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Triphenylmethylamides (TPMAs): Structure–activity relationship of compounds that induce apoptosis in melanoma cells

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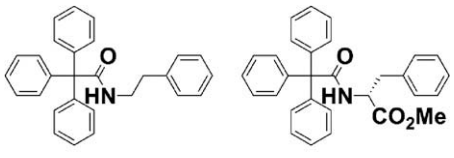
ABSTRACT

Triphenylmethylamides (TPMAs) have been previously identified as compounds that arrest cells in the G1-phase of the cell cycle and induce apoptotic death in melanoma cell lines in culture. Here we report the synthesis of a series of TPMA derivatives, allowing the structure–activity relationship of this class of molecules to be established. Several new compounds have been identified that induce death in UACC-62 and SK-MEL-5 human melanoma cell lines, including a compound with enhanced aqueous solubility.

Metastatic melanoma is a particularly aggressive form of cancer that is resistant to standard anticancer therapies. If identified early, melanoma (Stage I/II) primary tumors can be surgically resected with a >95% success rate.[1] In contrast, late-stage (Stage IV) metastatic melanoma is one of the most deadly forms of cancer, with the median survival of patients with distant metastases being 7–8 months.[2] The current standard of care for patients with late-stage melanoma is dacarbazine/temozolomide chemotherapy or immunotherapy involving interleukin-2/interferon-α.[3–5] Few patients respond positively to these treatments; sometimes tumor shrinkage is observed for a few months, after which tumor growth typically resumes.[5,6] Clearly, novel compounds and new biological targets are needed to address the limitations of current melanoma therapies.

Drugs that target rapidly dividing cells by interfering with DNA synthesis (S-phase arrest) or mitosis (M-phase arrest) such as taxol, etoposide, cisplatin, and doxorubicin have failed to demonstrate efficacy in large randomized trials with melanoma patients.[7] The resistance to DNA-targeted therapy may be due to down-regulation of DNA mismatch repair enzymes in melanoma.[8] Additionally, elevated levels of survivin, a protein that assists mitotic spindle formation permitting the evasion of the G2/M checkpoint, may allow melanoma to resist the effect of anti-mitotics.[9,10] Given these characteristics, we hypothesized that compounds inducing arrest in the G1-phase of the cell cycle might prove to be effective for the treatment of certain melanomas (and other cancers).[11,12] Towards this end, we recently identified the triphenylmethylamides (TPMAs) as a novel class of molecules that induce G1-phase arrest and apoptosis in several human melanoma cell lines in culture.[11] Two representative TPMAs, **4A** and **4BI**, are shown in Figure 1. These compounds also reduce cellular levels of the transcription factor NFκB in melanoma cell lines in culture.[11] In addition, the compound **4BI** is relatively non-toxic against bone marrow cells from healthy human donors,[11] and [^{11}C]labeled TPMAs have been synthesized for positron emission tomography (PET) imaging.[13] While the functionality on the nitrogen side of the amide has little influence on the potency of the TPMAs (amides based on phenethylamine, 4-methoxy-phenylamine, and 2-(3-methoxy-phenyl)-ethylamine are all potent inducers of apoptosis), the triphenylmethyl moiety is critical to activity as the diphenylmethylamides are largely inactive.[11]

Herein, we report our effort to optimize the anticancer properties of the TPMAs and to further define their structure–activity relationship (SAR). This study was performed through synthesis



4A 4BI

Figure 1. The TPMAs **4A** and **4BI** were previously identified as potent inducers of cell death in human melanoma cell lines in cell culture.

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of derivatives with functionalized triphenylmethyl moieties and extensions of the carbon chain between the triphenylmethyl moiety and the amide. Various triphenylmethyl-containing carboxylic acids were prepared (as described in Supporting information) and coupled with phenethylamine or D-phenylalanine methyl ester using benzotriazol-1-yloxytripyrrolidinophosphonium hexafluorophosphate (PyBOP)-mediated coupling reactions (Chart 1).

The desired TPMAs were obtained in moderate to excellent yields (72% average yield). The compounds were named based on the triphenylmethyl (first number), the number of methylenes between the triphenylmethyl and the amide (second number), and whether they were derivatives of phenethylamine or D-phenylalanine (**A** or **BI**). The TPMA derivatives synthesized were evaluated in cell culture against two human melanoma cell lines, SK-MEL-5 and UACC-62. Cells were incubated with the compound (at a range of concentrations) for 72 h, at which time growth inhibition was assessed by quantification of biomass using the sulforhodamine B assay.[14] To obtain the IC_{50} values, the compounds were assessed on three different occasions and the average IC_{50} values and standard deviations were determined (see Supporting information for dose–response curves).

As shown in Chart 1, introduction of functional diversity in the triphenylmethyl group considerably affected the biological properties of the TPMAs. With the exception of **5-0-A**, hydroxyl-bearing TPMAs (such as **4-0-A**, **4-0-BI**, **6-0-A, 6-0-BI**, **7-0-A and 7-0-BI**) were less potent than the unfunctionalized TPMAs **4A** and **4BI** despite being more soluble in aqueous solution.

Methyl ether-substituted TPMAs, **1-0-A** and **1-0-BI**, were slightly less potent than the unfunctionalized TPMAs **4A** and **4BI**. Increasing the number of methyl ether substitutions reduced the potency as evidenced by **2-0-A**, **2-0-BI, 3-0-A**, and **3-0-BI**. TPMAs **9-0-A** and **9-0-BI** bearing conformationally rigid triphenylmethyl motifs were up to 4-fold less active than **4A** and **4BI**, highlighting the importance of flexibility in the triphenylmethyl motif. Replacement of one of the phenyls with a 2-thiophene (**8-0-BI** and **8-0-A**) resulted in a moderate decrease in potency. Curiously, increasing the length of the carbon chain between the triphenylmethyl and the amide reduced the potency against UACC-62 cells while the activity against SK-MEL-5 cells remained unchanged as observed for **10-1-BI**, **10-2-A**, **10-2-BI**, **10-3-A**, and **10-3-BI**.

It was of interest to determine whether the TPMA derivatives share a similar mode of action to the unfunctionalized TPMAs **4A** and **4BI**, as it was recently shown that anticancer compounds with the triphenylmethyl motif can be placed into at least four different categories based on their mechanisms of action, with position of cell cycle arrest being a major discriminator.[12] Thus, several of the most potent compounds identified herein were tested for their ability to induce cell cycle arrest. For this assay, the rapidly dividing HL-60 cell line (human leukemia) was utilized. These cells were treated with 20 μM compound for 6 h, after which the percentage cell population distributed in the various phases of the cell cycle was determined using cell flow cytometry and propidium iodide staining. As shown in Figure 2, the TPMAs **1-0-A**, **2-0-A**, **5-0-A**,

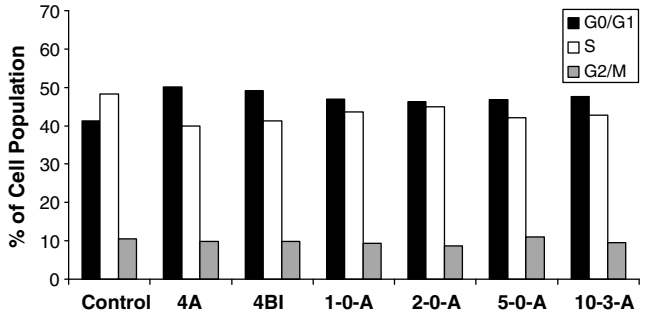
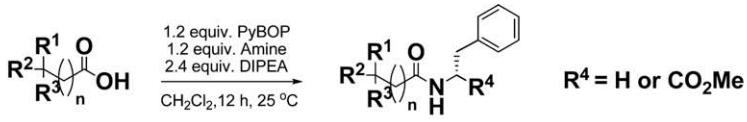


Figure 2. Cell cycle analysis of HL-60 cells treated with various TPMAs. Cells were incubated for 6 h in the presence of 20 μM compound, and then analyzed by propidium iodide staining and cell flow cytometry. Control indicates cells treated with vehicle only.



Compound	n	R^1	R^2	R^3	R^4	Yield (%)	SK-MEL-5 IC_{50} (μM)		UACC-62 IC_{50} (μM)	
***4A* (4BI)**	0	phenyl	phenyl	phenyl	*H* (CO_2Me)	*Ref 11* (Ref 11)	*5.8 ± 0.7*	(5.7 ± 1.7)	*8.3 ± 2.8*	(14 ± 2)
***1-0-A* (1-0-BI)**	0	4-methoxyphenyl	phenyl	phenyl	*H* (CO_2Me)	*81* (68)	*29 ± 13*	(6.1 ± 2.9)	*13 ± 8*	(27 ± 3)
***2-0-A* (2-0-BI)**	0	4-methoxyphenyl	4-methoxyphenyl	phenyl	*H* (CO_2Me)	*100* (75)	*14 ± 1*	(24 ± 1)	*20 ± 3*	(>100)
***3-0-A* (3-0-BI)**	0	4-methoxyphenyl	4-methoxyphenyl	4-methoxyphenyl	*H* (CO_2Me)	*98* (83)	*20 ± 4*	(49 ± 10)	*>100*	*(*>100)
***4-0-A* (4-0-BI)**	0	4-hydroxyphenyl	phenyl	phenyl	*H* (CO_2Me)	*57* (20)	*8.1 ± 1.0*	(8.1 ± 2.7)	*13 ± 5*	(21 ± 6)
5-0-A	0	3,4-dihydroxyphenyl	phenyl	phenyl	*H*	*45*	*4.2 ± 2.6*		*2.1 ± 1.2*	
***6-0-A* (6-0-BI)**	0	4-hydroxyphenyl	4-hydroxyphenyl	phenyl	*H* (CO_2Me)	*73* (71)	*8.6 ± 2.7*	(6.7 ± 1.9)	*47 ± 11*	*(*20 ± 2)
***7-0-A* (7-0-BI)**	0	4-hydroxyphenyl	4-hydroxyphenyl	4-hydroxyphenyl	*H* (CO_2Me)	*61* (42)	*36 ± 21*	(13 ± 5)	*71 ± 6*	(27 ± 6)
***8-0-A* (8-0-BI)**	0	2-thiophene	phenyl	phenyl	*H* (CO_2Me)	*89* (74)	*9.0 ± 3.3*	(12 ± 2)	*13 ± 5*	(21 ± 10)
***9-0-A* (9-0-BI)**	0	fluorenyl		phenyl	*H* (CO_2Me)	*99* (86)	*20 ± 6*	(23 ± 9)	*20 ± 2*	(27 ± 9)
***10-1-A* (10-1-BI)**	1	phenyl	phenyl	phenyl	*H* (CO_2Me)	*75* (37)	*17 ± 12*	(5.1±1.8)	*50 ± 16*	(>100)
***10-2-A* (10-2-BI)**	2	phenyl	phenyl	phenyl	*H* (CO_2Me)	*70* (92)	*5.5 ± 2.5*	(2.5 ± 0.9)	*50 ± 6*	(40 ± 5)
***10-3-A* (10-3-BI)**	3	phenyl	phenyl	phenyl	*H* (CO_2Me)	*83* (75)	*5.4 ± 3.6*	(5.8 ± 3.7)	*26 ± 2*	(45 ± 8)

Chart 1. Synthesis and biological evaluation of TPMA phenethylamine (**A**) and D-phenylalanine (**BI**) derivatives with functionalized triphenylmethyl groups and varying carbon chain lengths between the triphenylmethyl motif and amide. The values for phenethylamine derivatives are italicized while those for D-phenylalanine derivatives are in parentheses. Compounds **4A** and **4BI** were synthesized as previously reported.[11] Average IC_{50} values and standard deviations were determined from three independent experiments. Biomass was assessed by the sulforhodamine B assay after 72 h incubation in the presence of compound.

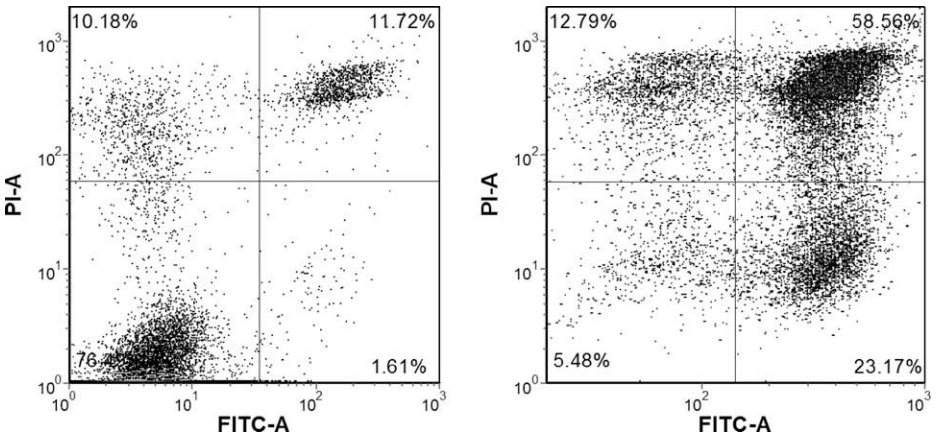


Figure 3. The TPMA **5-0-A** induces cell death via apoptosis. U-937 cells were treated with vehicle (left) or 100 μM **5-0-A** (right) for 48 h, and the cell populations were quantified using FITC-conjugated Annexin V (FITC-A) and propidium iodide (PI-A) staining together with cell flow cytometry.

and **10-3-A** all induce G1-phase arrest, similar to the parent TPMAs **4A** and **4BI**.

The TPMA **5-0-A**, which is slightly more potent than the unfunctionalized TPMAs **4A** and **4BI**, was further evaluated for its ability to induce apoptotic cell death as assessed by the externalization of phosphatidylserine that occurs prior to disruption of cell membrane integrity. Phosphatidylserine externalization was assessed by binding of FITC-conjugated Annexin V, and cell membrane integrity was assessed by exclusion of propidium iodide as measured by flow cytometry. As shown in Figure 3, U-937 human lymphoma cells treated for 48 h with 100 μM of TPMA **5-0-A** show a large shift to the early apoptotic quadrant (lower right; Annexin V positive/propidium iodide negative) indicating that **5-0-A**-induced G1-phase cell cycle arrest leads to apoptosis.

In order to assess whether the TPMAs **4A, 4BI**, **5-0-A**, and **10-2-BI** possess general anticancer properties, their ability to affect the viability of various cancer cell lines in cell culture was evaluated. As shown in Table 1, the TPMAs exhibited potent anti-proliferative activity against leukemia, lymphoma, and breast cancer cell lines in addition to melanoma. In contrast, the rat adrenal cancer PC-12 cell line was found to be fairly resistant to the effects of the TPMAs with **5-0-A** being the most potent (IC_{50} = 16 ± 4 μM).

Triphenylmethylamides (TPMAs) show promise as anticancer agents against human melanoma and other cancer cell lines in culture. Introduction of 3,4-dihydroxy functionality on the triphenylmethyl moiety modestly increased the potency but also increased the aqueous solubility; the solubility of compound **5-0-A** in phosphate-buffered saline (PBS, pH 7.4) is approximately 80 μM, compared to approximately 20 μM for **4BI**. Compounds having an extension of the carbon chain length between the triphenylmethyl motif and the amide, such as **10-2-BI**, showed similar abilities in inducing cell death in SK-MEL-5 cells as **4A** and **4BI**. This work adds to a growing body of literature showing that a variety of compounds possessing the triphenylmethyl group induce death in cancer cells, albeit by diverse mechanisms.[11,12,15–22] While the biological target of the TPMAs remains unknown, the structure–activity relationship described herein may prove useful for further optimization and development of triphenylmethyl-containing anticancer agents.

Table 1
Evaluation of TPMAs **4A**, **4BI**, **5-0-A** and **10-2-BI** against various cancer cell lines in culture

Cell line	Cancer type	**4A** (IC_{50}, μM)	**4BI** (IC_{50}, μM)	**5-0-A** (IC_{50}, μM)	**10-2-BI** (IC_{50}, μM)
SK-MEL-5	Melanoma (human)	5.8 ± 0.7	5.7 ± 1.7	4.2 ± 2.6	2.5 ± 0.9
UACC-62	Melanoma (human)	8.3 ± 2.8	14 ± 2	2.1 ± 1.2	40 ± 5
HL-60	Leukemia (human)	7.7 ± 1.6	6.2 ± 0.5	7.3 ± 1.2	6.0 ± 1.5
U-937	Lymphoma (human)	9.5 ± 1.1	5.9 ± 0.3	7.2 ± 1.8	6.8 ± 3.2
MCF-7	Breast (human)	7.1 ± 1.0	8.1 ± 1.1	8.0 ± 1.4	6.1 ± 1.0
PC-12	Adrenal (rat)	31 ± 11	85 ± 14	16 ± 4	74 ± 17

Cells were incubated in the presence of compound for 72 h and cell growth/viability was assessed as described in Supporting information. Average IC_{50} values and standard deviations were determined from three independent experiments.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2008.07.128.

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