Synthesis, characterization and biological evaluation of some derivatives of Ethyl-4-hydrazino-3, 4-dihydroquinazoline -2-carboxylate

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ABSTRACT

A facile synthesis of derivatives 2-carbethoxy-3,4-dihydro-4-chloro-quinazoline has been achieved by the cyclization of ethyl anthranilate and ethyl cyanoformate in the presence of dry hydrogen chloride gas then on treatment with hydrazine hydrate to yield ethyl—4-hydrazino-3,4-dihydro-quinazoline-2-carboxylate (Va-e). The structures of newly prepared compounds have been confirmed from IR, 'H NMR and mass spectral data. All the synthesized compounds have been screened for their antimicrobial and anti-inflammatory activities.

Keywords: Synthesis, Elemental analysis, Antimicrobial, Anti-inflammatory

INTRODUCTION

Quinazoline derivatives are well known compounds and are found to possess varied pharmacological activities. They are found to possess antibacterial, antifungal, antiviral, anti-tubercular, anti-inflammatory, estrogenic, antimalarial, bronchodilatory, anticancer, antihypertensive and analgesic etc. in other words, the quinazoline moiety is an important structural feature of many biologically active compounds. In view such reports, we now report the synthesis of some ethyl—4-hydrazino-3,4-dihydro -quinazoline-2-carboxylate (Va-e) and their antimicrobial and anti-inflammatory activity. Ethyl—4-hydrazino-3,4-dihydro -quinazoline-2-carboxylate (Va-e) were prepared by the cyclization of ethyl anthranilate and ethyl cyanoformate with 2-carbethoxy-3,4-dihydro-4-chloro-quinazoline in the presence of dry hydrogen chloride gas then on treatment with hydrazine hydrate.

MATERIALS AND METHODS

Melting points of newly synthesized compounds were determined by open capillary method and were uncorrected. The final products were purified by recrystallization and purity was checked by TLC. The IR spectra of the compounds were recorded on JASCO FT/IR-5300 spectrometer using KBr pellet. 'H NMR spectra were recorded BRUKER DPX-400 MHz on a spectrometer using TMS as internal standard. The spectra were obtained in chloroform and the chemical shift values are reported as values in ppm relative to TMS as internal standard. The Mass spectra were recorded on FENNIGAN MAT 70eV. Physical data of the compounds and percentage yield of various reactions are given in Table.1.

Synthesis of 2-carbethoxy-3,4-dihydro quinazolin-4-one (III)

A stream of dry hydrogen chloride gas was passed through a solution of 4.95 gm (0.03mol) of ethyl anthranilate and 3.0 gm (0.03mol) of ethyl cyanoformate in 90 ml of dioxane for 8 hours and the mixture was allowed to stand at room temperature for 12 hrs. The reaction mixture was heated under reflux for 3 hours, cool then poured in ice water and basified with sodium bicarbonate solution. The solid obtained was filtered with water and recrystallization from benzene. The purity of synthesized product was checked by TLC. Yield 4.6 gm (57.8%) of colorless crystalline compound, melting pt. 206-208°C.

Synthesis of dry hydrogen chloride gas:

HCl gas is produced by allowing concentrated sulphuric acid to react with lumps of fused ammonium chloride in a Kipp’s apparatus the gas was dried by passing through a Drehsel bottle containing concentrated sulphuric acid. The dried gas is used for the synthesis of 2-carbethoxy-3,4-dihydro quinazolin-4-one(III).

Synthesis of 2-carbethoxy-3,4-dihydro-4-chloro-quinazoline (IV)

0.01 mol of 2-carbethoxy-3,4-dihydroquinazoline-4-one(III) and 15 ml of POCl3, was refluxed for 5 hrs. The excess of POCl3 was removed under reduced pressure and neutralized with NaHCO3 solution and solid thus separated was washed with water, dried and recrystallized from ethanol. The purity of synthesized product was checked by TLC. Yield 56%, melting pt. 129-131°C.
**Table-1: Physical data of synthesized compounds Va-e**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Recrystallization Solvent</th>
<th>% yield</th>
<th>Melting Point (°C)</th>
<th>Molecular Formula</th>
<th>Molecular Weight (gm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Va</td>
<td>Chloroform</td>
<td>65</td>
<td>146-148°C</td>
<td>C_{9}H_{15}N_{4}O_{4}</td>
<td>259.23</td>
</tr>
<tr>
<td>Vb</td>
<td>Benzene</td>
<td>50</td>
<td>142-144°C</td>
<td>C_{9}H_{15}N_{4}O_{4}</td>
<td>243.22</td>
</tr>
<tr>
<td>Vc</td>
<td>Benzene</td>
<td>75</td>
<td>152-154°C</td>
<td>C_{9}H_{15}N_{4}O_{4}</td>
<td>318.33</td>
</tr>
<tr>
<td>Vd</td>
<td>Ethanol</td>
<td>62</td>
<td>147-149°C</td>
<td>C_{9}H_{15}N_{4}O_{4}</td>
<td>298.33</td>
</tr>
<tr>
<td>Ve</td>
<td>Ethanol</td>
<td>55</td>
<td>138-140°C</td>
<td>C_{9}H_{15}N_{4}O_{4}</td>
<td>242.23</td>
</tr>
</tbody>
</table>

**Synthesis of 2-carboxethoxy-3,4-dihydro-4-hydrazino-quinazoline (V)**

A mixture of 2-carboxethoxy-3,4-dihydro-4-chloro-quinazoline(IV) 0.01 mol and hydrazine hydrate 0.02 mol (1ml) in 30 ml ethanol was refluxed for 2 hrs. The resulting solution is concentrated, cooled and the solid thus separated was filtered, dried and recrystallized from ethanol. The purity of synthesized product was checked by TLC. Yield 69%, melting pt. 156-158°C.

**Synthesis of ethyl—4-hydrazino-3,4-dihydro -quinazoline-2-carboxylate (Va-e)**

Ethyl—4-hydrazino-3,4-dihydro -quinazoline-2-carboxylate (Va-e) were prepared by the cyclization of ethyl antranilate and ethyl cyanoformate with 2-carboxethoxy-3,4-dihydro-4-chloro-quinazoline in the presence of dry hydrogen chloride gas then on treatment with hydrazine hydrate.

**Ethyl 3-methyl-[1, 2, 4]-triazolo-[4,3-c]-quinazoline-5-carboxylate (Va):**

\[ IR (KBr, cm^{-1}): 3485 \text{ cm}^{-1} (\text{N-H Stretching}), 2922 \text{ cm}^{-1} (\text{C-H Stretching}), 1682 \text{ cm}^{-1} (\text{C=O Stretching}), 1605 \text{ cm}^{-1} (\text{Ar-C-H}), 1524 \text{ cm}^{-1} (\text{Ar-C=C}), 1554 \text{ cm}^{-1} (\text{Carboxylate}), 1460 \text{ cm}^{-1} (\text{C-N}). \]

1H NMR: δ 6.8-8.5 (m, 4H, Ar-H), 1.2-1.5 (s, 5H, -COOC₂H₅), 1.5-4.0 (s, 3H, -N=C-CH₃).

**Ethyl tetrazolo-[1,5-c]-quinazoline-5-carboxylate (Vb):**

\[ IR (KBr, cm^{-1}): 3416 \text{ cm}^{-1} (\text{N-H Stretching}), 2922 \text{ cm}^{-1} (\text{C-H Stretching}), 2163 \text{ cm}^{-1} (\text{N, Stretching}), 1660 \text{ cm}^{-1} (\text{C-N}), 1554 \text{ cm}^{-1} (\text{Carboxylate}), 1448 \text{ cm}^{-1} (\text{Ar-C=C}), 1375 \text{ cm}^{-1} (\text{Ar-C-N}), 1334 \text{ cm}^{-1} (\text{Ar-N-H}). \]

1H NMR: δ 6.8-8.5 (m, 4H, Ar-H), 1.2-1.5 (s, 5H, -COOC₂H₅).

**Ethyl 3-phenyl-[1,2,4]-triazolo[4,3-c]-quinazoline-5-carboxylate (Ve):**

\[ IR (KBr, cm^{-1}): 3428 \text{ cm}^{-1} (\text{N-H Stretching}), 3009 \text{ cm}^{-1} (\text{C-H Stretching}), 1687 \text{ cm}^{-1} (\text{C=O Stretching}), 1602 \text{ cm}^{-1} (\text{Ar-C-H}), 1582 \text{ cm}^{-1} (\text{Ar-C=C}), 1453 \text{ cm}^{-1} (\text{C-N}), 1326,1292 \text{ cm}^{-1} (\text{Ar-C-N}). \]

1H NMR: δ 6.8-8.5 (m, 9H, Ar-H), 1.2-1.5 (s, 5H, -COOC₂H₅), m/z=318 (M⁺).

**Table-2: antibacterial activity of synthesized compound Va-e**

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Comp V</th>
<th>Comp Ve</th>
<th>Comp Vb</th>
<th>Comp Vd</th>
<th>Std</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Diameter of Zone (MM)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>6</td>
<td>5</td>
<td>10</td>
<td>14</td>
<td>7</td>
</tr>
<tr>
<td>Bacillus subtilis</td>
<td>7</td>
<td>6</td>
<td>9</td>
<td>15</td>
<td>NA</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>5</td>
<td>6</td>
<td>12</td>
<td>12</td>
<td>7</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>7</td>
<td>NA</td>
<td>8</td>
<td>13</td>
<td>5</td>
</tr>
</tbody>
</table>

Standard drug: Ampicillin
NA = No activity at this amount of test compound or standard.

**Acute Toxicity Studies**

The acute toxicity test aims at establishing the therapeutic index, i.e., the ratio between the pharmacological effective dose and lethal dose on same strain and species (LD₅₀ / ED₅₀). In any screening programme, acute toxicity on mice is usually performed first. This is a test in which a single dose of drug is used animal on one occasion only for the determination of LD₅₀, median lethal dose (MLD), i.e., the dose which kills 50% of animal of a particular species. LD₅₀ value was determined by a 24 hours test using mice by oral route administration.

**Antimicrobial activity**

Previously liquefied Muller Hinton agar media was inoculated with the requisite quantity of the suspension of the microorganism. The suspension was added to the medium at a temperature between 40-50°C and the inoculated medium was poured immediately into dried Petri dish to occupy a depth of 3-4mm. The Petri dishes were sterilized at 160-170°C for 1 hr., before use. The paper disc (No. 2 Whatmann) was cut down into a small disc (6 mm in diameter) and sterilized in the hot air oven, and then impregnated with the test solutions and standard solution. The dried discs were placed on the surface of the medium.

After addition of all the drugs, Petri dished were left standing for 1 to 4 hrs at room temperature, as a period of pre-incubation diffusion to minimize the effects of variation in time between the applications of different solutions. All the Petri dishes were incubated for 24 hrs at the required temperature, i.e. 37°C for bacteria and 25°C for fungi. After incubation, the diameters of the circular inhibition zones were measured.

**Anti-inflammatory activity**

Anti-inflammatory activity of compounds Va-e was studied by Carrageenan-induced rat paw oedema model. Carrageenan; is a sulphated polysaccharides obtained from sea weed (Rhodophyceae), was used to induce inflammation. The animals were weighed and numbered. A mark was made on both the hind paws (right & left) just beyond tibio-tarsal junction, so that an every time the paw was dipped beyond tibio-tarsal junction, so that an every time the paw was dipped.
left hind paw to induce inflammation. The rats in control group (I) received Vehicle (0.25% CMC solution). While groups III to VII received test compounds Va-e orally in doses 100, 100, 100, 50, and 100mg/kg body weight respectively and group-II received Diclofenac sodium (10mg/kg) subcutaneously. The paw volumes average swelling of paws in group of test treated was compared with control groups (treated with vehicle) and the standard (Diclofenac sodium). Measurement of paw volume (ml) was made by mercury displacement technique using Plethysmograph in a time interval of 60 minutes and 180 minutes, after Carraggenan injection.

RESULTS AND DISCUSSION

The LD_{50} values for the synthesized Va-e were found to be 1000mg/kg, 1000mg/kg, 1000mg/kg, 500mg/kg and 1000mg/kg. 4-Hydradzo quinazolines derivatives are synthesized in good yields (60-70%). All the synthesized quinazolines derivatives have shown potent to weak antibacterial activity. Compounds Vd showed potent activity against Staphylococcus aureus and Bacillus subtilis. The compound Vc and Vd showed good activity against pseudomonas aeruginosa and Escherichia coli compared with the standard respectively. The compounds Va, Vb and Ve showed weak antibacterial activity against all microorganisms and also Vb and Ve compounds does not show activity against Escherichia coli and Bacillus subtilis respectively.

All the synthesized compounds exhibited excellent to potent antifungal activities Compounds Vb and Ve showed excellent activity against Apsergillus niger and Candida albicans respectively. Compound Va shows moderate activity against Apsergillus niger compared with the standard. The compounds Vc and Vd shows good antifungal activity against above organisms. The results are given in Table no 2 and 3.

All the synthesized quinazolines derivatives have shown poor to significant anti-inflammatory activity. Compound Va and Ve shows significant activity. The results of anti-inflammatory activity is given in Table no 4.

REFERENCE:

2. Robert C. Elder field Heterocyclic Compound, vol-06

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