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Improving both aqueous solubility and anti-cancer activity by assessing progressive lead optimization libraries

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Abstract

Thiazolidinone compounds **1–3** are lead compounds that have cytoselective toxicity toward non-small cell lung cancer (NSCLC) cells and drug-resistant NSCLC cells while showing low toxicity to normal human fibroblasts (NHFB). However, this class of compounds generally has a very low aqueous solubility (~0.1 μg/ml). In order to improve both solubility and anti-cancer activity, we designed and synthesized two lead-optimization libraries and investigated these libraries using simultaneous high-throughput solubility and cytotoxicity assays. By all-around modifications on R^1, R^2 and R^3 substitutions, consecutive library synthesis, and testing, we improved the aqueous solubility (5-fold improvement in solubility, from 0.1 to 0.5 μg/ml) and anti-cancer activity (10-fold improvement in EC_{50} from 0.72–0.98 μM to 0.08–0.16 μM) in the new lead thiazolidinone compound **31**.

Keywords

NSCLC; Thiazolidinone; Aqueous solubility; Cytotoxicity; Lead optimization

Because of its persistent low survival rate, lung cancer remains a top global threat.[1] Therapeutic agents for lung cancer, especially for drug-resistant non-small cell lung cancer (NSCLC) are urgently needed. We previously reported a series of thiazolidinone compounds[2] that targeted tubulin and heat shock proteins.[3,4] These compounds can inhibited growth of NSCLC cells and the drug-resistant NSCLC cells with EC_{50} values around 1.0 μM while exhibiting a low toxicity to normal human fibroblasts (>100 μM). However, these compounds generally have a low aqueous solubility. Solubility is crucial in the success of a drug candidate.[5] Compounds with low solubility not only cause problem for in vitro and in vivo assays, but also add significant burdens to drug development. In order to optimize lead compounds, exploring rapid and effective approaches for optimization of multi-parameters

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Supplementary data

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in a compound is necessary. This article reports our efforts to optimize both aqueous solubility and anti-cancer activity of leading thiazolidinone compounds using combinatorial lead optimization libraries.

In order to improve aqueous solubility of thiazolidinone compounds, we first incorporated more polar R^2 groups and diverse R^1 groups (Fig. 1) in a combinatorial lead-optimization library (Library **1**). Library **1** containing 25 members were synthesized using an existing method[2] with slight modifications (Section 1 in the SI). The crude yields of all products were ranging from 50% to 90%. All compounds were purified by column chromatography to a purity of ≥95% by LC/UV_{214nm} and characterized by ^{1}H NMR and ESI-MS (Section 2 in the SI).

Compounds in Library **1** were screened for their aqueous solubility using a high throughput solubility assay (Section 3 in the SI) and for their cytoselective toxicity in NSCLC cell line H460, drug-resistant NSCLC cell line $H460_{TaxR}$, and normal human fibroblasts (NHFB) using SRB assay. (Sections 4 and 5 in the SI). Since a single concentration of 10 μM best distinguishes the anti-cancer activities of this class of compounds from our preliminary experiments, we selected this concentration for an initial screening. The aqueous solubility results (Fig. 2A) showed that besides R^{2e} substitution (Compounds **5, 10** and **25**) there was little improvement in aqueous solubility for compounds in this library. The R^{2e} substitution with an amino group adds an H-donor, which is responsible for an improved solubility. Compounds in this group also show a lower computed log*P* than compounds with other substitutions (Section 6 in SI) consistent with the improved solubility. The cytotoxicity of these compounds to normal cell NHFB was generally low (Fig. 2B). However, only compounds **1, 2, 4, 11, 12, 21** and **22** exhibited good cytoselective anti-cancer effects in H460 and $H460_{TaxR}$ cells. But their solubility remained poor. Compounds with R^{2a} and R^{2b} ($–NMe_2$ and $–NEt_2$) groups have better cytoselective anti-cancer activities compared to those compounds with a ring structure (R^{2c}, R^{2d}, and R^{2e}, Section 6 in the SI). We found a consistent SAR trend with our previously finding that a $–NMe_2$ group at 4-position is required for optimal cytoselective anti-cancer activity,[2] while R^1 tolerates more diverse substitutions at various positions.

Compounds **5, 10** and **25** had the largest improvement in aqueous solubility. Substituting group at 4-position with ring (morpholine, *N*-methyl piperazine, piperazine) structures increased the number of hydrogen bond donors and acceptors in these molecules. The number of H-bonds was a key factor in determining the solubility.[6,7] Unfortunately, they all had a reduced anticancer activity. The altered number of the hydrogen bonds and hydrophobic regions in these molecules might cause a deviation from the optimal pharmacophore we discovered previously[2] in active compounds.

Since the purpose of this work was to optimize both aqueous solubility and cytoselective anti-cancer activity, the first library did not achieve our goal. To accomplish our original goal, we made another compounds library (Library **2**) to further explore the effects of R^3 modifications. By reviewing Library **1** screening results, we noticed that more active compounds in Library **1** had R^{1a} as a hydrogen atom. Therefore, we selected this group in designing Library **2**.

In Library **2**,we synthesized 10 compounds **26–35** using a similar synthesis protocol (Section 1 in SI). All compounds were also purified using column chromatography to a purity of ≥95% (LC/UV_{214nm}) and characterized by ^{1}H NMR and ESI-MS (Section 2 in SI). In order to compare effects of R^3, we also listed data from compounds **1–5** (R^{3a} = H) from Library **1** (Fig. 3). These compounds were different only in the R^3 position. Compounds **1–5** have R^3 substitution as a –H group, **26–30** as –Ph group, and **31–35** as –Me group.

The aqueous solubility results (Fig. 4A) showed that R^3 have played a more important role in determining compound solubility. Larger (R^{3b} = Ph) or smaller (R^{3a} = H) groups did not improve solubility. When R^{3c} = CH_3, the aqueous solubility of compounds exhibited improvements. This modification turned the nitrogen into a tertiary amine. Although a larger substitution like –Ph also turned the nitrogen into a tertiary amine, it simultaneously increases the log*P* of the molecule. Therefore, the aqueous solubility was not enhanced. Previous reports revealed that the tertiary amine substitution enhanced both the aqueous solubility[8,9] and the anti-cancer activity.[10,11] Compounds **31** and **32** also showed a potent cytoselective anti-cancer activity in H460 and $H460_{TaxR}$ cell lines at a single concentration (10 μM) of compounds. To explain why some compounds have the anti-cancer activity, we conducted a computational study generating a pharmacophore using 10 active compounds. The pharmacophore showed a requirement for two hydrogen bond acceptor regions and three hydrophobic regions. (Fig. 5) These pharmacophore features match well with what we previously reported on anti-cancer thiazolidinone compounds.[2]

To further evaluate effect of R^3 substitutions on compound's anti-cancer efficacy, we determined EC_{50} values of compounds in Library **2**. Physicochemical properties and experimental data for compounds with R^3 modifications were summarized in Table 1. Compound **31** exhibited the largest improvement compared to compound **1** in both aqueous solubility (5-fold) and cytoselective toxicity toward NSCLC cell line H460 (EC_{50} 0.08 μM) and its drug resistant variant $H460_{TaxR}$ (EC_{50} 0.16 μM) cells (Fig. 6A and B). It also exhibited less toxicity to normal cell NHFB (EC_{50} >100 μM) (Fig. 6A and B). Compound **32** did not improve solubility compared to **2** (Fig. 6C and D) while compound **33** did not maintain anti-cancer activity (EC_{50} 8.9 and 2.9 μM) although its solubility was significantly better than **3**. A computational pharmacophore study was conducted and results showed that the less active compounds such as **33, 34**, and **35** exhibited weak anti-cancer effects because they did not possess the unique pharmacophore we identified previously.[2]

Compounds with R^{3b} substitution (R^{3b} = Ph) all have larger *C*log*P* values. This resulted in a poor aqueous solubility due to increased hydrophobicity and a low anti-cancer activity due to unfavorable pharmacophore as discussed in previous sections. Compounds with R^{3c} substitution exhibited an improved solubility compared with compounds with R^{3a} substitution. This can be explained by the conversion of a secondary amine to a tertiary amine.[9] This modification also resulted in an improvement in anti-cancer activity (10-fold) showing a significant advance in this round of optimization.

In summary, using progressive optimization library approach, we improved both aqueous solubility and cytoselective anti-cancer activity of lead compound **1**. We demonstrate that lead optimization library approach combined with solubility and cytoselective toxicity

screenings is an effective approach to optimize both compound solubility and anti-cancer activity. Although further optimization, especially for solubility, is still needed, a new lead compound **31**, with a 5-fold enhanced solubility and 10-fold improved anti-cancer activity is a promising compound for further investigations.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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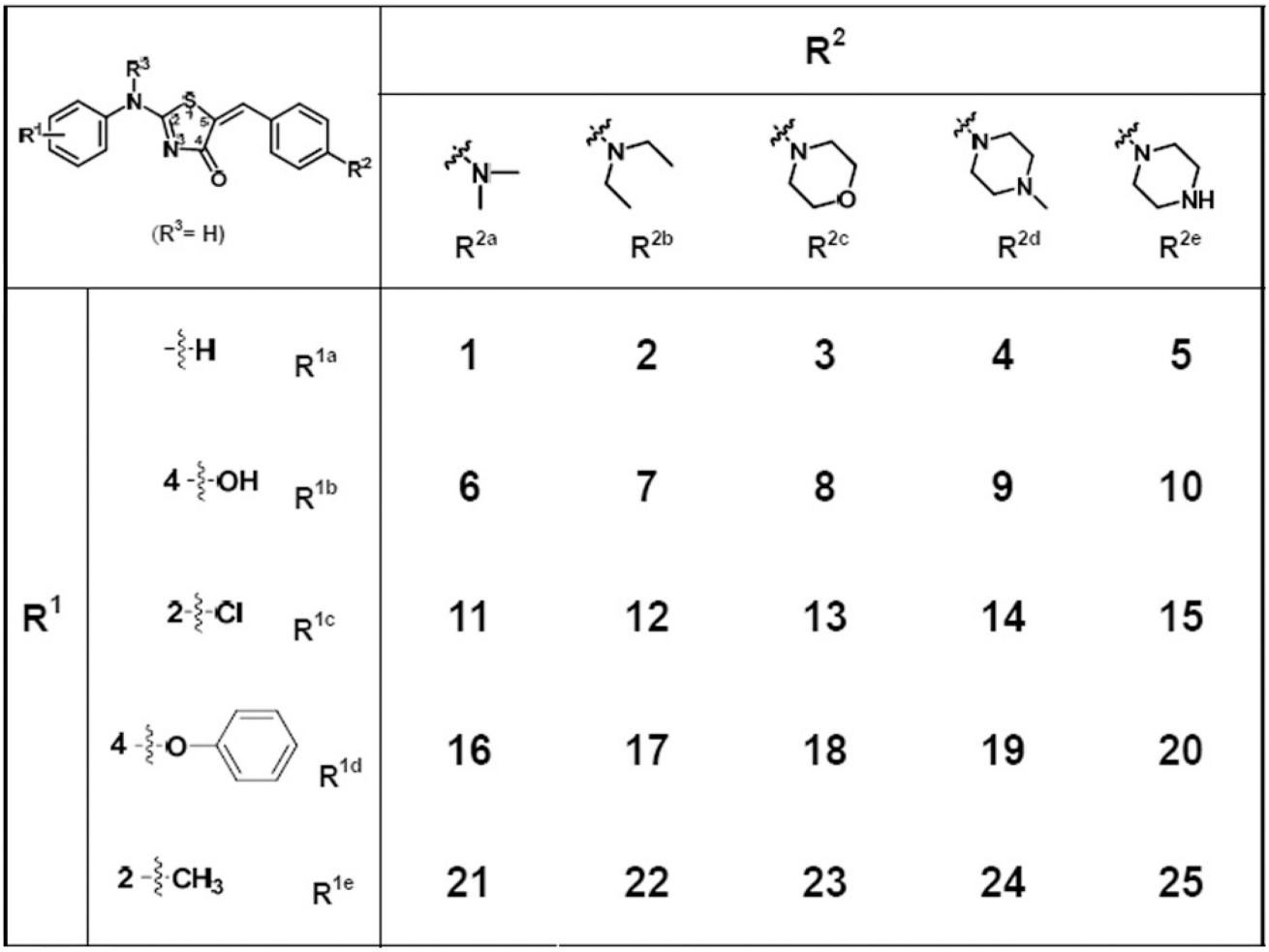

Figure 1.
Chemical structures of Library **1** (R^3 = H).

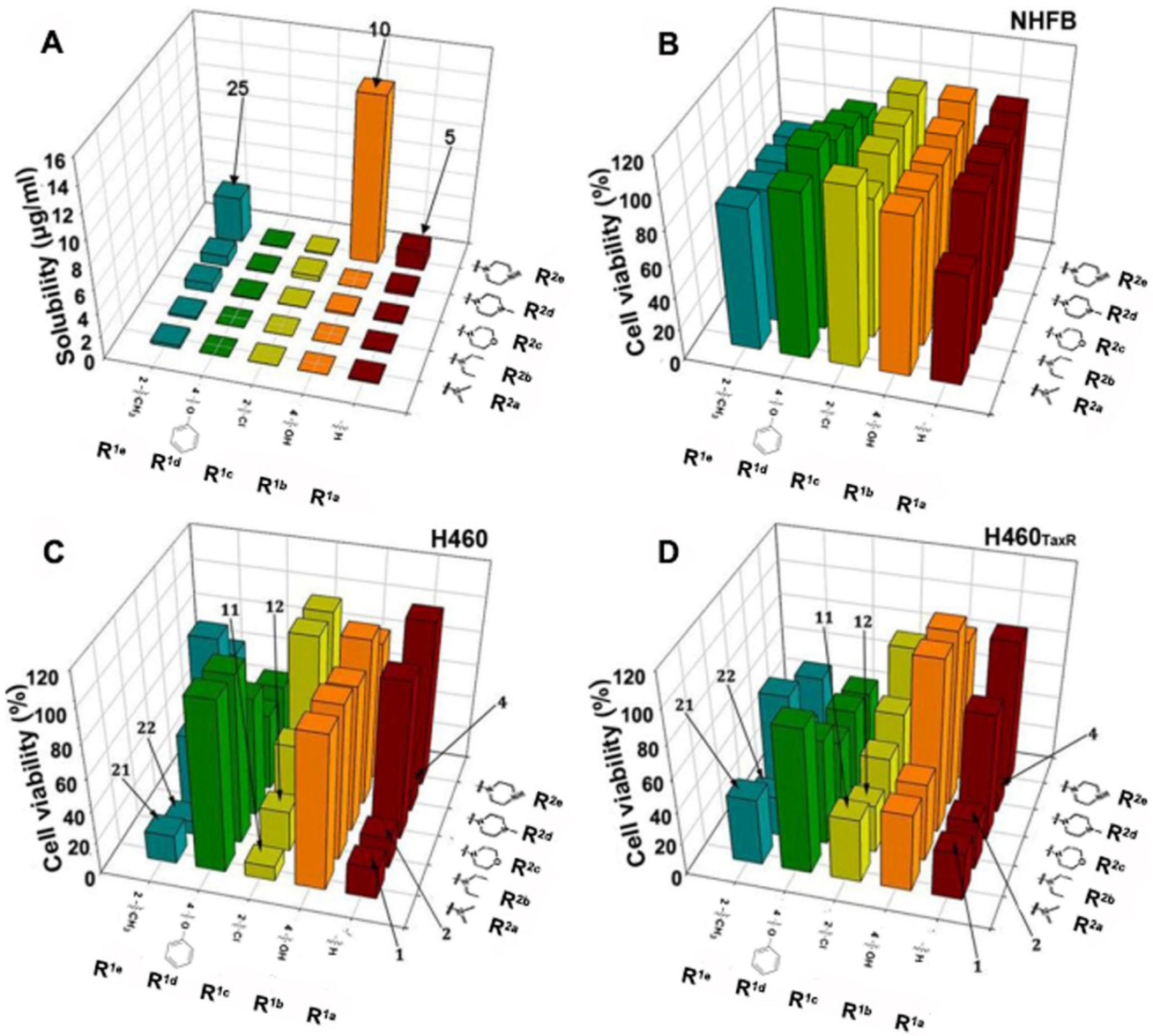

Figure 2.

Aqueous solubility and in vitro anti-cancer activities of compounds in Library **1** (R^3 = H). Aqueous solubility of compounds (A) was determined using a method described in Supporting information. Cytotoxicity of compounds in normal human fibroblast (NHFB) (B), H460 (C) and $H460_{TaxR}$ (D) were measured using SRB method with a compound concentration of 10 μM. Cell viability in DMSO was designated as 100%.

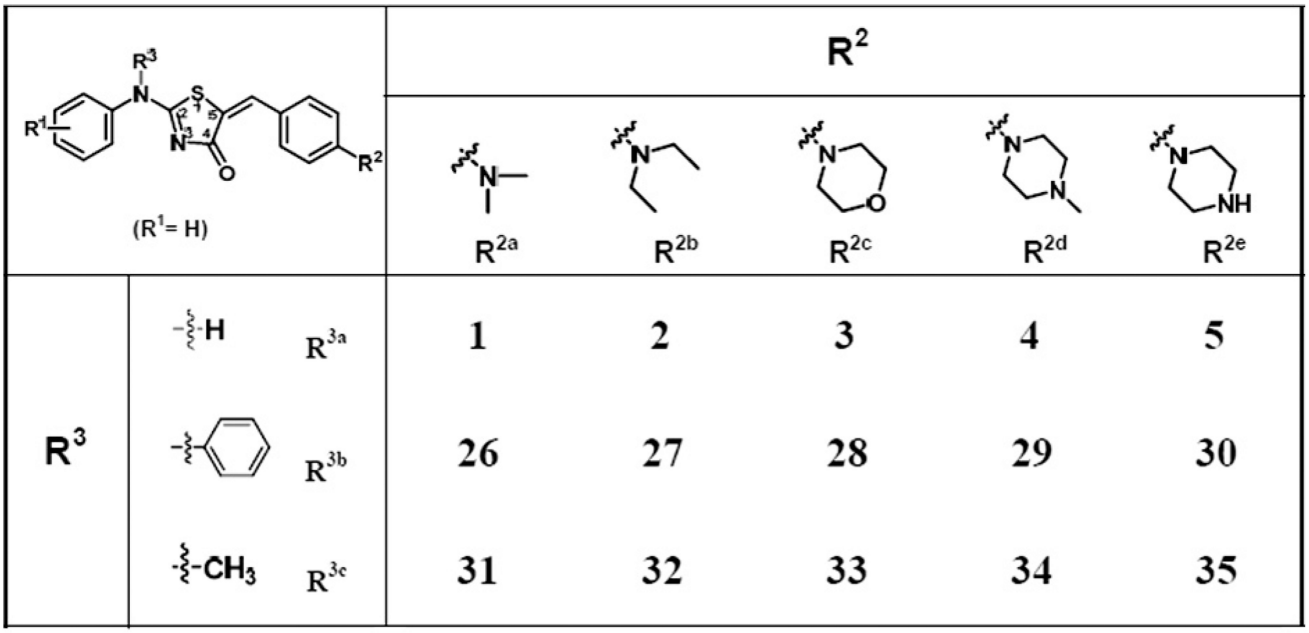

Figure 3.
Chemical structures of Library **2** (R^1 = H).

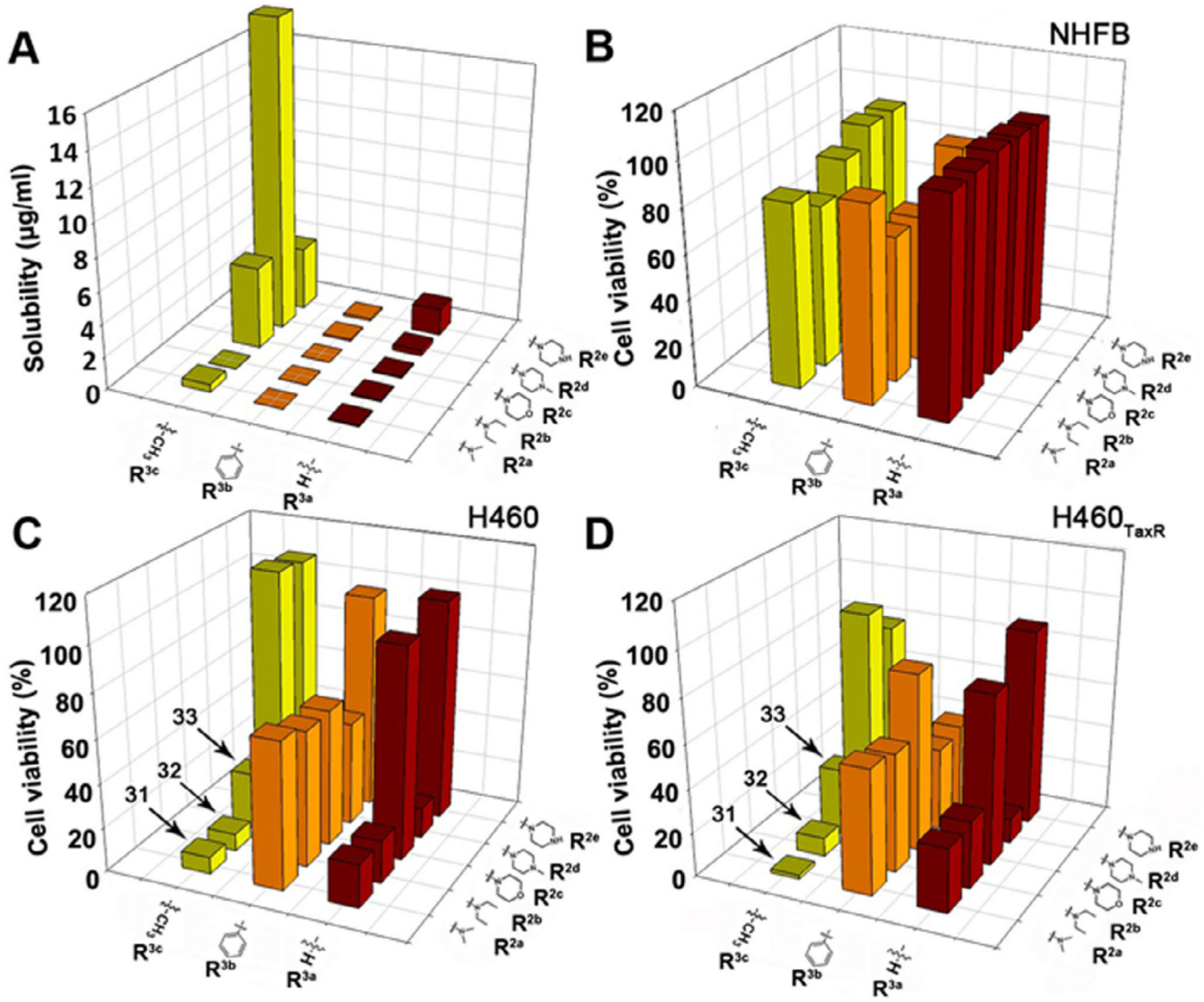

Figure 4.

Aqueous solubility and in vitro anti-cancer activities of compounds in Library **2** (R^1 = H). Aqueous solubility of compounds (A) was determined using a method described in Supporting information. Cytotoxicity of compounds in normal human fibroblast (NHFB) (B), H460 (C) and $H460_{TaxR}$ (D) were measured using SRB methods using with a compound concentration of 10 μM. Cell viability in DMSO was designated as 100%.

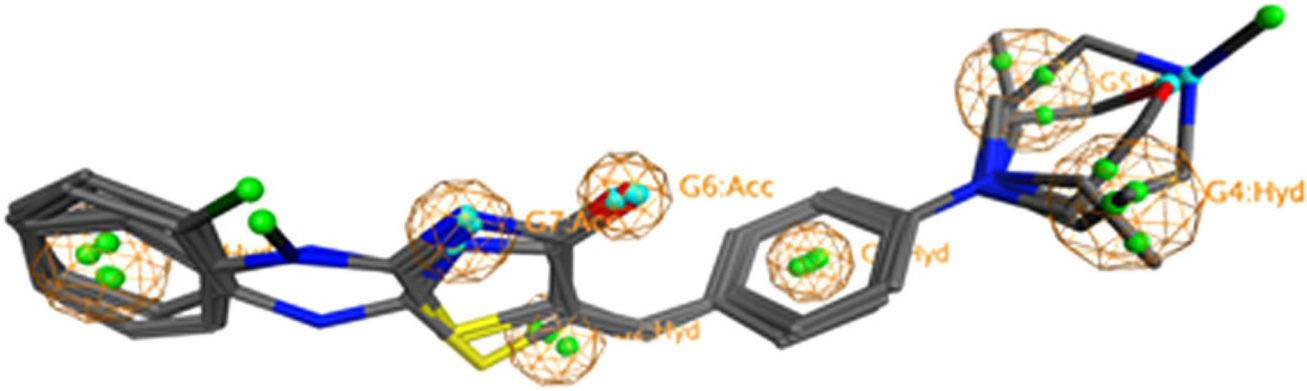

Figure 5.

Anti-cancer pharmacophore generated using the 10 active molecules. The graph shows the four most active (**2, 4, 12, 33**) compounds aligned. Green dots represent hydrophobic features; blue regions represent hydrogen bond acceptors.

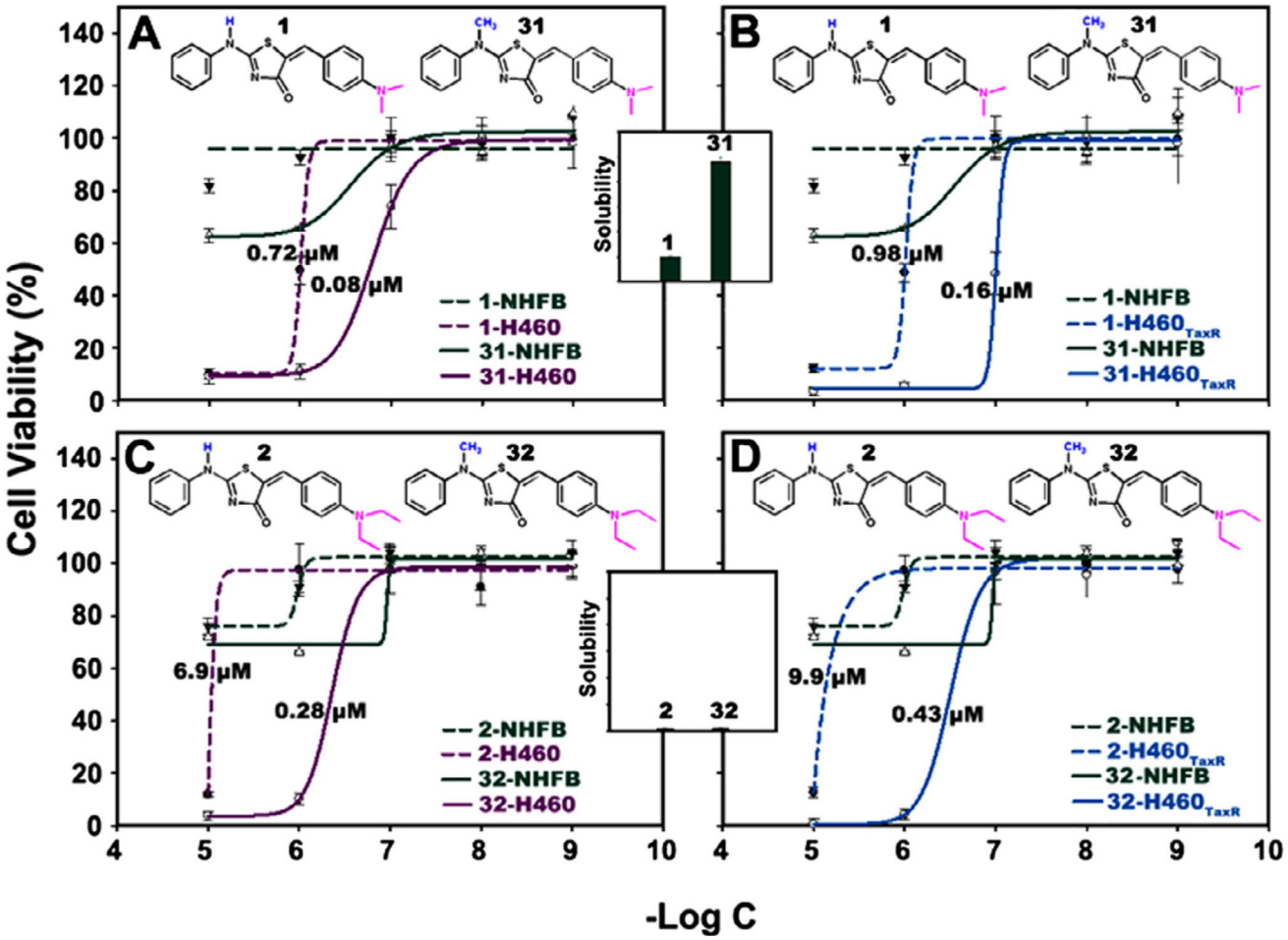

Figure 6.

Comparison of effects of R^3 on the cytotoxicity and solubility of compounds. Dose-dependent cytotoxicity and aqueous solubility for selected compounds with –H and −CH_3 as R^3 group in cell lines H460 (A, C) and $H460_{TaxR}$ (B, D). Aqueous solubility was shown in insets.

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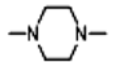
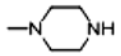
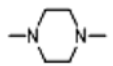
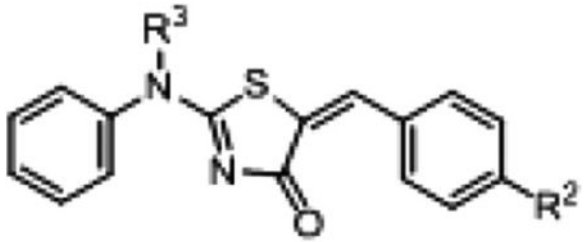
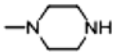
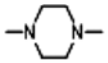
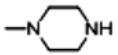
Table 1

Solubility and anti-cancer activity of compounds with diverse R^2 and R^3 substitutions

Compounds			*Clog P*[a]	Solubility[b] (μg/ml)	EC_{50}[c] (μM)		
	R^2	R^3			H460	$H460_{TaxR}$	NHFB
1	$-N(CH_3)_2$	–H	4.3	0.1	0.72 ± 0.02	0.98 ± 0.03	>100
2	$-N(CH_2CH_3)_2$	–H	5.3	0.1	6.9 ± 0.28	9.9 ± 0.35	>100
3		–H	3.6	0.1	>100	>100	>100
4		–H	4.1	0.35	1.4 ± 0.14	2.5 ± 0.46	6.4 ± 0.05
5		–H	3.5	1.68	>100	>100	>100
26	$-N(CH_3)_2$		5.7	0	>100	>100	>100
27	$-N(CH_2CH_3)_2$		6.8	0.01	>100	>100	>100
28			5.0	0.01	>100	>100	>100
29			5.6	0.11	>100	>100	>100

Compounds			Clog P^a	Solubility^b (μg/ml)	$EC_{50}{}^c$ (μM)		
							
	R^2	R^3			H460	$H460_{TaxR}$	NHFB
30			5.0	0.15	>100	>100	>100
31	$-N(CH_3)_2$	$-CH_3$	4.3	0.50	0.08 ± 0.06	0.16 ± 0.05	>100
32	$-N(CH_2CH_3)_2$	$-CH_3$	5.6	0.01	0.28 ± 0.05	0.43 ± 0.03	>100
33		$-CH_3$	3.5	5.06	8.9 ± 0.08	2.9 ± 0.05	>100
34		$-CH_3$	4.1	18.91	>100	>100	>100
35		$-CH_3$	3.5	3.89	>100	>100	>100

Note:

[a] *C*log *P* was computed by using Accord 6.1 (Accelrys, San Diego, CA, USA).

[b] Albendazole was used as negative control and carbamazepine, ranitidine and verapamil as positive controls for solubility assay.

[c] DMSO was used as a negative control in cytotoxicity assay.