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Short communication

Synthesis and evaluation of second generation Flex-Het scaffolds against the human ovarian cancer A2780 cell line

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ABSTRACT

Flexible Heteroarotinoids (Flex-Hets) are a class of substituted di-aryl compounds that exhibit potent anti-cancer activity without toxicity. They were derived from the more conformationally restricted, 2-atom linker Hets by substitution of the 2-atom linker with a 3-atom urea or thiourea linker, which conferred more potent inhibitory activity against cancer cell lines. The objectives of this structure activity relationship (SAR) study were to determine if a 4-atom acrylamide linker and various substitutions on the terminal aryl ring altered the anti-cancer activity of these second generation Flex-Het compounds compared to the parent Flex-Het compound, SHetA2, which has a thiourea linker and a nitro substituent. Biological activity was measured using a cytotoxicity assay of the human A2780 ovarian cancer cell line treated with a range of compound concentrations. Nitrogen-based substitutions on the terminal aryl group caused similar, but slightly reduced efficacies and potencies. Exceptions were systems that had a nitro group at the *para* position, the potencies of which were better than that of SHetA2 with efficacies that were only slightly reduced compared to SHetA2. Similarly, the potency of the system with a *para* dimethylamino group was greater than that of SHetA2. However, a 30% reduction in efficacy compared to SHetA2 was noted. While specific members with the 4-atom acrylamide linker did exhibit excellent potency, the efficacy was slightly below that of SHetA2. Thus, a gradient of activities was observed as the substituent on the aryl ring was altered.

1. Introduction

Ovarian cancer is the most lethal gynecological malignancy and represents 3% of all female cancers with 239,000 new cases detected per year and 152,000 deaths per year worldwide [1]. In the United States alone, the detection rate for ovarian cancer is around 21,980 per year, with 14,270 deaths reported annually [2]. The disease is also termed as the 'silent killer', as more than 80% of patients exhibit no symptoms if it is limited within the ovaries [3]. Over the last two decades, those 20% of patients diagnosed with early stage ovarian cancer had a survival rate of over 90% [3]. Although more effective surgery and treatment with optimized combinations of cytotoxic drugs have improved the survival rate over the past 5 years, the overall cure rate remains at only 30% [4]. Due to the distinctive biology of ovarian cells at the clinical, cellular and molecular level, the choice of treatment and effective drug options are still elusive. Thus, there is a great need to develop new drugs for the inhibition of ovarian cancer cells.

Retinoids and their synthetic analogues are known to modulate cell proliferation/homoeostasis and induce differentiation and apoptosis at the cellular level [5]. Modern medicinal chemistry shifted its focus to these retinoids as anticancer agents during late 1970s and early 1980s [6]. Despite the potential significance as anticancer agents, the scope of the retinoid drug scaffolds was limited due to high toxicity towards normal cells. To overcome the challenge of toxicity, various researchers focused on developing synthetic retinoid analogues with reduced toxicity and useful inhibitory activity. Over the years, we have developed a new class of synthetic heteroarotinoids, known as Flexible Heteroarotinoids (Flex-Hets), which function independently of retinoic acid receptors [7–11]. These Flex-Hets have exhibited promising inhibitory activity against various cancer cell lines and were not cytotoxic to normal cells [10]. Of all the Flex-Hets, SHetA2 (NSC721689), a sulfur heteroarotinoid anticancer compound, exhibited the most

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promising activity against various types of cancers [12]. SHetA2 exerted greater inhibitory activity on cancer cells, possessed excellent differentiation between benign and malignant cells, exhibited no mutagenicity, carcinogenicity, teratogenicity or toxicity and displayed a wide therapeutic window [7,9,10,13–18]. The differential apoptosis in normal *vs* cancerous cells was evident at the molecular level from the effect of SHetA2 on the heat shock protein HSPA9 (mortalin) and the Bcl-2 proteins [8,14]. Animal studies revealed that SHetA2 inhibited the growth of ovarian cancer cells without evidence of toxicity and did not induce skin irritation or teratogenicity [7,15,16]. SHetA2 was stable in plasma at 4 °C; however, concentrations decreased monoexponentially with increases in temperature similarly in mouse, rat, dog and human plasma [19]. The half live of SHetA2 in mouse plasma was 42.4 h at 22 °C and 12.7 h at 37 °C, and the major metabolites were hydroxylated structures and glutathione adducts [19,20]. The objective of the current work was to develop and evaluate second generation Flex-Hets containing the sulfur heteroarotinoid backbone tethered with a 4-atom acrylamide linker to enhance the activity of SHetA2 on ovarian cancer cells.

1.1. Rationale in drug design

The rationale for the second generation Flex-Hets is based on our knowledge of the relationships between Flex-Het retinoid structures and their anticancer activity (Fig. 1). Most synthetic retinoids are derived from the parent *all-trans* retinoic acid (1), which possesses anticancer activity, but also exerts high cytotoxicity against the normal cells [21,22]. To overcome this problem, various modifications were incorporated into the retinoid structure to reduce toxicity while maintaining its anticancer activity. Over the last decade, we introduced various alterations into the retinoid structure, including a cyclic heteroatom unit (**2, 3, 4, 5**) within the retinoid framework [23]. Individual structural variations changed the retinoic acid receptor activation profiles of the compounds [24]. The introduction of the heteroatom drastically reduced the *in vivo* toxicity when compared to the corresponding arotinoid structures lacking the heteroatom [25]. Similarly, various research groups proposed modifications to the linker units within the ring system (**6, 7, 8, 9**) to enhance the activity and reduce the toxicity [26–30]. It was noted that altering the linker units resulted in a marked increase in the inhibitory activity from **7** to **9**. These compounds revealed the relation between the linker within the retinoid structure and its anticancer activity. Multiple research groups demonstrated the anticancer activity of various retinoid structures, and yet, these compounds also exhibited high toxicity and carcinogenicity within normal cells. Recent progress by the Gurkan group demonstrated that structure **9**, which contains an acrylamide linker, enhanced the biological activity of the compounds within a related synthetic retinoid family [31]. Based on molecular modeling, we had previously predicted that allowing flexibility within the ring system of the molecule could specifically increase the H-bonding capabilities between the ligands and the binding pocket of retinoic acid receptors [10]. We found that structures with 3-atom linkers exhibited improved anti-cancer activity compared to 2-atom linkers presumably due, in part, to the increased flexibility of the molecule. Interestingly, two retinoid derivatives containing 4-atom linkers, like compound **9**, have been reported [31,32].

Building on these observations, we hypothesized that selected compounds with 4-atom linkers would possess increased flexibility and increased conjugation with the aromatic ring (Ring B), which might improve the efficacy of SHetA2. We subsequently designed and synthesized a new series of 11 compounds as second generation Flex-Hets incorporating a cinnamamide linked to a sulfur-containing heterocycle (Ring A). This scaffold was projected to enhance the biological activity of the related SHetA2 system and would, hopefully, reduce the toxicity of these synthetic, modified retinoids. We now report the synthesis and the biological activity of the second-generation Flex-Hets containing a 4-atom linker unit against the ovarian cancer cell line A2780. These new heteroarotinoids were synthesized and evaluated for activity against this cancer cell line and were appraised relative to SHetA2.

2. Results and discussion

2.1. Chemistry

The syntheses of second-generation Flex-Het analogues were performed in 5–7 steps. Initially, acetamidothiophenol (**11**) was treated with mesityl oxide (**12**) in the presence of triethylamine (Scheme 1). A 2-fold excess of mesityl oxide and base was required to perform this Michael reaction to afford the ketone product **13** in 70% yield [33]. Notably, this Michael addition gave higher yields only when the reactants were added portion-wise. A standard, one-time addition gave the products in lower yields along with recovered excess starting materials. Subsequent addition of methyllithium to **13** at low temperature led to the formation of tertiary alcohol **14**. Even in this reaction, the sequence of reactant addition played a critical role in the outcome. When the substrate was dissolved in tetrahydrofuran, followed by dropwise addition of methyllithium, a low conversion was observed, and product purification was difficult. With inverse addition, however, the reaction afforded a cleaner conversion of **13** to **14** and simplified the purification process. Dehydration-cyclization of **14** by the action of $AlCl_3$ in chlorobenzene generated the desired thiochroman derivative **15** in 89% yield. Refluxing **15** with 6 M HCl in methanol furnished the desired aminothiochroman in 95% yield as its hydrochloride salt **16**.

Aminothiochroman **16** was joined with cinnamic acid derivatives **17a**–**f** using standard coupling promoted by 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC·HCl), hydroxybenzotriazole (HOBt), and diisopropylethylamine (DIPEA) to generate the desired cinnamamides **18a**–**f** (Scheme 2). The 4-carboxycinnamamide derivative **18g** was prepared by saponification of **18a** using lithium hydroxide in aqueous tetrahydrofuran. The 4-aminocinnamamide congener **18h** was realized by reduction of the corresponding 4-nitro derivative **18b** using iron/ammonium chloride in ethanol. Furthermore, the amine **18g** was converted to amides **20a**–**c**, as illustrated, using the standard coupling protocol. The purity of the targets was evaluated with ^{1}H and ^{13}C NMR as well as elemental analysis. In order to anchor our spectral assignments, an X-ray structure determination was conducted for **18a**. With the exception of the fused tetrahydrothiopyran moiety, the perspective view of **18a** (Fig. 2) shows a nearly planar structure with good conjugation of the two aromatic rings with the acrylamide linker.

2.2. Biology

These second generation Flex-Hets were screened to identify structural features important for cancer cell inhibition activity. SHetA2 was used as a standard for comparison. The biological effects of the compounds were assessed using a cytotoxicity assay of the human A2780 ovarian cancer cell line (Table 1). The three most active compounds **18b**, **18f** and **20c** contained a nitro, a tertiary amino or an oxomorpholino group, respectively, as the aryl substituent (Fig. 3A). Compound **18b** with a nitro substituent, as in SHetA2, exhibited activity comparable to SHetA2. Replacement of the nitro substituent with a dimethylamino group (**18f**) reduced efficacy but not the potency. The oxomorpholino substituent (**20c**)

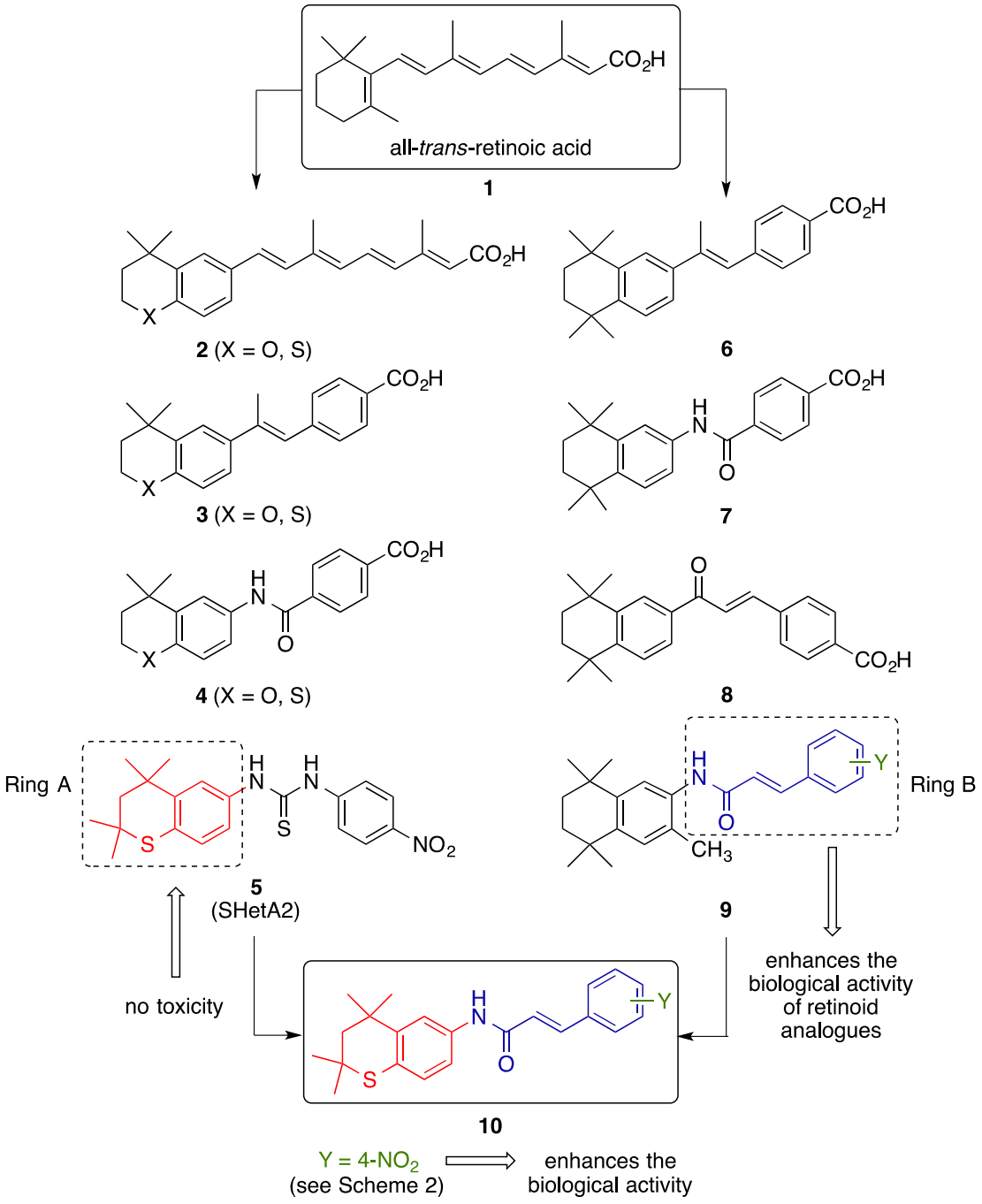
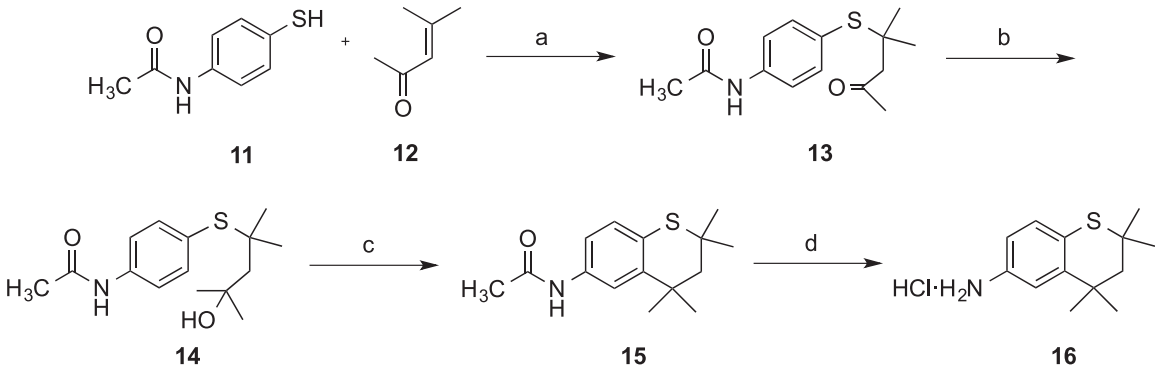


Fig. 1. Rational structure design of second generation Flex-Hets.

slightly reduced the potency and efficacy. The efficacy and potency were reduced to a greater extent when the substituent was an amino group (**18h**), a methylamide group (**20a**), or the related cyclopropylamide group (**20b**).

Compounds lacking a nitrogen atom at the *para* position exhibited potencies similar to SHetA2, but with 60–70% reduced efficacy (Fig. 3B). The ethyl ester derivative **18a** had only 43% efficacy compared to SHetA2, although the former was slightly more potent. Previous comparison of SHetA2 with the ethyl ester derivative SHetA3 (CO_2Et in place of NO_2) did not demonstrate improved potency in the context of thiourea linker containing compounds [34]. Interestingly, the related carboxylic acid derivative **18g** was completely devoid of activity. The CF_3 derivative **18c** was about 30% as effective as SHetA2, but had a *ca.* 3-fold higher potency. Compound **18d**, which possessed a pentafluorocinnamamide, was also about 30% as effective as SHetA2, and it had an almost 2-fold reduced potency. The dimethoxy compound **18e** exhibited about 37% of SHetA2 efficacy and a *ca.* 5-fold attenuated potency. The study demonstrated that various substitutions at the *para* position on the aryl moiety had highly variable effects on the anticancer activity of Flex-Hets. Compound **18b**, which was identical to SHetA2 except for the linker group, had similar but slightly reduced potency and efficacy, indicating the importance of the nitro group. The oxomorpholino derivative **20c** was less potent than SHetA2, but the efficacy was only 10% lower than that of SHetA2. Other substitutions containing nitrogen atoms exhibited similar but



[a]Reaction conditions: (a) TEA, $CHCl_3$, 62 °C; (b) CH_3Li, Et_2O, –50 °C; (c) $AlCl_3$, chlorobenzene, 80 °C; (d) 6 M HCl

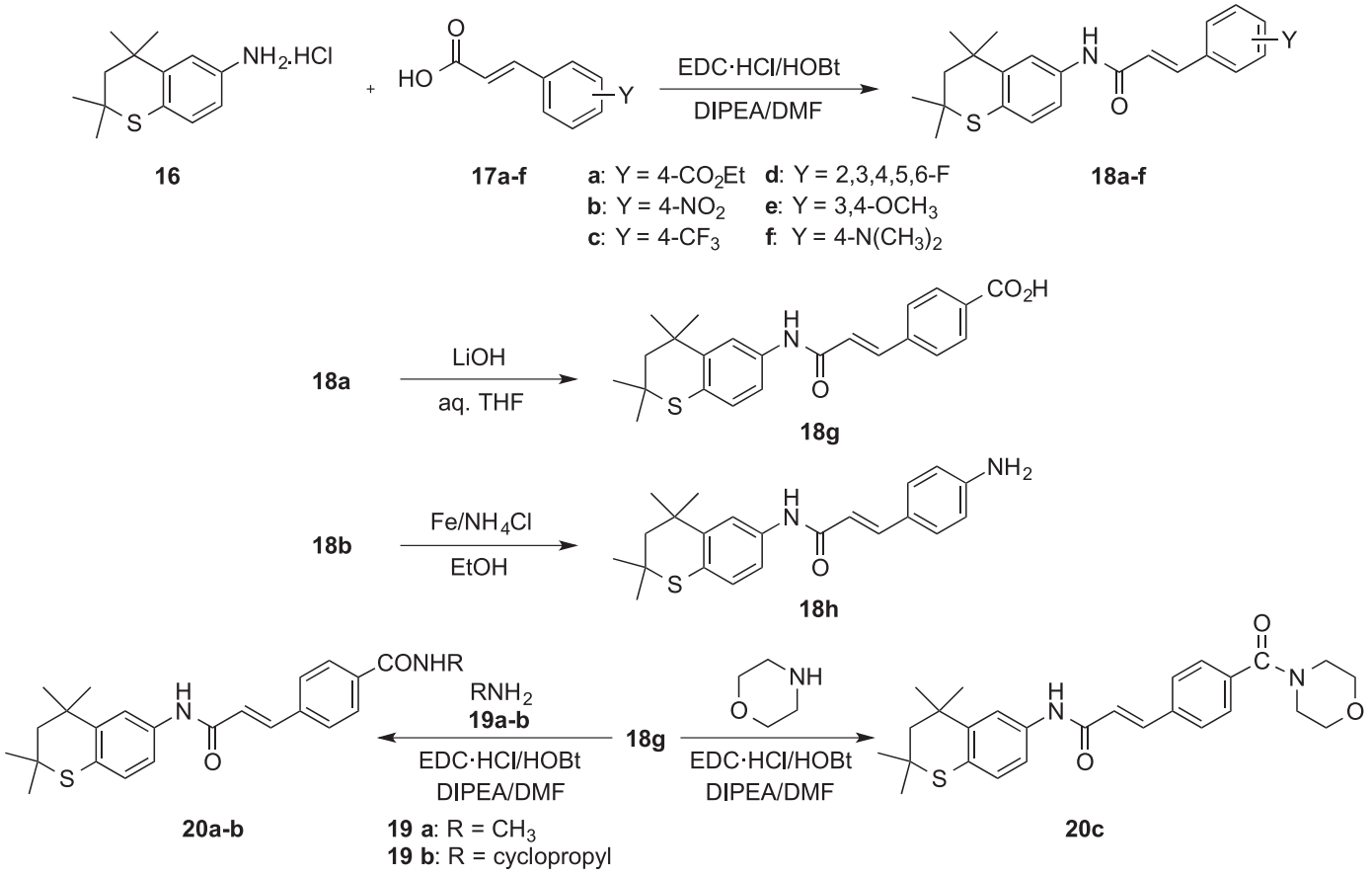
Scheme 1. [a]Synthesis of advanced intermediate **16**.

weaker activity, while substitutions lacking nitrogen atoms were the weakest, and the carboxyl group abolished activity. Recent studies revealed that SHetA2 binds to and disrupts protein interaction with mortalin [8,17].

An overall appraisal of the four best derivatives **18b**, **18f**, **20b**, and **20c** revealed that both a *para* electron donating group (**18f**-NH_2) and three *para* electron withdrawing groups (**18b**-NO_2; **20b**–(CO)–cyclopropylamino; **20c**–C(O)–morpholino) exhibited reasonable efficacy within the experimental limits, although potency varied within a less than 2-fold range. Compounds with the two larger *para* groups (**20b** and **20c**) had reduced potency compared to **18b** and **18f** and may reflect steric hindrance. All four of these compounds have polar components, and, thus, there is not a simple rationale for the observed activity. The data are summarized (Fig. 3) regarding the effects of each compound on the growth of the human A2780 ovarian cancer cell line.

3. Conclusions

In this study, a new series of second generation Flex-Hets containing acrylamide linkers was designed, synthesized and evaluated for biological activity against the human A2780 ovarian cancer



Scheme 2. Synthesis of new Flex-Hets **18a**–**h** and **20a**–**c**.

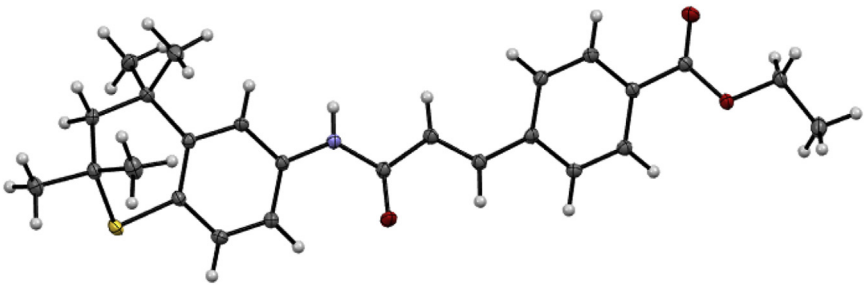


Fig. 2. X-ray structure of **18a**.

cell line. Changing from a 3-atom linker [NC(S)N] to a 4-atom linker [NC(O)C=C] in the target molecule resulted in a range of activities. Compound **18b** displayed activity comparable to that of the lead compound SHetA2. Additionally, it was revealed that the nitro group at the *para* position played an important role in maximizing biological activity. Interestingly, the derivative substituted with a carboxylic acid at the *para* position exhibited no activity. With the exception of the carboxylic acid, electron-withdrawing groups on the cinnamamide ring generally elicited greater activity compared to groups with electron donating ability.

4. Experimental section

4.1. Chemistry

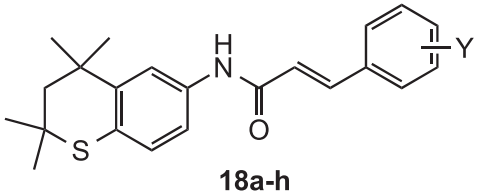
4.1.1. General methods

Commercial anhydrous *N,N*-dimethylformamide was stored under dry N_2 and transferred by syringe into reactions when needed. Tetrahydrofuran was dried over potassium hydroxide pellets and distilled from lithium aluminum hydride prior to use. All other commercial reagents and solvents were used as received. Unless otherwise indicated, all reactions were carried out under dry N_2 in oven-dried glassware. Reactions were monitored by thin layer chromatography (TLC, Analtech No 21521) using silica gel GF plates. Preparative separations were performed by column chromatography on silica gel (Davisil®, grade 62, 60–200 mesh) containing UV-active phosphor (Sorbent Technologies No UV-05) slurry packed into quartz columns. Band elution for all chromatographic separations was monitored using a hand-held UV lamp. Melting points were uncorrected. IR spectra were run as $CHCl_3$ solutions on NaCl disks. The ^{1}H- and ^{13}C-NMR spectra were measured in the indicated solvent at 400 MHz and 100 MHz, respectively, using tetramethylsilane as the internal standard with coupling constants (*J*) given in Hz.

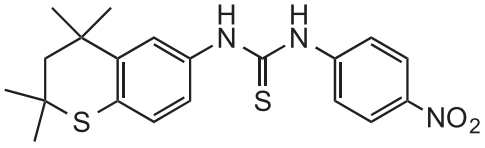
4.1.2. N-{4-[(2-Methyl-4-oxopentan-2-yl)thio]phenyl}acetamide (**13**)

To a stirred solution of acetamidothiophenol (**11**) (25.0 g, 149.7 mmol) in dry chloroform (200 mL) was added triethylamine (21.0 mL, 149.7 mmol), followed by addition of mesityl oxide (**12**) (17.0 mL, 149.7 mmol). The resulting slurry was heated to reflux (bath temperature 70 °C). Two additional portions of triethylamine (10.5 mL, 74.5 mmol) and mesityl oxide (8.6 mL, 74.5 mmol) were added at regular intervals of 4 h, and the resulting solution was refluxed for 16 h after the final addition. The resulting reaction mixture was cooled, filtered through Celite® and washed with chloroform (2 × 50 mL). The combined organic layers were washed with water (2 × 100 mL), saturated aqueous NaCl, dried ($MgSO_4$), and concentrated under vacuum to give a yellow oil. The crude reaction mixture was then purified by silica gel column

Table 1
Potency and efficacy of compounds at inhibiting ovarian cancer cell line growth.



18a-h
20a-c



5 (SHetA2)

Compound	Y	Potency IC_{50} (μM)	Efficacy (% SHetA2)	Maximal Activity (% growth inhibition)
Nitrogen-Substituted Flex-Hets				
5 (SHetA2)	4-NO_2	2.98 ± 0.10	100	93.17 ± 2.37
18b	4-NO_2	2.17 ± 1.69	97.29 ± 10.75	85.07 ± 4.10
18f	4-$N(CH_3)_2$	2.42 ± 0.14	71.33 ± 7.34	68.02 ± 10.27
18h	4-NH_2	5.18 ± 0.23	49.65 ± 0.34	52.02 ± 9.63
20a	4-N-methylamide	5.04 ± 0.46	52.22 ± 5.00	47.67 ± 5.48
20b	4-*N*-cyclopropylamide	5.52 ± 0.58	67.35 ± 5.50	45.11 ± 10.05
20c	4-oxomorpholino	4.64 ± 0.24	89.32 ± 0.35	77.47 ± 1.11
Non-Nitrogen-Substituted Flex-Hets				
18a	4-CO_2Et	1.49 ± 0.13	43.19 ± 5.01	40.37 ± 4.88
18c	4-CF_3	1.09 ± 0.22	30.73 ± 8.65	28.61 ± 7.66
18d	2,3,4,5,6-F	5.42 ± 0.36	31.16 ± 5.26	29.17 ± 5.33
18e	3,4-OCH_3	7.81 ± 0.23	37.07 ± 12.18	34.73 ± 11.44
18g	4-CO_2H	>1000	0	0

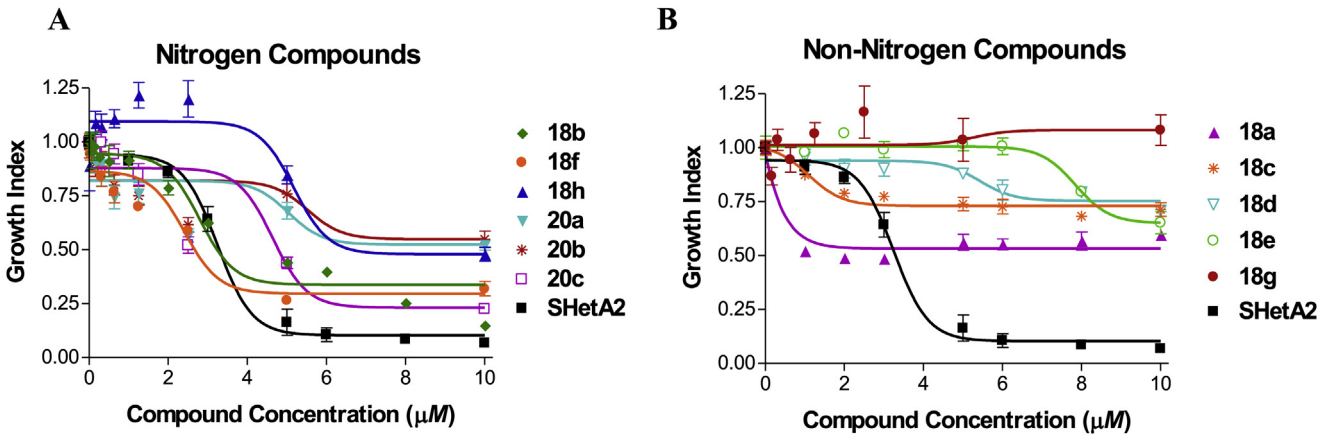


Fig. 3. Effects of new compounds relative to SHetA2 on the growth of the human A2780 ovarian cancer cell line. Cells were incubated with the compounds in triplicate for 72 h, and growth was assessed using the MTS assay. Graphs shown are the results of two independent experiments for compounds with nitrogen atom substituents (A) and other substituents exhibiting weaker activity (B).

chromatography eluted with dichloromethane:ethyl acetate (1:1) to afford **13** as a pale yellow solid, mp 49–51 °C (lit [33]. mp 46–49 °C); IR: 3310, 1699, 1676 cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.90 (br s, 1H), 7.53 (d, 1H, $J = 8.8$ Hz, 2H), 7.45 (d, $J = 8.8$ Hz, 2H), 2.65 (s, 2H), 2.19 (s, 3H), 2.15 (s, 3H), 1.36 (s, 6H); ^{13}C NMR ($CDCl_3$): δ 206.9, 168.6, 139.0, 138.3, 126.2, 119.6, 54.3, 47.0, 32.1, 28.0, 24.5.

4.1.3. *N-{4-[(4-Hydroxy-2,4-dimethylpentan-2-yl)thio]phenyl} acetamide (**14**)*

To a stirred solution of methyllithium in ether (198 mL, 316.5 mmol, 1.6 M) in tetrahydrofuran (300 mL) at −50 °C was added dropwise **13** (28 g, 105.5 mmol) in tetrahydrofuran (200 mL) over 30–45 min. The reaction mixture formed a white precipitate, which was slowly warmed to room temperature over a period of 3 h. Finally, the mixture was stirred at room temperature for 1 h. The reaction mass was then cooled to 0 °C, and the mixture was quenched by dropwise addition to ice water (150 mL). After adjusting the pH of the solution to pH 6-7 by addition of 6 M aqueous HCl, the solution was extracted with ethyl acetate (2 × 250 mL). The combined organic extracts were washed with saturated aqueous NaCl (1 × 150 mL), dried ($MgSO_4$), and concentrated under vacuum to afford a dark brown liquid. To the crude mixture was added chloroform (60 mL) with cooling to 0 °C for 1 h which afforded a yellow solid. The solid was then filtered and dried under vacuum to afford **14** (18g, 61%) as a pale yellow solid, mp 141–142 °C (lit [33]. mp 138–144 °C); IR: 3400, 3303, 1676 cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.68 (br, s, 1H), 7.52 (m, 4H), 3.50 (br s, 1H), 2.19 (s, 3H), 1.77 (s, 2H), 1.34 (s, 6H), 1.33 (s, 6H); ^{13}C NMR ($CDCl_3$): δ 168.4, 138.8, 138.1, 126.3, 119.6, 72.0, 52.0, 49.2, 32.2, 30.8, 24.6.

4.1.4. *N-(2,2,4,4-Tetramethylthiochroman-6-yl)acetamide (**15**)*

To a stirred solution of **14** (18 g, 63.9 mmol) in chlorobenzene (125 mL) at room temperature was added portion-wise anhydrous aluminum chloride (10.22 g, 76.7 mmol) over a period of 45 min, and reaction was refluxed for 90 min. The reaction mixture was cooled to room temperature and quenched with ice cold water (150 mL) to give a thick suspension. The solid was removed by filtration through Celite® and washed with ethyl acetate (2 × 100 mL). The layers were separated, and the aqueous layer was extracted with additional ethyl acetate (2 × 100 mL). The combined organic extracts were washed with saturated aqueous NaCl, dried ($MgSO_4$), and concentrated under vacuum to give a crude yellow oil. The crude product was purified on a silica gel column using hexanes:ethyl acetate (1:1) to afford the product **15** (15.0 g, 89%) as a pale yellow solid, mp 105–107 °C (lit [33]. mp 104–107 °C); IR: 3295, 1662 cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.60 (br s, 1H), 7.27 (d, 1H, $J = 2.3$ Hz), 7.20 (dd, $J = 8.2$, 2.3 Hz, 1H), 7.04 (d, $J = 8.2$ Hz, 1H), 2.16 (s, 3H), 1.92 (s, 2H), 1.39 (s, 6H), 1.35 (s, 6H); ^{13}C NMR ($CDCl_3$): δ 168.3, 143.4, 135.1, 128.4, 128.2, 118.7, 118.2, 54.4, 42.0, 35.7, 32.4, 31.4, 24.4.

4.1.5. *2,2,4,4-Tetramethylthiochroman-6-amine hydrochloride (**16**)*

To a stirred solution of **15** (15.0 g, 56.9 mmol) in methanol (75 mL) was added 6 M aqueous HCl (75 mL). The reaction mixture was heated to 90 °C for 1 h, followed by cooling to room temperature. The reaction was concentrated to 1/4 of its initial volume. The resulting crude mixture was cooled to 0 °C and maintained at this temperature for 1 h to yield a solid. The solid was filtered and dried under vacuum to afford **16** as a white solid (14.0 g, 95%), mp 208–209 °C; IR: 2922, 2853 cm^{-1}; ^1H NMR (DMSO-d_6): δ 10.08 (s, 2H), 7.48 (s, 1H), 7.22 (d, 1H, $J = 8.3$ Hz), 7.09 (d, 1H, $J = 8.3$ Hz), 1.94 (s, 2H), 1.42 (s, 6H), 1.40 (s, 6H); ^{13}C NMR (DMSO-d_6): δ 144.2, 132.0, 130.0, 129.1, 122.0, 121.2, 53.5, 42.6, 35.8, 32.6, 31.6.

4.2. *General procedure for synthesizing cinnamamides (**18a–f**)*

To a stirred solution of each cinnamic acid **17a–f** (0.5 mmol) in *N,N*-dimethylformamide (5 mL) was added diisopropylethylamine (1.5 mmol), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (0.55 mmol), and 1-hydroxylbenzotriazole (0.55 mmol). After stirring for 1 h at room temperature, 2,2,4,4-tetramethyl-6-aminothiochroman hydrochloride (**16**, 0.55 mmol) was added. The resulting mixture was stirred at room temperature for 12 h. The reaction mixture was poured into ice (approx. 100 g) and was stirred for 30 min. The aqueous layer was then extracted with ethyl acetate (3 × 100 mL). The combined organic layers were washed with saturated aqueous NaCl (2 × 50 mL), dried ($MgSO_4$), and concentrated under vacuum to afford the corresponding crude cinnamamide derivatives. The compounds were further purified by silica gel column chromatography eluted with hexanes:ethyl acetate (1:1) to afford the pure cinnamamides **18a–f**.

4.2.1. *Ethyl 4-{3-Oxo-3-[(2,2,4,4-tetramethylthiochroman-6-yl) amino]prop-1-en-1-yl}benzoate (**18a**)*

Yield: 195 mg (0.46 mmol, 92%) as a white solid, mp 184–185 °C; IR: 3288, 1716, 1646 cm^{-1}; ^1H NMR ($CDCl_3$): δ 8.05 (d, 2H, $J = 8.2$ Hz), 7.80 (br s, 1H), 7.78 (d, 1H, $J = 15.4$ Hz), 7.58 (m, 1H), 7.55

(d, 2H, J = 8.2 Hz), 7.30 (m, 1H), 7.10 (d, 1H, J = 8.4 Hz), 6.66 (d, 1H, J = 15.6 Hz), 4.39 (q, 2H, J = 7.0 Hz), 1.95 (s, 2H), 1.41 (s, 6H), 1.40 (t, 3H, J = 7.0 Hz), 1.40 (s, 6H); ^{13}C NMR ($CDCl_3$): δ 166.1, 163.2, 143.7, 140.9, 138.9, 135.1, 131.4, 130.1, 128.8, 128.6, 127.7, 123.1, 118.7, 118.1, 61.2, 54.4, 42.2, 35.8, 32.5, 31.6, 14.3. *Anal*. Calcd for $C_{25}H_{29}NO_3S$: C, 70.89; H, 6.90; N, 3.31. Found: C, 70.71; H, 6.76, N, 3.30.

4.2.2. 3-(4-Nitrophenyl)-N-(2,2,4,4-tetramethylthiochroman-6-yl) acrylamide (**18b**)

Yield: 188 mg (0.48 mmol, 95%) as a yellow solid, mp 212–213 °C; IR (cm^{-1}) 3281, 1662, 1521, 1340 cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 10.3 (s, 1H), 8.30 (d, 2H, J = 8.8 Hz), 7.89 (d, 2H, J = 8.8 Hz), 7.80 (d, 1H, J = 2.0 Hz), 7.70 (d, 1H, J = 15.8 Hz), 7.51 (dd, 1H, J = 8.4, 2.1 Hz), 7.05 (d, 1H, J = 8.4 Hz), 6.98 (d, 1H, J = 15.8 Hz), 1.92 (s, 2H), 1.37 (s, 6H), 1.35 (s, 6H); ^{13}C NMR (DMSO-d_6): δ 165.4, 143.5, 140.5, 139.5, 138.8, 137.2, 128.9, 128.5, 127.2, 126.5, 125.7, 118.6, 118.3, 54.1, 42.6, 35.9, 32.9, 31.9. *Anal*. Calcd for $C_{22}H_{24}N_2O_3S$: C, 66.64; H, 6.10; N, 7.07. Found: C, 66.28; H, 6.13, N, 7.03.

4.2.3. N-(2,2,4,4-Tetramethylthiochroman-6-yl)-3-[4-(trifluoromethyl)phenyl]acrylamide (**18c**)

Yield: 184 mg (0.44 mmol, 88%) as a white solid, mp 185–186 °C; IR: 3272, 1662, 1532, 1323 cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 10.3 (s, 1H), 8.31 (d, 2H, J = 8.8 Hz), 7.89 (d, 2H, J = 9.0 Hz), 7.80 (d, 1H, J = 2.1 Hz), 7.70 (d, 1H, J = 15.8 Hz), 7.52 (dd, 1H, J = 8.4, 2.2 Hz), 7.05 (d, 1H, J = 8.4 Hz), 7.00 (d, 1H, J = 15.8 Hz), 1.92 (s, 2H), 1.37 (s, 6H), 1.35 (s, 6H); ^{13}C NMR (DMSO-d_6): δ 163.5, 143.5, 139.5, 138.8, 137.2, 130.0 (q, J = 31.3 Hz), 129.0, 128.5, 127.2, 126.6 (q, J = 3.7 Hz), 125.8, 124.8 (q, J = 273.7 Hz), 118.6, 118.3, 54.2, 42.6, 36.0, 32.9, 31.9. *Anal*. Calcd for $C_{23}H_{24}F_3NOS$: C, 65.85; H, 5.77; N, 3.34. Found: C, 65.57; H, 5.79, N, 3.32.

4.2.4. 3-(2,3,4,5,6-Pentafluorophenyl)-N-(2,2,4,4-tetramethylthiochroman-6-yl)acrylamide (**18d**)

Yield: 207 mg (0.47 mmol, 94%) as a yellow solid, mp 143–144 °C; IR: 3276, 1664, 1523, 1498 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 7.80 (d, 1H, J = 2.0 Hz), 7.77 (d, 1H, J = 15.8 Hz), 7.39 (br s, 1H), 7.29 (dd, 1H, J = 8.4, 2.1 Hz), 7.12 (d, 1H, J = 8.4 Hz), 6.88 (d, 1H, J = 15.8 Hz), 1.95 (s, 2H), 1.42 (s, 6H), 1.40 (s, 6H); ^{13}C NMR ($CDCl_3$): 162.6, 145.6 (dm, J = 254.5 Hz), 143.7, 141.5 (dm, J = 264.6 Hz), 137.8 (dm, J = 254.5 Hz), 134.9, 129.2, 128.6, 128.5, 125.9, 118.8, 118.1, 110.7 (td, J = 13.1, 4.0 Hz), 54.3, 42.2, 35.8, 32.5, 31.6. *Anal*. Calcd for $C_{22}H_{20}F_5NOS$: C, 59.86; H, 4.57; N, 3.17. Found: C, 59.99; H, 4.75, N, 3.13.

4.2.5. 3-(3,4-Dimethoxyphenyl)-N-(2,2,4,4-tetramethylthiochroman-6-yl)acrylamide (**18e**)

Yield: 162 mg (0.41 mmol, 82%) as a white solid, mp 182–183 °C; IR: 3286, 1660, 1260 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 7.77 (br s, 1H), 7.68 (d, 1H, J = 15.4 Hz), 7.40 (br s, 1H), 7.23 (s, 1H), 7.07 (d, 2H, J = 8.4 Hz), 7.00 (s, 1H), 6.84 (d, 1H, J = 8.4 Hz), 6.43 (d, 1H, J = 15.4 Hz), 3.88 (s, 3H), 3.86 (s, 3H), 1.92 (s, 2H), 1.38 (s, 6H), 1.37 (s, 6H); ^{13}C NMR ($CDCl_3$): δ 164.4, 150.7, 149.1, 143.6, 141.9, 135.7, 128.5, 128.3, 127.7, 122.1, 118.9, 118.7, 118.1, 111.1, 109.9, 55.9, 55.8, 54.4, 42.2, 35.8, 32.5, 31.6. *Anal*. Calcd for $C_{24}H_{29}NO_3S \cdot 0.2H_2O$: C, 69.43; H, 7.14; N, 3.37. Found: C, 69.34; H, 7.05, N, 3.42.

4.2.6. 3-[4-(Dimethylamino)phenyl]-N-(2,2,4,4-tetramethylthiochroman-6-yl)acrylamide (**18f**)

Yield: 189 mg (0.48 mmol, 96%) as a yellow solid, mp 196–197 °C; IR: 3284, 1642, 1595, 1526 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 7.82 (br s, 1H), 7.69 (d, 1H, J = 15.2 Hz), 7.40 (d and obscured signal, 3H, J = 8.9 Hz), 7.24 (br s, 1H), 7.08 (d, 1H, J = 8.2 Hz), 6.65 (d and obscured signal, 2H, J = 9.0 Hz), 6.35 (d, 1H, J = 15.6 Hz), 3.00 (s, 6H), 1.93 (s, 2H), 1.40 (s, 6H), 1.39 (s, 6H); ^{13}C NMR ($CDCl_3$): δ 164.9, 151.5, 143.5, 142.6, 135.8, 129.5, 128.4, 127.8, 122.5, 118.6, 118.0, 115.3, 111.8, 54.4, 42.1, 40.1, 35.8, 32.5, 31.5. *Anal*. Calcd for $C_{24}H_{30}N_2OS$: C, 73.06; H, 7.66; N, 7.10. Found: C, 72.85; H, 7.70, N, 7.08.

4.2.7. 4-{3-Oxo-3-[(2,2,4,4-tetramethylthiochroman-6-yl)amino] prop-1-en-1-yl}benzoic acid (**18g**)

To a stirred solution of **18a** (0.1 g, 0.241 mmol) in THF (12 mL), was added lithium hydroxide (11.5 mg, 0.482 mmol) in water (8 mL), and the reaction was stirred for a 4 h at room temperature. The resulting mixture was concentrated under vacuum to 1/2 of its volume and further diluted with water (10 mL). The aqueous layer was washed with ethyl acetate (3 × 20 mL), and the resulting water layer was acidified to pH 1–2 with 6 M HCl. The final solution was extracted with ethyl acetate (3 × 20 mL). The combined organic extracts were washed with saturated aqueous NaCl (50 mL), dried ($MgSO_4$), and concentrated under vacuum to afford **18g** (88 mg, 95%) as a yellow solid, mp 219–220 °C; IR: 3600–2400, 1682 cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 13.1 (s, 1H), 10.3 (s, 1H), 8.00 (d, 2H, J = 8.0 Hz), 7.80 (d, 1H, J = 2.2 Hz), 7.73 (d, 2H, J = 8.1 Hz), 7.62 (d, 1H, J = 15.7 Hz), 7.49 (dd, 1H, J = 8.5, 2.2 Hz), 7.03 (d, 1H, J = 8.4 Hz), 6.91 (d, 1H, J = 15.7 Hz), 1.92 (s, 2H), 1.37 (s, 6H), 1.35 (s, 6H); ^{13}C NMR (DMSO-d_6): δ 167.3, 163.5, 143.4, 139.4, 139.2, 137.0, 131.8, 130.4, 128.3, 128.2, 127.0, 125.0, 118.4, 118.1, 54.0, 42.4, 35.8, 32.7, 31.7. *Anal*. Calcd for $C_{23}H_{25}NO_3S$: C, 69.85; H, 6.37; N, 3.54. Found: C, 69.53; H, 6.39, N, 3.53.

4.2.8. 3-(4-Aminophenyl)-N-(2,2,4,4-tetramethylthiochroman-6-yl)acrylamide (**18h**)

To a stirred solution of **18b** (0.45 g, 1.2 mmol) and iron powder (0.42 g, 7.5 mmol) in an ethanol:water mixture (4:1, 20 mL) was added NH_4Cl (0.15 g, 2.8 mmol), and the resulting mixture was refluxed for 18 h. The reaction mass was cooled to room temperature and filtered through a bed of Celite®. The Celite® was washed with ethanol (3 × 20 mL), and the solution was concentrated under vacuum at 45 °C to give 0.6 g of a yellow solid. The yellow solid was purified by silica gel column chromatography eluted with dichloromethane:ethyl acetate (7:3) to afford **18h** (0.42 g, 95%) as a yellow solid, mp 115–116 °C; IR: 3439, 3351, 3203, 1641, 1594 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 7.80 (br s, 1H), 7.67 (d, 1H, J = 15.2 Hz), 7.42–7.21 (complex, 4H), 7.09 (d, 1H, J = 8.2 Hz), 6.64 (d, 2H, J = 8.4 Hz), 6.35 (d, 1H, J = 15.2 Hz), 3.91 (br s, 2H), 1.94 (s, 2H), 1.40 (s, 6H), 1.39 (s, 6H); ^{13}C NMR ($CDCl_3$): δ 163.7, 148.4, 143.6, 142.4, 135.7, 129.7, 128.5, 128.1, 125.0, 118.7, 118.1, 116.5, 114.9, 54.4, 42.1, 35.8, 32.5, 31.6. *Anal*. Calcd for $C_{22}H_{26}N_2OS$: C, 72.09; H, 7.15; N, 7.64. Found: C, 71.74; H, 7.17; N, 7.61.

4.3. General procedure for synthesizing benzamides (**20a–c**)

To a stirred solution of acid **18g** (0.5 mmol) in *N*,*N*-dimethylformamide (5 mL) was added diisopropylethylamine (1.5 mmol), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (0.55 mmol), and 1-hydroxylbenzotriazole (0.55 mmol). After stirring for 1 h at room temperature, the corresponding amine (**19a,b** or **c**) (0.55 mmol) was added. The resulting mixture was stirred at room temperature for 12 h. The mixture was poured into ice (approx. 100 g) and stirred for 30 min. The aqueous layer was then extracted with ethyl acetate (3 × 100 mL). The combined organic extracts were washed with saturated aqueous NaCl (2 × 50 mL), dried ($MgSO_4$), and concentrated under vacuum to afford the crude benzamides. The compounds were further purified *via* a silica gel column chromatography using hexanes:ethyl acetate (1:1) to afford the desired pure benzamides **20a–c**.

4.3.1. N-Methyl-4-{3-oxo-3-[(2,2,4,4-tetramethylthiochroman-6-yl)amino]prop-1-en-1-yl}benzamide (**20a**)

Yield: 182 mg (0.445 mmol, 89%) as a yellow solid, mp 273–274 °C; IR: 1628, 1529, 1475, 1313 cm^{-1}; ^1H NMR (DMSO-d_6): δ 10.2 (s, 1H), 8.50 (d, 1H, J = 4.7 Hz), 7.89 (d, 2H, J = 8.2 Hz), 7.79 (d, 1H, J = 1.5 Hz), 7.69 (d, 2H, J = 8.2 Hz), 7.60 (d, 1H, J = 15.6 Hz), 7.49 (dd, 1H, J = 8.2, 1.6 Hz), 7.03 (d, 1H, J = 8.6 Hz), 6.88 (d, 1H, J = 15.6 Hz), 2.79 (d, 3H, J = 3.9 Hz), 1.91 (s, 2H), 1.35 (s, 6H), 1.34 (s, 6H); ^{13}C NMR (DMSO-d_6): δ 166.4, 163.5, 143.3, 139.4, 137.7, 137.1, 135.6, 128.3, 128.2, 128.0, 126.9, 124.3, 118.3, 118.1, 54.0, 42.4, 35.8, 32.7, 31.7, 26.7. *Anal.* Calcd for $C_{24}H_{28}N_2O_2S$: C, 70.56; H, 6.91; N, 6.86. Found: C, 70.28; H, 6.78, N, 6.83.

4.3.2. N-Cyclopropyl-4-{3-oxo-3-[(2,2,4,4-tetramethylthiochroman-6-yl)amino]prop-1-en-1-yl}benzamide (**20b**)

Yield: 202 mg (0.465 mmol, 93%) as a yellow solid, mp 228–229 °C; IR: 1635, 1531 cm^{-1}; ^1H NMR (DMSO-d_6): δ 10.2 (s, 1H), 8.48 (d, 1H, J = 4.3 Hz), 7.86 (d, 2H, J = 8.2 Hz), 7.77 (d, 1H, J = 2.0 Hz), 7.66 (d, 2H, J = 8.2 Hz), 7.58 (d, 1H, J = 15.6 Hz), 7.48 (dd, 1H, J = 8.6, 2.2 Hz), 7.01 (d, 1H, J = 8.6 Hz), 6.87 (d, 1H, J = 15.6 Hz), 2.86 (m, 1H), 1.89 (s, 2H), 1.34 (s, 6H), 1.32 (s, 6H), 0.68 (m, 2H), 0.56 (m, 2H); ^{13}C NMR (DMSO-d_6): δ 167.0, 163.3, 143.1, 139.1, 137.5, 136.9, 135.3, 128.1, 127.7, 126.7, 124.1, 118.1, 117.8, 53.7, 42.2, 35.5, 32.5, 31.5, 23.3, 6.0 (1 aromatic C unresolved). *Anal.* Calcd for $C_{26}H_{30}N_2O_2S$: C, 71.86; H, 6.96; N, 6.45. Found: C, 71.56; H, 6.84, N, 6.31.

4.3.3. 3-[4-(Morpholine-4-carbonyl)phenyl]-N-(2,2,4,4-tetramethylthiochroman-6-yl)acrylamide (**20c**)

Yield: 211 mg (0.455 mmol, 91%) as a yellow solid, mp 181–182 °C; IR: 1614, 1530, 1470, 1278, 1115 cm^{-1}; ^1H NMR (DMSO-d_6): δ 8.19 (br s, 1H), 7.87 (br s, 1H), 7.66 (d, 1H, J = 15.3 Hz), 7.44 (d, 2H, J = 8.2 Hz), 7.39 (d, 2H, J = 8.2 Hz), 7.34 (d, 1H, J = 7.0 Hz), 7.10 (d, 1H, J = 8.5 Hz), 6.48 (d, 1H, J = 15.3 Hz), 3.77–3.46 (m, 8H), 1.95 (s, 2H), 1.41 (s, 6H), 1.40 (s, 6H); ^{13}C NMR (DMSO-d_6): δ 170.0, 163.5, 158.1, 143.5, 140.2, 136.5, 136.0, 135.6, 128.5, 128.1, 127.5, 122.8, 118.7, 118.0, 66.8, 54.4, 42.1, 35.8, 32.5, 31.5 (1 aliphatic C unresolved). *Anal.* Calcd for $C_{27}H_{32}N_2O_3S$: C, 69.80; H, 6.94; N, 6.03. Found: C, 69.53; H, 6.96, N, 6.01.

4.4. X-ray structure determination of ethyl 4-{3-oxo-3-[(2,2,4,4-tetramethylthiochroman-6-yl)amino]prop-1-en-1-yl}benzoate (**18a**)

A colorless plate-shaped crystal of dimensions 0.270 × 0.220 × 0.020 mm was selected for structural analysis. Intensity data for this compound were collected using a diffractometer with a Bruker APEX ccd area detector [35] and graphite-monochromated Mo Kα radiation (λ = 0.71073 Å). The sample was cooled to 100(2) K. Cell parameters were determined from a non-linear least squares fit of 3137 peaks in the range 2.71 < θ < 30.68°. A total of 12,709 data were measured in the range 2.114 < θ < 30.721° using φ and ω oscillation frames. The data were corrected for absorption by the empirical method [36] giving minimum and maximum transmission factors of 0.954 and 0.996. The data were merged to form a set of 6264 independent data with R(int) = 0.0283 and a coverage of 99.3%.

The triclinic space group $P\bar{1}$ was determined by statistical tests and verified by subsequent refinement. The structure was solved by direct methods and refined by full-matrix least-squares methods on F^2 [37]. The positions of hydrogens bonded to carbons were initially determined by geometry and were refined using a riding model. The hydrogen bonded to the nitrogen was located on a difference map, and its position was refined independently. Non-hydrogen atoms were refined with anisotropic displacement parameters. Hydrogen atom displacement parameters were set to 1.2 (1.5 for methyl) times the isotropic equivalent displacement parameters of the bonded atoms. A total of 274 parameters were refined against 6264 data to give $wR(F^2) = 0.1222$ and $S = 1.000$ for weights of $w = 1/[\sigma^2(F^2) + (0.0560\ P)^2 + 0.4000\ P]$, where $P = [Fo^2 + 2Fc^2]/3$. The final $R(F)$ was 0.0466 for the 4573 observed, $[F > 4\sigma(F)]$, data. The largest shift/s.u. was 0.001 in the final refinement cycle. The final difference map had maxima and minima of 0.452 and −0.286 e/Å^3, respectively.

4.5. Biological methods

The compounds were dissolved in DMSO at a concentration of 0.01 M, except for **18d**, which was dissolved at 0.005 M due to low solubility. The human ovarian cancer cell line A2780 was plated in 96-well tissue culture dishes at a concentration of 2000 cells per well in RPMI medium supplemented with 10% fetal bovine serum and a mixture of antibiotics and antimycotics. The next day, the cultures were treated in triplicate with compound concentrations ranging from 0.16 μM to 10 μM. Control cultures were treated with DMSO solvent only. After 72 h of incubation, the CellTiter 96 Non-Rad Cell Proliferation Assay (Promega) was used to quantify the remaining metabolically living cells. After subtracting blank values, the optical density (OD) readout of the assay for the treated cultures was divided by the average OD of the control cultures to derive the Growth Index. GraphPad Prism Software was used to draw sigmoidal dose–response curves and derive the IC_{50} values of two independent experiments. The efficacy was determined by dividing the maximal % growth inhibition of the cultures treated with the test compounds by the maximal % growth inhibition of cultures treated with SHetA2. Compound **18a** appeared to have IC_{50} values at the lower limit of the compound concentrations tested, and thus additional experiments were performed with this sample at doses between 0 μM and 2 μM to refine the IC_{50} accuracy.

Supplementary data

Supplementary data related to this article—copies of spectra for **18a**–**h** and **20a**–**c** as well as X-ray details for **18a** (CCDC 1042350)—can be found at http://dx.doi.org/10.1016/j.ejmech.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2015.03.070.

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