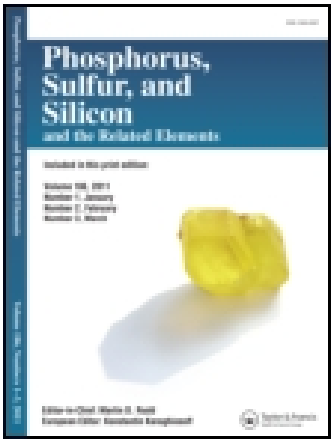

Phosphorus, Sulfur, and Silicon and the Related Elements
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Synthesis of N-[3,4-Dihydro-4-(acetoxymethyl)-2,2,4-trimethyl-2H-1-benzothiopyran-6-yl]-N′-(4-nitrophenyl)thiourea and N-[3,4-dihydro-4-(hydroxymethyl)-2,2,4-trimethyl-2H-1-benzothiopyran-6-yl]-N′-(4-nitrophenyl)thiourea, a Major Metabolite of N-(3,4-Dihydro-2,2,4,4-tetramethyl-2H-1-benzothiopyran-6-YL)-N′-(4-nitrophenyl)thiourea

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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

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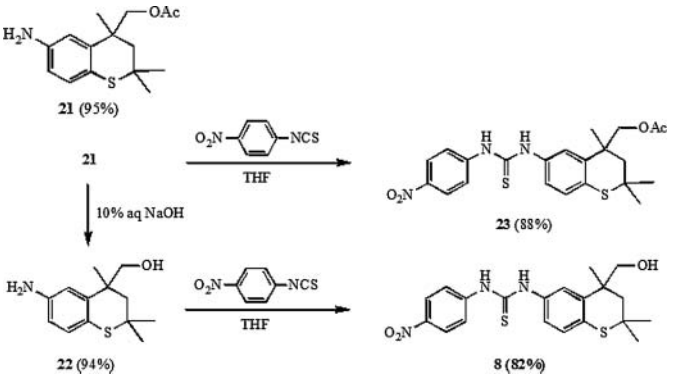
GRAPHICAL ABSTRACT



Abstract *The compound N-(3,4-dihydro-2,2,4,4-tetramethyl-2H-1-benzothiopyran-6-yl)-N′-(4-nitrophenyl)thiourea [NSC 726189] has exhibited strong anticancer activity in several*

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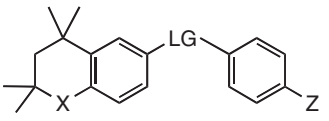
assays and cell lines and is under consideration for clinical trials for the possible treatment of kidney cancer. Since the heterocycle has not shown any significant toxicity in animal studies, it is conceivable that a metabolite could be the active agent. In a previous study, major metabolites were tentatively identified via analyses of materials extracted from rat and human liver microsomes, as well as extracts from rat urine, by the use of HPLC-UV and MS/MS. However, no metabolite has been obtained in pure form. This article outlines a de novo synthesis of a primary metabolite, namely N-[3,4-dihydro-4-(hydroxymethyl)-2,2,4-trimethyl-2H-1-benzothiopyran-6-yl]-N′-(4-nitrophenyl)thiourea. A prodrug of the metabolite was also prepared, namely N-[3,4-dihydro-4-(acetoxymethyl)-2,2,4-trimethyl-2H-1-benzothiopyran-6-yl]-N′-(4-nitrophenyl)thio-urea.

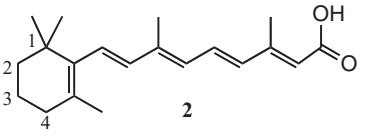
Keywords *N*-[3,4-Dihydro-4-(acetoxymethyl)-2,2,4-trimethyl-2*H*-1-benzothiopyran-6-yl]-*N*′-(4-nitrophenyl)thiourea; *N*-[3,4-dihydro-4-(hydroxymethyl)-2,2,4-trimethyl-2*H*-1-benzothiopyran-6-yl]-*N*′-(4-nitrophenyl)thiourea; multi-step synthesis

INTRODUCTION

Kidney cancer is known to have a high mortality rate due to its resistance to a variety of therapies.[1] The incidence of renal cancer appears to have increased over the last 50 years.[2] Preliminary evidence is available that demonstrates that certain heteroarotinoids can be effective in the treatment of such cancers.[3,4] The basic structural unit **1** was originally designed for heteroarotinoids as illustrated,[4] and was based, in part, on a structural relationship with *trans*-retinoic acid (**2**). Studies of the biological activity of **2** and isomers thereof revealed them to possess high toxicity and therefore limited utility.[5] Pioneering work in



1 X = O, S. NR; Z = CO_2H, CO_2R, SO_3H, NO_2; LG = Linking group; amide, alkene ester, urea, thiourea



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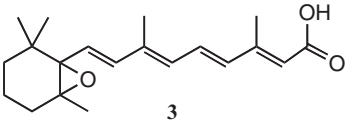

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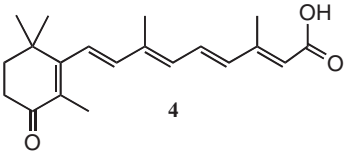

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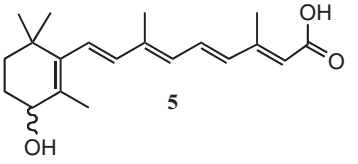

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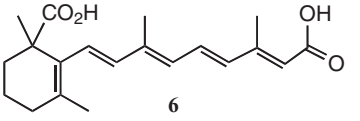

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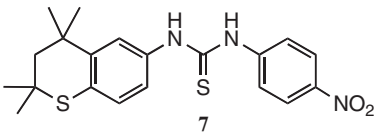

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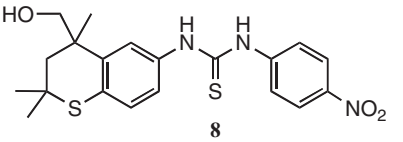
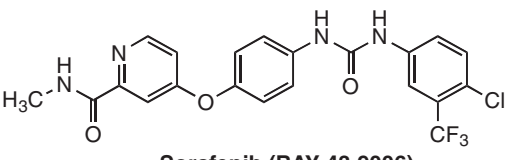


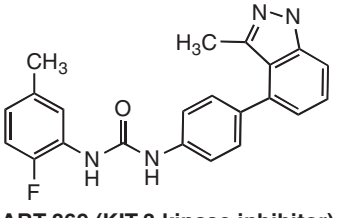
8

which heteroatoms were inserted into strategic positions within the molecular framework, as shown in **1**, reduced the toxicity significantly.[4,6] Since an aryl ring was also present, the term "heteroarotinoids" was established to identify such systems.[4,6] High toxicity associated with acid **2** has been presumed to arise from its metabolites, the major members of which are shown as **3**–**6**.[7] An initial objective was to ascertain if heteroarotinoids with a heteroatom at C-4 had reduced toxicity and could avoid oxidation at this position, which might lead to derivatives of **2**, such as **4** and **5**, which were toxic. The hypothesis that heteratoms do reduce toxicity has been validated.[4,6,8,9] Moreover, the addition of the benzene ring fused to the cyclohexyl unit in **1** would also prevent epoxidation, which produced toxic **3** from **2**. The versatility of heteroarotinoids in treating a variety of cancers is evident in that good activity has been observed against breast, head/neck, kidney, and lung cancers.[8–23]

It is significant to recall that the development of useful drugs has often required an improvement in hydrophilicity, which increases aqueous solubility, frequently without loss of biological activity.[24,25] Examples related to our system **7** are certain diarylurea multikinase inhibitors that inhibit tumor growth.[26,27] Sorafenib (BAY 43–9006) is such a compound that was developed by increasing the hydrophilicity of its precursor, which led to enhanced *in vivo* potency.[27–30] The ability of Sorafenib to prolong progression-free survival in recurrent kidney cancer patients with manageable toxicity led to its FDA approval.[31,32] However, the toxicity of this agent remains a problem.[32] Since *N*-(3,4-dihydro-2,2,4,4-tetramethyl-2*H*-1-benzothiopyran-6-yl)-*N*′-(4-nitrophenyl)-thiourea (**7**, NSC 726189) has shown high activity in the treatment of certain kidney cancerous cell lines without any significant toxicity in studies by the NCI,[22] the metabolites may not be toxic. The increased polarity of the metabolites could provide a number of pharmaceutical and pharmacokinetic advantages with increased aqueous solubility. Higher hydrophilicity correlates well with reduced lipid tissue uptake,[33] typically resulting in higher blood or plasma concentrations available for delivery to target tumor cells. Since **7** is a candidate for the potential treatment of kidney cancer,[22,34] the importance of meeting the Metabolites in Safety Testing (MIST) standards for metabolites of potential drugs is well documented.[35–38] One metabolite[39] of **7**, and the synthetic target molecule of this article, is *N*-[3,4-dihydro-4-(hydroxymethyl)-2,2,4-trimethyl-2*H*-1-benzothiopyran-6-yl]-*N*′-(4-nitrophenyl)thiourea (**8**). Urea derivatives as kinase inhibitors are well recognized with many such inhibitors being marketed by American Custom Chemicals (P.O. Box 910574, San Diego, CA 92191–0574, USA), including the examples Sorafenib and ABT-869, as systems with urea linkages between aryl groups as in **7** but with the latter having a thiourea group.



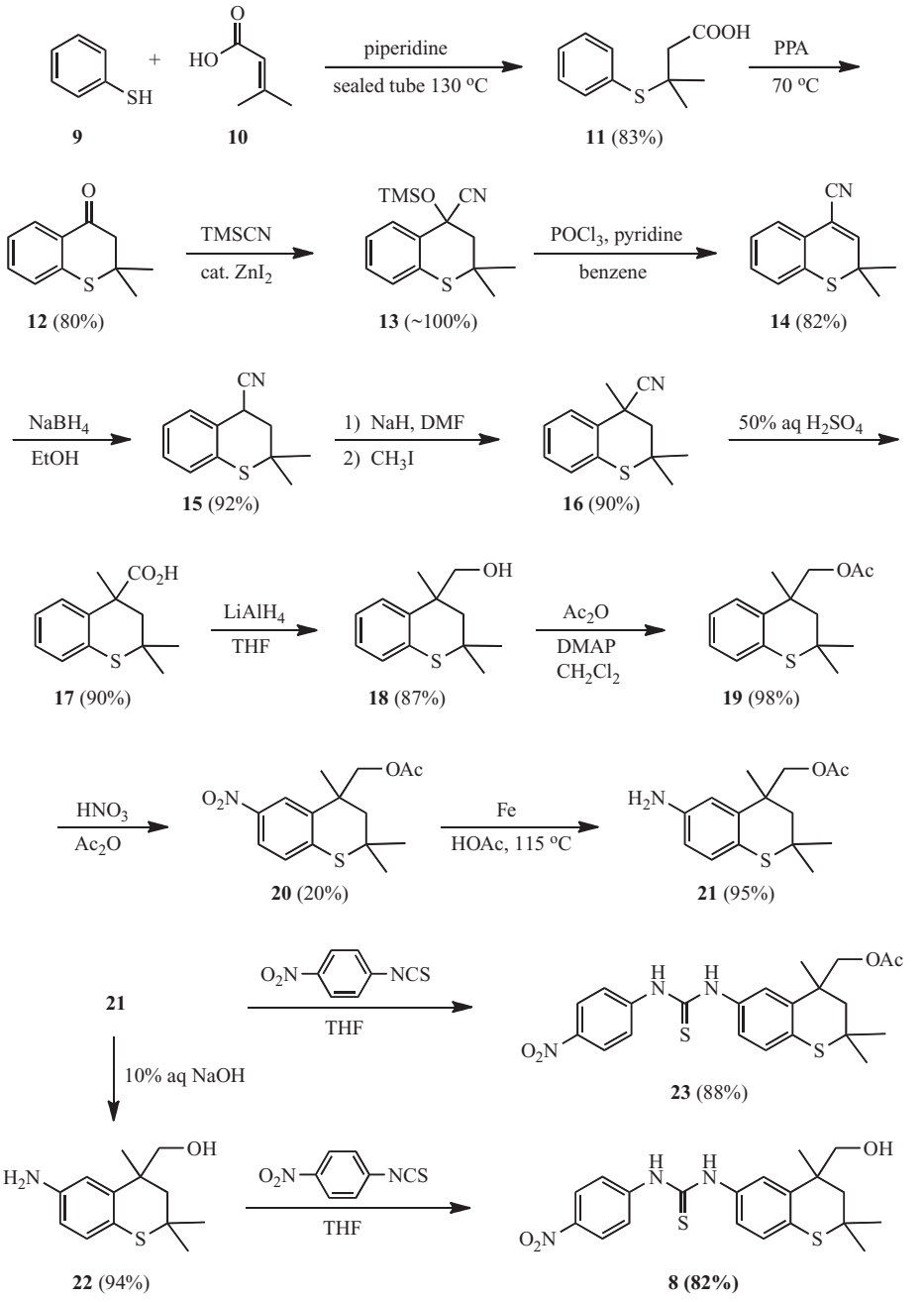
Sorafenib (BAY 43 9006)



ABT-869 (KIT-3 kinase inhibitor)
[CAS Number: 796967-16-3]

CHEMISTRY

Michael addition of thiophenol (**9**) to 3-methyl-2-butenoic acid in standard fashion gave 3-(phenylthio)-3-butenoic acid (**10**), which, when subjected to treatment with



Scheme 1

polyphosphoric acid, led to the known 2,2-dimethylthiochroman-4-one (**11**) in 66% overall yield (Scheme 1).[40] Conversion of **12** to the trimethylsilyl cyanohydrin **13** and treatment directly with phosphorus oxychloride in benzene/pyridine provided the unsaturated carbonitrile **14** in a yield of 82%.[41] Saturation of the double bond in **14** with sodium borohydride

generated **15**, which was smoothly alkylated to 2,2,4-trimethylthiochroman-4-carbonitrile (**16**, 83% overall yield). Hydrolysis of the nitrile group in **16** gave acid **17**, which was immediately reduced to the corresponding key intermediate alcohol **19** (78% for two steps). Acylation of **18** with acetic anhydride in methylene chloride in the presence of DMAP then gave the corresponding acetate intermediate **19** in near quantitative yield.[42]

Although the prior steps could be optimized to give very good to excellent yields, the nitration of **19** resulted in a modest return (∼20%) of the required nitroester **20** due to several side reactions. This experience had been noted previously[15] in nitration of the related 2,2,4,7-pentamethylthiochroman system, where such reactions markedly reduced the production of 2,2,4,4,7-pentamethyl-6-nitrothiochroman. Reduction of the nitro group in **20** using iron and acetic acid at 115°C afforded amine **21** in a near quantitative yield. Saponification of **21** was easily accomplished to give amino alcohol **22**. Treatment of **21** and **22** with 4-nitrophenylisothiocyanate in dry THF followed a similar pattern employed in the final step to create **7**,[15] and gave **23** and **8**, respectively, in high returns. Both crystalline products had very similar melting points, but a mixture melting point determination confirmed the different individual structures as did elemental analyses. Ester **23** can be considered as a prodrug for alcohol **8**.

RESULTS AND DISCUSSION

In view of the high activity[8–23] and low toxicity[9,15,22,43] of heteroarotinoids, as well as a structural relationship to diarylureas, which interact with many kinases, a theoretical investigation of the binding properties of **7** and **8** was initiated. Sorafenib is a well-known multitarget kinase inhibitor, inhibiting the isoforms of Raf, KIT, VEGFR, PDGFR, and others, which was approved for the treatment of advanced renal cell cancer (RCC) by the FDA in 2005. Moreover, the Raf family of kinases is the main target of Sorafenib, among which B-Raf is the most important member and an intensely studied anticancer target. Since **7** and **8** are structurally similar to Sorafenib, a preliminary assessment of their binding affinity and plausible binding mode was performed using molecular docking (employing Glide as the docking program) on **7** with the crystal structure of B-Raf (PDB code: 1UWJ). In the molecular docking study, Sorafenib was incorporated as a reference, although the modeling showed that the Glide score[44,45] of **7** (−7.7) was higher than that of Sorafenib (−10.8) and suggested that **7** might be less potent than sorafenib.[46] However, **7** was oriented in a similar manner to Sorafenib (as shown in Figure 1). Both the urea group in Sorafenib and the thiourea group in **7** formed critical H−bonds with Glu501 and Asp594. Thus, it is predictable that **7** and its metabolite **8** would inhibit B-Raf.

It is known that B-Raf is a good therapeutic target for new agents,[47] and so, it is predictable that metabolites of **7** would inhibit kinases, especially members of the Raf family. For experimental confirmation, **7** was submitted to Ambit Biosciences Corporation (4215 Sorrento Valley Blvd, San Diego, CA 92121, USA) for evaluation of kinase activity with 442 different human kinases. The technique is an active-site–dependent competition binding assay with an immobilized, active site directed ligand.[48] However, in the competitive binding studies, the percent control for **7** with B-Raf[48,49] was 87%, while that of its mutant [B-Raf-V600E] was 77%. In contrast, agent **7** exhibited a percent control of 2.6% with KIT-V559D, 5.2% with KIT, 11% with KIT-L576P, 17% with KIT-V559D,V6, and 12% with the kinase PDGFRB. Consequently, binding constant Kd values were determined for each kinase: KIT (820 nM), KIT-V559D (1300 nM), KIT-V559D,V6 (2700 nM), PDGFRB (2900 nM), and KIT-L576P (3800 nM).[46] Thus, activity at the nanomolar level, coupled with

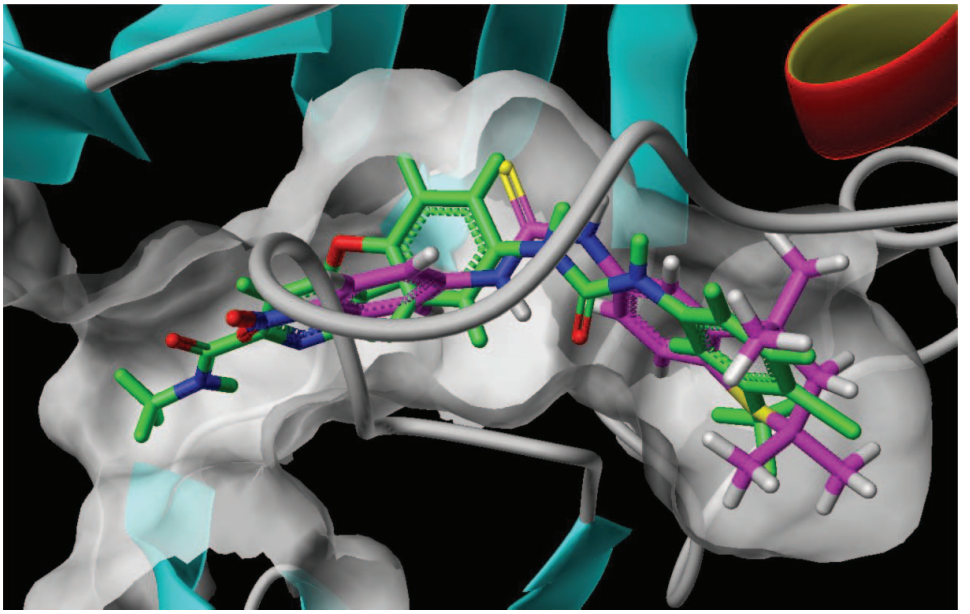


Figure 1 Sorafenib and **7** were docked into the binding sites of B-Raf. Sorafenib and **7** are shown in green stick and magnenta stick representation, respectively, and the binding pocket is shown as the white surface.

low toxicity,[9,15,22] suggests that **7** has a theoretical and experimental basis for development of an inhibitor of KIT. Sorafenib is also known to be an inhibitor of KIT (also known as c-KIT).[50]

Similar experiments with metabolite **8** revealed competitive inhibition with an immobilized, active-site–directed ligand in terms of percent control with values of 15% for KIT, 15% for KIT-V559D, and 17% for PDGFRB kinases. The corresponding Kd values were 1200 nM, 4100 nM, and 3400 nM, respectively. Thus, both **7** and **8** exhibited good inhibitory activity against the KIT kinase. As additional support of our work, molecular docking experiments of metabolite **8** with the crystal structure of kinase KIT (PDB code: 1t46) were conducted to explore a possible binding mode. In the complex of KIT with the known anticancer agent Imatinib, there exist four hydrogen bonds with residues Cys673, Thr670, Asp810, and Glu640.[47] As shown in Figure 2, the docking conformation of **8** occupied the same docking pocket[49] and adopted a similar binding mode to Imatinib by forming H-bond interactions with Glu640 and Asp810 and hydrophobic contacts with residues in the hydrophobic pocket. Moreover, Imatinib is selective for BCR-ABL, inhibiting its tyrosine kinase activity as well as several other kinases.[46] The Glide docking scores are a measure of the binding affinities of the ligands for the kinase.[44,47] The scores were −11.97 for Imatinib and −8.70 for metabolite **8**.[44] (The lower the score, the greater the affinity.) Consequently, potential useful activity is predicted for the metabolite **8**. It is noteworthy that KIT kinase mutants have shown resistance to Imatinib in gastrointestinal stromal tumor patients.[47] Thus, the continued search for new agents is a worthy goal, and the identification of **8** provides insight into the development of other KIT kinase inhibitors.

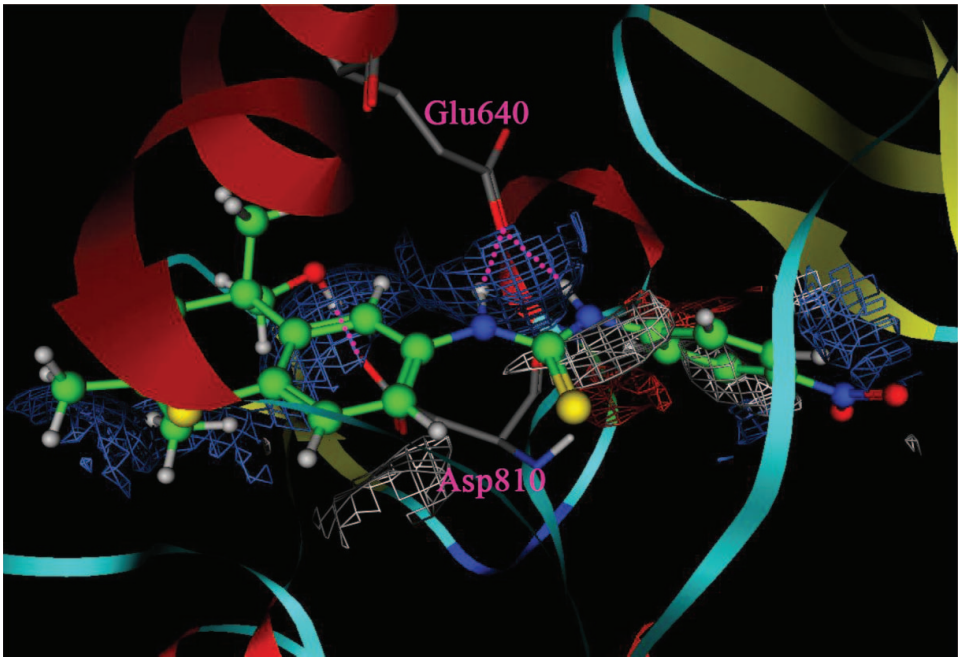


Figure 2 Plausible binding mode of **8** with the crystal structure of KIT by molecular docking. Agent **8** is in green stick and ball representation, and the magenta dashed lines represent the hydrogen bond interactions. The line surfaces in different colors represent hydrogen bond donor (blue), hydrogen bond acceptor (red), and hydrophobic (white) interactions, respectively.

CONCLUSIONS

The synthesis of the first metabolite of *N*-(3,4-dihydro-2,2,4,4-tetramethyl-2*H*-1-benzothiopyran-6-yl)-*N*′-(4-nitrophenyl)thiourea (NSC 726189) was accomplished, namely *N*-[3,4-dihydro-4-(hydroxymethyl)-2,2,4-trimethyl-2*H*-1-benzothiopyran-6-yl]-*N*′-(4-nitrophenyl)thiourea. In addition, the acetate derivative of the metabolite, namely *N*-[3 ,4-dihydro-4-(acetoxymethyl)-2,2,4-trimethyl-2*H*-1-benzothiopyran-6-yl]-*N*′-(4-nitrophenyl)-thiourea, was also prepared as a possible prodrug. The compounds await biological evaluation for anticancer activity.

EXPERIMENTAL

All reactions were run under dry nitrogen in oven-dried glassware. Reactions were monitored by thin layer chromatography on silica gel GF plates (Analtech No. 21521) using ultraviolet detection. Preparative separations were performed by flash column chromatography on silica gel (grade 62, 60–200 mesh) mixed with UV-active phosphor (Sorbent Technologies No. UV-5); band elution was monitored using a handheld UV lamp. Melting points were uncorrected. FT-IR spectra were taken as thin films on NaCl disks. Unless otherwise indicated, ^{1}H and ^{13}C NMR spectra were measured in $DCCl_3$ at 300 MHz and 75 MHz, respectively, and were referenced to internal TMS with coupling constants (*J*)

in Hz. Elemental analyses were provided by Atlantic Microlab (Norcross, GA). Tetrahydrofuran (THF) was dried over KOH pellets and distilled from $LiAlH_4$ under nitrogen. Commercial anhydrous *N,N*-dimethylformamide (DMF) was transferred by syringe into reactions where required. Other reagents were used as received.

N-[3,4-Dihydro-4-(hydroxymethyl)-2,2,4-trimethyl-2*H*-1-benzothiopyran-6-yl]-*N'*-(4-nitrophenyl)thiourea (8)

The alcohol (97 mg, 82%) was prepared via the general procedure employed for **23** using alcohol **22** (66 mg, 0.28 mmol) and 4-nitrophenyl isothiocyanate (54 mg, 0.30 mmol). The product was purified by recrystallization [H_3COH], mp 154–155°C. IR: 3435 cm^{-1}; ^1H NMR (CD_3OD): δ 8.24 (d, $J = 9.0$ Hz, 2 H, Ar-H), 7.83 (d, $J = 9.0$ Hz, 2 H, Ar-H), 7.56 (d, $J = 2.2$ Hz, 1 H, Ar-H), 7.18 (dd, $J = 8.2, 2.2$ Hz, 1 H, Ar-H), 7.13 (d, $J = 8.2$ Hz, 1 H, Ar-H), 3.63 (d, $J = 11.0$ Hz, 1 H, $C\underline{H}_2$-OH), 3.57 (d, $J = 11.0$ Hz, 1 H, $C\underline{H}_2$OH), 2.36 (d, $J = 14.3$ Hz, 1 H, CH_2-ring), 1.82 (d, $J = 14.3$ Hz, 1 H, CH_2-ring), 1.44 (s, 3 H, CH_3), 1.40 (s, 6 H, $(CH_3)_2$); the 2 NH and 1 OH exchanged with D_3COD. ^{13}C NMR (CD_3OD): δ 181.5, 147.2, 144.9, 141.5, 136.8, 133.2, 129.7, 125.3, 125.2, 123.7, 123.6, 72.2, 50.2, 42.9, 42.0, 31.6, 31.1, 27.7. *Anal*. Calcd for $C_{20}H_{23}N_3O_3S_2$: C, 57.53; H, 5.55; N, 10.06; S, 15.36. Found: C, 57.45; H, 5.52; N, 10.05; S, 15.23. TLC analysis, using 5% methanol in $HCCl_3$, showed one spot.

3-(Phenylthio)-3-methylbutanoic Acid (11)

A 200-mL, Pyrex pressure vessel (Chemglass No. CG-1880-R-112) was charged with 3-methyl-2-butenoic acid (**10**, 30.0 g, 0.30 mol), piperidine (27.0 g, 0.32 mol, 1.05 eq), and thiophenol (**9**, 33.0 g, 0.30 mol), and then sealed and heated at 130°C for a period of 24 h. The resulting mixture was cooled to room temperature and diluted with 1 L of ether. The ether layer was washed with 1 *N* HCl (3 × 200 mL), water (1×), and saturated NaCl (1×). The organic solution was dried (Na_2SO_4) and concentrated under vacuum to give a pale yellow solid. The compound was recrystallized (benzene:petroleum ether, 1:3) to give **11** (52.3 g, 83%) as an off-white solid, mp 70–72°C [lit[40] mp 71°C]. IR: 3700–2350, 1707 cm^{-1}; ^1H NMR: δ 10–11 (br s, 1 H, CO_2H), 7.57 (dd, $J = 7.7, 1.6$ Hz, 2 H, Ar-H), 7.36 (m, 3 H, Ar-H), 2.56 (s, 2 H, CH_2), 1.41 (s, 6 H, $C(CH_3)_2$); ^{13}C NMR: δ 177.0, 137.7, 131.1, 129.2, 128.7, 46.7, 46.4, 28.4. Previous spectra were at low resolution in CCl_4 and the yield was 87%.[40] Product purity was adequate to prepare **12**. The TLC showed one spot using 40% EtOAc in hexanes.

2,2-Dimethylthiochroman-4-one (12)

A 1-L, three-necked, round-bottomed flask, fitted with a mechanical stirrer, a condenser, and a nitrogen inlet was charged with polyphosphoric acid (400 g) and heated to 70°C using an oil bath. To the stirred polyphosphoric acid, **3** (50 g, 0.24 mol) was added, and the mixture was stirred at 70°C for 45 min. The reaction mixture was cooled to 0°C using an ice bath and then carefully added to ice-cold water. [*Caution*! The reaction is extremely exothermic.] The resulting solution was extracted with ether (3 × 600 mL). The combined organic extracts were washed with 1 *N* NaOH (1×) and saturated NaCl (1×), dried ($MgSO_4$), and concentrated under vacuum to afford a white solid. The compound was recrystallized [ethyl acetate:petroleum ether, 1:3] to give 36.5 g (80%) of ketone **12** as

a white solid, mp 67–68°C [lit[40] 66–68°C]. IR: 1686 cm^{-1}; ^1H NMR: δ 8.10 (dd, $J = 7.7$, 1.1 Hz, 1 H, Ar-H), 7.39 (td, $J = 8.0$, 1.6 Hz, 1 H, Ar-H), 7.22 (d, $J = 7.7$ Hz, 1 H, Ar-H), 7.16 (td, $J = 7.7$, 1.1 Hz, 1 H, Ar-H), 2.87 (s, 2 H, CH_2), 1.47 (s, 6 H, $C(CH_3)_2$); ^{13}C NMR: δ 194.8, 141.3, 133.6, 129.6, 128.6, 127.5, 124.6, 53.8, 44.6, 28.5. Only low resolution spectra taken in CCl_4 were previously reported along with a yield of 70%.[40] The product was used directly in the next reaction to prepare **14**. The TLC showed one spot using 40% EtOAc in hexanes.

2,2-Dimethyl-2*H*-thiochromene-4-carbonitrile (14)

A 250-mL, one-necked, round-bottomed flask, equipped with a magnetic stirrer, a condenser, and a nitrogen inlet was charged with ketone **12** (32.0 g 0.17 mol), trimethylsilyl cyanide (19.8 g, 25 mL, 0.20 mol, 1.2 eq), and anhydrous zinc iodide (40 mg). The resulting solution was stirred at room temperature for 24 h under nitrogen, at which time TLC indicated the reaction was complete. Trimethylsilyl cyanohydrin **13** was unstable to purification and was used directly in the next step. Spectral data for only slightly impure **13** were IR: 2210 (weak) cm^{-1}; ^1H NMR: δ 7.70 (dd, $J = 7.7$, 1.6 Hz, 1 H, Ar-H), 7.28–7.10 (complex, 3 H, Ar-H), 2.53 (d, $J = 14.0$ Hz, 1 H, CH_2), 2.47 (d, $J = 14.0$ Hz, 1 H, CH_2), 1.54 (s, 3 H, CH_3), 1.49 (s, 3 H, CH_3), 0.22 (s, 9 H, $C(CH_3)_3$); ^{13}C NMR: 133.4, 132.0, 129.5, 129.3, 127.8, 125.3, 121.6, 69.9, 51.5, 41.4, 31.3, 31.0, 1.2. TLC analysis, using 30% EtOAc in hexanes, indicated **13** was pure.

In a 500-mL, three-necked, round-bottomed flask, fitted with a magnetic stirrer, a reflux condenser, an addition funnel, and a drying tube was placed the crude trimethylsilyl cyanohydrin **13** dissolved in benzene (200 mL) containing pyridine (10 mL). To the resulting solution, phosphorus oxychloride (33.5 g, 20 mL, 0.22 mol) was added dropwise with stirring. After refluxing for 4 h, TLC indicated that the reaction was complete. The reaction mixture was carefully poured onto 500 g of crushed ice, and the mixture was extracted with ether (3 × 300 mL). The combined ether layers were washed with water (1×) and saturated NaCl (1×), dried ($MgSO_4$), and concentrated under vacuum. The crude product was further purified by flash chromatography on a silica gel column (50 cm × 5 cm) using increasing concentrations of ether (5%, 10%, 25%, 50%) in hexanes to give 27.5 g (82%) of **14** as a white solid, mp 62–63°C. IR: 2224 cm^{-1}; ^1H NMR: δ 7.59 (dd, $J = 6.0$, 2.7 Hz, 1 H, Ar-H), 7.36–7.18 (complex, 3 H, Ar-H), 6.56 (s, 1 H, =CH), 1.46 (s, 6 H, $C(CH_3)_2$); ^{13}C NMR: δ 146.8, 131.6, 129.7, 127.8, 126.9, 126.7, 126.0, 117.1, 113.5, 40.9, 28.2. TLC analysis, using 33% EtOAc in hexanes, indicated that **14** was pure.

2,2-Dimethylthiochroman-4-carbonitrile (15)

To a 500-mL, three-necked, round-bottomed flask fitted with a magnetic stirrer, a reflux condenser, and a nitrogen inlet, was added **14** (24.0 g, 0.12 mol) and absolute ethanol (150 mL). The mixture was heated for 10 min to effect solution. To the warm solution, sodium borohydride (2.25 g, 0.06 mol) was slowly added in small portions, and refluxing was continued for 45 min. The reaction mixture was evaporated under vacuum and purified by flash chromatography on a silica gel column (50 cm × 5 cm) using 50% ether in hexanes to give 22.3 g (92%) of **15** as a white solid, mp 64–65°C. IR: 2243 cm^{-1}; ^1H NMR: δ 7.51 (d, $J = 7.1$ Hz, 1 H, Ar-H), 7.24–7.10 (complex, 3 H, Ar-H), 4.12 (dd, $J = 11.0$, 5.2 Hz, 1 H, CH—CN), 2.35 (dd, $J = 13.7$, 5.2 Hz, 1 H, CH_2), 2.21 (dd,

$J = 13.7, 11.0$ Hz, 1 H, CH_2), 1.47 (s, 3 H, CH_3), 1.44 (s, 3 H, CH_3); ^{13}C NMR: δ 133.5, 128.6, 128.4, 127.7, 126.4, 125.1, 120.7, 41.9, 41.7, 30.9, 29.9, 29.4. TLC analysis, using 30% EtOAc in hexanes, indicated that **15** was pure.

2,2,4-Trimethylthiochroman-4-carbonitrile (16)

A 500-mL, three-necked, round-bottomed flask, equipped with a magnetic stirrer, a reflux condenser, and a nitrogen inlet, was charged with of oil-free NaH (3.11 g, from 5.19 g of a 60% mineral oil suspension, 0.13 mol) in dry DMF (50 mL). To this suspension, a solution of **7** (22.0 g, 0.11 mol) in dry DMF (50 mL) was added at room temperature. The resulting dark brown mixture was stirred at room temperature for 30 min, and then methyl iodide (16.9 g, 7.4 mL, 0.112 mol) was added dropwise over a period of 15 min. The reaction mixture was stirred for 30 min and then carefully quenched by addition of 25 mL of saturated NH_4Cl followed by extraction with ether (3×150 mL). The combined organic layers were washed with saturated NaCl ($1\times$), dried ($MgSO_4$), and concentrated under vacuum to give a 21.1 g (90%) of **16** as a pale yellow solid, mp 71–72°C. IR: 2231 cm^{-1}; ^{1}H NMR: δ 7.56 (m, 1 H, Ar-H), 7.20–7.14 (complex, 3 H, Ar-H), 2.52 (d, $J = 14.5$ Hz, 1 H, CH_2), 2.14 (d, $J = 14.5$ Hz, 1 H, CH_2), 1.80 (s, 3 H, $C(CH_3)$-CN), 1.59 (s, 3 H, CH_3), 1.44 (s, 3 H, CH_3); ^{13}C NMR: δ 132.8, 131.7, 128.7, 128.5, 128.2, 125.6, 124.5, 51.3, 41.4, 34.8, 30.8, 30.6, 28.2. TLC analysis, using 30% EtOAc in hexanes, indicated that **16** was pure.

2,2,4-Trimethylthiochroman-4-carboxylic Acid (17)

A 500-mL, one-necked, round-bottomed flask, equipped with a magnetic stirrer, a reflux condenser, and a nitrogen inlet, was charged with 200 mL of 50% aqueous H_2SO_4 and **16** (20.0 g, 0.09 mol). The resulting mixture was heated at 100°C for 2 h and then cooled to room temperature and slowly poured onto 250 g of crushed ice. The aqueous layer was extracted with ether:ethyl acetate (1:1; 3×200 mL). The combined organic layers were washed with water ($1\times$) and saturated NaCl ($1\times$), dried ($MgSO_4$), and concentrated under vacuum to give 19.5 g (90%) of **17** as a pale yellow solid, mp 136–137°C. The solid was spectroscopically pure and was used in the next step without further purification. IR: 3680–2360, 1699 cm^{-1}; ^{1}H NMR: δ 10.29 (br s, 1 H, CO_2H), 7.41 (m, 1 H, Ar-H), 7.22–7.14 (complex, 3 H, Ar-H), 2.82 (d, $J = 14.3$ Hz, 1 H, CH_2), 1.81 (d, $J = 14.3$ Hz, 1 H, CH_2), 1.66 (s, 3 H, $C(\underline{CH_3})$-CO_2H), 1.43 (s, 3 H, CH_3), 1.31 (s, 3 H, CH_3); ^{13}C NMR: δ 183.2, 136.6, 134.2, 128.7, 128.4, 127.3, 125.2, 50.5, 47.4, 42.6, 32.6, 29.4, 27.8. TLC of the product, using 50% EtOAc in hexanes, revealed one spot.

(2,2,4-Trimethylthiochroman-4-yl)methanol (18)

A 500-mL, three-necked, round-bottomed flask, fitted with a magnetic stirrer, a reflux condenser, and a nitrogen inlet, was charged with **17** (18.0 g, 0.08 mol) and dry THF (150 mL) at room temperature. To the resulting solution, 5.78 g (0.15 mol, 2 eq) of lithium aluminum hydride was slowly added in small portions (ca 20 mg) with stirring, and the reaction was allowed to stir for 4 h. (*Caution*! The use of larger portions of lithium aluminum hydride initially caused excessive "frothing.") The resulting gray suspension was slowly quenched with 20 mL of 10% NaOH solution. The reaction mixture turned to a turbid, white solution, which slowly formed a white precipitate. Filtration through Celite®

removed the solid, and the filtrate was concentrated under vacuum. The crude product was purified by flash chromatography on a silica gel column (30 cm × 5 cm) using 40% ethyl acetate in hexanes to give 14.7 g (87%) of **18** as a pale yellow oil, which was used directly to prepare **19**. IR: 3378 cm^{-1}; ^1H NMR: δ 7.36 (m, 1 H, Ar-H), 7.16–7.08 (complex, 3 H, Ar-H), 3.60 (d, $J = 10.8$ Hz, 1 H, $C\underline{H}_2OH$), 3.56 (d, $J = 10.8$ Hz, 1 H, $C\underline{H}_2OH$), 2.38 (d, $J = 14.0$ Hz, 1 H, CH_2), 1.78 (d, $J = 14.0$ Hz, 1 H, CH_2), 1.43 (s, 3 H, CH_3), 1.41 (s, 3 H, CH_3), 1.40 (s, 3 H, CH_3), 1.40 (br s, 1 H, $CH_2O\underline{H}$); ^{13}C NMR: δ 138.2, 134.6, 128.6, 127.2, 126.5, 125.1, 72.0, 49.3, 41.8, 40.7, 31.3, 30.6, 27.2. TLC analysis, using 30% EtOAc in hexanes, showed one spot.

(2,2,4-Trimethylthiochroman-4-yl)methyl Acetate (19)

A 250-mL, two-necked, round-bottomed flask, equipped with a magnetic stirrer, a condenser, and a nitrogen inlet, was charged with alcohol **18** (14.0 g, 0.06 mol), 4-dimethylaminopyridine (7.7 g, 0.06 mol), and dichloromethane (100 mL). A solution of 7.0 g (6.5 mL, 0.07 mol) of acetic anhydride in 5 mL of dichloromethane was added slowly over a period of 5 min. After stirring at room temperature for 3 h, TLC confirmed the absence of starting material. The reaction mixture was diluted with 100 mL of dichloromethane and washed with 2 *N* HCl (1×), water (1×), and saturated NaCl (1×), dried ($MgSO_4$), and concentrated under vacuum to give 16.3 g (98%) of **19** as a dark yellow oil, which was used without further purification. IR: 1742 cm^{-1}; ^1H NMR: δ 7.35 (m, 1 H, Ar-H), 7.17–7.07 (complex, 3 H, Ar-H), 4.18 (d, $J = 11.0$ Hz, 1 H, $C\underline{H}_2OC(O)CH_3$), 4.14 (d, $J = 11.0$ Hz, 1 H, $C\underline{H}_2OC(O)CH_3$), 2.23 (d, $J = 14.3$ Hz, 1 H, CH_2-ring), 2.03 (s, 3 H, C(O)-CH_3), 1.86 (d, $J = 14.3$ Hz, 1 H, CH_2-ring), 1.42 (2s, 6 H, $C(CH_3)_2$), 1.41 (s, 3 H, CH_3); ^{13}C NMR: δ 171.0, 138.0, 133.9, 128.5, 127.2, 126.7, 125.1, 71.9, 49.6, 41.6, 38.9, 31.3, 31.2, 27.3, 20.9. TLC analysis, using 30% EtOAC in hexanes, showed one spot.

(2,2,4-Trimethyl-6-nitrothiochroman-4-yl)methyl Acetate (20)

A 250-mL, three-necked, round-bottomed flask, equipped with a magnetic stirrer, an addition funnel, and a nitrogen inlet, was charged with **19** (10.0 g, 0.04 mol) and freshly distilled acetic anhydride (25 mL). The solution was cooled to −5°C (ice/salt bath), and a cold solution of 3.4 mL of concentrated nitric acid in 8.1 mL of acetic anhydride was added dropwise over 10 min. The reaction was stirred at −5°C for 90 min and then diluted with ether and washed with saturated $NaHCO_3$. The $NaHCO_3$ wash was back-extracted with 25 mL of dichloromethane (1×) and the combined organic layers were washed with water (1×) and saturated NaCl (1×), dried ($MgSO_4$), and concentrated under vacuum. The crude product was purified by flash chromatography on a silica gel column (30 cm × 2.5 cm) using increasing concentrations of ethyl acetate (5%, 10%, 25%, 50%) in hexanes to give 2.3 g (20%) of **20** as a viscous, yellow oil, which was used directly in the next step. IR: 1743, 1516, 1340 cm^{-1}; ^1H NMR: δ 8.29 (d, $J = 2.2$ Hz, 1 H, Ar-H), 7.95 (dd, $J = 8.2$, 2.2 Hz, 1 H, Ar-H), 7.28 (d, $J = 8.2$ Hz, 1 H, Ar-H), 4.20 (d, $J = 11.0$ Hz, 1 H, $C\underline{H}_2OC(O)CH_3$), 4.11 (d, $J = 11.0$ Hz, 1 H, $C\underline{H}_2OC(O)CH_3$), 2.23 (d, $J = 14.3$ Hz, 1 H, CH_2-ring), 2.05 (s, 3 H, $C(O)CH_3$), 1.92 (d, $J = 14.3$ Hz, 1 H, CH_2-ring), 1.52 (s, 3 H, CH_3), 1.47 (s, 3 H, CH_3), 1.44 (s, 3 H, CH_3); ^{13}C NMR: δ 170.7, 145.1, 144.1, 138.9, 128.6, 122.8, 121.6, 71.3, 48.9, 42.6, 39.1, 31.3, 31.2, 27.3, 20.8. TLC analysis, using 20% EtOAc in hexanes, revealed one spot.

(6-Amino-2,2,4-trimethylthiochroman-4-yl)methyl Acetate (21)

A modified literature procedure was used.[43] A 25-mL, three-necked, round-bottomed flask, equipped with a magnetic stirrer, a reflux condenser, and a nitrogen inlet, was charged with a mixture of **20** (0.50 g, 1.62 mol), acetic acid (8 mL), and 0.27 g (4.85 mmol, 3 eq) of iron powder (>100 mesh). This mixture was heated with stirring at 115°C (oil bath) until TLC indicated the absence of starting material (ca. 15 min). The crude mixture was cooled, transferred to a separatory funnel containing 50 mL of water, and extracted with ether (3 × 25 mL). The combined ether layers were washed with water (1×), saturated $NaHCO_3$ (3×), and saturated NaCl (1×), dried ($MgSO_4$), and concentrated under vacuum to give 0.43 g (95%) of **21** as pale brown liquid, which was used at once to generate **22**. IR: 3451, 3365, 3224, 1734 cm^{-1}; ^1H NMR: δ 6.97 (d, $J = 8.2$ Hz, 1 H, Ar-H), 6.73 (d, $J = 2.2$ Hz, 1 H, Ar-H), 6.51 (dd, $J = 8.2$, 2.2 Hz, 1 H, Ar-H), 4.19 (d, $J = 11.0$ Hz, 1 H, $C\underline{H}_2OC(O)CH_3$), 4.14 (d, $J = 11.0$ Hz, 1 H, $C\underline{H}_2OC(O)CH_3$), 3.61 (br s, 2 H, NH_2), 2.19 (d, $J = 14.3$ Hz, 1 H, CH_2-ring), 2.06 (s, 3 H, $C(O)CH_3$), 1.80 (d, $J = 14.3$ Hz, 1 H, CH_2-ring), 1.39 (s, 3 H, CH_3), 1.38 (s, 3 H, CH_3), 1.37 (s, 3 H CH_3); ^{13}C NMR: δ 171.1, 144.1, 139.4, 129.6, 122.2, 114.4, 114.2, 71.8, 49.8, 41.6, 39.1, 31.1, 29.6, 27.1, 20.9. TLC analysis, using 30% EtOAc in hexanes, showed one spot.

(6-Amino-2,2,4-trimethylthiochroman-4-yl)methanol (22)

A 25-mL, three-necked, round-bottomed flask, equipped with a magnetic stirrer, a reflux condenser, and a nitrogen inlet, was charged with 2 mL of 10% NaOH and 150 mg (0.54 mmol) of **21**. The flask was heated in an oil bath at 90°C for 30 min. The mixture was cooled to room temperature, adjusted to pH 9 (1 *N* HCl), and extracted with ether (2 × 25 mL). The combined ether layers were washed with water (1×) and saturated NaCl (1×), dried ($MgSO_4$), and concentrated under vacuum to give 120 mg (94%) of **22** as a viscous, brown oil, which was used without further purification. IR: 3344 cm^{-1}; ^1H NMR: δ 6.96 (d, $J = 8.2$ Hz, 1 H, Ar-H), 6.73 (d, $J = 2.2$ Hz, 1 H, Ar-H), 6.49 (dd, $J = 8.2$, 2.2 Hz, 1 H, Ar-H), 3.60 (d, $J = 11.0$ Hz, 1 H, $C\underline{H}_2OH$), 3.55 (d, $J = 11.0$ Hz, 1 H, $C\underline{H}_2OH$), 2.90 (br s, 2 H, NH_2), 2.30 (d, $J = 14.3$ Hz, 1 H, CH_2-ring), 1.72 (d, $J = 14.3$ Hz, 1 H, CH_2-ring), 1.39 (s, 3 H, CH_3), 1.36 (s, 3 H, CH_3), 1.34 (s, 3 H, CH_3) 1.25 (br s, 1 H, $CH_2O\underline{H}$); ^{13}C NMR: δ 144.0, 139.7, 129.7, 123.1, 114.4, 114.3, 71.9, 49.9, 41.8, 41.0, 31.2, 30.4, 27.1. TLC analysis, using 50% EtOAc in hexanes, showed one spot.

Synthesis of *N*-[3,4-Dihydro-4-(acetoxymethyl)-2,2,4-trimethyl-2*H*-1-benzothiopyran-6-yl]-*N*′-(4-nitrophenyl)thiourea (23)

A 25-mL, one-necked, round-bottomed flask, equipped with a magnetic stirrer, an addition funnel, and a nitrogen inlet, was charged with a solution of acetate **21** (79 mg, 0.28 mol) in dry THF (3 mL), and the flask was cooled to 0°C (ice bath). To this mixture, a solution of 54 mg (0.30 mmol) of 4-nitrophenyl isothiocyanate in 3 mL of dry THF was added over 5 min. After the addition, the reaction mixture was allowed to warm to room temperature, and stirring was continued for 24 h. The solvent was evaporated, and the residue was purified using flash chromatography on a silica gel column (25 cm × 2.5 cm) using 70% ether in hexanes to give **23** as pale yellow solid. The solid was further recrystallized [H_2CCl_2:pentane, 1:1] to give 114 mg (88%) of **23** as a yellow solid, mp 156–157°C. IR: 3291, 1727 cm^{-1}; ^1H NMR: δ 8.35 (br s, 1 H, NH), 8.20 (d, $J = 9.0$ Hz,

2 H, Ar-H), 7.95 (br s, 1 H, NH), 7.83 (d, $J = 9.0$ Hz, 2 H, Ar-H), 7.41 (d, $J = 2.2$ Hz, 1 H, Ar-H), 7.18 (d, $J = 8.2$ Hz, 1 H, Ar-H), 6.99 (dd, $J = 8.2$, 2.2 Hz, 1 H, Ar-H), 4.53 (d, $J =$ 11.0 Hz, 1 H, $C\underline{H}_2OC(O)CH_3$), 3.74 (d, $J = 11.0$ Hz, 1 H, $C\underline{H}_2OC(O)CH_3$), 2.48 (d, $J =$ 14.3 Hz, 1 H, $C\underline{H}_2$-ring), 1.87 (s, 3 H, $C(O)CH_3$), 1.86 (d, $J = 14.3$ Hz, 1 H, $C\underline{H}_2$-ring), 1.45 (s, 6 H, $C(CH_3)_2$), 1.40 (s, 3 H, CH_3); ^{13}C NMR: δ 179.6, 170.9, 144.6, 144.5, 139.3, 135.4, 132.2, 129.7, 126.0, 124.2, 124.1, 123.9, 72.6, 49.5, 42.1, 39.9, 30.9, 29.5, 27.3, 20.8. *Anal.* Calcd for $C_{22}H_{25}N_3O_4S_2$: C, 57.49; H, 5.48; N, 9.14; S, 13.95. Found: C, 57.63; H, 5.48; N, 9.09; S, 13.83. A mixture melting point determination with **8** and **23** gave a range of 144–151°C, confirming that the structures were different. TLC analysis, using 40% EtOAc in hexanes, showed one spot.

Theoretical Studies

Computational docking studies were performed employing Glide (version 5.6, Schrödinger suite 2010)[45] using the Maestro user interface to set up the glide docking and for visualization of the results. The Glide extra precision (XP) mode was used, and the docked conformations were ranked by a Glide scoring function (G-score). For each Glide run, 10,000 ligand poses, and at most 10 poses per ligand, were written out. The crystal structures of B-Raf (1UWJ.pdb)[50] and KIT (1T46.pdb) were downloaded from the PDB and then prepared within the "Protein Preparation Wizard" workflow in Maestro. This includes removal of the water and ligand, addition of hydrogens, and restrained energy minimization on hydrogens using the OPLS 2001 force field. After the preparation, Glide grids were generated by "Receptor Grid Generation," using bound ligands in the crystal complexes to define the centers and sizes of the boxes. Compounds **7** and **8** were first stretched in Sybyl 6.9, energy minimized with the hydrogens and Gasterger-Hückel charges added, and then prepared by Ligprep in Maestro before docking. After the docking, the top ranked conformations were chosen and exported with the crystal structures of the kinases for further analysis.

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