Research Paper

Synthesis and Anti-platelet Activity of 3-phenyl-4(1H)-quinolones

G. Aruna¹, T. Saritha Jyostna² and Garlapati Achaiah²*

¹Vanitha College of Pharmacy, Hyderabad-506 001, A.P., India.
² University College of Pharmaceutical Sciences, Kakatiya University, Warangal – 506 009, A.P., India.

ABSTRACT: A series of substituted 3-phenyl-4(1H)-quinolones and their 4-alkoxy derivatives have been prepared and evaluated for antiplatelet activity. Among the series compound IVb 1 with 5-ethyl substituent and 4-methoxy group showed greater potency (IC₅₀ = 13.29 µM) when compared to Aspirin (IC₅₀ = 28.10 µM).

Introduction

Thrombin-mediated platelet activation and aggregation are critical for arterial thrombosis in unstable angina and myocardial infarction [Ruggeri et al., 2002]. Human platelet expresses two thrombin receptors PAR1, the principal receptor, and PAR4, which serves as an auxiliary factor to signal platelet activation [Covic et al., 2000]. Platelets play a central role in normal hemostasis and are key participants in pathologic thrombosis due to their capacity to adhere to injured blood vessels and to accumulate at the place of injury. Major stimuli able to induce platelet activation including shape change (into spiny spheres), secretion and aggregation include: collagen, thrombin, and adenosine diphosphate (ADP). These items can bind to fibrinogen, aggregate, and release the contents of their intracellular granules including ADP and serotonin; ADP and arachidonic acid (AA) metabolites act as endogenous platelet activators for thromboxane A2 (Tx A2), intensify the extent of platelet aggregation, and provide positive feedback. Platelet adhesion and activation is a normal physiological response to the accidental rupture of blood vessels, and when such activity is uncontrolled may cause thromboembolic artery occlusion, acute coronary syndrome, and ischemic stroke.

The process of platelet adhesion to extracellular matrix consists in binding to various glycoprotein (GP) receptors mediated by von Willebrand factor (vWF) and collagen [Eriksson et al., 2002]. Factor Xa is a trypsin-like serine protease situated at the convergence of the surface-activated intrinsic and factor-activated extrinsic coagulation pathways. The prothrombinase complex is formed by factor Xa on the phospholipid surface with factor Va and calcium; it catalyzes the proteolysis of prothrombin to thrombin (factor IIa).

Thrombin is the main, final enzyme in the phospholipids coagulation system that leads to fibrin formation. It provides positive and negative feedback regulatory signal in the normal hemostasis, while in pathological conditions factor Xa provides catalytic activation of thrombin. Thus, the inhibition factor Xa affects the coagulation but not the platelet function. Therefore, the inhibition factor Xa may provide a novel, effective antithrombotic drug that provides no risk of bleeding. Recently, the inhibition factor Xa has been intensely investigated in order to replace the existing therapies in the treatment or prevention of thromboembolic disorders [Huang et al., 2003]. Overall, looking at existing therapies and drugs, it can be argued that only some of the thrombotic disorders can be treated efficiently at this moment. Limitations originate from the mechanisms of action, pharmacokinetics, side effect profile and route of administration [Costi et al., 2005]. The current therapies necessitate the development of new, better and safer antithrombotic drugs with different modes of action.

Certain quinolin-2(1H)-one (carbostyril) derivatives have been proved to possess antiplatelet, anti-inflammatory, anti-ulcer, vasodilatory, and phosphodiesterase inhibitory activities [Tominaga et al 1984, Fujioka et al 1992]. Studies on quinolin-2(1H)-one skeleton revealed that the activity was influenced not only by the kind of peripheral side chains but also by the position, 5-substituted quinolin-2(1H)-one exhibited the most potent positive inotropic effect among their positional isomers [Tominaga et al., 1987]. The quinolin-2(1H)-one moiety is versatile and the inhibitory activity of quinolin-2(1H)-one a-methylene-cbutyrolactones against arachidonic acid (AA)-induced platelet aggregation decreases in the order 7-substituted > 6-substituted > 8-substituted. Huang et al., 1998 reported a series of phenyl-4-quinolone possessed aspirin like antiplatelet activity. Among all those compounds, 3-phenyl-4-quinolones (R₃ =
Ph) showed the highest potency with IC$_{50}$ value of 0.17 µM when compared to aspirin IC$_{50}$ = 20.00 µM. 2-Phenyl-4-quinolone showed IC$_{50}$ of 9.3 µM next to 3-phenyl-4-quinolone. Later it was found that 5-alkyl derivative of 1-methyl-2-phenyl-4-quinolone showed improved potency in in vitro studies. This clearly indicated that -CH$_2$CH$_3$ substitution on 5$^{th}$ position of quinolone played a key role in enhancing activity. Similar increase in potency was observed in case of o-alkylated-2-phenyl-4-quinolones when compared to 2-phenyl-4-quinolone.

Since 3-phenyl-4-quinolone showed greater potency than 2-phenyl analogue and no work has been reported on the influence of ethyl substitution at 5 & 7-positions of 3-phenyl-4-quinolone moiety, it has been proposed to carryout the synthesis of 5 & 7-alkyl substituted 3-phenyl-4-quinolones and their corresponding o-methoxy (methoxy & ethoxy) derivatives and evaluate them for their activity on inhibition of platelet aggregation the findings are reported here is.

**Chemistry**

The preparation of 3-phenyl-quinolin-2(1H)-one derivatives is illustrated in Scheme I. Ethyl phenylacetate was treated with ethyl formate in presence of sodium hydride to obtain I as a viscous oil, which was further treated with substituted aniline to form II. Cyclisation of II with polyphosphoric acid afforded the 3-phenyl quinolones (III). Alkylation of 3-phenylquinolin-2(1H)-ones (III) with alkyl iodide under basic conditions gave O-alkyl phenyl quinolones (IV) in good yields. However, a minor quantity of 1-alkyl derivative was also obtained from the reaction of 3-phenylquinolin-2(1H)-one with alkyl iodide.
Antiplatelet activity

The antiplatelet activities were evaluated in washed sheep platelets. Sheep blood was collected from slaughter house into a flask containing required amount of trisodium citrate the platelet rich plasma was separated by centrifuging the citrate blood at 800 rpm for 10 minutes and the remaining blood was centrifuged further 300 rpm for 15 minutes to separate the platelet poor plasma. Mixture of 2 ml of platelet rich plasma and 0.5 ml of platelet poor plasma was taken in a cuvette and optical density was adjusted to 0.4 at 600 nm wavelength. To the solutions of appropriate concentrations of test compounds in test tubes, 2.5 ml of plasma was added and then appropriate amount of adrenaline solution was added such that the final concentration of plasma would be 5 μM, when the volume was made upto 10 ml, optical density was recorded after incubating for 5 minutes at 37± 1°C and mixing each time gently on a cyclomixer. The absorbance changes are monitored on a UV spectrophotometer using a technique originally developed. [O’Brien, 1963]

Results and discussion

Among all the compounds (3-phenyl-4-quinolones and 3-phenyl-4-alkoxy quinolines) tested, the compound IVb1 (R1 = C2H5; R = CH3) with 5-ethyl substituent and 4-methoxy group showed the highest potency with IC50 value of 13.29 μM in platelet aggregation inhibition assay, where as the corresponding 4-methoxy analg IVa1, without 5-ethyl substituent showed a little potency (IC50 = 17.72 μM). Thus compound IVb1 is about 1 and 1/2 times more potent than aspirin as platelet aggregation inhibitor. 4-Ethoxy compound, (IVb2: R = R1 = C2H5), showed comparable potency to that of 4-methoxy analog IVb1 with IC50 values 13.93 and 13.29 μM respectively. However ethyl substitution on 4-ethoxy compound (IVc1) resulted in complete loss of activity. Substitution of 3-phenyl 4(1H)-quinolines with ethyl group at 5th position resulted in a slight decrease in activity (IC50 values 13.93 and 13.29 μM respectively. However 1-methyl derivative (IVc2) with methyl iodide. Similarly, compounds IVa2, 4-ethoxy-3-phenyl-quinoline, IVb2, 5-ethyl-4-ethoxy-3-phenyl quinoline and IVc2, 6-ethyl-4-ethoxy-3-phenyl quinoline were prepared by adopting similar procedure from IIIa, IIIb and IIIc respectively by using ethyl iodide in place of methyl iodide. Formation of 1-methyl derivative, 6-ethyl-1-ethyl-3-phenyl-4-quinolone V, was confirmed based on its spectral data. All the compounds (IIa, IIb, IIc, IVa1, IVb1, IVa2, IVb2 & IVc2) have been purified by column chromatography (silica gel) using a mixture of pet.ether, ethyl acetate (1: 0.5) as eluent. They are characterized by spectral analyses (IR, 1H NMR & elemental analyses). Spectral data of the compounds are presented below:

**IIa**: IR KBr (cm-1) 3447 (-NH) and 1515 (C=O). 1H NMR spectrum (DMSO) (δ ppm): 1.16 (s, 3H, CH3), 6.7-7.4 (m, 10H, aromatic), 8.4 (1H, d, C-5), 7.8 (1H, s, C-2), 7.6-7.7 (2H, m, H-2',6'), 7.4-7.5 (2H, m, C-7, C-8), 7.0-7.3 (4H, m, H-3',4',5'). Elemental analysis: Anal. Calculated: C, 81.43%; H, 5.01%; N, 6.33%. Found: C, 81.39%; H, 5.02%; N, 6.29%.

**IIb**: IR KBr (cm-1) 3450 (-NH), 1510 (C=O). 1H NMR spectrum (DMSO) (δ ppm): 1.1 -1.45 (t, 3H, CH3), 3.0 - 3.50 (q, 2H, CH2), 7.17 - 7.21 (m, 4H, C-6, C-3', C-4', C-5'), 7.38-7.42 (m, 3H, C-8, C-2', C-6'), 7.60 - 7.61 (m, 1H, C-7), 7.77 (s, 1H, C-2), 11. (1H, br, N-H). Elemental analysis Anal. Calculated : C, 81.90%; H, 6.60%; N, 5.62%. Found : C, 81.89%; H, 6.48%; N, 5.59%.

**IVa1**: IR KBr (cm-1) 3450 (-NH), 1510 (C=O). 1H NMR spectrum (DMSO) (δ ppm): 1.1 -1.4 (t, 3H, CH3), 2.7 - 3.0 (q, 2H, CH2), 4.1 - 4.2 (s, 3H, OCH3), 7.17 - 7.21 (m, 4H, C-6, C-3', C-4', C-5'), 7.38-7.42 (m, 3H, C-8, C-2', C-6'), 7.0 - 7.1 (m, 1H, C-7), 7.77 (s, 1H, C-2). Elemental analysis Anal. Calculated : C, 82.10%; H, 6.51%; N, 5.32%. Found : C, 82.01%; H, 6.48%; N, 5.49%.

**IVb1**: IR KBr (cm-1) 1520 (N-H), 1050 (C-O). 1H NMR spectrum (CDCl3) (δ ppm): 1.1 -1.4 (t, 3H, CH3), 2.7 - 3.0 (q, 2H, CH2), 4.1 - 4.2 (s, 3H, OCH3), 7.17 - 7.21 (m, 4H, C-6, C-3', C-4', C-5'), 7.38-7.42 (m, 3H, C-8, C-2', C-6'), 7.0 - 7.1 (m, 1H, C-7), 7.77 (s, 1H, C-2). Elemental analysis Anal. Calculated : C, 82.10%; H, 6.51%; N, 5.32%. Found : C, 82.01%; H, 6.48%; N, 5.49%.

**IVb2**: IR (KBr) (cm-1) 1510 (N=O), 1070 (C-O). 1H NMR spectrum (CDCl3) (δ ppm): 1.2 - 1.5 (t, 3H, CH3), 1.6 - 1.8 (t, 3H, CH3), 2.4 - 2.7 (q, 2H, CH2), 4.0 - 4.2 (q, 2H, CH2), 7.17 - 7.21 (m, 4H, C-6, C-3', C-4', C-5'), 7.38 - 7.42 (m, 3H, C-8, C-2', C-6'), 7.60 -7.61 (m, 1H, C-7), 7.77 (s, 1H, C-2). Elemental analysis Anal. Calculated : C, 82.82%; H, 6.90%; N, 5.05%. Found : C, 82.21%; H, 6.88%; N, 5.01%.

**IVc1**: IR (KBr) (cm-1) 1530(N=C), 1060 (C-O). 1H NMR spectrum (DMSO) at (δ ppm): 1.6-1.8 (t, 3H, CH3), 1.9-2.0 (t, 3H, CH3), 2.2 - 2.4 (d, 2H, CH2), 4.19 - 4.25 (d, 2H, CH2), 7.4 - 8.0 (m, 9H, aromatic). Elemental analysis: Anal. Calculated C, 82.29%; H, 6.90%; N, 5.05%. Found : C, 82.21%; H, 6.88%; N, 5.01%.

**V**: IR KBr (cm-1) 1620 (C=O). 1H NMR spectrum (CDCl3) (δ ppm): 1.19 - 1.3 (t, 3H, CH2CH3), 2.5 - 2.73 (q, 2H, CH2CH3), 3.4 (s, 1H, OCH3), 7.8 - 7.94 (m, 9H, aromatic).

Materials and methods

**Synthesis of 4-alkoxy-3-phenyl quinoline derivatives**

**IIa** (2.21 g, 0.01 mol) was dissolved in dry dimethyl formamide (50 ml), and sodium hydride (80% in oil, 0.3 g, 0.01 mol) was added portion wise with stirring for 30 min at room temperature. Methyllodide (0.01 mol) was then added drop wise at 30-40°C. Stirring was continued for an additional 30 min, and the reaction mixture was poured into ice-water and extracted with chloroform. The organic layer was washed with water, dried over magnesium sulfate, and evaporated. The residue was purified by chromatography on silica gel, using benzene as eluent to get a colourless solid IVa1. Similarly the compound IVb1, 5-ethyl-4-methoxy-3-phenylquinoline was prepared from IIlb. However, 1-methyl derivative (V) was obtained from a reaction of IIIc with methyl iodide. Similarly, compounds IVa3, 4-ethoxy-3-phenyl-quinoline, IVb3, 5-ethyl-4-ethoxy-3-phenyl quinoline and IVc3, 6-ethyl-4-ethoxy-3-phenyl quinoline were prepared by adopting similar procedure from IIIa, IIIb and IIIc respectively by using ethyl iodide in place of methyl iodide. Formation of 1-methyl derivative, 6-ethyl-1-ethyl-3-phenyl-4-quinolone V, was confirmed based on its spectral data. All the compounds (IIa, IIb, IIc, IVa1, IVb1, IVa2, IVb2 & IVc2) have been purified by column chromatography (silica gel) using a mixture of pet.ether, ethyl acetate (1: 0.5) as eluent. They are characterized by spectral analyses (IR, 1H NMR & elemental analyses). Spectral data of the compounds are presented below:
Table 1 Inhibitory effect of 3-phenylquinolones (III) on platelet aggregation induced by adrenaline

<table>
<thead>
<tr>
<th>Compound</th>
<th>R₅</th>
<th>R₆</th>
<th>IC₅₀</th>
</tr>
</thead>
<tbody>
<tr>
<td>IIIa</td>
<td>H</td>
<td>H</td>
<td>14.05</td>
</tr>
<tr>
<td>IIIb</td>
<td>CH₂CH₃</td>
<td>H</td>
<td>15.53</td>
</tr>
<tr>
<td>IIIc</td>
<td>H</td>
<td>CH₂CH₃</td>
<td>NA</td>
</tr>
</tbody>
</table>

Table 2 Inhibitory effect of 3-phenyl-4-methoxy / ethoxy quinolines (IV/V) on platelet aggregation induced by adrenaline

<table>
<thead>
<tr>
<th>Compound</th>
<th>R</th>
<th>R₁</th>
<th>R₅</th>
<th>R₆</th>
<th>IC₅₀</th>
</tr>
</thead>
<tbody>
<tr>
<td>IVa₁</td>
<td>CH₃</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>17.72</td>
</tr>
<tr>
<td>IVa₂</td>
<td>CH₂CH₃</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>17.40</td>
</tr>
<tr>
<td>IVb₁</td>
<td>CH₃</td>
<td>CH₂CH₃</td>
<td>H</td>
<td>H</td>
<td>13.29</td>
</tr>
<tr>
<td>IVb₂</td>
<td>CH₂CH₃</td>
<td>CH₂CH₃</td>
<td>H</td>
<td>H</td>
<td>13.93</td>
</tr>
<tr>
<td>IVc₂</td>
<td>CH₂CH₃</td>
<td>H</td>
<td>CH₂CH₃</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>V</td>
<td>CH₃</td>
<td>H</td>
<td>CH₂CH₃</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Aspirin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>28.10</td>
</tr>
</tbody>
</table>

NA – No activity (i.e. their IC₅₀ > 25 µg/ml)

References


