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Original Article

CONSISE SYNTHESIS AND POTENTIAL ANTI-ANGIOGENIC ACTIVITY OF N-1 SUBSTITUTED INDOLYLCHALCONE HYBRIDS ON CHORIOALLANTOIC MEMBRANE (CAM) ASSAY

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ABSTRACT

Objective: Synthesis of N-1 Substituted Indolylchalcone Hybrids and evaluation of anti-angiogenic activity using Chorioallantoic Membrane (CAM) Assay.

Methods: Claisen-schmidt reaction is used for the synthesis of 30 Indolylchalcone hybrids, it involves condensation of N-1 substituted indole-3-carboxaldehyde and N1 substituted 2-acetyl-benzimidazole. The phase transfer catalyst, a green catalyst such as anhydrous potassium carbonate (K_2CO_3) and PEG-400 are used in the alkylation and arylation. All synthesized indolylchalcone hybrids were evaluated for their antiangiogenic activity by *in vivo*-chorioallantoic membrane (CAM) assay method.

Results: The synthesized indolylchalcone compounds are evaluated. The morphometric study was carried out as described by Melkonian *et al.* (2002). The Compounds with code C-2, I-1, I-2 are showing the more potent effect on the dose-dependent assay of CAM. The compounds with code C-1, C-3, E-1 to E-3, M-1 and M-5 shows the significant activity, however, though the compounds with code B-1, B-2, CL-1 and A-5 were showing antiangiogenic effect at 0.1 µM, but does not show any significant activity on dose-dependent assay of CAM.

Conclusion: The synthesized Indolylchalcones as shown in the graph possess very good dose-dependent anti-angiogenic activities. The potency of anti-angiogenetic activity shows that methyl>Ethyl>Cl-benzyl>Benzyl>Isobutyl. 2-acetyl benzimidazole analogs have possible future scope to develop as potent angiogenesis inhibitors.

Keywords: Angiogenesis, Indole chalcone, CAM assay

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INTRODUCTION

Angiogenesis or neovascularization is a combination of two Greek words, "angio" and "genesis" means vase and birth, respectively. Development of these blood capillaries, vessels plays a critical role in cell division, proliferation and movement [1]. More than 30 y ago, the scientist Folkman proposed that the role of these blood vessel to supply food, oxygen and nutrious to malignant tumor because of that growth was dependent on the development of tumor-associated blood vessels, a process called angiogenesis [2]. Targeting angiogenesis has proven to be an effective strategy in several malignant tumour not only colorectal, gastric and lung tumour but also breast, cervical and ovary tumour [3]. Anti-vascular approaches to cancer therapy may be primarily divided into antiangiogenesis inhibitors and vascular disrupting agents (VDAs) [4, 5].

The well-known marketed anti-angiogenic inhibitors drug, bevacizumab (Avastin) [6], and sorafenib (Nexavar, BAY 43-9006) and sunitinib malate (Sutent, SU11248) [7] have been approved by the United States Food and Drug Administration for the treatment of cancer patients. However, these marketed drugs has serious side effects, such as hypertension, bleeding and gastrointestinal perforation; they have been associated with currently available anti-VEGF agents, limiting their chronic use [8]. Hence, there is an urgent need to find a chalcone hybrid molecule that can be more potent towards cancerous cell and less toxic to normal cell but also may overcome the problem of multidrug-resistant strains.

It is well know that all type of heterocyclic ring comprises a very core active moiety or the pharmacophore [9-12]. Derivatives of nitrogen-containing fused heterocyclic compounds such as benzimidazole and indole core molecule have found number of application as an anticancer agent [13]. Literature survey reveals that there is interest in the indolylchalcone systems for use in cancer therapy and such indolylchalcone have the potential to improve the selectivity of chemotherapeutic agents and hence reduce unwanted side effects and multidrug resistance MDR syndrome [14, 15]. The well-known antiangiogenic drug sunitinib also has indole core, taking this in an account, chemistry of 3-substitution on indole moity shows that these has various biological activities and synthesis of such derivatives which has a skeleton that contains an indole cores and are also in chemistry of benzimidazole 2-substitution are valid for biological activities. Considering this in our study of rational research, we fused these indole and benzimidazole moieties with chalcone like structure (fig. 1). Thus three scaffolds moieties in our design are based on the diversity of these moieties in nature as well as in various marketed drugs.

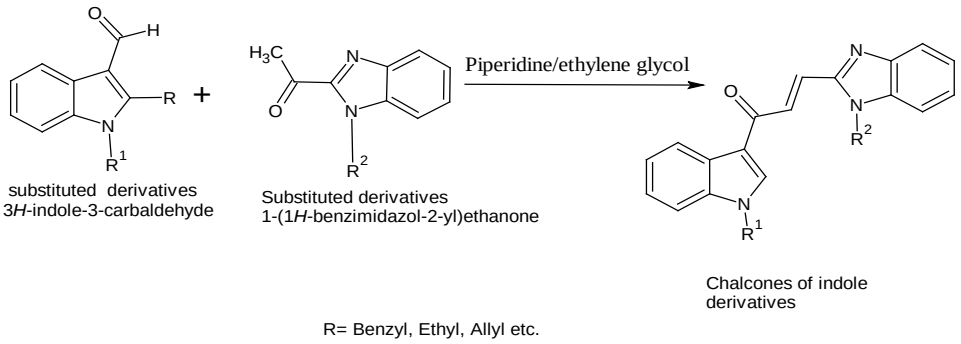
Now a day's 2-acetylbenzimidazole molecule and indole-3-carboxaldehyde are associated with a wide range of biological activities such as anticancer, anti-inflammatory, analgesic and anthelmintic etc. The N-substituted fused heterocyclic compounds are usually biologically active and may be used as potential therapeutic alternatives to the antitumor drug.

MATERIALS AND METHODS

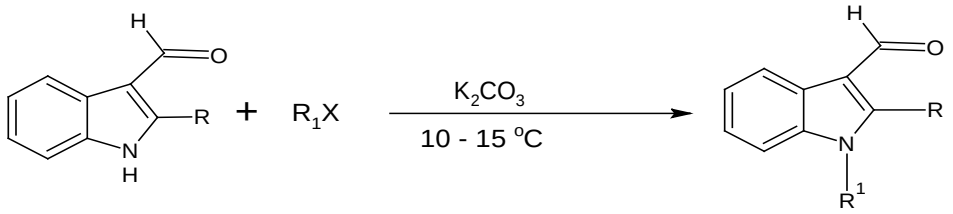
Synthesis of indolylchalcone

In this research study, a series of 30 Indolylchalcone hybrids were synthesized by Claisen-schmidt condensation of N-1 substituted indole-3-carboxaldehyde and N1 substituted 2-acetyl-benzimidazole. The reaction involved in the synthesis of indolylchalcone hybrids as shown in (Scheme 1). The N-alkylation/arylation, mono-methylation on indole-3-carboxaldehyde were achieved by using alkylating and arylating agents in the

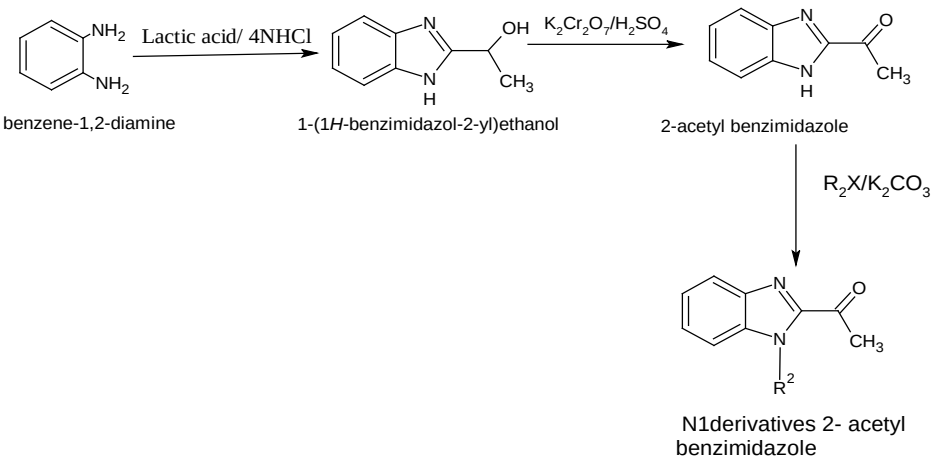
presence of phase transfer catalyst, a green catalyst such as anhydrous potassium carbonate (K_2CO_3) and PEG-400. The N1 substitution reaction on indole-3-carboxaldehyde and 2-aceyl-benzimidazole as shown in (scheme 1A and scheme 1B). The physicochemical properties and analytical data of synthesized indolylchalcone compounds are mentioned in table 1 and 2, respectively.



Scheme 1: Synthesis of indole chalcone



Scheme 1A: Synthesis of N-substituted indole-3-carbaldehyde derivatives



Scheme 1B: Synthesis of N-derivatives of 2-acetyl benzimidazole

Anti-angiogenic activity

Chicken egg chorioallantoic membrane assay (CAM)

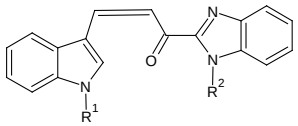
The fertile eggs of 6th day was procured from IPDP poultry Maqarba, Ahmedabad and Angiogenesis inhibitory activities of 30 indolylchalcone hybrids were evaluated using quantitative CAM assay [16-18], Out of which 15 Indolylchalcone hybrids shows most inhibitory effect on *in vivo* assay models of angiogenesis as shown in (table 3). The morphometric study was carried out as described by Melkonian *et al.* (2002). Further, these indolylchalcone hybrids also shows the dose-dependent percentage inhibition of antiangiogenic activity of tested compounds calculated as per given formula (fig. 1)

% inhibition = [(vessel number of untreated CAM-vessel number of CAM treated with test compounds)/vessel number of untreated CAM] x100

RESULTS

The Compounds with code C-2, I-1, I-2 are showing more potent effect on dose dependent assay of CAM when compared to standard. Compounds with code C-2 and I-2 consist of N-1 substitution on ethyl group on both the indole and benzimidazole. Compound with code I-1 is unsubstituted indole, which consists of N-1 substitution of iso-butyl moity on benzimidazole. The compounds with code C-1, C-3, E-1 to E-3, M-1 and M-5 shows the significant activity, however, though the compounds with code B-1, B-2, CL-1 and A-5 were showing antiangiogenic effect at 0.1 µM, but does not show any significant activity on dose-dependent assay of CAM.

Table 1: Physicochemical properties of synthesized indolylchalcone



S. No.	Name of the compound	R-1	R-2	Molecular weight	Molecular formula	% yield of chalcone reaction	Melting point	RF
1.	C-1	H	H	287.31532	$C_{18}H_{13}N_3O$	77	265 °C	0.6123
2.	C-2	Ethyl	H	315.36848	$C_{20}H_{17}N_3O$	65	213 °C	0.1711
3.	C-3	Benzyl	H	377.43786	$C_{25}H_{19}N_3O$	68	249 °C	0.45234
4.	C-4	2-Cl benzyl	H	411.8892	$C_{25}H_{18}ClN_3O$	85	223 °C	0.2105
5.	B-1	H	Benzyl	377.4376	$C_{25}H_{19}N_3O$	70	139 °C	0.2630
6.	B-2	Ethyl	Benzyl	405.49102	$C_{27}H_{23}N_3O$	69	155 °C	0.756
7.	B-3	Benzyl	Benzyl	467.5604	$C_{32}H_{25}N_3O$	72	183 °C	0.842
8.	B-4	2-Cl benzyl	Benzyl	502.00546	$C_{32}H_{24}ClN_3O$	75	171 °C	0.754
9.	E-1	H	Ethyl	315.36898	$C_{20}H_{17}N_3O$	55	205 °C	0.641
10.	E-2	Ethyl	Ethyl	343.42164	$C_{22}H_{21}N_3O$	60	177 °C	0.6728
11.	E-3	Benzyl	Ethyl	405.49102	$C_{27}H_{23}N_3O$	65	216 °C	0.5553
12.	E-4	2-Cl benzyl	Ethyl	439.93608	$C_{27}H_{22}ClN_3O$	77	149 °C	0.453
13.	CL-1	H	2-Cl benzyl	411.8892	$C_{25}H_{18}ClN_3O$	79	219 °C	0.2156
14.	CL-2	Ethyl	2-Cl benzyl	439.9360	$C_{27}H_{22}ClN_3O$	81	181 °C	0.7800
15.	CL-3	Benzyl	2-Cl benzyl	502.00546	$C_{32}H_{24}ClN_3O$	84	232 °C	0.8600
16.	CL-4	2-Cl benzyl	2-Cl benzyl	536.45052	$C_{32}H_{23}Cl_2N_3O$	68	226 °C	0.8039
17.	A-1	Allyl	H	327.37918	$C_{21}H_{19}N_3O$	65	122 °C	0.1428
18.	A-2	Allyl	Ethyl	355.43234	$C_{23}H_{23}N_3O$	68	101 °C	0.3564
19.	A-3	Allyl	Benzyl	417.50172	$C_{28}H_{25}N_3O$	71	132 °C	0.5870
20.	A-4	Allyl	2-Cl benzyl	451.94678	$C_{28}H_{24}ClN_3O$	60	139 °C	0.5612
21.	A-5	Allyl	Iso-Butyl	399.5279	$C_{25}H_{27}N_3O$	70	95 °C	0.4012
22.	M-1	Methyl	H	301.3419	$C_{19}H_{15}N_3O$	71	142 °C	0.1451
23.	M-2	Methyl	Ethyl	329.39506	$C_{21}H_{19}N_3O$	65	224 °C	0.4166
24.	M-3	Methyl	Benzyl	391.46444	$C_{26}H_{21}N_3O$	66	231 °C	0.4833
25.	M-4	Methyl	2-Cl benzyl	425.9095	$C_{26}H_{20}ClN_3O$	80	220 °C	0.6353
26.	M-5	Methyl	Iso-Butyl	357.4422	$C_{23}H_{23}N_3O$	79	141 °C	0.5833
27.	I-1	H	Iso-butyl	343.42164	$C_{22}H_{21}N_3O$	65	131 °C	0.6857
28.	I-2	Ethyl	Iso-butyl	371.4748	$C_{24}H_{25}N_3O$	70	116 °C	0.5421
29.	I-3	Benzyl	Iso-butyl	433.54418	$C_{29}H_{27}N_3O$	73	129 °C	0.5483
30.	I-4	2-Cl benzyl	Iso-butyl	467.94678	$C_{29}H_{26}ClN_3O$	68	125 °C	0.6140

Table 2: Analytical data of synthesized indolylchalcone

S. No.	Name of the compound	Wavenumber (cm^{-1})	Chemical shift (δ ppm)
1.	C-1	3424, 3226(NH str), 1639(C=O),1562(CH=CH Str),	7.26-7.30 (m, 4H, Ar-CH),7.5 (d, H, C-O-CH), 7.7(br, 2H, NH), 8.05 (t,3H, Ar) 8.26 (d, 1H CH=CH), 8.29(d, 2H, Ar)ppm.
2.	C-2	3224(NH Str) 3055, 2934(CH_2CH_3) 2978 (-CH_3-Str)1646(C=O) 1523(C=H Str)	1.55 (s,3H, CH_3) 4.24(m, 2H,CH_2) 7.75 (d, H, C-O-CH)7.29-7.33 (m, 4H, Ar-CH), 7.96(d, 1H, CH=CH), 8.13 (t, 3H, Ar-CH), 8.42(d,2H, Ar-CH), 10.71(br, 1H, NH) ppm
3.	C-3	3258(NH-str),3100,3058,(CH-str,Ar),1648(C=O),1528 (C=H Str),	5.50(s, 2H, CH_2), 7.51(d, H, C-O-CH), 7.25-7.36 (m, 8H, Ar-CH),7.81(d, 1H, CH=CH), 8.06 (t, 3H, Ar-CH), 8.23(d,2H, Ar-CH), 13.24(br, 1H, NH)ppm.
4.	C-4	3248(NH-Str), 3120,3058(CH str, Ar),1658 (C=O),1525(C=H Str),737 (C-Cl)	5.57(s, 2H, CH_2), 7.85(d, 1H, CH=CH)7.51(d, 1H, C-O-CH), 7.19-7.33 (m, 8H, Ar-CH), 8.10 (m, 4H, Ar-CH) 13.1(br, 1H, NH)ppm.
5.	B-1	3208(NH str),3068(CH str, Ar), 1650(C=O), 1530(CH=CH Str), 1477(C=N strAr)	3.46(s, 2H, CH_2), 6.03 (d, 1H, C-O-CH),7.77, (d, 1H, CH=CH) 7.19 to 7.77 (m, 8H, Ar-CH), 8.16(t,3H,Ar), 8.31(d,2H,Ar-CH), 12.87 (br, 1H, NH)ppm.
6.	B-2	3059,3032,3007(CH str, Ar) 2980(CH_2CH_3), 1650(C=O),1525(CH=CH Str).	1.50(t,3H, CH_3) 4.18(q, 2H,CH_2), 6.06(s,2H, Ar-CH_2) 7.21(d, 1H, C-O-CH), 7.60(d, 1H, CH=CH), 7.23-7.36 (m, 8H, Ar-CH) 7.85(t, 3H, Ar-CH), 7.96(d,2H, Ar-CH) ppm.
7.	B-3	3061,3031, (CH str, Ar) 2927(-CH_2), 1651(C=O) 1525(CH=CH Str).	5.47(s, 2H, CH_2-Ar), 5.71 (s,2H, CH_2-Ar), 6.32 (d, H, C-O-CH), 7.21(d, 1H, CH=CH), 6.39to 7.50 (m, 13H, Ar-CH), 8.04 (m,5H, Ar-CH) ppm.
8.	B-4	3061,3031(CHAr-str,),2927(CH_2Str), 1651(C=O), 1525(CH=CH str), 623(C-Cl)	5.45(s, 2H, CH_2-Ar), 6.00 (s,2H, CH_2-Ar), 6.75 (d, 1H, C-O-CH), 7.21(d, 1H, CH=CH), 7.24-7.63 (m, 14H, Ar-CH), 7.90-8.21(m,4H, Ar-CH) ppm.
9.	E-1	3229(NH str), 2961(CH_2CH_3), 1638(C=O),1433(C=C strAr),	1.38 (s, 3H, CH_3) 4.70(q, 2H,CH_2), 7.40 (d, H, C-O-CH), 7.26-7.41 (m, 4H, Ar-CH),7.50 (d, 1H, CH=CH),7.89(d, 2H, Ar-CH), 8.12 (t, 3H, Ar-CH), 11.82(br, 1H, NH)ppm.
10.	E-2	3053(CH str, Ar), 2934(CH_2CH_3), 2978(CH_2CH_3),1650(C=O), 1524 (CH=CH Str).	1.45(t, 3H, CH_3), 1.52(t, 3H, CH_3), 4.18(q, 2H, CH_2), 4.76(q, 2H, CH_2), 7.65(d, 1H, C-O-CH), 7.96 (d, H, CH=CH), 7.25-7.45 (m, 5H, Ar-CH), 8.21(t, 3H, Ar-CH) ppm.
11.	E-3	3090, 3059, (CH str, Ar), 2980(CH_2CH_3), 1736(CH-C-O), 1651(C=O), 1569, 1582 (CH=CH).	1.49(s, 3H, CH_3), 4.74 (q, 4H,-CH_2 CH-$_3$), 5.29(d, 2H,CH_2-Ar), 7.62(d, H, C-O-CH), 715(d, 2H,Ar-CH) 7.25-7.40 (m, 8H, Ar-CH), 7.93(d, 1H, CH=CH), 8.20 (t, 3H, Ar-CH) ppm.

12.	E-4	3441, 3057(CH str, Ar),2971(CH_2CH_3),2932,1654 (C=O),1084,623 (C-Cl), 592 (C=N)	1.51(t, 3H, CH_3), 4.76 (q,2H,-CH_2 CH_3), 5.44(d, 2H,CH_2-Ar),7.60 (d, H, C-O-CH),7.23-7.38 (m, 8H, Ar-CH), 7.92(d, 1H, CH=CH), 8.1-8.35(m, 4H,Ar)ppm.
13.	CL-1	3173(N-H str),3059, 2973, 2922,1775(CH-str), 1651(C=O), 1570(C=C), 737 (C-Cl)	6.05(s, 2H, CH_2-Ar), 6.40 (d, 1H, C-O-CH), 7.08 (d, 1H, CH=CH) 7.25-7.37 (m,8H, Ar) 7.94 (m, 4H, Ar-CH), 11.91(br, 1H, NH).
14.	CL-2	3093,3059(CH str, Ar), 1647(C=O),1525(C-H Str),5 733.5(C-Cl).	1.47 (t,3H, CH_3) 4.23(m, 2H, CH_2) 6.06(s, 2H, Ar-CH_2),6.42(d,1H, C-O-CH 7.13(d, 1H, CH=CH),), 7.23-7.54 (m, 8H, Ar-CH), 8.07(m, 4H, Ar-CH)
15.	CL-3	3054(CH str, Ar), 3029, 1656(C=O), 1569(CH=CH, Str), 739 (C-Cl)	5.51(s, 2H, CH_2-Ar), 5.54 (s,2H, CH_2-Ar) 6.75 (d, 1H, C-O-CH),) 7.41(d, 1H, CH=CH), 7.23-7.34(m, 12H,Ar-CH), 8.11 (m, 5H, Ar-CH).
16.	CL-4	3058(CH str, Ar), 1643(C=O),1522(C-H Str), 738(C-Cl)	5.58(s, 2H, CH_2-Ar), 6.08 (s,2H, CH_2-Ar), 6.39(d, 1H, C-O-CH), 6.89(d, 1H, CH=CH), 7.13-7.28 (m, 13H, Ar-CH), 7.96(m, 4H, Ar-CH)
17.	A-1	3254(N-H str), 3063,2931(CH str, Ar), 1649(C=O), 1525(C=C Str).	3.63(d, 2H, CH_2), 4.91 (d, 2H, N-CH_2), 5.24(m, 1H, CH=CH_2), 6.89(d, 1H, C-O-CH), 7.13-8.19 (m, 8H, Ar-CH) 7.77 (d,1H,CH=CH), 11.91(br, 1H, NH)ppm.
18.	A-2	3294,3092,3052,2980, (CH str, Ar) 1650(C=O), 1527(C=C Str), 1447(N-CH_2),	1.49 (s,3H, CH_3) 5·28(d, 2H, NCH_2), 5.98 (m, 1H, CH=CH_2),, 5·14 (s, 2H, CH_2), 7.63 (d, H, C-O-CH)7.94(d, 1H, CH=CH), 7.22-7.99(m, 8H, Ar-CH), 8.13(q, 2H, CH_2) ppm.
19.	A-3	3091, 3026, 3054, 2985(CH str, Ar), 1644(C=O), 1524(CH=CH Str),	4.7(s,2H,Ar-CH_2), 5.18 (m, 1H, = CH_2), 5·26 (d, 1H, =CH_2) 5·84(d, 2H, NCH_2), 7.77(d, H, C-O-CH), 8.02(d, 1H, CH=CH), 7.23-8.30(m, 13H, Ar-CH), 8.12(s, 2H, CH_2) ppm.
20.	A-4	3107,3058 (CH str, Ar), 1768(C=CH-C), 1650(C=O), 1527(CH=CH), 741(C-Cl).	4.7(s,2H,CH_2), 5.95 (m, 1H, CH= CH_2), 5·14(d, 2H, NCH_2), 5·18(d, 1H, =CH_2), 6.49 (d, H, C-O-CH), 7.00-7.69(m, 12H, Ar-CH) 7.98(d, 1H, CH=CH) ppm.
21.	A-5	3399, 3053(CH str, Ar), 2958, 2871,(CH_3CH_2str) 1687,1651(C=O), 1525(CH=CH Str),	0.92(s, 6H, CH_3-CH_3), 2.30(m,1H,CH=CH_2), 4.53(s, 2H,CH_2), 4.55(s,2H,CH_2), 5.95 (m, 1H, CH= CH_2), 5·14(d, 2H, NCH_2), 7.61 (d, H, C-O-CH),7.25-7.46(m, 6H, Ar-CH), 7.98(d, 1H, CH=CH), 8.12-8.25(t, 2H, Ar)ppm.
22.	M-1	3390(N-H str), 3213, 3062(CH str, Ar), 2937, 1647 (C=O), 1566, 1521 (CH=CH Str).	3.65 (s, 3H, CH_3), 7.34-7.53 (m, 4H, Ar-CH), 7.74 (d, H, C-O-CH) 8.02(d, 2H, Ar), 8.10(t, 3H, Ar), 8.25(d, 1H,CH=CH) 13.18 (br, 1H, NH)ppm.
23.	M-2	3430,3215, 3047(CH str, Ar),3101, 1648(C=O), 1528(CH=CH str), 1462(N-CH_2), 592 (C=CH)	1.44(t,3H,CH_3), 3.53(s,3H, CH_3),4.77(d,2H,CH_2), 7.53-7.55(m, 4H, Ar-CH), 7.66(d, 1H, C-O-CH), 7.88 (d, H, CH=CH),8.01-8.11 (m, 4H, Ar-CH) ppm.
24.	M-3	3093, 3055(CH str, Ar),2931(R-CH_3,str), 1647(C=O), 1527(CH=CH Str).	3.89(s,3H,CH_3), 7.31-7.48 (m, 8H, Ar-CH), 7.59(d, 1H, C-O-CH), 7.93 (d, H, CH=CH), 7.95-8.01 (m, 3H, Ar-CH), 8.11(d, 2H, Ar-CH)ppm.
25.	M-4	3402, 3202, 3286, 3096(CH str, Ar), 3055, 2987(R-CH_3), 1646(C=O),1527(CH=CH Str), 1441(N-CH2), 1064, 633(C-Cl)	3.89(s, 3H, CH_3), 6.08(s, 2H, CH_2), 6.38 (d, H, C-O-CH),7.24 (d, 1H, CH=CH), 7.35-7.49 (m, 8H, Ar-CH), 7.94-8.13(m, 4H, Ar-CH) ppm.
26.	M-5	3104, 3053(CH str, Ar), 2934, 1643(C=O), 1524(CH-CH Str).	; 0.90(s, 6H, CH_3-CH_3), 1.6 (s,3H, CH_3), 2.21(m, 1H, CH=CH_2), 4.57 (q, 2H,CH_2), 7.68(d, 1H, C-O-CH), 7.87 (d, H, CH=CH), 7.31-7.36 (m, 4H, Ar-CH), 8.02-8.11(m, 3H, Ar-CH) ppm.
27.	I-1	3176(NH-Str), 2955, 1898(CH str, Ar), 1634(C=O), 1519(CH=CH Str).	1.35(s,6H,CH_3-CH_3), 2.27(m,1H,CH=CH_2), 4.57(d, 2H,CH_2), 7.61 (d, H, C-O-CH), 7.87(d, 1H, CH=CH) 7.18-7.65 (m, 5H, Ar-CH), 7.96-8.16 (m, 4H, Ar-CH),11.86(br, 1H, NH) ppm.
28.	I-2	3368, 3107, 3053(CH str, Ar),1642(C=O),1522(CH=CH str)	0.92(s, 6H, CH_3-CH_3), 1.50 (t,3H, CH_3-CH_2), 2.36(m, 1H,CH=CH_2), 4.20 (q, 2H,CH_2),4.56(d, 2H, CH_2), 7.46 (d, H, C-O-CH), 7.25-7.40 (m, 5H, Ar-CH), 7.95 (d, 1H, CH=CH), 8.11-8.21(m, 3H,Ar-CH) ppm.
29.	I-3	3083,3056, 3029 (Ar-CH str), 1646(C=O), 1526(CH=CH Str).	0.65(d, 6H, CH_3-CH_3), 2.02(m, 1H, CH=CH_2), 4.39(d, 2H, CH_2), 5.3(s, 2H, CH_2-Ar),7.09-7.19(m, 9H, Ar-CH), 7.27(d, 1H, C-O-CH), 7.72(d, 1H, CH=CH), 7.77(d, 1H, Ar-CH), 7.97-8.22(t, 3H, Ar-CH)ppm.
30.	I-4	3294,3090(CH=CHAr),1650(C=O), 1525(CH=CH str), 625(C-Cl)	0.73(d, 6H, CH_3-CH_3), 2.05(m, 1H,CH=CH_2), 4.27(d, 1H, CH_2), 5.47(s, 2H, CH_2),6.77(d, 1H, C-O-CH), 7.10-7.40(m, 8H, Ar-CH), 7.77(d, 1H, CH=CH), 7.80-8.21(m, 4H,Ar-CH) ppm.

Table 3: The % inhibitory effect of antiangiogenic activity N-1 substituted indolylchalconehybrids and in a CAM assay in a concentration of 0.1 µM/egg

Code of compound	No% avascular CAM/Total N=6	Std. error of deviation	Code of compound	No % avascular CAM/Total N=6	Std. error of deviation	Code of compound	No% avascular CAM/Total N=6	Std. error of deviation
B-1	14/20(70%)	1.826	CL-3	2/20 (10%)	0.5774	A-1	6/20(30%)	1.390
B-2	13/20(65%)	1.390	CL-4	7/20(35%)	1.390	A-2	5/20(25%)	0.5774
B-3	8/20(40%)	1.390	E-1	18/20(90%)	0.5774	A-3	4/20(20%)	1.390
B-4	7/20(35%)	1.390	E-2	19/20(95%)	0.2582	A-4	3/20 (15%)	13.336
C-1	18/20(90%)	0.5774	E-3	15/20(75%)	1.155	A-5	14/20(70%)	0.8165
C-2	17/20(85%)	1.317	E-4	6/20(30%)	1.390	M-1	15/20(75%)	1.155
C-3	16/20(80%)	0.9309	I-1	16/20(80%)	0.9309	M-2	8/20(40%)	6.731
C-4	15/20(75%)	1.155	I-2	18/20(90%)	0.8165	M-3	7/20(35%)	0.8165
CL-1	17/20(85%)	1.317	I-3	7/20(35%)	0.8165	M-4	6/20(30%)	1.390
CL-2	5/20(25%)	1.317	I-4	6/20(30%)	1.390	M-5	16/20(80%)	0.7303

*SD (n=6)

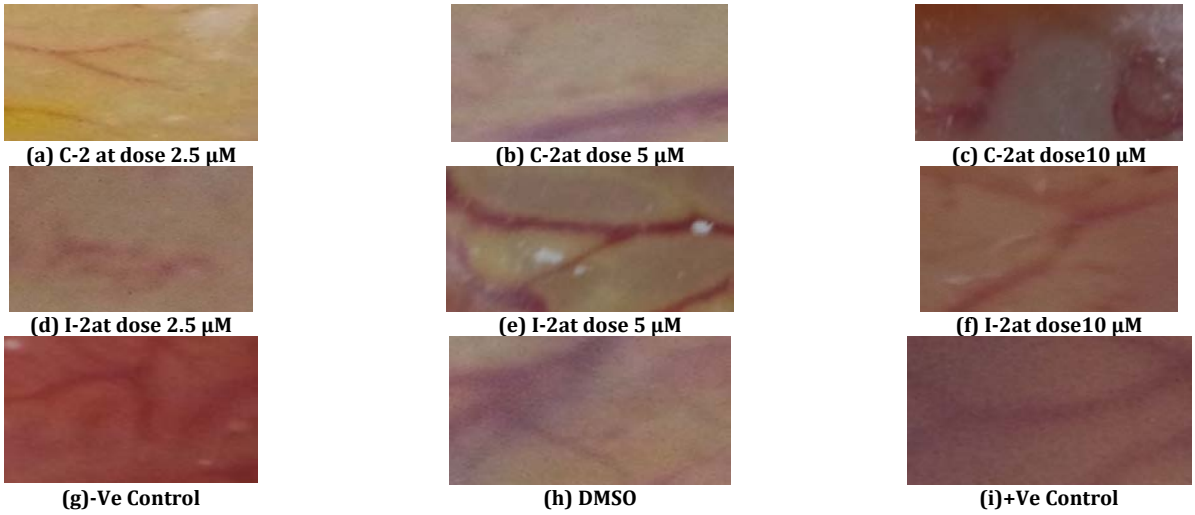


Fig. 1A: Effect of dose-dependent assay of synthesized indolylchalcone hybrids of benzimidazole on *in vivo* CAM angiogenesis: CAM were treated with different series of dose such as 2.5 µM, 5 µM and 10 µM treatment groups indolyl chalcone hybrids at day 8th, the percentage of inhibition of blood vesel formation, compared to untreated conrol, was determined. (a to i) Result suggests that indolyl chalcone hybrids compound code C-2 and I-2 inhibited angiogenesis expressed as (*p* =<0.001 *vs* DMSO)

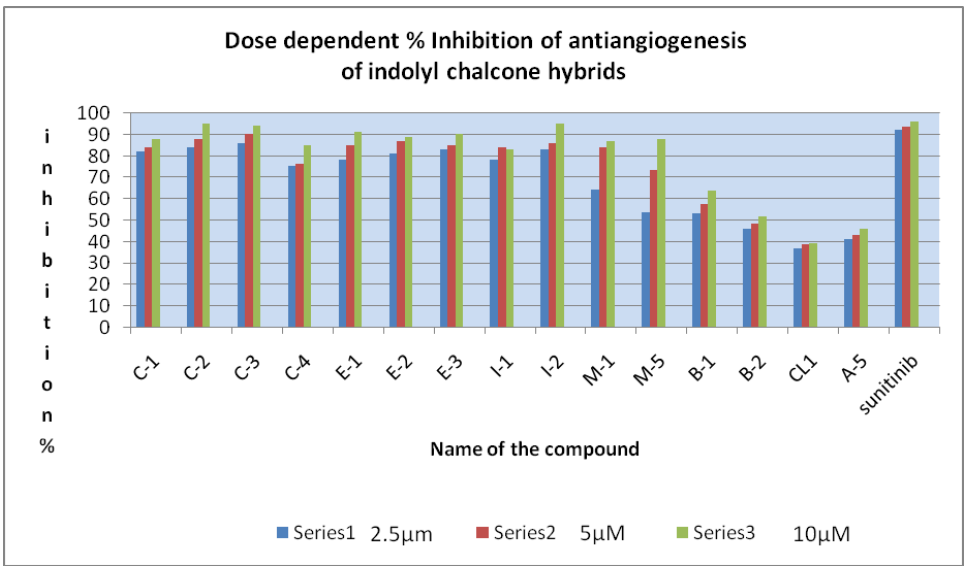


Fig. 1A: Effect of dose-dependent assay of synthesized indolylchalcone hybrids of benzimidazole on *in vivo* CAM angiogenesis

DISCUSSION

We found during our CAM Assay Study that there was noticeable decrease in number of blood vessels as compared with the negative control group, as shown in fig. 1A. Furthermore, One-way ANOVA was used to compare the means of groups. Results showed that the different series of dose such as 2.5µM, 5µM and 10µM treatment groups exhibited varying response based on their %inhibition on blood vessel count (fig. 1B). These newly synthesized indolylchalcone hybrids were statistically significant at P = 0.05. Considering this we can draw an inference that amongst 30 synthesized compounds 11 compounds of indolylchalcone hybrids has potent anti-angiogenic properties.

CONCLUSION

We have developed an inexpensive, simple, and eco-friendly synthesis of N-alkyl derivatives of two fused heterocyclic compounds of indole-3-carboxaldehyde and 2-acetylbenzimidazole. The 11 compounds of indolylchalcones as shown in graph possess very good dose-dependent anti-angiogenic activities. The N-1 substitution is valid on benzimidazole such as ethyl, benzyl and also 2-Cl substituted group on phenyl shows significant potent anticancer activity. The potency of anti-angiogenetic activity shows that methyl>Ethyl>Cl-benzyl>Benzyl>Isobutyl.

The present study suggests that N-1 substituted Indolylchalcone hybrids of 2-acetyl benzimidazole might be promising analogs of angiogenesis inhibitors to manage the uncontrolled neovascularization occurring during malignant tumor development.

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally to this study.

CONFLICT OF INTERESTS

Declared none

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