MICROWAVE ASSISTED SYNTHESIS OF *N*-(1-ISOPROPYL-2- OXOINDOLIN-5-YL) SUBSTITUTED AMIDE DERIVATIVES AND THEIR ANTIMICROBIAL ACTIVITY

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ABSTRACT

A series of *N*-(1-isopropyl-2-oxoindolin-5-yl)substituted amide derivatives **6a-h** has been synthesized with 5-amino-1-isopropylindolin-2-one using HATU and EDC.HCl under microwave conditions. All final compounds were characterized by different spectral techniques. These target compounds (**6a-h**) were assessed for their antimicrobial activity and showed significant activity. Among the compounds tested **6f**, **6h**, **6e**, **6g** and **6b** were found potent antimicrobial activities at concentrations 75 and 100 µg/mL.

Keywords: Benzimidazole, Heterocyclic, HATU, EDC.HCl.

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INTRODUCTION

In the continuous development of new drugs, some of the heterocyclic compounds have been found important source for development of new bioactive compounds. Among these, indolin-2-one derivatives possess a vital role such as antimicrobial,¹ anticancer,² antiproliferative³ and anti-inflammatory activities.⁴ The increasing significance in biological profile of indolin-2-one⁵⁻¹⁰ derivatives prompted us to the synthesis of new heterocyclic compounds which are pharmacologically active.

In recent years, radiation like microwaves (MWs) has been used to establish the reaction conditions for number of organic reactions.¹¹ MW technique provides simple, efficient, economical and safe mimicking to green chemistry. Reactions executed under MW condition ensure fast and good in yields than conventional heating. This irradiation method is alternative to the conventional method.

Inspired by above mentioned biological activities and the advantages of microwave irradiation over conventional method, herein we report the synthesis of the amide derivatives of indolin-2-one(**6a-h**) from 5-amino-1-isopropylindolin-2-one (**4**) and different acids (**5a-g**) under microwave conditions in high yields. These derivatives were screened for their antimicrobial activity against eight bacterial and three fungal species.

EXPERIMENTAL

Bruker AV-400 NMR spectrometer used for NMR spectra for CDCl₃ solutions. The chemical shifts were measured in ppm against internal TMS. ESI mass spectra were recorded on a QSTARXL hybrid MS system. TLC was carried out on Merck silica gel 60 F₂₅₄ TLC plates.

General Procedure for the Synthesis of Compounds 6a-h

Conventional Heating Method

To a stirred individual solutions of 5-amino-1-isopropylindolin-2-one (**4**) (0.2 g, 1.05mmol), substituted acids (**5a-h**) (1.05 mmol) in DMF (10 mL) was added EDC.HCl (1 mmol), HATU (1.2 mmol) and the reaction mixture was stirred for 12h at 80 °C. After complete conversion of the **4** into products as indicated

by the TLC the reaction mixture was poured over crushed ice, solid obtained was filtered, washed with excess of water and dried. The crude product obtained was purified with 100-200 silica mesh with 30% EtOAc/hexane as eluent.

Microwave Irradiation Method

To individual solutions of 5-amino-1-isopropylindolin-2-one (**4**) (0.2 g, 1.05 mmol), substituted acids (**5a-h**) (1.05 mmol) in dimethylformamide (10 mL) was added EDC.HCl (1 mmol), HATU (1.2 mmol) and the mixture was employed in a microwave tube and exposed to microwave irradiation at 180 W for 8–20 min. After consumption of the compound **4** into products. The reaction mixture was poured over crushed ice, solid obtained was filtered. The crude product obtained was purified with column chromatography using 100-200 silica mesh eluting with 30% EtOAc/hexane.

2-Iodo-N-(1-isopropyl-2-oxoindolin-5-yl)nicotinamide (**6a**)

Brown solid; M.P. 168-170 °C; ¹H NMR (400 MHz, CDCl₃) δ, ppm 1.46 (d, *J* = 4.72 Hz, 6H), 3.54 (s, 2H), 4.68-4.67 (m, *J* = 1H), 7.01 (d, *J* = 8.48 Hz, 1H), 7.50-7.38 (m, 2H), 7.67 (s, 1H), 8.39 (d, *J* = 4.49 Hz, 1H), 8.50 (d, *J* = 15.26 Hz, 1H), 8.67 (ddd, *J* = 9.75, 7.64, 1.82 Hz, 1H). ¹³C NMR (100 MHz, CDCl₃) δ, ppm 174.4, 159.7, 150.6, 143.5, 141.0, 131.5, 125.8, 122.7, 122.6, 120.3, 118.4, 116.4, 166.2, 109.7, 43.6, 36.0, 19.2. Anal. Calcd for: C₁₇H₁₆IN₃O₂: C, 48.47; H, 3.83; I, 30.13; N, 9.98; O, 7.60. Found: C, 48.41; H, 3.82; N, 9.96.

Scheme-1: Synthesis of N-(1-isopropyl-2-oxoindolin-5-yl) Substituted Amide Derivatives

2-Bromo-N-(1-isopropyl-2-oxoindolin-5-yl)nicotinamide(6b)

White solid; M.P. 158-159 °C; ^1H NMR (400 MHz, CDCl_3) δ , ppm 1.48 (d, $J = 4.32$ Hz, 6H), 3.58 (s, 2H), 4.66-4.56 (m, $J = 1$ Hz), 7.03 (d, $J = 8.42$ Hz, 1H), 7.56-7.33 (m, 2H), 7.67 (s, 1H), 8.36 (d, $J = 4.50$ Hz, 1H), 8.48 (d, $J = 15.11$ Hz, 1H), 8.62 (ddd, $J = 9.73, 7.65, 1.84$ Hz, 1H). ^{13}C NMR (100 MHz, CDCl_3) δ , ppm 174.3, 164.2, 151.2, 142.4, 141.1, 132.2, 123.4, 122.4, 122.6, 121.3, 118.8, 116.2, 108.7, 43.5, 35.9, 19.1. Anal. Calcd. for: $\text{C}_{17}\text{H}_{16}\text{BrN}_3\text{O}_2$: C, 54.56; H, 4.31; Br, 21.35; N, 11.23; O, 8.55. Found: C, 48.41; H, 4.30; N, 11.21.

6-Iodo-N-(1-isopropyl-2-oxoindolin-5-yl)picolinamide(6c)

Brownish solid; M.P. 171-173 °C; ^1H NMR (400 MHz, CDCl_3) δ , ppm 1.51-1.46 (m, 6H), 3.54 (s, 2H), 4.68 (d, $J = 7.02$ Hz, 1H), 7.01 (d, $J = 8.53$ Hz, 1H), 7.54 (dd, $J = 8.47, 2.01$ Hz, 1H), 7.67 (d, $J = 7.94$ Hz, 1H), 7.75 (t, $J = 7.73$ Hz, 2H), 8.24 (d, $J = 7.53$ Hz, 1H), 9.56 (s, 1H). ^{13}C NMR (100 MHz, CDCl_3) δ , ppm 173.49, 162.1, 151.36, 140.26, 131.00, 124.96, 121.36, 119.50, 117.28, 110.00, 77.33, 77.01, 76.31, 43.56, 36.21, 19.35. Anal. Calcd for: $\text{C}_{17}\text{H}_{16}\text{IN}_3\text{O}_2$: C, 48.47; H, 3.83; I, 30.13; N, 9.98; O, 7.60. Found: C, 48.46; H, 3.81; N, 9.97.

6-Bromo-N-(1-isopropyl-2-oxoindolin-5-yl)picolinamide(6d)

White solid; M.P. 198-200 °C; ^1H NMR (400 MHz, CDCl_3) δ , ppm 1.51-1.46 (m, 6H), 3.54 (s, 2H), 4.68 (d, $J = 7.04$ Hz, 1H), 7.01 (d, $J = 8.51$ Hz, 1H), 7.56 (dd, $J = 8.49, 2.02$ Hz, 1H), 7.67 (d, $J = 7.93$ Hz, 1H), 7.79 (t, $J = 7.75$ Hz, 2H), 8.26 (d, $J = 7.55$ Hz, 1H), 9.64 (s, 1H). ^{13}C NMR (100 MHz, CDCl_3) δ , ppm 174.50, 163.2, 150.47, 141.4, 139.97, 130.98, 125.86, 121.48, 119.48, 117.49, 109.84, 77.35, 77.03, 76.71, 43.66, 36.21, 19.35. Anal. Calcd for: $\text{C}_{17}\text{H}_{16}\text{BrN}_3\text{O}_2$: C, 54.56; H, 4.31; Br, 21.35; N, 11.23; O, 8.55. Found: C, 54.51; H, 4.29; N, 11.21.

N-(1-Isopropyl-2-oxoindolin-5-yl)-2-(4-(trifluoromethyl)phenyl)acetamide(6e)

White solid; M.P. 162-163 °C; ^1H NMR (400 MHz, CDCl_3) δ , ppm 1.46 (d, $J = 10.88$ Hz, 6H), 3.44 (s, 2H), 3.74 (d, $J = 21.89$ Hz, 2H), 4.63 (d, $J = 6.94$ Hz, 1H), 6.90 (d, $J = 8.45$ Hz, 1H), 7.26-7.17 (m, 1H), 7.45 (dd, $J = 15.04, 10.42$ Hz, 4H), 7.62 (d, $J = 7.88$ Hz, 2H). ^{13}C NMR (100 MHz, CDCl_3) δ , ppm 174.52, 168.31, 140.59, 138.68, 132.09, 129.69, 125.86, 125.73, 119.80, 117.95, 109.73, 77.36, 77.04, 76.73, 44.06, 43.67, 36.12, 19.28. Anal. Calcd for: $\text{C}_{20}\text{H}_{19}\text{F}_3\text{N}_2\text{O}_2$: C, 63.82; H, 5.09; F, 15.14; N, 7.44; O, 8.50. Found: C, 63.80; H, 5.08; N, 7.43.

N-(1-Isopropyl-2-oxoindolin-5-yl)-2-(4-(methylsulfonyl)phenyl)acetamide(6f)

White solid; M.P. 121-123 °C; ^1H NMR (400 MHz, CDCl_3) δ , ppm 1.45 (d, $J = 2.87$ Hz, 6H), 3.07 (s, 3H), 4.79-4.49 (m, 1H), 3.81 (s, 2H), 3.46 (s, 2H), 7.03-6.82 (m, 1H), 7.27 (s, 2H), 7.56 (d, $J = 4.58$ Hz, 3H), 7.94 (d, $J = 4.71$ Hz, 2H). ^{13}C NMR (100 MHz, CDCl_3) δ , ppm 174.45, 167.71, 141.18, 140.67, 130.32, 127.84, 119.74, 117.89, 77.35, 77.03, 76.72, 44.54, 43.65, 36.13, 19.31. Anal. Calcd for: $\text{C}_{20}\text{H}_{22}\text{N}_2\text{O}_2\text{S}$: C, 62.16; H, 5.74; N, 7.25; O, 16.56; S, 8.30. Found: C, 62.15; H, 5.73; N, 7.24.

N-(1-Isopropyl-2-oxoindolin-5-yl)-1-methyl-1H-pyrazole-5-carboxamide(6g)

White solid; M.P. 180-181 °C; ^1H NMR (400 MHz, CDCl_3) δ , ppm 1.46 (d, $J = 10.88$ Hz, 6H), 3.52 (s, 2H), 4.21 (d, $J = 8.67$ Hz, 3H), 4.67-4.68 (m, 1H), 6.66 (d, $J = 1.80$ Hz, 1H), 6.99 (d, $J = 8.48$ Hz, 1H), 7.39-7.30 (m, 1H), 7.51 (d, $J = 2.02$ Hz, 1H), 7.60 (s, 1H), 7.71 (s, 1H). ^{13}C NMR (100 MHz, CDCl_3) δ , ppm 174.5, 158.1, 140.7, 137.5, 135.2, 131.8, 125.7, 120.2, 118.2, 109.8, 106.5, 43.6, 39.8, 36.0, 19.2. Anal. Calcd for: $\text{C}_{16}\text{H}_{18}\text{N}_4\text{O}_2$: C, 64.41; H, 6.08; N, 18.78; O, 10.73. Found: C, 64.40; H, 6.07; N, 18.77.

N-(1-Isopropyl-2-oxoindolin-5-yl)-4-methylisoxazole-3-carboxamide(6h)

White solid; M.P. 190-191 °C; ^1H NMR (400 MHz, CDCl_3) δ , ppm 1.47 (t, $J = 7.00$ Hz, 6H), 2.56 (d, $J = 6.38$ Hz, 3H), 3.51 (s, 2H), 4.67-4.68 (m, 1H), 6.99 (t, $J = 6.01$ Hz, 1H), 7.30 (dd, $J = 8.47, 1.72$ Hz, 1H), 7.61-7.44 (m, 2H), 8.80 (s, 1H). Anal. Calcd for: $\text{C}_{16}\text{H}_{17}\text{N}_3\text{O}_3$: C, 64.20; H, 5.72; N, 14.04; O, 16.04. Found: C, 64.19; H, 5.71; N, 14.03.

BIOLOGICAL ASSAY

Bacterial and Fungal Strains

The bacterial strains were purchased from the Public Health England Culture Collections. Fungal strains were collected from the department of biotechnology, Chaitanya P.G.College (autonomous), Hanamkonda-506001, Telangana, India. All microbial strains stored at -80°C were streaked on Luria–Bertani (LB) agar plate and incubated at 37°C for 24h. Fewer numbers of isolated colonies were chosen from each plate and suspended in 5 mL of LB broth in a hygienic culture vessel. The vessel was plugged with cotton and gestated with gentle shaking (140 rpm) at 37°C for 20 hours.

Preparation of Inoculums

Following the protocol of the Kirby–Bauer disk diffusion assay,¹² four to five well-isolated colonies of the same morphological type were picked with an inoculating loop, transferred into 5 mL of nutrient broth, and incubated at 37°C for 24 h until slight noticeable turbidity appeared. The turbidity of the vigorously growing broth cultures was then adjusted with broth to a density equivalent to that of a 0.5 McFarland standard, and the resulting suspensions were used as the initial inocula in the assay.

Antimicrobial Assay

The initial inocula of the test organisms, 100 μL , were swabbed over the surface of the agar media (20 mL) in Petri dishes and let to be absorbed for 15 min. Wells, 8 mm in diameter, were made with a sterile cork borer in the seeded agar plates. Solutions of the test compounds in DMSO (100 μL ; c 75 and 100 mg/mL) were then loaded into the wells and incubated in the air at 37°C for 24 h. The inhibition zone diameter was measured with a zone reader (Antibiotic Zone Scale). The positive control was the standard drug Gentamicin.

RESULTS AND DISCUSSION

As depicted in Scheme-1, new derivatives of indolin-2-ones (**6a–h**) were synthesized in five steps protocol. The key intermediate 5-amino-1-isopropylindolin-2-one (**4**)¹³⁻¹⁵ was prepared from commercially available indoline-2,3-dione and 2-iodopropane in the presence of sodium hydride in DMF for reacting 10 min at room temperature to give 1-isopropylindoline-2,3-dione (**1**). Compound **1** was reduced in the presence of hydrazine hydride at 130°C , yielded 1-isopropylindolin-2-one (**2**).¹² Further, nitration of compound **2** was carried out by reacting with the nitrating mixture (sodium nitrate and Con. H_2SO_4) at room temperature for 30 min to yield 1-isopropyl-5-nitroindolin-2-one (**3**).¹³ The reduction of 1-isopropyl-5-nitroindolin-2-one (**3**) in the presence of iron and NH_4Cl in solvent mixture of ethanol and water (1:1) produced 5-amino-1-isopropylindolin-2-one (**4**).¹⁴ Structure of the compound **4** was confirmed with the already reported melting point $453\text{--}455^{\circ}\text{C}$.¹⁴ As we found amine (**4**) compound as a better precursor, the same has been taken in to design amide compounds based on the acid-amine coupling reaction. Different acids (**5a–h**) in DMF solvent were used for the acid-amine coupling reaction with 5-amino-indolin-2-one (**4**) in the presence of EDC.HCl and HATU under MW irradiation to get compounds **6a–h** in high yields. The conventional process to obtain the same compounds **6a–h** produced low yields and the reaction time also more than under MW irradiation (Table-1). The structures of all the newly synthesized compounds were characterized by ^1H NMR, ^{13}C NMR spectral data and MASS.

Table-1: *N*-(1-Isopropyl-2-oxoindolin-5-yl) Substituted Amide Derivatives

Entry	Compound	Room Temperature		MW	
		Time	Yield ^a	Time	Yield ^a
1.	<p style="text-align: center;">6a</p>	12 h	45%	10 min	88%

2.	6b	12 h	46%	10 min	89%
3.	6c	12 h	48%	17 min	87%
4.	6d	12 h	43%	15 min	89%
5.	6e	12 h	47%	20 min	86%
6.	6f	12 h	38%	20 min	82%
7.	6g	12 h	44%	20 min	79%
8.	6h	12 h	43%	20 min	80%

^aIsolated Yield

Table-2: In Vitro Antibacterial Activity of 6a-h

Compound	Conc. μg/mL	Zone of Inhibition (mm)							
		Gram-positive bacteria				Gram-negative bacteria			
		<i>M. tub</i>	<i>M. lut</i>	<i>B. sub</i>	MRSA	<i>P. aer</i>	<i>K. pne</i>	<i>E. coli</i>	<i>P. vul</i>
6a	75	17	13	15	13	14	16	19	20
	100	18	15	16	16	16	17	20	21

6b	75	21	19	19	18	19	19	23	24
	100	23	21	21	20	20	21	24	25
6c	75	14	11	13	09	12	13	17	17
	100	16	13	13	10	12	14	18	19
6d	75	18	16	18	17	16	18	21	20
	100	20	17	20	19	17	19	22	23
6e	75	25	23	25	24	23	21	26	25
	100	27	24	26	25	24	23	28	27
6f	75	28	25	27	29	26	24	29	28
	100	32	26	29	30	27	26	31	29
6g	75	23	22	23	20	22	20	25	24
	100	25	24	26	23	23	22	28	24
6h	75	27	23	26	27	24	23	27	27
	100	30	25	28	28	25	25	29	28
Zentamicin	75	29	27	30	31	28	27	31	29
	100	32	30	33	33	31	30	33	31

Mycobacterium tuberculosis (M. tub), *Micrococcus luteus* (M. lut), *Bacillus subtilis* (B. sub), Methicillin-resistant *Staphylococcus aureus* (MRSA), *Pseudomonas aeruginosa* (P. aer), *Klebsiella pneumonia* (K. pne), *Escherichia coli* (E. coli), *Proteus vulgaris* (P. vul), No Activity (NA).

Table-3: In Vitro Antifungal Activity of 6a-h

Compound	Conc. µg/mL	Zone of Inhibition (mm)		
		M. canis	E. floccosum	M. gypseum
6a	75	16	15	17
	100	18	16	19
6b	75	17	15	18
	100	21	17	19
6c	75	14	13	16
	100	15	14	16
6d	75	15	14	16
	100	17	15	17
6e	75	20	18	19
	100	23	19	19
6f	75	23	19	18
	100	25	20	19
6g	75	19	17	18
	100	22	18	20
6h	75	22	18	19
	100	25	19	20
Nystatin	75	25	20	19
	100	28	23	24

Microsporium canis (M. canis), *Epidermophyton floccosum* (E. floccosum), *Microsporium gypseum* (M. gypseum).

All the final target compounds (**6a-h**) were screened for their antibacterial activity against four Gram-positive and four-gram negative bacterial species and the analogs **6a-h** showed pronounced antibacterial activity compared to standard drug Gentamycin (Table-2). Among all the tested compounds **6f**, **6h**, **6e**, **6g** and **6b** showed promising zone of inhibition compared to all other compounds. These compounds (**6a-h**) were also assessed for their antifungal activity against three fungal species and the compounds **6f**, **6h**, **6e** and **6b** exhibited significant antifungal activity compared to standard drug Nystatin (Table-3).

CONCLUSION

A series of novel *N*-(1-isopropyl-2-oxoindolin-5-yl) substituted amide derivatives **6a-h** were synthesized using Microwave irradiation with short time of process and in high yields. All target compounds were characterized by spectral dat. Final derivatives (**6a-h**) were screened for their antimicrobial activity and

demonstrated moderate to good activity. Among all the compounds, **6f**, **6h**, **6e**, **6g** and **6b** were found potent antimicrobial activities at concentrations 75 and 100 µg/mL. These results positively encouraged us to further developing novel bioactive agents.

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