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Synthesis and Antibacterial Evaluation of new Ofloxacin-Chalcone derivatives Conjugates as Possible Mutual Prodrugs

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Abstract

Objectives:
To synthesize ofloxacin-chalcones conjugates as possible mutual prodrugs of enhanced antibacterial activity and evaluate this activity against different bacterial strains.

Methods:
Two chalcone derivatives have been synthesized using Claisen–Schmidt condensation of p-hydroxyacetophenone with p-fluorobenzaldehyde and p-bromobenzaldehyde using $SOCl_2$/EtOH as a catalyst to obtain finally a fluorinated chalcone derivative (FCD) and a brominated chalcone derivative (BCD) respectively.
These synthesized chalcones derivatives were esterified with ofloxacin to obtain two possible mutual prodrugs that evaluated for its antibacterial activity against gram-positive and gram negative bacteria in comparison with standard antibiotics: ofloxacin and ciprofloxacin using a disc diffusion method.

Results:
FTIR Spectroscopy, elemental microanalysis (CHN), and other physicochemical properties have been used to characterize the structure of the synthesized compounds.A moderate antibacterial activity of the synthesized chalcone derivatives (FCD and BCD) was obtained whereas their conjugates with ofloxacin were showed enhanced antibacterial activity in comparison with ofloxacin and ciprofloxacin using the culture and sensitivity test model on gram-positive and negative bacteria.

Conclusion:
Chalcones with their pronounced antibacterial activity can enhance the activity of Fluoroquinolones when conjugated with this class of antibiotics.

Keywords: Antibiotics, Fluoroquinolones, Ofloxacin, Chalcone derivatives, Mutual prodrugs.

INTRODUCTION

The Fluoroquinolones drugs are one of the most common therapeutic groups of agents used worldwide for their greater efficacy, a broader spectrum of antimicrobial activity and a better safety (1).

By entering bacteria through porin channels; Fluoroquinolones exhibit their antimicrobial activity by inhibition two important bacterial enzymes: DNA gyrase (bacterial topoisomerase II) and bacterial topoisomerase IV. Result in relaxation of supercoiled DNA by inhibition of the first bacterial enzyme that promotes DNA strand breakage. Whereas impacts chromosomal stabilization during cell division through inhibition of topoisomerase IV, thus interfering with the separation of newly replicated DNA. *Pseudomonas aeruginosa* as an example of gram-negative organisms, the inhibition of DNA gyrase is more significant than that of topoisomerase IV, while in *Streptococcus pneumoniae* as an example of gram-positive organisms, the opposite is true (1) (2).

The clinical efficacy of Fluoroquinolones family to treat infections especially urinary tract infections were has been threatened by growing bacterial strains that resist progressively since the 1990s. Furthermore, oxidative Stress induced by Fluoroquinolones led to complicated side effects in human beings. Recent studies suggested that co-administration of Fluoroquinolones with exogenous antioxidants might decrease the oxidative stress associated with the use of such agents (2) (3) (4) (5).

Chalcones are derived from 1,3-diphenyl-2-propene-1-one in which two aromatic rings conjugated together through a reactive three carbon α,β-unsaturated carbonyl system (–CO–CH=CH–), such system allow a completely delocalized π-electron system on both aromatic rings in such a way that this system has relatively low redox potentials and has a greater probability of undergoing electron transfer reactions (6). In addition, the colors of these compounds are attributed to the presence of α,β-unsaturated ketone chromophore and other auxochromes (7).

Chalcones are considered to be precursors of flavonoids which are a very large and widespread group of plant constituents that participate in the defense strategies by acting as antimicrobial, antifungal, and antioxidants agents. Thus, they have a great therapeutic potential for healing of diverse diseases. Chalcones being natural or synthetic are known to display a remarkable spectrum of pharmacological activities such as antibacterial, antioxidant, anti-inflammatory, analgesic, and other activities (8) (9) (10). They are also well known as valuable intermediates in the organic synthesis of many heterocycles that show a multitude of biological activities (11) (12) (13).

The antimicrobial activity of chalcones is being increasingly documented. Many research groups were concerned with either isolated or synthesized chalcones that possess antimicrobial activity. The presence of a reactive Enone moiety (α,β-unsaturated ketone) in chalcones was found to undergo conjugate addition with a nucleophilic group like a thiol group in an essential protein, thus partly contributing for their antimicrobial activity, which may be altered depending on the type and position of the substituents on the aromatic rings present in Chalcones (14) (15).

There are a number of strategies for the synthesis of chalcones that are based generally on the formation of the carbon-carbon bond of the enone moiety (16) (17) (18). The Claisen-Schmidt condensation is the most common strategy which involves using suitable condensing agents for condensation of an aromatic ketone with an aromatic aldehyde. therefore, a diversity of methods are available for the chalcones' synthesis that employs this kind of approach and the most important and simple one is the

condensation under acidic conditions (HCl) that is produced *in situ* in the $SOCl_2$/EtOH catalytic system and followed by dehydration to yield the predicted chalcone derivative (18).

Therefore, the present study was designed to synthesize two of appropriately substituted Chalcones, which on their conjugation with ofloxacin may serve as possible mutual prodrugs that prevent oxidative stress with possible synergistic antibacterial activity that devoid of bacterial resistance.

Therefore the co-administration of Fluoroquinolones with chalcones might decrease the bacterial resistance and oxidative stress as well as increasing the spectrum to cover a wide range of bacterial generations. However, there are potential advantages in giving together such agents having complementary activities, in the form of single chemical entity, i.e. mutual prodrugs which are designed to improve physicochemical properties and targeting of the parent drug. Furthermore, the prodrug designing of ofloxacin were devoted to masking some of it is side effects due to the chemical change that has been occurred to the structure.

MATERIALS AND METHODS

P-bromobezaldehyde, p-fluorobenzaldehyde, and p-hydroxyaceto-phenone were purchased from Himedia (India) while ofloxacin was purchased from Ampla chemicals (China), and the quality of all these chemicals together with the other ones used throughout the study are obtained from standard commercial sources were of analar grade and used without further purification. The melting points were determined by the open capillary method using Thomas Hoover (England) and were used uncorrected. Cooling of reactions when needed was done using a Julabo chiller VC (F30) (GMBH, Germany). Infra-red spectra were recorded in potassium bromide (KBr) disc on Shimadzu FTIR 8400 spectrophotometer (Japan), at the Faculty of Pharmacy, University of Kufa. Elemental microanalysis was performed at the Jordanian University using CHN Elemental Analyzer (Euro-vector EA3000A, Italy). The progress of the reaction was monitored by ascending thin layer chromatography which was run on Kieslgel GF_{254} (60) aluminum plates (E. Merck, Germany), which was used as well to check the purity of the product. The synthesized compounds were revealed either by derivatization or reactivity toward iodine vapor or by irradiation with UV_{254} light. Chromatograms were eluted using petroleum spirit (40-60): ethyl acetate (70:30) solvent system. Culture and sensitivity were performed at the Faculty of Girls Education, University of Kufa using disk diffusion method by the aid of Mueller Hinton Agar (MHA).

Chemical synthesis

1. **Synthesis of Chalcone derivatives (FCD) and (BCD)** (18) (19) (20)

Chalcone derivatives (FCD) and (BCD) were synthesized by Claisen-Schmidt condensation using $SOCl_2$/EtOH as a catalytic system. The general procedure to prepare these chalcone derivatives starting with substituted aromatic ketone and substituted aromatic aldehydes (Scheme 1).

To a stirred mixture of the p-hydroxyacetophenone (10 mmol/1.36 gm) and p-flourobenzaldehyde (10 mmol/1.07 ml) and p-bromobenzaldehde (10 mmol/1.85 gm) in 10 ml absolute ethanol; 0.6 ml of thionyl chloride was added in dropwise manner over a 10 min with vigorous stirring continued for 3-4 hr at room temperature. The solution turned deep red immediately; when stirred for 30 min, the mixture became coagulated. After completion of the reaction, the reaction mixture was allowed to stand overnight. Then reaction mixture was precipitated by the addition 25 ml of cold distilled water and the mixture was filtered; the obtained solid was washed successively with cold distilled water (3×30ml), cold absolute ethanol (3×10ml) and cold diethyl ether (3×10ml), and allowed to dry to get (*E*)-3-(4-fluorophenyl)-1-(4- hydroxyphenyl)prop-2-en-1-one (FCD) and (*E*)-3-(4-bromophenyl)-1-(4- hydroxyphenyl)prop-2-en-1-one (BCD) respectively. These products were recrystallized from absolute ethanol (Scheme 1).

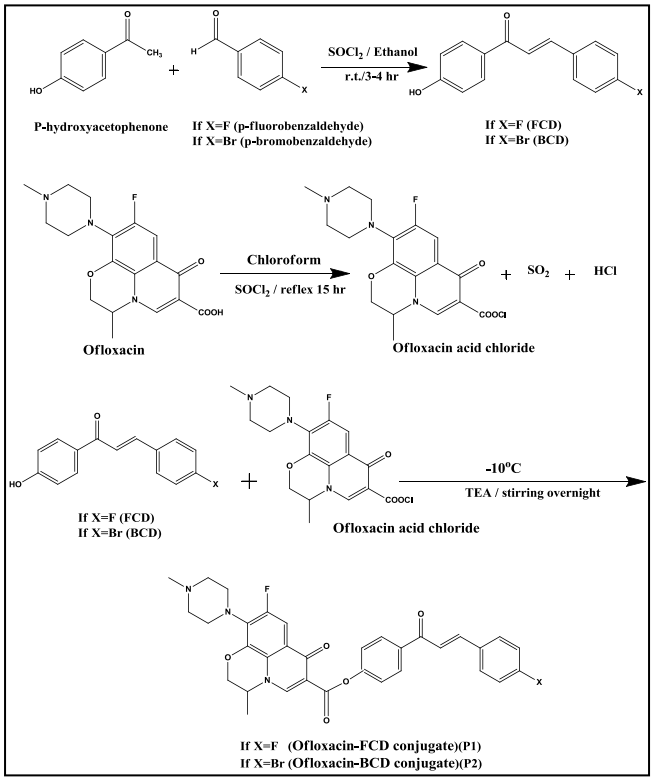
2. **Synthesis of Ofloxacin-chalcones conjugates (P1) and (P2)** (21)

Ofloxacin (10 mmol/2.96 gm) was dissolved in the minimum volume of dry chloroform, that was cooled down to (-15^{o}C). Then a thionyl chloride (16 mmol/1 ml) was added in dropwise manner over a period of 30 min with stirring while the temperature was kept (-15^{o}C).

After thionyl chloride addition was completed, the reaction mixture was refluxed for (24 hours) at (60-70^{o}C) with continuous stirring on a magnetic stirrer. The reaction progress was monitored by means of using a gas trap. Later the solvent was evaporated to dryness, re-dissolved in dry chloroform and evaporated. To ensure removal of all thionyl chloride, the process was repeated several times. Then the residue (ofloxacin acid chloride) was re-dissolved in (30 ml) of dry chloroform. The obtained acid chloride solution was added in dropwise manner to a mixture of either one of the following synthesized chalcone derivatives (10 mmol):

- FCD (2.42 gm)
- BCD (3.03 gm)

and triethylamine (10 mmol/ 1.4 ml) in dry dichloromethane (10 ml), previously cooled to (-10^{o}C), with constant stirring over a period of 1 hr while maintaining a constant temperature. The reaction mixture was stirred overnight, then washed with 5% v/v HCl (3×50 ml), 5% w/v NaOH (3×50 ml) and finally with distilled water (3×50 ml). The anhydrous sodium sulphate was used to dry an organic layer, filtered and the solvent was removed under reduced pressure to obtain the Ofloxacin-chalcones conjugates (P1 & P2), that were recrystallized from petroleum spirit (40-60) and ethyl acetate (Scheme 1).



Scheme 1: Synthetic diagram of FCD, BCD, ofloxacin-FCD conjugate (P1), and ofloxacin-BCD conjugate (P2). (21) (22) (23).

Evaluation of antibacterial activity

The synthesized compounds (FCD, BCD, P1, and P2) were screened for their *in vitro* antibacterial activity against some selected microorganisms (gram-positive *staphylococcus aureus* and gram-negative *Escherichia coli*) by measuring the zone of inhibition in mm. The evaluation of antibacterial activity was performed by filter paper disc plate method at concentration 1000 µg/mL and reported in table 3. Muller Hinton agar was employed as culture medium and DMSO was used as solvent control for antibacterial activity. Ofloxacin and ciprofloxacin were used as chemotherapeutic standards for the antibacterial activity evaluation.

RESULTS & DISCUSSION

Synthesis of Chalcone derivatives (FCD) and (BCD)

In table 1, FCD showed an excellent yield (92%) with a melting point around 192-194^{o}C, while showing a single spot with R_f value of 0.3 when eluted on a TLC plate using petroleum ether (40-60): ethyl acetate (70:30) solvent system. The spectral analysis in the UV region exhibited a λmax between 300-350 nm. The IR spectrum in KBr discs showed many characteristic absorption bands at *v* values (3134, 1649, 1608, 1161 cm^{-1}). The elemental microanalysis revealed that the C & H% were (74.968%) and (4.407%) respectively (Table 2).

In table 1, BCD also showed an excellent yield (80%) with a melting point around 182-184^{o}C, while showing a single spot with R_f value of 0.45 when eluted on a TLC plate using petroleum ether (40-60): ethyl acetate (70:30) solvent system. The spectral analysis in the UV region exhibited a λmax between 300-350 nm. The IR spectrum in KBr discs showed many characteristic absorption bands at *v* values (3125, 1655, 1610, 808 cm^{-1}). The elemental microanalysis revealed that the C & H% were (60.365%) and (3.707%) respectively (Table 2).

Synthesis of ofloxacin-chalcones conjugates (P1 & P2)

In table 1, ofloxacin-FCD conjugate (P1) showed a good yield (60%) with a melting point around 102-104^{o}C, while showing a spot with R_f value of 0.11 when eluted on a TLC plate using petroleum ether (40-60): ethylacetate (70:30) solvent system. The IR spectrum in KBr discs showed many characteristic absorption bands at *v* values (3064, 1732, 1614, 1159, and 1077 cm^{-1}). The elemental microanalysis revealed that the C & H & N% were C (69.754%), H (4.908%), and N (7.303%) respectively (Table 2).

In table 1, ofloxacin-BCD conjugate (P2) also showed a good yield (65%) with a melting point around 120-122^{o}C, while showing a spot with R_f value of 0.26 when eluted on a TLC plate using petroleum ether (40-60): ethylacetate (70:30) solvent system. The IR spectrum in KBr discs showed many characteristic absorption bands at *v* values (3043, 1737, 1608, 1155, 1071 cm^{-1}). The elemental microanalysis revealed that the C & H & N% were C (60.722%), H (4.358%), and N (6.334%) respectively (Table 2).

Table 1: Physicochemical data for the synthesized FCD, BCD, ofloxacin-FCD conjugate (P1), and ofloxacin-BCD conjugate (P2).

compound	Chemical name	Molecular formula	Molecular weight	Appearance	Yield (%)	M.P (^{o}C)	R_f
FCD	(*E*)-3-(4-fluoro phenyl)-1-(4-hydroxyphenyl)prop-2-en-1-one	$C_{15}H_{11}FO_2$	242	Pink powder	92	192-194	0.3
BCD	(*E*)-3-(4-bromo phenyl)-1-(4-hydroxyphenyl)prop-2-en-1-one	$C_{15}H_{11}BrO_2$	303	Red powder	80	182-184	0.45
P1	(*E*)-4-(3-(4-fluoro phenyl)acryloyl)phenyl 9-fluoro-3-methyl-10-(4-methyl piperazin-1-y1)-7-oxo-3,7-dihydro-2H-[1,4]oxazino[2,3,4-ij]quinolone-6-carboxulate	$C_{33}H_{29}F_2N_3O_5$	585	Dark brown powder	60	102-104	0.11
P2	(*E*)-4-(3-(4-bromophenyl)acryloyl)phenyl 9-fluoro-3-methyl-10-(4-methylpiperazin-1-yl)-7-oxo-3,7-dihydro-2H-[1,4]oxazino[2,3,4-ij]quinoline-6-carboxylate	$C_{33}H_{29}BrFN_3O_5$	645	Brown powder	65	120-122	0.26

Table 2: IR spectral data and CHN elemental analysis of the synthesized FCD, BCD, ofloxacin-FCD conjugate (P1), and ofloxacin-BCD conjugate (P2).

Sym.	IR Spectral data with its Interpretation	Elemental analysis (calculated) %		
		C	H	N
FCD	IR (KBr) *v* cm^{-1} 3134 (-OH), 1649 (C=O) ketone, 1608 (C=C) trans alkene, 1161 (C-F)	75.968 (74.37)	4.407 (4.58)	-
BCD	IR (KBr) *v* cm^{-1} 3125 (-OH), 1655 (C=O) ketone, 1610 (C=C) trans alkene, 808 (C-Br)	60.365 (59.43)	3.707 (3.66)	-
P1	IR (KBr) *v* cm^{-1} 3064 (aromatic), 1732 (C=O) ester, 1614 (C=C) trans alkene overlap with (C=O) stretching vibration of ketone* 1159 (C-N), 1077 (C-O-C) stretching vibration	69.754 (67.68)	4.908 (4.99)	7.303 (7.18)
P2	IR (KBr) *v* cm^{-1} 3043 (aromatic), 1737 (C=O) ester, 1608 (C=C) trans alkene overlap with (C=O) stretching vibration of ketone* 1155 (C-N), 1071 (C-O-C) stretching vibration	60.722 (61.31)	4.358 (4.52)	6.334 (6.50)

*The C=O stretching vibration of the target compounds (P1 and P2) was overlapped with that of the adjacent stretching vibration (C=C) of the trans alkene, which is in contrast to that shown by their original chalcones (FCD and BCD).

Antibacterial evaluation

The antibacterial evaluation revealed that the synthesized chalcones have a moderate to good potency in comparison to the standard drugs, whereas their conjugates with ofloxacin have superior antibacterial activity in comparison to these standards (Table 3).

Table 3: The antibacterial activity of the [FCD, BCD, ofloxacin, ciprofloxacin, ofloxacin-FCD conjugate (P1), and ofloxacin-BCD conjugate (P2)] against gram-positive and negative bacteria.

Compound	Zone of inhibition (in mm)	
	Escherichia coli	*Staphylococcus aureus*
	1000µg/ml	1000µg/ml
FCD	30	35
BCD	36	44
P1	51	60
P2	56	63
Ofloxacin	45	53
Ciprofloxacin	49	50

The Claisen-Schmidt condensation is an important C-C bond formation for the synthesis of 1,3-diaryl-2-propen-1-ones (chalcones). It is commonly carried out by using strong bases such as NaOH or KOH in polar solvents (MeOH or DMF) or the use of an acid as HCl, BF_3, B_2O_3, p-toluenesulfonic acid, etc (19). However, many of these methods had limited applications owing to their harsh reaction conditions, poisonous reagents, strongly acidic or basic conditions, prolonged reaction-times, poor yields and low selectivity. Therefore, several modifications had been made to overcome such problems. Accordingly, the present study was employed an efficient protocol for synthesizing chalcone derivatives using the $SOCl_2$/EtOH catalytic system. Such protocol gave an excellent yield of the intended product without formation of any side product in a short period of time. Thus, we follow this approach and synthesized (*E*)-3-(4-fluorophenyl)-1-(4-hydroxyphenyl) prop-2-en-1-one (FCD) and (*E*)-3-(4-bromophenyl)-1-(4-hydroxyphenyl) prop-2-en-1-one (BCD). The chemical structures of the all synthesized compounds were confirmed by chromatographic and spectral data. The melting point of the synthesized compounds was observed to be different from the melting points of the starting ingredients, which confirm the successful synthesis of the products. The purity of these synthesized compounds was checked by monitoring a single spot on the TLC plate which in turn proves its purity. The λmax of the chalones was observed between 300-350 nm and this indicates the presence of an α,β-unsaturated carbonyl moiety. The IR absorption bands at *v* values of the chalones also confirmed the presence of a conjugated carbonyl group (C=O), and (C=C) respectively. The synthesized (Ofloxacin-FCD and ofloxacin-BCD conjugates) showed several characteristic sharp bands in the IR region around 1732 cm^{-1}, and 1737 cm^{-1} respectively which indicate the presence of the C=O group of the formed esters, that is also confirmed by the disappearance of signals between 3000-3050 cm^{-1} of the O-H group of a carboxylic acid of ofloxacin (Table 2). The experimentally determined elemental microanalysis results of all the synthesized compounds were compatible with the calculated data. The percent deviations of the observed/calculated values were found to be within the limits of accurate analysis (Table 2).

FCD and BCD were prepared as intermediate compounds for synthesizing the various target conjugates with ofloxacin because of their marked antibacterial activity compared to that of ofloxacin in addition to their known antioxidant activity. Therefore, a synergistic antibacterial effect is achieved that may decrease the bacterial resistance and oxidative stress that associated with the use of the Fluoroquinolones (Table 3).

CONCLUSION

The obtained results proved that the synthesized chalcone derivatives have moderate to good antimicrobial effects against *Staphylococcus aureus* and *Escherichia coli*. Whereas, the synthesized ofloxacin conjugates have stronger inhibition zones than that of ofloxacin alone or correlated to ciprofloxacin (Table 3). These results suggest that these conjugates (ofloxacin-FCD and ofloxacin-BCD) can serve as possible mutual prodrugs and excellent templates for designing and further development as the commercial antibacterial agents. Further experiments are needed to elucidate their mechanism of action and their adverse and side effects.

REFERENCES

1. Karen Whalen(2015) Lippincott Illustrated Reviews: Pharmacology. Sixth Edition ed.; .
2. Katie J. Aldred, Robert J. Kerns, and Neil Osheroff.(2014) Mechanism of Quinolone Action and Resistance. Biochemistry. ; 53(10): p. 1565–1574.
3. Talla V., and Veerareddy P.(2011) Oxidative Stress Induced by Fluoroquinolones on Treatment for Complicated Urinary Tract Infections in Indian Patients. J Young Pharm. ; 3(4): p. 304–309.
4. Rampal S, Kaur R, Sethi R, Singh O, and Sood N.(2008) Ofloxacin-associated retinopathy in rabbits: role of oxidative stress. Hum Exp Toxicol. ; 27(5): p. 409-15.
5. Saraçoğlu A., Temel H., Ergun B., and Colak O.(2009) Oxidative stress-mediated myocardiotoxicity of ciprofloxacin and ofloxacin in juvenile rats. Drug Chem. Toxicol. ; 32(3): p. 238-42.
6. Chetana B. Patil, S. K. Mahajan, and Suvarna A. Katti. (2009)Chalcone: A Versatile Molecule. J. Pharm. Sci. & Res. ; 1(3): p. 11-22.
7. Sharma B., Agrawal S., and Gupta K..(2010) Chalcones: a new class of potential non-azo dyes. Int. J. Chem. Res. ; 1(1): p. 25-28.
8. Hatish Prashar, Anshul Chawla, Anil Kumar Sharma, and Rajeev Kharb. (2012)Chalcone as a versatile moiety for diverse pharmacological activities. IJPSR. ; 3(7): p. 1913-1927.
9. Xue Y., Zheng Y., An L., Zhang L., Qian Y., and Yu D et al. (2012)A theoretical study of the structure–radical scavenging activity of hydroxychalcones. Comput. Theor. Chem. ; 982: p. 74–83.
10. Santosh L. Gaonkar, and U. N. Vignesh.(2017) Synthesis and pharmacological properties of chalcones: a review. Research on chemical intermediates. ; 43(11): p. 6043–6077.
11. B. B. Chavan, A. S. Gadekar, P. P. Mehta, P. K. Vawhal, A. K. Kolsure, and A. R. Chabukswar. (2016)synthesis and Medicinal Significance of Chalcones- A Review. AJBPS. ; 6(56): p. 1-7.
12. Zhuang C., Zhang W., Sheng C., Zhang W., Xing C., and Miao Z.(2017) Chalcone: A Privileged Structure in Medicinal Chemistry. Chem. Rev. ; 117(12): p. 7762-7810.
13. Gomes M , Muratov E , Pereira M., Peixoto J., Rosseto L., Cravo P., Andrade C., and Neves B.. (2017)Chalcone Derivatives: Promising Starting Points for Drug Design. Molecules. ; 22(8): p. 1210.
14. Samer A. Hasan, Amer N. Elias, and Muthanna S. Farhan.(2015) Synthesis, characterization and antimicrobial evaluation of a series of. Der .Pharma. Chemica. ; 7(2): p. 39-42.
15. Nowakowska Z.(2007) A review of anti-infective and anti-inflammatory chalcones. Eur. J .Med. Chem. ; 42(2): p. 125-37.
16. Knoevenagel E. (1898 ???)Condensation von Malonsäure mit Aromatiachen Aldehyden durch Ammoniak und Amine. Chem Ber. ; 31(3): p. 2596–2619.
17. Li JJ. Meyer–Schuster rearrangement.(2006) In Name Reactions: A Collection of Detailed Reaction. 3rd ed. Berlin: Springer; .
18. Hu Z., Liu J., Dong Z., Guo L., Wang D., and Zeng P.(2004) Synthesis of chalcones catalyzedby $SOCl_2$/EtOH. J .Chem. Res. ; 35(34): p. 158-159.

19. Petrov O., Ivanova Y., and Gerova M.(2008) $SOCl_2$/EtOH: Catalytic system for synthesis of chalcones. Catal Commun. ; 9(2): p. 315-316.
20. Jayapal M., and Sreedhar N..(2010) Synthesis and characterization of 4-hydroxychalcones by aldol condensation using $SOCl_2$/EtOH. J. Curr .Pharm. Res. ; 2(4): p. 60-62.
21. Samer A. Hasan, and Amer N. Elias. (2014)Synthesis of new diclofenac derivatives by coupling with chalcone derivatives as possible mutual prodrugs. Int. J. Pharm. Pharm. Sci. ; 6(1): p. 239-245.
22. Rasheed A., and Ashok Kumar C..(2009) Synthesis, hydrolysis and pharmacodynamic profiles of novel prodrugs of mefenamic acid. Int .J. Curr. Pharm. Res. ; 1(1): p. 47-55.
23. Rasheed A., Theja I., Ashok Kumar C., Lvanya Y., Ravindra P., and Vamsee Krishna S.(2009) Synthesis, hydrolysis studies and pharmacodynamic profile of novel colon-specific mutual prodrug of Aceclofenac with amino acids. Der. Pharma .Chemica. ; 1(2): p. 59-71.