Design, Synthesis and Biological Screening of Novel 1,5-Diphenyl-3-(4-(trifluoromethyl)phenyl)-2-pyrazoline Derivatives

Fatih Tok¹ and Bedia Koçyiğit-Kaymakçıoğlu¹,*

¹ Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Marmara University, Istanbul-34668, Turkey
* Corresponding author: E-mail: bkaymakcioglu@marmara.edu.tr
Tel: +90-216-4142963/1023
Received: 04-01-2020

Abstract
1-Phenyl-5-substituted-3-(4-(trifluoromethyl)phenyl)-4,5-dihydro-1H-pyrazole derivatives were synthesized from chalcone derivatives. The structures of compounds were characterized by IR, 1H NMR spectroscopic methods and elemental analysis. All compounds were evaluated for their in vitro antioxidant activity using DPPH and ABTS methods, anti-inflammatory activity using lipoygenase inhibitory method and antidiabetic activity using the α-glucosidase inhibitory method. Especially, pyrazoline derivatives exhibited stronger anti-inflammatory activity than the reference drug indomethacin (IC₅₀: 50.45 µM) and their IC₅₀ values were in the range of 0.68 and 4.45 µM. In addition, the ADME properties of all chalcone and pyrazoline derivatives were calculated by Lipinski’s and Veber’s rules.

Keywords: 2-Pyrazoline; lipoxygenase enzyme; α-glucosidase; ABTS and DPPH

1. Introduction
Pyrazoline scaffolds bearing five-membered heterocyclic ring systems are used frequently in organic synthesis and medicinal chemistry because of their broad spectrum of activities.¹ Pyrazoline rings have important pharmacological and biological properties such as antioxidant, anti-inflammatory, analgesic, antimicrobial, antimalarial, antihypertensive, anticonvulsant, antidepressant, and anticancer.²–⁶ We studied the synthesis and antiproliferative activity of some pyrazoline compounds in our previous study.⁷ Pyrazolines exhibited these different activities by interacting with some receptors or enzymes. For example, ElBordiny et al. demonstrated in their study that pyrazolines are superior lipoxygenase enzyme inhibitors compared to the reference drug.⁸ Furthermore, Chaudhry et al. and Sathish et al. reported pyrazoline derivatives as alpha-glucosidase inhibitors.⁹,¹⁰ Additionally, many studies proved different activities of pyrazoline derivatives as receptor tyrosine kinase, topoisomerase 1, carbonic anhydrase and cholinesterase inhibitors. It was found that nitrogen atoms of the pyrazoline ring and at least one substitution with aryl moiety are essential for anti-inflammatory activity (Gawad et al., 2012).¹¹–¹⁵

![Figure 1. Chemical structure of designed compounds and celecoxib](image-url)
Several drug molecules carrying pyrazole core with different activities are currently available in the market, for example, antipyrine (analgesic), celecoxib (anti-inflammatory), pyrazofurin (antibiotic). It is known that fluorine substitution increases biological activity and metabolic-chemical stability (compared to C–H bond) in drug research. Furthermore, fluorine alters physicochemical properties and enhances binding affinity to target protein easily. We designed new molecules which are carrying pyrazoline core and trifluoromethyl substitution on the aromatic ring (Figure 1).

In this study, we synthesized chalcone derivatives from 4'-(trifluoromethyl)acetophenone in the first step. Then we synthesized new 2-pyrazoline derivatives from chalcones. We aimed to show different biological activities of pyrazoline derivatives. Therefore all synthesized compounds were screened for their antioxidant activity using DPPH and ABTS methods, anti-inflammatory activity using lipoxigenase inhibitory method, and antidiabetic activity using α-glucosidase inhibitory methods.

2. Experimental

2.1. Synthesis

Chemicals and solvents were obtained from Sigma Aldrich (St. Louis, MO, USA) or Merck (Darmstadt, Germany). The progress of the reaction was monitored via thin layer chromatography (TLC), performed on commercially available silica gel (Kieselgel 60, F254) coated aluminum sheets (Merck) by using developing systems: petroleum ether/acetone (60:40 v/v) as a solvent system. The visualization on TLC was done under ultraviolet (UV) light (λ = 254 nm). Melting points were determined by Schmelzpunktbestimmer SMP II apparatus. Infrared spectra were recorded on a Shimadzu FTIR 8400S Spectrometer and data was expressed in wave-number ν (cm⁻¹). Proton nuclear magnetic resonance (NMR) (400 MHz) spectra were recorded with a Bruker ACP 200 spectrometer (Bruker Corp., Billerica, MA, USA). Deuterated dimethylsulfoxide (DMSO-d₆) was used as the solvent and tetramethylsilane (TMS) as the internal standard. The chemical shift values (δ) were expressed in ppm. Elemental analyses (C, H and N) was performed on a CHNS-Thermo Scientific Flash 2000 (Waltham, MA, USA).

2.1.1. General Procedure for the Synthesis of Chalcone

1 mmol of 4'- (trifluoromethyl)acetophenone and equimolar quantities of substituted aromatic aldehydes were dissolved in methanol, then NaOH (50% water solution) was added to the reaction mixture. It was stirred at room temperature for 16 h and then poured into ice-cold water. The precipitated product was washed with water, filtered and recrystallized from methanol.

2.1.2. General Procedure for the Synthesis of Pyrazoline

10 mmol of chalcone derivatives, 10 mmol of phenylhydrazine hydrochloride and 10 mL of glacial acetic acid were put in a reaction flask. The content was refluxed and stirred for 12 h. Then, it was neutralized with dilute ammonia solution. The precipitate was washed with water, filtered and recrystallized from ethanol.

3-(2,6-Dimethylphenyl)-1-(4-(trifluoromethyl)phenyl)prop-2-en-1-one (1a)

White powder, yield 77%, mp 74.3–74.5 °C. IR (KBr) ν 3064, 2978, 2912, 1696, 1606, 1591, 1573, 1510, 1408, 1315, 1213, 1161, 1064, 835 cm⁻¹. ¹H NMR (400 MHz, DMSO-d₆) δ 2.38 (s, 6H, 2CH₃), 7.12–7.87 (m, 5H, Ar-H and CH=CH), 7.92 (d, J = 8.4 Hz, 2H, Ar-H) 8.27 (d, J = 8.4 Hz, 2H, Ar-H). Anal. Calcd for C₁₈H₁₃F₃O: C, 71.04; H, 4.97. Found: C, 70.82; H, 4.86%.

3-(2,6-Dichlorophenyl)-1-(4-(trifluoromethyl)phenyl)prop-2-en-1-one (1b)

Yellow powder, yield 65%, mp 62.3–62.7 °C. IR (KBr) ν 3072, 1696, 1606, 1573, 1508, 1467, 1408, 1305, 1213, 1161, 1064, 835 cm⁻¹. ¹H NMR (400 MHz, DM-SO-d₆) δ 7.41–8.24 (m, 9H, Ar-H and CH=CH). Anal. Calcd for C₁₈H₁₃Cl₂F₃O: C, 55.68; H, 2.63. Found: C, 55.48; H, 2.78.

3-o-Tolyl-1-(4-(trifluoromethyl)phenyl)prop-2-en-1-one (1c)

White powder, yield 75%, mp 89.0–89.8 °C. IR (KBr) ν 3063, 2978, 2987, 1683, 1595, 1510, 1462, 1410, 1317, 1211, 1161, 1064, 833 cm⁻¹. ¹H NMR (400 MHz, DM-SO-d₆) δ 2.42 (s, 3H, CH), 7.26–8.32 (m, 10H, Ar-H and CH=CH). Anal. Calcd for C₁₈H₁₃F₃O: C, 75.68; H, 2.63. Found: C, 75.56; H, 2.78.

3-(4-Isopropylphenyl)-1-(4-(trifluoromethyl)phenyl)prop-2-en-1-one (1d)

White powder, yield 81%, mp 84.7–85.3 °C. IR (KBr) ν 3063, 2960, 2928, 1660, 1600, 1579, 1510, 1408, 1319, 1215, 1165, 1066, 821 cm⁻¹. ¹H NMR (600 MHz, DMSO-d₆) δ 1.23 (d, J = 7.2 Hz, 6H, CH₃), 2.94 (m, 1H, CH), 7.35–8.33 (m, 10H, Ar-H and CH=CH). Anal. Calcd for C₁₈H₁₃F₃O: C, 71.69; H, 5.38. Found: C, 71.44; H, 5.46.

3-Phenyl-1-(4-(trifluoromethyl)phenyl)prop-2-en-1-one (1e)

White powder, yield 75%, mp 113.7–114.3 °C. IR (KBr) ν 3063, 1664, 1600, 1573, 1510, 1410, 1317, 1217, 1159, 1064, 839 cm⁻¹. ¹H NMR (600 MHz, DMSO-d₆) δ 7.44–8.35 (m, 11H, Ar-H and CH=CH). Anal. Calcd for
Toket al.: Design, Synthesis and Biological Screening of Novel...
1-Phenyl-5-p-tolyl-3-(4-(trifluoromethyl)phenyl)-4,5-dihydro-1H-pyrazole (2g)

Yellow powder, yield 80%, mp 123.4–123.7 °C. IR (KBr) ν 3027, 2924, 2852, 1614, 1597, 1552, 1498, 1321, 1244, 1168, 1064, 835 cm⁻¹. ¹H NMR (400 MHz, DMSO-d₆) δ 2.25 (s, 3H, CH₃), 3.16 (dd, Jax = 6.30 Hz, Jab = 17.91 Hz, 1H, Ha), 3.93 (dd, Jbx = 12.42 Hz, Jab = 17.91 Hz, 1H, Hb), 5.56 (dd, Jax = 6.31 Hz, Jab = 12.40 Hz, 1H, Hx), 6.72–7.19 (m, 9H, Ar-H). Anal. Calcd for C₂₃H₁₉F₃N₂: C, 72.62; H, 5.20; N, 7.57.

1-Phenyl-5-m-tolyl-3-(4-(trifluoromethyl)phenyl)-4,5-dihydro-1H-pyrazole (2h)

Yellow powder, yield 75%, mp 145.9–146.3 °C. IR (KBr) ν 3028, 2926, 1615, 1585, 1552, 1498, 1321, 1244, 1155, 1064, 835 cm⁻¹. ¹H NMR (400 MHz, DMSO-d₆) δ 2.24 (s, 3H, CH₃), 3.14 (dd, Jax = 6.61 Hz, Jab = 17.57 Hz, 1H, Ha), 3.95 (dd, Jbx = 12.24 Hz, Jab = 17.56 Hz, 1H, Hb), 5.50 (dd, Jax = 6.60 Hz, Jab = 12.24 Hz, 1H, Hx), 6.71–7.22 (m, 9H, Ar-H), 7.75 (d, J = 8.4 Hz, 2H, Ar-H), 7.91 (d, J = 8.4 Hz, 2H, Ar-H). Anal. Calcd for C₂₃H₁₉F₃N₂: C, 72.62; H, 5.03; N, 7.36. Found: C, 72.35; H, 4.49; N, 6.56.

1-Phenyl-5-(thiophen-2-yl)-3-(4-(trifluoromethyl)phenyl)-4,5-dihydro-1H-pyrazole (2i)

Orange powder, yield 77%, mp 128.8–129.4 °C. IR (KBr) ν 3097, 1618, 1591, 1529, 1479, 1315, 1220, 1166, 1064, 825 cm⁻¹. ¹H NMR (400 MHz, DMSO-d₆) δ 3.25 (dd, Jax = 12.33 Hz, Jab = 17.61 Hz, 1H, Ha), 3.93 (dd, Jbx = 12.33 Hz, Jab = 17.61 Hz, 1H, Hb), 5.96 (dd, Jax = 6.57 Hz, Jab = 12.33 Hz, 1H, Hx), 6.77–7.38 (m, 8H, Ar-H), 7.76 (d, J = 8.1 Hz, 2H, Ar-H), 7.91 (d, J = 8.1 Hz, 2H, Ar-H). Anal. Calcd for C₂₃H₁₅F₃N₂S: C, 64.50; H, 4.06; N, 7.52. Found: C, 64.14; H, 4.13; N, 7.47.

N,N-Dimethyl-4-(1-phenyl-3-(4-(trifluoromethyl)phenyl)-4,5-dihydro-1H-pyrazol-5-yl)aniline (2j)

Yellow powder, yield 75%, mp 130.1–130.3 °C. IR (KBr) ν 3059, 1635, 1591, 1533, 1483, 1321, 1259, 1168, 1064, 835 cm⁻¹. ¹H NMR (400 MHz, DMSO-d₆) δ 3.12 (dd, Jax = 6.56 Hz, Jab = 17.39 Hz, 1H, Hb), 6.26 (dd, Jax = 6.56 Hz, Jbx = 12.80 Hz, 1H, Hx), 6.71–8.00 (m, 16H, Ar-H). Anal. Calcd for C₂₆H₁₅F₃N₂: C, 74.99; H, 6.46; N, 6.73. Found: C, 75.43; H, 4.49; N, 6.56.

2. 2. Biological Studies

2. 2. 1. Antioxidant Activity

2. 2. 1. 1. DPPH Radical Scavenging Activity

Free radical scavenging capacity of chalcone and pyrazoline derivatives was carried out according to the methods described previously.²⁶,²⁷ Briefly, 1 mg of the compound was dissolved in DMSO and four different concentrations (0.250–0.048 µg/mL, approximately) were prepared. 190 µL methanol solution of DPPH (0.1 mM) was added on this mixture in a well of 96-well plate. The mixture was allowed to stand in the dark at room temperature for 30 min. Absorbance readings were carried out at 517 nm. The DPPH stock solution without compounds was taken as the negative control. The percentage of inhibition was calculated according to the following:

\[
\text{DPPH scavenging effect (\%)} = \frac{A_{\text{control}} - A_{\text{compound}}}{A_{\text{control}}} \cdot 100 \quad (1)
\]

\[A_{\text{control}}: \text{Absorbance of the control (containing all reagents except the synthesized compounds).}\]

\[A_{\text{compound}}: \text{Absorbance of the synthesized compounds.}\]

Tests were repeated in quadruplicate. Ascorbic acid was used as the positive control.

2. 2. 1. 2. ABTS Radical Scavenging Activity

ABTS radical cations were prepared by dissolving 7 mM ABTS and 4.9 mM potassium persulfate, allowing them to react for 16 h at room temperature in the dark. Then, the ABTS radical solution was diluted with 96% ethanol to an absorbance recorded at 734 nm. Four different concentrations of the analyzed compounds were prepared according to the above method. DPPH radical scavenging activity.²⁷ Absorbance readings were recorded at 734 nm. The percentage of inhibition was calculated according to the following:

\[
\text{ABTS scavenging effect (\%)} = \frac{A_{\text{control}} - A_{\text{compound}}}{A_{\text{control}}} \cdot 100 \quad (2)
\]

Tests were repeated in quadruplicate. Trolox was used as the positive control.

2. 2. 2. Anti-Inflammatory Activity

The anti-lipoxygenase activity was evaluated as described by Phosrithong and Nuchtavorn with slight modifications described by Yildirim et al.²⁸,²⁹ Four different concentrations of chalcone and pyrazoline derivatives...
were added to 250 μL of 0.1 M borate buffer pH 9.0, followed by the addition of 250 μL of type V soybean lipoxygenase solution in a buffer (20,000 U/mL). The mixture was incubated at 25 °C for 5 min and 1000 μL of 0.6 mM linoleic acid solution was added, mixed well and the change in absorbance at 234 nm was measured for 6 min. The percentage of inhibition was calculated from the following equation:

\[
\% \text{ inhibition} = \frac{A_{\text{control}} - A_{\text{compound}}}{A_{\text{control}}} \cdot 100
\]  

(3)

Tests were repeated in quadruplicate. Indomethacin was used as the positive control. The IC\textsubscript{50} values were determined as the concentration of the synthesized compounds required to inhibit lipoxygenase enzyme activity by 50%.

### 2.2.3. Antidiabetic Activity

#### 2.2.3.1. α-Glucosidase Inhibitory Assay

The α-glucosidase inhibitor activity was evaluated as described by Ramakrishna et al. with slight modifications described by Sen et al.\textsuperscript{30,31} 40 μL of 0.1 M sodium phosphate buffer (pH 6.8) was mixed with 10 μL of the synthesized compound at 37 °C. The mixtures were incubated at 25 °C for 10 minutes with 100 μL of α-glucosidase which was obtained from \textit{Saccharomyces cerevisiae}. Then, 50 μL of 5 mM \textit{p}-nitrophenyl-α-D-glucopyranoside (pNPG) which was prepared in the buffer, was added to the mixture. The resulting solution was incubated at 25 °C for 5 minutes again, and absorbance was recorded before and after incubation at 405 nm.

The percentage of inhibition was calculated from the following equation:

\[
\% \text{ inhibition} = \frac{A_{\text{control}} - A_{\text{compound}}}{A_{\text{control}}} \cdot 100
\]  

(4)

Tests were repeated in quadruplicate. Acarbose was used as the positive control. The IC\textsubscript{50} values were determined as the concentration of the synthesized compounds required to inhibit α-glucosidase enzyme activity by 50%.

#### 2.2.3.2. Statistical Analysis

The data were given as means ± standard deviations and analyzed by one-way analysis of variance (ANOVA) followed by the Tukey’s multiple comparison tests using GraphPad Prism 5. Differences between means at \( p < 0.05 \) level were considered significant.

### 3. Results and Discussion

#### 3.1. Chemistry

Pyrazoline derivatives were synthesized as depicted in Figure 2. Firstly, chalcone derivatives were obtained from 4’-(trifluoromethyl)acetophenone according to Claisen–Schmidt condensation reaction. Then, 2-pyrazoline derivatives were obtained from synthesized chalcones by refluxing in the presence of acidic medium (Figure 2). The structures of the compounds were confirmed by IR, \(^1\)H NMR spectroscopic methods and elemental analysis. Physicochemical and spectroscopic characterization of the chalcone derivatives 1e, f, g, i, j have been previously described.\textsuperscript{21–25}

IR spectra of pyrazoline derivatives afforded aromatic C–H (3097–3007 cm\textsuperscript{-1}) stretching, pyrazoline C=N stretching (1635–1614 cm\textsuperscript{-1}) and C–F stretching (1168–1118 cm\textsuperscript{-1}) bands. When \(^1\)H NMR spectra of pyrazoline derivatives were examined, three different characteristic signals belonging to the methylene group at position 4 (Ha and Hb) and the methine group at position 5 (Hx) of the pyrazoline ring were determined. These signals appeared as doublet of doublets due to ABX spin system in the structures (Figure 3). The Ha, Hb and Hx protons resonated at 3.04–3.25 ppm (\( J_{\text{ab}} = 17.28–17.91 \) Hz), 3.86–4.02 ppm (\( J_{\text{ax}} = 6.12–6.66 \) Hz) and 5.44–6.26 ppm (\( J_{\text{bx}} = 12.24–13.96 \) Hz), respectively. Aromatic CH protons were seen at 6.63–8.00 as multiplet. Especially ortho protons belonging to ar-
romatic ring (bearing trifluoromethyl substituent) were observed as doublet ($J = 8.1–8.4$ Hz) at $7.72–7.96$ ppm.

3.2. Biological Evaluation

All chalcone and pyrazoline derivatives were screened for in vitro antioxidant activity with DPPH and ABTS assays, anti-inflammatory activity with lipoygenase (LOX) inhibition assay and antidiabetic activity with α-glucosidase inhibition assay. All IC$_{50}$ values of compounds against reference standards were given as Table 1. Results demonstrated that all pyrazoline derivatives had a very strong anti-inflammatory activity with IC$_{50}$ values between $0.68 ± 0.07$ and $4.45 ± 0.25$ µM when compared to

| Compound | DPPH activity | ABTS activity | Anti-inflammatory activity | Anti-diabetic activity |
|----------|---------------|---------------|----------------------------|------------------------|
| 1a       | >1000         | >1000         | 128.40 ± 3.92$^f$         | 381.20 ± 9.29$^{c,d}$   |
| 1b       | >1000         | 852.80 ± 51.42$^c$ | 48.47 ± 2.29$^c$         | 460.50 ± 3.89$^{d,e}$   |
| 1c       | 211.60 ± 3.48$^b$ | 869.00 ± 64.4$^c$ | 121.50 ± 4.77$^f$       | 746.10 ± 20.95$^{h,i,j}$|
| 1d       | 389.50 ± 2.22$^{c,d,e}$ | 807.00 ± 16.88$^c$ | 79.35 ± 0.93$^d$        | 338.60 ± 29.3$^c$       |
| 1e       | 282.10 ± 15.92$^{b,c}$ | >1000         | 304.60 ± 1.61$^b$       | >1000                  |
| 1f       | >1000         | >1000         | 50.97 ± 2.75$^c$        | 377.40 ± 4.35$^{c,d}$   |
| 1g       | 286.10 ± 19.48$^{b,c}$ | 635.80 ± 11.45$^{h,c}$ | 105.90 ± 0.02$^c$      | >1000                  |
| 1h       | 321.30 ± 9.96$^{b,c}$ | >1000         | 38.42 ± 3.02$^b$       | >1000                  |
| 1i       | >1000         | 907.40 ± 26.80$^c$ | 85.13 ± 1.56$^d$      | >1000                  |
| 1j       | 494.00 ± 10.41$^{c,f,g,h,i}$ | 204.40 ± 4.29$^{a,b}$ | 193.60 ± 0.3$^{a,b}$   | >1000                  |
| 1k       | >1000         | >1000         | 83.07 ± 2.31$^d$       | 823.8 ± 0.43$^j$       |
| 2a       | 534.40 ± 5.02$^{h,i,j}$ | 136.30 ± 3.97$^a$ | 0.68 ± 0.07$^a$        | 81.09 ± 0.70$^{a,b}$   |
| 2b       | 586.10 ± 44.19$^i$ | 117.40 ± 0.18$^a$ | 0.76 ± 0.09$^a$        | 463.40 ± 6.50$^{d,e}$   |
| 2c       | 505.80 ± 23.04$^{g,h,i}$ | 127.00 ± 0.90$^a$ | 1.56 ± 0.08$^a$        | 613.80 ± 14.13$^{j,g}$  |
| 2d       | 342.90 ± 3.29$^{g,h,i}$ | 108.00 ± 2.92$^a$ | 2.15 ± 0.13$^a$        | 596.90 ± 18.00$^{f,g}$  |
| 2e       | 645.00 ± 22.0$^{d}$ | 130.10 ± 2.00$^a$ | 1.73 ± 0.04$^a$        | 519.50 ± 18.72$^{f,i}$  |
| 2f       | 524.20 ± 20.82$^{g,h,i}$ | 92.62 ± 5.45$^b$ | 3.11 ± 0.05$^a$        | 451.30 ± 13.76$^{c,d,e}$|
| 2g       | 898.90 ± 5.76$^k$  | 112.20 ± 2.12$^a$ | 4.45 ± 0.25$^a$        | 637.90 ± 1.67$^{f,g}$   |
| 2h       | >1000         | 150.90 ± 0.57$^a$ | 2.14 ± 0.11$^a$        | 657.30 ± 14.68$^{g,h}$  |
| 2i       | 855.60 ± 19.93$^k$ | 136.40 ± 0.15$^a$ | 4.41 ± 0.02$^a$        | 812.70 ± 5.13$^{i,j}$   |
| 2j       | >1000         | 57.13 ± 0.03$^a$ | 1.19 ± 0.05$^a$        | 201.10 ± 5.22$^{b}$    |
| 2k       | 464.80 ± 11.38$^{d,e,g}$ | 94.24 ± 0.86$^a$ | 1.22 ± 0.07$^a$        | 417.60 ± 10.53$^{c,d,e}$|

Ascorbic acid 14.56 ± 0.60$^a$ 12.67 ± 0.28$^a$ 50.45 ± 0.20$^c$ 62.04 ± 3.32$^a$

Troxol 12.67 ± 0.28$^a$ 50.45 ± 0.20$^c$

Indomethacin 12.67 ± 0.28$^a$ 50.45 ± 0.20$^c$

Acarbose 12.67 ± 0.28$^a$ 50.45 ± 0.20$^c$

* Each value in the table is represented as mean ± SD (n = 4). Different letter superscripts in the same column indicate significant differences (p < 0.05).
reference standard indomethacin \( (\text{IC}_{50} = 50.45 \pm 0.20 \ \mu\text{M}) \). Additionally, compound 2a inhibited 5-lipoxygenase activity by 99.73% at a concentration of 0.098 \( \mu\text{g/mL} \). Chalcone derivatives 1a–k at a concentration of 0.098 \( \mu\text{g/mL} \) inhibited lipoxygenase enzyme activity with the inhibition rate of 88.85–15.83% compared to the control (Figure 4).

Synthesized compounds exhibited low antioxidant activity. Only compound 2j showed the best antioxidant activity according to ABTS assay with IC\(_{50}\) value of 57.13 ± 0.03 \( \mu\text{M} \) compared to the reference standard trolox (12.67 ± 0.28 \( \mu\text{M} \)).

Antidiabetic activity of all compounds was evaluated by the \( \alpha \)–glucosidase inhibition assay. These results showed that compound 2a showed maximum \( \alpha \)–glucosidase inhibitory activity with the IC\(_{50}\) value of 81.09 ± 0.70 \( \mu\text{M} \) (IC\(_{50}\) for acarbose 62.04 ± 3.32 \( \mu\text{M} \)).

3.3. ADME Calculations

The prediction of ADME properties of the compounds is a very important development of new drug candidates. Therefore the druglike molecule was carried out by using the Lipinski rule of five and Veber rule.\(^{32-34}\) Calculations were performed using molinspiration online server. The screening results were presented in Table 2.

The molecular weights varied from 276.25 to 435.27 for the synthesized compounds which are lower than the maximum molecular weight of 500. All the compounds are having logP in the range of 3.48–6.22. The number of hydrogen bond acceptors of all compounds is 4 which is less than the maximum value of ten. On the other hand, all the compounds have zero hydrogen bond donors which must be less than the maximum value of five. Furthermore, the number of rotatable bonds is in the range of 4–5 which is lower than the maximum value of 10. Similarly the polar

Table 2. Predicted ADME, Lipinski and Veber parameters of the synthesized compounds.

| Compound | MW   | Lipinski rule of five | Veber rule | TPSA |
|----------|------|-----------------------|------------|------|
|          | IC\(_{50}\) | Log P | n-ON | n-OHNH | n-ROTB | | |
| 1a       | 304.31 | 4.78  | 4    | 0      | 4   | 17.07 |
| 1b       | 345.14 | 5.32  | 4    | 0      | 4   | 17.07 |
| 1c       | 290.28 | 4.55  | 4    | 0      | 5   | 17.07 |
| 1d       | 318.33 | 5.01  | 4    | 0      | 17.07 |
| 1e       | 276.25 | 4.32  | 4    | 0      | 17.07 |
| 1f       | 310.70 | 4.82  | 4    | 0      | 17.07 |
| 1g       | 290.28 | 4.55  | 4    | 0      | 17.07 |
| 1h       | 290.28 | 4.55  | 4    | 0      | 17.07 |
| 1i       | 282.28 | 3.48  | 4    | 0      | 45.31 |
| 1j       | 319.32 | 4.12  | 4    | 0      | 5   | 20.31 |
| 1k       | 326.31 | 5.04  | 4    | 0      | 4   | 17.07 |
| 2a       | 394.43 | 5.69  | 4    | 0      | 4   | 15.60 |
| 2b       | 435.27 | 6.22  | 4    | 0      | 4   | 15.60 |
| 2c       | 380.41 | 5.48  | 4    | 0      | 4   | 15.60 |
| 2d       | 408.46 | 5.89  | 4    | 0      | 5   | 15.60 |
| 2e       | 366.38 | 5.27  | 4    | 0      | 4   | 15.60 |
| 2f       | 400.82 | 5.75  | 4    | 0      | 4   | 15.60 |
| 2g       | 380.41 | 5.48  | 4    | 0      | 4   | 15.60 |
| 2h       | 380.41 | 5.48  | 4    | 0      | 4   | 15.60 |
| 2i       | 372.41 | 5.07  | 4    | 0      | 5   | 18.84 |
| 2j       | 409.45 | 5.07  | 4    | 0      | 4   | 15.60 |
| 2k       | 416.44 | 5.91  | 4    | 0      | 4   | 15.60 |

TPSA: Topological polar surface area, n-ON: number of hydrogen bond acceptors, n-OHNH: number of hydrogen bond donors, n-ROTB: number of rotatable bonds. Calculations were performed using Molinspiration online property calculation toolkit (http://www.molinspiration.com).
surface area of all synthesized compounds is indicated to be in the range of 15.60–45.31 Å² which is less than the maximum value of 140 Å². These values demonstrate that none of the synthesized compounds are violating the Lipinski and Veber rules.

4. Conclusion

New pyrazoline derivatives were synthesized from chalcone derivatives and the designed molecules were investigated for their drug-likeness properties which were defined as Lipinski and Veber rules. All compounds were tested for their antioxidant (DPPH and ABTS), anti-lipooxygenase and α-glucosidase inhibitory activities. These results showed that pyrazoline derivatives exhibited better activity than chalcone derivatives. Especially pyrazoline derivatives 2a-k showed very strong anti-lipooxygenase activity each with greater activity than reference drug indomethacin. Also, compounds 2j and 2a demonstrated good antioxidant and α-glucosidase inhibitory activity, respectively. These findings revealed that the pyrazoline core could lead to considerably active molecules.

Conflict of Interest

Authors declare no conflict of interest.

5. References

1. M. Asad, M. N. Arshad, S. A. Khan, M. Oves, M. Khalid, A. M. Asiri, A. A. C. Braga, J. Mol. Struct. 2020, 1201, 1–14. DOI:10.1016/j.molstruc.2019.127186
2. M. R. Bhosle, A. R. Deshmukh, S. Pal, A. K. Srivastava, R. A. Mane, Bioorg. Med. Chem. Lett. 2015, 25, 2442–2446. DOI:10.1016/j.bmcl.2015.03.068
3. C. Kharbanda, M. S. Alam, H. Hamid, K. Javed, S. Shafi, Y. Ali, P. Alam, M. A. Q. Pasha, A. Dhulap, S. Bano, S. Nazreen, S. Haider, Bioorg. Med. Chem. Lett. 2014, 24, 5298–5303. DOI:10.1016/j.bmcl.2014.09.044
4. D. S. Raghuvanshi, N. Verma, S. V. Singh, S. Khare, A. Pal, A. S. Negi, Bioorg. Chem. 2019, 88, 1–11. DOI:10.1016/j.bioorg.2019.102933
5. G. Turan-Zitouni, P. Chevallet, F. S. Kilç, K. Erol, Eur. J. Med. Chem. 2000, 35, 635–641. DOI:10.1016/S0223-5234(00)00152-5
6. C. Kharbanda, M. S. Alum, H. Hamid, K. Javed, S. Bano, A. Dhulap, Y. Ali, S. Nazreen, S. Haider, Bioorgan. Med. Chem. 2014, 22, 5804–5812. DOI:10.1016/j.bmcc.2014.09.028
7. F. Tok, B. L. Abas, Ö. Çevik, B. Koçyiğit-Kaymakçiąoğlu, Bioorg. Chem. 2020, 102, 104063. DOI:10.1016/j.bioorg.2020.104063
8. H. S. ElBordiny, M. M. El-Miligy, S. E. Kassab, H. Daabees, W. A. M. Ali, S. A. M. El-Hawash, Eur. J. Med. Chem. 2018, 145, 594–605. DOI:10.1016/j.ejmech.2018.01.026
9. F. Chaudhry, S. Naureen, R. Huma, A. Shaukat, M. Al-Rashidi, N. Asif, M. Ashraf, M. A. Munawar, M. A. Khan, Bioorg. Chem. 2017, 71, 102–109. DOI:10.1016/j.bioorg.2017.01.017
10. M. Sathish, G. Meenakshi, S. Xavier, S. Sebastian, S. Periandy, N. Ahmad, J. Jamalis, M. Rosli, H. Fun, J. Mol. Struct. 2018, 1164, 420–437. DOI:10.1016/j.molstruc.2018.03.004
11. B. Sever, M. D. Altimtop, M. O. Radwan, A. Ozdemir, M. Otsuka, M. Fujita, H. I. Ciftci, Eur. J. Med. Chem. 2019, 182, 1–17. DOI:10.1016/j.ejmech.2019.111648
12. B. Shu, Q. Yu, D. Hu, T. Che, S. Zhang, D. Li, Bioorg. Med. Chem. Lett. 2020, 30, 1–4. DOI:10.1016/j.bmcl.2019.126925
13. D. Moi, A. Nocentini, A. Deplano, G. Balboni, C. T. Supuran, V. Ornis, Eur. J. Med. Chem. 2019, 182, 1–11. DOI:10.1016/j.ejmech.2019.111638
14. A. Mumtaz, A. Majeed, S. Zaib, S. Rahman, S. Hameed, A. Saeed, H. Rafique, E. Mughal, A. Maalik, I. Hussain, I. Iqbal, Bioorg. Chem. 2019, 90, 1–9. DOI:10.1016/j.bioorg.2019.103036
15. N. M. A. Gawad, G. H. Hassan, H. H. Georgey, Int. J. Med. Chem. 2012, 21, 983–994. DOI:10.1007/s00044-011-9606-4
16. S. Viveka, P. Shama, G. K. Nagaraja, S. Ballav, S. Kerkar, Eur. J. Med. Chem. 2015, 101, 442–451. DOI:10.1016/j.ejmech.2015.07.002
17. P. Shah, A. D. Westwell, J. Enzym. Inhib. Med. Chem. 2007, 22, 527–540. DOI:10.1080/14756360701425014
18. K. Haranahalli, T. Honda, I. Ojima, J. Fluorine Chem. 2017, 217, 29–40. DOI:10.1016/j.jfluchem.2018.11.002
19. N. Beyhan, B. Kocyiigsawat-Kaymakcioglu, S. Gunur, F. Arisco, Arab. J. Chem. 2017, 10, 2073–2081. DOI:10.1016/j.arabjc.2013.07.037
20. A. Ahmad, A. Husain, S. A. Khan, M. Mujeeb, A. Bhandari, J. Saudi Chem. Soc. 2016, 20, 577–584. DOI:10.1016/j.jscs.2014.12.004
21. X. Huang, J. Li, X. Li, J. Wang, Y. Peng, G. Song, RSC. Adv. 2019, 9, 26419–26424. DOI:10.1039/C9RA05708K
22. T. T. Nguyen, G. D. Kortman, K. L. Hull, Organometallics, 2016, 35, 1713–1725. DOI:10.1021/acs.organomet.6b00111
23. D. Liu, J. Yu, J. Cheng, Tetrahedron, 2014, 70, 1149–1153. DOI:10.1016/j.tet.2013.12.077
24. W. Katsuaki, G. Takuya, O. Yuichi, S. Katsuhiko, A. Takahisa, A. Wolfram, H. Achim, L. Peter, Preparation of nematocidal pyrazoles, US Patent Number 6310049, date of patent October 30, 2001.
25. R. Shalaby, J. P. Petzer, A. Petzer, U. M. Ashraf, I. Alasmari, S. Kumarasamy, Y. Sari, A. Khalil, J. Enzym. Inhib. Med. Ch. 2019, 34, 863–876. DOI:10.1080/14756366.2019.1593158
26. Y. Zou, S. K. C. Chang, Y. Gu, S. Y. Qian, J. Agric. Food Chem. 2011, 59, 2268–2276. DOI:10.1021/jf104640k
27. A. Şen, Marmara Pharm. J. 2018, 22, 328–333. DOI:10.12991/mpj.2018.71
28. A. Yıldırım, A. Şen, A. Doğan, L. Bitiş, Int. J. Sec. Metabolite, 2019, 6, 211–222.
29. N. Phosrithong, N. Nuchtavorn, Eur. J. Integr. Med. 2016, 8, 281–285. DOI:10.1016/j.eujim.2015.10.002
30. R. Ramakrsihna, D. Sarkar, P. Schwarz, K. Shetty, *Ind. Crops Prod*. 2017, 107, 509–517.  
DOI: 10.1016/j.indcrop.2017.03.033

31. A. Sen, M. Kurkuoglu, I. Senkardes, L. Bitis, K. H. C. Baser, *J. Essent. Oil Bear. Pl.* 2019, 22, 1048–1057.  
DOI: 10.1080/0972060X.2019.1662333

32. R. Matsa, P. Makam, M. Kaushik, S.L. Hoti, T. Kannan, *Eur. J. Pharm. Sci.* 2019, 137, 1–12.  
DOI: 10.1016/j.ejps.2019.104986

33. C. A. Lipinski, *Drug Discov. Today Technol.* 2004, 1, 337–341.  
DOI: 10.1016/j.ddtec.2004.11.007

34. D. F. Veber, S. R. Johnson, H. Y. Cheng, B. R. Smith, K. W. Ward, K. D. Kopple, *J. Med. Chem.* 2002, 45, 2615–2623.  
DOI: 10.1021/jm020017n

**Povzetek**

Iz halkonskih derivatov smo sintetizirali serijo 1-fenil-5-substituiranih-3-(4-(trifluorometil)fenil)-4,5-dihidro-1H-pirazolov. Strukture spojin smo določili s pomočjo IR ter 1H NMR spektroskopskih metod in z elementno analizo. Za vse spojine smo določili in vitro antioksidativno aktivnost s pomočjo DPPH in ABTS metod, antiinflamatorno aktivnost s pomočjo metode inhibicije lipooksigenaze ter antidiabetično aktivnost s pomočjo metode inhibicije α-glukozidaze. Še zlasti pirazolinski derivati so izkazali visoke antiinflamatorne aktivnosti, celo večje kot referenčna učinkovina indometa-cin (IC_{50}: 50.45 µM) z IC_{50} vrednostmi v območju od 0.68 do 4.45 µM. V nadaljevanju smo določili še ADME lastnosti vseh halkonskih in pirazolinskih derivatov skladno s pravili Lipinskega in Vebra.