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Novel pyrazolyl-thiazoles: synthesis, characterization and study of their antidiabetic properties

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ABSTRACT

A novel series of 1,3-diphenyl-pyrazole-4-carboxylic acid and its thiazole derivatives were conveniently synthesized followed by coupling reaction to investigate their efficiency as α-amylase and α-glucosidase inhibiting agents. Initially, pyrazole esters were obtained by using various benzaldehydes as precursor and subsequently converted to respective acids in tetrahydrofuran solvent medium. All the acid key intermediates obtained were coupled with previously synthesized thiazole amines to yield title compound with good yield. The current attempt cultivated many advantages such as good yield, time quenching and easy work up. Structures of newly obtained compounds were characterized by spectral studies such as NMR, Mass, IR and elemental analytical techniques. Finally, all the prepared compounds were tested for their antidiabetic activity. Among the synthesized compounds in comparison with standard drug, compound 4c, 4e and 4j showed promising in vitro antidiabetic activity. Bioassay result suggest that the compound 4e may emerge as a potent antidiabetic agent in future drug design.

Keywords: *pyrazole-4-carboxylic acid; thiazole; in vitro antidiabetic; α-amylase and α-glucosidase.*

1. INTRODUCTION

DM (Diabetes mellitus) is a metabolic disorder symbolized by chronic hyperglycemia or intensified blood glucose levels with disturbances in carbohydrate, fat and protein metabolism resulting from absolute or relative lack of insulin secretion [1]. Diabetes, as one of the most common global diseases, affects about 200 million individuals worldwide and about 300 million people worldwide are at risk of diabetes [2]. The management of the blood glucose level is a censorious strategy in the restriction of diabetes complications. It is extensively accepted that the most important objective in the management of patients with diabetes mellitus is to achieve blood glucose levels as close to normal as possible.

First, the dietary carbohydrates should be broken down to monosaccharides by some specific gastrointestinal enzymes since the only monosaccharide can be absorbed from intestinal lumen. The key exzyme involved in the digestion of carbohydrates are α-Glucosidase and α-amylase. α- Amylase deteriorates complex dietary carbohydrates to oligosaccharides and disaccharides that are later converted into monosaccharide by α-glucosidase. Ultimately, liberated glucose is absorbed by the gut and later results in postprandial hyperglycemia (PPHG).

The inhibition of enzymes involved in the metabolism of carbohydrates can undoubtedly decrease the postprandial raise of blood glucose after a mixed carbohydrate diet by procrastinating the process of carbohydrate hydrolysis and absorption. The control of postprandial hyperglycemia is asignificant strategy in the management of diabetes mellitus, exclusively type II diabetes, and lessening chronic complications associated with the disease. Therefore, such enzyme inhibitors can be useful in the medication of type II diabetes [3].

Pyrazole is a five membered ring system with two nitrogen atom represents an important class of compounds not only for their theoretical interest but also for anti-inflammatory, analgesic, anti hypertensive, antipyretic, antibacterial and sedatives [4-6]. In fact, Celecoxib, a pyrazole derivative is now widely used in the market as antiinflammatory drug [7].

Thiazole is a 5-membered ring, in which two of the vertices of the ring are nitrogen and sulfur, and the other three are carbons. Hantzsch, who prepared a number of simple compounds containing thiazole rings, did the pioneering work in the field of thiazoles. Soon many other researchers picked up the lead resulting in a series of thiazole and related compounds. The thiazole moiety is a crucial part of various biologically active molecules [8-9] including Vitamin B1 (thiamine), Penicillin [10], Actithiazic acid [11] and epothilone [12]. In addition to these, various pyrazole and thiazole derivatives were synthesized and studied their hypoglycemic activity [13-20].

Although, the current antidiabetic drugs used in the clinical arena possess good therapeutic effects, but exist security problems while being administered in long-term, e.g. liver toxicity and weight increase and the like [21-22].

In the light of these facts and in continuation of our interest in the synthesis of heterocycles containing a multi-structure for biological activity [23], we thought of synthesizing a new class of heterocycles, wherein potent thiazole heterocycle is linked to pyrazole (via an amide bond) to see the additive effect of these rings towards the α-amylase and α-glucosidase inhibitory activity, which is the current passion being accomplished in most of the drug discoveries.

2. MATERIALS AND METHODS

2.1. General remarks.

Melting points were determined in open capillaries on a Buchi oil melting point apparatus and are uncorrected. Elemental analysis was carried out on an Elementor vairo-EL instrument. IR spectra were recorded with an IR spectrophotometer Avtar 370 FT-IR (Thermo Nicolet). ^{1}H NMR and ^{13}C NMR spectra were acquired on a Bruker Avance (400 MHz for proton and 100 MHz for carbon) instrument in DMSO-d6 or $CDCl_3$ and TMS was used as an internal reference. Mass spectrometry was performed with a Bruker-Franzen Esquire LC mass spectrometer unless otherwise stated. Flash column chromatography was carried out with Merck silica gel 60 (15–40 mm). Thin-layer chromatography (TLC) was carried out with aluminum sheets precoated with silica gel 60 F254 (0.2 mm, Merck). Chromatographic spots were visualized by UV light and/or with iodine. All commercial chemicals were used without further purification.

2.2. General procedure for the synthesis of 5-methyl-1,3-diphenyl-1*H*-pyrazole-4-carboxylic acid 2a:

In a two neck round bottomed flask ethyl 5-methyl-1,3-diphenyl-1*H*-pyrazole-4-carboxylate (1a, 0.306g, 1.00mmol) and 10% NaOH (10mL) in MeOH (10mL) was taken and refluxed for 4-6h. After the completion of the reaction (monitored by TLC), the reaction mass was evaporated to half of its volume and then acidified with dil. HCl with stirring to obtain 5-methyl-1,3-diphenyl-1*H*-pyrazole-4-carboxylic acid 2a as white solid in 95% yield (0.264g). The white solid thus separated was filtered, dried and it was soluble in Na_2CO_3. The small portion of the sample was taken and purified (recrystallized in hot EtOH) for analytical measurements and the remaining was taken into the next step without purification.

2.3. General procedure for the synthesis of 5-phenylthiazol-2-amine (3a):

The mixture of 2-bromo-1-phenylethanone (2.00g, 10.00mmol) and thiourea (11.00mmol) in anhydrous ethyl alcohol (20mL) was refluxed for 1h. After that, the solvent was removed and saturated aqueous $NaHCO_3$ was added to make the mixture basic (pH = 8-9). Then, the mixture was extracted with DCM (2x25mL) and the combined organic layers were collected and washed with brine and dried with $MgSO_4$. Later the solvent was removed and the resultant residue was stirred for 20 min with petroleum ether and filtered to afford 5a as buff colored solid in 97% yield (1.71g).

4-(4-chlorophenyl)thiazol-2-amine (3b):

Obtained from the mixture of 2-bromo-1-(4-chlorophenyl)ethanone (2.31g, 10.00mmol) and thiourea (11.00mmol) in anhydrous ethyl alcohol (20mL) as amorphous solid in 72% (1.50g) yield.

4-(4-methoxyphenyl)thiazol-2-amine (3c):

Obtained from the mixture of 2-bromo-1-(4-methoxyphenyl)ethanone (2.29g, 10.00mmol) and thiourea (11.00mmol) in anhydrous ethyl alcohol (20mL) as white solid in 77% (1.58g) yield.

4-(4-nitrophenyl)thiazol-2-amine (3d):

Obtained from the mixture of 2-bromo-1-(4-nitrophenyl)ethanone (2.44g, 10.00mmol) and thiourea (11.00mmol) in anhydrous ethyl alcohol (20mL) as brown solid in 67% (1.48g) yield.

4-(2-aminothiazol-4-yl)phenol (3e):

Obtained from the mixture of 2-bromo-1-(4-hydroxyphenyl)ethanone (2.15g, 10.00mmol) and thiourea (11.00mmol) in anhydrous ethyl alcohol (20mL) as white solid in 70% (1.34g) yield.

2.4. General procedure for the synthesis of 5-methyl-1,3-diphenyl-N-(4-phenylthiazol-2-yl)-1H-pyrazole-4-carboxamide (4a):

A solution of 5-methyl-1,3-diphenyl-1*H*-pyrazole-4-carboxylic acid (**2a**, 0.28g, 1.00mmol) in dry DCM (5mL) was cooled to 0°C and added EDC.HCl (1.20mmol) and HOBt (1.20mmol) under nitrogen atmosphere and stirred the reaction mixture at the same temperature for 0.5h. To this reaction mixture 5-phenylthiazol-2-amine (3a, 1.00mmol) was added and stirred at 0°C for 0.5h. The reaction mixture was slowly brought to room temperature and stirring continued for 6-8h. After completion of the reaction monitored by TLC, the reaction mixture was poured to ice cold water, extracted with ethyl acetate (2x25mL) and the combined organic phase was washed with brine, dried over anhydrous sodium sulfate. Ethyl acetate was removed under vacuum and the residue thus obtained was purified by recrystallization in ethyl alcohol afforded the corresponding compound (**4a**) as white amorphous solid with 88% yield (0.38g); IR (KBr): 3315 cm^{-1} (NH), 1665 cm^{-1} (amide C═O), 1620 cm^{-1} (C═N); ^{1}H NMR (400 MHz, DMSO-d6): δ 2.46 (s, 3H), 6.96 (s, 1H), 7.28-7.40 (m, 3H), 7.48-7.57 (m, 3H), 7.61-7.67 (m, 5H), 7.74-7.76 (d, 2H, J = 8.0 Hz), 7.82-7.84 (d, 2H, J = 8.4 Hz), 8.82 (s, 1H) (Figure 1); ^{13}C NMR (100 MHz, $CDCl_3$): δ 13.3, 108.3, 120.4, 125.5, 126.2, 127.4, 128.2, 129.6, 129.7, 129.8, 131.0, 131.9, 137.2, 143.2, 147.6, 155.0, 164.8, 166.0 (Figure 2); MS: m/z = 437.1 $[M^+H]^+$ (100%), 261.0, 234.9, 221.1 (Figure 3); Anal. % Calculated for $C_{26}H_{20}N_4OS$: C 71.54, H 4.62, N 12.83; Found: C 71.58, H 4.65, N 12.80.

3-(4-chlorophenyl)-N-(4-(4-chlorophenyl)thiazol-2-yl)-5-methyl-1-phenyl-1*H*-pyrazole-4-carboxamide (4b):

Obtained from 3-(4-chlorophenyl)-5-methyl-1-phenyl-1*H*-pyrazole-4-carboxylic acid (**2b**, 0.31g, 1.00mmol), EDC.HCl (1.20mmol), HOBt (1.20mmol) and 4-(4-chlorophenyl)thiazol-2-amine (3b, 0.21g, 1.00mmol) as white solid in 82% yield (0.41g); IR (KBr): 3310 cm^{-1} (NH), 1666 cm^{-1} (amide C═O), 1635 cm^{-1} (C═N); ^{1}H NMR (400 MHz, DMSO-d6): δ 2.43 (s, 3H), 6.88 (s, 1H), 7.33-7.45 (m, 5H), 7.54 (dd, 4H), 7.57 (dd, 4H), 8.86 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 13.4, 108.4, 120.5, 125.5, 127.4, 127.5, 129.4, 129.6, 129.8, 130.1, 130.2, 136.2, 143.2, 144.2, 147.6, 149.1, 155.1, 164.7, 166.2; MS: m/z = 509.2 [M+5], 507.3 [M+3], 505.1 $[M^+H]^+$ (100%), 269.1, 255.2, 229.1; Anal. % Calculated for $C_{26}H_{18}Cl_2N_4OS$: C 61.79, H 3.59, N 11.09; Found: C 61.81, H 3.56, N 11.12.

3-(4-methoxyphenyl)-N-(4-(4-methoxyphenyl)thiazol-2-yl)-5-methyl-1-phenyl-1*H*-pyrazole-4-carboxamide (4c):

Obtained from 3-(4-methoxyphenyl)-5-methyl-1-phenyl-1*H*-pyrazole-4-carboxylic acid (**2c**, 0.31g, 1.00mmol), EDC.HCl (1.20mmol), HOBt (1.20mmol) and 4-(4-methoxyphenyl)thiazol-2-amine (3c, 0.206g, 1.00mmol) as thick paste in 76% yield (0.38g); IR (KBr): 3320 cm^{-1} (NH), 1662 cm^{-1} (amide C═O), 1625 cm^{-1}(C═N) ; ^{1}H NMR (400 MHz, DMSO-d6): δ 2.47 (s, 3H), 3.77 (s, 3H), 3.80 (s, 3H), 6.87(s, 1H), 7.30-7.44 (m, 5H), 7.47-7.55 (m, 8H), 8.91 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 13.0, 108.6,

115.5, 115.7, 120.3, 124.8, 125.5, 127.2, 127.4, 129.2, 129.6, 130.1, 143.3, 144.2, 147.6, 155.1, 159.8, 164.8, 166.0; MS: m/z = 497.6 $[M^+H]^+$ (100%), 265.1, 251.1, 225.3, 191.3; Anal. % Calculated for $C_{28}H_{24}N_4O_3S$: C 67.72, H 4.87, N 11.28; Found: C 67.75, H 4.85, N 11.33.

5-methyl-3-(4-nitrophenyl)-N-(4-(4-nitrophenyl)thiazol-2-yl)-1-phenyl-1H-pyrazole-4-carboxamide (4d):

Obtained from 5-methyl-3-(4-nitrophenyl)-1-phenyl-1*H*-pyrazole-4-carboxylic acid (**2d**, 0.32g, 1.00mmol), EDC.HCl (1.20mmol), HOBt (1.20mmol) and 4-(4-nitrophenyl)thiazol-2-amine (3d, 0.22g, 1.00mmol) as brown amorphous solid in 67% yield (0.35g); IR (KBr): 3292 cm^{-1} (NH), 1685 cm^{-1} (amide C═O), 1645 cm^{-1} (C═N); ^{1}H NMR (400 MHz, DMSO-d6): δ 2.44 (s, 3H), 6.85 (s, 1H), 7.38-7.46 (m, 5H), 7.49-7.51 (d, 4H, J = 8.2 Hz), 7.56-7.59 (d, 4H, J = 8.2 Hz), 8.64 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 13.0, 108.7, 120.6, 125.2, 125.3, 125.5, 127.4, 129.0, 129.1, 129.6, 137.8, 143.0, 143.2, 144.2, 147.6, 150.2, 155.0, 164.8, 166.0; MS: m/z = 527.3 $[M^+H]^+$ (100%), 280.2, 266.1, 240.3, 206.1; Anal. % Calculated for $C_{26}H_{18}N_6O_5S$: C 59.31, H 3.45, N 15.96; Found: C 59.28, H 3.50, N 15.99.

3-(4-hydroxyphenyl)-N-(4-(4-hydroxyphenyl)thiazol-2-yl)-5-methyl-1-phenyl-1*H*-pyrazole-4-carboxamide (4e):

Obtained from 3-(4-hydroxyphenyl)-5-methyl-1-phenyl-1*H*-pyrazole-4-carboxylic acid (**2f**, 0.29g, 1.00mmol), EDC.HCl (1.20mmol), HOBt (1.20mmol) and 4-(2-aminothiazol-4-yl)phenol (3e, 0.19g, 1.00mmol) as thick oil in 72% yield (0.33g); IR (KBr): 3205 cm^{-1} (OH-phenolic), 3290 cm^{-1} (NH), 1685 cm^{-1} (amide C═O), 1610 cm^{-1} (C═N); ^{1}H NMR (400 MHz, DMSO-d6): δ 2.43 (s, 3H), 6.90 (s, 1H), 7.25-7.50 (m, 5H), 7.85 (dd, 4H), 7.93 (dd, 4H), 8.64 (s, 1H), 9.68 (s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 13.1, 108.6, 115.9, 120.6, 125.2, 125.5, 127.4, 128.2, 129.0, 129.6, 137.6, 143.0, 144.0, 147.2, 150.2, 154.8, 158.8, 164.8, 166.0; MS: m/z = 527.3 $[M^+H]^+$ (100%), 251.1, 237.1, 211.3, 117.2; Anal. % Calculated for $C_{26}H_{20}N_4O_3S$: C 66.65, H 4.30, N 11.96; Found: C 66.63, H 4.32, N 11.98.

5-methyl-N-(5-methylthiazol-2-yl)-1,3-diphenyl-1*H*-pyrazole-4-carboxamide (4f):

Obtained from 5-methyl-1,3-diphenyl-1*H*-pyrazole-4-carboxylic acid (**2a**, 0.28g, 1.00mmol), EDC.HCl (1.20mmol), HOBt (1.20mmol) and 5-methylthiazol-2-amine (3f, 0.11g, 1.00mmol) as cream color amorphous solid in 88% yield (0.33g); IR (KBr): 3300 cm^{-1} (NH), 1685 cm^{-1} (amide C═O), 1625 cm^{-1} (C═N); ^{1}H NMR (400 MHz, DMSO-d6): δ 2.43 (s, 3H), 2.47 (s, 3H), 6.99 (s, 1H), 7.20-7.75 (m, 10H), 8.62 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 13.0, 13.6, 108.4, 112.1, 125.5, 126.2, 127.4, 128.2, 129.6, 129.7, 129.8, 130.0, 131.9, 137.2, 143.2, 143.6, 147.6, 154.8, 161.8, 166.0; MS: m/z = 375.4 $[M^+H]^+$ (100%), 235.1, 221.1, 195.3; Anal. % Calculated for $C_{21}H_{18}N_4OS$: C 67.36, H 4.85, N 14.96; Found: C 67.40, H 4.89, N 14.92.

3-(4-chlorophenyl)-5-methyl-N-(5-methylthiazol-2-yl)-1-phenyl-1H-pyrazole-4-carboxamide (4g):

Obtained from 3-(4-chlorophenyl)-5-methyl-1-phenyl-1*H*-pyrazole-4-carboxylic acid (**2b**, 0.31g, 1.00mmol), EDC.HCl (1.20mmol), HOBt (1.20mmol) and 5-methylthiazol-2-amine (3f, 0.11g, 1.00mmol) as off white solid in 82% yield (0.33g); IR (KBr): 3302 cm^{-1} (NH), 1670 cm^{-1} (amide C═O), 1628 cm^{-1} (C═N); ^{1}H NMR (400 MHz, DMSO-d6): δ 2.43 (s, 3H), 2.47 (s, 3H), 6.96 (s, 1H), 7.14-7.45 (m, 5H), 7.60 (dd, 2H), 7.73 (dd, 2H), 8.68 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 13.1, 13.7, 108.4, 112.1, 125.5, 126.2, 127.4, 129.4, 129.6, 129.7, 129.8, 130.2, 136.2, 137.2, 143.2, 143.6, 147.5, 154.5, 161.8, 166.1; MS: m/z = 411.1 [M+3], 409.4 $[M^+H]^+$ (100%), 269.4, 255.2, 229.4; Anal. % Calculated for $C_{21}H_{17}ClN_4OS$: C 61.68, H 4.19, N 13.70; Found: C 61.75, H 4.14, N 13.72.

3-(4-methoxyphenyl)-5-methyl-N-(5-methylthiazol-2-yl)-1-phenyl-1*H*-pyrazole-4-carboxamide (4h):

Obtained from 3-(4-methoxyphenyl)-5-methyl-1-phenyl-1*H*-pyrazole-4-carboxylic acid (**2c**, 0.31g, 1.00mmol), EDC.HCl (1.20mmol), HOBt (1.20mmol) and 5-methylthiazol-2-amine (3f, 0.11g, 1.00mmol) as white amorphous solid in 80% yield (0.32g); IR (KBr): 3300 cm^{-1} (NH, 1665 cm^{-1} (amide C═O), 1635 cm^{-1} (C═N); ^{1}H NMR (400 MHz, DMSO-d6): δ 2.43 (s, 3H), 2.47 (s, 3H), 3.76 (s, 3H), 6.92 (s, 1H), 7.20-7.67 (m, 9H), 8.88 (s, 1H); ^{13}C NMR (100 MHz, CDCl3): δ 13.3, 13.8, 108.4, 112.1, 115.7, 124.8, 125.5, 126.2, 127.4, 129.2, 129.6, 129.7, 137.2, 143.2, 143.4, 147.5, 154.8, 159.8, 161.8, 166.0; MS: m/z = 405.5 $[M^+H]^+$ 265.2, 251.1, 225.3; Anal. % Calculated for $C_{22}H_{20}N_4O_2S$: C 65.33, H 4.98, N 13.85; Found: C 65.36, H 4.94, N 13.91.

5-methyl-N-(5-methylthiazol-2-yl)-1-phenyl-3-(p-tolyl)-1*H*-pyrazole-4-carboxamide (4i):

Obtained from 5-methyl-1-phenyl-3-(p-tolyl)-1*H*-pyrazole-4-carboxylic acid (2e, 0.29g, 1.00mmol), EDC.HCl (1.20mmol), HOBt (1.20mmol) and 5-methylthiazol-2-amine (3f, 0.11g, 1.00mmol) as buff color solid in 72% yield (0.27g); IR (KBr): 3298 cm^{-1} (NH), 1675 cm^{-1} (amide C═O), 1620 cm^{-1} (C═N); ^{1}H NMR (400 MHz, DMSO-d6): δ 2.43 (s, 3H), 2.47 (s, 3H), 6.92 (s, 1H), 7.37-7.52 (m, 5H), 7.87-7.89 (d, 2H, J = 8.8 Hz), 7.92-7.94 (d, 2H, J = 8.8 Hz), 8.82 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 13.0, 13.5, 108.4, 112.0, 125.3, 125.5, 126.2, 127.4, 129.1, 129.6, 129.7, 137.2, 137.8, 143.2, 143.6, 147.6, 150.2, 154.8, 161.7, 166.0; MS: m/z = 420.1 $[M^+H]^+$; Anal. % Calculated for $C_{21}H_{17}N_5O_3S$: C 60.13, H 4.09, N 16.70; Found: C 60.10, H 4.15, N 16.72.

3-(4-hydroxyphenyl)-5-methyl-N-(5-methylthiazol-2-yl)-1-phenyl-1*H*-pyrazole-4-carboxamide (4j):

Obtained from 3-(4-hydroxyphenyl)-5-methyl-1-phenyl-1*H*-pyrazole-4-carboxylic acid (**2f**, 0.29g, 1.00mmol), EDC.HCl (1.20mmol), HOBt(1.20mmol) and 5-methylthiazol-2-amine (3f, 0.11g, 1.00mmol) as white amorphous solid in 80% yield (0.31g); IR (KBr): 3300 cm^{-1} (NH), 3205 cm^{-1} (OH-phenolic), 1678 cm^{-1} (amide C═O), 1640 cm^{-1} (C═N); ^{1}H NMR (400 MHz, DMSO-d6): δ 2.47 (s, 3H), 2.49 (s, 3H), 7.01 (s, 1H), 7.40-7.45 (m, 5H), 7.70-7.85 (m, 4H), 8.62 (s, 1H), 9.72 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 13.0, 13.6, 108.4, 112.1, 115.9, 124.7, 125.5, 126.2, 127.4, 129.5, 129.6, 129.7, 137.2, 143.2, 143.7, 147.6, 154.8, 158.6, 161.8, 166.0; MS: m/z = 391.4 $[M^+H]^+$; Anal. % Calculated for $C_{21}H_{18}N_4O_2S$: C 64.60, H 4.65, N 14.35; Found: C 64.65, H 4.66, N 14.32.

2.5. Antidiabetic activity.

2.5.1. 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 0.25 g of soluble potato starch in 50 mL of deionized water for 15 min. The enzyme solution of concentration 0.5 unit/mL, was prepared by mixing 0.001 g of α-amylase (EC 3.2.1.1) in 100 mL of 20 mM sodium phosphate buffer (pH 6.9)

containing 6.7 mM NaCl. The compounds were dissolved in DMSO to give various concentrations. The color reagent contains 96 mM 3,5-dinitrosalicylic acid (20 mL), 5.31 M sodium potassium tartrate in 2 M sodium hydroxide (8 mL) and demineralized water (12 mL). One mL of each sample and 1 mL of enzyme solution was mixed in a glass tube and incubated at 25°C for 30 min. To 1 mL of this mixture 1 mL of starch solution was added and incubated at 25°C for 3 min. Then, added 1 mL of the color reagent to the closed tube and was placed on water bath at 85 °C for 15 min. The reaction mixture was removed from the water bath, cooled and diluted with 9 mL distilled water and the absorbance value determined at 540 nm in a spectrophotometer. Individual blanks will be prepared torectify the background absorbance. In this bioassay, prior to the addition of starch solution the color reagent solution should be added and then the tube should be placed into the water bath. The other bioassay procedures will be carried out as above. Controls should be conducted in an identical fashion replacing the samples with 1 mL DMSO. Acarbose solution will be used as positive control. The inhibition percentage of α-amylase was calculated by the following formula:

Inhibition of α-amylase % = 100 × (OD Control – OD Sample) /OD Control

2.5.2. 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, an enzyme solution (0.1 U) in 900 µl of sodium phosphate buffer in the final volume of 1 mL. Each compound 100 µg was dissolved in 20 µl of distilled water and added to the test mixture before adding the substrate. The blanksample contained whole test mixture and the compound without enzyme solution. Acarbose was used as positive control. The mixture solution was incubated at 37 °C for 30 min and the reaction was terminated by adding 3 volumes of NH_4OH solution. The absorbance at 405 nm was determined by spectrophotometer. The inhibition percentage of α-glucosidase was calculated by the following formula:

Inhibition of α-glucosidase % = (OD Control – OD Test / OD Control) x 100.

3. RESULTS

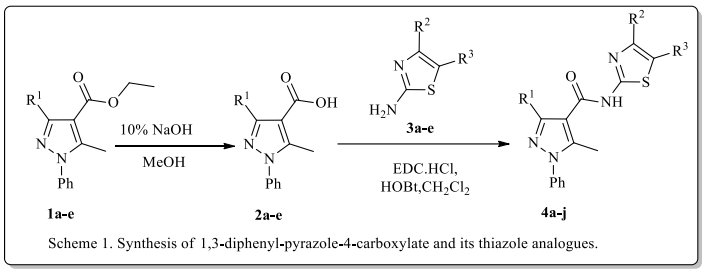
3.1. Chemistry.

The synthesis of our target pyrazole-4-carboxylic acid and its thiazole derivatives began with ethyl 5-methyl-1,3-diphenyl-1*H*-pyrazole-4-carboxylate [24] 1a-e as depicted in Scheme 1. The precursor **1** was converted into the 5-methyl-1,3-diphenyl-1*H*-pyrazole-4-carboxylic acid **2** by 10% NaOH solution in refluxing MeOH for 4h followed by acidification with dilute hydrochloric acid (pH ≈ 2). The 5-methyl-1,3-diphenyl-*N*-(4-phenylthiazol-2-yl)-1*H*-pyrazole-4-carboxamide **4** was achieved in one step coupling reaction between the acids **2** and 2-amino-thiazole **3** utilizing a peptide coupling reagents EDCI and HOBt in CH_2Cl_2.

The structures of newly synthesized compounds **2a-e** and **4a-j** were confirmed by their analytical and other spectral data. The compound **2a-e** exhibited the characteristic band at 3180-3200 cm^{-1} and 1710-1720 cm^{-1} due to the -OH and C═O stretching frequency of acid respectively. The formation of compounds **4a-j** was confirmed by the absence of peaks due to carboxylic acid and appearance of peaks at 3290-3320 cm^{-1} for amide –NH and 1660-1685 cm^{-1} for amide C═O functional groups. In ^1H NMR singlets at δ 12.30-12.45 for acidic –OH and 6.78-6.85 for C5-H of thiazole for products **2a-e** similarly at 6.90-7.01 C4-H of thiazole for products **4a-j** and peaks at 8.60-8.91 for –NH bond of all the compounds **4a-j** confirms the formation of products. The aryl group exhibited characteristic signals in the aromatic region of the spectrum. In ^{13}C NMR spectra, the absence of peak at δ 169.6 due to carbonyl group (C═O) of **2a-e** and appearance of peak at 166.0 (C═O amide) substantiated the formation of compounds **4a-j**. The mass spectrum of all the synthesized compounds showed molecular ion peak at M^{+1} corresponding to its molecular formula, which confirmed its chemical structure.

3.2. Biological Screening.

The treatment goal of diabetes patients is to maintain near normal levels of glycemic control, in both the fasting and post-prandial states. α-Amylase and α-glucosidase are pivotal enzymes which catalyses the hydrolysis of carbohydrates into simpler monosaccharides that are absorbed in the small intestine. The inhibition of their activity, in the digestive tract of humans, is considered to be effective to control diabetes by diminishing the absorption of glucose decomposed from starch by these enzymes [25-27]. Therefore, effective and nontoxic inhibition of α-amylase and α-glucosidase have long been sought.



Scheme 1. Synthesis of 1,3-diphenyl-pyrazole-4-carboxylate and its thiazole analogues.

In this study, we have synthesized the compounds **2a-e** and a hybrid molecule consists of pyrazole and thiazole linked through an amide bond **4a-j** and were screened in vitro for their antidiabetic activity by measuring the α-amylase and α-glucosidase inhibitory potential. Of all the compounds analyzed, compounds **4c, 4e** and **4j** demonstrated significant inhibitory effects on α-amylase and α-glucosidase. Among the compounds **4c, 4e** and **4j,** compound **4e** emerged as a potent inhibitor of both the enzymes. This may be attributed to the presence of electron donating –OH group on both the phenyl ring. This was further confirmed by the less activity of compound **4j** compare to **4e** which is having only one 4-OH substituted phenyl ring as shown in table 1. Other compounds **2c, 2e** and **4h** display moderate activity, whereas remaining compounds showed less activity. A preliminary examination from table 1 revealed that, 4-substituted phenyl ring attached to the thiazole nucleus is essential for the activity. Although compounds devoid of the phenyl ring on thiazole moiety showed less activity (table 1 entry 11-15), compound **4j** showed comparable activity with standard. This might be because of the H-bonding ability of the amide bond in addition to electron donating –OH group.

Table 1. Antidiabetic Activity[a] of **2a-e** and **4a-j**.

Sl No	R^1	R^2	R3	Product	IC_{50} values of α-amylase inhibition activity	IC_{50} values of α-glucosidase inhibition activity
1	C_6H_5	---	---	**2a**	45 µg/ml	40 µg/ml
2	4-Cl-C_6H_4	---	---	**2b**	40 µg/ml	40 µg/ml
3	4-CH_3O-C_6H_4	---	---	**2c**	30 µg/ml	25 µg/ml
4	4-NO_2-C_6H_4	---	---	**2d**	60 µg/ml	70 µg/ml
5	4-OH-C_6H_4	---	---	**2e**	30 µg/ml	30 µg/ml
6	C_6H_5	C_6H_5	H	**4a**	45 µg/ml	45 µg/ml
7	4-Cl-C_6H_4	4-Cl-C_6H_4	H	**4b**	40 µg/ml	45 µg/ml
8	4-CH_3O-C_6H_4	4-CH_3O-C_6H_4	H	**4c**	15 µg/ml	15 µg/ml
9	4-NO_2-C_6H_4	4-NO_2-C_6H_4	H	**4d**	40 µg/ml	40 µg/ml
10	4-OH-C_6H_4	4-OH-C_6H_4	H	**4e**	10 µg/ml	10 µg/ml
11	C_6H_5	H	CH_3	**4f**	45 µg/ml	45 µg/ml
12	4-Cl-C_6H_4	H	CH_3	**4g**	50 µg/ml	50 µg/ml
13	4-CH_3O-C_6H_4	H	CH_3	**4h**	30 µg/ml	25 µg/ml
14	4-NO_2-C_6H_4	H	CH_3	**4i**	50 µg/ml	55 µg/ml
15	4-OH-C_6H_4	H	CH_3	**4j**	20 µg/ml	25 µg/ml
16	Acarbose (+ ve control)				15 µg/ml	15 µg/ml

[a] Each value represents a mean of three replicates

4. CONCLUSIONS

In summary we have synthesized and studied the antidiabetic activity of a new series of 1,3-diphenyl-pyrazole-4-carboxylic acid and its thiazole analogues. Accordingly, these novel classes of compounds presented in our laboratory emerged as a potent α-amylase and α-glucosidase inhibitory agents. Among the synthesized compounds, compound 4e showed excellent in vitro antidiabetic activity in comparison with standard drug. Hence, it could be a promising drug candidate for the treatment of diabetes. Currently, investigations are underway to understand the mechanism and the results will be reported in due course.

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