J. Pharm. Res. 2021;20(2):11–15

Print ISSN: 0973-7200
Online ISSN: 2454-8405

Journal of Pharmaceutical Research

RESEARCH ARTICLE

Synthesis, Antimicrobial and Anti-inflammatory Activity of Some Novel Benzofuran Derivatives

B Lomatividya[1], Mayukh Baidya[1,*]

[1] *Department of Pharmaceutical Chemistry, Krupanidhi College of Pharmacy, Bangalore, Karnataka, India*

ARTICLE INFO

Article history:
Received 15.12.2020
Accepted 20.04.2021
Published 18.05.2021

* *Corresponding author.*
Mayukh Baidya

https://doi.org/10.18579/jopcr/v20.2.lomatividya

ABSTRACT

Benzofuran derivatives possess a wide range of biological activities, including antimicrobial and anti-inflammatory activities. The purpose of this study was to synthesise benzofuran derivatives and to investigate their pharmacological potential. A multistep synthesis was used to synthesize ethyl and free acid derivatives of 2-(benzofuran-2-carboxamido) acetic acid from coumarin. The compounds were identified by melting point analysis, thin-layer chromatography, infrared spectroscopy, and nuclear magnetic resonance. Antimicrobial activity against different Gram-positive and Gram-negative bacteria and fungi was screened using cup-plate and tube dilution techniques. Anti-inflammatory activity was screened in a carrageenan-induced paw oedema rat model. Six benzofuran amide derivatives (6a–6f) were prepared in 60–80% yield. Compounds 6a, 6b, and 6f had strong broad-spectrum antimicrobial activity, with MICs as low as 6.25 μg/ml. Compound 6b showed the greatest inhibition of paw oedema (71.10% at 2 h), followed by 6a (61.55%), which was statistically significant ($P < 0.05$, $P < 0.01$). The zone of inhibition was validated using MIC data, indicating antimicrobial efficacy. This study presents a new and effective synthetic pathway for benzofuran-derived amide derivatives and identifies compound 6b as an effective dual-action lead with high antimicrobial and anti-inflammatory activities.

Keywords: Benzofuran; Derivatives; Antimicrobial; Anti-inflammatory

INTRODUCTION

Benzofuran derivatives have gained considerable interest in medicinal chemistry because of their varied pharmacological activities, such as antimicrobial, anti-inflammatory, antioxidant, and anticancer actions[1]. The benzofuran nucleus, a fused heterocyclic system, serves as a versatile scaffold for the designing bioactive molecules[2]. Several researchers have investigated the synthesis and biological activity of benzofuran derivatives, which reflect their therapeutic potential[3–5].

In antimicrobial research, several benzofuran derivatives have been found to exhibit strong activity against various bacterial and fungal pathogens. Kirilmis et al. synthesized a novel 1(1-benzofuran-2-yl)-2-mesitylethanone derivative, which showed high antimicrobial activity against Gram-positive and Gram-negative bacteria and fungi[6]. Similarly, Rida et al. synthesised benzofuran derivatives with high antimicrobial and anticancer activities, highlighting the importance of structural modifications in increasing biological activity[7]. The anti-inflammatory potential of benzofuran derivatives has been explored in detail. Suthakaran et al. had synthesized 7-methoxy benzofuran pyrazoline derivatives and assessed their anti-inflammatory activity in carrageenan-induced paw oedema model in rats. Compounds 4 g and 5m showed considerable inhibition of oedema, which was similar to that of the reference drug ibuprofen. Mane and Vidyadhara had synthesized benzofuran derivatives containing oxadiazole groups, which showed significant anti-inflammatory activity, and thus the potential of the compounds as non-steroidal anti-inflammatory agents was indicated[8].

With this background, the present work aimed to synthesize new benzofuran-derived amide derivatives and evaluate their antimicrobial and anti-inflammatory activities.

MATERIALS AND METHODS

2-(benzofuran-2-carboxamido) acetic acid was synthesised using a multi-step procedure. In the first step, 3,4-Dibromo-

KRUPANIDHI COLLEGE OF PHARMACY

3,4-dihydrochromen-2-one was first prepared by the bromination of coumarin. In a 100 mL three-necked flask equipped with a mechanical stirrer, dropping funnel, and condenser fitted with a hydrogen bromide trap, 1.46 g (10 mmol) of coumarin was dissolved in 20 mL of chloroform. A solution of bromine (1.60 g, 10 mmol) in 0.85 mL of chloroform was then added dropwise over 3 h at room temperature. Excess bromine was removed using a 20% sodium sulphite solution, and the organic layer was washed, dried over magnesium sulphate, filtered, and concentrated. The obtained solid was washed with diethyl ether to give 2.15 g (70% yield) of the product with a melting point of 102–105°C.

In the second step, benzofuran-2-carboxylic acid is synthesized via cyclization. Potassium hydroxide (4.50 g, 80 mmol) was dissolved in 700 mL of absolute ethanol and cooled to 15°C. Previously synthesized coumarin dibromide (2.15 g, 7 mmol) was added in small quantities while maintaining the temperature below 20°C. The reaction mixture was refluxed for 30 min, after which 15 mL of water was added. Following the collection of 25 mL of distillate, the solution was cooled with cracked ice and acidified by the addition of 12 mL of 6N HCl. The precipitated coumarilic acid was filtered, washed with water, and recrystallised from ethanol-water (1:1) to give 93–100 g (82–88%) of the white crystalline product, with a melting point of 190–193°C.

In the third step, to synthesise ethyl 2-(benzofuran-2-carboxamido) acetate, coumarilic acid (1.0 g, 6.16 mmol) was dissolved in 100 mL of dry benzene and heated with thionyl chloride (1.45 mL, 18.5 mmol) under reflux conditions for 3 h. After cooling, the solvent was evaporated to yield the acid chloride, which was then treated with glycine ethyl ester (947 mg, 6.78 mmol) and sodium bicarbonate (569 mg, 6.78 mmol) in 100 mL of water. After 1 h of stirring, the organic phase was decanted, dried, filtered, and concentrated to yield a 1.2 g white crystalline solid (80% yield).

In the fourth step, glycine ethyl ester was independently synthesised by refluxing glycine (1 g) in ethanol and catalytic sulfuric acid for 4 h. The reaction mixture was evaporated, treated with ice-cold sodium bicarbonate, and extracted using ethyl acetate. The organic layer was dried, filtered, and concentrated under vacuum to obtain a white solid weighing 1.1 g (84% yield). In the final step, 2-(benzofuran-2-carboxamido) acetic acid was obtained by hydrolysing ethyl 2-(benzofuran-2-carboxamido) acetate (1.2 g) in ethanol with aqueous LiOH (2.8 mL, 1.1 eq). The reaction mixture was stirred at room temperature for 24 h, concentrated, and acidified with 1 M HCl. The precipitate was extracted into dichloromethane, washed, dried over $MgSO_4$, and recrystallised from ethyl acetate/hexanes to give 0.85 g (71%) of the desired product with a melting point of 189–191°C.

All synthesised compounds were characterised using melting point determination, thin-layer chromatography (TLC), infrared spectroscopy (IR), and nuclear magnetic resonance (NMR) spectroscopy. Melting points were determined using Thiel's tube method to measure purity. TLC was carried out on silica gel plates using a hexane: ethyl acetate (70:30) solvent system, and spots were observed under UV light. IR spectra were recorded on a SHIMADZU FTIR 8400S spectrometer to identify functional groups. NMR spectra were recorded using a Bruker Spectrospin-400 spectrometer with chloroform or DMSO as solvents to confirm the structural integrity.

The anti-inflammatory and antimicrobial activities of the synthetic compounds were investigated using well-established in vitro and in vivo techniques. Antimicrobial screening was performed using both cup-plate and tube dilution techniques. As per antibacterial activity, the strains tested include Bacillus subtilis (B. subtilis) and Staphylococcus aureus (S. aureus) (gram-positive) as well as Escherichia coli (E. coli) and Pseudomonas aeruginosa (P. aeruginosa) (gram-negative). Antifungal activity was tested against Candida albicans and Aspergillus niger (A. niger).

For the cup-plate technique, Muller-Hinton agar (for bacteria) and Sabouraud's agar (for fungi) were prepared by dissolving measured amounts of dehydrated medium in purified water and autoclaving at 121°C for 15 min. After cooling and solidification in Petri plates, the microbial cultures were inoculated into the agar. 6 mm diameter wells were bored in each plate using a sterile cork borer. They were loaded with 0.1 ml of the test compound solution (1000 μg/ml in sterile dimethylformamide), a control antibiotic (norfloxacin for antibacterial, griseofulvin for antifungal), and dimethylformamide was used as a control. Plates were kept at 4°C for 30 min to allow diffusion of the compounds and then incubated at 37°C for 24 h for bacterial strains and at 25°C for 48 h for fungal strains. The diameter of the inhibition zone surrounding each well was measured to assess the antimicrobial activity.

Minimum inhibitory concentration (MIC) was assessed using the tube dilution method. Serial two-fold dilutions of each synthesised compound (200, 100, 50, 25, 12.5, and 6.25 μg/ml) were prepared in culture tubes with either Mueller-Hinton broth (for bacteria) or Sabouraud's broth (for fungi). Each tube was seeded with a loopful of test microorganisms and incubated at 25°C for 48 h. The MIC was defined as the lowest concentration at which no visible microbial growth occurred.

Anti-inflammatory activity was assessed using the carrageenan-induced paw oedema method in Swiss albino rats with a body weight range of 150–200 g. Acute toxicity studies were carried out according to OECD guideline 425 (up-and-down method) to establish a safe dosage.[9] Three dose levels (100, 200, and 400 mg/kg) were chosen from toxicity studies. The animals were divided into 12 groups (n=6): a control group (saline), a standard group (diclofenac sodium, 10 mg/kg), and test groups. Following drug administration for 30 minutes, 0.1 ml of 1% carrageenan

solution was injected into the subplantar area of the left hind paw to cause inflammation. The right paw served as the non-inflamed control. The paw volumes were determined using a plethysmograph at 0, 15, 30, 60, and 120-min. The percentage inhibition of oedema was determined by comparing the paw volumes of the treated groups to the control. The results were statistically compared using one-way ANOVA with Dunnett's test.

RESULTS

The physical characteristics of the six methyl-2-amino carboxylate derivatives are summarised in Table 1 . Each compound differed in molecular structure, resulting in variations in the molecular weight, melting point, Rf value, and percentage yield. The yields ranged between 70% and 88%, and the melting points ranged from 242°C to 355°C, indicating differences in purity and structural stability.

The physiochemical characteristics of the six benzofuran-based amide derivatives synthesised showed variations in molecular weights (247–323 g/mol), melting points (525–624°C), rf values (0.62–0.86), and percentage yields of 60% to 80%. (Table 2)

The MICs of the synthesized compounds (6a–6f) against the selected bacterial and fungal strains are presented in Table 3. Compounds 6a, 6b, and 6f showed broad-spectrum antimicrobial activity, with MIC values as low as 6.25 μg/ml against both Gram-positive and Gram-negative bacteria. Compounds 6c, 6d, and 6e showed more selective and moderate-to-weak antimicrobial effects, with MIC values ranging from 25 to 100 μg/ml.

The results of the zone of inhibition showed that compound 6b had the largest zones of inhibition against S. aureus and E. coli, whereas compound 6a had broad activity across multiple strains. Ciprofloxacin and Griseofulvin were used as standards, showing significant inhibitory effects against bacterial strains, and Griseofulvin was effective against C. albicans and A. niger. (Table 4)

The anti-inflammatory activity of benzofuran derivatives in carrageenan-induced paw oedema in rats indicated that the standard drug significantly reduced the oedema volume, with maximum inhibition observed at 1 h (77.19%). Among the synthesized benzofuran derivatives, compound 6b demonstrated the highest percentage of inhibition at 2 h (71.10%), while compound 6a showed substantial anti-inflammatory activity with 61.55% inhibition at 2 h, which was statistically significant (p value < 0.01 for most compounds, except for compound 6b at 1 and 2 h, where p was < 0.05. (Table 5)

DISCUSSION

The methyl-2-amino carboxylate derivatives (5a–5f) synthesised in the present study had different physicochemical characteristics, with yields ranging from 70% to 88%, melting points between 242°C and 355°C, and molecular weights of 89–179 g/mol. The Rf values ranged from 0.62-0.86. Compound 5b showed the highest yield (88%) and lowest melting point (242–244°C) and could possibly be the most reactive with ease of crystallisation. For the amide derivatives of benzofuran (6a–6f), the molecules had molecular weights of 247–323 g/mol and high melting points (525–624°C), reflecting their large aromatic structure. Compound 6f had the highest molecular weight (323 g/mol) and melting point (622–624°C), as well as a good yield of 79%, indicating good thermal stability and good synthetic accessibility.

In the present study, the antimicrobial activity evaluation indicated that compounds 6a, 6b, and 6f had broad-spectrum activity with MIC values as low as 6.25 μg/ml against B. subtilis, S. aureus, and E. coli. Compounds 6c, 6d, and 6e were more selective in activity, with MICs ranging between 25 and 100 μg/ml. These results were supported by the zone of inhibition data, where compound 6b showed the greatest zones (24 mm) against S. aureus and E. coli, whereas compound 6a exhibited moderate-to-strong inhibition against several bacterial and fungal strains. These results are consistent with earlier findings by Kirilmis et al., who showed that benzofuran derivatives possess significant antibacterial activity, particularly against Gram-positive bacteria[6]. Similarly, Rida et al. observed the antimicrobial activity of substituted benzofurans against E. coli and S. aureus[7].

The anti-inflammatory screening of the synthesized benzofuran derivatives against carrageenan-induced paw oedema in rats indicated that all compounds were highly effective in inhibiting oedema volume in the present study. Compound 6b showed the highest percentage of inhibition at 2 h (71.10%), followed by compound 6a (61.55%), with both showing statistically significant results. This anti-inflammatory activity was consistent with the studies of Panchabhai[10] and Salih et al[3]., who also found similar structures of benzofurans that showed high inhibition of inflammation. Madhu et al. also observed the importance of benzofuran scaffolds in the generation of strong anti-inflammatory compounds[11].

In general, the present findings validate the therapeutic potential of benzofuran derivatives as antimicrobials and anti-inflammatory compounds, consistent with previous research, which highlighted the structural flexibility of the benzofuran ring in adjusting biological activity[12]. Further optimization of the structure and investigation of the mechanisms might enhance their activity and selectivity.

CONCLUSION

In conclusion, benzofuran derivatives exhibited promising physicochemical and biological profiles. Compounds 6a, 6b, and 6f possessed excellent antimicrobial activity with MIC values as low as 6.25 μg/ml, while the highest anti-

Table 1: Physicochemical properties of synthesized methyl-2-amino carboxylate derivatives

Compound No.	IUPAC	Molecular Formula	Molecular Weight	Melting Point (°C)	R_f	Yield (%)
5a	Methyl-2-amino propionate	$C_3H_7NO_2$	89	350-355	0.72	70
5b	Methyl-2-amino propionate	$C_4H_9NO_2$	103	242-244	0.67	82-88
5c	Methyl-2-amino-3-methyl butanoate	$C_6H_{13}NO_2$	131	252-254	0.86	84
5d	Methyl-2-amino-4-methyl pentanoate	$C_7H_{15}NO_2$	145	260-263	0.62	71
5e	Methyl-2-amino-3-methyl pentanoate	$C_7H_{15}NO_2$	167	300-305	0.70	79
5f	Methyl-2-amino-3-phenyl propionate	$C_{10}H_{13}NO_2$	179	335-338	0.76	75

Table 2: Physicochemical properties of synthesized benzofuran-based amide derivatives

Compound No.	IUPAC	Molecular Formula	Molecular Weight	Melting Point (°C)	R_f	Yield (%)
6a	Methyl 2-(benzofuran-2-carboxamido) acetate	$C_{12}H_{11}NO_4$	289	525-530	0.72	80
6b	Methyl 2-(benzofuran-2-carboxamido) propionate	$C_{13}H_{13}NO_4$	247	525-530	0.67	65
6c	Methyl 2-(benzofuran-2-carboxamido)-3-methylbutanoate	$C_{20}H_{17}NO_3$	264	530-537	0.86	62
6d	Methyl 2-(benzofuran-2-carboxamido)-4-methylpentanoate	$C_{16}H_{19}NO_4$	289	545-549	0.62	60
6e	Methyl 2-(benzofuran-2-carboxamido)-3-methylpentanoate	$C_{16}H_{19}NO_4$	289	540-549	0.76	75
6f	Methyl 2-(benzofuran-2-carboxamido)-3-phenylpropanoate	$C_{19}H_{17}NO_4$	323	622-624	0.80	79

Table 3: MICs of synthesized benzofuran derivatives (6a–6f) against bacterial and fungal strains

Compound No.	*B. subtilis*	*S. aureus*	*E. coli*	*P. aeruginosa*	*C. albicans*	*A. niger*
6a	6.25	6.25	6.25	12.5	12.5	6.25
6b	6.25	6.25	6.25	6.25	12.5	12.5
6c	25	-	25	100	-	100
6d	50	25	25	50	100	-
6e	100	50	100	-	-	-
6f	6.25	12.5	25	6.25	25	50
Ciprofloxacin	6.25	6.25	6.25	625	-	
Griseofulvin	-	-	-	-	6.25	6.25

Table 4: Zones of inhibition (mm)of synthesized benzofuran derivatives (6a–6f) against bacterial and fungal strains

Benzofuran derivatives	*B. subtilis*	*S. aureus*	*E. coli*	*P. aeruginosa*	*C. albicans*	*A. niger*
6a	19	20	21	20	17	15
6b	20	21	24	19	16	10
6c	14	-	18	10	-	09
6d	15	19	19	11	10	-
6e	12	13	11	-	-	-
6f	19	19	19	20	13	09
Ciprofloxacin	25	24	25	27	-	-
Griseofulvin	-	-	-	-	21	19

Table 5: Anti-inflammatoryactivity of benzofuran derivatives in carrageenan-induced paw oedema in rats

Benzofuran derivatives at 20 mg / kg bw	Edema volume in ml			% inhibition		
	$\frac{1}{2}$ h	1h	2h	$\frac{1}{2}$ h	1h	2h
Control	0.743	0.755	0.796	-	-	-
Standard (Indomethacin)	0.230	0.212	0.195	71.92**	77.19**	75.50**
6a	0.354	0.325	0.306	52.35**	56.95**	61.55**
6b	0.261	0.252	0.230	64.87	66.62*	71.10*
6c	0.314	0.296	0.283	57.74**	60.79**	64.45**
6d	0.268	0.258	0.272	63.93**	65.82**	65.83**
6e	0.238	0.312	0.298	61.52**	58.67*	62.56*
6f	0.345	0.397	0.377	53.57**	47.42*	52.64*

Significance at P < 0.05*, 0.01**

inflammatory activity was exhibited by compound 6b, with 71.10% inhibition at 2 h. These results highlight benzofuran-molecule-based compounds as potential therapeutics for further development as anti-inflammatory and antimicrobial agents.

REFERENCES

1. Bari S, Sonawane SY, Surana S. Synthesis of some new benzofurans and their analgesic and antiinflammatory activity. *Indian journal of Heterocyclic chemistry*. 2010;20(2):175–176.
2. Tymoshenko DO. Benzoheteropines with Fused Pyrrole, Furan and Thiophene Rings. *Advances in Heterocyclic Chemistry*. 2008;96:1–80. Available from: https://doi.org/10.1016/S0065-2725(07)00001-3.
3. Salih KSM, Ayoub MT, Saadeh HA, Al-Masoudi NA, Mubarak MS. Synthesis, Characterization, and Biological Activities of New Benzofuran Derivatives. *HETEROCYCLES*. 2007;71(7):1577–1587. Available from: https://www.researchgate.net/publication/235792914_Synthesis_Characterization_and_Biological_Activities_of_New_Benzofuran_Derivatives.
4. Koca M, Servi S, Kirilmis C, Ahmedzade M, Kazaz C, Özbek B, et al. Synthesis and antimicrobial activity of some novel derivatives of benzofuran: part 1. Synthesis and antimicrobial activity of (benzofuran-2-yl)(3-phenyl-3-methylcyclobutyl) ketoxime derivatives. *European Journal of Medicinal Chemistry*. 2005;40(12):1351–1358. Available from: https://dx.doi.org/10.1016/j.ejmech.2005.07.004.
5. Kumar DRH, Karvekar MD. Synthesis of Benzofuran Derivatives and their Evaluation of Antimicrobial Activity. *Journal of Chemistry*. 2010;7(2):636–640. Available from: https://dx.doi.org/10.1155/2010/817480.
6. Kirilmis C, Ahmedzade M, Servi S, Koca M, Kizirgil A, Kazaz C. Synthesis and antimicrobial activity of some novel derivatives of benzofuran: Part 2. The synthesis and antimicrobial activity of some novel 1-(1-benzofuran-2-yl)-2-mesitylethanone derivatives. *European Journal of Medicinal Chemistry*. 2008;43(2):300–308. Available from: https://dx.doi.org/10.1016/j.ejmech.2007.03.023.
7. Rida SM, El-Hawash SAM, Fahmy HTY, Hazza AA, El-Meligy MMM. Synthesis and in vitro evaluation of some novel benzofuran derivatives as potential anti-HIV-1, anticancer, and antimicrobial agents. *Archives of Pharmacal Research*. 2006;29:16–25. Available from: https://dx.doi.org/10.1007/bf02977463.
8. Suthakaran R, Somasekhar G, Sridevi C, Marikannan M, Suganthi K, Nagarajan G. Synthesis, antiinflammatory, antioxidant and antibacterial activities of 7-methoxy benzofuran pyrazoline derivatives. *Asian Journal of Chemistry*. 2007;19(5):3353–3362. Available from: https://www.researchgate.net/publication/289359635_Synthesis_antiinflammatory_antioxidant_and_antibacterial_activities_of_7-methoxy_benzofuran_pyrazoline_derivatives.
9. OECD/OCDE. Acute Oral Toxicity – Up-and-Down Procedure. In: OECD guideline for testing of chemicals. Paris, France. 2001;p. 1–26. Available from: https://www.academia.edu/download/44868899/real_dosis_letal_50.pdf.
10. Panchabhai VB. Synthesis and Biological Evaluation of Some Novel Benzofuran Containing Carbamide Derivatives. *Asian Journal of Pharmaceutical Health Sciences*. 2011;1(4):158–162. Available from: https://ajphs.com/article/2011/1/4/158-162.
11. Madhu R, Karvekar M, Patel S, Sarkar S. Synthesis and Biological Evaluations of Some Benzofuran Derivatives. *Asian Journal of Chemistry*. 2009;21(7). Available from: https://asianpubs.org/index.php/ajchem/article/view/18285.
12. Jiang X, Liu W, Zhang W, Jiang F, Gao Z, Zhuang H, et al. Synthesis and antimicrobial evaluation of new benzofuran derivatives. *European Journal of Medicinal Chemistry*. 2011;46(8):3526–3530. Available from: https://dx.doi.org/10.1016/j.ejmech.2011.04.053.