Two Novel Triazolo-Thiadiazepines: Synthesis, Stereostructure, Antimicrobial and Cytotoxic Activity

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Abstract: Two novel triazolo-thiadiazepines, 2-(4-chlorophenyl)-4-[2-(4-chlorophenyl)ethenyl]—7-(9-decenyl)-2,3-dihydro-1,2,4- triazolo [3,4-b] [1,3,4] thiadiazepine (**4**) and 2,4-bis (4-chlorophenyl)-7-(9-decenyl)-2,3-dihydro-1,2,4-triazolo[3,4-b] [1,3,4]thiadiazepine (**7**) are synthesized from 3-(9-decenyl)-4-amino-5-mercapto-1,2,4-triazole (**1**) using 1,5-bis(4-chlorophenyl)pent-1,4-dien-3-one (**2**) in the former and 4,4'-dichlorochalcone (**5**) in the latter. The uncyclized adducts, 1,5-bis(4-chlorophenyl)-5-[3-(9-decenyl)-4-amino-5-thio-1,2,4-triazolyl]pent-1-en-3-one (**3**) and 1,3-bis(4-chlorophenyl)-3-[3-(9-decenyl)-4-amino-5-thio-1,2,4-triazolyl]propan-1-one (**6**) are also respectively obtained with them. The reaction of (**1**) with 1,3-bis(2-thienyl)propen-1-one (**8**) yielded only the uncyclized adduct, 1,3-bis(2-thienyl)-3-[3-(9-decenyl)-4-amino-5-thio-1,2,4-triazolyl]propan-1-one (**9**) as the sole product. The triazole (**1**) is prepared from commercially available 10-undecenoic acid by reaction with thiocarbohydrazide at 170°C. The α, β -unsaturated enones, 1,4-dien-3-one (**2**), dichlorochalcone (**5**), and thienyl propenone (**8**) are respectively obtained by condensing acetone with p-chlorobenzaldehyde(molar ratio,1:2), p-chloroacetophenone with p-chlorobenzaldehyde and 2-acetylthiophene with thiophene-2-aldehyde(molar ratios, 1:1) in the presence of 2.5 equivalents of sodium hydroxide.The synthesized compounds (**1**), (**4**), (**7**), and (**9**) are screened for *in vitro* antimicrobial and cytotoxic activities. These compounds showed potent antibacterial activity against "Gram negative" bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*) but no activity could be demonstrated against Gram positive bacterial strains.Streostuctures are established using IR, DCI-MS, ^{1}H- and ^{13}C- NMR spectra.

Keywords: Substituted triazole, α,β-unsaturated enones, uncyclized adducts, triazolo-thiadiazepines, antimicrobial activity, cytotoxic activity.

1. INTRODUCTION

The occurrence of long alkyl chain substituted heterocyclic compounds and their analogues in plants [1-3] has inculcated the interest in the synthesis of such heterocycles. The interest in 4-amino-5-mercapto-1,2,4-triazoles and their N-bridged heterocyclic derivatives for medical applications is also increasing strongly on account of their wide spectrum biological potentialities as anthelmintic [4], antihypertensive [5], antitubercular[6], antibacterial [7], anti-inflammatory [8], anti-HIV [9], antiviral [10], inhibition of cholinesterase [11], analgesic [12] and anticonvulsant [13]. The 1, 2, 4-triazole nucleus has been incorporated into a wide variety of therapeutically interesting drugs [14] including H_1/H_2-Histamine receptor blockers, cholinesterase active agents, CNS stimulants, antianxiety agents and sedatives. Also [1, 3, 4]-thiadiazepine derivatives are associated with a varied range of biological activities as antimicrobial [15], anticancer [16], herbicidal [17], antiviral and anti-HIV [18], anticholinergic and antidepressant [19], anaesthetic and calcium antagonist [20], choleretics [21] and ACE inhibitory [22] activity. Keeping in view, the importance of biological potentiality of these heterocycles mentioned above , we have undertaken this problem. The present paper deals with the synthesis, stereostructure, antimicrobial and cytotoxic activity of two novel compounds, 2-(4-chlorophenyl)-4-[2-(4-chlorophenyl)ethenyl]-7-(9-decenyl)-2,3-dihydro-1,2,4-triazolo[3,4-b][1,3,4]thiadiazepine (**4**) and 2,4-bis(4-chlorophenyl)-7-(9-decenyl)-2,3-dihydro-1,2,4-triazolo[3,4-b][1,3,4] thiadiazepine (**7**) along with their two uncyclized adducts, 1,5-bis(4-chlorophenyl)-5-[3-(9-decenyl)-4-amino-5-thio-1,2,4-triazolyl] pent-1-en-3-one (**3**) and 1,3-bis(4-chlorophenyl)-3-[3-(9-decenyl)-4-amino-5-thio-1,2,4-triazolyl] propan-1-one (**6**) from 3-(9-decenyl)-4-amino-5-mercapto-1,2,4-triazole (**1**) using 1,5-bis(4-chlorophenyl)pent-1,4-diene-3-one (**2**) in the former and 4,4'-dichlorochalcone (**5**) in the latter. The reaction of (**1**) with 1,3-bis(2-thienyl)propen-1-one (**8**) yielded only the uncyclized adduct, 1,3-bis(2-thienyl)-3-[3-(9-decenyl)-4-amino-5-thio-1,2,4-triazolyl]propan-1-one (**9**) as the sole product. The triazole (1) is prepared by fusing 10-undecenoic acid with thiocarbohydrazide at 170° C. The α, β- unsaturated enones, 1,4-dien-3-one (**2**), dichlorochalcone (**5**), and thienyl propenone (**8**) are prepared by condensing acetone with p-chlorobenzaldehyde (molar ratio,1:2), p-chloroacetophenone with p-chlorobenzaldehyde, and 2-acetylthiophene with thiophene-2-aldehyde (molar ratios, 1:1) respectively in the presence of 2.5

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equivalents of sodium hydroxide.The synthesized compounds (**1**), (**4**), (**7**) and (**9**) are evaluated for antimicrobial activity against four bacteria, *Escherichia coli*, *Pseudomonas aeruginosa*("Gram negative") , *Bacillus subtilis*, and *Staphylococcus aureus*("Gram positive") and one fungus, *Candida albicans* by Agar well diffusion method.All the compounds documented potent antibacterial activity against Gram negative bacterial strains but no activity could be demonstrated against Gram positive bacterial strains. Screening results of these compounds are also summarized for cytotoxic activity against 3-cell lines of three types of human cancers: lung, breast and CNS.

2. EXPERIMENTAL

2.1. Chemistry

Reagents and solvents were of commercial grade and were used without further purification. 10-Undecenoic acid was also of commercial grade. Column chromatography was performed on silica gel (60-120 mesh LR, 25049). Melting points were determined on Koffler hot-plate apparatus and are uncorrected. IR spectra were recorded on a Perkin Elmer 621 spectrophotometer using the KBr disc technique. ^{1}H-NMR (δ ppm) spectra were recorded on a Varian Unity 400 spectrometer in acetone-d_6 with TMS as the internal standard. ^{13}C-NMR spectra were recorded on a Varian Unity 100 spectrometer in acetone-d_6 . DCI-mass spectra were recorded in a Ribermag RI0-10B quadrupole mass spectrometer using ammonia as reagent gas. The splitting patterns of ^{1}H-NMR were designated as follows: s:singlet; d: doublet; t: triplet; m: multiplet; dd: double doublet; tdd: triplet double doublet; brs: broad singlet; brp: broad pentet.

2.1.1. 3-(9-Decenyl)-4-amino-5-mercapto-1,2,4-triazole (1)

Compound (**1**) was prepared from 10-undecenoic acid according to the reported method [23] as white crystalline needles, 7.078g (85.6%), m.p. 82°C, R_f 0.63 (pet. ether-EtOAc, 8:2 v/v). **IR KBr**(cm^{-1}): 3250, 3100 (NH_2), 2900 (CH_2), 2350 (SH), 1630 (C=N), 1610 (C=C), 1550, 1470, 1410, 1370, 1280, 1230, 940,890, 720. **^{1}H-NMR (acetone-d6, δppm)**: 12.25 (1H, SH), 5.18 (2H, s, NH_2), 2.73 (2H, t, J=7.51Hz, H-1'), 1.75 (2H, br p, J=7.46Hz, H-2'), 1.33 (10H, br s, H-3'-7'), 2.03 (2H, m, H-8'), 5.81 (1H, tdd, J=6.78, 10.26, 17.03Hz , H-9'), 4.90 (1H, dd, J=10.26, 2.20Hz, H_E-10'), 4.98 (1H, dd, J=17.21, 2.20Hz, H_Z-10'). **^{13}C-NMR (acetone-d_6,δppm)**: 153.34 (C-3), 168.26 (C-5), 25.40 (C-1'), 26.68 (C-2'), 29.40-30.20 (C-3'-7'), 34.40 (C-8'), 139.82 (C-9'), 114.61 (C-10'). **DCI-MS (NH_3), m/z (%)**: 254 (M^+, 7.1), 255 [$(M+1)^+$, 100.0], 256 (25.3), 257 (9.6), 143 (9.2), 144 (3.6), 130 (17.3), 131 (3.1).

2.1.2. 1,5-Bis(4-chlorophenyl)pent-1,4-dien-3-one (2)

It was prepared following a literature procedure [24] by condensing acetone with p-chlorobenzaldehyde (molar ratio, 1:2) in the presence of 2.5 equivalents of sodium hydroxide as bright yellow crystalline flakes, 710mg (72.0%), m.p 176 °C, R_f 0.72 (pet.ether-EtOAc, 8:2 v/v). **^{1}H-NMR (acetone-d_6,δppm)**: 7.28 (2H, d, J=16.0Hz, H-2/H-4), 7.70 (2H, d, J=16.0Hz, H-1/H-5), 7.73 (4H, d, J=8.50Hz, 2×Ar-2,6), 7.45 (4H, d, J=8.50Hz, 2×Ar-3,5).

2.1.3. 4,4'-Dicholrochalcone (5)

Compound (**5**) was prepared following a reported procedure [24] by condensing p-chloroacetophenone with p- chlorobenzaldehyde in equimolar ratio in the presence of 2.5 equivalents of sodium hydroxide, as light yellow crystalline needles in 90% yields, m.p. 154^o C. R_f o.73 (pet.ether (60-80^o)- benzene, 1:1 v/v).

2.1.4. 1,3-Bis(2-thienyl)propen-1-one (8)

It was prepared following the literature procedure [24] by condensing 2-acetylthiophene with thiophene-2-aldehyde in equimolar ratio, in the presence of 2.5 equivalents of sodium hydroxide as light yellow crystalline needles, 765 mg (78%), m.p. 89°C, R_f 0.63 (pet.ether-EtOAc, 8:2 v/v). **^{1}H-NMR (acetone-d_6, δppm)**: 7.48 (1H, d, J=15.41Hz, H-2), 7.93 (1H, d, J=15.41Hz, H-3), 7.67 (1H, d, J=5.04Hz, Ar-3), 7.27(1H, dd, J=5.04,3.82 Hz, Ar-4), 8.11(1H, d, J=3.82Hz, Ar-5), 7.60 (1H, d, J=4.73Hz, Ar'-3), 7.17(1H, dd, J=4.73,3.67,Hz, Ar-4'), 7.91(1H, d J=3.67Hz, Ar'-5). **^{13}C-NMR (acetone-d_6,δppm)**: 181.80 (C-1), 121.36 (C-2), 140.95 (C-3), 146.68 (Ar-C-2), 144.36(Ar'-C-2), 136.58 (Ar-C-5), 133.06 (Ar'-C-5), 129.34-134.25 (Ar-C-3, Ar'-C-3, Ar-C-4, Ar'-C-4). **DCI-MS (NH_3), m/z (%)**: 221/222 [(M+1)/(M+2), 100.0/20.4], 220 (14.0), 191 (3.7), 137 (3.8), 111 (7.2).

2.1.5. 1,5-Bis(4-chlorophenyl)-5-[3-(9-decenyl)-4-amino-5-thio-1,2,4-triazolyl]pent-1- en-3-one (3) and 2-(4-chlorophenyl)-4-[2-(4-chlorophenyl)ethenyl]-7-(9-decenyl)]-2,3-dihydro-1,2,4-triazolo[3,4-b][1,3,4] thiadiazepine (4). [Reaction of 1,2,4-triazole (1) with 1,5-bis(4-chlorophenyl)pent-1,4-dien-3-one (2)]

A mixture of the triazole (**1**) (50mg, 1.96 mmol) and 1,5-bis(4-chlorophenyl)pent-1,4-dien-3-one (**2**) (590

mg, 1.96 mmol) in dry benzene (20ml) was refluxed with stirring on an oil bath for 33 h in presence of p-TsOH (5 mg), collecting the generated water in an azeotropic collector and monitoring the progress of the reaction(TLC). TLC examination of the reaction mixture revealed the presence of two spots. The reaction mixture was cooled, washed with water and the organic phase was dried over Na_2SO_4 .The solvent was distilled off under diminished pressure and the residue left was subjected to column chromatography (silica gel, pet. ether-EtOAc, 8:2 v/v as eluent) which afforded (**3**) as glassy flakes, 416 mg (38.2%), m.p. 84°C, R_f 0.71 (pet. ether-EtOAc, 8:2 v/v). **^{1}H-NMR(acetone-d_6, δppm)** and **^{13}C-NMR(acetone-d_6,δppm)** values are given in Table **1**. **DCI-MS (NH_3), m/z (%)**: 557[$(M+1)^+$, 35.4], 558 (10.3), 559 (19.6), 560 (8.7), 561 (7.8), 301 (5.1), 302 (15.5), 303 (73.3), 304 (22.9), 305 (47.0), 306 (10.4), 307 (8.3), 267 (5.5), 254 (20.8), 255 (100.0), 256 (17.6), 257 (6.6), 204 (4.3), 165 (28.8), 167 (9.2), 143 (9.2), 137 (4.8), 130 (15.7), 101 (4.1), 102 (11.0).

Further elution of the column using (pet. ether-EtOAc, 6:4 v/v) followed by crystallization (benzene-acetone) afforded (**4**) as yellow crystalline globules, 460 mg (47.5%), R_f 0.30 (pet. ether-EtOAc, 8:2 v/v), m.p 161°C. **^{1}H-NMR(acetone-d_6 ,δppm)** and **^{13}C-NMR(acetone-d_6,δppm)** values are given in Table **2**. **DCI-MS (NH_3), m/z (%)**: 538 (M^+, 10.2), 539 [$(M+1)^+$, 100.0], 540 (45.1), 541 (75.7), 542 (28.9), 543 (18.7), 544 (5.9), 415 (7.6), 416 (5.9), 417 (5.8), 300 (16.5), 301 (5.9), 302 (30.6), 303 (8.5), 304 (16.5), 255 (6.8), 240 (25.1), 128 (5.9), 115 (6.3), 102 (9.3).

2.1.6. 1,3-Bis(4-chlorophenyl)-3-[3-(9-decenyl)-4-amino-5-thio-1,2,4-triazolyl] propan-1-one (6) and 2,4-bis(4-chlorophenyl)-7-(9-decenyl)-2,3-dihydro-1,2,4-triazolo[3,4-b][1,3,4]thiadiazepine, (7) [Reaction of the triazole (1) with 4,4'-dichlorochalcone (5)]

To a solution of the triazole (**1**) (500mg, 1.96 mmol) in dry benzene (20ml) was added 4,4'-dichlorochalcone (**5**) (540mg, 1.96 mmol) and refluxed with stirring for 28 h in presence of p-TsOH (5 mg), collecting the generated water in an azeotropic collector. The reaction mixture was then worked up as described above for compounds (**3**) & (**4**). The residue left was chromatographed over a silica gel column using pet. ether-EtOAc, (8:2 v/v) as an eluent,which yielded (**6**) as colourless oily liquid, 384mg (37.0%), R_f 0.63 (pet. ether-EtOAc, 8:2 v/v). **IR KBr(cm^{-1})**: 3178.55, 3074.26 (NH_2), 2927.0 (CH), 1689.17 (C=O), 1639.66 (C=N), 1590.04 (C=C), 1571.40, 1491.79, 1426.78, 1402.11, 1360.60, 1308.68, 1260.38, 1205.25, 1174.16, 1091.68, 1014.26, 991.38, 961.79, 910.10, 827.98, 783.54, 723.97, 629.04, 531.33, 462.77.**^{1}H-NMR (acetone-d_6,δppm)**: 5.14 (2H, s, NH_2), 6.40 (1H, dd, J=9.08, 5.58Hz, H-3), 3.60 (1H, dd, J=17.10, 5.58Hz, H-2_{up}), 4.20 (1H, dd, J=17.10, 9.80Hz, H-2_{dn}), 2.68 (2H, t, J=7.46Hz, H-1''), 1.64 (2H, br p, J=7.33Hz, H-2''), 1.23 (10H, br s, H-3''-7''), 1.98 (2H, m, H-8''), 5.70 (1H, tdd, J=6.28, 10.22, 17.08Hz, H-9''), 4.88 (1H, dd, J=10.22, 2.28Hz, H_E-10''), 4.97 (1H, dd, J=17.08, 2.28Hz, H_Z-10''), 7.20 (2H, d, J=8.55Hz, H-Ar-2, 6), 7.42 (2H, d, J=8.55Hz, H-Ar-3,5), 7.84 (2H, d, J=8.42Hz, H-Ar'-2,6), 7.70 (2H, d, J=8.42Hz, H-Ar'-3,5). **^{13}C-NMR(acetone-d_6,δppm)**: 152.60 (C-3'), 166.58 (C-5'); 54.64((C-3), 44.48 (C-2), 192.08 (C-1), 25.50 (C-1''), 26.78 (C-2''), 29.20-30.40 (C-3''-7''), 34.48 (C-8''), 139.40 (C-9''), 114.62 (C-10''), 129.62-138.20 (C-Ar+Ar'). **DCI-MS (NH_3), m/z (%)**: 277 (16.0), 278 (4.2), 279 (9.2), 280 (3.1), 276 (13.3), 275 (10.0), 255 (21.2), 256 (7.0), 257 (3.1), 242 (26.0), 243 (8.9), 221 (8.0), 200 (7.2), 196 (14.0), 157 (10.0), 143 (28.3), 130 (67.8), 128 (24.0), 130 (67.8), 128 (24.0), 103 (67.8), 128 (24.0), 103 (4.8), 83 (9.0).

Further elution of the column using pet. ether-EtOAc (6:4 v/v) as eluent yielded **(7**) as an yellow solid, which was recrystallised from benzene as pale yellow crystalline globules, 540 mg (51.9%), m.p 148°C, R_f 0.32 (pet. ether-EtOAc, 8:2 v/v). **^{1}H-NMR (acetone-d_6,δppm)**: 3.27 (1H, dd, J=13.91, 12.08Hz, H-3_{up}), 3.66 (1H, dd, J=13.91, 4.76Hz, H-3_{dn}), 5.27 (1H, dd, J=12.08, 4.76Hz, H-2), 2.89 (2H, t, J=7.51Hz, H-1''), 1.82 (2H, br p, J=7.46Hz, H-2''), 1.29 (10H, br s, H-3''-7''), 2.00 (2H, m, H-8''), 5.79 (1H, tdd, J=6.78, 10.29, 17.21Hz, H-9''), 4.89 (1H, dd, J=10.26, 2.20Hz, H_E-10''), 4.97 (1H, dd, J=17.21, 2.20Hz, Hz-10''), 8.19 (2H, d, J=8.79Hz, H-Ar'-2,6), 7.61 (2H, d, J=8.79Hz, H-Ar'-3,5), 7.41 (2H, d, J=8.61Hz, H-Ar -2,6), 7.56 (2H, d, J=8.61Hz, H-Ar-3,5). **^{13}C-NMR (acetone-d_6,δppm)**: 156.09 (C-9), 170.18 (C-7), 143.05 (C-4), 38.42 (C-3), 53.72 (C-2), 25.55 (C-1''), 27.36 (C-2''), 29.20-30.40 (C-3''-7''), 34.42 (C-8''), 139.83 (C-9''), 114.63 (C-10''), 130.44 (C-Ar'-2,6), 129.67 (C-Ar'-3,5), 130.03 (C-Ar-2,6), 129.39 (C-Ar-3,5), 142.31 (C-Ar'-1), 134.24 (C-Ar-1), 138.81 (C-Ar'-4), 134.39 (C-Ar-4). **DCI-MS (NH_3), m/z (%)**: 512 (M^+, 6.2), 513 [$(M+1)^+$, 100.0], 514 (40.7), 515 (72.1), 516 (25.2), 517 (18.2), 518 (5.4), 273 (3.9), 274 (23.7), 275 (7.4), 276 (54.3), 277 (12.6), 278 (27.8), 279 (6.7), 280 (6.8), 238 (3.9), 239 (3.7), 240 (28.6), 241 (7.4), 242 (5.7), 128 (5.1), 115 (7.9), 117 (5.1), 102 (9.0), 93 (6.4).

2.1.7. 1, 3-Bis(2-thienyl)-3-[3-(9-decenyl)-4-amino-5-thio-1,2,4-triazolyl]propan-1-one (9) [Reaction of the triazole (1) with 1,3-bis(2-thienyl) propen-1-one (8)]

A mixture of the triazole (**1**) (500 mg, 1.96 mmol) and 1, 3-bis (2-thienyl)propen-1-one (**8**) (432 mg, 1.96 mmol) in dry benzene (20 ml) was refluxed for 28 h in presence of catalytic amount of p-TsOH (5 mg) as mentioned above. The reaction mixture on TLC examination (silica gel G, pet. ether- EtOAc, 8:2 v/v) showed the presence of only one spot even on prolonging the reaction time.The reaction mixture was worked up as usual and the residue on purification by column chromatography (silica gel, pet. ether-EtOAc, 8:2 v/v) afforded **(9)** as pale yellow oily liquid, 343mg (68.6%), R_f 0.69 (pet.ether-EtOAc, 8:2 v/v). **^{1}H-NMR (acetone-d_6,ppm)**: 5.21 (2H, s, NH_2), 6.79 (1H, dd, J=9.01, 5.80Hz, H-3), 3.82 (1H, dd, J=16.94, 5.80Hz, H-2_{up}), 4.25 (1H, dd, J=16.94, 9.01Hz, H-2_{dn}), 2.71 (1H, t, J=7.47Hz, H-1''), 1.68 (2H, br p, J=7.32Hz, H-2''), 1.28 (10H, br s, H-3''-7''), 2.02 (2H, m, H-8''), 5.81 (1H, tdd, J=6.72, 10.23, 17.09Hz, H-9''), 4.91 (1H, dd, J=10.22, 2.29Hz, H_E-10''), 4.99 (1H, dd, J=17.24, 2.29Hz, H_Z-10''), 7.90 (1H, dd, J=4.89, 1.22Hz, H-Ar'-5), 7.24 (1H, dd, J=4.88, 3.81Hz, H-Ar'-4), 8.03 (1H, dd, J=3.81, 1.22Hz, H-Ar'-3), 7.22 (1H, dd, J=3.51, 1.22, H-Ar-5), 6.97 (1H, dd, J=5.03, 3.51Hz, H-Ar-4), 7.36 (1H, dd, J=5.03, 1.22, H-Ar-3). **^{13}C-NMR (acetone-d_6,δppm)**: 152.51 (C-3'), 167.48 (C-5'), 54.03 (C-3), 44.25 (C-2), 189.06 (C-1), 25.18 (C-1''), 26.6 (C-2''), 29.20-30.40 (C-3''-7''), 34.45 (C-8''), 139.89 (C-9''), 114.64 (C-10''), 126.30-144.78 (Ar+Ar'). **DCI-MS (NH_3), m/z (%)**: 475 (M^+, 12.5), 476 (4.4), 254 (6.8), 255 (67.1), 256 (11.9), 257 (4.1), 220 (11.5), 221 (100.0), 222 (15.3), 223 (11.3), 137 (3.6), 130 (5.7), 111 (32.0).

2.2. Biological Activity

2.2.1. In Vitro Antimicrobial Activity

The synthesized compounds (**1**), ((**4**), (**7**) and (**9**) were screened *in vitro* for antimicrobial activity by agar well diffusion method against four antibacterial and one antifungal organisms. The antimicrobial activity of these test compounds was assayed on nutrient agar medium [Hi-Media Lab. Pvt. Mumbai, India]. The antifungal activity was tested using Sabouraud dextrose agar medium [Hi-Media, Lab. India] by agar well diffusion method [25-27]. Briefly 0.1 ml of the diluted inoculum (10^6 CFU ml^{-1}) of test organism was spread on NA/SDA (Nutrient Agar/ Sabouraud dextrose Agar) plates. Wells of 8 mm diameter were punctured into the agar medium and filled separately with 100µl of compound (250 µg ml^{-1} solvent blank and 100 µg ml^{-1} chloramphenicol antibiotic) to which the test bacteria were sensitive. Fluconazole at the concentration of 100µg ml^{-1} was used as the control against Candida albicans. The plates were incubated for 18 h at 37°C. Antimicrobial activity was evaluated by measuring the zone of inhibition against the test organisms.

2.2.2. Cytotoxic Activity Against Human Tumor Cells

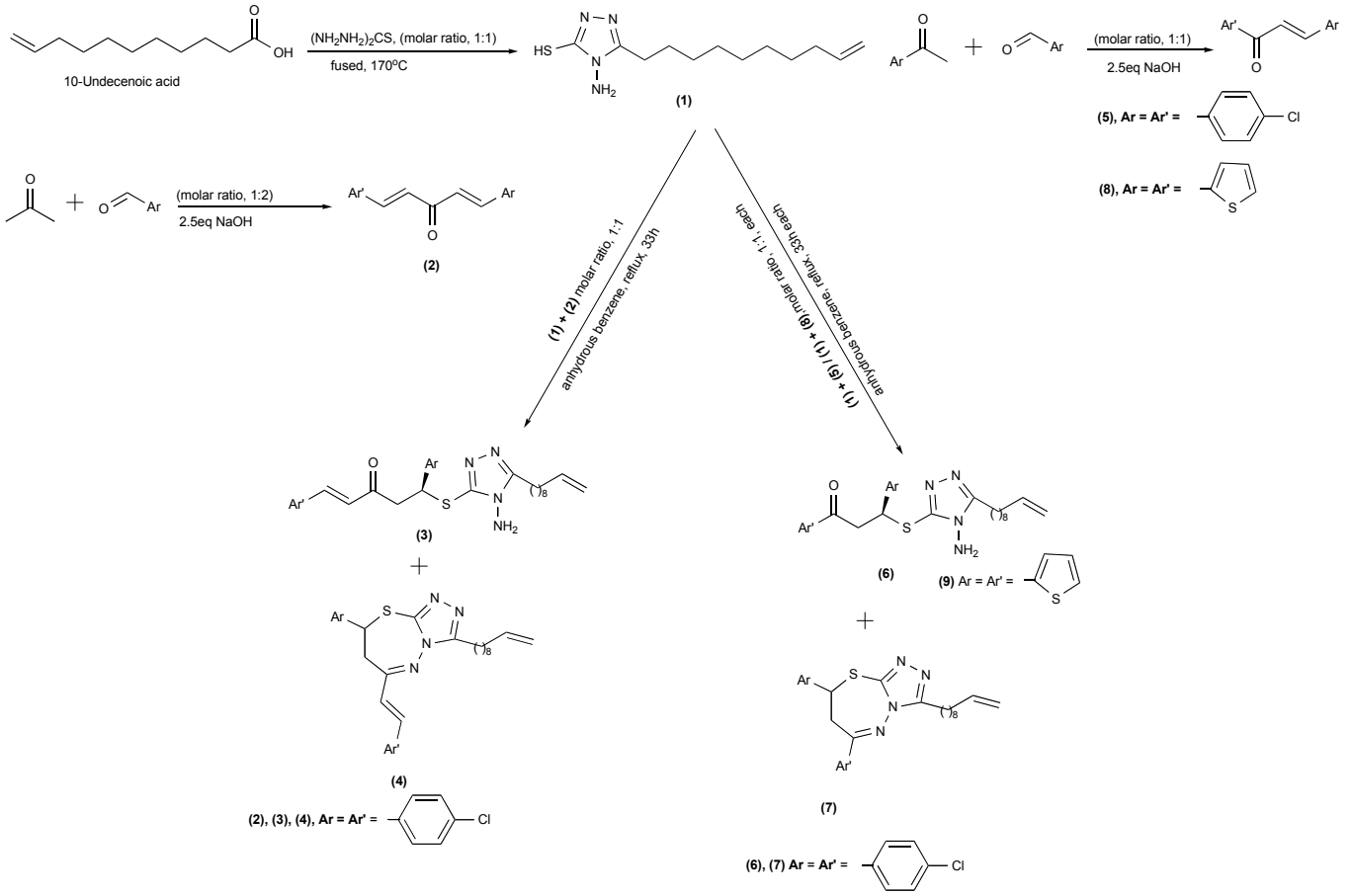
The compounds (**1**), (**4**), (**7**) and (**9**) were evaluated in a three cell lines panel of three types of human cancers: Breast (MCF7), Lung (NCI-H460) and CNS (SF-268) in one dose primary anticancer assay at a single concentration, 1×10^{-4} M. The results in Table **4** showed that these compounds were inactive against the above mentioned cancers at this single concentration.

3. RESULTS AND DISCUSSION

3.1. Chemistry

The synthesis of compounds (**3**), (**4**), (**6**), (**7**) and (**9**) has been performed in two steps. The 1,2,4-triazole (**1**) was first prepared following a published procedure [23] by fusing commercially available 10-undecenoic acid with thiocarbohydrazide (molar ratio, 1:1) at 170°C, as white crystalline needles in 85% yield. The compound (**1**) was then separately reacted with 1, 5-bis (4-chlorophenyl)pent-1,4-dien-3-one (**2**), 4,4'-dichloro-chalcone (**5**) and 1,3-bis(2-thienyl)propen-1-one (**8**) (molar ratio, 1: 1, in each case) in dry benzene in presence of p-TsOH, monitoring (TLC) and refluxing the reaction mixtures with azeotropic removal of water. The products on chromatography over a silica gel column using pet.ether (60-80°C)-EtOAc(8:2v/v) as eluent, yielded the respective compounds (Scheme **1**). It is noteworthy that reaction of the triazole (**1**) with 1,3-bis(2-thienyl)propen-1-one (**8**) yielded only the uncyclized adduct (**9**) as the sole product.The α, β-unsaturated enones, 1,4- dien-3-one(**2**), dichloro-chalcone(**5**), and thienyl propenone (**8**) were prepared prior to this reaction following literature procedure [24] by condensing acetone with p-chlorobenzaldehyde (molar ratio, 1:2), p-chloroacetophenone with p-chlorobenzaldehyde, and 2-acetylthiophene with thiophene-2-aldehyde(molar ratios, 1:1) respectively in the presence of 2.5 equivalents of sodium hydroxide.

The structures and stereochemistry of (**1**), (**3**), (**6**), and (**9**) have been confirmed by IR, DCI-MS, ^{1}H-NMR and ^{13}C-NMR spectra (spectral data of representative



Scheme 1: Synthesis of (**3**)+(**4**), (**6**)+(**7**) and (**9**) by reactions of (**1**)+(**2**), (**1**)+ (**5**) and (**1**)+(**8**) respectively.

compound (**3**) are shown in Table **1** and that of (**1**), (**6**), and (**9**) in **Section 2**). IR spectrum of (**1**) showed characteristic absorption peaks corresponding to N-H, C-H, S-H, C=N and C =C groups and that of (**6**) to N-H, C-H, C=O, C=N, C=C, phenyl, S-CH and C-Cl groups. The S-H peak was found to be absent here. This indicated that the SH group of (**1**) must be involved in the nucleophilic addition to β-Carbon of α,β-unsaturated enone group(CO-CH=CH-) of (**5**) forming (**6**). DCI-MS spectra of (**1**), (**3**) and (**9**) showed characteristic $[M+1]^+$ peaks corresponding to their molecular weights. The compound (**6**) which was subjected to EI-MS spectrum could not show molecular ion peak but its fragment ions confirmed its molecular weight. The molecular weights of (**3**), (**6**), and (**9**) correspond to the sum of the molecular weights of the triazole(**1**) and the respective enones (**2**), (**5**), and (**8**), showing that the former three compounds are the adducts of the latter two compounds, triazole(**1**) and enone. The assignments of all the signals to individual H- or C-atoms have been made on the basis of their typical chemical shift values, coupling constants, and relative integrations.The ^{1}H-NMR spectrum of (**1**) showed signals at δ 12.25 ppm and δ 5.18 ppm for the protons of SH and NH_2 groups respectively. The signal for the thiol group(SH) proton at δ12.25ppm was found to be absent in ^{1}H-NMR spectra of (**3**), (**6**), and (**9**).This also showed that the SH group of (**1**) has undergone nucleophilic addition at the β-carbons of α, β- enones group of (**2**), (**5**), and (**8**) forming the uncyclized adducts (**3**), (**6**) and(**9**) respectively.The ^{1}H-NMR and ^{13}C-NMR spectra of (**3**), (**6**), and (**9**) also revealed that they are formed by the nucleophilic addition of thiol (SH) group of the 1,2,4-triazole (**1**) at the β –carbon of the α, β- enone group of (**2**), (**5**), and (**8**) respectively as the methylene and methine protons at C-4 and C-5 formed after the addition, couple with each other giving two double doublet signals of C-4 protons, one for H-4_{up} and the other for H-4_{dn} and one double doublet signals for C-5 proton.The ^{1}H-NMR spectra of (**1**), (**3**), (**6**), and (**9**) showed signals for the terminal olefinic protons as two double doublets for H-10 protons, one for 10- H_E and the other for 10-H_Z , and triple double doublets for H-9 proton. Also, ^{13}C-NMR spectra showed characteristic signals for the C-3 and C-5 carbons of the 1,2,4- triazole ring. The other signals were attributed to the 9-decenyl side chain carbons. The ^{1}H-NMR and ^{13}C-NMR spectra of (**3**) confirmed the

existence of a trans olefinic α, β- enone group (Table **1**). Based on the above spectral evidence, the preferred stereostructures of (**1**) is shown in Figure **1** and that of (**3**), (**6**) and (**9**) are in Figure **2**.

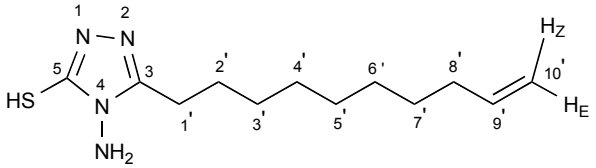


Figure 1: Structure of triazole (**1**).

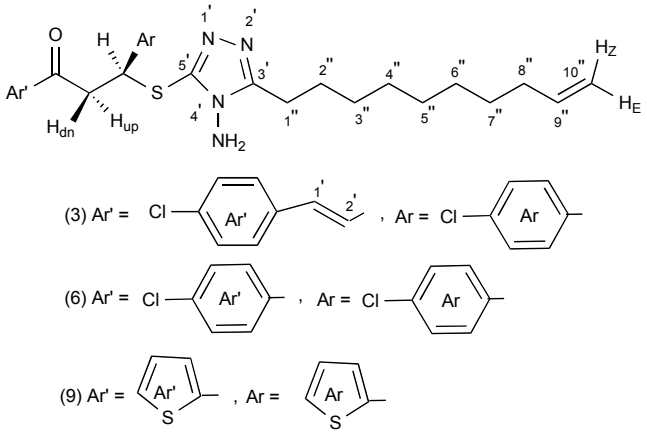


Figure 2: Structures of (**3**), (**6**) and (**9**).

The stereostructures of (**4**) and (**7**) have been established by DCI-MS, ^{1}H-NMR and ^{13}C-NMR spectra (spectral data of (**4**) are shown in Table **2** and that of (**7**) in Section 2). The assignments of all the signals to individual H- and C-atoms have been performed from their typical chemical shift values, coupling constants, relative integrations and by comparison wih the spectra of their precursors (**3**) and (**6**) respectively. DCI-MS spectra showed $[M + 1]^{+}$ peaks confirming their molecular weights which are equivalent to the molecular weights of the respective precursors (**3**) and (**6**) minus 1 mole of water, showing that their NH_2 group has undergone intramolecular nucleophilic cyclocondensation with the carbonyl group forming seven membered thiadiazepine ring containing compounds (**4**) and (**7**). The ^{1}H-NMR and ^{13}C- NMR spectral data are in strong agreement with the formation of condensed 1,2,4-triazolo-thiadiazepine rings. The H-4/C-4 signals of (**3**) have shifted to higher fields from δ_H 3.47ppm (H-4_{up}), 3.97ppm (H-4_{dn})/δc 45.05ppm (C-4) to δ_H 3.07ppm (H-3_{up}), 3.52ppm (H-3_{dn})/δc 36.92ppm (C-3) in (**4**). Similary, the methine proton/carbon signals at C-5 of (**3**) have shifted to higher fields from δ_H 6.43ppm/ δ_C 57.69ppm to δ_H 5.22ppm/δ_C 53.59ppm in (**4**). In a similar way, H-2/C-2 signals of (**6**) have shifted to higher fields from δ_H

Table 1: ^{1}H-NMR and ^{13}C-NMR Spectral Data of (3) in Acetone-d_6

^{1}H-NMR Assignment	δ(ppm)	Integration	Multiplicity	J (Hz)	^{13}C-NMR Assignment	δ(ppm)
NH_2	5.12	2H	s		5	57.69
5	6.43	1H	dd	$J_{5,4dn}$9.31, $J_{5,4up}$ 5.50	4	45.05
4up	3.47	1H	dd	$J_{4up,4dn}$ 17.25, $J_{4up,5}$ 5.50	3	196.04
4dn	3.97	1H	dd	$J_{4up,4dn}$17.25, $J_{4dn,5}$ 9.31	2’	127.64
2’	6.90	1H	d	$J_{2,1}$ 16.33	1’	142.08
1’	7.67	1H	d	$J_{1,2}$ 16.33	3’	152.46
1’’	2.73	2H	t	$J_{1'',2''}$7.48	5’	167.62
2’’	1.70	2H	br p	J≈7.36	1’’	25.21
3’’-7’’	1.25	5x2H	br s		2’’	26.70
8’’	2.00	2H	m		3’’-7’’	29.20-30.40
9’’	5.80	1H	tdd	$J_{9'',8''}$6.71, $J_{9'',HE}$ 10.22, $J_{9'',Hz}$ 17.09	8’’ 9’’	45.05 139.35
10’’-H_E	4.90	1H	dd	$J_{HE,9''}$10.22, $J_{HE,Hz}$ 2.29	10’’	114.64
10’’-H_Z	4.98	1H	dd	$J_{Hz,9''}$17.09, $J_{HE,Hz}$ 2.29	Ar + Ar’’	129.29-139.85
Ar-2,6	7.36	2H	d	$J_{Ar\text{-}2,6,Ar\text{-}3,5}$ 8.54		
Ar-3,5	7.46	2H	d	$J_{Ar\text{-}2,6,Ar\text{-}3,5}$ 8.54		
Ar’-2,6	7.72	2H	d	$J_{Ar'\text{-}2,6,Ar'\text{-}3,5}$ 8.39		
Ar’-3,5	7.49	2H	d	$J_{Ar'\text{-}2,6,Ar'\text{-}3,5}$ 8.39		

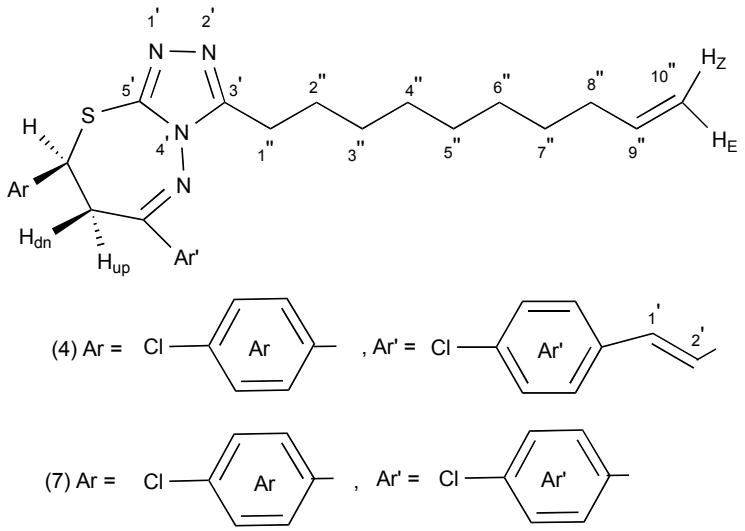


Figure 3: Structures of (**4**) and (**7**).

3.60ppm (H-2_{up}), 4.20ppm (H-2_{dn})/δ_C 44.48ppm (C-2) to δ_H 3.27ppm (H-3_{up}), 3.66ppm (H-3_{dn})/δc 38.42ppm (C-3) in (**7**). Similarly, the methine proton/carbon signals at C-3 of (**6**) have shifted to higher fields from δ_H 6.40ppm /δ_C 54.64ppm to δ_H 5.27ppm (H-2) /δc 53.72ppm (C-2) in (**7**) due to formation of the ring.On the basis of above spectral findings, structures and preferred conformation of (**4**) and (**7**) deduced are shown in Figure **3**.

3.2. Study for Biological Activity

The newly synthesized compounds were screened for *in vitro* antimicrobial (antibacterial and antifungal) against four antibacterial strains and one antifungal strain. These compounds were also evaluated for their cytotoxic activity against malignant human tumor cells.

3.2.1. In Vitro Antimicrobial Activity

The *in vitro* antimicrobial activity against four antibacterial organisms, Staphylococcus aureus (IOA-106), Bacillus subtilis (MTCC-121 laboratory isolate), Escherichia coli (U.P. -2566), and Pseudomonas aeruginosa (IOA-110) and one antifungal organism, Candida albicans (SC-5314 laboratory isolate) of the synthesized compounds (**1**), (**4**), (**7**) and (**9**) were evaluated by agar well diffusion method [25-27]. The results for the antimicrobial study of the tested

Table 2: ^{1}H-NMR and ^{13}C-NMR Spectral Data of (4) in Acetone-d_6

^{1}H-NMR Assignment	δ(ppm)	Integ-ration	Multi-plicity	J (Hz)	^{13}C-NMR Assignment	δ(ppm)
3_{up}	3.07	1H	dd	$J_{3up,3dn}$ 13.73, $J_{3up,2}$ 11.75	9a	171.27
3_{dn}	3.52	1H	dd	$J_{3up,3dn}$ 13.73, $J_{3dn,2}$ 4.89	7	155.84
2	5.22	1H	dd	$J_{2,3up}$11.75, $J_{2,3dn}$ 4.89	4	143.16
1’	7.27	1H	d	$J_{1',2'}$16.48	3	36.92
2’	7.66	1H	d	$J_{2',1'}$16.48	2	53.59
1’’	2.83	2H	t	$J_{1'',2''}$7.47	1’	126.76
2’’	1.79	2H	br p	J≈7.44	2’	140.69
3’’-7’’	1.29	5x2H	br s		1’’	25.51
8’’	2.00	2H	m		2’’	27.36
9’’	5.78	1H	tdd	$J_{9'',8''}$6.71, J_{9'',H_E}10.22, J_{9'',H_Z} 17.09	3’’-7’’ 8’’	29.20-30.40 34.42
H_E-10’’	4.88	1H	dd	$JH_{E,9''}$10.22, $JH_{E,}$ H_Z 2.29	9’’	139.81
H_Z-10’’	4.96	1H	dd	$JH_{Z,9''}$17.09, $JH_{E,}$ H_Z 2.29	10’’	114.59
Ar + Ar’	7.42-7.76	2x4H	m		Ar +Ar’	129.47-130.39

Table 3: Antimicrobial Activity of Some of the Synthesized Compounds by Agar well Diffusion Method

Test Compounds	Effective concentration µg/well	Antimicrobial activity in terms of zone of inhibition in mm				
		SA	BS	EC	PA	CA
(**1**)	250	-	-	10	12	-
(**4**)	250	-	-	18	21	-
(**7**)	250	-	-	14	16	-
(**9**)	250	-	-	15	20	-
Chloram-phenicol	100µg/well	25	20	24	30	-
Fluconazole	100µg/well	-	-	-	-	25

SA, *Staphylococcus aurens*; BS, *Bacillus subtilis*; EC, *Escherichia coli*; PA, *Pseudomonas aeruginosa*; CA, *Candida albicans*.

compounds against the test organisms are depicted in Table **3**. The antibacterial activity against" Gram negative" bacteria (*E. coli* and *P. aeruginosa*) was deduced in all compounds. However, no activity could be detected against" Gram positive" bacteria (*S. aureus* and *B. subtilis*). Compounds also lacked antifungal (anticandidal) activity. It is interesting to note that the compound (**4**), which has an additional olefinic bond at C-4 of the thiadiazepine ring as compared to (**7**) showed enhanced activity against "Gram negative" bacteria and is nearly comparable to that of the antibiotic drug, chloramphenicol. Effective concentration of these compounds was 250 µg per well. Further exploration requires detailed study on exact mode of interaction of these peculiar compounds with "Gram negative" and "Gram positive" bacteria. *In vivo* protection and possible toxicity data on these compounds are to be generated further.

3.2.2. Cytotoxic Activity Against Malignant Human Tumor Cells

Out of the newly synthesized compounds, (**1**), (**4**), (**7**) and (**9**) were selected by the National Cancer Institute (NCI) developmental therapeutic program for the *in vitro* cell line screening to investigate their antitumor activity. The compounds were first evaluated as one dose primary anticancer assay in a three cell lines panel consisting of three types of human cancers: breast (MCF7), lung (NCI-H460) and CNS (SF-268) [28, 29]. In the screening protocol, each cell line was inoculated and preincubated for 24-28 h on a microtiter plate. Test agents were then added at a single concentration (1×10^{-4} M) and the culture incubated for further 48 h. End point determinations were made with alamar blue. Results for each test agent were reported as the percent growth of the treated cells when compared to the untreated control cells. Compounds that reduced the growth of any one of the cell lines to approximately 32% or less are considered to be active. The preliminary screening results are shown in Table **4** according to which these compounds demonstrated to be inactive as the percentage growth of the treated cells are the above 32 %.

4. CONCLUSIONS

In conclusion, the aim of the present research work was to synthesize some novel long alkyl chain substituted condensed triazolo-thiadiazepines and to evaluate their antimicrobial and antitumor activities. It has been achieved by synthesizing two new compounds, 2-(4-chlorophenyl)-4-[2-(4-chlorophenyl) ethenyl]-7-(9-decenyl)-2,3-dihydro-1,2,4- triazolo [3,4-b] [1,3,4] thiadiazepine (**4**) and 2,4-bis (4-chlorophenyl)-7-

Table 4: Cytotoxic Activity of the Compounds Against 3-Cell Lines of Human Cancers

Test Compounds	Concentration	Retardation of growth (%)			Activity
		MCF7 (Breast)	NCI-H460 (Lung)	SF-268 (CNS)	
(**1**)	1×10^{-4}M	69	93	95	Inactive
(**4**)	1×10^{-4}M	72	98	112	Inactive
(**7**)	1×10^{-4}M	79	99	106	Inactive
(**9**)	1×10^{-4}M	63	99	90	Inactive

(9-decenyl)-2,3-dihydro-1,2,4-triazolo[3,4-b][1,3,4]thiadiazepine (**7**) from 3-(9-decenyl)-4-amino-5-mercapto-1,2,4-triazole (**1**) using 1,5-bis(4-chlorophenyl)pent-1,4-dien-3-one (**2**) in the former and 4,4'-dichlorochalcone (**5**) in the latter. The respective uncyclized adducts, 1,5-bis (4-chlorophenyl) – 5 –[3-(9-decenyl) -4- amino-5-thio-1,2,4-triazolyl] pent-1-en-3-one (**3**) and 1,3 –bis (4-chlorophenyl) -3-[3-(9-decenyl)-4-amino-5-thio-1,2,4 triazolyl] propan-1-one (**6**) were also obtained with them in low yields. The reaction of the triazole (**1**) with 1,3-bis(2-thienyl) propen-1-one (**8**) yielded only the uncyclized adduct, 1,3-bis(2-thienyl)-3-[3-(9-decenyl)-4-amino-5-thio-1,2,4-triazolyl] propan-1-one (**9**) as the sole product.The triazole (**1**) and the α ,β- unsaturated enones, (**2**),(**5**) and (**8**) were prepared following the reported procedures.All the tested ompounds (**1**), (**4**), (**7**) and (**9**) manifested significant antibacterial activity against "Gram negative" bacteria.However, no such activity could be deduced against "Gram positive" bacteria.These compounds also lacked antifungal activity. It is noteworthy that the compound (**4**), which has an additional olefinic bond at C-4 of thiadiazepine ring as compared to (**7**) showed enhanced activity against "Gram negative" bacteria and is nearly comparable to that of the drug, chloramphenicol. The compounds (**1**), (**4**), (**7**) and (**9**) were also screened for cytotoxic activity in a three cell lines panel against three types of human cancers: breast, lung and CNS at a single concentration (1×10^{-4} M). These compounds showed no cytotoxic activity at this single concentration. Further investigations for other biological assays are required to explore their potentialities in future.

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