Original Article

Synthesis and *in-vitro* Antioxidant Activity of Novel Schiff Bases and Azetidines Derived from Phenyl Urea Derivatives

Veera Raghavulu Nagavolu[1], Shankarananth Velusamy[1]*, Sridhar Chechugari[1], Prasanna Raju Yalavarthi[2], Manasa Karani[1]

[1]Department of Pharmaceutical Chemistry, Sri Padmavathi School of Pharmacy, Tirupati, Andhra Pradesh, INDIA.
[2]Department of Pharmaceutics, Sri Padmavathi School of Pharmacy, Tirupati, Andhra Pradesh, INDIA.

ABSTRACT

Objective: The Schiff bases and azetidines are important intermediates used in synthesis of several therapeutics and medicinally contributing molecules. This research was focused on synthesis of Schiff bases and azetidines, characterization and subsequent evaluation of their *in-vitro* antioxidant potentials. **Methods:** In this work, the Schiff bases and azetidines were derived from phenyl urea derivatives. They were tested qualitatively for melting point and characterized by TLC, FTIR, 1H-NMR, ^{13}C-NMR and GCMS for their chemical structures. The Schiff bases and azetidines were screened for *in-vitro* antioxidant activity by employing hydrogen peroxide free-radical inhibition method. **Results:** The compounds 1-[(1E)-2-(3-fluorophenyl) ethylidene]-3-(4- ethoxyphenyl) urea and 3-chloro-2-(3-fluorophenyl)-N-(4-methoxyphenyl)-4-oxoazetidine-1-carboxamide were derived by nucleophilic addition and cycloaddition reactions and had displayed moderate to significant antioxidant effects compared to ascorbic acid. Thus, medicinally and chemically important Schiff bases and azetidines were synthesized successfully. **Conclusion:** The present research can be a base to explore further simple and easy means of other synthetic schemes in processing of complex and potent bulk chemicals as well as medicinal agents.

Key words: Antimicrobial, Arylamine, Chloroacetyl chloride, FTIR, GCMS, Hydrogen-peroxide.

INTRODUCTION

Schiff bases are used as substrates in the preparation of industrially and biologically active compounds via ring closure, cycloaddition and replacement reactions.[1] Moreover, Schiff bases are known to have biological activities such as antimicrobial,[2,3,4,5] antifungal,[6] antitumor[7,8,9] and herbicide.[10] Schiff bases are the products of condensation of arylamines and carbonyl compounds. They are quite stable and versatile intermediates for preparation of a number of important medicinal compounds. Applying principle of condensation on Schiff bases along with chloroacetyl chloride and triethylamine in the presence of 1, 4-dioxan, azetidines are produced. Azetidines are widely used in the areas of fine chemicals and medical substrates. Azetidines principally possess anti-inflammatory, analgesic,[11,12] antibacterial,[13,14] antifungal,[15,16] antitumour[17,18] and anti-malarial[19,20] activities. So far, several synthetic methods have been tabled for the synthesis of Schiff bases and azetidines. However, most of them were not very successful due to their limitations such as low-yield, high-cost and extensive-recrystallization. Therefore, the pursuance of convenient and practically feasible methods for preparation of these compounds still remain an active area of research.

With this background, the research was aimed to explore an efficient method to synthesize Schiff bases and azetidines.

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Correspondence:
Dr. Shankarananth Velusamy,
Sri Padmavathi School of Pharmacy, Tiruchanur, Tirupati-517503, INDIA.
Mobile: +91-9908391835
E-mail: shankraja2004@rediffmail.com

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Experimental section

General

Mps (uncorrected) were recorded on a GUNA digital melting point apparatus. FTIR spectra were recorded on an SHIMADZU 440 FTIR spectrometer in the range 4000-400 cm^{-1}. ^{1}H-NMR and ^{13}C-NMR spectra were recorded in DMSO-d_6 solution using TMS as an internal reference standard on a Bruker-Avance-III 300 NMR spectrometer of 300 MHz. GC-MS spectra were recorded on a JEOL GC MATE II GC-MS1000 X spectrometer. All reagents used were analytical grade.

Synthesis of phenyl urea derivatives from substituted anilines

A mixture of aromatic substituted anilines (0.5 M) and urea (2 M) were taken in a calibrated round bottom flask and the contents were dissolved in a mixture of 50 ml distilled water, 4 ml hydrochloric acid and 4 ml glacial acetic acid and refluxed for 30 min. Then it was cooled and poured into ice bath. Substituted phenyl urea derivatives were obtained as a precipitate. Then it was filtered and kept for drying.

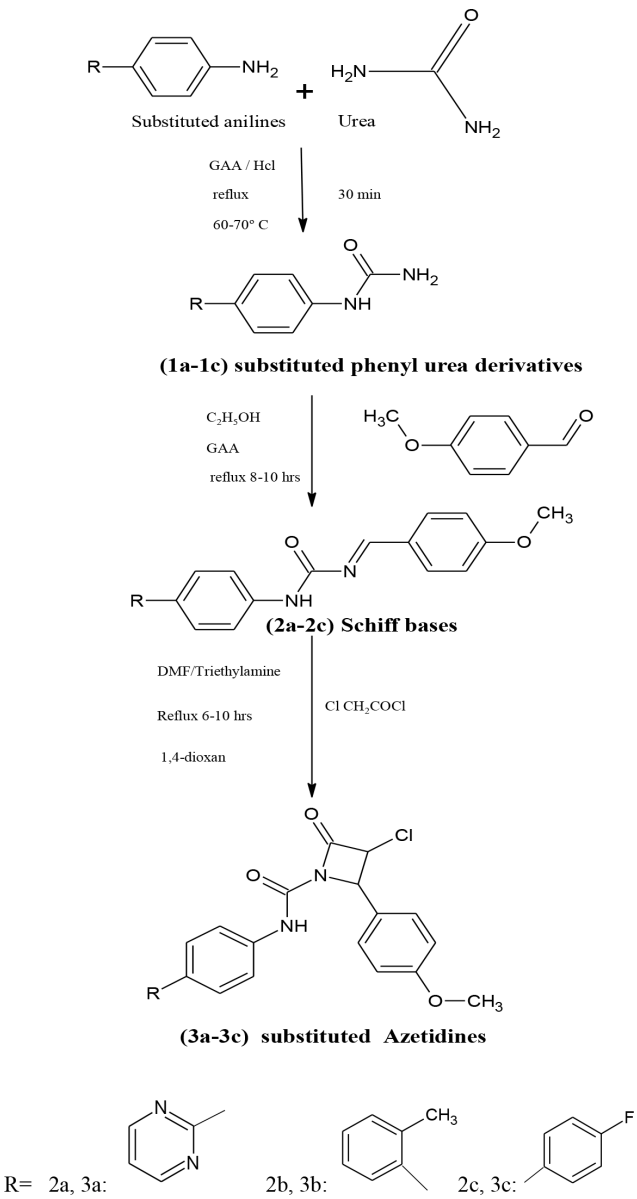
Synthesis of Schiff's bases from phenyl urea derivatives

A mixture of phenyl urea derivatives (0.01 M), 30 ml of ethanol, 2 ml of glacial acetic acid and anisaldehyde (0.01 M) were taken in a round bottom flask and refluxed for 10 h. Then it was cooled with help of ice-bath. Schiff's bases were obtained as crude product. Then it was filtered and kept for drying. It was then recrystallized by using ethanol to get crystalline product. The completed reaction was confirmed by thin layer chromatography using chloroform: methanol (8:2) as solvent system. Then it was further used without purification.

Synthesis of substituted azetidines from Schiff's bases

Schiff's base (0.01M) was slowly added to 8 ml of N, N-dimethyl form amide and stirred for few min in a graduated flask. Chloroacetyl chloride (0.01M), triethyl amine and 10 ml of 1, 4-dioxan was added to it and refluxed for 10 h. After refluxing, the reaction mixture was poured in ice-mixture to get crude azetidines. The crude product was filtered and kept for drying. It was then recrystallized by using ethanol to get crystalline product. The completed reaction was confirmed by thin layer chromatography using chloroform: methanol (9:1) as solvent system.

Evaluation of in-vitro antioxidant activity



Scheme 1: Synthesis of title compounds.

Synthesized compounds were evaluated for *in-vitro* antioxidant activity by hydrogen peroxide free radical inhibition method. Test samples were prepared with methanol at 12.5, 25, 50 and 100 µg/ml concentrations. About 2 mmol/l concentration hydrogen peroxide solution was prepared using phosphate buffer saline. Both test and standard drug (ascorbic acid) solutions were added to hydrogen peroxide solution (0.6 ml) and incubated for 10 min. The antiradical power was assessed by measuring a decrease in the absorbance of hydrogen peroxide solution at 230 nm. The experiment was repeated for three independent samples and mean of antioxidant potentials in terms of the percent of inhibition (%) of free radical production from hydrogen peroxide solution was computed. A plot of percentage

inhibition against concentration was drawn and IC_{50} was calculated.[21]

RESULTS AND DISCUSSION

Characterization

All the synthesized compounds (2a-2c) and (3a-3c) were purified by successive recrystallisation using ethanol. The purity of the synthesized compounds was examined by TLC. The structures of synthesized compounds were elucidated by spectral data of FTIR, ^{1}H-NMR, ^{13}C-NMR and GCMS.

1-(4-methoxyphenyl)-3-[(1E)-2-(pyrimidin-5-yl) ethylidene] urea (2a)

In this reaction scheme, a nucleophilic addition was observed between substituted aromatic amine (phenyl urea derivative) and carbonyl compound (anisaldehyde). Powdered compounds (white) (Schiff base) were obtained with yield of 80%, melting range of 240-242°C and R_f value of 0.87. The principal functional groups were appeared as OCH_3 (2952-3074 cm^{-1}), C=N (1572-1624 cm^{-1}), C=O (1690-1670 cm^{-1}), aromatic summation at 1670-2000 cm^{-1} and N-H stretching at 3340-3370 cm^{-1} in the FTIR spectra. The ^{1}H-NMR spectra displayed chemical shifts as: δ 8.0 ppm (s, N-H), 2.6 ppm (s, methylene), δ 3.73 ppm (s, CH3, methyl), δ 7.12, 6.77 ppm (benzene) *J*=7.96. The ^{13}C-NMR spectra displayed chemical shift as: δ 55.9 ppm (CH_3, aliphatic), δ 33.8 ppm (s, CH_2, aliphatic), δ 114.5, 122.6 ppm (s, CH, benzene), δ 154.9, 157.0 ppm (s, CH, pyrimidine), δ 130.4 ppm (s, C, pyrimidine), δ 162.6 ppm (s, C, amide) whereas GC-MS-EI (m/e, relative intensity, %); 271 (M^++1).

N-(4-methoxyphenyl)-N'-[(1E)-2-(4-methylphenyl) ethylidene]urea (2b)

These Schiff bases were yielded due to nucleophilic addition reaction. Compound yield was 90% as white powder with melting range of 300-302°C and 0.90 as R_f value. Spectra of FTIR (cm^{-1}) indicated the principal functional groups as OCH_3 (2948-3067), C=N (1568-1618), C=O (1690-1710), aromatic summation at 1670-2000 and N-H stretching at 3340-3360. Chemical shifting was observed as δ 8.0 ppm (s, N-H), 2.6 ppm (s, methylene), δ 3.73 and 2.3 ppm (s, CH_3, methyl), δ 6.94 ppm (benzene) in the spectra of ^{1}H-NMR and *J*=7.96. ^{13}C NMR spectra obtained with chemical shift of δ 24.3, 55.9 ppm (CH_3, aliphatic), δ 33.8 ppm (s,CH_2, aliphatic), δ 114.5,122.6,129.0 ppm (s, CH, benzene), δ 162.6 ppm (s, C, amide), δ 128.2,134.5 ppm (s, C, benzene) GC-MS-EI (m/e, relative intensity, %); 283 (M^++1).

1-[(1E)-2-(3-fluorophenyl) ethylidene]-3-(4-methoxyphenyl) urea (2c)

Reaction between substituted aromatic amine and carbonyl compound was envisaged by nucleophilic addition. White powdered compound (94% yield) was obtained with melting range of 260-262°C and R_f value of 0.82. FTIR spectral data (cm^{-1}) indicated the functional groups as OCH_3 (2945-3065), C=N (1565-1605), C=O (1690-1710), aromatic summation at (1670-2000) and N-H stretching at 3330-3420. ^{1}H-NMR Spectra obtained with chemical shift of δ 8.0 ppm (s, N-H), 2.6 ppm (s, methylene), δ 3.73 ppm (s, CH_3, methyl), δ 7.12, 6.83 and 6.77 ppm (benzene) and *J*=7.96. ^{13}C-NMR spectra was observed with chemical shift of δ 55.9 ppm (CH_3, aliphatic), δ 33.8 ppm (s,CH_2, aliphatic), δ 114.5,122.6,124.7,130.3 ppm (s, CH, benzene), δ 162.6 ppm (s, C, amide), δ 128.2,156.3 ppm (s, C, benzene) δ 112.5, 116.1 (s, CH, benzene(F)) GC-MS-EI (m/e, relative intensity, %); 287 (M^++1).

3-chloro-N-(4-methoxyphenyl)-2-oxo-4-(pyrimidin-5-yl) azetidine-1-carboxamide (3a)

Cycloaddition reaction between imine (Schiff base) and ketene was attributed to derive these compounds. About 75% of compound was yielded as white powder and exhibited melting range of 500-502°C, R_f value 0.76. The FTIR (cm^{-1}) data indicated that the principal functional groups as OCH_3 at 2950-3075, C=N at 1570-1620, C=O at 1700-1720, C-Cl at 768-618, aromatic summation at 1680-2000 an N-H stretching at 3350-3450. Chemical shifting was observed in ^{1}H-NMR spectra at δ 6.0 ppm (s, N-H), 3.49 and 3.24 ppm (s, propiolactam), δ 3.73 ppm (s,CH_3, methyl), δ 5.47 ppm (CH-methine ,α Cl) δ 7.5 and 6.75 ppm (aromatic rings) whereas *J*=7.96. ^{13}C-NMR spectra displayed the chemical shift as: δ 55.9 ppm (CH_3, aliphatic), δ 56.7, 61.4 ppm (CH, aliphatic) δ 114.5, 122.6 ppm (s, CH, benzene), δ 147.6 ppm (s, C, N-urea) δ 154.5, 155.8 ppm (s, CH, pyrimidine), δ 136.9 ppm (s, C, pyrimidine), δ 163.9 ppm (s, C, amide) GC-MS-EI (m/e, relative intensity, %); 333(M^++1).

3-chloro-N-(4-methoxyphenyl)-2-(4-methylphenyl)-4-oxoazetidine-1-carboxamide (3b)

Cycloaddition reaction was interpreted in this scheme. White powdered compound was obtained with yield of 85% having melting range in between 391-393°C with R_f value of 0.92. FTIR spectral data (cm^{-1}) includes OCH_3 (2948-3073), C=N (1580-1600), C=O (1702-1716), C-Cl (754-577), aromatic summation at 1670-2000 and N-H stretching at 3355-3455; spectra of ^{1}H-NMR was observed with shifting at δ 6.1 ppm (s, N-H), 3.48 and 3.24 ppm (s, propiolactam), δ 3.74 ppm (s,CH_3, methyl),

δ 5.44 ppm (CH-methine ,α Cl) δ 7.4 and 6.72 ppm (aromatic rings); whereas *J*=7.66. Chemical shifting of ^{13}C-NMR was obtained at δ 24.3,55.9 ppm (CH_3, aliphatic), δ 56.7, 61.4 ppm (CH, aliphatic) δ 114.5, 122.6, 126.9, 128.9 ppm (s, CH, benzene), δ 147.6 ppm (s, C, N-urea) δ 136.4,156.3 ppm (s, C, benzene) δ 163.9 ppm (s, C, amide) GC-MS-EI (m/e, relative intensity, %); 345 (M^++1).

3-chloro-2-(3-fluorophenyl)-N-(4-methoxyphenyl)-4-oxoazetidine-1-carboxamide (3c)

A white powder with 95% yield was obtained with underlying principle of cycloaddition reaction. The resulting compounds had melting range of 402-404°C and R_f value 0.86. The FTIR (cm^{-1}) spectral data demonstrated the functional groups as OCH_3 (2952-3078), C=N (1585-1606), C=O (1705-1718), C-Cl (743-613), aromatic summation at 1678-2000 and N-H stretching at 3360-3458. The ^{1}H-NMR spectra indicated the chemical shifting with δ 6.1 ppm (s, N-H), δ 3.47 and 3.23 ppm (s, propiolactam), δ 3.72 ppm (s, CH_3, methyl), δ 5.45 ppm (CH-methine, α Cl) δ 7.3-6.70 ppm (aromatic rings) and *J*=6.96. ^{13}CNMR spectral data obtained at δ 55.9 ppm (CH_3, aliphatic), δ 56.7,61.4 ppm (CH, aliphatic) δ 113.5,114.5,122.6,130.2 ppm (s, CH, benzene), δ 147.6 ppm (s, C, N-urea), δ 136.4,156.3 ppm (s, C, benzene), δ 145.1,162.1 ppm (s, C, benzene(F)) δ 163.9 ppm (s, C, amide) GC-MS-EI (m/e, relative intensity, %); 349 (M^++1).

FTIR spectra

The characteristic vibration was present for OCH_3 at 2950-3075cm^{-1}, C=N at 1570-1620 cm^{-1}, C=O at 1700-1720 cm^{-1}, C-Cl at 750-580 cm^{-1}, aromatic summation at 1680-2000 cm^{-1} and N-H stretching at 3350-3450 cm^{-1}.

^{1}H-NMR spectra

In the ^{1}H-NMR spectrum, chemical shift was observed at δ 8.0 ppm (s, N-H secondary amides). In all title compounds, the above shift was appeared, along with δ 3.49, 3.24 ppm (s, propiolactam). All title compounds were having the chemical shift at δ 3.73 ppm (s, CH_3, methyl). The differently substituted aromatic rings result in the protons appearing at δ 7.5-6.75 ppm.

^{13}C-NMR spectra

Chemical shift was observed in the ^{13}CNMR spectra at δ 163.9 ppm (s, C, amide). In all the title compounds, the above shift was appeared along with δ 113.5, 114.5, 122.6 and 130.2 ppm (s, CH, benzene). The title compounds had exhibited chemical shifts at δ 55.9 ppm (CH_3, aliphatic), δ 56.7, 61.4 ppm (CH, aliphatic).

In-vitro antioxidant activity

The free radical inhibition activity of the synthesized title compounds was compared to that of the standard ascorbic acid. The synthesized compounds had displayed moderate to significant antioxidant activity.

CONCLUSION

The novel Schiff bases and azetidines were derived successfully from phenyl urea derivatives. The Schiff bases were synthesized by nucleophilic addition whereas azetidines were derived by the cycloaddition reaction. The compounds obtained were characterized for their optimal physicochemical principles and functional chemistry by applying advanced spectroscopic and chromatographic tools. The synthesized compounds have displayed antioxidant potential against hydrogen peroxide free radical. Thus, important intermediates like Schiff bases and therapeutically important moieties like azetidines were synthesized successfully by condensation process. It is therefore concluded that the present research is useful to explore many of simple and easy means of synthetic schemes in processing of complex and potent chemicals as well as therapeutic moieties.

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CONFLICT OF INTEREST

The article content has no declarations of interest.

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ABBREVIATIONS USED

DMSO: dimethyl sulfoxide; **FTIR:** Fourier-transform infrared spectroscopy; **GCMS:** Gas chromatography–mass spectrometry; **NMR:** Nuclear magnetic resonance; **TMS:** Tetramethylsilane; **TLC:** Thin-layer chromatography.

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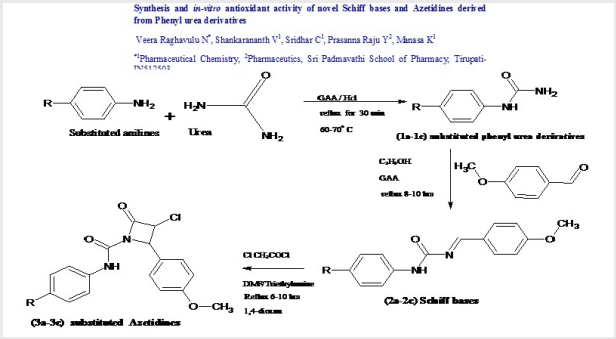
PICTORIAL ABSTRACT



SUMMARY

- Schiff bases and azetidines were synthesized for antioxidant potentials by nucleophilic addition and cycloaddition reactions respectively.
- Thus, the present research is useful to explore many of simple and easy means of synthetic schemes in processing of complex and potent chemicals as well as therapeutic moieties.

About Authors

Dr. Shankarananth Velusamy: PhD (Pharm Chem): He is Associate Professor of Pharmaceutical Chemistry at Sri Padmavathi School of Pharmacy, Tirupati, India. His main research focus is on synthesis of novel APIs and Intermediates with simple and robust approaches. He published his research findings in referred journals and presented his innovative research outcomes at many scientific forums.

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