DESIGN, SYNTHESIS, MOLECULAR DOCKING, AND EVALUATION OF CHROMONE BASED TETRAZOLE DERIVATIVES

ANUJA CHOPRA1,2, LAKHWINDER SINGH2*, KAPOOR VK3, RICHA DHINGRA4, NEELIMA DHINGRA4

1Research Scholar, IKG Punjab Technical University, Jalandhar- Kapurthala Highway, Near Pushpa Gujral Science City, Kapurthala[144601] Punjab, India. 2Department of Pharmaceutical Sciences, G. H. G. Khalsa College of Pharmacy, Ludhiana (141104), Punjab, India. 3Department of Applied Science, GGC College of Engineering, Mohali (140307), Punjab, India. 4Department of Pharmaceutical Sciences, University Institute of Pharmaceutical Sciences, Panjab University, Chandigarh (160014), India. Email: lakhwinder.pharma@yahoo.com

ABSTRACT

Objectives: The objective of this research work was to design, synthesize, study the molecular docking, and evaluate the antimicrobial activity of some novel substituted 2-(Phenylamino)-3-(1H-tetrazol-5-yl)-4H-chromen-4-one derivatives (12a-h).

Methods: In the present work, 3-Formylchromones were transformed into pharmacologically active substituted 2-(Phenylamino)-3-(1H-tetrazol-5-yl)-4H-chromen-4-one derivatives (12a-h) through a multistep reaction. Initially, synthesis of the substituted 4-Oxo-2-(phenylamino)-4H-chromone-3-carbaldehydes (9a-h) was carried out using substituted acetonaphenones (6a-h) as starting material and by employing an earlier reported method (1,3-dipolar cycloadduction reaction). Then, these synthesized compounds were converted into respective oximes (10a-h) through a stepwise addition of neutral or anionic azide species to furnish final substituted 2-(Phenylamino)-3-(1H-tetrazol-5-yl)-4H-chromen-4-one derivatives (12a-h). All the newly synthesized compounds (12a-h) and a reference compound (ciprofloxacin) were docked into the active site of TyrRS (PDB: 1JIK) by means of the BioPredicta module of VLife MDS. The synthesized compounds (12a-h) were also evaluated in vitro for their antibacterial (against Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa, and Escherichia coli bacterial strains) and antifungal activities (against Aspergillus niger and Candida albicans fungal strains) using Zone of Inhibition method.

Results: The formation of substituted 2-(Phenylamino)-3-(1H-tetrazol-5-yl)-4H-chromen-4-one derivatives (12a-h) was confirmed through their spectral analysis, that is, 1H-NMR, 13C-NMR, and Mass spectroscopy. During docking study, the recorded molecular binding interactions revealed that the newly synthesized compounds (12a-h) interacted well with binding site of the enzyme. The synthesized compounds were also evaluated in vitro for their antibacterial (against S. aureus, B. subtilis, P. aeruginosa, and E. coli bacterial strains) and antifungal activities (against A. niger and C. albicans fungal strains). All the synthesized compounds exhibited moderate-to-potent antimicrobial activities.

Conclusions: All the synthesized compounds exhibited moderate-to-potent antimicrobial activity.

Keywords: Chromone, VilsmeierHaack, Antibacterial, Antifungal, 2-Anilino-3-formylchromones, Tetrazole, DNA gyrase.

INTRODUCTION

Chromones are the heterocyclic compounds demonstrating high degree of structural diversity. They constitute the largest and most varied family of organic compounds [1]. They are known to display a remarkable spectrum of pharmacological activities, including antitumor [2], anti-inflammatory [3], antibacterial [4], antifungal [5], antioxidant [6], anti-HIV [7], vasodilation [8], antiviral [9] and anti-allergic [10] activities, etc.

As per the available literature reports, karangin (1) and pongaglabol (2) from plant Pongamia pinnata show antibacterial activity against Shigella dysenteriae and Staphylococcus aureus, respectively. 3-Hydroxy-2-(1-phenyl-3-arly-4-pyrazolyl) chromones (3,4) show antifungal activity against three phytopathogenic fungi [11], namely, Helminthosporium species, Fusarium oxysporum, and Alternaria alternata. Chromones are very prone to chemical transformations such as photocycloaddition, photodimerization, photosomerization, photorearrangement, photo-oxidation reduction, and photocyclization which involve both n→π* and π→π* transitions [12,13]. Further, chromones and bischromones (especially their 3-alkoxy derivatives) have also proven themselves as interesting substrates to study the mechanism of photochemical reactions [14].

Among their derivatives, 3-formylchromones (5) bear a unique name for being part of molecular structure of several naturally occurring and pharmacologically active heterocyclic compounds [15,16].

Further, 3-Formylchromones are used as synthons in the synthesis of various heterocyclic analogs [17]. The presence of three strongly electrophilic centers (C-2 and C-4 of the chromone system as well as the carbon of the formyl group) in their structure facilitates their use in this regard and makes the chromone moiety [18] pharmacologically active.

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Despite tremendous research which has been done on chromones and 3-formylchromones, modern techniques are employed to synthesize their new derivatives with a focus to get improved pharmacological activities. Keeping in view the above observations, it was decided to synthesize some novel substituted 2-(Phenylamino)-3-(1H-tetrazol-5-yl)-4H-chromene-4-one derivatives (12a-h, Scheme 1) through reactions of various substituted 3-Formylchromones (7a-h) with different chemical reagents and evaluate them for their antimicrobial activities.

MATERIALS AND METHODS

Materials

Solvents, starting materials, and reagents were purchased from commercial suppliers and used after purification. All the solvents were purified by the standard procedure before use.

The melting points of all the synthesized compounds were measured on a liquid paraffin bath in open glass capillary tubes using Digital Melting point apparatus by Nutronics Popular Ltd. The reaction progress and product purity were checked by thin-layer chromatography using silica gel-G-coated glass plates (TLC plates) which were visualized by exposure to iodine vapors. IR spectra were recorded on Perkin-Elmer 882 model spectrometer using KBr pellets. Frequencies were recorded in wave number. ¹H NMR and ¹³C NMR spectra were obtained on Bruker Avance II (300 MHz) NMR spectrometer for solutions in CDCl₃/DMSO-d₆ using tetramethylsilane as internal reference. All chemical shifts are reported in parts per million (ppm) and coupling constant (J) values in Hertz. The mass spectra were recorded on Q-TOF Micromass (liquid chromatography-mass spectrometry) instrument.

Methods

First, synthesis of the substituted 4-Oxo-2-(phenylamino)-4H-chromone-3-carbaldehydes (9a-h) was carried out starting from substituted acetophenones (6a-h) through earlier reported method [19-22]. In the next step, obtained substituted 4-Oxo-2-(phenylamino)-4H-chromene-3-carbaldehydes (9a-h) were again dissolved in ethanol, and hydroxylamine was added to this solution. The mixture was refluxed for 15 min and further stirred at room temperature for 1 hr. On completion of the reaction (monitored by TLC using hexane-ethyl acetate gradient, 9:1 v/v), the required substituted 4-Oxo-2-(phenylamino)-4H-chromone-3-carbaldehyde oximes (10a-h) were obtained as solid precipitates and were purified through recrystallization using a mixture of hexane and chloroform (9:1 v/v). Further, these 4-Oxo-2-(phenylamino)-4H-chromone-3-carbaldehyde oximes (10a-h) were mixed with acetic anhydride and refluxed for 4 h with continuous stirring. The hot reaction mixture was then poured into crushed ice leading to the formation of precipitates of compounds 11a-h (TLC monitored, hexane: ethyl acetate [9:1 v/v]) which were then obtained by filtration [23] and further purified by recrystallization using glacial acetic acid. Finally, the obtained nitrile derivatives (11a-h) were dissolved in ice-cooled tetrahydrofuran (THF). Aluminum chloride (AlCl₃) was added to this solution. After some time, sodium azide was added to the solution. The overall reaction mixture was stirred for 10 h, followed by cooling in ice bath for 2–3 h. Later, 5 ml dilute HCl was added to it which led to the formation of crystals of final products (12a-h) which were then collected by filtration and were purified by recrystallization using hexane and chloroform (9:1 v/v, Scheme 1). The reaction conditions are summarized in Table 1 given below:

Molecular docking studies

A computational ligand-target docking approach was used to analyze structural complexes of the DNA gyrase (target) with these chromone-based heterocyclic ligands to understand the structural basis of the protein-ligand specificity [24,25]. All the newly synthesized compounds (12a-h) and a reference compound (ciprofloxacin) were docked [26] into

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\text{Scheme 1: Reactions conditions}
\]
THF: Tetrahydrofuran, RT: Room temperature

the active site of TyrRS (PDB: 1JJK) by means of the BioPredicta module of VLife MDS. Before interpretation and analysis of interactions, correct ligand pose assessment generally remains an important criterion for the optimal binding affinity prediction using scoring functions. Through this docking study, all the ligand poses were visually inspected. All the docked ligands were scored using the lower Dock Score function, and the pose of each binding mode that matched the assumed binding mode was considered valid and put to the separate set (valid poses). The best pose of each was identified for subsequent analysis.

Pharmacological activity

The obtained compounds, that is, substituted 2-(Phenylamino)-3-(1H-tetrazol-5-yl)-4H-chromen-4-one derivatives (12a-h) were evaluated for their in vitro antimicrobial activities against different strains of bacteria (S. aureus, Bacillus subtilis, Pseudomonas aeruginosa, and Escherichia coli) and fungi (Aspergillus niger and Candida albicans) as per the reported methods [13,27].

RESULTS AND DISCUSSION

The results of synthetic work have revealed that maximum yield of compounds (12a-h) was obtained in the cases where chromone nucleus was bearing electron-withdrawing groups at C-6 or C-7. All the synthesized compounds were evaluated by spectral analysis, that is, H-NMR, 13C-NMR, and Mass spectroscopy. The spectral data of the synthesized compounds 12a-h are given below:

2-(Phenylamino)-3-(1H-tetrazol-5-yl)-4H-chromen-4-one (12a)

Reaction of 4-Oxo-2-(phenylamino)-4H-chromone-3-carbonitrile (11a, 1.7 g) with ice-cooled THF (4 ml), AlCl₃ (0.5 g), and sodium azide (0.5 g) was carried out, and compound 12a was obtained as brownish yellow crystals “Yield 1.02 g (60%),” mp 183-194°C; C₁₂H₁₀N₂O₂ molecular weight 305 g, and solubility DMSO.

1H-NMR: (400 MHz, DMSO-d₆), δ ppm (J, Hz)
7.24-6.75 (5H, m, C₅H₃), 7.54-7.40 (3H, m, Ar-chromone); and 7.84-7.82 (1H, J= 8 Hz, d, C₇-chromone).  

13C-NMR: (400 MHz, DMSO-d₆), δ ppm
163.3 (C-2), 130.2 (C-3), 124.7 (C-4, C-6), 173.7 (C-4), 153.5 (C-8), 131.9 (C-5), 122.6 (C-6), 133.9 (C-7), 117.0 (C-8), 75.9 (5’ tetrazole), 135.5 (C-1’), 116.1 (C-2’-6’), 129.6 (C-3’-5’), and 118.9 (C-4’).

Mass: M’ m/z
323 (M⁺ + C₁₂H₁₀O₂), FCl) and 304 (M⁺ + C₁₂H₁₀O₂), FCl) (100).

6-Fluoro-2-(phenylamino)-3-(1H-tetrazol-5-yl)-4H-chromen-4-one (12b)

Reaction of 6-Fluoro-2-(phenylamino)-4H-chromone-3-carbonitrile (11b, 0.900 g) with ice-cooled THF (4 ml), AlCl₃ (0.5 g), and sodium azide (0.5 g) was carried out, and compound 12b was obtained as brownish yellow crystals “Yield 0.774 g (85%).” mp 229-240°C; C₁₂H₁₀N₂O₂ molecular weight 323 g, and solubility DMSO.

1H-NMR: (400 MHz, DMSO-d₆), δ ppm (J, Hz)
2.52 (1H, s, tetrazole); 3.65 (1H, s, NHC₅H₅); 7.43-7.15 (5H, m, C₅H₃); 7.84-7.54 (3H, m, Ar-chromone); and 7.84-7.82 (1H, J= 8 Hz, d, C₇-chromone).

13C-NMR: (400 MHz, DMSO-d₆), δ ppm
164.3 (C-2), 78.4 (C-3), 124.7 (C-4, C-6), 125.6 (C-4’),152.7 (C-8a); 115.1 (C-5), 171.1 (C-6), 122.0 (C-7), 118.3 (C-8), 79.1 (5’ tetrazole); 135.5 (C-1’), 116.2 (C-2’-6’), 129.5 (C-3’-5’), and 118.8 (C-4’).

Mass: M’ m/z
323 (M⁺ + C₁₂H₁₀O₂N₅F) and 284 (M⁺ + C₁₂H₁₀O₂N₅F).

6-Fluoro-7-chloro-2-(phenylamino)-3-(1H-tetrazol-5-yl)-4H-chromen-4-one (12c)

Reaction of 6-Fluoro-7-chloro-2-(phenylamino)-4H-chromone-3-carbonitrile (11c, 0.721 g) with ice-cooled THF (4 ml), AlCl₃ (0.5 g), and sodium azide (0.5 g) was carried out, and compound 12c was obtained as brownish yellow crystals “Yield 0.556 g (72%).” mp233-245°C; C₁₂H₁₀N₂O₂Cl molecular weight 357 g, and solubility DMSO.

1H-NMR: (400 MHz, DMSO-d₆), δ ppm (J, Hz)
2.53 (1H, s, tetrazole); 3.36 (1H, s, NHC₅H₅); 7.62-7.16 (5H, m, C₅H₃); 8.03-8.01 (1H, d, J= 8 Hz, C₇-chromone); and 8.27-8.25 (1H, J= 4 Hz, t, C₇-chromone).

13C-NMR: (400 MHz, DMSO-d₆), δ ppm
163.1 (C-2), 79.2 (C-3), 124.2 (C-4, C-6), 122.5 (C-4’), 138.1 (C-8); 128.7 (C-5), 124.6 (C-6), 129.4 (C-7), 120.2 (C-8), 78.6 (5’ tetrazole); 135.2 (C-1’), 116.0 (C-2’-6’), 129.1 (C-3’-5’), and 118.5 (C-4’).

Mass: M’ m/z
357 (M⁺ + C₁₂H₁₀O₂N₅Cl), 313 (M⁺ + C₁₂H₁₀O₂N₅Cl), 198 (M⁺ + C₁₀H₆O₃Cl), and 254 (M⁺ + C₁₀H₆O₃Cl).

6-Bromo-2-(phenylamino)-3-(1H-tetrazol-5-yl)-4H-chromen-4-one (12d)

Reaction of 6-Bromo-2-(phenylamino)-4H-chromone-3-carbonitrile (11d, 0.946 g) with ice-cooled THF (4 ml), AlCl₃ (0.5 g), and sodium azide (0.5 g) was carried out, and compound 12d was obtained as brownish yellow crystals “Yield 0.651 g (69%).” mp 208-220°C; C₁₂H₁₀N₂O₂Br molecular weight 384 g, and solubility DMSO.

1H-NMR: (400 MHz, DMSO-d₆), δ ppm (J, Hz)
2.30 (1H, s, tetrazole); 3.38 (1H, s, NHC₅H₅); 7.24-6.75 (5H, m, C₅H₃); 7.54-7.40 (3H, m, Ar-chromone); and 7.82-7.80 (1H, J= 4 Hz, d, C₇-chromone).

Table 1: The percentage age yield, reaction conditions are summarized in following table

| Serial number | Compound number | X | Y | Solvent | Reaction condition | Product (percentage yield) |
|---------------|----------------|---|---|---------|-------------------|---------------------------|
| 1             | 12a            | H | H | THF     | Stirring RT, 10 h | 60                        |
| 2             | 12b            | F | H | THF     | Stirring RT, 10 h | 85                        |
| 3             | 12c            | F | Cl| THF     | Stirring RT, 10 h | 72                        |
| 4             | 12d            | Br| H | THF     | Stirring RT, 10 h | 65                        |
| 5             | 12e            | Cl| H | THF     | Stirring RT, 10 h | 64                        |
| 6             | 12f            | H | Cl| THF     | Stirring RT, 10 h | 92                        |
| 7             | 12g            | NO₂| H | THF    | Stirring RT, 10 h | 62                        |
| 8             | 12h            | H | NO₂| THF    | Stirring RT, 10 h | 82                        |
Table 2: D score of synthesized compounds (12a-h)

| Serial number | Compound number | X  | Y  | D-scores   |
|---------------|----------------|----|----|------------|
| 1             | 12a            | H  | H  | −69.674574 |
| 2             | 12b            | F  | H  | −72.68212  |
| 3             | 12c            | F  | Cl | −68.884006 |
| 4             | 12d            | Br | H  | −64.574891 |
| 5             | 12e            | Cl | H  | −70.17632  |
| 6             | 12f            | H  | NO2| −67.160354 |
| 7             | 12g            | NO2| H  | −67.72237  |
| 8             | 12h            | H  | NO2| −71.268687 |

Molecular docking analysis

The results of the docking simulation study represented as D-Score are shown in Table 2. The recorded binding interactions revealed that all the newly synthesized compounds interacted well with binding site of enzyme. Further, it was also observed that the number of the substituent groups and their respective positions on the aryl moiety affects the orientation and binding pattern of the compounds in the binding pocket of the receptor. Based on the experimental results of in vitro and in vivo investigations, the detailed interaction analysis was performed on selected compound, that is, 12a, 12b, and 12c. Most stable conformers of 12a, 12b, and 12c (namely, LP-2, LP-4, and LP-5) afforded −69.674 to −72.682 and −64.574 D score values, respectively, as compared to the reference compound, ciprofloxacin, that exhibited D score value of 43.93 against TyrRS (PDB: 1IK). Table 3 presents the potential interactions such as hydrogen bonding, aromatic interactions, Van der Waal, and hydrophobic ones between the protein and the synthesized compounds 12a, 12b, and ciprofloxacin, respectively.

In compound 12a, (2-Phenylaminophenyl)-3-(1H-tetrazol-5-yl)-4H-chromen-4-one (12c) which is an unsubstituted derivative, chromen-4-one group was found involved in hydrogen bonding with GLN 196A having force distance of 1.77Å, along with the hydrogen bonding between its fluoro group and GLN 193A amino acid at a force distance of 1.77Å, as shown in Fig. 1(B). The bonding between its fluoro group and GLN 193A amino acid at a force distance of 1.77Å, as shown in Fig. 1(B). The bonding between the protein and the compound was found involved in hydrogen bonding with GLN 196A having force distance of 1.77Å, along with the hydrogen bonding between its fluoro group and GLN 193A amino acid at a force distance of 1.77Å, as shown in Fig. 1(B).
observed in its binding with residue HIS 50Å, at the bond distance of 3.95Å. Further, the highest D score of the 12b is in agreement with its \textit{in vitro} antibacterial and antifungal activity results. Similarly, 12c, the disubstituted Halo derivative of 4H-chromen-4-one nucleus, was found to interact with residue Arg 88Å through hydrogen bonding involving N-atom of the tetrazole ring (force distance of 1.56Å) and with Gln 196Å residue through C=O group of chromen-4-one nucleus. Aromatic interactions have also been observed between the residues HIS 50Å and 2-(phenylamino) of chromen-4-one nucleus, having a bond distance of 3.91Å.

**Pharmacological activity**

Results of their antibacterial and antifungal activities [28] of synthesized compounds (12a-h) are shown in Table 4 and 5, respectively. Most of the compounds exhibited potent antibacterial as well as antifungal activity against all the microbes tested as compared to the standard drugs used (i.e., ciprofloxacin and fluconazole for bacterial and fungal strains, respectively).

The results revealed (Table 4) that compounds 12a, 12b, and 12e presented themselves as more effective against both Gram-positive and Gram-negative bacteria. However, compounds 12d, 12f, 12g, and 12h emerged as more effective against Gram-positive bacteria. Compound 12c was found to be more effective only against Gram-negative bacteria. It has been concluded from Kirby–Bauer disc diffusion method that the most active compounds, that is, 12a, 12b, and 12e are the broad-spectrum candidates, and other lesser active drugs, that is, 12c, 12d, 12f, 12 g, and 12h are narrow-spectrum contenders.

The results showed (Table 5) that compounds 12b and 12f were effective against both \textit{C. albicans} and \textit{A. niger} by Kirby–Bauer disc diffusion method. Compounds 12a, 12d, 12g, and 12h are narrow-spectrum analogs.

**CONCLUSIONS**

Novel chromone-based tetrazole derivatives (12a-h) were designed, synthesized, docked, and evaluated for their \textit{in vitro} antibacterial and antifungal activities against various bacterial (Gram-positive and Gram-negative) and fungal strains, respectively.
The docking studies thus revealed that derivatives with electron-withdrawing groups play a critical role in drug-receptor interactions as exemplified by the fluoro derivative with good D score. (6-Fluoro-2-(phenylamino)-3-(1H-tetrazol-5-yl)-4H-chromen-4-one), 12b (displayed good number of interaction at lesser bond distance with receptor as compared to the 12a), and 12c molecule, respectively, indicating 12b molecule binds more strongly with the receptor with lesser distance as compared to another one. The docking studies predicted almost the same behavior as was observed in *in vitro* and *in vivo* biological evaluations for the different substituted groups among the derivatives. Overall, a good correlation was observed between the docking study and biological evaluation of active compound.

The obtained compounds substituted 2-{Phenylamino}-3-(1H-tetrazol-5-yl)-4H-chromen-4-one derivatives (12a-h) were found to be potent against different bacterial strains (S. aureus, B. subtilis, P. aeruginosa, and E. coli) and fungal strains (A. niger and C. albicans) when compared with standard drug ciprofloxacin for bacterial strains and fluconazole for fungal strains. The compounds 12a, 12b, and 12e were active against *S. aureus*, *B. subtilis*, *P. aeruginosa*, and *E. coli*, respectively, at minimum inhibitory concentration (MIC) 30 µg/ml when compared with standard drug ciprofloxacin and rest compounds 12c active against *S. aureus*, *B. subtilis*, and *P. aeruginosa* at MIC 30 µg/ml; 12f active against *S. aureus* and *B. subtilis* at MIC 30 µg/ml; 12g active against *S. aureus* and *B. subtilis* at MIC 30 µg/ml; 12h and 12d active against *S. aureus*, *B. subtilis*, and *P. aeruginosa* at MIC 50 µg/ml, respectively, when compared with standard drug ciprofloxacin. Similarly, compound 12b was active against *A. niger* and *C. albicans*, respectively, at MIC 30 µg/ml when compared with standard drug fluconazole. The rest compounds 12a, 12d, 12e, and 12h were active against *C. albicans*, respectively, at MIC 30 µg/ml and 12c, 12f, and 12g against *C. albicans*, respectively, at MIC 50 µg/ml when compared with standard fluconazole. These molecules can potentially serve as useful "lead" compounds for further development.

**AUTHOR'S CONTRIBUTION**

Dr. Lakhwinder Sing and Anuja Chopra make contributions to the conception, design, and implementation of the research to the analysis of the results and to the writing of the manuscript. Dr. Lakhwinder Singh helped to supervise the work and gave final approval of the written manuscript. Dr. NeelimalaDhingra and RichaDhingra work on the molecular docking studies.

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**CONFLICTS OF INTERESTS**

All authors have none to declare.

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