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Synthesis, Characterization and *In vitro* Antimicrobial Activity of Aspartoyl Derivatives

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Authors' contributions

This work was carried out in collaboration between all authors. Author KP designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors KJ and WKE managed the analyses of the study. Author KP managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: This research focused on synthesizing antifungal N-tosyl-L-aspartoyl derivatives with the aim of relating the structure with the expected biological activities. Elucidation of compounds that had good yiel and were active was done.
Study Design: The antimicrobial activities of aspartic acid derivatives was determined against five fungal isolates using the *in vitro* model.
Place and Duration of Study: The experiments were carried out in Makerere University, Department of Chemistry and Department of Botany, between 2012 and 2014.
Methodology: Synthesis N-tosyl-L-aspartic acid was prepared by reacting p-tosyl chloride with L-aspartic acid in sodium hydroxide-ether mixture. The anhydride was prepared by refluxing N-Tosyl-L-aspartic acid with acetic anhydride. N-tosyl-L-aspartoylamino acids were obtained using 1:1 mole of the tosyl derivatives with amino acids: glycine, L- alanine, L-Leucine, L-valine and L-tyrosine. N-tosyl-L-aspartoylamino acid methyl esters were prepared by the action of thionyl chloride in methanol on N-tosyl-L-aspartoylamino acid derivatives. N-tosyl-L-aspartoylaniline derivatives and

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N-tosyl-L-aspartoyl-p-amino benzoic acid were achieved by refluxing the amines and p-amino benzoic acid in glacial acetic acid with the anhydride. The acid chloride was synthesized by refluxing N-tosyl-L-aspartoyl-p-amino benzoic acid in thionyl chloride. Stirring the acid chloride with appropriate amino acid in triethylamine-benzene mixture yielded the N-tosyl-L-aspartoyl-p-aminobenzoylamino acids. Esterifying these derivatives with Methanol in thionyl chloride afforded the methyl esters. The acid azide was prepared by stirring sodium azide in dry benzene with N-tosyl-L-aspartoyl-p-aminobenzoyl chloride. N-tosyl-L-aspartoylamino phenyl ureas were obtained by Curtius rearrangement by coupling of acid azides with appropriate amino acids in dry benzene. The structures of the products were elucidated using micro- and IR-spectral analyses. Confirmation of the structures was done by ^{1}H NMR (Nuclear Magnet Resonance) spectroscopy at 60 MHz with TMS (Tetramethylsaline) as internal standard and elemental analysis.

Results: Thirty three compounds were tested for their biological activity against five fungal isolates, *Candida albicans, Fusarium solani, Fusarium moniliforme, Penicillium expansum* and *Cladosporium cladosporioides*. Eighteen compounds showed activity on either *Candida albicans* or *Fusarium*, while all the derivatives showed no antimicrobial activity on the three fungal isolates *Fusarium moniliforme*, *Penicillium expansum* and *Cladosporium cladoporioides*. All the synthesised compounds were tested against selected micro-organisms. These included: *Candida albicans, Fusarium solani, Fusarium moniliforme, Penicillin expansum, Cladosporium cladosporioides*. The antimicrobial properties of derivatives were assayed *in vitro* by agar disc diffusion method. The fungal isolates were locally isolated from rice porridge, milled Pakistan rice and from millet powder while the culture media was prepared using Potato Dextrose Agar (PDA).

Conclusion: The study showed that changing the chemical structures of the synthesized compounds (1-33) resulted in change of biological activity, when the structure of the compound was altered. This proves that they could be of practical pharmaceutical application.

Keywords: Antimicrobial activity; N-tosyl-L-aspartoyl; L-aspartic acid; in vitro antifungal activity.

1. INTRODUCTION

Considering the previous study [1] in which some biologically active derivatives from L-glutamic acid were reported, further studies were done on other corresponding derivatives from L-aspartic acid. L-aspartic acid derivatives have shown to exhibit antimicrobial activity just like those from L-glutamic acid. Aspartic acid is present in proteins and biologically active polypeptides as aspartyl or asparaginyl residue. Glucagon, the angiotensins and eledoisin contain one or several residues of aspartic acid. Oxytocin, Vasopressin, insulin and tryrocidin A and B contain asparaginyl residues. In bacitracin an aspartyl residue is linked through its α- and ß- carbonyl groups. The molecule contains an additional residue of D-asparagine. Asparagine has been used for the synthesis of many naturally occurring polypeptides, since it offers certain advantages over preparative procedures required for peptides that contain the aspartyl residue [2]. N-Protected aspartic anhydrides and N-protected derivatives of aspartic acid that are selectively substituted at the α- and ß- carboxyl group are used as starting materials for the synthesis of aspartoyl peptides. Bergman and Zervas [3] prepared carbobenzoxy-aspartic anhydride by heating carbobenzoxy-L-aspartic acid with acetic anhydride. Cleavage of the anhydride with amino acid esters lead to the formation of aspartoyl peptides. Some N^2-tetrabromophthaloyl-L-aspartoylamino acids have been synthesized by condensation of N^2-tetrabromophthaloyl-L-aspartic anhydride with appropriate amino acid in glacial acetic acid. Esterification of amino acid derivatives in methanol/thionyl chloride medium gave the methyl esters. Fourteen of the synthesized compounds were found to have antimicrobial activity against a number of microorganisms [4]. The purpose of this study was to synthesize aspartoyl derivatives and elucidate the structures with the aim of determining their antimicrobial activity towards different fungal isolates.

2. MATERIALS AND METHODS

2.1 Materials and Apparatus

Chemicals and solvents used were of analytical grade and did not need any more purification. The melting points (℃) of the all compounds were determined by the open tube capillary method and recorded uncorrected. The purity of the compounds was determined using thin layer chromatography (TLC). The infrared (IR) spectra were determined using infrared spectrophotometer, while elemental analysis

using elemental analyzers by combustion in a stream of oxygen. The gaseous products were converted to nitrogen which was detected using thermal conductivity detectors. The ^1H Nuclear Magnetic Resonance (NMR) spectra of the derivatives were determined in $CDCl_3$ with TMS as standard using a 90 MHz instrument. All the derivatives of N-tosyl-L-aspartic acid (3-33) were synthesized from N-tosyl-L-aspartic anhydride (2) as starting material, shown in the general reaction (scheme 1).

2.2 Synthesis of N-tosyl-L-aspartic Acid (1)

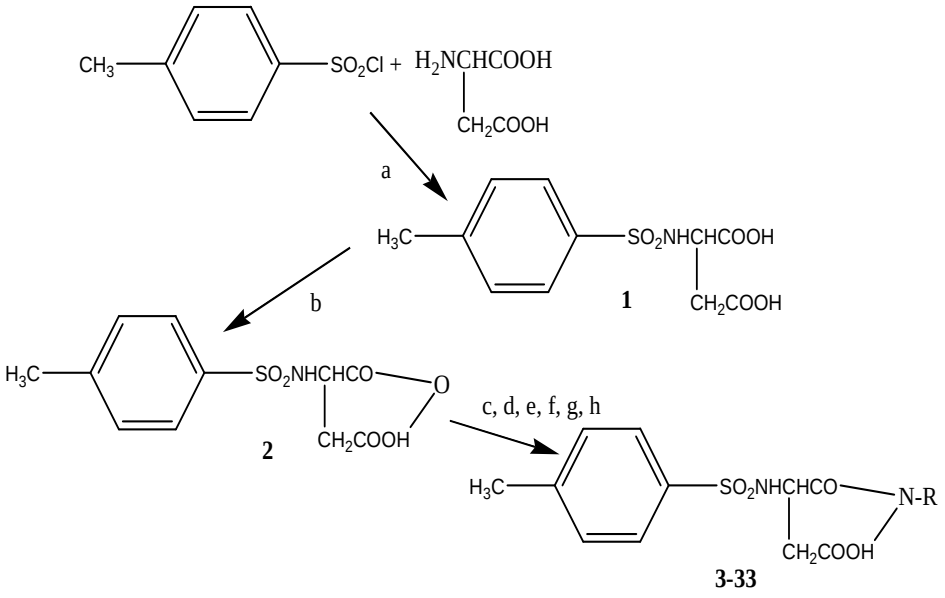
The p-tosyl chloride (0.05 mol, 9.53 g) in ether (15 ml) was added drop wise during 45 min. to a mixture of L-aspartic acid (0.05mol, 6.65 g) in sodium hydroxide (70 ml) at room temperature while stirring. The reaction mixture was maintained at room temperature until complete addition then it was further stirred for 4 hours. The resulting solution was acidified with 1M HCl using Congo red indicator (pH 5) and concentrated by evaporation. The precipitate was filtered, washed with cold water and purified by recrystallizing from acetic acid-water mixture. The resulting compound was used as a starting material that gave physical properties that were in agreement with those reported earlier [5-7]. The melting points of the compounds were determined using the capillary tube method.

2.3 Synthesis of N-tosyl-L-aspartic Anhydride (2)

The above compound was prepared from N-tosyl-L-aspartic acid (1, 0.03 mol.) which was added to acetic anhydride (10 ml), refluxed for one hour. The product precipitated, was washed with petroleum ether 40°-60℃ and then dried. The anhydride was recrystallized from benzene-petroleum ether 40℃-60℃ followed by determination of the melting point of the compound and percentage yield. This compound was used as a starting material for the synthesis of all the derivatives of N-tosyl-L-aspartic acid, (Table 2).

2.4 Procedure for the General Synthesis of N-tosyl-L-aspartoylamino Acids

N-tosyl-L-aspartic anhydride (2) (0.01 mol, 2.54 g) was added to the appropriate amino acid (0.011 mol.) in glacial acetic acid (20 ml) and refluxed for 6-8 hours. The solid products (3-7) were filtered and purified by recrystallization from acetic acid-water mixture, followed by determination of melting points and percentage yields.



Scheme 1. General reaction scheme for the synthesis of N-tosyl-L-aspartic acid derivatives

a = $NaOH_{(aq)}$ /Ether, b = Acetic anhydride, c = Glacial acetic acid, d = $SOCl_2$, e = Triethylamine /benzene; f = CH_3OH / $SOCl_2$, g = NaN_3 / dry benzene, h = Dry benzene

2.5 Procedure for the General Synthesis of N-tosyl-L-aspartoylamino Acid Methyl Esters (8-12)

Each of the N-tosyl-L-aspartoylamino acids (3-7, 0.01 mol.) was dissolved in absolute methanol (20 ml), cooled to -10℃ and pure thionyl chloride (7.9 ml, 0.11 mol.) added drop wise over one hour. The temperature of the reaction mixture was kept at -5℃ during the addition and stirring was continued for an additional 3 hours at room temperature. The reaction mixture was left for 24 hours at room temperature and the solvent evaporated in *vacuo*. Other portions of absolute methanol were added together and evaporated several times. The residual materials were recrystallized from ethanol, followed by determination of the melting points and percentage yields.

2.6 Procedure for the General Synthesis of N-tosyl-L-aspartoyl-substituted Anilines and N-tosyl-L-aspartoyl-p-Amino Benzoic Acid Derivatives

The anhydrides (2, 0.05 mol.) in glacial acetic acid (20 ml) was added to aniline derivatives (0.05 mol.) or amino benzoic acid (0.05 mol.) and refluxed for 6-8 hours. The crude products (13-16) were recrystallized from dioxane, and then the melting points and percentage yields of the compounds were determined.

2.7 Synthesis of N-tosyl-L-aspatoyl-p-aminobenzoyl Chloride (17)

N-tosyl-L-aspartoyl-p-aminobenzoic acid derivative (16, 0.01 mol.) with thionyl chloride (5 ml) were refluxed for two hours. The resulting mixture was cooled, filtered and the solid product recrystallized form acetic acid, after which the melting point and percentage yield were determined.

2.8 Procedure for the General Synthesis of N-tosyl-L-aspartoyl-p-amino Benzoylamino Acids

The acid chloride (17, 0.002 mol.) was stirred with appropriate amino acids (0.002 mol.) in triethylamine /benzene mixture for 3 hours. The solid products (18-22) were washed with petroleum-ether 40℃-60℃, recrystallized from acetic-water mixture, after which percentage yields and melting points were determined.

2.9 Procedure for the General Synthesis of N-tosyl-L-aspartoyl-p-aminobenzoylamino Acid Methyl Esters (23-27)

Each of the N-tosyl-L-aspartoyl-p-aminobenzoylamino acid derivatives (18-22, 0.01 mol.) was dissolved in absolute methanol, cooled to −10℃ and pure thionyl chloride (7.9 ml, 0.11 mol.) added drop wise over one hour. The temperature of the reaction mixture was kept at −5℃ during the addition and stirring was continued for 3 hours. The resulting mixture was left overnight at room temperature and the solvent evaporated in *vacuo*. Absolute methanol was added and evaporated several times. The precipitates were recrystallized from ethanol-water mixture, followed by determination of melting points and percentage yields.

2.10 Synthesis of N-tosy-L-aspartoyl-p-amino Benzoic Acid Azide (28)

N-tosyl-L-aspartoyl-p-aminobenzoyl chloride (17, 0.01 mol.) was refluxed with sodium azide (0.01 mol, 0.65 g) in dry benzene with stirring for three hours. The reaction mixture was cooled, the product filtered and recrystallized from dioxane, followed by determination of melting point and percentage yield.

2.11 Procedure for the General Synthesis of N-tosyl-L-aspartoyl-p-aminophenyl Ureas

The acid azide (28, 0.01 mol.) was refluxed for one hour to undergo Curtius rearrangement to form the isocynate intermediate after which appropriate amino acids were added and refluxed for additional 3 hours. The resulting solid compounds (29-33) were filtered and recrystallized from ethanol/water (29), dioxane (30) and acetic/water (31-33), followed by determination of melting points and percentage yields. All the physical data of the synthesised compounds are represented in (Table 2).

2.12 Biological Screening

The antimicrobial properties of derivatives (1-33) were assayed *in vitro* by agar disc diffusion method [8-12]. All the synthesised derivatives were tested against five selected micro-organisms. These included: *Candida albicans, Fusarium solani, Fusarium moniliforme, Penicillin expansum, Cladosporium cladosporioides*. The

fungal isolates were locally isolated from rice porridge, milled Pakistan rice and from millet powder. The culture media was prepared using Potato Dextrose Agar (PDA) that was prepared using manufactures" instructions for purposes of culturing fungi. Spread plate method was used to culture 100 µl of the microbial suspension that was introduced into the Petri dishes. Filter paper discs (5 mm) were impregnated with the compounds to test for antifungal activity. Sterile discs (5 mm diameter) were soaked in the compounds (made by dissolving 300 mg of the compounds in 1000 µl of methanol) air dried and placed on the spread plates at reasonable distances. Discs impregnated with methanol and air dried were used as negative controls and Fluconazole (Pfizer Ltd., UK batch 30) as positive controls. The plates were then incubated at 35℃ for 24 h. This was replicated three times for each pathogen. The fungi was cultured by taking 100 µl from the broth and spreading on PDA. The culture was incubated at 25℃ for 72 h. The cork borer was used to pick a section of the young mycelium which was placed at the centre of the PDA plate and the dry discs which were impregnated with 100 µl of the plant extracts placed at a distance around the inoculum mycelium. The inoculum was incubated at 25℃ (+2℃) for five days. Fluconazole and dry discs treated with methanol were also used as positive and negative controls respectively. All tests were performed in triplicate and the microbial growth inhibition was determined by measuring the average diameter of the inhibition zone (ADIZ) using a transparent ruler. The plates were then incubated at 25℃ (+2℃) for five days and the average diameter of the inhibition zone (ADIZ) was determined from the centre of filter paper disc for the biologically active compounds. The appropriate solvents for these compounds were also assessed for their biological activity against the fungi. All the solvents used: ethanol, acetic acid, dioxane and water showed no activity against all the fungal isolates tested.

3. RESULTS AND DISCUSSION

3.1 Spectroscopic Analysis

Several simple asparagine and aspartic acid derivatives have been reported to possess some antitumor activity [13] while N, N-dibenzyl asparagine and N^2-phthaloylaspartylamine derivatives have shown significant anticancer activity [14]. Some N^2-tetrachlorophthaloyl-DL-aspartoyl dipeptide methyl esters have been prepared using the carbodiimide (DCC) method. All N^2-tetrachlorophthaloyl-DL-aspartoyl dipeptide methyl esters prepared using this method have been reported to possess high antimicrobial properties against a number of microorganisms and fungi with minimal inhibitory concentration values ranging from 15 to 50 mg / ml [15].

Elemental analysis, IR spectra, spot tests and chromatographic studies of the starting material (1) and products were in agreement with that reported earlier [16]. Removal of p-tosyl group of derivative (1) using $Na/Liq.NH_3$ gave a ninhydrin positive spot confirming the structure.

The IR spectrum of the synthesised derivatives (3-7) showed bands at (ν_{max} in cm^{-1}) 3400-2400 (O-H), 2130 (over tone aromatic), 1326, 1310 (S=O), 1700 (COOH), 1252 (C-O), 600, 866, 787 (=C-H, aromatic) and other bands in agreement with the proposed structures (Fig. 1). The IR spectrum of the synthesised compounds (8-12) showed bands at (ν_{max} in cm^{-1}); 3466 (N-H), 3010 (C-H; aromatic), 2910 (C-H, aliphatic), 1666 (C=O ester), 1300 (S= O), which were in accordance with the structures (8-12). The IR Spectrum of the above compounds (13-15) showed bands at (ν_{max} in cm^{-1}): 3492 (N-H), 2136 (over tone, aromatic), 1640 (C=O), 1530, 1333 (N=O), 1066 (C-Cl) and other bands characteristic of the structures (13-15). Derivatives (23-27) showed bands at ((ν_{max} in cm^{-1}); 3466 (N-H), 3066 (C-H, aromatic), 2938 (C-H, aliphatic), 1440 (S=O), 1653 (C=O), confirming the presence of the functional groups of the compounds (23-27).

The IR spectrum of the derivatives (29-33) showed bands at ((ν_{max} in cm^{-1}): 3492 (N-H), 3034 (C-H, aromatic) 1666 (C=O), 1333, 1306 (S=O) and other bands in agreement with the structures (29-33).

All the remaining compounds (1, 2, 28 and 16-22) gave IR spectra consistent with their assigned structures, which supported their chemical structures.

Considering the elemental analysis for nitrogen, the values calculated and those found were in close agreement. This confirmed the structures of the synthesised derivatives of the acid (Table 2).

Compound 5 gave a ^1H NMR spectrum which had: singlet at 10.2 δ due to -OH and -NH, peaks in the range (7.0 - 8.0) δ are due to the 4 protons on the benzene ring for the carbon atoms 19, 20, 22, 23; each is split into a doublet. Singlet at 2.2 δ is for

the protons on carbon atom 25 and the doublet at 3.75 δ is that for the protons on carbon atom 4. The triplet at 3.7 δ is for the proton on carbon atom 3, while the doublet at 4.75 δ is due to the proton on carbon atom 6. The multiplet at 2.5 δ is for the proton on carbon atom 7 and doublet at 1.0 δ is due to the protons on carbon atom 8 and 16. The ^{1}H NMR Spectra were in agreement with the structure of compound 5 (Fig. 2).

Derivative number 17 had a singlet at 2.2 δ were for the protons on carbon atom 27 and the peaks in the range (3.9- 4.7) δ were due to the protons on carbon atoms 3 and 4. Between 7.2 δ - 7.9 δ the peaks are due to the protons on carbon atoms 21, 22, 24 and 25 of the benzene ring each of which was split into a doublet. The peaks between 7.98δ - 8.5 δ are due to the protons on carbon atoms 7, 8, 11 and 10, where each is split into a doublet. This is in agreement with the structure proposed for compound 17.

3.2 Antifungal Activity

Derivatives which had a diameter of greater than zero inhibition zones against one or more of the micro-organisms were considered to be active. The antifungal properties of N-tosyl-L-aspartic acid derivatives (1-33) were examined *in vitro* against fungal micro-organisms. The antifungal activity of the synthesized compounds (1–33) at 0.2 g/l concentration, against the micro-organism is shown in (Table 1). The results shown in (Table 1), suggest that of the 33 compounds tested against the five fungal isolates: *Candida albicans, Fusarium solani, Fusarium moniliforme, Penicillium expansum* and *Cladosporium cladosoporioides*, fifteen were biologically inactive while eighteen were able to inhibit two of the tested micro-organisms (Table 1).

The derivatives (2, 3, 12, 17, 20, 22, 23 and 26-33) which were omitted from (Table 1) were found to be inactive against *Candida albicans* and *Fusarium Solani*. All the structures of compounds (1-33) had no activity on the three tested micro-organisms: *Fusarium moniliforme, Penicillium expansum* and *cladosporium cladosporioides.* N-tosyl aspartoyl-L-alanine (4) with inhibition diameter of 6 mm and N-tosyl aspartoyl-L- valine (5) of diameter 8 mm were found to be slightly active against *Candida albicans*. Derivative (5) possessed activity on *Fusarium solani* with inhibition zone of diameter 7 mm.

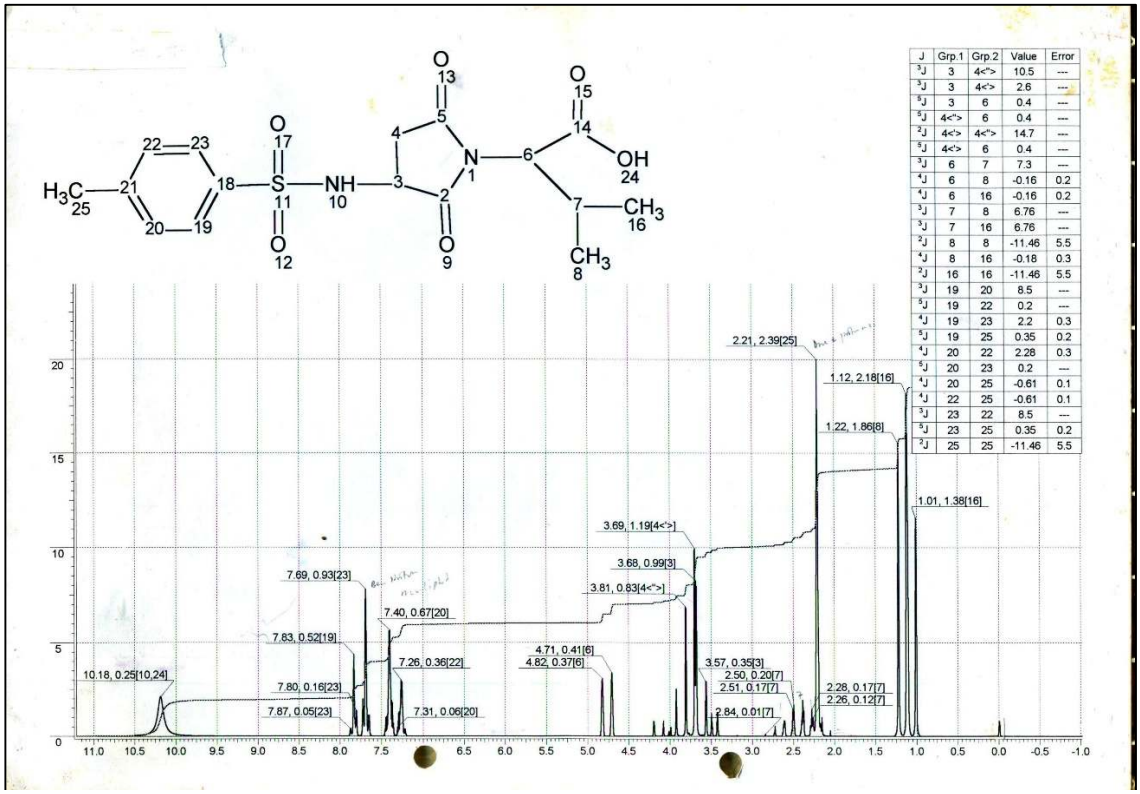


Fig. 1. ^{1}H NMR spectrum of compound 5

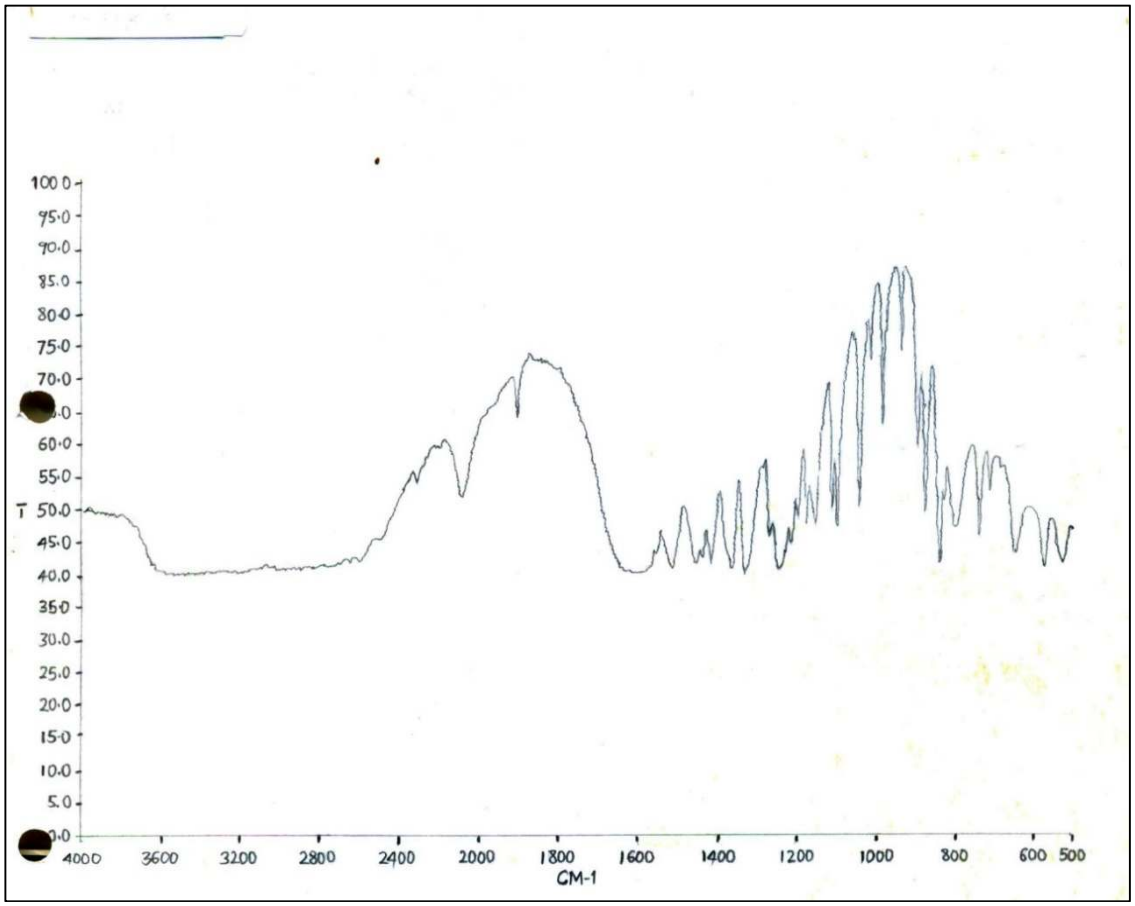


Fig. 2. IR spectrum of compound of 5

Compound 6 (N-tosyl aspartoyl-L-Lencine) with diameter of 7 mm had slight activity on *Fusarium Solani*, but showed no effect on *Candida albicans*. N-tosyl-L-aspartoyl-L-tyrosine (compound 7) with inhibition zone of diameter 6 mm was found to be slightly active on *Candida albicans*, but had no effect on *Fusarium solani.* The N-tosyl-L-aspartoyl-(glycine, L-alanine, L-valine,L-Lencine) methyl esters (8-12) with diameters of 7 mm, 9 mm, 9 mm, 7 mm respectively showed increase in activity, possessed antifungal properties against *Candida albicans* but had no effect on *Fusarium Solani*. N-tosyl-L-aspartoyl-(4-chloro, 2-chloro-4-nitro, and 4-chloro-2-nitro.) amino phenyl derivatives (13-15) with diameters of 6 mm, 7 mm and 8 mm as inhibition zones, affected the growth of only *Candida albicans* while they were found to be inactive against *Fusarium solani.*

N-tosyl-L-aspartoyl-p-aminobenzoic acid (16) with diameter of 7 mm as inhibition zone was found to have activity on only *Candida albicans* as compared to *Fusarium solani* where the compound showed no activity. The N-tosyl-L-spartoylamino benzoylglycine (18) of diameters 6 mm, and 18 mm was found to affect growth on both *Candida albicans* and *Fasarium solani* respectively. This showed an increase in activity, while N-tosyl-L-aspartoylamino benzoyl-(-L-alanine, -L-Lencine) compounds (19 and 21) with diameters 9 mm and 8 mm, had activity on *Candida albicans*, but were found to be only slightly active on *Fusarium solani*, thus showing a decrease in activity as compared to derivative (18). The compounds related to N-tosyl-L-aspartoyl-p-aminobenzoyl-(-L-alanine, L-valine) methyl esters (24 and 25) with inhibition zones of diameter 10 mm and 11 mm respectively displayed antifungal properties on *Candida albicans* and *Fusarium solani*, ranging from slight activity to moderate activity showing an increase in activity as compared to derivatives (19 and 21).

Table 1. Antifungal activities of the N-tosyl-L-aspartic acid derivatives (1-33)

Name of compound	Inhibition zones (mm)				
	Candida albicans	*F. solani*	*F. moniliforme*	*P. expansum*	*C. cladosoporioides*
1. N-tosyl-L-aspartic acid	0	0	0	0	0
2. N-tosyl-L-aspartic anhydride	0	0	0	0	0
3. N-tosyl-L-aspartoyl glycine	0	0	0	0	0
4. N-tosyl-L-aspartoyl-L-alanine	6	0	0	0	0
5. N-tosyl-L-aspartoyl-L-valine	8	7	0	0	0
6. N-tosyl-L-aspartoyl-L-leucine	0	7	0	0	0
7. N-tosyl-L-aspartoyl-L-tyrosine	6	0	0	0	0
8. N-tosyl-L-aspartoyl glycine methyl ester	7	0	0	0	0
9. N-tosyl-L-aspartoyl-L-alanine methyl ester	9	0	0	0	0
10. N-tosyl-L-aspartoyl-L-valine methyl ester	9	0	0	0	0
11. N-tosyl-L-aspartoyl-L-leucine methyl ester	7	0	0	0	0
12. N-tosyl-L-aspartoyl-L-tyrosine methyl ester	0	0	0	0	0
13. N-tosyl-L-aspartoyl-4-chloro aniline	6	0	0	0	0
14. N-tosyl-L-aspartoyl-2-chloro-4-aniline	7	0	0	0	0
15. N-tosyl-L-aspartoyl-4-chloro-2-aniline	8	0	0	0	0
16. N-tosyl-L-aspartoyl-p-amino benzoic acid	7	0	0	0	0
17. N-tosyl-L-aspatoyl-p-amino benzoyl chloride	0	0	0	0	0
18. N-tosyl-L-aspartoyl-p-amino benzoyl glycine	6	8	0	0	0
19. N-tosyl-L-aspartoyl-p-amino benzoyl-L-alanine	0	9	0	0	0
20. N-tosyl-L-aspartoyl-p-amino benzoyl-L-valine	0	0	0	0	0
21. N-tosyl-L-aspartoyl-p-amino benzoyl-L-leucine	0	8	0	0	0
22. N-tosyl-L-aspartoyl-p-amino benzoyl-L-tyrosine	0	0	0	0	0
23. N-tosyl-L-aspartoyl-p-amino benzoyl glycine methyl ester	0	0	0	0	0
24. N-tosyl-L-aspartoyl-p-amino benzoyl-L-alanine methyl ester	7	7	0	0	0
25. N-tosyl-L-aspartoyl-p-amino benzoyl-L-valine methyl ester	10	11	0	0	0
26. N-tosyl-L-aspartoyl-p-amino benzoyl-L-leucine methyl ester	0	0	0	0	0
27. N-tosyl-L-aspartoyl-p-amino benzoyl-L-tyrosine methyl ester	0	0	0	0	0
28. N-tosyl-L-aspartoyl-p-amino benzoic acid azide	0	0	0	0	0
29. N-tosyl-L-aspartoyl-p-amino phenyl glycine urea	0	0	0	0	0
30. N-tosyl-L-aspartoyl-p-amino phenyl-L-alanine urea	0	0	0	0	0
31. N-tosyl-L-aspartoyl-p-amino phenyl-L-valine urea	0	0	0	0	0
32. N-tosyl-L-aspartoyl-p-amino phenyl-L-leucine urea	0	0	0	0	0
33. N-tosyl-L-aspartoyl-p-amino phenyl-L-tryrosine urea	0	0	0	0	0

Table 2. Physical data of N-tosyl-L-aspartic acid derivatives (2-33)

Compound number	R	Yield %	M.P. (°C)	Molecular formula	Elemental analysis of nitrogen	
					Calc. (%)	Found (%)
2	-O-	48.00	230-232	$C_{11}H_{11}NO_5S$	4.95	4.88
3	Gly-	81.60	208-210	$C_{13}H_{14}N_2O_6S$	8.59	8.56
4	L-Ala-	63.30	278-280	$C_{14}H_{11}N_2O_6S$	8.24	8.28
5	L-Val-	59.20	280-282	$C_{16}H_{20}N_2O_6S$	7.61	7.60
6	L-Leu-	88.70	270-272	$C_{17}H_{22}N_2O_6S$	7.33	7.29
7	L-Tyr-	84.80	288-290	$C_{20}H_{20}N_2O_7S$	6.48	6.50
8	-Gly-OMe	91.30	231-233	$C_{14}H_{16}N_2O_6S$	8.24	8.20
9	-L-Ala-OMe	72.00	223-225	$C_{15}H_{28}N_2O_6S$	7.91	7.86
10	-L-Val-OMe	82.40	219-221	$C_{17}H_{22}N_2O_6S$	7.33	7.35
11	-L-Leu-OMe	79.00	235-237	$C_{18}H_{24}N_2O_6S$	7.07	7.00
12	-L-Tyr-OMe	60.50	217-219	$C_{21}H_{22}N_2O_6S$	6.51	6.56
13	4- Cl-C_6H_4-NH_2-	89.30	185-187	$C_{17}H_{15}O_4SCl$	7.40	7.36
14	2-Cl-4-NO_2-C_6H_3-NH_2-	55.00	203-205	$C_{17}H_{14}N_3O_6SCl$	9.92	9.89
15	4-Cl-2-NO_2-C_6H_3-NH_2-	83.50	198-200	$C_{17}H_{14}N_3O_6SCl$	9.92	9.96
16	4-C_6H_4-COOH-NH_2-	62.80	238-240	$C_{18}H_{16}N_2O_6S$	7.22	7.20
17	4-C_6H_4-COCl-NH_2-	45.60	215-217	$C_{18}H_{15}N_2O_5SCl$	6.89	6.90
18	4-C_6H_4-CO-Gly-NH_2-	56.30	222-224	$C_{20}H_{19}N_3O_7S$	9.44	9.36
19	4-C_6H_4CO-L-Ala-NH_2-	78.60	230-232	$C_{21}H_{21}N_3O_7S$	9.15	9.20
20	4-C_6H_4-CO-L-Val-NH_2-	72.00	236-238	$C_{23}H_{25}N_3O_7S$	8.62	8.57
21	4-C_6H_4-CO-L-Leu-NH_2-	67.00	226-228	$C_{24}H_{27}N_3O_7S$	8.38	8.40
22	4-C_6H_4-CO-L-Try-NH_2-	53.00	229-231	$C_{27}H_{25}N_3O_8S$	7.62	7.59
23	4-C_6H_4-CO-Gly-OMe-NH_2-	70.20	218-220	$C_{21}H_{21}N_3O_7S$	9.15	9.10
24	4-C_6H_4-CO-L-Ala-OMe-NH_2-	68.70	224-226	$C_{22}H_{23}N_3O_7S$	8.88	8.70
25	4-C_6H_4-CO-L-Val-OMe-NH_2-	52.00	243-245	$C_{24}H_{27}N_3O_7S$	8.38	8.32
26	4-C_6H_4-CO-L-Leu-OMe-NH_2-	58.00	236-238	$C_{25}H_{29}N_3O_7S$	8.16	8.20
27	4-C_6H_4-CO-L-Try-OMe-NH_2-	56.00	233-235	$C_{28}H_{27}N_3O_7S$	7.65	7.51
28	4-C_6H_4-CON_3-NH_2-	44.50	215-217	$C_{18}H_{15}N_5O_5S$	16.95	16.88
29	4-C_6H_4-NHCO-Gly-NH_2-	71.50	204-206	$C_{20}H_{20}N_4O_7S$	12.17	12.09
30	4-C_6H_4-NHCO-L-Ala-NH_2-	51.20	199-201	$C_{21}H_{22}N_4O_7S$	11.81	11.65
31	4-C_6H_4-NHCO-L-Val-NH_2-	46.00	221-223	$C_{23}H_{26}N_4O_7S$	11.16	11.11
32	4-C_6H_4-NHCO-L-Leu-NH_2-	42.00	211-213	$C_{24}H_{28}N_4O_7S$	10.86	10.90
33	4-C_6H_4-NHCO-L-Try-NH_2-	59.10	203-205	$C_{27}H_{2\backslash 6}N_4O_7S$	9.89	9.80

Considering the corresponding compounds from earlier studies of L- glutamic acid [1], the N-tosyl-L-aspartic acid with inhibition diameter of 6 mm lower activity than the N-tosyl-L-glutamic acid with diameter of 8 mm both were on *Candida albicans*. For both N-tosyl-L-aspartic and N-tosyl-L-glutamic anhydride there was no activity on all the tested fungal isolates. The of N-tosyl-L-aspartoylamino acids had four compounds that showed activity: This included compounds number (4) that had activity on only *Candida albicans* with diameter of 6 mm. Compound number (5) had activity on two fungal isolates, *Candida albicans* and *Fusarium solani* with diameters of 8 mm and 7 mm respectively. Compound number (6) was active on only *Fusarium solani* with diameter of 7 mm while compound (7) that had diameter of 6 mm was also active on only *Candida albicans*. The N-tosyl-L-glutamoylamino acids activity was shown on only one compound that was N-tosyl-L-glutamoyl-L-alanine with inhibition diameter of 6 mm on *Candida albicans.* The results show that the corresponding compounds from L-glutamic acid were active than those from L-aspartic acid.

For the N-tosyl-L-aspartoylamino acid methyl esters (8-12) activity was observed on only one fungal isolate, *Candida albicans* for compounds (8, 9, 10, 11). This showed decreased activity as compared with corresponding compounds from N-tosyl-L-glutamoylamino acid methyl esters that showed activity on two fungal isolates *Candida albicans* and *Fusarium solani.* The N-tosyl-L-aspartoyl-substituted anilines (13, 14, 15) had lower activity as compared to the N-tosyl-L-glutamoyl-substituted anilines that had activity on all the five fungal isolates. N-tosyl-L-aspartoyl-p-amino benzoic acid with diameter of 7 mm had activity on only *Candida albicans* which showed increased activity as compared to N-tosyl-L-glutamoyl-p-amino benzoic acid which no activity on any fungal isolates. For N-tosyl-L-aspatoyl-p-aminobenzoyl chloride no activity was observed on any fungal isolate tested however there was an increase in activity with the corresponding N-tosyl-L-glutamoyl-p-aminobenzoyl chloride that showed activity on two fungal isolates *Candida albicans* and *Fusarium solani*. Considering the N-tosyl-L-aspartoyl-p-amino benzoylamino acids compounds there was decreased activity because two fungal isolates showed activity as compared to N-tosyl-L-glutamoyl-p-amino benzoylamino acids were one compound (N-tosyl-L-aspartoyl-p-amino benzoyl-L-tyrosine) showed activity on all the fungal isolates tested. N-tosyl-L-aspartoyl-p-aminobenzoylamino acid methyl esters (23-27) showed increased activity on two fungal isolate as compared to N-tosyl-L-glutamoyl-p-aminobenzoylamino acid methyl esters where only one compound showed activity on one fungal isolate. No activity was observed N-tosy-L-aspartoyl-p-amino benzoic acid azide and N-tosyl-L-aspartoyl-p-aminophenyl ureas. The corresponding N-tosy-L-glutamoyl-p-amino benzoic acid also showed no activity however N-tosyl-L-glutamoyl-p-aminophenyl-L-leucine urea showed activity on only *Candida albicans*.

In the previous study of glutamic acid derivatives [1], thirteen compounds showed activity on all the above tested fungal isolates compared to aspartic acid derivatives where eighteen had activity on only two. This shows that compounds from glutamic acid have high activity than aspartic acid derivatives.

The figures represent the average diameters of the inhibition zones (ADIZ). 0 = inactive, up to 9 mm = slightly active, 10-19 mm, moderately active, 20 mm and above = highly active. Compounds (2, 3, 12, 17, 20, 22, 23, 26-33) had no effect on the tested micro-organisms. None of the 33 compounds of N-Tosyl-L-aspartic acid derivatives had effect on the three fungal isolates: *Fusarium moniliforme, Penicillium expansum* and *Cladosporium cladoporioides.*

4. CONCLUSION

This study showed that N-tosyl-L-aspartic acid derivatives can be used as a starting point for further research to carry out synthesis of other biologically active compounds that can find application in medicine and pharmacy.

Eighteen compounds showed activity on only Yeast or Fusarium, while all the derivatives showed no antimicrobial activity on the three fungal isolates *Fusarium moniliforme, Penicillium expansum and Cladosporium cladoporioides*. The antimicrobial activities shown by some of N-tosyl-L-aspartic acid derivatives were due to structural changes of various compounds which led to increase, decrease or complete loss in biological activity.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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