RESEARCH ARTICLE

Synthesis and biological evaluation of amino acid and peptide conjugates of rhein derivatives

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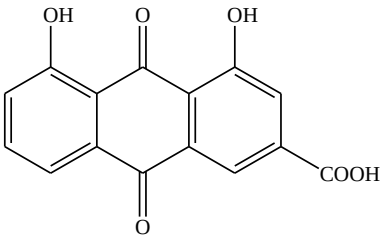
ABSTRACT

Rhein is an aromatic compound, provides basic framework for number of structural and functional units in plants and animals. Incorporation of amino acids and peptides into the aromatic congeners results in compounds with potent bioactivities. The experimental work involves: synthesis of rhein from barbaloin via formation of aloe-emodin and then diacerein from rhein. The peptide derivatives were synthesized by the coupling of rhein and diacerein with amino acid methyl esters/ dipeptides under continuous stirring for 36 hours. All the synthesized compounds were identified on the basis of Rf values, IR and ^{1}HNMR spectral data. All the synthesized compounds were screened for antimicrobial activity at 40, 80 and 160 µg/ml. The antibacterial activity of synthesized compounds was determined against bacterial strains viz. *E. coli* and *S. aureus* using ciprofloxacin as standard. The results revealed that rhein possess potent activity than diacerein and methyl esters of rhein/diacerein exhibited more activity as compared to dipeptides. The antifungal activity was also determined against fungal strains viz. *C. albicans* and *A. niger* using fluconazole as standard and results revealed that degree of inhibition increased as rhein/rhein methyl esters/dipeptide and decreased as diacerein/ diacerein methyl ester/dipeptide. Comparison of antibacterial and antifungal screening data suggested that rhein exhibits good antimicrobial activity profile than substituted rhein (diacerein).

Keywords: aromatic, rhein, peptides, antimicrobial

INTRODUCTION

Rhein (1, 8-dihydroxyanthraquinone-3-carboxylic acid) is a natural product having an anthraquinone scaffold found in *Rheum* species. It is also known as cassic acid, monorhein and rheic acid. It is an anthraquinone glycoside. Rhein and its analogues are a large class of compounds that have attracted their interest for a long time due to their therapeutic value. Rhein is found naturally in free state and as glucoside. It is obtained from *Rheum emodi* (Indian rhubarb) or *Rheum webbianum* (Chinese rhubarb) belonging to family Polygonaceae. Also, it is found in leaflets of *Cassia anngustifolia* as a minor constituent belonging to family Leguminosae. [1] Rhein is one of the important aromatic compounds. It has tricyclic structure consisting of anthraquinone moiety two –OH groups at 1, 8 positions and one carboxylic group at 3-position. [2] Rhein derivatives are known to be associated with broad spectrum of biological activities like anticancer, [3-4] hypoglycemic [5] and inhibition of IL-1[6].



Peptides are made of amino acids linked into linear chain with overall length up to 100 amino acids. Peptides are short polymers of amino acid monomers linked by peptide bonds. Every peptide has N-terminus and C-terminus residue on the ends of peptide. Peptides are synthesized by coupling the carboxyl group or C-terminus of one amino acid to the amino group or N-terminus of another. Thus, keeping in mind the pharmacological potential of rhein as well as taking advantage of biodegradability and biocompatibility of peptides, peptide derivatives of rhein was prepared to increase therapeutic efficacy and to decrease adverse effect. In present research, compound or moiety coupled with different peptide methyl ester using DCC and NMM to afford peptide derivatives. [7] Peptide derivatives of rhein and its derivatives show good antibacterial activity and antifungal activity.

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The newly synthesized rhein peptide derivatives were characterized by TLC, IR and NMR analysis.

MATERIALS AND METHODS

The chemicals used were obtained from Spectrochem Pvt. Ltd, Mumbai and S d fine-chem Limited, Mumbai. All the melting points were determined by open capillary method and uncorrected. The reactions were monitored by TLC on silica gel G plates using Chloroform: Methanol as developing solvent system in the ratio of 9: 1 and brown spots was detected on exposure to iodine vapours in tightly closed chamber. IR spectrum of compounds in KBr pellet was recorded on a FTIR-RXI spectrophotometer (PERKIN ELMER). ^{1}HNMR Spectra of compounds was recorded on Bruker NMR spectrometer in deuterium-substituted chloroform using TMS as internal Standard (Chemical Shift in δ ppm). The biological activity of Rhein, diacerein and its methylester/ dipeptide/ tripeptide/ tetrapeptide on different bacterial and fungal strains were tested using ciprofloxacin (MIC: 40 µg/ml) as antibacterial standards and fluconazole (MIC: 40 g/ml) as antifungal standard.

Synthesis of Boc-amino acids:

L-amino acid (20mmol) was dissolved in 1 mmol/litre sodium hydroxide (20ml) and isopropanol (20ml). Ditert-butylpyrocarbonate (Boc) (26mmol) in isopropanol (10ml) was added followed by 1 mmol/litre sodium hydroxide (20ml) to the resulting solution. The solution was stirred at room temperature for 2 hrs, washed with light petroleum ether (b.p 40-60°C) (20ml), acidified to pH 3.0 with 1 mmol/litre H_2SO4 and finally extracted with chloroform (3x20ml).The organic layer was dried over anhydrous sodium sulphate and evaporated under reduced pressure to give the crude product, which was crystallized from chloroform and petroleum ether (b.p 40-60 ºC) to get semi-solid mass of compound.

Synthesis of L-amino acid methyl ester hydrochlorides:

Thionyl chloride (1.4ml, 20mmol) was slowly added to methanol (100ml) at 0°C and L-amino acid (2.3g, 20mmol) was added to above solution. The resulting mixture was refluxed for 12 hrs at 70°C. Methanol was evaporated and the residue was triturated with ether at 0°C, until excess dimethyl sulphate was removed. The crude solid was crystallized from methanol and ether at 0°C to give pure amino acid methyl ester.

Synthesis of linear peptide fragments:

Amino acid methyl ester hydrochloride/peptide methyl ester (10mmol) was dissolved in chloroform (20mL). To this, N-methylmorpholine (21mmol) was added at 0 °C and the reaction mixture was stirred for 15 minutes. Boc-amino acid/peptide (10mmol) in chloroform (20mL) and N, N-dicyclohexylcarbodiimide (10 mmol) were added with stirring. After 36hrs, the reaction mixture was filtered and the residue was washed with chloroform (30mL) and added to the filtrate. The filtrate was washed with 5% sodium bicarbonate and saturated sodium chloride solutions. The organic layer was dried over anhydrous sodium sulphate, filtered and evaporated in vacuum. The crude product was crystallized from a mixture of chloroform and petroleum ether followed by cooling at 0 °C. Deprotection at carboxyl terminal was done by adding lithium hydroxide (0.36 g, 15mmol) to a solution of Boc-di/tripeptide methyl ester (10mmol) in tetrahydrofuran: Water (1: 1, 36ml) at 0 °C. Resulting mixture was stirred at RT for 1 hr and then acidified to pH 3.5 with 1N H2SO4. The aqueous layer was extracted with diethyl ether (3 × 25mL). The combined organic extracts were dried over anhydrous sodium sulphate and concentrated under reduced pressure. The crude product was crystallized from methanol and ether to get pure Boc-di/tripeptides. Deprotection at amino terminal was accomplished by treatment of Boc di/ tripeptides (10 mmol) dissolved in chloroform (15mL) with trifluoroacetic acid (2.28g, 20mmol). The resulting solution was stirred at RT for 1 hr, washed with saturated sodium bicarbonate solution (25mL). The

organic layer was dried over anhydrous sodium sulphate and concentrated under reduced pressure. The crude product was purified by crystallization from chloroform and petroleum ether (B.p. 40-60 °C) to get pure di/tri/tetrapeptide methyl esters.

Synthesis of 9, 10-dihydro-4, 5-dihydroxy-9, 10-dioxo-anthracene-2-carboxylic acid (Rhein)

Synthesis of aloe-emodin from barbaloin (1a)

10 g of barbaloin was added to an acidic solution comprising of a mixture of 250 ml of conc.HCl with 750 ml of water. 500 ml of a 20% aqueous solution of $FeCl_3$ solution was added to the above acidic solution and resulting mixture was added to the round bottom flask. Toluene, about 300 ml was added to the above solution and the biphasic mixture refluxed for 8 hrs at 100 ± 2^{0}C to yield crystals of compound **1a.**

Synthesis of 9, 10-dihydro-4, 5-dihydroxy-9, 10-dioxo-anthracene-2-carboxylic acid (Rhein) (1b)

Sodium nitrite 1.275 g was dissolved in 7 ml of H_2SO_4, the solution was heated to about 120^{0}C for oxidation of compound **1a**. Compound **1a**, 0.5 g added in parts to this mixture over a period of 30 mins. The reaction mixture was kept at this temperature for 5 hrs. At the end of 5 hrs, the reaction mixture was poured into ice to get orange brown precipitates. The precipitates so formed was filtered and dried to obtain crude compound **1b**. This was then dissolved in sodium carbonate solution pH below 9.5 and extracted with organic solvent (toluene). Compound 32b again regenerated from sodium bicarbonate solution using hydrochloric acid. The precipitates are then filtered, washed, dried and recrystallized from methanol to obtain pure crystals of compound **1b** (3.5g, 65.6%)

Synthesis of 9, 10-dihydro-4, 5-dihydroxy-9, 10-dioxo-anthracene-2-carboxylic acid peptide derivatives (2-3):

To a mixture of compound amino aci methtl ester/ Dipeptide methyl ester (10 mmol) in THF (75 ml), 2.3 ml of NMM was added at 0 °C with stirring. Rhein(10 mmol) in THF (75 ml) and DCC (2.1 g, 10 mmol) were added to the above mixture and stirring was done for 36 h. After 36 h, the reaction mixture was filtered and residue was washed with THF (50 ml). Then, filtrate was washed with 5% sodium hydrogen carbonate and saturated sodium chloride solution (30 ml) and dried over anhydrous Na2SO4, filtered and evaporated in vacuum. The crude product obtained was crystallized from a mixture of chloroform and n-hexane followed by cooling at 0^o C to give pure compound **(2-3).**

Synthesis of 9, 10-dihydro-4, 5-diacetyloxy-9, 10-dioxo-anthracene-2-carboxylic acid (4)

Compound **(1b)** 10 g was dissolved in acetic anhydride (2 ml) and the solution was cooled at 0^{0}C. Then, H_2SO_4 was added (7.2 ml) and the reaction mixture was warmed at 3^{0}C. After completion of the reaction (4-5 hrs), the reaction mixture was poured into distilled water at 4^{0}C and the precipitates were formed. The precipitates so formed was filtered, washed with distilled water and recrystallized from ethanol to give pure semi-solid compound **7** (4.2g, 74%).

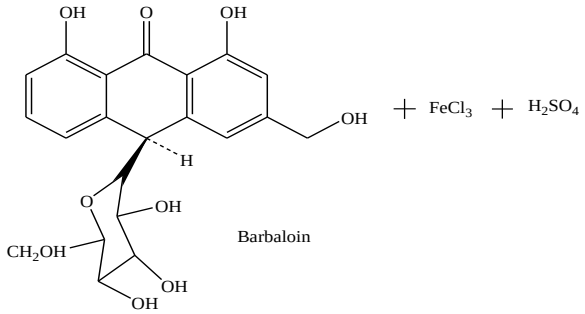
Synthesis of 9, 10-dihydro-4, 5-diacetyloxy-9, 10-dioxo-anthracene-2-carboxylic acid peptide derivatives (5-6)

To a mixture of compound amino aci methtl ester/ Dipeptide methyl ester (10 mmol) in THF (75 ml), 2.3 ml of NMM was added at 0 °C with stirring. Rhein(10 mmol) in THF (75 ml) and DCC (2.1 g, 10 mmol) were added to the above mixture and stirring was done for 36 h. After 36 h, the reaction mixture was filtered and residue was washed with THF (50 ml). Then, filtrate was washed with 5% sodium hydrogen carbonate and saturated sodium chloride solution (30 ml) and dried over anhydrous Na2SO4, filtered and evaporated in vacuum. The crude product obtained was crystallized from a mixture of chloroform and n-hexane followed by cooling at 0^0 C to give pure compound **(5-6).**

Characterizations of synthesized compounds were done by TLC, IR and NMR techniques and corresponding values of synthesized compounds are listed in table 1.

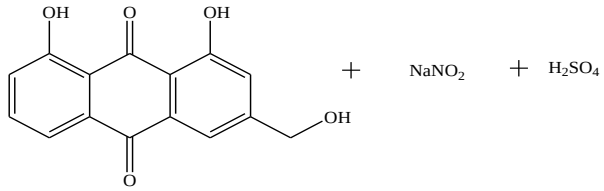
SCHEME OF WORK

Scheme 1



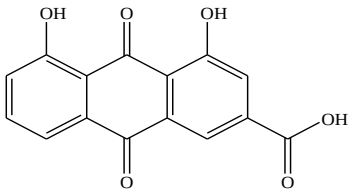
Barbaloin

Reflux
8hrs

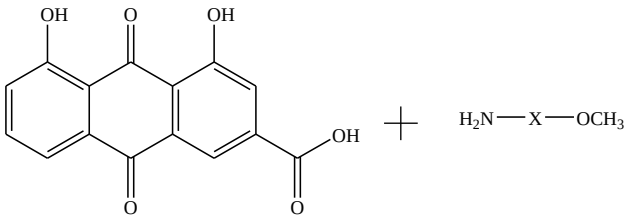


Aloe-emodin

Reflux
5hrs



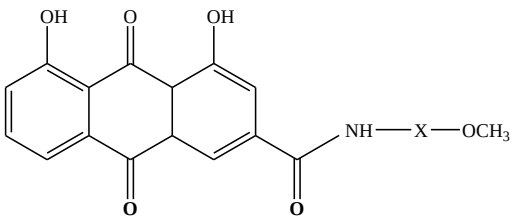
Rhein (1b)



1b

DCC
NMM

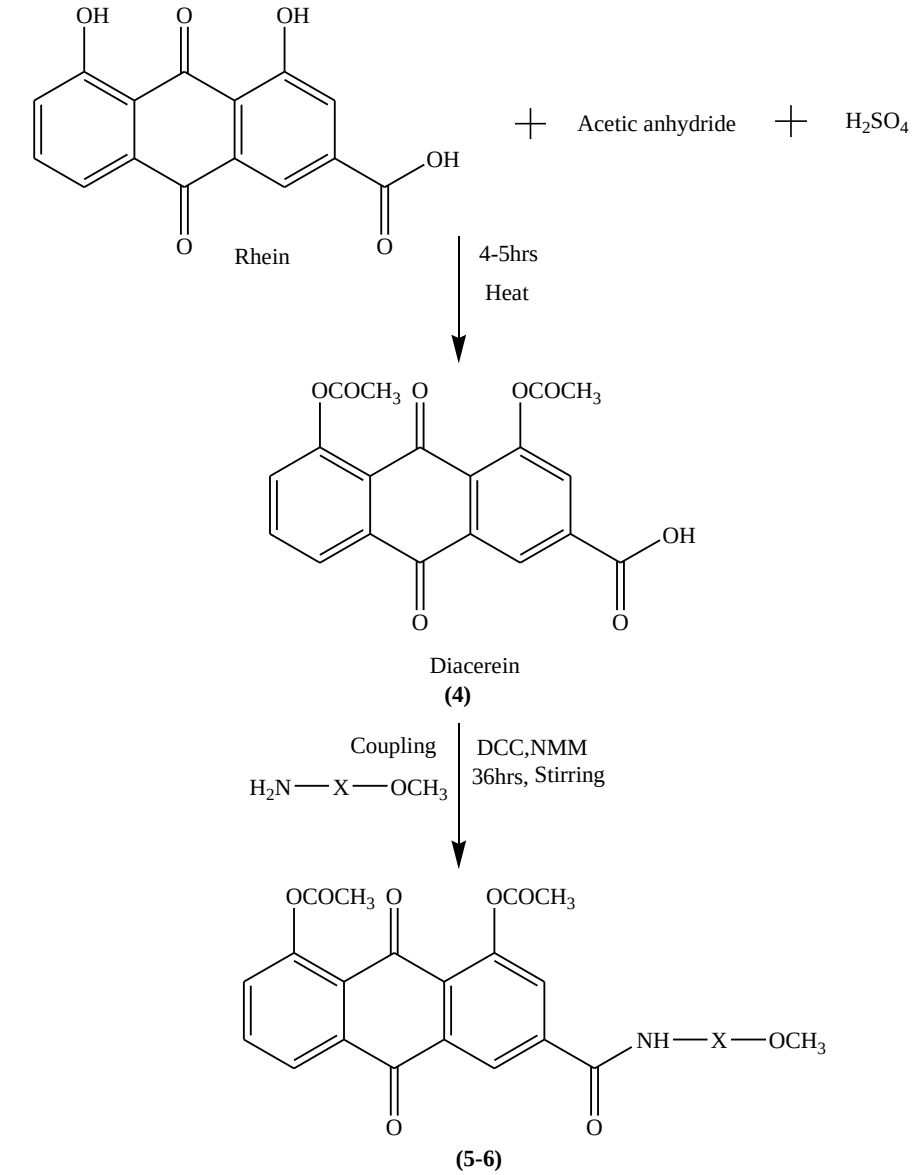
36hrs
Stirring
coupling



2-3

X= Arg **(2),** Ala-Trp **(3)**

SCHEME 2



X= Arg **(5),** Ala-Trp **(6)**

Biological activity:

Antimicrobial activity

An antibacterial and antifungal agent is a substance that kills or inhibits the growth of bacteria or fungi respectively. Both activities can be determined by two methods i.e. turbidimetric method, disc diffusion method. The synthesized peptide derivatives were screened for antibacterial activity against *Escherichia coli* (Gram-negative bacteria) and *Staphylococcus aureus* (Gram-positive bacteria) ,antifungal activity against fungal strain *Aspergillus niger* and *Candida albicans* using modified Kirby-Bauer disc diffusion method. All synthesized compounds were dissolved separately to prepare stock solution of 1mg ml^{-1} using dimethyl sulphoxide (DMSO) and chloroform as solvent. From this stock solution further dilutions of concentration 40, 80 and 160 µg ml^{-1} were prepared respectively. [8-9]

Firstly, nutrient agar medium was prepared by using the different ingredients mentioned above and then this medium was poured into the petri plates. Petri plates were inoculated with bacterial suspension and incubated at 37^0C for 24 hrs. After 24 hours, petri plates were taken out from incubator. Each petri plate was divided into five equal portions along the diameter to place one disc. Three discs of test samples 40, 80 and 160 µg ml^{-1} were placed on three portions together and one disc with standard drug

ciprofloxacin (40µg ml^{-1}) and a disc impregnated with the solvent (DMSO and Chloroform) as negative control. The test samples tested at the concentration 40, 80, 160µg ml^{-1} were checked, incubated and the diameters obtained for the test samples were compared with the diameter obtained with standard drug ciprofloxacin and fluconazole at 40µg ml^{-1}.

MIC values of synthesized compounds are listed in table 2 and in table 3.The results of antibacterial activity are shown in fig. 1&2 and antifungal activity are shown in fig 3& 4.

RESULTS AND DISCUSSION

Table 1: TLC, IR, NMR ranges of compounds

Cmpds	IR ranges (KBr) υ cm-1	NMR ranges(DMSO) δ ppm	TLC Rf value
1b	3420 (O-H stretch, aromatic), 3063 (O-H stretch, COOH), 3032 (C-H stretch, aromatic), 1696 (C=O stretch, carbonyl), 1652 (C=O stretch, COOH), 1456 (C=C stretch, aromatic)	12.0 (s, 1H, COOH), 10.92 (s, 2H, OH), 7.0-8.2(m, 5H, aromatic) ppm	0.48
2	3425 (O-H stretch, aromatic), 3365 (N-H stretching, 2^0 amide), 3335 (N-H stretching, amine), 3020 (C-H stretch, aromatic), 1735 (C=O stretching, ester), 1698 (C=O stretch, carbonyl), 1680 (C=O stretching, 2^0 amide), 1531 (N-H bending, amide), 1455 (C=C stretch, aromatic), 1286 (C-O stretching, ester) .	10.96 (s, 2H, OH), 8.24 (s, 1H, NH, amide), 7.4-8.0 (m, 5H, aromatic), 4.4 (m, 2H, CH_2, Arg), 3.79 (s, 3H, OCH_3), 2.48 (m, 2H, CH_2, Arg), 1.94 (s, 4H, NH, Arg), 1.86 (m, 2H, CH_2, Arg), 1.56 (m, 2H, CH_2, Arg) ppm	0.46
3	3424 (O-H stretch, aromatic), 3402 (N-H stretching, 2^0 amide), 3235 (N-H stretching, amine), 3042 (C-H stretch, aromatic), 1734 (C=O stretching, ester), 1685 (C=O stretching, 2^0 amide), 1688 (C=O stretch, carbonyl), 1532 (N-H bending, amide), 1480 (C=C stretch, aromatic), 1286 (C-O stretching, ester)	10.98 (s, 2H, OH), 8.03 (s, 2H, NH, amide), 7.15-7.18 (m, 5H, Trp), 7.2-8.0 (m, 5H, aromatic), 4.30-4.36 (m, 1H, α-H's, Trp), 4.20-4.24 (m, 1H, CH, Ala), 3.70 (s, 1H, NH, Trp), 3.53 (s, 3H, OCH_3), 3.24-3.26 (m, 2H, β-H's, Trp), 1.42 (d, 3H, CH_3, Ala)	0.50
4	3250 (O-H stretch, COOH), 3026 (C-H stretch, aromatic), 1735 (C=O stretching, esters), 1720 (C=O stretch, COOH), 1698 (C=O stretch, carbonyl), 1482 (C=C stretch, aromatic).	12.2 (s, 1H, COOH), 7.8-8.4 (m, 5H, aromatic), 2.69(s, 6H, $OCOCH_3$) ppm.	0.27
5	3410 (N-H stretching, 2^0 amide), 3335 (N-H stretching, amine), 3020 (C-H stretch, aromatic), 1736 (C=O stretching, esters), 1690 (C=O stretch, carbonyl), 1686 (C=O stretching, 2^0 amide), 1652, 1562 (N-H bending, amide), 1482 (C=C stretch, aromatic), 1186 (C-O stretching, ester)	7.84 (s, 1H, NH, amide), 7.2-8.0 (m, 5H, aromatic), 4.2-4-6 (m, 2H, CH_2, Arg), 3.52 (s, 3H, OCH_3), 2.68 (s, 6H, $OCOCH_3$), 2.46-2.48 (m, 2H, CH_2, Arg), 1.92 (s, 4H, NH, Arg), 1.86 -1.90 (m, 2H, CH_2, Arg), 1.58-1.78 (m, 2H, CH_2, Arg),	0.24
6	3412 (N-H stretching, 2^0 amide), 3335 (N-H stretching, amine), 3012 (C-H stretch, aromatic), 1746 (C=O stretching, ester), 1680 (C=O stretching, 2^0 amide), 1668 (C=O stretch, carbonyl), 1532 (N-H bending, amide), 1492 (C=C stretch, aromatic), 1286 (C-O stretching, ester)	8.14 (s, 2H, NH, amide), 7.8-8.4 (m, 5H, Trp), 7.2-7.9 (m, 5H, aromatic), 4.34-4.38 (m, 1H, α-H's, Trp), 4.4-4.8 (m, 1H, CH,Ala), 3.74 (s, 3H, OCH_3), 3.68(s, 1H, NH, Trp) , 3.24-3.26 (m, 2H, β-H's, Trp), 2.70 (s, 6H, $OCOCH_3$) , 1.46 (d, 3H, CH_3, Ala) ppm.	0.26

Table 2: Antibacterial activity of different compounds against *S.aureus and E.coli*

Synthesized compounds	*Staphylococcus aureus*		*Escherichia coli*	
	MIC (µg/ml)	Zone of inhibition (mm)	MIC (µg/ml)	Zone of inhibition (mm)
1b	80	4.0	80	3.2
2	40	3.4	40	2.9
3	40	3.0	80	2.8
4	160	2.5	160	3.5
5	40	3.1	80	3.0
6	80	2.4	80	1.9
Ciprofloxacin(std)	40	4.6	40	4.5

From the table 1 this seems that all compounds give different MIC against *S.aureus* bacterial strain. But compounds **2, 3 and 5** are most effective against S.aureus and compound 2 is most effective against E.coli gives MIC at 40 µg/ml (shown in fig. 1 and 2)

Table 3: Antifungal activity of different compounds against *A. niger*

Synthesized Compounds	*Aspergillus niger*		*Candida albicans*	
	MIC (µg/ml)	Zone of inhibition (mm)	MIC (µg/ml)	Zone of inhibition (mm)
1b	80	3.0	80	2.2
2	40	2.9	40	2.3
3	80	2.6	40	2.1
4	80	2.8	160	2.8
5	40	2.3	40	1.8
6	80	2.3	80	2.1
Fluconazole (std)	40	4.8	40	4.9

From table 2, this seems that all compounds give different MIC against *A. niger* and C. albicans fungal strain. But compounds **2 and 5** are most effective against A.niger and compound 2, 3 and 5 are most effective against C.albicans and gives MIC at 40 µg/ml (Shown in fig. 3 and 5)

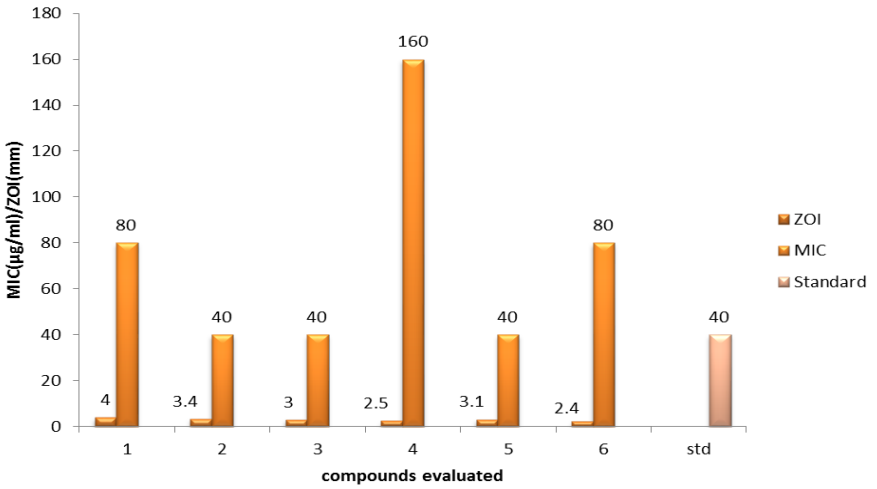


Fig 1: Comparison of minimum inhibitory concentration (µg/ml)/Zone of inhibition of standard Ciprofloxacin and synthesized derivatives in case of *S.aureus*.

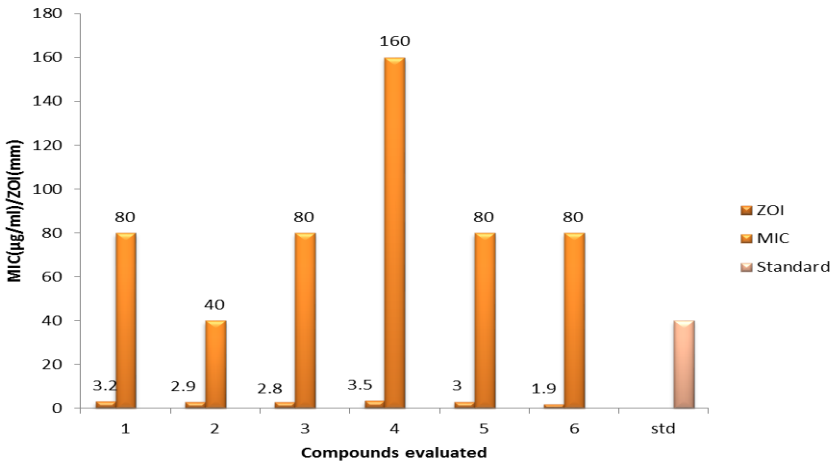


Fig 2: Comparison of minimum inhibitory concentration (µg/ml)/ Zone of inhibition of standard Ciprofloxacin and synthesized derivatives in case of *E.coli*

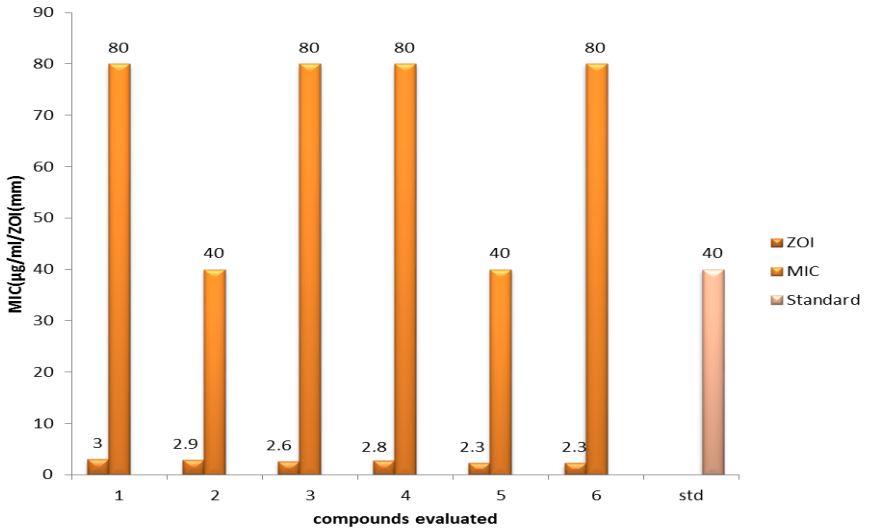


Fig 3: Comparison of minimum inhibitory concentration (µg/ml)/ Zone of inhibition of standard Fluconazole and synthesized derivatives in case of *A.niger.*

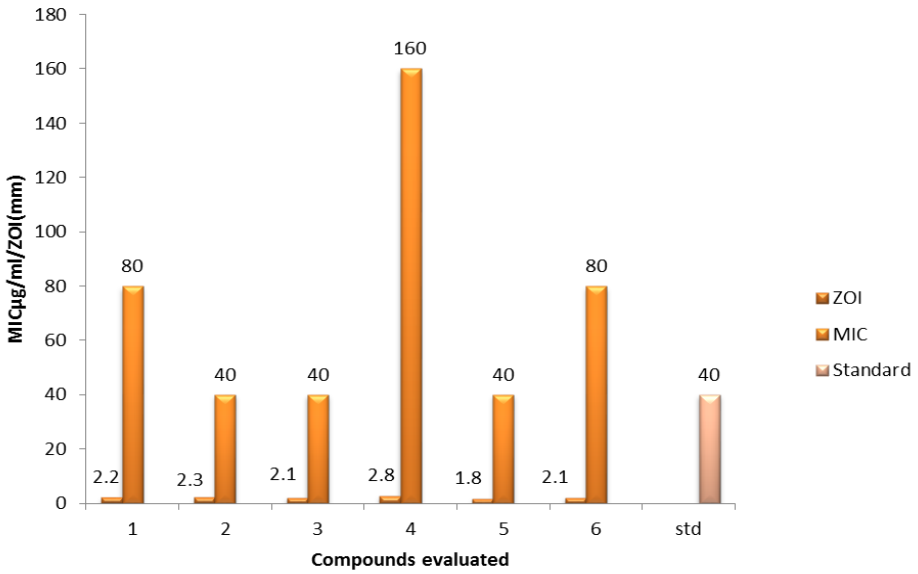


Fig 4: Comparison of minimum inhibitory concentration (µg/ml)/ Zone of inhibition of standard Fluconazole and synthesized derivatives in case of *C.albicans.*

CONCLUSION

Synthesis of all peptide derivatives were carried out successfully via coupling reaction with good yields. N, N-dicyclohexylcarbodiimide proved to be a good coupling agent both economically and yield wise. The title compounds were synthesized by coupling of rhein and diacerein with amino acid methyl esters/ dipeptides in the presence of N, N-dicyclohexylcarbodiimide as a coupling agent and N-methylmorpholine as a base under continuous stirring for 36 hrs. All synthesized peptide derivatives were identified on the basis of melting point range, Rf values, and IR and ^{1}H NMR spectral data confirmed the identity of the synthesized compounds. Rhein and its peptide derivatives showed the presence of characteristic absorption bands in the region between 1680-1630 cm^{-1}, 1750-1730 cm^{-1} respectively and in NMR data the compounds show peaks of esters and amides in the range between 3.7-4.1, 7-8.5 ppm respectively.Rhein and its methylester /dipeptide derivatives all showed mild to moderate antimicrobial activity. From the antibacterial studies, we concluded that newly synthesized peptide derivative **2,3,5** exhibits highest activity against *S. aureus;* compounds and compound **2** exhibits good activity against *E. coli.* at 40 µg/ml concentration. In case of fungal strains,

compound **2,5** exhibits highest activity against *C. albicans*; compound **2,3,5** exhibits potent activity against *A. niger.*

REFERENCES

1. Xiaokang Z. Synthesis and hypoglycaemic activity evaluation of rhein amide derivatives. Med. Chem. Res. 2012; 21: 417- 420.
2. Mohammed S. The pharmacological actions of naturally occurring 1, 8-dihydroxy anthraquinone derivatives. World J. Agri. Sci. 2008; 4 (4): 495-505.
3. Yong Z. Rhein lysinate inhibits cell growth by modulating various mitogen activated protein kinase in cervical cancer cells. Oncology lett. 2011; 2: 129-133.
4. Yang X. Novel rhein analogues as potential anticancer agents. Med. Chem. Res. 2011; 6: 2294-2301.
5. Xiaokang Z. Synthesis and hypoglycaemic activity evaluation of 7-alkoxyl rhein. Med. Chem. Res. 2012; 21: 421-427.
6. Moldovan F. Diacerein and rhein reduce the ICE-induced IL-1β and IL-8 activation in human osteoarthritic cartilage. Osteoarthritis Res. Soc. Int. 2000; 8: 186-196.
7. Bharathi K., Haseena Banu B. Applications of Peptide Coupling Reagents – An Update. Int. J. Pharma. Sci. Rev. Res. 2011; 8-11.
8. Carolina H. Antifungal free acids: a review science against microbial pathogens. Comm. Curr. Res. Tech. Adv. 2011; 4: 61-71.
9. Dahiya R., K. Ramninder. Synthesis and biological screening of a novel series of 3, 4, 5.-trisubstituted Phenoxyacetic acid analogues Aus. J. Basic. App. Sci. 2007; 1(4): 525-532.

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