EURASIAN JOURNAL OF MEDICAL AND NATURAL SCIENCES
Innovative Academy Research Support Center
IF = 9.2 www.in-academy.uz/index.php/ejmns

CYTOTOXIC EVALUATION OF SMALL DRUG-LIKE MOLECULES (ASPIRIN ANALOGUES) AGAINST HUMAN COLORECTAL CANCER CELLS

Dodiyev Akram Maxmudovich
Tashkent pharmaceutical institute
*e-mail: akram.dodiev01@gmail.com
https://doi.org/10.5281/zenodo.17580524

ARTICLE INFO

Received: 05th November 2025
Accepted: 10th November 2025
Online: 11th November 2025

KEYWORDS

Colorectal cancer, aspirin analogues, cytotoxic evaluation, H630 WT cell lines.

ABSTRACT

Colorectal cancer remains one of the leading causes of morbidity and mortality worldwide, highlighting the urgent need for new and effective anticancer agents. This study evaluated the cytotoxic activity of eight aspirin analogues (A1, A2, B1, B2, C1, C2, D1, D2) against human colorectal cancer H630 WT cell lines. The results showed that the degree of growth inhibition varied depending on the structural differences among the compounds. The analogues A2, B1, B2, and D2 demonstrated the most pronounced cytotoxic activity, significantly reducing cell viability. These findings suggest that especially A2, B2, and D2 are promising candidates for further investigation as potential anticancer agents in the treatment of colorectal cancer.

Introduction

Colorectal cancer (CRC) is one of the most serious and deadly malignancies worldwide, accounting for a significant portion of cancer-related deaths. Its incidence continues to rise globally, making it a major public health concern and emphasizing the urgent need for effective preventive and therapeutic strategies [1,2]. Current treatments, including surgery, chemotherapy, and targeted therapies, have improved patient outcomes, but they are often limited by low selectivity, drug resistance, and serious adverse effects. These limitations highlight the necessity to develop novel anticancer agents that are highly effective, safer, and capable of addressing the complex mechanisms involved in tumor progression [1,4].

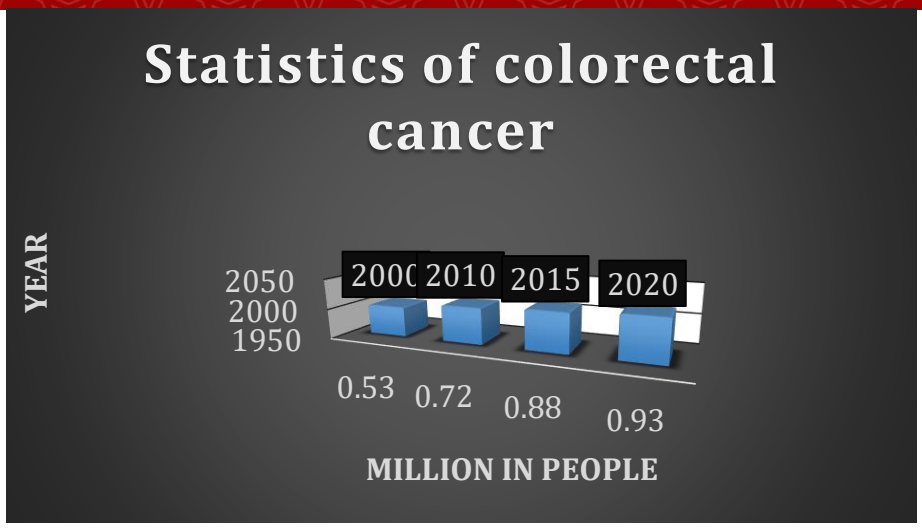

Figure 1: Death statistics of colorectal cancer from 2000 to 2020.

According to (Figure 1) the number of colorectal cancer deaths by 2040 may approach 1.3 million individuals, indicating that developing a new revolutionary treatment is an important challenge [2].

Aspirin (acetylsalicylic acid) has been widely investigated for its potential in CRC prevention and therapy due to its multifaceted biological effects. It reduces inflammation and lowers the risk of colorectal cancer primarily through the inhibition of cyclooxygenase (COX) enzymes, especially COX-1 and COX-2, which play a critical role in tumorigenesis and inflammation [3,5].Beyond its anti-inflammatory action, aspirin is involved in the complex modulation of apoptotic signalling pathways, inhibits angiogenesis, and exhibits immunomodulatory effects, making it a promising therapeutic agent in the context of CRC [6,8]. Clinical studies have confirmed that long-term aspirin use significantly reduces the risk of colorectal and gastrointestinal cancers. For example, aspirin treatment for six years or more was associated with a 15% reduction in CRC risk and a 19% reduction in gastrointestinal cancers. Large-scale population studies, including analyses of over 660,000 participants in the US Cancer Prevention Program, demonstrated that regular aspirin intake (at least 16 times per month) reduced colon cancer mortality by 40% over six years, and daily doses of 325 mg for at least five years were linked to a lower incidence of CRC [11, 13].

Despite these promising effects, the clinical application of aspirin is limited by dose-dependent adverse reactions, particularly gastrointestinal bleeding and ulceration. These side effects have prompted researchers to focus on **aspirin analogues**, small drug-like molecules designed to maintain or enhance anticancer activity while minimizing toxicity [7]. Studies indicate that even minor structural modifications of aspirin can significantly alter pharmacodynamics and pharmacokinetics, thereby increasing anticancer activity and reducing undesirable effects [2,8]. Specific analogues, such as fumaryldiaspirin (PN517) and benzoyl salicylates (PN524, PN528, PN529), have shown higher cytotoxicity against ovarian cancer and colorectal cancer cell lines compared to aspirin itself [14]. Furthermore, preclinical studies using derivatives dissolved in DMSO demonstrated strong inhibitory effects on tumor cell growth, with IC50 values below 2 μM for CRC cell lines, highlighting their potential therapeutic value [12].

The limitations of aspirin, combined with the promising results from analogue studies, underline the urgent need to develop new derivatives with enhanced anticancer efficacy and improved safety profiles [9]. This has led to a strategic focus on designing, synthesizing, and systematically evaluating novel aspirin analogues with diverse chemical structures, capable of exerting strong cytotoxic effects while minimizing side effects. Such studies aim not only to advance therapeutic options for CRC but also to expand understanding of the molecular mechanisms underlying drug efficacy, pharmacokinetics, and pharmacodynamics [15].

Based on the current literature, it is clear that aspirin and its analogues represent a promising avenue for CRC therapy. By systematically designing and evaluating structurally diverse derivatives, researchers hope to identify compounds with superior anticancer potential, providing a basis for safer and more effective clinical interventions. This study aims to create a comprehensive library of aspirin analogues, assess their cytotoxic activity against CRC cell lines, and investigate their chemical and physical properties to establish a detailed profile of their molecular behaviour. Ultimately, this research contributes to the ongoing effort to develop innovative, effective, and low-toxicity treatments for colorectal cancer, offering new hope for patients and advancing the field of cancer therapeutics [6,10].

Research aim: The major goal of this study aspirin analogues against colorectal cancer cell lines. This work aims to discover analogues with higher cytotoxic action by methodically changing the chemical structure of aspirin, contributing to the discovery of novel therapeutic medicines for colorectal cancer therapy.

Research objectives: Cytotoxic Evaluation: The primary purpose of this research is to carry out a full and comprehensive evaluation of the cytotoxic activity of the aspirin analogues. A variety of CRC cell lines will be systematically treated to varied doses of these analogues using the reliable and time-tested MTT technique. The findings of this investigation will be cytotoxicity data, which will provide crucial insight into each analogue's potential anticancer effectiveness.

Materials and Methods: Dulbecco's Modified Eagle's Medium (DMEM) and fetal bovine serum (FBS) were purchased from PAA (Somerset, UK) or Sigma-Aldrich (Dorset, UK). Precision Plus Protein Colour standards and nitrocellulose membranes were used for protein analysis. 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), dimethyl sulfoxide (DMSO), and propidium iodide were obtained from Sigma-Aldrich. All other chemicals and reagents were sourced from Sigma-Aldrich and Acros Organics.

CODE	NAME OF CHEMICAL STRUCTURES
A1	2-Benzoyloxy-5-bromobenzoic acid.
A2	2-Benzoyloxy-5-chlorobenzoic acid.
B1	5-Chloro-2-{[(4-chlorophenyl)carbonyl]oxy}benzoic acid.
B2	5-Bromo-2-(4-chlorobenzoyl)oxybenzoic acid.
C1	2-Bromo-5-hydroxybenzoic acid.
C2	2-Chloro-5-hydroxybenzoic acid.

D1	5-Bromo-2-(3-methylbenzoyl)oxybenzoic acid.
D2	5-Chloro-2-(3-methylbenzoyl)oxybenzoic acid.

Table 1: Molecules investigated in the present study.

Before adding compounds to cells, they were prepared as a stock (0.5 M) in DMSO. The cytotoxic impact of aspirin and the other new substances was assessed using a modified MTT test. In 96-well microplates, 60 cells were grown in each well. The culture medium was discarded 72 hours after the initial seeding, and after that, the cells were incubated with a medium containing drugs at the required concentration and incubated for the tested time at 37°C. Then replaced with medium containing 200 l of 0.5 mg/ml MTT per well and incubated for four hours at 37°C then replaced with 200 l of DMSO and was incubated for 10 minutes at 37°C to develop a coloured formazan complex. A visible plate reader (Thermo Scientific) was used to measure absorbance at 540 nm. Viability was reported as the percentage of treated cells relative to the cells in control wells.

Results of absorbance reading and graphs: Statistical data and graphs obtained during the cytotoxicity of H 630 WT cells products are presented below in the form of tables and graphs.

Code		Absorbance 540nm									
		1	2	3	4	5	6	7	8	9	10
A1	A	0.113	1.243	2.471	2.460	3.823	2.315	2.586	2.357	1.875	2.366
	B	0.167	1.614	2.141	2.378	3.745	2.505	2.586	2.408	2.033	2.151
	C	0.081	1.356	2.052	2.751	2.246	2.473	2.284	2.141	2.567	3.089
A2	D	0.216	1.167	2.715	2.842	2.214	2.218	2.540	3.380	2.049	2.640
	E	0.201	1.223	2.327	2.583	2.249	2.509	2.409	2.755	2.359	2.983
	F	0.067	0.141	2.583	2.575	2.298	2.335	2.575	1.875	1.570	3.464

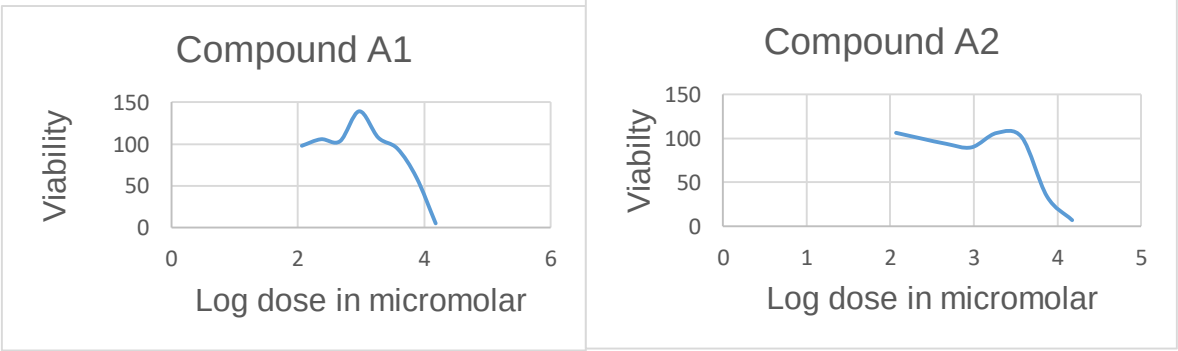

Figure 6A: Table and graphs of preparations A1 and A2 studied in this research.

Code		Absorbance 540nm									
		1	2	3	4	5	6	7	8	9	10
B1	A	0.067	0.283	1.500	3.073	2.641	2.100	2.152	1.799	1.932	2.311
	B	0.207	0.234	2.001	2.463	2.317	2.086	2.141	2.373	2.084	2.271
	C	0.075	0.230	1.972	0.520	2.288	2.390	2.179	2.089	2.282	2.404

B2	D	0.074	0.206	0.776	2.632	2.455	2.337	2.242	2.272	2.105	2.266
	E	0.137	0.262	1.924	2.707	2.585	2.413	2.329	1.961	2.186	2.210
	F	0.088	0.118	1.784	2.556	1.969	2.018	2.598	2.294	1.983	2.493

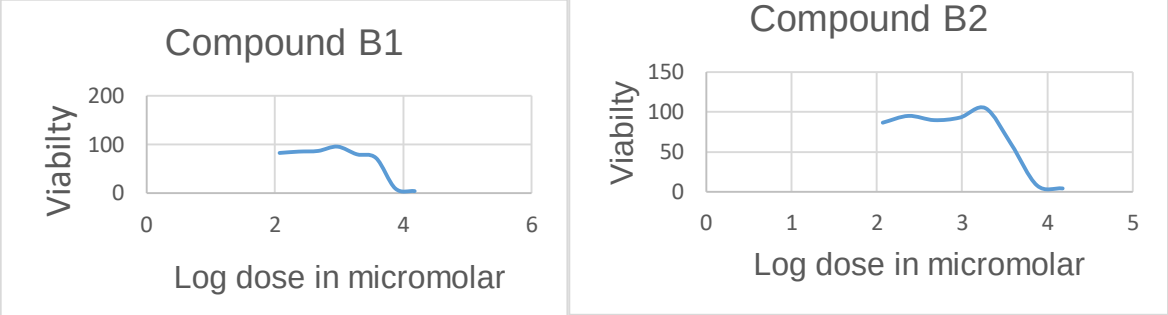

Figure 6B: Table and graphs of preparations B1 and B2 studied in this research.

Code		Absorbance 540nm									
		1	2	3	4	5	6	7	8	9	10
C1	A	0.677	1.926	2.341	2.143	2.924	2.633	2.503	2.398	2.413	2.373
	B	0.922	2.118	2.256	1.975	2.662	2.422	2.239	2.254	2.224	2.191
	C	0.685	2.454	2.895	1.959	2.716	2.503	2.519	2.414	1.861	2.039
C2	D	0.291	1.474	2.232	2.856	2.910	2.682	2.694	2.311	2.231	1.904
	E	0.301	1.587	2.221	2.351	2.579	2.776	2.233	2.549	1.987	2.171
	F	0.230	1.635	2.067	1.903	1.954	1.685	1.864	1.879	1.885	1.737

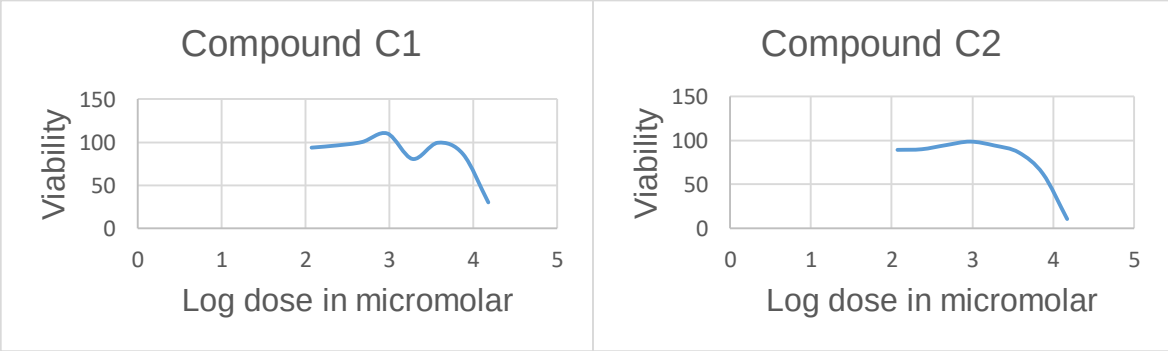

Figure 6C: Table and graphs of preparations C1 and C2 studied in this research.

Code		Absorbance 540nm									
		1	2	3	4	5	6	7	8	9	10
D1	A	0.212	0.076	2.059	2.032	2.210	1.937	1.851	2.083	2.295	2.013
	B	0.135	1.205	0.170	2.339	2.243	2.122	2.194	2.050	1.952	2.311
	C	0.192	1.405	2.320	2.814	2.314	2.363	2.236	2.465	1.973	2.358
D2	D	0.245	0.281	2.374	2.455	2.325	2.185	2.387	2.359	2.208	1.829
	E	0.137	0.183	1.265	3.014	2.418	2.386	2.336	2.494	2.504	2.158
	F	0.187	0.242	2.291	2.708	2.549	2.834	2.049	2.277	2.600	1.948

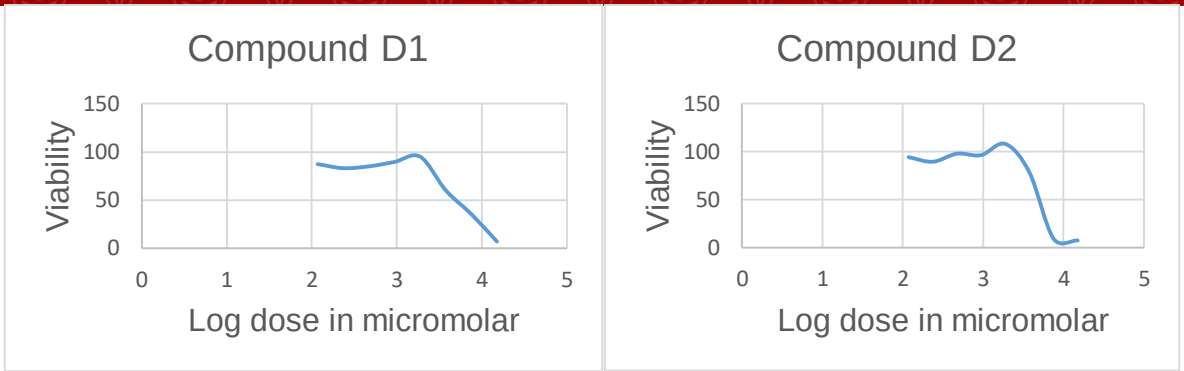

Figure 6D: Table and graphs of preparations D1 and D2 studied in this research.

Results of Cytotoxicity. *Sample Description:* The eight different aspirin analogues (A1, A2, B1, B2, C1, C2, D1, D2) at the heart of this scientific inquiry were strictly using well-established techniques. These analogues were subjected to a comprehensive cytotoxicity assessment using the H630 WT colorectal cancer cell line.

Code	Type of compounds	IC50 (μM)	Antilog
A1	2-Benzoyloxy-5-bromobenzoic acid	3,8	6309,573
A2	**2-Benzoyloxy-5-chlorobenzoic acid**	3,6	3981,072
B1	**5-Chloro-2-{[(4-chlorophenyl)carbonyl]oxy}benzoic acid**	3,7	5011,872
B2	**5-Bromo-2-(4-chlorobenzoyl)oxybenzoic acid**	3,6	3981,072
C1	**2-Bromo-5-hydroxybenzoic acid**	4,1	12589,25
C2	**2-Chloro-5-hydroxybenzoic acid**	4	10000
D1	**5-Bromo-2-(3-methylbenzoyl)oxybenzoic acid**	3,8	6309,573
D2	**5-Chloro-2-(3-methylbenzoyl)oxybenzoic acid**	3,7	5011,872

Table 2: IC50 values (mM) for compounds using the MTT assay to colorectal cancer cell line (H 630 WT).

A comprehensive analysis of the ability to inhibit the rate of development of a specific biological target in eight aspirin analogues, A1, A2, B1, B2, C1, C2, D1 and D2 was part of the main task assigned to this study. Determination of the required concentration of each analogue to inhibit the target by 50%, namely the IC50 value, was necessary for quantitative analysis (Table 2).

Several aspirin analogues show activity against colorectal cancer cells. The cytotoxic evaluation of the analogues on H630 WT colorectal cancer cell lines was comprehensively

investigated in this research. The goal of the analysis was to identify the potential of these analogues as anticancer therapies. The growth inhibition percentages were utilized as quantitative measurements to indicate the degree of cytotoxicity demonstrated by each analogue. The findings for compounds A2, B1, B2, and D2 were particularly outstanding, indicating considerable cytotoxic potential with growth inhibition. Importantly, this remarkable discovery was frequently replicated throughout the experiment, increasing the data's robustness.

Discussion: The congruence of outcomes reflects the study's primary aim – exploring the potential anticancer properties of novel aspirin analogues against colorectal cancer. The cytotoxic evaluations collectively provide insights into the analogues' effectiveness and chemical profiles. The effect of hydroxy substitution. The IC50 values of the C1 and C2 analogues containing hydroxy substitutions are equal to 4.1 and 4.0 μM. These values are higher than those of their analogues, including benzoyloxy substitutions. This means that hydroxyl groups reduce the inhibitory ability of compounds since they create spatial distortions and electronic effects that interfere with interaction with the target

Conclusion: Summing up the results of our study, was concluded that the effectiveness of the inhibitory action of compounds is determined by structural motives and specific substituents. The limitations of this study that we have recognized may become opportunities for further research projects to contribute to the creation of medicines with high efficiency, which can be achieved by determining the structure of compounds and mechanisms of action. Colorectal cancer cell lines H630 WT were used to determine the cytotoxic potential of analogues. The high efficiency of inhibiting the growth of cancer cells by compounds A1, B1, B2, and D1 focuses on promising indicators of their cytotoxic activity, and subsequent studies are necessary to confirm the antitumor activity of these analogues about colorectal cancer. The volume of knowledge in the field of developing advanced approaches in the treatment of colorectal cancer was supplemented with data obtained during the successful evaluation of aspirin analogues and the results of this study can become the basis for future research aimed at creating new treatment approaches and complementing existing methods.

References:

1. Australian Journal of General Practice. (2023). *Chemoprevention*. [online] Available at: https://www1.racgp.org.au/ajgp/2018/december/chemoprevention-a-new-concept-for-cancer-preventio.
2. Bashir, A.I.J. (2017). A novel mechanism for the anti-cancer activity of aspirin and its analogues. *wlv.openrepository.com*. [online] Available at: https://wlv.openrepository.com/handle/2436/620956.
3. Bowel Cancer UK. (2019). *Aspirin | Reduce your risk | About bowel cancer*. [online] Available at: https://www.bowelcanceruk.org.uk/about-bowel-cancer/risk-factors/reducing-your-risk/aspirin/.

4. Chan, A.T., Ogino, S. and Fuchs, C.S. (2009). Aspirin use and survival after diagnosis of colorectal cancer. *JAMA*, [online] 302(6), pp.649–58. doi:https://doi.org/10.1001/jama.2009.1112.

5. Chheda, P.B. (2014). *Assessment of the in vitro efficacy of aspirin and aspirin analogues in combination with standard chemotherapeutics in glioma cell lines*. [online] clok.uclan.ac.uk. Available at: https://clok.uclan.ac.uk/11162/.

6. Claudius, A.-K., Kankipati, C.S., Kilari, R.S., Hassan, S., Guest, K., Russell, S.T., Perry, C.J., Stark, L.A. and Nicholl, I.D. (2014). Identification of aspirin analogues that repress NF-κB signalling and demonstrate anti-proliferative activity towards colorectal cancer in vitro and in vivo. *Oncology Reports*, [online] 32(4), pp.1670–1680. doi:https://doi.org/10.3892/or.2014.3373.

7. Din, F.V.N., Theodoratou, E., Farrington, S.M., Tenesa, A., Barnetson, R.A., Cetnarskyj, R., Stark, L., Porteous, M.E., Campbell, H. and Dunlop, M.G. (2010). Effect of aspirin and NSAIDs on risk and survival from colorectal cancer. Gut, [online] 59(12), pp.1670–1679. doi:https://doi.org/10.1136/gut.2009.203000.

8. Garcia-Albeniz, X. and Chan, A.T. (2011). Aspirin for the prevention of colorectal cancer. *Best Practice & Research Clinical Gastroenterology*, [online] 25(4-5), pp.461–472. doi:https://doi.org/10.1016/j.bpg.2011.10.015.

9. International Aspirin Foundation (2020). The Chemistry of Aspirin | *The International Aspirin Foundation*. [online] www.aspirin-foundation.mtcdevserver4.com. Available at: https://www.aspirin-foundation.com/history/chemistry/#:~:text=Aspirin%20is%20prepared%20by%20chemical.

10. Ismail, N.I., Othman, I., Abas, F., H. Lajis, N. and Naidu, R. (2020). The Curcumin Analogue, MS13 (1,5-Bis(4-hydroxy-3- methoxyphenyl)-1,4-pentadiene-3-one), Inhibits Cell Proliferation and Induces Apoptosis in Primary and Metastatic Human Colon Cancer Cells. *Molecules*, 25(17), p.3798. doi:https://doi.org/10.3390/molecules25173798.

11. Kankipati, C.S., Perry, C.J. and Nicholl, I.D. (2014). Abstract 335: Aspirin, di-aspirin and their toxicity to colorectal cancer. *Cancer Research*, 74(19_Supplement), pp.335–335. doi:https://doi.org/10.1158/1538-7445.am2014-335.

12. Kim, B., Park, S.J., Hong, S.P., Cheon, J.H., Kim, W.H. and Kim, T.I. (2015). The effect of prediagnostic aspirin use on the prognosis of stage III colorectal cancer. *International journal of clinical and experimental medicine*, [online] 8(8), pp.13435–45. Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4612965/

13. Nicholl, I. (2011). Activity of aspirin analogues and vanillin in a human colorectal cancer cell line. *Oncology Reports*. doi:https://doi.org/10.3892/or.2011.1320.

14. uclahealth.org. (2021). *Should you take aspirin to prevent colorectal cancer?* [online] Available at: https://www.uclahealth.org/news/should-you-take-aspirin-to-prevent-colorectal-cancer#:~:text=But%20for%20certain%20people%2C%20taking.

15. Sleda, M.A., Albasrawi, H.K. and Timmons, S.C. (2018). Synthesis of Aspirin Analogs for Anticancer and Antibacterial Testing. *The FASEB Journal*, 32(S1). doi:https://doi.org/10.1096/fasebj.2018.32.1_supplement.531.8.