Anti-inflammatory Activities of Novel Derivatives of Hydrazide-Hydrazone

Aakashdeep*, Parveen Kumar, N.Kannappan, Mahesh Kumar, Parbhakar Verma

aG.V.M College of Pharmacy, Sonepat, Haryana, India
bAnnamalai University, Chidambaram, TamilNadu, India
cMaharishi Dayanand University, Rohtak, Haryana, India

* Corresponding author. Tel.: +919443878647
E-mail address: kannappan70@yahoo.co.in (N.Kannappan)

Summary
Several biphenyl-4-carboxylic acid hydrazide-hydrazone have been synthesised. These hydrazones derivatives were characterized by CHN analyses, IR, and 1H NMR spectral data. All the compounds were evaluated for their anti-inflammatory activity. All compound exhibited anti-inflammatory activity at the dose 10mg/kg.

Keywords: Synthesis, Hydrazide hydrazone, anti-inflammatory activity.

Introduction
Hydrazide-hydrazone have been demonstrated to possess anticonvulsant [1], antidepressant[2], anti-inflammatory [3], antimalarial [4], antimycobacterial[5], anticancer [6], and antimicrobial [7-10]. These reports prompted us to synthesize the novel derivatives of biphenyl-4-carboxylic acid hydrazide hydrazone. In the present work biphenyl-4-carboxylic acid hydrazides were condensed with substituted aromatic aldehydes to yield the target products. The structures of all compounds have been evaluated by elemental analysis and spectral analysis (IR and 1H NMR). All the compounds have been evaluated for their anti-inflammatory activity.

Experimental
The purity of the synthesized compounds were ascertained by thin layer chromatography on silica gel G in various solvent systems using iodine vapors as detecting agent. Melting points were determined by Toshniwal Melting Point in open capillary tubes and are uncorrected. Elemental analyses were done using Carlo Erba 1106 CHN Analyzer. Infra-red spectra were recorded on Perkin Elmer Spectrum RXI FTIR spectrophotomer in KBr phase. Proton NMR spectra were recorded on Bruker Avance II 400 NMR Ultra Shield Spectrometer using tetramethyl silane as internal standard.
Synthesis of biphenyl-4-carboxylic acid hydrazide hydrazone

(a) Synthesis of biphenyl-4-carboxylic acid methyl ester

A mixture of (0.25 M) biphenyl-4-carboxylic acid and excess of Methanol (250 ml) with 1mL of sulphuric acid was refluxed for 3-4 hrs. in 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 using chloroform: benzene: glacial acetic acid (3:1:1).

Yield is 84.43%, Rf is 0.73, m.p. is 176-178 °C.

(b) Synthesis of biphenyl-4-carboxylic acid hydrazide

A mixture of (0.2 M) biphenyl-4-carboxylic acid methyl ester and excess of hydrazine hydrate (0.30 M) ethanol (250 ml) was refluxed for about 3 hrs and cooled. The solid was separated by filtration and recrystallized from ethanol to afford biphenyl-4-carboxylic acid hydrazide. The purity of compound was checked by single spot TLC using chloroform: benzene: glacial acetic acid (3:1:1).

Yield is 86.79%, Rf is 0.69, m.p. is 182-183 °C.

(c) Synthesis of biphenyl-4-carboxylic acid hydrazide hydrazone

A mixture of (0.025 M) biphenyl-4-carboxylic acid hydrazide and required aromatic aldehydes (0.025 M) was refluxed in methanol (50 ml) in the presence of a catalytic amount of glacial acetic acid for about 2 hrs. The mixture was cooled; the solid was separated by filtration and recrystallized from methanol to give the corresponding hydrazide hydrazones. The purity of compound was checked by single spot TLC using chloroform: benzene: glacial acetic acid (3:1:1).

Chemistry

The reaction between biphenyl-4-carboxylic acid and methanol in the presence of sulphuric acid yielded corresponding methyl ester of biphenyl-4-carboxylic acid, which on reaction with hydrazine afforded the corresponding hydrazides in appreciable yield. Further the hydrazides were condensed with substituted aldehydes to yield the title compounds (Scheme 1). The physicochemical data of synthesized compounds are presented in Table 1.

1. Biphenyl-4-Carboxylic acid-(benzylidene)-hydrazide

IR (cm\(^{-1}\), KBr): 3252-3072 (NH-H\(_2\)), 3055 (C–H str aromatic), 1647 (C=O str amide1), 1612-1472 (C=C str aromatic), 1445(C=N str). \(^1\)HNMR, \(\delta\)ppm (DMSO): 8.12 (s, 1H, CH=N), 7.92-7.24 (m 9H, Ar, H), 8.05 (s, 1H, NH), 782-7.54 (m 5H, Ar) Anal.: Calcd. For C\(_{20}\)H\(_{16}\)N\(_2\)O; C, 79.98; H, 5.37; N, 9.33. Found C, 79.96; H, 5.40; N, 9.31.

2. Biphenyl-4-Carboxylic acid (3-nitro-benzylidene)-hydrazide

IR (cm\(^{-1}\), KBr): 3228-3142 (NH-H\(_2\)), 3046 (C–H str aromatic), 1655 (C=O str amide1), 1606-1471(C=C str aromatic), 1441(C=N str). \(^1\)HNMR, \(\delta\)ppm (DMSO): 8.82-792. (m 4H, Ar, H), 8.15 (s, 1H, CH=N), 8.03 (s, 1H, NH), 782-7.54 (m 9H, Ar) Anal.: Calcd. For For Anal.: Calcd. For C\(_{20}\)H\(_{16}\)N\(_3\)O\(_3\); C, 69.56; H, 4.38; N, 12.17. Found C, 69.60; H, 4.40; N, 12.20.
3. Biphenyl-4-Carboxylic acid (3-hydroxy-benzylidene)-hydrazide

IR (cm$^{-1}$, KBr): 3354-3252 (NH-NH$_2$), 3047 (C–H str aromatic), 1643 (C=O str amide1), 1632-1463 (C=C str aromatic), 1449(C=N str), 1412 (C-O-H bending).

$^1$HNMR, δ ppm (DMSO): 8.24 (s, 1H, CH=N), 8.12-7.84 (m 9H, Ar, H), 8.05 (s, 1H, NH), 7.53-7.44 (m 4H, Ar), 3.48 (s, 1H, -C-O-H).

Anal.: Calcd. For Anal.: Calcd. For C$_{20}$H$_{16}$N$_2$O$_2$; C, 75.93; H, 5.10; N, 8.86. Found C, 75.98; H, 5.15; N, 8.89.

4. Biphenyl-4-Carboxylic acid (4-hydroxy-benzylidene)-hydrazide

IR (cm$^{-1}$, KBr): 3359-3263 (NH-NH$_2$), 3053 (C–H str aromatic), 1633 (C=O str amide1), 1642-1454 (C=C str aromatic), 1454(C=N str), 1404 (C-O-H bending), 1HNMR, δ ppm (DMSO): 8.17 (s, 1H, CH=N), 8.09-7.87 (m 9H, Ar, H), 8.01 (s, 1H, NH), 7.51-7.47 (m 4H, Ar), 3.98 (s, 1H, -C-O-H).

Anal.: Calcd. For Anal.: Calcd. For C$_{20}$H$_{16}$N$_2$O$_2$; C, 75.93; H, 5.10; N, 8.86. Found C, 75.91; H, 5.06; N, 8.82.

5. Biphenyl-4-Carboxylic acid (4-fluoro-benzylidene)-hydrazide

IR (cm$^{-1}$, KBr): 3299-3182 (NH-NH$_2$), 3062 (C–H str aromatic), 1646 (C=O str amide1), 1622-1471 (C=C str aromatic), 1446(C=N str), 1221 (C–F). 1HNMR, δ ppm (DMSO): 8.25 (s, 1H, CH=N), 8.11-7.64 (m 9H, Ar, H), 8.03 (s, 1H, NH), 7.72-7.59(m 4H, Ar) Anal.: Calcd. For Anal.: Calcd. For C$_{20}$H$_{15}$FN$_2$O; C, 75.46; H, 4.75; N, 8.80. Found C, 75.50; H, 4.79; N, 8.82.

Anti-inflammatory Screening

Anti-inflammatory activity of all title compounds was carried out by carrageenan-induced rat paw edema test as described by Winter et al. [11]. Carrageenan-induced rat paw edema test- Albino rats of either sex (150-200 g) were divided into different groups, each containing six individuals. Animals were fasted for 12 h before experiment and only water was allowed. While the first group was a control one and received vehicle sodium CMC (0.5 % w/v) 0.5 mL per rat, the second group received diclofenac sodium 10 mg kg$^{-1}$ body mass. All the remaining groups received orally the test compounds at the same dose. All the suspensions for oral dose were prepared in the vehicle mentioned above and administered in a constant volume of 0.5 ml per rat. After one hour of the administration of the test compound and diclofenac sodium 10 mg kg$^{-1}$ body mass. All the remaining groups received orally the test compounds at the same dose. All the suspensions for oral dose were prepared in the vehicle mentioned above and administered in a constant volume of 0.5 ml per rat. After one hour of the administration of the test compound and diclofenac sodium 0.1 ml of 1% w/v suspension of carrageenan was injected in to the subplanatar of left paw of control and test animals. Immediately, the paw volume was measured using plethysmometer (initial paw volume), there after the paw volume was measured every half an hour till three hours. The difference between initial and subsequent readings gave the edema volume for the corresponding time. Percentage inhibition was calculated shown in table.2

Result and Discussion

The synthesized compounds were characterized by IR, $^1$H-NMR and elemental analytical data was found to be in good agreement with the calculated values. The newly synthesised compounds were subjected to preliminary testing for their anti-inflammatory activity in comparison with the reference drug, diclofenac sodium. The percentage reduction in the inflammation (i.e. reduction in the left hand paw edema volume of the animals) 2 h and 4 h after administration of carrageenan was recorded. All the compounds showed a tendency to causing a reduction in edema. However amongst all compounds tested, compound 2 was found to exhibit high activity.
Table 1

Physiochemical data of title compounds.

<table>
<thead>
<tr>
<th>Compounds no.</th>
<th>Ar</th>
<th>Molecular Formula</th>
<th>Molecular Weight</th>
<th>Melting Points (°C)</th>
<th>%Yields</th>
<th>Rf</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>C₆H₅</td>
<td>C₂₀H₁₆N₂O</td>
<td>300.33</td>
<td>204</td>
<td>81.33</td>
<td>0.66</td>
</tr>
<tr>
<td>2</td>
<td>3-NO₂C₆H₄</td>
<td>C₂₀H₁₅N₃O₃</td>
<td>345.35</td>
<td>250</td>
<td>77.24</td>
<td>0.82</td>
</tr>
<tr>
<td>3</td>
<td>3-OHC₆H₄</td>
<td>C₂₀H₁₆N₂O₂</td>
<td>316.35</td>
<td>257</td>
<td>79.73</td>
<td>0.61</td>
</tr>
<tr>
<td>4</td>
<td>4-OHC₆H₄</td>
<td>C₂₀H₁₆N₂O₂</td>
<td>316.35</td>
<td>251</td>
<td>84.20</td>
<td>0.69</td>
</tr>
<tr>
<td>5</td>
<td>4-FC₆H₄</td>
<td>C₂₀H₁₃FN₂O</td>
<td>318.34</td>
<td>232</td>
<td>75.40</td>
<td>0.81</td>
</tr>
</tbody>
</table>

Scheme 1
Table 2

Anti-inflammatory activity of title compounds.

<table>
<thead>
<tr>
<th>Compounds no.</th>
<th>Ar</th>
<th>Dose (10mg/kg)</th>
<th>% Anti-inflammatory activity 2hr</th>
<th>4hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>C₆H₅-</td>
<td>10</td>
<td>28.18</td>
<td>33.97</td>
</tr>
<tr>
<td>2</td>
<td>3-NO₂C₆H₄</td>
<td>10</td>
<td>31.73</td>
<td>45.06</td>
</tr>
<tr>
<td>3</td>
<td>3-OHC₆H₄</td>
<td>10</td>
<td>28.82</td>
<td>34.90</td>
</tr>
<tr>
<td>4</td>
<td>4-OHC₆H₄</td>
<td>10</td>
<td>29.73</td>
<td>34.61</td>
</tr>
<tr>
<td>5</td>
<td>4-FC₆H₄</td>
<td>10</td>
<td>30.60</td>
<td>37.68</td>
</tr>
<tr>
<td>Standard drug (Diclofenac sodium)</td>
<td></td>
<td></td>
<td>54.18</td>
<td>79.26</td>
</tr>
</tbody>
</table>

References