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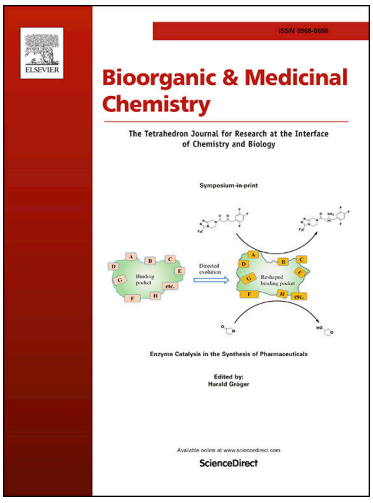


# Tetrahydroquinoline units in flexible heteroarotinoids (Flex-Hets) convey anti-cancer properties in A2780 ovarian cancer cells

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## Graphical Abstract

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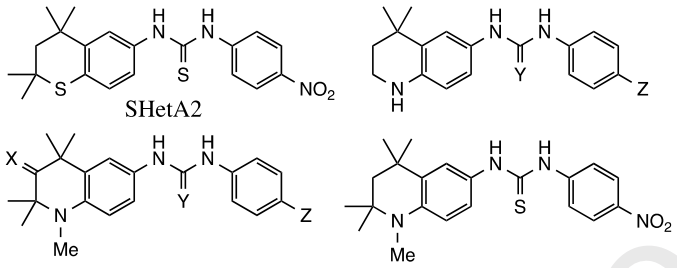


Flex-Hets related to SHetA2 that contain a tetrahydroquinoline ring have been synthesized and evaluated for their activity against the A2780 ovarian cancer cell line.

X = O or H, OH; Y = S or O;
Z = $NO_2$, $CF_3$, $OCF_3$ or $NH_2$

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# Tetrahydroquinoline units in flexible heteroarotinoids (Flex-Hets) convey anti-cancer properties in A2780 ovarian cancer cells

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ABSTRACT

SHetA2 (NSC 721689), our lead Flex-Het anti-cancer agent, consists of a thiochroman (Ring A) and a 4-nitrophenyl (Ring B) linked by a thiourea bridge. In this work, several series of new analogs having a tetrahydroquinoline (THQ, Ring A) unit connected by a urea or thiourea linker to a 4-substituted phenyl (Ring B) have been prepared and evaluated relative to SHetA2 in terms of binding affinity with mortalin and inhibition of A2780 ovarian cancer cells. Six of the derivatives equaled or exceeded the efficacy shown by SHetA2. Compounds **1a-d** (series 1), lacking a methyl on the Ring A nitrogen and the *gem*-dimethyls on the adjacent carbon, showed only weak activity. Salt **2**, the quaternized *N*,*N*-dimethyl iodide salt analog of **1a**, also possessed very modest growth inhibition in the cell line studied. Series 3 compounds, which had a C3 ketone and an *N*-methyl replacing the sulfur in Ring A, were most successful. Compound **3a** [Ring A = 1,2,2,4,4-pentamethyl-3-oxo-1,2,3,4-tetrahydroquinolin-6-yl; urea linker; Ring B = 4-nitrophenyl] had slightly lower potency ($IC_{50}$ 3.8 μM), but better efficacy (94.8%) than SHetA2 ($IC_{50}$ 3.17 μM, efficacy 84.3%). In addition, **3c** and **3d** [urea and thiourea linkers, respectively; Ring B = 4-(trifluoromethyl)phenyl] and **3e** and **3f** [urea and thiourea linkers, respectively; Ring B = 4-(trifluoromethoxy)phenyl] were also evaluated since these agents possessed electron-withdrawing groups with H-bonding capability. All displayed good activity. Compounds **3c** and **3e** showed improvement in both potency and efficacy compared to SHetA2. In general, when the linker group between Rings A and B was a urea, efficacy values slightly exceeded those with a thiourea linker in the carbonyl-containing THQ systems **3a-g**. In contrast, when Ring A possessed the 1,2,2,4,4-pentamethyl-3-hydroxytetrahydroquinolin-6-yl unit (**4a-f**, series 4), very modest potency and efficacy were observed. Model compound **5**, an exact *N*-methyl THQ analog of SHetA2, demonstrated less potency ($IC_{50}$ 4.5 μM), but improved efficacy (91.7%). Modeling studies were performed to rationalize the observed results.



## 1. Introduction

Mortalin (PDB ID: 3N8E) is a highly conserved heat-shock protein that exists in multiple subcellular locations. Its overexpression causes cell proliferation and inhibits apoptosis by binding to proteins such as p53, Bcl-2 and p66shc. The target of the current work is mortalin, wherein we compare the docking affinity and activity of nineteen new analogs with that of SHetA2. Earlier, we demonstrated that the Flex-Het SHetA2 [1-(4-nitrophenyl)-3-(2,2,4,4-tetramethylthiochroman-6-yl)amino)-thiourea, NSC 721689] binds to mortalin and causes the release of these proteins to initiate apoptosis in ovarian cancer cells.[1] Flex-Hets are a promising family of heterocycles which have displayed strong anti-cancer activity in a number of cell lines.[2] Decreased toxicity,[3] strong discriminatory ability between malignant and benign cells,[4] and defined structure-activity relationships[4] have been observed in selected examples. SHetA2 has induced apoptosis of cancer cells in specific ovarian,[5-7] lung[8,9] and kidney systems.[10] The current study is focused on ovarian cancer which is often a lethal gynecological event in women with a survival rate of about 30%.[11] SHetA2 has exhibited strong activity against ovarian cancer *in vitro*[5-7,12,13] and has demonstrated low toxicity and good pharmacokinetics in an

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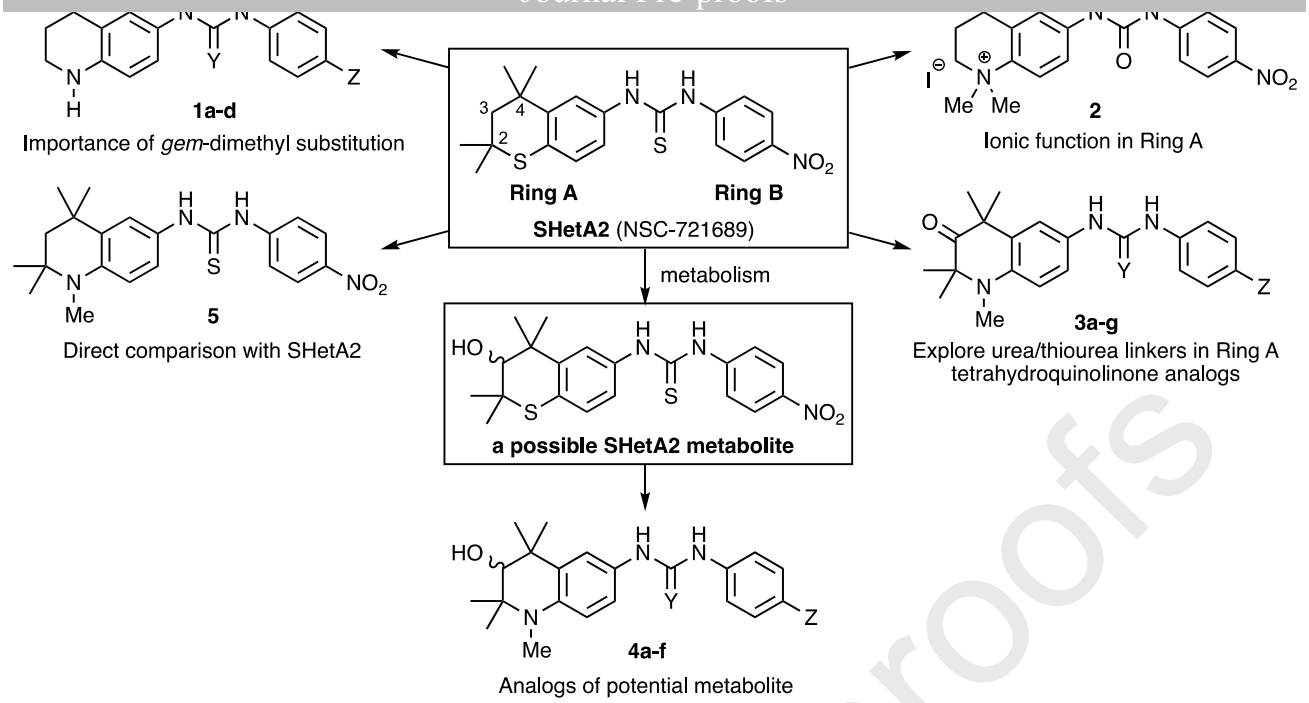



**Figure 1**. Target systems in nitrogen-containing heteroarotinoids

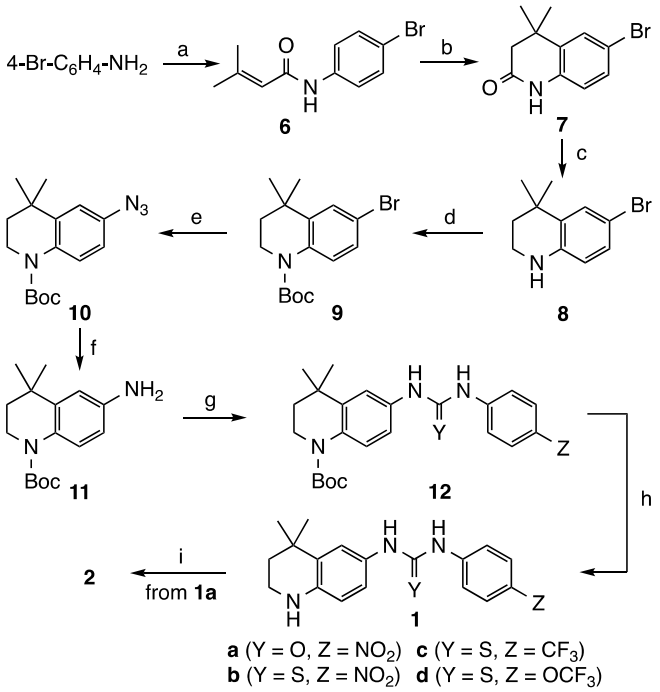
investigation with rats and dogs.[14] The present study sought to evaluate nitrogen analogs of our lead compound against ovarian cancer.

Nitrogen occurs in a very high percentage of FDA approved pharmaceuticals.[15] The nitrogen atom renders compounds weakly basic with good H-bonding properties, allowing it to bind to a wide range of proteins. Specifically, the tetrahydroquinoline (THQ) framework is found in diverse natural products and in chemotherapeutic targets to modulate pharmacodynamics and/or pharmacokinetic properties. Thus, THQ analogs of SHetA2 are reasonable candidates (Figure 1) for evaluation in ovarian cancer screens. In this study, we have studied the impact of replacing the thiochroman ring (Ring A) with a THQ. Compounds **1a-d** (series 1) assessed THQ derivatives lacking the *gem*-dimethyl adjacent to an unsubstituted nitrogen. Compound **2** was a *N*,*N*-dimethyl quaternized THQ linked *via* a urea group to a 4-nitrobenzene ring. Moreover, since several SHetA2 metabolites have been identified as Ring A hydroxylated SHetA2 in both mouse and rat plasma,[16] the current project also investigated the activity of THQ analogs with a carbonyl group (**3a-g**, series 3) and an alcohol group (**4a-f,** series 4) on C3 of Ring A, linked by urea and thiourea functions to Ring B bearing various substituents at C4. Finally, compound **5**, a direct THQ analog of our lead compound, was prepared to assess the effect of replacing the sulfur in Ring A with an alkylated nitrogen.

## 2. Results and Discussion

### *2.1. Chemistry*

The syntheses of compounds **1a-d** and **2** were initiated from 4-bromoaniline as depicted in Scheme 1. Formation of amide **6** with 3-methylbut-2-enoyl chloride, Friedel-Crafts cyclization to lactam **7** and carbonyl reduction gave bromide **8** in good yield (51%, 3 steps). Boc protection of the THQ nitrogen to give **9** was accomplished in high yield (92%). The C6 nitrogen was introduced using an L-proline-promoted, CuI-catalyzed Ullmann reaction[17] to create azide **10**. The azide was not purified but was instead immediately reduced to amine **11** (62%, 2 steps). Removal of the Boc group from **12** under acidic conditions gave

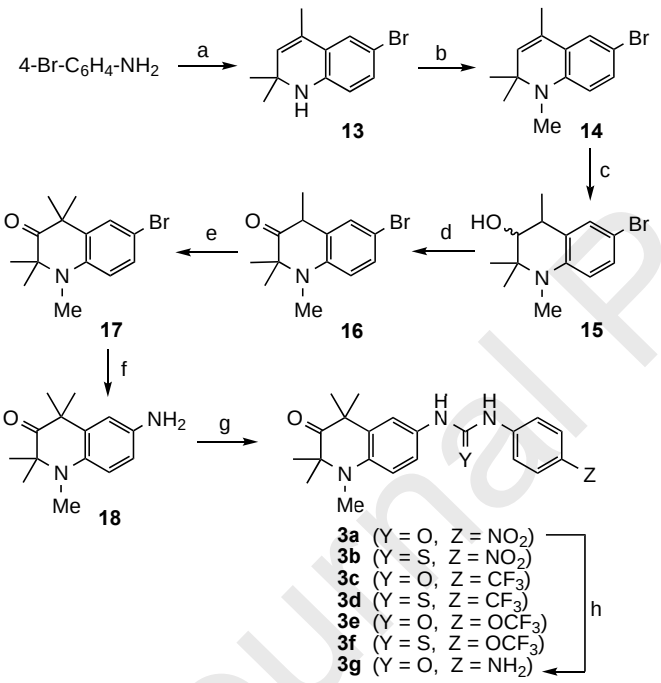



a) 3-methylbut-2-enoyl chloride, $CHCl_3$/reflux, 5 h; b) $AlCl_3$, DCE, 83 °C; c) $H_3B$:DMS, toluene, 0 °C to 110 °C; d) *n*-BuLi, $Boc_2O$, THF, –78 °C to 23 °C; e) $NaN_3$, NaOH, DMF, L-proline, 90 °C; f) $H_2$, Pd/C, MeOH, 23 °C; g) YCN-$C_6H_4$-4-Z, THF, 23 °C; h) TFA, DCM, RT; i) Me-I, DMF, $Cs_2CO_3$, 23 °C.

**Scheme 1**. Synthesis of **1a-d** (series 1) and **2**

men
crystallization from a mixture of pentane/ether. Adding excess methyl iodide to **1a** (Y = O, Z = $NO_2$) in DMF in the presence of $Cs_2CO_3$ with stirring for 24 hours at room temperature (23 °C) afforded salt **2** (62%).

An entry to members of series 3 is delineated in Scheme 2. A modified Skraup reaction between 4-bromoaniline and acetone promoted by bismuth(III) trifluoromethanesulfonate gave the dihydroquinoline **13** (62%).[18] A solution of **13** in DMF was carefully treated with sodium hydride, followed by a 4-fold excess of methyl iodide in DMF at 15 °C, and the solution was allowed to warm slowly to 23 °C. Stirring for an additional 18 hours produced **14** (82%). Alcohol **15** (57%) was generated by hydroboration of the double bond in **14**.[19] Oxidation of alcohol **15** *via* a Swern procedure led to the tetrahydroquinolinone **16**, which was methylated immediately to afford **17** (62%, 2 steps). Utilizing a pressure vessel, a mixture of **17**, copper iodide, L-proline, DMF and aqueous ammonia was heated to produce aniline **18** (65%).[17] A selection of isocyanates and isothiocyanates were then added to **18** to give analogs **3a-f** (60-78%). The final member of the series, **3g**, was prepared by reduction of **3a** with Fe/$NH_4Cl$ in aq EtOH at reflux (84%).[20]

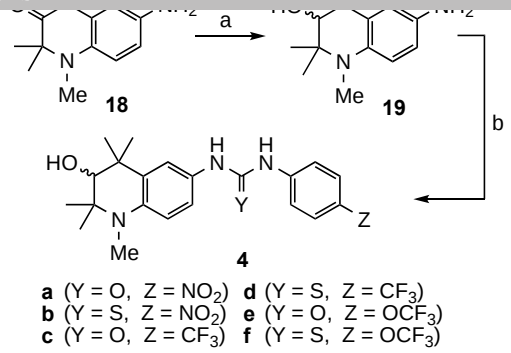



a) acetone, $Bi(OTf)_3$, reflux; b) NaH, $H_3C$-I, DMF, 15 °C to 23 °C, 24 h; c) $H_3B$:THF, THF, 10-15 °C; 3 M NaOH, 30% $H_2O_2$, 23 °C; d) $(COCl)_2$, DMSO, TEA, DCM, –60 °C to –20 °C; e) LiHMDS, $H_3C$-I, THF, –50 °C to 23 °C; f) CuI, L-proline, aq $NH_3$, DMF, 110 °C; g) YCN-$C_6H_4$-4-Z, THF, 23 °C; h) Fe, $NH_4Cl$, EtOH/$H_2O$, reflux.

**Scheme 2**. Synthesis of **3a-g** (series 3)

To access series 4, intermediate **18** was reduced to alcohol **19** (82%) with $LiAlH_4$ in THF at 23 °C (Scheme 3). Addition of iso(thio)cyanates to **19** in THF at 0 °C, followed by warming to 23 °C, generated **4a-f**.

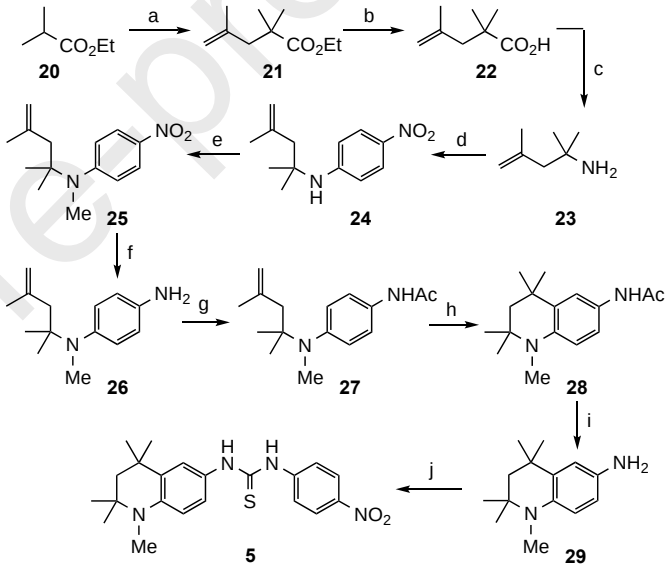
Model system **5** was prepared as shown in Scheme 4. A modification of the method of Walborsky[21] was employed to prepare **23** and involved deprotonation of ethyl isobutyrate (**20**) with LDA at –78 °C and alkylation with 3-iodo-2-methylpropene to yield unsaturated ester **21** (64%).[22a] Saponification of **21** and




a) $LiAlH_4$, THF, 0 °C to 23 °C; b) YCN-$C_6H_4$-4-Z, THF, 23 °C.

**Scheme 3**. Synthesis of **4a-f** (series 4)

neutralization gave acid **22** (96%).[22b,c] Treatment of this acid with diphenyl phosphoryl azide then produced amine **23** (72%). $S_NAr$ reaction of **23** with 1-fluoro-4-nitrobenzene led to **24** (45%), which was *N*-methylated to **25** and reduced[20] to give amine **26** (94%, 2 steps). Acylation of the amino group in **26**




a) LDA, THF, –78 °C; $H_2C$=C($CH_3$)$CH_2$I, –78 °C to 23 °C; b) NaOH, MeOH, 60 °C to 70 °C; $H_3O^+$; c) TEA, PhH; $(PhO)_2P(O)N_3$, 0 °C to 23 °C, then reflux; d) 1-F-$C_6H_4$-4-$NO_2$, DMSO, 80 °C; e) NaH, MeI, DMF, 23 °C; f) Fe, $NH_4Cl$, EtOH/$H_2O$, reflux; g) AcCl, pyridine, 23 °C; h) $AlCl_3$, DCM, –78 °C to 23 °C; i) 70% $H_2SO_4$, reflux; 30% NaOH; j) 4-SCN-$C_6H_4$-$NO_2$, THF, 23 °C.

**Scheme 4**. Synthesis of **5**

gave **27** (97%) and subsequent ring closure[23] yielded **28** (53%). Deacylation of **28** generated **29** (92%) which was reacted with 4-nitrophenyl isothiocyanate to afford **5** (90%). All of the compounds (**1**-**5**) were solids with sharp melting points and were fully characterized by IR, $^1H$ NMR and $^{13}C$ NMR analyses. Final products were also confirmed by MS and elemental analyses.

*2.2. Biology and Modeling Studies*

The biological activities of all compounds were validated by screening with A2780 human ovarian cancer cells (Table 1). The half-maximal inhibitory concentration ($IC_{50}$) and percent efficacy, defined as the maximal percent inhibition of cancer cell growth for all compounds, revealed a range of growth inhibition. The Pearson correlation coefficient between the two parameters was –0.82. This highly negative correlation is consistent with the

**Tab** the binding free energy (ΔG) and dissociation constant ($K_d$) values for each compound docked to the mortalin substrate-binding domain.

| Cpd | Y | Z | $IC_{50}$ (μM) | Efficacy (%) | –ΔG (kcal/mole) | $K_d$ (μM) |
|---|---|---|---|---|---|---|
| **SHetA2** | S | $NO_2$ | 3.17 ± 0.05 | 84.3 ± 0.7 | 8.5 | 0.6 |
| **1a** | O | $NO_2$ | 6.9 ± 0.2 | 17.1 ± 1.2 | 8.2 | 1.1 |
| **1b** | S | $NO_2$ | 7.1 ± 0.3 | 17.8 ± 1.6 | 7.9 | 1.6 |
| **1c** | S | $CF_3$ | 6 ± 0.2 | 42 ± 3 | 7.5 | 3.3 |
| **1d** | S | $OCF_3$ | 7.1 ± 0.8 | 24 ± 2 | 7.2 | 5.4 |
| **2** | O | $NO_2$ | 6.6 ± 0.3 | 22 ± 4 | 8.5 | 0.7 |
| **3a** | O | $NO_2$ | 3.8 ± 0.1 | 94.8 ± 2.2 | 8.9 | 0.3 |
| **3b** | S | $NO_2$ | 4.4 ± 0.2 | 91.4 ± 1.7 | 8.2 | 1.1 |
| **3c** | O | $CF_3$ | 2.58 ± 0.1 | 90.1 ± 1.4 | 8.0 | 1.5 |
| **3d** | S | $CF_3$ | 3.9 ± 0.1 | 90.8 ± 2.0 | 7.9 | 1.6 |
| **3e** | O | $OCF_3$ | 2.4 ± 0.2 | 91.3 ± 1.3 | 7.9 | 1.8 |
| **3f** | S | $OCF_3$ | 5.4 ± 0.6 | 76 ± 8 | 7.7 | 2.4 |
| **3g** | O | $NH_2$ | 7.7 ± 1.4 | 24 ± 4 | 8.2 | 1.1 |
| **4a** | O | $NO_2$ | 8.4 ± 1.9 | 26 ± 4 | 8.0 | 1.6 |
| **4b** | S | $NO_2$ | 10 ± 5 | 25 ± 4 | 7.7 | 2.3 |
| **4c** | O | $CF_3$ | 6.7 ± 0.5 | 25 ± 5 | 8.4 | 0.7 |
| **4d** | S | $CF_3$ | 7.6 ± 0.7 | 56.1 ± 2.4 | 7.7 | 2.3 |
| **4e** | O | $OCF_3$ | 7.8 ± 0.2 | 23.6 ± 3.3 | 8.2 | 1.1 |
| **4f** | S | $OCF_3$ | 13.1 ± 6.1 | 15.3 ± 3.3 | 7.6 | 2.9 |
| **5** | S | $NO_2$ | 4.5 ± 0.1 | 91.7 ± 0.4 | 8.7 | 0.5 |

expectation that a compound with a smaller $IC_{50}$ is more effective in competing for the receptor site than its substrate protein(s).[24]

To better assess the dose-response results, data in Table 1 were visualized by plotting the efficacy against the $IC_{50}$ (Figure 2). In this plot, a sigmoid curve was sketched to capture the main

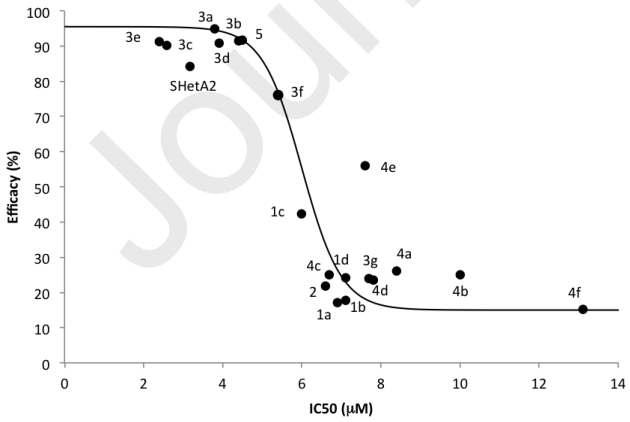



**Figure 2.** Assessment of the efficacy to $IC_{50}$ relation for all compounds.

trend in the data. Most compounds with an $IC_{50}$ below 5 μM demonstrated efficacy comparable to or better than that of SHetA2. Moreover, a small $IC_{50}$ also usually indicates a stronger affinity for the compound to bind with its protein receptor. The data suggest that a compound must have an affinity above a certain threshold for its receptor in order to effectively initiate the destruction of cancer cells. This threshold is very likely determined by interaction between the receptor and its substrate protein(s), which is(are) competitively displaced by the synthetic ligand. The most desirable properties of a molecule are a combination of the smallest $IC_{50}$ and the highest efficacy. A small $IC_{50}$ is advantageous when translating from single-layered cell cultures to real tissues where a lower concentration of the compound may be available. Flex-Hets **3a-e** showed higher efficacy than SHetA2, with **3a** exhibiting the highest efficacy of 94.8%. In addition, two other members of series 3 also had low $IC_{50}$ values, 2.58 μM for **3c** and 2.4 μM for **3e**, which were better than SHetA2 (3.17 μM).

The interactions of the compounds with the mortalin substrate binding domain (SBD) were studied by virtual docking with Autodock.[25] The binding free energies (ΔG) and dissociation constants ($K_d$), where a more negative ΔG and a smaller $K_d$ value reflect a stronger binding affinity, are reported in Table 1. The Pearson correlation coefficient between the $K_d$ and $IC_{50}$ gave a weak positive correlation of 0.32. This weak correlation could possibly derive from the relatively low accuracy of ΔG and $K_d$ obtained using virtual docking. Figure 3 demonstrated a roughly linear relationship between $IC_{50}$, which was obtained from cell-based assays, with the dissociation constant $K_d$ calculated from the binding free energy (see Experimental section).

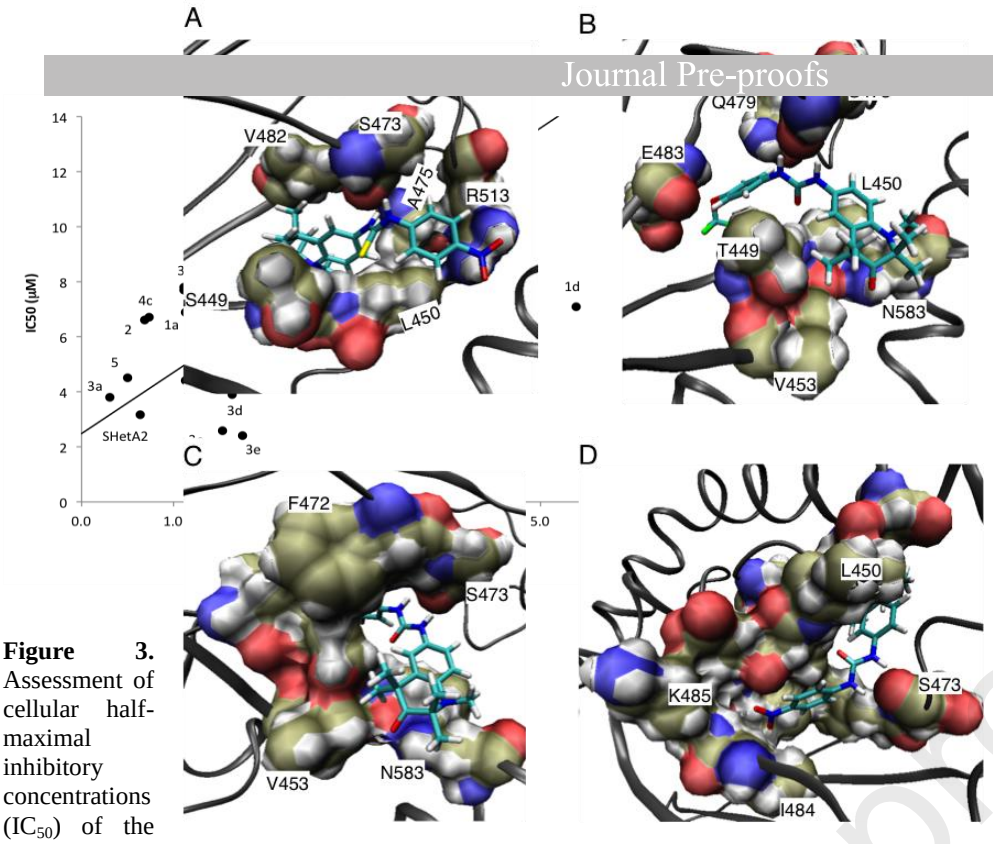



**Figure 3.** Assessment of cellular half-maximal inhibitory concentrations ($IC_{50}$) of the compounds and their calculated biochemical dissociation constants ($K_d$) with the mortalin substrate-binding domain. The data were linearly fit with a slope of 2.2 ± 0.4 and an intercept of 2.5 ± 1.7 μM.

The $IC_{50}$ depends on the cellular concentration of the receptor (mortalin in this case) which is higher than $K_d$, obeying $IC_{50} = [R]_0/2 + K_d(1+[S]/K_M)$, where $[R]_0$ is the receptor concentration, [S] is the concentration of the substrate protein being competitively displaced by the drug, and $K_M$ is the concentration of the substrate protein at which the receptor achieves half activity.[24] The intercept (2.5 ± 1.7 μM) of the fitting line indicated that the mortalin concentration in A2780 cancer cells was roughly 5 μM and, for this reason, it might be difficult to obtain $IC_{50}$ values much lower than 2.5 μM in A2780 cells for these analogs.

Analogs in series 1 and 4, as well as compound **2**, do not bind well with mortalin, and this likely reflects the reduced hydrophobicity of ring A in these derivatives. Derivatives in series 3 and compound **5** have an affinity for mortalin which is the focus of our binding studies. Protein-ligand interactions are illustrated in Figure 4 for three of the best compounds–**5**, **3e** and **3a**. The docking pose of compound **5** (Figure 4A) was very similar to that of SHetA2,[26] with the negatively polarized $NO_2$ group being attracted to the positively charged side chain of R513 (arginine at position 513 of the mortalin polypeptide chain), the backbone C=O of S473 (serine) being hydrogen-bonded to the thiourea linker, and Ring A with methyl groups residing in the hydrophobic part of the pocket formed by the

**Figure 4.** Molecular docking of compounds **5** (A), **3e** (B), and **3a** (C, D) to the SBD of mortalin at the peptide-binding pocket. Amino acids in contact with the compounds are shown using a surface drawing method, with the compounds as sticks. Carbon atoms of the amino acids are tan; carbon atoms of the new compounds are cyan, nitrogen is blue, oxygen is red, sulfur is yellow, and fluorine is green. Docking poses of **5**, **3a** and **3e** are also directly compared with SHetA2 in Figures S1-S3 in the SI.

methyl groups of residues V482 (valine), L450 (leucine) and A475 (alanine). The docking pose of compound **3e** (Figure 4B) was opposite to that of SHetA2. This might be a consequence of the slightly reduced hydrophobicity in Ring A with the addition of oxygen and nitrogen atoms. In addition to the hydrogen bonds involving the linker and S473 (serine), there were two more hydrogen bonds formed between the $OCF_3$ oxygen and the E483 (glutamic acid) backbone amide NH, and between the C=O in Ring A and the N583 (asparagine) side chain amide. The docking pose of **3a** (Figures 4C and 4D) was also opposite to that of SHetA2. In addition to the hydrogen bonds between the urea linker NH groups and S473 (serine), the linker C=O formed a hydrogen bond with the L450 (leucine) backbone NH, and the $NO_2$ group formed a hydrogen bond with the K485 (lysine) backbone NH. Other residues marked in Figures 4C and 4D, including V453 (valine), F472 (phenylalanine), I484 (isoleucine), and N583 (asparagine), were in van der Waals contact with the ligand molecule. Finally, compound **3g** possessed a hydrogen bond donating $NH_2$ group in place of the hydrogen bond accepting $NO_2$, and this likely decreased its ability to bind to mortalin.

Since SHetA2 binds to mortalin and interferes with mortalin-p53 interactions,[1] a brief discussion on the function of p53 seems warranted. The SBD of mortalin was shown to associate with tumor protein p53 in a concentration dependent manner.[27] The p53 tumor suppressor protein has been described as the "guardian of the genome" due to its role in conserving stability by preventing genome mutation.[28] Since p53 plays a major role in cell cycle arrest and apoptosis,[29] the loss of p53 function leads to immortalization of human cells.[30] Thus, investigations on the regulation of p53 function and its reactivation are paramount to identifing new cancer therapeutics. Prior findings have indicated that alterations of p53 have a significant role in ovarian cancer, including a predilection to the disease in some patients, which inferred a possible mechanism for somatic mutations leading to ovarian cancer.[29] In addition, p53 is nonfunctional in some tumors by cytoplasmic sequestration of its binding proteins, such as Bcl-2, hsp70/mortalin, Parc, PML, and cytoskeleton proteins.[31] It was reported that mortalin causes cytoplasmic sequestration of p53 by binding its carboxy terminal amino acid residues.[32] Similar research has demonstrated that mortalin binds p53 and inactivates its apoptotic function in stressed cancer cells and is therefore considered to be an important target.[33,34]

SHetA2 binds strongly to mortalin ($K_d$ = 0.6 μM), anchored by very strong hydrogen bonding between the Ring B $NO_2$ group and R513. Compound **5** ($K_d$ = 0.5 μM), a direct THQ analog of the lead compound, adopts a similar pose (See Figure S1) with the same robust hydrogen bonding. By analogy, and based on the current docking results, we infer that compound **5** would be able to displace or block p53, Bcl-2, p66shc and other client proteins from mortalin, similar to SHetA2.[1] The analogs in series 3 show varying $K_d$ values (0.3-2.4 μM) and are also effective at binding to mortalin. Analog **3a** (4-$NO_2$ on Ring B) adopts a different pose, almost the opposite of that observed for SHetA2, in binding mortalin (see Figure S2). The small $K_d$ (0.3 μM) suggests an even tighter association, indicating that hydrogen bonding between the $NO_2$ and the K485 peptide NH, which stabilizes this interaction, is very significant. Analogs **3c** (4-$CF_3$ on Ring B) and **3e** (4- $OCF_3$ on Ring B) have higher $K_d$ values (1.5 μM and 1.8 μM, respectively), but show greater efficacy. This result likely derives from these compounds also adopting an opposite binding orientation (see Figure S3) which, while not as strong, still effectively impedes client protein binding. Thus, compounds with both poses are effective in competing for the protein binding site on mortalin resulting in higher cytoplasmic concentrations of p53 and other proteins that can initiate apoptosis in ovarian cancer cells.

## 3. Conclusion

Several Flex-Hets having a THQ-based Ring A, linked by means of a urea or thiourea to a C4-substituted phenyl (Ring B), have been prepared and evaluated relative to our lead compound SHetA2 in terms of binding affinity to mortalin and inhibition of A2780 ovarian cancer cells. Six of the derivatives equaled or exceeded the efficacy shown by SHetA2. For compounds **1a-d**, the nitrogen analogs of SHetA2, without a methyl on the Ring A nitrogen or the *gem*-dimethyl groups at C2, showed only weak activity. These compounds possess significant polarity with additional H-bonding capability in Ring A, which could adversely affect the interaction of the compound with the active site of mortalin. Compound **2**, the quaternized *N*,*N*-dimethyl iodide salt of **1a**, also lacked significant percent growth inhibition and efficacy in the cell line studied and has similar issues with polarity and H-bonding. Structures incorporating a 1,2,2,4,4-pentamethyl-3-oxo-1,2,3,4-tetrahydroquinolin-6-yl unit, linked by urea and thiourea functions to a 4-nitrophenyl (**3a** and **3b**, respectively), displayed better growth inhibition than SHetA2, but with a slightly lower potency (larger $IC_{50}$) than SHetA2. In these structures, the Ring A nitrogen and the C3 carbonyl are shielded by methyls, which would reduce the H-bonding capabilities of the these groups. In addition to compounds with $NO_2$ at C4 of Ring B, the corresponding analogs with $CF_3$ (**3c** and **3d**) and $OCF_3$ (**3e** and **3f**) were also evaluated and found to exhibit high growth inhibition. Urea-linked compounds **3c** and **3e** displayed better potency and efficacy than our lead compound. Binding studies on **3a** and **3e** revealed that these analogs adopt a pose opposite to that of SHetA2, but still interfere with client protein binding to mortalin. Furthermore, in the C=O containing THQ systems **3a-f**, the efficacy was modestly higher when the linker group between Rings A and B was the more rigid urea versus a thiourea. Interestingly, conversion of the H-bond accepting $NO_2$ of **3a** to a H-bond donor $NH_2$ (**3g**) at C4 of Ring B abolished nearly all activity. In contrast, for compounds **4a-e**, having an OH at C3 of Ring A, the efficacy of growth inhibition varied only slightly, with all being less effective than the C=O analogs. In these structures, H-bonding is again possible with the Ring A alcohol, which possesses an O–H bond that extends beyond the steric influence of the adjacent methyls. Finally,

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examined. As expected, this system has similar electronics to the lead compound, binds mortalin with a nearly identical orientation and exhibits significant activity.

## 4. Experimental

### *4.1. Chemistry*

#### *4.1.1. General methods*

Commercial anhydrous *N*,*N*-dimethylformamide (DMF) was stored under dry $N_2$ and was transferred by syringe into reactions when needed. Tetrahydrofuran (THF) was distilled from $LiAlH_4$ prior to use. All other commercial reagents and solvents were used as received. Unless otherwise indicated, all reactions were carried out under dry $N_2$ in oven-dried glassware. The HCl and NaOH solutions, saturated $NH_4Cl$, 5% $NaHCO_3$, 10% $Na_2CO_3$, saturated $Na_2SO_4$ and saturated NaCl used in work-up procedures were aqueous solutions. Reactions were monitored by thin layer chromatography (TLC, Analtech No. 21521) using silica gel GF plates. Preparative separations were performed by column chromatography on silica gel (Davisil™, grade 62, 60-200 mesh) containing UV-active phosphor (Sorbent Technologies No. UV-05) slurry packed into quartz columns. Band elution for all chromatographic separations was monitored using a hand-held UV lamp. Melting points were uncorrected. IR spectra were run as $CHCl_3$ solutions or as nujol mulls on NaCl disks. Both $^1H$ and $^{13}C$ NMR spectra (400 or 300 MHz, and 101 or 75 MHz, respectively) were measured in the indicated solvents using tetramethylsilane as the internal standard with coupling constants (*J*) given in Hz. Unit resolution mass spectra were obtained using a Shimadzu LCMS-2010EV system operating with an electrospray source. Elemental analyses (± 0.4%) were performed by Atlantic Microlabs, Norcross, GA.

### *4.2. Synthesis of **1a-d** (series 1)*

#### *4.2.1 N-(4-Bromophenyl)-3-methylbut-2-enamide (**6**)*

A solution of 3-methylbut-2-enoyl chloride (3.4 mL, 29.0 mmol) in $CHCl_3$ (25 mL) was added dropwise to a stirred solution of 4-bromoaniline (10 g, 58.1 mmol) in $CHCl_3$ (250 mL). The resulting cloudy reaction mixture was refluxed for 5 h, then cooled to room temperature (23 °C) and filtered through Celite. The filtrate was washed with 1 M HCl (100 mL), saturated $NaHCO_3$ and saturated NaCl, then dried ($MgSO_4$), filtered and concentrated under vacuum. The resulting material was recrystallized from ethanol to afford **6** as a white solid (5.5 g, 21.8 mmol, 75%), mp 118-119 °C. IR: 3294, 1663, 1643, 826 $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$): δ 7.42 (m, 4H, ArH), 7.23 (br s, 1H, NH), 5.69 (s, 1H, =CH), 2.21 (s, 3H, $CH_3$), 1.89 (s, 3H, $CH_3$); $^{13}C$ NMR (101 MHz, $CDCl_3$): δ 165.0, 154.4, 137.3, 131.9, 121.3, 118.3, 116.5, 27.5, 20.0.

#### *4.2.2. 6-Bromo-4,4-dimethyl-3,4-dihydroquinolin-2(1H)-one (**7**)*

To a stirred solution of **6** (5.0 g, 19.6 mmol) in 1,2-dichloroethane (50 mL) was added $AlCl_3$ (3.9 g, 29.5 mmol) portion-wise, and the mixture was heated to reflux (83 °C) for 1 h. The reaction was cooled to 0 °C, quenched with ice-cold water (20 mL), filtered through Celite, and the filter cake was washed with DCM (2 × 50 mL). The organic phase was separated, washed with saturated $NaHCO_3$ and saturated NaCl, then dried ($MgSO_4$), filtered and concentrated under vacuum. The crude material was purified by column chromatography eluted with increasing concentrations of ether in hexanes to afford **7** as a brown solid (3.8 g, 14.9 mmol, 76%), mp 151-153 °C. IR: 3201, 1681, 1488, 1368 $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$): δ 9.36 (s, (d, *J* = 8.4 Hz, 1H, ArH), 2.48 (s, 2H, $CH_2$), 1.34 (s, 6H, $C(CH_3)_2$); $^{13}C$ NMR (101 MHz, $CDCl_3$): δ 171.2, 135.1, 134.6, 130.4, 127.7, 117.5, 116.1, 44.9, 34.1, 27.5.

#### *4.2.3. 6-Bromo-4,4-dimethyl-1,2,3,4-tetrahydroquinoline (**8**)*

To a stirred, ice-cooled solution of **7** (3.5 g, 13.8 mmol) in distilled toluene (35 mL) was added dropwise borane-dimethyl sulfide complex (1.4 mL, 14.4 mmol), and the mixture was refluxed (110 °C) for 3 h. The reaction was cooled to 23 °C and quenched carefully by dropwise addition of 10% $Na_2CO_3$ (10 mL). The resulting biphasic mixture was stirred at 23 °C for 15 min, and the layers were separated. The organic phase was dried ($MgSO_4$), filtered and concentrated under vacuum to give **8** as a colorless oil (3.0 g, 12.4 mmol, 90%). IR: 3414, 1495, 1282 $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$): δ 7.29 (d, *J* = 2.3 Hz, 1H, ArH), 7.07 (dd, *J* = 8.3, 2.3 Hz, 1H, ArH), 6.52 (d, *J* = 8.5 Hz, 1H, ArH), 3.33 (t, *J* = 5.8 Hz, 2H, $CH_2$), 1.76 (t, *J* = 5.8 Hz, 2H, $CH_2$), 1.29 (s, 6H, $C(CH_3)_2$), NH not observed; $^{13}C$ NMR (101 MHz, $CDCl_3$): δ 140.4, 133.7, 129.5, 129.4, 117.1, 110.6, 38.4, 36.4, 32.0, 30.8.

#### *4.2.4. tert-Butyl 6-bromo-4,4-dimethyl-3,4-dihydroquinoline-1(2H)-carboxylate (**9**)*

A THF (50 mL) solution containing **8** (3.0 g, 12.4 mmol) was cooled to –78 °C, and 2.5 M *n*-BuLi (6 mL, 15.0 mmol) was added dropwise over a period of 30 min. The solution was stirred for 30 min, and then di-*tert*-butyl dicarbonate (3.3 g, 14.9 mmol) in THF (15 mL) was added dropwise over a period of 30 min. The reaction mixture was slowly allowed to warm to 23 °C with stirring for 18 h, and then it was cooled to 0 °C. The mixture was quenched by dropwise addition of saturated $NH_4Cl$ solution (20 mL). The layers were separated, and the aqueous phase was extracted with ether (2 × 50 mL). The combined organic extracts were dried ($MgSO_4$), filtered, concentrated and purified by column chromatography (ether in hexanes gradient) to provide **9** as a brown oil (3.8 g, 11.4 mmol, 92%). IR: 1679, 1483, 1367, 1152 $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$): δ 7.52 (d, *J* = 6.6 Hz 1H, ArH), 7.36 (d, *J* = 2.4 Hz, 1H, ArH), 7.21 (dd, *J* = 6.6, 2.3 Hz, 1H, ArH), 3.72-3.69 (m, 2H, $CH_2$), 1.74-1.71 (m, 2H, $CH_2$), 1.51 (s, 9H, $C(CH_3)_3$), 1.28 (s, 6H, $C(CH_3)_2$); $^{13}C$ NMR (101 MHz, $CDCl_3$): δ 153.6, 140.2, 136.3, 128.7, 128.6, 126.0, 116.4, 81.1, 41.6, 38.1, 33.4, 29.9, 28.4.

#### *4.2.5. tert-Butyl 6-azido-4,4-dimethyl-3,4-dihydroquinoline-1(2H)-carboxylate (**10**) and tert-butyl 6-amino-4,4-dimethyl-3,4-dihydroquinoline-1(2H)-carboxylate (**11**)*

A mixture of **9** (3.5 g, 10.3 mmol), sodium azide (1.3 g, 20.6 mmol), CuI (0.2 g, 1.03 mmol), L-proline (0.35 g, 3.1 mmol), NaOH (0.12 g, 3.14 mmol) and ethanol/water (7:3, 20 mL) was heated to 90 °C in a 35-mL Chemglass pressure vessel (No. CG-1880-02) for 18 h.[17] The reaction mixture was cooled to 23 °C, filtered through Celite and the filter cake was washed with EtOAc (50 mL). The organic phase was washed with water (2 × 30 mL) and saturated NaCl, dried ($MgSO_4$), and concentrated under vacuum to provide **10** as a brown oil. This oil was quickly dissolved in methanol (100 mL), transferred to a 250-mL, round-bottomed flask, and 10% Pd/C (0.3 g) was added under a $N_2$ atmosphere. The reaction vessel was flushed with $H_2$ gas, and stirred under $H_2$ (1 atm, balloon) at 23 °C for 6 h. The catalyst was removed by filtration through Celite and washed with methanol (25 mL). The filtrate was concentrated and purified by column chromatography (hexanes:ether; 4:1) to afford **11** (1.8 g, 6.4 mmol, 62%) as a brown oil. IR: 3448, 3362, 1685, 1503, 1380 $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$): δ 7.28 (d, *J* = 1.6 Hz,

1H, Hz, 1H, ArH), 3.75-3.72 (m, 2H, $CH_2$), 1.76-1.73 (m, 2H, $CH_2$), 1.52 (s, 9H, $C(CH_3)_3$), 1.30 (s, 6H, $C(CH_3)_2$), $NH_2$ not observed; $^{13}C$ NMR (101 MHz, $CDCl_3$): δ 154.0, 142.1, 139.1, 128.7, 125.5, 113.1, 112.2, 80.3, 41.6, 38.8, 30.2, 28.5, 28.4**.**

*4.2.6. General procedure to prepare (thio)ureas and remove the Boc group*

To a stirred solution of **11** (0.2 g, 0.72 mmol) in THF (5 mL) was added various iso(thio)cyanates (0.72 mmol) in THF (2 mL) dropwise at 23 °C. The mixture was stirred until TLC analysis indicated that **11** was completely consumed. The solvent was evaporated under vacuum to give the Boc-protected (thio)urea derivatives **12a-d**. To each Boc-protected compound (**12a**, **b**, **c** or **d**) in dichloromethane (DCM, 5 mL) was slowly added trifluoroacetic acid (200 µL, 2.6 mmol), and the mixture was stirred until TLC indicated the absence of starting material. The solvent was removed under vacuum. To remove residual TFA, two additional portions of DCM (2 × 10 mL) were added and then removed under vacuum. Water (20 mL) was added to the resulting residue, and the mixture was washed with ether (2 × 20 mL). The aqueous layer was basified using $NaHCO_3$ powder and extracted with EtOAc (3 × 20 mL). Combined organic extracts were washed with water (2 × 20 mL), saturated NaCl (20 mL), dried ($MgSO_4$), filtered and concentrated under vacuum. Recrystallization of the crude products from pentane/ether (3:7) afforded pure **1a-d.**

*4.2.7. 1-(4,4-Dimethyl-1,2,3,4-tetrahydroquinolin-6-yl)-3-(4-nitrophenyl)urea (**1a**)*

Yellow solid (153 mg, 0.45 mmol, 63%), mp 219-220 °C; IR (nujol): 1698, 1548, 1180, 851 $cm^{-1}$; $^1H$ NMR (400 MHz, DMSO-$d_6$): δ 9.23 (s, 1H, NH), 8.36 (s, 1H, NH), 8.16 (d, *J* = 8.9 Hz, 2H, ArH), 7.66 (d, *J* = 8.9 Hz, 2H, ArH), 7.18 (s, 1H, ArH), 6.91 (d, *J* = 8.5 Hz, 1H, ArH), 6.39 (d, *J* = 8.5 Hz, 1H, ArH), 5.54 (s, 1H, NH), 3.16 (s, 2H, $CH_2$), 1.61 (s, 2H, $CH_2$), 1.22 (s, 6H, $C(CH_3)_2$); $^{13}C$ NMR (101 MHz, DMSO-$d_6$): δ 152.6, 147.4, 141.0, 129.4, 127.6, 125.6 (2C), 119.5, 118.7, 117.6, 114.1, 37.8, 37.3, 31.9, 31.3; MS(ESI): *m/z* 341 ($M+H^+$). *Anal.* Calcd. for $C_{18}H_{20}N_4O_3$: C, 63.52; H, 5.92; N, 16.46. Found: C, 63.36; H, 6.08; N, 16.51.

*4.2.8. 1-(4,4-Dimethyl-1,2,3,4-tetrahydroquinolin-6-yl)-3-(4-nitrophenyl)thiourea (**1b**)*

Red solid (164 mg, 0.46 mmol, 64%), mp 150-152 °C; IR (nujol): 3333, 1509, 1334, 1263 $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$): δ 8.19 (d, *J* = 8.6 Hz, 2H, ArH), 7.78 (d, *J* = 8.6 Hz, 2H, ArH), 7.71 (obscured signal, 2H, ArH, NH), 7.09 (s, 1H, NH), 6.88 (d, *J* = 8.3 Hz, 1H, ArH), 6.50 (d, *J* = 8.3 Hz, 1H, ArH), 4.19 (br s, 1H, NH), 3.39-3.36 (m, 2H, $CH_2$), 1.76-1.74 (m, 2H, $CH_2$), 1.30 (s, 6H, $C(CH_3)_2$); $^{13}C$ NMR (101 MHz, $CDCl_3$): δ 179.5, 144.28, 144.25, 144.1, 131.6, 125.1, 124.8, 124.4, 123.3, 122.7, 114.9, 38.2, 36.2, 32.0, 30.6; MS(ESI): *m/z* 357 ($M+H^+$) *Anal.* Calcd. for $C_{18}H_{20}N_4O_2S$: C, 60.65; H, 5.65; N, 15.72. Found: C, 60.53; H, 5.82; N, 15.87.

*4.8.9. 1-(4,4-Dimethyl-1,2,3,4-tetrahydroquinolin-6-yl)-3-(4-(trifluoromethyl)phenyl)thiourea (**1c**)*

Yellow solid (169 mg, 0.44 mmol, 61%), mp 104-105 °C; IR (nujol): 3346, 1512, 1324 $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$): δ 7.67 (br s, 1H, NH), 7.65 (d, *J* = 8.9 Hz, 2H, ArH), 7.58 (coincident d, *J* = 8.9 Hz, 2H, ArH and s, 1H, NH), 7.11 (d, *J* = 2.4 Hz, 1H, ArH), 6.89 (dd, *J* = 8.4, 2.4 Hz, 1H, ArH), 6.49 (d, *J* = 8.4 Hz, 1H, ArH), 4.02 (br s,1H, NH), 3.36 (t, *J* = 5.9 Hz, 2H, $^{13}C$ NMR (101 MHz, $CDCl_3$): δ 180.1, 143.9, 141.4, 131.5, 127.4 (q, *J* = 32.9 Hz), 125.9 (br), 125.2, 124.9, 123.9 (q, *J* = 271.5 Hz), 123.8, 114.8, 38.9, 36.3, 32.0, 30.6 (1 aromatic C unresolved); MS(ESI): *m/z* 380 ($M+H^+$). *Anal.* Calcd. for $C_{19}H_{20}F_3N_3S$: C, 60.14; H, 5.31; N, 11.07. Found: C, 60.28; H, 5.12; N, 11.28.

*4.2.10. 1-(4,4-Dimethyl-1,2,3,4-tetrahydroquinolin-6-yl)-3-(4-(trifluoromethoxy)phenyl)thiourea (**1d**)*

Yellow solid (164 mg, 0.42 mmol, 58%), mp 69-71 °C; IR (nujol): 3348, 3186, 1509, 1257 $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$): δ 7.69 (br s, 1H, NH), 7.49 (d, *J* = 9.0 Hz, 2H, ArH and s, 1H, NH), 7.18 (d, *J* = 9.0 Hz, 2H, ArH), 7.11 (d, *J* = 2.4 Hz, 1H, ArH), 6.89 (dd, *J* = 8.0, 2.4 Hz, 1H, ArH), 6.48 (d, *J* = 8.0 Hz, 1H, ArH), 4.20 (br s, 1H, NH), 3.35 (t, *J* = 5.5 Hz, 2H, $CH_2$), 1.74 (t, *J* = 5.5 Hz, 2H, $CH_2$), 1.29 (s, 6H, $C(CH_3)_2$); $^{13}C$ NMR (101 MHz, $CDCl_3$): δ 180.4, 146.7, 143.8, 136.8, 131.4, 126.1, 125.2, 124.9, 124.0 (br), 121.3, 120.4 (q, *J* = 257.4 Hz), 114.8, 38.2, 36.3, 31.9, 30.7; MS(ESI): *m/z* 396 ($M+H^+$). *Anal.* Calcd. for $C_{19}H_{20}F_3N_3OS$: C, 57.71; H, 5.10; N, 10.63. Found: C, 57.56; H, 5.23; N, 10.37.

*4.3. Synthesis of model compound **2***

*4.3.1. 1,1,4,4-Tetramethyl-6-(3-(4-nitrophenyl)ureido)-1,2,3,4-tetrahydroquinolin-1-ium iodide (**2**)*

To a stirred solution of **1a** (200 mg, 0.59 mmol) in DMF (5 mL) in a 15 mL Chemglass pressure vessel (No. CG-1880-01) was added $Cs_2CO_3$ (390 mg, 1.20 mmol) and methyl iodide (1.0 mL, 16.0 mmol). The vessel was closed, and the reaction was stirred at 23 °C for 24 h. The vessel was unsealed, water (5 mL) was added, and the solid was filtered. The crude solid was stirred with ethanol (10 mL) for 15 min and filtered to provide **2** as a yellow solid (185 mg, 0.37 mmol, 62%), mp 249-251 °C. IR ($CHCl_3$): 3297, 3260, 1724, 1598, 843 $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$): δ 9.59 (s, 1H, NH), 9.21 (s, 1H, NH), 8.21 (d, *J* = 8.9 Hz, 2H, ArH), 7.88 (d, *J* = 9.2 Hz, 1H, ArH), 7.71 (d, *J* = 8.9 Hz, 2H, ArH), 7.67 (s, 1H, ArH), 7.51 (d, *J* = 9.2 Hz, 1H, ArH), 3.89 (m, 2H, $CH_2$), 3.56 (s, 6H, $N^+(CH_3)_2$), 2.11 (m, 2H, $CH_2$), 1.36 (s, 6H, $C(CH_3)_2$); $^{13}C$ NMR (101 MHz, $CDCl_3$): δ 151.4, 145.4, 140.7, 139.7, 139.4, 135.0, 124.5, 121.3, 117.4, 117.2, 116.7, 59.4, 56.1, 31.6, 30.5, 26.8; MS(ESI): *m/z* 370 ($M+H^+$). *Anal.* Calcd. for $C_{20}H_{25}IN_4O_3$: C, 48.40; H, 5.08; N, 11.29. Found: C, 48.65; H, 5.23; N, 11.52.

*4.4. Synthesis of **3a-g** (series 3)*

*4.4.1. 6-Bromo-2,2,4-trimethyl-1,2-dihydroquinoline (**13**)*

Bismuth(III) trifluoromethanesulfonate (19.0 g, 30.0 mmol) was added to a solution of 4-bromoaniline (25.0 g, 145 mmol) in acetone (500 mL), and the mixture was stirred at reflux for 3 days.[18] The solvent was removed under vacuum, and the residue was partitioned between ether (300 mL) and water (200 mL). The layers were separated, and the aqueous phase was extracted with ether (2 x 100 mL). The combined organic extracts were washed with saturated NaCl and evaporated under vacuum. The crude product was purified by column chromatography (ether in hexanes gradient) to afford **13** as a brown solid (23 g, 89.9 mmol, 62%), mp 83-85 °C. IR: 3382, 1486, 1257, 806 $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$): δ 7.13 (d, *J* = 2.2 Hz, 1H, ArH), 7.05 (dd, *J* = 8.4, 2.2 Hz, 1H, ArH), 6.31 (d, *J* = 8.4 Hz, 1H, ArH), 5.33 (s, 1H, =CH), 3.71 (br s, 1H, NH), 1.95 (s, 3H, $=CCH_3$), 1.26 (s, 6H, $C(CH_3)_2$); $^{13}C$ NMR (101 MHz, $CDCl_3$): δ 142.2, 130.7, 129.4, 127.6, 126.2, 123.4, 114.3, 108.6, 51.9, 30.9, 18.4.

*4.4.2.* Sodium hydride (4.5 g of a 60% dispersion in mineral oil, 113.0 mmol) was added to DMF (190 mL) under $N_2$, and the mixture was cooled to 15 °C. A solution of **13** (19.0 g, 75.3 mmol) in DMF (75 mL) was added dropwise. The mixture was stirred for 30 min, and then methyl iodide (42.6 g, 18.7 mL, 300 mmol) in DMF (75 mL) was added dropwise. The reaction mixture was allowed to warm to 23 °C gradually and was then stirred for 18 h. The crude reaction mixture was added to water and extracted with ether (2 × 100 mL). The combined organic extracts were washed with saturated NaCl, dried ($MgSO_4$), filtered and concentrated to provide the **14** (16.4 g, 62 mmol, 82%) as a yellow oil. IR: 1488, 1406, 797 $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$): δ 7.16 (dd, *J* = 8.4, 2.4 Hz, 1H, ArH), 7.11 (d, *J* = 2.4 Hz, 1H, ArH), 6.37 (d, *J* = 8.4 Hz, 1H, ArH), 5.32 (d, *J* = 1.5 Hz, 1H, =CH), 2.76 (s, 3H, $NCH_3$), 1.95 (s, 3H, $=CCH_3$), 1.29 (s, 6H, $C(CH_3)_2$); $^{13}C$ NMR (101 MHz, $CDCl_3$): δ 144.2, 131.2, 130.9, 127.3, 125.8, 125.2, 112.2, 108.4, 56.3, 30.7, 27.1, 18.5.

*4.4.3. 6-Bromo-1,2,2,4-tetramethyl-1,2,3,4-tetrahydroquinolin-3-ol (**15**)*

A 1.0 M borane:THF solution (97.0 mmol, 97 mL) was added dropwise to an ice-cooled solution of **14** (13.0 g, 48.8 mmol) in THF (250 mL), and the mixture was stirred at 15 °C for 6 h. A 1:1 solution of THF/$H_2O$ (60 mL) was added dropwise to the reaction mixture over 30 min, followed by dropwise addition of 3 M NaOH (50 mL) over 30 min. To this mixture was added 30% aqueous hydrogen peroxide (16.0 mL), and stirring was continued at 23 °C for 2 h. The crude reaction mixture was poured into water and extracted with EtOAc (3 × 150 mL). The combined organic layers were washed with saturated $NaHCO_3$ and saturated NaCl, dried ($Na_2SO_4$), filtered and concentrated under reduced pressure. The crude product was purified by flash chromatography (hexane/EtOAc, 7:3) to afford **15** as a colorless oil (7.9 g, 27.8 mmol, 57%). IR: 3406, 1589, 1490 $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$): δ 7.24 (d, *J* = 2.4 Hz, 1H, ArH), 7.17 (dd, *J* = 8.8, 2.4 Hz, 1H, ArH), 6.47 (d, *J* = 8.8 Hz, 1H, ArH), 3.27 (d, *J* = 9.4 Hz, 1H, *CH*OH), 2.79 (s, 3H, $NCH_3$), 2.76-2.68 (m, 1H, *CH*$CH_3$); 1.90 (br s, 1H, OH), 1.41 (d, *J* = 6.8 Hz, 3H, $CH_3$), 1.36 (s, 3H, $CH_3$), 1.01 (s, 3H, $CH_3$); $^{13}C$ NMR (101 MHz, $CDCl_3$): δ 144.3, 129.9, 129.7, 128.2, 113.8, 109.0, 58.0, 36.1, 31.6, 24.9, 18.1, 17.0.

*4.4.4. 6-Bromo-1,2,2,4-tetramethyl-1,2,3,4-tetrahydroquinolin-3-one (**16**) and 6-Bromo-1,2,2,4,4-pentamethyl-1,4-dihydro-quinolin-3(2H)-one (**17**)*

DMSO (2.3 mL, 31.7 mmol) was added dropwise to a solution of oxalyl chloride (1.4 mL, 17.3 mmol) in DCM (60 mL) at –60 °C, and the resulting mixture was stirred for 10 min. This mixture was transferred *via* cannula to a solution of **15** (4.1 g, 14.4 mmol) in DCM (60 mL) at –60 °C. The mixture was stirred for 15 min, and then triethylamine (10 mL, 72.0 mmol) was added dropwise over 30 min. The reaction was stirred for 1 h and quenched by dropwise addition of water (20 mL). The mixture was stirred while warming to 23 °C, and the layers were separated. The organic layer was washed with water (2 × 20 mL), dried ($MgSO_4$), filtered and concentrated to give **16**. The crude product was used directly for the next step without further purification.

To a solution of **16** (*ca*. 14.4 mmol from above) in THF (20 mL) was added dropwise 1.3 M lithium bis(trimethylsilyl)amide in THF (15.4 mL, 20.0 mmol) over 10 min at –50 °C. The reaction was allowed to warmed to –20 °C, iodomethane (3.55 g, 1.56 mL, 25.0 mmol) in THF (20 mL) was added dropwise, and The reaction mixture was poured into ice-cold water and extracted with EtOAc (2 × 50 mL). The combined organic layers were washed with saturated NaCl, dried ($Na_2SO_4$), filtered and concentrated under vacuum. Purification by column chromatography (hexanes/EtOAc, 3:2) gave **17** (2.6 g, 8.9 mmol, 62%) as a colorless oil. IR: 1719, 1486 $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$): δ 7.33 (dd, *J* = 8.6, 2.3 Hz, 1H, ArH), 7.29 (d, *J* = 2.3 Hz, 1H, ArH), 6.08 (d, *J* = 8.6 Hz, 1H, ArH), 2.83 (s, 3H, $NCH_3$), 1.45 (s, 6H, $C(CH_3)_2$), 1.28 (s, 6H, $C(CH_3)_2$); $^{13}C$ NMR (101 MHz, $CDCl_3$): δ 214.4, 144.6, 132.7, 130.4, 127.5, 115.7, 112.4, 64.2, 47.6, 30.9, 23.3, 22.9.

*4.4.5. 6-Amino-1,2,2,4,4-pentamethyl-1,4-dihydroquinolin-3(2H)-one (**18**)*

Into a 200-mL Chemglass pressure vessel (No. CG-1880-R-03) was added **17** (1.9 g, 6.42 mmol), copper iodide (0.61 g, 3.2 mmol), L-proline (0.74 g, 6.42 mmol), DMF (4.0 mL) and aqueous ammonia (19.0 mL). The reaction mixture was heated to 110 °C for 24 h, and then cooled to 23 °C and finally quenched with water (150 mL). The resulting mixture was extracted with EtOAc (3 × 100 mL), and the combined extracts were dried ($MgSO_4$), filtered and concentrated under vacuum. Purification by column chromatography (EtOAc in hexanes gradient) gave **18** (0.9 g, 3.9 mmol, 65%) as a brown oil. IR: 3422, 3357, 1711, 1501 $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$): δ 6.66-6.60 (m, 3H, ArH), 3.35 (br s, 2H, $NH_2$), 2.78 (s, 3H, $NCH_3$), 1.44 (s, 6H, $C(CH_3)_2$), 1.25 (s, 6H, $C(CH_3)_2$); $^{13}C$ NMR (101 MHz, $CDCl_3$): δ 215.8, 139.6, 138.6, 132.0, 115.0, 114.5, 112.7, 64.3, 47.7, 31.0, 23.0, 22.9.

*4.4.6. 1-(4-Nitrophenyl)-3-(1,2,2,4,4-pentamethyl-3-oxo-1,2,3,4-tetrahydroquinolin-6-yl)urea (**3a**)*

The general procedure for the preparation of iso(thio)ureas was used. To a stirred solution of **18** (0.2 g, 0.86 mmol) in THF (5 mL) was added dropwise a series of iso(thio)cyanates (0.86 mmol) in THF (2 mL) at 23 °C. Stirring was continued until TLC indicated the reaction was complete. The solvent was evaporated under vacuum, the residue was purified by column chromatography (EtOAc in hexanes gradient), and the product was crystallized from ether in pentane (3:7). Orange solid (0.25 g, 0.64 mmol, 74%), mp 200-201 °C; IR (nujol): 1718, 1655, 1556 $cm^{-1}$; $^1H$ NMR (400 MHz, DMSO-$d_6$): δ 9.35 (s, 1H, NH), 8.73 (s, 1H, NH), 8.18 (d, *J* = 8.0 Hz, 2H, ArH), 7.68 (d, *J* = 8.0 Hz, 2H, ArH), 7.35 (s, 1H, ArH), 7.31 (d, *J* = 8.7 Hz, 1H, ArH), 6.82 (d, *J* = 8.7 Hz, 1H, ArH), 2.79 (s, 3H, $NCH_3$), 1.39 (s, 6H, $C(CH_3)_2$), 1.20 (s, 6H, $C(CH_3)_2$); $^{13}C$ NMR (101 MHz, DMSO-$d_6$): δ 213.6, 151.5, 146.0, 140.4, 140.2, 131.1, 129.6, 124.5, 118.2, 116.7, 115.4, 113.7, 63.1, 46.5, 30.2, 22.2, 22.1; MS(ESI): *m/z* 397 ($M+H^+$). *Anal.* Calcd. for $C_{21}H_{24}N_4O_4$: C, 63.62; H, 6.10; N, 14.13. Found: C, 63.39; H, 6.26; N, 14.27.

*4.4.7. 1-(4-Nitrophenyl)-3-(1,2,2,4,4-pentamethyl-3-oxo-1,2,3,4-tetrahydroquinolin-6-yl)thiourea (**3b**)*

Yield: 0.23 g (0.56 mmol, 65%) as a yellow solid, mp 145-147 °C; IR (nujol): 3308, 1715, 1532, 1498, 1332 $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$): δ 8.20 (d, *J* = 8.7 Hz, 2H, ArH), 7.83 (s, 1H, NH), 7.75 (d, *J* = 8.7 Hz, 2H, ArH), 7.69 (s, 1H, NH), 7.21 (dd, *J* = 8.6, 2.4 Hz, 1H, ArH), 7.14 (d, *J* = 2.4 Hz, 1H, ArH), 6.88 (d, *J* = 8.5 Hz, 1H, ArH), 2.91 (s, 3H, $NCH_3$), 1.45 (s, 6H, $C(CH_3)_2$), 1.32 (s, 6H, $C(CH_3)_2$); $^{13}C$ NMR (101 MHz, $CDCl_3$): δ 213.6, 179.4, 145.7, 144.5, 144.0, 132.7, 127.0, 125.7, 124.5, 123.0, 122.8, 115.1, 64.4, 47.7, 31.1, 23.7, 23.0; MS(ESI): *m/z* 413 ($M+H^+$). *Anal*. Calcd. for $C_{21}H_{24}N_4O_3S$: C, 61.15; H, 5.86; N, 13.58. Found: C, 61.38; H, 5.52; N, 13.37.

*4.4.* *lin-6-yl)-3-(4-(trifluoromethyl)phenyl)urea (**3c**)*

Brown solid (0.28 g, 0.67 mmol, 78%), mp 198-199 °C; IR (nujol): 3328, 1720, 1656 $cm^{-1}$; $^1H$ NMR (400 MHz, DMSO-$d_6$): δ 8.78 (s, 1H, NH), 8.52 (s, 1H, NH), 7.54 (d, *J* = 8.5 Hz, 2H, ArH), 7.32-7.24 (complex, 4H, ArH), 6.79 (d, *J* = 8.6 Hz, 1H, ArH), 2.82 (s, 3H, $NCH_3$), 1.38 (s, 6H, $C(CH_3)_2$), 1.19 (s, 6H, $C(CH_3)_2$); $^{13}C$ NMR ($CDCl_3$): δ 213.7, 151.8, 143.1, 140.1, 131.4, 129.6, 125.4 (q, *J* = 3.5 Hz), 124.1 (q, *J* = 272.5 Hz), 120.8 (q, *J* = 32.0 Hz), 118.1, 117.1, 115.3, 113.7, 63.1, 46.5, 30.2, 22.2 (2C) ; MS(ESI): *m/z* 420 ($M+H^+$). *Anal.* Calcd. for $C_{22}H_{24}F_3N_3O_2$: C, 63.00; H, 5.77; N, 10.02. Found: C, 63.12; H, 5.59; N, 10.29.

*4.4.9. 1-(1,2,2,4,4-Pentamethyl-3-oxo-1,2,3,4-tetrahydroquinolin-6-yl)-3-(4-trifluoromethyl)phenyl)thiourea (**3d**)*

Brown solid (0.25 g, 0.58 mmol, 67%), mp 149-151 °C; IR (nujol): 3291, 3206, 1716, 1615, 1324 $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$): δ 7.88 (s, 1H, NH), 7.65 (s, 1H, NH), 7.61 (d, *J* = 9.1 Hz, 2H, ArH), 7.59 (d, *J* = 9.1 Hz, 2H, ArH), 7.22 (dd, *J* = 8.5, 2.6 Hz, 1H, ArH), 7.15 (d, *J* = 2.4 Hz, 1H, ArH), 6.86 (d, *J* = 8.5 Hz, 1H, ArH), 2.90 (s, 3H, $NCH_3$), 1.48 (s, 6H, $C(CH_3)_2$), 1.32 (s, 6H, $C(CH_3)_2$); $^{13}C$ NMR (101 MHz, $CDCl_3$): δ 214.0, 179.8, 145.3, 141.1, 132.3, 127.8 (q, *J* = 32.6 Hz), 127.7 (br), 126.1 (q, *J* = 2.7 Hz), 125.6, 124.1, 123.9 (q, *J* = 272.0 Hz), 122.7, 115.0, 64.4, 47.6, 31.1, 23.6, 23.0; MS(ESI): *m/z* 436 ($M+H^+$). *Anal.* Calcd. for $C_{22}H_{24}F_3N_3OS$: C, 60.67; H, 5.55; N, 13.09. Found: C, 60.94; H, 5.23; N, 13.28.

*4.4.10. 1-(1,2,2,4,4-Pentamethyl-3-oxo-1,2,3,4-tetrahydroquinolin-6-yl)-3-(4-(trifluoromethoxy)phenyl)urea (**3e**)*

Brown solid (0.27 g, 0.62 mmol, 72%), mp 191-192 °C; IR (nujol): 3318, 1716, 1648 $cm^{-1}$; $^1H$ NMR (400 MHz, DMSO-$d_6$): δ 9.01 (s, 1H, NH), 8.61 (s, 1H, NH), 7.65 (d, *J* = 8.8 Hz, 2H, ArH), 7.61 (d, *J* = 8.8 Hz, 2H, ArH), 7.32 (d, *J* = 2.4 Hz, 1H, ArH), 7.30 (dd, *J* = 8.5, 2.4 Hz, 1H, ArH), 6.80 (d, *J* = 8.6 Hz, 1H, ArH), 2.79 (s, 3H, $NCH_3$), 1.39 (s, 6H, $C(CH_3)_2$), 1.19 (s, 6H, $C(CH_3)_2$); $^{13}C$ NMR (101 MHz, DMSO-$d_6$): δ 213.7, 152.1, 141.8, 140.0, 138.7, 131.6, 129.6, 121.1, 119.6 (q, *J* = 255.2 Hz), 118.6, 117.9, 115.2, 113.7, 63.1, 46.5, 30.2, 22.1 (2C); MS(ESI): *m/z* 436 ($M+H^+$). *Anal.* Calcd. for $C_{22}H_{24}F_3N_3O_3$: C, 60.68; H, 5.56; N, 9.65. Found: C, 60.79; H, 5.76; N, 9.83.

*4.4.11. 1-(1,2,2,4,4-Pentamethyl-3-oxo-1,2,3,4-tetrahydroquinolin-6-yl)-3-(4-(trifluoromethoxy)phenyl)thiourea (**3f**)*

Brown solid (0.24 g, 0.52 mmol, 60%), mp 83-85 °C; IR (nujol): 3291, 3213, 1716, 1501 $cm^{-1}$; $^1H$ NMR (400 MHz, DMSO-$d_6$): δ 9.76 (s, 1H, NH), 9.72 (s, 1H, NH), 7.56 (d, *J* = 8.8 Hz, 2H, ArH), 7.30 (coincident d, *J* = 8.8 Hz, 2H, ArH and dd, *J* = 8.7, 2.1 Hz, 1H, ArH), 7.24 (d, *J* = 2.1 Hz, 1H, ArH), 6.83 (d, *J* = 8.7 Hz, 1H, ArH), 2.81 (s, 3H, $NCH_3$), 1.37 (s, 6H, $C(CH_3)_2$), 1.21 (s, 6H, $C(CH_3)_2$); $^{13}C$ NMR (101 MHz, DMSO-$d_6$): δ 213.5, 178.9, 143.8, 142.0, 138.3, 130.8, 129.1, 124.6, 123.3, 120.5, 120.2, 119.5 (q, *J* = 255.7 Hz), 113.3, 63.2, 46.4, 30.3, 22.4, 22.1; MS(ESI): *m/z* 452 ($M+H^+$). *Anal.* Calcd. for $C_{22}H_{24}F_3N_3O_2S$: C, 58.52; H, 5.36; N, 9.31. Found: C, 58.29; H, 5.12; N, 9.07.

*4.4.12. 1-(4-Aminophenyl)-3-(1,2,2,4,4-pentamethyl-3-oxo-1,2,3,4-tetrahydroquinolin-6-yl)urea (**3g**)*

To a stirred suspension of **3a** (120 mg, 0.30 mmol) and iron powder (electrolytic, 106 mg, 1.88 mmol, ≥100 mesh) in ethanol:water (4:1, 6.0 mL) was added $NH_4Cl$ (48 mg, 0.90 The reaction was cooled and filtered through Celite. The Celite was washed with ethanol (3 × 5 mL), and the filtrate was concentrated under vacuum at 45 °C to give a brown solid. Recrystallization of the solid from ether-pentane gave pure **3g** (92 mg (0.25 mmol, 84%) as a brown solid, mp 135-137 °C. IR (nujol): 3297, 1713, 1642, 1601, 1502 $cm^{-1}$; $^1H$ NMR (400 MHz, DMSO-$d_6$): δ 8.26 (s, 1H, NH), 8.00 (s, 1H, NH), 7.38-7.22 (complex, 2H, ArH), 7.05 (d, *J* = 8.2 Hz, 2H, ArH), 6.76 (d, *J* = 8.6 Hz, 1H, ArH), 6.50 (d, *J* = 8.2 Hz, 2H, ArH), 4.76 (br s, 2H, $NH_2$), 2.77 (s, 3H, $NCH_3$), 1.37 (s, 6H, $C(CH_3)_2$), 1.18 (s, 6H, $C(CH_3)_2$); $^{13}C$ NMR (101 MHz, DMSO-$d_6$): δ 213.8, 152.6, 143.2, 139.5, 132.4, 129.5, 128.2, 120.0, 117.4, 114.7, 113.7, 113.5, 63.1, 46.5, 30.2, 22.2, 22.1; MS(ESI): *m/z* 367 ($M+H^+$). *Anal.* Calcd. for $C_{21}H_{26}N_4O_2$: C, 68.83; H, 7.15; N 15.29. Found: C, 68.66; H, 7.26; N, 15.07.

*4.5. Synthesis of **4a-f** (series 4)*

*4.5.1. General procedure to synthesize **4a-f** (series 4)*

To a stirred solution of **18** (0.2 g, 0.86 mmol) in THF (10 mL) was added portion-wise lithium aluminum hydride (65.0 mg, 1.72 mmol) at 0 °C. The mixture was stirred at 23 °C for 4 h, quenched with saturated $Na_2SO_4$ at 0 °C, filtered through Celite and extracted with EtOAc (20 mL). The organic extract was washed with water, saturated NaCl, dried ($Na_2SO_4$), filtered and concentrated to give **19** as a brown oil. The oil was dissolved in THF (5 mL), and the solution was added dropwise at 23 °C to a solution of an iso(thio)cyanate (0.86 mmol) in THF (5 mL). When TLC analysis indicated the disappearance of **19**, the reaction mixture was concentrated under vacuum and purified by column chromatography (EtOAc in hexanes gradient). Concentration of the major fraction and crystallization from DCM/ether mixture (2:8) afforded **4a-f**.

*4.5.2. 1-(3-Hydroxy-1,2,2,4,4-pentamethyl-1,2,3,4-tetrahydroquinolin-6-yl)-3-(4-nitrophenyl)urea (**4a**)*

Yellow solid (0.27 g, 0.69 mmol, 80%), mp 215-217 °C; IR (nujol): 3473, 3251, 1659, 1556 $cm^{-1}$; $^1H$ NMR (400 MHz, DMSO-$d_6$): δ 9.27 (s, 1H, NH), 8.51 (s, 1H, NH), 8.17 (d, *J* = 8.8 Hz, 2H, ArH), 7.67 (d, *J* = 8.8 Hz, 2H, ArH), 7.27 (s, 1H, ArH), 7.11 (d, *J* = 8.6 Hz, 1H, ArH), 6.51 (d, *J* = 8.8 Hz, 1H, ArH), 5.17 (d, *J* = 6.4 Hz, 1H, OH), 3.23 (d, *J* = 6.4 Hz, 1H, *CH*OH), 2.71 (s, 3H, $NCH_3$), 1.26 (s, 3H, $CH_3$), 1.23 (s, 3H, $CH_3$), 1.16 (s, 3H, $CH_3$), 1.06 (s, 3H, $CH_3$); $^{13}C$ NMR (101 MHz, DMSO-$d_6$): δ 151.5, 146.2, 140.0, 139.8, 132.1, 128.0, 124.5, 117.9, 117.1, 116.5, 111.4, 78.2, 57.7, 37.2, 30.9, 28.8, 26.5, 22.9, 17.6; MS(ESI): *m/z* 399 ($M+H^+$). *Anal.* Calcd. for $C_{21}H_{26}N_4O_4$: C, 63.30; H, 6.58; N, 14.06. Found: C, 63.62; H, 6.81; N, 14.16.

*4.5.3. 1-(3-Hydroxy-1,2,2,4,4-pentamethyl-1,2,3,4-tetrahydroquinolin-6-yl)-3-(4-nitrophenyl)thiourea (**4b**)*

Yellow solid (0.22 g, 0.53 mmol, 62%), mp 161-163 °C; IR (nujol): 3444, 1645, 1377 $cm^{-1}$; $^1H$ NMR (400 MHz, DMSO-$d_6$): δ 10.1 (s, 1H, NH), 10.0 (s, 1H, NH), 8.17 (d, *J* = 8.7 Hz, 2H, ArH), 7.81 (d, *J* = 8.7 Hz, 2H, ArH), 7.23 (s, 1H, ArH), 7.13 (d, *J* = 8.7 Hz, 1H, ArH), 6.54 (d, *J* = 8.8 Hz, 1H, ArH), 5.21 (d, *J* = 6.3 Hz, 1H, OH), 3.23 (d, *J* = 6.4 Hz, 1H, *CH*OH), 2.74 (s, 3H, $NCH_3$), 1.25 (s, 6H, $C(CH_3)_2$), 1.15 (s, 3H, $CH_3$), 1.09 (s, 3H, $CH_3$); $^{13}C$ NMR (101 MHz, DMSO-$d_6$): δ 177.9, 146.0, 141.4, 141.3, 131.5, 127.4, 123.7, 122.2, 121.3, 120.5, 110.9, 77.9, 57.9, 37.1, 31.0, 28.5, 26.8, 22.9, 17.9; MS(ESI): *m/z* 415 ($M+H^+$). *Anal.* Calcd. for $C_{21}H_{26}N_4O_3S$: C, 60.85; H, 6.32; N, 13.52. Found: C, 60.58; H, 6.42; N, 13.71.

*4.5.4. 1-(3-Hydroxy-1,2,2,4,4-pentamethyl-1,2,3,4-tetrahydro-quinolin-6-yl)-3-(4-(trifluoromethyl)phenyl)urea (**4c**)*

Brown solid (0.26 g, 0.61 mmol, 71%), mp 213-215 °C; IR (nujol): 3308, 1647, 1605 $cm^{-1}$; $^1H$ NMR (400 MHz, DMSO-$d_6$): δ 8.92 (s, 1H, NH), 8.38 (s, 1H, NH), 7.64 (d, *J* = 8.7 Hz, 2H, ArH), 7.60 (d, *J* = 8.7 Hz, 2H, ArH), 7.25 (d, *J* = 2.3 Hz, 1H, ArH), 7.10 (dd, *J* = 8.7, 2.3 Hz, 1H, ArH), 6.50 (d, *J* = 8.7 Hz, 1H, ArH), 5.16 (d, *J* = 6.4 Hz, 1H, OH), 3.22 (d, *J* = 6.4 Hz, 1H, *CH*OH), 2.70 (s, 3H, $NCH_3$), 1.26 (s, 3H, $CH_3$), 1.22 (s, 3H, $CH_3$), 1.16 (s, 3H, $CH_3$), 1.06 (s, 3H, $CH_3$); $^{13}C$ NMR (101 MHz, DMSO-$d_6$): δ 151.9, 143.3, 139.6, 132.1, 128.4, 125.4 (q, *J* = 4.4 Hz), 124.0 (q, *J* = 271.3 Hz), 120.6 (q, *J* = 31.9 Hz), 117.7, 117.0, 116.9, 111.4, 78.2, 57.7, 37.2, 30.9, 28.8, 26.5, 22.9, 17.6; MS(ESI): *m/z* 422 ($M+H^+$). *Anal.* Calcd. for $C_{22}H_{26}F_3N_3O_2$: C, 62.70; H, 6.22; N, 9.97. Found: C, 62.58; H, 6.48; N, 10.12.

*4.5.5. 1-(3-Hydroxy-1,2,2,4,4-pentamethyl-1,2,3,4-tetrahydro-quinolin-6-yl)-3-(4-(trifluoromethyl)phenyl)thiourea (**4d**)*

Brown solid (0.23 g, 0.52 mmol, 60%), mp 105-107 °C; IR (nujol): 3345, 1615, 1501 $cm^{-1}$; $^1H$ NMR (400 MHz, DMSO-$d_6$): δ 9.79 (s, 2H, 2NH), 7.73 (d, *J* = 8.5 Hz, 2H, ArH), 7.64 (d, *J* = 8.5 Hz, 2H, ArH), 7.20 (s, 1H, ArH), 7.11 (d, *J* = 8.7 Hz, 1H, ArH), 6.53 (d, *J* = 8.7 Hz, 1H, ArH), 5.20 (d, *J* = 6.4 Hz, 1H, OH), 3.23 (d, *J* = 6.4 Hz, 1H, *CH*OH), 2.74 (s, 3H, $NCH_3$), 1.25 (s, 3H, $CH_3$), 1.24 (s, 3H, $CH_3$), 1.15 (s, 3H, $CH_3$), 1.09 (s, 3H, $CH_3$); $^{13}C$ NMR (101 MHz, DMSO-$d_6$): δ 178.4, 143.1, 141.3, 131.5, 127.6, 124.8 (q, *J* = 3.6 Hz), 123.9 (q, *J* = 252.2 Hz), 122.8 (q, *J* = 32.0 Hz), 122.4, 121.9, 121.5, 110.9, 77.9, 57.9, 37.1, 31.0, 28.5, 26.8, 22.9, 17.8; MS(ESI): *m/z* 438 ($M+H^+$). *Anal.* Calcd. for $C_{22}H_{26}F_3N_3OS$: C, 60.39; H, 5.99; N, 9.60. Found: C, 60.28; H, 6.12; N, 9.47.

*4.5.6. 1-(3-Hydroxy-1,2,2,4,4-pentamethyl-1,2,3,4-tetrahydro-quinolin-6-yl)-3-(4-(trifluoromethoxy)phenyl)urea (**4e**)*

Brown solid (0.25 g, 0.57 mmol, 66%), mp 188-189 °C; IR (nujol): 3467, 3310, 1648, 1554 $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$): δ 8.68 (s, 1H, NH), 8.28 (s, 1H, NH), 7.53 (d, *J* = 8.7 Hz, 2H, ArH), 7.26 (s, 1H, ArH), 7.23 (d, *J* = 8.7 Hz, 2H, ArH), 7.09 (dd, *J* = 8.7, 2.5 Hz, 1H, ArH), 6.49 (d, *J* = 8.8 Hz, 1H, ArH), 5.16 (d, *J* = 6.4 Hz, 1H, OH), 3.22 (d, *J* = 6.4 Hz, 1H, *CH*OH) 2.70 (s, 3H, $NCH_3$), 1.26 (s, 3H, $CH_3$), 1.22 (s, 3H, $CH_3$), 1.16 (s, 3H, $CH_3$), 1.05 (s, 3H); $^{13}C$ NMR (101 MHz, DMSO-$d_6$): δ 152.2, 141.7, 139.5, 138.8, 132.1, 128.6, 121.0, 119.6 (q, *J* = 255.1 Hz), 118.5, 117.7, 117.0, 111.5, 78.2, 57.7, 37.2, 30.9, 28.8, 26.5, 22.9, 17.5; MS(ESI): *m/z* 438 ($M+H^+$). *Anal.* Calcd. for $C_{22}H_{26}F_3N_3O_3$: C, 60.40; H, 5.99; N, 9.61. Found: C, 60.28; H, 6.15; N, 9.38.

*4.5.7. 1-(3-Hydroxy-1,2,2,4,4-pentamethyl-1,2,3,4-tetrahydro-quinolin-6-yl)-3-(4-(trifluoromethoxy)phenyl)thiourea (**4f**)*

Brown solid (0.24 g, 0.52 mmol, 60%), mp 97-99 °C; IR (nujol): 3419, 1605, 1501 $cm^{-1}$; $^1H$ NMR (400 MHz, DMSO-$d_6$): δ 9.62 (s, 1H, NH), 9.55 (s, 1H, NH), 7.57 (d, *J* = 8.6 Hz, 2H, ArH), 7.29 (d, *J* = 8.6 Hz, 2H, ArH), 7.17 (s, 1H, ArH), 7.08 (d, *J* = 8.8 Hz, 1H, ArH), 6.52 (d, *J* = 8.8 Hz, 1H, ArH), 5.19 (d, *J* = 6.4 Hz, 1H, OH), 3.22 (d, *J* = 6.4 Hz, 1H, *CH*OH), 2.73 (s, 3H, $NCH_3$), 1.25 (s, 3H, $CH_3$), 1.24 (s, 3H, $CH_3$), 1.14 (s, 3H, $CH_3$), 1.09 (s, 3H, $CH_3$); $^{13}C$ NMR (101 MHz, DMSO-$d_6$): δ 179.8, 144.8, 142.4, 139.5, 132.6, 128.7, 125.5, 123.7, 122.7, 121.5, 121.3 (q, *J* = 255.5 Hz), 112.0, 79.0, 58.9, 38.2, 32.0, 29.6, 27.8, 23.9, 18.9; MS(ESI): *m/z* 454 ($M+H^+$). *Anal.* Calcd. for $C_{22}H_{26}F_3N_3O_2S$: C, 58.26; H, 5.78; N, 9.27. Found: C, 58.34; H, 5.57; N, 9.31.

*4.6.1. Ethyl 2,2,4-trimethyl-4-pentenoate (**21**)*

A 1-L, three-necked, round-bottomed flask was charged with a solution of diisopropylamine (15.9 g, 21.9 mL,15.7 mmol) in freshly distilled THF (200 mL). The solution was cooled to –70 °C, and then *n*-butylllithium (2.5 M, 63.0 mL, 157.5 mmol) was added to the solution. After 5 min, ethyl isobutyrate (**20**, 14.0 g, 120.5 mmol) was added dropwise. Stirring was continued for 1 h at –78 °C. A solution of 3-iodo-2-methylpropene (21.9 g, 12.9 mL, 120.5 mmol) in 100 mL of THF was added dropwise, and the mixture was stirred overnight while warming to 23 °C. The reaction mixture was poured into a mixture of ice and 1 *M* HCl, and the product was extracted with ether (3 × 100 mL). The combined ether extracts were washed with saturated NaCl, dried ($MgSO_4$), filtered, and concentrated. Vacuum distillation afforded **21** (12.8 g, 64%) as a colorless liquid, bp 50 ºC (2 mm) [lit[22a] bp 72-74 °C (14 mm)]. IR: 3078, 1729, 1645, 895 $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$): δ 4.79 (s, 1H, =CH), 4.65 (s, 1H, CH), 4.08 (q, *J* = 7.1 Hz, 2H, O$CH_2$$CH_3$), 2.31 (s, 2H, $CH_2$), 1.66 (s, 3H, =$CCH_3$), 1.25 (t, *J* = 7.1 Hz, 3H, $OCH_2CH_3$), 1.17 (s, 6H, $C(CH_3)_2$); $^{13}C$ NMR (75 MHz, $CDCl_3$): δ 177.7, 142.3, 113.9, 60.1, 48.2, 41.7, 25.4, 23.3, 13.9.

*4.6.2. 2,2,4-Trimethyl-4-pentenoic acid (**22**)*

A 250-mL, round-bottomed flask was charged with **21** (11.2 g, 66.6 mmol) in 25 mL of MeOH, and then 20% NaOH (30 mL) was added, and the mixture was refluxed overnight at 60-70 °C. After cooling to 23 °C, 50 mL of water was added, and the mixture was acidified with 1 M HCl. The product was extracted with ether (3 × 100 mL), and the combined ether extracts were washed with saturated NaCl, dried ($MgSO_4$), filtered, and concentrated. Vacuum distillation afforded acid **22** (9.04 g, 96%) as a colorless liquid, bp 83 °C (2 mm). The IR and $^1H$ NMR data matched those previously reported.[22c] IR: 3077, 1703, 1644, 895 $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$): δ 11.2 (s, 1H, $CO_2H$), 4.82 (s, 1H, =CH), 4.70 (s, 1H, =CH), 2.34 (s, 2H, $CH_2$), 1.71 (s, 3H, =$CCH_3$), 1.21 (s, 6H, $C(CH_3)_2$); $^{13}C$ NMR (75 MHz, $CDCl_3$): δ 185.3, 142.6, 114.8, 48.5, 42.3, 25.7, 23.9.

*4.6.3. 2,4-Dimethyl-4-penten-2-amine (**23**)*

The method of Walborsky was adapted.[21] A 100-mL, three-necked, round-bottomed flask was charged with **22** (2.2 g, 15.6 mmol) and TEA (2.9 g, 4.0 mL, 28.6 mmol) in dry benzene (25 mL). The flask was cooled to 0 ºC (ice bath), and diphenyl phosphoryl azide (6.3 g, 23.2 mmol) was added dropwise with stirring. The reaction mixture was stirred at 0 °C for 1 h, then at 23 °C for 1 h, and finally heated at reflux for 3 h ($N_2$ was evolved). The solution was cooled, ether (250 mL) was added, and the organic layer was washed with water (3 × 50 mL). The organic layer was dried ($Na_2SO_4$), filtered, and concentrated. To the concentrated residue was added a mixture of 15% HCl (10 mL) and AcOH (10 mL), and the mixture was stirred overnight at 23 °C. Water was added, and the aqueous layer was washed with ether (3 × 50 mL). The aqueous layer was cooled (ice bath), basified by dropwise addition of 10% NaOH solution, and the product was extracted with ether (3 × 50 mL). The combined ether extracts were washed with water, saturated NaCl, and then dried (KOH). The solvent was evaporated to give **23** (1.2 g, 72%) as a light yellow liquid, which was used without purification in the next step. IR: 3355, 2967, 1639, 891 $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$): δ 4.91 (s, 1H, =CH), 4.72 (s, 1H, =CH), 2.10 (s, 2H, $CH_2$), 1.82 (s, 3H, =$CCH_3$), 1.32 (br s, 2H, $NH_2$), 1.13 (s, 6H, $C(CH_3)_2$); $^{13}C$ NMR (75 MHz, $CDCl_3$): δ 143.4, 114.8, 52.8, 46.5, 31.3, 25.5.

A 35-mL Chemglass pressure vessel (No. CG-1880-02), equipped with a magnetic stirrer, was charged with the amine **23** (2.2 g, 19.7 mmol) and 1-fluoro-4-nitrobenzene (2.5 g, 17.7 mmol) in DMSO (15 mL). The vessel was sealed under $N_2$ and heated at 80 °C for 48 h. After cooling to 23 °C, the vessel was unsealed, water (100 mL) was added, and the product was extracted with ether (3 × 50 mL). The combined extracts were washed with water and saturated NaCl, dried ($Na_2SO_4$), filtered and concentrated to give a yellow oil, which was chromatographed using 5-15% ether in hexanes to afford **24** (1.8 g, 45%) as a yellow oil. IR: 3379, 1599, 1531, 1368, 834 $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$): δ 8.05 (d, *J* = 9.3 Hz, 2H, ArH), 6.61 (d, *J* = 9.3 Hz, 2H, ArH), 4.94 (s, 1H, =CH), 4.72 (s, 1H, =CH), 4.63 (br s, 1H, NH), 2.46 (s, 2H, $CH_2$), 1.76 (s, 3H, $=CCH_3$), 1.44 (s, 6H, $C(CH_3)_2$); $^{13}C$ NMR (75 MHz, $CDCl_3$): δ 152.7, 142.2, 136.3, 126.6, 116.3, 113.3, 54.3, 48.4, 28.8, 24.9.

*4.6.5. N-(2,4-Trimethyl-4-penten-2-yl)-N-methyl-4-nitroaniline (**25**)*

A 50-mL, round-bottomed flask was charged with **24** (70 mg, 0.3 mmol) in 5 mL of DMF, and sodium hydride (80 mg of a 60% mineral oil dispersion, 2.0 mmol) was added under $N_2$. The mixture was stirred for 5 min, methyl iodide (3.0 g, 0.6 mmol) was added dropwise, and stirring was continued overnight at 23 °C. The crude reaction mixture was added to aqueous $NH_4Cl$ (10%, 10 mL), and the product was extracted with ether (3 × 10 mL). The ether extracts were washed with water and saturated NaCl, dried ($MgSO_4$), filtered, and concentrated to give a yellow oil, which was chromatographed using 10-20% ether in hexane to afford **25** (70 mg, 99%) as a yellow oil. IR: 1591, 1502, 1307, 837 $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$): δ 8.06 (d, *J* = 9.3 Hz, 2H, ArH), 6.95 (d, *J* = 9.3 Hz, 2H, ArH), 4.91 (s, 1H, =CH), 4.75 (s, 1H, =CH), 3.02 (s, 3H, $NCH_3$), 2.47 (s, 2H, $CH_2$), 1.72 (s, 3H, $=CCH_3$), 1.41 (s, 6H, $C(CH_3)_2$); $^{13}C$ NMR (75 MHz, $CDCl_3$): δ 156.6, 142.7, 137.4, 124.8, 119.7, 115.5, 59.6, 47.2, 37.5, 28.6, 24.6.

*4.6.6. $N^1$-(2,2,4-Trimethyl-4-penten-2-yl)-$N^1$-methyl-1,4-benzenediamine (**26**)*

A 250-mL, round-bottomed flask was charged with **25** (2.0 g, 8.1 mmol), iron powder (electrolytic, 3.0 g, 53.7 mmol, ≥100 mesh) and $NH_4Cl$ (1.0 g, 18.6 mmol) in 100 mL of EtOH:$H_2O$ (4:1).[20] The mixture was heated at reflux for 2 h. The reaction mixture was filtered through Celite, treated with saturated $NaHCO_3$ (100 mL), and extracted with EtOAc (3 × 100 mL). The combined extracts were washed with saturated NaCl, dried ($Na_2SO_4$), filtered, and concentrated to afford a brown residue (**26**, 1.67 g, 95%), which was spectroscopically pure and used directly for the next reaction step. IR: 3348, 1551 $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$): δ 6.97 (d, *J* = 7.8 Hz, 2H, ArH), 6.58 (d, *J* = 8.7 Hz, 2H, ArH), 4.84 (s, 1H, =CH), 4.71 (s, 1H, =CH), 3.81 (s, 2H, $NH_2$), 2.72 (s, 3H, $NCH_3$), 2.22 (s, 2H, $CH_2$), 1.81 (s, 3H, $=CCH_3$), 1.06 (s, 6H, $C(CH_3)_2$); $^{13}C$ NMR (75 MHz, $CDCl_3$): δ 144.1, 143.5, 129.5, 115.0, 114.6, 113.5, 58.2, 46.5, 37.3, 25.7, 25.3.

*4.6.7. N-[4-(2,4-Dimethyl-4-penten-2-yl)methylamino)phenyl]-acetamide (**27**)*

To a stirred mixture of **26** (1.0 g, 4.7 mmol) and pyridine (20 mL) in a 200-mL, round-bottomed flask was added dropwise acetyl chloride (3.3 g, 3.0 mL, 42.1 mmol). Stirring was continued at 23 °C for 2-3 h. The crude reaction mixture was added to water (100 mL), and the product was extracted with ether (3 × 50 mL). The combined extracts were washed with water and saturated NaCl, dried ($MgSO_4$), filtered, and concentrated, to give a brown residue, which was purified by chromatography using 30% ether in hexane to afford **27** (1.2 g, 97%) as a light yellow solid, mp 74-75 °C. IR: 3297, 1663, 887 $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$): δ 7.45 (s, 1H, ArH), 7.37 (d, *J* = 7.1 Hz, 2H, ArH), 7.09 (d, *J* = 7.1 Hz, 2H, ArH), 4.86 (s, 1H, =CH), 4.73 (s, 1H, =CH), 2.75 (s, 3H, $NCH_3$), 2.23 (s, 2H, $CH_2$), 2.15 (s, 3H, $C(O)CH_3$), 1.82 (s, 3H, $=CCH_3$), 1.08 (s, 6H, $C(CH_3)_2$); $^{13}C$ NMR (75 MHz, $CDCl_3$): δ 168.7, 147.6, 143.9, 134.3, 128.8, 120.0, 114.7, 58.1, 46.6, 37.1, 26.0, 25.2, 24.8.

*4.6.8. N-(1,2,2,4,4-Pentamethyl-1,2,3,4-tetrahydroquinolin-6-yl)acetamide (**28**)*

The basic procedure of Faure was followed.[23] A mixture of $AlCl_3$ (5.0 g, 37.4 mmol) in 75 mL of DCM in a 200-mL, three-necked, round-bottomed flask was cooled at –78 °C. Amide **27** (0.9 g, 3.9 mmol) in 15 mL of DCM was added dropwise. Stirring was continued overnight while gradually warming to 23 °C. The process was monitored by TLC until the starting material was consumed. The reaction mixture was poured onto crushed ice, and the aqueous phase was extracted with DCM (3 × 50 mL). The combined organic extracts were washed with saturated $NaHCO_3$, water, saturated NaCl, dried ($MgSO_4$), filtered, and concentrated to give a brown residue, which was chromatographed using 5-20% EtOAc in hexane to afford semisolid **28** (0.48 g, 53%). IR: 3289, 1655, 1610, 806 $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$): δ 8.08 (s, 1H), 7.28-7.22 (m, 2H, ArH), 6.52 (d, *J* = 8.2 Hz, 1H, ArH), 2.72 (s, 3H, $NCH_3$), 2.08 (s, 3H, $C(O)CH_3$), 1.74 (s, 2H, $CH_2$), 1.26 (s, 6H, $C(CH_3)_2$), 1.19 (s, 6H, $C(CH_3)_2$); $^{13}C$ NMR (75 MHz, $CDCl_3$): δ 169.0, 142.9, 134.4, 128.3, 125.4, 123.8, 120.1, 54.5, 53.2, 32.8, 31.7, 31.5, 27.8, 24.5.

*4.6.9. 1,2,2,4,4-Pentamethyl-1,2,3,4-tetrahydroquinolin-6-amine (**29**)*

A 25-mL, round-bottomed flask was charged with amide **28** (0.15 g, 0.6 mmol) and 10 mL of 70% (v/v) of $H_2SO_4$, and the resulting mixture was refluxed overnight. After cooling to 23 °C, water (10 mL) was added, and the mixture was basified with 30% NaOH. The amine was extracted with EtOAc (3 × 15 mL). The combined organic extracts were dried ($Na_2SO_4$), filtered and concentrated to give **29** (0.15 g, 92%) as a brown residue. This compound was used without further purification. IR: 3336, 1623 $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$): δ 6.64 (s, 1H, ArH), 6.50 (s, 2H, ArH), 3.64 (s, 2H, $NH_2$), 2.69 (s, 3H, $NCH_3$), 1.74 (s, 2H, $CH_2$), 1.28 (s, 6H, $C(CH_3)_2$), 1.17 (s, 6H, $C(CH_3)_2$); $^{13}C$ NMR (75 MHz, $CDCl_3$): δ 139.3, 137.2, 135.8, 114.7, 114.2, 114.0, 53.4 (2C), 32.1, 31.7, 27.4 (2C).

*4.6.10. 3-(1,2,2,4,4-Pentamethyl-1,2,3,4-tetrahydroquinolin-6-yl)-1-(4-nitrophenyl)thiourea (**5**)*

A 50-mL, round-bottomed flask was charged with amine **29** (100 mg, 0.46 mmol) in dry THF (5 mL). To the stirred solution at 23 °C was added dropwise a solution of 4-nitrophenyl isothiocyanate (82 mg, 0.50 mmol) in THF (2 mL), and stirring was continued overnight. The solvent was removed, and the mixture was subjected to silica gel chromatography using 20-30% ether in hexanes to afford **5** (164 mg, 90%) as orange crystals, mp 151-153 °C. IR: 3335, 1596, 1500, 1332, 1263, 851 $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$): δ 8.17 (d, *J* = 9.3 Hz, 2H, ArH), 7.97 (s, 1H, ArH), 7.78 (d, *J* = 8.8 Hz, 2H, ArH and s,1H, ArH), 7.08 (d, *J* = 2.2 Hz, 1H, ArH), 7.02 (dd, *J* = 8.8, 2.2 Hz, 1H, ArH), 6.63 (d, *J* = 8.8 Hz, 1H, ArH), 2.83 (s, 3H, $NCH_3$), 1.82 (s, 2H, $CH_2$), 1.32 (s, 6H, $C(CH_3)_2$), 1.29 (s, 6H, $C(CH_3)_2$);

$^{13}C$ 135.4, 124.9, 124.4, 123.2 (br), 123.1, 122.7, 112.8, 54.6, 52.0, 31.51, 31.45, 31.0, 28.0; MS(ESI): *m/z* 399 ($M+H^+$). *Anal.* Calcd. for $C_{21}H_{26}N_4O_2S$: C, 63.28; H, 6.57; N, 14.06. Found: C, 63.06; H, 6.58; N, 13.92.

*5. Biology*

The compounds were dissolved in DMSO at a concentration of 0.01 M. The human ovarian cancer cell line A2780 was plated in 96-well tissue culture dishes at a concentration of 3000 cells per well in RPMI medium, supplemented with 10% fetal bovine serum and a mixture of antibiotics and antimycotics. The next day, the cultures were treated in triplicate with compound concentrations ranging from 2 μM to 8 μM. For compound **5**, additional experiments were performed with concentrations of 10 μM and a series of two-fold dilutions to 156 nM. Control cultures were treated with DMSO solvent only. After 72 h of incubation, the CellTiter 96 Non-Rad Cell Proliferation Assay (Promega) was used to quantify the remaining metabolically living cells. After subtracting blank values, the optical density (OD) readouts of the assay for the treated cultures were normalized with the average OD of the control cultures. For each compound, the experiment was repeated at least twice, resulting in a minimum of 6 dose-response curves. For SHetA2, 24 response curves were available for statistical fitting parameters. A custom program was written in GNU Octave, a free software compatible with Matlab, to fit the dose-response curves with a four-parameter sigmoid function, extracting the $IC_{50}$ and efficacy (the maximal % growth inhibition) parameters.

*6. Theoretical docking methods*

AutoDock 4.2[25] was used to dock the compounds to the substrate binding domain of mortalin (PDB ID: 3N8E). For the compounds, ChemSketch™ (Advanced Chemistry Development, Inc. ADC/Labs, Toronto, Canada) was used to generate the SMILES notations, which were subsequently converted to PDB files with initial three-dimensional coordinates using OpenBabelGUI.[35] AutoDockTool (ADT)[25] was then employed to add partial charges to the protein and ligands and to set rotatable bonds for the ligands. Only polar hydrogens were retained in the molecules. Gasteiger partial charges and solvation parameters were assigned. The search space of 44 Å × 47 Å × 41 Å was slightly larger than the protein molecule, and the grid spacing was 0.375 Å. Autogrid was run first to prepare the coordinate system, and then the Lamarckian genetic algorithm was applied with a population size of 150 and 25 million maximum evaluations. The minimum empirical binding free energies (ΔG) between the compounds and the receptor are reported Table 1. The dissociation constants $K_d$ were calculated from the binding free energy using the relation $\Delta G = -RT \ln(K_d)$, where R is the gas constant. All dockings were performed on an iMac computer with a 2.4 GHz Intel Core i3 processor and 4 GB RAM.

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The authors declare no competing interests.

**Supplemental data**

Supplementary data related to this article—copies of $^1H$ and $^{13}C$ NMR spectra for all final compounds—can be found at xxx

**References**

1. Benbrook, D.M.; Nammalwar, B; Long, A.; Matsumoto, H.; Singh, A.; Bunce, R.A.; Berlin. K.D. SHetA2 interference with mortalin binding to p66shc and p53 identified using drug-conjugate magnetic microspheres. *Invest. New Drugs*, **2014**, *32*, 412-423.

2. (a) Nammalwar, B.; Berlin, K.D.; Bunce, R.A. SHetA2–A minireview of a promising anti-cancer agent. *J. SciMed Chem*. **2013**, *1*, 1-6. (b) Liu, S.; Zhou, G.; Lo, S.N.H.; Louie, M.; Rajagopalan V. SHetA2, a new cancer-preventive drug candidate. Chapter 3 in *Anti-cancer drugs, nature synthesis and cell*, 2016; In Tech D.O.O., Janeza Trdine 9, 51000 Rijeka, Croatia, ISBN: 978-953-51-2814-4. (c) Nammalwar, B.; Bunce, R.A. 1,2,3,4-Tetrahydroquinolines, 2,3-dihydro-4(1*H*)-quinolinones and 4(1*H*)-quinolinones using domino reactions. *Molecules*, **2014**, *19*, 204-232.

3. Benbrook, D.M.; Madler, M.M.; Spruce, L.W.; Birckbichler, P.J.; Nelson, E.C.; Subramanian, S.; Weerasekare, G.M.; Gale, J.B.; Patterson, M.K.; Wang, B.; Wang, W.; Lu, S.; Rowland, T.C.; DiSivestro, P.; Lindamood, C.; Hill, D.L.; Berlin, K.D. Biologically active heteroarotinoids exhibit anticancer activity and decreased toxicity. *J. Med. Chem*., **1997**, *40*, 3567-3583.

4. Liu, S.; Brown, C.W.; Berlin, K.D.; Dhar, A.; Guruswamy, S.; D. Brown, D.; Benbrook, D.M. 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.

5. Dhar, A.; Liu, S.; Klucik, J.; Berlin, K.D.; Madler, M.M.; Lu, S.; Ivey, R.T.; Zacheis, D.; Brown, C.W.; Nelson, E.C.; Birckbichler, P.J.; Benbrook, D.M. Synthesis, structure-activity relationships, and RAR γ-ligand interactions of nitrogen heteroarotinoids. *J. Med. Chem*., **1999**, *42*, 3602-3614.

6. Le, T.C.; Berlin, K.D.; Benson, S.D.; Nelson, A.C.; Benbrook, D.M.; Eastman, M.; Bell-Eunice, G. Unusual hetero-arotinoids with anti-cancer activity against ovarian cancer cells. *Open Med. Chem*., **2007**, *1*, 11-23.

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

8. Lin, Y.-D.; Chen, S.; Yue, P.; Zou, W.; Benbrook, D.M.; Liu, S.; Le, T.C.; Berlin, K.D.; Khuri, F.R.; Sun, S.-Y. 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.

9. Lin, Y.; Liu, X.; Yue, P.; Benbrook, D.M.; Berlin, K.D.; Khuri, F.R.; Sun, S.-Y. Involvement of C-flip and surviving down-regulation in flexible heteroarotinoid-induced apoptosis in lung cancer cells. *Mol. Cancer Ther*., **2008**, *7*, 3556-3565.

10. C.; Lightfoot, S.; He, F.; Benbrook, D.M. Development of flexible-heteroarotinoids (Flex-Hets) for kidney cancer. *Mol. Cancer Ther*., **2009,** *8*, 1227-1238.

11. Bast, R.C.; Hennessy, B.; Mills, C.V. The biology of ovarian cancer: New opportunities for translation. *Nat. Rev. Cancer*, **2009**, *9*, 415-428.

12. Benbrook, D.M.; Kamelle, S.A.; Guruswamy, S.B.; Lightfoot, S.A.; Hannafon, B.N.; Rutledge, T.L.; Gould, N.S.; Dunn, S.T.; Berlin, K.D. Flexible heteroarotinoids (Flex-Hets) exhibit improved therapeutic ratios as anti-cancer agents over retinoic acid receptor agonists. *Invest. New Drugs*, **2005**, *23*, 417-428.

13. Gnanasekaran, K.K.; Benbrook, D.M.; Nammalwar, B.; Thavathiru, E.; Bunce, R.A.; Berlin, K.D. 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.

14. (a) Kabirov, K.K.; Kapetanovic, M.; Benbrook, D.M.; Dinger, N.; Mankovskaya, I.; Zakharov, A.; Detrisac, C.; Pereira, M.; Martin-Jimenez, T.; Onua, E.; Banerjee, A.; van Breemen, R.B.; Nikolic, D.; Chen, L.; Lyubimov, A.V. Oral toxicity and pharmacokinetic studies of SHetA2, a new chemopreventive agent in rats and dogs. *Drug Chem. Toxicol*., **2012,** *36*, 284-295. (b) Sharma, A.; Benbrook, D.M.; Woo, S. Pharmacokinetics and interspecies scaling of a novel, orally bioavailable anti-cancer drug, SHetA2. *PLOS One*, **2018**, *13*, Article 4. https://doi.org/10.1371/journal.pone.0194046.

15. Vitaku, E.; Smith, D.T.; Njardarson. J.T. Analysis of the structural diversity, substitution patterns, and frequency of nitrogen heterocycles among U.S. FDA approved pharmaceuticals. *J. Med. Chem.*, **2014**, *57*, 10257-10274.

16. Liu, Z.; Zhang, Y.; Hua, Y.F.; Covey, J.M.; Benbrook, D.M.; Chan, K.K. Metabolism of a sulfur-containing heteroarotinoid anticancer agent, SHetA2, using liquid chromatography/tandem mass spectrometry. *Rapid Commun. Mass Spec.*, **2008**, *22*, 3371-3381.

17. Zhu, W.; Ma. D. Synthesis of aryl azides and vinyl azides via proline-promoted CuI-catalyzed coupling reactions. *Chem. Commun*. **2004**, 888-889.

18. Yadav, J.S.; Reddy, B.V.S.; Premalatha, K.; Murty, M.S.R. $Bi(OTf)_3$-catalyzed condensation of 2,2-DMP with aromatic amines. A rapid synthesis of 2,2,4-trimethyl-1,2-dihydroquinolines. *J. Mol. Catal. A: Chem*., **2007**, *271*, 161-163.

19. Eda, M.; Kuroda, T.; Kaneko, S.; Aoki, Y.; Yamashita, M.; Okumura, C.; Ikeda, Y.; Ohbora, T.; Sakaue, M.; Koyama, N.; Aritomo, K. Synthesis and biological evaluation of cyclopentaquinoline derivatives as nonsteroidal glucocorticoid receptor antagonists. *J. Med. Chem*. **2015**, *58*, 4918-4926.

20. Zhao, G.; Souers, A.J.; Voorbach, M.; Falls, H.D.; Droz, B.; Brodjian, S.; Lai, Y.Y.; Iyengar, R.R.; Gao, J.; Judd, A.S.; Wagaw, S.H.; Ravn, M.M.; Engstrom, K.N.; Lynch, J.K.; Mulhern, M.; Freeman, J.; Dayton, B.D.; Wang, X.; N. Grihalde, N.; Fry, D.; Beno, D.W.A.; Marsh, K.C.; Su, Z.; Diaz, G.J.; Collins, C.A.; Sham, H.; Reilly, R.M.; Brune, M.E.; Kym, P.R. Validation of diacyl glycerolacyltransferase I as a novel target for the treatment of obesity and dyslipidemia using a potent and selective small molecule inhibitor. *J. Med. Chem.*, **2008**, *51*, 380-383.

Pankowski, J. Attempts to trap radicals formed in solution by a magnesium surface. *J. Org. Chem*., **1992**, *57*, 6188-6191.

22. (a) Gaudemar, M. Préparation d'esters γ-ethylenic par allylation du réactif de Reformatsky en presence de sels de cuivre. *Tetrahedron Lett*., **1983**, *24*, 2749-2752. (b) Acid **22** has a few reported properties, which mostly agreed with our compound, see Chapius, C.; Saint-Leger, C. Alternative synthesis of 2-hydroxy-3,5,5-trimethylcyclopent-2-en-1-one. *Helv. Chim. Acta*, **2010**, *93*, 111-117. (c) Hayashi, Y.; Nishizawa, M.; Sakan, T. Studies on the sesquiterpenoids of *hypolepis punctata* mett–II. The total synthesis of hypacrone. *Tetrahedron*, **1977**, *33*, 2513-2519.

23. Faure, R.; Pommier, A.; Pons, J.M.; Rajzmann, M.M.; Santelli, M. Formation of 2-cyclohexenones by Friedel-Crafts acylation of alkenes with β,γ-ethylenic acyl halides. *Tetrahedron*, **1992**, *48*, 8419-8430.

24. Cha, S. Tight-binding inhibitors—I: Kinetic behavior. *Biochem. Pharm*., **1975**, *24*, 2177-2185.

25. Morris, G.M.; Garrett, M.; Huey, R.; Lindstrom, W.; Sanner, M.F.; Belew, R.K.; Goodsell, D.S.; Olson., A.J. AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility. *J. Comp. Chem.*, **2009**, *30*, 2785-2791.

26. Watts, F.M. Jr; Pouland, T.; Bunce, R.A.; Berlin, K.D.; Benbrook, D.M.; Mashayekhi, M.; Bhandari, D. Zhou, D.. Activity of oxygen-versus sulfur-containing analogs of the Flex-Het anticancer agent SHetA2. *Eur. J. Med. Chem*., **2018**, *158*, 720-732.

27. Iosefson, O.; Azem. A. Reconstitution of the mitochondrial Hsp70 (mortalin)-p53 interaction using purified proteins–identification of additional interacting regions, *FEBS Lett*., **2010**, *584*, 1080-1084.

28. Levine., A.J. p53: The cellular gatekeeper for growth and division. *Cell*, **1997**, *88*, 323-331.

29. Duncan, E.L.; Wadhwa, R.; Kaul, S.C. Senescence and immortalization of human cells. *Biogerontology*, **2000**, *1*, 103-121.

30. Kaul, S.C.; Wadhwa, R.; Sugihara, T.; Obuchi, T.K.; Komatsu, Y.; Mitsui, Y. Identification of genetic events involved in early steps of immortalization of mouse fibroblasts. *Biochim. Biophys. Acta*, **1994**, *1201*, 389-396.

31. Kypryjanczyk, J.; Thor, A.D.; Beauchamp, R.; Merritt, V.; Edgerton, S.M.; Bell, D.A.; Yandell D.W. p53 gene mutations and protein accumulation in human ovarian cancer. *Proc. Natl. Acad. Sci. USA*, **1993,** *40*, 4961-4965.

32. Tao, W.; Levine, A.J. Nucleocytoplasmic shuttling of oncoprotein Hdm2 is required for Hdm2-mediated degradation of p53. *Proc. Natl. Acad. Sci. USA*, **1999**, *96*, 3077-3080.

33. Wadhwa, R.; Yaguchi, T.; Hasan, Md.K.; Mitsui, Y.; Reddal, R.R.; Kaul, S.C. Hsp70 family member, mot-2/mthsp70/GRP75, binds to the cytoplasmic sequestration domain of the p53 protein. *Exp. Cell Res*., **2002**, *274*, 246-253.

34. Lu, J.; Lee, N.P.; Kaul, S.C.; Lan, F.; Poon, R.T.P.; Wadhwa, R.; Luk, J.M. Mortalin-p53 interaction in cancer cells is stess dependent and constitutes a selective target for cancer therapy. *Death and Differentiation* **2011**, *18*, 1046-1056.

35. Vandermeersch, T.; Hutchinson, G.R. Open Babel: An open chemical toolbox. *J. Cheminform.*, **2011**, *3*, 33.

**Highlights**

1. Nineteen new tetrahydroquinoline (THQ)-based SHetA2 analogs have been prepared
2. Pentamethyl-THQ analogs bearing a carbonyl at C3 were most active
3. A direct analog of SHetA2 with a pentamethyl-THQ unit was also very active
4. Modeling shows that C3 carbonyl THQs have an opposite binding pose to SHetA2
5. Six derivatives showed greater efficacy than SHetA2