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Study of the influence of N-alkylation of 2-benzylthiopyrimidines on the growth of two bacteria of medical interest

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Abstract

The present work highlights the synthesis and effect of introducing substituents on the nitrogen at position -1 of five 2-benzylthiopyrimidine derivatives (6a-e). The compounds (6a-e) were obtained by condensation of 2-thiopyrimidines (4) with benzyl halides in the presence of a base. As for the N-alkylated/arylated-2thiobenzylpyrimidines (8a-k), they were obtained by nucleophilic substitution reaction on nitrogen in basic medium. All the synthesized compounds were characterized by 1H proton, 13C carbon NMR spectroscopic analyses and high resolution mass spectrometry. All compounds were subjected to antibacterial testing on two multidrug resistant strains of Escherichia coli and Staphylococcus aureus. The results revealed that compounds 6a, 6b, 6c, 6d and 6e showed no minimum inhibitory concentration on either *E. coli* or *S. aureus*. However, compounds 8b, 8c, 8d and 8f showed a MIC on *S. aureus*1174. As for the bacterial strain *E. coli* 1178, only compound 8h showed a minimum inhibitory concentration. N-alkylation/arylation enhanced the antibacterial effect of some 2-benzylthiopyrimidine derivatives.

Keywords: 2-Thiopyrimidine; 2-Benzylthiopyrimidine; N-alkylation; Antibacterial activity

1. Introduction

Bacterial infections are nowadays considered as a major public health threat, certainly because of the resistance of bacteria to antibiotics [1, 2]. In response to this major public health problem, our study focused on the pyrimidine ring. Derivatives of 3, 4-dihydropyrimidines have received much attention from many chemists due to their wide range of therapeutic and pharmacological properties, such as antiviral [3], antitumoral, antibacterial and antifungal [4], anti-inflammatory [5]. Studies have shown that several dihydropyrimidine-containing alkaloids such as batzelladine alkaloids, have been shown to be potent inhibitors of HIVgp-120-CD4 [6, 7]. More recently, beginelli compounds have been classified as calcium channel blockers [8, 9], antioxidant molecules [10] and radical scavengers [11-12]. The work of some authors such as Barbosa et al. showed that dihydropyrimidines substituted on the nitrogen were recognized as very effective for the treatment of Alzheimer's disease [13]. The importance of these compounds has led chemists to propose methods of synthesis. It is in this sense that Dallinger and Kappe synthesized N-alkylated dihydropyrimidine derivatives by Mitsunobu reaction [14]. As for Singh et al. they proposed a method of N-alkylation catalyzed by an inorganic strong base [15]. In this work, N-alkylated compounds were synthesized by treating 2-benzylpyrimidine derivatives in the presence of potassium carbonate. After obtaining these compounds, the influence of the introduction

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of substituents on the nitrogen of 2-benzylthiopyrimidine derivatives was studied by determining the antibacterial parameters (MIC and MBC) on two multidrug resistant strains of *E. coli* and *S. aureus*.

2. Material 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 75MHz or 400 and 101 MHz or 600 and 151MHz, respectively, in $CDCl_3$, DMSO and Acetone solutions. Chemical shifts are reported in ppm on the $\boldsymbol{\delta}$ scale. Multiplicities are described as s (singulet), d (doublet), dd (doublet of doublet), t (triplet), q (quartet), 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 analyser.

2.1.2 Materials of Biology

Microbial strains

The microbial support is composed of clinical strains of *E. coli* (Gram negative bacteria) and *S. aureus* (Gram positive bacteria). These strains were provided by the Laboratoire National de la Santé Publique (LNSP) of Cote d'Ivoire. These strains are all pathogenic and multi-resistant. *S. aureus* strains are resistant to: Amoxicillin, Ampicillin, Oxacillin, Ceftazidine, Fosfomycin, Vancomycin and Cefsulodine and those of *E. coli*, are all resistant to: Amoxicillin, Ampicillin, Ceftriazone, Fosfomycin, Cefsulodine. The culture media used are: Mueller-Hinton broth (Oxoid) and Mueller-Hinton agar (Lab.Conda s.a). The synthetic products are composed of eight 2-thiopyrimidine derivatives (**6a-e**) and (**8a-k**). The solvents used for solubilization of the chemicals were dimethylsulfoxide (DMSO) and distilled water.

2.2 Methods

2.2.1 Methods of chemistry

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.

General procedure for the synthesis of N-alkyl 2-thiobenzylpyrimidines derivatives 8a-k

To a solution of 2-benzylsulfanyl-pyrimidine (1 eq, 0.55 mmol) in 5 mL of dimethylformamide (DMF), potassium carbonate (K_2CO_3) was added (6 eq, 3.3 mmol). The mixture was stirred at room temperature for 1 hour, then benzyl / ethyl chloride or bromide (4eq, 2.2 mmol) were added dropwise and the reaction mixture was heated to 70 °C. At the end of the reaction, the mixture was cooled to room temperature, neutralized with a dilute solution of HCl (2M). The precipitate formed was filtered, dried and purified by silica column chromatography to give compounds 3a-k.

2.3 Products characterizations

2.3.1 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, *Jab = 13.4 Hz*, 1Ha, S-CH_2), 4.28 (d, *Jba = 13.4 Hz*, 1Hb, 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, CDCl3) 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.

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

Yield = 94%; ^{1}H NMR (300 MHz, DMSO-d6) δ (ppm) 7.56 - 6.89 (m, 9H, H_{Ar}), 5.59 (s, 1H, CH), 4.90 (d, *Jab = 16.3 Hz*, 1Ha, S-CH_2), 4.38 (d, *Jba = 16.3 Hz*, 1Hb, 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-d6) 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.

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

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, Jab = 13.4 Hz, 1Ha, S-CH_2), 4.13 - 4.09 (m, 3H, Hb, 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.

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

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, Jab = 13.6 Hz, 1Ha, S-CH_2), 4.13 (d, *Jba = 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.

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

Yield = 55%, ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 11.26 (s, 1H, NH), 7.66 (d, *J = 8.0 Hz*, 2H, HAr), 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, *Jab = 14.3 Hz*, 1Ha, S-CH_2), 4.21 (d, *Jba = 14.3 Hz*, 1Hb, 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.

2.3.6 Ethyl 1-benzyl-2-((4-chlorobenzyl) sulfanyl)-6-methyl-4-phenyl-1, 4-dihydropyrimidine-5- carboxylate 8a

Yield = 55 %, ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 7.40 – 7.21 (m, 12H, H_{Ar}), 7.11 (dd, *J = 13.4, 8.4 Hz*, 2H; H_{Ar}), 5.17 (s, 1H, CH), 4.84 (d, *J = 16.1 Hz,* 1H, N-CH_2), 4.49 (d, *J = 13.7 Hz*, 1H, S-CH_{2a}) 4.33 (d, *J = 13.7 Hz*, 1H, CH_{2b}), 4.13 – 3.96 (m, 3H, N-CH_2, CH_2-O), 2.44 (s, 3H, CH_3), 1.17 (t, *J = 10.4 Hz*, 3H, CH_3; ^{13}C NMR (75 MHz, CDCl3) δ (ppm) 166.65, 160.66, 153.75, 141.77, 136.37, 133.67, 133.06, 130.56, 129.02, 128.88, 128.78, 128.68, 128.65, 128.61, 128.21, 127.36, 126.32, 104.80, 60.07, 51.41, 35.30, 22.95, 14.29; HRMS (ESI): Calc for $C_{28}H_{28}ClN_2O_2S$ (M+H) +: 491.1832 found: 491.1835

2.3.7 Ethyl 1-(4-chlorobenzyl)-2-((4-chlorobenzyl) sulfanyl)-6-methyl-4-phenyl-1,4- dihydropyrimidine-5-carboxylate 8b

Yield = 65%. ^{1}H NMR (300 MHz, $CDCl_3$) δ(ppm) 7.5- 7.1 (m, 13H, H_{Ar}), 5.22 (s, 1H, CH), 4.91 (d, *J = 16.3 Hz*, 1H, N-CH), 4.51 (d, *J = 13.8 Hz*, 1H, S-CH_{2a}), 4.34 (d, *J = 13.8 Hz*, 1H, S-CH_{2b}), 4.10 (d, *J = 16.3 Hz*, 1H, N-CH_2), 4.08 – 3.91 (m, 2H, CH_2-O), 2.44 (s, 3H, CH_3), 1.14 (t, *J = 7.0 Hz*, 3H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm) 166.71, 161.01, 142.13, 136.35, 135.16, 133.07, 130.57, 128.85, 128.59, 128.12, 127.98, 127.43, 127.33, 104.49, 60.00, 59.68, 51.95, 35.22, 23.02, 14.18; HRMS (ESI): Calc for $C_{28}H_{27}Cl_2N_2O_2S$ (M+H) +: 525. 1543 found: 525.1548.

2.3.8 Ethyl 2-((4-chlorobenzyl) sulfanyl)-1-ethyl-6-methyl-4-phenyl-1, 4-dihydropyrimidine-5- carboxylate 8c

Yield =66%. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 7.40 – 7.23 (m, 9H, H_{Ar}), 5.36 (s, 1H ; CH), 4.51 (d, *J = 13.6 Hz*, 1H, S-CH_{2a}), 4.31 (d, *J = 13.6 Hz*, 1H, S-CH_{2b}), 4.23 – 4.05 (m, 2H, CH_2-O), 3.49 (dq, *J = 14.4, 7.1 Hz*, 1H, N-CH_2, 3.29 (dq, *J = 14.4, 7.1 Hz*, 1H, N-CH_2), 1.26 (t, *J = 7.1 Hz*, 3H, CH_3), 1.15 (t, *J = 7.1 Hz*, 3H, CH_3);^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm) 166.94, 160.73, 154.47, 143.27, 136.50, 133.05, 130.56, 127.94, 127.02, 104.18, 60.59, 59.73, 44.70, 34.81, 23.13, 14.41, 13.44; HRMS (ESI): Calc for $C_{23}H_{26}ClN_2O_2S$ (M+H) +: 429. 1752 found: 429.1757.

2.3.9 Ethyl 1-benzyl-2-(benzylsulfanyl)-6-methyl-4-phenyl-1, 4-dihydropyrimidine-5-carboxylate 8d

Yield = 54%. ^{1}H NMR (300 MHz, CDCl3) δ (ppm) 7.46 – 7.16 (m, 15H, H_{Ar}), 5.23 (s, 1H, CH), 4.95 (d, *J = 16.0 Hz*, 1H, N-CH_2), 4.59 (d, *J = 13,4 1H*, S-CH_{2a}), 4.45 (d, *J = 13,4,1Hz*, S-CH_{2b}), 4.14 – 3.98 (m, 3H, N-CH_2, CH_2-O), 2.46 (s, 3H, CH_3), 1.15 (t, *J = 7.1 Hz*, 3H, CH_3);^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm) 166.79, 161.45, 154.43, 142.29, 137.58, 135.25, 129.23, 128.82, 128.57, 128.35, 128.05, 127.92, 127.47, 127.43, 127.33, 104.38, 59.83, 59.62, 51.90, 36.11, 23.13, 14.19; HRMS (ESI): Calc for $C_{28}H_{28}N_2NaO_2S$ (M+Na) +: 479. 2583 found: 479.2588.

2.3.10 Ethyl 2-(benzylsulfanyl)-1-ethyl-6-methyl-4-phenyl-1, 4-dihydropyrimidine-5-carboxylate 8e

Yield = 70%. ^{1}H NMR (300 MHz, CDCl3) δ (ppm) 7.51 – 7.23 (m, 10H, H_{Ar}), 5.38 (s, 1H, CH), 4.58 (d, *J = 13.4 Hz*, 1H, S-CH_{2a}), 4.42 (d, *J=13.4Hz*, 1H, S-CH_{2b}), 4.23 – 4.08 (m, 2H, CH_2-O), 3.52 (dq, *J = 14.4, 7.2 Hz*, 1H, N-CH2), 3.38 – 3.25 (dq, *J = 14.4, 7.2 Hz*, 1H, N-CH_2), 2.42 (s, 3H, CH_3), 1.27 (t, *J = 7.1 Hz*, 3H, CH_3), 1.16 (t, *J = 7.1 Hz*, 3H, CH_3);^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm) 167.00, 161.26, 143.36, 137.63, 129.24, 128.54, 127.92, 127.33, 127.07, 104.09, 60.62, 59.72, 44.76, 35.73, 23.17, 14.37, 13.45; HRMS (ESI) : Calc for $C_{23}H_{27}N_2O_2S$ (M+H) +: 395.2187.found : 395.2191

2.3.11 Ethyl 1-benzyl-2-(benzylsulfanyl)-6-methyl-4-(p-tolyl)-1, 4-dihydropyrimidine-5-carboxylate 8f

Yield = 54%. ^{1}H NMR (300 MHz, $CDCl_3$) 7.50 – 7.07 (m, 14H, H_{Ar}), 5.18 (s, 1H, CH), 4.94 (d, *J = 16.0 Hz*, 1H, N-CH_2), 4.55 (d, *J = 13.4 Hz*, 1H, S-CH_{2a}), 4.45 (d, *J = 13.4 Hz*, 1H, S-CH_{2b}), 4.15 – 3.93 (m, 3H, N-CH_2, CH_2-O), 2.45 (s, 3H, CH_3), 2.35 (s, 3H, CH_3), 1.15 (t, *J = 7.1 Hz*, 3H, CH3);^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm) 166.79, 161.35, 154.25, 139.39, 137.78, 137.62, 135.31, 129.23, 129.21, 128.79, 128.49, 127.87, 127.42, 127.40, 127.30, 104.44, 59.59, 59.52, 51.74, 36.08, 23.13, 21.19, 14.19; HRMS (ESI): Calc for $C_{29}H_{31}N_2O_2S$ (M+H) + : 471.2557. found: 471.2561.

2.3.12 Ethyl 2-(benzylsulfanyl)-1-ethyl-6-methyl-4-(p-tolyl)-1, 4-dihydropyrimidine-5-carboxylate 8g

Yield = 66%. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) δ 7.46 – 7.27 (m, 5H, H_{Ar}), 7.18 (dd, *J = 40.2, 8.0 Hz*, 4H, H_{Ar}), 5.33 (s, 1H, CH), 4.56 (d, *J = 13.4 Hz*, 1H, S-CH_{2a}), 4.40 (d, *J = 13.4 Hz*, 1H, S-CH_{2b}) , 4.22 – 4.07 (m, 2H, CH_2-O), 3.50 (dq, *J = 14.3, 7.2 Hz*, 1H, N-CH_2), 3.30 (dq, *J = 14.3, 7.2 Hz*, 1H, N-CH_2), 1.27 (t, *J = 7.1 Hz,* 3H, CH_3), 1.16 (t, *J = 7.1 Hz*, 3H, CH_3);^{13}C NMR (75 MHz, CDCl3) δ (ppm) 167.03, 161.09, 154.65, 140.57, 137.66, 129.23, 129.20, 128.52, 127.30, 104.10, 60.21, 59.67, 44.61, 35.69, 23.22, 21.17, 14.45, 13.45; HRMS (ESI) : Calc for $C_{24}H_{29}N_2O_2S$ (M+H) +: 409.2714 found : 409.2719.

2.3.13 Ethyl 1-benzyl-2-((4-chlorobenzyl) sulfanyl)-6-methyl-4-(p-tolyl)-1, 4-dihydropyrimidine-5- carboxylate 8h

Yield = 62%. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 7.40 – 7.10 (m, 13H, H_{Ar}), 5.19 (s, 1H, CH), 4.91 (d, J = 16.0 Hz, 1H, N-CH_2), 4.51 (d, *J = 13.7 Hz*, 1H, S-CH_{2a}), 4.34 (d, *J = 13.7 Hz*, 1H, S-CH_{2b}), 4.15 – 3.99 (m, 3H, N-CH_2, CH_2-O), 2.44 (s, 3H, CH_3), 2.36 (s, 3H, CH_3), 1.16 (t, *J = 7.1 Hz*, 3H, CH_3); ^{13}C NMR (75 MHz, CDCl3) δ (ppm) 166.77, 160.84, 154.06, 139.31, 137.87, 136.49, 135.24, 133.06, 104.47, 59.65, 51.81, 35.24, 23.08, 21.20, 14. 27; HRMS (ESI): Calc for $C_{29}H_{29}ClN_2NaO_2S$ (M+Na) +: 527. 2035 found: 527.2040.

2.3.14 Ethyl 2-((4-chlorobenzyl) sulfanyl)-1-ethyl-6-methyl-4-(p-tolyl)-1, 4-dihydropyrimidine-5- carboxylate 8i

Yield = 70%. ^{1}H NMR (300 MHz, CDC_{l3}) δ (ppm) 7.39 – 7.25 (m, 4H, H_{Ar}), 7.24 – 7.06 (m, 4H, H_{Ar}), 5.31 (s, 1H, CH), 4.50 (d, *J = 13.6 Hz*, 1H, S-CH_{2a}), 4.30 (d, *J = 13.6 Hz*, 1H, S-CH_{2b}), 4.21 – 4.03 (m, 2H, CH_2-O), 3.48 (dq, *J = 14.4, 7.2 Hz*, 1H, N-CH_2), 3.28 (dq, *J = 14.4, 7.1 Hz*, 1H, N-CH_2), 2.38 (s, 3H, CH_3), 2.33 (s, 3H, CH_3), 1.26 (dd, *J = 8.9, 5.4 Hz*, 3H, CH_3), 1.14 (t, *J = 7.1 Hz*, 3H, CH_3).^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm) 167.04, 162.08, 160.69, 145.86, 140.39, 137.66, 136.68, 130.56, 129.20, 128.59, 126.98, 104.32, 60.30, 59.76, 44.59, 34.80, 23.20, 21.15, 14.35, 13.41; HRMS (ESI): Calc for $C_{24}H_{27}ClN_2NaO_2S$ (M+Na) +: 465. 1841 found: 465.1837.

2.3.15 Ethyl 1-benzyl-2-((4-(methoxycarbonyl) benzyl) sulfanyl)-6-methyl-4-(p-tolyl)-1, 4- dihydropyrimidine-5-carboxylate 8j

Yield = 68%. ^{1}H NMR (300 MHz, CDCl3) δ (ppm) 8.02 – 7.98 (m, 2H, H_{Ar}), 7.47 (d, *J = 8.3 Hz*, 2H, H_{Ar}), 7.44 – 7.07 (m, 9H, H_{Ar}), 5.17 (s, 1H, CH), 4.90 (d, *J = 16.0 Hz*, 1H, N-CH_2), 4.58 (d, *J = 13.7 Hz*, 1H, S-CH_{2a}), 4.40 (d, *J = 13.7 Hz*, 1H, S-CH_{2b}), 4.14 – 3.98 (m, 3H, N-CH_2, CH_2-O), 3.95 (s, 3H, CH_3), 2.42 (s, 3H, CH_3), 2.35 (s, 3H, CH_3), 1.14 (dd, *J = 9.3, 4.9 Hz*, 3H, CH_3);^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm) 167.04, 160.66, 154.00, 143.62, 137.87, 135.24, 129.73, 129.26, 129.18, 128.83, 127.68, 127.37, 127.31, 127.01, 104.17, 65.41, 59.66, 52.15, 51.74, 35.57, 23.02, 21.21, 14.17; HRMS (ESI) : Calc for $C_{31}H_{33}N_2O_4S$ (M+H) +: 529.2532 found : 529.2534.

2.3.16 Ethyl 1-ethyl-2-((4-(methoxycarbonyl) benzyl) sulfanyl-6-methyl-4-(p-tolyl)-1, 4- dihydropyrimidine-5-carboxylate 8k

Yield = 55%. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 8.04 – 7.46 (m, 4H, H_{Ar}), 7.24 – 7.04 (m, 4H, H_{Ar}), 5.31 (s, 1H, CH), 4.60 (d, *J = 13.7 Hz*, 1H, S-CH2a), 4.40 (d, *J = 13.7 Hz*, 1H, S-CH_{2b}), 4.19 – 4.06 (m, 2H, CH_2-O), 3.94 (s, 3H, CH_3- O), 3.49 (dq, *J = 14.4, 7.1 Hz*, 1H, N-CH_2), 3.29 (dq, *J = 14.4, 7.1 Hz*, 1H, N-CH_2), 2.38 (s, 3H, CH_3), 2.32 (s, 3H, CH_3), 1.25 (dd, *J = 9.1, 5.1 Hz*, 3H, CH_3), 1.14 (dd, *J = 8.6, 5.7 Hz*, 3H, CH_3).13C NMR (75 MHz, $CDCl_3$) δ (ppm) 166.93, 160.36, 143.40, 140.31, 137.70, 129.76, 129.22, 126.98, 104.35, 60.36, 59.79, 52.10, 44.65, 35.16, 22.98, 21.15, 14.34, 13.45; HRMS (ESI): Calc for $C_{31}H_{33}N_2O_4S$ (M+H) + :529. 2241. found: 529.2244.

2.4 Biological Materials

The microbial support consisted of clinical strains of *E. coli* (Gram-negative bacteria) and *S. aureus* (Gram-positive bacteria). These strains were provided by the Laboratoire National de la santé Publique (LNSP) of Cote d'Ivoire. These strains were all pathogenic and multi-resistant. *S. aureus* strains was resistant to: Amoxicillin, Ampicillin, Oxacillin, Ceftazidine, Fosfomycin, Vancomycin and Cefsulodine and those of *E. coli*, was all resistant to: Amoxicillin, Ampicillin, Ceftriazone, Fosfomycin, Cefsulodine. The culture media used was: Mueller-Hinton broth (Oxoid) and Mueller-Hinton agar (Lab.Conda s.a). The synthetic products were composed of eight 2-alkyl sulfanyl-pyrimidine (3a-k) derivatives. The solvents used for solubilization of chemicals products was dimethylsulfoxide (DMSO) and distilled water.

2.5 Biological methods

In this study, the antibacterial activity evaluation was performed according to the macro dilution technique reported by T. Marc et. al., [16] and Moroh J-L. et. al [17], with some modifications. A series of dilutions of the synthetic chemical products in Muller Hinton broth was carried out followed by a colony count on agar medium. Thus, a stock solution of 1000 µg/mL of chemical products was prepared by dissolving 0.1g of powder of each synthetic chemical product in 100 ml of a DMSO/water mixture (50/50). The stock solution of 1000 µg/mL of DMSO/water (50/50) mixture was incorporated into Muller Hinton broth in six test tubes to make final dilutions with concentrations rang from: 500; 250; 125; 62.5; 31.250 to 15.6250 µg/mL with a dilution factor of 1/2. Each tube contains 10 mL of the chemical product stock solution/Muller Hinton broth mixture. Control tubes, i.e. without the synthetic chemical product and containing only Muller Hinton broth, was also prepared. All these solutions (media without chemical synthesis product and media with chemical synthesis product) was autoclaved 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 *E. coli* and *S. aureus* strains and adjusted by opacimeter with the Mac Farland standard. All media (test and control) was inoculated with 0.2 ml of the inoculum and incubated at 37°C for 18 - 24 hours. The tests were repeated 3 times. The contents of the different tubes were then transferred to petri dishes containing 15 mL of Mueller-Hinton agar. This operation consisted of taking 1 mL of the broth contained in the tubes and adding it to 9 mL of sterile distilled water to obtain a 10-1 dilution. 1 mL of the 10-1 dilution was taken again and added to 9 mL of sterile distilled water to obtain the 10-2 dilution. This operation was repeated until a 10-4 dilution solution was obtained. A bactericidal control was performed by plating 0.1 mL of the 10°, 10^{-1}, 10^{-2}, 10^{-3} and 10^{-4} dilutions on 15 mL of Mueller-Hinton agar in a petri dish and incubating at 37°C. This operation was repeated three (3) times for each compound and each germ. After 24 hours of observation, the number of colonies was counted directly. It is expressed as a percentage of inhibition compared to the control.

- **MIC:** This is the lowest concentration of synthetic chemical that inhibits 99% of bacteria calculated compared to the control.
- **MBC:** Minimum Bactericidal Concentration

3. Results and discussion

3.1 Chemistry

The synthesis of the new thiopyrimidine compounds (**6a-e**) was achieved by reacting 2-thiopyrimidines (4) with benzyl halides in the presence of a base [18] (Figure 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 [19]. 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 molecules were obtained with yields between 51% - 94. The method of N-alkylation/arylation consists to treat 2- thiopyrimidine with a base, thus creating an amide ion which will subsequently react with an appropriate carbon chain to lead to N-alkyl derivatives [20, 21]. Compounds (8a-k) were obtained using the second method by condensation of 2-alkylsulfanyl-pyrimidine with ethyl or benzyl chloride in the presence of potassium carbonate (K_2CO_3) under reflux of dimethylformamide (DMF) (Figure 1). All synthesized compounds were characterized by ^1H, ^{13}C NMR spectra and HRMS. The ^{1} H NMR spectrum of the 2-benzylthio-pyrimidines (**6a-e**) 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-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. Concerning ^1H NMR spectrum for compound (8a-k), the two protons of the methylene group linked to the nitrogen atom (N-CH_2) of 1-ethyl-2-benzylsulfanyl-pyrimidine appeared in two multiplets. Around 3.49 ppm, we observed the first quadruplet doublet corresponding to a hydrogen with the coupling constants *Jpq = 14.4 Hz*; *Jp- (3H) = 7.2 Hz*. The second hydrogen appeared also in the quadruplet doublet form at 3.30 ppm with the coupling constants Jqp = 14.4 Hz; Jq- (3H) = 7.2 Hz. Likewise, these twice protons in 1- benzyl-2-benzylsulfanyl-pyrimidines, two others signal were observed. The first one

was a doublet, it fitted around 4.90 ppm with a coupling constant *Jpq = 16 Hz* for a proton of the methylene group (N-CH_2) and the second one was confused in the multiplet detected in the fitting region of signal between 4.14 and 3.99 ppm.

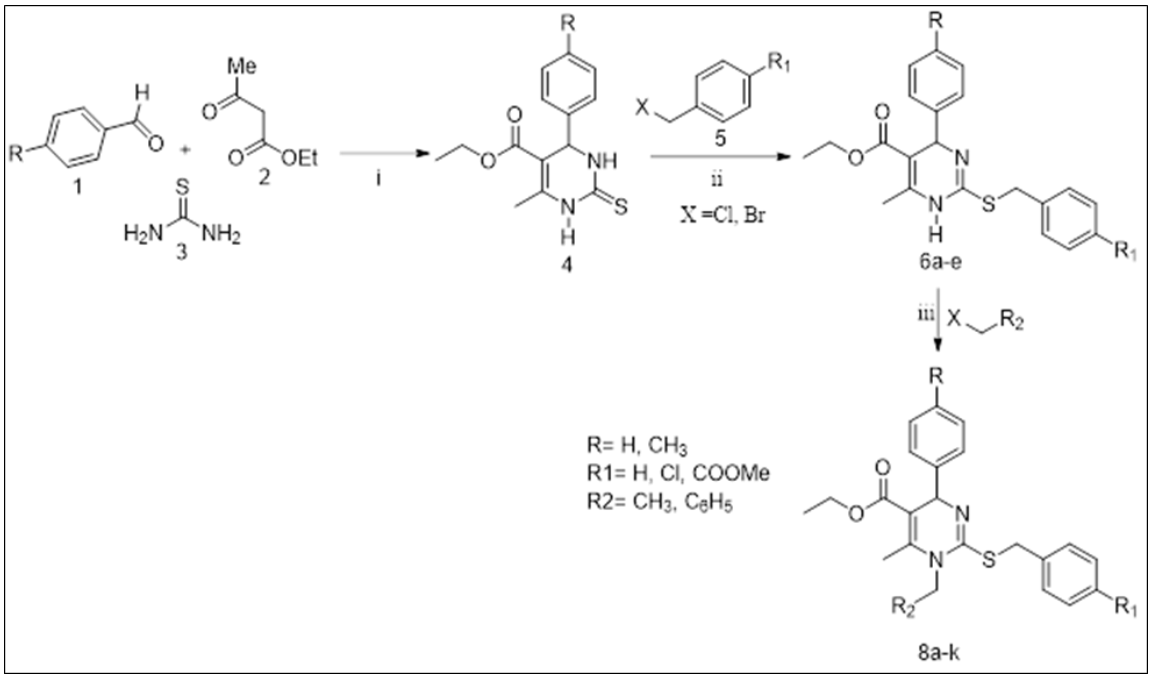

Figure 1 Synthesis of compounds (6a-e) and (8a-k). Reagents and operating conditions: (i): HCl/ethanol (reflux); (ii) K2CO3/DMF, r.t, (iii) K2CO3/DMF, 70˚C

3.2 Biology

Table 1 Antibacterial parameters of N-alkyl-2-benzylthiopyrimidines (MIC and MBC)

	S. aureus1174				***E. coli 1178***			
Compounds	**MIC (µg/mL)**	**MBC (µg/mL)**	**MBC/ MIC**	**Power or Capacity**	**MIC (µg/mL)**	**MBC (µg/mL)**	**MBC/ MIC**	**Power or Capacity**
6a	-	-	-	-	-	-	-	-
6b	-	-	-	-	-	-	-	-
6c	-	-	-	-	-	-	-	-
6d	-	-	-	-	-	-	-	-
6e	-	-	-	-	-	-	-	-
8b	125	250	2	bactericidal	-	-	-	-
8c	500	500	1	Bactericidal	-	-	-	-
8d	250	500	2	Bactericidal	-	-	-	-
8e	-	-	-	-	-	-	-	-
8f	125	250	2	bactericidal	-	-	-	-
8g	-	-	-	-	-	-	-	-
8h	-	-	-	-	500	500	1	bactericidal
8i	-	-	-	-	-	-	-	-
8j	-	-	-	-	-	-	-	-
8k	-	-	-	-	-	-	-	-

-: Not determined.

The antibacterial parameters (MIC and MBC) were determined by the liquid microdilution method. These parameters are recorded in the Table below. The results of the antibacterial effect of 2-benzylthiopyrimidines (6a-e) on *S. aureus* show that these compounds showed no minimum inhibitory concentration (MIC). On *E. coli*, also these compounds revealed no minimum inhibitory concentration. When the ethyl or benzyl groups were introduced on the nitrogen, the N-alkylated compounds thus obtained (8b, 8c, 8d and 8f) showed a minimum inhibitory concentration (MIC) on *S. aureus* with respective values of 125, 500, 250 and 125µg/mL. On *E. coli*, only compound 8h showed a minimum inhibitory concentration (MIC = 500 µg/mL). Also, the different MBCs were determined and then the MBC/MIC ratios of compounds 8b, 8c, 8d and 8f on *S. aureus1174* showed that they all have a bactericidal power. For the *E. coli 1178* bacterial strain, only compound 8h showed bactericidal power. The existence of MICs for compounds 8b, 8c, 8d and 8f shows that the N-alkyl/aryl compounds were more active than their precursors 6a, 6b and 6c. This shows that N-alkylation or N-arylation improved the antibacterial activity of the 2-thiopyrimidine derivatives. The majority of the active compounds came from N- arylation. This could be due to the fact that the benzyl group is more electron rich.

4. Conclusion

This work synthesized and studied the influence of N-alkylation on the antibacterial activity of 2-thiopyrimidine derivatives. The 2-benzylthiopyrmidine derivatives (**6a-e**) were with a yield ranging from 51 to 94%. As for the twelve N-substituted 2-benzylthiopyrimidine derivatives, they were obtained in yields ranging from 54 to 70%. All these compounds were characterized by ^{1}H, ^{13}C NMR and high resolution mass spectrometry. The results of antibacterial tests showed that N-alkylation/arylation enhanced the antibacterial effect of some 2-benzylthiopyrimidine derivatives. The introduction of the aryl group on the nitrogen at position -1 of 2-benzylthiopyrimidines improved the antibacterial activity more and this is due to the fact that it is more electron rich. It is therefore an interesting avenue of structural modification in the search for more effective antibacterial compounds.

Compliance with ethical standards

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Disclosure of conflict of interest

No potential conflict of interest was reported by the author(s).

Author contributions

A. P.A. performed the syntheses, D.Z. and A.T. participated in the design and direction of the project, E.A and C.L.A.K. conceptualized the biological study and methodology. S.C., write the paper and supervised the project. All authors have read and agreed to the published version of the manuscript.

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