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Synthesis of Caffeic Acid Derivatives: Identification of (*E*)-*N*-(4-Cyanobenzyl)-3-(3,4-dihydroxyphenyl)acrylamide as an Anticancer Agent against Human Cervical Cancer Cells

B. SHWETHA[1], M.S. SUDHANVA[2], N.R. THIMMEGOWDA[3], B.M. ANIL KUMAR[2], G.S. JAGADEESHA[3], H. GUDDAPPA[1], D.S. PRASANNA[1], R. DINESH[1], R. SHOBITH[2,*] and C.S. ANANDAKUMAR[1,*]

[1]Department of Applied Sciences, Visvesvaraya Technological University, Center for Postgraduate Studies, Bengaluru Region, Muddenahalli, Chikkaballapur District-562101, India
[2]Adichunchanagiri Institute for Molecular Medicine, Adichunchanagiri University, B.G. Nagara-571448, India
[3]Government S.K.S.J. Technological Institute (Affiliated to Visvesvaraya Technological University), K.R. Circle, Bangalore-560001, India

*Corresponding authors: E-mail: ; shobithrangappa@gmail.com; csanandakumar@gmail.com

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A novel series of natural compound-caffeic acid derivatives were synthesized by coupling with different substituted amines and alkyl halides in an effort to enhance the anticancer activity and explore the structure-activity relationship. The structures of the compounds were determined by ^{1}H NMR and mass spectroscopy analysis. Compounds were evaluated for inhibition against HeLa-cervical cancer cell proliferation and results revealed that compound (*E*)-*N*-(4-cyanobenzyl)-3-(3,4-dihydroxyphenyl)acrylamide **(SHC5)** exhibited potent antiproliferative activity with 5.2 µM concentration and it is further confirmed by Hoechst/PI double staining and Annexin V/PI double staining assay. Further, compound **SHC5** was screened against other cancer cell lines namely K562, Jurkat, HCT116 and MiaPaCa2 to test the specificity of the molecule and found to be ineffective.

Keywords: Caffeic acid derivatives, Anticancer agents, Cervical cancer, Natural compound derivatives.

INTRODUCTION

In the biological and pharmaceutical fields, natural products are known to play a crucial role as a important source of developing medicines. Up to 80% of people still rely predominantly on the traditional medicines, World Health Organization (WHO) reports approximately 40% of all medicines are either natural compounds or their semi-synthetic derivatives. Natural products continue to produce a fair share of new clinical candidates and medications despite competition from other drug discovery methods [1-3]. Due to their high chemical diversity and pharmacological qualities, natural products and their analogues make them favourable structures for the discovery of new drugs and continue to inspire new discoveries in biology, chemistry and medicines. In some instances, semi-synthetic derivatives have higher biological activities [4,5]. Natural product research has recently gained more attention as a result of the failure of alternative drug discovery methods to produce numerous key molecules in important therapeutic areas. The pharmaceutical industry may leverage the new structures that are being investigated in natural product research as models when developing new medications. Natural products have been and will continue to be major sources of novel pharmaceutical ingredients.

Design, synthesis and identification of structurally novel anticancer agent's by derivatization of natural compounds may lead to non-toxic drug candidates with selective antitumoral potencies [6-11]. Therefore, the structural modification of natural products is needed to develop novel compounds with specific properties and many significant drugs have been derived from natural sources by structural optimization of natural compounds [12] and some of them are listed in Fig. 1.

Caffeic acid is a bioactive natural compound which is present in coffee, wine, tea and popular medicines such as propolis and is widely consumed in human diet. In recent years, substantial research on caffeic acid and its derivatives has shown a wide range of biological activity and better medicinal uses. It has been proved in many biological investigations that caffeic acid and its derivatives show a wide range of biological

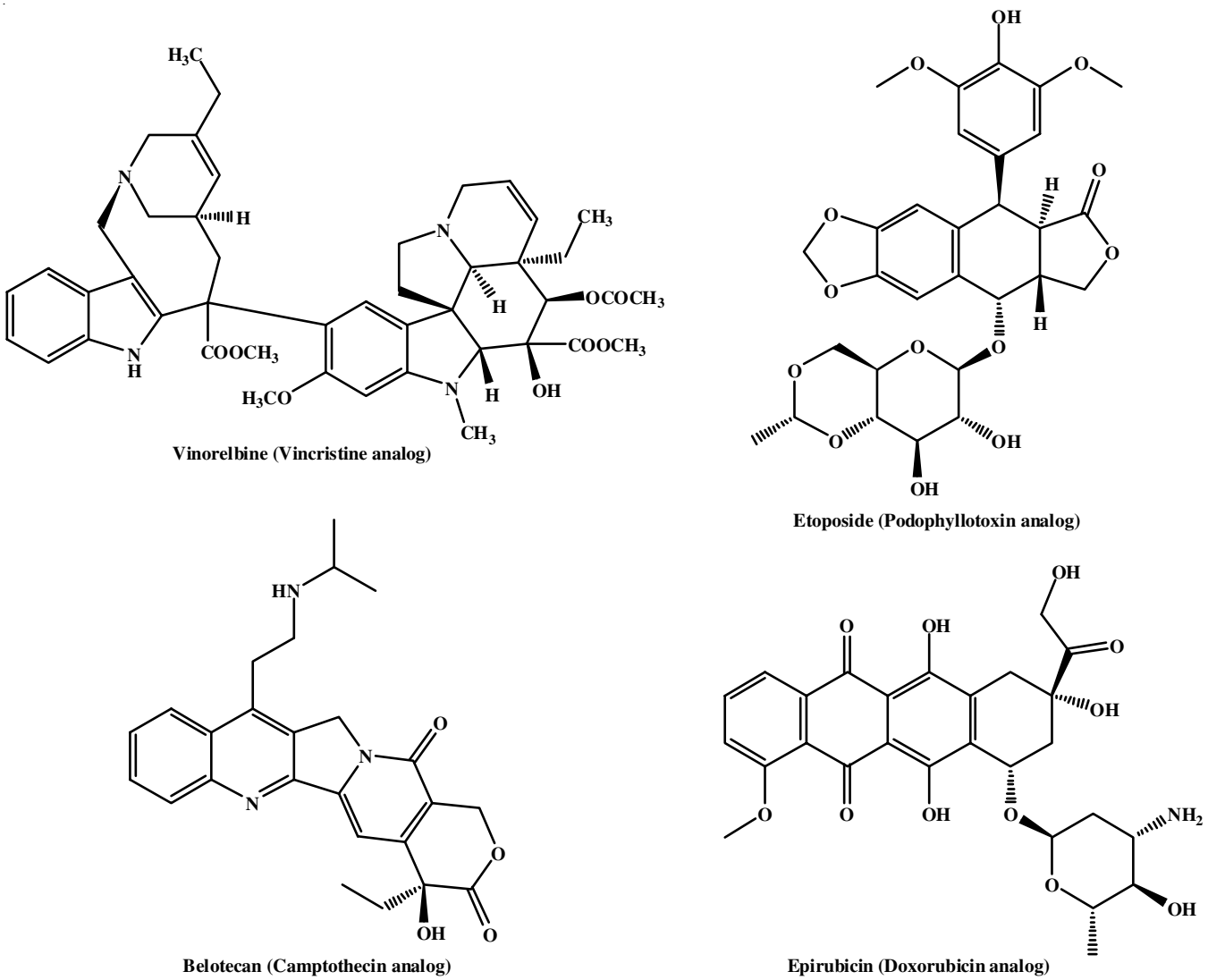


Fig. 1. Structures of anticancer drugs derived from natural compounds

properties *viz*. antimicrobial [13-19], antioxidant [20-24], anti-inflammatory [25-29], antidiabetic [30-35] and potential anticancer activity [36-43].

Previous reports demonstrated the importance of derivatization of natural compounds in drug discovery. Hence in our continuation of effort to find novel anticancer agents, we have focused on the synthesis of novel bioactive natural compound-caffeic acid derivatives and evaluation of their anticancer effect to identify structurally novel caffeic acid derivative as anticancer agent.

EXPERIMENTAL

Starting materials, substituted amines, other reagents and solvents were purchased from commercial sources and used as received unless stated otherwise. The materials and methods used for the reaction monitoring, purification, characterization and MTT cell proliferation assay of synthesized caffeic derivatives were followed as reported earlier [44].

Synthesis of (*E*)-ethyl 3-(3,4-diethoxy phenyl) acrylate (2): To a solution of caffeic acid (**1**) (5.0 g, 27.75 mmol, 1.0 equiv) in dimethyl formamide (50 mL) was added potassium carbonate (23.01 g, 166.51 mmol, 6 equiv.) and stirred for 30 min at room temperature and then ethyl bromide (12.29 mL, 138.75 mmol, 5 equiv.) was added to the reaction mixture and further the reaction mixture was stirred at room temperature for 10 h. The reaction progress was monitored by TLC. Upon completion, the organic phase was extracted with ethyl acetate (150 mL × 3), the combined organic phase was washed with water and saturated brine. The organic phase was dried over anhydrous sodium sulphate, solvent was filtered and concentrated under *vacuo* to get compound **2** as yellow solid (R_f = 0.71 with mobile phase ethyl acetate:hexane 3:1) with 89% yield (6.53 g).

Synthesis of (*E*)-ethyl 3-(3,4-diethoxy phenyl) acrylic acid (3): Compound (*E*)-ethyl 3-(3,4-diethoxyphenyl)acrylate (6 g, 22.6 mmol, 1 equiv.) was taken in round bottomed flask in mixture of THF:methanol:water (2:1:1 v/v, 10 v, 60 mL) was added $LiOH{\cdot}H_2O$ (3 equiv.), then the reaction mixture was stirred at 80 ºC for 2 h. The reaction progress was monitored by TLC. Upon completion the reaction, mixture was concentrated partially to remove THF and methanol. The residual aqueous layer was diluted with water and extracted with ethyl

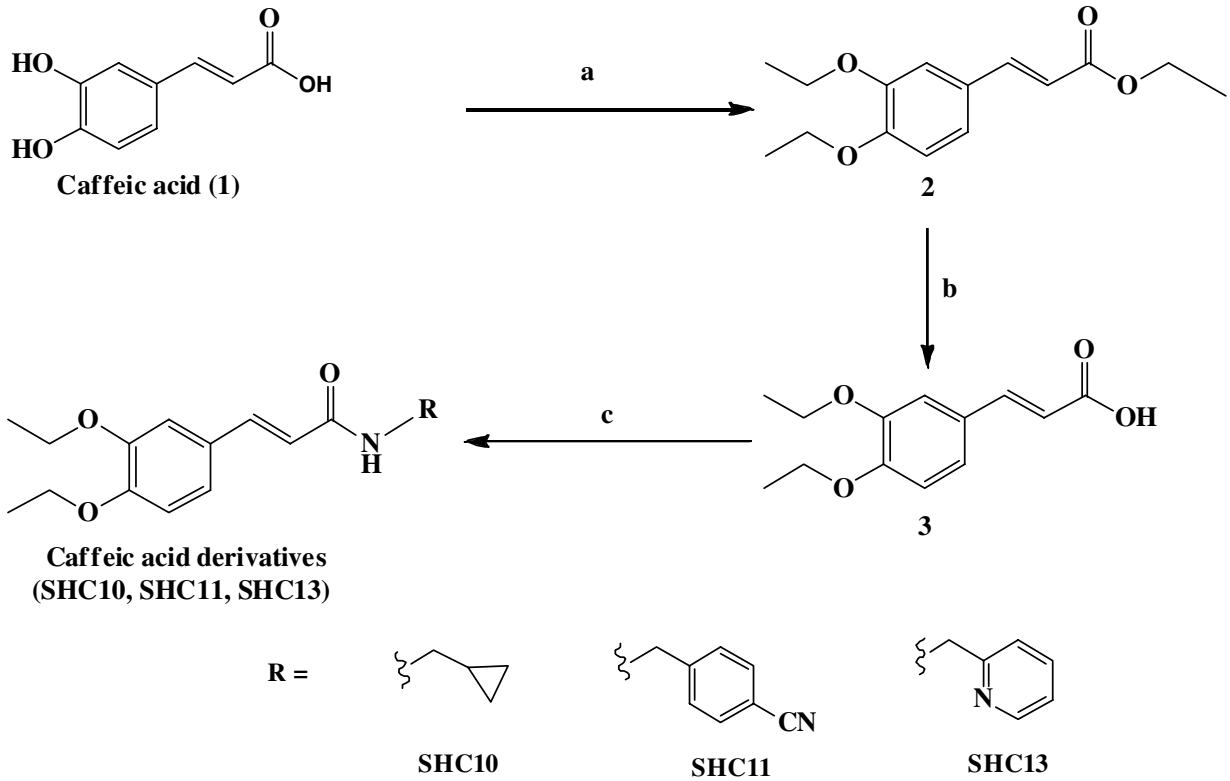
acetate to remove impurities and collected the aqueous layer. The collected aqueous layer was acidified by adding 1.0 N HCl solution, which was precipitated, filtered and washed with distilled water and dried under *vacuo* to obtain compound **3** as yellow solid (R_f = 0.75 with mobile phase ethyl acetate: hexane 2:1) with 82% yield (4.38 g).

Synthesis of SHC10, SHC11 and SHC13 (General procedure A): To a well stirred solution of compound **3** (250 mg, 1.059 mmol, 1 equiv.) in DMF solvent (2.5 mL, 10 vol), the coupling agent EDCI·HCl (203 mg, 1.059 mmol, 1 equiv.) and HOBt (143 mg, 1.06 mmol, 1 equiv.) were added and the mixture was stirred for 20 min at room temperature and then amine (1 equiv., 1.06 mmol) was added to the reaction mixture and stirred at room temperature for 20 min after that DIPEA (0.36 mL, 2.12 mmol, 2 equiv.) was added and then the reaction mixture was further stirred for 16 h at room temperature. The reaction progress was monitored by TLC. Upon completion, the organic phase was extracted with ethyl acetate (25 mL × 3). The combined organic phase was washed with water and brine solution and dried over anhydrous sodium sulphate, then the solvent was filtered and concentrated under *vacuo* to get crude compounds **SHC10**, **SHC11** and **SHC13**. The obtained crude compounds were further purified by silica gel column chromatography using ethyl acetate and hexane mixture as mobile phase (**Scheme-I**).

Synthesis of (*E*)-*N*-(cyclopropylmethyl)-3-(3,4-diethoxyphenyl)acrylamide (SHC10): Following general procedure A, compound **SHC10** was obtained from compound **3** (250 mg, 1.06 mmol, 1 equiv.) and cyclopropylmethanamine (75 mg (92 µL), 1.06 mmol, 1 equiv.) as white solid in 68% yield (208 mg) (R_f = 0.72 in EtOAc:hexane 3:2 v/v);^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.56-7.52 (d, 1H, Ar-H), 7.06-7.03 (m, 2H, Ar-H), 6.84-6.82 (t, 1H, -CONH), 6.28-6.25 (d, 1H, -C=C), 4.13-4.07 (m, 4H, -OCH_2), 3.25-3.22 (m, 2H, -*N*-CH_2), 1.47-1.43 (m, 6H, -CH_3), 1.02-0.98 (m, 1H, -CH), 0.54-0.50 (m, 2H, -C-CH_2), 0.25-0.21 (m, 2H, -C-CH_2). ESI/HRMS (*m/z*): Calculated for $[C_{17}H_{23}NO_3][M + H]^+$: 289.1678; Found *m/z*: 290.2406 $[M+H]^+$.

Synthesis of (*E*)-*N*-(4-cyanobenzyl)-3-(3,4-diethoxyphenyl)acrylamide (SHC11): Following general procedure A, compound **SHC11** was obtained from compound **3** (250 mg, 1.06 mmol, 1 equiv.) and 4-(aminomethyl)benzonitrile (140 mg, 1.06 mmol, 1 equiv., which was obtained from 4-(aminomethyl)benzonitrile hydrochloride by neutralizing HCl salt using $NaHCO_3$) pale brown solid in 85% yield (295 mg) (R_f = 0.5 in EtOAc:hexane 2:1 v/v); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.62 (s, 1H, Ar-H), 7.82-7.80 (d, 2H, Ar-H), 7.48-7.38 (m, 3H, Ar-H), 7.16-7.10 (m, 2H, -C=C), 6.99-6.97 (t, 1H, -CONH), 6.58-6.54 (d, 1H, Ar-H), 4.48-4.46 (d, 2H, -NH-CH_2), 4.07-4.05 (t, 4H, -OCH_2), 1.35-1.32 (t, 6H, -CH_2-CH_3). ESI/LCMS (*m/z*): Calculated for $[C_{17}H_{23}NO_3][M + H]^+$: 350.16; Found *m/z*: 351.1 $[M+H]^+$.

Synthesis of (*E*)-3-(3,4-dimethoxyphenyl)-*N*-(pyridin-2-ylmethyl)acrylamide (SHC13): Following general procedure A, compound **SHC13** was obtained from compound **3**



Reagents and conditions: (a) Etyl bromide (5 equiv.), K_2CO_3 (6 equiv.), DMF (10 vol.), RT, 10 h; **(b)** LiOH (3 equiv.), THF:MeOH:water [(2:1:1) (10 vol.)]; **(c)** R-NH_2 (1 equiv.), EDC·HCl (1 equiv.), HOBt (1 equiv.), DIPEA (2.5 equiv.), DMF (10 vol.), 0 °C-RT, 16 h

Scheme-I: Synthesis of caffeic acid derivatives (**SHC10**, **SHC11** and **SHC13**)

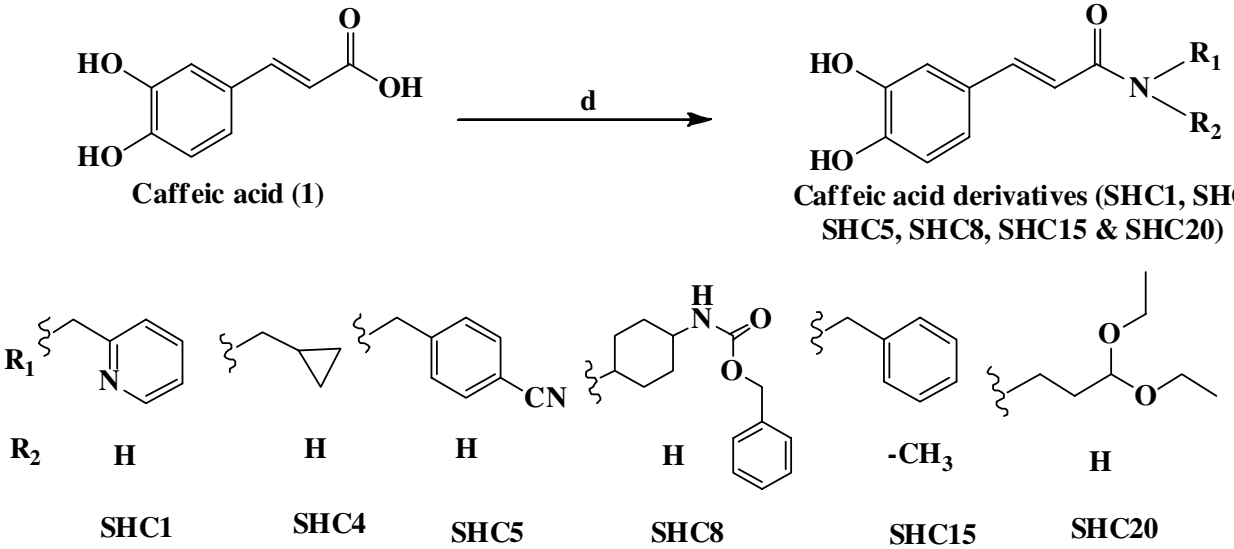
(250 mg, 1.06 mmol, 1 equiv.) and pyridin-2-ylmethanamine (115 mg (110 µL), 1.06 mmol, 1 equiv.), as pale yellow solid in 72% yield (250 mg) (R_f = 0.4 in EtOAc:hexane 3:1 v/v); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.56-8.55 (d, 1H, Ar-H), 7.77-7.75 (t, 1H, Ar-H), 7.59-7.56 (m, 1H, Ar-H), 7.40-7.29 (m, 1H, Ar-H), 7.27-7.21 (m, 1H, Ar-H), 7.07-7.05 (m, 1H, Ar-H), 6.85-6.83 (t, 1H, -CONH), 6.40-6.36 (d, 1H, Ar-H), 4.73-4.71 (t, 2H, -$NHCH_2$),4.14 -4.08 (t, 4H, -OCH_2), 1.49-1.44 (m, 6H, -CH_2CH_3). ESI/HRMS (*m/z*): Calculated for [$C_{19}H_{22}N_2O_3$]-[M+H]$^+$: 326.1630; Found *m/z*: 327.2391[M+H]$^+$.

Synthesis of SHC1, SHC4, SHC5, SHC8, SHC15 and SHC20 (General procedure B): To a well stirred solution of caffeic acid (500 mg, 2.77 mmol, 1 equiv.) in DMF solvent (5 mL, 10 vol.), The coupling agent EDC·HCl (532 mg, 2.77 mmol, 1 equiv.) and HOBt (375 mg, 2.77 mmol, 1 equiv.) were added and the mixture was stirred for 20 min at room temperature and then amine (1 equiv., 2.77 mmol) was added to the reaction mixture and stirred at room temperature for 20 min after that DIPEA (0.69 mL, 5.54 mmol, 2 equiv.) was added and then the reaction mixture was further stirred for 16 h at room temperature. The reaction progress was monitored by TLC. Upon completion, the reaction mass was quenched with 1 N HCl and extracted with ethyl acetate (50 mL × 3) (in case of **SHC1**: aqueous layer was collected and neutralized with saturated $NaHCO_3$ solution and then extracted with ethyl acetate). The combined organic phase was washed with water and brine solution and dried over anhydrous Na_2SO_4, then the solvent was filtered and concentrated under *vacuo* to get crude compounds **SHC1**, **SHC4**, **SHC5**, **SHC8**, **SHC15** and **SHC20**. The obtained crude compounds were further purified by silica gel column chromatography using ethyl acetate and hexane mobile phase (**Scheme-II**).

Synthesis of (*E*)-3-(3,4-dihydroxyphenyl)-*N*-(pyridin-2-ylmethyl)acrylamide (SHC1): Following general procedure B, compound **SHC1** was obtained from caffeic acid (500 mg, 2.77 mmol, 1 equiv.) and pyridin-2-ylmethanamine(2-picolylamine) (299 mg (288 µL), 2.77 mmol, 1 equiv.), as pale yellow solid in 55% yield (412 mg) (R_f = 0.2 in EtOAc: hexane 3:1 v/v); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.60-8.57 (t, 1H, Ar-H), 8.48-8.47 (d, 1H, Ar-H), 7.74-7.70 (m, 1H, Ar-H), 7.32-7.27 (m, 2H, Ar-H), 7.24-7.21 (m, 1H, Ar-H), 6.988-6.984 (d, 1H, -CONH), 6.86-6.84 (m, 1H, Ar-H), 6.76-6.74 (m, 1H, Ar-H), 6.49-6.45 (m, 1H, Ar-H), 4.48-4.47 (t, 2H, -CH_2); ESI/HRMS (*m/z*): Calculated for [$C_{15}H_{14}N_2O_3$][M+H]$^+$: 270.1044; Found *m/z*: 271.1207 [M+H]$^+$.

Synthesis of (*E*)-*N*-(cyclopropylmethyl)-3-(3,4-dihydroxyphenyl)acrylamide (SHC4): Following general procedure B, compound **SHC4** was obtained from caffeic acid (500 mg, 2.77 mmol, 1 equiv.) and cyclopropylmethanamine (197 mg (240 µL), 2.77 mmol, 1 equiv.), as pale brown semi-solid in 51% yield (330 mg) (R_f = 0.7 in DCM:MeOH 9:1 v/v); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.92 (s, 1H, Ar-H), 7.26-7.20 (d, 1H, Ar-H), 6.92 (t, 1H, -CONH), 6.92-6.90 (m, 1H, Ar-H), 6.74-6.72 (m, 1H, Ar-H), 6.37-6.33 (m, 1H, Ar-H), 3.04-3.01 (m, 2H, NH-CH_2), 0.92-0.91 (m, 1H, -CH), 0.41-0.27 (m, 2H, -C-CH_2), 0.17-0.13 (m, 2H, -C-CH_2); ESI/HRMS (*m/z*): Calculated for [$C_{13}H_{15}NO_3$][M+H]$^+$: 233.1052; Found *m/z*: 234.1238[M+H]$^+$.

Synthesis of (*E*)-*N*-(4-cyanobenzyl)-3-(3,4-dihydroxyphenyl)acrylamide (SHC5): Following general procedure B, compound **SHC5** was obtained from caffeic acid (500 mg, 2.77 mmol, 1 equiv.) and 4-(aminomethyl)benzonitrile (366 mg, 2.77 mmol, 1 equiv., which was obtained from 4-(aminomethyl)benzonitrile hydrochloride by neutralizing HCl salt using $NaHCO_3$), as off-white solid in 63% yield (513 mg) (R_f = 0.8 in DCM:MeOH 8:2 v/v); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.36(s, 1H, -OH), 9.17 (s, 1H, -OH), 8.60 (s, 1H, Ar-H), 7.78-7.76 (t, 2H, Ar-H), 7.45-7.44 (t, 2H, Ar-H), 7.32-7.28 (d, 1H, Ar-H), 6.97 (t, 1H, -CONH), 6.86-6.84 (m, 1H, Ar-H), 6.75-6.74 (m, 1H, Ar-H), 6.42-6.38 (d,1H, Ar-H),4.45(t, 2H, -NH-CH_2); ESI/HRMS (*m/z*): Calculated for [$C_{17}H_{14}N_2O_3$][M+H]$^+$: 294.1004; Found *m/z*: 295.1215[M+H]$^+$.



Reagents and conditions:

(d) R-NH_2 (1 equiv.), EDC·HCl (1 equiv.), HOBt (1 equiv.), DIPEA (2.5 equiv.), DMF (10 vol.), 0 °C-RT, 16 h.

Scheme-II: Synthesis of caffeic acid derivatives **SHC1**, **SHC4**, **SHC5**, **SHC8**, **SHC15** and **SHC20**

Synthesis of (*E*)-benzyl(4-(3-(3,4-dihydroxyphenyl)-acrylamido)cyclohexyl)carbamate (SHC8): Following general procedure B, compound **SHC8** was obtained from caffeic acid (500 mg, 2.77 mmol, 1 equiv.) and benzyl (4-amino-cyclohexyl)carbamate (733 mg, 2.77 mmol, 1 equiv., as pale yellow solid in 58% yield (660 mg) (R_f = 0.6 in EtOAc:hexane 2:1 v/v); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.85-7.83 (d, 1H, -CONH), 7.33-7.28 (d, 7H, Ar-H & -C=C), 7.20-7.16 (d, 2H, Ar-H), 6.90 (s, 1H, -CONH), 6.80-6.78 (d, 1H, Ar-H), 6.72-6.70 (d, 1H, Ar-H),6.30-6.26 (d, 1H, -C=C), 4.98 (2H, s, -CH_2), 3.53 (s, 1H, -CH), 3.25 (s, 1H, -CH),1.82-1.80 (d, 4H, -CH_2), 1.23 (s, 4H, -CH_2). ESI/HRMS (*m/z*): Calculated for [$C_{23}H_{26}N_2O_5$]-[M-H]$^-$: 410.1842; Found *m/z*: 409.2127[M-H]$^-$.

Synthesis of (*E*)-*N*-benzyl-3-(3,4-dihydroxyphenyl)-*N*-methylacrylamide (SHC15): Following the general procedure B, compound **SHC15** was obtained from caffeic acid (500 mg, 2.77 mmol, 1 equiv) and *N*-methyl-1-phenylmethanamine (366 mg (360 µL), 2.77 mmol, 1 equiv., as pale brown solid in 65% yield (510 mg). (R_f = 0.7 in EtOAc:hexane 2:1 v/v); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.93 (s, 1H, –OH), 9.92 (s, 1H, -OH), 7.41-7.21 (10H, m, Ar-H &-C=C), 6.98-6.78 (1H, m, -CONH), 4.78-4.60 (d, 2H,-CH_2), 3.07-2.89 (d, 3H, -CH_3). ESI/HRMS (*m/z*): Calculated for [$C_{17}H_{17}NO_3$][M+H]$^+$: 284.1208; Found *m/z*: 284.1950[M+H]$^+$.

Synthesis of (*E*)-*N*-(3,3-diethoxypropyl)-3-(3,4-dihydroxyphenyl)acrylamide (SHC20): Following general procedure B, compound **SHC20** was obtained from caffeic acid (500 mg, 2.77 mmol, 1 equiv.) and 3,3-diethoxypropan-1-amine (408 mg (450 µL), 2.77 mmol, 1 equiv., as pale brown liquid in 52% yield (445 mg) (R_f = 0.4 in EtOAc:hexane 2:1 v/v); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.70-9.30 (s, 2H, –OH), 7.24-6.75 (m, 5H, Ar-H & -C=C), 4.54-4.53 (t, 1H, -CONH), 3.59-3.47 (m, 2H, -N-CH_2), 3.46-3.34 (m, 4H, -O-CH_2), 3.20-3.17 (m, 2H, -CH_2), 1.71-1.69 (t, 1H, -O-CH-O-), 1.13-1.04 (m, 6H, -CH_3). ESI/LCMS (*m/z*): Calculated for [$C_{16}H_{23}NO_5$][M–H]$^-$: 308.15; Found *m/z*: 308.1 [M–H]$^-$.

Synthesis of (*E*)-diethyl 2,2′-((4-(3-(2-ethoxy-2-oxo-ethoxy)-3-oxoprop-1-en-1-yl)-1,2-phenylene)*bis*(oxy))-diacetate (SHC21): To a solution of caffeic acid (500 mg, 2.77 mmol, 1 equiv.) in acetone (5 mL) was added Cs_2CO_3 (5.415 g, 16.62 mmol, 6 equiv.) and stirred for 30 min at 70 ºC and cooled to room temperature and then ethyl bromoacetate (1.536 mL, 13.85 mmol, 5 equiv.) was added to the reaction mixture and further the reaction mixture was stirred at room temperature for 24 h. The reaction progress was monitored by TLC. Upon completion, the solvent acetone removed by rotary evaporator and then extracted with ethyl acetate (50 mL × 3), the combined organic phase was washed with water, saturated brine. The organic phase was dried over anhydrous sodium sulphate, solvent was filtered and concentrated under *vacuo* to get crude compound **SHC21** and then pure compound was obtained by recrystallizing with hexane and ether and finally with ethanol as white solid in 63% yield (765 mg) (R_f = 0.8 with mobile phase ethyl acetate:hexane 1:1) (**Scheme-III**). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.65-7.61 (d, 1H, Ar-H), 7.584-7.580 (d, 1H, Ar-H), 7.51-7.30 (t, 1H, Ar-H), 6.96-6.94 (d, 1H, Ar-H), 6.69-6.65 (d, 1H, Ar-H), 4.89-4.8 8 (d, 4H, -OCH_2), 4.77 (s, 2H, -COOH), 4.21-4.13 (m, 6H, -COOH), 1.24-1.19 (m, 9H, -OCH_2CH_3).

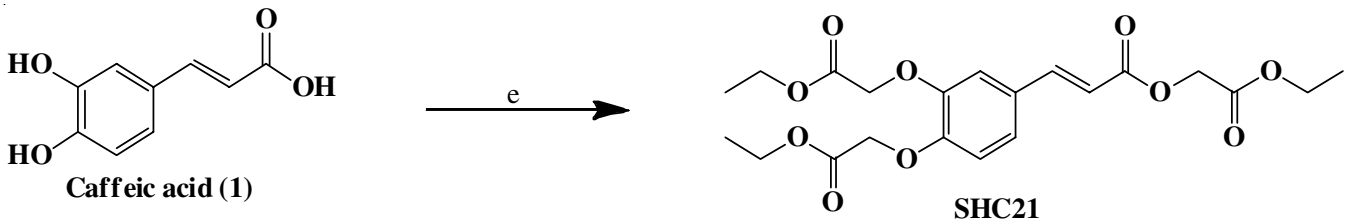
MTT cell proliferation assay: MTT assay was carried out for compound **SHC5** by following the reported procedure [44] with the concentration of 1, 10, 25 and 50 µM.

Apoptotic analysis by Annexin V/PI double staining: Annexin V/PI double staining assay was performed as described by Rangappa *et al*. [44], In brief, HeLa cells were seeded at 1×10^5 cells per well in 6 well plate and allowed to attach overnight and treated with increased concentration of compound **SHC5** and incubated for 48 h and subjected to apoptotic analysis by Annexin V/PI double staining.

Apoptotic analysis by Hoechst/PI staining: Hoechst/PI double staining assay was performed as described by Rangappa *et al*. [44], In brief, HeLa cells were seeded at 1×10^5 cells per well in 6 well plate and allowed to attach overnight and treated with increased concentration of compound **SHC5** and incubated for 48 h and subjected to apoptotic analysis by Hoechst/PI double staining.

RESULTS AND DISCUSSION

Herein, a series of novel amides and ester of caffeic acid is synthesized (**Schemes I-III**). The caffeic acid (**1**) converted to (*E*)-ethyl 3-(3,4-diethoxyphenyl)acrylate (**2**) by reacting with ethyl bromide in presence of K_2CO_3 as base in DMF solvent *via* nucleophilic substitution reaction with 89% yield. Compound **2** converted to (*E*)-3-(3,4-diethoxyphenyl)acrylic acid (**3**) by basic hydrolysis of ester group using LiOH base in methanol, water and tetrahydrofuran solvent with 82% yield. The final compounds **SH10**, **SHC11** and **SHC13** were obtained by condensation reaction between activated carboxylic acid **3** and different substituted amines using EDC·HCl and HOBt coupling reagents and DIPEA as base in DMF solvent with yield ranges from 68-85%.



Caffeic acid (1) **SHC21**

Reagents and conditions:(e) Ethyl 2-bromoacetate (5 equiv.), Cs_2CO_3 (6 equiv.), acetone (10 vol.), 70 °C-RT, 24 h

Scheme-III: Synthesis of caffeic acid derivative **SHC21**

Antiproliferative effect of compound SHC5: Uncontrolled cell proliferation is a hallmark process of cancer cells to form mass of tumor. Abrogation of proliferative potential of anticancer drug is very crucial to regress the stubborn cancer cells. Synthesized molecules were screened against HeLa-cervical cancer cells to assess the antiproliferative effect (Table-1), where compound **SHC5** displayed a significant antiproliferative activity against HeLa cells in 5.22 µM concentrations, which was unveiled by MTT assay (Fig. 2).

TABLE-1
CELL GROWTH INHIBITION (IC_{50}) OF DERIVATIVES AGAINST HeLa CELLS

Compound	IC_{50} (µM)	Compound	IC_{50} (µM)
SHC1	>25	**SHC11**	>25
SHC4	>25	**SHC13**	>25
SHC5	5.22	**SHC15**	>25
SHC8	>25	**SHC20**	>25
SHC10	>25	**SHC21**	>25

SHC5 treatment instigates chromatin condensation: Chromatin condensation is an early process before induction of apoptosis, chromatin fragmentation followed by break down of cellular proteins marks the initiation of apoptosis. Compound **SHC5** treatment against cervical cancer cells also displayed similar results in Hoechst/PI double staining. Cells displayed distorted and shrink nucleus, which was unveiled in Hoechst stained nucleus of cervical cancer cells (Fig. 3).

Apoptosis: Induction of cell death in cancer cell is very crucial to force the cancer to undergo death. Anticancer drugs induce cell death by various mechanisms to force cell to undergo apoptosis, compound **SHC5** treatment against cervical cancer cells also exhibited similar effect on HeLa cells and force them to undergo cell death. Annexin V/PI double staining clearly

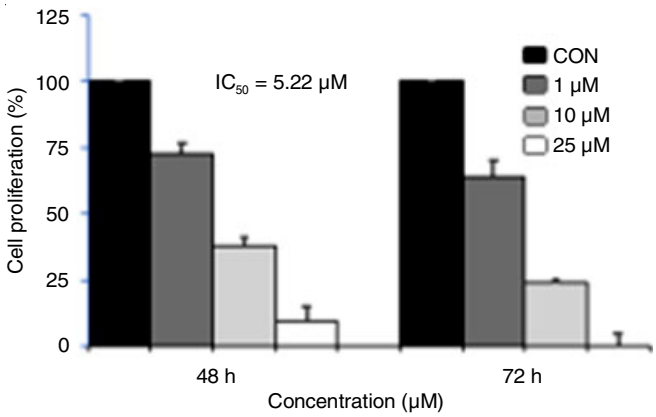


Fig. 2. Antiproliferative potential of **SHC5** was tested by seeding HeLa cells on 96 well plate and was treated with increasing concentration of **SHC5**. After 48 h and 72 h of treatment, cell proliferation was assessed by treating with MTT dye exclusion

unveils the induction of apoptosis after **SHC5** treatment (Fig. 4). Cells stained with green fluorescence indicates early apoptotic cells and cell stained with both green and red indicates late apoptotic cells.

Further, we screened SHC5 molecule against other cancer cell lines namely K562, Jurkat, HCT116 and MiaPaCa2 to test the specificity of the molecule and found to be ineffective (Table-2). The potent SHC5 molecule was characterized by other experiments against cervical cancer.

Conclusion

A series of novel caffeic acid derivatives were synthesized and screened against HeLa-cell line to test their inhibition. It was found that compound **SHC5** has shown a good inhibition with IC_{50} 5.2 µM concentration. Further, **SHC5** were screened against other cancer celllines namely K562, Jurkat, HCT116

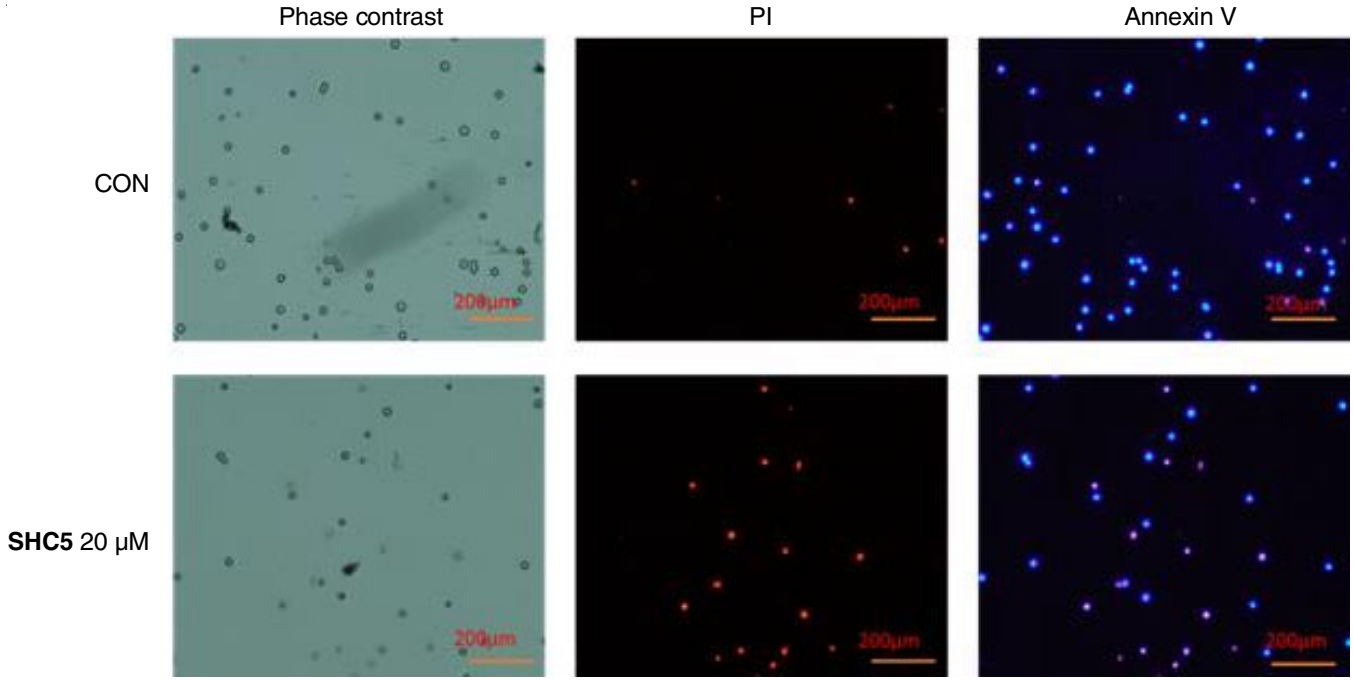


Fig. 3. Hela cells were seeded in two 60 mm dishes; one dish of cells was treated with vehicle control (DMSO) another with 20 µm SHC5 molecule. After the treatment cells were checked for chromatin condensation by employing Hoechst/PI staining

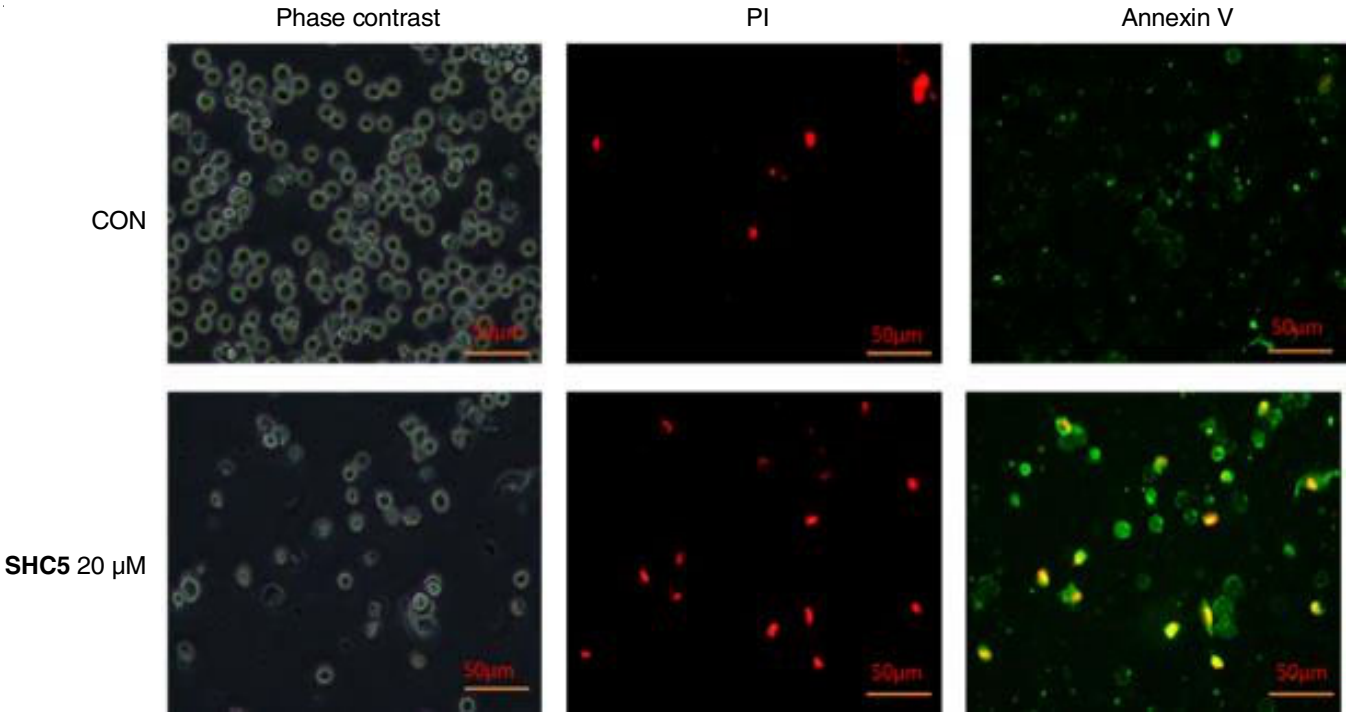


Fig. 4. HeLa cells were seeded to two 60 mm dishes, one plate of cells received vehicle control as treatment and another dish received 20 µm **SHC5** after 24 h cell were checked for early apoptosis and late apoptosis using Annexin V/PI staining

TABLE-2
CELL GROWTH INHIBITION (IC_{50}) OF **SHC5** AGAINST K562, JURKAT, HCT116 AND MiaPaCa2

Cell lines	IC_{50} (µM)
K562	>25
Jurkat	>25
HCT116	>25
MiaPaCa2	>25

and MiaPaCa2 to test the specificity of the molecule but its ineffective. These result indicated that compound **SHC5** has more specifically inhibiting cervical cancer cells. The structure activity relationship (SAR) study reveals that the caffeic acid substituted with 4-(aminomethyl)benzonitrile (**SHC5**) exhibit considerable inhibition of cancer cell growth at 5.22 µM (IC_{50}) but other derivatives did not exhibit considerable inhibition. Thus, the substituent with nitrile group at *p*-position of benzylamine is crucial to exhibit anticancer activity. This preliminary SAR study will help to design and synthesize library of molecules to find a better caffeic acid based novel anticancer agents.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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