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Anticancer and Antioxidant Activities of Chroman Carboxamide Analogs

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ABSTRACT Based on our previous work, a series of chroman carboxamide analogs **(5a-t)**, which were first used as antiepileptic agents, were evaluated for their anticancer and antioxidant potencies. The majority of the compounds displayed good to potent anticancer activity on the MCF-7 breast cancer cell line. The compounds **5k** (GI_{50}=40.9 μM) and **5l** (GI_{50}=41.1 μM) showed the highest potency among the series. Antioxidant activity was determined by the 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) and hydrogen peroxide radical scavenging methods. The majority of compounds were found to be more active than standard drug Trolox. The compound **5e** (93.7% inhibition) showed the highest DPPH radical scavenging activity and compound **5d** (83.2% inhibition) showed the highest hydrogen peroxide radical scavenging activity. These results indicate that it might be a novel concept to explore high-affinity inhibitors with excellent anticancer and antioxidant potency.

KEYWORDS Anticancer, Antioxidant, Chroman carboxamide, 2,2-Diphenyl-1-picryl-hydrazyl-hydrate, Hydrogen peroxide, MCF-7.

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INTRODUCTION

Chroman derivatives constitute an important class of heterocyclic compounds with several kinds of biological activities such as antimicrobials,[1] anti-HIV,[2] antidiabetic,[3] antioxidant,[4] anti-breast cancer,[5,6] and antiepileptic agents.[5] Several comparative pharmacological investigations of the chroman derivatives have shown it to have good antioxidant and anticancer activities with low side effects and less toxicity.[7,8] Resistance to drugs is increasing at an alarming rate and has surfaced as one of the pre-eminent public health concerns. Presently, a large number of therapeutic agents are available, but resistance and toxicity of the standard drug treatments limit the capability of chemotherapy. Thus, it becomes a challenge for the researchers.

In our previous work, chroman carboxamide analogs were synthesized and tested for their antiepileptic activity.[9] In view of the findings, as mentioned above, and as a continuation of our effort to identify some new activities of these analogs, we hereby reported the antioxidant and anticancer activities of chroman carboxamide analogs.

Chroman carboxamide compounds having a substitution at 2nd and 6th positions seemed pertinent, keeping in view anti-breast cancer and antioxidant diseases.[10,11] Literature studies revealed that chroman compounds having amide linkage showed potent anti-breast cancer activity.[6] The presence of the methyl groups on benzene ring and carbonyl (>C=O) group of anhydride also found to be favorable for the activity.[6,12,13] **Figure 1** represents the design strategy of chroman carboxamide analogs **(5a-t)** as anti-breast cancer and antioxidant agents.

RESULTS AND DISCUSSION

Chemistry

The synthetic route of targeted compounds [**5a-t**, **Table 1**] is presented in **Scheme 1**, as given in our recently published work.[9] Synthetic and spectroscopic evidence confirmed all compounds **(5a–t)** according to the previously reported procedure.

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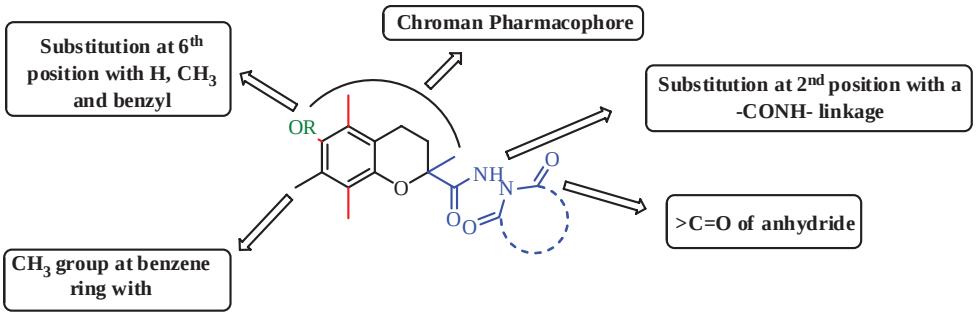
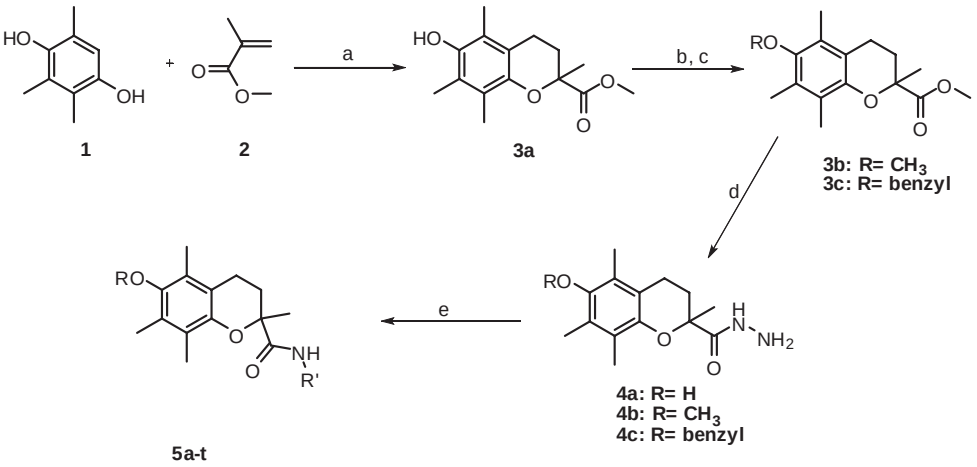


Figure 1: Design strategy of chroman analogs



Scheme 1: Synthetic pathway for compounds 5a-t. (a) (HCHO)n, $[CH_3(CH_2)_3]_2NH$, CH_3COOH, reflux, 20 h; (b) dimethyl sulfate, K_2CO_3, CH_3COCH_3, 50°C, 24 h (3b); (c) benzyl bromide, DMF, K_2CO_3, RT, 12 h (3c); (d) $NH_2NH_2.H_2O$, C_2H_5OH, reflux, 10 h; (e) Different anhydrides, CH_3COOH, reflux, 2-4 h

In vitro antioxidant activity

2,2-Diphenyl-1-picryl-hydrazyl-hydrate (DPPH) free radical scavenging assay

DPPH is a stable radical with a deep purple color whose reaction with other radicals or reducing agents leads to loss of its color. All compounds **(5a-t)** were tested for DPPH free radical scavenging assay. Free radical scavenging activity indicated as a percentage of the control shows the extent of reduction in DPPH radical absorption, achieved by each compound **[Table 2]**. A high percentage in a decrease of DPPH absorption indicates a high reactivity of compounds with DPPH, hence higher hydrogen atom donating activity, that is, a higher antioxidant activity.

Most of the compounds revealed significant DPPH radical scavenging action (<70% inhibition) at 100 µg/ml concentration. Among all compounds, pyridyl ring containing compound **5e** showed the highest percentage of inhibition (93.7%). Six compounds **5a, 5b, 5e, 5g, 5h, and 5i** were found to reduce DPPH more than the standard drug, Trolox (74% inhibition), compound **5m** (70%), also possessed a satisfactory level of inhibition.

Hydrogen peroxide scavenging assay

Hydrogen peroxide itself is not very reactive, but sometimes it can be toxic due to the elevated hydroxyl radicals in the cells. All compounds **(5a-t)** were tested for *in vitro* hydrogen peroxide radical scavenging activity. The ability of the compounds to effectively scavenge hydrogen peroxide radical was expressed as percentage inhibition with respect to control.

The scavenging ability of nine compounds **5a, 5d, 5g, 5k, 5n, 5o, 5r, 5s, and 5t** were found to be better, compared to the standard compound, Trolox [78.9%; **Table 2**]. The compound **5l** showed the lowest activity (70.4%) among the series. Among all electronegative groups (chloro, bromo, and fluorine), the fluorine group displayed better activity and showed the highest potency (83.2%). The absorbance and percentage inhibition of all the compounds **(5a-t)** are summarized in **Table 2**.

In vitro anticancer screening

The compounds **(5a-t)** were tested for *in vitro* anticancer activity against human breast cancer cells (MCF-7) using sulforhodamine B (SRB) assay. The growth inhibition (GI_{50}) concentration for every compound was estimated with reference to a control sample, which shows the concentration that causes 50% inhibition in cell growth after 48 h of incubation in the presence of the drug. The GI_{50} is calculated from sigmoidal dose-response curves and presented in **Table 3**. The activity data of Adriamycin (ADR) were included as a reference.

Among all compounds examined, six compounds (**5b, 5c, 5h, 5i, 5l,** and **5r**) showed GI_{50} in the range of 42.1–50.1 µM and four compounds (**5j, 5m, 5q,** and **5t**) showed GI_{50} in the

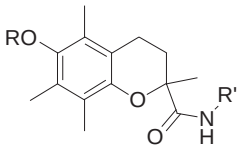
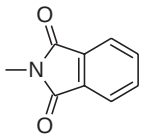
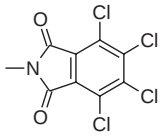
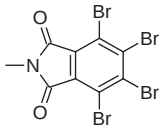
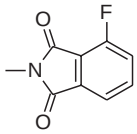
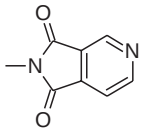
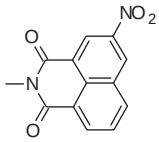
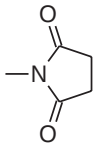
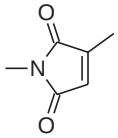
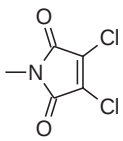
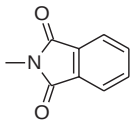
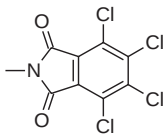
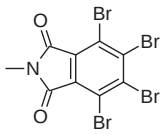
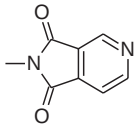
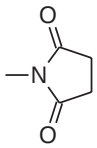
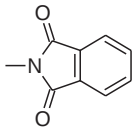
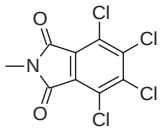
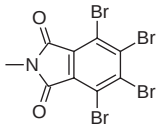
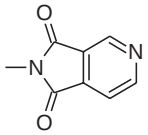
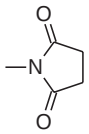
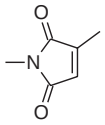


Table 1: Compounds (5a-t) differing in the substitution at R'

Compd.[a, b]	R	R'	Reaction time (h)	Mol. formula	Mol. weight	Yield (%) [c]	MP (°C)	R_f [d]
5a	**H**		2	$C_{22}H_{22}N_2O_5$	394.42	91	230	0.6
5b	**H**		2	$C_{22}H_{18}C_{l4}N_2O_5$	532.20	90	283	0.5
5c	**H**		2	$C_{22}H_{18}Br_4N_2O_5$	710.00	91	270	0.5
5d	**H**		2	$C_{22}H_{21}FN_2O_5$	412.41	68	218	0.4
5e	**H**		4	$C_{21}H_{21}N_3O_5$	395.41	70	220	0.6
5f	**H**		4	$C_{26}H_{23}N_3O_7$	489.48	72	140	0.7
5g	**H**		2	$C_{18}H_{22}N_2O_5$	346.38	85	236	0.6
5h	**H**		3	$C_{19}H_{22}N_2O_5$	358.39	86	218	0.5
5i	**H**		3	$C_{18}H_{18}C_{l2}N_2O_5$	413.25	71	170	0.4
5j	**-CH_3**		2	$C_{23}H_{24}N_2O_5$	408.45	88	210	0.6

(*Contd...*)

Table 1: (*Continued*)

Compd.[a, b]	R	R'	Reaction time (h)	Mol. formula	Mol. weight	Yield (%) [c]	MP (°C)	R_f[d]
5k	**-CH_3**		2	$C_{23}H_{20}C_{l4}N_2O_5$	546.22	81	228	0.4
5l	**-CH_3**		2	$C_{23}H_{20}Br_4N_2O_5$	724.03	80	258	0.5
5m	**-CH_3**		3	$C_{22}H_{23}N_3O_5$	409.45	78	185	0.4
5n	**-CH_3**		3	$C_{19}H_{24}N_2O_5$	360.40	75	170	0.6
5o	**-$CH_2C_6H_5$**		4	$C_{29}H_{28}N_2O_5$	484.58	69	190	0.5
5p	**-$CH_2C_6H_5$**		3	$C_{29}H_{24}C_{l4}N_2O_5$	622.32	64	190	0.5
5q	**-$CH_2C_6H_5$**		3	$C_{29}H_{24}Br_4N_2O_5$	800.13	66	220	0.5
5r	**-$CH_2C_6H_5$**		4	$C_{28}H_{27}N_3O_5$	485.53	63	168	0.4
5s	**-$CH_2C_6H_5$**		3	$C_{25}H_{28}N_2O_5$	436.50	61	120	0.6
5t	**-$CH_2C_6H_5$**		3	$C_{26}H_{28}N_2O_5$	448.51	62	140	0.7

[a]Reagents and conditions: 5a-t (1 mmol), 4a-c (1 mmol), acetic acid (10 ml). [b]Product was confirmed by the analysis FT-IR, ^{1}H NMR, ^{13}C NMR, and mass spectra. [c]Yield refers to pure compounds after column chromatography. [d]R_f=EtOAc/Hexane (40:60)

range of 55.9–71.3 μM on the MCF-7 breast cancer cells. All synthesized compounds exhibited less cytotoxic activity than standard drug, ADR (GI_{50}<0.1 μM). The most potent cytotoxic activity was exhibited by the 6-methoxy group-containing compounds **5k** and compound **5l** (GI_{50}=41.1 μM). The presence of a 6-benzyloxy group at benzene ring of chroman showed the promising activity (**5o, 5q, 5r, and 5t)**, whereas some compounds (**5d, 5e, 5f, and 5g**) having 6-hydroxyl

Table 2: *In vitro* antioxidant activity of compounds (5a-t)

Test compound	Conc. (μg/ml)	DPPH		H_2O_2	
		Absorption	% Inhibition	Absorption	% Inhibition
5a	100	0.060	80.0	0.168	80.3
5b	100	0.029	90.3	0.191	77.6
5c	100	0.139	53.3	0.211	75.2
5d	100	0.091	69.7	0.143	83.2
5e	100	0.019	93.7	0.185	78.3
5f	100	0.160	46.7	0.252	70.4
5g	100	0.033	89.0	0.164	80.8
5h	100	0.034	88.7	0.188	78.0
5i	100	0.077	74.3	0.200	76.5
5j	100	0.098	67.3	0.196	77.0
5k	100	0.105	65.0	0.173	79.7
5l	100	0.141	53.0	0.189	77.8
5m	100	0.090	70.0	0.181	78.8
5n	100	0.125	58.3	0.149	82.5
5o	100	0.114	62.0	0.172	79.8
5p	100	0.175	41.7	0.190	77.7
5q	100	0.126	58.0	0.188	77.9
5r	100	0.099	67.0	0.152	82.2
5s	100	0.112	62.7	0.166	80.5
5t	100	0.130	56.7	0.177	79.2
Trolox	100	0.016	74.0	0.180	78.9

group at benzene ring of chroman showed least activities (GI_{50}>100 μM). It was observed that compounds **(5a-t)** containing anhydride moieties were active.

EXPERIMENTAL

In vitro antioxidant assay

DPPH free radical scavenging assay

DPPH scavenging activity was measured according to the procedure described by Ghorbani *et al*., 2018.[14] 0.1 ml of synthesized compounds (100 μg/ml) and Trolox (100 μg/ml) were added to 3 ml of a 0.004% methanolic solution of DPPH. The absorbance of all compounds **(5a-t)** and Trolox was determined at 517 nm using UV spectrophotometer after 30 min of incubation. All experiments were performed in triplicate.

The percentage inhibition of activity was estimated by using the formula $[(A_0-A_1)/A_0] \times 100$, where A_1 and A_0 are the absorbance of the sample and control, respectively.

Hydrogen peroxide (H_2O_2) radical scavenging activity

The hydrogen peroxide scavenging assay was executed by following the known method.[14] A solution of hydrogen peroxide (40 mmol/L) was prepared in 0.15 M phosphate buffer (pH 7.4, 2.38 g disodium hydrogen phosphate, 0.19 g of potassium dihydrogen phosphate, and 8 g of sodium chloride were dissolved in 100 ml of distilled water). Test compounds (100 μg/ml) and Trolox (standard drug) were added to the 0.6 ml hydrogen peroxide solution. The absorbance of hydrogen peroxide at 230 nm was determined after 10 min against a blank solution containing phosphate buffer without hydrogen peroxide. The above-mentioned formula was used to calculate the percentage of hydrogen peroxide scavenging achieved by the test compounds and standard.

Table 3: GI_{50} of compounds (5a-t) on MCF-7 cell line

Compound	GI_{50} (μM)	Compound	GI_{50} (μM)
5a	>100	**5k**	40.9
5b	45.0	**5l**	41.1
5c	45.4	**5m**	61.7
5d	>100	**5n**	98.8
5e	>100	**5o**	48.5
5f	>100	**5p**	>100
5g	>100	**5q**	72.0
5h	42.7	**5r**	42.1
5i	50.1	**5s**	98.6
5j	55.9	**5t**	71.3
		ADR	<0.1

GI_{50}=Concentration of drug causing 50% inhibition of cell growth

In vitro anticancer screening by SRB assay

Advanced Centre for Treatment Research and Education in Cancer (ACTREC), Navi Mumbai, India, performed the *in vitro* anticancer activity (human breast cancer cell line MCF-7). All compounds **(5a-t)** were dissolved in dimethyl sulfoxide (DMSO) to give a stock solution of 10 μg/ml from which further

dilutions (10^{-7}, 10^{-6}, 10^{-5}, and 10^{-4} M) in culture medium were prepared. Control cultures were treated with DMSO alone.

The cytotoxic activity was measured *in vitro* on MCF-7 cell line by using SRB stain assay.[15] Cells were placed in 96 multiwell plates (104 cell/well) for 24 h before treatment with the test compounds. Different concentrations of the test compounds (10^{-7}, 10^{-6}, 10^{-5}, and 10^{-4} M) were added to the cell monolayer. Triplicate wells were prepared for each individual dose. Monolayer cells were then incubated with test compounds for 48 h at 37°C and in an atmosphere of 5% CO_2. After 48 h, cells were fixed, cleaned, and stained with SRB stain. Excess stain was cleaned with acetic acid and attached stain was recovered with Tris EDTA buffer. Color intensity was calculated in an ELISA reader. The relationship between surviving fraction and drug concentration is plotted and GI_{50} (the concentration required for 50% inhibition of cell growth) was calculated for each compound.

CONCLUSION

The objective of the present study was to evaluate the anticancer and antioxidant potency of our previously reported chroman carboxamide analogs **(5a-t)**. The best results for anticancer activity on MCF-7 cell lines were found in the 6-methoxy group-containing compounds **5k** (GI_{50}=40.9 μM) and **5l** (GI_{50}=41.1 μM). The benzyloxy group-containing compounds were found to be most active. All compounds were also tested for their antioxidant activity by DPPH and H_2O_2 method. The majority of compounds showed a free radical scavenging effect. Most of the compounds were found to be more active than standard drug Trolox. Therefore, these compounds can be used as the lead for further related pharmacological activities.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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