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Synthesis and biological evaluation of some new triazolo[1,5-*a*]quinoline derivatives as anticancer and antimicrobial agents

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In the present study, versatile multifunctional unreported triazolo[1,5-*a*]quinoline derivatives were prepared. Compounds **1–19** were synthesized by adopting appropriate synthetic routes and were pharmacologically evaluated for their *in vitro* anticancer activity against human cancer cell lines: hepatocellular liver carcinoma (HEPG2) and Caucasian breast adenocarcinoma (MCF-7), in addition to their antibacterial and antifungal activities. Compound **4** demonstrated strong inhibitory effects against breast cancer (MCF-7), whereas compounds **8** and **19** exhibited moderate activity against breast carcinoma cell line MCF-7. Compounds **16** and **19** gave moderate activity against liver carcinoma cell line HEPG2. The antimicrobial activity of the prepared compounds was tested against bacteria and fungi. Among them, the results of antimicrobial activity indicated that compounds **4**, **9**, **11**, **13**, **15**, **17**, **18** and **19** were the most active compounds. Compound **4** exhibited strong activity against *Fusarium* sp., whereas compounds **9**, **11**, **15**, **17**, **18** and **19** showed high activity against *Escherichia coli*. More specifically, compound **17** displayed a high inhibitory effect against *Bacillus cereus*, *Escherichia coli* and *Rhizoctonia* sp.

1. Introduction

Cancer chemotherapy has entered a new era of molecularly targeted therapeutics, which are highly selective and not associated with the serious toxicities of conventional cytotoxic drugs.[1] Quinoline is a heterocyclic scaffold of paramount importance to the human race. Several quinoline derivatives isolated from natural resources or prepared synthetically are significant with respect to medicinal chemistry and biomedical use.[2,3] In addition, a series of compounds derived from quinoline derivatives have been synthesized as potential anti-HIV agents.[4] Some quinolines showed significant similarity to some novel antifungal agents; therefore, they were screened for potential antifungal activity.[5,6] However, new quinoline derivatives display a wide spectrum of potent herbicidal[7,8] and anti-tumor activities.[9] The anti-microbial and anti-malarial activities of quinoline derivatives have been also evaluated.[10,11] Recently, quinolines and isoquinolines have been reported to possess pharmacological and clinical effects such as anti-viral,[12] anti-leishmanial,[13] anti-tuberculosis,[14] anti-inflammatory,[15] and anticancer agents,[16] as well as inhibitors for HIV-1 integrase.[17] However, the value of synthetic quinoline-based antimalarials has been seriously eroded in recent years, mainly as a result of the development and spread of resistant parasites (Winstanley and Warhurst).[18,19] Chloroquine, primaquine and mefloquine have been mainstays of malaria chemotherapy, in addition to lavendamycin, streptonigrin and streptonigrone, which have broad antimicrobial activity.

On the other hand, coumarins, an old class of benzopyrene compounds, are widely used in the pharmaceutical industry as precursor molecules in the synthesis of various anti-coagulant,[20] anti-HIV,[21] anti-tumor[22] and anti-hypertensive agents.[23] These observations aroused interest in the investigation of this structural class, and it seemed desirable to prepare and test some new triazolo[1,5-*a*]quinoline derivatives for their evaluation as anticancer and antimicrobial agents because of the aforementioned findings and due to our interest in the synthesis of a wide range of new heterocyclic compounds for biological screening.[24–29]

2. Results and discussion

The synthetic protocols of the targeted compounds are depicted in Schemes 1–3. Coumarin derivatives are important and fruitful starting materials used for synthesizing heterocyclic compounds of pharmaceutical importance. Therefore, we are

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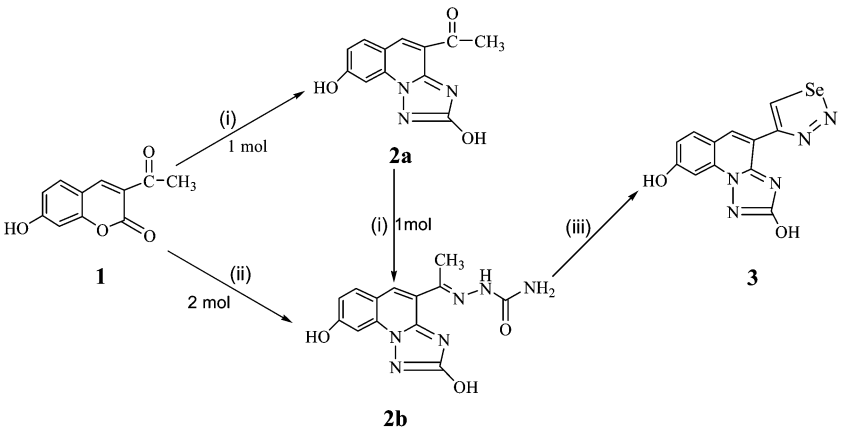


Scheme 1 Synthesis of 4-(6*H*-1,2,3-selenadiazol-4-yl)-[1,2,4]triazolo[1,5-*a*]quinoline-2,8-diol derivative **3**. Reagents and conditions: (i) 1 mol semicarbazide, sodium acetate, reflux. (ii) 2 mol semicarbazide, sodium acetate, reflux. (iii) Selenium dioxide, acetic acid, reflux.

interested in synthesizing a variety of new heterocyclic triazolo [1,5-*a*]quinoline derivatives from the coumarin moiety.

Compound **1** was synthesized *via* the condensation reaction of 4-hydroxysalicylaldehyde with ethyl acetoacetate in the presence of 4 N HCl to afford the corresponding product identified as 3-acetyl-7-hydroxy-2*H*-chromen-2-one (**1**), which was used as a starting material. The synthesis of this product was accomplished through a straightforward adaptation of the previously reported synthesis by Furniss *et al.* and Singh *et al.*[30,31] The reaction of compound **1** with one mole of semicarbazide in the presence of sodium acetate[32] led to the formation of 1-(2,8-dihydroxy-[1,2,4]triazolo[1,5-*a*]quinolin-4-yl)-ethanone (**2a**). The structure of compound **2a** was confirmed by spectroscopic data and elemental analysis. The IR spectrum of product **2a** indicated the presence of two bands (OH groups) at 3400 and 3430 cm^{-1}, one band (C=O group) at 1690 cm^{-1}, in addition to two bands at 1640 and 1645 cm^{-1} due to the presence of two C=N groups. The ^{1}H-NMR spectrum of compound **2a** showed two OH protons at δ 11.15 and 11.40 ppm, in addition to aromatic protons at δ 6.90–7.25 ppm and disappearance of the lactone band. The mass spectrum of **2a** showed a compound with *m*/*z* at 243 [M^+].

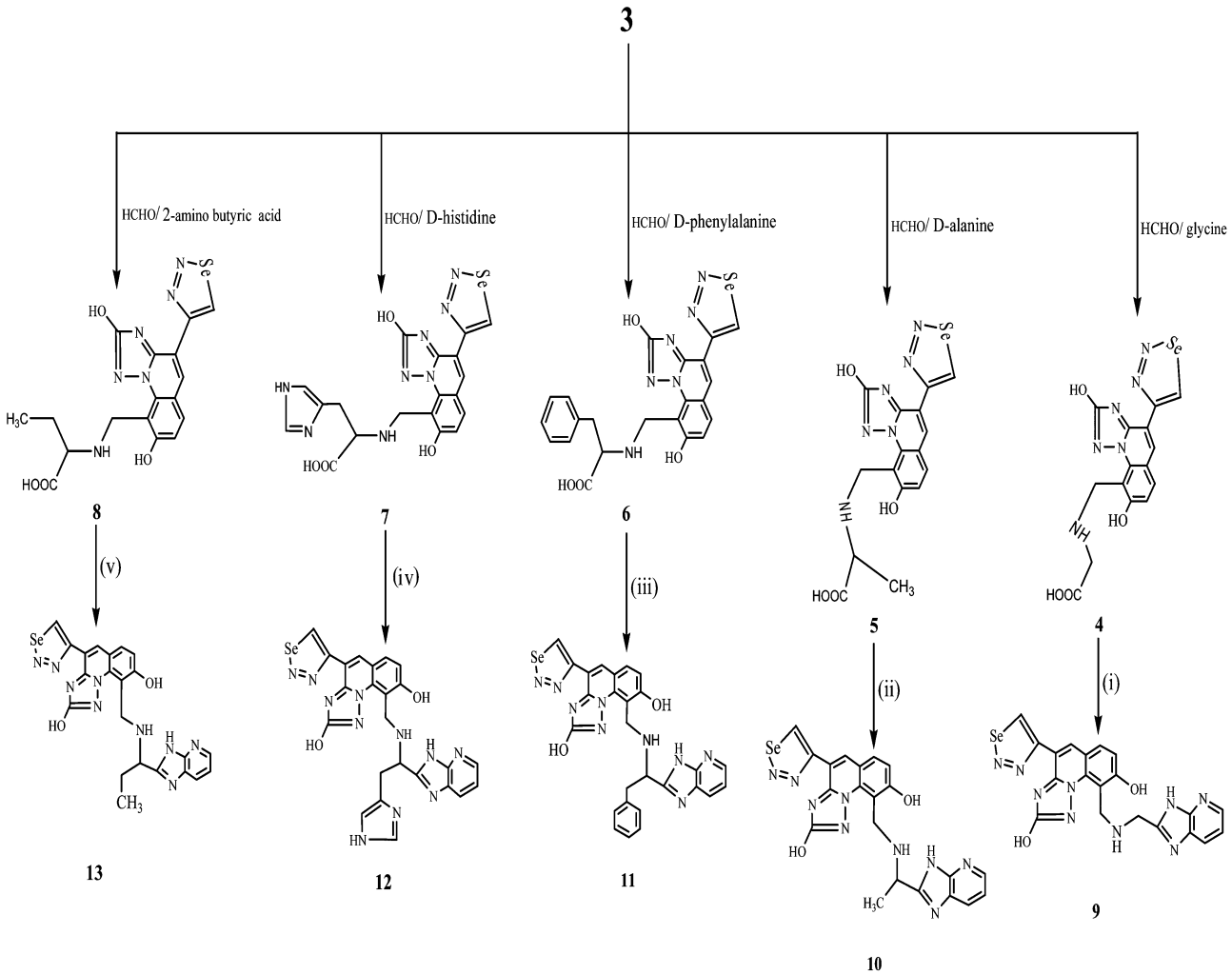
The target product **2a** reacted with another one mole of semicarbazide in sodium acetate to afford the corresponding product 2-(1-(2,8-dihydroxy-[1,2,4]triazolo[1,5-*a*]quinolin-4-yl)-ethylidene)hydrazinecarboxamide (**2b**). The IR spectrum of compound **2b** indicated the presence of two OH groups at 3445 and 3455 cm^{-1}, an NH absorption band at 3340 cm^{-1} and an absorption band at 3286 cm^{-1} (NH_2). In addition, the IR spectrum showed a C=O amide band at 1688 cm^{-1} and two absorption bands at 1635 and 1645 cm^{-1} (C=N groups), in addition to a peak in the mass spectrum with *m*/*z* at 300 [M^+].

The use of selenium dioxide as an oxidative agent is very well documented.[33,34] The oxidative cyclization of compound **2b** with selenium dioxide using acetic acid gave the corresponding 4-(6*H*-1,2,3-selenadiazol-4-yl)-[1,2,4]triazolo[1,5-*a*]quinoline-2,8-diol (**3**). The IR spectrum of compound **3** revealed absorption bands at 3435 and 3445 cm^{-1} (two OH groups), 1635 and 1644 cm^{-1} (C=N groups), in addition to an absorption band at 1575 cm^{-1} corresponding to N=N. The ^{1}H-NMR spectrum of compound **3** revealed the presence of multiple signals between δ 6.90–7.20 ppm (aromatic protons), in addition to two signals at 11.10 and 11.30 ppm corresponding to two OH protons (*cf.* experimental data).

Furthermore, when compound **3** was allowed to react with formaldehyde and amino acids, such as D-glycine, D-alanine, D-phenylalanine, D-histidine, and 2-aminobutyric acid in the presence of absolute ethanol and TEA, it led to the formation of the corresponding 2-((2,8-dihydroxy-4-(6*H*-1,2,3-selenadiazol-4-yl)-[1,2,4]triazolo[1,5-*a*]quinolin-9-yl)methylamino) carboxylic acid derivatives (**4–8**) (Scheme 2). The structures of compounds **4–8** were established on the basis of elemental analysis and spectral data (*cf.* experimental data). As an example, the IR spectrum of compound **4** showed absorption bands at 3335–3450 cm^{-1} (two OH groups), another absorption band at 1715 cm^{-1} (C=O acid), in addition to a band at 1640 cm^{-1} (C=N). The ^{1}H-NMR spectrum of compound **4** indicated the presence of signals at δ 11.00–11.40 ppm for OH protons, an NH proton at δ 9.80 ppm and aromatic protons between δ 6.80–7.00 ppm. The IR spectrum of compound **5** exhibited absorption bands at 3448–3458 cm^{-1} (attributed to the OH groups), 1700 cm^{-1} (corresponding to C=O acid) and 1570 cm^{-1} (for N=N group).

The ^{1}H-NMR spectrum of compound **6** showed the presence of signals between δ 6.86–7.45 ppm for aromatic protons, at δ 9.40 ppm for NH proton, and at δ 11.10, 11.28 and 11.50 ppm for OH protons.

The formation of 9-(1-(3*H*-imidazo[4,5-*b*]pyridin-2-yl)-4-(1,2,3-selenadiazol-4-yl)-[1,2,4]triazolo[1,5-*a*]quinoline-2,8-diol) derivatives **9–13** was achieved *via* condensation and cyclization reactions of the open compounds **4–8** with 2,3-diaminopyridine in the presence of anhydrous pyridine (*cf.* Scheme 2). The structures of the synthesized compounds **9–13** were confirmed by their corrected elemental analyses as well as spectral data. As an example, compound **9** can be structurally elucidated from analytical analysis and spectral data as follows: the ^{1}H NMR spectrum showed signals at δ 3.95 ppm, 4.20 ppm for two CH_2 protons, δ 6.80–7.10 ppm for aromatic protons, at δ 9.70 and 10.80 ppm for NH protons, and δ 11.40 and 11.58 ppm for OH



Scheme 2 Synthesis of 2-((2,8-dihydroxy-4-(6*H*-1,2,3-selenadiazol-4-yl)-[1,2,4]triazolo[1,5-*a*]quinolin-9-yl)methylamino) carboxylic acid derivatives (**4–8**) and 9-((1-(3*H*-imidazo[4,5-*b*]pyridin-2-yl)-4-(1,2,3-selenadiazol-4-yl)-[1,2,4]triazolo[1,5-*a*]quinoline-2,8-diol derivatives (**9–13**). Reagents and conditions: amino acids, *i.e.*, D-glycine, D-alanine, D-phenylalanine, D-histidine, or 2-aminobutyric acid; formaldehyde; absolute ethanol; TEA and reflux at 60 °C. (i–v) 2,3-Diaminopyridine, dry pyridine, stirring at room temperature for 1 h, then reflux.

protons. The IR spectrum displayed an absorption band at 3335–3359 cm^{-1} for NH groups and at 3445–3460 cm^{-1} for OH groups (*cf.* experimental data).

The IR spectrum of compound **10** showed absorption bands at 3438 and 3447 cm^{-1} (2 OH), 3332 and 3348 cm^{-1} (2 NH), 1640 cm^{-1} (C=N) and 1570 cm^{-1} (N=N).

The ^1H NMR spectrum of compound **13** revealed the presence of multiple signals between δ 6.86–7.45 ppm for aromatic protons and two signals at 9.50 and 10.78 ppm for two NH protons, in addition to two bands at 11.45 and 11.56 ppm for two OH protons (*cf.* experimental data).

When compound **2a** reacted with amino acids such as D-tyrosine, D-phenylalanine or D-histidine and formaldehyde in the presence of absolute ethanol and TEA under reflux conditions, it gave the corresponding 2-((2,8-dihydroxy-4-acetyl-[1,2,4]-triazolo[1,5-*a*]quinolin-9-yl)methylamino) carboxylic acid derivatives (**14–16**) (Scheme 3). The structures of compounds **14–16** were confirmed by their spectral data and elemental analyses. The IR spectra displayed characteristic absorption bands at 3420–3450 cm^{-1} (OH groups), 3380–3395 cm^{-1} (NH groups), in addition to absorption bands at 1710–1725 cm^{-1} (C=O acid) and at 1640 and 1645 cm^{-1} (C=N group).

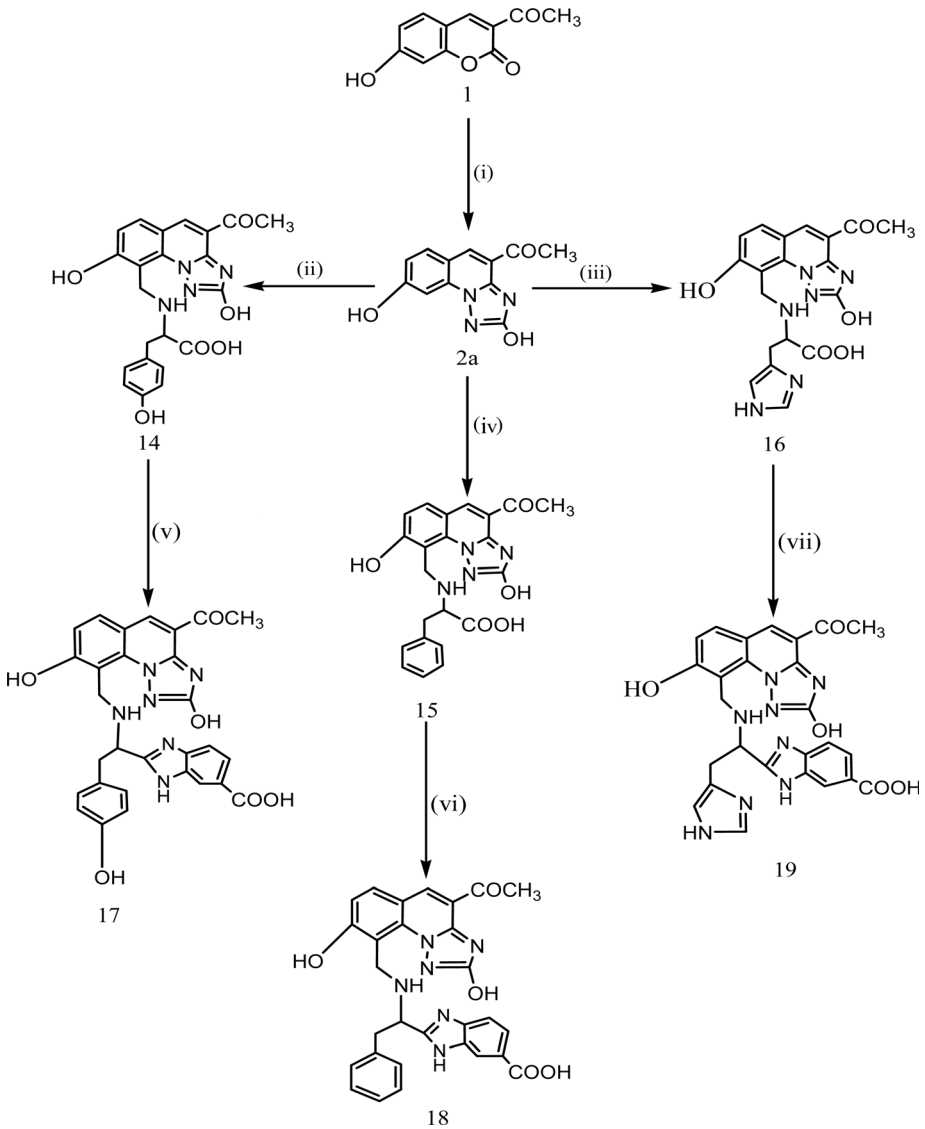
Finally, compounds **14–16** were readily condensed and cyclized to the corresponding 2-(2,8-dihydroxy-4-acetyl-[1,2,4] triazolo[1,5-*a*]quinolin-9-yl)methylamino-9-(benzimidazole-carboxylic acid) derivatives (**17–19**) upon treatment with 3,4-diaminobenzoic acid in the presence of anhydrous pyridine (*cf.* Scheme 3). The structures of compounds **17–19** were deduced from their elemental analyses and spectral data.

The IR spectra of compounds **17–19** indicated the presence of OH absorption bands at 3415–3465 cm^{-1}, NH absorption bands at 3370–3395 cm^{-1}, and CO carboxylic acid at 1710–1728 cm^{-1}.

3. Experimental

3.1 General

Reagents used for synthesis were purchased from Sigma-Aldrich and Merck. All melting points were determined using



Scheme 3 Synthesis of 2-((2,8-dihydroxy-4-acetyl-[1,2,4]triazolo[1,5-*a*]quinolin-9-yl)methylamino) carboxylic acid derivatives (**14–16**) and 2-((2,8-dihydroxy-4-acetyl-[1,2,4]triazolo[1,5-*a*]quinolin-9-yl)methylamino-9-(benzimidazole-carboxylic acid) derivatives (**17–19**). Reagents and conditions: (i) semicarbazide, absolute ethanol, TEA, reflux; (ii) D-tyrosine, HCHO, absolute ethanol, reflux; (iii) D-phenylalanine, HCHO, absolute ethanol, TEA, reflux; (iv) D-histidine, HCHO, absolute ethanol, TEA, reflux at 60 °C; (v–vii) 3,4-diaminobenzoic acid, anhydrous pyridine, reflux.

an electrothermal capillary melting point apparatus (Stuart, SMP30, UK) and are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were measured in DMSO-d_6 using a JEOL-300 spectrometer (USA), and chemical shifts were expressed as ppm against TMS as an internal reference. Mass spectra were obtained using a GC-MS Finnigan spectrometer (USA) at 70 eV. The IR spectra (4000–400 cm^{-1}) were recorded from thin films using KBr pellets in a Jasco FT/IR 300 E Fourier transform infrared spectrophotometer (JASCO Corporation, Tokyo, Japan). Elemental analyses were performed at the micro analytical laboratory of Cairo University, Cairo, Egypt. The monitoring of the progress of all reactions and homogeneity of the synthesized compounds was carried out by TLC (silica gel pre-coated aluminum cards with fluorescent indicator at 254 nm, Merck, Germany). Visualization was performed by illumination with a UV light source.

3.2 Synthesis

Synthesis of 3-acetyl-7-hydroxy-2*H*-chromen-2-one (**1**) was performed by the modified procedure of Furniss *et al.* and Singh *et al.*[30,31]

Synthesis of 1-(2,8-dihydroxy-[1,2,4]triazolo[1,5-*a*]quinolin-4-yl)ethanone (2a) and 2-(1-(2,8-dihydroxy-[1,2,4]triazolo[1,5-*a*]-quinolin-4-yl)ethylidene)hydrazinecarboxamide (2b)

General procedure. A solution of semicarbazide (0.01 mol) and crystalline sodium acetate (0.02 mol) in water (10 ml) was added with stirring to a solution of compound **1** (0.01 mol) in absolute ethanol (50 ml) containing a catalytic amount of glacial acetic acid (1 ml). Stirring was continued for 4–5 h. The reaction mixture was poured onto crushed ice and then was cooled in a refrigerator overnight. During the procedure the reaction progress was monitored by TLC (eluent: *n*-hexane–ethyl acetate:

2 : 1). The precipitated solid was filtered, washed with cold water, dried under vacuum and then crystallized from absolute ethanol to afford compound **2a**.

*1-(2,8-Dihydroxy-[1,2,4] triazolo[1,5-a]quinolin-4-yl)ethanone (**2a**).* Yield: 75%. m.p. 198–200 °C. ^{1}H-NMR: (300 MHz, DMSO): δ 2.26 (3H, s, CH_3), 6.90–7.25 (4H, m, Ar-H aromatic proton), 11.15 (1H, br s, OH-triazole), 11.40 (1H, br s, Ar-OH). ^{13}C NMR (DMSO-d_6): δ 20.90 (CH_3), 110.90, 115.80, 117.90, 119.80, 122.90, 127.00, 130.00, 135.80, 165.90, 168.00 (C=N), 171.60 (C=O). IR (KBr): ν = 3400, 3430 (2OH), 1690 (CO), 1640, 1645 (2C=N) cm^{-1}. MS: m/z = 243 [M$^+$]; anal. calcd for $C_{12}H_9N_3O_3$: C, 59.25; H, 3.70; N, 17.28; found: C, 59.29; H, 3.73; N, 17.24.

2-(1-(2,8-Dihydroxy-[1,2,4]triazolo[1,5-*a*]quinolin-4-yl)ethylidene)hydrazinecarboxamide (**2b**) was prepared according to the same procedure as **2a**.

*2-(1-(2,8-dihydroxy-[1,2,4]triazolo[1,5-a]quinolin-4-yl)ethylidene)-hydrazinecarboxamide (**2b**).* Yield 78%; m.p. 170–172 °C; ^{1}H-NMR (300 MHz, DMSO-d_6): δ 2.18 (3H, s, CH_3), 6.40 (2H, s, NH_2), 6.80–7.00 (4H, m, Ar-H aromatic proton), 10.30 (1H, br s, NH, D_2O-exchangeable), 11.25 (1H, br s, OH-triazole proton), 11.45 (1H, br s, OH-aromatic proton), ^{13}C NMR (DMSO-d_6): δ 22.10 (CH_3), 110.80, 111.90, 115.80, 117.90, 118.80, 122.59, 130.20, 134.60, 161.90, 166.20 (C=N), 175.60 (C=O); IR (KBr) ν = 3440, 3445 (2 OH), 3340 (NH), 3286 (NH_2), 3165 (CH aromatic), 1688 (C=O amide), 1635, 1645 (2C=N), 1630 (C=C) cm^{-1}; MS: m/z = 300 [M$^+$]; anal. calcd for $C_{13}H_{12}N_6O_3$. C, 52.00; H, 4.03; N, 27.99; found C, 52.08; H, 4.10; N, 28.06.

*Synthesis of 4-(6H-1,2,3-selenadiazol-4-yl)-[1,2,4]triazolo[1,5-a]-quinoline-2,8-diol (**3**).* A solution of compound **2b** (5 mmol) in glacial acetic acid (15 ml) was warmed to 80 °C with stirring, then selenium dioxide (0.55 g, 5 mmol) was added dropwise during a period of 30 min and stirring was continued for a further 4 h (TLC control). After completion of the reaction, the reaction mixture was filtered to remove the deposited selenium. The filtrate was poured onto crushed ice and the obtained solid was filtered off and washed thoroughly with cold water, sodium carbonate solution and water (10%). The obtained product after drying was recrystallized from absolute ethanol to give compound **3**.

*4-(6H-1,2,3-Selenadiazol-4-yl)-[1,2,4]triazolo[1,5-a]quinoline-2,8-diol (**3**).* Yield 74%; m.p. 200–202 °C; ^{1}H-NMR (300 MHz, DMSO-d_6): δ 5.90 (1H, s, CH-selenadiazol), 6.90–7.20 (4H, m, Ar-H aromatic proton), 11.10 (1H, br s, OH-triazole proton), 11.30 (1H, br s, OH-aromatic proton); ^{13}C NMR (DMSO-d_6): δ 114.60, 116.90, 117.90, 118.90, 122.60, 124.50, 126.90, 131.50, 135.80, 136.90, 156.30, 158.90 (C=N); IR (KBr) ν = 3435, 3445 (2 OH), 3170 (CH aromatic), 1635, 1644 (C=N), 1633 (C=C), 1575 (N=N) cm^{-1}; MS: m/z = 332 [M$^+$ − 1]; anal. calcd for $C_{12}H_7N_5O_2Se$: C, 43.38; H, 2.10; N, 21.09; Se, 23.77; found: C, 34.34; H, 2.14; N, 21.02; Se, 23.72.

Synthesis of 2-((2,8-dihydroxy-4-(6*H*-1,2,3-selenadiazol-4-yl)-[1,2,4]triazolo[1,5-*a*]quinolin-9-yl)methylamino) carboxylic acid derivatives (4–8)

General procedure. A mixture of compound **3**, amino acids (0.05 mmol), *i.e.*, D-glycine, D-alanine, D-phenylalanine, D-histidine, or 2-aminobutyric acid, and formaldehyde (1.25 mmol) in absolute ethanol (30 ml) containing TEA was refluxed at 60 °C with stirring for 5–6 h. The completion of the reaction was confirmed by TLC (eluent: *n*-hexane–ethyl acetate: 2 : 1). The product was then cooled to room temperature. The solvent was evaporated under vacuum and the precipitated solid obtained was dried to afford compounds **4–8**.

*2-((2,8-Dihydroxy-4-(6H-1,2,3-selenadiazol-4-yl)-[1,2,4]triazolo-[1,5-a]quinolin-9-yl)methylamino)acetic acid (**4**).* Yield 72%; m.p. 180–182 °C; ^{1}H-NMR (300 MHz, DMSO-d_6): δ 4.30 (2H, s, *CH_2*–), 4.50 (2H, d, *CH_2*–), 5.80 (1H, s, CH selenadiazol proton), 6.80–7.00 (3H, m, Ar-H), 9.80 (1H, br s, *NH*–, D_2O-exchangeable), 11.00 (1H, br s, CO*OH*), 11.20 (1H, br s, OH-triazole proton), 11.40 (1H, br s, OH-aromatic proton); ^{13}C NMR (DMSO-d_6): δ 39.90 (CH_2), 41.90 (CH_2), 110.60, 113.90, 118.50, 119.60, 121.80, 125.20, 127.90, 131.50, 134.80, 136.90, 156.60, 159.80 (C=N), 178.80 (CO acid); IR (KBr) ν = 3420, 3435, 3450 (3 OH), 3168 (CH aromatic), 1715 (CO carboxylic acid), 1640 (C=N), 1630 (C=C), 1570 (N=N) cm^{-1}; anal. calcd for $C_{15}H_{12}N_6O_4Se$: C, 42.97; H, 2.88; N, 20.05; Se, 18.83; found: C, 43.07; H, 2.94; N, 20.10; Se, 18.87.

*2-((2,8-Dihydroxy-4-(6H-1,2,3-selenadiazol-4-yl)-[1,2,4]triazolo-[1,5-a]quinolin-9-yl)methylamino)propanoic acid (**5**).* Yield 70%; m.p. 190–192 °C; ^{1}H-NMR (300 MHz, DMSO-d_6): δ 2.20 (3H, d, *CH_3*–), 4.10 (2H, s, *CH_2*–), 5.65 (1H, s, CH selenadiazol proton), 5.99 (1H, q, *CH*–), 6.90–7.10 (3H, m, Ar-H), 9.50 (1H, br s, *NH*–CH, D_2O-exchangeable), 11.20 (1H, br s, CO*OH*), 11.40 (1H, br s, *OH*-triazole), 11.60 (1H, br s, OH-aromatic); ^{13}C NMR (DMSO-d_6): δ 24.90 (CH_3), 40.90 (CH_2), 50.60 (CH), 111.60, 114.90, 116.50, 118.60, 121.80, 124.50, 126.80, 131.50, 134.80, 136.90, 158.60, 161.80 (C=N), 168.80 (CO acid); IR (KBr) ν = 3430, 3440, 3455 (3 OH), 3165 (CH aromatic), 1700 (CO carboxylic acid), 1658 (C=N), 1635 (C=C), 1570 (N=N) cm^{-1}; anal. calcd for $C_{16}H_{14}N_6O_4Se$: C, 44.35; H, 3.26; N, 19.40; O, 14.77; Se, 18.22; found: C, 44.39; H, 3.32; N, 19.48; Se, 18.27.

*2-((2,8-Dihydroxy-4-(6H-1,2,3-selenadiazol-4-yl)-[1,2,4]triazolo-[1,5-a]quinolin-9-yl)methylamino)-3-phenylpropanoic acid (**6**).* Yield 71%; m.p. 150–152 °C; ^{1}H-NMR (300 MHz, DMSO-d_6): δ 3.82 (2H, d, CH_2), 4.30 (2H, d, *CH_2*–), 5.70 (1H, s, CH selenadiazol proton), 5.95 (2H, t, *CH_2*–), 6.86–7.12 (3H, m, Ar-H aromatic proton), 7.29–7.45 (5H, m, Ar-H), 9.40 (1H, br s, NH, D_2O-exchangeable), 11.10 (1H, br s, CO*OH*), 11.28 (1H, br s, *OH*-triazole), 11.50 (1H, br s, OH aromatic); IR (KBr) ν = 3415, 3430, 3440 (3 OH), 3169 (CH aromatic), 1715 (CO carboxylic acid), 1650 (C=N), 1636 (C=C), 1575 (N=N) cm^{-1}; anal. calcd for $C_{22}H_{18}N_6O_4Se$: C, 51.87; H, 3.56; N, 16.50; Se, 15.50; found: C, 51.80; H, 3.6; N, 16.55; O; Se, 15.56.

*2-((2,8-Dihydroxy-4-(6H-1,2,3-selenadiazol-4-yl)-[1,2,4]triazolo-[1,5-a]quinolin-9-yl)methylamino)-3-(1H-imidazol-4-yl)propanoic acid (**7**).* Yield 71%; m.p. 140–142 °C; ^{1}H-NMR (300 MHz, DMSO-d_6): δ 3.85 (2H, s, *CH_2*–), 4.15 (2H, d, *CH_2*–), 5.60 (1H, s, CH selenadiazol proton), 5.90 (1H, t, *CH*–), 6.10 (1H, s, –*CH*–NH imidazole proton), 6.60 (1H, s, CH-imidazole proton), 6.90–7.18 (3H, m, Ar-H), 9.60 (1H, br s, CH_2–*NH*, D_2O-exchangeable), 10.40 (1H, br s, NH, imidazole D_2O-exchangeable), 11.20 (1H, br s, CO*OH*), 11.50 (1H, br s, OH-triazole), 11.70 (1H, s, OH-aromatic); IR (KBr) ν = 3420, 3437, 3446 (3 OH), 3164 (CH aromatic), 1710 (CO carboxylic acid), 1658 (C=N), 1633 (C=C), 1573 (N=N) cm^{-1}; anal. calcd for $C_{19}H_{16}N_8O_4Se$: C, 45.70; H,

3.23; N, 22.44; O, 12.82; Se, 15.81; found: C, 45.78; H, 3.29; N, 22.50; Se, 15.88.

*2-((2,8-Dihydroxy-4-(6H-1,2,3-selenadiazol-4-yl)-[1,2,4]triazolo-[1,5-a]quinolin-9-yl)methylamino)butanoic acid (**8**).* Yield 68%; m.p. 130–132 °C; 1H-NMR (300 MHz, DMSO-d_6): δ 2.18 (3H, t, CH_3), 3.85 (2H, s, CH_2–), 4.15 (2H, m, CH_2–), 5.55 (1H, t, *CH*–), 5.70 (1H, s, CH selenadiazol proton), 6.80–7.10 (3H, m, Ar-H), 9.20 (1H, br s, *NH*–, D_2O-exchangeable), 11.15 (1H, br s, OH), 11.35 (1H, br s, OH-triazole), 11.58 (1H, br s, OH aromatic); ^{13}C NMR (DMSO-d_6): δ 20.75 (CH_3), 39.10 (CH_2), 42.19 (CH_2), 51.80 (CH), 112.60, 113.80, 117.50, 118.90, 119.60, 124.80, 125.20, 126.90, 131.50, 134.80, 136.90, 152.60, 159.30 (C=N), 170.80 (CO acid); IR (KBr) ν = 3415, 3430, 3440 (3 OH), 3168 (CH aromatic), 1720 (CO carboxylic acid), 1650 (C=N), 1638 (C=C), 1578 (N=N) cm^{-1}; anal. calcd for $C_{17}H_{16}N_6O_4Se$: C, 45.65; H, 3.61; N, 18.79; Se, 17.60; found: C, 45.72; H, 3.68; N, 18.84; Se, 17.69.

Synthesis of 9-(1-(3*H*-imidazo[4,5-*b*]pyridin-2-yl)-4-(1,2,3-selenadiazol-4-yl)-[1,2,4]triazolo[1,5-*a*]quinoline-2,8-diol) derivatives (9–13)

General procedure. A mixture of compounds **4–8** (0.01 mmol) and 2,3-diaminopyridine (0.01 mmol) was dissolved in dry pyridine (10 ml) with stirring at room temperature for 1 h, and then heated under reflux for 5–6 h. The completion of the reaction was confirmed by TLC (eluent: petroleum ether (60–80)–ethyl acetate: 9 : 1). The reaction was cooled to room temperature, and then poured into crushed ice containing HCl (2 ml of 10% HCl). The formed precipitate was allowed to settle down overnight at room temperature, and then filtered, dried under vacuum and recrystallized from absolute ethanol to produce compounds **9–13**.

*9-(((3H-Imidazo[4,5-b]pyridin-2-yl)methylamino)methyl)-4-(1,2,3-selenadiazol-4-yl)-[1,2,4]triazolo[1,5-a]quinoline-2,8-diol (**9**).* Yield 74%; m.p. 166–186 °C. ^{1}H-NMR (300 MHz, DMSO-d_6): δ 3.95 (2H, s, CH_2–), 4.20 (2H, s, CH_2–), 5.86 (1H, s, CH-selenadiazol proton), 6.80–7.12 (3H, m, Ar-H aromatic), 7.20–7.48 (3H, m, Ar-H-pyridine), 9.70 (1H, br s, NH, D_2O-exchangeable), 10.80 (1H, br s, NH, D_2O-exchangeable), 11.42 (1H, br s, OH-triazole), 11.58 (1H, br s, OH-aromatic); IR (KBr) ν = 3445, 3460 (2 OH), 3335, 3359 (2 NH), 1654 (C=N), 1630 (C=C), 1576 (N=N) cm^{-1}; MS: m/z = 491[M$^+$ − 1]; anal. calcd for $C_{20}H_{15}N_9O_2Se$: C, 48.79; H, 3.07; N, 25.60; Se, 16.04; found: C, 48.70; H, 3.15; N, 25.69; O; Se, 16.10.

*9-((1-(3H-Imidazo[4,5-b]pyridin-2-yl)ethylamino)methyl)-4-(1,2,3-selenadiazol-4-yl)-[1,2,4]triazolo[1,5-a]quinoline-2,8-diol (**10**).* Yield 75%; m.p. 177–179 °C. ^{1}H-NMR (500 MHz, DMSO-d_6): δ 2.18 (3H, s, CH_3), 4.25 (2H, s, CH_2–), 5.20 (1H, q, –CH–), 5.65 (1H, s, CH-selenadiazol), 6.90–7.18 (3H, m, Ar-H aromatic proton), 7.15–7.32 (3H, m, Ar-H-pyridine), 9.60 (1H, br s, NH, D_2O-exchangeable), 10.55 (1H, br s, NH, D_2O-exchangeable), 11.20 (1H, br s, OH-triazole), 11.40 (1H, br s, OH-aromatic); IR (KBr) ν = 3438, 3447 (2 OH), 3332, 3348 (2 NH), 1640 (C=N), 1633 (C=C), 1570 (N=N) cm^{-1}; MS: m/z = 505 [M$^+$ − 1]; anal. calcd for $C_{21}H_{17}N_9O_2Se$: C, 49.81; H, 3.38; N, 24.89; Se, 15.59; found: C, 49.86; H, 3.32; N, 24.84; Se, 15.53.

*9-((1-(3H-Imidazo[4,5-b]pyridin-2-yl)-2-phenylethylamino)methyl)-4-(1,2,3-selenadiazol-4-yl)-[1,2,4]triazolo[1,5-a]quinoline-2,8-diol (**11**).* Yield 72%; m.p. 124–126 °C. ^{1}H-NMR (300 MHz, DMSO-d_6): δ 3.90 (2H, s, CH_2–NH), 4.15 (2H, d, –CH–CH_2), 5.58 (1H, t, –CH–), 5.70 (1H, s, CH-selenadiazol proton), 6.75–7.00 (3H, m, Ar-H aromatic proton), 7.10–7.25 (5H, m, Ar-H aromatic proton), 7.35–7.55 (3H, m, Ar-H-pyridine), 9.68 (1H, br s, NH, D_2O-exchangeable), 10.50 (1H, br s, NH, D_2O-exchangeable), 11.25 (1H, s, OH-triazole), 11.45 (1H, s, OH-aromatic); IR (KBr) ν = 3445, 3455 (2 OH), 3356, 3368 (2 NH), 1649 (C=N), 1635 (C=C), 1572 (N=N) cm^{-1}; MS: m/z = 581 [M$^+$ − 1]; anal. calcd for $C_{27}H_{21}N_9O_2Se$: C, 55.67; H, 3.63; N, 21.64; O, 5.49; Se, 13.56; found: C, 55.72; H, 3.68; N, 21.64; Se, 13.62.

*9-((1-(3H-Imidazo[4,5-b]pyridin-2-yl)-2-(1H-imidazol-4-yl)ethylamino)methyl)-4-(6H-1,2,3-selenadiazol-4-yl)-[1,2,4]triazolo[1,5-a]-quinoline-2,8-diol (**12**).* Yield 73%; m.p. 184–186 °C. ^{1}H-NMR (300 MHz, DMSO-d_6): δ 3.90 (2H, s, CH_2–), 4.15 (2H, d, CH_2–), 5.40 (1H, t, –CH–), 5.60 (1H, s, CH-selenadiazol), 5.90 (1H, s, –CH-imidazole proton), 6.10 (1H, s, –CH-imidazole proton), 6.80–7.15 (3H, m, Ar-H aromatic proton), 7.29–7.58 (3H, m, Ar-H), 9.50 (1H, br s, NH-imidazole, D_2O-exchangeable), 9.69 (1H, br s, NH, D_2O-exchangeable), 10.58 (1H, br s, NH, D_2O-exchangeable), 11.27 (1H, br s, OH-triazole), 11.48 (1H, br s, OH-aromatic proton); IR (KBr) ν = 3433, 3445 (2 OH), 3348, 3360 (2 NH), 1650 (C=N), 1635 (C=C), 1574 (N=N) cm^{-1}; MS: m/z = 570 [M$^+$ − 2]; anal. calcd for $C_{24}H_{19}N_{11}O_2Se$: C, 50.36; H, 3.35; N, 26.92; O, 5.59; Se, 13.79; found: C, 50.31; H, 3.39; N, 26.99; Se, 13.85.

*9-((1-(3H-Imidazo[4,5-b]pyridin-2-yl)propylamino)methyl)-4-(1,2,3-selenadiazol-4-yl)-[1,2,4]triazolo[1,5-a]quinoline-2,8-diol (**13**).* Yield 73%; m.p. 160–160 °C. ^{1}H-NMR (300 MHz, DMSO-d_6): δ 1.38 (3H, t, CH_3), 3.85 (2H, s, CH_2–), 4.28 (2H, m, CH_2–), 5.55 (1H, t, *CH*–), 5.77 (1H, s, CH-selenadiazol), 6.86–7.20 (3H, m, Ar-H aromatic proton), 7.30–7.45 (3H, m, Ar-H-pyridine proton), 9.50 (1H, br s, NH, D_2O-exchangeable), 10.78 (1H, br s, NH, D_2O-exchangeable), 11.45 (1H, br s, OH-triazole), 11.56 (1H, br s, OH aromatic); IR (KBr) ν = 3446, 3458 (2 OH), 3352, 3368 (2 NH), 1659 (C=N), 1638 (C=C), 1578 (N=N) cm^{-1}; MS: m/z = 518 [M$^+$ − 2]; anal. calcd for $C_{22}H_{19}N_9O_2Se$: C, 50.77; H, 3.68; N, 24.22; O, 6.15; Se, 15.17; found: C, 50.72; H, 3.74; N, 24.25; Se, 15.23.

Synthesis of 2-(2,8-dihydroxy-4-acetyl-[1,2,4]triazolo[1,5-*a*]-quinolin-9-yl) carboxylic acid derivatives (14–16)

General procedure. A mixture of 1-(2,8-dihydroxy-[1,2,4]triazolo[1,5-*a*]quinolin-4-yl)ethanone **2a** (0.01 mol), an amino acid, *i.e.*, D-tyrosine, D-phenylalanine or D-histidine (0.01 mol), and formaldehyde (0.01 mol) in absolute ethanol (30 ml) containing triethylamine (TEA, 1 ml) was refluxed with stirring for 5–6 h at 60 °C. The reaction mixture was monitored by TLC (eluent: *n*-hexane–ethyl acetate: 2 : 1). The solvent was then evaporated under vacuum, and the precipitate obtained was filtered and dried under vacuum to afford compounds **14–16.**

*2-((2,8-Dihydroxy-4-acetyl-[1,2,4]triazolo[1,5-a]quinolin-9-yl)-methylamino)-3-(4-hydroxyphenyl)propanoic acid (**14**).* Yield: 65%; m.p. 86–90 °C. ^{1}H-NMR (300 MHz, DMSO): δ 2.18 (3H, s, CH_3), 4.10 (2H, d, CH_2–), 4.28 (2H, s, CH_2–), 5.60 (1H, t, *CH*–), 6.70–6.95 (3H, m, Ar-H), 7.30–7.50 (4H, m, Ar-H), 9.30 (1H, br s, *NH*–), 11.25 (1H, br s, C_6H_4–*OH*), 11.46 (1H, br s, CO*OH*), 11.55 (1H, br s, OH-triazole), 11.65 (1H, br s, Ar-OH); IR (KBr) ν =

3420–3450 (4 OH), 3390 (NH), 1710 (CO carboxylic acid), 1640, 1645 (C=N) cm^{-1}; MS: m/z = 236 [M^+, 36%]; anal. calcd for $C_{22}H_{20}N_4O_6$: C, 60.55; H, 4.58; N, 12.84; found: C, 60.50; H, 4.52; N, 12.76.

*2((2,8-Dihydroxy-4-acetyl-[1,2,4]triazolo[1,5-a]quinolin-9-yl)-methylamino)-3-phenylpropanoic acid (**15**).* Yield: 67%; m.p. 160–162 °C. ^{1}H-NMR (300 MHz, DMSO): δ 2.18 (3H, s, CH_3), 4.35 (2H, d, CH_2–), 4.48 (2H, s, CH_2–), 5.58 (1H, t, *CH*–), 6.75–6.97 (3H, m, Ar-H), 7.40–7.65 (5H, m, Ar-H), 9.45 (1H, br s, *NH*–), 10.99 (1H, br s, CO*OH*), 11.30 (1H, br s, OH-triazole), 11.60 (1H, s, Ar-*OH*); IR (KBr) ν = 3395 (NH), 3410, 3430, 3450 (3 OH), 1715 (CO carboxylic acid), 1630, 1640 (2C=N) cm^{-1}; anal. calcd for $C_{22}H_{20}N_4O_5$: C, 62.85; H, 4.76; N, 13.33; found: C, 62.78; H, 4.71; N, 13.36.

*2-((2,8-Dihydroxy-4-acetyl-[1,2,4]triazolo[1,5-a] quinolin-9-yl)-methylamino)-3-(1H-imidazol-4-yl)propanoic acid (**16**).* Yield: 64%; m.p. 233–235 °C ^{1}H-NMR (300 MHz, DMSO): δ 2.35 (3H, s, CH_3), 4.20 (2H, d, CH_2–), 4.35 (2H, d, CH_2–), 4.55 (1H, m, *CH*–), 5.80 (1H, s, *CH*–NH-imidazole), 7.15–7.38 (3H, m, Ar-H), 8.78 (1H, s, CH=N-imidazole), 9.50 (1H, br s, *NH*–), 10.90 (1H, br s, CO*OH*), 11.30 (1H, br s, OH-triazole), 11.50 (1H, br s, Ar-OH), 11.80 (1H, br s, *NH*-imidazole); IR (KBr) ν = 3415, 3430, 3445, (3 OH), 3385, 3395 (2 NH), 1718 (CO carboxylic acid), 1645, 1640, 1630 (3C=N), 1250 (C=C) cm^{-1}; anal. calcd for $C_{19}H_{18}N_6O_5$: C, 55.60; H, 4.39; N, 20.48; found: C, 55.66; H, 4.32; N, 20.41.

Synthesis of 2-(2,8-dihydroxy-4-acetyl-[1,2,4]triazolo[1,5-*a*]-quinolin-9-yl)methylamino-9-(1*H*-benzimidazole-carboxylic acid) derivatives (17–19)

General procedure. A mixture of compounds **14**, **15** or **16** (0.01 mol) and 1,2-diamino-4-benzoic acid (0.01 mol) in dry pyridine (10 ml) was stirred at room temperature for about 30 minutes. The solution was then heated under reflux for 5–6 h at 80 °C. The completion of the reaction was confirmed by TLC (eluent: petroleum ether (60–80)–ethyl acetate: 2 : 1). The reaction mixture was then cooled to room temperature and poured into crushed ice, neutralized by hydrochloric acid (10%). The product was collected by filtration, dried and recrystallized from ethanol to give the desired products **17–19**.

*2-((2,8-Dihydroxy-4-acetyl-[1,2,4]triazolo[1,5-a]quinolin-9-yl)-methylamino)-3-(1H-4-methyl-benzo-4-yl)-9-(1H-benzoimidazole-carboxylic acid) (**17**).* Yield: 68%; m.p. 138–141 °C. ^{1}H-NMR (300 MHz, DMSO): δ 2.25 (3H, s, CH_3), 4.20 (2H, d, CH_2–), 4.45 (2H, s, CH_2–), 5.58 (1H, t, *CH*–), 6.79–6.95 (3H, m, Ar-H), 7.10–7.30 (3H, m, Ar-H), 7.36–7.55 (4H, m, Ar-H), 9.38 (1H, br s, *NH*–), 9.80 (1H, br s, NH-benzimidazole), 10.90 (1H, br s, Ar–*OH*), 11.10 (1H, br s, CO*OH*), 11.30 (1H, br s, OH-triazole), 11.70 (1H, s, Ar-*OH*); IR (KBr) ν = 3425, 3440, 3450, 3465 (4 OH), 3387, 3395 (2 NH), 1728 (CO carboxylic acid), 1650, 1645, 1640 (3C=N) cm^{-1}. MS: m/z = 550 [$M^+ - 2$]; anal. calcd for $C_{29}H_{24}N_6O_6$: C, 63.04; H, 4.34; N, 15.21; found: C, 63.09; H, 4.26; N, 15.25.

*2-((2,8-Dihydroxy-4-acetyl-[1,2,4]triazolo[1,5-a]quinolin-9-yl)-methylamino)-3-(1H-benzo-4-yl)-9-(1H-benzimidazole-carboxylic acid) (**18**).* Yield: 63%; m.p. 211–215 °C. ^{1}H-NMR (300 MHz, DMSO): δ 2.26 (3H, s, CH_3), 4.30 (2H, d, CH_2–), 4.48 (2H, s, CH_2–), 5.70 (1H, t, *CH*–), 6.65–6.90 (3H, m, Ar-H), 7.10–7.25 (3H, m, Ar-H), 7.35–7.60 (5H, m, Ar-H), 9.48 (1H, br s, *NH*–), 9.80 (1H, br s, NH-benzimidazole), 10.95 (1H, br s, CO*OH*), 11.45 (1H, br s, OH-triazole), 11.60 (1H, br s, Ar-OH); IR (KBr) ν = 3430, 3440, 3455 (3 OH), 3380, 3390 (2 NH), 1720 (CO carboxylic acid), 1649, 1645, 1640 (3C=N) cm^{-1}. MS: m/z = 534 [$M^+ - 2$, 21%]; anal. calcd for $C_{29}H_{24}N_6O_5$: C, 64.92; H, 4.47; N, 15.67; found: C, 64.85; H, 4.42; N, 15.61.

*2-((2,8-Dihydroxy-4-acetyl-[1,2,4]triazolo[1,5-a]quinolin-9-yl)-methylamino)-3-(1H-imidazol-4-yl)-9-(1H-benzoimidazole-carboxylic acid) (**19**).* Yield 63%; m.p. 173–175 °C. ^{1}H-NMR (300 MHz, DMSO): δ 2.19 (3H, s, CH_3), 4.10 (2H, d, CH_2–), 4.45 (2H, s, CH_2–), 5.83 (1H, t, *CH*–), 6.45 (1H, s, *CH*-imidazole), 6.85–7.20 (3H, m, Ar-H), 7.30–7.50 (3H, m, Ar-H), 8.80 (1H, s, CH=N-imidazole), 9.57 (1H, br s, *NH*–), 10.90 (1H, br s, CO*OH*), 11.40 (1H, br s, OH-triazole), 11.65 (1H, br s, NH-benzimidazole), 11.80 (1H, br s, Ar-OH), 11.90 (s, 1H, NH-imidazole); IR (KBr) ν = 3415, 3430, 3445, (3 OH), 3370, 3380, 3395 (3 NH), 1710 (CO carboxylic acid), 1635, 1640, 1644, 1648 (C=N), 1240 (C=C) cm^{-1}. MS: m/z = 524 [$M^+ - 2$]; anal. calcd for $C_{26}H_{22}N_8O_5$: C, 59.31; H, 4.18; N, 21.29; O, 15.21; found: C, 59.28; H, 4.15; N, 21.30; O, 15.22.

4. Anticancer activity

4.1 Cytotoxic effect on human cell lines HEPG2 and MCF7

The antitumor activities of the synthesized compounds were tested for their cytotoxic activity against two different cell lines: a liver carcinoma cell line (HEPG2) and a human breast carcinoma cell line (MCF-7). The antitumor activities were evaluated using doxorubicin as a reference cytotoxic drug for both cell lines. The most promising compounds that gave 60% cytotoxicity or more at a concentration of 100 mg ml^{-1} were subjected to comprehensive evaluation to calculate their IC_{50} values (Tables 1 and 2). Cell culture cytotoxicity assays were carried out as described previously.[35]

Studies concerning the cytotoxicity effect towards the HEPG2 cell line compared to the reference drug doxorubicin showed that compounds **16** and **19** exhibited moderate activity. Results for the cytotoxicity effect towards the human breast carcinoma cell line MCF-7 compared to the reference drug doxorubicin indicated that compound **4** was the most potent compound tested, while compounds **19** and **8** exhibited moderate activity, and compounds **17**, **12** and **7** exhibited a weak cytotoxic effect in both cell lines.

4.2 Biological screening: *in vitro* assay for anticancer activity

The synthesized compounds were supplied to the Bioassay-Cell Culture Laboratory, National Research Center, Cairo, Egypt, for *in vitro* primary antitumor screening on hepato-cellular carcinoma (HEPG2) and Caucasian breast adenocarcinoma (MCF7) (American Type Culture Collection). Cell viability was assessed by the mitochondrial-dependent reduction of yellow MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) to purple formazan.[36]

Procedure. All the following procedures were performed in a sterile area using a laminar flow cabinet bio-safety class II level (Baker, SG403INT, and Sanford, ME, USA). Cells were suspended in RPMI 1640 medium for HEPG2 and MCF7. The media are supplemented with 1% antibiotic–antimycotic

Table 1 *In vitro* cytotoxic effect of the newly synthesized compounds against hepatocellular carcinoma cell line (HEPG2) at 100 μg ml^{-1} and IC_{50} value of promising compounds

Compound	IC_{50} [a] (μg ml^{-1})	% cytotoxicity at 100 μg ml^{-1}
4	—	13.6% at 100 μg ml^{-1}
5	—	29.3% at 100 μg ml^{-1}
6	—	0% at 100 μg ml^{-1}
7	—	0% at 100 μg ml^{-1}
8	—	0% at 100 μg ml^{-1}
14	—	40.2% at 100 μg ml^{-1}
15	—	0% at 100 μg ml^{-1}
16	54.5	87.5% at 100 μg ml^{-1}
17	—	33.3% at 100 μg ml^{-1}
19	62.5	89.2% at 100 μg ml^{-1}
DMSO	—	1% at 100 μg ml^{-1}
Negative control	—	0%
Doxorubicin	21.6	

[a] IC_{50} is defined as the concentration which results in a 50% decrease in cell number as compared with that of the control structures in the absence of an inhibitor.

Table 2 *In vitro* cytotoxic effect of the newly synthesized compounds against breast adenocarcinoma cell line (MCF-7) at 100 μg ml^{-1} and IC_{50} value of promising compounds

Compound	IC_{50}[a] (μg ml^{-1})	% cytotoxicity at 100 μg ml^{-1}
4	41.4	93.9% at 100 μg ml^{-1}
5	—	20.7% at 100 μg ml^{-1}
6	—	14.1% at 100 μg ml^{-1}
7	80.9	64.3% at 100 μg ml^{-1}
8	62.0	85.3% at 100 μg ml^{-1}
9	—	30.9% at 100 μg ml^{-1}
10	—	16.1% at 100 μg ml^{-1}
11	—	5.7% at 100 μg ml^{-1}
12	76.4	68.3% at 100 μg ml^{-1}
13	—	11.2% at 100 μg ml^{-1}
17	74.1	67.1% at 100 μg ml^{-1}
18	—	50.9% at 100 μg ml^{-1}
19	52.1	83.2% at 100 μg ml^{-1}
DMSO	—	3% at 100 μg ml^{-1}
Negative control	—	0%
Doxorubicin	26.1	

[a] IC_{50} is defined as the concentration which results in a 50% decrease in cell number as compared with that of the control structures in the absence of an inhibitor.

mixture (10 000 U ml^{-1} potassium penicillin, 10 000 μg ml^{-1} streptomycin sulfate and 25 μg ml^{-1} amphotericin B), 1% L-glutamine and 10% fetal bovine serum and maintained at 37 °C under 5% CO_2.

Cells were batch cultured for 10 days, then seeded at a concentration of 10×10^3 cells per well in fresh complete growth medium in 96-well microtiter plastic plates at 37 °C for 24 h under 5% CO_2 using a water jacketed carbon dioxide incubator (Sheldon, TC2323, Cornelius, OR, USA). Media was aspirated, fresh medium (without serum) was added and cells were incubated either alone (negative control) or with different concentrations of sample to give final concentrations of 100, 50, 25, 12.5, 6.25, 3.125, 0.78 and 1.56 μg ml^{-1}. After 48 h of incubation, the medium was aspirated, 40 μl of MTT salt (2.5 μg ml^{-1}) were added to each well and incubated for a further 4 h at 37 °C under 5% CO_2. To stop the reaction and dissolve the formed crystals, 200 μl of 10% sodium dodecyl sulphate (SDS) in deionized water was added to each well and incubated overnight at 37 °C. A positive control that is composed of 100 μg ml^{-1} was used as a known cytotoxic natural agent that gives 100% lethality under the same conditions.[37]

The absorbance was then measured using a microplate multi-well reader (Bio-Rad Laboratories Inc., Model 3350, Hercules, CA, USA) at 595 nm and a reference wavelength of 620 nm. Statistical significance was tested between samples and a negative control (cells with vehicle) using an independent *t*-test by the SPSS 11 program. DMSO is the vehicle used for dissolution of plant extracts, and its final concentration on the cells was less than 0.2%. The percentage of change in viability was calculated according to the formula:

$$((\text{Reading of extract/reading of negative control}) - 1) \times 100.$$

A probate analysis was carried out for IC_{50} determination using the SPSS 11 program.

The percent inhibition of cell viability was determined with reference to the control values. The data were subjected to linear regression analysis and the regression lines were plotted for the best fit. The IC_{50} (inhibition of cell viability) concentrations were calculated using the respective regression equation.

From the results obtained in Tables 1 and 2, it was observed that some of the tested compounds showed significant activity against HEPG2 and MCF-7 cancer cell lines, where some compounds showed moderate to low activity.

The results of the antitumor screening allow for the following assumptions about the structure–activity relationship (SAR) of the synthesized compounds.

For the cytotoxic activity against HEPG2 (liver carcinoma cell line): the results show that compounds **19** and **16** were almost comparable with doxorubicin (Table 1), which may be correlated with the presence of the imidazolyl moiety in both compounds. For the cytotoxic activity against MCF-7 (cells from breast cancer):

The cytotoxic activity of the synthesized tested compounds **4**, **19**, **8**, **17** and **12** against MCF-7 ranged from potent to moderate activity in a descending order, respectively. Compounds **4** and **8** are open chain substituted triazolo methylamino carboxylic acids, whereas compound **19** comprises an imidazole as well as a benzimidazole moiety.

Compounds **12** and **17** are both related to compound **19** as they contain the benzoimidazole carboxylic acid, which might be attributed to the cytotoxic activity.

Compound **7** is also related to compounds **4** and **8** being a triazolo methylamino carboxylic acid, but the activity might be less due to the absence of the open chain noticed in compounds **4** and **8**.

Therefore, the appreciable cytotoxicity of the triazolo quinolines, especially compounds **19** and **16**, against the HEPG2 cell

line may be attributed to the imidazole moieties as well as the benzimidazole moiety.

We can also relate the cytotoxicity of compounds **4** and **8** against breast cancer cell line MCF-7 to the aliphatic moiety of the amino carboxylic acid; the steric hindrance of the imidazole moiety in compound **7** may contribute to its weaker activity.

5. Antimicrobial activity

The antibacterial and antifungal activities of the synthesized compounds were tested *in vitro* in comparison with ampicillin as a reference drug using the standard agar disc diffusion method against five bacterial species and four fungal species. The antibacterial and antifungal activities were carried out in the Microbial Chemistry Department, National Research Center, using the diffusion plate method. A filter paper sterilized disc saturated with a measured quantity (25 μl) of each sample (1 mg ml^{-1} final concentration) was placed on a plate (9 cm diameter) containing a solid bacterial medium (nutrient agar) or a fungal medium (potato dextrose agar) that had been seeded with the spore suspension of the tested organism. After incubation at 37 °C for 24 h for bacteria (in case of fungi, at 25 °C for 72 h), the diameter of the clear zone of inhibition surrounding the sample was taken as a measure of the inhibitory power of the sample against the particular tested organism (% inhibition = sample inhibition zone (mm)/plate diameter × 100).

All the tested compounds were solubilized in DMSO as a solvent that has zero inhibition activity.[38–41]

The antibacterial activities of the tested compounds were examined with Gram positive bacteria, *Bacillus cereus* and *Staphylococcus aureus*, and Gram negative bacteria *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella typhimurium*. Antifungal activity was examined against *Candida albicans*, *Fusarium* sp., *Rhizoctonia* sp., and *Mucor* sp.

The obtained results were compared with the reference antibiotic ampicillin that was purchased from the Egyptian market (Pfizer Company, Cairo).

The results revealed that the compounds showed varying degrees of inhibition against the tested microorganisms.

As shown in Tables 3 and 4, the synthesized compounds showed different antimicrobial and antifungal activities.

Table 3, representing results obtained for compounds **4–13**, shows that compounds **9** and **11** exhibited strong antibacterial activities against Gram negative bacterium *Escherichia coli* reaching a 20 mm and a 25 mm clear zone, respectively, exceeding the activity of the reference antibiotic ampicillin (15 mm), which is a good result of medical value.

Table 4 shows the results obtained for compounds **14–19**. Compound **15** showed strong antibacterial activity against *Escherichia coli* (Gram negative stain bacterium) with double the value of the reference antibiotic used reaching a 30 mm clear zone. Thus, we can report this novel compound as an excellent formula against Gram negative highly pathogenic *Escherichia coli* and stronger than compounds **9** and **11**.

Compounds **18** and **19** exhibited good inhibitory activity against Gram negative bacterium *Escherichia coli*. These results are of great value for pharmaceutical and biological control applications.

The outer membrane of Gram negative bacteria comprises a complex liposaccharide (LPS) that acts as an endotoxin, protects the bacteria from several antibiotics, dyes, and detergents and provides these bacteria with resistance to lysozyme and penicillin. However, alternative medicinal treatments such as lysozyme with EDTA and the antibiotic ampicillin have been developed to combat the protective outer membrane of some pathogenic Gram-negative organisms. Other drugs can also be used, the significant ones being chloramphenicol, streptomycin, and nalidixic acid (Fig. 1).

Based on our results in this research paper we introduce compounds **15**, **11** and **9** as effective agents against the Gram negative pathogen *Escherichia coli* and suggest applying pharmacological drug tests on them.

Compound **13**, on the other hand, exhibited high antibacterial activity against Gram positive bacterium *Staphylococcus aureus*, which was almost equal to the activity of the reference antibiotic ampicillin.

Compound **17** was the most active against Gram positive bacterium *Bacillus cereus*; moreover, it exhibited a good inhibitory effect against Gram negative bacterium *Escherichia coli*.

Table 3 The antibacterial and antifungal activities of the tested compounds **4**, **5**, **6**, **7**, **8**, **9**, **10**, **11**, **12** and **13**

Inhibition zone diameter (mm per mg sample)												
Compound number												
Microorganism	Gram stain reaction	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	Reference antibiotic ampicillin
Bacillus cereus	Positive	5	0	5	3	5	0	0	0	10	0	25
Escherichia coli	Negative	3	0	10	0	0	20	0	25	0	12	15
Pseudomonas aeruginosa	Negative	0	0	12	11	10	0	9	0	15	0	45
Staphylococcus aureus	Positive	10	0	8	0	9	0	0	0	10	28	30
Salmonella typhimurium	Negative	12	10	9	8	0	0	0	0	0	0	40
Fusarium sp.	Fungus	35	0	15	0	16	0	0	0	0	0	25
Rhizoctonia sp.	Fungus	0	0	0	0	0	0	22	0	0	22	28
Mucor sp.	Fungus	0	0	0	0	0	0	0	0	0	0	25
Candida albicans	Fungus	25	12	12	10	10	0	10	0	12	10	35

Table 4 The antibacterial and antifungal activities of the tested compounds **14**, **15**, **16**, **17**, **18** and **19**

Inhibition zone diameter (mm per mg sample)								
Compound number								
Microorganism	Gram stain reaction	**14**	**15**	**16**	**17**	**18**	**19**	Reference antibiotic ampicillin
Bacillus cereus	Positive	8	0	10	35	0	0	25
Escherichia coli	Negative	10	30	11	15	15	15	15
Pseudomonas aeruginosa	Negative	0	0	0	0	0	0	45
Staphylococcus aureus	Positive	0	0	0	25	0	0	30
Salmonella typhimurium	Negative	0	0	0	0	0	18	40
Fusarium sp.	Fungus	0	0	0	0	0	0	25
Rhizoctonia sp.	Fungus	0	15	0	32	0	10	28
Mucor sp.	Fungus	0	0	10	0	0	0	25
Candida albicans	Fungus	0	0	10	0	0	0	35

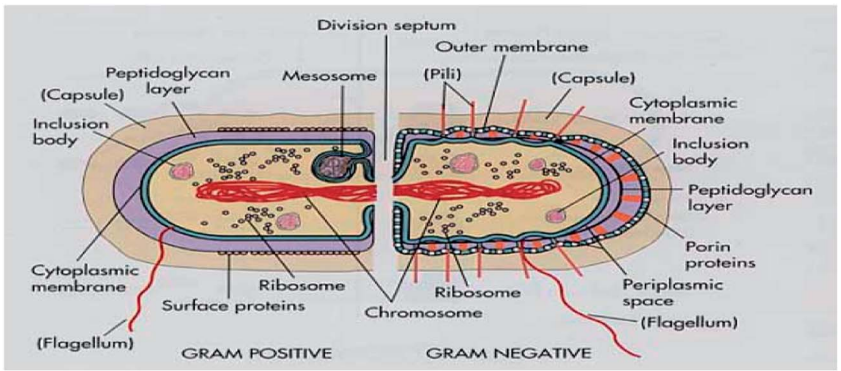


Fig. 1 Gram positive and Gram negative bacteria: a Gram positive bacterium has a thick layer of peptidoglycan (left). A Gram negative bacterium has a thin peptidoglycan layer and an outer membrane (right) (http://www.digitalproteus.com).

Compound **17** also exhibited an antifungal activity against *Rhizoctonia* sp. This compound has the advantage of distinct effects on both tested bacterial and fungal pathogens.

Gram-positive bacteria lack an outer membrane but are surrounded by layers of peptidoglycan many times thicker than those found in Gram-negative bacteria (Fig. 1).

Special importance must be given to novel agents having the ability to inhibit the growth of such pathogens to overcome the pathogens' resistance to normally used antibiotics.

Compound **4** showed superior antifungal activity (against *Fusarium* sp.) that was achieved by a clear zone reaching 35 mm. The same compound **4** displayed good activity against the *Candida albicans* pathogen. This compound may affect an essential component of the fungal cell membrane and thus inhibit its growth. The three major groups of antifungal agents in clinical use, *i.e.*, azoles, polyenes, and allylamine/thiocarbamates, all owe their antifungal activities to inhibition of the synthesis of or direct interaction with ergosterol, which is the predominant component of the fungal cell membrane (Parks and Casey, 1996).[42]

6. Conclusion

Quinoline and its derivatives have always attracted both synthetic and biological chemists because of their diverse chemical and pharmacological properties. The present study reports an efficient and convenient synthesis of a new series of selenadiazoles and triazolo[1,5-*a*]quinoline derivatives. The synthesized compounds were screened in order to evidence anticancer and antimicrobial activities. Some of them such as compound **4** exhibited strong inhibitory effects against breast cancer (MCF-7); compounds **8** and **19** showed moderate significant cytotoxic activities towards the tested cell lines, in particular with the liver carcinoma cell line HEPG2; and compounds **16** and **19** gave moderate activity. Compounds **4–19** were bio-assayed *in vitro* against four kinds of phytopathogenic fungi (*Fusarium* sp., *Rhizoctonia* sp., *Mucor* sp. and *Candida albicans*) and five Gram positive and Gram negative microorganisms (*Bacillus cereus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhimurium* and *Staphylococcus aureus*). The results showed that some of the synthesized compounds exhibited strong antifungal activities, among which compounds **4** and **17** displayed more potent activities against *Fusarium* sp. and *Rhizoctonia* sp. Compounds **9** and **11** exhibited high activity against *Escherichia coli*, whereas compound **13** exhibited high activity against *Staphylococcus aureus*. Compound **17** is the most active compound against *Bacillus cereus*, *Escherichia coli* and *Rhizoctonia* sp., and compound **15** gave the highest activity against *Escherichia coli*, but compounds **18** and **19** also displayed high activity against *Escherichia coli*.

Conflict of interest

The author(s) confirms that this article content has no conflicts of interest.

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