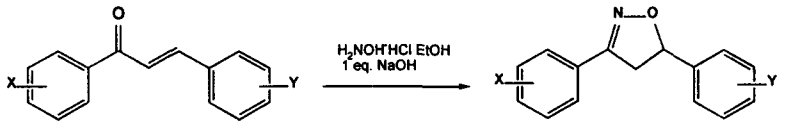
Table 1.

Aldehydes	Acetophenones
2-furaldehyde	acetophenone
3-(4-*t*-butylphenoxy)benzaldehyde	3'-methylacetophenone
3-(3-trifluoromethylphenoxy)benzaldehyde	4'-methylacetophenone
3-(4-methylphenoxy)benzaldehyde	3',4'-dimethylacetophenone
3-(3,4-dichlorophenoxy)benzaldehyde	4'-ethylacetophenone
3-bromobenzaldehyde	4'-*t*-butylacetophenone
3-furaldehyde	4'-cyclohexylacetophenone
5-ethyl-2-furaldehyde	3'-methoxyacetophenone
5-methylfurfural	4'-methoxyacetophenone
4-ethylbenzaldehyde	4'-ethoxyacetophenone
2,5-dimethylbenzaldehyde	3',4'-dimethoxyacetophenone
2-thiophenecarboxaldehyde	2'-methylacetophenone
3-thiophenecarboxaldehyde	4'-*n*-butylacetophenone
4-bromo-2-thiophenecarboxaldehyde	2-acetyl-5-methylfuran
4- *n*-butoxybenzaldehyde	2-acetylfuran
3,4-dichlorobenzaldehyde	2-acetyl-1-methylpyrrole
m-anisaldehyde	2-acetyl-3-methylthiophene
4-isopropylbenzaldehyde	2'-trifluoromethylacetophenone
4-propoxybenzaldehyde	2'-fluoro-6'-trifluoromethyl-acetophenone
3-methyl-*p*-anisaldehyde	2',4',6'-trimethylacetophenone
6-methyl-2-pyridinecarboxaldehyde	2'-methoxyacetophenone
1,4-benzodioxan-6-carboxaldehyde	2',4'-dimethoxyacetophenone
5-methyl-2-thiophenecarboxaldehyde	2',5'-dimethoxyacetophenone
benzaldehyde	2',6'-dimethoxyacetophenone
3-(4-methyoxyphenoxy)-benzaldehyde	2'-fluoro-4'-methoxyacetophenone
3,5-dimethoxybenzaldehyde	2',3',4'-trimethoxyacetophenone
4-*t*-butylbenzaldehyde	4'-chloroacetophenone
3,4-dimethoxybenzaldehyde	1,4-benzodioxan-6-yl methyl ketone
3-phenoxybenzaldehyde	4'-morpholinoacetophenone
4-bromobenzaldehyde	4'-piperidinoacetophenone
o-tolualdehyde	3',4'-methylenedioxyacetophenone
3-fluoro-*p*-anisaldehyde	3',5'-dimethoxyacetophenone
2,6-difluorobenzaldehyde	
4-ethoxybenzaldehyde	
4-fluorobenzaldehyde	
2,4-dichlorobenzaldehyde	
4-chlorobenzaldehyde	
4-phenoxybenzaldehyde	
m-tolualdehyde	
p-tolualdehyde	

The goal of the program was now to successively develop chemistries that would allow access to various manifolds of five- and six-membered ring systems using the chalcone synthons. The first chalcone based library involved the treatment of the basis set with hydroxylamine hydrochloride under basic conditions to form the isoxazoline ring system shown in figure 2.[2] While the third element of diversity for this library was equal to one (hydroxylamine), the library still contained over one thousand analogs due to the size of the initial chalcone library. Similar chemistry was performed using hydrazine hydrate to prepare a library of

pyrazoline derivatives, however upon storage these molecules were observed to partially air oxidize to mixtures of the desired product and the corresponding aromatic diarylpyrazoles. Efforts to drive the oxidation of the pyrazoline/pyrazole mixtures to completion did not provide satisfactory results and were discontinued.

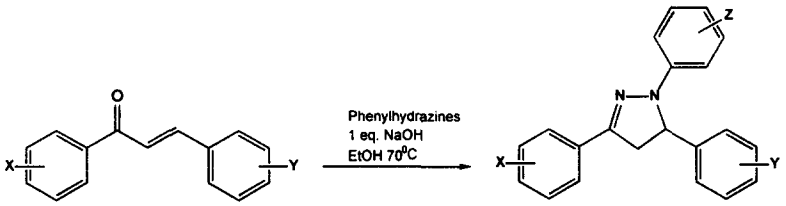
Figure 2.



1280 1280

The next series of libraries based on the pyrazoline scaffold were prepared by the condensation of the chalcone basis set with various phenylhydrazines.[2] The resultant N-phenyl pyrazolines seen in figure 3 are quite stable and do not suffer from air oxidation upon storage. Arrays were prepared using six different phenylhydrazines (Z = H, 4-OMe, 3-CF_3, 4-F, 4-*i*-Pr, and 3,4-$(CH_3)_2$) affording 7680 individual analogs.

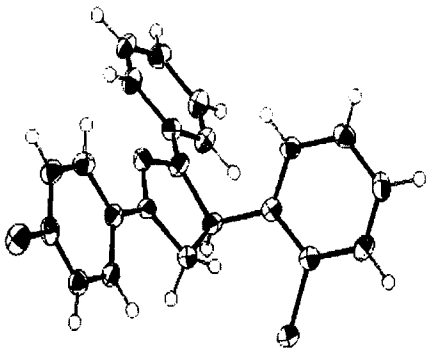
Figure 3.



1280 7680

The original assignment of the N-phenyl-pyrazoline structure shown was based on the NMR spectrum which clearly showed three alkyl protons with appropriate vicinal and geminal coupling constants. This initial regiochemical and tautomeric assignment was verified by X-ray crystallographic structure determination for a representative constituent of the library. The ORTEP representation of the diffraction data is shown in figure 4.

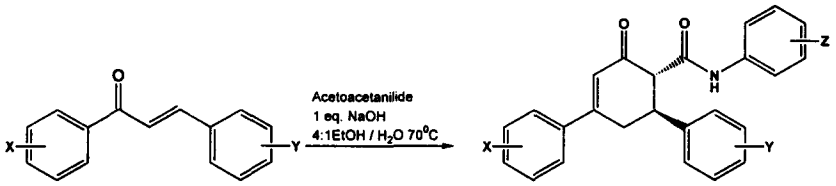
Figure 4.



Single crystal X-ray structure of N-phenyl-3-(4-fluorophenyl)-5-(2-chlorophenyl)-pyrazoline (ORTEP representation)

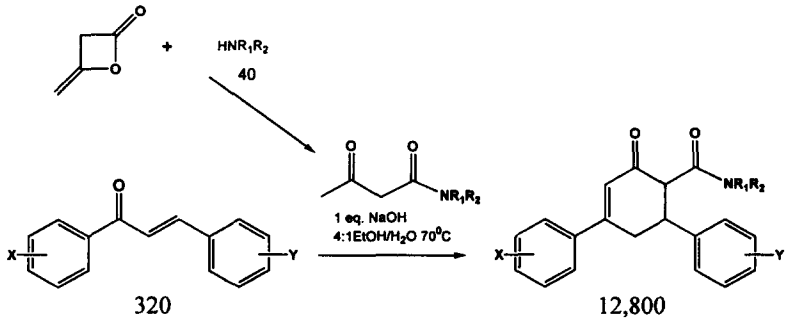
Concurrent with the efforts to prepare five-membered ring scaffolds, several methods that would afford a central six-membered ring scaffold using the chalcone building block set were under investigation. It was found that the Michael addition of β-dicarbonyl compounds to the chalcone library proceeded very well and that if ethylacetoacetate or an acetoacetanilide were used that the Michael adduct would then undergo Robinson ring closure to afford cyclohexenone derivatives of the type shown in figure 5.[3]

Figure 5.



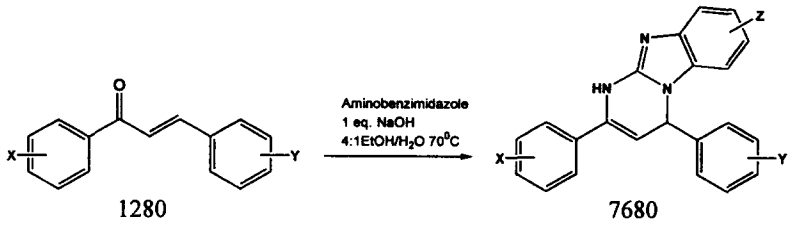
The preparation of these materials is very economical with respect to the reagents used. The same equivalent of sodium hydroxide that was used to prepare the chalcone serves as the base for the Michael addition and in the subsequent Robinson ring annulation. It is important to note that this series of reactions results in the formation of three different carbon-carbon bonds. These derivatives contain two chiral centers and the possibility of isolating the products as diastereomeric mixtures was originally a concern, however under the reaction conditions used only the thermodynamically favored *trans* adducts were obtained. Arrays using six acetoacetanilides (where Z = H, 4-Cl, 2-Me, 2-OMe, 4-OMe, and 4-phenyl) were prepared for a total of 7680 analogs. This series of molecules was extended to incorporate further diversity by the creation of an acetoacetamide building block set. This was accomplished by the condensation of forty primary and secondary amines with diketene as shown in figure 6. Due to the potentially overwhelming size of this array, only 320 of our 1280 chalcone building blocks were crossed with the forty acetoacetamides to afford a library of 12,800 analogs.

Figure 6.



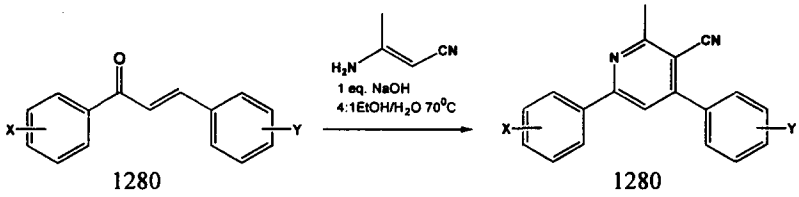
Another series of compounds that were prepared containing a central six-membered ring arose from exposure of the chalcone basis set to a 2-aminobenzimidazole under basic conditions to afford the diphenyl-tetrahydropyrimidines shown in figure 7.[4] Once again the chalcone basis set was crossed with six reagents to afford an array of 7680 derivatives. The six 2-aminobenzimidazoles used include the parent system (Z = H, 5,6-dichloro, 5,6-dimethyl, 5-methoxy, 5-fluoro, and 5-trifluoromethylaminobenzimid-azoles). The asymmetrically substituted aminobenzimidazoles afforded mixtures of regioisomers.

Figure 7.



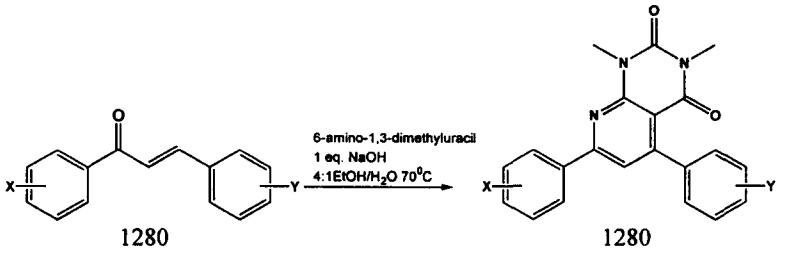
Having prepared the acetoacetanilide and aminobenzimidazole conjugates, further investigation of other 1,3-dinucleophiles that would afford 6-membered ring scaffolds in a regioselective manner seemed warranted. One of the first examples that was attempted was based on the Hantzsch synthesis of dihydropyridines.[5] Treatment of chalcones with enamino esters, such as ethyl 3-aminocrotonate, under standard Hantzsch cyclization conditions (EtOH, reflux) provided the desired adducts in only modest yields, however, it was observed that 3-aminocrotononitrile underwent the desired condensation with the chalcone basis set to afford the 2,3,4,6-tetrasubstituted pyridines shown in figure 8.

Figure 8.



It was also found that cyclic-enamino systems such as those shown in figures 9 and 10 afforded fused ring diphenyl-pyridyls in high yield. The condensation to form the dihydropyridine adducts expected from a Hantzsch synthesis in this case afforded the fully oxidized (aromatic) pyridyl product presumably due to the highly conjugated system formed. Interestingly, a group interested in the synthesis of nicotinic acid derivatives reports that derivatives of this general type required forcing conditions with several equivalents of reagent to effect the transformation on solid phase while another reference says that syntheses of similar heterocycles using unsubstituted aminouracil could not be effected in solution due to the lack of reactivity of the enamine.[5,6] A library of 1280 amino-dimethylaminouracil/chalcone conjugates was prepared.

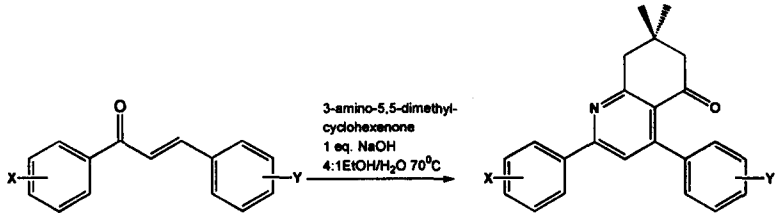
Figure 9.



In analogy to the uracil array described, 3-amino-5,5-dimethylcyclohexenone and other related cyclic enamines, have been successfully used as 1,3-dinucleophiles to afford a library of 7680 diphenylpyridyl derivatives. Cyclic 3-amino-*alpha*, *beta*-unsaturated carbonyl systems are readily generated by the treatment

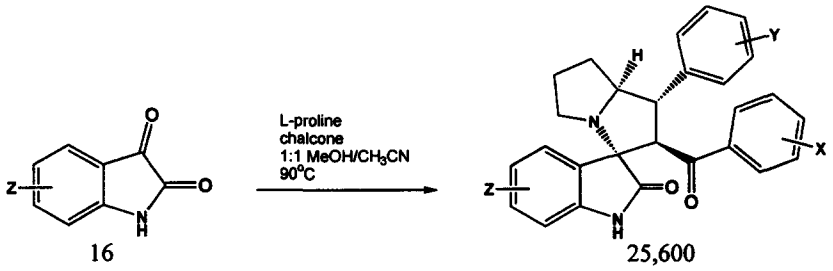
of 1,3-dicarbonyls with ammonia. Consequently, the potential library size for this series was quite large. The aminohexenone reagent set used for this array was prepared from 1,3-cyclohexanedione, 1,3-cycloheptanedione, 5-methyl-1,3-cyclohexanedione, 5-isopropyl-1,3-cyclohexane-dione, and 5-phenyl-1,3-cyclohexanedione.

Figure 10.



The most intriguing library prepared to date that has included the chalcone array as a building block set is the preparation of the spiro-polyheterocycles shown in figure 11. Isatins are cyclic-dicarbonyl amides whose chemistry revolves around the reactive nature of the *alpha*-carbonyl. The combination of this aldehyde equivalent with an amino acid, such as proline shown, followed by concomitant decarboxylation results in the formation of a 1,3-dipole of sufficient reactivity that addition to the recalcitrant dipolarophile chalcone occurs without the aid of Lewis acids.[7,8] With twenty available isatin analogs, twenty naturally occurring amino acids and approximately 1000 chalcones, the complete library of these analogs would number approximately 500,000. An array of 25,600 analogs was prepared from 80 chalcones, 20 amino acids, and 16 isatins (Table 2).

Figure 11.

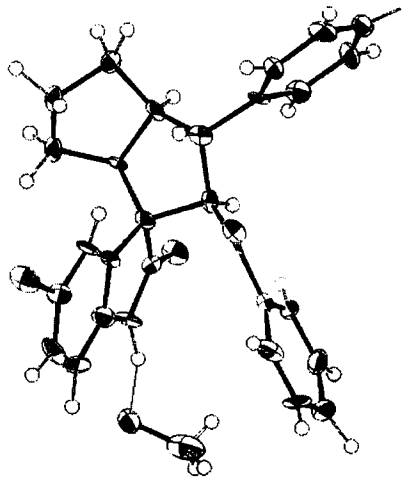


The products were isolated as single racemates where four new stereo-centers had been generated in one transformation. The initial regiochemical assignments of addition were based on NMR data and from analogous systems that had been reported in the literature, however single crystal X-ray analysis of the adduct from 5-bromoisatin, proline, and chalcone (X = Y = H) demonstrated that the regiochemistry of addition was the reverse of that predicted.[9] The ORTEP representation of the diffraction data is shown in figure 12.

Table 2.

Isatins	Amino Acids
5-fluoroisatin	sarcosine
isatin	L-valine
1-methylisatin	L-methionine
5-methylisatin	L-methionine sulfoxide
5-nitroisatin	L-methionine sulfone
5-iodoisatin	L-alanine
1phenylisatin	L-glutamine
5-chloro-7-methylisatin	L-threonine
5,7-dimethylisatin	D-serine
5-bromoisatin	L-phenylalanine
5-chloroisatin	glycine
5-trifluoromethoxyisatin	L-leucine
1-benzylisatin	O-benzyl-D,L-serine
1-(3-chloro)-benzylisatin	O-methyl-L-tyrosine
1-(4-methoxy)-benzylisatin	L-isoleucine
1-allylisatin	L-proline
	4-hydroxy-L-proline
	R-thiazolidinecarboxylic acid
	L-tryptophan
	L-phenylglycine

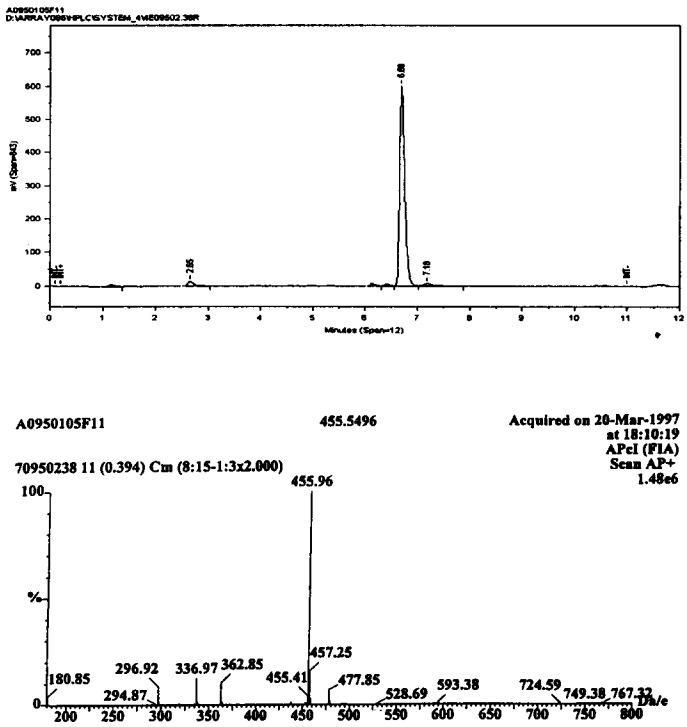
Figure 12.



Single crystal X-ray structure of a spiro[pyrrolidine-2,3'-oxindole].
Unit cell contains one methanol of crystallization
(ORTEP representation)

Automated parallel synthesis is supported by a sophisticated high-throughput analytical laboratory and all of the single compound arrays described above meet quality control standards based on MS and HPLC analyses as shown in figure 13 below. For each library, a QC pattern containing 25% of the library was analyzed by reverse phase HPLC, using light scattering detection, and flow injection mass spectrometry. For each library, the average purity by HPLC was greater than 85%. Each QC well also afforded a strong molecular ion (APCI, chemical ionization, N_2) of the desired mass to help insure that the major component was the target product.

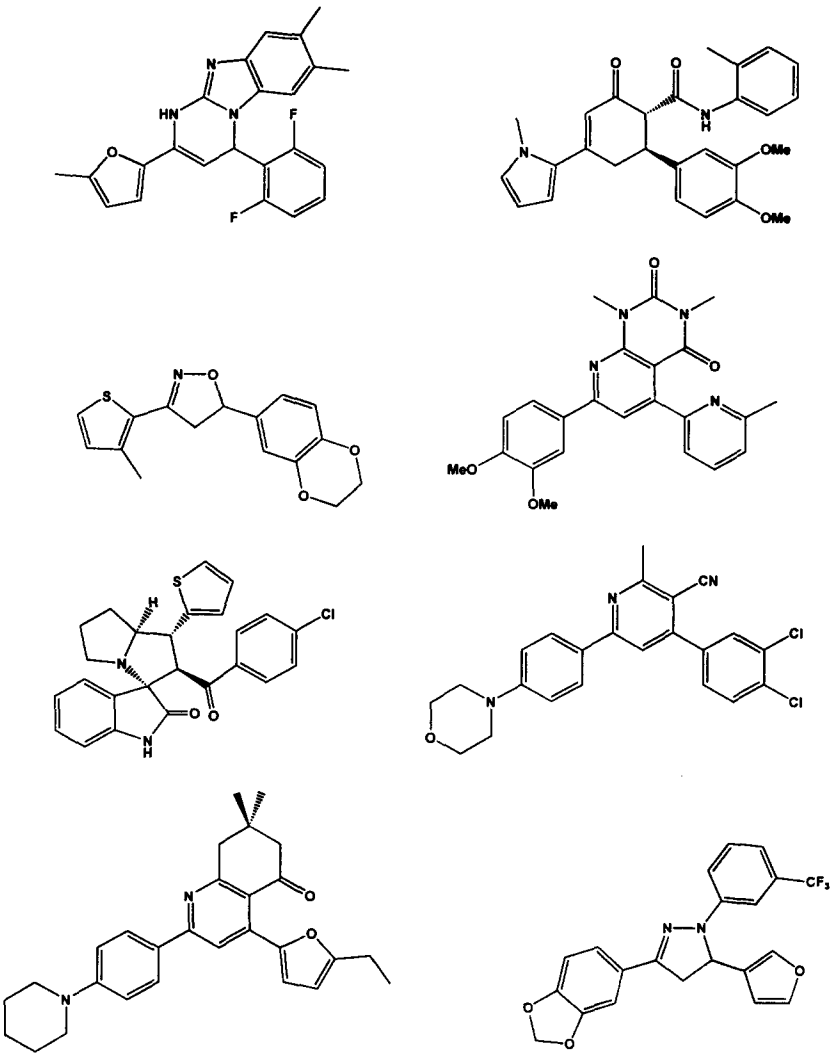
Figure 13.



Representative analytical data from the chalcone based arrays.
(Product from figure 5, X = 2,4-$(MeO)_2$, Y = 4-CH_2CH_3, Z = H)

This report has disclosed a variety of structurally different libraries whose synthesis arose from a common building block set. Five and six-membered ring system scaffolds were prepared from several different types of reactions and an array of 1280 chalcones. Work for the preparation of seven membered ring systems analogous to the well known diazepin/oxazepin family of pharmacophores is ongoing. Libraries of this type along with their biological data will be reported when appropriate. In summary, in this one modular building block program we have prepared ~74,000 compounds in nine structural variations, including the parent chalcones, and illustrate the diversity of the chalcone-derived family of arrays in figure 14 with a "portrait" including an individual member of each group. When scanned against the MDDR database (MDL-Drug Data Report) of 87,500 pharmacologically active compounds, the eight compound classes represented below elicited over 700 hits that contained common core substructures. These various materials possessed biological activity over a wide range of therapeutic areas, a result that augurs well for discovery screening.

Figure 14.



EXPERIMENTAL:

General:

Instruments:
Array layout, fluid handling, array production, reaction workup, quality control and replication/shipping were all performed using the Automated Molecular Assembly Plant (AMAP™) facility at ArQule. This facility is an integrated set of robotic workstations that perform stepwise unit operations in a 96 well format.[10]

^{1}H NMR spectra were obtained on a Gemini 300 MHz Varian NMR system. Deuterochloroform (99.8% *d*, Aldrich) used for NMR spectra was stored over activated 4Å molecular sieves and K_2CO_3. Chemical shifts in

chloroform-*d* are reported relative to residual chloroform (7.26 ppm). DMSO-d_6 (99.9% *d*, Aldrich) used for NMR spectra was obtained in ampules that were used immediately upon opening. Chemical shifts in DMSO-d_6 are reported relative to residual DMSO (2.50 ppm). NMR data are reported as follows: chemical shift (multiplicity, integration) where s = singlet, d = doublet, t = triplet, q = quartet and m = multiplet. Low resolution mass spectra were obtained on a VG quadrapole unit using flow-through injection. Ionization of the compounds was achieved either by electrospray (ES+) or chemical ionization (APCI, N_2) techniques. X-ray crystallographic analyses were performed by Dr. John Huffman of Indiana University. Data were collected using a standard moving crystal, moving detector technique with fixed background counts at each extreme of the scan. All data were corrected for Lorentz and polarization effects, and equivalent data were then averaged to yield a unique set of intensities. Technical detail about the crystallography apparatus and manipulable models of the X-ray structures reported in this paper can be found at the Indiana University Molecular Structure Center Web-site (www.iumsc.indiana.edu) in reports #97703 and 97704. HPLC was performed using Shimadzu 10A analytical chromatography systems fitted with YMC reverse phase (C18, 3 micron, 3 x 100 mm) columns. The retention times reported in this experimental were obtained using the following conditions. Injection of five microliters of a one milligram per milliliter solution of the reaction product in acetonitrile was monitored at 254 nM. The flow rate was 0.75 mL per minute. The linear gradient elution profile for the acetonitrile / water eluent was from 20 to 100% acetonitrile over ten minutes. The elution solvents contained 0.1% TFA.

Chemicals:

All reagents were used as purchased unless otherwise specified. The water used in the synthesis of the chalcone basis set (NaOH solution) was obtained from a Millipore water purification system where the deionization and organic-free cartridges routinely provided water whose resistance was greater than 17.8 MΩ-cm^{-1}.

The experimentals described below are for one representative well in each library:

Preparation of the chalcone basis set (figure 1):

To a reaction vial in a 96 well reaction block at room temperature was added 200 μL of a 0.25 M solution of 4-methoxy-acetophenone in absolute ethanol and 200 μL of a 0.25 M solution of an benzaldehyde in absolute ethanol. To the reaction mixture was added 100 μL of a 0.5 M solution of sodium hydroxide in Ultrapure water. The reaction mixture was capped, shaken to ensure mixing, and then allowed to sit overnight at room temperature. Upon completion, if the following reaction was to use the equivalent of base present, the reaction was concentrated to dryness *in vaccuo* to afford the product as a solid. If the equivalent of base was not required for the ensuing chemistry, the reactions were quenched with 100 μL of a 0.5 M solution of HCl in Ultrapure water. The reaction mixture was shaken to ensure mixing and then concentrated to dryness *in vaccuo* to afford the product as a solid. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.1 (d, 2H), 7.9-7.3 (m, 7H), 7.0 (d, 2H) 3.9 (s, 3H) ppm. M.S. (ES+) *m/z* 239.1 [M^++H] amu. Retention time = (8.15 min.).

Preparation of isoxazoline library (figure 2):

To a reaction vial containing 50 μmoles of chalcone and 50 μmoles of NaOH as a solid was added 400 μL absolute ethanol. To the reaction mixture was added 200 μL of a 0.25 M solution of hydroxylamine hydrochloride in absolute ethanol. The reaction mixture was capped, shaken to ensure mixing and then allowed to heat at 80 °C for 12 hours. The reaction mixture was concentrated to dryness *in vaccuo* to afford the product as a solid. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.5-7.2 (m, 10H), 5.7 (dd, 1H), 3.85 (dd, 1H), 3.4 (dd, 1H) ppm. M.S. (ES+) *m/z* 224 [M^++H] amu. Retention time = (6.30 min.).

Preparation of N-phenylpyrazoline library (figure 3):

To a reaction vial containing 50 μmoles of chalcone and 50 μmoles of NaOH as a solid was added 400 μL absolute ethanol. To the reaction mixture was added 200 μL of a 0.25 M solution of phenylhydrazine in absolute ethanol. The reaction mixture was capped, shaken to ensure mixing and then allowed to heat at 70°C for 8 hours. Upon completion the reactions were cooled to room temperature were quenched with 100 μL of a 0.5 M solution of HCl in Ultrapure water. The reaction mixture was shaken to ensure mixing and then concentrated to dryness *in vaccuo* to afford the product as a solid. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.75 (d, 2H), 7.5-7.2 (m, 10H), 7.1 (d, 2H), 6.8 (t, 1H), 5.3 (dd, 1H), 3.85 (dd, 1H), 3.15 (dd, 1H) ppm. M.S. (ES+) *m/z* 298.7 [M^++H] amu. Retention time = (9.61 min.).

Preparation of cyclohexenone library (figure 5):

To a reaction vial containing 50 μmoles of chalcone and 50 μmoles of NaOH as a solid was added 400 μL absolute ethanol. To the reaction mixture was added 200 μL of a 0.25 M solution of acetoacetanilide in absolute ethanol. The reaction mixture was capped, shaken to ensure mixing and then allowed to heat at 80 °C for 16 hours. Upon completion the reactions were cooled to room temperature were quenched with 100 μL of a 0.5 M solution of HCl in Ultrapure water. The reaction mixture was shaken to ensure mixing and then concentrated to dryness *in vaccuo* to afford the product as a solid. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.0 (s, 1H), 7.5-7.0 (m, 14H), 6.6 (s, 1H), 4.2 (ddd, 1H), 3.7 (d, 1H), 3.4 (dd, 1H), 3.1 (dd, 1H), ppm. M.S. (ES+) *m/z* 368 [M^++H] amu. Retention time = (9.21 min.)

Preparation of tetrahydropyrimidine library (figure 7):

To a reaction vial containing 50 μmoles of chalcone and 50 μmoles of NaOH as a solid was added 400 μL absolute ethanol. To the reaction mixture was added 200 μL of a 0.25 M solution of 2-aminobenzimidazole in absolute ethanol. The reaction mixture was capped, shaken to ensure mixing and then allowed to heat at 80°C for 16 hours. Upon completion the reactions were cooled to room temperature were quenched with 100 μL of a 0.5 M solution of HCl in Ultrapure water. The reaction mixture was shaken to ensure mixing and then concentrated to dryness *in vaccuo* to afford the product as a solid. ^{1}H NMR (DMSO-d_6, 300 MHz) δ 10.1 (s broad, 1H), 7.7 (m, 3H), 7.4 (m, 8H), 7.0 (t, 1H), 6.9 (m, 2H), 6.4 (d, 1H), 5.3 (d, 1H) ppm. M.S. (ES+) *m/z* 324.2 [M^++H] amu. Retention time = (5.28 min.)

Preparation of 3-cyanopyridine library (figure 8):

To a reaction vial containing 50 μM of chalcone and 50 μM of NaOH as a solid was added 400 μL absolute ethanol. To the reaction mixture was added 200 μL of a 0.25 M solution of acetonitrile dimer in absolute ethanol. The reaction mixture was capped, shaken to ensure mixing and then allowed to heat at 70 °C for 6 hours. Upon completion the reactions were cooled to room temperature were quenched with 100 μL of a 0.5 M solution of HCl in Ultrapure water. The reaction mixture was shaken to ensure mixing and then concentrated to dryness *in vaccuo* to afford the product as a solid. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.1 (d, 2H), 7.7 (m, 3H), 7.5 (m, 6H), 2.9 (s, 3H) ppm. M.S. (ES+) *m/z* 271 [M^++H] amu. Retention time = (7.26 min.)

Preparation of uracil-chalcone adduct library (figure 9):

To a reaction vial containing 50 μM of chalcone and 50 μM of NaOH as a solid was added 400 μL absolute ethanol. To the reaction mixture was added 200 μL of a 0.25 M solution of 1,3-dimethyl-4-amino-uracil in absolute ethanol. The reaction mixture was capped, shaken to ensure mixing and then allowed to heat at 80 °C for 16 hours. Upon completion the reactions were cooled to room temperature were quenched with 100 μL of a 0.5 M solution of HCl in Ultrapure water. The reaction mixture was shaken to ensure mixing and then concentrated to dryness *in vaccuo* to afford the product as a solid. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.2 (d, 2H),

7.5 (m, 7H), 7.3 (d, 2H), 3.9 (s, 3H), 3.4 (s, 3H) ppm. M.S. (ES+) *m/z* 344 [M^++H] amu. Retention time = (7.69 min.)

Preparation of pyridotetralone library (figure 10):

To a reaction vial containing 50 μM of chalcone and 50 μM of NaOH as a solid was added 400 μL absolute ethanol. To the reaction mixture was added 200 μL of a 0.25 M solution of 3-amino-5,5-dimethyl-2-cyclohexene-1-one in absolute ethanol. The reaction mixture was capped, shaken to ensure mixing and then allowed to heat at 80°C for 12 hours. Upon completion the reactions were cooled to room temperature were quenched with 100 μL of a 0.5 M solution of HCl in Ultrapure water. The reaction mixture was shaken to ensure mixing and then concentrated to dryness *in vaccuo* to afford the product as a solid. ^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.2 (s, 1H), 7.5-7.2 (m, 10H), 3.2 (s, 2H), 2.6 (s, 2H), 1.1 (s, 6H) ppm. M.S. (ES+) *m/z* 328.5 [M^++H] amu. Retention time = (7.06 min.)

Preparation of spiro-pyrrolizidine library (figure 11):

To a reaction vial was added 200 μL of a 0.25 M solution of isatin in dioxane, 200 μL of a 0.25 M aqueous solution of proline and 400 μL of a 0.125 M solution of chalcone in dioxane. The reaction mixture was capped, shaken to ensure mixing and then allowed to heat at 80 °C for 16 hours. Upon completion the reactions were cooled to room temperature and then concentrated to dryness *in vaccuo* to afford the product as a solid. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.4 (s broad, 1H), 7.6-7.0 (m, 13H), 6.6 (d, 1H), 4.9 (d, 1H), 4.3 (ddd, 1H), 3.9 (dd, 1H), 2.7 (m, 2H), 2.2-1.7 (m, 4H) ppm. M.S. (ES+) *m/z* 409 [M^++H] amu. Retention time = (4.40 min.)

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References:

1) *Organic Syntheses*, Collective Volume I, pg 78, *Benzalacetophenone.*
2) Khalil, Z. H.; Yanni, A. S.; Abdel-Hafez, A. A.; Khalaf, A. A., *J. Indian Chem. Soc.*,**1990**, *67*, 821.
3) Yousef, M. Y.; Sofan, M. A.; Etman, H. A.; Metwally, H. A., *J. Indian Chem. Soc.*,**1990**, *67*, 55.
4) Nawrocka, W., *Pol. J. Chem.*,**1996**, *70*, 193.
5) Patel, D. V.; Gordeev, M. F.; Englund, B. P.; Gordon, E. M., in *Molecular Diversity and Combinatorial Chemistry: Libraries and Drug Discovery, Chpt. 7*, ACS Press **1996**, Chaiken, I. M.; Janda, K. D., Eds.
6) Kajino, M.; Meguro, K., *Heterocycles*,**1990**, *31*, 2153.
7) Fokas, D.; Ryan, W. J.; Casebier, D. S.; Coffen, D. L., *Tetrahedron Lett.*, **1998**, *39*, 000.
8) Aly, M. F.; Younes, M. I.; Metwally, S. A. M., *Tetrahedron.*,**1994**, *50*, 3159.
9) Grigg, R.; Iddle, J.; McKeenin, P.; Surendrakumar, S., *J. Chem. Soc., Perkin Trans I.*, **1988**, 2703-2713.
10) Rivero, R.A.; Greco, M. N.; Maryonoff, B. E., *Equipment for the High Throughput Organic Synthesis of Chemical Libraries.*, in *A Practical Guide to Combinatorial Chemistry.*, **1997**, American Chemical Society, Czarnik, A. W. and Hobbs DeWitt, S. Eds.