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SYNTHESIS AND ANTI-CANCER EVALUATION OF N- HETEROCYCLIC SCHIFF BASES

Sania Mehreen[1], Freeha Hafeez[*2], Muhammad Suleman[3], Farah Ashraf[4], Khadija Hamid[4], Amina Tahir[5]

[1,*2,3,4,5]*Department of Chemistry, Riphah international University Faisalabad Campus, Faisalabad, Punjab. Pakistan. 44000*

[*2]freeha.hafeez@riphahfsd.edu.pk

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Corresponding Author: *
Freeha Hafeez

Abstract

Schiff base moiety is found to exist as a potent pharmacological scaffold in a number of the drugs. The biological schiff bases preparation process along with analysis work demonstrates both the demand for new anti-cancer drugs and their wide array of biological activities. The structure identification of these derivatives required ^{1}HNMR, ^{13}CNMR, FT-IR and mass spectrometry analysis. A standard hemolysis assay combined with a thrombolysis assay was used to evaluate the cytotoxic potential of the manufactured novel compounds against breast carcinoma MCF-7 cells in the laboratory. The 3-phenylprop-2-en-1-imine derivatives demonstrated various levels of cytotoxic effect toward MCF-7 cells through viability tests. Compound 6e showed the highest potency as a lead compound because it reached 33.66±0.66µg/mL cell viability concentration and is promising for scientific advancement.

INTRODUCTION

Breast cancer, a common type in women that is diagnosed in about 2.3 million women every year (Sun et al., 2017; Waks & Winer, 2019). Even with the presence of various medications for cancer handling, there is a broad interest in forming new and efficient chemotherapeutics in this area (Sartaj et al., 2020; Sykula, 2018). Heterocycles containing azomethine functional group such as schiff bases are notable scaffolds in medicine and display a comprehensive array of pharmacological potential (Ghorafi et al., 2020; Kerru et al., 2020).

Schiff bases retrieved from different heterocyclic compounds depict a wide array of biological activities including anti-bacterial, anti-inflammatory and anti-cancer (Abd El-Halim et al., 2018; Ashraf et al., 2011). The firm heterocyclic backbone performs a profound role in diverse pharmacologically relevant compounds including sulfa drugs those that are FDA-approved anticancer drugs (fig. 1). Both Schiff bases and their metal complexes have been shown to be effective against bacterial infections and tumors through research. (Podolski-Renić et al., 2024; Tülüce et al., 2022).

A nitrogen atom in the azomethine group with a sp^2 hybridized orbital holding only one electron pair greatly impacts both its chemical properties and its influence on biochemistry. It is asserted that the nitrogen atom of azomethine interacts with functioning centers of cell constituents by forming a hydrogen bond, interrupting normal

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cell processes and leading to the destruction of enzymatic efficiency in cancerous cells. This positions Schiff base as a promising target for the identification of anticancer agents (Ghorafi et al., 2020; Matela, 2020).

As a result, scientists are more active in developing and manufacturing effective medicines using schiff base substances for breast cancer treatment.

MATERIAL AND METHOD

General experimental part

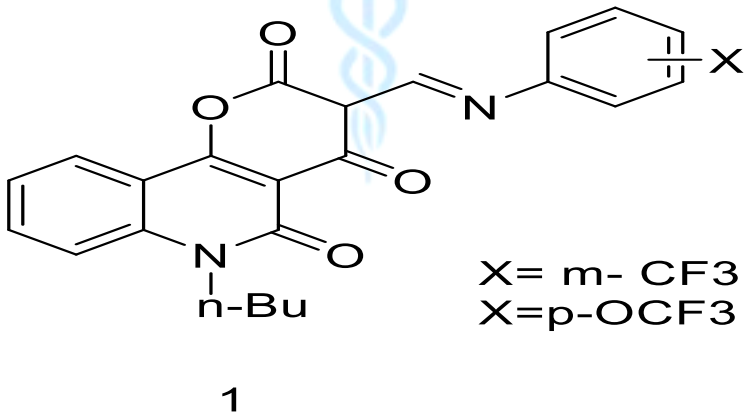
Chemicals, cinnamon aldehyde, anilines, methanol were obtained from Sigma- Aldrich, Alfa-Aesar (Germany), Daejung and Scharlau by local supplier. Natural extract (Lemon) was obtained from garden that was used as catalyst. The melting points of the targeted derivatives were determined by using Gallenkamp equipment. Derivatives which were synthesized were characterized using ^{13}C NMR and ^{1}H NMR spectra on a Bruker spectrometer functioning at frequencies of 100 MHz and 500 MHz, correspondingly. For FT-IR, a Bruker Fourier-transform spectrometer was employed.

General preparation of schiff bases

All in separate, stir the mixture containing aldehyde, aniline and 10mL methanol, adding a little natural catalyst into the shaken water inside the ultrasonic container at 60°C for about 1 hour. After 1 to 2 minutes of ultrasound, the TLC analysis revealed that the necessary separation was completed. The substance that has accumulated is purified with water to give the pure product you want. The final materials were re-crystallized from ethanol only when it was needed.

Preparation of N-heterocyclic schiff bases derivatives

Stirred cinnamon aldehyde (eq) (0.01g, 0.075mmol) together with aniline derivatives 3a-e (0.07g, 0.075mmol) through methanol solution (10mL) at 60°C during one hour using an ultrasonic container. Upon completion of reaction the reaction mixture underwent ultrasonic treatment for 1 to 2 minutes before obtaining pure product by filtering it with water. When final materials needed purification, they were re-crystallizing from ethan



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Fig 1. Schiff based anti-cancer drug with fluorine and fluoroalkyl moieties

Cytotoxic assay

The schiff bases derivatives 4a-e was investigated against cancer cell MCF-7. Appropriate cancer cells were kept at 37 °C in Dulbecco's Modified Eagles medium (DMEM) containing antibiotics, supplemented with 10% Fetal Bovine Serum (FBS) in a humidified, 5% CO2 environment. Cell viability was evaluated in these derivatives using a standard MTT assay. Synthesized derivative compounds were mixed with DMSO (di-methyl-sulfoxide) for use. 48 hours of treatment was done for cancer cells at a final DMSO concentration of 0.05%. These DMSO treated cells were selected as a control in the experiments discussed here. The medium was added to the cells and incubated 37°C for four

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hours. We examined the absorbance with a solution of formazan crystals in DMSO. Measurements were made at 570 nm using a thermo scientific microplate reader. We measured cell viability as a percentage of total cells.

Statistical analysis

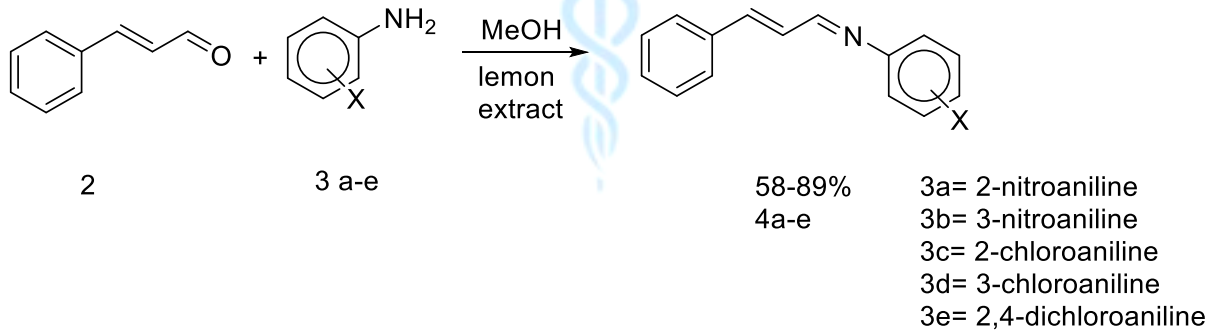
The software, Microsoft Excel 2016, was used to gather data, and each test process was performed a minimum of three times. The results were presented in form of mean ± SD.

Results

Ultrasonication of cinnamon aldehyde with anilines 3a-3e in methanol with few drops of lemon extract produced imine derivatives 4a-4e in 58-89% yield (Premalatha & Santhi, 2014)(El-Faham et al., 2016) as displayed in scheme 1.

Table 1: spectral characterization of compound 4a-e

Compound	M.P. (°C)	Yield %	FT-IR (cm-1) Vmax / 1HNMR (500 MHz, CDCl3)/ MS (EI) (m/z)
4a	172 °C	58	C-H (3223), C=N (1654), C-C (881), N=O (1523)/ d, 1H, J= $3H_2$, C-H (6.63), d, 1H, J= $3H_2$, -CH=N (8.41), m, 4H, Ar-H (7.18-7.59)
4b	168 °C	62	C-H (3223), C=N (1654), C-C (881), N=O (1523)/ d, 1H, J= $3H_2$, C-H (6.63), d, 1H, J= $3H_2$, -CH=N (8.41), m, 4H, Ar-H (7.12-7.61)
4c	176 °C	67	C-H (3214), C=N (1652), C-C (877), C-Cl (757)/ m, 1H, J= $3H_2$, C-H (6.99), d, 1H, J= $3H_2$, -CH=N (8.50), m, 4H, Ar-H (7.08-7.54)
4d	179 °C	75	C-H (3315), C=N (1666), C-C (891), C-Cl (786)/ m, 1H, J= $3H_2$, C-H (6.58), d, 1H, J= $3H_2$, -CH=N (8.43), m, 4H, Ar-H (6.99-7.49)
4e	166 °C	89	C-H (3125), C=N (1676), C-C (889), C-Cl (797)/ m, 1H, J= $3H_2$, C-H (6.61), d, 1H, J= $4H_2$, -CH=N (8.49), m, 3H, Ar-H (7.11-7.52)



Scheme 1: synthesis of derivatives 4a-e

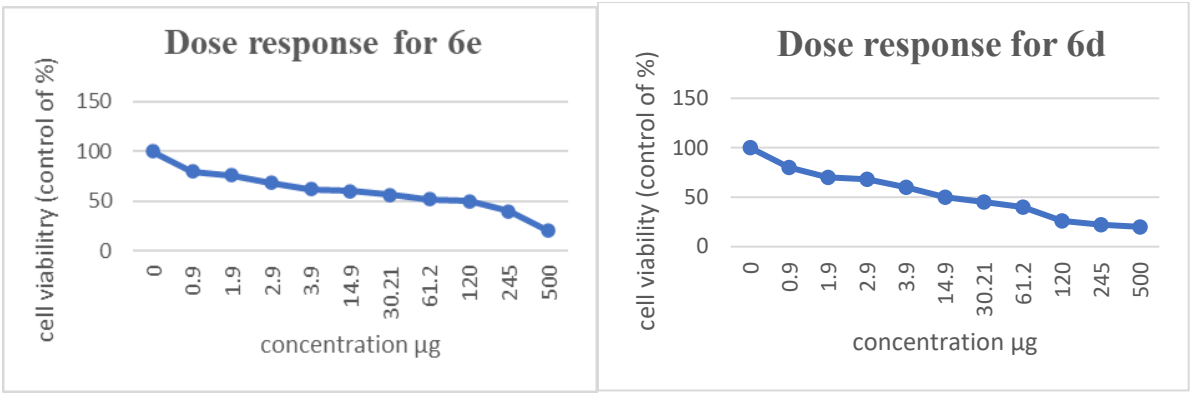
DISCUSSION

Spectral studies of compound 4c

The structure elucidation of the derivative 4c was done using Fourier transform infra-red (FT-IR), ^{1}HNMR (proton), ^{13}CNMR (carbon) NMR spectroscopy (fig. 2). The IR showed the absorption bands at 757 cm^{-1} for carbon single-bond chlorine, at 1652 cm^{-1} for C=N, at 877 cm^{-1} for C-C and at 3214 cm^{-1} for methine. For ^{1}HNMR of 4c gave multiplet at 6.63 ppm for methine of schiff base. Hydrogens of -CH=N were given as duplet at 8.41 ppm. Multiplet signals are seen in range 6.31-7.55 ppm for Ar-H. ^{13}CNMR spectrum of 4c had characteristic peak at 163.7 ppm for C=N. Peaks of C1-C3 carbons are seen at value of chemical shift of 135.2 ppm; C2-C4 is visible at

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126.8 ppm. The C5-C7 and C6-C8 carbons of morpholine were found at 128.6 and 119.9 ppm respectively.



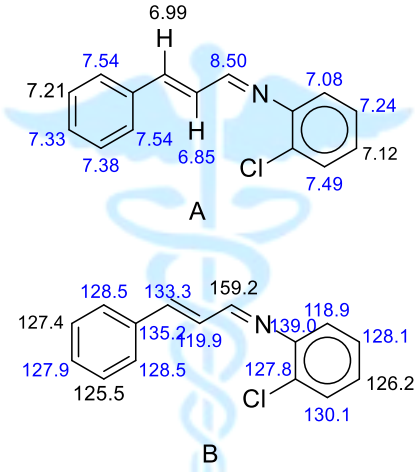


Fig 2. [1]HNMR A and [13]CNMR B Chemical Schiff values (ppm) of compound

Table 2: Cytotoxic potential of analogues 4a-e against MCF-7 (Breast cancer cell)

Derivatives	MCF-7 (% Cell viability ± SD)
4a	68.891±3.24
4b	49.78±0.63
4c	33.66±0.66
4d	47.22±4.09
4e	97.72±1.37

Cytotoxic activity

The anti-cancer potential for the synthesized derivatives 4a-4e was tested for the MCF-7 cell line. The effect of different substituents in the phenyl ring was tested to obtain the information on cytotoxic action of synthesized analogues. Table 2 shows the result for anti-proliferative activity and releases that in general, the insertion of the electron-donating group into the substituent in the phenyl ring facilitated the enhancement of the anti-cancer potential of the synthesized derivative. From synthesized derivatives, compounds 4c and 4d displayed an enhanced cycle-toxicity. The viability of cells treated with 100µg/mL of compound 4c was 33% and compound 4d was

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47% which was less than the other synthesized analogues. One way-ANOVA analysis of variance was applied for comparison of cell viability values for different compounds (table 2). Compounds 4c and 4d were examined for dose response by using various doses viz. 0.9-500µg. Maximum results observed was 500 µg concentration of 4c and 4d (fig.3).

CONCLUSION

Schiff bases, a familiar nucleus which is used to synthesize many compounds with anticancer potential. Successful synthesis and investigation of anti-cancer potential of N-heterocyclic schiff bases 4a-4e was carried out. Among the various synthesized derivatives, compound 4c even at low concentration (0.9 µg) of anti-cancer potency was found significantly for MCF-7 (Human breast carcinoma), which can be best possible improved by considering the results (of present study). Due to our result of structure activity relationship, more modifications can be made over N-heterocyclic schiff bases derivatives for the formation of highly potent biologically active piperazine based agents.

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