Novobiocin analogues with second-generation noviose surrogates

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Abstract

Hsp90 is a promising therapeutic target for the treatment of cancer. Novobiocin is the first Hsp90 C-terminal inhibitor ever identified and recent structure-activity relationship studies on the noviose sugar identified several commercially available amines as suitable surrogates. In an effort to further understand this region of the molecule, analogues containing various N'-amino substituents were prepared and evaluated against two breast cancer cell lines for determination of their efficacy. Compound **37j** manifested the most potent anti-proliferative activity from these studies and induced Hsp90-dependent client protein degradation at mid nano-molar concentrations.

Keywords

Heat shock protein 90; Hsp90 inhibitors; Novobiocin analogues; Breast cancer

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The 90 kDa heat shock protein is an ATP-dependent chaperone that belongs to the GHKL superfamily.[1] It is one of the most abundant molecular chaperones in the cytosol and promotes the folding, activation and stabilization of more than 200 client proteins, approximately 50 of which are directly associated with cell growth and/or signaling pathways.[2] Malignant or mutated oncogenic proteins, such as Her-2, Raf-1, Akt, CDK4, Src, Flt-3, hTert, c-Met, etc, are distributed amongst the six hallmarks of cancer and are highly dependent upon the Hsp90 protein folding machinery for their ability to promote cell survival, proliferation, and adaptation.[3] In contrast to the homodimeric chaperone that is present in normal cells, Hsp90 exists as a heteroprotein super-chaperone complex in cancer cells and is bound to oncogenic proteins that are sensitive to physiological stress.[4] In such scenarios, Hsp90 inhibition results in the simultaneous disruption of multiple oncogenic pathways and eventually leads to cancer cell death, while largely sparing normal cells. As a consequence, Hsp90 has emerged as a promising therapeutic target for the treatment of cancer.

Hsp90 contains three highly conserved domains: the 25 kDa N-terminus is responsible for ATPase activity, the 35 kDa middle domain is utilized for substrate recognition, and the 12

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kDa C-terminus elicits dimerization and co-chaperone binding.[5] The N-terminal domain has been extensively studied and 16 small molecules targeting this region have been evaluated in clinical trials.[6,7] However, detriments such as heat shock induction and cytostatic activity associated with N-terminal inhibition has limited their potential use against cancer.[8] However, some small molecules that bind to the C-terminal domain do not induce the pro-survival heat shock response, and in some cases, even cause Hsp70 and Hsp90 degradation.[9–12] Because the Hsp90 C-terminus is responsible for mediating interactions with co-chaperones such as HOP (Hsp70-Hsp90 organization protein) and the immunophilins (eg. FK506 binding protein) to facilitate client protein maturation,[13,14] small molecule modulation of this region exhibits activities not observed with N-terminal inhibitors.[11,15]

Novobiocin was first identified as an Hsp90 C-terminal inhibitor in 2000 by Neckers and coworkers.[16] Subsequent modification to novobiocin has led to the elucidation of structure-activity relationships and analogues that exhibit superior inhibitory activity.[17–21] Deletion of both the 4-hydroxy substituent on the coumarin ring and the 3′-carbamoyl group on noviose resulted in DHN2, which transformed novobiocin from a DNA gyrase inhibitor to a selective Hsp90 inhibitor (Figure 1).[22] Subsequent replacement of the synthetically complex noviose sugar with readily available amines led to molecules represented by **NA-1** (novobiocin analogue 1, Figure 1) and **NA-2** (novobiocin analogue 2) that manifest increased anti-proliferative activity and solubility.[21] During the course of studies aimed at the modification of these amines, Huang and coworkers developed a three-dimensional quantitative structure-activity relationship (3D-QSAR) model that suggested modifications to the amine may improve anti-proliferative activity.[23] Consequently, second generation amino-analogues were designed, synthesized and evaluated against two breast cancer cell lines.

SAR generated from novobiocin analogues suggested that a three-carbon linker between the 7-coumarin phenol and the amine was optimal.[21] Although the anti-proliferative activity of **NA-1** and **NA-2** suggested flexibility of the amine moiety may be required, increasing the number of rotatable bonds is generally considered detrimental, due to entropic penalties. Therefore, novobiocin analogues containing rigid heterocyclic piperidine and pyrroridine derivatives were synthesized and evaluated. As illustrated in Figure 2, the piperidine or pyrrolidine ring system was linked to the 7-phenolic oxygen by 1–3 carbons.

These amines were assembled with the coumarin core in modular fashion utilizing Boc-protected secondary amines (**1** and **3**) or tertiary amines (**2** and **4**), which underwent Mitsunobu esterification with phenol **5** to afford **6–9**.[21] Subsequent hydrogenolysis to unmask the amine allowed for amide coupling with 4-(chlorocarbonyl)-2-(3-methylbut-2-en-1-yl)phenyl acetate (**10**) in the presence of pyridine afforded novobiocin C-linked heterocyclic analogues **11–14**. Acid-catalyzed deprotection of **11** and **13** resulted in secondary amines **15** and **16**. Finally, hydrolysis of ester **12** and **14–16** generated phenols **17–20** (Scheme 1).

Along with the C-linked heterocyclic derivatives (**12**, **14**, **15–20**), *N*-linked heterocycles (**29–33**) were also synthesized (Scheme 2). Compound **23** was regarded as a versatile

intermediate and was prepared in 3 steps. Benzyl (7-hydroxy-8-methyl-2-oxo-2H-chromen-3-yl)carbamate (**5**) was treated with ten equivalents of 1,3-dibromopropane (**21**) in the presence of potassium carbonate to afford alkyl bromide **22**. Subsequent hydrogenolysis followed by amide coupling with acid chloride **10** produced the corresponding amide **23**. Displacement of the bromide in **23** with amines **24**–**28** in *N*,*N*-dimethylformamide and simultaneous hydrolysis of the phenolic ester gave amines **29**–**33**.

Upon construction of the C- and N-linked heterocyclic novobiocin analogues, these molecules were evaluated for anti-proliferative activity against SKBr3 (estrogen receptor negative, Her2 over-expressing breast cancer cells) and MCF-7 (estrogen receptor positive breast cancer cells) cell lines. As shown in Table 1, the anti-proliferative activities manifested by C-linked heterocyclic analogues (**12**–**20**) were similar to **NA-1**, suggesting that various ring structures are accommodated within the binding site. Interestingly, the secondary amino analogues exhibit greater inhibitory activity than the corresponding tertiary amines (**16** *vs* **12**, **19** *vs* **18**), which is further exacerbated against the MCF-7 cell line. Although piperizine analogues (**29** and **30**) maintained activity comparable to **NA-1**, compounds **31** and **32** were inactive at the highest concentration tested. However, shrinking the ring size to five atoms (**33**) restored activity, suggesting that hydrophobicity is limited in this region and that the inclusion of heteroatoms is necessary for further extension.

Data from the first set of compounds suggest there is limited hydrophobic space, however, further extension is possible, but requires an H-bond donor (*e.g.*, **30**), which cannot be replaced with a hydrophobic moiety (**32**) or an H-bond acceptor (**31**). In addition, Huang and co-workers suggested in their 3D-QSAR study that steric bulk *N*-substitution of **NA-2** could lead to increased anti-proliferative activity.[23] Therefore, analogues containing various linear and branched *N*-substitutions were pursued in an effort to maintain both the hydrophobicity and the H-bond donor properties. As shown in Scheme 3, compound **23** was treated with primary and secondary amines (**34a**–**34o**) to produce the corresponding secondary and tertiary amine derivatives containing various linear or branched chains (**35a**–**35k**). Similarly, treatment of **23** with amines **34l**–**34o** afforded compounds **35j**–**35o** that contain various H-bond properties to further verify whether H-bond donors or acceptors were favored.

Anti-proliferative activity manifested by these analogues was evaluated against SKBr3 and MCF-7 cell lines as previously described. As shown in Table 2, a bulky group (up to 3 linear carbons, **35a**, **35b**, **35d,** and **35f**–**35k** except **35i**) was generally well-tolerated in the binding site. It appears as though this hydrophobic chain can be linear (**35a**, **35b**, and **35d**) or branched (**35f**–**35h**, **35j** and **35k**). The diethylamine (**35c**) and diisopropylamine (**35i**) analogues exhibited decreased anti-proliferative activity against SKBr3 cells, while the diisopropylamine analogue manifested no activity (**35e**), indicating a limited amount of space is available. Glycine ester analogue **35l** and amide analogue **35n** exhibited decreased anti-proliferative activity against SKBr3 cells or MCF-7 cells while alcohol **35o** retained activity against both cancer cell lines, supporting further extension into the binding site requires hydrogen bond donors. The significantly decreased activity of glycine acid analogue **35m** may result from limited cell membrane penetration.

Although none of the first two sets of compounds showed significantly improved anti-proliferative activity, it is clear that steric bulk is tolerated in the binding site. Therefore, a set of **NA-2** derivatives containing various rings (**37a**–**37j**) were prepared in a similar synthetic sequence, as described in Scheme 4.

The anti-proliferative data (Table 3) shows that cyclohexyl (**37a** and **37b**), benzyl (**37e**), substituted benzyl (**37f** and **37g**), and bicyclic alkyls (**37i** and **37j**) are tolerated in the binding site. Aniline-containing analogues were inactive (**37c** and **37d**), possibly due to the altered hydrogen bonding property manifested by amine due to the incorporation of the phenyl ring. Compound **37j**, which contained an *N*-adamantyl substitution, manifested the most potent anti-proliferative activity against both SKBr3 and MCF-7 breast cancer cell lines, indicating that significant space is available, but the molecule must contain a free H-bond donor in the form of a secondary amine. In addition, **37j** exhibited lower activity against normal cells (MRC5, 5.42 μM; HMLE, 15.05 μM), thus provide a considerable therapeutic window for the treatment of cancer.

To confirm these novobiocin analogues that contain amino groups in lieu of noviose manifest inhibitory activity through Hsp90 inhibition, western blot analyses of cell lysates following the administration of most active compound, **37j**, were performed. As shown in Figure 3, the Hsp90-dependent client proteins, Raf and Akt, were degraded in MCF-7 cells in a concentration-dependent manner upon treatment with **37j**. The non-Hsp90-dependent protein, actin, was not degraded upon the administration of **37j**, indicating selective degradation of Hsp90-dependent proteins. In accordance with previous studies, Hsp90 levels remained constant at all concentrations tested, demonstrating that **37j** does not induce the pro-survival heat shock response.

In conclusion, a library of novobiocin analogues containing a second generation of amino-appendages was designed, synthesized and evaluated. The results showed that a variety of bulky *N*-alkyl groups are well tolerated and can lead to compounds with efficacious activity against breast cancer cells. Compound **37j**, a secondary amino derivative containing an *N*-adamatyl group, manifested the most potent anti-proliferative activity, which through western blot analyses confirmed this compound inhibits the Hsp90 protein folding machinery.

Acknowledgments

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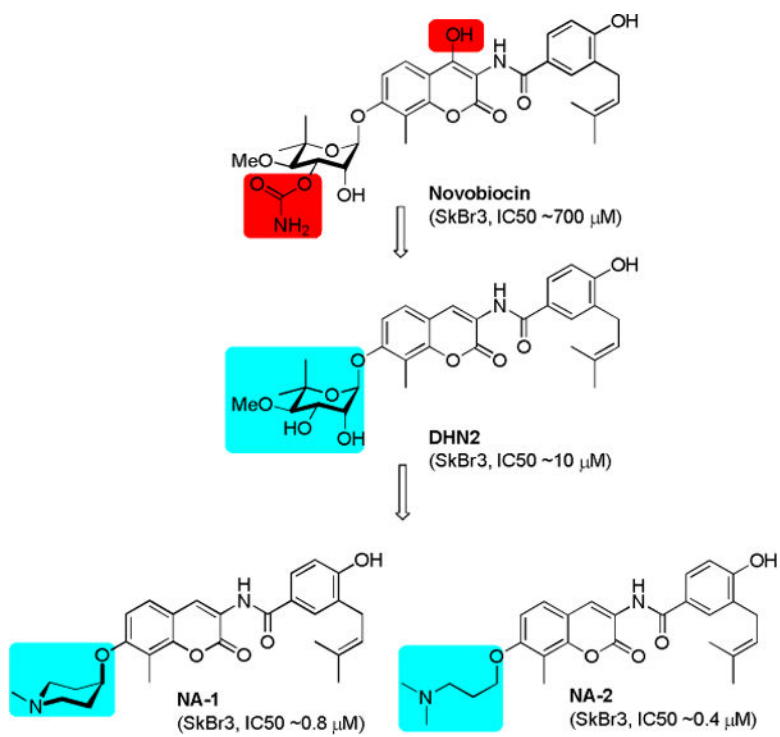
Figure 1.
Evolution of novobiocin analogues.
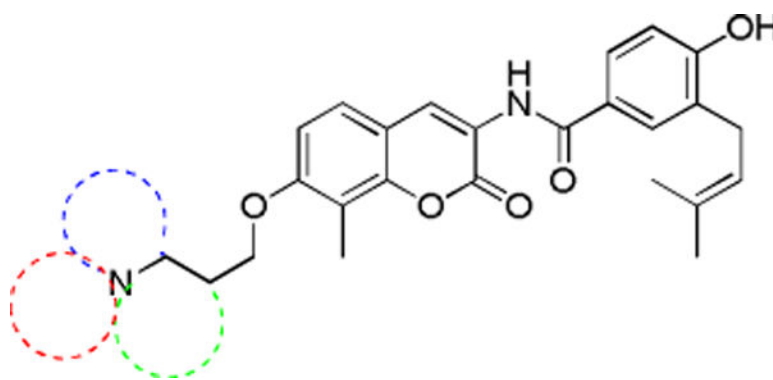
**Figure 2.**
Proposed cyclic amino derivatives of novobiocin
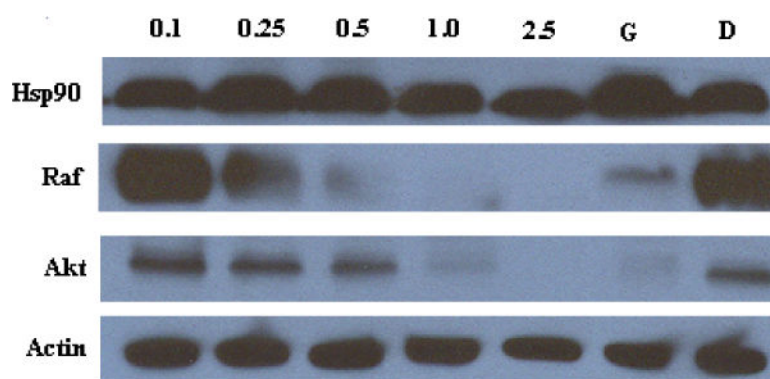
Figure 3.
Western blot analyses of MCF-7 cell lysates for Hsp90 client protein degradation after 24 h incubation. Concentrations (in μM) of **37j** are indicated above each lane. Geldanamycin (G, 500 nM) and DMSO (D) were employed respectively as positive and negative controls.
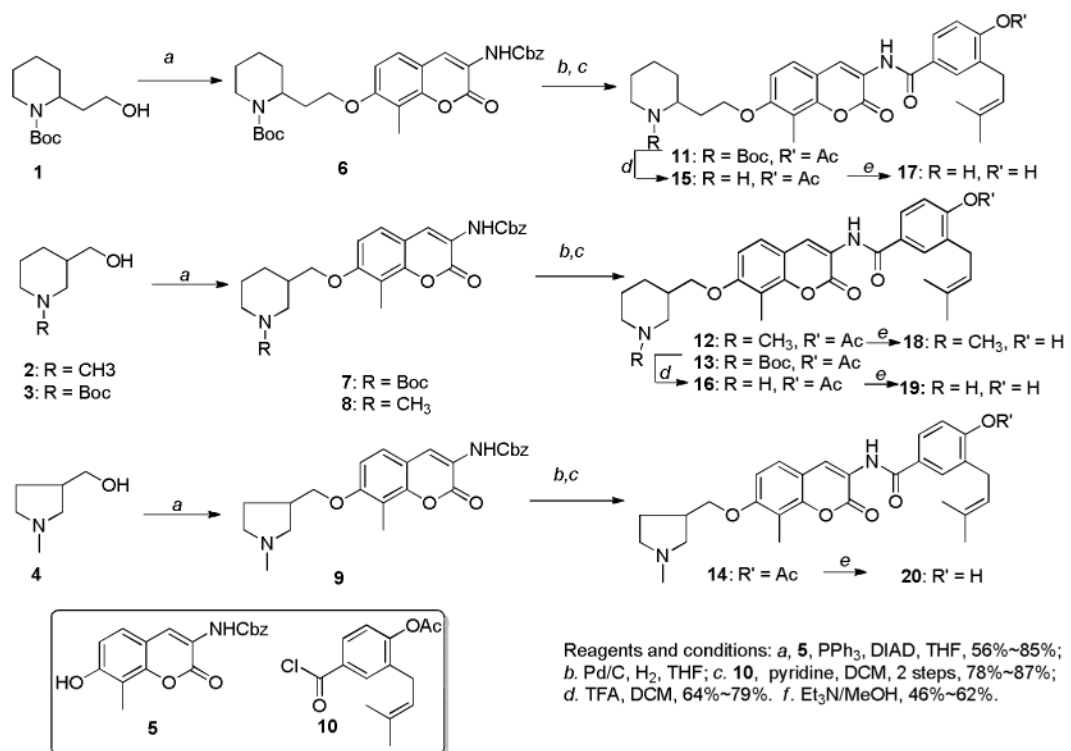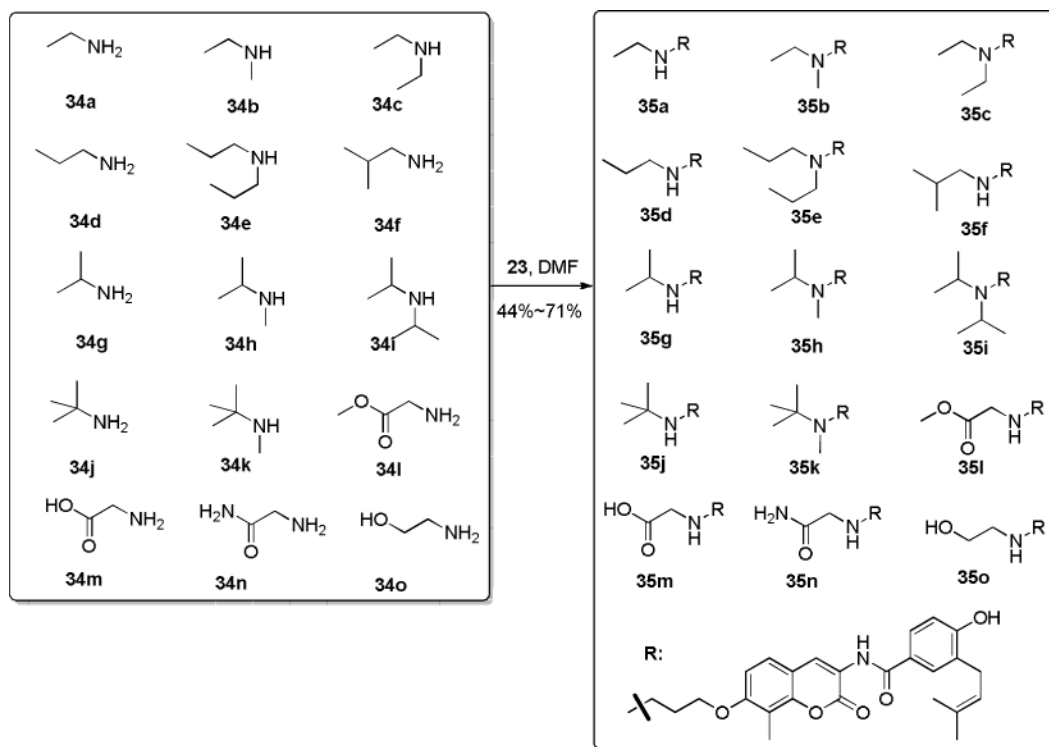
Scheme 1.
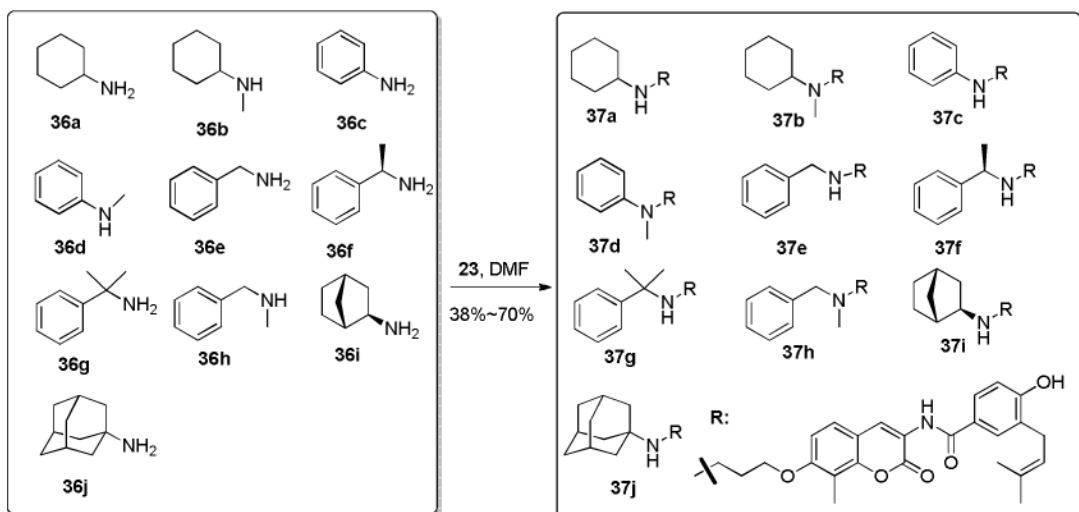Synthesis of C-linked heterocyclic derivatives of novobiocin
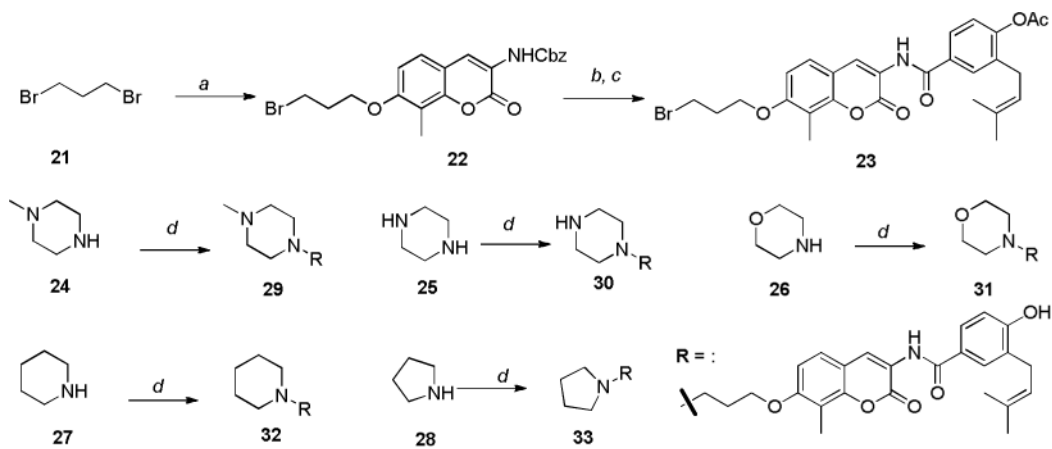
Reagents and conditions: *a.* **5**, sealed tube, reflux, 48 h, 39%; *b,* Pd/C, THF, H$_2$; *c.* **10**, pyridine, DCM. 2 steps, 93% *d.* **23**, DMF, room temperature, overnight, 75~83%

Scheme 2.
Synthesis of terminal cycloamine derivatives of novobiocin

Scheme 3.
Synthesis of **NA-2** analogues with linear and branched chains.

Scheme 4.
Synthesis of **NA-2** derivatives with cyclic *N*-substitutions

Table 1

Anti-proliferative activities **NA-1** analogues.

	R	R′	SKBr3 (μM)	MCF-7 (μM)
KU-398		H	0.76±0.17[a]	1.09±0.10
12		OAc	0.74±0.01	1.65±0.11
14		OAc	0.88±0.09	1.88±0.45
15		OAc	0.64±0.06	0.71±0.03
16		OAc	0.62±0.04	0.82±0.07
17		H	0.64±0.06	0.71±0.03
18		H	1.30±0.63	2.64±0.45
19		H	0.60±0.01	0.79±0.10
20		H	0.92±0.56	1.55±0.04
29		H	1.01±0.03	1.20±0.12
30		H	0.82±0.02	0.93±0.21
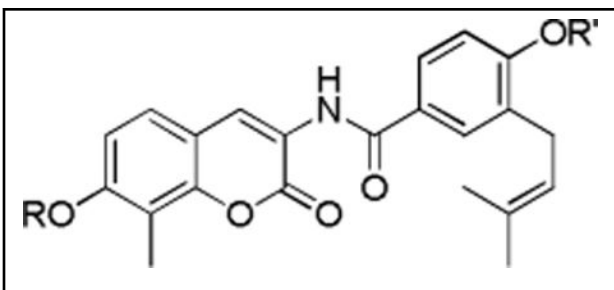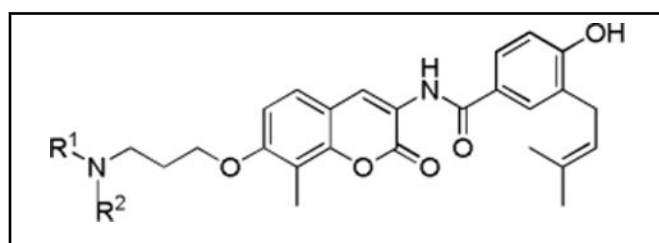
	R	R′	SKBr3 (μM)	MCF-7 (μM)
31		H	>50	>50
32		H	>50	>50
33		H	0.72±0.01	0.99±0.04

[a]Values represent mean ± standard deviation for at least two separate experiments performed in triplicate
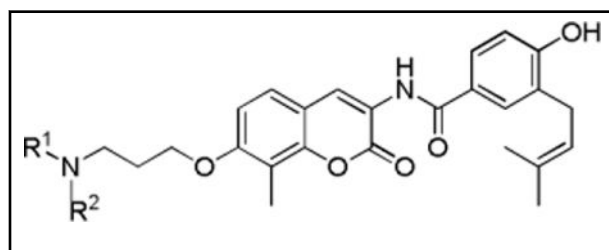
Table 2

Anti-proliferative activities of **NA-2** analogues

	R^1	R^2	SKBr3 (µM)	MCF-7 (µM)
KU-407	CH$_3$	CH$_3$	0.44±0.02[a]	1.35±0.38
35a	CH$_2$CH$_3$	H	0.72±0.34	0.98±0.35
35b	CH$_2$CH$_3$	CH$_3$	0.73±0.06	2.19±0.50
35c	CH$_2$CH$_3$	CH$_2$CH$_3$	4.90±0.42	6.39±0.04
35d	CH$_2$CH$_2$CH$_3$	H	0.99±0.03	0.98±0.00
35e	CH$_2$CH$_2$CH$_3$	CH$_2$CH$_2$CH$_3$	>50	>50
35f	CH$_2$CH(CH$_3$)$_2$	H	1.03±0.02	1.32±0.06
35g	CH(CH$_3$)$_2$	H	0.75±0.26	0.74±0.16
35h	CH(CH$_3$)$_2$	CH$_3$	0.57±0.13	1.05±0.06
35i	CH(CH$_3$)$_2$	CH(CH$_3$)$_2$	1.60±0.02	1.31±0.39
35j	C(CH$_3$)$_3$	H	0.66±0.06	0.95±0.10
35k	C(CH$_3$)$_3$	CH$_3$	0.88±0.10	1.14±0.11
35l	CH$_2$COOMe	H	1.06±0.11	2.82±0.27
35m	CH$_2$COOH	H	7.57±2.86	18.11±5.35
35n	CH$_2$CONH$_2$	H	2.29±0.47	1.46±0.35
35o	CH$_2$CH$_2$OH	H	0.61±0.06	1.56±0.09

[a] Values represent mean ± standard deviation for at least two separate experiments performed in triplicate

Table 3

Anti-proliferative activity of **NA-2** derivatives with cyclic *N*-substitutions

	R¹	R²	SKBr3 (μM)	MCF-7 (μM)
37a	Cyclohexyl	H	0.66±0.12*a*	1.07±0.35
37b	Cyclohexyl	CH₃	0.97±0.03	1.08±0.07
37c	Phenyl	H	>50	>50
37d	Phenyl	CH₃	>50	>50
37e	Benzyl	H	0.47±0.16	0.91±0.01
37f	(R)-1-phenylethyl	H	1.00±0.31	1.45±0.20
37g	Cumenyl	H	0.96±0.01	1.07±0.01
37h	Benzyl	CH₃	10.73±0.49	11.52±4.16
37i		H	0.77±0.13	1.03±0.12
37j	Adamantyl	H	0.31±0.04	0.32±0.03

*a*Values represent mean ± standard deviation for at least two separate experiments performed in triplicate