Supplementary Materials

Article

In silico Analysis and Experimental Evaluation of Ester Prodrugs of Ketoprofen for Oral Delivery: With a View to Reduce Toxicity

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Characterization of ketoprofen ester prodrugs

Ketoprofen ester prodrugs were synthesized by esterification reaction using three alcohols involving reagent grade methanol, ethanol and propanol in order to obtain methyl 2-(3-benzoyl phenyl) propanoate, ethyl 2-(3-benzoyl phenyl) propanoate and propyl 2-(3-benzoyl phenyl) propanoate as finish products. After synthesis, chromatographically purified and isolated compounds were provided good yields of about 66–84% (Table S1). Ester prodrugs of ketoprofen were analysed by ^{1}HNMR and IR spectroscopic techniques to characterize their chemical groups. ^{1}HNMR spectrum (Figure S1) synthesized by esterification of ketoprofen and methanol showed the presence of a broad singlet signal at 3.497 ppm for and multiplet signal at 7.249–7.322 ppm corresponding to methoxy proton ($-OCH_3$) and aromatic protons (ArH), respectively, whereas the IR spectrum (Figure S2) showed characteristic bands signifying carbonyl C=O and aromatic ArC=C groups. The rest of the two prodrugs synthesized from esterification of ketoprofen with ethanol and propanol were also found to show characteristic signals and bands in ^{1}HNMR and IR analysis, indicating their identity as ethyl 2-(3-benzoyl phenyl) propanoate and propyl 2-(3-benzoyl phenyl) propanoate (Table S2). All the data offered by ^{1}HNMR and IR spectra were meet the characteristic functional groups and anticipated chemical structures of the three distinct ester prodrugs of ketoprofen. Solubility of ketoprofen prodrugs was increased significantly compared to ketoprofen (0.5 μg/mL). Among the three prodrugs, ethyl ester derivative was found to have the lowest solubility. Moreover, the permeability parameter, or Log P (octanol-water partition coefficient) of synthesized prodrugs was also increased compared to that of the parent drug ketoprofen (3.18) (Table S1).

In Silico Toxicity Analysis

SwissADME software was used to estimate the affinity of synthesized prodrugs to be substrate of P-gp or inhibitor CYP isoenzymes; the results are represented in Table S3. Methyl 2-(3-benzoyl phenyl) propanoate showed no affinity for these proteins and isoenzymes, whereas the rest of the two prodrugs showed inhibitory effects on CYPs isoenzymes. Acute toxicity were specific organ toxicity of synthesized prodrugs was predicted by ProTox-II webserver. Acute toxicity data (Table S2) indicated that the toxicity class of prodrugs shifted to a safer zone (toxicity class: 4) from a narrow zone (toxicity class: 2) of active ketoprofen. Predicted LD_{50} values for the prodrugs were > 30 fold greater than that of active drug. Besides, organ toxicity data indicated (Table S2) that hepatotoxicity was not found in the prodrug, whereas active ketoprofen was hepatotoxic. Any other organ toxicities, such as mutagenicity, cytotoxicity, immunotoxicity and others listed in Table S2, were absent in methyl 2-(3-benzoyl phenyl) propanoate. Ethyl 2-(3-benzoyl phenyl) propanoate and propyl 2-(3-benzoyl phenyl) propanoate were found to bind with the estrogen receptor alpha (ER) whereas, other organ toxicities were absent.

Table S1. Characterization of methyl, ethyl and propyl ester prodrugs of ketoprofen synthesized by esterification reaction
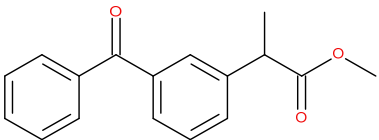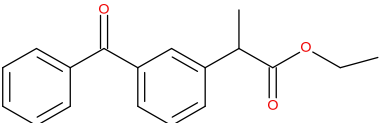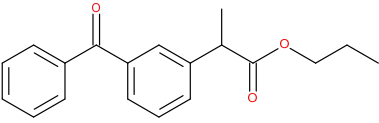
Chemical name	Structure	% Yield	^{1}HNMR data	IR data	Solubility (μg/mL)	Log P
Methyl 2-(3-benzoyl phenyl) propanoate		84	δ 3.49 (s, 3H, OCH$_3$), 7.32-7.24 (m, 3H, ArH), 7.43-7.38 (m, 3H, ArH), 7.64-6.53 (m, 2H, ArH)	1735.93 and 1658.78 cm^{-1} (C=O bond), 1597.06 and 1581.63 cm^{-1} (ArC=C bond)	31.6 ± 3.28	3.47
Ethyl 2-(3-benzoyl phenyl) propanoate		72	δ 1.09 (t, 3H, CH$_2$CH$_3$), 4.00 (q, 2H, OCH$_2$), 7.32–7.25 (m, 3H, ArH), 7.49-7.26 (m, 3H, ArH), 7.53-7.61 (m, 2H, ArH)	1732.08 and 1658.78 cm^{-1} (C=O bond), 1597.06 and 1581.63 cm^{-1} (ArC=C bond)	39.6 ± 1.24	3.92
Propyl 2-(3-benzoyl phenyl) propanoate		66	δ 0.88 (t, 3H, CH$_2$CH$_3$), 1.46 (d, 3H, CHCH$_3$), 3.99 (q, 1H, CH), 4.05 (t, 2H, OCH$_2$), 7.40-7.32 (m, 3H, ArH), 7.59-7.44 (m, 2H, ArH), 7.72-7.68 (m, 3H, ArH)	1732.08 and 1658.78 cm^{-1} (C=O bond), 1597.06 and 1581.63 cm^{-1} (ArC=C bond)	34.9 ± 4.18	4.43

Table S2. Predicted LD_{50} values, toxicity class and organ toxicity of synthesized ester prodrugs of ketoprofen

Organ toxicity	Ketoprofen	ME	EE	PE
LD_{50} (mg/kg)	45	1550	1550	1550
Toxicity class	2	4	4	4
Hepatotoxicity	+	-	-	-
Carcinogenicity	-	-	-	-
Immunotoxicity	-	-	-	-
Mutagenicity	-	-	-	-
Cytotoxicity	-	-	-	-
Aryl hydrocarbon receptor	-	-	-	-
Androgen receptor	-	-	-	-
Androgen receptor ligand binding domain	-	-	-	-
Estrogen receptor alpha	-	-	-	-
Estrogen receptor ligand binding domain	-	-	-	-
Peroxisome proliferator activated receptor gamma	-	-	-	-
Nuclear factor (erythroid-derived 2)-like 2/antioxidant responsive element	-	-	-	-
Heat shock factor response element	-	-	-	-
Mitochondrial membrane potential	-	-	-	-
Phosphoprotein (tumor suppressors) p53	-	-	-	-
ATPase family AAA domain-containing protein 5	-	-	-	-

ME: Methyl 2-(3-benzoyl phenyl) propanoate, EE: Ethyl 2-(3-benzoyl phenyl) propanoate, PE: Propyl 2-(3-benzoyl phenyl) propanoate. + and – indicate active and inactive respectively.

Table S3. Interactions of ester prodrugs with physiologically important transporters and enzymes accountable for induction of pathogenicity

Compound	P-gp substrate	CYP1A2 inhibitor	CYP2C19 inhibitor	CYP2C9 inhibitor	CYP2D6 inhibitor	CYP3A4 inhibitor
Ketoprofen	No	No	No	No	No	No
ME	No	No	No	No	No	No
EE	No	Yes	Yes	No	Yes	No
PE	No	Yes	Yes	No	Yes	No

ME: Methyl 2-(3-benzoyl phenyl) propanoate, EE: Ethyl 2-(3-benzoyl phenyl) propanoate, PE: Propyl 2-(3-benzoyl phenyl) propanoate
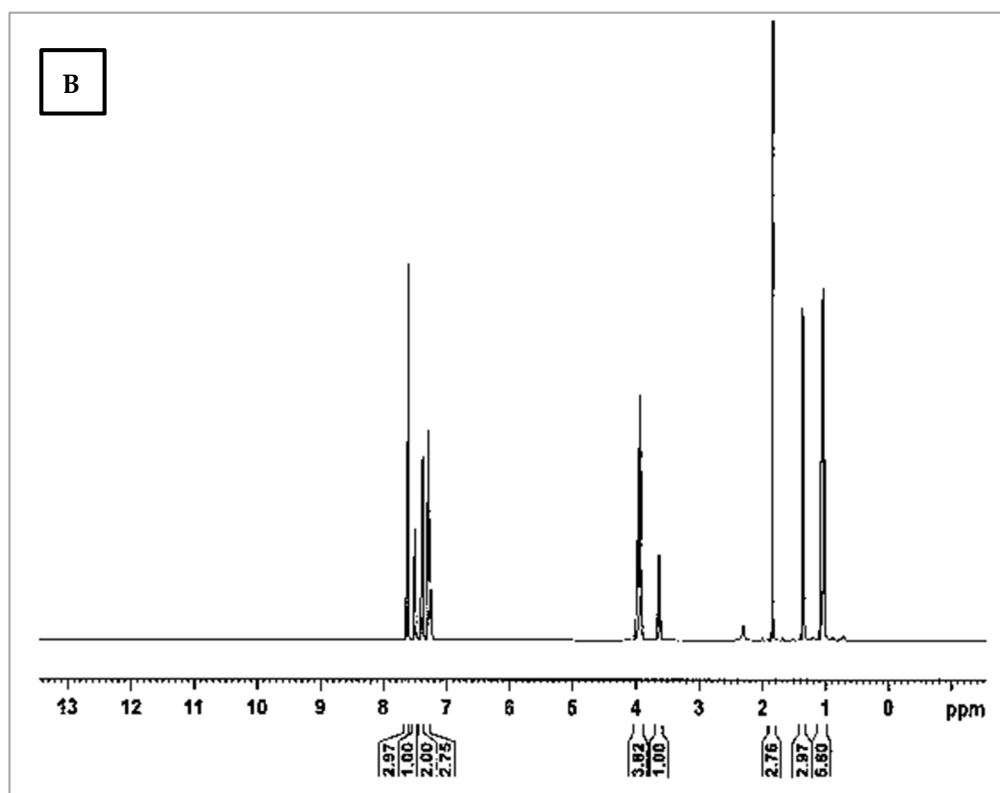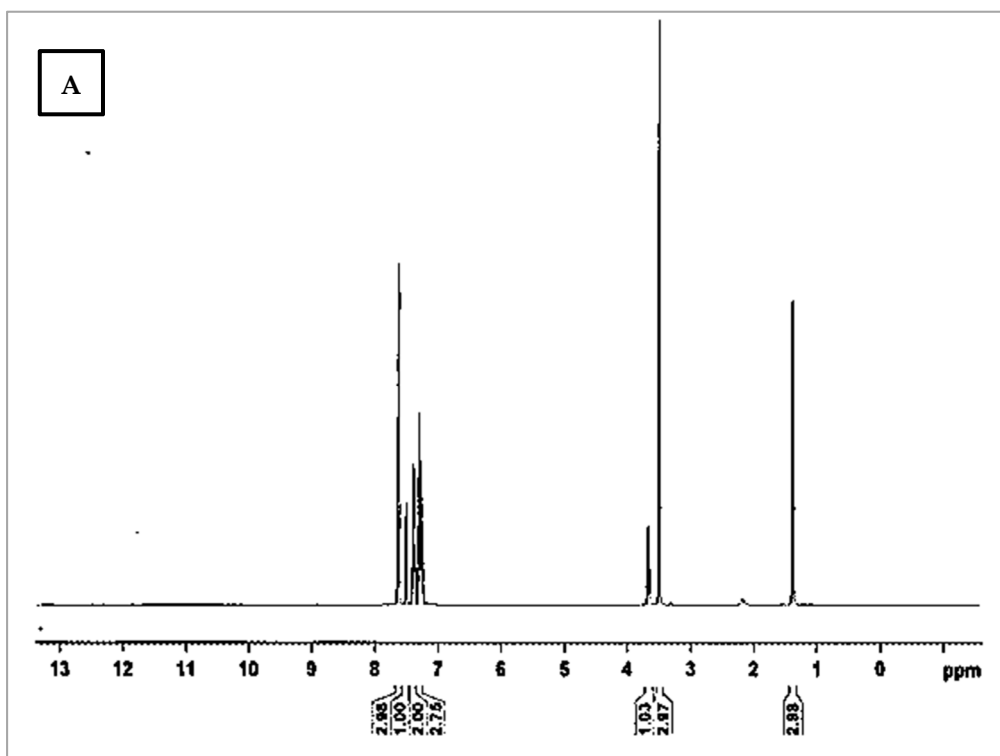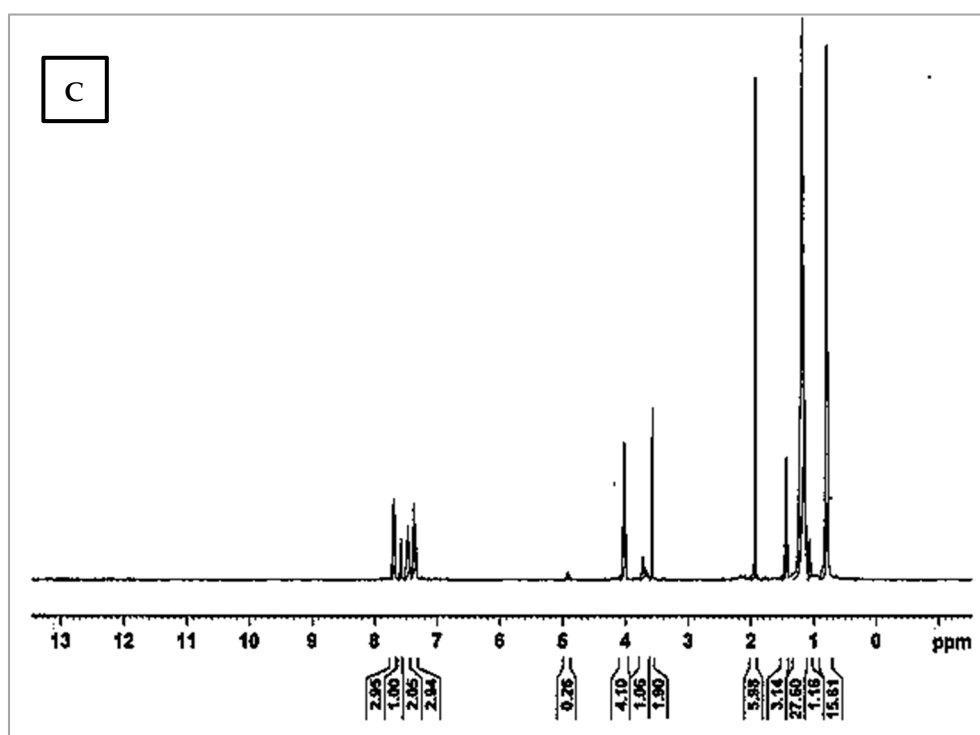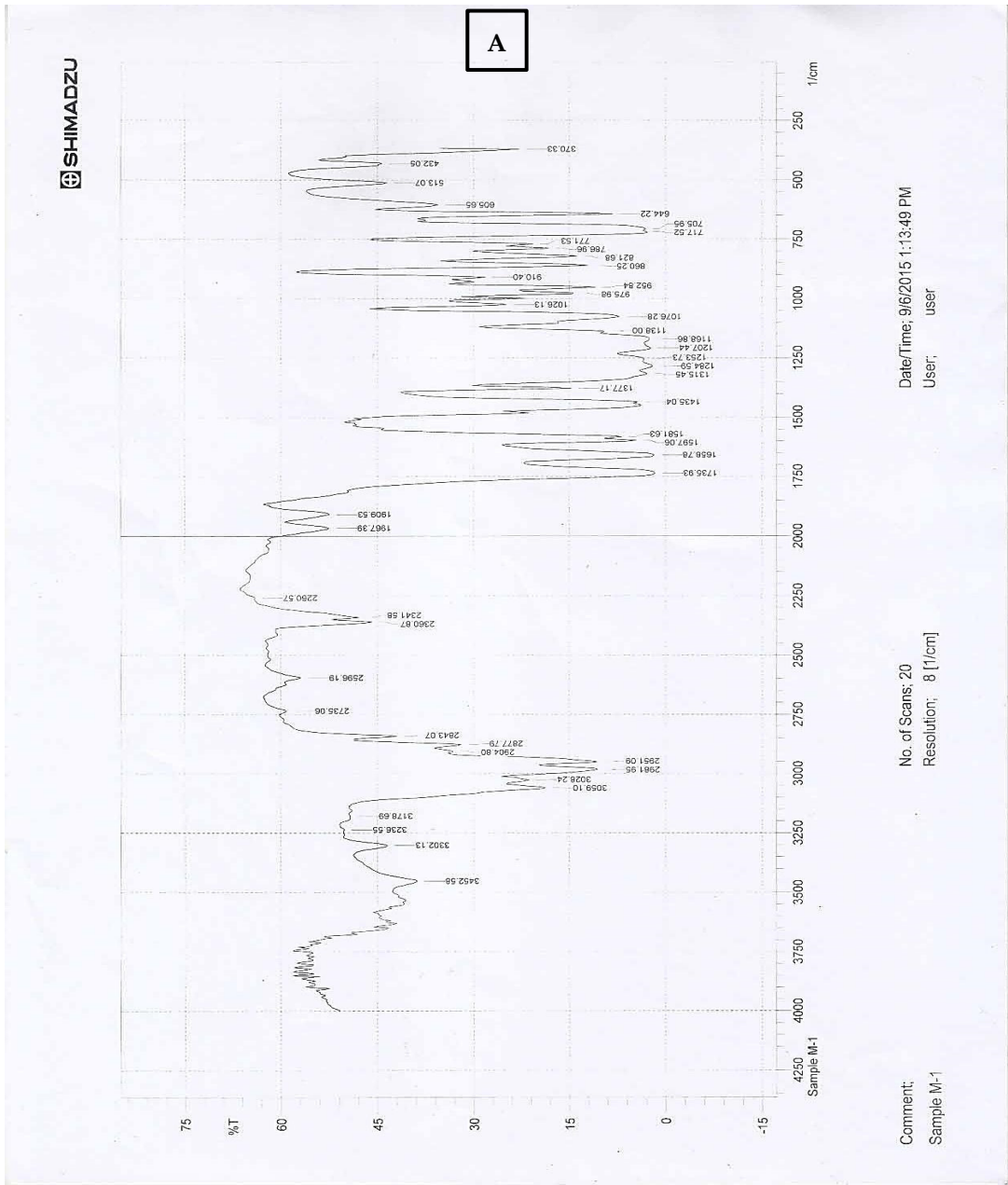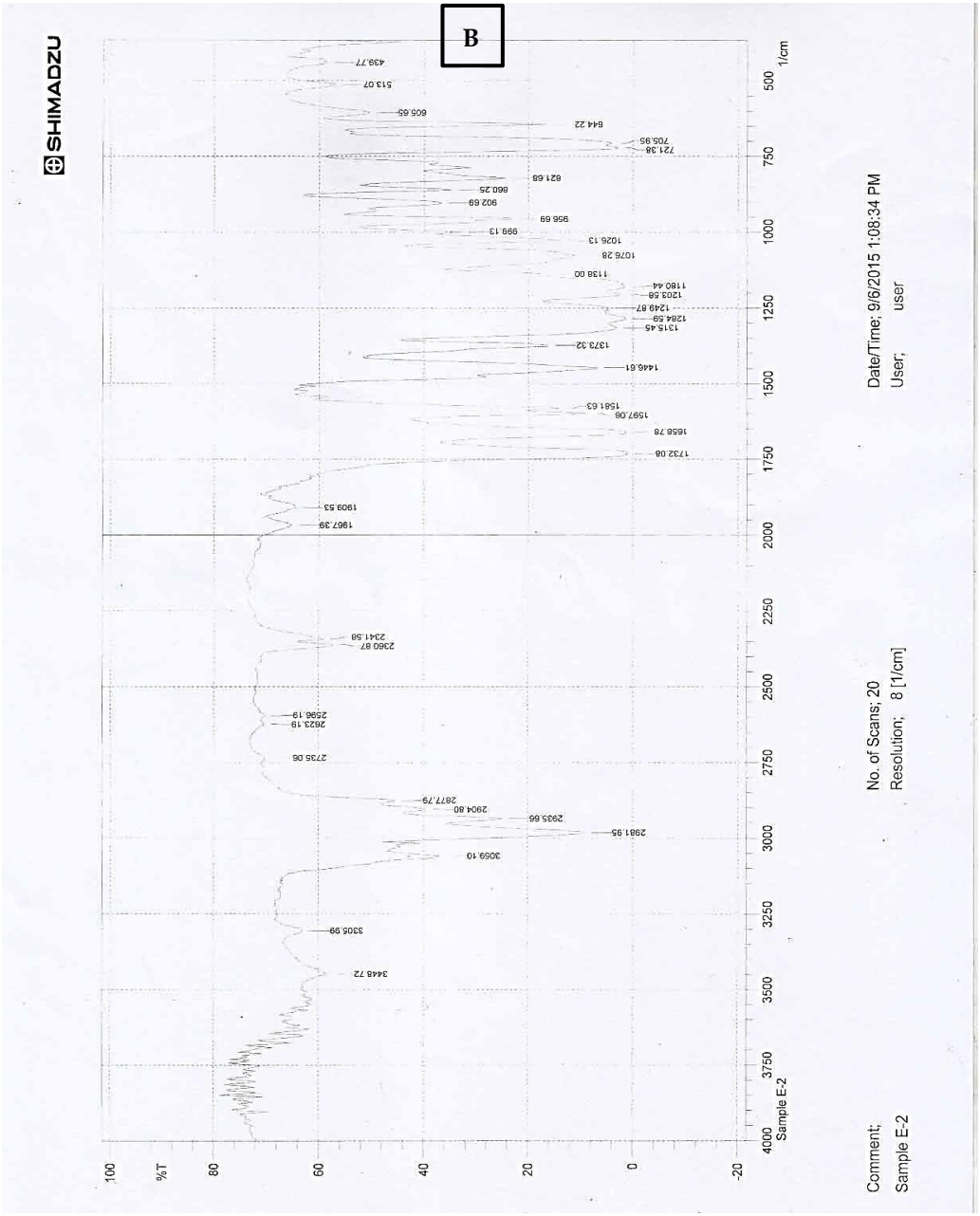
Figure S1. ^{1}HNMR spectra of synthesized prodrugs of ketoprofen; (**A–C**) represented methyl 2-(3-benzoyl phenyl) propanoate, ethyl 2-(3-benzoyl phenyl) propanoate and propyl 2-(3-benzoyl phenyl) propanoate respectively.

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Figure S2. IR spectra of synthesized prodrugs of ketoprofen; (**A–C**) represented methyl 2-(3-benzoyl phenyl) propanoate, ethyl 2-(3-benzoyl phenyl) propanoate and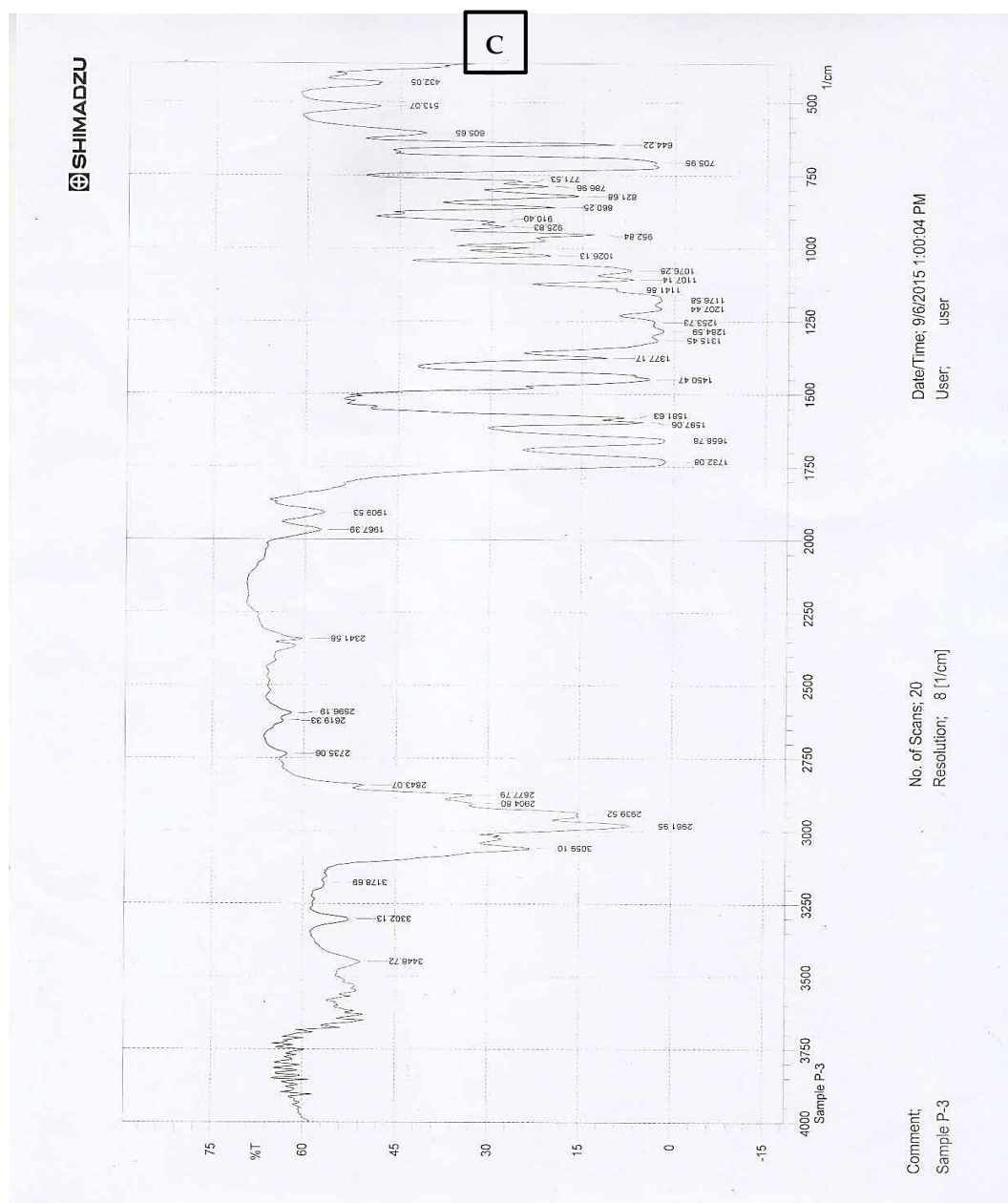 propyl 2-(3-benzoyl phenyl) propanoate respectively.