# Preparation of Sulfapyridine Prodrugs and their Nanoscale Self-assemblies for the Management of Rheumatoid Arthritis

## Introduction

Rheumatoid arthritis (RA), the most prevalent systemic inflammatory disease, that not only affects the joints, but also includes extra-articular manifestations, therefore must be regarded as syndrome [1]. In the past three decades, therapeutic resources for the management of RA have grown tremendously, which include disease modifying anti-rheumatic drugs i.e., DMARDs, both of synthetic and of biological origin, non-steroidal anti-inflammatory drugs (NSAIDs), and glucocorticoids [2]. Despite of extensive research, management of RA is still challenging because of unfavorable properties of existing medications such as instability, poor aqueous solubility, low cell permeability, and rapid metabolism, all contributing towards the low bioavailability of drug at the target site, thereby require frequent administration and may lead to toxicity [3].

Novel drug delivery system is an excellent strategy to improve pharmacokinetic properties of various drugs with diverse physiochemical properties [4, 5]. Among the various delivery systems explored, the major cluster of research has been accomplished through the use of drug nanocarriers *e.g.* hydrogels [6], liposomes [7], dendrimers [8] and polymeric nanoparticles/nanospheres/nanocapsules [9]. These delivery systems not only shield the cargo drug, but also effectively transport molecules to the targeted sites, leading to improvement of bioavailability and efficacy, in addition to solubility and stability [10]. However inefficient drug loading/releasing pattern and biosafety issues of these delivery systems still remain a challenging task [11]. A prodrug based self-assembled nanostructures that spontaneously form supramolecular structures in aqueous media via hydrophilic and hydrophobic interactions have opened up new avenues in targeted drug delivery. The nanoscale structure provides protection to the drug during storage and administration, and fulfill the strategic role that excipient play in conventional drug development process [12]. With enormous advantages, self-assembling prodrugs exhibit high drug loading capacity, passive targeting ability, prevent premature leakage, reduce renal clearance, extends circulation half-life and improved cellular uptake [13].

Recently, stimuli-responsive nanoplatforms triggered by endogenous (such as pH, ROS, GSH, enzymatic, etc.) or exogenous (such as magnetic fields, light, electric pulses, etc.) stimuli have been developed for targeted delivery of drugs[14]. The synovial fluid of rheumatic patients contains elevated levels of a series of enzymes including matrix metalloproteinases (MMPs), cysteine protease, and hyaluronidase [15]. Moreover, an increased level of reactive oxygen species in RA patients as a result of a hypoxic and inflammatory situation [16], and weakly acidic microenvironment (pH less than 6) [17] can be used as trigger to release the drug from nanoparticles at the target side for sustained action. Based on the above hypothesis, we propose to give a second life to the long-abandoned drug, sulfapyridine, which, due to its high toxicity index could not be explored for anti-inflammatory potential [4]. We propose to synthesize amphiphilic prodrugs of sulfapyridine by conjugating with a pro-moieties via enzyme- and acid-sensitive amide

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bond, which is expected to form self-assembled nanostructures in aqueous environment. Intra-articular administration of these systems is anticipated to prolong the retention of drug in the target site by virtue of their size as well as two-steps release of the entrapped drugs (disassembly and then lysis of prodrug to release the active drug). Moreover, the prodrugs are expected to be converted to parent drug in the presence of proteases overexpressed in synovial fluid of RA patients, which will be further catalyzed by slight acidic environment of synovial fluid. Hence, the dual stimuli-responsive amphiphilic prodrug nanoparticles are expected to provide the sustained release of drug, thereby prolonged therapeutic effect with less frequent dose administration [18]. The rationale of the proposed work has been depicted in Figure 1.

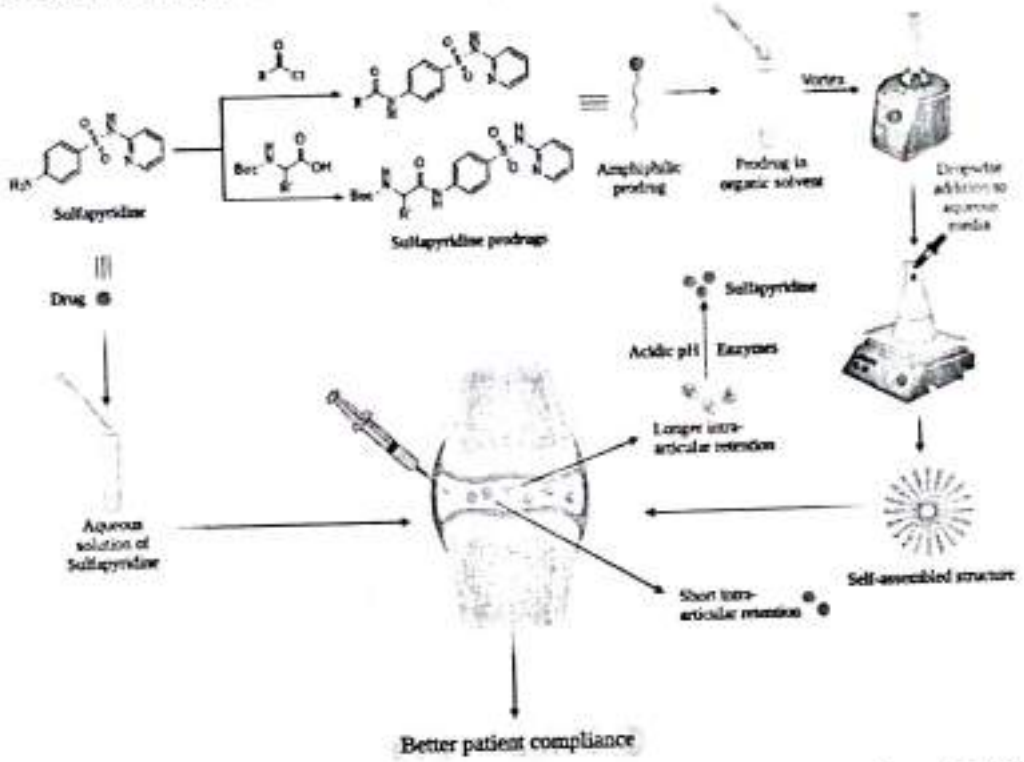


**Figure 1**: Rationale of better therapeutic efficacy of self-assembled nanostructures of amphiphilic prodrugs

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**Objectives**

- Synthesis and characterization of amide prodrugs of sulfapyridine
- Analytical method development of sulfapyridine and its prodrugs
- Thermodynamic equilibrium solubility studies and partition coefficient
- Assessment and characterization of prodrugs for self-assembly in aqueous solutions
- Hydrolysis kinetics of prodrugs
  - Chemical (non-enzymatic) hydrolysis in buffers
  - Enzymatic hydrolysis
  - Stability in biorelevant media, simulated synovial fluid
  - Hydrolysis in human plasma
  - Hydrolysis in human synovial fluid
- *In vivo* anti-arthritic activity of supramolecular prodrugs in rats
  - Body weight recording
  - Measurement of paw volume, joint diameter, and thermal hyperalgesia
  - Biochemical estimations (RBC, WBC, Hb, platelet count, haematocrit and ESR
  - Estimation of inflammatory mediators (ILs, TNF-α)
  - Radiographical study of joints of hind paws
  - Histopathological examination

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## Materials and Methods

### A. Synthesis and characterization of sulfapyridine prodrugs

Amphiphilic prodrugs of sulfapyridine were synthesized by its reaction with various saturated and unsaturated acid halides in anhydrous conditions. Briefly, sulfapyridine was dissolved in dimethyl formamide (DMF) and potassium carbonate was added to the solution. The flask was kept on magnetic stirrer and the respective acid chloride was added to the drug solution drop-wise while the solution will be being stirred continuously. The temperature of the flask was maintained at 15-20°C during the addition. After addition of acid chloride, the flask was closed with calcium chloride guard tube and the reaction mixture was stirred at room temperature till the completion of reaction. The progress of reaction was monitored by thin layer chromatography. After completion of the reaction, excess of water was added and the reaction mixture was filtered by vacuum filtration. The crude product was washed with plenty of water 2-3 times for complete removal of DMF. The synthesized amide prodrugs were purified using silica gel column chromatography.

Amino acid prodrugs of sulfapyridine were synthesized by using Boc-protected amino acids. The coupling of Boc-amino acids with sulfapyridine was carried by using *N,N'*-dicyclohexylcarbodiimide as coupling agent. All the synthesized prodrugs were purified by column chromatography and characterized by various analytical techniques such as IR, Mass, NMR, DSC, XRD and SEM. The general scheme for the synthesis of sulfapyridine prodrugs is depicted in Figure 2.

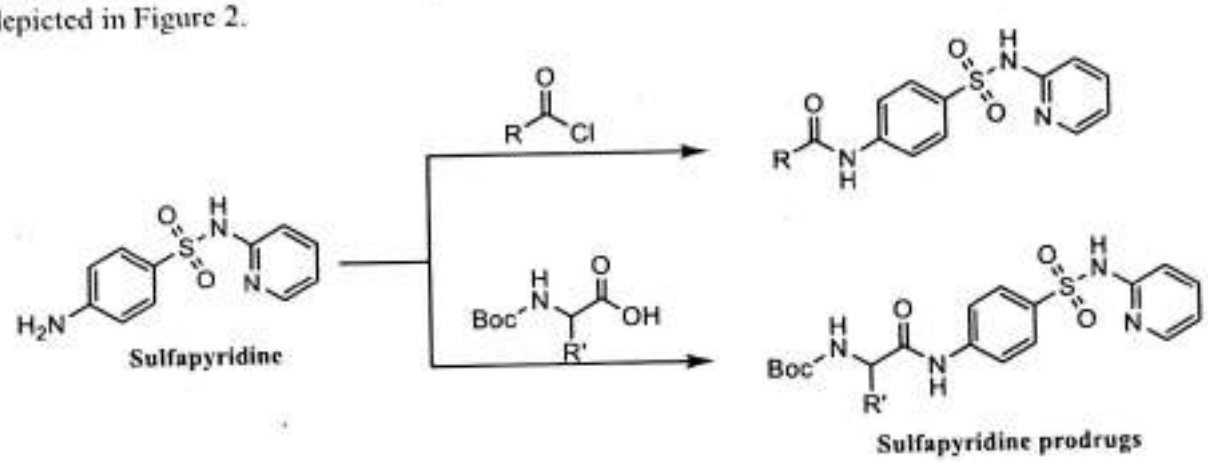


**Figure 2:** General scheme for the synthesis of amide prodrugs.

### B. Analytical method development

HPLC Shimadzu LC20AD, fitted with quaternary solvent manager, binary pump, online degasser and photodiode array (PDA) detector was used for the method development.

The analyses of Sulfapyridine prodrugs were carried out at an ambient temperature using reverse phase Nucleodur® C18 (250 x 4.6 mm, 5 µm) column. The mobile phase consisted of 0.1% v/v formic acid and acetonitrile. The mobile phase was filtered through 0.22 µm nylon membrane filter

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and delivered at a constant flow rate of 1 mL/min. The injection volume was 25 µL and the analytes were detected by UV at 265 nm.

**C. Physicochemical characterization of prodrugs**

Equilibrium solubility and partition coefficient of the synthesized prodrugs as well as of sulfapyridine will be determined by shake-flask experiments.

Briefly, an excess of compound will be added to vials containing 5 ml of solvent or simulated synovial fluid (SSF). The solution containing solid excess of the sample will be then capped, and stirred at 37 ± 1°C temperature for 24 h. The equilibrated samples will be centrifuged at 8000 rpm for 10 minutes at 37 ± 1°C in order to remove the undissolved drug. The clear supernatant will be taken out with a fine pipette from the saturated solution, filtered through 0.22 µm filter and diluted with appropriate solvent (maintained at 37 ± 1°C), if necessary. The concentration of drug in each aliquot will be measured by HPLC.

Partition coefficients of sulfapyridine and prodrugs will be determined using *n*-octanol and SSF. The two phases of the liquid system will be well saturated with each other for 24 h on a mechanical shaker before use. These pre-saturated solvents will be used to determine the partition coefficient of the compounds. A known quantity of sample will be added to 20 mL of pre-saturated *n*-octanol and SSF mixture (1:1) and kept on mechanical shaker at 37 ± 1°C for 24 h and 100 rpm. The flask will be kept undisturbed for 2 h for complete separation of both the phases and drug content in both organic and aqueous phase will be determined by HPLC.

**D. Self-assembly of prodrugs in water**

Prodrugs will be dissolved in organic solvent and then rapidly injected into water or buffers of various *p*H to observe their solubility. The solution will be further dialyzed using water/buffer to remove organic solvent. The resultant solution will be analyzed by microscope to identify the prodrugs capable of forming self-assembled structures in aqueous media.

**E. Characterization of self-assembled structures**

The morphology and hydrodynamic diameters of self-assembled nanostructures will be investigated by transmission electron microscopy and dynamic light scattering particle size analyzer respectively.

**F. HPLC determination of hydrolysis rates of prodrug-assembled nanoparticles**

The hydrolysis of self-assembled prodrugs will be evaluated by HPLC in the presence of:

- Buffers (*p*H 1-13)
- Enzymes (esterase, elastase and collagenase)
- Simulated synovial fluid
- Human plasma and synovial fluid

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## G. Anti-arthritic activity

The anti-arthritic activity of self-assembled nanoparticles will be evaluated in rats using complete Freund's adjuvant-induced arthritis model. Anti-arthritic potential of treatment groups will be evaluated on the basis of various parameters such as paw volume, joint diameter, pain threshold and body weight on day 0 and thereafter every fourth day till day 28. On day 28, blood will be withdrawn by retro-orbital puncture and used for estimation of various biochemical parameters (serum aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase and total protein levels), hematological parameters (RBC count, WBC count, Hb, platelets, ESR and serum RF) and serum inflammatory mediators such as TNFα and IL-6. Radiographs of joints of hind paws of rats will be taken using X-ray tube assembly. Finally, histopathology of the joins will be carried out to observe the morphology of joint tissue.

The summary of the proposed work has been given in Figure 3.

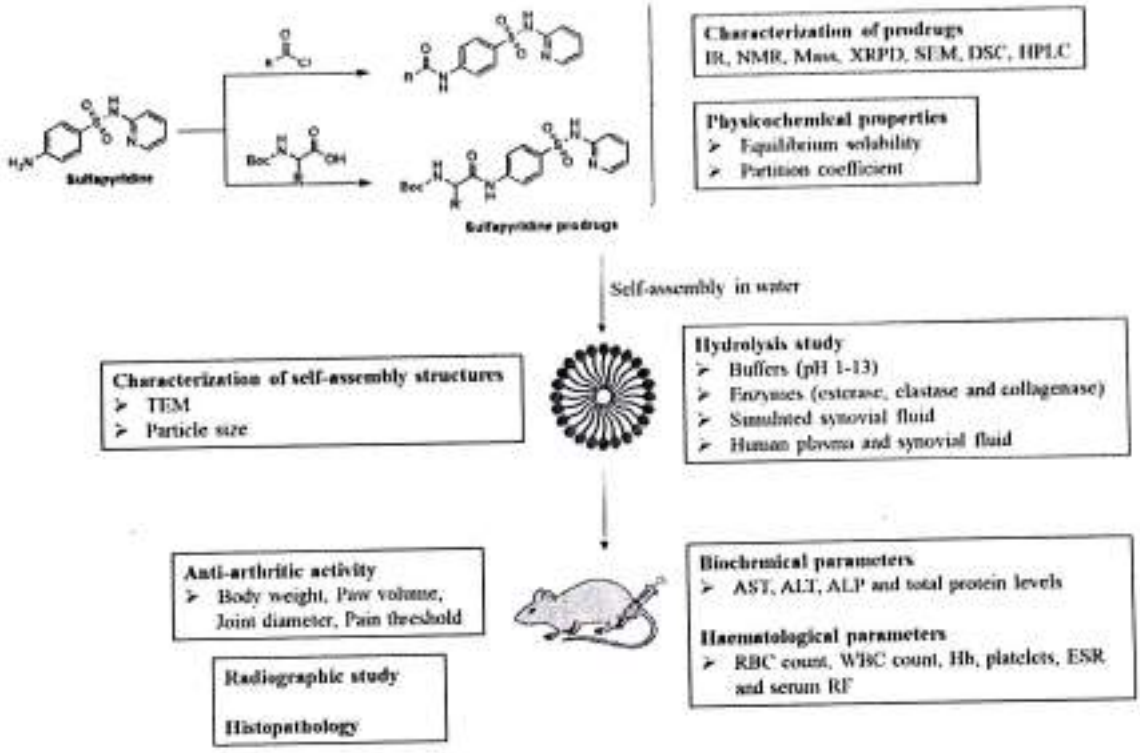


**Figure 3:** Summary of the proposed work

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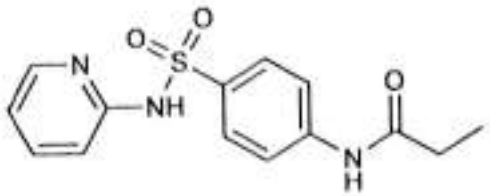
**Results**

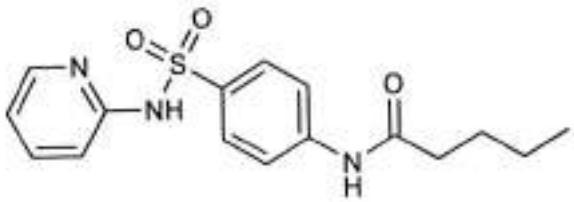
**1. Characterization of synthesized prodrugs**

***N-(4-(N-(pyridin-2-yl)sulfamoyl)phenyl)propionamide (SP-PROP)***



Cream white powder; Yield: 30%; UV absorption maxima:265nm; m.p. (DSC) 213.89°C; $^1$H NMR (400 MHz, DMSO) δ 10.23 (s, 1H, amide-H), 8.00 (d, *J* = 4.6 Hz, 1H, Ar-H), 7.86 – 7.59 (m, 5H, Ar-H), 7.12 (d, *J* = 8.6 Hz, 1H, Ar-H), 6.96 – 6.72 (m, 1H, Ar-H), 2.33 (q, *J* = 7.5 Hz, 2H, $CH_2$), 1.06 (t, *J* = 7.5 Hz, 3H, $CH_3$); $^{13}$C NMR (500 MHz, DMSO): 172.48, 152.75, 143.88, 142.61, 139.84, 135.07, 127.68, 118.30, 115.78, 113.29, 29.46, 9.32; IR (KBr) ν: 3321 (N-H str. $SO_2NH$/CONH), 2924 (C-H str. ArH), 2811 (C-H str. aliphatic), 1670 (C=O str. amide), 1629 and 1460 (C=C ArH), 1597 (N-H bending CONH), 1531 (N-H bending $SO_2NH$), 1384 (S=O asymm. str.), 1280 (C-N str.), 1135 (S=O symm. str.), 1109 (para oop), 786 (S-N str.); Mass (m/z): 306.15 ($M+H^+$)

***N-(4-(N-(pyridin-2-yl)sulfamoyl)phenyl)pentanamide (SP-VAL)***



Cream white powder; Yield: 28.44%; UV absorption maxima:265nm; m.p.(DSC) 187.83°C ; $^1$H NMR (400 MHz, DMSO) δ 10.22 (s, 1H, amide-H), 8.01 (d, *J*= 4.4 Hz, 1H,Ar-H), 7.76 (m, 5H,Ar-H), 7.12 (d, *J* = 8.4 Hz, 1H, Ar-H), 7.00 – 6.79 (m, 1H, Ar-H), 2.34 (t, *J* = 7.2 Hz, 2H,$CH_2$), 1.66 –1.51 (m, 2H, $CH_2$), 1.31 (m, 2H, $CH_2$), 0.88 (t, *J* = 7.2 Hz, 3H, $CH_3$). $^{13}$C NMR (500 MHz, DMSO): 171.77, 152.74, 143.98, 142.57, 139.83, 135.11, 127.67, 116.32, 115.84, 113.27, 36.04, 26.95, 21.64, 13.57; IR (KBr) ν: 3298 (N-H str. $SO_2NH$/CONH), 2926 (C-H str. ArH), 2858 (C-H str. aliphatic), 1665 (C=O str. amide), 1628 and 1460 (C=C ArH), 1595 (N-H bending CONH), 1532 (N-H bending $SO_2NH$), 1381 (S=O asymm. str.), 1272 (C-N str.), 1133 (S=O symm. str.), 1110 (para oop), 787 (S-N str.); Mass (m/z): 334.25 ($M+H^+$)

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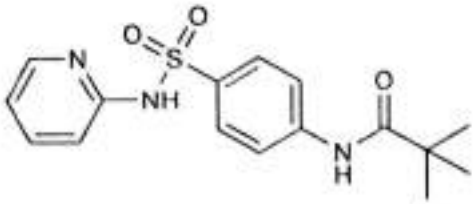
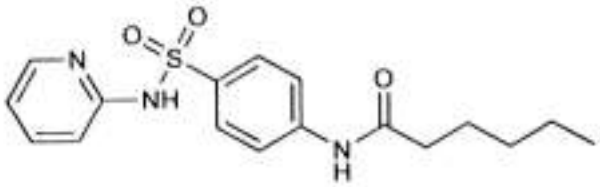
***N-(4-(N-(pyridin-2-yl)sulfamoyl)phenyl)pivalamide (SP-PIVA)***



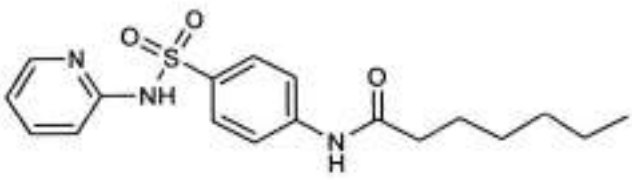
Cream white powder; Yield: 58.71%; UV absorption maxima:265 nm; m.p. (DSC) 232.80°C; $^1$H NMR (400 MHz, DMSO) δ 9.52 (s, 1H, amide-H), 7.97 (d, *J* = 24.0 Hz, 1H, Ar-H), 7.80 – 7.54 (m, 5H, Ar-H), 7.12 (d, *J* = 8.6 Hz, 1H, Ar-H), 6.87 (t, *J* = 6.3 Hz, 1H, Ar-H), 1.20 (s, 9H); $^{13}$C NMR (500 MHz, DMSO): 176.83, 152.72, 143.98, 142.72, 139.81, 135.25, 127.39, 119.39, 115.88, 113.24, 39.42, 26.87; IR (KBr) ν: 3331 (N-H str. $SO_2NH$/CONH), 2921 (C-H str. ArH), 2851 (C-H str. aliphatic), 1677 (C=O str. amide), 1629 and 1459 (C=C ArH), 1590 (N-H bending CONH), 1518 (N-H bending $SO_2NH$), 1383 (S=O asymm. str.), 1278 (C-N str.), 1132 (S=O symm. str.), 999 (para oop), 779 (S-N str.); Mass (m/z): 334.15 ($M+H^+$)

***N-(4-(N-(pyridin-2-yl)sulfamoyl)phenyl)hexanamide (SP-HEX)***



Cream white powder; Yield: 73%; UV absorption maxima:265nm; m.p. (DSC) 198.96°C ; $^1$H NMR (400 MHz, DMSO) δ 10.21 (s, 1H,amide-H), 8.02 (d, *J* = 4.4 Hz, 1H, Ar-H), 7.89 – 7.62 (m, 5H, Ar-H), 7.12 (d, *J* = 8.6 Hz, 1H, Ar-H), 6.96 – 6.61 (m, 1H, Ar-H), 2.31 (t, *J* = 7.4 Hz, 2H, $CH_2$), 1.67 – 1.47 (m, 2H, $CH_2$), 1.42 – 0.95 (m, 4H, $CH_2$), 0.86 (t, *J* = 6.9 Hz, 3H,$CH_3$). $^{13}$C NMR (500 MHz, DMSO): 171.79, 152.77, 143.90, 142.60, 139.81, 135.13, 127.70, 118.35, 115.82, 113.28, 36.32, 30.72, 24.52, 21.76, 13.80; IR (KBr) ν: 3320 (N-H str. $SO_2NH$/CONH), 2927 (C-H str. ArH), 2857 (C-H str. aliphatic), 1668 (C=O str. amide), 1630 and 1461 (C=C ArH), 1596 (N-H bending CONH), 1528 (N-H bending $SO_2NH$), 1383 (S=O asymm. str.), 1271 (C-N str.), 1136 (S=O symm. str.), 1110 (para oop), 771 (S-N str.); Mass (m/z): 348.30 ($M+H^+$)

***N-(4-(N-(pyridin-2-yl)sulfamoyl)phenyl)Heptanamide (SP-HEPT)***



Cream white powder; Yield: 34%; UV absorption maxima:265nm; m.p. (DSC) 192.82°C; $^1$H NMR (400 MHz, DMSO) δ 10.23 (s, 1H,amide-H), 8.02-8.03 (d, J = 4.3 Hz, 1H,Ar-H), 7.68 – 7.82 (m, 5H,Ar-H), 7.12-7.14 (d, J = 8.6 Hz, 1H,Ar-H), 6.85 – 6.88 (m, 1H,Ar-H), 2.30-2.34 (t, J

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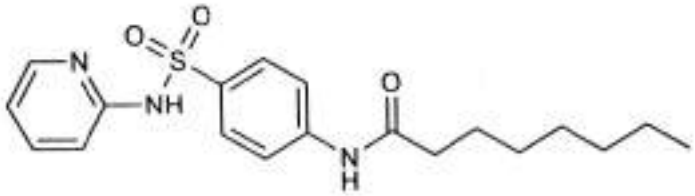
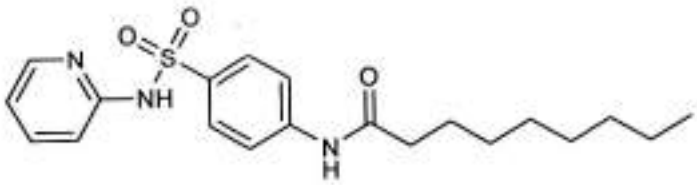
= 7.4 Hz, 2H,$CH_2$), 1.53-1.58 (m, 2H, $CH_2$), 1.26 (m, 6H), 0.83-0.86 (t, J = 8.9, 4.6 Hz, 3H, $CH_3$). $^{13}$C NMR (500 MHz, DMSO): 171.77, 152.76, 142.55, 139.85, 139.05, 135.15, 127.68, 118.32, 115.79, 113.29, 36.33, 30.88, 28.15, 24.78, 21.84, 13.79; IR (KBr) v: 3330 (N-H str. $SO_2NH$/CONH), 2924 (C-H str. ArH), 2858 (C-H str. aliphatic), 1699 (C=O str. amide), 1629 and 1462 (C=C ArH), 1593 (N-H bending CONH), 1523 (N-H bending $SO_2NH$), 1379 (S=O asymm. str.), 1276 (C-N str.), 1131 (S=O symm. str.), 1110 (para oop), 781 (S-N str.); Mass (m/z): 362.5 ($M+H^+$)

***N-(4-(N-(pyridin-2-yl)sulfamoyl)phenyl)octanamide (SP-OCT)***



Cream white powder; Yield: 89%; UV absorption maxima:265nm; m.p. (DSC) 218.47°C; $^1$H NMR (400 MHz, DMSO) δ 10.21 (s, 1H, amide-H), 8.02 (d, $J$ = 4.4 Hz, 1H, Ar-H), 7.83 – 7.56 (m, 5H, Ar-H), 7.12 (d, $J$ = 8.6 Hz, 1H, Ar-H), 7.02 – 6.87 (m, 1H, Ar-H), 2.31 (t, $J$ = 7.4 Hz, 2H, $CH_2$), 1.67 – 1.50 (m, 2H, $CH_2$), 1.25 (m, 8H), 0.85 (t, $J$ = 6.9 Hz, 3H, $CH_3$).$^{13}$C NMR (500 MHz, DMSO): 171.76, 152.76, 143.95, 142.56, 139.81, 135.11, 127.68, 118.32, 115.79, 113.26, 36.33, 31.01, 28.44, 28.31, 24.82, 21.92, 13.79; IR (KBr) v: 3324 (N-H str. $SO_2NH$/CONH), 2921 (C-H str. ArH), 2851 (C-H str. aliphatic), 1700 (C=O str. amide), 1629 and 1462 (C=C ArH), 1593 (N-H bending CONH), 1526 (N-H bending $SO_2NH$), 1381 (S=O asymm. str.), 1276 (C-N str.), 1129 (S=O symm. str.), 1001 (para oop), 779 (S-N str.); Mass (m/z): 376.17 ($M+H^+$)

***N-(4-(N-(pyridin-2-yl)sulfamoyl)phenyl)nonanamide (SP-NON)***



Cream white powder; Yield: 43.398%; UV absorption maxima:265nm; m.p. (DSC) 210.82°C; $^1$H NMR (400 MHz, DMSO) δ 10.23 (s, 1H, amide-H), 8.00 (d, $J$ = 4.5 Hz, 1H, Ar-H), 7.86 – 7.56 (m, 5H, Ar-H), 7.12 (d, $J$ = 8.6 Hz, 1H, Ar-H), 6.93 – 6.58 (m, 1H, Ar-H), 2.30 (t, $J$ = 7.4 Hz, 2H, $CH_2$), 1.65 – 1.42 (m, 2H, $CH_2$), 1.24 (d, $J$ = 8.5 Hz, 10H), 0.83 (t, $J$ = 6.8 Hz, 3H, $CH_3$).$^{13}$C NMR (500 MHz, DMSO): 171.76, 152.73, 144.14, 142.56, 139.92, 135.10, 127.67, 118.31, 113.25, 36.32, 31.10, 28.60, 28.48, 28.43, 24.81, 21.93, 13.80; IR (KBr) v: 3331(N-H str. $SO_2NH$/CONH), 2922 (C-H str. ArH), 2854 (C-H str. aliphatic), 1696 (C=O str. amide), 1629 and 1459 (C=C ArH), 1592 (N-H bending CONH), 1532 (N-H bending $SO_2NH$), 1379 (S=O asymm. str.), 1271 (C-N str.), 1135 (S=O symm. str.), 1001 (para oop), 774 (S-N str.); Mass (m/z): 390.25 ($M+H^+$)

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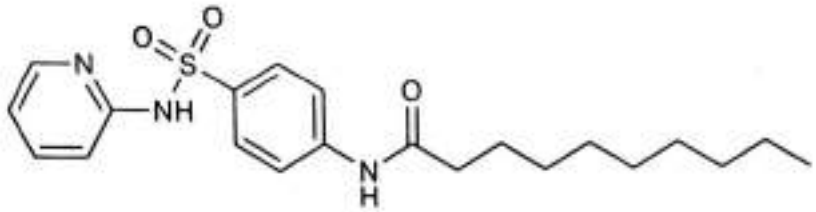
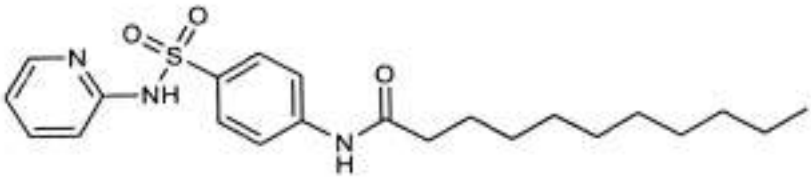
***N-(4-(N-(pyridin-2-yl)sulfamoyl)phenyl)decanamide (SP-DEC)***



Cream white powder; Yield: 40.00%; UV absorption maxima: 265nm; m.p. (DSC) 183.91°C; $^1$H NMR (400 MHz, DMSO) δ 10.21 (s, 1H,amide-H), 8.02 (d, $J$ = 4.5 Hz, 1H, Ar-H), 7.86 – 7.59 (m, 5H, Ar-H), 7.12 (d, $J$ = 8.6 Hz, 1H, Ar-H), 6.98 – 6.73 (m, 1H, Ar-H), 2.31 (t, $J$ = 7.4 Hz, 2H, $CH_2$), 1.57 (m, 2H, $CH_2$), 1.25 (m, 12H), 0.84 (t, $J$ = 6.8 Hz, 3H, $CH_3$); $^{13}$C NMR (500 MHz, $CDCl_3$): 172.46, 154.24, 142.46, 141.82, 141.71, 135.96, 127.76, 119.48, 115.74, 114.73, 37.76, 31.84, 29.46, 29.40, 29.29, 29.27, 25.54, 22.64, 14.08; IR (KBr) ν: 3335 (N-H str. $SO_2NH$/CONH), 2924 (C-H str. ArH), 2850 (C-H str. aliphatic), 1696 (C=O str. amide), 1630 and 1459 (C=C ArH), 1592 (N-H bending CONH), 1531 (N-H bending $SO_2NH$), 1381 (S=O asymm. str.), 1244 (C-N str.), 1135 (S=O symm. str.), 957 (para oop), 774 (S-N str.); Mass (m/z): 404.20 ($M+H^+$)

***N-(4-(N-(pyridin-2-yl)sulfamoyl)phenyl)undecanamide (SP-UNDEC)***



Cream white powder; Yield: 56%; UV absorption maxima: 265 nm; m.p. (DSC) 147.12 °C. $^1$H NMR (400 MHz, DMSO) δ 10.21 (s, 1H, amide-H), 8.02 (d, $J$ = 3.9 Hz, 1H, Ar-H), 7.74 (m, 5H, Ar-H), 7.12 (d, $J$ = 8.5 Hz, 1H, Ar-H), 6.87 (m, 1H, Ar-H), 2.31 (t, $J$ = 7.2 Hz, 2H, $CH_2$), 1.56 (m, 2H, $CH_2$), 1.23 (m, 14H), 0.83 (t, $J$ = 7.2 Hz, 3H, $CH_3$); $^{13}$C NMR (500 MHz, $CDCl_3$): 172.31, 154.07, 142.70, 141.73, 141.58, 136.00, 127.83, 119.44, 115.91, 114.68, 37.81, 31.87, 29.57, 29.51, 29.40, 29.29, 25.52, 22.66, 14.09; IR (KBr) ν: 3306 (N-H str. $SO_2NH$/CONH), 2918 (C-H str. ArH), 2848 (C-H str. aliphatic), 1665 (C=O str. amide), 1630 and 1463 (C=C ArH), 1595 (N-H bending CONH), 1526 (N-H bending $SO_2NH$), 1360 (S=O asymm. str.), 1285 (C-N str.), 1136 (S=O symm. str.), 1034 (para oop), 774 (S-N str.); Mass (m/z): 418.35 ($M+H^+$)

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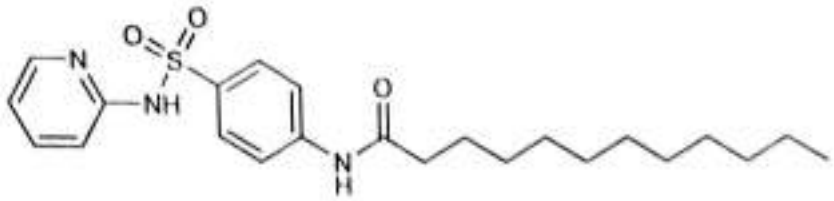
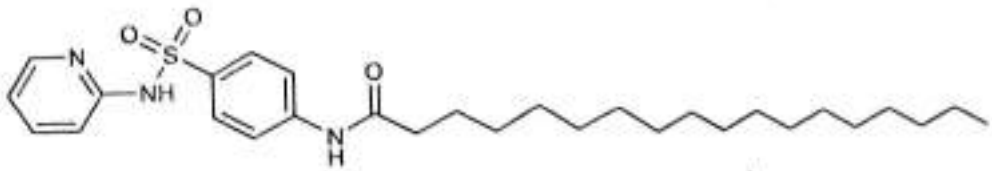
***N-(4-(N-(pyridin-2-yl)sulfamoyl)phenyl)dodecanamide (SP-DODEC)***



Cream white powder; Yield: 37.00%; UV absorption maxima: 265nm; m.p. (DSC) 150.61°C.$^1$H NMR (400 MHz, $CDCl_3$) δ 8.38 (d, $J$ = 5.1 Hz, 1H, Ar-H), 8.18 (b, 1H, Amide-H), 7.78 – 7.44 (m, 6H, Ar-H), 6.87 (m,1H, Ar-H), 2.40 (t, $J$ = 7.2 Hz, 2H, $CH_2$), 1.71 (m, 2H, $CH_2$), 1.36 – 1.12 (m, 16H), 0.87 (t, $J$ = 6.8 Hz, 3H, $CH_3$); $^{13}$C NMR (500 MHz, $CDCl_3$): 172.26, 154.03, 142.85, 141.70, 141.48, 136.02, 127.84, 119.42, 115.96, 114.65, 37.82, 31.89, 29.63, 29.60, 29.51, 29.41, 29.32, 29.30, 25.52, 22.67, 14.10; IR (KBr) ν: 3341 (N-H str. $SO_2NH$/CONH), 2918 (C-H str. ArH), 2849 (C-H str. aliphatic), 1692 (C=O str. amide), 1629 and 1462 (C=C ArH), 1596 (N-H bending CONH), 1516 (N-H bending $SO_2NH$), 1383 (S=O asymm. str.), 1285 (C-N str.), 1131 (S=O symm. str.), 939 (para oop), 791 (S-N str.); Mass (m/z): 432.23 ($M+H^+$)

***N-(4-(N-(pyridin-2-yl)sulfamoyl)phenyl)stearamide (SP-STYL)***



Cream white powder; Yield:45.54%; UV absorption maxima: 265nm. $^1$H NMR (400 MHz, DMSO) δ 10.21 (s, 1H, amide-H), 8.02 (b, 1H, Ar-H), 7.69 – 7.81 (m, 5H, Ar-H), 7.11-7.13 (d, J = 7.5 Hz, 1H, Ar-H), 6.86 (m, 1H, Ar-H), 2.31 (b, 2H, $CH_2$), 1.56 (m, 2H, $CH_2$), 1.22 (m, 28H), 0.84 (b, 3H, $CH_3$). $^{13}$C NMR (500 MHz, DMSO): 174.35, 151.74, 142.55, 140.67, 139.96, 135.08, 127.66,118.30, 115.48, 113.24, 39.08, 38.91, 36.32, 33.54, 31.16, 28.89, 28.61, 28.57, 28.48, 28.42, 24.80, 24.37, 21.96, 13.82; IR (KBr) ν: 3332 (N-H str. $SO_2NH$/CONH), 2915 (C-H str. ArH), 2848 (C-H str. aliphatic), 1697 (C=O str. amide), 1630 and 1465 (C=C ArH), 1596 (N-H bending CONH), 1526 (N-H bending $SO_2NH$), 1389 (S=O asymm. str.), 1291 (C-N str.), 1136 (S=O symm. str.), 1110 (para oop), 775 (S-N str.); Mass (m/z): 516.3 ($M+H^+$)

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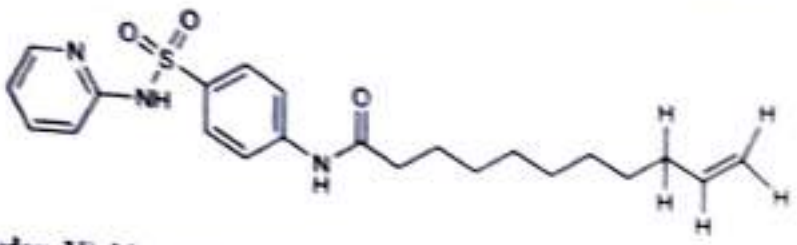
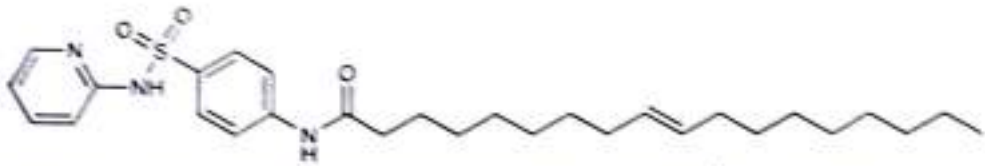
***N*-(4-(*N*-(pyridin-2-yl)sulfamoyl)phenyl)undec-10-enamide (SP-10UNDEC)**



Cream white powder; Yield: 49.01%; UV absorption maxima: 265nm; m.p. (DSC) 108.84°C. $^1$H NMR (400 MHz, DMSO) δ 10.35 (s, 1H , amide-H), 8.01-8.02 (d, J = 4.7 Hz, 1H , Ar-H), 7.67 – 7.81 (m, 5H , Ar-H), 7.11-7.13 (d, J = 8.6 Hz, 1H , Ar-H), 6.85-6.88 (m, J = 6.2 Hz, 1H, Ar-H), 5.72-5.82 (m, 1H), 4.90-5.00 (m, 2H, $CH_2$), 3.56-3.63 (m, 1H), 3.09-3.14 (m, 1H), 2.34 (t, J = 7.4 Hz, 2H), 1.25-2.01 (m, 12H). $^{13}$C NMR (500 MHz, DMSO):171.81, 152.75, 142.62, 139.84, 138.68, 135.08, 127.63, 118.31, 115.83, 114.49, 113.27, 36.29, 33.03, 28.59, 28.45, 28.33, 28.10, 24.82; IR (KBr) ν: 3323 (N-H str. $SO_2NH$/CONH), 2924 (C-H str. ArH), 2853 (C-H str. aliphatic), 1691 (C=O str. amide), 1629 and 1460 (C=C ArH), 1591 (N-H bending CONH), 1528 (N-H bending $SO_2NH$), 1384 (S=O asymm. str.), 1271 (C-N str.), 1130 (S=O symm. str.), 1110 (para oop), 774 (S-N str.); Mass (m/z): 416.6 ($M+H^+$)

***(E)*-*N*-(4-(*N*-(pyridin-2-yl)sulfamoyl)phenyl)octadec-9-enamide (SP-OLE)**



Yellowish powder; Yield:27%; UV absorption maxima: 265nm; $^1$H NMR (400 MHz, DMSO) δ 10.21 (s, 1H, amide-H), 8.01-8.02 (d, J = 4.3 Hz, 1H, Ar-H), 7.67 – 7.81 (m, 5H, Ar-H), 7.11-7.13 (d, J = 8.6 Hz, 1H, Ar-H), 6.85 – 6.88 (m, 1H, Ar-H), 5.27 – 5.64 (m, 2H, Alkenyl-H), 2.29-2.32 (t, J = 7.4 Hz, 2H, $CH_2$), 1.96-1.97 (d, J = 5.1 Hz, 4H), 1.54 – 1.58 (m, 2H, $CH_2$), 1.21-1.26 (m, 20H), 0.80-0.84 (t, J = 6.8 Hz, 3H, $CH_3$). $^{13}$C NMR (500 MHz, DMSO):171.70, 152.75, 143.89, 142.57, 135.09, 129.51, 129.47, 118.29, 115.92, 113.23, 36.31, 31.14, 28.96, 28.55, 28.52, 28.48, 28.46, 28.36, 26.44, 24.80, 21.95, 13.79; IR (KBr) ν: 3336 (N-H str. $SO_2NH$/CONH), 2920 (C-H str. ArH), 2851 (C-H str. aliphatic), 1668 (C=O str. amide), 1629 and 1460 (C=C ArH), 1597 (N-H bending CONH), 1525 (N-H bending $SO_2NH$), 1383 (S=O asymm. str.), 1280 (C-N str.), 1136 (S=O symm. str.), 1109 (para oop), 795 (S-N str.); Mass (m/z): 514.4 ($M+H^+$)

The representative spectra for the characterization of *N*-(4-(*N*-(pyridin-2-yl)sulfamoyl)phenyl)decanamide has been dipicted in Figure 4.

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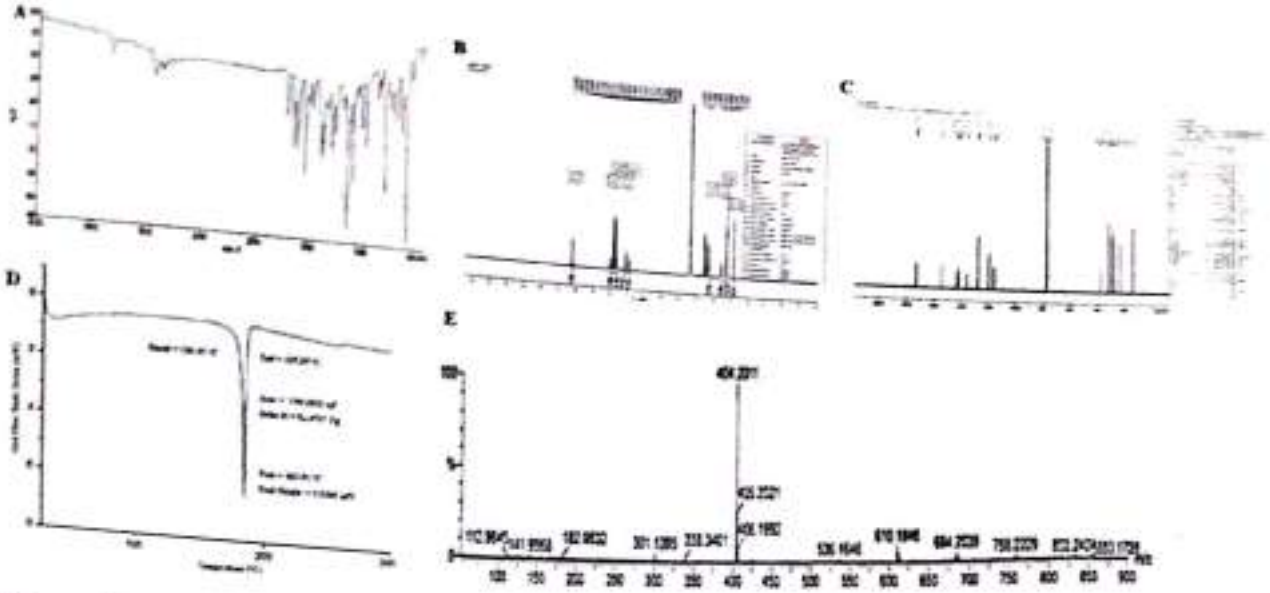

**Figure 4:** Characterization of *N*-(4-(*N*-(pyridin-2-yl)sulfamoyl)phenyl)decanamide. (A) IR spectra (B) $^{1}H$ NMR (C) $^{13}C$ NMR (D) DSC (E) Mass spectra

## 2. Purity analysis by HPLC

The purity of the synthesized prodrugs was analyzed by HPLC using acetonitrile and formic acid (0.1% v/v) in gradient elution. All the synthesized prodrugs were found to have purity of more than 94% (Table 1) and the respective chromatograms have been given in Figure 5.

**Table 1:** Percentage purity of synthesized prodrugs determined by HPLC

| Compound code | % Purity |
|---|---|
| SP-PROP | 97.92% |
| SP-VAL | 95.529% |
| SP-PIVA | 98.91% |
| SP-HEX | 94.077% |
| SP-HEPT | 99.478% |
| SP-OCT | 97.639% |
| SP-NON | 97.443% |
| SP-DEC | 97.69% |
| SP-UNDEC | 98.344% |
| SP-DODEC | 99.75% |
| SP-STYL | 96.878% |
| SP-10UNDEC | 98.690% |
| SP-OLE | 95.165% |

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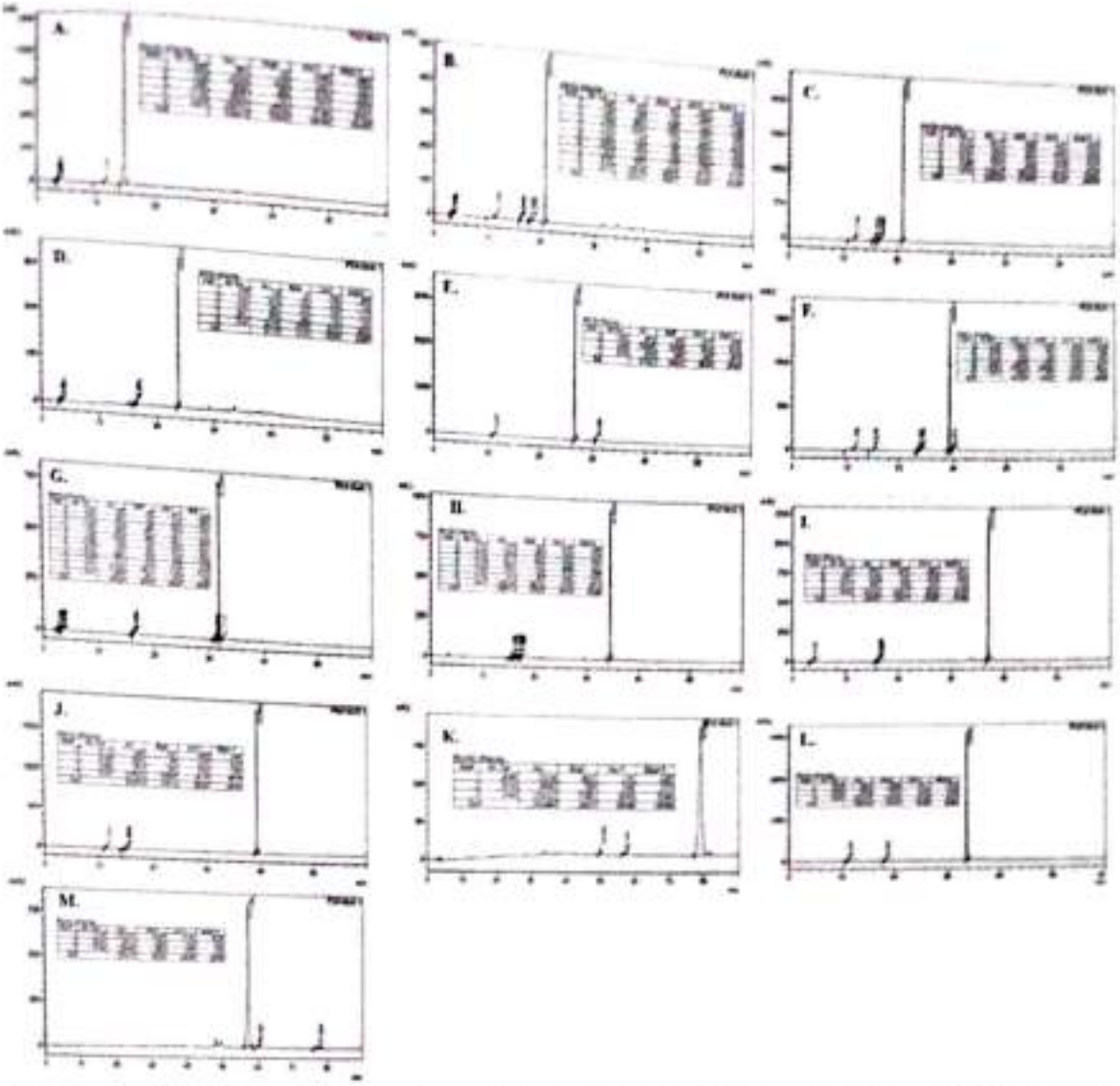

**Figure 5**: HPLC chromatogram of (A) SP-PROP (B) SP-VAL (C) SP-PIVA (D) SP-HEX (E) SP-HEPT (F) SP-OCT (G) SP-NON (H) SP-DEC (I) SP-UNDEC (J) SP-DODEC (K) SP-STYL (L) SP-10UNDEC (M) SP-OLE for purity analysis

## 3. Analytical method development

Isocratic methods were developed for pre-formulation studies i.e., determination of solubility and partition coefficient of synthesized prodrugs and sulfapyridine. The mobile phase used was acetonitrile and formic acid (0.1% v/v) but in different ratios for various prodrugs. The other chromatographic conditions such as flow rate, column, and detection wavelength were same. The methods were validated as per ICH Q2R1 guidelines.

### *3.1 Linearity*

The linearity of the developed method was established by analysing each dilution five times and mean data was recorded. Further, linearity curve was prepared by plotting the area of peak against the concentration of the respective solution injected. The slope, intercept and regression coefficient

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values were determined by least squares linear regression analysis. The linearity curves for various prodrugs have been given in Figure 6. The other parameters of validation have to be carried out.

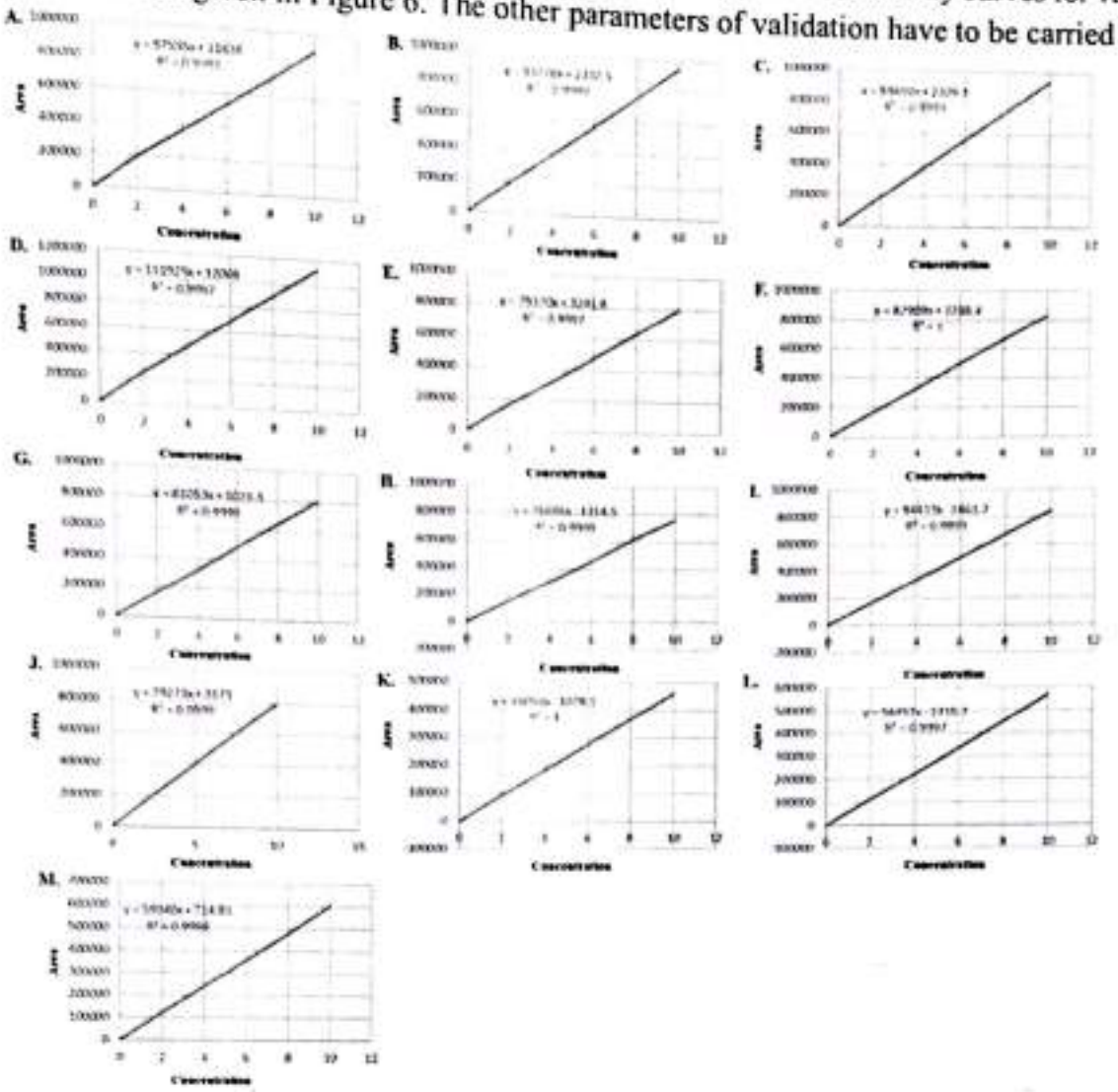

**Figure 6**: Linearity curves of (A) SP-PROP (B) SP-VAL (C) SP-PIVA (D) SP-HEX (E) SP-HEPT (F) SP-OCT (G) SP-NON (H) SP-DEC (I) SP-UNDEC (J) SP-DODEC (K) SP-STYL (L) SP-10UNDEC (M) SP-OLE for purity analysis

### Statistical Analysis

The data were analysed with Microsoft Excel 2021 and expressed as mean ± SEM.

### Discussion

The structures of amide prodrugs were confirmed by NMR, IR and mass. The formation of sulfapyridine prodrugs was confirmed by appearance of amide proton in the range of 8-10 ppm in NMR spectra. The aromatic protons were deshielded to higher value (6-8 ppm) due to the electron withdrawing nature of amide bond. Signals in the range of 1-2 ppm in NMR spectra were attributed

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to the aliphatic protons of alkyl chain. In $^{13}C$ NMR, the appearance of signal around 170-172 ppm indicated the presence of carbonyl carbon in prodrugs. The signals due to alkyl chain were observed at δ value less than 40 ppm. Mass spectra confirmed that the amide prodrugs were synthesized successfully. The appearance of amide band in the IR spectra confirmed the successful synthesis of sulfapyridine prodrugs. The UV absorption maxima of the all the prodrugs was found to be same i.e., 265 nm, which is due to the similarity in the chromophore. The percentage purity of the synthesized prodrugs, as determined by HPLC, was found to be greater than 94%.

**Impact of the research in the advancement of knowledge or benefit to mankind:**

- Development of self-assembling supramolecular nanostructure of sulfapyridine prodrugs that are expected to exhibit prolonged effect than the other regimes, thereby will be an economical, patient friendly therapeutic approach for the management of arthritis.
- Bio-labile pro-drugs of sulfapyridine will open the doors for the re-positioning of this abandoned drug for intra-articular delivery.

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