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Design, Synthesis and Biological Evaluation of Thiophene Based Pyrimidin-4-one Derivatives as New Type of Antimicrobial Agents

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In the present investigation, a novel series of 3-(benzylidene-amino)-2-methyl-5-phenyl-3*H*-thieno [2,3-*d*]pyrimidin-4-one and its derivatives (**5a-i**) were synthesized by condensation reaction from the final intermediate, 3-amino-2-methyl-5-phenyl-3*H*-thieno[2,3-*d*]pyrimidin-4-one (**4**). The synthesis of the title compounds commenced from commercially available 2-amino-4-phenyl-thiophene-3-carboxylic acid ethyl ester (**1**) and by involving 2-amino-4-phenyl-thiophene-3-carboxylic acid (**2**) and 2-methyl-5-phenyl-thieno[2,3-*d*][1,3]oxazin-4-one (**3**) as reactive intermediates. The chemical structures of synthesized compounds were characterized by IR, ^{1}H NMR, mass spectral data and elemental analysis. The final compounds were used to screen for their antifungal activity against two strains of fungi.

Keywords: Thiophene, Pyrimidin-4-ones, Antimicrobial activities.

INTRODUCTION

Pyrimidines represent an important class of heterocycles and their structural framework is not only a key constituent of nucleic bases, alkaloids and numerous pharmacophores with variety of potent biological activities. Pyrimidines occupy a distinct and unique place in medicine, large array of pyrimidine non-nucleoside derivatives possess a variety of pharmacological properties include anticancer [1], antiviral [2], antibacterial [3], antifungal [4], antiprotozoal [5], antihypertensive [6], antihistaminic [7], anti-inflammatory [8] and central nervous activities [9]. In view of the above mentioned findings and following our work on the synthesis of new heterocyclic compounds we here are aimed at reporting the preparation of a new class of 3-(benzylidene-amino)-2-methyl-5-phenyl-3*H*-thieno [2,3-*d*]pyrimidin-4-one and its derivatives (**5a-i**) by involving 2-amino-4-phenyl-thiophene-3-carboxylic acid ethyl ester (**1**) as starting material.

EXPERIMENTAL

All the reagents and solvents were used as purchased without further purification. Melting points were determined on a Fisher-Johns melting point apparatus and are uncorrected. Crude products were purified by column chromatography on silica gel of 60-120 mesh. IR spectra were obtained on a Perkin-Elmer BX serried FTIR 5000 spectrometer using KBr pellet. NMR spectra were recorded on a Varian 300 MHz spectrometer for ^{1}H NMR. The chemical shifts were reported as ppm down field using TMS as an internal standard. Mass spectra were recorded on a VG-Micromass 7070H spectrometer operating at 70 eV.

Synthesis of 2-amino-4-phenyl-thiophene-3-carboxylic acid (2): A mixture of ethyl 2-amino-4-phenyl-thiophene-3-carboxylic acid ethyl ester (**1**) (0.01 mol) and sodium hydroxide (1.25 g) in ethanol (15 mL) was heated at reflux temperature for 4 h. After completion of the reaction (monitored by the TLC), the reaction mixture was cooled and poured in ice-cold water (25 mL), then acidified with conc. HCl to get crude solid and it filtered, dried and recrystallized from ethanol to achieve pure 2-amino-4-phenyl-thiophene-3-carboxylic acid (**2**).

Synthesis of 2-methyl-5-phenyl-thieno[2,3-*d*][1,3]oxazin-4-one (3): A solution of 2-amino-4-phenyl-thiophene-3-carboxylic acid (**2**) (0.01 mol) and acetic anhydride (5 mL) was refluxed for 6 h with constant stirring. After accomplishment of the reaction (examined by the TLC), the mixture was cooled, poured into ice-cold water to form crude product. It is collected by filtration, washed with cold water, dried and recrystallized from ethyl acetate to achieve 2-methyl-5-phenyl-thieno[2,3-*d*][1,3]oxazin-4-one (**3**).

Synthesis of 3-amino-2-methyl-5-phenyl-3*H*-thieno-[2,3-*d*]pyrimidin-4-one (4): A mixture of 2-methyl-5-phenyl-

thieno[2,3-*d*][1,3]oxazin-4-one (**3**) (0.01 mL) and hydrazine hydrate (0.05 mol) in ethanol (10 mL) was prepared at room temperature. Then the reaction mixture was heated at reflux temperature on uniform stirring for 6 h. After achievement of the reaction (scanned by the TLC), the resulted mixture cooled and precipitated after poured in ice-cold water. The crude product was filtered and washed with cold water, dried and recrystallized with ethanol to get pure 3-amino-2-methyl-5-phenyl-3*H*-thieno[2,3-*d*]pyrimidin-4-one (**4**).

Synthesis of 3-(benzylidene-amino)-2-methyl-5-phenyl-3*H*-thieno [2,3-*d*]pyrimidin-4-one (5a): A solution of 3-amino-2-methyl-5-phenyl-3*H*-thieno[2,3-*d*]pyrimidin-4-one (**4**) (0.01 mol) and benzaldehyde (0.01 mol) in acetic acid (10 mL) was prepared at ambient temperature. Then the reaction mixture is stirred uniformly for 14 h at reflux temperature. After fulfillment of the reaction (examined by the TLC), the mixture was cooled and poured in ice-cold water and it is collected by filtration, washed with cold water, recrystallized from ethyl acetate to obtain 3-(benzylidene-amino)-2-methyl-5-phenyl-3*H*-thieno[2,3-*d*]pyrimidin-4-one (**5a**). Similar procedure is followed to prepare other compounds of this series **5b-i**.

Spectral data

2-Amino-4-phenyl-thiophene-3-carboxylic acid (2): Yield: 68 %, m.p.: 123-125 °C, IR (KBr, ν_{max}, cm^{-1}): 3314 (N-H, NH_2), 3036 (C-H, Ar), 1718 (C=O), 1654 (C=C, Ar), 1272 (C-O), 865 (C-S); ^{1}H NMR (300 MHz, DMSO-d_6): δ 11.84 (s, 1H, COOH), 7.82 (s, 1H, thiophene), 7.70-7.45 (m, 5H, Ar-H), 7.36 (s, 2H, NH_2). MS: 219 *m/z* (M^+). Elemental analysis: Calculated for $C_{11}H_9NO_2S$: C 60.26, H 4.14, N 6.39, S 14.62. Found: C 58.65, H 4.08, N 6.12, S 14.14.

2-Methyl-5-phenyl-thieno[2,3-*d*][1,3]oxazin-4-one (3): Yield: 70 %, m.p.: 131-133 °C, IR (KBr, ν_{max}, cm^{-1}): 3030 (C-H, Ar), 2970 (C-H, CH_3), 1742 (C=O), 1660 (C=C, Ar), 1570 (C=N), 1269 (C-O), 854 (C-S); ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.77 (s, 1H, thiophene), 7.75-7.52 (m, 5H, Ar-H), 2.74 (s, 3H, CH_3). MS: 243 *m/z* (M^+). Elemental analysis: Calculated for $C_{13}H_9NO_2S$: C 64.18, H 3.73, N 5.76, S 13.18. Found: C 62.65, H 3.54, N 5.61, S 12.98.

3-Amino-2-methyl-5-phenyl-3*H*-thieno[2,3-*d*]pyrimidin-4-one (4): Yield: 77 %, m.p.: 154-156 °C, IR (KBr, ν_{max}, cm^{-1}): 3235 (N-H, NH_2), 3010 (C-H, Ar), 2958 (C-H, CH_3), 1685 (C=O), 1616 (C=C), 1597 (C=N), 862 (C-S); ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.85 (s, 2H, NH_2), 7.79-7.55 (m, 5H, Ar-H), 7.73 (s, 1H, thiophene), 2.81 (s, 3H, CH_3). MS: 257 *m/z* (M^+). Elemental analysis: Calculated for $C_{13}H_{11}N_3OS$: C 60.68, H 4.31, N 16.33, S 12.46. Found: C 58.67, H 4.21, N 15.87, S 12.12.

3-(Benzylidene-amino)-2-methyl-5-phenyl-3*H*-thieno-[2,3-*d*]pyrimidin-4-one (5a): Yield: 71 %, m.p.: 160-162 °C, IR (KBr, ν_{max}, cm^{-1}): 3048 (C-H, Ar), 2966 (C-H, CH_3), 1672 (C=O), 1625 (C=C, Ar), 1584 (C=N), 858 (C-S); ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.80 (s, 1H, thiophene), 7.71-7.42 (m, 10H, Ar-H), 7.52 (s, 1H, N=CH), 3.79 (s, 3H, CH_3). MS: 345 *m/z* (M^+). Elemental analysis: Calculated for $C_{20}H_{15}N_3OS$: C 69.54, H 4.38, N 12.17, S 9.28. Found: C 67.36, H 4.28, N 11.87, S 9.09.

2-Methyl-3-[(2-methyl-benzylidene)amino]-5-phenyl-3*H*-thieno[2,3-*d*]pyrimidin-4-one (5b): Yield: 74 %, m.p.: 144-146 °C, IR (KBr, ν_{max}, cm^{-1}): 3064 (C-H, Ar), 2974 (C-H, CH_3), 1668 (C=O), 1632 (C=C, Ar), 1588 (C=N), 848 (C-S); ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.77 (s, 1H, thiophene), 7.73-7.50 (m, 9H, Ar-H), 7.48 (s, 1H, N=CH), 2.66 (s, 3H, CH_3), 2.45 (s, 3H, CH_3). MS: 359 *m/z* (M^+). Elemental analysis: Calculated for $C_{21}H_{17}N_3OS$: C 70.17, H 4.77, N 11.69, S 8.92. Found: C 68.74, H 4.58, N 11.05, S 8.41.

2-Methyl-3-[(2-methoxy-benzylidene)amino]-5-phenyl-3*H*-thieno[2,3-*d*]pyrimidin-4-one (5c): Yield: 75 %, m.p.: 158-160 °C, IR (KBr, ν_{max}, cm^{-1}): 3060 (C-H, Ar), 2944 (C-H, CH_3), 1665 (C=O), 1640 (C=C, Ar), 1581 (C=N), 1235 (C-O), 855 (C-S); ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.83 (s, 1H, thiophene), 7.70-7.53 (m, 9H, Ar-H), 7.51 (s, 1H, N=CH), 2.62 (s, 3H, OCH_3), 2.54 (s, 3H, CH_3). MS: 375 *m/z* (M^+). Elemental analysis: Calculated for $C_{21}H_{17}N_3O_2S$: C 67.18, H 4.56, N 11.19, S 8.54. Found: C 65.47, H 4.39, N 10.84, S 8.30.

2-Methyl-3-[(2-chloro-benzylidene)amino]-5-phenyl-3*H*-thieno[2,3-*d*]pyrimidin-4-one (5d): Yield: 69 %, m.p.: 120-122 °C, IR (KBr, ν_{max}, cm^{-1}): 3048 (C-H, Ar), 2968 (C-H, CH_3), 1665 (C=O), 1644 (C=C, Ar), 1580 (C=N), 859 (C-S); ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.74 (s, 1H, thiophene), 7.72-7.49 (m, 9H, Ar-H), 7.55 (s, 1H, N=CH), 2.61 (s, 3H, CH_3). MS: 379 *m/z* (M^+). Elemental analysis: Calculated for $C_{20}H_{14}N_3OSCl$: C 63.24, H 3.71, Cl 9.33, N 11.06, S 8.44. Found: C 61.36, H 3.58, Cl 9.06, N 10.75, S 8.19.

2-Methyl-3-[(4-chloro-benzylidene)amino]-5-phenyl-3*H*-thieno[2,3-*d*]pyrimidin-4-one (5e): Yield: 66 %, m.p.: 114-116 °C, IR (KBr, ν_{max}, cm^{-1}): 3058 (C-H, Ar), 2965 (C-H, CH_3), 1658 (C=O), 1648 (C=C, Ar), 1584 (C=N), 865 (C-S); ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.82 (s, 1H, thiophene), 7.79-7.58 (m, 5H, Ar-H), 7.54 (d, 2H, *J* = 7.0 Hz, Ar-H), 7.51 (s, 1H, N=CH), 7.40 (d, 2H, *J* = 7.0 Hz, Ar-H), 2.66 (s, 3H, CH_3). MS: 379 *m/z* (M^+). Elemental analysis: Calculated for $C_{20}H_{14}N_3OSCl$: C 63.24, H 3.71, Cl 9.33, N 11.06, S 8.44. Found: C 61.36, H 3.58, Cl 9.06, N 10.75, S 8.19.

2-Methyl-3-[(2-bromo-benzylidene)amino]-5-phenyl-3*H*-thieno[2,3-*d*]pyrimidin-4-one (5f): Yield: 72 %, m.p.: 150-152 °C, IR (KBr, ν_{max}, cm^{-1}): 3064 (C-H, Ar), 2956 (C-H, CH_3), 1665 (C=O), 1642 (C=C, Ar), 1580 (C=N), 861 (C-S); ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.85 (s, 1H, thiophene), 7.79-7.51 (m, 9H, Ar-H), 7.53 (s, 1H, N=CH), 2.68 (s, 3H, CH_3). MS: 424 *m/z* (M^+). Elemental analysis: Calculated for $C_{20}H_{14}N_3OSBr$: C 56.61, H 3.33, Br 18.83, N 9.90, S 7.56. Found: C 54.69, H 3.26, Br 18.08, N 9.67, S 7.48.

2-Methyl-3-[(4-bromo-benzylidene)amino]-5-phenyl-3*H*-thieno[2,3-*d*]pyrimidin-4-one (5g): Yield: 67 %, m.p.: 132-134 °C, IR (KBr, ν_{max}, cm^{-1}): 3045 (C-H, Ar), 2971 (C-H, CH_3), 1668 (C=O), 1640 (C=C, Ar), 1584 (C=N), 868 (C-S); ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.85 (s, 1H, thiophene), 7.69-7.38 (m, 5H, Ar-H), 7.49 (d, 2H, *J* = 7.3 Hz, Ar-H), 7.42 (s, 1H, N=CH), 7.21 (d, 2H, *J* = 7.3 Hz, Ar-H), 2.47 (s, 3H, CH_3). MS: 424 *m/z* (M^+). Elemental analysis: Calculated for $C_{20}H_{14}N_3OSBr$: C 56.61, H 3.33, Br 18.83, N 9.90, S 7.56. Found: C 54.69, H 3.26, Br 18.08, N 9.67, S 7.48.

2-Methyl-3-[(2-nitro-benzylidene)amino]-5-phenyl-3*H*-thieno[2,3-*d*]pyrimidin-4-one (5h): Yield: 73 %, m.p.:

147-149 °C, IR (KBr, ν_{max}, cm^{-1}): 3066 (C-H, Ar), 2954 (C-H, CH_3), 1663 (C=O), 1649 (C=C, Ar), 1577 (C=N), 1542 (N=O), 855 (C-S); ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.80 (s, 1H, thiophene), 7.71-7.37 (m, 9H, Ar-H), 7.59 (s, 1H, N=CH), 2.62 (s, 3H, CH_3). MS: 390 *m/z* (M^+). Elemental analysis: Calculated for $C_{20}H_{14}N_4O_3S$: C 61.53, H 3.61, N 14.35, S 8.21. Found: C 59.87, H 3.52, N 13.98, S 8.07.

2-Methyl-3-[(4-nitro-benzylidene)amino]-5-phenyl-3*H*-thieno[2,3-*d*]pyrimidin-4-one (5i): Yield: 66 %, m.p.: 131-133 °C, IR (KBr, ν_{max}, cm^{-1}): 3055 (C-H, Ar), 2948 (C-H, CH_3), 1645 (C=O), 1653 (C=C, Ar), 1584 (C=N), 1548 (N=O), 863 (C-S); ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.79 (s, 1H, thiophene), 7.72-7.41 (m, 5H, Ar-H), 7.57 (d, 2H, *J* = 7.6 Hz, Ar-H), 7.37 (s, 1H, N=CH), 7.19 (d, 2H, *J* = 7.6 Hz, Ar-H), 2.54 (s, 3H, CH_3). MS: 390 *m/z* (M^+). Elemental analysis: Calculated for $C_{20}H_{14}N_4O_3S$: C 61.53, H 3.61, N 14.35, S 8.21. Found: C 59.87, H 3.52, N 13.98, S 8.07.

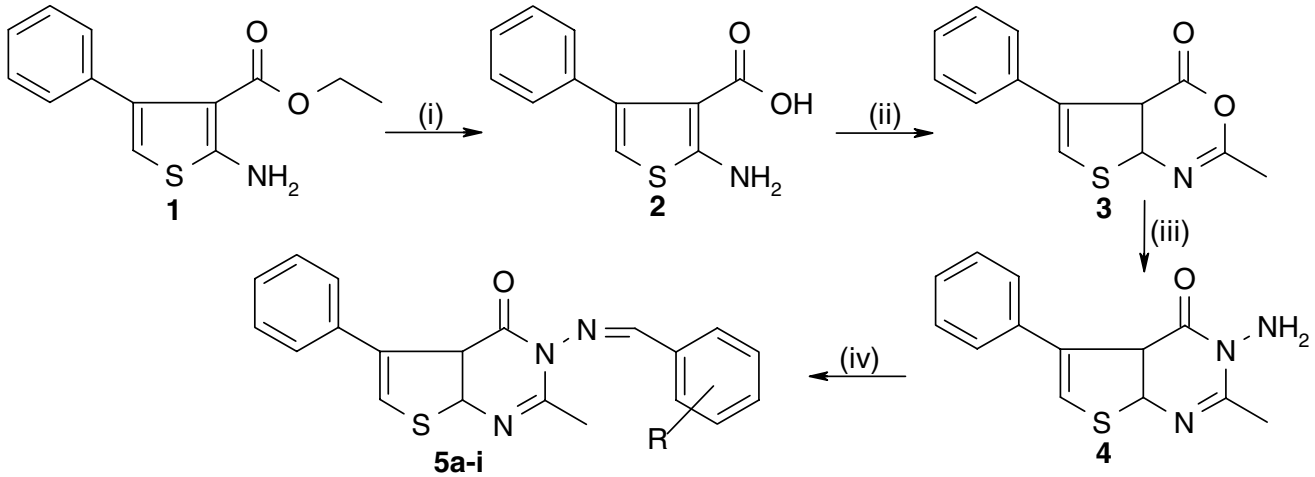
Antifungal activity: Compounds, 3-(benzylidene-amino)-2-methyl-5-phenyl-3*H*-thieno[2,3-*d*]pyrimidin-4-one and its derivatives (**5a-i**) were screened for their antifungal activity against two fungal organisms such as *Aspergillus niger* and *Pencilium chrosogenum* in dimethyl sulfoxide (DMSO) by broth dilution method [10]. Potato dextrose agar (Hi-media) was dissolved and distributed in 25 mL quantities in 100 mL conical flasks and were sterilized in an autoclave at 121 °C (151 lbs/sq.in) for 20 min. The medium was inoculated at 1 % level using 48 h old cultures of the test organism mentioned above aseptically in to sterile petridish and allowed to set at room temperature for about 0.5 h. In size of 4 inches petridish, four cups of 8 mm diameter at equal distance were made in each plate. In each plate, one cup was used for control *i.e.* dimethyl sulfoxide (DMSO), other for standard fluconazole with 100 µg/mL. Other two cups with concentrations of test compound i.*e*. 50 and 100 µL solutions. The plates thus prepared were left for 1.5 h in refrigerator for diffusion. After incubation for 48 h at 25 °C, plates were examined for inhibition zones. The experiments were performed in duplicate and the average diameters of the zones of inhibition measured were recorded.

RESULTS AND DISCUSSION

We have designed, synthesized and characterized a novel series of compounds, 3-(benzylidene-amino)-2-methyl-5-phenyl-3*H*-thieno [2,3-*d*]pyrimidin-4-one and its derivatives (**5a-i**) in good yields. The synthesis of title compounds commenced from commercially available 2-amino-4-phenyl-thiophene-3-carboxylic acid ethyl ester (**1**) and by including 2-amino-4-phenyl-thiophene-3-carboxylic acid (**2**), 2-methyl-5-phenyl-thieno[2,3-*d*][1,3]oxazin-4-one (**3**) and 3-amino-2-methyl-5-phenyl-3*H*-thieno[2,3-*d*]pyrimidin-4-one (**4**) as intermediates (**Scheme-I**).

The initial intermediate **2** has been prepared from raw material **1** on hydrolysis reaction in presence of catalytic amount sodium hydroxide in ethanol solvent under reflux for 4 h on uniform stirring. The next intermediate **3** was afforded from the cyclization followed by condensation between compound **2** with acetic anhydride at reflux temperature on constant stirring for 6 h. Then compound **3** is turned into final intermediate **4** on condensation reaction with hydrazine hydrate in ethanol solvent under reflux for 6 h with constant stirring. Finally, the target compounds, 3-(benzylidene-amino)-2-methyl-5-phenyl-3*H*-thieno [2,3-*d*]pyrimidin-4-one and its derivatives (**5a-i**) were synthesized in good yields from the condensation of compound **4** with different aromatic aldehydes in acetic acid on reflux for 14-18 h on steady stirring. The chemical structures of the newly prepared compounds were confirmed by their IR, ^{1}H NMR, mass spectral data and elemental analysis.

Synthesized compounds **5a-i** performed significant to moderate antifungal activity with a degree of variation at both 0.05 mL (µg) and 0.1 mL (100 µg) concentration level. Compounds **5d** and **5e** exhibited high activity and compounds **5h** and **5i** possessed maximum activity (Fig. 1). The outstanding properties of this new class of antifungal substances deserve further investigation in order to clarify the mode of action at molecular level, responsible for the activity observed. More extensive study is also warranted to determine additional physico-chemical and biological parameters to have a deeper



Scheme-I: (i) NaOH, EtOH, reflux, 4 h; (ii) Ac_2O, reflux, 6 h; (iii) $NH_2NH_2.H_2O$, EtOH, reflux, 6 h; (iv) ArCHO, AcOH, reflux, 14-16 h; **5a-i** a) H; b) 2-CH_3; c) 2-OCH_3; d) 2-Cl; e) 4-Cl; f) 2-Br; g) 4-Br; h) 2-NO_2; i) 4-NO_2

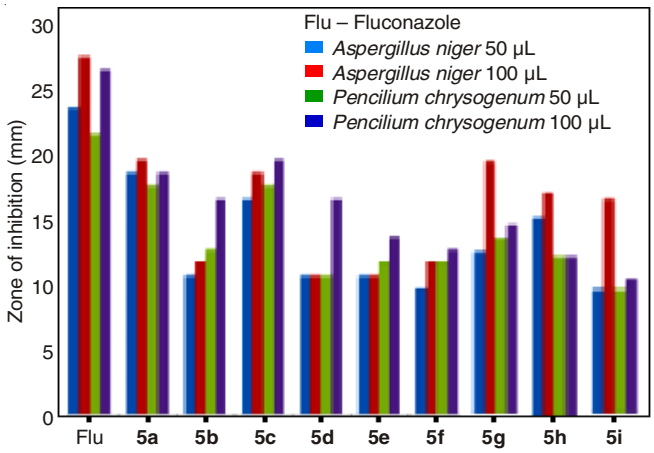


Fig. 1

insight into structure-activity relationship and to optimize the effectiveness of this series of molecules.

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