SYNTHESIS OF BIOLOGICALLY ACTIVE BRIDGED HEAD NITROGEN HETEROCYCLES FROM INEXPENSIVE STARTING SUBSTRATES

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Abstract

One of the most pressing issues facing modern medicine is the development of treatments for bacterial strains that have developed resistance to existing antibiotics. A total of seven thiadiazolo[20,30]imidazo [4,5-b]quinoxalines and their derivatives were synthesised with the intention of using them as antibacterial agents. The compounds' structures were determined by infrared nuclear magnetic resonance (NMR), mass spectrometry, and elemental analysis. Disc diffusion and agar streak dilution were used to test the newly synthesised compounds for anti-microbial activity against a variety of bacteria. There was a significant inhibitory impact seen in all of the synthetic substances.

Keywords: Nitroquinoxaline; thiadiazole; antibiotic; antimycotic

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1. Introduction

Synthetic organic chemistry, and heterocyclic chemistry in particular, advances like wildfire when new ideas and better preparative methods are blown onto the flames.[1] Inorganic chemists' areas of study continue to broaden as new molecules are discovered each year. Anything from naturally occurring molecules to outlandish atomic arrangements created to test the ever-evolving ideas of structure theory can be found among these compounds. [2] As a result, the ability to build more complex organic compounds from less simple components through a succession of rational operations is at the very heart of organic chemistry. Heterocycles make up the vast majority of organic substances. In the wild, you can find them just about anywhere. Heterocyclic compounds have fundamental roles in all of life's fundamental biochemical processes. All living cells rely on a variety of nutrients, including the pyrimidine and purine bases of DNA, the essential amino acids, the vitamins and co-enzyme precursors, the B12 and E families of vitamins, the photosynthesizing pigment chlorophyll, the oxygen transport agent haemoglobin, the hormones, and most of the sugars.[3] A variety of heterocyclic-ring-containing natural compounds have been identified to have pharmacological activity. Most synthetic heterocycles have medicinal value as pain relievers, sedatives, vasopressor modulators, insecticides, herbicides, or rodenticides. In addition to their employment as vulcanization accelerators and anti-oxidants in the rubber industry, a wide variety of synthetic heterocyclic compounds are employed as dye stuffs, co-monomers, solvents, photographic sensitizers and developers, and more. [4]The target system's known and unknown properties should be taken into account before commencing any concrete efforts in planning a synthesis. The findings from one set of experiments often lead to the development of a new set of chemicals or the exploration of alternative synthesis strategies. [5]To keep up with the latest discoveries and advancements in the field of bio-active molecules, as well as to arm oneself with the synthetic technique and quantitative structure activity relationships (QSAR), a literature survey is performed. Numerous nitrogen and sulfur-containing heterocycles, both simple and N-bridged, are shown to possess potent biological activity.

One can find uses for the 1,2,4-triazole-derived N-bridged heterocycles in medicine, agriculture, and industry. Several triazolothiadiazines have been found to have diuretic, herbicidal, fungicidal, and central nervous system depressive properties.[6] There are additional chemicals that are utilised as antisecretory agents4 and as photographic couplers. Since ancient times, the chemistry of chalcones and their heterocyclic analogues has been considered a fascinating area of research.[7] In order to construct heterocyclic rings, chalcones and their derivatives are frequently employed. The heterocyclic systems that are deduced from chaicone analogues have several uses in medicinal chemistry due to their wide range of biological actions.[8]

It is widely accepted that the study of heterocyclic molecules is one of the most promising areas of research within organic chemistry. Heterocyclic compounds are ubiquitous in the natural world and absolutely necessary for all forms of life. [9]All living cells rely on them for proper metabolism. Numerous heterocyclic compounds with pharmacological activity are in widespread therapeutic usage. Heterocyclic molecules with nitrogen, sulphur, and oxygen have played a huge role in the development of new medicines. A great many new heterocyclic medicines enter pharmacopoeias each year. [10]Their physicochemical characteristics were clearly exhibited by the size and kind of ring structures, as well as the effective substituent groups of the mother scaffold. When it comes to medicine, heterocyclic compounds play an important role as antibiotics, anti-inflammatories, anti-fungals, and even tumour suppressors. Resistance of harmful bacteria and fungus towards available antimicrobial medicine is rapidly becoming a serious problem worldwide, and the design of novel heterocyclic compounds to cope with resistant bacteria and fungi has become one of the most important fields of antibacterial and antifungal research. So it has become more difficult and demanding for chemists and pharmacists to find new and effective antibacterial and antifungal agents.[11]

2. Materials and Methods

The solvents used were all of the highest quality and were purchased from reputable companies including SD fine chemicals (Mumbai, India), Loba Chemie, and Merck (Mumbai, India). Without any adjustments, melting points were measured in open glass capillary tubes. Thin layer chromatography (TLC) plates made of Silica gel G (Merck) were used to frequently test the purity of the compounds. TLC spots were observed using a UV lamp and an iodine chamber. The KBr pellets IR spectra were recorded by a (BIO-RAD FTS) FT-IR spectrophotometer. The Bruker DPX-300 NMR spectrometer was used to acquire 1H NMR spectra in $CDCl_3$ with tetramethylsilane (TMS) serving as the internal standard, and the Bruker 125 MHz spectrometer was used to acquire ^{13}C NMR spectra. Chemical changes were reported on a parts per million (ppm) scale. Through the use of electron impact ionisation, mass spectra were acquired on a

JEOL-SX-102 device. The Perkin Elmer model 240C analyser was used to conduct elemental studies, and the results were within 0.4% of the theoretical values for carbon, hydrogen, and nitrogen.

Microorganisms and medium

The National Chemical Laboratory in Pune provided all of the test micro-organisms used in the antimicrobial screening. The paper disc diffusion method was used to test the produced chemical for antibacterial activity. The chemicals were dissolved in DMSO, and negative control experiments using the solvent at the same quantities were run to make sure it didn't have any influence on bacterial growth or enzyme activity. Bacillus cereus ATCC 11778 and Staphylococcus aureus ATCC 9144 were used to test the produced compounds for antibacterial activity (Pseudomonas aeruginosa ATCC 2853and Escherichia coli ATCC 25922). Synthesized compounds' antifungal properties were tested against two different types of fungus (Aspergillus fumigatus ATCC 46645and Aspergillus niger ATCC 9029). The agar streak dilution method was also used to calculate the minimum inhibitory concentrations (MIC) of the substances. For antibacterial activity, all plates were incubated at 37 + 0.5 C for 24 hours, while for antifungal activity, all plates were incubated at 25 degrees Celsius for 72 hours on Sabouraud dextrose agar (SDA). Compounds' zones of inhibition were evaluated on a centimetre scale. To achieve a final density of 5* 10^5 cfu/ml, the chosen strains were inoculated into nutrient agar.

General procedure for the synthesis of 2,3-dichloro-6,7- dinitroquinoxaline

For 90 minutes, 1.62 grammes (0.01 mol) of 2,3-dichloro-6,7-dinitroquinoxaline-6,7-dintroquinoxaline-2,3-dione 2 were refluxed in a mixture of 6 millilitres (ml) of freshly distilled phosphorus oxychloride and 0.5 millilitres (ml) of dimethyl formamide. Pure was obtained by cooling the reaction mixture by pouring it into ice water while stirring, then filtering, washing, drying, and recrystallizing it from ethyl acetate and petroleum ether.

An Overview of the Synthesis of the Named Compound

30 ml of ethanol and 250 ml of RBF were combined with 1.44 grammes (0.005 mol) of 2, 3-dichloro-6,7-dinitro quinoxaline (compound 3), 0.005 mol of substituted thiadiazole derivatives (4a-g), and 0.82 grammes (0.01 mol) of sodium acetate. After five hours of refluxing the mixture, it was poured onto ice to form a precipitate. The appropriate solvents were removed, and then it was filtered, dried, and recrystallized. The spectrum analyses and TLC separations demonstrated that the materials obtained through this method and isolation were of high purity. Insoluble in water, but soluble in dimethyl sulfoxide and dimethylformamide, the substances in the title were discovered.

Antimicrobial effects

Using a Paper Disc Diffusion Method

When the petridish reached a thickness of about 3–4 mm, the medium was inoculated (1 ml/100 ml of medium) with the suspension of the chosen microbe (matched to McFarland barium sulphate standard) and then autoclaved at 120 ^{0}C for 30 minutes to sterilise it. After the solidified medium had been pre-incubated for 1 hour at room temperature, paper impregnated with the test chemicals (100, 150, and 200 lg/ml in dimethyl formamide) was placed on top of it. For 24 hours at 37 degrees Celsius and 72 hours at 25 degrees Celsius, they were cultured for antibacterial and antifungal activity, respectively.

Lowest effective dose

The agar streak dilution method was used to determine the MIC of each drug. Before the sterile agar medium (nutrient agar for antibacterial activity and Sabouraud's dextrose agar for antifungal activity) was hardened to a depth of 3-4 mm in a petridish, a stock solution of the synthesised chemical (100 lg/ml) in dimethyl formamide was made. Microorganisms were plated at 5 *105 cfu/ml and cultured at 37 C for 24 and 25 C for 72 hours for bacteria and fungi, respectively.

3. Results

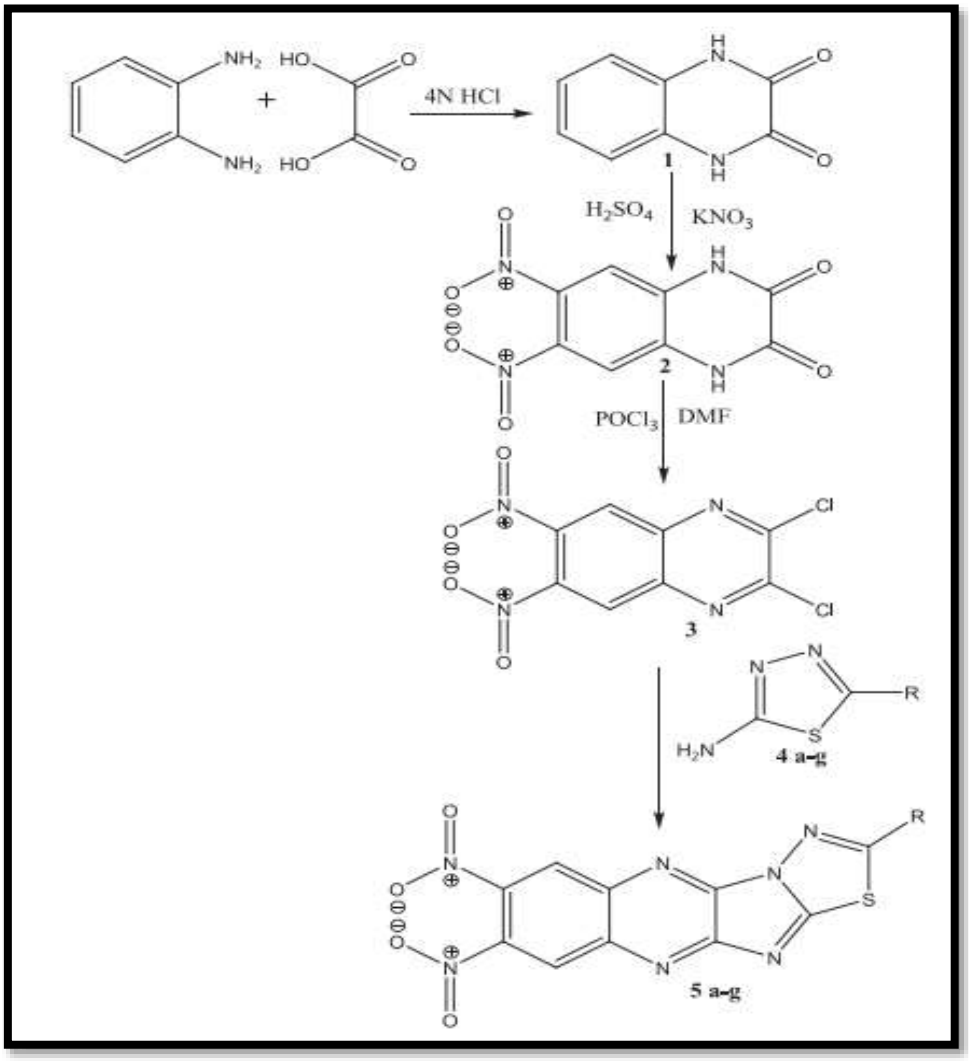

Fig 1. Synthesis of intermediates compounds.

Table 1. Physicochemical data of the synthesized compounds (5a-5g).

Compound	R	Mf	Mp	% yield
5a	C_6H_5	$C_{16}H_7N_7O_4S$	102-104	74
5b	4-$C_6H_4NH_2$	$C_{16}H_8N_8O_4S$	122-125	81
5c	2-C_6H_4Cl	$C_{16}H_6ClN_7O_4S$	113-116	79
5d	$2C_6H_4OH$	$C_{16}H_7N_7O_5S$	129-131	78
5e	3,5 $C_6H_2(NO_2)_2$ 2-OH	$C_{16}H_5N_9O_9S$	119-121	86
5f	C_6H_5CH—CH-	$C_{18}H_9N_7O_4S$	99-102	71
5g	CF_3	$C_{11}H_2F_3N_7O_4S$	154-156	72

Table 2. MIC (lg/ml) of synthesized compounds (5a–5g)

Compound	S.aureus	B.cereus	E.coli	P.aeruginosa	A.niger	A.fumigatus
5a	31.26	15.64	31.26	15.64	31.26	15.64
5b	31.26	15.64	31.26	31.26	31.26	15.64
5c	31.26	>62.7	31.26	>62.7	>62.7	>62.7
5d	7.82	7.82	31.26	7.82	31.26	7.82
5e	>62.7	31.26	>62.7	>62.7	31.26	31.26
5f	>62.7	>62.7	31.26	31.26	31.26	>62.7
5g	31.26	31.26	>62.7	31.26	>62.7	>62.7
Ciprofloxacin	15.64	7.82	15.64	7.82	-	-
Fluconazole	-	-	-	-	7.82	15.64

4. Discussions

Seven different chemicals were synthesised using this method. Spectroscopic, elemental, and spectroscopic studies, as well as infrared and nuclear magnetic resonance spectroscopy, were used to characterise all of the substances. [12]Synthesis was initiated with O-phenylene diamine and oxalic acid. After preparing quinoxaline dione 1 by reaction, we nitrated it using a nitrating mixture of potassium nitrate and sulphuric acid to obtain 2. Phosphorus oxychloride and dimethyl formamide are then used to transform this into dichloro compound 3. [13]The synthesised molecule matched the assigned structures in spectroscopic data (IR, ^{1} H NMR, mass spectra, and elemental analyses). All of the produced compounds had signature peaks in their infrared spectra, each one representing the presence of a different group. The IR absorption peaks at 1620 cm1 (C-NO_2) and 741 cm1 (C-Cl stretching) provide evidence that the 2,3-dichloro-6,7-dinitroquinoxaline ring has formed. [14]The production of 4a-g is further confirmed by the appearance of a singlet in its ^{1} H NMR spectra at 3.85 ppm for two protons, which may be assigned to the NH_2 group. The absence of peaks at 3400-3300 and approximately 760-740 cm1 in the IR spectra of compounds 5a-g indicates that NH and C-Cl are not present in the molecule; the appearance of M + 2 peaks was seen for compound 5c in the mass spectra. Each chemical produced was tested for its antimicrobial (antibacterial and antifungal) activity against a variety of species using the paper disc diffusion method, as detailed in the experimental section. [15] Standard concentrations of the antibiotic ciprofloxacin (100 lg/ml/disc) and the antifungal drug fluconazole (100 lg/ml/disc) were used to make comparisons, while the solvent DMF served as a control.

5. Conclusions

From o-phenylenediamine, thiadiazolo imidazo[4,5-b]quinoxalines were created. Instruments such as infrared spectroscopy, proton nuclear magnetic resonance, mass spectrometry, and elemental analysis were used to characterise them. After testing the synthesised compounds against four different microorganisms (two gram-positive bacteria like B. cereus and S. aureus, two gram-negative bacteria like E. coli and P. aeruginosa, and two antifungal organisms like A. niger and A. fumigatus), it was determined that only the unsubstituted and electron donating group containing molecules have shown better antimicrobial activity when compared to electron withdrawing compound. Good antibacterial and antifungal activity was found for compounds 5a, 5b, and 5d out of all the produced compounds; moderate activity was found for the remaining compounds. This demonstrates that the presence of

an electron-donating group or an unsubstituted group in the molecule contributed to its enhanced antibacterial activity.

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