Microwave Assisted Solution Phase "Libraries from Libraries" Approach towards the Synthesis of Allylic and C-Cyclopropylalkylamides

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Abstract

A microwave assisted "Libraries from Libraries Approach" was used to synthesize an intermediate 20 member library and expanded 100 member library of allylic and *C*-cyclopropylalkyl amides. The biological activity of these derivatives was evaluated in an assay for competitive binding to the estrogen receptor (ERα), revealing three potent compounds. Our data supports the finding that allylic amides and cyclopropylalkylamides represent novel scaffolds for ERα-targeting agents.

Introduction

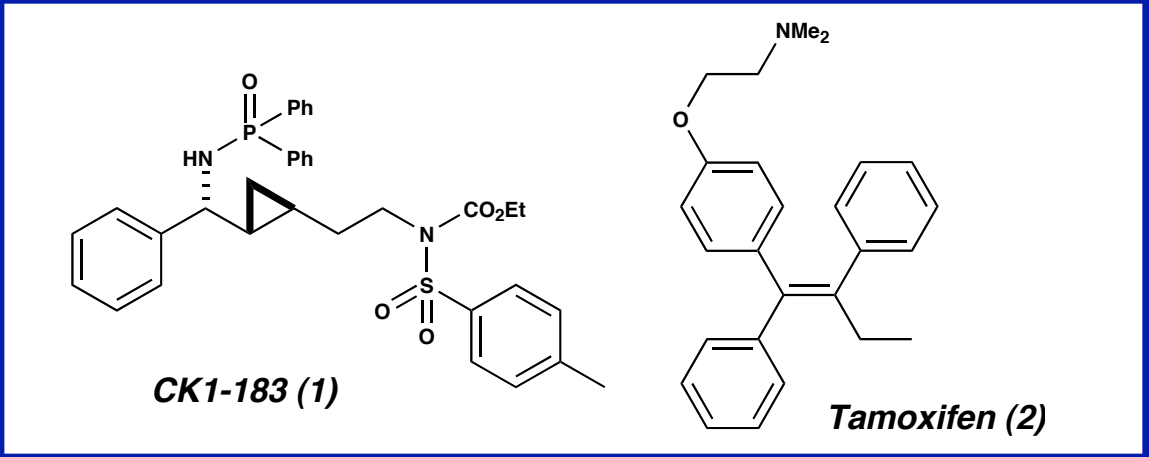


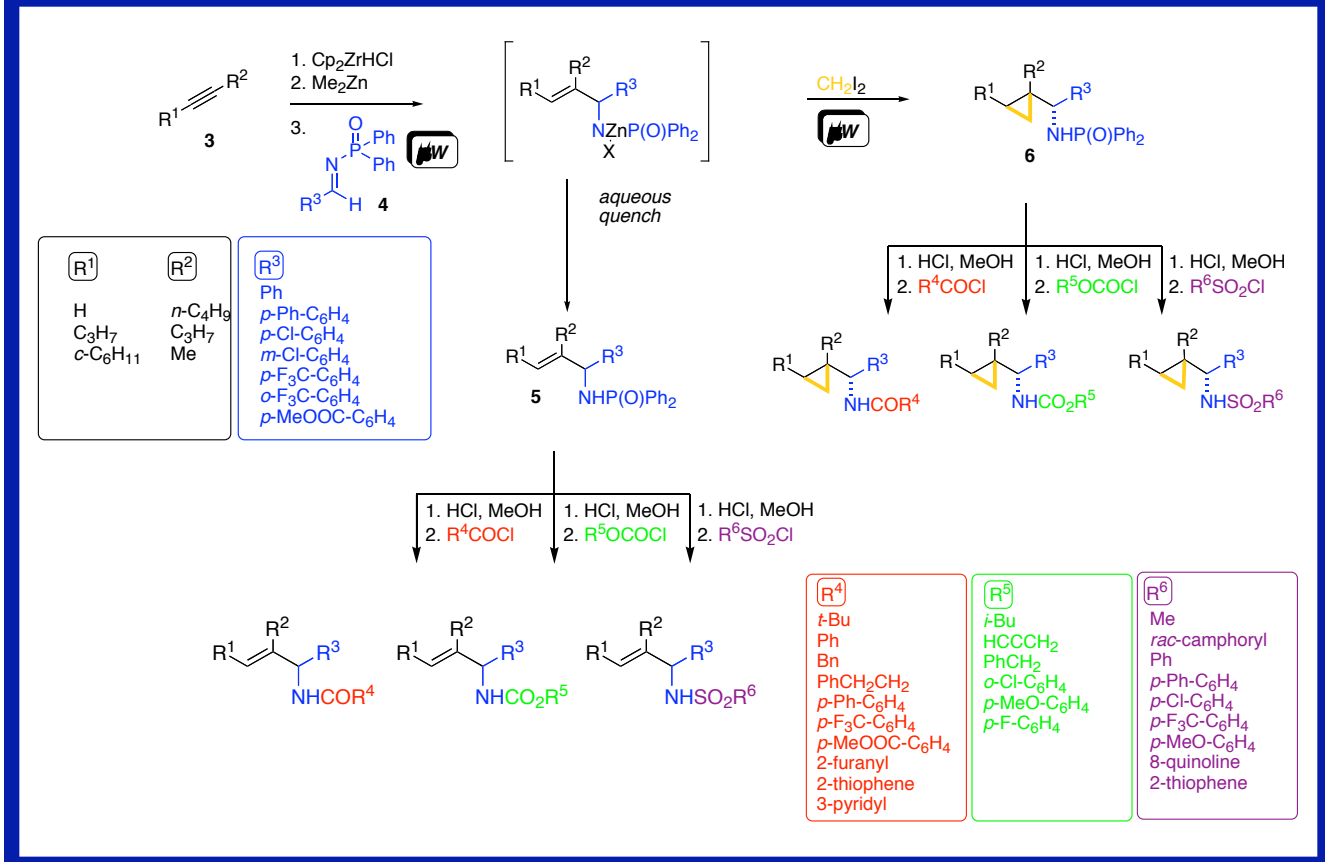
Figure 1: Both the clinically used tamoxifen (2) and CK1-183 (1) demonstrate similar effects at ERα and selective antiproliferative activity

Library based on Assay Hit of the Wipf Group

In a preliminary screen of allylic, homoallylic and *C*-cyclopropylalkyl amides obtained by classical solution phase synthesis[1] in our laboratory, a potentially antiestrogenic compound, **CK1-183**, was identified. **CK1-183** was found to have antiestrogenic activity at ERα comparable to that of Tamoxifen (clinically used to treat breast cancer in postmenopausal women) (Figure 1). We envisioned that an expanded focused library of analogues of **CK1-183** may provide sufficient structure-activity relationship (SAR) data to understand the antiestrogenic activity of this new lead structure. We wanted to prepare 20 allylic amides and *C*-cyclopropylalkyl amides and expand this to a 100 member library by parallel *N*-acylation, -carbamoylation and -sulfonation.

Our one-pot multi-component preparation of phosphinoyl amides from alkenyl zirconocenes, diphenylphosphinoylimines, dimethylzinc and diiodomethane provides access to a library of intermediates from commercially available or readily synthesized starting materials. However, the original classical protocol needed to generate the library required long reaction times, i.e. 3-15 h.

Microwave Accelerated Library Synthesis



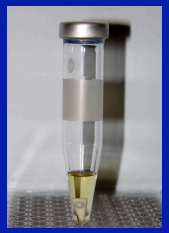
Scheme 1: "Libraries from Libraries Approach" using a Divergent MCR for the preparation of potential Antiestrogens.

We have recently reported the microwave accelerated hydrozirconation and aldimine addition sequence in toluene for the synthesis of allylic amides **5**, reducing reaction times from **3-15 h** to less than **30 min**.[2] This microwave methodology can also be used to accelerate the synthesis of *C*-cyclopropylalkylamides in CH_2Cl_2.

Library Protocol

We used a matrix of three alkynes **3** and seven phosphinoylimines **4** to prepare the 20 phosphinoyl amides **5** and **6** (Scheme 1). An automated Emrys Optimizer single mode microwave reactor was used to perform the serial productions on a ca. 200 mg scale. All library members were purified with a CombiFlash Companion and analyzed by reversed phase chromatography and ^{1}H NMR. Allylic amides **5a-g** were formed in an average yield of 55% and a mean purity of 97% (Figure 2 and Table 1). The yields and purities of cyclopropanes **6a-m** were lower (46% and 80% respectively). Sluggish cyclopropanations could be improved by adding additional diiodomethane (10 equiv) and dimethylzinc (6 equiv).

Emrys Optimizer microwave



Intermediate Library

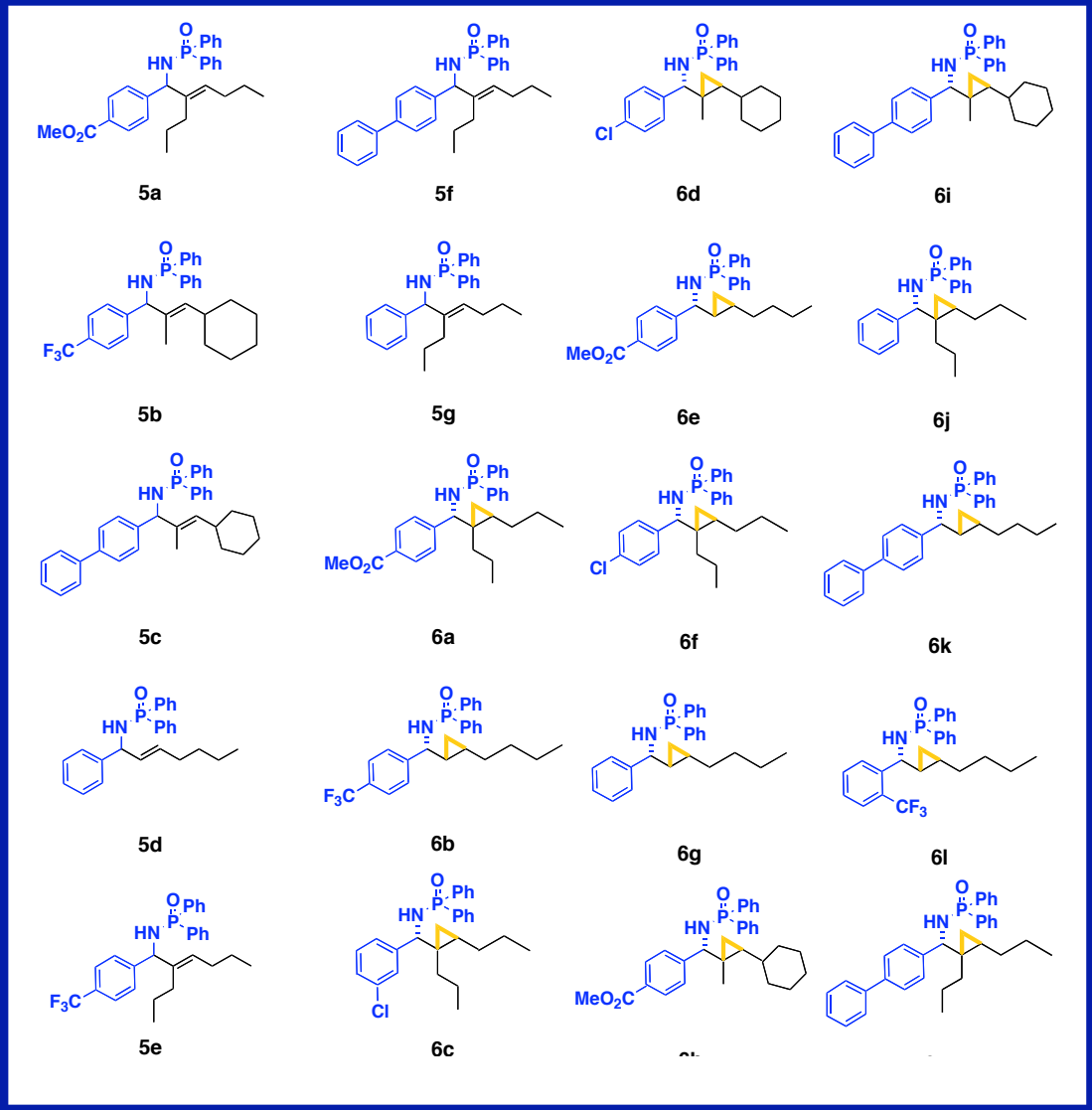


Figure 2: Structures of the intermediate library: All samples are racemic.

Intermediate Library

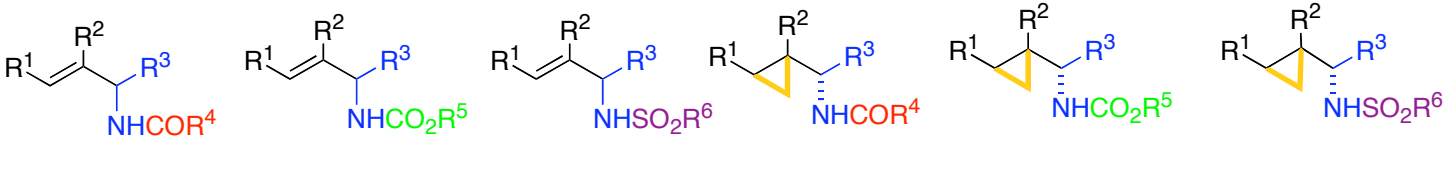
entry	product	yield [%][a]	LC Purity [%][b]	MS [m/z][c]	entry	product	yield [%][a]	LC Purity [%][b]	MS [m/z][c]
1	**5a**	76	100	475.2	11	**6d**	32[g]	80	478.5
2	**5b**	10[d]	96	498.4	12	**6e**	18[f,i]	73	462.4
3	**5c**	40[d]	93	506.3	13	**6f**	33[f]	92	466.4
4	**5d**	58[h]	91	390.3	14	**6g**	49[f]	80	404.3
5	**5e**	68	100	485.0	15	**6h**	73[d,i]	50	502.3
6	**5f**	65	100	494.2	16	**6i**	80[d,i]	78	520.2
7	**5g**	68	100	418.3	17	**6j**	63	92	432.2
8	**6a**	75	100	489.0	18	**6k**	34[d,i]	95	480.4
9	**6b**	30[f,i]	92	472.3	19	**6l**	31[e,i]	67	472.4
10	**6c**	45[f]	85	466.3	20	**6m**	33[h]	78	508.4

[a]Isolated yields of purified products based on aldimine. [b]Purity determined by HPLC peak area integration at 210 nm. [c] LCMS (EI) analysis. [d]Based on two reactions. [e]Based on three reactions. [f]Based on four reactions. [g]Based on five reactions. [h]Based on six reactions. [i]Additional Me_2Zn (6 equiv) and CH_2I_2 (10 equiv) were added to the reaction mixture.

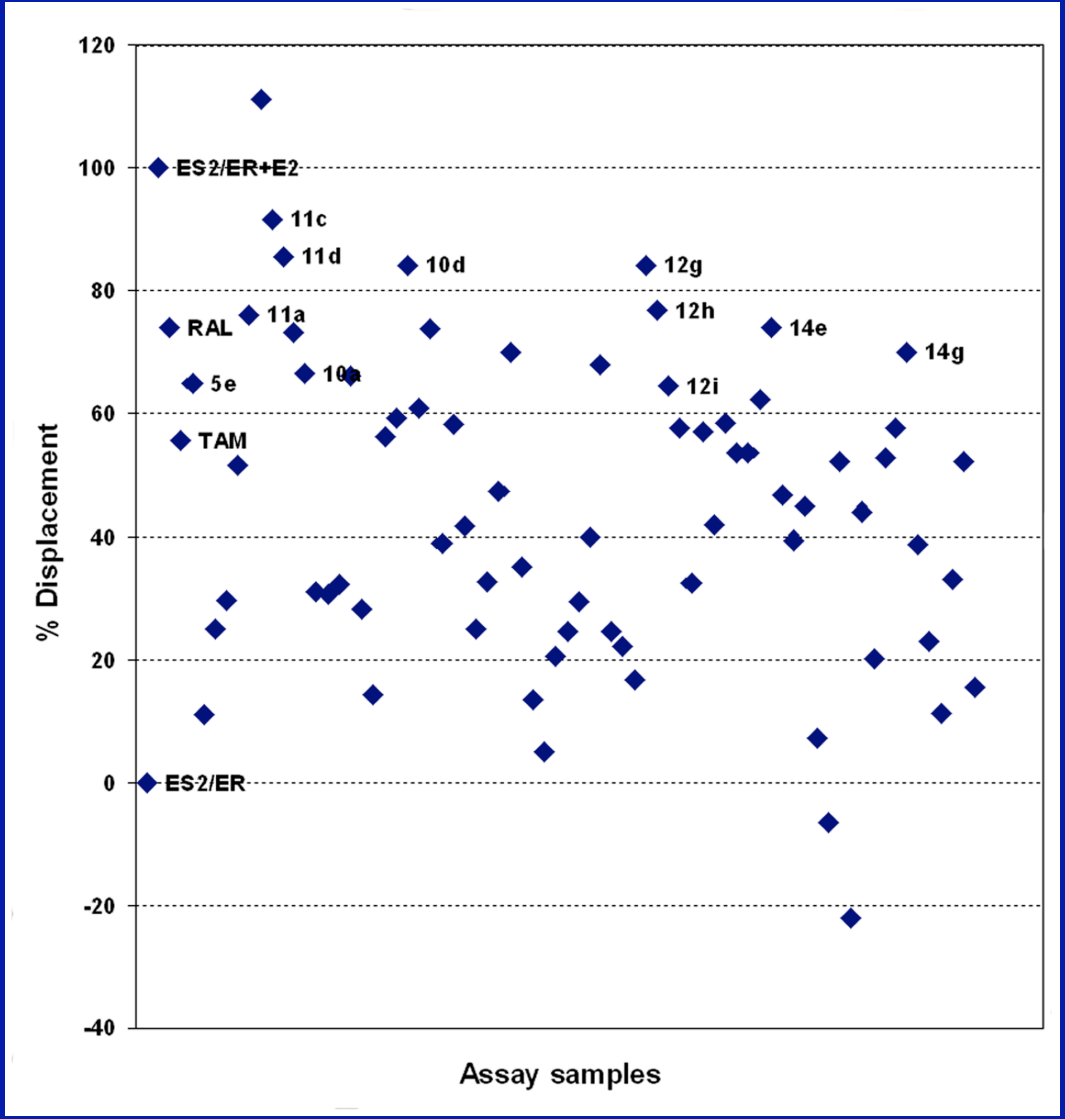
Table 1: Yields and purities of the intermediate library

One Hundred Member Expanded Library Protocol

The 100-member expanded library was generated in parallel using a Radleys Greenhouse Carousel. In batches of twenty-four, the phosphinoyl group of the intermediate library was removed by treatment with 2 M HCl/MeOH. After solvent evaporation with a blowdown evaporator the residues were treated with 2 equiv of ten different acyl chlorides, six carbamoyl chlorides and nine sulfonyl chlorides, 3 equiv of DIPEA and 10 mol% DMAP. After stirring for 1 h at RT, the reaction mixtures were purified in parallel and analyzed by LC-MS with UV detection at 210 and 220 nm. The product was formed in every case with an average isolated yield of 51% and a purity >80% for 85 out of 100 samples.



ERα targeted hits



The biological activity of seventy library members with LC-MS purities >85% by 210 nm UV detection were evaluated using a commercially available in vitro fluorescence polarization-based homogenous ER α binding competition assay (Figures 3 and 4). Eleven compounds were identified as hits and further tested in the MCF-7 proliferative assay (Figure 5). Criteria for hit selection were: (1) >50% displacement at 200 nM, and (2) displacement was concentration dependent.

Figure 3. The ERα competitor assay (Panvera) was performed according to manufacturer's recommendations with some modifications. Library compounds were screened at 3 concentrations (5, 1 and 0.2 μM) in duplicate.

ERα Competitive Assay

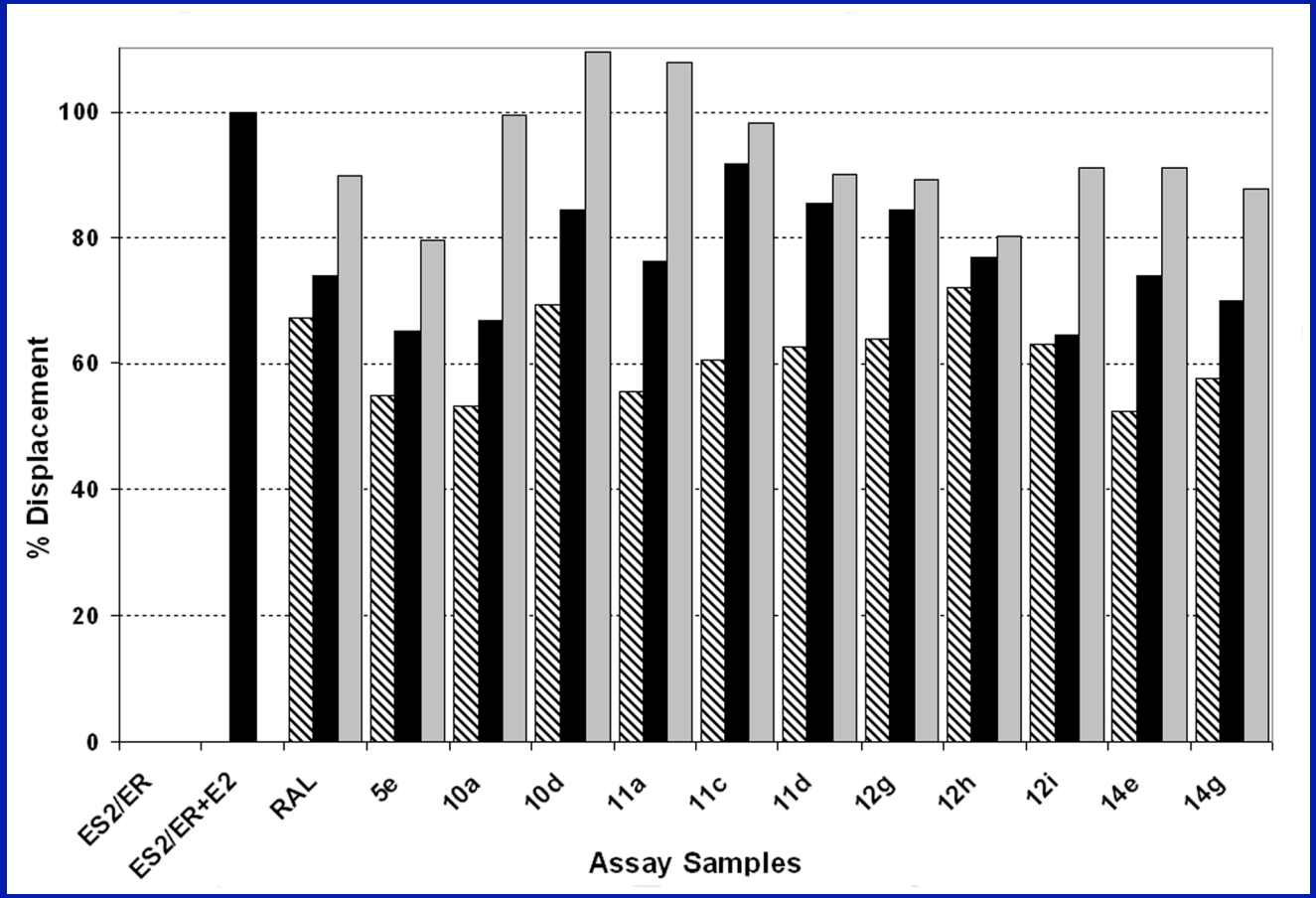
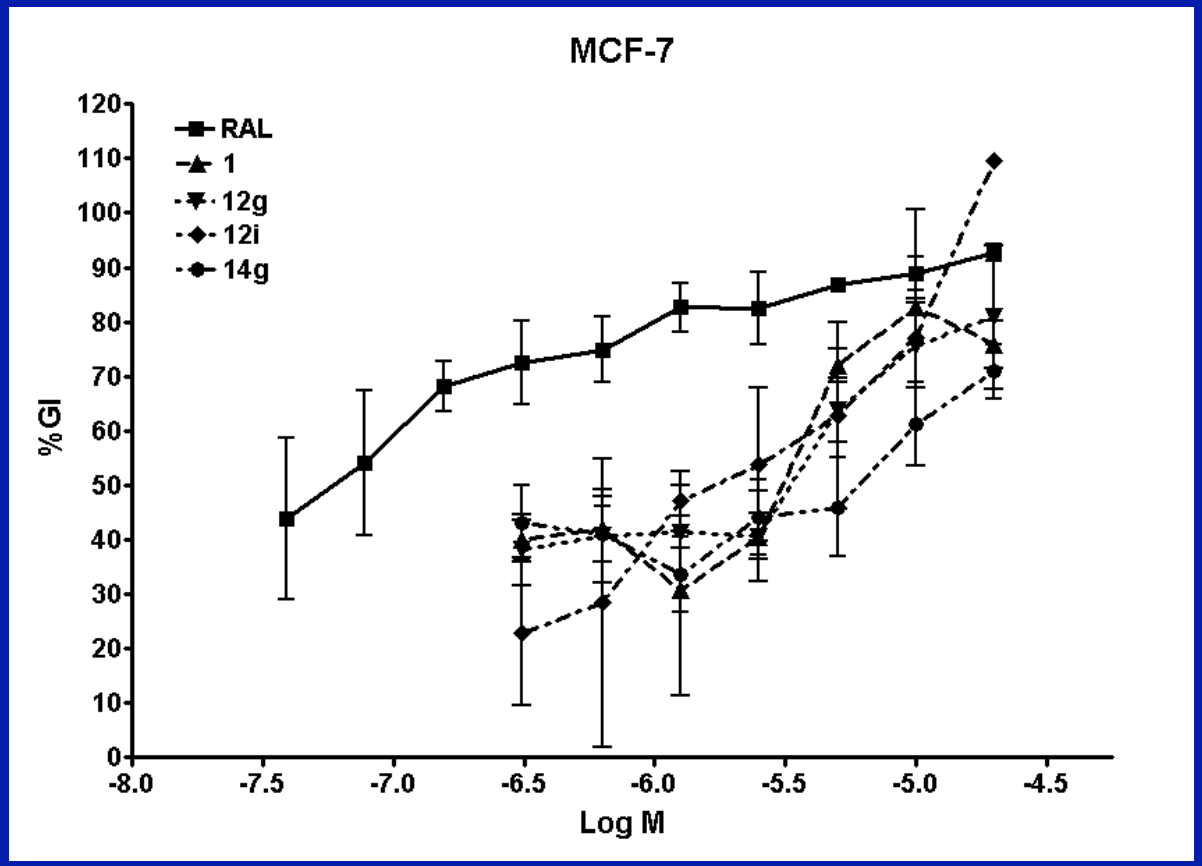
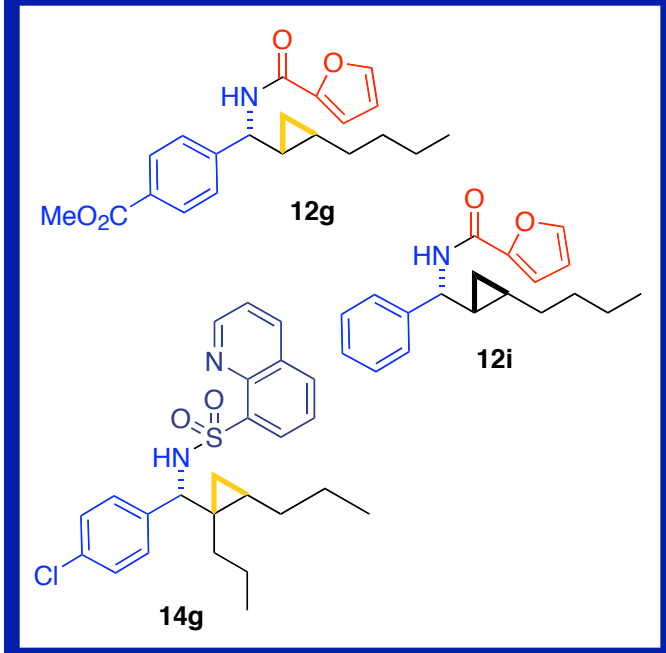


Figure 4. Hits from in vitro screen. Compounds that showed >50% displacement and concentration dependence were classified as hits. The data represents the percent of competition (%I), calculated based on the following formula: $\% I = [(mP_0 - mP)/(mP_0 - mP_{100})] \times 100$, where mP_0 is mP value for 0% competition as referred to high polarization of fluorescently labeled estradiol complexed to ERα (ER/ES2 complex); mP_{100} is mP value for 100% competition, as referred to low polarization in presence of 1μM E2; and mP is fluorescence polarization in the presence of test compounds.

MCF Proliferative Assay





Three new lead compounds

Figure 5. Three library members inhibit E2 induced proliferation of MCF-7 cells. Shown are means ± SD (N = 4). Statistical Graph Pad 4 software was used to estimate GI50 values from non-linear best curve fit (p=0.9 > 0.05): **Tam** (3.0 μM), compound **96** (4.5 μM), compound **98** (4.7 μM), compound **110** (>50 μM), and compound **123** (2.0 μM).

Conclusion

We have developed an expeditious and robust divergent multi-component reaction (DMCR) method combining the advantages of microwave reaction acceleration and combinatorial technologies with a "libraries from libraries" concept[3] to create a 20 member allylic amide and *C*-cyclopropylalkylamide library and an expanded 100 member library. An earlier, structurally novel hit in an antiestrogenic nuclear receptor (ERα) assay served as a lead structure for this targeted array. Seventy library members were screened for their ability to compete with fluorescently labeled 17β-estradiol for binding to human ERα. Eleven hits were identified from this screen. Compounds **12g**, **12i** and **14g** showed comparable or better activity in the ERα competitor assay than raloxifene, as well as the ability to inhibit E2-induced proliferation of MCF-7 cells. IC_{50}s were as follows: 2.7±0.8 μM (**12g**), 3.7±2.8 μM (**12i**), and 5.7±2.0 μM (**14g**). QikProp analysis of the three new lead compounds show that the ADMET properties of these compounds fall within the 95% range of similar values for known drugs. Exceptions to this are the *c*LogP and aqueous solubility values for **14g** and the aqueous solubility for **12g**. These compounds are currently under further biochemical and cell-based assay investigation. The biological evaluation supports the finding that allylic amides and cyclopropylalkylamides represent attractive novel scaffolds for the development of ERα targeting agents.

References

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3. Ostresh, J. M.; Husar, Gt. H.; Blondelle, S. E.; Dorner, P. A.; Weber, R. A.; Houghten, R. A. *Proc. Nat. Acad. Sci. USA* **1994**, *91*, 11138-11142.

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