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A Divergent Synthesis and *in vitro* Biological Evaluation of Novel Pyrazolyl Chalcones and Pyrazolyl Flavones

TANJIMA TARIQUE LASKAR

Department of Pharmaceutical Sciences, University of Science and Technology Meghalaya, Ri-Bhoi, Baridua-793101, India

Corresponding author: Fax: +91 3844 224243; E-mail: tanjimatarique.12@gmail.com

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A total of sixteen flavonoids, eight each of pyrazolyl chalcones [**IM-3(a-h)**] and pyrazolyl flavones [**IM-4(a-h)**] were synthesized and tested for *in vitro* antidiabetic, antiarthritic, anticoagulant, anthelmintic, anti-inflammatory and antioxidant activities by various methods such as α-amylase inhibitory method, egg albumin denaturation method, prothrombin time method, paralysis and death time method, bovine serum denaturation method and DPPH radical scavenging activity method, respectively. Structural analysis of the synthesized chalcone and flavone derivatives was carried out by various characterization techniques such as IR, ^{1}H NMR, ^{13}C NMR, LC-MS and elemental analysis. Out of all the synthesized derivatives **IM-3a** containing dibenzyloxy groups had displayed the most promising activity in all the *in vitro* analysis with IC_{50} value ranging from 59.33 ± 10.26-144.44 ± 11.16 µg/mL. Compound **IM-3a** may prove to be a potential therapeutic option owing to its strong antidiabetic, antiarthritic, anticoagulant, anthelmintic, anti-inflammatory and and antioxidant properties.

Keywords: Antidiabetic, Antiarthritic, Anticoagulant, Anthelmintic, Anti-inflammatory, Antioxidant.

INTRODUCTION

Flavonoids belong to an indispensable class of plant-based secondary metabolites which are widely found in stems, roots, fruits, vegetables, grains, bark, flowers, wine, tea and certain beverages. Within the class of secondary metabolites, flavonoids consist of more than 9000 reported structures and among which chalcones and flavones constitute one of the largest subgroups of this class [1]. Chalcones and flavones are considered to be a compelling anchor for devel-opment of various potential drug candidates and it is considered as a cardinal component in a variety of nutraceutical, pharmaceutical, medicinal and cosmetic applications. Chalcones are generally low molecular weight (300-600 g/mol), non-chiral and highly lipophilic (Log P ≈ 5-7) coloured molecules which are also known as benzalacetophenone, phenyl styryl ketone γ-oxo-α,γ-diphenyl-α-propylene, α-phenyl-β-benzoylethylene, β-phenylacrylophenone or benzylidene acetophenone. They belong to the class of plant flavonoids with a C_6-C_3-C_6 skeletal system where the three carbon aliphatic chain is used as an adjunct between two aromatic ring system [2]. Structurally, chalcones are α,β-unsaturated ketones contains a reactive keto-ethylenic group (–CO-CH=CH-) and two aromatic rings, which are linked by an aliphatic three-carbon α,β-unsaturated carbonyl system and have a chemical scaffold of 1,3-diaryl-2-propen-1-one that exists as either trans or cis isomers with the *trans*- isomer being more stable thermodynamically [3].

Structurally, flavones are a class of flavonoids, which are based on the backbone of 2-phenylchromen-4-one (2-phenyl-1-benzopyran-4-one) with the molecular formula $C_{15}H_{10}O_2$. These polyphenolic compounds are ubiquitous in wide variety of plants in which they occur as free form, *e.g. O*-glycosides, *C*-glycosides, *O*-methylated, *C*-methylated, isoprenylated, hydroxylated or methylenedioxy substituted derivatives [4]. Flavones consist of functional groups including a carbonyl group and a conjugated double bond with unsaturation at C_2 and C_3 position [5]. Chalcones and flavones are reported to possess a broad range of significant therapeutic potentials including antitubercular [6], antioxidant [7,8], anti-inflammatory [9], antiviral [10], anticancer [11,12], antiestrogenic [13], anti-acetylcholinesterase [14], antimalarial [15], antipigmentation [16], antibacterial [17,18], antidiabetic [19], antinociceptive [20], antiulcerogenic [21], antiallergic [22], antiplatelet [23],

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antifungal [24], cardiovascular protective [25] and anticonvulsant [26] properties.

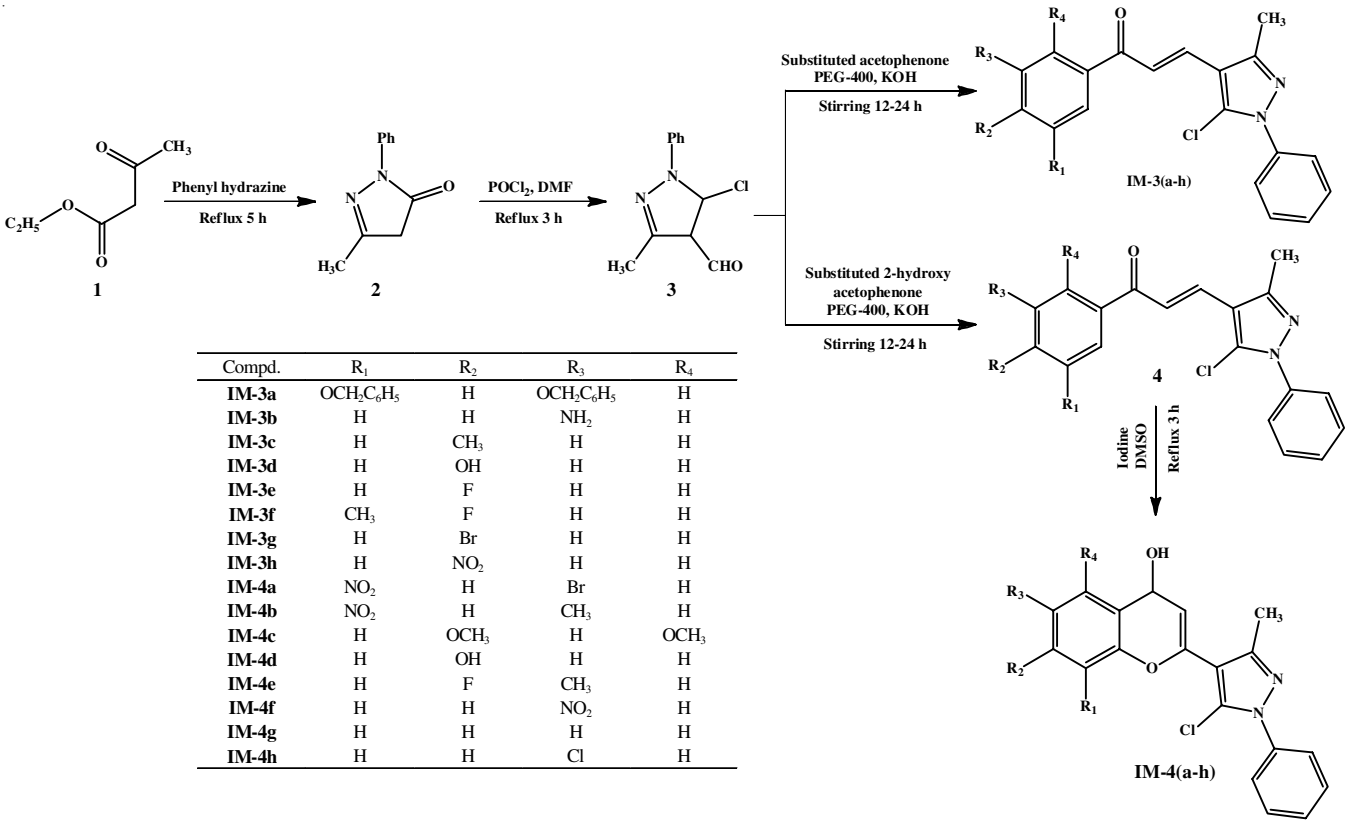
Increasing evidence suggests that nitrogen containing heterocyclic compounds particularly pyrazoles are biologically potent and active and exhibits a wide range of therapeutic activities [27,28]. The association of pyrazoles with chalcones and flavones are anticipated to augment the therapeutic potential of the designed compounds. In light of these biological findings, studies on the therapeutic ability of the compounds containing chalcones and flavones fused with pyrazole nucleus can contribute to the discovery of new and more effective derivatives. In current work, a new series of pyrazolyl chalcone and flavone derivatives containing different substituted functional groups and moieties were synthesized and evaluated for *in vitro* antidiabetic, antiarthritic, anticoagulant, anti-inflammatory, anthelmintic and antioxidant activities by various methods such as α-amylase inhibitory method, egg albumin denaturation method, prothrombin time method, paralysis and death time method, bovine serum denaturation method and DPPH radical scavenging activity method, respectively.

EXPERIMENTAL

All chemicals and reagents, which were used in this protocol were purchased from Sigma-Aldrich, USA. The purity of chemicals and reagents, which were employed for synthesis of intermediate and benzimidazole derivatives were checked by TLC analysis. Melting point of the synthesized compounds were determined in open capillary tube and are uncorrected. Moreover, the structures of all derivatives were confirmed *via* spectroscopic techniques like IR, ^{1}H NMR (400 MHz), ^{13}C NMR and LC-MS spectroscopy.

Synthesis of pyrazolyl chalcones series IM-3(a-h) and pyrazolyl flavones series IM-4(a-h): All the compounds in series **IM-3(a-h)** and **IM-4(a-h)** were synthesized according to the standard procedures as outlined in **Scheme-I**. The reaction proceeded *via* several steps. The first step involved the reaction of ethyl acetoacetate and phenyl hydrazine to yield intermediate **1** using Knorr pyrazole synthesis reaction. Chlorination as well as conversion of ketone group of intermediate **1** to aldehyde group in the second step in presence of $POCl_3$ and DMF yielded the intermediate **2** following the Vilsmeier-Haack reaction. In next step, intermediate **2** underwent Claisen- Schmidt condensation reaction with different substituted acetophenones as well as 2-hydroxy substituted acetophenones in presence of KOH/NaOH in solvent PEG-400/methanol to yield different substituted pyrazolyl chalcones **IM-3(a-h)** and intermediate **3**. The final step for the synthesis of flavones involved the oxidative cyclization of intermediate **3** in presence of catalytic amount of iodine in DMSO to yield pyrazolyl flavone derivatives **IM-4(a-h)**.

(2*E*)-1-(3,5-Dibenzyloxyphenyl)-3-(5-chloro-3-methyl-1-phenyl-1*H*-pyrazol-4-yl)prop-2-en-1-one (IM-3a): Dark yellow solid; yield: 78%; m.p.: 223-225 ºC; IR (KBr, ν_{max}, cm^{-1}): 3039.55 (aromatic-CH), 1159.50 (C-O-C), 1543.84 (C=C), 1678.90 (C=O), 1595.56 (pyrazole-C=N), 2924.60 (methyl-CH), 746.42 (pyrazole-Cl); ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 7.62 (d, *J* = 16 Hz, 1H, H-18), 7.59 (d, 2H, *J* = 4 Hz, H-10) 7.46



Compd.	R_1	R_2	R_3	R_4
IM-3a	$OCH_2C_6H_5$	H	$OCH_2C_6H_5$	H
IM-3b	H	H	NH_2	H
IM-3c	H	CH_3	H	H
IM-3d	H	OH	H	H
IM-3e	H	F	H	H
IM-3f	CH_3	F	H	H
IM-3g	H	Br	H	H
IM-3h	H	NO_2	H	H
IM-4a	NO_2	H	Br	H
IM-4b	NO_2	H	CH_3	H
IM-4c	H	OCH_3	H	OCH_3
IM-4d	H	OH	H	H
IM-4e	H	F	CH_3	H
IM-4f	H	H	NO_2	H
IM-4g	H	H	H	H
IM-4h	H	H	Cl	H

Scheme-I: Synthesis of pyrazolyl chalcones **IM-3(a-h)** and pyrazolyl flavones **IM-4(a-h)**

(d, 2H, *J* = 8.5 Hz, benzyl-H), 6.68 (s, 1H, H-6) 5.18 (s, 1H, CH_2-benzyl), 2.49 (s, 3H, H-23); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm: 188.7, 161.2, 150.1, 146.2, 138.5, 136.6, 136.4, 134.3, 130.1, 129.7, 127.9, 126.9, 124.7, 115.8, 70.6, 14.1; LC-MS: *m/z* 535.03 [M+]; Anal. calcd. (found) % for $C_{33}H_{27}ClN_2O_3$: C, 74.08 (74.08); H, 5.09 (5.10); Cl, 6.63 (6.62); N, 5.24 (5.25); O, 8.97 (8.99).

(2*E*)-1-(3-Aminophenyl)-3-(5-chloro-3-methyl-1-phenyl-1*H*-pyrazol-4-yl)prop-2-en-1-one (IM-3b): Light yellow solid; yield: 69%; m.p.: 184-186 ºC; IR (KBr, ν_{max}, cm^{-1}): 3426.05 (aromatic-NH), 3069.00 (aromatic-CH), 2968.42 (methyl-CH), 1626.46 (C=C), 1676.03 (C=O), 1483.49 (pyrazole-C=N), 688.94 (pyrazole-Cl); ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 7.61 (d, *J* = 12 Hz, 1H, H-18), 7.60 (d, 2H, *J* = 4 Hz, H-10), 7.32 (d, *J* = 8 Hz, H-4), 6.98 (s, 1H, H-3), 6.23 (s, 2H, H-26), 2.49 (s, 3H, H-23); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm: 189.6, 149.8, 148.5, 146.2, 139.2, 137.4, 135.1, 133.5, 129.4, 127.9, 126.5, 124.4, 121.6, 119.2, 114.3, 14.1; LC-MS: *m/z* 338.80 [M+1]; Anal. calcd. (found) % for $C_{19}H_{16}ClN_3O$: C, 67.56 (67.47); H, 4.77 (4.71); Cl, 10.50 (10.56); N, 12.44 (12.49); O, 4.74 (4.76).

(2*E*)-3-(5-Chloro-3-methyl-1-phenyl-1*H*-pyrazol-4-yl)-1-(4-methylphenyl)prop-2-en-1-one (IM-3c): Light yellow solid; yield: 73%; m.p.: 181-183 ºC; IR (KBr, ν_{max}, cm^{-1}): 3063.25 (aromatic-CH), 2925.32, 2854.20 (methyl-CH), 1675.31 (C=O), 1597.72 (C=C), 1523.01 (pyrazole-C=N), 764.38 (pyrazole-Cl *str.*); ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 7.95 (d, *J* = 16 Hz, H-3), 7.61 (d, *J* = 12 Hz, 1H, H-18), 7.60 (d, 2H, *J* = 4 Hz, H-10), 2.49 (s, 3H, H-23), 2.36 (s, 3H, H-25); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm: 189.67, 149.6, 146.2, 144.7, 138.2, 134.1, 133.3, 129.47, 126.9, 124.9, 121.6, 114.7, 21.6, 14.1; LC-MS: *m/z* 336.81 [M+]; Anal. calcd. (found) % for $C_{20}H_{17}ClN_2O$: C, 71.32 (71.42); H, 5.09 (5.13); Cl, 10.53 (10.34); N, 8.32 (8.39); O, 4.75 (4.70).

(2*E*)-3-(5-Chloro-3-methyl-1-phenyl-1*H*-pyrazol-4-yl)-1-(4-hydroxyphenyl)prop-2-en-1-one (IM-3d): Dark yellow solid; yield: 74%; m.p.: 184-186 ºC; IR (KBr, ν_{max}, cm^{-1}): 3431.79 (aromatic-OH), 3056.79 (aromatic-CH), 2922.45 (methyl-CH), 1615.68 (C=O), 1550.31 (C=C), 1524.73 (pyrazole-C=N), 756.47 (pyrazole-Cl); ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 8.00 (d, *J* = 16 Hz, H-3), 7.61 (d, *J* = 12 Hz, 1H, H-18), 7.60 (d, 2H, *J* = 4 Hz, H-10), 5.37 (s, 1H, H-25), 2.49 (s, 3H, H-23); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm: 189.7, 166.8, 149.2, 145.6, 137.2, 133.3, 132.4, 131.1, 129.8, 127.9, 126.6, 124.7, 117.6, 114.7, 14.0; LC-MS: *m/z* 339.78 [M+1]; Anal. calcd. (found) % for $C_{19}H_{15}ClN_2O_2$: C, 67.36 (67.39); H, 4.46 (4.49); Cl, 10.46 (10.47); N, 8.27 (8.20); O, 9.45 (9.41).

(2*E*)-3-(5-Chloro-3-methyl-1-phenyl-1*H*-pyrazol-4-yl)-1-(4-fluorophenyl)prop-2-en-1-one (IM-3e): Dark yellow solid; yield: 56%; m.p.: 186-188 ºC; IR (KBr, ν_{max}, cm^{-1}): 3065.41 (aromatic-CH), 2959.80 (methyl-CH), 1622.15 (C=O), 1589.82 (C=C), 1546.71 (pyrazole-C=N), 1166.68 (aromatic-F), 753.60 (pyrazole-Cl); ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 7.85 (d, *J* = 20 Hz, H-3), 7.63 (d, *J* = 16 Hz, 1H, H-18), 7.60 (d, 2H, *J* = 4 Hz, H-10), 2.49 (s, 3H, H-23), ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm: 189.6, 168.8, 149.7, 145.9, 137.6, 133.9, 133.4, 131.7, 129.5, 127.3, 126.3, 116.3, 117.6, 114.9, 14.0; LC-MS: *m/z* 340.77 [M+]; Anal. calcd. (found) % for $C_{19}H_{14}ClFN_2O$: C, 66.97 (66.93); H, 4.14 (4.24); Cl, 10.40 (10.40); F, 5.57 (5.47); N, 8.22 (8.28); O, 4.69 (4.63).

(2*E*)-3-(5-Chloro-3-methyl-1-phenyl-1*H*-pyrazol-4-yl)-1-(4-fluoro-3-methyl-1-phenyl)prop-2-en-1-one (IM-3f): Light yellow solid; yield: 75%; m.p.: 188-190 ºC; IR (KBr, ν_{max}, cm^{-1}): 3057.51 (aromatic-CH), 2922.45 (methyl-CH), 1659.50 (C=O), 1599.16 (pyrazole-C=N), 1539.53 (C=C), 1002.89 (aromatic-F), 751.45 (pyrazole-Cl); ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 7.66 (d, *J* = 16 Hz, H-3), 7.62 (d, *J* = 16 Hz, 1H, H-18), 7.60 (d, 2H, *J* = 4 Hz, H-10), 2.49 (s, 3H, H-23), 2.37 (s, 3H, H-24); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm: 189.7, 167.8, 149.2, 145.2, 137.3, 133.9, 133.1, 131.4, 129.5, 128.9, 127.8, 126.6, 124.5, 115.3, 114.3, 14.0; LC-MS: *m/z* 354.80 [M+]; Anal. calcd. (found) % for $C_{20}H_{16}ClFN_2O$: C, 67.70 (67.73); H, 4.55 (4.51); Cl, 9.99 (9.97); F, 5.35 (5.38); N, 7.90 (7.91); O, 4.51 (4.53).

(2*E*)-1-(4-Bromophenyl)-3-(5-chloro-3-methyl-1-phenyl-1*H*-pyrazol-4-yl)prop-2-en-1-one (IM-3g): Dark yellow solid; yield: 69%; m.p.: 192-194 ºC; IR (KBr, ν_{max}, cm^{-1}): 3061.10 (aromatic-CH), 2923.88 (methyl-CH), 1674.59 (C=O), 1607.06 (C=C *str.*), 1481.34 (pyrazole-C=N), 760.07 (pyrazole-Cl), 602.74 (aromatic-Br); ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 8.01 (d, *J* = 8 Hz, H-3), 7.63 (d, *J* = 16 Hz, 1H, H-18), 7.60 (d, 2H, *J* = 4 Hz, H-10), 2.49 (s, 3H, H-23); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm: 189.6, 149.6, 145.1, 137.2, 133.6, 132.1, 130.4, 129.5, 128.9, 127.3, 126.3, 124.8, 114.6, 14.0; LC-MS: *m/z* 402.68 [M+1]; Anal. calcd. (found) % for $C_{19}H_{14}ClBrN_2O$: C, 56.81 (56.88); H, 3.51 (3.57); Br, 19.89 (19.81); Cl, 8.83 (8.89); N, 6.97 (6.93); O,3.98 (3.91).

(2*E*)-3-(5-Chloro-3-methyl-1-phenyl-1*H*-pyrazol-4-yl)-1-(4-nitrophenyl)prop-2-en-1-one (IM-3h): Light yellow solid; yield: 70%; m.p.: 187-189 ºC; IR (KBr, ν_{max}, cm^{-1}): 3062.54 (aromatic-CH), 2982.6 (methyl-CH), 1674.59 (C=O), 1600.59 (C=C), 1373.58 (N=O), 1262.23 (pyrazole-C-N), 763.66 (pyrazole-Cl); ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 8.43 (d, *J* = 24 Hz, H-3), 7.61 (d, *J* = 12 Hz, 1H, H-18), 7.60 (d, 1H, *J* = 4 Hz, H-10), 2.47 (s, 3H, H-23); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm: 190.1, 153.9, 149.8, 146.1, 144.3, 137.3, 133.4, 130.2, 129.6, 127.9, 126.4, 124.9, 124.1, 114.6, 14.0; LC-MS: *m/z* 367.78 [M+]; Anal. calcd. (found) % for $C_{19}H_{14}ClN_3O_3$: C, 62.05 (62.15); H, 3.84 (3.89); Cl, 9.64 (9.69); N, 11.43 (11.45); O, 13.05 (13.01).

6-Bromo-2-(5-chloro-3-methyl-1-phenyl-1*H*-pyrazol-4-yl)-8-nitro-4*H*-1-benzopyran-4-one (IM-4a): Brown; yield: 78%; m.p.: 191-193 ºC; IR (KBr, ν_{max}, cm^{-1}): 3073.31 (aromatic-CH), 2915.98 (methyl-CH), 1645.13 (C=O), 1582.63 (C=C), 1357.77 (N=O), 1107.05 (C-O-C), 849.87 (pyrazole-Cl) 585.50 (aromatic-Br); ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 8.65 (s, 1H, H-6), 8.30 (s, 1H, H-3), 6.74 (s, 1H, H-11), 7.62 (d, 1H, *J* = 16 Hz, H-24), 2.45 (s, 3H, H-21); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm: 177.3, 163.8, 153.1, 150.2, 141.9, 140.8, 137.2, 133.4, 133.2, 129.8, 127.3, 126.4, 124.2, 118.2, 114.6, 110.1, 14.3; LC-MS: *m/z* 460.66 [M+]; Anal. calcd. (found) % for $C_{19}H_{11}BrClN_3O_4$: C, 49.54 (49.50); H, 2.41 (2.46); Br, 17.35 (17.30); Cl, 7.70 (7.71); N, 9.12 (9.12); O, 13.89 (13.84).

2-(5-Chloro-3-methyl-1-phenyl-1*H*-pyrazol-4-yl)-6-methyl-8-nitro-4*H*-1-benzopyran-4-one (IM-4b): Brown;

yield: 71%; m.p.: 189-191 ºC; IR (KBr, ν_{max}, cm^{-1}): 3073.31 (aromatic-CH), 2922.45, 2860.67 (methyl-CH), 1653.04 (C=O), 1625.02 (C=C), 1360.65 (N=O), 1583.35 (pyrazole-C=N), 1268.63 (C-O-C), 756.47 (pyrazole-Cl); ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 8.23 (s, 1H, H-6), 7.82 (s, 1H, H-3), 6.71 (s, 1H, H-11), 7.62 (d, 1H, *J* = 16 Hz, H-24), 2.45 (s, 3H, H-21), 2.37 (s, 1H, H-9); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm: 177.4, 163.6, 150.7, 149.6, 138.9, 137.6, 134.1, 133.4, 130.6, 129.8, 129.3, 126.7, 124.4, 124.1, 114.7, 110.6, 20.9, 14.3; LC-MS: *m/z* 396.79 [M+1]; Anal. calcd. (found) % for $C_{20}H_{14}ClN_3O_4$: C, 60.69 (60.64); H, 3.57 (3.51); Cl, 8.96 (8.92); N, 10.62 (10.66); O, 16.17 (16.19).

2-(5-Chloro-3-methyl-1-phenyl-1*H*-pyrazol-4-yl)-5,7-dimethoxy-4*H*-1-benzopyran-4-one (IM-4c): Dark yellow; yield: 77%; m.p.: 189-191 ºC; IR (KBr, ν_{max}, cm^{-1}): 3064.69 (aromatic-CH), 2923.17 (methyl-CH), 2857.79 (methoxy-CH), 1622.86 (C=O), 1545.28 (C=C), 1206.91 (C-O), 1575.02 (pyrazole-C=N), 1102.02 (C-O-C), 754.32 (pyrazole-Cl); ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 7.62 (d, 1H, *J* = 16 Hz, H-24), 6.34 (s, 1H, H-5), 6.71 (s, 1H, H-11), 3.83 (s, 1H, H-10, H-8), 2.47 (s, 3H, H-21); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm: 177.2, 164.5, 163.8, 161.2, 158.9, 149.1, 137.6, 133.4, 129.8, 126.8, 124.5, 114.6, 110.5, 107.7, 96.8, 96.2, 55.9, 14.3; LC-MS: *m/z* 396.82 [M+]; Anal. calcd. (found) % for $C_{20}H_{14}ClN_3O_4$: C, 63.56 (63.51); H, 4.32 (4.39); Cl, 8.93 (8.98); N, 7.06 (7.09); O, 16.13 (16.18).

2-(5-Chloro-3-methyl-1-phenyl-1*H*-pyrazol-4-yl)-7-hydroxy-4*H*-1-benzopyran-4-one (IM-4d): Dark yellow; yield: 79%; m.p.: 185-187 ºC; IR (KBr, ν_{max}, cm^{-1}): 3444.72 (aromatic-OH), 3056.79 (aromatic-CH), 2918.14 (methyl-CH), 1616.40 (C=O), 1611.86 (C=C), 1099.87 (C-O-C), 1521.24 (pyrazole-C=N), 753.60 (pyrazole-Cl); ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 7.96 (s, 1H, H-3), 7.62 (d, 1H, *J* = 16 Hz, H-24), 6.71 (s, 1H, H-11), 6.45 (s, 1H, H-5), 5.33 (s, 1H, H-8), 2.47 (s, 1H, H-21); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm: 177.5, 165.5, 163.4, 158.6, 149.3, 137.6, 133.7, 129.8, 128.8, 126.2, 124.5, 115.4, 114.6, 110.3, 101.7, 14.3; LC-MS: *m/z* 352.77 [M+]; Anal. calcd. (found) % for $C_{19}H_{13}ClN_2O_3$: C, 64.69 (64.61); H, 3.71 (3.78); Cl, 10.05 (10.09); N, 7.94 (7.97); O, 13.61 (13.66).

2-(5-Chloro-3-methyl-1-phenyl-1*H*-pyrazol-4-yl)-6-methyl-4*H*-1-benzopyran-4-one (IM-4e): Dark yellow; yield: 79%; m.p.: 185-187 ºC; IR (KBr, ν_{max}, cm^{-1}): 3061.91 (aromatic-CH), 2924.60, 2924.14 (methyl-CH), 1621.41 (C=O), 1621.81 (C=C), 1097.71 (C-O-C), 1527.29 (pyrazole-C=N), 753.60 (pyrazole-Cl); ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 7.68 (d, *J* = 28 Hz, H-6), 7.62 (d, 1H, *J* = 16 Hz, H-24), 7.43 (s, 1H, H-3), 6.71 (s, 1H, H-11), 2.47 (s, 1H, H-21), 2.39 (s, 1H, H-9); ^{13}C NMR (101 MHz, $CDCl_3$) δ pp: 177.2, 163.6, 153.6, 149.4, 138.6, 137.9, 133.3, 133.1, 129.8, 126.4, 124.8, 124.3, 120.4, 114.1, 110.5, 21.7, 14.3; LC-MS: *m/z* 350.79 [M+]; Anal. calcd. (found) % for $C_{20}H_{15}ClN_2O_2$: C, 68.48 (68.39); H, 4.31 (4.30); Cl, 10.11 (10.17); N, 7.99 (7.91); O, 9.12 (9.13).

2-(5-Chloro-3-methyl-1-phenyl-1*H*-pyrazol-4-yl)-6-nitro-4*H*-1-benzopyran-4-one (IM-4f): Dark yellow; yield: 81%; m.p.: 187-189 ºC; IR (KBr, ν_{max}, cm^{-1}): 3061.82 (aromatic-CH), 2926.04 (methyl-CH), 1716.26 (C=O), 1615.68 (C=C), 1522.13 (pyrazole-C=N), 1491.40 (aromatic-N=O), 1095.56 (C-O-C), 1527.29 (pyrazole-C=N), 755.76 (pyrazole-Cl); ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 8.27 (s, 1H, H-3), 7.62 (d, 1H, *J* = 16 Hz, H-24), 6.71 (s, 1H, H-11), 2.47 (s, 1H, H-21); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm: 177.5, 163.6, 162.4, 149.2, 142.6, 137.2, 133.3, 131.1, 129.2, 126.2, 124.8, 124.3, 114.1, 110.2, 14.3; LC-MS: *m/z* 38.796 [M+1]; Anal. calcd. (found) % for $C_{19}H_{12}ClN_3O_4$: C, 59.78 (59.71); H, 3.17 (3.19); Cl, 9.29 (9.24); N, 11.01 (11.71); O, 16.76 (16.72).

2-(5-Chloro-3-methyl-1-phenyl-1*H*-pyrazol-4-yl)-4*H*-1-benzopyran-4-one (IM-4g): Dark yellow; yield: 83%; m.p.: 171-173 ºC; IR (KBr, ν_{max}, cm^{-1}): 3065.41 (aromatic-CH), 2920.20 (methyl-CH), 1623.43 (C=O), 1641.94 (C=C), 1096.28 (C-O-C), 1547.49 (pyrazole-C=N), 752.88 (pyrazole-Cl); ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 8.08 (d, *J* = 16 Hz, H-3), 7.62 (d, 1H, *J* = 16 Hz, H-24), 6.71 (s, 1H, H-11), 2.47 (s, 1H, H-21); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm: 177.7, 163.1, 156.6, 149.4, 138.6, 137.9, 135.7, 133.3, 129.3, 126., 125.8, 124.3, 123.9, 123.3, 116.5, 114.1, 110.5, 14.3; LC-MS: *m/z* 336.77 [M+]; Anal. calcd. (found) % for $C_{19}H_{13}ClN_2O_2$: C, 67.76 (67.79); H, 3.89 (3.81); Cl, 10.53 (10.55); N, 8.32 (8.32); O, 9.50 (9.52).

6-Chloro-2-(5-chloro-3-methyl-1-phenyl-1*H*-pyrazol-4-yl)-4*H*-1-benzopyran-4-one (IM-4h): Light yellow; yield: 79%; m.p.: 174-176 ºC; IR (KBr, ν_{max}, cm^{-1}): 3063.97 (aromatic-CH), 2927.48 (methyl-CH), 1676.74 (C=O), 1640.82 (C=C), 1192.28 (C-O-C), 1523.72 (pyrazole-C=N), 765.10 (aromatic-Cl), 696.13 (pyrazole-Cl); ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 8.07 (d, *J* = 12 Hz, H-3), 7.62 (d, 1H, *J* = 16 Hz, H-24), 6.71 (s, 1H, H-11), 2.47 (s, 1H, H-21); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm: 177.4, 163.6154.6, 149.9, 137.6, 136.4, 133.3, 130.5, 129.8, 129.1, 126.6, 125.3, 124.6, 119.1, 114.3, 110.7, 14.3; LC-MS: *m/z* 336.77 [M+]; Anal. calcd. (found) % for $C_{19}H_{12}Cl_2N_2O_2$: C, 61.47 (61.47); H, 3.26 (3.20); Cl, 19.10 (19.11); N, 7.55 (7.59); O, 8.62 (8.68).

Biological activity: Synthesized compounds were tested for their *in vitro* antidiabetic, antiarthritic, anticoagulant, anthelmintic, anti-inflammatory and antioxidant activities by various methods such as α-amylase inhibitory method, egg albumin denaturation method, prothrombin time method, paralysis and death time method, bovine serum denaturation method and DPPH radical scavenging activity method respectively. All the analysis were performed in triplicate to obtain an accurate and precise result. The IC_{50} values for the compounds at different concentrations were determined from plots of percent inhibition *versus* log inhibitor concentration and were calculated from the mean inhibitory values by non-linear regression analysis.

***In vitro* antidiabetic activity:** Stock solution of test compounds was prepared by dissolving various concentrations of test compounds (100, 300 and 500 µg/mL) in 1 mL of DMSO. A total of 250 µL of stock solution of test compounds was placed in a tube containing 250 µL of 0.02 M sodium phosphate buffer (pH 6.9) and 25 µL of α-amylase solution. The solution was pre-incubated at room temperature (28 ± 2 ºC) for 10 min. A 250 µL of 1% starch solution was added in 0.02 M sodium phosphate buffer (pH 6.9) and then it was further incubated at 25 ºC for 10 min. The reaction mixture was quenched by adding 400 µL of DNSA (3,5-dinitrosalicylic acid) colour reagent. The test

tubes were boiled for 5 min in a water bath until the yellowish orange colour was developed and then the tubes were cooled to room temperature. The reaction mixture was diluted with 5 mL of distilled water and the absorbance was measured at 540 nm using UV-visible spectrophotometer. Acarbose was used as standard α-amylase inhibitor drug and blank solution was prepared in the same manner by replacing the test solution with distilled water. The α-amylase inhibitory activity of each test solution is expressed as percent inhibition, which can be calculated as follows [29]:

$$\text{Inhibition (\%)} = \frac{A_{control} - A_{extract}}{A_{control}} \times 100$$

***In vitro* antiarthritic activity:** *In vitro* antiarthritic activity was carried out using egg albumin protein denaturation method in which 2 mL of several concentrations (100, 300 and 500 µg/mL) of compounds were dissolved in ethanol and 0.2 mL of egg albumin as well as phosphate buffer 2.8 mL (pH = 6.5) were added. Reaction mixture was incubated for at 37 ºC for 20 min followed by heating at 70 ºC for 5 min and then cooled to room temperature. Same quantity of egg albumin and phosphate buffer was used for diclofenac solution as standard and for negative control test solution was replaced with 2 mL distilled water and absorbance was measured at 660 nm using UV spectrophotometer. The percentage inhibition was calculated by following formula [30]:

$$\text{Inhibition (\%)} = \frac{A_C - A_S}{A_C} \times 100$$

where A_C = absorption of the control sample, A_S = absorption of the test sample.

***In vitro* anticoagulant activity:** Blood samples were drawn and placed separately in containers containing 3.8% trisodium citrate solution to prevent the clotting process. In order to separate the blood cells from plasma, centrifugation (15 min at 3000 rpm) was carried out and pure platelet plasma (ppp) was obtained for prothrombin time test. After centrifugation, 0.2 mL plasma, 0.1 mL of methanolic solution of different concentration of compounds (100, 300 and 500 mg/mL) and 0.3mL of $CaCl_2$ (25 mM) were added together in a clean fusion tube and incubated at 37 ºC in water bath. Warfarin was taken as a standard drug for the experiment. For control, methanolic solution of compounds was replaced by same volume of 0.9% saline water. The clotting time of blood was recorded with a stopwatch by tilting the test tubes every 5 seconds and it is called as prothrombin time [31].

***In vitro* anthelmintic activity:** Earthworms (3-5 cm × 0.1-0.2 cm) from moist soil were taken and washed with normal saline water. The earthworms were used in this study due to its anatomical and physiological resemblance with the intestinal roundworm parasites of human beings. All the test solutions and standard drug solution were prepared freshly before starting the experiment using 5% DMF solution. Six groups of earthworms of approximately equal size were taken and kept into 25 mL solutions of three different concentrations (25, 50, 100 mg/mL) in petri dishes containing 5% of DMF solution. Albendazole was used as reference drug for this study. Determination of time of paralysis and time of death of the worm were noted down. The time for paralysis was noted when no movement was observed upon vigorous shaking of worms. Time for death of worms was recorded after ascertaining that the worms neither moved when shaken vigorously nor when dipped in warm water (50 ºC) followed by fading away of their skin colours [32].

***In vitro* anti-inflammatory activity:** The reaction mixtures consist of varying concentrations (100, 300 and 500 µg/mL) of test compounds and reference drug indomethacin. Bovine serum albumin (BSA, 1% w/v) and phosphate buffer saline (pH 6.4) were added separately to the test solutions. The reaction mixtures were incubated at 37 ºC for 20 min and the temperature was increased to 70 ºC for 5 min for denaturation of proteins. After cooling, turbidity was measured at 660 nm using UV-visible spectrophotometer. A blank control solution was prepared in the same manner by replacing the test solution with phosphate buffer solution. The percentage inhibition of BSA denaturation can be calculated as stated below [33]:

$$\text{Inhibition of BSA denaturation (\%)} = \left(1 - \frac{A_2}{A_1}\right) \times 100$$

where A_1 = absorbance of the control and A_2 = absorbance of the test sample.

***In vitro* antioxidant activity:** The DPPH (2,2-diphenyl-1-picrylhydrazyl) solution was prepared by dissolving 4 mg of DPPH in 100 mL of methanol and then the solution was stirred for 30 min. The standard solution of Ascorbic acid was prepared in methanol in 100, 300 and 500 µg/mL while sample solutions was prepared in DMSO at the same concentrations. After addition of 1.5 mL of DPPH solution to 0.75 mL of sample and standard solutions, it was allowed to react for 30 min in the dark condition. Samples absorbance measurements were evaluated with a UV-vis spectrophotometer at fixed wavelength of 517 nm. Blank sample was prepared by adding methanol to DPPH solution. After incubation, decrease in absorption was measured at a wavelength of 517 nm. Percent radical scavenging activity of samples was determined in comparison with reference drug ascorbic acid by using the following formula [34,35]:

$$\text{Antioxidant activity (\%)} = \left(\frac{A_{control} - A_{test}}{A_{control}}\right) \times 100$$

where $A_{Control}$ = absorbance of the control, A_{test} = absorbance of the samples.

RESULTS AND DISCUSSION

In view of the importance of the bioactive chalcones and flavones, it was thought to incorporate pyrazole ring as well as different substituted functional groups into the chalcone and flavone moiety and to evaluate the effect of pyrazole ring and various substituents on the biological activities of chalcones and flavones. In this work, the pyrazolyl chalcones and flavones were synthesized using Knorr pyrazole synthetic reaction followed by Vilsmeier-Haack reaction and Claisen Schmidt condensation reaction whereas as the additional step involving oxidative cyclization was carried out to obtain flavone derivatives which is shown in **Scheme-I**.

Biological activity

Antidiabetic activity: The results indicate that compound **IM-3a** had exhibited the best anti α-amylase activity at a concentration of 500 µg/mL with IC_{50} value of 144.44 ± 11.16 and compound **IM-3g** had showed the least inhibitory activity (Table-1). The present findings also revealed that there was a dose dependent increase in percentage inhibitory activity against α-amylase by all the test solutions. The present study suggested that compound **IM-3a** could be useful for management of post-prandial hyperglycaemia and can exhibit potent antidiabetic activity.

TABLE-1
PERCENT INHIBITION OF WHEAT α-AMYLASE BY THE SYNTHESIZED COMPOUNDS FOR *in vitro* ANTIDIABETIC ACTIVITY

Compd.	Inhibition (%)			
	100 µg/mL	300 µg/mL	500 µg/mL	IC_{50} value (µg/mL)
IM-3a	47	63	89	144.44 ± 11.16
IM-3b	37	61	82	211.11 ± 11.29
IM-3c	35	48	63	319.14 ± 14.11
IM-3d	25	38	69	354.54 ± 10.06
IM-3e	29	49	76	288.68 ± 13.18
IM-3f	29	55	79	265.33 ± 9.13
IM-3g	19	37	64	388.88 ± 8.92
IM-3h	22	39	75	335.22 ± 13.17
IM-4a	27	49	76	294.55 ± 11.19
IM-4b	18	36	70	366.66 ± 8.76
IM-4c	27	38	70	346.51 ± 6.83
IM-4d	30	57	81	252.94 ± 12.11
IM-4e	31	54	82	255.55 ± 14.15
IM-4f	24	51	78	292.59 ± 10.16
IM-4g	35	57	87	225.63 ± 7.63
IM-4h	31	55	83	251.28 ± 12.15
Acarbose	51	67	90	101.68 ± 12.16

Antiarthritic activity: In this work, all the synthesized compounds impeded egg albumin protein denaturation in a dose dependent manner with remarkable effect observed at the highest concentration. The percent inhibition and IC_{50} value of the compounds plotted as a function of concentration in comparison with diclofenac is shown in Table-2. The results indicate that compound **IM-3a** had exhibited the best anti-arthritic activity at a concentration of 500 µg/mL with IC_{50} value of 59.33 ± 10.26 and compound **IM-4a** had showed the least inhibitory activity. The results suggest that there was a dose dependent increase in percentage inhibitory activity against egg albumin denaturation by all the test solutions. Therefore, compound **IM-3a** could be useful for management of arthritis and can act as an effective antiarthritic agent.

Anticoagulant activity: The anticoagulant activity was carried out to evaluate the effect of pyrazolyl chalcones and pyrazolyl flavones as anticoagulants in blood samples of normal healthy volunteers by using principles of coagulation time by prothrombin method. All the test solution of compounds inhibited platelet aggregation with compound **IM-3a** being the most potent anticoagulant compound with coagulation time of 49.22 ± 1.45 min at 500 mg/mL and compound **IM-3f** had displayed the least activity (Table-3). Therefore, it can be concluded that compound **IM-3a** can be a potential source of platelet inhibitor and can act as an effective anticoagulant agent.

TABLE-2
PERCENT INHIBITION OF EGG ALBUMIN DENATURATION BY THE SYNTHESIZED COMPOUNDS FOR *in vitro* ANTIARTHRITIC ACTIVITY

Compd.	Inhibition (%)			
	100 µg/mL	300 µg/mL	500 µg/mL	IC_{50} value (µg/mL)
IM-3a	54	71	90	59.33 ± 10.26
IM-3b	53	69	88	71.42 ± 11.20
IM-3c	45	58	73	176.18 ± 9.20
IM-3d	35	48	68	295.96 ± 12.28
IM-3e	47	69	79	112.5 ± 16.10
IM-3f	45	65	82	148.64 ± 11.40
IM-3g	49	52	87	166.66 ± 16.50
IM-3h	46	59	75	162.06 ± 13.30
IM-4a	26	39	66	363.33 ± 8.33
IM-4b	40	56	73	223.236 ± 14.21
IM-4c	43	58	77	190.20 ± 11.22
IM-4d	50	67	83	97.975 ± 16.27
IM-4e	47	64	83	137.033 ± 15.25
IM-4f	51	68	86	90.51 ± 10.22
IM-4g	49	65	81	112.5 ± 16.18
IM-4h	47	61	79	145.87 ± 12.18
Diclofenac	55	73	92	47.78 ± 16.20

TABLE-3
PROTHROMBIN TIME DETERMINATION BY THE SYNTHESIZED COMPOUNDS FOR ANTICOAGULANT ACTIVITY

Compd.	Prothrombin time (Mean ± SD) (min)		
	100 µg/mL	300 µg/mL	500 µg/mL
IM-3a	19.12 ± 1.31	31.11 ± 1.17	49.22 ± 1.45
IM-3b	11.15 ± 1.03	25.33 ± 1.06	36.56 ± 0.55
IM-3c	16.11 ± 1.31	28.06 ± 1.13	43.05 ± 0.76
IM-3d	15.18 ± 1.17	27.06 ± 1.53	34.34 ± 1.09
IM-3e	13.34 ± 1.02	25.54 ± 0.09	33.08 ± 1.11
IM-3f	7.43 ± 1.13	17.43 ± 0.10	23.33 ± 0.07
IM-3g	9.07 ± 1.13	21.44 ± 1.34	30.32 ± 0.06
IM-3h	12.56 ± 0.08	24.55 ± 0.13	31.32 ± 0.56
IM-4a	18.52 ± 1.15	30.42 ± 0.56	47.31 ± 1.18
IM-4b	17.34 ± 1.13	29.05 ± 0.14	45.26 ± 1.35
IM-4c	8.11 ± 1.16	18.25 ± 1.36	25.55 ± 1.45
IM-4d	16.44 ± 1.17	28.23 ± 0.54	44.46 ± 0.08
IM-4e	18.01 ± 1.12	29.55 ± 0.39	45.43 ± 1.18
IM-4f	16.45 ± 1.15	27.34 ± 1.16	43.56 ± 1.15
IM-4g	16.12 ± 1.14	26.54 ± 1.15	43.01 ± 0.09
IM-4h	17.45 ± 0.34	27.09 ± 1.03	43.58 ± 1.13
Warfarin	19.66 ± 1.30	33.23 ± 1.29	53.32 ± 1.19

Anthelmintic activity: In current *in vitro* study, different concentration of test solution of compounds produced a statistically significant anthelmintic activity and among which compound **IM-3a** had exhibited the most promising activity which is comparable with the conventional standard anthelmintic drug albendazole. The *in-vitro* anthelmintic activity of the synthesized compounds was characterized by a decrease in motility followed by paralysis and death of earthworms (Pheretima Poshtuma).

All the compounds had displayed appreciable results and the compound **IM-3a** was found to be the most potent compound with a death time of 11 ± 0.53 min (Table-4). Therefore, compound **IM-3a** has the potential to contribute in controlling gastrointestinal parasite infection in ruminants and human beings.

TABLE-4
PARALYSIS AND DEATH TIME TAKEN BY THE SYNTHESIZED COMPOUNDS FOR ANTHELMINTIC ACTIVITY

Compd.	Conc. (mg/mL)	Time taken (min)	
		Paralysis	Death
IM-3a	25	27 ± 0.12	31 ± 0.16
	50	16 ± 0.53	24 ± 0.12
	100	6 ± 0.37	11 ± 0.53
IM-3b	25	47 ± 0.17	51 ± 0.56
	50	36 ± 0.43	41 ± 0.32
	100	29 ± 0.27	35 ± 0.23
IM-3c	25	36 ± 0.27	39 ± 0.36
	50	24 ± 0.23	31 ± 0.42
	100	15 ± 0.29	19 ± 0.33
IM-3d	25	31 ± 0.52	36 ± 0.56
	50	20 ± 0.43	26 ± 0.52
	100	10 ± 0.56	15 ± 0.55
IM-3e	25	36 ± 0.57	39 ± 0.56
	50	24 ± 0.43	31 ± 0.52
	100	15 ± 0.59	19 ± 0.56
IM-3f	25	34 ± 0.51	38 ± 0.53
	50	26 ± 0.48	30 ± 0.54
	100	11 ± 0.44	16 ± 0.43
IM-3g	25	49 ± 0.1	55 ± 0.51
	50	39 ± 0.48	44 ± 0.37
	100	32 ± 0.29	35 ± 0.21
IM-3h	25	52 ± 0.11	57 ± 0.56
	50	39 ± 0.41	46 ± 0.39
	100	33 ± 0.2	38 ± 0.29
IM-4a	25	43 ± 0.17	47 ± 0.56
	50	32 ± 0.43	36 ± 0.30
	100	25 ± 0.20	29 ± 0.21
IM-4b	25	46 ± 0.19	50 ± 0.16
	50	34 ± 0.23	38 ± 0.36
	100	28 ± 0.21	34 ± 0.24
IM-4c	25	43 ± 0.19	47 ± 0.23
	50	30 ± 0.43	36 ± 0.35
	100	25 ± 0.56	20 ± 0.29
IM-4d	25	37 ± 0.26	39 ± 0.38
	50	25 ± 0.21	30 ± 0.40
	100	16 ± 0.21	19 ± 0.37
IM-4e	25	29 ± 0.50	34 ± 0.56
	50	19 ± 0.45	24 ± 0.52
	100	9 ± 0.57	14 ± 0.51
IM-4f	25	41 ± 0.11	45 ± 0.53
	50	30 ± 0.46	33 ± 0.36
	100	23 ± 0.29	27 ± 0.21
IM-4g	25	39 ± 0.17	43 ± 0.56
	50	30 ± 0.43	34 ± 0.31
	100	23 ± 0.21	26 ± 0.21
IM-4h	25	37 ± 0.20	39 ± 0.55
	50	24 ± 0.28	30 ± 0.43
	100	16 ± 0.51	19 ± 0.47
Albendazole	25	26 ± 0.22	30 ± 0.36
	50	16 ± 0.33	22 ± 0.42
	100	5 ± 0.26	9 ± 0.43

Anti-inflammatory activity: All the test compounds showed concentration dependent inhibition of bovine serum albumin protein denaturation with substantial inhibitory effect. Out of all the compounds, compound **IM-3a** had showed the highest percentage inhibition at a concentration of 500 µg/mL with IC_{50} value of 117.68 ± 6.36 µg/mL and showed the comparable results with standard drug indomethacin (Table-5).

TABLE-5
% OF BOVINE SERUM DENATURATION BY THE SYNTHESIZED COMPOUNDS FOR *in vitro* ANTI-INFLAMMATORY ACTIVITY

Compd.	% Inhibitory Concentration			
	100 µg/mL	300 µg/mL	500 µg/mL	IC_{50} value (µg/mL)
IM-3a	45	78	94	117.68 ± 6.36
IM-3b	34	59	78	236.36 ± 4.89
IM-3c	39	63	83	193.93 ± 9.13
IM-3d	42	65	86	169.70 ± 13.11
IM-3e	38	57	74	229.66 ± 10.14
IM-3f	36	56	73	245.94 ± 15.66
IM-3g	34	53	70	274.07 ± 12.11
IM-3h	31	49	65	319.61 ± 9.76
IM-4a	40	63	86	186.95 ± 13.12
IM-4b	26	39	61	391.42 ± 16.10
IM-4c	23	34	57	441.17 ± 10.35
IM-4d	47	66	88	134.14 ± 16.13
IM-4e	49	79	91	80.95 ± 18.11
IM-4f	41	65	82	176.41 ± 5.34
IM-4g	28	42	61	376.76 ± 7.68
IM-4h	41	64	79	180.70 ± 11.12
Indomethacin	52	74	93	75.60 ± 10.13

Antioxidant activity: In the *in-vitro* antioxidant activity, free-radical scavenging compounds possessing antioxidant property reacts to DPPH to form DPPHH which causes decolorization (from purple to a yellow hue) of the compounds due to increase in the number of electrons. It can be observed from Table-6 that compound **IM-3a** had displayed the highest percentage of inhibition 88% with IC_{50} value of 134.92 ± 10.23 µg/mL.

Conclusion

Synthesis of pyrazolyl chalcones and pyrazolyl flavones was carried out using various substituents containing halogens, methyl, methoxy, hydroxy, benzyloxy and nitro groups and the synthesized compounds were evaluated for their *in vitro* antidiabetic, antiarthritic, anticoagulant, anthelmintic, anti-inflammatory and antioxidant activities by various methods such as α-amylase inhibitory method, egg albumin denaturation method, prothrombin time method, paralysis and death time method, bovine serum denaturation method and DPPH radical scavenging activity method respectively. It is concluded from the results that substituents on the skeleton of pyrazolyl chalcones and pyrazolyl flavones are responsible for their potent activities and the compound particularlycompound **IM-3a** had exhibited the most promising action in almost all the *in vitro* activities owing to the presence of dibenzyloxy groups in its skeleton. Further *in vivo* testing will be necessary to confirm the obtained results and evaluate risks, side effects and future

TABLE-6
% OF DPPH RADICAL SCAVENGING ACTIVITY BY THE SYNTHESIZED COMPOUNDS FOR *in vitro* ANTIOXIDANT ACTIVITY

Compd.	Inhibitory (%)			
	100 µg/mL	300 µg/mL	500 µg/mL	IC_{50} value (µg/mL)
IM-3a	46	68	88	134.92 ± 10.23
IM-3b	33	49	61	333.32 ± 11.13
IM-3c	35	53	63	295.24 ± 10.15
IM-3d	30	41	52	463.63 ± 16.33
IM-3e	34	52	64	300 ± 15.17
IM-3f	36	50	62	310.26 ± 14.11
IM-3g	32	53	60	323.85 ± 10.13
IM-3h	36	54	65	277.01 ± 16.63
IM-4a	40	59	76	207.41 ± 7.18
IM-4b	50	76	91	82.18 ± 12.16
IM-4c	30	48	57	374.07 ± 9.16
IM-4d	47	63	81	139.21 ± 13.19
IM-4e	44	61	79	170.48 ± 10.56
IM-4f	38	56	72	237.25 ± 15.13
IM-4g	48	62	82	135.29 ± 10.21
IM-4h	46	59	79	162.63 ± 14.13
Ascorbic acid	52	77	93	65.85 ± 5.14

applicability. Therefore, *in vivo* evaluation of the compounds is imperative prior to their clinical use.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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