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# A FACILE AND CONVENIENT SYNTHESIS OF AURONES: AN INTRAMOLECULAR OXIDATIVE CYCLISATION CATALYSED BY MERCURY (II) IONS AND THEIR ANTIMICROBIAL, ANTIOXIDANT ACTIVITIES

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## ABSTRACT

Some novel biologically active Aurones **(3a-d)** were synthesized by oxidative cyclization of α-β unsaturated ketones (chalcones) with mercuric acetate in presence of DMSO. Chalcones **(2a-d)** were prepared by treatment of benzyloxy benzaldehyde **(1)** with different substituted acetophenones **(1a-d)** by Claisen-Schmidt condensation. The structures of the newly synthesized compounds were established on the basis of elemental analysis and spectral methods such as IR, $^1$H NMR and mass spectra. Antioxidant activities and zone of inhibition for these Aurones was determined. *E. coli, S. aureus, B. thurengienesis* and *E. aerogenes* were used as bacterial strains compared with Chloramphenicol as a reference drug. Synthesized Aurones showed moderate to good antibacterial and antioxidant activity.

**KEY WORDS:** Aurones, chalcones, mercuric acetate, antioxidant activity.

## INTRODUCTION

Aurones 2-benzylidenebenzofuran-3(2H)-ones, are a class of flavonoids,[1] called anthochlor pigments[2] found in fruits and flowers where they function as phytoalexins against infections and contribute to the yellow pigmentation of plant parts.[3] The molecule contains a benzofuran element associated with a benzylidene linked in position 2 into a 5-membered ring instead of the 6-membered ring. There are two isomers of the molecule, with (*E*) and (*Z*) configurations. Analogy with flavonoids suggests that Aurones constitute an interesting class of heterocycles due to their synthetic versatility and exhibit anti-cancer activity as well as a variety of other pharmacological activities including anti-inflammatory and anti-viral properties.[4-7] Aurones are obtained from chalcones by aurone synthase as well as through the biosynthesis of other flavonoids. Literature survey reveals that aurones has been synthesized using different methods.[8-12] Mercuric acetate is well known for highly regio selective and stereo specific oxymercuration of olefins. Oxidative cyclisation of 2'-hydroxy chalcones leading to Aurones; ready availability of a number of more elaborated 2'-hydroxy chalcones and search for new potential antimicrobial compounds, prompted us to verify and study the scope of the reaction. Thus, we present here the synthesis and characterization of some novel 2'-hydroxy-chalcones and their oxidative cyclization with mercuric acetate in presence of DMSO to form aurones and simultaneously carry out their *in vitro* biological and antioxidant activity.

## MATERIAL AND METHODS

The melting points were recorded in open capillary in paraffin bath and are uncorrected. IR spectra were recorded on a Shimadzu IR Spectrophotometer (KBr, v max in $cm^{-1}$). $^1$H NMR spectra are recorded on a Bruker AM 400 instrument (400 MHz) using tetramethylsilane (TMS) as an internal reference and DMSO-$d_6$ as solvent. Chemical Shifts are given in parts per million (ppm). Positive-ion Electro Spray Ionization (ESI) mass spectra were obtained with a Waters Micromass Q–TOF Micro, Mass Spectrophotometer. Elemental (CHN) analysis was done using Thermo Scientific (Flash-2000), the compounds were analysed for carbon, hydrogen and nitrogen and the results obtained are in good agreement with the calculated values. Chemicals used for the synthesis were of AR grade of Merck, S.D.Fine and Aldrich. The reactions were monitored by E. Merck TLC aluminum sheet silica $gel_{60}F_{254}$ and visualizing the spot in UV Cabinet and iodine chamber.

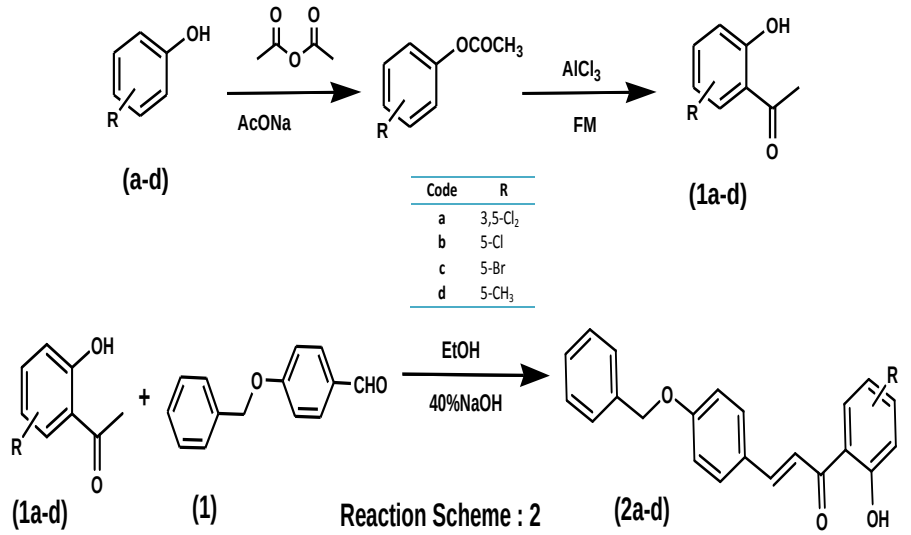
## EXPERIMENTAL PROCEDURE

**General procedure for the synthesis of 3-[4-(benzyloxy)phenyl]-1-(substitiuted-2-hydroxyphenyl)prop-2-en-1-one (2a-d):** 2-hydroxy acetophenone **1a** (10mmole) (Scheme **1**) and benzyloxy benzaldehyde (**1**, 10mmole) were dissolved in minimum quantity of ethanol and solution was heated till the solid

get dissolved. Aqueous 70% sodium hydroxide solution (10mL) was added drop wise with constant stirring. The mixture was further stirred mechanically at room temperature to obtain dark orange mass and kept overnight, acidified by 1:1 hydrochloric acid. The solid obtained was filtered, washed and recrystallized from glacial acetic acid to get **2a** (Scheme **2**).

Similarly, **2b-d** were synthesised from **b-d** by extending the same procedure followed for **2a**.

**Reaction Scheme : 1**




**Reaction Scheme : 2**

**3-(4-(benzyloxy)phenyl)-1-(3,5-dichloro-2-hydroxyphenyl)prop-2-en-1-one (2a):** Orange coloured crystals; mp 162-164˚C; yield 77%; M. F. $C_{22}H_{17}0_3Cl_2$.

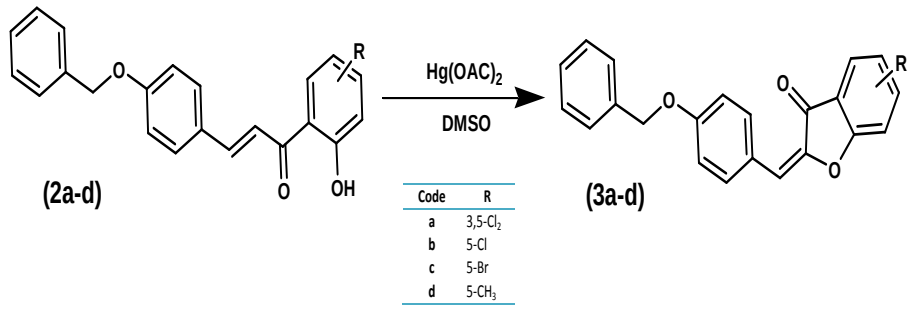
**3-(4-(benzyloxy)phenyl)-1-(5-chloro-2-hydroxyphenyl)prop-2-en-1-one (2b):** Orange coloured crystals; mp 131-133˚C; yield 60%; M. F. $C_{22}H_{17}0_3Cl$.

**3-(4-(benzyloxy)phenyl)-1-(5-bromo-2-hydroxyphenyl)prop-2-en-1-one (2c):** Orange coloured crystals; mp 142-144˚C; yield 70%; M. F. $C_{22}H_{17}0_3Br$; Recrystallizing solvent: Glacial acetic acid; IR(KBr, $\nu_{max}$): 3061 (-OH), 3036 (ArH), 2875, 2937 ($CH_2$), 1634 (C=O), 1556, 1508 (C=C) $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$): δ 12.65 (s, 1H, -OH), 5.19 (s, 2H, -$CH_2$), 6.94-8.40 (m, 14H, ArH; -CH=CH-) ppm; MS: *m/z* 411 $[M+2]^+$, 412 $[M+3]^+$; Calculated: C, 64.71; H, 4.16 Found: C, 63.008; H, 4.136.

**3-(4-(benzyloxy)phenyl)-1-(5-methyl-2-hydroxyphenyl)prop-2-en-1-one (2d):** Orange coloured crystals; mp 134-136˚C; yield 68%; M. F. $C_{23}H_{20}O_3$.

**General procedure for the synthesis of 2-[4-(benzyloxy) benzylidene]-substituted benzofurane-3(2H)-one (3a-d): 2a (**10mmol), mercuric acetate (10mmole) and DMSO (20mL) were taken in round bottom flask. The reaction mixture was refluxed for 3h. It was diluted with cold water, filtered and recrystallized from ethanol to obtain **3a** (Scheme **3**). Similarly, **3b-d** were synthesised from **b-d** by following the same procedure followed for **3a**.

**Reaction Scheme : 3**




**2-(4-(benzyloxy)benzylidene)-5,7-dichlorobenzofuran-3(2H)-one (3a):** Yellow coloured crystals; mp 187-188˚C; yield 97%; M. F. $C_{22}H_{14}0_3Cl_2$; Recrystallizing solvent: Ethanol; IR(KBr, $\nu_{max}$): 3060, 3067 (ArH), 2865, 2917 ($CH_2$), 1644,1703 (C=O), 1598 (C=C), 1006,1129(C-O-C) $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$): δ 5.21 (s, 2H, -$CH_2$), 7.08-8.26 (m, 14H, ArH; -CH=CH-) ppm;

MS: *m/z* 397 $[M+H]^+$, Calculated: C, 66.52 H, 3.55 Found: C, 65.418; H, 3.667.

**2-(4-(benzyloxy)benzylidene)-5-chlorobenzofuran-3(2H)-one (3b):** Yellow coloured crystals; mp 166-167˚C; yield 82%; M. F. $C_{22}H_{15}0_3Cl$; Recrystallizing solvent: Ethanol

**2-(4-(benzyloxy)benzylidene)-5-bromobenzofuran-3(2H)-one (3c) :** Yellow coloured crystals; mp 148-150˚C; yield 78%; M. F. $C_{22}H_{15}0_3Br$; Recrystallizing solvent: Ethanol

**2-(4-(benzyloxy)benzylidene)-5-methylbenzofuran-3(2H)-one (3d) :** Yellow coloured crystals; mp 158-160˚C; yield 97%; M. F. $C_{23}H_{20}0_3$; Recrystallizing solvent: Ethanol

## ANTIMICROBIAL ACTIVITY

The novel synthesized heterocyclic compounds (3a-d) as were screened for their *in vitro* antimicrobial activity using cup plate agar disc-diffusion method against two Gram positive bacterial strains, *B. thurengienesis* and *S. aureus* and two Gram negative strains, *E. coli* and *E. aerugenes.* Chloramphenicol was used as standard drug for bacteria.

**General procedure: Determination of zone of inhibition by agar disc-diffusion method**

Test solutions were prepared with known weight of compound in DMSO and half diluted suitably to give the resultant concentration of 31-500µg/mL. Whatmann no. 1 sterile filter paper discs (6 mm) were impregnated with solution and allowed to dry at room temperature. *In vitro* antibacterial activity was determined by using Mueller Hinton Agar obtained from Himedia Ltd., Mumbai. Petri plates were prepared by pouring 10 ml of Mueller Hinton Agar for bacteria containing microbial culture was allowed to solidify. The discs were then applied and the plates were incubated at 37˚C for 24h (bacteria) and the inhibition zone was measured as diameter in four directions and expressed as mean. The results were compared using Chloramphenicol as a standard antibacterial agent. The results of antibacterial activity (i.e. Zone of inhibition in mm) of some of the synthesized compounds are given in the Table **1**.

## ANTIOXIDANT ACTIVITY

**Reducing power**[13]

The reducing power in-vitro model was used to evaluate antioxidant activity according to the method of Oyaizu (Oyaizu, 1986). This method is based on the principle of increase in the absorbance of the reaction mixture, indicates increase in the antioxidant activity hence increasing reducing power of the samples. In this method antioxidant compound gives a colored complex with potassium ferricyanide, trichloroacetic acid and ferric chloride, which is measured at 700 nm.

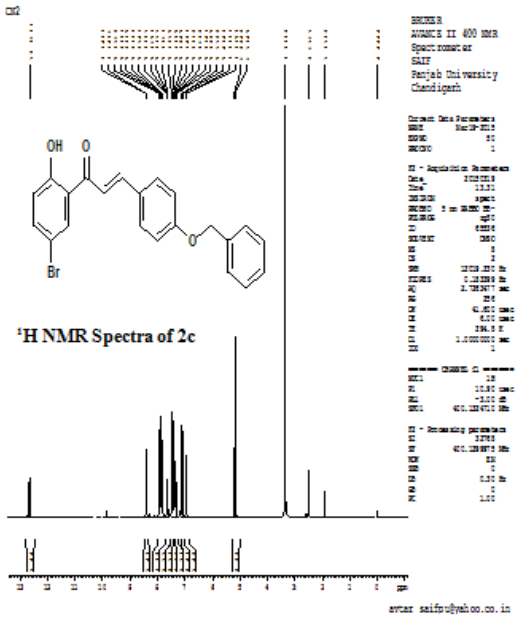
**Procedure**

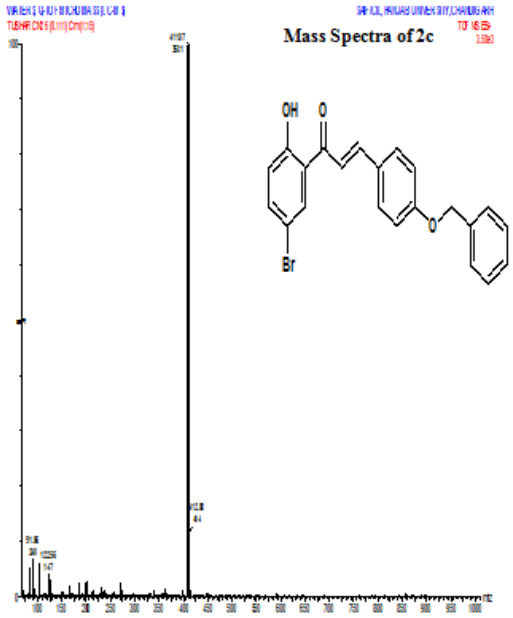
The standard drug and test compounds were dissolved in dimethyl formamide so as to get different concentrations (20µg/mL to 100µg/mL). This was mixed with 2.5mL of (pH 6.6) 0.2 mol phosphate buffer and 2.5mL of 1 % potassium ferricyanide. The mixture was incubated at 50°C for 20 minutes. 2.5mL of 10 % trichloroacetic acid was added to the mixture, which was then centrifuged for 10 minutes at 1000 rpm. 2.5mL upper layer of solution was mixed with 2.5mL of distilled water and 0.5mL of 0.1% ferric chloride. The absorbance was measured at 700nm. The absorbance of the blank was also measured in similar manner. The results were compared with ascorbic acid, which was used as a reference standard antioxidant. Antioxidant activities of some representative compounds are given in Table **2.**

## RESULTS AND DISCUSSION

The synthesis of the novel compounds **2a-d** and **3a-d** is described in reaction schemes. The reactions were monitored by TLC. The identities of the newly synthesized compounds have been established on the basis of their elemental analysis and spectral data[14] such as IR, $^1$H NMR and Mass spectral studies.

The reaction of substituted 2-hydroxy acetophenones **(1a-d)** with benzyloxy benzaldehyde **(1)** in presence of 40% NaOH afforded (**2a-d)** by Claisen-Schimidt condensation. $FeCl_3$ test for **2c** gave violet colouration showing the presence of Phenolic–OH. The IR spectra of **2c** showed stretching bands at 1556 and1508 $cm^{-1}$ due to the disappearance of $–CH_3$ group and appearance of –CH=CH-, similarly the $^1$H NMR spectrum showed multiplet in the range of δ 6.94 to 8.40 ppm due to fourteen protons confirms the formation of **2c** which was further confirmed by mass spectrum with a molecular ion peak at *m/z* 411 $[M+2]^+$ is in agreement with the molecular formula $C_{22}H_{17}O_3Br$.

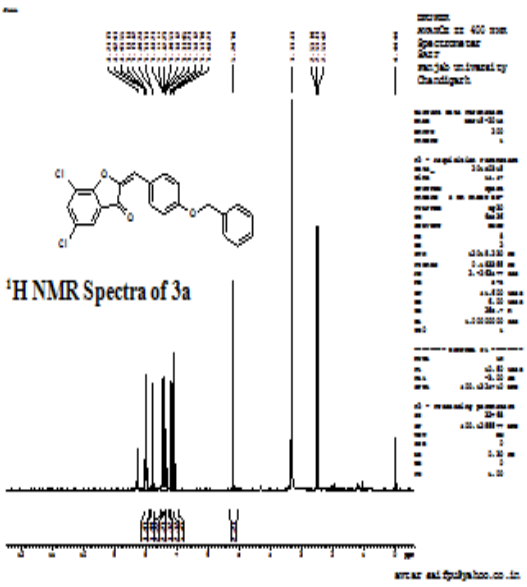


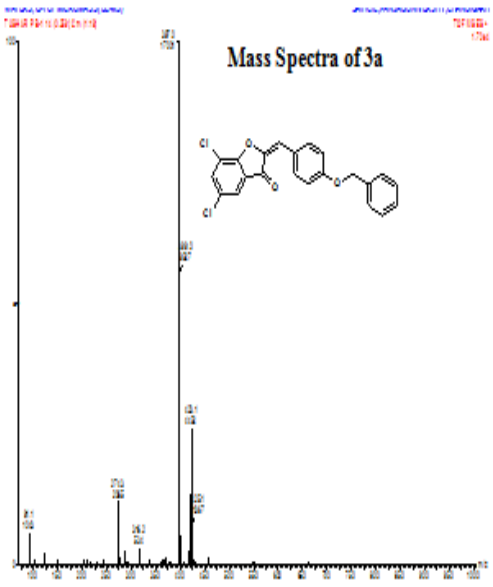




The uses of different chalcones for the synthesis of aurones have been investigated. The presence of bromo, chloro and methyl groups in different position of benzene ring of the chalcones and the use of mercuric acetate resulted in synthesis of new aurones with significantly high yield.

Reaction of substituted 2-hydroxy chalcones (**2a-d)** with mercuric acetate in presence of DMSO yielded corresponding aurones **(3a-d)**. $FeCl_3$ test for **3a** was found to be negative; similarly the structure of **3a** was confirmed from its spectral data.

The $^1$H NMR spectra in **3a** showed a singlet at δ 5.21 ppm for two $-CH_2$ protons and a multiplet for twelve protons due to ArH and =CH which shows that the chalcones has been cyclized and a five membered ring containing oxygen atom is formed. Mass spectrum with a molecular ion peak at *m/z* 397 $[M+H]^+$ confirms its molecular formula $C_{22}H_{14}O_3Cl_2$.




**ANTIMICROBIAL ACTIVITY**

Synthesized aurones i.e., **(3a-d)** were screened for antimicrobial activity. Table no. **1**, shows the inhibition zone calculated at different concentrations from 31-500 µg/mL using Chloramphenicol as the standard drug. Data obtained revealed that the test compound **3a, 3b** and **3c** are highly active against the *B. thurengienesis* while **3d** against *E. coli* and *S. aureus*, **3c** is highly active against *E. areogenes* while it is moderately active at some concentrations. Whereas rest of the compounds possess poor or found to be inactive against some concentrations.

**TABLE 1: ANTIBACTERIAL ACTIVITY OF AURONES**

| Compd. No | Code of the compound | Zone of inhibition in mm Antibacterial activity | | | | | Chloramphenicol |
|---|---|---|---|---|---|---|---|
| | | | Gm +ve | | Gm –ve | | |
| | | *Conc.µg/mL* | *S.aureus* | *B. Thurengienesis* | *E.coli* | *E.aerogenes* | |
| 1. | 3a | 31 | - | - | 7 | - | 30 |
| | | 125 | 12 | - | 6 | - | 27 |
| | | 250 | 11 | 14 | 10 | - | 21 |
| | | 500 | 13 | 22 | 11 | 4 | 20 |
| 2. | 3b | 31 | - | 11 | 11 | - | 21 |
| | | 125 | - | 17 | - | 11 | 16 |
| | | 250 | 9 | - | - | 17 | 16 |
| | | 500 | 10 | 21 | 10 | - | 20 |
| 3. | 3c | 31 | 11 | 10 | 6 | 11 | 18 |
| | | 125 | 6 | - | 12 | 4 | 17 |
| | | 250 | - | 18 | - | - | 11 |
| | | 500 | 8 | 10 | 11 | 14 | 16 |
| 4. | 3d | 31 | - | - | 10 | 7 | 16 |
| | | 125 | 14 | - | 8 | 10 | 17 |
| | | 250 | 19 | 7 | - | - | 16 |
| | | 500 | - | 9 | 15 | - | 15 |

**ANTIOXIDANT ACTIVITY**

Aurones **(3a-d)** were assessed for their *in vitro* antioxidant activities using free radical reducing power method at various concentrations. Ascorbic acid was used as a reference standard. Increase in the absorbance of the tested compounds, indicates increase in the antioxidant activity hence increasing reducing power of the samples. The antioxidant activity of the tested compounds **3c** and **3d** compared with ascorbic acid possesses significant activity as shown in Fig. 1. The results reveal that **3a** and **3b** showed moderate activity when compared to control.

**TABLE 2 : ANTIOXIDANT ACTIVITY OF AURONES**

| Sr No. | Compound Code | Absorbance | | | | | % increase in absorbance | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 20 µg/ml | 40 µg/ml | 60 µg/ml | 80 µg/ml | 100 µg/ml | 20 µg/ml | 40 µg/ml | 60 µg/ml | 80 µg/ml | 100 µg/ml |
| 1 | Control | 0.205 | | | | | - | | | | |
| 2 | Standard (Ascorbic acid) | 0.231 | 0.276 | 0.280 | 0.304 | 0.308 | 12.68 | 34.63 | 36.58 | 48.29 | 50.24 |
| 3 | 3a | 0.11 | 0.145 | 0.156 | 0.162 | 0.177 | 12 | 45 | 56 | 62 | 77 |
| 4 | 3b | 0.135 | 0.145 | 0.168 | 0.175 | 0.198 | 32 | 45 | 68 | 75 | 98 |
| 5 | 3c | 0.143 | 0.167 | 0.178 | 0.177 | 0.199 | 43 | 67 | 78 | 77 | 99 |
| 6 | 3d | 0.153 | 0.157 | 0.171 | 0.187 | 0.200 | 53 | 57 | 71 | 87 | 100 |

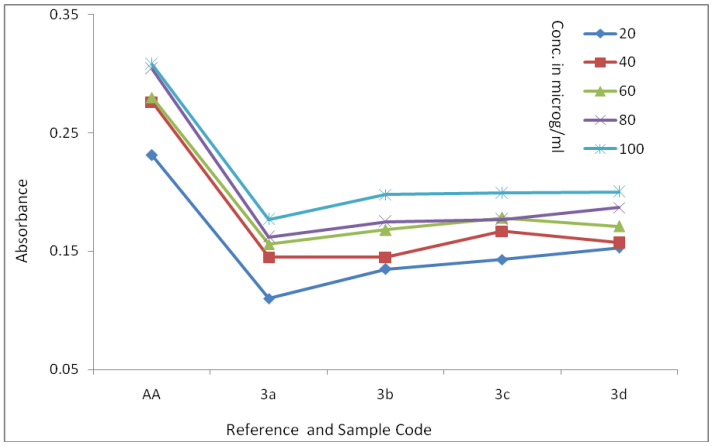



**FIG 1: REDUCING POWER OF THE SYNTHESISED AURONES AS COMPARED WITH THE STANDARD ASCORBIC ACID AT CONCENTRATIONS FROM 20-100µG/ML**

## CONCLUSION

Substituted Aurones **(3a-d)** were successfully synthesized in good yields. Their purity and conformation was checked by melting point and from spectral data. Antimicrobial screening of these compounds was found to possess moderate to good activity against selected strains of bacteria. Similarly, they possess good Antioxidant activity.

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