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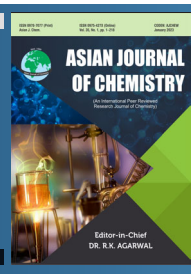

Synthesis and Anticancer Activity of Novel Thiazolo-Coumarin Derivatives

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As a starting point for creating new inhibitor scaffolds, a molecular hybridization approach was applied in designing the novel molecules using coumarin-thiazole hybrids as mPGES-1 enzyme inhibitors. Thus, in present work, the novel thiazolo coumarin derivatives (**8-35**) were synthesized and characterized by ^{1}H NMR, IR and ESI-MS spectra. All the synthesized molecules (**8-35**) were also investigated for their anticancer activity on MCF-7 (breast cancer), caco-2 (colon cancer), HeLa (Cervix cancer) cell lines. Studies revealed that compounds (**8-14**) and (**22-28**) showed inhibition (IC_{50}) at different concentrations of 12.5, 50, 100 µg/mL and more than 100 µg/mL.

Keywords: Thiazole, Coumarin, Cancer, mPGES-1 inhibitors, Molecular hybridization.

INTRODUCTION

Normally, an abnormal and unregulated cell proliferation that happens throughout the body is referred to as cancer. The most serious and deadly disease, cancer is capable of invading/spreading by a wide range of processes and resulting in a variety of cancers in both sexes and people of all ages. The latter phase of metastasis, which accounts for a large portion of cancer-related death [1]. According to WHO, cancer alone was responsible for almost 10 million deaths in 2020, making it one of the leading causes of death globally. Both the incidence and mortality of cancer are rapidly increasing globally and this trend is visible in both adults and young people, including children. About 1.9 million new cases of cancer have been identified as of 2022 and there have been 609,360 cancer-related fatalities in the USA [2-4].

In most cancer types, inflammation, cancer development and malignant progression are intimately related. An ongoing inflammatory response impairs immunity, which further creates the perfect environment for growth and metastasis. Prostaglandin E2 (PGE2) increases the growth of cancer cells and encourages tumor-favouring M2 polarization of tumor-associated macrophages (TAMs) [5,6]. Glutathione-dependent mPGES-1, a microsomal membrane protein with a molecular weight of approximately 16 KDa and 152 amino acids, is in charge of producing PGE2 during the inflammatory process [7]. The suppression of mPGES-1 can be employed in conjunction with already available standard chemotherapies, targeted treatments or immunotherapies, opening up novel cancer therapeutic options [8,9].

Among heterocyclic derivatives, coumarin and its derivatives showed significant biological activities [10-12]. Further scientific reports provide evidences that coumarin can inhibit human cancer cell lines *in vitro* and showed anti-proliferative activity in many mammalian cancers *in vivo* [13,14]. In recent works, coumarin were used as ligands in the formation of metal complexes concentrating on their use as anticancer agents [15-21]. Similarly, another heterocyclic compound thia-zole also contains both nitrogen and sulphur atoms, exhibiting numerous pharmacological activities and makes it as a most suitable, effective and widely used pharmacophore nucleus in several pharmaceutical drugs, due to its potential therapeutic benefits [22-27].

Several coumarin-thiazole hybrids were examined for their biological activity by medicinal chemists and some of them showed beneficial effects [28-30]. There are few drugs possessing coumarin-thiazole hybrids, which are currently being used commercially in the market for different therapeutic applic-

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ations. These findings enhanced our interest in looking for thiazole substituted possible pharmacologically active leads. In this study, hybridization approach is applied to discover novel coumarin-thiazole hybrids as mPGES-1 enzyme inhibitors. Substitution of coumarin at the 3rd and 6th positions with different pharmacophore like 2-aminothiazole ring, thiomorpholine group, acyclic analogs, thiazolidinone analogs) of them, few hybrids showed good selective, growth inhibitory actions against cancer cell lines MCF-7, HepG2 and SW480 [31-36].

EXPERIMENTAL

All the chemicals and solvents were procured from the S.D. Fine Chemicals Ltd., Mumbai and Avra Chemicals Pvt. Ltd. New Delhi and used without further purification. After recrystallization with aqueous ethanol, the purity of the synthesized derivatives was assessed using a single spot in TLC. The uncorrected melting points were noted using an ANALAB digital melting point instrument. The ^{1}H & ^{13}C NMR spectra were captured using DMSO-d_6 as solvent, with TMS serving as internal standard. Shimadzu FT-IR spectrophotometer and discs containing 1% KBr were used to obtain the FTIR spectra. An Agilent 1100 series mass spectrometer was used to record the mass spectra.

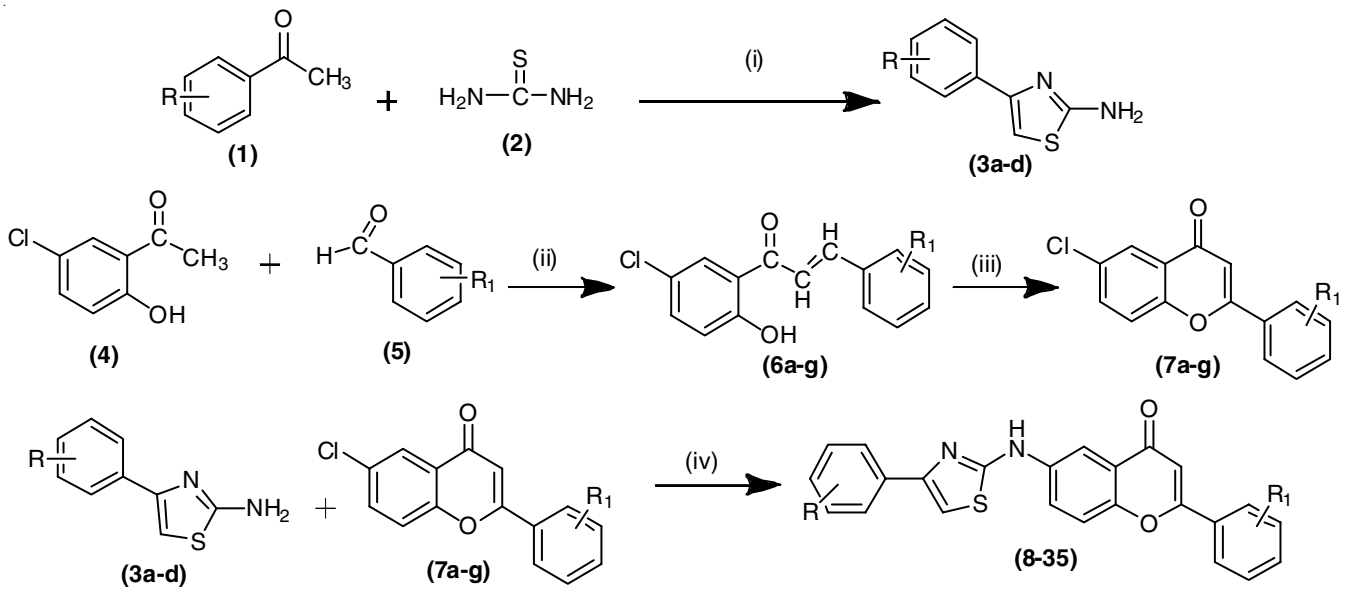
Synthesis of 4-(substituted phenyl)thiazol-2-amine (3a-d): Refluxed a mixture of various 4-substituted acetophenone (**1**, 0.1 mol), thiourea (**2**, 0.2 mol) and iodine (0.1 mol) on a water bath for 3-5 h. The hydroiodide was separated by washing with ether then the filtered solution and dried. The finished product was dissolved in hot water, filtered while still warm and the clear solution that was produced was neutralized with a strong ammonia solution. Filtered, fully water-washed and recrystallized, the separated solid.

Synthesis of chalcone derivatives (6a-g): Chalcones derivatives were synthesized by Claisen-Schmidt condensation reaction. Equimolar amounts (0.001 mol) of cold solution of 1-(5-chloro-2-hydroxyphenyl)ethenone (**4**) and various aldehydes (**5**, 0.001 mol) were dissolved in the minimum amount of alcohol. To a above mixture, 7.5 mL of 50% alcoholic KOH solution (0.003 mol) was added dropwise slowly and shaked occasionally for 24 h at room temperature. After completion of reaction, the reaction mixture was poured into crushed ice and acidified with dil. HCl resulting in the precipitation of desired intermediate compounds **6a-g**. The solid separated was filtered, dried and recrystallized using mixture mobile phase of ethyl acetate and hexane (1:1).

Synthesis of flavone derivatives (7a-g): A mixture of ice-cold solution of chalcones (**6a-g**, 0.01 mol) and conc. H_2SO_4 (50 mL) was stirred for 4-5 h at below room temperature during the entire reaction time. The resulting solution was transferred into crushed ice with vigorous stirring to obtain precipitate. To this precipitate, 20 mL of 5% K_2CO_3 solution was added and refluxed at 100 ºC for 1 to 2 h and the pH of resulting mixture 4-5 was adjusted with conc. HCl. The solid of various flavone derivatives (**7a-g**) was obtained, filtered, dried and purified with suitable solvent.

Synthesis of titled compounds (8-35): Equimolar (0.01 mol) of intermediate compounds 4-(substituted phenyl)thiazol-2-amine (**3a-d**) and flavone derivatives (**7a-g**) were dissolved in acetone (40 mL) and refluxed for 6-7 h. Periodically, alkaline pH was maintained by adding Na_2CO_3 solution (10%). Then the mixture was cooled and poured into crushed ice. The solid separated was filtered, washed with water, dried and recrystallized with absolute ethanol (**Scheme-I**).

2-Phenyl-6-((4-phenylthiazol-2-yl)amino)-4*H*-chromen-4-one (8): Yield: 78%; m.p.: 188-191 ºC; *m.w.*: 396.09. IR (KBr, ν_{max}, cm^{-1}): 3316 (NH), 1726 (C=O), 1641 (C=C), 1514, 1461, 1053 (characteristic of thiazole nucleus), 1370 (C-O); ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 7.13 (s, 1H, CH), 7.25–7.95 (m, 8H, Ar-H), 3.30 (s, 1H, -NH, thiazol-2-amine), 6.76 (s, 1H, CH-thiazole), 7.10 (s, 1H, CH-chromenone), 7.18–7.76 (m, 13H, Ar-H). ^{13}C NMR: 101.1, 104.7, 116.6, 117.1, 129.5, 130.2, 131.2, 133.4, 133.9, 134.8, 135.8, 138.2, 144.7, 151.8,



Scheme-I: Preparation of thiazolo coumarin derivatives [Reagents and conditions (i) I_2, reflux 3-5 h; (ii) EtOH, alc. KOH, dil. HCl 24 h; (iii) Conc. H_2SO_4, 4-5 h, 5% K_2CO_3, 1-2 h; (iv) acetone 6-7 h] (for R and R_1 please refer Table-1)

161.2, 164.4, 179.5. Exact mass: 396.09; found M+1 = 397.23; Elemental analysis of m.f.: $C_{24}H_{16}N_2O_2S$: Calcd. (found) %: C, 72.71 (72.70); H, 4.07 (4.10); N, 7.07 (7.10); S, 8.09 (8.10).

2-(4-Chlorophenyl)-6-((4-phenylthiazol-2-yl)amino)-4*H*-chromen-4-one (9): Yield: 69%; m.p.: 202-206 ºC; *m.w.*: 430.91. IR (KBr, ν_{max}, cm^{-1}): 3311 (NH), 1722 (C=O), 1639 (C=C), 1518, 1459, 1051 (characteristic of thiazole nucleus), 1374 (C-O), 775 (Ar-Cl); ^{1}H NMR (DMSO-d_6), δ ppm: 3.32 (s, 1H, -NH, thiazol-2-amine), 6.74 (s, 1H, CH-thiazole), 7.12 (s, 1H, CH-chromenone), 7.21-7.85 (m, 12H, Ar-H). Exact mass: 430.05; found M+1 = 431.23 *m/z*; Elemental analysis of $C_{24}H_{15}N_2O_2SCl$: Calcd. (found) %: C, 66.90 (66.91); H, 3.51 (3.50); Cl, 8.23 (8.25); N, 6.50 (6.52); S, 7.44 (7.45).

2-(4-Fluorophenyl)-6-((4-phenylthiazol-2-yl)amino)-4*H*-chromen-4-one (10): Yield: 72%; m.p.: 194-198 ºC, *m.w.*: 414.45; IR (KBr, ν_{max}, cm^{-1}): 3324 (NH), 1729 (C=O), 1642 (C=C), 1512, 1463, 1055 (characteristic of thiazole nucleus), 1372 (C-O), 987 (Ar-F); ^{1}H NMR (DMSO-d_6), δ ppm: 3.38 (s, 1H, -NH, thiazol-2-amine), 6.80 (s, 1H, CH-thiazole), 7.15 (s, 1H, CH-chromenone), 7.25–7.92 (m, 12H, Ar-H). Exact mass: 414.08; found M+1 = 415.23 *m/z*; Elemental analysis of $C_{24}H_{15}N_2O_2SF$: Calcd. (found) %: C, 69.55 (69.56); H, 3.65 (3.64); F, 4.58 (4.55); N, 6.76 (6.72); S, 7.74 (7.75).

2-(4-Hydroxyphenyl)-6-((4-phenylthiazol-2-yl)amino)-4*H*-chromen-4-one (11): Yield: 65%; m.p.: 172-176 ºC; *m.w.*: 412.46. IR (KBr, ν_{max}, cm^{-1}): 3319 (NH), 1736 (C=O), 1639 (C=C), 1518, 1461, 1049 (characteristic of thiazole nucleus), 1367 (C-O); ^{1}H NMR (DMSO-d_6), δ ppm: 3.31 (s, 1H, -NH, thiazol-2-amine), 6.78 (s, 1H, CH-thiazole), 7.09 (s, 1H, CH-chromenone), 7.19-7.81 (m, 12H, Ar-H), 11.81 (s, 1H, -OH); Exact mass: 412.09; found found M+1 = 413.52 *m/z*; Elemental analysis of $C_{24}H_{16}N_2O_3S$: Calcd. (found) %: C, 70.40 (69.82); H, 4.25 (4.10); N, 6.57 (6.58); S, 7.52 (7.48).

6-((4-Phenylthiazol-2-yl)amino)-2-(*p*-tolyl)-4*H*-chromen-4-one (12): Yield: 71%; m.p.: 185-189 ºC; *m.w.*: 410.49. IR (KBr, ν_{max}, cm^{-1}): 3325 (NH), 1725 (C=O), 1644 (C=C), 1520, 1458, 1058 (characteristic of thiazole nucleus), 1361 (C-O); ^{1}H NMR (DMSO-d_6), δ ppm: 3.12 (s, 3H, -CH_3), 3.35 (s, 1H, -NH, thiazol-2-amine), 6.71 (s, 1H, CH-thiazole), 7.14 (s, 1H, CH-chromenone), 7.24-7.86 (m, 12H, Ar-H); Exact mass: 410.11; found M+1 = 411.12 *m/z*; Elemental analysis of $C_{25}H_{18}N_2O_2S$: Calcd. (found) %: C, 69.89 (69.90); H, 3.91 (3.90); N, 6.79 (6.80); S, 7.77 (7.78).

2-(4-Methoxyphenyl)-6-((4-phenylthiazol-2-yl)amino)-4*H*-chromen-4-one (13): Yield: 62%; m.p.: 142-146 ºC; *m.w.*: 426.49. IR (KBr, ν_{max}, cm^{-1}): 3332 (NH), 1731 (C=O), 1641 (C=C), 1525, 1451, 1055 (characteristic of thiazole nucleus), 1368 (C-O); ^{1}H NMR (DMSO-d_6), δ ppm: 3.32 (s, 1H, -NH, thiazol-2-amine), 3.58 (s, 3H, -OCH_3), 6.81 (s, 1H, CH-thiazole), 7.21–7.79 (m, 12H, Ar-H). Exact mass: 426.10; found M+1 = 427.36 *m/z*; Elemental analysis of $C_{25}H_{18}N_2O_3S$: Calcd. (found) %: C, 70.40 (70.41); H, 4.25 (4.27); N, 6.57 (6.55); S, 7.52 (7.50).

2-(4-Nitrophenyl)-6-((4-phenylthiazol-2-yl)amino)-4*H*-chromen-4-one (14): Yield: 71%; m.p.: 165-169 ºC; *m.w.*: 441.46. IR (KBr, ν_{max}, cm^{-1}): 3321 (NH), 1738 (C=O), 1645 (C=C), 1538 (Ar-NO_2), 1530, 1448, 1062 (characteristic of thiazole nucleus), 1359 (C-O); ^{1}H NMR (DMSO-d_6), δ ppm: 3.38 (s, 1H, -NH, thiazol-2-amine), 6.73 (s, 1H, CH-thiazole), 7.20-7.81 (m, 12H, Ar-H); Exact mass: 441.08; found M+1 = 442.20 *m/z*; Elemental analysis of $C_{24}H_{15}N_3O_4S$: Calcd. (found) %: C, 65.30 (65.31); H, 3.42 (3.45); N, 9.52 (9.56); S, 7.26 (7.30).

6-((4-(4-Hydroxyphenyl)thiazol-2-yl)amino)-2-phenyl-4*H*-chromen-4-one (22): Yield: 62%; m.p.: 166-170 ºC; *m.w.*: 412.46. IR (KBr, ν_{max}, cm^{-1}): 3325 (NH), 3218 (Ar-OH), 1742 (C=O), 1632 (C=C), 1538, 1434, 1032 (characteristic of thiazole nucleus), 1338 (C-O); ^{1}H NMR (DMSO-d_6), δ ppm: 3.32 (s, 1H, -NH, thiazol-2-amine), 6.75 (s, 1H, CH-thiazole), 7.21-7.78 (m, 12H, Ar-H), 12.12 (s, 1H, -OH); Exact mass: 412.09; found M+1 = 413.62 *m/z*; Elemental analysis of $C_{24}H_{16}N_2O_3S$: Calcd. (found) %: C, 69.89 (69.90); H, 3.91 (3.95); N, 6.79 (6.80); S, 7.77 (7.78).

2-(4-Chlorophenyl)-6-((4-(4-hydroxyphenyl)thiazol-2-yl)amino)-4*H*-chromen-4-one (23): Yield: 69%; m.p.: 145-149 ºC*m.w.*: 446.91; IR (KBr, ν_{max}, cm^{-1}): 3334 (NH), 3217 (Ar-OH), 1748 (C=O), 1639 (C=C), 1535, 1431, 1040 (characteristic of thiazole nucleus), 1331 (C-O); ^{1}H NMR (DMSO-d_6), δ ppm: 3.39 (s, 1H, -NH, thiazol-2-amine), 6.62 (s, 1H, CH-thiazole), 7.17-7.85 (m, 11H, Ar-H), 12.20 (s, 1H, -OH); Exact mass: 446.05 found M+1 =447.01; Elemental analysis of $C_{24}H_{15}N_2O_3SCl$: Calcd. (found) %: C, 64.50 (64.55); H, 3.38 (3.40); Cl, 7.93 (7.95); N, 6.27 (6.30); S, 7.17 (7.20).

2-(4-Fluorophenyl)-6-((4-(4-hydroxyphenyl)thiazol-2-yl)amino)-4*H*-chromen-4-one (24): Yield: 60%; m.p.: 162-166 ºC; *m.w.*: 430.45 IR (KBr, ν_{max}, cm^{-1}): 3342 (NH), 3211 (Ar-OH), 1739 (C=O), 1642 (C=C), 1531, 1438, 1048 (characteristic of thiazole nucleus), 1336 (C-O), 1016 (Ar-F); ^{1}H NMR (DMSO-d_6), δ ppm: 3.32 (s, 1H, -NH, thiazol-2-amine), 6.78 (s, 1H, CH-thiazole), 6.98-7.78 (m, 11H, Ar-H), 11.92 (s, 1H, -OH); Exact mass: 430.08; found M+1 = 431.20 *m/z*; Elemental analysis of $C_{24}H_{15}N_2O_3SF$: Calcd. (found) %: C, 66.97 (66.98); H, 3.51 (3.55); F, 4.41 (4.42); N, 6.51 (6.52); S, 7.45 (7.48).

2-(4-Hydroxyphenyl)-6-((4-(4-hydroxyphenyl)thiazol-2-yl)amino)-4*H*-chromen-4-one (25): Yield: 67%; m.p.: 185-189 ºC; *m.w.*: 428.46. IR (KBr, ν_{max}, cm^{-1}): 3351 (NH), 3219 (Ar-OH), 1745 (C=O), 1639 (C=C), 1535, 1441, 1035 (characteristic of thiazole nucleus), 1341 (C-O); ^{1}H NMR (DMSO-d_6), δ ppm: 3.32 (s, 1H, -NH, thiazol-2-amine), 6.78 (s, 1H, CH-thiazole), 6.98-7.78 (m, 11H, Ar-H), 11.85 (s, 2H, -OH); Exact mass: 428.08; found M+1 = 429.35 *m/z*; Elemental analysis of $C_{24}H_{16}N_2O_4S$: Calcd. (found) %: C, 67.28 (67.30); H, 3.76 (3.80); N, 6.54 (6.55); S, 7.48 (7.45).

6-((4-(4-Hydroxyphenyl)thiazol-2-yl)amino)-2-(*p*-tolyl)-4*H*-chromen-4-one (26): Yield: 72%; m.p.: 153-157 ºC; *m.w.*: 426.49; IR (KBr, ν_{max}, cm^{-1}): 3347 (NH), 3221 (Ar-OH), 1742 (C=O), 1641 (C=C), 1539, 1445, 1039 (characteristic of thiazole nucleus), 1337 (C-O); ^{1}H NMR (DMSO-d_6), δ ppm: 3.16 (s, 3H, -CH_3), 3.28 (s, 1H, -NH, thiazol-2-amine), 6.82 (s, 1H, CH-thiazole), 7.12-8.12 (m, 11H, Ar-H), 11.96 (s, 1H, -OH); ^{13}C NMR: 25.6, 106.4, 107.3, 114.6, 115.2, 117.6, 126.7, 127.7, 128.2, 129.7, 130.2, 132.1, 132.9, 140.1, 141.5, 148.9, 151.4, 160.5 163.2, 165.8, 180.2. Exact mass: 426.10; found M+1 = 427.23 *m/z*: Elemental analysis of $C_{25}H_{18}N_2O_3S$: Calcd. (found) %: C, 70.40 (70.42); H, 4.25 (4.26); N, 6.57 (6.56); S, 7.52 (7.55).

6-((4-(4-Hydroxyphenyl)thiazol-2-yl)amino)-2-(4-methoxyphenyl)-4*H*-chromen-4-one (27): Yield: 67%; m.p.: 173-177 ºC; *m.w.*: 442.49. IR (KBr, ν_{max}, cm^{-1}): 3338 (NH), 3228 (Ar-OH), 1737 (C=O), 1639 (C=C), 1529, 1437, 1029 (characteristic of thiazole nucleus), 1332 (C-O); ^{1}H NMR (DMSO-d_6), δ ppm: 3.32 (s, 1H, -NH, thiazol-2-amine), 3.57 (s, 3H, $-CH_3$), 6.78 (s, 1H, CH-thiazole), 7.25-7.89 (m, 11H, Ar-H), 12.09 (s, 1H, -OH); Exact mass: 442.10; found M+1 = 443.21 *m/z*; Elemental analysis of $C_{25}H_{18}N_2O_4S$: Calcd. (found) %: C, 67.86 (67.90); H, 4.10 (4.12); N, 6.33 (6.35); S, 7.25 (7.30).

6-((4-(4-Hydroxyphenyl)thiazol-2-yl)amino)-2-(4-nitrophenyl)-4*H*-chromen-4-one (28): Yield: 63%; m.p.: 213-217 ºC; *m.w.*: 457.46. IR (KBr, ν_{max}, cm^{-1}): 3340 (NH), 3231 (Ar-OH), 1732 (C=O), 1645 (C=C), 1541 (Ar-NO_2), 1535, 1441, 1035 (characteristic of thiazole nucleus), 1338 (C-O); ^{1}H NMR (DMSO-d_6), δ ppm: 3.32 (s, 1H, -NH, thiazol-2-amine), 6.78 (s, 1H, CH-thiazole), 7.25-7.89 (m, 11H, Ar-H), 12.09 (s, 1H, -OH); Exact mass: 457.07; found M+1 = 458.23 *m/z*; Elemental analysis of $C_{24}H_{15}N_3O_5S$: Calcd. (found) %: C, 63.01 (62.92); H, 3.31 (3.21); N, 9.19 (9.16); S, 7.01 (7.10).

Anticancer activity: The synthesized compounds (**8-35**) were tested for their potential for inhibiting the cancer growth on different cell lines *e.g*. MCF-7 breast cancer cell lines, caco-2 colon cancer cell lines, HeLa-Cervix cancer cell lines at different concentrations.

Caco-2 activity: Fetal bovine serum (10%) was added to Dulbecco's modified Eagle's medium (DMEM) to boost the culture of Caco-2 cells, 1% penicillin, streptomycin and glutamine. Additionally, 1% NEAA and sodium pyruvate were added to the cell media. To maintain a high level of P-glycoprotein 1 (P-gp), the cells were grown for 24 h in new culture medium (DMEM) with 2 µg/mL doxorubicin.The cells were grown in a 95% humidified environment at 37 ºC with 5% CO_2. Experiments were conducted on the cells obtained between passage numbers 30 and 50 in the logarithmic growth phase.

Cell toxicityin Caco-2 cells: The MTT assay test was used to assess the Caco-2 cells' vitality.The cells (5×10^4 cells/well) were seeded in 96-well plates overnight. Set of different concentrations of compounds were prepared through serial dilution method were added and were incubated for 24 h at 37 ºC. After removing the growth media from each well, 100 µL MTT (0.5 mg/mL) was added and incubated at 37 ºC for an additional 4 h. The growth media was then taken out and the formazan formed was dissolved in 150 µL of DMSO. A wavelength of 570 nm measured the optical density after the plates were shaken at room temperature for 5 min. Graphpad Prism programme was used to determine the IC_{50} values.

HeLa activity

Cell culture: Human cervical cancer (HeLa) cells were grown in DMEM with 10% (v/v) foetal calf serum, 100 µg/mL streptomycin, 100 U/mL penicillin as supplemental ingredients. Cultures were maintained at 37 ºC in a humid incubator with a 5% CO_2 environment. The following tests were conducted using confluent cells.

MTT cell proliferation: In brief, HeLa cells in logarithmic growth phase were seeded into 96-well plates at 1×10^4 cells/well followed by incubation at 37 ºC for 24 h to allow attachment. The cells were then treated with different concentrations of the compounds prepared using serial dilution method for 24, 48 or 72 h. Six wells were included in each group. MTT (20 L of 5 mg/mL) was applied to each well and then incubated at 37 ºC for 4 h. After discarding the supernatant, the formazan were gently shaken for 10 min in 150 µL of DMSO to dissolve them. On a microplate reader, absorbance (A) was measured at 490 nm upon dissolution. From all experimental samples, background absorbance of the medium devoid of cells was subtracted. Percent viability (%) was determined using below formula:

$$\text{Viability (\%)} = \frac{\text{Drug treated group}}{\text{Control group}} \times 100$$

The assay was repeated three times and the mean (± SEM) was used to express them.

MCF-7 activity: In DMEM media supplemented with 10% FBS and 1% penicillin-streptomycin, MCF-7 cells were grown. The cultures were grown at 37 ºC in a humidified and contained 5% CO_2. Every 2 days, the medium was replaced and cells were passed through a 1:4 dilution.

MTT assay: Using MTT assay, the anticancer activity was assessed. In short, cells were cultivated in RPMI medium and raised at 37 ºC in an incubator with humidified 5% CO_2. 100 µL of cells (5 × 103/100 µL) were seeded on a 96-well cell culture plate and incubated for 24 h at 37 ± 0.5 ºC with 5% CO_2.

A concentration of 250 µg mL^{-1} in 0.5% DMSO was prepared by serially dilution in RPMI of 100 µL of compounds dissolved in DMSO. RPMI medium was replaced by RPMI media with different concentration of compounds and cells were subsequently cultured with the new medium for 2 days at 37 ± 0.5 ºC in a humidified 5% CO_2 incubator. After the completion of incubation period, media was withdrawn and 20 µL of 0.5% w/v MTT, dissolved in the phosphate buffered saline, was added to each well. The wells were then incubated for a further 4 h at 37 ± 0.5 ºC in a humidified 5% CO_2 incubator. About 100 µL of DMSO was added to each well to dissolve formazan crystals after the 4 h of incubation. Using a microplate reader, the absorbance at 550 nm was measured.

Drug-likeness properties: Further, drug-likeness properties of the synthesized compounds which show activity against different cell lines were also studied. The pharmacokinetic profiles of the molecules were predicted using Osiris property explorer.

RESULTS AND DISCUSSION

Novel thiazolo-coumarin derivatives were synthesized in three steps. In first step, intermediate compounds (**3a-d**) were synthesized by reacting 4-substituted acetophenone and thiourea by using iodine as catalyst and refluxed for 3-5 h. In next step, chalcone derivatives (**6a-g**) were synthesized through Claisen-Schmidt condensation reaction with equimolar quantities of 1-(5-chloro-2-hydroxyphenyl)ethenone and different aromatic aldehydes, further this chalcone derivatives were reacted with H_2SO_4 to undergo cyclization resulting in the

formation of flavone derivatives (**7a-g**). In final step, the equimolar quantities of intermediate compounds (substituted phenyl)-thiazol-2-amine derivatives (**3a-d**) and flavone derivatives (**7a-g**) were dissolved in acetone and refluxed by maintaining the pH on basic side using Na_2CO_3 solution (10%) resulting in the formation of final set of compounds (**8-35**) (**Scheme-I**). The compounds synthesized showed good yields with high purity. The structure of the synthesized compounds was characterized and confirmed by different spectral techniques.

In IR spectra, the typical charateristic bands at 3316 cm^{-1} ν(NH), 1706 cm^{-1} ν(C=O), 1641 cm^{-1} ν(C=C), 1514, 1461, 1053 (characteristic of thiazole nucleus) and 1370-1330 cm^{-1} range (C-O) streching bands were all oberved in all of the synthesized compounds (**8-35**). Compounds **9** and **10** exhibited aryl halide (Ar-Cl and Ar-F) charateristics streching bands at 775 and 987 cm^{-1}. Proton assignments in ^1H NMR spectra. The appearance of N-H signal as a singlet at δ 12.10 ppm, the chromenon C-H single proton as a signal between δ 7.0-7.2 ppm, a new multiplet between δ 7.18-7.92 ppm confirmed the formation of aromatic protons, clearly indicating the smooth cyclization of final compounds. Further, the titled compounds were confirmed by mass spectra (*m/z* values).

Anticancer activity: The synthesized compounds **8-35** were evaluated further for their biological activity. Compound **9** showed an IC_{50}value of 12.5 µg/mL against Caco-2 cell lines and compound **13** showed IC_{50}value of 12.5 µg/mL for MCF-7 cell lines and 25 µg/mL against Caco-2 and HeLa cells. Compound **14** shown to be more potent against MCF-7, Caco-2 and HeLa cells lines at IC_{50} at 12.5 µg/mL. Compound **10** showed IC_{50} at 25 µg/mL against HeLa and 50 µg/mL against MCF-7 and Caco-2 cell lines. The significant activity was due to the presence ofelectron donating group like methoxy group at 4th position and electron withdrawing groups like chloro, flouro and nitro at 4th position of aromatic ring attached to the coumarin moiety.

Compound **23** showed IC_{50} value of 12.5 µg/mL against Caco-2 and 25 µg/mL for MCF-7 and HeLa cells lines, whereas compound **24** showed IC_{50} value of 25 µg/mL against HeLa and 50 µg/mL for Caco-2 and MCF-7 cell lines (Table-1). This may be due to the presence of OH group at the 4th position of aromatic ring attached to thiazole moiety and presence of electron withdrawing groups like chloro and flouro at 4th position of aromatic ring attached to the coumarin moiety. This significant anticancer activity was due to the molecular hybridization of coumarin and thiazole incorporated hybrids with different substituents. The biological activities data of the synthesized compounds suggests the importance of chloro, flouro, methoxy and nitro groups favoured the activity, this confirms that proposed study have identified new scaffolds as inhibitors of mPGES-1 enzyme inhibitors. The pharmacokinetic profiles of the molecules were predicted using Osiris property explorer and the values are summarized in Table-2.

TABLE-1
ANTICANCER ACTIVITY OF SYNTHESIZED THIAZOLO-COUMARIN DERIVATIVES (**8-35**) ON DIFFERENT CANCER CELL LINES

Compd. code	R	R_1	Log P	IC_{50} (µg/mL)		
				MCF-7	Caco-2	HeLa
8	4-H	4-H	6.36	50	100	100
9	4-H	4-Cl	6.91	25	12.5	25
10	4-H	4-F	6.51	50	50	25
11	4-H	4-OH	5.97	nd	nd	100
12	4-H	4-CH_3	6.84	>100	>100	>100
13	4-H	4-OCH_3	6.32	12.5	25	25
14	4-H	4-NO_2	5.15	12.5	12.5	12.5
15	4-Cl	4-H	6.91	–	–	–
16	4-Cl	4-Cl	7.47	–	–	–
17	4-Cl	4-F	7.07	–	–	–
18	4-Cl	4-OH	6.52	–	–	–
19	4-Cl	4-CH_3	7.40	–	–	–
20	4-Cl	4-OCH_3	6.79	–	–	–
21	4-Cl	4-NO_2	5.72	–	–	–
22	4-OH	4-H	5.97	50	>100	>100
23	4-OH	4-Cl	6.52	25	12.5	25
24	4-OH	4-F	6.12	50	50	25
25	4-OH	4-OH	5.58	50	100	100
26	4-OH	4-CH_3	6.45	>100	>100	>100
27	4-OH	4-OCH_3	5.84	25	50	25
28	4-OH	4-NO_2	4.38	12.5	50	12.5
29	4-NO_2	4-H	5.04	–	–	–
30	4-NO_2	4-Cl	5.43	–	–	–
31	4-NO_2	4-F	5.09	–	–	–
32	4-NO_2	4-OH	4.26	–	–	–
33	4-NO_2	4-CH_3	5.50	–	–	–
34	4-NO_2	4-OCH_3	5.31	–	–	–
35	4-NO_2	4-NO_2	4.77	–	–	–

MCF-7 = Breast; Caco-2 = Colon; HeLa = Cervix; nd = not done

TABLE-2
PREDICTIVE DRUG LIKELINESS PROPERTIES OF SYNTHESIZED COMPOUNDS USING OSIRIS PROPERTY EXPLORER

Hit molecules	log P	log S	TPSA (Å)	Drug likeness	HBA	HBD	Number of stereocenters	Number of rotatable bonds	Drug-score	Mutagenic	Tumorigenic	Irritant	Reproductive effective
8	6.28	-6.83	79.46	2.82	4	1	0	4	0.53	In-Act	In-Act	In-Act	In-Act
9	6.88	-7.57	79.46	2.69	4	1	0	4	0.46	In-Act	In-Act	In-Act	In-Act
10	6.38	-7.15	79.46	0.05	4	1	0	4	0.39	In-Act	In-Act	In-Act	In-Act
11	5.93	-6.54	99.69	2.91	5	2	0	4	0.54	In-Act	In-Act	In-Act	In-Act
12	6.62	-7.18	79.46	2.52	4	1	0	4	0.49	In-Act	In-Act	In-Act	In-Act
13	6.21	-6.85	88.69	2.66	5	1	0	5	0.51	In-Act	In-Act	In-Act	In-Act
14	5.19	-7.29	125.2	-2.38	7	1	0	5	0.33	In-Act	In-Act	In-Act	In-Act
22	5.93	-6.54	99.69	2.98	5	2	0	4	0.54	In-Act	In-Act	In-Act	In-Act
23	6.54	-7.27	99.69	2.71	5	2	0	4	0.47	In-Act	In-Act	In-Act	In-Act
24	6.03	-6.85	99.69	0.07	5	2	0	4	0.41	In-Act	In-Act	In-Act	In-Act
25	5.59	-6.24	119.9	2.64	6	3	0	4	0.56	In-Act	In-Act	In-Act	In-Act
26	6.28	-6.88	99.69	2.54	5	2	0	4	0.50	In-Act	In-Act	In-Act	In-Act
27	5.86	-6.55	108.9	2.67	6	2	0	5	0.52	In-Act	In-Act	In-Act	In-Act
28	4.48	-7.00	145.5	-2.36	8	2	0	5	0.34	In-Act	In-Act	In-Act	In-Act

Conclusion

In summay, novel thiazole-coumarin hybrid compounds were synthesized, characterized and investigated for their anticancer activity on MCF-7 (breast cancer), caco-2 (colon cancer), HeLa (Cervix cancer) cell lines. Compounds **9**, **10**, **14** and **23** showed significant anticancer activity on different cancer cell lines at concentrations of 12.5 and 50 µg/mL. The significant anticancer activity was due to the molecular hybridization of coumarin and thiazole and incorporation of different substituents such as electron donating group like methoxy, chloro, flouro and nitro at 4th position of aromatic ring attached to the coumarin moiety and chloro and flouro at 4th position of aromatic ring attached to the coumarin moiety of hybrid for compounds **9**, **10**, **13** and **14**. Whereas compounds **23** and **24** showed activity due to the presence of OH group at 4th position of aromatic ring attached to thiazole moiety and presence of electron withdrawing groups like chloro and flouro at the 4th position of aromatic ring attached to the coumarin moiety. This confirms that proposed study has identified new scaffolds as inhibitors of mPGES-1 enzyme inhibitors. Further scrutiny and screening of this inhibitors can lead promising candidates as inhibitors of mPGES-1 against treatment of various cancers.

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CONFLICT OF INTEREST

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

REFERENCES

1. M. Akram, M. Iqbal, M. Daniyal and A.U. Khan, *Biol. Res.*, **50**, 33 (2017); https://doi.org/10.1186/s40659-017-0140-9
2. H. Sung, J. Ferlay, R.L. Siegel, M. Laversanne, I. Soerjomataram, A. Jemal and F. Bray, *CA Cancer J. Clin.*, **71**, 209 (2021); https://doi.org/10.3322/caac.21660
3. R.L. Siegel, K.D. Miller, H.E. Fuchs and A. Jemal, *CA Cancer J. Clin.*, **72**, 7 (2022); https://doi.org/10.3322/caac.21708
4. E. Ricciotti and G.A. FitzGerald, *Arterioscler. Thromb. Vasc. Biol.*, **31**, 986 (2011); https://doi.org/10.1161/ATVBAHA.110.207449
5. M. Murakami, Y. Nakatani, T. Tanioka and I. Kudo, *Prostaglandins Other Lipid Mediat.*, **68–69**, 383 (2002); https://doi.org/10.1016/S0090-6980(02)00043-6
6. G. O'Callaghan and A. Houston, *Br. J. Pharmacol.*, **172**, 5239 (2015); https://doi.org/10.1111/bph.13331
7. S. Donnini, F. Finetti, E. Terzuoli, L. Bazzani and M. Ziche, *Immunopathol. Dis. Therap.*, **5**, 223 (2014); https://doi.org/10.1615/ForumImmunDisTher.2015014095
8. B. Waltenberger, K. Wiechmann, J. Bauer, P. Markt, S. M Noha, G. Wolber, J.M. Rollinger, O. Werz, D. Schuster and H. Stuppner, *J. Med. Chem.*, **54**, 3163 (2011); https://doi.org/10.1021/jm101309g
9. V. Comunanza, *EBioMedicine*, **33**, 14 (2018); https://doi.org/10.1016/j.ebiom.2018.06.008
10. A. Carneiro, M.J. Matos, E. Uriarte and L. Santana, *Molecules*, **26**, 501 (2021); https://doi.org/10.3390/molecules26020501
11. M.A. Gouda, M.A. Salem and M.H. Helal, *Curr. Bioactive Comp.*, **16**, 818 (2020); https://doi.org/10.2174/1573407215666190405154406
12. F.G. Medina, J.G. Marrero, M. Macías-Alonso, M.C. González, I. Córdova-Guerrero, A.G. Teissier García and S. Osegueda-Robles, *Nat. Prod. Rep.*, **32**, 1472 (2015); https://doi.org/10.1039/C4NP00162A
13. E.K. Akkol, Y. Genç, B. Karpuz, E. Sobarzo-Sánchez and R. Capasso, *Cancers*, **12**, 1959 (2020); https://doi.org/10.3390/cancers12071959
14. K.V. Sairam, B.M. Gurupadayya, R.S. Chandan, D.K. Nagesha and B. Vishwanathan, *Curr. Drug Dev.*, **13**, 186 (2016); https://doi.org/10.2174/1567201812666150702102800
15. F. Annunziata, C. Pinna, S. Dallavalle, L. Tamborini and A. Pinto, *Int. J. Mol. Sci.*, **21**, 4618 (2020); https://doi.org/10.3390/ijms21134618.
16. D. Feng, A. Zhang, Y. Yang and P. Yang, *Arch. Pharm.*, **353**, 1900380 (2020); https://doi.org/10.1002/ardp.201900380

17. I. Fotopoulos and D. Hadjipavlou-Litina, *Med. Chem.*, **16**, 272 (2020); https://doi.org/10.2174/1573406415666190416121448
18. X.F. Song, J. Fan, L. Liu, X.F. Liu and F. Gao, *Arch. Pharm.*, **353**, 2000025 (2020); https://doi.org/10.1002/ardp.202000025
19. T. Al-Warhi, A. Sabt, E.B. Elkaeed and W.M. Eldehna, *Bioorg. Chem.*, **103**, 104163 (2020); https://doi.org/10.1016/j.bioorg.2020.104163
20. A. Carneiro, M.J. Matos, E. Uriarte and L. Santana, *Molecules*, **26**, 501 (2021); https://doi.org/10.3390/molecules26020501
21. S. Nayak and S.L. Gaonkar, *Mini Rev. Med. Chem.*, **19**, 215 (2019); https://doi.org/10.2174/1389557518666180816112151
22. M.T. Chhabria, S. Patel, P. Modi and P.S. Brahmkshatriya, *Curr. Top. Med. Chem.*, **16**, 2841 (2016); https://doi.org/10.2174/1568026616666160506130731
23. A. Arshad, H. Osman, M.C. Bagley, C.K. Lam, S. Mohamad and A.S.M. Zahariluddin, *Eur. J. Med. Chem.*, **46**, 3788 (2011); https://doi.org/10.1016/j.ejmech.2011.05.044
24. R.G. Kalkhambkar, G.M. Kulkarni, H. Shivkumar and R.N. Rao, *Eur. J. Med. Chem.*, **42**, 1272 (2007); https://doi.org/10.1016/j.ejmech.2007.01.023
25. B.P. Mallikarjuna, B.S. Sastry, G.V. Suresh Kumar, Y. Rajendraprasad, S.M. Chandrashekar and K. Sathisha, *Eur. J. Med. Chem.*, **44**, 4739 (2009); https://doi.org/10.1016/j.ejmech.2009.06.008
26. L. Shao, X. Zhou, Y. Hu, Z. Jin, J. Liu and J. Fang, *Synth. React. Inorg. Met.-Org. Nano-Met. Chem.*, **36**, 325 (2006); https://doi.org/10.1080/15533170600651405
27. F. Chimenti, S. Carradori, D. Secci, A. Bolasco, P. Chimenti, A. Granese and B. Bizzarri, *J. Heterocycl. Chem.*, **46**, 575 (2009); https://doi.org/10.1002/jhet.110
28. F. Annunziata, C. Pinna, S. Dallavalle, L. Tamborini and and A. Pinto, *Int. J. Mol. Sci*., **21**, 4618 (2020); https://doi.org/10.3390/ijms21134618
29. H. Singh, J.V. Singh, K. Bhagat, H.K. Gulati, M. Sanduja, N. Kumar, N. Kinarivala and S. Sharma, *Bioorg. Med. Chem.*, **27**, 3477 (2019); https://doi.org/10.1016/j.bmc.2019.06.033
30. U. Salar, K.M. Khan, S. Chigurupati, S. Syed, S. Vijayabalan, A. Wadood, M. Riaz, M. Ghufran and S. Perveen, *Med. Chem.*, **15**, 87 (2019); https://doi.org/10.2174/1573406414666180903162243
31. J. Das, P. Chen, D. Norris, R. Padmanabha, J. Lin, R. Moquin, Z. Shen, L.S. Cook, A.M. Doweyko, S. Pitt, S. Pang, D.R. Shen, Q. Fang, H.F. de Fex, K.W. McIntyre, D.J. Shuster, K.M. Gillooly, K. Behnia, G.L. Schieven, J. Wityak and J.C. Barrish, *J. Med. Chem.*, **49**, 6819 (2006); https://doi.org/10.1021/jm060727j
32. A. Ayati, T. Oghabi Bakhshaiesh, S. Moghimi, R. Esmaeili, K. Majidzadeh-A, M. Safavi, L. Firoozpour, S. Emami and A. Foroumadi, *Eur. J. Med. Chem.*, **155**, 483 (2018); https://doi.org/10.1016/j.ejmech.2018.06.015
33. P. Mutai, G. Breuzard, A. Pagano, D. Allegro, V. Peyrot and K. Chibale, *Bioorg. Med. Chem.*, **25**, 1652 (2017); https://doi.org/10.1016/j.bmc.2017.01.035
34. T.S. Chitre, M.K. Kathiravan, K.G. Bothara, S.V. Bhandari and R.R. Jalnapurkar, *Chem. Biol. Drug Design*, **78**, 826 (2011); https://doi.org/10.1111/j.1747-0285.2011.01200.x
35. K.S. Jain, J.B. Bariwal, M.S. Phoujdar, M.A. Nagras, R.D. Amrutkar, M.K. Munde, R.S. Tamboli, S.A. Khedkar, N.C. Vidyasagar, R.H. Khiste, V.V. Dabholkar and M.K. Kathiravan, *J. Heterocycl. Chem,*, **46**, 178 (2009); https://doi.org/10.1002/jhet.30
36. N.S. Vyawahare, A.A. Hadambar, A.S. Chothe, R.R. Jalnapurkar, A.M. Bhandare and M.K. Kathiravan, *J. Chem. Biol.*, **5**, 35 (2012); https://doi.org/10.1007/s12154-011-0067-5