Synthesis and Antimicrobial Evaluation of 2-Aminobenzophenone Linked 1,4-Dihydropyridine Derivatives

PENGYING LI¹, KETKI SAHORE², JIANJUN LIU^{1,*} and RAJESH K. SINGH^{2,*}

¹Forestry College, Northwest A and F University, Yangling 712100, P.R. China

²Department of Pharmaceutical Chemistry, Shivalik College of Pharmacy (Under Local Govt. Dept. Punjab) Nangal, Distt. Rupnagar-140126, Punjab, India

*Corresponding authors: E-mail: jianjun2013Liu@126.com; rksingh244@gmail.com

Received: 17 February 2014;

Accepted: 19 April 2014;

Published online: 28 July 2014;

AJC-15680

A series of novel aminobenzophenone linked 1,4-dihydropyridines (1,4-DHPs) hybrids were synthesized by incorporating aminobenzophenone moiety on 1,4-dihydropyridines scaffold (**5a-f**) and characterized by IR, ¹H NMR and CHN elemental analysis. The synthesized compounds were tested for their *in vitro* antibacterial activity against the Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Escherichia coli*) bacteria. Among all the synthesized compounds, compounds **5b**, **5c** and **5e** have shown the maximum activity in their group whereas compounds **5a**, **5d**, **5f** have shown the minimum activity.

Keywords: 1,4-Dihydropyridine, 2-Aminobenzophenone, Pharmacophore, Antimicrobial activity.

INTRODUCTION

Nitrogen heterocyclic frameworks are prevalent in pharmaceuticals and biologically functional molecules. Among various nitrogen heterocyclics, 1,4-dihydropyridines (1,4-DHP) scaffold is one of the most versatile pharmacophores since it has been found as the central core in many pharmaceuticals¹⁻⁷ and so considered as most "privileged structure" in heterocyclic chemistry that, when appropriately substituted can exert potent and diverse pharmacological actions as shown in Fig. 1.

Fig. 1. Pharmacological activities exhibited by dihydropyridine derivatives

Recent studies have revealed that 1,4-DHPs exhibit potent antimicrobial activities⁸⁻¹³ and lots of works have been going on focusing 1,4-dihydropyridines synthesis as antibacterial chemotherapeutic regimen (Fig. 2).

Fig. 2. Different derivatives of 1,4-dihydropyridine nucleus having good antimicrobial activities

2-Aminobenzophenone derivatives are important compounds in organic chemistry because of their application in heterocyclic synthesis and medicines¹⁴. Some heterocyclic compounds containing 2-aminobenzophenone moiety possess anti-inflammatory¹⁵, anti-tumor¹⁶, antimitotic¹⁷, anti-viral¹⁸ and skeletal muscle relaxant activities¹⁹. More recently, some

research workers identified the antimicrobial potential of benzophenone nucleus and much research has been focused on the synthesis of its various derivatives for antibacterial and antifungal activities^{20,21}.

The combination of two pharmacophores in a single molecule may enable synergistic interaction, where the efficacy of a single agent is enhanced by the addition of a second compound. In view of these observations, it was decided to synthesize aminobenzophenone linked 1,4-dihydropyridines derivatives by incorporating aminobenzophenone pharmacophore on 1,4-dihydropyridines scaffold (Fig 3.) and screen them for their level of antimicrobial activity in the hope to obtain potent antibacterial agents.

Fig. 3. Design of aminobenzophenone linked 1,4-dihydropyridine derivatives as possible antimicrobial agents

EXPERIMENTAL

The melting points were determined on Veego-programmable melting point apparatus (microprocessor based) and are uncorrected. Infrared (IR) spectra were obtained with on a Perkin Elmer 882 Spectrum and RXI, FT-IR model using a potassium bromide pellets (KBr) in cm^{-1} . ^1H NMR spectra were obtained using Bruker AC-400 F, 400 MHz spectrometer and are reported in parts per million (ppm), downfield from tetramethylsilane (TMS) as internal standard. To monitor the reactions, as well as to establish the identity and purity of reactants and products, thin layer chromatography was performed on microscopic slides (2×7.5 cm), which were coated with silica gel G and activated at 110°C for 0.5 h. The plates were developed by exposure to iodine vapours. All the chemicals used in the synthesis are of analytical grade. Anhydrous sodium sulphate was utilized as drying agents. All the solvents were dried and freshly distilled prior to use according to standard procedure. The starting compound 2-(chloroacetamido)-5-chlorobenzophenone and substituted 1,4-dihydropyridines were earlier synthesized in our lab by conventional as well as microwave irradiation technique^{19,22,23}.

General method for the synthesis of dimethyl/diethyl-4/3(substituted)-2-oxoethoxy)-4-methoxyphenyl)-2,6-dimethyl-1, 4-dihydropyridine-3,5-dicarboxylate (5a-5f)

Method A: A mixture of 2-(chloroacetamido)-5-chlorobenzophenone (1.23 g, 4 mmol), potassium fluoride (0.116 g, 2 mmol) and dry powdered potassium carbonate (0.7 g, 5 mmol) in dry DMF (15 mL) was stirred at room temperature for 1 h. A solution of substituted 1,4-dihydropyridines (1 mmol) in dry DMF (5 mL) was added into the above mixture and it was stirred at room temperature for 24 h. The reaction mixture

was poured onto ice water (100 mL) and extracted with EtOAc (3×50 mL). The organic extracts were combined, washed with ice water, dried over Na_2SO_4 and evaporated *in vacuo* to dryness. Residue was recrystallised from EtOH to get products (5a-f).

Method B: A mixture of substituted 1,4-dihydropyridines (2 mmol) and anhydrous potassium carbonate (0.7 g, 5 mmol) in ethyl methyl ketone (50 mL) was refluxed for 2 h with stirring. To this, 2-(chloroacetamido)-5-chlorobenzophenone (1.23 g, 4 mmol) was added and the reaction mixture was refluxed for another 6 h. The resulting slurry was filtered off and the solvent was recovered under reduced pressure to obtain oily residues which was recrystallized from ethanol to get products (5a-f).

Dimethyl 4-[4-{2-((2-benzoyl-4-chlorophenyl)amino)-2-oxoethoxy}-3-methoxyphenyl]-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (5a): Yield = **method A**: 71 %, **method B** 65 %, m.p. $112-115^\circ\text{C}$; $R_f = 0.62$ [chloroform:methanol] [9:1], IR (KBr, ν_{max} , cm^{-1}): 3350 (Sec N-H str. of DHP), 3088 (aromatic C-H str.), 2984 (C-H alkanes str.), 1681 ($>\text{C}=\text{O}$ str. ester), 1488 (C=C aromatic ring), 1214 (C-O str.) and 750 (C-Cl). ^1H NMR (CDCl_3) δ (ppm): 2.33 (s, 6H, $2 \times \text{CH}_3$), 3.64 (s, 2H, OCH_2), 3.65 (s, 6H, $2 \times \text{COOCH}_3$), 3.83 (s, 3H, $-\text{OCH}_3$), 4.93 (s, 1H, $-\text{CH}$ of DHP), 5.63 (s, 1H, NH of DHP) and 6.71-7.74 (m, 11H, ArH), 8.61 (s, 1H, NH). Anal. calcd. for $\text{C}_{33}\text{H}_{31}\text{N}_2\text{O}_8\text{Cl}$: C % 64.02, H % 5.05, N % 4.53; Found: C % 64.22, H % 5.18, N % 4.58

Dimethyl 4-[3-{2-((2-benzoyl-4-chlorophenyl)amino)-2-oxoethoxy}phenyl]-2,6-dimethyl-1, 4-dihydropyridine-3,5-dicarboxylate (5b): Yield = **method A** 68 %, **method B** 62 %, m.p. $163-165^\circ\text{C}$; $R_f = 0.68$ [chloroform:methanol] [9:1] IR (KBr, ν_{max} , cm^{-1}): 3329 (N-H str.), 3091 (ar C-H str.), 3000 (C-H str. alkanes), 1681 ($>\text{C}=\text{O}$ str. amide), 1484 (C=C str. ar. ring), 1348 (C-N), 1227 (C-O str. ester) and 759 (C-Cl). ^1H NMR ($\text{DMSO}-d_6$) δ (ppm): 2.27 (s, 6H, $2 \times \text{CH}_3$), 3.58 (s, 6H, $2 \times \text{COOCH}_3$), 3.94 (s, 2H, OCH_2), 4.63 (s, 1H, $-\text{CH}$ of DHP), 4.9 (s, 1H, NH of DHP), 6.89-8.06 (m, 12H, ArH), 10.6 (s, 1H, NH). Anal. calcd. for $\text{C}_{32}\text{H}_{29}\text{N}_2\text{O}_7\text{Cl}$: C % 65.25, H % 4.96, N % 4.76; Found: C % 65.58, H % 5.12, N % 4.62

Diethyl 4-[4-{2-((2-benzoyl-4-chlorophenyl)amino)-2-oxoethoxy}-3-methoxyphenyl]-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (5c): Yield = **method A** 67 %, **method B** 60 %, m.p. $128-130^\circ\text{C}$; $R_f = 0.85$ [chloroform:methanol] [9:1] IR (KBr, ν_{max} , cm^{-1}): 3332 (N-H str. of DHP), 3061 (ar. C-H str.), 2976 (C-H str. alkanes), 1691 ($>\text{C}=\text{O}$ str. ester), 1596 (C=C Ar), 1505 (N-H bend), 1212 (C-O str. ester) and 748 (C-Cl). ^1H NMR (CDCl_3) δ (ppm): 1.22-1.25 (m, 6H, $2 \times \text{OCH}_2\text{CH}_3$), 2.32 (s, 6H, $2 \times \text{CH}_3$), 3.83 (s, 5H, $-\text{OCH}_3 + \text{OCH}_2$), 4.07-4.10 (q, 4H, $2 \times \text{OCH}_2\text{CH}_3$), 4.94 (s, 1H, CH of DHP), 5.54 (s, 1H, NH of DHP), 6.84-7.82 (m, 11H, ArH), 8.60 (s, 1H, NH). Anal. calcd. for $\text{C}_{35}\text{H}_{35}\text{N}_2\text{O}_8\text{Cl}$: C % 64.96, H % 5.45, N % 4.33; Found: C % 65.24, H % 5.70, N % 4.45

Diethyl 4-[4-{2-((2-benzoyl-4-chlorophenyl)amino)-2-oxoethoxy}phenyl]-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (5d): Yield = **method A** 74 %, **method B** 64 %, m.p. $158-161^\circ\text{C}$; $R_f = 0.82$ [chloroform:methanol] [9:1] IR (KBr, ν_{max} , cm^{-1}): 3347 (N-H str.), 2984 (C-H str. alkanes), 1659 ($>\text{C}=\text{O}$ str. amide), 1486 (C=C str. ar. ring), 1333 (C-N), 1228 (C-O str. ester) and 745 (C-Cl). ^1H NMR ($\text{DMSO}-d_6$)

δ (ppm): 1.05-1.09 (t, 6H, $2 \times \text{OCH}_2\text{CH}_3$), 2.18 (s, 6H, $2 \times \text{CH}_3$), 3.35 (s, 2H, OCH_2), 3.89-3.98 (m, 4H, $2 \times \text{OCH}_2\text{CH}_3$), 4.68 (s, 1H, CH of DHP), 6.49-7.48 (m, 12H, ArH), 8.29 (s, 1H, NH). Anal. calcd. for $\text{C}_{34}\text{H}_{33}\text{N}_2\text{O}_7\text{Cl}$: C% 66.18, H% 5.39, N% 4.54; Found: C% 66.58, H% 5.66, N% 4.58

Diethyl 4-[3-{2-((2-benzoyl-4-chlorophenyl)amino)-2-oxoethoxy}-4-methoxyphenyl]-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (5e): Yield = **method A** 72%, **method B** 65 %, m.p. 198-200 °C; R_f = 0.65 [chloroform: methanol] [9:1] IR (KBr, ν_{max} , cm^{-1}): 3356 (N-H str. of DHP), 3090 (aromatic C-H str.), 2958 (C-H str. alkanes), 1696 (ester C=O), 1486 (aromatic C=C), 1214 (ether C-O), 780 (C-Cl). ^1H NMR (CDCl_3) δ (ppm): 1.23-1.28 (m, 6H, $2 \times \text{OCH}_2\text{CH}_3$), 2.27 (s, 3H, CH_3), 2.88 (s, 3H, CH_3), 3.77-3.82 (s, 5H, $\text{OCH}_3 + \text{OCH}_2$), 4.04-4.1 (q, 4H, OCH_2CH_3), 4.58 (s, 1H, CH of DHP), 4.89 (s, 1H, NH of DHP), 6.66-7.72 (m, 11H, ArH), 8.62 (s, 1H, NH). Anal. calcd. for $\text{C}_{35}\text{H}_{35}\text{N}_2\text{O}_8\text{Cl}$: C% 64.96, H% 5.45, N% 4.33; Found: C% 63.87, H% 5.89, N% 4.68.

Dimethyl 4-[3-{2-((2-benzoyl-4-chlorophenyl)amino)-2-oxoethoxy}-4-methoxyphenyl]-2,6-dimethyl-1,4-dihydro-

pyridine-3,5-dicarboxylate (5f): Yield = **method A** 69 %, **method B** 62 %, m.p. 165-167 °C; R_f = 0.63 [chloroform: methanol] [9:1] IR (KBr, ν_{max} , cm^{-1}): 3345 (N-H str. of DHP), 2982 (C-H str. alkanes), 1673 (ester C=O), 1487 (Ar C=C), 1228 (ether C-O), 759 (C-Cl). ^1H NMR (CDCl_3) δ (ppm): 2.27 (s, 6H, $2 \times \text{CH}_3$), 3.54 (s, 2H, OCH_2), 3.61 (s, 6H, $2 \times \text{COOCH}_3$), 3.81 (s, 3H, $-\text{OCH}_3$), 4.58 (s, 1H, CH of DHP), 5.70 (s, 1H, NH of DHP), 6.68-7.75 (m, 11H, ArH), 8.62 (s, 1H, NH). Anal. calcd. for $\text{C}_{33}\text{H}_{31}\text{N}_2\text{O}_8\text{Cl}$: C% 64.02, H% 5.05, N% 4.53; Found: C% 64.22, H% 5.16, N% 4.60

RESULTS AND DISCUSSION

The reaction pathway used for the synthesis of title compounds have been shown in **Scheme-I**. The final compounds were prepared by two methods (**methods A and B**) to optimize the yield. **Method A** includes the stirring at room temperature of 2-(chloroacetamido)-5-chlorobenzophenone (**4**) and substituted 1,4-dihydropyridines (**3a-f**) in the presence of potassium carbonate and sodium iodide in a minimum quantity of DMF and by refluxing 2-(chloroacetamido)-5-chlorobenzophenone

	5a	5b	5c	5d	5e	5f
X		H			OCH ₃	OCH ₃
Y	OCH ₃		OCH ₃	H		
R	CH ₃	CH ₃	C ₂ H ₅	C ₂ H ₅	C ₂ H ₅	CH ₃

Reagents & conditions: (i) Ammonium acetate, EtOH, reflux for 24 h (iia) **Method A:** K₂CO₃, KI and DMF, stirring at RT for 24 h (iib) **Method B:** K₂CO₃, ethyl methyl ketone, reflux with stirring for 6 h.

Scheme-I: Chemical reactions for the synthesis of title compounds

TABLE-1
ZONE OF INHIBITION (IN mm) OF *Staphylococcus aureus* AND *Escherichia coli*
OF TEST COMPOUNDS AT A CONCENTRATION OF 50, 100, 150 AND 200 µG/ML

Compounds	Zone of inhibition (mm)							
	<i>Staphylococcus aureus</i>				<i>Escherichia coli</i>			
	50 (µg/mL)	100 (µg/mL)	150 (µg/mL)	200 (µg/mL)	50 (µg/mL)	100 (µg/mL)	150 (µg/mL)	200 (µg/mL)
Amoxicillin	12	13	17	18	10	12	15	18
5a	2	4	7	6	2	2	8	6
5b	3	5	8	8	3	4	9	8
5c	2	6	9	7	2	2	8	6
5d	2	4	8	6	2	3	9	5
5e	2	5	10	7	2	3	11	7
5f	2	3	7	6	2	4	6	6

(4) and (3a-f) in ethyl methyl ketone as solvent in the presence of potassium carbonate as catalyst by **method B**. It was observed that from both methods, **method A** was found to give the better yield. The structures of the synthesized compounds were determined by IR, NMR and CHN elemental studies.

Antibacterial activity of synthesized compounds was tested by Agar plate diffusion method²⁴ against two bacterial strains: *Staphylococcus aureus* (Gram+ve) (ATCC-29737) and *Escherichia coli* (Gram-ve) (ATCC-8739) procured from IMTECH, Chandigarh. After the setting of media, 2 mL of bacterial culture was poured in Petri dishes and sprayed over agar media. Different concentrations of test compound (50, 100, 150 and 200 µg/mL) were placed in each hole of the Petri dish. The plates were incubated at 37 °C for 24 h for bacteria strains. The diameter of zones of inhibition formed around the disc was measured after 24 h for the bacteria plates. Amoxicillin was used as standard or positive control while DMSO was used as a solvent and as negative controls. Although none of the synthesized compounds have shown to have good antibacterial activity. However (compounds **5b**, **5c**, **5e**) have shown the maximum activity in their group whereas (compounds **5a**, **5d**, **5f**) have shown the minimum activity (Table-1).

Conclusion

A series of 2-aminobenzophenone linked 1,4-dihydropyridine derivatives were synthesized, characterized and finally evaluated for their antibacterial activity but none of the compounds have exhibited significant antibacterial activity. The study revealed that 1,4-dihydropyridines nucleus is a rich source of exploitation. Hence further modification of 1,4-dihydropyridines and 2-aminobenzophenone moiety by substitution of different groups at different positions may provide more potent antibacterial agents in future.

ACKNOWLEDGEMENTS

The authors are thankful to Principal and College Managing Committee, Shivalik College of Pharmacy, Nangal, India for providing the necessary facilities to carry out the research work. Thanks are also due to Panjab University, Chandigarh, India for cooperation in getting the spectral data and CHN elemental studies.

REFERENCES

- R.R. Poondra, R.V. Nallamelli, C.L.T. Meda, B.N.V. Srinivas, A. Grover, J. Muttathathula, S.R. Voleti, B. Sridhar, M. Pal and K.V.L. Parsa, *Bioorg. Med. Chem. Lett.*, **23**, 1104 (2013).
- R. Boer and V. Gekeler, *Drugs Future*, **20**, 499 (1995).
- V. M. Briukhanov, *Exp. Clin. Pharmacol.*, **57**, 47 (1994).
- S. Gullapalli and P. Ramarao, *Neuropharmacology*, **42**, 467 (2002).
- S. Bahekar and D. Shinde, *Acta Pharm.*, **52**, 281 (2002).
- R. León, C. Ríos, J. Marco-Contelles, O. Huertas, X. Barril, F.J. Luque, M.G. López, A.G. García and M. Villarroya, *Bioorg. Med. Chem.*, **16**, 7759 (2008).
- J. Marco-Contelles, R. León, C. de los Ríos, A. Samadi, M. Bartolini, V. Andrisano, O. Huertas, X. Barril, F.J. Luque, M.I. Rodríguez-Franco, B. López, M.G. López, A.G. García, M. do Carmo Carreiras and M. Villarroya, *J. Med. Chem.*, **52**, 2724 (2009).
- S. Ulloora, R. Shabaraya, R. Ranganathan and A.V. Adhikari, *Eur. J. Med. Chem.*, **70**, 341 (2013).
- G.A. Wachter, M.C. Davis, A.R. Martin and S.G. Franzblau, *J. Med. Chem.*, **41**, 2436 (1998).
- V.L.R. Sana, V.R. Katla, S. Chennamsetty, A. Shaik and N.R. Chamarthi, *Der Pharm. Sinica*, **4**, 10 (2013).
- A.M. Vijesh, A.M. Isloor, S.K. Peethambar, K.N. Shivananda, T. Arulmoli and N.A. Isloor, *Eur. J. Med. Chem.*, **46**, 5591 (2011).
- Y.L.N. Murthy, A. Rajack, M.T. Ramji, J. Jesonbabu, C. Praveen and K.A. Lakshmi, *Bioorg. Med. Chem. Lett.*, **22**, 6016 (2012).
- V. Niraimathi, A.J. Suresh, S. Latha and S.C. Bala, *Int. J. Pharm. Pharm. Sci.*, **4**, 212 (2012).
- D.A. Walsh, *Synthesis*, 677 (1980).
- E.R. Ottosen, M.D. Sørensen, F. Björklund, T. Skak-Nielsen, M.S. Fjording, H. Aaes and L. Binderup, *J. Med. Chem.*, **46**, 5651 (2003).
- S. Cortez-Maya, E. Cortes, S. Hernández-Ortega, T. Ramirez Apan and M. Martínez-García, *Synth. Commun.*, **42**, 46 (2012).
- J.P. Liou, C.W. Chang, J.S. Song, Y.N. Yang, C.F. Yeh, H.Y. Tseng, Y.K. Lo, Y.L. Chang, C.M. Chang and H.P. Hsieh, *J. Med. Chem.*, **45**, 2556 (2002).
- P. Cheng, Q. Zhang, Y.B. Ma, Z.-Y. Jiang, X.M. Zhang, F.X. Zhang and J.J. Chen, *Bioorg. Med. Chem. Lett.*, **18**, 3787 (2008).
- R.K. Singh, S. Devi and D.N. Prasad, *Arab. J. Chem.*, doi:10.1016/j.arabjc.2011.11.013.
- L. Sun, J. Wu, M. Luo, X. Wang, M. Pan, Z. Gou and D. Sun, *Molecules*, **16**, 9739 (2011).
- A. Sakunpak and P. Panichayupakaranant, *Food Chem.*, **130**, 826 (2012).
- P. Kaur, H. Sharma, R. Rana, D.N. Prasad and R.K. Singh, *Asian J. Chem.*, **24**, 5649 (2012).
- P. Kaur and R.K. Singh, *Chem. Sci. Trans*, **2**(S1), S295 (2013).
- Indian Pharmacopoeia, Government of India, Ministry of Health and Welfare, Published by Controller of publication, New Delhi, II, A105 (1996).