Synthesis of Novel 2-(Substituted amino)alkylthiopyrimidin-4(3H)-ones as Potential Antimicrobial Agents

Mohamed I. Attia 1, Ali A. El-Emam 1, Abdulghafoor A. Al-Turkistani 1, Amany L. Kansoh 2 and Nasser R. El-Brollosy 1,3,*

1 Department of Pharmaceutical Chemistry, College of Pharmacy, King Saud University, P. O. Box 2457, Riyadh 11451, Saudi Arabia
2 Microbial Chemistry Department, Genetic Engineering and Biotechnology Division, National Research Centre, Giza 12622, Egypt
3 Department of Chemistry, Faculty of Science, Tanta University, Tanta 31527, Egypt

* Author to whom correspondence should be addressed; E-Mail: brollosy@yahoo.com; Tel: +966-114-673-765, Fax: +966-114-676-220.

Received: 29 November 2013; in revised from: 13 December 2013 / Accepted: 16 December 2013 / Published: 27 December 2013

Abstract: 5-Alkyl-6-(substituted benzyl)-2-thiouracils 3a,c were reacted with (2-chloroethyl)diethylamine hydrochloride to afford the corresponding 2-(2-diethylamino)ethylthiopyrimidin-4(3H)-ones 4a,b. Reaction of 3a–c with N-(2-chloroethyl)pyrrolidine hydrochloride and/or N-(2-chloroethyl)piperidine hydrochloride gave the corresponding 2-[2-(pyrrolidin-1-yl)ethyl]-thiopyrimidin-4(3H)-ones 5a–c and 2-[2-(piperidin-1-yl)ethyl]thiopyrimidin-4(3H)-ones 6a,b, respectively. Treatment of 3a–d with N-(2-chloroethyl)morpholine hydrochloride under the same reaction conditions formed the corresponding 2-[2-(morpholin-4-yl)ethyl]thiopyrimidines 6c–f. On the other hand, 3a,b were reacted with N-(2-bromoethyl)phthalimide and/or N-(3-bromopropyl)phthalimide to furnish the corresponding 2-[2-(N-phthalimido)ethyl]-pyrimidines 7a,b and 2-[3-(N-phthalimido)-propyl]pyrimidines 7c,d, respectively. Compounds 3a–d, 4a,b, 5a–c, 6a–f and 7a–d were screened against Gram-positive bacteria (Staphylococcus aureus ATCC 29213, Bacillus subtilis NRRL 4219 and Bacillus cereus), yeast-like pathogenic fungus (Candida albicans ATCC 10231) and a fungus (Aspergillusniger NRRL 599). The best antibacterial activity was displayed by compounds 3a, 3b, 4a, 5a, 5b, 6d, 6f, 7b and 7d, whereas compounds 4b, 5b, 5c, 6a, 6b and 6f exhibited the best antifungal activity.
Keywords: 2-thiouracils; pyrimidin-4(3H)-ones; alkylation; antibacterial activity; anti-fungal activity

1. Introduction

In chemotherapy pyrimidines are considered as privileged structures with a large spectrum of biological activities. They are known very widely in Nature since they are components of RNA and DNA. The chemotherapeutic efficacy of pyrimidines may be due to their ability to inhibit vital enzymes responsible for nucleic acid biosynthesis such as reverse transcriptase, dihydrofolate reductase, uridine and thymidine phosphorylase, as well as thymidylate synthetase. Several pyrimidine derivatives exhibit diverse pharmacological activities as antiviral [1–9], anti-inflammatory [10–12], and antimalarial agents [13–15]. Many pyrimidines have been demonstrated to possess anticancer [16–21], antituberculosis [22] and anti-allergic [23] activities. Moreover, several pyrimidine derivatives have been reported as antithyroid [24] and antimicrobial agents [25–30], as well as human thymidine and uridine phosphorylase inhibitors [31–33].

In a previous study [34], we synthesized a series of 2-(substituted amino)ethylthiopyrimidines analogues of S-DABOs to be screened as reverse transcriptase inhibitors against human immunodeficiency virus (HIV-1). We found it of interest to evaluate the antimicrobial activity for such pyrimidine derivatives. In the present work, and as a part of our continuing interest in the chemistry of pyrimidines [30,34–42], the synthesis and antimicrobial evaluation of some novel 2-(substituted amino)alkylthiopyrimidin-4(3H)-one derivatives have been investigated.

2. Results and Discussion

2.1. Chemistry

5-Alkyl-6-(substituted benzyl)-2-thiouracils 3a–d were prepared, as described in our previous work [34,42], by reaction of (substituted phenyl)acetonitrile 1 with the appropriate ethyl 2-bromoesters 2 in anhydrous THF in the presence of zinc dust, followed by treatment of the β-ketoesters thus formed with thiourea in the presence of sodium ethoxide. Compounds 3a and 3c were reacted with (2-chloroethyl) diethylamine hydrochloride in DMF in the presence of anhydrous potassium carbonate to afford 6-(4-chlorobenzyl)-2-(2-diethylamino)ethylthio-5-methylpyrimidin-4(3H)-one (4a) [34] and 6-(3,4-dimethoxybenzyl)-2-(2-diethylamino)ethylthio-5-ethylpyrimidin-4(3H)-one (4b) in good yields (Scheme 1).

6-(4-Chlorobenzyl)-5-methyl-2-[2-(pyrrolidin-1-yl)ethyl]thiopyrimidin-4(3H)-one (5a) [34], 6-(4-chlorobenzyl)-5-ethyl-2-[2-(pyrrolidin-1-yl)ethyl]thiopyrimidin-4(3H)-one (5b) [34] and 6-(3,4-dimethoxybenzyl)-5-ethyl-2-[2-(pyrrolidin-1-yl)ethyl]thiopyrimidin-4(3H)-one (5c) were obtained, respectively, in good yields, on reaction of compounds 3a, 3b and/or 3c with N-(2-chloroethyl)pyrrolidine hydrochloride in the presence of anhydrous potassium carbonate in DMF (Scheme 2). Alkylation of 3b and/or 3c with N-(2-chloroethyl)piperidine hydrochloride in DMF containing potassium carbonate gave 6-(4-chlorobenzyl)-5-ethyl-2-[2-(piperidin-1-yl)ethyl]thiopyrimidin-4(3H)-one (6a) [34] and 6-(3,4-dimethoxybenzyl)-5-ethyl-2-[2-(piperidin-1-yl)ethyl]thiopyrimidin-
4(3H)-one (6b) in 77% and 72% yields, respectively. Reaction of compounds 3a–d with N-(2-chloroethyl)morpholine hydrochloride under the same reaction conditions formed the corresponding 2-[2-(morpholin-4-yl)ethyl]thiopyrimidines 6c–f in 63%–74% yields (Scheme 2).

Scheme 1. Synthesis of compounds 3a–d and 4a,b.

On the other hand, compounds 3a and 3b were treated with N-(2-bromoethyl)phthalimide and/or N-(3-bromopropyl)phthalimide in the presence of potassium carbonate in DMF to furnish the corresponding 2-[2-(N-phthalimido)ethyl]pyrimidines 7a,b and 2-[3-(N-phthalimido)propyl]-pyrimidines 7c,d in 69%, 71% and 62%, 64% yields, respectively (Scheme 2).
2.2. Antimicrobial Testing

The antimicrobial activities of the synthesized compounds, 3a–d, 4a,b, 5a–c, 6a–f and 7a–d (200 µg/10 mm disc) as well as the reference drugs, ampicillin and clotrimazole, were screened against yeast-like pathogenic fungus (Candida albicans ATCC 10231), fungus (Aspergillus niger NRRL 599) and Gram-positive bacteria (Staphylococcus aureus ATCC 29213, Bacillus subtilis NRRL 4219 and Bacillus cereus) which are important human pathogenic microorganisms. A Diameter of Inhibition Zone (DIZ) assay [43] was performed to evaluate the preliminary antimicrobial potential of the test compounds against the test organisms and the results are given in Table 1.

Table 1. Antimicrobial activity of compounds 3a–d, 4a,b, 5a–c, 6a–f and 7a–d, the broad spectrum antibacterial drug ampicillin and the antifungal drug clotrimazole against Gram-positive bacteria (Staphylococcus aureus ATCC 29213, Bacillus subtilis NRRL 4219 and Bacillus cereus), yeast-like pathogenic fungus (Candida albicans ATCC 10231) and fungus (Aspergillus niger NRRL 599).

| Comp. No. | Staphylococcus aureus | Bacillus subtilis | Bacillus cereus | Candida albicans | Aspergillus niger |
|-----------|-----------------------|-------------------|-----------------|-----------------|-----------------|
| 3a        | 22                    | 17                | 21              | 15              | -               |
| 3b        | 30                    | 18                | 18              | 22              | -               |
| 3c        | -                     | -                 | -               | -               | -               |
| 3d        | -                     | -                 | -               | -               | -               |
| 4a        | 30                    | 12                | 12              | -               | 13              |
| 4b        | -                     | -                 | -               | 21              | 25              |
| 5a        | 30                    | 15                | 13              | 13              | -               |
| 5b        | 27                    | 13                | 15              | 32              | 13              |
| 5c        | -                     | -                 | -               | 35              | 31              |
| 6a        | 23                    | -                 | -               | 30              | 21              |
| 6b        | 23                    | -                 | -               | 20              | 25              |
| 6c        | 24                    | -                 | -               | -               | -               |
| 6d        | 27                    | 18                | 12              | 15              | -               |
| 6e        | -                     | 12                | 12              | -               | -               |
| 6f        | 21                    | 15                | 13              | 25              | 24              |
| 7a        | 21                    | -                 | -               | 22              | -               |
| 7b        | 25                    | 13                | 12              | 12              | -               |
| 7c        | -                     | -                 | -               | 13              | 18              |
| 7d        | 24                    | 13                | 16              | 12              | -               |
| Ampicillin| 35                    | 38                | 35              | 38              | 40              |
| Clotrimazole|                    |                   |                 |                 |                 |

(−): Inactive (inhibition zone < 10 mm).

The synthesized compounds showed varying degrees of inhibition zones against the tested microorganisms. The antibacterial results revealed that compounds 3a, 3b, 4a, 5a, 5b, 6a–d, 6f, 7a, 7b and 7d showed strong activity (growth inhibition zones > 18 mm against one or more of the tested microorganisms), compound 6e exhibited weak activity (growth inhibition zone 10–13 mm), while
compounds 3c, 3d, 4b, 5c and 7c showed no antibacterial activity (growth inhibition zones < 10 mm). Concerning the antifungal results, compounds 3b, 4b, 5b, 6a, 6b, 6f and 7a exhibited strong activity, compounds 3a, 6d and 7c showed moderate activity (growth inhibition zones 14–18 mm), compounds 4a, 5a, 5b, 6d, 6f, 7b and 7d showed weak activity, whereas no antifungal activity was noticed for compounds 3c, 3d, 5c and 6e. In general, the best antibacterial activity was displayed by compounds 3a, 3b, 4a, 5a, 5b, 6d, 6f, 7b and 7d. Compounds 4b, 5b, 5c, 6a, 6b and 6f exhibited the best antifungal activity, whereas compounds 3c and 3d showed no activity against the test organisms. Gram-positive bacteria, *Staphylococcus aureus*, and the yeast-like, *Candida albicans*, are considered the most sensitive among the tested microorganisms. The synthesized test compounds showed no activity against Gram negative pathogens, *Escherichia coli* and *Pseudomonas aeruginosa*. Although several compounds showed strong antibacterial and antifungal activities, none of them were found to be superior to the reference drugs. Compounds 3a, 3b, 4a, 5a, 5b, 6b, 6d, 6f, 7b and 7d displayed a relatively broad spectrum activity, accordingly, their MIC values were determined. The MIC values for compounds 3a, 3b, 4a, 5a, 5b, 6b, 6d, 6f, 7b and 7d against the most sensitive tested microorganisms, *Staphylococcus aureus* and *Candida albicans* are represented in Table 2.

### Table 2. The minimal inhibitory concentration (MIC, µg/mL) values for compounds 3a, 3b, 4a, 5a, 5b, 6b, 6d, 6f, 7b and 7d against the most sensitive tested microorganisms, *Staphylococcus aureus* and *Candida albicans*.

| Compound No. | The minimal inhibitory concentration (MIC, µg/mL) *a* |
|--------------|------------------------------------------------------|
|              | *Staphylococcus aureus* | *Candida albicans* |
| 3a           | 100                     | 100                  |
| 3b           | 25                      | 25                   |
| 4a           | 25                      | ND                   |
| 5a           | 25                      | 100                  |
| 5b           | 25                      | 25                   |
| 6b           | 100                     | 50                   |
| 6d           | 25                      | 100                  |
| 6f           | 50                      | 50                   |
| 7b           | 50                      | 100                  |
| 7d           | 50                      | 100                  |
| Ampicillin   | 6.0                     |                      |
| Clotrimazole |                        | 6.0                  |

* *The lowest concentration of the test compound that inhibits the growth of microorganism (µg/mL).*  
* ND: not determined.

According to the above results, the antimicrobial activity seemed to be dependent on the nature of substituents. Compounds containing a 4-chlorobenzyl substituent at C-6 of the pyrimidine ring showed the best antibacterial activity, whereas, the best antifungal results were given by compounds containing 2-(pyrrolidin-1-yl)ethylthio and 2-(piperidin-1-yl)ethylthio substituents at C-2 of the pyrimidine ring. Concerning compounds 7a–d, the ethyl group at C-5 of the ring was found to improve the antimicrobial activity.
3. Experimental

3.1. General

Melting points (°C) were measured in open glass capillaries using a Branstead 9100 Electrothermal melting point apparatus and are uncorrected. NMR spectra were obtained on a Bruker AC 500 Ultra Shield NMR spectrometer (Fällanden, Switzerland) operating at 500.13 MHz for $^1$H and 125.76 MHz for $^{13}$C, the chemical shifts are expressed in δ (ppm) downfield from tetramethylsilane (TMS) as internal standard; coupling constants (J) are expressed in Hz and signals are expressed as s (singlet), d (doublet), t (triplet), q (quartet), or m (multiplet). Electrospray ionization mass spectra (ESI-MS) were recorded on an Agilent 6410 Triple Quad tandem mass spectrometer (Santa Clara, CA, USA) at 4.0 kV for the positive ions. The progress of reactions was monitored by TLC (DC-alufolio 60 F254) from Merck, and visualization with ultraviolet light (UV) at 365 and 254 nm. For column chromatography Merck silica gel (0.040–0.063 mm) was used. The tested microorganisms were obtained from MIRCIN Cairo, Faculty of Agriculture, Ain Shams University, Cairo, Egypt. Bacteria, fungi and yeast-like fungi were cultivated on agar media of nutrient, Czapek’s dox and malt–extract, respectively. The reference drugs ampicillin trihydrate (CAS 7177-48-2) and clotrimazole (CAS 23593-75-1) were obtained from Sigma-Aldrich Chemie GmbH (Taufkirchen, Germany). Compounds 3a–d, 4a, 5a,b and 6a were reported in our previous studies [34,42].

3.2. General Procedure for Preparation of 2-(Substituted amino)ethylthiopyrimidines 4b, 5c and 6b–f

To a solution of the appropriate compound 3a–d (1 mmol) in anhydrous DMF (5 mL), was added anhydrous potassium carbonate (0.304 g, 2.2 mmol) followed by the appropriate 2-chloroethyl substituted amine hydrochloride (1.1 mmol). The mixture was stirred at room temperature for 24 h, then was diluted with H$_2$O (100 mL) and extracted with diethyl ether (3 × 50 mL). The combined organic extract was washed with H$_2$O (3 × 50 mL), dried (MgSO$_4$) and evaporated under reduced pressure. The residue was chromatographed on silica gel column with CHCl$_3$ to afford the target compounds.

**6-(3,4-Dimethoxybenzyl)-2-[2-(diethylamino)ethyl]thio-5-ethylpyrimidin-4(3H)-one (4b)** White solid. M.p.: 119–120 °C, Yield: 0.287 g (71%). $^1$H-NMR (CDCl$_3$): δ = 0.89 (t, 3H, $J = 7.0$ Hz, CH$_3$), 1.00–1.04 (m, 6H, 2 × CH$_3$), 2.41 (q, 2H, $J = 7.0$ Hz, CH$_2$), 2.71–2.74 (m, 4H, 2 × CH$_2$), 2.89–2.91 (m, 2H, CH$_2$), 3.03–3.06 (m, 2H, CH$_2$), 3.67 (s, 2H, CH$_2$), 3.77 (s, 2H, OCH$_3$), 3.78 (s, 2H, OCH$_3$), 6.70 (bs, 2H, H arom.), 6.77 (s, 1H, H arom.), 11.71 (bs, 1H, NH). $^{13}$C-NMR (CDCl$_3$): δ = 10.06 (CH$_3$), 10.16 (CH$_3$), 13.40 (CH$_3$), 36.27 (CH$_2$), 39.78 (CH$_2$), 47.05 (2 × CH$_2$), 54.62 (CH$_3$), 55.88 (2 × OCH$_3$), 120.68 (C-5), 111.02, 112.22, 119.14, 130.93, 147.63, 148.84 (C arom.), 157.59 (C-6), 161.32 (C-4), 164.69 (C-2). ESI-MS, m/z (Rel. Int.): 406 (M + H$^+$, 78).

**6-(3,4-Dimethoxybenzyl)-5-ethyl-2-[2-(pyrrolidin-1-yl)ethyl]thio-5-ethylpyrimidin-4(3H)-one (5c)** White solid. M.p.: 143–145 °C, Yield: 0.274 g (68%). $^1$H-NMR (DMSO-d$_6$): δ = 0.92 (t, 3H, $J = 7.5$ Hz, CH$_3$), 1.68–1.71 (m, 4H, 2 × CH$_2$), 2.37 (q, 2H, $J = 7.5$ Hz, CH$_2$), 2.52–2.55 (m, 4H, 2 × CH$_2$), 2.68 (t, 2H, $J = 6.0$ Hz, CH$_2$), 3.20 (t, 2H, $J = 6.0$ Hz, CH$_2$), 3.70 (s, 2H, OCH$_3$), 3.72 (s, 2H, OCH$_3$), 3.84 (s, 2H, CH$_2$), 6.72–6.88 (m, 3H. H arom.). $^{13}$C-NMR (DMSO-d$_6$): δ = 13.40 (CH$_3$), 18.14 (CH$_2$),
Molecules 2014, 19, 285

23.08 (CH2), 28.72 (CH2), 35.11 (CH2), 54.96 (CH2), 55.39 (OCH3), 55.48 (OCH3), 120.52 (C-5), 111.83, 112.89, 120.57, 131.06, 147.29, 148.50 (C arom.), 159.97 (C-6), 161.20 (C-4), 164.01 (C-2). ESI-MS, m/z (Rel. Int.): 404 (M + H+, 90).

6-(3,4-Dimethoxybenzyl)-5-ethyl-2-[2-(piperidin-1-yl)ethyl]thiopyrimidin-4(3H)-one (6b) White solid. M.p.: 127–129 °C, Yield: 0.301 g (72%). 1H-NMR (CDCl3): δ = 0.88 (t, 3H, J = 7.5 Hz, CH3), 1.45–1.47 (m, 2H, CH2), 1.78–1.80 (m, 4H, 2 × CH2), 2.39 (q, 2H, J = 7.5 Hz, CH2), 2.50–2.52 (m, 4H, 2 × CH2), 2.73 (t, 2H, J = 5.0 Hz, CH2), 2.99 (t, 2H, J = 5.0 Hz, CH2), 3.75 (s, 2H, CH2), 3.77 (s, 3H, OCH3), 3.79 (s, 3H, OCH3), 6.70 (s, 2H, H arom.), 6.79 (s, 1H, H arom.), 11.73 (s, 1H, NH). 13C-NMR (CDCl3): δ = 13.23 (CH3), 18.91 (CH2), 24.01, 24.43, 55.44 (C piperidin.), 36.21 (CH2), 39.80 (CH2), 55.90 (2 × OCH3), 61.57 (CH2), 122.64 (C-5), 111.09, 112.23, 120.71, 130.88, 147.33, 148.52 (C arom.), 157.07 (C-6), 162.85 (C-4), 164.51 (C-2). ESI-MS, m/z (Rel. Int.): 418 (M + H+, 85).

6-(4-Chlorobenzyl)-5-methyl-2-[2-(morpholin-4-yl)ethyl]thiopyrimidin-4(3H)-one (6c) White solid. M.p.: 158–159 °C, Yield: 0.280 g (74%). 1H-NMR (DMSO-d6): δ = 1.95 (s, 3H, CH3), 2.33 (t, 4H, J = 4.5 Hz, 2 × CH2), 2.46 (t, 2H, J = 7.0 Hz, CH2), 3.13 (t, 2H, J = 7.0 Hz, CH2), 3.54 (t, 4H, J = 4.5 Hz, 2 × CH2), 3.84 (s, 2H, CH2), 7.24, 7.32 (2 × d, 4H, J = 8.5 Hz, H arom.), 12.61 (s, 1H, NH). 13C-NMR (DMSO-d6): δ = 10.31 (CH3), 26.70 (CH2), 34.17 (CH2), 52.86, 65.97 (C morpholin.), 57.35 (CH2), 115.19 (C-5), 128.13, 130.00, 130.58, 137.24 (C arom.), 157.15 (C-6), 159.80 (C-4), 163.34 (C-2). ESI-MS, m/z (Rel. Int.): 380 (M + H+, 100).

6-(4-Chlorobenzyl)-5-ethyl-2-[2-(morpholin-4-yl)ethyl]thiopyrimidin-4(3H)-one (6d) White solid. M.p.: 133–135 °C. Yield: 0.271 g (69%). 1H-NMR (DMSO-d6): δ = 0.94 (t, 3H, J = 7.5 Hz, CH3), 2.25 (s, 3H, CH3), 2.31–2.39 (m, 6H, 3 × CH2), 2.58 (t, 2H, J = 7.0 Hz, CH2), 3.15 (t, 2H, J = 7.0 Hz, CH2), 3.51 (t, 4H, J = 4.5 Hz, 2 × CH2), 3.79 (s, 2H, CH2), 7.07, 7.11 (2 × d, 4H, J = 8.0 Hz, H arom.), 12.49 (s, 1H, NH). 13C-NMR (DMSO-d6): δ = 13.16 (CH3), 18.02 (CH2), 20.51 (CH3), 26.62 (CH2), 30.59 (CH2), 52.82, 65.96 (C morpholin.), 57.32 (CH2), 121.27 (C-5), 128.09, 130.64, 130.78, 137.24 (C arom.), 157.20 (C-6), 159.44 (C-4), 162.77 (C-4). ESI-MS, m/z (Rel. Int.): 394 (M + H+, 100).

5-Ethyl-6-(4-methylbenzyl)-2-[2-(morpholin-4-yl)ethyl]thiopyrimidin-4(3H)-one (6e) White solid. M.p.: 137–139 °C. Yield: 0.236 g (63%). 1H-NMR (DMSO-d6): δ = 0.90 (t, 3H, J = 7.5 Hz, CH3), 2.25 (s, 3H, CH3), 2.31–2.39 (m, 6H, 3 × CH2), 2.58 (t, 2H, J = 7.0 Hz, CH2), 3.15 (t, 2H, J = 7.0 Hz, CH2), 3.51 (t, 4H, J = 4.5 Hz, 2 × CH2), 3.79 (s, 2H, CH2), 7.07, 7.11 (2 × d, 4H, J = 8.0 Hz, H arom.), 12.49 (s, 1H, NH). 13C-NMR (DMSO-d6): δ = 13.16 (CH3), 18.02 (CH2), 20.51 (CH3), 26.62 (CH2), 30.59 (CH2), 52.82, 65.96 (C morpholin.), 57.32 (CH2), 121.27 (C-5), 128.09, 130.64, 130.78, 137.24 (C arom.), 157.20 (C-6), 159.44 (C-4), 162.77 (C-4). ESI-MS, m/z (Rel. Int.): 374 (M + H+, 100).

6-(3,4-Dimethoxybenzyl)-5-ethyl-2-[2-(morpholin-4-yl)ethyl]thiopyrimidin-4(3H)-one (6f) White solid. M.p.: 151–152 °C, Yield: 0.276 g (66%). 1H-NMR (DMSO-d6): δ = 0.90 (t, 3H, J = 7.5 Hz, CH3), 2.30–2.39 (m, 6H, 3 × CH2), 2.58 (t, 2H, J = 7.0 Hz, CH2), 3.21 (t, 2H, J = 7.0 Hz, CH2), 3.57 (t, 4H, J = 4.5 Hz, 2 × CH2), 3.70 (s, 3H, OCH3), 3.72 (s, 3H, OCH3), 3.76 (s, 2H, CH2), 6.72–6.87 (m, 3H, H arom.), 12.54 (s, 1H, NH). 13C-NMR (DMSO-d6): δ = 13.32 (CH3), 18.06 (CH2), 26.68 (CH2), 52.83, 65.98 (C morpholin.), 55.36 (OCH3), 55.42 (OCH3), 57.44 (CH2), 117.23 (C-5), 111.76, 112.85, 120.57,
131.29, 147.27, 148.45 (C arom.), 158.34 (C-6), 162.20 (C-4), 162.43 (C-2). ESI-MS, m/z (Rel. Int.): 420 (M + H\(^+\), 100).

3.3. General Procedure for Preparation of 2-[2-(N-Phthalimido)ethyl]thiopyrimidin-4(3H)-ones 7a,b and 2-[3-(N-Phthalimido)propyl]thiopyrimidin-4(3H)-ones 7c,d

Anhydrous potassium carbonate (0.152 g, 1.1 mmol) was added to a solution of the appropriate compound 3a,b (1 mmol) in DMF (5 mL), followed by addition of N-(2-bromoethyl)phthalimide and/or N-(3-bromopropyl)phthalimide (1.1 mmol). The reaction mixture was stirred at room temperature for 24 h and worked up as described above for the preparation of compounds 4–6.

6-(4-Chlorobenzyl)-5-methyl-2-[2-(N-phthalimido)ethyl]thiopyrimidin-4(3H)-one (7a) White solid. M.p.: 251–253 °C, Yield: 0.302 g (69%). ¹H-NMR (DMSO-\(d_6\)): \(\delta = 1.90\) (s, 3H, CH\(_3\)), 3.34 (t, 2H, J = 6.0 Hz, CH\(_2\)), 3.82 (s, 2H, CH\(_2\)), 3.87 (t, 2H, J = 6.0 Hz, CH\(_2\)), 7.32–7.33 (m, 4H, H arom.), 7.83–7.88 (m, 4H, H arom.). ¹³C-NMR (DMSO-\(d_6\)): \(\delta = 10.27\) (CH\(_3\)), 28.12 (CH\(_2\)), 30.59 (CH\(_2\)), 36.61 (CH\(_2\)), 115.11 (C-5), 123.00, 128.15, 130.64, 130.83, 131.41, 134.36, 137.18 (C arom.), 159.13 (C-6), 162.21 (C-4), 163.11 (C-2), 167.60 (CO). ESI-MS, m/z (Rel. Int.): 440 (M + H\(^+\), 18).

6-(4-Chlorobenzyl)-5-ethyl-2-[2-(N-phthalimido)ethyl]thiopyrimidin-4(3H)-one (7b) White solid. M.p.: 203–204 °C, Yield: 0.322 g (71%). ¹H-NMR (DMSO-\(d_6\)): \(\delta = 0.87\) (t, 3H, J = 7.5 Hz, CH\(_3\)), 2.37 (q, 2H, J = 7.5 Hz, CH\(_2\)), 3.35 (t, 2H, J = 6.0 Hz, CH\(_2\)), 3.80 (s, 2H, CH\(_2\)), 3.86 (t, 2H, J = 6.0 Hz, CH\(_2\)), 7.33–7.34 (m, 4H, H arom.), 7.83–7.89 (m, 4H, H arom.), 12.71 (s, 1H, NH). ¹³C-NMR (DMSO-\(d_6\)): \(\delta = 13.03\) (CH\(_3\)), 18.00 (CH\(_2\)), 28.07 (CH\(_2\)), 30.60 (CH\(_2\)), 36.71 (CH\(_2\)), 115.19 (C-5), 123.00, 128.12, 130.70, 130.83, 131.40, 134.36, 137.50 (C arom.), 162.34 (C-4), 163.22 (C-2), 167.60 (CO). ESI-MS, m/z (Rel. Int.): 454 (M + H\(^+\), 31).

6-(4-Chlorobenzyl)-5-methyl-2-[3-(N-phthalimido)propyl]thiopyrimidin-4(3H)-one (7c) White solid. M.p.: 234–235 °C, Yield: 0.282 g (62%). ¹H-NMR (DMSO-\(d_6\)): \(\delta = 1.85–1.91\) (m, 2H, CH\(_2\)), 1.93 (s, 3H, CH\(_3\)), 3.02 (t, 2H, J = 6.5 Hz, CH\(_2\)), 3.60 (t, 2H, J = 6.5 Hz, CH\(_2\)), 3.74 (s, 2H, CH\(_2\)), 7.19, 7.25 (2 × d, 4H, J = 8.0 Hz, H arom.), 7.80–7.85 (m, 4H, H arom.). ¹³C-NMR (DMSO-\(d_6\)): \(\delta = 10.29\) (CH\(_3\)), 26.97 (CH\(_2\)), 28.13 (CH\(_2\)), 30.59 (CH\(_2\)), 36.37 (CH\(_2\)), 115.32 (C-5), 122.88, 128.03, 130.53, 130.74, 131.55, 134.22, 137.18 (C arom.), 157.86 (C-6), 162.71 (C-4), 163.67 (C-2), 167.87 (CO). ESI-MS, m/z (Rel. Int.): 454 (M + H\(^+\), 20).

6-(4-Chlorobenzyl)-5-ethyl-2-[3-(N-phthalimido)propyl]thiopyrimidin-4(3H)-one (7d) White solid. M.p.: 197–198 °C, Yield: 0.298 g (64%). ¹H-NMR (DMSO-\(d_6\)): \(\delta = 0.93\) (t, 3H, J = 7.5 Hz, CH\(_3\)), 1.84–1.89 (m, 2H, CH\(_2\)), 2.41 (q, 2H, J = 7.5 Hz, CH\(_2\)), 3.02 (t, 2H, J = 6.5 Hz, CH\(_2\)), 3.59 (t, 2H, J = 6.5 Hz, CH\(_2\)), 3.76 (s, 2H, CH\(_2\)), 7.21, 7.26 (2 × d, 4H, J = 8.0 Hz, H arom.), 7.80–7.85 (m, 4H, H arom.), 12.74 (s, 1H, NH). ¹³C-NMR (DMSO-\(d_6\)): \(\delta = 13.11\) (CH\(_3\)), 18.01 (CH\(_2\)), 26.97 (CH\(_2\)), 28.09 (CH\(_2\)), 30.59 (CH\(_2\)), 36.34 (CH\(_2\)), 115.69 (C-5), 122.88, 128.00, 130.60, 130.74, 131.55, 134.21, 137.47 (C arom.), 158.98 (C-6), 161.92 (C-2), 163.43 (C-4), 167.87 (CO). ESI-MS, m/z (Rel. Int.): 468 (M + H\(^+\), 17).
3.4. Determination of the Antimicrobial Activity by the Agar Disc-Diffusion Method [43]

Sterile nutrient, Czapek’s dox and malt extract agar media were inoculated, separately, with 100 µL cell suspension of the chosen microorganism, bacteria, fungi and yeast-like fungi, respectively, and poured into Petri-dishes (20 cm diameter). The test compounds (200 µg/10 mm diameter disc) were placed onto the surface of the agar Petri-dishes. The antimicrobial activities were expressed as the diameter of the growth inhibition zone in mm.

3.5. Determination of Minimal Inhibitory Concentration (MIC) [44]

The minimal inhibitory concentrations (MICs) of the test compounds were determined using serial dilutions technique. Different concentrations ranging 50.0–200.0 µg/mL for each compound in dimethyl sulphoxide (DMSO) were placed on filter paper disc (1 cm diameter). The discs were deposited on the surface of inoculated agar plates and kept at low temperature before incubation which favours diffusion over microbial growth to detect the inhibition zone clearly. The plates were incubated at 30 °C for 24 h for bacteria and yeast and for 48 h for fungi.

4. Conclusions

In the present study, several 2-(substituted amino)alkylthiopyrimidin-4(3H)-ones were synthesized and screened against Gram-positive bacteria (Staphylococcus aureus ATCC 29213, Bacillus subtilis NRRL 4219 and Bacillus cereus), yeast-like pathogenic fungus (Candida albicans ATCC 10231) and fungus (Aspergillus niger NRRL 599) which are important human pathogenic microorganisms. Most of the test compounds showed good antimicrobial activities.

Acknowledgments

The authors would like to extend their sincere appreciation to the Deanship of Scientific Research at King Saud University for its funding of this research through the Research Group Project No. RGP-VPP-274.

Conflicts of Interest

The authors declare no conflict of interest.

References

1. Mitsuya, H.; Yarchoan, R.; Broder, S. Molecular targets for AIDS therapy. Science 1990, 249, 1533–1544.
2. Miyasaka, T.; Tanaka, H.; Baba, M.; Hayakawa, H.; Walker, R.T.; Balzarini, J.; de Clercq, E. A novel lead for specific anti-HIV-1 agents: 1-[(2-hydroxyethoxy)methyl]-6-(phenylthio)thymine. J. Med. Chem. 1989, 32, 2507–2509.
3. Hopkins, A.L.; Ren, J.; Tanaka, H.; Baba, M.; Okamato, M.; Stuart, D.I.; Stammers, D.K. Design of MKC-442 (emivirine) analogues with improved activity against drug-resistant HIV mutants. J. Med. Chem. 1999, 42, 4500–4505.
4. Malik, V.; Singh, P.; Kumar, S. Unique chlorine effect in regioselective one-pot synthesis of 1-alkyl-/allyl-3-(o-chlorobenzyl) uracils: Anti-HIV activity of selected uracil derivatives. Tetrahedron 2006, 62, 5944–5951.

5. Gazivoda, T.; Raic-Malic, S.; Marjanovic, M.; Kralj, M.; Pavelic, K.; Balzarini, J.; de Clercq, E.; Mintas, M. The novel C-5 aryl, alkenyl and alkynyl substituted uracil derivatives of L-ascorbic acid: Synthesis, cytostatic, and antiviral activity evaluations. Bioorg. Med. Chem. 2007, 15, 749–758.

6. Novikov, M.S.; Buckheit, R.W., Jr.; Temburnikar, K.; Khandazhinskaya, A.L.; Ivanov, A.V.; Seley-Radtke, K.L. 1-Benzyl derivatives of 5-(arylamino)uracils as anti-HIV-1 and anti-EBV agents. Bioorg. Med. Chem. 2010, 18, 8310–8314.

7. Novikov, M.S.; Valuev-Elliston, V.T.; Babkov, D.A.; Paramonova, M.P.; Ivanov, A.V.; Gavryushov, S.A.; Khandazhinskaya, A.L.; Kochetkov, S.N.; Pannecouque, C.; Andrei, G.; et al. N1,N3-disubstituted uracils as nonnucleoside inhibitors of HIV-1 reverse transcriptase. Bioorg. Med. Chem. 2013, 21, 1150–1158.

8. Sakakibara, N.; Hamasaki, T.; Baba, M.; Demizu, Y.; Kurihara, M.; Irie, K.; Iwai, M.; Asada, E.; Kato, Y.; Maruyama, T. Synthesis and evaluation of novel 3-(3,5-dimethylbenzyl)uracil analogs as potential anti-HIV-1 agents. Bioorg. Med. Chem. 2013, 21, 5900–5906.

9. Novikov, M.S.; Babkov, D.A.; Paramonova, M.P.; Ozerov, A.A.; Chizhov, A.O.; Andrei, G.; Snoeck, R.; Balzarini, J.; Seley-Radtke, K.L. Synthesis and anti-HCMV activity of 1-[ω-(phenoxy)alkyl]uracil derivatives and analogues thereof. Bioorg. Med. Chem. 2013, 21, 4151–4157.

10. Isobe, Y.; Tobe, M.; Inoue, Y.; Isobe, M.; Tsuchiya, M.; Hayashi, H. Structure and activity relationships of novel uracil derivatives as topical anti-inflammatory agents. Bioorg. Med. Chem. 2003, 11, 4933–4940.

11. Evaldsson, C.; Ryden, I.; Uppugunduri, S. Anti-inflammatory effects of exogenous uridine in an animal model of lung inflammation. Inter. Immunopharmacol. 2007, 7, 1025–1032.

12. Keche, A.P.; Hatnapure, G.D.; Tale, R.H.; Rodge, A.H.; Birajdar, S.S.; Kamble, V.M. A novel pyrimidine derivatives with aryl urea, thiourea and sulfonamide moieties: Synthesis, anti-inflammatory and antimicrobial evaluation. Bioorg. Med. Chem. Lett. 2012, 22, 3445–3448.

13. Agarwal, A.; Srivastava, K.; Puri, S.K.; Chuahan, P.M. Synthesis of 2,4,6-trisubstituted pyrimidines as antimalarial agents. Bioorg. Med. Chem. 2005, 13, 4645–4650.

14. Agarwal, A.; Srivastava, K.; Puri, S.K.; Chuahan, P.M. Antimalarial activity of 2,4,6-trisubstituted pyrimidines. Bioorg. Med. Chem. Lett. 2005, 15, 1881–1883.

15. Singh, K.; Kaur, H.; Chibale, K.; Balzarini, J. Synthesis of 4-aminoquinoline-pyrimidine hybrids as potent antimalarials and their mode of action studies. Eur. J. Med. Chem. 2013, 66, 314–323.

16. Xie, F.; Zhao, H.; Zhao, L.; Lou, L.; Hu, Y. Synthesis and biological evaluation of novel 2,4,5-substituted pyrimidine derivatives for anticancer activity. Bioorg. Med. Chem. Lett. 2009, 19, 275–278.

17. El-Deeb, I.M.; Lee, S.H. Design and synthesis of new anticaner pyrimidines with multiple-kinase inhibitory effect. Bioorg. Med. Chem. 2010, 18, 3860–3874.

18. Prachayasittikul, S.; Worachartcheewan, A.; Nantasenamat, C.; Chinworrungsee, M.; Sornsongkhram, N.; Ruchirawat, S.; Prachayasittikul, V. Synthesis and structure-activity relationship of 2-thiopyrimidine-4-one analogs as antimicrobial and anticaner agents. Eur. J. Med. Chem. 2011, 46, 738–742.
19. Tsoukala, E.; Agelis, G.; Dolinsek, J.; Botic, T.; Cencic, A.; Komiotis, D. An efficient synthesis of 3-fluoro-5-thio-xylofuranosyl nucleosides of thymine, uracil and 5-fluorouracil as potential antitumor or/and antiviral agents. *Bioorg. Med. Chem.* 2007, 15, 3241–3247.

20. Manta, S.; Tsoukala, E.; Tzioumaki, N.; Kiritsis, C.; Balzarini, J.; Komiotis, D. Synthesis of 4,6-dideoxy-3-fluoro-β-d-glucopyranosyl analogues of 5-fluorouracil, N6-benzyl adenine, uracil, thymine, N4-benzoyl cytosine and evaluation of their antitumor activities. *Bioorg Chem.* 2010, 38, 48–55.

21. Lauria, A.; Patella, C.; Abbate, I.; Martorana, A.; Almerico, A.M. An unexpected Dimroth rearrangement leading to aneled thieno[3,2-d][1,2,3]triazolo[1,5-a]pyrimidines with potent antitumor activity. *Eur. J. Med. Chem.* 2013, 65, 381–388.

22. Matyugina, E.; Khandazhinskaya, A.; Chernousova, L.; Andreevskaya, S.; Smirnova, T.; Chizhov, A.; Karpenko, I.; Kochetkov, S.; Alexandrova, L. The synthesis and antituberculosis activity of 5'-nor carbocyclic uracil derivatives. *Bioorg. Med. Chem.* 2012, 20, 6680–6686.

23. Tobe, M.; Isobe, Y.; Goto, Y.; Obara, F.; Tsuchiya, M.; Matsui, J.; Hirota, K.; Hayashi, H. Synthesis and biological evaluation of CX-659S and related compounds for their inhibitory effects on the delayed-type hypersensitivity reaction. *Bioorg. Med. Chem.* 2000, 8, 2037–2047.

24. Bhabak, K.P.; Bhowmick, D. Synthesis and structural characterization of some trisulfide analoges of thiouracil-based antithyroid drugs. *J. Mol. Struct.* 2012, 1022, 16–24.

25. Sriharsha, S.N.; Satish, S.; Shashikanth, S.; Raveesha, K.A. Design, synthesis and antibacterial activity of novel 1,3-thiazolidine pyrimidine nucleoside analogues. *Bioorg. Med. Chem.* 2006, 14, 7476–7481.

26. Semenov, V.E.; Vošchina, A.D.; Toroptzova, E.M.; Kulik, N.V.; Zobov, V.V.; Giniyatullin, R.K.; Mikhailov, A.S.; Nikolaev, A.E.; Akamsin, V.D.; Reznik, V.S. Antibacterial and antifungal activity of acyclic and macrocyclic uracil derivatives with quaternized nitrogen atoms in spacers. *Eur. J. Med. Chem.* 2006, 41, 1093–1101.

27. Svenstrup, N.; Kuhl, A.; Ehler, K.; Habich, D. Improved synthesis of antibacterial 3-substituted 6-anilouracils. *Bioorg. Med. Chem. Lett.* 2008, 18, 3215–3218.

28. Al-Abdullah, E.S.; Al-Obaid, A.M.; El-Deeb, O.A.; Habib, E.E.; El-Emam, E.E. Synthesis of novel 6-phenyl-2,4-disubstituted pyrimidine-5-carbonitriles as potential antimicrobial agents. *Eur. J. Med. Chem.* 2011, 46, 4642–4647.

29. Krim, J.; Grunewald, C.; Taourirte, M.; Engels, J.W. Efficient microwave-assisted synthesis, antibacterial activity and high fluorescence of 5-benzimidazolyl-2'-deoxyuridines. *Bioorg. Med. Chem.* 2012, 20, 480–486.

30. Al-Deeb, O.A.; Al-Turkistani, A.A.; Al-Abdullah, E.S.; El-Brollosy, N.R.; Habib, E.E.; El-Emam, A.A. Pyrimidine-5-carbonitriles-part III: Synthesis and antimicrobial activity of novel 6-(2-substituted propyl)-2,4-disubstituted pyrimidine-5-carbonitriles. *Heterocycl. Commun.* 2013, 19, 411–419.

31. Orr, G.F.; Musso, D.L.; Boswell, G.E.; Kelly, J.L.; Joyner, S.S.; Davis, S.T.; Baccanari, D.P. Inhibition of uridine phosphorylase: Synthesis and structure-activity relationships of aryl-substituted 5-benzyluracils and 1-[(2-hydroxyethoxy)methyl]-5-benzyluracils. *J. Med. Chem.* 1995, 38, 3850–3856.
Molecules 2014, 19

32. Murray, P.E.; McNally, V.A.; Lockyer, S.D.; Williams, K.J.; Stratford, I.J.; Jaffar, M.; Freeman, S. Synthesis and enzymatic evaluation of pyridinium-substituted uracil derivatives as novel inhibitors of thymidine phosphorylase. *Bioorg. Med. Chem.* 2002, 10, 525–530.

33. Focher, F.; Ubiali, D.; Pregbolato, M.; Zhi, C.; Gambino, J.; Wright, G.E.; Spadari, S. Novel nonsubstrate inhibitors of human thymidine phosphorylase, a potential target for tumor-dependent angiogenesis. *J. Med. Chem.* 2000, 43, 2601–2607.

34. El-Brollosy, N.R.; Al-Omar, M.A.; Al-Deeb, O.A.; El-Emam, A.A.; Nielsen, C. Synthesis of novel uracil non-nucleosides analogues of 3,4-dihydro-2-alkylthio-6-benzyl-4-oxopyrimidines and 6-benzyl-1-ethoxyxymethyl-5-isopropyluracil. *J. Chem. Res.* 2007, 525–530.

35. El-Brollosy, N.R.; Jorgensen, P.T.; Dahan, B.; Boel, A.M.; Pedersen, E.B.; Nielsen, C. Synthesis of Novel N-1 (allyloxymethyl) Analogues of 6-Benzyl-1-(ethoxymethyl)-5-isopropyluracil (MKC-442, Emivirine) with Improved Activity Against HIV-1 and its Mutants. *J. Med. Chem.* 2002, 45, 5721–5726.

36. El-Brollosy, N.R.; Pedersen, E.B.; Nielsen, C. Synthesis of novel MKC-442 analogues with potent activities against HIV-1. *Arch. Pharm. Pharm. Med. Chem.* 2003, 336, 236–241.

37. El-Essawy, F.A.; El-Brollosy, N.R.; Pedersen, E.B.; Nielsen, C. Synthesis of new uracil non-nucleoside derivatives as potential inhibitors of HIV-1. *J. Heterocycl. Chem.* 2003, 40, 213–217.

38. Wamberg, M.; Pedersen, E.B.; El-Brollosy, N.R.; Nielsen, C. Synthesis of 6-arylviny analogues of the HIV drugs SJ-3366 and Emivirine. *Bioorg. Med. Chem. Life Sci.* 2004, 12, 1141–1149.

39. El-Brollosy, N.R.; Nielsen, C.; Pedersen, E.B. Synthesis of N-1-(indanyloxymethyl) and N-1-(4-Hydroxybut-2-enyloxymethyl) analogues of the HIV drug Emivirine and GCA-186. *Monatsh. Chem.* 2005, 136, 1247–1254.

40. Sorensen, E.R.; El-Brollosy, N.R.; Jorgensen, P.T.; Pedersen, E.B.; Nielsen, C. Synthesis of 6-(3,5-dichlorobenzyl) derivatives as isosteric analogues of the HIV drug 6-(3,5-dimethylbenzyl)-1-(ethoxymethyl)-5-isopropyluracil (GCA-186). *Arch. Pharm. Chem. Life Sci.* 2005, 338, 299–304.

41. El-Brollosy, N.R.; Sorensen, E.R.; Pedersen, E.B.; Sanna, G.; LaColla, P.; Loddo, R. Synthesis and antiviral evaluation of 6-(trifluoromethylbenzyl) and 6-(fluorobenzyl) analogues of HIV drugs emivirine and GCA-186. *Arch. Pharm. Chem. Life Sci.* 2008, 341, 9–19.

42. El-Brollosy, N.R.; Al-Deeb, O.A.; El-Emam, A.A.; Pedersen, E.B.; LaColla, P.; Collu, G.; Sanna, G.; Loddo, R. Synthesis of novel uracil non-nucleoside derivatives as potential reverse transcriptase inhibitors of HIV-1. *Arch. Pharm. Chem. Life Sci.* 2009, 342, 663–670.

43. Penna, C.A.; Marino, S.G.; Gutkind, G.O.; Clavin, M.; Ferraro, G.; Martino, V. Antimicrobial activity of *Eupatorium* species growing in Argentina. *J. Herbs. Spices Med. Plants* 1998, 5, 21–28.

44. Wilkins, T.D.; Holdeman, J.J.; Abramson, I.J.; Moore, W.E.C. Standardized single-disc method for antibiotic susceptibility testing of anaerobic bacteria. *Antimicrob. Agents Chemother.* 1972, 1, 451–455.

*Sample Availability*: Samples of the compounds 3a–d, 4a,b, 5a–c, 6a–f and 7a–d are available from the corresponding author.

© 2013 by the authors; licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution license (http://creativecommons.org/licenses/by/3.0/).