In vitro Antimicrobial Activity of Some Synthetic Isoindolinone and Isoquinolinone Derivatives

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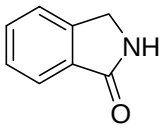
ABSTRACT: A total of six *N*-substituted benzamides, eight isoindolinone derivatives and six isoquinolinone analogs have been screened against five Gram positive and twelve Gram negative bacteria as well as four human fungal pathogens. From the antimicrobial screening, it is evident that 2-iodo-*N*-subustituted tetrahydro-1-oxo isoquinoline-3-carboxylic acids showed very prominent activity at a concentration 200 µg/disc, while the *N*-subtituted-3-alkyl isoindolin-1-one acetates showed weak to moderate activity. At the same time, 2-iodo-*N*-subustituted benzamides revealed very poor activity.

Key words: 2-iodo-*N*-substituted benzamide, Isoindolinone, Isoquinolinone, Antimicrobial assay, Disc diffusion.

INTRODUCTION

Most antibiotics are costly and not affordable to the majority of the patients in developing countries. Antibiotic resistance further compromises with the effectiveness of treatment. Many bacteria including those producing common infections of throat, lungs, skin and urinary tract are becoming resistant to the commonly available antibiotics, leading to increasing treatment failures, sufferings and death.[1-7] In this regard, development of new synthetic antimicrobial agents is a pressing need of the time.

Isoindolinone **1**, Isoquinolinone **2** and their derivatives are found to have antileukemic, antiinflammatory, antipsychotic and antiulcerent properties.[8-18] In this investigation, some of these derivatives were screened against some pathogenic microorganisms for antimicrobial activity.



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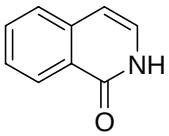

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The aim of the present study was both to explore their effect on the tested pathogens and to find lead compounds having potent antimicrobial activity.

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MATERIALS AND METHODS

General experimental procedure. Melting points were determined in open capillary tubes on Gallenkamp (England) melting point. IR spectra were recorded on a Shimadzu FTIR spectrophotometer and

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UV spectra were recorded in dry EtOH with a Shimadzu visible spectrophotometer. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker DPX-400 spectrophotometer (400 MHz) using tetramethylsilane as internal reference. Analytical thin-layer chromatography (TLC) was performed on precoated silica gel 60F-254 (E. Merck) and the spots were visualized with UV light. Column chromatography was performed on silica gel (60-120 mesh). Elemental analyses (C, H, N) were carried out on a Perkin-Elmer 240C Analyser. Bis(triphenyl-phosphine) palladium(II) chloride, acrylic esters and other reagents were purchased from E. merck (Germany) and Fluka (Switzerland).

Synthesis of Isoindoline and isoindolinone derivatives. The compounds used in the present study were synthesized according to the following procedures.[18]

Synthesis of 2-iodo-*N*-substituted benzamides (10-15). 2-iodo-*N*-substituted benzamides (**10-15**) were prepared from 2-iodobenzoic acids obtained through Sandmeyer reaction of anthranilic acid.[19] 2-iodobenzoic acid was converted to 2-iodobenzoylchloride by heating with PCl_5 at 80°C for 2 hr. 2-iodobenzoyl chloride (3.0 g) was dissolved in dry benzene (20 mL) under nitrogen atmosphere and cooled in ice bath. To the resulting mixture, a solution of primary amine (2.0 equiv) in dry benzene (10 mL) was added slowly with stirring. The residue obtained by filtration was washed with dilute HCl (3 x 50 mL), saturated $NaHCO_3$ solution (3 x 50 mL) and distilled water (3 x 50 mL) and finally the residue was washed with ether (2 x 25 mL). The crystallization was done from ethanol to yield 2-iodo-*N*-substituted benzamides **10-15** (scheme-1).

2-Iodo-*N*-*p*-chlorobenzyl benzamide 11. Colourless needle; m.p. 164–165°C; IR: ν_{max} (KBr) 3276.8, 3059.9, 3029.0, 2921.0, 2845, 1647.1, 1584.4, 1488.9 cm^{-1}; UV (EtOH): λ_{max} 326.4, 305.2, 275.4, 227.6 and 208.0 nm; ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.58 (d, 2H, *J*=4.08 Hz, –CH_2), 6.16 (br. s, 1H–NH), 7.10 (d, 1H, *J*=7.09 Hz, Ar-H), 7.26–7.37 (m, 6H, Ar-H) and 7.85 (d, 1H, *J*=7.49 Hz, Ar-H). Anal. Calcd for $C_{14}H_{11}NOClI$: C, 45.25; H, 2.98; N, 3.76. Found: C, 45.01; H, 3.12; N, 3.95.

Synthesis of *N*-substituted-3-alkyl isoindolinone esters (22-29). A mixture of 2-iodo-*N*-substituted benzamides **10-15** (0.5 g, 1.55 mmol), bis(triphenyl phosphine)palladium(II) chloride (0.038 g, 3.5 mol%) and triethylamine (0.625 g, 4 equiv) were stirred in dimethyl formamide (10 mL) under nitrogen atmosphere for 1 h. Then alkyl acrylates (**16–19**) (0.57 g, 3 equiv) was added to the reaction mixture. The solution was heated at 80°C for 23 hr. The progress of the reaction was monitored by TLC over F_{254} silica gel (*n*-hexane-chloroform 1:1). After completion of the reaction, the mixture was then evaporated to dryness under reduced pressure and the residue was extracted with chloroform (3 x 50 mL). The combined chloroform extracts was washed with distilled water (3 x 50 mL), dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure to obtain reddish gum. The latter was purified by chromatography on a column of silica gel (60-120 mesh) with *n*-hexane-chloroform 1 : 3 and chloroform (100%). *N*-substituted-3-alkyl isoindolinone esters (**22-29**) and small amount of deiodinated products **36-41** were obtained.

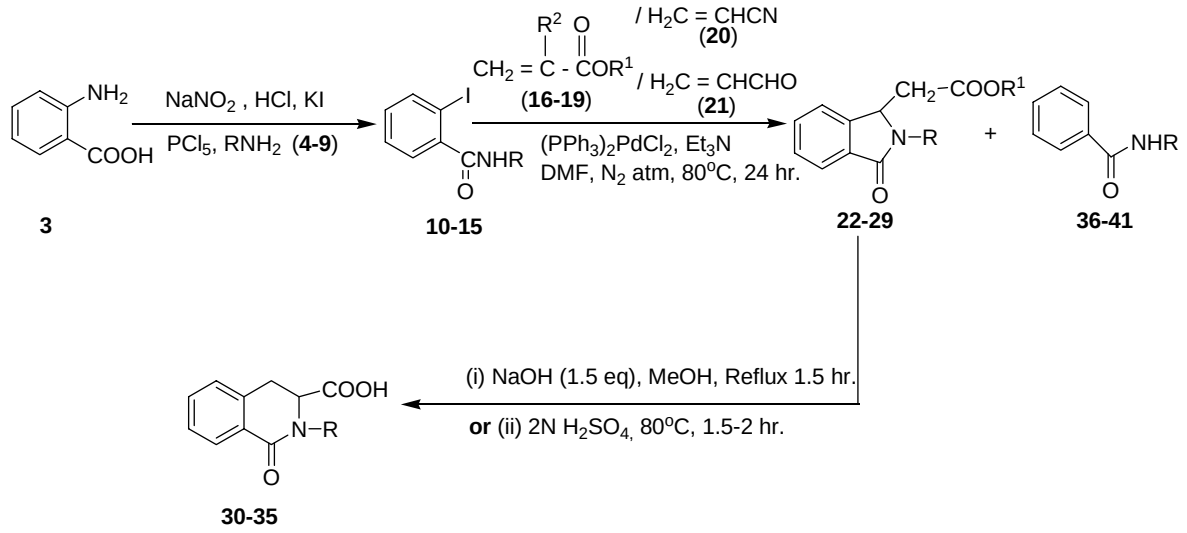
***N*-*p*-methyl phenyl-3-methyl isoindolin-1-one acetate 28**. Light yellow liquid; IR: ν_{max} (CCl_4) 1739.7, 1707.8, 1550.7, 1515.9 and 1380.9 cm^{-1}; UV (EtOH): λ_{max} 245.80 (log ε 3.771) and 206.20 (log ε 3.631) nm; ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.35 (s, 3H, Ar-CH_3), 2.50 (dd, 1H, *J*=8.52, 16.06 Hz, H-2'), 2.92 (dd, 1H, *J*=4.1, 16.14 Hz, H-2'), 3.60 (s, 3H, OCH_3), 5.52 (dd, 1H, *J*=4.02, 8.4 Hz, H-3), 7.22 (d, 2H, *J*=8.9 Hz, Ar-H), 7.40 (d, 2H, *J*=8.16 Hz, Ar-H), 7.47–7.58 (m, 3H, Ar-H) and 7.91 (d, 1H, *J*=7.45 Hz, Ar-H). Anal. Calcd for $C_{18}H_{17}NO_3$: C, 73.21; H, 5.80; N, 4.74. Found: C, 73.50; H, 5.65; N, 4.88.

Synthesis of *N*-substituted-1,2,3,4-tetrahydro-1-oxoisoquinolinoe-3-carboxylic acids (30-35). The mixture of *N*-substituted-3-alkyl isoindolin-1-one acetate 22-29 (200 mg) and NaOH (1.5 equiv) in MeOH (10 mL) was heated under refluxing condition for 1.5 hr. After removal of solvent from the mixture, the residue was diluted with water (25 mL) and

filtered. The filtrate upon neutralization with dilute HCl acid was extracted with chloroform (3x50 mL) and dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. Crystallization from *n*-hexane/ethyl acetate mixture afforded colourless solid compounds **30-35**.

***N*-phenyl-1,2,3,4-tetrahydro-1-oxoisoquinoline-3-carboxylic acid 30.** Colourless solid; m.p. 184–185°C; IR: ν_{max} (KBr) 1730, 1650, 1600, 1500 and 1420 cm^{-1}; UV (EtOH): λ_{max} 274.8 (log ε 4.01) and 228.6 (log ε 4.12) nm; ^{1}H NMR (400 MHz, d_6-DMSO): 2.60 (dd, 1H, *J*=8.00, 16.00 Hz, H-4 ax), 2.92 (dd, 1H, *J*=4.00, 16.00 Hz, H-4 eq), 5.72 (dd, 1H, *J*=4, 8 Hz, H-3), 7.16–8.12 (m, 9H, Ar-H) and 12.40 (br s, 1H, CO2H); ^{13}C NMR (100 MHz, d_6-DMSO): 36.82 (C-4), 57.91 (C-3), 123.79, 124.08, 124.76, 126.32, 129.42, 129.79, 132.55, 133.13, 137.50, 145.57 (Ar-C), 167.01, (CON) and 171.82 (CO_2H). Anal. Calcd for $C_{16}H_{13}NO_3$: C, 71.90; H, 4.90; N, 5.24. Found: C, 71.77; H, 5.03; N, 5.36.

Scheme-1:



Compounds				R	Compounds	R^1	R^2	Compounds	R	R^1
4	**10**	**34**	**36**	CH_3	**16**	C_4H_9	H	**22**	C_6H_5	C_4H_9
5	**11**	**35**	**37**	$CH_2C_6H_4Cl$–*p*	**17**	C_2H_5	H	**23**	$C_6H_4CH_3$–*p*	C_4H_9
6	**12**	**30**	**38**	C_6H_5	**18**	CH_3	H	**24**	$C_6H_4OCH_3$–*p*	C_4H_9
7	**13**	**31**	**39**	$C_6H_4CH_3$–*p*	**19**	CH_3	CH_3	**25**	C_6H_4Cl–*p*	C_4H_9
8	**14**	**32**	**40**	$C_6H_4OCH_3$–*p*				**26**	$C_6H_4CH_3$–*p*	C_2H_5
9	**15**	**33**	**41**	C_6H_4Cl–*p*				**27**	$C_6H_4OCH_3$–*p*	C_2H_5
								28	$C_6H_4CH_3$–*p*	CH_3
								29	$C_6H_4OCH_3$–*p*	CH_3

Antimicrobial screening. The antimicrobial activity of the test compounds was determined by the disc diffusion method.[20] The samples were dissolved separately in chloroform and applied to sterile filter paper discs at a concentration of 200 µg/ disc. The standard test microorganisms were collected from the Microbiology Laboratory of the Institute of Nutrition and Food Sciences (INFS), University of Dhaka, Bangladesh.

RESULTS AND DISCUSSION

A total of six 2-iodo-*N*-substituted benzamides (**10-15**), eight isoindolinone derivatives (**22-29**) and six isoquinolinones (**30-35**) have been tested for antimicrobial activity against five Gram positive and twelve Gram negative bacteria as well as four human fungal pathogens. From the antimicrobial screening (Table 1), it is evident that 2-iodo-*N*-subustituted- 1, 2,3,4-tetrahydro-1-oxoisoquinoline-3-carboxylic acids (**30-35**) showed very prominent activity. On the other hand, *N*-subtituted-3-alkyl isoindolin-1-one acetates (**22-29**) showed weak to moderate activity, while 2-iodo-*N*-subustituted benzamides (**10-15**) demonstrated weak inhibition of microbial growth.

Table 1. Antimicrobial activity of compounds 10-15, 22-29 and 30-35

Test microorganisms	Diameter of zone of inhibition (mm)																				
	Compounds no.																				
	10	11	12	13	14	15	22	23	24	25	26	27	28	29	30	31	32	33	34	35	Kan
Gram positive bacteria																					
Bacillus cereus	-	9	-	-	-	7	7	-	10	-	8	8	9	7	11	14	15	13	9	14	26
Bacillus megaterium	-	-	-	-	7	-	9	-	9	-	8	10	8	-	11	10	16	13	10	14	21
Bacillus subtilis	-	-	-	-	-	-	8	-	10	-	7	9	9	-	9	8	13	13	7	12	23
Sarcina lutea	NT	NT	NT	NT	NT	NT	7	-	7	-	-	8	9	-	NT	NT	NT	NT	NT	NT	24
Staphylococcus aureus	-	11	-	-	-	7	10	-	9	7	9	9	9	-	-	-	12	11	-	13	22
Gram negative bacteria																					
Aeromonas hydrophilia	NT	NT	NT	NT	NT	NT	9	-	9	-	7	8	8	-	NT	NT	NT	NT	NT	NT	20
Escherichia coli	-	-	-	-	-	-	8	-	8	-	-	9	9	-	-	-	9	-	-	7	20
Pseudomonas aepuginosa	-	-	-	-	-	-	-	-	9	-	7	7	8	-	8	8	7	7	8	7	23
Salmonella paratyphi spp							8	-	9	-	6	9	8	-	NT	NT	NT	NT	NT	NT	24
Salmonella paratyphi A	-	-	-	-	-	9	10	-	7	-	7	8	8	-	8	8	12	10	10	12	21
Salmonella paratyphi C	NT	NT	NT	NT	NT	NT	8	-	9	-	7	9	10	9	12	12	13	-	12	-	23
Shigella boydii	NT	NT	NT	NT	NT	NT	-	7	10	7	7	8	8	-	-	-	-	-	-	-	23
Shigella dysenteriae	-	-	-	-	-	-	8	-	9	-	-	-	9	-	-	-	-	-	-	-	23
Shigella flexneri	-	-	-	-	-	-	7	-	9	7	-	7	9	-	-	-	-	-	-	-	23
Shigella sonnei	NT	NT	NT	NT	NT	NT	9	9	9	7	9	8	11	7	-	-	-	-	-	-	25
Vibrio mimicus	-	-	-	-	-	-	9	-	10	7	-	9	8	-	12	-	-	-	12	10	22
Vibrio parahemolyticus	-	-	-	-	-	-	9	-	7	7	-	9	7	8	-	10	-	-	-	-	22
Fungi																					
Aspergillus niger	-	-	-	-	-	-	NT	NT	NT	NT	NT	NT	NT	NT	7	10	7	9	8	10	22
Candida albicans	10	-	-	10	-	12	-	NT	NT	-	-	-	-	-	-	8	10	8	-	12	19
Rhizopus oryzae	-	-	-	-	-	-	8	-	8	8	7	8	7	-	-	-	-	-	-	9	24
Saccharo myces cerevaceae	-	-	-	-	-	8	-	-	-	-	-	-	-	-	-	7	12	12	-	12	24

'-' indicates no sensitivity or zone of inhibition lower than 6 mm and NT refers to "Not Tested", due to scarcity of samples.

In the screeing, the growth of *B. megaterium* and *B. cereus* was strongly inhibited by the compound **32**. It also showed moderate activity against *B. subtilis, S. paratyphi* C*, S. paratyphi A* and *S. aureus.* In case of fungi, it showed weak to moderate activity having average zone size 7-12 mm.

At the same time, compound **35** exhibited strong inhibition of growth of *B. cereus* and *B. megaterium* having the same zone size of 14 mm. The growth of *S. aureus, S. paratyphi* A *and B. subtilis* was moderately inhibited. In case of fungi, it showed weak to moderate activity having zone of inhibition 9-12 mm.

On the other hand, the growth of *B. cereus*, *B. megaterium* and *B. subtilis* was moderately inhibited by compound **33**. The average zone inhibition was 9-12 mm for this compound.

The growth of *B. cereus* was strongly inhibited by compound **31** having the zone size 14 mm. It also showed moderate activity against *S. paratyphi C.* In case of fungi, it demonstrated weak to moderate activity having average zone size, 7-10 mm.

However, the compound **30** showed weak to moderate activity against *S. paratyphi C, V. mimicus, B. cereus and B. megaterium.* It showed very poor activity against the growth of fungi.

The growth of *S. paratyphi* C and *V. mimicus* was moderately inhibited by compound **34**. Besides, compound **28** showed moderate inhibition of growth of *S. paratyphi* C and *S. sonnei*. Compound **24** also showed moderate activity against *B. cereus, B. subtilis, S. boydii* and *V. mimicus.* In case of fungi, the growth of *C. albicans* was moderately inhibited by the compound **15**. *C. albicans* was also inhibited moderately by the compounds **10** and **13** revealed moderate growth inhibitory activity of *C. albicans.*

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