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Synthesis and Evaluation of Antibacterial Properties of Chalcones Derived from Thiophene-2-Carbaldehyde

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Chalcone is a simple chemical structure which is present in most of the naturally occurring substances. Many chalcone derivatives are synthesized by Claisen-Schmidt condensation reaction. It is a subject of great research opportunity due to numerous biological activities and convenient synthesis of chalcones. This study aims on synthesizing different α, β unsaturated ketones (chalcones) containing thiophene from thiophene-2-carbaxldehyde and different substituted acetophenones, further evaluating antibacterial activity of synthesized compounds. The synthesized compounds are characterized for their spectral study. From the antibacterial study it was observed that the compounds bearing electron withdrawing group, electron releasing group exhibited excellent to moderate antibacterial activity respectively. These results showed that chalcones incorporated with thiophene have better scopes for further development of the antimicrobial agents.

Keywords: Chalcones; thiophene; claisen-schmidt condensation; E.coli; antibacterial.

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1. INTRODUCTION

The field of medicinal chemistry is well advanced with the discovery of variety of drug molecules with different organic and heterocyclic moieties with various substitutions for the various diseases caused by pathogens by suitably focusing on the target site. Currently we have number of antimicrobial agents to treat the different infectious diseases [1]. There are challenges associated with anti-microbial agents, as most of the micro-organisms develop resistance against current antibiotics/antibacterial agents; hence there is a scarcity of new antimicrobial agents globally. As we know, infectious disease is the reason for one-half of death especially in the developing countries. Hence there is a need for effective, novel and inexpensive antimicrobial agents [2].

During the study for such alternatives, compounds having broad spectrum biological activities like chalcones and its derivatives were highly considered. This led to the research and development of chalcones as it is a multifunctional molecule since it possesses various biological activities in a single structure like anti-inflammatory, antimicrobial, antiviral, antioxidant, anticancer, immunomodulatory, antitubercular, analgesic, antihyperglycemic, antiplatelet etc., [2].

Chalcones are also known by the terms benzal acetophenone and benzylideneacetophenone. Chalcone or chalconoids describes compounds with aromatic ketone which form the central core for a variety of important biological compounds, which are known collectively as chalcones. In chalcones 2 aromatic rings are joined by a three-carbon aliphatic alpha beta unsaturated carbonyl chain. The interconnected chain has highly electrophilic alpha beta unsaturation and conjugated double bonds [3]. Molecules possessing such system have relatively low redox potentials and have a greater probability of undergoing electron transfer reactions. They mainly obtained by the condensation reaction between the aromatic aldehydes and acetophenones in the presence of acid /base catalyst (Claisen Smith condensation) [4].

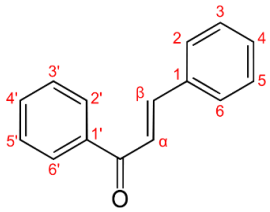

Fig. 1. General chemical structure of chalcones

Chalcones and its analogues have found its significant role in the development of new medicinal agents. Thus, it has become an object of interest in synthesis and research [5]. Recent studies on chalcones documented its use for the treatment of microbial diseases, stomach cancer, food additives, CVS disorders and cosmetic formulation preparation [6,7].

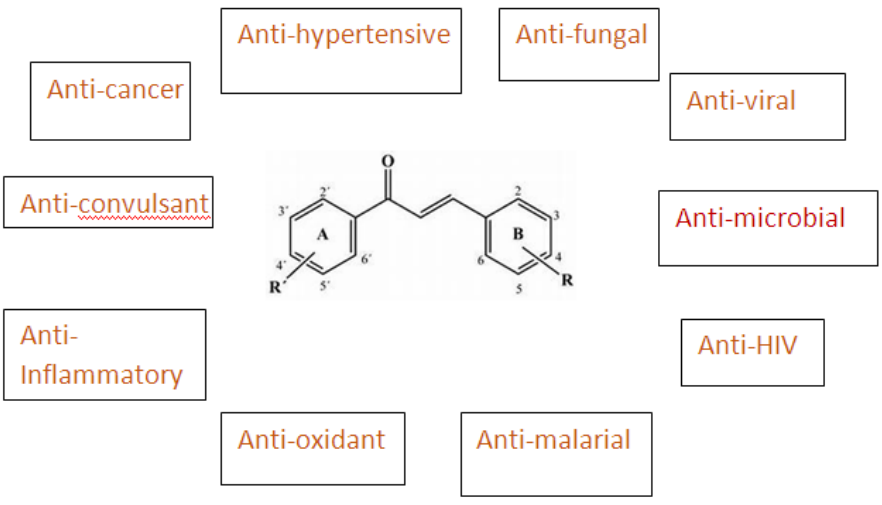

Fig. 2. Biological importance of chalcones

As thiophene is an active, potential 5-membered, 'S' containing hetero cyclic ring commonly present as building block in the most of the drugs, the present study we have considered thiophene incorporated chalcones containing different substitution to find a better advantageous anti-bacterial molecule with improved activity and lower side effect.

2. MATERIALS AND METHODS

All the reactions were carried out under specified laboratory conditions. All the synthetic work was done by procuring laboratory grade reagents and analytical grade solvents. All the aromatic substituted acetophenones were obtained commercially. The products were purified by recrystallization using suitable solvents. Melting points were determined by Digital melting point apparatus and were uncorrected.

2.1 Procedure for the Synthesis of Thiophene Incorporated Chalcones

An equimolar mixture of substituted acetophenone (0.01mol) and thiophene-2-carbaldehyde (0.01 mol) was taken in iodine flask with 30 ml ethanol as solvent and aqueous solution of KOH was added to reaction mixture. Further it was kept in magnetic stirrer and reaction mixture was stirred for 6-8 hours. The mixture is then poured into crushed ice and acidified with conc. HCl. The chalcone derivatives precipitates out which was then filtered using whatman filter paper. Further purification of compounds was done by recrystallization using ethanol [8,9].

Scheme

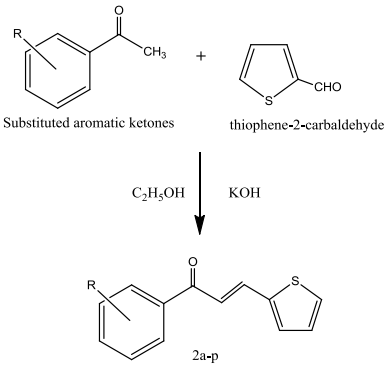

Fig. 3. Synthesis of substituted α-β unsaturated ketones (2a-p)
R= H, p-F, p-NO_2, p-NH_2, m-NO_2, m-CH_3

2.2 *In-vitro* Antibacterial Studies

The *in-vitro* antibacterial study was performed by tube dilution and cup plate method. In this study we have taken gram negative organism Escherichia coli to evaluate antibacterial properties of novel synthesized compounds (2a-f). Ciprofloxacin was used as standard reference drug. Fresh 24h old bacterial culture is used for the activity.

2.3 Tube Dilution Method

This method is mainly used to determine MIC, it is the minimum or lowest concentration of a compound which prevents the growth of a bacteria. Two- fold dilutions of the compounds and standard drugs were prepared using DMSO ranging from 15.625-500µg/ml (A1-A6). Both the positive control (Nutrient broth+ solvent inoculated with culture, to determine the effect of solvent) and negative control (Nutrient broth+ solvent, to ensure that there is no growth and media is sterile) (A7 and A8). All the tubes were kept for incubation for 24h in incubators at 37^0C. The inhibitory concentration was noted by visual observation after 24h [10,11,12,13].

2.4 Cup Plate Method

We have selected two concentrations of the compounds based on the minimum inhibitory concentration from the tube dilution method (40µg/ml and 100µg/ml). Solution of the compounds and standard drug was prepared by dissolving 1mg each in 1ml of DMSO (concentration= 1000 µg/ml). Volumes of 40 µL and 100µL (0.04ml and 0.1ml) is used for the testing. Same volume of the solvent is used as control. A liquid agar medium was prepared by mixing the nutrient agar with water and sterilizing it in an autoclave for 15 min at 121^0C. The freshly prepared liquid agar medium was immediately poured into each of the Petri plate and allowed to solidify. The plates were inoculated with fresh 24h culture using and L-shaped spreader. The sterile borer was used to make wells in the medium. The sample solution of particular concentration was added into the wells. Procedure is repeated for the standard drug. The plates were then incubated for 24 hours at 37^0C. After incubation, the zone of inhibition was observed and measured in mm [14,15,16,17].

3. RESULTS

3.1 Characterization of Synthesized Compounds

3.1.1 (2*E*)-1-(4-fluorophenyl)-3-(thiophen-2-yl)prop-2-en-1-one(2a)

yellow powder, yield:68%, M.P: 76-78^{0}C. IR (KBr,cm^{-1}) : 1580.47(C=C, aliphatic), 1655.31(C=O), 1190.52(C-F), 3459.92(C-H stretch, aromatic), 1500.54(C=C aromatic), 1025.8(C-H bend), 703(C-S-C). ^{1}H NMR(DMSO, δ ppm): 7.47(d, 1H, CH of olefin), 7.32(d, 1H, CH of olefin), 6.98(m, 3H), 7.85 (d, 1H, Ar-H), 7.54 (d, 1H, Ar-H), 6.86 (d, 1H, Ar-H), 6.39 (d, 1H, Ar-H). Mass (LC-MS, m/z): 232.31 (M^+).

3.1.2 (2*E*)-1-(4-aminophenyl)-3-(thiophen-2-yl)prop-2-en-1-one(2b)

IR (KBr,cm^{-1}): yellow powder, yield:72%, M.P: 80-82^{0}C**.** 3214.92(N-H stretch), 1573.52(C=C), 1644.01(C=O), 3746.49(C-H stretch, aromatic), 1044.51(C-H bend), 1409(C=C, aromatic), 701(C-S-C). ^{1}H NMR(DMSO, δ ppm): 7.38(d, 1H, CH of olefin), 7.23(d, 1H, CH of olefin), 6.92(m, 3H), 7.88 (d, 1H, Ar-H), 7.59 (d, 1H, Ar-H), 6.88 (d, 1H, Ar-H), 6.41 (d, 1H, Ar-H), 4.94(s, 2H, NH_2). Mass (LC-MS, m/z): 229.24 (M^+).

3.1.3 (2*E*)-1-(4-methylphenyl)-3-(thiophen-2-yl)prop-2-en-1-one(2c)

IR (KBr, cm^{-1}): creamish white powder, yield:85%, M.P: 84-86^{0}C**.** 1584.37(C=C aliphatic), 1655.88(C=O), 1361(C=C aromatic), 3819.46(C-H stretch, aromatic), 1013.23(C-H bend),714.62(C-S-C). ^{1}H NMR(DMSO, δ ppm): 7.38(d, 1H, CH of olefin), 7.23(d, 1H, CH of olefin), 6.92(m, 3H), 7.88 (d, 1H, Ar-H), 7.59 (d, 1H, Ar-H), 6.88 (d, 1H, Ar-H), 6.41 (d, 1H, Ar-H), 3.789(s, 3H, CH_3). Mass (LC-MS, m/z): 228.12 (M^+).

3.1.4 (2*E*)-1-(3-nitrophenyl)-3-(thiophen-2-yl)prop-2-en-1-one(2d)

IR (KBr,cm^{-1}): yellow powder, yield:81%, M.P: 132-134^{0}C**.** 152.4(C=C aliphatic), 1656.28(C=O), 1346.08(C=C aromatic), 3649.59(C-H stretch), 1084.55(C4 bend), 716.01(C-S-C), 1569.86 and 1420.52(NO_2). ^{1}H NMR(DMSO, δ ppm): 7.43(d, 1H, CH of olefin), 7.32(d, 1H, CH of olefin), 6.89(m, 3H), 7.82 (d, 1H, Ar-H), 7.55 (d, 1H, Ar-H), 6.85 (d, 1H, Ar-H), 6.44 (d, 1H, Ar-H).Mass (LC-MS, m/z): 259.34 (M^+).

3.1.5 (2*E*)-1-phenyl-3-(thiophen-2-yl)prop-2-en-1-one(2e)

white powder, yield: 58%, M.P: 64-66^{0}C**.** IR (KBr,cm^{-1}): 1585.04(C=C aliphatic), 1654.86(C=O), 1445.64(C=C aromatic), 3368.42(C- H stretch), 1032.57(C-H bend), 685.21(C-S-C). ^{1}H NMR(DMSO, δ ppm): 7.59(d, 1H, CH of olefin), 7.45(d, 1H, CH of olefin), 6.74(m, 3H), 7.86-7.51 (m, 5H, Ar-H). Mass (LC-MS, m/z): 214.09 (M^+).

3.1.6 (2*E*)-1-(4-nitrophenyl)-3-(thiophen-2-yl)prop-2-en-1-one (2f)

yellow powder, yield:81%, M.P: 146-148^{0}C**.** 152.4(C=C aliphatic), 1656.28(C=O), 1346.08(C=C aromatic), 3649.59(C-H stretch), 1084.55(C4 bend), 716.01(C-S-C), 1569.86 and 1420.52(NO_2). ^{1}H NMR(DMSO, δ ppm): 7.48(d, 1H, CH of olefin), 7.36(d, 1H, CH of olefin), 6.84(m, 3H), 7.80 (d, 1H, Ar-H), 7.15 (d, 1H, Ar-H), 6.65 (d, 1H, Ar-H), 6.49 (d,1H, Ar-H).Mass (LC-MS, m/z): 259.41(M^+).

Table 1. Physical data of synthesized compounds 2a-f

Name	R	Molecular formula	Molecular weight	Melting point (^{0}C)	Percentage Yield
2a	4-F	$C_{13}H_9OSF$	232	76-78	68
2b	4-NH_2	$C_{13}H_{11}NOS$	229	80-82	72
2c	4-CH_3	$C_{14}H_{12}SO$	228	84-86	85
2d	3-NO_2	$C_{13}H_9SNO_3$	259	132-134	81
2e	H	$C_{13}H_{10}SO$	214	64-66	58
2f	4-NO_2	$C_{13}H_9SNO_3$	259	146-148	89

3.2 Anti-Microbial Studies

3.2.1 Tube dilution method

Table 2. Minimum inhibitory concentration of 2a-f and Ciprofloxacin

	Compounds					
Concentration (µg/ml)	**2a**	**2b**	**2c**	**2d**	**2e**	**2f**
250	-	-	-	-	-	-
125	-	-	-	-	-	-
62.5	-	-	+	-	+	-
31.25	+	-	+	+	+	+
15.625	+	+	+	+	+	+
7.8125	+	+	+	+	+	+
MIC(µg/ml) against E.coli	62.5	31.25	125	62.5	125	31.25

(+) indicates presence of growth, (-) indicates absence of growth, MIC- Minimum concentration of compounds required for the inhibition of growth

Fig. 4. Evaluation of MIC by Tube dilution method Cup plate method

Table 3. Zone of inhibition produced by compound 2a-f and ciprofloxacin

Compound	**Zone of inhibition in mm**	
Concentration(µg/ml)	**40**	**100**
2a	9	15
2b	13	19
2c	7	11
2d	**12**	**21**
2e	5	9
2f	**14**	**24**
Standard (ciprofloxacin)	19	26
Control	2	2

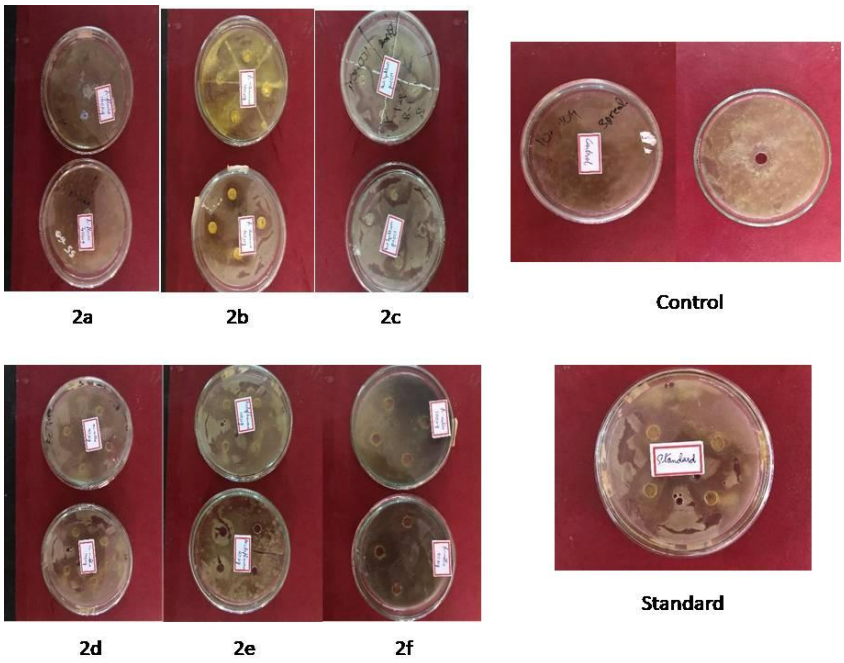

Fig. 5. Zone of inhibition by cup plate method

4. DISCUSSION

The synthesis of thiophene incorporated chalcones were synthesised by scheme 1 using conventional method of synthesis of chalcones i.e., Claisen Schmidt condensation using substituted acetophenones and thiophene-2-carboxaldehyde. All the synthesized compound were purified by recrystallization using ethanol and characterized by IR, NMR and Mass spectroscopy. We have observed the important peaks for different functional groups in IR like C=C (aliphatic, aromatic) C=O, CH stretch and bend, C-S-C and for important functional groups like F, NO_2, NH_2 and CH_3. In NMR spectra, hydrogen's attached to the carbon atoms of the olefin linkage gave prominent peaks, peaks for hydrogen's attached to aromatic ring were also observed. In mass spectrum, M^+ peak was observed for all the compounds. The physical properties of all the newly synthesized compounds are given in Table 1.

All the synthesized chalcones were evaluated for *in-vitro* anti-bacterial potency against gram negative bacteria i.e., E. coli by tube dilution and cup plate method using ciprofloxacin as the reference standard.

All the tested compounds showed minimum inhibitory concentration of 62.5, 31.25, 125, 62.5, 125, 31.25 and15.625 µg/ml respectively for 2a, 2b, 2c, 2d, 2e, 2f and ciprofloxacin given in Table 2. Whereas in the positive control test tube was turbid, which indicates the growth of organism, this tells us that prepared medium supports the growth of organism. In the negative control the no growth was observed which ensures the sterility of the media. Out of all six compounds 2b, 2f, 2a, 2d exhibited comparatively better inhibition with lower MIC, where the inhibition by compound 2b and 2f were found to be impressive (Figure 4). Based on MIC, two fixed doses of the compounds were selected i.e., 40 µg /ml and 100 µg/ml for further evaluation by cup plate method.

All the six test compounds along with standard were evaluated further by cup plate method. After 24hours of incubation, zone of inhibition produced by the compounds were measured. (It was measured along with well diameter) (Figure 5). Out of all the six tested compounds 2d and 2f exhibited excellent antibacterial activity where zone of inhibition produced was 12mm, 21mm and 14mm, 24mm which were comparable to standard ciprofloxacin, where zone of inhibition was 19mm and 26mm in two different concentration respectively. 2a and 2b exhibited moderate and 2c and 2e exhibited mild anti-bacterial activity as given in Table 3, where zone of inhibition produced by control was 2mm. Out of all the compounds 2f has exhibited excellent antibacterial activity which can be considered as promising molecule was further evaluation.

5. CONCLUSION

The main objective of this particular project was to synthesize the novel thiophene incorporated chalcones and evaluation of their antimicrobial properties. Out of the synthesized compounds, molecules bearing electron withdrawing group, electron releasing group and electronegative atom exhibited excellent to moderate antibacterial activity. These results showed that these synthesized chalcones incorporated with thiophene especially 2f, 2d, 2a and 2b are the promising molecules with antibacterial properties have better scope for further development of the antibacterial; agents and potency of these compounds are required to confirm further by *in-vivo* screening.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Elisha IL, Botha FS, McGaw LJ, Eloff JN. The antibacterial activity of extracts of nine plant species with good activity against Escherichia coli against five other bacteria and cytotoxicity of extracts. BMC complementary and alternative medicine. 2017;17(1):1-10.
2. Kumar SK, Hager E, Pettit C, Gurulingappa H, Davidson NE and Khan SR: Design, synthesis, and evaluation of novel boronic-chalcone derivatives as antitumor agents: J Med Chem 2003; 46(14): 2813-15.
3. Shu-Zhen Hua, Jian-Guang Luo, Xiao-Bing Wang, Jun-Song Wang, Ling-Yi Kong. Two novel monoterpene–chalcone conjugates isolated from the seeds of *Alpinia katsumadai.* Science direct. 2009;19(10):2728-30.
4. Sharma N, Mohanakrishnan D, Sharma U K, Kumar R, Richa, Sahal D et al. Design, Economical synthesis and antiplasmodial evaluation of vanillin derived allylated chalcones and their marked synergism with artemisinin against chloroquine resistant strains of Plasmodium falciparum. Eur J Med Chem. 2014;79: 350-68
5. Tekale S, Mashele S, Pooe O, Thore S, Kendrekar P, Pawar R. Biological role of chalcones in medicinal chemistry. InVector-Borne Diseases-Recent Developments in Epidemiology and Control. 2020.
6. C Dias Tielas, E Grana, M.J.Riegosa, A.M.Sanchez Moreira. Biological activities and Novel applications of chalcones. Dimensions publications.2016; 34(3): 606-16.
7. Domingues J N, Leon C, Rodrigues J, Rosenthal P J et al. Synthesis and Evaluation of new antimalarial phenylurenyl chalcone derivatives. J. Med.Chem. 2005;48(10),3654-58
8. Chavan BB, Gadekar AS, Mehta PP, Vawhal PK, Kolsure AK, Chabukswar AR. Synthesis and medicinal significance of chalcones-A review. Asian journal of biomedical and pharmaceutical sciences. 2016;6(56):01-07.
9. Vu Quoc T, Tran Thi Thuy D, Dang Thanh T, Phung Ngoc T, Nguyen Thien V, Nguyen Thuy C, Van Meervelt L. Some chalcones derived from thiophene-3-carbaldehyde: synthesis and crystal structures. Acta Crystallographica Section E: Crystallographic Communications. 2019; 75(7):957-63.
10. Arasavelli AM, Sharma GV, Vidavalur S. Design, synthesis, and anticancer activity of novel aryl/heteroaryl chalcone derivatives. Heterocyclic Communications. 2016; 22(1):1-5.
11. Lagu SB, Yejella RP, Bhandare RR, Shaik AB. Design, synthesis, and antibacterial and antifungal activities of novel trifluoromethyl and trifluoromethoxy substituted chalcone derivatives.Pharmaceuticals.2020; 13(11): 375.
12. Cao Z, Yang J, Xu R, Song O, Zhang X, Liu H et al. Design, synthesis and evaluation of 4′-OH-flurbiprofen chalcone hybrids as potential multifunctional agents of

Alzheimer's disease treatment. Bioorganic and medicinal chemistry 2018; 26(5): 1102-15.
13. Burmaoglu S, Algul O, Gobek A, Anil D A et al. Design of potent fluoro substituted chalcones as antimicrobial agents. J Enzyme Inhib Med Chem 2017; 32(1): 490-5.
14. Božić DD, Milenković M, Ivković B, Cirković I. Antibacterial activity of three newly-synthesized chalcones & synergism with antibiotics against clinical isolates of methicillin-resistant Staphylococcus aureus. The Indian journal of medical research. 2014;140(1):130.
15. Shakhatreh MA, Al-Smadi ML, Khabour of, Shuaibu FA, Hussein EI, Alzoubi KH. Study of the antibacterial and antifungal activities of synthetic benzyl bromides, ketones, and corresponding chalcone derivatives. Drug design, development and therapy. 2016; 10:3653.
16. Prasad YR, Rao AL, Rambabu R. Synthesis and antimicrobial activity of some chalcone derivatives. E-Journal of Chemistry. 2008; 5(3):461-6.
17. B.A. Baviskar, B.B. Baviskar, M.R. Shiradkar, U.A. Deokate, S.S. Khadabadi. Synthesis and Antimicrobial activity of some novel Benzimidazolyl chalcones. E Journal of Chemistry 2009;6(1): 196-200.

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