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Indian Journal of Forensic and Community Medicine

Journal homepage: www.ipinnovative.com

Original Research Article

Design, synthesis and evaluation of chalcones as anti-microbial agent

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ARTICLE INFO

Article history:
Received 09-09-2020
Accepted 10-09-2020
Available online 29-10-2020

Keywords:
Chalcones
Antimicrobials
Polymerization
Dimethyl sulphoxide

ABSTRACT

2, 4-dichloro-5'fluoro-1-ene-2-(4-hydroxyphenyl) phenone (EHP) was synthesized using 4-hydroxybenzaldehyde and 2,4-dichloro-5-fluoro acetophenone (DFA). 4-[3-(2, 4-dichloro-5-fluorophenyl)-3-oxoprop-1-en-1-yl]phenyl acrylate (EAP) was prepared by reacting EHP with acryloyl chloride using ethyl methyl ketone (EMK) as a solvent in the presence of triethylamine (TEA) as a base at $0\text{-}5^0$. Poly (EAP) was prepared by solution polymerization technique using benzoyl peroxide (BPO) as a free radical initiator and EMK as a solvent at 70 ± 1^0. EHP, EAP and poly (EAP) were characterized by Infrared, proton NMR and ^{13}C NMR techniques. Anti-microbial activity of the DFA, EHP, EAP and poly (EAP) were tested on three different ATCC microorganisms (E.coli ATCC 25922, Pseudomonas aeruginosa ATCC 27853 and Salmonella Typhi ATCC 6539). The minimum concentration of the drug needed to inhibit the growth of the bacteria was estimated using photometry assay method.

1. Introduction

Chalcones and their derivatives were used extensively in various fields such as photo resisting polymer and biology. The anti-bacterial and anti-fungal activities of these type of chalcones were reported. Chalcone derivative (2–hydroxy chalcone) was tested as inhibitor of inflammatory mediators generation by Ballesteros et al (1995)[1] and they reported that the inhibition rate was followed concentration – dependence way. Also, these chalcones exhibit anti-inflammatory effect in mice. Anti-fungal activity of hydroxy chalcones was studied by Tsuchiya et al (1994) using candida species as a fungal agent, and they found certain hydroxy chalcones effectively used to treat candidosis disease. Modification of the α, β-double bond in chalcone was studied by Simon et al (1998)[2] and found that the modification gives marginal effect on anti-protozoa activity. Biological activity of wide variety of chalcone derivatives were reported by Shubhasish et al (2001)[3] with a view to develop efficient anti-fungal / anti-bacterial active polymer conjugates, we have taken up this work to investigate the biological activity of the chalcone polymers containing fluorine and chlorine as a biologically active site. Though it was reported that DFA found to be active against several microbes, the chalcone derivative of the DFA to the best of our knowledge was not investigated. In this article, we have synthesized chalcone derivative of DFA using 4-hydroxybenzaldehyde, acrylated product of the chalcone EAP and poly (EAP). Anti-microbial activities of all these three compounds were tested on different ATCC strain bacteria. 4-Hydroxybenzaldehyde and acryloyl chloride were used as received from Aldrich chemicals. DFA was obtained as a gift sample from INSPEC Laboratory, Chennai. Triethylamine from SD fine chemicals was freshly distilled before use for acrylation.

2. Experimental

Nicolet FT-IR spectrophotometer model 20 DXB was used and the spectra were recorded using kbr pellet. ^{1}H and ^{13}C NMR spectra of the samples were run on a Bruker FT-NMR spectrophotometer operating at 320 mhz using $CDCl_3$ as a solvent and tetramethylsilane (TMS) as an internal reference.

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https://doi.org/10.18231/j.ijfcm.2020.026

2.1. Synthesis of 2, 4-Dichloro-5'-flouro-1-ene-2-(4-hydroxyphenyl) phenone (EHP)

0.025 mole (5.18g) of DFA and 0.025 mole (3.05g) of 4-hydroxybenzaldehyde were charged into a clean conical flask containing 30ml of ethanol. To this solution, 4g of sodium hydroxide dissolved in 20ml of distilled water was added in drop wise at room temperature. The contents of the flask were stirred for 20h at room temperature. The mixture was neutralized using dilute hydrochloric acid. The precipitate was filtered, washed with cold water and dried in vacuo at 40°C. Melting point =102°C. Yield = 6.9g (88.7%).

IR (cm^{-1}): 3367 (-OH stretch), 3020 (-CH aromatic stretch), 2986 (-CH aliphatic stretch), 1680 and 1652 (cis and trans C=O stretch), 1592 (CH=CH stretch), 1555 and 1441 (aromatic). ^{1}H NMR (d,ppm): 9.3 (1H,b), 7-8 (6H,m), 6.3 (1H,d) and 5.9 (1H,d). ^{13}C NMR (d,ppm): 1753 (C=O of ester), 1676 and 1658 (cis and trans of C=O stretch), 1594 (CH=Ch stretch), 504 and 1457 (aromatic stretch). ^{1}H NMR (d,ppm): 0.9-1.3 (3H,m), 6.1(1H,d), 6.3 (1H,d) and 7-8 (6H,m).

2.2. Synthesis of 4-[3- (2, 4 dichloro-5-fluorophenyl) -3-oxoprop-1-en-1-yl] phenyl acrylate (EAP)

0.01 mol (2.98g) of EHP and 0.012 mole (1.67 ml) of triethylamine were taken into a clean 500ml round bottom flask containing 150ml of ethyl methyl ketone fitted with a mechanical stirrer. 0.012 moel (0.95ml) of acryloyl chloride was Charged into a pressure equalizer containing 30 ml of ethyl methyl ketone. To the contents of the round bottom flask, acryloyl chloride was added slowly drop wise with constant stirring in such a way that the temperature maintained at 0-5°C. After the completion of addition, the contents of the flask were stirred for a period of 3h at room temperature and filtered. The filtrate was washed with water and the organic layer was dried with anhydrous sodium carbonate. The product was obtained by evaporating the solvent. Melting point = 84°C. Yield = 2.9g (78.8%).

IR (cm^{-1}) 3063 (aromatic CH stretch), 2993 (aliphatic CH stretch), 1748 (C=O of ester), 1680 and 1665 (cis and trans C=O stretch), 1636(CH=CH stretch), 1596 (CH=CH stretch), 1504 and 1463 (aromatic stretch). ^{1}H NMR (d,ppm): 7-8(6H,m) 6.4 (1H,d), 6.2 (3H,m) and 5.9 (1H,d). 13C NMR ((d,ppm): 190,166,157,155, 152,143,110-135. (Figure 1)

3. Results and Discussion

3.1. Antimicrobial activity

Poly (EAP) was synthesized using a free radical solution polymerization method (Scheme). 1g of EAP and 0.05g of BPO (5% w/w) were taken in a polymerization tube containing 10 ml. of EMK. The polymerization tube containing the reaction mixture was kept at 70 ±1°C in a thermostatic water bath for a period of 24h. Poly (EAP) was precipitated into large excess of methanol. IR (cm^{-1}):3065 (aromatic CH stretch), 2993 (aliphatic CH stretch), Hinton broth was received from Himedia. Dimethylsulphoxide (DMSO) was received from Merck and used as such. Working solutions were prepared according to the procedures prescribed by the National Committee for Clinical Laboratory Standards (NCCLS) (1991). Stock solution (10mg/mL) of each compound in DMSO was diluted with DMSO (1:10 at least) and subsequently diluted in sterile buffer (1:1).[4]

3.2. Average of three values were taken

The drugs were tested by the disc-diffusion method given by Bauer et al., (1966)[5] for the activity of drug against microbes. 100 μl of the diluted bacterial culture was spread on a sterilized Muller-Hinton broth agar plates. Then 6mm diameter disks impregnated with the drug to be tested (100 μl) were placed on a Muller-Hinton broth agar plated. The plate was incubated at 37°C for 24^{h} under aerobic condition. The zone of inhibition was measured and the inhibition zone which exceeds 10mm was taken for calculating the minimum inhibitory concentration value (MIC).

MIC's were calculated by the method described by Sham and Washington (1991) using Muller-Hinton broth medium.[6] Concentration ranging from 0.015 to 250 μg/ml was tested for each drug using approximately 10^{7} cells/ml inoculums. The growth of the bacteria was determined by measuring the turbidity test after 24^{h} of incubation at 37°C. All the experiments were done in triplicate.

The MICs value reported in the Table 1 shows that the DFA, EHP, EAP and poly(EAP) were active against the tested microbes such as E.coli, Pseudomonas aeruginosa and Salmonella Typhi. E.coli and Salmonella Typhi were significantly inhibited by all the three compounds. Pseudomonas aeruginosa was markedly inhibited by EAP and EHP. Among the three tested drugs, poly (EAP) is showing higher anti-microbial activity in the case of E.coli (MIC value 93.3 μg/ml) and Salmonella Typhi (MIC=110 μg/ml) whereas it shows low activity on Pseudomonas aeruginosa (MIC value > 250 μg/ml). All three tested microorganism belongs to the species gram negative bacteria. These results confirm that the compound synthesized was active against the three tested organisms and the anti-microbial activity of the polymer based product was higher in two organisms namely, E.coli and Salmonella Typhi. Novel chalcone type molecule EHP was synthesized using 4-hydroxybenzaldehyde and 2, 4 – dichloro – 5 – flouro acetophenone. Monomer EAP was prepared from EHP using acryloyl chloride (Scheme 1). Poly(EAP) (Scheme) was prepared using EHP as a parent compound. All the compounds and the polymer were characterized by IR, NMR techniques. The anti-microbial activity of the polymer was done using minimum inhibitory concentration

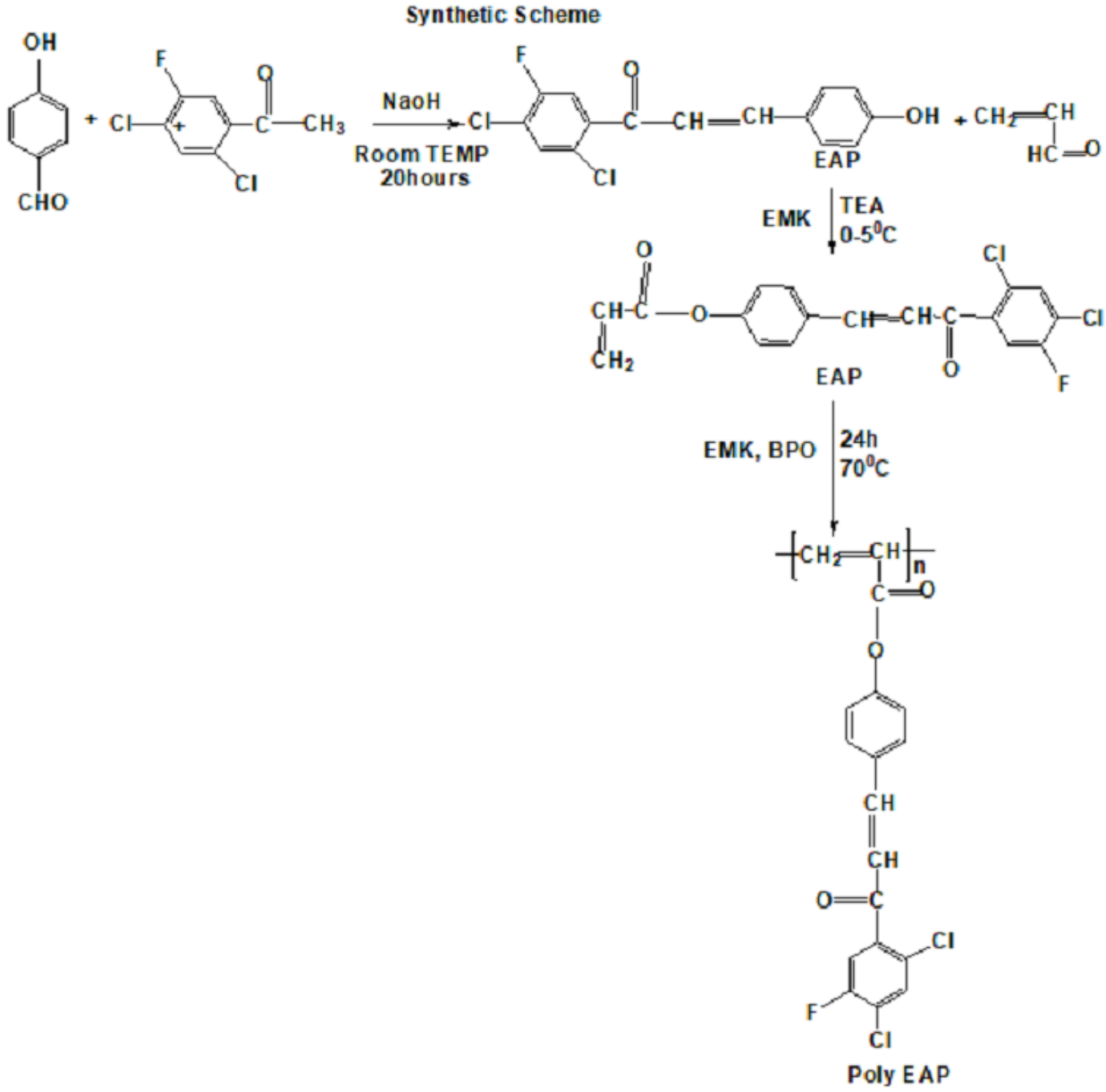

Fig. 1:

Table 1: Minimum inhibitory concentration (MIC) of DFA, EHP, EAP and poly (EAP) against ATCC bacterial strains

Strains	Minimum Inhibitory Concentration (mg/ml) [a]			
	DFA	EHP	EAP	Poly (EAP)
E.coli ATCC 25922	>250	153 ± 5.8	200 ± 10	93 ± 5.8
Pseudomonas aeruginosa ATCC 27853	>250	200 ± 10	197 ± 5.8	>250
Salmonella Typhi ATCC 6439	>190 ± 6	233 ± 5.8	240 ± 10	110 ± 10

(MIC) method.

4. Conclusion

2, 4-dichloro-5' fluoro-1-ene-2-(4-hydroxyphenyl) phenone (EHP), 4-[3-(2, 4-dichloro-5-fluorophenyl)-3-oxoprop-1-en-1-yl]phenyl acrylate (EAP), Poly (EAP) and DFA has been synthesized & characterized as per the procedure descried in the experimental section. The anti-microbial activity of four synthesized compounds [DFA, EHP, EAP and poly (EAP)] has been tested on gram positive and gram negative microorganisms such as E.coli, pseudomonas aeruginosa and Salmonella Typhi microbes. Among the screened compounds, the compound Poly (EAP) has shown a significantly higher activity against the E.Coli and Salmonella Typhi and moderate activity against Pseudomonas aeruginosa whereas, EHP, DFA and EAP have shown a moderately higher activity towards all the three microorganisms such as E.Coli, Salmonella typhi and Pseudomonas aeruginosa respectively.

5. Source of Funding

None.

6. Conflict of Interest

None.

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Cite this article: Manivannan S. Design, synthesis and evaluation of chalcones as anti-microbial agent. *Indian J Forensic Community Med* 2020;7(3):120-123.