Research Paper

Synthesis and Cytotoxic Studies of Undecenoic Acid-based Schiff's Base Derivatives Bearing 1,2,4-Triazole Moiety

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A series of novel 1,2,4-triazole-based schiff's base derivatives were synthesized by condensing undecenoic triazole compound with various substituted benzaldehydes. Compounds so synthesized were thoroughly characterized using ^{1}H nuclear magnetic resonance, ^{13}C nuclear magnetic resonance, high resolution mass spectrometry and Fourier transform-infrared spectroscopy. Cytotoxicity of these compounds was tested *in vitro* on three cancer cell lines, mouse melanoma cancer cells (B16 F10), human colon cancer cells (HCT-15), human ovarian cancer cell line (SKOV3) and normal mouse embryonic fibroblasts (NIH-3T3) using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay. The results showed that most of the compounds exhibited cytotoxicity against the tested cell lines. Among the tested compounds, 6g and 6n showed significant cytotoxicity against B16 F10 cells and compounds 6i, 6n, 6o, 6p and 6q against HCT-15 cell line. The Schiff's base derivative 6g, appeared to be the most effective on B16 F10 cancer cells due to bromo substitution on the 4th position of the phenyl ring. Altogether, the cytotoxicity of these compounds observed against cancer cells indicate anticancer potential.

Key words: Undecenoic acid, 1,2,4-triazole, schiff's base, anticancer, cytotoxicity

Cancer continues to be a serious health problem in the developed and the developing countries. It is now the most common cause of death worldwide. The current human lifestyle has played a key role in the increasing rate of cancer. Therefore, continuous efforts are needed to discover novel compounds with improved selectivity and activity by chemical modifications. 1,2,4-Triazole nucleus represent an important class of heterocyclic compounds and their derivatives are characterized with a broad spectrum of biological activities including antibacterial, antifungal[1], antitubercular[2], anticancer[3], anticonvulsant[4], antiinflammatory[5], analgesic[6] and molluscicidal properties[7-10]. Various studies reported synthesis and pharmacological activities of 4-amino-1,2,4-triazole-5-thione moiety. This core structural unit was present in a diverse molecules that showed a wide range of activities namely, antifungal[11], antibacterial[12-16], anticancer[17,18], antitubercular[19], antiinflammatory[20], antimolluscicidal[21], antiviral[22,23] and antioxidant[24].

In addition, Schiff's bases form an important class of organic compounds exhibiting a wide range of applications. Schiff's bases have also gained importance in medicinal and pharmaceutical fields due to a broad spectrum of activities that these compounds exhibited, which included antiinflammatory[25-28], analgesic[26-29], antimicrobial[30,31], anticonvulsant[32], antitubercular[33], anticancer[34,35], antioxidant[36] and anthelmintic[37]. Apart from the above mentioned biological activities, Schiff's bases have also been used as catalysts, intermediates in organic synthesis, dyes, pigments, polymer stabilizers and corrosion inhibitors[38]. Schiff's base metal complexes also showed greater biological activity than free organic compounds[39].

On the other hand, fatty acids and their derivatives are known to possess antimicrobial[40,41], antifungal[42], and pesticidal[43] activities. Undecenoic acid was found to exhibit several biological activities such as

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antifungal, antibacterial and antiviral. The derivatives of undecenoic acid also affect cellular processes related to cancer[44-46]. Literature revealed that a variety of modified fatty acid were promising molecules in cancer prevention and have the potential to treat cancers[44,47,48]. In the light of these interesting biological activities associated with 1,2,4-triazole, Schiff's bases and undecenoic acid, it was planned in the present study to synthesize some novel Schiff's base derivatives bearing 1,2,4-triazole and undecenoic moieties and to evaluate the cytotoxicity possessed by these derivatives towards cancer and normal cells.

MATERIALS AND METHODS

All the chemicals used in these schemes were of analytical grade, which were obtained from different commercial sources and used without any further purification. Reactions were monitored on micro TLC plates (coated with TLC grade silica gel, obtained from Merck). Column chromatography was performed using silica gel (100-200 mesh) procured from Qualigens (India) using freshly distilled solvents. All ^{1}H-nuclear magnetic resonance (NMR) and ^{13}C-NMR spectra were recorded with a Bruker Avance (for ^{1}H-NMR at 300 MHz, 400 MHz, 500 MHz and for ^{13}C-NMR at 75 MHz, 100 MHz, 125 MHz) spectrometer, using TMS δ=0 ppm and δ 77.00 ppm as internal standard for chemical shifts (δ) in $CDCl_3$ at 25°. The chemical shift values are presented in ppm (parts per million) units. Mass spectra were recorded with high resolution mass spectra (HRMS). IR spectra were recorded in chloroform on a Perkin-Elmer Fourier-transform infrared spectroscopy (FTIR) spectrum BX.

Synthesis of methyl undec-10-enoate (2):

To a stirred solution of undec-10-enoic acid (73.45 mmol) in methanol (100 ml), a few drops of concentrated H_2SO_4 was added. The reaction mixture was refluxed for 10 h. Progress of the reaction was monitored by micro TLC. After completion of the reaction, methanol was removed under reduced pressure and water was added and the title compound was extracted with ethyl acetate, dried over anhydrous sodium sulphate and concentrated under vacuum to afford the title compound. ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm)=5.75-5.85 (m, -CH=CH_2-, 1H), 4.91-5.01 (m, -CH=CH_2-, 2H), 3.66 (s, -OCH_3, 3H), 2.28-2.31 (t, -CH_2-, *J*=7.4 Hz, 2H), 2.01-2.06 (m, -CH_2-, 2H), 1.59-1.65 (m, -CH_2-, 2H), 1.26-1.39 (m, -$(CH_2)_5$-, 10H). electrospray ionization (ESI)-MS: $[M+H]^+$ *m/z*=199.

Synthesis of undec-10-enehydrazide (3):

To a stirred solution of methyl undec-10-enoate (2) (59.93 mmol) in ethanol (90 ml), hydrazine hydrate (269.68 mmol) was added. The reaction mixture was refluxed for about 10 h. Progress of the reaction was monitored by micro TLC. After completion of reaction, the solvent was evaporated under reduced pressure, ice water (50 ml) was added and the mixture was stirred for 15 min. The solid obtained was filtered and dried under vacuum to yield undec-10-enehydrazide as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm)=5.75-5.85 (m, -CH=CH_2-, 1H), 4.91-5.01 (m, -CH=CH_2, 2H), 2.67-3.01 (broad-s, -NH_2, 2H), 2.12-2.16 (t, -CH_2-, *J*=7.3 Hz, 2H), 2.00-2.06 (m, -CH_2-, 2H), 1.59-1.66 (m, -CH_2-, 2H), 1.25-1.38 (m, -$(CH_2)_5$-, 10H). ESI-MS: $[M+H]^+$ *m/z*=199.

Synthesis of potassium 2-(undec-10-enoyl) hydrazine-1-carbodithioate (4):

Potassium hydroxide pellets (106.54 mmol) were dissolved in ethanol (40 ml). To this solution, undec-10-enehydrazide (53.27 mmol), carbon disulphide (117.19 mmol) were added successively and the contents were stirred at room temperature for 8 h. Progress of the reaction was monitored by micro TLC. After completion of the reaction, diethyl ether (100 ml) was added to the reaction mixture and stirred for 10 min. After filtration, potassium 2-(undec-10-enoyl) hydrazine-1-carbodithioate was obtained as an off-white solid.

Synthesis of 4-amino-5-(dec-9-en-1-yl)-4H-1,2,4-triazole-3-thiol (5):

Hydrazine hydrate (45.38 mmol) was added to potassium 2-(undec-10-enoyl) hydrazine-1-carbodithioate (45.38 mmol) and the contents were refluxed for 5 h. Progress of the reaction was monitored by micro TLC. After completion of reaction, the reaction mixture was acidified with concentrated hydrochloric acid. The obtained precipitate was filtered and dried under vacuum to obtain the crude compound, which was subjected to silica gel column chromatography and the required product was eluted in a solvent mixture (hexane:EtOAc, 85:15, v/v) as an off white solid. ESI-MS: $[M+H]^+$ *m/z*=255.

General procedure for the synthesis of benzylideneamine derivatives (6a-6t):

To a stirred solution of compound 5 (4.84 mmol) in ethanol (10 ml), benzaldehyde (1 equivalent) and two

to three drops of concentrated H_2SO_4 were added and the contents were refluxed for 6 h. The reaction was monitored by micro TLC. After completion of reaction, the solvent was removed under reduced pressure. To the residue, 5 ml of ice water was added, stirred for 5 min, and the precipitated solid was filtered and dried under vacuum. The compounds were purified by silica gel column chromatography using ethyl acetate and hexane as a solvent mixture.

4-Bromo-2-(((3-(dec-9-en-1-yl)-5-mercapto-4H-1,2,4-triazol-4-yl)imino)methyl)phenol (6a):

The crude compound was subjected to silica gel column chromatography and the required product was eluted in a solvent mixture (hexane:EtOAc, 70:30, v/v) as an off white solid with 81 % yield. ^{1}H-NMR data (500 MHz, $CDCl_3$): δ (ppm)=10.37 (s, -SH, 1H), 10.26 (s, -CH=N-, 1H), 7.52-7.56 (m, Ar-H, 2H), 6.96 (d, Ar-H, *J*= 8.6 Hz, 1H), 5.75-5.84 (m, -CH=CH_2-, 1H), 4.91-5.00 (m, -CH=CH_2-, 2H), 2.74-2.77 (t, -CH_2-, *J*=7.4 Hz, 2H), 2.00-2.04 (m, -CH_2-, 2H), 1.71-1.77 (m, -CH_2-, 2H), 1.25-1.40 (m, -$(CH_2)_5$-, 10H); ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm)=159.7, 155.9, 149.7, 137.1, 134.2, 127.4, 119.1, 118.0, 117.1, 112.6, 109.4, 31.7, 29.8, 27.2, 27.0, 26.8, 24.0, 23.0, 20.6; IR ($CHCl_3$ ν_{max} cm^{-1}): 3412, 2854, 2254, 2128, 1652, 1420, 1115, 1026, 825, 764, 625; HR-MS (ESI) *m/z* [M-H$^+$]: 435.13823.

5-(Dec-9-en-1-yl)-4-((4-nitrobenzylidene)amino)-4H-1,2,4-triazole-3-thiol (6b):

The crude compound was subjected to silica gel column chromatography and the required product was eluted in a solvent mixture (hexane:EtOAc, 75:25, v/v) as a light yellow solid with 79 % yield. ^{1}H-NMR data (500 MHz, $CDCl_3$): δ (ppm)=10.92 (s, -CH=N-, 1H), 8.35 (d, Ar-H, *J*=8.5 Hz, 2H), 8.03 (d, Ar-H, *J*=8.5 Hz, 2H), 5.75-5.83 (m, -CH=CH_2-, 1H), 4.91-5.00 (m, -CH=CH_2-, 2H), 2.83-2.86 (t, -CH_2-, *J*=7.4 Hz, 2H), 2.00-2.05 (m, -CH_2-, 2H), 1.74-1.80 (m, -CH_2-, 2H), 1.25-1.43 (m, -$(CH_2)_5$-, 10H); ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm)= 162.2, 156.2, 153.1, 149.7, 139.0, 130.4, 129.1, 124.1, 114.1, 33.7, 31.8, 29.2, 28.9, 28.8, 25.9, 24.9, 22.6; IR ($CHCl_3$ ν_{max} cm^{-1}): 3398, 2923, 2854, 2109, 1581, 1525, 1468, 1345, 1275, 1110, 844, 749; HR-MS (ESI) *m/z* [M+H$^+$]: calc for $C_{19}H_{26}O_2N_5S$ is 388.18017 found 388.17969 ($C_{28}H_{41}FN_2O_4Na$).

2,4-Dichloro-6-(((3-(dec-9-en-1-yl)-5-mercapto-4H-1,2,4-triazol-4-yl)imino)methyl)phenol (6c):

The crude compound was subjected to silica gel column chromatography and the required product was eluted in a solvent mixture (hexane:EtOAc, 70:30, v/v) as an off white solid with 73 % yield. ^{1}H-NMR data (400 MHz, $CDCl_3$): δ (ppm)=10.69 (s, -SH, 1H), 10.60 (s, -CH=N-, 1H), 7.54 (s, Ar-H, 1H), 7.38 (s, Ar-H, 1H), 5.75-5.84 (m, -CH=CH_2-, 1H), 4.91-5.01 (m, -CH=CH_2-, 2H), 2.75-2.78 (t, -CH_2-, *J*=7.4 Hz, 2H), 2.00-2.05 (m, -CH_2-, 2H), 1.72-1.79 (m, -CH_2-, 2H), 1.25-1.43(m, -$(CH_2)_5$-, 10H); ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm)=162.6, 161.9, 153.7, 151.6, 139.0, 133.9, 130.7, 124.9, 123.1, 117.8, 114.1, 33.7, 31.8, 29.1, 28.9, 28.8, 25.6, 25.0, 22.6; IR ($CHCl_3$ ν_{max} cm^{-1}): 3401, 2926, 2854, 2110, 1603, 1459, 1273, 1161, 1024, 738; HR-MS (ESI) *m/z* [M-H$^+$]: 425.14937.

4-Chloro-2-(((3-(dec-9-en-1-yl)-5-mercapto-4H-1,2,4-triazol-4-yl)imino)methyl)phenol (6d):

The crude compound was subjected to silica gel column chromatography and the required product was eluted in a solvent mixture (hexane:EtOAc, 70:30, v/v) as an off white solid with 76 % yield. ^{1}H-NMR data (400 MHz, $CDCl_3$): δ (ppm)=10.37 (s, -SH, 1H), 10.24 (s, -CH=N-, 1H), 7.39-7.41 (m, Ar-H and -OH, 3H), 7.00 (d, Ar-H, *J*=8.5 Hz, 1H), 5.75-5.85 (m, -CH=CH_2-, 1H), 4.92-5.00 (m, -CH=CH_2-, 2H), 2.73-2.77 (t, -CH_2-, *J*=7.4 Hz, 2H), 2.00-2.04 (m, -CH_2-, 2H), 1.72-1.78 (m, -CH_2-, 2H), 1.25-1.43 (m, -$(CH_2)_5$-, 10H); ^{13}C NMR (125 MHz, $CDCl_3$): δ (ppm)=163.8, 162.3, 158.1, 151.5, 139.0, 134.2, 132.2, 124.9, 119.0, 117.0, 114.1, 33.7, 31.8, 29.1, 28.9, 28.2, 25.7, 25.1, 22.6; IR ($CHCl_3$ ν_{max} cm^{-1}): 3149, 2927, 2855, 1597, 1479, 1425, 1352, 1276, 1166, 1118, 1016, 915, 828, 660; HR-MS (ESI) *m/z* [M+H$^+$]: calc for $C_{19}H_{26}ON_4ClS$ is 393.15104 found 393.15140 ($C_{19}H_{26}ON_4ClS$).

5-(Dec-9-en-1-yl)-4-((3-nitrobenzylidene)amino)-4H-1,2,4-triazole-3-thiol (6e):

The crude compound was subjected to silica gel column chromatography and the required product was eluted in a solvent mixture (hexane:EtOAc, 75:25, v/v) as a light yellow solid with 73 % yield. ^{1}H-NMR Data (400 MHz, $CDCl_3$): δ (ppm)=10.86 (s, -CH=N-, 1H), 8.72 (s, Ar-H, 1H), 8.36-8.39 (m, Ar-H, , 1H), 8.14-8.16 (d, Ar-H, *J*=7.7 Hz, 1H), 7.67-7.71 (t, Ar-H, 1H), 5.74-5.84 (m, -CH=CH_2-, 1H), 4.93-5.00 (m, -CH=CH_2-, 2H), 2.84-2.88 (t, -CH_2-, *J*=7.4 Hz, 2H), 1.99-2.04 (m, -CH_2-, 2H), 1.74-1.82 (m, -CH_2-, 2H), 1.25-1.44 (m, -$(CH_2)_5$-, 10H); ^{13}C NMR (125 MHz, $CDCl_3$): δ (ppm)=162.0, 156.7, 153.0, 148.6, 139.0, 134.5, 134.1, 130.0, 126.3, 122.7, 114.0, 33.6, 31.7, 29.1, 28.9, 28.7, 25.9, 24.9,

22.5; IR ($CHCl_3$ ν_{max} cm^{-1}): 3106, 2926, 2854, 1613, 1584, 1536, 1414, 1352, 1287, 1094, 808, 734; HR-MS (ESI) *m/z* [M+H$^+$]: calc for $C_{19}H_{26}O_2N_5S$ is 388.18017 found 388.17969 ($C_{19}H_{26}O_2N_5S$).

5-(Dec-9-en-1-yl)-4-((2,4,6-trifluorobenzylidene) amino)-4H-1,2,4-triazole-3-thiol (6f):

The crude compound was subjected to silica gel column chromatography and the required product was eluted in a solvent mixture (hexane:EtOAc, 80:20, v/v) as an off white solid with 65 % yield. ^{1}H-NMR data (500 MHz, $CDCl_3$): δ (ppm)=10.84 (s, -CH=N-, 1H), 6.78-6.81 (m, Ar-H, 2H), 5.76-5.84 (m, -CH=CH_2-, 1H), 4.91-5.00 (m, -CH=CH_2-, 2H), 2.77-2.80 (t, -CH_2-, *J*=7.4 Hz, 2H), 2.00-2.05 (m, -CH_2-, 2H), 1.70-1.76 (m, -CH_2-, 2H), 1.25-1.41 (m, -$(CH_2)_5$-, 10H); ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm)=165.8, 163.9, 163.1, 161.9, 161.0, 153.2, 150.3, 139.1, 114.1, 107.7, 101.3, 33.7, 31.8, 29.2, 29.0, 28.8, 25.9, 25.0, 22.6; IR ($CHCl_3$ ν_{max} cm^{-1}): 3409, 3071, 2922, 2852, 1640, 1596, 1442, 1349, 1276, 1175, 1125, 1050, 847, 746.

4-((4-Bromobenzylidene)amino)-5-(dec-9-en-1-yl)-4H-1,2,4-triazole-3-thiol (6g):

The crude compound was subjected to silica gel column chromatography and the required product was eluted in a solvent mixture (hexane:EtOAc, 75:25, v/v) as an off white solid with 69 % yield. ^{1}H-NMR data (500 MHz, $CDCl_3$): δ (ppm)=10.49 (s, -CH=N-, 1H), 7.73 (d, Ar-H, *J*=8.5 Hz, 1H), 7.63 (d, Ar-H, *J*=8.5 Hz, 1H), 5.77-5.84 (m, -CH=CH_2-, 1H), 4.92-5.00 (m, -CH=CH_2-, 2H), 2.79-2.82 (t, -CH_2-, *J*=7.4 Hz, 2H), 2.00-2.04 (m, -CH_2-, 2H), 1.72-1.78 (m, -CH_2-, 2H), 1.25-1.45 (m, -$(CH_2)_5$-, 10H); ^{13}C NMR (125 MHz, $CDCl_3$): δ (ppm)=162.0, 159.1, 152.9, 139.0, 132.2, 131.5, 129.9, 127.0, 114.1, 33.7, 31.8, 29.2, 29.0, 28.8, 25.9, 25.0, 22.6; IR ($CHCl_3$ ν_{max} cm^{-1}): 3406, 3109, 3062, 2923, 2853, 1589, 148, 1418, 1283, 1172, 1070, 1011. 820; ESI-MS: *m/z* at 421 [M-H]$^+$.

2-(((3-(Dec-9-en-1-yl)-5-mercapto-4H-1,2,4-triazol-4-yl)imino)methyl)phenol (6h):

The crude compound was subjected to silica gel column chromatography and the required product was eluted in a solvent mixture (hexane:EtOAc, 70:30, v/v) as an off white semi solid with 74 % yield. ^{1}H-NMR data (500 MHz, $CDCl_3$): δ (ppm)=10.27 (s, -CH=N-, 1H), 7.43-7.48 (m, Ar-H, 2H), 7.00-7.06 (m, Ar-H, 2H), 5.75-5.84 (m, -CH=CH_2-, 1H), 4.91-5.00 (m, -CH=CH_2-, 2H), 2.75-2.78 (t, -CH_2-, *J*=7.4 Hz, 2H), 2.00-2.04 (m, -CH_2-, 2H), 1.72-1.78 (m, -CH_2-, 2H), 1.25-1.41 (m, -$(CH_2)_5$-, 10H); ^{13}C NMR (125 MHz, $CDCl_3$): δ (ppm)=166.0, 162.3, 159.7, 151.5, 139.0, 134.6, 133.5, 120.1, 117.4, 116.0, 114.1, 33.7, 31.8, 29.1, 28.9, 28.8, 25.8, 25.1, 22.6; IR ($CHCl_3$ ν_{max} cm^{-1}): 3401, 2924, 2853, 2108, 1605, 1464, 1297, 1153, 756; HR-MS (ESI) *m/z* [M-H$^+$]: 357.22718.

4-((4-Chlorobenzylidene)amino)-5-(dec-9-en-1-yl)-4H-1,2,4-triazole-3-thiol (6i):

The crude compound was subjected to silica gel column chromatography and the required product was eluted in a solvent mixture (hexane:EtOAc, 75:25, v/v) as an off white solid with 79 % yield. ^{1}H-NMR data (400 MHz, $CDCl_3$): δ (ppm)=10.47 (s, -CH=N-, 1H), 7.81 (d, Ar-H, *J*=8.5 Hz, 2H), 7.47 (d, Ar-H, *J*=8.5 Hz, 2H), 5.74-5.84 (m, -CH=CH_2-, 1H), 4.91-5.01 (m, -CH=CH_2-, 2H), 2.79-2.83 (t, -CH_2-, *J*=7.4 Hz, 2H), 2.00-2.05 (m, -CH_2-, 2H), 1.71-1.79 (m, -CH_2-, 2H), 1.25-1.41 (m, -$(CH_2)_5$-, 10H); ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm)=161.7, 159.2, 152.8, 139.0, 138.4, 131.0, 129.7, 129.2, 114.1, 33.7, 31.8, 29.2, 28.9, 28.8, 25.9, 25.0, 22.6; IR ($CHCl_3$ ν_{max} cm^{-1}): 3156, 2925, 2854, 1591, 1472, 1278, 1089, 1014, 825, 516; HR-MS (ESI) *m/z* [M+H$^+$]: calc for $C_{19}H_{26}N_4ClS$ is 377.15612 found 377.15589 ($C_{19}H_{26}N_4ClS$).

4-((3-Bromo-4-methoxybenzylidene)amino)-5-(dec-9-en-1-yl)-4H-1,2,4-triazole-3-thiol (6j):

The crude compound was subjected to silica gel column chromatography and the required product was eluted in a solvent mixture (hexane:EtOAc, 75:25, v/v) as an off white solid with 68 % yield. ^{1}H-NMR data (500 MHz, $CDCl_3$): δ (ppm)=10.27 (s, -CH=N-, 1H), 8.12 (d, Ar-H, *J*=1.9 Hz, 1H), 7.72-7.74 (m, Ar-H, 1H), 6.98 (m, Ar-H, 1H), 5.75-5.83 (m, -CH=CH_2-, 1H), 4.91-5.00 (m, -CH=CH_2-, 2H), 3.98 (m, OCH_3, 3H),2.79-2.82 (t, -CH_2-, *J*=7.4 Hz, 2H), 2.00-2.04 (m, -CH_2-, 2H), 1.73-1.78 (m, -CH_2-, 2H), 1.25-1.41(m, -$(CH_2)_5$-, 10H); ^{13}C NMR (125 MHz, $CDCl_3$): δ (ppm)=161.8, 159.2, 159.0, 152.8, 139.0, 132.7, 130.2, 126.3, 114.1, 112.6, 111.6, 56.4, 33.7, 31.8, 29.2, 29.0, 28.8, 25.9, 25.0, 22.6; IR ($CHCl_3$ ν_{max} cm^{-1}): 3381, 2922, 2852, 2109, 1591, 1497, 1469, 1415, 1265, 1160, 1050, 1018, 914, 804, 771, 577; ESI-MS: *m/z* at 451 [M+H]$^+$.

5-(Dec-9-en-1-yl)-4-((3-fluorobenzylidene)amino)-4H-1,2,4-triazole-3-thiol (6k):

The crude compound was subjected to silica gel column chromatography and the required product was eluted in

a solvent mixture (hexane:EtOAc, 75:25, v/v) as an off white solid with 79 % yield. ^{1}H-NMR data (500 MHz, $CDCl_3$): δ (ppm)=10.56 (s, -CH=N-, 1H), 7.58-7.62 (m, Ar-H, 2H), 7.44-7.48 (m, Ar-H, 1H), 7.22-7.25 (m, Ar-H, 1H), 5.75-5.84 (m, -CH=CH_2-, 1H), 4.91-5.00 (m, -CH=CH_2-, 2H), 2.80-2.82 (t, -CH_2-, *J*=7.4 Hz, 2H), 2.00-2.04 (m, -CH_2-, 2H), 1.73-1.79 (m, -CH_2-, 2H), 1.24-1.42 (m, -$(CH_2)_5$-, 10H); ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm)=164.2, 162.1, 158.7, 153.0, 139.1, 134.8, 130.6, 125.2, 119.4, 114.2, 114.1, 33.7, 31.8, 29.2, 29.0, 28.8, 25.9, 25.0, 22.6; IR ($CHCl_3$ ν_{max} cm^{-1}): 3406, 3148, 2921, 2853, 1612, 1578, 1496, 1414, 128, 1266, 1159, 1001, 906. 786; HR-MS (ESI) *m/z* [M+H$^+$]: calc for $C_{19}H_{26}N_4FS$ is 361.18567 found 361.18475 ($C_{19}H_{26}N_4FS$).

4-((2-Bromobenzylidene)amino)-5-(dec-9-en-1-yl)-4H-1,2,4-triazole-3-thiol (6l):

The crude compound was subjected to silica gel column chromatography and the required product was eluted in a solvent mixture (hexane:EtOAc, 75:25, v/v) as an off white solid with 81 % yield. ^{1}H-NMR data (500 MHz, $CDCl_3$): δ (ppm)=10.97 (s, -CH=N-, 1H), 8.10 (d, Ar-H, *J*=8.3 Hz, 1H), 7.68 (d, Ar-H, *J*=9.1 Hz, 1H), 7.35-7.43 (m, Ar-H, 2H), 5.75-5.84 (m, -CH=CH_2-, 1H), 4.91-5.00 (m, -CH=CH_2-, 2H), 2.81-2.84 (t, -CH_2-, *J*=7.4 Hz, 2H), 2.00-2.04 (m, -CH_2-, 2H), 1.73-1.79 (m, -CH_2-, 2H), 1.25-1.43 (m, -$(CH_2)_5$-, 10H); ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm)=161.9, 159.2, 152.9, 139.0, 133.4, 133.1, 132.2, 127.9, 127.6, 126.4, 114.1, 33.7, 31.8, 29.2, 28.9, 28.8, 25.9, 25.0; IR ($CHCl_3$ ν_{max} cm^{-1}): 3149, 2925, 2853, 1639, 1585, 1465, 1416, 1355, 1277, 1122, 1024, 909, 756; HR-MS (ESI) *m/z* [M+H$^+$]: calc for $C_{19}H_{26}N_4BrS$ is 421.10561 found 421.10510 ($C_{19}H_{26}N_4BrS$).

5-(Dec-9-en-1-yl)-4-((2-methoxybenzylidene)amino)-4H-1,2,4-triazole-3-thiol (6m):

The crude compound was subjected to silica gel column chromatography and the required product was eluted in a solvent mixture (hexane:EtOAc, 75:25, v/v) as an off white solid with 86 % yield. ^{1}H-NMR data (500 MHz, $CDCl_3$): δ (ppm)=10.75 (s, --SH, 1H), 10.50 (s, -CH=N-, 1H), 8.06 (dd, Ar-H, 1H), 7.48-7.51 (t, Ar-H, 1H), 7.02-7.05 (t, Ar-H, 1H), 6.98 (d, Ar-H, *J*=8.3Hz, 1H), 5.75-5.84 (m, -CH=CH_2-, 1H), 4.90-5.00 (m, -CH=CH_2-, 2H), 3.91 (s, -OCH_3, 3H), 2.77-2.81 (t, -CH_2-, *J*=7.4 Hz, 2H), 2.00-2.04 (m, -CH_2-, 2H), 1.73-1.77 (m, -CH_2-, 2H), 1.25-1.40 (m, -$(CH_2)_5$-, 10H); ^{13}C NMR (125 MHz, $CDCl_3$): δ (ppm)=161.8, 159.6, 158.7, 152.6, 139.0, 133.9, 126.9, 121.0, 120.7, 114.0, 111.4, 55.8, 33.7, 29.6, 29.2, 28.9, 28.8, 25.9, 25.0; IR ($CHCl_3$ ν_{max} cm^{-1}): 3344, 2926, 2853, 2108, 1682, 1589, 1456, 1284, 1166, 997, 874, 785, 683; HR-MS (ESI) *m/z* [M+H$^+$]: calc for $C_{20}H_{29}N_4OS$ is 373.20566 found 373.20474 ($C_{20}H_{29}N_4OS$).

5-(Dec-9-en-1-yl)-4-((4-fluorobenzylidene)amino)-4H-1,2,4-triazole-3-thiol (6n):

The crude compound was subjected to silica gel column chromatography and the required product was eluted in a solvent mixture (hexane:EtOAc, 75: 25, v/v) as an off white solid with 72 % yield. ^{1}H-NMR data (500 MHz, $CDCl_3$): δ (ppm)=10.69 (s, -SH, 1H), 10.40 (s, -CH=N-, 1H), 7.89 (d, Ar-H, *J*=8.5 Hz, 2H), 7.19 (d, Ar-H, *J*=8.5 Hz, 2H), 5.75-5.83 (m, -CH=CH_2-, 1H), 4.91-5.00 (m, -CH=CH_2-, 2H), 2.79-2.82 (t, -CH_2-, *J*=7.4 Hz, 2H), 2.00-2.04 (m, -CH_2-, 2H), 1.72-1.78 (m, -CH_2-, 2H), 1.25-1.41 (m, -$(CH_2)_5$-, 10H); ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm)=164.0, 161.9, 159.6, 152.9, 139.0, 130.8, 128.8, 116.3, 114.1, 33.7, 31.8, 29.2, 29.0, 28.8, 25.9, 25.0, 22.6; IR ($CHCl_3$ ν_{max} cm^{-1}): 3392, 2927, 2855, 2108, 1603, 1511, 1466, 1413, 1277, 1235, 1154, 910. 837; HR-MS (ESI) *m/z* [M+H$^+$]: calc for $C_{19}H_{26}N_4FS$ is 361.18567 found 361.18476 ($C_{19}H_{26}N_4FS$).

4-((5-Chloro-2-nitrobenzylidene)amino)-5-(dec-9-en-1-yl)-4H-1,2,4-triazole-3-thiol (6o):

The crude compound was subjected to silica gel column chromatography and the required product was eluted in a solvent mixture (hexane:EtOAc, 75:25, v/v) as a light yellow solid with 71 % yield. ^{1}H-NMR data (500 MHz, $CDCl_3$): δ (ppm)=11.20 (s, -SH, 1H), 10.17 (s, -CH=N-, 1H), 8.13 (d, Ar-H, *J*=8.6 Hz, 1H), 8.05 (s, Ar-H, 1H), 7.65 (m, Ar-H, 1H), 5.76-5.83 (m, -CH=CH_2-, 1H), 4.91-5.00 (m, -CH=CH_2-, 2H), 2.79-2.82 (t, -CH_2-, *J*=7.4 Hz, 2H), 2.00-2.04 (m, -CH_2-, 2H), 1.72-1.78 (m, -CH_2-, 2H), 1.25-1.42 (m, -$(CH_2)_5$-, 10H); ^{13}C NMR (125 MHz, $CDCl_3$): δ (ppm)=162.3, 155.2, 152.9, 146.7, 140.4, 139.0, 131.8, 129.9, 129.2, 126.4, 114.1, 33.7, 31.8, 29.2, 28.9, 28.8, 25.9, 24.9, 22.6; IR ($CHCl_3$ ν_{max} cm^{-1}): 3404, 2926, 2854, 2108, 1563, 1528, 1465, 1414, 1341, 1270, 1109, 854; HR-MS (ESI) *m/z* [M+H$^+$]: calc for $C_{19}H_{25}O_2N_5ClS$ is 422.14120 found 422.13967 ($C_{19}H_{25}O_2N_5ClS$).

5-(Dec-9-en-1-yl)-4-((2-nitrobenzylidene)amino)-4H-1,2,4-triazole-3-thiol (6p):

The crude compound was subjected to silica gel column

chromatography and the required product was eluted in a solvent mixture (hexane:EtOAc, 75:25, v/v) as a light yellow solid with 68 % yield. ^{1}H-NMR data (400 MHz, $CDCl_3$): δ (ppm)=11.04 (s, -SH, 1H), 10.64 (s, -CH=N-, 1H), 8.08-8.14 (m, Ar-H, 2H), 7.67-7.78 (m, Ar-H, 2H), 5.75-5.84 (m, -CH=CH_2-, 1H), 4.91-5.00 (m, -CH=CH_2-, 2H), 2.78-2.80 (t, -CH_2-, *J*=7.4 Hz, 2H), 1.99-2.05 (m, -CH_2-, 2H), 1.71-1.79 (m, -CH_2-, 2H), 1.24-1.41 (m, -$(CH_2)_5$-, 10H); ^{13}C NMR (125 MHz, $CDCl_3$): δ (ppm)=162.1, 157.2, 152.8, 148.6, 139.0, 133.6, 132.0, 129.6, 127.9, 124.8, 114.0, 33.6, 31.8, 29.1, 28.9, 28.7, 25.9, 24.9, 22.6; IR ($CHCl_3$ ν_{max} cm^{-1}): 3110, 3066, 2927, 2855, 1640, 1585, 1528, 1413, 1347, 1280, 1169, 996, 854, 737; HR-MS (ESI) *m/z* [M+H$^+$]: calc for $C_{19}H_{26}O_2N_5S$ is 388.18017 found 388.17932 ($C_{19}H_{26}O_2N_5S$).

5-(Dec-9-en-1-yl)-4-((2-fluorobenzylidene)amino)-4H-1,2,4-triazole-3-thiol (6q):

The crude compound was subjected to silica gel column chromatography and the required product was eluted in a solvent mixture (hexane:EtOAc, 75:25, v/v) as an off white solid with 64 % yield. ^{1}H-NMR data (500 MHz, $CDCl_3$): δ (ppm)=10.72 (s, -CH=N-, 1H), 8.06 (t, Ar-H, 1H), 7.50-7.54 (m, Ar-H, 1H), 7.24-7.27 (t, Ar-H, 1H), 7.17-7.19 (t, Ar-H, 1H), 5.77-5.83 (m, -CH=CH_2-, 1H), 4.91-4.99 (m, -CH=CH_2-, 2H), 2.80-2.83 (t, -CH_2-, *J*=7.4 Hz, 2H), 2.00-2.04 (m, -CH_2-, 2H), 1.73-1.79 (m, -CH_2-, 2H), 1.25-1.40 (m, -$(CH_2)_5$-, 10H); ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm)=163.7, 162.0, 154.5, 152.9, 139.0, 134.1, 127.3, 124.5, 120.7, 116.3, 114.1, 33.7, 31.8, 29.2, 29.0, 28.8, 25.9, 25.0, 22.6; IR ($CHCl_3$ ν_{max} cm^{-1}): 3409, 3107, 2924, 2852, 2108, 1613, 1582, 1415, 1285, 1101, 876, 764; HR-MS (ESI) *m/z* [M+H$^+$]: calc for $C_{19}H_{26}N_4S$ is 361.18567 found 361.18476 ($C_{19}H_{26}N_4S$).

5-(Dec-9-en-1-yl)-4-((3-(trifluoromethoxy) benzylidene)amino)-4H-1,2,4-triazole-3-thiol (6r):

The crude compound was subjected to silica gel column chromatography and the required product was eluted in a solvent mixture (hexane:EtOAc, 75:25, v/v) as an off white solid with 72 % yield. ^{1}H-NMR data (300 MHz, $CDCl_3$): δ (ppm)=13.56 (s, -SH, 1H), 10.60 (s, -CH=N-, 1H), 7.74-7.80 (m, Ar-H, 2H), 7.60 (d, Ar-H, *J*=9.1 Hz, 1H), 7.38-7.54 (m, Ar-H, 2H), 5.71-5.84 (m, -CH=CH_2-, 1H), 4.89-4.99 (m, -CH=CH_2-, 2H), 2.80-2.82 (t, -CH_2-, *J*=7.4 Hz, 2H), 2.00-2.04 (m, -CH_2-, 2H), 1.69-1.79 (m, -CH_2-, 2H), 1.25-1.43 (m, -$(CH_2)_5$-, 10H); ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm)=160.1, 156.9, 150.4, 147.8, 137.3, 133.5, 129.6, 129.4, 126.1, 122.8, 118.0, 112.8, 32.0, 30.1, 27.5, 27.2, 27.1, 24.4, 23.3, 20.9; IR ($CHCl_3$ ν_{max} cm^{-1}): 3425, 2927, 2254, 2128, 1650, 1415, 1257, 1217, 1163, 1026, 1005, 825, 764; HR-MS (ESI) *m/z* [M+H$^+$]: calc for $C_{20}H_{26}ON_4F_3S$ is 427.17739 found 427.17586 ($C_{20}H_{26}ON_4F_3S$).

5-(Dec-9-en-1-yl)-4-((5-fluoro-2-nitrobenzylidene) amino)-4H-1,2,4-triazole-3-thiol (6s):

The crude compound was subjected to silica gel column chromatography and the required product was eluted in a solvent mixture (hexane:EtOAc, 75:25, v/v) as a light yellow solid with 75 % yield. ^{1}H-NMR data (500 MHz, $CDCl_3$): δ (ppm)=11.23 (s, -SH, 1H), 10.88 (s, -CH=N-, 1H), 8.21-8.24 (m, Ar-H, 1H), 7.77-7.79 (m, Ar-H, 1H), 7.34-7.38 (m, Ar-H, 1H), 5.75-5.83 (m, -CH=CH_2-, 1H), 4.90-5.00 (m, -CH=CH_2-, 2H), 2.80-2.83 (t, -CH_2-, *J*=7.4 Hz, 2H), 2.00-2.04 (m, -CH_2-, 2H), 1.73-1.79 (m, -CH_2-, 2H), 1.24-1.43 (m, -$(CH_2)_5$-, 10H); ^{13}C NMR (125 MHz, $CDCl_3$): δ (ppm)=165.8, 163.8, 162.3, 155.1, 152.9, 144.7, 139.0, 128.0, 118.9, 116.1, 114.1, 33.7, 31.8, 29.2, 28.9, 28.8, 25.9, 24.9, 22.6; IR ($CHCl_3$ ν_{max} cm^{-1}): 3147, 2926, 2854, 1577, 1529, 1471, 1422, 1344, 1277, 1161, 856, 753; HR-MS (ESI) *m/z* [M+H$^+$]: calc for $C_{19}H_{25}O_2N_5FS$ is 406.17075 found 406.16944 ($C_{19}H_{25}O_2N_5FS$).

5-(Dec-9-en-1-yl)-4-((3-methoxybenzylidene)amino) -4H-1,2,4-triazole-3-thiol (6t):

The crude compound was subjected to silica gel column chromatography and the required product was eluted in a solvent mixture (hexane:EtOAc, 75:25, v/v) as an off white solid with 83 % yield. ^{1}H-NMR data (500 MHz, $CDCl_3$): δ (ppm)=10.84 (s, -SH, 1H), 10.35 (s, -CH=N-, 1H), 7.38-7.43 (m, Ar-H, 3H), 7.07-7.10 (m, Ar-H, 1H), 5.75-5.83 (m, -CH=CH_2-, 1H), 4.91-5.00 (m, -CH=CH_2-, 2H), 3.87 (s, -OCH_3, 3H), 2.80-2.83 (t, -CH_2-, *J*=7.4 Hz, 2H), 2.00-2.04 (m, -CH_2-, 2H), 1.73-1.79 (m, -CH_2-, 2H), 1.25-1.42 (m, -$(CH_2)_5$-, 10H); ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm)=161.8, 161.0, 159.8, 152.8, 139.0, 133.8, 129.9, 121.8, 118.7, 114.1, 112.4, 55.3, 33.7, 29.2, 28.9, 28.8, 25.9, 25.0; IR ($CHCl_3$ ν_{max} cm^{-1}): 3077, 2927, 2854, 1619, 1577, 1480, 1456, 1428, 1272, 1071, 1003, 931, 788, 750; HR-MS (ESI) *m/z* [M+H$^+$]: calc for $C_{20}H_{29}ON_4S$ is 373.20566 found 373.20460 ($C_{20}H_{29}ON_4S$).

MTT assay:

Cell viability of the synthesized compounds was determined using MTT assay, a colorimetric method[49],

in which the yellow colored 3-(4,5-di-methylthiazol-2-yl)-2,5-diphenyltetrazolium bromide dye is reduced to purple colored formazan crystals by mitochondrial dehydrogenase enzymes. The measure of reduction of MTT to formazan crystals directly correlates with the cell viability. This assay is well-established to determine the cytotoxic nature of drugs. Briefly, the cells were cultured in Dulbecco's modified Eagle's medium in a humidified incubator maintained at 37°. Further, the cells were seeded at a density of 10×10^3 cells per well in 96-well plates and grown for 24 h. The cells were then incubated with the series of compounds for 48 h. After incubation period, MTT (0.5 mg/ml) was added to each well of the plate by removing the old media and incubated in dark for 4 h. The *in situ* formed formazan crystals were solubilized to a purple color dye by adding dimethyl sulfoxide (DMSO):methanol (1:1; v/v) solvent mixture to the wells and kept on the shaker for homogeneous mixing for a few minutes. The absorbance of the samples was measured at 570 nm using a Synergy H1 multimode plate reader. All the experiments were carried out in triplicate and the results were expressed as normalized viability.

RESULTS AND DISCUSSION

Twenty target compounds were synthesized as outlined in fig. 1. The starting material undec-10-enoic acid was converted to the methyl ester (2) by reacting with few drops of concentrated sulphuric acid in methanol. This ester was treated with hydrazine hydrate to yield undec-10-enehydrazide (3). Undec-10-enehydrazide was reacted with carbon disulfide in presence of potassium

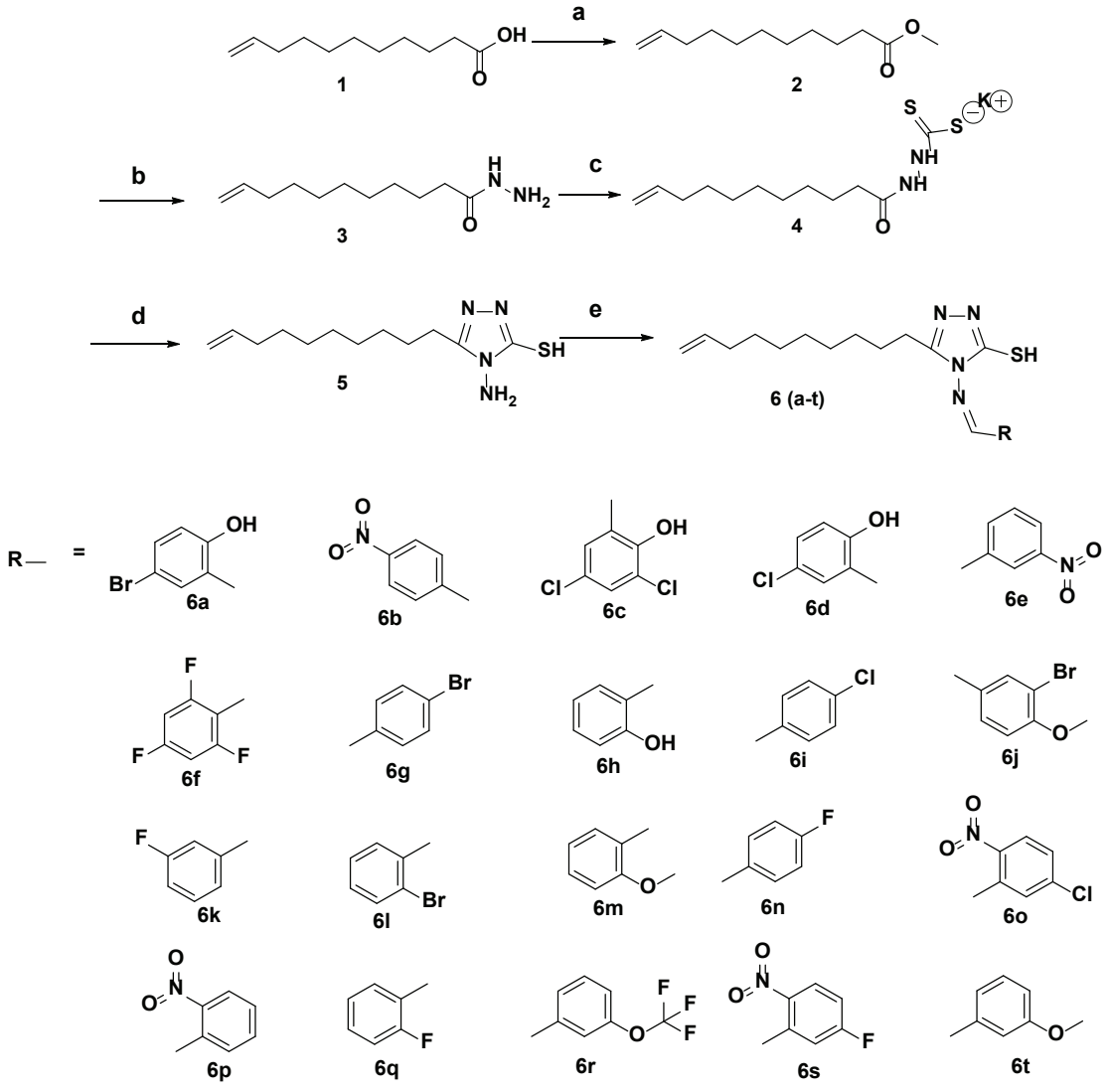

Fig. 1: Reagents and conditions
(a) MeOH, H_2SO_4, reflux, 10 h; (b) hydrazine hydrate, EtOH, reflux, 10h; (c) CS_2, KOH, EtOH, RT, 8 h; (d) hydrazine hydrate, reflux, 5 h; (e) aldehyde, EtOH, H_2SO_4, reflux, 6 h

hydroxide in ethanol to afford potassium 2-(undec-10-enoyl)hydrazine-1-carbodithioate (4). The triazole (5) was obtained by refluxing the potassium 2-(undec-10-enoyl)hydrazine-1-carbodithioate in presence of hydrazine hydrate. Further, a new series of Schiff's base derivatives (6a-t) were prepared by treating triazole with equimolar amounts of the selected benzaldehydes in presence of catalytic quantity of sulphuric acid and ethanol under reflux conditions. The synthesized compounds were characterized by ^{1}H, ^{13}C NMR, ESI-MS, HRMS and IR spectral analysis.

MTT assay was performed in order to determine the cytotoxic effect of synthesized compounds on cancerous and normal cells. The cytotoxic effect of the synthesized compounds (6a-t) (1 and 10 µM) was determined against different cancer cell lines, B16 F10 (mouse melanoma cell line), HCT-15 (human colon cancer cells), SKOV3 (human ovarian cancer cell line) and a normal cell line NIH-3T3 (mouse embryonic fibroblasts) using MTT assay. The results of the MTT assay on various cell lines are shown in fig. 2. The effect of the synthesized compounds on colon cancer cells (HCT-15) is shown in fig. 2A. Among all the tested samples, compounds 6i, 6n, 6o, 6p and 6q were found to be cytotoxic to HCT-15 cells (fig. 2A). DMSO served as the vehicle control and doxorubicin (DOX: 2.5 µM) served as the positive control. In all the experiments untreated control cells were designated as UT. Fig. 2B demonstrated the cytotoxic effect of the compounds on the viability of mouse melanoma cells (B16 F10). Among the tested compounds, 6g and 6n exhibited significant cytotoxicity against B16 F10 cells (fig. 2B) and particularly compound 6g exhibited better cytotoxicity against B16 F10, which might be due to enhanced uptake of 6g by B16 F10 cancer cells and it is noteworthy that 6g has a bromo substitution at 4th position. The cytotoxicity of compounds 6i, 6n, 6o, 6p and 6q might be attributed to the presence of 4-chloro, 4-fluoro, 5-chloro-2-nitro, 2-nitro and 2-fluoro substitutions, respectively.

The effect of the synthesized compounds on the viability of SKOV3 ovarian cancer cells is shown in fig. 2C, which surprisingly demonstrated that none of the compounds exhibited cytotoxicity to SKOV3 cancer cells. All the above results support the observation that similar compounds show difference in their cytotoxic potential towards various cancer cell types as reported in the literature[50]. In spite of effective cytotoxic nature of commercial anticancer agents towards cancer cells, severe cytotoxicity towards normal cells limits their clinical translation. In order to check the effect of the synthesized compounds on normal cells, MTT assay was performed using NIH 3T3 mouse embryonic fibroblasts and the results are

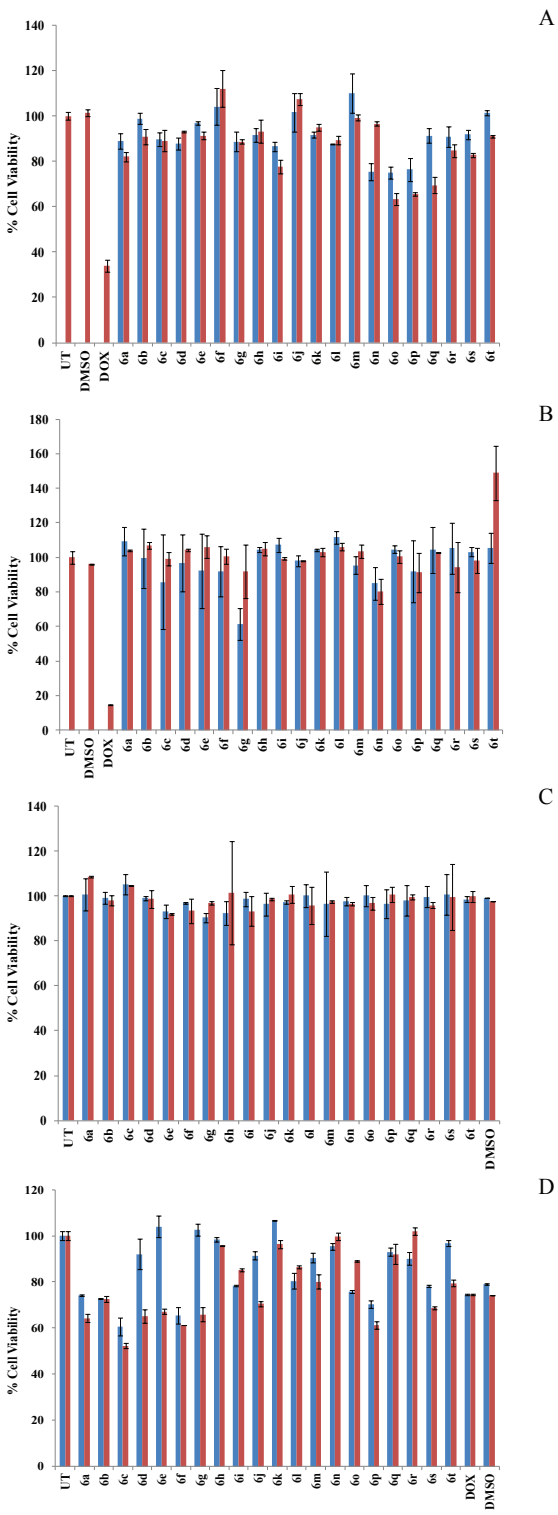

Fig. 2: Determination of cytotoxic effect of compounds 6a-6t using MTT assay
A. HCT-15 cells viability, B. B16 F10 cells viability, C. SKOV3 cells viability, D. NIH 3T3 cells viability, ■ 1 µM, ■ 10 µM

shown in fig. 2D. According to the assay results, the active compounds, which were cytotoxic to cancer cells (6g, 6n and 6q) were found nontoxic to normal cells (NIH 3T3), especially at low concentration (fig. 2D), and this observation makes them ideally suited for further development.

In conclusion, a series of novel 1,2,4-triazole-based Schiff's base heterocycles were synthesized. Cytotoxicity of all the synthesized compounds was tested against three cancer cells and a normal cell line. In this study, 4-bromo, 4-chloro, 4-fluoro, 5-chloro-2-nitro, 2-nitro and 2-fluoro substitution-based Schiff's base derivatives exhibited significant cytotoxicity; however, 4-bromo derivative exhibited more selective cytotoxicity to cancer cells. Majority of these active compounds were non-toxic to the normal cells, which makes them suitable for further development. Taken together, the observations of cytotoxic nature of these compounds against cancer cells, indicate preliminary anticancer properties.

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Conflict of interest:

There are no conflicts of interest.

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