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Synthesis, Characterization and Biological Evaluation of Novel Saccharin–Thiadiazole Derivatives as Potential Antimicrobial Agen

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Abstract

A new series of saccharin–propane hybrid derivatives was successfully synthesized through a multistep synthetic pathway. Initially, substituted benzoic acid derivatives were reacted with thiosemicarbazide in the presence of phosphorus oxychloride ($POCl_3$) to yield 2-amino-5-(substituted phenyl)-1,3,4-thiadiazole intermediates, designated as L1–L3. Subsequently, these intermediates were treated with 1-chloro-3-bromopropane to afford the corresponding chloropropyl derivatives M1–M3. In the final step, nucleophilic substitution with sodium saccharin produced the desired saccharin-containing compounds S1–S3. The structures of the synthesized compounds were confirmed using Fourier Transform Infrared Spectroscopy (FT-IR) and Nuclear Magnetic Resonance spectroscopy (^1H NMR and ^13C NMR). The antimicrobial potential of compounds S1–S3 was evaluated against three bacterial strains and the fungal strain Candida albicans. Antimicrobial activity was assessed using the agar diffusion technique, and the minimum inhibitory concentration (MIC) values were determined. Among the produced derivatives, substance S2 showed the best inhibitory action against Pseudomonas aeruginosa, while compounds S1, S2, and S3 revealed significant antimicrobial activities against the other examined bacteria. Also, MIC testing in nutritional broth media indicated that substance S2 has a minimum inhibitory concentration of 62.5 µg/mL with P. aeruginosa and 31.2 µg/mL with Candida albicans. The safety profile of substance S2 was further studied by EDTA-tube assay and blood agar hemolysis test. The results obtained showed that the substance was not found to be poisonous and it was biocompatible at high level within the measured concentration range of (1000,500, 250, 125, 62.5) µg/mL.

Keyword: 1,3,4-Thiadiazole derivatives; Saccharin-based compounds; Heterocyclic hybrids; Antibacterial activity; Antifungal activity; Broad-spectrum antimicrobial agents

1. Introduction

Saccharin is considered one of the oldest artificial sweeteners, which was found in the late 19th century. It has a significant sweetening capacity compared to sucrose, despite carrying nearly little calories. It is commonly utilized in the dietary and pharmaceutical sectors for diabetics or for individuals who are on reduced calorie diets. It is also characterized by its thermal and chemical stability, making it suitable for use in cooking and processed products. Despite past controversy surrounding its safety, several global health authorities, such as the Food and Drug Administration (FDA) and the World Health Organization (WHO), have confirmed that it is safe within the permissible limits for daily consumption [1]. Saccharin is chemically a sulfonamide derivative with the molecular formula C7H5NO3S. It is characterized by its lack of metabolism in the body, being excreted unchanged in the urine, which explains its low calorific value. It is also used in oral care products such as toothpaste and mouthwash because it does not cause tooth decay. Recent studies have shown interest in investigating its effects on the gut microbiome, with some findings suggesting a possible impact on the balance of gut bacteria; however, these effects are still under investigation [2]. Saccharin, one of the first artificial sweeteners discovered in 1879, is an organic compound used as a sugar substitute due to its high sweetening power, which is approximately 300–500 times greater than sucrose, while being

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virtually calorie-free. Classified as a non-nutritive sweetener, it is not metabolized by the body and has become a staple in the food and pharmaceutical industries, particularly in products for diabetics and weight loss programs, because it does not directly affect blood glucose levels [3].

Saccharin is characterized by its high thermal and chemical stability, making it suitable for use in cooking and food processing without losing its effectiveness. Chemically, it is a sulfonamide derivative with the molecular formula C7H5NO3S and is usually found in the form of salts such as sodium saccharin to increase solubility. A large proportion of it is absorbed by the body without undergoing metabolic processes, and is excreted as is through urine, which explains why it does not contribute to energy production [4]. In terms of safety, there has been a lot of recent research on saccharin and international authorities, like the European Food Safety Authority (EFSA), have concluded that consumption at the recommended daily intake (ADI) levels is safe. Indeed, this limit has been increased according to current estimates that incorporate data up to 2024. These studies reveal that the actual consumption levels of most populations are substantially below the ADI, hence maintaining its long-term safety. [5]. However, the influence of saccharin on the gut flora has been studied in recent studies. Some research points to potential effects of artificial sweeteners on gut flora and possible links to alterations in metabolic systems like glucose tolerance. However, results are not clear yet, as previous human research have not demonstrated any significant changes in microbial diversity when taken within the ADI, highlighting the need for more long-term studies [6]. In its free form saccharin is not very soluble in water, but it is commonly utilized in the form of salts (e.g. sodium saccharin) to boost solubility. It is resilient at high temperatures and different pH levels which makes it excellent for food preparation. The presence of a benzene ring connected to a sulfonamide group provides it distinct electrical characteristics that affect its chemical processes, especially the ability to establish hydrogen bonds with the taste receptors [7]. Saccharin attaches to sweet taste receptors (T1R2 / T1R3) on the tongue, which sends nerve impulses to the brain that are perceived as sweet . Recent studies have demonstrated that saccharin can influence indirect metabolic pathways such as insulin secretion or neural signals associated to satiety, however these effects are dose- and individual-dependent [8]. Recent study has shown that artificial sweeteners like saccharin may change the makeup of the gut flora, and in so doing impact glucose metabolism. Yet, subsequent clinical trials (2023-2024) have produced conflicting outcomes. Some studies have shown no significant impact when taken in limitations, therefore the effect is dependent on the dose and the individual conditions [9]. Recently, saccharin has been re-assessed by international authorities and has been reaffirmed as safe within the recommended daily intake (ADI). Toxicology data have also been revised in the light of new investigations and past worries about cancer have been proved to be non-applicable to people. Saccharin is therefore regarded to be one of the most investigated sweeteners for safety [10]. The recent scientific evidence has prompted international scientific and regulatory authorities, including the European Food Safety Authority, to evaluate the safety of saccharin. The assessment found no evidence that saccharin causes genetic harm or raises the risk of cancer in people. Thus, the Acceptable Daily Intake (ADI) was revised to 9 mg/kg body weight and it was stated that the current levels of exposure in the population are within safe limits, such that there is no health risk when taken at recommended amounts [11]. 1,3,4-thiadiazole is a five-membered heterocyclic ring with two nitrogen and one sulfur atom, and is regarded an essential basic structure in medicinal chemistry. This chemical has garnered extensive interest due to its many biological qualities since it is a part of variety of compounds possessing pharmacological activities such as antibacterial, antifungal, anti-inflammatory and even anticancer substances [12]. The wide range of biological activity of 1,3,4-thiadiazole derivatives was attributed to the capacity of these molecules to interact with numerous intracellular biological targets. Recently, research has been concentrated on the design of new and better derivatives of this chemical with better efficacy and lower toxicity. It is also being investigated for its uses in the development of medications that target enzymes and proteins involved in chronic illnesses such as cancer and neurological disorders. In addition, this chemical is employed as a significant precursor in organic synthesis for preparation of complex compounds of commercial and medicinal importance [13]. 1,3,4-Thiadiazole is one of the most significant five-membered heterocyclic compounds in organic chemistry. Its aromatic ring has two nitrogen atoms and one sulfur atom. This structure offers it distinct electrical and chemical capabilities. This molecule is an important scaffold in current drug design and has received much interest in pharmaceutical chemistry because of its capacity to make strong interactions with biological targets inside the body [14]. This molecule is significant because of its efficacy as a pharmacophore. Its compounds have been shown to possess a wide range of biological properties, including antibacterial, antifungal, antiviral, anti-inflammatory and anti-tumor actions. Besides, structural alterations of the base ring enable the synthesis of more potent and selective compounds. It is an essential platform for the design of novel medications [15]. Recent studies (2024-2025) show that 1,3,4-thiadiazole derivatives are increasingly being utilized in the development of anticancer drugs. New compounds have been synthesized which have shown significant inhibitory activity against a number of cancer cell lines including breast and lung cancer. They have also been employed in the construction of enzyme inhibitors acting on microbiological processes in cancer cells, which further increases their potential as a promising therapeutic component [16]. Moreover, this chemical has been used for the synthesis of novel medicines especially against drug resistant microorganisms. Its derivatives have been shown to inhibit important bacterial enzymes such as peptide deformylase and have been shown to be effective against both gram-positive and gram-negative bacteria. Some

derivatives have also demonstrated action as inhibitors of enzymes related to diabetes such as α-glucosidase, opening the door to its usage in the treatment of chronic conditions [17]. In industry 1,3,4-thiadiazole is an essential intermediate in organic synthesis. It participates in the development of complex compounds with medicinal and agricultural uses. Its electrical characteristics allow the design of molecules with high biological activity. Thus this chemical is one among the most researched heterocyclic compounds in contemporary science and still the topic of much future study [15]. Modern methods of synthesis of 1,3,4-thiadiazole derivatives include sophisticated techniques including microwave-assisted synthesis, solventless synthesis and multicomponent processes. The building of the ring is done with precursor materials such as thiosmicarbazide and hydrazides to rapidly and efficiently synthesize a wide variety of derivatives, which improves yield and reduces the environmental effect [18]. Thiadiazole derivatives have been shown to offer great potential in advanced cancer therapy due to their many biological activities and attractive structural features . Recent investigations are more and more directed to the incorporation of thiadiazole frameworks into certain therapeutic systems in order to increase the drugs specificity for malignant cells, with few side effects on normal tissues. Moreover, the thiadiazole-based compounds have shown the capacity to interact with the important cellular components such as DNA, enzymes and regulatory proteins which are implicated in tumor growth. Recent investigations have also focused on their incorporation into nanomedicine platforms such as nanoparticles and smart drug delivery systems for enhanced bioavailability, controlled release and targeted accumulation in the tumor tissues. These results imply that thiadiazole derivatives might be possibilities for developing more effective and safer anticancer treatments [19]. Recent investigations have demonstrated that the biological activity of thiadiazole derivatives depends strongly on the kind of substituents connected to the ring. In the presence of electron withdrawing and electron donating groups, anticancer or antibacterial activities. The pharmacological efficiency of the modifications at the locations 2 and 5 of the ring was similarly considerable and employed for the creation of precision-targeted medications [20]. Recent investigations 2024 have shown that 1,3,4-Thiadiazole compounds have substantial biological activity, functioning as powerful inhibitors of various key intracellular enzymes and pathways. These targets include kinases and vascular endothelial growth factor receptors (VEGFRs), important for the development of cancer cells and tumor angiogenesis. Inhibition of these pathways slows down the multiplication of aberrant cells, which makes these chemicals potential candidates for the development of targeted treatments of cancer and chronic disorders [21]. 1,3,4-Thiadiazole is an essential heterocyclic framework in medicinal chemistry because to its various chemical characteristics and wide range of biological actions, including antibacterial, anti-inflammatory and anticancer effects. This is due to its capacity to interact with various biological targets in the body and the simplicity of modification of its chemical structure to generate new derivatives with greater potency and superior pharmacokinetic qualities [22]. Recent research trends show that future research on 1,3,4-thiadiazole derivatives will be focused on the development of more selective and targeted medications, notably in the treatment of cancer, neurological and inflammatory diseases. This research also attempts to enhance the pharmacokinetic features such as absorption, bioavailability, and to minimize toxicity and side effects , so boosting their potential for clinical application as effective and safe therapeutic agents [23]. From structure-activity relationship (SAR) investigations, it has been seen that the biological activity of 1,3,4-thiadiazole derivatives is highly dependent on the kind and location of the substituents attached to the ring. Electron-withdrawing groups were discovered to promote antibacterial activity by better contact with biological targets, whereas electron-donating compounds may increase anticancer activity by enhancing binding to target enzymes and proteins. Changes at the 2- and 5-positions of the ring are also important determinants determining the activity and bioselectivity of these molecules [24]. Thiadiazole compounds have anticancer action through different molecular pathways. These chemicals may act by disturbing DNA synthesis and cell proliferation, inhibiting important enzymes such as kinases and carbonic anhydrase, and modulating biosignature pathways implicated in cancer, such as PI3K/Akt and MAPK pathways. In addition, several thiadiazole derivatives are known to cause oxidative stress and apoptosis in cancer cells and to decrease tumor growth and survival [25]. Antimicrobial agents are a heterogeneous collection of compounds that aim to inhibit the development or survival of harmful microorganisms such as bacteria, fungus and parasites. Their mode of action involves disruption of important cellular processes, such as cell wall production, protein synthesis, nucleic acid replication and metabolism. Antimicrobial medications have been a cornerstone of contemporary medicine due to their ability to treat infectious illnesses, dramatically improving patient outcomes and lowering infection-related morbidity and death worldwide [26]. Antibiotics are medicines that kill germs or stop them from growing. Their antibacterial effect is achieved via interfering with crucial cellular targets including creation of cell wall, protein production, nucleic acid replication and critical metabolic processes. The therapy of infectious illnesses has been revolutionized by antibiotics, but the indiscriminate and overuse of these drugs has spurred the establishment of antimicrobial resistance, a serious concern to global public health [27]. Antimicrobials are a key component of contemporary medicine, utilized to manage a wide range of pathogens, including bacteria and fungus. Their success is based on the capacity to target essential components of the microbial cell such as the cell wall, ribosomes and nucleic acid manufacturing pathways and hence impede development or kill the pathogen. Their usage has largely contributed to reduction of mortality due to infectious infections [28]. Antimicrobial agents are a broad class of medicinal drugs that are used to inhibit the growth or destroy harmful microorganisms including bacteria, viruses, fungus and parasites. They exhibit their antimicrobial function by several routes including disruption of cell wall

construction, inhibition of key enzymes, interference with protein creation or repression of nucleic acid replication. Because of these activities antimicrobial medicines continue to be important tools in the prevention and treatment of infectious disorders [29].

Antibiotics are one of the most essential therapeutic agents in the contemporary medicine. Antibiotics target critical components of bacterial cells such as a cell wall, ribosomes, and DNA replication processes. Proper administration of antibiotics can effectively prevent bacterial infections including Staphylococcus aureus, Acinetobacter baumannii and other harmful strains. However, abuse or misuse may result in antibiotic resistance, treatment failure, and significant adverse effects [30]. Microbial infections are a global health problem, especially in hospitals and in the immunocompromised. Gram-positive bacteria such as Staphylococcus aureus , Gram-negative bacteria such as Pseudomonas aeruginosa and Acinetobacter baumannii and Candida fungus cause a wide variety of diseases from skin and wound infections to major systemic infections. Knowing the features of these organisms and their mechanisms of resistance to contemporary therapies is vital in order to design successful methods for infection control [31]. *Staphylococcus aureus* is a Gram-positive coccus that is frequently seen in clusters resembling grapes. It is a major human pathogen, able to colonize several body locations and cause a wide spectrum of infections, from moderate skin and soft tissue infections to severe invasive illnesses including bacteremia, osteomyelitis and infective endocarditis . *S. aureus* is harmful because it produces several virulence factors such as toxins, adhesins and enzymes that help in invading tissue and escaping from the immune system. Of particular concern in the clinic is the rise of methicillin-resistant *Staphylococcus aureus* (MRSA), which is resistant to most β-lactam medicines . Management of MRSA infections frequently involves the use of alternative medicines such as vancomycin or linezolid and antimicrobial susceptibility testing to guide therapy[32]. Staphylococcus aureus is a frequent source of infection leading to disease from moderate to severe. Some types, like MRSA, are resistant to several antibiotics, leaving few treatment alternatives. Resistant instances are treated with medicines such as vancomycin, whilst susceptible strains respond to standard antibiotics such as penicillins [33]. Pseudomonas aeruginosa is an opportunistic gram-negative bacillus ubiquitous in natural and health care contexts. It is a major source of hospital acquired diseases such respiratory tract infections, urinary tract infections and wound or burn infections especially in immunocompromised individuals. This pathogen is of clinical importance mainly because of its extraordinary capacity to develop antibiotic resistance by several pathways including biofilm formation, efflux pumps and production of β-lactamase enzymes. Therefore, the best results of therapy should be obtained based on susceptibility testing for antimicrobial therapy [34]. Pseudomonas aeruginosa is a Gram-negative, rod-shaped bacterium, flagellated, and producing pigments like pyocyanin. It may live in many locations including hospitals and stagnant water increases the chances of it being spread. Pseudomonas aeruginosa causes a wide spectrum of illnesses including respiratory tract infections (especially in cystic fibrosis patients), urinary tract infections, wound and burn infections. This bacterium is resistant to antibiotics by producing beta-lactamases and active transporter propellants and forming biofilms that hinder drug effectiveness. Treatment varies on the findings of susceptibility testing and may include piperacillin-tazobactam, ceftazidime, or aminoglycosides. [35].

Pseudomonas aeruginosa is an opportunistic bacteria responsible for devastating infections, especially in hospitalized patients. It has great resistance to antibiotics because of the presence of drug efflux pumps and the production of biofilm which lowers the efficacy of therapy. It can be treated with Beta Lactam and Fluoroquinolone antibiotics although the efficacy depends on the resistance pattern of each isolate [36]. Actinobacter is a prevalent source of hospital-acquired illnesses and is a rod-shaped, non-motile, gram-negative bacteria that may live for lengthy durations on surfaces. The bacteria cause septicemia, lung infections, wound infections and urinary tract infections, especially in patients on mechanical ventilation. Actinobacter is resistant to numerous medicines including carbapenems which makes treatment more difficult. Thus, doctors typically use polymyxins, aminoglycoside or combination treatment depending on the findings of susceptibility testing [37,38]. Acinetobacter baumannii is one of the most deadly causes of hospital-acquired infections due to its great ability to develop antibiotic resistance. It causes infections of the respiratory system and of wounds and is increasingly resistant to carbapenems, making it harder to treat. Sometimes colistin is used as a last resor [39]. Candida fungi are yeast or filamentous fungus that are commonly found on the skin, mucosal membranes and in the bowels. Candida albicans is the most prevalent form. These fungi cause mouth thrush, vaginal yeast infections and candidiasis (infection of blood and internal organs) in immunocompromised persons. Some strains are resistant to antifungal agents like fluconazole, especially in long term usage. Treatment varies on the kind and degree of infection and includes azoles such as fluconazole, echinocandins such as caspofungin or amphotericin B for severe infections [40] The newborn period is a particularly vulnerable time for infants to develop Candida colonization and infection, as their immune system is not yet fully mature. Vertical transmission from mother can occur during passing through the birth canal, in case of maternal infection with Candida. Moreover, several environmental and clinical conditions (e.g., extended exposure to antibiotics, artificial feeding, and hospitalization) may promote fungal overgrowth and increase the chance of infection in early life [41]. Candida albicans is a prevalent opportunistic fungal pathogen of superficial and invasive infections in immunocompromised patients. Oral candidiasis appears as white plaques on the mucosa which are removable leaving an erythematous surface behind. The illness is due to an overgrowth of fungi after changes in

host immunity or in the usual oral flora. The mainstay of therapy is antifungal medications such as fluconazole but the rise of resistance strains has become an increasing therapeutic challenge. [42]. Laboratory methods to determine antimicrobial effectiveness include the minimum inhibitory concentration (MIC) test and the disc diffusion test. Efficacy is pathogen type and genetic characteristic dependent and is also dependent on the capacity of the microbe to develop resistance mechanisms such as modification of the drug target or production of enzymes that inactivate the medication. Hence, the right therapy needs to be chosen following susceptibility testing findings, in order to assure treatment success [43]. Microorganisms range greatly in their sensitivity to antimicrobial drugs and antimicrobial susceptibility testing is a vital tool for the selection of the most appropriate therapy. The improper and excessive use of antimicrobial medications has hastened the development of antimicrobial resistance, which is acknowledged as one of the most severe risks to global public health [44]. Toxicity is the capacity of a chemical substance or agent to harm an organism upon exposure to it in certain doses, either acutely or chronically. The toxicity of a substance relies on several aspects, such as the dose, the length of exposure, the method of entrance into the body, and the physiological features of the organism. This is why the same drug may be safe in low concentrations but poisonous in large concentrations. This is the concept of " the dose makes the poison " [45].

The toxic effects may occur at several levels of the organism, affecting the whole body systemically or a particular tissue or organ locally. Toxicity may also be various creatures including human, animals, plants and even microbes and is frequently employed in the medical, pharmacy, and environmental research [46]. Cytotoxicity refers to the harm that cells suffer upon exposure to hazardous chemicals. These compounds interfere with the structure or essential activities of cells, for example by damaging the cell membrane or by stopping metabolism. Cytotoxicity tests are frequently used to evaluate the safety of medications and chemicals. Cytotoxicity tests are a significant indication of early biological effects before organ level damage [47]. Organ toxicity can affect individual organs of the body, which is called organ toxicity. A good example of this is hepatotoxicity, where the liver is one of the most susceptible organs because of its function to metabolize toxins. Other kinds include nephrotoxicity that damages the kidneys. These effects can lead to malfunction, which can be reversible or irreversible depending on the intensity of exposure [48]. Toxicity is not confined to the biological world. It is used figuratively at times to refer to adverse consequences in social systems such as familial ties or poisonous communal surroundings. While not scientific, this usage does show the broad influence and pervasiveness of the phrase in other domains and the need to know it in its specific scientific context [49]. This research seeks to design and synthesize new derivatives of propanesaccharin linked to the 1,3,4-thiadiazole ring, while evaluating their bioavailability as antimicrobials by studying their effect against three types of clinical bacteria, as well as a type of fungus such as Candida albicans, to determine their efficacy as potential therapeutic compounds [50].

2. Materials and methods

2.1. The synthesis of the derivatives

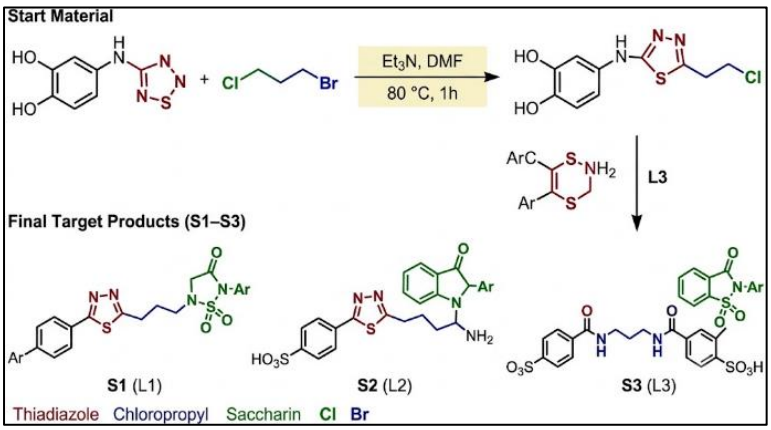

Figure 1 Saccharin Compound Analysis **: (**i) $POCl_3$, reflux 3 h, H_2O, reflux 4 h, KOH. (ii) 1-Chloro-3-Bromo propane, triethyl amine, DMF, Stitred at 80 c for are hour ,(iii) Sodium saccharin, DMF, reflux 3 h

The synthesis of the derivatives (S1-S3) are shown in the in the scheme below. The F.T-IR spectral data recorded on F.T-IR-8400 Fourier Transform Infrared Spectrophotometer SHIMADZU using potassium bromide disc.1H-NMR and 13C-NMR were recorded on Bruker Ultra Shield, 500MHz, using DMSO as solvent and TMS as internal standard. Melting

points (0C) recorded on hot stage Gallen Kamp melting point apparatus and were uncorrected. Chemical names follow the IUPAC nomenclature. Starting materials were used without purification.

2.2. Synthesis of 2-Amino-5-(substituted phenyl)-1,3,4-thiadiazole Derivatives (L1).

A series of 2-amino-5-(substituted phenyl)-1,3,4-thiadiazole derivatives (L1) were synthesized via cyclization of thiosemicarbazide with the corresponding substituted aromatic carboxylic acids. Thiosemicarbazide (1.8 g, 0.02 mol) and the appropriate substituted aromatic carboxylic acid (0.02 mol) were introduced into a round-bottom flask, followed by the gradual addition of phosphorus oxychloride (10 mL) under constant stirring.The reaction mixture was heated under reflux for (3 h), after which distilled water (25 mL) was added cautiously with continuous stirring. Refluxing was then maintained for a further (4 h) to ensure completion of the cyclization process. Upon completion, the reaction mixture was allowed to cool to ambient temperature and subsequently neutralized using a 10% aqueous potassium hydroxide solution until approximately neutral pH was reached.The solid product formed was isolated by filtration, washed several times with distilled water to remove inorganic residues, and purified by recrystallization from ethanol. The required thiadiazole derivatives (L1) were produced in good yields as crystalline solids, dehydrating and ring-closing reagent phosphorus oxychloride has been used for the cyclodehydration of thiosemicarbazide with substituted aromatic carboxylic acids to synthesize the 1,3,4-thiadiazole heterocyclic ring system, which is the synthetic route for the target compounds [51].

2.3. 2-Amino-5-(2,4-dihydroxy benzoic acid)-1,3,4-thiadiazole (L1)

light brown powder with a yellowish tinge, yield (88%), m.p 260-263°C, R_f = 0.43; F.T-IR (KBr disk, cm^{-1}) 3288-3139, 3080, 1630, 1580, 1429.

2.4. Synthesis of 1-chloro-3-(5-(substituted phenyl)-1,3,4-thiadiazol-2-yl-amino) propane (M_1-M_2).

The proper 2-amino-5-(substituted phenyl)-1,3,4-thiadiazole derivative (0.01 mol) was dissolved in dry N,N-dimethylformamide (DMF, 30 mL) with constant stirring. To this solution 1-bromo-3-chloropropane (0.01 mol) was added dropwise then triethylamine (0.01 mol) was added as acid scavenger. The reaction mixture was stirred at 50 °C for 1 h to facilitate the nucleophilic substitution process.Upon completion of the reaction, the mixture was poured slowly into distilled water (35 mL) with vigorous stirring. The resulting precipitate was collected by filtration, washed thoroughly with distilled water, and dried under reduced pressure. The crude product was subsequently purified by recrystallization from ethanol to afford the corresponding 1-chloro-3-(5-(substituted phenyl)-1,3,4-thiadiazol-2-ylamino)propane derivatives (M1–M2) as pure crystalline solids. [52].

2.4.1. 1-chloro-3-(5-(2,4-dihydroxy benzoic acid)-1,3,4-thiadiazol-2-yl-amino) propane (M1)

Pale Yellow powder, yield (91%), m.p 212-214 °C, R_f = 0.39; F.T-IR (KBr disk, cm^{-1}) 3296, 2956.

2.4.2. 1-chloro-3-(5-(Sulfanilicacide)-1,3,4-thiadiazol-2-yl-amino) propane (M2)

white crystal powder, yield (87%), m.p 160-163°C, R_f = 0.42; F.T-IR (KBr disk, cm^{-1}) 3270, 2985.

2.4.3. Synthesis of 1-N-saccharin-3-(5-substituted phenyl)-1,3,4-thiadiazole-2-yl-amino) propane (S1-S3).

Sodium saccharin (0.01 mol) was dissolved in N,N-dimethylformamide (DMF, 25 mL) under continuous stirring. Subsequently, the corresponding intermediate compounds (M1–M3) (0.01 mol) were added portion-wise to the reaction mixture. The resulting mixture was heated under reflux for 3 h to facilitate the nucleophilic substitution reaction and the formation of the desired saccharin-linked derivatives.Upon completion of the reaction, the mixture was allowed to cool to room temperature and then poured into distilled water (25 mL) with continuous stirring. The precipitated solid was collected by filtration, washed thoroughly with distilled water to remove residual solvent and inorganic impurities, and dried under reduced pressure. The crude products were purified by recrystallization from ethanol to afford the target compounds S1–S3 as pure crystalline solids. [53].

2.5. 1-(N-saccharin)-3-(5-(2,4-dihydroxy benzoic acid phenyl)-1,3,4-thiadiazol-2-yl-amino) propane (S1)

Light Brown powder, yield (81%), m.p 230-232°C, R_f = 0.46; F.T-IR (KBr disk, cm^{-1}) 3531 , 3336-3259 , 3101-3066, 1645 , 1587 , 1546,1460,1334,1147, . ^{1}H-NMR (DMSO-d_6, 500 MHz, δ) 3.40 (t_2, 2H, CH2), 2.05 (m, 2H, CH2), 3.85 (t_1, 2H, CH2), 7.85-8.15 (m, 4H), 7.4 (s, 1H,-NH-),12.10(s,1H,-OH). ^{13}C-NMR (DMSO-d_6, 125 MHz, δ)26, 38, 46, 103, 108-110, 120, 123,138,148,155,161, 168.

2.6. 1-(N-saccharin)-3-(5-(-4 Sulfophenyl)-1,3,4-thiadiazol-2-yl-amino) propane (S2)

light yellowpowder, yield (86%), m.p 246-248°C, Rf = 0.48; F.T-IR (KBr disk, cm^{-1}) 3529 , 3425 ,3383,3012, 2939-2951 , 1645 , 1598 , 1332,1149,1118 . ^{1}H-NMR (DMSO-d_6, 500 MHz, δ) 3.45 (t_2, 2H, CH2), 2.1 (m, 2H, CH2), 6.8 (t_1, 2H, CH2), 7.75 (m, 8H), 7.5, (s, 1H),11.5(br s,1H,SO,3H). ^{13}C-NMR (DMSO-d_6, 125 MHz, δ) 26,38, 46, 114, 121-123, 132, 136, 147,149,156,167.

2.7. 1-(N-saccharin)-3-(5-(1,3-disaccarine propane phenyl)-1,3,4-thiadiazol-2-yl-amino) propane (S3)

Pale Yellow powder, yield (85%), m.p 195-197°C, R_f = 0.43; F.T-IR (KBr disk, cm^{-1}) 3531 , 3334, 3101,3066 , 1645 , 1585 , 1458,1334,1255,1147,1118. ^{1}H-NMR (DMSO-d_6, 500 MHz, δ) 2.18 (t_2, 2H, CH2), 2.02 (m, 2H, CH2), 3.38 (t_1, 2H, CH2), 7.8-8.2 (m, 8H), 7.2 (s, 1H). ^{13}C-NMR (DMSO-d6, 125 MHz, δ) 27, 42, 45, 120-144, 121, 124,132,134,137,144,159,164, 168.

2.8. Biological activity

2.8.1. In Vitro Antimicrobial and Cytotoxicity Assays

Microbial Strains and Inoculum Preparation

The antimicrobial efficacy of the newly synthesized compounds (S1–S3) was evaluated against a clinical panel of pathogenic microorganisms, including *Staphylococcus aureus* (Gram-positive), *Pseudomonas aeruginosa* and *Acinetobacter baumannii* (Gram-negative), and the fungal strain *Candida albicans*. Clinical isolates were validated via standard morphological and biochemical procedures. Inocula were calibrated to a 0.5 McFarland turbidity standard (sim 1 \times 10^5 \ CFU/mL) and inoculated at a 1% v/v ratio into sterile growth media under aseptic conditions to ensure a uniform agar depth of 3–4 mm [56].

2.8.2. Antimicrobial Susceptibility and MIC Determination

The inhibitory profiles were initially screened using the agar well diffusion technique on Mueller-Hinton Agar (MHA) [54]. Quantitative determination of the Minimum Inhibitory Concentration (MIC) was established using both the agar dilution method [55] and the broth macrodilution method in Nutrient Broth, strictly following the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines [57]. Stock solutions of the derivatives were prepared in dimethyl sulfoxide (DMSO) at a standard concentration of 100\mg/mL} to generate progressive gradients. For comparative baselines, four standard reference drugs were employed as positive controls: Meropenem (*S. aureus*), Polymyxin B (*P. aeruginosa*), Doxycycline (*A. baumannii*), and Fluconazole (*C. albicans*). The MIC was defined as the lowest concentration that completely prevented visible microbial reproduction [1, 2, 58].

2.8.3. In Vitro Cytotoxicity Screening

To evaluate the biocompatibility of compounds **S1–S3**, *in vitro* cytotoxicity was quantitatively screened across a descending series of concentrations (1000, 500, 250, 125, and 62.5 \mg/mL). Cell viability thresholds and fluid-based visual alterations were monitored via the tube macro-dilution approach [59]. Furthermore, cytocompatibility was confirmed using the solid-phase culture dish method via the agar-overlay technique to spatially assess cell monolayer integrity and map zones of cellular degradation [60].

3. Results and discussion

3.1. 1-N-saccharin-3-(5-substituted phenyl)-1,3,4-thiadiazole-2-yl-amino) propane (S1-S3)

The synthesis involved the reaction of an appropriate carboxylic acid with thiosemicarbazide in the presence of POCl3 followed by reflux with water to form compounds (A1). The compounds (A1) were reacted with 1-Chloro-2-Bromo propane in the presence of DMF to form (B1-B3). The (B1-B3) were reacted with sodium saccharin in DMF to form the target derivatives (S1-S3). The compounds purified by multiple recrystallization from ethanol. All the synthesized compounds were characterized by their melting point, F.T.IR, 1H-NMR and 13C-NMR. The target derivatives, 1-(N-saccharin)-3-((5-substituted phenyl)-1,3,4-thiadiazol-2-yl)amino)propane (S1–S3), were successfully synthesized via a well-designed, multi-step sequential pathway. The synthetic protocol relied on utilizing stable heterocyclic scaffolds to build the final pharmacophore cores, in accordance with established methodologies for constructing hybrid thiadiazole-saccharin systems [61, 62].

The initial phase involved the construction of the core heterocycle, where 2-amino-5-(substituted phenyl)-1,3,4-thiadiazoles (L1) were prepared by the cyclization of appropriate aromatic carboxylic acids with thiosemicarbazide. The reaction was effectively promoted by phosphorus oxychloride POCl3 as dehydrating agent under reflux and then hydrolyzed with water [63]. Then, the nucleophilic substitution of (L1) with 1-bromo-3-chloropropane in dimethylformamide (DMF) was performed easily to provide the alkylated chlorinated intermediates (M1-M2) by virtue of the increased leaving ability of the bromo group. Finally, the target compounds (S1–S3) were synthesized by nucleophilic displacement of the terminal chlorine atom in (M1–M2) with sodium saccharin in DMF medium [61]. Crude target products were separated and recrystallized many times from ethanol to achieve optimum chemical purity. The structural identification and characterization of all the novel derivatives (S1– S3) and their antecedents were convincingly validated by a combination of physical and spectroscopic tools. These included assessment of melting point, Fourier-transform infrared spectroscopy (FT-IR), high resolution proton (H-NMR) and carbon (C-NMR) nuclear magnetic resonance spectroscopies, all of which gave conclusive support for the success of the suggested synthesis process.

3.2. Antimicrobial activity

The recently synthesized compounds (S1–S3) were subjected to biological effectiveness by in vitro antimicrobial screening against a panel of harmful bacteria. This panel consisted of two Gram-negative bacterial strains (Pseudomonas aeruginosa and Acinetobacter baumannii), one Gram-positive bacterial strain (Staphylococcus aureus) and one fungus strain (Candida albicans). Standardized Agar Well Diffusion Method was used to do the main screening for the zones of inhibition. Minimum Inhibitory Concentrations (MICs) were determined thereafter to get the lowest concentration of the produced compounds which may totally inhibit the observable development of the microorganisms. Table 1 summarizes quantitatively the results of the antimicrobial tests of the substances (S1-S3) in a systematic way.

Table 1 The results of the inhibition zone test of antimicrobial activity (MIC (µg/ml) for the synthesized compounds (S1-S3)

Comp. No.	**Concentration (µg /mL)**	**In vitro activity-zone of inhibition in mm (MIC in µg/mL)**							
		Gram- negative				**Gram positive**		**Fungi**	
		P. aeruginos	MIC µg/mL	*A. baumannii*	MIC µg/ml	*S. aureus*	MIC µg/ml	*C. albicans*	MIC µg/ml
S1	1000	18	59.3	25	63	18	65	15	62.34
	500	24		20		18		20	
	250	12		22		18		20	
	125	11		15		14		15	
	62.5	0		20		12		12	
S2	1000	13	60.5	16	58.7	12	66	15	61.10
	500	18		18		16		12	
	250	14		24		15		14	
	125	14		13		13		12	
	62.5	0		14		12		14	
S3	1000	12	61.7	14	58.12	10	63	12	60
	500	21		20		10		14	
	250	10		16		14		14	
	125	13		12		12		13	
	62.5	0		13		13		12	
poly mexin B(PB)	300	20	-----	-----	------	-----	-------	-----	-----

Doxycyclin(DO)	30	----	-----	40	-------	-----	------	------	------
Meropenem(MEM)	10	-----	-----	-----	------	13	----	-----	------
Fluconzole	120	----	----	-----	------	-----	-----	10	----
DMSO	absolute	0	-----	0	-------	0	-------	0	-----

The synthesized saccharin derivatives were classified into two major groups, i.e. high efficacy and low efficacy based on the inference of the efficiency of the standard antagonist Polymyxin B (PB) against *P. aeruginosa*. Among the produced group compound S3 showed the best efficacy at the maximum concentration of 1000 µg /Ml. Compound S2 and compound S1 showed lesser efficacy profile. The high toxicity of the symmetrical bis-saccharin core in S3 at this stock level is first based on a dense concentration gradient that forces the molecule across the cellular envelope. On dilution to a concentration of 500 µg/Ml, a clear change in behavior was seen. A reasonably good effectiveness was found for compound S2 as it maintained consistent growth suppression. In contrast, both S1 and S3 started to lose most of their antibacterial activity. This suggests that the 4-sulfophenyl group in S2 starts to play a significant role in the enhanced anti-P. aeruginosa activity with dilution, providing an ideal amphiphilic balance for cell membrane penetration.At 250 µg /Ml and below (125)µg /Ml, compound S2 securely occupied the high efficacy grid, achieving its best performance and retaining its high sensitivity. On the other hand, the substances S1 and S3 were fully classified into the poor effectiveness group as they reached the bacterial resistance threshold too early.Here, the detailed dilution route proves that the hydrophilic 2,4-dihydroxyphenyl moiety of S1 or the rigid symmetric bissaccharin architecture of S3 are of limited long-term effectiveness in diffusing through the defensive lipid-rich outer walls of P. aeruginosa upon serial dilutions when compared to the very stable, beautifully balanced sulfophenyl-modified analogue (S2) µg /Ml.By induction of the potency of Meropenem (Me), the compounds demonstrated remarkable activity against *S. aureus*. S1 1000 and 500.µg /Ml were more effective than S2 (moderate effectiveness) and both were more effective than S3 (moderate efficacy). At 250.µg /Ml S1 was better, whereas S2 and S3 were clearly examined at moderate effectiveness. 125 µg /Ml, the order of S1 higher than S2 (moderate effectiveness) higher than S3 (moderate efficacy).

Conversely at 62.5.µg /Ml a behavioural inversion was noticed where S3 became the most powerful and showed higher efficacy than S1 and S2 (which reduced to moderate efficacy).This suggests that the 2,4-dihydroxyphenyl group in S1 dominates at high concentrations while the rigid symmetrical bis-saccharin core of S3 confers an outstanding lipophilic advantage at very low doses, which is maximized by cell wall penetration through the thick peptidoglycan layer of S. aureus on dilution.

The synthesized derivatives exhibited a remarkable gradient of activity against A. baumannii by inference based on the known activity of Doxycycline (Do). S1 (high efficacy) at 1000.µg /Ml showed higher efficacy than S3 (moderate efficacy) and both were higher than S2 which showed low efficacy. S2 and S3 showed a moderate efficacy profile, while S1 showed a greater efficacy than S2 and S3 at 500.µg /Ml. S1 and S2 were more effective than S3 at 250.µg /Ml, whereas S3 was considered to have moderate effectiveness. The most effective agent was found to be S1 at 125.µg /Ml with better efficacy than S2 which was higher than S3. Finally, S2 showed greater effectiveness than S3 at 62.5.µg /Ml. The continuous deviation indicates that the 1,3,4-thiadiazole core conjugated with the 2,4-dihydroxyphenyl group in S1 offers a decisive boost. Its free hydroxyl groups are strong electron donors and thus can densely hydrogen bond with bacterial target porins to maintain maximal potency at reduced doses. Derivatives from Fluconazole (Fl) exhibited concentration dependent inhibition against C. albicans. S1 demonstrated great efficacy at 1000.µg /Ml, higher than S2 and S3 which showed moderate efficacy. At 500.µg /Ml, S1 alone remained superior with better efficacy than S2 (moderate efficacy) while S3 reduced to low efficacy. S1 and S2 showed better efficiency at 250. µg /Ml as compared to S3. Even at 125. µg /Ml, S1 was still the most powerful with better efficiency than S2 and S3. Finally, all derivatives achieved the resistance threshold at 62.5 µg /Ml. This confirms that the 1,3,4-thiadiazole core bearing the 2,4-dihydroxyphenyl group at S1 provides a key improvement, where the free hydroxyl groups promote strong hydrogen-bonding with the fungal cell wall, maximizing ergosterol disruption and maintaining efficacy at lower dosages. The results of these compounds, which show the lowest effective concentration, are shown in Table (2).

Table 2 The lowest effective concentration of the prepared compounds towards the isolates under study

Comp. No	Concentration (µg /mL)			
	P. aeruginos	***A. baumannii***	***S. aureus***	***C.albicans***
S1	500	62.5	62.5	62.5
S2	125	62.5	62.5	125
S3	500	125	31.2	125

4. Conclusion

This study demonstrates the successful synthesis of novel saccharin–thiadiazole hybrids (S^1–S^3), with derivative S^1 exhibiting outstanding, broad-spectrum antimicrobial efficacy and the lowest MIC values against both Gram-negative pathogens and *Candida albicans*. Furthermore, the *in vitro* cytotoxicity assays confirmed the high cytocompatibility and biosafety profiles of these newly fabricated chemical entities across graded concentrations. Consequently, these findings establish compound S_2 as a promising, safe pharmaceutical scaffold for the future development of potent antimicrobial and antifungal therapeutic agents.

Compliance with ethical standards

Acknowledgments

All authors have contributed according to their speciality to the work.

Disclosure of conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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