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Synthesis, Molecular Modeling and Anticancer Activity of Novel 1,2,3-Triazole Hybrids-a Click Chemistry Approach

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ABSTRACT

A novel series of 1,4-disubstituted 1,2,3-triazole derivatives (3a-3m) were one pot synthesized via cupper nanoparticles azide-alkyne click chemistry, and evaluated for their lung cancer (A549), prostate cancer (PC-3) inhibitory activity. Meanwhile, the activity of compounds containing 1,2,3-triazoles was higher than that of reference compounds. Out of these all, compound 3a showed most notable anticancer activity against prostate cancer cell line with IC_{50} value of 8.08 µM and 16.18 µM in A549, respectively. In particular, hybrid compound 3f was found to be the most potent derivative with IC_{50} values of 15.46 µM against one strain lung cancer cell line and was found to be more selective against prostate cancer (PC-3). Further the compounds 3g, 3e and 3b has been moderate tested for its anticancer activity and its inhibitory activity against A549 and PC-3 cell lines. Furthermore 3c,3k was found to be non-toxic against cell lines when screened for toxicity. To elucidate the interaction mechanism between the active potential binding site of nitrate reductase protein and its inhibitor, the docking-based molecular dynamics simulation (MD) and lamarckian genetic algorithm (LGA) calculations was selected for freezing, default parameters used in autodock 4.2. In the present investigation we focused mainly on the binding energy, hydrogen bonds and distance between the protein and ligand. The results show that compound 3f was potently bound to TYR248, LYS273 with highest score -6.96.

Keywords: $CuFe_2O_4$ nanoparticles; 1,2,3-triazoles; Anticancer activity; Molecular docking studies; XRD studies

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INTRODUCTION

Cancer is a group of heterogenious disease, commenly characterized by uncontrolled cell division that can further progress towards mortality through cancer cells an acquiring ability to invade other vital organs. After cardiovascular disease, cancer is the most life threatening disease that causes one out of eight deaths world-wide. An estimated 13.2 million cancer related deaths and ~21.4 million new cancer cases are expected by 2030 [1]. National institutes of health (NIH) has projected medical expenditures to reach $158 billion in the year 2020 with total cost estimates of $895 billion worldwide. On the otherhand, prostate cancer is the second leading cause of cancer deaths in men, contributing to ~15% of the total number of new cases diagnosed in 2012 [2]. Lung cancer is among the most common cancers and has resulted in the highest mortality rate in the world [3]. The incidence of these two cancers has significantly increased in developed countries like United States abd is constantly raising in developing countries [4]. Triazoles constitute an important class of nitrogen heterocycles, which display an ample spectrum of biological activities and are widely employed as pharmaceuticals and agrochemicals. 1,2,3-triazoles play a key role in many bioactive molecules/drugs and are bioisosteres of amide bonds due to structural and electronic similarity and are more stable against metabolic degradation [5,6]. medicinally, they have been shown to possess a wide range of diverse interesting pharmaceutical properties such as reported to exhibit anticancer [7,8], antimicrobial [9], anti-HIV [10], antimalarial [11], anti-inflammatory [12], antifungal [13], antiallergic [14], antiepileptic [15],

antileishmanial [16], exceptional gamma amino butyric acid [17] and glycosidase inhibitors [18], photographic elements agrochemicals [19] antituberculosis [20] and antihelmintic activities [21].

In recent years, 1,2,3-triazoles generated using click chemistry [22,23] further, click chemistry also played a significant role in new drug discovery because of azide-alkyne cyclo addition (1,3-dipolar cyclization) of bioactive compounds/units that are covalently linked in the generation of newer substrates [24]. Triazole skeleton possesses moderate dipolar character, rigidity and stability under *in vivo* conditions which qualifies them lead frameworks in drug design [25]. Particularly, Jabeen et al. have recently reported that 1,4-disubstituted-1,2,3-triazoles showed promising activity against α-glucosidase (1) [26], and 1,2,3-triazole bearing anti-cancer drugs carboxyamidotriazole (2), combretastatin A4-1,2,3-triazole (3), coronopilin-1,2,3-triazole conjugates (4), naphthalimide-1,2,3-triazole conjugates (5), antitubercular activity (6), and fluconazole analogues (7) is now available in the market (Figures 1 and 2). Over the last few years, the molecular hybridization approach has received considerable attention in discovery of new biological agents which containing two or more bioactive pharmacophores. Based on these things mentioned above, and in continuation of our interest in the synthesis of a novel series of 1,4-disubstituted 1,2,3-triazole derivatives by cupper nanoparticles catalyzed azide-alkyne click chemistry and evaluated for their anticancer (lung cancer, prostate cancer inhibitors) activity. In this paper, we describe results of nanocopper studies carried out by means of the following techniques: scanning electron microscopy (SEM), energy dispersive X-ray analysis (EDX). Studies carried out by SEM and EDX, global techniques, enabled not only a detailed examination of copper powder morphology, but also its chemical composition analysis. Furthermore, molecular docking was also performed to understand the interaction modes of these compounds with the active site of anticancer agents.

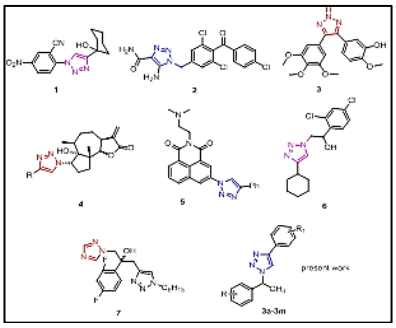
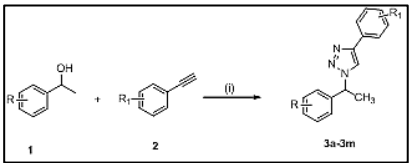


Figure 1: Chemical structures of some inhibitors containing 1,2,3-triazole rings



Scheme 1: Reagents and conditions: (i) $CuFe_2O_4$ nanoparticles, $TMSN_3$/rt, 5 hr, 1,4-dioxane

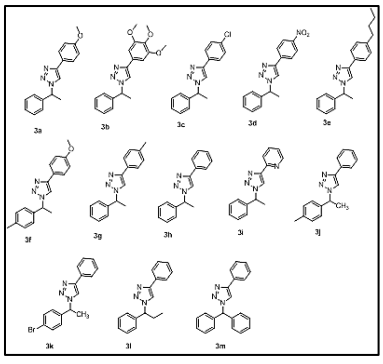


Figure 2: Design strategy for new linked 1,4-disubstituted triazoles (3a-3m)

EXPERIMENTAL SECTION

Materials and Methods

Chemistry

All the chemicals were purchased from S.D Fine Chemicals, Sigma-Aldrich and Pvt. Ltd. India used as received. The other chemicals, solvents were obtained from commercial sources and purified using standard methods. ACME silica gel (100-200 mesh) was used for column chromatography. The IR spectra of these compounds were recorded on a perkin-elmer, spectrum GX FTIR spectrometer. The ^{1}H NMR, ^{13}C NMR spectra were recorded on a varian-300 MHz, bruker-advance 400 MHz spectrometer. Chemical shifts are reported in ppm, using TMS (δ=0) as an internal standard in $CDCl_3$. ESI mass spectra were recorded on a finnigan LCQ advantage max spectrometer.

General Experimental Procedure for One-Pot Synthesis Compounds (3a-3m)

A combine of aromatic alcohol (1.2 mmol), $CuFe_2O_4$ nanoparticles (3.2 mol%) and trimethylsilyl azide (2.6 mmol) in 1,4-dioxane (6 mL) was stirred at 75°C for 4 hr. After completion consumption of the alcohol as indicated by TLC, aromatic alkynes (1.4 mmol) and water (5 mL) was added and continued the reaction. After integration of the reaction, the mixture was waterish and extracted with ethyl acetate (3 × 10 mL). The united organic layers were dried over anhydrous $MgSO_4$, concentrated in vacuo and purified by column chromatography on silica gel to allow the authentic product. The product was well characterized by ^{1}H NMR, ^{13}C NMR and mass spectroscopic analysis.

4-(4-methoxyphenyl)-1-(1-phenylethyl)-1H-1,2,3-triazole (3a)

IR ($\bar{\upsilon}$, cm^{-1}) 3050 (=CH str), 2920 (-CH str), 2890 (-CH str), 1624 (N=N str), 1605, 1575, 1492 (ArC=C str), 1105 (C-O-C str). ^{1}H NMR (300 MHz, $CDCl_3$) δ_{ppm} 7.65 (d, 2H, *J* = 8.7 Hz, ArH), 7.47 (s, 1H, ArH), 7.28-7.35 (m, 5H, ArH), 6.86 (d, 2H, *J* = 8.5 Hz, ArH), 5.80 (q, 1H, *J* = 7.2 Hz, $CHCH_3$), 3.80 (s, 3H, OCH_3), 2.03 (d, 3H, *J* = 7.0 Hz, $CHCH_3$). ^{13}C NMR (75 MHz, $CDCl_3$) δ_{ppm} 159.66, 140.75, 129.12, 128.52, 126.90, 125.50, 118.52, 114.20, 54.32, 60.20, 21.86. ESI- MS (*m/z*): 280.1 $(M+1)^+$.

1-(1-phenylethyl)-4-(3,4,5-trimethoxyphenyl)-1H-1,2,3-triazole (3b)

IR ($\bar{\upsilon}$, cm^{-1}) 3040 (=CH str), 2930 (-CH str), 2885 (-CH str), 1622 (N=N str), 1590, 1523, 1485 (Ar-C=C str), 1120 (C-O-C str). ^{1}H NMR (300 MHz, $CDCl_3$) δ_{ppm} 7.65 (s, 1H, ArH), 7.47 (s, 1H, ArH), 7.25-7.38 (m, 5H, ArH), 6.89 (s, 1H, ArH), 4.85 (q, 1H, *J* = 7.0 Hz, $CHCH_3$), 3.68 (s, 9H, OCH_3), 1.98 (d, 3H, *J* = 7.2 Hz, $CHCH_3$), ^{13}C NMR (75 MHz, $CDCl_3$) δ_{ppm} 158.65, 139.75, 129.15, 128.50, 127.91, 126.52, 118.52, 115.22, 61.22, 53.30, 20.85. ESI-MS (*m/z*): 339.15 $(M+1)^+$.

4-(4-chlorophenyl)-1-(1-phenylethyl)-1H-1,2,3-triazole (3c)

IR ($\bar{\upsilon}$, cm^{-1}) 3052 (=CH str), 2940 (-CH str), 1615 (N=N str), 1600, 1524, 1445 (Ar-C=C str), 824 (C-Cl str). ^{1}H NMR (500 MHz, $CDCl_3$) δ_{ppm} 7.85 (d, 2H, *J* = 8.5 Hz, ArH), 7.65 (d, 2H, *J* = 8.0 Hz, ArH), 7.52 (m, 2H, ArH), 7.47 (s, 1H, ArH), 7.05-7.24 (m, 3H, ArH), 4.20 (q, 1H, *J* = 6.5 Hz, $CHCH_3$), 1.92 (d, 3H, *J* = 7.0 Hz, $CHCH_3$), ^{13}C NMR (100 MHz, $CDCl_3$) δ_{ppm} 148.05, 144.42, 134.32, 130.35, 129.85, 125.90, 117.52, 63.22, 19.55. ESI- MS (m/z): 283.1 $(M+1)^+$.

4-(4-nitrophenyl)-1-(1-phenylethyl)-1H-1,2,3-triazole (3d)

IR ($\bar{\upsilon}$, cm^{-1}) 3065 (=CH str), 2952 (-CH str), 1622 (N=N str), 1608, 1542, 1475 (Ar-C=C str). ^{1}H NMR (300 MHz, $CDCl_3$) δ_{ppm} 8.08 (d, 2H, *J* = 7.5 Hz, ArH), 7.68 (s, 1H, ArH), 7.60 (d, 2H, *J* = 8.0 Hz, ArH), 7.42 (m, 2H, ArH), 7.15-7.29 (m, 3H, ArH), 3.98 (q, 1H, *J* = 7.2 Hz, $CHCH_3$), 1.90 (d, 3H, *J* = 7.0 Hz, $CHCH_3$), ^{13}C NMR (100 MHz, $CDCl_3$) δ_{ppm} 147.90, 146.75, 143.78, 136.85, 130.82, 128.68, 126.28, 124.12, 64.85, 19.65. ESI -MS (m/z): 294.2 $(M+1)^+$.

4-(4-butylphenyl)-1-(1-phenylethyl)-1H-1,2,3-triazole (3e)

IR ($\bar{\upsilon}$, cm^{-1}) 3048 (=CH str), 2928 (-CH str), 2906 (-CH str), 1615 (N=N str), 1605, 1542, 1465 (Ar-C=C str). ^{1}H NMR (500 MHz, $CDCl_3$) δ_{ppm} 7.61 (d, 2H, *J* = 7.8 Hz, ArH), 7.51 (s, 1H, ArH), 7.26-7.36 (m, 3H, ArH), 7.25 (m, 2H, ArH), 7.05 (d, 2H, *J* = 8.2 Hz, ArH), 4.76 (q, 1H, *J* = 7.2 Hz, $CHCH_3$), 2.56 (t, 2H, *J* = 7.9 Hz, CH_2CH_3), 2.01 (d, 3H, *J* = 7.2 Hz, $CHCH_3$), 1.24-1.33 (m, 4H, *J* = 3.6 Hz, CH_2CH_2), 0.84 (t, 3H, *J* = 6.5 Hz, CH_2CH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ_{ppm} 147.85, 142.96, 140.02, 128.99, 128.76, 128.47, 128.06, 126.06, 125.59, 118.00, 60.19, 35.67, 31.42, 30.98, 22.49, 21.27, 13.96. ESI-MS (*m/z*): 305.2 $(M+1)^+$.

4-(4-methoxyphenyl)-1-(1-(p-tolyl)ethyl)-1H-1,2,3-triazole (3f)
IR ($\bar{\upsilon}$, cm^{-1}) 3048 (=CH str), 2935 (-CH str), 2896 (-CH str), 1620 (N=N str), 1618, 1589, 1458 (Ar-C=C str), 1089 (C-O-C str). ^{1}H NMR (300 MHz, $CDCl_3$) δ_{ppm} 7.62 (d, 2H, *J* = 8.5 Hz, ArH), 7.45 (s, 1H, ArH), 7.30 (d, 2H, *J* = 7.8 Hz, ArH), 7.18-7.25 (m, 5H, ArH), 4.80 (q, 1H, *J* = 7.2 Hz, $CHCH_3$), 3.81 (s, 3H, OCH_3), 2.16 (d, 3H, *J* = 7.2 Hz, $CHCH_3$). ^{13}C NMR (100 MHz, $CDCl_3$) δ_{ppm} 160.60, 148.75, 144.52, 130.36, 128.86, 125.91, 122.78, 116.58, 114.81, 64.23, 55.84, 19.62. ESI- MS (*m/z*): 280.10 $(M+1)^+$.

1-(1-phenylethyl)-4-(p-tolyl)-1H-1,2,3-triazole (3g)
IR ($\bar{\upsilon}$, cm^{-1}) 3051 (=CH str), 2964 (-CH str), 2884 (-CH str), 1620 (N=N str), 1618, 1589, 1458 (Ar-C=C str). ^{1}H NMR (300 MHz, $CDCl_3$) δ_{ppm} 7.61 (d, 2H, *J* = 7.6 Hz, ArH), 7.50 (s, 1H, ArH), 7.25-7.36 (m, 5H, ArH), 7.11 (d, 2H, *J* = 7.5 Hz), 5.75 - 5.85 (q, 1H, *J* = 7.2 Hz, $CHCH_3$), 2.26 (s, 3H, $ArCH_3$), 2.01 (d, 3H, *J* = 7.2 Hz, $CHCH_3$). ^{13}C NMR (75 MHz, $CDCl_3$) δ_{ppm} 147.85, 140.02, 137.87, 129.43, 129.03, 128.53, 127.92, 126.56, 125.59, 118.0, 60.22, 21.30, 21.24. ESI MS (*m/z*): 264.01 $(M+1)^+$.

4-phenyl-1-(1-phenylethyl)-1H-1,2,3-triazole (3h)
IR ($\bar{\upsilon}$, cm^{-1}) 3046 (=CH str), 2943 (CH str), 2785 (CH str), 1608 (N=N str), 1598, 1512, 1478 (Ar-C=C str). ^{1}H NMR (500 MHz, $CDCl_3$): δ_{ppm} 7.74 (d, 2H, *J* = 8.0 Hz, ArH), 7.54 (s, 1H, ArH), 7.22-7.38 (m, 5H, ArH), 7.08 (m, 3H, ArH), 5.80 (q, 1H, *J* = 7.5 Hz, $CHCH_3$), 2.02 (d, 3H, *J* = 7.6 Hz, $CHCH_3$), ^{13}C NMR (75 MHz, $CDCl_3$): δ_{ppm} 147.45, 140.89, 130.68, 128.90, 128.72, 128.55, 128.10, 126.52, 125.61, 118.22, 61.26, 20.89. ESI-MS (*m/z*): 250 $(M+H)^+$.

2-(1-(1-phenylethyl)-1H-1,2,3-triazol-4-yl)pyridine (3i)
IR ($\bar{\upsilon}$, cm^{-1}) 3052 (=CH str), 2976 (CH str), 1625 (C=N str), 1616 (C=N str), 1602, 1548, 1496 (Ar-C=C str). ^{1}H NMR (300 MHz, $CDCl_3$) δ_{ppm} 8.93 (s, 1H, ArH), 8.76 (d, 1H, *J* = 4.5 Hz, ArH), 8.10 (d, 1H, *J* = 8.3 Hz), 7.57 (s, 1H, ArH), 7.45 (d, 1H, *J* = 8.5 Hz), 7.29-7.35 (m, 5H, ArH), 5.76 (q, 1H, *J* = 7.5 Hz, $CHCH_3$), 2.01 (d, 3H, *J* = 7.6 Hz, $CHCH_3$), ^{13}C NMR (75 MHz, $CDCl_3$) δ_{ppm} 149.25, 147.93, 135.41, 133.52, 128.01, 127.52, 125.53, 124.24, 118.42, 60.25, 21.20. ESI MS (*m/z*): 251.2 $(M+1)^+$.

4-phenyl-1-(1-(p-tolyl)ethyl)-1H-1,2,3-triazole (3j)
IR ($\bar{\upsilon}$, cm^{-1}) 3045 (=CH str), 2976 (CH str), 2942 (CH str), 1632 (C=N str), 1598, 1523, 1485 (Ar-C=C str). ^{1}H NMR (400 MHz, DMSO-d_6) δ_{ppm} 7.60 (d, 2H, *J* = 8.0 Hz, ArH), 7.52 (s, 1H, ArH), 7.27-7.38 (m, 5H, ArH), 7.12 (d, 2H, *J* = 8.1 Hz, ArH), 5.76 (q, 1H, *J* = 7.0 Hz, $CHCH_3$), 2.35 (s, 3H, $ArCH_3$), 2.03 (d, 3H, *J* = 7.6 Hz, $CHCH_3$). ^{13}C NMR (100 MHz, $CDCl_3$) δ_{ppm} 147.77, 139.91, 137.89, 129.43, 128.96, 128.52, 127.93, 126.59, 125.57, 117.94, 60.19, 21.34, ESI MS (*m/z*): 264 $(M+1)^+$.

1-(1-(4-bromophenyl)ethyl)-4-phenyl-1H-1,2,3-triazole (3k)
IR ($\bar{\upsilon}$, cm^{-1}) 3024 (=CH str), 2962 (CH str), 1642 (C=N str), 1608, 1576, 1453 (Ar-C=C str), 735 (C-Br str). ^{1}H NMR (300 MHz, DMSO-d_6) δ_{ppm} 7.72 (d, 2H, *J* = 8.0, ArH), 7.56 (s, 1H, ArH), 7.48 (d, 2H, *J* = 8.2 Hz, ArH), 7.28-7.39 (m, 3H, ArH), 7.19 (d, 2H, *J* = 8.4 Hz, ArH), 5.72 (q, 1H, *J* = 7.5 Hz, $CHCH_3$), 2.01 (d, 3H, *J* = 7.5 Hz, $CHCH_3$). ^{13}C NMR (75 MHz, $CDCl_3$) δ_{ppm} 147.75, 139.26, 132.26, 128.77, 128.65, 128.18, 127.98, 125.71, 120.19, 118.21, 59.70, 21.24. ESI MS (*m/z*): 329 $(M+2)^+$.

4-phenyl-1-(1-phenylpropyl)-1H-1,2,3-triazole (3l)
IR ($\bar{\upsilon}$, cm^{-1}) 3033 (=CH str), 2945 (CH str), 2876 (CH str), 1632 (C=N str), 1602, 1546, 1494, (Ar-C=C str). ^{1}H NMR (300 MHz, $CDCl_3$) δ_{ppm} 7.75 (d, 2H, *J* = 7.5 Hz), 7.59 (s, 1H, ArH), 7.40-7.46 (m, 3H, ArH), 7.30-7.37 (m, 5H, ArH), 5.42 (t, 1H, *J* = 6.7 Hz), 2.28-2.63 (m, 2H, CH_2CH_3), 0.98 (t, 3H, *J* = 7.6 Hz, CH_2CH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ_{ppm} 147.64, 138.83, 130.68, 128.94, 128.70, 128.50, 127.02, 125.59, 118.52, 66.85, 28.36, 11.0. ESI MS (*m/z*): 264.1 $(M+1)^+$.

1-benzhydryl-4-phenyl-1H-1,2,3-triazole (3m)
IR ($\bar{\upsilon}$, cm^{-1}) 3078 (Ar=CH str), 2984 (CH str), 1636 (C=N str), 1615, 1576, 1498 (Ar-C=C str). ^{1}H NMR (300 MHz, $CDCl_3$) δ_{ppm} 7.95 (s, 1H, ArH), 7.80 (d, 2H, ArH), 7.28-7.50 (m, 12H, ArH), 6.73 (s, 1H, CH). ^{13}C NMR (75 MHz, $CDCl_3$) δ_{ppm} 147.49, 138.09, 130.51, 128.71, 128.53, 125.66, 119.57, 77.42, 77.00, 76.57, 68.09. ESI MS (*m/z*): 312.1 $(M+1)^+$.

In vitro Anticancer Activity

In vitro anti-cancer activity of the test compounds was tested using MTT colorimetric assay [27,28] as per ATCC protocol. Cell lines that were used for testing *in vitro* cytotoxicity included PC-3 derived from human prostate adenocarcinoma cells (ATCC No. CRL-1435), A549 derived from human lung carcinoma cells (ATCC No. CCL-185) which were procured from American Type Culture Collection, Manassas, VA, USA. PC-3 supplemented with 10% new born calf serum (NBCS), 100 IU/mL penicillin, 100 mg/ml streptomycin and 2 mM-glutamine. Cell lines were maintained at 37°C in a humidified 5% CO_2 incubator (Thermo scientific). Lung cancer cell line A549 was maintained in DMEM medium supplemented with 10% new born calf serum, along with 1% non-essential amino acids, 0.2% sodium bicarbonate, 1% sodium pyruvate and 1% antibiotic mixture (10,000 U penicillin and 10 mg streptomycin per mL). Cell lines were processed by initial trypsinization to detach the adhered cells and followed by centrifugation to get cell pellet. Fresh media was added to the pellet to make a cell count using haemocytometer and plate 100 µL of media with cells ranging from 5,000-6,000 per well in a 96-well plate. The plate was incubated overnight in CO_2 incubator for the cells to adhere and regain its shape. After 24 hr cells were treated with the test compounds at 25 µM diluted using the media to deduce the percentage inhibition on cancer cells and human normal cells. The cells were incubated for 48 hr to assay the effect of the test compounds on different cell lines. Zero hour reading was noted down with untreated cells and also control with 1% DMSO to subtract further from the 48 hr reading. After 48 hr incubation, cells were treated by MTT (4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) dissolved in PBS (5 mg/ml) and incubated for 3-4 hr at 37°C. The formazan crystals thus formed were dissolved in 100 µL of DMSO and the viability was measured at 540 nm on a multimode reader (spectra max) (Figure 3and Table 1).

Table 1: *In vitro* anticancer activity of the target compounds 3a-3m[a]

Entry	Cytotoxicity % of inhibition at 25 µM	
	PC-3	A549
3a	8.08	16.18
3b	23.28	52.68
3c	-12.27	46.27
3d	38.21	-5.23
3e	18.28	25.24
3f	11.64	15.46
3g	12.48	64.12
3h	68.45	23.48
3i	55.12	58.12
3j	45.29	26.13
3k	-6.78	18.25
3l	46.28	52.19
3m	22.46	67.13
b	c	d

[a] Cytotoxic activity against two human cancer cell lines (prostate, lung); [b] Doxorubicin; [c] Doxorubicin IC_{50} is 2.0 µM.; [d] Doxorubicin IC_{50} is 3.2 µM.

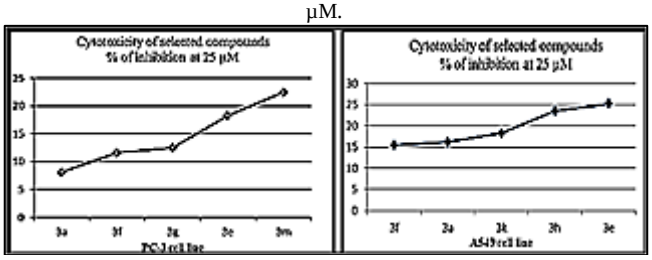


Figure 3: Cytotoxicity of target compounds

Molecular Docking Studies

The ligands were sketched in sybyl 6.7 and saved it in mol2 format [29]. All the sketched molecules were converted to energy minimized 3D structures by using gasteiger-huckel charges [30] for in silico protein–ligand docking using autodock tools. Each molecule was docked separately. Initially the molecule was loaded, torsions were set and saved it in PDBQT format. All the hetero atoms were removed from the 3IVX. PDB (crystal structure of pantothenate synthetase in complex with 2-(2-(benzofuran-2-yl-sulfonylcarbamoyl)-5-methoxy-1H-indol-1-yl)acetic acid) [31] to make complex receptor free of any ligand before docking [32]. The PDB was also saved in PDBQT format. All calculations for protein-ligand flexible docking were performed using the Lamarckian Genetic Algorithm (LGA)

method. A grid box with the dimensions of X: 15.137, Y: 17.850 and Z: -3.573 Å, with a default grid spacing of 0.375 Å was used. The best conformation was chosen with the lowest docked energy [33-36] after the docking search was completed. The interactions of 3IVX protein and ligand conformations, including hydrogen bonds and the bond lengths were analyzed. Molecular docking study was performed by using AUTODOCK 4.2 which was a suite of automated docking tools and was used to predict the affinity, activity, binding orientation of ligand with the target protein and to analyze best conformations [37-39], the protein with all the 12 compounds were loaded individually into ADT and evaluate ten finest conformations. In the present investigation we focused mainly on the binding energy, hydrogen bonds, and distance between the protein and ligand (Table 2 and Figure 4).

Table 2: Molecular docking studies of title compounds (3a-3m)

S.No	Protein id	Coordinates	Interacting amino acids	Score In (Kcal/Mol)	KI
3a	2ZCS	X:56.026;Y:5.999;Z:60.238	ARG45	-5.24	143.58 µM
3b	2ZCS	X:56.026;Y:5.999;Z:60.238	LYS20, ARG171	-6.38	20.64 µM
3c	2ZCS	X:56.026;Y:5.999;Z:60.238	NO INTERACTIONS	-5.5	93.3 µM
3d	2ZCS	X:56.026;Y:5.999;Z:60.238	ARG171, LYS273	-7.53	3.03 µM
3e	2ZCS	X:56.026;Y:5.999;Z:60.238	LYS273	-6.18	29.65 µM
3f	2ZCS	X:56.026;Y:5.999;Z:60.238	TYR248, LYS273	-6.96	7.85 µM
3g	2ZCS	X:56.026;Y:5.999;Z:60.238	LYS20	-6.57	15.24 µM
3h	2ZCS	X:56.026;Y:5.999;Z:60.238	LYS273	-6.57	15.3 µM
3i	2ZCS	X:56.026;Y:5.999;Z:60.238	Lys160	-4.28	43.12 µM
3j	2ZCS	X:56.026;Y:5.999;Z:60.238	Lys160, Val187	-6.78	75.26 µM
3k	2ZCS	X:56.026;Y:5.999;Z:60.238	Val287, Met195	-5.45	7.85 µM
3l	2ZCS	X:56.026;Y:5.999;Z:60.238	NO INTERACTIONS	-4.23	18.25 µM
3m	2ZCS	X:56.026;Y:5.999;Z:60.238	His44	7.20	14.48 µM

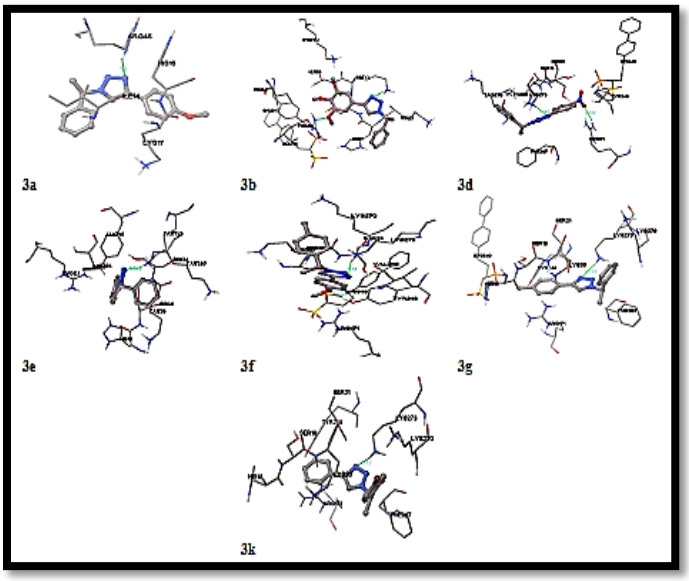


Figure 4: Docked conformations of active site target compounds

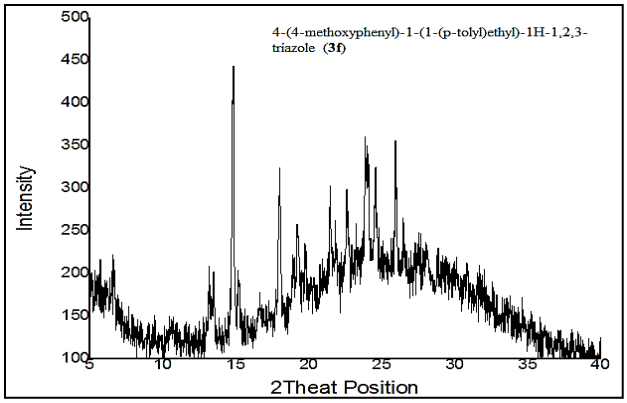


Figure 5: XRD study of compound 3f

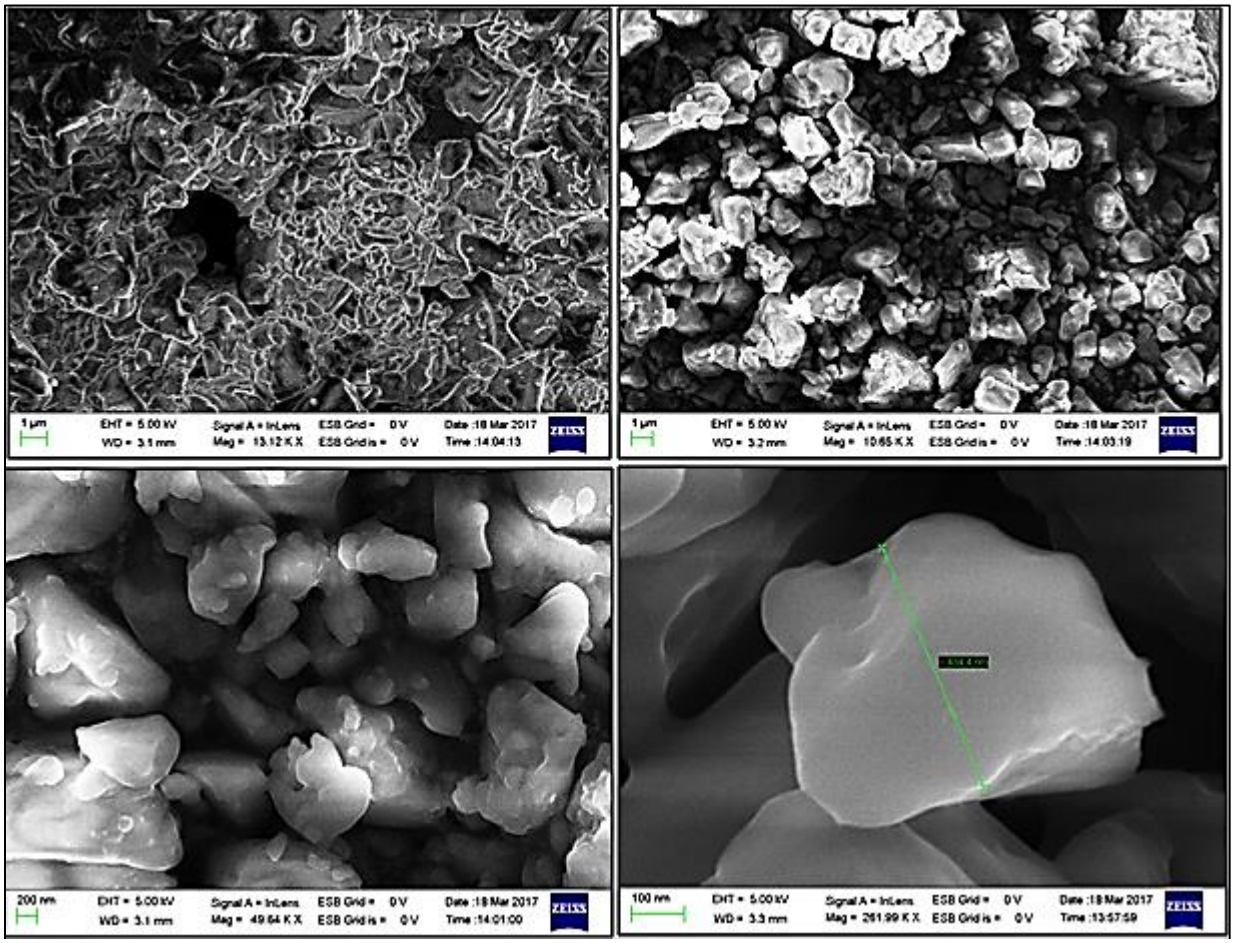


Figure 6: SEM pictures of compound (3f) powder granulate deposited on a carbon strip

XRD Studies

Figures 5 and 6 shows typical results of the studies of compound (3f) powder deposited on a carbon strip by means of SEM. Part (a) of the figure represents the view of the sample 475 at 300 × magnification which stands for examining the area of 800 × 800 μm^2 surface. Around the examined area, one can notice the presence of objects of sizes within 200 μm to 300 μm. Those objects consist of tiny particles, as can be proved by SEM studies results gathered on one of the particles.

RESULTS AND DISCUSSION

Chemistry

Synthesis of 1,4-disubstituted triazoles (3a-3m) is outlined in Scheme 1. To a solution of trimethylsilyl azide (1.4 g, 2.6 mmol), $CuFe_2O_4$ nanoparticles (0.6 g, 3.2 mol%), 1-phenylethanol 1 (1.0 g, 1.2 mmol) and aromatic alkynes 2a-2m (1.2 g, 1.4 mmol) dissolved in 5 mL H_2O. After completion of addition, the reaction mixture was reflux 60 min. Then the reaction mixture was poured into ice water and extracted with ethyl acetate. Following this, dried the organic layer over magnesium sulphate and evaporated to yield 4-(4-methoxyphenyl)-1-(1-phenylethyl)-1H-1,2,3-triazole (3a) shown in (Scheme 1 and Figure 1). Our subject induce with the conversion of an aromatic alcohol with trimethylsilyl azide into a alkyl azide center which, without isolation, was reacted with phenyl acetylene to provide the 1,3-dipolar cyclo-addition product 1,4-disubstituted 1,2,3-triazoles in an one-pot exertion using nano $CuFe_2O_4$ as a catalyst. H_2O plays an important role in this reaction for check of excess trimethylsilyl azide, accumulation of copper acetylide from nano $CuFe_2O_4$ and acetylene without any amine base. Chemical structures of the proposed compounds were in full agreement as supported by ^{1}H NMR, ^{13}C NMR, IR and HRMS. Structures of the title compounds were elucidated by spectral data. For instance, the ^{1}H NMR timescale of 3e showed two triplets at δ 0.84 and 2.56 ppm due to CH_3 proton of the CH_2CH_3. The total ten aromatic hydrogen resonance signals appeared as one singlet, two doublets and two multiplets range δ 7.05 to 7.61 ppm. The single peak of CH proton as quartet of CH–CH_3– was observed at 4.76 ppm and δ 2.01 for doublet for three hydrogen's in $CHCH_3$ at *J* value 7.2 Hz respectively. The total number of protons matched perfectly with its structure. Moreover, in their ^{13}C NMR spectra, the number of signals equals the number of different carbons in the molecule. The resonance signals in ^{13}C NMR spectra at *δ* 147.85, (C-1) ppm and 142.96 (C-5) ppm validated the construction of 1,2,3-triazole core and *δ* at 128.99, 128.76, 128.47, 128.06, 126.06, 125.59, 118.00 ppm for aromatic carbons. The IR spectrum of 3e displayed characteristic absorption bands at υ_{max} 3048 cm^{-1}, 2928 cm^{-1} aromatic =CH, aliphatic CH stretching's, 1615 cm^{-1} for -N=N- and 1605, 1542, 1465 cm^{-1} (Ar-C=C-) evidenced the presence of cyclic 1,4-disubstituted triazole frame work. Further m/z 305.2 $(M+H)^+$ molecular ion peak in the mass spectrum confirmed their molecular weight.

Anticancer Activity

In vitro cytotoxicity of the title compounds was assessed by employing MTT colorimetric assay as per ATCC protocol[1] at 25 µM against a panel of cancer cell lines namely prostate (PC-3) and lung (A549). Doxorubicin was used as standard and the results are summarized in Table 1. From the close inspection of the data, it is obvious that the compounds 3a, 3f, 3g exhibited higher anti-cancer activity against both the cell lines. All the compounds demonstrated better activity than the parent scaffold, except 3d for lung cancer and 3c, 3k for prostate cells. Present study revealed that among the tested cell lines, PC-3 cells are more sensitive to all the tested compounds than A549 cells. Among the tested compound 3a displayed promising activity and products 3f, 3g, 3e demonstrated moderate to good activity against A549 cells. Homologation of 3a (4-ome) to 3f (me, 4-ome) in turn to 3g (4-methyl) and then to 3e (4-n-butyl) decreased the activity profile for prostate cells and similar pattern was not observed for lung cancer cell line indicating the selectivity of these compounds to a particular cell line and for the homologues series 3e (4-n-butyl) and 3f (me, 4-ome) increment of activity was observed for both the cells. For the series 3c, 3k introduction of halogens, nitro (Cl, Br, NO_2) led to the decrement of activity for both the cell lines tested. For the analogues 3a, 3f, 3g and 3k the activity trend followed the pattern of the +I effect of the substituent higher than the –I Inductive effect the activity for lung, prostate cancer cell line. It is well documented that cytotoxic drugs doxorubicin and mitoxantrone show anticancer activity against A549, PC-3 cells by the generation of reactive oxygen species, thus causing apoptosis through the expression of caspase-3. Hence, it is believed that the synthesized compounds may act as effective anticancer agents by the similar mechanism discussed above.

Molecular Docking Studies

The molecular docking studies have been carried out to evaluate the anti-cancer activity of 1,4-disubstituted-1,2,3-triazoles (3a-3m) using autodock 4.2 software and all the hetero atoms were removed from the 3IVX PDB (crystal structure of pantothenate synthetase in complex with 2-(2-(benzofuran-2-yl-sulfonylcarbamoyl)-5-methoxy-1H-indol-1-yl)acetic acid). The compound 3d shows highest binding energy with two amino acid interactions ARG171, LYS273 with binding energy ΔG = -7.53 and dissociation constant in 3.03 µM. In Figure 4 illustrates some of the synthesized compounds docked poses and compound 3f, 3m exhibits binding energy shows -6.96, 7.20 and KI 7.85, 14.48 µM values and interactions amino acids with TYR248, LYS273, His44 respectively. Further the compound 3c, 3l has no interacting in any amino acids. Almost all the target compounds show good binding energy, Π-Π interactions, vanderwall interactions etc., (Figure 4). The hydrogen bonding distance of all the molecules with

proteins is less than 2.0 Å. The docking score and interactions of final compounds are against the 3IVX protein illustrated in Table 2and Figure 4 and the order of the docking score is 3d > 3f > 3m > 3g > 3h > 3b > 3a etc.

CONCLUSION

In conclusion, an efficient method for the synthesis of 1,4-disubstituted 1,2,3-triazoles from a variety of benzylic alcohol/allylic alcohol, trimethylsilyl azide and terminal alkynes has been developed via a simple one-pot, two-step procedure involving the azidation of alcohols, followed by 1,3-dipolar cycloaddition with terminal alkynes using nano CuO as the catalyst. A series of novel triazoles were evaluated for their *in vitro* anticancer activity. Having +I groups like 4-ome, 4-methyl etc., containing compounds 3a, 3g, 3e were found to be most active among the screened compounds and has been identified as a lead compound, further studies under way to optimize the best pharmacophore. Additionally, the compound 3d has showed best score -7.53 in docking protocol respectively. The most active compound 3f, 3m in screening has exhibited good docking score respectively. The hydrogen bonding distance of all the molecules with proteins is less than 2.0 Å. In future research, these 1,2,3-triazole derivatives will be synthesized and screen for their *in vitro* anti-cancer activity [(PDB code 3IVX, software (Autodock 4.2)].

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