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Original Research Article

Comprehensive investigation of two substituted benzimidazoles: Design, synthesis, biological assessment, and characterization

Gopinathan Narasimhan[1]*, Gokul kumar K[1], B Balalji[1], Azhagu Naachiar[1], Khiren S[1], Harikrishnan N[1]

[1]*Dept. of Pharmaceutical Chemistry, Faculty of pharmacy Dr. MGR Educational and Research Institute, Chennai, Tamil Nadu, India*

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ABSTRACT

Background: Benzimidazole is a bicyclic molecule that is mostly used in medicinal chemistry. The 2-substituted benzimidazoles that are now on the market include omeprazole, flubendazole, mebendazole, and albendazole. Antioxidants are compounds that impede the oxidation process, which is the chemical process that produces free radicals. A computational technique called "in-silico screening" is used in drug discovery to effectively estimate a molecule's likelihood of interacting with a target of interest. Antihelminthics are a class of antiparasitic drugs that either kill or paralyze internal parasites like parasitic worms, all without endangering the host.
Materials and Methods: A standard procedure was used to create the 2-substituted benzimidazole. PyRx software was used to do the in-silico screening for anthelmintic drugs. The DPPH and ABTS methods were used to measure the antioxidant activity.
Results: Melting point, TLC, and IR spectra were used to confirm the structures of the produced compounds. The binding affinity for sulphosalicylic acid in the anthelmentic in silico screening was -7.7. Salicylic acid demonstrated excellent antioxidant activity, as seen by its IC50 values of less than 5 mg/ml for the ABTS method and 37 mg/ml for the DPPH method.
Conclusion: Overall, the combination of theoretical and practical yield data, along with melting points, ATR Spectra and Rf values, offers valuable insights into the synthesis and characterization of chemical compounds. The salicylic acid derivative exhibits good antioxidant activity by both the methods. The Sulphosalicylic acid derivative shows prominent In-silico anthelmintic activity result hence the synthesized compounds can be tested for anthelmintic activity in future.

1. Introduction

The main focus of medicinal chemistry is designing and creating pharmaceutical drugs with the desired therapeutic activity and intended use. Benzimidazole belongs to a significant class of biologically active heterocyclic compounds and plays a major role in medicinal chemistry. One of the earliest nitrogen heterocycles known to science. This bicyclic compound consists of the fusion of benzene and imidazole. Benzimidazoles are a group of molecules that showed potential for use in various pharmacological purposes. Since its derivatives have a variety of biological activities, including antioxidant, antimicrobial, anthelmintic, anticancer, antihypertensive, antineoplastic, anti-inflammatory, analgesic, antifungal, and antiviral properties, the chemistry and pharmacology of benzimidazole are of great interest to medicinal chemistry. A large variation of 2-replaced benzimidazole was found

* *Corresponding author*.
E-mail address: gopipharmacist@rediffmail.com (G. Narasimhan).

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to contain anti-inflammatory, antispasmodic, antihistamine, antimicrobial, anti-tumor, and cyclooxygenase inhibitor activity. Various 2-substituted benzimidazole compounds include Mebendazole, Flubendazole, Parbendazole, Albendazole, Pimobendan, and Omeprazole.

Molecular docking is a key tool. Predicting the dominant binding mode of a ligand with a protein having a known three-dimensional structure is the objective of ligand-protein docking.[1,2] For candidate ligands being docked into a protein target with high binding energies, a scoring function is used. The macromolecule serves as the protein receptor in this process. The ligand molecule, which can function as an inhibitor, is a micromolecule. A class of antiparasitic medications known as anthelmintics, or antihelminthics, eliminates internal parasites such as parasitic worms from the body by either killing or stunning them, all without posing a serious risk to the host. They are also called "vermifuges" and "vermicides". Toxicants, such as anthelmintics, must be specific to the parasite. Certain biological activity is indicated by the presence of multiple phytochemical contents and strong antioxidant activity observed in anthelmentics. Antioxidants are substances that inhibit the production of free radicals through the chemical process of oxidation. The target parasite can affect the effectiveness of benzimidazole anthelmintics; whipworms are less responsive to these drugs than Ascaris nematodes. The β-tubulin isotypes of soil-transmitted helminths and ascarids were identified, and phylogenetic analysis revealed that the ascarids have a similar repertoire of seven β-tubulin isotypes.[3–5]

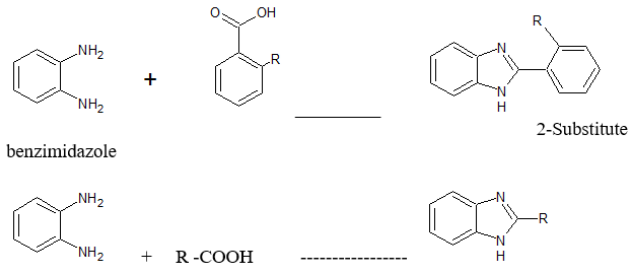


Figure 1: Scheme of the work

2. Materials and Methods

2.1. Procedure for synthesis

The process of synthesizing 2-substituted benzimidazole was done by using 1.08 g of ortho-phenylene diamine, which was kept in the Round Bottom Flask (RBF). The contents were weighed accordingly, as follows: 1.38 g salicylic acid, 2.18 g sulfosalicylic acid, 2.12 g 3,5-dinitrobenzoic acid, 0.75 g glycine, and 0.92 g thioglycolic acid. Then, ethanol was added to the RBF. The porcelain chips were also added in small quantities to avoid overheating or bumping. Then the RBF was connected to the reflux condenser with outlet and inlet pipes and a continuous water supply. It was kept in the heating mantle at a constant temperature for 2-3 hours. After the process of heating the compound, the sample was poured into the ice water, and then sodium hydroxide was added drop by drop to obtain the product. The same process was repeated for all the compounds for the synthesis.

2.2. Determination of DPPH Method:[4]

The product sample of each compound is weighed at 10 mg separately and diluted with ethanol to make 10 ml using a standard flask. The extracted compound was then made in various concentrations. The concentrations were obtained by serial dilution for each compound, ranging from 1000μg/ml, 500μg/ml, 250μg/ml, 125μg/ml, 62.5μg/ml, 31.25μg/ml, 15.625μg/ml, and 7.81μg/ml. Here, we're applying the DPPH method to determine the antioxidant properties of the product. The ascorbic acid is taken as the standard solution with different concentrations of 100μg/ml, 200μg/ml, 300μg/ml, 400μg/ml, and 500μg/ml by serial dilution. Ethanol is taken as the control. A test tube containing 1 ml of the extracted compound and 1 ml of the freshly made DPPH solution was filled with the mixture. The free radical property of the compound was indicated by a colour change from violet to yellow. Then it is determined by using the spectrophotometer and measuring the absorbance at 517 nm. After the absorbance, the percentage inhibition and IC50 values were determined.

2.3. Determination of ABTS Method

The phosphate buffer solution with a pH of 6.8 was prepared in a 100-ml volumetric flask. [Preparation of phosphate buffer solution pH-6.8: 0.1 g of KH2PO4, 0.2 g of K2HPO4, and 0.85 g of NaCl were dissolved in distilled water up to 100 ml in the volumetric flask]. Then 0.0662 g of ammonium per sulfate was added to the phosphate buffer solution in the volumetric flask and made up to 100 ml. 0.3841 g of ABTS was weighed and mixed with the above prepared buffer solution up to 10 ml in the volumetric flask, making the ABTS stock solution.

The product sample of each compound is weighed at 10 mg separately and diluted with ethanol to make 10 ml using a standard flask. The extracted compound was then made in various concentrations. The concentrations were obtained by serial dilution for each compound, ranging from 1000μg/ml, 500μg/ml, 250μg/ml, 125μg/ml, 62.5μg/ml, 31.25μg/ml, 15.625μg/ml, and 7.81μg/ml. Here, we're applying the ABTS method to determine the antioxidant properties of the product. The ascorbic acid is taken as the standard solution with different concentrations of 100μg/ml, 200μg/ml, 300μg/ml, 400μg/ml, and 500μg/ml by serial dilution. Ethanol is taken as the control. Various test tubes

were taken, each containing 1 ml of product of different concentrations, and 1 ml of ABTS solution was filled with the mixture. The free radical property of ABTS is that the dark blue color disappears when it combines with an antioxidant compound. Then it is determined by using the spectrophotometer and measuring the absorbance at 734 nm. After the absorbance, the percentage inhibition and IC50 values were determined.

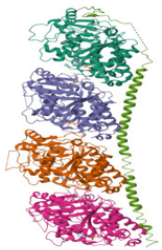
2.4. In-Silico Screening[2]

2.4.1. Ligand prepation

The structures of the product were drawn using the chemsketch software. Then the smile notations were extracted using structure using an online SMILES translator. SMILES notations were converted to PDB format. The file format designated as "PDB" was selected for the output. Three-dimensional coordinates and the SMILES representation "Kekule" were selected. The ligand in PDB file format is downloaded.

2.4.2. Protein preparation

1SA0 TUBULIN-COLCHICINE: STATHMIN-LIKE DOMAIN COMPLEX was downloaded from the protein data bank in the PDB format. PDB DOI: https://doi.org/10.2210/pdb1SA0/pdb



Method: X-RAY DIFFRACTION
Resolution: 3.58 Å
R-Value Free: 0.249
R-Value Work: 0.232

Figure 2: PDB structure of protein ISA0

2.4.3. Molecular docking

This study deals with the evaluation of the anthelmintics using 2-substitued benzimidazole using in silico docking studies. In-silico docking studies were carried out using PyRx software. Three important parameters, like binding energy, inhibition constant, and intermolecular energy, were determined.

2.4.4. Lipinski rule of 5 analyzer[6]

The analysis of the Lipinski Rule of 5 for the product sample was performed using the online website scfbio@iitd.

The PDB format of the chosen product was selected and uploaded.

The pH was set at 7.

The chosen file format was submitted.

After submitting the file, the results table will be provided along with the mass, hydrogen bond donor, hydrogen bond acceptors, log P, and molar refractivity.

3. Results

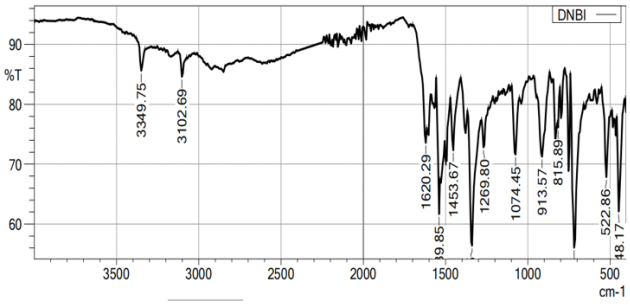
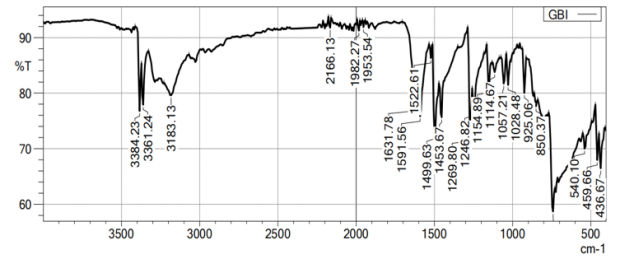
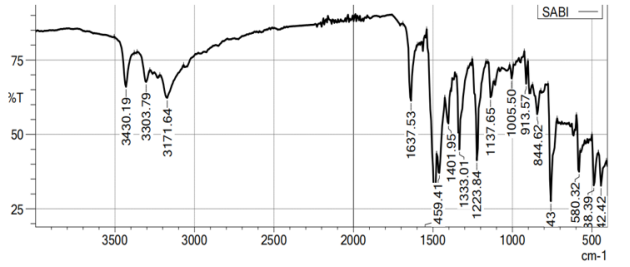


Figure 3: ATR of dinitrobenzoic acid derivative



The NH stretch was obtained at the 3384.23 cm−1 and 3361.24 cm−1 peaks and the aromatic CH stretch was obtained at 3183.13 cm−1.

Figure 4: ATR of glycine derivative



The NH stretch was obtained at 3430.19 cm−1 and 3303.79 cm−1. The aromatic CH stretch was obtained at 3171.64 cm−1.

Figure 5: ATR of salicylic acid derivative

4. Discussion

The dinitrobenzoic acid derivative had a binding affinity of –6.2. The benzimidazole part had pi-pi stacking with the residue TRY A 407. The benzene fused with imidazole formed a pi-alkyl interaction with the residue VAL A 182, and the phenyl part formed a pi-alkyl interaction with

Table 1: Theoretical and practical yield of compounds

Compounds	Theoretical Yield	Practical Yield	Melting point	Rf
Salicylic Acid	2.1g	0.042g	320°C	0.12
Sulphosalicylic Acid	2.9g	4.420g	340°C	0.42
Dinitrobenzoic Acid	2.8g	2.901g	280°C	0.6
Thioglycolic Acid	1.64g	2.417g	260°C	0.45
Glycine	1.47g	2.054g	310°C	0.54

Table 2: Analysis of compounds through Lipinski's Rule of Five

Compound	Molecularweight <500	H-bonddonors <5	H-bondacceptors <10	LogP <5	Molar refractivity (40-130)	Number ofLipinski′srule violation
Salicylic Acid	210.236	2	2	2.936	63.195	0
Sulfosalicylic Acid	290.3	3	4	2.182	73.009	0
Dinitrobenzoic acid	284.231	1	5	3.045	74.839	0
Glycine	147.181	2	2	1.022	43.985	0
Thioglycolic Acid	164.233	2	2	1.993	48.784	0

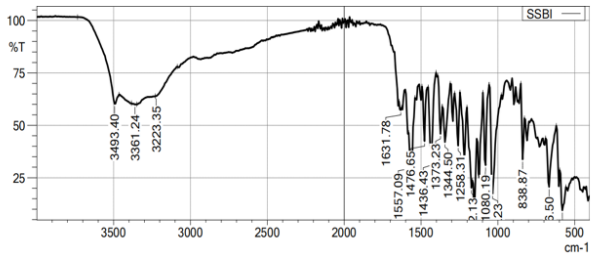
Table 3: Molecular docking studies of the compounds against the target

Ligand	Binding Affinity	No of hydrogen bond	Interaction
Glycine	5.2	Two hydrogen bond GlU A 411	Pi-Pi stacking Pi-alkyl
Thioglycolic Acid	4.4	Nil	Pi-Pi stacking Pi-alkyl
Dinitrobenzoic Acid	6.2	Nil	Pi-Pi stacking Pi-alkyl
Salicylic Acid	6.4	Nil	Pi-Pi stacking, pi-alkyl and pi-sigma
SulphoSalicylic Acid	6.5	One -ARG A 105	Pi-Pi stacking, pi-alkyl and pi-sigma
Standard Albendazole	5.2	Nil	Pi-Pi stacking, pi-alkyl and pi-sigma, Pi-sulfur

Table 4: Antioxidant activity of compounds

S.NO	Compound	IC50 Value Mcg/ml DPPH method	IC50 Value Mcg/ml ABTS method
1	Salicylic Acid	37	3.5
2	Sulphosalicylic Acid	Above 200	Above 200
3	Dinitrobenzoic Acid	190	Above 200
4	Thioglycolic Acid	130	180
5	Glycine	360	Above 150
6	Ascorbic acid	60	1.5

the residue ALA A 100. Salicylic acid derivatives had a binding affinity of -6.4. The benzimidazole part had pi-pi stacking with the residue TRY A 407 . The benzene fused with imidazole formed a pi-alkyl interaction with the residue VAL A 182, and the phenyl part formed a pi-alkyl interaction with the residue ALA A 100. The phenyl part of the derivative had a pi-sigma interaction with the residue ALA A 100. The derivative of sulphosalicylic acid exhibited a binding affinity of -6.5 towards the target 1SA0. It shared a single 2.87 A hydrogen bond with residue ARG A 105. There was pi-pi stacking in the benzimidazole portion with residue TRY A 407. Pi-alkyl interactions were established by the benzene fused with imidazole with residue VAL A 182 and the phenyl portion with residue ALA A 100. The derivative's phenyl component interacted pi-sigma with the residue ALA A 100. With target 1SA0, the glycine derivative had a binding affinity of -5.2. Two hydrogen bonds had been created. Two hydrogen bonds were established between the free hydrogens and the residue GLU A 411. It measured 3.31 and 3.36 A in length, respectively.



The NH stretch was obtained at 3493.40 cm−1 and an Intra-molecular OH stretch was obtained at 3361.24 cm−1. The aromatic CH stretch was obtained at 3223.35 cm−1.

Figure 6: ATR of sulphosalicylic acid derivative

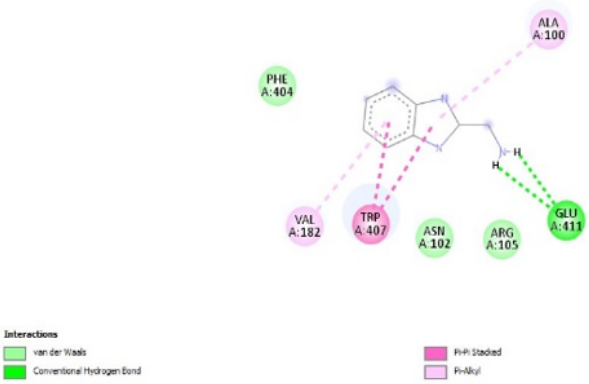


Figure 7: The glycine derivative interact with the target

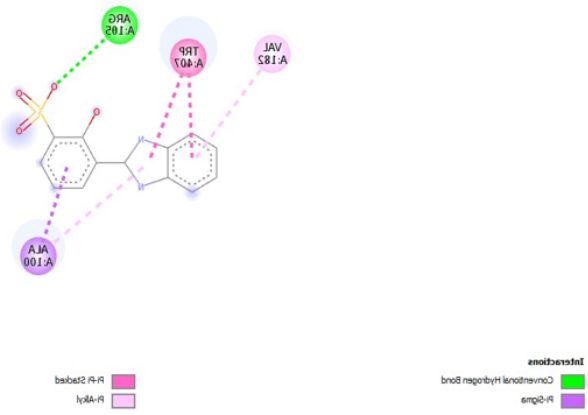


Figure 8: The sulphosalicylic acid derivative interaction with target

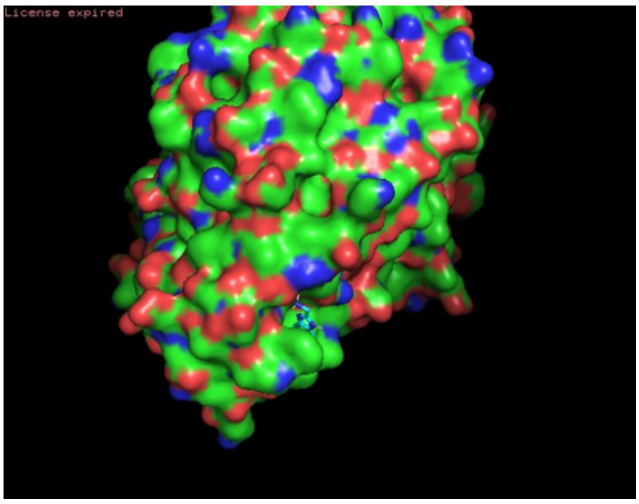


Figure 9: The sulphosalicylic acid derivative interaction with target

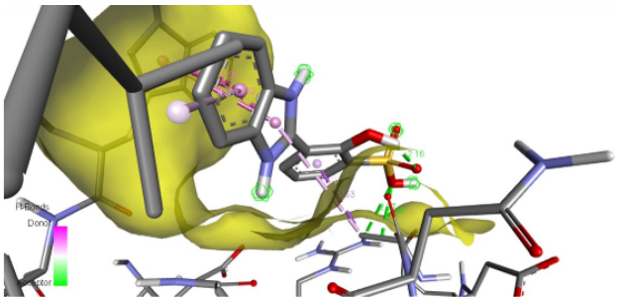


Figure 10: The sulphosalicylic acid derivative formed hydrogen bond with the target

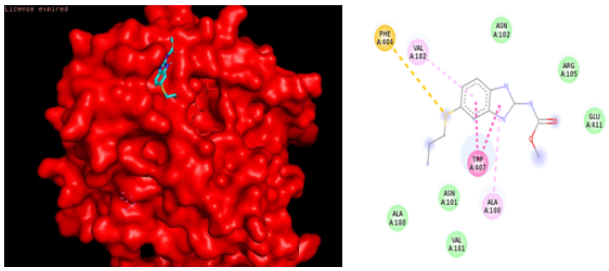


Figure 11: Thioglycolic acid derivative interaction with the target

There was pi-pi stacking in the benzimidazole portion with residue TRY A 407. Pi-alkyl interactions were established by the benzene fused with imidazole with residue VAL A 182 and the phenyl portion with residue ALA A 100.A derivative of thioglycolic acid had a binding affinity of -4.4. There was pi-pi stacking in the benzimidazole portion with residue TRY A 407. Pi-alkyl interactions were established by the benzene fused with imidazole with residue VAL A 182 and the phenyl portion with residue ALA A 100.A derivative of dinitrobenzoicacid

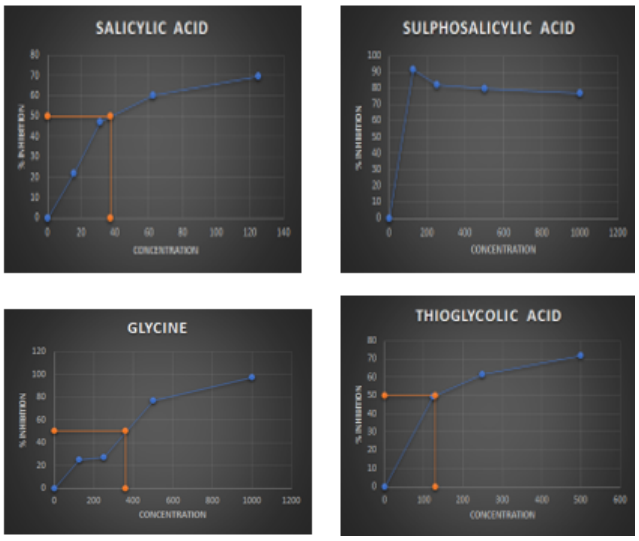


Figure 12: Antioxidant activity of compounds using DPPH Method

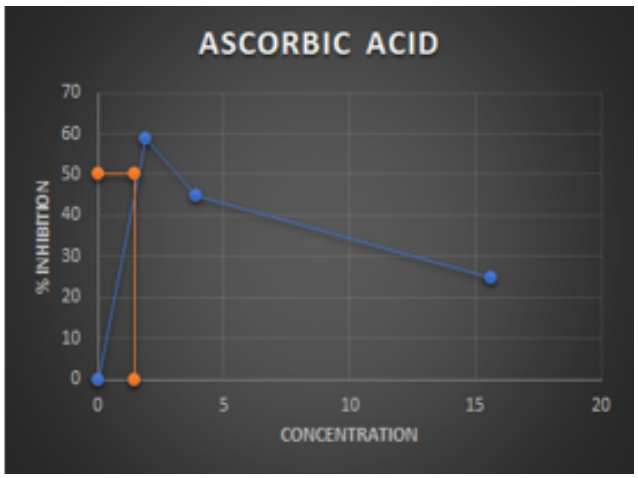


Figure 13: Antioxidant activity of ascorbic acid

had a binding affinity of -6.2. There was pi-pi stacking in the benzimidazole portion with residue TRY A 407.Pi-alkyl interactions were established by the benzene fused with imidazole with residue VAL A 182 and the phenyl portion with residue ALA A 100. The derivative of salicylic acid demonstrated a binding affinity of -6.4. The residue TRY A 407 in the benzimidazole portion displayed pi-pi stacking. Pi-alkyl interactions were established by the benzene fused with imidazole with residue VAL A 182 and the phenyl portion with residue ALA A 100. The derivative's phenyl component interacted pi-sigma with the residue ALA A 100.With a binding affinity of -4.4, the derivative of thioglycolic acid exhibited pi-pi stacking in the benzimidazole portion, containing the residue TRY A 407. Pi-alkyl interactions were established by the benzene fused with imidazole with residue VAL A 182 and the

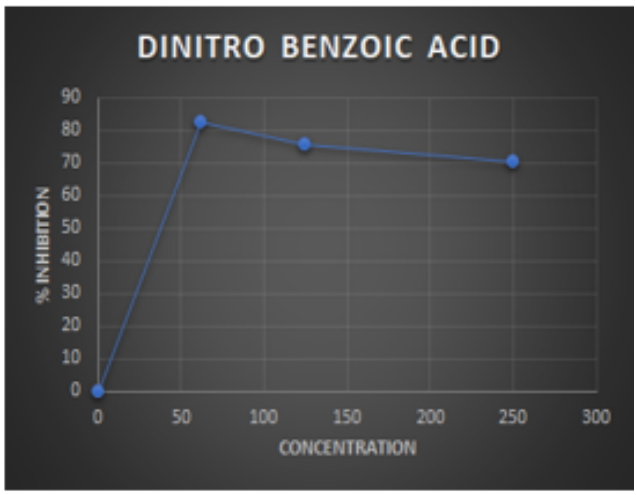


Figure 14: Antioxidant activity of compounds using ABTS method

phenyl portion with residue ALA A 100.TRY A 407 was the residue with pi-pi stacking in the benzimidazole portion, while albendazole had a binding affinity of -5.4. Pi-alkyl interactions were established by the benzene fused with imidazole with residue VAL A 182 and the phenyl portion with residue ALA A 100. Pi-sulfur interaction was observed with PHE A 404.Overall, these results highlight the importance of specific molecular interactions, such as pi-pi stacking, hydrogen bonding, and pi-alkyl interactions, in dictating the binding affinities and specificities of the studied derivatives with their target proteins. Understanding these interactions is essential for rational drug design and optimization efforts aimed at developing potent and selective therapeutic agents.

Theoretical and practical yields of compounds provide insight into the efficiency of chemical reactions and the practicality of synthesis processes. In this table, we observe the theoretical and practical yields of five different compounds, along with their melting points and Rf values. The theoretical yield of salicylic acid was 2.1 g, but the practical yield was significantly lower at 0.042 g. This discrepancy suggests issues with the synthesis process or purification methods. The practical yield of sulphosalicylic acid exceeded its theoretical yield, indicating a successful synthesis with a practical yield of 4.420 g compared to a theoretical yield of 2.9 g. The melting point and Rf value align with the expected characteristics of this compound. Both the theoretical and practical yields of dinitrobenzoic acid were close, indicating a relatively efficient synthesis process. The melting point and Rf value also match the expected properties of this compound. The practical yield of thioglycolic acid exceeded its theoretical yield, indicating a successful synthesis process with a practical yield of 2.417 g compared to a theoretical yield of 1.64 g. The melting point and Rf value align with the expected characteristics of this

compound. Similar to thioglycolic acid, the practical yield of glycine also exceeded its theoretical yield, indicating a successful synthesis process with a practical yield of 2.054g compared to a theoretical yield of 1.47g. The melting point and Rf value align with the expected properties of this compound.

The interpretation of ATR (attenuated total reflectance) spectra involves analyzing the absorption bands to identify functional groups present in a molecule. Here's an interpretation based on the provided peaks: Peaks observed at 3384.23 cm^{-1} and 3361.24 cm^{-1} correspond to the stretching vibrations of N-H bonds. These peaks indicate the presence of amine or amide functional groups in the molecule. The presence of two peaks suggests that there may be different types of N-H bonds present, such as primary, secondary, or tertiary amines, or different types of amide groups. The peak observed at 3183.13 cm^{-1} corresponds to the stretching vibration of aromatic C-H bonds. This peak indicates the presence of aromatic rings in the molecule. Aromatic rings typically absorb in this region due to the stretching vibrations of the C-H bonds within the ring structure. Overall, based on the ATR interpretation, the molecule likely contains both amine or amide functional groups and aromatic rings. This information can be useful for further structural elucidation and characterization of the compound.

The Lipinski rule, also known as the Rule of Five, is a guideline in drug discovery and medicinal chemistry used to assess the drug-likeness of chemical compounds. It helps predict whether a compound is likely to be orally bioavailable based on its physicochemical properties. The table provided lists several compounds along with their molecular weight, hydrogen bond donors, hydrogen bond acceptors, LogP (octanol-water partition coefficient), molar refractivity, and the number of Lipinski's rule violations. All compounds listed have molecular weights well below the threshold of 500, hydrogen bond donors, and acceptors, which is favorable according to Lipinski's rule. All the compounds listed have LogP values below 5, indicating good membrane permeability. The compounds listed fall within this range of 40-130, further supporting their drug-likeness. This suggests that all the compounds are likely to have good oral bioavailability based on their physicochemical properties.

The table presents the antioxidant activity of various compounds measured using two different methods: DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)) assays. Antioxidant activity is expressed as the IC50 value, which represents the concentration of the compound required to scavenge 50% of free radicals in the assay. Salicylic acid shows moderate antioxidant activity, with IC50 values of 37 μg/ml in the DPPH method and 3.5 μg/ml in the ABTS method. Its ability to scavenge free radicals indicates its potential as an antioxidant agent. Sulphosalicylic acid exhibits weak antioxidant activity, as indicated by IC50 values above 200 μg/ml in both the DPPH and ABTS methods. This suggests that it may not be effective in scavenging free radicals at the concentrations tested. Dinitrobenzoic acid shows moderate antioxidant activity with an IC50 value of 190 μg/ml in the DPPH method. However, its activity in the ABTS method is not determined due to the IC50 value being above 200 μg/ml. Thioglycolic acid demonstrates moderate to strong antioxidant activity, with IC50 values of 130 μg/ml in the DPPH method and 180 μg/ml in the ABTS method. Its ability to scavenge free radicals indicates its potential as an antioxidant agent. Glycine shows moderate antioxidant activity with IC50 values of 360 μg/ml in the DPPH method and above 150 μg/ml in the ABTS method. While it exhibits some antioxidant activity, it is less potent compared to other compounds tested. Ascorbic acid, known as vitamin C, serves as a positive control and demonstrates strong antioxidant activity, with IC50 values of 60 μg/ml in the DPPH method and 1.5 μg/ml in the ABTS method. Its potent scavenging ability confirms its well-established role as an antioxidant.

5. Conclusion

The comparison between theoretical and practical yields provides valuable information about the efficiency and success of chemical synthesis processes. Discrepancies between theoretical and practical yields, as seen in the case of salicylic acid, suggest potential issues with the synthesis or purification methods that need to be addressed. On the other hand, compounds like sulphosalicylic acid, dinitrobenzoic acid, thioglycolic acid, and glycine demonstrated successful synthesis processes, with practical yields either matching or exceeding their theoretical values. This indicates the effectiveness of the synthesis methods used for these compounds. Overall, the combination of theoretical and practical yield data, along with melting points and Rf values, offers valuable insights into the synthesis and characterization of chemical compounds, aiding in process optimization and quality control efforts in chemical research and manufacturing. The ATR spectra confirmed the compounds' functional groups.

The compounds listed in the table adhere to Lipinski's rule, indicating favorable drug-like properties such as appropriate molecular weight, hydrogen bond donors and acceptors, LogP values, and molar refractivity. These properties suggest that the compounds have the potential to be orally bioavailable and may be suitable candidates for further drug development or medicinal chemistry studies. Overall, adherence to Lipinski's rule enhances the likelihood of successful drug discovery and development by identifying compounds with favorable pharmacokinetic properties.

The antioxidant activity of the compounds varies, with some showing moderate to strong scavenging abilities against free radicals. Salicylic acid and thioglycolic acid exhibit moderate antioxidant activity, while ascorbic acid demonstrates strong antioxidant activity, as expected. However, sulphosalicylic acid and glycine show weak to moderate antioxidant activity, indicating their limited efficacy as antioxidants. Further studies could explore the mechanisms underlying the antioxidant properties of these compounds and their potential applications in therapeutic or nutraceutical formulations. Additionally, structure-activity relationship studies may help identify structural features that contribute to enhanced antioxidant activity, aiding in the design of novel antioxidant agents.

6. Source of Funding

None.

7. Conflict of Interest

None.

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Author biography

Gopinathan Narasimhan, Professor

Gokul kumar K, Student

B Balalji, Student

Azhagu Naachiar, Student

Khiren S, Student

Harikrishnan N, Principal

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