RESEARCH PAPER

Synthesis and antibacterial activity of N-(3-(4-(1,3-dioxolan-2-yl) phenyl)-1,2,4-thiadiazol-5-yl) benzamide derivatives

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Abstract:

Introduction: The emergence of multidrug resistant bacteria has rendered the infections such as pneumonia, tuberculosis etc difficult to treat. Antimicrobial resistance increases the cost of treatment as well as disease associated morbidity and mortality. Hence, it is necessary to develop antibacterial drugs with unique structure and mechanism of action.

Objectives: The study was conducted with these objectives: (1) To synthesize N-(3-(4-(1,3-dioxolan-2-yl) phenyl)-1,2,4-thiadiazol-5-yl) benzamides,

(2) To evaluate their activity against E. coli, Pseudomonas aeruginosa, Proteus mirabilis and Bacillus cereus.

Method: N-(3-(4-(1,3-dioxolan-2-yl) phenyl)-1,2,4-thiadiazol-5-yl) benzamides (1,2,4 thiadiazoles) were synthesized by a cost effective process and 1H and 13C NMR and LC-MS confirmed their characteristics. They were assessed for activity against E. coli, Pseudomonas aeruginosa, Proteus mirabilis and Bacillus cereus by agar well diffusion method.

Results: Twelve novel 1,2,4-thiadiazole derivatives (7a-7l) were synthesized with good yield ranging from 70-88%. Many of these compounds had good activity against the tested bacterial strains.

Conclusion: A series of 1,2,4-thiadiazole derivatives (7a-7l) were synthesized by a cost effective process. Some of these derivatives have good activity against *E. coli*, *Bacillus cereus* and Pseudomonas aeruginosa. Some compounds have activity comparable to ciprofloxacin against *Proteus mirabilis.*

Keywords: 1,2,4 thiadiazole, synthesis, antibacterial activity, Proteus mirabilis

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Introduction:

Antibiotics have revolutionized the management of various bacterial infections and saved millions of lives. However, the development of multidrug-resistant bacterial strains, due to antibiotic abuse and misuse, has become a serious threat.[1] In 2019, diseases associated with antimicrobial resistance (AMR) have resulted in the death of approximately 4.95 million people.[2,3] Due to AMR, infections such as pneumonia, tuberculosis, gonorrhoea, septicaemia etc have become difficult to treat. AMR results in lengthier hospital stay, expensive medical treatments and increased mortality.[4] Hence, it is essential to develop novel antibacterial drugs with potent activity and non-standard mechanism of action.[5]

The nitrogen containing compounds have unique structure, chemical properties and diverse biological activities. Thiadiazoles contain a five membered heterocyclic ring with one sulphur and two nitrogen atoms and are used as a core component in the development of new drugs with antimicrobial, antibacterial, antifungal, anticancer, anti-inflammatory activities etc.[6] Among the thiadiazoles, 1,3,4 thiadiazole isomers are extensively studied and their biological activities are reported.[7-11] However, there is insufficient data about the 1,2,4 thiadiazole isomers.[12]

1,2,4-Thiadiazole containing compounds are reported to have application in the treatment of leukemia, as allosteric modulators, cathepsin B inhibitors, dual 5-lipo-oxygenase and cyclo-oxygenase inhibitors etc.[13] The cephalosporins – cefozopran, ceftobiprole and ceftaroline and fezolinetant – a neurokinin-3 inhibitor are examples of commercial drugs which contain 1,2,4 thiadiazole.[14] Since there is paucity of data on the synthesis and biological activities of 1,2,4 thiadiazole isomers and they possess the potential to be explored as novel drugs, the present study was planned to synthesize novel N-(3-(4-(1,3-dioxolan-2-yl) phenyl)-1,2,4-thiadiazol-5-yl) benzamides (1,2,4 thiadiazoles) and to screen them for their activity against E. coli, Pseudomonas aeruginosa,

Proteus mirabilis and Bacillus cereus by agar well diffusion method.

Materials and Methods:

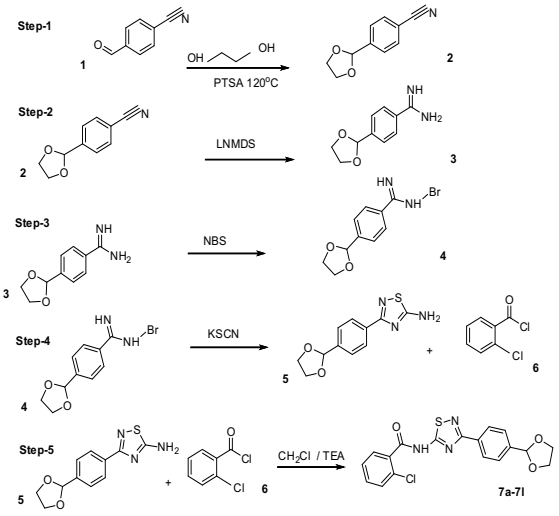
Chemicals and Reagents:

Reagents such as Toulene, Ethanol and dry THF were acquired from Spectrochem private limited, Mumbai, India. Rest of the study reagents were procured from Sigma-Aldrich chemical co. Nitrogen atmosphere was utilized to conduct the reactions. Excess solvents were removed using Rotavapor. 1H and 13C Nuclear magnetic resonance (NMR) spectra was assessed at 400 MHz with Bruker AMX 400 instrument. The IR spectra values were obtained using Perkin-Elmer 1600 series FTIR spectrometer. Liquid chromatography- mass spectrometry (LCMS) data was estimated with Agilent 1200 series LC and Micro mass zQ spectrometer. Silica gel (230–400 mesh) was utilized for Column chromatography. The melting points were noted with Buchi melting point apparatus B-540. The elemental analysis was done with Varian instrument VARIO EL3 series.

Synthesis of novel 1,2,4-thiadiazole derivatives (7a-7l):

Pyridine (0.096g, 1.2 mmol) was mixed with 3-(4-(1,3-dioxolan-2yl) phenyl)-1,2,4-thiadiazol-5-amine **(5)** (0.1g, 0.4 mmol) in dichloromethane (5 ml), and it was cooled to 0˚C. The corresponding acid chloride **(6)** (0.07 g, 0.4 mmol) in dichloromethane was mixed dropwise. The resultant mass was stirred at reaction time for 16 hr and Dichloromethane (50 ml) was added. Water and saturated brine solution were used to wash the mixture and later dried over anhydrous sodium sulfate and subsequently concentrated under reduced pressure. The residue obtained was purified with column chromatography utilizing ethyl acetate and petroleum ether to obtain corresponding N-(3-(4-(1,3 dioxolan-2-yl) phenyl)-1,2,4-thiadiazol-5-yl) benzamides **(7a-7l).** The novel compounds were characterized with LCMS and NMR data. (Scheme 1)

Scheme 1: Synthesis of novel 1,2,4-thiadiazole derivatives (7a-7l)



PTSA - p-Toluenesulfonic acid
LNMDS -Lithiumbis(trimethylsilyl)amide
NBS - N-Bromosuccinimide
TEA - Triethanolamine

Pharmacological Activity:

Ethical clearance: The study was approved by the institutional ethics committee, Islamiah College, Vaniyambadi, Tamil Nadu, India (Ref No. IEC/IC/31/2022-23).

Antibacterial Activity: The antibacterial activity of the novel 1,2,4 thiadiazole compounds (7a-7l) was screened against three gram negative bacterial strains - *E. coli (ATCC- 25922), P. aeruginosa (ATCC- 27853)* and *P mirabilis (ATCC- 29906)* and one gram positive bacteria – *B. cereus (ATCC – 11778)* by agar well diffusion method.

Preparation of Inoculums:

The bacterial stock cultures were maintained on nutrient agar slopes at 4°C. A loopful of stock cultures cells were transferred to test tubes containing Mueller-Hinton Broth (MHB) at 25°C to prepare the active cultures. Fresh MHB was utilized to dilute the cultures till optical densities of 2 x 10^6 colony-forming units were obtained.

Agar well Diffusion Method:

The 1,2,4 thiadiazole compounds (7a-7l) were assessed for their antibacterial activity and compared with ciprofloxacin (100 µg/ml) as reference standard. A volume of the bacterial inoculum was spread over the Muller Hinton agar surface to prepare lawn culture. Using a well cutter, wells were cut in the plates aseptically and 30µl of 1,2,4 thiadiazole compounds was added to these well with sterilized micropipettes. Overnight incubation was done at 37°C. Control experiments with ciprofloxacin were conducted similarly. The antibacterial activity of the 1,2,4 thiadiazole compounds was determined at 37°C after 18-24 hrs by measuring the growth inhibition zone. All experiments were repeated thrice and the mean of these determinations was noted. Zone of inhibition >8 mm was considered the cut off value for antibacterial activity.[15]

Statistical analysis: Descriptive statistics was used to summarize the data and data was

compiled and tabulated using MS excel (2013 version).

Results:

Synthesis and Yield of 1,2,4 thiadiazole Derivatives:

Twelve novel N-(3-(4-(1,3-dioxolan-2-yl) phenyl)-1,2,4-thiadiazol-5-yl) benzamides (7a-7l) were prepared using **Scheme 1**. The compounds had good yield ranging from 70% (7l) to 88% (7g).

Antibacterial Activity:

The antibacterial activity of the 1,2,4 thiadiazole derivatives (7a-7l) was evaluated against - *E. coli, Pseudomonas aeruginosa, Proteus mirabilis* and *Bacillus cereus*. Ciprofloxacin was the standard drug used for comparison. The antibacterial activity was measured as a zone of growth inhibition in mm. The values displayed are the mean of three determinations with range less than 5% of mean. Zone of inhibition >8 mm was considered the cut off value for antibacterial activity.[15] Many of the 1,2,4 thiadiazole compounds exhibited good activity against *E. coli,* P. aeruginosa, and *B. cereus*. Compounds 7a and 7l showed activity comparable to ciprofloxacin against P. mirabilis. (Table 1)

Table 1: Antibacterial activity of 1,2,4-thiadiazole derivatives (7a-7l)

Compound	E. coli	P. aeruginosa	P. mirabilis	B. cereus
7a	8	3	10	6
7b	7	NZ	7	NZ
7c	11	6	4	7
7d	NZ	8	NZ	3
7e	12	11	8	12
7f	13	10	3	11
7g	11	NZ	NZ	9
7h	9	7	5	6
7i	NZ	5	7	7
7j	8	6	8	NZ
7k	12	14	6	13
7l	13	10	9	11
Ciprofloxacin	15	16	9	15

Zone of inhibition in mm. *NZ: No zone of inhibition.*

Discussion:

Antibiotics have a vital role in reducing the morbidity and mortality of life threatening bacterial infections. However, the emergence of multidrug resistant bacteria and their accelerated global spread is a cause of concern.[16] These organisms are developing novel resistance mechanisms which are related to the chemical structure of antibiotics and their mechanism of antibacterial activity.[17] Hence, there is a need to design new drugs with antibacterial activity having unique structure and novel mechanism of activity. In this study, we have synthesized novel 1,2,4-thiadiazole derivatives and screened them for antibacterial activity.

Twelve novel 1,2,4-thiadiazoles (7a-7l) were prepared using Scheme 1, which is a cost effective process. These compounds had good yield ranging from 70% to 88%. LCMS and NMR data was used to confirm the characteristics of these novel compounds. The activity of these compounds (7a-7l) was evaluated against three Gram negative bacteria - *E. coli, P. aeruginosa, P. mirabilis* and one Gram positive bacteria – *B. cereus*. E. coli is a normal human gut flora but it can cause hospital or community acquired infections such as urinary tract infections, pneumonia, septicaemias etc. Antibiotic resistance among E. coli is an important public health problem.[18] P. aeruginosa is a nosocomial pathogen which has the propensity for biofilm formation on medical devices, implants etc and ability to develop resistance against aminoglycosides, fluoroquinolones, carbapenems and tetracyclines.[19] P. mirabilis has urease activity, ability to form biofilms and is a cause of various infections, most important being urinary tract infections. Hence the development of antibiotic resistance among P. mirabilis is a cause of concern.[20] B. cereus is an important foodborne pathogen with toxin producing ability. With the development of antibiotic resistance among B. cereus, there is a concern about food safety and public health.[21] Hence, in the present study, the antibacterial activity of 1,2,4 thiadiazole compounds was evaluated against these bacterial strains because of their public health importance.

Among the 1,2,4 thiadiazole compounds, compounds 7f and 7l exhibited good activity against *E. coli*, compound 7k against P. aeruginosa, and compound 7k against *B. cereus* respectively. Compound 7a and 7l showed activity comparable to ciprofloxacin against P. mirabilis. Sharma M et al and Anstis DG et al have reported the synthesis and isolation of 1,2,4 thaidiazole derivatives.[22,14] Zang Y et al have described the design, synthesis and antibacterial activity of 1,2,4 thiadiazole derivatives.[23]

The variable antibacterial activity of the 1,2,4 thiadiazole compounds against the tested bacterial strains could be as a result of the differences in the cell wall structure of gram positive and negative organisms and the differences in the structures of the novel 1,2,4 thiadiazole compounds themselves. The novel 1,2,4 thiadiazole derivatives have the potential to be developed into new antibacterial drugs.

However, further studies are necessary to explain the mechanism of action of these derivatives.

Conclusion:

A series of novel 1,2,4-thiadiazole derivatives (7a-7l) were synthesized by a cost-effective method with good yield. Some of these derivatives have good activity against *E. coli*, *Bacillus cereus* and Pseudomonas aeruginosa. Some compounds have activity comparable to ciprofloxacin against *Proteus mirabilis*.

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Authorship: All authors have made significant contributions to the work's conception, design, data acquisition, analysis, or interpretation;

Drafting or critically revising for intellectual content;

Final approval of the version to be published; and Accountability for all work aspects, ensuring integrity and resolving questions.

Ethical approval: The study was approved by the institutional ethics committee, Islamiah College, Vaniyambadi, Tamil nadu, India (Ref No. IEC/IC/31/2022-23).

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Conflicts of interest: None declared

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