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Synthesis and *In Vitro* Screening of Novel Heterocyclic Compounds as Potential Breast Cancer Agents

Narsimha Reddy Penthala[1], Thirupathi Reddy Yerramreddy[2], Nikhil Reddy Madadi[1], Vijayakumar Sonar[2] and Peter A. Crooks[1]
[1]*Department of Pharmaceutical Sciences, College of Pharmacy, University of Arkansas for Medical Sciences, Little Rock, Arkansas*
[2]*Department of Pharmaceutical Sciences, College of Pharmacy, University of Kentucky, Lexington*
USA

1. Introduction

Breast cancer is one of the most common non-cutaneous type of cancer in women in worldwide and a leading cause of cancer-related deaths, and is increasing year by year in almost every areas of the globe. Breast cancer is commonly classified into the following two major types: (1) non-invasive breast cancer (cancer cells are confined within the duct and lobules) and (2) invasive breast cancer (cancer cells invade through the walls of the duct or lobules and infiltrate the surrounding tissues). Various kinds of treatments are available for breast cancer, such as chemotherapy, radiotherapy and hormone therapy (Ragaz, 2009). Many indole derivatives are reported as potent breast cancer agents, such as aplysinopsin analogs and indole-3-carbinols. Aplysinopsins are indole-derived marine natural products. The parent aplysinopsin was isolated for the first time (Kazlauskas, et al, 1977) as the major metabolite of eight Indo-Pacific sponge species, which are representatives of the genus *Thorecta*. The *N*-1-unsubstituted aplysinopsins have generated considerable interest due to their potentially useful medicinal properties (Dobroslawa, et al, 2009). Aplysinopsin has been reported as a potent cytotoxic agent against the Kβ-cell line and methyl-aplysinopsins against L-1210 and Kβ-cell lines has been reported as potent cytotoxic agents (Hollenbeak & Schmitz, 1977), and the anticancer activities of aplysinopsin and methyl-aplysinopsins against both 1210- and Kβ-cells has also been reported (Kondo *et al*. 1994). Indole-3-carbinol, a phytochemical derived from cruciferous vegetables such as broccoli and Brussel sprouts, exhibits potent antiproliferative effects against human breast cancer cells and has been shown to decrease metastatic spread of tumors in experimental animals (Brew, et al., 2010). From the above observations and as part of a program for the development of small molecules as potential anticancer treatments (Thirupathi Reddy, et al., 2010 and Narsimha Reddy et al., 2010), we initiated a drug discovery program to identify novel benzyl-aplysinopsin analogs as potent breast cancer agents.

Combretastins are plant products from the South African tree *Combretum caffrum*. This compound was found to inhibit tubulin polymerization, and competitively inhibit the

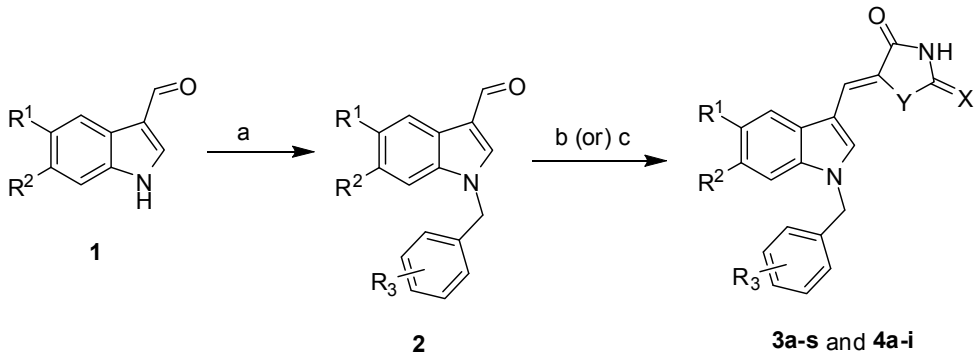
binding of radiolabeled colchicines to tubulin. Investigation of combretastatins revealed that combrestastatin A-4 was active against multidrug resistant (MDR) cancer cell lines (McGown, et al., 1990; Lin, et al., 1988 and Cushman, et al 1991). Combretastatin (A-4), as well as its trans-isomer and a number of related substances, has been found to cause mitotic arrest in cells in culture at cytotoxic concentrations. *trans*-Tetrahydroxystilbene and a number of other related substances were also reported to be cytotoxic agents (Lin, et al 1988). Also, many natural products possessing a trimethoxybenzene ring, e.g., colchicines, and podophyllotoxins, were found to be potent cytotoxic agents that exert their antitumor property by their antitubulin character. In view of the known antitubulin activity of combretstatin, a large number of its derivatives have been synthesized and evaluated for antitubulin activity. Some of the structural modifications carried out on these molecules are emerging as potent breast cancer agents. These observations encouraged us to also investigate combretastatin analogs as potent breast cancer agents.

Both the naturally occurring stilbene derivatives i.e. resveratrol (*trans*-3,5,4'-trihydroxystilbene) and structurally related combretastatin analogs have been reported as cytotoxic agents. Resveratrol is one of the phytoalexins widely distributed in plants products such as grapes, berries, peanuts, and red wine. Resveratrol has recently been reported as a potential chemotherapeutic agent due to its striking inhibitory effects on cellular events associated with cancer initiation, promotion, and progression (Jang, et al., 1997). In addition, there are several reports on the anti-cancer activity of resveratrol in animal models (Baur, et al., 2006). Resveratrol interacts with multiple molecular targets *in vitro*, and exhibits cytotoxic effects against breast, skin, gastric, colon, esophageal, prostate, and pancreatic cancer cells, and leukemia cells (Baur, et al., 2006). Based on the anticancer activity of resveratrol analogs we also focused on the synthesis several resveratrol analogs as potent breast cancer agents.

2.1 Synthesis and evaluation of *in vitro* anticancer activity of (*Z*)-5-((*N*-benzyl-1*H*-indol-3-yl) methylene) imidazolidine-2,4-diones and (*Z*)-5-((1-benzyl-1H-indol-3-yl) methylene)thiazolidine-2,4-diones

A series of novel substituted (*Z*)-5-((*N*-benzyl-1*H*-indol-3-yl)methylene)imidazolidine-2,4-diones and (*Z*)-5-((1-benzyl-1*H*-indol-3-yl)methylene)thiazolidine-2,4-diones that incorporate a variety of substituents in both the indole and *N*-benzyl moieties, and that are considered structurally related to the natural product, aplysinopsin, were synthesized utilizing aldol condensation chemistry under both microwave irradiation and conventional heating methodologies (**Scheme 1**). A library of simple and substituted *N*-benzylindole-3-carboxaldehydes were synthesized in 85-90% yield by reacting the corresponding indole-3-carboxaldehydes with various substituted benzyl halides under phase-transfer catalytic (PTC) conditions using triethylbenzyl ammonium chloride (TEBA) and 50% w/v aqueous NaOH solution in dichloromethane (**Scheme 1**). Aldol condensation of the appropriate *N*-benzylindole-3-carboxaldehyde with hydantoin or 2-iminothiazolidin-4-one, in the presence of CH_3COOH and ammonium acetate and utilizing both microwave irradiation and conventional heating methods (**Scheme 1**), afforded a series of novel simple and substituted (*Z*)-5-((*N*-benzyl-1*H*-indol-3-yl)methylene)imidazolidine-2,4-diones (**3a-s**) and (*Z*)-5-((1-benzyl-1*H*-indol-3-yl) methylene) thiazolidine-2,4-diones (**4a-i**) (Table 1). Microwave irradiation using an open vessel in a domestic microwave oven (1100 W; Kenmore) at atmospheric pressure afforded product yields in the range of 85–93% in 30–60s. All the synthesized compounds were fully characterized by ^{1}H NMR, ^{13}C NMR. The resulting analogs were evaluated for their anti-proliferative activity against a panel of human MCF-7, MDA-MB-

231/ATCC, HS-578T, BT-549, T-47D, MDA-MB-468 breast cancer cell lines (**Tables 1-3**). Compound (*Z*)-methyl-1-(4-cyanobenzyl)-3-((2,5-dioxoimidazolidin-4-ylidene) methyl)-1*H*-indole-6-carboxylate (**3n**) exhibited GI_{50} values of 970nM against HS 578T breast cancer cells, and afforded GI_{50} values ranging from 0.97μM-4.1μM against HS 578T, MCF-7, MDA-MB-231/ATCC, BT-549, T-47D, MDA-MB-468 (**Table 3**) (Narsimha Reddy et al, 2011). Two other analogs, (*Z*)-5-((1-(2-bromobenzyl)-1*H*-indol-3-yl)methylene) imidazolidine-2,4-dione (**3f**) and (*Z*)-5-((1-benzyl-5-methyl-1*H*-indol-3-yl)methylene) imidazolidine-2,4-dione (**3j**) exhibited IC_{50} values of 4.4μM and 5.2μM against MCF-7 cells (**Table 2**) (Narsimha Reddy et al, 2011). From the series of 2-iminothiazolidin-4-ones the compound *(Z)*-5-((5-chloro-1-(4-fluorobenzyl)-1*H*-indol-3-yl)methylene)-2-iminothiazolidin-4 one (**4c**) exhibited growth inhibition (GI_{50}= 3.7μM) against cultured HS 578T cancer cells (**Table 3**).



Scheme 1. Reagents and conditions: (a) appropriate benzylhalide, aqueous NaOH solution, triethylbenzylammonium chloride, DCM, RT; (b) Method A: hydantoin (or), 2-iminothiazolidin-4 one, ammonium acetate in acetic acid, microwave irradiation, 40-50 seconds, 87-95% yield; (c) Method-B: hydantoin (or) 2-iminothiazolidin-4 one, ammonium acetate in acetic acid, 120-125 ^{0}C, 8-12 hours, 74-85% yield.

S. No.	R^1	R^2	R^3	X	Y
3a	H	H	H	O	NH
3b	H	H	*p*-CN	O	NH
3c	H	H	*p*-NO_2	O	NH
3d	H	H	*p*-Cl	O	NH
3e	H	H	*p*-$COOCH_3$	O	NH
3f	H	H	*o*-Br	O	NH
3g	H	H	*p*-F	O	NH
3h	Cl	H	H	O	NH
3i	Br	H	*p*-OCH_3	O	NH
3j	CH_3	H	H	O	NH

S. No.	R^1	R^2	R^3	X	Y
3k	Br	H	H	O	NH
3l	OCH_3	H	H	O	NH
3m	H	H	*p*-CH_3	O	NH
3n	H	$COOCH_3$	*p*-CN	O	NH
3o	$COOCH_3$	H	*p*-CN	O	NH
3p	H	H	*p*-CN	O	NH
3q	H	H	*H*	O	NH
3r	H	Cl	H	O	NH
3s	H	OCH_3	H	O	NH
4a	H	H	*o*-F	NH	S
4b	Cl	H	*o*-F	NH	S
4c	Cl	H	*p*-F	NH	S
4d	Br	H	*o-F*	NH	S
4e	Br	H	*p*-F	NH	S
4f	H	$COOCH_3$	*p*-CN	NH	S
4g	H	$COOCH_3$	*p*-$COOCH_3$	NH	S
4h	$COOCH_3$	H	*p*-CN	NH	S
4i	$COOCH_3$	H	*p*-$COOCH_3$	NH	S

Table 1. List of *N*-benzyl aplysinopsin analogs synthesized (**3a-s and 4a-i**).

Entry	Breast cancer cell lines	
	MCF-7 (IC_{50})	MDA-231 (IC_{50})
3a	27.3	15.7
3b	14.1	17.6
3c	10.9	23.3
3d	21.4	25.3
3e	15.4	18.4
3f	4.4	21.8
3g	22.3	32.8
3h	9.2	18.0
3i	16.4	23.2
3j	5.2	20.1
3k	7.6	12.4
3l	7.2	7.0
3m	20.2	22.1

Table 2. IC_{50} values for simple and substituted *N*-benzyl aplysinopsin analogs (**3a-m).**

Breast Cancer Panel/cell line	**3n**		**4c**	
	GI_{50}	LC_{50}	GI_{50}	LC_{50}
MCF7	3.55	>100	>100	>100
MDA-MB-231/ATCC	3.32	>100	6.45	>100
HS 578T	0.97	>100	3.67	>100
BT-549	1.24	>100	98.8	>100
T-47D	1.50	>100	>100	>100
MDA-MB-468	4.14	>100	>100	>100

[a]GI_{50}: 50% Growth inhibition, concentration of drug resulting in a 50% reduction in net protein increase compared with control cells. [b]LC_{50}: Lethal concentration, concentration of drug lethal to 50% of cells

Table 3. IC_{50} values for compounds **3n** and **4c.**

2.2 Synthesis and *in vitro* cytotoxicity evaluation of (*Z*)-2-amino-5-(1-benzyl-1*H*-indol-3-yl) methylene-1-methyl-1*H*-imidazol-4(5*H*)-ones

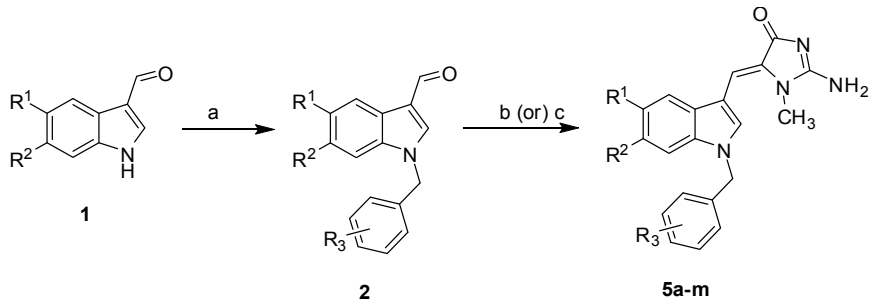
Another series of substituted (*Z*)-2-amino-5-(1-benzyl-1*H*-indol-3-yl)methylene-1-methyl-1*H*-imidazol-4(5*H*)-ones were synthesized (**Scheme 2**) utilizing similar aldol condensation chemistry. Simple and substituted *N*-benzylindole-3-carboxaldehydes were synthesized in 85-90% yield by reacting the corresponding indole-3-carboxaldehydes with various substituted benzyl halides under phase-transfer catalytic (PTC) conditions using triethylbenzyl ammonium chloride (TEBA) and 50% w/v aqueous NaOH solution in dichloromethane. Aldol condensation of the appropriate *N*-benzylindole-3-carboxaldehyde with creatinine, in the presence of CH_3COOH and sodium acetate and utilizing both microwave irradiation and conventional heating methods (**Scheme 2**), afforded a series of novel simple and substituted (*Z*)-2-amino-5-(1-benzyl-1*H*-indol-3-yl)methylene-1-methyl-1*H*-imidazol-4(5*H*)-ones.

Evaluation of analogs these analogs for *in vitro* cytotoxicity activity against MCF-7, MDA-MB-231/ATCC, HS-578T, BT-549, T-47D, MDA-MB-468 human breast cancer cell lines is shown in **Table 4** (Narsimha Reddy et al, 2010). From this series, compounds, (*Z*)-2-amino-5-((1-(2-bromobenzyl)-1*H*-indol-3-yl)methylene)-1-methyl-1*H*-imidazol-4-(5H)-one (**5e**) and (*Z*)-methyl 4-((3-((2-amino-1-methyl-4-oxo-1*H*-imidazol-5(4H)-ylidene)methyl)-1*H*-indol-1-yl)methyl)benzoate (**5f**) showed good growth inhibition with GI_{50} values ranging from 1.5μM-4μM, and LC_{50} values ranging from 5.7 μM->100 μM (**Table 4**).

Breast Cancer Panel/ cell lines	Compound **5e** Molar concentrations		Compound **5f** Molar concentrations	
	GI_{50}	LC_{50}	GI_{50}	LC_{50}
MCF7	2.45×10^{-6}	7.78×10^{-5}	2.25×10^{-6}	4.81×10^{-5}
MDA-MB-231/ATCC	2.01×10^{-6}	1.43×10^{-5}	4.07×10^{-6}	4.67×10^{-5}
HS 578T	2.17×10^{-6}	$>1.0 \times 10^{-4}$	3.97×10^{-6}	$>1.00 \times 10^{-4}$
BT-549	1.73×10^{-6}	$>1.0 \times 10^{-4}$	2.05×10^{-6}	$>1.00 \times 10^{-4}$
T-47D	1.50×10^{-6}	5.74×10^{-6}	1.68×10^{-6}	1.31×10^{-5}
MDA-MB-468	1.63×10^{-6}	6.78×10^{-6}	1.69×10^{-6}	6.90×10^{-6}

[a]GI_{50}: 50% Growth inhibition, concentration of drug resulting in a 50% reduction in net protein increase compared with control cells. [b]LC_{50}: Lethal concentration, concentration of drug lethal to 50% of cells

Table 4. IC_{50} values for compounds **3e** and **3f.**



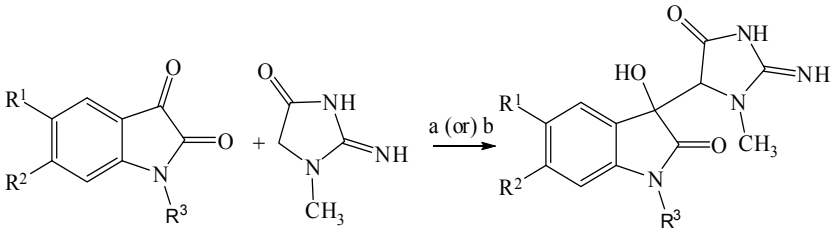
5a	R^1=R^2 = R^3= H	**5g**	R^1=H, R^2=H, R^3=*p*-F
5b	R^1=H, R^2=H, R^3=*p*-CN	**5h**	R^1=Cl, R^2=R^3=H
5c	R^1=H, R^2=H, R^3=*p*-NO_2	**5i**	R^1=Br, R^2=H, R^3=OCH_3
5d	R^1=H, R^2=H, R^3=*p*-Cl	**5j**	R^1=CH_3, R^2=R^3=H
5e	R^1=H, R^2=H, R^3= *o*-Br	**5k**	R^1=Br, R^2=R^3=H
5f	R^1= H, R^2=H, R^3=*p*-$COOCH_3$	**5l**	R^1=OCH_3, R^2= R^3=H
		5m	R^1=H, R^2=H, R^3=*p*-CH_3

Scheme 2. Synthesis of (*Z*)-2-amino-5-(1-benzyl-1*H*-indol-3-yl)methylene-1-methyl-1*H*-imidazol-4(5*H*)-one analogs: Reagents and conditions (a) appropriate benzyl halide, aqueous NaOH solution, triethylbenzyl ammonium chloride, DCM, RT; (b) Creatinine (1.1 mol. eq), NaOAc (1.2 mol. eq), AcOH, MWI, 30-60 sec; (c) Creatinine (1.1 mol. eq), NaOAc (1.2 mol. eq), AcOH, reflux, 7-10 h.

2.3 Synthesis and *invitro* cytotoxicity evaluation of *N*-alkyl-3-hydroxy-3-(2-imino-3-methyl-5-oxo-imidazolidin-4-yl)indolin-2-one

A third series of *N*-alkyl-3-hydroxy-3-(2-imino-3-methyl-5-oxo-imidazolidin-4-yl)indolin-2-one analogs that incorporated a variety of substituents in both the isatin phenyl and *N*-benzyl moieties were also synthesized. These novel analogs (**8a-w**) were prepared by condensation of the appropriate substituted *N*-alkyl isatin with creatinine, in the presence of sodium acetate and acetic acid via both microwave irradiation and conventional heating methodologies (**Scheme 3)** (Narsimha Reddy et al, 2010). Of these two methods, microwave irradiation was found to be advantageous over conventional heating, since the product yields were 83-94% for the former method, but only 70-83% for the latter method. In addition, the time course of the reaction was very fast using microwave irradiation (20-40 sec) compared to 6-8 h for conventional heating. The simple and *N*-alkyl substituted isatins (**6a-j**) were all prepared utilizing literature methods (Macpherson, et al., 2007; Jacobs, et al., 1948 and Shindikar, et al., 2006). All the synthesized compounds were characterized by ^{1}H-NMR and ^{13}C-NMR spectrometry. The geometry of the hydroxyl position in the representative compounds **8a, 8b** and **8t** was established as *trans* to the 4′-methyne hydrogen from X-ray crystallographic data (Narsimha Reddy et al, 2009). From the X-ray diffraction and ^{1}H-NMR data, analogs **8a-8w** were mixtures of *RR* and *SS* isomers. This is consistent with the mechanism of the aldol condensation reaction of **6** with **7**, which proceeds via the formation of the *E*-enolate, as per the Zimmerman–Traxler model, which favors *anti* products, and is predicted to lead to the formation of equimolar *RR* and *SS* enantiomers. We also determined from the crystal

structures of **8a, 8b** and **8t** that the 3-hydroxy group was *trans* to the 4′-methyne hydrogen, which may explain the inability of these analogs to undergo facile dehydration. The cytotoxicity data on these analogs is provided in **Table 5** (Narsimha Reddy et al, 2010). Two analogs, 3-hydroxy-3-(2-imino-3-methyl-5-oxoimidazolidin-4-yl)-1-(4-methoxy benzyl)indolin-2-one (**8n**) and 5-chloro-3-hydroxy-3-(2-imino-3-methyl-5-oxoimidazolidin-4-yl)-1-(4-methoxybenzyl)indolin-2-one (**8o**), showed growth inhibition with GI_{50} values ranging from 2μM-60μM. Substitution of a methoxy group at the 4th position of *N*-benzyl group (**8n**) increases the activity over the breast cancer cells. Further the substitution of chloro group at 5th position of *N*-benzyl *p*-methoxy isatin (**8n**) increased the activity.



Scheme 3. Reagents and conditions: (a) Method A: sodium acetate in acetic acid, microwave irradiation, 20-40 seconds, 83-94% yield; (b) Method-B: sodium acetate in acetic acid, 115-120 ^{0}C, 6-8 hours, 70-83% yield.

Compound	R^1	R^2	R^3
8a	H	H	H
8b	F	H	H
8c	Cl	H	H
8d	Br	H	H
8e	Br	Br	H
8f	NO_2	H	H
8g	H	H	$-CH_3$
8h	F	H	$-CH_3$
8i	Cl	H	$-CH_3$
8j	Br	H	$-CH_3$
8k	H	H	-Bz
8l	Cl	H	-Bz
8m	Br	H	-Bz
8n	H	H	4-OCH_3 Bz
8o	Cl	H	4-OCH_3 Bz
8p	Br	H	4-OCH_3 Bz
8q	H	H	4-Cl Bz
8r	H	H	4-$COOCH_3$Bz
8s	H	H	4-CN Bz
8t	H	H	$-C_6H_5$

Compound	R^1	R^2	R^3
8u	H	H	$-COCH_3$
8v	Cl	H	$-COCH_3$
8w	H	H	$-SO_2C_6H_5$

Breast Cancer Panel/cell lines	Compound **8o** Molar concentrations		Compound **8n** Molar concentrations	
	GI_{50}	LC_{50}	GI_{50}	LC_{50}
MCF7	3.22	>100	6.93	>100
MDA-MB-231/ATCC	8.55	>100	19.4	>100
HS 578T	2.04	>100	60.3	>100
BT-549	2.98	54.9	8.45	97.0
T-47D	13.5	>100	32.0	>100
MDA-MB-468	2.29	>100	11.6	>100

[a]GI_{50}: 50% Growth inhibition, concentration of drug resulting in a 50% reduction in net protein increase compared with control cells. [b]LC_{50}: Lethal concentration, concentration of drug lethal to 50% of cells

Table 5. IC_{50} values for compounds **8o** and **8n.**

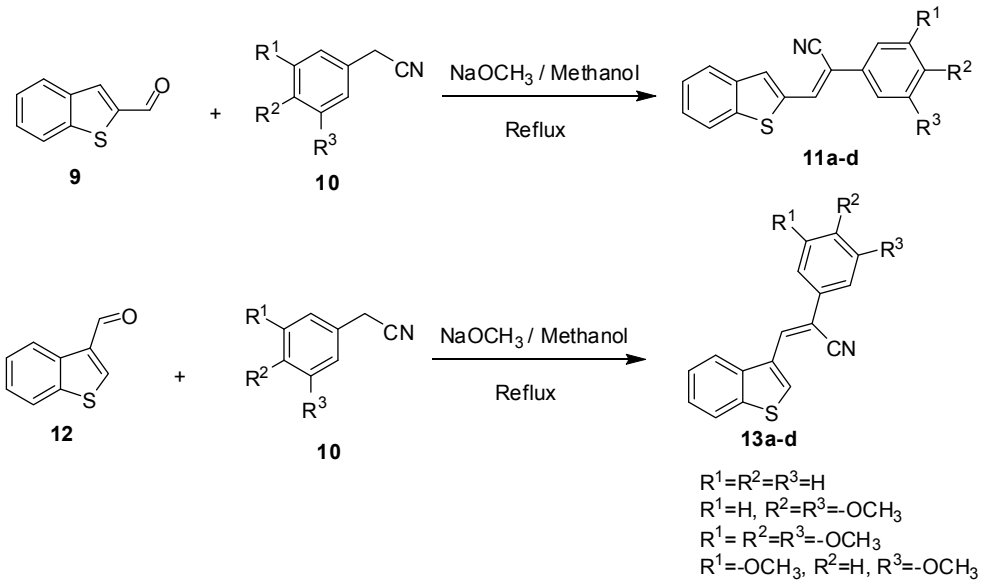
2.4 Synthesis and *in vitro* cytotoxicity evaluation of benzo[*b*]thiophene phenyl acrylontriles as novel combretastatin analogs

A series of combretastatin analogs were prepared via the reaction of benzo[b]thiophene-2-carbaldehyde or benzo[b]thiophene-3-carbaldehyde with simple and substituted benzyl cyanides in 5% sodium methoxide methanol (**Scheme 4**) and evaluated for their *in vitro* cytotoxicity against a panel of human MCF-7, MDA-MB-231/ATCC, HS-578T, BT-549, T-47D, breast cancer cell lines (**Table 6**). Two analogs, (*Z*)-3-(benzo[*b*]thiophen-3-yl)-2-(3,4-dimethoxy phenyl)acrylonitrile (**11b**), and (*Z*)-3-(benzo[*b*]thiophen-3-yl)-2-(3,4,5-trimethoxyphenyl) acrylonitrile (**11c**) showed very potent growth inhibitory properties against four breast cancer cell lines utilized (MCF7, MDA-MB-231/ATCC, HS 578T, BT-549), with GI_{50} values ranging from 28nm-269nm. benzo[b]thiophene-2-carbaldehyde series of compounds (11a-d) generally exhibits greater potency than the benzo[b]thiophene-3-carbaldehyde series of compounds (13a-d) over all the breast cancer cell lines screened.

Breast Cancer Panel/cell line	Compound **11b** Molar concentrations		Compound **11c** Molar concentrations	
	GI_{50}	LC_{50}	GI_{50}	LC_{50}
MCF7	$47.8x10^{-9}$	>100.0	$40.2 x10^{-9}$	>100.0
MDA-MB-231/ATCC	$269 x10^{-9}$	>100.0	$33.0 x10^{-9}$	>100.0
HS 578T	$30.0 x10^{-9}$	>100.0	$42.7 x10^{-9}$	>100.0
BT-549	$28.3 x10^{-9}$	>100.0	$36.6 x10^{-9}$	>100.0
T-47D	>100.0	>100.0	$27.2 x10^{-6}$	>100.0

[a]GI_{50}: 50% Growth inhibition, concentration of drug resulting in a 50% reduction in net protein increase compared with control cells. [b]LC_{50}: Lethal concentration, concentration of drug lethal to 50% of cells

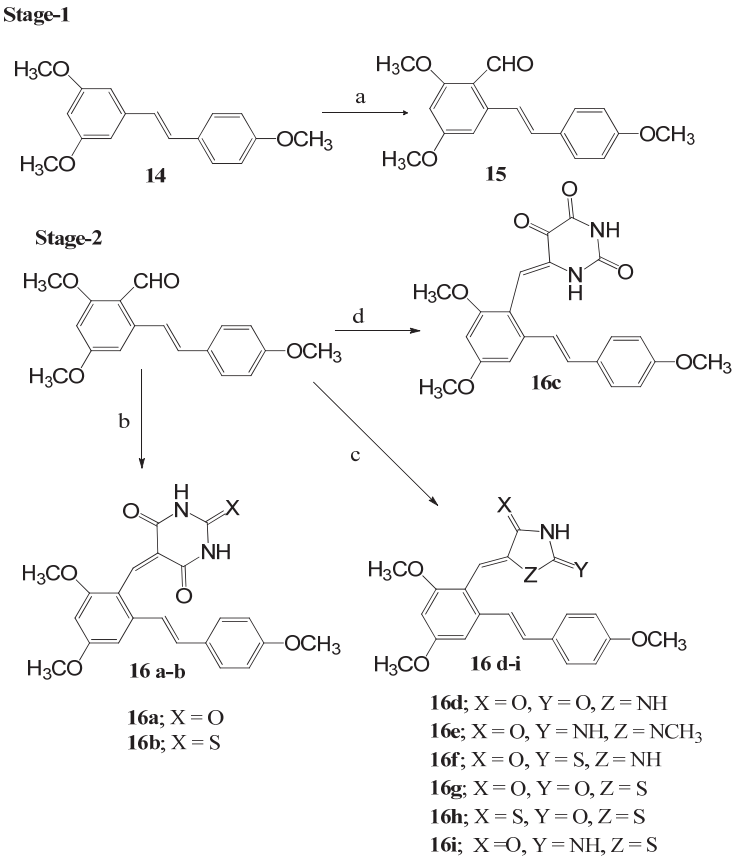
Table 6. IC_{50} values for compounds **11b** and **11c.**



Scheme 4. Synthesis of simple and substituted benzo[*b*]thiophene phenyl acrylontriles (**11a-d** and **13a-d**).

2.5 Synthesis and *in vitro* cytotoxicity evaluation of resveratrol analogs

A series of novel resveratrol analogs were synthesized (**Scheme 5**) and evaluated for their anti-proliferative activity against MCF-7 and MDA-231 breast cancer cells (**Table 7**). The initial step in the synthesis of resveratrol analogs **16a-i** is the synthesis of the common intermediate *trans*-2-formyl-3,4′,5-trimethoxystilbene (**15**), which was prepared via formylation of (*E*)-1,3-dimethoxy-5-(4-methoxystyryl)benzene (**14**) with a slight excess of phosphorous oxychloride ($POCl_3$) in dimethyl formamide (DMF) at 0 ^oC in 69% yield (Xian, et al, 2007). The novel resveratrol analogs **16a-i** were then prepared by aldol condensation of resveratrol-2-carboxaldehyde with the appropriate active methylene compound, utilizing a variety of reaction conditions, i.e., ammonium acetate in acetic acid under microwave irradiation (MWI), by refluxing the reactants in ethanol, or by stirring the reaction at ambient temperature in methanol. The synthetic routes to the resveratrol analogs **16a-i** are illustrated in **Scheme 5**. Compounds **16a-i** were fully characterized by ^1H-NMR and ^{13}C-NMR spectrometry. The geometry of the double bond was established as *Z*, based on NMR spectrometric data. The X-ray crystallographic data of the representative compound **16e** confirmed the *Z*-geometry in this analog (Madadi, et al, 2010). The most potent compound, (*Z*)-5-(2,4-dimethoxy-6-(4-methoxystyryl)benzylidene)-2-imino-1-methylimidazolidin-4-one (**16e**), had IC_{50} values of 0.99μM against MDA-231 cancer cell lines. Compound (*Z*)-6-(2,4-dimethoxy-6-(4-methoxystyryl)benzylidene)-dihydropyrimidine-2,4,5(3H)-trione (**16c**) had an IC_{50} value of 1.28μM against the MCF-7 cell line.



Scheme 5. Synthesis of resveratrol analogs **16a-i**; reagents and conditions: (a) $POCl_3$, DMF, 0 ^oC, 69% yield; (b) barbituric acid or thiobarbituric acid, methanol, RT, 6 hrs, 95-96% yield; (c) five membered active methylene compound, NH_4OAc, AcOH, MWI, 1-2 min, 94-97% yield; (d) isobarbituric acid, ethanol, reflux, 4 hrs, 90% yield.

Entry	Breast cancer cell lines	
	MCF-7 (IC_{50})	MDA-231 (IC_{50})
16a	11.8	5.57
16b	>40	7.59
16c	1.28	12.3
16d	7.93	4.24
16e	14.8	0.99
16f	2.40	10.3
16g	3.96	10.1
16h	1.99	4.09

Table 7. IC_{50} values (μM) for resveratrol analogs **16a-h**.

The resveratrol analog, (*E*)-5-(2,4-dimethoxy-6-(4-methoxystyryl)benzylidene)-2-iminothiazolidin-4-one (**16i**) was also evaluated for its *in vitro* cytotoxicity against a panel of human MCF-7, MDA-MB-231/ATCC, HS-578T, BT-549, T-47D, breast cancer cell lines (**Table 8**). This compound showed growth inhibition (GI_{50} values ranging from 1.4μM-3.9μM) and cytotoxicity (LC_{50} values ranging from 7.1μM-65.7μM) amongst four out of five of the breast cancer cell lines utilized.

Breast Cancer Panel/cell line	GI_{50}	LC_{50}
MCF7	1.44	7.89
MDA-MB-231/ATCC	2.76	65.7
HS 578T	2.55	>100
BT-549	3.91	49.8
T-47D	1.66	7.26
MDA-MB-468	1.66	7.10

[a]GI_{50}: 50% Growth inhibition, concentration of drug resulting in a 50% reduction in net protein increase compared with control cells. [b]LC_{50}: Lethal concentration, concentration of drug lethal to 50% of cells

Table 8. Growth inhibition **(**GI_{50}/ μM)[a] and cytotoxicity (LC_{50}/ μM)[b] data of (*Z*)-2-amino-5-(2,4-dimethoxy-6-(4-methoxystyryl) benzylidene)thiazol-4(5*H*)-one (**16i**).

3. Conclusion

In conclusion, novel *N*-benzyl aplysinopsin, combretastatin and resveratrol analogs have been synthesized and evaluated for their anticancer activity against a number of breast cell lines. In the N-benzyl aplysinopsin series, the analog (*Z*)-methyl-1-(4-cyanobenzyl)-3-((2,5-dioxoimidazolidin-4-ylidene)methyl)-1*H*-indole-6-carboxylate (**3n**) emerged as promising lead compound for further structural optimization studies. The novel combretastatin analogs, (*Z*)-3-(benzo[*b*]thiophen-3-yl)-2-(3,4-dimethoxyphenyl)acrylonitrile (**11b**), and (*Z*)-3-(benzo[*b*]thiophen-3-yl)-2-(3,4,5-trimethoxy phenyl)acrylonitrile (**11c**) were shown to be potent cytotoxic agents against breast cancer cell lines in culture and worthy of further evaluation in animal models of breast cancer. From the library of novel resveratrol analogs, the creatinine analog, (*Z*)-5-(2, 4-dimethoxy-6-(4-methoxystyryl)benzylidene)-2-imino-1-methylimidazolidin-4-one (**16e**) was considered as a lead analog for subsequent structural optimization as a potential agent for the treatment of breast cancer.

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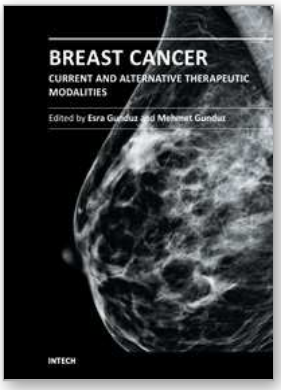
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Cancer is the leading cause of death in most countries and its consequences result in huge economic, social and psychological burden. Breast cancer is the most frequently diagnosed cancer type and the leading cause of cancer death among females. In this book, we discussed various therapeutic modalities from signaling pathways through various anti-tumor compounds as well as herbal medicine for this deadly cancer. We hope that this book will contribute to the development of novel diagnostic as well as therapeutic approaches.

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InTech Europe
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
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InTech China
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

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