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$ZnBr_2$-SiO_2 catalyzed green synthesis of tetrazoles: Molecular docking and antioxidant activity studies

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ABSTRACT

A series of 5-substituted and 1,5-disubstituted tetrazoles were synthesized in high yields from various biologically active substituted nitriles with sodium azide under heterogeneous catalysed ($ZnBr_2$-SiO_2) [2+3] cycloaddition conditions. This reaction gave an excellent yield in the presence of catalytic amount of 0.2 g of $ZnBr_2$-SiO_2, glycerol solvent system under microwave irradiation conditions. All the prepared compounds were characterized by elemental analysis ^{1}H NMR, ^{13}C NMR, FT-IR, and mass spectral data. The newly synthesized compounds were investigated for their respective molecular target using molecular docking studies. The results reveal that compounds 5a, 5c, 5e and 3e have conferred with multi target property. The compounds 5a, 5c and 5e have shown the highest binding affinities of -10.1, -9.7 and -10.6 with reverse transcriptase, -8.5, -8.2 and -8.9 with Aurora B, respectively. The compounds 5a, 5e and 3e have shown -8.9, -8.5 and 8.4 with Aromatase, respectively. In addition, the antioxidant activity data reveals that all the compounds showed good antioxidant activity, particularly the compounds 3d, 5d, and 5e exhibited promising radical scavenging activity.

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1. Introduction

Tetrazole moiety is a 5-membered cyclic ring containing four nitrogen atoms and one carbon atom. The structure of the tetrazole ring may be considered unusual. The nitrogen content in an unsubstituted tetrazole (CN_4H_2) is 80% of the total weight of the molecule, the largest percentage among the stable unsubstituted heterocyclic systems. Despite the extremely high percentage of nitrogen, the unsubstituted tetrazole and its derivatives are relatively stable on heating or under microwave irradiation, and also in the presence of various chemical reagents (oxidants, acids, bases, alkylating agents, dienophiles, *etc.*) [1]. Tetrazoles have attracted considerable interest in recent years due to their utility in various fields [2]. The tetrazole moiety is resistant to metabolic degradation, chemical oxidants and possesses greater lipophilicity. There is a close similarity between the acidic character of the tetrazole group and carboxylic acid group. This character inspires number of researchers to synthesize substituted tetrazoles as potential medicinal agents, materials as explosives, information recording systems, ligands in coordination chemistry and also as precursors to a variety of nitrogen-containing compounds. Hence, synthetic methodologies leading to the replacement of -CO_2H groups by -CN_4H groups in biologically active molecules are of major relevance [3]. Tetrazole forms a fundamental core of many drug candidates which are used for the treatment of many diseases. For example tetrazole substituted proline such as LY300020, known for its relatively potent, highly selective systemically-active AMPA receptor agonist (Figure 1) [4]. Some tetrazole compounds are extensively used in models for anxiety, mediated by its unspecific interaction with a number of receptors in the CNS [5,6], and mannose mimitics have been reported to be inhibitors of mannosidase [7,8].

In addition, tetrazole derivatives have been investigated in diverse areas such as anti-arrhythmic [9], anti-diabetic [10], antifungal [11], anti-allergic [12] and neurodegenerative diseases [13].

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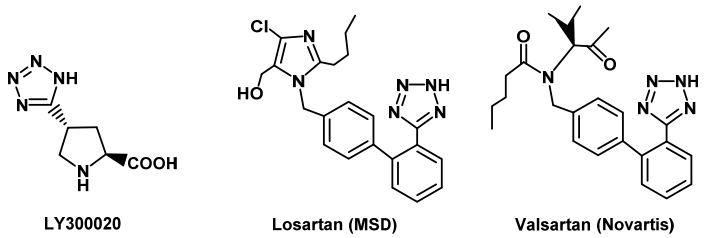


Figure 1. Some biologically active tetrazole derivatives.

Angiotensin II (AII) is the octapeptide respon-sible for the peripheral effects of the rennin-angiotensin system [14] which include the regulation of blood pressure and volume homeostasis. The first nonpeptide angiotensin receptor antagonist released in the market namely Losartan followed by Valsartan [15]. Tetrazoles also show significant applications in agriculture, herbicides, fungicides [16], and also in photo imaging [17]. Two nitrogen molecules and large amount of energy has liberated by the decomposition of tetrazole due to its high enthalpy formation. Therefore, derivatives of tetrazoles are explored as explosives, propellant components and gas generators [18]. In addition, a variety of tetrazole-based moieties has excellent coordination properties and is able to form stable complexes with various metal ions [19,20]. This ability is successfully used in analytical chemistry for the removal of heavy metal ions from liquids and in chemical systems [21].

The growth of tetrazole chemistry has been gained promising significance due to the wide range of applications of tetrazole derivatives. Hence, intensive interest has been focused recently not only in the incorporation of this entity in bioactive molecules but also in the development of new methodologies for the production of these derivatives in high yields. A number of methods have been reported for the preparation of tetrazoles, among; one of the major convenient synthetic routes to tetrazole formation is the [3+2] cycloaddition of azide to corresponding nitriles [22]. Many synthetic methods were introduced for this transformation, requires amine salts [23], strong Lewis acid [24,25] and toxic metals [25]. The generated hydrazoic acidin this transformation is highly toxic and explosive. Now a days, Sharpless and his co-workers synthesized an innovative and safe procedure for the preparation of tetrazoles using stoichiometric proportions of Zn (II) salts in water [26,27].

However, many of the established approaches for this [3+2] cycloaddition reaction are still limited in their use by the lack of generality, the harsh reaction conditions such as high temperatures and long reaction times, or the multi-step procedures required. Recent days, the main goals of *Green Chemistry* are for the improvement of selectivity process, to increase the use of starting materials, and to replace hazardous reagents with eco-friendly ones. Organic reactions with non-toxic organic solvents have attracted a great interest in both academic and industrial applications due to their beneficial effects on rates and selectivity of important organic transformations [27]. Further, heterogeneous organic reactions have proven to be useful to the chemists in the laboratory and industry. The activity and selectivity of a reagent dispersed on the surface of a support improves the effective surface area of the reagent significantly and expected to perform more effectively than the individual reagents. Hence, solid supported heterogeneous acid catalysts are unique and they become more popular over the last two decades. The recyclability of a few of these solid supports renders these processes into truly eco-friendly green protocols. Recently, several organic transformations are effectively catalysed by silica gel supported Lewis acid catalysts in organic synthesis. Also, in recent years, microwave irradiation has been playing a prominent role in promoting a wide variety of reactions in organic synthesis [28,29] and provides a number of advantages over the conventional heating techniques such as improved reaction yield; shorten the reaction time and easy work-up procedure.

2. Experimental

2.1. Chemistry

By considering the vast applications of tetrazole derivatives in various fields, the researchers have been focused considerable interest for the synthesis of new kind of heterocyclic molecules having tetrazole entity as a core and development of new methodologies to synthesize them in simple fashion compound **3a-e** and **5a-e** (Scheme 1 and 2).

After, adopting the developed procedure, we synthesized 1,5-disubstituted tetrazole derivatives of zidovudine **5a-e** (Scheme 2) in good yields by reacting azide of zidovudine with substituted nitriles.

2.2. Instrumentation

In part of our research programme, the authors have tried to develop an alternative heterogeneous catalysed ($ZnBr_2$-SiO_2) green procedure for the synthesis of tetrazoles **3a-e** in microwave irradiation and conventional conditions using a green solvent, glycerol which is an inexpensive, easy to handle, thermally stable, non-toxic and performs many useful organic transformations under mild reaction conditions.

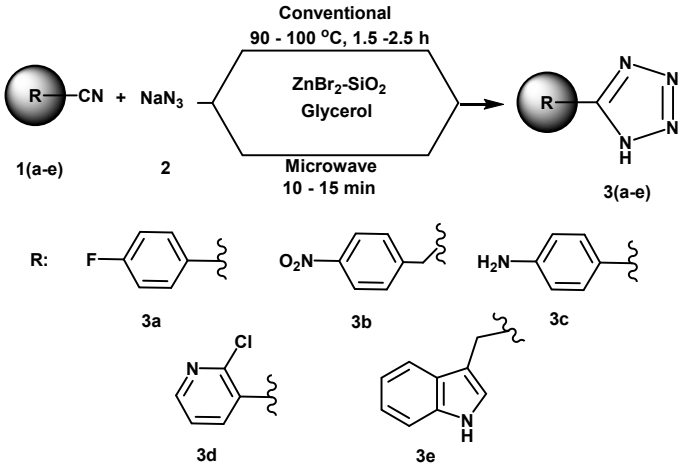
All chemicals used for synthesis were commercially available, AR grade and were used as such received from Sigma-Aldrich, Merck and Sd. Fine Chem. Analytical thin-layer chromatography (TLC) was carried out on pre-coated plates and spots were visualized with UV light. IR spectra were recorded on Bruker Alpha FT-IR spectrophotometer. $^1H/^{13}C$ NMR spectra were recorded on a Bruker 400 MHz NMR spectrometer operating at 400 MHz for ^1H NMR and 100 MHz for ^{13}C NMR in DMSO-d_6. TMS was used as an internal standard for ^1H and ^{13}C NMR spectra. Mass spectra were recorded on LC-MS Shimadzu, Japan (Positive mode). Elemental analyses were performed on a ThermoFinnigan Instrument.

2.3. Synthesis

2.4. General procedure for the synthesis of tetrazole derivatives

2.4.1. Conventional method

4-Fluorobenzonitrile (**1a**) (1.0 mmol), sodium azide **2** (1.5 mmol) and $ZnBr_2$-SiO_2 (0.2 g) were taken into a 50 mL round-bottomed flask containing 5 mL of glycerol. The reaction mass was stirred for 1.5 h at 110 °C and examined the progress of reaction by TLC.



Scheme 1

After completion of the reaction, the reaction mass was treated with 10 mL of HCl (0.1 N) and then extracted with ethyl acetate (3 × 15 mL). The combined organic layer was washed with water and dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to obtain the crude product, 5-(4-fluorophenyl)-1*H*-tetrazole, **3a**. The crude product was purified by column chromatography using 40% ethyl acetate:*n*-hexane as an eluent. The above procedure was followed for the synthesis of rest of the other compounds.

2.4.2. Microwave irradiation method

4-Fluorobenzonitrile (**1a**) (1.0 mmol), sodium azide (**2**) (1.5 mmol) and $ZnBr_2$-SiO_2 (0.2 g) were taken into a 50 mL round-bottomed flask containing 5 mL of glycerol. The reaction mass was irradiated under microwave radiations using catalyst systems (CATA-4R-Scientific Microwave Oven) instrument, at 465 Watts and examined the progress of reaction by TLC (Ethyl acetate:hexane, 1:3, *v*:*v*). After completion of the reaction, the reaction mass was treated with 10 mL of HCl (0.1 N) and then extracted with ethyl acetate (3 × 15 mL). The combined organic layer was washed with water and dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to obtain the crude product, 5-(4-fluoro phenyl)-1*H*-tetrazole (**3a**)**.** It was purified by column chromatography using 40% ethyl acetate:*n*-hexane as an eluent. We followed the same procedure for the preparation of remaining compounds.

5-(4-Fluorophenyl)-1H-tetrazole (**3a**): Color: Yellowish white. Yield: 89%. M.p.: 77-79 °C. FT-IR (KBr, ν, cm^{-1}): 3213 (N-H, str), 2994 (C-H, str), 1647 (C=N, str), 1476 (N=N, str), 1094 (C-F, str). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 8.03 (d, 2H, *J* = 7.6 Hz, Ar-H), 7.16 (d, 2H, *J* = 7.6 Hz, Ar-H), 3.53 (br s, 1H, NH, overlap with solvent). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 162.8, 156.2 ($C_{tetrazole}$), 130.2, 128.5, 118.2. LC-MS (Positive, *m/z* (%)):165 $[M+H]^+$ (100). Anal. calcd. for $C_7H_5FN_4$: C, 51.22; H, 3.07; N, 34.13. Found: C, 51.16; H, 3.05; N, 34.07%.

5-(4-Nitrobenzyl)-1H-tetrazole (**3b**): Color: Yellow. Yield: 90%. M.p.: 79-81 °C. FT-IR (KBr, ν, cm^{-1}): 3201 (N-H, str), 2928 (C-H, str), 1602 (C=N, str), 1510 (-NO_2, Assym. str), 1433 (N=N, str), 1334 (-NO_2, str). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 8.24 (d, *J* = 7.2 Hz, 2H, Ar-H), 7.49 (d, 2H, *J* = 7.2 Hz, Ar-H), 3.65 (br s, 1H, NH, overlap with solvent), 3.92 (s, 2H, CH_2). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 159.7 ($C_{tetrazole}$), 147.9, 141.4, 133.5, 124.9, 29.6. LC-MS (Positive, *m/z* (%)): 206 $[M+H]^+$ (100). Anal. calcd. for $C_8H_7N_5O_2$: C, 46.83; H, 3.44; N, 34.13. Found: C, 46.79; H, 3.42; N, 34.07%.

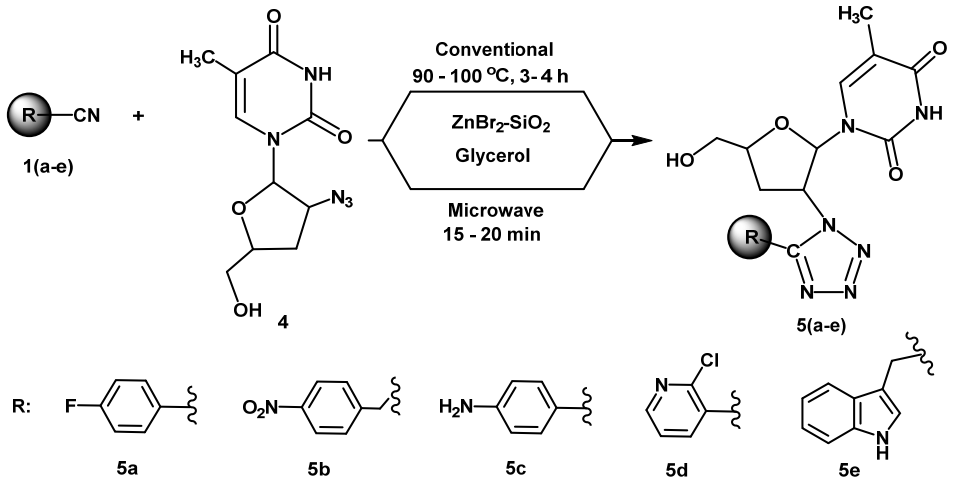
4-(1H-Tetrazol-5-yl)aniline (**3c**): Color: Light brown. Yield: 83%. M.p.: 80-82 °C. FT-IR (KBr, ν, cm^{-1}): 3354 (N-H, str), 3239 (N-H, str), 2958 (C-H, str), 1605 (C=N, str), 1452 (N=N, str). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 7.83 (d, 2H, *J* = 7.6 Hz, Ar-H), 7.08 (d, 2H, *J* = 7.2 Hz, Ar-H), 4.99 (s, 2H, NH_2), 3.66 (br s, 1H, NH, overlap with solvent). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 155.9 ($C_{tetrazole}$), 147.2, 130.5, 121.9, 118.1. LC LC-MS (Positive, *m/z* (%)): 162 $[M+H]^+$ (100). Anal. calcd. for $C_7H_7N_5$: C, 52.17; H, 4.38; N, 43.45. Found: C, 52.12; H, 4.34; N, 43.39%.

2-Chloro-3-(1H-tetrazol-5-yl)pyridine (**3d**): Color: Light yellow. Yield: 88%. M.p.: 79-81 °C. FT-IR (KBr, ν, cm^{-1}): 3367 (N-H, str), 3066 (C-H, str), 1618 (C=N, str), 1483 (N=N, str), 735 (C-Cl, str). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 8.54 (d, *J* = 6.4 Hz, 1H, Ar-H), 8.14 (d, *J* = 8.0 Hz, 1H, Ar-H), 7.99-8.01 (m, 1H, Ar-H), 3.65 (br s, 1H, NH, overlap with solvent). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 164.1 ($C_{tetrazole}$), 148.0, 143.4, 136.1, 134.7, 123.5. LC-MS (Positive, *m/z* (%)): 184 $[M+H+2]^+$ (32.7), 182 $[M+H]^+$ (100).

3-((1H-tetrazol-5-yl)methyl)-1H-indole (**3e**): Color: Light orange. Yield: 86%. M.p.: 85-87 °C. FT-IR (KBr, ν, cm^{-1}): 3390, 3253 (N-H, str), 3053 (C-H, str), 1621 (C=N, str), 1453 (N=N, str). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 10.63 (s, 1H, NH, Indole), 7.44 (d, *J* = 7.6 Hz, 1H, Ar-H), 7.27 (d, *J* = 7.2 Hz, 1H, Ar-H), 7.25 (s, 1H, Ar-H), 6.99-7.10 (m, 2H, Ar-H) 4.31 (s, 2H, CH_2), 3.74 (br s, 1H, NH, overlap with solvent). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 162.4 ($C_{tetrazole}$), 137.7, 128.5, 124.6, 123.5, 122.7, 120.5, 115.1, 109.3, 28.3. LC-MS (Positive, *m/z* (%)): 200 $[M+H]^+$ (100). Anal. calcd. for $C_{10}H_9N_5$: C, 60.29; H, 4.55; N, 35.16. Found: C, 60.23; H, 4.48; N, 35.05%.

1-(4-(5-(4-Fluorophenyl)-1H-tetrazol-1-yl)-5-(hydroxymethyl)tetrahydrofuran-2-yl)-5-methylpyrimidine-2,4(1H,3H)-dione (**5a**): Color: Yellow. Yield: 86%. M.p.: 91-93 °C. FT-IR (KBr, ν, cm^{-1}): 3489 (N-H, str), 3382 (O-H, str), 2238 (=C-H, str), 1662 (C=O), 1616 (C=N, str), 1452 (N=N, str), 1159 (C-O), 1086 (C-F, str), 1083 (C-N, str). ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 10.56 (s, 1H, NH, Pyrimidine), 7.73 (d, 2H, *J* = 6.4 Hz, Ar-H), 7.55 (s, 1H, pyrimidine), 7.23 (d, 2H, *J* = 6.8 Hz, Ar-H), 5.53 (m, 1H, furan), 5.18 (m, 1H, furan), 4.92 (m, 1H, OH), 4.20 (m, 1H, furan), 3.54 (m, 2H, furan) 2.37 (m, 2H, C*H*$_2$OH), 2.26 (s, 3H, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 163.5, 162.5, 154.3($C_{tetrazole}$), 150.7, 136.1, 129.6, 121.9, 116.7, 110.9, 85.1, 74.8, 62.4, 57.5, 37.6, 23.1. LC-MS (Positive, *m/z* (%)): 389 $[M+H]^+$ (100). Anal. calcd. for $C_{17}H_{17}FN_6O_4$: C, 52.58; H, 4.41; N, 21.64. Found: C, 52.45; H, 4.43; N, 21.48%.

1-(5-(Hydroxymethyl)-4-(5-(4-nitrobenzyl)-1H-tetrazol-1-yl)tetrahydrofuran-2-yl)-5-methylpyrimidine-2,4(1H,3H)-dione (**5b**): Color: Brown. Yield: 88%. M.p.: 90-92 °C.



Scheme 2

FT-IR (KBr, ν, cm^{-1}): 3453 (N-H, str), 3320 (O-H, str), 2245 (=C-H, str), 1675 (C=O), 1598 (C=N, str), 1464 (N=N, str), 1378 (-NO_2, str), 1175 (C-O), 1086 (C-N, str). ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 10.79 (s, 1H, NH, Pyrimidine), 7.90 (d, 2H, *J* = 6.8 Hz, Ar-H), 7.62 (s, 1H, pyrimidine), 7.49 (d, 2H, *J* = 6.0 Hz, Ar-H), 5.83 (m, 1H, furan), 4.81 (m, 1H, OH), 4.54 (s, 2H, CH_2), 4.16 (m, 1H, furan), 4.01 (m, 1H, furan), 3.56 (m, 2H, furan), 2.39 (m, 2H, C*H*$_2$OH), 2.17 (s, 3H, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 163.2, 151.4 ($C_{tetrazole}$), 149.3, 144.9, 141.7, 136.5, 132.0, 123.4, 111.3, 85.0, 75.2, 62.1, 57.3, 37.0, 30.5, 18.1. LC-MS (Positive, *m/z* (%)): 430 $[M+H]^+$ (100). Anal. calcd. for $C_{18}H_{19}N_7O_6$: C, 50.35; H, 4.46; N, 22.83. Found: C, 50.25; H, 4.34; N, 22.56%.

1-(4-(5-(4-Aminophenyl)-1H-tetrazol-1-yl)-5-(hydroxymethyl)tetrahydrofuran-2-yl)-5-methylpyrimidine-2,4(1H,3H)-dione (**5c**): Color: White. Yield: 80%. M.p.: 95-97 °C. FT-IR (KBr, ν, cm^{-1}): 3485 (N-H, str), 3445 (N-H, str), 3325 (O-H, str), 2196 (=C-H, str), 1640 (C=O), 1645 (C=N, str), 1413 (N=N, str), 1164 (C-O), 1074 (C-N, str). ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 10.87 (s, 1H, NH, Pyrimidine), 7.90 (d, 2H, *J* = 6.8 Hz, Ar-H), 7.47 (s, 1H, pyrimidine), 6.75 (m, 2H, Ar-H), 5.49 (m, 1H, furan), 5.33 (s, 2H, NH_2) 4.57 (m, 1H, OH), 4.02 (m, 1H, furan), 3.92 (m, 1H, furan), 3.78 (m, 2H, CH_2), 2.59 (m, 2H, furan), 2.37 (s, 3H, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 163.4, 154.6 ($C_{tetrazole}$), 150.5, 145.5, 136.2, 127.8, 117.2, 114.7, 109.9, 84.5, 75.4, 62.4, 57.1, 37.7, 15.8. LC-MS (Positive, *m/z* (%)): 386 $[M+H]^+$ (100). Anal. calcd. for $C_{17}H_{19}N_7O_4$: C, 52.98; H, 4.97; N, 25.44. Found: C, 52.54; H, 4.88; N, 25.16%.

1-(4-(5-(2-Chloropyridin-3-yl)-1H-tetrazol-1-yl)-5-(hydroxymethyl)tetrahydrofuran-2-yl)-5-methylpyrimidine-2,4(1H,3H)-dione (**5d**): Color: Light yellow. Yield: 83%. M.p.: 94-96 °C. FT-IR (KBr, ν, cm^{-1}): 3476 (N-H, str), 3286 (O-H, str), 2208 (=C-H, str), 1645 (C=O), 1629 (C=N, str), 1476 (N=N, str), 1155 (C-O),1068 (C-N, str), 785 (C-Cl, str). ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 10.34 (s, 1H, NH, Pyrimidine), 8.20-7.68 (m, 3H, Ar-H), 7.33 (s, 1H, pyrimidine), 5.80 (m, 1H, furan), 4.70 (m, 1H, OH), 4.15 (m, 1H, furan), 4.05 (m, 1H, furan), 3.45 (m, 2H, CH_2), 2.50 (m, 2H, furan), 2.40 (s, 3H, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 163.0, 154.1 ($C_{tetrazole}$), 151.2, 147.4, 142.0, 136.3, 135.5, 134.9, 122.9, 109.7, 85.4, 74.8, 62.0, 55.8, 36.1, 16.8. LC-MS (Positive, *m/z* (%)): 406 $[M+H]^+$ (100). Anal. calcd. for $C_{16}H_{16}ClN_7O_4$: C, 47.36; H, 3.97; N, 24.16. Found: C, 47.16; H, 3.79; N, 24.01%.

1-(4-(5-((1H-Indol-3-yl)methyl)-1H-tetrazol-1-yl)-5-(hydroxymethyl)tetrahydrofuran-2-yl)-5-methylpyrimidine-2,4(1H,3H)-dione (**5e**): Color: Yellowish White. Yield: 85%. M.p.: 93-95 °C. FT-IR (KBr, ν, cm^{-1}): 3492-3216 (N-H, str), 3391 (O-H, str), 2214 (=C-H, str), 1660 (C=O), 1600 (C=N, str), 1447 (N=N, str), 1180 (C-O), 1095 (C-N, str). ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 10.98 (s, 1H, NH, Pyrimidine), 10.57 (s, 1H, NH, Indole), 7.86-7.43 (m, 4H, Ar-H), 7.46 (s, 1H, pyrimidine), 7.20 (s, 1H, CH, Indole), 5.84 (m, 1H, furan), 4.76 (m, 1H, OH), 4.12 (m, 1H, furan), 3.98 (m, 1H, furan), 3.80 (m, 2H, CH_2), 3.61 (s, 2H, CH_2) 2.48 (m, 2H, furan), 2.35 (s, 3H, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 163.5, 151.1 ($C_{tetrazole}$), 150.2, 136.8, 136.1, 127.9, 123.5, 121.4, 119.8, 118.6, 111.7, 110.5, 108.0, 85.1, 75.5, 62.4, 57.8, 37.1, 25.5, 14.9. LC-MS (Positive, *m/z* (%)): 424 $[M+H]^+$ (100). Anal. calcd. for $C_{20}H_{21}N_7O_4$: C, 56.73; H, 5.00; N, 23.16. Found: C, 56.46; H, 4.97; N, 23.03%.

2.5. Molecular modelling

2.5.1. Protein preparations

Different potential drug targets such as Reverse transcriptase (PDBID:1FKB), Aurora B (PDBID:2FBY) and Aromatase (PDBID:3EQM) were retrieved from Brookhaven Protein Data Bank (PDB). Hydrogens were added to proteins and energy was minimized using Gromacs96 force field. These molecules were subjected molecular docking studies in order to validate best lead molecules.

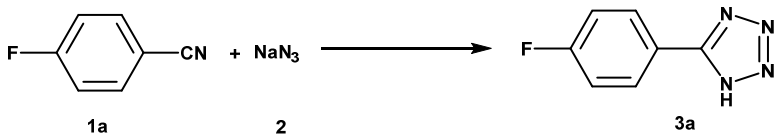
2.5.2. Molecular docking

Initially, 3D structures of tetrazole derivatives were drawn and energy minimized with Universal Force Field (UFF) using conjugate-gradient algorithm with 200 run iterations. Molecular docking was carried out with Lamarkian genetic Algorithm (GA) using AutodockVina 4.0 [30] with PyRx [31]. The following docking parameters were set such as the number of populations is 150, maximum number of energy evaluations are 25000, maximum number of generations are 27,000, top individuals is set to 1 to survive to next generation, gene mutation rate is 2, Crossover rate is 0.8, Cauchy beta is 1.0 and GA window size is 10.0, respectively. The grid was set to according to the active site of proteins and exhaustiveness at 8. The good docked molecules were selected on the basis of docking score and binding energies. Further, binding interacttions, bond angles and bond lengths were analysed using PyMol [32].We also assessed the pharmacological properties for the title compounds using Lipinski rule of five [33].

Table 1. Optimization of the reaction condition for the synthesis of tetrazole derivative **3a** [a].

Entry	Solvent	Catalyst	Temperature (°C)	Time	Yield (%)
1	THF	No catalyst	65	36 h	<25
2	Toluene	No catalyst	90	30 h	<36
3	1,4-dioxane	No catalyst	110	24 h	<35
4	H_2O	No catalyst	110	28 h	44
5	DMSO	No catalyst	120	24 h	30
6	DMF	No catalyst	120	28 h	<39
7	PEG-400	No catalyst	100	24 h	46
8	Glycerol	No catalyst	110	5.5 h	72
9	Glycerol	$CuCl_2$ (10 mol%)	100	5.5 h	73
10	Glycerol	$FeCl_3$ (10 mol%)	100	5.5 h	75
11	Glycerol	$CeCl_3.7H_2O$ (10 mol%)	100	3.5 h	76
12	Glycerol	$InBr_3$ (10 mol%)	100	3.5 h	78
13	Glycerol	$ZnCl_2$ (10 mol%)	100	2.5 h	78
14	Glycerol	$ZnBr_2$ (10 mol%)	100	2.5 h	82
15	Glycerol	$ZnCl_2$-SiO_2 (0.2 g)	100	1.5 h	83
16	Glycerol	$ZnBr_2$-SiO_2 (0.2 g)	100	1.5 h	86
17	Glycerol	$ZnBr_2$-SiO_2 (0.2 g) [b]	---	10 min	91
18	Glycerol	$ZnBr_2$-SiO_2 (0.2 g) [c]	---	10 min	92

[a]The substances 4-fluorobenzonitrile (**1a**) and sodium azide (**2**) were considered as models for optimization of the reaction.
[b] Model reaction carried out under microwave irradiation conditions at 465 Watts power.
[c] Model reaction carried out under microwave irradiation conditions at 490 Watts power.



Scheme 3

2.5.3. Antioxidant activity

The radical scavenging activity for the synthesized compounds was determined by using DPPH, NO and H_2O_2 methods [34-37].

3. Results and discussion

3.1. Chemistry

Initially, we have focused our attention on the optimization of experimental conditions for the synthesis of 5-substituted 1*H*-tetrazoles through [3+2] cycloaddition reaction. The substrates 4-fluorobenzonitrile (**1a**) and sodium azide (**2**) were considered as models (Scheme 3).

In the optimization of reaction conditions, the model reaction was examined in different solvents like THF, toluene, dioxane, H_2O, DMSO, DMF, PEG-400 and glycerol under thermal conditions at different temperatures without using any catalyst (Entry 1-8, Table 1). Moderate to less yield of the product, 5-(4-fluorophenyl)-1*H*-tetrazole (**3a**) was observed in these solvents, among increasing the cycloaddition product by changing the solvents from H_2O to glycerol. However, high yield of the product was observed in glycerol as compared with other solvents, so it was optimized as a solvent. In an effort to develop better reaction conditions, aforesaid model reaction was tested in different Lewis acid catalysts such as $CuCl_2$ (10 mol%), $FeCl_3$ (10 mol%), $CeCl_3.7H_2O$ (10 mol%), $ZnCl_2$ (10 mol%), $ZnBr_2$ (10 mol%), $InBr_3$ (10 mol%) and SiO_2-$ZnCl_2$ (0.2 g) and SiO_2-$ZnBr_2$ (0.2 g) and the results are tabulated in Table 1 (Entry 9-16). The observations revealed that when the reaction was run in glycerol solvent and SiO_2-$ZnBr_2$ catalyst afforded high yield of the product as compared with other catalyst conditions. Also, $ZnBr_2$ and SiO_2-$ZnCl_2$ afforded good yields of the product but somewhat low yield as compared with SiO_2-$ZnBr_2$. Therefore, SiO_2-$ZnBr_2$ was chosen as the catalyst to carry out the reaction. To our interest and based on our promising results under microwave conditions, the optimized reaction was also examined in the microwave irradiation condition, interestingly; some significant enhancement in the yield of product was observed in very less reaction time (Entry 17-18, Table 1).

After optimization of the reaction conditions, the generality of the reaction was investigated using different nitriles to afford the 5-substituted 1*H*-tetrazoles, **3a-e** (Table 2) under conventional and microwave conditions. The observations disclosed that the electron-withdrawing groups substituted nitriles afforded high yields of the products as compared with electron-donating substituted nitriles.

After optimization of the method, the developed procedure was adopted for the synthesis of 1,5-disubstituted terazole derivatives of zidovudine **5a-e** (Table 2) in high yields by reacting azide of zidovudine with substituted nitriles.

3.2. Biological activity

3.2.1. Molecular docking studies

Keeping in view of down fall of single target therapeutics, multi-target approaches could be more effective which allow the discovery of new classes of multi targeting drugs with fewer side effects and less toxicity. In order to optimize the multi target property of synthesized title compounds, we have performed molecular docking studies [30-32] with three potential drug targets such as reverse transcriptase, aurora B and aromatase which play a major role causing the AIDS and cancers respectively.Molecular docking studies with reverse transcriptase (PDBID:1FKB), Aurora B (PDBID: 2BFY) and aromatase (PDBID: 3eqm) which reveals that compound **5a**, **5c** and **5e** have shown highest binding affinities of -10.1, -9.7 and -10.6 Kcal/mol for reverse transcriptase whereas -8.5, -8.2 and -8.9 Kcal/mol for Aurora B (Table 3). Compound **5a** has formed three interactions with reverse transcriptase viz., hydroxymethyl group of furan interacted with hydroxyl group of Tyr318, 4C=O of pyrimidine interacted with Lys101 and fluorophenyl formed arene-arene interactions with Trp229, respectively, on the other handtwo interactions with aurora B such as N atom of tetrazole moiety with Ala173 and 2C=O group of the pyrimidine with Lys103 and formed only one interaction with aromatase viz., hydroxymethyl group of furan interacted with basic side of Arg115 (Figure 2-4).

Table 2. Physical properties of the synthesized tetrazoles **3a-e** and **5a-e**.

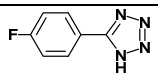
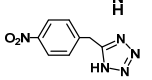
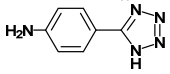
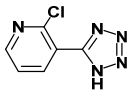
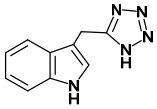
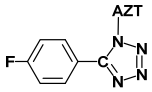
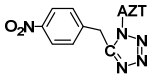
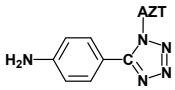
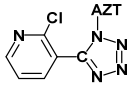
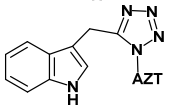
Compound	Product	Conventional conditions		Ultrasonication conditions	
		Time	Yield (%)	Time	Yield (%)
3a		1.5 h	89	10 min	94
3b		1.5 h	90	10 min	94
3c		2.5 h	83	15 min	89
3d		2.0 h	88	15 min	92
3e		2.5 h	86	15 min	90
5a		3.5 h	86	17 min	91
5b		3.0 h	88	15 min	92
5c		4.5 h	80	20 min	87
5d		3.0 h	83	17 min	90
5e		3.5 h	85	20 min	91

Table 3. Binding affinities of the synthesized compounds with reverse transcriptase, aromatase and Aurora B.

S. No	Compound	Binding affinity (ΔG) (Kcal/mol)		
		Reverse transcriptase(PDBID:1FKP)	Aromatase(PDBID:3eqm)	Aurora B(PDBID:2bfy)
1	3a	-7.6	-7.3	-6.3
2	3b	-7.3	-7.9	-6.5
3	3c	-6.9	-7.1	-6.1
4	3d	-7.1	-7.1	-6.0
5	3e	-8.6	-8.4	-7.1
6	5a	-10.1	-8.9	-8.5
7	5b	-1.3	-1.3	-1.1
8	5c	-9.7	-8.5	-8.2
9	5d	-2.1	-2.1	-2.0
10	5e	-10.6	-6.5	-8.9

Compound **5c** exhibited two interactions with reverse transcriptase such as NH_2 of phenyl formed interactions with Asn103 and Pro236, three interactions with aurora B viz., NH_2 of phenyl group formed Ala173, hydroxymethyl group of furan interacted with Lys101 and 3NH of pyrimidine and one interaction with aromatase such as NH of tetrazole with Leu477 (Figure 2-4). Compound **5e** has conferred interactions with reverse transcriptase such as 1NH of tetrazole formed arene-cationic with Trp229 and 4C=O group of the pyrimidine formed interaction with Lys101, two bonds formed with aurora B such as N atom of tetrazole and hydroxymethyl group of furan formed two interactions with Lys122 (Figure 2 and 3). In addition, **3e** showed the best binding affinity of -8.6 Kcal/mol for reverse transcriptase and formed one arene-cationic interaction with Arg115 (Figure 4).

3.2.1.1. Lipinski rule of five

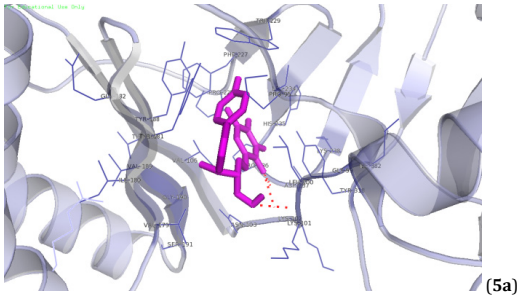
Open source program like OSIRIS Property Explorer [33] was employed to compute drug relatedproperties of the newly synthesized compounds. This server predicts the properties liketoxicity risk assessment, cLogP value, Molecular weights, Solubility, Drug-Likeness prediction andoverall Drug-Likeness score. All the compounds are found to be well satisfied with these properties and are tabulated in Table 4. H-bond donor, H-bond acceptor and cLogP or partition coefficient values were predicted to be less than five, less than ten and less than five respectively. This indicates that good absorption and distribution of drug in the body. All the title compounds showed good drug-likeness and displayed best drug score.

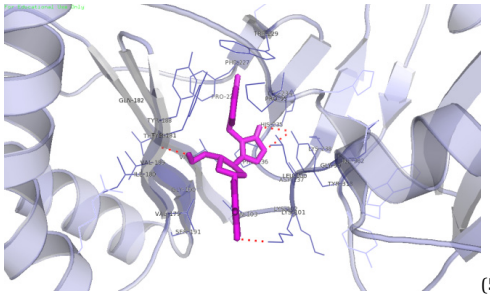
3.2.2. Antioxidant activity

The newly synthesized 5-substituted-1*H*-tetrazoles (**3a-e**) and tetrazole derivatives of AZT (**5a-e**) were screened for their antioxidant activity by radical scavenging activity methods (DPPH, NO and H_2O_2) including half-maximal inhibitory concentrations (IC_{50}).

Table 4. Determination of Lipinski's rule of five properties such as Molecular Weight (MW), cLog P, Topology of Polar Surface Area (TPSA), H-bond donor (H-Don) and H-bond Acceptor (H-Acc) for the synthesized compounds.

S. No	Compound	MW	cLog P	TPSA	H-Don	H-Acc
1	3a	164.00	1.000	54.40	1	3
2	3b	191.15	0.700	100.20	1	3
3	3c	161.16	-1.560	76.60	2	3
4	3d	181.58	0.910	67.35	1	4
5	3e	201.23	-1.880	69.68	3	2
6	5a	388.30	0.679	122.40	2	7
7	5b	444.40	0.163	162.70	3	9
8	5c	385.30	0.122	148.49	3	7
9	5d	433.80	0.410	135.30	2	8
10	5e	424.40	0.360	139.50	4	6


(5a)


(5c)

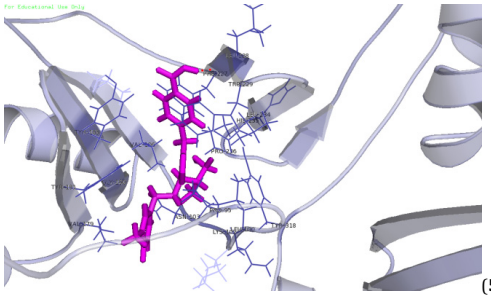
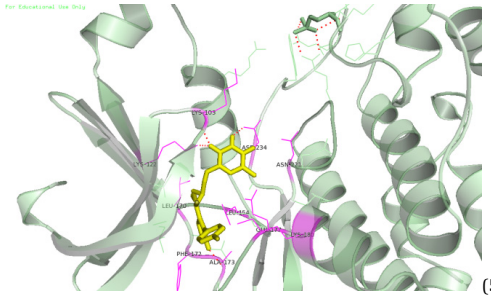
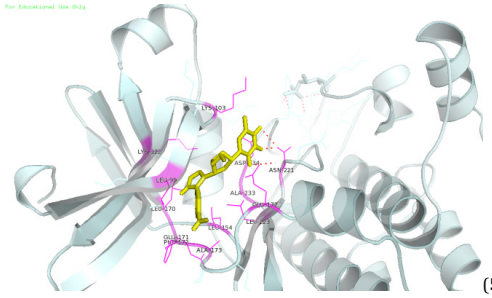

(5e)

Figure 2. Binding interactions of the best docket compounds within the active pocket of reverse transcriptase (PDBID: 1FKP).


(5a)


(5c)

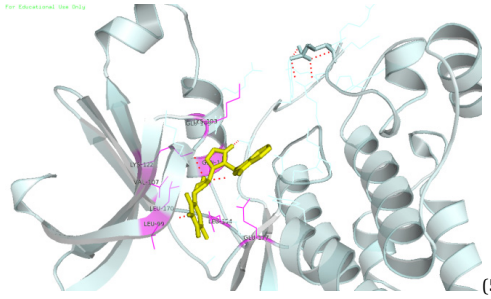

(5e)

Figure 3. Best binding mode of the best docked compounds aligned with in the active pocket of Aurora B (PDBID: 2bfy).

3.2.2.1. 1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging method

The 1,1-diphenyl-2-picrylhydrazyl (DPPH) [34,35] has been widely used to evaluate the free radical scavenging capacity of the synthesized antioxidants. The strong absorption maximum at 517 nm was observed in the DPPH due to the presence of radical.All the newly synthesized tetrazole derivatives **3a**-**e**, **5a**-**e**, and standard antioxidant, Butylated hydroxytoluene (BHT) were prepared in different concentrations (25, 50, 75 and 100 µg/mL) in methanol and homogeneity of the test samples were attained using magnetic stirrer. DPPH solution was prepared in methanol and adjusted the concentration to 0.004% (*w*:*v*) by adding methanol. The DPPH solution (4 mL, 0.004% (*w*:*v*)) was added to aliquot of standard solution and tested samples solution (1 mL of each) of various concentrations in a set of test tubes and shaken vigorously. Recorded the room temperature and kept for 30 min. in the dark to complete the reaction. The absorbance values of the tested samples were recorded against blank at 517 nm. The antioxidant, BHT was used as positive control. The scavenging capacity of DPPH radicals were calculated using the following equation. The experiment was repeated in triplicate and the average values are shown in Figure 5.

$$\% \text{ of Scavenging} = [Abs_{DPPH} - Abs_{sample} / Abs_{DPPH}] \times 100 \quad (1)$$

where Abs_{DPPH} is the absorbance of the control (DPPH solution without the test compound solution) and Abs_{sample} is the absorbance of the test sample (DPPH solution with the test compound solution).

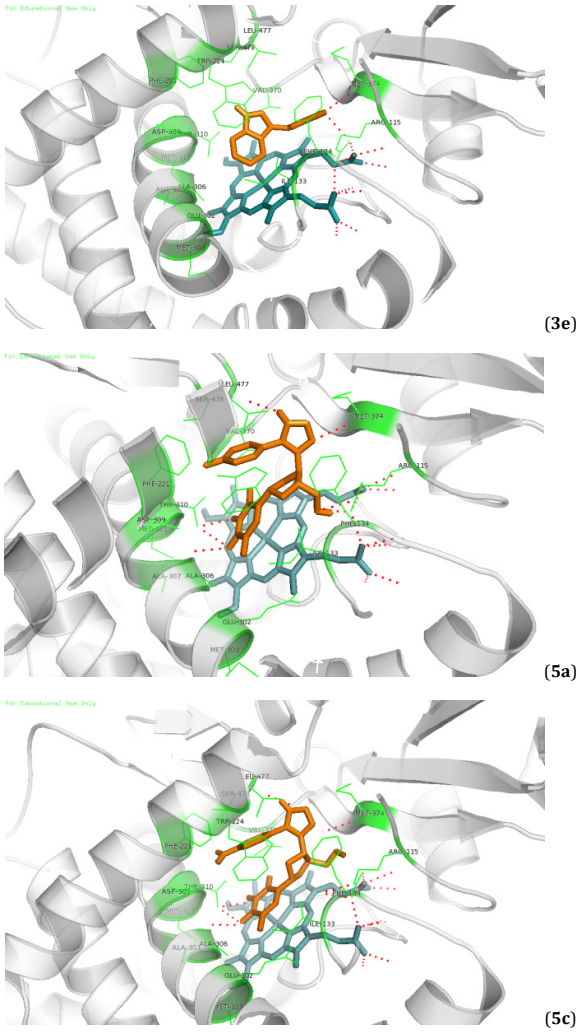


Figure 4. Binding interactions of the best docking lead molecules within the active pocket of Aromatase (PDBID: 3eqm).

3.2.2.1.1. Half maximal inhibitory concentration (IC_{50})

The half maximal inhibitory concentration (IC_{50}) was evaluated for the title compounds **3a-e** and **5a-e** using DPPH method and butylated hydroxytoluene was used as a standard drug. The results are presented in Table 5.

Table 5. IC_{50} values of the synthesized compounds **3a-e** and **5a-e**.

S. No	Compound	IC_{50}
1	3a	30.48±0.54
2	3b	42.37±0.18
3	3c	56.48±0.93
4	3d	27.63±1.04
5	3e	47.38±0.68
6	5a	28.59±0.42
7	5b	44.24±0.24
8	5c	68.03±0.71
9	5d	25.87±0.68
10	5e	24.33±0.18
11	Std.	22.92±0.43

3.2.2.2. Nitric oxide (NO) radical scavenging method

Marcocci *et al.* [36] modified method was employed for investigation of the nitric oxide radical scavenging activity of the title compounds. All the newly synthesized compounds and natural antioxidant, Ascorbic acid in various concentrations (25, 50, 75 and 100 µg/mL) were prepared in methanol and the homogeneous solutions were achieved by stirring on magnetic stirrer. Nitric oxide radicals (NO) were generated from 1 mL of sodium nitroprusside (10 mM) and 1.5 mL of phosphate buffer saline (0.2 M, pH = 7.4) were added to different concentrations of the test compounds in a set of test tubes and incubated for 150 min at 25 °C. 1 mL of the reaction mixture was treated with 1 mL of Griess reagent (1% sulfanilamide, 3% H_3PO_4 and 0.1% naphthylethylenediamine dihydrochloride). The absorbance of the chromophore was measured at 546 nm. Ascorbic acid was used as a positive control. The scavenging capacity of NO radicals was calculated. The experiment was carried out in triplicate and the results are presented in Figure 6.

3.2.2.3. Hydrogen peroxide scavenging method

The hydrogen peroxide radical scavenging activity of the tested samples was determined according to the protocol of Nabavi *et al.* [37]. Hydrogen peroxide (40 mM) solution was prepared in phosphate buffer (pH = 7.4) and its concentration was determined by using a spectrophotometer. Different concentrations (25, 50, 75 and 100 µg/mL) of standard antioxidant, ascorbic acid and synthesized compounds were prepared in DMSO.

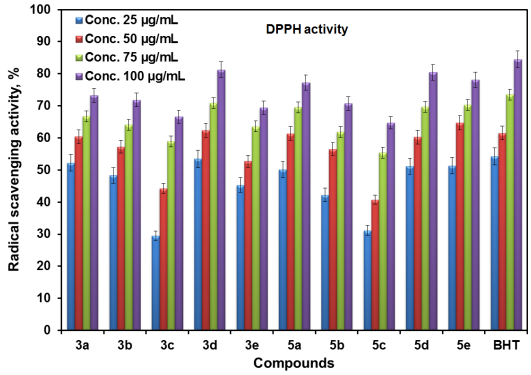


Figure 5. Antioxidant activity of tetrazoles **3a-e** and **5a-e** by DPPH method.

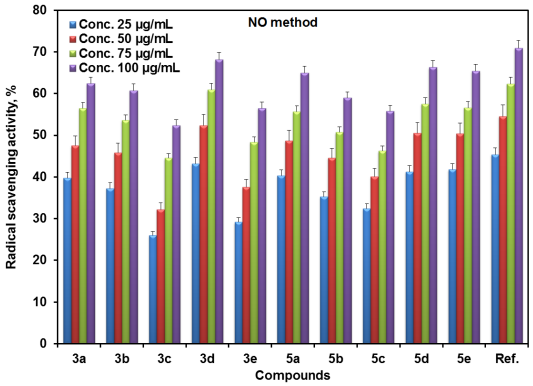


Figure 6. Antioxidant activity of tetrazoles **3a-e** and **5a-e** by NO method (Ref.: Ascorbic acid).

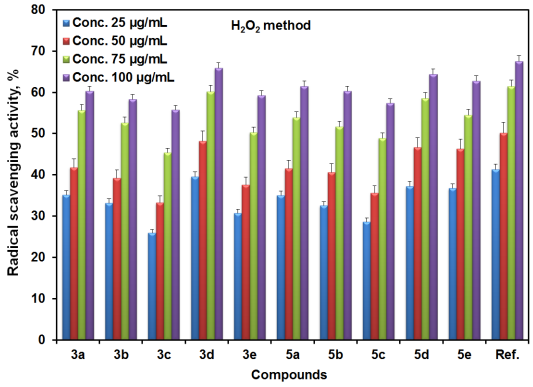


Figure 7. Antioxidant activity of tetrazoles **3a-e** and **5a-e** by H_2O_2 method (Ref.: Ascorbic acid).

Hydrogen peroxide solution (0.6 mL, 40 mM) was added to different concentrations of the test compounds in a set of test tubes. The absorbance values of these test compounds at 230 nm were examined after ten minutes against a blank solution. The experiment was carried out in triplicate and the results are presented in Figure 7.

It was found from the antioxidant bio-screening data that the simple tetrazole compound, **3d** (27.63 µg/mL) bearing 2-chloro-3-pyridyl ring and zidovudine tetrazole derivatives, **5d** (25.87 µg/mL) having 2-chloro-3-pyridyl ring, and **5e** (24.33 µg/mL) connected to indole ring exhibited promising antioxidant activity, almost closer to that of the standard, BHT (22.92 µg/mL) in all the tested methods. Majority of the tetrazole derivatives of AZT showed good antioxidant activity as compared with remaining simple tetrazole derivatives.

4. Conclusions

In the present investigation, an efficient heterogeneous $ZnBr_2$-SiO_2 catalysed green synthetic method was developed for the synthesis of 5-substituted and 1,5-disubstituted tetrazole derivatives in glycerol solvent under conventional and microwave irradiation conditions. The advantages of this method are, green solvent system, high yield of the product, less reaction time and reusability of catalyst. High yields of the products in less reaction time were observed under microwave irradiation conditions (10-20 min) as compared with conventional conditions (1.5-2.5 h). Antioxidant and molecular docking studies were performed for the title compounds. Compounds **3d**, **5d** and **5e** have shown potent

antioxidant activity while compounds **5a**, **5c**, **5e** and **3e** have shown good binding affinities.

Acknowledgements

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