Synthesis of combretastatin analogues with their potent anticancer activity

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ABSTRACT

There is a growing interest in the development of newer and effective antimitotic agents because cancer is a major public health burden in both developed and developing countries. Combretastatin, the most powerful antimitotic agent, is isolated from the bark of the South African Willow tree Combretum caffrum. Combretastatin and its analogues are very powerful antimitotic agents which show their potential biological activities. A series of combretastatin analogues were synthesized and further evaluated for in-vitro cytotoxic activity against a panel of human cancer cell lines. Structures of newly synthesized compounds have been confirmed by using different spectral techniques. All compounds were screened, which showed significant activity against particular cell lines. Biological evaluation of the compounds reflects that all the synthesized compounds show appreciable activity.

Keywords: Combretastatin; Antimitotic; Potent activity; Cell lines; in-vitro.

INTRODUCTION

Combretastatin isolated from the bark of the South African Willow tree- Combretum caffrum acts as a powerful antimitotic agent. It categorized under stilbene family which inhibits the polymerization of tubulin by binding to the colchicine binding site. It show the activity against human cell lines including MDR cancer cell lines (Hamilton, A.C. et al., 1997, Holley, J et al., 1998, Farnsworth, N.R. et al., 1991). Its phosphate ester sodium salts are also use as leading compound for synthesis of many anticancer compounds (Fransworth, N.R. et al., 1988). Anti-angiogenic drugs are those which stop the growth of new blood vessels rather than blocking the existing blood supply to the cancer cells therefore combretastatin is not acts as anti-angiogenic drug (Chaplin, D.J. et al., 1999, West, C.M. et al., 2004) whereas this act by inhibiting polymerization of microtubulues specifically at the colchicine binding site.

The “butterfly model”, which is an essential requirement for the anticancer activity is because of the two aromatic rings present in the moiety of these drugs are look like the wings of a butterfly and fits properly to the binding site of these anticancer drugs. In these stilbenes, two aromatic rings separated by an olefinic bond. Ring-A is very essential for its activity having trimethoxy group (Cushman, M et al., 1991, Cushman, M et al., 1992) whereas Ring-B allow for modifications. cis form of combretastatin is essential requirement for its potent activity whereas trans form reduces its activity. cis form on storage converts into trans form therefore there are so many projects are still going on to freeze cis form(Ter Haar,E et al.,1996). The present work contain 4-nitropheny lacetic acid ring in place of 3-hydroxy-4-methoxy ring, which were synthesized by condensation of 4-nitrophenylacetic acid with appropriate aromatic aldehyde at a high temperature.

The synthesized compounds were evaluated for cytotoxic activity against SF-295(CNS), A-549 (Lung), IGR-OV-1 (Ovary), HOP-62 (Lung), DU-145 (Prostate) (Monks A et al., 1991, Skehan P et al., 1990).

MATERIALS AND METHODS

IR spectra (KBr discs) were recorded on a THERMONICOLET-380 FT-IR spectrophotometer. Proton magnetic resonance spectra were recorded on dpx200 NMR spectrometer using tetramethylsilane (TMS) as the internal standard. Chemical shifts have been expressed

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Reagents used: (a) 4-nitrophenylaceticacid (b) substituted benzaldehyde (c) aceticanhydride (d) triethylamine

in δ values downfield from TMS. Multiplicity of NMR signals is designated as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), b (broad). Silica gel 60-120 mesh was used for column chromatography.

SYNTHESIS
4-nitrophenylacetic acid analogues (S-5 and S-6) of combretastatin were synthesized by using a mixture of 4-nitrophenylacetic acid and substituted aldehydes with triethylamine and acetic anhydride. Then the reaction mixture was acidified with 35% aqueous HCl and re-crystallized from ethanol (95%) to give pure acids.

CHARACTERIZATION
Characterization of synthesized compounds was carried out by using ¹H NMR, IR and Mass spectrum. The synthesized compounds were pure and their cis-configuration (J = 6-15Hz) were also identified on the basis of NMR data. The physical data of these compounds are described in table 2.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Ring B</th>
</tr>
</thead>
<tbody>
<tr>
<td>S5</td>
<td>2,3-dichlorobenzaldehyde</td>
</tr>
<tr>
<td>S6</td>
<td>2,5-dimethoxybenzaldehyde</td>
</tr>
</tbody>
</table>

PHARMACOLOGICAL STUDIES
Pharmacological evaluation of synthesized compounds were carried out by using SF-295(CNS), A-549 (Lung), IGR-OV-1 (Ovary), HOP-62 (Lung), DU-145 (Prostate) cell lines. The compounds were submitted for the anticancer activity to IIIM Jammu. The human cancer cell lines were procured from National Cancer Institute, Frederick, U.S.A. Cells were grown in tissue culture flasks in complete growth medium (RPMI-1640 medium with 2mM glutamine, pH 7.4, supplemented with 10% fetal calf serum, 100 µg/ml streptomycin and 100 units/ml penicillin) in a carbon dioxide incubator (37°C, 5% CO₂, 90% RH) (Monks, A et al., 1991, Skehan, P et al., 1990).

RESULTS AND DISCUSSION
The aim of study was to synthesized the more potent analogues of combretastatin by replacing one of the aromatic ring of combretastatin by 4-nitrophenylacetic acid. These compounds (S-5, S-6) were synthesized by the condensation of 4-Nitrophenylacetic acid with different substituted aldehydes.

S-5: This compound showed significant anticancer activity against A-549 (Lung), IGR-OV-1 (Ovary) cell lines. Two chloride atom attached to this compound was responsible for its activity.

S-6: This showed potent anticancer activity against A-549(Lung), DU-145(prostate) cell lines. Its two dimethoxy groups attached to it are responsible for its activity.

The activity of the compounds must be because of presence of more periphery charges in the space of the compound. The yield of these compounds ranges from 53-63%. NMR analysis of these synthetics showed that the cis isomers were formed. Screening of synthetics for cytotoxic activity revealed that all compounds showed significant response against A-549, IGR-OV-1, DU-145 cell lines taking Adriamycin, Mitomycin C and Paclitaxel as standard. These analogues show their potential cytotoxic activity by inhibiting the polymerization of microtubules. This is because of their butterfly model which is very essential for their anticancer activity.

(a) 2-(4-nitrophenyl)-3-(2,3-dichlorophenyl) acrylacid:
Table 2: Physical data of compounds

<table>
<thead>
<tr>
<th>Compound</th>
<th>IUPAC Names</th>
<th>Molecular Formula</th>
<th>Melting Point (°C)</th>
<th>Yield %</th>
</tr>
</thead>
<tbody>
<tr>
<td>S5</td>
<td>2-(4-nitrophenyl)-3-(2,3-dichlorophenyl)acrylic acid</td>
<td>C₁₅H₁₂O₄N</td>
<td>197-199°C</td>
<td>63</td>
</tr>
<tr>
<td>S6</td>
<td>2-(4-nitrophenyl)-3-(2,5-dimethoxyphenyl)acrylic acid</td>
<td>C₁₇H₁₆O₄N</td>
<td>170-172°C</td>
<td>53</td>
</tr>
</tbody>
</table>

Table 3: Pharmacological activity

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Cell Line Type</th>
<th>Conc.</th>
<th>CNS SF-295</th>
<th>Lung A-549</th>
<th>Ovary IGR-OV-1</th>
<th>Lung HOP-62</th>
<th>Prostate DU-145</th>
</tr>
</thead>
<tbody>
<tr>
<td>S-5</td>
<td>1x10⁻³M</td>
<td>66</td>
<td>86</td>
<td>70</td>
<td>33</td>
<td>34</td>
<td></td>
</tr>
<tr>
<td>S-6</td>
<td>1x10⁻³M</td>
<td>48</td>
<td>78</td>
<td>43</td>
<td>28</td>
<td>75</td>
<td></td>
</tr>
<tr>
<td>Adriamycin</td>
<td>1x10⁻³M</td>
<td>68</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Mitomycin C</td>
<td>1x10⁻³M</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>55</td>
</tr>
<tr>
<td>Paclitaxel</td>
<td>1x10⁻³M</td>
<td>-</td>
<td>63</td>
<td>60</td>
<td>59</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

IR(KBr, ucm⁻¹): 3429(OH),1535,1347(NO₂), 1585(C=O), MS, M⁺ at m/z : 335

(b) 2-(4-nitrophenyl)-3-(2,5-dimethoxyphenyl)acrylic acid:

¹HNMR (200 MHz,CDCl₃): 6.51(1H,d,J=8.3Hz,H-4'),6.66(1H,d,J=8.3Hz,H-3'), 6.82(1H,d,J=8.3Hz,H-6'), 7.68(2H,d,J=8.2Hz,H-2'',6''), 8.11(1H,s,H-3), 8.19(2H,d,J=8.2Hz,H-3'',5''), IR(KBr,μcm⁻¹):3435(OH), 1598(C=O), 1512, 1342(NO₂), MS, M⁺ at m/z : 329

CONCLUSION

From the above spectral and pharmacological data it was concluded that S-5 and S-6 showed potential anticancer activity against A-549, IGR-OV-1 and A-549, DU-145 cell lines respectively taking Adriamycin, Mitomycin C and Paclitaxel as standard.

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