Volume 7, Issue 1, 2017, 1901 - 1912 ISSN 2069-5837

Biointerface Research in Applied Chemistry

www.BiointerfaceResearch.com

Original Research Article Open Access Journal

Received: 28.12.2016 / Revised: 20.01.2017 / Accepted: 10.02.2017 / Published on-line: 15.02.2017

Novel 1,3,4-oxadiazole tethered pyrazolyl-isoxazoles: synthesis, characterization and pharmacological screening

Kumbaradoddi B Umesha[1,*], Shridevi D Doddramappa[2], Chandra[3], Nagarakere S Lingegowda[4], Javarasetty Chethan[5], Srikantamurthy Ningaiah[4,*]

[1] Department of Chemistry, Yuvaraja's College, University of Mysore, Mysuru-570 005
[2] Department of Studies in Chemistry, Manasagangotri, University of Mysore, Mysuru-570 006
[3] Department of Studies in Physics, Manasagangotri, University of Mysore, Mysuru-570 006
[4] Department of Chemistry, Vidyavardhaka College of Engineering, Gokulam, Mysuru-570 002
[5] Department of Studies in Biotechnology, Manasagangotri, University of Mysore, Mysuru-570 006, India
*corresponding author e-mail address: ***srijmn@vvce.ac.in, kbu68umesha@rediffmail.com***

ABSTRACT

A novel series of 2-(5-methyl-1,3-diphenyl-1*H*-pyrazole-4-yl)-5-(5-methyl-3-phenyl-isoxazole-4-yl)-[1,3,4]-oxadiazoles were synthesized from the respective 5-methyl-*N*′-(5-methyl-1,3-diphenyl-1*H*-pyrazole-4-carbonyl)-3-phenylisoxazole-4-carbohydrazides using $POCl_3$ at 120°C or by oxidative cyclization using Burgess reagent as oxidant. Newly synthesized compounds were characterized by analytical and spectral (IR, ^{1}H NMR, ^{13}C NMR and LC–MS) methods. The synthesized compounds were evaluated for their antioxidant, antimicrobial and antidiabetic activity and were compared with standard drugs.

Keywords: *Pyrazole; 1,3,4-oxadiazoles; Isoxazole; Antioxidant activity; Antimicrobial activity; Antidiabetic activity.*

1. INTRODUCTION

Antioxidants play an important role in resisting oxidative damage induced by free radicals and ROS (Reactive Oxygen Species). To keep the mammalian cells in a healthy condition, the balanced oxidants (ROS) generation and detoxification is important during cellular metabolism. When a cell fails to detoxify the excessive ROS generated, they enter into a state of oxidative stress and are damaged [1]. High level of ROS can cause mutation [2] and also damage cell structure, nucleic acids, membrane lipids and proteins [3] Oxidative stress on a cell due to high concentration of ROS can lead to a variety of disorders including cancer, neurodegenerative disorder, atherosclerosis and aging [4]. Many studies have suggested that antioxidants or other compounds that can neutralize free radicals may be of pivotal interest in the prevention of vascular diseases and some forms of cancer.

The prevalence of microbial infections has augmented dramatically in recent years [5]. Resistance to antimicrobial agents has resulted in morbidity and mortality from treatment failures and increased health care costs [6]. Thus, researchers are focused on the development of more efficacious drugs for use in the clinical arena [7]. Identification of novel structure leads for designing new, potent and broad spectrum antimicrobial agents remains a major challenge for medicinal chemistry researchers.

DM (Diabetes mellitus) is a metabolic disorder resulting from absolute or relative lack of insulin secretion [8] and affects approximately 200 million individuals worldwide [9]. The control of postprandial hyperglycemia is a captious approach in the management of diabetes mellitus, especially type II diabetes and reducing chronic complications associated with the disease. Therefore, such enzyme inhibitors can be useful in the treatment of type II diabetes [10].

Survey of the literature revealed that linked heterocyclic compounds containing two or more rings like pyrazole incorporated thiazole [11], thiadiazole [12], 1,2,4-oxadiazole [13], 1,2,4-triazole and benzoxazoles [14] were synthesized and showed an enhancement of pharmacological effect. Also several biologically active pyrazolyl-1,3,4-oxadiazole analogues have been reported [15-18]. Substituted 1,3,4-oxadiazoles [19] pyrazoles [20] and isoxazoles [21] all being bioactive, if they are linked together, the tri-heterocyclic compounds obtained could have better biological activity.

Fortified by these observations and in continuation of our research work on the synthesis and pharmacological screening of heterocyclic compounds comprising multi-structure [13, 22, 23] we thought of synthesizing a new class of heterocycles, wherein potent 1,3,4-oxadiazole moiety is tethered between pyrazole and isoxazole moiety to see the additive effect of these rings towards the pharmacological activity, which is the present-day urge being accomplished in most of the drug discoveries.

2. EXPERIMENTAL SECTION

2.1. General methods. All chemicals were obtained from commercial suppliers and used without further purification. Melting points were determined in open capillaries on a Buchi oil melting point apparatus and are uncorrected. Reactions were monitored by using thin layer chromatography (TLC) on aluminum sheets precoated with silicagel 60 F_{254} (0.2 mm, Merck). Chromatographic spots were visualized by UV light and/or with iodine. For column chromatography, silicagel of 100-200 mesh size was used. ^{1}H NMR spectra were acquired on a Bruker Avance 400 MHz instrument in DMSO-d_6 or $CDCl_3$ and

TMS was used as an internal reference. ^{13}C NMR spectra were recorded on a Bruker AMX-400 (100.6 MHz) with complete proton decoupling. Chemical shifts are reported in ppm from tetramethylsilane with the solvent as the internal reference ($CDCl_3$: δ 77.0 ppm). All novel compounds were characterized by LC-MS, and gave satisfactory results in agreement with the proposed structure. LC-MS data were obtained using electrospray ionization (positive mode) on a C-18 column at a flow rate 0.2 mL/ min using MeOH/water (90:10) as eluent. LC-MS M+H signals were consistent with expected molecular weight for all reported products.

2.2. General procedure for the preparation of benzaldehyde oximes. Solution of hydroxylamine hydrochloride (2.0g) and crystallized sodium acetate (2.5g) in distilled water (10 ml) was mixed with solution of aldehyde (1.0g) in ethyl alcohol (5 ml). It was then warmed for 5 to 10 minutes and cooled in ice water. The precipitated solid was filtered and recrystallized from methanol and in agreement with the literature value [24].

***2.3. General procedure for the synthesis of ethyl-5-methyl-1,3-diphenyl-1H-pyrazole-4-carboxylate* (1a) [25].** To a stirred solution of benzaldehyde (0.106 g, 1.00 mmol) in EtOH (5 mL) was added phenyl hydrazine (0.119 g, 1.00 mmol, 1.1 equiv). After stirring at room temperature for 15 min, the benzaldehyde phenyl hydrazone was formed based on TLC analysis. $Hg(OAc)_2$ (0.478g, 1.5 mmol, 1.5 equiv) in 5ml EtOH and Ethyl but-2-ynoate (0.224g, 2.00 mmol, 2.0 equiv) were added simultaneously to the reaction mixture from two separate droppers. The contents were then allowed to stir at room temperature for 30 min (1.0 hr total). On completion of the reaction, the reaction mixture was extracted with ethyl acetate (3 × 5 mL). The combined organic layer was washed with 1M KBr solution (in order to remove mercury salts), with brine, dried over anhydrous Na_2SO_4 and then concentrated under reduced pressure. The crude product was purified by column chromatography (*n*-hexane / EtOAc 95/05) two fractions which on evaporation, **1a** obtained as white solids (Yield 0.277g, 96%), mp 102-104 °C; ^1H NMR (400 MHz, $CDCl_3$): δ 1.15 (t, J = 7.2 Hz, 3H), 2.41 (s, 3H), 4.13 (q, J = 7.2 Hz, 2H), 7.29-7.36 (m, 4H), 7.41-7.45 (m, 4H), 7.49-7.57 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 12.7, 14.1, 60.1, 110.6, 125.8, 127.6, 128.2, 128.7, 129.2, 129.4, 133.1, 138.8, 144.7, 153.6, 164.2; MS *m/z* 307.6 $(M+H)^+$; Anal. Calc. for $C_{19}H_{18}N_2O_2$: C, 74.50; H, 5.90; N, 9.14; Found: C, 74.56; H, 5.91; N, 9.10.

***2.4. General procedure for the synthesis of 5-methyl-1,3-diphenyl-1H-pyrazole-4-carbohydrazide* (2a).** An oven-dried two neck round bottomed flask was charged with Pyrazole-4-carboxylate (**1a**, 1.0 g, 1.00 mmol) and 98% hydrazine hydrate (2mL) in EtOH (10mL). The mixture was stirred at reflux for 2 h. After the completion of the reaction the solvent was evaporated in vacuum. The residual mass was extracted into ether (25 ml), washed successively with water (2 x 25 ml) and dried over anhydrous sodium sulphate, evaporation of the solvent afforded the respective **2a** as off white solid (Yield 0.71 g, 75%); ^1H NMR (400 MHz, $CDCl_3$) δ 2.49 (s, 3H), 7.33-7.40 (m, 3H), 7.42-7.47 (m, 1H), 7.50-7.61 (m, 6H) 7.98 (s, 2H) 8.04 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 13.00, 109.8, 125.7, 127.6, 128.6, 128.9, 129.2, 129.4, 132.2, 138.4, 146.1, 154.0, 168.4. MS: m/z = 293.3 $[M+H]^+$; Anal. % Calculated for $C_{17}H_{16}N_4O$: C 69.85, H 5.52, N 19.17; Found: C 69.82, H 5.50, N 19.21.

2.5. General procedure for the synthesis of 5-methyl-3-phenyl-isoxazole-4-carboxylic acid ethyl esters (3a). In a typical reaction, a mixture of benzaldehyde oxime (**a**, 1.45g, 12.0 mmole), excess of freshly distilled ethyl acetoacetate (2.6g, 20.0 mmole) and CAT (3.93g, 14.0 mmole) in ethyl alcohol (20 ml) were stirred at -10°C for about 3-4 hours. The reaction was monitored by TLC and continued till the disappearance of starting material and aldoxime. After the usual workup, the product isoxazole **3a** was obtained as light oil. (Yield 1.99 g, 72%). The isoxazole **3a** showed in IR (Nujol): 3005 cm^{-1} (C-H), 1688 cm^{-1} (C=O), 1609 cm^{-1} (C=C); ^1H NMR ($CDCl_3$): δ 1.35 (t, 3H, $OCH_2\underline{C}H_3$), 2.37 (s, 3H, $\underline{C}H_3$), 4.35 (q, 2H, $O\underline{C}H_2CH_3$), 7.36-7.42 (d, 2H, 3,5-Ar'-H), 7.28 (s, 1H, 4-Ar-H), 7.51-7.64 (s, 2H, 2,6-Ar'-H); MS (relative abundance): m/e for $C_{13}H_{13}NO_3$, 232 (M+1, 100), 202 (11), 158 (44), 144 (29), 119 (19), 103 (70); Anal. Calc. C, 67.52, H, 5.67, N, 6.06%; Found: C, 67.42, H, 5.53, N, 5.99%.

The same procedure was used in all cases (See supplementary information).

2.5a 3-(4-methoxy-phenyl)-5-methyl-isoxazole-4-carboxylic acid ethyl ester (3b). Obtained from 4-methoxy benzaldehyde oxime (**b**, 1.81g, 12.00 mmole), freshly distilled ethyl acetoacetate (**2**, 2.34g, 18 mmole) and CAT (3.94g, 14.0 mmole) in ethyl alcohol as an oil in 70% (2.19g) yield. IR (Nujol): 3010 cm^{-1} (C-H), 1690 cm^{-1} (C=O), 1612 cm^{-1} (C=C); ^1H NMR ($CDCl_3$): δ 1.34 (t, 3H, $OCH_2\underline{C}H_3$), 2.38 (s, 3H, CH_3), 3.75 (s, 3H, OCH_3), 4.32 (q, 2H, $O\underline{C}H_2CH_3$), 6.89-6.96 (t, 2H, 3,5-Ar'-H) 7.42-7.58 (d, 2H, 2,6-Ar'-H); Mass Spectrum (MS) (relative abundance): m/e for $C_{14}H_{15}NO_4$, 262 (M+1, 100), 232(12.0), 188 (45.0), 174 (30), 149 (22), 133 (74); Anal. Calc. C, 64.36, H, 5.79, N, 5.36%; Found: C, 64.24, H, 5.60, N, 5.29%.

2.5b 3-(3,4-dimethoxy-phenyl)-5-methyl-isoxazole-4-Carboxylic acid ethyl ester (3c). Obtained from 3,4-dimethoxy benzaldehyde oxime (**c,** 1.81g, 10 mmole), freshly distilled ethyl acetoacetate (**2**, 2.60g, 20.0 mmole) and CAT (3.94g, 14.0 mmole) in ethyl alcohol as an oil in 74% (2.15g) yield. IR (Nujol): 3015 cm^{-1} (C-H), 1692 cm^{-1} (C=O), 1611 cm^{-1} (C=C); ^1H NMR ($CDCl_3$): δ 1.32 (t, 3H, $OCH_2\underline{C}H_3$), 2.39 (s, 3H, CH_3), 3.79-3.82 (s, 6H, OCH_3), 4.31 (q, 2H, $OCH_2\underline{C}H_3$), 6.78 (s, 1H, 5-Ar'-H), 6.90 (s, 1H, 2-Ar'-H), 6.98 (s, 1H, 6-Ar'-H); MS (relative abundance): m/e for $C_{15}H_{17}NO_5$ 292 (M+1, 100), 262 (13), 218 (46), 204 (29), 179 (18), 163 (70); Anal. Calc. C, 61.85, H, 5.88, N, 4.81%; Found: C, 61.55, H, 5.65, N, 4.75%.

2.5c 5-methyl-3-(3,4,5-trimethoxy-phenyl)-isoxazole-4-Carboxylic acid ethyl ester (3d). Obtained from 3,4,5-trimethoxy benzaldehyde oxime (**d**, 2.11g, 10.0 mmole), ethyl acetoacetate (**2**, 2.0g, 16 mmole) and CAT (3.94g, 14.0 mmole) in ethyl alcohol as an oil in 70% (2.24g) yield. IR (Nujol): 3005 cm^{-1} (C-H), 1701 cm^{-1} (C=O), 1602 cm^{-1} (C=C); ^1H NMR ($CDCl_3$): δ 1.35 (t, 3H, $OCH_2\underline{C}H_3$), 2.32 (s, 3H, CH_3), 3.78-3.86 (s, 9H, OCH_3), 4.25 (q, 2H, $O\underline{C}H_2CH_3$), 6.49-6.68 (s, 2H, 2,6-Ar'-H); MS (relative

abundance): m/e for $C_{16}H_{19}NO_2$, 322 (M+1, 100), 292 (12), 248 (44), 234 (29), 209 (18), 193 (73); Anal. Calc. C, 59.81, H, 5.96, N, 4.36%; Found: C, 59.75, H, 5.80, N, 4.25%.

2.5d 3-(4-chloro-phenyl)-5-methyl-isoxazole-4-Carboxylic acid ethyl ester (3e). Obtained from 4-chloro benzaldehyde oxime (**e**, 1.86g, 12.0 mmole), ethyl acetoacetate (**2**, 2.34g, 18 mmole) and CAT (3.94g, 14.0 mmole) in ethyl alcohol as an oil in 72% (2.28g) yield. IR (Nujol): 3022 cm^{-1} (C-H), 1686 cm^{-1} (C=O), 1609 cm^{-1} (C=C); ^{1}H NMR ($CDCl_3$): δ 1.36 (t, 3H, $OCH_2\underline{C}H_3$), 2.31 (s, 3H, CH_3), 4.31(q, 2H, $O\underline{C}H_2CH_3$), 7.37-7.48 (d, 2H, 3,5-Ar'-H) 7.48-7.62 (d, 2H, 2,6-Ar'-H); MS (relative abundance): m/e for $C_{13}H_{12}NO_3Cl$, 266 (M+1, Cl^{37}, 33), 264 (M+1, Cl^{35}, 100) 237 (13), 193 (46), 178 (31), 153 (16), 137 (70); Anal. Calc. C, 58.77, H, 4.55, N, 5.27%; Found: C, 58.59, H, 4.43, N, 5.11%.

2.5e 3-(4-N,N-dimethylamino-phenyl)-5-methyl-isoxazole-4-Carboxylic acid ethyl ester (3f). Obtained from 4-*N,N*-dimethylamino benzaldehyde oxime (**f**, 1.64g, 10.0 mmole), ethyl acetoacetate (**2**, 2.08g, 16 mmole) and CAT (3.94g, 14.0 mmole) in ethyl alcohol as an oil in 74% (2.02g) yield. IR (Nujol): 3026 cm^{-1} (C-H), 1678 cm^{-1} (C=O), 1606 cm^{-1} (C=C); ^{1}H NMR ($CDCl_3$): δ 1.30 (t, 3H, $OCH_2\underline{C}H_3$), 2.32 (s, 3H, CH_3), 2.82 (s, 6H, *N*-CH_3), 4.24 (q, 2H, $O\underline{C}H_2CH_3$), 6.66-6.78 (d, 2H, 3,5-Ar'-H), 7.28-7.36 (d, 2H, 2,6-Ar'-H); MS (relative abundance): m/e for $C_{15}H_{18}N_2O_3$, 275 (M+1, 100), 245 (10), 201 (44), 187 (32), 162 (19), 146 (70); Anal. Calc. C, 65.64, H, 6.60, N, 10.18%; Found: C, 65.36, H, 6.38, N, 10.04%.

2.5f 5-methyl-3-p-tolyl-isoxazole-4-Carboxylic acid ethyl ester (3g). Obtained from 4-methyl benzaldehyde oxime (**g**, 1.35g, 10.0 mmole), ethyl acetoacetate (**2**, 2.34g, 18 mmole) and CAT (3.94g, 14.0 mmole) in ethyl alcohol as an oil in 72% (1.76g) yield. IR (Nujol): 3032 cm^{-1} (C-H), 1692 cm^{-1} (C=O), 1618 cm^{-1} (C=C); ^{1}H NMR ($CDCl_3$): δ 1.32 (t, 3H, $OCH_2\underline{C}H_3$), 2.30 (s, 3H, CH_3), 2.34 (s, 3H, Ar'-CH_3), 4.28 (q, 2H, $O\underline{C}H_2CH_3$), 7.24-7.30 (d, 2H, 3,5-Ar'-H) 7.42-7.50 (d, 2H, 2,6-Ar'-H); MS (relative abundance): m/e for $C_{14}H_{15}NO_3$, 246 (M+1, 100), 216 (12), 172 (46), 158 (30), 133 (20), 117 (72); Anal. Calc. C, 68.54, H, 6.16, N, 5.70%; Found: C, 68.24, H, 6.04, N, 5.56%.

2.6. General procedure for the synthesis of 5-methyl-3phenyl-isoxazole-4-carboxylic acid–N-(5-methyl-1,3-diphenyl-1H-pyrazole-4-carbonyl)-hydrazide (4a). 5-methyl-1,3-diphenyl-1*H*-pyrazole-4-carboxylic acid hydrazide (**2a,** 1.50g, 5.0 mmol) reflux with 5-methyl-3-phenyl-isoxazole-4-carboxylic acid ethyl ester (**3a,** 1.15g, 5.0 mmol) on water bath using absolute alcohol (25 ml) as a solvent for about 4-5 hours. The progress of the reaction was monitored by TLC. After completion of the reaction the solvent was evaporated in vacuum. The residual mass was extracted into ether (25 ml), washed successively with water (2 x 25 ml) and dried over anhydrous sodium sulphate, evaporation of the solvent afforded 5-methyl-3-phenyl-isoxazole-4-carboxylic acid–*N*-(5-methyl-1,3-diphenyl-1*H*-pyrazole-4-carbonyl)-hydrazide (**4a**, 1.14g) as a light yellow solid in 70% yield, m.p. 168-170°C. The isoxazole-pyrazole hydrazide **4a** showed IR (Nujol): 3345-3468 cm^{-1} (NH), 1659-1698 cm^{-1} (C=O); ^{1}H NMR ($CDCl_3$): δ 2.30 (s, 3H, isoxazole -CH_3), 2.79 (s, 3H, pyrazole -CH_3), 7.15-7.56 (m, 15H, Ar-H), 8.01 (s, 1H, NH) 8.03 (s, 1H, NH); ^{13}C NMR (100 MHz $CDCl_3$): δ 11.3, 12.4, 110.8, 114.0, 120.9, 126.2, 127.5, 128.7, 129.0, 129.2, 129.3, 132.0, 140.4, 151.9, 152.2, 162.4, 164.8, 168.2; MS (Relative intensity): m/e for $C_{28}H_{23}N_5O_3$; 478 (M+1, 100), 276 (64), 201 (58); Anal. Calc. C, 70.43, H, 4.85, N, 14.67%; Found: C, 70.32, H, 4.68, N, 14.54%.

The same procedure was used in all cases (See supplementary information).

2.6a 3-(4-methoxy-phenyl)-5-methyl-isoxazole-4-carboxylic acid-N-[3-(4-methoxy-phenyl)-5-methyl-1-phenyl-1H-pyrazole-4-carbonyl]-hydrazide (4b). Obtained from 3-(4-methoxy-phenyl)-5-methyl-1-phenyl-1*H*-pyrazole-4-carbonyl-hydrazide (**2b**, 1.60g 5.0 mmol) and 3-(4-methoxy-phenyl)-5-methyl-isoxazole-4-carboxylic acid ethyl ester (**3b**, 1.30g, 5.0 mmol) in absolute alcohol as a light yellow solid in 68% yield, m.p. 176-178°C. The isoxazole-pyrazole hydrazide **4b** showed IR (Nujol): 3368-3482 cm^{-1} (NH), 1672-1702 cm^{-1} (C=O); ^{1}H NMR ($CDCl_3$): δ 2.34 (s, 3H, isoxazole -CH_3), 2.68 (s, 3H, pyrazole -CH_3), 3.73 (s, 6H, 2-OCH_3), 7.02-7.64 (m, 13H, Ar-H), 8.01 (s, 1H, NH) 8.03 (s, 1H, NH); ^{13}C NMR (100 MHz $CDCl_3$): δ 11.3, 12.4, 55.3, 110.6, 113.8, 121.3, 124.9, 125.3, 126.2, 128.5, 129.3, 141.4, 152.9, 153.6, 160.1, 162.4, 164.8, 168.4; MS (Relative intensity): m/e for $C_{30}H_{27}N_5O_5$; 538 (M+1, 100), 306 (60), 231 (52); Anal. Calc. C, 67.03, H, 5.05, N, 13.02%; Found: C, 67.00, H, 5.00, N, 13.0%.

2.6b 3-(3,4-dimethoxy-phenyl)-5-methyl-isoxazole-4-carboxylic acid-N-[3-(3,4-dimethoxy-phenyl)-5-methyl-1-phenyl-1H-pyrazole-4-carbonyl]-hydrazide (4c). Obtained from 3-(3,4-dimethoxy-phenyl)-5-methyl-1-phenyl-1H-pyrazole-4-carbonyl-hydrazide (**2c**, 1.70g 5.0 mmol) and 3-(3,4-dimethoxy-phenyl)-5-methyl-isoxazole-4-carboxylic acid ethyl ester (**3c**, 1.50g, 5.0 mmol) in absolute alcohol as a light yellow solid in 66% yield, m.p. 162-164°C. The isoxazole-pyrazole hydrazide **4c** showed IR (Nujol): 3388-3490 cm^{-1} (NH), 1684-1710 cm^{-1} (C=O); ^{1}H NMR ($CDCl_3$): δ 2.38 (s, 3H, isoxazole -CH_3), 2.76 (s, 3H, pyrazole -CH_3), 3.75-3.84 (s, 12H, 4-OCH_3), 6.86-7.66 (m, 11H, Ar-H), 8.02 (s, 2H, NH); ^{13}C NMR (100 MHz $CDCl_3$): δ 11.2, 12.8, 55.6, 108.2, 111.4, 111.6, 114.0, 120.4, 124.4, 126.0, 126.2, 129.1, 141.2, 149.2, 150.2, 153.0, 153.4, 162.2, 164.6, 168.0; MS (Relative intensity): m/e for $C_{32}H_{31}N_5O_7$; 598 (M+1, 100), 336 (66), 261 (58); Anal. Calc. C, 64.30, H, 5.22, N, 11.72%; Found: C, 64.28, H, 5.20, N, 11.68%.

2.6c 3-(3,4,5-trimethoxy-phenyl)-5-methyl-isoxazole-4-carboxylic acid-N-[3-(3,4,5-trimethoxy-phenyl)-5-methyl-1-phenyl-1H-pyrazole-4-carbonyl]-hydrazide (4d). Obtained from 3-(3,4,5-trimethoxy-phenyl)-5-methyl-1-phenyl-1*H*-pyrazole-4-carbonyl-hydrazide (**2d**, 1.90g 5.0 mmol) and 3-(3,4,5-trimethoxy-phenyl)-5-methyl-isoxazole-4-carboxylic acid ethyl ester (**3d**, 1.60g, 5.0 mmol) in absolute alcohol as a light yellow solid in 70% yield, m.p. 144-146°C. The isoxazole-pyrazole hydrazide **4d** showed IR (Nujol): 3368-3486 cm^{-1} (NH), 1672-1708 cm^{-1} (C=O); ^{1}H NMR ($CDCl_3$): δ 2.35 (s, 3H, isoxazole -CH_3), 2.80 (s, 3H, pyrazole -CH_3), 3.80-3.94 (s, 18H, 4-OCH_3), 6.86-7.42 (m, 9H, Ar-H), 8.02 (s, 2H, NH); ^{13}C NMR (100 MHz $CDCl_3$): δ 11.4, 12.8, 56.2, 60.6, 100.2, 111.4, 114.1, 123.1, 126.4, 127.6, 129.1, 139.0, 141.2, 153.1, 153.2, 153.6, 162.4, 164.6, 168.2; MS (Relative intensity): m/e for $C_{34}H_{35}N_5O_9$; 658 (M+1, 100), 366

(62), 291 (48); Anal. Calc. C, 62.09, H, 5.36, N, 10.64%; Found: C, 62.00, H, 5.34, N, 10.60%.

2.6d 3-(4-chloro-phenyl)-5-methyl-isoxazole-4-carboxylic acid-N-[3-(4-chloro-phenyl)-5-methyl-1-phenyl-1H-pyrazole-4-carbonyl]-hydrazide (4e). Obtained from 3-(4-chloro-phenyl)-5-methyl-1-phenyl-1*H*-pyrazole-4-carbonyl-hydrazide (**2e**, 1.63g 5.0 mmol) and 3-(4-chloro-phenyl)-5-methyl-isoxazole-4-carboxylic acid ethyl ester (**3e**, 1.30g, 5.0 mmol) in absolute alcohol as a light yellow solid in 64% yield, m.p. 138-140°C. The isoxazole-pyrazole hydrazide **4e** showed IR (Nujol): 3392-3422 cm^{-1} (NH), 1690-1710 cm^{-1} (C=O); ^1H NMR ($CDCl_3$): δ 2.30 (s, 3H, isoxazole -CH_3), 2.72 (s, 3H, pyrazole -CH_3), 7.02-7.42 (m, 13H, Ar-H), 8.01 (s, 1H, NH) 8.03 (s, 1H, NH); ^{13}C NMR (100 MHz $CDCl_3$): δ 11.6, 12.7, 111.4, 114.2, 123.1, 126.4, 127.4, 129.1, 129.3, 131.3, 134.3, 141.4, 153.2, 153.8, 162.4, 164.6, 168.4; MS (Relative intensity): m/e for $C_{28}H_{21}Cl_2N_5O_3$; 546 (M+1, 100), 310 (58), 201 (46); Anal. Calc. C, 61.54, H, 3.86, N, 12.82%; Found: C, 61.50, H, 3.82, N, 12.78%.

2.6e 3-(4-N,N-dimethylamino-phenyl)-5-methyl-isoxazole-4-carboxylic acid-N-[3-(4-N,N-dimethylamino-phenyl)-5-methyl-1-phenyl-1H-pyrazole-4-carbonyl]-hydrazide (4f). Obtained from 3-(4-*N,N*-dimethylamino-phenyl)-5-methyl-1-phenyl-1*H*-pyrazole-4-carbonyl-hydrazide (**2f**, 1.65g 5.0 mmol) and 3-(4-*N,N*-dimethylamino-phenyl)-5-methyl-isoxazole-4-carboxylic acid ethyl ester (**3f**, 1.35g, 5.0 mmol) in absolute alcohol as a light yellow solid in 66% yield, m.p. 153-155°C. The isoxazole-pyrazole hydrazide **4f** showed IR (Nujol): 3412-3466 cm^{-1} (NH), 1692-1724 cm^{-1} (C=O); ^1H NMR ($CDCl_3$): δ 2.32 (s, 3H, isoxazole -CH_3), 2.68 (s, 3H, pyrazole -CH_3), 2.85-2.90 (s, 12H, 2 N(CH_3)$_2$), 6.60-7.42 (m, 13H, Ar-H), 8.01 (s, 1H, NH) 8.03 (s, 1H, NH); ^{13}C NMR (100 MHz $CDCl_3$): δ 11.6, 12.8, 41.4, 111.9, 112.8, 113.8, 118.5, 122.6, 125.1, 126.4, 128.0, 129.2, 141.1, 153.2, 153.8, 155.8, 162.6, 164.6, 168.1; MS (Relative intensity): m/e for $C_{32}H_{33}N_7O_3$; 564 (M+1, 100), 319 (64), 244 (50); Anal. Calc. C, 68.18, H, 5.90, N, 17.40%; Found: C, 68.10, H, 5.82, N, 17.28%.

2.6f 5-methyl-3-p-tolyl-isoxazole-4-carboxylic acid-N-(5-methyl-1-phenyl-3-p-totyl-1H-pyrazole-4-carbonyl)-hydrazide (4g). Obtained from 5-methyl-1-phenyl-3-*p*-tolyl-1*H*-pyrazole-4-carbonyl)-hydrazide (**2g**, 1.52g 5.0 mmol) and 5-methyl-3-*p*-tolyl-isoxazole-4-carboxylic acid ethyl ester (**3g**, 1.22g, 5.0 mmol) in absolute alcohol as a light yellow solid in 72% yield, m.p. 160-162°C. The isoxazole-pyrazole hydrazide **4g** showed IR (Nujol): 3392-3434 cm^{-1} (NH), 1688-1708 cm^{-1} (C=O); ^1H NMR ($CDCl_3$): δ 2.28 (s, 3H, isoxazole -CH_3), 2.40 (s, 6H, 2 Ar-CH_3), 2.82 (s, 3H, pyrazole -CH_3), 7.06-7.42 (m, 13H, Ar-H), 8.01 (s, 1H, NH) 8.03 (s, 1H, NH); ^{13}C NMR (100 MHz $CDCl_3$): δ 11.6, 12.8, 21.2, 111.8, 113.8, 125.1, 125.8, 126.2, 126.4, 128.8, 129.6, 130.1, 130.4, 132.1, 141.4, 153.0, 154.0, 162.8, 164.6, 168.0; MS (Relative intensity): m/e for $C_{30}H_{27}N_5O_3$; 506 (M+1, 100), 290 (66), 215 (52); Anal. Calc. C, 71.27, H, 5.37, N, 13.85%; Found: C, 71.14, H, 5.24, N, 13.76%.

2.7. General procedure for the synthesis of 2-(5-methyl-1,3-diphenyl-1H-pyrazole-4-yl)-5-(5-methyl-3-phenyl-isoxazole-4yl)-[1,3,4]-oxadiazoles (5a).

5-methyl-3phenyl-isoxazole-4-carboxylic acid–*N*-(5-methyl-1,3-diphenyl-1*H*-pyrazole-4-carbonyl)-hydrazide (**4a,** 2.38g, 5.0 mmol) was refluxed with phosphorous oxy chloride (20 ml) for about 7-8 hours on water bath. The progress of the reaction was monitored by TLC. After the completion of the reaction the residual mass was extracted into ether (25 ml) and washed successively with water (2 X 20 ml) and dried over anhydrous sodium sulphate. Evaporation of the solvent afforded crude solid substance gave one major spot with R_f value 0.54. The purification was done by column chromatography using chloroform : acetone (7:1) as eluent, which afforded the expected product 2-(5-methyl-1,3-diphenyl-1*H*-pyrazole-4-yl)-5-(5-methyl-3-phenyl-isoxazole-4yl)-[1,3,4]-oxadiazoles (**5a**) in 68 % yield, m.p. 122-124°C. The oxadiazoles **5a** showed; IR (Nujol): 1622-1666 cm^{-1} (C=N), 1602-1616 cm^{-1} (C=C); ^1H NMR ($CDCl_3$): δ 2.35 (s, 3H, isoxazole-CH_3), 2.80 (s, 3H, pyrazole-CH_3), 7.20-7.52 (m, 15H, Ar-H); ^{13}C NMR (100 MHz $CDCl_3$): δ 11.3, 12.7, 104.8, 111.2, 123.1, 126.0, 127.8, 128.6, 129.3, 129.4, 131.8, 133.0, 136.0, 139.7, 148.2, 154.5, 163.0, 165.6; MS (Relative intensity): m/e for $C_{28}H_{21}N_5O_2$; 460 (M+1, 100), 301 (72), 233 (60), 158 (40); Anal. Calc. C, 73.19, H, 4.60, N, 15.24%; Found: C, 73.08, H, 4.48, N, 15.12%.

The same procedure was used in all cases (See supplementary information).

2.7a 2-[3-(4-methoxy-phenyl)-5-methyl-isoxazole-4-yl]-5-[3-(4-methoxy-phenyl)-5-methyl-1-phenyl-1H-pyrazole-4-yl]-[1,3,4]-oxadiazole (5b). Obtained from 3-(4-methoxy-phenyl)-5-methyl-isoxazole-4-carboxylic acid-*N*-[3-(4-methoxy-phenyl)-5-methyl-1-phenyl-1*H*-pyrazole-4-carbonyl]-hydrazide (**4b**, 2.65g, 5.0 mmol) and phosphorous oxy chloride (20 ml) as a light yellow solid in 65% yield, m.p. 162-164°C. The oxadiazoles **5b** showed; IR (Nujol): 1644-1668 cm^{-1} (-C=N-), 1608-1620 cm^{-1} (-C=C-); ^1H NMR ($CDCl_3$): δ 2.29 (s, 3H, isoxazole-CH_3), 2.78 (s, 3H, pyrazole-CH_3), 3.82-3.88 (s, 6H, 2-OCH_3), 6.82-7.32 (m, 13H, Ar-H); ^{13}C NMR (100 MHz $CDCl_3$): δ 11.4, 12.8, 55.8, 104.5, 111.0, 114.8, 124.2, 125.0, 125.3, 126.2, 128.8, 129.3, 136.0, 139.8, 148.2, 154.6, 160.8, 162.9, 165.6; MS (Relative intensity): m/e for $C_{30}H_{25}N_5O_4$; 520 (M+1, 100), 331 (76), 263 (58), 188 (44); Anal. Calc. C, 69.35, H, 4.85, N, 13.48%; Found: C, 69.26, H, 4.74, N, 13.30%.

2.7b 2-[3-(3,4-dimethoxy-phenyl)-5-methyl-isoxazol-4-yl]-5-[3-(3,4-dimethoxy-phenyl)-5-methyl-1-phenyl-1H-pyrazole-4-yl]-[1,3,4]-oxadiazole (5c). Obtained from 3-(3,4-dimethoxy-phenyl)-5-methyl-isoxazole-4-carboxylic acid-*N*-[3-(3,4-dimethoxy-phenyl)-5-methyl-1-phenyl-1*H*-pyrazole-4-carbonyl]-hydrazide (**4c**, 2.99g, 5.0 mmol) and phosphorous oxy chloride (20 ml) as a light yellow solid in 68% yield, m.p. 158-160°C. The oxadiazoles **5c** showed; IR (Nujol): 1632-1644 cm^{-1} (C=N), 1614-1626 cm^{-1} (C=C); ^1H NMR ($CDCl_3$): δ 2.34 (s, 3H, isoxazole-CH_3), 2.74 (s, 3H, pyrazole-CH_3), 3.82-3.88 (s, 12H, 4-OCH_3), 6.72-7.22 (m, 11H, Ar-H); ^{13}C NMR (100 MHz $CDCl_3$): δ 11.7, 13.0, 55.6, 55.7, 104.8, 109.1, 111.0, 111.1, 120.8, 123.2, 126.2, 126.3, 129.3,

135.8, 139.4, 148.3, 149.8, 150.1, 154.4, 163.1, 165.8; MS (Relative intensity): m/e for $C_{32}H_{29}N_5O_6$; 580 (M+1, 100), 361 (72), 293 (60), 218 (40); Anal. Calc. C, 66.30, H, 5.03, N, 12.08%; Found: C, 66.18, H, 4.88, N, 12.00%.

2.7c 2-[3-(3,4,5-trimethoxy-phenyl)-5-methyl-isoxazol-4-yl]-5-[3-(3,4,5-trimethoxy-phenyl)-5-methyl-1-phenyl-1H-pyrazole-4-yl]-[1,3,4]-oxadiazole (5d). Obtained from 3-(3,4,5-trimethoxy-phenyl)-5-methyl-isoxazole-4-carboxylic acid-*N*-[3-(3,4,5-trimethoxy-phenyl)-5-methyl-1-phenyl-1*H*-pyrazole-4-carbonyl]-hydrazide (**4d**, 3.25g, 5.0 mmol) and phosphorous oxy chloride (20 ml) as a light yellow solid in 64% yield, m.p. 164-166°C. The oxadiazoles **5d** showed IR (Nujol): 1638-1652 cm^{-1} (C=N), 1616-1634 cm^{-1} (C=C); ^{1}H NMR ($CDCl_3$): δ 2.26 (s, 3H, isoxazole -CH_3), 2.72 (s, 3H, pyrazole -CH_3), 3.76-3.86 (s, 18H, 6-OCH_3), 6.46-7.08 (m, 9H, Ar-H); ^{13}C NMR (100 MHz $CDCl_3$): δ 11.6, 13.1, 56.4, 58.4, 100.3, 104.2, 111.0, 123.1, 126.4, 127.5, 130.1, 135.8, 139.2, 139.7, 148.3, 153.1, 154.6, 163.3, 165.8; MS (Relative intensity): m/e for $C_{34}H_{33}N_5O_8$; 640 (M+1, 100), 391 (70), 323 (66), 248 (46); Anal. Calc. C, 63.83, H, 5.20, N, 10.95%; Found: C, 63.64, H, 5.08, N, 10.80%.

2.7d 2-[3-(4-chloro-phenyl)-5-methyl-isoxazol-4-yl]-5-[3-(4-chloro-phenyl)-5-methyl-1-phenyl-1H-pyrazole-4-yl]-[1,3,4]-oxadiazole (5e). Obtained from 3-(4-chloro-phenyl)-5-methyl-isoxazole-4-carboxylic acid-*N*-[3-(4-chloro-phenyl)-5-methyl-1-phenyl-1*H*-pyrazole-4-carbonyl]-hydrazide (**4e**, 2.25g, 5.0 mmol) and phosphorous oxy chloride (20 ml) as a light yellow solid in 65% yield, m.p. 136-138$^{°C}$. The oxadiazoles **5e** showed; IR (Nujol): 1622-1644 cm^{-1} (C=N), 1608-1626 cm^{-1} (C=C); ^{1}H NMR ($CDCl_3$): δ 2.34 (s, 3H, isoxazole-CH_3), 2.80 (s, 3H, pyrazole-CH_3), 6.93-7.22 (m, 13H, Ar-H); ^{13}C NMR (100 MHz $CDCl_3$): δ 11.6, 13.1, 104.6, 110.9, 125.0, 126.2, 129.1, 129.3, 130.2, 131.2, 134.1, 136.4, 139.8, 148.1, 154.6, 163.5, 165.6; MS (Relative intensity): m/e for $C_{28}H_{19}Cl_2N_5O_2$; 520 (M+1, 100), 335 (76), 267 (60), 192 (42); Anal. Calc. C, 63.65, H, 3.62, N, 13.25%; Found: C, 63.54, H, 3.50, N, 13.12%.

2.7e 2-[3-(4-N,N-dimethylamino-phenyl)-5-methyl-isoxazole-4-yl]-5-[3-(4-N,N-dimethylamino-phenyl)-5-methyl-1-phenyl-1H-pyrazole-4-yl]-[1,3,4]-oxadiazole (5f): Obtained from 3-(4-*N,N*-dimethylamino-phenyl)-5-methyl-isoxazole-4-carboxylic acid-*N*-[3-(4-*N,N*-dimethylamino-phenyl)-5-methyl-1-phenyl-1*H*-pyrazole-4-carbonyl]-hydrazide (**4f**, 2.30g, 5.0 mmol) and phosphorous oxy chloride (20 ml) as a light yellow solid in 68% yield, m.p. 144-146°C. The oxadiazoles **5f** showed IR (Nujol): 1612-1630 cm^{-1} (C=N), 1602-1618 cm^{-1} (C=C); ^{1}H NMR ($CDCl_3$): δ 2.26 (s, 3H, isoxazole -CH_3), 2.74 (s, 3H, pyrazole -CH_3), 2.80-2.94 (s, 12H, 2 $N(CH_3)_2$), 6.82-7.18 (m, 13H, Ar-H); ^{13}C NMR (100 MHz $CDCl_3$): δ 11.8, 13.1, 41.2, 104.4, 111.0, 113.1, 120.8, 122.2, 125.2, 126.1, 128.4, 129.2, 136.1, 140.2, 148.2, 154.8, 155.2, 163.3, 165.7; MS (Relative intensity): m/e for $C_{32}H_{31}N_7O_2$; 546 (M+1, 100), 344 (72), 376 (56), 201 (38); Anal. Calc. C, 70.44, H, 5.72, N, 17.96%; Found: C, 70.34, H, 5.60, N, 17.82%.

2.7f 2-(5-methyl-3-p-tolyl-isoxazole-4-yl)-5-(5-methyl-1-phenyl-3-p-tolyl-1H-pyrazole-4-yl)-[1,3,4]-oxadiazole (5g): Obtained from 3-(4-*N,N*-dimethylamino-phenyl)-5-methyl-isoxazole-4-carboxylic acid-*N*-[3-(4-*N,N*-dimethylamino-phenyl)-5-methyl-1-phenyl-1*H*-pyrazole-4-carbonyl]-hydrazide (**4g**, 2.30g, 5.0 mmol) and phosphorous oxy chloride (20 ml) as a light yellow solid in 68% yield, m.p. 144-146°C. The oxadiazoles **5g** showed IR (Nujol): 1630-1644 cm^{-1} (C=N), 1610-1620 cm^{-1} (C=C); ^{1}H NMR ($CDCl_3$): δ 2.28 (s, 3H, isoxazole -CH_3), 2.48-2.54 (s, 6H, 2Ar-CH_3), 2.82 (s, 3H, pyrazole -CH_3), 6.98-7.22 (m, 13H, Ar-H); ^{13}C NMR (100 MHz $CDCl_3$): δ 11.6, 13.1, 21.2, 104.6, 111.0, 125.0, 125.6, 126.4, 129.1, 129.4, 129.7, 130.2, 131.7, 136.0, 139.8, 148.2, 154.8, 163.8, 165.8; MS (Relative intensity): m/e for $C_{30}H_{25}N_5O_2$; 488 (M+1, 100), 315 (76), 247 (50), 172 (46); Anal. Calc. C, 73.90, H, 5.17, N, 14.36%; Found: C, 73.84, H, 5.04, N, 14.26%.

2.8. **Biological assay of newly synthesized 4(a-g) and 5(a-g) derivatives.**

2.8a DPPH radical scavenging assay. The free radical scavenging property of the samples **4(a-g)** and **5(a-g)** was determined by DPPH method. The DPPH radical solution was prepared in methanol. The reaction mixture contained 5 µL of test samples and 95 µL of DPPH (300 µM) in methanol. Different concentrations of test samples were prepared and were used for DPPH radical scavenging activity. The reaction for scavenging $DPPH^{·}$ radical was carried out at 37ºC in dark for 30 min and the absorbance was recorded at 517 nm (Spectra max 340, Molecular devises). Percent radical scavenging activity was determined by comparison with a solvent treated control. Ascorbic acid was used as positive control. Percent scavenging effect was determined by the following equation.

$$\textbf{(\%) Inhibition} = [(A_c - A_s)/A_c] \times 100$$

Where, A_c= mean absorption of control, A_s= mean absorption of sample

The IC_{50} value was derived from the % inhibition at different concentration.

2.8b Measurement of reducing power. The reducing power of samples **4(a-g)** and **5(a-g)** was determined according to the method[31] of Yen and Chen. The samples (100-500 µg/mL) were mixed with an equal volume of 0.2 M phosphate buffer, pH 6.6 and 1 % potassium ferricyanide. The mixture was incubated at 50 °C for 20 min. Then an equal volume of 10 % trichloroacetic acid was added to the mixture and then centrifuged at 1000 rpm for 10 min. The upper layer of solution was mixed with distilled water and 0.1 % ferric chloride at a ratio of 1:1:2 and the absorbance were measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power. The blank was also carried out in similar manner. For all the above antioxidant methods, experiments were done in triplicate and average is taken, the % inhibition at different concentration was calculated by the following formula.

$$\textbf{(\%) Inhibition} = A_c - A_s \times 100 / A_c$$

Where, A_c= mean absorption of control, A_s= mean absorption of sample

The IC_{50} value was derived from the % inhibition at different concentration.

Statistical analysis: All the experiments were carried out in triplicates (n = 3) and the results are expressed as mean ± standard deviation (SD).

2.8c Antimicrobial activity. The newly synthesized compounds **4(a-g)** and **5(a-g)** were screened in vitro for their antibacterial activity by disc diffusion and microdilution method. The antibiotic *Tetracycline* and *Nystatin* were used as positive reference to determine the sensitivity of each microbial species tested. The test bacteria maintained on the nutrient agar (NA) medium at 37 °C. Briefly, a suspension of the test microorganism (0.1 ml of 108 cells/mL) was spread on the solid media plates. Filter paper discs (6 mm in diameter) were impregnated with synthetic compounds dissolved in dimethylformamide (DMF) at the concentration 50 and 100µg/mL and placed on the inoculated plates and after allowing at 4°C for 2 h, they were incubated at 37°C for 24 h. The diameters of the inhibition zones were measured in millimetres.

The smallest amount of synthesized compounds or standard (*Tetracycline*) antibiotic needed to inhibit the visible growth of a test microorganism (MIC) and the lowest concentration of an antibiotic required to kill a particular bacterium/fungi (MBC/MFC). Generally the antimicrobials are considered as bactericidal/fungicidal if the MBC/MFC is not greater than four times the MIC. The results are compiled in **Table 2–4**. In all the determinations tests were performed in six replicate and the results were taken as a mean of at least three determinations.

The microdilution method was used to evaluate the minimum inhibitory concentration (MIC) of all the synthesized compounds. Minimum inhibitory concentration (MIC) was measured by determining the smallest amount of synthesized compounds **4(a-g)** and **5(a-g)** or standard (*Tetracycline*) antibiotic needed to inhibit the visible growth of a test microorganism after 24 hours incubation periods at 37 °C. This was done using 96-well plates, the assay plates were filled with Mueller-Hinton broth medium (MHB) containing different concentrations of compounds, tetracycline or negative control (DMSO) and the test microorganisms (109 CFU/mL). The compounds were stable in the Nutrient agar and Potato dextrose agar. It can be seen in both solvent control and negative control (only organism without any treatment).

The MIC for fungal strains was performed using 96-well plate. The fungi were maintained on potato dextrose agar (PDA) medium at 28 °C. Each well contained potato dextrose broth (PDB), different concentration of compounds, Nystatin or negative control (DMSO) and the test fungal strains (105 CFU/mL). Incubation was performed at room temperature (18-20 °C) for 48 hours. Minimal bactericidal concentration (MBC) was determined by transferring and spreading the treated culture broth of the wells containing the concentrations equal to or higher than the MIC on agar plates. The lowest concentration of the compounds or the standard antibiotics required to completely destroy test microorganisms (no growth on the agar plate) after incubation at 37 °C for 24 hours (bacteria) and room temperature at (18-20 °C) for 48 hours (yeasts) was reported as MBC and minimal fungicidal concentration (MFC).

2.8d Inhibition of α-amylase activity. The α-amylase inhibitory activity for each compound was determined based on the colorimetric assay using Acarbose as the reference compound. The starch solution (0.5% w/v) was obtained by stirring and boiling 0.25g of soluble potato starch in 50mL of deionized water for 15 min. The enzyme solution (0.5 unit/mL) was prepared by mixing 0.001g of α-amylase (EC 3.2.1.1) in 100mL of 20 mM sodium phosphate buffer (pH 6.9) containing 6.7 mM sodium chloride. The compounds were dissolved in DMSO to give various concentrations. The color reagent contains 96 mM 3,5-dinitrosalicylic acid (20mL), 5.31 M sodium potassium tartrate in 2 M sodium hydroxide (8mL) and deionized water (12mL). One mL of each sample and 1mL of enzyme solution was mixed in a tube and incubated at 25°C for 30 min. To 1mL of this mixture 1mL of starch solution was added and incubated at 25°C for 3 min. Then, 1mL of the color reagent was added and the closed tube was placed on water bath at 85°C. After 15 min, the reaction mixture was removed from the water bath, cooled and diluted with 9mL distilled water and the absorbance value determined at 540 nm in a spectrophotometer. Individual blanks will be prepared for correcting the background absorbance. In this case, the color reagent solution should be added prior to the addition of starch solution and then the tube placed into the water bath. The other procedures will be carried out as above. Controls should be conducted in an identical fashion replacing the samples with 1mL DMSO. Acarbose solution will be used as positive control. The inhibition percentage of α-amylase was calculated by the following formula:

$$\text{Inhibition of } \alpha\text{-amylase } \% = 100 \times (\text{ODControl} - \text{ODSample}) / \text{ODControl}$$

2.8e Inhibition of α-glucosidase activity. The enzymatic activity of α-glucosidase was determined colorimetrically by monitoring the release of *p*-nitrophenol from the appropriate *p*-nitrophenol glycoside substrate. The assay mixture for these experiments contained 5µ M PNPG, enzyme solution (0.1 U) in 900 µl of sodium phosphate buffer in the final volume of 1mL. Each compound 100 µg was dissolved in 20 µl of distilled water and added to the test mixture before adding the substrate. Blank sample contained whole test mixture and the compound without enzyme solution. Acarbose was used as positive control. The mixture was incubated at 37°C for 30 min. the reaction was terminated by adding 3 volumes of NH4OH solution. The absorbance at 405 nm was determined by spectrophotometer. The inhibition percentage of α-glucosidase was calculated by the following formula:

$$\text{Inhibition of } \alpha\text{-glucosidase } \% = (OD_{Control} - OD_{Test} / OD_{Control}) \times 100.$$

2.8f Molecular docking. Automated docking was used to assess the appropriate binding orientations and conformations of the ligand molecules with different protein inhibitors. A Lamarkian genetic algorithm method implemented in the program AutoDock 4.2, was employed. For docking calculations, Gasteiger

charges were added and the rotatable bonds were set by the AutoDock tools and all torsions were allowed to rotate. Polar hydrogen atoms were added and Kollaman charges were assigned to the protein using AutoDock tools (ADT). The grid maps were generated by Autogrid program. Each grid was centered at the active pocket of the proteins and grid parameters were specified separately. In all the cases, we have used grid maps with a grid box size of 55×55×55 Å3 points with a grid-point spacing of 0.375 Å. The Lamarckian genetic algorithm, the pseudo-Solis and Wets methods were applied for minimization using default parameters. The docking protocol for rigid and flexible ligand docking consisted of 10 independent Genetic Algorithm (GA) runs per ligand. The docking results for a given macromolecule ligand pair mainly comprised of the intermolecular interaction energies including inhibition constant, hydrogen bond interaction energy, van der Waals forces, electrostatic energy and ligand efficiency. The lowest binding energy of proteinligand complex has been considered to be the best. The details of dock score results of the different pyrazole derivatives are given in **Table 6**.

2.8g Preparation of ligands and macromolecules. All ligand molecules **4(a-g)** and **5(a-g)** were drawn and the structure was analyzed by using ChemDraw Ultra 12.0. The compounds are converted to 3D structure using Openbable open access software tool. Energy minimization was performed by employing Dundee PRODRG server [33]. α-Amylase proteine was retrieved from the Protein Data Bank [34]. The protein target was selected based on their best appropriate ligand interactions. The water molecules, co-factors and ligands were removed from the protein structure and then checked for polar hydrogen atom in the macromolecules.

3. RESULTS SECTION

3.1. ***Chemistry.*** The compounds ethyl-5-methyl-1,3-diphenyl-1*H*-pyrazole-4-carboxylates **1(a–g)** were synthesized as reported earlier[24] (See fig 1 for ORTEP diagram of compound **1a**). The compounds **1(a–g)** were converted to respective 5-methyl-1,3-diphenyl-1*H*-pyrazole-4-carbohydrazides **2(a–g)** by simple procedure that involves; reacting **1(a–g)** with hydrazine hydrate in refluxing EtOH for 2h.[22] After the reaction goes to completion (monitored through thin layer chromatography), the compounds **2(a–g)** thus obtained were taken for the next step without isolation.

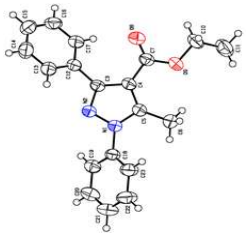
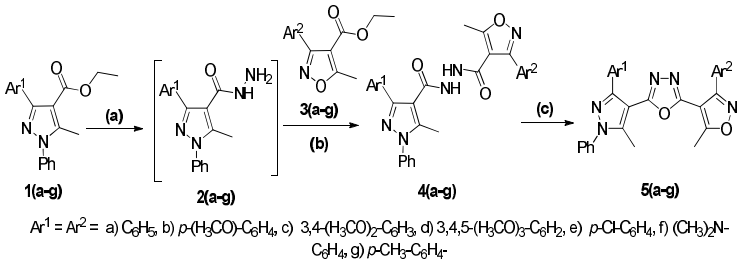


Figure 1. Perspective diagram of the molecules (**1a**) with 50% probability displacement ellipsoids.

The compounds **2(a–g)** obtained were then condensed with the alcoholic solution of isoxazoles **3(a–g)** (for the synthesis of **3(a-g)**, see the supporting information) for about 4-5 hours to produce the respective 5-methyl-3-phenyl-isoxazole-4-carboxylic acid-*N*-(5-methyl-1,3-diphenyl-1*H*-pyrazole-4-carbonyl)-hydrazide **4(a–g)**. The structure of newly synthesized pyrazolyl-isoxazole hydrazides **4(a–g)** were confirmed by IR, ^{1}H NMR, mass spectral studies and elemental analysis. In IR spectra, the ester carbonyl stretching frequency at 1678-1701 cm^{-1} was found absent but hydrazide carbonyl frequency at 1659-1700 cm^{-1} and (NH) frequency at 3368-3490 cm^{-1} were shown. In ^{1}H NMR spectra, it shows the absence of ethoxy protons in the region δ 4.24-4.35 ppm, (q, 2H, –O*CH*$_2$–CH$_3$) and triplet (t, 3H, –OCH$_2$–*CH*$_3$) in the region δ 1.30-1.36 ppm and also the absence of NH$_2$ peak at δ 7.98 ppm and appearance of two peaks at δ 8.01 and δ 8.03 for CONH protons confirms the formation of product **4(a–g)**.

The synthesis of target compound 2-(5-methyl-1,3-diphenyl-1*H*-pyrazole-4-yl)-5-(5-methyl-3-phenyl-isoxazole-4-yl)-[1,3,4]-oxadiazoles **5(a–g)** from the respective carbohydrazide **4(a–g)** is as depicted in Scheme 1. For the conversion of substituted pyrazolyl-isoxazole hydrazide **4a** to **5a**, initially we heated the compound **4a** with phosphorousoxychloride at 120°C which gave the respective compound **5a** in moderate yield. On the other hand, dehydration followed by cyclization in presence of Burgess reagent yielded the corresponding **5(a–g)** in good quality and yield (Scheme 1).



Scheme 1. Synthesis of 1,3,4-oxadiazole tethered pyrazolyl-isoxazole. Reagents and conditions: (a) NH_2NH_2, EtOH, 80°C. (b) EtOH, reflux (c) $POCl_3$, 120°C or Burgess Reagent.

The structures of newly synthesized compounds **5(a–g)** were confirmed by IR, ^{1}H NMR, mass spectral studies and elemental analysis. For instance, in IR spectra, the absence of (NH) frequency at 3368-3490 cm^{-1} and appearance of (C═N) frequency at 1640-1600 cm^{-1} substantiated the formation of product. The compounds also showed the characteristic IR absorption bands at 1570-1460 cm^{-1} for (C═C), 1260-1285 cm^{-1} for (C–N) and 1240-1230 cm^{-1} for (C–O). In ^{1}H NMR spectra, the disappearance of broad singlets at δ 8.01 and δ 8.03 for two (CONH) protons confirms the formation of cyclized 1,3,4-oxadiazole ring. The methyl and aryl moiety exhibited characteristic signals in the expected region of the spectrum. Finally, all the pyrazolyl-isoxazolo-1,3,4-oxadiazoles **5(a–g)** showed a molecular ion peak at M + 1 corresponding to their molecular formula, which confirmed their chemical structure.

3.2. Biological activity.

3.2a Antioxidant Activity. The synthesized compounds **5(a–g)** were screened for their in vitro antioxidant activity by DPPH radical scavenging assay [26] and reducing power determination [27]. The free radical scavenging is considered a good in vitro model and is widely used to conveniently assess

antioxidant efficacy. The result of in vitro antioxidant activity of synthesized compound is summarized in **Table 1**. The investigation of antioxidant screening revealed that some of the tested compounds showed moderate to good antioxidant activity. The interaction of pyrazolyl-isoxazolo-1,3,4-oxadiazoles **5(a–g)** with stable DPPH free radical indicates their free radical scavenging ability.

Among the synthesized compounds 4(a–g) and 5(a–g), the compounds **4d**, **4e**, **4f**, **5d**, **5e** and **5f** showed good interaction with the DPPH radical. This could be due the substitution on benzene ring like; methoxy group in **4d** and **5d**, chlorine group in **4e** and **5e**, and dimethyl amine groups on **4f** and **5f** respectively. The maximum antioxidant activity of compounds was observed in the following order **4f** > **5f** > **4e** > **5e** > **4d** > **5d**. Strikingly, the compound **4f** showed more propitious DPPH RSA as compared to that of standard ascorbic acid. This could be due the presence of electron donating *N,N*-dimethyl amine group on *p*-position of benzene. Interestingly, the hydrated open forms of 1,3,4-oxadiazole compounds **4(a–g)** showed better DPPH RSA than the compounds **5(a–g)**. The presence of stable amide bonds might add on to the hydrogen-bond donating capacity to DPPH radical thus increasing in activity. Notably the compounds **4e** and **5e** has shown very good antioxidant activity by reducing power determination. This may be due to two halogen atoms on benzene rings. All other compounds showed moderate to good IC_{50} value compare to standard by DPPH and reducing power determination.

Table 1. Antioxidant activity (IC_{50} values) of tested samples **4(a-g)** and **5(a-g).**

Compounds	Ar^1	Ar^2	IC_{50} (Mean ± SD) µg/mL	
			% DPPH radical scavenging assay	Reducing power determination
4a	C_6H_5	C_6H_5	63 ± 0.016	77 ± 0.098
4b	4–OCH_3–C_6H_4	4–OCH_3–C_6H_4	46 ± 0.025	47 ± 0.145
4c	3,4–$(OCH_3)_2$–C_6H_3	3,4–$(OCH_3)_2$–C_6H_3	48 ± 0.088	50 ± 0.112
4d	3,4,5–$(OCH_3)_3$–C_6H_2	3,4,5–$(OCH_3)_3$–C_6H_2	43 ± 0.030	46 ± 0.116
4e	4–Cl–C_6H_4	4–Cl–C_6H_4	36 ± 0.091	28 ± 0.186
4f	4–$(CH_3)_2$N-C_6H_4	$(CH_3)_2$N-C_6H_4	27 ± 0.092	39 ± 0.079
4g	4–CH_3–C_6H_4	4–CH_3–C_6H_4	52 ± 0.098	54 ± 0.203
5a	C_6H_5	C_6H_5	74 ± 0.086	77 ± 0.098
5b	4–OCH_3–C_6H_4	4–OCH_3–C_6H_4	46 ± 0.091	47 ± 0.165
5c	3,4–$(OCH_3)_2$–C_6H_3	3,4–$(OCH_3)_2$–C_6H_3	48 ± 0.088	50 ± 0.138
5d	3,4,5–$(OCH_3)_3$–C_6H_2	3,4,5–$(OCH_3)_3$–C_6H_2	45 ± 0.080	47 ± 0.106
5e	4–Cl–C_6H_4	4–Cl–C_6H_4	40 ± 0.091	29 ± 0.198
5f	4–$(CH_3)_2$N-C_6H_4	$(CH_3)_2$N-C_6H_4	32 ± 0.073	38 ± 0.156
5g	4–CH_3–C_6H_4	4–CH_3–C_6H_4	52 ± 0.077	54 ± 0.131
Ascorbic acid	---	---	34 ± 0.083	32 ± 0.115

SD = standard deviation (Average of three determination)

3.2b Antimicrobial Activity. The synthesized compounds were evaluated for in vitro antimicrobial activity against *Bacillus mycoides* (MTCC 645), *Staphylococcus aureus* (MTCC 96), (gram-positive bacteria), *Escheria coli* (MTCC 724), *Klebsiella pneumonia* (MTCC 3384), (gram-negative bacteria) and three fungi *Aspergillus flavus* (MTCC 873), *Aspergillus niger* (MTCC 281) and *Trichoderma viridae* (MTCC 167) by disc diffusion [28] and microdilution method [29].

Table 2. Antimicrobial activity of 1,3,4-oxadiazole tethered pyrazolyl-isoxazoles **4(a-g)** and **5(a-g)**

Com-pounds	Antibacterial activitya											
	Gram positive						Gram negative					
	B. mycoides			S. aureus			E. coli			K. pneumonia		
	25 µg/ml ± SD	50 µg/ml ± SD	100 µg/ml ± SD	25 µg/ml ± SD	50 µg/ml ± SD	100 µg/ml ± SD	25 µg/ml ± SD	50 µg/ml ± SD	100 µg/ml ± SD	25 µg/ml ± SD	50 µg/ml ± SD	100 µg/ml ± SD
4a	8 ± 0.10	12 ± 0.12	20 ± 0.20	8 ± 0.12	14 ± 0.20	24 ± 0.22	10 ± 0.20	20 ± 0.15	22 ± 0.14	8 ± 0.22	14 ± 0.20	22 ± 0.12
4b	9 ± 0.12	13 ± 0.18	20 ± 0.06	8 ± 0.20	16 ± 0.12	22 ± 0.12	6 ± 0.23	20 ± 0.20	24 ± 0.20	6 ± 0.14	18 ± 0.10	25 ± 0.14
4c	7 ± 0.16	14 ± 0.16	22 ± 0.12	4 ± 0.11	12 ± 0.16	22 ± 0.l0	8 ± 0.15	22 ± 0.14	28 ± 0.22	6 ± 0.24	15 ± 0.22	23 ± 0.10
4d	6 ± 0.20	16 ± 0.10	26 ±0.18	6 ± 0.12	16 ± 0.18	25 ± 0.16	9 ± 0.20	21 ± 0.10	27 ± 0.10	10 ± 0.14	19 ± 0.18	24 ± 0.16
4e	4 ± 0.12	16 ± 0.08	22 ± 0.09	9 ± 0.12	13 ± 0.22	20 ± 0.26	10 ± 0.17	20 ± 0.22	26 ± 0.22	8 ± 0.10	18 ± 0.08	26 ± 0.16
4f	10 ± 0.10	18 ± 0.08	25 ± 0.11	7 ± 0.12	15 ± 0.08	23 ± 0.22	9 ± 0.20	23 ± 0.26	26 ± 0.14	9 ± 0.24	14 ± 0.16	22 ± 0.20
4g	7 ± 0.20	17 ± 0.14	20 ± 0.12	5 ± 0.04	12 ± 0.08	20 ± 0.14	8 ± 0.24	21 ± 0.14	27 ± 0.13	5 ± 0.16	16 ± 0.20	23 ± 0.16
5a	9 ± 0.18	14 ± 0.12	20 ± 0.14	6 ± 0.11	14 ± 0.06	24 ± 0.20	9 ± 0.10	22 ± 0.16	28 ± 0.10	9 ± 0.10	18 ± 0.20	24 ± 0.14
5b	8 ± 0.22	18 ± 0.22	22 ± 0.22	6 ± 0.22	14 ± 0.15	23 ± 0.14	6 ± 0.15	21 ± 0.22	27 ± 0.15	8 ± 0.10	18 ± 0.12	25 ± 0.10
5c	8 ± 0.14	17 ± 0.24	22 ± 0.18	8 ± 0.22	16 ± 0.24	21 ± 0.08	6 ± 0.19	21 ± 0.10	22 ± 0.18	10 ± 0.08	14 ± 0.10	22 ± 0.12
5d	12 ± 0.09	23 ± 0.20	30 ± 0.20	10 ± 0.13	19 ± 0.09	29 ± 0.16	14 ± 0.24	25 ± 0.24	31 ± 0.20	14 ± 0.16	22 ± 0.09	30 ± 0.10
5e	6 ± 0.12	16 ± 0.16	22 ± 0.20	8 ± 0.12	15 ± 0.08	23 ± 0.22	11 ± 0.21	20 ± 0.12	28 ± 0.24	10 ± 0.16	20 ± 0.10	24 ± 0.10
5f	10 ± 0.15	20 ± 0.12	29 ± 0.23	10 ± 0.18	17 ± 0.10	27 ± 0.04	11 ± 0.30	24 ± 0.24	29 ± 0.21	12 ± 0.12	20 ± 0.10	26 ± 0.21
5g	8 ± 0.12	18 ± 0.20	25 ± 0.30	6 ± 0.21	14 ± 0.08	22 ± 0.10	6 ± 0.24	20 ± 0.12	24 ± 0.10	10 ± 0.12	16 ± 0.18	22 ± 0.16
Tetra-cycline	10 ± 0.20	22 ± 0.22	30 ± 0.18	9 ± 0.20	18 ± 0.10	29 ± 0.14	12 ± 0.24	22 ± 0.08	28 ± 0.18	12 ± 0.12	20 ± 0.16	28 ± 0.10

a Zone of inhibition (diameter in mm) (Mean six replicate ± standard deviation).

The antibiotic *Tetracycline* and *Nystatin* were used as positive reference. The smallest amount of synthesized compounds or standard antibiotic was required to inhibit the visible growth of a test microorganism (MIC) and the lowest concentration of an antibiotic required to kill a particular bacterium/fungi (MBC/MFC) analysis were determined and the results are summarized in **Table 2–4** (See supporting information).

The results revealed that, compounds **5d**, **5e**, **5f**, **4d**, **4e** and **4f** exhibited propitious antimicrobial activity and the activity was in the order **5d**>**5f**>**4d**>**4f**. Among the newly synthesized compounds, the compound **5d** emerged as a promising broad spectrum anti-bacterial agent this may be due to the presence of three $-OCH_3$ group on benzene. While the gram negative strains were inhibited by the compounds **5e** and **4e** which contain –Cl group at para position of benzene ring. The compound **5g** containing $-CH_3$ group were moderately active against bacterial strains but it possess good antifungal activity. The compounds **5(a–g)** were most active against microbes compare to hydrated open forms of 1,3,4-oxadiazoles **4(a–g)** which are active against DPPH radical.

Table 3. Inhibitory zone (diameter) mm of synthesized compounds against tested fungal strains

Compounds	Antifungal activity								
	A. flavus			*A. niger*			*T. viridae*		
	25 µg/ml ± SD	50 µg/ml ± SD	100 µg/ml ± SD	25 µg/ml ± SD	50 µg/ml ± SD	100 µg/ml ± SD	25 µg/ml ± SD	50 µg/ml ± SD	100 µg/ml ± SD
4a	9 ± 0.12	13 ± 0.20	15 ± 0.08	10 ± 0.12	13 ± 0.18	20 ± 0.16	10 ± 0.12	15 ± 0.12	20 ± 0.18
4b	12 ± 0.09	15 ± 0.10	18 ± 0.10	13 ± 0.12	15 ± 0.16	20 ± 0.24	9 ± 0.16	15 ± 0.18	18 ± 0.20
4c	12 ± 0.16	15 ± 0.20	19 ± 0.12	10 ± 0.14	12 ± 0.12	16 ± 0.10	8 ± 0.22	13 ± 0.16	17 ± 0.10
4d	9 ± 0.16	12 ± 0.16	18 ± 0.14	13 ± 0.12	15 ± 0.10	22 ± 0.08	10 ± 0.16	14 ± 0.20	17 ± 0.16
4e	8 ± 0.10	11 ± 0.16	16 ± 0.12	12 ± 0.10	16 ± 0.12	20 ± 0.12	11 ± 0.16	14 ± 0.10	16 ± 0.20
4f	12 ± 0.12	15 ± 0.24	19 ± 0.22	9 ± 0.16	13 ± 0.14	18 ± 0.22	11 ± 0.22	15 ± 0.16	18 ± 0.14
4g	9 ± 0.20	14 ± 0.16	18 ± 0.12	12 ± 0.14	15 ± 0.12	21 ± 0.18	9 ± 0.14	13 ± 0.22	17 ± 0.14
5a	11 ± 0.15	16 ± 0.13	19 ± 0.22	11 ± 0.12	16 ± 0.22	21 ± 0.22	9 ± 0.10	14 ± 0.20	19 ± 0.10
5b	8 ± 0.14	11 ± 0.12	15 ± 0.16	12 ± 0.16	16 ± 0.16	20 ± 0.26	7 ± 0.16	12 ± 0.18	16 ± 0.10
5c	10 ± 0.22	15 ± 0.16	19 ± 0.20	13 ± 0.14	17 ± 0.19	22 ± 0.14	8 ± 0.22	12 ± 0.10	17 ± 0.22
5d	12 ± 0.10	11 ± 0.06	17 ± 0.10	10 ± 0.14	16 ± 0.10	22 ± 0.22	10 ± 0.10	15 ± 0.06	19 ± 0.21
5e	15 ± 0.14	18 ± 0.12	22 ± 0.16	14 ± 0.10	18 ± 0.20	24 ± 0.22	14 ± 0.10	18 ± 0.16	20 ± 0.18
5f	8 ± 0.10	12 ± 0.18	16 ± 0.12	12 ± 0.16	15 ± 0.10	20 ± 0.12	10 ± 0.16	15 ± 0.06	18 ± 0.12
5g	14 ± 0.10	16 ± 0.18	20 ± 0.20	16 ± 0.10	20 ± 0.18	24 ± 0.13	12 ± 0.14	16 ± 0.14	22 ± 0.16
Nystatin	15 ± 0.20	16 ± 0.10	20 ± 0.14	15 ± 0.09	18 ± 0.14	25 ± 0.16	12 ± 0.14	18 ± 0.20	22 ± 0.16

[a] Zone of inhibition (Mean six replicate ± standard deviation).

Table 4. The minimal inhibitory concentration (MIC), minimal bactericidal concentration (MBC) and minimal fungicidal concentration (MFC) in µg/mL of synthesized compounds against tested strains

Compounds	Antibacterial activity								Antifungal activity					
	Gram positive				Gram negative									
	B. mycoides		*S. aureus*		*E. coli*		*K. pneumonia*		*A. flavus*		*A. niger*		*T. viridae*	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MFC	MIC	MFC	MIC	MFC
4a	35	205	40	195	35	195	30	200	20	260	25	245	25	250
4b	30	200	25	190	25	175	25	200	25	255	20	240	25	250
4c	30	210	25	195	30	180	40	200	35	260	30	260	30	260
4d	25	175	20	180	30	180	25	185	30	245	30	260	30	240
4e	40	185	40	180	40	200	30	190	20	245	25	255	30	255
4f	40	190	30	200	35	220	25	195	30	285	35	260	35	265
4g	35	185	30	205	30	215	35	200	30	280	30	245	30	260
5a	25	200	35	210	35	195	40	185	25	270	25	265	25	245
5b	35	215	25	190	45	200	30	175	20	265	20	270	25	250
5c	25	205	30	185	40	185	25	170	20	260	35	280	20	255
5d	15	150	20	160	15	150	15	155	25	245	30	245	25	240
5e	35	180	30	180	30	185	30	175	15	200	15	205	15	200
5f	20	160	20	165	20	155	15	160	15	200	20	195	20	205
5g	35	200	35	200	30	185	25	180	25	255	25	260	30	240
Tetracycline	10	125	10	130	12	130	8	125	-	-	-	-	-	-
Nystatin	-	-	-	-	-	-	-	-	10	160	15	150	15	175

[a] (Mean six replicate ± standard deviation).

3.2c Antidiabetic Activity. The newly synthesized compounds **4(a–g)** and **5(a–g)** were also screened *in vitro* for their antidiabetic activity by measuring the α-amylase and α-glucosidase inhibitory potential. α-amylase and α-glucosidase are crucial enzymes for hydrolysis of carbohydrates into simpler monosaccharides that are

absorbed in the small intestine. The inhibition of these enzymes slow down the process of absorption of glucose decomposed from starch by these enzymes there by play an important role in controlling the diabetes [30-32]. Therefore, efficient inhibitors of α-amylase and α-glucosidase have long been sought. The IC_{50} values of tested compounds **4(a–g)** and **5(a–g)** on α-amylase and α-glucosidase are showed in **Table 5**.

From the **table 5** it is concluded that, the hydrated open forms of 1,3,4-oxadiazoles **4(a–g)** showed better activity when compare to the 1,3,4-oxadiazoles **5(a–g)**. Among the synthesized compounds, compound **4d** which contains $-OCH_3$ group on the phenyl ring of both the pyrazole as well as isoxazole moieties emerged as a potent inhibitor of both the enzymes. Of the newly prepared 1,3,4-oxadiazoles **5(a–g)**, the compound **5f** showed excellent inhibitory potential. The compounds **4b**, **4f**, **5d** and 5f showed good to potent antidiabetic activity. While the remaining compounds possesses moderate activity. This result was also supported by the molecular docking studies as discussed below. For simplicity, we report the docking poses for the most active compounds **4b**, **4d**, **4f** and **5f** only (Figure. 2).

Table 5. Antidiabetic Activity[a] of synthesized compounds **5(a-g)** and **4(a-g).**

Product	IC_{50} values of α-amylase inhibition activity	IC_{50} values of α-glucosidase inhibition activity
4a	65 µg/ml	100 µg/ml
4b	20 µg/ml	40 µg/ml
4c	65 µg/ml	110 µg/ml
4d	10 µg/ml	25 µg/ml
4e	80 µg/ml	125 µg/ml
4f	25 µg/ml	50 µg/ml
4g	55 µg/ml	80 µg/ml
5a	50 µg/ml	75 µg/ml
5b	40 µg/ml	65 µg/ml
5c	60 µg/ml	95 µg/ml
5d	40 µg/ml	60 µg/ml
5e	60 µg/ml	105 µg/ml
5f	35 µg/ml	60 µg/ml
5g	60 µg/ml	90 µg/ml
Acarbose (+ ve control)	15 µg/ml	15 µg/ml

[a] Each value represents a mean of three replicates

Docking of the drug molecule with receptor is a rational strategy helps to expedite the drug designing process. In order to gain more insight into the interaction between these new series of compounds **4(a–g)** and **5(a–g)** with α-amylase and α-glucosidase, molecular docking studies were performed. As in vitro study of compounds **4(a–g)** and **5(a–g)** showed high inhibition activity against α-amylase when compared to α-glucosidase, α-amylase was selected for molecular docking study.

The molecular docking was performed and analyzed using AutoDock 4.2. A Lamarkian genetic algorithm method implemented in the program suite was employed to identify appropriate binding modes and conformation of the ligand molecules. Gasteiger charges were added and the rotatable bonds were set by the AutoDock tools and all torsions were allowed to rotate. Polar hydrogen atoms were added and Kollaman charges were assigned to the protein using AutoDock tools (ADT). All the compounds **4(a–g)** and **5(a–g)** were found to have minimum binding energy ranging from -8.69 to -10.83 kJ/mol with α-amylase (PDB Code: 1PPI). Among the molecules tested for docking study, 3-(3,4,5-trimethoxy-phenyl)-5-methyl-isoxazole-4-carboxylic acid-*N*-[3-(3,4,5-trimethoxy-phenyl)-5-methyl-1-phenyl-1*H*-pyrazole-4-carbonyl]-hydrazide **4d** showed minimum binding energy of -10.83 kJ/mol with ligand efficiency of -0.23. Most of the residues that are in close proximity to the inhibitor are hydrophobic in nature. The ligand molecules, **4b**, **4d**, **4f** and **5f** revealed binding energy of -10.14, -10.83, -10.06 and -10.04 kJ/mol, with ligand efficiency of -0.25, -0.23, -0.24 and -0.24, respectively. These molecules were completely wrapped by active site amino acid residues at the active site pocket region as shown in Fig. 2. Similarly, molecules **4d**, **4f** and **5f** were found to show hydrogen bond interaction with active site amino acid residues Gly 306, His 201 and His 305 at a distance of (1.792), (1.974 and 2.117) and (2.199) Å, respectively as shown in Fig. 2.

Table 6. The dock score results of synthesized compounds **5(a-g)** and **4(a-g)** with α-amylase (PDB Code: 1PPI)

Compounds	Binding Energy (kJ mol^{-1})	Ligand Efficiency	Inhibition Constant	vdW+H-bond+desolv energy	No. of H- bonds	Bonding residues	Bond Length (Å)
4a	-9.4	-0.26	128.08	-10.02	1	1PPI:A: HIS305:HD1	2.146
4b	-10.14	-0.25	37.2	-10.81	-	-	-
4c	-9.15	-0.22	197.75	-13.35	1	1PPI:A:GLY306:HN	1.972
4d	-10.83	-0.23	11.54	-13.68	1	1PPI:A:GLY306:HN	1.792
4e	-8.69	-0.23	428.9	-9.67	-	-	-
4f	-10.06	-0.24	42.16	-11.04	2	1PPI:A: HIS305:HD1 1PPI:A:HIS201:HE2	2.117 1.974
4g	-9.87	-0.31	58.35	-10.21	2	1PPI:A: HIS305:HD1 1PPI:A:ASP300:OD2	1.801 2.093
5a	-9.91	-0.28	54.7	-10.76	2	1PPI:A: GLY306:HN 1PPI:A:HIS201:HE2	2.021 2.215
5b	-9.97	-0.26	48.92	-11.76	2	1PPI:A: HIS305:HD1 1PPI:A:GLY306:HN	2.083 2.12
5c	-9.61	-0.22	90.6	-11.83	1	1PPI:A:GLY306:HN	2.151
5d	-9.99	-0.21	47.19	-12.7	2	1PPI:A: HIS299:HE2 1PPI:A:GLY306:HN	2.151 2.011
5e	-9.33	-0.25	144.27	-10.5	1	1PPI:A:HIS201:HE2	2.016
5f	-10.04	-0.24	43.71	-11.94	1	1PPI:A:GLY306:HN	2.199
5g	-9.66	-.0.26	83.67	-11.09	2	1PPI:A: HIS305:HD1 1PPI:A:GLY306:HN	2.165 1.851

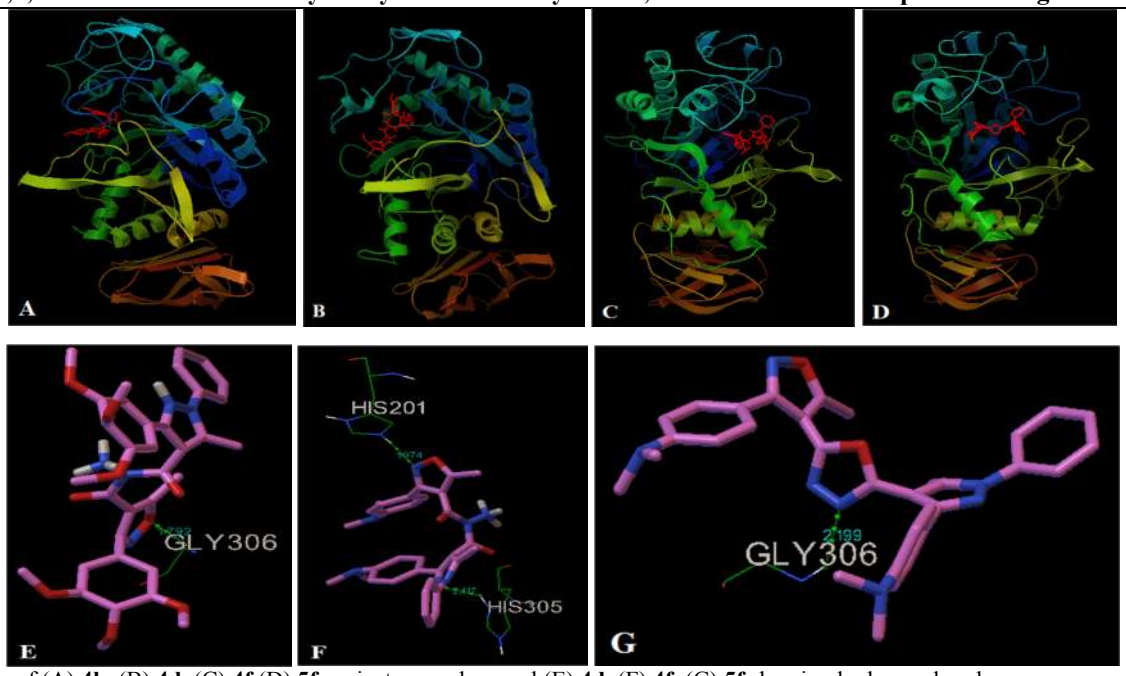


Figure 2. Docking of (A) **4b**, (B) **4d**, (C) **4f** (D) **5f** against α-amylase and (E) **4d**, (F) **4f**, (G) **5f** showing hydrogen bond.

The docking study results showed that the molecules **4(a–g)** and **5(a–g)** have good inhibition constant, vdW + Hbond + desolv energy with best RMSD value. The details of docked score results of the molecules with *α*-amylase (PDB Code: 1PPI) are given in the **Table 6**. Since the newly synthesized tri-heterocyclic compounds were better encased in the active site of the enzymes due to their conformation and hydrogen bonding ability, the augmented activity was observed compared to biheterocycles reported by our team.

4. CONCLUSIONS

In conclusion, we have synthesized a novel series of 1,3,4-oxadiazole tethered pyrazolyl-isoxazole **5(a–g)** and these compounds have been investigated for their in vitro antioxidant, antimicrobial and antidiabetic activity. Subsequently, these novel classes of compounds presented in our laboratory have emerged as potent pharmacological agents. Among the synthesized compounds, compound **4f**, **5d** and **4d** showed excellent antioxidant, antimicrobial and antidiabetic activity respectively in comparison with standard drugs. In vivo and cytotoxicity investigations of the active compounds are necessary to fully assess the efficacy of these compounds.

5. REFERENCES

[1] Sorg O. C. R., Oxidative stress: a theoretical model or a biological reality?, *Biology.* 327**,** 649, **2004.**

[2] Halliwell B., Gutteridge J. M. C., Oxidative stress, in *Free Radicals in Biology and Medicine* (3rd ed.) (Oxford University Press, New York) p. 246, **1999**

[3] Valko M., Rhodes C. J., Monocol J., Izakovic M and Mazur M., Free radicals, metals and antioxidants in oxidative stress-induced cancer, *Chemico-Biological Interactions*, 1, 160, **2006.**

[4] Bandgar B. P., Adsul L. K., Chavan H. V., Jalde S. S., Shringare S. N., Shaikh R., Meshram R. J., Gacche R. N., Masand V., Synthesis, biological evaluation, and docking studies of 3-(substituted)-aryl-5-(9-methyl-3-carbazole)-1*H*-2-pyrazolines as potent anti-inflammatory and antioxidant agents, *Bioorganic and Medicinal Chemistry Letters*, 22, 5839, **2012**.

[5] Perea S., Patterson T. F., Antifungal resistance in pathogenic fungi, *Clinical Infectious Diseases,* 35, 1073, **2002**.

[6] Giulia M., Luisa M., Paola F., Silvia S., Angelo R., Luisa M., Francesco B., Roberta L., Chiara M., Valeria M., Paolo L. C., Elena T., Synthesis, antimicrobial activity and molecular modeling studies of halogenated 4-[1*H*-imidazol-1-yl(phenyl)methyl]-1,5-diphenyl-1*H*-pyrazoles, *Bioorganic and Medicinal Chemistry*, 12, 5465, **2004**.

[7] Vincent T. A., Current and future antifungal therapy: new targets for antifungal agents, *Journal of Antimicrobial Chemotherapy,* 44, 151, **1999**.

[8] World Health Organization Consultation: Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus, *Report of a WHO Consultation Geneva*, **1999**.

[9] Mc Cune L. M., Johns T., Antioxidant activity in medicinal plants associated with the symptoms of diabetes mellitus used by the indigenous peoples of the North American boreal forest, *Journal of Ethnopharmacology,* 82, 197, **2002**.

[10] Rhabasa L. R., Chiasson J. L., in *International textbook of diabetes mellitus*. (Vol 1, 3rd ed.) (UK: John Wiley and Sons Ltd) p 901, **2004**.

[11] Venkat Ragavan R., Vijayakumar V., Suchetha Kumari N., Synthesis and antimicrobial activities of novel 1,5-diaryl pyrazoles, *European Journal of medicinal Chemistry,* 45, 1173, **2010**.

[12] Bekhit A. A., Ashour H. M. A., Ghany Y. S. A., Bekhit A. E. D. A., Baraka A., Synthesis and biological evaluation of some thiazolyl and thiadiazolyl derivatives of 1*H*-pyrazole as anti-inflammatory antimicrobial agents, *European Journal of medicinal Chemistry*, 43, 456, **2008**.

[13] Ningaiah S., Bhadraiah U. K., Keshavamurthy S., Javarasetty C., Novel pyrazoline amidoxime and their 1,2,4-oxadiazole analogues: synthesis and pharmacological screening., *Bioorganic and Medicinal Chemistry Letters*, 23, 4532, **2013**.

[14] Vijesh A. M., Isloor A. M., Shetty P., Sundershan S., Fun H. K., New pyrazole derivatives containing 1,2,4-triazoles and benzoxazoles as

potent antimicrobial and analgesic agents, *European Journal of medicinal Chemistry*, 62, 410, **2013.**

[15] Prakash O., Kumar M., Kumar R., Sharma C., Aneja K. R., Hypervalent iodine(III) mediated synthesis of novel unsymmetrical 2,5-disubstituted 1,3,4-oxadiazoles as antibacterial and antifungal agents, *European Journal of medicinal Chemistry,* 45, 4252, **2010**.

[16] Hong-Shui L., Yong-Sheng X., Wei-Yong L., Zhong-Liang G., Bao-Xiang Z., Hong-Shui L., Yong-Sheng X., Wei-Yong L., Zhong-Liang G., Bao-Xiang Z., Synthesis, X-Ray Crystal Structures and Optical Properties of Novel Substituted Pyrazoly 1,3,4-Oxadiazole Derivatives, *Journal of Fluorescence.* 21, 1797, **2011**.

[17] Puthiyapurayil P., Poojary B., Chikkanna C., Buridipad S. K., Design, synthesis and biological evaluation of a novel series of 1,3,4-oxadiazole bearing N-methyl-4-(trifluoromethyl)phenyl pyrazole moiety as cytotoxic agents, *European Journal of medicinal Chemistry,* 53**,** 203, **2012**.

[18] Horrocks P., Pickard M. R., Parekh H., Patel S. P., Ravindra B. P., Synthesis and biological evaluation of 3-(4-chlorophenyl)-4-substituted pyrazole derivatives, *Organic and Biomolecular Chemistry,* 11, 4891, **2013**.

[19] Gaonkar S. L., Rai K. M. L., Prabhuswamy B., Synthesis and antimicrobial studies of a new series of 2-{4-[2-(5-ethylpyridin-2-yl)ethoxy]phenyl}-5-substituted-1,3,4-oxadiazoles, *European Journal of medicinal Chemistry,* 41, 841, **2006**.

[20] Pimenova E. V., Voronina E. V., Antimicrobial activity of pyrazoles and pyridazines obtained by interaction of 4-aryl-3-aryl-hydrazono-2,4-dioxobutanoic acids and their esters with hydrazines, *Journal of Pharmaceutical Chem*istry, 35, 18, **2001**.

[21] Bondock S., Fadaly W., Metwally M. A., Enaminonitrile in heterocyclic synthesis: synthesis and antimicrobial evaluation of some new pyrazole, isoxazole and pyrimidine derivatives incorporating a benzothiazole moiety, *European Journal of medicinal Chemistry,* 44, 4813, **2009**.

[22] Ningaiah S., Bhadraiah U. K., Shridevi D. D., Keshavamurthy S., Javarasetty C., Novel pyrazole integrated 1,3,4-oxadiazoles: Synthesis, characterization and antimicrobial evaluation, *Bioorganic and Medicinal Chemistry Letters*, 24, 245, **2014**.

[23] Shridevi D. D., Rai K. M. L., Srikantamurthy N., Chandra, Chethan J., Novel 5-functionalized-pyrazoles: Synthesis, characterization and pharmacological screening, *Bioorganic and Medicinal Chemistry Letters*, 25, 3671, **2015**.

[24] A. I. Vogel's, *Text Book of Practical Organic Chemistry*. (5th ed.) (Longman ELBS Publications UK) a) p. 1258 b) p. 1334, **1989**.

[25] Srikantamurthy N., Shridevi D. D., Chandra, Mahendra M., Shubakara K., Umesha K. B., One-Pot Tandem Synthesis of Tetrasubstituted Pyrazoles via 1,3-Dipolar Cycloaddition Between Aryl Hydrazones and Ethyl But-2-ynoate, *Synthetic Communications,* 44, 2222, **2014**.

[26] Blois M. S., Antioxidant Determinations by the Use of a Stable Free Radical, *Nature*. 181, 1199, **1958**.

[27] Oyaizu M., Studies on products of browning reaction--antioxidative activities of products of browning reaction prepared from glucosamine, *The Japanese Journal of Nutrition and Dietetics,* 44, 307, **1986**.

[28] O'Donnell M. J., in: I. Ojima (Ed.), *Catalytic Asymmetric Synthesis*, (VCH Publishers, New York, Chapter 8) p. 389, **1993**.

[29] Shioiri T., in: Sasson Y., Neumann R., (Eds.), *Handbook of Phase-Transfer Catalysis*, (Blackie Academic & Professional, London, Chapter 14) p 462, **1997**.

[30] Andrews J. M., BSAC standardized disc susceptibility testing method (version 7), *Journal of Antimicrobial Chemotherapy,* 62, 256, **2008**.

[31] Zgoda J. R., Porter J. R., A Convenient Microdilution Method for Screening Natural Products Against Bacteria and Fungi, *Pharmaceutical Biology*. 39, 221, **2001**.

[32] Hara Y and Honda M., The Inhibition of α-Amylase by Tea Polyphenols, *Agricultural and Biological Chemistry*. 54, 1939, **1990**.

[33] Schuttelkopf A. W., van Aalten D. M. F., PRODRG: a tool for high-throughput crystallography of protein-ligand complexes. *Acta Crystallographica section D Biological Crystallography*. 60, 1355, **2004**.

[34] RCSB Protein Data Bank (http://www.rcsb.org/pdb/home/home.do).

6. ACKNOWLEDGEMENTS

One of the authors (K.B.U) is grateful to minor research project (128/MRP/UGC-SWRO/2009 Dated: 10-04-09) UGC, New Delhi, for providing the necessary fund to carry out the research at University of Mysore.