Chapter 3

SHetA2, a New Cancer-Preventive Drug Candidate

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Abstract

SHetA2 (NSC 721689) is a novel synthetic flexible heteroarotinoid that has promising cancer-preventive activity, and has exhibited growth inhibition on 60 cancer cell lines in vitro, along with ovarian, lung, and kidney cancers *in vivo*. It binds and interferes with the function of a molecular chaperone, mortalin, leading to mitochondrial swelling and mitophagy that induce apoptosis in cancer cells without harming normal cells. It showed minimal toxicity in preclinical studies and thus is now in Phase-0 clinical trial. This chapter summarizes its evolution, synthesis, structure-activity relationship, mechanism of action, pharmacokinetics, and potential clinical applications in last 12 years. It also provides insights into designing more potent and safer SHetA2 analogs for future cancer-preventive drug development.

Keywords: SHetA2, flexible heteroarotinoids, apoptosis, cancer prevention, ovarian cancer

1. Introduction

Cancer-preventive agents are biologics, dietary supplements, nutrients or marketed drugs used to reduce the risk of developing cancer or prevent the recurrence of cancer [1]. The cancer-preventive actions of these agents are mediated by a variety of proposed mechanisms, which include inhibition of oxidative and inflammatory stress, immunomodulatory action, induction of cell differentiation and apoptosis, and inhibition of cell growth and angiogenesis. Hundreds of natural and synthetic compounds have been shown to inhibit cancer cell growth including vitamins (vitamins A, D, and E), organosulfur compounds (brassinin, sulforaphane,

and isothiocyanates), minerals (calcium and selenium), phytoestrogens (resveratrol), flavonoids (genistein and quercetin), synthetic compounds (retinoids, sulforamate, and prenylated flavonoid analogs) [2], and marketed drugs (tamoxifen, raloxifene, finasteride, aspirin, sulindac, difluoromethylornithine, and metformin) [3]. Drug development for cancer prevention imposes distinct challenges beyond those associated with drug development for cancer therapy. One of the challenges is that cancer preventive agents must have higher therapeutic index. Nevertheless, the potential to intervene prior to the cancer developing is an attractive strategy to fight cancers [4].

A novel class of anticancer agents that has demonstrated such qualities is the flexible heteroarotinoids (Flex-Hets). Flex-Hets, derived from the retinoids, have exhibited potential anticancer activities in various cancer cell lines, while displaying minimal toxicity to normal cells. Among these compounds, SHetA2 [(4-nitrophenyl)amino][2,2,4,4-tetramethylthiochroman-6-yl]amino]methane-thione) exhibited the greatest growth inhibitory efficacy and potency against various cancer cell lines and was chosen as a lead compound for further development [5–7]. SHetA2 has been shown to interfere with mortalin binding to p53 and p66 Src homologous-collagen homologue (p66shc) leading to apoptosis in cancer cells [8]. Furthermore, it has been shown to induce both intrinsic [9] and extrinsic apoptotic pathway [10], cause cell cycle arrest [11], induce differentiation [7], and inhibit angiogenesis [12] in cancer cells, while displaying negligible toxicity in animal models [13]. Hence, SHetA2 was regarded as a novel class of promising anticancer agent that selectively targeted the cancer cells. Consequently, it was evaluated in preclinical development for cancer prevention through the National Cancer Institute (NCI) Rapid Access to Intervention Development (RAID) and Rapid Access to Preventive Intervention Development (RAPID) program [6]. Studies in rats and dogs showed that no toxicity was observed in any of the tested dosage groups. The no-observed-adverse-effect-level (NOAEL) for SHetA2 was not established and was considered to be above 1500 mg/kg/day in dogs [13]. The therapeutic window for administrative safety with SHetA2 was determined to be 25- to 150-folds above *in vivo* effective doses. As a result, it is currently undergoing Phase-0 clinical trials through RAID [14]. This chapter will provide a comprehensive review of SHetA2, including its design and development, and possible molecular targets and mechanisms of action and its potential clinical applications, based on the literature published thus far.

2. History of retinoid development

2.1. Retinoids

Retinoids, including natural retinoic acid (RA) and its synthetic derivatives, are a group of promising anticancer agents that have shown both chemotherapeutic and chemopreventive potential in both animals and humans [15–17]. They exhibit therapeutic properties by activating the retinoic acid receptors (RARs) and the retinoid X receptors (RXRs) [18]. Each receptor has three subtypes (-α, -β, -γ), and they belong to a family of proteins that regulate the transcription of genes responsible for a variety of metabolic, developmental, and differentia-

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tion pathways in cells and tissues [19]. Retinoids have been shown to inhibit growth, and induce differentiation and apoptosis of cancer cells both *in vitro* and *in vivo* [20, 21]. Naturally occurring retinoids, all-*trans*-retinoic acid (ATRA, **Figure 1**, **1**), 9-*cis*-retinoic acid (**Figure 1**, **2**), and 13-*cis*-retinoic acid (**Figure 1**, **3**) have been shown to display significant anticancer activities [22]. Synthetic retinoids, such as *N*-(4-hydroxyphenyl) retinamide (4HPR, fenretinide, **Figure 1**, **4**), are also effective inducers of apoptosis [23]. However, the use of retinoids as chemotherapeutics has been hampered by their local and systemic toxicities and side effects. Chronic retinoid treatment can lead to teratogenicity and toxicities to the skin, mucus membranes, hair, eyes, gastrointestinal system, liver, endocrine system, kidneys, and bone [24]. These toxicities are found to be associated with the activation of the nuclear retinoid receptors [25, 26].

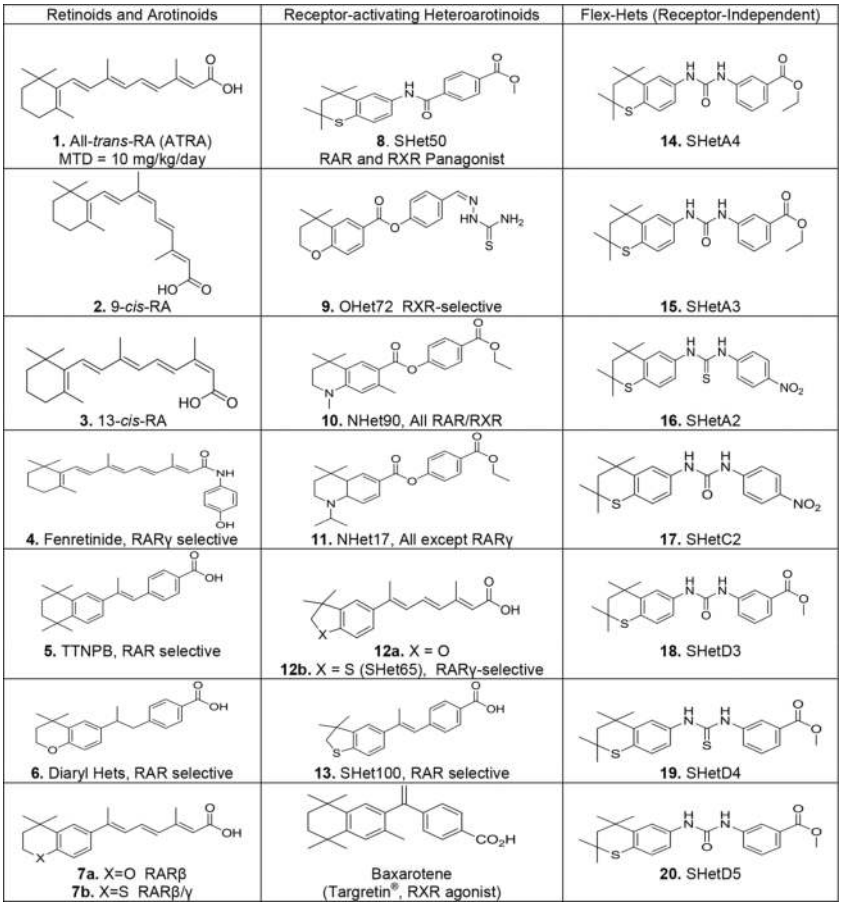

Figure 1. Chemical structures of retinoids, heteroarotinoids, and flexible-heteroarotinoids.

2.2. Arotinoids

Recognizing the potential chemopreventive and chemotherapeutic effects of retinoids, chemists have tried to design and synthesize new retinoid derivatives that express high cytotoxic potential with lower toxicity. The rationale is that by developing compounds that selectively activate only one retinoid receptor subtype, the associated toxicities could be reduced. For example, RXR-selective compounds were thought to have great potential for pharmacological use against tumors and other diseases due to a large number of ligand activated receptors whose activities involve dimerization with RXR receptors [19]. Bexarotene (Targretin®, with a one-carbon linker), is a selective RXR agonist and the first synthetic arotinoid approved for the treatment of all stages of cutaneous T-cell lymphoma [27]. RARγ, selectively expressed at high levels in the skin, has been shown to be the mediator of retinoid activity [28]. Early efforts to increase the therapeutic ratio of retinoids included increasing the structure rigidity by incorporating a double-bond between the two aromatic rings, leading to the development of arotinoids with two atom linker. For example, TTNPB (**Figure 1**, **5**), an arotinoid with significant anti-tumor activity [29] was found to be selective for RAR receptors [30]. Unfortunately, intolerable toxicities observed in animal models limited its clinical utility [29].

2.3. Heteroarotinoids

Subsequent structural modifications involved the addition of one heteroatom (O, N, and S) in the cyclic ring of the arotinoids to block its oxidation into toxic metabolites. This resulted in the development of a new class of compounds called the heteroarotinoids (Hets, **Figure 1**, **6**–**12**). These Hets exhibited similar biological activities to retinoids, but with significantly reduced toxicities [29, 31].

2.4. Flexible heteroarotinoids

Different linkers were placed between the two aryl groups of the Hets to increase their rigidity, providing a more specific fit into each receptor's binding pocket. Two-atom linkers, such as amide (**Figure 1**, **8**) and esters (**Figure 1**, **9**–**11**) were synthesized, and they showed varying degree of receptor selectivity. Interestingly, Hets with either the three-atom urea (**Figure 1**, **14**, **17**, **18**) or thiourea (**Figure 1**, **15**, **16**, **19**, **20**) linker demonstrated significant anticancer activity without activating any of the retinoid receptors [6]. These compounds showed significant growth inhibitory activities against the ovarian cancer cell lines: Caov-3, OVCAR-3, and SK-OV-3, while exhibiting low activities against normal and benign cells. Moreover, due to the lack of RAR/RXR activation, these compounds did not exhibit the associated toxicities observed with other retinoids. Among these compounds, SHetA2 (**Figure 1**, **16**) demonstrated the greatest potency against the aforementioned ovarian cancer cell lines at concentrations ranging from 0.2–3.7 μM. Since both urea and thiourea linkers are somewhat flexible in nature, these compounds were termed flexible heteroarotinoids (Flex-Hets). The evolution of SHetA2 is shown in **Figure 2**.

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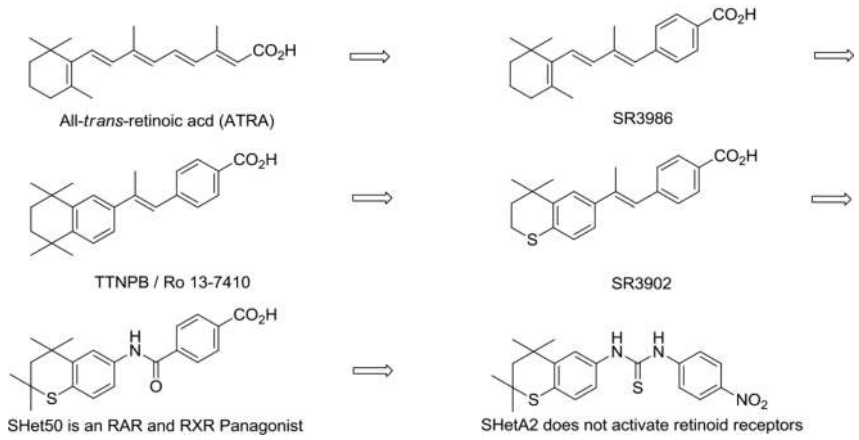

Figure 2. The evolution of SHetA2 from all *trans* retinoic acid (ATRA).

3. SHetA2, a Flex-Het

Flex-Hets have shown cancer-preventive activity by regulating apoptosis, cell growth, and differentiation in multiple types of cancer cell lines. They display significantly greater growth inhibition activities against ovarian cancer cells than both normal and benign ovarian cells [6]. Among the Flex-Hets, SHetA2 has shown to induce the highest levels of apoptosis in multiple cancer cell types, while retaining the differential resistance in normal cells [5]. SHetA2 functions independently of retinoid receptors [32] and therefore, lacks the toxicities associated with conventional retinoids. More importantly, SHetA2 differentially induces apoptosis in cancer cells while sparing normal cells [6]. All 60 cancer cell lines in NCI human tumor panel were sensitive to the growth inhibition activities of SHetA2 at micromolar concentrations [5] (**Table 1**). Despite this wide range of activity against cancer cells, normal and benign human ovarian, endometrial, and oral cultures were resistant to SHetA2-induced apoptosis and growth inhibition [6, 9]. In a recent study, SHetA2 was tested to be negative across three genetic toxicology assays, namely the *in vitro Salmonella-Escherichia coli* mutagenicity assay, Chinese hamster ovary cell chromosome aberration assay (CHO-CA), and *in vivo* mouse bone marrow micronucleus assay [34]. These assays are recommended by the International Conference on Harmonization (ICH), US Food and Drug Administration (FDA), and other regulatory agencies for the detection of mutagenicity by chemicals [35]. These negative results showed that SHetA2 was neither mutagenic nor genotoxic. In addition, SHetA2 exhibited no evidence of toxicity in animal models including dogs [13], and it did not induce skin irritation or teratogenicity [5, 36]. Encouraged by these promising results, SHetA2 was evaluated by NCI's RAID program for preclinical development as a cancer therapeutic agent (Application 196, Compound NSC 726189), and by RAPID program as a chemopreventive agent. Since SHetA2 showed no toxicity in preclinical studies, it is now in Phase-0 clinical trial.

GI_{50} Mean Graph for SHetA2 (NSC 726189) [33]			
Cancer panel	Cell line	Log GI_{50} (SHetA2)	Log GI_{50} (ATRA)
Leukemia	CCRF-CEM	−4.9	−4.2
	HL-60(TB)	−4.5	−4.7
	K-562	−5.0	−4.3
	MOLT-4	−4.7	−4.3
	RPMI-8226	−4.5	−5.7
	SR	−4.9	−4.2
Nonsmall-cell lung carcinoma	A549/ATCC	−4.9	−4.3
	EKVX	−4.9	−4.0
	HOP-62	−4.7	−4.2
	HOP-92	−4.7	−4.0
	NCI-H226	−4.9	−4.3
	NCI-H23	−4.9	−4.2
	NCI-H322M	−5.0	−4.0
	NCI-H460	−4.6	−4.2
	NCI-H522	−5.0	−4.2
Colon cancer	COLO 205	−4.9	−4.2
	HCC-2998	−5.4	−4.2
	HCT-116	−4.8	−4.2
	HCT-15	−4.9	−4.2
	HT29	−4.6	−4.2
	KM12	−5.0	−4.3
	SW-620	−4.8	−4.2
CNS (gliomas)	SF-268	−4.6	−4.0
	SF-295	−5.0	−4.2
	SF-539	−5.0	−4.2
	SNB-19	−4.7	−4.2
	SNB-75	−4.8	−4.0
	U251	−5.0	−4.2
Melanoma	LOX IMVI	−4.9	−4.4
	MALME-3M	−5.6	−4.3
	M14	−4.7	−4.2
	MDA-MB-435	−4.9	−4.2
	SK-MEL-2	−5.0	−4.4
	SK-MEL-28	−4.8	−4.2
	SK-MEL-5	−5.0	−4.2
	UACC-257	−4.9	−4.2
	UACC-62	−5.0	−4.0
Ovarian cancer	IGROV1	−5.0	−4.0

	OVCAR-3	−5.0	−4.2
	OVCAR-4	−4.8	−4.0
	OVCAR-5	−4.8	−4.0
	OVCAR-8	−4.8	−4.2
	NCI/ACR-RES	−4.9	−4.4
	SK-OV-3	−4.8	−4.0
Renal cancer	786-0	−4.8	−4.2
	A498	−5.0	−4.0
	ACHN	−5.0	−4.2
	CAKI-1	−4.9	−4.2
	RXF 393	−4.7	−4.0
	SN12C	−5.0	−4.2
	TK-10	−5.0	−4.1
	UO-31	−4.9	−4.0
Prostate cancer	PC-3	−4.0	−4.3
	DU-145	−4.9	−4.2
Breast cancer	MCF7	−4.5	−4.4
	MDA-MB-231	−5.0	−4.3
	HS 578T	−4.6	−4.0
	BT-549	−4.9	−4.2
	T-47D	−4.8	−6.3

Lower values indicate greater inhibition of cell growth.

Table 1. Growth inhibition values (GI_{50}) of NCI's human tumor cell line panel expressed in log scale.

4. Synthesis of SHetA2

The synthesis of SHetA2 mainly involved the preparation of the intermediate, aminothiochroman (**Figures 3** and **4**). Early synthetic procedure (**Figure 3**) involved using Fe/HOAc to reduce the nitro group with a yield of 40% [6]. The nitration reaction was the problematic step with only a 26% yield, since many additional by-products were formed in this reaction. In order to address this issue, Tallent et al. employed a nitrogen-containing starting material (4-acetamidobenzenethiol) [37] (**Figure 4**). They used methyllithium instead of methylmagnesium bromide to form the intermediate carbinol. In the cyclization procedure to form the thiochroman, chlorobenzene was used as solvent with a 58% yield avoiding the use of flammable and noxious CS_2. This method circumvented the low-yielding nitration step and the subsequent reduction step to generate the amine group of aminothiochroman. The improved synthesis was shorter and afforded a 5-fold higher overall yield. The final product, SHetA2, was formed by reacting the aminothiochroman with 4-nitrophenyl isothiocyanate in tetrahydrofuran (**Figure 4**).

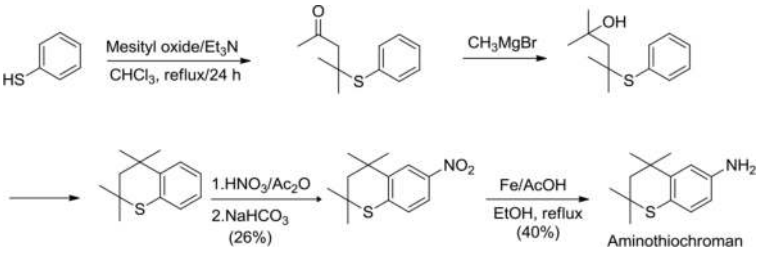

Figure 3. Synthesis of aminothiochroman.

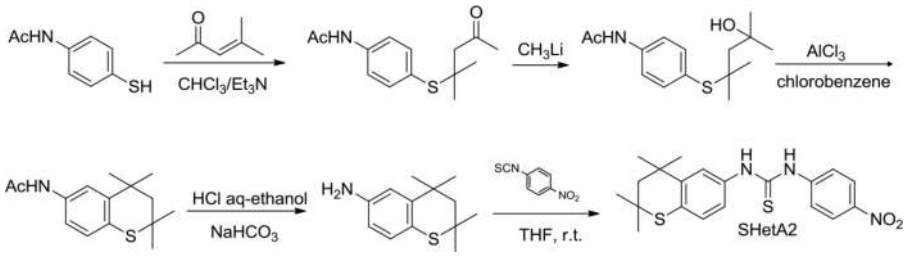

Figure 4. Improved synthesis of aminothiochroman and SHetA2.

5. Structure-activity relationship

Structure-activity relationship (SAR) of SHetA2 and its derivatives has been valuable to identify important structural modifications that have contributed to its anticancer potency and selectivity, and to guide the design of more potent and less toxic SHetA2 analogs. **Figure 2** illustrates the structural evolution of SHetA2 from ATRA. The goal was to increase the selectivity of retinoids towards RAR/RXR subtypes, so as to reduce the associated toxicities while retaining its anticancer activities. It has been shown that RAR-specific ligands can rescue *Raldh2*$^{-/-}$ embryos as effectively as ATRA, whereas RXR ligand showed no effect [36]. One strategy was to conformationally restrict the double-bonds of RA to allow a better fit into a specific receptor subtype by incorporating aromatic rings. For example, SR3986 (**Figure 2**) was developed with an aromatic ring from ATRA. Subsequently, TTNPB (**5**), the lead arotinoid, was modified with a diaryl group to increase its rigidity, and it was found to be selective for RAR receptors and 10 times more potent as compared to ATRA [38]. Unfortunately, this compound also exhibited a 10,000-fold increase in toxicity, which limited its clinical usage [29].

In order to reduce toxicity, a benzylic carbon in the tetrahydronaphthalene was replaced with a heteroatom (O, N, and S). The purpose was to prevent benzylic (metabolic) oxidation which could result in toxic metabolites. This single modification resulted in Hets (**Figure 1**, **6–12**) with similar biological activities to RA but significantly reduced toxicities [29, 31]. An example was

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the diaryl heteroarotinoid (**Figure 1**, **6**), a RAR-selective retinoid derivative. It differed from TTNPB by an oxygen heteroatom, but exhibited a significant decrease in toxicity, increasing the maximum tolerated dose (MTD) by 3000-fold as compared to TTNPB (**Figure 1**, **5**) [39]. Other monoaryl heteroarotinods (**Figure 1**, **7a**, **7b**) were also evaluated, and revealed a 3-fold decrease in toxicity along with a decreased ability to activate the RAR receptors when compared to ATRA (**Figure 5**) [40, 30].

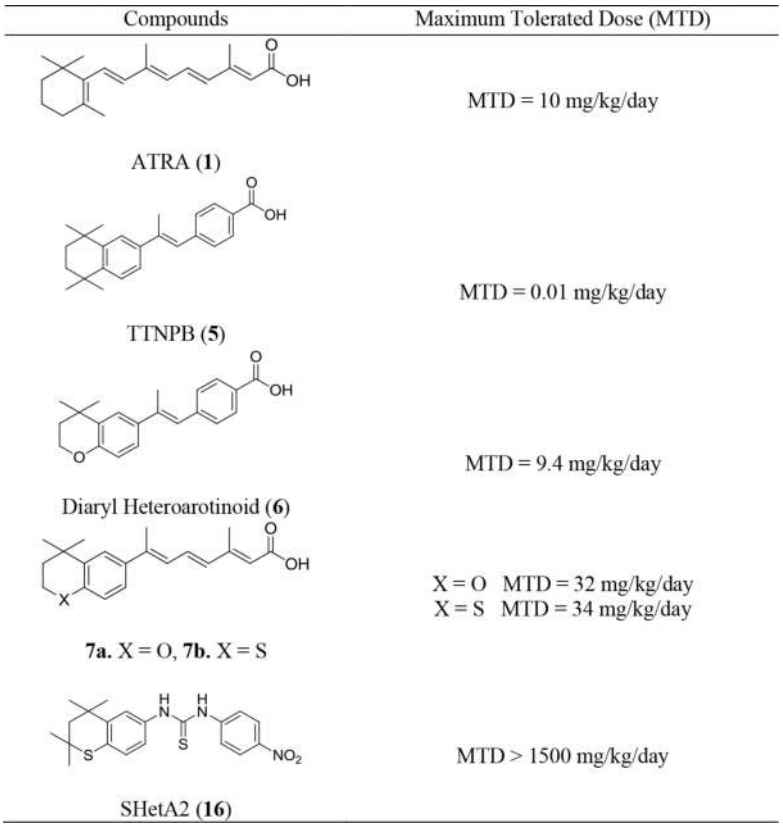

Figure 5. Maximum tolerated dose (MTD) for retinoids and their derivatives.

5.1. Thiochroman rings

SAR studies have identified structures containing six-membered ring (**Figure 1**, **7a**, **7b**) tend to confer increased RARβ selectivity over five-membered ring systems (**Figure 1**, **12a**, **12b**), while sulfur heteroatom confers a greater RARγ selectivity over oxygen atom [19]. The thiochroman ring system is flexible and has been shown to induce apoptosis to a greater extent than a rigid planar quinoline unit [41]. These findings highlight the important role played by

the thiochroman ring in enhancing the activity of Flex-Hets. Therefore, the thiochroman ring forms one of the fundamental moieties of SHetA2 and its analogs (14–20).

5.2. Two-atom linkers

To further increase the selectivity for each RAR and RXR receptors, various linkers were placed between the two aryl groups of the Hets by modifying their structure rigidity. Two-atom linker compounds such as amide (**Figure 1**, **8**) and ester (**Figure 1**, **9**) were reported [42, 43]. Compound (**8**) was found to be a receptor panagonist, while compound (**9**) was RXR selective. Both showed significant growth inhibitory activities against head and neck cancer using a tumor xenograft mouse model [42]; however, only compound (**8**) induced apoptosis in ovarian cancer cells. This indicated that RXR activation is sufficient to inhibit tumor growth, while activation of both RAR and RXR are required for the maximum activity, at the expense of toxicity. Other two ester-linked compounds (**Figure 1**, **10**, **11**) were found to activate both RARs and RXRs [44]. On the other hand, these ester-linked Hets appeared to only induce growth inhibition but not apoptosis.

5.3. Three-atom linkers

Three-atom linkers have also been reported in the literature (**Figure 4**, **21**, **22**). It was suggested that the presence of a three-atom linker may increase RAR selectivity [45], and the ability for linkers to form hydrogen bonds may enhance RARγ selectivity [46]. As such, a series of urea/thiourea derivatives were synthesized and evaluated (**Figure 1**, **14**–**20**) [6, 7]. Unlike conventional retinoids, these Flex-Hets were able to induce selective and potent apoptotic activity in cancer cells independent of RAR/RXR activation [44]. The only retinoid activity retained by Flex-Hets is the ability to induce differentiation and reverse the cancerous phenotype.

The X-ray crystal structure of the Flex-Hets showed that a unique lattice network formed through extensive intermolecular H-bonding between the NH and the oxygen of the highly polarized C=O urea linker in another molecule, whereas this was not observed in the C=S thiourea derivatives. It suggested that the urea derivatives may be more active than its thiourea counterpart. This observation is also supported by their *in vitro* growth inhibition activities where the urea derivative, SHetC2 (**17**), demonstrated to be slightly more potent (EC_{50}= 1.02 μM) than its thiourea counterpart, SHetA2 (**16**) (EC_{50}= 1.72 μM) [41].

Regardless, the fact remains that millions of dollars have been invested by the NCI RAID and RAPID programs in the preclinical development of SHetA2, and many studies involving animal models have shown that SHetA2 is a potent and selective inducer of apoptosis with no significant toxicities. While SHetC2 lacks these extensive studies, it does show potential as the next chemopreventive drug candidate following SHetA2. These results indicate that the inclusion of three-atom urea/thiourea linker is critical to induce potent anticancer activities independent of RAR/RXR activation as observed in these Flex-Hets. **Tables 2** and **3** summarize the effects of the structural modifications on the growth inhibition by these derivatives against various cancer cell lines reported to-date.

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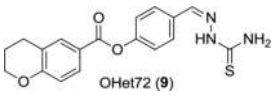
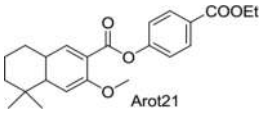
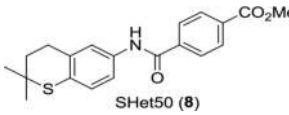
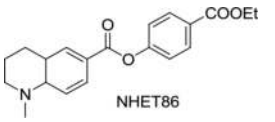
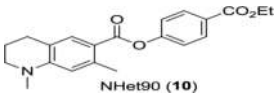
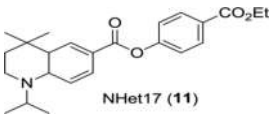
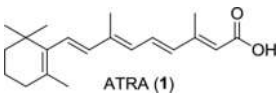
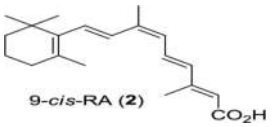
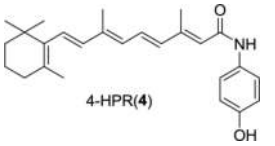
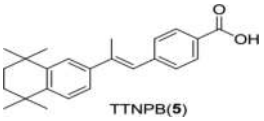
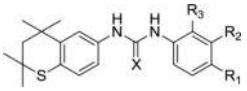
Compound	Cell growth inhibition													
	Renal (%)		Ovarian cancer (%)			NE EC_{50} (μM)	Vulvar cancer (%)		Cervical cancer (%)				HN cancer (%)	
	Cancer	Normal												
	1	1	1	2	3	NE	1	2	1	2	3	4	1	2
 OHet72 (**9**)	–	–	–	–	–	–	–	–	–	–	–	–	19	18
 Arot21	–	–	–	–	–	–	–	–	–	–	–	–	15	12
 SHet50 (**8**)	–	–	–	–	–	–	–	–	–	–	–	–	64	37
 NHET86	–	–	0	–	1	–	34	14	40	25	–	–	12	13
 NHet90 (**10**)	–	–	5	–	0	–	81	84	26	16	–	–	9	7
 NHet17 (**11**)	–	–	6	–	2	–	47	25	25	13	–	–	5	15
 ATRA (**1**)	6.5	16	–	–	–	–	–	–	–	–	–	–	–	52
 9-*cis*-RA (**2**)	–	–	25	–	31	>10	38	31	24	28	45	47	–	51
 4-HPR(**4**)	–	–	62	43	65	>10	–	–	–	–	–	–	–	74

Compound	Cell growth inhibition																	
	Renal (%)		Ovarian cancer (%)			NE EC_{50} (μM)	Vulvar cancer (%)		Cervical cancer (%)				HN cancer (%)	
	Cancer	Normal												
 TTNPB(**5**)	–	–	11	–	19	–	34	19	–	–	–	–	74	–

Growth inhibition (%) for renal cancer cell lines (1) Caki-1 and (2) 786-0; Normal renal cells (1) HK-2 and (2) RTC91696 [23]. Growth Inhibition (%) for ovarian cancer cell lines (1) CAOV-3, (2) OVCAR-3, and (3) SKOV-3 [6]. EC_{50} values for 50% growth inhibition for ovarian cancer cell line (4) A2780, and Normal endometrial cells (NE) [6, 41]. Growth inhibition (%) for cervical cancer cell lines (1) SiHa, (2) CC-1, (3) C33a, and (4) HT-3 [5]. Growth inhibition (%) for head and neck squamous cell cancer cell lines (HN) (1) SCC-2 [43], and (2) SCC-38 [32, 42]. Growth inhibition (%) for vulvar cancer cell lines (1) SW954 and (2) SW962 from Ref. [43].
"–" indicates no data available.

Table 2. Structural modifications of Hets, and their effects on cancer cell growth.

	 General Structure of Flex-Het				Cell growth inhibition													
					Renal cancer (%)		Renal normal (%)		Ovarian cancer (%)				NE	Cervical cancer (%)				HN cancer (%)
													EC_{50}(μM)					
	X	**R_1**	**R_2**	**R_3**	**1**	**2**	**1**	**2**	**1**	**2**	**3**	**4**	**NE**	**1**	**2**	**3**	**4**	**1**
14	O	CO_2Et	H	H	69	45	26	40	40	69	47	2.8	4.5	67	59	85	76	42
15	S	CO_2Et	H	H	56	54	35	28	24	58	42	2.9	2.7	58	42	84	65	39
16	S	NO_2	H	H	84	72	51	37	55	67	45	1.7	3.0	68	58	87	92	81
17	O	NO_2	H	H	86	79	53	52	–	–	–	1.0	2.3	–	–	–	–	–
18	O	CO_2Me	H	H	57	62	26	46	–	–	–	–	–	–	–	–	–	–
19	S	CO_2Me	H	H	39	45	44	38	–	–	–	–	–	–	–	–	–	–
20	S	H	CO_2Me	H	34	42	48	–	–	–	–	–	–	–	–	–	–	–
SHetA19	S	NO_2	H	Me	–	–	–	–	32	65	51	–	–	–	–	–	–	68

Growth inhibition (%) for renal cancer cell lines (1) Caki-1 and (2) 786-0; Normal renal cells (1) HK-2 and (2) RTC91696 [23]. Growth Inhibition (%) for ovarian cancer cell lines (1) CAOV-3, (2) OVCAR-3, and (3) SKOV-3 [6]. EC_{50} values for 50% growth inhibition for ovarian cancer cell line (4) A2780, and Normal endometrial cells (NE) [6, 41]. Growth inhibition (%) for cervical cancer cell lines (1) SiHa, (2) CC-1, (3) C33a, and (4) HT-3 [5]. Growth inhibition (%) for head and neck squamous cell cancer cell lines (HN) (1) SCC-2 [43], and (2) SCC-38 [32, 42]. Growth inhibition (%) for vulvar cancer cell lines (1) SW954 and (2) SW962 from Ref. [43].
"–" indicates no data available.

Table 3. Structural modifications of Flex-Hets, and their effects on cancer cell growth.

Substitutions on the phenyl group have also been evaluated [7]. The nitro (NO_2) substitution (**Table 3**, **16**, **17**) consistently exhibited greater growth inhibitory and apoptotic activity than

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their methyl (**Table 3**, **18–20**) or ethyl ester counterparts (**Table 3**, **14**, **15**). This suggests that the nitro substitution may have enhanced the overall activity of the compound. Collectively, for the Flex-Hets, the thiochroman ring and nitro substitution are important for enhancing the anticancer activity, while the thiourea linker is crucial for RAR/RXR independent and selective anticancer activities against cancer cells.

5.4. Three-atom linker with thiochroman ring replaced

In order to expand the potential clinical applications, we designed, synthesized, and evaluated nine *p*-nitrodiarylthiourea analogs in breast (MCF-7, T-47D, MDA-MB-453) and prostate (DU-145, PC-3, LNCaP) cancer cell lines for their anticancer activities. Majority of our compounds were able to inhibit the growth of these six cancer cell lines at low micromolar concentrations. Compound **23** (**Figure 6**) was found to be the most potent anticancer agent in this series with GI_{50} values of 3.16 μM for MCF-7, 2.53 μM for T-47D, 4.77 μM for MDA-MB-453 breast cancer lines and 3.54 μM for LNCaP prostate cancer cell line. These GI_{50} values were comparable to the parent compound, SHetA2 [47].

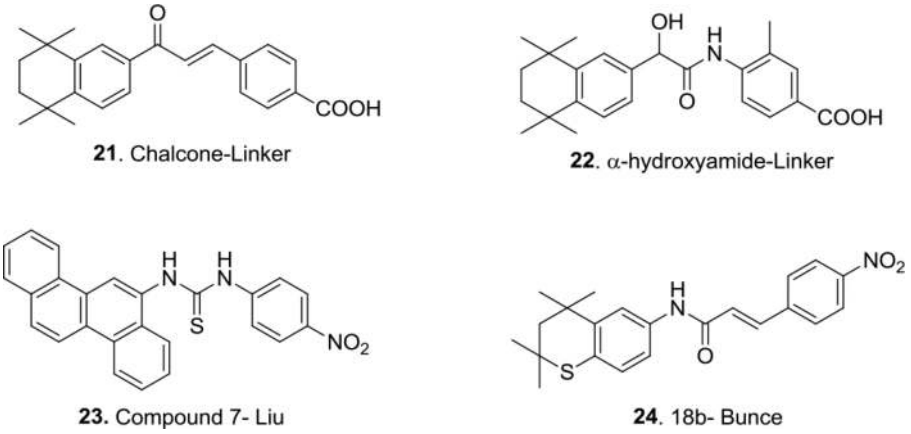

Figure 6. Three- and four-atom linkers in retinoid derivatives.

5.5. Four-atom linker

Another approach to the structure modification of SHetA2 was to keep the thiochroman ring but replace the thiourea linker with a 4-atom acrylamide linker NC(O)C=C and various substitutions on the terminal aryl ring. When evaluated in a cytotoxicity assay of the human A2780 ovarian cancer cell line, results indicate that activity of 4-nitro phenyl analogs are comparable to that of SHetA2 with efficacies slightly reduced compared to SHetA2 [48]. The α,β-unsaturated acrylamide may contribute to its increase in potency and the compound **24** (**Figure 6**) is the example.

6. Mechanism of action

The biological targets and SHetA2's mechanism of action have been extensively studied. Coimmunoprecipitation experiments demonstrated that SHetA2 interfered with the binding of mortalin, a molecular chaperone to p53 and p66 Src homologous-collagen homolog (p66shc) in A2780 ovarian cancer cell line, leading to mitochondrial swelling and mitophagy and finally apoptosis [8]. SHetA2 is also involved in modulating other cellular processes including cell-growth, differentiation, and angiogenesis.

6.1. Induction of apoptosis

6.1.1. Intrinsic pathway

SHetA2 is shown to selectively induce apoptosis in various cancer cell lines by targeting the mitochondria [32]. Exposure of SHetA2 to squamous carcinoma cells resulted in a decrease in mitochondrial permeability transition, followed by the release of cytochrome *c* into the cytoplasm, activation of caspase-3, and the induction of intrinsic apoptotic pathway. Further studies proposed that SHetA2-mediated mitochondrial swelling involved lowering the levels of anti-apoptotic proteins such as Bcl-XL and Bcl-2 in A2780 ovarian cancer cell [9]. However, the pro-apoptotic Bax expression was unaffected by SHetA2, suggesting SHetA2 may regulate the levels of Bcl-2 to promote apoptosis [9]. On the other hand, exposure of SHetA2 to normal ovarian or endometrial cells was found to increase both Bcl-XL and Bcl-2 protein levels. The up-regulation of these anti-apoptotic proteins may provide the cytoprotective effects necessary to block SHetA2-induced apoptosis in normal cells [9].

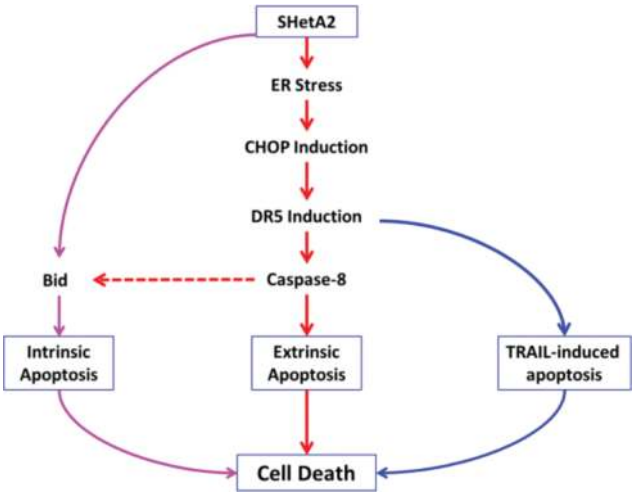

Figure 7. Pathways involving SHetA2-induced apoptosis.

6.1.2. Extrinsic pathway (death receptor pathway)

Exposure of non-small cell lung cancer cells (NSCLC) to SHetA2 revealed the induction of the extrinsic apoptotic pathway involving the death receptor 5 (DR5) [49]. It was shown that SHetA2-enhanced DR5 expression through the enhanced binding of CAAT/enhancer-binding protein homologous protein (CHOP) to its binding site located in the 5′-flanking region of the DR5 gene. Since CHOP is highly inducible during endoplasmic reticulum (ER) stress, this finding suggests that SHetA2 may act as an inducer of ER stress. The induction of DR5 expression leads to caspase 8-dependent apoptosis. Moreover, the induction of DR5 was shown to enhance tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)-induced apoptosis (**Figure 7**). Further studies found that down-regulation of cellular FLICE-inhibitory protein (c-FLIP), and to a lesser extent of survivin, were involved in SHetA2-induced apoptosis, as well as enhancement of TRAIL-initiated apoptosis [50]. c-FLIP is a major inhibitor of the extrinsic apoptotic pathway [51], while survivin modulates both intrinsic and extrinsic apoptotic pathways [52].

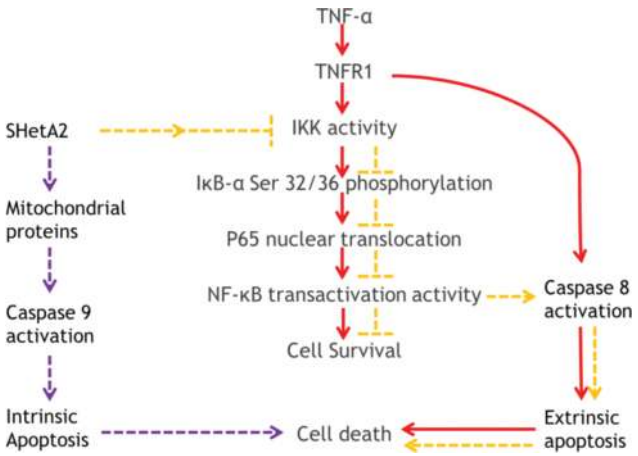

Figure 8. Working model for the apoptotic pathway involving NF-kB in ovarian cancer cells.

TRAIL and its receptors have also attracted much attention recently because TRAIL preferentially induces apoptosis in malignant cells while sparing most normal cells [53]. SHetA2 was found to sensitize ovarian carcinomas that are resistant to these DR ligands, without harming the normal cells [54, 55]. This suggests a possible synergistic effect Further investigation identified that downregulation of NF-kB transactivation activity by SHetA2 was involved in this sensitizing effect, triggering extrinsic apoptosis in cancer cells resistant to certain chemotherapeutics [55]. SHetA2 was shown to repress the upstream IkB kinase (IKK) activity that resulted in NF-kB downregulation. Apart from DR ligands, SHetA2 has also shown its chemosensitizing effects against cisplatin-resistant ovarian cancer through p53-independent pathways [6, 56]. However, such enhancement of sensitivities was not observed in resistant

uterine cancer cell lines for a number of chemotherapeutics in combination with SHetA2 treatment (**Figure 8**) [57].

	Apoptotic pathways affected by SHetA2		
	Possible biological targets	Effect	Reference
Intrinsic	↓ GSH	Redox regulation	[7]
	↓ *Bcl-2*	Antiapoptotic	[9]
	↓ *Bcl-XL*	Antiapoptotic	[9]
	↓ Survivin	Antiapoptotic	[50]
	↑ Caspase-9	Proapoptotic	[49]
	↑ Caspase-3	Proapoptotic	[32]
	↑ Bid	Proapoptotic	[49]
Extrinsic	↑ Caspase-8	Proapoptotic	[10]
	↑ Bip/GRP78, IRE1α, ATF4, XBP1	ER stress	[49]
	↑ DR-5	Cell death	[55]
	↑ CHOP	Cell death	[49]
	↓ IKK	Cell survival	[55]
	↓ NF-kB	Cell survival	[50]
	↓ c-FLIP	Antiapoptotic	[50]
Cell cycle pathways			
	↓ Cyclin D1	G_1 arrest	[11]
	↓ AP-1	Cell-cycle progression	[62]
Differentiation induction pathways			
	↑ E-Cadherin	Differentiation	[7]
	↑ MUC1	Differentiation	[44]
Antiangiogenic pathways			
	↑ Thrombospondin (TSP-4)	Antiangiogenic	[12]
	↓ Thymidine Phosphorylase (TP)	Angiogenic	[12]
	↓ VEGF	Angiogenic	[12]
Signal transduction pathways			
	↓ KIT kinase (c-KIT)	Cell death	[63]

Table 4. Possible biological pathways affected by SHetA2 and its anticancer activities.

6.1.3. ROS and GSH

Increased reactive oxygen species (ROS) level was initially thought to be responsible for SHetA2-induced apoptosis. Multiple studies have documented the ROS generation along with

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mitochondrial swelling and the release of cytochrome *c* in various cancer cell lines [6, 32]. Studies have also shown that SHetA2 is able to form adducts with glutathione (GSH) [7, 58]. This was reflected by the generation of ROS as reported in several pharmacokinetic studies [58, 59]. GSH is essential for cell survival. Adduct formation with GSH causes GSH depletion, leading to accumulation of ROS resulting in oxidative mitochondrial damage, ultimately causing cell death [60]. Although the exact role of GSH depletion in apoptosis is still controversial, it remains an early hallmark in the progression of cell death in numerous cell types [61]. Therefore, adduct formation between SHetA2 and GSH could be vital to the induction of apoptosis, as indicated by the ROS generation. However, further studies suggested that ROS generation appeared to be a consequence of, and not a cause for, mitochondrial swelling and apoptosis induced by SHetA2 treatment [7, 9]. Addition of GSH did not attenuate SHetA2-induced apoptosis [55]. It was also noted that the cellular GSH level was in the μM range, while μM SHetA2 is sufficient to kill cells, indicating that GSH depletion and ROS accumulation are not the only mechanisms of action. These findings suggest that SHetA2 probably acts through several mechanisms of action to bring about its apoptotic effect, along with other anticancer activities, such as cell cycle arrest and induction of differentiation (**Table 4**).

6.2. Induction of cell-cycle arrest and differentiation

Apart from apoptosis, SHetA2 repression of NF-kB expression also initiates a series of events that can lead to cell-cycle arrest and cell differentiation. SHetA2-induced Cyclin D1 degradation in both Caki-1 renal cancer and normal HK-2 cell lines results in the accumulation of cells in the G_0-G_1 phase [7]. Cyclin D1 degradation alone has shown to be sufficient in inducing G_1 cell cycle arrest [11]. Cyclin D1 transcription can be induced by NF-kB through multiple NF-kB-binding sites in the Cyclin D1 promoter, which is consistent with the observed down regulation of Cyclin D1. Also, SHetA2 can induce cellular differentiation in kidney [7], as well as in ovarian cancer organotypic cultures and xenografts [5]. Treatment of these cancer cells shows that 1 μM SHetA2 is sufficient to reverse the cancerous phenotype depending on the status of the cells. However, at higher concentrations of SHetA2, apoptosis would dominate over differentiation. The mechanism for differentiation induction is likely to be associated with the upregulation of E-Cadherin in renal cancer cells observed with the repression of NF-kB [7]. Decrease or loss of nuclear E-Cadherin expression is associated with poor prognosis in kidney cancer, as it confers the ability to migrate and invade [64]. Therefore, up-regulation or restoration of E-Cadherin's function has been one of the therapeutic goals for anticancer treatments.

6.3. Antiangiogenic activity

Microarray analysis have suggested that SHetA2 treatment results in significant up-regulation of thrombospondin-4 (TSP-4), and down-regulation of thymidine phosphorylase (TP) in A2780 ovarian cancer cells. TSP-4 and TP protein levels followed those of the mRNA. TSP-4 was hypothesized to be antiangiogenic due to the presence of type-III repeats which is also found in TSP-1. The type-III repeats in TSP-1 has been reported to inhibit the binding of fibroblast growth factor-2 to endothelial cells, a process that leads to endothelial cell proliferation *in vitro* [65]. As for TP, it has found to be angiogenic through its ability to convert thymidine to thymine

and the angiogenic 2-deoxy-$_D$-ribose-1-phosphate, as well as its metabolite, deoxy-$_D$-ribose. Protein levels of the angiogenic vascular endothelial growth factor A (VEGF) was also found to be downregulated despite its up-regulated mRNA level upon SHetA2 treatment. Interestingly, both mRNA and protein levels of the angiogenic basic fibroblast growth factor (bFGF) were also up-regulated, but its effect was deemed limited due to brief up-regulation of the gene only after hours of treatment with high concentration of SHetA2 [12]. Hence, the net effect of SHetA2 on these various proteins is antiangiogenic, and is supported by the decrease of endothelial tube formation observed in a number of cancer cell lines as well as human umbilical vascular endothelial cells (HUVECs) [12].

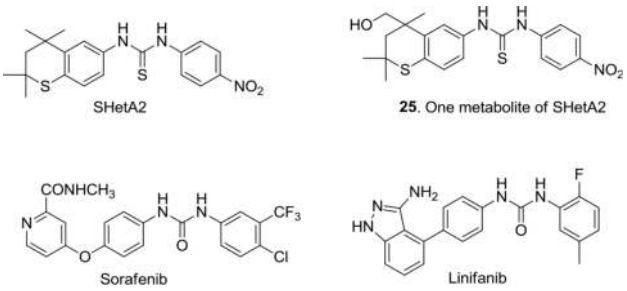

Figure 9. Structural comparison of SHetA2 and its metabolite with sorafenib and linifanib.

6.4. Kinase inhibitory activity

SHetA2 is also being evaluated for its ability to inhibit kinase activity based on its structural similarity to sorafenib and linifanib (**Figure 9**) [63]. Sorafenib is an FDA approved diarylurea multikinase inhibitor that inhibits tumor growth, while linifanib is a KIT-3 kinase inhibitor [66, 67]. All three compounds consist of a three atom urea or thiourea linker between two aromatic rings, of which this structural conformation is found to be vital for the formation of key H-bonds within the binding pockets of several kinases, including B-Raf, BCR-ABL, and KIT [63]. Upon evaluation with 442 different human kinases, SHetA2(**Figure 9**) has exhibited good binding affinity for KIT kinase (binding constant, Kd = 820 nM). This indicates that SHetA2 is a potential candidate for kinase inhibitor development. More importantly, one of the metabolites of SHetA2 (**Figure 9**, **25**) has also shown comparable binding affinity for KIT kinase (Kd = 1200 nM) [63]. This suggests that other metabolites of SHetA2 may also be active, which could have acted via different mechanisms of action and contributed to the various anticancer effects observed, making it a versatile chemotherapeutic agent.

7. Metabolism of SHetA2

Using liquid chromatography and tandem mass spectroscopy, four GSH adducts were identified along with four mono- and dihydroxylated SHetA2 metabolites [58]. At least one of

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these metabolites (**Figure 9**, **25**) has been deemed active against KIT kinase as mentioned earlier. Apart from the hydroxylated metabolites, other metabolites of SHetA2 were also detected *in vivo* [58, 59]. These metabolites may be produced upon formation of GSH adducts. The proposed mechanism for the formation of GSH adduct is shown in **Figure 10**. Subsequently, the GSH adduct could undergo further reactions that may result in the cleavage of the

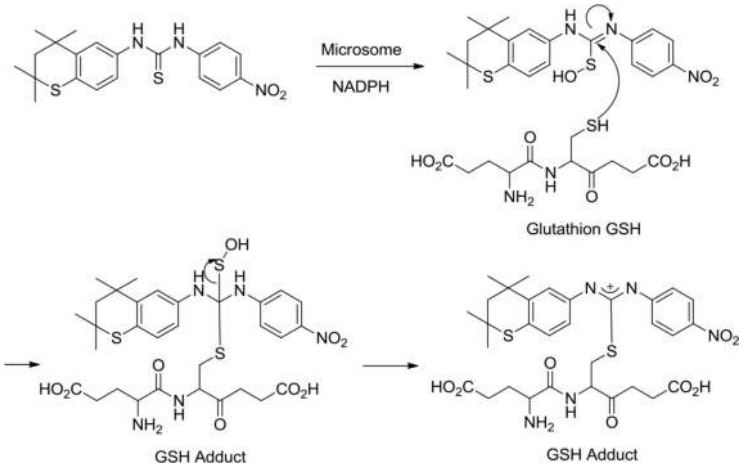

Figure 10. Proposed mechanism for the GSH adduct formation of SHetA2.

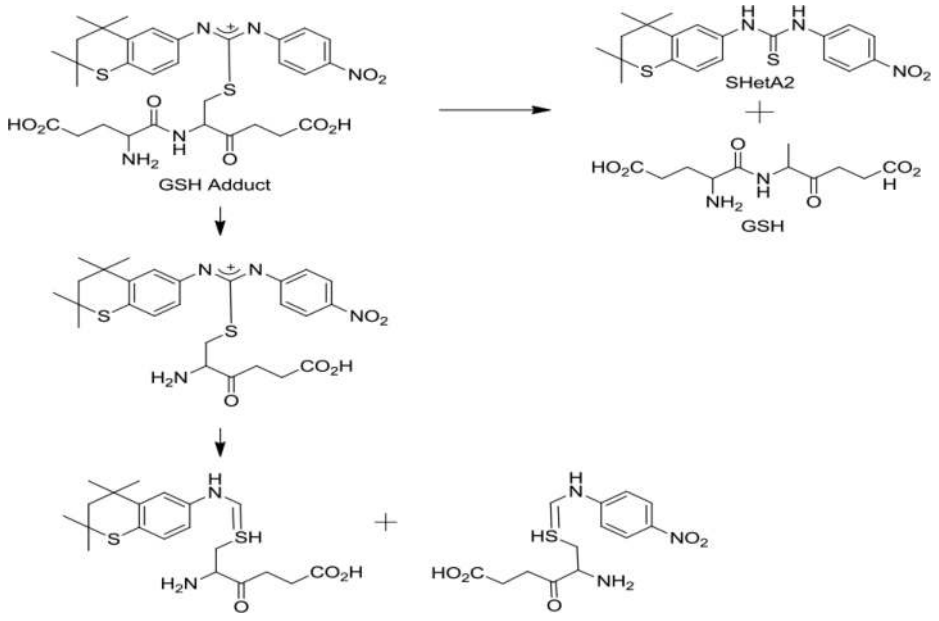

Figure 11. Proposed mechanism for the formation of metabolites following the formation of GSH adducts.

thiourea linker, yielding two metabolites that were also detected *in vivo* [58]. The proposed mechanism for the formation of these metabolites is shown in **Figure 11**.

Given that ShetA2 has several mechanisms of action involving various cellular targets, these findings suggest the possibility for SHetA2 to be metabolized into several active metabolites, each targeting a different molecular pathway. This is further supported by a recent *in vivo* study where the monohydroxy SHetA2 (**25**) was found to be the major metabolite of SHetA2 in rat plasma, and it was detected at a much higher concentration than the parent compound after oral and intravenous administration [13].

8. Pharmacokinetics

Pharmacokinetic studies using HPLC/UV of SHetA2 in mice(**Table 5**) have shown that pharmacokinetic profile of this compound is favorable for future development [59].

	PK	SHetA2
1	Oral bioavailability at 20 mg/kg	15%
	Oral bioavailability at 60 mg/kg	19%
2	Urinary excretion (%)	Not reported
3	Bound to mouse and human plasma proteins at μM concentrations	99.3–99.5%
4	Total body clearance (L/h/kg)	1.8
5	Volume distribution (L/kg) at steady state (*V*dss)	20.8
6	Half-life in mouse plasma	12.7 h
	Half-life in the mouse	Detectable after 60 h after IV administration
	Half-life in human plasma (once a day dosing proposed)	12 h
7	Peak time (plasma concentrations of 10 μM following i.v. bolus dose)	5 min
8	Peak concentration (IV 20 mg/kg dose of SHetA2 to mice)	10 μM after 5 min
	Maxima mean plasma concentration in the mouse (PO)	0.79 μM at 2 h
		2.35 μM at 3 h

Table 5. Pharmacokinetic data of SHetA2 [59].

9. Possible clinical applications

SHetA2 is now in Phase-0 clinical trial for ovarian cancer chemoprevention. However, since it inhibits growth of most cancers *in vitro* and *in vivo*, it may potentially be used for the prevention and treatment of other cancers which are listed in **Table 6**.

Cancer type	Findings	References
Ovarian cancer	G_1-phase cell cycle arrest through cyclin D1 degradation and apoptosis. Reduced cell survival, and increased sensitivity to TRAIL- and TNFα-induced apoptosis. Induced glandular differentiation reversed cancerous phenotype.	[14]
Uterine cancer • Endometrial carcinoma (HEC-1-A) • MMT (MES-SA) • uterine sarcoma (SK-UT-1)	SHetA2 decreased survival of all three cell lines, but did not increase sensitivities of cell lines to chemotherapeutic drugs.	[57]
Lung cancer (NSCLC)	Increased sensitivity to TRAIL-induced apoptosis through modulation of c-FLIP and upregulation of DR5. Inhibits growth, triggers ER stress.	[5, 49]
Head and neck cancer (HNSCC)	Apoptosis in 8 HNSCC cell lines.	[32]
Cervical cancer	Growth inhibition by micromolar concentrations.	[5]
Kidney cancer	Induced G_1 cell cycle arrest in Caki-1 and normal HK-2 cell lines; decreased cyclin D expression; induces apoptosis.	[7]

Table 6. Possible anti-cancer clinical applications of SHetA2 and documented results from different studies.

10. Conclusion

SHetA2 has exhibited anticancer activity in 60 NCI cancer cells and has shown favorable results in inhibiting various types of cancer growth *in vivo*, particularly ovarian cancer. It exerts various chemopreventive and chemotherapeutic activities through its ability to induce apoptosis and Differentiation, and inhibit angiogenesis and cell growth. It promotes mitochondrial swelling and mitophagy leading to apoptosis of cancer cells, while sparing normal cells. Once the targets are validated, tweaking of existing Flex-Hets or synthesis of newer related analogs may offer greater specificity and improved anticancer activity. Preclinical studies in animals have shown that SHetA2 has high efficacy with minimal toxicity and has a good pharmacokinetic profile. This provides the foundation for developing a novel class of more effective, chemo-preventive and anticancer drugs with a better therapeutic window.

Abbreviations

ATRA: All t*rans* retinoic acid
c-FLIP: Cellular FLICE-inhibitory protein
DR5: Death receptor 5

EC50:	50% Effective concentration
FDA:	US Food and Drug Administration
Flex-Hets:	Flexible heteroarotinoids
GI50:	50% Growth inhibition
GSH:	Glutathione
NCI:	National Cancer Institute
MTD:	Maximum tolerated dose
RA:	Retinoic acid
RAR:	Retinoic acid receptor
RAID:	Rapid access to intervention development
RAPID:	Rapid access to preventive intervention development
ROS:	Reactive oxygen species
RXR:	Retinoid X receptor
SAR:	Structure activity relationship
SHetA2:	[(4-Nitrophenyl)amino][2,2,4,4-tetramethylthiochroman-6-yl]amino]methane-thione
TP:	Thymidine phosphorylase
TRAIL:	Tumor necrosis factor-related apoptosis-inducing ligand
TSP-4:	Thrombospondin-4
TTNPB:	(E)-4-[2-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthylenyl)-1-propenyl] benzoic acid

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References

[1] Perloff M, Steele VE: Early-phase development of cancer prevention agents: Challenges and opportunities. Cancer Prev Res. 2013; 6:379–383.

[2] Naithani R, Huma LC, Moriarty RM, McCormick DL, Mehta RG: Comprehensive review of cancer chemopreventive agents evaluated in experimental carcinogenesis models and clinical trials. Curr Med Chem. 2008; 15:1044–1071.

[3] Bode AM, Dong Z: Cancer prevention research – then and now. Nat Rev Cancer. 2009; 9:508–516.

[4] Kummar S, Doroshow JH: Phase 0 trials: Expediting the development of chemoprevention agents. Cancer Prev Res. 2011; 4:288–292.

[5] Benbrook DM, Kamelle SA, Guruswamy SB, Lightfoot SA, Rutledge TL, Gould NS, Hannafon BN, Dunn ST, Berlin KD: Flexible heteroarotinoids (Flex-Hets) exhibit improved therapeutic ratios as anti-cancer agents over retinoic acid receptor agonists. Invest New Drugs. 2005; 23:417–428.

[6] Liu S, Brown CW, Berlin KD, Dhar A, Guruswamy S, Brown D, Gardner GJ, Birrer MJ, Benbrook DM: Synthesis of flexible sulfur-containing heteroarotinoids that induce apoptosis and reactive oxygen species with discrimination between malignant and benign cells. J Med Chem. 2004; 47:999–1007.

[7] Liu T, Masamha CP, Chengedza S, Berlin KD, Lightfoot S, He F, Benbrook DM: Development of flexible-heteroarotinoids for kidney cancer. Mol Cancer Ther. 2009; 8:1227–1238.

[8] Benbrook DM, Nammalwar B, Long A, Matsumoto H, Singh A, Bunce RA, Berlin KD: SHetA2 interference with mortalin binding to p66shc and p53 identified using drug-conjugated magnetic microspheres. Invest New Drugs. 2014; 32:412–423.

[9] Liu T, Hannafon B, Gill L, Kelly W, Benbrook D: Flex-Hets differentially induce apoptosis in cancer over normal cells by directly targeting mitochondria. Mol Cancer Ther. 2007; 6:1814–1822.

[10] Moxley KM, Chengedza S, Benbrook DM: Induction of death receptor ligand-mediated apoptosis in epithelial ovarian carcinoma: The search for sensitizing agents. Gynecol Oncol. 2009; 115:438–442.

[11] Masamha CP, Benbrook DM: Cyclin D1 degradation is sufficient to induce G1 cell cycle arrest despite constitutive expression of cyclin E2 in ovarian cancer cells. Cancer Res. 2009; 69:6565–6572.

[12] Myers T, Chengedza S, Lightfoot S, Pan Y, Dedmond D, Cole L, Tang Y, Benbrook DM: Flexible heteroarotinoid (Flex-Het) SHetA2 inhibits angiogenesis in vitro and in vivo. Invest New Drugs. 2009; 27:304–318.

[13] Kabirov KK, Kapetanovic IM, Benbrook DM, Dinger N, Mankovskaya I, Zakharov A, Detrisac C, Pereira M, Martin-Jimenez T, Onua E, et al.: Oral toxicity and pharmacokinetic studies of SHetA2, a new chemopreventive agent, in rats and dogs. Drug Chem Toxicol. 2013; 36:284–295.

[14] Available from: https://projectreporter.nih.gov/project_info_description.cfm?projectnumber=1R01CA196200-01A1 [accessed: 07/18/2016].

[15] Evans TR, Kaye SB: Retinoids: present role and future potential. Br J Cancer. 1999; 80:1–8.

[16] Lotan R: Retinoids in cancer chemoprevention. FASEB J. 1996; 10:1031–1039.

[17] Leid M, Retinoids. In: Abraham DJ, editor. Burger's Medicinal Chemistry and Drug Discovery. Vol 4, 6th ed. John Wiley & Sons, Inc. Hoboken, NJ. 2003:317-358.

[18] Smith W, Saba N: Retinoids as chemoprevention for head and neck cancer: where do we go from here? Crit Rev Oncol Hematol. 2005; 55:143–152.

[19] Benbrook DM: Refining retinoids with heteroatoms. Mini Rev Med Chem. 2002(3); 277–283

[20] Gottardis MM, Lamph WW, Shalinsky DR, Wellstein A, Heyman RA: The efficacy of 9-cis retinoic acid in experimental models of cancer. Breast Cancer Res Treat. 1996; 38:85–96.

[21] Shalinsky DR, Bischoff ED, Lamph WW, Zhang L, Boehm MF, Davies PJ, Nadzan AM, Heyman RA: A novel retinoic acid receptor-selective retinoid, ALRT1550, has potent antitumor activity against human oral squamous carcinoma xenografts in nude mice. Cancer Res. 1997; 57:162–168.

[22] Gale JB: Recent advances in the chemistry and biology of retinoids. Prog Med Chem. 1993; 30:1–55.

[23] Dawson MI, Fontana JA: The peptidomimetic, 1-adamantyl-substituted, and flex-het classes of retinoid-derived molecules: structure-activity relationships and retinoid receptor-independent anticancer activities. Mini Rev Med Chem. 2010; 10:455–491.

[24] Silverman AK, Ellis CN, Voorhees JJ: Hypervitaminosis A syndrome: a paradigm of retinoid side effects. J Am Acad Dermatol. 1987; 16:1027–1039.

[25] Garattini E, Gianni M, Terao M: Retinoid related molecules an emerging class of apoptotic agents with promising therapeutic potential in oncology: pharmacological activity and mechanisms of action. Curr Pharm Des. 2004; 10:433–448.

[26] Sun SY, Lotan R: Retinoids and their receptors in cancer development and chemoprevention. Crit Rev Oncol Hematol. 2002; 41:41–55.

[27] Henney JE: From the Food and Drug Administration. JAMA. 2000; 283 (9):1131

[28] Chen S, Ostrowski J, Whiting G, Roalsvig T, Hammer L, Currier SJ, Honeyman J, Kwasniewski B, Yu KL, Sterzycki R, et al.: Retinoic acid receptor gamma mediates topical retinoid efficacy and irritation in animal models. J Invest Dermatol. 1995; 104:779–783.

[29] Dawson MI, Hobbs PD, Derdzinski K, Chan RL, Gruber J, Chao WR, Smith S, Thies RW, Schiff LJ: Conformationally restricted retinoids. J Med Chem. 1984; 27:1516–1531.

[30] Benbrook DM, Subramanian S, Gale JB, Liu S, Brown CW, Boehm MF, Berlin KD: Synthesis and characterization of heteroarotinoids demonstrate structure specificity relationships. J Med Chem. 1998; 41:3753–3757.

[31] Waugh KM, Berlin KD, Ford WT, Holt EM, Carrol JP, Schomber PR, Thompson MD, Schiff LJ: Synthesis and characterization of selected heteroarotinoids. Pharmacological activity as assessed in vitamin A deficient hamster tracheal organ cultures. Single-crystal X-ray diffraction analysis of 4,4-dimethylthiochroman-6-yl methyl ketone 1,1-dioxide and ethyl (E)-p-[2-(4,4-dimethylthiochroman-6-yl)propenyl]benzoate. J Med Chem. 1985; 28:116–124.

[32] Chun KH, Benbrook DM, Berlin KD, Hong WK, Lotan R: The synthetic heteroarotinoid SHetA2 induces apoptosis in squamous carcinoma cells through a receptor-independent and mitochondria-dependent pathway. Cancer Res. 2003; 63:3826–3832.

[33] NCI 60 cell line screen dose response data for compound NSC 726189. Available from: http://dtp.nci.nih.gov/dtpstandard/servlet/dwindex?searchtype=NSC&chemname-boolean=and&outputformat=html&searchlist=726189&Submit=Submit

[34] Doppalapudi RS, Riccio ES, Davis Z, Menda S, Wang A, Du N, Green C, Kopelovich L, Rao CV, Benbrook DM, et al.: Genotoxicity of the cancer chemopreventive drug candidates CP-31398, SHetA2, and phospho-ibuprofen. Mutat Res. 2012; 746:78–88.

[35] Cimino MC: Comparative overview of current international strategies and guidelines for genetic toxicology testing for regulatory purposes. Environ Mol Mutagen. 2006; 47:362–390.

[36] Mic F, Molotkov A, Benbrook DM, Duester G: Retinoid activation of retinoic acid receptor but not retinoid X receptor is sufficient to rescue lethal defect in retinoic acid synthesis. Proc Natl Acad Sci U S A. 2003; 100:7135–7140.

[37] Tallent CR, Twine CE, Risbood P, Seltzman HH: An improved preparation of 2,2,4,4-tetramethyl-6-amiothiochroman, a key intermediate to urea and thiourea heteroarotinoids for anticancer studies. Org Prep Proced Int. 2007; 39:96–101.

[38] Jong L, Lehmann JM, Hobbs PD, Harlev E, Huffman JC, Pfahl M, Dawson MI: Conformational effects on retinoid receptor selectivity. 1. Effect of 9-double bond geometry on retinoid X receptor activity. J Med Chem. 1993; 36:2605–2613.

[39] Benbrook DM, Madler MM, Spruce LW, Birckbichler PJ, Nelson EC, Subramanian S, Weerasekare GM, Gale JB, Patterson MK, Jr., Wang B, et al.: Biologically active heteroarotinoids exhibiting anticancer activity and decreased toxicity. J Med Chem. 1997; 40:3567–3583.

[40] Willhite CC, Jurek A, Sharma RP, Dawson MI: Structure-affinity relationships of retinoids with embryonic cellular retinoic acid-binding protein. Toxicol Appl Pharmacol. 1992; 112:144–153.

[41] Le TC, Berlin KD, Benson SD, Eastman MA, Bell-Eunice G, Nelson AC, Benbrook DM: Heteroarotinoids with anti-cancer activity against ovarian cancer cells. Open Med Chem J. 2007; 1:11–23.

[42] Zacheis D, Dhar A, Lu S, Madler MM, Klucik J, Brown CW, Liu S, Clement F, Subramanian S, Weerasekare GM, et al.: Heteroarotinoids inhibit head and neck cancer cell lines in vitro and in vivo through both RAR and RXR retinoic acid receptors. J Med Chem. 1999; 42:4434–4445.

[43] Dhar A, Liu S, Klucik J, Berlin KD, Madler MM, Lu S, Ivey RT, Zacheis D, Brown CW, Nelson EC, et al.: Synthesis, structure-activity relationships, and RARgamma-ligand interactions of nitrogen heteroarotinoids. J Med Chem. 1999; 42:3602–3614.

[44] Guruswamy S, Lightfoot S, Gold M, Hassan R, Berlin KD, Ivey RT, Benbrook DM: Effects of retinoids on cancerous phenotype and apoptosis in organotypic cultures of ovarian carcinoma. J Natl Cancer Inst. 2001; 93:516–525.

[45] Bernard BA, Bernardon JM, Delescluse C, Martin B, Lenoir MC, Maignan J, Charpentier B, Pilgrim WR, Reichert U, Shroot B: Identification of synthetic retinoids with selectivity for human nuclear retinoic acid receptor gamma. Biochem Biophys Res Commun. 1992; 186:977–983.

[46] Klaholz BP, Mitschler A, Belema M, Zusi C, Moras D: Enantiomer discrimination illustrated by high-resolution crystal structures of the human nuclear receptor hRAR-gamma. Proc Natl Acad Sci U S A. 2000; 97:6322–6327.

[47] Liu S, Louie MC, Rajagopalan V, Zhou G, Ponce E, Nguyen T, Green L: Synthesis and evaluation of the diarylthiourea analogs as novel anti-cancer agents. Bioorg Med Chem Lett. 2015; 25:1301–1305.

[48] Gnanasekaran KK, Benbrook DM, Nammalwar B, Thavathiru E, Bunce RA, Berlin KD: Synthesis and evaluation of second generation Flex-Het scaffolds against the human ovarian cancer A2780 cell line. Eur J Med Chem. 2015; 96:209–217.

[49] Lin YD, Chen S, Yue P, Zou W, Benbrook DM, Liu S, Le TC, Berlin KD, Khuri FR, Sun SY: CAAT/enhancer binding protein homologous protein-dependent death receptor 5 induction is a major component of SHetA2-induced apoptosis in lung cancer cells. Cancer Res. 2008; 68:5335–5344.

[50] Lin Y, Liu X, Yue P, Benbrook DM, Berlin KD, Khuri FR, Sun SY: Involvement of c-FLIP and survivin down-regulation in flexible heteroarotinoid-induced apoptosis and enhancement of TRAIL-initiated apoptosis in lung cancer cells. Mol Cancer Ther. 2008; 7:3556–3565.

[51] Irmler M, Thome M, Hahne M, Schneider P, Hofmann K, Steiner V, Bodmer JL, Schroter M, Burns K, Mattmann C, et al.: Inhibition of death receptor signals by cellular FLIP. Nature. 1997; 388:190–195.

[52] Altieri DC: Validating survivin as a cancer therapeutic target. Nat Rev Cancer. 2003; 3:46–54.

[53] Kelley SK, Ashkenazi A: Targeting death receptors in cancer with Apo2L/TRAIL. Curr Opin Pharmacol. 2004; 4:333–339.

[54] Moxley K, Chengedza S, Benbrook DM: Induction of death receptor ligand -mediated apoptosis in epithelial ovarian carcinoma: the search for sensitizing agents. Gynecol Oncol. 2010; 115:438–442.

[55] Chengedza S, Benbrook DM: NF-kappaB is involved in SHetA2 circumvention of TNF-alpha resistance, but not induction of intrinsic apoptosis. Anticancer Drugs. 2010; 21:297–305.

[56] Guruswamy S, Benbrook DM: Retinoids chemosensitize ovarian cancer cell lines to cisplatin independent of nuclear receptors and p53. Am J Pharmacol Toxicol. 2006; 1:87–93.

[57] Hyde J, Benbrook DM: Sensitivities of uterine adenocarcinoma, mixed mullerian tumor (MMT) and sarcoma cell lines to chemotherapeutic agents and a flex-het drug. Am J Pharmacol Toxicol. 2006; 1:83–86.

[58] Liu Z, Zhang Y, Hua YF, Covey JM, Benbrook DM, Chan KK: Metabolism of a sulfur-containing heteroarotionoid antitumor agent, SHetA2, using liquid chromatography/tandem mass spectrometry. Rapid Commun Mass Spectrom. 2008; 22:3371–3381.

[59] Zhang Y, Hua Y, Benbrook DM, Covey JM, Dai G, Liu Z, Chan KK: High performance liquid chromatographic analysis and preclinical pharmacokinetics of the heteroarotinoid antitumor agent, SHetA2. Cancer Chemother Pharmacol. 2006; 58:561–569.

[60] Trachootham D, Zhou Y, Zhang H, Demizu Y, Chen Z, Pelicano H, Chiao PJ, Achanta G, Arlinghaus RB, Liu J, et al.: Selective killing of oncogenically transformed cells through a ROS-mediated mechanism by beta-phenylethyl isothiocyanate. Cancer Cell. 2006; 10:241–252.

[61] Franco R, Schoneveld OJ, Pappa A, Panayiotidis MI: The central role of glutathione in the pathophysiology of human diseases. Arch Physiol Biochem. 2007; 113:234–258.

[62] Benbrook DM, Lightfoot S, Ranger-Moore J, Liu T, Chengedza S, Berry WL, Dozmorov I: Gene expression analysis of biological systems driving an organotypic model of endometrial carcinogenesis and chemoprevention. Gene Regul Syst Bio. 2008; 2:21–42.

[63] Nammalwar B, Bunce RA, Benbrook DM, Lu T, Li H-F, Chen Y-D, Berlin KD: Synthesis of N-[3,4-dihydro-4-(acetoxymethyl)-2, 2, 4-trimethyl-2 H-1-benzothiopyran-6-yl]-N′-(4-nitrophenyl) thiourea and N-[3, 4-dihydro-4-(hydroxymethyl)-2, 2, 4-trimethyl-2 H-1-benzothiopyran-6-yl]-N′-(4-nitrophenyl) thiourea, a major metabolite of N-(3, 4-dihydro-2, 2, 4, 4-tetramethyl-2 H-1-benzothiopyran-6-YL)-N′-(4-nitrophenyl) thiourea. Phosphorus Sulfur Silicon. 2010; 186:189–204.

[64] Howard EW, Camm KD, Wong YC, Wang XH: E-cadherin upregulation as a therapeutic goal in cancer treatment. Mini Rev Med Chem. 2008; 8:496–518.

[65] Margosio B, Rusnati M, Bonezzi K, Cordes BL, Annis DS, Urbinati C, Giavazzi R, Presta M, Ribatti D, Mosher DF, et al.: Fibroblast growth factor-2 binding to the thrombospondin-1 type III repeats, a novel antiangiogenic domain. Int J Biochem Cell Biol. 2008; 40:700–709.

[66] Wilhelm S, Carter C, Lynch M, Lowinger T, Dumas J, Smith RA, Schwartz B, Simantov R, Kelley S: Discovery and development of sorafenib: a multikinase inhibitor for treating cancer. Nat Rev Drug Discov. 2006; 5:835–844.

[67] Albert DH, Tapang P, Magoc TJ, Pease LJ, Reuter DR, Wei RQ, Li J, Guo J, Bousquet PF, Ghoreishi-Haack NS, et al.: Preclinical activity of ABT-869, a multitargeted receptor tyrosine kinase inhibitor. Mol Cancer Ther. 2006; 5:995–1006.