Heliyon 6 (2020) e04956

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Synthesis of arylidene hydrazinylpyrido[2,3-*d*]pyrimidin-4-ones as potent anti-microbial agents

Reda M. Abdelhameed [a], Osama M. Darwesh [b], Mahmoud El-Shahat [c,*]

[a] *Applied Organic Chemistry Department, Chemical Industries Research Division, National Research Centre, Scopus affiliation ID 60014618, 33 EL Buhouth St., Dokki, Giza, 12622, Egypt*

[b] *Department of Agricultural Microbiology, Agricultural Research Division, National Research Centre, Scopus affiliation ID 60014618, 33 EL Buhouth St., Dokki, Giza, 12622, Egypt*

[c] *Photochemistry Department, Chemical Industries Research Division, National Research Centre, Scopus affiliation ID 60014618, 33 EL Buhouth St., Dokki, Giza 12622, Egypt*

ARTICLE INFO

Keywords:
Organic chemistry
Pharmaceutical chemistry
Pyrido[2,3-*d*]pyrimidin-4-one
Hydrazinylpyrido[2,3-*d*]pyrimidin-4-one
Anti-bacterial activity
Anti-fungal activity

ABSTRACT

Combination of arylidene hydrazinyl moiety with pyrido[2,3-*d*]pyrimidin-4-one skeleton in compounds **7–26** results in the output of unprecedented anti-microbial agents. Arylidene hydrazinyl based on Pyrido[2,3-*d*]pyrimidin-4-one analoges **7–26** prepared by the treatment of [2,3-*d*]pyrimidin-4-ones **6a**,**b** with various aromatic aldehydes. The antimicrobial action for recently synthesized compounds was considered towards gram positive bacterial species (*Staphylococcus aurous* ATCC- 47077; *Bacillus cereus* ATCC-12228), gram negative bacterial species (*Escherichia coli* ATCC-25922; *Salmonella typhi* ATCC-15566) and *Candida albicans* ATCC-10231 as fungal strains. The antimicrobial action expanded by expanding the electron donating group in position 2 and 5 for Pyrido[2,3-*d*]pyrimidin-4-one core. Derivatives **13**, **14**, **15**, **16** and **12**; individually appeared hopeful anti-microbial action towards all strains utilized with inhibition zone higher than that of standard reference drug with lowest MIC.

1. Introduction

Chemistry for hydrazinyl derivatives are growing interest [1, 2, 3, 4]. Hydrazinyls are extensively considered as promising reactions intermediates which can be readily undergo various reactions through ring closure [5, 6]. Hydrazinyl moiety possessing heterocyclic compounds displaying a wide spectrum of pharmacological activities which depend on the nature of incorporated substituent. Hydrazinyl derivatives display biological interesting as antioxidant [4, 7], anti-inflammatory [8], anti-convulsant [9], analgesic [10], antimicrobial [11, 12], anticancer [13], antiprotozoal [14], antiparasitic [15, 16], cardioprotective [17, 18], antitubercular [19] and anti-HIV [20, 21]. Hydrazinyls derivative are used as common drug diseases for malaria [22], tuberculosis [23] and mental disorders.

Heterocyclic derivatives builded on Pyrido[2,3-*d*]pyrimidine backbone have received much attention of synthetic chemist in the last few decades and still study is going on [24, 25, 26]. Pyrido[2,3-*d*]pyrimidine cores have been cited as a great nucleus with enormous area for pharmaceutical action value such as antimicrobial [27, 28, 29], anti-inflammatory [30, 31], antitumor [32, 33, 34] and antiviral [35, 36]. Moreover, some of pyrido[2,3-*d*]pyrimidines displayed phosphodiesterase inhibitory [37].

One of the most public health problems is considered in the raise resistance of known antibiotic and it can interpret why remediation for bacterial and fungal infections is disappointing to save countless millions of human lives [38]. So, development of unprecedented drugs with antibacterial and antifungal potency is a significant solution to beat drug-resistance troubles [39]. Researchers and scientists around the world work hard to develop new materials or derivatives for facing the pathogenic microbe's distribution in the environment [40, 41, 42]. These materials may be produced by microbial [43]or plant sources [44]as well as chemical synthesis [45, 46, 47].

According to which we mentioned high; we attempts to combine hydrazine function group and pyrido[2,3-*d*]pyrimidin-4(*3H*)-one together with a view to obtain more potent antimicrobial analogues. As a part of our program aims to develop synthesis of new biologically active heterocycles [45, 48, 49, 50, 51], In this study we are targeted to synthesize certain new pyrido[2,3-*d*]pyrimidin-4(*3H*)-ones with thiophene

* Corresponding author.
E-mail address: mahmoudelshahat@gmail.com (M. El-Shahat).

https://doi.org/10.1016/j.heliyon.2020.e04956
Received 17 July 2020; Received in revised form 15 August 2020; Accepted 14 September 2020

nucleus at position 7 and various substituted at position 5 bearing arylidene hydrazinyl groups and study their antimicrobial influence.

2. Results and discussion

2.1. Chemistry

Pyrido[2,3-*d*]pyrimidines analogue preparation starting from *α*,*β*-unsaturated ketones is proceeded through an electrophilic attack on the position 5 of the pyrimidine derivatives like 2,4-diamino-pyrimidin-6(1*H*)-one, 6-amino-1-ethyl-1*H*-pyrimidine-2,4-dione, 6-aminouracil, 6-amino-2-thiouracil and 6-amino-1,3-dimethyluracil [52, 53]. 3-(Aryl)-1-(thiophen-2-yl)prop-2-en-1-ones **3a**, **b** heated with 6-amino-2, 3-dihydro-2-thioxopyrimidin-4(1*H*)-one (**4**) in presence of dimethylformamide as a solvent to give 5-(aryl)-2,3-dihydro-7-(thiophen-2-yl)-2-thioxopyrido[2,3-*d*]pyrimidin-4(1*H*)-ones (**5a, b**), respectively (Figure 1). The constitute of thioxopyrido[2,3-*d*] pyrimidin-4(1*H*)-ones **5** were supported by the principle of its spectroscopic data; IR and ^{1}H-NMR spectra elucidated characteristic peaks attributed to the pyrimidine NH groups and absences of amino group significant peak. Additionally, NMR spectra display signals for both of Pyridine proton and carbons; respectively (c.f. experimental, Figure 1).

2-Thioxopyridopyrimidines **5** undergoes reacting with hydrazine hydrate afforded the corresponding 2-hydrazinylpyrido[2,3-*d*]pyrimidin-4(3H)-ones **6** in excellent yield (Scheme 1). The IR spectrum of latter derivatives presented new peaks corresponding to hydrazinyl group with absence for one peak belong to pyrimidine ring NH peaks. ^{1}H NMR data of compound **6a,b** exhibited new two exchangeable signals refer to hydrazinyl protons with disappearance for one NH pyrimidine ring signal. Furthermore; ^{13}C NMR data of compound **6a,b** showed absence for characteristic significant signal for C=S group(c.f. experimental, Figure 1).

2-Hydrazinylpyrido[2,3-*d*]pyrimidin-4(3H)-ones **6** is regards as vital starting material for preparation of many diverse pyrido[2,3-*d*]

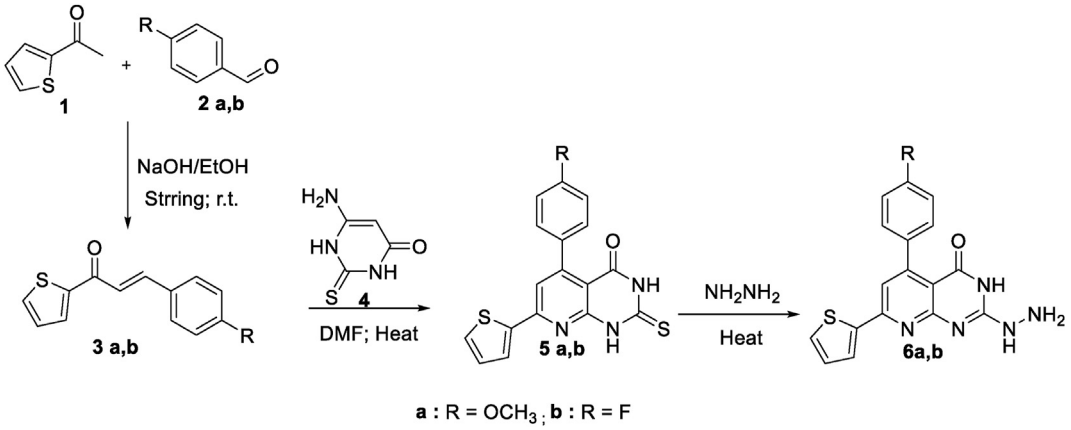

Figure 1. Pathway for preparation of 2-hydrazinylpyrido[2,3-*d*]pyrimidin-4(3*H*)-ones **6a**,**b**.

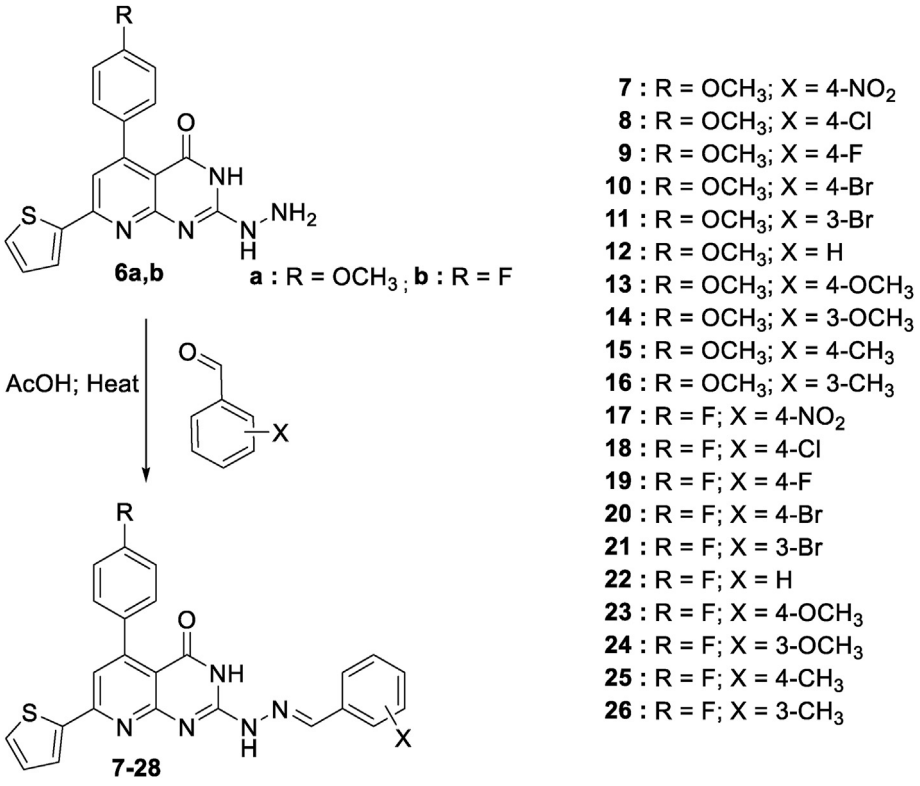

Figure 2. Synthetic pathways for preparation of arylidene hydrazinyl pyrido [2,3-*d*] pyrimidin-5-ones **7-26**.

Table 1. Anti-microbial activity of new synthesized Pyrido[2,3-*d*]pyrimidin-4-one **5**a,b; **6**a,b and hydrazinyl Pyrido[2,3-*d*]pyrimidin-4-one **7-26** derivatives (mm)*.

Compounds No.	Gram-positive bacteria		Gram-negative bacteria		Fungi
	St.	B.C.	E.C.	Salm.	C. Alb.
5a	12	11	12	13	12
6a	17	16	13	17	18
6b	-	-	12	13	16
9	21	19	23	21	28
12	26	20	25	27	31
13	35	33	40	35	35
14	35	32	35	30	35
15	28	23	27	30	38
16	25	25	30	21	25
23	21	19	23	26	30
24	18	19	22	25	28
25	18	20	19	20	21
26	18	17	20	20	28
Ampicillin	25	20	16	19	9
Vancomycin	14	15	15	17	15

* The diameter of the well (6 mm) is included.

pyrimidines derivative. The existence of a hydrazinyl group in compounds **6** has been assured by condensation with diverse aromatic aldehydes. So, A series of 2-(2-arylidenehydrazinyl)-5-(aryl)-7-(thiophen-2-yl)pyrido[2,3-*d*]pyrimidin-4(3H)-ones **7-26** derivatives prepared by reaction of compounds **6** with various aromatic aldehydes in presence of a acid produced the corresponding arylidene hydrazinyl derivatives **7-26** with high yield values. The structure of arylidene hydrazinyl derivatives **7-26** were elucidated via all possible spectral analysis. Both of IR and ^{1}H-NMR spectra of compounds **7-26** presented the absence of the amino group signals for 2-hydrazinylpyrido[2,3-*d*]pyrimidin-4(*3H*)-ones analoges besides existence of signals assigned to the benzylic proton and new arylidene SP^2 protons in ^{1}H-NMR spectra. ^{13}C-NMR spectra for **7-26** derivatives showed signals characteristic for new arylidene SP^2 carbon atoms (c.f. experimental, Figure 2; Figures S1–S40).

2.2. Anti-microbial activity

One of the main health attention globally is the microbial infections that occurred by multi-drug resistant microbial species. In particular, these infections are occured via the gram positive bacteria, *Staphylococcus aureus* and species of the genus *Enterococcus* in hospitals and communities. The semi-synthetic streptogramins, quinupristin/dalfopristin and daptomycin as some of most applicable antibacterial agents are disadvantageous with serious side effects. These side effects have challenged to the researchers to improve a new modern potent biocidal agents [54].

In this study, series of certain pyrido[2,3-*d*]pyrimidin-4-one **5**a,b; **6**a,b and hydrazinyl pyrido[2,3-*d*]pyrimidin-4-ones **7-26** analogue have been prepared to evaluate their biocidal activities against gram positive bacteria *Staphylococcus aurous* ATCC- 47077 (St.), *Bacillus cereus* ATCC-12228 (B.C.), and gram negative bacteria species *Escherichia coli* ATCC-25922 (E.C.), *Salmonella typhi* ATCC-15566 (Salm.), in addition to fungi strain namly; *Candida albicans* ATCC-10231 (C. Alb.). Antimicrobial activity results were illustrated in Table 1 and Figure 3. Antibacterial activity varied between all tested compounds.

The anti-bacterial and antifungal efficiency of the recently prepared pyrido[2,3-*d*]pyrimidin-4-ones and hydrazinylpyrido[2,3-*d*]pyrimidin-4-ones are illustrated in Table (1). From the data, it is observed that, compound **6a** which contain 4-methoxyphenyl group in position 5 revealed a higher antimicrobial activity than compound **6b** which contain 4- fluorophenyl group in position 5; compound **5a** show higher activity than **6b** due to the existence of electron donating group in position 5 and less activity than **6a** which contain the same electron donating group in the same position due to presence of hydrazinyl group in position 2 in compound **6a** rather than **5a**; the compounds **5a**; **6a,b** display moderate antibacterial and antifungal activity when compared to reference drugs. The hydrazinyl pyrido[2,3-*d*]pyrimidin-4-one analogues **12-16** revealed excellent antimicrobial activity action against all strain used when compared with common reference drugs within higher inhibition zones. All of hydrazinylpyrido[2,3-*d*]pyrimidin-4-one analogues **12-16** contain the same electron donating group in position 5 (R = OCH_3) also contain various electron donating group at phenyl ring in position 2; Compound **12** has no substitution on phenyl ring (X = H); Compound **13** has substitution in para position of phenyl ring (X = 4-OCH_3); Compound **14** has substitution in meta position of phenyl ring (X = 3-OCH_3); Compound **15** has substitution in para position of phenyl ring (X = 4-CH_3); Compound **16** has substitution at the meta position of phenyl ring (X = 3-CH_3). Presence of substituent in phenyl ring in position 2 for hydrazinylpyrido[2,3-*d*]pyrimidin-4-one analogues play vital role to increase both of anti bacterial and antifungal action; The activities against all the used microbial strains increased by increasing electron donating in position 2 so compound activity order is 13 > 14> 15 > 16 >12 > reference common drugs.

The hydrazinylpyrido[2,3-*d*]pyrimidin-4-one analogues **9** and **23-26** revealed good antimicrobial activity action against all strain used when compared with common reference drugs with inhibition zone equal or slightly less than reference drugs. hydrazinylpyrido[2,3-*d*]pyrimidin-4-

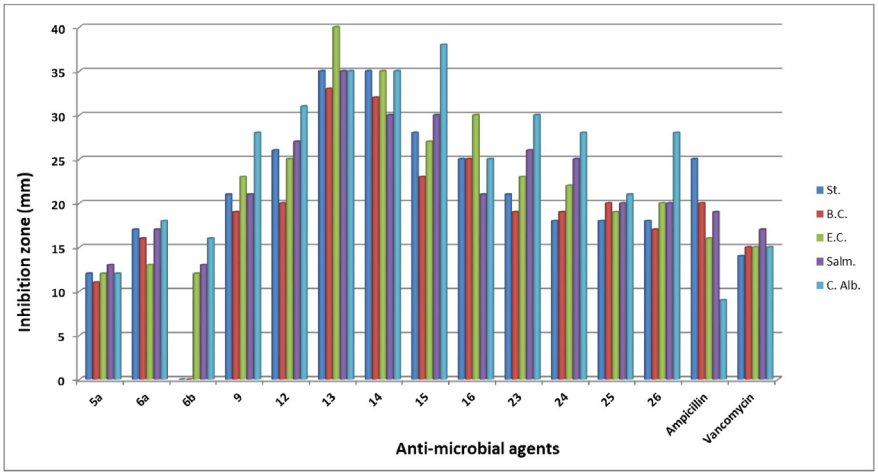

Figure 3. Anti-microbial activity of new synthesized Pyrido[2,3-*d*]pyrimidin-4-one **5**a,b; **6**a,b and hydrazinyl pyrido[2,3-*d*]pyrimidin-4-one **7-26** derivatives.

Table 2. Minimum Inhibition Concentration (ppm) of new synthesized Pyrido[2,3-*d*]pyrimidin-4-one **5**a,b; **6**a,b and hydrazinyl Pyrido[2,3-*d*]pyrimidin-4-one **7-26** derivatives.

Compounds No.	Gram-positive bacteria		Gram-negative bacteria		Fungi
	St.	B.C.	E.C.	Salm.	C. Alb.
5a	100	100	100	100	100
6a	66.7	66.7	100	66.7	40
6b	200	200	100	100	66.7
9	25	25	18.2	25	16.7
12	16.7	25	16.7	16.7	13.3
13	10	13.3	8	10	10
14	10	13.3	10	13.3	10
15	16.7	20	16.7	13.3	8
16	20	20	13.3	25	20
23	25	40	16.7	20	13.3
24	40	40	20	20	16.7
25	40	25	25	25	25
26	40	66.7	25	25	16.7

one derivative **9** has electron donating group in position 5 (R = OCH_3) and electron withdrawing substitution in para position of phenyl ring (X = 4-F); while hydrazinyl pyrido[2,3-*d*]pyrimidin-4-one derivatives **23-26** have electron withdrawing group in position 5 (R = F) with various electron donating group in phenyl ring at position 2; Compound **23** has substitution in para position of phenyl ring (X = 4-OCH_3); Compound **24** has substitution in meta position of phenyl ring (X = 3-OCH_3); Compound **25** has substitution in para position of phenyl ring (X = 4-CH_3); Compound **26** has substitution in meta position of phenyl ring (X = 3-CH_3). Existence of electron donating group in position 5 raise the anti microbial action so the activity of compound **9** > **23-26**; while the activity of compounds **23-26** which contain electron withdrawing group improved by increased electron donating substitute in position 7 i.e activity order 23 > 24> 25 > 66 >12.

Minimal Inhibitory Concentration (MIC) of the examined novel Pyrido[2,3-*d*]pyrimidin-4-one and hydrazinylpyrido[2,3-*d*]pyrimidin-4-one nucleus was studied after utilizing various concentrations of each new derivatives and studied about the least concentration gave inhibition of the studied pathogenic microbes growth. The results of this part are illustrated in Table 2. These results indicated that some derivatives need concentrations over 200 ppm to effect on microbial growth. Furthermore, derivatives 13; 14 and 15 required 8–10 ppm of concentration to kill the studied microbes. For all indications, the MIC of the studied novel derivatives is depended on both of the class of derivative and the type of microbes. The MIC diversified among all new synthetic derivatives and between pathogenic microbes into the same derivative.

2.3. Stracture activity relationship (SAR)

The synthesis of novel high bioactive pyridopyrimidine heterocyclic derivatives is important because pyrimidine derivatives are commercially important and they have unique application in medicinal drugs. However, it is still very challenging to develop suitable pyridopyrimidine derivatives capable of producing high biological activity. This work gives some details about how we can improve the antibacterial and antifungal activity for pyrido[2,3-*d*]pyrimidin-4-one. We introduce arylidene hydrazinyl moiety to pyrido[2,3-*d*]pyrimidin-4-one framework and study the effect of substituted electron donating/withdrawing group in positions number 2 and 5. compound **6a** which contain (4-OMe.C_6H_4) group in position 5 (R = OMe) revealed a higher antimicrobial activity than compound **6b** which contain (4-F.C_6H_4) group in position 5 (R = F); compound **5a** (R = OMe; R' = SH) show higher activity than **6b** (R = F; R' = $NHNH_2$) due to existence of electron donating group in position 5 and less activity than **6a** (R = OMe; R' = $NHNH_2$) which contain the same electron donating group in the same position due to presence of (-$NHNH_2$) group in position 2 in compound **6a** rather than **5a**; the compounds **5a**; **6a,b** show moderate both of antibacterial and antifungal action when compared to reference drugs (Ampicillin and Vancomycin) Figure (4a). The hydrazinylpyrido[2,3-*d*]pyrimidin-4-one analogues **12-16** revealed excellent antimicrobial activity action against all strain when compared with common reference drugs within higher inhibition zones. All of hydrazinylpyrido[2,3-*d*]pyrimidin-4-one analogues **12-16** contain the same electron donating group in position 5 (R = OMe) but contain electron donating substituted group at phenyl ring in position 2; Compound **12** has no substitution on phenyl ring (X = H); Compound **13** has substitution at para position of phenyl ring (X = 4-OMe); Compound **14** has substitution at meta position of phenyl ring (X = 3-OMe); Compound **15** has substitution at para position of phenyl ring (X = 4-Me); Compound **16** has substitution at meta position of phenyl ring (X = 3-Me). The substituent at phenyl ring in position 2 for hydrazinyl Pyrido[2,3-*d*]pyrimidin-4-one analogues play vital role to enhancement both of anti

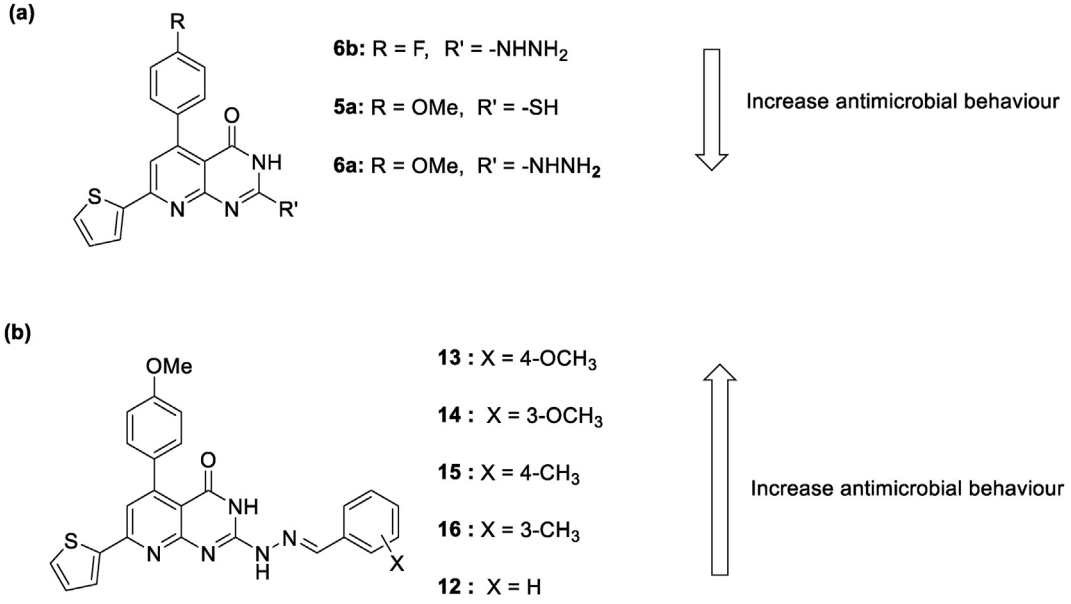

Figure 4. Summary of SAR of (a) pyrido[2,3-*d*]pyrimidin-4(3*H*)-ones derivatives; (b) arylidene hydrazinyl pyrido [2,3-*d*] pyrimidin-5-ones derivatives.

bacterial and antifungal action; The activities against all the used microbial strains increased by increasing electron donating in position 2; so compound activity order is **13** > **14**> **15** > **16** >**12** > reference common drugs Figure (4b).

3. Conclusion

This study represents a simple and proper route for the preparation of certain Pyrido[2,3-*d*]pyrimidin-4-one **5**a,b; **6**a,b and hydrazinylpyrido [2,3-*d*]pyrimidin-4-one **7-26** derivatives. The antimicrobial potency was estimated against four bacterial species and one fungal strain. Compounds **13**, **14**, **15**, **16** and **12**; respectively showed promising antimicrobial potency which showed the highest activities when compared with the standard reference drug. Incorporation of electron donating group into pyrido[2,3-*d*]pyrimidin-4-one in both of position 5 and phenyl ring in position 2 resulted in the production of superior anti-candidal laborers.

4. Experimental

4.1. Chemistry

4.1.1. General

Melting points are determined by utilizing digital melting point apparatus (Electro-Thermal IA 9100; Büchi, Flawil, Switzerland). Infrared spectra are listed on a Perkin-Elmer 1600 FTIR (Perkin-Elmer, Waltham, MA, USA) discs. NMR spectra are resolved on a Jeol-Ex-300 NMR spectrometer (JEOL, Tokyo, Japan) and chemical shift was represented as part per million; (δ values, ppm) against TMS as internal reference. Chemical shifts for both of ^{1}H and ^{13}C are pointed to the solvent signal (DMSO) at 2.50 and 39.52 ppm, respectively. Data are presented as follows: chemical shift, integration, multiplicity (br = broad, s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet) and coupling constant in Hertz (Hz). Mass spectra are hold at 70 eV with a Finnigan SSQ 7000 spectrometer (Thermo Electron Corporation, Madison, WI, USA) utilizing EI and the values of *m/z* are expressed by Dalton. Elemental analyses are done on a Perkin-Elmer 2400 analyzer (Perkin-Elmer) with agreeable range (±0.30) of the calculated values. Monitoring of the reaction and purity of new compounds was achieved by using thin layer chromatography on silica gel pre-coated aluminum sheets (type 60 F254, Merck, Darmstadt, Germany). All chemical reagents and solvents were purchased from Aldrich (Munich, Germany). Compounds **2** [54]; **3a,b** [55] and **5a,b** [11] were synthesized according to their previous method.

4.1.2. General procedure for preparation of compounds *6a,b*

Under solvent free reaction condition; heated an equal molar ratio of hydrazine hydrate (99%) and 2-thioxopyridopyrimidine **5a**,**b** for 2h. Then stand the reaction at room temperature to cool. Collect the formed precipitate after cooling and re-crystallized from EtOH: DMF mixture (v: v; 2:1).

*4.1.2.1. 2-hydrazinyl-5-(4-methoxyphenyl)-7-(thiophen-2-yl)pyrido[2,3-d] pyrimidin-4(3H)-one (**6a**).* Yield 92%, mp 266–268 °C; IR (KBr, υ, cm^{-1}): 3344(NH_2); 32895(NH); 3222(NH), 1677 (C=O). ^{1}H NMR (DMSO-d_6, 300 MHz): δ (ppm) 3.78 (s, 3H, OCH_3), 5.40 (br s, 2H, NH_2; D_2O exchangeable), 6.14 (br s, 1H, NH; D_2O exchangeable), 6.95–6.99 (m, 2H, thiophene-H+ pyrimidine NH; D_2O exchangeable), 7.17–7.34 (m, 5H, 4 Ar-H + pyridine-H), 7.46 (d, J = 3.8 Hz, 1H, thiophene -H), 7.50 (d, J = 3.5 Hz, 1H, thiophene-H). ^{13}C NMR (75 MHz, DMSO) δ = 160.15, 153.09, 150.00, 141.23, 132.11, 129.00, 128.66, 127.80, 127.80, 126.37, 124.55, 123.76, 115.31, 114.17, 101.14, 55.33. MS, m/z (%): 365 (M^+, 88); Anal. Calcd. for $C_{18}H_{15}N_5O_2S$ (365.09): C, 59.17; H, 4.15; N, 19.18; S, 8.77. Found: C, 58.90; H, 3.89; N, 18.94; S, 8.50.

*4.1.2.2. 5-(4-fluorophenyl)-2-hydrazinyl-7-(thiophen-2-yl)pyrido[2,3-d] pyrimidin-4(3H)-one (**6b**).* Yield 87%, mp 277–279 °C; IR (KBr, υ, cm^{-1}): 3340(NH_2); 3295(NH); 3220(NH), 1689 (C=O). ^{1}H NMR (DMSO-d_6, 300 MHz): δ (ppm) 5.47 (br s, 2H, NH_2; D_2O exchangeable), 6.19 (br s, 1H, NH; D_2O exchangeable), 6.97–7.00 (m, 2H, thiophene-H+ pyrimidine NH; D_2O exchangeable), 7.19–7.37 (m, 5H, 4 Ar-H + pyridine-H), 7.49 (d, J = 3.8 Hz, 1H, thiophene -H), 7.52 (d, J = 3.5 Hz, 1H, thiophene-H). ^{13}C NMR (75 MHz, DMSO) δ = 159.97, 153.49, 150.10, 141.90, 132.53, 129.08, 128.94, 127.88, 126.97, 124.75, 123.97, 115.67, 114.34, 101.27. MS, m/z (%): 353 (M^+, 69); Anal. Calcd. for $C_{17}H_{12}FN_5OS$ (353.07): C, 57.78; H, 3.42; N, 19.83; S, 9.07. Found: C, 57.55; H, 3.16; N, 19.58; S, 8.80.

4.1.3. Common method for synthesis of compounds *7–26*

In 15 ml glacial acetic acid; heated an equal molar ratio of compounds **6** and diversified aldehydes under reflux condition for 4h. Then stand the reaction at room temperature to cool. Collect the formed precipitate after cooling and re-crystallized from EtOH: DMF mixture (v: v; 1:2).

*4.1.3.1. 5-(4-methoxyphenyl)-2-(2-(4-nitrobenzylidene)hydrazinyl)-7-(thiophen-2-yl)pyrido [2,3-d]pyrimidin-4(3H)-one(**7**).* Yield 65%; m.p. over 310 °C; IR (KBr, υ, cm^{-1}): 3382 and 3371(2NH), 1685(C=O). ^{1}H NMR (DMSO-d_6, 300 MHz): δ (ppm) 3.82 (s, 3H, OCH_3), 6.96 (d, J = 8.8 Hz, 2H, Ar-H), 7.21 (td, J = 5.3, 3.7 Hz, 1H, Thiophene-H), 7.37–7.45 (m, 3H, 2Ar-H + Pyridine-H), 7.53–7.61 (m, 2H, Ar-H), 7.78 (dd, J = 12.7, 4.5 Hz, 2H, Ar-H), 8.01 (d, J = 3.5 Hz, 1H, Thiophene-H), 8.06 (d, J = 3.8 Hz, 1H, Thiophene-H), 8.37 (s, 1H, N=CH), 11.51 (s, 1H, NH; D_2O exchangeable), 11.91 (brs, 1H, NH; D_2O exchangeable). ^{13}C NMR (75 MHz, DMSO) δ = 160.55, 159.25, 153.10, 151.17, 148.46, 143.77, 143.08, 137.84, 136.97, 136.13, 132.09, 131.43, 130.59, 130.19, 129.16, 128.77, 127.17, 122.30, 116.02, 112.85, 108.02, 55.18. MS, m/z (%): 498 (M^+, 47); Analysis calc. for $C_{25}H_{18}N_6O_4S$ (498.52): C, 60.23; H, 3.64; N, 16.87; S, 6.43. Found: C, 59.96; H, 3.39; N, 16.64; S, 6.18.

*4.1.3.2. 2-(2-(4-chlorobenzylidene)hydrazinyl)-5-(4-methoxyphenyl)-7-(thiophen-2-yl) pyrido[2,3-d]pyrimidin-4(3H)-one(**8**).* Yield 67%; m.p.over 310 °C; IR (KBr, υ, cm^{-1}): 3379 and 3368(2NH), 1677(C=O).^{1}H NMR (DMSO-d_6, 300 MHz): δ (ppm) 3.79 (s, 3H, OCH_3), 6.96 (d, J = 8.7 Hz, 2H,Ar-H), 7.18–7.27 (m, 3H, 2Ar-H + Thiophene-H), 7.45–7.50 (m, 3H, 2Ar-H + Pyridine-H), 7.76 (d, J = 4.9 Hz, 1H, Thiophene-H), 7.90 (d, J = 8.7 Hz, 2H, Ar-H), 8.01 (d, J = 3.2 Hz, 1H, Thiophene-H), 8.06 (s, 1H,N=CH), 11.27 (s, 1H,NH; D_2O exchangeable), 11.70 (s, 1H,NH; D_2O exchangeable). ^{13}C NMR (75 MHz, DMSO) δ = 164.65, 160.87, 160.60, 160.17, 155.38, 152.15, 151.16, 143.92, 135.92, 130.74, 130.63, 129.21, 128.75, 127.92, 127.01, 115.61, 114.35, 114.01, 55.28. MS, m/z (%): 487 (M^+, 35), 489(M^++2, 11); Analysis calc. for $C_{25}H_{18}ClN_5O_2S$ (487.96): C, 61.54; H, 3.72; Cl, 7.26; N, 14.35; S, 6.57. Found: C, 61.32; H, 3.47; Cl, 7.06; N, 14.11; S, 6.37.

*4.1.3.3. 2-(2-(4-fluorobenzylidene)hydrazinyl)-5-(4-methoxyphenyl)-7-(thiophen-2-yl) pyrido[2,3-d]pyrimidin-4(3H)-one (**9**).* Yield 68%; m.p.over 310 °C; IR (KBr, υ, cm^{-1}): 3380 and 3374(2NH), 1682(C=O). ^{1}H NMR (DMSO-d_6, 300 MHz): δ (ppm) 3.81 (s, 3H, OCH_3), 6.96 (d, J = 8.2 Hz, 2H, Ar-H), 7.18–7.27 (m, 3H, 2Ar-H + Thiophene-H), 7.42–7.51 (m, 4H, Ar-H), 7.62 (s, 1H,Pyridine-H), 7.77 (d, J = 4.9 Hz, 1H, Thiophene-H), 8.03 (d, J = 3.8 Hz, 1H, Thiophene-H), 8.11 (s, 1H, N=CH), 11.39 (s, 1H, NH; D_2O exchangeable), 11.83 (s, 1H, NH; D_2O exchangeable). ^{13}C NMR (75 MHz, DMSO) δ = 163.64, 160.93, 160.41, 159.54, 155.29, 152.18, 151.21, 143.83, 135.78, 130.78, 130.67, 129.58, 128.77, 128.05, 120.63, 115.73, 114.36, 114.08, 111.91, 107.99, 55.31. MS, m/z (%): 471 (M^+, 56); Analysis calc. for $C_{25}H_{18}FN_5O_2S$ (471.12): C, 63.68; H, 3.85; N, 14.85; S, 6.80. Found: C, 63.41; H, 3.55; N, 14.61; S, 6.55.

*4.1.3.4. 2-(2-(4-bromobenzylidene)hydrazinyl)-5-(4-methoxyphenyl)-7-(thiophen-2-yl) pyrido[2,3-d]pyrimidin-4(3H)-one (**10**).* Yield 62%; m.p. over 310 °C; IR (KBr, υ, cm^{-1}): 3379 and 3366(2NH), 1680(C=O). ^{1}H NMR (DMSO-d_6, 300 MHz): δ (ppm) 3.80 (s, 3H, OCH_3), 6.97 (d, J = 8.7 Hz, 2H,Ar-H), 7.18–7.28 (m, 3H, 2Ar-H + Thiophene-H), 7.45–7.51 (m, 3H, 2Ar-H + Pyridine-H), 7.77 (d, J = 4.9 Hz, 1H, Thiophene-H), 7.90 (d, J = 8.7 Hz, 2H, Ar-H), 8.02 (d, J = 3.2 Hz, 1H, Thiophene-H), 8.06 (s, 1H, N=CH), 11.28 (s, 1H, NH; D_2O exchangeable), 11.70 (brs, 1H, NH; D_2O exchangeable). ^{13}C NMR (75 MHz, DMSO) δ = 163.51, 160.73, 160.46, 155.25, 152.02, 151.03, 143.78, 135.78, 130.60, 130.60, 130.50, 129.07, 128.61, 127.78, 126.87, 115.47, 114.21, 114.21, 113.92, 113.87, 55.15. MS, m/z (%): 531 (M^+, 39), 533 (M^+ +2, 35); Analysis calc. for $C_{25}H_{18}BrN_5O_2S$ (531.04): C, 56.40; H, 3.41; N, 13.15; S, 6.02. Found: C, 56.14; H, 3.15; N, 12.88; S, 5.77.

*4.1.3.5. 2-(2-(3-bromobenzylidene)hydrazinyl)-5-(4-methoxyphenyl)-7-(thiophen-2-yl) pyrido[2,3-d]pyrimidin-4(3H)-one(**11**).* Yield 54%; m.p. over 310 °C; IR (KBr, υ, cm^{-1}): 3387 and 3370(2NH), 1679(C=O). ^{1}H NMR (DMSO-d_6, 300 MHz): δ (ppm) 3.81 (s, 3H, OCH_3), 6.93–6.98 (m, 2H, Ar-H), 7.20 (td, J = 5.3, 3.7 Hz, 1H, Thiophene-H), 7.32–7.41 (m, 3H,2 Ar-H + Pyridine-H), 7.54 (d, J = 6.0 Hz, 1H, Ar-H), 7.60 (s, 1H, Ar-H), 7.77 (dd, J = 12.7, 4.5 Hz, 2H, Ar-H), 8.00 (d, J = 3.5 Hz, 1H, Thiophene-H), 8.07 (d, J = 3.5 Hz, 1H, Thiophene-H), 8.37 (s, 1H, N=CH), 11.50 (s, 1H, NH; D_2O exchangeable), 11.91 (brs, 1H, NH; D_2O exchangeable). ^{13}C NMR (75 MHz, DMSO) δ = 160.91, 159.25, 153.10, 151.17, 148.45, 148.42, 142.69, 132.09, 131.46, 131.42, 130.58, 130.19, 129.16, 128.76, 127.71, 127.17, 122.29, 116.02, 112.84, 108.02, 55.18. MS, m/z (%): 531 (M^+, 22), 533(M^+, 20); Analysis calc. for $C_{25}H_{18}BrN_5O_2S$ (531.04): C, 56.40; H, 3.41; N, 13.15; S, 6.02. Found: C, 56.19; H, 3.14; N, 12.92; S, 5.77.

*4.1.3.6. 2-(2-benzylidenehydrazinyl)-5-(4-methoxyphenyl)-7-(thiophen-2-yl)pyrido[2,3-d] pyrimidin-4(3H)-one (**12**).* Yield 41%; m.p. over 310 °C; IR (KBr, υ, cm^{-1}): 3387 and 3369 (2NH), 1677(C=O). ^{1}H NMR (DMSO-d_6, 300 MHz): δ (ppm) 3.81 (s, 3H, OCH_3), 6.95–6.98 (m, 2H, Ar-H), 7.18–7.20 (m, 3H, Ar-H), 7.29 (t, J = 7.6 Hz, 1H, Thiophene-H), 7.37–7.40 (m, 2H, Ar-H), 7.44 (s, 1H, Pyridine-H), 7.71 (d, J = 7.9 Hz, 1H, Thiophene-H), 7.76 (dd, J = 6.6, 1.5 Hz, 2H, Ar-H), 8.00 (dd, J = 3.8, 1.2 Hz, 1H, Thiophene-H), 8.11 (s, 1H,N=CH), 11.27 (brs, 2H, 2NH; D_2O exchangeable). ^{13}C NMR (75 MHz, DMSO) δ = 160.68, 159.24, 153.12, 150.68, 137.62, 134.38, 131.34, 130.70, 130.69, 130.43, 130.32, 130.18, 129.94, 128.76, 128.44, 128.43, 127.37, 125.02, 124.17, 115.74, 112.84, 55.19. MS, m/z (%): 453 (M^+, 11); Analysis calc. for $C_{25}H_{19}N_5O_2S$ (453.13): C, 66.21; H, 4.23; N, 15.43; S, 7.08. Found: C, 65.94; H, 3.98; N, 15.18; S, 6.84.

*4.1.3.7. 2-(2-(4-methoxybenzylidene)hydrazinyl)-5-(4-methoxyphenyl)-7-(thiophen-2-yl) pyrido[2,3-d]pyrimidin-4(3H)-one (**13**).* Yield 55%; m.p.over 310 °C; IR (KBr, υ, cm^{-1}): 3379 and 3368 (2NH), 1677(C=O). ^{1}H NMR (DMSO-d_6, 300 MHz): δ (ppm) 3.81 (s, 6H, 2OCH_3), 6.96 (d, J = 8.8 Hz, 4H, Ar-H), 7.19–7.22 (m, 2H, Thiophene-H + Pyridine-H), 7.38 (d, J = 8.7 Hz, 4H, Ar-H), 7.57 (s, 1H, N=CH), 7.81 (dd, J = 5.0, 1.2 Hz, 1H, Thiophene-H), 8.06 (dd, J = 3.8, 1.2 Hz, 1H, Thiophene-H), 12.31 (s, 1H, NH; D_2O exchangeable), 12.99 (s, 1H, NH; D_2O exchangeable). ^{13}C NMR (75 MHz, DMSO) δ = 175.31, 159.56, 158.64, 154.80, 153.34, 152.79, 142.44, 131.70, 130.87, 130.37, 130.26, 129.03, 128.92, 117.69, 112.91, 107.43, 55.24. MS, m/z (%): 483 (M^+, 33); Analysis calc. for $C_{26}H_{21}N_5O_3S$ (483.14): C, 64.59; H, 4.37; N, 14.47; S, 6.62. Found: C, 64.34; H, 4.11; N, 14.25; S, 6.37.

*4.1.3.8. (2-(2-(3-methoxybenzylidene)hydrazinyl)-5-(4-methoxyphenyl)-7-(thiophen-2-yl) pyrido[2,3-d]pyrimidin-4(3H)-one (**14**).* Yield 47%; m.p.over 310 °C; IR (KBr, υ, cm^{-1}): 3384 and 3370(2NH), 1681(C=O). ^{1}H NMR (DMSO-d_6, 300 MHz): δ (ppm) 3.82 (s, 6H, 2OCH_3), 6.94–6.99 (m, 3H, Ar-H), 7.19 (dd, J = 5.2, 3.7 Hz, 1H, Thiophene-H), 7.36–7.44 (m, 5H, Ar-H), 7.61 (s, 1H, Pyridine-H), 7.76 (d, J = 4.9 Hz, 1H, Thiophene-H), 8.01 (d, J = 2.8 Hz, 1H, Thiophene-H), 8.10 (s, 1H, N=CH), 11.31 (brs, 1H, NH; D_2O exchangeable), 11.79 (brs, 1H, NH; D_2O exchangeable). ^{13}C NMR (75 MHz, DMSO) δ = 161.35, 159.56, 159.24, 153.11, 130.68, 130.18, 129.60, 128.76, 120.63, 116.03, 115.43, 115.14, 112.84, 55.33, 55.19. MS, m/z (%): 483 (M^+, 15); Analysis calc. for $C_{26}H_{21}N_5O_3S$ (483.14): C, 64.59; H, 4.39; N, 14.47; S, 6.62. Found: C, 64.35; H, 4.10; N, 14.22; S, 6.38.

*4.1.3.9. 5-(4-methoxyphenyl)-2-(2-(4-methylbenzylidene)hydrazinyl)-7-(thiophen-2-yl) pyrido[2,3-d]pyrimidin-4(3H)-one (**15**).* Yield 54%; m.p.over 310 °C; IR (KBr, υ, cm^{-1}): 3378 and 3365(2NH), 1679(C=O). ^{1}H NMR (DMSO-d_6, 300 MHz): δ (ppm) 2.34 (s, 3H, CH_3), 3.82 (s, 3H, OCH_3), 6.97 (d, J = 7.7 Hz, 2H, Ar-H), 7.22–7.31 (m, 3H, 2Ar-H + Thiophene-H), 7.38–7.44 (m, 3H, 2Ar-H + Pyridine-H), 7.75–7.87 (m, 3H, 2Ar-H + Thiophene-H), 8.01–8.10 (m, 2H, Thiophene-H + N=CH), 11.27 (brs, 2H, 2NH; D_2O exchangeable). ^{13}C NMR (75 MHz, DMSO) δ = 159.24, 154.23, 138.31, 134.96, 134.19, 131.37, 131.34, 130.70, 130.43, 130.18, 128.76, 128.44, 127.89, 127.37, 124.17, 112.37, 55.19, 20.98. MS, m/z (%): 467 (M^+, 22); Analysis calc. for $C_{26}H_{21}N_5O_2S$ (467.14): C, 66.79; H, 4.53; N, 14.98; S, 6.86. Found: C, 66.54; H, 4.28; N, 14.70; S, 6.59.

*4.1.3.10. 5-(4-methoxyphenyl)-2-(2-(3-methylbenzylidene)hydrazinyl)-7-(thiophen-2-yl) pyrido[2,3-d]pyrimidin-4(3H)-one (**16**).* Yield 50%; m.p. over 310 °C; IR (KBr, υ, cm^{-1}): 3376 and 3362(2NH), 1674(C=O). ^{1}H NMR (DMSO-d_6, 300 MHz): δ (ppm) 2.34 (s, 3H,CH_3), 3.82 (s, 3H,O CH_3), 6.95–6.98 (m, 2H,Ar-H), 7.20 (dd, J = 5.0, 3.7 Hz, 2H, Ar-H), 7.30 (t, J = 7.6 Hz, 1H, Thiophene-H), 7.38–7.41 (m, 2H, Ar-H), 7.44 (s, 1H, Pyridine-H), 7.71 (d, J = 7.9 Hz, 1H, Thiophene-H), 7.76 (dd, J = 6.6, 1.5 Hz, 2H, Ar-H), 8.01 (dd, J = 3.8, 1.2 Hz, 1H, Thiophene-H), 8.11 (s, 1H, N=CH), 11.28 (brs, 2H, 2NH; D_2O exchangeable). ^{13}C NMR (75 MHz, DMSO) δ = 159.24, 154.23, 138.30, 134.95, 134.36, 131.34, 130.69, 130.42, 130.18, 128.76, 128.44, 127.37, 124.16, 112.36, 55.19, 20.98. MS, m/z (%): 467(M^+, 13); Analysis calc. for $C_{26}H_{21}N_5O_2S$ (467.14): C, 66.79; H, 4.53; N, 14.98; S, 6.86. Found: C, 66.55; H, 4.28; N, 14.74; S, 6.60.

*4.1.3.11. 5-(4-fluorophenyl)-2-(2-(4-nitrobenzylidene)hydrazinyl)-7-(thiophen-2-yl) pyrido [2,3-d]pyrimidin-4(3H)-one (**17**).* Yield 72%; m.p.over 310 °C; IR (KBr, υ, cm^{-1}): 3384 and 3368(2NH), 1675(C=O). ^{1}H NMR (DMSO-d_6, 300 MHz): δ (ppm) 6.97 (d, J = 8.8 Hz, 2H, Ar-H), 7.20 (dd, J = 5.2, 3.6 Hz, 1H, Thiophene-H), 7.37–7.45 (m, 3H, 2Ar-H + Pyridine-H), 7.56 (d, J = 8.8 Hz, 2H, Ar-H), 7.78 (d, J = 8.6 Hz, 2H, Ar-H), 8.01 (d, J = 3.5 Hz, 1H, Thiophene-H), 8.06 (d, J = 3.8 Hz, 1H, Thiophene-H), 8.37 (s, 1H, N=CH), 11.51 (s, 1H, NH; D_2O exchangeable), 11.91 (s, 1H, NH; D_2O exchangeable). ^{13}C NMR (75 MHz, DMSO) δ = 164.82, 163.71, 161.51, 153.46, 145.90, 137.78, 130.65, 128.78, 128.63, 128.43, 128.05, 126.37, 125.02, 114.35, 114.07. MS, m/z (%): 486 (M^+, 63); Analysis calc. for $C_{24}H_{15}FN_6O_3S$ (486.09): C, 59.26; H, 3.12; N, 17.27; S, 6.58. Found: C, 58.99; H, 2.88; N, 17.01; S, 6.33.

*4.1.3.12. 2-(2-(4-chlorobenzylidene)hydrazinyl)-5-(4-fluorophenyl)-7-(thiophen-2-yl)pyrido [2,3-d]pyrimidin-4(3H)-one (**18**).* Yield 74%; m.p.over 310300 °C; IR (KBr, υ, cm^{-1}): 3386 and 3370(2NH), 1680(C=O). ^{1}H NMR (DMSO-d_6, 300 MHz): δ (ppm) 7.19–7.33 (m, 4H, Ar-H), 7.47–7.51 (m, 4H, 2Ar-H + Pyridine-H + Thiophene-H), 7.72 (d, J = 3.8 Hz, 1H, Thiophene-H), 7.78 (d, J = 6.0 Hz, 2H, Ar-H), 8.03 (d, J = 3.8 Hz, 1H, Thiophene-H), 8.09 (s, 1H, N=CH), 11.31 (brs, 1H, NH; D_2O exchangeable), 11.77 (brs, 1H, NH; D_2O exchangeable). ^{13}C NMR (75 MHz, DMSO) δ = 165.10, 163.99, 161.79, 152.29, 146.18, 138.06, 131.05, 130.94, 130.52, 129.06, 128.71, 128.33, 126.65, 125.29, 114.63, 114.35. MS, m/z (%): 475 (M^+, 40), 477(M^++2, 13); Analysis calc. for $C_{24}H_{15}ClN_5OS$ (475.07): C, 60.57; H, 3.18; Cl, 7.45; N, 14.72; S, 6.74. Found: C, 60.33; H, 2.93; Cl, 7.19; N, 14.44; S, 6.45.

*4.1.3.13. 2-(2-(4-fluorobenzylidene)hydrazinyl)-5-(4-fluorophenyl)-7-(thiophen-2-yl) pyrido[2,3-d]pyrimidin-4(3H)-one (**19**).* Yield 70%; m.p.over 310 °C; IR (KBr, υ, cm^{-1}): 3380 and 3362(2NH), 1678(C=O). ^{1}H NMR (DMSO-d_6, 300 MHz): δ (ppm) 6.96 (d, J = 8.8 Hz, 4H, Ar-H), 7.20 (dd, J = 5.0, 3.8 Hz, 2H, Ar-H), 7.37–7.40 (m, 4H, 2Ar-H + Thiophene-H + Pyridine-H), 7.57 (s, 1H, N=CH), 7.81 (dd, J = 5.0, 1.2 Hz, 1H, Thiophene-H), 8.06 (dd, J = 3.8, 1.2 Hz, 1H, Thiophene-H), 12.31 (s, 1H, NH; D_2O exchangeable), 12.99 (s, 1H, NH; D_2O exchangeable). ^{13}C NMR (75 MHz, DMSO) δ = 163.92, 161.18, 160.69, 155.67, 152.46, 151.54, 144.05, 137.16, 136.05, 132.39, 131.06, 130.95, 130.86, 129.43, 129.06, 128.33, 127.47, 122.56, 116.21, 114.65, 114.36, 108.23. MS, m/z (%): 459 (M^+, 37); Analysis calc. for $C_{24}H_{15}F_2N_5OS$ (459.10): C, 62.74; H, 3.29; N, 15.24; S, 6.98. Found: C, 62.49; H, 3.02; N, 14.97; S, 6.72.

*4.1.3.14. 2-(2-(4-bromobenzylidene)hydrazinyl)-5-(4-fluorophenyl)-7-(thiophen-2-yl) pyrido[2,3-d]pyrimidin-4(3H)-one (**20**).* Yield 73%; m.p.over 310 °C; IR (KBr, υ, cm^{-1}): 3379 and 3363(2NH), 1675(C=O). ^{1}H NMR (DMSO-d_6, 300 MHz): δ (ppm) 7.24 (d, J = 8.0 Hz, 2H, Ar-H), 7.48 (d, J = 7.8 Hz, 2H, Ar-H), 7.58–7.66 (m, 3H, 2 Ar-H + Thiophene-H), 7.84 (d, J = 7.8 Hz, 2H, Ar-H), 7.98 (s, 1H, Pyridine-H), 8.03–8.16 (m, 2H, Thiophene-H), 10.00 (s, 1H, N=CH), 10.67 (s, 1H, NH; D_2O exchangeable), 11.50 (s, 1H, NH; D_2O exchangeable). ^{13}C NMR (75 MHz, DMSO) δ = 161.56, 160.58, 155.31, 152.17, 143.99, 139.46, 137.34, 133.86, 132.33, 130.75, 130.63, 129.74, 129.60, 129.30, 129.14, 128.75, 128.02, 127.60, 114.34, 114.06. MS, m/z (%): 519 (M^+, 11) 521(M^++2, 10); Analysis calc. for $C_{24}H_{15}BrFN_5OS$ (519.02): C, 55.39; H, 2.91; N, 13.46; S, 6.16. Found: C, 55.14; H, 2.66; N, 13.20; S, 5.90.

*4.1.3.15. 2-(2-(3-bromobenzylidene)hydrazinyl)-5-(4-fluorophenyl)-7-(thiophen-2-yl) pyrido[2,3-d]pyrimidin-4(3H)-one (**21**).* Yield 61%; m.p. over 310 °C; IR (KBr, υ, cm^{-1}): 3377 and 3362(2NH), 1675(C=O). ^{1}H NMR (DMSO-d_6, 300 MHz): δ (ppm) 7.18–7.27 (m, 3H, Ar-H), 7.35 (t, J = 7.8 Hz, 1H, Thiophene-H), 7.46–7.56 (m, 4H, 3Ar-H + Pyridine-H), 7.76 (d, J = 5.2 Hz, 1H, Thiophene-H), 7.81 (d, J = 8.0 Hz, 1H, Ar-H), 8.02 (d, J = 3.9 Hz, 1H, Thiophene-H), 8.08 (s, 1H, Ar-H), 8.37 (s, 1H, N=CH), 11.56 (brs, 1H, NH; D_2O exchangeable), 11.84 (brs, 1H, NH; D_2O exchangeable). ^{13}C NMR (75 MHz, DMSO) δ = 163.92, 161.18, 160.69, 155.67, 152.46, 151.53, 144.05, 137.16, 136.05, 132.39, 131.06, 130.95, 130.86, 129.43, 129.06, 128.33, 127.47, 122.56, 116.21, 114.65, 114.36. MS, m/z (%): 519 (M^+, 19), 521(M^++2, 17); Analysis calc. for $C_{24}H_{15}BrFN_5OS$ (519.02): C, 55.40; H, 2.90; N, 13.46; S, 6.16. Found: C, 55.11; H, 2.66; N, 3.19; S, 5.91.

*4.1.3.16. 2-(2-benzylidenehydrazinyl)-5-(4-fluorophenyl)-7-(thiophen-2-yl)pyrido[2,3-d] pyrimidin-4(3H)-one (**22**).* Yield 72%; m.p. over 310300 °C; IR (KBr, υ, cm^{-1}): 3386 and 3370(2NH), 1680(C=O). ^{1}H NMR (DMSO-d_6, 300 MHz): δ (ppm) 7.17–7.31 (m, 6H,5Ar-H + Thiophene-H), 7.45–7.50 (m, 3H, 2Ar-H + Pyridine-H), 7.71 (d, J = 8.2 Hz, 1H, Thiophene-H), 7.76 (d, J = 6.0 Hz, 2H, Ar-H), 8.02 (d, J = 3.8 Hz, 1H, Thiophene-H), 8.08 (s, 1H, N=CH), 11.30 (brs, 1H, NH; D_2O exchangeable), 11.76 (brs, 1H, NH; D_2O exchangeable). ^{13}C NMR (75 MHz, DMSO) δ = 164.82, 163.71, 161.51, 153.46, 145.90, 137.78, 130.77, 130.66, 130.43, 128.78, 128.43, 128.05, 128.04, 126.37, 125.02, 114.93, 113.61. MS, m/z (%): 441(M^+, 30); Analysis calc. for $C_{24}H_{16}FN_5OS$ (441.11): C, 65.30; H, 3.65; N, 15.85; S, 7.26. Found: C, 65.05; H, 3.39; N, 15.66; S, 7.00.

*4.1.3.17. 5-(4-fluorophenyl)-2-(2-(4-methoxybenzylidene)hydrazinyl)-7-(thiophen-2-yl) pyrido[2,3-d]pyrimidin-4(3H)-one (**23**).* Yield 65%; m.p.over 310 °C; IR (KBr, υ, cm^{-1}): 3380 and 3370(2NH), 1675(C=O). ^{1}H NMR (DMSO-d_6, 300 MHz): δ (ppm) 3.79 (s, 3H, OCH_3), 6.96 (d, J = 8.7 Hz, 2H, Ar-H), 7.17–7.26 (m, 3H, 2 Ar-H + Thiophene-H), 7.44–7.50 (m, 3H, 2 Ar-H + Pyridine-H), 7.75 (d, J = 4.9 Hz, 1H, Thiophene-H), 7.89 (d, J = 8.7 Hz, 2H, Ar-H), 8.01 (d, J = 3.2 Hz, 1H, Thiophene-H), 8.05 (s, 1H, N=CH), 11.27 (s, 1H, NH; D_2O exchangeable), 11.69 (brs, 1H, NH; D_2O exchangeable). ^{13}C NMR (75 MHz, DMSO) δ = 163.49, 160.87, 160.60, 155.38, 152.15, 151.16, 143.92, 135.92, 130.74, 130.63, 129.21, 128.75, 127.92, 127.01, 115.61, 114.35, 114.01, 55.28. MS, m/z (%): 471 (M^+, 21); Analysis calc. for $C_{25}H_{18}FN_5O_2S$ (471.12): C, 63.68; H, 3.85; N, 14.85; S, 6.80. Found: C, 63.40; H, 3.57; N, 14.65; S, 6.53.

*4.1.3.18. 5-(4-fluorophenyl)-2-(2-(3-methoxybenzylidene)hydrazinyl)-7-(thiophen-2-yl) pyrido[2,3-d]pyrimidin-4(3H)-one (**24**).* Yield 52%; m.p.over 310 °C; IR (KBr, υ, cm^{-1}): 3386 and 3370(2NH), 1678(C=O). ^{1}H NMR (DMSO-d_6, 300 MHz): δ (ppm) 3.81 (s, 3H, OCH_3), 6.96 (d, J = 8.2 Hz, 1H, Ar-H), 7.18–7.27 (m, 3H, 2 Ar-H + Thiophene-H), 7.33 (d, J = 7.9 Hz, 1H, Ar-H), 7.42–7.51 (m, 4H, Ar-H), 7.61 (s, 1H, Pyridine-H), 7.77 (d, J = 4.9 Hz, 1H, Thiophene-H), 8.02 (d, J = 3.8 Hz, 1H, Thiophene-H), 8.10 (s, 1H, N=CH), 11.39 (brs, 1H, NH; D_2O exchangeable), 11.82 (brs, 1H, NH; D_2O exchangeable). ^{13}C NMR (75 MHz, DMSO) δ = 163.64, 160.92, 160.40, 159.54, 152.18, 151.21, 135.78, 130.78, 130.66, 129.58, 128.77, 128.05, 120.63, 115.73, 114.36, 114.07, 111.91, 55.31. MS, m/z (%): 471 (M^+, 13); Analysis calc. for $C_{25}H_{18}FN_5O_2S$ (471.12): C, 63.68; H, 3.85; N, 14.85; S, 6.80. Found: C, 63.42; H, 3.55; N, 14.62; S, 6.55.

*4.1.3.19. 5-(4-fluorophenyl)-2-(2-(4-methylbenzylidene)hydrazinyl)-7-(thiophen-2-yl)pyrido [2,3-d]pyrimidin-4(3H)-one (**25**).* Yield 49%; m.p.over 310 °C; IR (KBr, υ, cm^{-1}): 3378 and 3365(2NH), 1674(C=O). ^{1}H NMR (DMSO-d_6, 300 MHz): δ (ppm) 2.33 (s, 3H, CH_3), 7.20–7.31 (m, 4H, Ar-H), 7.39–7.58 (m, 3H, 2Ar-H + Thiophene-H), 7.78–7.86 (m, 4H, 2Ar-H + Pyridine-H + Thiophene-H), 8.02–8.10 (m, 2H, Thiophene-H + N=CH), 11.36 (brs, 2H, 2NH; D_2O exchangeable). ^{13}C NMR (75 MHz, DMSO) δ = 161.56, 160.58, 155.31, 152.17, 143.98, 139.46, 137.34, 133.86, 132.33, 130.75, 130.63, 129.74, 129.60, 129.14, 128.75, 128.02, 127.60, 114.34, 114.06, 21.08. MS, m/z (%): 455 (M^+, 28); Analysis calc. for $C_{25}H_{18}FN_5OS$ (455.12): C, 65.92; H, 3.98; N, 15.38; S, 7.04. Found: C, 65.68; H, 3.72; N, 15.11; S, 6.80.

*4.1.3.20. 5-(4-fluorophenyl)-2-(2-(3-methylbenzylidene)hydrazinyl)-7-(thiophen-2-yl)pyrido [2,3-d]pyrimidin-4(3H)-one (**26**).* Yield 41%; m.p.over 310 °C; IR (KBr, υ, cm^{-1}): 3374 and 3362(2NH), 1671(C=O). ^{1}H NMR (DMSO-d_6, 300 MHz): δ (ppm) 2.35 (s, 3H,CH_3), 7.19–7.32 (m, 5H,4Ar-H+ pyridine-H), 7.46–7.51 (m, 3H, 2Ar-H+ thiophene-H), 7.72 (d, J = 8.2 Hz, 1H, thiophene-H), 7.77 (d, J = 6.0 Hz, 2H, Ar-H), 8.03 (d, J = 3.8 Hz, 1H, thiophene-H), 8.09 (s, 1H, N=CH), 11.31 (brs, 1H, NH; D_2O exchangeable), 11.77 (brs, 1H,NH; D_2O exchangeable). ^{13}C NMR (75 MHz, DMSO) δ = 164.65, 159.47, 151.85, 145.90, 137.78, 130.76, 130.65, 130.42, 128.77, 128.42, 128.04, 127.94, 126.37, 125.01, 114.35, 114.07, 20.96. MS, m/z (%): 455 (M^+, 10); Analysis calc. for $C_{25}H_{18}FN_5OS$ (455.12): C, 65.92; H, 3.98; N, 15.38; S, 7.04. Found: C, 65.67; H, 3.73; N, 15.15; S, 6.77.

4.2. Anti-microbial activity

The antimicrobial action of the tested samples were examined against some targeted pathogenic microorganisms gained from the American kind culture collection (ATCC; Rockville, MD, USA). The tested organisms were *Staphylococcus aurous* ATCC- 47077 (St.), *Bacillus cereus* ATCC-12228 (B.C.), *Escherichia coli* ATCC-25922 (E.C.), *Salmonella typhi* ATCC-15566 (Salm.) and *Candida albicans* ATCC-10231 (C. Alb.). The stock cultures of pathogens utilized in this study were keeped on nutrient agar slants at 4 °C. The Agar well diffusion procedure was employed to study the antimicrobial activities of the samples according to the method described [56, 57]. Reference antibacterial drugs ampicillin and vancomycin were estimated for their antibacterial and antifungal potency and compared with the tested samples. Seventy microliters of bacterial and yeast cells (10^6 CFU/mL) were spread on plates of nutrient agar media.

The wells (6 mm diameter) were excavated on the injected agar plates then 100 μl of the samples and its derivatives suspended in DMSO, were added up to the wells. The reference antibiotics disks (10 and 30 μg/disk of ampicillin and vancomycin, respectively) were potted onto surface of agar inoculated plates. The plates were kept at 4 °C for 2h before incubation to permit diffusion to occur. The plates were kept at 37 °C for 24 h except yeast strain that were incubated at 28 °C for 24h then followed by measure of the diameter of the inhibition zone (mm), and this was replicate for five times and the average was taken [56, 58, 59, 60, 61].

4.2.1. Minimum inhibition concentration (MIC)

The MIC was known as the concentration at which the bacteria and yeast do not presented visible growth with regard to the positive control and was done for new synthesized samples with a little modulation for previous reported procedure [62]. In summarized, serial dilutions were prepared for the examined materials dissolved in DMSO. 150 μL of double strength Mueller Hinton broth medium were loaded in each well of the 96 well micro liter plate followed up by 150 μL of the 2-fold appropriate concentration and mixed well to gain the final concentration. After 24h broth cultures of the screened bacterial and yeast strains prepared as an inoculums of 5 % (V/V) (OD = 0.5 McFarland standard) was inoculated into the respective wells. For the positive growth control, the same inoculums size of each test strain was inoculated in wells that didn't including any of the screened materials. DMSO solution was evaluated as negative control. The plates were statically incubated for 24 h at 37 °C. 30 μL of prepared solution (0.18 %) was added to each well to work as an electron acceptor and the inhibition of bacterial growth was easily visible as a dark blue well and the presence of growth was noticed by existence of red, pink or purple color.

4.2.2. Statistical analysis

Statistical analyses were accomplished utilizing GraphPad Prism 5.0 (Graph Pad Software, LaJolla, CA). In One-way model ANOVA, the detected variance is divided into sections due to different explanatory variables. A level of P less than 0.05 was count to be statistically significant.

Declarations

Author contribution statement

Reda M. Abdelhameed: conceived and designed the experiments; analyzed and interpreted the data; contributed reagents, materials, analysis tools or data; wrote the paper.

Osama M. Darwesh: performed the experiments; analyzed and interpreted the data; contributed reagents, materials, analysis tools or data.

Mahmoud El-Shahat: conceived and designed the experiments; performed the experiments; analyzed and interpreted the data; contributed reagents, materials, analysis tools or data; wrote the paper.

Funding statement

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Competing interest statement

The authors declare no conflict of interest.

Additional information

Supplementary content related to this article has been published online at https://doi.org/10.1016/j.heliyon.2020.e04956.

References

[1] R.A. Kudirka, A.E. Albers, R.M. Barfield, D. Rabuka, Hydrazinyl-indole Compounds and Methods for Producing a Conjugate, Google Patents, 2019.
[2] M. Foroughi Kaldareh, M. Mokhtary, M. Nikpassand, Deep eutectic solvent mediated one-pot synthesis of hydrazinyl-4-phenyl-1, 3-thiazoles, Polycycl. Aromat. Comp. (2019) 1–8.
[3] G. Hussein, S. Swafy, O. Ebian, Adsorption of uranium from sulfate leach liquor using rice straw impregnated with chlorophenyl hydrazinyl pyrazoloquinazolinon, Separ. Sci. Technol. 54 (11) (2019) 1764–1777.
[4] U. Salar, K.M. Khan, S. Chigurupati, S. Syed, S. Vijayabalan, A. Wadood, M. Riaz, M. Ghufran, S. Perveen, New hybrid scaffolds based on hydrazinyl thiazole substituted coumarin; as novel leads of dual potential; in vitro α-amylase inhibitory and antioxidant (DPPH and ABTS radical scavenging) activities, Med. Chem. 15 (1) (2019) 87–101.
[5] A.-I. Pricopie, I. Ionuț, G. Marc, A.-M. Arseniu, L. Vlase, A. Grozav, L.I. Găină, D.C. Vodnar, A. Pîrnău, B. Tiperciuc, Design and synthesis of novel 1, 3-thiazole and 2-hydrazinyl-1, 3-thiazole derivatives as anti-Candida agents: in vitro antifungal screening, molecular docking study, and spectroscopic investigation of their binding interaction with bovine serum albumin, Molecules 24 (19) (2019) 3435.
[6] S. Chuprakov, R.A. Kudirka, J.M. McFarland, A.W. Garafalo, D. Rabuka, Hydrazinyl-Substituted Heteroaryl Compounds and Methods for Producing a Conjugate, Google Patents, 2019.
[7] A. Grozav, I.-D. Porumb, L. Găină, L. Filip, D. Hanganu, Cytotoxicity and antioxidant potential of novel 2-(2-((1H-indol-5yl) methylene)-hydrazinyl)-thiazole derivatives, Molecules 22 (2) (2017) 260.
[8] R. Sreepriya, S.S. Kumar, V. Sadasivan, S. Biju, S.S. Meena, Synthesis, characterization & biological studies of Mn (II), Fe (III) and Co (II) complexes of (Z)-1, 5-dimethyl-4-(2-(2-oxopropylidene) hydrazinyl)-2-phenyl-1H-pyrazol-3 (2H)-one, J. Mol. Struct. 1201 (2020) 127110.
[9] K.Z. Łączkowski, N. Konklewska, A. Biernasiuk, A. Malm, K. Sałat, A. Furgała, K. Dzitko, A. Bekier, A. Baranowska-Łączkowska, A. Paneth, Thiazoles with cyclopropyl fragment as antifungal, anticonvulsant, and anti-Toxoplasma gondii agents: synthesis, toxicity evaluation, and molecular docking study, Med. Chem. Res. 27 (9) (2018) 2125–2140.
[10] N.M.H. Khalifa, M.A. Al-Omar, Naphthyridinyl Hydrazine Derivatives as Potent Peripheral Analgesic Agents, Google Patents, 2018.
[11] A. Biernasiuk, M. Kawczyńska, A. Berecka-Rycerz, B. Rosada, A. Gumieniczek, A. Malm, K. Dzitko, K.Z. Łączkowski, Synthesis, antimicrobial activity, and determination of the lipophilicity of ((cyclohex-3-enylmethylene) hydrazinyl) thiazole derivatives, Med. Chem. Res. 28 (11) (2019) 2023–2036.
[12] S. Paliwal, S. Sharma, J. Dwivedi, A. Mishra, Synthesis of novel substituted phenyl-3-hydrazinyl-quinoxaline-2-amine derivatives: evaluation of antimicrobial activity and its molecular docking studies, J. Heterocycl. Chem. 54 (6) (2017) 3689–3695.
[13] I. Jęśkowiak, M. Mączyński, J. Trynda, J. Wietrzyk, S. Ryng, The 5-hydrazino-3-methylisothiazole-4-carboxylic acid, its new 5-substituted derivatives and their antiproliferative activity, Bioorg. Chem. (2019) 103082.
[14] L. Popov, A. Zubenko, L. Fetisov, Y.D. Drobin, A. Klimenko, A. Bodryakov, S. Borodkin, I. Melkozerova, The synthesis of (1, 3, 4-Oxadiazol-2-yl) acrylic acid derivatives with antibacterial and protistocidal activities, Russ. J. Bioorg. Chem. 44 (2) (2018) 238–243.
[15] M.V. de Oliveira Cardoso, G.B. de Oliveira Filho, L.R.P. de Siqueira, J.W.P. Espíndola, E.B. da Silva, A.P. de Oliveira Mendes, V.R.A. Pereira, M.C.A.B. de Castro, R.S. Ferreira, F.S. Villela, 2-(phenylthio) ethylidene derivatives as anti-Trypanosoma cruzi compounds: structural design, synthesis and antiparasitic activity, Eur. J. Med. Chem. 180 (2019) 191–203.
[16] G. Subramanian, C.B. Rajeev, C.D. Mohan, A. Sinha, T.T. Chu, S. Anusha, H. Ximei, J.E. Fuchs, A. Bender, K.S. Rangappa, Synthesis and in vitro evaluation of hydrazinyl phthalazines against malaria parasite, Plasmodium falciparum, Bioorg. Med. Chem. Lett 26 (14) (2016) 3300–3306.
[17] X.Z. Zheng, J.L. Zhou, J. Ye, P.P. Guo, C.S. Lin, Cardioprotective effect of novel sulphonamides-1, 3, 5-triazine conjugates against ischaemic–reperfusion injury via selective inhibition of MMP-9, Chem. Biol. Drug Des. 88 (5) (2016) 756–765.
[18] M. Bigdeli, M. Sabbaghan, M. Esfahanizadeh, F. Kobarfard, S. Vitalini, M. Iriti, J. Sharifi-Rad, Synthesis of imine congeners of resveratrol and evaluation of their anti-platelet activity, Molbank 1 (2019) M1039, 2019.
[19] F. Turkan, A. Cetin, P. Taslimi, İ. Gulçin, Some pyrazoles derivatives: potent carbonic anhydrase, α-glycosidase, and cholinesterase enzymes inhibitors, Archiv der pharmazie 351 (10) (2018) 1800200.
[20] R. Meleddu, S. Distinto, A. Corona, G. Bianco, V. Cannas, F. Esposito, A. Artese, S. Alcaro, P. Matyus, D. Bogdan, (3Z)-3-(2-[4-(aryl)-1, 3-thiazol-2-yl] hydrazin-1-ylidene)-2, 3-dihydro-1H-indol-2-one derivatives as dual inhibitors of HIV-1 reverse transcriptase, Eur. J. Med. Chem. 93 (2015) 452–460.
[21] A. Rauf, M.K. Kashif, B.A. Saeed, N.A. Al-Masoudi, S. Hameed, Synthesis, anti-HIV activity, molecular modeling study and QSAR of new designed 2-(2-arylidenehydrazinyl)-4-arylthiazoles, J. Mol. Struct. 1198 (2019) 126866.
[22] M. Sharma, P. Prasher, An epigrammatic status of the 'azole'-based antimalarial drugs, RSC Med. Chem. (2020).
[23] M.A. Lopes-Ortiz, M.R. Panice, E.B. de Melo, J.P.A. Martins, V.P. Baldin, C.T.A. Pires, K.R. Caleffi-Ferracioli, V.L.D. Siqueira, R.B. de Lima Scodro, M.H. Sarragiotto, Synthesis and anti-Mycobacterium tuberculosis activity of imide-β-carboline and carbomethoxy-β-carboline derivatives, Eur. J. Med. Chem. 187 (2020) 111935.
[24] F. Jalili, M. Zarei, M.A. Zolfigol, S. Rostamnia, A.R. Moosavi-Zare, SBA-15/PrN (CH2PO3H2) 2 as a novel and efficient mesoporous solid acid catalyst with phosphorous acid tags and its application on the synthesis of new pyrimido [4, 5-b]

quinolones and pyrido [2, 3-d] pyrimidines via anomeric based oxidation, Microporous Mesoporous Mater. 294 (2020) 109865.
[25] S.R. Ambati, J.L. Patel, S. Gudala, K. Chandrakar, S. Penta, S. Mahapatra, S. Banerjee, Synthesis of novel coumarinyl-pyrido [2, 3-d] pyrimidine-2, 4-diones using task-specific magnetic ionic liquid,[AcMIm] FeCl4 as catalyst, Synth. Commun. 50 (1) (2020) 104–111.
[26] R.A. Azzam, G.H. Elgemeie, R.R. Osman, Synthesis of novel pyrido [2, 1-b] benzothiazole and N-substituted 2-pyridylbenzothiazole derivatives showing remarkable fluorescence and biological activities, J. Mol. Struct. 1201 (2020) 127194.
[27] J.I. DeGraw, R.L. Kisliuk, Y. Gaumont, C.M. Baugh, Antimicrobial activity of 8-deazafolic acid, J. Med. Chem. 17 (4) (1974) 470–471.
[28] J. Matsumoto, S. Minami, Pyrido [2, 3-d] pyrimidine antibacterial agents. 3. 8-alkyl-and 8-vinyl-5, 8-dihydro-5-oxo-2-(1-piperazinyl) pyrido [2, 3-d] pyrimidine-6-carboxylic acids and their derivatives, J. Med. Chem. 18 (1) (1975) 74–79.
[29] N. Suzuki, Synthesis of antimicrobial agents. V. Synthesis and antimicrobial activities of some heterocyclic condensed 1, 8-naphthyridine derivatives, Chem. Pharm. Bull. 28 (3) (1980) 761–768.
[30] H. Huang, D.A. Hutta, H. Hu, R.L. DesJarlais, C. Schubert, I.P. Petrounia, M.A. Chaikin, C.L. Manthey, M.R. Player, Design and synthesis of a pyrido [2, 3-d] pyrimidin-5-one class of anti-inflammatory FMS inhibitors, Bioorg. Med. Chem. Lett 18 (7) (2008) 2355–2361.
[31] M. Youssouf, P. Kaiser, G. Singh, S. Singh, S. Bani, V. Gupta, N. Satti, K. Suri, R. Johri, Anti-histaminic, anti-inflammatory and bronchorelaxant activities of 2, 7-dimethyl-3-nitro-4H pyrido [1, 2-a] pyrimidine-4-one, Int. Immunopharm. 8 (7) (2008) 1049–1055.
[32] H.B. El-Nassan, Synthesis and antitumor activity of novel pyrido [2, 3-d][1, 2, 4] triazolo [4, 3-a] pyrimidin-5-one derivatives, Eur. J. Med. Chem. 46 (6) (2011) 2031–2036.
[33] E.M. Grivsky, S. Lee, C.W. Sigel, D.S. Duch, C.A. Nichol, Synthesis and antitumor activity of 2, 4-diamino-6-(2, 5-dimethoxybenzyl)-5-methylpyrido [2, 3-d] pyrimidine, J. Med. Chem. 23 (3) (1980) 327–329.
[34] A. El-Sayed, N. Khaireldin, M. El-Shahat, E. El-Hefny, M. El-Saidi, M. Ali, A. Mahmoud, Antiproliferative activity for newly heterofunctionalized pyridine analogues, Ponte 72 (7) (2016) 106–118.
[35] M.N. Nasr, M.M. Gineinah, Pyrido [2, 3-d] pyrimidines and pyrimido [5′, 4′: 5, 6] pyrido [2, 3-d] pyrimidines as new antiviral agents: synthesis and biological activity, Arch. Pharmazie 335 (6) (2002) 289–295.
[36] A. El-Sayed, M. El-Shahat, S. Rabie, E. Flefel, D. Abd-Elshafy, New pyrimidine and fused pyrimidine derivatives: synthesis and anti hepatitis a virus (HAV) evaluation, Int. J. Pharm. 5 (2015) 69–79.
[37] G. Nam, C.M. Yoon, E. Kim, C.K. Rhee, J.H. Kim, J.H. Shin, S.H. Kim, Syntheses and evaluation of pyrido [2, 3-d] pyrimidine-2, 4-diones as PDE 4 inhibitors, Bioorg. Med. Chem. Lett 11 (5) (2001) 611–614.
[38] M. López-Pueyo, F. Barcenilla-Gaite, R. Amaya-Villar, J. Garnacho-Montero, Antibiotic multiresistance in critical care units, Med. Intensiva (English Edition) 35 (1) (2011) 41–53.
[39] E.M. Flefel, W.I. El-Sofany, H.M. Awad, M. El-Shahat, First synthesis for bis-spirothiazolidine derivatives as a novel heterocyclic framework and their biological activity, Mini Rev. Med. Chem. 20 (2) (2020) 152–160.
[40] S. Lu, R. Qiu, J. Hu, X. Li, Y. Chen, X. Zhang, C. Cao, H. Shi, B. Xie, W.-M. Wu, Prevalence of microplastics in animal-based traditional medicinal materials: widespread pollution in terrestrial environments, Sci. Total Environ. 709 (2020) 136214.
[41] H.E. Emam, o.m. darwesh, R.M. Abdelhameed, Protective cotton textiles via amalgamation of cross-linked zeolitic imidazole framework, Ind. Eng. Chem. Res. (2020).
[42] R.M. Abdelhameed, O.M. Darwesh, J. Rocha, A.M. Silva, IRMOF-3 biological activity enhancement by post-synthetic modification, Eur. J. Inorg. Chem. 2019 (9) (2019) 1243–1249.
[43] C. Freitas, T. Glatter, S. Ringgaard, The release of a distinct cell type from swarm colonies facilitates dissemination of Vibrio parahaemolyticus in the environment, ISME J. 14 (1) (2020) 230–244.
[44] S. Gouda, R.G. Kerry, G. Das, J.K. Patra, Synthesis of nanoparticles utilizing sources from the mangrove environment and their potential applications: an overview, nanomaterials in plants, algae and microorganisms, Elsevier (2019) 219–235.
[45] R.M. Abdelhameed, H.A. El-Sayed, M. El-Shahat, A.A. El-Sayed, O.M. Darwesh, Novel triazolothiadiazole and triazolothiadiazine derivatives containing pyridine moiety: design, synthesis, bactericidal and fungicidal activities, Curr. Bioact. Compd. 14 (2) (2018) 169–179.
[46] A.E. Rashad, A.H. Shamroukh, M.A. El-Hashash, A.F. El-Farargy, N.M. Yousif, M.A. Salama, N. Abdelwahed, M. El-Shahat, 1, 3-Bis (4-chlorophenyl)-2, 3-epoxy-propanone as synthons in synthesis of some interesting potential antimicrobial agents, Organic Chemistry, Indian J. 9 (7) (2013) 287–294.
[47] M. Salama, M. El-Shahat, E. Elhefny, A. El-Sayed, A novel fused pyridopyrimidine derivatives: synthesis and characterization, Int. J. Pharm. 5 (2015) 53–58.
[48] E.M. Flefel, W.A. Tantawy, W.I. El-Sofany, M. El-Shahat, A.A. El-Sayed, D.N. Abd-Elshafy, Synthesis of some new pyridazine derivatives for anti-HAV evaluation, Molecules 22 (1) (2017) 148.
[49] E.M. Flefel, W.I. El-Sofany, R.A. Al-Harbi, M. El-Shahat, Development of a novel series of anticancer and antidiabetic: spirothiazolidines analogs, Molecules 24 (13) (2019) 2511.
[50] M. El-Shahat, M.A. Salama, A.F. El-Farargy, M.M. Ali, D.M. Ahmed, Effective pharmacophore for CDC25 phosphatases enzyme inhibitors: newly synthesized bromothiazolopyrimidine derivatives, Mini Rev. Med. Chem. (2020).
[51] E.M. Flefel, W.I. El-Sofany, M. El-Shahat, A. Naqvi, E. Assirey, Synthesis, molecular docking and in vitro screening of some newly synthesized triazolopyridine, pyridotriazine and pyridine–pyrazole hybrid derivatives, Molecules 23 (10) (2018) 2548.
[52] R.S. Dongre, A.R. Bhat, J.S. Meshram, Anticancer activity of assorted annulated pyrimidine: a comprehensive review, Am. J. PharmTech Res. 4 (1) (2014) 138–155.
[53] J. Quiroga, B. Insuasty, A. Sanchez, M. Nogueras, H. Meier, Synthesis of pyrido [2, 3-d] pyrimidines in the reaction of 6-amino-2, 3-dihydro-2-thioxo-4 (1H)-pyrimidinone with chalcones, J. Heterocycl. Chem. 29 (5) (1992) 1045–1048.
[54] P. Crepaldi, B. Cacciari, M.-C. Bonache, G. Spalluto, K. Varani, P.A. Borea, I. von Kügelgen, K. Hoffmann, M. Pugliano, C. Razzari, 6-Amino-2-mercapto-3H-pyrimidin-4-one derivatives as new candidates for the antagonism at the P2Y12 receptors, Bioorg. Med. Chem. 17 (13) (2009) 4612–4621.
[55] M. Fares, R.A. Eladwy, A. Nocentini, S.R.A. El Hadi, H.A. Ghabbour, A. Abdel-Megeed, W.M. Eldehna, H.A. Abdel-Aziz, C.T. Supuran, Synthesis of bulky-tailed sulfonamides incorporating pyrido [2, 3-d][1, 2, 4] triazolo [4, 3-a] pyrimidin-1 (5H)-yl) moieties and evaluation of their carbonic anhydrases I, II, IV and IX inhibitory effects, Bioorg. Med. Chem. 25 (7) (2017) 2210–2217.
[56] A. Bauer, Antibiotic susceptibility testing by a standardized single disc method, Am. J. Clin. Pathol. 45 (1966) 149–158.
[57] Z.M. Alvand, H.R. Rajabi, A. Mirzaei, A. Masoumiasl, Ultrasonic and microwave assisted extraction as rapid and efficient techniques for plant mediated synthesis of quantum dots: green synthesis, characterization of zinc telluride and comparison study of some biological activities, New J. Chem. 43 (38) (2019) 15126–15138.
[58] H.R. Rajabi, R. Naghiha, M. Kheirizadeh, H. Sadatfaraji, A. Mirzaei, Z.M. Alvand, Microwave assisted extraction as an efficient approach for biosynthesis of zinc oxide nanoparticles: synthesis, characterization, and biological properties, Mater. Sci. Eng. C 78 (2017) 1109–1118.
[59] Z.M. Alvand, H.R. Rajabi, A. Mirzaei, A. Masoumiasl, H. Sadatfaraji, Rapid and green synthesis of cadmium telluride quantum dots with low toxicity based on a plant-mediated approach after microwave and ultrasonic assisted extraction: synthesis, characterization, biological potentials and comparison study, Mater. Sci. Eng. C 98 (2019) 535–544.
[60] S. Mirsadeghi, H. Zandavar, M. Rahimi, H.F. Tooski, H.R. Rajabi, M. Rahimi-Nasrabadi, E. Sohouli, B. Larijani, S.M. Pourmortazavi, Photocatalytic reduction of imatinib mesylate and imipenem on electrochemical synthesized Al2W3O12 nanoparticle: optimization, investigation of electrocatalytic and antimicrobial activity, Colloid. Surface. Physicochem. Eng. Aspect. 586 (2020) 124254.
[61] R.M. Abdelhameed, M. Abu-Elghait, M. El-Shahat, Hybrid three MOFs composites (ZIF-67@ ZIF-8@ MIL-125-NH2): enhancement the biological and visible-light photocatalytic activity, J. Environ. Chem. Eng. (2020) 104107.
[62] P.C.T. Hannan, Guidelines and recommendations for antimicrobial minimum inhibitory concentration (MIC) testing against veterinary mycoplasma species, Vet. Res. 31 (4) (2000) 373–395.