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Synthesis and anti-hyperglycemic evaluation of novel carboximidamides derived from cyanamides

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ABSTRACT

Carboximidamides **4-10** and 4,5-dihydro-1*H*-imidazol-2-amines **11, 12** linked with pyrimidine moiety were obtained *via* reaction of *N*-(pyrimidin-2-yl)cyanamides **1-3** with amines such as; morpholine, piperidine, piperazines and/or ethylenediamine, respectively. *N*-(4,6-Dimethylpyrimidin-2-yl)morpholine-4-carboximidamide **4** is subjected to react with 4-methylbenzenesulphonyl-, benzoyl-, and/or terephthaloyl chloride to give the corresponding *N*-substituted carboximidamides **13-16**. The structure of new compounds was confirmed by using FT-IR and NMR spectral data. X-ray single crystal diffraction analysis of **15** revealed that the structure belongs to the monoclinic system and has a chair conformation in a morpholine ring. DMSO and carboximidamides **6, 8** caused a significant decrease in the serum level of glucose, comparing with the diabetic-treated group (streptozotocin-treated group). Furthermore they restored the serum levels of ALT, AST, triglyceride and cholesterol (Biomarker of liver function) to the levels similar or slightly higher than of the negative control group. Also, the biomarkers of kidney function (serum urea and creatinine) were restored to the level urea or slightly higher than their negative control on the administration of DMSO and carboximidamides **6, 8**. So it can be concluded that, DMSO and carboximidamides **6, 8** may be used as ameliorative agents against streptozotocin-induced a pathological effects on blood glucose, liver function and kidney function. Morever, it can be stated that these compounds may be more effective in reducing the pathological effects of streptozotocin-induced diabetes than that of metformin.

1. Introduction

Type 2 diabetes mellitus (T2DM) is a disease that is characterized by raised blood glucose levels and it accounts for at least 90% of all cases of diabetes [1,2]. For this purpose, two groups of oral anti-hyperglycemic drugs such as sulphonyl ureas and biguanides have been used in the treatment of T2DM [3]. In spite of the worse outcomes of most sulfonylureas, the newer agents do not appear to increase the risk of death, heart attacks or strokes [4]. The biguanides such as phenformin, buformin and metformin were first discovered in the 1950s, (Fig. 1) [5]. But as a result of the lactic acidosis that is associated with the use of phenformin and buformin have since been discontinued during the 1970s [6] and therefore metformin (rarely causes lactic acidosis) was remained the only biguanide that is available for commercial use to treatment T2MD [7,8].

Carboximidamides have been previously prepared [9–16] and they exhibit pharmacological activities such as; antihypoglycemic [17], Tuberculostatic, antimicrobial [15,18,19], antileishmanial [20] antidegenerative [21], antitumor [22], anti-HIV [23] and antiplatelet [24]. Their derivatives were evaluated in *vitro* as dual COXs/LOX inhibitors [16] and as a pre-clinical development candidate for the treatment of migraine [25] and Melanoma [26].

There are several synthetic routes were schemed for preparation of carboximidamide derivatives *via*: (i) The reaction of ammonia/$HCl_{(g)}$, sulphonamides, or aliphatic/aromatic amines with substrates containing nitrile group [10,12,18–20]; (ii) The reaction of carboximidoyl chlorides with aliphatic/aromatic

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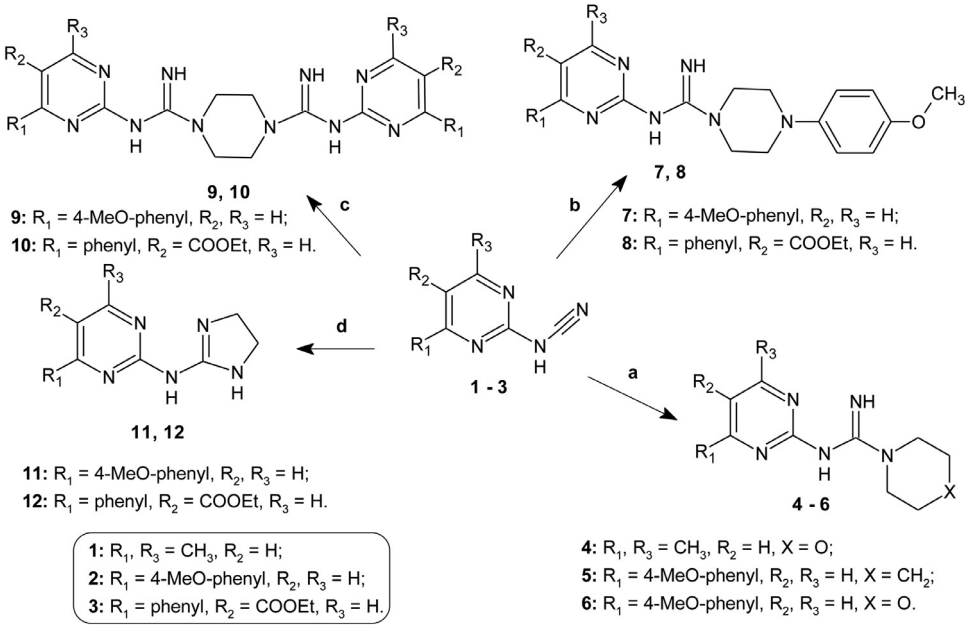
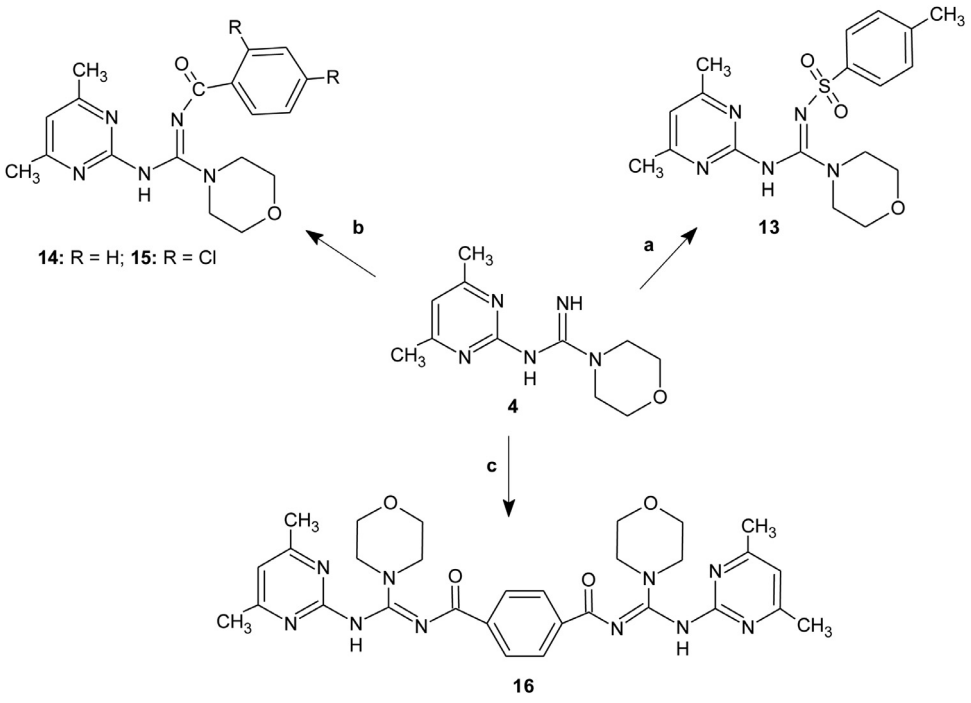


Scheme 1. Synthesis of carboximidamides **4-10** and 4,5-dihydro-1*H*-imidazol-2-amines **11, 12**. Reagents and conditions: (a) *sec. amines*: morpholine or piperidine, H_2O, r.t., 12 hrs.; (b) 1-(4-methoxyphenyl)piperazine, *iso*-PrOH, reflux, 8 hrs; (c) piperazine, *iso*-PrOH, reflux, 8 hrs.; (d) ethylenediamine, dioxane, reflux, 7 hrs.



Scheme 2. Synthesis of morpholine-4-carboximidamides **13-16**. Reagents and conditions: (a) 4-methylbenzenesulphonyl chloride, TEA, dry dioxane, 80 °C, 7 hrs.; (b,c) acid chlorides: benzoyl chloride, 2,4-dichlorobenzoyl chloride or terephthaloyl chloride, TEA, dry dioxane, r.t., 7 hrs.

amines [27]; (iii) The reaction of aminoguanidine with ketones [28], β-diketones [29], enones [13] or pyrimidines [30]; (iv) The reaction of aryl amidoximes with compounds containing carboxylic group [9,16,31]; (v) One-pot three-component reaction of *o*-aminobenzhydrazide, 2-pyridine/pyrazinecarbonitrile and aromatic aldehydes [32]; (vi) One-pot Cu-catalyzed multicomponent reaction of benzylamine/aniline derivatives, trichloroacetonitrile, phenylacetylene and sodium azide [11]; (vii) Three-component reaction of carbodiimides, isocyanides, and trimethylsilyl azide [33]; (viii) Other miscellaneous methods [14,34,35].

Undoubtedly, cyanamide derivatives having both imino (NH) and cyano (CN) groups can undergo reactions with various nucleophilic and/or electrophilic reagents [36–40]. These findings motivated us in the present work to design and synthesize new carboximidamides **4-10** and 4,5-dihydro-1*H*-imidazol-2-amines **11,12** *via* reaction of cyanamides with different aliphatic amines using various solvents: water, *iso*-propanol and/or dioxane, (Scheme 1). The greatest advantage in this preparation method is that, all new obtained products having pyrimidinyl moiety in their structures linked directly with the target carboximidamide fragment, and they can undergo another substitution using different elec-

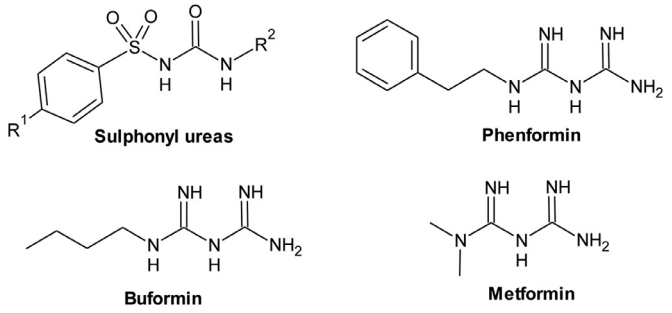


Fig. 1. Sulphonylureas and biguanides agents.

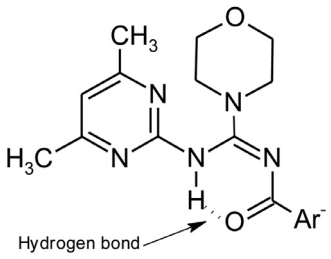


Fig. 2. Formation of hydrogen bond for compounds **14-16**.

trophiles. Thus the obtained carboximidamide **4** is subjected to react with benzoyl-, 4-methylbenzenesulphonyl- and/or terephthaloyl chloride, (Scheme 2). During our research into new antidiabetic agents, the new two carboximidamides **6,8** of the highest hypoglycemic effect were selected using online PASS Inet [41] and screened in *vivo* in mice as anti-hyperglycemic activity. Moreover, single crystal X-ray studies were performed to identify the molecular structure of the prepared compounds, and they provided us the essential chemical information such as; stereochemistry, bond distances, angles and packing of the molecules in the crystal [42].

2. Results and discussion

2.1. Chemistry

The starting materials, *N*-(pyrimidin-2-yl)cyanamides **1-3** were prepared according to the methods that were previously reported [38,43,44]. In the present work, we succeeded to synthesize some new carboximidamides **4-10** and 4,5-dihydro-1*H*-imidazol-2-amines **11, 12**, (Scheme 1). Reaction of *N*-(pyrimidin-2-yl)cyanamides **1-3** with 2-fold molar excess of *sec.* amines such as; morpholine and/or piperidine gave the corresponding *N*-(substituted pyrimidin-2-yl)morpholine-4-carboximidamides **4, 6** and/or *N*-(substituted pyrimidin-2-yl)piperidine-4-carboximidamide **5**, respectively. The reaction was carried out at room temperature *via* nucleophilic addition of NH group at cyano cyanamide group in water as a protic solvent. While, reaction of cyanamides **2, 3** with each of 1-(4-methoxyphenyl)piperazine as 1:1 molar ratio and/or piperazine as 2:1 molar ratio was achieved by refluxing for 8 hrs in *iso*-propanol to give the corresponding *N*-(substituted pyrimidin-2-yl)piperazine-1-carboximidamides **7, 8** and N^1,N^4-bis(substituted pyrimidin-2-yl)piperazine-1,4-dicarboximidamides **9, 10**, respectively. While, the reaction of compounds **2, 3** with ethylenediamine as bidentate nucleophile, gave *N*-(4,5-dihydro-1*H*-imidazol-2-yl)pyrimidin-2-amines **11** and **12**, respectively, (Scheme 1).

The structures of the newly synthesized compounds **4-12** were confirmed by their spectral IR, ^{1}H-, ^{13}C NMR and elemental analyses, (*see* **Experimental and Supplementary Material**). IR spectra of compounds **4-12** showed the absence of characteristic absorption band corresponding to C≡N group and appeared new bands for N-H group and C-H aliphatic. Their ^{1}H NMR spectra showed new signals corresponding to NH, methylene aliphatic protons in aliphatic region and other signals characteristic for each one. While ^{13}C NMR spectra showed new CH_2 groups at δ 24.7-66.4 ppm. *N*-(4,6-Dimethylpyrimidin-2-yl)morpholine-4-carboximidamide **(4)** was reacted with 4-methylbenzenesulfonyl chloride, benzoyl chloride, 2,4-dichlorobenzoyl chloride and/or terephthaloyl chloride in dry dioxane in the presence of triethylamine (TEA) to give the corresponding *N*-[4-methylbenzenesulfonyl]-*N'*-(4,6-dimethylpyrimidin-2-yl) morpholine-4-carboximidamide **(13)**, *N*-[(4,6-dimethylpyrimidin-2-ylamino)(morpholin-4-yl)methylidene]benzamide **(14)**, 2,4-dichloro-*N*-[(4,6-dimethylpyrimidin-2-ylamino)(morpholin-4-yl) methylidene]benzamide **(15)** and *N,N'-bis*-[(4,6-dimethylpyrimidin-2-ylamino)(morpholin-4-yl)methylidene]terephthalamide **(16)**, (Scheme 2).

The structures of the newly carboximidamides **13-16** were confirmed based on their spectral (IR, ^{1}H, ^{13}C NMR) and elemental analysis data, further confirmed by single-crystal X-ray crystallography for **15** (*see* **Experimental and Supplementary Material**). IR spectra of compounds **13-16** showed the absence of characteristic absorption band corresponding to N-H group and appeared new bands for C-H aromatic, while the characteristic bands for C=O and initial NH groups in compounds **14-16** did not appear due to the formation of intramolecular hydrogen bond (Fig. 2) as confirmed by X-ray single crystal results, (*see X-ray crystallography section*, Fig. 5). Their ^{1}H NMR spectra showed new signals corresponding to aromatic and methyl protons . Also ^{13}C NMR spectra showed the increasing of aromatic carbon signals.

Table 1
X-ray Diffraction details of compound **15**.

Crystal data	Compound 15
Chemical formula	$C_{18}H_{19}Cl_2N_5O_2$
M_r	408.29
Crystal system, space group	Monoclinic, $P2_1/n$
Temperature (K)	298
a, b, c (Å)	10.8590 (7), 15.5100 (8), 11.6550 (8)
β (°)	97.570 (3)
V (Å^3)	1945.9 (2)
Z	4
Radiation type	Mo $K\alpha$
μ (mm^{-1})	0.36
Crystal size (mm)	0.49 × 0.40 × 0.30
Refinement	
$R[F^2 > 2\sigma(F^2)]$, $wR(F^2)$, S	0.047, 0.072, 1.00
No. of reflections	1049
No. of parameters	244
H-atom treatment	H-atom parameters constrained
$\Delta\rho_{max}$, $\Delta\rho_{min}$ (e Å^{-3})	0.37, -0.32

2.2. X-ray crystallography

The X-ray crystallographic analysis revealed that, the compound **15** crystallized in a monoclinic system with four molecules in the unit cell, as given in Table 1. The molecular structure of the compound **15** is depicted as ORTEP view with the atom numbering scheme in Fig. 3. The results showed that the structure centered mainly on carboximidamide moiety linked with carbonyl group, where N13 attached with 4,6-dimethylpyrimidine, C5 with dichlorophenyl ring and the heterocyclic morpholine ring connected to C10.

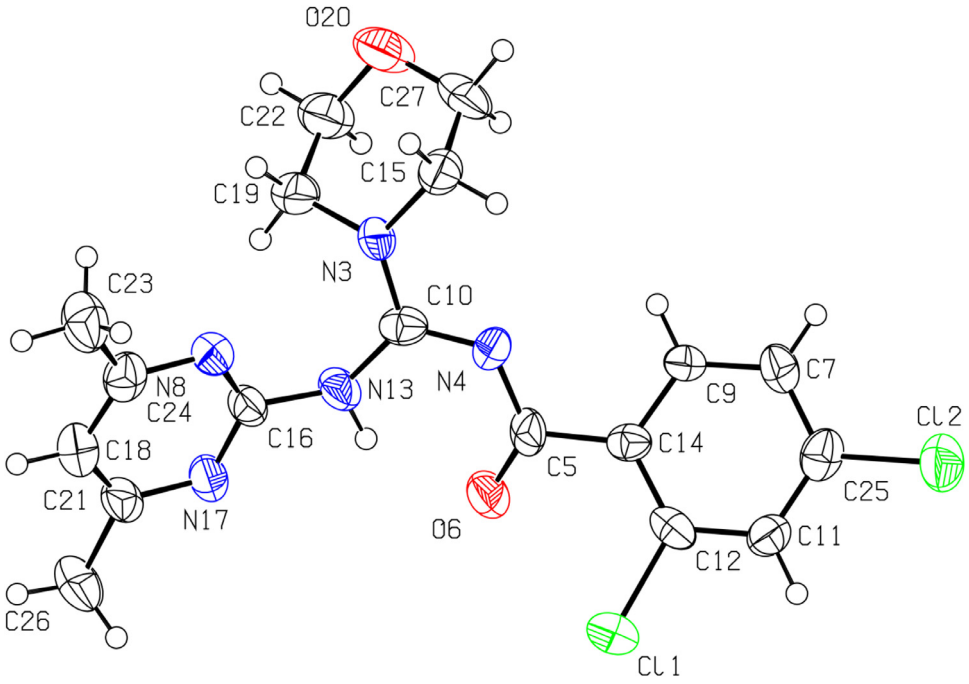


Fig. 3. A view of the structure of **15**, showing the atom-labelling scheme. Displacement ellipsoids are drawn at the 50% probability level.

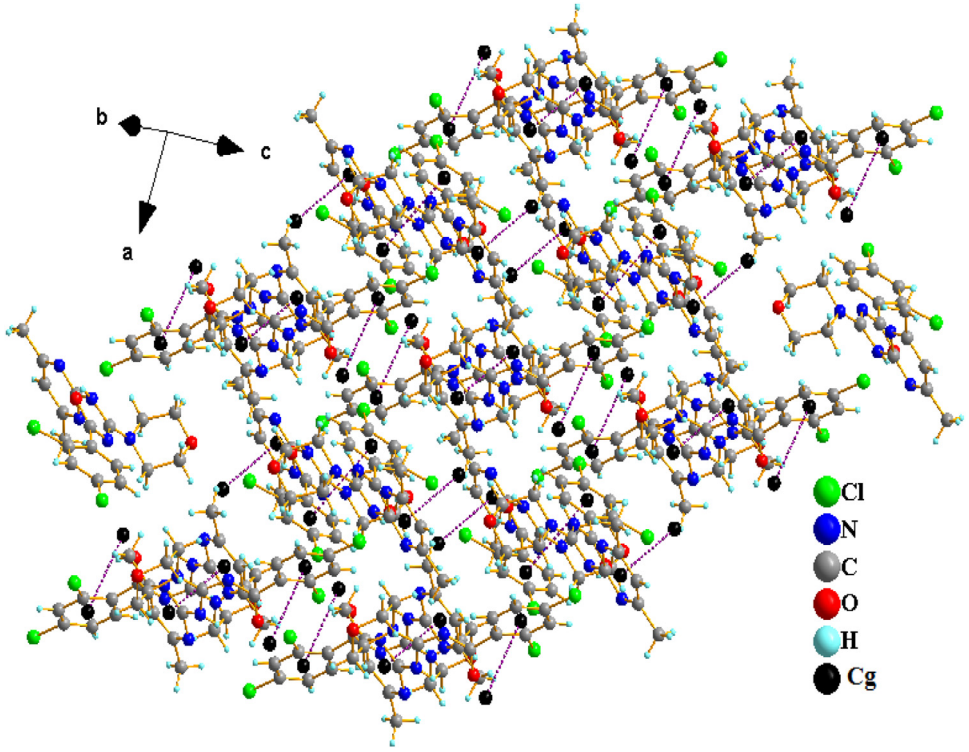


Fig. 4. A view of packing diagram shows the Cg-Cg interaction (dashed lines) of the title compound **15**.

Planar configuration of the individual rings of the structure was noticed, where the least-squares plane passing through N8-C24—C18- C21-N17-C16 (4,6-dimethylpyrimidine ring) and C14-C9-C7-C25-C11-C12 (dichlorophenyl ring) showed planar configuration, with maximum deviation of -0.022(7) Å for C14. However, morpholine ring revealed non planarity where O20 and N3 deviated 0.651(5) Å and 0.603(1) Å respectively from the plane passing through the other constituent atoms (C19- C15- C22-C27). Ring puckering and conformation analyses showed that the ring puckered and suggested chair conformation, as shown in Fig. 4 [45,46].

In the compound **15**, the crystal packing formed networks (Fig. 4), and no classic intermolecular hydrogen bonds were observed. However, intra-hydrogen-bonding interactions were noticed (Table 2). The crystal packing is further stabilized by a pi-ring Cg (I)...Cg (J) stacking intermolecular interactions, where Cg refers to the center of gravity of the ring, Cg(2) and Cg(3) refer to the rings 4,6-dimethylpyrimidine and dichlorophenyl, respec-

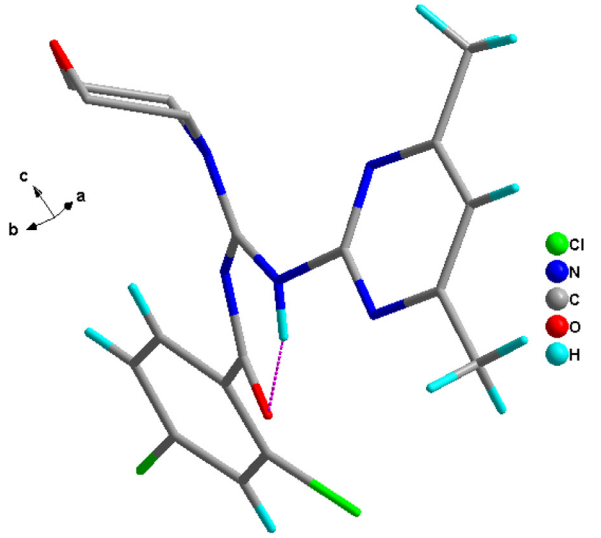


Fig. 5. A view of the compound **15** structure showing the chair configuration and H13..O6 intra hydrogen bond with dashed line (some hydrogen atoms are hidden for best view).

Table 2
Hydrogen-bond geometry (Å, °) for the title compound **15**.

D—H···*A*	*D*—H	H···*A*	*D*···*A*	*D*—H···*A*
N13—H13···O6	0.86	1.92	2.623 (10)	138
C19—H191···C16	0.98	2.52	3.132 (10)	120

tively. The distances 3.318(4) Å for Cg(2)-Cg(2)i and 3.908(4) Å for Cg(3)...Cg(3)ii (symmetry codes i= -x,-y,-z and ii= -x,1-y,-z) h (Fig. 4). No solvent molecules have been found in the crystal packing.

2.3. Biological activity

2.3.1. Blood glucose

Relative to the negative control, streptozotocin caused a highly significant ($p < 0.05$) increase in the level blood glucose. But, DMSO and compounds **6, 8** resulted in a highly significant ($p < 0.05$) decrease in the blood glucose level, in comparison with the positive control group. Relative to the negative control, DMSO and compounds **6, 8** resulted in a highly significant ($p < 0.05$) increase in the blood glucose level (Fig. 6).

The influence of injection of the above diabetic treated group for another four days with DMSO, compounds **6, 8** (repeated dose) and metformin for only one day on the blood glucose level was also examined. Relative to the negative control, streptozotocin (positive control), DMSO, compounds **6, 8** and metformin led to a highly significant increase ($p < 0.05$) in the blood glucose level. However, DMSO, compounds **6, 8** and metformin led to a highly significant decrease ($p < 0.05$) in the blood glucose level, in comparison with positive control. But, the decreased in the blood glucose level as a result of the effect of compound **8** was pronounced, which reduced the blood glucose level to the third, as compared with that of the positive control (Fig. 7).

2.3.2. Liver function

2.3.2.1. Serum ALT level. As indicated in Fig. 8, streptozotocin (positive control), DMSO and compounds **6, 8** caused a highly significant ($p < 0.05$) increase in serum level of ALT, relative to negative control. In comparison with positive control, DMSO and compounds **6, 8** caused a highly significant ($p < 0.05$) decrease in the serum level of ALT. It should be noted that, the serum level of ALT

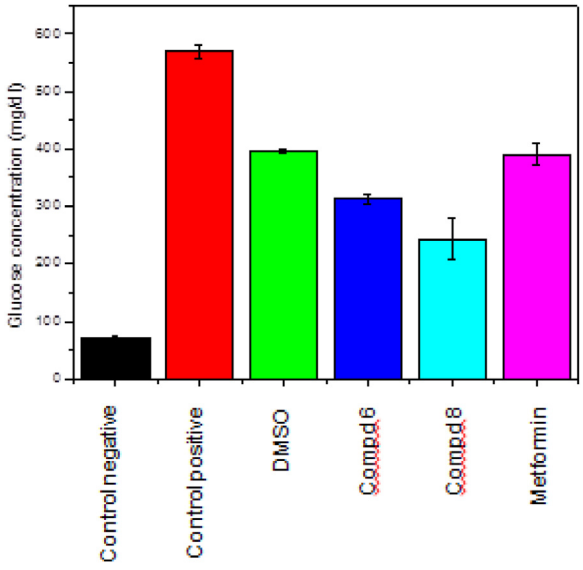


Fig. 6. Effect of DMSO, compounds **6, 8** (as a single dose) and metformin on glucose concentration (mg/dl) in the serum of albino rats (*Rattus rattus*).

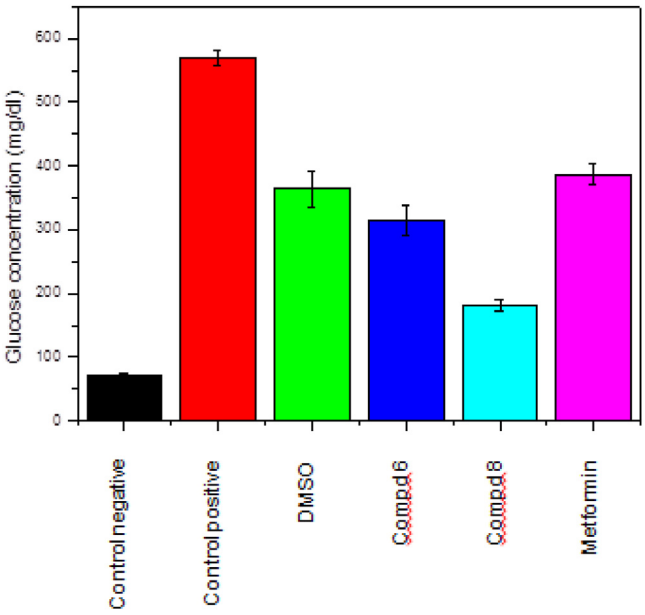


Fig. 7. Effect of DMSO, compounds **6, 8** (as repeated dose) and metformin on glucose concentration (mg/dl) in the serum of albino rats (*Rattusrattus*).

of positive control was very highly significant increase than negative control. Also, the influence of DMSO on the serum level of ALT was more pronounced. It caused a very highly significant ($p < 0.05$) decrease in the serum level of ALT than the positive control and the other compounds **6, 8**.

2.3.2.2. Serum aspartate transaminase (AST). As illustrated in Fig. 9, streptozotocin (positive control), DMSO and compounds **6, 8** resulted in a highly significant increase ($p < 0.05$) in the serum level of AST, relative to the negative control. But, these treatments (DMSO and compounds **6, 8**) resulted in a very highly significant ($p < 0.05$) decrease in the serum level of AST, relative to positive control (streptozotocin).

2.3.2.3. Serum triglycerides. Streptozotocin led to a very highly significant increase ($p < 0.05$) in the serum concentration of triglycerides, in comparison with the negative control. Also, DMSO led to

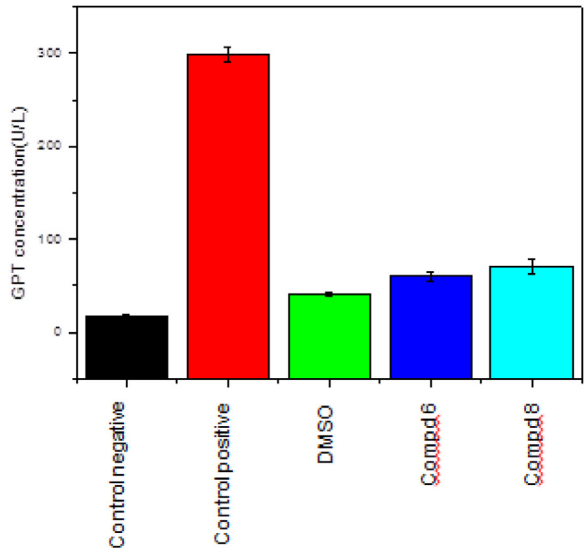


Fig. 8. Effect of DMSO and compounds **6, 8** (as repeated dose) on ALT concentration(U/L) in the serum of albino rats (*Rattusrattus*).

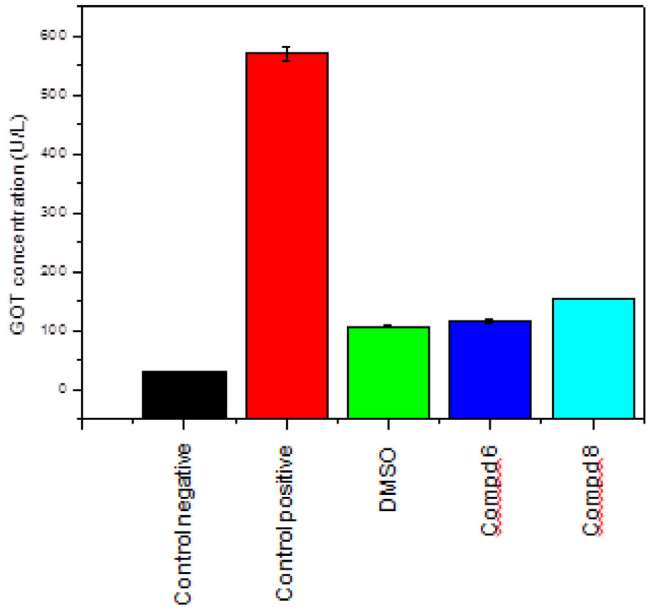


Fig. 9. Effect of DMSO and compounds **6, 8** (as repeated dose) on AST concentration(U/L) in the serum of albino rats (*Rattusrattus*).

a significant ($p < 0.05$) increase in the serum level of triglycerides, while compounds **6, 8** led to a significant ($p < 0.05$) decrease in the serum level of triglyceride, relative to that of the negative control. However, DMSO and compounds **6, 8** caused a very highly significant ($p < 0.05$) decrease in the serum level of triglyceride, relative to the positive control (Fig. 10).

2.3.2.4. Serum cholesterol. As illustrated in Fig. 11, streptozotocin, DMSO and compounds **6, 8** resulted in a highly significant ($p < 0.05$) increase in the serum level of cholesterol relative to the negative control. Also, DMSO and compounds **6, 8** resulted in a highly significant increase ($p < 0.05$) in the serum level of cholesterol relative to that of the positive control.

2.3.3. Kidney function

2.3.3.1. Serum urea level. Streptozotocin caused a very highly significant increase in the serum concentration of urea, relative to

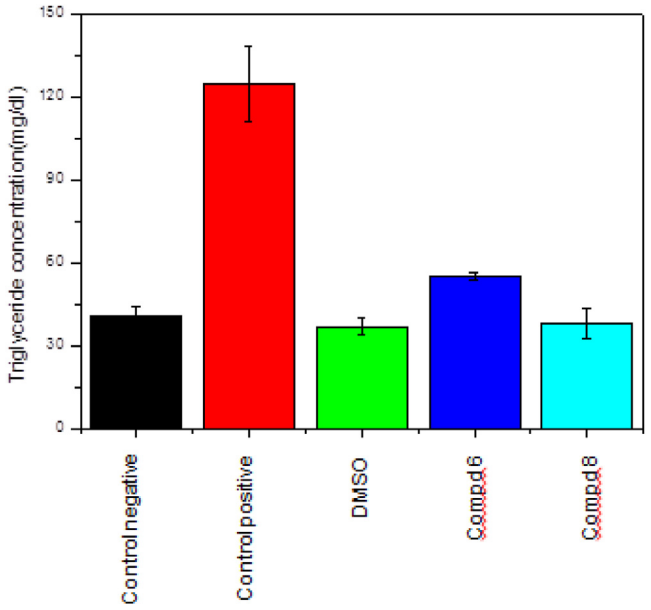


Fig. 10. Effect of DMSO and compounds **6, 8** (as repeated dose) on triglyceride concentration in the serum of albino rats (*Rattusrattus*).

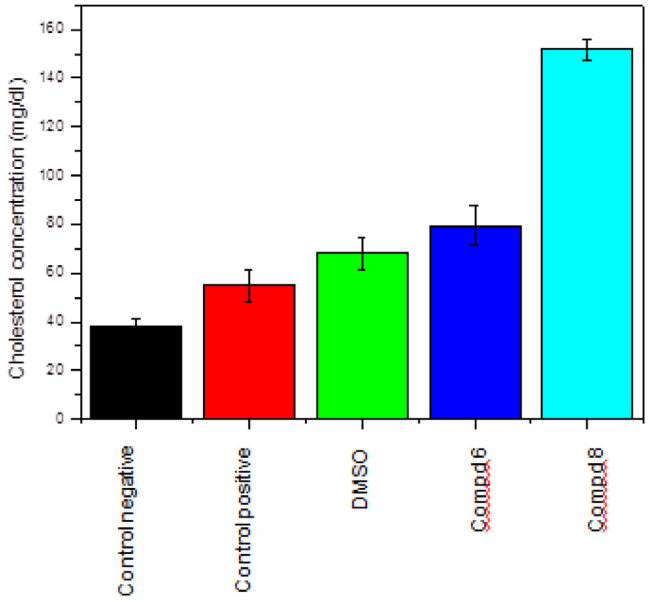


Fig. 11. Effect of DMSO and compounds **6, 8** (as repeated dose) on cholesterol concentration (mg/dl) in the serum of albino rats (*Rattusrattus*).

that of the negative control. Also, DMSO and compounds **6, 8** caused a significant ($p < 0.05$) increase in the serum level of urea relative to the negative control. However, DMSO and compounds **6, 8** resulted in a highly significant ($p < 0.05$) decrease in the serum level of urea, relative to the positive control (Fig. 12).

2.3.3.2. Serum creatinine. As indicated in Fig. 13, streptozotocin (positive control) resulted in a very highly significant ($p < 0.05$) increase in the serum level of creatinine, in comparison with that of the negative control. But, DMSO and compounds **6, 8** resulted in a significant ($p < 0.05$) increase in the serum level of creatinine, relative to the negative control, also. On the other hand, DMSO and compounds **6, 8** caused a highly significant ($p < 0.05$) decrease in the creatinine level, in comparison with the positive control (streptozotocin).

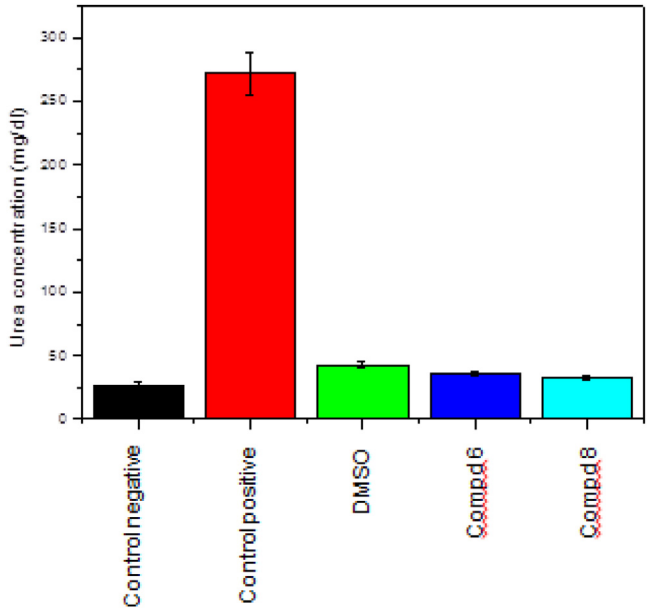


Fig. 12. Effect of DMSO and compounds **6, 8** (as repeated dose) on urea concentration (mg/dl) in the serum of albino rats (*Rattusrattus*).

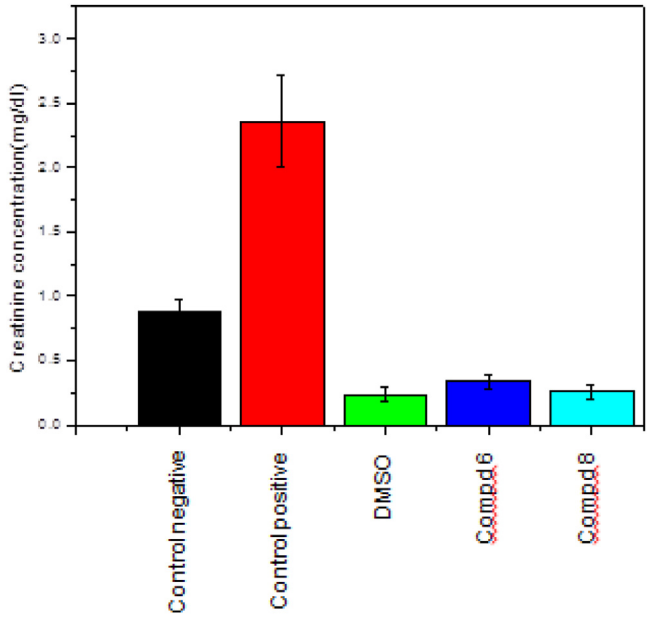


Fig. 13. Effect of DMSO and compounds **6, 8** (as repeated dose) on creatinine concentration (mg/dl) in the serum of albino rats (*Rattusrattus*).

3. Conclusion

A novel series of carboximidamides and 4,5-dihydro-1*H*-imidazol-2-amines was synthesized by the reaction of *N*-(pyrimidin-2-yl)cynamides with secondary amines and/or ethylenediamine. The utility of *N*-(4,6-dimethylpyrimidin-2-yl)morpholine-4-carboximidamide as building block for synthesis a novel class of *N*-acylated derivatives was also investigated. It should be noted that streptozotocin, as expected resulted in a highly significant increase in the blood glucose. Moreover, it resulted in a highly increase in the serum level of ALT, AST, urea, creatinine, triglyceride and cholesterol. On the other hand, treatments by DMSO and compounds **6, 8** caused a highly significant decrease in the serum level of parameters examined, except cholesterol which showed a significant increase under the effect of the treatments used in the current study, relative to that of the positive control.

4. Materials and methods

4.1. Chemistry

4.1.1. General information

All information about reagents, reactions, spectral apparatus: FT-IR, ^{1}H NMR, ^{13}C NMR and elemental analyses were recorded in **Supporting Information**.

4.1.2. General procedure for synthesis of compounds 4-6

A mixture of cyanamides **1, 2** (5 mmol) and secondary amines namely: piperidine and/or morpholine (10 mmol) was stirred in distilled water (50 mL) at room temperature for 12 hrs. The obtained crude product was filtered, washed with distilled water, dried and recrystallized from *iso*-propanol.

N-(4,6-Dimethylpyrimidin-2-yl)morpholine-4-carboximidamide (4):

Yield 85 %; white solid; m.p.: 193-195°C. IR (ATR) ν_{max} 3268, 3156, 3027, 2968, 2927, 2845, 1623 cm^{-1}. ^{1}H NMR δ: 9.39 (br. s, 2H, 2NH), 7.01 (s, 1H, $CH_{arom.}$), 3.69 (s, 4H, $2CH_2$), 3.63 (s, 4H, $2CH_2$), 2.42 (s, 6H, $2CH_3$). Anal. Calcd. for $C_{11}H_{17}N_5O$ (235.28): C, 56.15; H, 7.28; N, 29.77. Found: C, 56.03; H, 7.15; N, 29.62.

N-[4-(4-Methoxyphenyl)pyrimidin-2-yl]piperidine-1-carboximidamide (5):

Yield 81 %; white solid; m.p.: 196°C. IR (ATR) ν_{max} 3296, 3150, 3002, 2930, 2850, 1609 cm^{-1}. ^{1}H NMR δ: 8.42, 8.41 (d, J = 2.8 Hz, 1H, $CH_{pyrimidine}$), 8.30 (br. s, 2H, 2NH), 8.02, 8.00 (d, J = 7.8 Hz, 2H, $CH_{arom.}$), 7.18, 7.17 (d, J = 2.8 Hz, 1H, $CH_{pyrimidine}$), 7.07, 7.05 (d, J = 7.8 Hz, 2H, $CH_{arom.}$), 3.84 (s, 3H, OCH_3), 3.59 (s, 4H, 2($\underline{CH_2}$)-N), 1.62 (s, 2H, CH_2), 1.54 (s, 4H, $2CH_2$). ^{13}C NMR δ: 166.6, 163.1, 161.7, 158.0, 157.4, 130.1, 128.7, 114.6, 106.8, 55.8, 45.1, 26.0, 24.7. Anal. Calcd. for $C_{17}H_{21}N_5O$ (311.38): C, 65.57; H, 6.80; N, 22.49. Found: C, 65.81; H, 6.64; N, 22.58.

N-[4-(4-Methoxyphenyl)pyrimidin-2-yl]morpholine-4-carboximidamide (6):

Yield 83 %; beige solid; m.p.: 286-288°C. IR (ATR) ν_{max} 3334, 3165, 3045, 2964, 2899, 2857, 1605 cm^{-1}. ^{1}H NMR δ: 8.45, 8.44 (d, J = 5.0 Hz, 3H, $CH_{pyrimidine}$ + 2NH), 8.04, 8.02 (d, J = 8.4 Hz, 2H, $CH_{arom.}$), 7.24, 7.23 (d, J = 5.0 Hz, 1H, $CH_{pyrimidine}$), 7.07, 7.05 (d, J = 8.4 Hz, 2H, $CH_{arom.}$), 3.83 (s, 3H, OCH_3), 3.65-363 (m, 4H, $2CH_2$), 3.58-3.56 (m, 4H, $2CH_2$). ^{13}C NMR δ: 166.4, 163.2, 161.7, 158.1, 157.8, 130.0, 128.7, 114.6, 107.3, 66.4, 55.8, 44.7. Anal. Calcd. for $C_{16}H_{19}N_5O_2$ (313.35): C, 61.33; H, 6.11; N, 22.35. Found: C, 61.16; H, 6.32; N, 22.24.

4.1.3. General procedure for synthesis of compounds 7 and 8

An equimolar amount of cyanamides **2, 3** (5 mmol) and 1-(4-methoxyphenyl)piperazine (5 mmol, 0.96 g) was refluxed in iso-propanol (50 mL) for about 8 hrs. After completion of reaction (monitored by TLC), the reaction mixture was cooled to room temperature and the product was filtered, washed with *iso*-propanol. The products **7** and **8** were used without further purification.

4-(4-Methoxyphenyl)-N-[4-(4-methoxyphenyl)pyrimidin-2-yl]piperazine-1-carboximidamide (7):

Yield 82 %; beige solid; m.p.: 184°C. IR (ATR) ν_{max} 3314, 3163, 3039, 2992, 2956, 2904, 2830, 1610 cm^{-1}. ^{1}H NMR δ: 8.46, 8.45 (d, J = 4.7 Hz, 2H, $CH_{pyrimidine}$ + NH), 8.05, 8.03 (d, J = 8.8 Hz, 2H, $CH_{arom.}$), 7.24, 7.22 (d, J = 5.1 Hz, 1H, $CH_{pyrimidine}$), 7.08, 7.06 (d, J = 8.8 Hz, 2H, $CH_{arom.}$), 6.96, 6.94 (d, J = 9.0 Hz, 2H, $CH_{arom.}$), 6.86, 6.83 (d, J = 9.0 Hz, 2H, $CH_{arom.}$), 3.84 (s, 3H, OCH_3), 3.77-3.75 (t, J = 4.4 Hz, 4H, $2CH_2$), 3.70 (s, 3H, OCH_3), 3.4 (br. s, 1H, NH), 3.08-3.06 (t, J = 4.3 Hz, 4H, $2CH_2$). ^{13}C NMR δ: 166.2, 163.2, 161.8, 158.1,

157.5, 153.7, 145.7, 129.9, 128.8, 118.3, 114.9, 114.7, 107.3, 55.8, 50.2, 44.4, 44.3. Anal. Calcd. for $C_{23}H_{26}N_6O_2$ (418.49): C, 66.01; H, 6.26; N, 20.08. Found: C, 66.27; H, 6.18; N, 20.34.

Ethyl 2-{[imino(4-(4-methoxyphenyl)piperazin-1-yl)methyl] amino}-4-phenylpyrimidine-5-carboxylate (8):

Yield 79 %; white solid; m.p.: 174–176°C; IR (ATR) ν_{max} 3312, 3178, 3043, 2974, 2890, 2820, 1723, 1622 cm^{-1}. ^1H NMR δ: 8.83 (s, 1H, $CH_{pyrimidine}$), 8.57 (br. s, 2H, 2NH), 7.49-7.43 (m, 5H, $CH_{arom.}$), 6.97, 6.94 (d, J = 8.9 Hz, 2H, $CH_{arom.}$), 6.86, 6.83 (d, J = 8.9 Hz, 2H, $CH_{arom.}$), 4.11-4.05 (q, J = 7.1 Hz, 2H, $C\underline{H}_2CH_3$), 3.79-3.77 (t, J = 4.1 Hz, 4H, $2CH_2$), 3.70 (s, 3H, OCH_3), 3.07-3.05 (t, J = 4.1 Hz, 4H, $2CH_2$), 1.06-1.02 (t, J = 7.1 Hz, 3H, CH_3). ^{13}C NMR δ: 166.9, 166.2, 166.0, 160.4, 158.1, 153.8, 145.6, 139.4, 129.7, 128.8, 128.3, 118.4, 114.7, 113.3, 60.8, 55.7, 50.2, 44.2, 14.1. Anal. Calcd. for $C_{25}H_{28}N_6O_3$ (460.52): C, 65.20; H, 6.13; N, 18.25. Found: C, 65.39; H, 6.34; N, 17.97.

4.1.4. General procedure for synthesis of compounds 9 and 10

A mixture of cyanamides **2, 3** (5 mmol) and piperazine (2.5 mmol, 0.22 g) was refluxed in *iso*-propanol (50 mL) for about 8 hrs. After completion of reaction, the reaction mixture was cooled to room temperature and the product was filtered, washed with *iso*-propanol and recrystallized from *iso*-propanol - DMF.

N^1,N^4-Bis{[4-(4-methoxyphenyl)pyrimidin-2-yl]}piperazine-1,4-bis-carboximidamide (9):

Yield 69 %; white solid; m.p.: 280–282°C. IR (ATR) ν_{max} 3265, 3166, 3009, 2965, 2905, 1625 cm^{-1}. ^1H NMR δ: 8.46 (s, 2H, $2CH_{pyrimidine}$), 8.37 (s, 4H, 4NH), 8.05, 8.03 (d, J = 6.5 Hz, 4H, $4CH_{arom.}$), 7.23 (s, 2H, $2CH_{pyrimidine}$), 7.08, 7.07 (d, J = 6.5 Hz, 4H, $4CH_{arom.}$), 3.86 (s, 6H, $2OCH_3$), 3.72 (s, 8H, $4CH_2$). ^{13}C NMR δ: 166.4, 163.3, 161.8, 158.1, 157.6, 130.1, 128.7, 114.7, 107.3, 55.8, 43.9. Anal. Calcd. for $C_{28}H_{30}N_{10}O_2$ (538.60): C, 62.44; H, 5.61; N, 26.01. Found: C, 62.31; H, 5.37; N, 26.25.

Diethyl N^1,N^4-bis(carbonimidoylimino)piperazine-bis[(4-phenyl-pyrimidin-2-yl)-5-carboxylate] (10):

Yield 62 %; white solid; m.p.: 150–152°C. IR (ATR) ν_{max} 3370, 3140, 3057, 2982, 2930, 1723, 1624 cm^{-1}. ^1H NMR δ: 8.83 (s, 2H, $2CH_{pyrimidine}$), 8.56 (br. s, 4H, 4NH), 7.47 (s, 10H, $CH_{arom.}$), 4.09 (s, 4H, $2C\underline{H}_2CH_3$), 3.74 (s, 8H, $4CH_{2piperazine}$), 1.04 (s, 6H, $2CH_3$). ^{13}C NMR δ: 167.0, 166.2, 166.0, 160.3, 158.3, 139.5, 129.6, 128.7, 128.3, 113.5, 60.7, 43.7, 14.1. Anal. Calcd. for $C_{32}H_{34}N_{10}O_4$ (622.67): C, 61.72; H, 5.50; N, 22.49. Found: C, 61.88; H, 5.45; N, 22.43.

4.1.5. General procedure for synthesis of compounds 11 and 12

A mixture of cyanamides **2, 3** (5 mmol) and ethylenediamine (10 mmol, 0.68 mL) was refluxed in dioxane (50 mL) for about 7 hrs. After completion of reaction (monitored by TLC), the reaction mixture was cooled to room temperature and the product was filtered, washed with dioxane and recrystallized from *iso*-propanol.

N-(4,5-Dihydro-1H-imidazol-2-yl)-4-(4-methoxyphenyl) pyrimidin-2-amine (11):

Yield 62 %; beige solid; m.p.: 114–116°C. IR (ATR) ν_{max} 3246, 3058, 2953, 2883, 2835, 1625 cm^{-1}. ^1H NMR δ: 8.41-8.40 (d, J = 4.7 Hz, 1H, $CH_{pyrimidine}$), 8.06, 8.04 (d, J = 8.1 Hz, 4H, $2CH_{arom.}$ + 2NH), 7.20, 7.18 (d, J = 4.7 Hz, 1H, $CH_{pyrimidine}$), 7.07, 7.05 (d, J = 8.1 Hz, 2H, $2CH_{arom.}$), 3.84 (s, 3H, OCH_3), 3.57 (s, 4H, $2CH_2$). ^{13}C NMR δ: 166.9, 163.5, 163.2, 161.7, 158.1, 130.1, 128.8, 114.6, 107.2, 55.8, 42.0. Anal. Calcd. for $C_{14}H_{15}N_5O$ (269.30): C, 62.44; H, 5.61; N, 26.01. Found: C, 62.17; H, 5.70; N, 26.16.

Ethyl 2-(4,5-dihydro-1H-imidazol-2-ylamino)-4-phenylpyrimidine-5-carboxylate (12):

Yield 56 %; beige solid; m.p.: 136–138°C. IR (ATR) ν_{max} 3315, 3176, 3063, 2985, 2935, 2854, 1725, 1644 cm^{-1}. ^1H NMR δ: 8.78, 8.69 (2 s, 1H, $CH_{pyrimidine}$), 7.44 (s, 7H, $5CH_{arom.}$ + 2NH), 4.08-4.02 (q, J = 7.0 Hz, 2H, $C\underline{H}_2CH_3$), 3.57 (s, 2H, CH_2), 3.30 (s, 2H, CH_2), 1.04-1.00 (t, J = 7.0 Hz, 3H, CH_3). ^{13}C NMR δ: 168.5, 166.0, 164.1, 161.6, 160.3, 139.2, 129.6, 128.8, 128.1, 112.6, 60.6, 42.1, 14.1. Anal. Calcd. for $C_{16}H_{17}N_5O_2$ (311.33): C, 61.72; H, 5.50; N, 22.49. Found: C, 61.55; H, 5.58; N, 22.38.

4.1.6. General procedure for synthesis of compounds 13 - 16

A mixture of carboximidamide **4** (5 mmol, 1.18 g), triethylamine (5 mmol, 0.7 mL) and 4-methylbenzenesulfonyl-, benzoyl-, 2,4-dichlorobenzoyl chloride (5 mmol) and/or terephthaloyl chloride (2.5 mmol, 0.5 g) was stirred in dry dioxane (50 mL) at room temperature (except in case 4-methylbenzenesulphonyl chloride at 80°C) for about 7 hrs. After completion of reaction (monitored by TLC), the reaction mixture was added into distilled water and the formed product was filtered, washed with distilled water, dried and recrystallized from ethanol.

N-[4-Methylbenzenesulfonyl]-N'-(4,6-dimethylpyrimidin-2-yl)morpholine-4-carboximidamide (13):

Yield 74 %; beige solid; m.p.: 208–210°C. IR (ATR) ν_{max} 3244, 3079, 3017, 2975, 2903, 2852, 1600, 1259 cm^{-1}. ^1H NMR δ: 8.90 (br. s, 1H, NH), 7.53-7.51 (d, J = 7.5 Hz, 2H, $CH_{arom.}$), 7.16-7.14 (d, J = 7.5 Hz, 2H, $CH_{arom.}$), 6.76 (s, 1H, $CH_{pyrimidine}$), 3.62 (s, 4H, $2CH_2$), 3.45 (s, 4H, $2CH_2$), 2.27 (s, 3H, CH_3), 2.26 (s, 6H, $2CH_{3pyrimidine}$). ^{13}C NMR δ: 168.2, 157.5, 153.0, 142.0, 141.1, 129.3, 126.4, 114.2, 65.9, 47.4, 23.6, 21.2. Anal. Calcd. for $C_{18}H_{23}N_5O_3S$ (389.47): C, 55.51; H, 5.95; N, 17.98. Found: C, 55.59; H, 5.78; N, 17.78.

N-[(4,6-Dimethylpyrimidin-2-ylamino)(morpholin-4-yl)methylidene]benzamide (14):

Yield 76 %; beige solid; m.p.: 150–152°C. IR (ATR) ν_{max} 3055, 2986, 2918, 2870, 1603 cm^{-1}. ^1H NMR δ: 10.70 (s, 1H, NH), 8.03-7.42 (m, 5H, $CH_{arom.}$), 6.72 (s, 1H, $CH_{pyrimidine}$), 3.73 (s, 4H, $2CH_2$), 3.65 (s, 4H, $2CH_2$), 2.17 (s, 6H, $2CH_3$). ^{13}C NMR δ: 175.4, 168.0, 158.3, 154.4, 138.1, 131.7, 129.4, 128.2, 114.2, 66.3, 47.1, 23.5. Anal. Calcd. for $C_{18}H_{21}N_5O_2$ (339.39): C, 63.70; H, 6.24; N, 20.64. Found: C, 63.99; H, 6.01; N, 20.59.

2,4-Dichloro-N-[(4,6-dimethylpyrimidin-2-ylamino) (morpholin-4-yl)methylidene]benzamide (15):

Yield 79 %; brown crystal; 152–154°C. IR (ATR) ν_{max} 3019, 2959, 2924, 2842, 1602 cm^{-1}. ^1H NMR δ: 10.46 (br. s, 1H, NH), 7.86 (s, 1H, $CH_{arom.}$), 7.53–7.43 (m, 2H, $CH_{arom.}$), 6.74 (s, 1H, $CH_{pyrimidine}$), 3.69 (s, 4H, $2CH_2$), 3.62 (s, 4H, $2CH_2$), 2.23 (s, 6H, $2CH_3$). ^{13}C NMR δ: 173.2, 167.9, 158.4, 154.5, 136.8, 135.0, 133.2, 133.0, 130.0, 127.1, 114.3, 66.3, 47.1, 23.6. Anal. Calcd. for $C_{18}H_{19}Cl_2N_5O_2$ (408.28): C, 52.95; H, 4.69; N, 17.15. Found: C, 52.57; H, 4.71; N, 17.23.

N,N'-Bis-[(4,6-dimethylpyrimidin-2-ylamino)(morpholin-4-yl)methylidene]terephthalamide (16):

Yield 77 %; beige solid; m.p.: 258–260°C. IR (ATR) ν_{max} 3055, 2972, 2916, 2850, 1607 cm^{-1}. ^1H NMR δ: 10.68 (br. s, 2H, 2NH), 8.04 (s, 4H, $CH_{arom.}$), 6.72 (s, 2H, $2CH_{pyrimidine}$), 3.73 (s, 8H, $4CH_2$), 3.65 (s, 8H, $4CH_2$), 2.16 (s, 12H, $4CH_3$). ^{13}C NMR δ: 174.9, 168.0, 158.3, 154.7, 129.3, 128.9, 114.2, 66.8, 47.2, 23.5. Anal. Calcd. for $C_{30}H_{36}N_{10}O_4$ (600.67): C, 59.99; H, 6.04; N, 23.32. Found: C, 60.31; H, 6.14; N, 23.22.

4.2. X-ray single crystal diffraction

An appropriate crystal for single crystal X-ray study of compound **15** has been selected, and mounted onto thin glass fibers. X-Ray single crystal diffraction data were collected at room temperature (298 K) on an Enraf-Nonius 590 diffractometer with a Kappa CCD detector using graphite monochromated Mo-Kα (λ = 0.71073Å) radiation, at National Research Center, Egypt [47]. Reflection data has been recorded in the rotation mode using the ϕ and ω scan technique with 2θmax = 33. In the absence of significant anomalous scattering, Friedel pairs have been merged. Changes in illuminated volume were kept to a minimum, and were taken into account by the multi-scan inter-frame scaling [48,49].

Unit cell parameters were determined from least-squares refinement with θ in the range $2.910 \leq \theta \leq 33$. The structure of **15** was solved by direct methods using SHELX [50] implemented in CRYSTALS program suit [51]. The refinement was carried out by full-matrix least-squares method on the positional and anisotropic temperature parameters of all non-hydrogen atoms based on F^2 using CRYSTALS package [52]. All hydrogen atoms were positioned geometrically and were initially refined with soft restraints on the bond lengths and angles to regularize their geometry (C—H in the range 0.93–0.98 and N—H in the range 0.86–0.89) and Uiso(H) (in the range 1.2-1.5 times Ueq of the parent atom). Then, the positions were refined with riding constraints [53]. The general-purpose crystallographic tool PLATON [54] was used for the structure analysis and presentation of the results. The molecular graphics were done using DIAMOND [55] program. Details of the data collection conditions and the parameters of the refinement process are given in Table 1.

The full crystallographic information can be obtained free of charge using deposit number CCDC 1582055, via http://www.ccdc.cam.ac.uk/conts/retrieving.html or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: (+44) 1223-336-033; or e-mail: deposit@ccdc.cam.ac.uk.

4.3. Biological activity

4.3.1. Chemicals

Streptozotocin – induced diabetic drug and dimethyl sulphoxide (DMSO) were obtained from Merck Company Germany. *N*-[4-(4-Methoxyphenyl)pyrimidin-2-yl]morpholine-4-carboximidamide **6** and ethyl 2-{[imino(4-(4-methoxyphenyl)piperazin-1-yl)methyl] amino}-4-phenylpyrimid-ine-5-carboxylate **8** were prepared in our organic lab.

4.3.2. Experimental animals

This study was carried out on 30 male albino rats (*Rattus rattus*) approximately 8-10 weeks old, their weights ranging from 160-180 gm. The animals were obtained from the animal house of Zoology Department, Faculty of Science, Sohag University. They were housed in stainless cages at room temperature, five rats each and acclimatized to laboratory condition two weeks before the experiment and fed commercial pallet rat food. Food and water were available *add libitum*. Lightin221g cycle of 12 hours (light/dark) was also in consideration.

4.3.3. Experimental design

After the acclimation period, 25 rats were injected with streptozotocin for successive three days. On the fourth day the blood glucose were measured after two hours of injection, then experimental animals were randomly divided into 6 groups (5 rats each): ***Group 1 (G1)***, this group served as a negative control, received only water and food; ***Group2 (G2)***, This group served as a positive control which was injected with 50 mg/kg of streptozotocin; ***Group 3(G3)***, were injected with 1.5 ml of DMSO; ***Group 4 (G3)***, were injected with 50 ppm of compound **6; *Group5 (G5)***: were injected with 50 ppm of compound **8**.

After measuring the serum glucose in the four treated groups (G2,G3,G4 and G5), the four treated groups were injected again with the same treatments. On the fourth day, the blood glucose was measured. Thereafter, the sixth group was injected with 71 ppm of metformin. The day after (fifth), the animals of each group were scarified and the blood samples were collected in a hard glass tube, then allowed to clot for 30 minutes at room temperature, the serum was pipette off the clot and placed into clean tube and stored at -20°C until use.

4.3.4. Biochemical analysis

The activities of some biochemical parameters representing liver and kidney functions were measured in the blood serum of rats colorimetrically. The blood glucose concentration was measured using *Caraway & Walts* (1987) and *Howantiz & Howantiz* (1984) methods [56,57]; An alanine aminotransferase (ALT) and Aspartate Aminotransferase (AST) were measured using *Bergmeyer & Bernt* (1974) methods [58,59] and serum triglycerides concentration was measured according to the method of *Bablock et al.*, (1988) [60]. For kidney function, Serum urea level was measured according to the methods of *Kaplan* (1984) and *Tabacco et al.*, (1979) [61,62]; and serum creatinine level was measured according to *Bartles et al.* (1972) and *Cook* (1971) methods [63,64].

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

CRediT authorship contribution statement

Amr H. Moustafa: Conceptualization, Formal analysis, Visualization, Supervision, Writing - review & editing. **Walaa W. Ahmed:** Methodology, Data curation, Funding acquisition. **Ahmed Khodairy:** Project administration, Supervision, Writing - original draft, Supervision. **Ahmed F. Mabied:** Software, Formal analysis, Writing - original draft. **Ahmed Moustafa:** Investigation, Methodology, Supervision. **Mohamed F. El-Sayed:** Validation, Writing - original draft, Resources.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.molstruc.2021.129888.

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