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Synthesis and Pharmacological Evaluation of Some Chalcone Derivatives

G.R. Shendarkar[1*], D.M. Savant[2], K.D. Badole[2], G.S. Waghmare[3]

[1]Department of Pharmacognosy, Nanded Pharmacy College, Nanded-431602, Maharashtra, India
[2]Department of Pharmacology, Nanded Pharmacy College, Nanded-431602, Maharashtra, India.
[3]Department of Chemistry, Yeswant Mahavidyalaya, Nanded-431602, Maharashtra, India.

***Corres.author:gshendarkar@gmail.com**
***Tel +91 02462 286631; Fax: +91 02462 254445**

Abstract: Noval chalcone derivatives have been synthesized by condensation reaction of equimolar quantity of aldehyde with acetophenone in presence of aqueous alcoholic alkali. The structures of these newely synthesized compounds have been confirmed by spectral data. The synthesized compounds are screened for *in vitro* antioxidant activity on four different models namely Diphenyl Picryl hydrazine, Nitric oxide, Hydrogen peroxide and Reducing power assay. Ascorbic acid was used as standard.
Keywords: Chalcones, aldehydes, acetophenone, antioxidant activity.

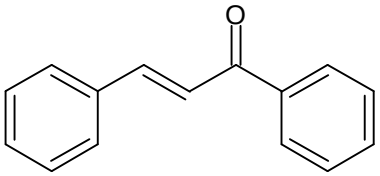
INTRODUCTION :

Heterocyclic synthesis has emerged as powerful technique for generating new molecules useful for drug discovery[1]. Heterocyclic compounds provide scaffolds on which pharmacophore can arrange to yield potent and selected drugs[2].Chalcone is an aromatic ketone that forms the central core for a variety of important biological compounds which are collectively known as chalcones. (fig.1)

The different structure a modification of chalcones has reported by various researchers[3].In fact, not many structural templates can claim association with such a diverse range of pharmacological activities. Some substituted chalcones and their derivatives have been reported to possess cytotoxic, antitumor, anti-inflammatory, antiplasmodial, immuno suppressant and antioxidant activities[4].

Chalcones are also intermediates in biosynthesis of flavonoids, which are substances wide spared in plants and with an array of biological activities[5].

Fig.1: Structure of Chalcone Nucleus



An antioxidant is a molecule capable of inhibiting the oxidation of other molecules. Oxidation is a chemical reaction that transfers electrons or hydrogen from a substance to an oxidizing agent. Oxidation reactions can produce free radicals. In turn, these radicals can start chain reactions. When the chain reaction occurs in a cell, it can cause

damage or death to the cell. Antioxidants terminate these chain reactions by removing free radical intermediates, and inhibit other oxidation reactions[6].

Chalcones basic structure includes two aromatic rings are linked by an, β-unsaturated carbonyl group, a unique template associated with very diverse application[7].Due to presence of reactive keto, vinylenic group, chalcone and their analogue have been reported to antioxidant[8].

The present paper deals with synthesis of different noval chalcones followed by evaluation of their antioxidant potential by using various antioxidant methodologies.

EXPERIMENTAL:

1. MATERIALS AND METHODS

Chalcones were synthesized from acetophenone and aromatic aldehyde by claisen-schmidt condensation reaction. Various physicochemical technique such as elemental .analysis, Mass, IR, have been employed to assign the structure of newly synthesized chalcones.The reaction were carried out with analytical reagent grade chemicals. The organic solvents were crystallized before reuse. The melting points were measured in open capillary tube on a Buchi 530 melting point apparatus and were uncorrected. The IR Spectra were recorded on a Parkin-Elmer 1600 series FTIR spectrophotometer in KBr pallets. Mass spectra were measured with a Shimadzu GC-MS-QP5000 spectrophotometer. The homogeneity of the compounds was monitored by ascending thin layer chromatography (TLC) on silica gel G (Merck) coated aluminum plates, visualization by iodine vapor.

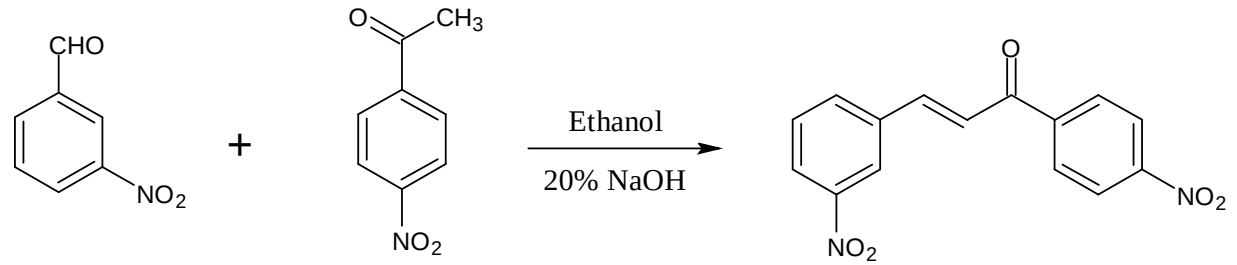
Synthesis of (*E*)-3-(3-nitrophenyl)-1-(4-nitrophenyl)prop-2-en-1-one (DMS-1)

Equimolar quantities of 3-nitro benzaldehyde and 1-(4-nitrophenyl) ethanone in minimum quantity of ethanol were dissolved. 20% NaOH in water was added in portion at room temperature. The reaction flask was kept in magnetic stirrer to about 3-4 hrs and kept overnight. The content of the flask were then acidified by dil.HCL and poured over crushed ice. The solid obtained was filtered & Wash with cold water & dried. **(Scheme-1)**

Synthesis of (*E*)-3-(4-methoxyphenyl)-1-(4-nitrophenyl)prop-2-en-1-one (DMS-2)

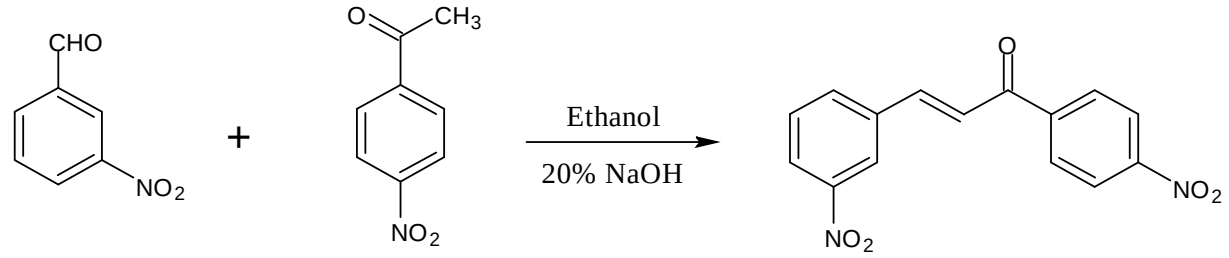
Equimolar quantities of 4-methoxy benzaldehyde and 1-(4-nitrophenyl) ethanone in minimum quantity of ethanol were dissolved. 20% NaOH in water was added in portion at room temperature. The reaction flask was kept in magnetic stirrer to about 3-4 hrs and kept overnight. The content of the flask were then acidified by dil.HCL and poured over crushed ice. The solid obtained was filtered & Wash with cold water & dried. **(Scheme-2)**

Scheme- 1: Synthesis of (*E*)-3-(3-nitrophenyl)-1-(4-nitrophenyl)prop-2-en-1-one (DMS-1)



3-nitrobenzaldehyde 1-(4-nitrophenyl)ethanone (*E*)-3-(3-nitrophenyl)-1-(4-nitrophenyl)prop-2-en-1-one

Scheme- 2 Synthesis of (*E*)-3-(4-methoxyphenyl)-1-(4-nitrophenyl)prop-2-en-1-one (DMS-2)



3-nitrobenzaldehyde 1-(4-nitrophenyl)ethanone (*E*)-3-(3-nitrophenyl)-1-(4-nitrophenyl)prop-2-en-1-one

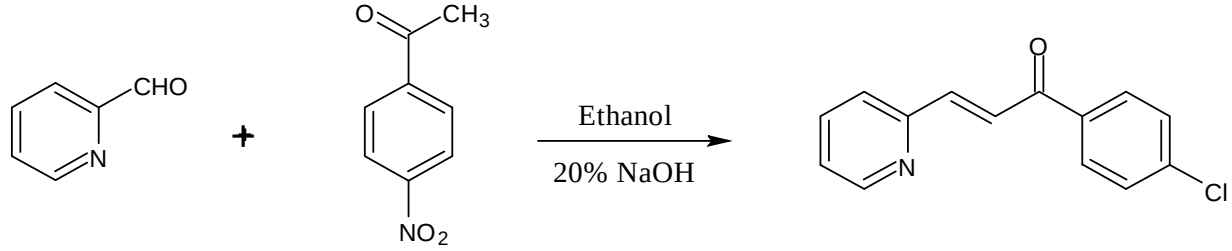
Synthesis of (*E*)-1-(4-chlorophenyl)-3-(pyridin-2-yl)prop-2-en-1-one (DMS-3)

Equimolar quantities of Picolinaldehyde and 1-(4-chlorophenyl) ethanone in minimum quantity of ethanol were dissolved. 20% NaOH in water was added in portion at room temperature. The reaction flask was kept in magnetic stirrer to about 3-4 hrs and kept overnight. The content of the flask were then acidified by dil.HCL and poured over crushed ice. The solid obtained was filtered & Wash with cold water & dried. **(Scheme-3)**

Synthesis of (*E*)-1-(4-chlorophenyl)-3-(2-hydroxyphenyl) prop-2-en-1-one (DMS-4)

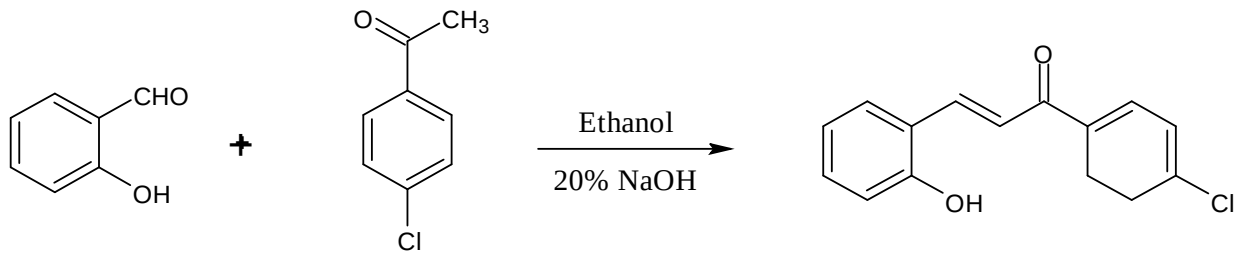
Equimolar quantities of 2-hydroxy benzaldehyde and 1-(4-nitrophenyl) ethanone in minimum quantity of ethanol were dissolved. 20% NaOH in water was added in portion at room temperature. The reaction flask was kept in magnetic stirrer to about 3-4 hrs and kept overnight. The content of the flask were then acidified by dil.HCL and poured over crushed ice. The solid obtained was filtered & Wash with cold water & dried.**(Scheme-4)**

Scheme- 3: Synthesis of (*E*)-1-(4-chlorophenyl)-3-(pyridin-2-yl)prop-2-en-1-one (DMS-3)



Picolinaldehyde 1-(4-chlorophenyl)ethanone (*E*)-1-(4-chlorophenyl)-3-(pyridin-2-yl)prop-2-en-1-one

Scheme- 4: Synthesis of (*E*)-1-(4-chlorophenyl)-3-(2-hydroxyphenyl) prop-2-en-1-one (DMS-4)



2-hydroxybenzaldehyde 1-(4-chlorophenyl)ethanone (*E*)-1-(4-chlorophenyl)-3-(2-hydroxyphenyl)prop-2-en-1-one

2.ANTIOXIDANT ACTIVITY

2.1 DPPH FREE RADICAL SCAVENGING ASSAY

The antioxidant activity of synthesized chalcones derivatives were evaluated by using DPPH free radical Scavenging assay[9,10,11].

The reaction mixture (3.0 ml) consists of 1 ml of 0.135mM DPPH solution in methanol was mixed with 1 ml of drug solution and 1.0 ml of methanol. The reaction mixture was vortexed and left in the dark at room temperature for 30 min. The various concentration of compounds (10, 20,30,40,50,100 µg/ml) were prepared. A reaction mixture without test sample was served as control. The absorbance was measured at 517 nm and (%) inhibition was calculated against control[12,13,14].

$$(\%)\ \text{inhibition} = \frac{A_{control} - A_{test}}{A_{control}} \times 100$$

Where,

$A_{control}$: absorbance of control.

A_{test}: absorbance of test.

The antioxidant activity of the test compounds is expressed comparing with ascorbic acid as standard and the (%) inhibition is shown in (**Table No.01).**

2.2 NITRIC OXIDE RADICAL SCAVENGING (NO) ASSAY

3.0 ml of 10 mM sodium nitroprusside in phosphate buffer is added to 2.0 ml of drug solution and reference compound in different concentrations. The resulting solutions are then incubated at 25°C for 60 min.The various concentration of compounds (10, 20,30,40,50,100 µg/ml) were prepared.

A similar procedure is repeated with methanol as blank, which serves as control. To 5.0 ml of the incubated sample, 5.0 ml of Griess reagent (1% sulphanilamide, 0.1% naphthyethylene diamine dihydrochloride in 2% H3PO3) is added and absorbance of the chromophore formed is measured at 540 nm. Percent inhibition of the nitrite oxide generated is measured by comparing the absorbance values of control and test preparations. Ascorbic acid can be used as a positive control[15, 16, 17].

$$(\%)\ \text{inhibition} = \frac{A_{control} - A_{test}}{A_{control}} \times 100$$

Where,

$A_{control}$: absorbance of control.

A_{test}: absorbance of test.

The antioxidant activity of the test compounds is expressed comparing with ascorbic acid as standard and the (%) inhibition is shown in (**Table No.02).**

Table 1:- DPPH Scavenging assay method

Compound Code	Absorbance (mean±SEM; 517 nm)$						% Inhibition at 100 µg/ml
↓	10 µg/ml	20 µg/ml	30 µg/ml	40 µg/ml	50 µg/ml	100 µg/ml	
DMS-1	0.52±0.0005	0.47±0.0005	0.32±0.0005	0.30±0.0005	0.28±0.0005	0.20±0.0005	83.91
DMS-2	0.58±0.0005	0.46±0.0005	0.41±0.0005	0.36±0.0005	0.33±0.0005	0.27±0.0005	78.68
DMS-3	0.72±0.0005	0.62±0.0005	0.57±0.0005	0.53±0.0005	0.49±0.0005	0.30±0.0005	75.59
DMS-4	0.69±0.0005	0.57±0.0005	0.48±0.0005	0.41±0.0005	0.33±0.0005	0.28±0.0005	77.18
STD	0.65±0.0005	0.61±0.0005	0.48±0.0005	0.43±0.0005	0.29±0.0005	0.20±0.0005	84.07

$ - Values are mean ± SEM of three determinants; **STD** - Ascorbic Acid

Table 2:- Nitric oxide radical inhibition method

Compound Code	Absorbance (mean±SEM; 517 nm)$						% Inhibition at 100 µg/ml
↓	10 µg/ml	20 µg/ml	30 µg/ml	40 µg/ml	50 µg/ml	100 µg/ml	
DMS-1	0.041±0.005	0.035±0.005	0.029±0.0005	0.024±0.0005	0.019±0.0005	0.015±0.0005	78.26
DMS-2	0.047±0.005	0.041±0.005	0.037±0.005	0.029±0.0005	0.023±0.0005	0.018±0.0005	73.91
DMS-3	0.045±0.005	0.043±0.005	0.039±0.005	0.038±0.005	0.032±0.005	0.025±0.0005	61.53
DMS-4	0.043±0.005	0.037±0.005	0.030±0.005	0.027±0.0005	0.022±0.0005	0.016±0.0005	75.38
STD	0.044±0.005	0.037±0.005	0.028±0.0005	0.020±0.0005	0.016±0.0005	0.013±0.0005	81.15

STD - Ascorbic Acid

2.3 HYDROGEN PEROXIDE SCAVENGING ACTIVITY

1ml of test extract solution [prepared in phosphate buffered saline (PBS)] with different concentrations were incubated with 0.6 ml of 4mM H_2O_2 solution (prepared in PBS) for 10 min. The absorbance of the solution was observed at 230 nm against a blank solution.. The H_2O_2 radical scavenging activity is calculated by formula-

$$(\%)\ \text{inhibition} = \frac{A_{control} - A_{test}}{A_{control}} \times 100$$

Where,

$A_{control}$: absorbance of control.

A_{test}: absorbance of test.

The antioxidant activity of the test compounds is expressed comparing with ascorbic acid as standard and the (%) inhibition is shown in (**Table No.03).**

2.4 REDUCING POWER ASSAY

1.0 ml drug solution is mixed with 2.5 ml of phosphate buffer (0.2M, pH 6.6) and 2.5 ml of 1% potassium ferricyanide. The mixture was then incubated at 50°C for 20 min.

Thereafter, 2.5 ml of 1% trichloroacetic acid (10%) was added to the reaction mixture, which was then centrifuged for 10 min at 3000 rpm. The upper layer of solution (2.5 ml) is mixed with 2.5 ml of distilled water and 0.5 ml of FeCl3 solution (0.1%).The absorbance is measured at 700 nm. Ascorbic acid was used as standard. The assays were carried out in triplicate and the results were expressed as Mean values ± SEM. Increased absorbance values indicate a higher reducing power.

Table 3:- Hydrogen Peroxide Scavenging Activity

Compound Code	Absorbance (mean±SEM; 517 nm)$						% Inhibition at 100 µg/ml
↓	10 µg/ml	20 µg/ml	30 µg/ml	40 µg/ml	50 µg/ml	100 µg/ml	
DMS-1	3.01±0.0005	2.71±0.0005	2.21±0.0005	2.11±0.0005	1.90±0.0005	1.62±0.0005	55.04
DMS-2	3.32±0.0005	2.95±0.0005	1.91±0.0005	1.80±0.0005	1.72±0.0005	1.51±0.0005	60.22
DMS-3	3.01±0.0005	2.34±0.0005	1.91±0.0005	1.72±0.0005	1.52±0.0005	1.40±0.0005	62.90
DMS-4	3.01±0.0005	2.81±0.0005	2.33±0.0005	2.11±0.0005	1.82±0.0005	1.71±0.0005	53.33
STD	2.23±0.0005	1.99±0.0005	1.87±0.0005	1.71±0.0005	1.51±0.0005	1.26±0.0005	66.71

STD - Ascorbic Acid

Table 4:- Reducing Power Assay Method

Sr. No.	Test Comp	Test/ Standard µg/ml	Reducing Power Abs at 700 nm
1-a	DMS-1	5	0.034
1-b		10	0.044
1-c		15	0.072
1-d		20	0.101
2-a	DMS-2	5	0.073
2-b		10	0.086
2-c		15	0.098
2-d		20	0.119
3-a	DMS-3	5	0.056
3-b		10	0.087
3-c		15	0.097
3-d		20	0.100
4-a	DMS-4	5	0.029
4-b		10	0.036
4-c		15	0.058
4-d		20	0.118
STD	AA	20	0.156

AA / **STD** - Ascorbic Acid

RESULT AND DISCUSSION

All the above synthesized compounds were tested for there *in vitro* antioxidant activity on four different models namely Diphenyl Picryl hydrazine, Nitric oxide, Hydrogen peroxide and Reducing power assay method.

As from the results in the tables, it could be seen that most of the compounds showed significant antioxidant activity. The highest reducing power activity was observed in compound DMS-4 is probably due to presence of Cl group in acetophenic & pyridine in aldehydic moiety of chalcone. The order of activity regarding substitution on chalconyl group is Cl > NH2 > Cimameldehyde > H[21, 22].

In DPPH method, compound DMS-1 and DMS-2 showing most potent activity with Percentage inhibition 83.91 and 75.59 respectively. Similarly, compound DMS-1and DMS-4 showed potent activity by nitric oxide scavenging method with Percentage inhibition 78.26 and 75.38 respectively. In hydrogen peroxide scavenging method compound DMS-2and DMS-3 showing significant activity with Percentage inhibition 60.22 and 62.90 respectively when compared with standard ascorbic acid.

CONCLUSION:

Most of the synthesized chalcones were potential lead for antioxidant activity. On the basis of observed results, it may be concluded that the substitution favors the activity. The antioxidant potential of chalcones is known to be influenced to a great extent by the two aryl structures, i.e. substituents on two aryl rings of chalcone molecule and their substitution pattern.

Especially the hydroxyl substituent is one of the key groups to enhance greatly the antioxidant activity of chalcone mainly due to its easy conversion to phenoxy radical through the hydrogen atom transfer mechanism. This phenoxy radical formation may be central to antioxidant potential of chalcones. In fact the hydroxyl substituent are wide spread among chalcones from synthetic source. There by, a number of structurally diverse chalcone including phenolic chalcones have been prepared & evaluated for antioxidant activity.

REFERENCES:

1. Hermakens P.H, Ottenheijn H.C , Ress D.C., Solid phase organic reaction II; A review of literature,1997, 53, 5643.
2. Gorden E, Barrett R.W, Dower W. J, Folder S.P., Applications of combinatorial technologies to drug discover, J. Medchem., 1994, 37, 1485.
3. Nielsen S.B, Christensen S. F, Cruciani G, Kharazmi A., Journal of Medchem.,1998, 41, 4819.
4. Go. M. L, Wu X , Liu X.L., Med. Chem. 2005, 12, 483.
5. Naik K.M , Nandibewoor S.T., Highly sensitive analysis of the chalcone in human plasma and urine by RP-High Performance liquid chromatography, 2011, 4, 1.
6. Dabbas M, Suganuma T, Kitahara K, Hou D, Fujii M., Cytotoxic, antioxidant and antibacterial activities of *Varthemia iphionoides* Boiss extracts, J. Ethno pharmacol.2008, 287-293.
7. Maria J , Gozalez Moa , Marcos Mandado, et al ., QTAIM electron density study of natural chalcones, Chemical physics letter, 2007,446.
8. Ruby John Anto, K.Sukumaran, Girija Kuttan, M.N.A. Roa, et al.,Anticancer & antioxidant activity of synthetic chalcones & related compounds., Cancer letters, 1995 , 97, 33-37.
9. Sanchez Morenco , larraui A , Saura calixtof., A procedure to measure the antiradical efficiency of polyphenols, J. Sci.FoodAgri, 1998, 76, 270.
10. Oktay M, Gulein I, Kufreviolglu I, Labenson Wiss U., Determination of *In-vitro* antioxidant activity of fennel (Foeniculum vulgare) seed extracts, Technol.2003, 36, 263-71.
11. Tagashira M, Ohtake Y., A new antioxidative 1,3-Benzodiazole from Melissa officinalis, Planta med.1998, 64, 555-558.
12. Velavan S, Nagulendra K, Mahesh R, V.Hazeena Begum., *In-Vitro* antioxidant activity of asparagus racemosus root, Pharmacognosy magazine, 973-1296.
13. Tiwari Ashok K., Imbalance in antioxidant defence & human disease, multiple approach of natural antioxidant therapy, Current Science, 2001, 81, 1179-1187.
14. Lime yua , Mouming Zhaoa , Jin Shui Wnaga , Chun Cuia , Bao Yangb , Yueming Jiangb , Qianzhong Zholoa., Antioxidant immune modulatory and anti-breast cancer Activities of phenolic extract from pine (Pinus Massoniana) bark., Innovative food science & emerging Technologies 2008, 9(1) , 122-128.
15. Green L.C, Wagner D. A, Gligowski J, Sleipper P.L, Tamenbaum S.R., Analysis of nitrite nitrile & 15 N in biological fluids, Anal. Biol. Chem. 1982, 131-136.
16. Bade J.D, Jaju A. H, Aher A. N, Pal S. C., *In-vitro* antioxidant & free radical scavenging activity of ethyl extract of Delonix regia Rafin Flowers, Asian journal of chemistry, 2009, 21(2), 1323-1329.
17. Kyoko Nakagawa Goto, Koji Yamada, et al., Synthesis and evaluation of Dietary antioxidant, Bio-organic & Medicinal chemistry Letters, 2007, 17, 5204-5209.
18. Ruch R. J, Cheng S. J, Klaunig J. E , Carcinogenesis, 1989, 10, 1003.
19. Oyaizu M , Jan J. N., 1986, 44, 307-315.
20. Khanam S, Shivprasada H. N, Devi K , Ind. J. Pharm. Edu. Res, 2004, 38(4), 180-183.
21. Venkatshwarlu S, Ramchandra M.S, Krishnaraju A.V, Subbaraju G. V., Indian Journal of chemistry, 2005, 45(B), 252-257.
22. Flynn D. L, Belliotti T. R, Boctor A. M, Connor D.J, Kostlan C.R , Nies D.E, Ortuine D. F, Schrier J. D, Siracar J. C , Journal Med. Chem, 1991, 34, 518-525.

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