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Synthesis and Evaluation of 1,2,4-Triazole Derivatives for Antioxidant, Anti-inflammatory, Cytotoxicity and QSAR Analysis

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A series of novel 4-amino-5-substituted-1,2,4-triazole-3-thiol derivatives (**B_1-B_{18}**) were synthesized and characterized by spectral analysis. Equimolar portions of thiocarbohydrazide and different acids (substituted aryl/heteroaryl/aliphatic) were fused to synthesize the title compounds. The compounds were evaluated for cytotoxicity, *in vitro* anti-inflammatory activity and antioxidant activities. Cytotoxicity studies highlighted **B_4** (2,4-dichloro analog) as the potent cytotoxic molecule with IC_{50} value of 20.35 µM against MCF-7 cell line compared to cisplatin (IC_{50} = 12.06 µM). **B_4** (2,4-dichloro), **B_{18}** (oleayl) and **B_{14}** (2-hydroxy) showed significant membrane-stabilizing activity with IC_{50} values < 35 µM, whereas **B_{11}** (3,4-dimethoxy), **B_4** (2,4-dichloro) and **B_{14}** (2-hydroxy) displayed moderate proteinase inhibitory activity with IC_{50} values < 72 µM. Compounds possessing phenolic hydroxyl group (**B_{12}–B_{14}**) demonstrated an appreciable antioxidant activity in the studied antioxidant models. QSAR analysis revealed the important contribution of molecular connectivity, ionization potential and mass of the compounds for optimum cytotoxicity. Present results suggested compound **B_4** as a potential lead molecule to design novel and potent cytotoxic and anti-inflammatory agents.

Keywords: 1,2,4-Triazoles, Antioxidant activity, Anti-inflammatory activity, Cytotoxicity activity, QSAR analysis.

INTRODUCTION

Breast cancer is one of the most lethal cancers affecting women of age 30-70 worldwide. Majority of the contributing element to breast cancer-related death is still metastasis [1,2]. Doxorubicin, cyclophosphamide and taxanes are drugs primarily used to treat high-risk breast cancer patients [3]. 1,2,4-Triazole ring is considered as a privileged scaffold to develop safer anti-cancer agents. Triazoles are associated with varied biological activities including cytotoxicity. 1,2,4-Triazoles inhibit various potential cancer-related target enzymes and proteins such as thymidine phosphorylase, kinases, topoisomerase, tankyrase, tubulin and methionine aminopeptidase leading to cytotoxicity [4,5].

4/5-Substituted-1,2,4-triazole-3-thiol derivatives were synthesized by several researchers for antifungal and antimicrobial activities [6-8]. In search of selective antibreast cancer agents, Boraei *et al*. [9] designed novel 1,2,4-triazole-thiones and alkylsulfanyl substituted triazoles as inhibitors of nuclear protein poly(ADP-ribose) polymerase-1 (PAPI-1). 1,2,4-Triazole ring was tethered with a 1,2,3-triazole scaffold to inhibit, cyclin-dependent kinase 2 (CDK-2), validated target to develop anticancer agents [10]. 1,2,4-Triazole remain as privileged scaffold to design anticancer agents specific to particular cell lines.

Quantitative structure-activity relationships (QSAR) studies identify potential moieties and structural characteristics that favour biological activity or toxicity in a group of structurally related compounds. Statistically significant QSAR models suggest suitable structural modifications and assist in the lead optimization step of the drug discovery process [11,12]. Molecular descriptors depict important structural, physico-chemical

characteristics of a chemical compound. Literature survey show that QSAR models were constructed to correlate the molecular descriptors of chemical structures and their cytotoxicity towards different cell lines [13-15]. Molecular docking predicts target-ligand binding affinity based on theoretical calculations. In earlier attempts, a series of 4-amino-5-substituted-1,2,4-triazole-3-thiol derivatives were designed showed promising binding affinity towards the targets related to breast cancer and inflammation [16]. Given the promising results, the designed 4-amino-5-substituted-1,2,4-triazole-3-thiol derivatives were synthesized and studied for their biological effects.

EXPERIMENTAL

The physico-chemical properties such as melting points were checked using Sigma melting point apparatus and are uncorrected. The FTIR were recorded using Bruker FT-IR spectrophotometer, whereas ^{1}H NMR spectra were recorded on Bruker Avance, 400 MHz, while mass spectra were obtained VG Autospec MS using ESI mode positive ion trap detector.

Synthesis of thiocarbohydrazide: For synthesizing thiocarbohydrazide, firstly, hydrazine hydrate (0.5 mmol) was cooled in ice with stirring. Then carbon disulfide (0.1 mmol) was added dropwise to the cold hydrazine hydrate solution. Around 70-80 mL of water was added and the reaction mixture was refluxed for 2 h at ~95 ºC. On cooling, thiocarbohydrazide was precipitated from the solution, which was filtered [17].

Synthesis of 4-amino-5-(substituted phenyl)-4*H*-1,2,4-triazole-3-thiols

General procedure for the synthesis of B_1: Equimolar quantities (0.01 mol) of thiocarbohydrazide and benzoic acid were taken in a China dish and triturated well. The reaction mixture was fused in a round bottom flask in an oil bath till the mixture starts melting and the same reaction temperature (~95 ºC) was maintained for 1-2 h with occasional stirring. After cooling, mixture was washed with water and then with 5% w/v solution of sodium bicarbonate, filtered and dried. The compound was recrystallized from ethanol [18]. A similar procedure was adopted for synthesizing other derivatives (**B_2–B_{18}**). Substituted benzoic acids, heteroaryl and aliphatic carboxylic acids were used in place of benzoic acid (**Scheme-I**).

Spectral data of title compounds

4-Amino-5-phenyl-4*H*-1,2,4-triazole-3-thiol (B_1): m.f.: $C_8H_8N_4S$, m.p.: 126-128 ºC, yield: 52%; R_f: 0.80 (T:E:M 2:2:1); FT-IR (KBr, ν_{max}, cm^{-1}): 3346.66 (N-H *str.*), 2835.84 (S-H *str.*), 1656.32 (C=N *str.* of 1,2,4-triazole), 1462.60 (C-N *str.*), 1274.25 (N-N-C *str.*); ^{1}H NMR ($CDCl_3$-d_6, 400 MHz) δ (ppm): 5.68 (s, 2H, NH_2), 7.52-7.82 (m, 5H, Ar-H), 13.60 (s, 1H, SH); MS (EI) *m/z*: 194 $[M+2H]^+$.

4-Amino-5-(4-chlorophenyl)-4*H*-1,2,4-triazole-3-thiol (B_2): m.f.: $C_8H_7N_4SCl$, m.p.: 198-200 ºC, yield: 53%; R_f: 0.75 (E:H:M 3:1:2); FT-IR (KBr, ν_{max}, cm^{-1}): 3349.13 (N-H *str.*), 2849.93 (S-H *str.*), 1661.38 (C=N *str.* of 1,2,4-triazole), 1460.68 (C-N *str.*), 1279.58 (N-N-C *str.*); ^{1}H NMR ($CDCl_3$-d_6, 400 MHz) δ (ppm): 5.64 (s, 2H, NH_2), 7.49-7.89 (m, 4H, Ar-H), 13.52 (s, 1H, SH)MS (EI) *m/z*: 227 $[M+H]^+$.

4-Amino-5-(2-chlorophenyl)-4*H*-1,2,4-triazole-3-thiol (B_3): m.f.: $C_8H_7N_4SCl$, m.p.: 142-144 ºC, yield: 41%; R_f: 0.76 (T:E:M 2:2:1); FT-IR (KBr, ν_{max}, cm^{-1}): 3326.37 (N-H *str.*), 2613.16 (S-H *str.*), 1613.16 (C=N *str.* of 1,2,4-triazole), 1553.70 (C-N *str.*), 1353.32 (N-N-C *str.*); ^{1}H NMR ($CDCl_3$-d_6, 400 MHz) δ (ppm): 5.59 (s, 2H, NH_2), 7.25-7.79 (m, 4H, Ar-H), 13.66 (s, 1H, SH); MS (EI) *m/z*: 227 $[M+H]^+$.

4-Amino-5-(2,4-dichlorophenyl)-4*H*-1,2,4-triazole-3-thiol (B_4): m.f.: $C_8H_6N_4SCl_2$, m.p.: 150-152 ºC, yield: 68%; R_f: 0.70 (E:H:M 2:1:3); FT-IR (KBr, ν_{max}, cm^{-1}): 3324.37 (N-H *str.*), 2960.14 (S-H *str.*), 1615.05 (N-H bend, NH_2); 1583.51 (C=N *str.* of 1,2,4-triazole), 1461.05 (C-N *str.*), 1279.55 (N-N-C *str.*); ^{1}H NMR ($CDCl_3$-d_6, 400 MHz) δ (ppm): 5.50 (s, 2H, NH_2), 7.56-7.74 (m, 4H, Ar-H), 13.62 (s, 1H, SH); MS (EI) *m/z*: 261 $[M]^+$.

4-Amino-5-(4-chloro-3-nitrophenyl)-4*H*-1,2,4-triazole-3-thiol (B_5): m.f.: $C_8H_6N_5O_2SCl$, m.p.: 128-130 ºC, yield: 28%; R_f: 0.80 (E:H:M 2:1:1); FT-IR (KBr, ν_{max}, cm^{-1}): 3348.81 (N-H *str.*), 2918.75 (S-H *str.*), 1574.52 (C=N *str.* of 1,2,4-triazole); 1460.22 (C-N *str.*), 1284.38 (N-N-C *str.*), ^{1}H NMR ($CDCl_3$-d_6, 400 MHz) δ (ppm): 5.54 (s, 2H, NH_2), 8.54-8.69 (m, 3H, Ar-H), 13.62 (s, 1H, SH)MS (EI) *m/z*: 227 $[M+H]^+$.

4-Amino-5-(4-nitrophenyl)-4*H*-1,2,4-triazole-3-thiol (B_6): m.f.: $C_8H_7N_5O_2S$, m.p.: 108-111 ºC; yield: 33%; R_f:0.78 (E:H:M 2:1:1); FT-IR (KBr, ν_{max}, cm^{-1}): 3358.34 (N-H *str.*), 2862.96 (S-H *str.*), 1668.28 (C=N *str.* of 1,2,4-triazole), 1466.52 (C-N *str.*), 1262.18 (N-N-C *str.*); ^{1}H NMR ($CDCl_3$-d_6, 400 MHz) δ (ppm): 5.56 (s, 2H, NH_2), 8.01-8.40 (m, 4H, Ar-H), 13.64 (s, 1H, SH)MS (EI) *m/z*: 227 $[M+H]^+$.

4-Amino-5-(3,5-dinitrophenyl)-4*H*-1,2,4-triazole-3-thiol (B_7): m.f.: $C_8H_6N_6O_4S$, m.p.: 116-118 ºC; yield: 41%; R_f: 0.81 (T:E:M 2:2:1); FT-IR (KBr, ν_{max}, cm^{-1}): 3342.75 (N-H *str.*), 2911.73 (S-H *str.*), 1578.56 (C=N *str.* of 1,2,4-triazole), 1452.28 (C-N *str.*); ^{1}H NMR ($CDCl_3$-d_6, 400 MHz) δ (ppm): 5.52 (s, 2H, NH_2), 7.54-7.74 (d, 3H, Ar-H), 13.62 (s, 1H, SH); MS (EI) *m/z*: 283 $[M+H]^+$.

4-Amino-5-(*p*-tolyl)-4*H*-1,2,4-triazole-3-thiol (B_8): m.f.: $C_9H_{10}N_4S$, m.p.: 122-124 ºC; yield: 38%; R_f: 0.73 (E:H:M 2:1:1); FT-IR (KBr, ν_{max}, cm^{-1}): 3346.25 (N-H *str.*), 2868.92 (S-H *str.*), 1660.21 (C=N *str.* of 1,2,4-triazole), 1462.14 (C-N

Scheme-I: Synthetic route of 4-amino-5-(substituted phenyl)-4*H*-1,2,4-triazole-3-thiols (B_1-B_{18}) (For R: please refer Table-1)

str.); ^{1}H NMR ($CDCl_3$-d_6, 400 MHz) δ (ppm): 2.35 (s, 3H, CH_3), 5.56 (s, 2H, NH_2), 7.44-8.34 (m, 4H, Ar-H), 13.66 (s, 1H, SH); ^{13}C NMR ($CDCl_3$-d_6, 300 MHz) δ (ppm): 127.6, 125.7, 129.5, 131.7 (aromatic ring), 153.4 (C_5 -triazole), 167.5 (C_3 -triazole), 21.3 (methyl carbon); MS (EI) *m/z*: 227 $[M+H]^+$.

4-Amino-5-(4-aminophenyl)-4*H*-1,2,4-triazole-3-thiol (B_9): m.f.: $C_8H_9N_5S$, m.p.: 124-125 ºC; yield: 56%; R_f:0.78 (T:E:M 2:2:1); FT-IR (KBr, ν_{max}, cm^{-1}): 3306.37 (N-H *str.*), 2673.16 (S-H *str.*), 1613.16 (C=N *str.* of 1,2,4-triazole), 1453.70 (C-N *str.*); ^{1}H NMR ($CDCl_3$-d_6, 400 MHz) δ (ppm): 5.48 (s, 2H, N-NH_2), 5.85 (s, 2H, Ar-NH_2), 7.45-7.86 (m, 4H, Ar-H), 13.40 (s, 1H, SH); MS (EI) *m/z*: 207 $[M]^+$.

4-Amino-5-(4-methoxyphenyl)-4*H*-1,2,4-triazole-3-thiol (B_{10}): m.f.: $C_9H_{10}N_4OS$, m.p.: 112-114 ºC; yield: 38%; R_f: 0.72 (T:E:M 3:1:2); FT-IR (KBr, ν_{max}, cm^{-1}): 3348.82 (N-H *str.*), 2919.42 (S-H *str.*), 1561.53 (C=N *str.* of 1,2,4-triazole), 1448.60 (C-N *str.*); ^{1}H NMR ($CDCl_3$-d_6, 400 MHz) δ (ppm): 3.72 (s, 3H, OCH_3); 5.58 (s, 2H, NH_2), 7.40-7.82 (m, 4H, Ar-H), 13.96 (s, 1H, SH); MS (EI) *m/z*: 223 $[M+H]^+$.

4-Amino-5-(3,4-dimethoxyphenyl)-4*H*-1,2,4-triazole-3-thiol (B_{11}): m.f.: $C_{10}H_{12}N_4O_2S$, m.p.: 156-158 ºC; yield: 37%; R_f: 0.74 (E:H:M 2:1:1); FT-IR (KBr, ν_{max}, cm^{-1}): 3342.87 (N-H *str.*), 2916.35 (S-H *str.*), 1568.57 (C=N *str.* of 1,2,4-triazole), 1470.17 (C-N *str.*); ^{1}H NMR ($CDCl_3$-d_6, 400 MHz) δ (ppm): 3.64 (s, 3H, OCH_3); 3.97 (s, 3H, OCH_3); 5.54 (s, 2H, NH_2), 7.26-7.61 (m, 3H, Ar-H), 13.91 (s, 1H, SH); MS (EI) *m/z*: 252 $[M]^+$.

2-(4-Amino-5-mercapto-4*H*-1,2,4-triazol-3-yl)-phenol (B_{12}): m.f.: $C_8H_8N_4OS$, m.p.: 118-120 ºC; yield: 38%; R_f: 0.68 (E:H:M 2:1:2); FT-IR (KBr, ν_{max}, cm^{-1}): 3358.34 (N-H *str.*), 2862.72 (S-H *str.*), 1668.36 (C=N *str.* of 1,2,4-triazole), 1468.66 (C-N *str.*); ^{1}H NMR ($CDCl_3$-d_6, 400 MHz) δ (ppm): 5.72 (s, 2H, NH_2), 7.40-7.86 (m, 4H, Ar-H), 9.68 (s, 1H, OH); 13.55 (s, 1H, SH); MS (EI) *m/z*: 208 $[M]^+$.

5-Amino-2-(4-amino-5-mercapto-4*H*-1,2,4-triazol-3-yl)phenol (B_{13}): m.f.: $C_8H_9N_5S$, m.p.: 130-131 ºC; yield: 47%; R_f:0.82 (E:H:M 3:1:1); FT-IR (KBr, ν_{max}, cm^{-1}): 3350.90 (O-H *str.*), 3198.49 (N-H *str.*), 2817.84 (S-H *str.*), 1618.08 (C=N *str.* of 1,2,4-triazole), 1389.06 (C-N *str.*); ^{1}H NMR ($CDCl_3$-d_6, 400 MHz) δ (ppm): 5.24-5.67 (s, 4H, NH_2), 7.26-7.61 (m, 3H, Ar-H), 13.32 (s, 1H, SH); MS (EI) *m/z*: 223 $[M]^+$.

2-(4-Amino-5-mercapto-4*H*-1,2,4-triazol-3-yl)-4,6-dinitrophenol (B_{14}): m.f.: $C_8H_6N_6O_5S$, m.p.: 180-182 ºC; yield: 44%; R_f:0.73 (E:H:M 3:1:1.5); FT-IR (KBr, ν_{max}, cm^{-1}): 3352.26 (N-H *str.*), 2854.84 (S-H *str.*), 1665.25 (C=N *str.* of 1,2,4-triazole), 1460.22 (C-N *str.*); ^{1}H NMR ($CDCl_3$-d_6, 400 MHz) δ (ppm): 5.68 (s, 2H, NH_2), 7.42-7.84 (m, 2H, Ar-H), 9.62 (s, 1H, OH); 13.64 (s, 1H, SH); MS (EI) *m/z*: 298 $[M]^+$.

4-Amino-5-styryl-4*H*-1,2,4-triazole-3-thiol (B_{15}): m.f.: $C_{10}H_{10}N_4S$, m.p.: 102-105 ºC; yield: 33%; R_f: 0.78 (E:H:M 3:1:1); FT-IR (KBr, ν_{max}, cm^{-1}): 3381.83 (N-H *str.*), 2822.40 (S-H *str.*), 1619.68 (C=N *str.* of 1,2,4-triazole), 1580.65 (C=C *str.*), 1451.73 (C-N *str.*); ^{1}H NMR ($CDCl_3$-d_6, 400 MHz) δ (ppm): 5.56 (s, 2H, NH_2), 6.34 (d, 2H, J= 12 Hz, olefinic); 7.29-7.89 (m, 5H, Ar-H), 13.42 (s, 1H, SH); MS (EI) *m/z*: 218 $[M]^+$.

4-Amino-5-(pyridine-2-yl)-4*H*-1,2,4-triazole-3-thiol (B_{16}): m.f.: $C_7H_7N_5S$, m.p.: 140-141 ºC; yield: 36%; R_f: 0.72 (E: H:M 3:2:2); FT-IR (KBr, ν_{max}, cm^{-1}): 3385.72 (N-H *str.*), 2828.56 (S-H *str.*), 1621.76 (C=N *str.* of 1,2,4-triazole), 1455.40 (C-N *str.*); ^{1}H NMR ($CDCl_3$-d_6, 400 MHz) δ (ppm): 5.71 (s, 4H, NH_2), 7.35-8.61 (m, 4H, Ar-H), 13.62 (s, 1H, SH)MS (EI) *m/z*: 218 $[M]^+$.

4-Amino-5-heptadecyl-4*H*-1,2,4-triazole-3-thiol (B_{17}): (m.f.: $C_{20}H_{40}N_4S$, m.p.: 146-148 ºC; yield: 68%; R_f: 0.79 (E:H:M 3:1:2); FT-IR (KBr, ν_{max}, cm^{-1}): 3255.47 (N-H *str.*), 2640.25 (S-H *str.*), 1576.99 (C=N *str.* of 1,2,4-triazole), 1465.46 (C-N *str.*); ^{1}H NMR ($CDCl_3$-d_6, 400 MHz) δ (ppm): 1.00 (t, 3H, CH_3), 1.6-1.92 (m, 30H, $(CH_2)_{15}$), 2.90 (t, 2H, CH_2-C=N), 5.91 (s, 2H, NH_2), 13.43 (s, 1H, SH); MS (EI) *m/z*: 356 $[M+2H]^+$.

4-Amino-5-(heptadec-9-en-1-yl)-4*H*-1,2,4-triazole-3-thiol (B_{18}): m.f.: $C_{20}H_{38}N_4S$, m.p.: 186-187 ºC; yield: 35%; R_f: 0.81 (T:E:M 3:2:1); FT-IR (KBr, ν_{max}, cm^{-1}): 3264.56 (N-H *str.*); 2646.24 (S-H *str.*), 1582.86 (C=N *str.* of 1,2,4-triazole), 1460.68 (C-N *str.*); ^{1}H NMR ($CDCl_3$-d_6, 400 MHz) δ (ppm): 1.00-2.48 (m, 32H, $(CH_2)_{16}$); 2.96 (d, 3H, CH_3); 5.96 (s, 2H, NH_2), 13.48 (s, 1H, SH); MS (EI) *m/z*: 352 $[M]^+$.

***In vitro* cytotoxicity-MTT assay:** Tetrazolium salts undergo reductionand dehydrogenation to form formazan crystals. At first, 96-well plates were seeded with cell lines (density of 5000 cells/well) and filled with fresh growth media (100 µL) and incubated overnight at room temperature. Then the sample solutions with different concentrations (5, 10, 25, 50, 75 and 100 µM) were added to each well and again incubated for 48 h. After that, fresh media (100 µL) mixed with MTT (0.5 mg/mL) solution was added and subsequently incubated for 3 h. Once formazan crystals were observed (purple precipitate), immediately the cells were dissolved in DMSO and optical density was determined at 570 nm. The assay was performed using MCF-7, HeLa and A549 cell lines with three individual experiments [19]. The IC_{50} values were generated for each cell using origin software (www.originlab.com). The formula used for calculation:

$$\text{Inhibition (\%)} = \frac{\text{Control} - \text{Test}}{\text{Control}} \times 100$$

***In vitro* anti-inflammatory activity**

Proteinase inhibition: Proteinases play a key role in tissue inflammation. The test was performed as per the modified method [20]. Trypsin (0.06 mg), Tris-HCl buffer (pH 7.4, 1 mL of 20 mM) and 1 mL test sample of various concentrations (5, 10, 20, 40, 80 and 100 µM were mixed and incubated at 37 ºC for 5 min. Then 1 mL of 0.8% (w/v), casein was added and again incubated for 20 min. Then, before centrifugation, 2 mL of 70% perchloric acid was added and later the absorbance was measured at 210 nm for the supernatant. The percentage of inhibition was determined using the below given equation:

$$\text{Inhibition (\%)} = \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{test}}}{\text{Abs}_{\text{control}}} \times 100$$

Inhibition of albumin denaturation method: Albumin denaturation is associated with inflammation process and several diseases [21]. The method involves reaction between 2 mL of test solutions (10, 20, 40, 80 & 100 µM), 0.4 mL of 1% aqueous

solution of egg albumin and 2.8 mL of phosphate buffer saline. Once thoroughly mixed, the contents were incubated at 37 ºC for 20 min and then heated at 70 ºC for 5 min. Upon cooling, turbidity was developed in the sample solutions which were measured at 660 nm. Diclofenac was used as a reference drug. The experiments were performed in triplicate and the percentage inhibition was calculated by using the following formula:

$$\text{Inhibition of albumin denaturation } (\%) = \frac{A_{control} - A_{test}}{A_{control}} \times 100$$

***in vitro* Antioxidant activity**

DPPH stable free radical scavenging activity: The antioxidant activity was measured as reported method [22]. Corresponding test compound and DPPH were dissolved in ethanol each to a concentration of 100 µM. Test solution and DPPH solution (2 mL each) were mixed thoroughly and kept aside for a period for approximately 20 min. Solvent ethanol and DPPH solution (2 mL each) were mixed to obtain negative control absorbance. The instrument was set using ethanol solution as blank. After incubation period, the absorbance was measured at 517 nm at room temperature. Ascorbic acid was used as a positive control. Radical scavenging ability of the synthesized compounds was screened using a range of concentrations (5, 10, 20, 40 and 80 µM). The experiment was performed in triplicates. The formula used for calculation:

$$\text{Inhibition } (\%) = \frac{\text{Control} - \text{Test}}{\text{Control}} \times 100$$

Nitric oxide (NO) scavenging activity: The NO scavenging ability was investigated using the method as described by Sreejayan *et al.* [23]. A combination of solution of sodium nitroprusside (5 mM) and phosphate buffer pH 7.4 produces nitrite ions. In the presence of antioxidant agents, the nitrite ions get scavenged and few ions will be available to form coloured solution with Griess reagent. For production of nitrite ions, approximately 5 h are required. Hence first, test solutions were mixed with buffered saline incubated at 25 ºC for 5 h. Again 2 mL of this reaction mixture was diluted with 2 mL of Griess reagent and the absorbance was measured at 546 nm.

The formula used for calculation:

$$\text{Inhibition } (\%) = \frac{\text{Control} - \text{Test}}{\text{Control}} \times 100$$

2,2′-Azino-*bis*(3-ethyl benzothiazoline-6-sulfonic acid) diammonium salt (ABTS) reduction assay: A stock solution of ABTS (7 mM) was prepared using 2.45 mM potassium persulfate in distilled water. Around 16 h was required for the generation of ABTS free radical cation. Then the reaction mixture was diluted with ethanol to get an absorbance of 0.700 at 734 nm. The test compounds (1 mL, 100 µM) were mixed with 1 mL of ABTS solution and the absorbance was measured at 734 nm. The BHT was used as reference compound [24]:

$$\text{Reduction of ABTS } (\%) = \frac{Abs_{control} - A_{test}}{A_{control}} \times 100$$

QSAR studies: In this work, QSAR models were constructed for the synthesized compounds using Small Dataset Modeler to find a relationship between cytotoxicity and the molecular properties [25]. For that, the IC_{50} values obtained in the MTT assay were converted to PIC_{50} values. Four potent anticancer agents (afatinib, lapatinib, testosterone, norethisterone) and their IC_{50} values against breast cancer cell lines were included in the QSAR analysis.

As a starting step, the chemical structures (18 synthesized compounds & four anticancer agents) were drawn and optimized in the ChemDraw tool. For all of them, molecular descriptors (1444) were computed with PaDEL software for calculating 1D, 2D 3D descriptors and molecular fingerprints [26]. Once it is over, suitable descriptors were selected using the V-WSP algorithm to remove unsuitable and redundant descriptors [27]. At this point, structures were further curated (removing duplicate structures, structural and response outliers) with small dataset curator. To derive a PLS-based QSAR model from this small data set using double-cross validation (DCV), it was submitted to small dataset modeler.

In Small Dataset Modeler, the training set is devided into "n" calibration and validation sets for QSAR model generation *via* inner loop validation. It is possible to derive QSAR models based on partial least square analyses (PLS)/multiple linear regression (MLR) using Small dataset Modeler. The number of components for the PLS models can be user/tool-defined based on the Q^2 (LOO) values. In this work, PLS based models were generated and validated.

QSAR model validation: The measure of goodness-of-fit, model stability and productivity analysis assess quality of the QSAR models. Important internal validation parameters used in this study are R^2, Q^2, scaled average R^2_m-LOO (leave-one-out), scaled delta R^2_m-LOO, mean absolute error (MAE) and SD. The external validation parameters include Q^2_{F1}, Q^2_{F2}, R^2_m (test) metrics, MAE, concordance correlation coefficient (CCC) and SD [25].

Applicability domain (AD): Applicability domain (AD) can be defined as the chemical space boundaries where QSAR model has reliable performance. AD insures the reliability of newer/external predictions in a QSAR model. If the predicted compound is inside the AD, it refers that the predictions are reliable and in a safer zone of chemical space (predicted compounds are structurally similar to the training set). In present work, the standardization approach was used to determine AD [28].

RESULTS AND DISCUSSION

A series of 4-amino-5-(substituted)-1*H*-1,2,4-triazole-3-thiols (**B_1–B_{18}**) was synthesized by fusion of thiocarbohydrazide and various substituted aromatic/heteroaryl/aliphatic acids The IR spectra displayed bands (3385-3200 cm^{-1}) due to N-H *str.* and bands (3099.24-2832.62 cm^{-1}). The absorption bands confirmed the presence of thiol group (SH) in the region around 2828 cm^{-1} and C=N *str.* of 1,2,4-triazole was observed at 1670-1570 cm^{-1}. The ^1H NMR spectra of the compounds displayed singlets at around δ 5.24-5.96 ppm due to free amino group, multiplets at δ 7.22-7.89 ppm for aromatic hydrogens and singlets at δ 13.32-13.96 ppm due to thiol hydrogen. The compounds' mass spectra showed the $[M]^+$ and $[M+H]^+$ peaks

as the molecular ion peaks for most of the compounds at their characteristic *m/z* values.

Cytotoxicity: All the synthesized compounds were designed to involve various substitutions on phenyl ring at the 5th position of 1,2,4-triazole nucleus and its replacement with heteroaryl, styryl and some aliphatic moieties. The results showed that 2,4-dichloro phenyl ring strongly favoured cytotoxicity, while other alterations exhibited compounds with moderate to poor activity. Cytotoxicity of the synthesized 1,2,4-triazoles was evaluated by MTT assay using breast cancer cell lines (MCF-7), cervical cancer cell lines (HeLa) and lung cancer cell lines A549.

Among the eighteen tested compounds 2,4-dichloro analog (**B_4**) exhibited promising cytotoxicity against breast cancer cell line (MCF-7) with IC_{50} value of 20.35 μM, while cisplatin showed the highest activity with IC_{50} value of 12.06 μM. When the phenyl group was substituted with chlorine atoms at the second and fourth positions, cytotoxicity was very significant, whereas substitution with chlorine atoms at the third or fourth position cytotoxicity was reduced (**B_3**, IC_{50} = 531.72 μM; **B_2**, IC_{50} = 811.68 μM), indicating the importance of chlorine atom and its position on the phenyl ring. Among the compounds possessing halogens, 2,4-dichloro derivative (**B_4**) is the only compound that exhibited marked cytotoxicity. Similarly, with the nitro group's substitution at the third and fifth positions, moderate cytotoxicity was noticed, whereas the substitution at only the fourth position led to a less active compound (**B_6**, IC_{50} = 664.85 μM). The free amino group at the fourth position of phenyl ring decreased the compound's cytotoxicity, but with an amino group at the fifth position and the hydroxy group at the second position, cytotoxicity was moderate. These results indicated the variable effects of electron releasing groups on the cytotoxicity of title compounds.

On the other hand, the introduction of hydroxyl (**B_{12}**), methyl (**B_8**), or methoxy (**B_{10}**) groups furnished moderately active compounds. The results also indicated that when the phenyl ring is replaced with a heteroaryl ring or long aliphatic chain, it led to poor cytotoxicity. The low cytotoxic potential of oleayl (**B_{18}**, IC_{50} = 245.00 μM) and stearyl derivatives (**B_{17}**, IC_{50} = 246.33 μM) indicated that the presence of long-chain alkyl groups is not favourable for activity (Table-1).

Compounds **B_4** and **B_8**, which displayed good potency against the MCF-7 cell line, were tested for their efficacy against cervical cancer cell lines (HeLa) and lung cancer cell lines (A 549). These two compounds showed IC_{50} values of 430.27 and 119.74 μM (towards HeLa cell lines) and 104.73 and 295.53 (towards A 549 cell lines) respectively, whereas the standard drug cisplatin showed IC_{50} value of 5.59 μM (HeLa cell lines) and 4.96 μM (A 549 cell lines) indicating poor cytotoxicity towards the HeLa and A 549 cell lines.

TABLE-1
In vitro CYTOTOXICITY OF 4-AMINO-5-(SUBSTITUTED PHENYL)-4*H*-1,2,4-TRIAZOLE-3-THIOLS (**B_1-B_{18}**) ON DIFFERENT CELL LINES BY MTT ASSAY

Compd.	R	MCF-7 IC_{50} (μM)	HeLa* IC_{50} (μM)	A549* IC_{50} (μM)
B_1	C_6H_5	148.30	NT	NT
B_2	4-Cl·C_6H_4	811.68	NT	NT
B_3	2-Cl·C_6H_4	531.72	NT	NT
B_4	2,4-$(Cl)_2$·C_6H_3	20.35	430.27	104.73
B_5	4-Cl,3-NO_2·C_6H_3	764.57	NT	NT
B_6	4-NO_2·C_6H_4	664.85	NT	NT
B_7	3,5-$(NO_2)_2$·C_6H_3	174.21	NT	NT
B_8	4-CH_3·C_6H_4	147.86	119.74	295.53
B_9	4-NH_2·C_6H_4	739.79	NT	NT
B_{10}	4-OCH_3·C_6H_4	385.20	NT	NT
B_{11}	3,4-$(OCH_3)_2$·C_6H_3	560.86	NT	NT
B_{12}	2-OH·C_6H_3	185.91	NT	NT
B_{13}	2-OH,5-NH_2,C_6H_3	294.31	NT	NT
B_{14}	2-OH, 3,5-$(NO_2)_2$·C_6H_2	174.92	NT	NT
B_{15}	Styryl	217.15	NT	NT
B_{16}	2-Pyridyl	236.86	NT	NT
B_{17}	Stearyl	246.33	NT	NT
B_{18}	Oleayl	245.00	NT	NT
Standard	Cisplatin	12.06	5.59	4.96

*Selected compounds were tested against HeLa and A549 cell lines

TABLE-2
In vitro ANTI-INFLAMMATORY ACTIVITY OF 4-AMINO-5-(SUBSTITUTED PHENYL)-4*H*-1,2,4-TRIAZOLE-3-THIOLS (**B_1-B_{18}**)

Compound	Proteinase inhibition[a] IC_{50} (μM)	Albumin denaturation inhibiton[a] IC_{50} (μM)
B_1	159.90 ± 8.05	118.81± 5.64
B_2	117.03 ± 6.23	80.32 ± 2.06
B_3	–	–
B_4	67.73 ± 2.91	23.16 ± 1.66
B_5	110.97 ± 1.13	36.75 ± 1.68
B_6	–	96.13 ± 3.83
B_7	98.40 ± 1.14	–
B_8	100.92 ± 2.59	48.48 ± 2.01
B_9	–	–
B_{10}	99.04 ± 2.80	–
B_{11}	58.33 ± 1.97	–
B_{12}	–	116.60 ± 3.72
B_{13}	–	84.89 ± 10.20
B_{14}	71.34 ± 1.71	33.83 ± 1.99
B_{15}	107.35 ± 3.69	50.73 ± 1.86
B_{16}	132.19 ± 2.46	97.04 ± 2.97
B_{17}	83.62 ± 2.74	47.19 ± 1.88
B_{18}	94.01 ± 3.22	32.30 ± 0.85
Diclofenac	38.84 ± 1.3	42.72 ± 1.67

Note: All values are mean ± SD of three replicates.

Anti-inflammatory studies

Proteinase inhibition: Among the tested compounds, **B_{11}** (3,4-diOCH_3) exhibited promising inhibitory activity on proteinase (IC_{50}, 58.33 μM), followed by **B_{14}** (2-OH-3,5-diNO_2) with IC_{50} value of 71.34 μM. **B_{10}** (4-OCH_3) and **B_8** (4-CH_3) displayed IC_{50} values 99.04 and 100.92 μM, respectively. The standard drug diclofenac showed IC_{50}, 38.84 μM (Table-2).

The compounds substituted with electron-withdrawing groups such as **B_4** (2, 4-Cl_2) showed IC_{50} value of 67.73 μM. Similar potencies were observed with **B_7** (3,5-diNO_2) and **B_5** (4-Cl, 3-NO_2), which displayed 98.40 and 110.97 μM, respectively. Compound **B_2** (4-Cl) displayed moderate proteinase inhibitory activity with IC_{50} values of 117.03 μM. **B_{17}** (stearyl) and **B_{18}** (oleayl) possessed better IC_{50} values 83.62 and 94.01

μM, respectively than that of the unsubstituted derivative **B_1** (159.90 μM). The remaining compounds showed weak activity. From the results, it was observed that electron-releasing groups and phenolic substitution played a significant role in the ability for proteinase inhibition.

Inhibition of albumin denaturation: The majority of the compounds were active in this model and **B_4** (2,4-Cl_2) exhibited the highest activity with IC_{50}, 23.16 μM. Compounds with nitro functionality such as **B_{14}** (2-OH, 3,5-$(NO_2)_2$) and **E_5** (4-Cl, 3-NO_2) showed IC_{50} values of 33.83 and 36.75 μM, respectively. These compounds exhibited higher inhibitionthan standard Diclofenac (IC_{50}, 42.72 μM). Moderate activity was found with **B_8** (4-CH_3) and **B_{15}** (styryl) with IC_{50} values of 48.48 and 50.73 μM, respectively. When the aromatic ring is replaced with long-chain fatty acid residues, especially oleayl moiety (**B_{18}**), anti-inflammatory activity was significant (IC_{50}, 32.30 μM). The remaining derivatives showed weak activity with IC_{50} values ranging from 80.00-116.00 μM (Table-2). A perusal of the data obtained in proteinase inhibition and inhibition of albumin denaturation methods suggested that synthesized compounds have promising albumin denaturation inhibitory properties and weaker proteinase inhibitory activities. A comparison of results in both the models suggested a similar activity profile of **B_4** being the promising one followed by **B_{14}**.

In previous studies, 1,2,4-triazoles were examined for *in vivo* anti-inflammatory activity and the importance of hydroxy, methyl and methoxy groups has been explored. Present findings followed previous studies indicating the importance of similar functionalities [29,30].

Antioxidant studies

DPPH radical scavenging activity: Among all the synthesized compounds, **B_{12}** (2-OH) showed the highest antioxidant activity with IC_{50} value of 26.02 μM, followed by **B_{14}** (2-OH-3,5-diNO_2) with IC_{50} value of 41.09 μM. These compounds showed better radical scavenging activity than ascorbic acid (IC_{50} = 45.50 μM). Compounds **B_{13}** (2-OH-5-NH_2) and **B_{11}** ((3,4-diOCH_3) also showed appreciable antioxidant activity with IC_{50} values of 53.67 and 58.15 μM, respectively (Table-3). Moderate antioxidant activity was observed with compounds **B_4** (2,4-diCl), **B_6** (4-NO_2) and **B_5** (4-Cl-3-NO_2) with IC_{50} values 83.77, 84.53 and 88.34 μM, respectively. Effect of substituent groups on DPPH stable free radical scavenging is electron-releasing > electron-withdrawing > styryl > 2-pyridyl > aliphatic chains.

NO scavenging activity: In the NO scavenging assay, compound **B_{11}** (3,4-diOCH_3) exhibited the promising NO radical scavenging activity with IC_{50}, 13.95 μM, followed by **B_{10}** (4-OCH_3), **B_{12}** (2-OH), **B_8** (4-CH_3), **B_{14}** (2-OH-3,5-diNO_2) and **B_{13}** (2-OH-5-NH_2) that showed IC_{50} values, 22.83, 24.83, 32.78, 36.89 and 52.63 μM, respectively. All these derivatives displayed remarkable antioxidant activity when compared to the standard ascorbic acid, IC_{50} 58.71 μM. Moderate antioxidant activity was observed with **B_2** (4-Cl) and **B_5** (4-Cl-3-NO_2), with IC_{50} values of 70.80 and 78.13 μM, respectively. Replacement of phenyl ring with oleayl (IC_{50} = 62.67 μM) and stearyl (IC_{50} = 76.11 μM) moieties was found to be supportive of the nitric oxide trapping activity (Table-3). The results also suggest that the presence of electron-donating groups such as methoxy, hydroxyl and methyl on the phenyl ring at position 5 of 1,2,4-triazole nucleus furnished the effective antioxidant molecules. Effect of substituent groups on NO radical scavenging is electron releasing > styryl > electron withdrawing > aliphatic chains > 2-pyridyl.

TABLE-3
In vitro ANTIOXIDANT ACTIVITY OF 4-AMINO-5-(SUBSTITUTED PHENYL)-4*H*-1,2,4-TRIAZOLE-3-THIOLS (**B_1-B_{18}**)

Compound	DPPH reduction IC_{50} (μM)[d]	Nitric oxide scavenging IC_{50} (μM)[d]	ABTS reduction IC_{50} (μM)[d]
B_1	104.30 ± 4.5	81.21± 4.3	148.96 ± 6.9
B_2	46.88 ± 2.1	70.80 ± 4.8	74.54 ± 7.2
B_3	241.64 ± 3.0	–	–
B_4	83.77 ± 1.3	–	75.81 ± 6.2
B_5	88.34 ± 2.1	78.13 ± 0.8	–
B_6	84.53 ± 3.1	158.22 ± 8.5	96.26 ± 2.2
B_7	171.7 ± 3.1	–	–
B_8	99.46 ± 3.5	32.78 ± 1.9	58.69 ± 1.1
B_9	124.93 ± 3.7	–	64.22 ± 5.6
B_{10}	98.23 ± 8.2	22.83 ± 2.6	57.24 ± 2.5
B_{11}	58.15 ± 2.7	13.95 ± 1.8	47.51 ± 2.1
B_{12}	26.02 ± 4.9	24.83 ± 3.7	23.9 ± 2.0
B_{13}	53.67 ± 2.1	52.63 ± 4.3	44.90 ± 3.1
B_{14}	41.09 ± 1.1	36.89 ± 1.1	35.03 ± 1.8
B_{15}	91.40 ± 2.1	17.06 ± 2.2	50.26 ± 7.2
B_{16}	97.68 ± 5.1	175.75 ± 7.2	52.02 ± 1.8
B_{17}	–	76.11 ± 1.6	81.87 ± 4.8
B_{18}	–	62.67 ± 1.8	83.51 ± 6.0
Standard	45.50 ± 2.0 (Ascorbic acid)	58.71 ± 2.5 (Ascorbic acid)	32.68 ± 2.0 (BHT)

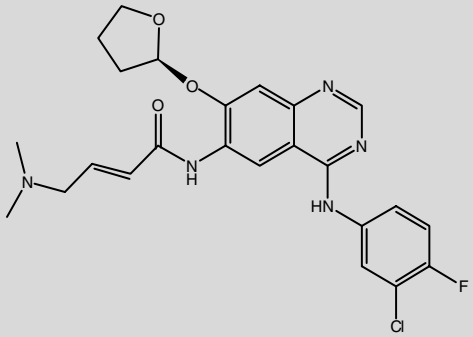
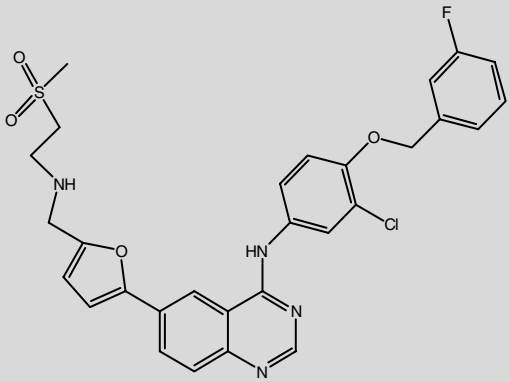
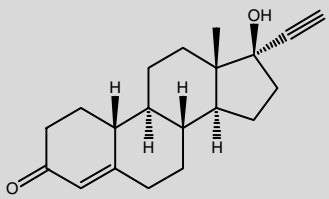
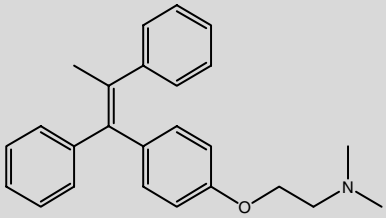
Note: All values are mean ± SD of three replicates.

ABTS reduction assay: Compound **B_{12}** (2-OH) exhibited higher antioxidant activity (IC_{50}, 23.90 μM) than BHT (IC_{50}, 32.68 μM). Phenolic derivatives **B_{14}** (2-OH-3,5-diNO_2) and **B_{13}** (2-OH-5-NH_2) also showed appreciable scavenging effects, with IC_{50} values of 35.03 and 44.90 μM, respectively. Compounds **B_{10}** (4-OCH_3), **B_8** (4-CH_3) and **B_9** (4-NH_2) exhibited moderate to significant antioxidant activities with IC_{50} values of 57.24, 58.69 and 64.22 μM, respectively. Replacement of phenyl ring with styryl (IC_{50}, 50.26 μM) or 2-pyridyl (IC_{50}, 52.02 μM) groups and with aliphatic chains **B_{17}** and **B_{18}** (IC_{50} values, 81.87 and 83.51 μM, respectively) resulted in an enhancement in the ABTS scavenging activity (Table-3).

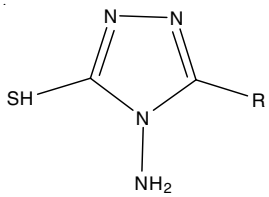
Overall, compound **B_{12}** (2-OH) exhibited promising antioxidant activity, followed by compound **B_{14}** (2-OH-3,5-diNO_2), where compounds **B_{15}**, **B_{17}** and **B_{18}** (styryl, stearyl and oleayl) were unable to exhibit significant activity in DPPH and ABTS assays but showed good activity against NO free radical.

QSAR studies: QSAR model correlates physico-chemical properties of the data set of structurally related compounds with biological activity. The data set of 22 compounds (Table-4) were submitted to PaDEL software to obtain 1444 descriptors (1D, 2D, molecular fingerprints) and among which 792 significant descriptors were selected by the V-WSP algorithm. A small dataset curator was applied to check data suitability such

TABLE-4
DATASET OF COMPOUNDS USED TO DERIVE QSAR MODELS

S. No.	R	m.w. (g/mol)	IC_{50} (μM)	PIC_{50}
1	C_6H_5	192.25	148.30	3.8288
2	4-Cl·C_6H_4	226.69	811.68	3.0906
3	3-Cl·C_6H_4	226.69	531.72	3.2743
4	2,4-$(Cl)_2$·C_6H_3	261.14	20.35	4.6914
5	4-Cl, 3-NO_2·C_6H_3	271.69	764.57	3.1165
6	4-NO_2·C_6H_4	237.24	664.85	3.1772
7	3,5-$(NO_2)_2$·C_6H_3	282.24	174.21	3.7589
8	4-CH_3·C_6H_4	206.27	147.86	3.8301
9	4-NH_2·C_6H_4	207.26	739.79	3.1308
10	4-OCH_3·C_6H_4	222.27	385.20	3.4143
11	3,4-$(OCH_3)_2$·C_6H_3	252.30	560.86	3.2511
12	2-OH·C_6H_4	208.25	185.91	3.7306
13	2-OH,5-NH_2·C_6H_3	223.26	294.31	3.5311
14	2-OH,3,5-$(NO_2)_2$·C_6H_2	298.24	174.92	3.7571
15	CH_2=CH·C_6H_5	218.28	217.15	3.6255
16	2-Pyridyl	193.24	236.86	3.6632
17	Stearyl	368.63	246.33	3.6084
18	Oleoyl	366.57	245.00	3.6108
19		485.95	0.031	7.5086
20		581.07	0.0102	7.9913
21		298.43	46	4.3372
22		357.50	7.3	5.1366

as removing duplicate structures and detecting structural and response outliers. The dataset was submitted to small dataset modeler for QSAR model generation and validation. Small Dataset Modeler provided various PLS-derived QSAR models in which two models were found to be statistically significant (Table-5). Both the models fulfilled the criteria for statistical significance, accuracy, robustness and predictive ability (internal and external). The validation metrics obtained for model 2 were superior compared to model 1. The difference between R^2 and Q^2 was 0.03 indicating over fitting (test set) and for model 1, the value was 0.02 suggesting good fitting.



R= C_6H_5, various substituted phenyl, 2-pyridyl, stearyl, oleayl

Total number of compounds = 18

The obtained PLS equation is (for number of components = 2):

$$pIC_{50} = 4.378 - 0.141*AATS0e + 0.116*ATSC4p + 0.012*fragC \quad (1)$$

n = 21, $R^2 = 0.881$ $Q^2 = 0.851$.

$$pIC_{50} = -3.067 - 0.019*AATSC7m + 3.487*SpMin1\_Bhe + 12.395*GGI10 \quad (2)$$

n = 20, $R^2 = 0.893$ $Q^2 = 0.871$.

The first equation was derived with 21 compounds as the afatinib structure was removed as response outlier (IC_{50} = 0.031 μM). When compounds **2** and **20** were removed, model 2 was generated. The lapatinib structure with IC_{50} 0f 0.0102 μM was removed as a response outlier and compound **2** was removed as an activity cliff.

Equation 1 revealed the positive contribution of fragment complexity and polarizability and the negative contribution of electronegativity. Fragment complexity depends on the number of non-hydrogen atoms and the number of bonds. A lower number of bonds with increased number of heteroatoms enhance the frag C values [31]. The higher cytotoxicity values of standard anticancer agents can be correlated with high frag C values (376.11.139.02 and 163.02) and low cytotoxicity values of the synthesized compounds with decreased frag C values (range of 25.05 to 61.12). Polarizability of a molecule increases with the insertion of heteroatoms and polarizability is inversely related to electronegativity. Polarizability initiates electronic interactions between molecules. Lack of polarizability and high electronegativity might be a reason for the low activity profile of all the synthesized compounds (**1-18**). Table-6 shows the descriptors values obtained for the dataset of compounds. The poor cytotoxicity of compounds **5** and **9** can be well explained using equation 1; frag C is low, polarizability is low and electronegativity is high compared to other compounds.

Model 2 was found to be superior concerning predictive ability. Three descriptors showed a significant correlation with the cytotoxicity of the molecules. SpMin1_Bhe and GGI10 positively contributed to the activity while AATSC7m was found to be unfavourable for the activity. Among the obtained descriptors, GGI10 was found to be the most effective descriptor for correlating the compounds with their anticancer activity.

Model 2 revealed the favourable contribution of GGI10 and SpMin1_Bhe. GGI10 addresses the GGI10 topological charge index by order of 10. Topological indices are promising molecular descriptors to predict important physico-chemical and biological activities. The connectivity of the atoms present in a molecule governs its properties. Researchers successfully used topological indices to identify hits and leads even when there was no information about the mechanism of action was available (antihistamines, anti-inflammatory agents, anticancer agents). In the obtained QSAR model, GGI10 has a positive charge, suggesting the higher value for GGI10 might increase the cytotoxicity. Table-4 presents the values of descriptors, GGI110 values are zero or near to zero for the synthesized compounds (**1-15**) except for compound **16**, **17** compared to the anticancer drugs in the study. The introduction of aliphatic chain as a substituent group increases GGI10 value and might increase the cytotoxicity as in the case of compounds **17** and **18**.

The second descriptor SpMin1_Bhe has a positive charge, indicating the higher value will favour the activity. In the

TABLE-5
INTERNAL AND EXTERNAL VALIDATION METRICS OBTAINED IN SMALL DATASET MODELER

No. of descriptors	R2	Q2	Scaled average rm2	Scaled delta rm2	MAE (100% data; train)	SD (100% data; train)	MAE (95% data; train)	SD (95% data; train)	MAE + 3*SD (95% data; train)	No. of compounds
3	0.881	0.851	0.809	0.073	0.236	0.292	0.165	0.182	0.71	21 (t-18,s-3)
3	0.893	0.871	0.827	0.077	0.235	0.262	0.187	0.151	0.641	20 (t-17,s-3)

TABLE-6
PaDEL DESCRIPTORS CORRELATED WITH ANTICANCER ACTIVITY

Name of the descriptor	Type (2D/3D)	Detailed information of the descriptor
Frag C	2D	Fragment complexicity
AATS0e	2D	Average Broto-Moreau autocorrelation - lag 0/weighted by Sanderson electronegativities
ATSC4p	2D	Centred Broto-Moreau autocorrelation of lag 4 weighted by polarizability
AATSC7m	2D	Average centeredBroto-Moreau autocorrelation - lag 7/weighted by mass
SpMin1_Bhe	2D	The smallest absolute eigenvalue of Burden modified matrix—n 1/weighted by relative first ionization potential.
GGI10	2D	Topological charge index of order 10

dataset compounds **16** and **17** have low ionization potential compared to others. The third descriptor which showed a less significant contribution was AATSC7m. In the equation, it has a negative charge. If the value is lowered, it might increase the cytotoxicity of the triazole analogs. The obtained models suggest introduction of substituent groups like aliphatic chain with heteroatoms similar to the afatinib and lapatinib structures. These results might be used to predict cytotoxicity of 1,2,4-triazoles before synthesis and also to design potent cytotoxic 1,2,4-triazoles. These findings may help in the development of 1,2,4-triazole based anticancerous compounds.

Conclusion

A series of novel compounds were synthesized containing 4-amino-4*H*-1,2,4-triazole-3-thiol ring as a main pharmacophore unit. A range of diverse aromatic and few aliphatic substituent groups were introduced at the 5th position to establish the structure activity relationship (SAR) for antibreast cancer activity. Perusal of the data illustrated the significance of 3,4-dichloro phenyl ring (IC_{50} value of 20.35 μM) at the 5th position. The compound exhibited good proteinase inhibitory action and antioxidant ability. The QSAR models correlated the cytotoxicity of the title compounds with topology, ionization potential and mass of the compounds. The obtained models suggest introduction of substituent groups like small aliphatic chain with heteroatoms similar to the afatinib (dimethylamino-*trans*-but-2-enamido group) and lapatinib (methylsulfonylethyl-amino) structures at the 5th position.

CONFLICT OF INTEREST

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

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