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A Promising Approach to Target Colorectal Cancer Using Hybrid Triarylmethanes

Ameni Hadj Mohamed,^[a, b] Christophe Ricco,^[a] Aline Pinon,^[c] Nathalie Lagarde,^[a] Elizabeth Goya-Jorge,^[d] Hadley Mouhsine,^[e] Moncef Msaddek,^[b] Bertrand Liagre,^{*,[c]} and Maité Sylla-Iyarreta Veitia^{*,[a]}

Aiming to create an innovative series of anti-colorectal cancer agents, we designed in this work hybrid triarylmethane compounds. Three hybrid triarylmethanes and their corresponding *N*-oxide analogues were successfully synthesized using an efficient procedure that involved connecting two triarylmethane molecules, through mono-, bi-, and triethylene glycol fragments. In our pursuit to develop more soluble molecules, we synthesized a hybrid triarylmethane featuring a lysine-based spacer through a convergent strategy involving 7 steps. All hybrid compounds were assessed for their antiproliferative activity on human HT-29 and HCT116 colorectal cancer (CRC) cell lines. Three pyridine *N*-oxide analogs demonstrated

notable antiproliferative potential among the set of tested compounds, with IC₅₀ values ranging from 18 to 24 μM on both human CRC cell lines analyzed. A cytotoxicity study conducted on murine fibroblasts revealed that these three active compounds were not toxic at the IC₅₀ values, indicating their suitability for further drug development. A docking study was conducted on two representative compounds, one for each series and protein kinase B (AKT) was identified as a potential target of their in anti-cancer effects. A computational drug-likeness study predicted favourable oral and intestinal absorption efficiency.

1. Introduction

In drug discovery, molecular hybridization stands as a prominent strategy, that involves the combination of two or more pharmacophoric fragments, each carrying distinct functionalities within a molecular structure. This approach paves the way for the creation of multifunctional hybrid molecules exhibiting enhanced biological activity.^[1–4] The strategy of developing

hybrid molecules breaks away from traditional monotherapy towards the adoption of dual and multi-target therapy strategies.^[5] By incorporating multiple pharmacophore moieties, this method allows for multiple biological activities, modified selectivity profiles, varied modes of action, and the potential generation of drug candidates for the treatment of multifactorial diseases such as cancer, Alzheimer's disease, AIDS, malaria, and cardiovascular diseases.^[6–8] Indeed, several examples in the literature emphasize the capacity of hybridization to improve biological activity and enhance multi-targeting in comparison to non-hybrid compounds.^[5,9–12]

Triarylmethane (TAM) molecules are renowned for their distinctive structural scaffolds, characterized by worthwhile biological properties. Indeed, some of these compounds have been successfully used for therapeutic applications.^[13–19] The noteworthy biological activities of TAMs have positioned them as attractive targets in medicinal chemistry.^[20] Some TAMs are broadly described in the literature as anti-infectious,^[21–27] anti-inflammatory^[28,29] and anti-cancer agents.^[14,18,19,30–33] Recognizing their paramount importance, our research efforts have been consistently dedicated to the exploration of these compounds for years.^[34,35]

Colorectal cancer (CRC) is a significant global health burden, with its incidence steadily rising over recent decades. According to the World Health Organization (WHO), CRC is the third most commonly diagnosed cancer worldwide and the second leading cause of cancer-related deaths, claiming over 900,000 lives annually.^[36–39] This malignancy primarily affects the colon and rectum, with risk factors including age, family history, dietary habits, and lifestyle factors such as smoking and physical inactivity.^[40] Despite the advancements in early detection and treatment modalities, CRC remains a serious threat, requiring

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ongoing research efforts to develop more effective therapeutic strategies.

Current therapeutic approaches for CRC typically involve a combination of surgery, chemotherapy, radiotherapy, and targeted therapies. In chemotherapy, drugs such as 5-fluorouracil (5-FU), trifluoridin, irinotecan, oxaliplatin and leucovorin are used as monotherapy or combination therapy in the treatment of different stages of CRC (Figure 1).

Regarding targeted therapies, two categories of monoclonal antibodies have been approved by the Food and Drug Administration (FDA), including vascular endothelial growth factor (VEGF) inhibitors like bevacizumab (Avastin®), and epidermal growth factor receptor (EGFR) inhibitors such as cetuximab (Erbix®) and panitumumab (Verbitix®). While these therapeutic approaches have improved patient outcomes and survival rates, challenges such as treatment resistance, toxicity, and disease recurrence persist.^[38,41] Therefore, there is an urgent need to explore and develop new therapeutic strategies to address these limitations.

Building on our longstanding interest in TAMs, we have developed in this work hybrid compounds by linking two TAM units with pegylated or peptide linkers, aiming to achieve interesting anti-CRC activity and improved physicochemical properties.

2. Results and Discussion

2.1. Synthesis of Hybrid Triarylmethanes

The synthesis of hybrid triarylmethanes followed a three-step sequence involving the synthesis of the TAM skeleton, the linker synthesis, and finally the coupling of TAM with the linker.

First, mono, di and triethylene glycols **1a–c** were tosylated using tosyl chloride **2** in the presence of trimethylamine in THF

at room temperature. Ditosylated PEG **3** ($n = 1, 2$, and 3) were obtained in 62 %, 70 %, and 76 % yields, respectively.

Pegylated hybrid TAMs were synthesized by a substitution reaction between the tosylated PEG and TAM **4**, previously prepared according to the literature.^[34,42] The reaction performed in the presence of K_2CO_3 in DMF at 80 °C, successfully led to the corresponding hybrids TAMs **5a–c** in good yields of 85 %, 73 % and 85 % respectively (Scheme 1).

The introduction of the *N*-oxide moiety has been highly considered in medicinal chemistry programs. *N*-oxides considerably improve the physicochemical properties such as solubility and the affinity with some receptor sites.^[29,43–46] Therefore, pegylated hybrid TAMs **5a–5c** were *N*-oxidized using *m*-chloroperbenzoic acid (*m*-CPBA) in dichloromethane at room temperature using experimental conditions previously standardized in our laboratory.^[47] The expected *N*-oxides TAMs **6a–6c** were obtained in good yields between 60 % and 74 % (Scheme 1).

Lysine residues, known for their versatility, play an important role in medicinal chemistry. They assist targeted drug delivery, enhance protein-drug interactions, and can improve drug pharmacokinetics. They are key components in designing multivalent ligands for complex diseases. Moreover, lysine-rich sequences within cell-penetrating peptides facilitate intracellular drug delivery.^[48–52]

The hybrid compound containing a bis-lysine linker was synthesized using a convergent strategy. First, the synthesis of TAM fragment by an alkylation reaction was carried out using 4-((4-methoxyphenyl)(pyridin-2-yl)methyl)phenol **4** and dibromomethane in the presence of K_2CO_3 and KI in acetonitrile leading to product **7** in 73 % yield. Then, the aminated TAM **8** was prepared by Gabriel reaction using phthalimide in dimethylformamide (DMF) at 70 °C. After hydrazine treatment in ethanol, the TAM **8** was obtained in 85 % yield (Scheme 2).^[53]

Secondly, the synthesis of the amino acid linker started with protection of the carboxylic acid group of the (L)-Fmoc-Lys-Boc-

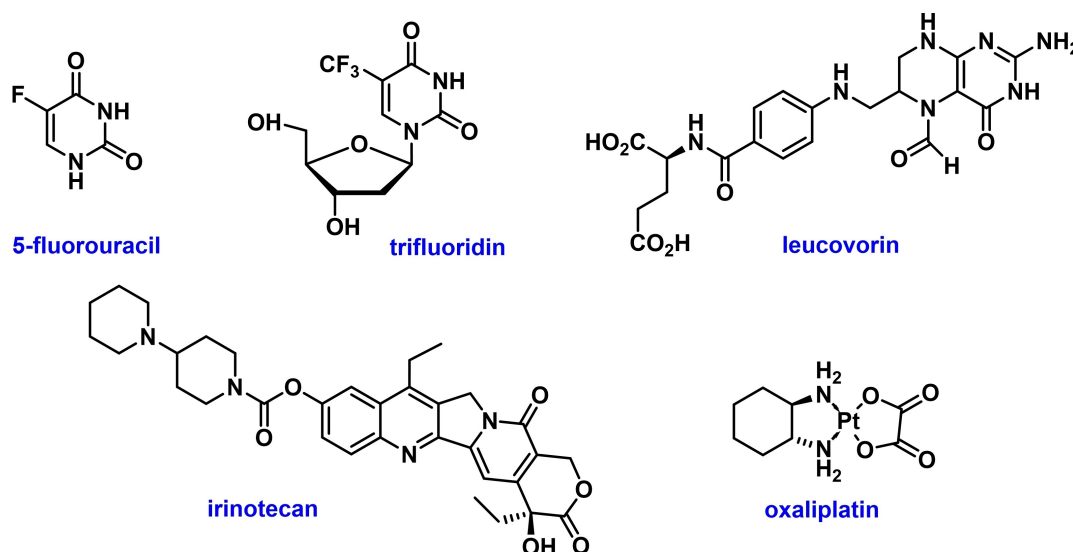
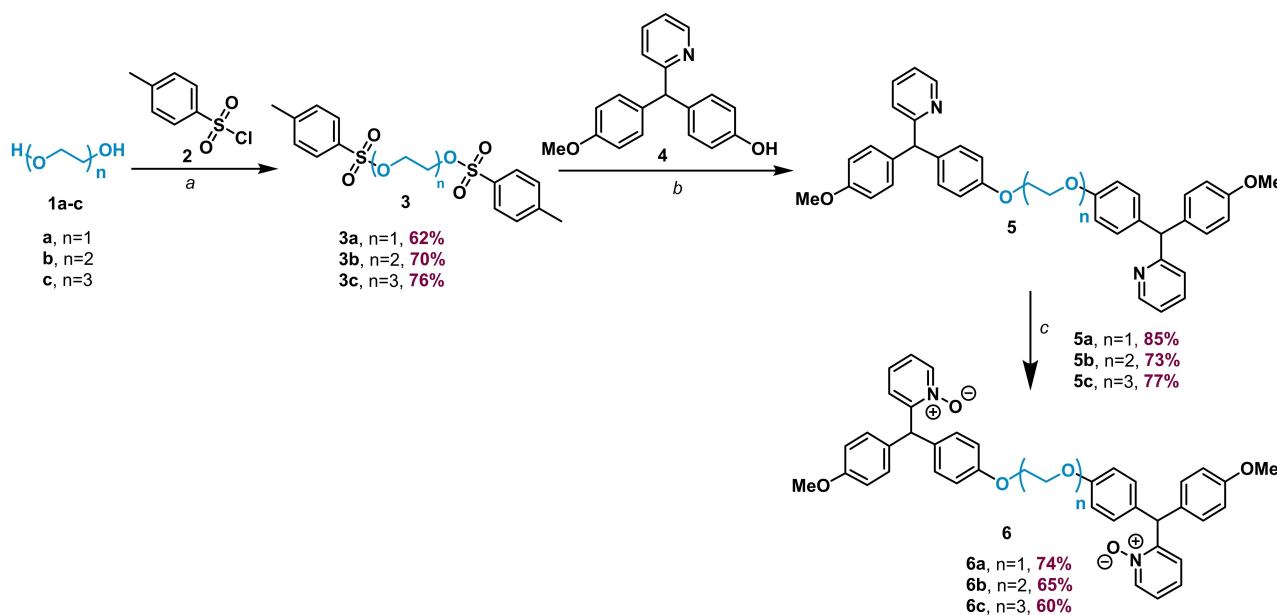
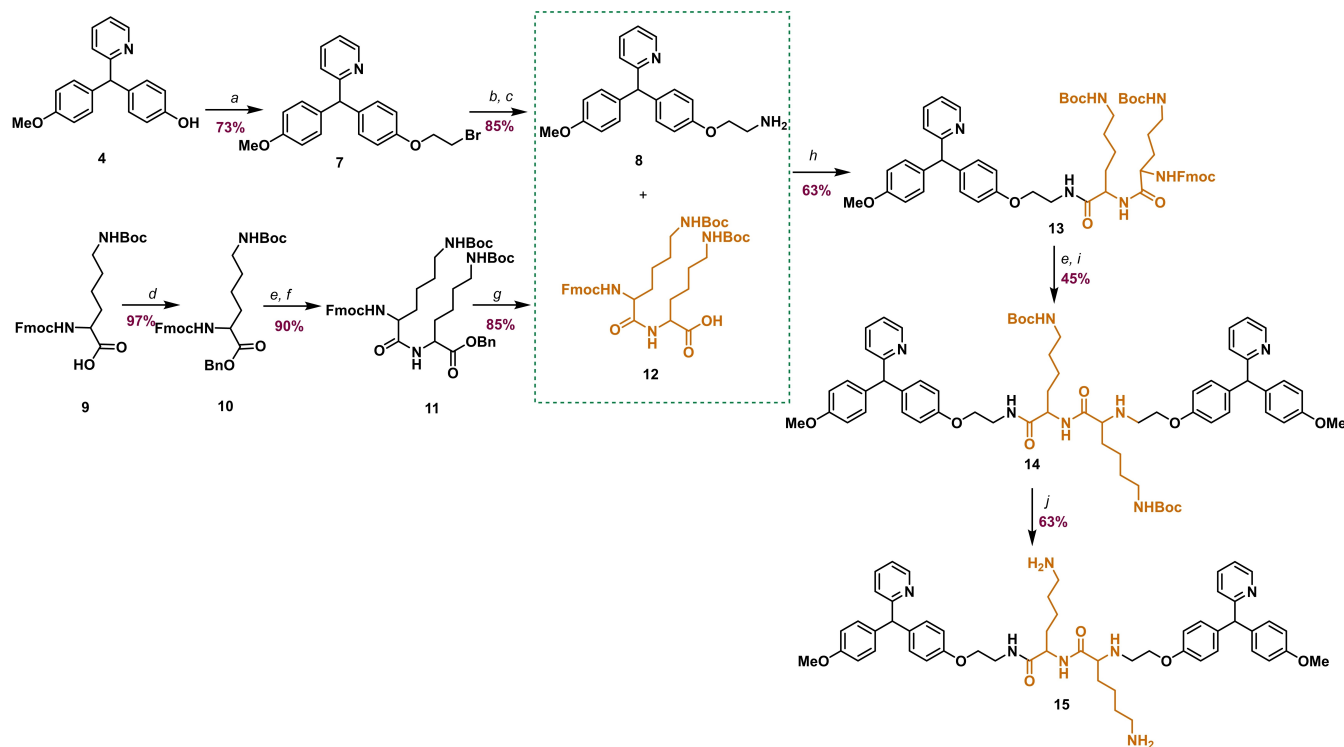


Figure 1. Small molecules used in first- or second-line chemotherapy for the CRC treatment.



Scheme 1. Synthesis of hybrid PEG TAM a) NEt_3 , THF, r.t.; b) K_2CO_3 , DMF, 80°C ; c) *m*-CPBA, DCM, r.t.



Scheme 2. Synthesis of dipeptide linked hybrid TAMs a) dibromoethane, K_2CO_3 , KI, ACN, reflux; b) potassium phthalimide, DMF, 70°C ; c) hydrazine, ethanol, 0°C to r.t.; d) BnBr, K_2CO_3 , DMF, r.t.; e) piperidine, DMF, r.t.; f) Fmoc-Lys(Boc)-OH, EDCI, HOBT, DCM, r.t.; g) Et_3SiH , Pd/C 10%, MeOH, r.t.; h) EDCI, HOBT, DCM, r.t.; e) piperidine, DMF, r.t.; i) triethylmethane 7, K_2CO_3 , KI, acetone, 60°C ; j) TFA, r.t.

OH **9** to the corresponding benzyl ester **10** in 97% yield.^[54] The treatment of (L)-Fmoc-Lys-Boc-OBn **10** with piperidine in DMF allows the deprotection the amine group which through peptide coupling with a lysine using hydroxybenzotriazole (HOBT) and ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDCI) leads to the dipeptide **11** in excellent yield of 90%.^[55] Benzyl

ester group of compound **11** was then deprotected by hydrogenolysis reaction using triethylsilane in the presence of Pd/C in methanol which successfully afforded compound **12** in 85% yield (Scheme 2).

To prepare the hybrid compound **14**, TAM **8** was coupled with bis-lysine linker **12** in the presence of EDCI/HOBT giving

the expected compound **13** in 63% yield. After Fmoc deprotection of compound **13**, the resulting TAM was subsequently reacted with a second triarylmethane unit **7** in a nucleophilic substitution reaction in the presence of K_2CO_3 and KI in acetone. The expected TAM-bi-lysine-TAM hybrid **14** was obtained in 45% yield. Finally, the deprotection of Boc groups using trifluoroacetic acid led to the expected product **15** in 63% yield.

Some studies have demonstrated that TAM can degrade in the presence of light, oxygen, heat, and humidity. Some research has specifically described the photooxidative cleavage of the central C-phenyl bond by singlet oxygen attack.^[56–58] We were interested in assessing the stability of hybrid compounds **5a–c** and their *N*-oxide analogs **6a–c** in deuterated DMSO, a solvent commonly used in biological studies. Solutions of the corresponding TAM in DMSO were placed in NMR tubes and kept at room temperature for 1 hour, then evaluated over 5 weeks. Stability was monitored by NMR spectroscopy by measuring the intensity of the singlet of the vinyl proton H1 of each TAM. No modifications were observed after 5 weeks (see Supplementary Data). These results indicate that TAMs are stable either in solid or oily state, or in solution.

2.2. Evaluation of Anticancer Activity

A series of synthesized TAM hybrids was *in vitro* tested to evaluate their potential as anticancer agents. An initial screening was carried out using human HT-29 and HCT116 cell lines with 7 hybrid molecules. According to the obtained results, the most promising candidates were selected for additional *in vitro* and *in silico* investigations.

2.2.1. Evaluation of the Antiproliferative Activity

The newly synthesized TAM hybrids were assessed for their *in vitro* anticancer activity against human CRC cells HT-29 and HCT116, following the established procedures outlined in our previous work.^[47,59] First, six TAM hybrids (**5a–c**) and (**6a–c**) and the TAM hybrid featuring a lysine-based spacer (**15**) were screened for their induced impact on cell viability using the 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assay at a concentration of 50 μ M over 48 h. The hybrid compounds, **5a–c**, did not show any important inhibition of cell viability at concentrations below 50 μ M. Conversely, their respective *N*-oxide analogs (**6a–c**) exhibited significant inhibition of cell viability in both HT-29 and HCT116 CRC cell lines (Figure 2). Subsequently, compounds **6a–c** were evaluated in a dose-response assay on both human CRC cell lines to determine their median inhibitory concentration (IC_{50}) at concentrations ranging from 1 to 50 μ M (1, 10, 20, 30, 40, and 50 μ M) over a 48 h incubation period. Among the most frequently used conventional chemotherapy drugs, particularly for treating CRC, 5-FU and irinotecan were chosen as positive controls,^[60] and their anticancer activity was evaluated following the same procedure (Table 1).

The inhibition of cell proliferation induced by the synthesized hybrid TAM enabled some structure–activity relationship analysis. Compounds **5a–c** did not show inhibition of cell proliferation at concentrations below 50 μ M. These findings align with prior results reported for some PEG derivatives of TAM within our research group.^[26] Furthermore, we observed that the length of the pegylated chain has no influence on the

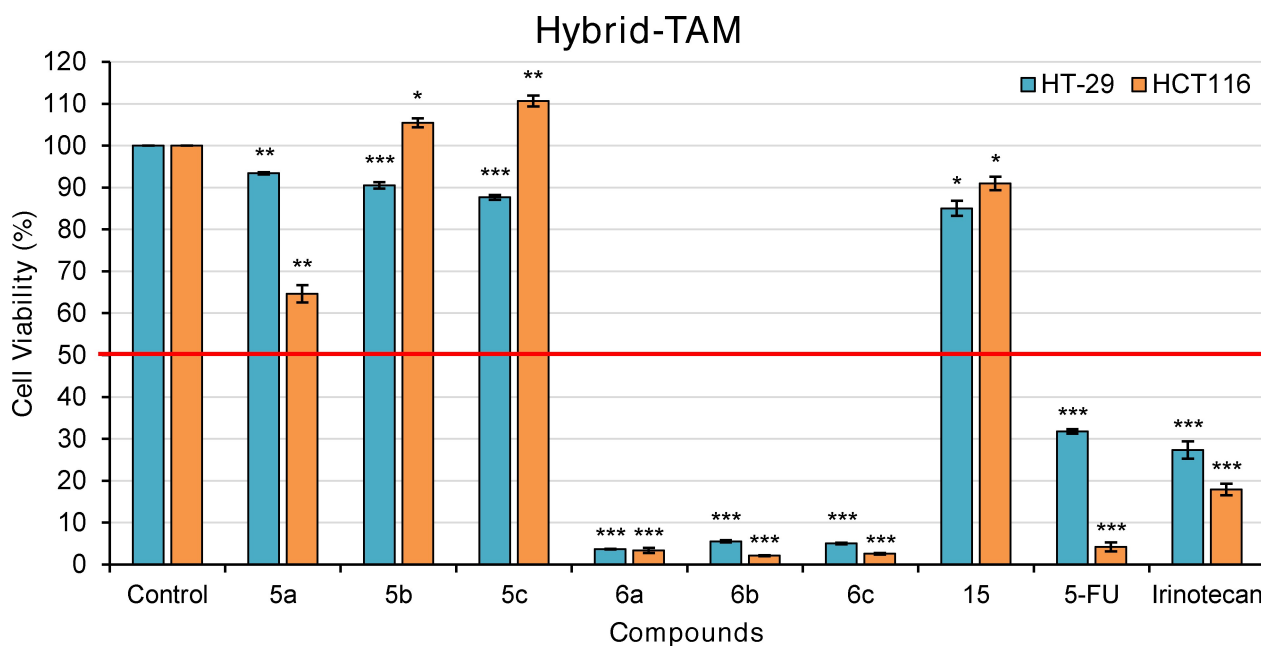
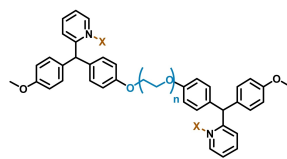
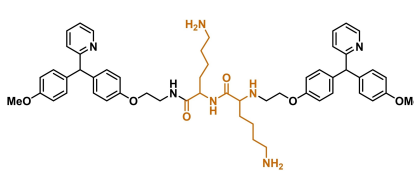


Figure 2. Screening of TAM hybrids on human HT-29 and HCT116 CRC cell lines viability. Cell viability (%) after exposure to hybrid compounds (**5a–c**, **6a–c** and **15**), as well as 5-FU and irinotecan used as reference compounds, was measured by MTT assay. All compounds were assayed at 50 μ M for 48 h. Each chart represents the mean percentage \pm SEM from at least three independent experiments. Cell viability lower than 50% was considered cytotoxic. * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ significantly different from the control.

Table 1. Half-maximal inhibitory concentration (IC_{50}) values of synthesized hybrid TAM compounds against the human CRC cell lines HT-29 and HCT116.

Structure	n	X	ID	$IC_{50} \pm SEM$ (μM) ^[a]	
				HT-29	HCT116
	n=1	–	5a	> 50	> 50
		X=O	6a	18.32 ± 1.34	23.55 ± 0.96
	n=2	–	5b	> 50	> 50
		X=O	6b	22.52 ± 0.78	24.52 ± 0.77
	n=3	–	5c	> 50	> 50
		X=O	6c	23.43 ± 0.72	24.69 ± 0.86
			15	> 50	> 50
5-FU				17.29 ± 1.03	2.55 ± 0.47
irinotecan				30.50 ± 1.24	18.78 ± 0.94

[a] Cells were treated with different concentrations of the compounds for 48 h and IC_{50} values (μM) are indicated as the mean \pm SEM of at least three independent experiments. Cell viability was measured by the MTT assay as described in the Experimental section. 5-FU and irinotecan were used as reference compounds.

antiproliferative activity of the tested compounds and the IC_{50} values consistently remain comparable.

The introduction of *N*-oxide moiety increases dramatically the antiproliferative activity against HT-29 and HCT116 CRC cell lines. Compounds **6b** and **6c** exhibit comparable effects on both CRC cell lines. The best results were obtained with compound **6a** with IC_{50} 18 μM for HT-29 cells and 23 μM for HCT116 cells (Table 1). These results suggest the substantial positive impact of *N*-oxide moiety on the antiproliferative activity. Interestingly, the IC_{50} of compound **6a** on HT-29 cells was similar to that of 5-FU and even lower than that of irinotecan, while it remained higher than the reference compounds for HCT116 cells. Finally, the hybrid TAM **15** bearing a peptide linker did not exhibit antiproliferative activity in either of the human CRC cell lines evaluated.

2.2.3. Non-cancerous Cell Line Viability

The MTT colorimetric assay was employed to assess the cell viability of the L929 cell line (murine fibroblasts) in the presence of the hybrid TAM synthesized. The emphasis was placed on showcasing the selective effectiveness of hybrid TAMs against cancer cells. Overall, these findings revealed that the compounds demonstrated negligible toxicity towards normal cell lines, as evidenced by cell survival percentages consistently hovering around 100%.

The hybrid TAMs featuring a PEG moiety **5a–c** did not demonstrate any cytotoxicity with cell survival rates consistently reaching 100%. Meanwhile, the *N*-oxide analogs **6a–c** showed a slight cytotoxic effect at high concentrations (50 to 100 μM). None of the compounds exhibited toxicity at concentrations

equal to or below 25 μM . The corresponding cell survival percentage are shown in the supplementary data section (SI-II).

2.3. In Silico Studies

2.3.1. Molecular Modeling

In order to study the impact of the *N*-oxidation in the biological interactions, we performed a docking study on one hybrid TAM (compound **5a**) and its corresponding *N*-oxide analog (compound **6a**). The predicted binding mode of compounds **5a** and **6a** in the 8 investigated potential targets: Protein kinase B (AKT), Extracellular signal-regulated kinases (ERK-1, ERK-2), poly-ADP-ribose-polymerase (PARP), Mitogen-activated protein kinases (MAPK11, MAPK13, MAPK14) and caspase-3 were obtained using a protein-ligand docking approach. Analyzing the predicted binding modes of compounds **5a** and **6a** in potential targets enabled us to identify the AKT protein as the most promising biological target. Interestingly, we obtained an identical outcome in a previous study conducted with another series of TAM compounds.^[61] Both compounds exhibit a predicted binding mode very similar to that of the native bound ligand in the AKT PDB structure (Figure 3A). They aligned well within the AKT binding site with the native ligand, displaying identical hydrophobic interactions with AKT residues (W80, I84, L210, L264, V270, Y272, D292) and maintaining a π -stacking interaction with W80. The native bound ligand in the AKT PDB structure forms a hydrogen bond with residue K297, an interaction similarly observed only with compound **5a** (Figure 3B). Both compounds **5a** and **6a** form two additional hydrogen bonds, one with residue R273, and the other respectively with N54 and W80. Additionally, while both the

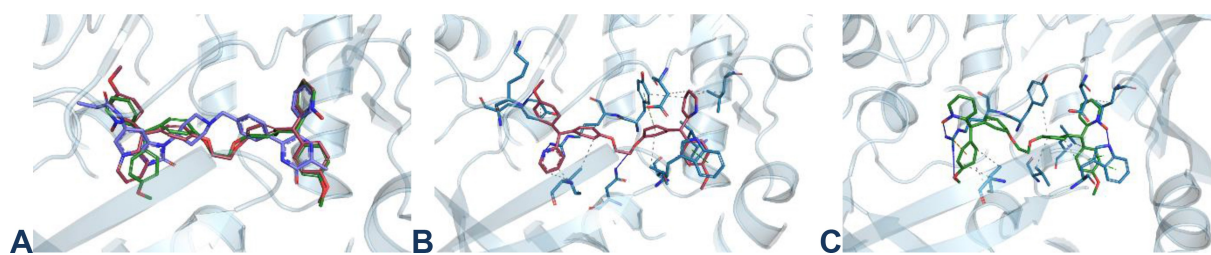


Figure 3. Predicted binding modes of compounds **5a** (in claret) and **6a** (in green) in the AKT binding site (PDB ID: 6S9W) (A) in comparison with the 6S9W co-crystallized ligand (in blue) with detailed information about the interactions established between compounds **5a** (B) and **6a** (C) in the AKT binding site obtained using PLIP (plain line blue: HB; green dotted lines: π -stacking; grey dotted lines: hydrophobic, orange dotted lines: π -cation).

native bound ligand and compound **6a** interact with residue R273 via a π -cation interaction (Figure 3C), this interaction is absent in compound **5a** predicted binding mode. If AKT is confirmed as the experimental target of the presented compounds, the π -cation interaction could elucidate the impact of the *N*-oxide moiety on their biological activity.

2.3.2. ADME Profile and Drug-likeness

Predictive results of the ADME properties and drug-likeness of the three hybrid TAMs (**6a**, **6b** and **6c**) that demonstrated a

potential antiproliferative activity in cancer cell lines are presented in Table 2.

The three studied hybrid TAMs (**6a**, **6b**, **6c**) were anticipated to possess significant membrane-crossing capabilities owing to their hydrophobic nature, as evidenced by the results of the Caco-2 and MDCK cell permeabilities, brain/blood partition coefficient, and human oral absorption models, shown in Table 2. However, their poor water solubility poses a considerable challenge. Thus, implementing innovative solutions such as nanoformulations, prodrug design, pH-dependent formulations, as previously proposed,^[26] may be imperative to improve solubility, stability, and efficacy in subsequent formulation stages.

Table 2. ADME properties and drug-likeness of hybrid TAMs predicted using QuikProp-Maestro software.

Parameter	Description	Recommended values	ADME predictions Hybrid TAMs		
			6a	6b	6c
dipole	Dipole moment of the molecule	1.0–12.5	7.843	9.696	12.592
Ppolrz	Predicted polarizability (cubic angstroms)	13.0–70.0	70.618	68.456	80.092
PSA	Van der Waals surface area of polar nitrogen and oxygen atoms and carbonyl carbon atoms.	7.0–200.0	65.725	80.067	92.396
SASA	Total solvent accessible surface area (SASA) (square angstroms)	300–1000	1051.294	971.104	1240.242
donorHB	Estimated hydrogen bonds donated by the solute to water molecules in an aqueous solution.	0.0–6.0	0	0	0
accptHB	Estimated number of hydrogen bonds that would be accepted by the solute from water molecules in an aqueous solution.	2.0–20.0	5	6.7	8.4
PlogPo/w	Predicted octanol/water partition coefficient.	–2.0–6.5	10.138	9.331	10.515
PlogS	Predicted aqueous solubility.	–6.5–0.5	–11.405	–8.507	–12.228
PPCaco	Predicted apparent Caco-2 cell permeability (nm/sec).	< 25 poor > 500 great	3700.783	3605.446	2388.584
PlogBB	Predicted brain/blood partition coefficient.	–3.0–1.2	–0.803	–0.899	–1.532
QPPMDCK	Predicted apparent MDCK cell permeability (nm/sec).	< 25 poor > 500 great	2035.205	1978.594	1267.872
Percent Human Oral Absorption	Predicted human oral absorption on 0 to 100% scale. The prediction is based on a quantitative multiple linear regression model.	> 80% is high < 25% is poor	100	100	100
QPlogKp	Predicted skin permeability, log K_p .	–8.0––1.0	1.513	1.162	1.727
Rule Of Five ^[a]	Number of violations of Lipinski's rule of five	Max 4	2	2	2
Rule Of Three ^[b]	Number of violations of Jorgensen's rule of three.	Max 3	2	2	2

[a] Lipinski's Rules: mol MW < 500, QPlogPo/w < 5, donorHB \leq 5, acctpHB \leq 10. Compounds that satisfy these rules are considered drug-like. (The "five" refers to the limits, which are multiples of 5).
[b] Jorgensen's Rules: QPlogS > –5.7, QP PCaco > 22 nm/s, # Primary Metabolites < 7. Compounds with fewer (and preferably no) violations of these rules are more likely to be orally available.

3. Experimental Section

3.1. Chemistry

All reagents were obtained from commercial sources unless otherwise noted and used as received. Heated experiments were conducted using thermostatically controlled heating mantles and were performed under an atmosphere oxygen-free in oven-dried glassware when necessary. Analytical Thin Layer Chromatography (TLC) monitored the reactions. TLC was performed on aluminum sheets precoated silica gel plates (60 F254, Merck). TLC plates were visualized using irradiation with light at 254 nm. Flash column chromatography (FCC) was carried out when necessary, using silica gel 60 (particle size 0.040–0.063 mm, Merck).

Melting points were recorded on a Kofler hot block Heizbank type 7841 and were uncorrected. The structures of the products were checked by comparison of NMR, IR and MS data and by the TLC behavior. ^1H and ^{13}C -NMR spectra were recorded on a Bruker BioSpin GmbH spectrometer 400 MHz, at room temperature. Chemical shifts are reported in δ units, parts per million (ppm). Coupling constants (J) are measured in hertz (Hz). Splitting patterns are designed as follows: s, singlet; d, doublet; dd, doublet of doublets; m, multiplet; t, triplet; td, triplet of doublet; ddd: doublet of doublet of doublet. DEPT experiments and various 2D techniques such as COSY, HSQC and HMBC were used to establish the structures and to assign the signals. GC-MS analyses were performed with an Agilent 689 0 N instrument equipped with a dimethyl polysiloxane capillary column (12 m \times 0.20 mm) and an Agilent 5973N MS detector-column temperature gradient 80–300 °C (method 80): 80 °C (1 min); 80 °C to 300 °C (12.05 °C/min); 300 °C (2 min). Low resolution mass spectra (ESI-LRMS) were performed from ionization by electrospray on a Waters Micromass ZQ2000. Infrared spectra were recorded over the 400–4000 cm^{-1} range with an Agilent Technologies Cary 630 FTIR/ATR/ ZnSe spectrometer. High-resolution mass spectra (HRMS) analyses were acquired on a Thermo Scientific LTQ Orbitrap mass spectrometer.

3.1.1 1,2-bis(4-((4-methoxyphenyl)(pyridin-2-yl)methyl)phenoxy)ethane 5a

To a solution of TAM 7 (472 mg; 1.62 mmol; 2.0 eq.) in anhydrous DMF (5 mL) was added K_2CO_3 (560 mg; 4.05 mmol; 5.0 eq.). The mixture was stirred at 80 °C for 1 h, under argon. Then a solution of ethyleneglycol ditosylate 4a (300 mg, 0.81 mmol, 1.0 eq.) in dry DMF (5 mL) was added dropwise and the mixture was stirred at 80 °C for 24 h. The mixture was diluted with water (100 mL) and extracted with EtOAc (3 \times 100 mL). The combined organic layer was washed twice with brine (100 mL). Then dried over MgSO_4 , filtered and the solvent was removed in a vacuum. The crude product was purified by FCC on silica gel (MeOH/DCM 99:1; 98.5:1.5) to give the desired compound 5a (321 mg; 85%) as an off-white amorphous solid. The starting material 7 was also recovered (111 mg). TLC: (DCM/MeOH (98:2)), R_f : 0.33 ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ (ppm): 8.51–8.53 (m, 2H, H12), 7.7 (td, 2H, $J_{10-11, 10-9}=7.7$ Hz, $J_{10-12}=1.9$ Hz, H10), 7.19–7.21 (m, 4H, H9, H11), 7.08–7.12 (m, 8H, H3, H7, H15, H19), 6.83–6.91 (m, 8H, H4, H6, H16, H18), 5.55 (s, 2H, H1), 4.24 (s, 4H, H20), 3.70 (s, 6H, H13). ^{13}C NMR (100 MHz, $\text{DMSO}-d_6$) δ (ppm): 163.1 (C8), 157.6 (C17), 156.7 (C5), 149.1 (C12), 136.7 (C10), 135.7 (C2), 135.4 (C14), 129.9 (C3, C7, C15, C19), 123.4 (C9), 121.5 (C11), 114.2 (C16, C18), 113.6 (C4, C6), 66.3 (C20), 56.5 (C1), 55.0 (C13). IR ν (cm^{-1}): 3043, 3002 ($\nu_{\text{Csp}2-\text{H}}$); 2931 ($\nu_{\text{Csp}3-\text{H}}$); 2859 (ν_{OMe}); 1608, 1585, 1507 and 1462 ($\nu_{\text{C}-\text{C}}$); 1236 ($\nu_{\text{asym C}-\text{O}-\text{C}}$); 1174 ($\nu_{\text{sym C}-\text{O}-\text{C}}$); 748 ($\delta_{\text{Csp}2-\text{H p-substitution}}$). LRMS: (ES $^+$, CV=30): 609.25 [M+H] $^+$; 631.23 [M+Na] $^+$. HRMS (ESI) m/z: [M+H] $^+$ Calcd for $\text{C}_{40}\text{H}_{36}\text{N}_2\text{O}_4\text{H}$ 609.2748. Found 609.2748.

3.1.2 2,2'-(((oxybis(ethane-2,1-diyl))bis(oxy))bis(4,1-phenylene))bis((4-methoxyphenyl)methylene) dipyridine 5b

To a solution of TAM 7 (422 mg; 1.44 mmol; 2.0 eq.) in anhydrous DMF (5 mL) was added K_2CO_3 (500 mg; 3.62 mmol; 5.0 eq.). The mixture was stirred at 80 °C for 1 h, under argon. Then a solution of diethyleneglycol ditosylate 4b (300 mg; 0.72 mmol, 1.0 eq.) in dry DMF (5 mL) was added dropwise and the mixture was stirred at 80 °C for 24 h. The mixture was diluted with water (100 mL) and extracted with EtOAc (3 \times 100 mL). The combined organic layer was washed twice with brine (100 mL). Then dried over MgSO_4 , filtered and the solvent was removed under a vacuum. The crude product was purified by flash chromatography on silica gel (MeOH/DCM: 99/1) to give the desired compound 5b (270 mg; 73%) as a yellow oil. The starting material 7 was also recovered (90 mg). TLC: (DCM/MeOH (98:2)), R_f : 0.29 ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ (ppm): 8.50–8.52 (m, 2H, H12), 7.7 (td, 2H, $J_{10-11, 10-9}=7.7$ Hz, $J_{10-12}=1.9$ Hz, H10), 7.18–7.22 (m, 4H, H9, H11), 7.05–7.10 (m, 8H, H3, H7, H15, H19), 6.82–6.86 (m, 8H, H4, H6, H16, H18), 5.53 (s, 2H, H1), 4.04–4.06 (m, 4H, H20), 3.76–3.78 (m, 4H, H21), 3.70 (s, 6H, H13). ^{13}C NMR (100 MHz, $\text{DMSO}-d_6$) δ (ppm): 163.1 (C8), 157.6 (C17), 156.8 (C5), 149.1 (C12), 136.7 (C10), 135.5 (C2), 135.4 (C14), 129.9 (C3, C7, C15, C19), 123.4 (C9), 121.5 (C11), 114.2 (C16, C18), 113.6 (C4, C6), 69.0 (C21), 67.1 (C20), 56.5 (C1), 55.0 (C13). IR ν (cm^{-1}): 3049, 3002 ($\nu_{\text{Csp}2-\text{H}}$); 2930 ($\nu_{\text{Csp}3-\text{H}}$); 2876 (ν_{OMe}); 1608, 1585, 1508 and 1462 ($\nu_{\text{C}-\text{C}}$); 1241 ($\nu_{\text{asym C}-\text{O}-\text{C}}$); 1175 ($\nu_{\text{sym C}-\text{O}-\text{C}}$); 750 ($\delta_{\text{Csp}2-\text{H p-substitution}}$). LRMS: (ES $^+$, CV=30): 653.28 [M+H] $^+$; 675.28 [M+Na] $^+$. HRMS (ESI) m/z: [M+H] $^+$ Calcd for $\text{C}_{42}\text{H}_{40}\text{N}_2\text{O}_5\text{H}$ 653.3010. Found 653.3009.

3.1.3 1,2-bis(2-(4-((4-methoxyphenyl)(pyridin-2-yl)methyl)phenoxy)ethoxy)ethane 5c

To a solution of TAM 7 (381 mg; 1.31 mmol; 2.0 eq.) in anhydrous DMF (5 mL) was added K_2CO_3 (451 mg; 3.27 mmol; 5.0 eq.). The mixture was stirred at 80 °C for 1 h, under argon. Then a solution of triethyleneglycol ditosylate 4c (300 mg; 0.65 mmol, 1.0 eq.) in dry DMF (5 mL) was added dropwise and the mixture was stirred at 80 °C for 24 h. The mixture was diluted with water (100 mL) and extracted with EtOAc (3 \times 100 mL). The combined organic layer was washed twice with brine (100 mL). Then dried over MgSO_4 , filtered and the solvent was removed under a vacuum. The crude product was purified by flash chromatography on silica gel (MeOH/DCM 99:1) to give the desired compound 5c (350 mg; 77%) as a yellow oil, starting TAM (50 mg) has been recovered. TLC: DCM/MeOH (98:2), R_f : 0.29 ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ (ppm): 8.50–8.52 (m, 2H, H12), 7.71 (td, 2H, $J_{10-11, 10-9}=7.7$ Hz, $J_{10-12}=1.9$ Hz, H10), 7.18–7.23 (m, 4H, H9, H11), 7.06–7.10 (m, 8H, H3, H7, H15, H19), 6.83–6.86 (m, 8H, H4, H6, H16, H18), 5.53 (s, 2H, H1), 4.01–4.04 (m, 4H, H20), 3.70–3.72 (m, 10H, H13, H21), 3.59 (s, 4H, H22). ^{13}C NMR (100 MHz, $\text{DMSO}-d_6$) δ (ppm): 163.1 (C8), 157.6 (C17), 156.8 (C5), 149.1 (C12), 136.7 (C10); 135.5 (C2); 135.4 (C14); 129.9 (C3, C7, C15, C19); 123.4 (C9); 121.5 (C11); 114.2 (C16, C18), 113.6 (C4, C6), 69.9 (C22), 69.0 (C21), 67.0 (C20), 56.5 (C1), 55.0 (C13). IR ν (cm^{-1}): 3050, 3002 ($\nu_{\text{Csp}2-\text{H}}$); 2930 ($\nu_{\text{Csp}3-\text{H}}$); 2876 (ν_{OMe}); 1608, 1586, 1506 and 1464 ($\nu_{\text{C}-\text{C}}$); 1241 ($\nu_{\text{asym C}-\text{O}-\text{C}}$); 1175 ($\nu_{\text{sym C}-\text{O}-\text{C}}$); 750 ($\delta_{\text{Csp}2-\text{H p-substitution}}$). LRMS: (ES $^+$, CV=30): 697.30 [M+H] $^+$; 719.27 [M+Na] $^+$. HRMS (ESI) m/z: [M+H] $^+$ Calcd for $\text{C}_{44}\text{H}_{44}\text{N}_2\text{O}_6\text{H}$ 697.3272. Found 697.327.

3.1.4 2-((4-methoxyphenyl)(4-(2-(4-((4-methoxyphenyl)(pyridin-2-yl)methyl)phenoxy)ethoxy)phenyl)methyl)pyridine 1-oxide 6a

To a solution of 5a (164 μmol ; 100 mg) in anhydrous DCM (2 mL) under argon was added *m*-CPBA (77%) (986 μmol ; 221 mg). The reaction mixture was stirred at room temperature for 2 h, under argon. Then, the reaction mixture was quenched by the addition of a solution of KOH (40%) until pH 8–9. The medium was diluted

with water (5 mL) and then extracted three times with DCM (10 mL). The combined organic layers were dried over MgSO_4 , filtered and concentrated. The crude product was purified by FCC on silica gel (100% to 95% DCM/MeOH) to afford the desired compound **6a** as an amorphous white solid (74%; 78 mg). TLC: 5% MeOH/DCM, R_f : 0.36 ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ (ppm): 8.24–8.26 (m, 2H, H12), 7.24–7.34 (m, 4H, H11, H10), 6.87–6.97 (m, 18H, H9, H3, H4, H6, H7, H15, H16, H18, H19), 6.03 (s, 2H, H1), 4.27 (s, 4H, H20), 3.72 (s, 6H, H13). ^{13}C NMR (400 MHz, $\text{DMSO}-d_6$) δ (ppm): 158.0 (C5), 157.0 (C17), 153.2 (C8), 139.1 (C12), 133.1 (C2), 132.7 (C14), 129.9 (C3, C7, C15, C19), 126.5 (C9), 124.5 (C10), 124.4 (C11), 114.5 (C16, C18), 113.9 (C4, C6), 66.3 (C20), 55.0 (C13), 48.1 (C1). IR ν (cm^{-1}): 3035 ($\nu_{\text{Csp}^2-\text{H}}$); 2932 ($\nu_{\text{Csp}^3-\text{H}}$); 2850 (ν_{OMe}); 1607, 1585, 1506 and 1462 ($\nu_{\text{C}=\text{O}}$); 1286 ($\nu_{\text{N}-\text{O}}$); 1232 ($\nu_{\text{asym C}-\text{O}-\text{C}}$); 1174 ($\nu_{\text{sym C}-\text{O}-\text{C}}$); 727 ($\delta_{\text{Csp}^2-\text{H p-substitution}}$). LRMS: (ES +, CV = 30): 641.19 [M + H] $^+$; 663.18 [M + Na] $^+$. HRMS (ESI) m/z : [M + Na] $^+$ Calcd for $\text{C}_{40}\text{H}_{36}\text{N}_2\text{O}_6\text{Na}$ 663.2466. Found 663.2462.

3.1.5. 2-((4-methoxyphenyl)(4-(2-(2-(4-((4-methoxyphenyl)(pyridin-2-yl)methyl)phenoxy)ethoxy)ethoxy)phenyl)methyl)pyridine 1-oxide **6b**

To a solution of **5b** (153 μmol ; 100 mg) in anhydrous DCM (2 mL) under argon was added *m*-CPBA (77%) (921 μmol ; 206 mg). The reaction mixture was stirred at room temperature for 3 h, under argon. Then, the reaction mixture was quenched by the addition of a solution of KOH (40%) until pH 8–9. The medium was diluted with water (5 mL) and then extracted three times with DCM (10 mL). The combined organic layers were dried over MgSO_4 , filtered and concentrated. The crude product was purified by flash column chromatography on silica gel (100% to 95% DCM/MeOH) to afford the desired compound **6b** as an amorphous white solid (65%; 68 mg). TLC: 5% MeOH/DCM; R_f : 0.32 ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ (ppm): 8.23–8.25 (m, 2H, H12), 7.30–7.34 (m, 2H, H11), 7.23–7.27 (m, 2H, H10), 6.92–6.96 (m, 10H, H9, H3, H7, H15, H19), 6.86–6.89 (m, 8H, H4, H6, H16, H18), 6.02 (s, 2H, H1), 4.06–4.09 (m, 4H, H20), 3.78–3.80 (m, 4H, H21), 3.72 (s, 6H, H13). ^{13}C NMR (100 MHz, $\text{DMSO}-d_6$) δ (ppm): 158.0 (C5), 157.2 (C17), 153.3 (C8), 139.1 (C12), 132.9 (C2), 132.8 (C14), 129.9 (C3, C7, C15, C19), 126.5 (C9), 124.5 (C10), 124.4 (C11), 114.5 (C16, C18), 113.6 (C4, C6), 69.0 (C21), 67.1 (C20), 55.1 (C13), 48.0 (C1). IR ν (cm^{-1}): 3066 ($\nu_{\text{Csp}^2-\text{H}}$); 2930 ($\nu_{\text{Csp}^3-\text{H}}$); 2876 (ν_{OMe}); 1608, 1586, 1507 and 1425 ($\nu_{\text{C}=\text{O}}$); 1280 ($\nu_{\text{N}-\text{O}}$); 1236 ($\nu_{\text{asym C}-\text{O}-\text{C}}$); 1175 ($\nu_{\text{sym C}-\text{O}-\text{C}}$); 726 ($\delta_{\text{Csp}^2-\text{H p-substitution}}$). LRMS: (ES +, CV = 30): 685.25 [M + H] $^+$; 707.24 [M + Na] $^+$. HRMS (ESI) m/z : [M + H] $^+$ Calcd for $\text{C}_{42}\text{H}_{40}\text{N}_2\text{O}_7\text{H}$ 685.2908. Found 685.2906.

3.1.6. 2-((4-methoxyphenyl)(4-(2-(2-(2-(4-((4-methoxyphenyl)(pyridin-2-yl)methyl)phenoxy)ethoxy)ethoxy)ethoxy)phenyl)methyl)pyridine 1-oxide **6c**

To a solution of **5c** (172 μmol ; 120 mg) in anhydrous DCM (2 mL) under argon was added *m*-CPBA (77%) (1.03 mmol; 232 mg). The reaction mixture was stirred at room temperature for 3 h, under argon. Then, the reaction mixture was quenched by the addition of a solution of KOH (40%) until pH 8–9. The medium was diluted with water (5 mL) and then extracted three times with DCM (10 mL). The combined organic layers were dried over MgSO_4 , filtered and concentrated. The crude product was purified by FCC on silica gel (100% to 95% DCM/MeOH) to afford the desired compound **6c** as an amorphous yellow solid (60%; 75 mg). TLC: (5% MeOH/DCM), R_f : 0.29 ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ (ppm): 8.23–8.25 (m, 2H, H12), 7.29–7.33 (m, 2H, H11), 7.23–7.27 (m, 2H, H10), 6.92–6.96 (m, 10H, H9, H3, H7, H15, H19), 6.86–6.89 (m, 8H, H4, H6, H16, H18), 6.02 (s, 2H, H1), 4.03–4.05 (m, 4H, H20), 3.71–3.73 (m, 10H, H13, H21), 3.59 (s, 4H, H22). ^{13}C NMR (100 MHz, $\text{DMSO}-d_6$) δ

(ppm): 158.0 (C5), 157.2 (C17), 153.3 (C8), 139.1 (C12), 132.9 (C2), 132.8 (C14), 129.9 (C3, C7, C15, C19), 126.5 (C9), 124.5 (C10), 124.4 (C11), 114.5 (C16, C18), 113.9 (C4, C6), 69.9 (C22), 69.0 (C21), 67.1 (C20), 55.1 (C13), 48.0 (C1). IR ν (cm^{-1}): 3064 ($\nu_{\text{Csp}^2-\text{H}}$); 2927 ($\nu_{\text{Csp}^3-\text{H}}$); 2873 (ν_{OMe}); 1608, 1586, 1508 and 1458 ($\nu_{\text{C}=\text{O}}$); 1280 ($\nu_{\text{N}-\text{O}}$); 1238 ($\nu_{\text{asym C}-\text{O}-\text{C}}$); 1176 ($\nu_{\text{sym C}-\text{O}-\text{C}}$); 725 ($\delta_{\text{Csp}^2-\text{H p-substitution}}$). LRMS: (ES +, CV = 30): 729.26 [M + H] $^+$; 751.26 [M + Na] $^+$. HRMS (ESI) m/z : [M + Na] $^+$ Calcd for $\text{C}_{44}\text{H}_{44}\text{N}_2\text{O}_8\text{Na}$ 751.2990. Found 751.2987.

3.1.7. tert-butyl(5-(6-((tert-butoxycarbonyl)amino)-2-((2-(4-((4-methoxyphenyl)(pyridin-2-yl)methyl)phenoxy)ethyl)amino)hexanamido)-6-((2-(4-((4-methoxyphenyl)(pyridin-2-yl)methyl)phenoxy)ethyl)amino)-6-oxohexyl)carbamate **14**

Under argon, 78 mg of 2-((4-(2-bromoethoxy)phenyl)(4-methoxyphenyl)methyl)pyridine **7** (0.098 mmol) was refluxed with K_2CO_3 (0.98 mmol, 10 eq, 136.5 mg) and KI (9.8 μmol , 0.1 eq, 16.26 mg) in acetone (5 mL) during 1 h. Then, a solution of compound **13** (0.108 mmol, 1.1 eq, 43 mg) in acetone (1 mL) was added dropwise to the mixture. The reaction mixture was refluxed for 36 h and controlled by TLC. The mixture was concentrated under a vacuum, then washed with water and extracted with DCM. The crude product was purified by FCC (DCM/MeOH.NH $_3$ (7N): 98/2) to afford the desired product **14** as an orange solid with 45% yield. TLC: DCM/MeOH.NH $_3$ (7N): 95/5, R_f : 0.6 ^1H NMR (400 MHz, CDCl_3) δ (ppm): 8.57–8.47 (m, 2H, H29), 7.57–7.49 (m, 2H, H27), 7.07 (ddd, $J_{28-29} = 7.6$ Hz, $J_{28-27} = 4.9$ Hz, $J_{28-26} = 1.2$ Hz, 2H, H28), 7.02–6.92 (m, 10H, H26, H14, H16, H20, H24), 6.80–6.71 (m, 8H, H13, H17, H21, H23), 5.52 (s, 2H, H18), 4.70–4.41 (m, 1H, H2), 4.01–3.85 (m, 4H, H11), 3.71 (s, 6H, H30), 3.11–2.84 (m, 5H, H10, H10', H2'), 1.96–1.93 (m, 4H, H3, H3'), 1.84–1.69 (m, 4H, H5, H5'), 1.58–1.48 (m, 26H, H9, H9', H4, H4'). ^{13}C NMR (100 MHz, CDCl_3) δ (ppm): 175.36 (C1, C1'), 159.64 (C25), 158.24 (C22), 156.27 (C12), 154.9 (C7, C7'), 149.41 (C29), 136.83 (C27), 134.44 (C19), 134.13 (C15), 130.41 (C14, C16, C20, C21), 123.75 (C26), 121.49 (C28), 113.98 (C13, C17, C21, C23), 82.28 (C8), 66.45 (C11, C11'), 60.20 (C2'), 57.86 (C2'), 57.15 (C18), 55.22 (C30), 52.44 (C2), 39.8 (C6, C6'), 38.66 (C10, C10'), 31.51 (C3'), 29.54 (C5, C5'), 28.58 (C9, C9'), 26.20 (C3'), 22.02 (C4, C4'). LRMS: (ES +, CV = 30) m/z : 1106.07 [M-2H] $^+$; 1008.12 [M-C $_5$ H $_9$ O $_2$ +2H] $^+$; 853.87 [M-C $_2$ H $_24$ O $_6$ +Na] $^+$; 831.87 [M-C $_2$ H $_24$ O $_6$ +H] $^+$. IR ν (cm^{-1}): 3340 ($\nu_{\text{N}-\text{H}}$); 3062 ($\nu_{\text{Csp}^2-\text{H}}$); 2931 ($\nu_{\text{Csp}^3-\text{H}}$); 2859 (ν_{OMe}); 1714 ($\nu_{\text{C}=\text{O}}$ carbamate); 1609, 1588 and 1467 and 1434 ($\nu_{\text{C}=\text{O}}$); 1510 ($\delta_{\text{N}-\text{H}}$); 1366 ($\nu_{\text{C}-\text{N}}$); 1247 ($\nu_{\text{asym C}-\text{O}-\text{C}}$); 1175 ($\nu_{\text{sym C}-\text{O}-\text{C}}$); 722 ($\delta_{\text{Csp}^2-\text{H p-substitution}}$).

3.1.8. 6-amino-N-(6-amino-1-((2-(4-((4-methoxyphenyl)(pyridin-2-yl)methyl)phenoxy)ethyl)amino)-1-oxohexan-2-yl)-2-((2-(4-((4-methoxyphenyl)(pyridin-2-yl)methyl)phenoxy)ethyl)amino)hexanamide **15**

37 mg of TAM-Lys (Boc)TAM **14** (0.033 mmol) was stirred in TFA (2 mL) for 3 hours. After reaction completion, the mixture was concentrated under a vacuum and then neutralized with a saturated aqueous solution of NaOH until pH = 6. The mixture was extracted with DCM, dried under MgSO_4 and concentrated under vacuum. The crude product was purified by FCC (DCM/MeOH.NH $_3$ (7N): 90/10). The desired product **15** was afforded as a yellow oil with a 63% yield. TLC: DCM/MeOH.NH $_3$ (7N): 90/10 R_f : 0.11 ^1H NMR (400 MHz, CDCl_3) δ 8.59 (d, $J_{26-25} = 4.8$ Hz, 2H, H26), 7.80–7.68 (m, 2H, H24), 7.62–7.48 (m, 2H, H23), 7.17–7.09 (m, 2H, H25), 7.05 (d, $J_{17-18, 11-10, 13-14, 21-20} = 8.0$ Hz, 8H, H17, H21, H11, H13), 6.86–6.77 (m, 8H, H10, H14, H18, H20), 5.61–5.54 (s, 2H, H15), 4.41–4.26 (m, 1H, H2), 4.06–3.91 (m, 4H, H8, H8'), 3.77 (s, 6H, H27), 3.67–3.66 (m, 3H, H2, H7), 3.25–3.14 (m, 2H, H7), 2.68–2.62 (m, 4H, H6), 2.32–2.29 (m, 2H, H3), 2.08–1.97 (m, 2H, H3'), 1.82–1.75 (m, 4H, H4, H4'), 1.61–61

(m, 4H, H5, H5'). ^{13}C NMR (100 MHz, CDCl_3) δ (ppm): 171.98 (C1, C1'), 158.24 (C19), 157.18 (C9), 149.66 (C26), 136.55 (C24), 134.51 (C16), 134.01 (C12), 130.34 (C11, C13, C17, C21), 123.70 (C23), 121.12 (C25), 113.95 (C10, C14, C18, C20), 66.65 (C8), 65.47 (C2), 57.43 (C15), 55.38 (C27), 53.57 (C2), 41.62 (C6, C6'), 38.71 (C7, C7'), 31.08 (C3'), 29.84 (C5, C5'), 26.05 (C3'), 22.83 (C4, C4'). IR ν (cm^{-1}): 3299 ($\nu_{\text{N-H}}$); 3060 ($\nu_{\text{Csp}2\text{-H}}$); 2924 ($\nu_{\text{Csp}3\text{-H}}$); 2855 (ν_{OMe}); 1656 ($\nu_{\text{C=O}}$); 1608, 1565 and 1508 and 1462 ($\nu_{\text{C=C}}$); 1369 ($\nu_{\text{C-N}}$); 1244 ($\nu_{\text{asym C-O-C}}$); 1176 ($\nu_{\text{sym C-O-C}}$); 737 ($\delta_{\text{Csp}2\text{-H p-substitution}}$).

3.2. Biology

3.2.1. Materials

DMEM medium, RPMI 1640 medium, fetal bovine serum (FBS), L-glutamine and penicillin-streptomycin were purchased from Gibco BRL – Fisher Scientific (Illkirch, France). 5-FU, irinotecan, MTT and DMSO were obtained from Sigma-Aldrich – Merck (Lyon, France).

3.2.2. Cell Lines, Cell Culture and Treatment

Human CRC HT-29 and HCT116 cell lines were purchased from the American Type Culture Collection (ATCC – LGC Standards, Molsheim, France). Cells were grown in DMEM medium for HT-29 cells and RPMI 1640 medium for HCT116 cells, supplemented with 10% FBS, 1% L-glutamine and 100 U/mL penicillin and 100 $\mu\text{g}/\text{mL}$ streptomycin. Cultures were maintained in a humidified atmosphere containing 5% CO_2 at 37 °C. Stock solutions in DMSO were used at 10^{-2} M for reference compounds (5-FU, irinotecan), compounds **5a–c** and **6a–c** or 5×10^{-3} M for compound **15**, and then diluted in culture medium to obtain the appropriate final concentrations.

3.2.3. Antiproliferative Assay

The antiproliferative effect of all compounds was determined using MTT assays. Briefly, cells were seeded in 96-well microplates at 8×10^3 cells/well for HT-29 cells and 5×10^3 cells/well for HCT116 cells and grown for 24 h in appropriate culture medium prior to exposure or not at first to compounds (5-FU, irinotecan, **5a–c**, **6a–c** and **15**) at 50 μM , then with concentration ranges from 1 to 50 μM only for 5-FU, irinotecan and compounds **6a–c**. The same amount of vehicle (percentage of DMSO did not exceed 0.5%) was added to control cells. After 48 h of treatment, MTT (5 g/L in PBS) was added and incubated for another 3 h, before adding 100 μL DMSO. Then the optical density was detected with a microplate reader at 550 nm and IC_{50} values were calculated for all compounds.

3.2.4. Statistical Analysis

Data are expressed as the arithmetic means \pm standard error of the mean (SEM) of at least three separate experiments. Statistical significance was evaluated by the two-tailed unpaired Student's *t*-test and expressed as: * $p < 0.05$; ** $p < 0.01$ and *** $p < 0.001$.

3.3. Computational Study

3.3.1. Molecular Modeling

3.3.1.1. Protein and Compounds Preparations

Protein structures were extracted from the Protein Data Bank (PDB)^[62]: AKT (PDB ID: 6S9W), ERK-1 (PDB ID: 4QTB), ERK-2 (PDB ID:

6SLG), PARP (PDB ID: 4ZZZ), MAPK11 (PDB ID: 3GP0) MAPK13 (PDB ID: 5EK0), MAPK14 (PDB ID: 5XYY) and caspase-3 (PDB ID: 6CKZ). The structure of ERK-2 was superimposed on the structure of the ERK-1 isoform. Protein structures were prepared for the docking study using the DockPrep tool from UCSF Chimera^[63] and MGL tools.^[64]

Three dimensional structures of compound **5a** and **6a** were generated using RDKit ("RDKit: Open-source cheminformatics. <https://www.rdkit.org>"). The protonation at pH 7.4 was achieved using the cxcalc plugin of the Chemaxon suite^[65] and the conformers were converted in.pdbqt format, required for the docking study.

3.3.1.2. Docking Study, Protein-Ligand Interactions Analysis and SuperPred

The smina software with the vinao scoring function was used to achieve the docking study. For each potential target, a box was defined to delimit the search space. The size of the box was set to 20 $\text{\AA} \times 20 \text{\AA} \times 20 \text{\AA}$ for all the proteins and appropriate *x*, *y*, *z* grid center coordinates were defined for each target: –12.729, –15.248, 13.193 for AKT, 36.683, –54.826, 49.926 for ERK-1 and ERK-2, 63.412, 6.484, 9.593 for PARP, 19.438, 60.811, 23.394 for MAPK11, –13.813, 10.465, –27.357 for MAPK13, 1.425, –32.820, –30.348 for MAPK14 and 39.023, 11.547, 71.322 for caspase-3.

The predicted binding mode of compounds **5a** and **6a** in each one of the 5 protein binding sites was analyzed using the protein-ligand interaction (PLIP) webserver^[66] (<https://plip-tool.biotec.tu-dresden.de/plip-web/plip/index>).

3.3.2. ADME Profile and Drug-Likeness

ADME properties and drug-likeness for three selected hybrid TAMs were predicted using the Molecular Properties Panel within the QikProp v5.9 platform integrated into SCHRÖDINGER Maestro v11.9 software.^[67] Ligand energy structure minimization was performed using the OPLS3e force field method,^[68] employing the conjugate gradient method Polak-Ribier (PRCG) with a convergence threshold of 0.05 and a maximum of 2500 iterations.

4. Conclusion

The present study illustrates the potential of molecular hybridization as a strategy for designing multifunctional compounds with improved anticancer activity. The synthesis of TAM hybrids, bearing pegylated and peptide linkers, allowed the creation of compounds with significant antiproliferative effects against human colorectal cancer cell lines. Notably, the introduction of *N*-oxide moiety further enhanced the antiproliferative activity. Docking studies revealed promising binding interactions of the *N*-oxide synthesized compounds with potential biological targets, particularly AKT. Additionally, the ADME profile and drug-likeness predictions underscored the membrane-crossing capabilities of the hybrid TAMs, despite some challenges related to poor water solubility. Overall, these findings highlight the potential of hybrid TAMs as promising candidates for further development as anticancer agents, requiring further investigations and optimizations to overcome solubility limitations and enhance therapeutic efficacy.

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Conflict of Interests

The authors have no conflicts of interest to declare.

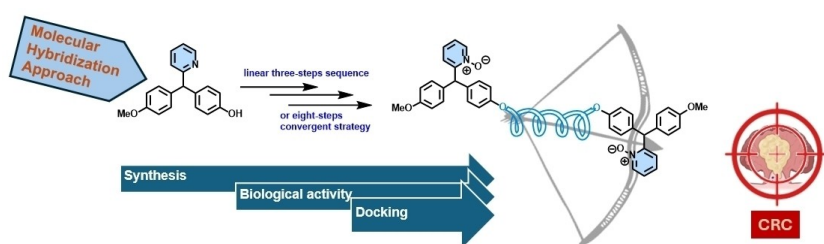
Keywords: Triarylmethanes · Molecular hybridization · Colorectal cancer · Pegylated linkers · Pyridine *N*-oxide

- [1] V. S. Gontijo, F. P. D. Viegas, C. J. C. Ortiz, M. de Freitas Silva, C. M. Damasio, M. C. Rosa, T. G. Campos, D. S. Couto, K. S. Tranches Dias, C. Viegas, *Curr. Neuropharmacol.* **2019**, *18*, 348.
- [2] A. C. G., R. Gondru, Y. Li, J. Banothu, *Eur. J. Med. Chem.* **2022**, *227*, 113921.
- [3] A. Fakhriolaii, F. Abedinifar, P. Salehi Darjani, M. Mohammadi-Khanaposhtani, B. Larijani, N. Ahangar, M. Mahdavi, *BMC Chem.* **2023**, *17*, 80.
- [4] A. K. A. Bass, M. S. El-Zoghbi, E.-S. M. Nageeb, M. F. A. Mohamed, M. Badr, G. E.-D. A. Abu-Rahma, *Eur. J. Med. Chem.* **2021**, *209*, 112904.
- [5] L. Scotti, F. Mendonça-Junior, M. Scotti, *Curr. Top. Med. Chem.* **2017**, *17*, 957.
- [6] S. B. Tsogoeva, *Mini-Rev. Med. Chem.* **2010**, *10*, 773.
- [7] B. Meunier, *C. R. Chim.* **2011**, *14*, 400.
- [8] W. J. Geldenhuys, M. B. H. Youdim, R. T. Carroll, C. J. Van der Schyf, *Prog. Neurobiol.* **2011**, *94*, 347.
- [9] L. Y. Ma, B. Wang, L. P. Pang, M. Zhang, S. Q. Wang, Y. C. Zheng, K. P. Shao, D. Q. Xue, H. M. Liu, *Bioorg. Med. Chem. Lett.* **2015**, *25*, 1124.
- [10] S. Sandhu, Y. Bansal, O. Silakari, G. Bansal, *Bioorg. Med. Chem.* **2014**, *22*, 3806.
- [11] H. L. Qin, Z. W. Zhang, R. Lekkala, H. Alsulami, K. P. Rakesh, *Eur. J. Med. Chem.* **2020**, *193*, 112215.
- [12] L. Fang, B. Kraus, J. Lehmann, J. Heilmann, Y. Zhang, M. Decker, *Bioorg. Med. Chem. Lett.* **2008**, *18*, 2905.
- [13] N. Ikarashi, K. Baba, T. Ushiki, R. Kon, A. Mimura, T. Toda, M. Ishii, W. Ochiai, K. Sugiyama, *Am. J. Physiol. Gastrointest. Liver Physiol.* **2011**, *301*, 887.
- [14] M. Feve, M. Zenyou-Meyer, M. Haiech, J. Chneiweiss, H. Kilhoffer, M. Mameni, S. Hibert, *Bisacodyl and Analogues as Drugs for Treating Cancer*, **2012**, WO 2012/168885 A2.
- [15] H. J. Park, H. J. Jung, M. J. Ho, D. R. Lee, H. R. Cho, Y. S. Choi, J. Jun, M. Son, M. J. Kang, *Eur. J. Pharm. Sci.* **2017**, *102*, 172.
- [16] S. Li, L. Wang, J. Jiang, P. Tang, Q. Wang, D. Wu, H. Li, *Carbohydrate polymers* **2015**, *134*, 82.
- [17] N. Ikarashi, K. Baba, T. Ushiki, R. Kon, A. Mimura, T. Toda, M. Ishii, W. Ochiai, K. Sugiyama, *Am. J. Physiol. Gastrointest. Liver. Physiol.*, **2011**, 887.
- [18] J. Dong, F. J. Aulestia, S. Assad Kahn, M. Zeniou, L. G. Dubois, E. A. El-Habr, F. Daubeuf, N. Tounsi, S. H. Cheshier, N. Frossard, M. P. Junier, H. Chneiweiss, I. Néant, M. Moreau, C. Leclerc, J. Haiech, M. C. Kilhoffer, *Biochim. Biophys. Acta Mol. Cell Res.* **2017**, *1864*, 1018.
- [19] M. Zeniou, M. Fève, S. Mameri, J. Dong, C. Salomé, W. Chen, E. A. El-Habr, F. Bousson, M. Sy, J. Obszynski, A. Boh, P. Villa, S. A. Kahn, B. Didier, D. Bagnard, M. P. Junier, H. Chneiweiss, J. Haiech, M. Hibert, M. C. Kilhoffer, C. Heeschen, *PLoS One* **2015**, *10*, e0134793.
- [20] R. Kshatriya, P. Shelke, S. Mali, G. Yashwantrao, A. Pratap, S. Saha, *ChemistrySelect* **2021**, *6*, 6230.
- [21] K. Sumoto, N. Mibu, K. Yokomizo, M. Uyeda, *Chem. Pharm. Bull.* **2002**, *50*, 298.
- [22] N. Mibu, K. Yokomizo, M. Uyeda, K. Sumoto, *Chem. Pharm. Bull.* **2005**, *53*, 1171.
- [23] J. L. Douglas, M. L. Panis, E. Ho, K. Lin, S. H. Krawczyk, D. M. Grant, R. Cai, S. Swaminathan, T. Cihlar, *J. Virol.* **2003**, *77*, 5054.
- [24] M. Cushman, S. Kanamathareddy, E. De Clercq, D. Schols, M. E. Goldman, *J. Med. Chem.* **1991**, 337.
- [25] J. L. Douglas, M. L. Panis, E. Ho, K. Lin, S. H. Krawczyk, D. M. Grant, R. Cai, S. Swaminathan, X. Chen, T. Cihlar, *Antimicrob. agents chemother.* **2005**, *49*, 2460.
- [26] C. Ricco, F. Abdmouleh, C. Riccobono, L. Guenineche, F. Martin, E. Goya-Jorge, N. Lagarde, B. Liagre, M. Ben Ali, C. Ferroud, M. El Arbi, M. S. I. Veitia, *Bioorg. Chem.* **2020**, *96*, 103591.
- [27] K. H. Buchel, W. Draber, E. Regel, M. Plempel, *Arzneimittelforschung.* **1972**, *22*, 1260.
- [28] E. Goya-Jorge, C. Rampal, N. Loones, S. J. Barigye, L. E. Carpio, R. Gozalbes, C. Ferroud, M. Sylla-Iyarreta Veitia, R. M. Giner, *Eur. J. Med. Chem.* **2020**, *207*, 112777.
- [29] M. S.-I. Veitia, D. S. Mota, V. Lerari, M. Marin, R. M. Giner, L. V. Muro, Y. R. Guerra, F. Dumas, C. Ferroud, P. A. M. de Witte, A. D. Crawford, V. J. Aran, Y. M. Ponce, *Curr. Top. Med. Chem.* **2017**, *17*, 2866.
- [30] A. G. Johnson, M. M. Tranquilli, M. R. Harris, E. R. Jarvo, *Tetrahedron Lett.* **2015**, *56*, 3486.
- [31] M. R. Lewis, P. P. Goland, *Cancer Res.* **1953**, *13*, 130.
- [32] B. L. H. Taylor, M. R. Harris, E. R. Jarvo, *Angew. Chemie Int. Ed.* **2012**, *51*, 7790.
- [33] K. Srivastava, R. Sharma, R. Mishra, A. K. Balapure, S. R. Murthy, G. Panda, *Bioorg. Med. Chem.* **2006**, *14*, 1497.
- [34] A. Hadj Mohamed, M. Görmen, M. El Arbi, M. Msaddek, M. S.-I. Veitia, *MDPI MOL2NET'18, Conf. Mol. Biomed. Comput. Sci. Eng. 4th ed.* **2019**.
- [35] M. Görmen, M. S. I. Veitia, F. Trigui, M. El Arbi, C. Ferroud, *J. Organomet. Chem.* **2015**, *794*, 274–281.
- [36] <https://www.who.int/news-room/fact-sheets/detail/cancer-n.d>.
- [37] <https://gco.iarc.fr/today/en/dataviz/pie?mode=cancer&group-populations=1&cancers=39,n.d>.
- [38] A. S. Adebayo, K. Agbaje, S. K. Adesina, O. Olajubutu, *Pharmaceutics* **2023**, *15*, 1.
- [39] H. Sung, J. Ferlay, R. L. Siegel, M. Laversanne, I. Soerjomataram, A. Jemal, F. Bray, *Cancer J. Clin.* **2021**, *71*, 209.
- [40] A. C. S. Medical and Editorial Content Team, *Am. Cancer Soc.*, cancer.org | 1.800.227.2345 **2018**.
- [41] E. Van Cutsem, A. Cervantes, R. Adam, A. Sobrero, J. H. Van Krieken, D. Aderka, E. Aranda Aguilar, A. Bardelli, A. Benson, G. Bodoky, F. Ciardiello, A. D'Hoore, E. Diaz-Rubio, J.-Y. Douillard, M. Ducreux, A. Falcone, A. Grothey, T. Gruenberger, K. Haustermans, V. Heinemann, P. Hoff, C.-H. Köhne, R. Labianca, P. Laurent-Puig, B. Ma, T. Maughan, K. Muro, N. Normanno, P. Österlund, W. J. G. Oyen, D. Papamichael, G. Pentheroudakis, P. Pfeiffer, T. J. Price, C. Punt, J. Ricke, A. Roth, R. Salazar, W. Scheithauer, H. J. Schmoll, J. Taberner, J. Taïeb, S. Tejpar, H. Wasan, T. Yoshino, A. Zaanan, D. Arnold, *Ann. Oncol. Off. J. Eur. Soc. Med. Oncol.* **2016**, *27*, 1386.
- [42] M. Fereidoonzhad, M. Niazi, M. Shahmohammadi Beni, S. Mohammad, Z. Faghhi, Z. Faghhi, H. R. Shahsavari, *ChemMedChem* **2017**, *12*, 456.
- [43] A. Ghaleb, A. Aoudate, H. B. El Ayouchia, M. Aarjane, H. Anane, S. E. Stiriya, *J. Biomol. Struct. Dyn.* **2022**, *40*, 143.
- [44] B. Saikia, R. Khatioda, P. Bora, B. Sarma, *CrystEngComm* **2016**, *18*, 8454.
- [45] G. Xu, Z. Liu, X. Wang, T. Lu, R. L. Desjarlais, T. Thieu, J. Zhang, Z. H. Devine, F. Du, Q. Li, C. M. Milligan, P. Shaffer, P. E. Cedervall, J. C. Spurlino, C. F. Stratton, B. Pietrak, L. M. Szewczuk, V. Wong, R. A. Steele, W. Bruinzeel, M. Chintala, J. Silva, M. D. Gaul, M. J. Macielag, R. Nargund, *J. Med. Chem.* **2022**, *65*, 10419.
- [46] A. M. Mfuh, O. V. Larionov, *Curr. Med. Chem.* **2015**, *22*, 2819.
- [47] A. Hadj Mohamed, A. Pinon, N. Lagarde, E. G. Jorge, H. Mouhsine, M. Msaddek, B. Liagre, S. Veit, *RSC Med. Chem.* **2023**, *13*, 54.
- [48] G. Bort, S. Catoen, H. Borderies, A. Keksi, S. Ballet, G. Louin, M. Port, C. Ferroud, *Eur. J. Med. Chem.* **2014**, *87*, 843.
- [49] M. S. Kang, T. W. S. Kong, J. Y. X. Khoo, T. P. Loh, *Chem. Sci.* **2021**, *12*, 13613.
- [50] Y. Tian, Q. Lin, *Chimia (Aarau)*. **2018**, *72*, 758.
- [51] N. Jain, S. W. Smith, S. Ghone, B. Tomczuk, *Pharm. Res.* **2015**, *32*, 3526.
- [52] J. Fiala, Z. Kukačka, P. Novák, *J. Proteomics* **2020**, *218*, 103716.
- [53] J. Masuda, S. Kondo, Y. Matsumoto, M. Yamanaka, *ChemistrySelect* **2018**, *3*, 6112.

- [54] X. Du, J. Qian, Y. Wang, M. Zhang, Y. Chu, Y. Li, *Bioorg. Med. Chem.* **2019**, *27*, 2784.
- [55] X. Du, J. Qian, Y. Wang, M. Zhang, Y. Chu, Y. Li, *Bioorg. Med. Chem.* **2019**, *27*, 2784.
- [56] G. S. Egerton, A. G. Morgan, *J. Soc. Dye. Colour.* **1970**, *86*, 242.
- [57] S. Nakagawa, K. Sakakibara, H. Gotoh, *Dye Pigment* **2016**, *124*, 130.
- [58] C. Weyermann, D. Kirsch, C. C. Vera, B. Spengler, *J. Forensic Sci.* **2009**, *54*, 339.
- [59] A. Hadj Mohamed, A. Pinon, N. Lagarde, C. Ricco, E. Goya-Jorge, H. Mouhsine, M. Msaddek, B. Liagre, M. S.-I. Veitia, *RSC Med. Chem.* **2024**, *15*, 660.
- [60] B. Frerker, F. Bock, M. L. Cappel, S. Kriesen, G. Klautke, G. Hildebrandt, K. Manda, *Int. J. Mol. Sci.* **2023**, *24*, 10385.
- [61] A. Hadj Mohamed, A. Pinon, N. Lagarde, C. Ricco, E. Goya-Jorge, H. Mouhsine, M. Msaddek, B. Liagre, M. Veitia, *RSC Med. Chem.* **2023**, *15*, 660.
- [62] I. M. L. Saur, R. Panstruga, P. Schulze-Lefert, *Nat. Rev. Immunol.* **2021**, *21*, 305.
- [63] E. F. Pettersen, T. D. Goddard, C. C. Huang, G. S. Couch, D. M. Greenblatt, E. C. Meng, T. E. Ferrin, *J. Comput. Chem.* **2004**, *25*, 1605.
- [64] A. Allouche, *J. Comput. Chem.* **2012**, *32*, 174.
- [65] <https://chemaxon.com/>, n.d.
- [66] M. F. Adasme, K. L. Linnemann, S. N. Bolz, F. Kaiser, S. Salentin, V. J. Haupt, M. Schroeder, *Nucleic Acids Res.* **2021**, *49*, 530.
- [67] L. L. C. Schrödinger Release 2019-4; Schrödinger, LLC: New York, NY **2019**.
- [68] K. Roos, C. Wu, W. Damm, M. Reboul, J. M. Stevenson, C. Lu, M. K. Dahlgren, S. Mondal, W. Chen, L. Wang, R. Abel, R. A. Friesner, E. D. Harder, *J. Chem. Theory Comput.* **2019**, *15*, 1863.

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RESEARCH ARTICLE



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between 18 and 24 μM . Computational studies identified (AKT) as a target and predicted favorable drug-likeness properties. These results highlight the potential of hybrid TAMs as anticancer agents.

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A Promising Approach to Target Colorectal Cancer Using Hybrid Triaryl-methanes

