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AMINO ACIDS BASED SULPHONAMIDES AND THEIR HYBRIDIZATION WITH SUBSTITUTED PIPERAZINE AND METRONIDAZOLE DRUG; SYNTHESIS, CHARACTERIZATION BY ^{1}H NMR, ^{13}C NMR SPECTROSCOPY AND PHYSICAL PARAMETERS

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ABSTRACT

Sulfonamide and carboxamide functionalities are vital in the development of bioactive molecules due to their broad pharmacological relevance. In this study, a series of amino acid-based sulfonamides were synthesized and further hybridized with metronidazole and substituted piperazine to enhance antibacterial activity. The synthetic approach involved ester and amide linkages using carbodiimide-mediated coupling reactions in the presence of DMAP. The target molecules were obtained in moderate to good yields and characterized by ^{1}H NMR, ^{13}C NMR, and physical parameters. These novel hybrids were designed based on the molecular hybridization strategy to potentially overcome resistance mechanisms and enhance drug-like properties. The chemical diversity introduced through different R-groups is expected to contribute toward improved antimicrobial efficacy. The synthesized compounds are promising candidates for further biological evaluation against bacterial strains.

KEYWORDS: Sulfonamides; Carboxamides; Amino acids; Metronidazole; Substituted piperazine; Molecular hybridization; Antibacterial agents; Esterification; Amide bond formation; EDC/DMAP coupling; NMR spectroscopy; Antimicrobial resistance; Drug design; Bioactive molecules.

1. INTRODUCTION

The growing resistance of bacteria to conventional antibiotics poses a critical challenge to public health worldwide, with the World Health Organization identifying antimicrobial resistance as one of the top 10 global health threats of the 21st century[1], The decline in efficacy of traditional therapies has underscored the urgent need for the development of novel chemotherapeutic agents with improved potency, reduced toxicity, and novel mechanisms of action.[2]Among the strategies in modern medicinal chemistry, molecular hybridization has emerged as a promising approach.[3] It involves the covalent linkage of two or more bioactive pharmacophores into a single molecular framework to generate hybrid compounds with synergistic or complementary biological properties[4], This method often results in enhanced pharmacodynamic profiles, improved selectivity, reduced side effects, and the potential to overcome drug resistance by targeting multiple biological pathways simultaneously.[5] For instance, the hybridization of quinolone and oxazolidinone scaffolds has yielded compounds with dual antibacterial action, showing increased efficacy against resistant bacterial strains.[6]

Metronidazole, a 5-nitroimidazole derivative, is well established for its activity against anaerobic bacteria and protozoal pathogens through DNA strand breakage upon nitro group reduction under anaerobic conditions.[7,8] It remains an essential component of treatment regimens

for infections such as *Trichomonas vaginalis*, *Entamoeba histolytica*, and *Clostridium difficile*.[9] However, its prolonged clinical use has been associated with several limitations, including neurotoxicity**,** gastrointestinal side effects, and the emergence of resistant microbial strains, particularly in *Helicobacter pylori* and anaerobic Gram-negative bacilli.[10]

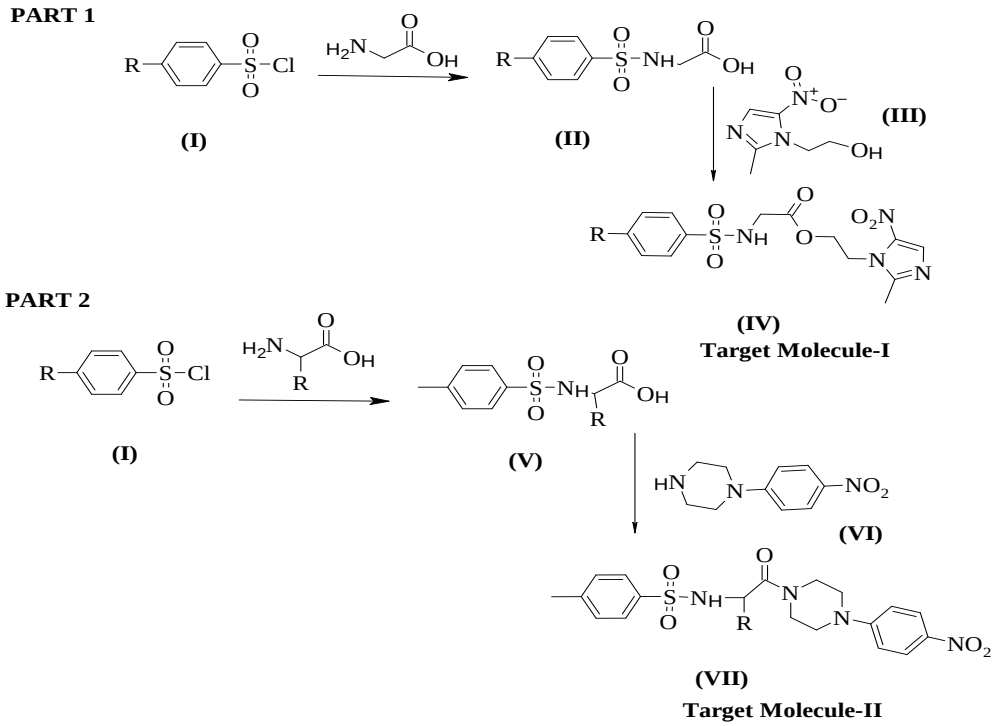
On the other hand, sulfonamides, the first class of synthetic antimicrobial agents[11], exert their bacteriostatic action by mimicking *p*-aminobenzoic acid (PABA) and inhibiting the enzyme dihydropteroate synthase, thereby disrupting folate biosynthesis in microbes.[12] Despite the availability of newer antibiotics, sulfonamides continue to serve as crucial scaffolds in drug design due to their structural modifiability and broad-spectrum activity.[13] For example, hybrid molecules such as sulfonamide-triazole conjugates have shown improved potency and selectivity against *Mycobacterium tuberculosis* and Gram-negative bacteria.[14,,15]

Amino acids, due to their intrinsic biocompatibility, chiral centers, and capacity to form hydrogen bonds, serve as ideal linkers or side chains in hybrid molecules.[16] Their inclusion can improve water solubility, enhance cellular uptake, and introduce site-specific targeting through transporter recognition.[17] Amino acid-based prodrugs, such as valacyclovir (valine ester of acyclovir), have demonstrated significantly increased oral bioavailability compared to their parent drugs, emphasizing the utility of amino acids in improving pharmacokinetic profiles.[18,19]

In this study, we synthesized a series of amino acid-based sulfonamides and hybridized them with metronidazole and substituted piperazine using ester and amide bond-forming strategies. Substituted piperazine, a core moiety in many antipsychotics and antimicrobials, was chosen for its diverse biological activity and potential to enhance antimicrobial potency through additional receptor interaction. The designed hybrids were structurally characterized using spectroscopic techniques and evaluated for their potential antibacterial activity, with the goal of exploring structure-activity relationships and identifying promising candidates for further pharmacological investigation.

2. Chemistry

Modification of pendant hydroxyl group of metronidazole is a common strategy to develop novel antibacterial agents.[20] Since Sulfonamides and its derivatives are important pharmacophores that were reported as potent antibacterial agents against resistant and non-resistant strains.[21] We planned the synthesis of two different Target Molecules (compounds **IV** & **VII**). Synthesis of Target Molecule I (part1, scheme1.1) starts with the commercially available different sulphonyl chlorides (**I**) reacted with Glycine to afford different sulfonamides (**II**). These sulfonamides then reacted with Metronidazole (**III**) to yield the desired hybrid molecule (**IV**). Similarly, synthesis of Target Molecule II (part2, scheme1.1) starts with the commercially available *p*-toluene sulfonyl chloride (**I**) reacted with different amino acids to afford sulfonamides which then reacted with substituted piperazine (**VI**) to yield amide linked target compound (**VII**). The synthetic strategy is shown in Scheme: 1.1



Scheme 1.1: Synthesis of Amino acids-based sulfonamides and their hybridization with metronidazole and substituted piperazine.

3. RESULTS AND DISCUSSION

3.1. Sequential Synthesis Strategy

In response to the alarming rise in multi-drug resistant bacterial strains, a sequential synthetic approach was adopted to construct novel hybrid molecules combining established pharmacophores with enhanced biological potential. The synthetic pathway involved two major parts.

Part I focused on the synthesis of ester-linked conjugates of sulfonamides with metronidazole

Part II involved amide-linked hybrids of sulfonamides with substituted piperazine.

The synthetic plan began with the construction of sulfonamide intermediates, which were then functionalized through esterification and amidation steps to afford the target molecules. The complete synthetic sequence is depicted in **Scheme 1.1**, outlining the design of **Target Molecule I and II**.

3.2. Part I

A series of 2-(4-substituted phenylsulfonamido)acetic acids were synthesized as core intermediates using a green chemistry protocol. The reaction involved the coupling of substituted sulfonyl chlorides (**I**) with glycine in aqueous medium, employing sodium carbonate as a base to neutralize the in situ generated HCl. After completion, the reaction mixture was acidified to pH 3 using dilute HCl, and the resulting products were purified through recrystallization to afford sulfonamides in excellent yields (87–92%). These sulfonamide intermediates were subsequently functionalized via esterification with metronidazole using carbodiimide coupling chemistry. EDC·HCl was employed as the coupling reagent, with DMAP serving as an acyl-transfer catalyst. The reaction was carried out in a DCM/DMF solvent system under an inert atmosphere for 24 hours. Post-reaction workup involved the removal of the urea by-product and excess DMAP through aqueous extraction, followed by recrystallization from chloroform. The resulting ester-linked conjugates (**IV**) were obtained as off-white solids in moderate yields (52–58%).

^{1}H NMR confirmed the disappearance of the acidic proton and appearance of characteristic signals for the imidazole moiety at δ 8.04 ppm (H-4) and δ 2.50 ppm (CH_3-imidazole). The methylene protons adjacent to oxygen (H-7) and nitrogen (H-6) resonated at δ 4.51 and δ 4.30 ppm, respectively.

^{13}C NMR data supported ester formation, with a deshielded carbonyl carbon at δ 169.1 ppm, and methyl carbons at δ 14.4 and 21.3 ppm.

3.3. Part II

Amino acid-based sulfonamides were synthesized by reacting *p*-toluenesulfonyl chloride with different amino acids such as glycine, alanine, and phenylalanine, following a method analogous to the previously described sulfonamide synthesis. The reactions proceeded smoothly in aqueous medium, yielding the desired carboxylic acid derivatives (**V**) in excellent yields ranging from 85% to 91%. These sulfonamides served as key intermediates for further amidation reactions with substituted piperazine derivatives (**VI**). Carboxamide formation was carried out under mild conditions using EDC·HCl as the coupling reagent and DMAP as the catalyst in a DCM/DMF solvent system. The reaction mixtures were stirred at room temperature under an inert atmosphere for 24 hours. Upon completion, the urea by-product and residual reagents were removed by solvent extraction, and the crude products were purified via silica gel column chromatography to afford the final carboxamides (**VII**) as yellow solids in yields ranging from 62% to 69%.

^{1}H NMR spectrum, the aromatic region displayed a doublet at δ 8.06 ppm corresponding to the phenyl ring protons (H-13, H-13a') attached to the piperazine moiety, while another set of doublets at δ 7.68 ppm represented protons (H-2, H-2a') of the sulfonamide-linked aromatic ring. A singlet at δ 7.72 ppm corresponded to the sulfonamide proton (H-1a), and a multiplet at δ 3.51 ppm indicated the presence of eight protons on the piperazine ring (H-8, H-8a', H-9, H-9a'). A methyl singlet was also observed at δ 2.36 ppm, confirming the presence of a *para*-methyl group on the aromatic ring.

^{13}C NMR spectrum, the amide carbonyl carbon appeared at δ 166.4 ppm, and the ipso-carbon of the phenyl ring attached to piperazine was observed at δ 154.7 ppm. Piperazine ring carbons resonated between δ 43.7 and 46.2 ppm, while the methyl carbon was detected at δ 21.4 ppm. The combined spectral data confirmed the successful formation of the desired carboxamide structure.

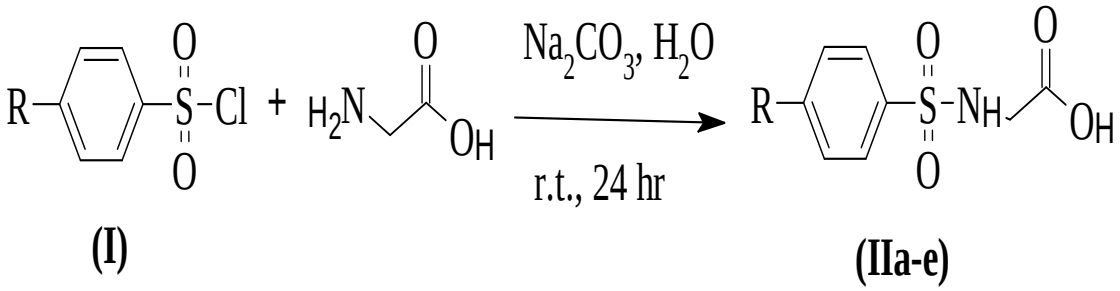
4. Experimental

All experimental procedures were carried out using thoroughly cleaned and dried glassware. Organic solvents were dried and distilled prior to use, and reactions in non-aqueous media were conducted under a nitrogen atmosphere. Anhydrous sodium sulfate was employed to remove residual moisture. Reaction progress was monitored using Thin Layer Chromatography (TLC) on pre-coated silica gel-60 F254 plates (0.2 mm thickness). UV-active compounds were visualized at 254 nm, while UV-inactive species were detected using spray reagents such as ninhydrin, *p*-anisaldehyde, and potassium permanganate. Carboxamide purification was achieved via flash column chromatography on silica gel (200–300 mesh). Melting points were determined using a Gallenkamp MPD350.BM3.5 apparatus, while structural elucidation was performed by ^{1}H and ^{13}C NMR spectroscopy on Bruker Avance instruments (300 MHz and 75 MHz, respectively), with chemical shifts (δ) reported in ppm and coupling constants (J) in Hz. For moisture-sensitive reactions, solvents were dried accordingly: DMF was

vacuum-distilled over calcium hydride and stored over 4 Å molecular sieves[22]; DCM was refluxed over CaH_2, distilled, and stored similarly[23]; and THF was refluxed with sodium/benzophenone under inert atmosphere until a dark blue color confirmed complete drying.[24] Ninhydrin and *p*-anisaldehyde staining solutions were freshly prepared and protected from light for effective detection of amino and ester functionalities, respectively.

4.1. Part I

4.1.1. General procedure for the synthesis of Sulfonamide Carboxylic acid (IIa-e)



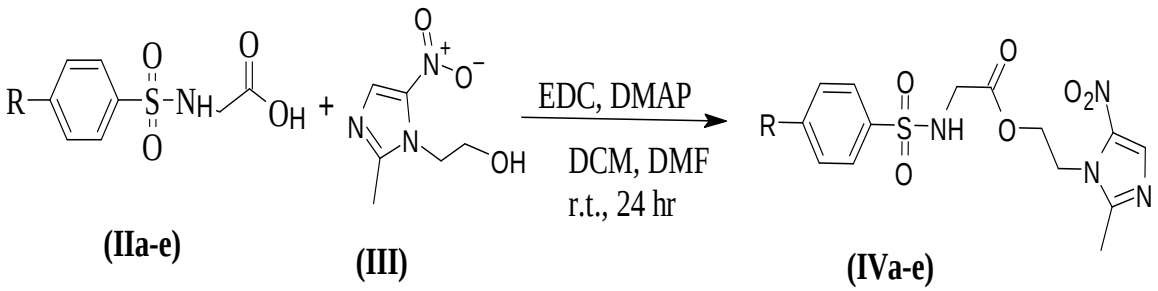
To an aqueous combination of an amino acid (10 mmol) and Na_2CO_3 (12 mmol) in water (50 mL), *p*-substituted benzenesulfonyl chloride (12 mmol) was added over the 48 course of 15 minutes. Following complete addition of reagents, reaction mixture in the flask was allowed to stir for the time period of 4-6 hours at ambient temperature before being acidified with dilute HCl. The precipitates obtained were filtered, purified, dried properly and then recrystallized using ethylacetate-*n*-hexane to get the desired product.[25]

Table 1.1: Physical & Spectral data of derivatives of 2-(4-substituted phenylsulfonamido) acetic acid (IIa-e)

Compd.	R	Colours	R*f**	Melting points (ºC)	Yield (%)
IIa	$-CH_3$	White	0.43	146-147 (Lit. 147)	92
IIb	$-OCH_3$	White	0.41	143-149 (Lit. 149)	90
IIc	$-NO_2$	Off White	0.38	156-159 (Lit. 157)	87
IId	-Cl	White	0.40	124-131 (Lit. 128)	88
IIe	Naphthyl	Off white	0.45	161-163(Lit. 164)	89

*($CHCl_3$: MeOH::9:1) on pre coated silica gel plates $60F_{254}$ visualized under UV light at 254 nm

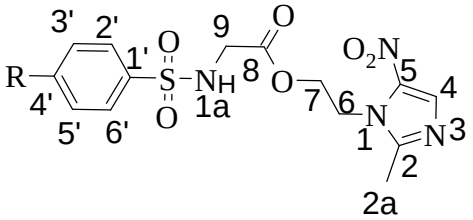
4.1.2. General procedure for the synthesis of Ester linked conjugates of metronidazole and sulfonamide carboxylic acid (IVa-e)



1 equivalent of 2-(4-substitutedphenylsulfonamido)acetic acid (**IIa-e**) was dissolved in DCM and DMF (10:1), then 0.2 equivalent of DMAP, 1 equivalent of metronidazole bearing OH moiety (**III**) and 1 equivalent of EDC were added in the reaction flask and allowed to stir in an inert atmosphere for 24 hours at the room temperature. After the completion of reaction, the urea and DMAP were removed by solvent extraction with ethyl acetate under acidic conditions. The pale yellow precipitates obtained were recrystallized using chloroform to afford the pure product (**IVa-e**) as off white crystals.

Physical and spectral data of synthesized Ester linked conjugates of Metronidazole and Glycine based Sulfonamides (**IVa-e**) is given in Table (1.2).

Table 1.2: Physical and spectral data of Labelled Ester linked conjugates of Metronidazole and Glycine based Sulfonamides (IVa-e)

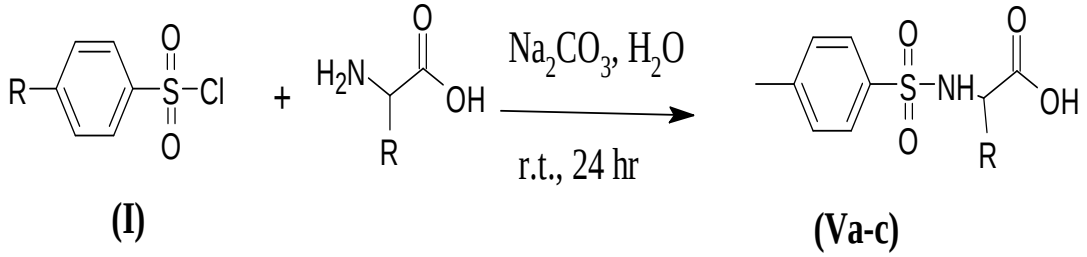


Compd.	R	R_{f*}	Colors	Melting Points (°C)	Yield (%)	^{1}H NMR (300 MHz, DMSO-d6): δ (ppm)	^{13}C NMR (75 MHz, DMSO-d6)
IVa	$-CH_3$	0.40	Off white	184-186	58	8.13 (1H, t, 3J =6 Hz, H-1a), 8.04 (1H, s, H-4), 7.61 (2H, d, H-2'& 6'), 7.34 (2H, d, H-3'& 5'), 4.51 (2H, t, 3J =4.8 Hz, H-7), 4.30 (2H, t, 3J =4.8 Hz, H-6), 3.60 (2H, d,3J =6 Hz, H-9), 2.43 (3H, s, H-2a), 2.36 (3H, s, H-4'a) ;	δ (ppm) 169.1 (C-8), 152.1 (C-2), 143.2 (C-5), 138.8 (C-4'), 138.0 (C-1'), 133.5 (C-4), 129.9 (C-3'&5'), 126.9 (C-2'&6'), 63.5 (C-7), 45.0 (C-9), 44.0 (C-6), 21.3 (C-4'a), 14.4 (C-2a)
IVb	$-OCH_3$	0.38	White	192-195	55	7.95 (1H, s, H-4), 7.75 (2H, d, H-2'& 6'), 6.96 (2H, d, H-3'& 5'), 5.56 (1H, t, H-1a), 4.57 (2H, t, 3J=4.8 Hz, H-7), 4.41 (2H, t, 3 J=4.8 Hz, H-6), 3.87 (3H, s, H-4'a), 3.72 (2H, d, 3J=6 Hz, H-9), 2.51 (3H, s, H-2a)	168.7 (C-8), 163.1 (C-4'), 150.9 (C-2), 138.3 (C-5), 132.6 (C-1'), 130.6 (C-4), 129.3 (C-2'& 6'), 114.3 (C-3'& 5'), 63.6 (C-7), 55.6 (C-4'a), 44.8 (C-9), 43.9 (C-6), 14.2 (C-2a)
IVc	$-NO_2$	0.33	White	180-182	56	8.65 (1H, s, H-4), 8.77 (2H, m, H-3'& 5'), 8.01 (3H, m, H-2',6'& 1a), 4.51 (2H, t, 3J=4.8 Hz, H-7), 4.33 (2H, t, 3J=4.8 Hz, H-6), 3.77 (2H, s, H-9), 2.42 (3H, s, H-2a)	169.1 (C-8), 152.1 (C-2), 150.0 (C-4'), 146.7 (C-1'), 138.8 (C-5), 133.5 (C-4), 128.4 (C-2'& 6'), 124.89 (C-3'& 5')), 63.6 (C-7), 45.0 (C-9), 44.0 (C-6), 14.4 (C-2a)
IVd	-Cl	0.35	White	207-210	52	8.36 (1H, t, 3J=6 Hz, H-1a), 8.05 (1H, s, H 4), 7.80 (2H, m, H-2' & 6'), 7.62 (2H, m, H-3' & 5'), 4.52 (2H, t, 3J=4.9 Hz, H-7), 4.33 (2H, t, 3J=4.9 Hz, H-6), 3.68 (2H, d, 3J=6 Hz, H-9), 2.44 (3H, s, H-2a)	169.1 (C-8), 152.1 (C-2), 140.4 (C-1'), 138.8 (C-5), 137.8 (C-4'), 133.4 (C-4), 129.6 (C-3'& 5'), 128.9 (C-2'& 6'), 63.5 (C-7), 45.07 (C-9), 44.0 (C-6), 14.4 (C-2a)
IVe	Naphthyl	0.39	Off white	217-221	52	8.80 (1H, m, H-1'), 8.40 (1H, m, H-4'), 8.13 (1H, m, H-3'), 8.00 (2H, m, H-5', 8'), 7.81 (1H, s, H-4), 7.74 (1H, s, H-1a), 7.59 (2H, m, H-6',7'), 4.56 (2H, t, 3J =4.9 Hz, H-7), 4.44 (2H, t, 3J=4.9 Hz, H-6), 3.81 (2H, s, H-9), 2.48 (3H, s, H-2a)	152.1 (C-2), 138.8 (C-5), 137.9 (C-4a'), 134.6 (C-2'), 133.5 (C-8a'), 132.0 (C-4), 129.7 (C 4'), 129.5 (C-5'), 129.2 (C-8'), 128.2 (C-6'), 128.0 (C-7'), 127.6 (C-1'), 122.6 (C-3'), 63.5 (C-7), 44.9 (C-9), 44.1 (C-6), 14.3 (C-2a)

*($CHCl_3$: MeOH::9:1) on pre coated silica gel plates $60F_{254}$ visualized under UV light at 254 nm

4.2. Scheme II:

4.2.1. General procedure for the synthesis of *N*-(*p*-toluene sulfonyl)amino acids (Va-c)



(I) **(Va-c)**

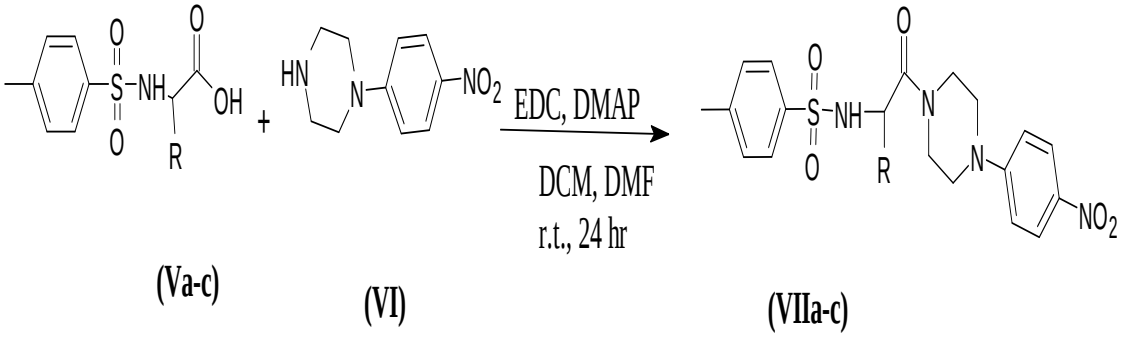
To an aqueous combination of an amino acid (10 mmol) and Na_2CO_3 (12 mmol) in water (50 mL), *p*-toluenesulfonyl chloride (12 mmol) was added slowly over the course of 15 minutes. Following complete addition of reagents, the reaction mixture in the flask was allowed to stir for the time period of 4-6 hours at ambient temperature before being acidified with dilute HCl. The precipitates obtained were filtered, purified, dried, and then recrystallized from ethyl acetate-n-hexane to get the desired product.[25]

Table 1.3: Physical data of amino acids based sulfonamides (Va-c)

Compd.	R_f*	Colors	Melting Points (ºC)	Yield (%)
Va	0.43	White	146-148 (Lit. 146-147)	91
Vb	0.40	White	135-137 (Lit.138-139)	85
Vc	0.59	White	164-169 (Lit.164-165)	88

*($CHCl_3$: MeOH::9:1) on pre coated silica gel plates $60F_{254}$ visualized under UV light at 254 nm

4.2.2. General procedure for the synthesis of Carboxamides (VIIa-c)

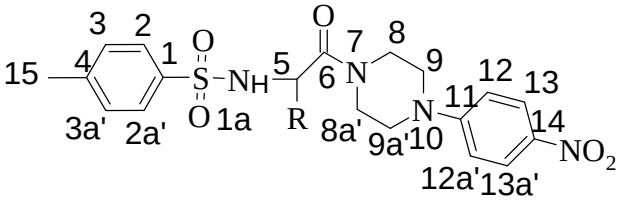


(Va-c) **(VI)** **(VIIa-c)**

1 equivalent of *N*-(*p*-Toluenesulfonyl)-amino acid was dissolved in DCM and DMF (10:1), then 0.2 equivalent of DMAP, 1 equivalent of an amine and 1 equivalent of EDC were added in the flask containing reaction mixture and allowed to stir in an inert atmosphere for 24 hours at the room temperature. After the completion of reaction, the urea and DMAP were removed by solvent extraction with ethyl acetate under acidic conditions. Carboxamides were further purified by flash column chromatography employing silica gel as stationary phase and *n*-hexane:ethyl acetate as mobile phase.

Physical and spectral data of synthesized Carboxamides (**VIIa-c**) is given in Table (1.4).

Table 1.4: Physical and spectral data of Labelled Carboxamides (VIIa-c)



Compd.	R	R_f*	Colors	Melting Points (ºC)	Yield (%)	^{1}H NMR (300 MHz, DMSO-d6): δ (ppm)	^{13}C NMR (75 MHz, DMSO-d6)
VIIa	H	0.33	Yellow	216-218	69	8.06 (2H, d, H-13 & 13a'), 7.68 (3H, m, H 2,2a' & 1a), 7.36 (2H, d, H-3 & 3a'), 6.99 (2H, d, H-12 & 12a'), 3.75 (2H, d, 3J =6 Hz, H-5), 3.51 (8H, m, H-8,8a'& 9,9a'), 2.36 (3H, s, H-15)	166.4 (C-6), 154.7 (C-11), 143.1 (C-4), 137.8 (C-14), 137.4 (C-1), 129.9 (C-3 & 3a'), 127.1 (C-2 & 2a'), 126.2 (C-13 & 13a'), 112.9 (C-12& 12a'), 46.2 & 46.0 (C-9 & 9a'), 44.4 & 43.7 (C-8 & 8a'), 41.4 (C-5), 21.4 (C-15)
VIIb	CH_3	0.39	Yellow	254-257	62	8.06 (2H, m, H-13 & 13a'), 7.66 (2H, m, H-2 & 2a'), 7.40 (2H, m, H-3 & 3a'), 7.01 (2H, m, H-12 & 12a'), 4.11 (1H, q, 3J=6 Hz, 55 H-5), 4.00 (1H, s, H-1a), 3.88 (2H, t, 3J=3 Hz, H-8 & 8a'), 3.65 (2H, t, H-8 & 8a'), 3.21 (4H, t, 3J=3 Hz, H-9 & 9a'), 2.43 (3H, s, H-15), 1.29 (3H, d, 3J=3 Hz, H-5a)	169.8 (C-6), 155.3 (C-11), 140.5 (C-4), 137.1 (C-1), 136.9 (C-14), 130.1 (C-2 & 2a'), 128.5 (C-3 & 3a'), 125.8 (C-13 & 13a'), 112.9 (C-12& 12a'), 53.3 (C-5), 47.9 (C-9 & 9a'), 46.8 (C-8 & 8a'), 21.5 (C-15), 18.7 (C-5a)
VIIc	C_7H_7	0.41	Yellow	268-273	63	8.04 (2H, m, H-13 & 13a'), 7.66 (2H, m, H-2 & 2a'), 7.39 (4H, m, H-3 & 3a'), 7.18 (5H, m, H-7b, 7b', 8b, 8b' & 9b), 7.01 (2H, m, H-12 & 12a'), 6.46 (1H, s, H-1a), 4.44 (1H, t, 3J=6 Hz, H-5), 3.69 (2H, t, H-8, 8a'), 3.39 (2H, t, H-8, 8a'), 3.21 (4H, m, H-9, 9a'), 2.91 (2H, dd, 3J=4.5 Hz, H-5a), 2.13 (3H, s, H-15)	171.0 (C-6), 155.1 (C-11), 141.6 (C-4), 139.1 (C-1), 137.3 (C-14), 137.3 (C-6b), 129.1 (C-7b & 7b'), 128.9 (C-8b & 8b'), 128.5 (C-3 & 3a'), 127.0 (C-9b), 126.6 (C-2 & 2a'), 125.9 (C-13 & 13a'), 112.5 (C-12 & 12a'), 61.3 (C-5), 47.9 (C-9 & 9a'), 46.3 (C-8 & 8a'), 38.3 (C-5a), 21.1 (C 15)

*(n-hex:EtOAc::1:1) on pre coated silica gel plates $60F_{254}$ visualized under UV light at 254nm

5. CONCLUSIONS

Sulfonamides and metronidazole scaffold has been investigated for many important biological properties. Utility of these important cores instigated the present study. Amino acids-based sulfonamides were used as core substrates. Different substituted sulfonamides were synthesized in aqueous medium and purified through recrystallization and obtained in good yields. Synthesized sulfonamides were functionalized in different ways by developing ester linkage with pendent hydroxyl group of metronidazole and amide linkage using substituted piperazine. Esters and carboxamides were synthesized using acyl transfer reagents EDC and DMAP. These acyl transfer reagents play a significant role in the activation of carboxylic acids. Carboxamides were purified through column chromatography. The synthesized compounds with different R-groups were obtained in moderate yield and then characterized by ^{1}H NMR, ^{13}C NMR spectroscopy and their physical parameters were also determined.

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