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DESIGN, SYNTHESIS AND ANTIDIABETIC ACTIVITY OF SOME NEW 4-AMINO (OR 6-OXO)-2-METHYL/BENZYLTHIO (OR SUBSTITUTED AMINO) PYRIMIDINE DERIVATIVES

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تم فى هذا البحث تصميم وتشييد بعض المشتقات الجديدة من ٢ الكيل ثيو (أو مستبدل أمينو) ٤ أمينو ٥ سيانو ٦ مستبدل فينيل بيريميدين (**5a-l**) و ٢ الكيل ثيو (أو مستبدل أمينو) ٥ سيانو ٦،١ ثنائى هيدرو ٦ أوكسو ٤ مستبدل فينيل بيريميدين (**6a-l**) من خلال تفاعل ثيوميثيل (بنزيل) أملاح أيزوثيوريا (**1a,b**) مع مشتقات بنزيلدين مالونونيتريل (**2a-c**) معطيا المركبات (**5a-f**). وبتفاعل المركبات (**5a-c**) مع الأمينات المناسبة (**4a,b**) تم الحصول على المركبات (**5g-l**) ومن ناحية أخرى ومن خلال اجراء التفاعلات ولكن باستخدام ايثيل الفا سيانو سينامات تم تحضير المركبات (**6a-f**) والتى بتفاعلها مع الأمينات المناسبة تم الحصول على المركبات (**6g-l**). تم إثبات التراكيب البنائية للمركبات الجديدة المحضرة باستخدام كلا من تحاليل الأشعة تحت الحمراء و طيف الكتلة وكذلك الرنين النووى المغناطيسي للهيدروجين. هذا وقد تم اختبار تأثير المركبات الجديدة كخافضات لمستوى السكر على حيوانات التجارب وقد اظهرت الدراسة نتائج جيدة لبعض المركبات بالمقارنة بعقار الميتفورمين كدواء مرجعى.

*A new series of 4-amino-5-cyano-2-methyl/benzylthio (or substituted amino) 6-substituted phenyl pyrimidines **5a-l**, and 2-methyl/benzylthio (or substituted amino)-5-cyano-1,6-dihydro-6-oxo-4-(substitutedphenyl) pyrimidines **6a-l** was prepared. The reaction of S-methyl (or benzyl) isothiourea salts **1a,b** with benzylidenemalononitriles **2a-c** afforded compounds **5a-f**. Reaction of compounds **5a-c** (R= methylthio) with the appropriate amines **4a,b** (cyclohexylamine or 2-phenylethylamine) afforded 4-amino-2-substituted amino-5-cyano-6- (substituted phenyl) pyrimidines **5g-l**. On the other hand, reaction of S-methyl (or benzyl) isothiourea salts **1a,b** with* ethyl α-*cyanocinnamates **3a-c** afforded compounds **6a-f**. Reaction of compounds **6a-c** (R= methylthio) with the appropriate amines **4a,b** afforded 2-substituted amino-5-cyano-4-oxo-6-(substituted phenyl) pyrimidines **6g-l**. The purity of the new compounds was checked by TLC and elucidation of their structures was confirmed by IR, ^{1}H-NMR, and mass spectrometry along with elemental microanalyses. All the target compounds were evaluated for their in-vivo antidiabetic effects in rates in comparison with metformin as a reference drug.*

INTRODUCTION

Diabetes mellitus is a group of disorders of carbohydrate metabolism results from body's failure to produce insulin (type 1, insulin dependent diabetes mellitus IDDM) or insulin resistance (type 2, non-insulin dependent diabetes mellitus NIDDM) through altered secretion, decreased insulin activity, or a combination of both factors and characterized by hyperglycaemia[1]. As the disease progresses tissue or vascular damage ensues leading to severe complications such as retinopathy, nephropathy, neuropathy, cardiovascular disease, and foot ulceration[1-3].

Four categories of oral hypoglycemic agents are now available: insulin secretagogues (sulfonylureas, meglitinides, and D-phenyl-

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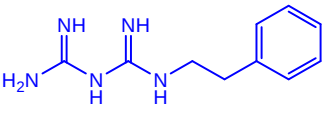
alanine derivatives), biguanides, thiazolidine-diones, and α-D-glucosidase inhibitors[2&3].

The biguanide antidiabetic such as **I** and **II** are a class of oral antihyperglycemic drugs used in the treatment of type 2 diabetes mellitus. They are given to supplement treatment by diet modification when such modification has not proved effective on its own. In addition, because biguanides are not associated with weight gain they are preferred in obese patients. Although sulfonylureas may be preferred in non-obese patients, a biguanide is often added or given instead to patients who are not responding to a sulfonylurea[4].

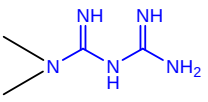
The main adverse effects of biguanides are GIT adverse effects, may be weight loss, absorption of various substances including vitamin B12, lactic acidosis specially with phenformin **I** may be fatal and effects on the cardiovascular system[2,4&5].

Additionally, aminopyrimidines have been reported for broad range of biological activities like antibacterial[6], analgesic[7], non-nucleoside HIV-1 reverse transcriptase inhibitory activity[8] and antiprotozoans[9-11].

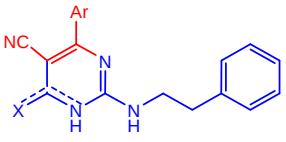
Search for new safer anti-diabetic agents is still a challenge for medicinal chemists. Encourage by the aforementioned data, the main objective of the present study is to synthesize different rigid cyclic biguanide analogues **IIIa-c** sharing a common functional group, a biguanide or its analogue in their structures. Also the study involves a preliminary screening of the target compounds for their oral antidiabetic effects.



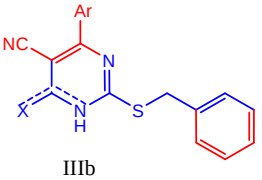
I, Phenformin



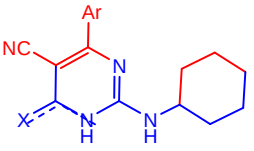
II, Metformin



IIIa



IIIb



IIIc

EXPERIMENTS

Chemistry

Experiments

Melting points were determined on an electrothermal melting point apparatus (Stuart Scientific Co.) and were uncorrected. The purity of the new compounds was checked by TLC and elemental microanalyses for carbon, hydrogen, and nitrogen were performed at the microanalytical center, Faculty of Science, Cairo University, and Assiut University Central Lab, Assiut. ^{1}H-NMR spectra were run on Varain Em-360L NMR spectrophotometer (60 MHz) (Varian USA) at the Faculty of Pharmacy, Assiut University otherwise stated using DMSO-d_6 as a solvent relative to TMS as an internal standard. IR spectra were recorded as KBr disks on Shimadzue IR 200-91527 Spectrophotometer at the Faculty of Pharmacy, Assiut University. Mass spectra were performed with JEOL JMS600, Assiut University Central Lab, Assiut.

Yields given are those of crude products and crystalization solvent was ethanol otherwise stated. Ethyl cyanoacetate was purchased from s.d.fine limited Co., malononitrile was purchased from Acros Co., phenylethylamine was purchased from Aldrich Co., cyclohexylamine was purchased from Prolabo Co. All other chemicals and solvents are of reagents grade.

General procedure for synthesis of 4-amino-2-alkylthiopyrimidines (5a-f)

A reaction mixture of different benzylidine malononitriles **2a-c** (10 mmol), S-alkylisothiourea salts **1a,b** (11.00 mmol) and sodium acetate anhydrous (2.50 g, 24.40 mmol) in pyridine (50 mL) was refluxed for 3 hrs.The reaction mixture was poured into water, acidified with dilute hydrochloric acid. The formed precipitate was filtered and crystallized from ethanol.

4-Amino-2-methylthio-6-phenylpyrimidine-5-carbonitrile (5a)

Yield, 75%; m.p., 156ºC; IR (ν cm^{-1}), 3345, 2160, 1640, 1524; ^{1}H-NMR (δ ppm), 8.00-7.35 (m, 5H, Ar-H); 3.90-3.30 (bs, 2H, exchangeable NH_2); 2.56 (s, 3H, SCH_3)[11].

4-Amino-6-(4-chlorophenyl)-2-methylthio-pyrimidine-5-carbonitrile (5b)

Yield, 74%; m.p., 166ºC; IR (ν cm^{-1}), 3365, 2180, 1648, 1535; ^{1}H-NMR (δ ppm), 8.10-7.35 (m, 4H, Ar-H,); 3.70-3.35 (bs, 2H, exchangeable NH_2); 2.50 (s, 3H, SCH_3)[11].

4-Amino-2-methylthio-6-(*p*-tolyl) pyrimidine-5-carbonitrile (5c)

Yield, 72%; m.p., 196ºC; IR (ν cm^{-1}), 3360, 2190, 1634, 1518; ^{1}H-NMR (δ ppm), 7.90-7.00 (m, 4H, Ar-H); 3.7-3.3 (bs, 2H, exchangeable NH_2); 2.60 (s, 3H, SCH_3); 2.30 (s, 3H, CH_3)[12&13].

4-Amino-2-benzylthio-6-phenylpyrimidine-5-carbonitrile (5d)

Yield, 72%; m.p., 120ºC; IR (ν cm^{-1}), 3385, 2180, 1622, 1517; ^{1}H-NMR (δ ppm), 8.0-7.10 (m, 10H, Ar-H); 4.40 (s, 2H, CH_2); 3.90 (bs, 2H, exchangeable NH_2); Anal. Calcd. for $C_{18}H_{14}N_4S$: H, 4.43; N, 17.60. Found: H, 4.22; N, 17.55; MS (70eV, EI): m/z (%) (M. Wt. 318.4): M^+ (318.65, 13.1%), M^++1 (319.64, 1.7%), and $C_4H_8N_2]^+$ (83.90, 100%).

4-Amino-2-benzylthio-6-(4-chlorophenyl) pyrimidine-5-carbonitrile (5e)

Yield, 70%; m.p., 177ºC; IR (ν cm^{-1}), 3405, 2190, 1649, 1610; ^{1}H-NMR (δ ppm), 8.00-7.10 (m, 9H, Ar-H); 4.40 (s, 2H, CH_2); 3.40 (bs, 2H, exchangeable NH_2); Anal. Calcd. for $C_{18}H_{13}ClN_4S$: C, 61.27; H, 3.71; N, 15.88. Found: C, 61.30; H, 3.55; N, 15.48; MS (70eV, EI): m/z (%) (M. Wt. 352.84): M^+ (352.07, 100%), M^++1 (353.28, 4.4%), M^++2 (354.07, 24.7%), and $C_4H_8N_2]^+$ (84.00, 73.8%).

4-Amino-2-benzylthio-6-(*p*-tolyl) pyrimidine-5-carbonitrile (5f)

Yield, 70%; m.p., 162ºC; IR (ν cm^{-1}), 3365, 2175, 1616, 1516; ^{1}H-NMR (δ ppm), 8.06-7.00 (m, 9H, Ar-H); 4.37 (s, 2H, CH_2); 3.36 (bs, 1H, exchangeable NH_2); 2.45 (s, 3H, CH_3). Anal. Calcd. for $C_{19}H_{16}N_4S$: C, 68.65; H, 4.85; N, 16.85. Found: C, 69.01; H, 4.82; N, 16.88.; MS (70eV, EI): m/z (%) (M. Wt. 332.42): M^+ (332.39, 34.00%), M^++1 (333.26, 8.3%), M^++2 (334.04, 3.6%), and $C_4H_6N_2]^+$ (83.97, 100%).

General procedure for synthesis of 2-alkyl-thio-4-oxopyrimidines (6a-f)

A reaction mixture of different ethyl α-cyanocinnamates **3a-c** (10 mmol), S-alkylisothiourea salts **1a,b** (11.0 mmol) and anhydrous sodium acetate (2.0 g, 24.4 mmol) in dimethylformamide (50 mL) was heated at 70°C for 1 hr. The reaction mixture was poured into ice water. The formed precipitate was filtered and crystallized from ethanol.

2-(Methylthio)-1,6-dihydro-6-oxo-4-phenyl-pyrimidine-5-carbonitrile (6a)

Yield, 75%; m.p., 274ºC; IR (ν cm^{-1}), 3460, 2190, 1649, 1529; ^{1}H-NMR (400 MHz, δ ppm), 8.08-7.76 (m, 2H, Ar-H); 7.72-7.48 (m, 3H, Ar-H); 2.66 (s, 3H, SCH_3)[12].

4-(4-Chlorophenyl)-1,6-dihydro-2-(methyl-thio)-6-oxopyrimidine-5-carbonitrile (6b)

Yield, 80%; m.p., 272ºC; IR (ν cm^{-1}), 3370, 2190, 1649, 1540; ^{1}H-NMR (400 MHz, δ ppm), 8.28-7.80 (m, 2H, Ar-H); 7.82-7.33 (m, 2H, Ar-H); 2.85 (s, 3H, SCH_3)[12].

2-(Methylthio)-1,6-dihydro-6-oxo-4-(*p*-tolyl) pyrimidine-5-carbonitrile (6c)

Yield, 72%; m.p., 296ºC; IR (ν cm^{-1}); 3405, 2195, 1638, 1522; ^{1}H-NMR (400 MHz, δ ppm), 8.00-7.70 (m, 2H, Ar-H); 7.70-7.40 (m, 2H, Ar-H); 2.66 (s, 3H, SCH_3), and 2.45 (s, 3H, CH_3)[13].

2-Benzylthio-1,6-dihydro-6-oxo-4-phenyl-pyrimidine-5-carbonitrile (6d)

Yield, 70%; m.p., 200ºC; IR (ν cm^{-1}), 3390, 2195, 1642, 1527; ^{1}H-NMR (DMSO-d_6), 8.16-7.36 (m, 10H, Ar-H); 6.80-5.35 (bs, 1H, exchangeable NH); 4.65 (s, 2H, CH_2); Anal. Calcd. for $C_{18}H_{13}N_3OS$: C, 67.69; H, 4.10; N, 13.16. Found: C, 68.00; H, 4.04; N, 13.07.; MS (70eV, EI): m/z (%) (M. Wt. 319.38): M^+ (319.67, 0.50%) and M^+-1 (318.58, 64.9%).

2-Benzylthio-4-(4-chlorophenyl)-1,6-di-hydro-6-oxopyrimidine-5-carbonitrile (6e)

Yield, 73%; m.p., 255ºC; IR (ν cm^{-1}), 3450, 2190, 1638, 1524; ^{1}H-NMR (δ ppm), 8.10-7.00 (m, 9H, Ar-H); 6.25-5.50 (bs, 1H, exchangeable NH); 4.50 (s, 2H, CH_2); Anal. Calcd. for $C_{18}H_{12}ClN_3OS$: C, 61.10; H, 3.42; N, 11.88. Found: C, 61.23; H, 3.12; N, 11.58; MS (70eV, EI): m/z (%) (M. Wt. 353.83): M^+

(353.56, 12.40%), M^++1 (354.56, 37.0%), M^++2 (355.56, 5.3%), M^+-1 (352.57, 63.4%) and $C_4H_9N_2]^+$ (83.95, 100%).

2-Benzylthio-1,6-dihydro-6-oxo-4-(*p*-tolyl) pyrimidine-5-carbonitrile[8] (6f)

Yield, 73%; m.p., 224ºC; IR (ν cm^{-1}), 3405, 2195, 1630, 1523; ^{1}H-NMR (δ ppm), 8.06-7.00 (m, 9H, Ar-H); 5.70-4.00 (bs, 3H, exchangeable NH and CH_2); 2.35 (s, 3H, CH_3). MS (70eV, EI): m/z (%) (M. Wt. 333.41): M^+ (333.59, 12.20%), M^++1 (334.11, 6.0%), M^++2 (335.23, 1.6%), and $C_4H_9N_2]^+$ (84.00, 100%).

General procedure for synthesis of 4-amino (oxo)-2-substituted aminopyrimidines (5g-l) and (6g-l)

2-Methylthiopyrimidines **5a-c** or **6a-c** (5.0 mmol) was heated under reflux overnight with 5 mL of the appropriate amine. The reaction mixture was cooled, diluted with water and acidified with dilute hydrochloric acid. The formed precipitate was filtered and crystallized from appropriate solvent.

4-Amino-2-(cyclohexylamino)-6-phenyl-pyrimidine-5-carbonitrile (5g)

Cryst. solvent, hexane; Yield, 58%; m.p., 88ºC; IR (ν cm^{-1}), 3452, 2370, 1649, 1614; ^{1}H-NMR (400 MHz, δ ppm), 7.77-7.01 (m, 5H, Ar-H); 3.67-3.97 (bs, 3H, exchangeable NH and NH_2); 2.66-0.83 (bm, 11H, c.Hex-H); Anal. Calcd. for $C_{17}H_{19}N_5$: C, 69.60; H, 6.53; N, 23.87. Found: C, 69.35; H, 6.50; N, 24.10; MS (70eV, EI): m/z (%) (M. Wt. 293.37): M^+ (293.69, 17.80%), M^+-1 (292.64, 68.4%), and M^+-$C_6H_{11}]^+$ (210.67, 100%).

4-Amino-6-(4-chlorophenyl)-2-(cyclohexyl-amino) pyrimidine-5-carbonitrile (5h)

Cryst. solvent, hexane; Yield, 62%; m.p., 96ºC; IR (ν cm^{-1}), 3445, 2370, 1664, 1510; ^{1}H-NMR (δ ppm), 8.35-7.11 (m, 4H, Ar-H); 4.50-3.30 (bs, 3H, exchangeable NH and NH_2); 2.60-1.00 (bm, 11H, c.Hex-H); Anal. Calcd. for $C_{17}H_{18}ClN_5$: C, 62.29; H, 5.53; N, 21.36. Found: C, 62.22; H, 5.43; N, 21.66; MS (70eV, EI): m/z (%) (M. Wt. 327.81): M^+ (327.23, 42.80%), M^++1 (328.17, 3.3%), M^++2 (329.46, 15.4%), and M^+-$C_4H_{11}N_2]^+$ (245.08, 100%).

4-Amino-2-(cyclohexylamino)-6-(*p*-tolyl) pyrimidine-5-carbonitrile (5i)

Cryst. solvent, hexane; Yield, 60%; m.p., 93ºC; IR (ν cm^{-1}), 3325, 2175, 1641, 1607; ^{1}H-NMR (δ ppm), 8.00-6.80 (m, 4H, Ar-H); 4.25-3.60 (bs, 3H, exchangeable NH and NH_2); 2.50 (s, 3H, CH_3); 2.10-1.00 (bm, 11H, c.Hex-H); Anal. Calcd. for $C_{18}H_{21}N_5$: C, 70.33; H, 6.89; N, 22.78. Found: C, 70.20; H, 6.65; N, 22.47; MS (70eV, EI): m/z (%) (M. Wt. 307.39): M^+ (307.52, 2.80%), M^++1 (308.68, 1.2%), M^++2 (309.88, 0.5%), and M^+-$C_6H_{11}]^+$ (224.91, 100%).

4-Amino-2-(phenethylamino)-6-phenyl-pyrimidine-5-carbonitrile (5j)

Cryst. solvent, ethanol; Yield, 67%; m.p., 122ºC; IR (ν cm^{-1}), 3345, 2175, 1639, 1539; ^{1}H-NMR (δ ppm), 7.90-7.20 (m, 10H, Ar-H); 3.90-3.20 (bm, 5H, exchangeable NH and NH_2 and $NH\underline{CH_2}$); 2.95 (t, 2H, CH_2); Anal. Calcd. for $C_{19}H_{17}N_5$: C, 72.36; H, 5.43; N, 22.21. Found: C, 72.62; H, 5.32; N, 22.55; MS (70eV, EI): m/z (%) (M. Wt. 315.37): M^+ (315.60, 4.40%), M^++1 (316.56, 0.3%), M^+-1 (314.75, 32.4%), and M^+-$C_7H_7]^+$ (223.89, 100%).

4-Amino-6-(4-chlorophenyl)-2-(phenethyl-amino) pyrimidine-5-carbonitrile (5k)

Cryst. solvent, ethanol; Yield, 70%; m.p., 161ºC; IR (ν cm^{-1}), 3410, 2195, 1649, 1608; ^{1}H-NMR (δ ppm), 8.45-8.10 (bs, 1H, exchangeable NH), 8.00-7.00 (m, 9H, Ar-H); 6.00-5.10 (bs, 2H, exchangeable NH_2); 3.63 (t, 2H, $NH\underline{CH_2}$); 2.90 (t, 2H, CH_2); Anal. Calcd. for $C_{19}H_{16}ClN_5$: C, 65.24; H, 4.61; N, 20.02. Found: C, 65.10; H, 4.55; N, 20.21; MS (70eV, EI): m/z (%) (M. Wt. 349.82): M^+ (349.12, 27.50%), M^++1 (350.61, 4.3%), M^++2 (351.23, 9.70%), and M^+-$C_7H_7]^+$ (257.95, 100%).

4-Amino-2-(phenethylamino)-6-(*p*-tolyl) pyrimidine-5-carbonitrile (5l)

Cryst. solvent, ethanol; Yield, 70%; m.p., 156ºC; IR (ν cm^{-1}), 3365, 2180, 1642, 1539; ^{1}H-NMR (δ ppm), 8.00-7.00 (m, 9H, Ar-H); 4.00-3.30 (bs, 5H, exchangeable NH and NH_2 and CH_2); 3.20 (t, 2H, $NH\underline{CH_2}$); 2.66 (s, 3H, CH_3); Anal. Calcd. for $C_{20}H_{19}N_5$: C, 72.93; H, 5.81; N, 21.26. Found: C73.05; H, 5.76; N, 21.34; MS (70eV, EI): m/z (%) (M. Wt. 329.40): M^+ (329.40, 26.80%), M^++1 (330.13,

6.50%), M^++2 (331.07, 0.4%), M^+-1 (328.24, 1.7%), and M^+-$C_7H_7]^+$ (237.98, 100%).

2-(Cyclohexylamino)-1,6-dihydro-6-oxo-4-phenylpyrimidine-5-carbonitrile (6g)

Cryst. solvent, ethanol; Yield, 60%; m.p., 292ºC; IR (ν cm^{-1}), 3390, 2190, 1646, 1598; ^{1}H-NMR (δ ppm), 11.20-10.10 (bs, 1H, exchangeable NHCO), 8.10-7.20 (m, 5H, Ar-H); 4.25-3.40 (bs, 1H, exchangeable NH); 2.10-1.00 (bm, 11H, c.Hex-H); Anal. Calcd. for $C_{17}H_{18}N_4O$: C, 69.37; H, 6.16; N, 19.03. Found: C, 70.03; H, 6.00; N, 19.13; MS (70eV, EI): m/z (%) (M. Wt. 294.35): M^+ (294.57, 2.90%), M^+-1 (293.54, 81.00%), and M^+-$C_6H_5]^+$ (211.63, 100%).

4-(4-Chlorophenyl)-2-(cyclohexylamino)-1,6-dihydro-6-oxopyrimidine-5-carbonitrile (6h)

Cryst. solvent, ethanol; Yield, 58%; m.p., 237ºC; IR (ν cm^{-1}), 3375, 2195, 1652, 1602; ^{1}H-NMR (δ ppm), 11.00-10.00 (bs, 1H, exchangeable NHCO), 8.00-7.00 (m, 4H, Ar-H); 4.20-3.55 (bs, 1H, exchangeable NH); 2.10-1.00 (bm, 11H, c.Hex-H); Anal. Calcd. for $C_{17}H_{17}ClN_4O$: C, 62.10; H, 5.21; N, 17.04. Found: C, 62.13; H, 5.20; N, 17.15; MS (70eV, EI): m/z (%) (M. Wt. 328.80): M^+ (328.81, 19.90%), M^++1 (329.78, 26.90%), M^++2 (330.85, 5.60%), and M^+-$C_6H_{11}]^+$ (245.81, 100%).

2-(Cyclohexylamino)-1,6-dihydro-6-oxo-4-(*p*-tolyl) pyrimidine-5-carbonitrile (6i)

Cryst. solvent, ethanol; Yield, 60%; m.p., 246ºC; IR (ν cm^{-1}), 3450, 2195, 1635, 1602; ^{1}H-NMR (δ ppm), 11.10-10.45 (bs, 1H, exchangeable NHCO), 7.90-6.90 (m, 4H, Ar-H); 4.20-3.50 (bs, 1H, exchangeable NH); 2.45 (s, 3H, CH_3); 2.10-1.00 (bm, 11H, c.Hex-H); Anal. Calcd. for $C_{18}H_{20}N_4O$: C, 70.11; H, 6.54; N, 18.17. Found: C, 69.95; H, 6.45; N, 18.10; MS (70eV, EI): m/z (%) (M. Wt. 308.38): M^+ (308.80, 8.20%), M^++1 (309.72, 2.60%), M^++2 (310.69, 0.4%), and M^+-$C_6H_{11}]^+$ (225.86, 100%).

2-(Phenethylamino)-1,6-dihydro-6-oxo-4-phenylpyrimidine-5-carbonitrile (6j)

Cryst. solvent, ethanol; Yield, 72%; m.p., 296ºC; IR (ν cm^{-1}), 3295, 2180, 1652, 1602; ^{1}H-NMR (400 MHz, δ ppm), 11.80-10.45 (bs, 1H, exchangeable NHCO), 8.72-7.19 (m, 10H, Ar-H); 4.10-3.25 (bm, 3H, exchangeable NH and $\underline{CH_2}$NH); 2.86 (t, 2H, CH_2); Anal. Calcd. for $C_{19}H_{16}N_4O$: C, 72.13; H, 5.10; N, 17.71. Found: C, 72.30; H, 5.00; N, 17.83; MS (70eV, EI): m/z (%) (M. Wt. 316.36): M^+ (316.02, 43.00%), M^++1 (317.07, 6.60%), M^++2 (318.00, 2.60%), and M^+-$C_7H_7]^+$ (224.97, 100%).

4-(4-Chlorophenyl)-1,6-dihydro-6-oxo-2-(phenethylamino) pyrimidine-5-carbonitrile (6k)

Cryst. solvent, ethanol; Yield, 70%; m.p., 216ºC; IR (ν cm^{-1}), 3405, 2180, 1649, 1610; ^{1}H-NMR (δ ppm), 10.90-10.00 (bs, 1H, exchangeable NHCO), 8.20-7.00 (m, 9H, Ar-H); 5.80-4.85 (bs, 1H, exchangeable NH); 3.86 (t, 2H, NH$\underline{CH_2}$); 3.52 (t, 2H, CH_2); Anal. Calcd. for $C_{19}H_{15}ClN_4O$: C, 65.05; H, 4.31; N, 15.97. Found: C, 65.21; H, 4.22; N, 16.00; MS (70eV, EI): m/z (%) (M. Wt. 350.80): M^+ (350.73, 11.80%), M^++1 (351.12, 0.80%), M^++2 (352.71, 4.40%), M^+-2 (348.80, 30.80%), and M^+-$C_7H_{13}]^+$ (257.79, 100%).

2-(Phenethylamino)-1,6-dihydro-6-oxo-4-(*p*-tolyl) pyrimidine-5-carbonitrile (6l)

Cryst. solvent, ethanol; Yield, 74%; m.p., 232ºC; IR (ν cm^{-1}), 3385, 2175, 1658, 1601; ^{1}H-NMR (δ ppm), 11.10-10.20 (bs, 1H, exchangeable NHCO), 8.20-6.810 (m, 9H, Ar-H); 4.80-4.00 (bs, H, exchangeable NH); 3.55 (t, 2H, NH$\underline{CH_2}$); 2.85 (t, 2H, CH_2); 2.40 (t, 3H, CH_3); Anal. Calcd. for $C_{20}H_{18}N_4O$: C, 72.71; H, 5.49; N, 16.96. Found: C, 72.55; H, 5.60; N, 17.11; MS (70eV, EI): m/z (%) (M. Wt. 330.38): M^+ (330.83, 5.10%), M^++1 (331.70, 1.90%), M^+-1 (329.69, 12.4%), and $C_4H_8N_2]^+$ (83.89, 100%).

Biology

Anti-diabetic activity of compounds 5d-l, 6d-l, and metformin

Biological screening was carried out at the Department of Pharmacology, Faculty of Pharmacy, Alazhar University-Assiut branch-Assiut. Male Wistar albino rats weighing 100-120 g were used for this study. The animals were obtained from Animal House, Faculty of Medicine, Assiut University (Assiut, Egypt) and were fed a standard diet with water *ad libitum*. Experiments were conducted in accordance with the guidelines for animal care

of the United States Naval Medical Research Centre, Unit No. 3, Abbaseya, Cairo, Egypt. During the study, the rats were maintained at a 12-hrs light : dark cycle. They were fasted for 24 hrs before the experiments but had free access to drinking water.

Animals were divided into four groups of six rats each. Group I control normal animals were treated with vehicle while Group II diabetic rats treated with vehicle. Group III diabetic rats treated with metformin as a reference drug at 100 mg/kg body weight/day orally by gastric intubation to the rats for 7 days[14]. Group IV diabetic rats treated with compounds at 100 mg/kg body weight/day orally for 7 days. Fasting blood sugar was estimated on overnight fasted rats on first day and after 7 days.

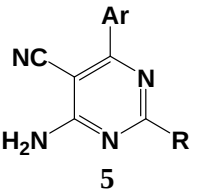
RESULTS AND DISCUSSION

Chemistry

The target compounds 2-benzylthio (substituted amino)-4-amino-5-cyano-6-(substituted phenyl) pyrimidines **5d-l**, and 2-benzylthio (substituted amino)-5-cyano-1,6-dihydro-6-oxo-4-(substituted phenyl) pyrimidines **6d-l** (Fig. 1) were prepared according to Scheme 1.

Compounds **5a-c** were prepared according to reported procedures through reaction of S-methylisothiourea sulfate **1a**[15] with benzylidenemalononitriles **2a-c**[16&17] by reflux in pyridine and in presence of anhydrous sodium acetate[18].

The chemical structure of compounds **5a-c** was confirmed using IR, ^{1}HNMR, spectroscopy and comparison with the reported data[12,16&17]. ^{1}H-NMR spectra of compounds **5a-c** revealed the appearance of NH_2 signals at 3.90-3.30 ppm and the SCH_3 signal at about 2.50 ppm. IR spectra of compounds **5a-c** showed broad bands at 3345-3365 cm^{-1} due to presence of NH_2 group, in addition to, the characteristic strong bands at 2160-2190 cm^{-1} of the cyano group. Reaction of S-benzylisothiourea hydrobromide **1b** with benzylidenemalono-nitriles **2a-c** afforded 4-aminopyrimidines with 2-benzylthio substituent **5d-f**. ^{1}H-NMR spectra of compounds **5d-f** revealed the appearance of a singlet signal at 4.40 at ppm corresponding to $\underline{CH_2}Ph$, and NH_2 signals at 3.90-3.36 ppm. IR spectra of compounds **5d-f** showed broad bands at 3405-3385 cm^{-1} (ν NH_2). MS data of compound **5d-f** showed molecular ion peaks at 318.65, 352.82, and 332.3 corresponding to the their molecular weights, respectively.



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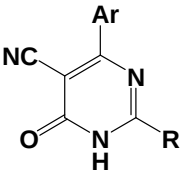

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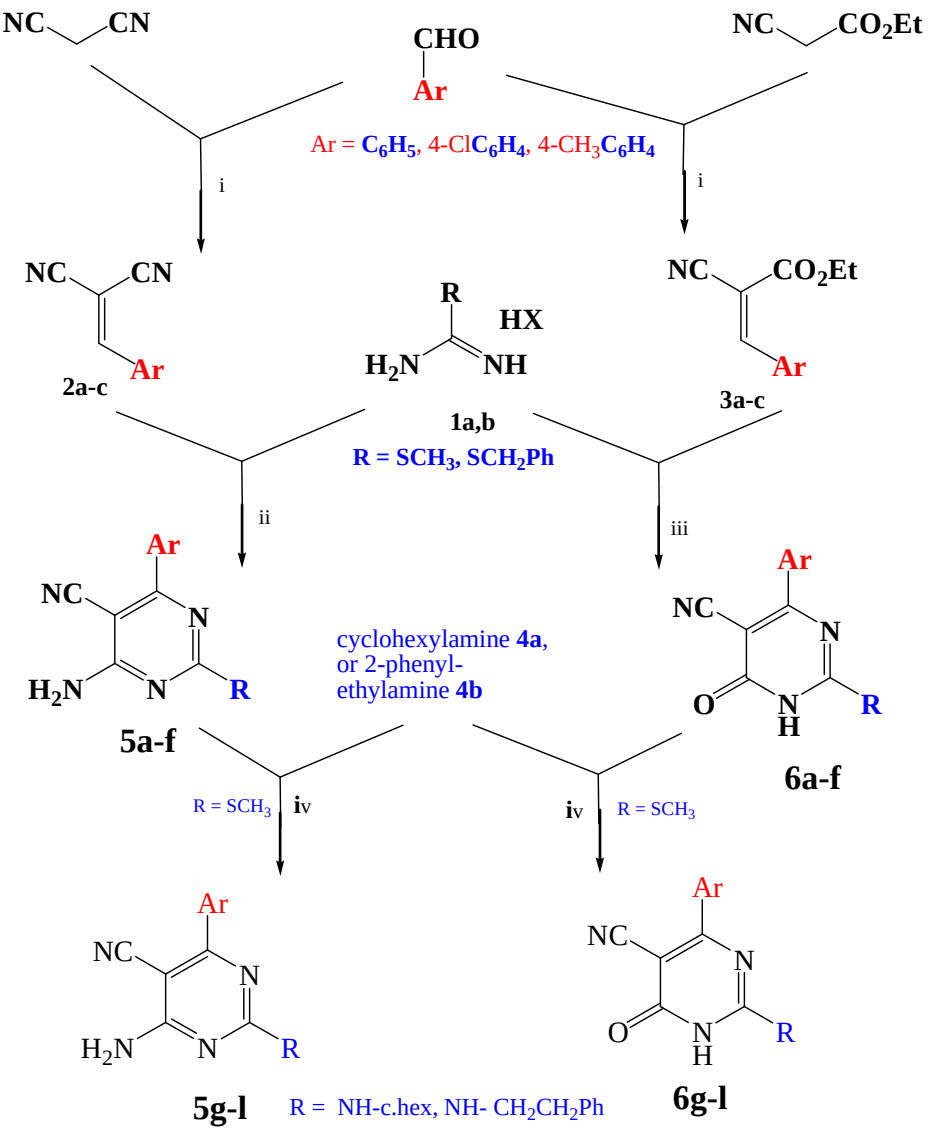
No	Ar	R	No	Ar	R
5a	C_6H_5-	SCH_3	**6a**	C_6H_5-	SCH_3
5b	4-Cl-C_6H_4-	SCH_3	**6b**	4-Cl-C_6H_4-	SCH_3
5c	4-CH_3-C_6H_4-	SCH_3	**6c**	4-CH_3-C_6H_4-	SCH_3
5d	C_6H_5-	SCH_2Ph	**6d**	C_6H_5-	SCH_2Ph
5e	4-Cl-C_6H_4-	SCH_2Ph	**6e**	4-Cl-C_6H_4-	SCH_2Ph
5f	4-CH_3-C_6H_4-	SCH_2Ph	**6f**	4-CH_3-C_6H_4-	SCH_2Ph
5g	C_6H_5-	NH-c.hexyl	**6g**	C_6H_5-	NH-c.hexyl
5h	4-Cl-C_6H_4-	NH-c.hexyl	**6h**	4-Cl-C_6H_4-	NH-c.hexyl
5i	4-CH_3-C_6H_4-	NH-c.hexyl	**6i**	4-CH_3-C_6H_4-	NH-c.hexyl
5j	C_6H_5-	$NHCH_2CH_2Ph$	**6j**	C_6H_5-	$NHCH_2CH_2Ph$
5k	4-Cl-C_6H_4-	$NHCH_2CH_2Ph$	**6k**	4-Cl-C_6H_4-	$NHCH_2CH_2Ph$
5l	4-CH_3-C_6H_4-	$NHCH_2CH_2Ph$	**6l**	4-CH_3-C_6H_4-	$NHCH_2CH_2Ph$

Fig. 1: Compounds **5a-l**, and **6a-l**.



Reagents and conditions: i, ethanol, piperidine, heat; ii, pyridine, sodium acetate, reflux; iii, dimethylformamide, sodium acetate, heat 70ºC; iv, reflux.

Scheme 1: Synthesis of compounds **5a-l** and **6a-l**.

Heating under reflux of compounds **5a-c** (R= methylthio) with excess of the appropriate amines afforded 2-substituted amino derivatives[12]. By this way, the reaction of cyclohexylamine **4a** with **5a-c** afforded 4-amino-2-cyclohexylamino-5-cyano-6-(substituted) phenylpyrimidines **5g-i** with the elimination of the good leaving methylthio group.

^{1}H-NMR spectra of compounds **5g-i** revealed the appearance of NH_2, NH as broad signals at 4.30-3.60 ppm, and cyclohexyl protons as broad multiplet at 2.50-1.00 ppm, with disappearance of methylthio signals. IR spectra of compounds **5g-i** showed broad bands at 3325-3452 cm^{-1} (NH_2 and NH).

In analogy, heating under reflux of compounds **5a-c** (R= methylthio) with excess 2-phenylethylamine **4b** afforded 4-amino-2-phenylethylamino-5-cyano-6-(substituted phenyl) pyrimidines **5j-l**.

^{1}H-NMR spectra of compounds **(5j-l)** revealed the appearance of NH_2, NH as a broad signal incorporated with $N\underline{CH_2}CH_2Ph$ protons that appeared as a broad multiplet at 4.00-3.30 ppm., and $NCH_2\underline{CH_2}Ph$ protons appear as a triplet at about 3.0 ppm. IR spectra of compounds **5j-l** showed broad bands at 3345-3410 cm^{-1} (NH_2 and NH).

MS data of compounds **5g-l** showed molecular ion peaks at 293.69, 327.23, 307.52, 315.60, 349.12, and 329.40, corresponding to their molecular weights, respectively.

On the other hand, reaction of S-methylisothiourea sulfate **1a** with ethyl α-cyanocinnamates **3a-c**[5&6] afforded derivatives **6a-c**[12&13]. ^{1}H-NMR spectra of compounds **6a-c** showed a singlet signal of SCH_3 at about 2.66 ppm. IR spectra of compounds **6a-c** showed broad bands at 3370-3460 cm^{-1} (NH).

Similarly, reaction of S-benzylisothiourea hydrobromide **1b** with ethyl α-cyano-cinnamates **3a-c** afforded derivatives **6d-f**. Their ^{1}H-NMR spectra revealed the appearance of NH signal as a broad signal at about 6.80-5.30 ppm, and S$\underline{CH_2}$Ph protons appeared as a singlet at about 4.65 ppm. IR spectra of compounds **6d-f** showed broad bands at 3390-3450 cm^{-1} (NH). MS data of compounds **6d-f** showed molecular ion peaks at 319.67, 353.56, and 333.59 corresponding to their molecular weights, respectively.

Reaction of compounds **6a-c** (R= methylthio) with cyclohexylamine **4a** afforded 2-cyclohexylamino-5-cyano-4-oxo-6-(substituted phenyl) pyrimidines **6g-i**. Their ^{1}H-NMR spectra revealed the appearance of a broad signal in the off set region for the amidic NH protons at 11.20-10.00 ppm, a broad singlet at 4.20-3.40 ppm (NH) and cyclohexyl protons appeared as a broad multiplet at 2.10-1.00 ppm, and disappearance of methylthio signals. IR spectra of compounds **6g-i** showed broad bands at 3375-3450 cm^{-1} (ν 2NH).

Additionally, reaction of compounds **6a-c** (R= methylthio) with 2-phenylethylamine **4b** afforded 2-phenylethylamino-5-cyano-4-oxo-6-(substituted phenyl) pyrimidines **6j-l**. Their ^{1}H-NMR spectra revealed the appearance of a broad signal in the off set region for the amidic NH protons at 11.80-10.00 ppm, a broad singlet at 5.80-4.00 ppm (NH), N$\underline{CH_2}CH_2$Ph as a triplet at 3.86-3.55 ppm, and $NCH_2\underline{CH_2}$Ph appeared as triplet at 3.52-2.85 ppm. IR spectra of compounds **6j-l** showed broad bands at 3295-3450 cm^{-1} (ν 2NH).

MS data of compounds **6g-l** showed molecular ion peaks at 294.57, 328.81, 308.80, 316.02, 350.73, and 330.83 corresponding to their molecular weights, respectively. Moreover, the fragmentation patterns for all new compounds are in accordance with their expected chemical structures.

Biology

Antidiabetic activity

Eighteen new compounds, **5d-l**, and **6d-l** were evaluated for their antidiabetic effect in comparison to metformin according to the reported methods[14&18]. The assessment of the antidiabetic activity for the aforementioned compounds was carried out by measuring the percentage decrease in the mean blood glucose for rats previously induced with type II diabetes mellitus non-insulin dependent diabetes mellitus (NIDDM).

Induction of type II diabetes mellitus was induced in overnight fasted male Wistar albino rats weighing 100-120 g, with a single injection of alloxan monohydrate (150 mg/kg body weight), dissolved in sterile normal saline, by intraperitoneal route[18]. Blood samples to measure fasting blood glucose (FBG) were obtained by tail vein puncture of all groups of rats, glucose levels were determined on different days using a (Accu-Chek GO, Ireland) blood glucose monitoring system (Johonson and Johonson Company, Germany). At the fifth day the development of diabetes was confirmed. The rats found with permanent NIDDM (FBG > 200 mg/dl) were used for the antidiabetic study.

Animals were treated orally with 100 mg/kg body weight/day of the tested compounds in comparison to metformin as a reference drug and other rats were treated with a vehicle as a control for 7 days, The fasting blood sugar was estimated on overnight fasted rats on first day and after 7 days.

The results, table 1 and figure 2, indicated that compounds **5d-f**, 2-benzylthio-4-aminopyrimidine derivatives, showed weak or no decrease in the mean blood glucose for rats previously induced with type II diabetes mellitus. The percentage reduction of FBG was improved to about 15-21% with compounds **6d-f** (2-benzylthio-4-oxopyrimidine derivatives).

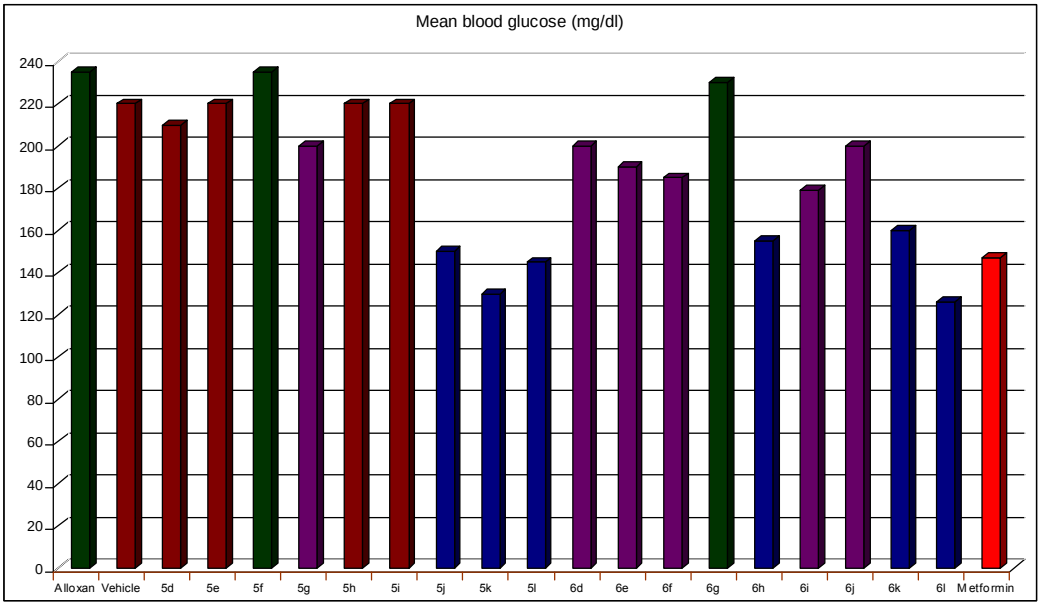
On the other hand, compounds **5g-i**, 4-amino-2-cyclohexylamino pyrimidine derivatives, showed 6-15% decrease of the percentage in the mean blood glucose, while with derivatives **6g-i**, 2-cyclohexylamino-4-oxopyrimidines, the activity showed up to 34%

Table 1: Anti-diabetic activity of compounds **5d-l**, **6d-l**, and metformin.

No	Ar	R	Mean blood glucose (mg/dl)	% Reduction
Alloxan monohydrate			235±9***	
Vehicle			220±17***	6%
5d	Ph	SCH_2Ph	210±8***	11%
5e	4-Cl.Ph	SCH_2Ph	220±16***	6%
5f	4-CH_3.Ph	SCH_2Ph	235±4***	0%
5g	Ph	NH-c.hexyl	200±18***	15%
5h	4-Cl.Ph	NH-c.hexyl	220±17***	6%
5i	4-CH_3.Ph	NH-c.hexyl	220±17***	6%
5j	Ph	$NHCH_2CH_2Ph$	150±9**	36%
5k	4-Cl.Ph	$NHCH_2CH_2Ph$	130± 9*	45%
5l	4-CH_3.Ph	$NHCH_2CH_2Ph$	145±21***	38%
6d	Ph	SCH_2Ph	200±18***	15%
6e	4-Cl.Ph	SCH_2Ph	190±12***	19%
6f	4-CH_3.Ph	SCH_2Ph	185±9***	21%
6g	Ph	NH-c.hexyl	230±12***	2%
6h	4-Cl.Ph	NH-c.hexyl	155±11**	34%
6i	4-CH_3.Ph	NH-c.hexyl	179±8***	24%
6j	Ph	$NHCH_2CH_2Ph$	200±29***	15%
6k	4-Cl.Ph	$NHCH_2CH_2Ph$	160±16**	32%
6l	4-CH_3.Ph	$NHCH_2CH_2Ph$	126±14**	46%
Metformin			147±6.7**	37%

* Significant difference at P< 0.05 vs. control value (student's-t-test).
** Significant difference at P< 0.01 vs. control value (student's-t-test).
*** Significant difference at P< 0.001 vs. control value (student's-t-test).

Fig. 2: Anti-diabetic activity of compounds **5d-l** and **6d-l** and metformin.



reduction in FBG level except for compound (**6g**) bearing unsubstituted phenyl at position 6, showed only 2% reduction in FBG.

Moreover, the activity was markedly increased with compounds, 4-amino-2-phenylethylamino pyrimidine derivatives, **5j-l**, especially **5k** which has 6- *(p*-chlorophenyl) moiety giving 45% reduction in comparison to metformin that showed only 37% decrease in FBG. In addition, compounds **6j-l** with 2-phenylethylamino-4-oxo moieties, the activity was also markedly increased especially compound **6l** with 6- *(p*-methylphenyl) which showed 46% decrease in FBG activity comparing to metformin**.** It is noteworthy to mention that, compounds **5j, 5l, 6h** and **6k** gave comparable results as the reference dug while compounds **5k** and **6l** are considered promising lead compounds as antidiabetic agents as they gave 45% and 46% reduction in FBG level respectively in comparison to metformin as a reference drug**.**

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