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Synthesis and Characterization of New Prodrug Polymers and Study of Their Biological Activity

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Abstract : The term drug refers to any substance whose administration results in a recognisable change in the body. Although these drugs are highly effective in their action as discovered, their activity, efficiency are affected by in vivo conditions and they also have the ability to cause a variety of side effects and toxicity to the body. To solve these problems, prodrugs were devised. Prodrugs, also called as metabolites of drugs or targeted drugs, are being very regularly used in pharmaceutical region. Prodrugs are biologically transformable agents which are derived from active drug molecules. Actually a prodrug is synthesised by conjugating an inert molecule or compound to an active drug. In vivo the bond between these two molecules is broken leading to release of the drug molecule. These prodrugs usually in vivo undergo enzymatic or chemical transformation which leads to release of the active drug molecule which shows its specific activity. In the process of discovering lead molecules and their development into potential drug molecules, the role of these prodrugs is becoming extensively established. This is because these prodrugs have the ability for enhancement of various properties of a drug like its biopharmaceutical, physicochemical, and pharmacokinetic also. In conventional drug formulations various problems related to drug's solubility, side effects or toxicity, absorption, excretion, delayed activity or low effective concentration at target site etc are faced frequently. Prodrugs strategy is considered to be having the preferred characteristics providing an advantage over the conventional methods. Prodrugs are usually synthesised to improve oral bioavailability, selectivity, specificity, sustained blood plasma levels of a drug molecule and it also improves the physical characteristics of the drug and protects it from immediate degradation in vivo.
In the present study prodrugs of ciprofloxacin and norfloxacin have been synthesised. Compound M was used for synthesising ciprofloxacin prodrug and norfloxacin prodrug with the use of N,N'-Dicyclohexylcarbodiimide and 4-Dimethylaminopyridine. These prodrugs were then polymerised using Nitrogen Benzoyl peroxide. These two polymeric prodrugs were then evaluated for potential antibacterial and anticancer activity in vitro. In antibacterial activity it was observed that as compared to original drug molecules, these prodrugs were observed to more bactericidal. In anticancer activity performed using cervical cancer cell lines (HeLa), these two polymeric prodrugs showed high activity as compared to cisplatin as a standard.
Keyword : Prodrug, Polymer, Ciprofloxacin, Norfloxacin, Anti bacterial activity, Anti Cancer activity, DNA Cleavage Studies.

Introduction

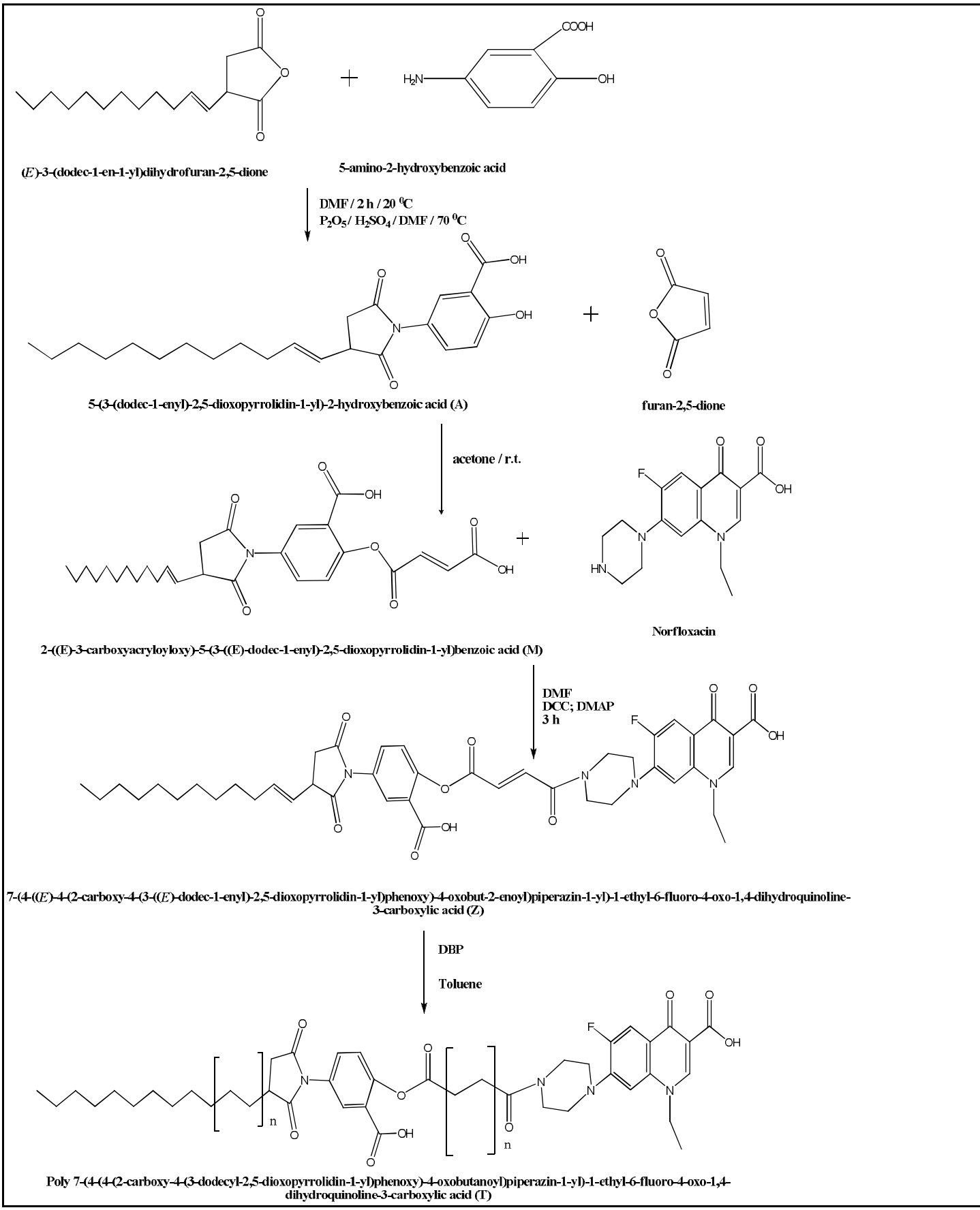
The main concern of modern science is to develop novel drugs and improve present drug's therapeutic efficiency. The traditional drug forms such as tablets, injections etc., and their delivery routes such as oral, intravenous, skin application etc., may have side effects or toxicity in some or the other forms which makes them inadvisable [1, 2]. The other main problem with traditional drugs are its variable concentration with time; as a result drug has to be administered repeatedly with high dosage. This variable concentration can be due to a drug's physicochemical properties like low in vivo half life, decreased water solubility which in turn causes low drug availability at desired site [3]. These problems can be avoided mainly by two approaches: one is to design new drugs with desirable properties; and the other is to use new delivery systems for conventional drugs [4].

As discovering and synthesis new drugs is a difficult process and there is a chance that these drugs can also have the limitations as those of conventional drugs, the second approach is the main focus of scientific community [5]. Various drug delivery techniques are being developed from the past few years which will have potential positive effects on improvement of pharmacokinetic and pharmacodynamic characteristics of drugs [6]. There are various techniques like alteration of the actual drug or conjugations of the drug to another carrier molecule. In all these techniques polymer prodrug synthesis has been rapidly growing technique [7].

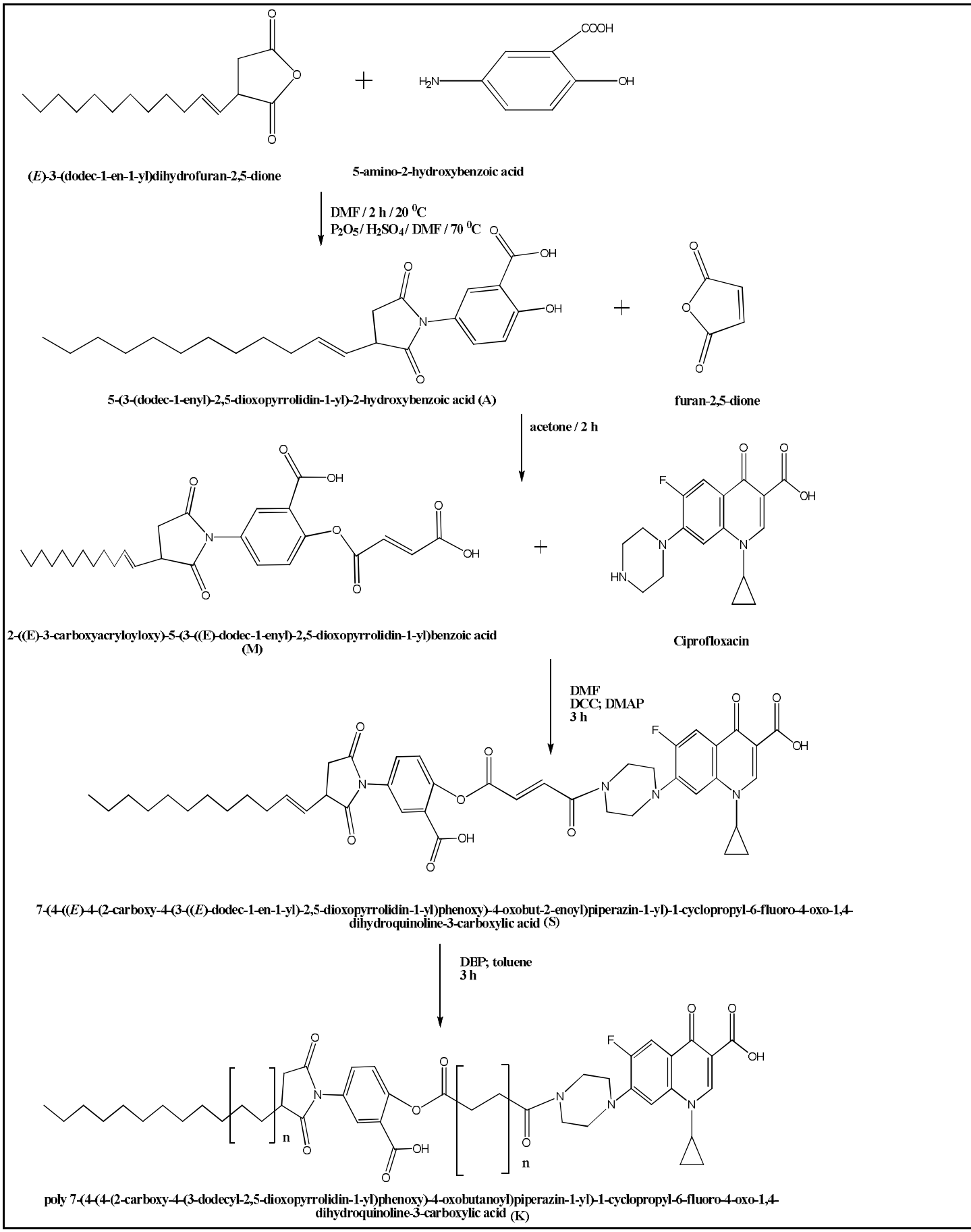
Prodrugs are a combination of drugs and their carrier molecule. Prodrugs are inactive forms of a drug [8]. These inactive forms are administered through various routes in vivo. In the body, these are metabolised to remove the carrier molecule and release the active drug molecule. The main modifications which can be performed in these carrier molecules is that these can be made site specific, can be made to specifically metabolised at a particular tissue or by a particular enzyme [9]. Albert [10] and Harper [11] first devised the concept of prodrug for drug delivery. These carrier molecules can assist the drug to have more biological half life, more drug concentration at target site, targeted action and extended consistent drug levels in vivo which are the limitations faced by many drugs [12]. As a result of extensive research, many carrier molecules have been developed which are specific in their action and have potential to be used in combination with drug molecules [13].

A combination of a drug molecule with a polymer forms a polymeric prodrug. There were various polymers made and were suggested carrier molecules in which the drug molecule can be implanted. In 1975, Prof. H. Ringsdorf developed the model for polymeric prodrug for the very first time making the potential of polymers to act as prodrug molecules evident [14]. The model proposed can be divided into five parts: polymeric backbone, the drug, the spacer, the targeting group and the solubilising agent. Each component has a specific role to play for the drug action. This polymeric backbone used can be inert in action or can be biodegradable. Spacer is used for specifying the target site and the rate at which the drug is being released from the prodrug which can be either through hydrolysis or enzymatic action [15, 16]. The prerequisites for prodrug synthesis is that the drug and polymer molecule should be covalently bonded together and this bond should not get broken until the drug reaches it target site. In case of drug being incorporated, it should be potent as there is limitation on the amount of drug that can be implanted, must have an essential functional group through which the polymer molecule can bind to it and the drug should be stable inside the polymer and should not get diffused out of the conjugate till target site is reached [17]. The targeting group assists in carrying the polymer prodrug to target site of action. The criteria used for targeting a prodrug can use targets like receptors expressed, antibody mediated and binding affinities [18].

The two drugs considered in this study, ciprofloxacin and norfloxacin, belong to fluoroquinolones family [19]. These two drugs are essentially used as antibacterial agents. These drugs exhibit cytotoxicity mainly by inhibition of enzyme topoisomerase II, also called as DNA gyrase [20]. This enzyme is essential in inducing negative supercoils in DNA in the processes like DNA replication, transcription and damage repair [21]. As this enzyme is inhibited, DNA can longer support these processes as a result cell dies. Although having advantages, these drugs have severe side effects which are tendinitis, side effects on nervous system etc. To overcome these limitations, polymeric prodrugs of these molecules have to be synthesised. These prodrugs are expected to have increased potential activity and also minimised side effects.



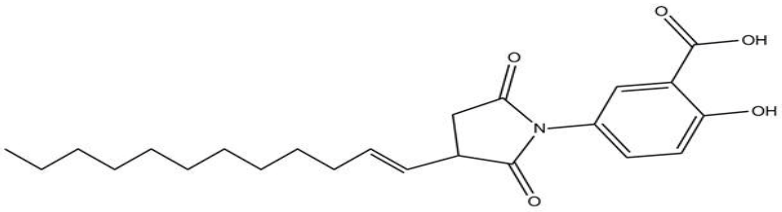
Scheme 1: Synthetic scheme for compound "T"



Scheme 2: Synthetic scheme for compound "K"

Experimental

Synthesis of 5-(3-(dodec-1-enyl)-2,5-dioxopyrrolidin-1-yl)-2-hydroxybenzoic acid (A):



The compound 5-amino-2-hydroxy benzoic acid (0.15 mM) and dodecenyl succinic anhydride (0.15 mM) were dissolved in DMF (50 ml) in two separate round bottom flasks to yield solution A and B, respectively. Solution B was added drop wise into solution A to give solution C. Solution C was kept in a water bath and stirred the solution completely at 20 ^{o}C for 2 h. Phosphorus pentoxide (P_2O_5) 12 grams was dissolved in H_2SO_4 (10 ml) and DMF (50 ml) the mixture was added drop wise to the solution C and stirred for 2 h at 70^{o}C. The mixture was kept chilled in the ice bath and poured into cold waterand formed product was filtered. A brown color semisolid wasobtained.

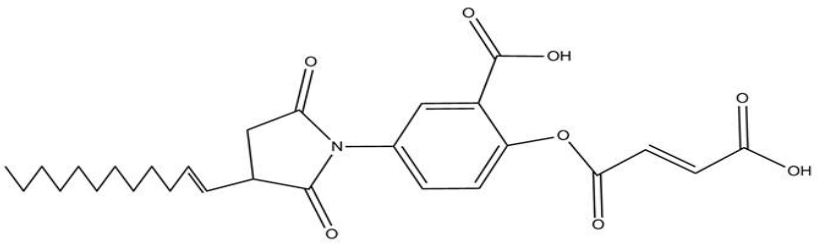
^{1}H NMR ($CDCl_3$) (500 MHz):

δ 0.85 – 2.05 (m, 19H), 2.19 – 2.21 (m, 2H), 2.74 – 3.00 (m, 2H), 4.90 – 5.10 (m, 1H), 5.20 – 5.40(m, 2H), 7.05 (s, 1H), 7.30(bs, 1H)

IR (neat):

3447, 2957, 1772, 1710, 1176, 667 cm^{-1}

Synthesis of 3-carboxyacryloyloxy-5-(3-((E)-dodec-1-enyl)-2,5-dioxopyrrolidin-1-yl)benzoic acid (M):



In a round bottom flask compound **(A)** (0.038mM) was dissolved in acetone (50ml) and in other round bottomed flask malic anhydride (0.038mM) was dissolved in acetone (20ml). These two solutions were mixed in a single flask to obtain the mixture of two compounds. The resulting mixture was left for 2 h under stirring.The solvent was removed under reduced pressure to afford compound **(M)** as brown color semi solid.

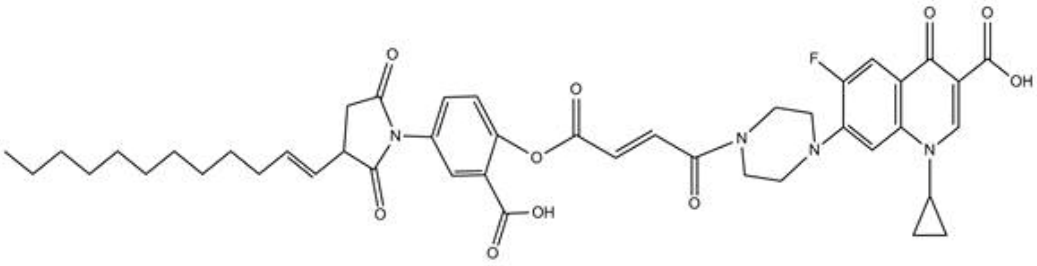
^{1}H NMR ($CDCl_3$)(500 MHz):

δ 0.85 – 2.05(m,19), 2.19 – 2.21 (m,2H), 2.74 – 3.00 (m,2H), 4.95 – 5.05 (m,1H), 5.20 – 5.40(m,2H), 6.40 (m,2H), 7.15(m,1H), 7.70 -

IR (neat):

3560, 3370, 2957, 1778, 1706, 1177, 695 cm^{-1}

Synthesis of ciprofloxacin prodrug (S):

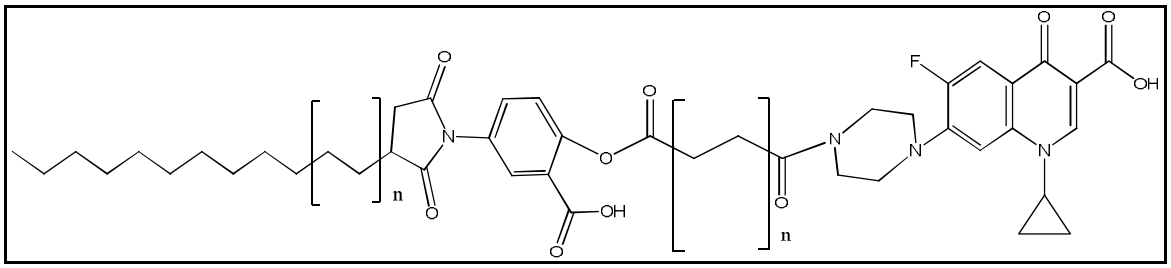


Acid compound **(M)** 3-carboxyacryloyloxy)-5-(3-((E)-dodec-1-enyl)-2,5-dioxopyrrolidin-1-yl) benzoic acid (0.0019 mM) was dissolved in DMF solvent (10 ml) and to this solution were added DCC and DMAP. This mixture was kept under magnetic stirrer for 30 minutes. In a round bottomed flask ciprofloxacin (0.0019 mM) was dissolved in DMF solvent and this was added drop wise to the acid mixture. The resulting mixture was kept under magnetic stirrer for 2 hours. A precipitate was obtained. Purification of crude compound by using silica gel column chromatography afforded compound**(S)** as an off white solid.

IR (neat):

3490, 2958, 1707, 1625, 1456, 1186, 1080, 802 cm^{-1}

Polymeric ciprofloxacin prodrug(K):

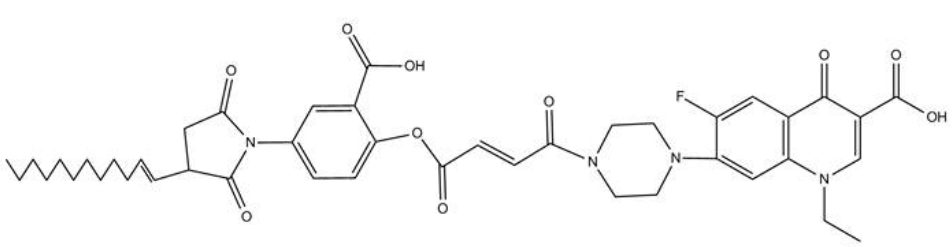


In a round bottomed flask compound **(S**) was dissolved in toluene (70ml). Under nitrogen,benzoyl peroxide (0.05grams) was added to the compound **(S).** The resulting mixture was kept in a water bath for 4 hours at 90^{o}C. A precipitate was formed. The final polymeric prodrug **(K)** was obtained when filtered the precipitate.

IR (KBr):

3370, 3560, 2996, 1730, 1625, 1455, 1188, 1037, 835 cm^{-1}

Synthesis of Norfloxacinprodrug (Z):



Acid compound (**M**) 3-carboxyacryloyloxy)-5-(3-((E)-dodec-1-enyl)-2,5-dioxopyrrolidin-1-yl) benzoic acid (0.0019 mM) was dissolved in DMF solvent (10 ml) and mix this solution with DCC and DMAP. This mixture was kept under magnetic stirrer for 30 minutes. In a round bottomed flask norfloxacin (0.0019 mM)

was dissolved in DMF solvent, this was added drop wise to the acid mixture. The resulting mixture was kept under magnetic stirrer for 2 hr. A precipitate was obtained. Purification of the crude compound by column chromatography Compound **(Z)** was obtained.

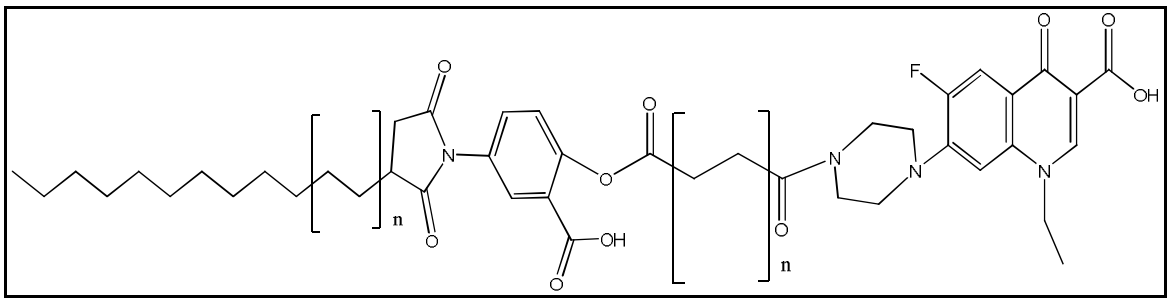
^{1}H NMR (DMSO-d6) (400MHz):

δ 0.85-1.90(m,24), 2.70-2.90(m, 2H), 3.35-3.62 (m, 9H), 4.45-4.51 (m,2H), 6.93 (m, 1H), 7.25 (m, 1H), 7.60(m,1H), 7.95 (m, 2H), 8.96 (s, 1H), 15.25 (bs, 2H) .

IR (KBr):

3412, 2960, 1774, 1710, 1629, 1490, 1359, 1101, 771 cm^{-1}

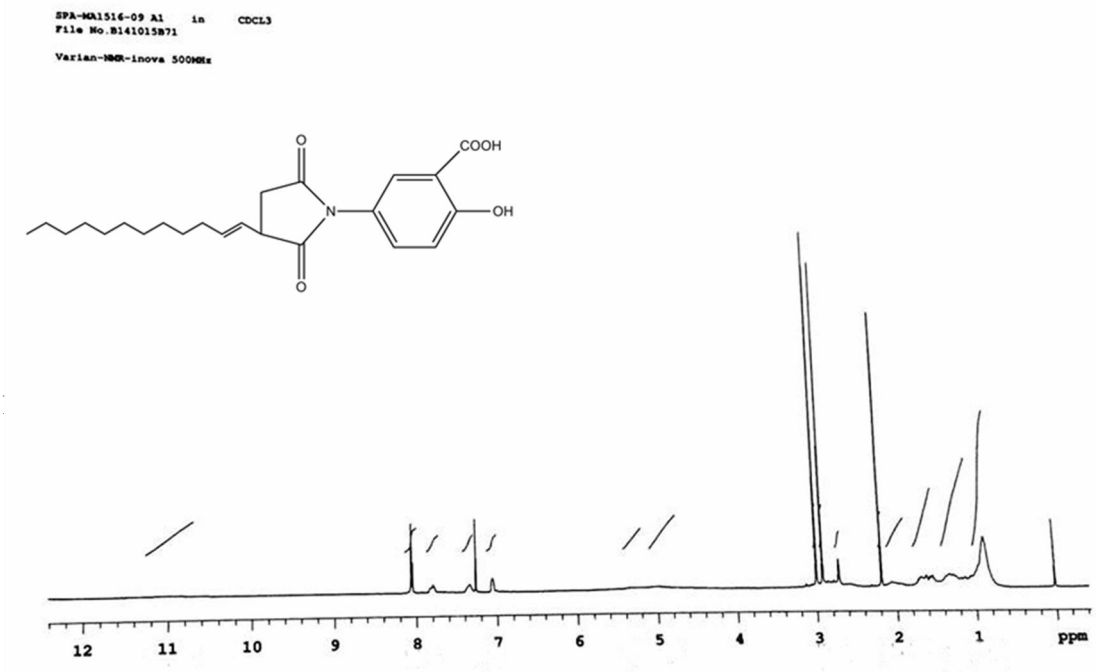
Polymeric Norfloxacinprodrug(T):

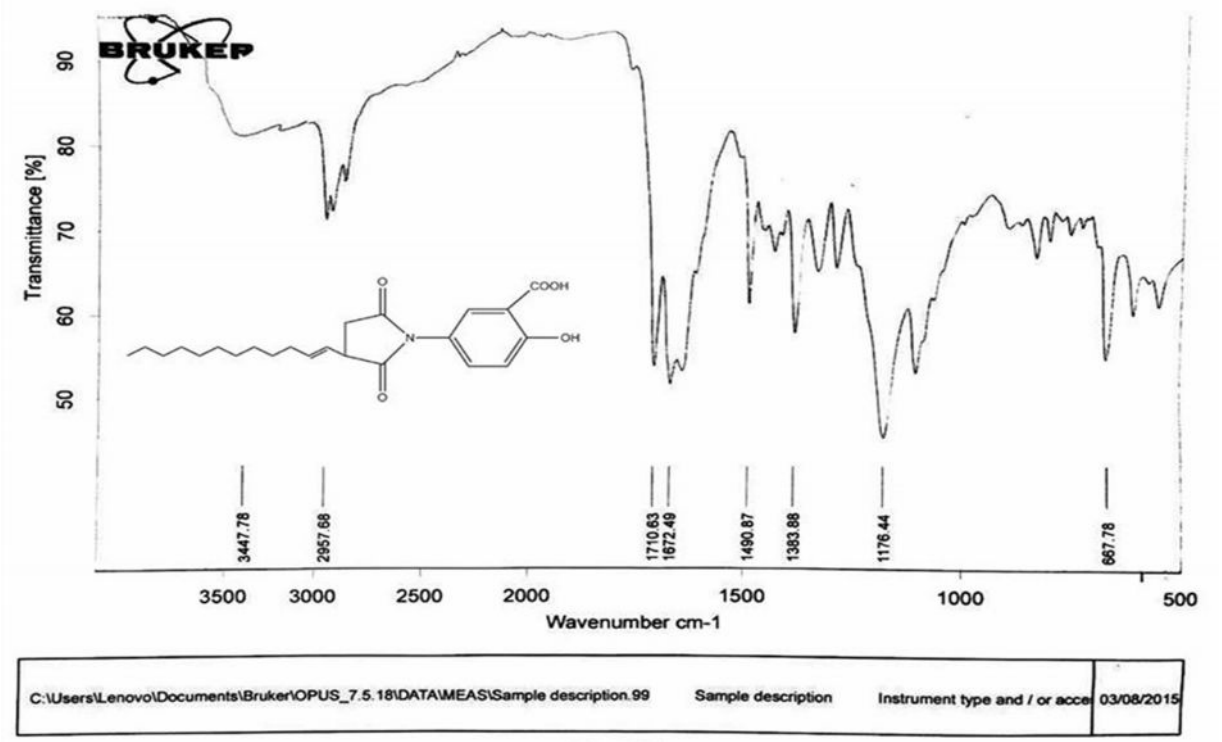


In a round bottomed flask Compound **(Z)** was dissolved in Toluene (70 ml). Under Nitrogen, Benzoyl peroxide (0.05 grams) was added to the compound **(Z)**. The resulting mixture was kept in a water bath for 4 h at 90 ^{o}C. A precipitate was formed. The final polymeric prodrug **(T)** was obtained when filtered the precipitate**.**

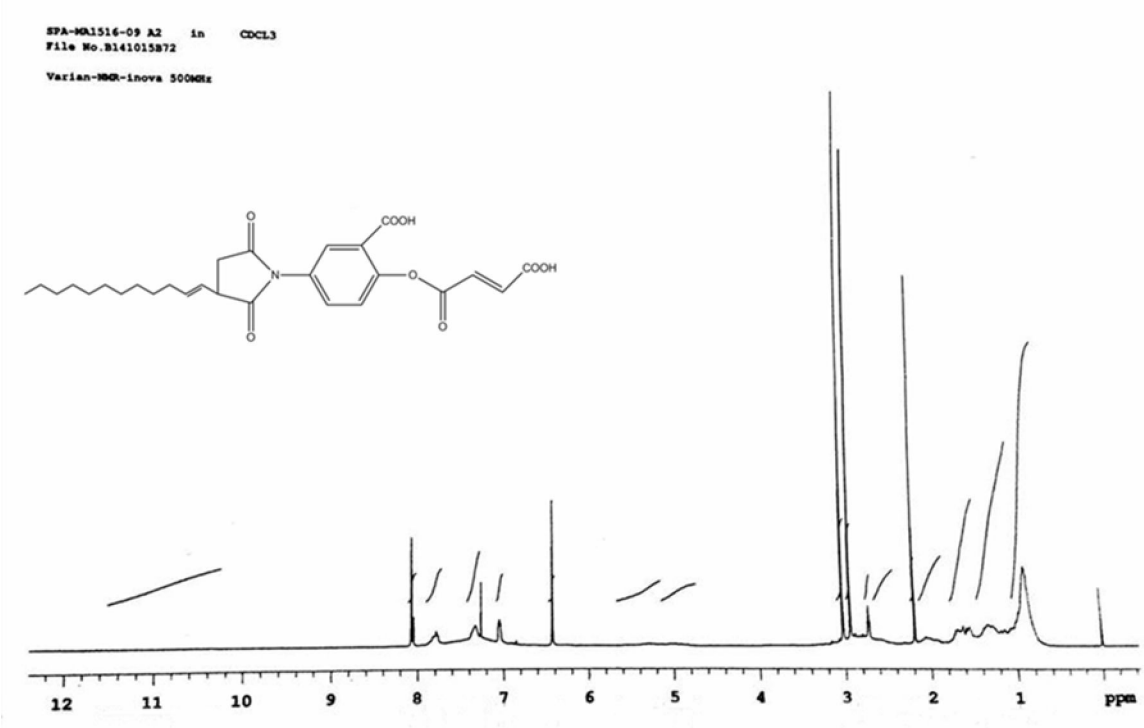
IR (KBr):

3352, 2960, 1686, 1606, 1520, 1349, 1064, 816 cm^{-1}

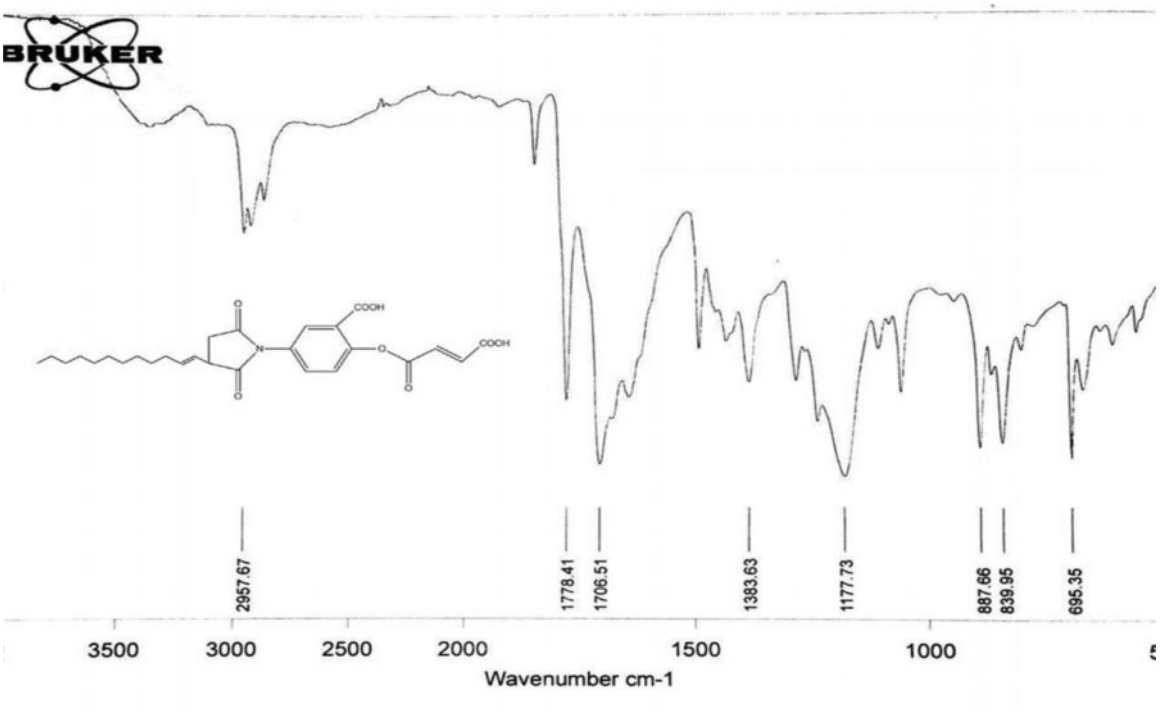




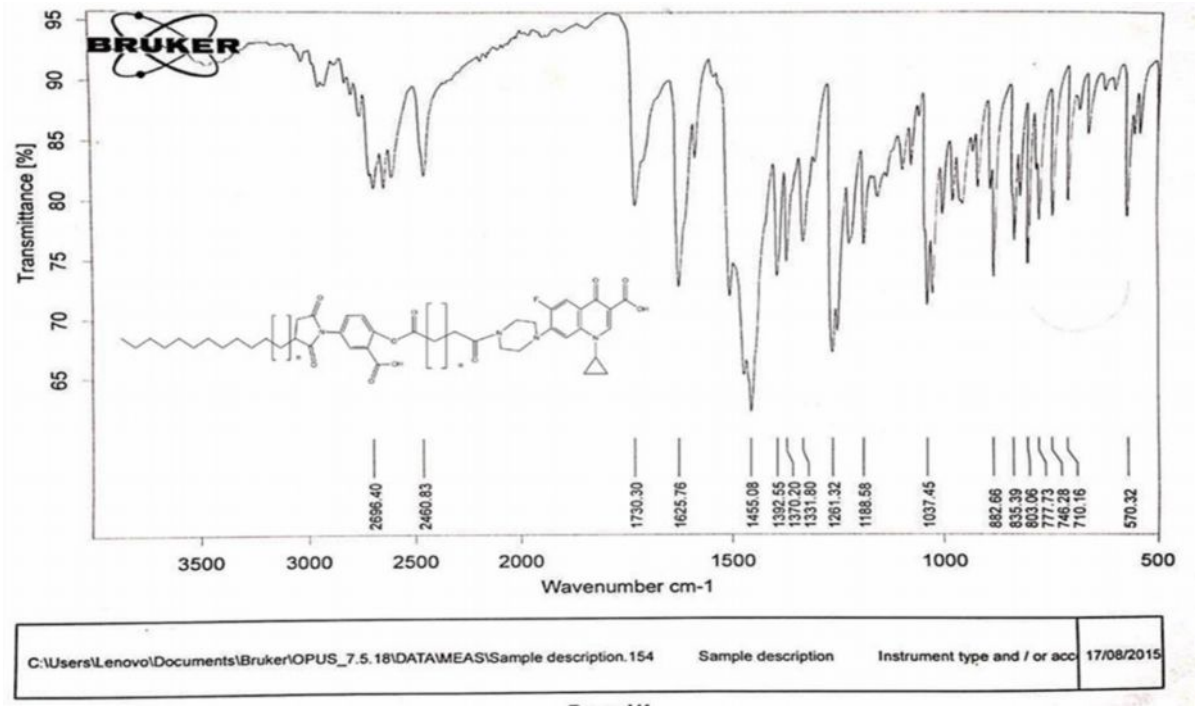
IR Spectrum of compound A neat



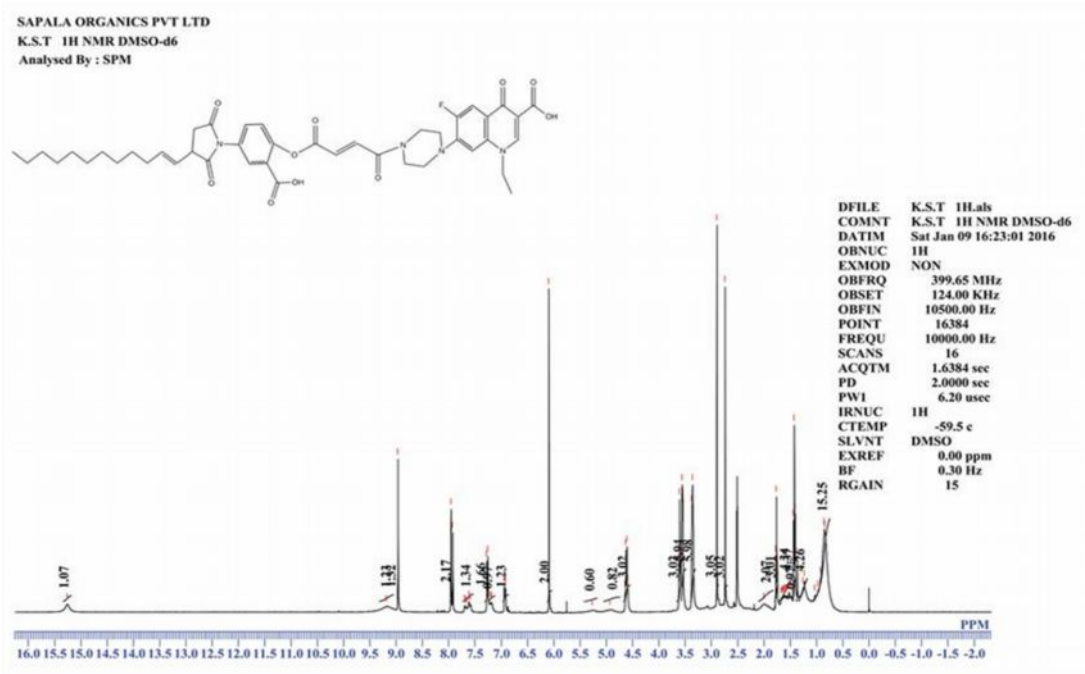
^{1}HNMR of Compound M, $CDCl_3$, 500 MHz



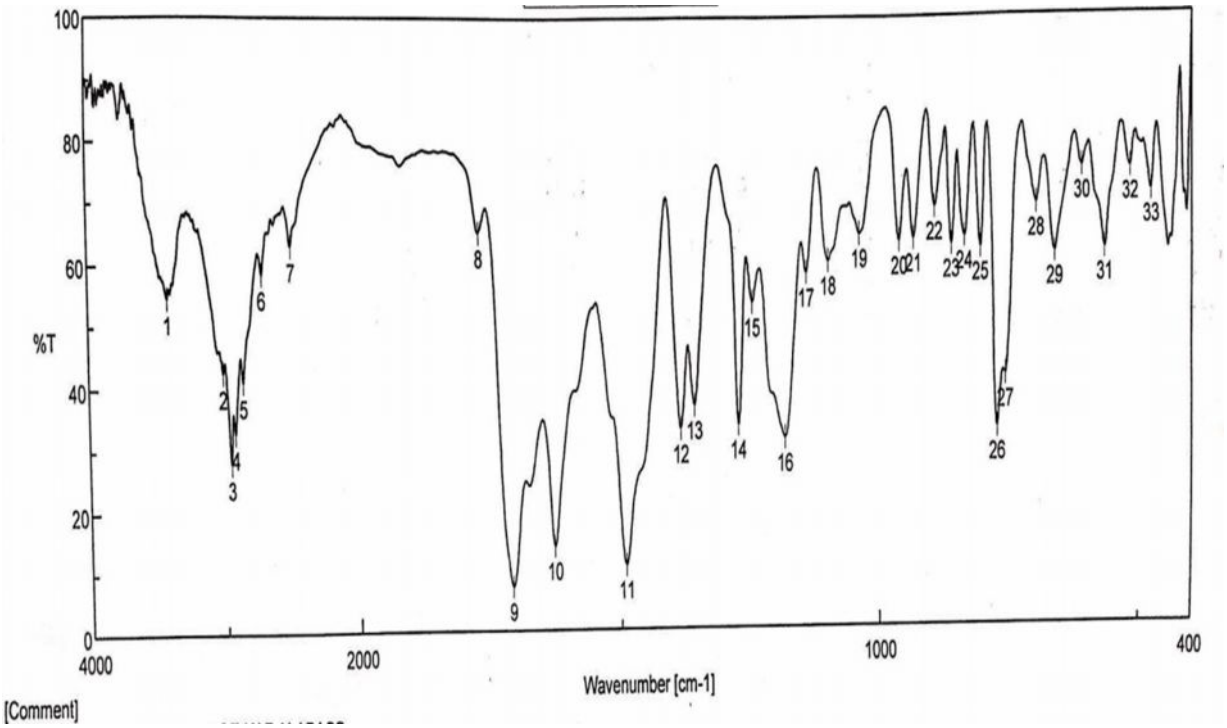
IR Spectrum of compound Mneat



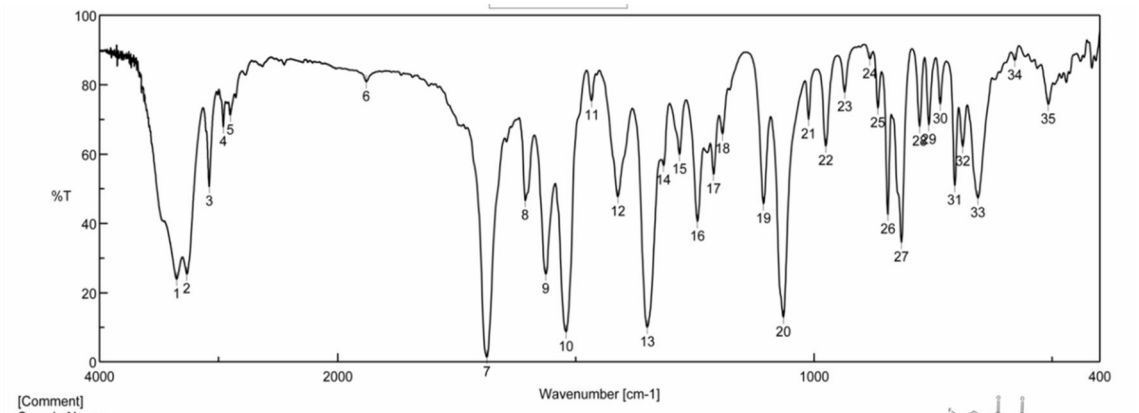
IR Spectrum of compound K neat



[1]HNMR of Compound Z, DMSO-d_6, 400 MHz



IRSpectrum of compound Z,KBR



[Comment]
Sample Name
Comment KBr Pellet
User GBR
Division QC
Company SAPALA ORGANICS PVT LTD

[Data Information]
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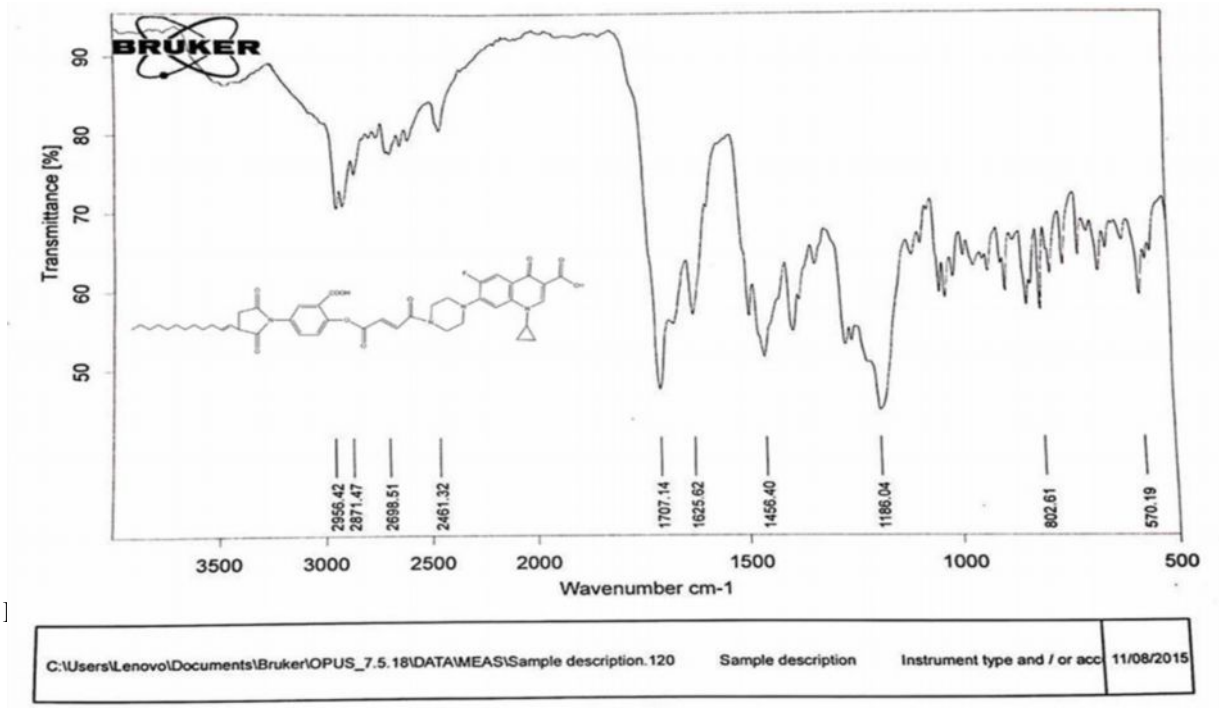
Data array type Linear data array
Horizontal Wavenumber [cm-1]
Vertical %T
Start 349.053 cm-1
End 7800.65 cm-1
Data pitch 0.964233 cm-1
Data points 7729

Result of Peak Picking

No.	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity
1	3352.6	23.866	2	3265.9	25.332	3	3078.8	50.614	4	2960.2	67.851	5	2903.3	71.196
6	1940.0	80.788	7	1686.4	1.349	8	1606.4	46.561	9	1563.0	25.337	10	1520.6	8.692
11	1466.6	75.388	12	1411.6	47.645	13	1349.9	10.075	14	1316.2	56.713	15	1282.4	59.892
16	1244.8	40.472	17	1211.1	54.199	18	1191.8	65.829	19	1106.0	45.672	20	1064.5	12.861
21	1011.5	69.992	22	974.8	62.326	23	935.3	77.845	24	882.3	87.422	25	865.9	73.302
26	844.7	42.562	27	816.7	34.558	28	778.1	67.928	29	758.9	68.434	30	734.7	74.418
31	704.9	50.918	32	687.5	62.145	33	655.7	47.292	34	578.5	87.054	35	508.2	74.341

IRSpectrum of compound T,KBR



Results

Ciprofloxacin and Norfloxacin polymeric prodrugs were synthesized in the laboratory and the activity of these drugs has been checked for anti-bacterial activity, anti-cancer activity and DNA cleavage. The results of the activity are mentioned below.

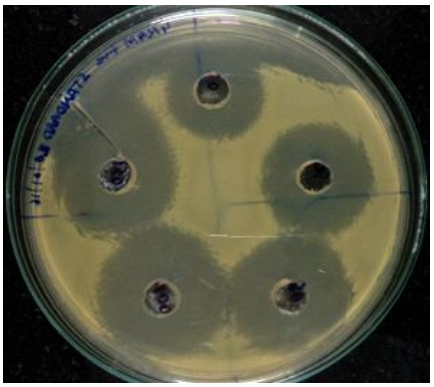
Anti-Bacterial Activity:

Agar Well Diffusion Assay

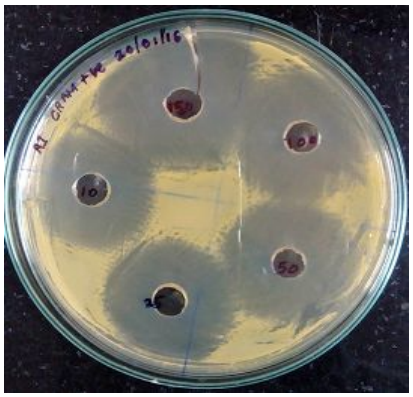
Gram Positive Strain : Staphylococcus aureus
Gram Negative Strain : E.Coli
Standard Drug for Gram Positive : Norfloxacin
Standard Drug for Gram Negative : Ciprofloxacin

Strain: Gram Positive

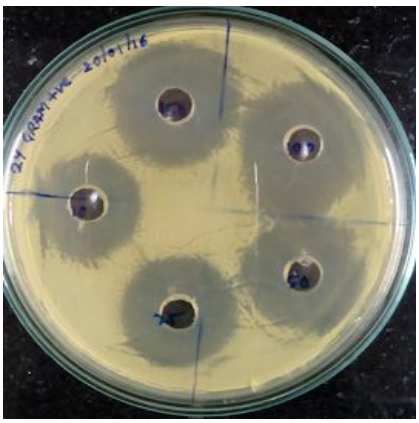
Compound/ Concentration (µg)	**10 Zone of Inhibition (mm)**	**25 Zone of Inhibition (mm)**	**50 Zone of Inhibition (mm)**	**100 Zone of Inhibition (mm)**	**150 Zone of Inhibition (mm)**
Gram Positive	9	10	12	13	13
K	11	12	13	13	16
T	12	13	13	14	14



Standard Gram Positive



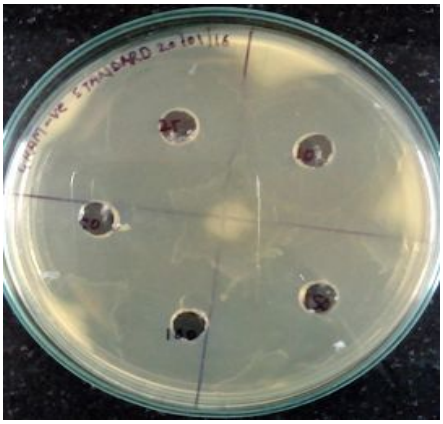
Compound: K



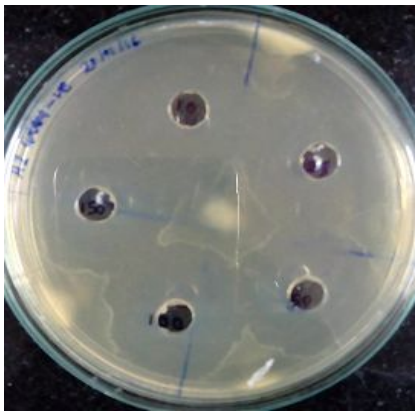
Compound: T

Strain: Gram Negative

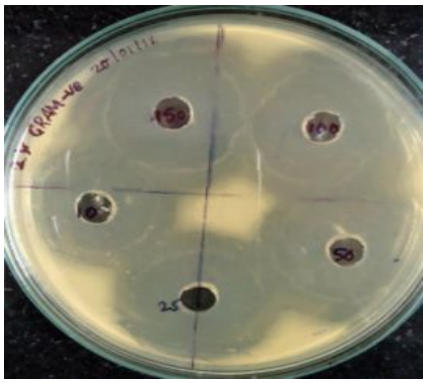
Compound/ Concentration (µg)	**10** **Zone of Inhibition (mm)**	**25** **Zone of Inhibition (mm)**	**50** **Zone of Inhibition (mm)**	**100** **Zone of Inhibition (mm)**	**150** **Zone of Inhibition (mm)**
Gram Negative	10	10	11	11	12
K	11	12	12	13	16
T	8	8	9	9	9



Standard Gram Negative



Compound: K



Compound: T

Discussion:

In the standard gram positive bacteria zone of inhibition was observed high at 100ug and 150 µg concentration and the compounds **K** and **T** the zone of inhibition was observed high when compared to standard at 150 µg concentration whereas in gram negative 150 µg concentration high inhibition was observed the compounds **K** and **T** the zone of inhibition was observed high when compared to standard at 150 µg concentration.

Anti-Cancer Activity:

Maintenance of cell line:

The HeLa cervical cancer cell lines were purchased from NCCS, Pune. The cells were maintained in DMEM supplemented with 10 % FBS and the antibiotics penicillin/streptomycin (0.5 mL^{-1}), in atmosphere of 5% CO_2 /95% air at 37 ^oC. For the MTT assay, HeLa cells were plated in 96 well plate at 5.0 X 10^3 cells were per well in culture medium and incubated overnight at 37 ^oC.

HeLa cell viability:

Cell viability was evaluated by the MTT Assay with three independent triplicate experiments of six concentrations of compounds (5, 10, 25, 50 75 and 100 µM). After 24 hrs of incubation, each treatment was withdrawn and MTT solution (0.5 mg / mL^{-1}) was added to each well and plates were incubated at 37 ^oC for 3 hrs. At the end of incubation time, precipitates are formed as a result of the reduction of the MTT salt to chromophoreformazan crystals by the cells with metabolically active mitochondria. The optical density of solubilized crystals in DMSO was measured at 560 nm on a microplate reader.

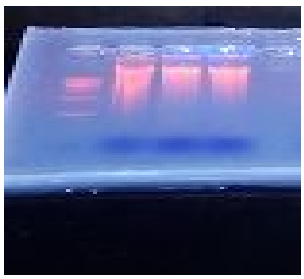
IC 50 Values:

S.NO	COMPOUND	IC 50 µM
1	K	9.28
2	T	27.38
3	Cisplatin standard	4.6

Discussion:

The compounds were treated with Breast cancer cell lines and MTT assay was done but the compound A1 was showing anti-cancer activity and the compound 2Y did not show any anti-cancer activity it was confirmed with standard Cisplatin.

DNA Cleavage Studies:



Sl.No	**Well**	**Sample Order**
1	M	Marker
2	1	Control (only CT-DNA)
3	2	Compound 1
4	3	Compound 2

DNA Cleavage is measured by relaxation of supercoiled DNA to nicked circular conformation and linear conformation. During electrophoresis process supercoiled DNA will migrate faster when compared with DNA in nicked and linear confirmations. The above figure illustrates the gel electrophoresis experiment do not show any apparent cleavage in the presence of H_2O_2 when compared with Control DNA.

Discussion

Prodrugs can be defined as the pharmacologically inactive forms of a drug in which an active drug molecule is bonded to a material or molecule. This conjugation is biologically reversible in vivo, where the drug molecule is released for an efficient activity. Prodrugs are already established as an essential tool required for novel drug designing and their development. It is estimated that worldwide there are 5-7% drugs are prodrugs and prodrugs are being implemented in initial steps of drug designing. Designing and synthesis of prodrugs is seen to be capable of avoiding complications which are related to a drug's stability, lipophilicity/hydrophilicity, absorption, distribution, metabolism, excretion, toxicity (ADMET) and site selectivity and target specificity. Prodrug design strategy is basically developed to optimise a drug's delivery, bioavailability, stability, solubility and all other criterions as per the requirement. These prodrugs also increases the drug concentration at the selective site of its action by modifying drug related physical and chemical properties. Any newly synthesised drug will have some or the other unwanted or toxicity causing properties which can be either biological or pharmaceutical. In precise manner, a prodrug reduces the disadvantages of conventional drugs and improves their clinical application by altering their biological and physico-chemical properties.

In the present study, two polymeric prodrugs of ciprofloxacin and norfloxacin are synthesised. These polymeric prodrugs were characterised for their potential antibacterial and anticancer activity. These prodrugs showed an improved efficiency and efficacy in both these tested parameters when compared with standards respectively. As in conclusion it can be said that preparation of polymeric prodrug forms of these two standard antibiotics have reduced their limitations and helped in improvement of activity.

This study along with many other studies in the concerned field have proven that all the desirable parameters are improved by the use of prodrugs, in turn minimising the undesirable side effects and toxicity. Presently extensive research is carried to make these prodrugs efficiently site or target specific. This specificity can be induced by conjugating the prodrug molecule with a protein or an antibody which is specific to the cell to be effected. There is also report that site specificity of a prodrug can be enhanced by use of enzyme or carrier proteins delivery in conjugation with prodrug molecule. So, more techniques can be evaluated by collaborating different fields of science for efficient development of prodrug targeting methods.

References

1. Rang HP (1995) Pharmacology. (3rdedn), Churchill Livingstone, Edinburgh.
2. Harvey RA, Champe PC (1992) Lippincott's Illustrated Reviews: Pharmacology. J.B. Lippincott, Philadelphia.
3. Bowman WC, Rand MJ, West GB (1975) Textbook of Pharmacology. (7thedn), Blackwell Scientific, Oxford.
4. Anderson JH, Kim SW (1986) Advantages in Drug Delivery Systems, Elsevier, Amsterdam.
5. Giammona G, Cavallaro G, Fontana G, Pitarresi G, Carlisi B (1998) Coupling of the antiviral agent zidovudine to polyaspartamide and in vitro drug release studies. J Control Release 54: 321-331.
6. Reddy KR (2000) Controlled-release, pegylation, liposomal formulations: new mechanisms in the delivery of injectable drugs. Ann Pharmacother 34: 915-923.
7. Duncan R (2003) The dawning era of polymer therapeutics. Nat Rev Drug Discov 2: 347-360.
8. Patil SJ and Shirote PJ. Prodrug approach: an effective solution to overcome side-effects. Int J Med Pharm Sci. 2011;1(7):1-13.
9. Silverman R. Prodrug and drug Delivery, System In the organic chemistry of drug design and drug action, New York, Academic Press; 1992. p. 352-401.
10. Albert A (1958) Chemical aspects of selective toxicity. Nature 182: 421-422.
11. Harper NJ (1959) Drug latentiation. J Med Pharm Chem 1: 467-500.
12. Azori M (1987) Polymeric prodrugs. Crit Rev Ther Drug Carrier Syst 4: 39-65.
13. Hemant NJ (1988) Recent advances in drug delivery systems: Polymeric prodrugs. Pharmaceutical technologies 118-130.
14. Hoste K, De Winne K, Schacht E (2004) Polymeric prodrugs. Int J Pharm 277: 119-131.
15. Etrych T, Chytil P, Jelinkova M, Rihova B, Ulbrich K (2002) Synthesis of HPMA copolymers containing doxorubicin bound via a hydrazone linkage: Effect of spacer on drug release and in vitro cytotoxicity. MacromolBiosci 2: 43-52.

16. Etrych T, Jelínková M, Ríhová B, Ulbrich K (2001) New HPMA copolymers containing doxorubicin bound via pH-sensitive linkage: synthesis and preliminary in vitro and in vivo biological properties. J Control Release 73: 89-102.
17. Duncan R (1987) Sustained and controlled drug delivery systems. In Robinson JR, Lee VHL, Controlled drug delivery: fundamentals and applications, Marcel Dekker, New York, NY, 581-621.
18. Allen TM (2002) Ligand-targeted therapeutics in anticancer therapy. Nat Rev Cancer 2: 750-763.
19. Oliphant CM, Green GM; Green (February 2002). "Quinolones: a comprehensive review". *Am Fam Physician*65 (3): 455–64.
20. Drlica K, Zhao X, K; Zhao, X (1 September 1997). "DNA gyrase, topoisomerase IV, and the 4-quinolones". *MicrobiolMolBiol Rev.*61 (3): 377–92.
21. Wigley, D.B.; Davies, G.J.; Dodson, E.J.; Maxwell, A; Dodson, G (1991). "Crystal structure of an N-terminal fragment of the DNA gyrase B protein". *Nature*351 (6328): 624–629.

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