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SYNTHESIS AND ANTIMICROBIAL ACTIVITY OF 4-[3,4-INDOLO-2,5-DIAZA-BICYCLO[4,3,1]-DECA-1(9),2,4,6(10),7-PENTENE-8-CARBONYL AMINO] BENZOYL PROLINES

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ABSTRACT: A series of 4-[3,4-indolo-2,5-diaza-bicyclo[4,3,1]-deca-1(9),2,4,6(10),7-pentene-8-carbonyl amino] benzoyl prolines were synthesized from 4-amino benzoic acid **(1)**. The newly synthesized compounds were characterized on the basis of elemental analysis, IR, and ^{1}H NMR. All the synthesized compounds were tested for their antibacterial activities against Gram + and Gram – bacteria, and antifungal activities. Antimicrobial and antifungal activities of the final compounds have been evaluated and all the compounds have shown significant inhibition of bacterial and fungal growth.
KEYWORDS: Proline, N-substituted isatin, antimicrobial activity.

INTRODUCTION

There is an increasing demand for the preparation of new antimicrobial agents due to developing resistance towards conventional antibiotics. A number of biological activities such as antibacterial[1-6], antifungal[1, 3,4,6,7], antiviral activities[1, 4] have been associated with N-substituted isatin attached with various amino acids containing compounds. Therefore we have synthesized a new class of combinational molecules in which all these moieties are present and evaluated for antimicrobial and antifungal activities. The structures of the compounds synthesized were assigned on the basis of elemental analysis and spectral analysis.

EXPERIMENTAL

GENERAL

Melting points were determined in open capillaries and are uncorrected. The IR spectra were recorded on a BioRad TS Spectrophotometer. The ^{1}H NMR spectra were recorded on a Brucker DRX- 300 MHz spectrometer using DMSO-d_6 as a solvent and tetramethylsilane (TMS) as an internal standard and expressed in δ ppm. Purity of synthesized compounds was checked by TLC using Silica gel G. Spots were exposed in an iodine chamber.

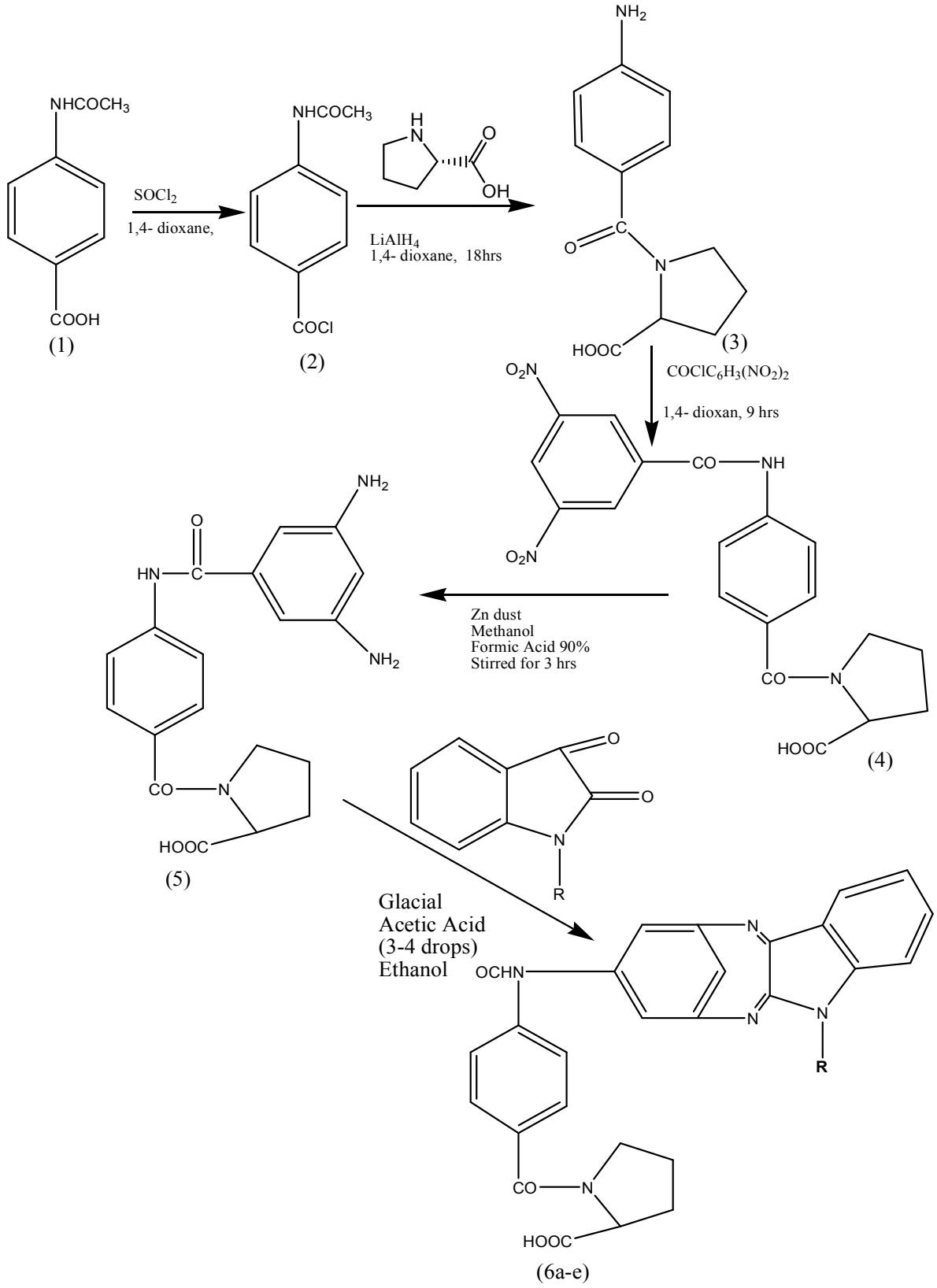
SYNTHESIS OF 4-ACETAMIDO BENZOYL CHLORIDE (2)

4-acetamido benzoic acid **(1)** (0.036 mole) was dissolved in 1, 4 dioxane and thionyl chloride (0.04 mole) was added drop wise and mixture refluxed for 10h. The reaction mixture was poured in cold water then filtered, dried and recrystallised from 1, 4-dioxane to give compound **(2)**. The completion of reaction was monitored by TLC and purified by column chromatography on silica gel (60-120 mesh) eluting with ethyl acetate: methanol (5: 3).

Rf Value: 0.62; λ_{max}: 155.

Yield: 52%; m.p. 220^{0}C; IR (KBr cm^{-1}), 1790 (C=O), 775-740 (C-Cl); ^{1}H-NMR δ 4.00 (s, 2H, NH_2 Ar), 6.67-7.82(m, 4H, Ar),Anal.Calcd. for C_7H_6NOCl: C 54.04, H 3.89, N 9.00 Found: C 54.01, H 3.82, N 9.13 %.

SCHEME 1. SYNTHESIS OF 4-[3,4-INDOLO-2,5-DIAZA-BICYCLO[4,3,1]-DECA-1(9),2,4,6(10),7-PENTENE-8-CARBONYL AMINO] BENZOYL PROLINES.



SYNTHESIS OF 1-(4- AMINO BENZOYL)-PYRROLIDINE-2-CARBOXYLIC ACID (3)

Para acetamido benzoyl chloride (0.018 mole) was reduced to para amino benzoyl chloride with lithium aluminium hydride. The resulting product was dissolved in 1, 4-dioxane to yield solution A. L-Proline (0.021 mole) was dissolved in 20 ml 0.1 N NaOH to yield solution B. Both the solutions A and B were added, reaction mixture was refluxed for 18h.The resulting mixture was poured in 1N HCl then the crude product was filtered, dried and recrystallised from ethanol to furnish compound **(3)**.

The completion of reaction was monitored by TLC and purified by column chromatography on silica gel (60-120 mesh) eluting with ethyl acetate: methanol (5: 3).

Rf Value: 0.61; λ_{max}: 234.

Yield: 42%;m.p. 286^{0}C; IR (KBr cm^{-1}), 1740(C=O), 3710 (OH); ^{1}H-NMR δ: 4.0 (s, 2H, NH_2 Ar), 3.45(m 2H),1.76(m 2H) 1.81(m 2H),3.98(CH pyrrolidine)11.0(s,1H,COOH pyrrolidine), Anal.Calcd. for $C_{12}H_{14}N_2O_3$: C 61.53, H 6.02, N 11.96 Found: C 61.63, H 6.04, N 11.87 %.

SYNTHESIS OF 1-[4-(3, 5-DINITROBENZOYL) AMINOBENZOYL]-PYRROLIDINE-2-CARBOXYLIC ACID (4)

Compound **(3)** dissolved in 0.1 N NaOH (15 ml) and a solution of 3, 5-dinitrobenzoyl chloride (0.008 mole) in 1, 4-dioxane was added and reaction mixture refluxed for 10h. The reaction mixture was poured in 1N HCl and then product was filtered, dried and recrystallised from methanol to yield compound **(4)**. The completion of reaction was monitored by TLC and purified by column chromatography on silica gel (60-120 mesh) eluting with ethyl acetate: methanol (5: 3).

Rf Value: 0.63; λ_{max}: 428

Yield: 78%; m.p. 272^{0}C; IR (KBr cm^{-1}): 1745 (C=O), 3440 (NH), 1670 (CONH);^{1}H-NMR δ: 8.32 (s,1H, NHAr), 11.12 (s, 1H, COOH),Anal.Calcd. for $C_{19}H_{16}N_4O_8$: C 53.27, H 3.76, N 13.08 Found: C 53.29, H 3.78, N 13.11 %.

SYNTHESIS OF 1-[4(3, 5-DIAMINOBENZOYL) AMINOBENZOYL]-PYRROLIDINE-2-CARBOXYLIC ACID (5)

Compound **(4)** (0.004 mole) and zinc dust (0.011 mole) dissolved in methanol and formic acid 3 ml (90%) was added drop wise and mixture was stirred for 3h. The mixture was then filtered and organic layer was removed under reduced pressure. The resulting solid residue was dissolved in ether and excess of formic acid was removed by washing with saturated sodium chloride solution and solvent was removed under reduced pressure and recystallised from methanol to afford **(5).** The completion of reaction was monitored by TLC and purified by column chromatography on silica gel (60-120 mesh) eluting with ethyl acetate: methanol (5: 3).

Rf Value: 0.78; λ_{max}: 368

Yield: 38%; m.p. 165^{0}C; IR (KBr cm-1): 1748 (C=O), 3440 (NH), 1678 (CONH); ^{1}H-NMR δ: 8.23 (s, NH Ar), 4.45(s, 2H, NH2 Ar), 4.40 (s, 2H, NH2 Ar) 11.10 (s, 1H, COOH pyrrolidine), Anal.Calcd. for $C_{19}H_{20}N_4O_4$: C 61.95,H 5.47, N 15.21 Found: C 61.87, H 5.67, N 15.24 %.

SYNTHESIS OF 4-[3, 4-INDOLO-2, 5-DIAZA-BICYCLO [4, 3, 1]-DECA-1(9), 2, 4, 6(10), 7-PENTENE-8-CARBONYL AMINO] BENZOYL PROLINES(6a-e):

GENERAL PROCEDURE:

A solution of various N-substituted isatin (0.0046 mole) in 30 ml ethanol prepared then a mixture of compound **(5)** (0.004 mole) in 20 ml 0.1 N NaOH and phosphorous pentoxide (0.012 mole) were added immediately. The resulting mixture was refluxed for 6h then poured into 1N HCl. The mixture was filtered, dried and recrystallised from methanol to yield **(6a-e)**. The completion of reaction was monitored by TLC and purified by column chromatography on silica gel (60-120 mesh) eluting with ethyl acetate: methanol (5: 3).

4-[3,4-INDOLO-2,5-DIAZA-BICYCLO[4,3,1]-DECA-1(9),2,4,6(10),7-PENTENE-8-CARBONYL AMINO]-BENZOYL PROLINE (6a)

Rf Value: 0.63; λ_{max} :495

Yield: 32%; m.p.: 209^{0}C; IR (KBr cm^{-1}): 1613, 3433, 3300, 1533, and 1289,^{1}H-NMR δ: 7.08(s,NH indole), 6.92(1H, NH Ar), 3.44(m 2H),1.77(m 2H) 1.76(m 2H),3.92(CH pyrrolidine)11.02(s,1H,COOH pyrrolidine;Anal. Calcd. For $C_{26}H_{21}N_5O_4$: C, 66.80, H, 4.53, N, 14.98 Found: C, 66.78, H 4.51, N 14.94 %.

4-[3,4-(N-METHYL) INDOLO-2,5-DIAZA-BICYCLO[4,3,1]-DECA-1(9),2,4,6(10),7-PENTENE-8-CARBONYL AMINO]- BENZOYL PROLINE (6b)

Rf Value:0.63; λ_{max} :509

Yield: 21%; m.p.: 180^{0}C; IR (KBr cm-1): 1614, 3430, 3320, 1540, and 1280,^{1}H-NMR δ: 6.94(1H,NHAr),3.34(m 2H),1.77(m 2H) 1.86(m 2H),3.72(CH pyrrolidine)11.07(s,1H,COOH pyrrolidine,3.79 (3H,N-CH_3 indole)Anal. Calcd. For $C_{27}H_{23}N_5O_4$: C 67.35, H 4.81, N 14.54, Found: C 67.37, H 4.83, N 14.49%.

4-[3,4-(N-METHYLENE-1-PYRROLE) INDOLO-2,5-DIAZA-BICYCLO[4,3,1]-DECA-1(9),2,4,6(10),7-PENTENE-8-CARBONYL AMINO]- BENZOYL PROLINE (6c)

Rf Value: 0.15; λ_{max} :592

Yield: 24%; m.p.: 95^{0}C; IR (KBr cm-1): 1610, 3400, 3340, 1560, and 1240;^{1}H-NMR δ: 6.94(1H,NHAr),3.43(m 2H),1.57(m 2H) 1.83(m 2H),3.92(CH pyrrolidine)11.03(s,1H,COOH pyrrolidine),1.26(2H,methylene pyrrole); Anal. Calcd. For$C_{31}H_{26}N_6O_6$: C 68.12, H 4.79, N 15.38, Found: C 68.16, H 4.72, N 15.40 %.

4-[3,4-(N-METHYLENE-4-METHYL PIPERAZIN-1-YL) INDOLO-2,5-DIAZA-BICYCLO[4,3,1]-DECA-1(9),2,4,6(10),7-PENTENE-8-CARBONYL AMINO]-BENZOYL PROLINE (6d)

Rf Value:0.25; λ_{max} : 620

Yield: 21%; m.p.: 107^{0}C; IR (KBr cm-1): 1620, 3460, 3290, 1560, and 1235;^{1}H-NMR δ: 6.94(1H,NHAr),3.43(m 2H),1.57(m 2H) 1.83(m 2H),3.92(CHpyrrolidine)11.03(s,1H,COOH pyrrolidine),1.27(2H,methylene),3.24(t,4H,piperazine);Anal.Calcd.For $C_{32}H_{33}N_7O_4$: C 66.31, H 5.74, N 16.91 Found: C 66.29, H 5.77, N 16.87 %.

4-[3,4-(N-METHYLENE-3-METHYL PIPERAZIN-1-YL) INDOLO-2,5-DIAZA-BICYCLO[4,3,1]-DECA-1(9),2,4,6(10),7-PENTENE-8-CARBONYL AMINO]-BENZOYL PROLINE (6e)

Rf Value:0.33; λ_{max} :620

Yield: 26 %; m.p.: 110^{0}C; IR (KBr cm-1): 1633, 3443, 3279, 1570, and 1268; ^{1}H-NMR δ: 6.93(1H,NHAr)3.30(m,2H,),1.63(m,2H,),1.79(m2H),4.10 (CH,pyrrolidine),11.01(s,1H,COOH pyrrolidine),1.26(2H,methylene),3.24(t,4H,piperazine), 3.86(q, 1H, NH), 2.28(d, 3H, CH_3, piperazine) ;Anal. Calcd. For $C_{32}H_{33}N_7O_4$: C 66.31, H 5.74, N 16.91, Found: C 66.30, H 5.76, N 16.93 %.

RESULTS AND DISCUSSION

SYNTHESIS

4-[3,4-indolo-2,5-diaza-bicyclo[4,3,1]-deca-1(9),2,4,6(10),7-Pentene-8-carbonylamino]benzoyl prolines **(6a-e)** have been synthesized by reaction between 4-amino benzoic acid **(1)** and thionyl chloride in 1,4-dioxane at reflux for 10h which afforded 4-aminobenzoyl chloride**(2)**. In the IR spectrum of compound **(2)** bands in the range of 1790 cm^{-1} were obtained due to C=O stretching in COCl group and disappearance of the IR band at 3240 cm^{-1} due to OH group and the appearance of a new band at 775-740cm^{-1} due to C-Cl bond.

Compound **(2)** on treatment with proline in 1, 4 – dioxane yielded 1-(4-aminobenzoyl)-pyrrolidine-2-carboxylic acid **(3)**.In the IR spectrum of compound **(3)** C=O stretching (attached with lactam ring) was observed at1740cm^{-1} which was observed at 1790cm^{-1} in compound **(2)**.In the ^{1}H NMR spectrum signals were found at δ 3.45 (multiplet), 1.76(multiplet), 1.81(multiplet),3.98(CH pyrrolidine)11.0(singlet,COOH pyrrolidine) which showed the attachment of proline amino acid. Compound **(3)** on reaction with 3, 5 dinitrobenzoyl chloride gave 1-[4-(3, 5-dinitrobenzoyl) aminobenzoyl]-pyrrolidine-2-carboxylic acid **(4)** on reduction afforded 1-[4(3, 5-diaminobenzoyl) aminobenzoyl]-pyrrolidine-2-carboxylic acid **(5)**.The final compounds **(6a-e)** were produced by the reaction of compound **(5)** and various N-substituted isatin. Formation of the final compounds **(6a-e)** was confirmed by the absence of NH_2 stretching at 3140cm^{-1} and also by absence of proton signals at δ 4.45(singlet ,2H,NH_2 Ar), 4.40(singlet ,2H,NH_2 Ar) in the compounds .These reactions are summarized in Scheme1. The purity of compounds was monitored by TLC.

ANTIMICROBIAL ACTIVITIES

The antimicrobial activities of the synthesized compounds **(6a-e)** were determined by the agar diffusion technique [8, 9]. The organism tested were *Staphylococcus aureus* (NCIM 2492), *Bacillus subtilis* (NCIM 2493), *Bacillus Stearothermophilus*(NCIM 2328),*Escherichia coli* (NCIM) for antibacterial activity and *Aspergillus niger*(NCIM 590), *Candida albicans*(NCIM 347) for antifungal activity. The agar media were inoculated with test organisms and a solution of the tested compound in DMSO (250 µg/ml) was placed separately in cups (8mm diameter) in the agar medium. Streptomycin (25µg), Ampicillin (5 µg/ml) and Fluconazole (300 µg/ml) were used as a reference for the antibacterial and antifungal activities respectively. The inhibition zones were measured after 24 hr incubation. The results of the antimicrobial activity tests are summarized in Table 2. Most of the synthesized compounds were found to possess varied antimicrobial activities towards all the microorganisms used with minimum inhibitory concentration (MIC).

CONCLUSION

A new series of 4-[3,4-indolo-2,5-diaza-bicyclo[4,3,1]-deca-1(9),2,4,6(10),7-pentene-8-carbonyl amino] benzoyl prolines were synthesized by the steps mentioned in experimental part. The structure of the synthesized compounds was confirmed by IR and ^{1}HNMR method. All the compounds were evaluated for antibacterial and antifungal activity. Compounds have shown promising antibacterial and antifungal activity. Therefore these can be further investigated.

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TABLE 1 .SUBSTITUENTS OF COMPOUNDS (6a-e)

COMPOUND	SUBSTITUENTS (R)
6a	hydrogen
6b	methyl
6c	1-ethyl-2,3-dihydro-1*H*-pyrrole
6d	1-ethyl-4-methylpiperazine
6e	1-ethyl-3-methylpiperazine

TABLE 2: ANTIMICROBIAL ACTIVITY OF SOME SYNTHESIZEDCOMPOUNDS (6a-e)

Compound	S. aureus (NCIM 2492)	B. subtilis (NCIM 2493)	B. stearothermophilus (NCIM 2328)	E. coli (NCIM)	A.niger (NCIM 590)	C.albicans (NCIM 347)
6a	+++	+++	++	++	++	+++
6b	+++	++	+++	++	++	++
6c	++	++	++	+	++	-
6d	++	+++	+++	+	+	++
6e	+++	++	++	-	+	++

Key:
- **-** **Inactive (inhibition zone<0.50 mm)**
- **+** **Less active (inhibition zone 0.50-2 mm)**
- **++** **Moderately active (inhibition zone 2- 3.00 mm)**
- **+++** **Highly active (inhibition zone 3-4.00 mm)**

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