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ORIGINAL PAPER

Synthesis and biological evaluation of novel hybrid compounds derived from gallic acid and the 2-aminothiophene derivatives

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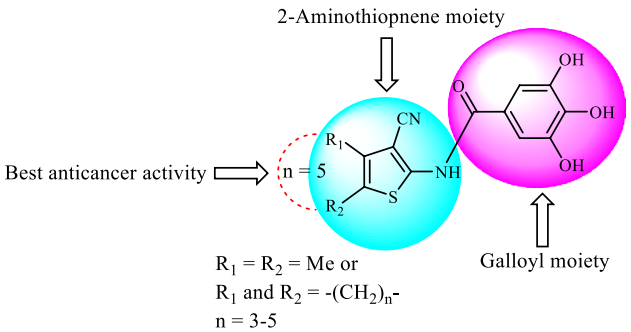
Abstract

Gallic acid (GA) and its benzamide derivatives have a wide variety of biological activities, such as antimicrobial, antioxidant, anticancer. In this study, we have reported the synthesis of some new hybrid compounds comprised of the 2-aminothiophene and GA moieties and evaluation of their cytotoxic activities against HeLa (cervical cancer), HCT116 (human colon cancer), and FT (fibroblast) cell lines as well as antimicrobial activities against some Gram-positive and Gram-negative bacteria. The reaction of some 2-aminothiophene derivatives (previously prepared from the Gewald reaction) with galloyl chloride having the acetylated hydroxyl groups and the subsequent deprotection of the hydroxyl groups gave the desired hybrid compounds. Then, the antimicrobial activity of the compounds was evaluated using disc diffusion and minimum inhibitory concentration assays. Finally, the MTT assay was carried out to evaluate the cytotoxicity of the synthesized compounds on the mentioned cell lines. The structure of the synthesized compounds was elucidated by conventional spectroscopic methods such as NMR, FT-IR, and UV–Vis spectroscopy. All compounds prevented the growth of *Staphylococcus coagulase* more than the positive control of chloramphenicol, and one compound was more sensitive to the growth of *Klebsiella pneumonia* compared to the standard antibiotic. All compounds showed acceptable activity against cancer cells. The highest activity was observed against HeLa with an IC_{50} value of 3.2 µg/mL for compound **3d** and against HCT116 with IC_{50} of 59.4 µg/mL for **3b**. The high anticancer activity of compound **3d** against HeLa allows us to consider it as a good lead compound for the development of new potent anticancer agents for the treatment of cervical cancer.

Graphic abstract



Keywords Synthesis · Biological evaluation · Anticancer activity · Antimicrobial activity · 2-Aminothiophenes · Gallic acid

Introduction

3,4,5-trihydroxybenzoic acid or gallic acid is a polyhydroxy phenolic compound that can be found in various plants such as gallnuts, sumac, tea leaves, oak bark, green tea, apple

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peels, grapes, strawberries, pineapples, bananas, lemons [1, 2]. Gallic acid and its esters, in turn, are hydroxybenzoic derivatives used as antioxidant additives in both food and pharmaceutical industry, E-310 (propyl gallate) and E-311 (octyl gallate), which are known to protect against oxidative damage induced by reactive oxygen species (ROS), as hydroxyl radicals or hydrogen peroxide, and reactive sulphur species (RSS) [3, 4]. Moreover, gallic acid derivatives are known to cause apoptosis in tumour cell lines and to inhibit lymphocyte proliferation [5]. However, the mechanism by which gallic acid analogues induce apoptosis in some cell lines is not yet completely understood, probably involving the paradoxical generation of ROS, which interferes with the homoeostatic redox balance of the cell [6–8]. It is also a fact that these compounds are excellent inhibitors of protein tyrosinase kinases (PTKs) [9, 10].

The chemistry of 2-aminothiophenes has received much attention upon their convenient availability through their most versatile synthetic method, namely the Gewald reaction [11]. An important feature of 2-aminothiophenes is that they can serve as good precursors for the synthesis of biologically active thiophene-derived heterocycles, conjugates, and hybrids [12]. For example, olanzapine is a 2-aminothiophene-derived medication that is used as antipsychotic for the treatment of schizophrenia and bipolar disorder [13]. Moreover, the 2-aminothiophene scaffolds exhibit diverse pharmacological activities in medicinal chemistry, including anticancer [14, 15], antimicrobial [14, 16, 17], and antiviral activities [15, 18].

Herein, we report the synthesis and biological evaluation of four novel hybrid compounds comprised of one gallic acid moiety and one 2-aminothiophene nucleus. Their antimicrobial activities against three Gram-positive and four Gram-negative bacteria were studied. Also, cytotoxicity activities of the hybrid compounds on the HCT116, HeLa, and FT cell lines were carried out and determined by the MTT assay method.

Materials and methods

General

All chemicals were used as received without further purification. Solvents were redistilled prior to use. All reactions were monitored by TLC on commercially available precoated glass plates (silica gel 60 F_{254}, Merck), and the plates were visualized in UV light (254 nm). Melting points were recorded on an Electrothermal-9200 melting point apparatus and are uncorrected. FT-IR spectra were measured using a Shimadzu 8400 FT-IR instrument. ^{1}H NMR and ^{13}C NMR spectra were obtained using a Bruker 300 MHz NMR spectrophotometer with TMS as the internal reference. UV–Vis spectroscopy was determined using a Shimadzu UV-1601 PC instrument. Elemental analyses were performed on a Costech ECS 4010 CHNS analyser.

General procedure for the preparation of the substituted 2-aminothiophene-3-carbonitriles (1a-d)

These compounds were prepared according to literature procedures with slight modification [19, 20]. Briefly, morpholine (5 mL, 60 mmol) was slowly added to a mixture of ketone (50 mmol), malonodinitrile (3.3 g, 50 mmol), and sulphur (1.6 g, 50 mmol) in ethanol (75 mL) at 30 °C. Then, the mixture was stirred for 8 h at this temperature. After completion, the reaction mixture was filtered, and the resulting filtrate was poured into cold water, and allowed to stand for 1 h. Finally, the precipitate was filtered, washed with cold aqueous ethanol (50%), and recrystallized from ethanol to give the desired Gewald product.

2-Amino-4,5-dimethylthiophene-3-carbonitrile (1a)

Yield: 45%; brown crystals; M.p. 136–138 °C (Lit. [21] 141-142 °C).

2-Amino-5,6-dihydro-4H-cyclopentathiophene-3-carbonitrile (1b)

Yield: 90%; brown crystals; M.p. 146–148 °C (Lit. [22] 144-145 °C).

2-Amino-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carbonitrile (1c)

Yield: 97%; cream crystals; M.p. 145–146 °C (Lit. [20] 147-148 °C).

2-Amino-5,6,7,8-tetrahydro-4H-cyclohepta[b]thiophene-3-carbonitrile (1d)

Yield: 95%; light brown needles; M.p. 120–122 °C (Lit. [22] 117-119 °C).

3,4,5-Triacetoxybenzoic acid (2a)

A solution of gallic acid (5.1 g, 30 mmol) in acetic anhydride (20 mL) was refluxed for 3 h. The solution was then poured into hot water (100 mL). During cooling time, compound **2a** was crystallized. After filtering and washing with water, the white crystals were dried *in vacuo*. Yield: 95%; M.p. 173–174 °C; FT-IR (KBr disc) ν_{max}:2400–3200 (OH), 1782 and 1699 (CO), 1605 (CC), 1178 (CO) cm^{-1}; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 2.30 (s, 6H), 2.33 (s, 3H), 7.75 (s, 2H), 13.38 (s, 1H) ppm; ^{13}C NMR (DMSO-d_6, 100 MHz):

δ 19.8, 20.3, 121.9, 128.9, 138.2, 143.2, 165.3, 166.9, 168.0 ppm.

3,4,5-Triacetoxybenzoyl chloride (2b)

Acid **2a** (5.9 g, 20 mmol) was refluxed with thionyl chloride (6 mL) for 1 h. The excess of thionyl chloride was removed by evaporating under reduced pressure. After drying, the pale yellow residue was washed with the dried diethyl ether. The raw product **2b** was recrystallized in xylene. Yield: 91%; white crystals; M.p. 102–104 °C; FT-IR (KBr disc) ν_{max}: 3092 and 2939 (CH), 1784 and 1762 (CO), 1605 (CC), 1180 (CO), 740 (CCl) cm^{-1}; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 2.31 (s, 6H), 2.32 (s, 3H), 7.89 (s, 2H) ppm.

General procedure for the synthesis of the hybrid compounds (3a-d)

A solution of compound **1** (8 mmol) and triethylamine (1.6 mL) in the dried dichloromethane (40 mL) was slowly added to a solution of **2b** (2.5 g, 8 mmol) in dried dichloromethane (40 mL), and the mixture was stirred at 0 °C for 2 h. Then, the mixture was washed with water (3 × 40 mL), dried over anhydrous sodium sulphate, evaporated to dryness. The obtained residue was re-dissolved in dichloromethane (40 mL), and hydrazine hydrate (1.3 mL) was slowly added to the solution and stirred for 1 h. The mixture was washed with water (3 × 40 mL), dried over anhydrous sodium sulphate, and evaporated to dryness. Finally, the recrystallization using water–methanol yielded compound **3**.

N-(3-cyano-4,5-dimethylthiophen-2-yl)-3,4,5-trihydroxybenzamide (3a)

Yield: 48%;brown precipitate; M.p. 292–294 °C; FT-IR (KBr disc) $\tilde{\nu}_{max}$: 3311 (NH), 3242 (OH), 2984 (CH), 2222 (CN), 1620 (CO), 1610 (CC), 1219 (CO), 1036 (CN) cm^{-1}; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 2.12 (s, 3H), 2.25 (s, 3H), 6.96 (s, 2H), 9.32 (br. s, 3H), 11.25 (s, 1H) ppm; ^{13}C NMR (DMSO-d_6, 75 MHz) δ 12.6, 12.6, 97.6, 99.9, 108.1, 115.3, 122.5, 125.6, 129.2, 138.4, 146.0, 146.1165.3 ppm; UV–Vis (DMSO): λ_{max} 461 nm; Anal. Calcd for $C_{14}H_{12}N_2O_4S$: C, 55.26; H, 3.97; N, 9.21; S, 10.53. Found: C, 55.61; H, 3.80; N, 8.86; S, 10.17.

N-(3-cyano-5,6-dihydro-4H-cyclopenta[b]thiophen-2-yl)-3,4,5-trihydroxybenzamide (3b)

Yield: 43%; mustard-yellow precipitate; M.p. 262–264 °C; FT-IR (KBr disc) $\tilde{\nu}_{max}$: 3298 (OH), 3296 (NH), 2922 (CH), 2220 (CN), 1652 (CO), 1606 (CC), 1207 (CO), 1032 (CN) cm^{-1}; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 2.36 (qui, J = 6.0 Hz, 2H), 2.74 (t, J = 6.0 Hz, 2H), 2.85 (t, J = 6.0 Hz, 2H), 6.97 (s, 2H), 9.04 (s, 1H), 9.36 (s, 2H), 11.31 (s, 1H) ppm; ^{13}C NMR (DMSO-d_6, 75 MHz) δ 27.9, 28.1, 29.6, 91.8, 108.2, 115.2, 122.5, 134.9, 138.8, 141.7, 146.1, 152.1, 165.4 ppm; UV–Vis (DMSO): λ_{max} 462 nm; Anal. Calcd for $C_{15}H_{12}N_2O_4S$: C, 56.95; H, 3.82; N, 8.86; S, 10.13. Found: C, 57.28; H, 3.68; N, 8.51; S, 9.76.

N-(3-cyano-4,5,6,7-tetrahydrobenzo[b]thiophen-2-yl)-3,4,5-trihydroxybenzamide (3c)

Yield: 45%; pale yellow precipitate; M.p. 267–268 °C; FT-IR (KBr disc) $\tilde{\nu}_{max}$: 3379 (NH), 3286 (OH), 2849 (CH), 2216 (CN), 1662 (CO), 1612 (CC), 1207 (CO), 1036 (CN) cm^{-1}; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 1.76 (s, 4H), 2.58 (s, 4H), 7.00 (s, 2H), 9.06 (s, 1H), 9.40 (s, 2H), 11.34 (s, 1H) ppm; ^{13}C NMR (DMSO-d_6, 75 MHz) δ 22.4, 23.3, 24.0, 24.1, 94.5, 108.2, 115.8, 124.0, 127.1, 130.9, 137.9, 145.9, 150.6, 165.9 ppm; UV–Vis (DMSO): λ_{max} 462 nm; Anal. Calcd for $C_{16}H_{14}N_2O_4S$: C, 58.17; H, 4.27; N, 8.48; S, 9.70. Found: C, 58.64; H, 4.12; N, 8.18; S, 9.36.

N-(3-cyano-5,6,7,8-tetrahydro-4H-cyclohepta[b]thiophen-2-yl)-3,4,5-trihydroxybenzamide (3d)

Yield: 40%; cream precipitate; M.p. 262–264 °C; FT-IR (KBr disc) $\tilde{\nu}_{max}$: 3345 (NH), 3290 (OH), 2847 (CH), 2214 (CN), 1626 (CO), 1608 (CC), 1207 (CO), 1036 (CN) cm^{-1}; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 1.60 (qui, J = 6.0 Hz, 4H), 1.78–1.81 (m, 2H), 2.65–2.70 (m, 4H), 6.99 (s, 2H), 9.01 (s, 1H), 9.34 (s, 2H), 11.30 (s, 1H) ppm; ^{13}C NMR (DMSO-d_6, 75 MHz) δ 27.5, 28.2, 28.9, 29.0, 31.9, 97.1, 108.1, 116.3, 124.0, 130.8, 135.7, 138.0, 145.9, 148.8, 166.0 ppm; UV–Vis (DMSO): λ_{max} 457 nm; Anal. Calcd for $C_{17}H_{16}N_2O_4S$: C, 59.29; H, 4.68; N, 8.13; S, 9.31. Found: C, 59.71; H, 4.47; N, 7.86; S, 8.93.

Antimicrobial activity

Three Gram-positive bacteria of Staphylococcus aureus ATCC25923, Enterococcus faecalis ATCC14506, Staphylococcus coagulase, and four Gram-negative bacteria including Escherichia coli ATCC25922, Klebsiella pneumonia ATCC13883, Proteus vulgaris ATCC33420, Pseudomonas aeruginosa ATCC27853 were used as the tested strains. All the microorganisms were obtained from the microbiology laboratory culture collection of Sabzevar Medical Science University.

Disc diffusion assay

The assay was carried out according to a previous study [23]; first, a plate of Mueller–Hinton agar medium was inoculated by the microorganism suspension (1×10^8 CFU/mL) of

microorganism. Then, a paper disc with a diameter of 6 mm, which had been impregnated with 30 µg of the compounds (3 × 10 µL of the solution of the compound with a concentration of 1 mg/mL), was placed on the inoculated agar. The plates were incubated at 37 °C for 24 h. The diameter of inhibition zones against the microorganisms was measured and reported as the antibacterial activity of the synthesized compounds. The assay was carried out in triplicate for each strain. Chloramphenicol 30 µg was used as a positive control. The values were reported in terms of inhibition levels percentage (%). The inhibition level was measured by dividing the inhibition zone diameter of the samples with that of the antibiotic (positive control) as following:

$$\text{Inhibition level (\%)} = (\text{Inhibition zone diameter of the sample}) / (\text{Inhibition zone diameter of the antibiotic}) \times 100$$

Antimicrobial activity was categorized as strong for inhibition level ≥ 70%, moderate for inhibition level 50–70%, and weak for inhibition level < 50% [24].

Minimum inhibitory concentration assay

The minimum inhibitory concentration (MIC) assay was run on the bacteria strains that showed sensitivity to the compounds in the disc diffusion method. For this purpose, a 96-well plate (8 × 12 wells) was filled with 100 µL of the culture media Mueller–Hinton broth (MHB); the first well was charged with 100 µL of DMSO solution of the synthesized compounds; then, 100 µL from each of their serial dilutions was transferred into consecutive wells; each well was charged with 50 µL of the MHB and 50 µL of the bacteria inoculums described earlier. The final volume in each well was 200 µL with concentrations of 1.00, 0.50, 0.25, 0.125, 0.062, 0.0312, 0.0156, and 0.0078 mg/mL for each compound and positive control of chloramphenicol. Each plate was used for one microorganism, respectively. Three wells in the last line of the plate were used as growth controls by filling the wells with 50 µL of inoculums and 150 µL of MHB; also, three wells were used as a negative control by filling them with 200 µL of MHB. The covered plates were incubated at 37.5 °C for 24 h in an incubator. The turbidity of each well was then observed and recorded. The minimum inhibitory concentration was assessed as the minimum concentration that resulted in no visible growth. All tests were carried out in triplicate [23].

Cells culture and treatment

Human cervical carcinoma cell line (HeLa), human colon cancer cell line (HCT116), and human embryonic kidney (HEK293-FT) cells were purchased from Pasteur Institute, Iran. The cell lines were cultured in DMEM (GIBCO) and were kept in medium supplemented with 10% FBS and antibiotics (100 U/mL penicillin G, 100 µg/mL streptomycin) at 37 °C in a humidified incubator with 5% CO_2.

Cytotoxicity assay

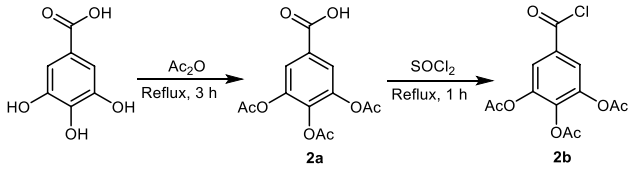
3-(4,5-dimethylthi-azol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method was used to determine the effects of the synthesized compounds on the cytotoxicity of HeLa, HCT116, and FT cell lines. Briefly, 5×10^3 cells/well were evenly distributed and incubated in 96-well plates (SPL Life Sciences) overnight. The cells were treated with the synthesized compounds at concentrations of 5, 10, 30, 50, 75,100, 130, 170, 200 µg/mL and negative control and then incubated for 24 h. Subsequently, the medium in each well was replaced with 20 µL MTT (5 mg/mL in PBS) and incubated at 37 °C for 4 h. The purple-blue formazan precipitate was dissolved in 100 µL DMSO, and the optical density was measured at a wavelength of 492 nm and a reference wavelength of 630 nm on a 96-well plate reader. The IC_{50} values were calculated as the concentration of compounds **3a-d** that caused a 50% inhibition of cell viability. Data were analysed using a GraphPad prism program (Graphpad Software Inc., San Diego, CA, USA) to determine the IC_{50} values of each compound independently.

Results and discussion

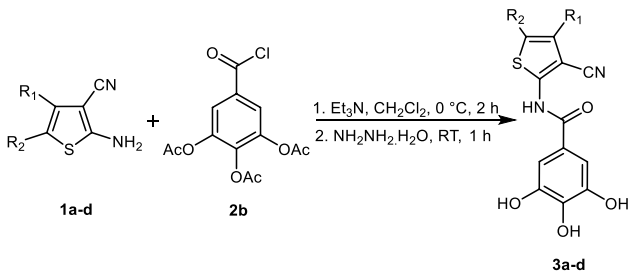
Synthesis

During our previous studies on the Gewald synthesis of 2-aminothiophenes [21, 25], we found numerous reports regarding the biological activities of the 2-aminothiophene derivatives in the literature such as anticancer, antimicrobial, antiviral, anti-tubercular, anti-parasitic activity, and also a prior molecule in modern drug design [26–28]. On the other hand, the various biological activities of gallic acid and its derivatives including anti-tumourigenic, anti-inflammatory, antioxidant, anticancer, antiviral, and antimutagenic have been extensively reported in the recent years [29–32]. These studies motivated us to design and synthesize new compounds consisting of a galloyl group and a 2-aminothiophene unit joined by an amide linkage and then examine whether the connection of these active moieties together would cause a synergic effect on their biological activities. Therefore, 2-aminothiophene-3-carbonitrile derivatives **1a-d** as one component of the desired hybrid compounds were synthesized through the Gewald three-component reaction

Scheme 1 Synthesis of 2-aminothiophene-3-carbonitriles **1a-d**



Scheme 2 Synthesis of compounds **2a** and **2b**



Scheme 3 Synthesis of the hybrid compounds **3a-d**

of 2-butanone and three cyclic ketones, and malononitrile (Scheme 1).

To fit the other component (galloyl group) for the linkage, the phenolic hydroxyl groups of gallic acid were acetylated to prevent them from participating in the linkage step. The fully acetylated acid was then activated with thionyl chloride to obtain the corresponding acid chloride (Scheme 2). Finally, the two components were linked together via a nucleophilic substitution reaction in the presence of triethylamine, and in situ deprotection of the hydroxyl groups with hydrazine hydrate yielded the desired products **3a-d** (Scheme 3).

Antibacterial activity

The antibacterial activity of compounds **3a-d** is shown in Table 1. According to the results, the compounds prevented the growth of all Gram-positive bacteria including *S. aureus*, *S. coagulase,* and *E. faecalis*. In disc diffusion assay, the compounds were recognized as a weak antibacterial agent against *S. aureus* and *E. faecalis* due to their inhibition level values (less than 50%); however, the compounds inhibited the growth of *S. coagulase* more than positive control (chloramphenicol) and they were known as strong agent against the bacterium. The maximum activity was obtained for **3b** with inhibition level of 120.8 ± 2.3 followed by **3a** (104.3 ± 2.0), **3c** (103.9 ± 2.6), and **3d** (101.3 ± 2.6). Similar results were collected for the MIC assay. **3b** inhibited the strain of *S. coagulase* with the lowest concentration of

Table 1 Antimicrobial activity of hybrid compounds using disc diffusion (DD test) and minimum inhibition concentration (MIC) assays

Microorganisms	Test	3a	3b	3c	3d	Positive control[a]
Gram-positive						
S. aureus	DD %	26.7 ± 0.4[b]	31.2 ± 1.5	35.1 ± 1.1	35.3 ± 1.1	23.2 ± 0.3[c]
	MIC mg/mL	0.41 ± 0.14	0.21 ± 0.07	0.21 ± 0.07	0.21 ± 0.07	0.03 ± 0.01
S. coagulase	DD %	104.3 ± 2.0	120.8 ± 2.3	103.9 ± 2.6	101.3 ± 2.6	7.7 ± 0.3
	MIC mg/mL	0.15 ± 0.01	0.10 ± 0.03	0.15 ± 0.01	0.15 ± 0.01	0.41 ± 0.14
E. faecalis	DD %	33.8 ± 0.7	33.2 ± 0.9	42.8 ± 1.2	38.0 ± 0.7	21.4 ± 0.4
	MIC mg/mL	0.25 ± 0.00	0.25 ± 0.00	0.17 ± 0.07	0.25 ± 0.00	0.05 ± 0.01
Gram-negative						
E. coli	DD %	NA[d]	NA	NA	NA	19.8 ± 0.7
	MIC mg/mL	NT[e]	NT	NT	NT	0.05 ± 0.01
K. pneumoniae	DD %	51.1 ± 1.0	36.3 ± 0.6	30.5 ± 0.9	65.2 ± 0.8	20.3 ± 0.6
	MIC mg/mL	0.25 ± 0.00	0.17 ± 0.07	0.41 ± 0.14	0.17 ± 0.07	0.03 ± 0.01
P. vulgaris	DD %	59.9 ± 1.2	82.7 ± 1.6	109.3 ± 1.6	91.7 ± 1.2	12.9 ± 0.4
	MIC mg/mL	0.17 ± 0.07	0.17 ± 0.07	0.10 ± 0.03	0.15 ± 0.01	0.03 ± 0.01
P. aeruginosa	DD %	NA	NA	NA	NA	24.7 ± 0.8
	MIC mg/mL	NT	NT	NT	NT	0.05 ± 0.01

[a]Chloramphenicol

[b]Values are presented as means ± SD ($n = 3$)

[c]The results of positive control in disc diffusion assay were reported in terms of mm

[d]Non-active

[e]Not tested

0.10 ± 0.03 mg/mL. The compounds inhibited two strains out of 4 Gram-negative microorganisms including *K. pneumonia* and *P. vulgaris*. The samples were inactive against *E. coli* and *P. aeruginosa*. Maximum inhibition level was obtained for **3c** against *P. vulgaris* (109.3 ± 1.6) followed by **3d** (91.7 ± 1.2), **3b** (82.7 ± 1.6), and **3a** (59.9 ± 1.2). A similarity in the results was observed for MIC assay; **3c** represented the highest activity (0.10 ± 0.03 mg/mL) followed by **3d** (0.15 ± 0.01 mg/mL), **3b**, and **3a** (0.17 ± 0.07 mg/mL).

Cytotoxicity

The cytotoxicity evaluation of the synthesized compounds was measured by the MTT assay method on three cell lines including HeLa, HCT116, and FT cells. The MTT results showed that the cytotoxicity of the compounds significantly increases in a dose-dependent manner (Fig. 1). The IC_{50} values are shown in Table 2. Compound **3d** with a cycloheptane ring incorporated into its structure exhibited excellent anticancer activity against HeLa cell line, while it had weak cytotoxicity on FT cells. Recently, Schiavon et al. have reported high anticancer activities against the HeLa cell line for a thiophene derivative which is structurally similar to compounds **3d** with respect to the thiophene moiety [33]. Therefore, it seems that the fusion of the cycloheptane ring to the 2-aminothiophene ring gives a nucleus that would have strong anticancer activity against HeLa cells.

Recently in a review work by Subramanian et al., molecular mechanisms of the anticancer activity of gallic acid and its derivatives have been comprehensively surveyed. According to the report, the activity of the compounds is related to the induction apoptosis through different mechanisms including generation of reactive oxygen species (ROS), suppression, and promotion of oncogenes, regulation of apoptotic and anti-apoptotic proteins, cell cycle arrest, and inhibition of matrix metalloproteinases (MMPs), depending upon the type of cancer studied [34]. As reported there, gallic acid induces cell lysis in the HeLa cells. Cell death is accompanied by the loss of mitochondrial membrane potential in the HeLa cells. Also, an increase in the ROS generation and GSH depletion in the HeLa cells treated with gallic acid has been observed [35].

Conclusion

Four novel hybrid compounds consist of gallic acid, and some 2-aminothiophene derivatives were synthesized, and their biological activities evaluated. The compounds inhibited the growth of *S. coagulase* more than the positive control (chloramphenicol). The maximum activity was obtained for **3b** with an inhibition level of 120.8 ± 2.3 and 0.10 ± 0.03 mg/mL in the disc diffusion and MIC assays, respectively; followed by **3c** against *P. vulgaris* (109.3 ± 1.6 for inhibition level and 0.10 ± 0.03 mg/mL for MIC). Moreover, the anticancer activities of compounds **3a-d** and gallic

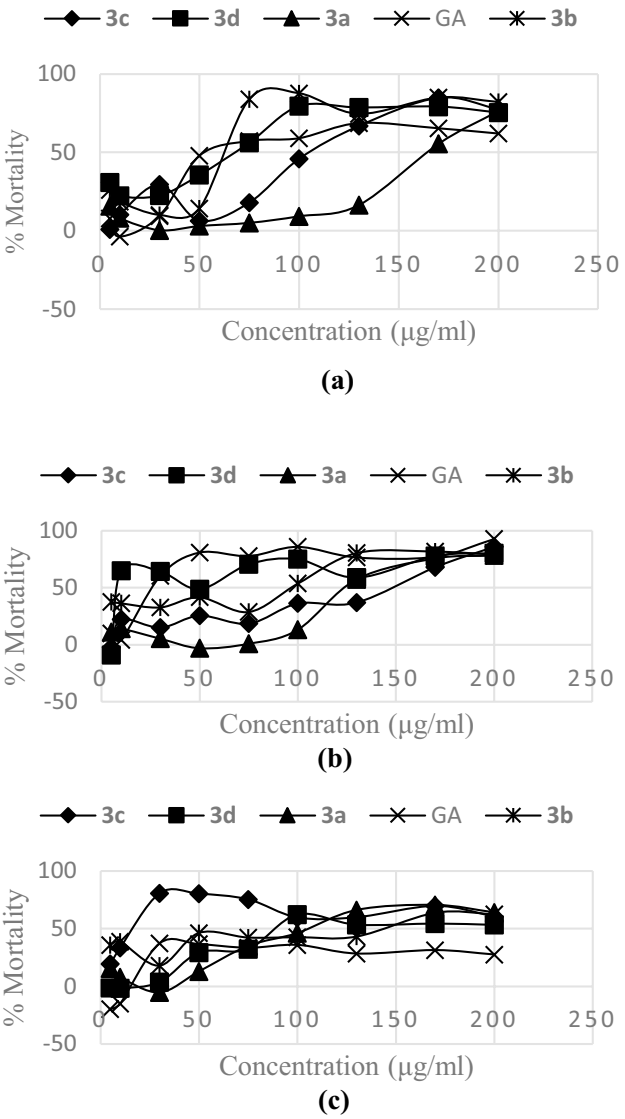


Fig. 1 Cytotoxicity activity of compounds 3a-d and gallic acid (GA) on the HCT116 (**a**), HeLa (**b**), and FT (**c**) cell lines

Table 2 In vitro anticancer activity data (µg/mL) of compounds 3a-d and gallic acid

Compound	HeLa (IC50)	HCT116 (IC50)	FT (IC50)
3a	120.1	162.7	107.4
3b	100.8	59.4	132.6
3c	165.4	102.2	10.7
3d	3.2	68.9	74.5
Gallic acid	26.3	41.8	NA[a]

[a]Non-active

acid (for comparison purpose), on the HCT116, HeLa, and FT cell lines, were determined. The results showed that compound **3d** with a seven-membered ring fused to the thiophene ring had an excellent activity ($IC_{50} = 3.2$ μg/mL) against HeLa cell line while **3c** having a six-membered ring exhibited the lowest activity ($IC_{50} = 165.4$ μg/mL). Therefore, it seems that compound **3d** could be considered as a good lead for designing of the anticancer agents targeting cervical cancer.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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