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Synthesis and evaluation of the anti-proliferative activity of diaryl-3-pyrrolin-2-ones and fused analogs

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ABSTRACT

Analogs containing a central 3-pyrrolin-2-one core with different methoxyphenyl and/or indole substituents were prepared and tested for anti-proliferative activity in U-937 cells. The most efficacious analogs were non-rigid, (non-fused) contained methoxyaryl groups located at the 4-position, and contained either methoxyaryl or indole groups located at the 3-position. Both the number of methoxy groups contained in the substituents and the particular location of the indole rings with respect to the lactam carbonyl had significant affects on anti-proliferative activity. This work provides a framework to better understand structure-activity relationships for inducing anti-proliferative activity in diaryl heterocyclic scaffolds.

Certain structural motifs are repeatedly found in biologically active small molecules. These reoccurring substructures[1] or scaffolds[2] were first coined as "privileged structures"[3–6] by Evans. Privileged structures provide a backbone upon which chemical motifs can be added to alter ligand specificity and activity. The presence of two proximal aryl rings is part of three common privileged structural motifs (Fig. 1): biphenyl, diarylmethane, and vicinal diaryl (two aryl groups located vicinal on a central ring). Diaryl-containing privileged structures have proven their importance in a number of drug discovery programs.[6]

Numerous biologically active molecules contain a privileged structure of a central nitrogen heterocycle substituted with two adjacent aryl groups (vicinal diaryl substructure) (Fig. 2).[7] Two such nitrogen heterocyclic ring systems, maleimides and 3-pyrrolin-2-ones, have been studied in some detail. Polymethoxylated maleimides (*e.g.*, **2**),[8] 3-pyrrolin-2-ones (*e.g.*, **3**),[9] and pyrroles (not shown)[10,11] have been investigated as *cis*-constrained analogs of the promising anti-cancer agent, combretastatin A-4.[12] SB-216763 (**4**) is an ATP-competitive inhibitor of glycogen synthase kinase.[13] Bisindolemaleimide (**5**)[14] is a potent inhibitor of protein kinase C, which has inspired a large number of follow-up studies on bisindolemaleimide analogs.[15–17] Indole-substituted arylmaleimides (*e.g.*, **6**)[18] and aryl-3-pyrrolin-2-ones (*e.g.*, **7**)[19] have shown anti-angiogenic activity, while fused bisindolemaleimide **8** (arcyriaflavin A) and 3-pyrrolin-2-one **9** (K-252c) are natural products with demonstrated antiviral[20] and protein kinase inhibitory activity,[21] respectively. Common aryl substituents found across this sample of biologically active nitrogen maleimides and 3-pyrrolin-2-ones include polymethoxyaryl groups and indole rings.

Building on our expertise in preparing aryl-substituted 3-pyrrolin-2-ones,[22–25] we systematically studied the effects of changing the aryl groups around the central 3-pyrrolin-2-one ring on cancer cell viability. In our study (Fig. 3), we prepared a small library of analogs that differed in the following ways: (i) analogs with different numbers (and locations) of aryl groups and methoxy substituents around the aryl periphery; (ii) analogs with indole groups located at different positions; and (iii) cyclized analogs (central ring fusion).

We started by preparing 4-aryl-substituted 3-pyrrolin-2-ones from known tetramic acid tosylate **10** (Scheme 1).[23] This starting material has previously been proven to be viable in Suzuki-Miyaura cross-coupling reactions. Indeed, treatment of **10** with arylboronic and Pd(dppf)Cl_2 in the presence of Cs_2CO_3 gave **11c** and **11d** in modest yields.

The preparation of 3,4-diaryl-3-pyrrolin-2-ones started with the synthesis of 3-aryl- and 3-indolyltetramic acids **15** adapting our recently published method (Scheme 2).[25] The three-step procedure included DCC-mediated amide coupling of arylacetic acids **12**

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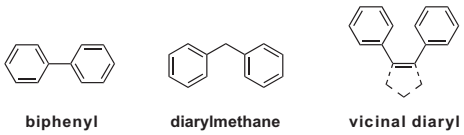
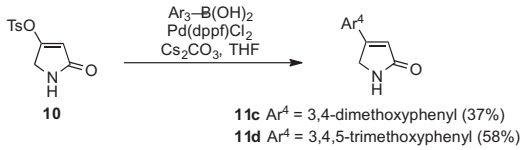


Fig. 1. "Privileged" diaryl-containing structural motifs.

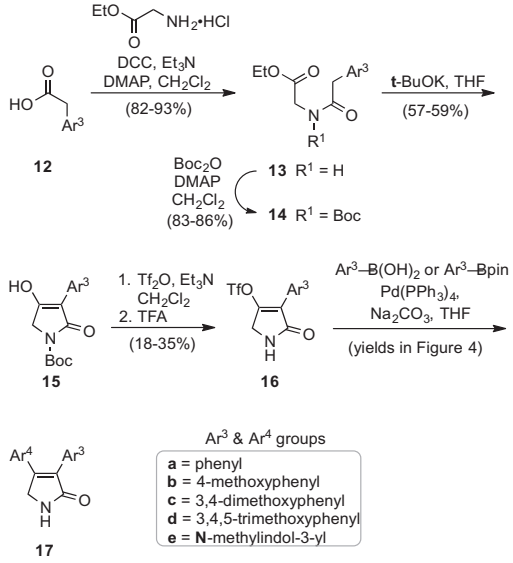


Scheme 1. Synthesis of 4-aryl-3-pyrrolin-2-ones.

with ethyl glycinate giving amidoesters **13**, Boc-protection to **14** with Boc_2O promoted by DMAP, and a Dieckmann cyclization to tetramic acids **15** promoted by *t*-BuOK. The tetramic acids **15** were then converted into the corresponding tetramic acid triflates **16** by treatment with Tf_2O followed by removal of the Boc-protecting group with TFA. Suzuki-Miyaura cross-coupling of **16** with arylboronic acids or arylboronic acid pinacol esters gave the desired 3,4-diaryl-3-pyrrolin-2-ones **17** (Fig. 4). Given that there are two different aryl/heteroaryl rings present in most of the analogs, the compound numbers assigned to each analog include two letters with each letter denoting one of the aryl rings. For example, **17ea** is an analog with a "*N*-methylindol-3-yl" substituent at the 3-position and a "phenyl" substituent at the 4-position.

We have previously found that electron-rich diaryl-substituted 3-pyrrolin-2-ones can be transformed into their corresponding ring fused analogs using the oxidant, phenyliodine(III)bis(trifluoroacetate) (PIFA).[25] Using this strategy, benzo[*a*]carbazole **18ec** was prepared using an oxidative cyclization of **17ec** (Scheme 3). Treatment of **17ec** with PIFA and $BF_3{\cdot}Et_2O$ at −40 °C gave **18ec** in 70% yield. An additional ring fused analog, **18cc**, was available from a prior study.[25]

To determine the potency of the various analogs, compounds were tested on the human promonocytic cell line, U-937,[26] via MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay over 48 h.[27] IC_{50} values were defined as the analog concentration at which cell viability reduced to 50% compared to mock-treated cells. The biological data for the compounds are presented in a graphical format in two figures: (i) indole-containing analogs (Fig. 5) and (ii) polymethoxyaryl-containing analogs (Fig. 6). The analogs with the most potent IC_{50} values within each figure included one indole-containing analog, **17ec** (10 μM ± 2), and two polymethoxyaryl-containing analogs, **17bb** (13 μM ± 2)



Scheme 2. Synthesis of 3,4-diaryl-3-pyrrolin-2-ones.

and **17dd** (11 μM ± 3). The IC_{50} values for these three compounds were statistically equivalent ($p > 0.35$).

In examining the results with the indole-containing analogs (Fig. 5), it is clear that the location of the indole substituent on the 3-pyrrolin-2-one central core ring impacts cell viability. Analogs with indole substitution at C4, **11e**[28] and **17ce**, were inactive, as defined as an $IC_{50} > 100$ μM. Two of the analogs with indole substitution at C3, **17ec** (10 μM ± 2) and **17ed** (38 μM ± 9), showed

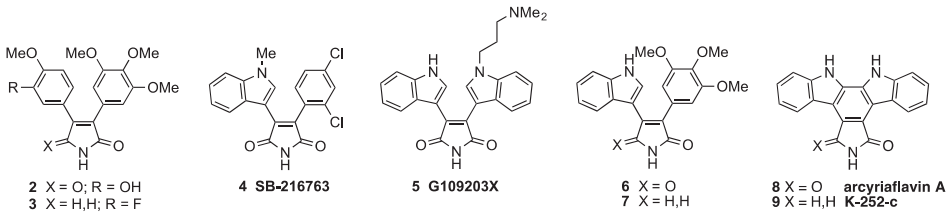


Fig. 2. Biologically active diaryl-substituted maleimides and 3-pyrrolin-2-ones.

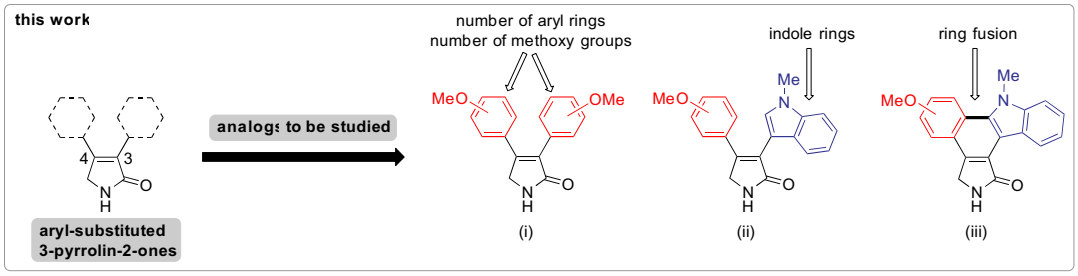


Fig. 3. Proposed aryl-substituted 3-pyrrolin-2-one targets for anti-proliferative studies.

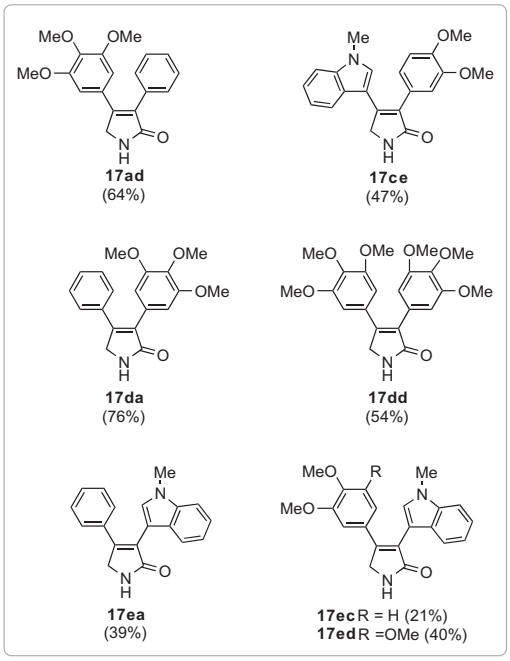
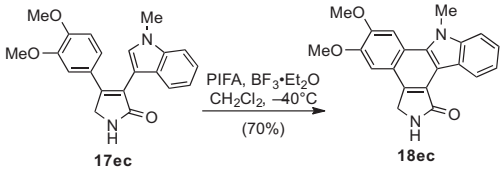


Fig. 4. 3,4-Diaryl-3-pyrrolin-2-one analogs synthesized.



Scheme 3. PIFA-mediated oxidative cyclization.

activity. The inactivity of **17ea** indicated that a polymethoxyaryl substituent at C4 was required. The number of methoxy groups also appears consequential as dimethoxy analog **17ec** was marginally more active than **17ed** ($p = 0.091$). Finally, the fused analog **18ec** showed lower activity compared to unfused **17ec** ($p < 0.02$). This indicates that a rigid cyclic structure is less active than a more flexible (non-ring fused) structure.

To explore the importance of the indole ring and to further analyze how varying the number of methoxyaryl substitutions impacts function, a series of polymethoxyaryl-containing analogs were also explored. The results for these compounds were less straight-forward (Fig. 6). Mono-substituted analogs, **11c** and **11d**, showed no activity. On the other hand, all of the 3,4-disubstituted 3-pyrrolin-2-ones showed activity. In comparing trimethoxyaryl-containing analogs, bis(trimethoxyphenyl) analog **17dd** (11 μM ± 3) was more active than mono(trimethoxyphenyl) analogs **17da** (33 μM ± 3) and **17ad** (84 μM ± 8) ($p \leqslant 0.004$). Decreasing the number of methoxy groups around the periphery had differential effects. Analog **17cc** (57 μM ± 13), which contains four methoxy groups, had much lower activity compared to **17dd** ($p < 0.05$). On the other hand, **17bb** (13 μM ± 2) with two methoxy groups had statistically the same activity as the analog with six methoxy groups **17dd** (11 μM ± 3) ($p > 0.55$). As seen previously, ring fusion was detrimental to activity. Fused analog **18cc** showed no activity.

MTT assays are an indirect measure of cell viability via mitochondrial activity and have been shown to vary in correlation with actual cell counts, depending on the analog tested.[29] To confirm the validity of the MTT findings, direct live cell counts over 48 h were measured using analog IC_{50} concentrations (Fig. 7); if the activity is analogous then approximately 50% of the cells should be viable after 48 h. We chose **17ec** and **17dd** to study live cell counts given their low IC_{50} values and their representation of the two divergent classes of analogs studied. By 48 h (the same incubation length for the MTT assay), 61% ± 6 of **17ec** and 51% ± 15 of **17dd** treated cells were alive, confirming the reliability and consistency of the MTT assay for analogs with varying structural attributes.

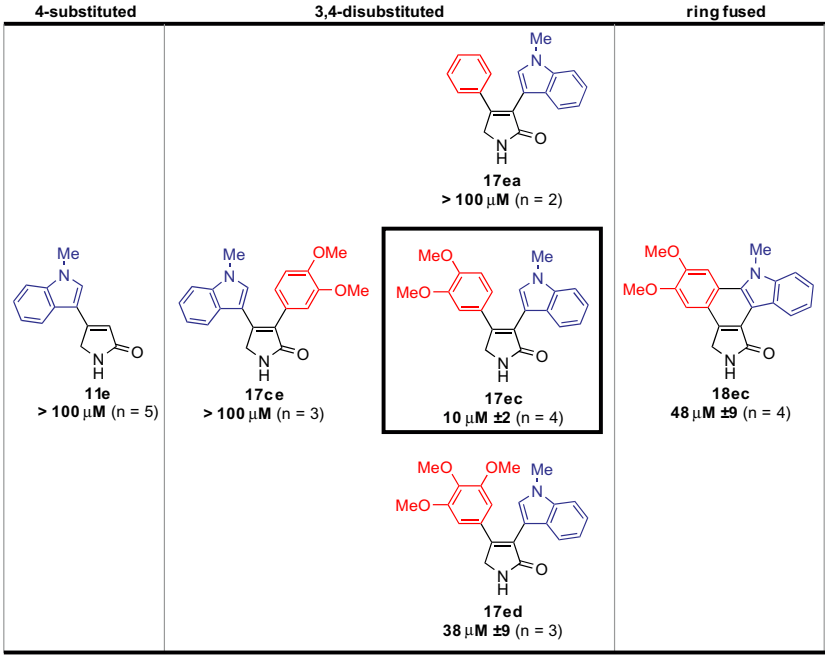


Fig. 5. Cytotoxicity results of indole-containing analogs on U-937 cells. U-937 cells were treated with compounds for 48 h followed by MTT treatment. Averages and standard errors based on a minimum of three independent runs with eight replicates each; n = number independent runs.

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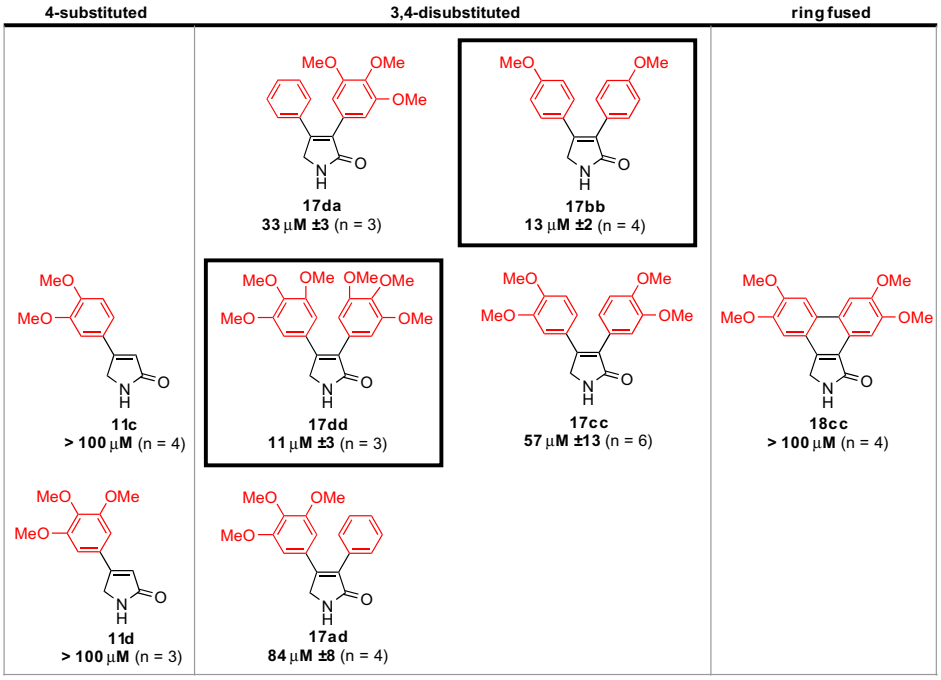

Fig. 6. Cytotoxicity results of polymethoxyaryl-containing analogs on U-937 cells. U-937 cells were treated with compounds for 48 h followed by MTT treatment. Averages and standard errors based on a minimum of three independent runs with eight replicates each; n = number independent runs.

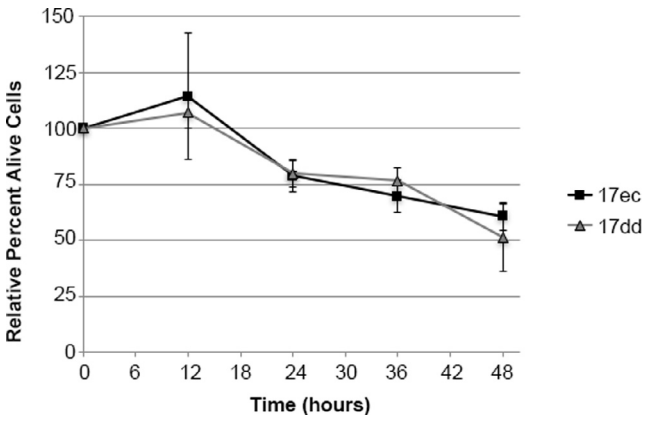

Fig. 7. Direct cell counts of analogs on U-937 cells. U-937 cells were treated with compounds for 48 h at their IC_{50} value as determined by MTT assay. Samples were removed periodically and direct cell viability was measured by trypan exclusion. Percentage alive was calculated relative to mock-treated cells. Averages and standard errors based on two independent runs, each in triplicate.

In summary, we found two themes in the data. First, flexibility of the molecule was critical as cyclization eliminated or significantly reduced activity (**17ec** > **18ec**; **17cc** > **18cc**). Second, substitution at the C4 position alone was insufficient for function as 4-aryl-substituted 3-pyrrolin-2-ones (**11e**, **11c**, **11d**) failed to induce cytotoxicity. Overall, from the two structurally related but distinct classes, three analogs were found to have low μM IC_{50} values representing: indole-substitution at C3 represented by **17ec**; and methoxyaryl-substitution at both C3 and C4 (bis-aryl analogs) represented by **17bb** and **17dd**. With regard to the C3-indole analog class, trimethoxy-substituted analog **17ed** was less active compared to dimethoxy-substituted analog **17ec**. On the other hand, with regard to the bis-aryl class of analogs, the dimethoxy-substituted analog (**17cc**) was much less active than either the trimethoxy-substituted analog (**17dd**) or methoxy-substituted analog (**17bb**). These findings may reflect that the two classes of compounds target different cellular proteins, and future studies identifying analog targets may clarify the initial structure-function findings.

Acknowledgments

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A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2016.11.076.

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