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Synthesis, biological evaluation and molecular docking studies of 2-amino-3,4,5-trimethoxyaroylindole derivatives as novel anticancer agents

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ABSTRACT

A series of novel 2-amino-3,4,5 trimethoxyaroylindole derivatives was synthesized and evaluated against selected human cancer cell lines of breast (MCF-7) and colon (HT-29). Introduction of an amino group at the C-2 position on ring A of 3,4,5-trimethoxyaroylindole derivatives resulted in novel compounds, i.e., 2-amino-3,4,5-trimethoxyaroylindole derivatives exhibiting excellent cytotoxic activity against human cancer cell lines. Substitution with methoxy group at R^6 in 2-amino-3,4,5-trimethoxyaroylindole **5d** exhibited excellent cytotoxic activity against MCF-7 (0.013 μM) and colon HT-29 (0.143 μM) indicating slightly higher potency than Combretastatin A-4. Molecular modeling studies of 2-amino-3,4,5-trimethoxyaroylindole derivatives have similar structural alignment as colchicine in protein (PDB code: 1SA0) and exhibited hydrogen bond interaction between *para* position of 3,4,5-trimethoxyphenyl ring with CYS 241 and N–H molecule of indole ring with Val 315 of receptor molecule.

Microtubules has been identified as an attractive and well established molecular targets for anticancer therapy because microtubule influences the crucial processes of cell, such as division, motility, shape maintenance and intracellular transport.[1,2] Combretastatin A-4 (CA-4) is a low molecular weight compound that binds to colchicine binding site causing impediment with microtubule assembly by prevention of tubulin polymerization.[3,4] A number of CA-4 analogues such as CA-4P, EPC2407 (Crolibulin), AVE8062 (ombrabulin), ABT-751 (E7010), OXi4503, T138067, BNC-105P, MPC-6827, etc. are in different phases of clinical trials.[5–7] CA-4 and its analogs have been divided into three major structural elements, i.e., ring A (trimethoxyphenyl ring), ring B (substituted phenyl ring) and the bridgehead linker. The substitution of ethylene bridge of CA-4 with a carbonyl group furnished a benzophenone type CA-4 analogue, named phenstatin. It possess significant anticancer activity in a variety of tumor models, indicating that carbonyl group as linker retain the non-planar character as CA-4.[6] Introduction of an amino group at the C-2 position on ring A of CA-4 and phenstatin analogues exhibited excellent cytotoxic activity in variety of human cell lines (Fig. 1) and tubulin polymerization inhibition. This introduction of amino group causes increased polarity leading to enhancement of the aqueous solubility. Structure–activity relationships (SAR) information indicated that the introduction of an amino group at the C-2 position on ring A of CA-4 and benzophenone analogues plays an important role in maximizing activity.[8–10]

Replacement of ring B to indole derivative prompted to synthesize a series of aroylindole based CA-4 analogs. A number of aroylindole derivatives have been designed and synthesized which exhibit cytotoxic activity on several cancer cell lines due to its excellent antitumor and antivascular activities.[11–16] BPR0L075 (6-methoxy-1*H*-indol-3-yl)(3,4,5-trimethoxyphenyl)methanone have exhibit excellent cytotoxic activity against a panel of cell lines then CA-4 (Fig. 1).[11–13] Introduction of hydroxy group at the C-2 position on ring A of aroylindole derivative exhibits marked antiproliferative activity against KB and MKN45 cells with IC_{50} values of 8.8 and 10.5 nM, respectively, binds strongly to the colchicine binding site and leads to inhibition of tubulin polymerization (Fig. 1).[17] An excellent anticancer activity of aroylindole based CA-4 analogs attracted considerable interest of medicinal chemists in the design and preparation of analogs as novel antitumor agents. Introduction of an amino group at the C-2 position on ring A of 3,4,5-trimethoxyaroylindole derivatives to synthesized 2-amino-3,4,5-trimethoxyaroylindole derivatives which exhibited excellent cytotoxic activity.

The 2-amino-3,4,5-trimethoxyaroylindole derivatives were synthesized by six step reaction sequence, starting with commercially available methyl 3,4,5-trimethoxy benzoate (**1**). The indole substituted 2-nitro-3,4,5-trimethoxyaroylindole derivatives (**4**)

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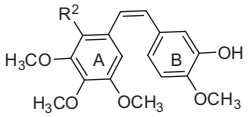
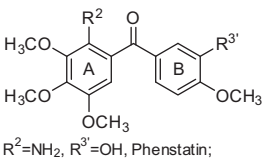
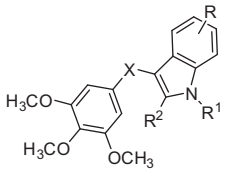


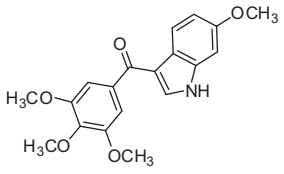
R^2=OH, Combretastatin A-4;
IC_{50}=57nM (HT-29).
R^2=NH_2, 2-amino Combretastatin A-4;
IC_{50} = 36nM (HT-29).



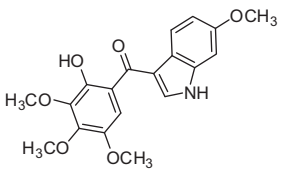
R^2=NH_2, $R^{3'}$=OH, Phenstatin;
IC_{50}=560nM (HT-29).
R^2=NH_2, $R^{3'}$=NH_2, 2-amino Phenstatin derivetive;
IC_{50} = 16nM (HT-29).



Aroylindole derivatives
X=C=O/CH_2;
R=subtitution at different position of indole ring;
R^1=H/CH_3/C_2H_5/CH_2COOH etc;
R^2=H/CH_3/$COOCH_3$/$COOC_2H_5$ etc.

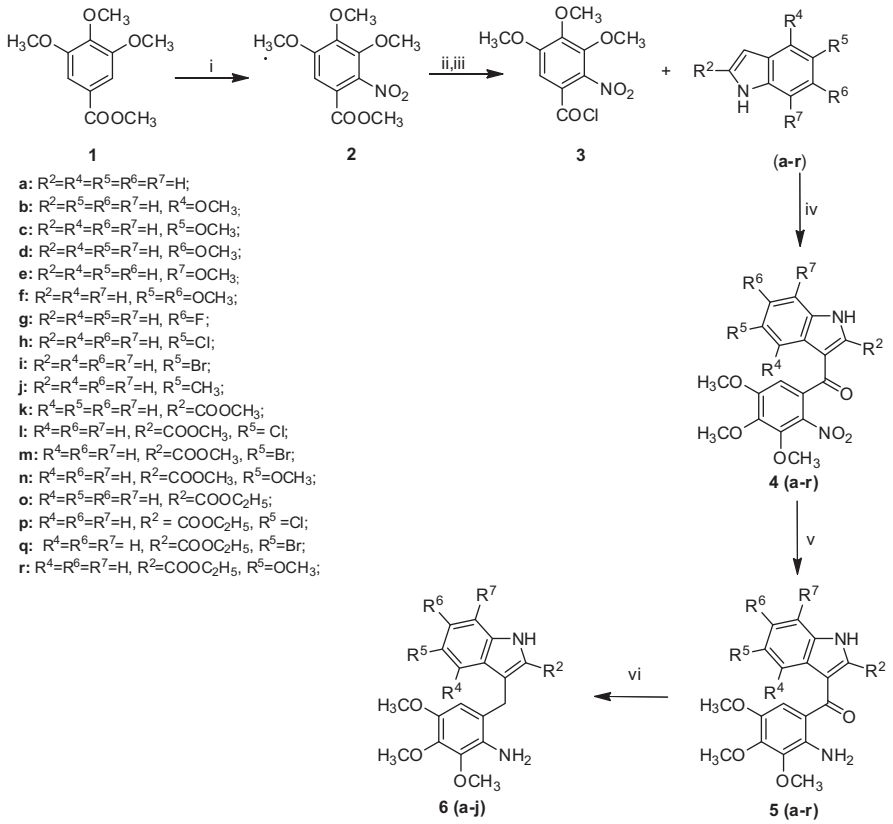


BPR0L075
IC_{50} nM = 7 (MCF-7)/23 (A549)/4 (MESSA)/4 (MKN45)/1 (NUGC)/4 (KB)/7 (H460)/1.1 (HT-29).
CA-4 IC_{50} nM = 8 (MCF-7)/117 (A549)/13 (MESSA)/ 82 (MKN45)/>10000 (NUGC)/6.2 (KB)/17 (H460).



(2-Hydroxy-3,4,5-trimethoxyphenyl)(6-methoxy-1 Hindol-3-yl)methanone
IC_{50} nM = 8.8 (KB)/10.5 (MKN45).
CA-4 IC_{50} nM = 2.5 (KB)/3.2 (MKN45).

Figure 1. Structure and biological activity of Combretastatin A-4, phenstatin and aroylindole derivatives.



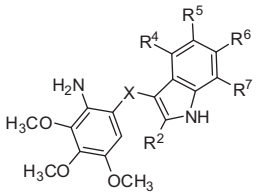
1 **2** **3** **(a-r)**

a: R^2=R^4=R^5=R^6=R^7=H;
b: R^2=R^5=R^6=R^7=H, R^4=OCH_3;
c: R^2=R^4=R^6=R^7=H, R^5=OCH_3;
d: R^2=R^4=R^5=R^7=H, R^6=OCH_3;
e: R^2=R^4=R^5=R^6=H, R^7=OCH_3;
f: R^2=R^4=R^7=H, R^5=R^6=OCH_3;
g: R^2=R^4=R^5=R^7=H, R^6=F;
h: R^2=R^4=R^6=R^7=H, R^5=Cl;
i: R^2=R^4=R^6=R^7=H, R^5=Br;
j: R^2=R^4=R^6=R^7=H, R^5=CH_3;
k: R^4=R^5=R^6=R^7=H, R^2=$COOCH_3$;
l: R^4=R^6=R^7=H, R^2=$COOCH_3$, R^5= Cl;
m: R^4=R^6=R^7=H, R^2=$COOCH_3$, R^5=Br;
n: R^4=R^6=R^7=H, R^2=$COOCH_3$, R^5=OCH_3;
o: R^4=R^5=R^6=R^7=H, R^2=$COOC_2H_5$;
p: R^4=R^6=R^7=H, R^2 = $COOC_2H_5$, R^5 =Cl;
q: R^4=R^6=R^7= H, R^2=$COOC_2H_5$, R^5=Br;
r: R^4=R^6=R^7=H, R^2=$COOC_2H_5$, R^5=OCH_3;

4 (a-r) **6 (a-j)** **5 (a-r)**

Scheme 1. Synthesis of 2-amino-3,4,5-trimethoxyaroylindole derivatives. Reagents and conditions: (i) HNO_3, 10–30 °C, stirring for 1–2 h, 26%; (ii) NaOH, EtOH, stirring for 2 h at 45–50 °C, 90%; (iii) CH_2Cl_2, $SOCl_2$, reflux for 1 h at 60 °C; (iv) $AlCl_3$, reflux for 3–5 h, 80–90 °C (Friedel–Craft acylation), 58–73%; (v) Sn, HCl, reflux at 70 °C, 10% K_2CO_3, 42–62%; (vi) $NaBH_4$, EtOH, reflux for 3 h, 32–54%.

Table 1
Structure, In vitro cell cytotoxic of compounds 2-amino-3,4,5-trimethoxyaroylindole derivatives (**5** (**a**–**r**) and **6** (**a**–**j**)) against MCF-7 and colon HT-29 cell lines



Compd	R^2	R^4	R^5	R^6	R^7	X	MCF-7 (GI_{50} μM[a])	HT-29 (GI_{50} μM[a])
5a	H	H	H	H	H	C=O	0.900	9.8
5b	H	OMe	H	H	H	C=O	>100	>100
5c	H	H	OMe	H	H	C=O	0.528	0.95
5d	H	H	H	OMe	H	C=O	0.013	0.143
5e	H	H	H	H	OMe	C=O	9.963	48.6
5f	H	H	OMe	OMe	H	C=O	38.45	24.4
5g	H	H	H	F	H	C=O	42.83	64.24
5h	H	H	Cl	H	H	C=O	0.321	4.85
5i	H	H	Br	H	H	C=O	0.360	3.83
5j	H	H	Me	H	H	C=O	12.35	>100
5k	COOMe	H	H	H	H	C=O	0.193	2.38
5l	COOMe	H	Cl	H	H	C=O	0.171	2.14
5m	COOMe	H	Br	H	H	C=O	0.124	1.94
5n	COOMe	H	OMe	H	H	C=O	0.102	1.24
5o	COOEt	H	H	H	H	C=O	0.480	5.63
5p	COOEt	H	Cl	H	H	C=O	0.590	6.84
5q	COOEt	H	Br	H	H	C=O	0.425	5.38
5r	COOEt	H	OMe	H	H	C=O	0.540	5.15
6a	H	H	H	H	H	CH_2	45.0	>100
6b	H	OMe	H	H	H	CH_2	NA	NA
6c	H	H	OMe	H	H	CH_2	32.8	>100
6d	H	H	H	OMe	H	CH_2	1.83	74.8
6e	H	H	H	H	OMe	CH_2	NA	NA
6f	H	H	OMe	OMe	H	CH_2	NA	NA
6g	H	H	H	F	H	CH_2	NA	NA
6h	H	H	Cl	H	H	CH_2	NA	NA
6i	H	H	Br	H	H	CH_2	NA	NA
6j	H	H	Me	H	H	CH_2	NA	NA
CA-4	–	–	–	–	–	–	0.025	0.260
BPR0L075	–	–	–	–	–	–	0.007[b]	0.001[c]

[a] GI_{50} = compound concentration required to inhibit 50% growth and data are expressed as the mean from the dose–response curves of at least three experiments.
[b] IC_{50} values taken from the literature for comparison.[11]
[c] IC_{50} values taken from the literature for comparison.[13]

were synthesized with 2-nitro-3,4,5-trimethoxy benzoyl chloride and various commercially available substituted indoles using Friedel–Craft acylation. It is well-known that indole ring readily undergoes electrophilic reactions particularly at the C-3 position with Lewis acids by Friedel–Craft acylation. The steps involved in the synthesis of 2-amino-3,4,5-trimethoxyaroylindole derivative (Scheme 1) includes (i) nitration of methyl 3,4,5-trimethoxy benzoate for the synthesis of **2**, (ii) base hydrolysis of ester for the synthesis of **3**, (iii) chlorination of acid with thionyl chloride, (iv) Friedel–Crafts acylation for the synthesis of 2-nitro-3,4,5-trimethoxyaroylindole for the synthesis of **4** (**a**–**r**), (v) Reduction of the nitro group for the synthesis of **5** (**a**–**r**), (vi) Reduction of carbonyl group for the synthesis of **6** (**a**–**j**).

The synthesized 2-amino-3,4,5 trimethoxyaroylindole compounds were evaluated for their anticancer activity against selected human cancer cell lines of breast (MCF-7) and colon (HT-29) using sulforhodamine B (SRB) method. The results of anticancer activity are expressed in terms of growth inhibition fifty (GI_{50} μM) values and are shown in Table 1. Unsubstituted 2-amino-3,4,5-trimethoxyaroylindole **5a** showed significant activity against MCF-7 and colon HT-29. Substitution at R^5 with electron withdrawing group such as chloro and bromo, i.e., **5h**, **5i**, **5l**, **5m**, **5p**, and **5q** increases the cytotoxic activity but electron donor such as methyl, i.e., **5j** significantly decreases the activity, while substitution with the methoxy group causes significant increase in activity. Substitution at R^6 with methoxy group **5d** exhibited excellent cytotoxic activity against MCF-7 (0.013 μM), colon HT-29 (0.143 μM) indicating slight higher potency than CA-4 while any substitution at R^4 and R^7 significantly decrease the activity. Substitution at R^2 position with methyl and ethyl carboxylate results in enhancement of the activity, i.e., **5k**–**5r**. Replacement of keto group at bridge (X) with methyl group cause considerable decline in activity i.e., **6** (**a**–**r**).

Molecular modeling studies were performed on Glide v5.8[18,19] (Schrodinger, LLC, New York, NY) to investigate the potential interactions between synthesized 2-amino-3,4,5-trimethoxyaroylindole derivative and protein (PDB code: 1SA0). All the 2-amino-3,4,5-trimethoxyaroylindole derivatives were docked to colchicines binding site of β tubulin (PDB 1SA0) for studying the binding mode of compounds for antitumor activity. 2-amino-3,4,5-trimethoxyaroylindole derivative (**5d**) possess similar structural alignment as colchicine in protein (PDB code: 1SA0) (Fig. 2a). 2-amino-3,4,5 trimethoxyaroylindole derivative (**5d**) showed critical interactions between Cys241, Val318 and *meta*, *para*-methoxy group at ring A. The hydrogen bond interaction was found between *para* position of trimethoxyphenyl ring with CYS 241 and N–H molecule of indole ring with Val 315 of receptor molecule (Fig. 2b).

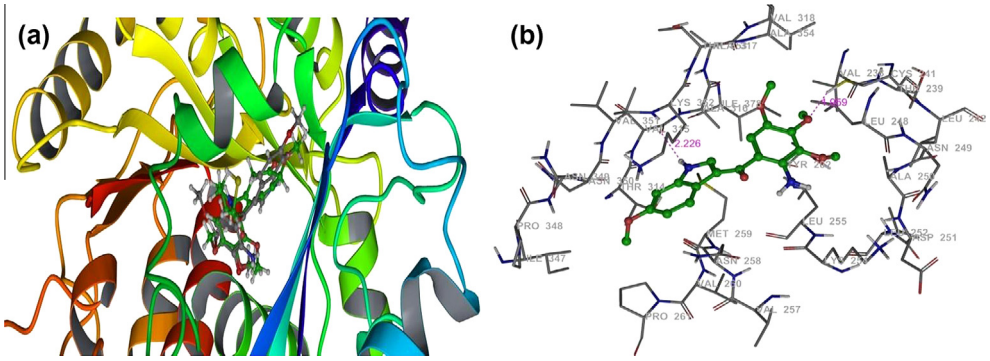


Figure 2. Docking of compound **5d** with protein (1SAO) on colchicine-binding site of β tubulin. (a) Docked pose alignments of **5d** (colored by atom) with colchicine (green) in the tubulin binding site. (b) XP Docking pose of **5d**, pink dotted lines represents hydrogen binding between *para* position of trimethoxyphenyl ring with CYS 241 and N–H molecule of indole ring with Val 315 of receptor molecule.

In conclusion, a series of novel 2-amino-3,4,5-trimethoxyaroylindole derivatives were synthesized and evaluated for their potential anticancer activity against selected human cancer cell lines of breast (MCF-7) and colon (HT-29) using sulforhodamine B (SRB) method. Introduction of an amino group at the C-2 position on ring A of 3,4,5-trimethoxyaroylindole derivatives leads to the formation of novel compounds, i.e., 2-amino-3,4,5-trimethoxyaroylindole derivatives possessing excellent cytotoxic activity. Molecular modeling studies of 2-amino-3,4,5-trimethoxyaroylindole derivatives exhibited similar structural alignment as colchicine in protein (PDB code: 1SA0) and hydrogen bond interaction was also observed between *para* position of trimethoxyphenyl ring with CYS 241 and N–H molecule of indole ring with Val 315 of receptor molecule. These results represent a noteworthy step regarding the design and development of novel 2-amino-3,4,5-trimethoxyaroylindoles with anticancer activity.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2016.03.081.

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