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Synthesis of retinoid enhancers based on 2-aminobenzothiazoles for anti-cancer therapy

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ABSTRACT

Indole-3-amides and dipeptides were produced from 2-aminobenzothiazoles using the PyBop peptide coupling reagent. These analogues were tested in anti-cancer cell viability assays against SH-SY5Y neuroblastoma and MDA-MB-231 breast adenocarcinoma cell lines, and were found to exhibit cytotoxic activities at concentrations ranging from 0.1 to 20 μM. These compounds were also found to act additively with a low dosage of 13-*cis*-retinoic acid in neuroblastoma cells. Then, using neuroblastoma cells transfected to stably overexpress the RARβ$_2$ gene, a SAR was developed for the indole-3-amides. Real-time PCR was also used to demonstrate their RARβ$_2$ agonistic activity.

1. Introduction

Neuroblastoma, an embryonal malignancy of the sympathetic nervous system, is the most common extracranial solid tumor in the pediatric population, accounting for approximately 7% of all childhood cancers.[1] Almost 50% of patients are diagnosed between the ages of 1 and 4, with a further 30% diagnosed before the age of 1. Approximately 80% of patients over 18 months present with metastatic disease and despite intensive treatment, including high-dose chemotherapy, surgery, radiotherapy, autologous bone marrow transplantation, retinoids and more recently immunotherapy, have a dismal 3-year event-free survival (EFS) rate of 38%.[2]

Retinoids (vitamin A derivatives) have preventative and therapeutic activity for many types of human cancers. The effective use of retinoids as differentiation reagents and tumor growth inhibitors has been well documented since the first use of all-*trans*-retinoic acid in the treatment of patients with acute promyelocytic leukemia (APL) in the mid 1980s.[3] Since then, a number of clinical trials have shown that treatment with natural and synthetic retinoids can induce tumor cell differentiation and reduce the occurrence of secondary malignancies.[4] Furthermore, treatment with isotretinoin (13-*cis*-retinoic acid) is currently the standard therapy for patients with high risk neuroblastoma in their first remission. However, due to the inherent toxicity associated with natural derivatives of vitamin A, the use of retinoids as chemotherapeutics is somewhat limited. Therefore, it is highly desirable to develop compounds which act synergistically with retinoids to enhance their activity in cancer cell lines, whilst minimizing teratogenic and mucocutaneous toxicities.[5]

The activity of retinoids is mediated mainly through their interactions with the nuclear retinoic acid receptors (RARs) and retinoid X receptors (RXRs). Each of these receptors has three subtypes (α, β and γ), with each subtype having a number of isoforms with differing affinities to retinoids and different biological functions. It has been demonstrated that a loss of RARβ$_2$ expression is associated with tumorigenesis and retinoid resistance. The observation that RARβ$_2$ was upregulated in patients after treatment with 13-*cis*-RA, and that increased expression of RARβ$_2$ was correlated with clinical response, suggested that RARβ$_2$ has an important role in suppressing carcinogenesis.[6] Recently developed receptor-specific retinoids and rexinoids have been shown to be less toxic and have enhanced anti-cancer effects.

In light of these recent results, our group performed an in silico pilot screen to identify potential RARβ$_2$ agonists and retinoid enhancers. In this paper, we discuss the synthesis, characterization and in vitro biological evaluation of a number of 2-substituted benzothiazoles based on structures identified as potential RARβ specific ligands from in silico screening.

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2. Results and discussion

2.1. Analysis of the ligands proposed via in silico methods

A subset of the Enamine small molecule library was screened using in silico methods, in order to identify small molecules capable of binding to the ligand binding domain of RARβ_2. This screening returned a series of 26 hits that were proposed as suitable ligands for RARβ_2. These ligands were then purchased from Enamine, and screened for in vitro activity against BE(2)-C neuroblastoma cells with the full-length human cDNA for RARβ cloned into the PvuII multi-cloning site of the pMEP4 episomal Epstein–Barr virus-based expression vector, as previously reported.[7,8] Briefly, cells were allowed to attach for 24 h in 96 well culture plates before being exposed to the ligands at a concentration of 5 μM for 96 h. Comparative values for cell viability in each well were determined by a Wallac 1420 Victor III spectrophotometer, which measured light absorbance in each well at 570 nm. The mean and standard error of mean (SEM) values of each experiment were determined after at least three replicates. Of the 26 compounds, 8 showed favorable in vitro activity, reducing the cell viability of the RARβ_2 transfected cells by at least 20% more than the empty vector (E.V.) control cells (Fig. 1). Of the eight compounds that showed favorable in vitro activity, compound T5497721 displayed the highest level of activity, reducing cell viability by 20% more than the next best compound.

Of the 26 hit compounds from the virtual screen, four contained either benzimidazole or benzothiazole moieties, with two of those also being included in the 8 with favorable activity. Benzothiazoles are a class of heterocyclic compounds found throughout nature in both plants and animals. Benzothiazole was first isolated from the volatile constituents of cranberries (*Vaccinium macrocarpon*), and since then many analogues have been isolated from bacteria, marine sponges and fireflies.[9,10] The biological properties, including anti-cancer activities, of 2-substituted benzothiazoles have been well documented.[11–14] Due to this precedent, the 2-substituted benzothiazole motif was incorporated as a conserved feature throughout our synthesis program.

Further analysis of the structures of the 8 favourable ligands revealed highly conserved linker functionality. Amide bonds were present in 4 of the 8 compounds, while 1,3,4-oxadiazoles, which are frequently used as isosteres for amides, were present in three more structures.[15] Compounds T5497721 and T5688216 (Fig. 2.) were selected from the 8 most effective compounds as leads for structural optimization. This selection was based upon the similarity between their structures, as they both contained the benzimidazole or benzothiazole motif, as well as the amide bonds that were prominent in the 8 most effective compounds. Furthermore, T5688216 shows striking resemblance to a glycine-phenylalanine dipeptide derivative. This structural similarity to a dipeptide was of interest as it offers many interesting possibilities for further functionalization using the many methods for peptide synthesis.

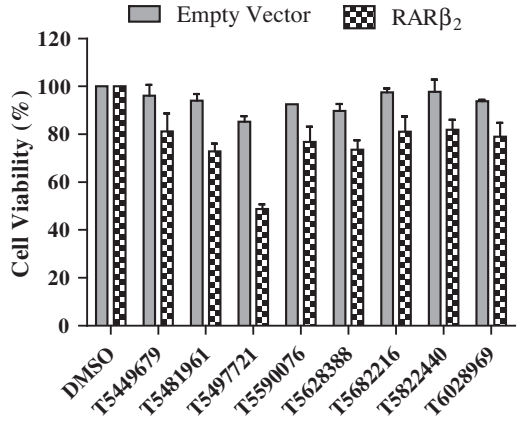


Figure 1. Cell viability of BE(2)-C transfected cells relative to empty vector control after 96 h treatment with 5 μM ligand.

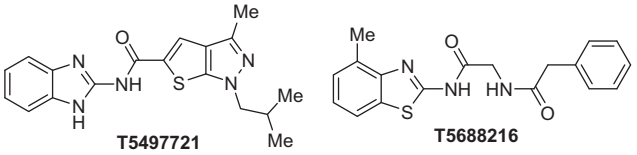


Figure 2. Structure of two compounds from in silico screening.

2.2. Synthesis of peptide-based ligands

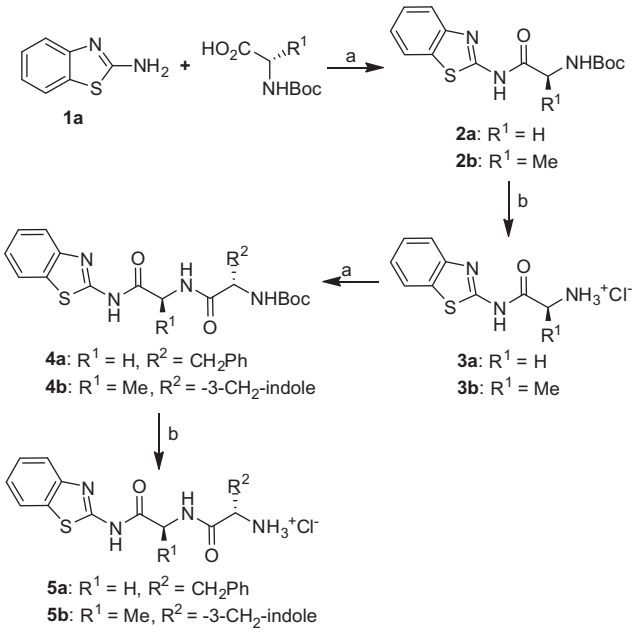
Due to the structural resemblance of compound T5682216 to a dipeptide motif, this structure was utilized as a general scaffold for the development of a series of dipeptide analogues. Solution phase peptide syntheses involving 2-aminobenzothiazole **1a** and similar systems utilizing a range of different conditions and coupling reagents have been reported. Luo et al. have used *N*,*N*′-dicyclohexylcarbodiimide (DCC) in the presence of *N*-hydroxybenzotriazole (HOBt) and *N*-methylmorpholine (NMM) to couple 2-aminobenzothiazole **1a** to *N*-Boc-glycine, followed by deprotection with dilute HCl in EtOAc.[16] Moriarty et al. reported the use of (Benzotriazol-1-yloxy)tripyrrolidino-phosphonium hexafluorophosphate (PyBop) in the presence of *N*,*N*-diisopropylethylamine (DIPEA) to couple benzothiazole **1a** to an *N*-Boc-protected amino acid.[17] The latter method was selected for this work due to its comparative ease as well as the reported success of PyBOP in difficult couplings.[18]

Hence, 2-aminobenzothiazole **1a** was coupled to *N*-Boc-protected glycine or L-alanine by PyBOP coupling in the presence of DIPEA in DCM to give the corresponding protected monopeptides **2a–b** in yields of 81% and 91% respectively. Cleavage of the Boc group was then achieved by stirring in a 4 M HCl in EtOAc solution to afford the amines **3a–b** as their hydrochloride salts in 90% and 91% yields, respectively. These systems were then further extended into dipeptides via the same PyBOP coupling method. Peptide **3a** was coupled to *N*-Boc-L-phenylalanine to afford **4a** in 61% yield, while peptide **3b** was coupled to *N*-Boc-L-tryptophan to give **4b** in 50% yield. Finally, dipeptides **4a–b** were de-protected under the same conditions as stated previously to afford the hydrochloride salts **5a–b** in 92% and 89% yield, respectively, (Scheme 1).

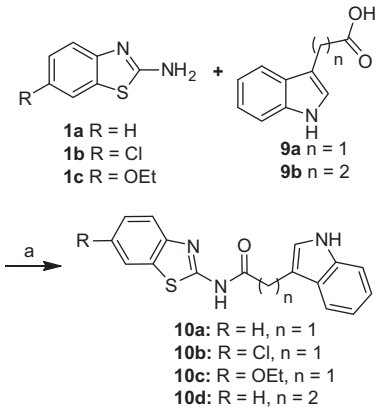
The ^{1}H NMR spectrum of (*S*)-2-amino-*N*-(2-(benzothiazol-2-ylamino)-2-oxoethyl)-3-phenylpropanamide hydrochloride **5a** (DMSO-d_6) exhibited a triplet at δ 9.21 (J = 5.6 Hz, 1H) corresponding to the phenylalanine amide proton and a doublet at δ 8.36 (J = 3.0 Hz, 3H) corresponding to the protonated amine group. For the benzothiazole ring, two doublets at δ 7.98 (J = 7.2 Hz, 1H) and δ 7.76 (J = 7.6 Hz, 1H) corresponded to H7 and H4, respectively, while two doublets of triplets at δ 7.44 (J = 1.3, 7.8 Hz, 1H) and δ 7.30 (J = 1.1, 8.4 Hz, 1H) corresponded to H5 and H6 respectively. It was observed that the other dipeptides gave similar coupling patterns for the benzothiazole ring and peptide chain. Furthermore, the DEPT-135 and broadband-decoupled ^{13}C NMR spectra of dipeptides **5a–b** displayed the appropriate number of CH and CH_2 resonances, confirming the production of the dipeptides.

2.3. Synthesis of indole amides

Indole **6** and its derivatives form a class of heterocyclic compounds that are found widely throughout nature. Both the naturally occurring molecules, as well as their synthetic analogues,



Scheme 1. Reagents and conditions: (a) DIPEA, PyBOP, DCM, 2 h, rt; (b) 4 M HCl/EtOAC, 4 h, rt.



Scheme 3. Reagents and conditions: (a) PyBOP, DIPEA, DCM, 2 h, rt.

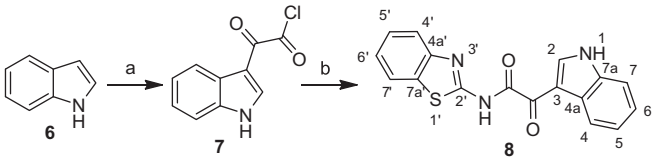
have been shown to possess a vast range of biological functions and properties, including anti-cancer activities. Due to the well-documented biological properties of indole, as well as our group's continuing interest in indole-related chemistry, we proceeded to develop 2-substituted benzothiazole derivatives that incorporated this heterocycle. Indole amides and glyoxylamides were explored due to their structural similarity to the initial 26 hit compounds.

The target indole amides were synthesized by two routes. In the first method, following the well-established procedure of Shaw, oxalyl chloride was added to a solution of indole **6** in Et_2O at 0 °C under a nitrogen atmosphere to produce α-oxo-1*H*-indole-3-acetyl chloride **7**.[19] This was subsequently condensed with benzothiazole **1a** in the presence of NEt_3 in DCM to afford the desired glyoxylamide *N*-(benzo[*d*]thiazol-2-yl)-2-(1*H*-indol-3-yl)-2-oxo acetamide **8** in 75% yield (Scheme 2).

The ^{1}H NMR spectrum of glyoxylamide **8** displayed three singlets at δ 12.86 (1H), δ 12.46 (1H) and δ 8.68 (1H) corresponding to the indole NH, glyoxylamide NH and H2 respectively. Two multiplets at δ 8.26 (J = Hz, 1H) and δ 7.58 (J = Hz, 1H) corresponded to H4 and H7, respectively, while a third multiplet at δ 7.33 (m, 2 H) were assigned to H5 and H6. Furthermore, two doublets at δ 8.07 (J = 7.9 Hz, 1H) and δ 7.84 (J = 7.7, 1H) corresponded to H7′ and H4′, respectively, while two doublets of triplets at δ 7.49 (J = 1.3, 7.2 Hz, 1H), δ 7.38 (J = 1.1, 7.9 Hz, 1H) corresponded to H5 and H6, respectively.

In order to access the indole amides, a second route was established in which benzothiazoles **1a–c** were coupled to indole-3-acetic acid **9a** and indole-3-propionic acid **9b** following the PyBOP coupling procedure as described previously (Scheme 3). Table 1 summarizes the yields of the indole amides.

The ^{1}H NMR spectrum of indole **10a** was similar to that of **8**, with the same aromatic patterns for both the indole and benzothiazole rings, but with the addition of a singlet at δ 3.93 (2H) corresponding to the CH_2 group of the amide linker. Indoles **10b–c** displayed different splitting patterns for the peaks of the benzothiazole ring due to the substitution at the 6-position. In the ^{1}H NMR spectra of **10b** and **10c**, the benzothiazole H4 proton was observed as a doublet at δ 7.39 and 7.54 ppm, respectively, the H5 proton appeared as a doublet at δ 7.65 and 6.98 ppm, respectively, while the H7 proton appeared as a singlet at δ 7.98 and 7.43 ppm, respectively.



Scheme 2. Reagents and conditions: (a) oxalyl chloride, Et_2O, 2 h, 0 °C; (b) benzothiazole **1a**, NEt_3, DCM, 4 h, rt.

2.4. Determination of the compounds in vitro cytotoxicity

The anti-proliferative activity of dipeptide **5a**, indoles **8** and **10a** was determined in the retinoid-sensitive SH-SY5Y neuroblastoma and retinoid-resistant MDA-MB-231 breast adenocarcinoma cell lines, using the Alamar blue (Resazurin) assay described earlier.[20,21] Briefly, cells were allowed to attach for 24 h in 96 well culture plates before being exposed to dilutions of the compounds for 96 h. Comparative values for cell viability in each well were determined by a Wallac 1420 Victor III spectrophotometer which measured light absorbance in each well at 570 nm. The mean and standard error of mean (SEM) values of each experiment were determined after at least three replicates. Figure 3 shows the dose-dependent response of the compounds on cell viability in each of the cell lines, relative to a DMSO treated control.

Among the compounds tested for their effect on cell viability, indole **10a** was found to be the most effective at reducing cell viability. At a concentration of 1 μM, indole **10a** reduced the cell viability of the SH-SY5Y and MDA-MB-231 cell lines by 55% and 58%, respectively. Interestingly, indole **8** was ineffective in the SH-SY5Y cell line, suggesting that the simple transition from a carboxylic amide (**10a**) to its corresponding glyoxylamide (**8**) results in diminished activity against cell viability. This suggests that the additional carbonyl on the linking chain may be restricting the ligands rotation to conformations that are less favorable for interactions with the RARβ$_2$ ligand binding domain. Similarly to indole **10a**, dipeptide **5a** demonstrated consistent, but lower levels of activity in both cell lines. Dipeptide **5a** displayed a reduction in the cell viability by approximately 50% at 20 μM in both cell lines.

In order to examine the relationship between structure, the effect on cell viability, and potential RARβ$_2$ activation, indole amides **10a–d** were used to treat the pMEP4 cloned BE(2)-C neuroblastoma cells used in the screening of the initial 26 ligands. The

Table 1
Indole amides based on 2-aminobenzothiazoles

Indole amide	R	*n*	Yields[a] (%)
10a	H	1	68
10b	Cl	1	70
10c	OEt	1	71
10d	H	2	73

[a] Yields of isolated pure product.

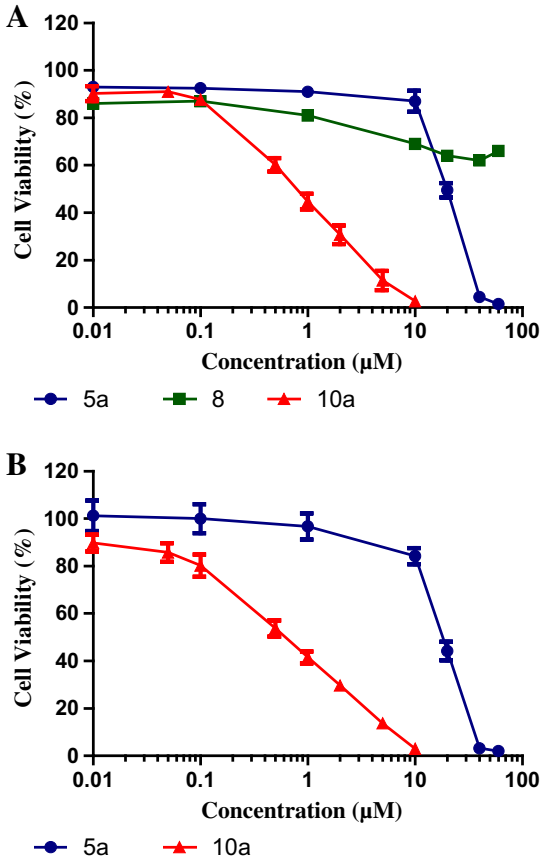


Figure 3. Cell viability of treated (A) SH-SY5Y and (B) MDA-MB-231 cell lines as a percentage of DMSO negative control after 96 h exposure.

screening was performed in a similar manner to that described earlier for the SH-SY5Y and MDA-MB-231 cell lines. Figure 4 shows a comparison of cell viability between the empty vector (E.V.) and RARβ$_2$ overexpressing cell line for a given concentration.

Indole **10a** showed quite promising results, maintaining a level of activity in the BE(2)-C cell lines similar to what was observed in the SH-SY5Y and MDA-MB-231 cell lines. In the empty vector cell line, indole **10a** reduced the cell viability by 38%, while it reduced the cell viability by 50% in the RARβ$_2$ overexpressing clone. It was interesting to observe that upon addition of a bulky, hydrophobic chlorine at the C6′ position to give indole **10b**, the effect on cell viability is reduced, giving reductions of 43% and 48% in the empty vector and RARβ2 cell lines respectively, at 20 μM. This is in contrast to previous findings made by other groups, where the addition of halogens at certain positions have increased RARβ selectivity.[22] Furthermore, upon substitution of an ethoxy group at the same position, giving indole **10c**, the activity is reduced further. Indole **10c** reduced the cell viability of the empty vector and RARβ$_2$ cell lines by 29% and 39%, respectively, suggesting that the C6′ position is not tolerant to modifications, particularly those which increase the bulk or hydrophobicity of the molecule.

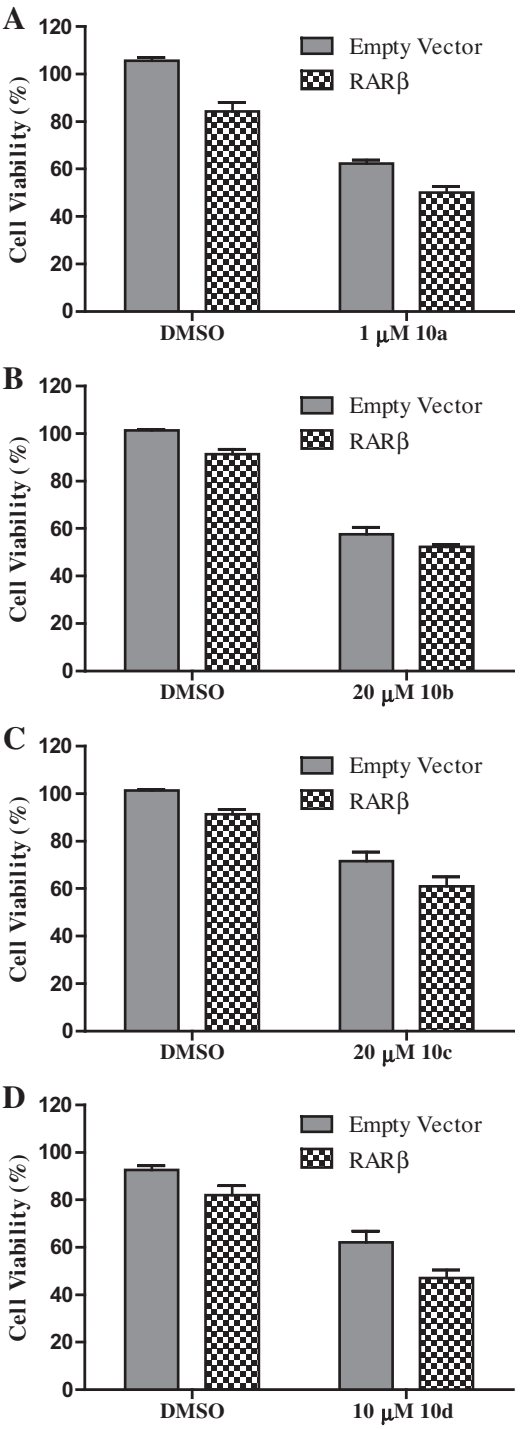


Figure 4. Cell viability of transfected BE(2)-C cell lines after treatment with (A) indole **10a**, (B) indole **10b**, (C) indole **10c** and (D) indole **10d**, as a percentage of negative control after 72 h exposure.

Additionally, when the linker of indole **10a** is increased in length by one CH_2 unit to give the corresponding propanamide **10d**, the effect on cell viability is again reduced. Indole 10d displays 38% and 53% reductions in the cell viability of the empty vector and RARβ$_2$ cell lines, respectively, at 10 μM. Whilst this increase in chain length does reduce the activity, it is not as dramatic as the reduction seen for substitution at the C6′ position.

As it was found to be the most effective of the tested compounds, indole **10a** was further tested for toxicity to the MRC-5 normal human lung fibroblast cell line (Fig. 5.). At a concentration of 1 μM,

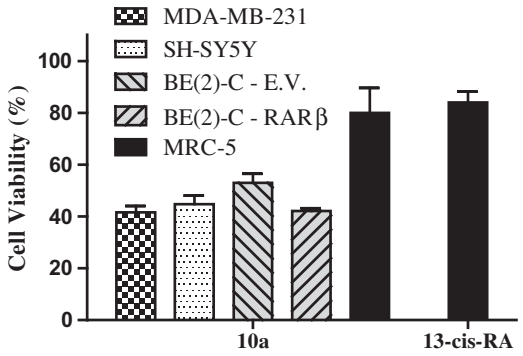


Figure 5. Comparative toxicity of indole **10a** and 13-*cis*-retinoic acid (13-*cis*-RA) in the tested cell lines at 1 μM after 96 h exposure.

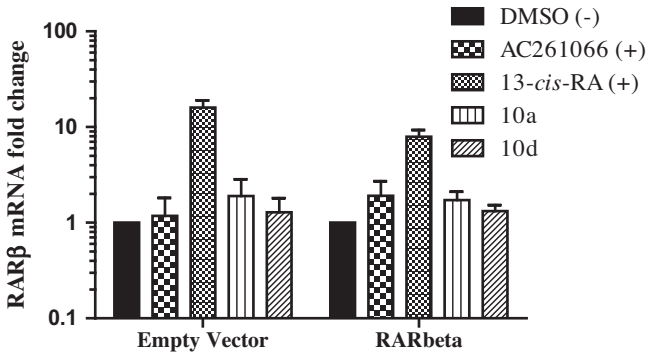


Figure 6. Comparative fold changes in RARβ_2 mRNA expression in pMEP4 cloned BE(2)-C cells after 24 h exposure to controls and indoles **10a** and **10d**.

indole **10a** reduced MRC-5 cell viability by 20%, which is at least 27% lower than its effects in any of the cancerous cell lines. However, it was observed that in the MRC-5 normal cell line, treatment with 13-*cis*-retinoic acid, also at 1 μM, produced only a 10% reduction in cell viability. This degree of specificity suggests that there is a therapeutic window, within which these indole derivatives may be utilized, however, further development is necessary to improve this specificity reduce their level of toxicity to normal cells.

2.5. Examination for enhancement of the retinoid signaling pathway

We next sought to determine if the newly synthesized compounds were able to enhance the retinoid signaling pathway. In order to examine this, two sets of experiments were envisaged. In the first, combinations of isotretinoin and the synthesized molecules would be examined for additive or synergistic enhancement of retinoid therapy, while the second examined the effect of the synthesized ligands on the level of RARβ_2 mRNA expression.

In order to determine whether these compounds acted to enhance the efficacy of retinoid treatment, dipeptide **5a** and indole **10a** were administered in combination with 13-*cis*-retinoic acid. SH-SY5Y cells were allowed to attach for 24 h in 96 well culture plates before relevant wells were exposed to 13-*cis*-retinoic acid at a concentration of 0.1 μM. This was followed 24 h later by exposure of relevant wells to dipeptide **5a** (10 μM) or indole **10a** (0.1 μM) for 96 h. Comparative values for cell viability in each well were determined by a Wallac 1420 Victor III spectrophotometer which measured light absorbance in each well at 570 nm. The mean and standard error of the mean (SEM) values of each experiment were determined after at least three replicates (Table 2).

It was observed that the combination of dipeptide **5a** and isotretinoin caused a reduction in cell viability of 57%, which was greater than the required additive value of 41%. This suggests that dipeptide **5a** is enhancing the efficacy of the retinoid treatment in the SH-SY5Y cell line. It was also observed that indole **10a**, in combination with isotretinoin, caused a reduction in cell viability of 40%, which was approximately equal to the required additive value of 39%. This suggests that combination therapies with the indole derivative may act additively with low doses of isotretinoin.

Table 2
Combination treatment of **5a** (10 μM) or **10a** (0.1 μM) with 13-*cis*-retinoic acid (0.1 μM; 13-*cis*-RA)

Compound	Dose (μM)	Reduction in cell viability (%)		
		Compound alone	13-*cis*-RA alone	Combination treatment
5a	10	13 ± 4	28 ± 2	57 ± 2
10a	0.1	12 ± 3	27 ± 2	40 ± 1

In order to determine the effect treatment with indole amides had on RARβ_2 mRNA expression, pMEP4 cloned BE(2)-C cells were allowed to attached to duplicate wells of 6 well culture plates for 24 h. The cells were then exposed to a DMSO solvent control, 13-*cis*-retinoic acid (10 μM), AC261066 (10 nM), an RARβ_2 selective agonist, indole **10a** (1 μM) or indole **10d** (10 μM), for 24 h.[23] RARβ_2 mRNA expression levels in the pMEP4 cloned BE(2)-C cell lines were assayed using a quantitative real time PCR technique (Fig. 6). Total RNA was isolated from cell lines using PureLink Mini Kit (Invitrogen). For each RT-PCR, 2 μg of total RNA was converted into cDNA using SuperScript III reverse transcriptase (Invitrogen). 2 μL of the reverse transcription reaction was then quantified by quantitative real time PCR using API-7500 Sequence Detection System and SYBR Green PCR Master Mix (Applied Biosystems). The quantitative results of RARβ_2 mRNA were normalized to β2 M mRNA from the same sample.

Upon treatment with indoles **10a** and **10d**, the level of RARβ_2 expression was observed to increase by 2.1 and 1.5 fold, respectively, in the RARβ_2 overexpressed cell line. This was much less than the 9.3 fold increase observed with 13-*cis*-retinoic acid treatment, but quite close to the 2.7 fold increase observed for the known RARβ_2 agonist AC261066. It was also interesting to note that the indole acetamide **10a** showed higher levels of RARβ_2 induction than the propanamide **10d**, which correlates to the observed difference in their effects on cell viability. This suggests that the indole amide derivatives are capable of causing RARβ_2 activation, and that their effect on cell viability may be linked to this activation. Examination of the levels of RARβ_2 activation at other concentrations is necessary to determine their maximal effect, as is measurement of RARα, RARγ and other RARβ isotype mRNA levels to determine their isotype selectivity.

3. Conclusion

The benzothiazole motif was identified as a promising structural scaffold for RARβ_2 agonists using virtual screening. A series of 2-substituted benzothiazoles was developed via simple peptide coupling reactions to amino acids and indol-3-yl acids. The indole amides displayed moderate effects on cell viability, and showed some selectivity towards cancerous cell lines. Furthermore, these compounds also increased levels of RARβ_2 mRNA production compared to a negative control. This data suggests that this class of compounds warrants further development.

4. Experimental section

4.1. Materials and methods

All reagents and solvents were obtained from commercial sources and purified if necessary. Melting points were measured

using a Mel-Temp melting point apparatus and are uncorrected. ^{1}H and ^{13}C NMR spectra were obtained on Bruker DPX300 (300 MHz). Mass spectra were recorded on either a Bruker FT-ICR MS (EI) or a Micromass ZQ2000 (ESI). Infrared spectra were recorded with a Thermo Nicolet 370 FTIR Spectrometer using KBr discs. Column chromatography was carried out using Merck 230–400 mesh ASTM silica gel, and preparative thin layer chromatography was performed using Merck silica gel 7730 60GF^{254}.

4.1.1. General procedure for the synthesis of *N*-Boc protected peptides (2a–d, 4a–d)

Benzothiazole **1** (1.1 mmol), the N-protected amino acid derivative (1 mmol) and PyBop (1 mmol) were dissolved in DCM (1 mL), and DIPEA (2 mmol, or 3 mmol if HCl salt) was added dropwise. The reaction mixture was stirred at rt for 1 h, and the solvent was evaporated. The residue was redissolved in EtOAc (20 mL) and washed with 5% $KHSO_4$ (2 × 50 mL), brine (50 mL), 5% $NaHCO_3$ (2 × 50 mL), again with brine (50 mL), dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The product was used in the next step without further purification.

4.1.2. *tert*-butyl 2-(Benzo[*d*]thiazol-2-ylamino)-2-oxoethylcarbamate (2a)

White solid yield: 91%; mp 177–179 °C; UV (MeOH): λ_{max} 269 (ε 4100 cm^{-1} M^{-1}), 297 (2400) nm; IR (KBr): ν_{max} 3433, 3177, 3064, 2976, 2931, 2860, 1698, 1598, 1536, 1500, 1435, 1367, 1268, 1243, 1163, 1052, 1022, 871, 762 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 12.38 (br s, 1H, NH), 7.98 (d, J = 7.8 Hz, 1H, H4), 7.74 (d, J = 8.1 Hz, 1H, H7), 7.43 (dt, J = 1.2, 8.7 Hz, 1H, H5), 7.30 (dt, J = 1.2, 8.4 Hz, 1H, H6), 7.19 (t, J = 1.8 Hz, 1H, CH_2NH), 3.90 (d, J = 6.3 Hz, 2H, CH_2), 1.40 (s, 9H, 3 × CH_3); ^{13}C NMR (75.6 MHz, DMSO-d_6): δ 166.4 (C═O), 157.2 (C2), 148.3 (C3a), 131.4 (C7a), 126.4 (C5), 123.9 (C6), 121.9 (C7), 120.8 (C4), 78.3 (C–O), 43.2 (CH_2), 28.2 (CH_3); HRMS (+ESI) *m/z* Calcd For $C_{14}H_{18}N_3O_3S$ $(M+H)^+$ 308.1063. Found 308.1058.

4.1.3. (*S*)-*tert*-Butyl 1-(benzo[*d*]thiazol-2-ylamino)-1-oxopropan-2-ylcarbamate (2b)

White solid, yield: 81%; mp 181–183 °C; UV (MeOH): λ_{max} 274 (ε 4500 cm^{-1} M^{-1}) 298 (3200) nm; IR (KBr): ν_{max} 3258, 3076, 2984, 2932, 1680, 1602, 1475, 1392, 1287, 1166, 1047, 1018, 961, 869, 750, 727 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): 12.39 (br s, 1H, NH), 7.97 (d, J = 7.8 Hz, 1H, H4), 7.74 (d, J = 7.7 Hz, 1H, H7), 7.43 (dt, J = 1.1, 7.6 Hz, 1H, H5), 7.30 (dt, J = 0.9, 7.1, Hz, 1H, H6), 4.28 (p, 1H, J = 7.1 Hz, CH), 1.37 (s, 9 H, 3 × CH_3), 1.28 (d, J = 7.2 Hz, 3H, CH_3); ^{13}C NMR (75.6 MHz, DMSO-d_6): δ 173.1 (C═O), 158.0 (C2), 155.3 (O-), 148.5 (C3a), 131.5 (C7a), 126.1 (C5), 123.6 (C6), 121.7 (C7), 120.5 (C4), 78.3 (C–O), 49.9 (CH), 29.1 (3 × CH_3), 17.5 (CH_3); HRMS (+ESI) *m/z* Calcd for $C_{15}H_{19}N_3NaO_3S$ $(M+Na)^+$ 344.1045. Found 344.1034.

4.1.4. (*S*)-*tert*-Butyl (1-((2-(benzo[*d*]thiazol-2-ylamino)-2-oxoethyl)amino)-1-oxo-3-phenylpropan-2-yl)carbamate (4a)

White solid, yield; 60%; mp 82-84 °C; UV (MeOH): λ_{max} 293 (ε 66200 cm^{-1} M^{-1}) 298 (76400) nm; IR (KBr): ν_{max} 3278, 3061, 2974, 2869, 1709, 1603, 1550, 1455, 1443, 1366, 1269, 1167, 1086, 1016, 757, 730, 699 cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 12.28 (s, 1H, NH), 8.41 (t, J = 5.5 Hz, 1H, CH_2NH), 7.98 (d, J = 7.2 Hz, 1H, H4), 7.75 (d, J = 7.7 Hz, 1H, H7), 7.44 (dt, J = 1.3, 8.7 Hz, 1H, H5), 7.31 (dt, J = 1.2, 8.4 Hz, 1H, H6), 7.24 (m, 5H, H2′, H3′, H4′, H5′, H6′), 4.24 (m, 1H, CH), 4.10 (d, 2H, J 5.0 Hz, $COCH_2NH$), 3.08 (m, 2H, $CHCH_2$), 1.30 (s, 9H, 3 × CH3); ^{13}C NMR (75.6 MHz, DMSO-d_6): δ 172.1 ((C═O)CH_2), 157.8 ((C═O)CH), 155.5 ((C═O)O), 148.5 (C3a), 137.5 (C1′), 131.5 (C7a), 129.3 (C3′, C5′), 128.1 (C2′, C6′), 126.4 (C4′), 126.1 (C5), 123.6 (C6), 121.7 (C7), 120.5 (C4), 78.3 ($C(CH_3)_3$), 56.2 (CH), 45.8 (CH_2), 36.8 ($CHCH_2$), 28.1 (CH_3); HRMS (+ESI) *m/z* Calcd for $C_{23}H_{27}N_4O_4S$ $(M+1)^+$ 455.1748. Found 455.1733.

4.1.5. (*S*)-*tert*-Butyl-1-(2-(benzo[*d*]thiazol-2-ylamino)-2-oxoethylamino)-3-(1*H*-indol-3-yl)-1-oxopropan-2-ylcarbamate (4b)

White solid, yield; 49%; mp 117–119 °C; UV (MeOH): λ_{max} 293 (ε 46700 cm^{-1} M^{-1}) 299 (61700) nm; IR (KBr): ν_{max} 3281, 3059, 2977, 1654, 1532, 1442, 1366, 1269, 1166, 1093, 1014, 962, 864, 743, 692 cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 12.45 (s, 1H, N^{1}H), 10.81 (s, 1H, N^{2}H), 8.39 (d, J = 6.4 Hz, 1H, CH_3CHNH), 7.98 (d, J = 7.7 Hz, 1H, H4), 7.76 (d, J = 7.8 Hz, 1H, H7), 7.65 (d, J = 7.7 Hz, 1H, H4′), 7.44 (t, J = 7.4 Hz, 1H, H6), 7.32 (d, J = 7.6 Hz, 1H, H7′), 7.29 (t, J = 6.3 Hz, 1H, H5), 7.16 (s, 1H, H3′), 7.05 (t, J = 7.1 Hz, 1H, H6′) 6.97 (t, J = 7.5 Hz, 1H, H5′), 4.59 (p, J = 6.9 Hz, 1H, CH_3CH), 4.36 (p, J = 7.3 Hz, 1H, $CHCH_2$), 3.02 (m, 2H, $CHCH_2$), 1.38 (d, J = 6.9 Hz, 3H, CH_3CH), 1.30 (s, 9H, 3 × CH_3); ^{13}C NMR (75.6 MHz, DMSO-d_6): δ 172.6 (CO), 170.8 (CO), 158.2 (C2), 155.6 (COO), 149.0 (C7a), 136.5 (C7a′), 131.9 (C4a), 127.9 (C4a′) 126.6 (C5), 124.3 (C6), 124.1 (C3), 122.2 (C7), 121.3 (C6′), 121.1 (C5′), 119.1 (C4′), 118.6 (C4), 111.7 (C7′), 110.4 (C3′), 78.5 (C–O) 60.2 (CH_2CH), 46.3 ($CHCH_3$), 26.3 (CH_2), 28.1 (3 × CH_3), 18.0 ($CHCH_3$); HRMS (+ESI) *m/z* Calcd for $C_{26}H_{30}N_5O_4S$ $(M+1)^+$ 508.2013. Found 508.2001.

4.1.6. General procedure for deprotection of the *N*-Boc group (3a-d, 5a-d)

A solution of 4 mol/L HCl/EtOAc (20 mL) was prepared by diluting a stock solution of 10 mol/L HCl with EtOAc. The N-protected product was added to the solution and stirred at rt for 4 h. Excess EtOAc and hydrogen chloride gas was removed under reduced pressure to give the peptide as the hydrochloride salt. The oily product formed was washed with Et_2O to yield the title compound as an off-white solid.

4.1.7. 2-Amino-*N*-(benzo[*d*]thiazol-2-yl)acetamide hydrochloride (3a)

White solid, yield: 90%; mp 177–179 °C; UV (MeOH): λ_{max} 289 (ε 102700 cm^{-1} M^{-1}), 297 (9900) nm; IR (KBr): ν_{max} 2964, 2921, 2582, 1731, 1589, 1568, 1460, 1325, 1191, 895 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.52 (br s, 3 H, NH_3Cl), 8.02 (d, J = 7.2 Hz, 1H, H4), 7.78 (d, J = 7.6 Hz, 1H, H7), 7.46 (dt, J = 1.3, 7.8 Hz, 1H, H5), 7.33 (dt, J = 1.2, 7.4 Hz, 1H, H6), 3.97 (d, J = 5.7 Hz, 2H, CH_2); ^{13}C NMR (75.6 MHz, DMSO-d_6): δ 166.4 (CO), 157.2 (C2), 148.3 (C3a), 131.4 (C7a), 126.3 (C5), 123.9 (C6), 121.9 (C7), 120.8 (C4), 40.6 (CH_2); HRMS (+ESI) *m/z* Calcd. for $C_9H_{10}N_3OS$ $(M+H)^+$ 208.0539. Found 208.0981.

4.1.8. (*S*)-2-Amino-*N*-(benzo[*d*]thiazol-2-yl)propanamide hydrochloride (3b)

White solid, yield: 91%; mp 181–183 °C; UV (MeOH): λ_{max} 203 (ε 12100 cm^{-1} M^{-1}), 221 (17100), 261 (6300) nm; IR (KBr): ν_{max} 3388, 2868, 1727, 1595, 1562, 1455, 1342, 1309, 1184, 1103, 760, 721, 654 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.70 (bs, 3H, NH_2), 8.01 (d, J = 7.8 Hz, 1H, H4), 7.77 (d, J = 7.8 Hz, 1H, H7), 7.45 (t, J = 6.9 Hz, 1H, H5), 7.33 (t, J = 6.9 Hz, 1H, H6), 4.24 (p, J = 6.9 Hz, 1H, CH), 1.52 (d, J = 6.9 Hz, 3H, CH_3); ^{13}C NMR (75.6 MHz, DMSO-d_6): δ 169.7 (CO), 157.4 (C2), 148.2 (C3a), 131.5 (C7a), 126.4 (C5), 124.0 (C6), 121.9 (C7), 120.7 (C4), 48.6 (CH), 16.7 (CH_3); HRMS (+ESI) *m/z* Calcd. for $C_{10}H_{12}N_3OS$ $(M+H)^+$ 222.0696. Found 222.0690.

4.1.9. (*S*)-2-Amino-*N*-(2-(benzo[*d*]thiazol-2-ylamino)-2-oxoethyl)-3-phenylpropanamide hydrochloride (5a)

White solid, yield: 90%; mp 186–188 °C; UV (MeOH): λ_{max} 286 (ε 12300 cm^{-1} M^{-1}), 288 (12600), 297 (11900) nm; IR (KBr): ν_{max} 3434, 3210, 2925, 2621, 1697, 1680, 1570, 1519, 1346, 1202,

1168, 753 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 9.21 (t, 1H, *J* 5.6 Hz, NH), 8.36 (s, 3H, NH_2), 7.98 (d, 1H, *J* 7.2 Hz, H4), 7.76 (d, 1H, *J* 7.6 Hz, H7), 7.44 (dt, 1H, *J* 7.8, 1.3 Hz, H5), 7.31 (m, 6H, H6, H2′, H3′, H4′, H5′, H6′), 4.12 (d, 3H, *J* 5.6 Hz, COC*H*$_2$NH, CH), 3.14 (m, 2 H, CHC*H*$_2$); ^{13}C NMR (DMSO-d_6): δ 168.7 (CO), 168.4 (CO), 157.6 (C2), 148.5 (C7a), 135.0 (C1′), 131.5 (C4a), 129.7 (C3′, C5′), 128.5 (C2′, C6′), 127.1 (C5′), 126.2 (C6), 123.7 (C5), 121.8 (C4), 120.6 (C7), 53.4 (CH), 42.2 (CO*C*H$_2$), 36.8 (CH*C*H$_2$); HRMS (+ESI): *m/z* Calcd. For $C_{18}H_{19}N_4O_2S$ $(M+H)^+$ 355.1229. Found 355.1222.

4.1.10. (*S*)-2-Amino-*N*-(2-(benzo[*d*]thiazol-2-ylamino)-2-oxoethyl)-3-(1*H*-indol-3-yl)propanamide hydrochloride (5b)

White solid, yield: 66%; mp 138–140 °C; UV (MeOH): λ_{max} 289 (ε 10000 cm^{-1} M^{-1}) nm; IR (KBr): ν_{max} 3225, 3057, 2976, 2914, 1665, 1601, 1544, 1456, 1442, 1267, 1152, 1098, 746 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 11.13 (s, 1H, N^{1}H), 9.28 (t, 1H, *J* 5.4 Hz, NH), 8.30 (br s, 2 H, NH2), 7.98 (d, 1 H, J 7.8 Hz, H4), 7.75 (d, 2 H, J 7.5 Hz, H7, H4′), 7.44 (t, 1H, *J* 7.2 Hz, H5), 7.33 (m, 3 H, H6, H2′, H7′), 7.08 (t, 1H, *J* 6.9 Hz, H5′), 6.99 (t, 1H, *J* 7.2 Hz, H6′), 4.55, (m, 2 H, CO*C*H$_2$NH), 4.17 (d, 1H, *J* 5.4 Hz, CH), 3.21 (m, 2H, CH*C*H$_2$). ^{13}C NMR (DMSO-d_6): δ 172.0 (CO), 168.5 (CO), 157.8 (C2), 148.5 (C7a), 136.3 (C7a′), 131.5 (C4a), 127.4 (C4a′), 126.2 (C6), 125.2 (C5), 123.7 (C2′), 121.8 (C4), 121.1 (C6′), 120.6 (C5′), 118.5 (C4′), 118.3 (C7), 111.5 (C7′), 106.7 (C3′), 52.4 (CHNH$_2$), 48.9 (*C*HCH$_3$), 25.9 (CH$_2$) 17.3 (CH$_3$); HRMS (+ESI): *m/z* Calcd. For $C_{21}H_{22}N_5O_2S$ $(M+H)^+$ 408.1489. Found 408.1479.

4.1.11. α-Oxo-1*H*-Indole-3-acetyl chloride (8)

Yellow solid, yield: 86%; mp 116–118 °C, lit. 117–119 °C.[24]

4.1.12. *N*-(Benzo[*d*]thiazol-2-yl)-2-(1*H*-indol-3-yl)-2-oxoacetamide (8)

Brown solid, yield: 75%; mp 294-296 °C. UV (DMSO): λ_{max} 336 (ε 18200 cm^{-1} M^{-1}) nm; IR (KBr): ν_{max} 3373, 3281, 1681, 1630, 1524, 1441, 1419, 1238, 1124, 823, 729 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 12.86 (bs, 1H, N^{1}H), 12.46 (br s, 1H, CONH), 8.68 (s, 1H, H2), 8.26 (m, 1 H, H4), 8.07 (d, *J* = 7.9 Hz, 1H, H4′), 7.84 (d, *J* = 7.7 Hz, 1H, H7′), 7.58 (m, 1H, H7), 7.49 (dt, *J* = 1.3, 7.2 Hz, 1H, H5′), 7.38 (dt, *J* = 1.1, 7.9 Hz, 1H, H6′), 7.33 (m, 2 H, H5, H6); ^{13}C NMR (75.6 MHz, DMSO-d_6): δ 180.2 (NHCO), 163.9 (CO), 157.8 (C2), 149.0 (C4a), 139.2 (C3′), 137.1 (C2′), 132.3 (C7a′), 126.8 (C5), 126.3 (C7a), 124.5 (C6), 124.3 (C7), 123.4 (C4a′), 122.3 (C5′), 121.7 (C6′), 121.4 (C4), 113.3 (C7′), 112.4 (C4′); HRMS (+ESI) *m/z* Calcd for $C_{17}H_{11}N_3NaO_2S$ $(M+Na)^+$ 344.0470. Found 344.0459.

4.1.13. General procedure for the synthesis of indole amides (10a–c)

Benzothiazole **1a–c** (1 mmol), indole-3-acetic acid **9** (1 mmol) and PyBop (1 mmol) were dissolved in DCM (1 mL), and DIPEA (3 mmol) was added dropwise. The reaction mixture was stirred at rt for 2 h. The solvent was evaporated and the residue was redissolved in EtOAc (20 mL) and washed with 5% $KHSO_4$ (2 × 50 mL), brine (50 mL), 5% $NaHCO_3$ (2 × 50 mL), again with brine (50 mL), dried over anhydrous Na_2SO4, filtered and concentrated under reduced pressure. The solid so obtained was purified by flash column chromatography with 2:3 EtOAc:hexane as the eluent.

4.1.14. *N*-(Benzo[*d*]thiazol-2-yl)-2-(1*H*-indol-3-yl)acetamide (10a)

Brown solid, yield: 53%; mp 209–211 °C. UV (MeOH): λ_{max} 289 (ε 17100 cm^{-1} M^{-1}), 298 (14400) nm; IR (KBr): ν_{max} 3373, 3163, 3062, 2960, 1747, 1693, 1599, 1529, 1450, 1396, 1316, 1261, 752, 727 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 12.54 (s, 1H, N^{1}H), 10.99 (s, 1H, NH), 7.95 (d, *J* = 7.2 Hz, 1H, H4), 7.74 (d, *J* = 7.6 Hz, 1H, H7), 7.61 (d, *J* = 7.8 Hz, 1H, H4′), 7.42 (t, *J* = 7.2 Hz, 1H, H5), 7.37 (d, *J* = 8.0 Hz, 1H, H7′), 7.32 (d, *J* = 2.4 Hz, 1H, H2′), 7.28 (t, *J* = 7.2 Hz, 1H, H7), 7.08 (t, *J* = 6.9 Hz, 1H, H5′), 7.00 (t, *J* = 6.8 Hz, 1H, H6′), 3.93 (s, 2H, CH$_2$); ^{13}C NMR (75.6 MHz, DMSO-d_6): δ 170.7 (CO), 158.1 (C2), 148.5 (C3a), 136.1 (C7a′), 131.4 (C7a), 127.1 (C3a), 126.1 (C5), 124.3 (C6), 123.5 (C2′), 121.7 (C7), 121.1 (C6′), 120.5 (C5′), 118.6 (C4, C4′), 111.5 (C7′), 107.2 (C3′), 32.4 (CH$_2$); HRMS (+ESI) *m/z* Calcd. for $C_{17}H_{14}N_3OS$ $(M+H)^+$ 308.0858. Found 308.0849.

4.1.15. *N*-(6-Chlorobenzo[*d*]thiazol-2-yl)-2-(1*H*-indol-3-yl) acetamide (10b)

Brown solid, yield: 70%; mp 199-201 °C. UV (MeOH): λ_{max} 291 (ε 25400 cm^{-1} M^{-1}) 304 (17700) nm; IR (KBr): ν_{max} 3349, 3177, 3057, 2968, 2914, 1685, 1595, 1533, 1446, 1267, 1099, 743 cm^{-1}; ^1H NMR (300 MHz, d_6-acetone): δ 10.23 (br s, 1H, NH), 7.98 (dd, *J* = 0.4, 2.2, Hz, 1H, H7), 7.69 (s, 1H, H2′), 7.65 (dd, *J* = 0.5 8.2 Hz, 1H, H5) 7.37 (m, 2H, H4′, H7′), 7.39 (dd, *J* = 2.2, 6.3 Hz, 1H, H4), 7.12 (dt, *J* = 1.3, 7.6 Hz, 1H, H5′), 7.04 (dt, *J* = 1.2, 6.9 Hz, 1H, H6′) 4.08 (d, *J* = 0.8 Hz, 2H, CH$_2$); ^{13}C NMR (75.6 MHz, d_6-acetone): δ 171.4 (CO), 159.6 (C2), 148.8 (C3a), 137.6 (C7a′), 134.7 (C7a), 129.2 (C3a′), 127.3 (C5), 125.2 (C3′), 122.7 (C6′), 122.5 (C7), 121.9 (C5′), 119.9 (C4′), 119.5 (C4), 112.3 (C7′), 108.3 (ArC), 33.7 (CH$_2$); HRMS (+ESI) *m/z* Calcd. for $C_{17}H_{13}ClN_3OS$ $(M+H)^+$ 342.0462. Found 342.0454.

4.1.16. *N*-(6-Ethoxybenzo[*d*]thiazol-2-yl)-2-(1*H*-indol-3-yl)acetamide (10c)

Brown solid, yield: 71%; mp 184–186 °C. UV (MeOH): λ_{max} 308 (ε 17900 cm^{-1} M^{-1}) nm; IR (KBr): ν_{max} 3393, 3177, 3048, 2976, 2926, 1688, 1605, 1548, 1458, 1263, 1220, 1152, 1061, 742 cm^{-1}; ^1H NMR (300 MHz, d_6-acetone): δ 10.22 (bs, 1H, N^{1}H), 7.68 (d, *J* = 7.3 Hz, 1H, H4′), 7.54 (d, *J* = 8.5 Hz, 1H, H4), 7.43 (s, 1H, H7), 7.41 (d, *J* = 5.3 Hz, 1H, H7′), 7.12 (dt, *J* = 1.1, 7.5 Hz, 1H, H6′), 7.04 (dt, *J* = 1.1, 7.6 Hz, 1H, H5′), 6.98 (dd, *J* = 2.58, 8.84 Hz, 1H, H5), 4.08 (q, *J* = 6.9 Hz, 2 H, CH$_3$*C*H$_2$) 4.04 (s, 2H, CH$_2$), 1.38 (t, *J* = 6.9 Hz, 3H, *C*H$_3$CH$_2$); ^{13}C NMR (75.6 MHz, d_6-acetone): δ 169.9 (CO), 156.4 (C6′), 155.7 (C4a′) 136.7 (C7a), 131.1 (C7a′) 126.9 (C4a), 124.4 (C6), 123.3 (C5), 121.4 (C4), 120.8 (C5′), 118.5 (C4′), 115.9 (C2), 111.8 (C7), 105.2 (C7′), 64.3 (*C*H$_2$CH$_3$), 33.6 (CH$_2$), 15.0 (CH$_3$);. HRMS (+ESI) *m/z* Calcd. for $C_{19}H_{18}N_3O_2S$ $(M+H)^+$ 352.1114. Found 352.1105.

4.1.17. *N*-(Benzo[*d*]thiazol-2-yl)-3-(1*H*-indol-3-yl)propanamide (10d)

White solid, yield: 73%; mp 224–226 °C. UV (MeOH): λ_{max} 295 (ε 73900 cm^{-1} M^{-1}) 299 (79000) nm; IR (KBr): ν_{max} 3374, 3141, 2960, 2922, 1706, 1598, 1541, 1492, 1454, 1444, 1348, 1270, 1232, 1190, 1154, 1106, 754, 735 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 12.36 (s, 1H, N^{1}H), 10.79 (s, 1H, CONH), 7.97 (d, *J* = 7.2 Hz, 1H, H4), 7.72 (d, *J* = 7.6 Hz, 1H, H7), 7.58 (d, *J* = 7.8 Hz, 1H, H 4′), 7.42 (dt, *J* = 1.3, 7.8 Hz, 1H, H5), 7.31 (dt, *J* = 1.1, 7.9 Hz, 1H, H6), 7.28 (d, *J* = 6.1 Hz, 1H, H7′), 7.13 (d, *J* = 2.3 Hz, 1H, H2′), 7.06 (dt, *J* = 1.2, 7.5 Hz, 1H, H6′), 6.97 (dt, *J* = 1.1, 7.4 Hz, 1H, H5′), 3.07 (t, *J* = 7.1 Hz, 2H, COCH$_2$), 2.87 (t, *J* 8.1 Hz, 2H, CH$_2$); ^{13}C NMR (75.6 MHz, DMSO-d_6): δ 172.0 (CO), 157.9 (C2), 148.5 (C3a), 136.2 (C7a), 131.4 (C7a), 126.9 (C3′a), 126.0 (C5), 123.4 (C6), 122.3 (C7), 121.7 (C6′), 121.0 (C5′), 120.4 (C4), 118.4 (C4′), 118.2 (C7′), 113.2 (C3′), 111.4(C7′), 36.2 (CO*C*H$_2$), 20.4(CH$_2$); HRMS (+ESI) *m/z* Calcd. for $C_{18}H_{16}N_3OS$ $(M+H)^+$ 322.1009. Found 322.1004.

4.2. Biological experiments

4.2.1. Cell biology techniques

The retinoic acid (RA)-sensitive SH-SY5Y neuroblastoma cell line was donated by Dr. J. Biedler (Memorial Sloan-Kettering Cancer Center, New York). The RA-resistant MDA-MB-231 breast

cancer cell line was purchased from the American Type Culture Collection. All cell lines were cultured under standard conditions at 37 °C in 5% CO_2 as an adherent monolayer in Dulbecco's modified Eagle's medium supplemented with L-glutamine (DMEM) (Invitrogen, USA) and 10% fetal calf serum (FCS) (Thermo Fisher Scientific).

4.2.2. Method for cell viability assays

Cell viability was measured by the standard Alamar blue assay, as previously described.[25] Briefly, cells were allowed to attach for 24 h in 6 replicate wells of 96-well culture plates. The cells were then continuously exposed to serial dilutions of the benzothiazole derivative for 96 h. Cell viability was determined by addition of 22 μL of Alamar blue reagent, recording comparative 0 h and 5 h values, using a Wallac 1420 Victor III spectrophotometer, which measured light absorbance in each well at 570 nm. The cell viability of each plate was calculated as a percentage compared to matched DMSO controls (0.5%). The mean (+/− SEM) is shown for three independent experiments.

4.2.3. Method for determining mRNA expression levels using quantitative real-time PCR

Cells were allowed to attach to duplicate wells of 6 well culture plates for 24 h before being exposed to a DMSO solvent control (0.05%), 13-*cis*-retinoic acid (10 μM), AC261066 (10 nM) an RARβ_2 selective agonist, indole **10a** (1 μM) or indole **10d** (10 μM), for 24 h.[23] Total RNA was isolated from cell lines using PureLink Mini Kit (Invitrogen). For each RT-PCR, 2 μg of total RNA was converted into cDNA using SuperScript III reverse transcriptase (Invitrogen). 2 μL of the reverse transcription reaction was then quantified by quantitative real time PCR using API-7500 Sequence Detection System and SYBR Green PCR Master Mix (Applied Biosystems). The quantitative results of RARβ_2 mRNA were normalized to β2 M mRNA from the same sample

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