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Chemistry, anti-diabetic activity and structural analysis of substituted dihydropyrimidine analogues

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ABSTRACT

In an effort to identify an anti-diabetic agent, a series of methyl/ethyl 4-(hydroxyphenyl)-6-methyl-2-oxo/thioxo-1,2,3,4 tetrahydropyrimidine-5-carboxylate analogues (4a-h) have been synthesized, purified, and characterized by using Fourier-Transform Infrared Spectroscopy (FT-IR) and NMR (^{1}H and ^{13}C). The synthesized compounds were screened for anti-hyperglycemic activity using Streptozotocin (STZ) induced diabetic rat model. The anti-hyperglycemic activity of dihydropyrimidine (DHPM) compound is mainly analyzed with the variation of substituents present on the phenyl ring and urea/thiourea group on pharmacophoric features. Further, the crystal structure and supramolecular characteristics of two compounds 4c and 4f were analyzed through a single-crystal X-ray method and the Hirshfeld Surface Analysis, which shows hydrogen bonding through N-H···O and N-H···S interactions with the formation of ring motif in the crystal structure. It is interesting to note that among the title compounds, the 4a, 4e, 4f, and 4g significantly displayed a better hypoglycemic effect *in vivo* rat model study.

1. Introduction

The metabolic complications like diabetes mellitus are increasing health problems of the modern world, and they are now one of the world's most prevalent non-communicable diseases. Type 2 diabetes mellitus (T2DM) is a disorder that causes hyperglycemia and glucose intolerance due to insufficient insulin secretion or insulin resistance. Complications resulting from insulin disorders such as cardiovascular disease, cerebrovascular disease, nephropathy, renal failure, ulceration, and amputation leads to lower life expectancy [1–4]. According to the International Diabetes Federation (IDF) Atlas report 2015, 415 million people were diagnosed worldwide and will increase to 642 million in 2040, according to prediction [5,6]. World Health Organization (WHO) has pointed out that the number of people with diabetes mellitus has quadrupled since 1980, and it is mainly type 2 diabetes mellitus (T2DM) [7]. Currently, in South East Asia (SEA), 39.3 million people are suffering from T2DM; this number will likely increase to 81.6 in 2025 estimated by epidemiologists [5]. Various factors have been identified to be responsible for the increased incidence of type II diabetes mellitus. They include obesity, which is a risk factor for type 2 diabetes mellitus along with high calories diet, an inactive lifestyle, both of which could precipitate the condition of hyperglycemia. In addition to diabetes, impaired glucose tolerance (IGT) has also been identified as a significant public health problem [6]. Nonetheless, as the disease burden gradually increases, there is a need for effective treatment and control of T2DM. Typically, pharmacological procedures are used to regulate T2DM by either reducing blood sugar levels or removing glucose from the gastrointestinal tract and insulin sensitivity [8]. However, it has been a challenging task to control blood glucose levels with oral hypoglycaemic agents such as sulfonylureas and thiazolidinediones, along with food, weight, and physical activity [9]. Currently, many orally administered hypoglycemic agents (OHAs) are used in addition to

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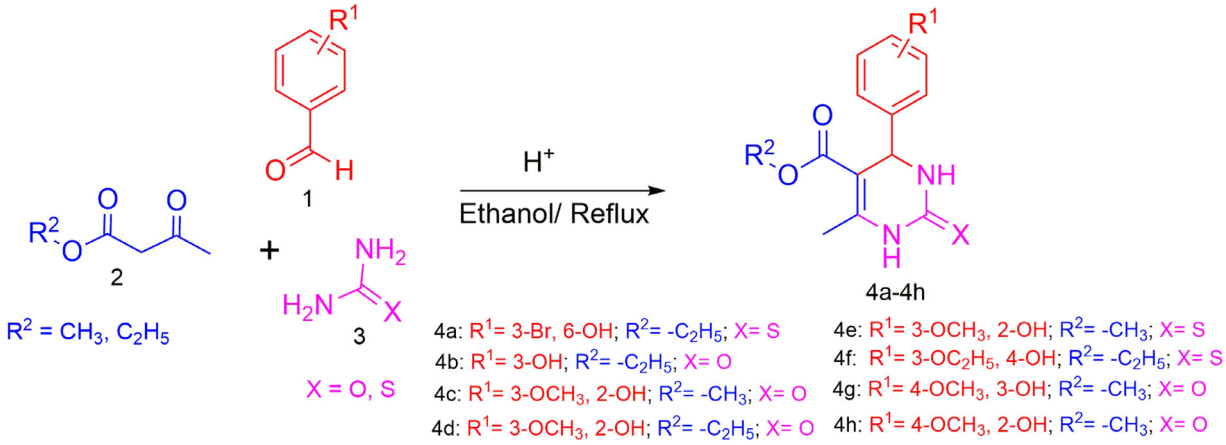

Scheme 1. The synthetic scheme followed for obtaining DHPM.

insulin to manage this scourge [10]. However, orally administered agents have many limitations, like adverse effects and high cost [11]. Besides, 30–40% of patients have a compliance problem [9]. In view of all these, there is need to formulate new drug molecules that will effectively reduce blood glucose with minimal side effects and enhance insulin level in the blood.

In this regard, we have formulated new DHPM derivatives by using the known synthetic method [12], with a view of adding an alternative agent to treat T2DM disease. The DHPM compounds have been reported to possess various pharmacological properties such as anti-microbial [13,14], antioxidant [15], anti-malarial [16,17], antitubercular [18–20], anti-mosquito [21], anti-diabetic [22], anthelmintic [23], anticancer [24], and larvicidal activity [25–27]. In our previous reports, we have remarked that the electron-withdrawing group on the phenyl part of dihydropyrimidine shows better larvicidal activity against *Anopheles arabiensis*. DHPM compounds are commercially available and widely used for the treatment of cardiovascular diseases such as hypertension [28], cardiac arrhythmias, and angina [29]. Besides, it is also known that dihydropyrimidine −5-carboxylate, also found in marine sources, play a potent role in HIVgp-120-CD4 inhibition [30]. Niguldipine, terazosin, and doxazosinare examples of commercially available DHPM drugs that are used in the treatment of benign prostatic hyperplasia (BPH) and cancers [31,32]. Streptozotocin (STZ) is an antibiotic that induces pancreatic islet β-cell destruction, which producing both type 1 diabetes mellitus (T1DM) and type 2 diabetes mellitus (T2DM) in a dose-dependent fashion [33,34]. Due to the significant biological activities observed with DHPM, the aim of this study is to synthesize a series of new DHPM derived compounds containing hydroxyl functional groups using the known synthetic method of Biginelli reaction (Scheme 1). Also, to screen these newly synthesized compounds for hypoglycemic activity using streptozotocin (STZ) -nicotinamide induced *in vivo* diabetic rat model for T2DM.

2. Materials and methods

2.1. Chemistry

All the chemicals for the synthesis were bought from Sigma-Aldrich and Tokyo Chemical Industry (TCI) Co., Ltd., whereas solvents were purchased from Fisher Scientific Corporation. The thin-layer chromatography (TLC) plate for analysis of reaction mixture was done on Merck aluminum plates (60 F_{254}) coated with silica gel. The product formed was confirmed by examining the spots on the TLC plate under ultra-violet (UV cabinet, BTI 1429) irradiation (Bio Technico India, Biotech, BTI-49) at 254 and 366 nm. The Merck silica gel of mesh size 100–120 was used for column chromatography to purify the product. Heidolph rotary evaporator was used to extract the solvent from the reaction mixture. The obtained compound has dried under vacuum oven (Bio Technics India). The melting points of synthesized compounds were determined using the DBK instrument, 230 V. The functional group present in the compounds were identified using Bruker FT-IR spectrophotometer (Bruker IFS 55 equinox). The purity of compounds was confirmed using Bruker NMR spectrometer ^{1}H (400 MHz) and ^{13}C (101 MHz) in DMSO solvents using TMS as an internal standard. The chemical shifts were recorded in ppm δ scale, coupling constant (J) values are given in hertz (Hz). The crystal structural data were collected on the Bruker D8 VENTURE Kappa Duo PHOTON II CPAD diffractometer with Mo micro-focused tube diffraction source (Mo Kα λ = 0.7103 Å), and data were refined using SHELXL 2018/3. The general method of synthesis and spectral information are available in the electronic supporting information file (ESI).

2.2. General experimental method for synthesis of DHPMcompounds(4a-4h)

A combination of equimolar substituted hydroxy aryl aldehyde (0.0125 mol), urea/thiourea (0.0125 mol) and β-ketoester (0.0125 mol) was taken in a 100 mL round bottom flask and refluxed into 30 mL of ethanol solvent with constant stirring, in the presence of concentrated hydrochloric acid in catalytic amount for overnight (24 h). The reaction mixture was monitored using TLC plate; after ensuring that the reaction contents were poured into ice-cold water. The obtained white precipitate was filtered and washed several times with distilled water to get the crude product.

The crude product and small amount of silica and DCM were added together and mixed homogenously for column chromatography. The column chromatography was performed using silica gel with the combination of ethyl acetate:pet-ether solvents (1:10) as an eluent. The purified eluent mixture was collected in a round bottom flask and evaporated under a rotary evaporator to obtain pure product and dried under a vacuum oven. The percentage yield of the compound obtained was more than 65%. Furthermore, a slow evaporation method was used to get the single crystal by dissolving the minimum amount (5 mg) of a compound in different solvents and characterized using different spectrophotometry techniques. The physicochemical constants of the substituted methyl/ethyl 4-(hydroxyphenyl)-6-methyl-2-oxo/thioxo-1,2,3,4 tetrahydropyrimidine-5-carboxylate analogues (4a-h) were listed in Table 1.

Table 1
The physicochemical constants of thesubstituted methyl/ethyl 4-(hydroxyphenyl)-6-methyl-2-oxo/thioxo-1,2,3,4 tetrahydropyrimidine-5-carboxylate analogues (4a-h).

Compound code	R^1	R^2	X	m.p. (°C)	(%) Yield
4a	3-Br, 6-OH	C_2H_5	S	95	80.52
4b	3-OH	C_2H_5	O	165	72.35
4c	3-OCH_3, 2-OH	CH_3	O	152	68.89
4d	3-OCH_3, 2-OH	C_2H_5	O	145	81.00
4e	3-OCH_3, 2-OH	CH_3	S	137	91.20
4f	3-OC_2H_5, 4-OH	C_2H_5	S	170	75.50
4g	4-OCH_3, 3-OH	CH_3	O	172	79.38
4h	4-OCH_3, 2-OH	CH_3	O	158	78.80

2.3. Ant-diabetic activity

2.3.1. Type 2 model of diabetes mellitus induction

This study was conducted with the use of Streptozotocin (STZ), an agent that is known experimentally to induce both type 1 diabetes mellitus (T1DM) and type 2 diabetes mellitus (T2DM) in a dose-dependent fashion in animal models [35]. Newly synthesized dihydropyrimidine compounds were evaluated for their anti-hyperglycemic activities in STZ induced diabetic mice according to the methods of Ghasemi et al., 2014 [36]. Diabetes was induced in mice after an overnight fast by a single intraperitoneal (IP) injection of nicotinamide (120 mg/kg body weight, dissolved in normal saline) 15 min before IP administration of STZ (50 mg/kg body weight, dissolved in citrate buffer, pH 4.5) (Sigma Aldrich, USA) [37]. The blood glucose levels were measured before and 72 h after nicotinamide-STZ injection, for confirmation of hyperglycemia and type 2 diabetes development. The mice that have a serum glucose level above 200 mg/dL, as well as with polydipsia, polyuria, and polyphagia, were selected and equally distributed into different diabetic treatment groups.

2.3.2. Experimental design

Male 57BL/6 mice (25–30 g) were purchased from Theodor Bilharzias Center, Cairo, Egypt. All experimental protocols were by the guidelines and standards of animal care as approved by the Ethical Committee for animal handling at Zagazig University (EC-AHZU). Mice were kept in cages at 20 ± 4 °C temperature with 12 h light/dark cycle and given free access to water and food. Animal's selection was performed randomly. Animals were allocated into 11 groups ($n = 6$); group 1 animals were administered with 0.5% CMC (0.5 mL) (vehicle); group 2 were the streptozotocin (STZ)/nicotinamide diabetic control (DC) group and untreated, group 3, diabetic animals were treated with gliclazide 50 mg/kg and act as a reference drug group. The rest of the groups of the diabetic animals were given the newly synthesized dihydropyrimidine compounds (50 mg/kg orally, daily for seven days). At the end of the experiment, their blood samples were collected after an overnight fast via retro-orbital plexus under ether anesthesia, and the level of fasting serum glucose was measured using diagnostic strips.

2.3.3. Data analysis

Data are hereby presented as mean ± standard deviation (SD). Anti-hyperglycemic were compared with control and standard drug treatment. One-way ANOVA followed by Dunnett's multiple comparisons test was performed using Graph Pad Prism version 8.2.0 for Windows, Graph Pad Software, San Diego, California USA. Also, Paired *t*-test was used to compare the percentage reductions in blood glucose levels amongst newly synthesized compounds. Levels of significance was determined at $p < 0.05$ -$p < 0.01$.

2.4. Crystal growth and single-crystal X-ray crystallographic study

The minimum amount (5 mg) of the synthesized (4a-h), purified compound was taken in 5 mL beaker and dissolved in different solvents and kept for a slow evaporation process at the optimum temperature to achieve good quality single crystals. The obtained single crystals were analyzed through Polarized Optical Microscope (Nikon H600L). We have successfully obtained good single crystal for compounds 4c and 4f for which single-crystal X-ray study was carried out. A Bruker D8 VENTURE Kappa Duo PHOTON II CPAD diffractometer was used to measure the single-crystal X-ray data. Intensity measurement was performed at 100 K temperature (using liquid nitrogen) with Mo micro-focused tube diffraction source (MoKα $\lambda = 0.7103$ Å). Data collection, integration, unit cell measurement, and crystal data absorption correction were performed using Bruker Apex III software [38], and data reduction was made by Bruker SAINT [39]. Direct technique SIR 2014 [40] has been used to solve crystal structures, and complete matrix least square method SHELXL 2018 [41] has been used to refine with WinGX (version 2018/3) application suit [42]. The absorption correction was implemented using SADABS. The crystallographic and refinement detail of compounds 4c and 4f are shown in Table 2.

2.5. Hirshfeld Surface analysis

The Hirshfeld surfaces and two dimensional (2D) fingerprint plots were developed using *Crystal Explorer* 17.5 application to visualize the intermolecular interaction in the crystal structure of 4c and 4f molecule [43]. On the Hirshfeld surfaces, distance d_e and d_i show the distances from the surfaces to the external nucleus and surface to the internal nucleus, respectively. The normalized contact distance (d_{norm}) appears on the surface as a white-red-blue color scheme, where the bright red spot indicates the shorter contact, the white area represents contacts around the van der Waals distances, and the blue region is uncontacted.

3. Results and discussion

3.1. Synthesis and reaction mechanism for the title compounds

The present research includes the synthesis of title compounds in ethanol as a solvent by the multi-component reaction between hydroxy aryl aldehyde, β-ketoester, and urea/thiourea, as shown in Scheme 1. The purity and structural elucidation of compounds (4a-4h) were revealed by using FT-IR, ^{1}H NMR, ^{13}C NMR. The peak range at 3300–3100 cm^{-1} and 1710–1640 cm^{-1} in FT-IR confirms the presence of N-H and amide/ester carbonyl group, respectively. The peak range at $\delta = 2.23$–2.28 ppm and $\delta = 2.28$–3.7 ppm in ^{1}H NMR confirms the presence methyl group at dihydropyrimidine and an ester methyl group, respectively. The methine hydrogen on the dihydropyrimidine ring is shown at the peak range of $\delta = 5.04$–5.50 ppm. The peak range at $\delta = 8.65$–9.86 ppm confirms the presence of the N-H hydrogen atom in the compound. The peak range at $\delta = 165$–176 ppm in ^{13}C NMR confirms the presence of carbonyl carbon in the title compounds.

3.2. Anti-diabetic activity

Results of hypoglycaemic activities of newly synthesized dihydropyrimidine analogues are presented in Fig. 1. These agents were given in equal dose as the standard drug (STD), gliclazide. Statistical analysis of the data obtained by one-way analysis of variance shows that there is a significant difference ($p < 0.01$) between the means of the treatment groups and the STZ group, indicating hypoglycaemic activity. Also, from the results, it shows that all

Table 2
Single crystal X-ray data and refinement detail for the compounds 4c and 4f.

DATA	4c	4f
Formula	$C_{14}H_{16}O_5N_2$	$C_{16}H_{20}O_4N_2S$
Formula weight	280.7	336.41
CCDC	2020234	2020235
Temperature/K	100	100
Wavelength(Å)	0.71073	0.71073
Crystal system	Monoclinic	Monoclinic
Space group	$P2_1/c$	$P2_1/c$
a(Å)	11.6727(74)	10.9797(6)
b(Å)	11.6512(65)	17.0410(11)
c(Å)	20.0564(7)	8.4730(4)
α (°)	90	90
β (°)	92.338(24)	107.125(2)
γ (°)	90	90
V (Å^3)	2725.42(39)	1515.05(10)
Z', Z	2,8	1, 4
Density (g cm^{-3})	1.39	1.47
μ (mm^{-1})	0.101	0.237
F (000)	1196.0	712
θ (min, max)	2.5, 28.8	2.4, 30.5
$h_{min,\ max,}\ k_{min,\ max,}\ l_{min,\ max.}$	(−15, 15)(−15, 13)(−26, 25)	(−13, 15)(−23, 21)(−12, 11)
No. of refl.	31722	20845
No of unique ref./Obs. ref.	7022/5543	4551/3592
No. parameters	534	256
$R_{all,}\ R_{obs}$	0.098, 0.076	0.080, 0.062
$wR_{all,}wR_{obs}$	0.174, 0.163	0.200, 0.184
$\Delta\rho_{min,\ max}$ (eÅ^{-3})	−0.35, 0.59	2.06, 0.93
G.O.O.F.	1.139	1.054

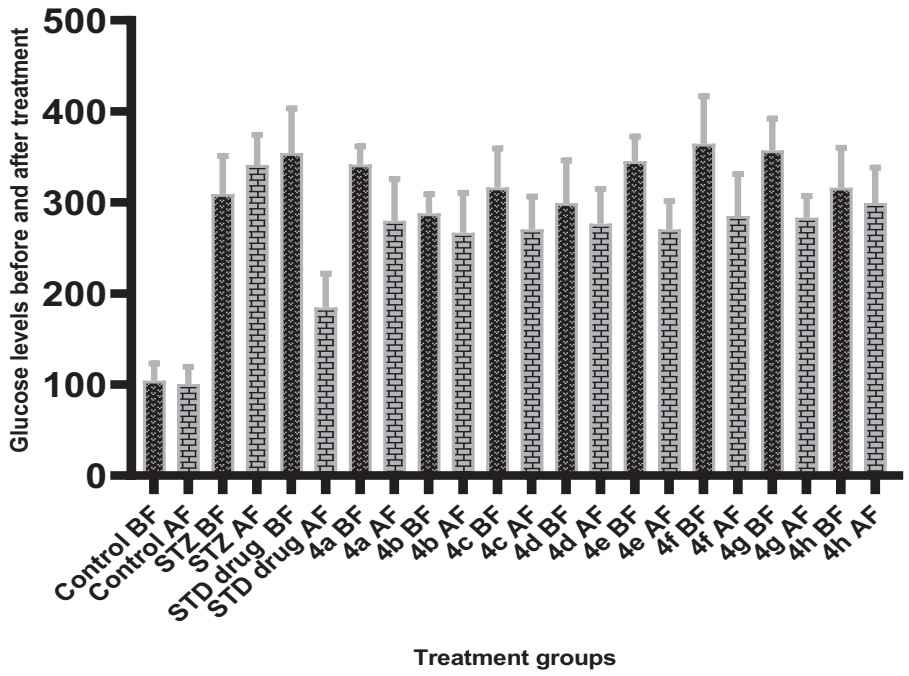

Fig. 1. Anti-diabetic activity of 4a-h on induced hyperglycemia in mice in comparison to STZ and STD (Standard drug).

the newly synthesized dihydropyrimidine compounds had an effect on the blood glucose levels post diabetic induction. The standard drug at the dose of 50 mg/kg showed a better lowering effect compared to the newly synthesized compounds (dose by dose). However, as represented in Fig. 2, the compounds 4a, 4e, 4f, and 4g significantly ($p < 0.05$) reduced the blood glucose levels when compared with STZ treated group and with other newly synthesized compounds. The study indeed has shown that the newly synthesized compounds of dihydropyrimidine possess hypoglycaemic potentials. The mean percentage reduction obtained for gliclazide was 47.9%, whereas that of 4a, 4e, 4f, and 4g were 18.06, 21.66, 21.76, and 20.64, respectively. Therefore, the attenuation of STZ induced hyperglycemia by these new compounds is a clear indication that they have the potential to lower high blood glucose levels. The details of the analysis result are listed in Table-S1 (ESI).

3.3. Analysis of the crystal structure of compounds 4c and 4f

Rod-shaped single crystals of 4c were obtained from Ethanol solvent by slow evaporation method at room temperature (Fig. S9). The crystal size of 0.26 × 0.18 × 0.10 mm^3 was chosen for single-crystal X-ray diffraction analysis, which shows that 4c crystallizes

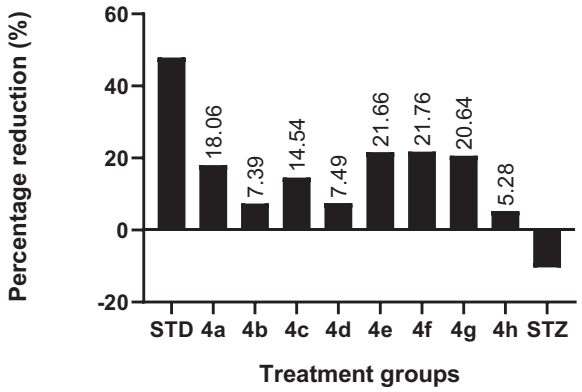

Fig. 2. Showing percentage reduction of glucose levels in diabetic induced mice by 4a-h in comparison to STZ and STD.

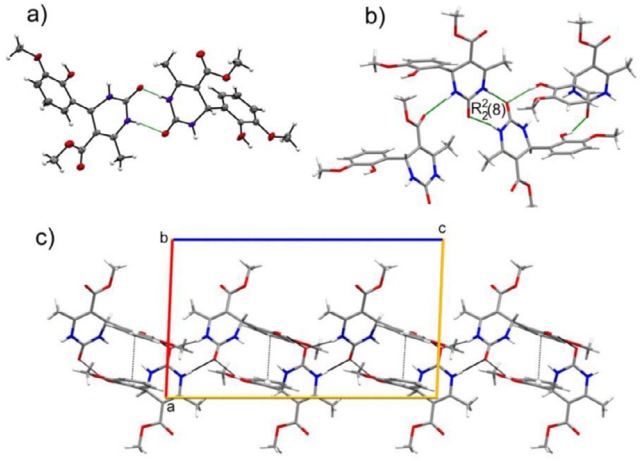

Fig. 3. (a) Oak Ridge Thermal Ellipsoid Plot (ORTEP) of compound 4c drawn at 50% probability) N–H···O hydrogen bonds form a dimer in the crystal packing)$\pi\cdots\pi$ interactions in the crystal packing along *bc* plane.

in the monoclinic space group $P2_1/c$ with eight molecules in the unit cell and two molecules in the asymmetric unit (Z=8, Z'=2). The dihedral angles between the planes of the six-membered dihydropyrimidine ring with its 2-hydroxy 3-methoxyphenyl ring and ester substituents are observed at 70.85° (4) and 16.75° (5), respectively for one molecule, whereas 89.57° (3) and 12.12° (12) are observed for the second molecule (Fig. 3a). In the crystal structure, pyrimidine carbonyl oxygen is involved in the generation of significant dimeric structure through two N-H···O hydrogen bonds, N2-H2N···O6(2.13 Å, 172°) and N4-H4N···O1 (2.09 Å, 168°) with the formation of $R_2^2(8)$ graph-set cyclic ring motif [44] (Fig. 3b). Further, the results of the dimer form chain which propagates along the *b*-axis. Besides the hydrogen bonding, the $\pi\cdots\pi$ interactions between the benzene ring of two individual DHPM molecule consolidate the molecular packing, which also strengthens the crystal packing along the *bc* plane (Fig. 3c). The crystallographic refinement details and intermolecular interactions are listed in Table-2 and Table-3, respectively.

The compound 4f crystallizes with block-shaped single crystals, which were obtained from ethanol solvent by slow evaporation method at room temperature (Fig. S10). Crystal size of $0.25\times0.14\times0.09 mm^3$) was taken for a single-crystal X-ray study, which crystallizes in the monoclinic space group $P2_1/c$ with four molecules in the unit cell and one molecule in the asymmetric unit (Z=4, Z'=1). The dihedral angle between hydropyrimidine ring with 4-hydroxy-3-ethoxy phenyl ring and ester group are observed at 80.31(0.8)° and 29.84(17)°, respectively (Fig. 4a). In the crystal structure, the N-H···S hydrogen bonds N1-H1···S1 [2.52 Å, 162°] and N2-H2···S1 [2.62 Å, 170°] form close ring dimer with graph set motif $R_2^2(8)$ (Fig. 4b). The 4-hydroxy hydrogen atom form hydrogen bond with ester oxygen through O4-H4A···O1 [2.14 Å, 130°] and form $R_2^2(20)$ ring motif. The molecule also forms C-H···π through C16-H16B···π [2.88 Å, 123°] and $\pi\cdots\pi$ interactions between the phenyl ring, which propagate along the *b*-axis (Fig. 4c). Further, the crystal packing also strengthens by C7-H7A···O4 [2.58 Å, 155°] and C13-H13···O2 [256 Å, 149°] intermolecular interaction in the crystal structure. The prominent intermolecular interaction present in the molecule is tabulated in Table 3.

3.4. Hirshfeld surface analysis

The Hirshfeld surface and 2D fingerprint plot were used to understand the role of intermolecular interactions present in their crystal structure. Here, we have developed the Hirshfeld surface for the compounds 4c and 4f to analyze the intermolecular interactions present in the molecule (Fig. 5a and 5b). The compound 4c shows red spot is due to N-H···O, O-H···O, C-H···O hydrogen bonding in the molecule, whereas S-H···O, O-H···O, C-H···O hydrogen bonding in 4f. Further, the existence of intermolecular interactions is clearly shown by the sharp spike in 2D fingerprint plots for both the compounds (Fig. 5c and 5d). The fingerprint plots show the H···H intermolecular contacts contribute relatively high for both the molecules, 45% and 47.5% for the compounds 4c and 4f, respectively (Fig. 5c and 5d). The percentage contribution of other intermolecular interactions in the title compound 4c are as follows: O···H/H···O (30.1%), C···H/H···C(16.3%), N···H/H···N(3%), C···C(2.5%), C···O/O···C(1.8%), O···O(1%), O···N/N···O(0.2%) and for the compound 4f are as follows: O···H/H···O(16.1%), S···H/H···S(15.3%), C···H/H···C(11.3%), C···O/O···C(3.2%), C···C(2.4%), N···H/H···N(2.3%), O···N/N···O(0.9%), O···O(0.9%). Fig. 6 showed that after H···H interactions, 4c shows O···H interaction as the major contribution, whereas 4f prefers equal contribution of O···H and S···H interactions in their molecular assembly.

The Cambridge Structural Database (CSD) (Conquest version 5.4; CCDC version 5.40) [45] shows that the crystal structures of the series of molecules (Scheme 1) were not yet reported elsewhere. However, similar type of other DHPM derivatives was reported [12]. Using urea and thiourea moiety, we have searched in CSD and observed that there are 121 DHPM has been reported. However, at different positions on the benzene ring, only the crystal structure of twenty DHPM derivatives has been reported with hydroxyl substituent. It is commonly observed that the DHPM with a hydroxyl group at 4th position on the benzene ring are crystallized with water and form hydrate in their crystal structures [46,47] whereas, the hydrate form was not being observed in the hydroxyl group at 2nd and 3rd position on the benzene ring of DHPM [48,49]. All the DHPM have shown a similar kind of synthon and form dimmer through N-H···O and N-H···S interaction. It is noted that only five crystal data on the benzene ring of DHPM are recorded with 2–hydroxy and all the compound form dimer through N-H···O interaction with one N-H bond and other N-H bond is free of any interaction [50,51]. However, in the reported crystal 4c and 4f, the $\pi\cdots\pi$ interaction is present in the crystal system, which is being absent in the CSD reported DHPM crystal. It is further noted the 4-OH with 3-ethoxy on benzene ring containing DHPM derivative not form dimer [52]; however, the dimer is observed in the reported crystal 4f through N-H···S short contact.

Analyzing the variation in their substituent's, it is observed that the reduction of glucose level from the blood is mainly influenced by the following reasons (i) thiourea analogues (4a, 4e, and 4f) showed prominent glucose reduction compared to other compounds (4b, 4c, 4d, and 4h); (ii) The *para*-methoxy and *meta*-

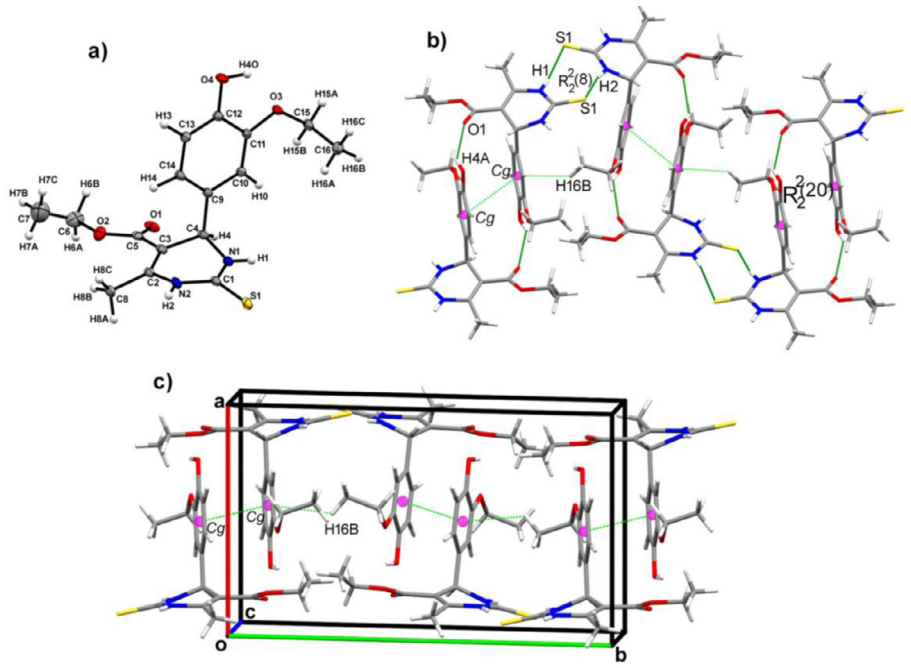

Fig. 4. (a) Oak Ridge Thermal Ellipsoid Plot (ORTEP) of compound 4f drawn at 50% probability, (b) the N–H···S hydrogen bonds and O–H···O form ring motif in the crystal structure, (c) the C–H···π connected through $\pi\cdots\pi$ interactions in the crystal packing along *b*-axis.

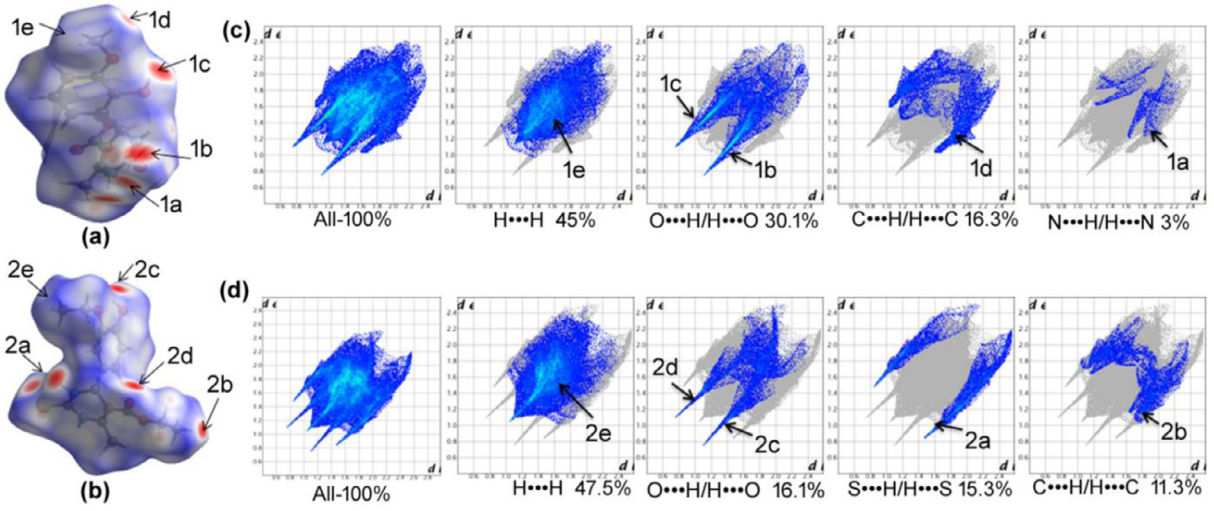

Fig. 5. (a) Hirshfeld surface of the compound 4c mapped over d_{norm}. (b) Hirshfeld surface of the compound 4f mapped over d_{norm}. (c) and (d) Two-dimensional fingerprint plots and relative contributions of various interactions to the Hirshfeld surface of the compounds 4c and 4f, respectively.

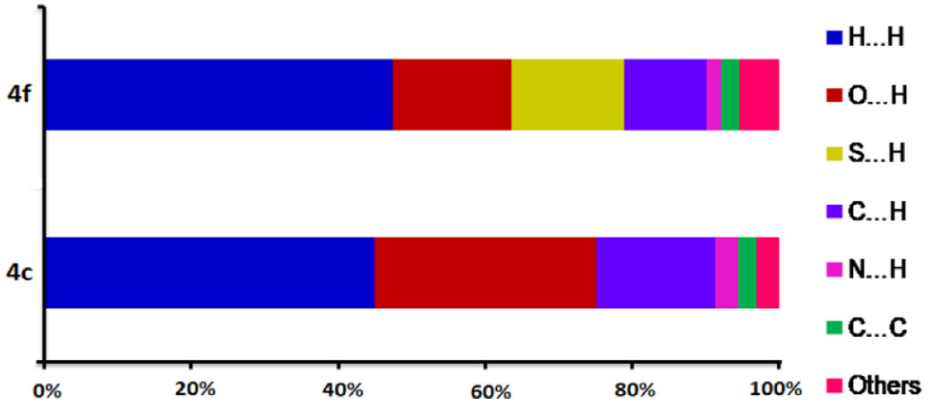

Fig. 6. Relative contributions of various intermolecular contacts to the Hirshfeld surface area in 4c and 4f.

Table 3
Intermolecular interaction of the title compounds 4c and 4f.

D-X···A	D-X(Å)	X···A(Å)	D···A(Å)	<D-X···A(°)	Symmetry code
4c					
N1-H1N···O7	0.85(3)	2.47(3)	3.268(3)	158(2)	x,y,z
N2-H2N···O6	0.84(3)	2.13(3)	2.965(3)	172(3)	1-x,1-y,-z
N3-H3N···O7	0.93(3)	2.54(3)	3.383(3)	150(3)	1-x,−1/2 + y,1/2-z
N4-H4N···O1	0.80(3)	2.09(3)	2.872(3)	168(3)	1-x,1-y,-z
O4-H4O···O6	0.85(4)	2.01(4)	2.826(3)	160(3)	1-x,1/2 + y,1/2-z
O9-H9O···O1	0.85(4)	2.08(4)	2.873(3)	154(3)	1-x,−1/2 + y,1/2-z
$\pi\cdots\pi$	–	3.79(5)	–	–	1- x,−1/2 + y,1/2-z
π= centroid between six member of C8-C9-C10-C11-C12-C13 and C22-C23-C24-C25-C26-C27 phenyl ring					
4f					
N1-H1···S1	0.80	2.52	3.2895(3)	162	x,1/2-y,−1/2 + z
N2-H2···S1	0.83	2.62	3.4405(2)	170	x,1/2-y,1/2 + z
O4-H4A···O1	0.90	2.14	2.7958(2)	130	1-x,-y,1-z
C7-H7A···O4	0.98	2.58	3.4889(2)	155	1-x,−1/2 + y,3/2-z
C13-H13···O2	1.01	2.56	3.4696(2)	149	1-x,-y,2-z
C16-H16B···π	0.98	2.88	3.5050(2)	123	x,1/2-y,−1/2 + z
$\pi\cdots\pi$	–	3.877	–	–	1-x,-y,1-z

π= centroid between six members of C9-C10-C11-C12-C13-C14.

hydroxy group, along with urea-based DHPM (4g) showed better anti-diabetic activity than others, however, it is lower activity than 4e and 4f in the absence of sulfur (thiourea) moiety; (iii) bromine at *meta* position instead of methoxy/methoxy group and a hydroxyl group at *ortho* position along with thiourea (4a) showed little reduction in its activity in comparison to 4e and 4f; (iv) ethyl or methyl substitution in ester part of this DHPM (4a-h) appears has no influence in its anti-diabetic activity. From their crystal structure analysis, 4f showed nearly same percentage of O···H (16.1%) and S···H (15.3%) interactions whereas, 4c preferred the dominate O···H (30.1%) interactions which clearly suggest that sulfur, because of large size and high polarizability in comparison to oxygen plays an important role for the enhancement of anti-diabetic activity.

4. Conclusion

A series of eight DHPM compounds (4a-h) have been synthesized and characterized successfully. The single-crystal study showed that 4c prefers to form dimer through strong N-H···O hydrogen bonding whereas, the crystal 4f form dimer through N-H···S hydrogen bonding. Both the crystal structures (4c and 4f) shows $\pi\cdots\pi$ interaction to consolidate the molecular packing, whereas 4f showed additional C-H···π interaction. The Hirshfeld surface analysis confirms the major contribution of H···O and S···H contacts in the crystal structure, which played a significant role to explain the strength and nature of intermolecular interaction associate in the crystal structure. Current structure-activity comparisons study revealed that the S···H interactions of thiourea derivative play major role in a significant lowering of glucose level in blood.. Further studies are required to improve the anti-diabetic activity and correlate the structure-activity relationship.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Keshab M. Bairagi: Conceptualization, Methodology, Software, Writing - original draft. **Nancy Safwat Younis:** Methodology, Validation, Formal analysis, Investigation. **Promise Madu Emeka:** Software, Validation, Formal analysis, Resources. **Katharigatta N. Venugopala:** Conceptualization, Methodology, Investigation, Resources, Supervision. **Osama I. Alwassil:** Methodology, Formal analysis, Investigation. **Hany Ezzat Khalil:** Formal analysis, Investigation, Resources. **Ekta Sangtani:** Software, Resources. **Rajesh G. Gonnade:** Data curation, Investigation. **Viresh Mohanlall:** Formal analysis, Methodology, Resources, Investigation. **Susanta K. Nayak:** Supervision, Project administration, Funding acquisition, Writing - original draft, Writing - review & editing.

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