Bioorganic & Medicinal Chemistry Letters 26 (2016) 2164–2169

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Dioxol and dihydrodioxin analogs of 2- and 3-phenylacetonitriles as potent anti-cancer agents with nanomolar activity against a variety of human cancer cells

Nikhil R. Madadi [a], Amit Ketkar [b], Narsimha R. Penthala [a], April C. L. Bostian [b], Robert L. Eoff [b], Peter A. Crooks [a,*]

[a] Department of Pharmaceutical Sciences, College of Pharmacy, University of Arkansas for Medical Sciences, Little Rock, AR 72205, USA
[b] Department of Biochemistry and Molecular Biology, College of Medicine, University of Arkansas for Medical Sciences, Little Rock, AR 72205, USA

ARTICLE INFO

Article history:
Received 23 February 2016
Revised 15 March 2016
Accepted 16 March 2016
Available online 17 March 2016

Keywords:
3,4-Methylenedioxycyanostilbenes
3,4-Ethylenedioxycyanostilbenes
Synthesis of cyanostilbenes
Anti-cancer activity
Molecular docking studies

ABSTRACT

A small library of (*Z*)-2-(benzo[*d*][1,3]dioxol-5-yl) and (*Z*)-2,3-dihydrobenzo[*b*][1,4]dioxin-6-yl analogs of 2- and 3-phenylacetonitriles has been synthesized and evaluated for their anti-cancer activities against a panel of 60 human cancer cell lines. The dihydrodioxin analog **3j** and dioxol analogs **5e** and **7e** exhibited the most potent anti-cancer activity of all the analogs synthesized in this study, with GI_{50} values of <100 nM against almost all of the cell lines in the human cancer cell panel. Of these three, only compound **3j** inhibited tubulin polymerization to any degree in vitro. The binding modes of **3j** and the structurally related tubulin-inhibitor **DMU-212** were determined by virtual docking studies with tubulin dimer. Compound **3j** docked at the colchicine-binding site at the dimer interface of tubulin. The Full-Fitness (FF) score of **3j** was observed to be substantially higher than **DMU-212**, which agrees well with the observed anti-cancer potency (GI_{50} values). The mechanism by which dioxol analogs **5e** and **7e** exert their cytotoxic effects remains unknown at this stage, but it is unlikely that they affect tubulin dynamics. Nevertheless, these findings suggest that both dioxol and dihydrodioxin analogs of phenylacrylonitrile may have potential for development as clinical candidates to treat a variety of human cancers.

In 1987, the US National Cancer Institute (NCI) identified the *cis*-stilbene analog, combretastatin A-4 (CA-4; Fig. 1, structure A), as the most potent anti-cancer agent of all the natural combretastatins isolated from the African bush willow, *Combretum caffrum*.[1] Early work showed that CA-4 targets tubulin and inhibits the proliferation of both murine and human cancer cells.[2] Unfortunately, subsequent reports have indicated that CA-4 possesses unfavorable properties, such as isomerization to the less active *trans*-stilbene isomer (Fig. 1, structure B) in solution, low water-solubility and vascular disruption,[3–5] which has precluded its development as a potential clinical candidate. Efforts to improve the drug-likeness properties of CA-4 have resulted in the discovery of a water-soluble prodrug, CA-4P, which is currently being evaluated in phase II/III clinical trials for activity in anaplastic thyroid carcinoma, non-small cell lung cancer, and ovarian cancer in combination with conventional standard of care cytotoxic drugs that include paclitaxel, carboplatin, and the antiangiogenic agent, brevacizumab.[6]

Recently our laboratory has reported on some novel *trans*-CA-4 (Fig. 1, structure B) analogs as potent inhibitors of tubulin polymerization with growth inhibitory activities superior to *cis*-CA-4.[4,5] These molecules can also be considered as structural analogs of resveratrol (Fig. 1, structure C), and include the anti-cancer agent **DMU-212** (Fig. 1, structure D), which binds to the colchicine binding site on tubulin. Our work has demonstrated that a trans double bond bearing a nitrile moiety can improve chemical stability and can serve as an effective replacement for the *cis*-olefinic moiety in CA-4 and its analogs.[5] More recently, we have reported on a series of diphenyl, 2-benzothio phenyl/phenyl, and 2-quinolinyl/phenyl acrylonitrile derivatives (Fig. 1, structures E, F, and G respectively) as potent anti-proliferative agents that have potential as anti-tubulin therapeutics for treatment of both solid and hematological tumors.[7]

In our current studies we have now synthesized a small library of thirty one substituted phenylacrylonitrile analogs that incorporate benzo[*d*][1,3]dioxol-5-yl and 2,3-dihydrobenzo[*b*][1,4]dioxin-6-yl moieties and have evaluated them against a panel of 60 human tumor cell lines. The most potent analogs identified have been evaluated as inhibitors of tubulin polymerization.

* Corresponding author. Tel.: +1 501 686 6495; fax: +1 501 686 6057.
E-mail address: pacrooks@uams.edu (P.A. Crooks).

http://dx.doi.org/10.1016/j.bmcl.2016.03.068
0960-894X/

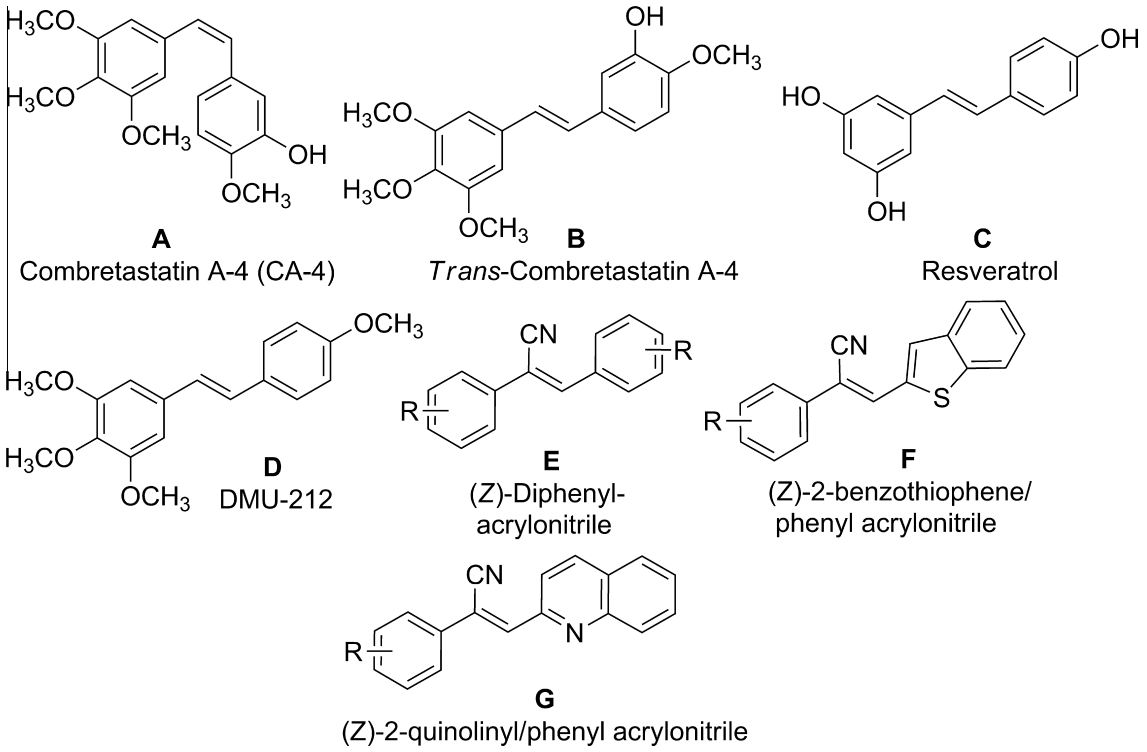


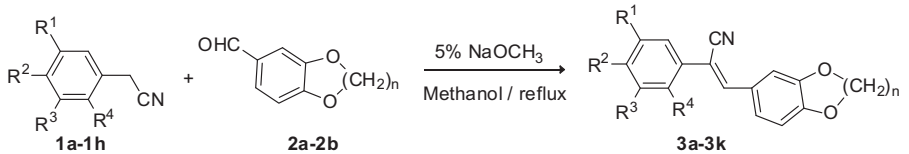
Figure 1. Structures of combretastatin A-4 (CA-4), *trans*-CA-4, **DMU-212**, (*Z*)-diphenyl, (*Z*)-2-benzothiophene/phenyl, and (*Z*)-2-quinolinyl/phenyl acrylonitrile derivatives.

An initial series of eleven (*Z*)-3-(benzo[*d*][1,3]dioxol-5-yl)-2-phenylacrylonitrile (**3a–3e**) and (*Z*)-3-(2,3-dihydrobenzo[*b*][1,4]-dioxin-6-yl-2-phenylacrylonitrile **(3f–3k)** analogs were synthesized by reacting benzo-[*d*][1,3]dioxole-5-carbaldehyde (**2a**) or 2,3-dihydrobenzo[*b*][1,4]dioxine-6-carbaldehyde (**2b**) with an appropriately substituted phenylacetonitrile (**1a–1h**) at reflux temperature in 5% sodium methoxide/methanol to yield the desired compounds in yields ranging from 70% to 95% (Scheme 1).[7,8]

A second series of twelve substituted (*Z*)-2-(benzo[*d*][1,3] dioxol-5-yl)-3-phenylacrylonitrile analogs (**5a–5l**) were synthesized by reacting appropriately substituted aromatic aldehydes (**4a–4l**) with 2-(benzo[*d*][1,3]dioxol-5-yl)acetonitrile (**1i**) at reflux temperature in 5% sodium methoxide/methanol. The reaction was carried out as described previously[7] for analogs **3a**–**3k**[8] to yield the desired compound in yields ranging from 80% to 90% (Scheme 2).

A third series of substituted (*Z*)-2-(benzo[*d*][1,3]dioxol-5-yl)-3-phenyl acrylonitrile analogs (**7a–7h**) were synthesized by reacting appropriate substituted aromatic and hetero aromatic aldehydes (**6a–6h**) with 2-(benzo[*d*][1,3]dioxol-5-yl)acetonitrile (**1i**) at reflux temperature in 5% sodium methoxide/methanol. The reaction was carried out as described previously[7] for analogs **3a**–**3k**[8] to yield the desired compound in yields ranging from 80% to 90% (Scheme 3).

The NCI employs an effective triage system for the submitted compounds based on duplicates already screened and ADME algorithm results, prior to selecting them for initial single dose and subsequent five dose screening assays.[9] The in vitro screening of the above compounds was carried out utilizing the procedure described by Rubinstein et al.[9–11] Of the thirty one phenylacetoni-



1a-1h

1a: $R^1 = R^3 = OCH_3$, $R^2 = R^4 =$ H
1b: $R^1 = R^2 = R^3 = OCH_3$, R^4 = H
1c: $R^1 = R^2 = R^3$ = H, $R^4 = OCH_3$
1d: $R^1 = R^3 = R^4$ = H, $R^2 = NO_2$
1e: $R^1 = R^3 = R^4$ = H, $R^2 = OCH_3$
1f: $R^1 = R^2 = R^3 = R^4$ = H
1g: $R^1 = R^2 = R^3$ = H, $R^4 = CF_3$
1h: $R^1 = R^2 = OCH_3$, $R^3 = R^4$ = H

2a-2b

2a: n=1
2b: n=2

3a-3k

3a: $R^1 = R^3 = OCH_3$, $R^2 = R^4$ = H, n=1
3b: $R^1 = R^2 = R^3 = OCH_3$, R^4 = H, n=1
3c: $R^4 = OCH_3$, $R^1 = R^2 = R^3$ = H, n=1
3d: $R^2 = NO_2$, $R^1 = R^3 = R^4$ = H, n=1
3e: $R^2 = OCH_3$, $R^1 = R^3 = R^4$ = H, n=1
3f: $R^1 = R^2 = R^3 = R^4$ = H, n=2
3g: $R^1 = R^3$ = H, $R^4 = CF_3$, n=2
3h: $R^1 = R^2 = OCH_3$, $R^3 = R^4$ = H, n=2
3i: $R^1 = R^3 = R^4$ = H, $R^2 = OCH_3$, n=2
3j: $R^1 = R^2 = R^3 = OCH_3$, R^4 = H, n=2
3k: $R^2 = NO_2$, $R^1 = R^3 = R^4$ = H, n=2

Scheme 1. Synthesis of (*Z*)-2-(benzo[*d*][1,3]dioxol-5-yl)-2-phenylacrylonitrile (**3a–3e**) and (*Z*)-2,3-dihydrobenzo[*b*][1,4]dioxin-6-yl-2-phenylacrylonitrile **(3f–3k)** analogs.