Acta Poloniae Pharmaceutica – Drug Research, Vol. 67 No. 3 pp. 255–259, 2010 ISSN 0001-6837
Polish Pharmaceutical Society

# DESIGN AND BIOLOGICAL EVALUATION OF BIPHENYL-4-CARBOXYLIC ACID HYDRAZIDE-HYDRAZONE FOR ANTIMICROBIAL ACTIVITY

AAKASH DEEP[1]*, SANDEEP JAIN[2], PRABODH CHANDER SHARMA[3]
PRABHAKAR VERMA[4], MAHESH KUMAR[4], and CHANDER PARKASH DORA[1]

[1]Department of Pharmaceutical Sciences, G.V.M. College of Pharmacy, Sonepat-131001, India
[2]Department of Pharm. Sciences, Guru Jambheshwar University of Science and Technology, Hisar-125001, India
[3]Institute of Pharmaceutical Sciences, Kurukshetra University, Kurukshetra-136119, India
[4]Institute of Pharmaceutical Sciences, Maharishi Dayanand University, Rohtak-124001, India

**Abstract:** Seven biphenyl-4-carboxylic acid hydrazide-hydrazones have been synthesized. These hydrazone derivatives were characterized by CHN analysis, IR, and $^1$H NMR spectral data. All the compounds were evaluated for their *in vitro* antimicrobial activity against two Gram negative strains (*Escherichia coli* and *Pseudomonas aeruginosa*) and two Gram positive strains (*Bacillus subtilis* and *Staphylococcus aureus*) and fungal strain *Candida albicans* and *Aspergillus niger.* All newly synthesized compounds exhibited promising results.

**Keywords**: synthesis, hydrazide-hydrazones, antimicrobial activity

Development of novel chemotherapeutic agents is an important and challenging task for the medicinal chemists and many research programs are directed towards the design and synthesis of new drugs for their chemotherapeutic usage. Hydrazone compounds constitute an important class for new drug development in order to discover an effective compound against multidrug resistant microbial infection. Hydrazide-hydrazones have been demonstrated to possess anticonvulsant (1), antidepressant (2), anti-inflammatory (3), antimalarial (4), antimycobacterial (5), anticancer (6), and antimicrobial (7-10) activities. These reports prompted us to synthesize the novel derivatives of biphenyl-4-carboxylic acid hydrazide hydrazone. The structures of all compounds have been evaluated by elemental analysis and spectral analysis (IR and $^1$H NMR). All the compounds have been screened for antimicrobial activity against two Gram positive bacteria *S. aureus*, *B. subtilis* and Gram negative bacteria *E. coli, P. aeruginosa* and also against two fungal strains *C. albicans* and A. *niger.* Some of the synthesized compounds showed good antimicrobial activity against these strains.

## EXPERIMENTAL

Melting points were determined in open capillary tubes and are uncorrected. Infra-red spectra were recorded on Perkin Elmer Spectrum RXI FTIR spectrophotomer in KBr phase. $^1$H NMR spectra were run on BRUKER spectrometer (300 MHz) using TMS as an internal standard. Elemental analyses were done using Carlo Erba 1106 CHN Analyzer. The purity of the synthesized compounds was ascertained by thin layer chromatography on silica gel G in solvent system chloroform : benzene : glacial acetic acid (3:1:1, v/v/v). using iodine vapors as detecting agent.

### Chemistry

The reaction between biphenyl-4-carboxylic acid **(I)** and methanol in the presence of sulfuric acid yielded corresponding methyl ester of biphenyl-4-carboxylic acid **(II),** which on reaction with hydrazine hydrate afforded the corresponding hydrazides **(III)** in appreciable yield. Further, the hydrazides were condensed with substituted aldehy-

* Corresponding author: mobile: +919896096727 ; e-mail: aakashdeep82@gmail.com

des to yield the title compounds (**IV**). Biphenyl-4-carboxylic acid hydrazones were characterized on the basis of the spectral and analytical studies.

**General method**

The title compounds were prepared in the following steps:

**Synthesis of biphenyl-4-carboxylic acid methyl ester (II)**

A mixture of (0.25 M) biphenyl-4-carboxylic acid (I) and an excess of methanol (250 mL) with 1 mL of sulfuric acid was refluxed for 3-4 h in round bottom flask (RBF). The mixture was cooled; the solid was separated by filtration and recrystallized from methanol. The purity of compound was checked by single spot TLC. Yield 84.43%, $R_f$ 0.73, m.p. 176-178$^oC$.

**Synthesis of biphenyl-4-carboxylic acid hydrazide (III)**

A mixture of (0.2 M) biphenyl-4-carboxylic acid methyl ester and an excess of hydrazine hydrate (0.30 M) in ethanol (250 mL) was refluxed for about 3 h and cooled. The solid was separated by filtration and recrystallized from ethanol to afford biphenyl-4-carboxylic acid hydrazide. The purity was checked by single spot TLC. Yield 86.79%, $R_f$ 0.69, m.p. 182-183 $^oC$.

**Synthesis of biphenyl-4-carboxylic acid hydrazide hydrazone (IVa-g)**

A mixture of (0.025 M) biphenyl-4-carboxylic acid hydrazide and required aromatic aldehyde (0.025 M) was refluxed in methanol (50 mL) in the presence of a catalytic amount of glacial acetic acid for about 2 h. The mixture was cooled; the solid was separated by filtration and recrystallized from methanol to give the corresponding hydrazide-hydrazones. The purity of the products was checked by single spot TLC.

By adopting similar type of procedures, and employing equimolar quantities of reactants, six compounds were synthesized. Physical and analytical data of the synthesized compounds are given in Table 1. Synthetic pathway for preparation of title compounds is shown in Scheme 1.

Biphenyl-4-carboxylic acid-(2-nitro-benzylidene)-hydrazide (**IVa**)

IR (KBr, $cm^{-1}$): 3301-3261 (NH-$NH_2$), 3077 (C–H, arom.), 1651 (C=O), 1609-1472 (C=C), 1667(C=N), 1544 (N–O). $^1$H NMR (300 MHz, DMSO-$d_6$, δ, ppm): 8.32-7.54 (m, 4H, Ar), 8.22 (s, 1H, CH=N), 8.09-7.29 (m 9H, Ar, H), 8.05 (s, 1H, NH). Analysis: for $C_{20}H_{15}N_3O_3$ calcd. C, 69.56; H, 4.38; N, 12.17%, found C, 69.58; H, 4.35; N, 12.19%.

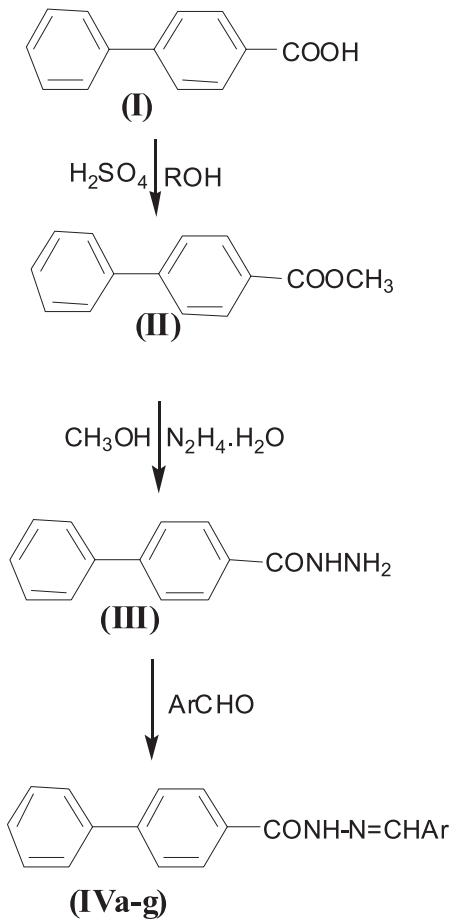
Biphenyl-4-carboxylic acid (2-chloro-benzylidene)-hydrazide (**IVb**)

R (KBr, $cm^{-1}$): 3312-3213 (NH-$NH_2$), 3033 (C–H, arom.), 1670 (C=O), 1607-1493 (C=C), 1661(C=N), 780-603 (C–Cl). $^1$H NMR (300 MHz, DMSO-$d_6$, δ, ppm): 8.29 (s, 1H, CH=N), 8.12-7.48 (m, 9H, Ar, H), 8.09 (s, 1H, NH), 7.36-7.27 (m 4H, Ar, H). Analysis.: for $C_{20}H_{15}ClN_2O$ calcd. C, 71.75, H, 4.52; N, 8.37%, found C, 71.78; H, 4.54; N, 8.33%.

Table 1. Physical data of title compounds.

| Compound no. | Ar | Molecular formula | Molecular weight | M. p. ($^oC$) | Yield % | $R_f$* |
|---|---|---|---|---|---|---|
| **IVa** | 2-$NO_2C_6H_4$ | $C_{20}H_{15}N_3O_3$ | 345.35 | 253-255 | 81.26 | 0.81 |
| **IVb** | 2-$ClC_6H_4$ | $C_{20}H_{15}ClN_2O$ | 334.80 | 252-253 | 78.45 | 0.79 |
| **IVc** | 3-$ClC_6H_4$ | $C_{20}H_{15}ClN_2O$ | 334.80 | 258-260 | 84.46 | 0.72 |
| **IVd** | 4-$ClC_6H_4$ | $C_{20}H_{15}ClN_2O$ | 334.80 | 261-262 | 83.20 | 0.83 |
| **IVe** | 3-$OCH_3C_6H_4$ | $C_{21}H_{18}N_2O_2$ | 330.38 | 205-207 | 82.62 | 0.88 |
| **IVf** | 4-$OCH_3C_6H_4$ | $C_{21}H_{18}N_2O_2$ | 330.38 | 204-205 | 81.21 | 0.74 |
| **IVg** | 3-$BrC_6H_4$ | $C_{20}H_{15}BrN_2O$ | 379.25 | 216-218 | 79.59 | 0.78 |

*for mobile phase: chloroform : benzene : glacial acetic acid (3:1:1, v/v/v).




Scheme 1. Preparation of biphenyl-4-carboxylic acid hydrazide-hydrazone (**IVa-g**).

Biphenyl-4-carboxylic acid (3-chloro-benzylidene)-hydrazide (**IVc**)

IR (KBr, $cm^{-1}$): 3436-3248 ($NH-NH_2$), 3027 (C–H, arom.), 1651 (C=O), 1635-1458 (C=C), 1649(C=N), 773-602 (C–Cl). $^1H$ NMR (300 MHz, DMSO-$d_6$, δ, ppm): 8.25 (s, 1H, CH=N), 8.14-7.67 (m 9H, Ar, H), 8.02 (s, 1H, NH), 7.53-7.44 (m 4H, Ar, H). Analysis: for $C_{20}H_{15}ClN_2O$ calcd. C, 71.75; H, 4.52; N, 8.37%, found C, 71.73; H, 4.55; N, 8.35%.

Biphenyl-4-carboxylic acid (4-chloro-benzylidene)-hydrazide (**IVd**)

IR (KBr, $cm^{-1}$): 3369-3272 ($NH-NH_2$), 3053 (C–H, arom.), 1648 (C=O), 1647-1451 (C=C), 1654 (C=N), 773-602 (C–Cl). $^1H$ NMR (300 MHz, DMSO-$d_6$, δ, ppm): 8.19 (s, 1H, CH=N), 8.11-7.77 (m 9H, Ar, H), 8.01 (s, 1H, NH), 7.55-7.41 (m 4H, Ar, H). Analysis: for $C_{20}H_{15}ClN_2O$ calcd. C, 71.75; H, 4.52; N, 8.37%, found C, 71.77; H, 4.49; N, 8.39%.

Biphenyl-4-carboxylic acid (3-methoxy-benzylidene)-hydrazide (**IVe**)

IR (KBr, $cm^{-1}$): 3437-3281 ($NH-NH_2$), 3027 (C–H, arom), 1649 (C=O), 1622-1475 (C=C), 1641(C=N), 1280-1253 (C–O–C), $^1H$ NMR (300 MHz, DMSO-$d_6$, δ, ppm): 8.21 (s, 1H, CH=N), 8.13-7.67 (m 9H, Ar, H), 8.02 (s, 1H, NH), 7.52-7.38 (m 4H, Ar, H), 3.48 ( s, 1H, -OCH). Analysis: for $C_{21}H_{18}N_2O_2$ calcd. C, 76.34; H, 5.49; N, 8.48%, found C, 76.36; H, 5.51; N, 8.50%.

Table 2. *In vitro* antibacterial activity of title compounds (**IVa-g**)

| Compound | Minimum inhibitory concentration (µg mL$^{-1}$) | | | |
|---|---|---|---|---|
| | *E. coli* (MTCC 40) | *P. aeruginosa* (MTCC 2453) | *S. aureus* (MTCC 121) | *B. subtilis* ( MTCC 96) |
| **IVa** | 2.50 | 2.50 | 1.25 | 2.50 |
| **IVb** | 0.31 | 1.25 | 0.62 | 0.31 |
| **IVc** | 1.25 | 2.50 | 1.25 | 1.25 |
| **IVd** | 1.25 | 2.50 | 1.25 | 2.50 |
| **IVe** | 1.25 | 0.62 | 0.62 | 2.50 |
| **IVf** | 2.50 | 1.25 | 2.50 | 1.25 |
| **IVg** | 1.25 | 0.62 | 1.25 | 0.62 |
| Ciprofloxacin | 0.01 | 0.25 | 0.15 | 0.12 |

Table 3. *In vitro* antifungal activity of the title compounds (**IVa-g**)

| Compounds | Minimum inhibitory concentration (µg mL$^{-1}$) | |
|---|---|---|
| | *C. albicans* (MTCC 8184) | *A. niger* (MTCC 8189) |
| **IVa** | 0.31 | 1.25 |
| **IVb** | 2.50 | 1.25 |
| **IVc** | 0.62 | 2.50 |
| **IVd** | 0.62 | 0.62 |
| **IVe** | 2.50 | 1.25 |
| **IVf** | 1.25 | 1.25 |
| **IVg** | 2.50 | 0.62 |
| Clotrimazole | 0.10 | 0.30 |

Biphenyl-4-carboxylic acid (4-methoxy-benzylidene)-hydrazide (**IVf**)

IR (KBr, cm$^{-1}$): 3369-3262 (NH-$NH_2$), 3037 (C–H, arom.), 1651 (C=O), 1631-1451 (C=C), 1613(C=N), 1288-1241 (C–O–C), $^1$H NMR (300 MHz, DMSO-$d_6$, δ, ppm): 8.17 (s, 1H, CH=N), 8.07-7.51 (m 9H, Ar, H), 8.02 (s, 1H, NH), 7.32-7.25(m 4H, Ar, H), 3.51 ( s, 1H, -OCH). Analysis: for $C_{21}H_{18}N_2O_2$ calcd. C, 76.34; H, 5.49; N, 8.48%, found C, 76.32; H, 5.45; N, 8.45%.

Biphenyl-4-carboxylic acid (3-bromo-benzylidene)-hydrazide (**IVg**)

IR (KBr, cm$^{-1}$): 3442-3352 (NH-$NH_2$), 3033 (C–H, arom.), 1668 (C=O), 1612-1454 (C=C), 1621(C=N), 553-531 (C–Br). $^1$H NMR (300 MHz, DMSO-$d_6$, δ, ppm): 8.11 (s, 1H, CH=N), 8.06-7.48 (m 9H, Ar, H), 8.01 (s, 1H, NH), 7.41-7.21(m 4H, Ar, H). Analysis: for $C_{20}H_{15}BrN_2O$ calcd. C, 63.34; H, 3.99; N, 7.39%, found C, 63.36; H, 3.95; N, 7.36%.

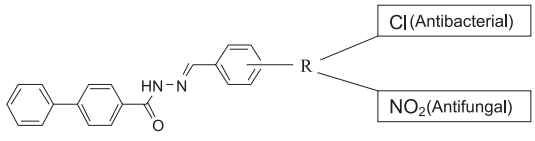
**Antimicrobial evaluation**

The synthesized compounds were evaluated for their *in vitro* antimicrobial activity against Gram positive bacteria: *S. aureus* (MTCC 121), *B. subtilis* (MTCC 96), Gram negative *E. coli* (MTCC 40), *P. aeruginosa* (MTCC 2453) and fungal strains: *C. albicans* (MTCC 8184) and A. *niger* (MTCC 8189). Antimicrobial activity was assessed by serial twofold dilution technique. Ciprofloxacin was used as a standard drug for antibacterial activity and clotrimazole was used as a standard drug for antifungal activity. All the compounds were dissolved

in dimethyl sulfoxide to give a concentration of 10 µg $mL^{-1}$. Twofold dilutions of test and standard compounds were prepared in double strength nutrient broth I.P. (bacteria) or Sabouraud dextrose broth I.P. (11) (fungi). The stock solution was serially diluted to give concentrations of 25-0.78 µg $mL^{-1}$ in nutrient broth. The inoculum size was approximately $10^6$ colony forming units (CFU)/mL. The tubes were incubated at 37 ± 1°C for 24 h (bacteria) and 25°C for 7 days (*A. niger*). After that, the inoculated culture tubes were macroscopically examined for turbidity. The culture tube showing turbidity (lower concentration) and the culture tube showing no turbidity (higher concentration) gave the minimum inhibitory concentration (MIC) for the compound. The MIC for antibacterial is given in Table 2 and MIC for antifungal is given in Table 3.

## RESULTS AND DISCUSSION

The results given in Tables 2 and 3 confirm that compounds with hydrazide-hydrazone units are potential antimicrobial agents. The structures of the synthesized compounds were confirmed by elemental analysis, IR spectra and $^1$H NMR spectral analysis. The data obtained were found to be in good agreement with the calculated values of proposed structures. From the analysis of structures and the activity displayed, some structure-activity relationships can be extracted. The structural requirements for antibacterial and antifungal activity are different for substituted hydrazides. This is evidenced by the fact that the most active antibacterial compound **IVb** showed the least antifungal activity and compound **IVa** being the most active antifungal compounds has shown the least antibacterial activity. The presence of electron-withdrawing groups (-$NO_2$, -Cl, -Br) on aromatic ring improved the antimicrobial activity of compounds.




## CONCLUSION

In conclusion, a series of substituted hydrazide derivatives have been synthesized and their *in vitro* antimicrobial activity was evaluated against six representative microorganisms. The results of antimicrobial study indicated that the presence of halogen moiety in aromatic ring improved antibacterial activity, whereas the presence of nitro group improved antifungal activity of substituted hydrazides.

## REFERENCES

1. Ragavendran J., Sriram D., Patel S., Reddy I., Bharathwajan N., Stables J., Yogeeswari P.: Eur. J. Med. Chem. 42, 146 (2007).
2. Ergenc N., Gunay N. S.: Eur. J. Med. Chem. 33, 143(1998).
3. Todeschini A.R., Miranda A.L., Silva C.M., Parrini S.C., Barreiro E.: J. Eur. J. Med. Chem. 33, 189 (1998).
4. Gemma S., Kukreja G., Fattorusso C., Persico M., Romano M. et al.: Bioorg. Med. Chem. Lett. 16, 5384 (2006).
5. Bijev A.: Lett. Drug. Des. Discov. 3, 506 (2006).
6. Gursoy E., Guzeldemirci-Ulusoy N.: Eur. J. Med. Chem. 42, 320 (2007).
7. Masunari A., Tavaris L.C.: Bioorg. Med. Chem. 15, 4229 (2007).
8. Loncle C., Brunel J., Vidal N., Dherbomez M., Letourneux Y.: Eur. J. Med. Chem. 39, 1067 (2004).
9. Kucukguzel S.G., Mazi A., Sahin F., Ozturk S., Stables J.P.: Eur. J. Med. Chem. 38, 1005 (2003).
10. Vicini P., Zani F., Cozzini P., Doytchinova I.: Eur. J. Med. Chem. 37, 553 (2002).
11. Pharmacopoeia of India, vol. II, p. A-88, Ministry of Health Department, Govt. of India, New Delhi 1996.

*Received: 04. 09. 2010*