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Synthesis and Evaluation of Imidazo[2,1-d][1,2,3,5]tetrazine-4(3*H*)-one Derivatives as Anticancer and Antimicrobial Agents

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A series of novel potent hetero aromatic nitrogen ring of imidazo[2,1-d][1,2,3,5]tetrazine-4(3H)-one derivatives (**1a**-**1i**) have been synthesized from 5-propylthio-2-diazo-2H-benzimidazole with substituted isocyanates. The synthesized compounds were structurally characterized by using various analytical and spectral techniques. *In vitro* microbial activities of the ligands were screened against some pathogenic bacterial and fungal species by modified well diffusion method using agar medium. All the synthesized compounds show significant biological activity as compared to the standard control drugs. Furthermore, antiproliferative activities of these compounds have been investigated against some breast (MCF-7), prostate (PC-3), lung (H1975) and colon (HCT-116) cancer cell lines. The compound (**1b**) showed a noticeable antiproliferative activity against the entire cell lines than the others.

Keywords: Imidazotetrazinones, Spectral studies, Microbial activity, Anticancer activity, Cytotoxicities.

INTRODUCTION

In recent year, design and synthesis of novel bioactive drug molecules having aromatic heterocyclic nitrogen bridged imidazole ring moieties show unique biological properties and play a crucial role in modern clinical and medicinal chemistry[1]. Now the biochemists are focused their interest against imidazotetrazinones and its substituted derivatives show remarkable anticancer activities against various cancer cell lines[2,3] and its lead compounds show an excellent *in vitro* and *in vivo* antitumor activities against a variety of cancer cell lines. Also, metozolomide have achieved in phase I and II clinical trials in chemotherapeutic field which shows cruel and unpredictable life-threatening toxicity to the human born marrow suppression[4] and deep platelet damage (thrombocytopenia) due to cross-linking of DNA strands[5,6]. Temozolomide (Temodal or Temodar) is used as a U.S. FDA approved anticancer prodrug for curing different types of cancers in human beings[7,8] and served as a molecular drug delivery device in the major groove of DNA[9]. Moreover, the monofunctional methylating series of temozolomide is used as a novel bio-effective clinical alternative to DTIC[10]. Due to this property, our main aim in this work is to synthesize the biologically active imidazo[2,1-d][1,2,3,5]tetrazine-4(3H)-one derivatives derived from 5-propylthio-2-aminobenzimidazole with a series of substituted isocyanides and structurally characterized with the help of various spectral and analytical studies. Moreover, the *in vitro* microbial activities of the ligands were tested against some pathogenic bacterial and fungal strains by modified well diffusion method using agar medium. Antiproliferative activities of these ligands have been investigated against some breast (MCF-7), prostate (PC-3), lung (H1975) and colon (HCT-116) cancer cell lines.

EXPERIMENTAL

All the chemicals and solvents used in this work were extra pure AnalaR grade. The solvents were purified according to the literature[11]. Melting point of the ligands was determined on Buchi-Tottoti open glass capillary tube and is uncorrected. Micro analytical (C, H and N) data were performed on Elementar Vario EL III CHNS analyzer. Molar conductance (Λ_M) of the ligands (1×10^{-3} mol solution in DMSO) was measured using an Elico CM 180 conductivity bridge by using KCl solution as calibrant. The IR spectra were recorded on KBr discs on a Perkin Elmer 100 FT-IR spectrometer (4000-400 cm^{-1}). The proton (^1H) and carbon (^{13}C) NMR spectral observation of the ligands were recorded on a Perkin Elmer R-32 spectrometer in $CDCl_3$ and DMSO-d_6 at 100 MHz with TMS as an internal

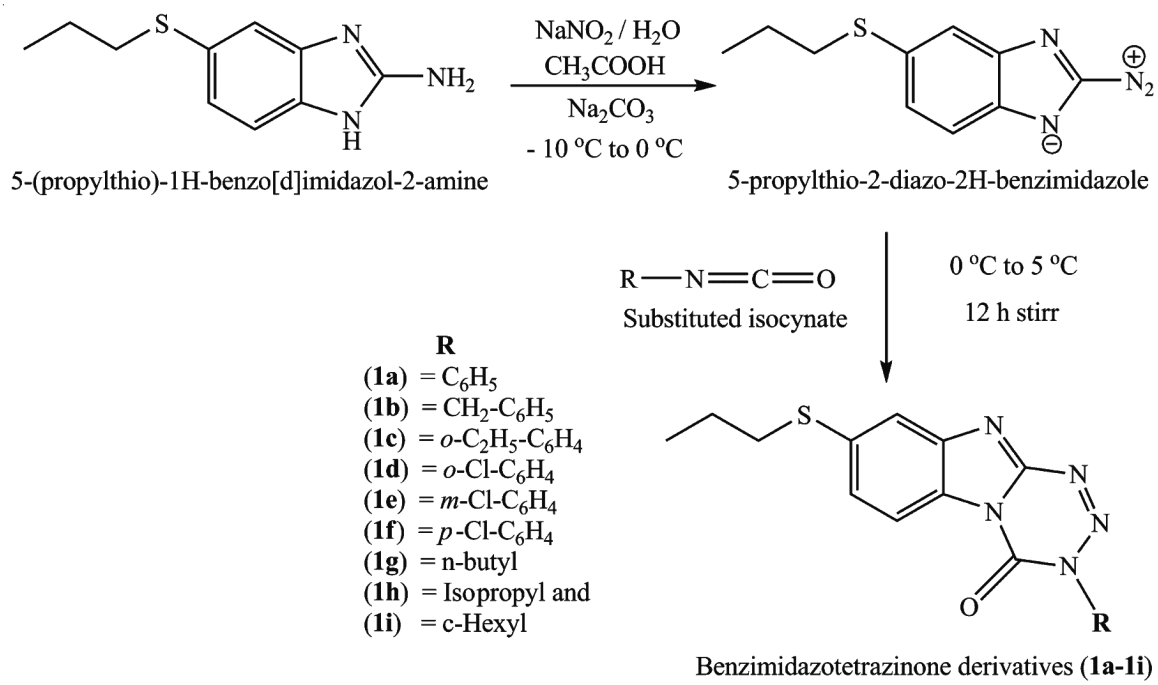
reference. The LC mass spectra of the ligands were determined on Agilent-1100 series mass spectrometer in ESI-MS mode. All the chemical reactions were performed under N_2 atmosphere using standard techniques. Column chromatography was performed with silica gel 230-400 mesh (Merck, India). Yield reported is the isolated yield after purification of the compounds.

Synthesis of benzimidazotetrazinone derivatives (1a-1i): To a solution of 5-propylthio-2-aminobenzimidazole (1 g, 4.82 mmol) in glacial acetic acid (6 mL) a solution of sodium nitrite (0.33 g, 4.82 mmol) in a small amount of water (1 mL) was added drop wise at -10 °C under nitrogen atmosphere. The mixture was neutralized at -10 to 0 °C with saturated Na_2CO_3 solution. The resulting solution was reduced to 1/3 of its original volume and kept aside. On standing, the brown coloured solid compound was collected by vacuum filtration, dried and stored in a dark place. The crude product, quickly shaken in cyclohexane and filtered off, gave a brown coloured 5-propylthio-2-diazo-2H-benzimidazole solid compound (yield: 50-60 %). To a this 5-propylthio-2-diazo-2H-benzimidazole solution (0.5 g, 2.3 mmol) in anhydrous dichloromethane (10 mL) was added drop wise to the different isocyanate (2.3 mmol) in anhydrous dichloromethane (10 mL) and the temperature of the medium was kept at -10 °C (**Scheme-I**). The reactions were allowed to reach at room temperature in the dark under a nitrogen atmosphere. The reaction mixture was stirred for 24 h, then removed the solvent under reduced pressure and kept aside. The crude product was purified by column chromatography to get a series of pure benzimidazotetrazinone derivatives (**1a-1i**).

3-Phenyl-4-oxo-8-propylthiobenzimidazo[2,1-d][1,2,3,5]tetrazine (1a): Colourless solid; Yield: 80 %; $C_{17}H_{15}N_5OS$; m.p.: 233 °C; IR (KBr, ν_{max} cm^{-1}): 1741.49 (CO); ^{1}H NMR (TMS, 100 MHz, DMSO-d_6) δ ppm: 0.94-0.98 (t, 3H,-C<u>H</u>$_3$), 1.45-1.68 (m, 2H,-C<u>H</u>$_2$-CH$_3$), 2.74-2.96 (m, 2H, -C<u>H</u>$_2$-CH$_2$-), 7.10-7.14 (t, 1H, ArH), 7.37-7.41 (t, 1H, ArH), 7.52-7.58 (m, 4H, ArH), 7.69-7.73 (t, 1H, ArH),7.83-7.85 (m, 1H, ArH), ^{13}C NMR (TMS, 100 MHz, DMSO-d_6) δ ppm: 153.38, 147.79, 139.42, 138.95, 137.61, 129.60, 129.13, 127.19, 124.11, 123.55, 118.80, 115.56, 33.77, 21.50, 13.25; LC-MS (*m/z*): 338.87 (M+1); Elemental analysis: Calcd.: C, 60.52; H, 4.48; N, 20.76; Found: C, 60.30; H, 4.55; N, 20.80; Λ_M (10^{-3} M in DMSO; ohm^{-1} cm^2 mol^{-1}): 11.62.

3-Benzyl-4-oxo-8-propylthiobenzimidazo[2,1-d][1,2,3,5]tetrazine (1b): Colourless solid, Yield: 65 %; $C_{18}H_{17}N_5OS$; m.p.: 153 °C (decom); IR (KBr, ν_{max} cm^{-1}): 1747.57 (CO) cm^{-1}; ^{1}H NMR (TMS, 100 MHz, DMSO-d_6) δ ppm: 0.92-0.96 (t, 3H,-C<u>H</u>$_3$), 1.46-1.56 (m, 2H,-C<u>H</u>$_2$-CH$_3$), 2.74-2.96 (m, 2H,S-C<u>H</u>$_2$-CH$_2$-),4.2-4.25 (d, 2H,-C<u>H</u>$_2$-Ar), 6.96 (d, 1H, ArH), 7.01-7.05 (t, 1H, ArH), 7.12-7.15 (m, 1H, ArH), 7.18-7.32 (m, 5H, ArH), ^{13}C NMR (TMS, 100 MHz, DMSO-d_6) δ ppm: 153.38, 146.83, 139.42, 137.61, 136.03, 129.60, 128.36, 128.03, 127.65, 123.55, 118.80, 115.56, 49.24, 33.77, 21.50, 13.25; LC-MS (*m/z*): 352.44 (M+1); Elemental analysis: Calcd.: C, 61.52; H, 4.88; N, 19.93; Found: C, 61.25; H, 5.05; N, 19.72. Λ_M (10^{-3} M in DMSO; ohm^{-1} cm^2 mol^{-1}): 10.41.

3-(2-Ethylphenyl)-4-oxo-8-propylthiobenzimidazo-[2,1-d][1,2,3,5]tetrazine (1c): Pale yellow colour powder, Yield: 60 %; $C_{19}H_{19}N_5OS$; m.p.:140 °C (decom); IR (KBr, ν_{max} cm^{-1}): 1741.49 (CO); ^{1}H NMR (TMS, 100 MHz, DMSO-d_6) δ ppm: 0.82-0.90 (t, 3H,-C<u>H</u>$_3$), 1.12-1.19 (t, 3H,-C<u>H</u>$_3$), 1.46-1.67 (m, 2H,-C<u>H</u>$_2$-CH$_3$), 2.6-2.7 (t, 2H,S-C<u>H</u>$_2$),2.8-2.9 (q, 2H,-C<u>H</u>$_2$-CH$_3$), 7.0 (t, 1H, ArH), 7.25-7.32 (m, 2H, ArH), 7.42-7.5 (m, 2H, ArH), 7.62-7.68 (m, 2H, ArH), ^{13}C NMR (TMS, 100 MHz, DMSO-d_6) δ ppm: 153.38, 147.56, 139.47, 139.43, 138.05, 137.61, 130.85, 129.60, 128.79, 128.13, 124.87, 123.55, 118.80, 115.56, 33.77, 24.76, 21.50, 14.05, 13.25; LC-MS (*m/z*): 366.37 (M+1); Elemental analysis: Calcd.:



Scheme-I: Synthetic root for the preparation of benzimidazotetrazinone derivatives

C, 62.44; H, 5.24; N, 19.16. Found: C, 62.40; H, 4.95; N, 19.10: Λ_M (10^{-3} M in DMSO; ohm^{-1} cm^2 mol^{-1}): 14.12.

3-(2-Chlorophenyl)-4-oxo-8-propylthiobenzimidazo-[2,1-d][1,2,3,5]tetrazine (1d): Colourless solid, Yield: 75 %; $C_{17}H_{14}N_5OSCl$; m.p.:235 °C (decom); IR (KBr, ν_{max} cm^{-1}): 1730.05 (CO); ^{1}H NMR (TMS, 100 MHz, DMSO-*d*$_6$) δ ppm: 0.92-0.96 (t, 3H,-C<u>H</u>$_3$), 1.45-1.62 (m, 2H, -C<u>H</u>$_2$-CH$_3$), 2.74-2.96 (m,2H,S-C<u>H</u>$_2$-), 6.94-7.05 (t, 1H, ArH), 7.20-7.34 (m, 3H, ArH), 7.42-7.58 (m, 3H, ArH), ^{13}C NMR (TMS, 100 MHz, DMSO-*d*$_6$) δ ppm: 153.38, 147.56, 139.42, 137.61, 136.72, 132.25, 129.81, 129.69, 129.60, 129.23, 123.55, 118.80, 115.56, 33.77, 21.50, 13.25; LC-MS (*m/z*): 372.93 (M+1); Elemental analysis: Calcd.: C, 54.91; H, 3.79; N, 18.83. Found: C, 54.62; H, 3.85; N, 18.95. Λ_M (10^{-3} M in DMSO; ohm^{-1} cm^2 mol^{-1}): 13.51.

3-(3-Chlorophenyl)-4-oxo-8-propylthiobenzimidazo-[2,1-d][1,2,3,5]tetrazine (1e): Colourless solid, Yield: 79 %; $C_{17}H_{14}N_5OSCl$; m.p.:248 °C (decom); IR (KBr, ν_{max} cm^{-1}): 1743.73 (CO); ^{1}H NMR (TMS, 100 MHz, DMSO-*d*$_6$) δ ppm: 0.98-1.09 (t, 3H, -C<u>H</u>$_3$), 1.42-1.68 (m, 2H, -C<u>H</u>$_2$-CH$_3$), 2.72-2.94 (t, 2H, S-C<u>H</u>$_2$-), 7.02-7.12 (t, 1H, ArH), 7.25-7.34 (m, 1H, ArH), 7.67-7.72 (m, 2H, ArH), 7.73-7.78 (m, 2H, ArH), ^{13}C NMR (TMS, 100 MHz, DMSO-*d*$_6$) δ ppm: 153.38, 147.79, 140.26, 139.42, 137.61, 132.91, 132.05, 129.61, 127.98, 123.88, 123.55, 121.55, 118.80, 115.56, 33.77, 13.25; LC-MS (*m/z*): 373.85 (*M+2*); Elemental analysis: Calcd.: C, 54.91; H, 3.79; N, 18.83. Found: C, 54.56; H, 3.90; N, 18.60. Λ_M (10^{-3} M in DMSO; ohm^{-1} cm^2 mol^{-1}): 12.58.

3-(4-Chlorophenyl)-4-oxo-8-propylthiobenzimidazo-[2,1-d][1,2,3,5]tetrazine (1f): Colourless solid, Yield: 68 %; $C_{17}H_{14}N_5OSCl$; m.p.:275 °C (decom); IR (KBr, ν_{max} cm^{-1}): 1733.66 (CO); ^{1}H NMR (TMS, 100 MHz, DMSO-*d*$_6$) δ ppm: 0.92-0.99 (t, 3H, -C<u>H</u>$_3$), 1.46-1.69 (m, 2H, -C<u>H</u>$_2$-CH$_3$), 2.74-2.96 (t, 2H, S-C<u>H</u>$_2$-), 7.10-7.15 (d, 1H, ArH), 7.45-7.52 (d, 2H, ArH), 7.62-7.73 (m, 4H, ArH), ^{13}C NMR (TMS, 100 MHz, DMSO-*d*$_6$) δ ppm: 153.38, 147.79, 139.42, 138.63, 137.61, 131.86, 129.61, 128.29, 125.63, 123.55, 118.80, 115.56, 33.77, 21.50, 13.25; LC-MS (*m/z*): 372.68 (M+1); Elemental analysis: Calcd.: C, 54.91; H, 3.79; N, 18.83. Found: C, 54.70; H, 3.85; N, 19.08. Λ_M (10^{-3} M in DMSO; ohm^{-1} cm^2 mol^{-1}): 13.26.

3-(*n*-Butyl)-4-oxo-8-propylthiobenzimidazo[2,1-d][1,2,3,5]tetrazine (1g): Colourless powder, Yield: 57 %; $C_{15}H_{19}N_5OS$; m.p.:178-180 °C (decom); IR (KBr, ν_{max} cm^{-1}): 1733.10 (CO); ^{1}H NMR (TMS, 100 MHz, DMSO-*d*$_6$) δ ppm: 0.92-0.98 (t, 6H, -C<u>H</u>$_3$), 1.45-1.56 (m, 2H, -C<u>H</u>$_2$-CH$_3$), 1.6-1.69 (m, 4H, -C<u>H</u>$_2$-CH$_2$-),2.74-2.96 (t, 2H, S-C<u>H</u>$_2$-), 3.10-3.18 (t, 2H, N-C<u>H</u>$_2$-CH$_2$-), 6.95(m, 1H, ArH), 7.10 (m, 2H, ArH), ^{13}C NMR (TMS, 100 MHz, DMSO-*d*$_6$) δ ppm: 153.38, 146.23, 139.42, 137.61, 129.61, 123.55, 118.80, 115.56, 44.41, 33.77, 30.61, 21.50, 20.03, 13.67, 13.25; LC-MS (*m/z*): 318.74 (M+1); Elemental analysis: Calcd.: C, 56.76; H, 6.03; N, 22.06. Found: C, 56.90; H, 6.38; N, 22.10. Λ_M (10^{-3} M in DMSO; ohm^{-1} cm^2 mol^{-1}): 11.08.

3-Isopropyl-4-oxo-8-propylthiobenzimidazo[2,1-d]-[1,2,3,5]tetrazine (1h): Yellow solid, Yield: 50 %; $C_{14}H_{17}N_5OS$; m.p.:142-144 °C (decom); IR (KBr, ν_{max} cm^{-1}): 1747.66 (CO); ^{1}H NMR (TMS, 100 MHz, DMSO-*d*$_6$) δ ppm: 0.90-0.94 (t, 3H, -C<u>H</u>$_3$), 0.95-1.08 (d, 6H, -CH-(C<u>H</u>$_3$)$_2$), 1.42-1.58 (m, 2H, -C<u>H</u>$_2$-CH$_3$), 2.74-2.83 (t, 2H, S-C<u>H</u>$_2$-), 3.6 (m, 1H, -C<u>H</u>-(CH$_3$)$_2$) 6.98 (d, 1H, ArH), 7.07 (d, 1H, ArH), 7.15 (s, 1H, ArH), ^{13}C NMR (TMS, 100 MHz, DMSO-*d*$_6$) δ ppm: 153.38, 143.88, 139.42, 137.61, 129.61, 123.55, 118.80, 115.56, 44.74, 33.77, 21.74, 21.50, 13.25; LC-MS (*m/z*): 304.44 (M+1); Elemental analysis: Calcd.: C, 55.42; H, 5.65; N, 23.08. Found: C, 55.10; H, 5.87; N, 23.18. Λ_M (10^{-3} M in DMSO; ohm^{-1} cm^2 mol^{-1}): 10.94.

3-Cyclohexyl-4-oxo-8-propylthiobenzimidazo[2,1-d][1,2,3,5]tetrazine (1i): Colourless solid, Yield: 72 %; $C_{17}H_{21}N_5OS$; m.p.:184-186 °C (decom); IR (KBr, ν_{max} cm^{-1}): 1720.56 (CO); ^{1}H NMR (TMS, 100 MHz, DMSO-*d*$_6$) δ ppm: 0.91-0.98 (t, 3H, -C<u>H</u>$_3$), 1.05-1.35 (m, 6H, -C$_6$H$_{11}$)1.45-1.54 (m, 2H, -C<u>H</u>$_2$-CH$_3$),1.56-1.66 (m, 2H, -C$_6$H$_{11}$) 1.67-1.78 (m, 2H, -C$_6$H$_{11}$) 2.76-2.84 (t, 2H, S-C<u>H</u>$_2$-), 3.13-3.18 (m, 1H, N-C<u>H</u>-),6.90 (m, 1H, ArH), 6.98 (m, 1H, ArH), 7.10 (m, 1H, ArH), ^{13}C NMR (TMS, 100 MHz, DMSO-*d*$_6$) δ ppm: 153.38, 144.89, 139.42, 137.61, 129.61, 123.55, 118.80, 115.56, 55.51, 33.77, 32.09, 25.05, 24.90, 21.50, 13.25; LC-MS (*m/z*): 344.71 (M+1); Elemental analysis: Calcd.: C, 59.45; H, 6.16; N, 20.39. Found: C, 59.74.; H, 6.55; N, 20.05. Λ_M (10^{-3} M in DMSO; ohm^{-1} cm^2 mol^{-1}): 14.06.

Microbial activity: *In vitro* microbial activities of benzimidazotetrazinone derivatives (3×10^{-3} M) in DMSO medium were tested against three Gram-positive bacterial species: *Bacillus subtilis*, *Staphylococcus saphyphiticus* and *Staphylococcus aureus,* two Gram-negative bacterial species: *Escherichia coli* and *Pseudomonas aeruginosa* using Muller Hinton nutrient agar (NA) and three fungal species: *Aspergillus niger, Enterobacter species* and *Candida albicans* using potato dextrose agar as medium were studied by modified well diffusion technique[12]. All the investigations were made in three replicates for each and the detailed procedure for measuring the zone of inhibition as described earlier[13,14]. The measured zone of inhibition (in mm) of each sample was compared with tetracycline (for antibacterial) and nystatin (for antifungal) control drugs respectively.

Antiproliferative activity: *In vitro* antiproliferative activity of benzimidazotetrazinone derivatives (**1a-1i**) were screened against human cancer cell lines like H1975 (non small lung), PC3 (Prostate), HCT116 (Colon) and MCF7 (Breast) by the MTT assay method[15]. In this assay method, to analyzes the ability of living cells to reduce the yellow dye of 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) to purple formazan product. The potential cytotoxicities of all the compounds were determined by measuring the percentage of cancers cells death/inhibition. The selected compounds were tested in various concentrations (0, 1, 5, 10, 20, 40, 80 and100 μM) against the human cancer cell lines and to determine IC_{50} values using non-linear regression (log conc. *v/s* % inhibition) by Graph pad Prism 5.

RESULTS AND DISCUSSION

The synthesized benzimidazotetrazinone derivatives (**1a-1i**) are air stable at room temperature, non-hygroscopic and insoluble in water and common organic solvents like benzene, acetone, petroleum ether, *etc*., but readily soluble in DMF, $CDCl_3$ and DMSO. The obtained result of micro elemental analysis (C, H, N) with their molecular formulae and various physico-chemical properties of the prepared derivatives were

summarized in the experimental part. From the C, H and N analysis, the obtained values are in good agreement with the calculated values. The observed low molar conductance values in DMSO solution (10^{-3} M) indicate that all the compounds are non-electrolytic in nature[16]. Fast atomic bombardment mass spectrum (FAB-MS) of the synthesized compounds show the molecular ion (*m/z*) peaks confirm the stoichiometry which is further confirmed by the observed analytical and their spectral data of the ligands. From the IR spectral data, the characteristic band at 1748-1720 cm^{-1} in all the compounds is due to the carbonyl (C=O) group of the tetrazine moiety[17].

^{1}H NMR and ^{13}C NMR spectra: Both the ^{1}H NMR and ^{13}C NMR spectra of the synthesized benzimidazotetrazinone derivatives (**1a-1i**) were recorded in $CDCl_3$ and DMSO-d_6 medium, tetramethylsilane (TMS) as internal standard (δ, in ppm) at room temperature. The representative ^{1}H NMR spectrum of compound **1h** was shown in Fig. 1. All the protons and carbons atoms in the structure are established to be in their predictable regions[18].

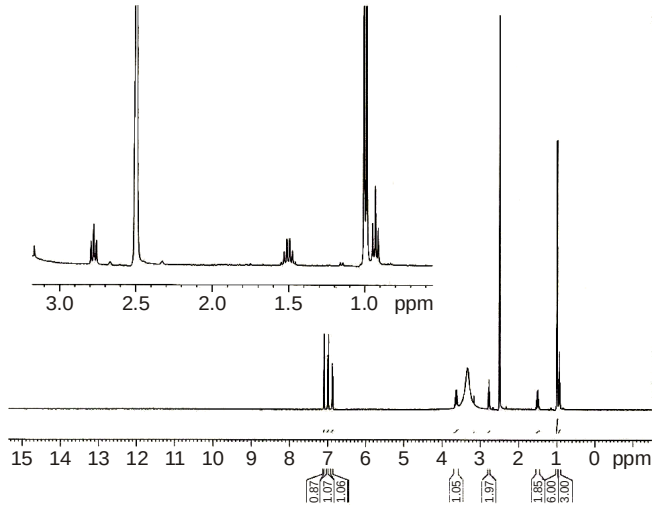


Fig. 1. ^{1}H NMR spectrum of compound (**1h**)

***In vitro* microbial activity:** *In vitro* microbial activities of the compounds in DMSO medium were tested against five pathogenic bacteria and three fungal strains by well diffusion method using agar as nutrient. Measured zone of inhibition (in mm) against the growth of bacterial and fungal for the above compounds are shown in Fig. 2.

On comparing the biological activities of the compounds with the commercially available standard drugs like tetracycline (antibacterial control) and nystatin (for antifungal) are used as control. The microbial activities of benzimidazotetrazinone derivatives (**1b, 1d, 1f and 1i**) show moderate activity against different types of microorganism as compared to the standard control drugs. Variations in the effectiveness of different biocidal species against microorganisms depend on the impermeability of the cell of the microbes or on differences in ribosome of microbial cells. Higher inhibition zones of synthesized molecules have been explained on the basis of Overtone's concept and Tweedy's chelation theory[19,20]. Also, it was established that the pathogenic bacterial species like *Bacillus subtilis, Staphylococcus aureus* and *Escherichia coli* show remarkable activities.

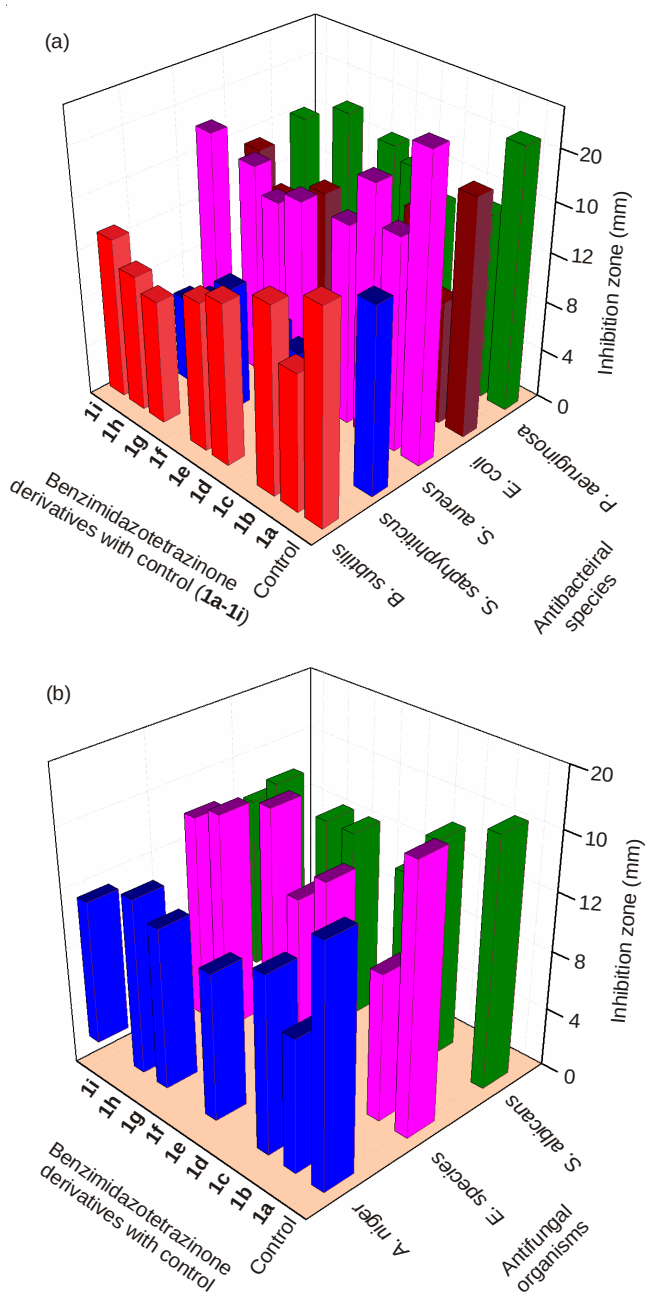


Fig. 2. *In vitro* (a) antibacterial and (b) antifungal activities of the compounds (**1a-1i**)

Antiproliferative activities: In the present study, the antiproliferative activity of the synthesized nine benzimidazotetrazinone derivatives (**1a-1i**), were selected for *in vitro* disease-oriented antitumor screenings against the four human tumor cell lines, including non-small lung, colon, prostate and breast cancers cell lines by the MTT assay. The potential cytotoxicities of all compounds were determined by measuring the percentage of cancers cells death/inhibition. The selected compounds were tested in various concentrations of 0, 1, 5, 10, 20, 40, 80,100 μM against human cancer cell lines (ATCC) *viz*., H1975 (non small lung), PC3 (Prostate), HCT116 (Colon) and MCF7 (Breast) to determine IC_{50} values using non-linear regression (log conc. *v/s* % inhibition) by Graph pad Prism 5.

The benzimidazotetrazinone derivatives **1a, 1c, 1d, 1e** and **1f** showed no significant activity, while the compounds (**1b**,

1g, **1h**, **1i**) showed antitumor activity against H1975, PC3, HCT116 and MCF7. Among the IC_{50} values of all tested compounds, **1b** showed 19.97 µM, 9.29 µM, 37.63 µM and 16.95 µM against H1975, PC3, MCF7 and HCT116 respectively. The compound **1h** showed IC_{50} 10.84 µM, 14.91 µM and 38.91 µM against H1975, MCF7 and HCT116 respectively. The compound **1g** and **1i** showed IC_{50} values of 8.84 µM and 36.13 µM against MCF7 respectively. The dose response curve and IC_{50} values of all tested compounds is shown in Fig. 3 and 4.

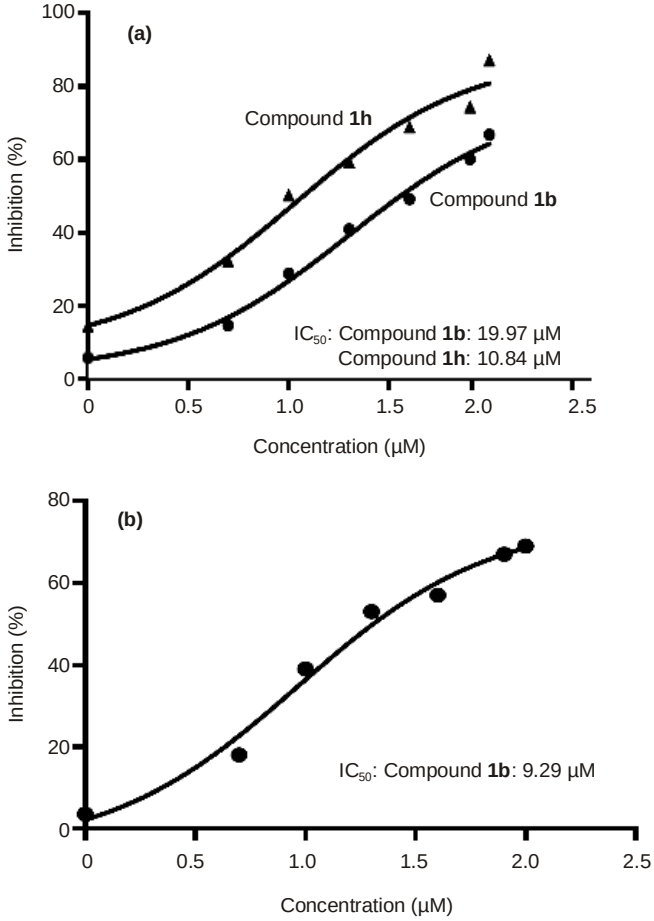
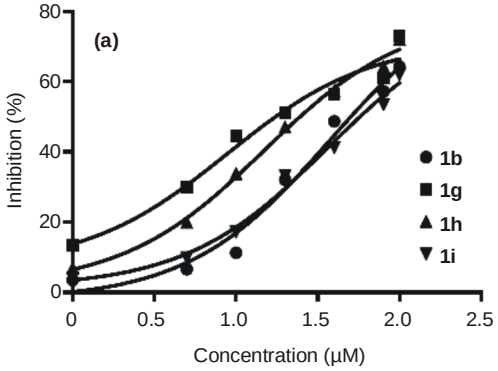


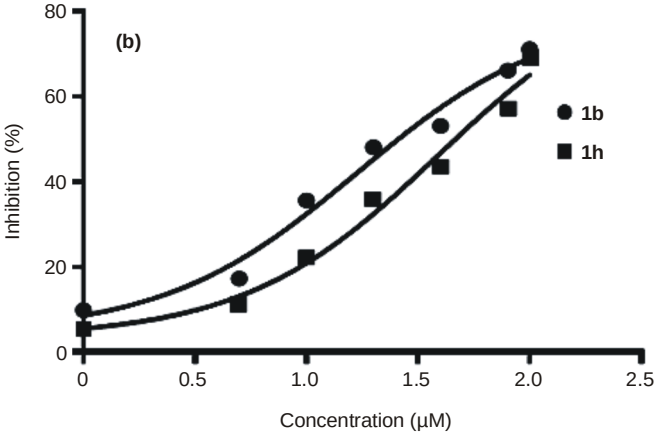
Fig. 3. Antiprolifeartive activity of (a) compounds (**1b** ane **1h**) on human non small lung carcinoma H1975 and (b) compound (**1b**) on human prostate carcinoma PC3

Conclusion

A highly efficient synthesis method is developed containing the new ring system benzimidazo [2,1-d][1,2,3,5]tetrazine-4(3H)-one by cycloaddition of isocyanates to the 5-propylthio-2-diazo-2H-benzimidazole. The tested new compounds **1b, 1g, 1h** and **1i** exhibited promising inhibitory activity in prostate, breast, lung, and colon cancer cells. The antimicrobial screening suggests that all the newly synthesized compounds, showed moderate to good activity against the tested organisms. Among the newly synthesized compounds (**1b, 1d, 1f** and **1i**) showed the most promising antibacterial and antifungal activity. Hence the fact that the compounds prepared in this study are chemically unrelated to the current medication, suggests that further work with similar analogues is clearly warranted.



	1b	1g	1h	1i
log (inhibitor) *vs.* response				
Best-fit values				
Bottom	89.04	71.70	79.27	80.68
Top	-2.302	7.199	1.606	1.306
log IC_{50}	1.575	0.9469	1.173	1.558
IC_{50}	37.63	8.849	14.91	36.13



	1b	1h
log (inhibitor) *vs.* response		
Best-fit values		
Bottom		88.90
Top		3.350
log IC_{50}		1.590
IC_{50}		38.91

Fig. 4. Antiprolifeartive activity of (a) compounds (**1b, 1g, 1h** and **1i**) on human breast carcinoma MCF7 and (b) compounds (**1b** and **1h**) on human colon carcinoma HCT116

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REFERENCES

1. H.H. Xu, X. Tao, Y.Q. Li, Y.Z. Shen and Y.H. Wei, *Polyhedron*, **33**, 347 (2012).
2. P. Diana, P. Barraja, A. Lauria, A. Montalbano, A.M. Almerico, G. Dattolo and G. Cirrincione, *Bioorg. Med. Chem.*, **11**, 2371 (2003).
3. D.A.F. Langnel, J. Arrowsmith and M.F.G. Stevens, *ARKIVOC*, 421 (2000).
4. J. Arrowsmith, S.A. Jennings, D.A.F. Langnel, R.T. Wheelhouse and M.F.G. Stevens, *J. Chem. Soc. Perkin Trans. I*, 4432 (2000).
5. P. Diana, A. Stagno, P. Barraja, A. Carbone, A. Montalbano, A. Martorana, G. Dattolo and G. Cirrincione, *ARKIVOC*, 1 (2009).
6. M.F.G. Stevens and E.S. Newlands, *Eur. J. Cancer*, **29**, 1045 (1993).
7. P. Barraja, P. Diana, A. Lauria, A. Montalbano, A.M. Almerico, G. Dattolo and G. Cirrincione, *Bioorg. Med. Chem.*, **13**, 295 (2005).
8. D. Liu, J.G. Yang, J. Cheng and L.X. Zhao, *Molecules*, **15**, 9427 (2010).
9. A.S. Clark, B. Deans, M.F.G. Stevens, M.J. Tisdale, R.T. Wheelhouse, B.J. Denny and J.A. Hartley, *J. Med. Chem.*, **38**, 1493 (1995).
10. M.F.G. Stevens, J.A. Hickman, S.P. Langdon, D. Chubb, L. Vickers, R. Stone, G. Baig, C. Goddard, N.W. Gibson, J.A. Slack, C. Newton, E. Lunt, C. Fizames and F. Lavelle, *Cancer Res.*, **47**, 5846 (1987).
11. D.D. Perrin, W.L.F. Armarego and D.R. Perrin, Purification of Laboratory Chemicals, Pergamon Press, Oxford (1980).
12. M.J. Pelczar, E.C.S. Chan and N.R. Krieg, *Microbiology, 5th ed.,* Blackwell Science, New York (1998).
13. J. Dharmaraja, T. Esakkidurai, P. Subbaraj and S. Shobana, *Spectrochim. Acta A*, **114**, 607 (2013).
14. P. Subbaraj, A. Ramu, N. Raman and J. Dharmaraja, *Spectrochim. Acta A*, **117**, 65 (2014).
15. T. Mosmann, *J. Immunol. Methods*, **65**, 55 (1983).
16. W.J. Geary, *Coord. Chem. Rev.*, **7**, 81 (1971).
17. K. Nakamoto, Infrared and Raman Spectra of Inorganic and Coordination Compounds, Wiley, New York (1986).
18. W.W. Simons, The Sadtler Handbook of Proton NMR Spectra, Sadtler Research Laboratories, Philadelphia, Sadtlerk (1978).
19. Y. Anjaneyulu and R.P. Rao, *Synth. React. Inorg. Met.-Org. Chem.*, **16**, 257 (1986).
20. B.G. Tweedy, *Phytopathology*, **55**, 910 (1964).