

Synthesis X-Ray Crystallography and Antimicrobial Activity of Protected and Deprotected Amides

¹SHER WALI KHAN, ¹JAVED HUSSAIN ZAIDI*, ²SAMMER YOUSUF,
²KHALID MOHAMMED KHAN*, ²NIDA AMBREEN, ³MOMIN KHAN,
⁴SHAHNAZ PERVEEN AND ⁵GHULAM ABBAS MIANA

¹*Department of Chemistry, Quaid-e-Azam University, Islamabad, 45320, Pakistan.*

²*H. E. J. Research Institute of Chemistry, International Center for Chemical and Biological Sciences, University of Karachi, Karachi-75270, Pakistan.*

³*Department of Microbiology, Institute of Basic Medical Sciences Khyber Medical University Peshawar, Peshawar, Pakistan.*

⁴*PCSIR Laboratories Complex, Karachi, Shahr-e-Dr. Salimuzzaman Siddiqui, Karachi-75280, Pakistan.*

⁵*Riphah Institute of Pharmaceutical Sciences, Riphah International University, 7th Avenue, G-7/4 Islamabad, Pakistan.*

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Summary: Twenty four chiral protected and deprotected amides were synthesized by using commercially available L-tartaric acid, having two asymmetric centers and C₂ axis of symmetry. In the synthetic sequence, diacid functionality of L-tartaric acid was protected as dimethyl ester and dihydroxy groups as acetonoid. The partial hydrolysis of acetonoid of dimethyl ester gives the corresponding monoesters. Monoester upon treatment with different substituted aromatic amines gave desired amides (**1a-1l**). Amides (**1a-1l**) afforded compounds (**2a-2l**) after reacting with acetyl chloride and methanol. All the compounds were purified and characterized by using sophisticated spectroscopic techniques including IR, ¹H-NMR, ¹³C-NMR and elemental analysis. The structure of compounds **1a** and **2l** were unambiguously obtained by X-ray crystallography. Protected (**1a-1l**) and deprotected amides (**2a-2l**) were tested for their antimicrobial activities at different concentrations against different strains of bacteria and fungi and found to be antimicrobial.

Key Words: L-Tartaric acid, protection and deprotection, amides, X-ray studies, antimicrobial activity.

Introduction

A growing concern about the continuing antibiotic-resistant infectious agents is compelling the pharmaceutical industry to search for novel antimicrobial agents [1]. A number of potential drug target has greatly increase and modern drug discovery is heavily focusing on rationally chosen targets [2-3]. Syntheses of new and more complex molecule for studying bio-molecular interactions activate the imaginative skill of synthetic chemists. Replacement of the oxygen with sulfur or nitrogen atom in the crown ether or by introducing functional groups *viz* amide, and ester in the ring would make them as models of protein-metal binding sites in biological systems [4-7]. Amide bonds are amongst the most stable attachments found in organic compounds and are probably the most common type of bonds employed in living organisms in the form of proteins and peptides. The amide bond is one of the most important linkages in organic chemistry and constitutes the key functional group in peptides, natural products, and pharmaceuticals [8]. Amides are usually prepared by thermolysis of tartaric acid with alkyl amines [9], by coupling of carboxylic acids and amines in the presence of coupling reagent [10] or by prior conversion of the carboxylic acid into a derivative [11]. Tartaric acid derivatives have been the subject of numerous studies including selective

ion complexation or ion transport [12], chiral recognition [13], molecular catalysis [14], asymmetric oxidation of prochiral sulfides [15] and as bio-inspired antifreeze additives [16]. Synthetic development of new macrocyclic peptide antibiotics such as biphenomycin B [17] and vancomycin type glycopeptides antibiotics [18] has brought dramatic changes during the last few years. Cyclic peptides with open pores are useful as transport vehicles for biologically important ions and neutral molecules [19]. Synthetic biphenyl based cyclic amides have been reported for anion complexation [21]. Cyclic tetra-amide receptors having barbiturate binding domain have also been reported [22]. Supramolecular amides are also used as molecular receptors [23] and in molecular recognition [24], some recently reported synthesis of permanent fluorescence sensing chiral fluorophoric macrocycles with antibacterial activity [25] and cyclophane amides with antiinflammatory activity have also been reported [26] but no carbazole based amide macrocycles have been reported. Synthesis of carbazolophane amides along with their antibacterial and antifungal activities has also been reported. An in-depth analysis of the comprehensive medicinal chemistry database revealed that the carboxamide group appears in more than 25% of known drugs. This can be expected, since

carboxamides are neutral, stable and have both hydrogen-bond accepting and donating properties [27]. In the continuation of our research work on biologically active heterocyclic molecules [28-30] and owing to the importance of amides in organic synthesis, we designed to synthesize some new biologically active amides based on L-tartaric acid motif. Protected amides (**1a-1l**) and deprotected amide (**2a-2l**) were tested for their antimicrobial activities at three different concentrations against different strains of bacteria and fungi. All the protected and deprotected amides are active against all the three fungal as well as bacterial strains. All the compounds were purified and were characterized by using spectroscopic techniques including IR, $^1\text{H-NMR}$, $^{13}\text{C-NMR}$ and elemental analysis. The structure of compound **1a** was confirmed by X-ray crystallography.

Results and Discussion.

Chemistry

For the designing and synthesis of biologically important amides we have started research work with selection of an inexpensive and commercially available starting material L-(+)-tartaric acid which has two chiral centers (Fig. 1).

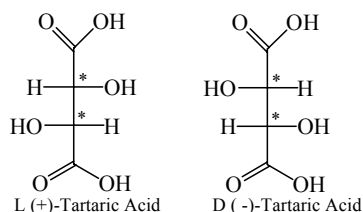


Fig. 1: Structure of L and D-Tartaric Acid.

Bifunctional L-Tartaric acid was protected and partially hydrolyzed to obtain monoamide [4, 21] as shown in Scheme-1.

The basic hydrolysis of dimethyl 2,2-dimethyl-1,3-dioxolane-4,5-dicarboxylate by using 1N NaOH, afford 5-(methoxycarbonyl)-2,2-dimethyl-1,3-dioxolane-4-carboxylic acid (monoester), which was treated with different substituted aromatic amines in dry chloroform as solvent and 1.2 equivalent *N,N*-dicyclohexylcarbodiimide (DCC) as dehydrating agent, the reaction mixture was stirred at room temperature for 8 h and was filtered to get rid of the byproduct *N,N*-dicyclohexyl urea. The crude was then purified by column chromatography using *n*-hexane/ethyl acetate as eluent. All the synthesized compounds were characterized by using

spectroscopic techniques like IR, $^1\text{H-NMR}$, $^{13}\text{C-NMR}$ spectroscopy and elemental analysis. The infrared absorption spectra of the synthesized amides (**1a-1l**) showed characteristic bands at 1738 and 1679 cm^{-1} for CO (ester) and CO (amide) stretching, respectively. The NH stretching frequencies for all the amides appear in the range of 3405-3395 cm^{-1} . The structures were further supported by ^1H and $^{13}\text{C-NMR}$ data. A one proton broad singlet appear at 8.40 ppm was attributed to NH proton. Two singlets at 3.82 and 1.56 ppm were appeared due to 3 protons of the methoxy group and 6 protons of the isopropylidene moiety. The aromatic protons showed doublet in *para* substituted anilines while *ortho* and *meta* substituted anilines showed multiplet and a singlet, respectively. In $^{13}\text{C-NMR}$ the quaternary carbon appeared at 113 ppm, carbonyl carbons of amide and ester were appear at 173 and 170 ppm respectively. Methoxy carbon appeared at 52 ppm while the two CH carbons showed signals at 76 and 75 ppm, respectively. Elemental analysis of these compounds, described in the experimental protocol further supported the structures of all the synthesized compounds. The assign structures was further supported by single crystal X-ray diffraction studies of amides 4*R*, 5*R*-Methyl 2,2-dimethyl-5-(*o*-tolylcarbamoyl)-1,3-dioxolane-4-carboxylate (**1g**) (Fig. 2).

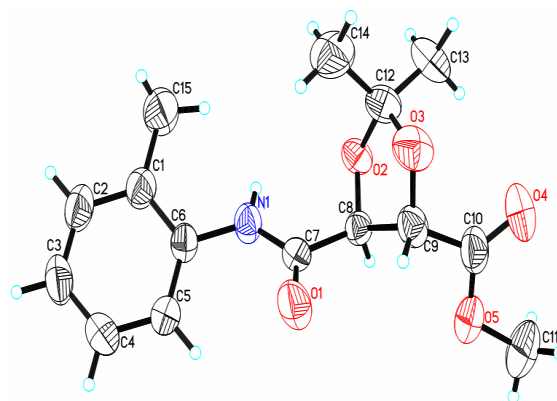
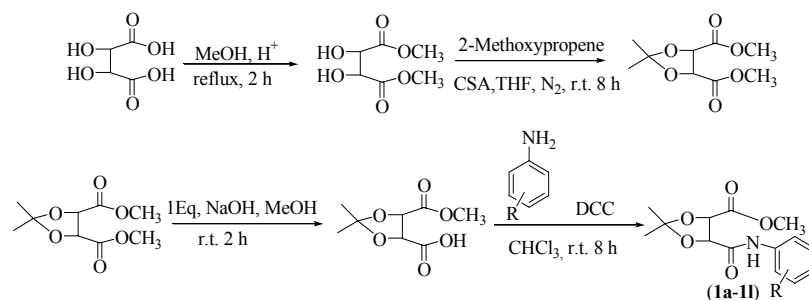


Fig. 2: X-ray crystal structure of 4*R*, 5*R*-Methyl 2,2-dimethyl-5-(*o*-tolylcarbamoyl)-1,3-dioxolane-4-carboxylate (**1g**). Crystal system, space group Orthorhombic, P212121. Selected bond lengths (Å) and angles (deg): O(2)-C(8) = 1.415(3), C(8)-C(9) = 1.526(3), C(7)-O(1) = 1.211(3), C(7)-N(1) = 1.326(3), C(2)-C(3) = 1.377(5), C(8)-O(2)-C(12) = 109.12(17), O(2)-C(8)-C(7) = 114.00(17), C(9)-C(8)-C(7) = 112.0(2), O(1)-C(7)-N(1) = 124.9(2), O(4)-C(10)-O(5) = 124.9(3), O(4)-C(10)-C(9) = 126.0(3).



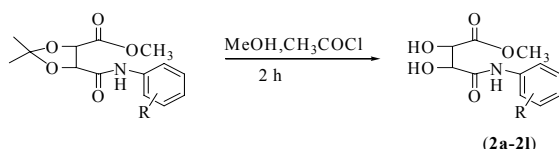
S. No.	Code	R	R _f values*	Yield (%)
1	1a	2-Trifluoromethylaniline	0.56	68
2	1b	3-Trifluoromethylaniline	0.73	70
3	1c	4-Trifluoromethylaniline	0.65	76
4	1d	2-Methoxyaniline	0.78	69
5	1e	3-Methoxyaniline	0.49	75
6	1f	4-Methoxyaniline	0.68	80
7	1g	2-Methylaniline	0.89	68
8	1h	3-Methylaniline	0.89	86
9	1i	4-Methylaniline	0.92	79
10	1j	2-Chloroaniline	0.77	73
11	1k	3-Chloroaniline	0.69	81
12	1l	4-Chloroaniline	0.68	77

*Solvent System: Ethyl acetate: *n*-Hexane (2:8)

Scheme-1: Synthesis of protected amides.

Deprotected Amides

All the protected amides were deprotected by using methanol and freshly distilled acetyl chloride. Acetyl chloride was added drop-wise and the progress of the reaction was monitored through thin layer chromatography to obtained compounds (2a-2l) Scheme-2.



S.No	Code	R	R _f values*	Yield (%)
1	2a	2-Trifluoromethylaniline	0.71	65
2	2b	3-Trifluoromethylaniline	0.51	78
3	2c	4-Trifluoromethylaniline	0.68	81
4	2d	2-Methoxyaniline	0.74	78
5	2e	3-Methoxyaniline	0.45	80
6	2f	4-Methoxyaniline	0.67	83
7	2g	2-Methylaniline	0.81	65
8	2h	3-Methylaniline	0.79	76
9	2i	4-Methylaniline	0.71	82
10	2j	2-Chloroaniline	0.91	85
11	2k	3-Chloroaniline	0.81	77
12	2l	4-Chloroaniline	0.66	87

*Solvent system: MeOH: Chloroform (2:8)

Scheme-2: Synthesis of deprotected amides.

The quantitative deprotection of the tartaric hydroxy groups was confirmed by ¹H-NMR and IR spectra which showed the disappearance of the ketal, resonance at 1.52 ppm and the presence of broad OH stretching bands at 3380 and 3400 cm⁻¹ (2a-2l). In ¹³C-NMR the disappearance of the quaternary carbon further supported the deprotection. Finally the assigned structure was supported by single X-ray

crystal data of 2*R*,3*R*-methyl-4-(*p*-toluidino)-2,3-dihydroxy-4-oxobutanoate (2i) in Fig. 3.

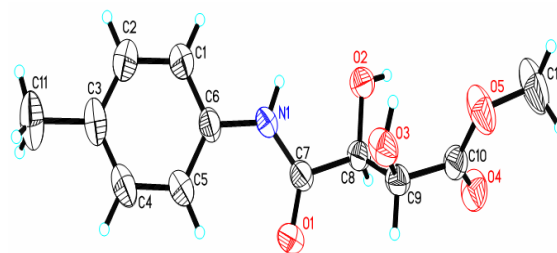


Fig. 3: X-ray crystal structure of 2*R*,3*R*-methyl-4-(*p*-toluidino)-2,3-dihydroxy-4-oxobutanoate (2i). Crystal system, space group Monoclinic, P21, Selected bond lengths (Å) and angles (deg): N(1)-C(7) = 1.3327(19), N(1)-C(6) = 1.4226(15), O(1)-C(7) = 1.2320(18), O(5)-C(10) = 1.323(2), O(2)-H(2A) = 0.8200, C(6)-C(5) = 1.388(2), C(2)-C(1)=1.386(2), C(7)-N(1)-C(6)= 127.67(12), C(6)-N(1)-H(1A) = 116.2, O(4)-C(10)-O(5) = 124.34(13), O(2)-C(8)-C(7) = 109.66(10), C(10)-C(9)-C(8) = 107.83(11). Torsion angles [deg]. C(12)-O(5)-C(10)-O(4) = 3.3(3), C(12)-O(5)-C(10)-C(9) = -174.45(18), C(7)-N(1)-C(6)-C(1) = 176.58(15), O(2)-C(8)-C(7)-O(1) = -173.70(14), C(11)-C(3)-C(4)-C(5) = 179.7(2).

Bioassay

All twenty-four synthetic protected amides (1a-1l) and deprotected (2a-2l) were screened for their *in vitro* antibacterial and antifungal potential against different strains of bacteria (*Enterobacter sp*, *Vibrio cholerae*, *Klebsiella sp*) and fungi (*Fausarium solani*, *Helminthosporium sativum*, and *Aspergillus niger*). The results are listed in Table-1 and Table-2.

Table-1: Antifungal activity of the protected (1a-1l) and deprotected amides (2a-2l) at three different concentrations against three fungal strains.

S. No.	Compounds	<i>Fausarium solani</i>			<i>Helminthosporium sativum</i>			<i>Aspergillus niger</i>		
		100%	80%	60%	100%	80%	60%	100%	80%	60%
1	1a, 2a	67, 70	41,43	33,40	65,78	28,32	29,33	70,72	38,44	17,23
2	1b, 2b	71, 88	33,44	22,55	70,77	34,33	22,32	71,77	28,34	10,22
3	1c, 2c	88, 91	70,72	15,21	89,91	16,22	20,22	78,89	23,44	22,43
4	1d, 2d	60, 67	46,56	31,28	64,68	33,21	29,32	53,54	33,34	21,32
5	1e, 2e	50, 68	48,50	45,54	57,60	22,24	17,22	40,43	33,45	23,45
6	1f, 2f	81, 89	30,33	29,41	79,88	08,22	06,15	68,70	41,55	22,34
7	1g, 2g	47, 57	45,56	13,22	46,43	56,67	40,45	57,63	45,60	33,55
8	1h, 2h	66, 72	52,56	30,34	58,67	27,37	25,33	38,43	34,40	25,50
9	1i, 2i	84,88	58,65	30,44	70,76	38,40	35,45	63,66	41,44	25,34
10	1j, 2j	67,70	43,55	39,51	62,66	48,66	38,48	67,69	33,53	33,48
11	1k, 2k	83,90	51,66	38,50	78,88	25,44	19,27	79,81	71,76	47,54
12	1l, 2l	73,78	65,59	71,77	70,77	50,55	34,55	53,56	30,47	31,41
	+Ve control	-	-	-	-	-	-	-	-	-
	Terbinafine	94	94	94	98	98	98	98	98	98

Table-2: Antibacterial activity^a of the protected (1a-1l) and deprotected amides (2a-2l) at three different concentrations against three bacterial strains

S. No.	Code	<i>Enterobacter sp</i>			<i>Vibrio cholerae</i>			<i>Klebsiella sp</i>		
		100%	80%	60%	100%	80%	60%	100%	80%	60%
1	1a, 2a	16,22	10,12	08,12	27,30	20,23	15,23	19,23	13,17	12,19
2	1b, 2b	14,21	05,22	11,22	22,26	13,14	21,22	23,25	10,13	09,13
3	1c, 2c	27,30	17,25	12,23	29,31	23,26	11,14	22,28	08,12	11,22
4	1d, 2d	17,27	13,22	11,21	22,23	18,21	14,23	19,21	12,22	10,21
5	1e, 2e	23,24	12,16	16,18	25,31	13,17	16,21	16,18	11,21	12,16
6	1f, 2f	26,30	12,18	17,19	18,21	10,14	12,17	28,30	23,28	22,18
7	1g, 2g	15,19	11,21	17,22	22,25	18,20	14,14	18,22	12,22	09,12
8	1h, 2h	22,23	15,22	06,10	23,24	15,21	12,22	27,29	18,21	14,21
9	1i, 2i	29,30	22,28	18,21	25,27	13,24	11,21	22,25	16,18	11,19
10	1j, 2j	19,22	12,24	10,23	26,29	23,29	18,22	22,29	13,22	14,17
11	1k, 2k	26,28	16,21	19,21	29,31	22,28	17,24	12,21	11,14	10,21
12	1l, 2l	21,29	19,23	19,23	17,22	13,21	11,20	23,23	17,22	16,18
	Chloramphenicol	32	25	34	32	25	34	32	25	34

^aZone diameter (Activity): Below 9 mm (no activity), 9-12 mm (non significant), 13-15 mm (low activity), 16-18 mm (good activity), above 18 mm (significant activity).

Antifungal Activity

All the compounds show good activity against *Fausarium solani*, *Helminthosporium sativum* and *Aspergillus niger* at 100% concentration. Compounds **1c**, (88%, 89%, 78%), **1f** (81%, 79%, 68%), **1i** (84%, 70%, 63%), and **1l** (73%, 70%, 53%) with substituents at *para* position showed significant activity against three fungal strains at 100% concentration. However, as the concentration decrease to 80% and 60% these compounds showed a clear decline in activity. Compounds **1a** and **1j** with the *ortho* substitutions of halogens showed (67%, 65%, 70%) and (67%, 62%, 67%) inhibition at 100% concentration in all the three strains of fungi, respectively, although compounds **1d** and **1g** with *ortho* methoxy and methyl substitutions showed only (60%, 64%, 53%) and (47%, 46%, 57%) inhibition against all three strains at 100% concentration, respectively. The activity of compounds **1b** (73%), **1e** (50%), **1h** (66%), and **1k** (83%) with *meta* substitution at 100% concentrations reveals that compounds **1b** and **1k** with *meta* fluoro and chloro substitutions showed significant activity. When the concentration decrease to 80% and 60% there is a decrease in activity is observed in all the compounds.

The results reveal that the same way of inhibition is observed in the activity of compounds of **2a-2l**. Compound **2c** with *para* fluoro substitution was found to be the most active compound showed inhibition 91%, 91%, 89%, at 100% concentration in all three strains. Compound **2f** with *para* methoxy showed 89%, 88%, 68% activity at 100% concentration. Compound **2i** and **2l** with *para* methyl and *para* chloro showed inhibition at 100% concentration (88%, 76%, 66%), (78%, 77%, 56%), respectively. Compounds **2b**, **2e**, **2h**, **2k** with *meta* substituents showed 88%, 68%, 72% and 90% inhibition for *Fausarium solani* and 70%, 57%, 58%, and 78% inhibition for *Helminthosporium sativum* and 71%, 40%, 38% and 79% inhibition for *Aspergillus niger* at 100% concentration, respectively. Compounds **2a**, **2d**, **2g**, **2j**, with *ortho* substitution displayed 88%, 68%, 72% and 90% inhibition for *Fausarium solani* 65% 64% 46%, and 62% inhibition *Helminthosporium sativum* and 70%, 53%, 57% and 67% inhibition for *Aspergillus niger* at 100% concentration, respectively. A declined in activity was observed in all the compounds when decreasing the concentration from 100% to 80% and 60%. On the basis of the above results, we can say that the compounds with halogens substitution at

ortho, *meta*, and *para* positions particularly when substituted at *para* and *meta* positions showed an excellent inhibitory potential against all the tested fungal strains and can be serve as a lead molecules for the further drug and medicinal research and studies.

Antibacterial Activity

All the twenty-four synthetic protected **1a-1l** and deprotected amides **2a-2l** were also tested for their antibacterial activity and the results are collected in Table-2. All the compounds demonstrated good activity against all the three (*Enterobacter sp*, *Vibrio cholerae*, *Klebsiella sp*) bacterial strains used at 100% concentration, the antibacterial activity of the compounds also decreases at 80% and 60% concentration, respectively. Compound **1c**, **1f**, **1i** and **1l** with substituents at *para* position show significant activity (27, 26, 29, 21, for *Enterobacter sp*, 29, 18, 25, 17 for *Vibrio cholerae*, and 22, 28, 22, 23 mm zone of inhibition, for *Klebsiella sp*, respectively) against three bacterial strains at 100% concentration, while the deprotected compounds **2c**, **2f**, **2i**, **2l**, (30, 30, 30, 29, for *Enterobacter sp*, 31, 21, 27, 22 for *Vibrio cholerae*, and 28, 30, 25, 23 mm zone of inhibition for *Klebsiella sp*, respectively) were found to be more active as compared to the protected amides. When the substitution amended with *ortho* substitutions displayed less activity against all the bacterial strains as it shown in protected amides **1a**, **1d**, **1g**, **1j**, (16, 17, 15, 19, for *Enterobacter sp*, 27, 22, 22, 26 for *Vibrio cholerae*, and 19, 19, 18, 22 mm zone of inhibition for *Klebsiella sp*, respectively) at 100% concentration. It is worth mentioning that deprotected amides showed much better activity as compared to the protected amides **2a**, **2d**, **2g**, **2j**, (22, 27, 19, 22, for *Enterobacter sp*, 30, 23, 25, 29 for *Vibrio cholerae*, and 23, 21, 22, 29 mm zone of inhibition for *Klebsiella sp*, respectively). When compare the *meta* substitution in protected amides it was found that **1b**, **1e**, **1h**, **1l**, (14, 23, 22, 21 for *Enterobacter. sp*, 22, 25, 23, 17, for *Vibrio cholerae*, and 23, 16, 27, 23 mm zone of inhibition for *Klebsiella sp*, respectively) less active than *ortho* and *para* protected and *meta* deprotected amides **2b**, **2e**, **2h**, **2l**, (21, 24, 23, 29 for *Enterobacter sp*, 26, 31, 24, 22 for *Vibrio cholerae*, and 25, 18, 29 and 23 mm zone of inhibition for *Klebsiella sp*, respectively) at 100% concentrations. Hence in the light of all the above finding it is confirmed that the deprotected amides are more active as compare to the protected amides and on the basis of substitution the *para* substituents is more active as compared to their *ortho* and *meta* counter parts while in the substituted

groups halogen substituted amides are found more potential inhibitors. The inhibition potential is going to be decrease as concentrations moving down from 100% to 80% and 60%, respectively.

General Experimental

All reactions were carried out in anhydrous conditions and under static pressure of nitrogen gas using rubber septa and three way stopcock. Solvent like ether was dried and distilled over sodium and benzophenone. Chloroform was dried by refluxing with phosphorus pentoxide and methanol dried over magnesium turnings and iodine crystals. Amines were dried by refluxing over potassium hydroxide. All the reactions were monitored through thin layer chromatography using pre-coated silica gel glass plates (layer thickness 0.25 mm, HF-254, E. Merck). Ethyl acetate: n-hexane and methanol: chloroform mixtures were used as eluent. Chromatograms were detected by using ultraviolet light (λ_{max} 254 and 365 nm). Column chromatography was performed on E. Merck silica gel (0.063 - 0.200 mm). IR spectra were recorded on Thermo scientific Nicolet Fourier Transform Infrared Spectrophotometer Model 6700. ¹H-NMR spectra were recorded on NMR Bruker apparatus at 300 MHz in CDCl₃. ¹³C-NMR spectra were recorded on NMR Bruker apparatus at 75 MHz. Tetramethylsilane (TMS) was used as internal reference. Chemical shifts are given in δ scale (ppm). Abbreviations s, d, and t have been used for singlet, doublet, and triplet, respectively. The optical rotations of the compounds were measured on ATAGO, AP-100 Automatic polarimeter. Single-crystal X-ray diffraction data were collected on a Bruker Smart APEX II, CCD detector diffractometer [31]. Data reductions were performed by using SAINT program. The structures were solved by direct methods [32], and refined by full-matrix least squares on F2 by using the SHELXTL-PC package [33]. The figures were plotted with the aid of ORTEP program [34].

Experimental

To carry out the synthesis of protected amides different aromatic primary amines and 5-(methoxycarbonyl)-2,2-dimethyl-1,3-dioxolane-4-carboxylic acid were used.

General Procedure for the Synthesis of Protected Amides (1a-1l)

In a 100 mL round-bottomed flask, 5-(methoxycarbonyl)-2,2-dimethyl-1,3-dioxolane-4-carboxylic acid (1 equivalent, 5 mmol, 1.02 g) and

N,N-dicyclohexylcarbodiimide (DCC) (1.2 equivalent, 06 mmol, 1.236 g) were placed under nitrogen and chloroform (40 mL) was added as a solvent. After half an hour substituted aniline (1 equivalent, 5 mmol) was added. The reaction mixture was stirred under nitrogen for eight hour, so that amide was synthesized. Dicyclohexyl urea was removed by extracting the reaction mixture with ethyl acetate or chloroform and water. Crude was purified on a silica gel column.

4R,5R-Methyl 2,2-dimethyl-5-((2-(trifluoromethyl)phenyl)carbamoyl)-1,3-dioxolane-4-Carboxylate (1a)

Yield: 68%, Yellowish oil, $[\alpha]_{\text{D}}^{25} = +1.23^{\circ}$ (15 mg / 20 mL) ethyl acetate, IR ν cm^{-1} : 3405 (-NH), 2928 (-CH stretching), 1738 (CO ester), 1679 (CO amide), 1592 (C=C), 1161 (C-O). $^1\text{H-NMR}$ (300 MHz, CDCl_3): δ (ppm) 8.40 (s, -NH), 7.64-7.57 (m, 4H, ArH), 4.31, (d, $J = 7.2$ Hz, 2H, -CH), 3.82 (s, 3H, -OCH₃), 1.57 s, 6H, (C-(CH₃)₂). $^{13}\text{C-NMR}$ (75 MHz, CDCl_3): δ (ppm): 173 (CO amide), 170 (CO ester), 133 (Ar-C-NH), 114 (Ar-CF₃), 112 (qt C), 76 (-CH amide), 75, -CH ester), 52 (-OCH₃), 26 (CH₃). Anal. Calc. For C₁₅H₁₆F₃NO₅ C, 51.88; H, 4.64; F, 16.41; N, 4.03; O, 23.03 Found C, 51.45; H, 3.96; F, 16.50; N, 4.09; O, 22.83.

4R,5R-Methyl 2,2-dimethyl-5-((3-(trifluoromethyl)phenyl)carbamoyl)-1,3-dioxolane-4-carboxylate (1b)

Yield: 70%, White crystalline solid, m.p. = 118-119 °C, $[\alpha]_{\text{D}}^{25} = +1.75^{\circ}$, IR ν cm^{-1} : 3336 (-NH-), 2992 (-CH stretching), 1741 (CO ester), 1683 (CO amide), 1540 (C=C), 1163 (C-O). $^1\text{H-NMR}$ (300 MHz, CDCl_3): δ (ppm) 8.47 (bs, -NH), 7.91 (s, 1H, Ar-H), 7.77-7.43 (m, 3H, Ar-H), 4.91 (d, $J = 6$ Hz, 2H, -CH), 3.85 (s, 3H -OCH₃), 1.56 (s, 6H, (C-(CH₃)₂)). $^{13}\text{C-NMR}$ (75 MHz, CDCl_3): δ (ppm) 170, (CO amide), 167, (CO ester), 137 (Ar-C-NH), 122 (Ar-CF₃), 113 (qt C), 77 (-CH amide), 76 (-CH ester), 53 (-OCH₃), 26 (CH₃). Anal. Calc. For C₁₅H₁₆F₃NO₅ C, 51.88; H, 4.64; F, 16.41; N, 4.03; O, 23.03 Found C, 51.45; H, 3.96; F, 16.50; N, 4.09; O, 22.83.

4R,5R-Methyl 2,2-dimethyl-5-((4-(trifluoromethyl)phenyl)carbamoyl)-1,3-dioxolane-4-carboxylate (1c)

Yield: 76%, White crystalline solid, m.p. = 115-117 °C $[\alpha]_{\text{D}}^{25} = +1.39^{\circ}$, IR ν cm^{-1} : 3347 (-NH-), 3009 (-CH stretching), 1758 (CO ester), 1686 (CO

amide), 1597 (C=C), 1185 (C-O). $^1\text{H-NMR}$ (300 MHz, CDCl_3): δ (ppm) 8.47 (bs, -NH), 7.73 (d, $J = 8.4$ Hz, 2H, Ar-H), 7.61, (d, $J = 8.4$ Hz, 2H, Ar-H), 4.91, (d, $J = 6$ Hz, 2H, -CH), 3.85 (s, 3H -OCH₃), 1.56 s, 6H, (C-(CH₃)₂). $^{13}\text{C-NMR}$ (75 MHz, CDCl_3): δ (ppm) 170, (CO amide), 167 (CO ester), 141 (Ar-C-NH), 125 (Ar-CF₃), 113 (qt C), 77 (-CH amide), 76 (-CH ester), 53 (-OCH₃), 26 (CH₃). Anal. Calc. For C₁₅H₁₆F₃NO₅ C, 51.88; H, 4.64; F, 16.41; N, 4.03; O, 23.03 Found C, 51.45; H, 3.96; F, 16.50; N, 4.09; O, 22.83.

4R,5R-Methyl 5-((2-methoxyphenyl)carbamoyl)-2,2-dimethyl-1,3-dioxolane-4-carboxylate (1d)

Yield: 69%, White crystalline solid, m.p. = 79-81 °C $[\alpha]_{\text{D}}^{25} = +0.65^{\circ}$, IR ν cm^{-1} : 3390, (-NH-), 2989, (-CH stretching 1739, (CO ester), 1688, (CO amide),), 1600 (C=C), 1209 (C-O). $^1\text{H-NMR}$ (300 MHz, CDCl_3): δ (ppm) 8.35 (bs, -NH), 7.52-7.47 (m, 4H, ArH), 4.94 (d, $J = 7.7$ Hz, 2H -CH), 3.95 (s, 3H Ar-OCH₃), 3.81 (s, 3H -OCH₃), 1.56 (s, 6H, (C-(CH₃)₂)). $^{13}\text{C-NMR}$ (75 MHz, CDCl_3): δ (ppm) 170 (CO amide), 167 (CO ester), 126 (Ar-C-NH), 113 9 (t, C), 77 (-CH amide), 76, -CH ester, 55, Ar-OCH₃, 52, -OCH₃, 26, CH₃. Anal. Calc. For C₁₅H₁₉NO₆ C, 58.25; H, 6.19; N, 4.53; O, 31.04 Found C, 57.95; H, 6.30; N, 4.50; O, 30.88.

4R,5R-Methyl 5-((3-methoxyphenyl)carbamoyl)-2,2-dimethyl-1,3-dioxolane-4-carboxylate (1e)

Yield: 75%, White crystalline solid, m.p. = 90-91 °C $[\alpha]_{\text{D}}^{25} = +1.34^{\circ}$, IR ν cm^{-1} : 3336 (-NH-), 2937 (-CH stretching), 1748, (CO ester), 1683, (CO amide), 1597, (C=C), 1154 (C-O). $^1\text{H-NMR}$ (300 MHz, CDCl_3): δ (ppm) 9.20 (bs, -NH), 7.48 (s, 1H, Ar-H), 7.47-7.30 (m, 3H, Ar-H), 4.93, (d, $J = 7.8$ Hz, 2H, -CH), 3.80 (s, 3H Ar-OCH₃), 3.78 (s, 3H -OCH₃), 1.49 (s, 6H, (C-(CH₃)₂)). $^{13}\text{C-NMR}$ (75 MHz, CDCl_3): δ (ppm) 170 (CO amide), 167 (CO ester), 137 (Ar-C-NH), 113 (qt C), 77 (-CH amide), 76 (-CH ester), 56 (Ar-OCH₃), 52 (-OCH₃), 26 (CH₃). Anal. Calc. For C₁₅H₁₉NO₆ C, 58.25; H, 6.19; N, 4.53; O, 31.04 Found C, 57.95; H, 6.30; N, 4.50; O, 30.88.

4R,5R-Methyl 5-((p-methoxyphenyl)carbamoyl)-2,2-dimethyl-1,3-dioxolane-4-carboxylate (1f)

Yield: 80%, White crystalline solid, m.p. = 109-110 °C $[\alpha]_{\text{D}}^{25} = +1.24^{\circ}$, IR ν cm^{-1} : 3357 (-NH-), 2989 (-CH stretching), 1747 (CO ester), 1681 (CO amide), 1509 (C=C), 1178 (C-O). $^1\text{H-NMR}$ (300 MHz, CDCl_3): δ (ppm) 8.21 (bs, -NH), 7.50 (d, $J = 9$

Hz, 2H Ar-H), 6.89 (d, $J = 8.7$ Hz, 2H Ar-H), 4.88 (d, $J = 7.8$ Hz, 2H -CH), 3.85 (s, 3H, Ar-OCH₃), 3.79 (s, 3H -OCH₃), 1.56 (s, 6H, (C-(CH₃)₂)). ¹³C-NMR (75 MHz, CDCl₃): δ (ppm) 170 (CO amide), 167 (CO ester), 129 (Ar-C-NH), 114 (qt C), 77 (-CH amide), 76 (-CH ester), 55 (Ar-OCH₃), 52 (-OCH₃), 26 (CH₃). Anal. Calc. For C₁₅H₁₉NO₆ C, 58.25; H, 6.19; N, 4.53; O, 31.04 Found C, 57.95; H, 6.30; N, 4.50; O, 30.88.

4R, 5R-Methyl 2,2-dimethyl-5-(o-tolylcarbamoyl)-1,3-dioxolane-4-carboxylate (1g)

Yield: 68%, White crystalline solid, m.p. = 120-121 °C [α]_D²⁵ = + 1.39°, IR ν cm⁻¹: 3393 (-NH-), 2998 (-CH stretching), 1754 (CO ester), 1693 (CO amide), 1552 (C=C), 1141 (C-O). ¹H-NMR (300 MHz, CDCl₃): δ (ppm) 8.30, (bs, -NH), 8.06-7.45 (m, 4H, Ar-H), 4.95 (d, $J = 8.8$ Hz, 2H -CH), 3.87 (s, 3H -OCH₃), 2.29 (s, 3H Ar-CH₃), 1.55 (s, 6H, (C-(CH₃)₂)). ¹³C-NMR (75 MHz, CDCl₃): δ (ppm) 170 (CO amide), 167 (CO ester), 134 (Ar-C-NH), 113 (qt C), 76 (-CH amide), 75 (-CH ester), 52 (-OCH₃), 26 (CH₃), 17 (Ar-CH₃). Anal. Calc. For C₁₅H₁₉NO₅ C, 61.42; H, 6.53; N, 4.78; O, 27.27 Found C, 60.90; H, 6.43; N, 4.94; O, 26.79.

4R, 5R-methyl 2,2-dimethyl-5-(m-tolylcarbamoyl)-1,3-dioxolane-4-carboxylate (1h)

Yield: 86%, White crystalline solid, m.p. = 122-123 °C [α]_D²⁵ = +1.32°, IR ν cm⁻¹: 3325 (-NH-), 2990 (-CH stretching), 1743 (CO ester), 1681 (CO amide), 1613 (C=C), 1157 (C-O). ¹H-NMR (300 MHz, CDCl₃): δ (ppm) 8.23 (bs, -NH), 7.44 (s, 1H, Ar-H), 7.38-6.99 (m, 3H, Ar-H), 4.91 (d, $J = 5$ Hz, 2H -CH), 3.87 (s, 3H -OCH₃), 2.36 (s, 3H Ar-CH₃), 1.58 (s, 6H, (C-(CH₃)₂)). ¹³C-NMR (75 MHz, CDCl₃): δ (ppm) 170 (CO amide), 167 (CO ester), 136 (Ar-C-NH), 112 (qt C), 77 (-CH amide), 76 (-CH ester), 52 (-OCH₃), 26 (CH₃), 21 (Ar-CH₃). Anal. Calc. For C₁₅H₁₉NO₅ C, 61.42; H, 6.53; N, 4.78; O, 27.27 Found C, 60.90; H, 6.43; N, 4.94; O, 26.79.

4R, 5R-Methyl 2,2-dimethyl-5-(p-tolylcarbamoyl)-1,3-dioxolane-4-carboxylate (1i)

Yield: 79%, White crystalline solid, m.p. = 117-118 °C [α]_D²⁵ = + 0.76°, IR ν cm⁻¹: 3348 (-NH-), 2969 (-CH stretching), 1739 (CO ester), 1669 (CO amide), 1596 (C=C), 1138 (C-O). ¹H-NMR (300 MHz, CDCl₃): δ (ppm) 8.21 (bs, -NH), 7.48 (d, $J = 6.9$ Hz, 2H Ar-H), 7.16 (d, $J = 8.1$ Hz, 2H Ar-H), 4.89 (d, $J = 6.2$ Hz, 2H -CH), 3.81 (s, 3H -OCH₃),

2.31 (s, 3H Ar-CH₃), 1.52 (s, 6H, (C-(CH₃)₂)). ¹³C-NMR (75 MHz, CDCl₃): δ (ppm) 170 (CO amide), 168 (CO ester), 134 (Ar-C-NH), 113 (qt C), 77 (-CH amide), 76 (-CH ester), 52 (-OCH₃), 25 (CH₃), 19 (Ar-CH₃). Anal. Calc. For C₁₅H₁₉NO₅ C, 61.42; H, 6.53; N, 4.78; O, 27.27 Found C, 60.90; H, 6.43; N, 4.94; O, 26.79.

4R,5R-Methyl5-((2-chlorophenyl)carbamoyl)-2,2-dimethyl-1,3-dioxolane-4-carboxylate (1j)

Yield: 73%, White crystalline solid, m.p. = 79-80 °C [α]_D²⁵ = + 2.22°, IR ν cm⁻¹: 3378 (-NH-), 2928 (-CH stretching), 1754 (CO ester), 1680 (CO amide), 1590 (C=C), 1142 (C-O). ¹H-NMR (300 MHz, CDCl₃): δ (ppm) 8.46 (bs, -NH), 8.43-7.38 (m, 4H, Ar-H), 4.95 (d, $J = 9$ Hz, 2H -CH), 3.87 (s, 3H -OCH₃), 1.54 (s, 6H, (C-(CH₃)₂)). ¹³C-NMR (75 MHz, CDCl₃): δ (ppm) 170 (CO amide), 167 (CO ester), 133 (Ar-C-NH), 129 (Ar-C-Cl), 113 (qt C), 77 (-CH amide), 76 (-CH ester), 52 (-OCH₃), 26 (CH₃). Anal. Calc. For C₁₄H₁₆ClNO₅ C, 53.60; H, 5.14; Cl, 11.30; N, 4.46; O, 25.50 Found C, 54.12; H, 5.09; Cl, 10.95; N, 4.65; O, 24.90.

4R,5R-Methyl5-((3-chlorophenyl)carbamoyl)-2,2-dimethyl-1,3-dioxolane-4-carboxylate (1k)

Yield: 81%, White crystalline solid, m.p. = 111-112 °C [α]_D²⁵ = + 2.78°, IR ν cm⁻¹: 3392 (-NH), 2951 (-CH stretching), 1752 (CO ester), 1682 (CO amide), 1586 (C=C), 1164 (C-O). ¹H-NMR (300 MHz, CDCl₃): δ (ppm) 7.96 (bs, -NH), 7.71 (s, 1H, Ar-H), 7.71-7.44 (m, 3H, Ar-H), 4.90 (d, $J = 5.2$ Hz, 2H -CH), 3.86 (s, 3H -OCH₃), 1.56 (s, 6H, (C-(CH₃)₂)). ¹³C-NMR (75 MHz, CDCl₃): δ (ppm) 170 (CO amide), 167 (CO ester), 137 (Ar-C-NH), 134 (Ar-C-Cl), 113 (qt C), 77 (-CH amide), 76 (-CH ester), 53 (-OCH₃), 26 (CH₃). Anal. Calc. For C₁₄H₁₆ClNO₅ C, 53.60; H, 5.14; Cl, 11.30; N, 4.46; O, 25.50 Found C, 54.12; H, 5.09; Cl, 10.95; N, 4.65; O, 24.90.

4R,5R-Methyl5-((4-chlorophenyl)carbamoyl)-2,2-dimethyl-1,3-dioxolane-4-carboxylate (1l)

Yield: 77%, White crystalline solid, m.p. = 89-90 °C [α]_D²⁵ = + 1.43°, IR ν cm⁻¹: 3348 (-NH-), 2984 (-CH stretching), 1755 (CO ester), 1680 (CO amide), 1592 (C=C), 1137 (C-O). ¹H-NMR (300 MHz, CDCl₃): δ (ppm) 8.29 (bs, -NH), 7.56 (d, $J = 6.9$ Hz, 2H Ar-H), 7.34 (d, $J = 6.9$ Hz, 2H Ar-H), 4.91 (d, $J = 6$ Hz, 2H -CH), 3.87 (s, 3H -OCH₃), 1.57 (s, 6H, (C-(CH₃)₂)). ¹³C-NMR (75 MHz, CDCl₃): δ

(ppm) 170 (CO amide), 167 (CO ester), 135 (Ar-C-NH), 129 (Ar-C-Cl), 113 (qt C), 77 (-CH amide), 76 (-CH ester), 53 (-OCH₃), 26 (CH₃);. Anal. Calc. For C₁₄H₁₆ClNO₅ C, 53.60; H, 5.14; Cl, 11.30; N, 4.46; O, 25.50 Found C, 54.12; H, 5.09; Cl, 10.95; N, 4.65; O, 24.90.

General Procedure for Deprotected Amides (2a-2l)

To the solution of the corresponding amide in MeOH (15 ml), acetyl chloride was added drop wise. After complete disappearance of the reactant the reaction mixture was concentrated on a rotary evaporator the mixture was extracted with EtOAc (4 × 15 ml). The organic layer was collected and dried with appropriate drying agent and concentrated on a rotary evaporator. The crude was then purified by column chromatography using methanol and chloroform as eluent.

2*R*,3*R*-Methyl-2,3-dihydroxy-4-oxo-4-(2-(trifluoromethyl)phenylamino)butanoate (2a)

Yield: 65%, White crystalline solid, m.p. = 130-132 °C [α]_D²⁵ = + 1.72°, IR ν cm⁻¹: 3479 (-OH), 3361 (-NH), 2926 (-CH stretching), 1726 (CO ester), 1693 (CO amide), 1591 (C=C), 1166 (C-O). ¹H-NMR (300 MHz, CDCl₃): δ (ppm) 9.37 (bs, -NH), 7.97-7.87 (m, 4H, ArH), 5.59 (d, *J* = 7.5 Hz, OH), 4.73 (dd, *J* = 7.5 Hz, 2H -CH), 3.77 (s, 3H -OCH₃); ¹³C-NMR (75 MHz, CDCl₃): δ (ppm) 172 (CO amide), 169 (CO ester), 135 (Ar-C-NH), 119 (CF₃), 73 (-CH amide), 72 (-CH ester), 51 (-OCH₃);. Anal. Calc. For C₁₂H₁₂F₃NO₅ C, 46.91; H, 3.94; F, 18.55; N, 4.56; O, 26.04 Found C, 47.03; H, 3.55; F, 18.30; N, 4.59; O, 26.20.

2*R*,3*R*-Methyl-2,3-dihydroxy-4-oxo-4-(3-(trifluoromethyl)phenylamino)butanoate (2b)

Yield: 78%, White crystalline solid, m.p. = 120-122 °C [α]_D²⁵ = + 1.75°, IR ν cm⁻¹: 3400 (-OH), 3308 (-NH), 2973 (-CH stretching), 1735 (CO ester), 1665 (CO amide), 1547 (C=C), 1176 (C-O). ¹H-NMR (300 MHz, CDCl₃): δ (ppm) 10.05 (bs, -NH), 8.26 (s, 1H, Ar-H), 7.99-7.87 (m, 3H, Ar-H), 5.58 (d, *J* = 7.5 Hz, OH), 4.55 (dd, *J* = 7.5 Hz, 2H -CH), 3.69 (s, 3H -OCH₃); ¹³C-NMR (75 MHz, CDCl₃): δ (ppm) 172 (CO amide), 171 (CO ester), 139 (Ar-C-NH), 116 (CF₃), 74 (-CH amide), 72 (-CH ester), 52 (-OCH₃); Anal. Calc. For C₁₂H₁₂F₃NO₅ C, 46.91; H, 3.94; F, 18.55; N, 4.56; O, 26.04 Found C, 47.03; H, 3.55; F, 18.30; N, 4.59; O, 26.20.

2*R*,3*R*-Methyl-2,3-dihydroxy-4-oxo-4-(4-(trifluoromethyl)phenylamino)butanoate (2c)

Yield: 81%, White crystalline solid, m.p. = 144-145 °C [α]_D²⁵ = + 1.23°, IR ν cm⁻¹: 3413 (-OH), 3304 (-NH-), 2957 (-CH stretching), 1735 (CO ester), 1670 (CO amide), 1597 (C=C), 1185 (C-O). ¹H-NMR (300MHz, CDCl₃): δ (ppm) 10.04 (bs, -NH), 7.98 (d, *J* = 8.4 Hz, 2H, Ar-H), 7.69 (d, *J* = 8.4 Hz, 2H, Ar-H), 6.11 (d, *J* = 6.9 Hz, OH), 4.54 (dd, *J* = 6.9 Hz, 2H -CH), 3.35 (s, 3H -OCH₃); ¹³C-NMR (75 MHz, CDCl₃): δ (ppm) 172 (CO amide), 171 (CO ester), 142 (Ar-C-NH), 119 (CF₃), 74 (-CH amide), 72 (-CH ester), 52 (-OCH₃); Anal. Calc. For C₁₂H₁₂F₃NO₅ C, 46.91; H, 3.94; F, 18.55; N, 4.56; O, 26.04 Found C, 47.03; H, 3.55; F, 18.30; N, 4.59; O, 26.20.

2*R*,3*R*-Methyl-2,3-dihydroxy-4-(2-methoxyphenylamino)-4-oxobutanoate (2d)

Yield: 78%, White crystalline solid, m.p. = 98-100 °C [α]_D²⁵ = + 0.86°, IR ν cm⁻¹: 3400 (-OH), 3371 (-NH), 2929 (-CH stretching), 1726 (CO ester), 1662 (CO amide), 1599 (C=C), 1174 (C-O). ¹H-NMR (300 MHz, CDCl₃): δ (ppm) 9.37 (bs, -NH), 8.31-8.28 (m, 4H, ArH), 5.58 (d, *J* = 7.5 Hz, OH), 4.89 (dd, *J* = 7.5 Hz, 2H -CH), 3.90 (s, 3H, Ar-OCH₃), 3.82 (s, 3H, -OCH₃). ¹³C-NMR (75 MHz, CDCl₃): δ (ppm) 172 (CO amide), 170 (CO ester), 148 (Ar-C-OCH₃), 126 (Ar-C-NH), 73 (-CH amide), 72 (-CH ester), 52 (-OCH₃); Anal. Calc. For C₁₂H₁₅NO₆ C, 53.53; H, 5.62; N, 5.20; O, 35.65 Found C, 53.77; H, 5.43; N, 4.91; O, 35.78.

2*R*,3*R*-Methyl-2,3-dihydroxy-4-(3-methoxyphenylamino)-4-oxobutanoate (2e)

Yield: 80%. White crystalline solid, m.p. = 98-99 °C [α]_D²⁵ = +2.45°, IR ν cm⁻¹: 3440 (-OH), 3314 (-NH-), 2998 (-CH stretching), 1739 (CO ester), 1667 (CO amide), 1595 (C=C), 1158 (C-O). ¹H-NMR (300 MHz, CDCl₃): δ (ppm) 9.37 (bs, -NH), 7.55-7.53 (m, 3H, Ar-H), 7.51 (s, 1H, Ar-H), 5.59 (d, *J* = 7.5 Hz, OH), 4.72 (dd, *J* = 7.5 Hz, 2H -CH), 3.78 (s, 3H, Ar-OCH₃), 3.76 (s, 3H -OCH₃); ¹³C-NMR (75 MHz, CDCl₃): δ (ppm) 172 (CO amide), 169 (CO ester), 160 (Ar-C-OCH₃), 139 (Ar-C-NH), 73 (-CH amide), 72 (-CH ester), 51 (-OCH₃); Anal. Calc. For C₁₂H₁₅NO₆ C, 53.53; H, 5.62; N, 5.20; O, 35.65 Found C, 53.77; H, 5.43; N, 4.91; O, 35.78.

2R,3R-Methyl-2,3-dihydroxy-4-(4-methoxyphenylamino)-4-oxobutanoate (2f)

Yield: 83%, White crystalline solid, m.p. = 111-113 °C [α]_D²⁵ = + 1.23°, IR ν cm⁻¹: 3390 (-OH), 3306 (-NH-), 2951 (-CH stretching), 1737 (CO ester), 1653 (CO amide), 1599 (C=C), 1179 (C-O). ¹H-NMR (300 MHz, CDCl₃): δ (ppm) 9.10 (bs, -NH), 7.69 (d, J = 7.6 Hz, 2H Ar-H), 6.91 (d, J = 7.6 Hz, 2H Ar-H), 5.58 (d, J = 7.5 Hz, OH), 4.89, dd, J = 7.5 Hz, 2H -CH), 3.89 (s, 3H, Ar-OCH₃), 3.80 (s, 3H -OCH₃); ¹³C-NMR (75 MHz, CDCl₃): δ (ppm) 172 (CO amide), 168 (CO ester), 156 (Ar-C-OCH₃), 131 (Ar-C-NH), 73 (-CH amide), 72 (-CH ester), 51 (-OCH₃); Anal. Calc. For C₁₂H₁₅NO₆ C, 53.53; H, 5.62; N, 5.20; O, 35.65 Found C, 53.77; H, 5.43; N, 4.91; O, 35.78.

2R,3R-methyl-4-(o-toluidino)-2,3-dihydroxy-4-oxobutanoate (2g)

Yield: 65%. White crystalline solid, m.p. = 122-124 °C [α]_D²⁵ = + 1.20°, IR ν cm⁻¹: 3390 (-OH), 3378, (-NH-), 2956 (-CH stretching), 1748 (CO ester), 1663 (CO amide), 1602 (C=C), 1162 (C-O). ¹H-NMR (300 MHz, CDCl₃): δ (ppm) 8.89 (bs, -NH), 8.52-8.49 (m, 4H, Ar-H), 5.58 (d, J = 7.5 Hz, OH), 4.71 (dd, J = 7.5 Hz, 2H -CH), 3.76 (s, 3H -OCH₃), 2.29 (s, 3H, Ar-CH₃); ¹³C NMR (75 MHz, CDCl₃): δ (ppm) 172 (CO amide), 169 (CO ester), 136 (Ar-C-NH), 130 (Ar-C-CH₃) 73 (-CH amide), 72 (-CH ester), 51 (-OCH₃); Anal. Calc. For C₁₂H₁₅NO₅ C, 56.91; H, 5.97; N, 5.53; O, 31.59 Found C, 56.70; H, 6.23; N, 5.41; O, 31.33.

2R,3R-Methyl-4-(m-toluidino)-2,3-dihydroxy-4-oxobutanoate (2h)

Yield: 76%, White crystalline solid, m.p. = 140-141 °C [α]_D²⁵ = + 1.87°, IR ν cm⁻¹: 3396 (OH), 3309, (-NH-), 2949, (-CH stretching), 1740, (CO ester), 1660, (CO amide), 1551, (C=C), 1172, (C-O). ¹H-NMR (300 MHz, CDCl₃): δ (ppm) 9.11 (bs, -NH), 7.53 (s, 1H, Ar-H), 7.50-7.48 (m, 3H, Ar-H), 6.11 (d, J = 7.5 Hz, OH), 4.72 (dd, J = 7.5 Hz, 2H -CH), 3.76 (s, 3H, -OCH₃), 2.31 (s, 3H, Ar-CH₃); ¹³C-NMR (75 MHz, CDCl₃): δ (ppm) 172 (CO amide), 169 (CO ester), 138 (Ar-C-NH), 137 (Ar-C-CH₃), 73 (-CH amide), 72 (-CH ester), 51 (-OCH₃); Anal. Calc. For C₁₂H₁₅NO₅ C, 56.91; H, 5.97; N, 5.53; O, 31.59 Found C, 56.70; H, 6.23; N, 5.41; O, 31.33.

2R,3R-Methyl-4-(p-toluidino)-2,3-dihydroxy-4-oxobutanoate (2i)

Yield: 82%, White crystalline solid, m.p. = 115-117 °C [α]_D²⁵ = + 1.17°, IR ν cm⁻¹: 3398 (OH); 3305 (-NH-), 2947 (-CH stretching), 1739 (CO ester), 1658 (CO amide), 1594 (C=C), 1123 (C-O); ¹H-NMR (300 MHz, CDCl₃): δ (ppm) 8.89 (bs, -NH), 7.51 (d, J = 8.1 Hz, 2H, Ar-H), 7.16 (d, J = 8.4 Hz, 2H, Ar-H), 5.59 (d, J = 6.9 Hz, OH), 4.67 (dd, J = 6.9 Hz, 2H -CH), 3.81 (s, 3H, -OCH₃), 2.32 (s, 3H, Ar-CH₃); ¹³C-NMR (75 MHz, CDCl₃): δ (ppm) 172 (CO amide), 169 (CO ester), 138 (Ar-C-NH), 137 (Ar-C-CH₃), 73 (-CH amide), 72 (-CH ester), 51 (-OCH₃). Anal. Calc. For C₁₂H₁₅NO₅ C, 56.91; H, 5.97; N, 5.53; O, 31.59 Found C, 56.70; H, 6.23; N, 5.41; O, 31.33.

2R,3R-Methyl-4-(2-chlorophenylamino)-2,3-dihydroxy-4-oxobutanoate (2j)

Yield: 85%, White crystalline solid, m.p. = 90-91 °C [α]_D²⁵ = + 1.12°, IR ν cm⁻¹: 3426 (OH), 3361 (-NH-), 2941 (-CH stretching), 1732 (CO ester), 1672 (CO amide), 1595 (C=C), 1125 (C-O) cm⁻¹; ¹H-NMR (300 MHz, CDCl₃): δ (ppm) 8.54 (bs, -NH), 8.21-7.98 (m, 4H, Ar-H), 5.58 (d, J = 7.5 Hz, OH), 4.72 (dd, J = 7.5 Hz, 2H -CH), 3.77 (s, 3H, -OCH₃); ¹³C-NMR (75 MHz, CDCl₃): δ (ppm) 172 (CO amide), 169 (CO ester), 134 (Ar-C-NH), 129 (Ar-C-Cl), 73 (-CH amide), 72 (-CH ester), 51 (-OCH₃); Anal. Calc. For C₁₁H₁₂ClNO₅ C, 48.28; H, 4.42; Cl, 12.95; N, 5.12; O, 29.23 Found C, 48.60; H, 4.30; Cl, 13.08; N, 5.34; O, 29.67.

2R,3R-Methyl-4-(3-chlorophenylamino)-2,3-dihydroxy-4-oxobutanoate (2k)

Yield: 77%, White crystalline solid, m.p. = 101-102 °C [α]_D²⁵ = + 2.12°, IR ν cm⁻¹: 3370 (OH), 3302 (-NH-), 2950 (-CH stretching), 1733 (CO ester), 1664 (CO amide), 1588 (C=C), 1125 (C-O); ¹H-NMR (300 MHz, CDCl₃): δ (ppm) 9.88 (bs, -NH), 7.95 (s, 1H, Ar-H), 7.84-7.79 (m, 3H, Ar-H), 6.11 (d, J = 7.5 Hz, OH), 4.51 (dd, J = 7.5 Hz, 2H -CH), 3.68 (s, 3H -OCH₃); ¹³C-NMR (75 MHz, CDCl₃): δ (ppm) 172, (CO amide), 171 (CO ester), 140 (Ar-C-NH), 133 (Ar-C-Cl), 74 (-CH amide), 72 (-CH ester), 52 (-OCH₃); Anal. Calc. For C₁₁H₁₂ClNO₅ C, 48.28; H, 4.42; Cl, 12.95; N, 5.12; O, 29.23 Found C, 48.60; H, 4.30; Cl, 13.08; N, 5.34; O, 29.67.

2R,3R-Methyl-4-(4-chlorophenylamino)-2,3-dihydroxy-4-oxobutanoate (2I)

Yield: 87%, White crystalline solid, m.p. = 110-112 °C [α]_D²⁵ = + 1.13°, IR ν cm⁻¹: 3380 (OH), 3300 (-NH-), 2948 (-CH stretching), 1737 (CO ester), 1660 (CO amide), 1593 (C=C), 1181 (C-O); ¹H-NMR (300 MHz, CDCl₃): δ (ppm) 9.81 (bs, -NH), 7.79 (d, *J* = 8.7 Hz, 2H Ar-H), 7.37 (d, *J* = 8.7 Hz, 2H, Ar-H), 5.59 (d, *J* = 7.5 Hz, OH), 4.50 (dd, *J* = 7.5 Hz, 2H -CH), 3.68 (s, 3H -OCH₃); ¹³C-NMR (75 MHz, CDCl₃): δ (ppm) 172 (CO amide), 170 (CO ester), 137 (Ar-C-NH), 128 (Ar-C-Cl), 74 (-CH amide), 72 (-CH ester), 52 (-OCH₃); Anal. Calc. For C₁₁H₁₂ClNO₅ C, 48.28; H, 4.42; Cl, 12.95; N, 5.12; O, 29.23 Found C, 48.60; H, 4.30; Cl, 13.08; N, 5.34; O, 29.67.

Material and Method

Assay for Antifungal Activity

In the present experiment three concentrations (100%, 80%, and 60%) of synthetic compounds were used to study the inhibition potential against three fungal and three bacterial strains.

The agar tube dilution method is used for determination of antifungal activity of extract [35]. DMSO was used as a control solvent, terbinafine was used as standard. The fungal strains were used in this study include *Fusarium solani*, *Helminthosporium sativum* and *Aspergillus niger*. Each fungal strain was maintained on Sabouraud dextrose agar (SDA) medium at 4 °C.

The samples for antifungal assay were prepared from initial stock of 100 mg of extract per ml of dimethyl sulfoxide (DMSO). Media for fungus was prepared by dissolving 6.5 g of SDA per 100 ml in distilled water pH was adjusted at 5.6. Test tubes were marked to 10 cm mark. The Sabouraud dextrose agar (Merck) dispensed as 4 mL volume into screw capped tubes or cotton plugged test tubes and was autoclaved at 121 °C for 21 minutes. Tubes were allowed to cool to 50 °C and non-solidified SDA was loaded with 67 μ L of compound pipette from the stock solution. This would give the final concentration of 200 μ g/mL of the pure compound in media. Tubes were then allowed to solidify in slanting position at room temperature. Three slants of the extract sample were prepared for each fungus species. The tubes containing solidified media and test compound were inoculated with 4 mm diameter

piece of inoculum, taken from a seven days old culture of fungus. One sample of each extract was prepared, which were used for positive control. Slants without extract were used for negative control. The test tubes were incubated at 28 °C for 7 days. Cultures were examined twice weekly during the incubation. Reading was taken by measuring the linear length of fungus in slant by measuring growth (mm) and growth inhibition was calculated with reference to negative control. The experiment was done on triplicate

Assay for Antibacterial Bioassay

Three strains of bacteria were used in the study *Enterobacter sp*, *Vibrio cholerae* and *Klebsiella sp*. Nutrient broth medium was prepared by dissolving 0.4 g of nutrient broth per 50 mL of distilled water for the growth of bacterial inocula; pH was adjusted at 7.0 and was autoclaved. Nutrient agar medium was prepared by dissolving 2.3 g agar in 100 ml of distilled water; pH was adjusted at 7.0 and was autoclaved at 121 °C.

The standard was prepared by adding 0.5 mL (0.048 M) barium chlorides to 99.5 ml (0.36 N) sulfuric acid. Barium sulfate turbidity standard (4-6 mL) and was taken in screw capped test tube and poured to inoculums till the inoculum give the same color as that of turbidity standard [36]. The organisms were maintained on nutrient agar medium at 4 °C. Centrifuged pallets of bacteria from 24 hours old culture in nutrient broth (SIGMA) of selected bacterial strains were mixed with physiological normal saline solution until a McFarland turbidity standard [10^6 colony forming unit (CFU) ml⁻¹] was obtained. Then this inoculum was used for seeding the nutrient agar.

Nutrient agar medium was prepared by adding nutrient agar (Merck) 2.3 g in 100 ml of distilled water; pH was adjusted at 7.0 and was autoclaved. It was allowed to cool up to 45 °C. Petri plates were prepared by pouring 75 ml of seeded nutrient agar and allowed to solidify. Four wells per plate were made with sterile cork borer (5 mm).

Using micropipette, 100 μ L of test solutions was poured in respective wells. These plates were incubated at 37 °C. After 24 hours of incubation the diameter of the clear zones of inhibitions was measured by a ruler. Antibacterial activity of two dilutions of each plant extract was determined against four bacterial strains.

Conclusion

All the protected and deprotected amides were found significant active at concentration of low % against used fungal and bacterial strains. The deprotected amides were found more active against fungal and bacterial strains as compared to protected amides. Compounds with halogen substitution showed potent activities against all the fungal and bacterial strains therefore, they may be served as lead compounds for further research in drug designing and medicinal chemistry in search of better antimicrobial agents.

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