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Short communication

Synthesis, antimicrobial and antiviral evaluation of certain thienopyrimidine derivatives

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Summary — A series of 2-substituted amino-3-aminocyclopenteno or cyclohexeno[*b*]thieno[2,3-*d*]-3,4-dihydropyrimidin-4-ones has been synthesized by reacting the corresponding thioureido derivatives with hydrazine hydrate. The thienoprimidine analogues obtained were further used to prepare their arylideneamino, thienotriazolopyrimidine or 2-methylthienotriazolopyrimidine derivatives. The prepared compounds were screened for antimicrobial, antiviral and cytotoxic activity. Some of the tested compounds showed promising activity. Detailed syntheses and spectroscopic and biological data are reported.

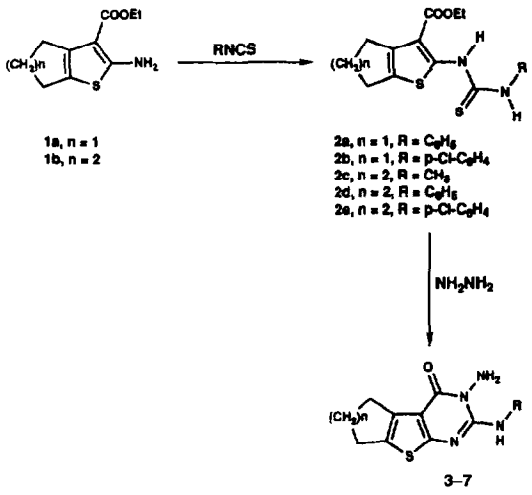
thienopyrimidine / antimicrobial activity / antiviral activity / cytotoxic activity

Introduction

The thienopyrimidine structure (a purine analogue) has recently received considerable attention. Many of its substituted derivatives, including thienotriazolopyrimidines [1], thienotetrazolopyrimidines [2], thienothiadiazolopyrimidine [3] and others, have pronounced antibacterial [4–7], antifungal [8], antiviral [9], antiinflammatory [10, 11] and herbicidal [12] activity. Derivatives of pyrazolopyrimidine, another purine isostere, have long been known to possess antitumor and cytostatic activity [13–17]. In view of these facts and as a continuation of the previous efforts carried out in our laboratory [18, 19], several cyclopenteno[*b*]thieno[2,3-*d*]-3,4-dihydropyrimidine-4-ones, cyclohexeno[*b*]thieno[2,3-*d*]-3,4-dihydropyrimidine-4-ones and their 1,2,3-triazolo[2,3-*a*]-5*H*-pyrimidine-5-one derivatives have been synthesized to explore their antimicrobial, antiviral and cytotoxic activity.

Chemistry

The starting materials ethyl 2-aminocyclopenteno and cyclohexeno[*b*]thiophene-3-carboxylate (**1a,b**) were prepared following the method of Gewald [20] *via* the reaction of either cyclopentanone or cyclohexanone, ethyl cyanoacetate and sulfur in the presence of diethylamine or morpholine. The reaction of **1a,b** with methyl, phenyl or *p*-chlorophenyl isothiocyanate yielded the corresponding thioureido derivatives **2a–e** [6, 21]. The 2-substituted amino-3-aminocyclopenteno or cyclohexeno[*b*]thieno[2,3-*d*]-3,4-dihydropyrimidin-4-ones (**3–7**) were prepared by prolonged heating of **2a–e** with hydrazine hydrate (table I, scheme 1).



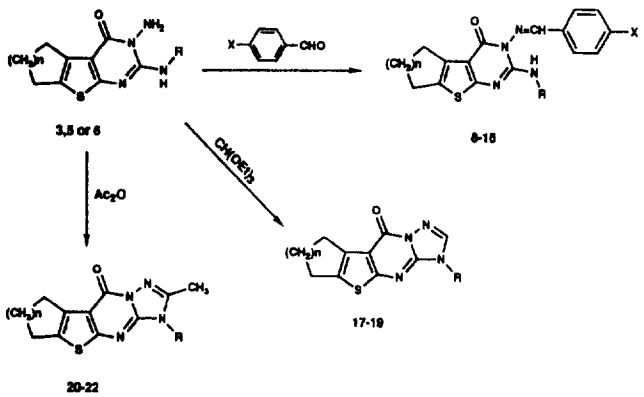
Scheme 1.

Table I. Recrystallization solvents, melting points, yields (%), and molecular formulae of the new compounds.

Compound	*n*	*R*	*X*	*Solvent*[a]	*Mp (°C)*	*Yield (%)*	*Molecular formula*
3	1	C_6H_5	–	E, C	192–194	45	$C_{15}H_{14}N_4OS$
4	1	*p*-ClC_6H_4	–	E	258–260	43	$C_{15}H_{13}ClN_4OS$
5	2	CH_3	–	E, C	243–245	55	$C_{11}H_{14}N_4OS$
6	2	C_6H_5	–	E, C	230–232	50	$C_{16}H_{16}N_4OS$
7	2	*p*-ClC_6H_4	–	E	254–256	48	$C_{16}H_{15}ClN_4OS$
8	1	C_6H_5	F	A	250–252	73	$C_{23}H_{17}FN_4OS$
9	1	C_6H_5	Br	A	238–240	68	$C_{23}H_{17}BrN_4OS$
10	1	C_6H_5	NO_2	A	263–265	79	$C_{23}H_{17}N_5O_3S$
11	2	CH_3	F	A	258–260	76	$C_{18}H_{17}FN_4OS$
12	2	CH_3	Br	A	252–255	65	$C_{18}H_{17}BrN_4OS$
13	2	CH_3	NO_2	A	270–273	80	$C_{18}H_{17}N_5O_3S$
14	2	C_6H_5	F	A	266–268	67	$C_{22}H_{19}FN_4OS$
15	2	C_6H_5	Br	A	246–247	70	$C_{22}H_{19}BrN_4OS$
16	2	C_6H_5	NO_2	A	250–253	82	$C_{22}H_{19}N_5O_3S$
17	1	C_6H_5	–	E	264–266	57	$C_{16}H_{12}N_4OS$
18	2	CH_3	–	E, C	257–260	65	$C_{12}H_{12}N_4OS$
19	2	C_6H_5	–	E	254–256	53	$C_{17}H_{14}N_4OS$
20	1	C_6H_5	–	E	265–267	78	$C_{17}H_{14}N_4OS$
21	2	CH_3	–	E, C	270–272	85	$C_{13}H_{14}N_4OS$
22	2	C_6H_5	–	E	281–283	80	$C_{18}H_{16}N_4OS$

[a]A = acetic acid, C = chloroform, E = ethanol.

Interaction of compounds **3**, **5** and **6** with a variety of aromatic aldehydes, triethyl orthoformate or acetic anhydride afforded the corresponding arylideneamino derivatives **8–16**, thienotriazolopyrimidines **17–19** and 2-methylthienotriazolopyrimidines **20–22**, respectively (table I, scheme 2).



Scheme 2.

Biological investigation and discussion

The antimicrobial screening of the synthesized compounds was undertaken using the agar diffusion assay [22]. Table II lists the screening results of the tested compounds against the Gram-negative bacteria *Pseudomonas aeruginosa* and *Escherichia coli* and the Gram-positive bacteria *Staphylococcus aureus*, *Bacillus subtilis*, in addition to the pathogenic fungi *Candida albicans*. The obtained data revealed that most of the tested compunds showed moderate activity towards the microorganisms used. Compound **3** proved to possess remarkable broad-spectrum potency against *P aeruginosa*, *S aureus*, *B subtilis* and *C albicans*, while compounds **4** and **5** exhibited moderate activity against *S aureus* and *B subtilis*, respectively. Compounds **6**, **7**, **15**, **16** and **22** are moderatly active against the Gram-positive bacteria. A minimum inhibitory concentration (MIC) experiment was performed for each of the active compounds with ampicillin, streptomycin and nystatin as positive controls. The results are shown in table II.

Antiviral testing was performed using Vero cells (cells isolated from the kidneys of green monkeys), herpes simplex and Aphidicolin as a positive control

Table II. Antibacterial and antimycotic activities of compounds **3–7**, **15**, **16** and **22** in terms of MIC (μg/ml) after 48 h.

Compound	*MIC (μg/ml)*			
	P aeruginosa	*S aureus*	*B subtilis*	*C albicans*
3	12.50	6.25	25.00	12.50
4	–	12.50	–	–
5	–	–	25.00	–
6	–	12.50	12.50	–
7	–	25.00	–	–
15	–	12.50	–	–
16	–	25.00	25.00	–
22	–	25.00	12.50	–
Ampicillin	1	1	1	–
Streptomycin	4	4	4	–
Nystatin	–	–	–	2

[25]. Compound **3** showed an 80% reduction of the viral plaque number while compounds **4**, **5**, **6**, **7** and **15** showed 40, 10, 30, 20 and 30% reduction respectively. Compounds **16** and **22** had no antiviral activity (table III).

Cultured mammalian cells have recently been used to determine the response of tumor cells isolated from cancer patients to various chemotherapeutic agents [23, 24] with high sensitivity (concentrations as low as 0.25–1.7 μg/ml). Mammalian cell culture systems have the benefit of being faster, less expensive and more sensitive than the use of live animals. Cytotoxicity assay [23, 24] results for the highly active

Table III. Antiviral activity of compounds **3–7**, **15**, **16** and **22**.

Compound	*% Reduction in number of plaques*	*Minimum antiviral concentration (μg/ml)*
Aphidicolin[a]	100	0.005
3	80	0.3
4	40	0.3
5	10	0.3
6	30	0.3
7	20	0.3
15	30	0.3
16	None	0.3
22	None	0.3

[a]Positive control.

Table IV. LC_{50}[a] values of the active compounds to 3T3 mouse fibroblast cells.

Compound	*LC_{50} (μg/ml)*
2	25
3	250
4	250
5	50
6	125
14	50
15	None[b]
21	None[b]

[a]LC_{50} values (concentration of compound resulting in 50% reduction in cell density) were estimated visually from triplicate cultures. [b]Toxicity was not observed up to 500 μg/ml.

antibacterial compounds showed that compound **3** was very toxic to 3T3 fibroblast cells, while compounds **4**, **5**, **6**, **7** and **15** showed moderate toxicity. Compounds **16** and **22** were devoid of any toxicity up to 500 μg/ml concentration (table IV).

Compound **3** exhibited broad-spectrum antibiotic and antiviral activities, with considerable cytotoxicity. Compounds **4**, **5**, **6**, **7**, **15**, **16** and **22** showed Gram-positive antibacterial potency. Only compounds **16** and **22** had a high safety margin. Efforts will be continued to explore the scope and limitation of activity for compound **3**.

Experimental protocols

Melting points (°C, uncorrected) were recorded on a Fisher-Johns apparatus. ^{1}H-NMR spectra were recorded on a Varian EM 360 (90 MHz) instrument using TMS as an internal standard (chemical shift in δ ppm). Microanalytical data (C, H, N) agreed with the proposed structures within ±0.4% of the theoretical values. Thin-layer chromatography was performed on precoated silica-gel plates (60-F 254, 0.2 mm) manufacturated by EM Sciences, Inc and shortwave UV light (254 nm) was used to detect the UV-absorbing compounds. The following organisms were used in the antimicrobial screening: *S aureus* ATCC 06538, *E coli* ATCC 10536, *P aeruginosa* ATCC 15442, *B subtilis* ATCC 6633, and *C albicans* ATCC 1023. The following materials were used in the cytotoxicity evaluation: hemocytometer (Bright line, AO instrument Co, Buffalo, NY, USA), inverted microscope (Nikon Inc, Instrument Division, Garden City, NY, USA), 96-well sterile tissue culture tray (Falcon products 3042 Microtest, Oxnard, CA, USA), Dulbecco's modified Eagles medium (Gibco, Grand Island, NY, USA) supplemented with 10% calf serum (Gibco) and antibiotics (Sigma Chemical Co), Trypsin (Sigma Chemical Co), Giemsa stain (Fisher Scientific Co, Fair lawn, NJ, USA).

Chemistry

2-Substituted amino-3-aminocyclopenteno or cyclohexeno[b]-thieno[2,3-d]-3,4-dihydropyrimidin-4-ones 3–7

A mixture of the appropriate thioureido derivative **2a–e** (0.01 mol) and hydrazine hydrate (10 g, 0.2 mol) was heated under reflux for 10 h. The solid that separated upon cooling was filtered, washed with water dried and recrystallized. ^{1}H NMR ($CDCl_3$) **3**: δ 2.2–2.6 (m, 2H, CH_2), 2.6–3.1 (m, 4H, CH_2), 4.5 (s, 2H, NH_2; exchangeable), 7.0–7.8 (m, 5H, ArH), 8.5 (s, 1H, NH; exchangeable). **4**: δ 2.1–2.5 (m, 2H, CH_2), 2.55–3.0 (m, 4H, CH_2), 5.5 (s, 2H, NH_2; exchangeable), 7.2–7.8 (q_{AB}, 4H, ArH), 9.6 (s, 1H, NH; exchangeable). **5**: δ 1.6–2.1 (m, 2H, CH_2), 2.6–3.1 (m, 6H, CH_2), 3.35 (s, 3H, CH_3), 4.4 (brm, 2H, NH_2; exchangeable), 8.6 (brs, 1H, NH; exchangeable). **6**: δ 1.7–1.95 (m, 2H, CH_2), 2.45–2.75 (m, 6H, CH_2), 7.1–7.8 (m, 7H, ArH and NH_2; exchangeable), 11.1 (brs, 1H, NH; exchangeable). **7**: δ 1.6–2.0 (m, 2H, CH_2), 2.4–2.8 (m, 6H, CH_2), 7.1–7.7 (q_{AB}, 4H, ArH), 8.2 (brm, 2H, NH_2; exchangeable), 11.2 (brs, 1H, NH; exchangeable).

2-Substituted amino-3-arylidenoaminocyclopenteno or cyclohexeno[b]thieno[2,3-d]-3,4-dihydropyrimidin-4-ones 8–16

A mixture of **3**, **5** or **6** (0.05 mol) and the appropriate aromatic aldehyde in acetic acid was heated under reflux for 4 h. The solid separated upon cooling was collected by filtration, dried and recrystallized. ^{1}H NMR: **8** ($CDCl_3$): δ 2.1–2.6 (m, 2H, CH_2), 2.6–3.0 (m, 4H, CH_2), 7.2–7.9 (m, 9H, ArH), 9.4 (s, 1H, CH-), 11.5 (brs, 1H, NH; exchangeable). **9** ($CDCl_3$): δ 2.2–2.6 (m, 2H, CH_2), 2.65–3.1 (m, 4H, CH_2), 7.3–7.8 (m, 9H, ArH), 9.3 (s, 1H, CH-), 10.5 (brs, 1H, NH; exchangeable). **10** (DMSO-d_6): δ 2.2–2.6 (m, 2H, CH_2), 2.7–3.2 (m, 4H, CH_2), 7.4–8.5 (m, 9H, ArH), 9.9 (s, 1H, CH-), 11.3 (brs, 1H, NH; exchangeable). **11** ($CDCl_3$): δ 1.6–2.0 (m, 2H, CH_2), 2.5–3.0 (m, 6H, CH_2), 3.5 (s, 3H, CH_3), 7.6–7.8 (q_{AB}, 4H, ArH), 8.1 (s, 1H, NH; exchangeable), 9.4 (s, 1H, CH-). **12** ($CDCl_3$): δ 1.65–1.85 (m, 2H, CH_2), 2.55–2.85 (m, 6H, CH_2), 3.55 (s, 3H, CH_3), 7.7–7.9 (qAB, 4H, ArH), 8.65 (s, 1H, NH; exchangeable), 9.6 (s, 1H, CH-). **13** (DMSO-d_6): δ 1.6–1.9 (m, 2H, CH_2), 2.5–3.0 (m, 6H, CH_2), 3.5 (s, 3H, CH_3), 7.7–8.6 (m, 5H, ArH and NH; exchangeable), 9.8 (s, 1H, CH-), **14** ($CDCl_3$): δ 1.55–1.8 (m, 2H, CH_2), 2.5–2.8 (m, 6H, CH_2), 7.2–8.0 (m, 9H, ArH), 9.3 (s, 1H, ArH), 9.3 (s, 1H, CH-), 10.9 (s, 1H, NH; exchangeable). **15** ($CDCl_3$): δ 1.6–2.0 (m, 2H, CH_2), 2.5–3.1 (m, 6H, CH_2), 7.2–7.9 (m, 10H, ArH, exchangeable), 9.4 (s, 1H, CH-). **16** (DMSO-d_6): δ 1.7–2.0 (m, 2H, CH_2), 2.5–3.1 (m, 6H, CH_2), 7.8–8.7 (m, 10H, ArH and NH; exchangeable), 9.9 (s, 1H, CH-).

1-Substituted cyclopenteno or cyclohexeno[b]thieno[2,3-d]-[1,2,4]triazolo[2,3-a]-5H-pyrimidin-5-ones 17–19

A mixture of **3**, **5** or **6** (0.012 mol), *p*-toluenesulfonic acid (3.4 g, 0.02 mol) and triethylorthoformate (45 ml) was heated under reflux for 12 h. The excess orthoformate was removed under vacuum and the obtained residue was triturated with ice-cold water, filtered, dried and recrystallized. ^{1}H NMR: **17** (DMSO-d_6): δ 2.1–3.1 (m, 6H, CH_2), 7.3–7.9 (m, 5H, ArH), 8.3 (s, 1H, 2-triazolo H). **18** ($CDCl_3$): δ 1.6–2.1 (m, 2H, CH_2), 2.6–3.2 (m, 6H, CH_2), 3.7 (s, 3H, CH_3), 8.2 (s, 1H, 2-triazolo H). **19** (DMSO-d_6): δ 1.5–2.0 (m, 2H, CH_2), 2.6–3.0 (m, 6H, CH_2), 7.2–7.8 (m, 5H, ArH), 8.2 (s, 1H, 2-triazolo H).

1-Substituted 2-methylcyclopenteno or cyclohexeno[b]thieno-[2,3-d][1,2,4]triazolo[2,3-a]-5H-pyrimidin-5-ones 20–22

A mixture of **3**, **5** or **6** (0.01 mol) and acetic anhydride (10 ml) was heated under reflux for 8 h. On cooling, the separated solid was filtered, dried and recrystallized. ^{1}H NMR, **20** (DMSO-d_6): δ 2.0–3.1 (m, 9H, CH_2 and CH_3), 7.1–7.8 (m, 5H, Ar). **21** ($CDCl_3$): δ 1.5–2.1 (m, 5H, CH_2 and CH_3), 2.5–3.1 (m, 6H, CH_2), 3.6 (s, 3H, CH_3). **22** (DMSO-d_6): δ 1.6–2.1 (m, 5H, CH_2 and CH_3), 2.5–3.05 (m, 6H, CH_2), 7.2–7.7 (m, 5H, ArH).

Antimicrobial testing

Nutrient agar plates were seeded using 0.1 ml of the diluted organism. Cylindrical plugs were removed from the agar using a sterile cork bore, and 100 µl of the tested compounds (1 mg/ml DMSO) and blank solvent were added to each well in triplicate. Plates of *P aeruginosa*, *E coli*, *S aureus* and *B subtilis* were incubated at 37°C. Those of *C albicans* was incubated at 30°C. After 24 h incubation the average diameter of the inhibition zone was measured in millimeters. MIC was determined using the simple dilution method [22].

Cytotoxicity assay

3T3 fibroblast cell supension was prepared by washing the cells from confluent 100 mm culture dish using Dulbecco's medium. The cells were then incubated under normal culture conditions (37°C, 5% CO_2 atmosphere) in a medium containing 0.05% trypsin for about 5 min until cells began to detach from substratum and drew off trypsin solution. The cells were then flushed from the substratum using fresh medium containing 20% calf serum. The number of the cells in the suspension was then counted using hemocytometer and the suspension was diluted to about 2 x 10^4 cells/ml. The investigated compounds were dissolved in 50% aqueous dimethylsulfoxide at 200 µg/ml. A 100 µl portion of the cell culture and a 100 µl portion of the drug solution were added to each well of a 96-well culture tray. (A preliminary range finding experiment was done with 1–10 dilution followed by another experiment with a low concentration gradient; the highest concentration of drug should contain less than 1% DMSO to eliminate the solvent toxicity.) The culture trays were then incubated for 3 or 4 d or until cells in control wells to approach confluency. The medium was decanted and each tray was blotted in inverted position with a paper towel. Cells were washed on a dish with additional serum-free medium, decanted and blotted again. Formol saline solution (200 µl) was added to each well and the tray was decanted after 20 min. Giemsa stain (200 µl) was added to each well and the tray was decanted after 30 min and then washed with water to remove excess stain. The LC_{50} (the drug concentration that kill 50% of the cells) was then estimated by visual examination of the cell density.

Antiviral screening

Microtiter trays with confluent cultures of Vero cells were inverted and the medium was shaken out. A serial dilution of sterile samples dissolved in the medium was then added (100 µl in each well). Trays wells were then inoculated with 30 plaque-forming units of herpes simplex-type 1 (HS-1) virus in 100 µl medium containing 10% (v/v) calf serum. In each tray, the last row of wells was reserved for controls consisting of Vero cells treated with virus but no test compounds. The trays were incubated under normal culture conditions (37°C, 5% CO_2 atmosphere) for 66 h. The trays were inverted onto a pad of paper towels and the remaining cells were rinsed carefully with the medium and fixed with 3.7% (v/v) formaldehyde in saline solution for 20 min. They were then rinsed with water, stained with 0.5% (w/v) crystal violet solution in 20% aqueous ethanol for 30 min and rinsed with water to remove excess stain. The trays were examined visually, and antiviral activity was estimated as the percentage reduction in the number of virus plaques.

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