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DESIGN, SYNTHESIS AND BIOLOGICAL EVALUATION OF NOVEL SUBSTITUTED CINNAMAMIDES OF 5-AMINO SALICYLIC ACID

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ABSTRACT
5-Aminosalicylic acid (5-ASA), known as mesalamine, is commonly used to treat various inflammatory conditions such as ulcerative colitis (UC), Crohn's disease (CD) and inflammatory bowel disease (IBD). Previous research has shown that the cinnamic acid and its derivatives are safe, effective and possess significant anti-inflammatory activity. Hence, the current work focuses on the preparation of some novel derivatives of 5-ASA by conjugating it with various substituted cinnamic acids through amide linkage. The compounds were characterised by physical and spectral data and were evaluated for *in vitro* antioxidant activity and anti-inflammatory activity. *In-silico* studies were performed to assess the drug-likeliness, anti-inflammatory activity and toxicity of the compounds using online computational tools. Compounds **6g** (3, 4, 5- OCH_3), and **6e** (4-OCH_3) showed strong antioxidant potential, while **6g** (3,4,5-OCH_3), **6b** (4-Cl), **6c** (4-F) and **6i** (4-$CH(CH_3)_2$) exhibited excellent anti-inflammatory effects. All derivatives complied with Lipinski's rule, suggesting good oral bioavailability and PASS/OSIRIS analyses indicated significant predicted anti-inflammatory activity with low toxicity risk. Compounds **6g, 6b, 6c** and **6i** emerged as promising lead candidates for the development of safe and effective anti-inflammatory agents.
Keywords: 5-Aminosalicylic Acid, *In Vitro* Antioxidant, *In Vitro* Anti-Inflammatory, *In Silico* Studies, Cinnamamide, Mesalamine.
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INTRODUCTION

5-Aminosalicylic acid (5-ASA) belongs to the class of aminosalicylic acids and is extensively used in the treatment of inflammatory conditions with minimal side effects.[1] Hence, several derivatives have been developed by coupling it with molecules such as amino acids[2-4], polymers[5], aromatic acid[6,7] and amines derivatives etc.[8-11] The main disadvantages of these derivatives include unknown safety profiles, toxicity and lack of pharmacological activity. Therefore, our search for a newer derivative of 5-ASA led to the identification of cinnamamides with a potent pharmacological profile.[12] Previous studies have demonstrated that cinnamamide derivatives containing a morpholine moiety exhibit notable antioxidant and antimicrobial activities.[13] In addition, several cinnamamide-based compounds have shown significant systemic anti-inflammatory effects[14–16] and therapeutic potential under specific pathological conditions such as inflammation associated with sepsis, acute lung injury[17], dermatitis[18] and myocardial ischemia.[19]

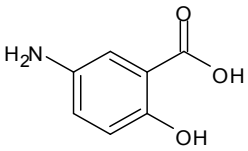

Fig.-1: 5-aminosalicylic Acid

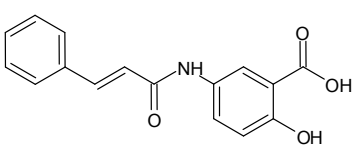

Fig.-2: Cinnamamide of 5-aminosalicylic Acid

Hence, the present study was designed to synthesize various substituted cinnamoyl conjugates of 5-ASA through amide linkage and evaluate their *in vitro* antioxidant activity and *in vitro* anti-inflammatory activity. Additionally, *in-silico* studies were planned to gain a better understanding of the

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physicochemical properties, predicted anti-inflammatory activity and toxicity of the title compounds using online computational tools.

EXPERIMENTAL

Materials and Methods

All chemicals were procured from Sigma Aldrich, Hi Media Laboratories and SR CHEM chemicals. Melting points were determined using open capillaries on a Bio Technics India-BTI-34 electric melting point apparatus and are uncorrected. Precoated alumina plates with silica gel were used to perform TLC, and spots were detected using a Bio Technics India-R/340/OB UV chamber. The IR spectra were recorded using KBr Pellets on a Bruker OPUS_7.5.18 Infrared spectrophotometer (cm^{-1}). ^{1}H and ^{13}C spectra were documented on SA-Varian 400MHz, SA-Agilent 400 MHz, Bruker® Avance 500 MHz and 100 MHz spectrometers in DMSO using TMS as internal standard (chemical shifts in δ ppm). Mass spectra were recorded on Shimadzu LCMS-8040 and Agilent SD/AD/INS/013, 015 LCMS using ESI.

Preparation of Novel Substituted Cinnamamides (6a-6m) of 5-ASA

Step 1: Preparation of Cinnamic Acids (3a-3m)

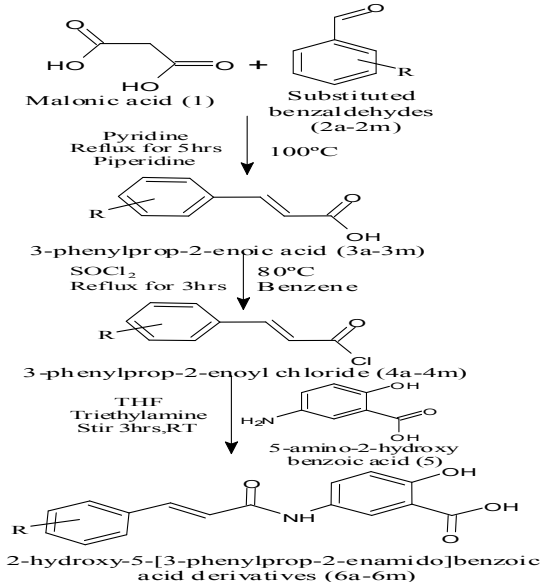
Various substituted aromatic benzaldehydes (0.015 mol) and malonic acid (0.035 mol) were added to a round-bottom flask containing a mixture of 7.5 mL of pyridine and 1–2 drops of piperidine and refluxed for 5 hours at 100°C. The reaction mixture was then poured into a solution of water and hydrochloric acid (pH 4.0) to remove excess pyridine. The resulting precipitate was separated by vacuum filtration, dried and recrystallised from ethanol.[20]

Step 2: Preparation of Cinnamoyl Chlorides (4a-4m)

Weighed quantities of substituted cinnamic acids (0.01 mol) and thionyl chloride (0.04 mol) were refluxed in dry benzene for 3 hours at 80°C. The excess thionyl chloride was removed by rotary evaporator, and the obtained cinnamoyl chloride was kept stable by adding a small amount of tetrahydrofuran.[21]

Step 3: Preparation of Cinnamamides (6a-6m)

To a solution of 5-ASA (0.01 mol) in THF (10 mL), substituted cinnamoyl chloride (0.01mol) in THF (10 mL) was added. A few drops of TEA were then added and stirred at RT for 3 hours. The excess solvent was removed by rotary evaporator, and ice-cold water was added to obtain a solid precipitate. The product was separated by vacuum filtration, dried and recrystallised from ethanol.[22]



Compound Code	R
6a	H
6b	4-Cl
6c	4-F
6d	4-Br
6e	4-OCH_3
6f	3,4-OCH_3
6g	3,4,5-OCH_3
6h	4-CH_3
6i	4-$CH(CH_3)_2$
6j	4-$C(CH_3)_3$
6k	4-CN
6l	4-CF_3
6m	4-NO_2

Fig.-3: Scheme Depicting the Synthesis of Novel Substituted Cinnamamides of Mesalamine
A Total of Thirteen Compounds Were Prepared using the Mentioned Procedure and were characterised by their Melting Point and Spectral Studies, including IR, ^{1}H NMR, ^{13}C NMR, and Mass Spectrometry

In-vitro **Antioxidant Activity**

DPPH Radical Scavenging Assay

Solutions of various test compounds at a concentration of 100 µM were added to 100 µM DPPH in 95% ethanol. Further procedure was carried out following the standard procedure.[23] Ascorbic acid and 5-ASA were used as reference standards. The percentage of scavenging activity was calculated by using the formula:

$$\text{Percentage Reduction of DPPH} = \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{test}}}{\text{Abs}_{\text{control}}} \times 100$$

Nitric Oxide Radical Scavenging Assay

Sodium nitroprusside (5 mM) in phosphate buffer saline (pH 7.4) was incubated with the test drug at a concentration of 100 µM, dissolved in 95% alcohol. Further procedure was carried out following the standard procedure.[23] Ascorbic acid and 5-ASA were used as reference standards. The % of scavenging activity was calculated using the formula.

$$\text{Percentage Nitric oxide scavenging activity} = \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{test}}}{\text{Abs}_{\text{control}}} \times 100$$

In-vitro **Anti-inflammatory Activity**

Protein Denaturation Method

Samples were prepared by mixing 2 mL of each test compound solution (100 µM), following the reported procedure.[24] 5-ASA served as the reference standard, and the percentage inhibition of protein denaturation was calculated using the formula

$$\text{Percentage Inhibition of denaturation} = \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{test}}}{\text{Abs}_{\text{control}}} \times 100$$

HRBC Membrane Stabilisation Method

For the assay, mixtures containing 0.5 mL of test compound solution (100 µM) were prepared following the reported procedure.[25] 5-ASA was used as a reference standard, and the percentage protection of HRBC was calculated using the formula:

$$\% \text{ Protection} = 100 - [(\text{OD}_{\text{test}} / \text{OD}_{\text{control}}) \times 100]$$

In-silico **Studies**

Molinspiration

The structures of the cinnamamides and the standard 5-ASA were generated, and their molecular properties, such as log P value, molecular weight, topological polar surface area (TPSA), hydrogen bond acceptor and donors, number of rotatable bonds and molecular volume, were calculated.[26,27]

PASS Analysis

PASS (Prediction of Activity Spectra for Substances) is a software tool designed to assess the biological potential of molecules. PASS can be employed to estimate the biological activity profiles of molecules before their preparation and pharmacological testing.[28,29]

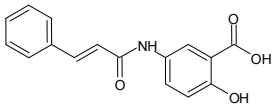
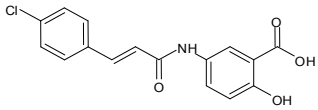
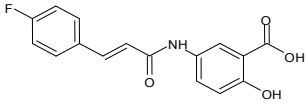
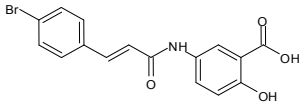
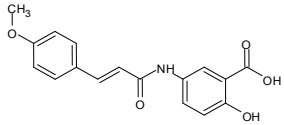
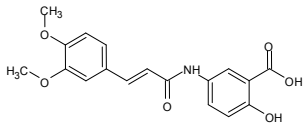
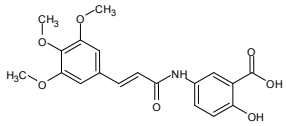
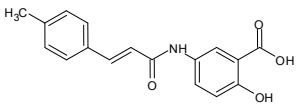
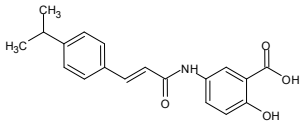
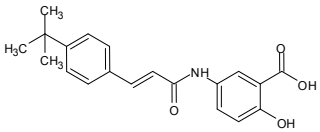
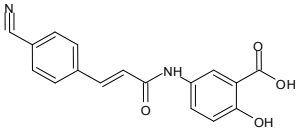
OSIRIS Property Explorer

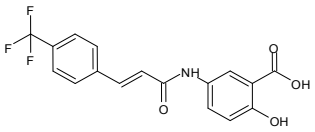
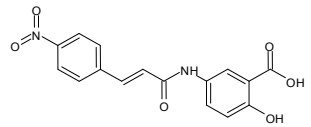
OSIRIS is a software tool employed in drug discovery and development to evaluate the drug-likeness, toxicity and other properties of chemical compounds.[30,31]

RESULTS AND DISCUSSION

Novel cinnamamides of 5-ASA **(6a-6m)** were synthesised by the amidation of 5-ASA with various substituted cinnamic acid chlorides. The substituted cinnamic acids were synthesised from substituted aromatic benzaldehydes and malonic acid in piperidine and pyridine. Subsequently, cinnamoyl chlorides were synthesised by treating the cinnamic acids with thionyl chloride. The cinnamic acid amide conjugates of 5-ASA were then obtained by stirring 5-ASA with cinnamoyl chlorides in THF in the presence of TEA. The reactions were monitored by TLC using a benzene: ethyl acetate (1:1), and the resulting compounds were purified from rectified spirit. The physical properties of the compounds are presented in Table-1.

Table-1: Physical Data of Novel 2-hydroxy-5-[3-phenylprop-2-enamido] Benzoic Acids (6a-6m)

S. No.	Compound Code	Structure of the compound	Molecular Formula	Molecular Weight	Melting point* (ºC)	Yield (%)	R_f**
1	6a		$C_{16}H_{13}NO_4$	283	210-212	81.2	0.45
2	6b		$C_{16}H_{12}NO_4Cl$	318	245-250	58.22	0.50
3	6c		$C_{16}H_{12}NO_4F$	301	250-252	80.00	0.54
4	6d		$C_{16}H_{12}NO_4Br$	362	230-235	81.81	0.38
5	6e		$C_{17}H_{15}NO_5$	313	215-220	77.21	0.42
6	6f		$C_{18}H_{17}NO_6$	343	190-192	91.10	0.52
7	6g		$C_{19}H_{19}NO_7$	373	85-90	78.20	0.61
8	6h		$C_{17}H_{15}NO_4$	297	190-195	78.70	0.30
9	6i		$C_{19}H_{19}NO_4$	325	220-225	82.35	0.43
10	6j		$C_{20}H_{21}NO_4$	339	235-240	84.40	0.56
11	6k		$C_{17}H_{12}N_2O_4$	308	225-230	78.65	0.50

12	6l		$C_{17}H_{12}NO_4F_3$	351	230-235	86.40	0.50
13	6m		$C_{16}H_{12}N_2O_6$	328	95-100	71.05	0.35

*Recrystallized from rectified spirit. **Solvent system: - benzene: ethyl acetate (1:1)

Spectral Data

6a: 2-Hydroxy-5-(3-phenylprop-2-enamido) benzoic acid

IR (KBr) **υ_{max}:** 3392 (br, NH str, OH str), 1677 (C=O str), 1620 (C=O str, amide I), 1560 (NH bend, amide II) cm^{-1}; ^1H (500 MHz) δ: 6.71-6.73 (d, 1H, COCH=CH), 6.84-6.88 (d, 1H, Ar), 7.39-7.45 (m, 3H, Ar), 7.52-7.55 (d, 1H, COCH=CH), 7.60-7.62 (d, 2H, Ar), 7.68-7.71 (dd, 1H, Ar), 8.04 (d, 1H, Ar), 10.14 (s, 1H, NH) ppm; ^{13}C (100 MHz) δ: 116.48, 118.15, 121.67,123.26, 125.31, 128.10 (2C), 129.38, 129.46, 130.02 (2C), 135.40, 139.59, 158.70, 163.44, 172.27 ppm; Mass m/z: 284 $(M+H)^+$.

6b: 5-[3-(4-chlorophenyl) prop-2-enamido]-2-hydroxybenzoic acid

IR (KBr) **υ_{max}:** 3445 (br, OH str), 3264 (NH str), 1680 (C=O str), 1621 (C=O str, amide I), 1541 (NH bend, amide II), 1086 (C-Cl str, Ar) cm^{-1}; ^1H (400 MHz) δ: 6.76-6.80 (d, 1H, COCH=CH), 6.86-6.88 (d, 1H, Ar), 7.46-7.60 (m, 2H, Ar; 1H, COCH=CH), 7.63-7.65 (d, 2H, Ar), 7.72-7.75 (dd, 1H, Ar), 8.13-8.14 (d, 1H, Ar), 10.14 (s, 1H, NH) ppm; ^{13}C (100 MHz) δ: 116.87, 120.82, 123.14, 126.48, 129.03 (2C), 129.35 (2C), 129.91, 130.43, 133.73, 134.09, 138.30, 157.55, 162.94, 171.86 ppm; Mass m/z: 318 (M), 320 $(M+2H)^+$.

6c: 5-[3-(4-fluorophenyl) prop-2-enamido]-2-hydroxybenzoic acid

IR (KBr) **υ_{max}:** 3440 (br, OH str), 3392 (NH str), 1677 (C=O str), 1620 (C=O str, amide I), 1544 (NH bend, amide II), 1224 (C-F str, Ar) cm^{-1}; ^1H (400 MHz) δ: 6.70-6.74 (d, 1H, COCH=CH), 6.90-6.92 (d, 1H, Ar), 7.26-7.30 (t, 2H, Ar), 7.54-7.58 (d, 1H, COCH=CH), 7.66-7.70 (dd, 2H, Ar), 7.74-7.77 (dd, 1H, Ar),8.17 (d, 1H, Ar), 10.16 (s, 1H, NH) ppm; ^{13}C (100 MHz) δ: 113.59, 115.88, 116.09, 117.06, 120.66, 122.12, 126.88, 129.83, 129.92, 130.71, 131.37, 138.60, 157.37, 161.59, 163.15, 171.75 ppm; Mass m/z: 302 $(M+H)^+$.

6d: 5-[3-(4-bromophenyl) prop-2-enamido]-2-hydroxybenzoic acid

IR (KBr) **υ_{max}:** 3444 (br, OH str), 3265 (NH str), 1677 (C=O str), 1620 (C=O str, amide I), 1542 (NH bend, amide II), 970 (C-Br str, Ar) cm^{-1}; ^1H (400 MHz) δ: 6.77-6.81 (d, 1H, COCH=CH), 6.88-6.90 (d, 1H, Ar), 7.51-7.59 (m, 2H, Ar; 1H, COCH=CH), 7.62-7.65 (dd, 2H, Ar), 7.74-7.76 (dd, 1H, Ar), 8.16 (d, 1H, Ar), 10.17 (s, 1H, NH) ppm; ^{13}C (100 MHz) δ: 113.85, 117.01, 120.14, 120.68, 122.87, 123.14, 126.72, 129.61, 130.16, 130.58, 131.84, 138.46, 142.60, 157.46, 162.95, 171.76 ppm; Mass m/z: 364 $(M+H)^+$.

6e: 2-hydroxy-5-[3-(4-methoxyphenyl) prop-2-enamido] benzoic acid

IR (KBr) **υ_{max}:** 3451 (br, OH str), 3260 (NH str), 1690 (C=O str), 1610 (C=O str, amide I), 1529 (NH bend, amide II), 1264 (C-O-C, Ar, asym), 1027 (C-O-C, Ar, sym) cm^{-1}; ^1H (400 MHz) δ: 3.80 (s, 3H, OCH_3), 6.61-6.65 (d, 1H, COCH=CH), 6.92-6.94 (d, 1H, Ar), 6.99-7.01 (d, 2H, Ar), 7.49-7.53 (d, 1H, COCH=CH), 7.56-7.58 (d, 2H, Ar), 7.76-7.79 (dd, 1H, Ar), 8.19-8.20 (d, 1H, Ar), 10.09 (s, 1H, NH) ppm; ^{13}C (100 MHz) δ: 55.29, 112.96, 114.47 (2C), 117.18, 119.62, 120.50, 127.17,127.30 (2C), 129.35, 131.11, 139.67, 157.16, 160.57, 163.64, 171.79 ppm; Mass m/z: 314 $(M+H)^+$.

6f: 5-[3-(3,4-dimethoxyphenyl) prop-2-enamido]-2-hydroxybenzoic acid

IR (KBr) **υ_{max}:** 3555, 3428 (br, OH str), 3288 (NH str), 1690 (C=O str), 1618 (C=O str, amide I), 1542 (NH bend, amide II), 1222 (C-O-C, Ar, asym), 1021 (C-O-C, Ar, sym) cm^{-1}; ^1H (400 MHz) δ: 3.79-3.83 (2s, 6H, $(OCH_3)_2$), 6.63-6.67 (d, 1H, COCH=CH), 6.92-6.94 (d, 1H, Ar), 7.02-7.04 (d, 1H, Ar), 7.16-7.23

(m, 2H, Ar), 7.48-7.52 (d, 1H, COCH=CH), 7.75-7.78 (dd, 1H, Ar), 8.21-8.22 (d, 1H, Ar), 10.09 (s, 1H, NH) ppm; ^{13}C (100 MHz) δ: 55.39, 55.55, 109.98, 111.75, 112.61, 117.23, 119.74, 120.30, 121.70, 127.18,127.45, 131.19, 140.00, 148.90, 150.34, 157.05, 163.57, 171.71 ppm; Mass m/z: 344 $(M+H)^+$.

6g: 2-hydroxy-5-[3-(3,4,5-trimethoxyphenyl) prop-2-enamido] benzoic acid
IR (KBr) υ_{max}**:** 3460 (br, OH str), 3330 (NH str), 1672 (C=O str), 1617 (C=O str, amide I), 1551 (NH bend, amide II), 1211 (C-O-C, Ar, asym), 998 (C-O-C, Ar, sym) cm^{-1}; ^{1}H (400 MHz) δ: 3.67-3.73 (s, 3H, OCH_3), 3.81-3.87 (s, 6H, $(OCH_3)_2$), 6.70-6.78 (d, 1H, COCH=CH), 6.94-7.03 (m, 3H, Ar), 7.49-7.55 (d, 1H, COCH=CH), 7.76-7.79 (dd, 1H, Ar), 8.24 (d, 1H, Ar), 10.19 (s, 1H, NH) ppm; ^{13}C (100 MHz) δ: 55.91, 56.04, 60.15, 105.11, 105.78, 112.54, 117.35, 118.55, 120.39, 121.53, 131.23, 138.91, 140.11, 144.25, 153.12, 153.16, 157.18, 163.43, 171.80 ppm; Mass m/z: 374 $(M+H)^+$.

6h: 2-hydroxy-5-[3-(4-methylphenyl) prop-2-enamido] benzoic acid
IR (KBr) υ_{max}**:** 3449 (br, OH str), 3254 (NH str), 1680 (C=O str), 1619 (C=O str, amide I), 1538 (NH bend, amide II) cm^{-1}; ^{1}H (400 MHz) δ: 2.33 (s, 3H, CH_3), 6.70-6.74 (d, 1H, COCH=CH), 6.93-6.96 (d, 1H, Ar), 7.24-7.26 (d, 2H, Ar), 7.50-7.56 (m, 2H, Ar), 7.57-7.58 (d, 1H, COCH=CH), 7.76-7.79 (dd, 1H, Ar), 8.21-8.22 (d, 1H, Ar), 10.15 (s, 1H, NH) ppm; ^{13}C (100 MHz) δ: 20.95, 112.55, 117.24, 120.40, 121.06, 127.29, 127.65 (2C), 129.59 (2C), 131.07, 131.94, 139.54, 139.86, 157.08, 163.40, 171.67 ppm; Mass m/z: 298 $(M+H)^+$.

6i: 2-hydroxy-5-{3-[4-(propan-2-yl) phenyl] prop-2-enamido} benzoic acid
IR (KBr) υ_{max}**:** 3451 (br, OH str), 3295 (NH str), 1680 (C=O str), 1619 (C=O str, amide I), 1552 (NH bend, amide II) cm^{-1}; ^{1}H (400 MHz) δ: 1.20-1.22 (d, 6H, $CH(CH_3)_2$), 2.88-2.93 (septet, 1H, CH), 6.61-6.63 (d, 1H, COCH=CH), 6.72-6.76 (d, 1H, Ar), 7.30-7.32 (d, 2H, Ar), 7.46-7.50 (d, 1H, COCH=CH), 7.51-7.53 (d, 2H, Ar), 7.56-7.58 (dd, 1H, Ar), 7.94-7.95 (d, 1H, Ar), 9.88 (s, 1H, NH) ppm; ^{13}C (100 MHz) δ: 23.68 (2C), 33.31, 115.57, 119.60, 121.54, 121.73, 123.94, 126.94 (2C), 127.66 (2C), 128.79, 132.57, 138.99, 150.10, 158.67, 162.90, 171.96 ppm; Mass m/z: 326 $(M+H)^+$.

6j: 5-[3-(4-*tert*-butylphenyl) prop-2-enamido]-2-hydroxybenzoic acid
IR (KBr) υ_{max}**:** 3461 (br, OH str), 3292 (NH str), 1690 (C=O str), 1621 (C=O str, amide I), 1554 (NH bend, amide II) cm^{-1}; ^{1}H (400 MHz) δ:1.23-1.29 (s, 9H, $(CH_3)_3$), 6.63-6.66 (d, 1H, COCH=CH), 6.74-6.78 (d, 1H, Ar), 7.44-7.58 (m, 4H, Ar; 1H, COCH=CH), 7.59-7.60 (dd, 1H, Ar), 7.99-8.00 (d, 1H, Ar), 9.94 (s, 1H, NH) ppm; ^{13}C (100 MHz) δ: 30.95 (3C), 34.52, 115.62, 119.39, 121.63, 121.83, 124.12, 125.76 (2C), 127.42 (2C), 128.99, 132.16, 138.92, 152.31, 158.49, 162.97, 172.24 ppm; Mass m/z: 340 $(M+H)^+$.

6k: 5-[3-(4-cyanophenyl) prop-2-enamido]-2-hydroxybenzoic acid
IR (KBr) υ_{max}**:** 3462 (br, OH str), 3367 (NH str), 2229 (CN str), 1679 (C=O str), 1610 (C=O str, amide I), 1539 (NH bend, amide II) cm^{-1}; ^{1}H (400 MHz) δ: 6.76-6.78 (d, 1H, COCH=CH), 6.96-7.00 (d, 1H, Ar), 7.58-7.62 (d, 1H, COCH=CH), 7.67-7.70 (d, 1H, Ar), 7.80-7.91 (m, 4H, Ar), 8.12 (s, 1H, Ar), 10.25 (s, 1H, NH) ppm; ^{13}C (100 MHz) δ: 111.45, 116.12,117.10, 118.68, 121.50, 124.91, 126.17, 128.31 (2C), 129.28, 132.85 (2C), 137.39, 139.55, 158.35, 162.32, 172.00 ppm; Mass m/z: 307 $(M-H)^-$.

6l: 2-Hydroxy-5-{3-[4-(trifluoromethyl) phenyl] prop-2-enamido} benzoic acid
IR (KBr) υ_{max}**:** 3448 (br, OH str), 3279 (NH str), 1676 (C=O str), 1610 (C=O str, amide I), 1545 (NH bend, amide II), 1329, 1205, 1112 (CF_3 str, Ar) cm^{-1}; ^{1}H (400 MHz) δ: 6.88-6.95 (m, 1H, COCH=CH; 1H, Ar), 7.36-7.41 (m, 1H, Ar), 7.61-7.68 (d, 1H, COCH=CH), 7.75-7.85 (m, 3H, Ar), 8.20 (d, 1H, Ar), 8.58 (s, 1H, Ar), 10.28 (s, 1H, NH) ppm; ^{13}C (100 MHz) δ: 116.22, 117.20, 121.27, 125.13, 125.38, 125.85, 128.22 (3C), 128.82, 129.09, 129.41, 137.59, 138.93, 158.27, 162.42, 172.00 ppm; Mass m/z: 352 $(M+H)^+$.

6m: 2-Hydroxy-5-[3-(4-nitrophenyl) prop-2-enamido] benzoic acid
IR (KBr) υ_{max}: 3461 (br, OH str), 3363 (NH str), 1677 (C=O str), 1620 (C=O str, amide I), 1500 (NH bend, amide II), 1475 (NO_2 str, asym), 1342 (NO_2 str, sym) cm^{-1}; ^{1}H (400 MHz) δ: 6.90-6.92 (d, 1H, COCH=CH), 6.95-6.99 (d, 1H, Ar), 7.65-7.69 (d, 1H, COCH=CH), 7.75-7.79 (dd, 1H, Ar), 7.87-7.90 (dd, 2H, Ar), 8.18-8.19 (d, 1H, Ar), 8.27-8.30 (d, 2H, Ar), 10.31 (s, 1H, NH) ppm; ^{13}C (100 MHz) δ: 113.91,

117.05, 120.81, 124.13 (2C), 126.58, 126.77, 128.69 (2C), 130.45, 137.31, 141.33, 147.57, 157.59, 162.46, 171.79 ppm; Mass m/z: 329 $(M+H)^+$. The IR spectra of compounds 6a–6m revealed broad bands in the range of 3555–3392 cm^{-1} corresponding to stretching vibrations of -OH groups. Characteristic absorption bands in the region of 3392–3254 cm^{-1} were attributed to N–H stretching of amide groups. Strong C=O stretching bands appeared between 1690–1676 cm^{-1}, while additional peaks at 1621–1610 cm^{-1} were characteristic of amide I stretching vibrations. The presence of amide II was further supported by N–H bending bands observed in the region of 1560–1500 cm^{-1}. ^1H NMR spectra of the title compounds provided clear evidence through a set of well-resolved aromatic proton signals occurring in the region of δ 6.72–8.58 ppm. Characteristic alkene protons (–CH=CH–) were noted as doublets in the range of δ 6.61–7.69 ppm. A distinct amide (CONH) proton resonance was detected commonly as a singlet at δ 9.88–10.31 ppm, confirming the formation of the amide group. Additional structural features were confirmed by the presence of methoxy protons in compounds 6e, 6f, and 6g and methyl protons in compounds 6h, 6i, and 6j. ^{13}C NMR spectra of the synthesised compounds-provided further confirmation of their proposed structures. Distinct signals observed in the range of δ 171.67–172.27 ppm correspond to carboxylic acid carbon atoms (COOH), while resonances at δ 163.64–167.43 ppm were attributed to the carbon linked to the phenolic hydroxyl group of the 5-ASA. Additional characteristic signals included methoxy carbons in compounds 6e, 6f, and 6g appeared in the range of δ 55.29–60.15 ppm. Methyl carbon signals were observed in compound 6h at δ 20.95 ppm, 6i at δ 23.68 (2C) and δ 33.31 ppm and in 6j at δ 30.95 (3C) and δ 34.52 ppm, indicating multiple methyl environments. The existence of a nitrile (CN) group in compound 6k was confirmed by the signal at δ 118.68 ppm. A distinct signal at δ 129.41 ppm validated the presence of a trifluoromethyl group in compound 6l. The observed peaks attributed to $(M+H)^+$ and $(M–H)^-$ ions in the mass spectra were consistent with the expected molecular formulae.

In vitro Antioxidant Activity

The *in vitro* antioxidant activity of the synthesised compounds (6a–6m) was evaluated at a concentration of 100 μM, and the results were compared with ascorbic acid and 5-ASA. The data is presented in Table-2.

Table-2: *In Vitro* Antioxidant Activity of Compounds (6a-6m) against DPPH and Nitric Oxide Radical Scavenging Assays

S. No.	Compound Code	Substituent	% Inhibition of DPPH at 100 μM*	% Scavenging by Nitric Oxide radical at 100 μM*
1	6a	-H	71.36	62.52
2	6b	4-Cl	73.59	60.25
3	6c	4-F	74.07	60.06
4	6d	4-Br	64.68	65.17
5	6e	4-OCH_3	74.22	70.24
6	6f	3,4-OCH_3	62.69	49.69
7	6g	3,4,5-OCH_3	88.78	73.71
8	6h	4-CH_3	65.39	61.65
9	6i	4-$CH(CH_3)_2$	67.54	61.26
10	6j	4-$C(CH_3)_3$	63.56	51.62
11	6k	4-CN	73.43	64.69
12	6l	4-CF_3	73.03	65.22
13	6m	4-NO_2	72.08	69.71
14	5-ASA	-	66.43	67.44
15	Ascorbic acid	-	73.11	57.36

*Average of triplicate measurement

The DPPH assay results revealed that the compounds showed moderate to high antioxidant activity with inhibition percentages ranging from 62.69% to 88.78%. Among them, compound **6g** (3, 4, 5- OCH_3) possessing electron donating substituent exhibited the highest activity (88.78%) suggesting a strong radical scavenging potential even higher than ascorbic acid (73.11 %) and 5-ASA (66.43%). Several other compounds **6b** (4-Cl), **6c** (-F), **6e** (4-OCH_3) and **6g** (3,4-OCH_3) also showed higher inhibition levels than

ascorbic acid and 5-ASA.The NO radical scavenging assay results revealed a varied range of *in vitro* antioxidant activity among the tested compounds. Compound **6g** (3, 4, 5- OCH_3) exhibited significant activity with 73.71% nitric oxide radical inhibition, higher than ascorbic acid (57.36%) and 5-ASA (67.44%). Other compounds with significant activity include **6e** (4-OCH_3; 70.24%) and **6m** (4-NO_2; 69.71%) indicating their strong nitric oxide scavenger capacity. Overall, the results highlight that analogues **6g** (3, 4, 5- OCH_3) and **6e** (4-OCH_3) exhibited superior radical scavenging activity in both the assays.

***In vitro* Anti-Inflammatory Activity**

The *in vitro* anti-inflammatory activity of the synthesised compounds (6a–6m) was evaluated at a concentration of 100 μM. The results were compared to the standard anti-inflammatory drug 5-ASA, as shown in Table-3.

Table-3: *In vitro* Anti-Inflammatory Activity of Compounds (6a-6m) by Protein Denaturation and HRBC Membrane Stabilisation Methods

S. No.	Compound Code	Substituent	% Inhibition of protein denaturation at 100 μM*	% Protection in HRBC membrane stabilisation method at 100 μM*
1	6a	-H	71.28	63.92
2	6b	4-Cl	79.71	71.79
3	6c	4-F	79.95	71.30
4	6d	4-Br	60.46	61.67
5	6e	4-OCH_3	71.92	63.74
6	6f	3,4-OCH_3	73.27	64.37
7	6g	3,4,5-OCH_3	85.92	76.72
8	6h	4-CH_3	58.39	62.48
9	6i	4-$CH(CH_3)_2$	80.11	71.26
10	6j	4-$C(CH_3)_3$	49.64	63.83
11	6k	4-CN	73.19	62.21
12	6l	4-CF_3	57.60	62.89
13	6m	4-NO_2	69.45	61.76
14	5-ASA	-	66.83	72.47

*Average of triplicate measurement

Compound **6g** (3,4,5-OCH_3) showed the highest inhibition of protein denaturation (85.92%) suggesting that an trisubstituted aromatic system may provide a balanced electronic environment crucial for anti-inflammatory efficacy. Compounds **6i** (H; 80.11%), **6c** (4-F, 79.95; 73.27%) and **6b** (4-Cl; 79.71%) enhance anti-inflammatory activity by improving electronic delocalization and lipophilic interactions. The substituted derivatives demonstrated significant anti-inflammatory potential compared to 5-ASA (66.83%).

The results of the HRBC membrane stabilization assay revealed that the compounds **6g** (3, 4, 5-OCH_3), **6b** (4-Cl), **6c** (4-F) and **6i** (4-$CH(CH_3)_2$) showed the highest percentage protection values of 71.79%, 71.30% and 71.26% respectively which are comparable to 5-ASA (72.47%).

Drug Likeliness

The molecular properties of all compounds (6a 6m) are compliant with drug-likeness criteria-(milogP ≤ 5, MW ≤ 500, HBA ≤ 10, HBD ≤ 5, n Violations ≤ 1),-indicating good potential for oral bioavailability. Physicochemical properties such as logP (3.01–5.08), molecular weight (283.28–373.36) and TPSA (86.62–132.45 Å²) showed moderate variation. Compounds with lower logP values (< 3.5), such as 6f, 6g and 6k, were expected to have better aqueous solubility, whereas compound 6j with a logP > 5 may exhibit lower solubility due to higher lipophilicity. All compounds had TPSA values below 140 Å², suggesting good ability for GI absorption. Compounds 6a–6m are predicted to display intestinal permeability.

Table-4: Physicochemical Properties of Compounds (6a-6m) Generated by Molinspiration Cheminformatics

S. No.	Compound Code	Substituent	milogP	TPSA	n atoms	M.W	HBA	HBD	nViolations	nrotb	Volume
1	6a	-H	3.38	86.62	21	283.28	5	3	0	4	249.27
2	6b	4-Cl	4.05	86.62	22	317.78	5	3	0	4	262.81
3	6c	4-F	3.54	86.62	22	301.27	5	3	0	4	254.21
4	6d	4-Br	4.18	86.62	22	362.18	5	3	0	4	267.16
5	6e	4-OCH_3	3.43	95.86	23	313.31	6	3	0	5	274.82
6	6f	3,4-OCH_3	3.02	105.09	25	343.33	7	3	0	6	300.37
7	6g	3,4,5-OCH_3	3.01	114.33	27	373.36	8	3	0	7	325.91
8	6h	4-CH_3	3.83	86.62	22	297.31	5	3	0	4	265.83
9	6i	4-$CH(CH_3)_2$	4.89	86.62	24	325.36	5	3	0	5	299.22
10	6j	4-$C(CH_3)_3$	5.08	86.62	25	339.39	5	3	1	5	315.46
11	6k	4-CN	3.13	110.42	23	308.29	6	3	0	4	266.13
12	6l	4-CF_3	4.27	86.62	25	351.28	5	3	0	5	280.57
13	6m	4-NO_2	3.33	132.45	24	328.28	8	3	0	5	272.61

MW = molecular weight; HBA = number of H-bond acceptors; HBD = number of H-bond donors; nrotb = number of rotatable bonds; TPSA = topological surface area.

The PASS analysis and OSIRIS property explorer data for the title compounds (6a-6m) and 5-ASA were presented in Table-5. The dataset presents the Pa value (probability of activity), significant in drug discovery, as it represents the estimated likelihood that a compound will exhibit a specific biological effect, such as anti-inflammatory activity, based on computational models and the safety profile of compounds. Most compounds showed promising anti-inflammatory potential, with Pa values ranging from 0.446 to 0.626; compounds 6i (4-CH $(CH_3)_2$) and 6j (4-$C(CH_3)_3$) exhibit the highest predicted activity (Pa > 0.6), suggesting stronger anti-inflammatory effects. Safety assessment indicates that nearly all compounds are low risk for mutagenicity, tumorigenicity, irritancy, and reproductive risk, with the exception of 6e (high risk for irritancy) and 6m (mutagenicity and tumorigenicity). The reference drug 5-ASA shows moderate predicted activity with some toxicity alerts.

Table-5: PASS Predicted Anti-Inflammatory Activity and Toxicity Risk Assessment by OSIRIS Property Explorer for the Compounds (6a-6m)

S. No.	Compound Code	Substituent	Pa value; anti-inflammatory activity	Mutagenic	Tumorigenic	Irritant	Reproductive effective
1	6a	-H	0.564	Green	Green	Green	Green
2	6b	4-Cl	0.549	Green	Green	Green	Green
3	6c	4-F	0.570	Green	Green	Green	Green
4	6d	4-Br	0.446	Green	Green	Green	Green
5	6e	4-OCH_3	0.541	Green	Green	Red	Yellow
6	6f	3,4-OCH_3	0.545	Green	Green	Green	Green
7	6g	3,4,5-OCH_3	0.541	Green	Green	Green	Green
8	6h	4-CH_3	0.564	Green	Green	Green	Green
9	6i	4-$CH(CH_3)_2$	0.626	Green	Green	Green	Green
10	6j	4-$C(CH_3)_3$	0.618	Green	Green	Green	Green
11	6k	4-CN	0.502	Green	Green	Green	Green
12	6l	4-CF_3	0.532	Green	Green	Green	Green
13	6m	4-NO_2	0.470	Red	Red	Green	Green
14	5-ASA	-	0.588	Yellow	Red	Green	Green

Pa: Probable activity; Red: Risk, Green: Low risk, Yellow: Medium risk

CONCLUSION

The novel cinnamamide derivatives of 5-ASA **(6a-6m)** were synthesized and characterized by the physical, spectral data, further evaluated for their *in vitro* antioxidant and anti-inflammatory potential. Compounds **6g** (3, 4, 5-OCH_3) and **6e** (4-OCH_3) displayed significant antioxidant effects and the

compounds **6g** (3,4,5-OCH_3), **6b** (4-Cl), **6c** (4-F) and **6i** (4-$CH(CH_3)_2$) exhibited strong anti-inflammatory activity. All compounds satisfied Lipinski's rule indicating good oral bioavailability and PASS/OSIRIS analyses predicted significant anti-inflammatory potential with low toxicity. Overall, **6g, 6b, 6c** and **6i** were found to be promising lead molecules for further development as potent anti-inflammatory agents.

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CONFLICT OF INTERESTS

The authors declare no conflict of interest, financial or otherwise.

AUTHOR CONTRIBUTIONS

The present work is related to *SDG-3: Good health and well-being*. All the authors contributed significantly to this manuscript, participated in reviewing/editing and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

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REFERENCES

1. H. Schram, M. I. Ugwoke, L. Buttafoco, US9511019B2, (2016)
2. Y.J. Jung, J.S. Lee, Y.M. Kim, *Journal of Pharmaceutical Sciences,* **89(5)**, 594(2000), https://doi.org/10.1002/(SICI)1520-6017(200005)89:5<594::AID-JPS5>3.0.CO;2-8.
3. Y.J. Jung, J.S. Lee, Y.M. Kim, *Journal of Pharmaceutical Sciences,* **90 (11),** 1767(2001), https://doi.org/10.1002/jps.1126.
4. Y.J. Jung, H.H. Kim, H.S. Kong, Y.M. Kim, *Archives of Pharmacal Research,* **26 (4)**, 264(2003), https://doi.org/10.1007/BF02976953.
5. N. Joon, K. Wooseong, L. Sunyoung, J. Seongkeun, Y. Jin-Wook, K. Min-Soo, J. Yunjin , *Journal of Drug Targeting*, **24(5)**, 468(2016), http://dx.doi org//10.3109/1061186X.2015.1087528
6. J. Jilani, M. Shomaf, Alzoubi, *Drug Design, Development and Therapy,* **7,** 691(2013), https://doi.org/10.2147/DDDT.S48636.
7. D.A. Kennedy, V. Nagarajan, F.R. Fronczek, M. Devocelle, *Journal of Organic Chemistry*, **76**, 9641(2011), https://pubs.acs.org/doi/10.1021/jo201358e.
8. K. Harveer, S. Jasmeen, *Bulletin of the Chemical Society of Ethiopia,* **28(3)**, 475(2014), http://dx.doi.org/10.4314/bcse.v28i3.17.
9. A.A.M. Abdel-Alim, A.N.A. El-Shorbagi, S.G. Abdel-Moty, H.H.M. Abdel-Allah, *Archives of Pharmacal Research*, **28(6)**, 637(2005), http://dx.doi.org/10.1007// BF02969351.
10. H. Abdu-Allah, S. Abdel-Moty, A. El-Shorbagi, Abdel-Alim, *Bulletin of Pharmaceutical Sciences,* **28(2)**, 237(2005), http://dx.doi.org/10.21608/bfsa. 2005.65398.
11. E. Carceller, J. Salas, M. Merlos, M. Giral, R. Ferrando, I. Escamilla, *Journal of Medicinal Chemistry*, **44**, 3001(2001), https://pubs.acs.org/doi/10.1021/jm000 72a019.
12. N. Gaikwad, S. Nanduri, Y.V. Madhavi, *European Journal of Medicinal Chemistry,* **181**, 111561(2019), https://doi.org/10.1016/j.ejmech.2019.07.064

13. G. Seelolla, P. Cheera, V. Ponneri, *Medicinal Chemistry* **4**, 778(2014), https://doi.org/10.4172/2161-0444.1000229
14. D. Ribeiro, C. Poença, C. Varela, J. Janela, E.J. Tavares da Silva, E. Fernandes, F.M.F.Roleira, *Bioorganic Chemistry,* **91**, 103179(2019), https://doi.org/10.1016/j.bioorg.2019.103179.
15. V. Geetika, K. Anup Chakraborty, K. Sarita, *World Journal of Biology Pharmacy and Health Sciences*, **14 (01),** 088(2023), https://doi.org/10.30574/wjbphs.2023.14.1.0164.
16. R. Marisa, Daniela, S J Joao, L V Carla, C C Sau, T Elisiario da, F Eduarda, M F R Fernanda M, *Food Chemistry,* **459**,140080(2024), https://doi.org/10.1016/j.foodchem.2024.140080
17. C. Gaozhi, Z. Yali, L. Xing, F. Qilu, W. Zhe, Lili Fu, Z. Liu, Yi Wang, Z. Yunjie, Li Xiaokun, L. Guang, *Journal of Medicinal Chemistry,* **59(6)**, 2436(2016), https://doi.org/10.1021/acs.jmedchem.5b01574
18. C. Eun-Ju, R. Young Bae, T. Yujiao, Bo Ram Kim, Woo Song Lee, Trishna Debnath, Meiqi Fan, Eun-Kyung Kim and Hyun-Su Lee, *Journal of Enzyme Inhibition and Medicinal Chemistry,* **34(1)**, 613(2019), https://doi.org/10.1080/14756366.2019.1569647
19. Z. Guangyuan, Zhangyue Ji, D. Yusen, W. Diya, P. Yajie, Shi Yangyang, Bo Wei, *International Immunopharmacology,* **136**, 112370(2024), https://doi.org/10.1016/j.intimp.2024.112370.
20. K. Changyu, K. Jaejeong, J. Sanghyun, C. Heeyeong, K. Hyun Young, Y. In-Soo, Y. Jin-Wook, J. Yunjin, *Pharmaceutics,* **15(1),** 41(2023), https://doi.org/10.3390/pharmaceutics15010041.
21. B. Narasimhan, D. Belsare, D. Pharande, V. Mourya, A. Dhake, *European Journal of Medicinal Chemistry*, **39**, 827(2004), https://doi:10.1016/j.ejmech.2004.06. 013.
22. P. Michele, P. Amedeo, B. Gianpiero, A. Battistina, A. Luciana, C. Riccardo, A. Loredana, F. Walter, F. Giuseppe, Giulia, *Archiv der Pharmazie, Medicinal Chemistry*, **333**, 17(2000), https://doi.org/10.1002/(SICI)1521-4184(200001)333:1%3C17::AID-ARDP17%3E3.3.CO;2-S.
23. A. Selim, Fatma El-Hag, R. Attia, S. Said, M. El-Gazzar. *Egyptian Journal of Chemistry*, **65(8)**, 163(2022), https://ejchem.journals.ekb.eg/article_245404.html.
24. M. Kuchana, D.R. Bethapudi, K.E. Ragini, S. Yoshitha, *Journal of Applied Pharmaceutical Science,* **9 (09)**, 105(2019), https://doi:10.7324/JAPS.2019. 90915.
25. R. C. Rakesh, M. H. Kallappa, *Royal Society Open Science,* **5**, 1(2018), https://doi.org/10.1098/rsos.172435.
26. T. K. Mohamed Saleem, A.K. Azeem, C. Dilip, C. Sankar, N.V. Prasanth, R. Duraisami, *Asian Pacific Journal of Tropical Biomedicine,* **1(2)**, 147(2011), https://doi.org/10.1016/s2221-1691(11)60014-2.
27. L. Sree Nath, S.A. Khan, A. Ahmad, *Scholars Academic Journal of Pharmacy,* **3(6),** 496(2014), www.molinspiration.com.
28. R. Pramely, L. Stephan, *Journal of Biochemical Technology*, **3(4)**, 375(2012), https://jbiochemtech.com/storage/models/article/IxTbkcdAjJga5E1dCbbCNtNO7CiibUBICZJiV32etSqDyvd0U5olJpmnK2nF/prediction-of-biological-activity-spectra-of-a-few-phytoconstituents-of-azadirachta-indicia-a-juss.pdf.
29. https://www.Way2drug.Com/pass online/.
30. A. S. Gondkar, V. K. Deshmukh, S. R. Chaudhari, *Drug Invention Today,* **5**, 175(2013), http://dx.doi.org/10.1016/j.dit.2013.04.004.
31. https://www.cheminfo.org/flavor/cheminformatics/Utility/Property_explorer/index.html.

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