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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Green synthesis and 3D pharmacophore study of pyrimidine and glucoside derivatives with in vitro potential anticancer and antioxidant activities

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Abstract

A facile and an efficient one-pot green synthesis of pyrimidine derivatives using the environmentally friendly Cerium(IV) ammonium nitrate (CAN) as catalyst and water as a solvent has been described. Some of the synthesized pyrimidines react with 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide in ethanol containing potassium hydroxide to give *S*-glucoside derivatives. The structures of the newly synthesized compounds were elucidated on the basis of their spectral and elemental analyses. In addition, selected derivatives of the products were screened for their anticancer activities against four tumor cell lines using MTT assay and the results showed that some of these compounds have potent cytotoxic effect, as concluded from their IC_{50} values. Molecular modeling studies including generation of a 3D pharmacophore model were carried out. The study showed high correlation with the experimental results. Antioxidant activity of the synthesized products was also investigated and most of them showed potent activity.

Keywords Pyrimidine · Cerium (IV) ammonium nitrate · *S*-glucoside · Pharmacophore · Anticancer · Antioxidant

Introduction

Pyrimidine derivatives gained great attention in the field of biological and pharmaceutical applications during the past decades owing to their remarkable anticancer (Geng et al. 2018; Chikhale et al. 2018; Tian et al. 2017), anti-inflammatory (Abdelgawad et al. 2018), antiviral (Okesli et al. 2017) antibacterial (Abdelghani et al. 2017; Salem et al. 2016; 2014; Maddila et al. 2016), antifungal (Zhang et al. 2016; Behalo 2009), antimalarial (Mane et al. 2014), and analgesic (Dinakaran et al. 2012) activities. Heterocycles bearing glucoside moiety were reported to exhibit potential pharmacological applications like antimicrobial (Corona et al. 2018), anticancer (Adiyala et al. 2018; Seo et al. 2017), anti-inflammatory (Abdelkhalek et al. 2017; Duarte et al. 2018) and antioxidant (Bozunovic et al. 2018) activities. On the other hand, Cerium (IV) ammonium nitrate (CAN) was reported to be used as an inexpensive and easily available catalyst of various organic reactions (Jaiprakash et al. 2008; Nikishin et al. 2017; Alaouia et al. 2018; Rodriguez et al. 2017; Kamal et al. 2011; Boddeti et al. 2012). Recently, CAN has been used in the synthesis of substituted 1,4-naphthoquinone derivatives by a carbon–carbon bond formation (Huang et al. 2017), substituted furans and oxadiazoles by carbon–oxygen bond formation (Undeela et al. 2014; Behalo 2016) and substituted pyrroles by carbon-nitrogen bond formation (Kamal et al. 2016). In light of these considerations and in continuation of our interest for synthesis of heterocycles and evaluations of their biological activities (Salem et al. 2016; 2014; 2008; 2007; Behalo et al. 2014; 2017; Madkour et al. 2001), the present work aims to develop a green and efficient method to synthesize new pyrimidine derivatives through Biginelli reaction using CAN as a catalyst, in

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addition to prepare pyrimidine-glucoside hybrids and evaluate their anticancer and antioxidant activity.

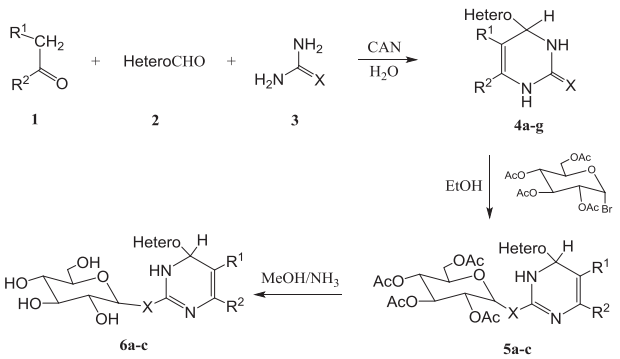
Results and discussion

Chemistry

Multicomponents reaction of ethyl acetoacetate with thiophene-2-carboxaldehyde and thiourea in the presence 10^{-4} mol of CAN as a catalyst and water as safe solvent afforded ethyl-6-methyl-4-(thiophen-2-yl)-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (**4a**) in good yield through Biginelli type reaction. The structure of product **4a** was confirmed on the basis of its elemental analyses and spectral data where IR spectrum showed absorption bands at 3420–3210 cm^{-1} and 1719 cm^{-1} corresponding to NH and CO, respectively. ^1H NMR showed signals at 10.46, 9.76, and 5.42 ppm attributed for 2NH and CH protons. Also, mass spectrum [Salem et al. 2013; 2012] showed molecular ion peak at $m/z = 282$ (M^+, 100%). We generalize the reaction conditions to synthesize several examples of pyrimidine derivatives with the aim of obtaining more potent antitumor and antioxidant molecules. Thus, treatment of heterocyclic carboxaldehyde (thiophene-2-carboxaldehyde and/or pyridine-3-carboxaldehyde) with a variety of active methylene compounds namely, ethyl acetoacetate, acetylacetone or 1,3-diphenyl-1,3-propandione and urea derivatives (urea, thiourea and guanidine) afforded pyrimidine derivatives **4a–g** (Scheme 1). The structural formulae of all products were confirmed on the basis of their elemental analyses and spectral data.

Based on the principles of green chemistry, we carried out the reactions in water owing to its use as inexpensive, nontoxic, nonflammable as well as green media. The reaction was performed also using CAN as a catalyst that provides shorter reaction times, better yields and simple work-up than conventional procedures.

Glucoside derivatives have occupied a unique position in the field of pharmaceutical applications [Ishioka et al. 2019, Chen et al. 2018]. Thus, glycosylation of pyrimidine derivatives **4e–g** with 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide was performed by both microwave irradiation and conventional method to afford the glucoside derivatives **5a–c** with better yields in case of microwave irradiation. The structures of the products were confirmed by IR spectra that showed absorption bands of CO at 1743–1662 cm^{-1}. Also, ^1H NMR spectra showed signals of acetyl groups and anomeric protons at 2.10–2.30 and 4.20–4.60 ppm. Synthesis of deacetylated glucoside derivatives **6a–c** was achieved upon treatment of glucoside derivatives **5a–c** with methanolic ammonia at room temperature, (Scheme 1). Treatment of pyrimidine **4a** with



4	R1	R2	Hetero	X
a	CH_3CH_2OCO	CH3	2-Thienyl	S
b	CH_3CH_2OCO	CH3	3-Pyridyl	S
c	CH_3CO	CH_3	2-Thienyl	S
d	CH_3CO	CH_3	3-Pyridyl	NH
e	CH_3CO	CH_3	3-Pyridyl	S
f	C_6H_5CO	C_6H_5	2-Thienyl	O
g	C_6H_5CO	C_6H_5	2-Thienyl	S

5, 6	a	b	c
Hetero	3-Pyridyl	2-Thienyl	2-Thienyl
X	S	O	S
R1	$COCH_3$	C_6H_5CO	C_6H_5CO
R2	CH_3	C_6H_5	C_6H_5

Scheme (1): Synthesis of pyrimidines 4a–g and their glucoside derivatives

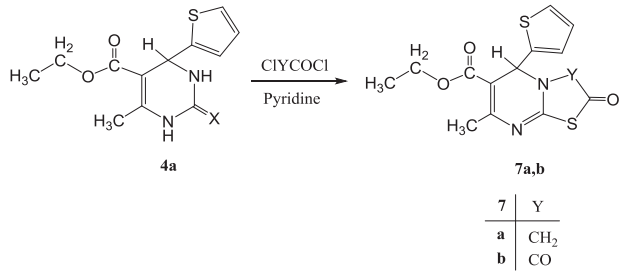
chloroacetylchloride or oxaloylchloride in pyridine afforded thiazolopyrimidine derivatives **7a,b** (Scheme 2).

On the other hand, treatment of thiophene-2-carboxaldehyde or pyridine-3-carboxaldehyde with ethyl cyanoacetate and thiourea in the presence of CAN gives pyrimidine nitriles **8a,b**. Alkaline hydrolysis of the pyrimidines **8a,b** by potassium hydroxide afforded the corresponding acid **9a,b**, disappearance of absorption band of nitrile at 2259 cm^{-1} and 2215 cm^{-1} confirm the conversion process of nitrile group to the corresponding acid, (Scheme 3).

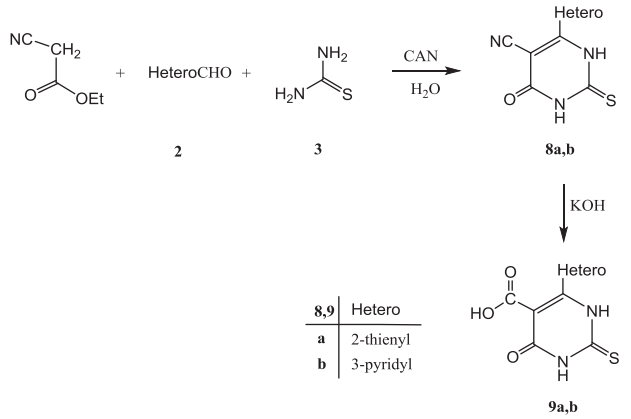
Similarly, treatment of thiophene-2-carboxaldehyde with 5,5-dimethylcyclohexane-1,3-dione (dimedone) and thiourea (or guanidine) afforded pyrimidines **10a,b**. Stirring of pyrimidines **10a,b** with thiophene-2-carboxaldehyde in a mixture of water and ethanol (60:40%) at room temperature gave compounds **11a,b** in excellent yield. Finally, treatment of pyrimidine derivatives **11a,b** with chloroacetylchloride or oxaloylchloride in pyridine afforded thiazolopyrimidine derivatives **12a,b** and **13a,b**, respectively (Scheme 4).

Cytotoxicity assay

Four human tumor cell lines namely; hepatocellular carcinoma (HePG-2), mammary gland (MCF-7), Colorectal carcinoma (HCT-116) and Human prostate cancer cell line (PC3) were used to determine the inhibitory effects of compounds on cell growth using the 3-[4,5-dimethylthiazole-2-yl]-2,5-diphenyl tetrazolium bromide (MTT) assay (Table 1). This colorimetric assay is based on the conversion of the yellow tetrazolium bromide to a purple formazan derivative by mitochondrial succinate dehydrogenase in viable cells [Mosmann 1983; Denizot, Lang 1986].



Scheme 2: Reaction of pyrimidine **4a** with chloroacetylchloride and oxaloylchloride



Scheme 3: Synthesis of tetrahydropyrimidine-5-carboxylic acid derivatives **9a,b**

Antiproliferative assay in vitro

The results of the cytotoxic activity in vitro were expressed as the IC50–concentration of the compound (in μM) that inhibits proliferation of the cells by 50% as compared to the untreated control cells. The IC values were separately calculated for each experiment and the mean values ± SD were calculated [c.f. supplementary data]. The results on the antiproliferative activity of pyrimidine derivatives are summarized in Table 1, along with the data of the anticancer drug, 5-fluorouracil (5-FU). The Cytotoxicity test of the pyrimidine derivatives and their chemically modified analogs indicated a synergistic effect of the substituents at the pyrimidine core structure. Among the synthesized compounds which exhibited very strong antiproliferative activity is **11a** with IC50: 5.56,7.88 μg against colorectal carcinoma (HCT-116) and the hepatocellular carcinoma (HepG-2) cell lines, respectively, which are comparable with the reference anticancer drug 5-FU with IC50: 8.10 and 9.90 μg for HepG-2 and HCT-116 cell lines, respectively. Compounds **4b** and **10b** also showed a similar comparable trend with 5-FU with a very strong antitumor effect on Human prostate cancer (PC3) and the hepatocellular carcinoma (HepG-2) cell lines with IC50: 8.94 and **9.39** μg respectively. On the other hand, compound **7a** showed a strong anticancer activity against all four carcinoma cell lines tested in this study with IC50: 15.47, 15.79,

Scheme 4: Synthesis of pyrimidine derivatives **10a, b–13a,b**

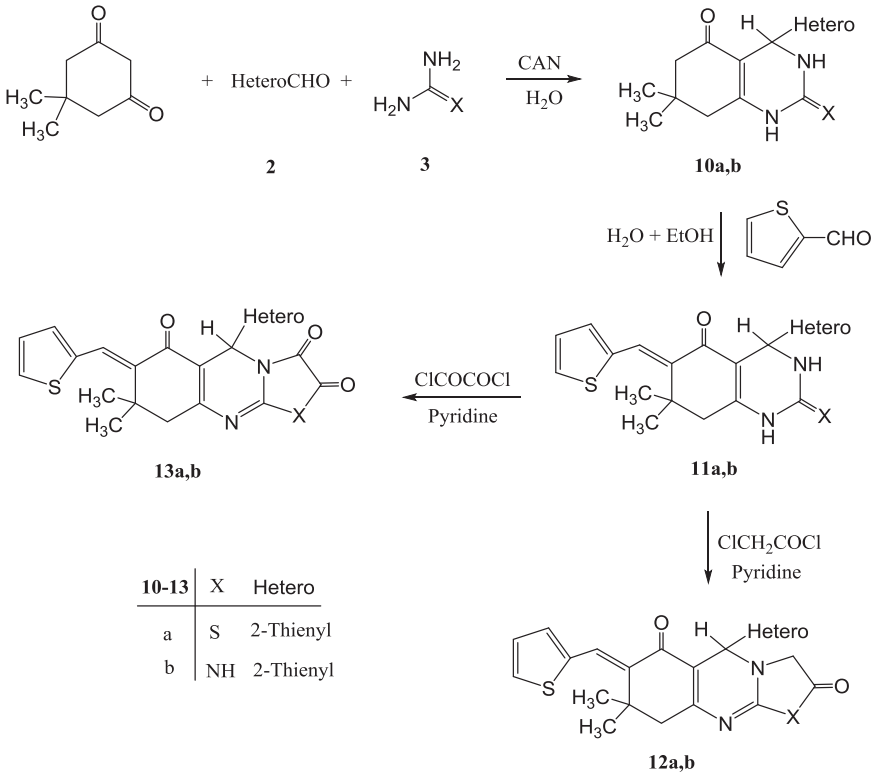


Table 1 Cytotoxic activity of some compounds against human tumor cells

Compounds	In vitro cytotoxicity IC_{50} (μg/mL)[a]			
	HePG2	MCF-7	HCT-116	PC3
5-FU	8.1 ± 0.24	6.3 ± 0.11	9.9 ± 0.20	11.0 ± 0.27
4a	83.5 ± 4.68	93.9 ± 5.64	97.1 ± 5.34	84.8 ± 5.41
4b	12.33 ± 1.3	18.05 ± 1.6	11.01 ± 1.1	8.94 ± 0.8
4e	>100	>100	>100	>100
4f	26.9 ± 1.87	42.4 ± 2.88	32.3 ± 3.10	35.9 ± 2.85
4g	55.38 ± 4.1	81.93 ± 5.1	74.73 ± 4.8	62.88 ± 4.6
5a	28.35 ± 2.3	32.12 ± 2.4	45.02 ± 3.2	35.16 ± 1.7
6a	33.12 ± 2.1	35.17 ± 2.2	40.09 ± 2.6	36.23 ± 3.5
6b	46.53 ± 2.1	38.23 ± 2.5	50.21 ± 2.7	42.15 ± 1.5
7a	16.34 ± 1.7	19.91 ± 1.8	15.79 ± 1.4	15.47 ± 1.5
7b	34.65 ± 3.1	35.77 ± 3.5	42.04 ± 3.7	45.93 ± 3.9
8a	74.6 ± 4.38	86.7 ± 5.15	61.0 ± 4.32	71.0 ± 4.56
8b	24.36 ± 2.4	29.80 ± 2.7	22.99 ± 1.9	32.52 ± 2.9
9a	26.7 ± 2.04	23.6 ± 1.31	20.6 ± 1.16	40.0 ± 3.68
10a	26.90 ± 2.2	38.39 ± 2.9	20.00 ± 1.7	32.39 ± 2.5
10b	9.39 ± 0.9	24.02 ± 2.0	10.51 ± 1.1	14.92 ± 1.4
11a	7.88 ± 0.8	39.90 ± 3.2	5.56 ± 0.6	51.81 ± 3.6
11b	49.69 ± 3.7	55.72 ± 3.9	28.27 ± 2.3	73.71 ± 4.5
12b	70.63 ± 4.1	11.34 ± 1.3	45.64 ± 3.3	13.80 ± 1.4

[a]IC50 (μg/ml): 1–10 (very strong). 11–20 (strong). 21–50 (moderate). 51–100 (weak) and above 100 (non-cytotoxic), 5-FU = 5-fluorouracil

Table 2 Antioxidant assay (ABTS)

Method	ABTS Abs(control)-Abs(test)/Abs (control) × 100	
Compound	Absorbance of samples	% inhibition
Control of ABTS	0.495	0
Ascorbic acid	0.051	89.70
4a	0.341	31.10
4b	0.367	25.40
4e	0.473	4.40
4f	0.364	26.50
4g	0.382	22.30
5a	0.29	42.10
6a	0.251	48.50
6b	0.288	42.40
7a	0.368	25.20
7b	0.377	23.40
8a	0.291	41.20
8b	0.371	24.60
9a	0.264	46.70
10a	0.246	50.00
10b	0.24	51.20
11a	0.345	29.90
11b	0.376	23.60
12b	0.154	68.70

16.34, and 19.91 μg for PC3, HCT-116, HePG-2, and MCF-7 cell lines, respectively. Moreover, compounds **10b** and **12b** showed a similar trend with IC50: 10.51, 11.34, 13.38, and 14.92 μg against MCF-7 and PC3 cancer cell lines for **10b** and **12b**, respectively. While the other compounds showed moderate to weak activities against all cell lines and no activity at all was observed for pyrimidine analog **4e**. In conclusion, pyrimidine core structure has been confirmed as a useful lead compound for the development of new anticancer agents. Our initial goal to prepare synthetic derivatives with higher anticancer activity could be achieved, resulting in several compounds with in vitro potent anticancer. Further variations in substituents and substitution pattern may be necessary to obtain more potent and selective compounds.

Antioxidant activity

Most of the synthesized products were evaluated for their antioxidant activity and their ability to inhibit oxidation in rat brain and kidney homogenates using 2,2^{-}-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assay. It was observed from data given in Table 2, compounds **10a**, **10b**, and **12b** exhibited the highest antioxidant activity while compounds **8a** and **9a** showed moderate activities in comparison with standard ascorbic acid.

Pharmacophore modeling

A pharmacophore is the ensemble of steric and electronic features necessary to ensure the optimal supramolecular interactions with a specific biological target, and to trigger or to block its biological response [Li et al. 2000].

Typical pharmacophore features indicate regions in a molecule that are hydrophobic, aromatic, can participate in hydrogen bonding (whether hydrogen bond donor or hydrogen bond acceptor), positive or negative ionizable, which permits pharmacophore generation, structural alignment, activity prediction and 3D database creation. It uses the catalyst HypoGen algorithm [Li et al. 2000] to derive SAR hypothesis models (pharmacophores) from a set of ligands with known activity values on a given biological target.

In this study, the 3D QSAR Pharmacophore Generation protocol (HypoGen protocol of CATALYST) was used with the Discovery Studio 2.5 software to generate ten predictive pharmacophore models via aligning different conformations, in which the molecules are likely to bind with the receptor pharmacophore models. The given hypothesis was combined with a known activitydata on hepatocellular carcinoma (HePG-2) cell line to create a 3D-QSAR model that identifies overall aspects of a molecular structure, which governs activity. Pharmacophores explain

the variability of bioactivity with respect to thegeometric localization of the chemical features present in themolecules.

Pharmacophore validation

In general, pharmacophoremodels are used as 3D queries to search chemical databases to identify new and highly potent drug leads. These pharmacophore models should be statistically significant, able to predict the activities of new chemical compounds and retrieve active compounds from the data base. The selected pharmacophore model was validated using these methods: cost analysis, activity prediction, and correlation coefficient.

HypoGen selects the best hypotheses by applying a cost analysis. The overall cost of each hypothesis is calculated by summing three cost factors: a weight cost, an error cost, and a configuration cost. HypoGen also calculates two the oretical costs, the null and fixed costs, which can be used to determine the significance of the selected hypotheses. The cost values of the optimized hypotheses should lie somewhere between these two costs. A larger difference between the fixed and null costs than that between the fixed and total costs signifies the quality of a pharmacophore model. The closer the cost value to the fixed cost and the further away it is from the null cost, the more statistically significant the hypothesis is believed to be.

Pharmacophore study results

In this study, pharmacophore models were generated using hydrogen bond acceptor (HBA), hydrogen bond donor (HBD), hydrophobic (HYP), positive ionizable (PosIon), and ring aromatic (RA) features from compounds **4a**, **4b**, **4e–g**, **7a-b**, **8a-b**, **9a**, **10a-b**, **11a-b**, and **12b**. Ten pharmacophore models were exported for further studies. All of the generated pharmacophore models contained at three chemical features. The best generated pharmacophore contained 2 HBA features and 1 HYP. The constraint distances and angles between the different features of the generated top pharmacophore are presented in Table 3 and Fig. (1).

The top pharmacophore hypothesis generated was developed with a total cost value of 54.97, null cost 63.05, and fixed cost 48.2. Further evaluation of the generated pharmacophore models was based on the correlation coefficient, which was found to be 0.80 that indicates the capability of the pharmacophore model to predict the activity of the training set compounds.

In addition to the cost analysis, the pharmacophore model was validated through the activity prediction of the synthesized structures as a training set. The predicted activities through the pharmacophore model are represented in Table 4 as well as their fit values. Interestingly; compound **11a** which showed the best biological activity has the highest fit value while compound **4e** which showed the worst biological activity has the lowest fit value.

Table 3 Constraint distances and angles between features of the generated top pharmacophore model

Constraint distances	(HBA1–HBA2): 7.018; (HBA1–HYP): 6.392; (HBA2–HYP): 4.62;
Constraint angles	(HBA1–HBA2–HYP): 62.7;

The best generated pharmacophore fitted with the synthesized compound **4b** and **11a**, is shown in Fig. 2. The pharmacophore features (hydrogen bond acceptors HBA1 &HBA2, and hydrophobic feature HYP) mapped with the synthesized compounds, as well as their fit values are represented in Table 5.

Conclusion

In summary, we have synthesized novel derivatives of pyrimidine derivatives on the basis of green chemistry principles (catalysis and multicomponent synthesis) using water as a solvent. Six glucoside derivatives were also synthesized. Most of the synthesized products were evaluated for their cytotoxic activity against four tumor cell lines, and it was observed that **4b, 7a**, **10b, 11a**, and **12b** showed the most potent cytotoxic effect against different cell lines, as concluded from their IC_{50} values. In addition, antioxidant activities of these compounds were screened and exhibited potent activities. Generation of a 3D pharmacophore model was also carried out. The predicated cytotoxic activities by the pharmacophore model were very close to those experimentally observed, indicating that pyrimidine derivatives are promising scaffolds for developing more active derivatives in view of their various biological properties.

Experimental

Materials and physical measurements

The chemical reagents were purchased from Sigma-Aldrich and the solvents were commercially available from El-Nasr chemicals Co. in analytical grade and were used without further purification. The reactions progress was checked by TLC (Kieselgel 60 F254, 0.20 mm, Merck).

Melting points were determined by the capillary tube method, and the thermometer was uncorrected. The reactions progress was checked by TLC, Mass spectra were obtained on an Agilent 1100 HPLC-MS instrument. ^{1}H NMR spectra were run in DMSO-d6, with TMS as the internal standard, on a Bruker ARX-300 instrument

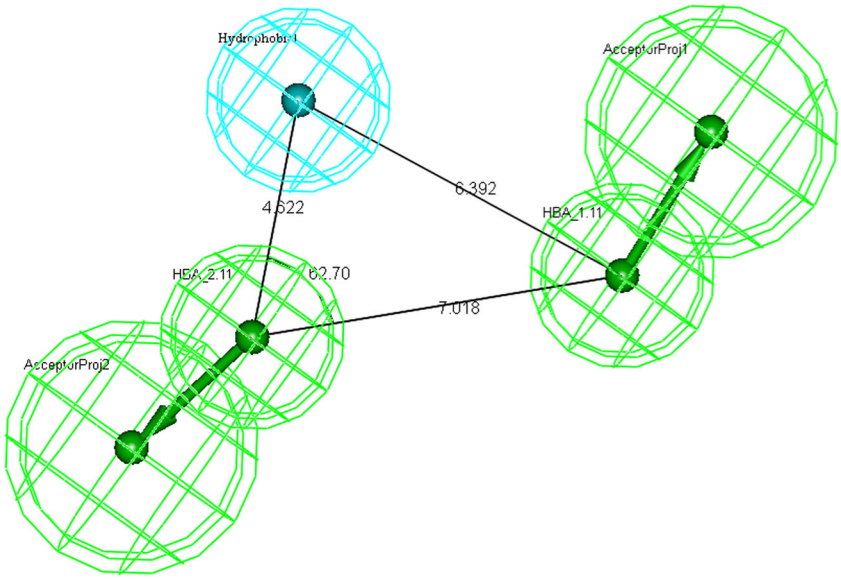


Fig. 1 Constraint distances and angles between features of the generated pharmacophore model with the considered features hydrogen bond acceptors (HBA1 & HBA2) colored in green, and hydrophobic (HYP) colored in cyan

Table 4 Fit values and predicted activities for the synthesized compounds mapped with the generated 3D-pharmacophore model

Cpd	Predicted activity IC_{50} (μg/ml)	Experimental activity IC_{50} (μg/ml)	Fit value
4a	56.39	83.5 ± 4.68	4.96
4b	17.33	12.33 ± 1.3	5.47
4e	117.91	>100	4.68
4f	45.17	26.9 ± 1.87	5.10
4g	51.60	55.38 ± 4.1	5.05
7a	15.44	16.34 ± 1.7	5.53
7b	35.27	34.65 ± 3.1	5.16
8a	56.16	74.6 ± 4.38	4.96
8b	41.63	24.36 ± 2.4	5.14
9a	21.25	26.7 ± 2.04	5.44
10a	17.01	26.90 ± 2.2	5.53
10b	14.23	9.39 ± 0.9	5.55
11a	11.14	7.88 ± 0.8	5.56
11b	23.02	49.69 ± 3.7	5.35
12b	77.61	70.63 ± 4.1	4.82

operating at 300 MHz. IR spectra (KBr disks) were recorded on a Bruker IFS 55 instrument. Elemental analysis was performed with a Carlo-Erba 1106 elemental analysis instrument.

General procedure for synthesis of pyrimidines 4a-g

A mixture of aromatic aldehyde (pyridin-3-carboxaldehyde or thiophen-2-carboxaldehyde) (1 mmol), active methylene compounds (1 mmol) namely ethyl acetoacetate, acetylacetone and 1,3-diphenyl-1,3-propandione, urea dreivatives (urea, thiourea or guanidine hydrochloride) (1 mmol), CAN (0.1 mmol) in distilled water (10 mL) was heated and stirred under reflux at 70 °C for 30 min. The formed solid product was filtered, washed with water, dried and recrystallized from the proper solvent to give pyrimidines **4a-g**.

Ethyl 6-methyl-4-(thiophen-2-yl)-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (4a)

Yield: 82%, M.p.: 132–134 °C, $C_{12}H_{14}N_2O_2S_2$ (282.38): Anal. Found: C, 50.87; H, 5.12; N, 9.83% Calc.: C, 51.04; H, 5.00; N, 9.92% IR (KBr, cm^{-1}): 3420-3210, *v* NH 1719, *v* CO; ^{1}H NMR (300 MHz, DMSO): *δ* 10.46, 9.76 (2brs, 2H, 2NH), 7.41-6.89 (m, 3H, thiophene H), 5.42 (s, 1H, CH), 4.07 (q, 2H, $CH_3\underline{CH_2}$), 2.27 (s, 3H, CH_3), 1.16 (t, 3H, $\underline{CH_3}CH_2$) ppm; ^{13}C NMR: 181.5 (C = S), 171.5 (CO), 158.5, 110.2 (pyrimidine C), 136.6, 128.5, 125.0, 123.5 (thiophene C), 62.2 (OCH_2), 47.9 (methine), 18.5 (CH_3), 14.8 (CH_3); MS: *m/z* = 282 (M^+, 100%).

Ethyl 6-methyl-4-(pyridin-3-yl)-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (4b)

Yield: 85%, M.p.: 150–152 °C, $C_{13}H_{15}N_3O_2S$ (277.34): Anal. Found: C, 56.23; H, 5.34; N, 15.01% Calc.: C, 56.30; H, 5.45; N, 15.15% IR (KBr, cm^{-1}): 3427-3230, *v* NH 1724, *v* CO; ^{1}H NMR (300 MHz, DMSO): *δ*11.23, 10.31 (2brs, 2H, 2NH), 7.52-6.99 (m, 4H, thiophene H), 5.33 (s, 1H, CH), 4.11 (q, 2H, $CH_3\underline{CH_2}$), 2.22 (s, 3H, CH_3), 1.09 (t, 3H, $\underline{CH_3}CH_2$) ppm; ^{13}CNMR: 179.5 (C = S), 168.5 (CO), 160.5, 107.5 (pyrimidine C), 153.3, 145.5, 130.5, 128.5, 123.7 (pyridine C), 63.5 (OCH_2), 49.5 (methine), 18.3, 15.5 (2 CH_3); MS: *m/z* = 277 (M^+, 20%);

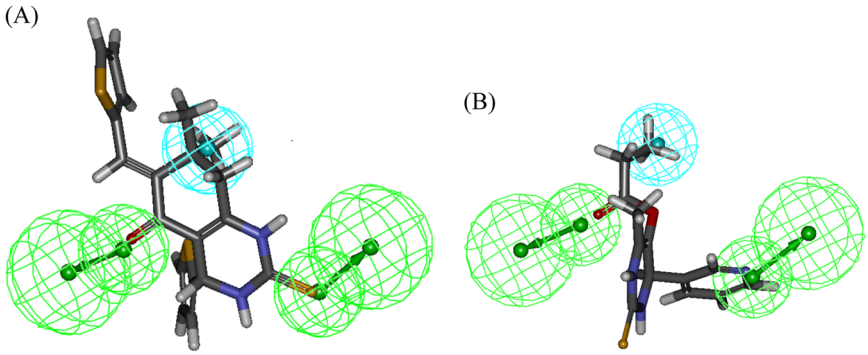


Fig. 2 The best generated pharmacophore hypothesis with the considered features, hydrogen bond acceptors (HBA1 and HBA2) colored in green, and hydrophobic (HYP) colored in cyan, with synthesized compound **11a** (**a**) and **4b** (**b**) fitted in the pharmacophore with a fit value 5.56 and 5.47, respectively

Table 5 The pharmacophore features hydrogen bond acceptors HBA1 and HBA2 and hydrophobic feature HYP mapped with the synthesized compounds as well as their fit values

Cpd	Fit value	HBA1	HBA2	HYP
4a	4.96	S of thiophene ring	Carbonyl of acetyl group	Methyl group
4b	5.47	N of pyrimidine ring	Carbonyl of acetyl group	Methyl of acetyl group
4e	4.68	S of pyrimidine ring	Carbonyl of keto group	Pyridine ring
4f	5.10	carbonyl of pyrimidine ring	Carbonyl of keto group	Thiophene ring
4g	5.05	S of pyrimidine ring	Carbonyl of keto group	Benzyl ring
7a	5.53	S of thiophene ring	S of thiazolpyrimidine ring	Methyl group
7b	5.16	Carbonyl of thiazolpyrimidine ring	Carbonyl of acetyl group	Methyl of acetyl group
8a	4.96	S of pyrimidine ring	Nitrile group	Thiophene ring
8b	5.14	S of pyrimidine ring	Nitrile group	Pyridine ring
9a	5.44	S of pyrimidine ring	Carbonyl of carboxylic group	Thiophene ring
10a	5.53	S of pyrimidine ring	Carbonyl of cyclohexyl group	Dimethyl group
10b	5.55	N of pyrimidine ring	Carbonyl of cyclohexyl group	Dimethyl group
11a	5.56	S of pyrimidine ring	Carbonyl of cyclohexyl group	Dimethyl group
11b	5.35	N of pyrimidine ring	Carbonyl of cyclohexyl group	Dimethyl group
12b	4.82	Carbonyl of thiazolpyrimidine ring	Carbonyl of cyclohexyl group	Dimethyl group

1-(6-Methyl-4-(thiophen-2-yl)-2-thioxo-1,2,3,4-tetrahydropyrimidin-5-yl) ethanone (4c)

Yield: 80%, M.p.: 147–149 °C, $C_{11}H_{12}N_2OS_2$ (252.36): Anal. Found: C, 52.23; H, 4.65; N, 11.03% Calc. C, 52.35; H, 4.79; N, 11.10% IR (KBr, cm^{-1}): 3424-3272, *v* NH 1665, *v* CO; ^{1}H NMR (DMSO, ppm): ^{1}H NMR (300 MHz, DMSO): δ 10.85, 9.64 (2brs, 2H, 2NH), 7.99-7.33 (m, 3H, thiophene H), 5.12 (s, 1H, CH), 2.45 (s, 3H, CH_3CO), 2.13 (s, 3H, CH_3) ppm; ^{13}C NMR: 188.0 (C = O), 180.5 (CS), 157.5, 108.8 (pyrimidine C), 136.5, 127.5, 126.2, 123.3 (thiophene C), 63.5 (OCH_2), 48.6 (methine), 23.5 (CH_3), 18.0 (CH_3); MS: $m/z = 252$ (M^+, 100%), 253 (M^{+1}, 18%).

1-(2-Imino-6-methyl-4-(pyridin-3-yl)-1,2,3,4-tetrahydropyrimidin-5-yl) ethanone (4d)

Yield: 79%, M.p.: 136–138 °C, $C_{12}H_{14}N_4O$ (230.27): Anal. Found: C, 62.68; H, 6.02; N, 24.39% Calc.: C, 62.59; H, 6.13; N, 24.33% IR (KBr, cm^{-1}): 3416, 3303, 3182, *v* NH, NH_2 1666 (CO); ^{1}H NMR (DMSO, ppm): ^{1}H NMR (300 MHz, DMSO): δ10.30 (brs, 1H, NH), 8.08 (s, 2H, NH_2), 7.85-7.12 (m, 4H, pyridine H), 4.76 (s, 1H, CH), 2.73 (s, 3H, CH_3CO), 2.22 (s, 3H, CH_3) ppm; ^{13}C NMR: 184 (CO), 158.3, 152.5, 112.5 (pyrimidine C), 148.5, 146.2, 131.5, 129.5, 126.4 (pyridine C), 49.5 (methine), 22.0, 18.5 (2 CH_3); MS: $m/z = 230$ (M^+, 60%).

1-(6-Methyl-4-(pyridin-3-yl)-2-thioxo-1,2,3,4-tetrahydropyrimidin-5-yl) ethanone (4e)

Yield: 86%, M.p.: 118–120 °C, $C_{12}H_{13}N_3OS$ (247.32): Anal. Found: C, 58.39; H, 5.17; N, 16.83% Calc.: C, 58.28; H, 5.30; N, 16.99% IR (KBr, cm^{-1}): 3424-3299, *v* NH 1670, *v* CO; ^{1}H NMR (300 MHz, DMSO): δ 10.39, 9.82 (2brs, 2H, 2NH), 7.87-6.93 (m, 4H, pyridine H), 5.53 (s, 1H, CH), 2.32 (s, 3H, CH_3CO), 2.21 (s, 3H, CH_3) ppm; ^{13}C NMR:188.5 (CO), 178.0 (CS), 158.5, 110.0 (pyrimidine C),

150.5, 148.2, 132.3, 128.5, 123.5 (pyridine C), 48.7 (methine), 23.0, 18.0 (2 CH_3).

5-Benzoyl-6-phenyl-4-(thiophen-2-yl)-3,4-dihydropyrimidin-2(1H)-one (4f)

Yield: 88%, M.p., 143–145 °C, $C_{21}H_{16}N_2O_2S$ (360.43): Anal. Found: C, 69.84; H, 4.35; N, 7.62% Calc.: C, 69.98; H, 4.47; N, 7.77% IR (KBr, cm^{-1}):3430-3245, *v* NH 1670, *v* CO; ^{1}H NMR (300 MHz, DMSO): 17.15, 10.21 (2brs, 2H, 2NH), 8.19-7.34 (m, 13H, phenyl and thiophene H), 4.88 (s, 1H, CH) ppm; ^{13}CNMR: 195.6 (C = O), 185.3 (NHCO), 93.2, 127.4, 128.8, 132.9, 133.6, 134.6, 136.2, 138.2 (unsaturated carbons), 49.4 (methine C); MS: $m/z = 360$ (M^+, 47%).

Phenyl(6-phenyl-4-(thiophen-2-yl)-2-thioxo-1,2,3,4-tetrahydropyrimidin-5-yl) methanone (4g)

Yield: 83%, M.p., 153–155 °C, $C_{21}H_{16}N_2OS_2$ (376.49): Anal. Found: C, 67.12; H, 4.32; N, 7.48%Calcd. for C: 66.99; H: 4.28; N: 7.44% IR (KBr, cm^{-1}): 3424-3157 *v* NH 1665, *v* CO; ^{1}H NMR (300 MHz, DMSO): 17.14, 10.13 (2brs, 2H, 2NH), 8.19-7.35 (m, 13H, phenyl and thiophene H), 4.88 (s, 1H, CH) ppm; ^{13}CNMR: 195.6 (CO), 185.3 (CS), 93.2, 127.4, 128.5, 128.7, 132.9, 133.6 (unsaturated carbons), 49.3 (methine C); MS: $m/z = 376$ (M^+, 63%).

Synthesis of glucosides 5a-c

Method A [Limousin et al. 1997]: 2,3,4,6-Tetra-*O*-acetyl-α-D-glucopyranosyl bromide (1 mmol) and potassium hydroxide (1 mmol) were mixed and pyrimidine **4e-g** was added. After 5 min under microwave irradiation, ethylacetate was added to the reaction mixture, filtered, and the filterate was concentrated under vacuum. The obtained residues were recrystallized to give glucoside derivatives **5a-c**.

Method B 2,3,4,6-Tetra-*O*-acetyl-α-D-glucopyranosyl bromide (1 mmol) was added to stirred solution of pyrmidine **4e-g** (1 mmol) in ethanol (30 mL) containing potassium hydroxide (1 mmol) and still stirring for 10 h at room temperature. The reaction mixture was filtered off, washed and recrystallized from ethanol to give glucosides **5a-c**.

2-(Acetoxymethyl)-6-((5-acetyl-6-methyl-4-(pyridin-3-yl)-1,4-dihydro pyrimidin-2-yl)thio)-3,4,5-triacetyl-β-D-glucopyranoside (5a)

Yield: 68%, M.p., 136–138 °C, $C_{26}H_{31}N_3O_{10}S$ (577.17): Anal. Found: C, 54.16; H, 5.51; N, 7.35%Calc.: C, 54.07; H, 5.41; N, 7.28% IR (KBr, cm^{-1}): 3433-3260, *v* NH 1743-1662, *v* CO; ^{1}H NMR (300 MHz, DMSO): δ 9.53 (brs, 1H, NH), 7.56-7.05 (m, 4H, pyridine H), 4.80 (s, 1H, CH), 4.72 (s, 2H, CH_2), 4.13–4.68 (m, 5H, 5 CH glucose), 2.65 (s, 3H, CH_3CO), 2.31 (s, 3H, CH_3); 1.95-2.20 (4 s, 12H, 4 CH_3CO), ppm; ^{13}C NMR: 192.3 ($COCH_3$), 171.2 (OCO), 159.5, 158.4, 112.5 (pyrimidine C), 150.4, 147.5, 134.5, 131.5, 123.8 (pyridine C), 78.5, 77.3, 73.3, 68.4, 65.5 (glucose C), 63.5 (OCH_2), 47.8 (methine), 26.5 (CH_3CO), 22.5 (CH_3COO), 18.5 (CH_3); MS: $m/z = 577$ (M^+, 30%), 578 (M^{+1}, 23%).

2-(Acetoxymethyl)-6-((5-benzoyl-6-phenyl-4-(thiophen-2-yl)-1,4-dihydropyrimidin-2-yl)oxy) -3,4,5-triacetyl-β-D-glucopyranoside (5b)

Yield: 71%, M.p., 152–154 °C, $C_{35}H_{34}N_2O_{11}S$ (690.19): Anal. Found: C, 60.78; H, 4.85; N, 4.01% Calc.: for C, 60.86; H, 4.96; N, 4.06% IR (KBr, cm^{-1}): 3385-3220 *v* (NH), 1735-1672 *v* (CO); ^{1}H NMR (300 MHz, DMSO): δ 9.86 (brs, 1H, NH), 8.02-7.11 (m, 13H, Ar H), 4.94 (s, 1H, CH), 4.77 (s, 2H, CH_2), 4.10–4.60 (m, 5H, 5 CH glucose), 2.07–2.28 (4s, 12H, 4 CH_3CO), ppm;^{13}C NMR: 190.2 (COC_6H_5), 171.2 (OCO), 155.5, 153.4, 108.3 (pyrimidine C), 139.2, 138.2, 133.5, 132.5, 128.7, 128.2, 127.6, 127.3, 126.5, 125.2, 124.8, 122.5 (phenyl and thiazole C), 80.5, 73.2, 72.5, 68.5, 66.8 (glucose C), 61.5 (OCH_2), 50.2 (methine), 21.3 (CH_3COO).

2-(Acetoxymethyl)-6-((5-benzoyl-6-phenyl-4-(thiophen-2-yl)-1,4-dihydropyrimidin-2-yl) thio)- 3,4,5-triacetyl-β-D-glucopyranoside (5c)

Yield: 67%, M.p., 144–145 °C, $C_{35}H_{34}N_2O_{10}S_2$ (706.17): Anal. Found: C, 59.35; H, 4.78; N, 3.88% Calc.: C, 59.48; H, 4.85; N, 3.96% IR (KBr, cm^{-1}): 3410-3245, *v* NH 1740-1683, *v* CO; ^{1}H NMR (300 MHz, DMSO): δ 10.12 (brs, 1H, NH), 8.10-7.25 (m, 13H, Ar H), 5.05 (s, 1H, CH), 4.90 (s, 2H, CH_2), 4.16–4.73 (m, 5H, 5 CH glucose), 1.97–2.25 (4s, 12H, 4 CH_3CO), ppm; ^{13}C NMR: 189.5 (COC_6H_5), 171.5 (OCO), 155.2, 152.5, 110.2 (pyrimidine C), 139.5, 138.4, 134.3, 131.5, 128.5, 128.2, 127.5, 126.5, 126.2, 125.5, 123.5, 122.2 (phenyl and thiazole C), 81.3, 74.5, 71.5, 69.2, 65.8 (glucose C), 64.2 (OCH_2), 49.3 (methine), 23.5 (CH_3COO).

General procedures for synthesis of deacetylated glucoside derivatives 6a–c

A solution of acetylated glucosides **5a–c** (1 mol) in methanol and ammonia (1:1) was stirred at room temperature for 6 h. After evaporation of solvent, the residue was dissolved in ethanol (15 mL) and left over night. The precipitated solid was filtered of and dried to give products 6a–c.

1-(6-Methyl-4-(pyridin-3-yl)-2-((-3,4,5-trihydroxy-6-(hydroxymethyl)-β-D-glucopyranosyl thio)-1,4-dihydropyrimidin-5-yl)ethan-1-one (6a)

Yield: 66%, M.p., 164–166 °C, $C_{18}H_{23}N_3O_6S$ (409.13): Anal. Found: C, 59.89; H, 4.93; N, 5.28% Calcd.: C, 60.06; H, 5.02; N, 5.36% IR (KBr, cm^{-1}):3430-3196, *v* OH, NH 1690-1675, *v* CO; ^{1}H NMR (300 MHz, DMSO): *δ* 11.30 (brs, 1H, NH), 7.48-7.07 (m, 4H, pyridine H), 5.10 (s, 1H, CH), 4.80 (s, 2H, CH_2), 4.91–5.22 (m, 4H, 4 OH), 3.60-4.32 (m, 5H, 5 CH glucose), 2.70 (s, 3H, CH_3CO), 2.18 (s, 3H, CH_3) ppm; ^{13}C NMR: 191.5 ($COCH_3$), 157.3, 158.5, 110.3 (pyrimidine C), 152.5, 148.2, 133.5, 132.3, 122.5 (pyridine C), 79.3, 77.5, 72.5, 67.3, 64.2 (glucose C), 65.5 (OCH_2), 47.3 (methine), 23.2 (CH_3CO), and 20.2 (CH_3).

Phenyl(6-phenyl-4-(thiophen-2-yl)-2-((-3,4,5-trihydroxy-6-(hydroxymethyl)-β-D-glucopyranosyloxy)-1,4-dihydropyrimidin-5-yl)methanone (6b)

Yield: 65%, M.p., 164–166 °C, $C_{27}H_{26}N_2O_7S$ (522.15): Anal. Found: C, 59.89; H, 4.93; N, 5.28%Calcd.: C, 60.06; H, 5.02; N, 5.36% IR (KBr, cm^{-1}): 3430-3196, *v* OH, NH 1690-1675, *v* CO; ^{1}H NMR (300 MHz, DMSO): *δ*11.12 (brs, 1H, NH), 7.98-6.99 (m, 13H, ArH), 5.03 (s, 1H, CH), 4.75 (s, 2H, CH_2), 4.82–5.18 (m, 4H, 4 OH), 3.70–4.45 (m, 5H, 5 CH glucose) ppm;^{13}CNMR:188.5 (COC_6H_5), 154.3, 153.5, 110.5 (pyrimidine C), 138.7, 136.5, 133.2, 132.5, 128.5, 128.0, 127.5, 126.3, 125.5, 123.5, 122.8, 121.5 (phenyl and thiazole C), 79.5, 74.3, 73.5, 68.8, 65.5 (glucose C), 62.8 (OCH_2), and 48.5 (methine).

Phenyl(6-phenyl-4-(thiophen-2-yl)-2-(((2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl) -β-D-glucopyranosylthio)-1,4-dihydropyrimidin-5-yl)methanone (6c)

Yield: 68%, M.p., 152–153 °C, $C_{27}H_{26}N_2O_6S_2$ (538.12): Anal. Found: C, 6.11; H, 4.79; N, 5.15% Calc.: C, 60.21; H, 4.87; N, 5.20% IR (KBr, cm^{-1}): 3413-3218, *v* OH, NH 1687-1676, *v* CO; ^{1}H NMR (300 MHz, DMSO): *δ* 10.75 (brs, 1H, NH), 8.02-6.97 (m, 13H, ArH), 4.94 (s, 1H, CH), 4.65 (s, 2H, CH_2), 4.73–5.32 (m, 4H, 4 OH), 4.03–4.48 (m, 5H, 5 CH glucose) ppm; ^{13}C NMR: 191.0 (COC_6H_5), 154.5, 148.3, 112.5 (pyrimidine C), 139.2 136.5, 133.5, 129.5, 128.2, 127.5, 127.0, 126.3, 125.8, 123.3, 122.5, 121.4 (phenyl and thiazole C), 80.5, 75.2, 72.5, 68.5, 65.5 (glucose C), 62.5 (OCH_2), 49.5 (methine); MS: *m/z* = 538 (M^+, 12%).

Synthesis of 7a,b

A mixture of pyrimidine **4a** (1 mmol) and chloroacetylchloride or oxaloyl chloride (1 mmol) in pyridine (2 mL) was heated under reflux for 4 h. After cooling, the reaction mixture was poured into crushed ice/HCl. The formed solid product was collected by filtration, washed, dried, and crystallized from proper solvent.

Ethyl-7-methyl-2-oxo-5-(thiophen-2-yl)-2,3-dihydro-5H-thiazolo[3,2-a]pyrimidine-6-carboxylate (7a)

Yield: 81%, M.p., 165–167 °C, Anal. Found: C, 52.02; H, 4.31; N, 8.56% Calc. $C_{14}H_{14}N_2O_3S_2$ (322.40): C, 52.16; H, 4.38; N, 8.69% IR (KBr, cm^{-1}):1724-1703, *v* CO 1649, *v* C = N; ^{1}H NMR (300 MHz, DMSO): 7.35-6.88 (m, 3H, thiophene H), 5.42 (s, 1H, CH), 4.02–4.10 (q, 2H, $CH_3\underline{CH_2}$), 2.22 (s, 3H, CH_3), 2.72 (s, 2H, $COCH_2$), 1.16 (t, 3H, $\underline{CH_3}CH_2$) ppm; ^{13}CNMR:191.0, 172.5 (2CO), 156.5, 153.2, 118.5 (pyrimidine C), 135.5, 127.2, 126.5, 122.5 (thiophene C), 63.5, 61.2 (2 OCH_2), 48.7 (methine), 18.0 (CH_3), 14.5 (CH_3); MS: *m/z* = 322 (M^+, 35%).

Ethyl 7-methyl-2,3-dioxo-5-(thiophen-2-yl)-2,3-dihydro-5H-thiazolo[3,2-a] pyrimidine-6-carboxylate (7b)

Yield: 79%, M.p., 182–184 °C, $C_{14}H_{12}N_2O_4S_2$ (336.38): Anal. Found: C, 49.86; H, 3.44; N, 8.15% Calc. C: 49.99; H: 3.60; N: 8.33% IR (KBr, cm^{-1}):1722-1703 *v* (CO), *v* 1646 (C = N); ^{1}H NMR (300 MHz, DMSO): 7.36-6.88 (m, 3H, thiophene H), 5.41 (s, 1H, CH), 4.03–4.09 (q, 2H, $CH_3\underline{CH_2}$), 2.22 (s, 3H, CH_3), 2.85 (s, 2H, $COCH_2$), 1.17 (t, 3H, $\underline{CH_3}CH_2$) ppm; ^{13}C NMR: 189.5, 175.3, 167.5 (3CO), 157.6, 148.5, 116.0 (pyrimidine C), 134.4, 128.5, 125.5, 121.5 (thiophene C), 62.3 (OCH_2), 51.3 (methine), 18.5 (CH_3), 14.2 (CH_3); MS: *m/z* = 336 (M^+, 82%).

General procedures for synthesis of pyrimidines 8a,b

The same procedures described before for synthesis of **4a–g**.

4-Oxo-6-(thiophen-2-yl)-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carbonitrile (8a)

Yield: 84%, M.p., 121–122 °C, $C_9H_5N_3OS_2$ (235.29): Anal. Found: C, 45.85; H, 2.19; N, 18.01% Calc. C, 45.94; H, 2.14; N, 17.86% IR (KBr, cm^{-1}): 3440-3230, *v* NH 2259, *v* C≡N 1683, *v* CO; ^{1}H NMR (300 MHz, DMSO): 12.82, 8.35 (2brs, 2H, 2NH), 7.90-7.13 (m, 3H, thiophene-H) ppm; ^{13}C NMR: 178.5 (CS), 172.2 (CO), 160.0, 111.5 (pyrimidine C), 133.5, 128.8, 123.5, 122.2 (thiophene C), 116.5 (CN).

4-Oxo-6-(pyridin-3-yl)-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carbonitrile (8b)

Yield: 82%, M.p., 96–97 °C, $C_{10}H_6N_4OS$ (230.25): Anal. Found: C, 52.01; H, 2.56; N, 24.18% Calc. C, 52.16; H, 2.63; N, 24.33% IR (KBr, cm^{-1}): 3394-3102, *v* NH 2215, *v*

C≡N 1668, *v*CO; ^{1}H NMR (300 MHz, DMSO): 12.82, 8.36 (2brs, 2H, 2NH), 7.92-7.12 (m, 4H, pyridine H) ppm; ^{13}CNMR:173.8 (CS), 168.5 (CO), 158.5, 115.3 (pyrimidine C), 156.5, 148.2, 130.5, 126.8, 125.5 (pyrimidine C), 114.5 (CN); MS: $m/z = 230$ (M^+, 67%).

General procedures for synthesis of acids 9a,b

Nitrile **8a,b** (1 mmol) was added to potassium hydroxide solution 10% (20 mL), the reaction mixture was stirred for 10 h. Acidification with HCl afforded solid products **9a,b** that collected by filtration, dried, and crystallized.

Yield: 78%, M.p., 129–131 °C, $C_9H_6N_2O_3S_2$ (254.28): Anal. Found: C, 42.35; H, 2.29; N, 10.88% Calc. C, 42.51; H, 2.38; N, 11.02% IR (KBr, cm^{-1}): 3432-3159, *v* NH, OH 1682-1664, *v* CO 1339, *v*CS; ^{1}H NMR (300 MHz, DMSO): 12.27 (s, 1H, OH, exchangeable), 11.38, 9.75 (2brs, 2H, 2NH), 7.62-7.15 (m, 3H, thiophene- H) ppm; ^{13}C NMR: 175.5 (CS), 171.5, 169.5 (2 CO), 158.2, 110.3 (pyrimidine C), 132.3, 126.5, 125.8, 123.0 (thiophene C); MS: $m/z =$ 254 (M^+, 100%), 255 (M^+, 28%).

4-Oxo-6-(pyridin-3-yl)-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylic acid (9b)

Yield: 80%, M.P., 115–116 °C, $C_{10}H_7N_3O_3S$ (249.24): Anal. Found: C, 48.11; H, 2.72; N, 16.80% Calc. C, 48.19; H, 2.83; N, 16.86%IR (KBr, cm^{-1}): 3385-3221, *v* NH, OH 1687-1660, *v* CO 1342, *v* CS; ^{1}H NMR (300 MHz, DMSO): 11.89 (s, 1H, OH, exchangeable), 11.07, 9.33 (2brs, 2H, 2NH), 7.56-7.11 (m, 4H, pyridine —H) ppm; MS: $m/z = 249$ (M^+, 55%).

General procedures for synthesis of quinazolines 10a,b

The same procedures described before for synthesis of **4a–g**.

7,7-Dimethyl-4-(thiophen-2-yl)-2-thioxo-2,3,4,6,7,8-hexahydroquinazolin-5(1H)-one (10a)

Yield: 86%, M.p., 133–134 °C, $C_{14}H_{16}N_2OS_2$ (292.42): Anal. Found: C, 57.42; H, 5.41; N, 9.45% Calc. C, 57.50; H, 5.52; N, 9.58% IR (KBr, cm^{-1}): 3417-3220, *v* NH1672, *v* CO; ^{1}H NMR (300 MHz, DMSO): 8.27, 8.13 (2brs, 2H, 2NH), 7.35-6.81 (m, 3H, thiophene H), 5.20 (s, 1H, CH), 2.62-2.12 (m, 4H, $2CH_2$), 1.01, 1.09 (2s, 6H, $2CH_3$) ppm**;** ^{13}C NMR: 185.8 (CO), 172.5 (CS), 158.3, 136.5, 128.2, 123.5, 118.2, 110.2 (thiophene and pyrimidine carbons), 56.5 (CH_2CO), 51.3 (methine C), 38.2 (CH_2), 32.5 ($\underline{C}(CH_3)_2$), 18.5 ($2CH_3$); MS: $m/z = 292$ (M^+, 48%), 293 (M^+, 56%).

2-Imino-7,7-dimethyl-4-(thiophen-2-yl)-2,3,4,6,7,8-hexahydroquinazolin-5(1H)-one (10b)

Yield: 83%, M.p., 142–143 °C, $C_{14}H_{17}N_3OS$ (275.37): Anal. Found: C, 61.00; H, 6.14; N, 15.21% Calc. C: 61.06; H: 6.22; N: 15.26% IR (KBr, cm^{-1}): 3384-3174, *v* NH, NH_2 1685, *v* CO; ^{1}H NMR (300 MHz, DMSO): 8.35 (brs, 1H, NH), 8.12 (s, 2H, NH_2), 7.42-6.88 (m, 3H, thiophene H), 4.93 (s, 1H, CH), 2.58-2.26 (m, 4H, $2CH_2$), 1.04, 0.99 (2s, 6H, $2CH_3$) ppm; ^{13}C NMR: 161.29 (CO), 153.16, 152.22, 136.42, 127.56, 124.13, 115.22, 104.93, (thiophene and pyrimidine carbons), 48.15 (methine C), 41.20 ($\underline{C}(CH_3)_2$), 62.54 (CH_2), and 13.93 ($2CH_3$).

General procedures for synthesis of 11a,b

A mixture of thiophene-2-carbaldehyde (1 mmol) and pyrimidine derivative **10a,b** in H_2O: EtOH (60:40%) (20 mL) was stirred and heated under reflux for 4 h. After cooling the solid product was filtered off and crystallized to give pure products **11a,b**.

7,7-Dimethyl-4-(thiophen-2-yl)-6-(thiophen-2-ylmethylene)-2-thioxo-2,3,4,6,7,8-hexahydro quinazolin-5(1H)-one (11a)

Yield: 85%, M.p., 147–148 °C, $C_{19}H_{18}N_2OS_3$ (386.55): Anal. Found: C, 58.88; H, 4.55; N, 7.16% Calc. C, 59.04; H, 4.69; N, 7.25% IR (KBr, cm^{-1}): 3304-3156 *v* NH 1670, *v* CO 1337, *v* CS; ^{1}H NMR (300 MHz, DMSO): 9.44, 8.81 (2brs, 2H, 2NH), 8.31 (s, 1H, CH = C), 7.63–6.97 (m, 6H, H), 5.12 (s, 1H, CH), 2.73 (s, 2H, CH_2), 1.05, 1.10 (2s, 6H, $2CH_3$) ppm**;** ^{13}C NMR: 183.5 (CO), 175.2 (CS), 163.3, 158.5, 152.2, 136.3, 131.5, 130.6, 129.4, 128.8, 126.2, 122.5, 121.3, 108.5 (pyrimidine carbons, thiophene and CH = C), 53.6 (methine C), 46.5 (CH_2), 30.8 ($\underline{C}(CH_3)_2$), 21.3 ($2CH_3$);MS: m/z = 386 (M^+, 100%).

2-Imino-7,7-dimethyl-4-(thiophen-2-yl)-6-(thiophen-2-ylmethylene)-2,3,4, 6,7,8-hexahydroquinazolin-5(1H)-one (11b)

Yield: 80%, M.p., 160–162 °C, $C_{19}H_{19}N_3OS_2$ (369.50) Anal. Found: C, 61.68; H, 5.05; N, 11.28% Calc.: C, 61.76; H, 5.18; N, 11.37% IR (KBr, cm^{-1}):3432-3155, *v* NH, NH_2 1670, *v* CO 1336, *v*CS**;** ^{1}H NMR (300 MHz, DMSO): 10.27, 9.36 (2brs, 2H, 2NH), 8.28 (s, 1H, CH = C), 7.58- 7.02 (m, 6H, Ar-H), 4.98 (s, 1H, CH), 2.70 (s, 2H, CH_2), 1.02, 1.08 (2s, 6H, $2CH_3$) ppm**;** ^{13}CNMR:181.5 (CO), 161.5, 158.2, 153.6, 148.4, 136.5, 133.2, 131.5, 128.6, 128.1, 127.3, 123.7, 121.1, 106.5 (pyrimidine carbons, thiophene, C = NH, and CH = C), 48.2 (methine C), 42.5 (CH_2), 35.3 ($\underline{C}(CH_3)_2$), 18.5 ($2CH_3$); MS: $m/z = 369$ (M^+, 72%).

Synthesis of 12a,b and 13a,b

A mixture of pyrimidine **11a,b** (1 mmol) and chloroacetylchloride or oxaloyl chloride (1 mmol) in pyridine (5 mL) was heated under reflux for 4 h. After cooling, the reaction mixture was poured into crushed ice/HCl. The formed solid products **12a,b** and **13a,b** were collected by filtration, washed, dried, and crystallized from proper solvent.

8,8-Dimethyl-5-(thiophen-2-yl)-7-(thiophen-2-ylmethylene)-5,7,8,9-tetra hydro-6H-thiazolo[2,3-b]quinazoline-2,6(3H)-dione (12a)

Yield: 75%, M.p., 205–207 °C, $C_{21}H_{18}N_2O_2S_3$ (426.57): Anal. Found: C, 59.04; H, 4.12; N, 6.49% Calc.: C, 59.13; H, 4.25; N, 6.57% IR (KBr, cm^{-1}): 1720-1663, *v* CO; ^{1}H NMR (300 MHz, DMSO): 7.29-7.16 (m, 6H, thiophene H+ CH = C), 4.49 (s, 1H, CH), 2.29 (s, H, CH_2CO), 2.11 (s, 2H, CH_2), 1.03, 0.90 (2s, 6H, $2CH_3$) ppm; ^{13}C NMR: 190.2, 182.3 (2 CO), 160.1, 158.3, 153.5, 134.2, 132.2, 130.1, 128.4, 127.5, 126.5, 121.7, 120.2, 106.5 (pyrimidine carbons, thiophene and CH = C), 47.4 (methine C), 62.2, 44.3 (2 CH_2), 32.5 (**C**$(CH_3)_2$), 23.8 ($2CH_3$); MS: *m/z* = 426 (M^+, 33%), 427 (M^{+1}, 27%).

8,8-Dimethyl-5-(thiophen-2-yl)-7-(thiophen-2-ylmethylene)-5,7,8,9-tetrahydroimidazo[2,1-b]quinazoline -2,6(1H,3H)-dione (12b)

Yield: 80%, M.p., 186–187 °C, $C_{21}H_{19}N_3O_2S_2$ (409.52): Anal. Found: C, 61.52; H, 4,57; N, 10.18% Calc.: C, 61.59; H, 4.68; N, 10.26% IR (KBr, cm^{-1}): 3432-3235, *v* NH 1690 -1660, *v* CO; ^{1}H NMR (300 MHz, DMSO): 8.70 (brs, 1H, NH), 7.98-7.16 (m, 6H, thiophene H+ CH = C), 2.29 (s, H, CH_2CO), 2.05 (s, 2H, CH_2), 1.03, 0.92 (2s, 6H, $2CH_3$) ppm; MS: *m/z* = 409 (M^+, 100%).

8,8-Dimethyl-5-(thiophen-2-yl)-7-(thiophen-2-ylmethylene)-8,9-dihydro-5H-thiazolo[2,3-b] quinazoline-2,3,6(7H)-trione (13a)

Yield: 76%, M.p., 214–216 °C, $C_{21}H_{16}N_2O_3S_3$ (440.55): Anal. Found: C, 57.31; H, 3.69; N, 6.40% Calc. C, 57.25; H, 3.66; N, 6.36% IR (KBr, cm^{-1}): 1720-1675, *v*CO; ^{1}H NMR (300 MHz, DMSO): 7.72-7.08 (m, 6H, thiophene H+ CH = C), 4.65 (s, 1H, CH), 2.20 (s, 2H, CH_2), 1.08, 0.97 (2s, 6H, $2CH_3$) ppm; MS: *m/z* = 440 (M^+, 70%).

8,8-Dimethyl-5-(thiophen-2-yl)-7-(thiophen-2-ylmethylene)-5,7,8,9-tetrahydroimidazo[2,1-b] quinazoline-2,3,6(1H)-trione (13b)

Yield: 78%, M.p., 205–207 °C $C_{21}H_{17}N_3O_3S_2$ (423.51): Anal. Found: C, 59.43; H, 4.01; N, 9.85% Calc. C, 59.56; H, 4.05; N, 9.92. IR (KBr, cm^{-1}): 3382-3243, *v* NH 1703 -1681, *v* CO; ^{1}H NMR (300 MHz, DMSO): 9.23 (brs, 1H, NH), 7.88-7.25 (m, 6H, thiophene H+ CH = C), 2.13 (s, 2H, CH_2), 1.11, 1.05 (2s, 6H, $2CH_3$) ppm; ^{13}C NMR: 183.5, 178.2, 171.8 (3 CO), 162.3, 158.5, 153.8, 136.2, 133.6, 131.5, 128.6, 127.2, 126.3, 123.5, 122.3, 108.3 (pyrimidine carbons, thiophene and CH = C), 55.2 (methine C), 48.3 (CH_2), 33.3 (C$(CH_3)_2$), 21.2 ($2CH_3$); MS: *m/z* = 423 (M^+, 62%), 424 (M^{+1}, 45%).

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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References

Abdelgawad MA, Bakr RB, Azouz AA (2018) Novel *pyrimidine*-pyridine hybrids: Synthesis, cyclooxygenase inhibition, anti-inflammatory activity and ulcerogenic liability. Bioorg Chem 77:339–348

Abdelghani E, Said SA, Assy MG, Abdel Hamid AM (2017) Synthesis and antimicrobial evaluation of some new *pyrimidines* and condensed *pyrimidines*. Arabian J Chem 10:2926–2933

Abdelkhalek AA, Sharaf AMA, Rabie M, El-Subbagh HI (2017) Derivatives of Cucurbitacin-E-*glucoside* produced by Curvularia lunata NRRL 2178: anti-inflammatory, antipyretic, antitumor activities, and effect on biochemical parameters. Future J Pharm Sci 3(2):124–130

Adiyala PR, Tekumalla V, Sayeed IB, Nayak VL, Kamal A (2018) Development of pyrrolo[2,1-c][1,4]benzodiazepine β-*glucoside* prodrugs for selective therapy of cancer. Bioorg Chem 76:288–293

Alaouia S, Driowyaa M, Demangeb L, Benhidab R, Bougrina K (2018) Ultrasound-assisted facile one-pot sequential synthesis of novel sulfonamideisoxazoles using cerium (IV) ammonium nitrate (CAN) as an efficient oxidant in aqueous medium. Ultrason Sonochem 40:289–297

Behalo MS, El-Karim IG, Issac YA, Farag MA (2014) Synthesis of novel pyridazine derivatives as a potential antimicrobial agents. J Sulfur Chem 35(6):661–673

Behalo MS, Mele G (2017) Synthesis and evaluation of pyrido[2,3-d] pyrimidine and 1,8-naphthyridine derivatives as potential anti-tumor agents. J Heterocycl Chem 54(1):295–300

Behalo MS (2009) Synthesis and antimicrobial activities of some novel pyrido[2,3-d]pyrimidine derivatives. Phosphorus Sulfur Silicon Relat Elem 184(1):206–219

Behalo MS (2016) An efficient one-pot catalyzed synthesis of 2,5-disubstituted-1,3,4-oxadiazoles and evaluation of their antimicrobial activities. RSC Adv 6:103132–103136

Boddeti G, Bhagavathula SD, Yellajyosula LN (2012) Synthesis and applications of β-enamino carbonyl compounds. Org Commun 5 (3):105–119

Bozunovic J, Zivkovic S, Gasic U, Glamoclija J, Misic D (2018) In vitro and in vivo transformations of Centaurium erythraea

secoiridoid *glucosides* alternate their antioxidant and antimicrobial capacity. Ind Crops Prod 111:705–721

Chikhale PR, Thorat S, Choudhary RK, Gadewal N, Khedekar P (2018) Design, synthesis and anticancer studies of novel aminobenzazolyl pyrimidines as tyrosine kinase inhibitors. Bioorg Chem 77:84–100

Corona AT, Lobo-Sánchez MA, Perez JL, Zanella R, Cuchillo OV (2018) Green synthesis of copper (0) nanoparticles with cyanidine-O-3-*glucoside* and its strong antimicrobial activity. Materials Lett 211:266–269

Chen D, Chen R, Xie K, Duan Y, Dai J (2018) Production of acetophenone C-glucosides using an engineered C-glycosyltransferase in Escherichia coli. Tetrahedron Lett 59:1875–1878

Denizot F, Lang R (1986) Rapid colorimetric assay for cell growth and survival. Modifications to the tetrazolium dye procedure giving improved sensitivity and reliability. J Immunol Methods 22:271–277

Dinakaran S, Keloth K, Bomma V (2012) The heterocycle of diverse biological and pharmacological significance. Der Pharma Chemica Acta Cytol 4:255–265

Duarte LJ, Chaves VC, Nascimento MVP, Calvete E, Dalmarco EM (2018) Molecular mechanism of action of Pelargonidin-3-O-*glucoside*, the main anthocyanin responsible for the anti-inflammatory effect of strawberry fruits. Food Chem 247:56–65

Geng PF, Liu XQ, Zhao TQ, Wang CC, Liu HM (2018) Design, synthesis and in vitro biological evaluation of novel [1,2,3]triazolo[4,5-d]pyrimidine derivatives containing a thiosemicarbazide moiety. Eur J Med Chem 146:147–156

Huang G, Zhao HR, Meng QQ, Zhoua W, Zhanga QJ, Dong Y, Cuia J, Lia SS (2017) Cerium (IV) ammonium nitrate (CAN)-mediated regioselective synthesis and anticancer activity of 6-substituted 5,8-dimethoxy-1,4- naphthoquinone. Chin Chem Lett 28:1553–1558

Ishioka W, Oonuki S, Iwadate T, Nih K (2019) Resorcinol alkyl glucosides as potent tyrosinase inhibitors. Biorg Med Chem Lett 29:313–316

Jaiprakash NS, Nagnnath DK, Sandeep AK, Devanand BS (2008) Ceric ammonium nitrate catalysed three component one-pot efficient synthesis of 2,4,5-triaryl-1H-imidazoles. J. Chem. Sci. 120(5):463–467

Kamal A, Faazil S, Malik MS, Balakrishna M, Bajee S, Siddiqui MR, Alarifi A (2016) Convenient synthesis of substituted pyrroles via a cerium (IV) ammonium nitrate (CAN)-catalyzed Paal–Knorr reaction. Arab J Chem. 9:542–549

Kamal US, Abdulaziz A, Ramadan AM, Nasr KM, Haney AM (2011) Cerium (IV) ammonium nitrate (CAN) mediated reactions IV. A highly efficient synthesis of N,N'-diarylsubstituted formamidines in water at ambient temperature. Green Sustain Chem 1:92–97

Li H, Sutter J, Hoffmann R, HypoGen X (2000) An automated system for generating predictive 3D pharmacophore models, in pharmacophore perception, development, and use in drug design. International University Line, La Jolla, CA, p 171–187

Limousin C, Cléophax J, Petit A, Loupy A, Lukacs G (1997) Solvent-free synthesis of decyl dglycopyranosides under focused microwave irradiation. J Carbohydr Chem 16(3):327–342

Maddila S, Gorle S, Seshadri N, Lavanya P, Jonnalagadda SB(2016) Synthesis, antibacterial and antifungal activity of novel benzothiazole pyrimidine derivatives Arab J Chem 9(5):681–687

Madkour HMF, Salem MAI, Soliman EA, Mahmoud NF (2001) A facil one—pot syntheses and ani bacterial activity of aziridines and thiazines from 1,3- diaryl prop – 2- enones phosphorus, sulfur and silicon 170:15–27

Mane UR, Mohanakrishnan UR, Sahal D, Murumkar PR, Gridihar R, Yaday MR (2014) Synthesis and biological evaluation of some novel pyrido[1,2-a]pyrimidin-4-ones as antimalarial agents. Eur J Med Chem 79:422–435

Mosmann T (1983) Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods 65:55–63

Nikishin GI, Kapustina NI, Sokova LL, Bityukov OV, Terentev AO (2017) One-pot oxidative bromination—esterification of aldehydes to 2-bromoesters using cerium (IV) ammonium nitrate and lithium bromide. Tetrahedron Lett. 58:352–354

Okesli A, Khosla C, Bassik MC (2017) Human *pyrimidine* nucleotide biosynthesis as a target for antiviral chemotherapy. Curr Opin Biotechnol 48:127–134

Rodriguez R, Daniel E, Vicentes JC, Manuel N (2017) Synthesis of new 1,2-diaryl[2]benzopyrano[3,4-d]imidazol-5(1H)-one derivatives mediated by ceric a mmonium nitrate. Tetrahedron Lett 58:1487–1489

Salem MAI, Marzouk MI, Salem MS, Alshibani GA (2016) One-pot synthesis of 1,2,3,4-tetrahydropyrimidin-**2**(1 H)-thione derivatives and their biological activity. J Heterocycl Chem 53:545–553

Salem MAI, Madkour HMF, Marzouk MI, Azab ME, Mahmoud NF (2007) Utility of cyanothioacetamide in the synthesis of pyrazolo [4, **3**-c], Isoxazolo [4, 5-c], thieno [**2**, **3**-b] and furo [**2**, **3**-c] of thioxopyridine derivatives and their antibacterial activities eurasian. J Sci Res 2:161–169

Salem MAI, Madkour HMF, Marzouk MI, Azab ME, Mahmoud NF (2008) Pyrimidinethiones (part 1): utility of 2-thioxopyrimidin- 6-(1H) ones as ring transformer in the synthesis of fused bi- and tricyclic heterocyclic compounds and their potential biological activities Phosphorus Sulfur Silicon Relat Elem 18:2596–2605

Salem MAI, Marzouk MI, Mahmoud NF (2014) Synthesis of various fused pyrimidine rings and their pharmacological and antimicrobial evaluation. J Serb Chem Soc 79(9):1059–1073

Salem MA, Ali TE, Marzouk MI, Kamal A, Barghash R (2013) Electron ionization mass spectra of organophosphorus compounds Part III: mass spectrometric fragmentation of diethyl spiro [pyrimidino[5,3'][1,2]oxazole] phosphonate, diethyl (oxazolo [5,4-d]pyrimidine-4,6-dione) phosphonate and diethyl (pyrimidino[4,5-b][1,4]oxazine) phosphonate derivatives. Phosphorus Sulfur Silicon Relat Elem 188(8):1007–1018

Salem MA, Ali TE, Khider RE (2012) Electron ionization mass spectra of organophosphorus compounds Part II: mass spectrometric fragmentation of spiro(indene-2, 3'-pyrazole) phosphonates, spiro (indene-2,3'-pyrazole) phosphanylidene, spiro (1,2,4-diazaphosphole-3,2'-indenes) and (1,3-dioxo-2h-isoindolyl) phosphonate derivatives. Heteroatom Chem 23(3):254–264

Seo UM, Nguyen DH, Zhao BT, Min BS, Woo MH (2017) Flavanonol *glucosides* from the aerial parts of Agrimonia pilosa Ledeb. and their acetylcholinesterase inhibitory effects. Carbohydr Res 445:75–79

Tian C, Wang M, Han Z, Fang F, Zhang Z, Wang X, Liu J (2017) Design, synthesis and biological evaluation of novel 6-substituted pyrrolo [3,2-d] pyrimidine analogues as antifolate antitumor agents. Eur J Med Chem 138:630–643

Undeela S, Ramchandra JP, Menon RS (2014) A sequential synthesis of substituted furans from aryl alkynes and ketones involving a cerium(IV) ammonium nitrate (CAN)-mediated oxidative cyclization. Tetrahedron Lett 55:5667–5670

Zhang J, Peng J, Wang T, Wang P, Zhang Z (2016) Synthesis, crystal structure, characterization and antifungal activity of pyrazolo[1,5-a]pyrimidines derivatives. J. Mol. Struct 1120:228–233