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

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

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Phytopharmaceuticals in Drug Discovery

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.

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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

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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

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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.

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RESVERATROL: SOURCES

* Originally isolated by Takaoka from the roots of hellebore in 1940.
* Later, in 1963, from the roots of Japanese knotweed.
* 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.

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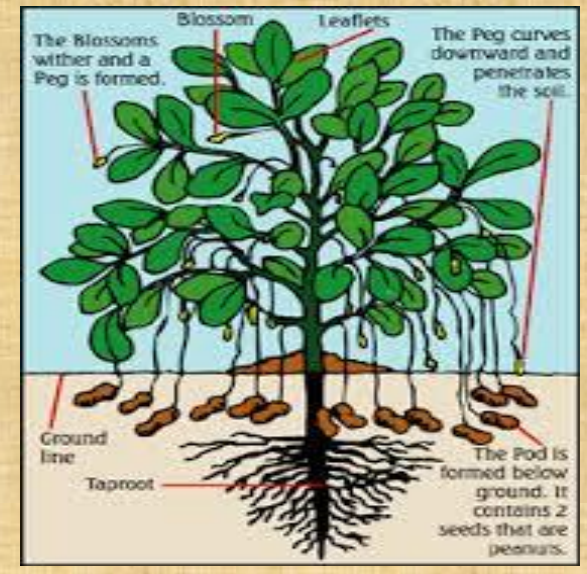
RESVERATROL: SOURCES

*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: SOURCES



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RESVERATROL CONTENT

Food	Serving	Total resveratrol (mg)/kg
Peanuts (raw)	1 c (146 g)	0.01 – 0.26
Peanuts (boiled)	1 c (180 g)	0.32 – 1.28
Peanut butter	1 c (258 g)	0.04 – 0.13
Red grapes	1 c (160 g)	0.24 – 1.25
Cocoa powder	1 c (200 g)	0.28 – 0.46

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Resveratrol extraction

- 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.

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RESVERATROL: THERAPEUTIC POTENTIAL

*Resveratrol interferes with all three stages of carcinogenesis including initiation, promotion and progression by acting through NF kB 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

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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 involvment 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.**

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Resveratrol Derivatives

*Dihydroresveratrol
*Epsillon vinniferin
*Pallidol
*Quadrangularin A
*Transdiptoindonesin (Dimer)
*Hopeaphenol (Tetramer)
*Piceatenol, Piceid, Pterostilbeine

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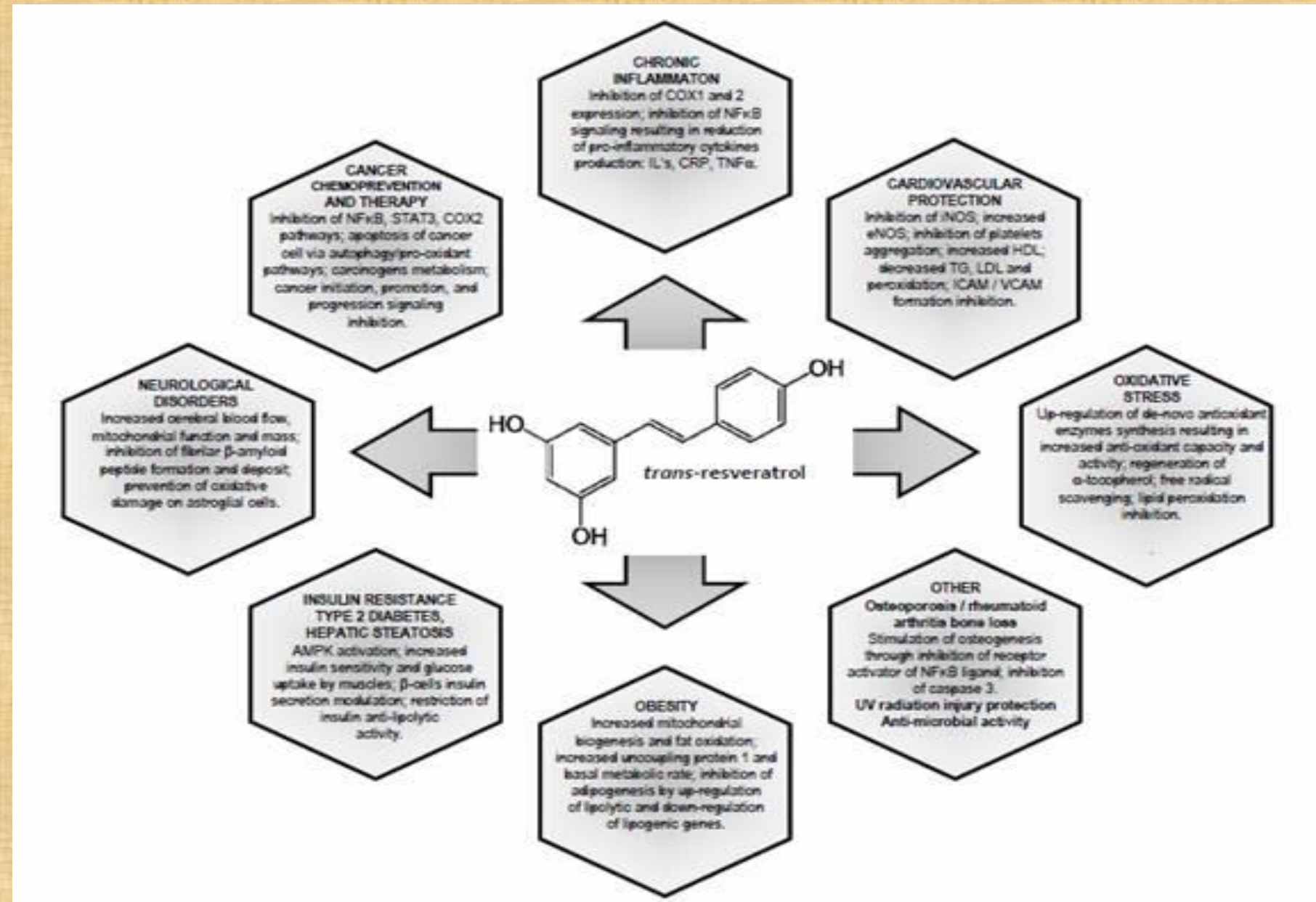
Resveratrol –Application summary



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.

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- 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.

•Resverateroll, a natural compound reported to possess a broad spectrum of biological activity with less side-effects.

• Due to its wide range of biological activity, resverateroll 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)

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- 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

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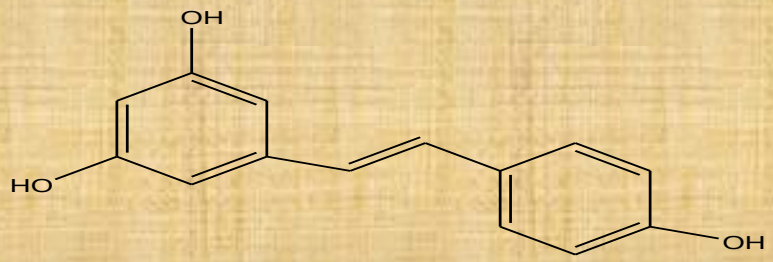
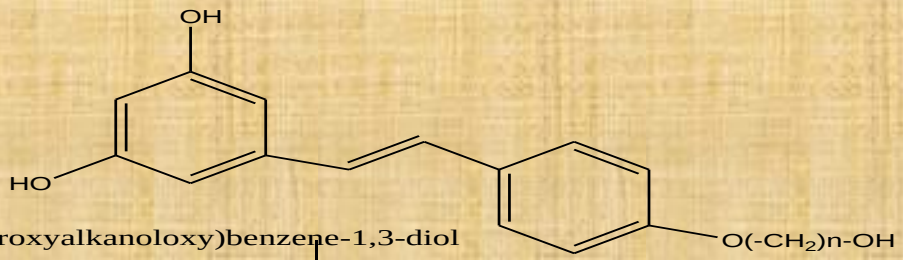
Synthetic General schemes: (For Series-1)



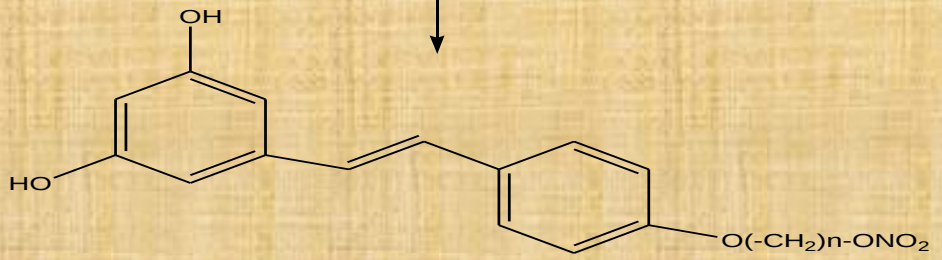
(*E*)-5-(4-hydroxystyryl)benzene-1,3-diol

$Cl\text{-}CH_2\text{-})n\text{-}OH$



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

$AC_2O/CHCl_3$ $NaNO_2/HNO_3$



(*E*)-5-(4-nitroxyalkanoloxy)benzene-1,3-diol

Scheme-1

When, n=1, –O-CH_2-ONO_2, (E)-5-(4-nitroxymethanoloxy)benzene-1,3-diol

n=2, –O-CH_2CH_2-ONO_2, (*E*)-5-(4-nitroxyethanoloxy)benzene-1,3-diol

n=3, –O-$CH_2CH_2CH_2$-ONO_2, (*E*)-5-(4-nitroxypropanoloxy)benzene-1,3-diol

n=4, –O-$CH_2CH_2CH_2CH_2$-ONO_2, (E)-5-(4-nitroxybutanoloxy)benzene-1,3-diol

n=5, –O-$CH_2CH_2CH_2CH_2CH_2$-ONO_2, (*E*)-5-(4-nitroxypentanoloxy)benzene-1,3-diol

n=6, , –O-$CH_2CH_2CH_2CH_2CH_2CH_2$-ONO_2, (*E*)-5-(4-nitroxyhexanoloxy)benzene-1,3-diol

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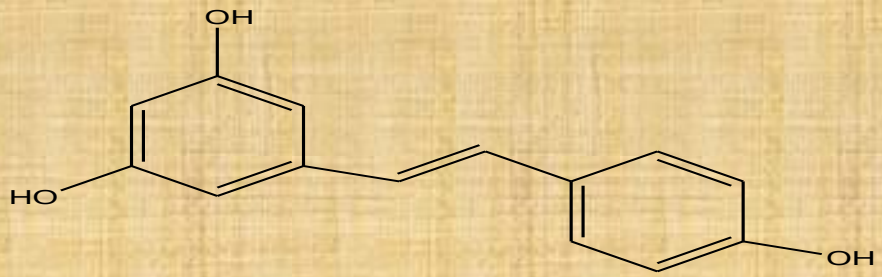
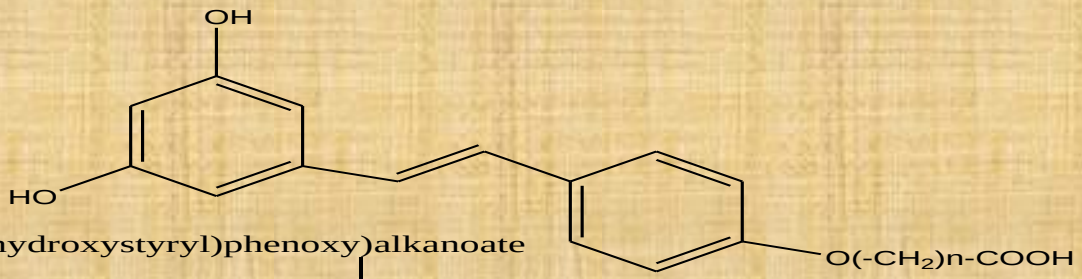
Scheme-2



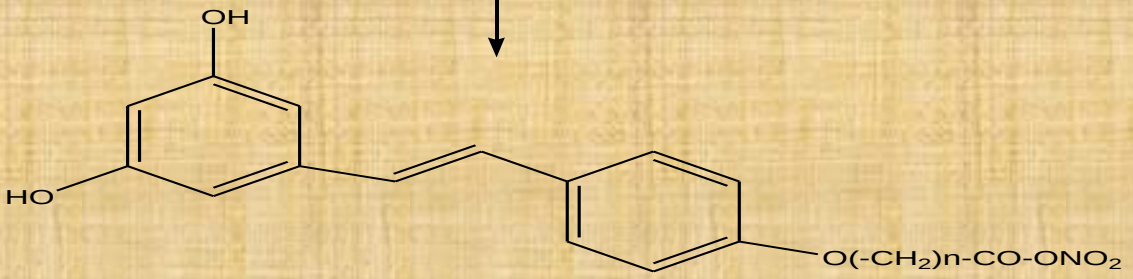
(*E*)-5-(4-hydroxystyryl)benzene-1,3-diol

$Cl\text{-}(\text{-}CH_2)n\text{-}COOH$



(E)-methyl 4-(4-(3,5-dihydroxystyryl)phenoxy)alkanoate

$AC_2O/CHCl_3$ $NaNO_2/HNO_3$



(E)-methyl 4-(4-(3,5-dihydroxystyryl)phenoxy)nitroalkanoate

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)nitrobutanoat

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

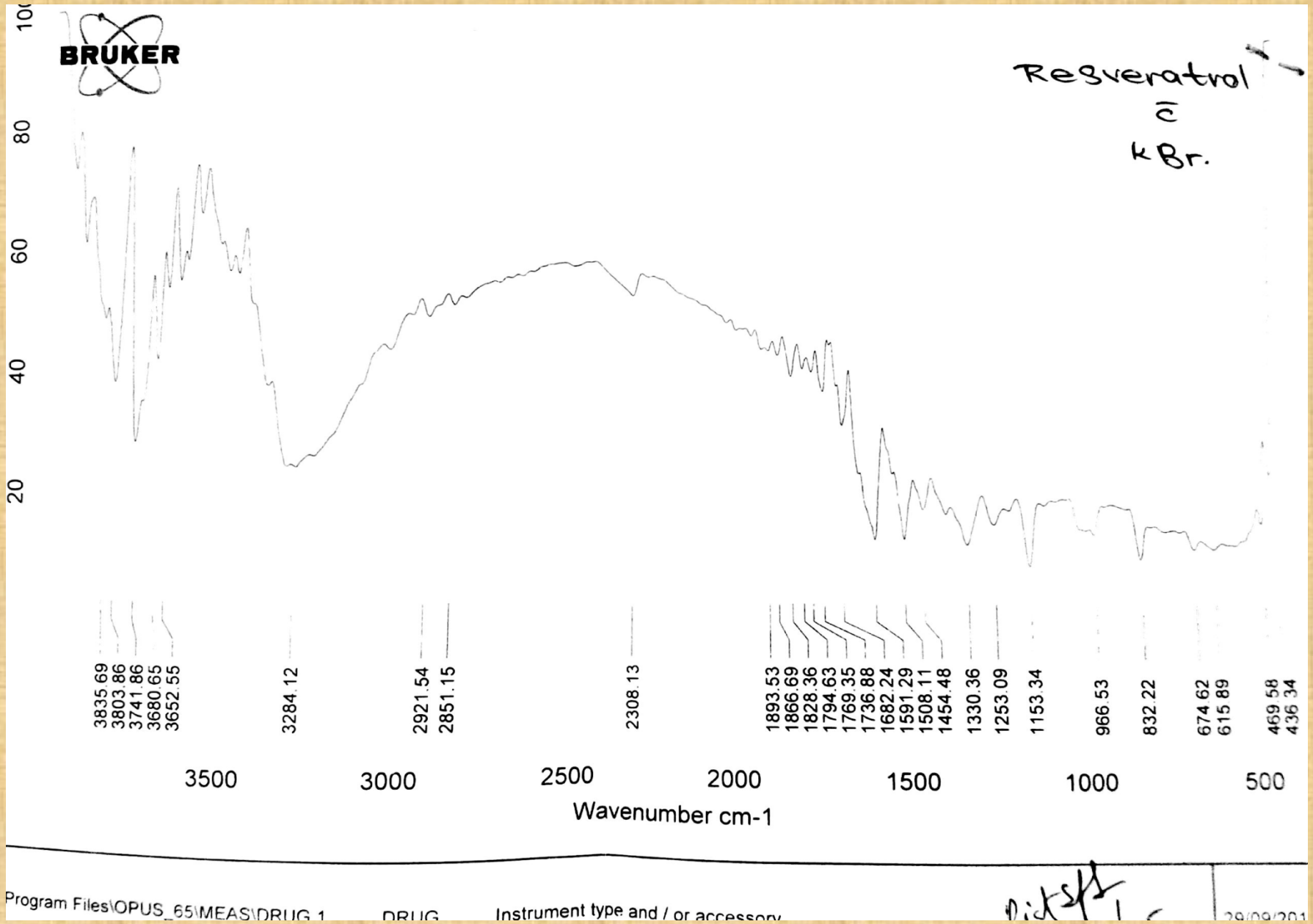
  Physical State: Fine powder

  Solubility: Freely soluble in Ethanol

- Spectral Characetrisation:

  λmax of Resveratrol in Ethanol: 340 nm

IR in KBr:


BRUKER
Resveratrol
in
KBr.
100
80
60
40
20
3835.69
3803.86
3741.86
3680.65
3652.55
3284.12
2921.54
2851.15
2308.13
1893.53
1866.69
1828.36
1794.63
1769.35
1736.88
1682.24
1591.29
1508.11
1454.48
1330.36
1253.09
1153.34
966.53
832.22
674.62
615.89
469.58
436.34
3500
3000
2500
2000
1500
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Wavenumber cm-1
Program Files\OPUS_65\MEAS\DRUG 1
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Instrument type and / or accessory

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Synthesis of Compound-1 of scheme-2

- Resveratrol = 22.8 mg for 0.001mole
- N-Chloroacetic acid = 9.0 mg for 0.001 mole
- Triethlamine = 101mg for 0.001 mole
- Acetone = 50 mL
- Benzene = 5 mL
- Resveratrol refluxed for 10 hrs with n-Chloractic acid and Triethlamine in actone.
- Further refluxed for 8hrs after adding benzene.

•% yield of recrystallised compound-1 in ethanol : 30%

•Colour: yellow

•Physical state: solid

•IR Spectral Characterisation:

•-CO-O stretching : 1174cm^{-1}

•C-O stretching : 1708cm^{-1}

•Aromatic =CH stretching : below 800cm^{-1}

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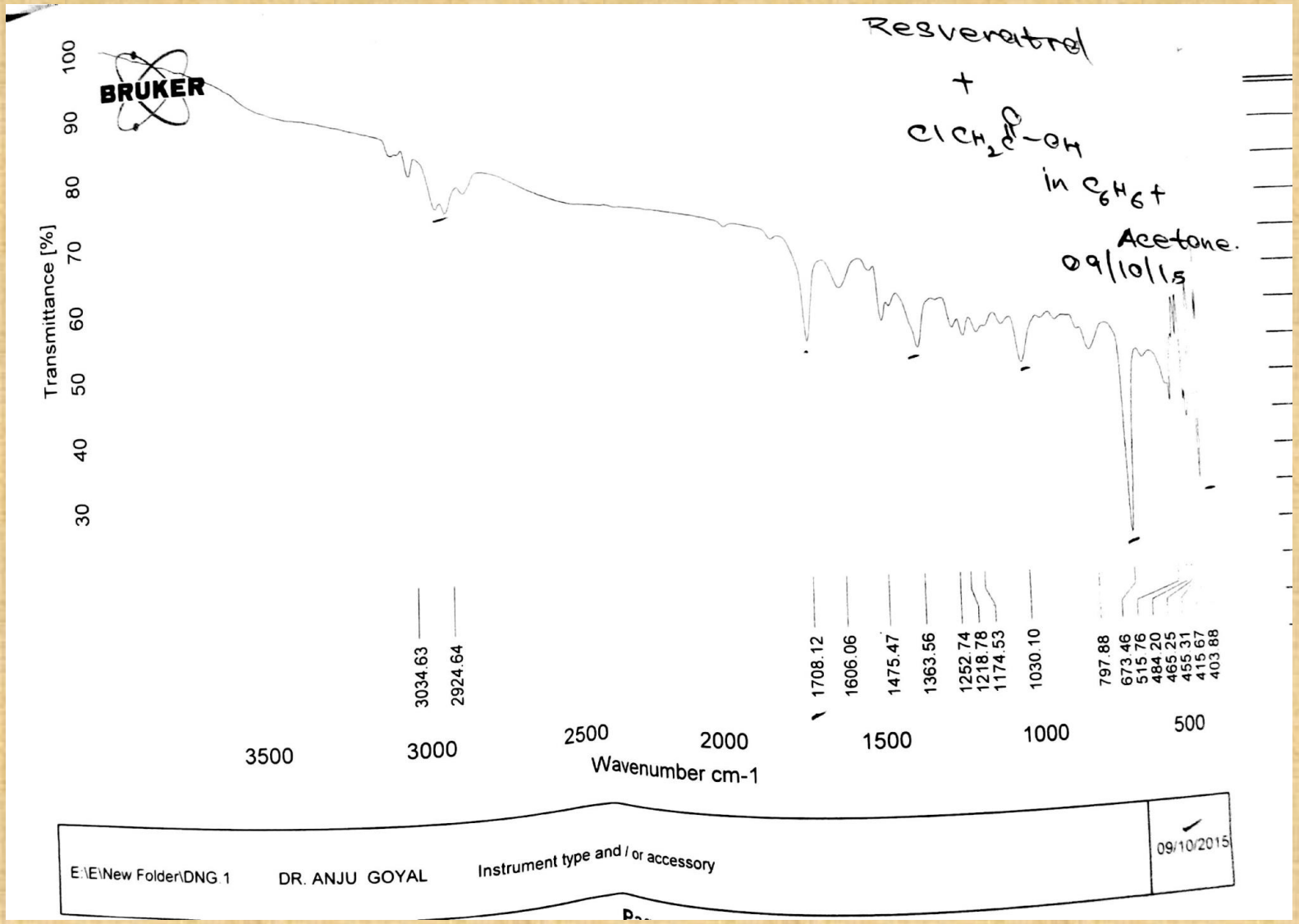

CHARACTERISATIONS AND PHARMACOLOGICAL PROFILING

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 componds 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.

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