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Research Article

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

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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-(1*H*-tetrazol-5-yl)-4*H*-chromen-4-one derivatives (12a-h).

Methods: In the present work, 3-Formylchromones were transformed into pharmacologically active substituted 2-(Phenylamino)-3-(1*H*-tetrazol-5-yl)-4*H*-chromen-4-one derivatives (12a-h) through a multistep reaction. Initially, synthesis of the substituted 4-Oxo-2-(phenylamino)-4*H*-chromone-3-carbaldehydes (9a-h) was carried out using substituted acetophenones (6a-h) as starting material and by employing an earlier reported method (1,3-dipolar cycloaddition reaction). Then, these synthesized compounds were converted into respective oximes (10a-h).The obtained oximes (10a-h) were further converted into nitriles (11a-h) which were finally subjected to concerted cycloaddition through stepwise addition of neutral or anionic azide species to furnish final substituted 2-(Phenylamino)-3-(1*H*-tetrazol-5-yl)-4*H*-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 stains) and antifungal activities (against *Aspergillus niger* and *Candida albicans* fungal strains) using Zone of Inhibition method.

Results: The formation of substituted 2-(Phenylamino)-3-(1*H*-tetrazol-5-yl)-4*H*-chromen-4-one derivatives (12a-h) was confirmed through their spectral analysis, that is, ^{1}H-NMR, ^{13}C-NMR, and Mass spectroscopy. During docking study, the recorded molecular binding interactions revealed that all 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 stains) 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*.

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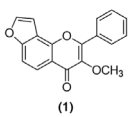
INTRODUCTION

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-aryl-4-pyrazolyl) chromones (3,4) show antifungal activity against three phytopathogenic fungi [11], namely, *Helminthosporium* species, *Fusarium oxysporum,* and *Alternaria alternata*.



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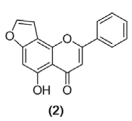

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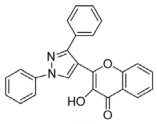

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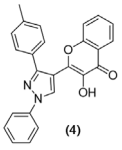

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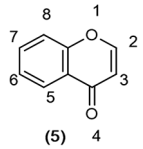
Chromones are very prone to chemical transformations such as photocycloaddition, photodimerization, photoisomerization, photorearrangement, photo-oxidation reduction, and photocyclization which involve both n→π* and π→π* transitions [12,13]. Further, chromones and bischromones (especially their 3-alkoxy 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].



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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.

Despite tremendous research which has been done on chromones and 3-formylchromones, still, 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-(1*H*-tetrazol-5-yl)-4*H*-chromen-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.

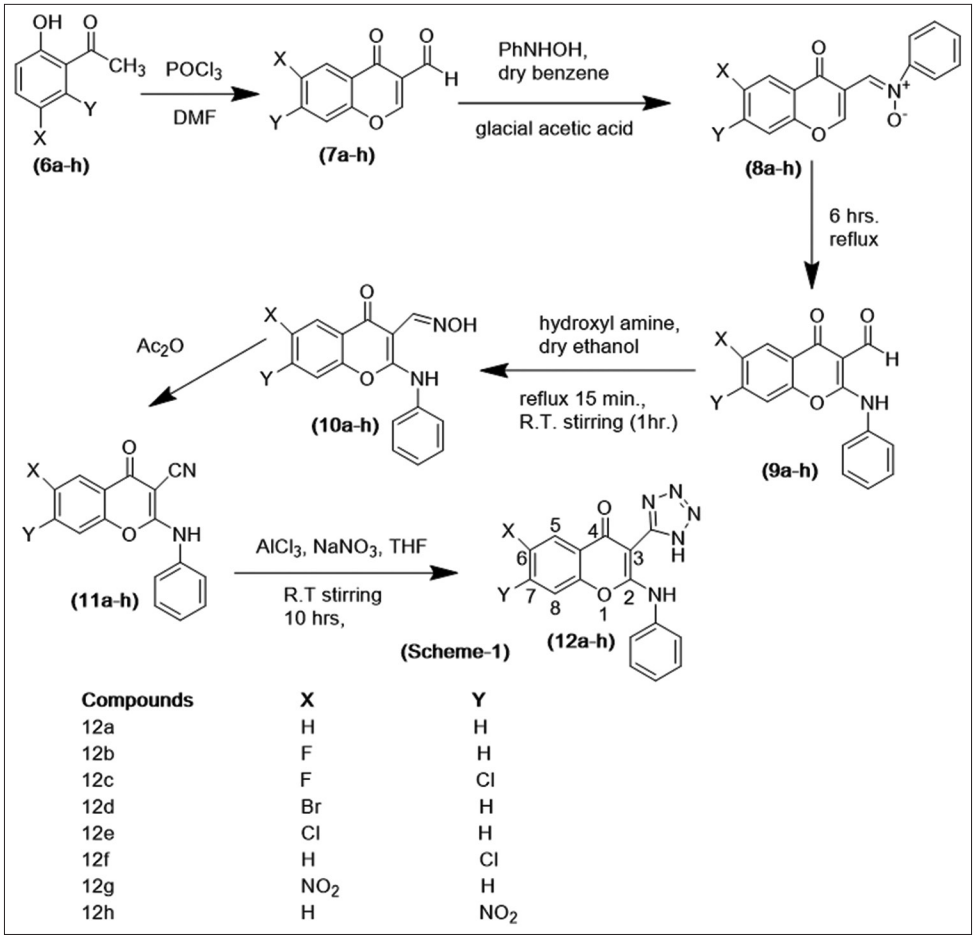
The melting points of all the synthesized compounds were measured on a liquid paraffin bath in open glass capillary tubes using Digital Melting point apparatus by Nutronics Popular Ltd. The reaction progress and product purity were checked by thin-layer chromatography using silica gel-G-coated glass 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. ^{1}H NMR and ^{13}C NMR spectra were obtained on Bruker Avance II (300 MHz) NMR spectrometer for solutions in $CDCl_3$/DMSO-d_6 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)-4*H*-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)-4*H*-chromone-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)-4*H*-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)-4*H*-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 over glacial acetic acid. Finally, the obtained nitrile derivatives (11a-h) were dissolved in ice-cooled tetrahydrofuran (THF). Aluminum chloride ($AlCl_3$) 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 (ciprofloaxin) were docked [26] into



Compounds	X	Y
12a	H	H
12b	F	H
12c	F	Cl
12d	Br	H
12e	Cl	H
12f	H	Cl
12g	NO_2	H
12h	H	NO_2

Scheme 1: Reactions conditions

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	NO_2	H	THF	Stirring RT, 10 h	62
8	12h	H	NO_2	THF	Stirring RT, 10 h	82

THF: Tetrahydrofuran, RT: Room temperature

the active site of TyrRS (PDB: 1JIK) 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 ligand 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-(1*H*-tetrazol-5-yl)-4*H*-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, ^{1}H-NMR, ^{13}C-NMR, and Mass spectroscopy. The spectral data of the synthesized compounds 12a-h are given below:

General procedure for the synthesis of 2-(phenylamino)-3-(1*H*-tetrazol-5-yl)-4*H*-chromen-4-one (12a-h)

The obtained compounds (11a-h) were dissolved in ice-cooled THF. $AlCl_3$ 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).

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

Reaction of 4-Oxo-2-(phenylamino)-4H-chromone-3-carbonitrile (11a, 1.7 g) with ice-cooled THF (4 ml), $AlCl_3$ (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, $C_{16}H_{13}N_5O_2$, molecular weight 305 g, and solubility DMSO.

^{1}H-NMR: (400 MHz, DMSO-d_6), δ ppm (*J, Hz*): 2.45 (1H, s, tetrazole); 3.73 (1H, s, NHC_6H_5); 7.21-6.76 (5H, m, C_6H_5); 7.61-7.30 (3Hs, m, Ar-chromone); 7.92-7.82 (IH, dd, *J*=8 Hz, C_5-chromone).

^{13}C-NMR: (400 MHz, DMSO-d_6),δ, ppm

163.3 (C-2), 79.2 (C-3), 124.7 (C-4, C=O), 173.7 (C-4_a), 153.5 (C-8_a), 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

305 (M+ $C_{16}H_{11}N_5O_2$); 238 (M+ $C_{15}H_{11}NO_2$); 210 (M+ $C_{13}H_{11}ON$); 128 (M+ C_8H_6O); and 94 (M+ C_6H_7N).

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

Reaction of 6-Flouro-2-(phenylamino)-4H-chromone-3-carbonitrile (11b, 0.900 g) with ice-cooled THF (4 ml), $AlCl_3$ (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, $C_{16}H_{12}O_2N_5F$, molecular weight 323 g, and solubility DMSO.

^{1}H-NMR: (400 MHz, DMSO-d_6),δ, ppm (J, Hz)

2.52 (1H, s, tetrazole); 3.65 (1H, s, NHC_6H_5); 7.43-7.15 (5H, m, C_6H_5); 7.84-7.54 (3H, m Ar-chromone); and 7.84-7.82 (1H, *J*=8 Hz, d, C_5-chromone).

^{13}C-NMR: (400 MHz, DMSO-d_6),δ, ppm

164.3 (C-2); 78.4 (C-3); 124.7 (C-4, C=O); 125.6 (C-4_a);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+ $C_{16}H_{10}O_2N_5F$) and 304 (M+ $C_{16}H_{10}O_2N_5$) (100).

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

Reaction of 6-Flouro-7-chloro-2-(phenylamino)-4*H*-chromone-3-carbonitrile (11c, 0.721 g) with ice-cooled THF (4 ml), $AlCl_3$ (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, $C_{16}H_{11}O_2N_5FCl$, molecular weight 357 g, and solubility DMSO.

^{1}H-NMR: (400 MHz, DMSO-d_6),δ, ppm (J, Hz)

2.53 (1H, s, tetrazole); 3.36 (1H, s, NHC_6H_5); 7.62-7.16 (5H, m, C_6H_5); 8.03-8.01 (1H, d, *J*=8 Hz, C_8-chromone); and 8.27-8.25 (IH, *J*=4 Hz, t, C_5-chromone).

^{13}C-NMR: (400 MHz, DMSO-d_6),δ, ppm

163.1 (C-2); 79.2 (C-3); 124.2 (C-4, C=O); 122.5 (C-4_a); 138.1 (C-8_a); 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+ $C_{16}H_9O_2N_5FCl$); 313 (M+ $C_{15}H_{io}N_5OCl$); 198 (M+ $C_{10}H_5O_2FCl$); and 254 (M+ $C_9H_5ON_5FCl$).

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

Reaction of 6-Bromo-2-(phenylamino)-4*H*-chromone-3-carbonitrile (11d, 0.946 g) with ice-cooled THF (4 ml), $AlCl_3$ (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, $C_{16}H_{12}O_2N_5Br$, molecular weight 384 g, and solubility DMSO. ^{1}H-NMR: (400 MHz, DMSO-d_6),δ ppm (*J, Hz*): 2.40 (1H, s, tetrazole); 3.38 (1H, s, NHC_6H_5); 7.24-6.75 (5H, m, C_6H_5); 7.54-7.40 (3H, m, Ar-chromone); and 7.82-7.80 (1H, *J*=4 Hz, d, C_5-chromone).

^{13}C-NMR: (400 MHz, DMSO-d_6),δ, ppm

163.1 (C-2); 77.4 (C-3); 124.1 (C-4, C=O); 126.1 (C-4_a); 169.1 (C-8_a); 133.9 (C-5); 117.0 (C-6); 135.5 (C-7); 118.9 (C-8); 76.7 (5' tetrazole); 135.2 (C-1"); 116.2 (C-2",6"); 129.2 (C-3",5"); and 118.4 (C-4").

Mass

Found, *m/z*:384 (M^+).(($C_{16}H_{10}O_2N_5Br$). Calculated, *m/z*:383.9.

6-Chloro-2-(phenylamino)-3-(1*H*-tetrazol-5-yl)-4*H*-chromen-4-one (12e)

Reaction of 6-Chloro-2-(phenylamino)-4*H*-chromone-3-carbonitrile (11e, 0.971 g) with ice-cooled THF (4 ml), $AlCl_3$ (0.5 g), and sodium azide (0.5 g) was carried out, and compound 12e was obtained as brownish yellow crystals "Yield 0.623 g (64)," mp 187-200°C, $C_{16}H_{12}N_5O_2Cl$, molecular weight 339 g, and solubility DMSO.

^{1}H-NMR: (400 MHz, DMSO-d_6),δ, ppm (J, Hz)

2.43 (1H, s, tetrazole); 4.01 (1H, s, NHC_6H_5); 7.63-7.08 (5H, m, C_6H_5); 8.02-7.64 (3H, m, Ar-chromone); and 8.25-8.21 (IH, *J*=8 Hz, d, C_5-chromone).

^{13}C-NMR: (400 MHz, DMSO-d_6),δ, ppm

163.0 (C-2); 78.6 (C-3); 124.3 (C-4, C=O); 125.7 (C-4_a); 153.5 (C-8_a); 129.6 (C-5); 129.2 (C-6); 135.3 (C-7); 118.4 (C-8); 78.0 (5' tetrazole); 135.2 (C-1"); 116.4 (C-2",6"); 129.4 (C-3",5"); and 118.8 (C-4").

Mass: M^+m/z

339 (M+ $C_{16}H_{10}N_5O_2Cl$); 311 (M+ $C_{15}H_{10}N_5OCl$); 237 (M+ $C_{15}H_{11}NO_2$); and 179 (M + $C_9H_5ClO_2$).

7-Chloro-2-(phenylamino)-3-(1*H*-tetrazol-5-yl)-4*H*-chromen-4-one (12f)

Reaction of 7-Chloro-2-(phenylamino)-4*H*-chromone-3-carbonitrile (11f, 0.643 g) with ice-cooled THF (4 ml), $AlCl_3$ (0.5 g), and sodium azide (0.5 g) was carried out, and compound 12f was obtained as brownish yellow crystals "Yield 0.589 g (92)", mp192-204°C, $C_{16}H_{12}N_5O_2Cl$, molecular weight 339 g, and solubility DMSO.

^{1}H-NMR: (400 MHz, DMSO-d_6),δ, ppm (J, Hz)

1.89 (1H, s, tetrazole); 3.37 (1H, s, NHC_6H_5); 7.63-7.15 (5H, m, C_6H_5); 7.63-7.60 (3H, m, Ar-chromone); and 8.01-7.90 (IH, *J*=9.6 Hz, d, C_5-chromone).

^{13}C-NMR: (400 MHz, DMSO-d_6),δ, ppm

163.1 (C-2); 79.2 (C-3); 124.2 (C-4, C=O); 122.5 (C-4_a); 138.1 (C-8_a); 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

Found, *m/z*:339 (M^+).(($C_{16}H_{10}N_5O_2Cl$). Calculated, *m/z*:339.5.

6-Nitro-2-(phenylamino) 3-(1*H*-tetrazol-5-yl)-4*H*-chromen-4-one (12g)

Reaction of 6-Nitro-2-(phenylamino)-4*H*-chromone-3-carbonitrile (11g, 0.920 g) with ice-cooled THF (4 ml), $AlCl_3$ (0.5 g), and sodium azide (0.5 g) was carried out, and compound 12g was obtained as brownish yellow crystals "Yield 0.572 g (62)," mp179°C–192°C, $C_{16}H_{12}N_6O_4$, molecular weight 350 g, and solubility DMSO.

^{1}H-NMR: (400 MHz, DMSO-d_6),δ, ppm (J, Hz)

2.51 (1H, s, tetrazole); 3.34 (1H, s, NHC_6H_5); 7.53-7.17 (5H, m, C_6H_5); 8.2-8.0 (3H, M, Ar-chromone); and 8.32-8.20 (IH, *J*=2.6 Hz, d, C_5-chromone).

^{13}C-NMR: (400 MHz, DMSO-d_6),δ, ppm

163.0 (C-2); 78.9 (C-3); 124.3 (C-4, C=O); 124.7 (C-4_a); 125.7 (C-8_a); 131.9 (C-5); 152.3 (C-6); 127.8 (C-7); 118.9 (C-8); 78.3 (5' tetrazole); 135.3 (C-1"); 116.3 (C-2",6"); 129.3 (C-3",5"); and 117.0 (C-4").

Mass: M^+m/z

350 (M + $C_{16}H_{10}N_6O_4$); 322 (M + $C_{15}H_{10}N_6O_3$); 280 (M + $C_{15}H_{10}N_2O_4$); 254 (M + $C_9H_6N_6O_3$); 230 (M + $C_{10}H_7N_5O_2$); and 206 (M + $C_9H_6N_2O_4$).

7-Nitro-2-(phenylamino)-3-(1*H*-tetrazol-5-yl)-4*H*-chromen-4-one (12h)

Reaction of 7-Nitro-2-(phenylamino)-4*H*-chromone-3-carbonitrile (11 h, 0.882 g) with ice-cooled THF (4 ml), $AlCl_3$ (0.5 g), and sodium azide (0.5 g) was carried out, and compound 12 h was obtained as brownish yellow crystals "Yield 0.723 g (82)", mp 185-198°C, $C_{16}H_{12}N_6O_4$, molecular weight 350 g, and solubility DMSO.

^{1}H-NMR: (400 MHz, DMSO-d_6),δ, ppm (J, Hz)

2.6 (1H, s, tetrazole); 3.36 (1H, s, NHC_6H_5); 7.52-7.18 (5H, m, C_6H_5); 8.02-7.80 (3H, m, Ar-chromone); and 8.25-8.21 (1H, *J*=2.6 Hz, d, C_5-chromone).

^{13}C-NMR: (400 MHz, DMSO-d_6),δ, ppm

163.2 (C-2); 79.0 (C-3); 124.6 (C-4, C=O); 132.8 (C-4_a); 153.5 (C-8_a); 134.1 (C-5); 116.1 (C-6); 152.3 (C-7); 114.5 (C-8); 78.3 (5' tetrazole); 135.3 (C-1"); 116.4 (C-2",6"); 129.7 (C-3",5"); and 118.7 (C-4").

Mass: M^+m/z

350 (M + $C_{16}H_{10}N_6O_4$) and 254 (M + $C_9H_6N_6O_3$).

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, −72.682, and −64.547 D score values, respectively, as compared to the reference compound, ciprofloxacin, that exhibited D score value of 43.93 against TyrRS (PDB: 1JIK). 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, 12c, and ciprofloxacin, respectively.

In compound 12a, (2-(Phenylamino)-3-(1*H*-tetrazol-5-yl)-4*H*-chromen-4-one) which is an unsubstituted derivative, chromen-4-one group was found to interact through hydrogen bonding with GLN 196A amino acid and through aromatic interactions with HIS 50A amino acid, assuring a bond distance of 1.74 and 3.95Å, respectively, whereas, in 6-Fluoro-2-(phenylamino)-3-(1*H*-tetrazol-5-yl)-4*H*-chromen-4-one (12b) that has a fluoro-substitution, C=O group of 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. Aromatic interactions were also

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	−72.682812
3	12c	F	Cl	−68.884069
4	12d	Br	H	−64.547849
5	12e	Cl	H	−70.176320
6	12f	H	Cl	−67.163534
7	12g	NO_2	H	−67.722374
8	12h	H	NO_2	−71.268687
Standard drug	Ciprofloxacin			−43.934875

Table 3: Indicates the potential interactions

Serial number	Compound number	X	Y	Ligand pose	D-score	Residues	Hydrogen	Hydro phobic	Aromatic
1	12a	H	H	LP2	−69.674574	GLN196Å, HIS 50Å	+	-	+
2	12b	F	H	LP4	−72.682812	GLN196Å, GLY 193Å, HIS 50Å	++	-	+
3	12c	F	Cl	LP3	−68.884069	ARG 88Å, GLY 196Å, HIS 50Å	++	-	+
4	Ciprofloxacin			LP1	−43.934875	ALA39Å, ASP 40Å, HIS 50Å	-	+++	_

Table 4: Antibacterial results of all compounds (12a-h) against *Staphylococcus aureus, Escherichia coli, Bacillus subtilis*, and *Pseudomonas aeruginosa* by Kirby–Bauer disc diffusion method

Synthesized compounds	Antibacterial activity			
	Minimum inhibitory concentration (µg/mL)			
	Staphylococcus aureus	*Escherichia coli*	*Bacillus subtilis*	*Pseudomonas aeruginosa*
12a	30	30	30	30
12b	30	30	30	30
12c	30	30	50	30
12d	50	100	50	50
12e	30	30	30	30
12f	30	50	30	100
12g	30	100	30	30
12h	50	100	50	50
Ciprofloxacin (standard)	30	30	30	30

Table 5: Antifungal results of compounds 12a-h against *Candida albicans* and *Aspergillus niger* by Kirby–Bauer disc diffusion method

Compounds	Antifungal activity	
	Minimum inhibitory concentration (µg/mL)	
	Candida albicans	*Aspergillus niger*
12a	30	50
12b	30	30
12c	50	30
12d	30	100
12e	30	100
12f	50	50
12g	50	30
12h	30	100
Fluconazole (standard)	30	30

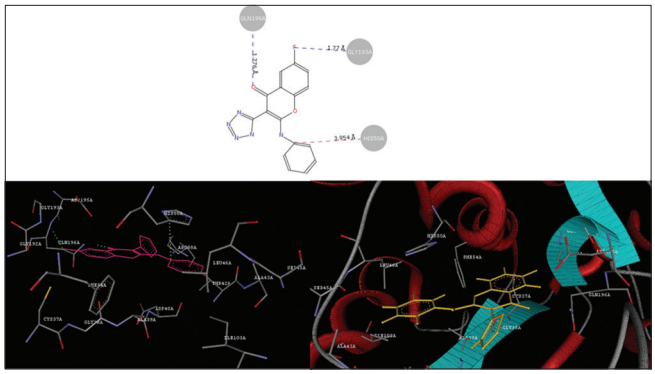


Fig. 1: Two-dimensional and three-dimensional representation of the compound 12b docked in pocket site of DNA gyrase, indicating different interactions involved with amino residue

observed in its binding with residue HIS 50A, at the bond distance of 3.95Å. Further, the highest D score of the 12b is in agreement with its *in vitro* antibacterial and antifungal activity results. Similarly, 12c, the disubstituted Halo derivative of 4*H*-chromen-4-one nucleus, was found to interact with residue Arg 88A through hydrogen bonding involving N-atom of the tetrazole ring (force distance of 1.56Å) and with Gln 196A residue through C=O group of chromen-4-one nucleus. Aromatic interactions have also been observed between the residues HIS 50A 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 Tables 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 analogs.

The results showed (Table 5) that compounds 12b and 12f were effective against both *C. albicans* and *A. niger.* Compounds 12a, 12d, 12e, and 12h were more effective against *C. albicans* only. Similarly, compounds 12c and 12g exhibited more potent activity against *A. niger* only. It has been concluded from Kirby–Bauer disc diffusion method that the most active drug, that is, 12b is broad spectrum in nature and the lesser active derivatives, that is, 12a, 12c, 12d, 12e, 12f, 12g, and 12h are narrow-spectrum contenders.

CONCLUSIONS

Novel chromone-based tetrazole derivatives (12a-h) were designed, synthesized, docked, and evaluated for their *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 flouro derivative with good D score. (6-Fluoro-2-(phenylamino)-3-(1*H*-tetrazol-5-yl)-4*H*-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-(1*H*-tetrazol-5-yl)-4*H*-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; and 12d and 12h 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. NeelimaDhingra 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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