Heterocyclic Letters
Vol. 7| No.3|733-744|May-July| 2017
ISSN : (print) 2231–3087 / (online) 2230-9632
CODEN: HLEEAI
http://heteroletters.org

SYNTHETIC STRATEGY OF NEW 3-CHLORO BENZO[B]THIOPHENE-2-CARBONYL LINKED PYRIMIDO[4,5-D]PYRIMIDINE SCHIFF, MANNICH BASES AND THEIR BIOLOGICAL EVALUATION

Krishna Kunwar Rathore*, Prakash Prajapat, Ram Chandra Senwar and Anita Mehta

Synthetic Organic Chemistry Laboratory, Department of Chemistry,
Mohanlal Sukhadia University, Udaipur-313001, Rajasthan, India
E-mail: krrishnarathore@yahoo.com

Abstract
The present work involves synthesis of 3-(3-chloro-benzo[b]thiophene-2-carbonyl)-7-[(morpholin-4-ylmethyl)-amino]- 5-phenyl-3*H*-pyrimido[4,5-d]pyrimidine-4-one **(5a-d)**, 3-(3-chloro-benzo[b]thiophene-2-carbonyl)-5-phenyl-7-[(piperidin-1-ylmethyl)-amino]-3*H*-pyrimido[4,5-d]pyrimidine-4-one **(6a-d)** and 7-(benzylidene-amino)-3-(3-chloro-benzo[b]thiophene-2-carbonyl)-5-phenyl-3*H*-pyrimido[4,5-d]pyrimidine-4-one **(8a-d)** derivatives using key intermediate compound 7-amino-5-phenylpyrimido[4,5-*d*]pyrimidin-4(3*H*)-one **(3)**. Newly synthesized compounds were confirmed by elemental analysis, FT-IR, ^{1}H-NMR, ^{13}C-NMR and mass spectral studies. The synthesized compounds were subjected to *in-vitro* antimicrobial screening against pathogenic strains of fungi and bacteria. Some of the derivatives were found to be equipotent or more potent than the standard drugs.

Keywords: antimicrobial, benzo[b]thiophene, pyrimidopyrimidine, mannich, schiff

Introduction

Heterocycles containing sulphur and nitrogen atoms in the core structure have occupied enormous significance in the field of chemistry. Pyrimidine and their derivatives play an essential role in the field of drugs and medicinal chemistry. A number of pyrimidine compounds have been claimed to have exciting bioactivity such as antitumor[i-iii], anti-inflammatory[iv-vi], analgesic[vii-viii], antihistamin[ix], antioxidant[x], and antimicrobial[xi-xiv]. In addition to their diverse biological, pyrimidines are known to play a crucial role in several processes of chemical and pharmacological importance as therapeutics in clinical applications.

Moreover, pyrimidopyrimidines are alternatively very essential pharmacodynamic heterocyclic nuclei that once included in several heterocyclic templates have currently been possessing broad spectrum of activities. Pyrimidopyrimidines, analogs of folic acid and also an important class of annulated uracil and thiouracil, have attracted attention of numerous researchers, because of their diverse range of biological activities[xv-xviii]. A number of Preclinical data from literature survey indicated that these heterocycles have shown good antimicrobial[xix], antitumour[xx-xxi], anti-inflammatory[xxii], antiallergic[xxiii] and antihypertensive[xxiv] activities.

Benzothiophene is the backbone of several important pharmaceuticals estabilished drugs such as Sertaconazole, Raloxifene and Zileuten (Figure 1). Over recent years benzothiophene compounds have acquired prominent significance because of their wide spectrum of biological activities such as antimicrobial, anti-inflammatory, anti-HIV, antiviral, antimalarial and antitumor[xxv-xxviii]. Benzo[b]thiophene and its derivatives are important, because they are broadly used in the preparation of, for example, pesticides[xxix-xxx], medicines[xxxi-xxxv], and semiconductors[xxxvi-xxxvii].

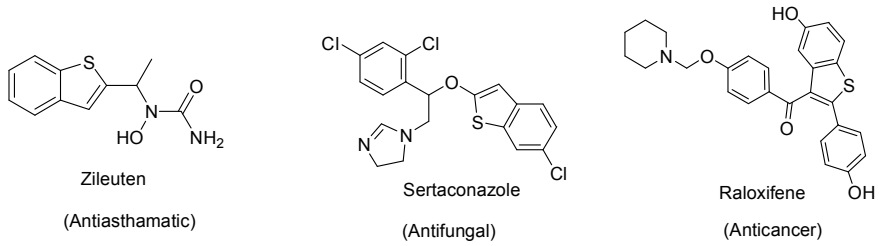


Figure1- Several benzo[b]thiophene containing pharmaceuticals Drugs

There is, however, another approach that has gained much consideration in the field of contemporary medicinal chemistry. It involves the combination of two biologically active pharmacophores into single hybrid unit with a dual mode of action. These novel hybrid molecules have the potential to improve safety, improve efficiency, be cost-effective and reduce the tendency to obtain resistance relative to the parent drugs[XXXVIII-XL]. Based on these prior observations, we postulated that a compound containing pyrimidopyrimidi ne and benzo[b]thiophene pharmacophore could be very effective for antimicrobial activity. Thus, the present paper describes the synthesis of some novel benzo[b]thiophene bearing pyrimidopyrimidine schiff and mannich bases that may be use as potent and effective biologically active agents. One of target compound having benzo[b]thiophene, pyrimidopyrimidine and morpholine in its structure is also compared with different drugs and bioactive agents (Figure 2).

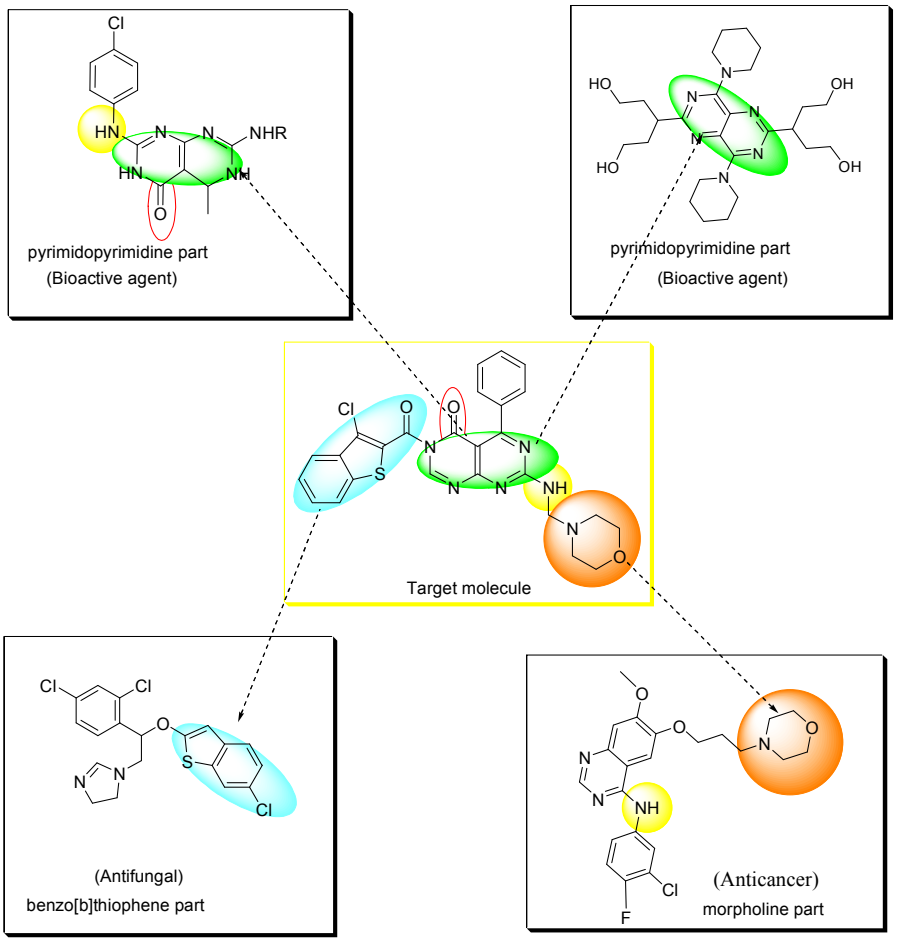


Figure 2. Structural comparison of the target compound with the different drugs and bioactive agents

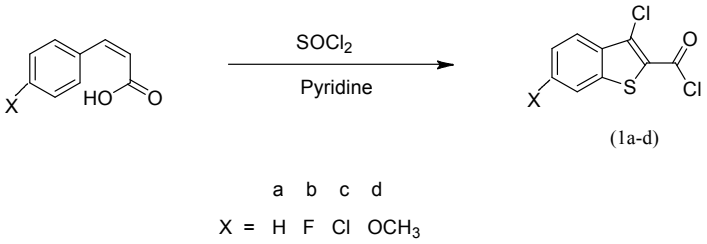
Result and discussion

Analytical results

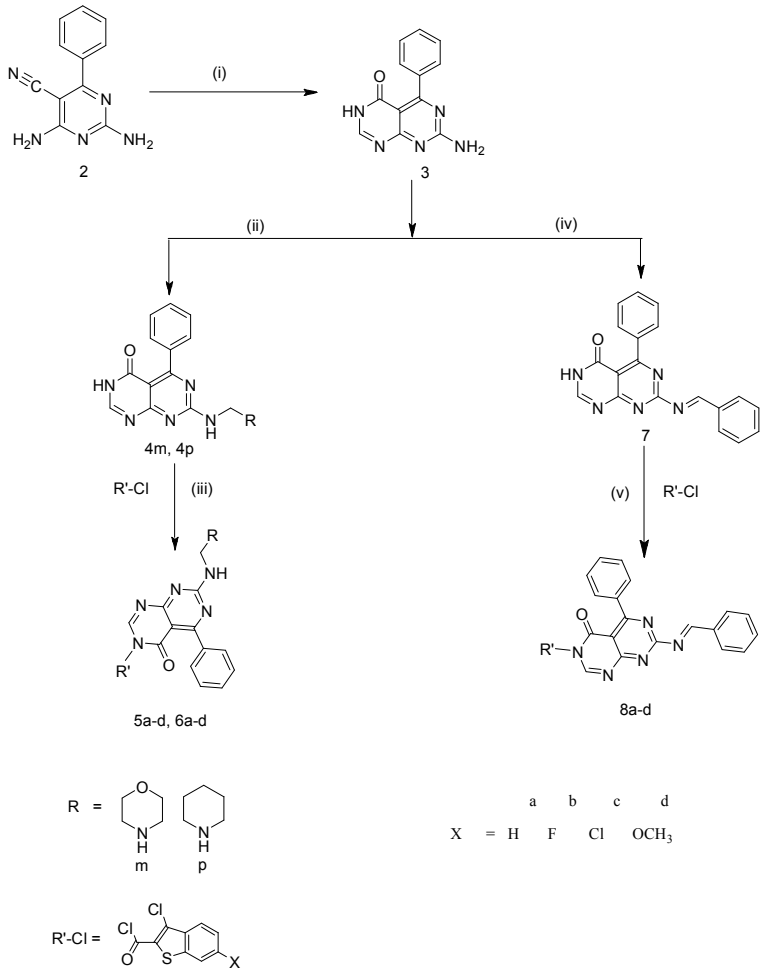
In this present work effort has been made to synthesize pyrimidopyrimidine based benzo[b]thiophene derivatives **5a-d, 6a-d** and **8a-d** using a multistep process (Scheme 2). All the compounds were purified by recrystallization method using appropriate solvent. 3-chloro-benzo[b]thiophene-2-carbonylchloride derivatives (**1a-d)** were prepared by literature method involving the treatment of substituted cinnamic acid with thionyl chloride in presence of pyridine as a catalyst[xli] (Scheme 1). Cyclization of the 2,4-diamino-6-phenylpyrimidine-5-carbonitrile[xlii] **(2)** upon reaction with formic acid in ethanol containing sulphuric acid led to fused 7-amino-5-phenylpyrimido[4,5-*d*]pyrimidin-4(3*H*)-one **(3)** in good yield. The IR spectrum of compound **3** showed the absence of absorption band corresponding to CN group but exhibited new absorption bands in range of 3260 cm^{-1} and 1671 cm^{-1} corresponding to NH and C=O group respectively. While its ^{1}H-NMR spectrum revealed new doublet at 9.10 ppm and at range of 8.44 ppm corresponding to NH proton and =CH respectively. Compound **3** was used as key intermediate for the synthesis of heterocyclic compounds of expected biological activity. In first route, formation of compounds **4m, 4p** is proposed to proceed through mannich reaction of **3** with formaldehyde and secondary amines *viz.* morpholine/pipridine. The assignment of structure of **4m, 4p** was supported by spectral data and elemental analysis. The

IR spectrum displayed new absorption band in the range of 2820-2950 cm^{-1} corresponding to CH_2 group. In addition, ^{1}H-NMR spectrum showed the absence of NH_2 group, whereas presence of the new N-CH_2-N doublet at 3.81-3.83 ppm. Multiplet in range at 2.11, 3.26 ppm of morpholine protons and at 2.21, 1.23 ppm of piperidine protons also confirmed the synthesis of compounds **4m, 4p** respectively. N-H hydrogen of compounds **4m, 4p** was replaced with 3-chloro-benzo[b]thiophene-2-carbonylchloride derivatives in presence of pyridine in catalytic amount to afford **5a-d** and **6a-d**. The IR spectrum revealed the presence of new absorption band at range of 760-786 cm^{-1} and 682-689 cm^{-1} due to chloro group and C-S-C bond respectively. In ^{1}H-NMR spectra doublet of NH proton was found missing.

In another route, reaction of **3** with benzaldehyde afforded aldamine **(7)** in good yield. The ^{1}H-NMR spectra of compound **7**, exhibited the singlet at 8.04 ppm corresponding to =CH group. The IR spectrum also revealed the absence of NH_2 group. Refluxing compound **7** with 6-substituted-3-chloro-benzo[b]thiophene-2-carbonylchloride derivatives in presence of pyridine afforded **8a-d**. The structures were established by elemental and spectral data. IR spectrum showed the new absorption band in range of 763-772 cm^{-1} due to chloro group. IR spectrum also exhibited C-S-C absorption band in the range of 678-690 cm^{-1}. NH proton doublet was disappeared in ^{1}H-NMR spectra in final products. The synthesis of compounds was further interpreted by the appearance of molecular ion peak in mass spectra.



Scheme 1. Synthesis of Benzo[b]thiophene derivatives



Reaction conditions -

(i) = HCOOH/H_2SO_4, 6 h reflux; (ii) = R, HCHO / EtOH, 12-15h reflux, mannich reaction; (iii) = DMF / Pyridine, 8-12 h reflux; (iv) = Benzaldehyde, Glacial Acetic acid, EtOH, 9 h reflux; (v) = EtOH / Pyridine, 7-9 h reflux

Scheme 2- Synthetic route to synthesize of derivatives 5a-d; 6a-d and 8a-d

In-vitro antimicrobial evaluation

The MICs of all the synthesized compounds were carried out by Broth Dilution Method according to National Committee for Clinical Laboratory Standards. Antibacterial activity was tested against Gram negative bacteria *E. coli* (MTCC 442) and two Gram positive bacteria *S. aureus* (MTCC 96), *S. pyogenus* (MTCC 443), with respect to ampicillin and chloramphenicol as a standard drug. For antifungal activity three fungal strains *C. albicans* (MTCC 227), *C. clavatus* (MTCC 1323) and *A. niger* (MTCC 282) were used in which greseofulvin and nystatin were used as a standard drug. DMSO was used as diluents to get desired concentration of drugs to test upon Standard bacterial strains. The observed minimum inhibitory concentrations (MICs μg/ml)) are given in (Table 1).

The results revealed that synthesized compounds **5a-d; 6a-d** and **8a-d** were showing significant activity against all the tested bacterial and fungal strains. Compounds carrying morpholine and benzo[b]thiophene rings have shown more pronounced activity as compared to the other derivatives. All the synthesized compounds were tested for their antibacterial activity. For

gram positive bacteria compounds **5b, 6d** and **8c** had equivalent activity against *S.aureus*, while compounds **5c, 6c** and **8d** showed excellent activity as compared to ampicillin (MIC = 250 µg/ ml). Compounds **5b** and **6c** were found equipotent to ampicillin (MIC = 100 µg/ ml) against *S. pyogenes*. For gram negative *E. coli* bacteria compounds **8b** and **8c** exhibited equally good activity whereas compound **5c** showed the highest activity as compared to standard drug. The investigation of antifungal activity revealed that compounds **5c, 5d and 6d** showed equipotent activity as compared to both griseofulvin (MIC = 100 µg/ ml) and nystatin (MIC = 100 µg/ ml) against *A. clavatus* and *A. niger*. For *C. albicans* compounds **5c, 6b, 6d, 8b and 8d** were Found to be more active against *C. albicans* as compared to grisofulvin (MIC = 500 µg/ ml), whereas compounds **5d, 6a** and **8c** were equally active.

Table 1 *In vitro* antimicrobial activity of 5a-d; 6a-d and 8a-d MICs (µg/ml)

Compound	Antibacterial activity			Antifungal activity		
	Gram +ve		Gram –ve			
	SA MTCC *96*	SP MTCC 443	EC MTCC 442	CA MTCC 227	AN MTCC 282	AC MTCC 1323
5a	500	250	500	1000	200	200
5b	**250**	**100**	1000	>1000	125	500
5c	**100**	125	**62.5**	**200**	**100**	**100**
5d	>1000	125	1000	**500**	**100**	**100**
6a	500	200	500	**500**	500	>1000
6b	500	200	250	**250**	200	500
6c	**125**	**100**	200	1000	125	200
6d	**250**	500	200	**250**	**100**	**100**
8a	1000	500	125	1000	200	>1000
8b	500	125	**100**	**200**	>1000	200
8c	**250**	200	**100**	**500**	250	250
8d	**200**	500	200	**250**	250	200
Ampicillin	250	100	100	-	-	-
Chloramphenicol	50	50	50			
Nystatin				100	100	100
Griseofulvin	-	-	-	500	100	100

**Bold numbers indicate more or equivalent potent compounds compared to standard drugs; SA, S. aureus; SP, S. pyogenes; EC, E. coli; CA, C. albicans; AC, A. clavatus; AN, A. niger; MTCC, microbial type culture collection*

Experimental

All chemicals were commercially procured and were used without further purification. Melting points were determined under open capillaries and were uncorrected. Purity of synthesized derivatives was checked by TLC using silica gel-N plate, ethyl acetate and n-hexane as

developing solvent, and spots were exposed in UV light. Perkin Elmer - Spectrum RX-FTIR Spectrophotometer was used to record IR spectra. ^{1}H and ^{13}C NMR spectra were recorded on Bruker Avance II NMR spectrometer (400 and 100 MHz, respectively) with DMSO-d_6 as solvent using tetramethylsilane (TMS) as an internal standard. The mass spectra were recorded on Waters, Micromass Q-TOF micro separation model. Elemental analysis was done on "Thermo Scientific (FLASH 2000) CHN Elemental Analyser."

General procedure for the synthesis 7-amino-5-phenylpyrimido[4,5-d]pyrimidin-4-one (3)

Compound **2** (0.211 g, 1.00 mmol), in formic acid (in excess) and concentrated sulphuric acid (1.2 ml) were heated under reflux for 6 h. The solution was cooled and poured into ice-water to give precipitate, which was filtered off, dried and recrystallised from ethanol.

Yield 76%; m.p. 256-258^{o}C; IR (KBr pellet) in cm^{-1}: 3420 and 3354 (NH_2), 3245 (NH), 3095 (aromatic =CH), 1670 (C=O), 1610 (cyclic C=N); ^{1}H NMR in δ (ppm): 7.01-7.52 (m, 5H, Ar-H), 8.44 (d, 1H, =CH), 9.10 (d, 1H, N-H), 5.17 (s, 2H, NH_2); ^{13}C NMR in δ (ppm): 108.9, 127.3, 128.2, 129.5, 135.8, 162.8, 165.7, 168.2, 171.4, 183.2; Calculated, %: For $C_{12}H_9N_5O$ in wt % C 60.25; H 3.79; N 29.27 and Found, %: C 60.17; H 3.82; N 29.32; MS, m/z: 239 [M^+].

General procedure for the synthesis of compounds (4m, 4p)

To a solution of compound **3** (0.239 g, 1.00 mmol), in ethanol, morpholine or piperidine (0.87 g or 0.85g, 1.00 mmol), and formaldehyde (1ml) were added. The reaction mixture was refluxed for 12-15 h. The progress of reaction was monitored by TLC. On cooling the obtained solid product was filtered and recrystallized from ethanol.

7-[(morpholin-4-ylmethyl)amino]-5-phenylpyrimido[4,5-d]pyrimidin-4-one (4m)

Yield 84%; m.p. 192-194^{o}C; IR (KBr pellet) in cm^{-1}: 3256 (NH), 3091 (aromatic =CH), 2820, 2950 (CH_2), 1678(C=O), 1622 (cyclic C=N), 1259 (C-O); ^{1}H NMR in δ (ppm): 7.08-7.54 (m, 5H, Ar-H), 8.41 (d, 1H, =CH), 9.16 (d, 1H, N-H), 4.14 (t, 1H, NH), 3.83 (d, 2H, CH_2), 2.11 (m, 4H, CH_2), 3.26 (m, 4H, CH_2); ^{13}C NMR in δ (ppm): 52.8, 72.9, 74.1, 108.6, 127.4, 128.8, 129.2, 135.7, 162.2, 165.3, 168.5, 171.7, 183.6; Calculated, %: For $C_{17}H_{18}N_6O_2$ (MW= 338.36) in wt % C 60.34; H 5.36; N 24.84 and Found, %: C 60.12; H 5.44; N 24.94; MS, m/z: 338 [M^+].

5-phenyl-7-[(piperidin-1-ylmethyl)amino]pyrimido[4,5-d]pyrimidin-4-one (4p)

Yield 80%; m.p. 186-188^{o}C; IR (KBr pellet) in cm^{-1}: 3263 (NH), 3079 (aromatic =CH), 2835, 2942 (CH_2), 1670 (C=O), 1616 (cyclic C=N); ^{1}H NMR in δ (ppm): 7.10-7.49 (m, 5H, Ar-H), 8.38 (d, 1H, =CH), 9.23 (d, 1H, N-H), 4.11 (t, 1H, NH), 3.81 (d, 2H, CH_2), 2.21 (m, 4H, CH_2), 1.23 (m, 6H, CH_2); ^{13}C NMR in δ (ppm) 31.7, 34.3, 42.5, 126.4, 127.1, 128.6, 135.2, 162.8, 165.9, 170.1, 171.5, 182.3; Calculated, %: For $C_{18}H_{20}N_6O$ (MW= 336.39) in wt % C, 64.27; H, 5.99; N, 24.98 and Found, %: C, 64.42; H, 5.80; N, 25.02; MS, m/z: 336 [M^+].

General procedure for the synthesis of compounds (5a-d, 6a-d)

To a mixture of compounds **4m/4p** (1.00 mmol) and 6-substituted-3-chloro-benzo[b]thiophene-2-carbonylchloride (1.00 mmol), in DMF (10 ml), pyridine was added in catalytic amount. The reaction mixture was refluxed for 8-12 h. Completion of the reaction was confirmed by TLC. The reaction mixture was left to cool and poured into crushed ice, filtered and washed with water. The product thus obtained was recrystallized with appropriate solvent.

3-(3-chloro-benzo[b]thiophene-2-carbonyl)-7-[(morpholin-4-ylmethyl)-amino]-5-phenyl-pyrimido[4,5-d]pyrimidine-4-one (5a)

Yield 72%; m.p. 213-215^{o}C; IR (KBr pellet) in cm^{-1}: 3260 (NH), 3088 (aromatic =CH), 2835 (C-H), 1675 (C=O), 1242 (C-O), 1612 (cyclic C=N), 686 (C-S-C), 764 (C-Cl); ^{1}H NMR in δ (ppm): 7.01-7.47 (m, 9H, Ar-H), 8.32 (s, 1H, =CH) 4.35 (t, 1H, NH), 3.51 (d, 2H, CH_2), 2.03 (m, 4H, CH_2), 3.27 (m, 4H, CH_2); ^{13}C NMR in δ (ppm): 52.3, 72.5, 74.7, 108.1, 121.4, 122.8, 123.2, 127.6, 128.2, 129.5, 133.7, 135.3, 142.8, 145.5, 152.3, 162.9, 165.7, 168.1, 169.1, 171.5,

183.4; Calculated, %: For $C_{26}H_{21}ClN_6O_3S$ (MW= 533) in wt % C 58.59; H 3.97; N 15.77 and Found, %: C 58.71; H 4.05; N 15.57; MS: m/z: 533 [M^+].

3-(3-chloro-6-fluoro-1-benzo[b]thiophene-2-carbonyl)-7-[(morpholin-4-ylmethyl)-amino]-5-phenyl-3H-pyrimido[4,5-d]pyrimidine-4-one (5b)

Yield 69%; m.p. >300^{o}C; IR (KBr pellet) in cm^{-1}: 3268 (NH), 3082 (aromatic =CH), 2830 (C-H), 1671(C=0), 1250 (C-O), 1608 (cyclic C=N), 684 (C-S-C), 770 (C-Cl); ^{1}H NMR in δ (ppm): 7.06-7.50 (m, 8H, Ar-H), 8.35 (s, 1H, =CH), 4.29 (t, 1H, NH), 3.51 (d, 2H, CH_2), 2.02 (m, 4H, CH_2), 3.31 (m, 4H, CH_2); ^{13}C NMR in δ (ppm): 51.7, 72.9, 73.5, 108.8, 121.1, 122.8, 123.7, 127.1, 128.4, 129.7, 133.2, 135.8, 143.2, 146.1, 152.5, 161.5, 166.2, 168.7, 169.0, 171.8, 182.6; Calculated, %: For $C_{26}H_{20}ClFN_6O_3S$ (MW= 550.99) in wt % C 56.68; H 3.66; N 15.25 and Found, %: C 56.61; H 3.78; N 15.20; MS: m/z: 551 [M^+].

3-(3,6-dichloro-1-benzo[b]thiophene-2-carbonyl)-7-[(morpholin-4-ylmethyl)-amino]-5-phenyl-3H-pyrimido[4,5-d]pyrimidine-4-one (5c)

Yield 74%; m.p. 224-226^{o}C;IR (KBr pellet) in cm^{-1}: 3262 (NH), 3081 (aromatic =CH), 2838 (C-H), 1668 (C=0), 1242 (C-O), 1610 (cyclic C=N), 682 (C-S-C), 768 (C-Cl); ^{1}H NMR in δ (ppm): 7.10-7.56 (m, 8H, Ar-H), 8.31 (s, 1H, =CH), 4.31 (t, 1H, NH), 3.46 (d, 2H, CH_2), 2.10 (m, 4H, CH_2), 3.28 (m, 4H, CH_2); ^{13}C NMR in δ (ppm): 52.5, 72.1, 74.5, 107.9, 121.8, 122.4, 123.7, 127.5, 128.8, 129.1, 133.3, 135.6, 142.2, 145.7, 152.5, 162.4, 165.9, 168.3, 169.5, 171.4, 183.1; Calculated, %: For $C_{26}H_{20}Cl_2N_6O_3S$ (MW= 567.44) in wt % C 55.03; H 3.55; N 14.81 and Found, %: C 55.17; H 3.48; N 14.74; MS: m/z: 567 [M^+].

3-(3-chloro-6-methoxy-1-benzo[b]thiophene-2-carbonyl)-7-[(morpholin-4-ylmethyl)-amino]-5-phenyl-3H-pyrimido[4,5-d]pyrimidine-4-one (5d)

Yield 78%; m.p. 238-240^{o}C; IR (KBr pellet) in cm^{-1}: 3256 (NH), 3080 (aromatic =CH), 2832 (C-H), 1670 (C=O), 1258 (C-O), 1622 (cyclic C=N), 689 (C-S-C), 765 (C-Cl); ^{1}H NMR in δ (ppm): 7.01-7.55 (m, 8H, Ar-H), 8.36 (s, 1H, =CH), 4.29 (t, 1H, NH), 3.52 (d, 2H, CH_2), 2.07 (m, 4H, CH_2), 3.21 (m, 4H, CH_2); ^{13}C NMR in δ (ppm): 52.3, 72.1, 74.8, 108.5, 121.6, 122.2, 123.8, 127.1, 128.2, 129.9, 133.3, 135.4, 142.3, 145.7, 152.6, 162.4, 165.3, 168.6, 169.6, 171.5, 183.7; Calculated, %: For $C_{27}H_{23}ClN_6O_4S$ (MW= 563.02) in wt % C 57.60; H 4.12; N 14.93 and Found, %: C 57.53; H 4.18; N 14.95; MS: m/z: 563 [M^+].

3-(3-chloro-benzo[b]thiophene-2-carbonyl)-5-phenyl-7-[(piperidin-1-ylmethyl)amino]-3H-pyrimido[4,5-d]pyrimidin-4-one (6a)

Yield 72%; m.p. 198-200^{o}C; IR (KBr pellet) in cm^{-1}: 3252 (NH), 3095 (aromatic =CH), 2847 (C-H), 1670 (C=O), 1614 (cyclic C=N), 678 (C-S-C), 778 (C-Cl); ^{1}H NMR in δ (ppm): 6.81-7.27 (m, 9H, Ar-H), 8.40 (s, 1H, =CH), 4.31 (t, 1H, NH), 3.57 (d, 2H, CH_2), 2.01 (m, 4H, CH_2), 1.22 (m, 6H, CH_2); ^{13}C NMR in δ (ppm): 24.7, 27.2, 51.5, 75.1, 108.8, 121.4, 122.3, 123.9, 127.5, 128.7, 129.1, 133.6, 135.8, 142.5, 146.2, 152.8, 162.4, 165.7, 168.9, 170.2, 171.6, 183.5; Calculated, %: For $C_{27}H_{23}ClN_6O_2S$ (MW= 531.02) in wt % C 61.07; H 4.37; N 15.83 and Found, %: C 61.24; H 4.41; N 15.62; MS: m/z: 531 [M^+].

3-(3-chloro--6-fluoro-1-benzo[b]thiophene-2-carbonyl)-5-phenyl-7-[(piperidin-1-ylmethyl)amino]-3H-pyrimido[4,5-d]pyrimidin-4-one (6b)

Yield 66%; m.p. 202-204^{o}C; IR (KBr pellet) in cm^{-1}: 3270 (NH), 3065 (aromatic =CH), 2830 (C-H), 1680 (C=O), 1620 (cyclic C=N), 689 (C-S-C), 762 (C-Cl); ^{1}H NMR in δ (ppm): 6.88-7.31 (m, 8H, Ar-H), 8.46 (s, 1H, =CH), 4.32 (t, 1H, NH), 3.62 (d, 2H, CH_2), 2.06 (m, 4H, CH_2), 1.21 (m, 6H, CH_2); ^{13}C NMR in δ (ppm): 24.3, 27.6, 51.2, 75.5, 108.1, 121.6, 122.6, 123.5, 127.2, 128.4, 129.8, 133.5, 135.3, 142.7, 146.6, 152.2, 162.8, 165.4, 169.3, 170.5, 171.4, 183.1; Calculated, %: For $C_{27}H_{22}ClFN_6O_3S$ (MW= 549.01) in wt % C 59.07; H 4.04; N 15.31 and Found, %: C 59.15; H 3.91; N 15.36; MS: m/z: 549 [M^+].

3-(3-chloro--6-chloro-1-benzo[b]thiophene-2-carbonyl)-5-phenyl-7-[(piperidin-1-ylmethyl)amino]-3H-pyrimido[4,5-d]pyrimidin-4-one (6c). Yield 82%; m.p. 220-222^{o}C; IR

(KBr pellet) in cm^{-1}: 3258 (NH), 3068 (aromatic =CH), 2848 (C-H), 1670 (C=O), 1612 (cyclic C=N), 670 (C-S-C), 770 (C-Cl); ^{1}H NMR in δ (ppm): 7.0-7.41 (m, 8H, Ar-H), 8.51 (s, 1H, =CH), 4.34 (t, 1H, NH), 3.58 (d, 2H, CH_2), 2.11 (m, 4H, CH_2), 1.23 (m, 6H, CH_2); ^{13}C NMR in δ (ppm): 24.5, 27.3, 51.5, 75.4, 108.6, 121.3, 122.8, 123.4, 127.3, 128.7, 129.5, 133.1, 135.5, 142.2, 146.4, 152.9, 162.1, 165.6, 169.8, 170.1, 171.8, 183.5; Calculated, %: For $C_{27}H_{22}Cl_2N_6O_2S$ (MW= 565.47) in wt % C 57.35; H 3.92; N 14.86 and Found, %: C 57.18; H 4.03; N 14.92; MS: m/z: 565[M^+].

3-(3-chloro--6-methoxy-1-benzo[b]thiophene-2-carbonyl)-5-phenyl-7-[(piperidin-1-ylmethyl)amino]-3H-pyrimido[4,5-d]pyrimidin-4-one (6d)

Yield 70%; m.p. 210-212^{o}C; IR (KBr pellet) in cm^{-1}: 3261 (NH), 3085 (aromatic =CH), 2838 (C-H), 1671 (C=O), 1605 (cyclic C=N), 682 (C-S-C), 786 (C-Cl); ^{1}H NMR in δ (ppm): 7.0-7.41 (m, 8H, Ar-H), 8.47 (s, 1H, =CH,) 4.44 (t, 1H, NH), 3.56 (d, 2H, CH_2), 2.05 (m, 4H, CH_2), 1.28 (m, 6H, CH_2); ^{13}C NMR in δ (ppm): 24.1, 27.6, 51.8, 75.2, 108.4, 121.8, 122.4, 123.7, 127.6, 128.2, 129.9, 133.4, 135.6, 142.6, 146.8, 152.3, 162.5, 165.1, 169.5, 170.7, 171.2, 183.3; Calculated, %: For $C_{28}H_{25}ClN_6O_3S$ (MW= 561.05) in wt % C 59.94; H 4.49; N 14.98 and Found, %: C 60.08; H 4.40; N 14.93; MS: m/z: 561 [M^+].

7-[benzylideneamino]-5-phenylpyrimido[4,5-d]pyrimidin-4(3H)-one (7)

A mixture of compound **3** (0.239 g, 1.00 mmol) and benzaldehyde (0.106g, 1.00 mmol) in ethanol in catalytic amount of glacial acetic acid was heated under reflux for 9 h and left to get cool. The reaction mixture was poured into crushed ice and product was filtered, washed with water and recrystallized with ethanol. Yield 88%; m.p. 180-182^{o}C; IR (KBr pellet) in cm^{-1}: 3087 (aromatic =CH), 3275 (NH), 1669 (C=O), 1616 (cyclic C=N); ^{1}H NMR in δ (ppm): 7.01-7.73 (m, 10H, Ar-H), 8.37 (d, 1H, =CH pyrimidine ring), 8.04 (s, 1H, =CH benzylidenimin), 9.01 (d, 1H, NH); ^{13}C NMR in δ (ppm): 116.8, 126.4, 127.2, 128.7, 132.4, 135.2, 163.5, 165.1, 170.5, 172.1, 181.3, 185.7; Calculated, %: For $C_{19}H_{13}N_5O$ (MW= 327.33) in wt % C 69.71; H 4.00; N 21.39 and Found, %: C 69.65; H 4.12; N 21.34; MS: m/z: 327 [M^+].

General procedure for the synthesis of compounds (8a-d)

In a solution of compound **7** (0.327g, 1.00 mmol) in ethanol 6-substituted-3-chloro-benzo[b]thiophene-2-carbonylchloride (1.00 mmol) was added. Pyridine was added as catalyst. After 7-9 h reflux the reaction mixture was poured into crushed ice with stirring. The solid product was filtered, dried and recrystallized from appropriate solvents.

7-(benzylidene-amino)-3-(3-chloro-benzo[b]thiophene-2-carbonyl)-5-phenyl-3H-pyrimido[4,5-d]pyrimidine-4-one (8a)

Yield 73%; m.p. 190-192^{o}C; IR (KBr pellet) in cm^{-1}: 3081 (aromatic =CH), 1672 (C=O), 690 (C-S-C), 763 (C-Cl); ^{1}H NMR in δ (ppm): 6.90-7.41 (m, 14H, Ar-H), 8.53 (s, 1H, =CH pyrimidine ring), 7.91 (s, 1H, =CH benzylidenimin); ^{13}C NMR in δ (ppm): 116.3, 121.8, 122.7, 123.5, 126.3, 127.3, 128.5, 132.4, 133.4, 135.8, 142.7, 145.3, 152.1, 163.2, 164.7, 169.3, 170.6, 171.6, 181.1, 183.9; Calculated, %: For $C_{28}H_{16}ClN_5O_2S$ (MW= 521.97) in wt % C 64.43; H 3.09; N 13.42 and Found, %: C 64.51; H 2.97; N 13.46; MS: m/z: 522 [M^+].

7-(benzylidene-amino)-3-(3-chloro-6-fluoro--benzo[b]thiophene-2-carbonyl)-5-phenyl-3H-pyrimido[4,5-d]pyrimidine-4-one (8b)

Yield 77%; m.p. 198-200^{o}C; IR (KBr pellet) in cm^{-1}: 3089 (aromatic =CH), 1668 (C=O), 686 (C-S-C), 772 (C-Cl); ^{1}H NMR in δ (ppm): 6.85-7.45 (13H, m, Ar-H), 8.55 (1H, s, =CH pyrimidine ring), 7.85 (1H, s, =CH benzylidenimin); ^{13}C NMR in δ (ppm): 116.9, 121.2, 122.1, 122.9, 126.7, 127.5, 128.1, 132.6, 133.7, 136.9, 142.1, 144.8, 152.6, 163.7, 165.1, 169.8, 170.2, 171.9, 181.4, 183.1; Calculated, %: For $C_{28}H_{15}ClFN_5O_2S$ (MW= 539.96) in wt % C 62.28; H 2.80; N 12.97 and Found, %: C 62.41; H 2.73; N 12.91; MS: m/z: 540 [M^+].

7-(benzylidene-amino)-3-(3,6-dichloro-benzo[b]thiophene-2-carbonyl)-5-phenyl-3H-pyrimido[4,5-d]pyrimidine-4-one (8c)

Yield 71%; m.p. 176-178^{o}C; IR (KBr pellet) in cm^{-1}: 3092 (aromatic =CH), 1670 (C=O), 694 (C-S-C), 768 (C-Cl); ^{1}H NMR in δ (ppm): 6.91-7.41 (13H, m, Ar-H), 8.48 (1H, s, =CH pyrimidine ring), 7.93 (1H, s, =CH benzylidenimin); ^{13}C NMR in δ (ppm): 117.4, 121.6, 122.2, 123.5, 127.7, 128.3, 129.4, 131.8, 133.7, 136.2, 142.3, 145.9, 152.8, 162.8, 164.1, 170.4, 171.1, 172.8, 180.8, 183.4; Calculated, %: For $C_{28}H_{15}Cl_2N_5O_2S$ (MW= 556.42) in wt % C 60.44; H 2.72; N 12.59 and Found, %: C 60.38; H 2.86; N 12.51; MS: m/z: 556[M^{+}].

7-(benzylidene-amino)-3-(3-chloro-6-methoxy-benzo[b]thiophene-2-carbonyl)-5-phenyl-3H-pyrimido[4,5-d]pyrimidine-4-one (8d)

Yield 68%; m.p. 189-191^{o}C; IR (KBr pellet) in cm^{-1}: 3085 (aromatic =CH), 1668 (C=O), 678 (C-S-C), 765 (C-Cl); ^{1}H NMR in δ (ppm): 6.89-7.41 (13H, m, Ar-H), 3.35 (3H, s, O-CH$_3$), 8.54 (1H, s, =CH pyrimidine ring), 7.91 (1H, s, =CH benzylidenimin); ^{13}C NMR in δ (ppm): 116.7, 121.3, 122.5, 123.6, 126.1, 128.7, 129.1, 132.6, 133.8, 135.4, 143.1, 145.7, 153, 163.5, 165.1, 169.8, 171.4, 172.7, 180.2, 185.5; Calculated, %: For $C_{29}H_{19}Cl_2N_5O_3S$ (MW= 552.00) in wt % C 63.10; H 3.29; N 12.69 and Found, %: C 63.23; H 3.35; N 12.50; MS: m/z: 552 [M^{+}].

Conclusion

In summary, our efforts are focused to synthesize benzo[b]thiophene clubbed pyrimidopyrimidine containing schiff and mannich bases. All the synthesized compounds were examined for biological activity with the aim of discovering innovative structure leads serving as potent antimicrobial agents. Some magnificent results have been obtained with the fused pyrimidopyrimidine scaffold. On biological screening, compounds **5c**, **6b, 6c, 6d** and **8d** showed an excillent antimicrobial activity than standard drug, whereas rest of the compounds showed moderate activity. Compound **5c** bearing morpholine and Cl substituted benzo[b]thiophene exhibited good activity against all the strains. It was concluded that the combination of two biologically active molecules into single hybrid unit, have the potential to obtain good pharmacological activity and pyrimidopyrimidine compounds bearing a benzo[b]thiophene nucleus have become an imperative area of antimicrobial drug research.

Acknowledgments

The authors are thankful to the Head, Department of Chemistry, M. L. Sukhadia University, Udaipur (Rajasthan) for providing laboratory facilities, the Director, SAIF and CIL, Chandigarh, India for providing spectral and analytical data, Microcare Laboratiry, Surat, India for providing antimicrobial results. One of the authors Ms Krishna Kunwar Rathore (NET-SRF) is thankful to UGC, New Delhi, for financial assistance.

References and notes

i. Al-Issa, S. A. *Saudi Pharma. J.* **2013**, 21, 305–316.
ii. Gangjee, A.; Vidwans, A.; Elzein, E.; McGuire, J. J.; Queener, S. F.; Kisliuk, R. L. *J. Med. Chem.* **2001**, 44, 1993-2003.
iii. Baraldi, P. G.; Pavani, M. G.; Nunez, M. *Bioorg. Med. Chem.* **2002**, 10, 449-456.
iv. Bhalgat, C. M.; Ali, M. I.; Ramesh, B.; Ramu G. *Arabian J. Chem.* **2014**, 7, 986–993.
v. Prajapat, P.; Talesara, G. L. *J. Heterocyclic Chem*. **2015**, DOI 10.1002/jhet.2471.
vi. Mohamed, M. S.; Awad, S. M.; Sayed, A. I. *Molecules.* **2010**, 15, 1882-1890.
vii. Hafez, H. N.; Abbas, H. A.; El-Gazzar, A. R. *Acta Pharm.* **2008**, 58, 359–78.

viii. Amin, K.M.; Hanna, M.M.; Abo-Youssef, H.E.; George, R.F. *Eur.J. Med. Chem.* **2009**, 44, 4572–4584.

ix. Rahaman, S. K. A.; RajendraPasad, Y.; Kumar, P.; Kumar, B. *Saudi Pharm. J.* **2009**, 17, 255–8.

x. Saundane, R. A.; Yarlakatti M.; Rayappa, K. *Med. Chem. Res.* **2012**, 21, 3809–3817.

xi. Patel, A. A.; Mehta, A. G. *J. Saudi Chem. Soc.* **2010**, 14, 203–8.

xii. Fathy, A. E.; Ashraf, H. F. A.; Gameel, A. M. E.; Moustafa, M. K. *Acta Pharm.* **2004**, 54, 13–26.

xiii. Chen, P. J.; Yang, A.; Gu, Y. F.; Zhang, X. S.; Shao, K. P.; Xue, D. Q.; He, P.; Jiang, T. F.; Zhang, Q. R.; Liu, H. M. *Bioorg. Med. Chem. Lett.* **2014**, 24(12), 2741-2743.

xiv. Salem, M. A.; Marzouk, M. I.; Mahmoud, N. F. *J. Serbian. Chem. Soc.* **2014**, 79(9), 1059-1073.

xv. Xiang, J.; Li, H.; Yang, K.; Yi, L.; Xu, Y.; Dang, Q.; Bai, X. *Mol. Divers.* **2012**, 16, 173–181.

xvi. Ahmed, A. F.; Ehab A. E. L.; Bondock, S.; Ahmed, S. *Synth. Commun.* **2008**, 38, 24, 4352-4368.

xvii. Mohamed, M. A. A.; Mahmoud, N. F. H.; Mohamed El-Saghie, A. M. *Chem. J.* **2012**, 02, 64-68.

xviii. José M. de la, T.; Manuel, N.; Eduardo, J. B.; Fernando, D.; Suvire, R. D.; Enriz, J. C. *ARKIVOC*. **2014**, (v), 42-63.

xix. Sharma, P.; Rane, N.; Gurram, V. K. *Bioorg. Med. Chem. Lett.* **2004**, 14, 4185–4190.

xx. Sanghvi, Y. S.; Larson, S. B.; Matsumoto, S. S.; Nord, L. D.; Smee, D. F.; Willis, R. C.; Avery, T. H.; Robins, R. K.; Revankar, G. R. *J. Med. Chem.* **1989**, 32, 629–637.

xxi. Solca, F. F.; Baum, A.; Kopt, E. L.; Dahmann, G.; Heider, K. Z.; Himmelsbach, F.; Jacques, C. A. *J. Pharmacol. Exp. Ther.* **2004**, 311, 502.

xxii. Karoui, A.; Allouche, F.; Deghrigue, M.; Agrebi, A.; Bouraoui, A.; Chabchoub, F. *Med. Chem. Res.* **2014**, 23, 1591–1598.

xxiii. Kitamura, N.; Kitamura, N.; Onishi, A. *Chem. Abstr.* **1984**, 104, 186439.

xxiv. Raddatz, P.; Raddatz, P.; Bergmann, R. *Chem. Abstr.* **1988**, 109, 54786.

xxv. De la Cruz, J. P.; Carrasco, T.; Ortega, G.; Sanchez de la Cuesta, F. *Lipids*. **1992**, 27, 192.

xxvi. Starčevi, K.; Kralj, M.; Piantanid, I.; Šuman, L.; Paveli, K.; Karminski-Zamol, G. *Eur. J. Med. Chem.* **2006**, 41, 925–939.

xxvii. Carballo, M.; Conde, M.; Tejedo, J.; Gualberto, A.; Jimenez, J.; Monteseirín, J.; Santa María, C.; Bedoya, F.J.; Hunt, S.W., III; Pintado, E. *Mol. Genet. Metab.* **2002**, 75, 360–368.

xxviii. Gadada, N.; Patchanita, T.; Runchana, K.; Waraporn, A.; Arithat, L.; Amorn, P. *J. Sulfur. Chem.* **2011**, 32, 235–247.

xxix. Gouda, T.; Shivaraj, J.; Salahuddin, M. d.; Shantakumar, S. M. *Asian. J. Chem.* **2009**, 21(7), 5171-5178.

xxx. Dadiboyena, S. *Eur. J. Med. Chem.* **2012**, 51, 17–34.

xxxi. Shinde, P. S.; Shinde, S. S.; Renge, A. S.; Patil, G. H.; Rode, A. B.; Pawar, R. R. *Lett. Org. Chem.* **2009**, 6(1), 8–10.

xxxii. Efange, S. M. N.; Mash, D. C.; Khare, A. B.; Ouyang, Q. J. *J. Med. Chem.* **1998**, 41(23), 4486–4491.

xxxiii. Burkamp, F.; Fletcher, S. R. *J. Hetero. Chem.* **2002**, 39(6), 1177–1187.

xxxiv. Galiano, S.; Erviti, O.; Perez, S.; Moreno, A.; Juanenea, L.; Aldana, I.; Monge, A. *Bioorg. Med. Chem. Lett.* **2004**, 14(3), 597–599.

xxxv. Mcdonnell, D. P.; Clemm, D. L.; Hermann, T.; Goldman, M. E.; Pike, J. W. *Mol. Endocrinol.* **1995**, 9(6), 659–669.

xxxvi. Moorthy, B. K. *J. Indian. Chem. Soc.* **1990**, 67(11), 909–911.
xxxvii. Wex, B.; Kaafarani, B. R.; Oliver, A. G.; Bauer, J. A. K.; Neckers, D. C. *J. Org. Chem.* **2003**, 68(21), 8258–8260.
xxxviii. Payne, M. M.; Odom, S. A.; Parkin, S. R.; Anthony, J. E. *Org. Lett.* **2004**, 6(19), 3325–3328.
xxxix. Walsh, J. J.; Bell, A. *Curr. Pharm. Des.* **2009**, 15, 2970.
xl. Meunier, B. *Polypharmacology in Drug Discovery*. **2012**, 423.
xli. Connor, D. T., Cetenko, W. A. *J. Med. Chem.* **1992**, 33, 958-65.
xlii. Rathore, K. K.; Senwar, R. C.; Mehta, A. *J. Applicable. Chem*. **2015**, 4, 1836-1843.

Received on April 10, 2017.