Design, Synthesis and Anticolorectal Cancer Activity of 2-Bromoalkoxyanthraquinones

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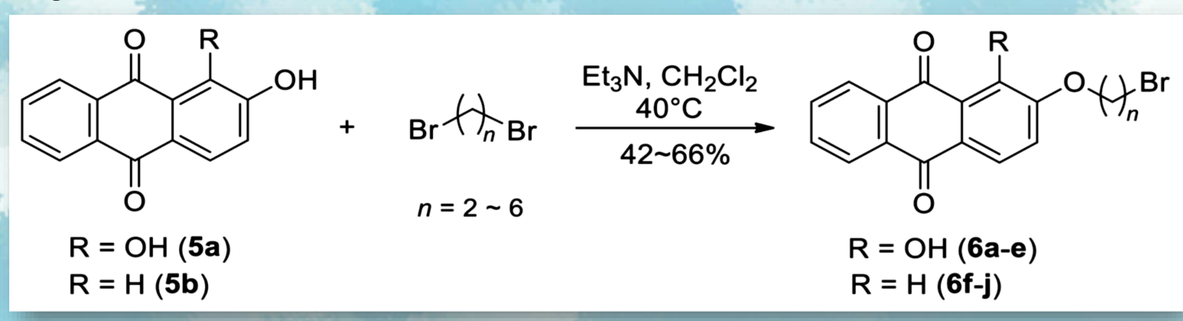
Introduction

Colorectal cancer (CRC) ranks as the third most prevalent cancer globally, with 1.4 million new cases and over half a million annual deaths reported. Genetic abnormalities, particularly KRAS and p53 mutations, play pivotal roles in CRC development . KRAS mutants exhibit resistance to anti-EGFR therapy, while p53 mutations correlate with poor responses to chemotherapy. The coexistence of KRAS and p53 mutations further diminishes survival rates in CRC patients with colorectal liver metastases resection. Consequently, targeting these mutated genes through new drug development is considered a potential treatment avenue.

Anthraquinones, tricyclic aromatic compounds, show promising pharmacological activities, with emodin demonstrating significant anticancer effects across various human cancers. Modifications of emodin and hydroxyanthraquinone have resulted in compounds displaying enhanced cytotoxicity. Having observed heightened cytotoxicity and selectivity in anthraquinones with a 1-OH group compared to those without in our prior work, we now delve into evaluating the combined impact of chain length and the 1-OH group on the anticancer properties of 2-bromoalkoxyanthraquinones in continuation of our earlier study. The study explores 2-bromoalkoxyanthraquinones bearing with and without a 1-OH group, featuring diverse alkoxy chain lengths (C2-C6). These compounds are synthesised via Williamson ether reaction and evaluated for their anticancer activities against colorectal cancer cells (HCT116 and HT29) and normal colon cells (CCD841 CoN) for comparison. Molecular docking explores interactions with putative p53 and KRAS targets, while *in silico* ADMET prediction assesses therapeutic potential.

Synthesis



Protein-Ligand Docking

Compounds			Binding Energy (kcal/mol)	
-	R	*n*	p53 (PDB: 7B4N)	KRAS (PDB: 6P0Z)
6a	OH	2	-6.2	-7.1
6b	OH	3	**-6.9**	**-7.6**
6c	OH	4	**-6.5**	**-7.3**
6d	OH	5	**-6.4**	**-7.3**
6e	OH	6	**-6.7**	**-7.8**
6f	H	2	-5.9	-7.0
6g	H	3	-6.0	-7.1
6h	H	4	-6.1	-7.1
6i	H	5	**-6.4**	**-7.4**
6j	H	6	**-6.3**	**-7.4**
QNN[a]	-	-	-4.7	-
GDP[b]	-	-	-	-8.2

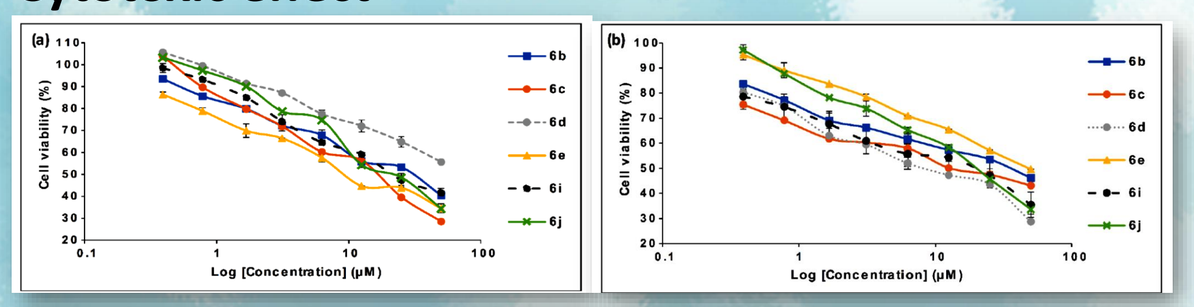
Abbreviations: [a]QNN = methylene quinuclidine, the bound substrate in the crystal structure of p53 (PDB: 7B4N); [b]GDP = guanosine diphosphate, the bound substrate in the crystal structure of KRAS (PDB: 6P0Z); The best binding energies were highlighted in bold.

Bioassays

Compounds	IC_{50} Value (μM)		
	HCT116	HT29	CCD841 CoN
6b	**3.83 ± 0.05**	13.76 ± 0.05*	> 50
6c	13.90 ± 0.04	15.91 ± 0.03	> 50
6d	11.80 ± 0.02*	44.0 ± 0.04	> 50
6e	24.70 ± 0.02*	**8.50 ± 0.05**	> 50
6i	20.47 ± 0.06	18.09 ± 0.02	> 50
6j	> 50	48.26 ± 0.05	> 50
DOX	0.17 ± 0.04	0.30 ± 0.06*	NA
5-FU	5.00 ± 0.04	0.20 ± 0.05	NA

Note: [a]Data was shown as mean ± SD from three independent experiments; *Indicates *p* value < 0.05 and considered significant; NA = not measured; DOX = doxorubicin hydrochloride; 5-FU = 5-fluorouracil.

Cytotoxic effect



HCT116 cells HT29 cells

Selectivity Index

-	Selectivity Index (SI)	
Compounds	CCD841 CoN/HCT116	CCD841 CoN/HT29
6b	**45.47**	23.29
6c	5.35	4.40
6d	9.47	2.54
6e	18.50	**36.74**
6i	3.85	4.51
6j	1.57	3.61

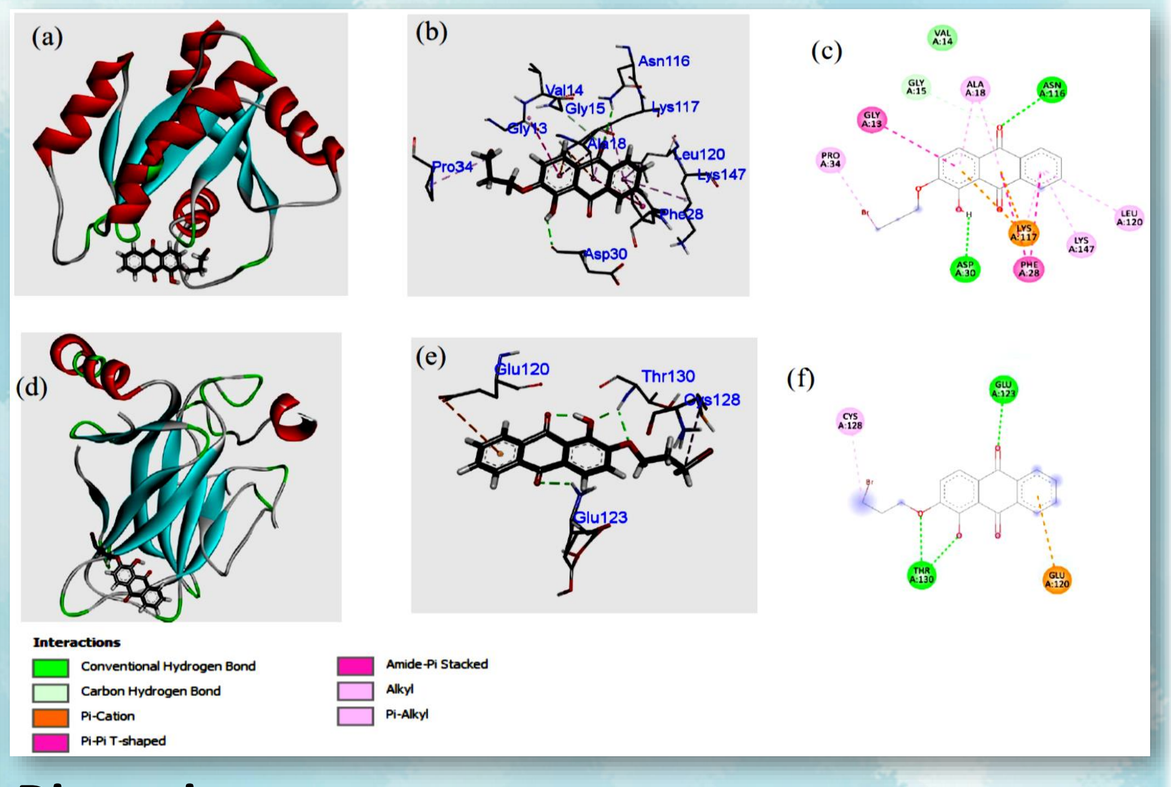
Note: [a]SI value was calculated based on the IC_{50} value of normal colon cells against the IC_{50} value of colorectal cancer cells. The bold indicates the highest selectivity index among tested compounds.

Predicted ADMET

Compounds	MW	HBD	HBA	RB	LogP	TPSA	GIA	LogS[f]	hERG[b,f]
6b	361.19	1	4	4	3.27	63.60	high	-5.27	safe
6e	403.27	1	4	7	4.25	63.60	high	-6.03	safe

Abbreviations: [a]MW: molecular weight; HBD: number of hydrogen bond donors; HBA: number of hydrogen bond acceptors; RB: number of rotatable bonds; LogP: calculated logarithm of octanol-water partition coefficient; TPSA: topological polar surface area (Å^2); GIA: Gastrointestinal absorption; LogS: aqueous solubility (mg/mL); [b]hERG: human ether-a-go-go-related gene; *determined using SwissADME; [f]determined using Chemaxon.

Docking of 6b against: KRAS (a-c); p52 (d-f)



Discussions

Molecular docking

- **6e** showed the best binding affinity to KRAS (-7.8 kcal/mol), close to the bound ligand GDP (-8.2 kcal/mol).
- **6b** exhibited the best binding affinity to p53 (-6.9 kcal/mol).
- **6b-e**, **6i**, and **6j** selected for biological evaluation based on comparable binding energies to co-crystallized ligands.
- **6b** exhibited binding site similarity to the co-crystallized ligand (QNN) in p53, forming hydrogen bonds, π-anion interactions, and stabilizing the protein-ligand complex.

Bioassays

- All tested 2-bromoalkoxyanthraquinones showed no significant cytotoxic effects on normal colon cells (CCD841 CoN) up to 50 μM.
- Compounds with shorter bromoalkoxy chains (C3-C4) exhibited higher cytotoxicity than those with longer chains (C5-C6).
- **6b** was the most cytotoxic against KRAS-mutated HCT116 cells, outperforming 5-FU.
- **6b** demonstrated high selectivity for HCT116 over CCD841 CoN (SI = 45.47).
- **6e** exhibited high selectivity for HT29 over CCD841 CoN (SI = 36.74) and the lowest IC50 value in p53 mutated HT29 cells.
- **6j** that lacking a 1-OH group was inactive, highlighting the importance of the 1-OH group for antiproliferation activity.

Predicted ADMET

- **6b** and **6e** adhere to Lipinski's rule without any violations, featuring fewer than three aromatic rings and moderate molecular flexibility.
- Despite good gastrointestinal absorption, both **6b** and **6e** exhibit poor water solubility (LogS > -5).
- The calculated Log P value for **6e** is slightly higher than the mean Log P value of marketed oral drugs, suggesting potential receptor promiscuity and poor bioavailability.
- Chemaxon's hERG classification model predicts both compounds to be safe.

Conclusions

- This study explored the impact of alkoxy chain length and 1-hydroxy group on 2-bromoalkoxy-anthraquinones' anticancer activity.
- Biological evaluation and *in silico* analysis corroborate findings.
- 1-hydroxy group is crucial for high cytotoxicity and hydrogen bond formation.
- Shorter alkoxy chains exhibit greater cytotoxicity.
- Compound 6b emerges as potent HCT116 cell inhibitor, surpassing 5-fluorouracil.
- 6b demonstrates high selectivity for HCT116, favorable lipophilicity, and efficient ligand metrics.
- *In vitro* and *in silico* results were aligned, suggesting 6b as a promising candidate for further preclinical evaluation and hit compound development.

Acknowledgements

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