

"Synthesis and Characterization of Substituted 3-(Arylamino)-1-phenylpyrrolidine-2,5-diones and Their Biological Evaluation"

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ABSTRACT

An assortment of substituted 3-(arylamino)-1-phenylpyrrolidine-2,5-diones were synthesized through an aza-Michael addition of substituted anilines to N-phenylmaleimide under mild reaction conditions. The developed methodology is simple, efficient and provides the desired products in good to excellent yields with a straightforward work up procedure. FT-IR, ¹H NMR, ¹³C NMR, and mass spectrometry were used to analyze the synthesized compounds, which confirmed the formation of the desired products. In addition to their synthesis and structural characterization, the biological potential of the synthesized compounds were evaluated. In the DPPH radical scavenging experiment, the compounds demonstrated strong antioxidant activity. Furthermore antifungal activity against *Penicillium chrysogenum* and *Aspergillus niger*, as well as antibacterial screening against *Escherichia coli* and *Staphylococcus aureus*, demonstrated significant inhibitory zones, suggesting promising bioactive qualities. Overall, the substituted 3-(arylamino)-1-phenylpyrrolidine-2,5-diones represent valuable heteroatom compounds with potential applications in biological and sustainable organic chemistry.

Keywords: Michael addition, Green synthesis, Bioactive, antioxidant & antimicrobial activity, heteroatom synthesis

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1. INTRODUCTION

One of the most significant processes in organic synthesis that forms carbon-carbon and carbon-heteroatom bonds is Michael addition. It is generally defined as the conjugate, 1,4-addition of a nucleophile to an electron-deficient α,β -unsaturated compound, commonly referred to as a Michael acceptor¹⁻³. Under comparatively mild reaction condition, the reaction offers an effective method for the creation of new σ -bonds and allows the insertion of various functional groups. The Michael reaction has become a basic transformation in contemporary organic synthesis due to its wide substrate scope, high synthetic utility, and superior functional group tolerance⁴⁻⁶. In the traditional Michael reaction, an activated alkene with electron-withdrawing groups like carbonyl, ester, nitrile and nitro functionalities combined with a carbon nucleophile, such as an enolate or stabilized carbanion^{7,8}. Michael addition can be broadly categorized into multiple types based on the type of nucleophile involved. C-Michael addition is the process of adding carbon nucleophiles to activated alkenes to create to new C-C bond. Nitrogen nucleophiles, such as primary and secondary amines, are added to electron-deficient alkenes in a process known as Aza-Michael addition, which creates new C-N bonds⁹⁻¹¹. Similar to this, S-Michael addition involves sulfur nucleophiles like

thiols and thiolates, whereas O-Michael addition happens when oxygen nucleophiles such alcohols and alkoxides take part in the conjugate addition¹²⁻¹⁴. There have also been reports of other heteroatom-based Michael addition, such as those based on phosphorus and selenium.

The aza Michael reaction has been drawn special attention among these transformations since it offers a straightforward and affordable pathway to β -amino carbonyl compounds and other molecules with nitrogen functionalization¹⁵⁻¹⁷. In a typical aza Michael reaction, the β -carbon of an α,β -unsaturated electron-deficient system is attacked by amine nucleophile, and the matching β -amino product is then produced by proton transfer. Maleates, fumarates, maleimides, nitriles, nitroalkenes, ester, α,β -unsaturated ketones and maleates are among the many Michael acceptors that can take part in this transition¹⁸⁻²⁰. Maleimides are particularly attractive Michael acceptors because the two electron-withdrawing carbonyl groups strongly activate the C-C double bond toward nucleophilic conjugate addition. Maleimide reactions with nitrogen nucleophiles thus offer a productive pathway to functionalized succinimide derivatives²¹⁻²³. Through an aza-Michael addition process, substituted 3-(arylamino)-1-phenylpyrrolidine-2,5-diones are

formed in the current work using N-phenylmaleimide as the Michael acceptor and substituted anilines as nitrogen nucleophiles. This conversion offers a practical method for adding structurally varied arylamino substituents to the succinimide framework and creating C-N linkages. Functionalized succinimide derivatives have been studied for a variety of biological activities, such as antibacterial, antifungal, antioxidant and enzyme inhibitory properties, in addition to their synthetic significance²⁴⁻²⁷. Molecular structure and the type of substituents bonded to the heteroatom framework have a significant impact on these biological characteristics. The link between molecular structure and biological activity can be better understood by integrating structural diversification by aza-Michael addition with biological assessment.

Thus, a series of substituted 3-(arylamino)-1-phenylpyrrolidine-2,5-diones were synthesized in this work by aza-Michael addition of substituted anilines with N-phenylmaleimide under mild reaction conditions. FT-IR, ¹H NMR, ¹³C NMR and mass spectrometry were used to characterize the produced compounds. Additionally, DPPH radical-scavenging, antibacterial and antifungal assays were used to explore their biological potential. Establishing a straightforward synthesis strategy for structurally varied succinimide derivatives and investigating their initial structure activity connections are the goals of the project.

Applications

In medical and pharmaceutical chemistry, N-containing heteroatom compounds are an important family of biologically active chemicals. They are useful scaffolds for the synthesis of physiologically active compounds because of their structure diversity, adjustable electronic characteristics and capacity to engage in H-bonding and other molecular interactions²⁸⁻³⁰. Numerous N-containing heteroatom have been shown to have antibacterial, antifungal, antiviral, antioxidant, anti-inflammatory, anticancer, analgesic and enzyme-inhibitory properties³¹⁻³⁴. Because of these various biological characteristics, heteroatom frameworks are crucial structure elements in the creation of novel medicinal medicines. Maleimide containing system have significant uses in chemical biology and bioconjugation. Maleimides have been widely used for the selective modification and labeling of peptides and proteins because of their strong reactivity towards thiol groups, especially cysteine residue. The significance of maleimide based link to therapeutic and diagnostic research has been demonstrated by their incorporation into antibody drug conjugates and other biomolecular structures³⁵.

Its effective radical scavenging action shows significant antioxidant characteristics, making it a promising candidate for use in pharmaceutical excipients, cosmetic formulations, and food preservation systems where oxidative damage prevention is critical^{36,37}. The compound also inhibits both Gram-positive and Gram-negative bacterial strains, indicating its potential as a lead molecule for antimicrobial drug development as well as incorporation into bioactive coatings used to prevent microbial contamination in food packaging materials and medical equipment^{38,39}. Furthermore, its antifungal action against pathogenic fungus broadens its range of applications to include pharmaceutical antifungal medicines, protective surface materials such as paints and polymers, and agricultural applications, particularly crop protection techniques^{40,41}. The approach is in line with sustainable chemistry principles and can be scaled up in environmentally aware industrial applications due to its mild ambient conditions, ethanol as a green solvent and in addition to its biological utility⁴².

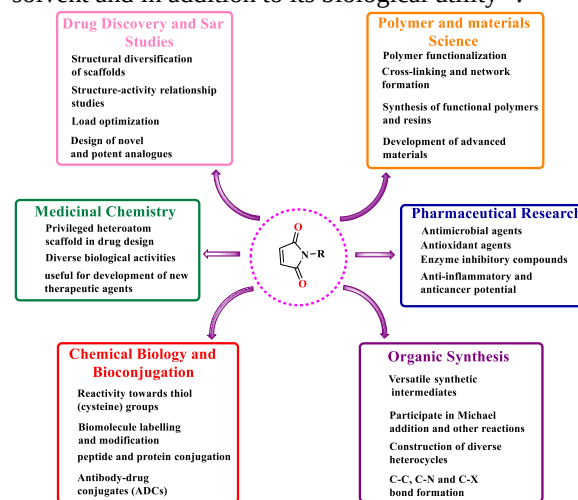


Fig.1 General properties and Biological Significance
In pharmaceutical research, the molecule shows promise as a potential prodrug scaffold, enzyme inhibitor, or anticancer lead^{43,44}. Maleimide-based adducts are prized in materials science as building blocks for hydrogels, high-performance polymers, and electronic devices including OLEDs and semiconductors^{45,46}. Additionally, these frameworks' high affinity for attaching to metal surfaces allows them effective as corrosion inhibitors, and their antibacterial properties may help treat wastewater and protect the environment^{47,48}. Their attractiveness for industrial scale-up is further increased by the addition of a sustainable, green synthesis technique⁴⁹. When taken as a whole, these characteristics make the synthesized adduct a biologically active substance as well as a flexible building block for use in innovative

materials, industry, healthcare, and environmental sustainability.

2. EXPERIMENTAL SECTION

2.1. General information

All of the substituted anilines and N-phenylmaleimide were bought from commercial vendors and used straight away. The reaction solvent used was analytical-grade ethanol. Using Merck silica gel 60-F-254 aluminium plates and thin-layer chromatography (TLC), the reaction's progress was tracked. On a Bruker Avance-III HD 400 MHz spectrometer, the ^1H and ^{13}C NMR spectra were captured with tetramethylsilane (TMS) as the internal standard and DMSO-d_6 and CDCl_3 as the solvent. Melting points are uncorrected and were measured in open capillary tubes. KBr discs were used on a Bruker ALPHA analyser to produce FTIR spectra. Mass spectra were recorded on a Xevo G2-S QToF (Waters, USA) mass spectrometer using the direct mass ESI/APCI technique. Elemental analyses (C, H, N) were performed on a PerkinElmer 2400 CHNS/O elemental analyzer to confirm the purity and composition of the synthesized compounds.

2.2. General procedure for the synthesis of substituted 3-(arylamino)-1-phenylpyrrolidine-2,5-diones derivatives (3a-3i)

A mixture of aniline derivatives (1 mmol) and N-phenylmaleimide (0.173 g, 1 mmol) were diluted in 5 mL of ethanol in a 25 mL round-bottom flask. The mixture was stirred continuously using a magnetic stirrer for 4-6hrs at room temperature (25°C). TLC (silica gel 60 F-254) was used to track the reaction's development. Following completion, the solvent was extracted at a lower pressure, and the residue was dissolved in 30 mL of ethyl acetate. After washing with water (2×15 mL) and brine (15 mL), the organic layer was dried over anhydrous Na_2SO_4 , filtered, and concentrated. To obtain the intended Michael adduct as a solid, the crude product was purified by column chromatography on silica gel with hexane/ethyl acetate (7:3) as the eluent. The crude product was subjected to purification by two different methods:

- Recrystallization method:** The pure product was obtained as a crystalline solid by filtering the residue, washing it with cold ethanol, and then recrystallizing it from hot ethanol.
- Column chromatography method:** An alternative method involved adsorbing the crude product onto silica gel and purifying it using column chromatography with hexane/ethyl acetate (gradient 7:3) as the eluent. The product was obtained by combining and concentrating fractions that contained the pure product under lower pressure.

2.3.3-((2,5-dimethylphenyl)amino)-1-phenylpyrrolidine-2,5-dione (3a)

The title compound was prepared following the general procedure using N-Phenylmaleimide (1mmol, 0.173gm) and 2,5-dimethylaniline (1mmol, 0.121gm) at r.t. (25°C). The desired product was obtained as a white/brown solid ; yield: 0.082gm, 87%; M.P.: $213-220^\circ\text{C}$; ^1H NMR (400 MHz, DMSO-d_6 / TMS, δ , ppm): 10.07 (s, 1H), 8.97– 6.45 (m, 8H, Ar-H), 4.34 (s, 1H), 3.44 (dd, $J = 17.9, 8.3$ Hz, 2H), 2.34 (s, 3H), 2.18 (s, 3H); $^{13}\text{C}\{^1\text{H}\}$ NMR (100 MHz, DMSO-d_6 / δ , ppm): 189.3, 182.8, 169.2, 139.3, 129.2, 123.8, 119.5, 52.2, 40.6, 39.4; IR(ν/cm^{-1}): 3217(N-H), 3020 (Ar–C–H), 1665, 1687 (C=O), 1603 (C=C), 1515 (N–H bending), 1478 (C–H bending), 1386 (C–N), 1286 (C–O), 627, 690 (Ar–C–H out-of-plane); HRMS (ESI) exact mass calcd for $\text{C}_{18}\text{H}_{18}\text{N}_2\text{O}_2$, (M + H), 295.15; Found: 295.1436.

2.4. 1-phenyl-3-(phenylamino) phenylpyrrolidine-2,5-dione (3b)

The title compound was prepared following the general procedure using N-Phenylmaleimide (1mmol, 0.173gm) and aniline (1mmol, 0.93gm) at r.t. (25°C). The desired product was obtained as a pale yellow solid ; yield: 0.033gm, 92%; M.P.: $208-213^\circ\text{C}$; ^1H NMR (400 MHz, DMSO-d_6 / TMS, δ , ppm): 10.08 (s, 1H), 6.67-7.22 (m, $J = 173.1, 139.0, 8.5, 4.2$ Hz, 10H, Ar-H), 4.49 (s, 1H), 3.22 (dd, $J = 16.5, 3.3$ Hz, 2H); $^{13}\text{C}\{^1\text{H}\}$ NMR (100 MHz, DMSO-d_6 / δ , ppm): 207.2, 177.0, 169.2, 147.8, 139.3, 132.9, 128.8, 123.8, 52.2, 31.2; IR(ν/cm^{-1}): 3212 (N–H), 3016 (Ar–C–H), 1668 and 1605 (C=O, imide), 1487 (C=C, aromatic), 1290 (C–N), 760 (C–H, Ar.); ; HRMS (ESI) exact mass calcd for $\text{C}_{16}\text{H}_{14}\text{N}_2\text{O}_2$, (M + H), 266.11; Found: 266.132.

2.5. 3-((2,5-dimethoxyphenyl)amino)-1-phenylpyrrolidine-2,5-dione (3c)

The title compound was prepared following the general procedure using N-Phenylmaleimide (1mmol, 0.173gm) and 2,5-dimethoxyaniline (1mmol, 0.153gm) at r.t. (25°C). The desired product was obtained as a black solid ; yield: 0.186gm, 86%; M.P.: $205-210^\circ\text{C}$; ^1H NMR (400 MHz, DMSO-d_6 / TMS, δ , ppm): 10.06 (s, 1H), 8.09 – 5.37 (m, 8H, Ar-H), 4.68 (s, 1H), 4.34 (dd, $J = 11.2, 3.5$ Hz, 2H), 3.64 (s, 3H), 3.56 (s, 3H); $^{13}\text{C}\{^1\text{H}\}$ NMR (100 MHz, DMSO-d_6 / δ , ppm): 189.4, 182.8, 169.2, 154.5, 141.4, 129.2, 123.8, 111.1, 100.2, 56.3, 52.2, 40.0; IR(ν/cm^{-1}): 3456 (N-H), 3217 (Ar-C-H), 3010, 2841 (C-H), 1662 (C=O, imide), 1600, 1502 (C=C, aromatic), 1215, 1172 (C-O-C, methoxy), 1030 (C-O stretch), 840 (C-H out of plane); HRMS (ESI) exact mass calcd for $\text{C}_{18}\text{H}_{18}\text{N}_2\text{O}_4$, (M + H), 327.14; Found: 327.1334.

2.6. 3-((2-amino-5-methylphenyl)amino)-1-phenylpyrrolidine-2,5-dione (3d)

The title compound was prepared following the general procedure using N-Phenylmaleimide (1mmol, 0.173gm) and 4-methyl-o-phenylenediamine (1mmol, 0.122gm) at r.t. (25°C). The desired product was obtained as a yellow solid ; yield: 0.089gm, 93%; M.P.: 205-210°C; ¹H NMR (400 MHz, DMSO-d₆/TMS, δ, ppm): 10.06 (s, 1H), 8.89 – 6.79 (m, 8H, Ar-H), 4.36 (s, 2H), 3.21 (dd, J = 16.4, 3.4 Hz, 2H), 2.67 (s, 1H), 2.46 (s, 3H); ¹³C{¹H} NMR (100 MHz, DMSO-d₆/ δ, ppm): 189.4, 172.5, 169.2, 139.3, 129.2, 119.5, 52.2, 40.0, 21.5; IR(ν/cm⁻¹): 3212 (br, N-H stretch), 3010 (br, N-H), 2855 (w, C-H), 1676 (s, C=O asym), 1625 (s, C=O sym), 1610 (N-H bend, C=C), 1490 (aromatic C=C), 1382 (C-H bend), 1254 (C-N), 753, 695 (aromatic C-H out of plane); HRMS (ESI) exact mass calcd for C₁₇H₁₇N₃O₂ (M + H), 296.34; Found: 296.3522.

2.7. 1-phenyl-3-(2-phenylhydrazineyl)pyrrolidine-2,5-dione (3e)

The title compound was prepared following the general procedure for using N-Phenylmaleimide (1mmol, 0.173gm) and phenylhydrazine (1mmol, 0.108gm) at r.t. (25°C). The desired product was obtained as a black/brown solid ; yield: 0.176gm, 87%; M.P.: 206-214°C; ¹H NMR (400 MHz, DMSO-d₆/TMS, δ, ppm): 10.06 (s, 1H), 8.85-6.89 (m, J = 335.9, 109.4, 90.5 Hz, 10H, Ar-H), 4.35 (s, 1H), 3.24 (s, 1H), 2.63 (dd, J = 37.7 Hz, 2H); ¹³C{¹H} NMR (100 MHz, DMSO-d₆/ δ, ppm): 189.4, 169.2, 139.3, 129.2, 119.5, 52.2, 40.0; IR(ν/cm⁻¹): 3219 (N-H stretch), 3010 (aromatic C-H), 1680 (C=O asym.), 1597 (C=O sym.), 1536 (aromatic C=C), 1480, 1284 (C-N), 1120 (C-O stretch.); HRMS (ESI) exact mass calcd for C₁₆H₁₅N₃O₂ (M + H), 282.13; Found: 282.1422.

2.8. 3-((2-aminophenyl)amino)-1-phenylpyrrolidine-2,5-dione (3f)

The title compound was prepared following the general procedure using N-Phenylmaleimide (1mmol, 0.173gm) and o-phenylenediamine (1mmol, 0.108gm) at r.t. (25°C). The desired product was obtained as a light pink solid ; yield: 0.0346gm, 92%; M.P.: 335-340°C; ¹H NMR (400 MHz, DMSO-d₆/TMS, δ, ppm): 10.07 (s, 1H), 8.42 – 6.18 (m, 9H, Ar-H), 4.36 (s, 2H), 3.22 (s, 1H), 2.68 (dd, J = 16.5, 3.5 Hz, 2H); ¹³C{¹H} NMR (100 MHz, DMSO-d₆/ δ, ppm): 189.4, 169.2, 139.3, 129.2, 123.8, 119.5, 100.0, 52.4, 39.8; IR(ν/cm⁻¹): 3222 (br, NH₂), 3014 (br, N-H), 2836 (Ar C-H), 1668, 1597 (C=O), 1536 (C=C/N-H bend), 1286 (C-N), 685 (Ar C-H, out of plane); HRMS (ESI) exact mass calcd for C₁₆H₁₅N₃O₂ (M + H), 282.13; Found: 282.1466.

2.9. 3-((4-hydroxyphenyl)amino)-1-phenylpyrrolidine-2,5-dione (3g)

The title compound was prepared following the general procedure using N-Phenylmaleimide (1mmol,

0.173gm) and 4-aminophenol (1mmol, 0.109gm) at r.t. (25°C). The desired product was obtained as a black solid ; yield: 0.164gm, 88%; M.P.: 215-220°C; ¹H NMR (400 MHz, DMSO-d₆/TMS, δ, ppm): 10.07 (s, 1H), 8.76 (s, 1H), 7.83 – 6.36 (m, 9H, Ar-H), 4.37 (s, 1H), 2.65 (dd, J = 16.3, 11.5 Hz, 2H); ¹³C{¹H} NMR (100 MHz, DMSO-d₆/ δ, ppm): 189.5, 182.8, 171.6, 154.7, 139.3, 129.4, 119.6, 86.4, 52.2, 40.0; IR(ν/cm⁻¹): 3236 (br, OH), 3063 (br, N-H), 1705 (C=O), 1616 (C=O), 1513 (C=C), 1201 (C-N/C-O, phenolic), 742, 691 (Ar C-H, out of plane); HRMS (ESI) exact mass calcd for C₁₆H₁₄N₂O₃ (M + H), 283.11; Found: 283.1233.

2.10. 3-((4-bromophenyl)amino)-1-phenylpyrrolidine-2,5-dione (3h)

The title compound was prepared following the general procedure using N-Phenylmaleimide (1mmol, 0.173gm) and 4-bromoaniline (1mmol, 0.109gm) at r.t. (25°C). The desired product was obtained as a white solid ; yield: 0.127gm, 91%; M.P.: 195-200°C; ¹H NMR (400 MHz, DMSO-d₆/TMS, δ, ppm): 10.08 (s, 1H), 9.13 – 6.44 (m, 9H, Ar-H), 4.37 (s, 1H), 2.68 (dd, J = 16.4, 11.1 Hz, 2H); ¹³C{¹H} NMR (100 MHz, DMSO-d₆/ δ, ppm): 188.6, 182.4, 169.1, 139.3, 129.2, 123.8, 119.5, 51.9, 40.4, 31.8; IR(ν/cm⁻¹): 3246 (N-H stretch.), 3026 (Ar-C-H), 2932 (C-H aliphatic), 1743, 1662 (C=O imide), 1602 (C=C Ar), 1485 (N-H bend), 1379 (C-O), 1259 (C-Br), 800, 693 (C-H out of plane); HRMS (ESI) exact mass calcd for C₁₆H₁₃BrN₂O₂ (M + H), 345.03; Found: 345.0657.

2.11. 3-((2,4-dimethylphenyl)amino)-1-phenylpyrrolidine-2,5-dione (3i)

The title compound was prepared following the general procedure using N-Phenylmaleimide (1mmol, 0.173gm) and 2,4-dimethylaniline (1mmol, 0.121gm) at r.t. (25°C). The desired product was obtained as a brown/white solid ; yield: 0.168gm, 89%; M.P.: 205-210°C; ¹H NMR (400 MHz, DMSO-d₆/TMS, δ, ppm): 10.07 (s, 1H), 9.43 – 6.17 (m, 8H, Ar-H), 4.35 (s, 1H), 3.22 (dd, J = 16.4, 3.5 Hz, 2H), 2.06 (s, 3H), 1.96 (s, 3H); ¹³C{¹H} NMR (100 MHz, DMSO-d₆/ δ, ppm): 189.4, 182.8, 169.2, 139.3, 129.2, 119.5, 52.2, 40.4, 20.6; IR(ν/cm⁻¹): 3222 (N-H stretch.), 1669, 1602 (C=O, imide), 1535 (C=C, Ar), 1478, 1382 (C-H bending, CH₃), 1286 (C-N stretch), 749, 692 (C-H out of plane, Ar); HRMS (ESI) exact mass calcd for C₁₈H₁₈N₂O₂ (M + H), 295.15; Found: 295.1766.

3. RESULT AND DISCUSSION

3.1. Optimization of Reaction Conditions

The solvent, temperature and reaction time were changed to optimize the reaction conditions (Table 1). The best reaction medium among the conditions examined was ethanol at room temperature, which produced the required product in a good isolated yield of 98% after 6h. Reaction efficiency was significantly reduced when using different solvents

"Synthesis and Characterization of Substituted 3-(Arylamino)-1-phenylpyrrolidine-2,5-diones and Their Biological Evaluation"

and reaction temperatures. Both raising the reaction temperature and extending the reaction time did not significantly improve the production of the product. For the production of the intended substituted 3-(arylamino)-1-phenylpyrrolidine-2,5-diones derivatives, ethanol at room temperature was therefore determined to be the ideal setting.

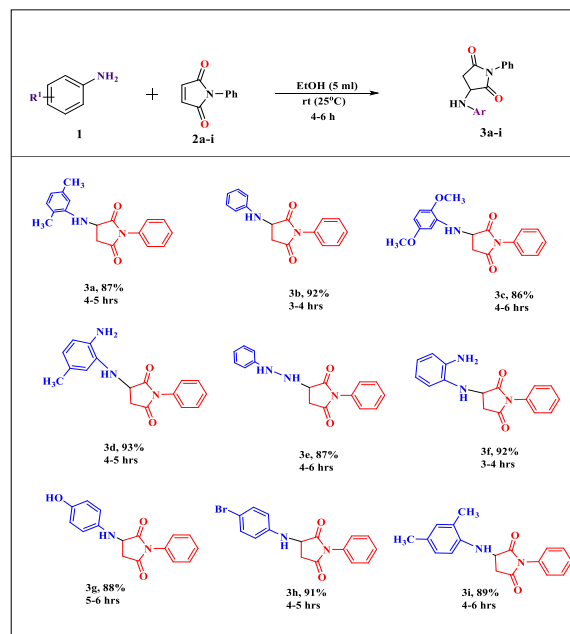
Table 1 The optimization of reaction conditions for synthesis of substituted 3-(arylamino)-1-phenylpyrrolidine-2,5-diones derivatives^a

S.N.	Solvent	Temperature (°C)	Time (h)	Yield ^b (%)
1.	EtOH	RT	24	Trace
2.	EtOH	RT	12	68
3.	EtOH	RT	6	92
4.	EtOH	RT	8	92
5.	MeOH	RT	10	81
6.	CH ₃ CN	RT	12	75
7.	CH ₂ Cl ₂	RT	15	60
8.	THF	RT	14	64
9.	Toluene	Reflux	10	70
10.	H ₂ O	RT	24	40

^aN-Phenylmaleimide (1 mmol), Substituted aniline (1 mmol), solvent (5 mL). ^bIsolated Yields.

3.2. Synthesis of substituted 3-(arylamino)-1-phenylpyrrolidine-2,5-diones derivatives

Using the optimized reaction conditions, a series of substituted 3-(arylamino)-1-phenylpyrrolidine-2,5-diones derivatives (3a-3i) were successfully synthesized from N-phenylmaleimide and various substituted anilines via aza Michael addition. The reaction showed a broad substrate scope and accommodation both electron donating and electron withdrawing substituents, affording the recrystallization and fully characterized by FTIR, ¹HNMR, ¹³CNMR and Mass spectrometry, confirming the successful formation of the target molecules.



Scheme 1 Synthesized and substrate scope of substituted 3-(arylamino)-1-phenylpyrrolidine-2,5-diones derivatives (3a-3i)

3.3. Biological Aspects

The combination presence of the maleimide and aniline moieties in the synthesized N-substituted succinimide derivative demonstrated extraordinary biological potential. With a DPPH radical scavenging ability of $197.03 \pm 2.32 \mu\text{g/mL}$, the chemical demonstrated significant antioxidant activity, demonstrating its effectiveness in lowering oxidative stress and neutralizing reactive oxygen species⁵⁰⁻⁵². Because of its antioxidant qualities, it is a viable option for uses in food preservation, cosmetic preparations, and medicinal compositions where oxidative stability is essential. Maleimide derivatives are also well known for their antifungal and antibacterial qualities, which result from their capacity to block essential enzyme systems and damage microbial cell membranes⁵³⁻⁵⁶. Through hydrogen bonding and π - π stacking, the succinimide ring promotes robust intermolecular interactions with microbial proteins, increasing biological activity. As a result, the synthesized chemical can be used as a useful scaffold to create multifunctional medicinal compounds with antibacterial and antioxidant properties.

3.3.1. Antibacterial activity

The Agar Well Diffusion method, a common microbiological technique, was used to perform the in-vitro antimicrobial assay. *Escherichia coli* (a Gram-negative bacterium) and *Staphylococcus aureus* (a Gram-positive bacterium) were tested for antibacterial activity using nutrient agar. Three

concentrations (25 mg/L, 50 mg/L, 75 mg/mL, and 100 mg/mL) were generated for each complex using 10% DMSO as the solvent for sample dilution. Before being evaluated, the plates were incubated for 24 hours at 37 °C. The typical antibiotic reference drug was ciprofloxacin at 1 mg/L⁵⁷. The [C₁₈H₁₈N₂O₂(Ak-34), C₁₆H₁₃BrN₂O₂ (Ak-45)] showed the strongest antibacterial action against both Gram-positive and Gram-negative bacterial strains, according to the results, which are summarized in Table 2. Figures 2, 3 and 4, respectively, show the antibacterial properties against *E. coli* and *S. aureus*. The Activity Index was calculated by comparing the

activity of samples with that of the traditional antibiotic drug.



Fig.2 Petri dishes showing activity against different bacterial strain

Table 2 Representation of the antibacterial properties against *E. coli* and *S. aureus*

Name of Sample	Inhibition zone (mm) at different conc. (mg/L)															
	Against <i>E. coli</i>								Against <i>S. aureus</i>							
	25		50		75		100		25		50		75		100	
	IZ	AI	IZ	AI	IZ	AI	IZ	AI	IZ	AI	IZ	AI	IZ	AI	IZ	AI
C ₁₈ H ₁₈ N ₂ O ₂ (Ak-34)	3	0.16	10	0.33	13	0.41	15	0.46	8	0.25	14	0.40	17	0.47	20	0.52
C ₁₆ H ₁₃ BrN ₂ O ₂ (Ak-45)	7	0.29	9	0.26	11	0.29	13	0.31	8	0.25	13	0.37	15	0.41	18	0.47
Standard (Cipro)	24		30		31		32		31		35		36		38	

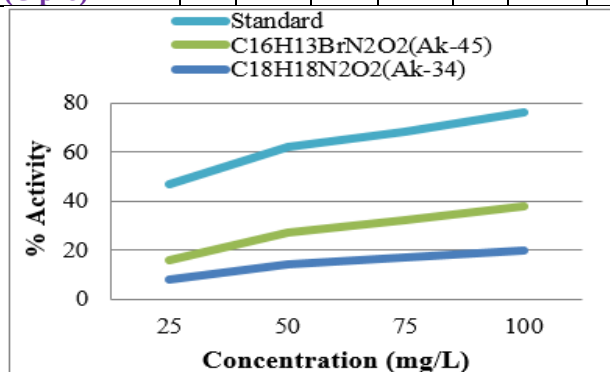


Fig. 3 potential against *E. coli*

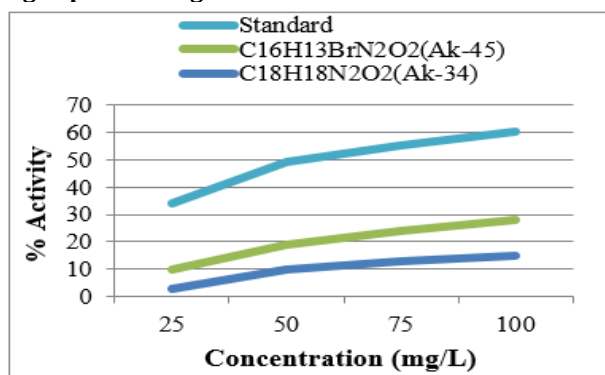


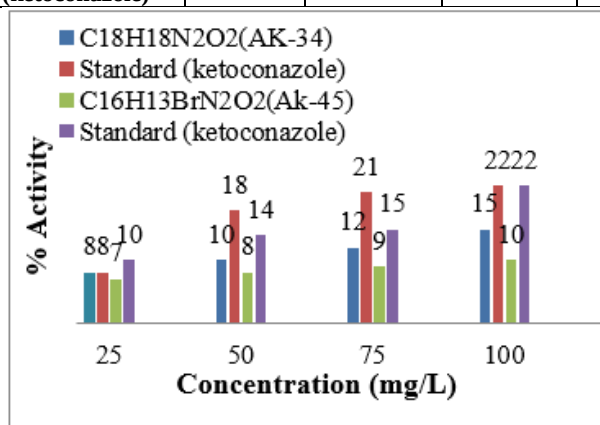
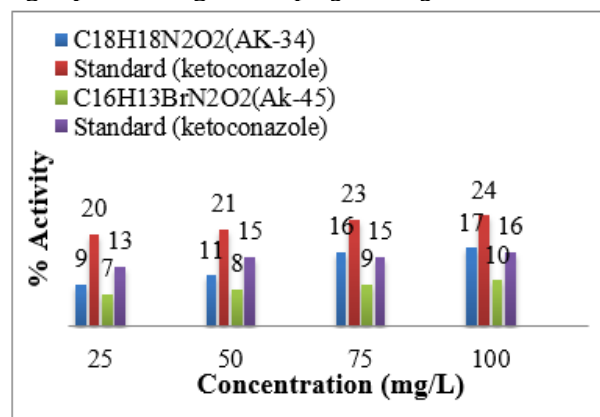
Fig. 4 potential against *S. Aureus*

3.3.2. Antifungal activity

At different doses (25 mg/L, 50 mg/L, 75 mg/mL, and 100 mg/mL) in dimethyl sulphoxide (DMSO), *Aspergillus niger* and *Penicillium chrysogenum* were tested against water, methanol, and pet ether extracts of *J. adhatoda* roots, stems, leaves, and flowers. Using the well diffusion method, the assessment was carried out in Potato Dextrose Agar (PDA) medium. with the outcomes shown in Table 3. The samples were incubated at 37°C for an entire day. Ketoconazole (1 mg/L) served as the positive control⁵⁸. According to the results, C₁₈H₁₈N₂O₂ (Ak-34) and C₁₆H₁₃BrN₂O₂ (Ak-45) showed much more antifungal activity than the other sample(Fig.5&6). The following formula was used to calculate the activity index (AI): IZ of the standard/IZ of the sample

Table 3: Representation of the Antifungal properties against *A.niger* and *P. chrysogenum*

Name of Sample	Inhibition zone (mm) at different conc. (mg/L)															
	Against <i>Aspergillus niger</i>								Against <i>Penicillium chrysogenum</i>							
	25		50		75		100		25		50		75		100	
	IZ	AI	IZ	AI	IZ	AI	IZ	AI	IZ	AI	IZ	AI	IZ	AI	IZ	AI
$C_{18}H_{18}N_2O_2$ (Ak-34)	8	1	10	0.55	12	0.57	15	0.68	9	0.45	11	0.52	16	0.69	17	0.70
Standard (ketoconazole)	8		18		21		22		20		21		23		24	
$C_{16}H_{13}BrN_2O_2$ (Ak-45)	7	0.7	8	0.57	9	0.6	10	0.45	7	0.53	8	0.53	9	0.6	10	0.62
Standard (ketoconazole)	10		14		15		22		13		15		15		16	

Fig. 5 potential against *Aspergillus niger*Fig. 6 potential against *Penicillium chrysogenum*

2.3.3. Antioxidant activity:

The antioxidant capacity of the sample was evaluated using the DPPH free radical scavenging activity assay. The isolated materials' ability to scavenge DPPH radicals was evaluated using the 1,1-diphenyl 2-picrylhydrazil (DPPH) method. 100 μ l aliquots of various concentrations (20-100 μ g/mL in DMSO) were combined with 3.9 ml of 0.1 mM DPPH (methanolic) solution. The liquid was thoroughly vortexed and then incubated in the dark for 30 minutes⁵⁹. Its optical density (OD) was measured at 517 nm using methanol as a blank. The ratio = (Abcontrol-Absample / Abcontrol) $\times$ 100 was used to calculate the radical scavenging activity. The finding results which are displayed in Table 4 and Fig. 7, show that both samples have significant antioxidant activity through the transfer of hydrogen atoms and electrons. Plotting a linear relationship between concentration and % inhibition allowed for the computation of IC₅₀ values. Each sample's antioxidant capacity was shown using an inhibition curve, which is defined as the concentration needed to reduce the generation of DPPH radicals by 50%.

Table 4 Displayed significant antioxidant activity in form of IC₅₀ values for the samples

Name of Sample	% Free radical scavenging activity at various concentrations (µg/ml)					Regression equation	IC ₅₀ value (µg/ml)
	20	40	60	80	100		
C₁₈H₁₈N₂O₂ (Ak-34)	14.16±0.3 9	17.28±0.3 3	23.08±0.1 5	27.31±0.1 9	29.40±0.2 6	Y=0.2025x+10.09	197.03±2. 32
C₁₆H₁₃BrN₂O (Ak-45)	8.18±0.08	17.04±0.2 6	17.92±0.1 3	23.15±0.2 3	25.44±0.1 7	Y=0.2029x +6.18	215.92±0. 40
Ascorbic acid	24.68±0.1 5	32.49±0.3 4	44.27±0.4 1	54.21±0.2 6	61.35±0.0. 3	Y=0.4707x+15.29	73.68±0.6 3

All experiments were conducted in triplicates and IZ values are represented as mean±SD

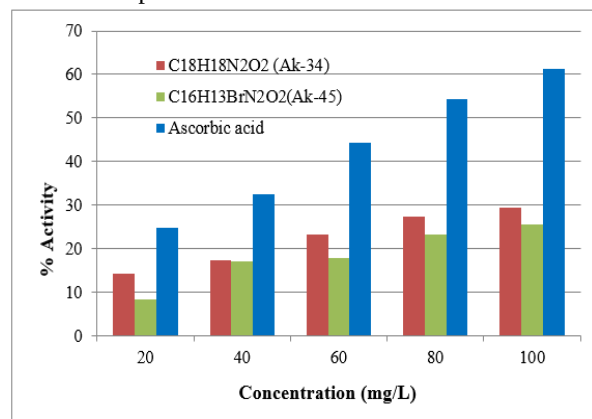


Fig. 7 illustration of antioxidant potential of reported compounds

4. CONCLUSION

A Series of substituted 3-(arylamino)-1-phenylpyrrolidine-2,5-dione derivatives were successfully synthesized through an aza Michael addition under mild reaction conditions. The protocol is operationally simple, environmentally friendly, and affords the target compounds in good to excellent yields with broad substrate scope. Structural characterization by FTIR, ¹H NMR, ¹³C NMR and mass spectrometry confirmed the formation of desired products. The synthesized compounds also exhibited promising biological activity, demonstrating the potential of this scaffold for sustainable route for the preparation of functionalized β-amino succinimide derivatives.

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