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Original Article

Synthesis, *in silico* and *in vitro* Evaluation of 1, 3, 4-Thiadiazole Derivatives Fused with Biphenyl Compound

Faruk Alam[1], Josef Yakin[1,*], Subham Ghosh[2], Surabhi Mandal[3], Moidul Islam Judder[4], Pynshailang Dkhar[2], Pratyushpal Kashyap[2]

[1]Department of Pharmaceutical Chemistry, Faculty of Pharmaceutical Science, Assam Down Town University, Panikhaiti, Narengi, Guwahati, Assam, INDIA.
[2]Department of Pharmaceutical Chemistry, NEPEDS College of Pharmaceutical Sciences, Gandhinagar, Tetelia, Sonapur, Assam, INDIA.
[3]Department of Pharmaceutical Chemistry, Himalayan Pharmacy Institute, Majhitar, Sikkim, INDIA.
[4]Department of Pharmaceutical Analysis, Royal School of Pharmacy, The Assam Royal Global University, Guwahati, Assam, INDIA.

ABSTRACT

Background: 1,3,4-thiadiazole nucleus is a flexible nucleus. There are numerous biological activities showed by this nucleus. Anticancer, anthelmintic, antimicrobial, anti-inflammatory, antioxidant; anti-HIV, anti- tubercular and anti-carbonic anhydrase are a few activities showed by 1,3,4-thiadiazole derivatives. This project study aimed to investigate the anthelmintic and *in silico* anticancer activity of derivatives of 1,3,4-thiadiazole. **Materials and Methods:** The UV visible spectrophotometer (Shimadzu UV-1900) has been used to determine λ_{max} of the synthesized molecules. The FT-IR (Bruker Alpha-II) spectrometer was used for IR spectra (4000-400 cm^{-1}) via the KBr disc method. Proton (1H) and Carbon-13 (13C) NMR spectra were recorded in $CDCl_3$ or (D6) DMSO at 300-500 MHz and 101-125 MHz, respectively, using the Bruker AV-III 400 spectrometer (Germany). High-Resolution Mass Spectra (HRMS) were obtained using the Xevo G2-XS QT of Mass Spectrometer (USA) with positive ESI mode at 70 eV. **Results:** The bonding energy for compounds 1, 2 and 3 were all in the range -42.929 to -48.909 kcal/mol. Results of the docking study shows interactions of compound 3 with the neighboring residues and showing significant anticancer activity. The presence of o-nitro, m-nitro and p-hydroxy groups makes compounds 1 (105.77%), 2 (131.67%) and 3 (155.54%) more potent and nearly equal in terms of inhibition percentage when compared to standard ascorbic acid. Compound-1 [5-([1,1'-biphenyl]-4-yl)-N -(2-nitrobenzylidene)- 1,3,4-thiadiazol-2-amine] showed significant anthelmintic activity. **Conclusion:** The biological profiles of these new generations of thiadiazoles would represent a fruitful matrix for further development of better medicinal agents.

Keywords: *In silico*, Synthesis, 1,3,4-Thiadiazole derivatives, Anthelmintic, Antioxidant.

Correspondence:
Mr. Josef Yakin
Department of Pharmaceutical Chemistry, Faculty of Pharmaceutical Science, Assam Down Town University, Panikhaiti, Narengi, Guwahati-781026, Assam, INDIA.
Email: joseph123yakin@gmail.com

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INTRODUCTION

One aromatic heterocyclic molecule that has drawn a lot of interest from the agrochemical and pharmaceutical sectors is thiadiazole due to its diverse pharmacological properties. A prominent and widely recognized example of heterocyclic nuclei is the 1,3,4-thiadiazole, which is a common and essential component of many different natural products and treatments. It is a five-membered ring having sulfur and nitrogen atoms. Originally discovered in 1898, the compound was later synthesized in pure form and has since been studied for its potential uses in different fields. In recent years, according to research, 1,3,4-thiadiazole might have a variety of pharmacological effects, such as anti-inflammatory,[1] anti-cancer,[2] and anti-convulsant properties.[3] Its chemical reactivity and structural versatility make this heterocyclic compound a valuable scaffold for developing new drugs with improved efficacy, selectivity and safety profiles. Despite the vast therapeutic potential of 1,3,4-thiadiazole, much remains unknown about its underlying mechanisms of action and its biomedical applications. Since the ring contains an N=C-S moiety, 1,3,4-thiadiazole is a versatile moiety with a broad range of action. With two or more heteroatoms, including one nitrogen atom, a five-membered ring structure is identified by the suffix -azole. Thiadiazoles are linked to a variety of biological activity, most likely as a result of the N=C-S- grouping 1. Because the 1,3,4-thiadiazole derivatives have a wide spectrum of biological actions, this investigation will concentrate on them. Numerous medications, such as acetazolamide,[4] butazolamide,[5] and sulfamethazole[6] are on the market and contain the 1,3,4-thiadiazole nucleus. In recent years, studies have suggested that fused 1,3,4-thiadiazole with biphenyl-4-carboxylic acid may

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have potential pharmacological applications, particularly in the fields of cancer[7] and inflammation.[8] Some experimental evidence indicated that biphenyl-4-carboxylic acid has anti-inflammatory, antioxidant[8] and anti-tumor[9] properties that may contribute to the development of new drugs with improved activities. Despite the exciting possibility for therapeutic uses of biphenyl-4-carboxylic acid, much remains unknown about its biological activities[10] and underlying mechanisms of action. 1,3,4-thiadiazole and biphenyl-4-carboxylic acid are among the compounds that have shown promising results in preclinical studies for their potential anti-cancer activity.[11,12] Some studies have indicated that these compounds may be able to inhibit cancer cell growth, migration and invasion. In addition, *in vitro* experiments have suggested that these compounds could be used for the treatment of different types of cancer, such as lung, colorectal and breast cancer. Despite the increasing interest in 1,3,4-thiadiazole and biphenyl-4-carboxylic acid as potential anti-cancer drugs, little is known about their mechanisms of action and their specificity. This work will provide valuable insights into the role of 1,3,4-thiadiazole and biphenyl-4-carboxylic acid in cancer treatment and facilitate the development of more efficient and targeted anti-cancer drugs. In low-income nations especially, parasitic infections are a leading cause of morbidity and death. Helminths, or parasitic worms, infect billions of people worldwide, causing chronic diseases such as schistosomiasis, ascariasis and hookworm infection. Drug resistance, the scarcity of suitable medications and the expense of treatment frequently make treating these illnesses difficult. Biphenyl-4-carboxylic acid and 1,3,4-thiadiazole have demonstrated potential as anthelmintic drugs,[13] which may help with some of these issues. This research aims to investigate the anthelmintic activity of 1,3,4-thiadiazole and biphenyl-4-carboxylic acid in order to determine their therapeutic potential against helminth infections and also this research will help create more accessible and cost-effective medicines by offering insightful information about the application of these substances as anthelmintic agents. This study aimed to investigate the anthelmintic and *in silico* anticancer activity of derivatives of 1,3,4-thiadiazole. By using advanced techniques, such as molecular docking and *in vivo* studies, we will gain a better understanding of the structure-activity relationship of this compound and its potential uses in different field. Although existing treatments such as chemotherapy and radiation therapy have contributed to improved outcomes for some patients, they often come with unwanted side effects and risks. Thus, there is an urgent need for developing novel and more efficient anti-cancer drugs.

MATERIALS AND METHODS

Experimental

Aromatic biphenyl carboxylic acid and thiosemicarbazide were subjected to customary strategies (Scheme 1). All blended mixes had to be liquefied for the reasons specified by the open capillary tube technique, which was communicated at °C. The data were regarded as uncorrected. Iodine was used as a visualization agent in thin layer chromatography, which measured the virtue of all produced compounds using 0.2 mm thick silica gel GF plates. The UV visible spectrophotometer (Shimadzu UV-1900) has been used to determine λ_{max} of the synthesized molecules. The FT-IR (Bruker Alpha-II) spectrometer was used for IR spectra (4000-400 cm^{-1}) via the KBr disc method. Proton (1H) and Carbon-13 (13C) NMR spectra were recorded in $CDCl_3$ or (D6) DMSO at 300-500 MHz and 101-125 MHz, respectively, using the Bruker AV-III 400 spectrometer (Germany). High-Resolution Mass Spectra (HRMS) were obtained using the Xevo G2-XS QT of Mass Spectrometer (USA) with positive ESI mode at 70 eV.

General procedure for preparation of 5-([1, 1'-biphenyl]-4-yl)-1, 3, 4-thiadiazol-2-amine[14]

At first the weight of biphenyl-4-carboxylic acid and thiosemicarbazide and transferred into a round bottom flux, and then 50 mL of ethanol and allowed to reflux. 5 mL of concentrated sulfuric acid was added dropwise and shaken after 30 min. The reflux process was carried out for about 1-3 hr. Finally, the obtained product was washed with water filtered dried, and recrystallized with ethanol. M. F: $C_{14}H_{11}N_3S$, M. Wt: 253.32 g/mol, M.P: 210-212°C, Yield: 91%, R_f: 0.85, Colour: White, Solubility: Ethanol (Soluble-20 mg/mL).

Compound 1: 5-([1,1'-biphenyl]-4-yl)-N -(2-nitrobenzylidene)-1,3,4- thiadiazol-2-amine

At first taken the weight of 5-([1,1'-biphenyl]-4-yl)-1,3,4-thiadiazol-2-amine and m-Nitro- benzaldehyde and transferred into a round bottom flux, then added 30 mL of ethanol and a few drops of conc. sulphuric acid and allowed to reflux for about 6-8 hr. Ultimately, ethanol was used to wash, filter, dry and recrystallize the resultant product. M. F: $C_{21}H_{14}N_4O_2S$, M. Wt: 386.43 g/mol, M.P: 208-209°C, Yield: 78%, R_f: 0.75, Colour: White crystals, Solubility: Ethanol (Soluble-25 mg/mL), IR(cm^{-1}): 1495 (C-N, st);1618 (Ar C=C st), 1372 (N=O, st), 694 (C-S-C, st); λ_{max} 280; 1HNMR(300 MHz, $CDCl_3$) δ (ppm): 8.85-8.67 (m, 1H), 8.20 (s, 1H), 7.71-7.36(m, 1H); 13C NMR (125 MHz, $CDCl_3$) δ (ppm): 121.49 (>C=N-), 149.60, 134.43, 129.53, 126.53, 124.76, 121.74, 121.49. HRMS (ESI-TOF) (m/z): $[M + H]^+$Calcd. for $C_{21}H_{14}N_4O_2S$: 386.0641; Found 386.2520.

Compound 2: 5-([1,1'-biphenyl]-4-yl)-N -(3-nitrobenzylidene)-1, 3, 4- thiadiazol-2-amine

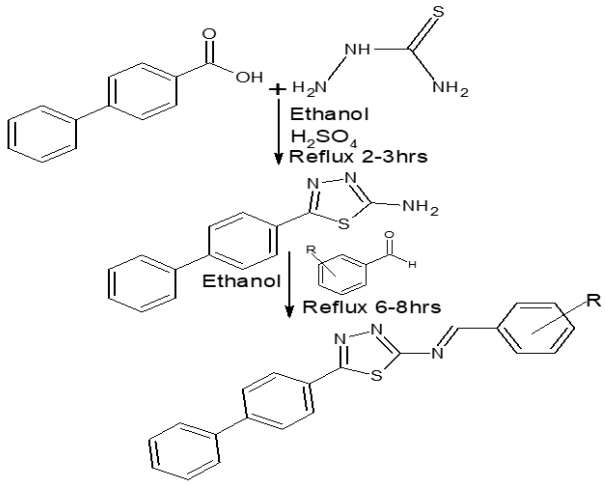
At first taken the weight of 5-([1,1'-biphenyl]-4-yl)-1,3,4-thiadiazol-2-amine and o-Nitrobenzaldehyde and transferred into a round bottom flux, then added 30 mL of ethanol and a few drops of conc. Sulphuric acid and allowed to reflux for about 6-8 hr. Finally, the obtained product was washed with ethanol and filtered and dried and recrystallized with ethanol. M.F: $C_{21}H_{14}N_4O_2S$, M.W: 386.43 g/mol, M.P:178-180°C,

Yield: 87%, R_f: 0.70, Colour: Yellow crystals, Solubility: Ethanol (Soluble, 25 mg/mL), IR(cm^{-1}): 1485 (C-N, st); 1595 (Ar C=C st), 1342 (N=O, st), 684 (C-S-C, st); λ_{max} 295; 1HNMR(300 MHz, $CDCl_3$) δ (ppm): 8.21-8.03 (m, 1H), 7.80-7.78 (s, 1H), 7.48-7.27(m, 1H); HRMS (ESI-TOF) (m/z): $[M-H]^+$ Calcd. for: $C_{21}H_{14}N_4O_2S$ 386.4312; Found: 385.9663.

Compound 3: 4-(((5-([1,1'-biphenyl]-4-yl)-1,3,4-thiadiazol-2- yl)-imino)-methyl)-phenol

At first taken the weight of 5-([1,1'-biphenyl]-4-yl)-1,3,4-thiadiazol-2-amine and p-Hydroxybenzaldehyde and transferred into a round bottom flux, then added 30 mL of ethanol and a few drops of conc. Sulphuric acid and allowed to reflux for about 6-8 hr. Finally, the obtained product was washed with ethanol and filtered and dried and recrystallized with ethanol (F. Alam *et al.*, 2015). M.F:$C_{21}H_{15}N_3OS$, M.Wt: 357.43 g/mol M.P:218-220°C, Yield: 92%, R_f: 0.80, Colour: Creamy white crystals, Solubility: Ethanol (Soluble, 25 mg/mL), IR(cm^{-1}): 3421(O-H, st),1476 (C-N, st), 1605 (Ar C=C st), 689,720 (C-S-C, st); λ_{max} 330; 1H NMR (400 MHz, DMSO-d6) δ (ppm): 8.01 (s, 1H), 7.51-7.36 (m, 1H), 7.86-7.84 (m, 1H), 6.65(s,1H), 5.13 (s, 1H, O-H);13C NMR (100 MHz, DMSO-d6) δ (ppm): 164.69(C-H), 160.68 (>C=N-),148.25, 132.99, 126.65, 121.69, 116.16, 114.61. HRMS (ESI-TOF) (m/z): $[M-H]^+$ Calcd. for: $C_{21}H_{15}N_3OS$ 357.43; Found: 357.1651.

Scheme



R= *m*-NO_2; *o*-NO_2; *p*-OH

In silico Anticancer Activity

Utilizing Earlotinib, the cytotoxicity of the recently synthesized heterocyclic compounds was assessed against EGRTs. The EGFR kinase domain structure (PDB ID: 2J6M), a human protein target, was the object of docking investigations using autodock 4.2 software[15] to evaluate the synthetic drugs' anti-cancer efficacy. Downloadable from the Protein Data Bank website (PDB ID: 2J6M) is the X-ray crystal structure of the epidermal growth factor receptor tyrosine kinase enzyme. There was ligand and protein regularization and optimization. To calculate the Root-Mean-Square Deviation (RMSD) of the crystal ligand's performance in the docking procedure, the enzyme's assigned active tyrosine kinase was re-docked. Next, by the described procedure,[16,17] molecular docking was used to apply compounds 1, 2 and 3 to the protein's ATP binding site. The results of *in silico* anticancer activity were assigned in Table 1, Figures 1 and 2.

In vitro Antioxidant Screening[14]

DPPH radical scavenging activity

One of the most widely used methods for characterizing antioxidants is the nitrogen-centered stable free radical 1,1-diphenyl-2-picrylhydrazyl (DPPH). Using a UV visible spectrophotometer (Shimadzu UV-1900), it is reversibly reduced, and the odd electron in the DPPH free radical yields a significant absorption maximum at λ 517 nm, which is purple in color. It is appropriate for spectrophotometer studies because of this feature. 1, 1-diphenyl-2-picrylhydrazine is produced when DPPH stable free radical combines with an antioxidant that scavenges radicals. Decolorization that results are stoichiometric about the quantity of electrons absorbed. Antioxidant properties have been measured using the change in absorbance that results from this reaction. The compounds' capacity to donate electrons or hydrogen atoms was assessed by measuring how well the purple DPPH radical-colored methanol solution bleached. The spectrophotometric test utilizes the stable DPPH radical as a reagent. Initially, a 4 mL solution of DPPH in methanol at a concentration of 0.004% (w/v) was prepared. Subsequently, 1 mL of various concentrations of the test compounds (10, 20, 40, 80, 160 µg/mL) dissolved in methanol was added to the DPPH solution. After a 30-minute incubation period at room temperature, the absorbance was measured at a wavelength of 517 nm against a blank. Ascorbic acid served as the standard compound in this experiment. The following formula was used to get the percent of Inhibition (I%) of free radical generation from DPPH.

$$I\% = [(A_{control} - A_{sample})/A_{control}] \times 100$$

The absorbance of the test substance (which contains both methanolic DPPH and ascorbic acid) is represented by A_{sample}, while the absorbance of the control reaction is denoted by $A_{control}$. The tests were run three times. The outcomes are shown in Figure 3.

Nitric oxide scavenging activity

The reaction mixture (6 mL) was incubated at 25°C for 150 min. It contained sodium nitroprusside (10 mM, 4 mL), phosphate buffer saline (pH 7.4, 1 mL), test samples or standard, ascorbic acid solution in dimethyl sulphoxide (1 mL) at different concentrations (10, 20, 40, 80, 160 µg/mL). Following incubation,

1 mL of sulphanillic acid reagent was added to 0.5 mL of reaction mixture containing nitrite ions. This mixture was thoroughly mixed and the diazotization process was allowed to stand for 5 min. After that, 30 min was spent in diffused light with 1 milliliter of naphthyl ethylene diamine dihydrochloride added and combined. A chromophore with a pink color produced. A Shimadzu UV-1900 UV visible spectrophotometer was used to measure the absorbance at λ 640 nm.[16] NO scavenging activity was calculated by the following equation:

$$\% \text{ NO} = [(\text{Acontrol} - \text{Asample})/\text{Acontrol}] \times 100$$

Where the absorbance of the test compound (which contains all reagents and test compound) is A_{sample}, then $A_{control}$ is the absorbance of the control reaction (which contains all reagents and ascorbic acid). Triologous tests were conducted. In Figure 4, the outcomes were allocated.

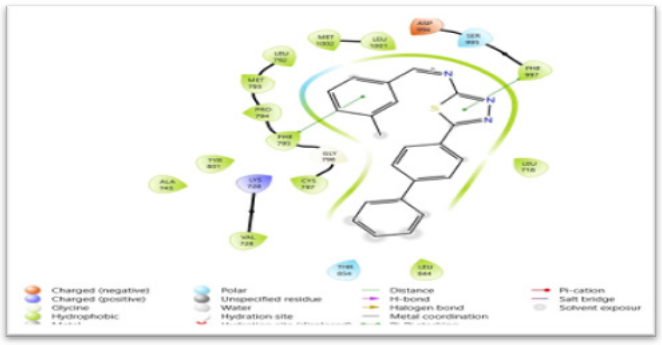
Anthelmintic Activity[18]

The new biphenyl-thiadiazole compounds that were produced underwent anthelmintic screening. The standard treatment was albendazole. For the anthelmintic study, adult earthworms *Eudrilus eugenia* were employed, which had been thoroughly cleaned of all faeces using normal saline. To obtain 0.075% w/v, 0.150% w/v and 0.225% w/v as standards, albendazole was diluted with regular saline and then added to Petri dishes. To achieve the same concentration as the standard, all test compounds were produced using the least amount of DMF possible and diluted to 15 mL with normal saline before being placed in Petri dishes. The standard is controlled by normal saline. In each Petri dish, six almost equal-sized earthworms were put in and allowed to come to room temperature. The duration it took for earthworms to become completely paralyzed and eventually die served as the basis for evaluating the compounds. The mean lethal time for each test compound was recorded and compared to that of the reference medication. The paralysis period referred to the time it took for the worms to cease movement. In some cases, external stimuli were applied to motionless worms to confirm their death, as live worms typically respond to such stimuli by moving. The mean lethal time and paralysis duration for earthworms exposed to various test compounds and the standard drug are presented in Figures 4 and 5, respectively.

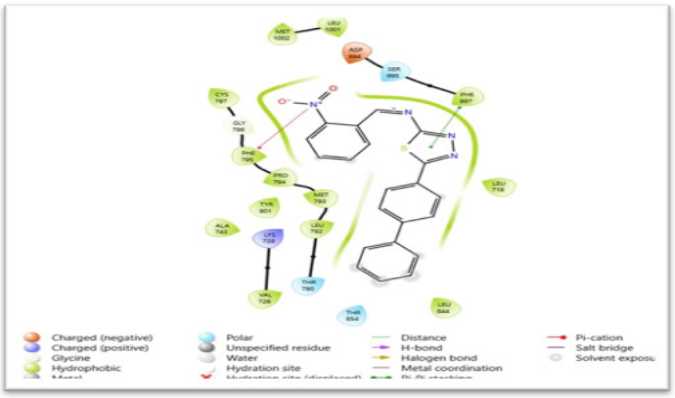
RESULTS AND DISCUSSION

Chemistry

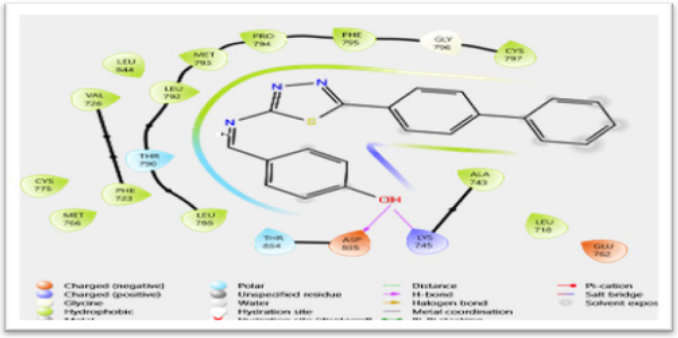
Upon concluding the experiment, it was found that the synthesized chemical compounds yielded favorable results. Their structures were elucidated using mass spectrometry, IR spectroscopy, TLC, melting point analysis and NMR spectroscopy. The synthetic methodology is detailed in Scheme 1. The IR spectra of the three compounds exhibited distinct absorption bands, providing essential information about their molecular structures and



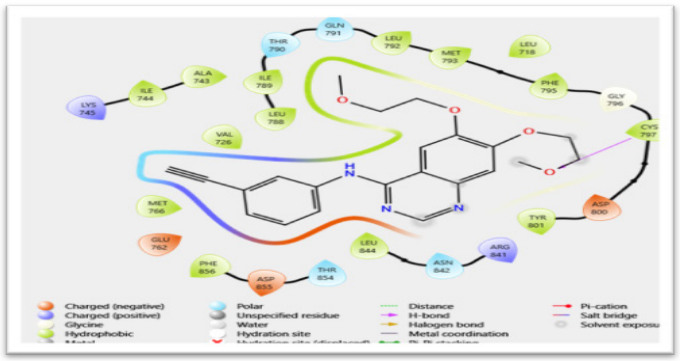
A. Compound 1



B. Compound 2



C. Compound 3



D. Erlotinib

Figure 1: 2D Visualization of anticancer properties test and standard compound.

functional groups. UV spectroscopy, a vital analytical technique for determining chemical composition and electronic structure, was utilized to further analyze the compounds. The UV spectra, recorded in the 200-400 nm range, revealed insights into the presence of conjugated systems, aromaticity and functional groups. Compound 1's UV spectrum showed absorption peaks at 320 nm and 250 nm, corresponding to the π-π* transition of the biphenyl group and the n-π* transition of the 2-nitrobenzylidene group, respectively. Similarly, Compound 2's UV spectrum featured peaks at 250 nm and 320 nm, with the 250 nm peak being more pronounced, indicating the 3-nitrobenzylidene group's stronger electron-withdrawing effect compared to the 2-nitrobenzylidene group. Compound 3's UV spectrum displayed three peaks at 220 nm, 280 nm and 340 nm, attributed to the n-π* transition of the phenol group, the π-π* transition of the biphenyl group and the n-π* transition of the thiazolidine ring, respectively.

In silico Docking Study

Using the X-ray crystallographic structure of EGFR (PDB ID: 2J6M), we next looked at how compounds 1, 2 and 3 interacted to better understand their efficacy and direct future SAR investigations. After the co-crystallized ligand Earlotinib was redocked into the EGFR pocket sites, the results showed that the docking score energies were -5.85 kcal/mol at a Root Mean Square Deviation (RMDS) value of 0.3. In contrast, compound 3 had a higher docking score, which is -7.187 at an RMDS value of 0.23, while compounds 1 and 2 have docking scores of -7.085 and -6.021, respectively, at RMDS values of 0.25 to 0.27. By introducing compounds 1, 2 and 3 into the ATP binding site of TKIs, the molecular docking was carried out. The bonding energy for compounds 1, 2 and 3 were all in the range -42.929 to -48.909 kcal/mol. We have compared the affinity and binding energy of our novel compounds towards the EGFR with the standard drug Erlotinib.

Antioxidant activity

Compounds 1 through 3 underwent antioxidant testing at various concentrations of 10 µg/mL, 20 µg/mL, 40 µg/mL, 80 µg/mL and 160 µg/mL using 2, 2-Diphenyl-1-Picrylhydrazyl (DPPH) and nitric oxide techniques. Figures 3 and 4 display the observed findings on the antioxidant activity of the drug-controlled compounds.

2, 2-Diphenyl-1-Picrylhydrazyl (DPPH) scavenging method

The presence of o-nitro, m-nitro and p-hydroxy groups makes compounds 1 (105.77%), 2 (131.67%) and 3 (155.54%) more potent and nearly equal in terms of inhibition percentage when compared to standard ascorbic acid, according to a comparison conducted at 160 µg/mL. As the concentration increased, there was a rise in the scavenging activity of DPPH•, as depicted in Figure 3.

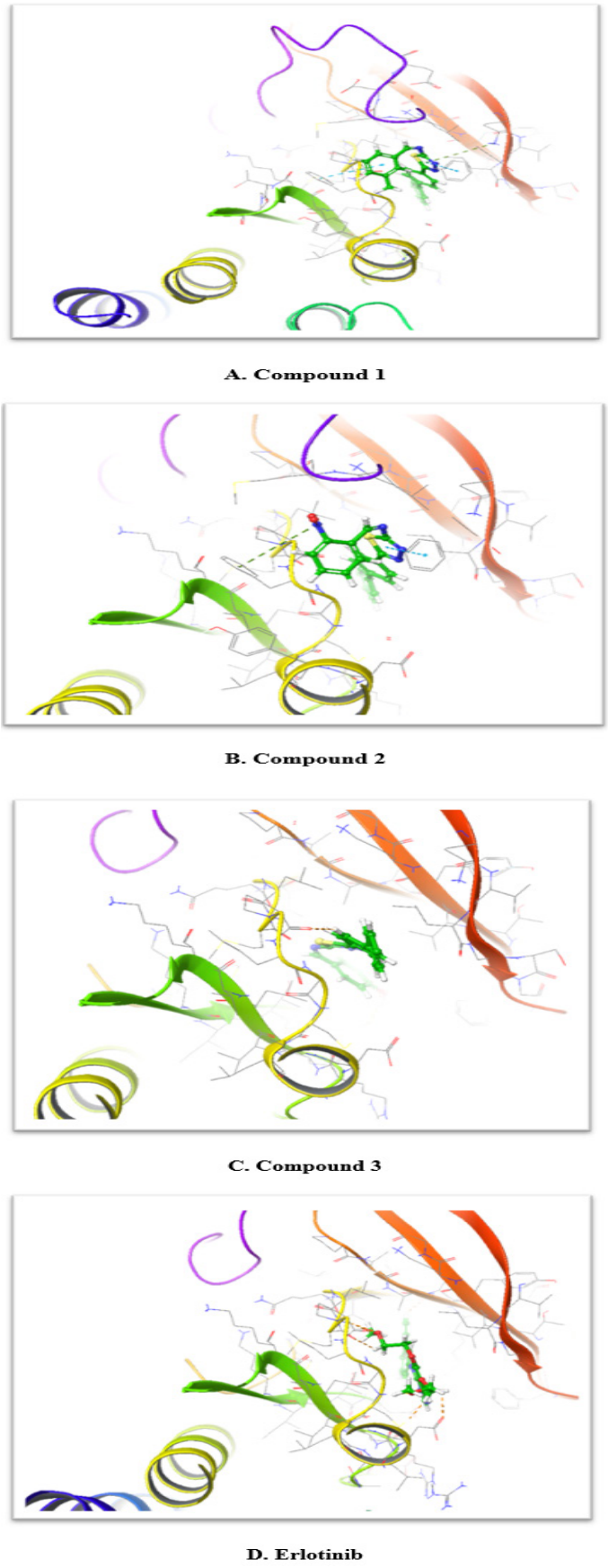


Figure 2: 3D Visualization of anticancer properties test and standard compound.

Table 1: Docking analysis of anti-cancer activity of synthesized compounds.

Ligand ID	Docking Score	Binding Energy (kcal/mol)	RMSD(Å)	Interaction
Compound 1	-7.085	-46.409	0,25 Å	Pi-Pi stacking, Pi-cation
Compound 2	-6.021	-42.929	0.27	Pi-Pi stacking
Compound 3	-7.187	-48.909	0.23	H-Bond
Erlotinib	-5.85	-51.218	0.30	H-Bond

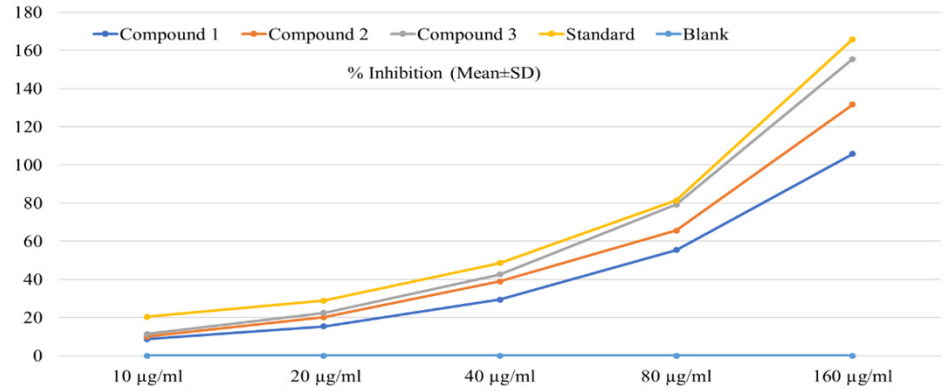


Figure 3: The synthesized compounds and standard were assessed for antioxidant activity using the DPPH scavenging method, measuring the percentage of DPPH radical scavenging activity. [Values are mean±SEM (*n*=3); Standard=Ascorbic acid].

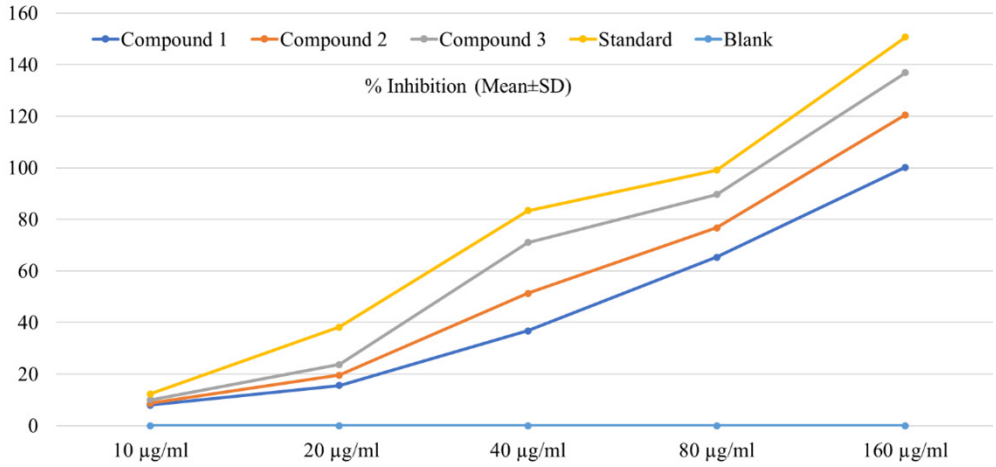


Figure 4: The antioxidant activity of the synthesized compounds and standard was evaluated using the NO scavenging method, measuring the percentage of NO radical scavenging activity. [Values are mean±SEM (*n*=3); Standard=Ascorbic acid].

Nitric oxide scavenging method

Component 3 showed the highest antioxidant activity of all the compounds examined, with an inhibition value of 136.91%; ascorbic acid, the reference component, had an inhibition value of 150.61%. At a concentration of 160 µg/mL, two more moderately active compounds, compound 1 and compound 2, demonstrated percentage inhibition values of 105.77% and 131.67%, respectively. In the increasing order of p-OH> m-NO_2> o-NO_2, the compounds exhibited activity that is comparable to the control (Figure 4).

Anthelmintic activity

The anthelmintic activity of the samples was assessed using *Eudrilus eugenia* earthworm. Water and saline solutions were used to make the desired concentration (5 mg/mL, 10 mg/mL) of drugs for testing. Albendazole was used here as standard and saline water was used as control; the paralysis time followed

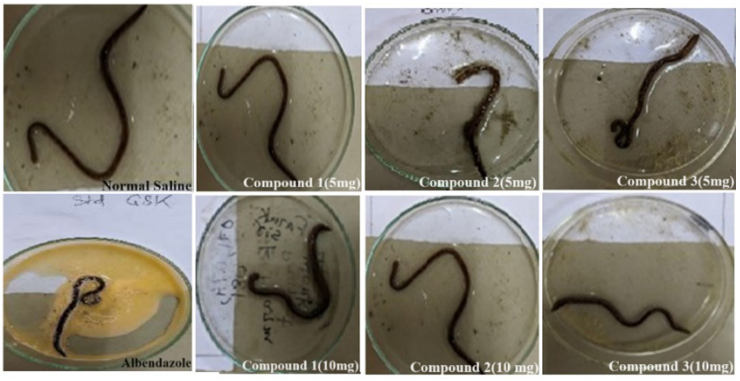


Figure 5: Anthelmintic activity of synthesized compounds.

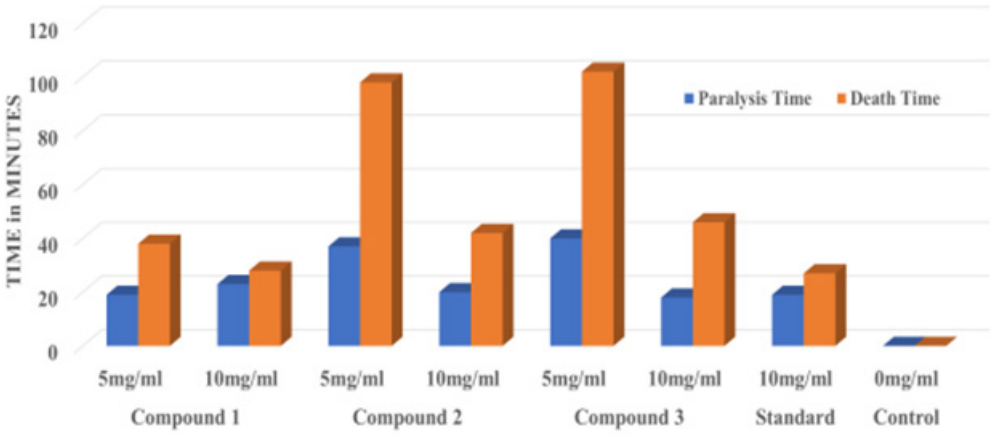


Figure 6: Anthelmintic activity of synthesized compounds.

by death time was recorded. Compound 1 showed prominent action at concentartion10 mg/mL was 31 min as compared with standard (30 min at 10 mg/mL) and the remaining shows mild to moderate (Figures 5 and 6).

DISCUSSION

The UV spectroscopy results reveal that the three compounds possess distinct electronic structures, as evidenced by their unique UV absorption spectra. Compounds 1 and 2, containing the nitrobenzylidene group, are more electron-withdrawing than Compound 3, which has a phenol group. This difference in electronegativity is reflected in their varied absorption maxima. An *in silico* docking study demonstrated that Compound 3 has a higher affinity for the EGFR, with a binding energy of -48.909 kcal/mol, compared to the standard drug Erlotinib, which has a binding energy of -51.218 kcal/mol. The docking results indicated that Compound 3 interacts effectively with neighboring residues, suggesting significant anticancer activity. In antioxidant evaluations using the DPPH scavenging method, Compound 3 exhibited the highest inhibition percentage at 155.54%, closely approaching the standard's 165.76%. Compounds 1 and 2 showed moderate inhibition levels compared to the standard. Specifically, Compound 3 achieved the highest antioxidant activity among the tested compounds, with an inhibition value of 136.91%, while ascorbic acid, the reference compound, had an inhibition value of 150.61%.

Additionally, Compound 1 [5-([1,1'-biphenyl]-4-yl)-N-(2-nitrobenzylidene)-1,3,4-thiadiazol-2-amine] demonstrated significant anthelmintic activity. This is attributed to the presence of the o-nitro group, which may stimulate ganglia in worms, causing tonic paralysis and expulsion of live worms, as well as interfering with carbohydrate metabolism.

CONCLUSION

The initial melting points of all compounds were determined using the open capillary tube method, ranging from 208°C to 220°C, respectively. After analysis of the compounds, we obtained from our synthesis we have found that compound 3 has higher affinity towards the EGFR and higher binding energy than the standard drug (Erlotinib) and these compounds can be used for the treatment of cancer disease. DPPH and NO free radical scavenging activity methods were used to assess the *in vitro* antioxidant activity of the compounds. Compound 1: 5-([1,1'-biphenyl]-4-yl)-N -(2-nitro

benzylidene) -1,3,4-thiadiazol-2-amine was found to have poor antioxidant activity when compared to ascorbic acid (standard). In accordance, with the data obtained from *in vivo* evaluation of the anthelmintic study, all the synthesized compounds-1 showed higher activity against earth warm whereas others showed moderate to fairly good activity when compared with Albendazole as a standard drug. The biological profiles of these new generations of thiadiazoles would represent a fruitful matrix for the further development of better medicinal agents.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

IR: Infrared spectroscopy; **TLC:** Thin Layer Chromatography; **MP:** Melting Point; **NMR:** Nuclear magnetic resonance; **UV:** Ultra Violet; **HRMS:** High-resolution mass spectrometry; **ESI-TOF:** Electrospray Ionization Time-of-Flight; **DMSO:** Dimethylsulfoxide; **EGRT:** Emission Guided Radiation Therapy; **EGFR:** Epidermal growth factor receptor; **DMF:** Dimethylformamide; **NO:** Nitric Oxide.

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