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New substituted 4*H*-chromenes as anticancer agents ★

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Abstract

As a continuation of our efforts to discover and develop small molecules as anticancer agents, we identified **GRI-394837** as an initial hit from similarity search on RGD and its analogs. Based on **GRI-394837**, we designed and synthesized a focused set of novel chromenes (**4a–e**) in a single step using microwave method. All five compounds showed activity in the nanomolar range (IC₅₀: 7.4–640 nM) in two melanoma, three prostate and four glioma cancer cell lines. The chromene **4e** is active against all the cell lines and particularly against the A172 human glioma cell line (IC₅₀: 7.4 nM). Interestingly, in vitro tubulin polymerization assay shows **4e** to be a weak tubulin polymerization inhibitor but it shows very strong cytotoxicity in cellular assays, therefore there must be additional unknown mechanism(s) for the anticancer activity. Additionally, the strong antiproliferative activity was verified by one of the selected chromene (**4a**) by the NCI 60 cell line screen. These results strongly suggest that the novel chromenes could be further developed as a potential therapeutic agent for a variety of aggressive cancers.

Keywords

Chromenes; RGD; Melanoma; Glioma; Prostate cancer; Tubulin polymerization inhibitors

Currently, one quarter of all deaths in the USA are caused by cancer.¹ Of the many cancer diseases, glioma, melanoma and prostate cancer are rapidly increasing in the United States. Despite intensive research, treatment options for these aggressive cancers are still very limited; therefore, there is an urgent need to find more effective chemotherapies. We have explored various pharmacophores such as 1,2,3,4-THI analogs (known as EDL compounds), 2-arylthiazolidine-4-carboxylic acid amides (ATCAA), 2-aryl-4-benzoylimidazoles (ABI),

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Supplementary data

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and 4-substituted methoxybenzoyl-arylthiazole (SMART) for glioma, melanoma and prostate cancers.²⁻⁹

Recently, Ruoslahti and co-workers have shown that co-administration of iRGD (Arginine-Glycine-Aspartic Acid) peptide with anticancer drugs increased the penetration and enhanced the efficacy of the anticancer drugs.¹⁰ In the present study we used RGD (**I**) and related analogs (Fig. 1) to perform the similarity searches against the University of Cincinnati, Drug Discovery Center (UC DDC) Library consisting of 362,000 compounds. Similarity searches were performed in Accelrys' Pipeline Pilot using 13 fingerprint types in parallel.¹¹⁻¹³ Analogs II, III, IV, and VI are fairly similar, so the search was performed on them as a group. Analogs V and VII were considered separately (Fig. 1). These similarity searches of the UC DDC Library contained very few compounds closely related to these analogs, so to augment this strategy, substructure searches for combinations of the fragments that are present across the structures above were performed (e.g., guanidine, piperidine, phenyl and acetic acid). From a total of 1549 compounds identified from these searches, a subset of 140 compounds were manually selected using Lipinski's rules and a visual perception of relatedness to the compounds of interest. Screening against melanoma cell lines in initial assay identified two compounds, 7-(4-bromocarbonyl-piperazin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,2,3,4-tetrahydro-quinoline-3-carboxylic acid (**GRI-102696**, a Ciprofloxacin analog) and 2-amino-4-(5-bromo-2-methoxy-phenyl)-7-hydroxy-4*H*-chromene-3-carbonitrile (**GRI-394837**) as initial hits (Fig. 2). **GRI-102696** being an alkylating agent, we turned our interest towards the **GRI-394837**, a chromene analog for the further development. Chromenes have been well studied as potent anticancer agents in the literature.¹⁴⁻¹⁶ This synthetically feasible scaffold served as a starting point in our program for finding potent new anticancer agents by designing and synthesizing a small set of chromenes and evaluating them against a panel of glioma, melanoma and prostate cancer cell lines.

We quickly developed a fast and efficient synthetic procedure to prepare these chromenes using microwave energy. A general synthesis of novel chromenes has been achieved by the cyclocondensation of 3-dimethylaminophenol, substituted naphthaldehydes and malonitrile (Scheme 1). We used the following general procedure for the synthesis of all five chromenes (**4a-e**): An equimolar mixture of a 3-dimethylaminophenol (**1**), substituted naphthaldehydes (**2**) and malonitrile (**3**) with 2-4 drops of triethylamine (TEA) was thoroughly mixed and irradiated at 150 °C using 300 psi pressure and 300 Watts power for 5 min in a CEM Discover Labmate Microwave Synthesizer (CEM Corporation, 3100 Smith Farm Rd, Matthews, North Carolina, USA). The reaction mixture was cooled and dissolved in dichloromethane. The mixture was subjected to flash chromatography to obtain the pure chromenes using ethyl acetate and hexanes (3:7).

We examined the antiproliferative activity of 4*H*-chromene analogues (**4a-e**) in two human metastatic melanoma cell lines (**A375** and **WM164**) and two human prostate cancer cell lines (**LnCap** and **PC3**) along with taxol resistant prostate cancer cell line PC3-TxR.^{8,17} Colchicine was used as a positive control. We used the standard sulforhodamine B (SRB) assay, which has been shown to provide very reliable results based on the literature¹⁸ and our own work.^{19,20} Briefly, cells seeded in round-bottom 96-well plates were exposed to a wide range of concentrations of testing compounds for 48 h before they were fixed with 10% trichloroacetic acid. After they were washed five times with water and air-dried overnight, cells were stained with SRB solution, and total proteins were measured at 560 nM with a BioTek ELx800 plate reader (BioTek Instruments, Winooski, VT). IC₅₀ (i.e., concentration that inhibited cell growth by 50% of DMSO-treated controls) values were obtained by nonlinear regression analysis with GraphPad Prism (GraphPad Software, San Diego, CA). Results are shown in Table 1.

All five compounds showed IC_{50} values in the nanomolar range (80–640 nM) for both melanoma and prostate cancer cell lines. This clearly shows that these chromenes are potent cytotoxic agents for both melanoma and prostate cancers (Table 1A). Compounds **4a** and **4b** are active against the taxol resistant prostate cancer cell line (**4a**: 0.19 μ M; **4b**: 0.21 μ M) and its parental cell line (**4a**: 0.42 μ M; **4b**: 0.39 μ M), indicating that these chromenes may have therapeutic advantages especially in prostate cancer. Compounds **4a** and **4b** have also shown good inhibition in the case of A375 melanoma cell line (**4a**: 0.13 μ M; **4b**: 0.14 μ M). Analogs **4c** and **4d** have shown moderate activity among all cell lines but improved activity toward the taxol resistant cell line compared to its parent cell line. The chromene **4e** is active against all the cell lines, particularly against the A375 melanoma cell line (0.08 μ M).

Stimulated by these results, we further selected two compounds (**4a** and **4e**) to assess their effectiveness in four human glioma cell lines (T98, U87, LN18, A172). The glioma cells and normal astrocytes were exposed to escalating concentrations of each chromene compound for five days and then subjected to the MTT assay. Cell viability data at each concentration were used to calculate IC_{50} values (Table 2). The chromene **4e** is active against all four human glioma cell lines and particularly against the A172 glioma cell line (IC_{50} = 7.4 nM). This clearly indicates that **4e** could be further developed therapeutically for brain cancer.

Since chromenes have been identified as tubulin destabilizers,²¹ we hypothesized that these new chromenes also interfere with tubulin polymerization. To test this hypothesis, we performed the in vitro tubulin polymerization assay with our most potent chromene **4e**. Briefly, bovine brain tubulin (>97% pure) was incubated with **4e** at concentrations of 10 and 20 μ M, respectively. Compound **4e** inhibits tubulin polymerization in a dose-dependent manner (Fig. 3). After 20 min of treatment, inhibition of tubulin polymerization was observed to extents of 29%, and 68% for **4e** at 10 μ M, and 20 μ M, respectively. Taxol is known to be a tubulin stabilizer, consistent with this, the growth of tubulin is enhanced by taxol as compared with vehicle control (DMSO). The tubulin polymerization assay clearly indicates that **4e** is a tubulin polymerization inhibitor.

Chromenes are known to inhibit tubulin polymerization by binding to the colchicine binding site in tubulin.²¹ Based on the tubulin polymerization study, we examined the potential binding modes of all five chromenes (**4a–e**) and their interactions with the receptor at the colchicine site in the tubulin dimer (PDB code: 1SA0) using Schrodinger Molecular Modeling Suite 2011 (Schrodinger LLC, New York, NY). Chromene derivatives (**4a–e**) were built and prepared using the LigPrep module and docked into colchicine site using the Glide module in Maestro. All the compounds showed fairly good glide scores varying from –9.6 for **4e** to –8.3 for **4a** (Table 1B). This result suggests potential strong interaction between these chromene analogs with the colchicine binding pocket, which is located at the interface of $\alpha\beta$ -dimers (red ribbon in Fig. 4). Their planar ring scaffold could overlap well with the fused ring system of colchicine in our docking model. Interestingly, substituents on the naphthalene ring could affect the calculated optimal bonding modes of these compounds. For **4e** (green tube in Figure 4), the one with the best glide score, its chromene head was buried into the pocket and the naphthalene ring with a relatively large group (*N,N*-dimethyl amino) was pushed outward towards the α -subunit (grey ribbon in Fig. 4). In contrast, compound **4b** (grey tube in Fig. 4), with a smaller group (bromine), showed an upside-down bonding mode where the naphthalene had been inserted to the pocket bottom and reached a similar position as the trimethoxy group of colchicine (blue ball & stick in Fig. 4).

Finally, the effectiveness of the scaffold is further confirmed in the NCI-60 single concentration (10 μ M) screening for selected chromene **4a**. Compound **4a** showed potent growth inhibition in 58 cancer cell lines, with best potency shown against melanoma, colon, ovarian, prostate and CNS cancers.²² In addition, the data are consistent with the mechanism

of action for compound **4a**, because compounds targeting tubulin polymerization are cytostatic instead of cytotoxic agents.

In conclusion, starting from virtual screening practice using RGD analogs, we identified a chromene analog as a hit compound. Synthesis and biological testing of a focused set of novel chromene analogs indicated that they are highly potent against variety types of aggressive cancers and have the potential to overcome drug resistance. Preliminary mechanism of action studies suggest that these novel chromenes interact with the colchicine binding site in tubulin. However, there seems to be a discrepancy between their relatively weak tubulin polymerization inhibition and their very strong cytotoxicity in cellular assays.²³ Therefore, there must be additional unknown mechanism(s) for their high anticancer activities. These results strongly support the further studies to elucidate their mechanism of actions and the development of chromenes as a potential therapeutic agent for varieties of aggressive cancers.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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22. See Supplementary data part D. NIC 60 Cell line screening data (Fig 5).
23. See Supplementary data part E. UV absorbance profile data (Fig 6).

Figure 1.
RGD (I) and related analogs (II-VII).

Figure 2.
Virtual screening for RGD and related analogs within UC DDC library.

Figure 3.

Effect of **4e** on tubulin polymerization in vitro. Tubulin (0.4 mg/assay) was exposed to 10, 20 μ M of **4e** and 5 μ M of taxol (vehicle control, 5% DMSO). Absorbance at 340 nM was monitored at 37 $^{\circ}$ C every minute for 20 min.

Figure 4.
Binding modes of **4b** (–) and **4e** (–) in the colchicine (–) binding site of tubulin.

Scheme 1.

Reagents and conditions: (a) Microwave irradiation, TEA (2–4 drops), 150 °C.

Table 1

Antiproliferative activity and glide docking scores of chromenes

Compound	A. Antiproliferative activity					B. Modeling Glide score
	IC ₅₀ ± SEM (μM)					
	LnCap	PC3-TxR	PC3	WM164	A375	
4a	0.25 ± 0.15	0.19 ± 0.04	0.42 ± 0.13	0.22± 0.07	0.13 ± 0.03	-8.3
4b	0.28 ± 0.07	0.21 ± 0.04	0.39 ± 0.08	0.22 ± 0.06	0.14 ± 0.02	-8.8
4c	0.76 ± 0.23	0.53 ± 0.11	0.43 ± 0.13	0.49 ± 0.23	0.44 ± 0.09	-8.9
4d	0.41 ± 0.12	0.29 ± 0.08	0.64 ± 0.23	0.47 ± 0.15	0.28 ± 0.05	-8.7
4e	0.14 ± 0.07	0.23 ± 0.03	0.22 ± 0.04	0.16 ± 0.03	0.08 ± 0.01	-9.6
Colchicine	0.02 ± 0.01	0.02 ± 0.01	0.01 ± 0.01	0.03 ± 0.02	0.02 ± 0.01	-13.4

Table 2

Cytotoxicity comparison of four glioma cell lines and normal astrocytes

Compound	IC ₅₀ ± SEM (nM)				
	T98G	U87	LN18	A172	Normal astrocytes
4a	NA	62.9 ± 2.0	80.4 ± 3.8	20.7 ± 1.7	9445 ± 1029
4e	12.5 ± 0.66	20.6 ± 0.46	13.3 ± 0.46	7.4 ± 0.05	2033 ± 146
Temozolamide	5.7e + 05 ± 2.91e + 04	4.9e + 04 ± 2.98e803	3.9e + 05 ± 4.46e + 04	6.5e + 04 ± 6.91e + 03	>1.0e + 06