Send Orders for Reprints to reprints@benthamscience.ae

Letters in Drug Design & Discovery, 2018, *15*, 1299-1307

RESEARCH ARTICLE

Synthesis, Biological Evaluation and Docking Studies of 1,3,4-oxadiazole Fused Benzothiazole Derivatives for Anticancer Drugs

Madala Subramanyam[a], Reddymasu Sreenivasulu[b], Rambabu Gundla[c], Mandava V.B. Rao[d] and Koya Prabhakara Rao[a,*]

[a]*New Generation Materials Lab (NGML), Department of Science and Humanities, Vignan's Foundation for Science Technology and Research VFSTR (A deemed to be University), Vadlamudi, Guntur-522 213, Andhra Pradesh, India;* [b]*Department of Chemistry, University College of Engineering, Jawaharlal Nehru Technological University, Kakinada - 533 003, Andhra Pradesh, India;* [c]*Department of Chemistry, School of Technology, GITAM University, Hyderabad, 502 102, Telengana, India;* [d]*Department of Chemistry, Krishna University, Machilipatnam-521001, Andhra Pradesh, India*

Abstract: ***Background*:** Hetero atom containing compounds are well studied class of organic compounds exhibits variety of properties and applications. Design and synthesis of new heterocyclic compounds are always of great interest in synthetic and medicinal organic chemistry. Benzothiazole or 2-aminobenzothiazole scaffold based derivatives were reported to display a wide range of biological activities including anticancer, anti-tubercular, antiviral, fungicidal, *etc*. On the other hand, 1,3,4-oxadiazoles were permit to increase their biological activities due to H-bonding with receptors. These derivatives possess diverse biological activities which include anticancer, antiviral, antifungal, antibacterial and antidepressant *etc*. Due to interesting biological activity information of about these hetero cyclic moieties, benzothiazole/2-aminobenzothiazole and 1,3,4-oxadiazoles moieties, we chose to design a new series of heterocyclic compounds by mimicking these two types of scaffolds in a single molecule for our study.

***Methods*:** The 1,3,4-oxadiazole linked benzothiazole derivatives were synthesized by condensation of, 2-(4-(5-(benzo[d]thiazol-2-yl)-1,3,4-thiadiazol-2-yl)-2,6-dimethoxyphenoxy)acetohydrazide and $POCl_3$ under reflux conditions. All these ten compounds structures were confirmed by spectral data ^1H & ^{13}C NMR, Mass, CHN analysis *etc*. Further, these compounds were evaluated for their anticancer activity against four human cancer cell lines, A549, MCF7, A375 and HT-29 in comparison to CA4 as a reference drug. We also carried out docking studies of these compounds in the Colchicines binding site of Tubulin (PDB_ID: 1SA0) using Glide docking tool indicated that the ligands show good interactions with active site residues.

***Results*:** A new series of 1,3,4-oxadiazole fused benzothiazole derivatives were synthesized successfully in totally six steps starting with 4-hydroxy-3,5-dimethoxybenzoyl chloride. All these newly synthesized compounds structures were confirmed by spectral studies and elemental analysis. As we designed for anticancer activity, they were assessed for their anticancer activity against four human cancer cell lines in comparison to a reference drug CA4. As expected, all the ten compounds exhibited anticancer activities against four cancer cell lines with half maximal inhibitory concentration (IC_{50}) values ranging from 0.01 μM to 12.3 μM. The docking studies indicated all the compounds exhibited good binding energies with the receptor.

***Conclusion*:** In this study we designed a new series heterocyclic compounds by mimicking two types of scaffolds benzothiazole/2-aminobenzothiazole and 1,3,4-oxadiazoles moieties in a single molecule based on their biological activity in the literature. They were synthesized successfully and molecular structures were confirmed by spectral studies. As expected, all the compounds exhibited anticancer activities against four cancer cell lines. This study can provide a roadmap for design and synthesis of new drug molecules for antitumor and anticancer activity.

ARTICLE HISTORY

Received: September 28, 2017
Revised: December 26, 2017
Accepted: February 12, 2018

DOI:
10.2174/1570180815666180219165119

Keywords: Anticancer activity, 2-aminobenzothiazole, antitumor agents, 1,3,4-oxadiazole, docking studies, cytotoxicity.

*Address correspondence to this author at the New Generation Materials Lab (NGML), Department of Science and Humanities, Vignan's Foundation for Science Technology and Research VFSTR (A deemed to be University), Vadlamudi, Guntur-522 213, Andhra Pradesh, India; Tel/Fax: +91-863-234-4762, +91-863-253-4468; E-mails: kprao2005@gmail.com, drkpr_sh@vignanuniversity.org

1. INTRODUCTION

Medicinal chemistry plays a substantial role to ascertain a relationship between biological activity and chemical structure [1-5] of the molecules. Hetro atom (N, O, S) containing compounds are a well studied class of organic

1570-1808/18 $58.00+.00

compounds exhibiting a variety of properties and applications (1-15). Design and synthesis of new heterocyclic compounds are always of great interest in synthetic and medicinal organic chemistry. They have exhibited various applications in the design of a variety of biologically active molecules [6-12] and also for unique physical properties [13-15].

The benzothiazole skeleton is a heterocyclic aromatic moiety found naturally in Luciferin (**1**) (Fig. **1**), which displays remarkable applications [16-21] in medicinal and material science. It is well known that, the benzothiazole moiety in Luciferin found in fireflies, is known for its light-emitting properties. On the other hand, the 2-aminobenzothiazole scaffold based derivatives were reported to display anticancer activity in human cancer cell lines [22-24]. Among them, benzothiazoles are a privileged fused heterocyclic scaffold, which exhibit a wide range of biological activities including anticancer [25, 26], antitubercular [27], anticonvulsant [28], antiinflammtory [29], antidiabetic [30], antimicrobial [31], antimalarial [32], antiviral [33], fungicidal [34], *etc*.

On the other hand, 1,3,4-oxadiazoles were permit to increase their biological activities due to H-bonding with receptors. These derivatives possess a variety of biological activities which include anticancer [35-38], antiviral [39], antifungal [40], antibacterial [41], anti-inflammatory [42], anti-anxiety [43], antitubercular [44] and antidepressant [45]. The Zibotentan (**2**) (Fig. **1**) was a 1,3,4-oxadiazole skeleton containing most important anticancer drugs available in the market.

Given the above interesting biological activity information of benzothiazole and 1,3,4-oxadiazoles moieties, we chose to design a new series of hetrocyclic compounds by mimicking these two scaffolds in a single molecule for our study. We report the synthesis of a series of ten 1,3,4-oxadiazole fused benzothiazole derivatives and their characterization by ^{1}H & ^{13}CNMR, CHNS and mass spectral analysis. Moreover, we also report their anticancer activity studies of these new compounds against four human cancer cell lines A549, MCF7, A375 and HT-29, which are also supported by docking studies at the active site of Tubulin receptor.

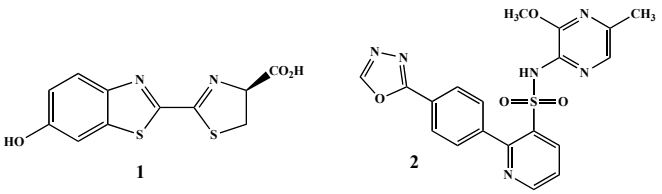


Fig. (1). Structures of Luciferin **1** and Zibotentan **2** drugs.

2. MATERIALS AND METHODS

2.1. General

All the chemicals and reagents used for this study were obtained from Sigma–Aldrich (St. Louis, MO, USA) and Lancaster (Alfa Aesar, Johnson Matthey Company, Ward Hill, MA, USA). They were used as procured without further purification. All the reactions of the present study were monitored by thin layer chromatography (TLC), attained on silica gel glass plates having 60 F-254, and visualization on TLC plates were observed by UV light [46]. ^{1}H and ^{13}C NMR spectra were recorded on Gemini Varian-VXR-unity (300 MHz) instrument and the chemical shift (δ) were reported in ppm downfield from internal tetramethysilane (TMS) standard [46]. Electrospray ionization (ESI) spectra were recorded on Micro mass, Quattro LC using ESI^{+} software package with capillary voltage of 3.98 kV and ESI mode was considered as positive ion trap detector [46]. CHN analysis was carried out using Vario Micro Cube Elementar instrument, Germany. The melting points were obtained with an electro-thermal melting point apparatus, and the values were reported without any further correction.

2.2. Synthesis

2.2.1. 4-Hydroxy-3,5-dimethoxybenzohydrazide (4)

A mixture of 4-hydroxy-3,5-dimethoxybenzoyl chloride (**3**) (18 g, 84.9 mmol) and hydrazine hydrochloride (8.7 g, 127.3 mmol) in dry THF and added triethyl amine (35 mL, 254.7 mmol) dropwise stirred at room temperature for 12 hours. The crude product was obtained by distilling off the excess of THF. The product was cooled, filtered and then washed with a little cold water yielded 15.3 g (87%). This product (**4**) was employed for the next step without any further purification. The spectral data of **4**, is as follows, Mp: 157^{o}C, ^{1}H NMR (300 MHz, DMSO-d6): δ 3.65 (s, 6H), 7.15 (s, 2H), 8.45 (brs, 1H), 9.74 (brs, 2H), 10.23 (brs, 1H); MS (ESI): 213 [M+H]$^{+}$, Elemental Anal. Calcd: C, 50.94; H, 5.70; N, 13.20; found: C, 50.91.01; H, 5.65; N, 13.14.

2.2.2. ***N'2-(4-Hydroxy-3,5-dimethoxybenzoyl)-1,3-benzothiazole-2-carbo hydrazide (6)***

A mixture of benzo[d]thiazole-2-carboxylic acid (**5**) (14 g, 78.1 mmol), EDCI (12.1 g, 78.1 mmol) and HOBt (105 mg, 0.72 mmol) in dry THF (50 mL) was stirred at room temperature for 15 minutes. To this reaction mixture, appropriate 4-hydroxy-3,5-dimethoxybenzohydrazide (**4**) (16.5 g, 78.1 mmol) was added and continued stirring at room temperature for 6 hours. The reaction contents were concentrated at reduced pressure, the solid 1,2-diacylhydrazine (**6**) was filtered off, washed with water and dried, yielded, 27.6 g, (95%), was used as such for the next step. The Spectral data for **6**, as follows, ^{1}H NMR (300 MHz, DMSO-d6): δ 3.76 (s, 6H), 6.98 (d, 1H, J = 8.05 Hz), 7.27 (s, 2H), 7.58-7.62 (m, 2H), 7.89 (d, 1H, J = 8.05 Hz), 9.78 (brs, 1H), 11.30 (brs, 1H); MS (ESI): 374 [M+H]$^{+}$; Elemental Anal. Calcd: C, 54.68; H, 4.05; N, 11.25; S, 8.59; found: C, 54.62; H, 4.01; N, 11.23; S, 8.52.

2.2.3. 4-[5-(1,3-Benzothiazol-2-yl)-1,3,4-thiadiazol-2-yl]-2,6-dimethoxyphenol (7)

A mixture of compound **6** (26 g, 69.7 mmol) and Lawesson's reagent (31 g, 76.6 mmol) in tetrahydrofuran (50 mL) was refluxed at 80^{o}C for 5 hours. After completion of the reaction as monitored by TLC, the crude product was adsorbed into silica gel and purified by column chromatography using ethyl acetate: hexane (7:3) as eluent to afford pure product **7**, 21.2 g, with 82% yield. The Spec-

tral data for **7**, as follows, ^{1}H NMR (300 MHz, DMSO-d6): δ 3.85 (s, 6H), 7.34 (s, 2H), 7.59-7.66 (m, 3H), 7.74 (d, 1H, *J* = 8.17 Hz), 10.07 (brs, 1H); MS (ESI): 372 $[M+H]^+$. Elemental Anal. Calcd: C, 54.97; H, 3.53; N, 11.31; S, 17.27; found: C, 54.95; H, 3.52; N, 11.28; S, 17.31.

2.2.4. Methyl 2-4-[5-(1,3-benzothiazol-2-yl)-1,3,4-thiadiazol-2-yl]-2,6-di methoxyphenoxyacetate (9)

The compound 4-[5-(1,3-benzothiazol-2-yl)-1,3,4-thiadiazol-2-yl]-2,6-dimethoxyphenol (**7**) (20 g, 53.9 mmol) was dissolved in 150 mL of dried acetone, followed by addition of methyl chloroacetate (**8**) (5.1 mL, 53.9 mmol) and K_2CO_3 (22.3 g, 161.7 mmol). The reaction mixture was heated under refluxed conditions for 6 hours. After completion of the reaction, K_2CO_3 was removed by filtration and the solvent was evaporated under vacuum to afford crude product. The crude product was purified by column chromatography with ethyl acetate/hexane (1:1) to afford pure compound 9, 22.8 g in 95%. The Spectral data for 9, as follows, Mp: 162-164^{o}C, ^{1}H NMR (300 MHz, DMSO-d6): δ 3.74 (s, 3H), 3.86 (s, 6H), 4.87 (s, 2H), 7.36 (s, 2H), 7.58-7.66 (m, 3H), 7.73 (d, 1H, *J* = 8.16 Hz); MS (ESI): 444 $[M+H]^+$; Elemental Anal. Calcd: C, 54.16; H, 3.86; N, 9.47; S, 14.46; found: C, 54.20; H, 3.90; N, 9.43; S, 14.41.

2.2.5. 2-4-[5-(1,3-Benzothiazol-2-yl)-1,3,4-thiadiazol-2-yl]-2,6-dimethoxy phenoxyethanohydrazide (10)

A mixture of methyl 2-4-[5-(1,3-benzothiazol-2-yl)-1,3,4-thiadiazol-2-yl]-2,6-dimethoxyphenoxyacetate (**9**) (20 g, 45.1 mmol) and hydrazine hydrate (42 mL, 135.4 mmol) in ethanol was refluxed for 6 hours. The crude product was obtained after distilling off with excess of ethanol, followed by cooling, filtering, and then washing with a little cold water. The product (**10**) was yielded 87% and employed in the next step without any further purification. The Spectral data for 10, is as follows, ^{1}H NMR (300 MHz, DMSO-d6): δ 3.88 (s, 6H), 4.88 (s, 2H), 7.56-7.64 (m, 3H), 7.69 (s, 2H), 7.73 (d, 1H, *J* = 8.17 Hz), 7.82 (brs, 1H), 8.13 (brs, 2H); MS (ESI): 444 $[M+H]^+$, Elemental Anal. Calcd: C, 51.46; H, 3.86; N, 15.79; S, 14.46; found: C, 51.43; H, 3.91; N, 15.77; S, 14.43.

2.2.6. 2-(4-[5-(1,3-Benzothiazol-2-yl)-1,3,4-thiadiazol-2-yl]-2,6-dimethoxy phenoxymethyl)-5-phenyl-1,3,4-oxadiazole (12a)

The compound 2-4-[5-(1,3-benzothiazol-2-yl)-1,3,4-thiadiazol-2-yl]-2,6-di methoxyphenoxyethanohydrazide (**10**) (500 mg, 1.12 mmol) was dissolved in $POCl_3$ (15 mL) and added benzoic acid (**11a**) (137 mg, 1.12 mmol). The reaction mixture was refluxed for 6 hours. After completion of reaction, it can be neutralized with aqueous $NaHCO_3$ and then workup with ethyl acetate. The organic layer can be evaporated through rotavapor and dried it with Na_2SO_4. The crude compound was purified by column chromatography with ethyl acetate/hexane (6:4) to afford compound **12a**, in 68% yield. Mp: 345–347^{o}C, ^{1}H NMR (300 MHz, DMSO-d6): δ 3.89 (s, 6H), 5.34 (s, 2H), 7.44 (s, 2H), 7.50-7.55 (m, 3H), 7.57-7.65 (m, 3H), 7.72 (d, 1H, *J* = 8.15 Hz), 7.85 (d, 2H, *J* = 8.13 Hz); ^{13}C NMR (75 MHz, DMSO-d6): δ 57.4, 68.5, 108.6, 123.4, 124.7, 127.3, 127.9, 128.2, 128.6, 130.2, 130.6, 131.7, 137.4, 143.8, 149.5, 151.3, 151.9, 154.3, 157.6, 159.4; MS (ESI): 530 $[M+H]^+$; Elemental Anal. Calcd: C, 58.97; H, 3.62; N, 13.22; S, 12.11; found: C, 58.91; H, 3.58; N, 13.18; S, 12.13.

Similar experimental procedure of **12a** was employed for all the remaining derivatives, **12b**-**12j** with yields between 59-85%. For spectral data (^{1}H & ^{13}C NMR and Mass), see supporting information Figs. **S1** to **S30**.

2.2.7. 2-(4-[5-(1,3-Benzothiazol-2-yl)-1,3,4-thiadiazol-2-yl]-2,6-dimethoxy phenoxymethyl)-5-(3,4,5-trimethoxyphenyl)-1,3,4-oxadiazole (12b)

The Spectral data for **12b**, as follows, 59% yield. Mp: 359–361^{o}C, ^{1}H NMR (300 MHz, DMSO-d6): δ 3.89 (s, 6H), 3.91 (s, 3H), 3.94 (s, 6H), 5.34 (s, 2H), 7.44 (s, 2H), 7.54-7.61 (m, 3H), 7.69 (d, 1H, *J* = 8.12 Hz), 7.75 (s, 2H); ^{13}C NMR (75 MHz, DMSO-d6): δ 57.6, 58.7, 61.3, 68.4, 105.4, 108.3, 123.4, 124.7, 126.4, 127.5, 127.9, 128.5, 137.6, 141.3, 143.6, 149.6, 151.2, 151.7, 154.7, 155.8, 157.7, 159.6, 160.6; MS (ESI): 620 $[M+H]^+$; Elemental Anal. Calcd: C, 56.21; H, 4.07; N, 11.30; S, 10.35; found: C, 56.25; H, 4.01; N, 11.28; S, 10.34.

2.2.8. 2-(4-[5-(1,3-Benzothiazol-2-yl)-1,3,4-thiadiazol-2-yl]-2,6-dimethoxy phenoxymethyl)-5-(4-methoxyphenyl)-1,3,4-oxadiazole (12c)

Spectral data for **12c**, as follows, 67% yield. Mp: 355–357^{o}C, ^{1}H NMR (300 MHz, DMSO-d6): δ 3.89 (s, 6H), 3.91 (s, 3H), 5.34 (s, 2H), 7.18 (d, 2H, J = 8.13 Hz), 7.43 (s, 2H), 7.54-7.61 (m, 3H), 7.70 (d, 1H, J = 8.10 Hz), 7.87 (d, 2H, J = 8.13 Hz); ^{13}C NMR (75 MHz, DMSO-d6): δ 56.5, 58.5, 68.4, 108.6, 117.5, 118.7, 123.4, 124.6, 124.9, 127.4, 127.8, 128.7, 137.5, 143.6, 149.7, 151.3, 151.8, 154.7, 154.9, 157.8, 159.7, 160.5, 160.9; MS (ESI): 560 $[M+H]^+$; Elemental Anal. Calcd: C, 57.95; H, 3.78; N, 12.51; S, 11.46; found: C, 57.97; H, 3.79; N, 12.55; S, 11.48.

2.2.9. 2-(4-[5-(1,3-Benzothiazol-2-yl)-1,3,4-thiadiazol-2-yl]-2,6-dimethoxy phenoxymethyl)-5-(4-chlorophenyl)-1,3,4-oxadiazole (12d)

Spectral data for **12d**, as follows, 85% yield. Mp: 361–363^{o}C, ^{1}H NMR (300 MHz, DMSO-d6): δ 3.89 (s, 6H), 5.34 (s, 2H), 7.44 (s, 2H), 7.54-7.61 (m, 3H), 7.69 (d, 1H, *J* = 8.12 Hz), 7.77 (d, 2H, *J* = 8.19 Hz), 7.85 (d, 2H, *J* = 8.19 Hz); ^{13}C NMR (75 MHz, DMSO-d6): δ 57.6, 68.6, 108.7, 123.6, 124.7, 127.6, 128.6, 128.9, 129.4, 129.8, 131.5, 137.5, 138.7, 143.6, 149.6, 151.2, 152.8, 154.6, 157.5, 159.7, 160.8; MS (ESI): 565 $[M+H]^+$; Elemental Anal. Calcd: C, 55.37; H, 3.22; N, 12.42; S, 11.37; found: C, 55.41; H, 3.20; N, 12.38; S, 11.36.

2.2.10. 2-(4-[5-(1,3-Benzothiazol-2-yl)-1,3,4-thiadiazol-2-yl]-2,6-dimethoxy phenoxymethyl)-5-(4-bromophenyl)-1,3,4-oxadiazole (12e)

Spectral data for **12e**, as follows, 81% yield. Mp: 364–366^{o}C, ^{1}H NMR (300 MHz, DMSO-d6): δ 3.89 (s, 6H), 5.34 (s, 2H), 7.44 (s, 2H), 7.56-7.78 (m, 6H), 7.86 (d, 2H, J = 8.20 Hz); ^{13}C NMR (75 MHz, DMSO-d6): δ 57.4, 68.5, 108.6, 123.9, 124.8, 126.5, 126.8, 127.4, 127.9, 128.3, 129.3, 133.4,

137.4, 143.6, 149.6, 151.3, 151.8, 154.7, 157.6, 159.6, 160.5; MS (ESI): 609 $[M+H]^+$; Elemental Anal. Calcd: C, 51.32; H, 2.98; N, 11.51; S, 10.54; found: C, 51.30; H, 3.00; N, 11.47; S, 10.55.

2.2.11. 2-(4-[5-(1,3-Benzothiazol-2-yl)-1,3,4-thiadiazol-2-yl]-2,6-dimethoxy phenoxymethyl)-5-(4-fluorophenyl)-1,3,4-oxadiazole (12f)

Spectral data for **12f**, as follows, 80% yield. Mp: 337–339°C, ^{1}H NMR (300 MHz, DMSO-d6): δ 3.89 (s, 6H), 5.34 (s, 2H), 7.32 (d, 2H, J = 8.09 Hz), 7.44 (s, 2H), 7.56-7.62 (m, 3H), 7.69 (d, 1H, J = 8.12 Hz), 7.84 (d, 2H, J = 8.09 Hz); ^{13}C NMR (75 MHz, DMSO-d6): δ 57.6, 68.5, 108.4, 117.4, 123.7, 124.4, 126.7, 127.5, 127.9, 128.4, 137.4, 143.3, 149.5, 151.5, 151.8, 154.7, 157.8, 159.5, 160.4, 160.9; MS (ESI): 548 $[M+H]^+$; Elemental Anal. Calcd: C, 57.03; H, 3.31; N, 12.79; S, 11.71; found: C, 57.02; H, 3.28; N, 12.78; S, 11.73.

2.2.12. 2-(4-[5-(1,3-Benzothiazol-2-yl)-1,3,4-thiadiazol-2-yl]-2,6-dimethoxy phenoxymethyl)-5-(4-nitrophenyl)-1,3,4-oxadiazole (12g)

Spectral data for **12g**, as follows, 85% yield. Mp: 358–360°C, ^{1}H NMR (300 MHz, DMSO-d6): δ 3.89 (s, 6H), 5.34 (s, 2H), 7.44 (s, 2H), 7.54-7.61 (m, 3H), 7.70 (d, 1H, J = 8.13 Hz), 7.83 (d, 2H, J = 8.22 Hz), 8.12 (d, 2H, J = 8.22 Hz); ^{13}C NMR (75 MHz, DMSO-d6): δ 57.8, 68.6, 108.7, 123.5, 124.8, 124.9, 126.4, 127.4, 127.8, 128.7, 136.5, 137.8, 143.6, 149.5, 149.8, 151.2, 151.8, 154.6, 157.8, 159.8, 160.9; MS (ESI): 575 $[M+H]^+$; Elemental Anal. Calcd: C, 54.35; H, 3.16; N, 14.63; S, 11.16; found: C, 54.32; H, 3.17; N, 14.58; S, 11.15.

2.2.13. 4-[5-(4-[5-(1,3-Benzothiazol-2-yl)-1,3,4-thiadiazol-2-yl]-2,6-dimethoxy phenoxymethyl)-1,3,4-oxadiazol-2-yl]benzonitrile (12h)

Spectral data for **12h**, as follows, 85% yield. Mp: 365–367°C, ^{1}H NMR (300 MHz, DMSO-d6): δ 3.89 (s, 6H), 5.34 (s, 2H), 7.44 (s, 2H), 7.54-7.63 (m, 3H), 7.70 (d, 1H, J = 8.14 Hz), 7.76 (d, 2H, J = 8.22 Hz), 7.85 (d, 2H, J = 8.22 Hz); ^{13}C NMR (75 MHz, DMSO-d6): δ 57.4, 68.5, 108.9, 115.6, 119.5, 123.4, 124.9, 127.3, 127.9, 128.6, 128.9, 134.4, 134.9, 137.6, 143.5, 149.7, 151.2, 151.8, 154.7, 157.8, 159.6, 160.9; MS (ESI): 555 $[M+H]^+$. Elemental Anal. Calcd: C, 58.47; H, 3.27; N, 15.15; S, 11.56; found: C, 58.45; H, 3.22; N, 15.16; S, 11.57.

2.2.14. 2-(4-[5-(1,3-Benzothiazol-2-yl)-1,3,4-thiadiazol-2-yl]-2,6-dimethoxy phenoxymethyl)-5-(4-methylphenyl)-1,3,4-oxadiazole (12i)

Spectral data for **12i**, as follows, 66% yield. Mp: 359–361°C, ^{1}H NMR (300 MHz, DMSO-d6): δ 2.35 (s, 3H), 3.89 (s, 6H), 5.34 (s, 2H), 7.28 (d, 2H, J = 8.08 Hz), 7.44 (s, 2H), 7.54-7.61 (m, 3H), 7.69 (d, 1H, J = 8.12 Hz), 7.76 (d, 2H, J = 8.08 Hz); ^{13}C NMR (75 MHz, DMSO-d6): δ 23.6, 57.8, 68.6, 108.9, 123.8, 124.7, 126.4, 127.3, 127.8, 128.2, 128.9, 131.4, 137.5, 141.3, 143.8, 149.7, 151.2, 151.8, 154.6, 157.8, 159.5, 160.6; MS (ESI): 544 $[M+H]^+$; Elemental Anal. Calcd: C, 59.65; H, 3.89; N, 12.88; S, 11.80; found: C, 59.66; H, 3.91; N, 12.88; S, 11.82.

2.2.15. 2-(4-[5-(1,3-Benzothiazol-2-yl)-1,3,4-thiadiazol-2-yl]-2,6-dimethoxy phenoxymethyl)-5-[4-(trifluoromethyl) phenyl]-1,3,4-oxadiazole (12j)

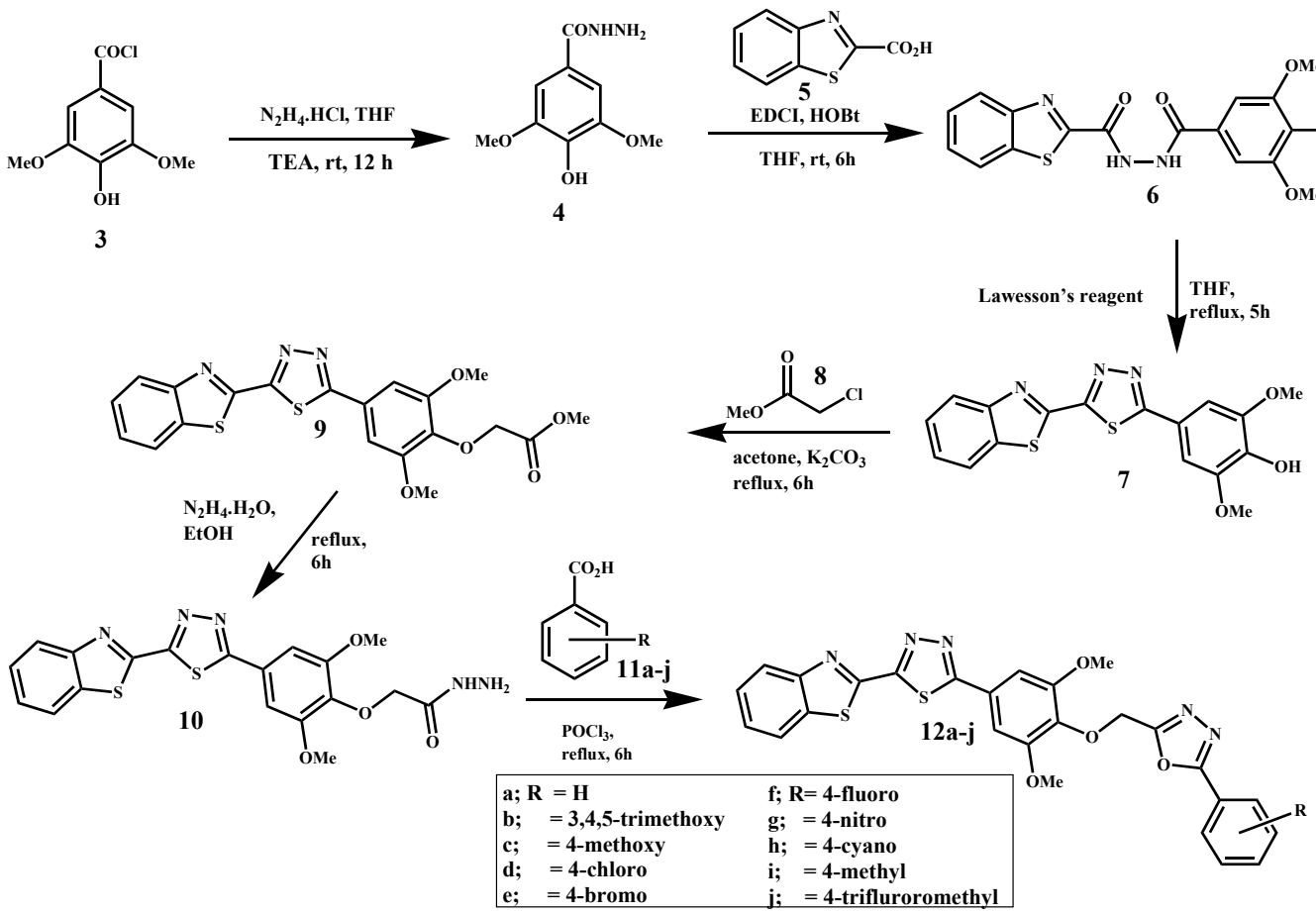
Spectral data for **12j**, as follows, 63% yield. Mp: 344–346°C, ^{1}H NMR (300 MHz, DMSO-d6): δ 3.89 (s, 6H), 5.34 (s, 2H), 7.44 (s, 2H), 7.54-7.61 (m, 3H), 7.70 (d, 1H, J = 8.14 Hz), 7.79 (d, 2H, J = 8.11 Hz), 8.13 (d, 2H, J = 8.11 Hz); ^{13}C NMR (75 MHz, DMSO-d6): δ 57.4, 68.6, 108.9, 121.4, 123.6, 124.8, 127.4, 127.9, 128.3, 129.5, 132.4, 137.5, 143.6, 149.5, 151.3, 151.9, 154.6, 157.6, 159.7, 160.9; MS (ESI): 598 $[M+H]^+$; Elemental Anal. Calcd: C, 54.27; H, 3.04; N, 11.72; S, 10.73; found: C, 54.32; H, 3.01; N, 11.70; S, 10.72.

2.3. MTT (3-(4,5-dimethylthiazol-2-yl)-2 5-diphenyltetrazolium Bromide) Assay

The cytotoxic activity of the compounds was determined using MTT assay. 1×10^4 cells/well were seeded in 200 ml Dulbecco's Modified Eagle's medium (DMEM), supplemented with 10% Fetal bovine serum (FBS) in each well of 96-well microculture plates and incubated for 24 hours at 37°C in a CO_2 incubator. Compounds, diluted to the desired concentrations in culture medium, were added to the wells with respective untreated cells. After 48 hours of incubation, 10 ml MTT was added to each well and the plates were further incubated for 4 hours. Then the supernatant from each well was carefully removed, formazan crystals were dissolved in 100 ml of Dimethyl sulfoxide (DMSO) and absorbance at 540 nm wavelength was recorded. Here, the anticancer activities of synthesized compounds were recorded over three replications at 2 μM concentration levels. Our target molecules having oxadiazole moiety lie in between two phenyl rings, structurally more similar to Combretastatin A4, prompted us to use as a standard drug over other commonly used drugs like Taxol derivative, such as paclitaxel and docetaxel.

2.4. Docking Studies

The binding mode of the compounds at the active site of Tubulin receptor was explored using Glide (Glide, Schrödinger, LLC, New York, NY, 2017). The PDB_ID 1SA0 was used for docking study [47-50]. Docking studies were executed with the solved 3D-structure of Tubulin (PDB_ID:1SA0), using Glide module in Schrodinger. The authenticity of this docking method to envisage the bioactive conformation was confirmed using the X-ray structure of Colcinines in complex. Co-crystal Colchicines was redocked into the active site of Tubulin receptor and the best predicted bound conformation having lowest docking energy was selected. The superimposition of the Glide docked pose of designed ligands with the co-crystal of 1SA0 exhibited good root mean square deviation (RMSD) value. The RMSD value between designed inhibitors and the reference molecule poses, 0.60 Å, showed a high docking authenticity of glide in terms of replicating the experimentally observed binding mode for the ligands [50].



Scheme 1. Synthesis of Oxadiazole fused benzothiazole derivatives.

2.5. Ligand Preparation

All the ligands for docking study were constructed using maestro software and later they were prepared for docking using lig-Prep module (Schrödinger, LLC, New York, NY, 2017) [50]. The ligand structures were submitted to the Polak-Ribiere Conjugate Gradient (PRCG) energy minimization using the OPLS 2005 force field until the energy difference between subsequent structures was reached to 0.001 kJ/molÅ. The possible tautomers of the ligands sustained original stereochemistry and were explored using Lig-Prep (Version 2.5, Schrodinger, LLC, New York, NY, 2011) [50].

2.6. Preparation of Protein Structures

The crystal structure of tubulin (PDB_ID: 1SA0) was considered in order to understand the insights of the binding modes of all the ligands [50]. The 3D structure of Tubulin was prepared using the Protein preparation wizard implemented in Maestro 9.5 (Schrodinger, LLC, New York, NY, 2011) by observing the following steps: (i) the missing side chains were added to the crystal structure by Schrodinger's Prime 3.0. Program. (ii) Hydrogen bonds were added and water molecules within 5 Å of the co-crystallized ligand were removed. (iii) Protonation states of entire systems were adjusted to the pH range of 7.0 +/– 4.0 using Epik. (iv) Hydrogen bond networks and flip orientations/tautomeric states of Gln, Asn, and His residues were optimized. (v) The geometry optimization was performed to a maximum RMSD value of 0.3 Å with the OPLS2005 force field [50].

2.7. Receptor Grid Generation

Grid was generated using receptor grid generation in Glide program [50]. The binding region was defined using a grid of centroid of the co-crystal ligand.

3. RESULTS AND DISCUSSION

3.1. Chemistry

A series of ten new oxadiazole fused benzothiazole derivatives (**12a-j**) were synthesized as described in Scheme **1**. In the first step, compound **4** was synthesized by taking starting material, 4-hydroxy-3,5-dimethoxybenzoyl chloride (**3**), which was coupled with hydrazine hydrochloride in the presence of triethylamine in tetrahydrofuran (**THF**) at room temperature, stirring for 12 hours to afford acid hydrazide derivative (**4**). In the 2nd step, this intermediate **4**, was coupled with benzo[d]thiazole-2-carboxylic acid (**5**) in the presence of 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide (**EDCI**), Hydroxybenzotriazole (**HOBt**) in dry THF at room temperature, stirring for 6 hours to afford compound **6**, in good yield. In the 3rd step, **6** was cyclized in the presence of Lawesson's reagent in THF and the reaction mixture was refluxed for 5 hours to afford pure 4-(5-(benzo[d]thiazol-2-

yl)-1,3,4-thiadiazol-2-yl)-2,6-dimethoxyphenol (**7**). Later, this intermediate **7**, was converted to etherification with methyl 2-chloroacetate (**8**) in the presence of K_2CO_3 in dry acetone by refluxed conditions for 6 hours to afford pure methyl 2-(4-(5-(benzo[d]thiazol-2-yl)-1,3,4-thiadiazol-2-yl)-2,6-dimethoxyphenoxy) acetate (**9**) [47]. In the 5th step, **9,** was reacted with hydrazine hydrate in ethanol at refluxed conditions for 6 hours to afford its hydrazide derivative, **10**. In the final step, compound **10** was cyclized with respective substituted aromatic acids (**11a-j**) in $POCl_3$ at refluxed conditions for 6 hours to afford targeted compounds (**12a-j**) (Scheme **1**).

3.2. Biological Evaluation (*In Vitro* Cytotoxicity)

As we designed and planned for *in vitro* cytotoxicity study, these ten newly synthesized compounds were assessed [47] for their anticancer activity against four human cancer cell lines, such as A549 (Lung cancer), MCF7 (Breast cancer), A375 (Melanoma cancer) and HT-29 (Colon cancer) in comparison to a reference drug CA4 and the results are listed in Table **1**. In fact, all the ten compounds exhibited anticancer activities against four cancer cell lines with half maximal inhibitory concentration (IC_{50}) values ranging from 0.01 μM to 12.3 μM. The compound **12b** exhibited exceptional anticancer activities on A549 and MCF-7 with IC_{50} values of 0.02 μM and 0.01 μM, respectively. While, the compound **12h** showed potent anticancer activities against A375 and MCF-7 with IC_{50} values of 0.01 μM and 0.02 μM, respectively. The above activities of **12b** and **12h** compounds are more potent than the standard drug, 'Combretastatin – A_4 and these two compounds could be potential candidates for cancer chemotherapy treatment. Moreover, the compounds **12a** and **12g** displayed anticancer activities against A375 and MCF-7 with IC_{50} values of 0.09 μM and 0.04 μM, respectively. Similarly, the compound **12i** showed good anticancer activity on MCF-7 and A375 with IC_{50} values of 0.15 μM and 0.19 μM, respectively. Whereas, the compounds **12d** and **12g** exhibited modest anticancer activities against A549 cancer cell line with IC_{50} values of 0.23 μM and 0.33 μM, respectively. Correspondingly, compound, **12a** on MCF-7 with an IC_{50} value of 0.22 μM and compound, **12b** on A375 with an IC_{50} value of 0.67 μM, respectively also displayed modest anticancer activities. All the remaining compounds showed less anticancer activities on the above mentioned four cancer cell lines.

3.3. Molecular Modelling and Drug Designing

To investigate the possible mode of binding of these new synthesised 1,3,4-Oxadiazole Fused Benzothiazole Derivatives (12a-j), we carried out docking studies of 12a-j in the Colchicines binding site of Tubulin (PDB_ID: 1SA0) using Glide docking tool. The docked ligand show good interactions with active site residues like Colchicines (co-crystal ligand): GLN247, IEU248, ASN249, ALA250, ASP251, LEU255, ALA256, ASN258, MET259, ASN349, VAL350. Among the above interactive residues of the receptor, ligands showed hydrogen bond network with ASN250. The active compound 12b also showed similar type of interactions with CYS241, LEU248, ALP25, LYS254, ASN258, ALA366 shown in Fig. **S31** (Supporting information). The drug like behavior of the 1,3,4-Oxadiazole Fused Benzothiazole Derivatives were assessed by *in silico* predictions. Some of the parameters such as molecular weight (MW), docking score, number of hydrogen bond donors and acceptors (HBD and HBA), number of rotatable groups (rotor) *etc*. are given in Table **2**. 12b compounds showed exceptional anticancer activities on A549 and MCF-7 with IC_{50} values of 0.02 μM and 0.01 μM (Table **1**), respectively, which also showed good docking score (-11.28), HBD (0) and HBA (10.5) values (Table **2**). Similarly, the compound 12h, showed potent anticancer activities against A375 and MCF-7 with IC_{50} values of 0.01 μM and 0.02 μM, respectively and also

Table 1. ***In vitro*** **cytotoxicity (IC_{50} μM) data of compounds 12a-j.**

Compound	A549	MCF-7	A375	HT-29
12a	1.90±0.11	0.22±0.01	**0.09±0.005**	8.70±0.31
12b	**0.02±0.001**	**0.01±0.007**	0.67±0.029	-
12c	3.78±0.23	10.4±0.45	9.34±0.35	-
12d	0.23±0.012	1.55±0.06	4.78±0.21	7.89±0.22
12e	6.23±0.34	7.10±0.33	-	-
12f	5.10±0.26	12.3±0.49	-	14.9±0.46
12g	0.33±0.015	**0.04±0.007**	1.22±0.06	2.45±0.012
12h	1.34±0.03	**0.02±0.001**	**0.01±0.0009**	1.89±0.09
12i	2.67±0.11	3.56±0.18	4.89±0.16	-
12j	1.77±0.09	0.15±0.007	0.19±0.08	1.45±0.03
CA4	0.11±0.004	0.18±0.01	0.21±0.008	0.93±0.01

CA4= Combretastatin-A4., "-" = not active.

Table 2. ***In silico*** **ADME docking properties of synthesized compounds 12a-j.**

S. No.	Molecule	Docking Score	M.W.	HBA	HBD	Rotatable Bond	Rule of 5 Violation
1	**12a**	-10.35	529.58	8.2	0	5	1
2	**12b**	-11.28	619.66	10.5	0	8	3
3	**12c**	-9.46	559.61	9.0	0	6	1
4	**12d**	-10.38	564.63	8.2	0	5	2
5	**12e**	-8.63	608.48	9.2	0	5	2
6	**12f**	-8.89	547.57	8.2	0	5	2
7	**12g**	-10.37	529.58	8.2	0	5	1
8	**12h**	-10.38	554.59	9.7	0	6	1
9	**12i**	-9.49	597.58	8.2	0	5	2
10	**12j**	-10.39	543.61	8.2	0	5	2
Ref. Cmpd	Colchicines	-10.8	431.50	7.0	1.8	7	0

showed good docking score, HBD and HBA. Whereas, moderate anticancer active compounds showed moderate docking values and *vice versa*.

CONCLUSION

In conclusion, we designed and synthesized a series of ten new compounds, 1,3,4-oxadiazole linked benzothiazole derivatives **(12a-j)** and their structures were confirmed by ^{1}H-NMR, ^{13}C-NMR, CHN analysis and mass spectral data. The synthesized compounds were screened for their anticancer activity against four human cancer cell lines A549 (Lung cancer), MCF7 (Breast cancer), A375 (Melanoma cancer) and HT-29 (Colon cancer) by MTT method. The combretastatin-A4 used as positive control and results are expressed in IC_{50} μM. Among them, compounds **12a, 12b, 12d, 12g, 12h** and **12j,** exhibited more potent activities superior to control drug. Docking studies of these compounds in the Colchicines binding site of Tubulin (PDB_ID: 1SA0) using Glide docking tool indicated that the ligands show good interactions with active site residues like Colchicines (co-crystal ligand): GLN247, IEU248, ASN249, ALA250, ASP251, LEU255, ALA256, ASN258, MET259, ASN349, VAL350. Among the above interactive residues of the receptor, ligands showed hydrogen bond network with ASN250. We strongly believe that, this study can provide a roadmap to design and synthesize new drug molecules for antitumor and anticancer activity applications.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS

Madala Subramanyam (M. S) acknowledges the VFSTR University for the research avenues and funding. M. S acknowledges Dr. G. Narasimha Rao, principal, JVR Govt. College, Sathupally for his constant support and encouragement for carrying his Ph. D. work. Koya Prabhakara Rao (K. P. Rao) acknowledges DST-SERB, for financial support, project for early career, project no. **EMR/2014/001114**.

SUPPLEMENTARY MATERIAL

Supplementary material is available on the publisher's web site along with the published article.

REFERENCES

[1] Remington, M.D. *The Science and Practice of Pharmacy*. 19th ed. MACK Publishing Company: Pennsylvania, **1995**.

[2] Gilchrist, T.L. *Heterocyclic Chemistry*, 3rd ed. Addison Wesley: England, **1997**.

[3] Reddy, N.B.; Burra, V.R.; Ravindranath, L.K.; Kumar, V.N.; Sreenivasulu, R.; Sadanandam, P. Synthesis and biological evaluation of benzimidazole fused ellipticine derivatives as anticancer agents. *Monatsh. Chem*., **2016**, *147*, 599-604.

[4] Padmaja, P.; Yedukondalu, M.; Sridhar, R.; Busi, S.; Rao, M.V.B. Synthesis and antimicrobial screening of novel 3, 5-disubstituted indazole derivatives. *Lett. Drug. Des. Disc*., **2013**, *10*, 625-631.

[5] Mehta, B.K.; Rambabu, D.; Raja, G.; Prasad, A.S.G.; Fang, J.-M.; Rao, M.V.B. Synthesis of novel macrocyclic tetraamides. *Lett. Drug. Des Disc*., **2014**, *11*, 756-761.

[6] Ahsan, M.J.; Choudhary, K.; Jadav, S.S.; Yasmin, S.; Ansari, M.Y.; Sreenivasulu, R. Synthesis, antiproliferative activity, and molecular docking studies of curcumin analogues bearing pyrazole ring. *Med. Chem. Res*., **2015**, *24*, 4166-4180.

[7] Gaudino, E.C.; Tagliapietra, S.; Martina, K.; Palmisano, G.; Cravotto, G. Recent advances and perspectives in the synthesis of bioactive coumarins. *RSC. Adv*., **2016**, *6*, 46394-46405.

[8] Srinivas, B.; Suryachandram, J.; Devi, Y.K.; Rao, K.P. Synthesis and antibacterial activity studies of 8,9-dihydro [7h] benzo 1,2,4-oxadiazoles and its coumarin derivatives. *J. Hetro. Cyclic. Chem*., **2017**, *54*, 3730-3734.

[9] Liu, J.; Shah, M.B.; Zhang, Q.; Stout, C.D.; Halpert, J.R.; Wilderman, P.R. Coumarin derivatives as substrate probes of mammalian

cytochromes P450 2B4 and 2B6: Assessing the importance of 7-alkoxyahain length, halogen substitution, and non-active site mutations. *Bio*. *Chem*., **2016**, *55*, 1997-2007.

[10] Yadlapalli, R.K.; Chourasia, O.P.; Jogi, M.P.; Podile, A.; Sridhar, P.R. Design, synthesis and *in vitro* antimicrobial activity of novel phenylbenzamido-aminothiazole-based azasterol mimics. *Med*. *Chem*. *Res*., **2013**, *22*, 2975-2983.

[11] Yadlapalli, R.K.; Chourasia, O.P.; Vemuri, K.; Sritharan, M.; Sridhar, P.R. Synthesis and *in vitro* anticancer and antitubercular activity of diarylpyrazole ligated dihydropyrimidines possessing lipophilic carbamoyl group. *Bioorg*. *Med*. *Chem*. *Lett*., **2012**, *22*, 2708-2711.

[12] Basha, N.M.; Lavanya, G.; Padmaja, A.; Padmavathi, V. Synthesis and antioxidant activities of acetamidomethylsulfonyl bis heterocycles oxazolyl/thiazolyl/imidazolyl-1,3,4-oxadiazoles. *Arch*. *Pharm*. *Chem*. *Life*. *Sci*., **2013**, *346*, 511-520.

[13] Rao, K.P.; Kusamoto, T.; Toshimitsu, F.; Inayoshi, K.; Kume, S.; Yamamoto, Y.; Sakamoto, R.; Nishihara, H. Double protonation of 1,5-Bis(triarylaminoethynyl)anthraquinone to form a paramagnetic pentacyclic dipyrylium salt. *J*. *Am*. *Chem*. *Soc*., **2010**, *132*, 12472-12479.

[14] Rao, K.P.; Kondo, M.; Sakamoto, R.; Kusamoto, T.; Kume, S.; Nihei, M.; Oshio, H.; Nishihara, H. Benzo[e]pyrene skeleton dipyrylium dication with a strong Donor-Acceptor-Donor interaction, and its two-electron reduced molecule. *Chem*. *Eur*. *J*., **2011**, *17*, 14010-14019.

[15] Sakamoto, R.; Rao, K.P.; Nishihara, H. Arylethynylanthraquinone and bis(arylethynyl)anthraquinone: Strong Donor-Acceptor interaction and proton-induced cyclization to form pyrylium and dipyrylium salts. *Chem*. *Lett*., **2011**, *40*, 1316-1326.

[16] Hastings, J.W. Chemistries and colors of bioluminescent reactions: A review. *Gene*, ***1996***, *173*, 5-11.

[17] Hastings, J.W. Biological diversity, chemical mechanisms, and the evolutionary origins of bioluminescent systems. *J*. *Mol*. *Evol*., **1983**, *19*, 309-321.

[18] Dunlap, J.C.; Hastings, J.W.; Shimomura, O. Crossreactivity between the light-emitting systems of distantly related organisms: Novel type of light-emitting compound. *Proc*. *Natl*. *Acad*. *Sci*. *USA*, **1980**, *77*, 1394-1397.

[19] Sreenivasulu, R.; Sujitha, P.; Jadav, S.S.; Ahsan, M.J.; Kumar, C.G.; Raju, R.R. Synthesis, antitumor evaluation, and molecular docking studies of indole-indazolyl hydrazide-hydrazone derivatives. *Monatsh*. *Chem*., **2017**, *148*, 305-314.

[20] Brancale, A.; Silvestrim, R. Indole, a core nucleus for potent inhibitors of tubulin polymerization. *Med*. *Res*. *Rev*., **2007**, *27*, 209-238.

[21] Singh, P.; Kaur, M.; Holzer, W. Synthesis and evaluation of indole, pyrazole, chromone and pyrimidine based conjugates for tumor growth inhibitory activities-development of highly efficacious cytotoxic agents. *Eur*. *J*. *Med*. *Chem*., **2010**, *45*, 4968-4982.

[22] Rezaei, Z.; Moghimi, S.; Javaheri, R.; Asadi, M.; Mahdavi, M.; Shabani, S.; Edraki, N.; Firuzi, O.; Safavi, M.; Amini, M.; Asadipour, A.; Zeinalzadeh, E.; Firoozpour, L.; Foroumadi, A. Synthesis and biological evaluation of 1,3,4-thiadiazole linked phthalimide derivatives as anticancer agents. *Lett*. *Drug*. *Des*. *Disc*., **2017**, *14*, 1138-1144.

[23] Reddy, P.; Lin, Y.; Chang, H. Synthesis of novel benzothiazole compounds with an extended conjugated system. *Arkivoc*, **2007**, *16*, 113-122.

[24] Piscitelli, F.; Ballatore, C.; Smith. A. Solid phase synthesis of 2-aminobenzothiazoles. *Bioorg*. *Med*. *Chem*. *Lett*., **2010**, *20*, 644-648.

[25] Huang, S.T.; Hsei, I.J.; Chen, C. Synthesis and anticancer evaluation of bis(benzimidazoles), bis(benzoxazoles), and benzothiazoles. *Bioorg*. *Med*. *Chem*., **2006**, *14*, 6106-6119.

[26] Akguna, H.; Yilmaza, D.U.; Atalay, R.C.; Gozen, D. A series of 2,4(1*H*,3*H*)-quinazolinedione derivatives: Synthesis and biological evaluation as potential anticancer agents. *Lett*. *Drug*. *Des*. *Disc*., **2016**, *13*, 64-76.

[27] Palmer, F.J.; Trigg, R.B.; Warrington, J.V. Benzothiazolines as antituberculous agents. *J*. *Med*. *Chem*., **1971**, *14*, 248-251.

[28] Kumar, P.; Shrivastava, B.; Pandeya, S.N. Tripathi, L.; Stables, J.P. Design, synthesis, and anticonvulsant evaluation of some novel 1, 3 benzothiazol-2-yl hydrazones/acetohydrazones. *Med*. *Chem*. *Res*., **2012**, *21*, 2428-2442.

[29] Kharbanda, C.; Alam, M.S.; Hamid, H.; Javed, K.; Bano, S.; Dhulap, A.; Ali, Y.; Nazreen, S.; Haider, S. Synthesis and evaluation of pyrazolines bearing benzothiazole as anti-inflammatory agents. *Bioorg*. *Med*. *Chem*., **2014**, *22*, 5804-5812.

[30] Pattan, S.R.; Suresh, Ch.; Pujar, V.D.; Reddy, V.V.K.; Rasal, V.P.; Koti, B.C. Synthesis of antidiabetic activity of 2-amino-[5`(4-sulphonylbenzedine)2,4-thiazolidinedione]-7chloro-6-floro benzothazole. *Ind*. *J*. *Chem*., **2005**, *44B*, 2404-2408.

[31] Singh, M.; Singh, S.K.; Gangwar, M.; Nath. G.; Singh, S.K. Design, synthesis and mode of action of some benzothiazole derivatives bearing an amide moiety as antibacterial agents. *RSC*. *Adv*., **2014**, *4*, 19013-19023.

[32] Burger, A.; Sawhey, S.N. Antimalarials. III. Benzothiazole amino alcohols. *J*. *Med*. *Chem*., **1968**, *11*, 270-273.

[33] Skinner, W.A.; Gualtiere, F.; Brody, G.; Fieldsteel, A.H. Antiviral agents. 1. Benzothiazole and benzoxazole analogs of 2-(alpha.-hydroxybenzyl)benzimidazole. *J*. *Med*. *Chem*., **1971**, *14*, 546-549.

[34] Guimaraes, C.R.W.; Boger, D.L.; Jorgensen, W.L. Elucidation of fatty acid amide hydrolase inhibition by potent α-Ketoheterocycle derivatives from monte carlo simulations. *J*. *Am*. *Chem*. *Soc*., **2005**, *127*, 17377-17384.

[35] Savariz, F.C.; Formagio, A.S.N.; Barbosa, V.A.; Foglio, M.A.; Carvalho, J.E.; Duarte, M.C.T.; Filho, B.P.D.; Sarragiotto, M.H. Synthesis, antitumor and antimicrobial activity of novel 1-substituted phenyl-3-[3-alkylamino(methyl)-2-thioxo-1,3,4-oxadiazol-5-yl] β-carboline derivatives. *J*. *Braz*. *Chem*. *Soc*., **2010**, *21*, 288-298.

[36] Gurdal, E.E.; Buclulgan, E.; Durmaz, I.; Cetin-Atalay, R.; Yarim, M. Synthesis and anticancer activity evaluation of some benzothiazole-piperazine derivatives. *Anticancer Agents Med*. *Chem*., **2015**, *15*, 382-389.

[37] Valente, S.; Trisciuoglio, D.; De Luca, T.; Nebbioso, A.; Labella, D.; Lenoci, A.; Bigogno, C.; Dondio, G.; Miceli, M.; Brosch, G.; Bufalo, DD.; Altucci, L.; Mai, A. 1,3,4-Oxadiazole-containing histone deacetylase inhibitors: Anticancer activities in cancer cells. *J*. *Med*. *Chem*., **2014**, *57*, 6259-6265.

[38] Abu-Zaied, M.; Nawwar, G.; Swellem, R.H.; El-Sayed, S.H. Synthesis and screening of new 5-substituted-1,3,4-oxadiazole-2-thioglycosides as potent anticancer agents. *Pharmacol*. *Phar*., **2012**, *3*, 254-261.

[39] Sangshetti, J.N.; Chabukswar, A.R.; Shinde, D.B. Microwave assisted one pot synthesis of some novel 2,5-disubstituted 1,3,4-oxadiazoles as antifungal agents. *Bioorg*. *Med*. *Chem*. *Lett*., **2011**, *21*, 444-448.

[40] Wani, M.Y.; Ahmad, A.; Shiekh, R.A.; Al-Ghamdi, K.J.; Sobral, A.J. Imidazole clubbed 1,3,4-oxadiazole derivatives as potential antifungal agents. *Bioorg*. *Med*. *Chem*., **2015**, *23*, 4172-4180.

[41] Raval, J.P.; Akhaja, T.N.; Jaspara, D.M.; Myangar, K.N.; Patel, N.H. Synthesis and *in vitro* antibacterial activity of new oxoethylthio-1,3,4-oxadiazole derivatives. *J*. *Saudi*. *Chem*. *Soc*., **2014**, *18*, 101-106.

[42] Nargund, L.V.G.; Reddy, G.R.N.; Hariprasad, V. Antiinflammatory activity of substituted 1,3,4-oxadiazoles. *J*. *Pharm*. *Sci*., **1994**, *83*, 246-248.

[43] Harfenist, M.; Heuser, D.J.; Joyner, C.T.; Batchelor, J.F.; White, H.L. Selective inhibitors of monoamine oxidase. 3. Structure-activity relationship of tricyclics bearing imidazoline, oxadiazole, or tetrazole groups. *J*. *Med*. *Chem*., **1996**, *39*, 1857-1863.

[44] Ahsan, M.J.; Samy, J.G.; Khalilullah, H.; Nomani, M.S.; Saraswat, P.; Singh, G.R. Molecular properties prediction and synthesis of novel 1,3,4-oxadiazole analogues as potent antimicrobial and antitubercular agents. *Bioorg*. *Med*. *Chem*. *Lett*., **2011**, *21*, 7246-7250.

[45] Ergun, Y.; Orhan, F.O.; Ozer, U.G.; Gisi, G. Synergistic effect of [1H-[1,2,4]Oxadiazole[4,3-a]quinoxalin-1-one] and antidepressant drugs in the mouse forced swimming test: Possible involvement of serotonergic pathway. *Eur*. *J*. *Pharmacol*., **2010**, *630*, 74-78.

[46] Madhavi, S.; Sreenivasulu, R.; Raju, R.R. Synthesis and biological evaluation of oxadiazole incorporated ellipticine derivatives as anticancer agents. *Monatsh. Chem*., **2017**, *148*, 933-938.

[47] Elokely, K.M.; Doerksen, R.J. Docking challenge: Protein sampling and molecular docking performance. *J. Chem. Inf. Model*., **2013**, *53*, 1934-1945.

[48] Friesner, R.A.; Banks, J.L.; Murphy, R.B.; Halgren, T.A.; Klicic, J.J.; Mainz, D.T.; Repasky, M.P.; Knoll, E.H.; Shelley, M.; Perry, J.K.; Shaw, D.E.; Francis, P.; Shenkin, P.S. Glide: A new approach for rapid, accurate docking and scoring. 1. Method and assessment of docking accuracy. *J. Med. Chem*., **2004**, *47*, 1739-1749.

[49] Reddy, P.N.; Padmaja, P.; Reddy, B.R.; Rambabu, G.; Kumar, M.P. Synthesis, molecular docking, antiproliferative, and antimicrobial activity of novel pyrano[3,2-*c*]carbazole derivatives. *Med. Chem. Res*., **2016**, *25*, 2093-2103.

[50] Agarwal, M.; Singh, V.; Sharma, S.C.; Sharma, P.; Ansari, Md.Y.; Jadav, S.S.; Yasmin, S.; Sreenivasulu, R.; Hassan, M.H. Design and synthesis of new 2,5-disubstituted 1,3,4-oxadiazole analogues as anticancer agents. *Med. Chem. Res*., **2016**, *25*, 2289-2303.