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Original article

Synthesis and structure–activity relationship of 2-thiopyrimidine-4-one analogs as antimicrobial and anticancer agents

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ABSTRACT

Considering that some thiopyrimidines were previously reported as potential therapeutics, the present study achieved novel analogs of bioactive 2-substituted thiopyrimidines-4-(3*H*)-ones *via* base catalyzed alkylation reaction of 2-thiouracil using alkyl and aralkyl bromides. The title compounds were 2-(1-butylthio)pyrimidine-4(3*H*)-one (**5a**), 2-(2-butylthio)pyrimidine-4(3*H*)-one (**5b**), 2-(cyclohexylmethylthio) pyrimidine-4(3*H*)-one (**5c**), 2-(benzylthio)pyrimidine-4(3*H*)-one (**5d**) and 2-(1-adamantylthio)pyrimidine-4(3*H*)-one (**5e**). Bioactivity tests revealed that thiopyrimidines **5a**, **5c**, **5d** and **5e** exhibited antimicrobial activity. The thiopyrimidine-4-one (**5c**) showed complete inhibition against *Streptococcus pyogenes* and *Branhamella catarrhalis* as well as antifungal action against *Candida albicans*. Significantly, the 1-adamantylthiopyrimidine (**5e**) was shown to be the most potent cytotoxic compound against multidrug-resistant small cell lung cancer (H69AR). Their structure–activity relationships were discussed.

1. Introduction

Pyrimidine constitutes an important component of nucleic acid and it is used as building blocks in pharmaceutics for the synthesis of antiviral, anticancer [1,2] antibacterial and antifungal [3] agents. Similarly, the related thiouracil derivatives are potential therapeutics e.g. as antivirals and anticancers [4,5]. In particular, 6-*n*-propyl-2-thiouracil (6-PTU, **1**) is an antithyroid drug [2] where its *S*-alkylation (**2**) and N3-alkylation (**4**) products (Fig. 1) have been recently reported as novel antibacterial, antimalarial and cytotoxic agents [6]. Thiouracils and their nucleoside analogs are found in natural sources; 2-thiouracil (2-TU, **3**) is generally present in *t*-RNA of *Escherichia coli* [7], 4-TU and 2-thiocytidine in other sources [7] including 4-thiouridine in bacterial and archaeal *t*-RNA [5]. In fact, 2-TU is a thyroid drug recognized as a highly specific melanoma seeker [7]. We bear in mind that novel small molecular bioactive 2-thiouracil analogs could be of interest and potential target compounds. To investigate for new lead candidates as medicinally important agents, therefore, a series of *S*-substituted 2-thiouracils **5** was designed and synthesized *via* an alkylation of 2-TU (Fig. 2) as well as tested for their antimicrobial, antimalarial and cytotoxic activities. Their structure and activity relationship were also examined.

2. Results and discussion

2.1. Chemistry

Title *S*-substituted thiouracils **5a**–**e** were achieved through the alkylation of 2-TU with alkylating agents (R–Br) in base catalysis (Et_3N or K_2CO_3). The results showed that the *S*-alkylation products; 2-(1-butylthio)pyrimidine-4(3*H*)-one (**5a**), 2-(2-butylthio)pyrimidine-4(3*H*)-one (**5b**), 2-(cyclohexylmethylthio)pyrimidine-4(3*H*)-one (**5c**), 2-(benzylthio)pyrimidine-4(3*H*)-one (**5d**) and 2-(1-adamantylthio) pyrimidine-4(3*H*)-one (**5e**) were obtained in 17.6–37.5%, where R = *n*-C_4H_9 gave the highest yield (37.5%) of *n*-butylthio analog **5a**. It is interested to note that bulky 1-adamantyl bromide (1-AdmBr,

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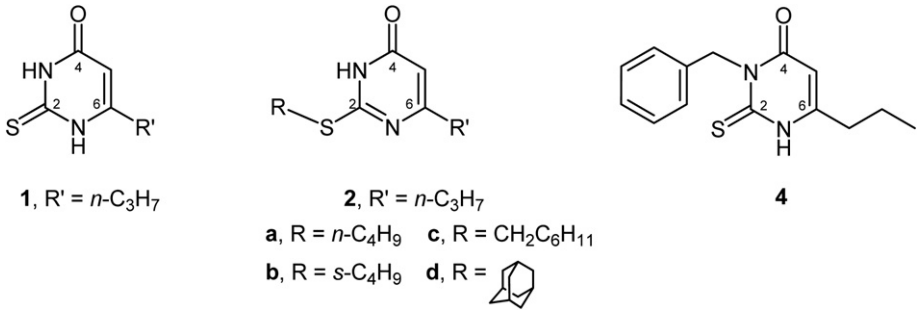

Fig. 1. Chemical structures of 2-thiouracil derivatives.

R = 1-Adm) produced 1-adamantylthiopyrimidine (**5e**) in a comparable yield to that of **5d** deriving from benzyl bromide (R = $CH_2C_6H_5$). Similar *S*-alkylation was observed for 6-*n*-propyl-2-thiouracil, except the reaction with benzyl bromide which furnished *N*-3 benzylation product [6]. In addition, *S*-alkylation of 6-methyl-2-thiouracil was also reported [8]. Previously, 2-TU reacted with chloroacetaldehyde or ketone to give *S*-alkylation product [9]. However, steric hindered *t*-butyl bromide did not react with 2-TU (as evidence by TLC chromatogram). Structures of the 2-thiopyrimidine-4-ones (**5a**–**e**) were determined by spectral data. The IR spectra showed strong CO absorption at 1652–1731 cm^{-1} and strong or sharp NH absorption in range 3200–3287 cm^{-1}. The 2-thiopyrimidines (**5a**–**e**) all displayed doublets of H-5 (δ 6.20–6.22) and H-6 (δ 7.83–7.88) with coupling constants of 6.4–6.6 Hz. ^{13}C NMR spectra showed C-4 carbonyls at δ 162.73–164.69 ppm and C-2 of thioimines at δ 160.98–162.55 ppm. From HMBC spectra H-6 proton of 2-thiopyrimidine analogs **5a**–**e** showed correlations with C-2, C-4 and C-5. On the other hand, H-1′ of the 2-thiopyrimidine analogs, except 1-adamantylthiopyrimidine **5e**, were also correlated with C-2, C-2′ and ArC. Such behaviour was not observed for the 2-thiopyrimidine **5e** due to C-1′ of 1-Adm group is a quaternary carbon lacking of H-1′. In addition, C-2 of the products **5a**–**e** appeared at δ 160.98–162.55 ppm where C-2 of the starting 2-TU displayed at 172.9 ppm. This confirms that *S*-alkylation reaction took place at a thione group. The mass spectra support the obtained *S*-alkylated 2-thiopyrimidines **5a**–**d** which all showed molecular ions $[M]^+$ or $[M + H]^+$ as their base peaks. Except the analog **5e** exhibited a fragment ion at *m*/*z* 135 (1-Adm) as the base peak. Such fragmentation is uniquely found in 1-adamantylthio analogs of pyridines [10–12] and pyrimidines [6]. However, the 2-thiopyrimidines **5b**, **5c** and **5e** are new *S*-alkylated 2-thiouracils.

2.2. Bioactivities

2.2.1. Antimicrobial activity

2-Thiouracil analogs **5a**–**e** were tested for antimicrobial activity against twenty-seven strains of microorganisms using the agar dilution method [13]. It was found that (Table 1) the tested analogs all displayed growth inhibition against *Streptoccus pyogenes* and *Branhamella catarrhalis* at concentration range 64–128 μg/mL. Significantly, *B. catarrhalis* was completely inhibited by cyclohexylmethylthiopyrimidine analog **5c** with a minimum inhibitory concentration (MIC) of 64 μg/mL. In addition, the compound **5c** also exhibited antigrowth activity against *S. pyogenes* with the MIC of 128 μg/mL. Complete inhibition of the *B. catarrhalis* was also noted for *n*-butylthiopyrimidine **5a** at 128 μg/mL. Interestingly, only the 2-thiopyrimidine compound **5c** showed growth inhibition (75%) against the diploid fungus, *Candida albicans* at 128 μg/mL. Previously, chlorothiouracil-hydroxamates were reported to exhibit growth inhibition against *C. albicans* [14]. However, antimicrobial activity of such 2-thiopyrimidines has not been reported. Thus, these 2-thiopyrimidines **5a** and **5c**–**e** represent a novel group of antimicrobials.

2.2.2. Antimalarial activity

2-Thiopyrimidine-4-one analogs **5a**–**e** were screened against *Plasmodium falciparum* chloroquine resistant (T9.94). Results revealed that the tested compounds were inactive antimalarials showing an $IC_{50} > 10^{-5}$ M, excepted **5b** was insoluble in the testing medium.

2.2.3. Cytotoxic activity

Cytotoxic activity of *S*-alkylated pyrimidine analogs **5a**–**e** was performed against eleven cancer cell lines using a modified method [15]. The results (Table 2) showed that *s*-butylthio- and benzylthiopyrimidines (**5b** and **5d**) were inactive toward all the tested cancer cells. No cytotoxic activity against HuCCA-1, MDA-MB 231, A549, HeLa, HCC-S102 and HL-60 cell lines were observed for all the tested analogs. Cyclohexylmethylthiopyrimidine analog **5c** selectively exhibited cytotoxic activity against P388 cell with the IC_{50} of 40.36 μg/mL 1-Adamantylthiopyrimidine-4-one (**5e**) exerted activity against many cancer cells; T47D, H69AR, HepG2 and P388 with the IC_{50} of 32.0, 35.0, 32.5 and 36.91 μg/mL, respectively. It is notable that **5e** is the most potent cytotoxic agent against H69AR with the IC_{50} of 35.0 μg/mL where the IC_{50} of the control etoposide is 30.0 μg/mL. This is presumably due to a high lipophilicity of the 1-Adm moiety which enhances its absorption to the cancer cells.

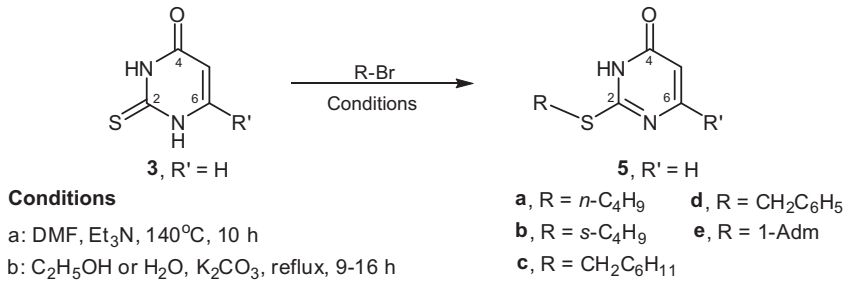

Fig. 2. *S*-alkylated thiouracil **5a**–**e** analogs.

Table 1
Antimicrobial activity[e] of 2-thiopyrimidine-4-ones **5a**–**e**.

Compound[f]	Activity	Inhibition (%)	
		64 μg/mL	128 μg/mL
5a	Active	0	25[a,c], 100[b]
5c	Active	100[b]	25[c], 75[d], 100[a]
5d	Active	0	25[a], 50[b]
5e	Active	0	25[a,c], 75[b]

Inhibition against [a]*S. pyogenes*, [b]*B. catarrhalis*, [c]*N. mucosa*, [d]*C. albicans*.
[e] Ampicillin at 10 μg/mL was used as a control of the antibacterial testing system; it showed 100% growth inhibition against *S. aureus* ATCC 25923, *B. subtilis* ATCC 6633, *S. epidermidis* ATCC 12228, *S. pyogenes*, *E. tarda*, *N. mucosa* and *B. catarrhalis*.
[f] Compound **5b** was not tested (insoluble in the testing medium).

This hydrophobic nature of 1-Adm moiety was found in a diverse group of bioactive compounds [16–18]. In addition, *n*-butylthiopyrimidine analog (**5a**) displayed activity against KB and T47D cells with the IC_{50} of 27.0 and 5.0 μg/mL, respectively. However, cytotoxic activity of **5a**–**e** has not been reported in the literature. Hence, the 2-thiopyrimidine analogs **5a**, **5c** and **5e** are new series of cytotoxic agents.

2.2.4. Structure–activity relationship

According to the results of bioactivities, it is noted that *S*-alkylated (R) 2-thiopyrimidine-4-ones with R = $CH_2C_6H_{11}$ (**5c**) provides better antimicrobial activity, against *B. catarrhalis*, than the other active compounds (**5a**, **5d** and **5e**). Whereas R = 1-Adm, the compound **5e** exerted cytotoxic action against H69AR cell with the significant IC_{50} value as compared to the reference drug. In comparison with the 2-thiopyrimidine-4-one analog **2** [6] bearing substituent (R′ = *n*-propyl) at C-6 position, the best antimicrobials was observed when R = 1-Adm (**2d**). The compound with an absence of *n*-propyl group at C-6 (**5e**, R = 1-Adm) also showed partial inhibition (75%) against the same organism (*B. catarrhalis*) at 128 μg/mL. This suggests that the best antimicrobial agent analog of 2-thiopyrimidine-4-one requires hydrophobic groups at both 2-thio and C-6 positions, like compound **2d** [6]. In addition, *S*-methylcyclohexyl derivative of 2-thiopyrimidine-4-one with substituent (R′ = *n*-C_3H_7, **2c**) or without substituent (R′ = H, **5c**) at C-6 provided the antimicrobial action against *B. catarrhalis* with the same MIC value. Apparently, *S*-methylcyclohexyl derivative of 2-thiopyrimidine-4-one analog containing substituent; R′ = *n*-C_3H_7 (**2c**) at C-6 position [6] displayed the highest cytotoxic activity against H69AR cell that was comparable to the activity of *S*-1-Adm analog (**5e**, R′ = H, absence of substituent at C-6). This is presumably due to a hydrophobic effect of sterically hindered 1-Adm group that enhances the penetration of compound **5e** to the cancer cell. Taken together of the *S*-alkylated (R) title compounds, the promising results were achieved when R = $CH_2C_6H_{11}$ and 1-Adm groups as seen for 2-thiopyrimidine-4-one compounds **5c**, **2c** and **5e**, **2d**, respectively. It was reported that methylcyclohexyl group when substituted to pyrimidine-2,4-dione e.g. 5-iodouracil afforded N1 and N1,N3-alkylated products with good cytotoxic activity [13]. Similarly, 1-Adm group containing thiopyridine derivatives were reported to be active antimicrobials [19], antioxidants [20], antimalarials, anticancers [21,22] and vasorelaxants [23]. In addition, 1-Adm moiety constituting thiotetrahydropyridines also exhibited antimicrobial and antioxidative activities [24,25]. From the results, 1-Adm and methylcyclohexyl are alkylated groups that could possibly be functionalized to other related heterocyclic rings in a search for seeking new bioactive compounds. Therefore, in relating their structures (analogs **5** and **2**) and bioactivities, it could be seen that either 1-Adm or methylcyclohexyl group is likely to be an important alkylated group for 2-thiopyrimidine-4-one analogs as bioactive compounds. In particular, 1-Adm analog of 2TU (**5e**) was shown to be the new and significant cytotoxic compound against H69AR cell. Such cytotoxic activity was also noted for the analog of 6PTU (**2c**) bearing methylcyclohexyl group at 2-thio function. Up to this point, it could be concluded that *S*-alkylation of the thyroid drugs (2TU and 6PTU) provided the analogs as the promising cytotoxic agent as well as the antimicrobials.

Table 2
Cytotoxic activity of 2-thiopyrimidine-4-ones **5a**–**e**.

Cell lines[a]	IC_{50} (μg/mL)[b,c]					
	5a	**5b**	**5c**	**5d**	**5e**	Etoposide[d]
KB	27.00	NA	>50	>50	>50	0.25
HuCCA-1	>50	>50	>50	>50	>50	4.00
MDA-MB 231	>50	NA	>50	>50	>50	0.24
T47D	5.0	NA	>50	>50	32.00	0.05
A549	>50	>50	>50	>50	>50	0.60
H69AR	>50	NA	50.0	>50	35.00	30.00
HeLa	>50	NA	>50	>50	>50	0.38
HepG2	>50	>50	>50	>50	32.5	21.00
HCC-S102	>50	NA	>50	>50	>50	6.00
HL-60	>50	NA	>50	>50	>50	0.85
P388	>50	NA	40.36	>50	36.91	0.12

NA: indicates not tested (insoluble in the testing medium).
[a] Cancer cell lines were human epidermoid carcinoma of the mouth (KB), human cholangiocarcinoma cancer cells (HuCCA-1), hormone-independent breast cancer cell line (MDA-MB231), hormone-dependent breast cancer cell line (T47D), human lung carcinoma cell line (A549), multidrug-resistant small cell lung cancer cell line (H69AR), cervical adenocarcinoma cell line (HeLa), human hepatocellular liver carcinoma cell line (HepG2), hepatocellular carcinoma cell line (HCC-S102), human promyelocytic leukemia cell line (HL-60), murine leukemia cell line (P388).
[b] When IC_{50} > 50 μg/mL denotes inactive compound.
[c] The assays were performed in triplicate.
[d] Etoposide was used as a reference drug.

3. Conclusion

2-TU reacted with alkyl or aralkyl bromides in the presence of Et_3N or K_2CO_3 to furnish exclusively *S*-substituted 2-thiouracils **5a**–**e** from which **5b**, **5c** and **5e** are new compounds. 2-Thiouracil analogs **5a** and **5c**–**e** exerted antigrowth activity against *S. pyogenes* and *B. catarrhalis* in concentration range of 64–128 μg/mL. Significantly, the cyclohexylmethylthiopyrimidine **5c** exhibited complete inhibition against *S. pyogenes* and *B. catarrhalis* with the MICs of 128 and 64 μg/mL, respectively. Only the analog **5c** that could show antifungal action against *C. albicans*. 1-Adamantylthiopyrimidines **5e** displayed cytotoxic activity against many cell lines; T47D, H69AR, HepG2 and P388. Significant activity of **5e** was observed against H69AR with the IC_{50} of 35.0 μg/mL, whereas the control drug; etoposide showing the IC_{50} of 30.0 μg/mL. All the tested 2-thiopyrimidines were shown to be inactive antimalarials. Their structure–activity relationships were examined and found that 1-Adm and $CH_2C_6H_{11}$ were important alkylated groups for 2-thiopyrimidine-4-one analogs producing significant bioactivities.

In conclusion, the study leads to the identification of novel antimicrobials (**5a** and **5c**–**e**) and cytotoxic agents (**5a**, **5c** and **5e**). Significantly, **5c** exhibits both antibacterial and antifungal actions, whereas, **5e** was found to be the most potent cytotoxic compound against multidrug-resistant small cell lung cancer (H69AR). The findings demonstrate a new potential for 2-thiopyrimidine-4-ones as lead compounds for further development as medicinal agents.

4. Materials and methods

4.1. General

Melting points were determined on an Electrothermal melting point apparatus (Electrothermal 9100) and are uncorrected. ^1H and ^{13}C NMR spectra were recorded on a Bruker AVANCE 300 NMR spectrometer (operating at 300 MHz for ^1H and 75 MHz for ^{13}C).

Infrared spectra (IR) were obtained on a Perkin Elmer System 2000 FTIR. Mass spectra were recorded on a Finnigan INCOS 50 and a Bruker Daltonics (micro TOF). Elemental analysis was performed on a Perkin Elmer Elemental analyzer 2400 CHN. Column chromatography was carried out using silica gel 60 (0.063–0.200 mm). Analytical thin layer chromatography (TLC) was performed on silica gel 60 PF_{254} aluminium sheets (cat. No. 7747 E., Merck).

Solvents were distilled prior to use. Chemicals for the synthesis and bioactivity testings were of analytical grade. Reagents for cell culture and assay were the following: RPMI-1640 (Rosewell Park Memorial Institute medium, Gibco and Hyclone laboratories, USA), HEPES (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid), L-glutamine, penicillin, streptomycin, sodium pyruvate and glucose (Sigma, USA), Ham's/F12 (Nutrient mixture F-12), DMEM (Dulbecco's Modified Eagle's Medium) and FBS (fetal bovine serum, Hyclone laboratories, USA), gentamicin sulfate (Government Pharmaceutical Organization, Thailand), MTT (3(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, Sigma–Aldrich, USA).

4.2. Synthesis of S-substituted 2-thiouracils **5a–e**

To a solution of 2-TU (**3**) and alkyl or aralkyl bromide in DMF (or water or ethanol) was added triethylamine (or K_2CO_3) and then heated under reflux for 10–16 h. The reaction was monitored by TLC, extracted and worked up as usual. The products were purified by silica gel column and recrystallized in methanol or dichloromethane/methanol.

4.2.1. 2-(1-Butylthio)pyrimidine-4(3H)-one (**5a**)

2-TU (3 mmol) reacted with *n*-BuBr (6 mmol) and K_2CO_3 (3 mmol) in C_2H_5OH (30 mL) to furnish 2-(1-butylthio)pyrimidine-4(3*H*)-one (**5a**) 0.21 g (37.5%); mp 89–90 °C (98–99 °C [26]), IR (KBr) υ_{max}: 3202, 2952, 1652, 1541 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.91 (t, 3H, J = 7.2 Hz, H-4′), 1.49 (sextet, 2H, J = 7.2 Hz, H-3′), 1.72 (quintet, 2H, J = 7.2 Hz, H-2′), 3.18 (t, 2H, J = 7.2 Hz, H-1′), 6.21 (d, 1H, J = 6.4 Hz, H-5), 7.85 (d, 1H, J = 6.4 Hz, H-6); ^{13}C NMR ($CDCl_3$): δ 12.93 (C-4′), 21.58 (C-3′), 28.92 (C-1′), 31.21 (C-2′), 110.30 (C-5), 153.69 (C-6), 162.04 (C-2), 162.73 (C-4); LRMS (EI): m/z (%) = 185 (100.00) $[M + H]^+$, 184 (6.60) $[M]^+$, 152 (11.45), 137 (23.17); HRMS (TOF): m/z $[M + H]^+$ calcd for $C_8H_{13}N_2OS$: 185.0743 found: 185.0749. Anal. Calcd. for $C_8H_{12}N_2OS$: C, 52.15; H, 6.56; N, 15.20. Found: C, 52.59; H, 6.26; N, 15.10.

4.2.2. 2-(2-Butylthio)pyrimidine-4(3H)-one(**5b**)

2-TU (5 mmol) reacted with *s*-BuBr (5 mmol) and K_2CO_3 (2.5 mmol) in H_2O (20 mL) to give 2-(2-butylthio)pyrimidine-4(3*H*)-one (**5b**) 0.16 g (17.6%); mp 90–91 °C; IR (KBr) υ_{max}: 3202, 2969, 2923, 2871, 1653 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.02 (t, 3H, J = 6.9 Hz, H-4′), 1.39 (d, 3H, J = 6.9 Hz, H-1′), 1.70 (quintet, 2H, J = 6.9 Hz, H-3′), 3.91 (sextet, 1H, J = 6.9 Hz, H-2′), 6.20 (d, 1H, J = 6.6 Hz, H-5), 7.84 (d, 1H, J = 6.6 Hz, H-6); ^{13}C NMR ($CDCl_3$): δ 11.32 (C-4′), 20.61 (C-1′), 29.23 (C-3′), 43.31 (C-2′), 110.78 (C-5), 154.7(C-6), 162.45 (C-2), 164.58 (C-4); LRMS (EI): m/z (%) = 185 (100) $[M + H]^+$, 184 (5.2) $[M]^+$, 152 (23.8), 137 (15.1), 124 (11.9); HRMS(TOF): m/z $[M + H]^+$ calcd for $C_8H_{13}N_2OS$: 185.0743 found: 185.0737. Anal. Calcd. for $C_8H_{12}N_2OS$: C, 52.15; H, 6.56; N, 15.20. Found: C, 52.46; H, 6.56; N, 15.57.

4.2.3. 2-(Cyclohexylmethylthio)pyrimidine-4(3H)-one (**5c**)

2-TU (5 mmol) reacted with cyclohexylmethyl bromide (5 mmol) in DMF (5 mL) and Et_3N (2 mL) to afford 2-(cyclohexylmethylthio) pyrimidine-4(3*H*)-one (**5c**) 0.20 g (17.8%); mp 150–151 °C; IR(KBr) υ_{max}: 3201, 2927, 2851, 1658 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.10–2.25 (m, H-2′, H-3′, H-4′, H-5′), 3.10 (d, 2H, J = 6.9 Hz, H-1′), 6.21 (d, 1H, J = 6.6 Hz, H-5), 7.84 (d, 1H, J = 6.6 Hz, H-6); ^{13}C NMR ($CDCl_3$): δ 25.92 (C-4′),26.14 (C-5′), 32.52 (C-3′), 37.39 (C-2′), 37.6 (C-1′), 110.84 (C-5),154.77 (C-6), 162.55 (C-2), 164.42 (C-4); LRMS(EI): m/z (%) = 226 (13.09) $[M + H]^+$, 225 (100.00) $[M]^+$, 178 (7.45), 129 (60.25); HRMS (TOF): m/z $[M + H]^+$: calcd for $C_{11}H_{17}N_2OS$: 225.1056 found: 225.1050. Anal. Calcd. for $C_{11}H_{16}N_2OS$: C, 58.90; H, 7.19; N, 12.49. Found: C, 58.41; H, 6.99; N, 12.39.

4.2.4. 2-(Benzylthio)pyrimidine-4(3H)-one (**5d**)

2-TU (5 mmol) reacted with benzyl bromide (5 mmol) in DMF (5 mL) and Et_3N (2 mL) to give 2-(benzylthio)pyrimidine-4(3*H*)-one (**5d**) 0.28 g (25.3%); mp 220–221 °C (193–195 °C [27], 174–175 °C [28]); IR(KBr) υ_{max}: 3200, 3071, 1731, 1504 cm^{-1}; ^1H NMR($CDCl_3$): δ 4.41 (s, 2H, H-1′), 6.22 (d, 1H, J = 6.5 Hz, H-5), 7.25–7.38 (m, 5H, Ar-H), 7.88 (d, 1H, J = 6.5 Hz, H-6); ^{13}C NMR ($CDCl_3$): δ 35.02(C-1′), 111.07(C-5), 127.78–129.21 (Ar-C), 135.74 (C-2′), 154.62 (C-6), 161.78 (C-2), 164.69 (C-4); LRMS (EI): m/z (%) = 219 (12.44) $[M + H]^+$, 218 (100.00) $[M]^+$, 185 (56.56), 91 (26.94); HRMS (TOF) m/z $[M + H]^+$ calcd. for $C_{11}H_{11}N_2OS$: 219.0587 found: 219.0582. Anal. Calcd. for $C_{11}H_{10}N_2OS$: C, 60.53; H, 4.62; N, 12.83. Found: C, 61.44; H, 4.60; N, 12.74.

4.2.5. 2-(1-Adamantylthio)pyrimidine-4(3H)-one (**5e**)

2-TU (10 mmol) reacted with 1-AdmBr (10 mmol) in DMF (8 mL) and Et_3N (2 mL). After chromatographic separation gave 2-(1-adamantylthio)pyrimidine-4(3*H*)-one (**5e**) 0.59 g (22.5%); mp 165–166 °C; IR (KBr) υ_{max}: 3287, 2915, 1667, 1565, 1526 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.67–2.26 (m,15H, 1-Adm-H), 6.22 (d, 1H, J = 6.6 Hz, H-5), 7.83 (d, 1H, J = 6.6 Hz, H-6); ^{13}C NMR ($CDCl_3$): δ 111.54 (C-5), 154.53 (C-6), 160.98 (C-2), 164.21 (C-4), 29.34–53.37 (1-Adm-C); LRMS(EI): m/z (%) = 263 (13.87) $[M + H]^+$, 262 (49.62) $[M]^+$, 261 (70.46), 135 (100.00); HRMS (TOF): m/z $[M + H]^+$ calcd for $C_{14}H_{19}N_2OS$: 263.1213 found: 263.1218. Anal. Calcd. for $C_{14}H_{18}N_2OS$: C, 64.09; H, 6.91; N, 10.68. Found: C, 65.06; H, 6.68; N, 10.66.

4.3. Bioactivities

4.3.1. Antimicrobial assay

Antimicrobial activity of the tested compounds was performed using the agar dilution method as previously described [13]. In brief, the tested compounds dissolved in DMSO were individually mixed with 1 mL Müller Hinton (MH) broth while the negative control was the MH broth without the tested compounds. The solution was then transferred to the MH agar solution to yield the final concentrations of 32–128 μg/mL. Twenty-seven strains of microorganisms as shown below, cultured in MH broth at 37 °C for 24 h, were diluted with 0.9% normal saline solution to adjust the cell density of 3×10^9 cell/mL. The microorganisms were inoculated onto each plate and further incubated at 37 °C for 18–48 h. Compounds which exerted high efficacy to inhibit cell growth of the organisms were determined. The DMSO was tested in parallel with the compounds and showed no effect on the tested organisms. Twenty-seven strains of tested microorganisms were gram negative bacteria: *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 700603, *Salmonella typhimurium* ATCC 13311, *Salmonella choleraesuis* ATCC 10708, *Pseudomonas aeruginosa* ATCC 15442, *Edwardsiella tarda*, *Shigella dysenteriae*, *Citrobacter freundii*, *Morganella morganii, Vibrio cholera*, *Vibrio mimicus*, *Aeromonas hydrophila*, *Plesiomonas shigelloides*, *Xanthomonas maltophilia*, *Neisseria mucosa*, *Branhamella catarrhalis*; gram positive bacteria: *Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* ATCC 12228, *Enterococcus faecalis* ATCC 29212, *Micrococcus lutens* ATCC 10240, *Corynebacterium diphtheriae* NCTC 10356, *Bacillus subtilis* ATCC 6633, *Streptococcus pyogenes*, *Listeria monocytogenes*, *Bacillus cereus*, *Micrococcus flavas* and diploid fungus (yeast): *Candida albicans*.

4.3.2. Antimalarial assay

Antimalarial activity of the tested compounds was evaluated against *P. falciparum* chloroquine resistant (T9.94) using the literature method [29].

Human erythrocytes (type O) infected with *P. falciparum* chloroquine resistant (T9.94) were maintained in continuous culture, according to the method described previously [30]. RPMI-1640 culture medium supplemented with 25 mM of HEPES, 40 mg/L gentamicin sulfate and 10 mL of human serum was used in continuous culture.

Initially, *P. falciparum* culture was synchronized by using sorbitol induced hemolysis according to the method of Lambros and Vanderberg [31] to obtain only ring stage-infected red blood cells and then incubated for 48 h prior to the drug testing to avoid effect of sorbitol. The experiments were started with synchronized suspension of 0.5–1% infected red blood cell during ring stage. Parasites were suspended with culture medium supplemented with 15% human serum to obtain 10% cell suspension. The parasite suspension was put onto a 96-well microculture plate; 50 μL in each well and then added 50 μL of various tested drug concentrations. These parasite suspensions were incubated for 48 h in the atmosphere of 5% CO_2 at 37 °C. The percents parasitemia of control and drug-treated groups were examined by microscopic technique using methanol-fixed Giemsa stained of thin smear blood preparation. The efficacy of the drugs were evaluated by determining the drug concentration that reduced parasite growth by 50% (IC_{50}).

4.3.3. Cytotoxic assay

Cytotoxic assay was performed using the modified method as previously described [15]. Cancer cells were grown in Ham's/F12 medium containing 2 mM L-glutamine supplemented with 100 U/mL penicillin, streptomycin and 10% FBS. Except HepG2 cell was grown in DMEM. Briefly, cell lines (Table 2) suspended in RPMI-1640 containing 10% FBS were seeded at 1×10^4 cells (100 μL) per well in a 96-well plate, and incubated in humidified atmosphere, 95% air, 5% CO_2 at 37 °C. After 24 h, additional medium (100 μL) containing the test compound and vehicle was added to a final concentration of 50 μg/mL, 0.2% DMSO, and further incubated for 3 days. Cells were subsequently fixed with 95% EtOH, stained with crystal violet solution, and lysed with a solution of 0.1 N HCl in MeOH, after which absorbance was measured at 550 nm, whereas HuCCA-1, A549 and HepG2 cells were stained by MTT. IC_{50} values were determined as the drug and sample concentrations at 50% inhibition of the cell growth.

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