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Synthesis and SAR Studies of Bis-Chromenone Derivatives for Anti-proliferative Activity against Human Cancer Cells

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Abstract

A novel family of 3-((4-oxo-4*H*-chromen-3-yl)methyl)-4*H*-chromen-4-one (bis-chromone) derivatives were designed, synthesized and studied for their anti-cancer activity using the XTT assay for the growth inhibition against various human cancer cells. Among them, 3-((5-(cyclohexylmethoxy)-4-oxo-4*H*-chromen-3-yl)methyl)-7-methoxy-4*H*-chromen-4-one and 3-((5-(cyclohexylmethoxy)-4-oxo-4*H*-chromen-3-yl)methyl)-7-hydroxy-4*H*-chromen-4-one showed micromolar level of *in vitro* anti-proliferative activity against human cancer cell lines. The SAR studies indicated bis-chromone as a basic scaffold to design anticancer agents. The 5-cyclohexylmethoxy on the first chromenone ring and electron donating group such as CH_3, OCH_3 or hydrogen bonding group (OH) on the other chromenone ring of bis-chromone increased the activity. However, saturation of one of chromenone to chromanone in bis-chromones decreased the activity.

Key words: Bis-chromone, Anti-cancer, Cytotoxicity.

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Cancer is a deadly disease which figures among the prominent causes of mortality worldwide[1] due to the lack of a breakthrough research in this field. Thus, discovery of anti-cancer drugs has become a desperate objective to be solved. Even though some potential candidates have been discovered, yet they lag behind due to the toxicity associated with them. Therefore, there is a need for the development of new anti-cancer agents which can overcome this problem.

Since the beginning of cancer research a large number of heterocyclic compounds have been discovered for their role in the treatment of various kinds of cancers.[2] Among them one of the important class is naturally occurring flavonoid which plays a crucial role in anti-cancer drug discovery.[3] Structural elucidation of flavonoid revealed chromen-4-one (chromone) connected phenyl ring at 2 or 3 position as a core structure. However, chromone motif seems to be vital for anti-cancer activity.[4-6] Therefore, a number of chromones derivatives have been designed, synthesized and studied for their anti-cancer activity.[7-9] Among them, some examples include a series of chromone derivatives **1**[10] bearing diverse dithiocarbamate moieties, 2-styrylchromone analogs **2**[11], 3-hydroxychromones **3**[12], geiparvarin analogs **4**[13] (chromone linked with 1,2,3-triazole residues), flavones **5**[14] bearing a *N*1-(flavon-7-yl)amidrazones incorporating *N*-piperazines and related congeners, 4*H*-chromen-1,2,3,4-tetrahydropyrimidine-5-carboxylate derivatives **6**[15] and chromone-based lavendustin analogs **7**[16]. Thus, the chromone scaffold has emerged as one of the most important synthetic analogs for their anti-cancer activity.

Although a large number of chromone analogs have been studied for their anti-cancer activity, bis-chromone analogs have never been investigated as anti-cancer agents. Accordingly, we have designed and synthesized bis-chromone derivatives **8 - 11** as shown in Figure 2 and their anti-cancer activities were measured against human prostate (PC-3), lung (NCI-H23),

breast (MDA-MB-231), colon (HCT-15), stomach (NUGC-3) and renal (ACHN) cancer cell lines.

Preparation of bis-chromone analogs **8** and **9** were outlined in Scheme 1. The α,β-unsaturated intermediates **14** were synthesized by the reaction of the substituted chromone aldehydes **12** with appropriate triphenyl phosphine salts **13**.[17,18] In order to obtain **16**, the reduction of double bond and additional debenzylation of **14** were initially attempted with 40 psi hydrogen gas in the presence of 20 % Pd/C in THF/methanol (1:1) at room temperature. However this reaction gave mixture of inseparable multiple products containing saturated chromone **15**. Thus the reaction conditions were changed to refluxing with cyclohexene[19] in the presence of 20 % Pd/C in THF/methanol (1:1). Five hour reflux of this reaction mixture partially reduced **14** to **16**, which was isolated by column chromatography with moderate yield. TLC monitoring of the reaction under transfer hydrogenation conditions indicated formation of chromanone **15** around seventh hour of reaction. The prolongation of reaction time to ten hours entirely gave chromanone **15**. Compound **16** was efficiently cyclized to bis-chromone **8** by the treatment with boron trifluoride diethyl etherate (6.5 equivalents), methanesulfonyl chloride (4.2 equivalent) and *N*,*N*-dimethylformamide at 50-60 ^{o}C. The same conditions were applied for the preparation of **9** from **15**. Compound **8b** was obtained from **16b** and subsequent methylation and benzylation reactions afforded **8c** and **8f**, respectively (Scheme 2).

The phosphorous ylide derivative **20**[20] was prepared from the chromone aldehyde **17** by reducing the aldehyde to the alcohol **18** in moderate yield using 9-BBN. The chromone alcohol was treated with tetrabromomethane in the presence of PPh_3 to afford the bromo methyl analog **19**. The bromo analog was reacted with PPh_3 to provide the corresponding phosphorous ylide **20** in good yield. Ylides thus formed was reacted with chromone aldehyde **12** as shown in Scheme 3 to get vinyl bridged bis-chromone **10** with an excellent yield. Using

same reaction condition for reduction of **14** (Pd/C, cyclohexene) gave only ethylene bridged compound **11**.

The *in vitro* anti-proliferative activities of compounds **8a - k**, **9 - 11** were measured against human cancer cell lines [prostate (PC-3), lung (NCI-H23), breast (MDA-MB-231), colon (HCT-15), stomach (NUGC-3) and renal (ACHN)] using XTT assay[21] as summarized with IC_{50} values in Table 1.

In the preliminary investigation, simple bis-chromone derivative **8a** (ACHN IC_{50} = 0.51), (HCT-15 IC_{50} = >10), (MDA-MB-231 IC_{50} = >10), (NCI-H23 IC_{50} = >1.40), (NUGC-3 IC_{50} = 7.77) and (PC-3 IC_{50} = 7.40) without any substitution interestingly showed sub micro-molar range activity. Thus the structure activity relationship of anticancer activity of bis-chromone analogs was further studied. We initially planned to study the effect of substituents' at position 5 of ring A of bis-chromone scaffold (Table 1). Hydroxyl analog **8b** showed similar potency to those of **8a**. Compound **8c** (ACHN IC_{50} = 0.34), (HCT-15 IC_{50} = 3.43), (MDA-MB-231 IC_{50} = >10), (NCI-H23 IC_{50} = >1.93), (NUGC-3 IC_{50} = 3.19) and (PC-3 IC_{50} = 3.49) substituted with electron donating methoxy group showed nearly two fold better activities. Interestingly, compounds **8a - c** showed potent anti-cancer activity against ACHN, NCl-H23, NUGC-3 and PC-3 cell lines. Increasing size of alkoxy group with isobutoxy (**8d**) and isopentyloxy (**8e**) reduced the activity. Both analogs only exhibited good activity against ACHN and PC-3 cell lines. However, the flat benzyloxy analog **8f** showed good activity not only against ACHN, PC-3 but also against NUGC-3 cell lines. These results gave the impression that bulkiness of substituent at position 5 of ring A of bis-chromone scaffold can be one of the factors for good activity. Therefore, the bulky cyclohexylmethoxy group (**8g**) at the same position was further introduced and this analog displayed the best activity almost against all cell lines. The above results confirmed our view point that increasing

hydrophobicity or bulkiness at this position is critical for the potent growth inhibitory activity against cancer cell lines.

In the next set of experiments, hydrophobic electron donating groups (CH_3, OCH_3), electron withdrawing group (Cl) and hydrogen bonding group (OH) at ring D of bis-chromones were introduced to study their effect on anti-cancer activity (Table 1). Interestingly, Substitution with electron donating groups as shown in **8h** (CH_3; CLogP: 6.2) and **8i** (OCH_3; CLogP: 5.8) exhibited potent anti-proliferative activity whereas the introduction of electron withdrawing group on **8j** (Cl; CLogP: 6.4) led to suppress activity. The presence of hydrogen bonding group on **8k** (OH; CLogP: 5.4) resulted (ACHN IC_{50} = 2.10), (HCT-15 IC_{50} = 2.11), (MDA-MB-231 IC_{50} = 3.22), (NCI-H23 IC_{50} = >2.15), (NUGC-3 IC_{50} = 3.22) and (PC-3 IC_{50} = 4.25) in good anti-cancer activity against all cancer cell lines. Saturation of the double bond of one of the chromone ring as shown in compound **9** reduced its activity compared to **8g**. To get more insight in the SAR of bis-chromones, methylene linker between chromenones in bis-chromone analog were increased to vinyl (**10**) or ethylene group (**11**). These analogs did not show any anti-cancer activity. The overall SAR study is represented in **Fig.2**.

To conclude, a novel family of bis-chromone derivatives were designed, synthesized and evaluated using XTT assay for growth inhibition against various human cancer cells. Among them compounds 3-((5-(cyclohexylmethoxy)-4-oxo-4H-chromen-3-yl)methyl)-7-methoxy-4H-chromen-4-one **(8i)** and 3-((5-(cyclohexylmethoxy)-4-oxo-4H-chromen-3-yl)methyl)-7-hydroxy-4H-chromen-4-one **(8k)** displayed the most potent anti-cancer activity. According to the SAR studies, the activity increases by increasing the bulkiness at position 5 of ring A and also by the introduction of electron donating group (CH_3 and OCH_3) and hydrogen bonding group (OH) at ring D. However, upon saturation of bis-chromones of **8** to **9** the activity decreased which indicates the critical importance of planar two chromenones of these bis-chromones for their the anti-proliferative activity. Moreover, increasing the chain length in-

between the chromone rings (n = 2) abolished the activity completely. It concludes that bis-chromone scaffold might serve as a lead molecule for finding a novel family of anti-cancer agents.

Acknowledgment

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Supplementary data

The detailed experimental part of this article can be found in the Supplementary data.

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Figures captions

Fig. 1. Synthetic anti-cancer chromones

Fig.2. Graphical representation of SAR design.

Table 1. *In vitro* anti-proliferative activity of bis-chromones **8a-k, 9, 10** and **11.**

Scheme 1: Synthesis of bis-chromones **8a-b, 8d-f, 8h-k, 9**

Reagents and conditions;

a) Methylene chloride, rt.; b) Pd/C, Cyclohexene, THF/ MeOH, reflux; c) BF_3/Et_2O, MsCl, DMF, 50 ^oC.

Scheme 2: Synthesis of Bis-chromones **8c** and **8f**

Reagents and conditions;

a) BF_3/Et_2O, MsCl, DMF, 50 ^oC; b) NaH, CH_3I, THF, rt. ; c) BnBr, K_2CO_3,Acetone, reflux.

Scheme 3: Synthesis of Bis-chromones **10** and **11**

Reagents;

a) Aluminum oxide, 2-Propanol, reflux; b) CBr_4/PPh_3 c) PPh_3, Methylene Chloride, reflux; d) Methylene chloride, rt.; e) Pd/C, Cyclohexene, THF, reflux.

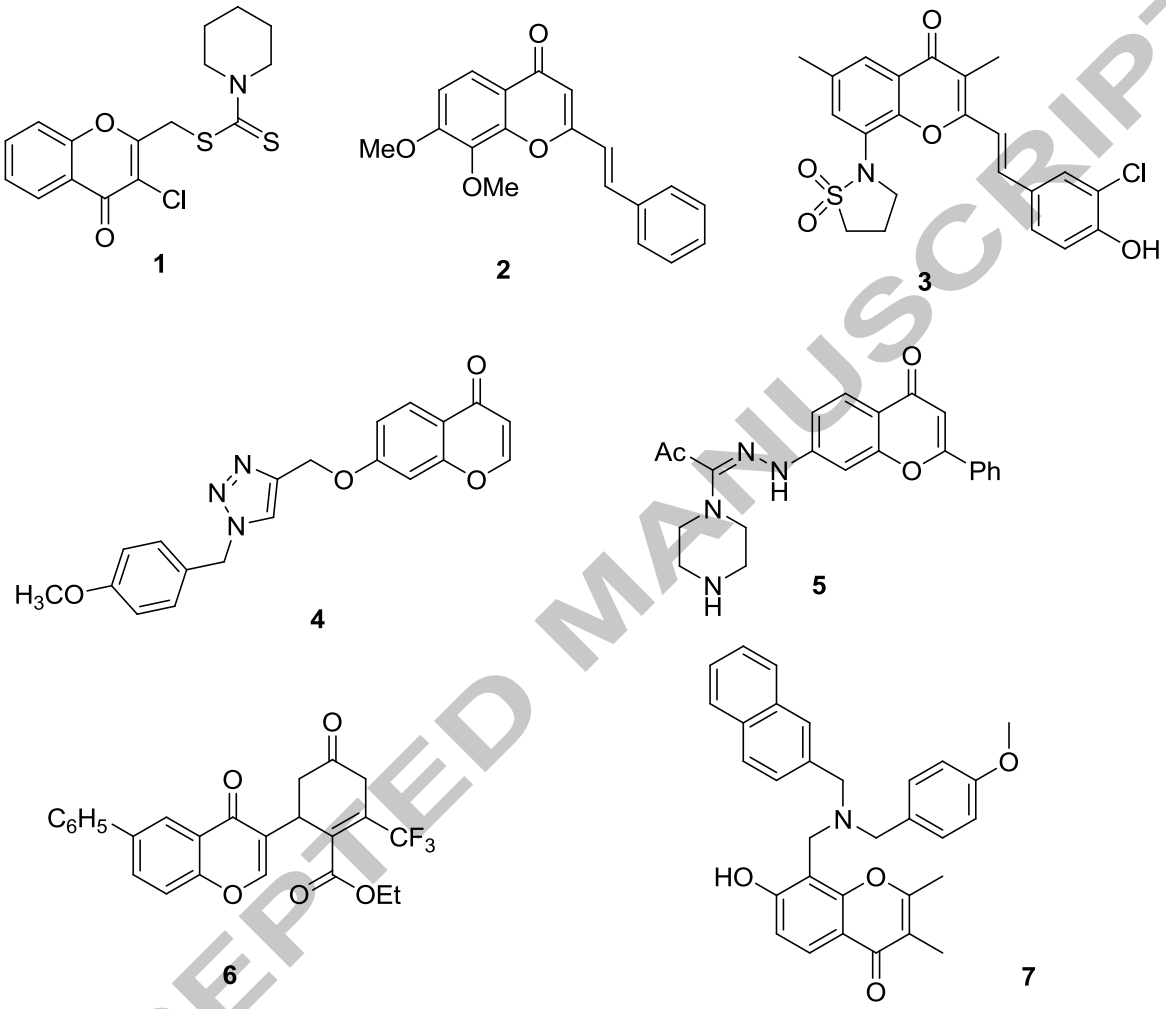


Fig. 1

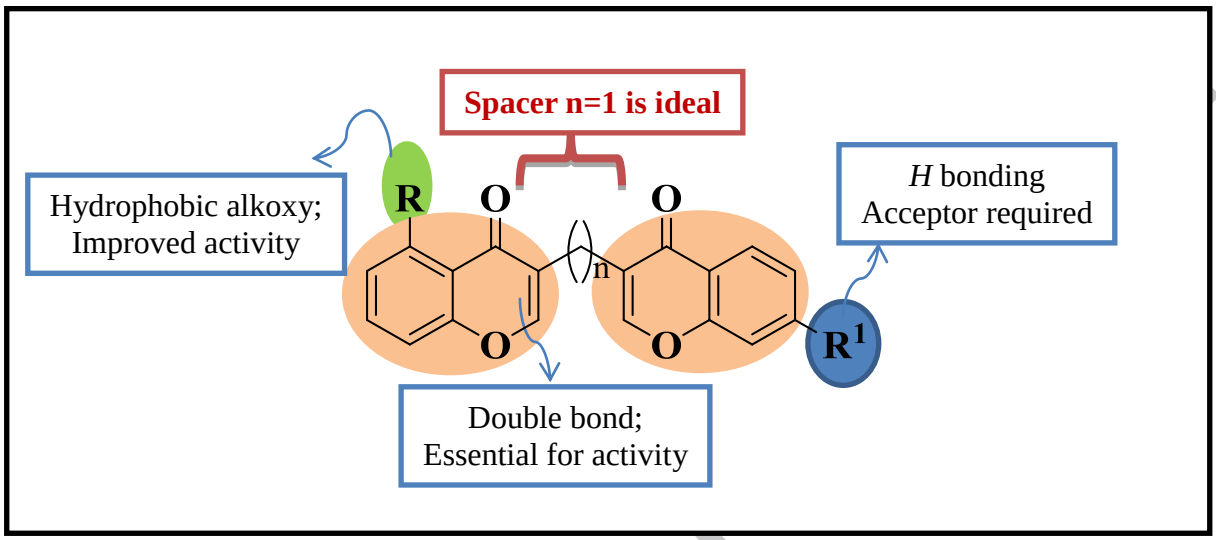
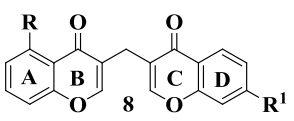


Fig. 2

Table 1



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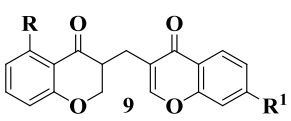

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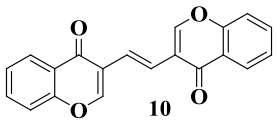

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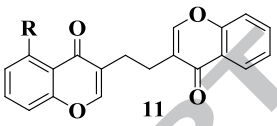
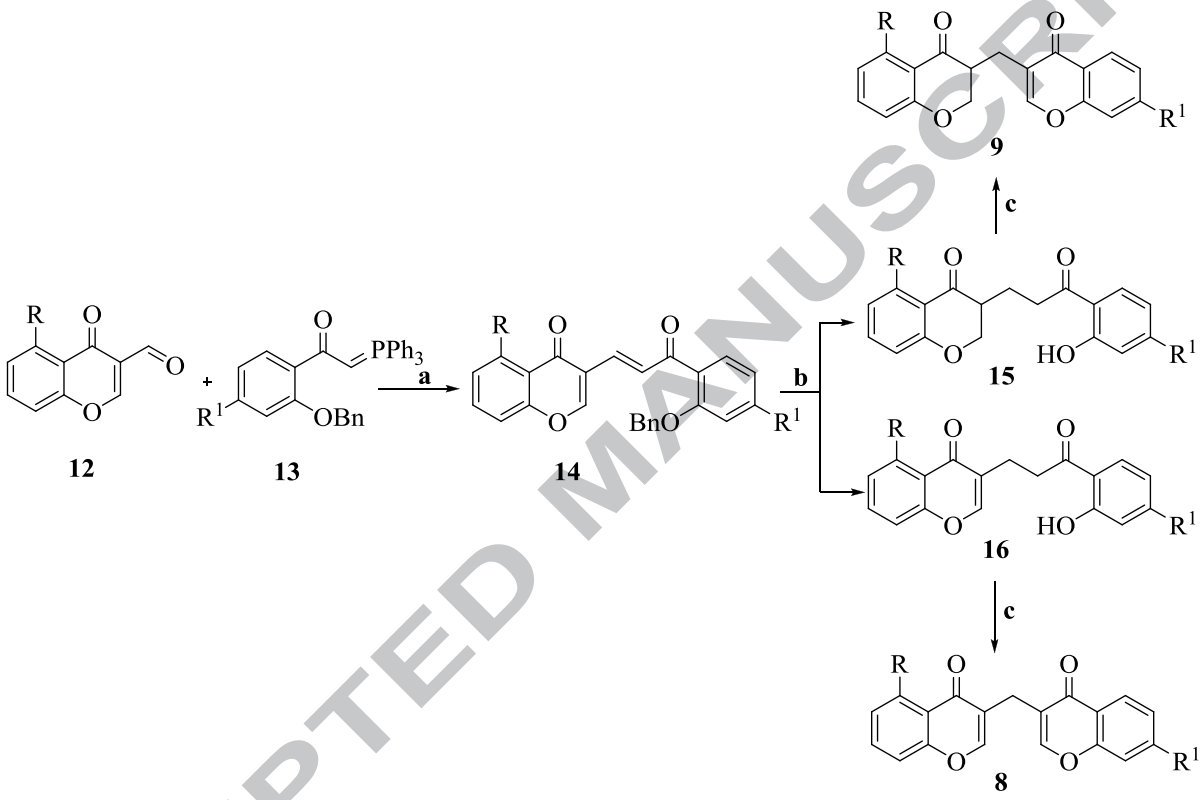


11

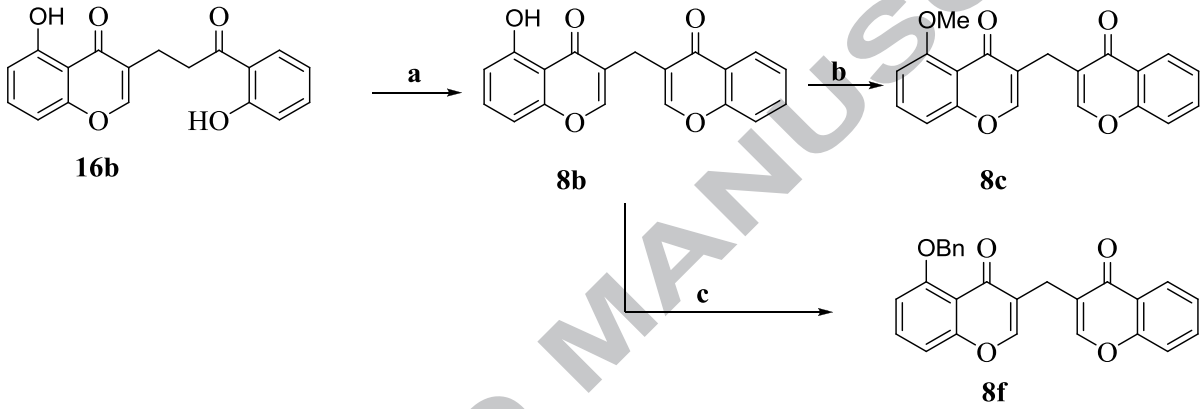
Comp. No.	R	R^1	$IC_{50}(\mu M)$[a]					
			ACHN	HCT15	MDA-MB231	NCI-H23	NUGC-3	PC-3
8a	H	H	0.51 ± 0.05	>10	>10	1.40 ± 0.09	7.77 ± 0.10	7.40 ± 0.57
8b	OH	H	0.74 ± 0.07	>10	>10	7.55 ± 0.35	3.27 ± 0.55	5.95 ± 0.24
8c	OMe	H	0.34 ± 0.04	3.43 ± 0.23	>10	1.93 ± 0.07	3.19 ± 0.14	3.49 ± 0.07
8d	IBO	H	1.46 ± 0.05	>10	>10	>10	>10	1.72 ± 0.01
8e	IPO	H	3.96 ± 0.24	>10	>10	>10	>10	>10
8f	BZO	H	3.99 ± 0.14	>10	>10	>10	7.22 ± 0.18	4.83 ± 0.34
8g	CHM	H	4.23 ± 0.09	4.24 ± 0.17	4.11 ± .22	4.13 ± 0.31	3.53 ± 0.10	4.51 ± 0.14
8h	CHM	CH_3	2.25 ± 0.11	2.22 ± 0.07	3.32±0.19	3.22 ± 0.20	2.15 ± 0.17	2.44 ± 0.21
8i	CHM	OMe	1.35 ± 0.05	1.45 ± 0.08	1.2 ± 0.10	1.32 ± 0.06	1.44 ± 0.07	1.12 ± 0.05
8j	CHM	Cl	>10	>10	>10	>10	>10	>10
8k	CHM	OH	2.10 ± 0.15	2.11 ± 0.11	3.22±0.18	2.15±0.07	3.22±0.10	4.25±0.13
9	CHM	H	8.20 ± 0.74	8.36±0.94	9.11±0.88	9.49±0.54	8.42±0.23	9.38±0.77
10	H	>10	>10	>10	>10	>10	>10	>10
11	H	>10	>10	>10	>10	>10	>10	>10
	Adriamycin		0.26±0.02	0.43±0.02	0.60±0.03	0.49±0.01	0.38±0.03	0.5±0.02

a) IC_{50} values are taken as a mean from 3 experiments. Mean ± SE

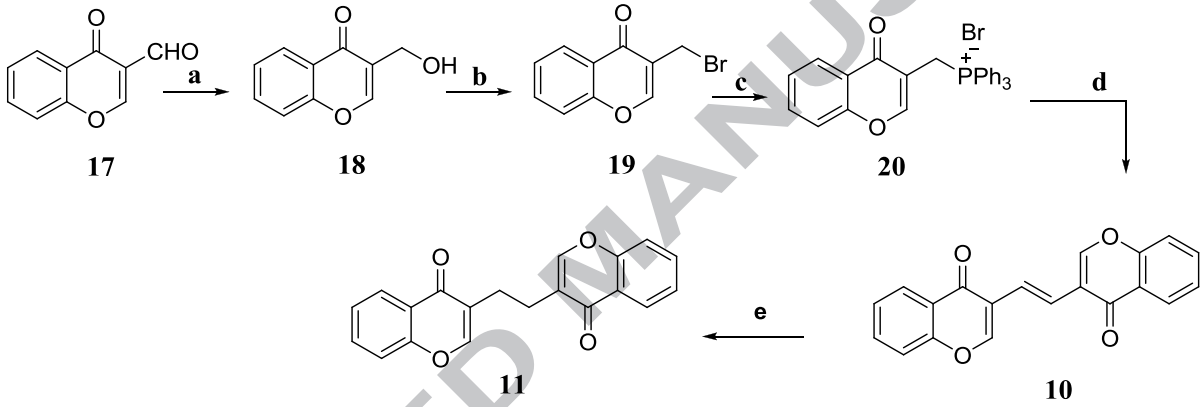
IBO = Isobutoxy , IPO = Isopentyloxy, BZO = Benzyloxy, CHM = Cyclohexyl methoxy,



Scheme 1



Scheme 2



Scheme 3

Synthesis and SAR Studies of Bis-Chromenone Derivatives for Anti-proliferative Activity against Human Cancer Cells

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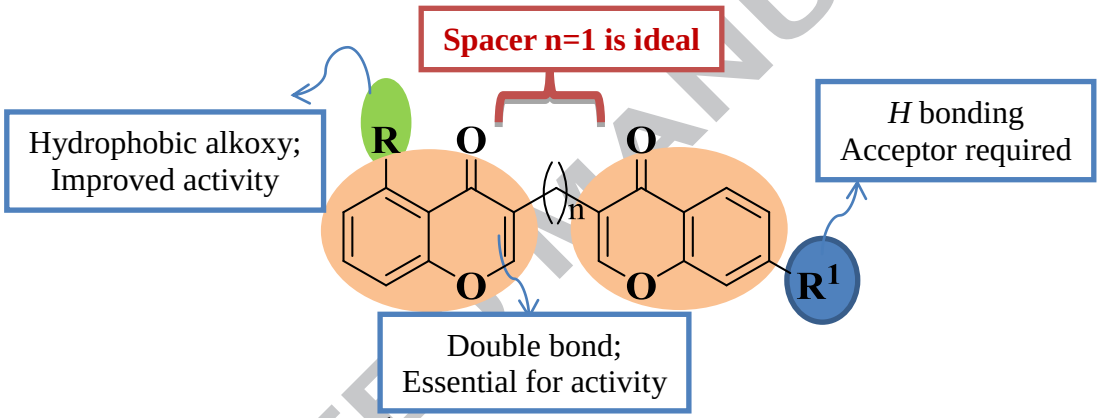
Graphical abstract



8i (R= Cyclohexylmethoxy, R^1= OMe): IC_{50} values (μM, cancer cell line) = 1.35(ACHN), 1.45 (HCT-15), 1.21 (MDA-MB-231), 1.32 (NCI-H23), 1.44 (NUGC-3), 1.12 (PC-3).