SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL ACTIVITIES OF SOME BENZOFUROPYRIMIDINE DERIVATIVES

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Received: 15 Mar 2013, Revised and Accepted: 30 Apr 2013

ABSTRACT

A series of new 8-bromo-3-[(phenylmethylidene)amino][1]benzofuro[3,2-][d]pyrimidin-4(3H)-one derivatives 4a-r have been synthesized by using ethyl 3-amino-5-bromo-1-benzofuran-2-carboxylate 1 as a starting material. The amino ester 1 was converted into its hydrazide 2 by reacting with hydrazine hydrate, which upon treatment with various aldehydes and ketones yielded corresponding Schiff bases 3a-r. The desired compounds 4a-r were obtained by reacting Schiff bases 3a-r with triethyl orthoformate. The structures of all the synthesized compounds were established by analytical data. Purity of the compounds exhibited considerable antimicrobial activities and compounds 4o and 4r were found to be excellent in scavenging DPPH radical at 50 μg and also retained almost same activity in 100 μg.

Keywords: Benzofuran, Fused pyrimidine, Antimicrobial, Antioxidant activity.

INTRODUCTION

The synthesis of benzofuran derivatives has received considerable attention due to their occurrence in large number of natural products [1] and synthetic pharmaceuticals [2]. Various benzofurans also find application as fluorescent sensors [3], oxidants [4], antioxidants, a variety of drugs and in other field of chemistry and agriculture [5]. The compounds encompassing pyrimidine moiety have been shown to be useful as bactericidal, antimalarial [6], analgesics [7], anti-hypertensive [8] and anti-tumor agents [9]. Pyrimidines and their ring-fused derivatives have a broad spectrum of biological activity; best known as the heterocyclic core of the nucleic acids. Fused heterocyclic structures containing pyrimidine and furan ring systems are well known to possess diverse biological activities such as antimicrobial [10, 11], anti-inflammatory [12], antiviral [13], anti-HIV [14] and antitumor [15]. Moreover the derivatives of benzofuro[3,2-d]pyrimidines are of great importance because they are associated with analgesic, anti-inflammatory and antimicrobial activities [16, 17]. Some of these derivatives are also reported to exhibited good antiocidal and blood sugar-lowering activities [18, 19]. Considering the importance of benzofuro[3,2-d]pyrimidines, some of the synthetic methodologies for these fused heterocycles have been reported [20, 21].

Inspired by these reports, it was planned to synthesize a new series of benzofuro[3,2-d]pyrimidines, i.e., various 8-bromo-3-[(1-phenylethylidene)amino][1]benzofuro[3,2-d]pyrimidin-4(3H)-ones (4a-r) and evaluate their antibacterial, antifungal and in-vitro antioxidant activities. The structures of the synthesized compounds were confirmed on the basis of IR, 1H NMR, mass spectral data and supported by analytical data.

MATERIALS AND METHODS

Melting points were determined in an open capillary melting point apparatus and are uncorrected. Purity of the compounds was checked by TLC on silica gel. The IR spectra were recorded on a Nicolet Impact 410 FT-IR Spectrophotometer, using KBr pellets. 1H NMR spectra were recorded on a Bruker-400 MHz spectrometer in CDCl3 or DMSO using TMS as an internal standard. High-resolution mass spectra (HRMS-ESI) were obtained on a Micromass Q-TOF Mass Spectrometer. All reagents were AR grade or chemically pure.

Synthesis of 3-amino-5-bromo-1-benzofuran-2-carboxhydrazide (2)

A mixture of ethyl 3-amino-5-bromo-benzofuran-2-carboxylate (1) (2.9 g, 0.01 mole) and hydrazine hydrate (4 ml, 99%) was heated under reflux in ethanol (5 ml) on a steam bath for 3 hours and the reaction was monitored by TLC. The reaction product was cooled thoroughly and colorless solid that separated and was collected by filtration. It was recrystallized from ethanol as colorless thin needles. Yield 74%; mp. 238-240°C.


The mixture of compound 2 (0.27 g, 1 mmol) and benzaldehyde (0.10 ml, 1 mmol) in DMF (4 ml) was refluxed at 110-120°C for about 3 hours. Progress of the reaction was monitored by TLC. After the completion of reaction, the reaction mixture was cooled to room temperature and added to ice cold water with constant stirring. The solid obtained was filtered, dried and recrystallized from ethanol.

The similar procedure was followed for the preparation of compounds 3b-r using substituted aldehydes and acetophenones.

3-Amino-5-bromo-N-{[phenylmethylidene]amino][1]benzofuro[2-carboxhydrazide (3a): IR νmax (KBr) cm^-1: 3422, 3298, 3065, 2975, 2919, 1668, 1840, 1691. 1H NMR (CDCl3) δ ppm (TMS): 5.1 (s, 1H), 7.2-7.7 (m, 8H); MS: m/z = 359 (M+H)+; 361 (M+H+2)+.

8-Bromo-3-[(1-phenylethylidene)amino][1]benzofuro[3,2-d]pyrimidin-4(3H)-one (4a): IR νmax (KBr) cm^-1: 3440, 3359, 1952, 1635, 1568. 1H NMR (CDCl3) δ ppm (TMS): 7.2 (s, 3H), 5.0 (s, NH), 11 (s, NH), 7.4-8.2 (m, 8H); MS: m/z = 373 (M+H)+, 375 (M+H+2)+.

General procedure for the synthesis of 8-bromo-3-[(1-phenylethylidene)amino][1]benzofuro[3,2-d]pyrimidin-4(3H)-ones (4a-k) and 8-bromo-3-[(1-phenylethylidene)amino][1]benzofuro[3,2-d]pyrimidin-4(3H)-ones (4l-r)

The compound 3a (0.5 g, 1.4 mmol) was refluxed with triethyl orthoformate (5 ml) for about 5 hours. Excess of triethyl orthoformate was removed by distillation under reduced pressure. The solid obtained was dried and recrystallized from ethanol.

The similar procedure was followed for the synthesis of compounds 3b-r.

8-Bromo-3-[(phenylmethylidene)amino][1]benzofuro[3,2-d]pyrimidin-4(3H)-one (4a):

IR νmax (KBr) cm^-1: 3058, 1697, 1611, 1566, 1525. 1H NMR (CDCl3) δ ppm (TMS): 7.2 (s, 1H), 8.4 (s, 1H), 7.4-8.2 (m, 8H); MS: m/z = 369 (M+H)+, 371 (M+H+2)+.
8-Bromo-3-[[2-chlorophenyl]methylidene]amino][1 benzofuro [3,2-d]pyrimidin-4(3H)-one (4f): IR *v*<sub>KBr</sub> (cm<sup>-1</sup>) v cm<sup>-1</sup>: 3059, 2961, 2910, 1669, 1657, 1529. 1H NMR (CDCl<sub>3</sub>) δ ppm (TMS): 9.2 (s, 1H), 8.7 (s, 1H), 7.5-8.2 (m, 7H). MS: m/z = 403 (M+H)<sup>+</sup>, 405 (M+H+2)<sup>+</sup>.

8-Bromo-3-[[2-nitrophenyl]methylidene]amino][1 benzofuro [3,2-d]pyrimidin-4(3H)-one (4d): IR *v*<sub>KBr</sub> (cm<sup>-1</sup>) v cm<sup>-1</sup>: 3085, 2963, 1698, 1613, 1571, 1528. 1H NMR (CDCl<sub>3</sub>) δ ppm (TMS): 9.7 (s, 1H), 8.9 (s, 1H), 7.5-8.3 (m, 7H). MS: m/z = 414 (M+H)<sup>+</sup>, 416 (M+H+2)<sup>+</sup>.

8-Bromo-3-[[4-methoxyphenyl]methylidene]amino][1 benzofuro [3,2-d]pyrimidin-4(3H)-one (4e): IR *v*<sub>KBr</sub> (cm<sup>-1</sup>) v cm<sup>-1</sup>: 3402, 3054, 2929, 2929, 1883, 1700, 1594, 1563, 1529. 1H NMR (CDCl<sub>3</sub>) δ ppm (TMS): 11.5 (s, -OH), 8.6 (s, 1H), 8.2 (d, 1H), 6.9-7.9 (m, 6H). MS: m/z = 399 (M+H)<sup>+</sup>, 401 (M+H+2)<sup>+</sup>.

Biological activity

Antimicrobial activity

Cultures of two gram-positive bacteria: *Bacillus subtilis* (AL009126) and *Staphylococcus aureus* (BX571856) and two gram-negative bacteria: *Escherichia coli* (AE014075) and *Pseudomonas aeruginosa* (AE004091) were used to investigate the antibacterial activity and *Aspergillus fumigatus* (CM000170) and *Candida albicans* (EAL02784) for the antifungal activity of the compounds (4a-r). The antimicrobial activity was assayed biologically using agar well diffusion method. In this method, wells of standard diameter were made in the nutrient agar medium and potato dextrose agar for the antibacterial and antifungal activity respectively containing standard microbial inoculums. The test compounds were introduced into the wells and diameter of the zone of inhibition was measured by antibiotic zone reader. The test was carried out in triplicates and average value of these as a zone of inhibition. The final values of the compounds were tested at a concentration of 50 μg/mL and 100 μg/mL in dimethyl sulfoxide as control against all the organisms. Ampicillin/Ciprofloxacin and Fluconazole were used as standards for comparison of antibacterial and antifungal activities, respectively at the same concentration as samples taken. The zone of inhibition was compared with the standard drugs after 24 h of incubation at 37 °C for antibacterial activity and 72 h at 25 °C for antifungal activity.

Evaluation of Anti-oxidant activity-DPPH radical scavenging method

The free radical scavenging capacities of the compounds 4a-r were determined by using DPPH (1, 1-diphenyl-2-picrylhydrazyl radical). The DPPH radical scavenging assay is the simplest method to measure the ability of antioxidants to intercept free radicals. Antioxidants react with DPPH, which is a stable free radical, and then scavenge this radical by converting it to 1, 1-diphenyl-2-picrylhydrazine due to their H-donating ability. The degree of DPPH free radical scavenging potential of the compounds 4a-r was determined. Freshly prepared DPPH (3 mL of 0.1% m/mole) was mixed with serial dilutions (50 μg and 100 μg) of compounds to a final volume of 4 mL and after 30 min of incubation in dark at room temperature, the absorbance was read at 517 nm using a spectrophotometer (Shimadzu UV-visible spectrophotometer, Japan). The DPPH control (containing no sample) was prepared using the same procedure. The inhibition concentration calculated using values obtained [22].

The capability to scavenge the DPPH- radical was calculated using the following equation:

DPPH Scavenging effect (%) = ([(A<sub>control</sub> - A<sub>sample</sub>) / A<sub>control</sub>]<sup>100</sup>

Where A<sub>control</sub> = The initial concentration of the stable DPPH radical without the test compound.

A<sub>sample</sub> = Absorbance of the remaining concentration of DPPH- in the presence of compound (4a-r).

Ascorbic acid, BHT (Butylated hydroxytoluene) and BHA (Butylated hydroxyanisole) have been used as standards.
RESULTS AND DISCUSSION

In the present work, the synthesis of fused bromo substituted benzofuro[3,2-d]pyrimidines associated with amine group was accomplished by series of the reactions as shown in the Scheme. In order to achieve synthesis of the desired compounds, ethyl 3-amino-5-bromo benzofuran-2-carboxylate (1) was used as starting material which was synthesized by a reported method [23]. It was converted into 3-amino-5-bromo-1-benzofuran-2-carbohydrazide (2) by refluxing with excess of hydrazine hydrate in ethanol. The condensation of hydrazide 2 with different aldehydes and acetophenones in DMF furnished corresponding Schiff bases 3a-r. The series of 3a-r compounds were treated with triethyloxthomiformate to yield 8-bromo-3-[[phenylethylidene] amino][1]benzofuro[3,2-d] pyrimidin-4(3H)-ones (4a-k) and 8-bromo-3-[[phenylethylidene] amino][1] benzofuro[3,2-d] pyrimidin-4(3H)-ones (4l-r). All the compounds synthesized were obtained in good yield, their corresponding melting points and analytical data shown in Table 1.

Whereas Ar,

\[ 4a = \text{C}_6\text{H}_5 \]
\[ 4b = \text{2-Cl C}_6\text{H}_4 \]
\[ 4c = \text{4-Cl C}_6\text{H}_4 \]
\[ 4d = \text{3-NO}_2 \text{C}_6\text{H}_4 \]
\[ 4e = \text{4-OCH}_3 \text{C}_6\text{H}_4 \]
\[ 4f = \text{4-0H} \text{C}_6\text{H}_4 \]

The characterization of the synthesized compounds were carried out by FT-IR, 1H NMR and mass spectral studies. The IR spectrum of hydrazide 2 exhibited absorption bands at 3402 cm\(^{-1}\), 3307 cm\(^{-1}\), 3201 cm\(^{-1}\) and 1856 cm\(^{-1}\) due to –NH, –NH\(_2\), =C-H (aromatic) and –C=O groups respectively. The 1H NMR spectrum of compound 2 showed three D2O exchangeable peaks as three singlets. One singlet appeared at δ 4.3 due to two protons of –NH-NH\(_2\) group, second singlet at δ 5.9 due to two protons at –NH\(_2\) group at C-3 and third singlet at δ 9.2 due to one proton at –NH–NH\(_2\) group respectively. Three aromatic protons appeared as one singlet at δ 8.1 and two doublets at δ 7.5 and δ 7.3. The mass spectrum of compound 2 exhibited molecular ion peak at m/z 270 (M+H\(^+\)) and at m/z 272 (M+H\(^+\)+2)\(^+\) of equal intensity as expected for the compounds containing bromine atom.

The conversion of hydrazide 2 into Schiff base 3a was evidenced by 1H NMR and mass spectral data. The 1H NMR spectrum exhibited a D2O exchangeable singlet at δ 5.1 assignable to protons of –NH\(_2\) group at C-3 and another D2O exchangeable singlet at δ 9.2 due to –NH proton. Disappearance of singlet at δ 4.3 due to –NH\(_2\) protons of hydrazide group and appearance of peak integrating for one proton at δ 9.2 due to –CH=N– group along with the multiplet integrating for eight protons at δ 7.2-7.7 confirmed the formation of Schiff base. Its mass spectrum containing two peaks at m/z 359 (M+H\(^+\)) and m/z 361 (M+H\(^+\)+2) supported the assigned structure.

The formation of 8-bromo-3-[[phenylethylidene] amino][1] benzofuro[3,2-d] pyrimidin-4(3H)-one 4a was established by conspicuous absence of singlets at δ 5.1 and δ 9.2 in 1H NMR spectrum indicating the involvement of –NH\(_2\) and –NH groups in cyclization. A proton of –CH of pyrimidine resulted in a peak as singlet at δ 9.5 whereas –CH=N– proton gave a singlet at δ 8.4 and aromatic protons appeared as multiplet between δ 7.4 and δ 8.4. As expected two molecular ion peaks at m/z 369 (M+H\(^+\)) and m/z 371 (M+H\(^+\)+2) were observed in its mass spectrum. Similarly, structures of all other compounds were confirmed and are given in experimental section.
The scavenging potentials of the antioxidant compound.

In order to evaluate the antioxidant activity and free radical scavenging potentials of compounds 4a-r were tested at 50 μg and 100 μg by the DPPH radical scavenging method. Antioxidant reacts with DPPH, which is a stable free radical, and converts it to 1,1-diphenyl-2-picrylhydrazine. The degree of discoloration indicates the scavenging potentials of the antioxidant compound. The experiment was done in triplicate and average value has taken as % of scavenging. On comparison of screened samples with standards ascorbic acid (A A), BHT and BHA, compounds 4a and 4r were shown to be excellent in scavenging DPPH radical at 50 μg and also retained almost same activity in 100 μg. But compound 4m showed appreciable scavenging activity compared to all derivatives. The results are results were graphically presented in Figure 1.
CONCLUSION

In conclusion, the synthesis of fused benzofuroypyrimidines was achieved by an efficient and simple approach. The results of present investigation clearly indicate that the antibacterial and antifungal activity vary with the species of organisms used and molecular structure. In most of the tested samples, they showed comparable fungicidal activity against Candida albicans than Aspergillus fumigatus and in case antibacterial activity most of the molecules screened were showed appreciable inhibition at 100 μg compared with lower concentrations. The compounds 4o and 4r showed promising free radical scavenging activity as compared with standards used.

ACKNOWLEDGEMENT

The authors wish to acknowledge the Chairman, Department of Chemistry and Department of Biochemistry, Kuvempu University, Shankaraghatta, Shimoga for providing the laboratory facility.

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