Synthesis and evaluation of novel spiro derivatives for pyrrolopyrimidines as anti-hyperglycemia promising compounds

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ABSTRACT

Pyrrolopyrimidin-4-ylidene-malononitriles IIa-d were prepared as important intermediates for preparation of a new series of spiro-pyrrolopyrimidines. These intermediates undergo cyclisation via reaction with acetone, guanidine hydrochloride or hydrazine hydrate. Elemental and spectroscopic evidences for the structures of these compounds are presented. The final compounds have been monitored for in vivo anti-hyperglycemic activity, compared with Amaryl as standard drug. Among 12 tested compounds, both spiro (pyrano IIIb and pyrazlo Va) derivatives exhibit promising anti-hyperglycemic activity.

Introduction

Diabetes mellitus (DM) is a severe metabolic complaint that has a significant influence on the health and feature of patients’ life. In 2013, 382 million adults were diagnosed with diabetes worldwide. This number is expected to grow to 592 million in 2035, of which 90% will have type 2 diabetes (non-insulin-dependent diabetes mellitus; T2D). Patients with T2D are 2–4 times more likely to have fatal or non-fatal coronary events or a stroke. Almost 70–80% of patients die from one of these two conditions. The International Diabetes Federation (IDF) listed Egypt among the world top 10 countries in the number of patients with diabetes. In Egypt, the prevalence of diabetes is around 15.56% among adults (age: 20 and 79 years), with an annual death of 86,478 related to diabetes.

Treatment of diabetic patients has been concentrated on dietary controlling and well-known anti-hyperglycemic like sulfonylureas, metformin and acarbose. Glimepiride (Amaryl®), Sanofi-Aventis, Gentilly, France), a sulfonylurea containing a pyrrole group, acting as anti-hyperglycemic drug. It indicated to treat type 2 diabetes through increase insulin production by the pancreas (Figure 1). Recently, urgent requisite to develop novel anti-hyperglycemic agents was observed.

Numerous adverse effects present anti-hyperglycemic were indicated such as hepatotoxicity, weight gain and hypoglycemia. Administration of dipeptidyl peptidase IV (DPP-IV) inhibitors to diabetic patients results in higher concentrations of endogenous glucagon-like peptide (GLP-1) lead to decrease in plasma glucose. Long-term treatment with a DPP-IV inhibitor reduced HbA1c (glycosylated haemoglobin), offered prospective improvement in insulin producing function of the pancreas.

DPP-IV inhibitors were validated to be active and safe compounds that control blood glucose. Vildagliptin, saxagliptin, DPP-IV inhibitors, (having pyrrole and fused pyrrole ring, are on the market in many countries. Gosogliptin, di-pyrrole containing DPP-IV inhibitors, has been reported in advanced clinical trials. A highly potent DPP-IV inhibitor with pyrrolopyrimidine was also reported (Figure 1).

In 2004, pyrazolopyrimidine APD668 was discovered by Arena pharmaceutics, was found to exhibition high in vivo activity compared to a known DPP-IV inhibitor. APD668 was found to be more potent on delaying the onset of hyperglycemia (Figure 2). Researchers at GlaxoSmithKline replacement of pyrazolopyrimidine ring system in APD668 with a dihydropyrrolopyrimidine scaffold, which were described as having therapeutic value for diabetes and associated conditions, obesity, glucose intolerance, insulin resistance and atherosclerosis (Figure 2).

Spiro-based heterocyclic systems, containing one carbon atom common to two rings, were found to be very motivating. The asymmetric nature of these compounds, due to the spiro carbon, found to be one of the important criteria of the biological activities. Several patents described spiroazetidine and spiroazetidinone derivatives as GPR119 receptor agonists for the treatment of diabetes (Figure 3).

Encouraged by the prominence of spiro containing compounds, and in maintenance of our research efforts, in this research, we are going to spot an aspect on the chemistry of some newly synthesised spiro-pyryllypyrimidine derivatives and estimate them for the anti-diabetic activities. The synthetic pathways approved for the synthesis of these compounds are revealed in Scheme 1.

Materials and methods

Synthesis of lead compounds

All commercial chemicals used as starting materials and reagents in this study were purchased from Merck (Darmstadt, Germany) and were of reagent grade. All melting points were uncorrected and measured using Electro-thermal IA 9100 apparatus (Shimadzu, Japan).
Approved DPP-IV inhibitors as type 2 diabetes medications containing a pyrrole moiety.

Figure 1. Pyrroles and pyrrolopyrimidines as anti-diabetic agents.

A highly potent DPP-IV inhibitor with pyrrolopyrimidine significantly improved metabolic stability

Figure 2. Pyrrolopyrimidines as anti-diabetic agents.

Figure 3. Spiro compounds as biological active scaffolds.
Japan); IR spectra were recorded as potassium bromide pellets on a Perkin-Elmer 1650 spectrophotometer (Waltham, MA, USA), Faculty of Science, Cairo University, Cairo, Egypt. \( ^1 \)H NMR spectra were determined on a Varian Mercury (300 MHz) spectrometer (Varian, UK) and chemical shifts were expressed as ppm against TMS as internal reference (Faculty of Science, Cairo University, Cairo, Egypt). Mass spectra were recorded on 70 eV (EI Ms-QP 1000 TMS as internal reference (Faculty of Science, Cairo University, Cairo, Egypt). IR spectra were recorded as potassium bromide pellets on a Perkin-Elmer 1650 spectrophotometer (Waltham, MA, USA), Faculty of Science, Cairo University, Cairo, Egypt. \( ^1 \)H NMR spectra were performed on (Merck) Silica gel 60 (particle size 0.06–0.20 mm). Compounds Ia–d were synthesised as reported\(^{32–34}\). The rest of compounds prepared in this paper were new and their structures were confirmed using spectral data.

**General procedure for the synthesis of compounds Ila–d**

Compounds Ia–d (0.01 mol) and malononitrile (0.06 g, 0.01 mol) were heated under reflux in dry ethanol (30 ml) for 8 h, cooled, poured onto ice-water to give precipitate which was filtered off, dried and recrystallised from methanol to give Ila–d.

2-(7-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)-5,6-diphenyl-7H-pyrrolo[2,3-d]pyrimidin-4-ylidene)-malononitrile (Ila). Yield: 73%; m.p.: 179–181. \( ^1 \)C NMR (DMSO-d6, 300 MHz) \( \delta \) (ppm): 5.4 (s, 2H, Ph–CH3), 6.8–8.0 (m, 15H, Ar–H ), 8.18 (s, 1H, C2–H), 8.9 (s, 1H, NH, D2O exchangeable); Anal. Calcd for \( C_{26}H_{19}N_7O \): C, 75.43; H, 5.14; N, 13.33%. Found: C, 75.80; H, 5.02; N, 13.61%.

2-(7-benzyl-5,6-diphenyl-3H-pyrrolo[2,3-d]pyrimidin-4-ylidene)-malononitrile (Iib). Yield: 56%; m.p.: 191–193°C; IR (KBr) \( \nu \) (cm\(^{-1}\)): 3347 (N=–H), 2212 (C=N), 1581 (C=N); MS (EI) \( m/z \): 404 (M\(^+\), 13.5%), 406 (M\(^+\)+2, 8.5%), 408 (M\(^+\)+4, 2.7%) \(^1 \)H NMR (DMSO-d6, 300 MHz) \( \delta \) (ppm): 6.8–7.8 (m, 9H, Ar–H ), 8.09 (s, 1H, C2–H), 8.83 (s, 1H, NH, D2O exchangeable); Anal. Calcd for \( C_{25}H_{18}N_6O \): C, 72.38; H, 2.72; N, 17.33%. Found: C, 72.05; H, 2.69; N, 17.71%.

**General procedure for the synthesis of compounds Illa–d**

A mixture of compounds Ila–d (0.02 mol), acetylacetone (2g, 0.02 mol) and pyridine (68 drops) was heated under reflux in dry ethanol (50 ml) for 8 h, concentrated, cooled and the separated compound was filtered off and recrystallised from methanol to give Illa–d.

5-acetyl-4-amino-7"-benzyl-6-methyl-5"',6'-diphenyl-spiro[3H-pyrrolo[2,3-d]pyrimidine-4',2'-pyran]-3-carbonitrile (Illia). Yield: 72%; m.p.: 187–189°C; IR (KBr) \( \nu \) (cm\(^{-1}\)): 3212–3345 (N=–H), 2199 (C=O), 1667 (C=O), 1598 (C=O), 1260 (C=O); MS (EI) \( m/z \): 525 (M\(^+\), 41%), \(^1 \)H NMR (DMSO-d6, 300 MHz) \( \delta \) (ppm): 2.23 (s, 3H, C6–CH3), 2.27 (s, 3H, COCH3), 5.8(s, 2H, Ph–CH3), 4.7 (brs, 2H, NH2, D2O exchangeable), 6.9–7.7 (m, 15H, Ar–H ), 8.3 (s, 1H, C2=–H), 8.8 (s, 1H, NH, D2O exchangeable); Anal. Calcd for \( C_{31}H_{28}N_7O_2 \): C, 75.43; H, 5.14; N, 13.33%. Found: C, 75.80; H, 5.02; N, 13.61%.

5-acetyl-4-amino-7"-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)-6-methyl-5"'-diphenyl-spiro[3H-pyrrolo[2,3-d]pyrimidine-4',2'-pyran]-3-carbonitrile (Illib). Yield: 55%; m.p.: 195–197°C; IR (KBr) \( \nu \) (cm\(^{-1}\)): 3250–3387 (N=–H), 2207 (C=C), 1678, 1693.

**Scheme 1. Synthetic pathway for preparation of II-V [reagents; i = NC–CH2–CN, ii = (CH3CO)2CH, iii = (NH2)2CCl, iv = NH2NH2, v = Me]**
5-acylamino-4-amino-7-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrrol-4-yl)-6-methyl-5-phenyl-spiro[3H-pyrrolo[2,3-dipyrimidine]-4,2-pyran]-3-carbonitrile (IIa). Yield: 65%; m.p.: 188–190 ºC; IR (KBr) v (cm⁻¹): 3145–3149 (N–H, NH2), 2123 (C≡N), 1661, 1688 (C=O), 1602 (C=N), 1305 (C=O); MS (EI) m/z: 717 (M⁺, 60%), 528 (M⁺–1, 31.4%), 1H NMR (DMSO-d₆, 300 MHz) δ (ppm): 2.2 (s, 3H, C₆H), 2.34 (s, 3H, COCH₃), 2.37 (s, 3H, CH₃), 3.48 (s, 3H, NCH₃), 4.82 (brs, 2H, NH₂, D₂O exchangeable), 7.0–7.9 (m, 11H, Ar–H), 8.36 (1H, C=CH₂–H). 8.9 (s, 1H, NH, D₂O exchangeable); Anal. Calcd for C₂₇H₂₂N₉O₅ (504.25): C, 68.26; H, 4.95; N, 17.98%. Found: C, 68.03; H, 5.20; N, 18.22%.

5-acylamino-4-amino-7-(3,4-dichlorophenyl)-6-methyl-5-phenyl-spiro[3H-pyrrolo[2,3-dipyrimidine]-4,2-pyran]-3-carbonitrile (IIId). Yield: 33%; m.p.: 203–205 ºC; IR (KBr) v (cm⁻¹): 3225–3419 (N–H, NH₂), 2222 (C≡N), 1709 (C=O), 1614 (C=O), 1312 (C=O); MS (EI) m/z: 857 (M⁺, 60%), 505 (M⁺–1, 20.3%), 507 (M⁺–3, 8.3%) 1H NMR (DMSO-d₆, 300 MHz) δ (ppm): 2.1 (s, 3H, C₆H), 2.41 (s, 3H, COCH₃), 4.8 (brs, 2H, NH₂, D₂O exchangeable), 6.9–7.8 (m, 9H, Ar–H), 8.4 (s, 1H, C=CH₂–H). 8.9 (s, 1H, NH, D₂O exchangeable); Anal. Calcd for C₃₀H₂₂N₉ClO (535.32): C, 68.26; H, 4.95; N, 17.98%. Found: C, 68.03; H, 5.20; N, 18.22%.

General procedure for the synthesis of compounds IVa–d
A mixture of compounds IIa–d (0.02 mol), guanidine (1.18g, 0.02 mol) and pyridine (6–8 drops) was heated under reflux in dry ethanol (50 ml) for 8 h, concentrated, cooled, and the separated compound was filtered off and recrystallised from methanol to give IVa–d.

2,4-diamino-7-benzyl-5′,6′-diphenyl-spiro[1H-pyrindine-6,4′-3H-pyrrol(2,3-dipyrimidine]-5-carbonitrile (IVA). Yield: 68%; m.p.: 195–197 ºC; IR (KBr) v (cm⁻¹): 3126–3419 (N–H, NH₂), 2221 (C≡N), 1618 (C≡N); MS (EI) m/z: 484 (M⁺, 61%), 1H NMR (DMSO-d₆, 300 MHz) δ (ppm): 2.52 (s, 2H, Ph–CH₂), 4.2–4.6 (brs, 4H, 2NH₂D₂O exchangeable), 6.9–8.1 (m, 16H, Ar–H), 8.33 (1H, C=CH₂–H). 8.9 (s, 1H, NH, D₂O exchangeable); Anal. Calcd for C₂₂H₂₀N₁₀O (484.44): C, 71.90; H, 4.96; N, 23.14%. Found: C, 71.75; H, 4.98; N, 23.42%.

2,4-diamino-7-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrrol-4-yl)-5′,6′-diphenyl-spiro[1H-pyrindine-6,4′-3H-pyrrol(2,3-dipyrimidine]-5-carbonitrile (IVb). Yield: 53%; m.p.: 197–199 ºC; IR (KBr) v (cm⁻¹): 3153–3320 (N–H, NH₂), 2227 (C≡N), 1698 (C=O), 1622 (C=N); MS (EI) m/z: 580 (M⁺, 23.9%), 1H NMR (DMSO-d₆, 300 MHz) δ (ppm): 2.3 (s, 3H, CH₃), 3.41 (3H, NCH₃), 4.2–4.45 (brs, 4H, 2NH₂D₂O exchangeable), 7.0–7.7 (m, 16H, Ar–H), 8.09 (s, 1H, C=CH₂–H). 8.94 (s, 1H, NH, D₂O exchangeable); Anal. Calcd for C₂₆H₂₁N₁₀O₅ (580.04): C, 68.28; H, 4.83; N, 24.14%. Found: C, 68.39; H, 5.09; N, 24.45%.

2,4-diamino-7-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrrol-4-yl)-5′,6′-diphenyl-spiro[1H-pyrindine-6,4′-3H-pyrrol(2,3-dipyrimidine]-5-carbonitrile (IVc). Yield: 43%; m.p.: 194–196 ºC; IR (KBr) v (cm⁻¹): 3239–3485 (N–H, NH₂), 2205 (C≡N), 1682 (C=O), 1598 (C≡N); MS (EI) m/z: 504 (M⁺, 22%), 1H NMR (DMSO-d₆, 300 MHz) δ (ppm): 2.2 (s, 3H, CH₃), 3.5 (s, 3H, NCH₃), 4.05–4.4 (brs, 4H, 2NH₂D₂O exchangeable), 6.8–7.8 (m, 12H, Ar–H), 8.2 (s, 1H, C=CH₂–H), 8.8 (s, 1H, NH, D₂O exchangeable); Anal. Calcd for C₂₉H₂₈N₁₀O₅ (504.25): C, 64.29; H, 4.76; N, 27.78%. Found: C, 63.93; H, 4.65; N, 27.40%.

2,4-diamino-7′-(3,4-dichlorophenyl)-5′-phenyl-spiro[1H-pyrindine-6,4′-3H-pyrrol(2,3-dipyrimidine]-5-carbonitrile (IVd). Yield: 35%; m.p.: 212–214 ºC; IR (KBr) v (cm⁻¹): 3209–3345 (N–H, NH₂), 2218 (C≡N), 1626 (C≡N), MS (EI) m/z: 462 (M⁺, 58%), 464 (M⁺+2, 18.3%), 466 (M⁺+4, 5.7%); 1H NMR (DMSO-d₆, 300 MHz) δ (ppm): 4.1–4.4 (brs, 4H, 2NH₂D₂O exchangeable), 6.9–8.0 (m, 10H, Ar–H), 8.23 (s, 1H, C=CH₂–H), 9.1 (s, 1H, NH, D₂O exchangeable); Anal. Calcd for C₂₉H₂₈N₁₀Cl₂ (462.34): C, 75.14; H, 3.46; N, 24.24%. Found: C, 75.08; H, 3.62; N, 24.53%.

General procedure for the synthesis of compounds Va–d
A mixture of compound IIa–d (0.02 mol), hydrazine hydrate (0.64g, 0.02 mol) and pyridine (6–8 drops) was heated under reflux in dry ethanol (50 ml) for 8 h, concentrated, cooled, and the separated compound was filtered off and recrystallised from methanol to give Va–d.
7-benzyl-4-methylsulfanyl-5,6-diphenyl-pyrrolo[2,3-d]pyrimidine (VIa).

General procedure for the synthesis of compounds VIa–d

A mixture of compounds Ia–d (0.02 mol) and methyl iodide (0.02 mol) was stirred in 10% NaOH solution at room temperature for 8 h, poured onto acidified ice-water to give a precipitate which was filtered off, dried and crystallised from methanol to afford compounds VIa–d.

7-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)-4-methylsulfanyl-5,6-diphenyl-pyrrolo[2,3-d]pyrimidine (VIa). Yield: 73%; m.p.: 187–189°C; IR (KBr) v (cm⁻¹): 1583 (C=–N); MS (EI) m/z: 407 (M⁺, 52.3%), 1H NMR (DMSO-d6, 300 MHz) δ (ppm): 3.12 (s, 3H, S–CH3), 3.27 (s, 3H, S–CH3), 6.8–7.8 (m, 9H, Ar–H), 8.4 (s, 1H, C–2H); Anal. Calcd for C21H15N7Cl2 (435.28): C, 57.93%; H, 3.45; N, 10.32%. Found: C, 57.78; H, 3.65; N, 10.70%.

7-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)-4-methylsulfanyl-5,6-diphenyl-pyrrolo[2,3-d]pyrimidine (VIIb). Yield: 51%; m.p.: 183–185°C; IR (KBr) v (cm⁻¹): 1708 (C=O), 1618 (C=N); MS (EI) m/z: 503 (M⁺, 73%), 1H NMR (DMSO-d6, 300 MHz) δ (ppm): 2.31 (s, 3H, S–CH3), 3.27 (s, 3H, S–CH3), 3.37 (s, 3H, N–CH3), 7.0–8.1 (m, 15H, Ar–H), 8.4 (s, 1H, C–2H); Anal. Calcd for C26H21N3S (407.32): C, 71.57; H, 4.97; N, 13.92%. Found: C, 71.82; H, 4.66; N, 13.77%.

7-(3,4-dichlorophenyl)-4-methylsulfanyl-5,6-diphenyl-pyrrolo[2,3-d]pyrimidine (VIIc). Yield: 41%; m.p.: 183–185°C; IR (KBr) v (cm⁻¹): 1596 (C=N), MS (EI) m/z: 385 (M⁺, 22%), 387 (M⁺+2, 7.3%), 389 (M⁺+4, 2.6%), 1H NMR (DMSO-d6, 300 MHz) δ (ppm): 3.3 (s, 3H, S–CH3), 6.8–7.8 (m, 9H, Ar–H), 8.3 (s, 1H, C–2H); Anal. Calcd for C19H13N3SCl2 (385.32): C, 59.22; H, 3.38; N, 10.91%. Found: C, 59.61; H, 3.56; N, 11.13%.

Biological screening

Animals

The complete progress of the experiment was conducted using male Wistar albino rats (200–250 g), delivered by the Institutional Breeding House, Egypt, reared and maintained in the animal house of the institution. The animals had free access to food and water ad libitum and maintained in a controlled environment under standard conditions of temperature and humidity with an alternating 12 h light and dark cycle for about a week for acclimatisation. The protocol of the study was approved by the Animal Ethics Committee of the Faculty of Pharmacy, Helwan University on November 2016. The study was conducted in accordance with the EC, directive 86/609/EEC for animal experiments.

Dose determination

Glimepiride (Amaryl) was used as a standard anti-diabetic (4 mg/kg) in 1% of gum acacia and administered orally. Equivalent doses of all derivatives were calculated according to their molecular weight (M.wt).

Assessment of improvement on oral glucose tolerance and blood glucose lowering activity: sucrose loaded normal rats (SLM)

Male albino Wistar rats (200–250 g) were chosen and kept back on an overnight fasting. Next morning, the blood glucose level (0 min) of each animal was stated by glucometer using glucostrips. The animals presenting their fasting blood glucose levels in the range of 60–80 mg/dL were selected and separated into one control group and 13 experimental groups with six animals in each. Each rat of experimental groups was given suspension of the test compounds made in 1% of gum acacia at a dosage of (4 mg/kg) for the standard drug Glimepiride and Equivalent doses of all derivatives.

The animals of the control group received vehicle (1.0% of gum acacia) only. Exactly 30 min post-administration of the test samples/vehicle, an oral sucrose load of 10 g/kg body weight (bw) was given to each animal and the blood glucose level of each animal were measured at 30, 60, 90 and 120 min. The percentages (%) decreased in blood glucose level were calculated conferring to the AUC method.

Streptozotocin-induced diabetic rats

Male albino Wistar rats (200–250 g) were designated for this study. Diabetes was prompted in the rats by intraperitoneally (i.p.) injecting freshly prepared solution of Streptozotocin (STZ) (Sigma–Aldrich, Co., MO; catalogue number: 1001062761) in ice cold 0.1 M citrate buffer (pH 4.5) at a dosage of 50 mg/kg bw. The blood glucose of each animal was tested after 48 h and animals displaying fasting blood glucose level ≥200 mg/dl were elected. These diabetic rats were unsystematically scattered into groups consisting of six animals in each.

Experimental design

Five groups (eight rats each) were used to investigate the antihyperglycemic effect of the derivatives which showed promising antihyperglycemic effect in SLM (compounds IIa, Va and IIb). Group 1: diabetic control and Group 2: diabetic and Glimepiride (Amaryl) (4 mg/kg) served as a reference anti-diabetic drug. Groups 3–5 were given the various pyrrole derivatives (compounds IIa, Va and IIb). The treated groups administered the standard drug (Amaryl) and different derivatives orally. For each group, blood samples were collected by tail nipping and blood glucose level was estimated at 0, 1, 2, 4 and 6 h after oral administration of the tested compounds using glucometer (Gluco Dr Super Sensor, All Medicus Co., Ltd., Anyang, Gyeonggi, Korea).

Statistical analysis

Data were represented as mean area under curve (AUC) ± SD. Significant differences between groups was tested using GraphPad InStat (Graph software Inc., V 3.05, Ralph Stahlman, Purdue University, Lafayette, IN). Appropriate graphs were plotted using Microsoft Excel 2016. p Value less than .05 was considered statistically significant.
Discussion

Chemistry

The synthetic route to compounds Ia–d was reported in our previous work. Amino-cyano-pyrroles 1 were reacted with HCO$_2$H to produce pyrrolopyrimidine-4-ones 2, which on react with POCl$_3$, 4-chloro-pyrrolopyrimidines 3 were obtained in good yield. 4-Chloro derivatives II on react with thiourea adapted to pyrrolopyrimidine-4-thiones I, To date, and to the best of our knowledge, formation of the 4-thione analogues has been reported numerous in literature, but not mechanistically explained. Herein, the proposed mechanism of the reaction was believed to proceed via initial nucleophilic attack by the thiol group of thiourea on C-4 of the pyrimidine ring with proton transfer to N-3 and the formation of the potentially unstable intermediate [A]. This intermediate lose carbodiimide and HCl to give the pyrrolopyrimidine-4-thione, as revealed (Figure 4).

For preparation of spiro-pyrrolopyrimidines III–V; pyrrolopyrimidine-4-ylidene-malononitrile IIa–d was accomplished by the reaction of Ia–d with malononitrile in absolute ethanol using same procedure reported on our previous work. On treat thione derivatives II with acetylacetone guanidine hydrochloride and/or hydrazine hydrate in ethanol, containing catalytic amount of pyridine, the corresponding spiro-pyrrolopyrimidine derivatives of pyrazole, pyrimidine or pyran III–V were afforded in good yield, as revealed in Scheme 1. All novel compounds were confirmed with spectroscopic analysis (MS, IR, $^1$H NMR and microanalysis).

Biological activities

Twelve of synthesised spiro-pyrrolopyrimidines and pyrrolopyrimidine-4-one were evaluated for their anti-hyperglycemic activity using both sucrose load model and streptozotocin models of diabetes. The synthesised compounds were assessed for their anti-hyperglycemic activity, which is comparable to Glimepiride (Amaryl) the standard anti-hyperglycemic drug, by comparing the mean area under the curve (AUC) for the blood glucose level between the different studied groups.

Among the 12 tested compounds; five compounds showed significant improvement (12.32%, 13.3%, 14.52%, 15.18% and 21.54%, respectively) on oral glucose tolerance post-sucrose-loaded normoglucemic rats compared to the sucrose-loaded untreated control, as revealed in Figure 5. From those active derivatives, treatment of derivatives IIIa, IIlb and Va only to STZ model of diabetes caused lowering on the blood glucose profile to the average

![Figure 4. Synthetic and mechanistic pathway for preparation of Pyrrolopyrimidine-4-thione Ia–d.](image-url)

![Table 1. Assessment of various treatments on oral glucose tolerance and blood glucose lowering activity in sucrose load model and diabetic rats.](table-url)
of (17.49%, 22.48% and 25.92%, respectively) compared to the diabetic control group, as depicted in Table 1.

Comparing the anti-hyperglycemic activity of these compounds to that of the reference anti-diabetic drug (Amaryl), compounds IIIa, Va and IIIb showed significant decrease in the blood glucose level (144.67%, 185.94% and 214.39%, respectively) compared to the activity of Amaryl, as shown in Figure 6. Studying these anti-hyperglycemic derivatives IIIa, Va and IIIb showed that the rats survived and showed no toxicity symptoms, as revealed in Table 1.

Active compounds were classified into two main sets: first, the 4-malononitrile derivative of pyrrolopyrimidines, namely, IIIa.b (Ar = benzyl and anti-pyrine). Also, the spiro derivatives containing pyrane ring IIIa,b, spiro-containing pyrazole ring Va.

Conclusions
We designated a direct and efficient synthesis of novel spiro-pyrrolopyrimidine, and estimated as anti-hyperglycemic agents. The structure activity analysis indicated that the pyrano IIIa,b displayed a significant anti-hyperglycemic activity profile compared to Amaryl. Pyrimidine group in IVa did not enrich the activity. The introduction of pyrazolo group to Va give rise to superior anti-hyperglycemic activity.

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