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In Vitro Antifungal Activity Screening of Some New Glutamoyl derivatives

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Abstract

This research was focused at synthesizing new antifungal N-tosyl-L-glutamoyl derivatives with the aim of relating the structure with the expected biological activities. Synthesis N-tosyl-L-glutamic acid was prepared by reacting p-tosyl chloride with L-glutamic acid in sodium hydroxide-ether mixture. The anhydride was prepared by refluxing N-tosyl-L-glutamic acid with acetic anhydride. N-tosyl-L-glutamoy lamino 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-glutamoylamino acid methyl esters were prepared by the action of thionyl chloride in methanol on N-tosyl-L-glutamoylamino acid derivatives. N-tosyl-L-glutamoylaniline derivatives and N-tosyl-L-glutamoyl-p-amino benzoic acid were achieved by refluxing the amines and p-aminobenzoic acid in glacial acetic acid with the anhydride. The acid chloride was synthesized by refluxing N-tosyl-L-glutamoyl-p-amino benzoic acid in thionyl chloride. Stirring the acid chloride with appropriate amino acid in triethylamine-benzene mixture yielded the N-tosyl-L-glutamoyl-p-aminobenzoylamino acids. Esterifying these derivatives with Methanol in thionylchloride afforded the methyl esters. The acid azide was prepared by stirring sodium azide in dry benzene with N-tosyl-L-glutamoyl-p-aminobenzoyl chloride. N-tosyl-L-glutamoylamino phenyl ureaswere obtained by Curtius rearrangement by coupling of acid azides with appropriate amino acids in dry benzene. The structures of synthesized derivatives were confirmed by IR spectroscopy, ^1H NMR spectroscopy at 90 MHz with TMS as internal standard and elemental analysis. The synthesized derivativeswere tested for their antifungal properties against five fungal isolates, Yeast, Fusariumsolani, Fusarium moniliforme, Penicillium expansum and cladosporiumcladosporioides. Twenty out of the 33 compounds of the N-Tosylglutamoyl derivatives had no activity on the tested micro-organisms, while 13 compounds were found to affect the growth of Yeast, Fusariumsolani, Fusarium moniliforme, Penicillium expansum and cladosporiumcladosporioides with varying degrees. The study showed that changing the structures of the synthesized compounds resulted in increased, decreased or a complete loss in biological activity, which proves that they could be of practical pharmaceutical application.

Keywords: Antifungal activity, N-tosyl-L-glutamoyl derivatives, L- glumatic acid.

Introduction

Glutamic acid is very widely distributed in animals, plants and bacteria. It is incorporated in proteins and peptides most commonly via an α-peptide bond, while the γ-carbonyl group is free (glutamyl) for example gastrin or present as amide (glutaminyl, for example oxytocin, Vasopressin, glucagon). Naturally occurring glutamylγ-peptides are known, examples are glutathione, ophthalmic acid, noropthalmic acid, hypoglutathione, and pteroylglutamic acid. Other glutamyl γ-peptides have been isolated from vegetable sources. Glutamyl α-or γ-peptides can be prepared from intramolecular cyclic derivatives of glutamic acid and from derivatives of N-protected glutamic acid and monsubstituted at the α- or γ- carbonyl group. Derivatives of N-protected glutamic acid are capable of forming intramolecular anhydrides or intramolecula rdiacyl imides. They may also cyclize with formation of γ-lactams for instance the derivatives of pyroglutamic acid (pyrrolid-5-one -2-carboxylic acid).

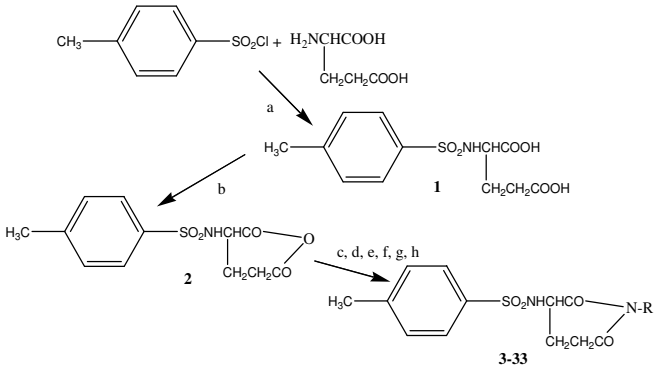
Glutamylα-or γ-peptides and derivatives selectively substituted at α-or γ-carbonyl group are directly accessible from intramolecular anhydrides and from pyroglutamic acid[1].N-Acylglutamic acid anhydrides are cleaved by aminolysis, hydrazinolysis and alcoholysis. These reactions lead to the formation of α-and γ- derivatives which cannot be separated satisfactorily in each case[2].

Bergmann and Zervas[1] first described carbobenzoxy-L-glutamic acid anhydride, which was prepared by heating a mixture of carbobenzoxy-L-glutamic acid and acetic anhydride. Le Quesne and Young[2] recommend that the reaction be carried out at room temperature to reduce danger of racemization. According to Le Quesne and Young[2], the opening of anhydride ring is governed by the type of cleaving agent and the solvent used.

Experiments by King; et al[3], have shown that the aminolysis and alcoholysis of phthalyl-L-glutamic acid anhydride lead to the formation of γ- derivatives with preservation of the optical activity. These results were confirmed by several authors. The purpose of the present study was to synthesize new glutamoyl derivatives with the aim of determining their antifungal activity towards different fungal isolates.

Material and Methods

Materials and apparatus: Chemicals and solvents used were of analytical grade and did not need any more purification. The melting points (^oC) 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). IR spectra were determined usinginfrared 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 NMR spectra of the derivatives were determined in $CDCl_3$ with TMS as standard using a 90 MHz instrument. All the derivatives ofN-tosyl-L-glutamic acid (**1-33**) were synthesized from N-tosyl-L-glutamic anhydride (**2)** as starting material, shown in the general reaction scheme-1.



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

Scheme-1

General reaction scheme for the synthesis of N-tosyl-L-glutamic acid derivatives

Synthesis of N-tosyl-L-glutamic acids (1): p-tosylchloride (0.05mol, 9.53g) in ether (15ml) was added dropwise during 45 min to a mixture of L-glutamic acid(0.05mol, 7.36g) in sodium hydroxide (70ml) 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 until acidic to Congo red indicator (pH 5) and concentrated by evaporation.The precipitate was filtered, washed with cold water and purified by recrystallizing from aceticacid- water mixture. This compound was used as a starting material. The physical properties of the compound was in agreement with those reported earlier[4,5,6].The melting points of the compounds were determined using the capillary tube method.

Synthesis of N-tosyl-L-glutamic anhydride (2): The above compound were prepared from N-tosyl-L-glutamic acid (**1**, 0.03mol) which was added to acetic anhydride (10ml), refluxed for one hour. The product precipitated, was washed with petroleum ether 40^0-60^0Cand then dried. The anhydride was recrystallized from benzene-petroleum ether 40^0-60^0C and the melting point of the compound was determined and percentage yield. This compound was used as a starting material for the synthesis of all the derivatives of N-tosyl-L-glutamic acid (table -2).

Procedure for the generalsynthesis of N-tosyl-L-glutamoyl aminoacids: N-tosyl-L-glutamic anhydride (**2**,0.01 mol, 2.68 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.

Procedure for the general synthesis of N-tosyl-L-glutamoyl amino acid methyl esters (8-12): Each of the N-tosyl-L-glutamoylamino acids (**3-7**, 0.01mol) was dissolved in absolute methanol (20ml), cooled to -10^{o}C and pure thionylchloride (7.9ml, 0.11mol) added dropwise over one hour. The temperature of the reaction mixture was kept at -5^{o}C 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 and re-evaporated several times and the residual materials were recrystallized from ethanol, followed by determination of the melting points and percentage yields.

Procedure for the general synthesis of N-tosyl-L-glutamoyl-substituted anilines and N-tosyl-L-glutamoyl-p-aminobenzoic acid derivatives: The anhydrides (**2**, 0.05 mol) in glacial acetic acid (20ml) was added to aniline derivatives (0.05mol) or amino benzoic acid (0.05mol) 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 weredetermined.

Synthesis of N-tosyl-L-glutamoyl-p-aminobenzoylchloride (17): N-tosyl-L-glutamoyl-p-aminobenzoic acid derivative (**16**,0.01mol) with thionyl chloride (5 ml) was 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.

Procedure for the general synthesis of N-tosyl-L-glutamoyl-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 40^0-60^0C, recrystallized from acetic-water mixture, after which percentage yields and melting points were determined.

Procedure for the general synthesis of N-tosyl-L-glutamoyl-p-aminobenzoylamino acid methyl esters (23-27): Each of the

N-tosyl-L-glutamoyl-p-aminobenzoylamino acid derivatives (**18-22**, 0.01mol) was dissolved in absolute methanol, cooled to -10^{o}C and pure thionyl chloride (7.9ml, 0.11mol) added dropwise over one hour. The temperature of the reaction mixture was kept at -5^{o}C 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 re-evaporated several times, the precipitates were recrystallized from ethanol-water mixture, followed by determination of melting points and percentage yields.

Synthesis of N-tosy-L-glutamoyl-p-amino benzoic acid azide (28): N-tosyl-L-glutamoyl-p-aminobenzoyl chloride (**17**, 0.01mol) was refluxed with sodium azide (0.01mol, 0.65g)in dry benzene with stirring for three hours. The reaction mixture was cooled, the product filtered and recrystallized from dioxane, followed by determination of meltingpoint and percentage yield.

Procedure for the general synthesis of N-tosyl-L-glutamoyl-p-aminophenylureas: The acid azide (**28**, 0.01mol) 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.

Biological screening: The antifungal properties of derivatives (**1-33**) were tested using the filter paper disc method[7-11]. All the synthesised derivatives were tested against few selected micro-organisms. These included: Yeast, *Fusariumsolani, Fusarium moniliforme, Penicillin expansum, Cladosporium-cladosporioides*. The fungal isolates were locally isolated from rice porridge, milled Pakistan rice and from millet powder. The filter paper disc (5 mm diameter) each loaded with 1 mg of the compounds dissolved in the appropriate solvent, were put on the surface of the agar plates seeded with the test fungus. The plates were then incubated at 25^{o}C (+2^{o}C) for five days and the average diameter of the inhibition zone (ADIZ) was determined for the biologically active compounds, figure-2. The appropriate solvents for these compounds were also assessed for their biological activity against the tested fungi. All the solvents used: ethanol, acetic acid, dioxane and water showed no activity against all the fungal isolates tested.

Results and Discussion

Several phthaloylamino acids and peptide derivatives were reported[12]. The reactions of these derivatives were intensively studied via different methods of peptide synthesis and a variety of the synthesised compounds possessed wide pharmacological applications[12]. The reactions of phthaloylamino acids or tosylaminoacids with different sulfonamides have been reported for their biological properties studied[13]. Moreover, their reactions with urea and thiourea for the aim of producing amino acid derivatives of hypoglycaemic activity or diuretic properties gave good results. Inhibitors such as sorbates, propionates, benzoates and other potential inhibitors have been studied from the point of their effects on growth of potentially toxic moulds and toxin production[14]. In addition, other substances such as antibiotic, matamycin and derivatives of essential oils, medicinal plants and spices have also been studied for their antifungal activities and effects on mycotoxin production[12]. Also a number of aromatic and hetrocyclicsulphonylamino acid and peptide derivatives[15] and some benzenesulphonyl-glycinederivatices[7,8] were reported to possess various biological activities.

Elemental analysis, IR spectra, spot tests and chromatographic studies of the starting materials (**1**) and products were in agreement with that reported earlier[4]. 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} incm^{-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. The IR spectrum of the synthesised compounds (**8-12**) showed bands at (ν_{max} incm^{-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 suggested.

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 ofthe structures proposed (figure-1).

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 proposed.

The IR spectrum of the derivatives (**29-33**) showed bands at ((ν_{max} incm^{-1}): 3492 (N-H), 3034 (C-H, aromatic) 1666 (C=O), 1333, 1306 (S=O) and other bands in agreement with the structures suggested. All the remaining compounds (**1, 2, 28** and **16-22**) gave IR spectra consistent with their assigned structures, thereby supporting their 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 acids (table-2).

For compound **13**, the singlet at 2.2δ is due to the protons on carbon atom 26 and the peaks between 7.0δ - 7.8δ are due to

the protons on carbon atoms 8, 9, 11, 12, 21, 22, 24 and 25 of the benzene rings. The peak at 4.3δ is due to the proton on carbon atom 3, which gives a triplet. Peaks between 2.3δ - 2.8δ are due to the protons on carbon atoms 4 and 5 which give a quartet and triplet respectively. The ^{1}HNMR spectrum was in agreement with the proposed structure of the derivative (figure-1). All the compounds which were synthesized (**1-33**) gave IR and ^{1}H NMR spectra confirmed the suggested structures.

Antifungal activity results: 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-glutamic acid derivatives (**1-33**) were examined *in vitro* against fungal micro-organisms. The photograph provided (figure-2) shows how the antifungal activity was determined. The spectral data of antifungal activity of the synthesized compounds (**1–33**) at 0.2g/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: Yeast, *Fusariumsolani, Fusarium moniliforme, Penicillium expansum* and *Cladosporium cladosoporioides* 13 showed antifungal properties on at least one of the micro-organisms tested.The activities ranged from slight to moderate depending on the compound and the fungal isolate tested (table-1). The compounds (**2, 3, 5-8, 13, 16, 19-21, 23, 24, 26-31** and **33**) showed no activity on the tested micro-organisms; these derivatives were not included in table-1.

Considering N-tosyl-L-glutamoyl alanine (**4**) with inhibition zone of diameter 6mm and N-tosyl-L-glutamoyl alanine methyl ester (**9**) with diameter of 9mm,both compounds were found to be slightly active against Yeast only, while they had no effect on, *Fusariumsolani, Fusariummoniforme, Penicillium expansum and Cladosporiumcladosporioides.*

The N-tosyl-L-glutamoyl-(L-valine, L-leucine,L-tyrosine), methyl esters (**10-12**) with diameters of 7mm and 8mm (**10**), 9mm (**11**) and 6mm and 8mm (**12**) as inhibition zones showed an increase in biological activity by having antifungal properties on both *FusariumSolani*and Yeast, but showed no biological activity on *Fusarium moniliforme, Penecilliumexpansum* and*Clasdoporiumcladosporioides.* The slight increase in activity could be due the introduction of theester group (COO-).

N-tosyl-L-glutamoyl-2-chloro-4-nitro-aniline (**14**) had no activity on Yeast, but was found to have slight activity on *Fusariumsolani, Fusarium moniliforme, Penicillium expansum* and *clasdosporium cladosporioides* with diameters of 7mm,6mm,7mm,8mm respectively as inhibition zones. This showed an increase in activity as compared to compounds (**10-12**) which had effect on only two micro-organisms. The increase in activity could have been as a result of the introduction of the chloro and nitro groups on the benzene ring.However N-tosyl-L-glutamoyl-4-chloro-2-nitro-aniline (**15**) was found to be active on only Yeast*, Fusarium moniliforme, Fusariumsolani* with diameters of 9mm, 10mm, 6mm and displayed no activity on *Penicillium expansum, cladosporium cladosporioides.* The slight decrease in activity was due changing of chloro and nitro groups at position 2 and 4 of the benzene ring. N-tosyl-L-glutamoyl-p-aminobenzoyl chloride (**17**) with diameters of 9mm,7mm and N-tosylamino benzoyl-L-alanine (**18**) with inhibition zones of diameter 6mm,7mm, both were found to show slight activity on Yeast and *Fusariumsolani* and had no response on *Fusarium moniliforme, Penicillium expansum and Cladosporium cladosporioides*.

Table-1
Antifungal activity of N-tosyl-L-glutamic acid derivatives (1-33)

Compound No.	Yeast	*Fusariumsolani*	*Fusarium moniliforme*	*Penicillium expansum*	*cladoporium cladosporioides*
34	8	0	0	0	0
37	6	0	0	0	0
42	9	0	0	0	0
43	7	8	0	0	0
44	9	9	0	0	0
45	6	8	0	0	0
47	0	7	6	7	8
48	9	10	6	0	0
50	9	7	0	0	0
51	6	7	0	0	0
55	12	12	7	9	7
58	6	0	0	0	0
65	9	0	0	0	0

Antifungal activity, 0 = inactive, 0 – 9 mm = slightly active, 10-19 mm = moderately active, ≥20 mm = highly active. Compounds (**2, 3, 5-8, 13, 16, 19-21, 23, 24, 26-31** and **33**) were inactive against the fungal isolates tested.

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

Compound number	R	Yield %	M.P. (C)	Molecular formula	Elemental analysis of nitrogen	
					Calc. (%)	Found (%)
2	-O-	71.50	240-242	$C_{11}H_{11}NO_5S$	5.20	5.11
3	Gly-	76.60	220-222	$C_{14}H_{16}NO_6S$	8.23	8.20
4	L-Ala-	65.50	260-262	$C_{15}H_{18}N_2O_6S$	7.91	7.58
5	L-Val-	40.00	252-254	$C_{17}H_{22}N_2O_6S$	7.33	7.16
6	L-Leu-	59.00	286-288	$C_{18}H_{24}N_2O_6S$	7.07	6.99
7	L-Tyr-	70.00	248-250	$C_{21}H_{22}N_2O_7S$	6.27	6.17
8	-Gly-OMe	60.00	249-251	$C_{15}H_{18}N_2O_6S$	7.91	7.86
9	-L-Ala-OMe	66.00	247-249	$C_{16}H_{20}N_2O_6S$	7.61	7.50
10	-L-Val-OMe	51.00	214-216	$C_{18}H_{24}N_2O_6S$	7.07	7.00
11	-L-Leu-OMe	84.30	242-244	$C_{19}H_{26}N_2O_6S$	6.83	6.77
12	-L-Tyr-OMe	73.30	239-241	$C_{22}H_{24}N_2O_6S$	6.31	6.18
13	4- Cl-C_6H_4-NH_2-	49.40	218-220	$C_{18}H_{17}O_4SCl$	7.13	6.99
14	2-Cl-4-NO_2-C_6H_3-NH_2-	45.00	206-208	$C_{18}H_{16}N_3O_6SCl$	9.60	9.37
15	4-Cl-2-NO_2-C_6H_3-NH_2-	80.80	216-218	$C_{18}H_{16}N_3O_6SCl$	9.60	9.50
16	4-C_6H_4-COOH-NH_2-	62.00	241-243	$C_{19}H_{18}N_2O_6S$	6.97	6.50
17	4-C_6H_4-COCl-NH_2-	73.60	221-223	$C_{19}H_{17}N_2O_5SCl$	6.66	6.47
18	4-C_6H_4-CO-Gly-NH_2-	39.60	199-201	$C_{21}H_{21}N_3O_7S$	9.15	9.01
19	4-C_6H_4CO-L-Ala-NH_2-	52.60	258-260	$C_{22}H_{23}N_3O_7S$	8.88	8.76
20	4-C_6H_4-CO-L-Val-NH_2-	46.70	201-203	$C_{24}H_{27}N_3O_7S$	8.38	8.29
21	4-C_6H_4-CO-L-Leu-NH_2-	50.20	233-235	$C_{25}H_{29}N_3O_7S$	8.16	8.10
22	4-C_6H_4-CO-L-Try-NH_2-	54.00	262-264	$C_{28}H_{27}N_3O_8S$	7.43	7.30
23	4-C_6H_4-CO-Gly-OMe-NH_2-	74.00	243-245	$C_{22}H_{23}N_3O_7S$	8.88	8.79
24	4-C_6H_4-CO-L-Ala-OMe-NH_2-	63.50	221-223	$C_{23}H_{25}N_3O_7S$	8.62	8.52
25	4-C_6H_4-CO-L-Val-OMe-NH_2-	81.00	227-229	$C_{25}H_{29}N_3O_7S$	8.16	8.07
26	4-C_6H_4-CO-L-Leu-OMe-NH_2-	78.00	232-234	$C_{26}H_{31}N_3O_7S$	7.94	7.81
27	4-C_6H_4-CO-L-Try-OMe-NH_2-	78.00	246-248	$C_{29}H_{29}N_3O_7S$	7.46	7.37
28	4-C_6H_4-CON_3-NH_2-	42.30	232-234	$C_{19}H_{17}N_5O_5S$	16.39	16.17
29	4-C_6H_4-NHCO-Gly-NH_2-	47.90	235-237	$C_{21}H_{22}N_4O_7S$	11.81	11.7
30	4-C_6H_4-NHCO-L-Ala-NH_2-	52.00	228-230	$C_{22}H_{24}N_4O_7S$	11.48	11.27
31	4-C_6H_4-NHCO-L-Val-NH_2-	63.00	230-232	$C_{24}H_{28}N_4O_7S$	10.85	10.49
32	4-C_6H_4-NHCO-L-Leu-NH_2-	58.40	225-227	$C_{25}H_{30}N_4O_7S$	10.57	10.21
33	4-C_6H_4-NHCO-L-Try-NH_2-	49.60	221-223	$C_{28}H_{28}N_4O_7S$	9.39	9.39

Only N-tosyl-L-glutamoyl-p-aminobenzoyl-L-tyrosine(**22**) with inhibition zones of diameters 12mm, 12mm, 7mm, 9mm, 7mm was found to affect growth on all the tested micro-organisms: Yeast, *Fusariumsolani, Fusarium moniliforme, Penicillium expansum and Cladosporium cladoporioides*. This compound showed an increase in biological activity as compared to all the other compounds above. This was due the presence of the hydroxyl group on the benzene ring of L-tyrosine.

Both N-tosyl-L-glutamoyl-p-aminobenzoyl-L-valine methyl ester (**25**) with diameter of 6mm as inhibition zone and N-tosyl-L-glutamoyl-p-aminobenzoyl-L-Lencine urea (**32**) having inhibition zone of diameter 9mm, had antifungal activity on only Yeast, while they were inactive against *Fusariumsolani, Fusarium moniliforme, Penicillium expansum and Cladosporium cladosporioides.* All the results of the antifungal properties of the above compounds are illustrated in table-1.

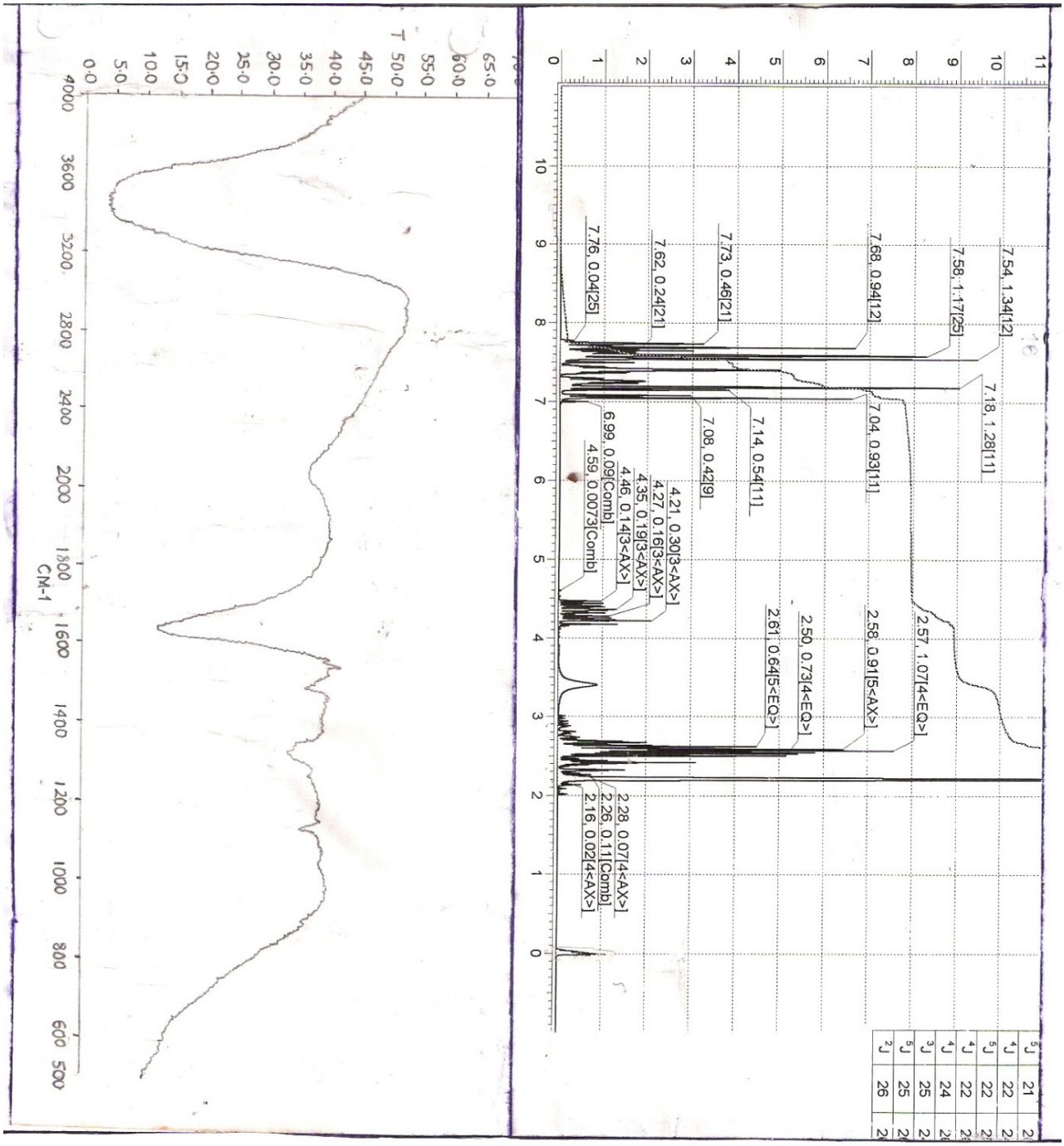
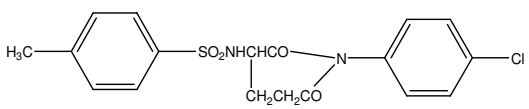


Figure-1
IR spectrum for compound 13 Confirmatory ^{1}HNMR spectrum for compound13



Scheme-1
Structure for Compound 13

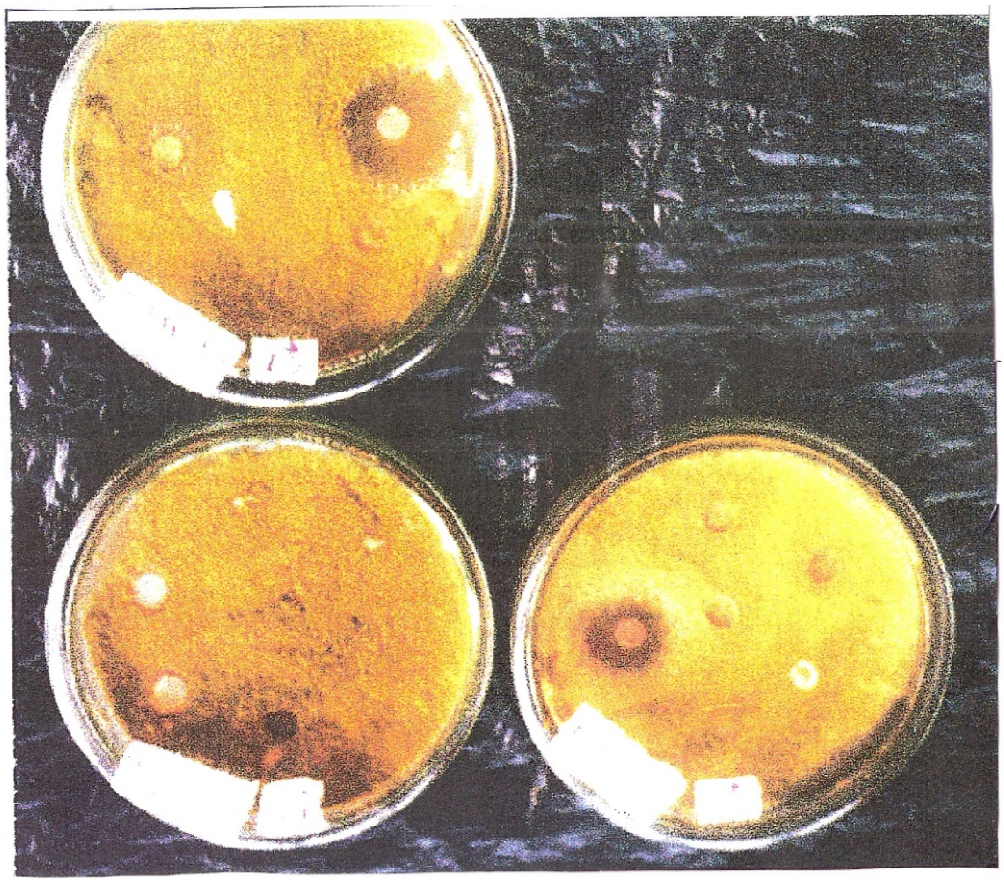


Figure-3
Antifungal Activity

Conclusion

This study showed that N-tosyl-L-glutamic 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. Eleven of the synthesised 33 compounds had antifungal activities against all the tested five antifungal isolates which included: Yeast, *Fusariumsolani, Fusarium moniliforme, Penicillium expansum, Cladosporium cladosporioides.* Findings also suggest that some derivatives showed no biological activity towards the tested fungal isolates. The antifungal activities shown by some of N-tosyl-L-glutamic acid derivatives were due to structural adjustments of various compounds which led to increase, decrease or complete loss in biological activity.

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