Research Article

Synthesis and Antimicrobial Activity of Benzothiophene Substituted Coumarins, Pyrimidines and Pyrazole as New Scaffold

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Accepted on: 01-04-2014; **Finalized on:** 30-09-2014.

ABSTRACT

In the present research manuscript, we described a series of new compounds containing coumarins, pyrimidines and pyrazole substituted benzothiophene derivatives 3a-f, 4, 5, and 7 have been synthesized from 2,4-diflurobenzonitrile and methyl thioglycolate as starting material. The structures of newly synthesized compounds have been characterized by IR, ^{1}H-NMR, MS spectral and elemental analysis. Some of the synthesized compounds have been found to exhibit better antibacterial and antifungal activities.

Keywords: Anti-microbial, Antiprotozoal, Benzothienopyrimidines, Coumarins, Pyrazole.

INTRODUCTION

Coumarin and its derivatives form an important class of benzopyrones found in nature. They are structural subunits in many complex natural products and have shown numerous biological activities such as antiviral[1], anticoagulant[2], antibacterial[3], antifungal[4], anti-HIV[5] and antihistamine[6]. Besides the wide biological applications of coumarin and its derivatives, the chemical literature also embodies their some applications from the material viewpoint such as cosmetics, optical brightening agents and dispersed fluorescent[7]. In view of the diverse and remarkable biological properties of coumarins, we thought of condensing with benzothiophene derivatives.

Nitrogen and sulfur containing heterocycles play an important role in diverse biological activities. Benzothienopyrimidines are structural analogs of biogenic purines and can be considered as potential nucleic acid antimetabolites[8,9]. Thienopyrimidines occupy a special position among fused pyrimidine compounds because of their great practical usefulness and many benzothienopyrimidines have been evaluated pharmacologically for their anticancer[10] anti-inflammatory[11], antihyperlipidemic[12] and antimicrobial[13] etc. In view of these observations, it was also thought to be of interest to synthesize benzothiophene fused with pyimidines by cyclising benzothiophene carbohydrazide.

Pyrazoles are five member ring heterocyclic compounds, have some structural features with two nitrogen atoms in adjacent position. These derivatives are the subject of many research studies due to their widespread potential biological activities such as anti-inflammatory[14], antiprotozoal[15], antibacterial[16], antihistaminic[17] and antidepressant[18].

Benzothiophene has increasingly been recognized as a pharmacophore that offers advantages including superior chemical and pharmacological stability, low intrinsic toxicity[19,20] and most importantly a rich chemistry that enables medicinal chemists to explore molecular diversity in a rapid fashion. In this context and as part of our continuing research program on the synthesis of sulfur containing heterocyclic compounds with potential pharmacological activities, we have designed the synthesis of some new benzothiophene substituted coumarins, pyrimidines, pyrazoles moieties.

MATERIALS AND METHODS

Melting points were determined in an open capillary and were uncorrected. IR spectra were recorded on Bruker alpha FT IR spectrophotometer, ^{1}H NMR spectra were measured on Bruker AV 400MHZ using $CDCl_3$ and DMSO as solvent. Chemical shifts were expressed in δ ppm. Mass spectra were performed on a Joel JMS-D 300 mass spectrometer operating at 70 eV. All the reactions were followed and checked by TLC, and further purification was done by column chromatography. All the reagents used were of AR grade and they were again purified by distillation.

Preparation of methyl-3-amino-6-fluoro-benzothiophene-2-carboxylate (1)

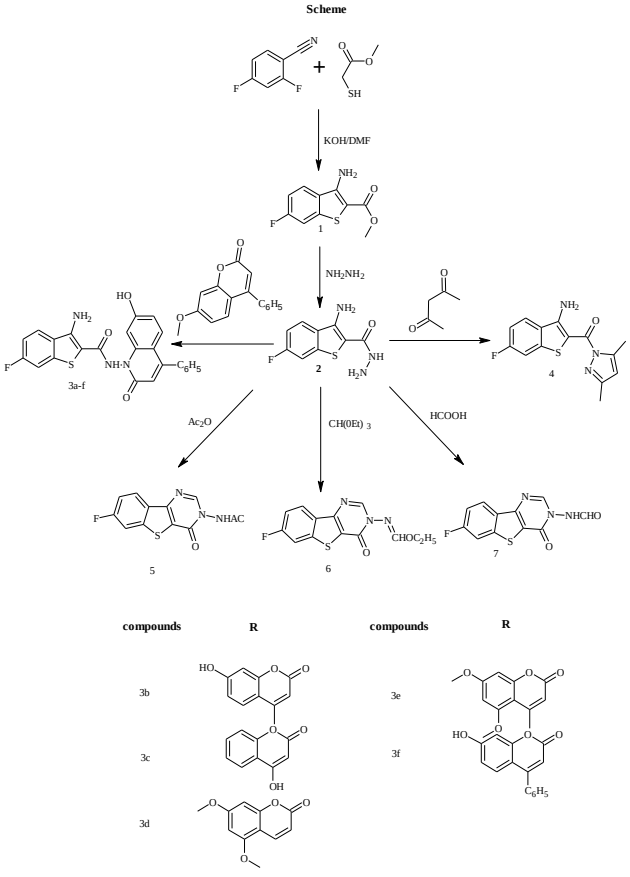
2, 4-Difluorobenzonitrile (4.17g, 0.03 mol) was added to stirred solution of methylthioglycolate (2.7ml, 0.03 mol) and potassium hydroxide (4.12g, 0.075 mol) in DMF refluxed at 75°C for 10 hrs. Completion of the reaction was monitored by TLC. After completion, the reaction mixture was cooled to room temperature and poured in to crushed ice. The pale yellow solid were separated out and filtered it. Washed with water, dried and purified through column chromatography by using ethyl acetate and n-hexane. Yield 84 %, mp 148-152 °C. solid (crystalline); IR (KBr) (ν_{max} cm^{-1}): 3430 (NH_2), 1651 (C=O). ^{1}H-NMR; DMSO-d6; 400 MHz; δ (ppm): 8.07-8.04 (1H, m), 7.77-7.74 (1H, m), 7.32-7.25 (1H, m), 6.78 (2H, bs), 3.92

(3H, s). MS: m/z 225. Anal.calcd for $C_{10}H_8FNO_2S$: C, 53.32. H, 3.58. N, 6.22. S, 14.23 Found: C, 53.28. H, 3.55. N, 6.19. S, 14.15 %. MS: m/z 225.

Preparation of 3-amino-6-fluoro-benzothiophene-2-carbohydrazide (2)

To a stirred solution of methyl-3-amino-6-fluoro-benzothiophene-2-carboxylate **1** (2.25g, 0.01 mol) in absolute alcohol (50ml) was added hydrazine hydrate (0.3ml, 0.01 mol) at room temperature. Then the reaction mixture was refluxed for 6 hrs. The completion of the reaction was monitored by TLC. After completion, the reaction mixture was cooled and poured into crushed ice. The solid separated was filtered, washed with water, dried and recrystalised by ethyl acetate. Yield 78 %, mp 180-184 °C. Solid (crystalline); IR (KBr) (ν_{max} cm^{-1}): 3422 (NH_2), 3396 (CONH), 1663 (C=O). ^{1}H-NMR; DMSO-d6; 400 MHz; δ (ppm): 8.17-8.11 (2H, m), 7.95-7.91 (1H, m), 7.52-7.45 (1H, m), 7.15 (2H, bs), 5.20 (2H, bs). MS: m/z 225. Anal.calcd for $C_9H_8FN_3OS$: C, 48.04. H, 3.57. N, 18.65. S, 14.23 Found: C, 47.49. H, 3.52. N, 18.61. S, 14.15 %. MS: m/z 225.

Scheme



Preparation of 3-amino-6-fluoro-N-(7-methoxy-2-oxo-4-phenylquinolin-1(2H)-yl)benzo[b]thiophene-2 carboxamide (3a)

An equimolar mixture of methyl-3-amino-6-fluoro-1-benzothiophene-2-carbohydrazide **2** (0.225g 0.001 mol) and 7-methoxy-4-phenylcoumarin (0.252g, 0.001 mol) in glacial acetic acid (20ml) refluxed for 12 hrs. Completion of the reaction was monitored by TLC. After the completion, the reaction mixture was poured in to crushed ice. Solid that separated out was filtered, dried and recrystalized from aqueous ethanol. Similarly, **3b-f** were prepared.

66 % Yield, mp 281-283 °C. Solid (crystalline); IR (KBr) (ν_{max} cm^{-1}): 3451 (NH_2), 1669 (C=O). ^{1}H-NMR: 400 MHz: DMSO-d6: δ (ppm): 11.23 (1H, bs), 8.27-8.23 (4H, m), 8.13-8.10 (4H. m), 7.90-7.98 7.50-7.39 (3H, m), 5.95 (1H, m), 4.75 (2H, bs), 3.85 (3H, s). Anal.calcd for $C_{25}H_{18}FN_3O_3S$: C, 65.41. H, 3.95. N, 9.51. S, 6.98 Found: C, 65.38. H, 3.85. N, 9.49. S, 6.95 %. MS: m/z 459.

3-Amino-6-fluoro N-(7-hydroxy-3-methyl-2-oxoquinolin-1(2H)-yl)benzo[b]thiophene-2-carboxamide (3b)

Yield 75 %, mp 262-264 °C. Solid (crystaline); IR (KBr) (ν_{max} cm^{-1}): 3445 (NH_2), 1662 (C=O). ^{1}H-NMR: 400 MHz: DMSO-d6: δ (ppm): 10.83 (1H, bs), 8.27-8.23 (2H, m), 7.80-7.75 (2H, m), 7.40-7.35 (2H, m), 6.70-6.68 (1H, m), 4.82 (2H, bs),2.33 (3H, s). Anal.calcd for $C_{19}H_{14}FN_3O_3S$: C, 59.58. H, 3.68. N, 10.97. S, 8.37. Found: C, 59.52. H, 3.52. N, 10.91. S, 8.28 %. MS: m/z 383.

3-Amino-6-fluoro N-(4-hydroxy-2-oxoquinolin-1(2H)-yl)benzo[b]thiophene-2-carboxamide (3c)

Yield 67 %, mp 235-237 °C. Solid (crystaline); IR (KBr) (ν_{max} cm^{-1}): 3440 (NH_2), 1660 (C=O). ^{1}H-NMR: 400 MHz: DMSO-d6: δ (ppm): 10.93 (1H, bs), 8.30-8.27 (2H, m), 7.85-7.82 (2H, m), 7.40-7.35 (2H, m), 6.81-6.78 (2H, m). 4.89 (2H, bs), Anal.calcd for $C_{20}H_{16}FN_3O_4S$: C, 58.16. H, 3.90. N, 10.17. S, 7.76. Found C, 58.10. H, 3.87. N, 10.11. S, 7.61 %. MS: m/z 413.

3-Amino-*N*-(5, 7-dimethoxy-2-oxoquinolin-1(2*H*)-yl)-6-fluoro-1-benzothiophene-2-carboxamide (3d)

Yield 84 %, mp 254-256 °C. Solid (crystaline); IR (KBr) (ν_{max} cm^{-1}): 3460 (NH_2), 1675 (C=O). ^{1}H-NMR: 400 MHz: DMSO-d6: δ (ppm): 10.80 (1H, bs), 8.25-8.22 (2H, m), 7.75-7.72 (2H, m), 7.35-7.30 (2H, m), 6.75-6.70 (2H, m), 4.75 (2H, bs), 3.85 (6H, s). Anal.calcd for $C_{18}H_{12}FN_3O_3S$: C, 58.59. H, 3.27. N, 11.38. S, 8.69. Found: C, 58.56. H, 3.23. N, 11.32. S, 8.65 %. MS: m/z 369.

3-Amino-6-fluoro N-(5-methoxy-4-methyl-2-oxoquinolin-1(2H)-yl)benzo[b]thiophene-2-carboxamide (3e)

Yield 63 %, mp 270-272°C. Solid (crystalline); IR (KBr) (ν_{max} cm^{-1}): 3465 (NH_2), 1680 (C=O). ^{1}H-NMR: 400 MHz: DMSO-d6: δ (ppm): 10.80 (1H, bs), 8.25-8.22 (2H, m), 7.75-7.72 (2H, m), 7.35-7.32 (1H, m), 6.75-6.70 (2H, m), 4.69 (2H, bs), 3.85 (6H, s), 2.52 (3H, s). Anal.calcd for $C_{20}H_{16}FN_3O_3S$: C, 60.50. H, 4.02. N, 10.58. S, 8.07. Found C, 60.35. H, 3.98. N, 10.55. S, 8.01 %. MS: m/z 397.

Preparation of 3-amino-6-fluoro-N-(7-hydroxy-2-oxo-4-phenylquinolin-1(2H)-yl)benzo[b]thiophene-2-carboxamide (3f)

Yield 70 %, mp 311-313 °C. Solid (crystalline); IR (KBr) (ν_{max} cm^{-1}): 3472 (NH_2), 1688 (C=O). ^{1}H-NMR: 400 MHz: DMSO-d6: δ (ppm): 11.23 (1H, bs), 8.27-8.23 (4H, m), 8.13-8.10 (4H. m), 7.90-7.98 (1H, m), 7.50-7.39 (4H, m),

4.94 (2H, bs). Anal.calcd for $C_{24}H_{16}FN_3O_3S$: C, 64.77. H, 3.62. N, 9.44. S, 7.20. Found C, 64.71. H, 3.58. N, 9.39. S, 7.15 %. MS: m/z 445.

Preparation of (3-amino-6-fluoro-1-benzothiophen-2-yl)(3, 5-dimethyl-1*H*-pyrazol-1-yl) methanone (4)

A sample of compound **2** (0.225 g 0.001 mol) and acetyl acetone (1ml, 0.01 mol) was heated under reflux for 1hour,.then ethanol (10ml) was added .and the mixture was refluxed for an additional 5 hours. After completion of the reaction, the reaction mixture was allowed to cool. The solid thus separated was collected and recrystalized from ethanol. Yield 73 %, mp 235-237 °C. Solid (amorphous); IR (KBr) (ν_{max} cm^{-1}): 3435 (NH_2), 1671 (C=O). ^{1}H-NMR: 400 MHz: DMSO-d6: δ (ppm): 8.30-8.27 (2H, m), 7.75-7.73 (1H, m), 6.75-6.70 (1H, m), 5.10 (2H, bs), 2.43(3H, s), 2.38 (3H, s). Anal.calcd for $C_{14}H_{12}FN_3OS$: C, 58.18. H, 4.18. N, 14.53. S, 11.09. Found C, 58.11. H, 4.15. N, 14.48. S, 10.95 %. MS: m/z 289.

Preparation of 6-fluoro *N*-(7-fluoro-4-oxo[1]benzothieno[3, 2-*d*]pyrimidin-3(4*H*)-yl)acetamide (5)

Acetic anhydride (10ml) was added to compound **2** (0.225g, 0.001 mol) and the reaction mixture was refluxed for 10 hrs on water bath. After completion of the reaction, it was cooled, poured in to crushed ice. The solid thus separated was filtered, and washed with water, dried and recrystallized from ethanol. It was purified by column chromatography. Yield 78 %, mp 210-212 °C. Solid (crystalline); IR (KBr) (ν_{max} cm^{-1}): 3030-3080 (C-H of Ar) 1645 (C=N), 1734 (C=O). ^{1}H-NMR: 400 MHz: DMSO-d6:δ (ppm): 8.47 (1H, s), 8.32-8.30 (1H, m), 7.60-7.58 (1H, m), 7.41-7.39 (1H, m), 2.13 (3H, s). Anal.calcd for $C_{10}H_4AcFN_3OS$: C, 26.11. H, 0.87. N, 9.13. S, 6.97. Found C, 26.7. H, 0.84. N, 9.10. S, 6.91 %. MS: m/z 460.

Preparation of ethyl (7-fluoro-4-oxo[1]benzothieno[3, 2-*d*]pyrimidin-3(4*H*)-yl)imidoformate (6)

A Sample of compound **2** (0.225g, 0.001 mol) in triethylorthoformate (10ml) was heated under reflux for 8 hours. Progress of the reaction was monitored by TLC. After completion, reaction mixture was allowed to cool and poured into cold water. The solid product was collected and recrystallized ethanol Yield 72 %, mp 234-236 °C. Solid (crystalline); IR (KBr) (ν_{max} cm^{-1}): 3020-3070 (C-H of Ar) 1630 (C=N), 1720 (C=O). ^{1}H-NMR: 400 MHz: DMSO-d6: δ (ppm): 8.71 (1H, s), 8.47 (1H, s), 8.27-8.26 (1H, m), 7.60-7.58 (1H, m), 7.41-7.39 (1H, m), 4.47-4.42 (2H, q, J=7.2 Hz), 1.48-1.44 (3H, t, J=7.2 Hz). Anal.calcd for $C_{13}H_{10}FN_3O_2S$: C, 53.65. H, 3.46. N, 14.43. S, 11.01. Found: C, 53.62. H, 3.43. N, 14.38. S, 10.85 %. MS: m/z 291.

Preparation of *N*-(7-fluoro-4-oxo [1]benzothieno[3, 2-*d*]pyrimidin-3(4*H*)-yl)formamide (7)

Formic acid (10ml) was added to compound 2 (0.225g, 0.001 mol) then the reaction mixture was refluxed for 6 hrs on water bath. After completion, the reaction mixture was cooled and poured in to crushed ice. The solid thus separated was filtered, washed with water, dried and recrystallized from ethanol. Again, compound was purified by column chromatography. Yield

69 %, mp 260-262 °C. Solid (crystalline); IR (KBr) (ν_{max} cm^{-1}): 3370 (NH), 3040-3090 (C-H of Ar) 1710, (C=O). ^{1}H-NMR: 400 MHz: DMSO-d6: δ (ppm): 10.42 (1H, s), 8.61 (1H, s), 8.51 (1H, s), 8.34-8.27 (1H, m), 8.16-8.14 (1H, m), 2.98 (1H, m). Anal.calcd for $C_{11}H_6FN_3O_2S$: C, 50.23. H, 2.29. N, 15.97. S, 12.19. Found C, 50.20. H, 2.25. N, 15.83. S, 12.16 %. MS: m/z 263.

RESULTS AND DISCUSSION

2,4-Difluorobenzonitrile on reacting with methyl thioglycolate in the presence of potassium hydroxide in DMF produced methyl-3-amino-6-fluoro-benzothiophene-2-corboxylate **1** in good yield which on treated with hydrazine hydrate to gave 3-amino-6-fluorbenzothiophene-2-carbohydrazide **2**. The compound 2 on reacting with substituted coumarins to gave different 3-amino-6-fluoro-N-substituted phenylquinolin-1(2H)-yl) benzothiophene-2-carboxamide **3a-f**, In confirmation, The IR spectrum of all these compounds exhibited a stretching frequency at 3440-3465 cm^{-1} for NH_2 group and sharp band at 1660-1680 cm^{-1} for C=O. further mass of these compounds confirms the formation of compound 3a-f. ^{1}H-NMR spectrum of compound **3a** showed a peak at δ 4.75 for two protons of NH_2 and 5.95 for one proton of OH group and remaining 12 protons in aromatic region. The compound **2** on refluxing with acetyl acetone to gave (3-amino-6-fluoro-1-benzothiophen-2-yl)(3, 5-dimethyl-1*H*-pyrazol-1-yl)methanone **4**. The compound 4 showed a stretching absorption band at 3410 cm-1 for NH_2 group and 1635 cm-1 for C=N group and 1667 for C=O Further, a molecular ion peak at m/z 289 in its mass spectrum was in agreement with the structure. ^{1}H-NMR spectrum of compound **4** showed NH_2 peak at δ 5.10 for two protons and δ 2.43 and 2.38 for six protons of two CH_3 groups, δ 6.20 for one hydrogen atom of pyrazole substituted benzothiophene and remaining 3 protons in aromatic region. In addition, we investigated reaction of the intermediate compound **2** with acetic anhydride, formic acid and triethylorthoformate, which undergo cyclization to give benzothienopyrimidines **5-7**. Formation of compound **5-7** was confirmed on the basis of its correct elemental analysis and spectral data. The IR spectrum 5 and 7 compounds exhibited a stretching at 3363 and 3378 cm^{-1} due to NH group. 1734 and 1710 for C=O respectively the compound 6 exhibited a peak 1630 for C=N and 1720 (C=O). ^{1}H-NMR spectrum of compound **7** showed NH peak at δ 2.98 for one proton, δ 10.42 one proton for aldehydes group and remaining 4 protons in aromatic region. Further, a molecular ion peak at m/z 263 in its mass spectrum was in agreement with the compound 7. The investigation of antibacterial and antifungal activities reveals that some of the synthesized compounds 3b, 3d, 3f and 6 have been found to exhibited moderate to good antimicrobial activity.

Antibacterial Activity

Some selected compounds were screened for their antibacterial activity against *Staphylococcus aureus, Escherichia coli, Salmonella paratyphi-A* and *Bacillus subtilis* and the activity was carried out using the cup-plate agar diffusion method[21]. The zone of inhibition was measured in millimeters. DMF was used as a vehicle. Chloramphenicol used as standard drugs for antibacterial activity. The compounds were tested at 100µg/mL concentration. The tested compounds were found to show moderate activity against all bacteria. The zones of inhibition are presented in Table 1.

Table 1: Antibacterial activity of the compounds

Compound	Diameter of zone of inhibition (in mm)			
	Staphylococcus aureus 100µg/ml	*Escherichia coli* 100µg/ml	*Salmonella paratyphi A* 100µg/ml	*Bacillus subtilis* 100µg/ml
1	14	12	11	12
2	13	11	13	15
3a	17	19	16	18
3b	23	13	22	25
3c	18	10	13	16
3d	24	15	20	23
3e	15	09	14	17
3f	14	15	15	19
4	17	12	12	18
5	16	17	17	13
6	25	16	25	24
7	16	13	14	19
DMF	00	00	00	00
Chloramphenicol	20	14	18	22

Table 2: Antifungal activity data of compounds

Compound	Diameter of zone of inhibition (in mm)			
	Aspergillus niger 100µg/ml	*Pencillium notatum* 100µg/ml	*Aspergillus fumigates* 100µg/ml	*Candiada albicans* 100µg/ml
1	15	14	13	13
2	17	16	15	16
3a	20	21	19	14
3b	26	28	28	20
3c	18	20	23	15
3d	19	17	20	16
3e	14	28	18	14
3f	27	26	29	26
4	15	18	19	13
5	23	21	16	18
6	28	19	27	23
7	21	17	19	17
DMF	00	00	00	00
Fluconazole	25	25	25	19

Antifungal Activity

Some selected compounds were screened for their antifungal activity against four species of fungi *Aspergillus niger, Aspergillus fumigates, Candida albicans* and *Pencillium notatums*. The activity was carried out using the cup-plate agar diffusion method[22]. The zone of inhibition was measured in millimeters. DMF was used as a vehicle. Fluconazole was used as standard drug for antifungal activity. The compounds were tested at 100µg/mL concentration. The tested compounds were found to show moderate activity against all fungi. The zones of inhibition are presented in Table 2.

CONCLUSION

In the present investigation, we have synthesized some novel benzo[*b*]thiophene derivatives carrying coumarins, pyrimidines, pyrazoles moieties compounds with better yields. And some of the compounds were displayed potential antibacterial and antifungal activities.

Acknowledgements: Authors are very thankful to the Principal, Sahyadri Science College, Shimoga, for providing laboratory facilities. Authors are also thankful to the Principal, Pharmacy College, Shimoga, for providing laboratory facilities to conduct biological activities. H.K. Nagesh is grateful to the Kuvempu University for financial support in the form of fellowship.

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Source of Support: Nil, **Conflict of Interest:** None.