Pharmacological Evaluation of Novel Semi-synthetic Nitric-Oxide Releasing derivatives of Resveratrol for their Biological Activities.

PATENT -- TEMP/E-1/22023/2015-DEL

Dr Sandeep Arora, Dr Anju Goyal, Kartik Sharma, Kapil Bajaj
(Dr Sandeep Arora) Prof and Director,
Chitkara College of Pharmacy, Chitkara University,
Rajpura-140401
The value of natural products in the treatment and prevention of human diseases can be assessed, according to Chin et al. using three criteria: 1) the rate of introduction of new chemical entities of a wide structural diversity, including their application as templates for semi synthetic and total synthetic modification; 2) the number of diseases treated or prevented by these substances; and 3) their frequency of use in the treatment of diseases.
Phytopharmaceuticals in Drug Discovery

• Though pharmaceutical industry appreciates the role of nature as the chief architect of natural products' libraries and respect the science therein, they fear carrying out research in the area. However, the rethinking on fresh strategies is on the verge of gaining prominence due to results of combinatorial chemistry and high throughput screening (HTS) being not highly productive in delivering potent chemical entities.
Phytopharmaceuticals in Drug Discovery

- Historically, most drugs have been derived from natural products, but there has been a shift away from their use with the increasing predominance of molecular approaches to drug discovery.
- Nevertheless, their structural diversity makes them a valuable source of novel lead compounds against newly discovered therapeutic targets. Technical advances in analytical techniques mean that the use of natural products is easier than before.
Phytopharmaceuticals in Drug Discovery

- It has long been recognized that natural-product structures have the characteristics of high chemical diversity, biochemical specificity and other molecular properties that make them favorable as lead structures for drug discovery, and which serve to differentiate them from libraries of synthetic and combinatorial compounds.
INTRODUCTION

• Resveratrol: Resveratrol (3, 5, 4’-trihydroxy-trans-stilbene) is a stilbenoid-a type of natural phenol, and a phytoalexin produced naturally by several plants in response to injury or when the plant is under attack by pathogens such as bacteria or fungi.
• Food sources of resveratrol include the skin of grapes, blueberries, raspberries, and mulberries.
RESVERATROL: SOURCES

* Originally isolated by Takaoka from the roots of hellebore in 1940.
* In 1992, presence in wine was suggested as the explanation for cardioprotective effects of wine.
* In grapes, trans-resveratrol is a phytoalexin found primarily in the skin & produced against the growth of fungal pathogens such as Botrytis cinerea.
* Its presence in Vitis vinifera grapes can also be constitutive, with accumulation in ripe berries of different levels of bound and free resveratrols, according to the genotype.
RESVERATROL: SOURCES

*Red wine contains between 0.2 and 5.8 mg/l, depending on the grape variety, while white wine has much less, because red wine is obtained when grapes are fermented with skin.*

*The resveratrol 3 glycosides are hydrolysed to Trans and cis Resveratrol and Muscadine grapes contain large quantities of Resveratrol.*

*One of the most promising sources is peanuts to the level of 2-5ug/gm when unsprouted and 11-25 grams when sprouted.*

*Other sources include, the fruit of the mulberry with skin, cocoa powder, baking chocolate, dark chocolate, with .5 to 2 mg/kg.
RESVERATROL: SOURCES
## RESVERATROL CONTENT

<table>
<thead>
<tr>
<th>Food</th>
<th>Serving</th>
<th>Total resveratrol (mg)/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peanuts (raw)</td>
<td>1 c (146 g)</td>
<td>0.01 – 0.26</td>
</tr>
<tr>
<td>Peanuts (boiled)</td>
<td>1 c (180 g)</td>
<td>0.32 – 1.28</td>
</tr>
<tr>
<td>Peanut butter</td>
<td>1 c (258 g)</td>
<td>0.04 – 0.13</td>
</tr>
<tr>
<td>Red grapes</td>
<td>1 c (160 g)</td>
<td>0.24 – 1.25</td>
</tr>
<tr>
<td>Cocoa powder</td>
<td>1 c (200 g)</td>
<td>0.28 – 0.46</td>
</tr>
</tbody>
</table>
• One gram of freeze-dried skins are treated as reported by Sun et al. (2006), with some modifications. Briefly, the berry skins were introduced into 40 mL of methanol containing 50 μL of hydrochloric acid and 250 μL of internal standard (trans-hydroxyl stilbene, 200 μg/mL in ethanol).
• After homogenisation for 1 min, the samples are maintained for 48 h in closed containers under stirring at room temperature in the dark.
• The extract containing polyphenols was obtained by centrifugation (5 000 g, 5 min) and the solid residue are washed twice with 5 mL of methanol.
• The washing solutions are added to the first supernatant and the mixture filtered with a 0.2 μm PTFE syringe filter.
• After adding 2 mL of water, the extract is almost completely evaporated to dryness under vacuum at 35°C. The dense residue obtained is suspended in 20 mL of water and stilbene compounds are extracted twice for 15 min with 10 mL of ethyl acetate.
RESVERATROL: THERAPEUTIC POTENTIAL

*Resveratrol interferes with all three stages of carcinogenesis including initiation, promotion and progression by acting through NF κB modulation, inhibition of Cytochrome P 450, and other mechanisms.
The action is also proposed to be due to induction of apoptosis, either Fas-Fas ligand mediated, or p53 and Cyclins and Cyclin dependent kinases mediated apoptosis.
It is however found to be useful in Neuronal cell degeneration and so useful in Huttington disease.
RESVERATROL: THERAPEUTIC POTENTIAL

* Resveratrol also significantly increases natural Testosterone production by selective estrogen receptor modulation and aromatase inhibition (68)
* Resveratrol increased intracellular glutathione levels via Nrf2-dependent upregulation of gamma glutamylcystein ligase in lung epithelial cells helping reduce smoke based stress injury.
* Another potentially important mechanism common to both resveratrol supplementation and caloric restriction is the modulation of autophagy with involvement of SIRT 1 and m TOR.
* The oxidative stress induced by ultraviolet radiation is one of the main causes for premature skin ageing. The photoprotective effects of several polyphenols known for their antioxidant properties, including resveratrol, has been studied
* The neuroprotective effects have been confirmed in several animal model studies and Sirtuin activation is a prominent effect.
Resveratrol Derivatives

* Dihydioresveratrol
* Epsillon vinniferin
* Pallidol
* Quadrangularin A
* Transdiptoindonesin (Dimer)
* Hopeaphenol (Tetramer)
* Piceatenol, Piceid, Pterostilbeine
Resveratrol – Application summary

- **Cancer Chemoprevention and Therapy**
  - Inhibition of NFκB, STAT3, COX2 pathways; apoptosis of cancer cell via autophagy/pro-oxidant pathways; carcinogens metabolism; cancer initiation, promotion, and progression signaling inhibition.

- **Chronic Inflammation**
  - Inhibition of COX1 and 2 expression; inhibition of NFκB signaling resulting in reduction of pro-inflammatory cytokines production: IL’s, CRP, TNFα.

- **Cardiovascular Protection**
  - Inhibition of iNOS; increased eNOS; inhibition of platelets aggregation; increased HDL; decreased TG, LDL and peroxidation; ICAM/VCAM formation inhibition.

- **Neurological Disorders**
  - Increased cerebral blood flow; mitochondrial function and mass; inhibition of fibrin β-amylol peptide formation and deposit; prevention of oxidative damage on astroglial cells.

- **Insulin Resistance Type 2 Diabetes, Hepatic Steatosis**
  - AMPK activation; increased insulin sensitivity and glucose uptake by muscles; β-cells insulin secretion modulation; restriction of insulin anti-lipolytic activity.

- **Obesity**
  - Increased mitochondrial biogenesis and fat oxidation; increased uncoupling protein 1 and basal metabolic rate; inhibition of adipogenesis by up-regulation of lipolytic and down-regulation of lipogenic genes.

- **Oxidative Stress**
  - Up-regulation of de-novo antioxidant enzymes synthesis resulting in increased anti-oxidant capacity and activity; regeneration of α-tocopherol; free radical scavenging; lipid peroxidation inhibition.

- **Other**
  - Osteoporosis / rheumatoid arthritis / bone loss
    - Stimulation of osteogenesis through inhibition of receptor activator of NFκB ligand; inhibition of caspase 3.
  - UV radiation injury protection
    - Anti-microbial activity
Nitric oxide

• Nitric oxide, or nitrogen oxide, also known as nitrogen monoxide, is a molecule with the chemical formula NO.
• It is a free radical.
• Nitric Oxide: involved in a multitude of physiological and pathophysiological states in mammals. For example, it is now known to be involved in the control of blood pressure neurotransmission, and in the immune defense system of the body.
• Nitric Oxide has low solubility in water and is unstable in the presence of various oxidants.
• Difficult to introduce as such into biological systems in a controlled or specific fashion. Therefore, development of chemical agents that release NO is important.
• Numerous compounds are available which show anti-inflammatory, analgesic, anti-ulcer, antimicrobial agents and antihyperlipidemic activity.
• Resveraterol, a natural compound reported to possess a broad spectrum of biological activity with less side-effects.
• Due to its wide range of biological activity, resveraterol has received a considerable interest in the field of drug discovery and therefore it constitutes a relevant synthetic target in Pharmaceutical Industry.
AIM OF STUDY

• To synthesize novel, semi-synthetic derivatives of resveratrol and nitric oxide.
• To disclose novel semi-synthetic derivatives which provide significant therapeutic activity.
Plan of Work

• To characterize authentic procured resveratrol by means of TLC, UV, IR etc.

• To characterize synthesised (E)-5-(4-hydroxyalkanoloxy) benzene-1,3-diol by the reaction of (E)-5-(4-hydroxystyryl)benzene-1,3-diol (Resveratrol) (I) with 4-chloroalkanols to yield the compounds (II) by means of chromatographic and spectrophotometric methods (Series-1)
To characterize synthesised (E)-5-(4-nitroxyalkanoloxy)benzene-1,3-diol (III) by the reaction of (E)-5-(4-hydroxystyryl)benzene-1,3-diol (Resveratrol) (I) with 4-chloroalkanols to yield the compounds (II) by means of chromatographic and spectrophotometric methods (Series-1).

To characterize synthesised (((E)-methyl 4-(4-(3,5-dihydroxystyryl)phenoxy)alkanoate (II) by the reaction
of (E)-5-(4-hydroxystyryl)benzene-1,3-diol (Resveratrol) (I) with n-chloroalkanoic acid to yield the compounds (II) by means of chromatographic and spectrophotometric methods (Series-2).
• To characterize synthesised (E)-5-(4-nitroxyalkanoloxy)benzene-1,3-diol (III) by the reaction of (E)-5-(4-hydroxystyryl)benzene-1,3-diol (Resveratrol) (I) with 4-chloroalkanols to yield the compounds (II) by means of chromatographic and spectrophotometric methods (Series-2).
To measure Nitric oxide release by using Grignard’s reagent.

• Pharmacological Evaluation:
  - Anti-inflammatory activity
  - Analgesic activity
  - Antimicrobial studies
  - Circulatory disorders
Synthetic General schemes:
(For Series-1)

(E)-5-(4-hydroxyalkanoloxy)benzene-1,3-diol

(E)-5-(4-hydroxystyrlyl)benzene-1,3-diol

Cl-CH₂-)n-OH

(E)-5-(4-hydroxyalkanoloxy)benzene-1,3-diol

AC₂O/CHCl₃

NaNO₂/HNO₃

(E)-5-(4-nitroxyalkanoloxy)benzene-1,3-diol
When, \( n=1 \), \(-O-\text{CH}_2\text{-ONO}_2\), (E)-5-(4-nitroxyethanoloxy)benzene-1,3-diol

\( n=2 \), \(-O-\text{CH}_2\text{CH}_2\text{-ONO}_2\), (E)-5-(4-nitroxypropanoloxy)benzene-1,3-diol

\( n=3 \), \(-O-\text{CH}_2\text{CH}_2\text{CH}_2\text{-ONO}_2\), (E)-5-(4-nitroxybutanoloxy)benzene-1,3-diol

\( n=4 \), \(-O-\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{-ONO}_2\), (E)-5-(4-nitroxypentanoloxy)benzene-1,3-diol

\( n=5 \), \(-O-\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{-ONO}_2\), (E)-5-(4-nitroxyhexanoloxy)benzene-1,3-diol

\( n=6 \), \(-O-\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{-ONO}_2\), (E)-5-(4-nitroxyhexanoloxy)benzene-1,3-diol
When, \( n=1 \), \(-O-CH_2-CO-ONO_2\), (E)-methyl 4-(4-(3,5-dihydroxystyryl)phenoxy)nitromethanoate

\( n=2 \), \(-O-CH_2CH_2-CO-ONO_2\), (E)-methyl 4-(4-(3,5-dihydroxystyryl)phenoxy)nitroethanoate

\( n=3 \), \(-O-CH_2CH_2CH_2-CO-ONO_2\), (E)-methyl 4-(4-(3,5-dihydroxystyryl)phenoxy)nitropropanoate

\( n=4 \), \(-O-CH_2CH_2CH_2CH_2-CO-ONO_2\), (E)-methyl 4-(4-(3,5-dihydroxystyryl)phenoxy)nitrobutanoate

\( n=5 \), \(-O-CH_2CH_2CH_2CH_2CH_2-CO-ONO_2\) (E)-methyl 4-(4-(3,5-dihydroxystyryl)phenoxy)nitropentanoate

\( n=6 \), \(-O-CH_2CH_2CH_2CH_2CH_2CH_2-CO-ONO_2\) (E)-methyl 4-(4-(3,5-dihydroxystyryl)phenoxy)nitrohexanoate
Work Done

• Resveratrol Characterization:
  - Colour: White
  - Physical State: Fine powder
  - Solubility: Freely soluble in Ethanol

• Spectral Characterisation:
  - $\lambda_{\text{max}}$ of Resveratrol in Ethanol: 340 nm

IR in KBr:
Synthesis of Compound-1 of scheme-2

- Resveratrol = 22.8 mg for 0.001 mole
- N-Chloroacetic acid = 9.0 mg for 0.001 mole
- Triethlamine = 101 mg for 0.001 mole
- Acetone = 50 mL
- Benzene = 5 mL

- Resveratrol refluxed for 10 hrs with n-Chloroacetic acid and Triethlamine in acetone.
- Further refluxed for 8 hrs after adding benzene.
• % yield of recrystallised compound-1 in ethanol : 30%

• Colour: yellow

• Physical state: solid

• IR Spectral Characterisation:
  • -CO-O stretching : 1174 cm^{-1}
  • C-O stretching : 1708 cm^{-1}
  • Aromatic =CH stretching : below 800 cm^{-1}
OTHER CHARACTERISATION WORK IN PROGRESS:
1. NMR
2. MP Corelations

PHARMACOLOGICAL SCREENING:

1. ANTIINFLAMMATORY & ANTINOCICEPTIVE:
   A. Adjuant induced Arthritis studies and lysosomal enzyme inhibition: Trials under process and positive
   B. ACETIC ACID INDUCED WRYTHING AND ANTINOCICEPTIVE ACTIVITY: TRIALS UNDER PROCESS AND POSITIVE
Future Plan of Work:

• Synthesis of all compounds of scheme -1 and 2.
• Chromatographic and Spectral characterisation of all the synthesized compound
• Detection of NO release by Griess Reagent.
• Pharmacological Evaluation of syntheised compounds.
References:


References:

Bhat KP, Lantvit D, Christov K, Mehta RG, Moon RC, Pezzuto JM; Lantvit; Christov; Mehta; Moon; Pezzuto (October 2001). "Estrogenic and antiestrogenic properties of resveratrol in mammary tumor models". Cancer Res. 61 (20): 7456–63.


Ghosh HS, McBurney M, Robbins PD; McBurney; Robbins (2010). SIRT1 negatively regulates the mammalian target of Rapamycin, PLoS ONE, 5 (2), e9199

Morselli E,


References:


Thank you for your kind Attention