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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Type II diabetes-related enzyme inhibition and molecular modeling study of a novel series of pyrazolone derivatives

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Abstract Inhibitors of alpha-amylase are targets for the development of novel drugs for the treatment of diabetes and obesity. Alpha amylase is an enzyme which increases the bio availability of glucose in the blood. Hence, the inhibition effects of alpha amylase of 2-[1-(4-isobutylphenyl)ethyl]-5-methyl-4-[2-(aryl-substituted)hydrazinylidene]-2,4-dihydro-3*H*-pyrazol-3-one (**4a–l**) were investigated, among them compounds **4d**, **4f**, **4a**, and **4g** have displayed good inhibitory activity. The compounds with significant results were further evaluated for their molecular modeling study using in silico method. The new series of compounds were synthesized by solvent-free microwave irradiation method and were characterized by spectral and analytical data.

Keywords Pyrazolone · Green technique · Antidiabetic · α-Amylase inhibition · In silico molecular modeling study

Introduction

Pyrazolone derivatives form an important class of compounds having significant pharmacological activities such as anti-inflammatory, antimicrobial (Bekhit *et al.*, 2010), analgesic (Hall *et al.*, 2008), angiotensin antagonists (Sharma and Kohli, 2013), cytotoxic agents (Xu *et al.*, 2013). Aryl pyrazoles are reported to have non-nucleoside HIV-I reverse transcriptase inhibitory activity (Genin *et al.*, 2000). Furthermore, pyrazoles with a wide array of substituted groups were reported to be selective inhibitors of cyclooxygenase (Reddy *et al.*, 2008) and also exhibit antidiabetic (Hassan *et al.*, 2011; Eduardo *et al.*, 2013), herbicidal (Wu *et al.*, 2012) properties. Coupling products of diazonium salts with compounds having active hydrogen are widely used as intermediates in the synthesis of variety of heterocyclic compounds (Oruç *et al.*, 2006).

Type 2 diabetes mellitus (T2DM) is a complex metabolic disorder results from the body's ineffective use of insulin (American Diabetes Association, 2012), leading to abnormal metabolism of not only glucose but also lipids and amino acids. The pharmacophoric moiety 3,5-disubstituted pyrazoles as antidiabetic agents was first reported by Wright *et al.* (1964). Based on this pharmacophore numerous pyrazole derivatives were synthesized and evaluated for antidiabetic activity. A number of pyrazole compounds have been cited in the literature that elicit antihyperglycemic effects, including 1,3-disubstituted pyrazoles, 1,3,5-trisubstituted pyrazoles, and pyrazolones (Bertrand *et al.*, 2002; Hassan *et al.*, 2011).

α-Amylase is an important key enzyme responsible for carbohydrate digestion. So inhibitors of α-amylase can effectively retard the digestion and assimilation at the early steps of starch digestion, and thus succeed in a significant delay of postprandial hyperglycemia and have a beneficial

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effect on insulin resistance (Michelle de Sales *et al.*, 2012). So α-amylase catalyze the hydrolysis of α-(1,4)-glycosidic linkages in starch and are considered to be one of the best targets for the development of type II diabetes therapeutic agents (Qin *et al.*, 2011). For this purpose inhibitors such as acarbose and voglibose are clinically used. However, they often cause severe gastrointestinal side effects such as abdominal pain, flatulence, and diarrhea (van de Laar *et al.*, 2005). Therefore, a number of studies have been conducted in the search for new α-amylase inhibitors (Matsui *et al.*, 2007).

Computational biology and bioinformatics play a major role in designing the drug molecules and have the potential of speeding up the drug discovery process. Molecular docking methods are commonly used for predicting binding modes and energies of ligands to proteins. Molecular docking of the drug molecule with the receptor gives important information about drug receptor interactions and is commonly used to find out the binding orientation of drug candidates to their target sites in order to predict the affinity and activity.

The driving force for microwave developments in organic synthesis by green techniques has many benefits because of their simplicity in operation, enhanced reaction rates, and greater selectivity (Jyothi *et al.*, 2007). The increase in requirement for environmentally clean technology that minimizes the production of waste (Escalante and Díaz-Coutiño, 2009) is another important criterion of green technology. Keeping in view of the above observations and in continuation of our search for biologically active heterocycles (Girisha *et al.*, 2012; Nithinchandra *et al.*, 2012), we herein report a new series of pyrazolone derivative synthesized by microwave irradiation technique. These new molecules were tested for α-amylase inhibition. Molecular docking simulations of compounds with significant amylase inhibition effects and acarbose to α-amylase were performed in order to gain functional and structural insight into the mechanism of inhibition.

Experimental

Microwave-assisted synthesis was carried out using Catalyst system microwave oven, CATA R ranging from power levels 1–9 at 140–700 W. Melting points were determined using the apparatus Innovative DTC-967A and are uncorrected. The IR spectra (in KBr pellets) were recorded on a Shimadzu FT-IR 157 spectrophotometer. The ^{1}H NMR spectra were recorded on a Bruker AMX-400 (400 MHz) NMR spectrometer using TMS as an internal standard. The chemical shifts are expressed in δ scale downfield from TMS and proton signals are indicated as s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet. The mass spectra were recorded either on a Waters UPLC mass spectrometer or API3000 LCMS instrument operating at 70 eV.

The synthesis of hitherto unreported title compounds was as outlined in Scheme 1. Synthesis of new pyrazolone derivatives using 2-[4-isobutylphenyl]propanoyl hydrazine and ethyl-2-[2-(aryl substituted)hydrazinylidene]-3-oxobutanoate by both conventional and microwave-assisted methods were carried out. Diazotisation of appropriately substituted anilines (**1a–l**) with nitrous acid gave the diazo compound. The in situ coupling of this diazo compound with ethyl aceto acetate in the presence of sodium acetate as catalyst gave the required ethyl-2-arylhydrazono-3-oxobutyrates (**3a–l**). The 2-[4-isobutylphenyl]propanoyl hydrazine were obtained from the esterification of 2-[4-isobutylphenyl]propanoic acid followed by hydrazinolysis with hydrazine hydrate as per the literature procedure (Nithinchandra *et al.*, 2012).

Characterization data of 2-[4-isobutylphenyl]propanoyl hydrazine is given below:

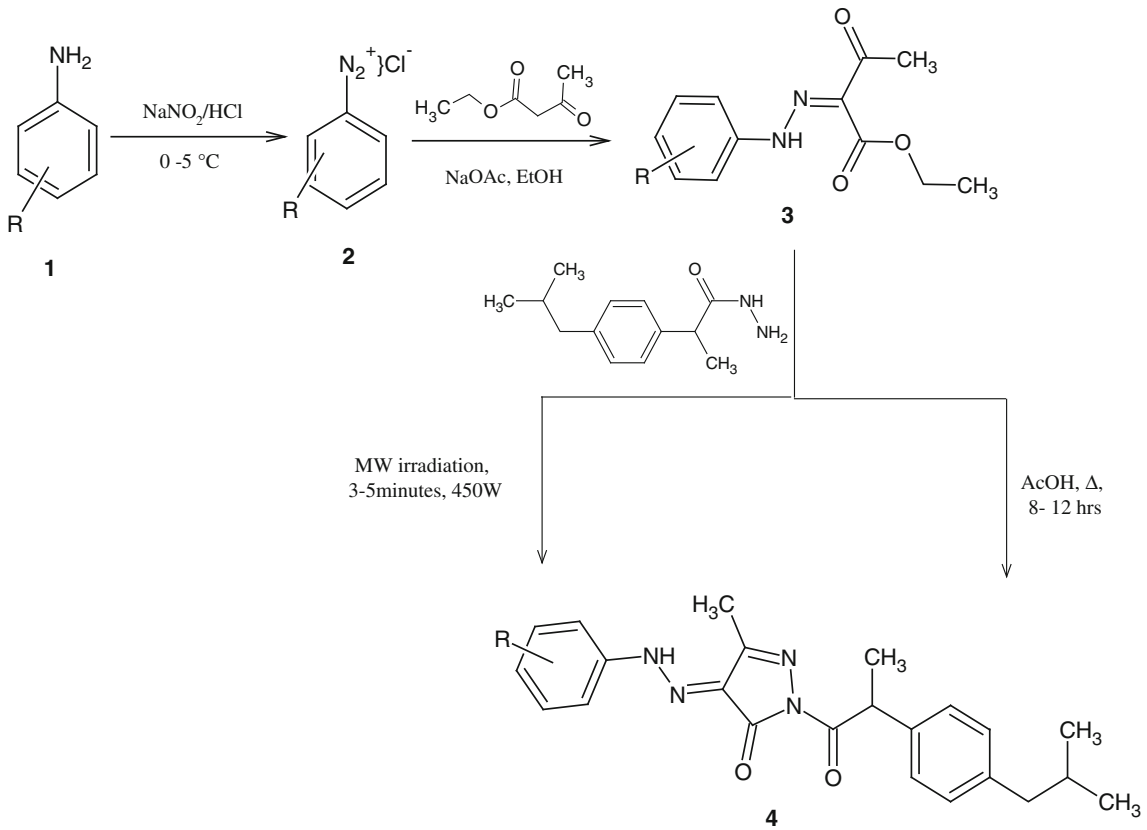
It was obtained as a white solid with mp 71–72 °C; Yield :80 %, IR (KBr) γ/cm^{-1}: 3,270.3 (N–H), 2,954.4 (C–H stretching), 1,686.2 (C=O stretching); ^{1}H NMR (DMSO-d_6), δ in ppm: 0.84 (d, 6H, J = 6.56 Hz, $(CH_3)_2$), 1.30 (d, 3H, J = 7 Hz, CH_3), 1.81–1.74 (m, 1H, CH–$(CH_3)_2$), 2.38 (d, 2H, J = 7.04 Hz, CH_2), 3.48 (q, 1H, CH), 4.16 (s, 2H, NH_2), 7.06 (d, 2H, J = 7.72 Hz, 3′,5′-Ib–Ar–H), 7.20 (d, 2H, J = 7.72 Hz, 2′,6′-Ib–Ar–H), 9.13 (s, 1H, NH); ^{13}C NMR (DMSO-d_6 100 MHz): δ 14.11 (isobutyl-$(CH_3)_2$), 19.15 (CH_3), 30.14 (isobutyl CH), 42.01 (isobutyl-CH_2), 43.06 (CH), 127.20 (C-4 of isobutylphenyl), 127.84 (C-3 and C-5 of isobutylphenyl), 131.16 (C-2 and C-6 of isobutylphenyl), 137.23 (C-1 of isobutylphenyl), 161.9 (C=O); LC–MS (*m*/*z*): 220.8 (M^+). Anal. Calcd. For $C_{13}H_{20}N_2O$: C, 70.87; H, 9.15; N, 12.72, Found: C, 70.85; H, 9.17; N, 12.70.

General procedure for the preparation of ethyl-2-arylhydrazono-3-oxobutyrate (**3a–l**)

Appropriately substituted amine **1** (0.01 mol) was dissolved in dilute hydrochloric acid (10 ml) and cooled to 0 °C in an ice bath. To this, a cold solution of sodium nitrite (0.02 mol) was added. The diazonium salt solution was filtered into a cold solution of ethyl acetoacetate (0.02 mol) and sodium acetate (0.02 mol) in 15 ml of aqueous ethanol. The separated solid was filtered, washed with water, and recrystallized from ethanol. Compounds prepared according to this procedure and their characterization data are given below:

*Ethyl-2-(4-chlorophenyl)hydrazono-3-oxobutyrate (**3a**)*

It was obtained as a yellowish solid with melting point 92–93 °C; Yield : 76 %; IR (KBr) γ/cm^{-1}: 3,315.7 (NH



Scheme 1 Synthetic protocols for the target compounds (**4a–l**)

stretch), 1,720 (C=O ester carbonyl), 1,680.1 (C=O stretching of keto group), 1,558.1(NH–N=C), 2,978.8 (CH stretch); ^{1}H NMR (DMSO-d_6 400 MHz) δ in ppm: 1.78 (t, 3H, CH_3), 2.42 (s, 3H, CH_3–C=O), 4.8 (q, 2H, CH_2), 7.23(d, 2H, ortho protons of 4-chlorophenyl), 7.50 (d, 2H, meta protons of 4-chlorophenyl), 12.06 (s, 1H, NH); ^{13}C NMR (DMSO-d_6 100 MHz) δ in ppm : 22.50 (ester CH_3), 24.91 (acetyl CH_3), 60.01 (CH_2), 165.21 (C=O of ester), 169.14 (C=O of acetyl), 120.21 (C-2 and C-6 of 4-chlorophenyl), 129.80 (C-3 and C-5 of 4-chlorophenyl), 132.61 (C-1 of 4-chlorophenyl), 136.21 (C-4 of 4-chlorophenyl), 140.31(C=N–); LC–MS (*m*/*z*): 269 (M^++1); Anal. Calcd. for $C_{12}H_{13}ClN_2O_3$: C, 53.64; H, 4.88; N, 10.43. Found: C, 53.66; H, 4.89; N, 10.41.

*Ethyl-2-(3-nitrophenyl)hydrazono-3-oxobutyrate (**3b**)*

Reddish brown solid with melting point 111–112 °C, Yield: 68 %; IR (KBr) γ/cm^{-1}: 3,325.4 (NH stretch), 1,730.5 (C=O ester carbonyl), 1,670.8 (C=O stretching of keto group), 1,554.8 (NH–N=C), 2,977.8 (CH stretch); 1,560 (NO_2 asymmetric stretch), 1,346 (NO_2 symmetric stretch); ^{1}H NMR (DMSO-d_6 400 MHz) δ in ppm: 1.84 (t, 3H, CH_3), 2.46 (S, 3H, CH_3–C=O), 4.45 (q, 2H, CH_2), 7.50–7.28 (m, 4H, 2′, 4′, 5′, and 6′ protons of 3-nitrophenyl), 12.16 (1H, NH); ^{13}C NMR (DMSO-d_6 100 MHz) δ in ppm : 23.41 (ester CH_3), 25.75 (acetyl CH_3), 59.11 (CH_2), 175.28 (C=O of acetyl), 167.17 (C=O of ester), 117.81 (C-5 of 3-nitrophenyl), 120.75 (C-6 of 3-nitrophenyl), 128.92 (C-4 of 3-nitrophenyl), 130.35 (C-2 of 3-nitrophenyl), 134.42 (C-1 of 3-nitrophenyl), 135.98 (C-3 of 3-nitrophenyl) 139.93 (C=N–); LC–MS (*m*/*z*): 280.3 (M^++1). Anal. Calcd. for $C_{12}H_{13}N_3O_5$: C, 51.61; H, 4.69; N, 15.05. Found: C, 51.63; H, 4.70; N, 15.03.

*Ethyl-2-(4-fluorophenyl)hydrazono-3-oxobutyrate (**3c**)*

It was obtained as a yellowish solid with melting point 99 °C; Yield : 75 %; IR (KBr) γ/cm^{-1}: 3,328.5 (NH stretch), 1,716.8 (C=O ester carbonyl), 1,677 (C=O stretching of keto group), 1,541.5 (NH–N=C), 2,988.7 (CH stretch); ^{1}H NMR (DMSO-d_6 400 MHz) δ in ppm: 1.88 (t, 3H, CH_3), 2.38 (s, 3H, CH_3–C=O), 4.76 (q, 2H, CH_2), 7.20 (d, 2H, ortho protons of 4-fluorophenyl), 7.69 (d, 2H, meta

protons of 4-fluorophenyl), 12.11 (1H, NH); ^{13}C NMR (DMSO-d_6 100 MHz) δ in ppm : 21.62 (ester CH_3), 25.22 (acetyl CH_3), 63.04 (CH_2), 170.25 (C=O of acetyl), 168.90 (C=O of ester), 116.67 (C-2 and C-6 of 4-fluorophenyl), 120.91 (C-3 and C-5 of 4-fluorophenyl), 128.25 (C-1 of 4-fluorophenyl), 135.91 (C-4 of 4-fluorophenyl), 139.96 (C=N–); LC–MS (*m/z*): 253.3 (M^++1); Anal. Calcd. for $C_{12}H_{13}FN_2O_3$: C, 57.14; H, 5.19; N, 11.11. Found: C, 57.16; H, 5.16; N, 11.09.

*Ethyl-2-(2,4-difluorophenyl)hydrazono-3-oxobutyrate (**3d**)*

Yellowish solid with melting point 109 °C. IR (KBr) γ/cm^{-1}: 3,309.4 (NH stretch), 1,735.5 (C=O ester carbonyl), 1,689.5 (C=O stretching of keto group), 1,561.5 (NH–N=C), 2,988.98 (CH stretch); ^{1}H NMR (DMSO-d_6 400 MHZ) δ in ppm: 1.98 (t, 3H, CH_3), 2.58 (s, 3H, CH_3–C=O), 4.86 (q, 2H, CH_2), 7.68–7.57 (m, 3H, 3′, 5′ and 6′ protons of 2,4-difluorophenyl), 12.81 (1H, NH); ^{13}C NMR (DMSO-d_6 100 MHz) δ in ppm: 24.41 (ester CH_3), 28.21 (acetyl CH_3), 59.63 (CH_2), 162.6 (C=O of acetyl), 160.25 (C=O of ester), 116.2 (C-6 of 2,4-difluorophenyl), 120.2 (C-5 of 2,4-difluorophenyl), 124.23 (C-3 of 2,4-difluorophenyl), 128.87 (C-1 of 2,4-difluorophenyl), 138.91 (C-4 of 2,4-difluorophenyl), 139.65 (C-2 of 2,4-difluorophenyl), 140.2 (C=N–); LC–MS (*m/z*): 271.3 (M^++1); Anal. Calcd. for $C_{12}H_{12}F_2N_2O_3$: C, 53.34; H, 4.48; N, 10.37. Found: C, 53.30; H, 4.46; N, 10.39.

*Ethyl-2-(2-anisyl)hydrazono-3-oxobutyrate (**3e**)*

Brownish yellow solid with melting point 114 °C; Yield: 70 %. IR (KBr) γ/cm^{-1}: 3,299.4 (NH stretch), 1,729.8 (C=O ester carbonyl), 1,689.1 (C=O stretching of keto group), 1,567.5 (NH–N=C), 2,992.9 (CH stretch); ^{1}H NMR (DMSO-d_6 400 MHz) δ in ppm: 1.88 (t, 3H, CH_3), 2.68 (s, 3H, CH_3–C=O), 4.77 (q, 2H, CH_2), 7.60–7.54 (m, 4H, 3′, 4′, 5′ and 6′ protons of 2-anisyl), 12.11 (s, 1H, NH); ^{13}C NMR (DMSO-d_6 100 MHz) δ in ppm: 22.1 (ester CH_3), 25.91 (acetyl CH_3), 50.15 (CH_2), 64.20 (OCH_3), 168.61 (C=O of acetyl), 158.20 (C=O of ester), 128.21 (C-4 of 2-anisyl), 132.63 (C-3 of 2-anisyl), 133.26 (C-5 of 2-anisyl), 134.42 (C-6 of 2-anisyl), 136.20 (C-1 of 2-anisyl), 138.20 (C-2 of 2-anisyl), 140.21 (C=N–); LC–MS (*m/z*): 265 (M^++1); Anal. Calcd. for $C_{13}H_{16}N_2O_4$: C, 59.08; H, 6.10; N, 10.60. Found: C, 59.10; H, 6.13; N, 10.62.

*Ethyl-2-(3-chloro-4-fluorophenyl)hydrazono-3-oxobutyrate (**3f**)*

Yellow solid with melting point 108 °C; Yield: 69 %. IR (KBr) γ/cm^{-1}: 3,301.4 (NH stretch), 1,722.6 (C=O ester carbonyl), 1,678.5 (C=O stretching of keto group), 1,560.5 (NH–N=C), 2,990.9 (CH stretch); ^{1}H NMR (DMSO-d_6 400 MHz) δ in ppm: 1.79 (t, 3H, CH_3), 2.61 (s, 3H, CH_3–C=O), 4.70 (q, 2H, CH_2), 7.70–7.59 (m, 3H, 2′, 5′ and 6′ protons of 3-chloro-4-fluorophenyl), 12.06 (1H, NH); ^{13}C NMR (DMSO-d_6 100 MHz) δ in ppm: 22.4 (ester CH_3), 26.2 (acetyl CH_3), 51.22 (CH_2), 171.61 (C=O of acetyl), 169.22 (C=O of ester), 120.32 (C-6 of 3-chloro-4-fluorophenyl), 122.51 (C-2 of 3-chloro-4-fluorophenyl), 129.32 (C-5 of 3-chloro-4-fluorophenyl), 132.27 (C-1 of 3-chloro-4-fluorophenyl), 137.80 (C-3 of 3-chloro-4-fluorophenyl), 141.44 (C-4 of 3-chloro-4-fluorophenyl), 142.28 (C=N–); LC–MS (*m/z*): 287 (M^++1); Anal. Calcd. for $C_{12}H_{12}ClFN_2O_3$: C, 50.27; H, 4.22; N, 9.77. Found: C, 50.29; H, 4.25; N, 9.79.

*Ethyl-2-(2-carboxyphenyl)hydrazono-3-oxobutyrate (**3g**)*

Yellow solid with melting point 84 °C, Yield 70 %; IR (KBr) γ/cm^{-1}: 3,311.3 (NH stretch), 1,719.8 (C=O ester carbonyl), 1,701.5 (acid C=O), 1,681.5 (C=O stretching of keto group), 1,564.2 (NH–N=C), 2,980.9 (CH stretch); ^{1}H NMR (DMSO-d_6 400 MHz) δ in ppm: 1.83 (t, 3H, CH_3), 2.58 (s, 3H, CH_3–C=O), 4.76 (q, 2H, CH_2), 7.78–7.71 (m, 4H, 3′, 4′, 5′, and 6′ protons of 2-carboxyphenyl), 12.08 (1H, NH), 13.16 (1H, OH); ^{13}C NMR (DMSO-d_6 100 MHz) δ in ppm: 24.10 (ester CH_3), 26.34 (acetyl CH_3), 54.91 (CH_2), 169.62 (C=O of acetyl), 165.27 (C=O of ester), 168.23 (C=O of carboxyl), 133.26 (C-5 of 2-carboxyphenyl), 134.24 (C-4 of 2-carboxyphenyl), 137.23 (C-6 of 2-carboxyphenyl), 138.21 (C-3 of 2-carboxyphenyl), 138.65 (C-1 of 2-carboxyphenyl), 139.89 (C-2 of 2-carboxyphenyl), 142.21 (C=N–); LC–MS (*m/z*): 279 (M^++1); Anal. Calcd. for $C_{13}H_{14}N_2O_5$: C, 56.11; H, 5.07; N, 10.07. Found: C, 56.13; H, 5.05; N, 10.05.

*Ethyl-2-(4-carboxyphenyl)hydrazono-3-oxobutyrate (**3h**)*

Reddish solid with melting point 102 °C, Yield: 71 %; IR (KBr) γ/cm^{-1}: 3,302.3 (NH stretch), 1,724.9 (C=O ester carbonyl), 1,718.1 (C=O acid carbonyl), 1,679.2 (C=O stretching of keto group), 1,564.6 (NH–N=C), 2,990.6 (CH stretch); ^{1}H NMR (DMSO-d_6 400 MHz) δ in ppm: 1.79 (t, 3H, CH_3), 2.49 (s, 3H, CH_3–C=O), 4.69 (q, 2H, CH_2), 7.48 (d, 2H, ortho protons of 4-carboxyphenyl), 7.64 (d, 2H, meta protons of 4-carboxyphenyl), 12.24 (1H, NH), 13.08 (1H, OH); ^{13}C NMR (DMSO-d_6 100 MHz) δ in ppm: 24.52 (ester CH_3), 26.91 (acetyl CH_3), 56.22 (CH_2), 169.29 (C=O of acetyl), 166.13 (C=O of ester), 167.11 (C=O of acid), 129.66 (C-2 and C-6 of 4-carboxylphenyl), 133.84 (C-3 and C-5 and 4-carboxylphenyl), 136.23 (C-1 of 4-carboxylphenyl), 138.35 (C-4 of 4-carboxyphenyl), 143.91 (C=N–); LC–MS (*m/z*): 279 (M^++1); Anal. Calcd. for $C_{13}H_{14}N_2O_5$: C, 56.11; H, 5.07; N, 10.07. Found: C, 56.09; H, 5.05; N, 10.09.

*Ethyl-2-(4-nitrophenyl)hydrazono-3-oxobutyrate (**3i**)*

Brown solid with melting point 124–125 °C, [literature (Ferguson *et al.*, 2008): 127.5 °C]; Yield : 68 %. IR (KBr) γ/cm^{-1}: 3,203.8 (NH stretch), 1,726.7 (C=O ester carbonyl), 1,679.1 (C=O stretching of keto group), 1,566.7 (NH–N=C), 1,532 (NO_2 asymmetric stretch), 1,328.6 (NO_2 symmetric stretch); ^{1}H NMR (DMSO-d_6 400 MHz): 1.78 (t, 3H, CH_3), 2.42 (s, 3H, CH_3–C=O), 4.46 (q, 2H, CH_2), 7.23(d, 2H, ortho protons of 4-nitrophenyl), 7.50 (d, 2H, meta protons of 4-nitrophenyl), 12.10 (1H, NH); ^{13}C NMR (DMSO-d_6 100 MHz) δ in ppm: 20.5 (ester CH_3), 23.9 (acetyl CH_3), 50.06 (CH_2), 168.24 (C=O of acetyl), 161.42 (C=O of ester), 132.22 (C-2 and C-6 of 4-nitrophenyl), 135.61 (C-3 and C-5 of 4-nitrophenyl), 136.81 (C-1 of 4-nitrophenyl), 137.12 (C-4 of 4-nitrophenyl), 148.31 (C=N–); LC–MS (*m*/*z*): 279 (M^+); Anal. Calcd. for $C_{12}H_{13}N_3O_5$: C, 51.61; H, 4.69; N, 15.05. Found: C, 51.59; H, 4.72; N, 15.07.

*Ethyl-2-(3-chlorophenyl)hydrazono-3-oxobutyrate (**3j**)*

Brownish yellow solid with melting point 86–88 °C, [literature (Ferguson *et al.*, 2008): 85 °C]; Yield : 72 %. IR (KBr) γ/cm^{-1}: 3,213.8 (NH stretch), 1,723.4 (C=O ester carbonyl), 1,677.3 (C=O stretching of keto group), 1,560.9 (NH–N=C); ^{1}H NMR (DMSO-d_6 400 MHz) δ in ppm: 1.69 (t, 3H, CH_3), 2.49 (s, 3H, CH_3–C=O), 4.44 (q, 2H, CH_2), 7.38–7.20 (m, 4H, 2′, 4′, 5′, and 6′ protons of 3-chlorophenyl); 12.70 (1H, NH); ^{13}C NMR (DMSO-d_6 100 MHz) δ in ppm: 23.0 (ester CH_3), 25.82 (acetyl CH_3), 50.31 (CH_2), 169.28 (C=O of acetyl), 165.13 (C=O of ester), 121.91 (C-5 of 3-chlorophenyl), 125.96 (C-6 of 3-chlorophenyl), 126.82 (C-4 of 3-chlorophenyl), 130.22 (C-2 of 3-chlorophenyl), 130.98 (C-1 of 3-chlorophenyl), 134.42 (C-3 of 3-chlorophenyl), 141.31 (C=N–); LC–MS (*m*/*z*): 269 (M^++1). Anal. Calcd. for $C_{12}H_{13}ClN_2O_3$: C, 53.64; H, 4.88; N, 10.43. Found: C, 53.66; H, 4.90; N, 10.45.

*Ethyl-2-(2-nitrophenyl)hydrazono-3-oxobutyrate (**3k**)*

Brownish yellow solid with melting point 111–112 °C; Yield: 71 %; IR (KBr) γ/cm^{-1}: 3,323.1 (NH stretch), 1,728.6 (C=O ester carbonyl), 1,673.7 (C=O stretching of keto group), 1.557.8 (NH–N=C), 1,544.4 (NO_2 asymmetric stretch), 1,346.5 (NO_2 symmetric stretch); ^{1}H NMR (DMSO-d_6 400 MHz) δ in ppm: 1.72 (t, 3H, CH_3), 2.44 (s, 3H, CH_3–C=O), 4.38 (q, 2H, CH_2), 7.44–7.30 (m, 4H, 3′, 4′, 5′, and 6′ protons of 2-nitrophenyl); 12.23 (s, 1H, NH); ^{13}C NMR (DMSO-d_6 100 MHz) δ in ppm: 21.94 (ester CH_3), 25.09 (acetyl CH_3), 51.84 (CH_2), 168.8 (C=O of acetyl), 162.2 (C=O of ester), 122.61 (C-5 of 2-nitrophenyl), 129.28 (C-4 of 2-nitrophenyl), 134.02 (C-6 of 2-nitrophenyl), 135.44 (C-3 of 2-nitrophenyl), 136.93 (C-1 of 2 -nitrophenyl), 137.92 (C-2 of 2-nitrophenyl), 141.02 (C=N–); LC–MS (*m*/*z*): 280 (M^++1); Anal. Calcd. for $C_{12}H_{13}N_3O_5$: C, 51.61; H, 4.69; N, 15.05. Found: C, 51.63; H, 4.67; N, 15.07.

*Ethyl-2-(4-anisyl)hydrazono-3-oxobutyrate (**3l**)*

Yellow solid with melting point 126–127 °C; Yield : 70 %. IR (KBr) γ/cm^{-1}: 3,317.3 (NH stretch), 1,730.7 (C=O ester carbonyl), 1,687.3 (C=O stretching of keto group), 1,557.8 (NH–N=C); ^{1}H NMR (DMSO-d_6 400 MHz) δ in ppm: 1.72 (t, 3H, CH_3), 2.44 (s, 3H, CH_3–C=O), 3.06 (s, 3H, OCH_3), 4.48 (q, 2H, CH_2), 7.44 (d, 2H, ortho protons of 4-anisyl), 7.66 (d, 2H, meta protons of 4-anisyl), 12.28 (s, 1H, NH); ^{13}C NMR (DMSO-d_6 100 MHz) δ in ppm : 21.98 (ester CH_3), 24.03 (acetyl CH_3), 51.09 (CH_2), 167.92 (C=O of acetyl), 163.14 (C=O of ester), 67.44 (OCH_3), 131.12 (C-3 and C-5 of 4-anisyl), 132.39 (C-2 and C-6 of 4-anisyl), 136.18 (C-4 of 4-anisyl), 136.72 (C-1 of 4-anisyl), 145.3 (C=N–); LC–MS (*m*/*z*): 265 (M^++1); Anal. Calcd. for $C_{13}H_{16}N_2O_4$: C, 59.08; H, 6.10; N, 10.60. Found: C, 59.07; H, 6.12; N, 10.61.

Procedure for the preparation of 2-[1-(4-isobutylphenyl)ethyl]-5-methyl-4-[2-(aryl substituted)hydrazinylidene]-2,4-dihydro-3H-pyrazol-3-one (**4a–l**)

(i) Microwave method

An equimolar mixture of ethyl-2-arylhydrazono-3-oxobutyrate (0.01 mol) and 2-[4-isobutylphenyl]propanoyl hydrazine (0.01 mol) were ground together to get a uniform mixture. For this 5–6 drops of glacial acetic acid was added and mixed well and it was kept inside a 100 ml round-bottom flask and subjected to microwave irradiation on a catalyst systems microwave oven operating at 490 W for about 3–5.5 min. Progress of the reaction was monitored by TLC. After the completion of reaction, the reaction mass was poured into crushed ice, solid obtained was filtered and recrystallized from ethanol–DMF mixture.

(ii) Conventional method

Ethyl-2-arylhydrazono-3-oxobutyrate (0.01 mol) was dissolved in 25 ml glacial acetic acid. To this a solution of 2-[4-isobutylphenyl]propanoyl hydrazine (0.01 mol) in glacial acetic acid was added and the mixture was refluxed for 8 h. Progress of the reaction was monitored by TLC. After the completion of the reaction it was cooled and allowed to stand overnight and the separated solid was filtered, dried and recrystallized from ethanol–DMF mixture.

Characterization data of the title compounds are given below:

*2-[1-(4-Isobutylphenyl)ethyl]-5-methyl-4-[2-(4-chlorophenyl)hydrazinylidene]-2,4-dihydro-3H-pyrazol-3-one (**4a**)*

Yellow solid, mp 270 °C, IR (KBr) γ/cm^{-1}: 3,153.3 (N–H), 2,957.6 (C–H), 1,708.7 (pyrazolone carbonyl), 1,690 (C=O), 1,542.2 (C=N); ^{1}H NMR (DMSO-d_6 400 MHz), δ in ppm: 0.83 (d, 6H, $J = 6.56$ Hz, $(CH_3)_2$), 1.43 (d, 3H, $J = 7$ Hz, CH_3), 1.83–1.73 (m, 1H, CH–$(CH_3)_2$), 2.21 (s, 3H, pyrazolone CH_3), 2.39 (d, 2H, $J = 7.16$ Hz, CH_2), 4.86 (q, 1H, CH), 7.10 (d, 2H, $J = 8.04$ Hz, 3′,5′-Ib–Ar–H), 7.24 (d, 2H, $J = 8.04$ Hz, 2′,6′-Ib–Ar–H), 7.49 (d, 2H, $J = 8.88$ Hz, ortho protons of 4-chlorophenyl), 7.68 (d, 2H, $J = 8.88$ Hz, meta protons of 4-chlorophenyl), 13.0 (s, 1H, NH); ^{13}C NMR ($CDCl_3$-d_6 100 MHz) δ in ppm: 12.01 (isobutyl-$(CH_3)_2$), 19.15 (CH_3), 22.40 (pyrazolone CH_3), 30.14 (isobutyl CH), 44.01 (isobutyl-CH_2), 45.06 (CH between pyrazolone and isobutylphenyl), 117.18 (C-4 of isobutylphenyl), 127.20 (C-3 and C-5 of isobutylphenyl), 127.84 (C-2 and C-6 of isobutylphenyl), 129.35 (C-1 of isobutylphenyl), 129.88 (C-2 and C-6 of 4-chlorophenyl), 131.68 (C-3 and C-5 of 4-chlorophenyl), 137.23 (C-1 of 4-chlorophenyl), 139.35 (C-4 of 4-chlorophenyl), 140.64 (C-5 of pyrazolone), 143.83 (C-4 of pyrazolone), 159.04 (C=O of pyrazolone), 161.35 (C=O between pyrazolone and isobutylphenyl); UPLC mass (*m*/*z*, %) 425.4 (M^++1, 100). Anal. Calcd. For $C_{23}H_{25}ClN_4O_2$: C, 65.01; H, 5.93; N, 13.19, Found: C, 65.03; H, 5.95; N, 13.17.

*2-[1-(4-Isobutylphenyl)ethyl]-5-methyl-4-[2-(3-nitrophenyl)hydrazinylidene]-2,4-dihydro-3H-pyrazol-3-one (**4b**)*

Yellow solid, mp 269 °C, IR (KBr) γ/cm^{-1}: 3,080.7 (N–H), 2,954.5 (C–H), 1,734.5 (pyrazolone carbonyl), 1,669.7 (C=O), 1,561.2 (C=N), 1,350.1 (NO_2 sym.), 1532.5 (NO_2 asym.); ^{1}H NMR (DMSO-d_6) δ in ppm: 0.84 (d, 6H, $J = 6.6$ Hz, $(CH_3)_2$), 1.44 (d, 3H, $J = 7$ Hz, CH_3), 1.80–1.75 (m, 1H, CH–$(CH_3)_2$), 2.23 (s, 3H, pyrazolone CH_3), 4.83 (q, 1H, CH), 2.39 (d, 2H, $J = 7.08$ Hz, CH_2), 7.11 (d, 2H, $J = 8.04$ Hz, 3′,5′-Ib–Ar–H), 7.25 (d, 2H, $J = 8.04$ Hz, 2′,6′-Ib–Ar–H), 7.71 (t, 1H, 5′-proton of 3-nitrophenyl), 8.02 (doublet of doublet, 1H, $J = 1.68$ and 8.12 Hz, 6′-proton of 3-nitrophenyl), 8.09 (doublet of doublet, 1H, $J = 1.44$ and 8.2 Hz, 4′-proton of 3-nitrophenyl), 8.51 (s, 1H, 2′-proton of 3-nitrophenyl), 13.08 (s, 1H, NH); ^{13}C NMR (DMSO-d_6) δ in ppm: 13.09 (isobutyl-$(CH_3)_2$), 19.91 (CH_3), 21.40 (pyrazolone CH_3), 31.44 (isobutyl CH), 41.31 (isobutyl-CH_2), 46.01 (CH between pyrazolone and isobutylphenyl), 121.18 (C-4 of isobutylphenyl), 126.20 (C-3 and C-5 of isobutylphenyl), 127.84 (C-2 and C-6 of isobutylphenyl), 128.91 (C-1 of isobutylphenyl), 129.98 (C-5 of 3-nitrophenyl), 131.16 (C-6 of 3-nitrophenyl), 134.23 (C-4 of 3-nitrophenyl), 134.98 (C-2 of 3-nitrophenyl), 133.88 (C-1 of 3-nitrophenyl), 138.22 (C-3 of 3-nitrophenyl), 140.62 (C-5 of pyrazolone), 142.4 (C-4 of pyrazolone), 159.04 (C=O of pyrazolone), 161.9 (C=O between pyrazolone and isobutylphenyl); UPLC mass (*m*/*z*, %) 436.4 (M^++1, 88). Anal. Calcd. For $C_{23}H_{25}N_5O_4$: C, 63.44; H, 5.79; N, 16.08, Found: C, 63.47; H, 5.81; N, 16.06.

*2-[1-(4-Isobutylphenyl)ethyl]-5-methyl-4-[2-(4-fluorophenyl)hydrazinylidene]-2,4-dihydro-3H-pyrazol-3-one (**4c**)*

Yellow solid, mp 277 °C, IR (KBr) γ/cm^{-1}: 3,251.3 (N–H), 2,868.1 (C–H), 1,726.7 (pyrazolone carbonyl), 1,685.7 (C=O), 1,541.9 (C=N); ^{1}H NMR (DMSO-d_6), δ in ppm: 0.83 (d, 6H, $J = 6.56$ Hz, $(CH_3)_2$), 1.43 (d, 3H, $J = 7$ Hz, CH_3), 1.73–1.83 (m, 1H, CH–$(CH_3)_2$), 2.21 (s, 3H, pyrazolone CH_3), 2.39 (d, 2H, $J = 7.16$ Hz, CH_2), 4.86 (q, 1H, CH), 7.10 (d, 2H, $J = 8.04$ Hz, 3′,5′-Ib–Ar–H), 7.24 (d, 2H, $J = 8.04$ Hz, 2′,6′-Ib–Ar–H), 7.17 (d, 2H, $J = 9.2$ Hz, ortho protons of 4-fluorophenyl), 7.48 (d, 2H, $J = 9.2$ Hz, meta protons of 4-fluorophenyl), 13.0 (S, 1H, NH);^{13}C NMR (DMSO-d_6) δ in ppm: 14.11 (isobutyl-$(CH_3)_2$), 20.15 (CH_3), 22.50 (pyrazolone CH_3), 33.14 (isobutyl CH), 43.51 (isobutyl-CH_2), 45.66 (CH between pyrazolone and isobutylphenyl), 120.33 18 (C-4 of isobutylphenyl), 129.60 (C-3 and C-5 of isobutylphenyl), 130.84 (C-2 and C-6 of isobutylphenyl), 131.35 (C-1 of isobutylphenyl), 133.88 (C-2 and C-6 of 4-fluorophenyl), 134.16 (C-3 and C-5 of 4-fluorophenyl), 136.73 (C-1 of 4-fluorophenyl), 137.31 (C-4 of 4-fluorophenyl), 141.62 (C-5 of pyrazolone), 143.4 (C-4 of pyrazolone), 160.04 (C=O of pyrazolone), 163.9 (C=O between pyrazolone and isobutylphenyl); LC–MS (*m*/*z*): 409 (M^++1). Anal. Calcd. For $C_{23}H_{25}FN_4O_2$: C, 67.63; H, 6.17; N, 13.72, Found: C, 67.65; H, 6.19; N, 13.74.

*2-[1-(4-Isobutylphenyl)ethyl]-5-methyl-4-[2-(2,4-difluorophenyl)hydrazinylidene]-2,4-dihydro-3H-pyrazol-3-one (**4d**)*

Yellow solid, mp 267 °C, IR (KBr) γ/cm^{-1}: 3,153.3 (N–H), 2,984.6 (C–H), 1,728.7 (pyrazolone carbonyl), 1,668.7 (C=O), 1,541.2 (C=N); ^{1}H NMR (DMSO-d_6), δ in ppm: 0.86 (d, 6H, $J = 6.56$ Hz, $(CH_3)_2$), 1.46 (d, 3H, $J = 7$ Hz, CH_3), 1.81–1.71 (m, 1H, CH–$(CH_3)_2$), 2.26 (s, 3H, pyrazole CH_3), 2.43 (d, 2H, $J = 7.18$ Hz, CH_2), 4.80 (q, 1H, CH), 7.12 (d, 2H, $J = 8.04$ Hz, 3′,5′-Ib–Ar–H), 7.26 (d, 2H, $J = 8.04$ Hz, 2′,6′-Ib–Ar–H), 7.05–7.00 (m, 2H, 5′ and 6′-protons of 2,4-difluorophenyl), 7.83 (m, 1H, 3′-proton of 2,4-difluorophenyl), 13.0 (s, 1H, NH); ^{13}C NMR (DMSO-d_6) δ in ppm:

13.64 (isobutyl-$(CH_3)_2$), 19.01 (CH_3), 20.30 (pyrazolone CH_3), 32.46 (isobutyl CH), 40.96 (isobutyl-CH_2), 45.31 (CH between pyrazolone and isobutylphenyl), 119.28 (C-4 of isobutylphenyl), 125.58 (C-3 and C-5 of isobutylphenyl), 126.20 (C-2 and C-6 of isobutylphenyl), 126.56 (C-1 of isobutylphenyl), 127.84 (C-6 of 2,4-difluorophenyl), 128.8 (C-5 of 2,4-difluorophenyl), 131.16 (C-3 of 2,4-difluorophenyl), 134.23 (C-1 of 2,4-difluorophenyl), 137.19 (C-4 of 2,4-difluorophenyl), 138.91 (C-2 of 2,4-difluorophenyl), 141.82 (C-5 of pyrazolone), 143.24 (C-4 of pyrazolone), 162.04 (C=O of pyrazolone), 164.93 (C=O between pyrazolone and isobutylphenyl); LC–MS (*m/z*): 426 (M^+). Anal. Calcd. For $C_{23}H_{24}F_2N_4O_2$: C, 64.78; H, 5.67; N, 13.14, Found: C, 64.76; H, 5.69; N, 13.17.

*2-[1-(4-Isobutylphenyl)ethyl]-5-methyl-4-[2-(2-anisyl)hydrazinylidene]-2,4-dihydro-3H-pyrazol-3-one (**4e**)*

It was obtained as a reddish brown solid, mp 248 °C, IR (KBr) γ/cm^{-1}: 3,173.3 (N–H), 2,957.6 (C–H), 1,719.7 (pyrazolone carbonyl), 1,668.7 (C=O), 1,542.2 (C=N); ^{1}H NMR (DMSO-d_6), δ in ppm: 0.83 (d, 6H, $J = 6.56$ Hz, $(CH_3)_2$), 1.43 (d, 3H, $J = 7$ Hz, CH_3), 1.83–1.73 (m, 1H, CH–$(CH_3)_2$), 2.21 (s, 3H, pyrazolone CH_3), 2.39 (d, 2H, $J = 7.16$ Hz, CH_2), 4.86 (q, 1H, CH), 3.91 (s, 3H, OCH_3), 7.10 (d, 2H, $J = 8.04$ Hz, 3′,5′-Ib–Ar–H), 7.24 (d, 2H, $J = 8.04$ Hz, 2′,6′-Ib–Ar–H), 7.79–6.99 (m, 4H, aromatic protons of 2-anisyl), 13.0 (s, 1H, NH); ^{13}C NMR (DMSO-d_6) δ in ppm: 13.04. (isobutyl-$(CH_3)_2$), 18.99 (CH_3), 21.40 (pyrazolone CH_3), 33.56 (isobutyl CH), 40.06 (isobutyl-CH_2), 44.68 (CH between pyrazolone and isobutylphenyl), 56.88 (OCH_3), 126.21 (C-4 of isobutylphenyl), 127.11 (C-3 and C-5 of isobutylphenyl), 128.14 (C-2 and C-6 of isobutylphenyl), 128.84 (C-1 of isobutylphenyl), 129.16 (C-4 of 2-anisyl), 132.11 (C-5 of 2-anisyl), 133.13 (C-3 of 2-anisyl), 133.91 (C-6 of 2-anisyl), 136.36 (C-2 of 2-anisyl), 136.78 (C-1 of 2-anisyl), 144.82 (C-5 of pyrazolone), 145.21 (C-4 of pyrazolone), 162.91 (C=O of pyrazolone), 164.93 (C=O between pyrazolone and isobutylphenyl); LC–MS (*m/z*): 421 (M^++1).Anal. Calcd. For $C_{24}H_{28}N_4O_3$: C, 68.55; H, 6.71; N, 13.32, Found: C, 68.58; H, 6.73; N, 13.35.

*2-[1-(4-Isobutylphenyl)ethyl]-5-methyl-4-[2-(3-chloro-4-fluorophenyl)hydrazinylidene]-2,4-dihydro-3H-pyrazol-3-one (**4f**)*

Yellow solid, mp 270 °C, IR (KBr) γ/cm^{-1}: 3,200.9 (N–H), 2,955.9 (C–H), 1,795.9 (pyrazolone carbonyl), 1,657.5 (C=O), 1,560.2 (C=N); ^{1}H NMR (DMSO-d_6 300 MHz), δ in ppm: 0.84 (d, 6H, $J = 6.6$ Hz, $(CH_3)_2$), 1.51 (d, 3H, $J = 7.26$ Hz, CH_3), 1.81–1.70 (m, 1H, CH–$(CH_3)_2$), 2.36 (s, 3H, pyrazolone CH_3), 2.39 (d, 2H, $J = 7.29$ Hz, CH_2), 4.31 (q, 1H, CH), 7.08 (d, 2H, $J = 8.1$ Hz, 3′,5′-Ib–Ar–H), 7.16 (d, 2H, $J = 8.04$ Hz, 2′,6′-Ib–Ar–H), 7.70–7.59 (m, 3H, 2′, 5′ and 6′-protons of 3-chloro-4-fluorophenyl), 13.06 (s, 1H, NH); ^{13}C NMR (DMSO-d_6) δ in ppm: 14.11. (isobutyl-$(CH_3)_2$), 19.99 (CH_3), 21.10 (pyrazolone CH_3), 31.36 (isobutyl CH), 39.26 (isobutyl-CH_2), 44.87 (CH between pyrazolone and isobutylphenyl), 122.31 (C-4 of isobutylphenyl), 123.51 (C-3 and C-5 of isobutylphenyl), 124.81 (C-2 and C-6 of isobutylphenyl), 126.14 (C-1 of isobutylphenyl), 130.93 (C-6 of 3-chloro-4-fluorophenyl), 131.11 (C-2 of 3-chloro-4-fluorophenyl), 131.76 (C-5 of 3-chloro-4-fluorophenyl), 132.91 (C-1 of 3-chloro-4-fluorophenyl), 134.23 (C-3 of 3-chloro-4-fluorophenyl), 136.98 (C-4 of 3-chloro-4-fluorophenyl), 141.82 (C-5 of pyrazolone), 143.24 (C-4 of pyrazolone), 162.04 (C=O of pyrazolone), 164.93 (C=O between pyrazolone and isobutylphenyl); UPLC-mass (*m/z*, %): 443.2 (M^+, 89 %). Anal. Calcd. For $C_{23}H_{24}ClFN_4O_2$: C, 62.37; H, 5.46; N, 12.65, Found: C, 62.39; H, 5.44; N, 12.68.

*2-[1-(4-Isobutylphenyl)ethyl]-5-methyl-4-[2-(2-carboxyphenyl)hydrazinylidene]-2,4-dihydro-3H-pyrazol-3-one (**4g**)*

Yellow solid, mp 272 °C, IR (KBr) γ/cm^{-1}: 3,158.3 (N–H), 2,947.6 (C–H), 1,729.3 (pyrazolone carbonyl), 1,719.2 (carboxyl C=O), 1,678.2 (C=O), 1,540.5 (C=N); ^{1}H NMR (DMSO-d_6), δ in ppm: 0.84 (d, 6H, $J = 6.58$ Hz, $(CH_3)_2$), 1.44 (d, 3H, $J = 7$ Hz, CH_3), 1.79–1.67 (m, 1H, CH–$(CH_3)_2$), 2.31 (s, 3H, pyrazolone CH_3), 2.43 (d, 2H, $J = 7.16$ Hz, CH_2), 4.80 (q, 1H, CH), 7.12 (d, 2H, $J = 8.04$ Hz, 3′,5′-Ib–Ar–H), 7.22 (d, 2H, $J = 8.04$ Hz, 2′,6′-Ib–Ar–H), 6.98–7.79 (m, 4H, aromatic protons of 2-carboxyphenyl), 12.81 (s, 1H, NH), 13.10 (s, broad, 1H, OH); ^{13}C NMR (DMSO-d_6) δ in ppm: 13.94. (isobutyl-$(CH_3)_2$), 19.09 (CH_3), 22.40 (pyrazolone CH_3), 33.86 (isobutyl CH), 40.96 (isobutyl-CH_2), 44.78 (CH between pyrazolone and isobutylphenyl), 119.91 (C-4 of isobutylphenyl), 120.46 (C-3 and C-5 of isobutylphenyl), 122.11 (C-2 and C-6 of isobutylphenyl), 127.31 (C-1 of isobutylphenyl), 128.11 (C-5 of 2-carboxyphenyl), 129.21 (C-4 of 2-carboxyphenyl), 129.86 (C-6 of 2-carboxyphenyl), 130.11 (C-3 of 2-carboxyphenyl), 132.93 (C-1 of 2-carboxyphenyl), 134.84 (C-2 of 2-carboxyphenyl), 143.82 (C-5 of pyrazolone), 146.21 (C-4 of pyrazolone), 161.91 (C=O of pyrazolone), 164.34 (C=O of carboxyl), 165.93 (C=O between pyrazolone and isobutylphenyl); LC–MS (*m/z*): 435 (M^++1). Anal. Calcd. For $C_{24}H_{26}N_4O_4$: C, 66.34; H, 6.03; N, 12.89, Found: C, 66.37; H, 6.00; N, 12.91.

*2-[1-(4-Isobutylphenyl)ethyl]-5-methyl-4-[2-(4-carboxyphenyl)hydrazinylidene]-2,4-dihydro-3H-pyrazol-3-one (**4h**)*

Yellow solid, mp 258 °C, IR (KBr) γ/cm^{-1}: 3,178.7 (N–H), 3,317.56 (O–H), 2,922.16 (C–H), 1,757.2 (carboxyl C=O),

1,720 (pyrazolone carbonyl), 1,614.42 (C=O), 1,543.05 (C=N); ^{1}H NMR (DMSO-d_6): 0.85 (d, 6H, $J = 6.56$ Hz, $(CH_3)_2$), 1.32 (d, 3H, $J = 7$ Hz, CH_3), 2.51–2.50 (m, 1H, CH–$(CH_3)_2$), 2.16 (s, 3H, pyrazolone CH_3), 2.89 (d, 2H, $J = 7.16$ Hz, CH_2), 4.86 (q, 1H, CH), 7.08 (d, 2H, $J = 8.08$ Hz, 3′,5′-Ib–Ar–H), 7.24 (d, 2H, $J = 8.00$ Hz, 2′,6′-Ib–Ar–H), 7.62 (d, 2H, $J = 8.64$ Hz, ortho protons of 4-carboxyphenyl), 7.98 (d, 2H, $J = 8.68$ Hz, meta protons of 4-carboxyphenyl), 12.87, (s, 1H, NH), 13.20 (s, broad, 1H, OH); ^{13}C NMR (DMSO-d_6) δ in ppm: 12.11 (isobutyl-$(CH_3)_2$), 19.69 (CH_3), 22.49 (pyrazolone CH_3), 30.54 (isobutyl CH), 44.61 (isobutyl-CH_2), 45.36 (CH between pyrazolone and isobutylphenyl), 120.18 (C-4 of isobutylphenyl), 128.75 (C-3 and C-5 of isobutylphenyl), 129.20 (C-2 and C-6 of isobutylphenyl), 129.62 (C-1 of isobutylphenyl), 130.84 (C-2 and C-6 of 4-carboxyphenyl), 131.16 (C-3 and C-5 of 4-carboxyphenyl), 136.23 (C-1 of 4-carboxyphenyl), 138.95 (C-4 of 4-carboxyphenyl), 140.62 (C-5 of pyrazolone), 142.11 (C-4 of pyrazolone), 163.04 (C=O of pyrazolone), 165.11 (C=O of carboxyl), 168.91 (C=O between pyrazolone and isobutylphenyl); LC–MS (*m*/*z*): 433.3 (M^+-1). Anal. Calcd. For $C_{24}H_{26}N_4O_4$: C, 66.34; H, 6.03; N, 12.89, Found: C, 66.32; H, 6.05; N, 12.87.

*2-[1-(4-Isobutylphenyl)ethyl]-5-methyl-4-[2-(4-nitrophenyl)hydrazinylidene]-2,4-dihydro-3H-pyrazol-3-one (**4i**)*

Yellow solid, mp 269 °C, IR (KBr) γ/cm^{-1}: 3,268.1 (N–H), 2,950.9 (C–H), 1,736.2 (pyrazolone carbonyl), 1,641.0 (C=O), 1,539.6 (C=N), 1,373.6 (NO_2 sym.), 1,506.2 (NO_2 asym.); ^{1}H NMR (DMSO-d_6), δ in ppm: 0.85 (d, 6H, $J = 6.6$ Hz, $(CH_3)_2$), 1.34 (d, 3H, $J = 7.12$ Hz, CH_3), 1.81–1.70 (m, 1H, CH–$(CH_3)_2$), 2.66 (s, 3H, pyrazolone CH_3), 2.41 (d, 2H, $J = 7.04$ Hz, CH_2), 4.64 (q, 1H, CH), 7.19 (d, 2H, $J = 7.76$ Hz, 3′,5′-Ib–Ar–H), 7.27 (d, 2H, $J = 8.04$ Hz, 2′,6′-Ib–Ar–H), 8.03 (doublet of doublet 2H, $J = 1.96$ and 7.04 Hz, ortho protons of 4-nitrophenyl), 8.51 (doublet of doublet, 2H, $J = 2$ and 7 Hz, meta protons of 4-nitrophenyl), 13.01 (s, 1H, NH); ^{13}C NMR (DMSO-d_6) δ in ppm: 12.01 (isobutyl-$(CH_3)_2$), 19.21 (CH_3), 22.43 (pyrazolone CH_3), 31.14 (isobutyl CH), 43.91 (isobutyl-CH_2), 45.86 (CH between pyrazolone and isobutylphenyl), 119.97 (C-4 of isobutylphenyl), 127.84 (C-3 and C-5 of isobutylphenyl), 128.20 (C-2 and C-6 of isobutylphenyl), 129.85 (C-1 of isobutylphenyl), 130.08 (C-2 and C-6 of 4-nitrophenyl), 132.11 (C-3 and C-5 of 4-nitrophenyl), 136.35 (C-1 of 4-nitrophenyl), 137.23 (C-4 of 4-nitrophenyl), 141.62 (C-5 of pyrazolone), 142.98 (C-4 of pyrazolone), 159.04 (C=O of pyrazolone), 164.9 (C=O between pyrazolone and isobutylphenyl);LC–MS (*m*/*z*): 436 (M^++1).Anal. Calcd. For $C_{23}H_{25}N_5O_4$: C, 63.44; H, 5.79; N, 16.08, Found: C, 63.47; H, 5.82; N, 16.06.

*2-[1-(4-Isobutylphenyl)ethyl]-5-methyl-4-[2-(3-chlorophenyl)hydrazinylidene]-2,4-dihydro-3H-pyrazol-3-one (**4j**)*

Yellow solid, mp 276 °C, IR (KBr) γ/cm^{-1}: 3,253.3 (N–H), 2,867.6 (C–H), 1,715.2 (pyrazolone carbonyl), 1,667.7 (C=O), 1,536.2 (C=N); ^{1}H NMR (DMSO-d_6), δ in ppm: 0.84 (d, 6H, $J = 6.56$ Hz, $(CH_3)_2$), 1.68 (d, 3H, $J = 7$ Hz, CH_3), 1.78–1.82 (m, 1H, CH–$(CH_3)_2$), 2.47 (s, 3H, pyrazolone CH_3), 2.36 (d, 2H, $J = 7.14$ Hz, CH_2), 4.80 (q, 1H, CH), 7.16 (d, 2H, $J = 8.06$ Hz, 3′,5′-Ib–Ar–H), 7.36 (d, 2H, $J = 8.08$ Hz, 2′,6′-Ib–Ar–H), 7.69 (m, 3H, 4′, 5′ and 6′-protons of 3-chlorophenyl), 8.36 (s, 1H, 2′-proton of 3-chlorophenyl), 13.0 (s, 1H, NH);^{13}C NMR (DMSO-d_6) δ in ppm: 13.19. (isobutyl-$(CH_3)_2$), 19.41 (CH_3), 21.44 (pyrazolone CH_3), 32.44 (isobutyl CH), 42.01 (isobutyl-CH_2), 45.01 (CH between pyrazolone and isobutylphenyl), 120.18 (C-4 of isobutylphenyl), 128.20 (C-3 and C-5 of isobutylphenyl), 128.92 (C-2 and C-6 of isobutylphenyl), 129.84 (C-1 of isobutylphenyl), 131.88 (C-5 of 3-chlorophenyl), 133.26 (C-6 of 3-chlorophenyl), 133.53 (C-4 of 3-chlorophenyl), 133.98 (C-2 of 3-chlorophenyl), 135.88 (C-1 of 3-chlorophenyl), 136.15 (C-3 of 3-chlorophenyl), 141.12 (C-5 of pyrazolone), 143.2 (C-4 of pyrazolone), 161.04 (C=O of pyrazolone), 163.95 (C=O between pyrazolone and isobutylphenyl) LC–MS (*m*/*z*): 425 (M^++1). Anal. Calcd. For $C_{23}H_{25}ClN_4O_2$: C, 65.01; H, 5.93; N, 13.19, Found: C, 64.99; H, 5.96; N, 13.21.

*2-[1-(4-Isobutylphenyl)ethyl]-5-methyl-4-[2-(2-nitrophenyl)hydrazinylidene]-2,4-dihydro-3H-pyrazol-3-one (**4k**)*

It was obtained as a yellow solid, mp 272 °C, IR (KBr) γ/cm^{-1}: 3,203.9 (N–H), 2,950.9 (C–H), 1,712.2 (pyrazolone carbonyl), 1,669.3 (C=O), 1,539.2 (C=N), 1,344.8 (NO_2 sym.), 1,539 (NO_2 asym.); ^{1}H NMR (DMSO-d_6), δ in ppm: 0.85 (d, 6H, $J = 6.56$ Hz, $(CH_3)_2$), 1.41 (d, 3H, $J = 7$ Hz, CH_3), 1.66–1.79 (m, 1H, CH–$(CH_3)_2$), 2.40 (s, 3H, pyrazolone CH_3), 2.37 (d, 2H, $J = 7.16$ Hz, CH_2), 4.78 (q, 1H, CH), 7.14 (d, 2H, $J = 8.04$ Hz, 3′,5′-Ib–Ar–H), 7.22 (d, 2H, $J = 8.04$ Hz, 2′,6′-Ib–Ar–H), 8.12–7.69 (m, 4H, aromatic protons of 2-nitrophenyl), 13.0 (s, 1H, NH); ^{13}C NMR (DMSO-d_6) δ in ppm : 12.31. (isobutyl-$(CH_3)_2$), 18.99 (CH_3), 22.89 (pyrazolone CH_3), 31.12(isobutyl CH), 43.61 (isobutyl-CH_2), 46.36 (CH between pyrazolone and isobutyl pheny), 120.96 (C-4 of isobutylphenyl), 121.91 (C-3 and C-5 of isobutylphenyl), 125.19 (C-2 and C-6 of isobutylphenyl), 126.31 (C-1 of isobutylphenyl), 128.11 (C-5 of 2-nitrophenyl), 129.31 (C-4 of 2-nitrophenyl), 130.16 (C-6 of 2-nitrophenyl), 131.01 (C-3 of 2-nitrophenyl), 131.93 (C-1 of 2-nitrophenyl), 132.11 (C-2 of 2-nitrophenyl), 142.82 (C-5 of pyrazolone), 145.21 (C-4 of

pyrazolone), 162.01 (C=O of pyrazolone), 165.93 (C=O between pyrazolone and isobutylphenyl); LC–MS (*m/z*): 436 (M^++1). Anal. Calcd. For $C_{23}H_{25}N_5O_4$: C, 63.44; H, 5.79; N, 16.08, Found: C, 63.46; H, 5.81; N, 16.11.

*2-[1-(4-Isobutylphenyl)ethyl]-5-methyl-4-[2-(4-anisyl)hydrazinylidene]-2,4-dihydro-3H-pyrazol-3-one (**4l**)*

Yellow solid, mp 279 °C, IR (KBr) γ/cm^{-1}: 3,201.3 (N–H), 2,954.8 (C–H), 1,795.4 (pyrazolone carbonyl), 1,655.6 (C=O), 1,560.7 (C=N); ^{1}H NMR (DMSO-d_6), δ in ppm: 0.83 (d, 6H, $J = 6.56$ Hz, $(CH_3)_2$), 1.58 (d, 3H, $J = 7.08$ Hz, CH_3), 1.80–1.74 (m, 1H, CH–$(CH_3)_2$), 2.65 (s, 3H, pyrazolone CH_3), 3.90 (s, 3H, OCH_3), 2.39 (d, 2H, $J = 7.08$ Hz, CH_2), 4.27 (q, 1H, CH), 7.08 (d, 2H, $J = 7.92$ Hz, 3′,5′-Ib–Ar–H), 7.22 (d, 2H, $J = 7.96$ Hz, 2′,6′-Ib–Ar–H), 7.33 (d, 2H, $J = 8.96$ Hz, ortho protons of 4-anisyl), 7.80 (d, 2H, $J = 8.92$ Hz meta protons of 4-anisyl), 12.90 (s, 1H, NH); ^{13}C NMR (DMSO-d_6) in ppm: 13.11 (isobutyl-$(CH_3)_2$), 20.01 (CH_3), 22.93 (pyrazolone CH_3), 32.11(isobutyl CH), 42.21 (isobutyl-CH_2), 44.96 (CH between pyrazolone and isobutylphenyl), 55.87 (OCH_3), 120.87 (C-4 of isobutylphenyl), 129.16 (C-3 and C-5 of isobutylphenyl), 129.64 (C-2 and C-6 of isobutylphenyl), 130.55 (C-1 of isobutylphenyl), 132.41 (C-2 and C-6 of 4-anisyl), 133.08 (C-3 and C-5 of 4-anisyl), 136.25 (C-1 of 4-anisyl), 137.02 (C-4 of 2-anisyl), 142.62 (C-5 of pyrazolone), 143.92 (C-4 of pyrazolone), 161.04 (C=O of pyrazolone), 164.56 (C=O between pyrazolone and isobutylphenyl); LC–MS (*m/z*): 421.5 (M^++1). Anal. Calcd. For $C_{24}H_{28}N_4O_3$: C, 68.55; H, 6.71; N, 13.32, Found: C, 68.57; H, 6.74; N, 13.35.

Biological evaluation

In vitro α-amylase inhibition

The α-amylase inhibitory activity of newly synthesized compounds was carried out using the method of Giancarlo *et al.* (2006), with slight modifications. It was performed by using 1 ml of enzyme solution (1 U/ml in 20 mM PBS of pH 6.9), 1 ml of different concentration (25, 50, 75 and 100 µg) of synthesized compounds in dimethyl sulfoxide (DMSO) was incubated at 25 °C, for 30 min, added 1 ml of 0.5 % of starch solution and tubes were further incubated for 3 min at 25 °C. To this, 1 ml of 96 mM of 3,5-dinitro salicylic acid solution (prepared by using 5.31 M sodium potassium tartrate in 2 M sodium hydroxide) was added. The reaction mixture was shaken well and closed tube was placed in a water bath at 85 °C for 15 min. The reaction mixture was diluted with 9 ml of distilled water and resulting solution was measured at 540 nm by spectrophotometer (Shimadzu-1800) to determine the inhibition of enzyme. Then, the enzyme was incubated with starch solution without adding any inhibitors which act as positive control. The inhibitory effect of the synthesized compound was compared with standard α-inhibitor Acarbose solution of same concentration. The inhibition percentage of α-amylase was assessed by the following formula:

$$\% \text{ of } I_{\alpha-\text{amylase}} = \left(\Delta A_{\text{control}} - \Delta A_{\text{sample}}\right)/\Delta A_{\text{control}} \times 100,$$

where $\Delta A_{\text{control}} = A_{\text{Test}} - A_{\text{Blank}}$,

$\Delta A_{\text{sample}} = A_{\text{Test}} - A_{\text{Blank}}$.

Acute toxicity and gross behavioral studies

The acute oral toxicity study for the organic compound **4a–l** was carried out following the OECD guidelines No. 420. Swiss albino male mice weighing 25–30 g were used for the evaluation. Each group consisting of 6 male mice (overnight fasted) was kept in the colony cage at 25 ± 2 °C with 55 % relative humidity and 12-h light/dark cycle was maintained. A specified fixed dose of 250, 500, 750, 1000, 1500, 2000, and 3000 mg/kg was selected and administered orally as a single dose as fine suspension prepared in saline using gum acacia powder. The acute toxic symptoms and the behavioral changes produced by the test compounds were observed continuously for 4th, 8th, 12th, and 24th h onset of toxic symptoms and gross behavioral changes were also recorded (Jaouhari *et al.*, 1999).

Simulation studies

X-ray crystallographic structure resolved at 2.0 Å of *Aspergillus oryzae* alpha-amylase complex with inhibitor acarbose (PDB: 7TAA) was obtained from protein databank. The structure 7TAA was simplified by removing HETATOMS, water molecules, and co-crystallized compounds. For all ligands (**4a**, **4d**, **4f**, **4g**, and acarbose) topology file and other force field parameters were generated using PRODRG server (van Aalten *et al.*, 1996). Flexible torsions for ligands were defined using AutoTors provided with AutoDock suite. Acarbose was redocked together with **4a**, **4d**, **4f**, and **4g** in the inhibitor binding site. The docking site for all ligands in 7TAA was considered at co-crystallized position of acarbose. PyRx 0.8 interface was used (Wolf, 2009) with grid box size 89 × 85 × 74, spacing 0.375, grid center 41.0450, 39.9533, 27.6100, and assigning 3 degrees of Freedom. The Lamarckian genetic algorithm was employed (Morris and Goodsell, 1998) with 10 conformations, for each ligand and best pose with the lowest binding energy was considered. For all the ligands binding energy and inhibition constant (Ki) were tabulated. Python molecular viewer

Table 1 Comparative data showing the yield and reaction time by conventional and microwave irradiation methods for the synthesis of pyrazolones (**4a–l**)

Comp. no.	Molecular formula (mol. wt.)	Conventional method		Microwave method	
		Yield (%)	Reaction time (h)	Yield (%)	Reaction time (min)
4a	$C_{23}H_{25}ClN_4O_2$ (424.92)	56	8	69	5.0
4b	$C_{23}H_{25}N_5O_4$ (435.47)	57	8	71	4.5
4c	$C_{23}H_{25}FN_4O_2$ (408.47)	55	10	69	5.5
4d	$C_{23}H_{24}F_2N_4O_2$ (426.46)	58	9	70	4.6
4e	$C_{24}H_{28}N_4O_3$ (420.50)	59	11	71	4.5
4f	$C_{23}H_{24}ClFN_4O_2$ (442.91)	61	10	74	5.5
4g	$C_{24}H_{26}N_4O_4$ (434.48)	60	9	75	5.0
4h	$C_{24}H_{26}N_4O_4$ (434.48)	62	13	77	5.0
4i	$C_{23}H_{25}N_5O_4$ (435.47)	60	10	78	4.5
4j	$C_{23}H_{25}ClN_4O_2$ (424.92)	59	10	74	5.3
4k	$C_{23}H_{25}N_5O_4$ (435.47)	60	13	75	4.8
4l	$C_{24}H_{28}N_4O_3$ (420.50)	61	14	77	4.4

(Michel, 1999) was used for docking confirmation representation.

Results and discussion

Chemistry

After optimization of the experimental conditions, 2-[1-(4-isobutylphenyl)ethyl]-5-methyl-4-[2-(aryl substituted)hydrazinylidene]-2,4-dihydro-3*H*-pyrazol-3-one was synthesized by the reaction of equimolar mixture of 2-[4-isobutylphenyl] propanoyl hydrazine and ethyl-2-arylhydrazono-3-oxobutyrate by both conventional heating and microwave irradiation method. The main advantage of microwave-mediated method is reduced time interval. In microwave irradiation method, the yields were high as compared to the yield obtained by conventional method. Microwave irradiation method facilitates the polarization of the reacting molecule causing reactions to occur at higher rate. A comparative study in terms of yield and reaction time is shown in Table 1. It is noteworthy that the reaction, which required 8–14 h in conventional method, was completed within 3.50–5.50 min in microwave system at power level of 490 W. Also yields have been remarkably improved from 55–62 to 69–78 %.

Pharmacology

In this study, we have evaluated the inhibitory effects of title compounds (**4a–l**) against α-amylase to elucidate the possible use of title compounds as anti-hyperglycemic agents. The inhibitory effect of the title compounds are shown in Table 2. The bar diagram representation of comparative activities of title compounds (**4a–l**) is shown in Fig. 1.

Table 2 In vitro alpha amylase inhibition activity of pyrazolone derivatives (**4a–l**)

Compounds	% of inhibition
4a	46.2 ± 1.3
4b	10.6 ± 0.9
4c	26.7 ± 1.5
4d	61.6 ± 1.3
4e	40.0 ± 0.8
4f	60.4 ± 1.6
4g	47.3 ± 1.5
4h	32.0 ± 2.1
4i	–
4j	10.5 ± 0.6
4k	40.7 ± 1.4
4l	16.3 ± 1.5
Acarbose	87.7 ± 1.2

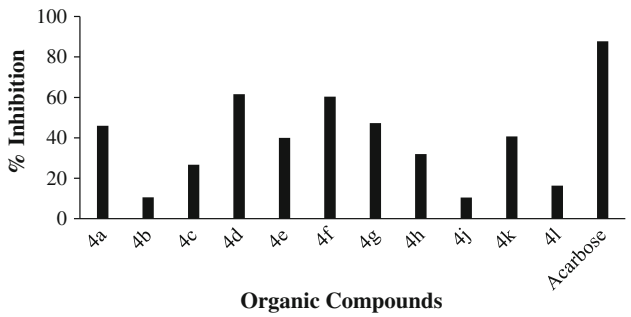


Fig. 1 Bar diagram showing α-amylase inhibition activity of compounds **4a–l**

Molecular docking simulation study of the synthesized compounds and acarbose was carried out in order to gain functional and structural insight into the mechanism of

Table 3 The binding energy (kJ mol^{-1}) and inhibition constant of **4a**, **4d**, **4f**, **4g**, and acarbose

Compound	Binding energy (kJ mol^{-1})	Inhibition constant (μM)
4a	−5.97	37.04
4d	−2.09	41.98
4f	−3.65	40.69
4g	−5.38	37.52
Acarbose	−1.6	66.63

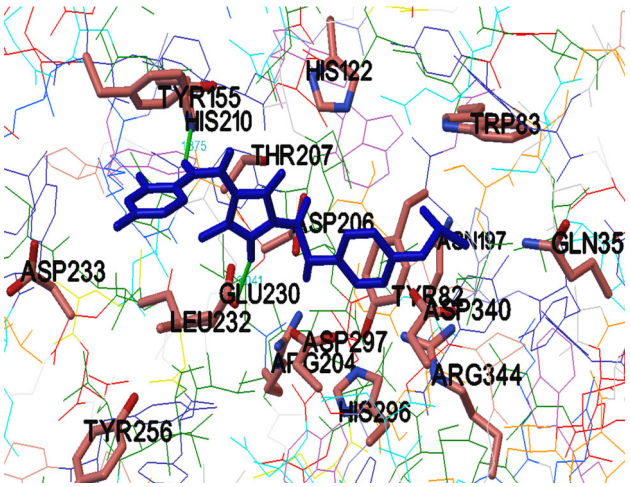


Fig. 2 **4d** docked in best of its conformation with α-amylase (i.e., 7TAA). Ligand **4d** shown in ball and stick model in *blue color*, amino acid residues in active pocket shown in ball and stick model as colored by rasmol view and remaining residues of the protein as lines colored by rasmol view. Hydrogen bonds represented as *green cylinders*. **4d** forms two hydrogen bonds in total, 1 bound with GLU230 with bond length 2.041 Å, and another with HIS210 with the bond length 1.875 Å (Color figure online)

inhibition. Auto Dock 4.2 suite was used as molecular docking tool. The docking of receptor α-amylase with newly synthesized candidate ligands exhibited well established binding energy in the receptor active pocket. The binding energy, inhibition constant of the compound **4a**, **4d**, **4f**, **4g**, and acarbose is shown in Table 3. It is observed that the compounds with good amylase inhibitory activity docked in best of its conformation with α-amylase (i.e., 7TAA) as shown in Fig. 2; and also acarbose docked in best of its conformation with α-amylase (i.e., 7TAA) is shown in Fig. 3.

The acute toxic symptoms and the behavioral changes produced by the test compounds were observed. The experimental studies revealed that these compounds were quite safe up to 250, 500, 750, 1000, and 2000 mg/kg, and no death of animals were recorded. Further, no significant gross behavioral changes were observed in experimental animals except in the 3,000 mg/kg of **4a**, **4b**, **4d**, **4j**, and **4l**

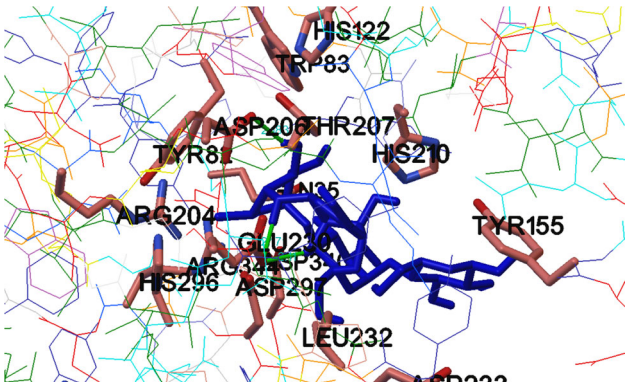


Fig. 3 Acarbose docked in best of its conformation with α-amylase (i.e., 7TAA). Ligand acarbose shown in ball and stick model as *blue color*, amino acid residues in active pocket shown in ball and stick model as colored by rasmol view and remaining residues of the protein as lines colored by rasmol view. Hydrogen bonds represented as *green cylinders*. Acarbose forms three hydrogen bonds in total, 2 bound in common with GLU230 with bond lengths 2.171 and 2.11 Å, and third bound with Asp206 with the bond length 2.183 Å (Color figure online)

compounds, which showed depression on the first day and death on second day.

Structure–activity relationship

Based on the nature of substituents each of the synthesized individual compounds showed sensitivity toward alpha amylase inhibition. With regard to SAR we observed that the substituent's present on phenyl ring remarkably results in the increase or decrease in the inhibition. Compounds **4d**, **4f**, **4a**, and **4g** exhibited good inhibitory activity against α-amylase as compared to the control enzyme inhibitor, whereas rest of the compounds showed weak inhibitory activity. Introduction of disubstituted halogen derivatives caused good inhibitory activity as observed in the case of 2,4-difluoro (**4d**) and 3-chloro-4-fluoro (**4f**) substituents. This may be due to the high electronegativity effect of the halogens. Also it is noticeable that difluoro derivative showed slight higher inhibition than chloro–fluoro substituent. Whereas, the compounds carrying 4-chloro (**4a**) and 2-carboxyl (**4g**) substituents showed moderate activity. But when the carboxyl group is present at para position it diminishes the activity compared to ortho position. Compounds with meta substituents like 3-nitro and 3-chloro groups showed very weak inhibition. Also the para-substituted groups such as 4-carboxyl, 4-methoxy, and 4-fluoro groups showed weak inhibition. In case of nitro group, ortho-substituted group showed moderate activity, but the para-substituted compound did not showed any activity.

Conclusion

Pyrazolones are highly versatile members of the heterocyclic compounds. They possess an array of interesting chemical and variety of biological activities. From the above studies it can be concluded that microwave technique is one of the promising technique of synthesis with better yield, lower reaction time, and provides green chemical pathway. Alpha amylase inhibition results of pyrazolone derivatives revealed that compounds with high electron withdrawing substituents showed good inhibitory activity, i.e., compounds **4d** and **4f** with dihalo substituent's showed good inhibition. In silico studies revealed that synthesized molecules **4a**, **4d**, **4f**, and **4g** showed good binding energy toward the target protein ranging from -2.09 to -5.97 kJ mol^{-1} and **4d** being the best among them. All the docking results showed the best RMS value, i.e., 0.0.

The preliminary in vitro anti-diabetic activity of these series of pyrazolone derivatives has shown that compounds (**4a**, **4d**, **4f**, and **4g**) have exhibited good α-amylase inhibition. The in silico molecular docking results are matching with the in vitro studies and they may be considered as good inhibitor of α-amylase enzyme.

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