Scientific Research Publishing

Open Journal of Medicinal Chemistry, 2021, 11, 27-39
https://www.scirp.org/journal/ojmc
ISSN Online: 2164-313X
ISSN Print: 2164-3121

Synthesis and Antibacterial Activities of New 2-(Benzylthio)pyrimidines and 2-(Benzimidazolylmethylthio)pyrimidines Derivatives

Achi Patrick-Armand[1], Coulibali Siomenan[1*], Zon Doumade[2], Timotou Adéyolé[2], Bolou Eric[3], Touré Daouda[4], Sissouma Drissa[1], Adjou Ané[1]

[1]Laboratoire de Constitution et Réaction de la Matière, UFR Sciences des Structures de la Matière et Technologie, Université Félix Houphouët Boigny de Cocody, Abidjan, Côte d'Ivoire
[2]Département de Mathématiques-Physique-Chimie, UFR des Sciences Biologiques, Université Peleforo Gon Coulibaly, Korhogo, Côte d'Ivoire
[3]Centre National de Floristique, Université Félix Houphouët-Boigny de Cocody, Abidjan, Côte d'Ivoire
[4]Département de Biochimie-Génétique, UFR des Sciences Biologiques, Université Peleforo Gon Coulibaly, Korhogo, Côte d'Ivoire
Email: *bsiomenan@yahoo.fr

How to cite this paper: Patrick-Armand, A., Siomenan, C., Doumade, Z., Adéyolé, T., Eric, B., Daouda, T., Drissa, S. and Ané, A. (2021) Synthesis and Antibacterial Activities of New 2-(Benzylthio)pyrimidines and 2-(Benzimidazolylmethylthio)pyrimidines Derivatives. *Open Journal of Medicinal Chemistry*, **11**, 27-39.
https://doi.org/10.4236/ojmc.2021.113003

Received: June 29, 2021
Accepted: September 15, 2021
Published: September 18, 2021

Abstract

A series of 2-(benzylthio)pyrimidines (**6a-l**) and 2-(benzimidazolylmethylthio)-pyrimidines derivatives (**6m** and **6n**) analogues of ethyl 2-(benzylthio)-6-methyl-4-phenyl-1,4-dihydropyrimidine-5-carboxylates and ethyl 2-(((1H-benzimidazol-2-yl)methyl)thio)-6-methyl-4-phenyl-1,4-dihydropyri-midine-5-carboxylates were prepared and evaluated for antibacterial activity. These compounds were obtained by condensation of 2-thiopyrimidines (**4**) with benzyl halides or 2-(chloromethyl)-*1H*-benzimidazole (**5**) in the presence of a base. All compounds were characterized by ^{1}H, ^{13}C and HRMS spectra. Out of fourteen, only eight compounds were screened against multi-resistant strains of *Escherichia coli* and *Staphylococcus aureus*. The results revealed that all of them were found to possess significant antibacterial activity against the germs tested. Compounds **6c**, **6d**, **6h** and **6m** were more active on *S. aureus* and compounds **6h** and **6m** more active on *E. coli*.

Keywords

2-Thiopyrimidine, Benzylthiouracil, Benzimidazole, Antibacterial Activity

1. Introduction

The Pyrimidine ring is a well-known heterocyclic compound found in the struc-

ture such as Uracil, cytosine and thymine. These bases are part of the composition of nucleosides forming DNA and RNA, like Uridine, which has Uralic as a nitrogen base (**Figure 1**). This Pyrimidine ring is part of a very important new class of heterocyclic compounds widely used as building blocks of pharmaceutical agents [1] [2]. It has many biological properties namely antiviral [3], antimicrobial [4], anti-tuberculosis [5], analgesic [6] and also is used as an antihypertensive [7] and antitumor agent [8]. Studies showed that poly-substituted Pyrimidines could be used as potential anti-tumor agents [9] [10] [11]. Indeed, the sulfur derivatives of Uracil which gave 2-thiouracils are used as anti-inflammatory and virucidal agents [12]. **Figure 1** shows 2-thiopyrimidines derivatives resulting from the replacement of the oxygen atom bonded to C-2 in the uridine base [13] by a sulfur atom and the replacement of the oxygen in the carbon C-4 by a phenyl group. A European patent [14] has revealed the importance of 2-thiopyrimdines derivatives in the preparation of cardiotonic drugs. Benzylthiouracil marketed under the name BASDENE is a synthetic antithyroid drug. Pathak *et al.* have evaluated and reported the existence of a primary activity of 2-thiopyrimidine derivatives against Mycobacterium tuberculosis [15]. Moreover, Biginelli in 1893, synthesized 2-thiopyrimidines [16] which demonstrated several pharmaceutical properties. The 2-thiopyrimidines was found to have excellent antimicrobial [17] [18] [19], antiviral [20], anti-inflammatory [21] and antitumor [22] properties. Therefore, 2-thiopyrimidines gained more attention from worldwide organic chemists [23]. Furthermore, the benzimidazole ring also had a panel of biological activities [24] [25] [26]. Among them, the most important which could be quoted are the antiviral [24], antiplasmodial [25] and anthelmintic [26] (**Figure 1**). Thus, pyrimidines or thiopyrimidines and benzimidazoles play an important role in cellular processes making these molecules valuable to become leads for new drug discoveries. To investigate new antimicrobial agents, we initiated the synthesis of new pyrimidines derivatives attaching benzylthio and benzimidazolylthio groups in position-2 and then evaluated their antibacterial activity.

2. Materials and Methods

2.1. Materials

2.1.1. Materials of Chemistry

Unless otherwise indicated, ^{1}H and ^{13}C NMR spectra were recorded at 300 and 75 MHz or 400 and 101 MHz or 600 and 151 MHz, respectively, in $CDCl_3$, DMSO-d_6 and Acetone-d_6 solutions. For ^{1}H NMR assignments, the chemical shifts are reported in ppm on the δ scale. The following notation is used for the ^{1}H NMR spectral splitting patterns: s (singlet), d (doublet), dd (doublet of doublet), t (triplet), q (quadruplet), m (multiplet) and further qualified as app (apparent), br (broad) coupling constants, J are reported in Hz. HRMS were measured in the electrospray (ESI) mode on a LC-MSD TOF mass analyzer.

2.1.2. Biological Materials

Microbial strains

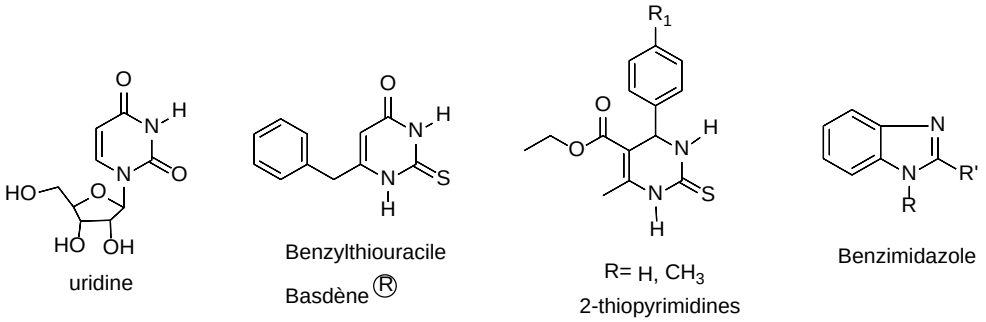


Figure 1. Structures of pyrimidine and benzimidazole biological molecules.

The microbial support was composed of clinical strains of *E. Coli* (Gram negative bacteria) and *S. aureus* (Gram positive bacteria). Strains were provided by the National Public Health Laboratory (LNSP) of Ivory Coast. These strains are all pathogenic and multi-resistants. Strains of *S. aureus* are resistant to: Amoxicillin, Ampicillin, Oxacillin, Ceftazidine, Fosfomycin, Vancomycin and Cefsulodine and those of *E. coli* were all resistant to: Amoxicillin, Ampicillin, Ceftriazone, Fosfomycin, Cefsulodin. The culture medium used was Mueller-Hinton broth (Oxoid) and Mueller-Hinton agar (Lab.Conda s.a). The synthetic compounds used in the experiments were eight 2-thiopyrimidine derivatives **(6a, 6b, 6c, 6d, 6h, 6j, 6k, 6m)**. Dimethylsulfoxide (DMSO) and distilled water were used as solvents for the solubilization of chemicals.

2.2. Methods

2.2.1. Methods of Synthesis

Method of synthesis of compounds 4a and 4b

12.5 mmol of thiourea, 13 mmol of benzaldehyde and 19 mmol of ethyl acetoacetate were dissolved in 10 mL of anhydrous ethanol and then ten drops of concentrated hydrochloric acid (37%) were added. The reaction mixture was stirred under reflux in ethanol for 2 h. After that time the reaction was quenched by addition of ice water. The resulting white precipitate obtained which was filtered and then washed with cold ethanol. The crystals obtained were purified by recrystallization in ethanol.

General procedure for the synthesis of compounds 6a-l

1 mmol of the 2-thiopyrimidine derivative was dissolved in 10 mL of dimethylformamide (DMF), then 1.5 mmol of potassium carbonate (K_2CO_3) were added. The reaction was stirred at room temperature and then 1.3 mmol of substituted benzyl chloride or bromide were added dropwise.

From 2 h to overnight, the reaction mixture was neutralized with a dilute solution of HCl (2 M). The precipitate formed was filtered and purified by silica gel chromatography with a mixture of ethyl acetate/ hexane. Compounds **6a-l** was obtained with yields between 50% to 94% yields.

Method of synthesis of compounds 6m and 6n

1.81 mmol of 2-thiopyrimidine were dissolved in 25 mL of tetrahydrofuran

(THF), then 2.89 mmol of triethylamine (Et_3N) were added. The mixture was stirred at room temperature for 15 min, then 2.2 mmol of 2-(chloromethyl)-*1H*-benzimidazole were added dropwise. After 24 h, solvent was evaporated and the organic layer was concentrated under vacuo. Then ice was added to the mixture. The precipitate formed was filtered and purified by silica gel chromatography with a mixture of ethyl acetate/ hexane. Compounds **6m** and **6n** were obtained with 60% and 55% yields respectively.

Products characterizations

Ethyl 2-(benzylthio)-6-methyl-4-phenyl-1,4-dihydropyrimidine-5-carboxylate 6a

Yield = 62%; **^{1}H NMR (300 MHz, $CDCl_3$)** δ (ppm) 11.20 (s, 1H, NH), 7.36 - 6.93 (m, 10H, H_{Ar}), 5.82 (s, 1H, CH), 4.91 (d, J_{ab} = *13.4 Hz*, 1H_a, S-CH_2), 4.28 (d, J_{ba} = *13.4 Hz*, 1H_b, S-CH_2), 4.22 - 3.82 (m, 2H, O-CH_2), 2.57 (s, 3H, CH_3), 1.14 (t, *J = 7.1 Hz*, 3H, CH_3).; **^{13}C NMR (75 MHz, $CDCl_3$)** δ (ppm): 164.01, 161.00, 142.95, 139.64, 132.94, 129.10, 129.03, 128.98, 128.51, 127.28, 106.20, 61.05, 54.71, 37.34, 17.28, 13.90. **HRMS (ESI):** Calc for $C_{21}H_{23}N_2O_2S$ $(M+H)^+$: 367.1576. Found: 367.1570.

Ethyl 2-((4-chlorobenzyl) thio)-6-methyl-4-phenyl-1,4-dihydropyrimidine-5-carboxylate 6b

Yield = 94%; ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm) 7.56 - 6.89 (m, 9H, H_{Ar}), 5.59 (s, 1H, CH), 4.90 (d, J_{ab} = *16.3 Hz*, 1H_a, S-CH_2), 4.38 (d, J_{ba} = *16.3 Hz*, 1H_b, S-CH_2), 4.10 - 3.99 (m, 2H, CH_2-O), 2.38 (s, 3H, CH_3), 1.08 (t, *J = 7.1 Hz*, 3H, CH_3); **^{13}C NMR (75 MHz, DMSO-d_6)** δ (ppm) 164.55, 144.31, 140.62, 135.11, 132.87, 130.96, 129.33, 128.92, 127.24, 104.89, 60.97, 54.98, 34.66, 17.42, 14.30; **HRMS (ESI):** Calc for $C_{21}H_{22}ClN_2O_2S$ $(M+H)^+$: 401.1017. Found: 401.1015.

Ethyl 6-methyl-2-((3-nitrobenzyl) thio)-4-phenyl-1,4-dihydropyrimidine-5-carboxylate 6c

Yield = 50%, ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 8.17 (s, 1H, H_{Ar}), 8.04 (d, *J = 8.0 Hz*, 1H, H_{Ar}), 7.57 (s, 1H, H_{Ar}), 7.34 (s, 2H, H_{Ar}), 7.31 - 7.11 (m, 4H, H_{Ar}), 5.56 (s, 1H, CH), 5.43 (d, *J = 4.5 Hz*, 1H_a, S-CH_2), 4.21 - 4.01 (m, 3H, H_b, CH_2-O), 2.38 (s, 3H, CH_3), 1.19 (t, *J = 7.1 Hz*, 3H, CH_3).; **^{13}C NMR (75 MHz, $CDCl_3$)** δ (ppm) 166.59, 165.65, 153.28, 148.12, 146.27, 143.70, 140.16, 135.24, 129.21, 128.73, 128.42, 127.98, 126.94, 126.62, 123.99, 122.24, 101.17, 60.06, 55.40, 33.64, 18.59, 14.29.; **HRMS (ESI):** Calc for $C_{21}H_{22}N_3O_4S$ $(M+H)^+$: 412.1716. Found: 412.1716.

Ethyl 6-methyl-2-((4-methylbenzyl) thio)-4-phenyl-1,4-dihydropyrimidine-5-carboxylate 6d

Yield = 57%, ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 7.36 - 7.27 (m, 4H, H_{Ar}), 7.19 - 7.11 (m, 5H, H_{Ar}), 5.25 (s, 1H, CH), 4.90 (d, J_{ab} = *13.3 Hz*, 1H_a, S-CH_2), 4.41 (d, J_{ba} = *13.3 Hz*, 1H_b, S-CH_2), 4.14 - 3.97 (m, 2H, O-CH_2), 2.39 (s, 3H, CH_3), 1.16 (t, *J = 9.2 Hz*, 3H, CH_3); **^{13}C NMR (101 MHz, $CDCl_3$)** δ (ppm) 166.72, 161.63, 154.80, 142.40, 137.64, 137.16, 134.38, 132.37, 129.47, 129.21, 129.14, 128.54, 128.01, 127.49, 59.73, 51.78, 36.28, 23.44, 21.19, 14.20; **HRMS**

(ESI): Calc for $C_{22}H_{25}N_2O_2S$ $(M+H)^+$: 381.1508. Found: 381.1506.

Ethyl 2-((3,5-dichlorobenzyl) thio)-6-methyl-4-phenyl-1,4-dihydropyrimidine-5-carboxylate 6e

Yield = 51%, ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm) 7.28 - 7.11 (m, 9H, H_Ar), 5.74 (s, 1H, -CH), 4.77 (d, J_{ab} = *12.3 Hz*, 1H_a, S-CH_2), 4.16 (d, J_{ba} = *12.3 Hz*, 1H_b, S-CH_2), 4.13 - 4.06 (m, 2H, CH_2-O), 2.51 (s, 3H, CH_3), 1.17 (t, *J = 7.1 Hz*, 3H, CH_3); **^{13}C NMR (151 MHz, $CDCl_3$);** δ (ppm) 165.12, 145.15, 141.30, 139.09, 135.01, 128.79, 128.42, 128.23, 127.37, 126.74, 104.72, 60.63, 34.52, 14.10.; **HRMS (ESI):** Calc for $C_{21}H_{20}Cl_2N_2NaO_2S$ $(M+Na)^+$: 457.0725. Found: 457.0729.

Ethyl 2-((4-(methoxycarbonyl) benzyl) thio)-6-methyl-4-phenyl-1,4-dihydropyrimidine-5-carboxylate 6f

Yield = 59%, ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 7.95 - 7.75 (m, 4H, H_{Ar}), 7.39 - 7.17 (m, 5H, H_{Ar},), 5.66 (s, 1H, -CH-), 4.47 (d, J_{ab} = *6.9 Hz*, 1H_a, S-CH_2), 4.14 (d, J_{ba} = *6.9 Hz*, 1H_b, S-CH_2), 4.13 - 4.06 (m, 2H, CH_2-O), 3.91 (s, 3H, CH_3-O), 2.32 (s, 3H, CH_3), 1.2 (t, *J = 7.1 Hz*, 3H, CH_3); .**^{13}C NMR (101 MHz, $CDCl_3$)** δ (ppm) 166.93, 166.73, 144.49, 143.15, 128.71, 128.39, 128.00, 126.96, 101.65, 59.86, 52.36, 34.64, 22.8, 14.37; **HRMS(ESI):** Calc for $C_{23}H_{24}N_2NaO_4S$ $(M+Na)^+$: 447.1283. Found: 447.1285.

Ethyl 2-(benzylthio)-6-methyl-4-(p-tolyl)-1,4-dihydropyrimidine-5-carboxylate 6g

Yield = 51%, ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 7.50 - 6.67 (m, 9H, H_{Ar}), 5.68 (s, 1H, CH), 4.36 (d, J_{ab} = *13.4 Hz*, 1H_a, S-CH_2), 4.13 - 4.09 (m, 3H, H_b, CH_2-O), 2.33 (s, 6H, 2 CH_3), 1.23 (t, *J = 7.1 Hz*, 3H, CH_3); **^{13}C NMR (75 MHz, $CDCl_3$)** δ (ppm) 167.02, 160.03, 146.63, 134.56, 129.36, 129.24, 128.93, 119.51, 112.52, 60.07, 54.71, 35.04, 21.29, 14.29.; **HRMS(ESI):** Calc for $C_{22}H_{25}N_2O_2S$ $(M+H)^+$: 381.1839. Found: 381.1842.

Ethyl 2-((4-chlorobenzyl) thio)-6-methyl-4-(p-tolyl)-1,4-dihydropyrimidine-5-carboxylate 6h

Yield = 75%, ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 7.26 - 7.04 (m, 8H, H_{Ar}), 5.63 (s, 1H, -CH), 4.36 (d, J_{ab} = *13.6 Hz*, 1H_a, S-CH_2), 4.13 (d, J_{ba} = *13.6 Hz*, 1H, S-CH_2), 4.10 - 4.00 (m, 2H, CH_2-0), 2.36 (s, 6H, 2 CH_3), 1.22 (t, *J = 7.1 Hz*, 3H, CH_3); **^{13}C NMR (75 MHz, $CDCl_3$)** δ (ppm) 167.04, 141.98, 136.26, 133.22, 130.37, 129.10, 128.54, 126.91, 60.06, 34.38, 21.28, 14.26.; **HRMS(ESI):** Calc for $C_{22}H_{23}ClN_2NaO_2S$ $(M+Na)^+$: 437.0923. Found: 437.0920.

Ethyl 6-methyl-2-((3-nitrobenzyl) thio)-4-(p-tolyl)-1,4-dihydropyrimidine-5-carboxylate 6i

Yield = 65%, ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 8.16 (m, 1H, H_{Ar}), 8.04 (d, *J* = 7.7 Hz, 1H, H_{Ar}), 7.58 (dd, *J* = 8.2, 6.8 Hz, 1H, H_{Ar}), 7.34 - 7.24 (m, 1H, H_{Ar},), 7.14 (d, *J* = 8.0 Hz, 2H, H_{Ar},), 7.05 (d, *J* = 7.8 Hz, 2H, H_{Ar}), 5.54 (S, 1H, CH), 4.49 (d, J_{ab} = *13.2 Hz*, 1H_a, S-CH_2), 4.22 - 4.01 (m, 3H, H_b, CH_2-O),), 2.32 (s, 6H, 2CH_3), 1.20 (t, *J = 7.1 Hz*, 3H, CH_3). ^{13}C **NMR (101 MHz, $CDCl_3$)** δ (ppm) 166.72, 148.04, 141.55, 140.26, 135.22, 129.15, 126.81, 124.00, 122.13, 59.88, 33.97, 21.10, 14.21. **HRMS (ESI):** Calc for $C_{22}H_{24}N_3O_4S$ $(M+H)^+$: 426.1938. Found: 426.1942.

Ethyl 6-methyl-2-((4-methylbenzyl) thio)-4-(p-tolyl)-1,4-dihydropyrimidine-5-carboxylate 6j

Yield = 67%, ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm) 11.06 (s, 1H, NH), 7.14 (d, *J = 7.9 Hz*, 2H, H_{Ar}), 7.07 (d, *J = 7.9 Hz*, 2H, H_{Ar}), 7.01 (d, *J = 7.9 Hz*, 2H, H_{Ar}), 6.84 (d, *J = 7.9 Hz*, 2H, H_{Ar}), 5.79 (s, 1H, -CH-), 4.80 (d, J_{ab} *= 13.3 Hz*, $1H_a$, S-CH_2), 4.28 (d, J_{ba} *= 13.3 Hz*, $1H_b$, S-CH_2), 4.15 - 3.98 (m, 2H, CH_2-O), 2.56 (s, 3H, CH_3), 2.31 (s, 3H, CH_3), 2.23 (s, 3H, CH_3), 1.13 (t, *J = 7.1 Hz*, 3H, CH_3).; **^{13}C NMR (151 MHz, $CDCl_3$)** δ (ppm) 163.95, 161.35, 143.03, 138.68, 138.11, 136.74, 129.75, 129.57, 129.44, 128.97, 127.20, 106.07, 60.94, 54.41, 36.88, 21.23, 21.16, 17.29, 14.01.; **HRMS (ESI):** Calc for $C_{23}H_{27}N_2O_2S$ $(M+H)^+$: 395.1276. Found: 395.1279.

Ethyl 2-((4-(3, 5-dichloro benzyl) thio)-6-methyl-4-(p-tolyl)-1,4-dihydropyrimidine-5-carboxylate 6k

Yield = 59%, ^{1}H NMR (400 MHz, DMSO-*d_6*) δ (ppm) 7.41 (dd, *J = 5.2, 3.5 Hz*, 1H, H_{Ar}), 7.33 (d, J = 1.6 Hz, 2H, H_{Ar}), 7.07 (d, *J = 8.1 Hz*, 2H, H_{Ar}), 6.99 (d, *J = 8.1 Hz*, 2H, H_{Ar}), 5.50 (s, 1H, -CH), 4.99 (d, J_{ab} *= 14.7 Hz*, $1H_a$, S-CH_2), 4.37 (d, J_{ba} *= 14.7 Hz*, $1H_b$, S-CH_2), 4.05 (q, *J = 7.1 Hz*, 2H, CH_3), 2.28 (s, 3H, CH_3), 2.09 (s, 3H, CH_3), 1.11 (t, *J = 7.1 Hz*, 3H, CH_3); **^{13}C NMR (101 MHz, DMSO-d6)** δ (ppm) 165.85, 163.25, 136.46, 134.76, 132.57, 131.44, 130.97, 129.20, 127.90, 126.69, 106.07, 65.94, 59.41, 31.07, 22.26, 18.11, 14.41; **HRMS(ESI):** Calc for $C_{22}H_{23}Cl_2N_2O_4S$ $(M+H)^+$: 449.2329. Found: 449.2327.

Ethyl 2-((4-(methoxycarbonyl) benzyl) thio)-6-methyl-4-(p-tolyl)-1,4-dihydropyrimidine-5-carboxylate 6l

Yield = 55%, ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 11.26 (s, 1H, NH), 7.66 (d, *J = 8.0 Hz*, 2H, H_{Ar}), 7.40 - 7.20 (m, 2H, H_{Ar}), 7.07 (d, *J = 8.0 Hz*, 2H, H_{Ar}), 6.98 (d, *J* = 7.9 Hz, 2H, H_{Ar}), 5.77 (s, 1H, -CH-), 5.22 (d, J_{ab} *= 14.3 Hz*, $1H_a$, S-CH_2), 4.21 (d, J_{ba} *= 14.3 Hz*, $1H_b$, S-CH_2), 4.13 - 3.98 (m, 2H, CH_2-O), 3.92 (s, 3H, CH_3-O), 2.59 (s, 3H, CH_3), 2.29 (s, 3H, CH_3), 1.13 (t, *J = 7.1 Hz*, 3H, CH_3); **^{13}C NMR (75 MHz, $CDCl_3$)** δ (ppm) 166.23, 163.82, 160.47, 142.69, 139.13, 138.51, 136.31, 129.93, 129.82, 129.70, 128.93, 127.14, 106.46, 61.13, 54.65, 52.19, 36.40, 21.09, 17.31, 14.02; **HRMS (ESI):** Calc for $C_{24}H_{27}N_2O_4S$ $(M+H)^+$: 439.1232. Found: 439.1229.

Ethyl 2-(((1H-benzo[d]imidazol-2-yl) methyl)thio)-6-methyl-4-phenyl-1,4-dihydropyrimidine-5-carboxylate 6m

Yield = 60%, ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 7.51 - 7.15 (m, 9H, H_{Ar}), 5.80 (s, 1H, CH), 4.53 (d, J_{ab} = 14.7 Hz, $1H_a$, S-CH_2), 4.22-4.07 (m, 2H, $1H_b$, S-CH_2, CH_2-O), 2.41 (s, 6H, $2CH_3$), 1.20 (t, *J = 7.1 Hz*, 3H, CH_3). **^{13}C NMR (75 MHz, Acetone-*d_6*);** δ (ppm) 166.22, 166.22, 152.08, 152.08, 152.08, 145.49, 145.49, 145.49, 128.49, 128.49, 127.10, 127.10, 126.19, 126.19, 121.96, 115.01, 100.80, 59.06, 58.84, 27.28, 17.94, 13.67; **HRMS (ESI):** Calc for $C_{22}H_{22}N_4NaO_2S$ $(M+Na)^+$: 429. 1407. Found: 429.1410.

Ethyl 2-(((1H-benzo[d]imidazol-2-yl) methyl) thio)-6-methyl-4-(p-tolyl)-1,4-dihydropyrimidine-5-carboxylate 6n

Yield = 55%, ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 7.75 - 6.97 (m, 8H, H_{Ar}), 5.76 (s, 1H, CH), 4.55 (d, J_{ab} = 14.7 Hz, $1H_a$, S-CH_2), 4.28 - 4.03 (m, 3H, H_b,

CH_2-O), 2.41 (s, 6H, $2CH_3$), 1.22 (t, *J = 6.8 Hz*, 3H, CH_3); **^{13}C NMR (75 MHz, Acetone-d_6).** δ (ppm) 165.99, 151.97, 142.64, 136.92, 129.25, 126.96, 121.86, 114.85, 59.38, 58.40, 20.30, 13.68; **HRMS(ESI):** Calc for $C_{23}H_{24}N_4NaO_2S$ $(M+Na)^+$: 443.2035. Found: 443.2038.

2.2.2. Biological Methods

In this study, the evaluation of antibacterial activity was carried out according to the macro dilution technique reported by Marc *et al.* [27] and Moroh *et al.* [28], with some modifications. A series of dilution in Muller Hinton broth of the synthetic chemicals was carried out followed by a colony count on agar medium. Thus a 1000 µg/mL mother solution of the chemical was prepared by dissolving 0.1 g of powder of each synthetic chemical in 100 ml of a DMSO/water mixture (50/50). The mother solution of 1000 µg/mL of the DMSO/water mixture (50/50) was incorporated into Muller Hinton broth in six test tubes to make final dilutions with concentrations ranging from: 500; 250; 125; 62.5; 31.250 to 15.6250 µg/mL with a dilution factor of 1/2. Each tube contained 10 mL of the mother chemical-Muller Hinton broth mixture. Control tubes, *i.e.* without synthetic chemicals and containing only Muller Hinton broth were also prepared. All these solutions (media without synthetic chemicals and media with synthetic chemicals) were sterilized in an autoclave for 15 min at 121˚C. Then a bacterial inoculum estimated at 106 CFU/mL was prepared from a 16 hour-old colony of multidrug resistant strains of *E. coli* and *S. aureus* and adjusted by opacimeter with the standard Mac Farland. All the media (tests and controls) were inoculated with 0.2 ml of the inoculums, then incubated at 37˚C for 18 to 24 hours. The tests were repeated 3 times. Then the contents of the different tubes were transferred into Petri dishes containing 15 mL of Mueller-Hinton agar. This operation consisted of taking 1 mL of the broth contained in the tubes and added to 9 mL of sterile distilled water in order to have a 10^{-1} dilution. 1mL of the 10^{-1} dilution is taken again and added to 9 mL of sterile distilled water to obtain the 10^{-2} dilution. This operation was repeated until to get a 10^{-4} dilution solution. A bactericidal test was carried out by inoculating 0.1 mL of dilutions of 10°, 10^{-1}, 10^{-2}, 10^{-3} and 10^{-4} by spreading on 15 mL of Mueller-Hinton agar, in a Petri box and incubated at 37˚C.

This operation was repeated three (3) times for each compound and each germ. After 24 hours of observation, the count of the colony count was done by direct counting. It is expressed as a percentage inhibition relative to the control. The method of calculation of the percentage inhibition could be summarized by the following formula:

$$\text{Rate of inhibition}\left(\text{Ti}\right)\left(\%\right) = \left(1-\frac{\text{mean number of colonies for a given concentration}\left(\text{CX}\right)}{\text{mean number of colonies for a growth indicator}\left(\text{TC}\right)}\right)\times 100$$

$$\text{Rate of inhibition}\left(\text{Ti}\right)\left(\%\right) = \left(1-\frac{\text{N}\left(\text{CX}\right)}{\text{N}\left(\text{TC}\right)}\right)\times 100$$

MIC: This is the lowest concentration of synthetic chemical that inhibits 99% of bacteria calculated compared to the control.

The IC_{50} was determined graphically from the sensitivity curve as a function of the concentrations of the synthetic chemicals.

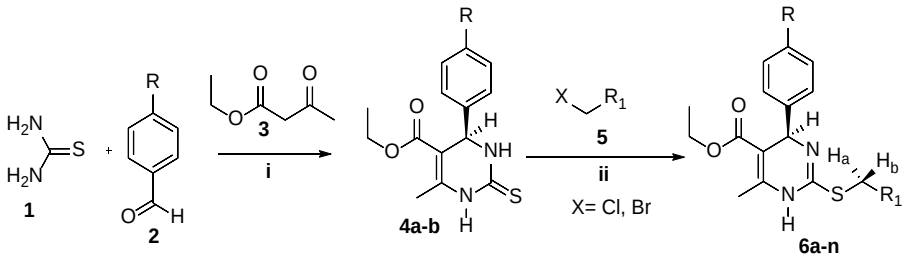
3. Results and Discussion

3.1. Chemistry

The synthesis of the new thiopyrimidine molecules (**6a-n**) was achieved by reacting 2-thiopyrimidines (**4**) with benzyl halides or 2-(chloromethyl)-*1H*-benzimidazole in the presence of a base [29] (Scheme 1). The starting 2-thiopyridines were obtained by a stereospecific process between ethyl acetoacetate, thiourea and *p*-alkylbenzaldehydes under reflux of ethanol in the presence of hydrochloric acid [16]. From the two precursors, we got twelve new 2-benzylthio-pyrimidines (**6a-l**) by nucleophilic substitution reaction between 2-thiopyrimidines (**4**) and benzyl halides in dimethylformamide (DMF) in the presence of potassium carbonate (K_2CO_3) as base. The originality of these compounds lied in introduction of diverse groups on the aromatic ring such as methyl (Me), fluorine (F), chlorine (Cl), methyl carboxylate (CO_2Me) and nitro (NO_2). The molecules were obtained with yields between 50% - 95%. Likewise, for the benzimidazole derivatives (**6m**, **6n**), we obtained them by interaction of 2-thiopyrimidines (**4a**, **4b**) with 2-(chloromethyl)-*1H*-benzimidazole in tetrahydrofuran (THF) in the presence of triethylamine such as base. Both compounds were obtained with 60% and 55% yields respectively. The ^1H NMR spectrum of the 2-benzylthio-pyrimidines (**6a-l**) revealed the presence of two doublets between 4.13 and 5.43 ppm that we attributed to the two protons of the methylene group of benzyl bond to the sulfur atom ($S\text{-}CH_2$). The appearance of twice protons in two doublets could be explained by the tetrahedral geometry of the molecule. Protons behave as if they were neighbors to an asymmetric carbon. For compounds 6m and 6n, the ^1H NMR spectrum revealed the presence of a doublet at 4.53 ppm corresponding to a proton of the methylene group ($CH_2\text{-}S$) and the second proton appeared between 4.28 and 4.03 ppm as a multiplet.

3.2. Biology

The average of the results obtained is given in Table 1.



Scheme 1. Synthesis of compounds 6a-n. Reagents and operating conditions: (i): HCl/ethanol (reflux); (ii) K_2CO_3/DMF or Et_3N/THF.

Table 1. Antibacterial activity of 2-benzylthiopyrimidines and 2-benzimidazolylmethylthiopyrimidines derivatives (rate of inhibition (Ti) and IC_{50}).

Concentration of compounds (µg/mL)		*S. aureus*1174		*E. coli* 1178	
		Ti (%)	IC_{50} (µg/mL)	Ti (%)	IC_{50} (µg/mL)
6a	500	91.16	26.04	82.35	29.68
	250	83.33		76.47	
	125	75		70.58	
	62.5	66.66		58.82	
	31.25	58.33		52.94	
	15.625	33.33		23.52	
6b	500	83.33	61.07	82.35	57.06
	250	75		70.58	
	125	66.66		64.7	
	62.5	50		52.94	
	31.25	41.66		35.29	
	15.625	25		23.52	
6c	500	100	25.83	70.58	116.55
	250	100		58.82	
	125	100		52.94	
	62.5	91.66		35.29	
	31.25	51.66		35.29	
	15.625	41.66		29.41	
6d	500	91.66	15.625	76.47	45.86
	250	91.66		76.47	
	125	83.33		70.58	
	62.5	83.33		52.94	
	31.25	66.66		47.05	
	15.625	50		41.17	
6h	500	91.66	15.625	94.11	13.02
	250	91.66		88.23	
	125	91.66		82.35	
	62.5	66.66		76.47	
	31.25	66.66		70.58	
	15.625	50		52.94	
6j	500	83	41.3	76.47	55.15
	250	75		70.58	
	125	66.66		64.7	
	62.5	58.33		52.94	
	31.25	48.5		47.05	
	15.625	41.66		35.29	

Continued

6k	500	83.33	8.27	76.47	211
	250	83.33		58.82	
	125	75		47.05	
	62.5	66.66		35.29	
	31.25	66.66		29.41	
	15.625	55.33		23.52	
6m	500	100	9.95	100	13.02
	250	83.33		88.23	
	125	83.33		82.35	
	62.5	83.33		76.47	
	31.25	75		70.58	
	15.625	56.66		52.94	

Data processing allowed to plot the sensitivity curves of the germs (inhibition rate as a function of the product concentrations) and the concentration at which the substances inhibit the germs by half (IC_{50}) was determined graphically.

Multidrug-resistant strains of both *E. coli* and *S. aureus* showed good sensitivity to the eight 2-thiopyrimidine derivatives with a better sensitivity to *S. aureus*. Compounds **6c**, **6d**, **6h** and **6m** were more active against *S. aureus*, while compounds **6h** and **6m** were more active against *E. coli*. The presence of the benzyl and benzimidazolyl groups in position-2 of 2-thiopyrimidines demonstrated good antibacterial activity. In this series of compounds, the presence of the nitro group (NO_2) at the 3-position and methyl group at the 4-position of the benzyl enhanced the biological activity on *S. aureus*. Likewise, the attachment of chloride group at the 4-position of the benzyl enhanced the inhibition activity on *E. coli*. Introduction of benzimidazole ring to the sulfur atom at position-2 of the pyrimidine ring enhanced the biological activity against both *S. aureus* and *E. coli*. Compound **6c** with the nitro group in position-3 on the phenyl was more active on *S. aureus* with a MIC = 125 μg/mL while compound **6m** was more active on both *S. aureus* and *E. coli* with a MIC = 500 μg/mL.

4. Conclusion

In this work, it is showed through a number of experimental evidence that between fourteen (14) new 2-thiopyrimidines derivatives (**6a-n**) obtained in yields ranging from 50% to 94%, eight of them were submitted to antibacterial tests on multidrug-resistant strains of *E. coli* and *S. aureus*. They showed good activities on both resistant bacteria. Moreover, compounds **6c** (MIC = 125 μg/mL) and **6m** (MIC = 500 μg/mL) were efficiently active on *S. aureus* and similarly compound **6m** (MIC = 500 μg/mL) was active on *E. coli*. Furthermore, these results validated our approach on the design of new 2-thiopyrimidine derivatives by the attachment of benzyl and benzimidazolyl groups in the 2 position. This ap-

proach could be an addressing contribution to the development of new antibiotics. The next challenge to access antibiotic-resistant is to bring the active compounds into a large spectrum of bacteria.

Acknowledgements

We wish to thank the laboratory (Laboratoire de Méthodologie et Synthèse de Produits Naturels) of the University of Quebec in Montreal (Canada) and the Laboratory LG2A of Jules Verne Picardie University (France) for providing us the chemical reagents and material for the spectroscopic analyzes.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Eicher, T., Hauptmann, S. and Speicher, A. (2003) The Chemistry of Heterocycles: Structures, Reactions, Synthesis, and Applications. John Wiley & Sons, Hoboken. https://doi.org/10.1002/352760183X

[2] Chiang, A.N., Valderramos, J.-C., Balachandran, R., Chovatiya, R.J., Mead, B.P., Schneider, C., Bell, S.L., Klein, M.G., Huryn, D.M., Chen, X.S., *et al.* (2009) Select Pyrimidinones Inhibit the Propagation of the Malarial Parasite, *Plasmodium falciparum*. *Bioorganic & Medicinal Chemistry*, **17**, 1527-1533. https://doi.org/10.1016/j.bmc.2009.01.024

[3] Clercq, E.D., Sakuma, T., Baba, M., Pauwels, R., Balzarini, J., Rosenberg, I. and Holý, A. (1987) Antiviral Activity of Phosphonylmethoxyalkyl Derivatives of Purine and Pyrimidines. *Antiviral Research*, **8**, 261-272. https://doi.org/10.1016/S0166-3542(87)80004-9

[4] Rajanarendar, E., Reddy, M.N., Murthy, K.R., Reddy, K.G., Raju, S., Srinivas, M., Praveen, B. and Rao, M.S. (2010) Synthesis, Antimicrobial, and Mosquito Larvicidal Activity of 1-aryl-4-methyl-3,6-bis-(5-methylisoxazol-3-yl)-2-thioxo-2,3,6,10*b*-tetrahydro-1*H*-pyrimido[5,4-*c*]quinolin-5-ones. *Bioorganic & Medicinal Chemistry Letters*, **20**, 6052-6055. https://doi.org/10.1016/j.bmcl.2010.08.060

[5] Virsodia, V., Pissurlenkar, R.R.S., Manvar, D., Dholakia, C., Adlakha, P., Shah, A. and Coutinho, E.C. (2008) Synthesis, Screening for Antitubercular Activity and 3D-QSAR Studies of Substituted N-phenyl-6-methyl-2-oxo-4-phenyl-1,2,3,4-tetrahydro-pyrimidine-5-carboxamides. *European Journal of Medicinal Chemistry*, **43**, 2103-2115. https://doi.org/10.1016/j.ejmech.2007.08.004

[6] Regnier, G., Canevari, R., Le Douarec, J., Holstorp, S. and Daussy, J. (1972) Triphenylpropylpiperazine Derivatives as New Potent Analgetic Substances. *Journal of Medicinal Chemistry*, **15**, 295-301. https://doi.org/10.1021/jm00273a600

[7] Farghaly, A.M., AboulWafa, O.M., Elshaier, Y.A., Badawi, W.A., Haridy, H.H. and Mubarak, H.A. (2019) Design, Synthesis, and Antihypertensive Activity of New Pyrimidine Derivatives Endowing New Pharmacophores. *Medicinal Chemistry Research*, **28**, 360-379. https://doi.org/10.1007/s00044-019-02289-6

[8] Wang, Z., Gao, Y., He, L., Sun, S., Xia, T., Hu, L., Yao, L., Wang, L., Li, D., Shi, H. and Liao, X. (2021) Structure-Based Design of Highly Potent Toll-Like Receptor 7/8 Dual Agonists for Cancer Immunotherapy. *Journal of Medicinal Chemistry*, **64**,

7507-7532. https://doi.org/10.1021/acs.jmedchem.1c00179

[9] Maquoi, E., Sounni, N. E., Devy, L., Olivier, F., Frankenne, F., Krell, H.-W., Grams, F., Foidart, J.-M. and Noël, A. (2004) Anti-Invasive, Antitumoral, and Antiangiogenic Efficacy of a Pyrimidine-2,4,6-trione Derivative, an Orally Active and Selective Matrix Metalloproteinases Inhibitor. *Clinical Cancer Research*, **10**, 4038-4047. https://doi.org/10.1158/1078-0432.CCR-04-0125

[10] Huang, M., Wang, Y., Collins, M., Mitchell, B.S. and Graves, L.M. (2002) A77 1726 Induces Differentiation of Human Myeloid Leukemia K562 Cells by Depletion of Intracellular CTP Pools. *Molecular Pharmacology*, **62**, 463-472. https://doi.org/10.1124/mol.62.3.463

[11] Von Bubnoff, N., Veach, D.R., Miller, W.T., Li, W., Sänger, J., Peschel, C., Bornmann, W.G., Clarkson, B. and Duyster, J. (2003) Inhibition of Wild-Type and Mutant Bcr-Abl by Pyrido-Pyrimidine-Type Small Molecule Kinase Inhibitors. *Cancer Research*, **63**, 6395-6404.

[12] Mojtahedi, M.M., Saidi, M.R., Shirzi, J.S. and Bolourtchian, M. (2002) Microwave Promoted Efficient Synthesis of Substituted Uracils and Thiouracils under Solvent-Free Conditions. *Synthetic Communications*, **32**, 851-855. https://doi.org/10.1081/SCC-120002693

[13] Sondhi, S.M., Goyal, R.N., Lahoti, A.M., Singh, N., Shukla, R. and Raghubir, R. (2005) Synthesis and Biological Evaluation of 2-Thiopyrimidine Derivatives. *Bioorganic & Medicinal Chemistry*, **13**, 3185-3195. https://doi.org/10.1016/j.bmc.2005.02.047

[14] Sochacka, E. and Frątczak, I. (2004) Efficient Desulfurization of 2-Thiopyrimidine Nucleosides to the Corresponding 4-Pyrimidinone Analogues Using Trans-2-(phenylsulfonyl)-3-phenyloxaziridine. *Tetrahedron Letters*, **45**, 6729-6731. https://doi.org/10.1016/j.tetlet.2004.07.052

[15] Salina, E., Ryabova, O., Kaprelyants, A. and Makarov, V. (2014) New 2-Thiopyridines as Potential Candidates for Killing both Actively Growing and Dormant *Mycobacterium tuberculosis* Cells. *Antimicrobial Agents and Chemotherapy*, **58**, 55-60. https://doi.org/10.1128/AAC.01308-13

[16] Biginelli, P. and Gazz, P. (1893) Synthesis of 3,4-Dihydropyrimidin-2(1H)-Ones. *Gazzetta Chimica Italiana*, **23**, 360-416.

[17] Bamba, F., Jin, J., Chaudhary, A.S., Tai, P.C. and Wang, B. (2021) Design, Synthesis, and Biological Evaluation of Pyrimidine Analogs as SecA Inhibitors. *Medicinal Chemistry Research*, **30**, 1334-1340. https://doi.org/10.1007/s00044-021-02717-6

[18] Bamba, F., Jin, J., Tai, P.C. and Wang, B. (2020) Synthesis and Biological Evaluation of Novel 4-oxo-5-cyano Thiouracil Derivatives as SecA Inhibitors. *Heterocyclic Communications*, **26**, 76-83. https://doi.org/10.1515/hc-2020-0100

[19] Maddila, S., Gorle, S., Seshadri, N., Lavanya, P. and Jonnalagadda, S.B. (2016) Synthesis, Antibacterial and Antifungal Activity of Novel Benzothiazole Pyrimidine Derivatives. *Arabian Journal of Chemistry*, **9**, 681-687. https://doi.org/10.1016/j.arabjc.2013.04.003

[20] Shigeta, S., Mori, S., Watanabe, F., Takahashi, K., Nagata, T., Koike, N., Wakayama, T. and Saneyoshi, M. (2002) Synthèse et activités anti-herpèsvirus d'analogues nucléosidiques 5-Alkyl-2-Thiopyrimidine. *Antiviral Chemistry and Chemotherapy*, **13**, 67-82. https://doi.org/10.1177%2F095632020201300201

[21] Mokale, S. N., Shinde, S. S., Elgire, R. D., Sangshetti, J. N. and Shinde, D. B. (2010) Synthesis and Anti-Inflammatory Activity of Some 3-(4,6-disubtituted-2-thioxo-1,2,3,4-tetrahydropyrimidin-5-yl) Propanoic Acid Derivatives. *Bioorganic & Medicinal Chemistry Letters*, **20**, 4424-4426. https://doi.org/10.1016/j.bmcl.2010.06.058

[22] Pathak, A.K., Pathak, V., Seitz, L.E., Suling, W.J. and Reynolds, R.C. (2004) Antimycobacterial Agents. 1. Thio Analogues of Purine. *Journal of Medicinal Chemistry*, **47**, 273-276. https://doi.org/10.1021/jm030389b

[23] Lal, K., Paliwal, L. and Bagade, M. (2018) Synthesis, Molecular Docking and Pharmacological Study of Some New Thiopyrimidine Derivatives. *Materials Today*: *Proceedings*, **5**, 15354-15360. https://doi.org/10.1016/j.matpr.2018.05.017

[24] Tonelli, M., Paglietti, G., Boido, V., Sparatore, F., Marongiu, F., Marongiu, E., La Colla, P. and Loddo, R. (2008) Antiviral Activity of Benzimidazole Derivatives. I. Antiviral Activity of 1-Substituted-2-[(Benzotriazol-1/2-yl)methyl]benzimidazoles. *Chemistry & Biodiversity*, **5**, 2386-2401. https://doi.org/10.1002/cbdv.200890203

[25] Ouattara, M., Sissouma, D., Yavo, W. and Kone, M.W. (2015) Synthèse et criblage antiplasmodial de quelques benzimidazolyl-chalcones. *International Journal of Biological and Chemical Sciences*, **9**, 1697-1710. https://doi.org/10.4314/ijbcs.v9i3.48

[26] Ouattara, M., Sissouma, D., Koné, M., Menan, H., Touré, S. and Ouattara, L. (2011) Synthesis and Anthelmintic Activity of Some Hybrid Benzimidazolyl-Chalcone Derivatives. *Tropical Journal of Pharmaceutical Research*, **10**, 767-775. https://doi.org/10.4314/tjpr.v10i6.10

[27] Marc, T., Gérard, W. and Denis, L. (2001) Classification des anti-inflamatoires dans Guide pharmacologie. In: *Guide Pharmacologie*. *Etudiants et professionnels paramédicaux*, 4th Edition, 126-128.

[28] Moroh, J.-L.A., Bahi, C., Dje, K., Loukou, Y.G. and Guede-Guina, F. (2008). Study of the Antibacterial Activity of Morinda Morindoides (Baker) Milne-Redheat (Rubiaceae) Acetatique Extract (ACE) on *In Vitro* Growth of Escherichia Coli Strains. *Bullentin de la société Royale des Sciences de Liège*, **77**.

[29] Severina, H.I., Skupa, O.O., Voloshchuk, N.I., Suleiman, M.M. and Georgiyants, V.A. (2019) Synthesis and Anticonvulsant Activity of 6-methyl-2-((2-oxo-2-arylethyl)-thio)pyrimidin-4(3*H*)-one Derivatives and Products of Their Cyclization. *Pharmacia*, **66**, 141-146. https://doi.org/10.3897/pharmacia.66.e38137