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Synthesis, Antimicrobial and Antifungal Activity of a New Class of Spiro Pyrrolidines

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Abstract—A new class of spiro pyrrolidines, dispiro[oxindole-cyclohexanone]pyrrolidines,[1] dispiro[oxindole-tetrahydronaphthalen-1-one]pyrrolidines,[2] dispiro[oxindole-arylidene-cyclohexanone]pyrrolidines,[3] dispiro[oxindole-hexahydro-indazole]pyrrolidines,[3] and spiro[butenolide]pyrrolidines,[4] have been screened for their antibacterial and antifungal activity against ten human pathogenic bacteria and four dermatophytic fungi. They were found to have antimicrobial and antifungal activity compounds against various pathogens except *Bacillus subtilis*. The spiro pyrrolidinines were synthesized by the regioselective 1,3-dipolar cycloaddition reaction of azomethine ylides generated either from isatin and sarcosine or from aziridine. The azomethine ylide so generated reacted with various dipolarophiles such as 2-arylidene-cyclohexanones, 2-arylidene-tetrahydronapthalen-1-ones, 2,6-bis(arylmethylidene)cyclohexanones and 3-arylidene-5-phenyl- butenolides.

Introduction

Heterocyclic compounds particularly five and six membered ring compounds have occupied a prominent place among various classes of organic compounds for their diverse biological activities.[5] Among a wide variety of heterocycles that have been explored for developing pharmaceutically important molecules, indoles and 1,3,4-oxadiazines have played an important role in medicinal chemistry.[6–8] Some of them have received considerable attention as potential antimicrobial agents.[9,10] Moreover, spiro[indole-pyrrolidine] ring systems acquired a special place in the heterocyclic field because it is a frequently encountered structural motif in many pharmacologically relevant alkaloids, as typified by vincristine, vinblastine and spirotryprostatins. Further structural classification divides this alkaloid family into several subgroups (e.g., Aspidosperma, Strychnos) among which oxindoles deserve mention.[11] Oxindole alkaloids have aroused a great deal of synthetic effort and with significant biological acitivity.[12,13] Arylidene-cyclohexanones, arylidene-tetrahydronaphthalen-1-ones,[14] bis(arylmethylidene)cyclohexanones[15] and arylidene-phenylbutenolides[16] have also drawn much attention due to their important pharmacological properties. In view of these observations and our continued interest in the synthesis of spiro pyrrolidines,[17–19] it was considered worthwhile to evaluate their antibacterial activity and antifungal activity. We have accomplished an efficient synthetic route to spiro pyrrolidines[1–4] by regioselective 1,3-dipolar cycloaddition reactions of azomethine ylides(1,3-dipoles) with various dipolarophiles. Herein we disclose the screening results of their antibacterial and antifungal activity of some of the compounds.

Results and Discussion

The 1,3-dipole (azomethine ylide) generated by the decarboxylative condensation of isatin and sarcosine reacted with dipolarophiles containing exocyclic double bond to afford novel spiro pyrrolidine ring systems.[20] Thus refluxing a solution of 2-arylidene-cyclohexanones **4–8** in methanol or 1,4-dioxan with isatin **1** and sarcosine **2** yielded a series of 1-*N*-methyl-spiro[2.3^1]oxindole-spiro[3.2^{11}]1^{11}-cyclohexanone-4-aryl-pyrrolidines **9–13** (Scheme 1). The reaction gave a single product in all cases, as evidenced by thin layer chromatography (TLC). The reaction afforded a series of novel spiro derivatives **9–13** through regioselective cycloaddition of azomethine ylide to the exocyclic double bond of the 2-arylidene-cyclohexanones in all cases. No trace of other regioisomer **14–18** was detected. The regio and

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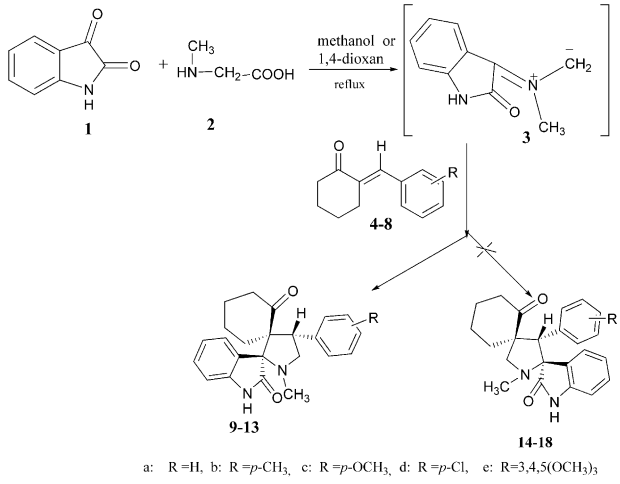
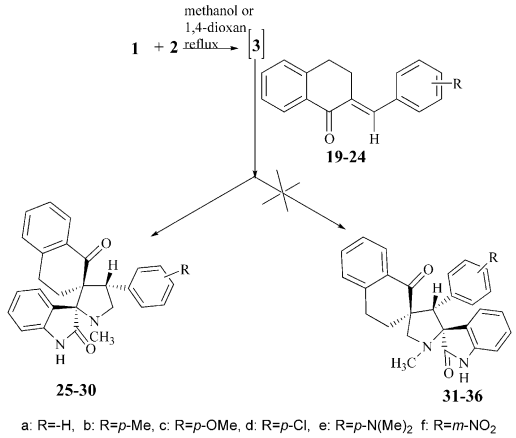
stereochemical outcome of the cycloaddition was determined by spectroscopic data.

Similarly the ylide **3** generated from **1** and **2** reacted with 2-arylidene-1,2,3,4-tetrahydronaphthalen-1-ones **19–24** in methanol or 1,4-dioxan to give a series of 1-*N*-methyl - spiro[2.3[1]]oxindole - spiro[3.2[11]]1[11],2[11],3[11],4[11], - tetrahydronaphthalen-1[11]-one-4-aryl-pyrrolidines **25--30** (Scheme 2). The reaction yielded a single product in all cases, as evidenced by TLC. The reaction afforded a series of novel spiro derivatives **25--30** through regioselective cycloaddition of azomethine ylide to the exocyclic double bond of the 2 - arylidene - 1,2,3,4 - tetrahydronaphthalen-1-ones in all cases. No trace of other regioisomer **31–36** was detected. The regio and stereochemical outcome of the cycloaddition was determined by spectroscopic data.

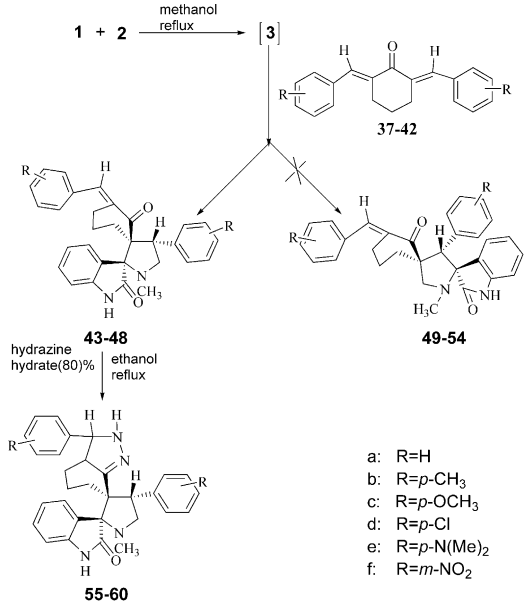
The same methodology is also applied to synthesize dispiro[oxindole-arylidenecyclohexanone]pyrrolidines. Refluxing a solution of 2,6-bis(arylmethylidene) cyclohexanones **37–42** in methanol with isatin **1** and sarcosine **2** afforded a series of 1-*N*-methyl-spiro[2.3[1]] oxindole-spiro[3.2[11]]6[11]-arylmethylidenecyclohexanone-4-aryl-pyrrolidines **43–48** (Scheme 3). The reaction gave a single product in all cases, as evidenced by TLC. The reaction afforded a series of novel spiro derivatives **43–48** through regioselective cycloaddition of azomethine ylide to one of the exocyclic double bonds of the bis(arylmethylidene)cyclohexanones in all cases. No trace of the other regioisomer **49–54** was detected. The regio and stereochemical outcome of the cycloaddition was determined by spectroscopic data and X-ray analysis of **43**.[3] After the formation of the mono adduct, the reaction failed to proceed to give bis adduct even with excess of 1,3-dipole and prolonged reaction time. Thus addition occurs at one of the exocyclic double bonds, while the other one remains unaffected. The dispiro[oxindole/ cyclohexanonone]pyrrolidines **43–48** were further annulated by using hydrazine hydrate in ethanol at reflux to give a series of novel 1-*N*-methyl-spiro[2.3[1]]oxindole - spiro[3.7[11]](3[11] - aryl)$\Delta^{111,711a}$ - hexa-



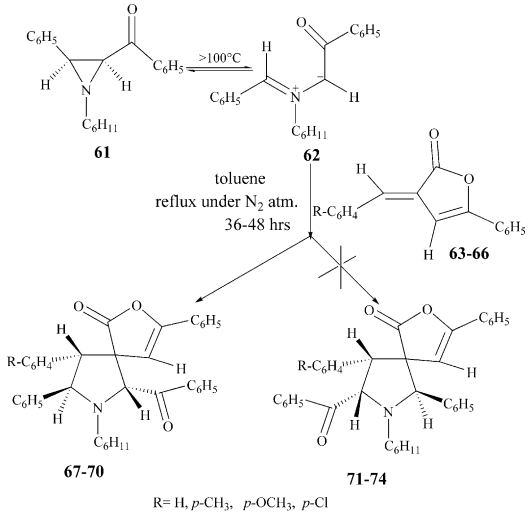
Scheme 1. (a) R = H, (b) R = *p*-CH_3 (c) R = *p*-OCH_3 (d) R = *p*-Cl, (e) R = 3,4,5$(OCH_3)_3$.



Scheme 2. (a) R = -H, (b) R = *p*-Me, (c) R = *p*-OMe, (d) R = *p*-Cl, (e) R = *p*-$N(Me)_2$ (f) R = *m*-NO_2.



Scheme 3.



Scheme 4.

hydro-2*H*-indazole-4-aryl-pyrrolidines **55–60**. The structure of each product was confirmed by spectroscopic data.

Aziridines undergo thermally induced electrocyclic ring opening to generate azomethine ylides which subsequently react with electron deficient olefins to yield pyrrolidines. Thus refluxing a solution of *cis*-3-benzoyl-1-cyclohexyl-2-phenylaziridine **61** with various (*E*)-3-arylidene-5-phenylbutenolides **63–66** in toluene resulted in the formation of novel spiro[butenolide]pyrrolidines **67–70** (Scheme 4). The reaction occurs by conrotatory ring opening of the *cis* aziridine **61** to generate the azomethine ylide **62**, which adds across the exocyclic double bond of the various (*E*)-3-arylidene-5-phenylbutenolides **63–66**. The reaction yielded a single product as evidenced by TLC and resulted in the formation of a series of novel 3-aryl-5-benzoyl-1-cyclohexyl-2-phenyl-pyrrolidine-spiro[4.3^1]5^1-phenylbutenolides **67–70**. We could not find even a trace of the other regioisomer **71–74** in all the cases that have been studied.

Biological Study

The structure–activity relationship of each series (schemes) was studied by changing the substituent in aryl ring of the products. The compounds **9**,**10**,**12** (Scheme 1), **25**, **26**, **29**, **30** (Scheme 2), **43**, **44**, **46**, **48**, **55**, **56**, **58**, **60** (Scheme 3) and **67**, **68** (Scheme 4) were screened for their antibacterial activity against the following pathogens *Shigella dysenteriae, Shigella boydii, Salmonella typhi-A, Salmonella typhi-H, Staphylococcus aureus, Escherichia coli, Bacillus subtilis, Pseudomonas aeriginosa, Proteus mirabilis* and *Klebsiella pneumoniae* and for their antifungal activity against the dermatophytic fungi *Microsporum gypseum, Microsporum nanum, Trichophyton mentagrophytes,* and *Trichophyton rubrum* at various concentrations from MIC level 5 to 200 μL.

Antibacterial activity

The results of the antimicrobial screening for the compounds **9**, **10**, **12** were given in Tables 1–3. From these data, it has been found that the compounds **9**, **10**, **12** showed good inhibition zone on the pathogens such as *Shigella boydii, Salmonella typhi-A, Salmonella typhi-H, Pseudomonas aeriginosa* while other pathogens showed moderate activity. The compound **9** showed good activity than the compounds **10**, **12**. It is clear that introduction of any substituent in the aryl ring does not make much change in activities.

The results of the antimicrobial screening for the compounds **25**, **26**, **29**, **30** were given in Tables 4–7. From these data, the compounds **25**, **26**, **29**, **30** showed good inhibition zone on the pathogens such as *Salmonella*

Table 1.

S. No.	Human pathogens	Concentration of compound **9**											
		A 50 μL		5 μL		50 μL		100 μL		150 μL		200 μL	
		I	%*I*	*I*	%*I*	*I*	%*I*	*I*	%*I*	*I*	%*I*	*I*	%*I*
1.	*Shigella dysenteriae*	28	31.1	2	2.2	10	11.1	12	13.3	13	14.4	15	16.6
2.	*Shigella boydii*	14	15.5	18	20	90	100	90	100	90	100	90	100
3.	*SalmonellaTyphi-A*	25	27.7	17	18.8	90	100	90	100	90	100	90	100
4.	*SalmonellaTyphi-H*	22	24.4	18	20	90	100	90	100	90	100	90	100
5.	*Staphylococcus aureus*	24	26.6	3	3.3	11	12.2	13	14.4	14	15.5	16	17.7
6.	*Escherichia coli*	27	30	—	—	8	8.9	10	11.1	11	12.2	14	15.5
7.	*Bacillus subtilis*	21	23.3	—	—	—	—	—	—	—	—	—	-
8.	*Pseudomonas aeruginosa*	17	18.8	17		90	100	90	100	90	100	90	100
9.	*Proteus mirabilis*	19	21.1	2	2.2	10	11.1	12	13.3	14	15.5	17	18.8
10.	*Klebsiella pneumoniae*	20	22.2	3	3.3	12	13.3	14	15.5	16	17.7	18	20

A-Antibiotic streptomycin; *I*—Inhibition zone;% I—Percentage of inhibition

Table 2.

S. No.	Human pathogens	Concentration of compound **10**											
		A 50 μL		5 μL		50 μL		100 μL		150 μL		200 μL	
		I	%*I*	*I*	%*I*	*I*	%*I*	*I*	%*I*	*I*	%*I*	*I*	%*I*
1.	*Shigella dysenteriae*	28	31.1	2	2.2	11	12.2	14	15.5	15	16.6	17	18.8
2.	*Shigella boydii*	14	15.5	—	—	10	11.1	12	13.3	13	14.4	15	16.6
3.	*Salmonella typhi-A*	25	27.7	18	20	90	100	90	100	90	100	90	100
4.	*Salmonella typhi-H*	22	24.4	18	20	90	100	90	100	90	100	90	100
5.	*Staphylococcus aureus*	24	26.6	3	3.3	13	14.4	15	16.6	17	18.8	19	21.1
6.	*Escherichia coli*	27	30	2	2.2	11	12.2	12	13.3	14	15.5	16	17.7
7.	*Bacillus subtilis*	21	23.3	—	—	—	—	—	—	—	—	—	—
8.	*Pseudomonas aeruginosa*	17	18.8	15	16.6	74	82.2	90	100	90	100	90	100
9.	*Proteus mirabilis*	19	21.1	—	—	10	11.1	11	12.2	12	13.3	14	15.5
10.	*Klebsiella pneumoniae*	20	22.2	2	2.2	12	13.3	14	15.5	15	16.6	17	18.8

typhi-A, Salmonella typhi-H, Pseudomonas aeriginosa, while other pathogens showed less activity.

The results of the antimicrobial screening for the compounds **43**, **44**, **46**, **48** were given in Tables 8–11. From these data, the compounds **43**, **44**, **46**, **48** showed good inhibitory property against *Shigella boydii, Salmonella typhi-A, Salmonella typhi-H, Pseudomonas aeriginosa*. The compound **48** showed good activity compared to **43**, **44**, **46**. It is clear that introduction of nitro group enhances the antibacterial activity.

The results of the antimicrobial screening for the compounds **55**, **56**, **58**, **60** were given in Tables 12–15. From the tables, the compounds **55**, **56**, **58**, **60** showed good inhibitory property against *Shigella dysenteriae, Shigella boydii, Salmonella typhi-A, Salmonella typhi-H, Escherichia coli* while other pathogens showed less activity. It is clear that introduction of any substituent does not make much change in activities.

The results of the antimicrobial screening for the compounds **67**, **68** were given in Tables 16 and 17. From these data, the compounds **67**, **68** showed good inhibition property against the pathogens *Shigella dysenteriae, Shigella boydii, Salmonella typhi-A, Salmonella typhi-H, Pseudomonas aeriginosa* while with other pathogens showed less activity. The compound **67** possessed

Table 3.

S. No.	Human pathogens	Concentration of compound **12**											
		A 50 μL		5 μL		50 μL		100 μL		150 μL		200 μL	
		I	%*I*	*I*	%*I*	*I*	%*I*	*I*	%*I*	*I*	%*I*	*I*	%*I*
1.	*Shigella dysenteriae*	28	31.1	—	—	10	11.1	11	12.2	13	14.4	15	16.6
2.	*Shigella boydii*	14	15.5	17	18.8	90	100	90	100	90	100	90	100
3.	*Salmonella typhi-A*	25	27.7	18	20	90	100	90	100	90	100	90	100
4.	*Salmonella typhi-H*	22	24.4	18	20	90	100	90	100	90	100	90	100
5.	*Staphylococcus aureus*	24	26.6	2	2.2	12	13.3	13	14.4	15	16.6	17	18.8
6.	*Escherichia coli*	27	30	—	—	10	11.1	11	12.2	13	14.4	14	15.5
7.	*Bacillus subtilis*	21	23.3	—	—	—	—	—	—	—	—	—	—
8.	*Pseudomonas aeruginosa*	17	18.8	19	21.1	90	100	90	100	90	100	90	100
9.	*Proteus mirabilis*	19	21.1	2	2.2	11	12.2	13	14.4	14	15.5	16	17.7
10.	*Klebsiella pneumoniae*	20	22.2	—	—	10	11.1	12	13.3	14	15.5	16	17.7

Table 4.

S. No	Human pathogens	Concentration of compound **25**											
		A 50 μL		5 μL		50 μL		100 μL		150 μL		200 μL	
		I	%*I*	*I*	%*I*	*I*	%*I*	*I*	%*I*	*I*	%*I*	*I*	%*I*
1.	*Shigella dysenteriae*	28	31.1	2	2.2	11	12.2	12	13.3	14	15.5	15	16.6
2.	*Shigella boydii*	14	15.5	—	—	10	11.1	12	13.3	13	14.4	15	16.6
3.	*Salmonella typhi-A*	25	27.7	18	20	90	100	90	100	90	100	90	100
4.	*Salmonella typhi-H*	22	24.4	17	18.8	90	100	90	100	90	100	90	100
5.	*Staphylococcus aureus*	24	26.6	3	3.3	14	15.5	16	17.7	17	18.8	19	21.1
6.	*Escherichia coli*	27	30	2	2.2	12	13.3	14	15.5	16	17.7	18	20
7.	*Bacillus subtilis*	21	23.3	—	—	—	—	—	—	—	—	—	—
8.	*Pseudomonas aeruginosa*	17	18.8	13	14.4	60	66.6	73	81.1	90	100	90	100
9.	*Proteus mirabilis*	19	21.1	3	3.3	13	14.4	15	16.6	17	18.8	18	20
10.	*Klebsiella pneumoniae*	20	22.2	2	2.2	12	13.3	14	15.5	16	17.7	17	18.8

Table 5.

S. No.	Human pathogens	Concentration of compound **26**											
		A 50 μL		5 μL		50 μL		100 μL		150 μL		200 μL	
		I	%*I*	*I*	%*I*	*I*	%*I*	*I*	%*I*	*I*	%*I*	*I*	%*I*
1.	*Shigella dysenteriae*	28	31.1	4	4.4	13	14.4	14	15.5	15	16.6	17	18.8
2.	*Shigella boydii*	14	15.5	—	—	9	11.1	11	12.2	13	14.4	14	15.5
3.	*Salmonella typhi-A*	25	27.7	18	20	90	100	90	100	90	100	90	100
4.	*Salmonella typhi-H*	22	24.4	16	17.7	90	100	90	100	90	100	90	100
5.	*Staphylococcus aureus*	24	26.6	2	2.2	12	13.3	14	15.5	15	16.6	16	17.7
6.	*Escherichia coli*	27	30	—	—	11	12.2	12	13.3	13	14.4	15	16.6
7.	*Bacillus subtilis*	21	23.3	—	—	—	—	—	—	—	—	—	—
8.	*Pseudomonas aeruginosa*	17	18.8	16	17.7	82	91.1	90	100	90	100	90	100
9.	*Proteus mirabilis*	19	21.1	—	—	11	12.2	13	14.4	14	15.5	16	17.7
10.	*Klebsiella pneumoniae*	20	22.2	—	—	10	11.1	12	13.3	13	14.4	15	16.6

greater activity compared to **68**. It is clear that introduction of any group in aryl ring decreases its activity against the pathogens.

Antibacterial activity testing by well diffusion method

The nutrient agar medium, 20 mL was poured into the sterile petri-dishes. To the solidified plates, wells were made using a sterile cork borer 10 mm in diameter. The 24 h subcultured bacteria was inoculated in the petri-plates, with a sterile cotton swab dipped in the nutrient broth medium. After inoculating, the compounds were dissolved separately with the chloroform solvent and poured in to the wells with varying concentrations ranging from 5, 50, 100, 150 and 200 μL using a micro-pipette. The plates were left over for 24 h at 37 °C. The antibiotic Streptomycin was used as a standard for comparative study.

The percentage of inhibition was calculated by the formula

$$\% \text{ inhibition} = \frac{\text{I (Diameter of the inhibition zone)}}{\text{90 (Diameter of the Petri-plate in mm)}} \times 100$$

Table 6.

S. No.	Human pathogens	Concentration of compound **29**											
		A 50 μL		5 μL		50 μL		100 μL		150 μL		200 μL	
		I	%*I*	*I*	%*I*	*I*	%*I*	*I*	%*I*	*I*	%*I*	*I*	%*I*
1.	*Shigella dysenteriae*	28	31.1	—	—	11	12.2	13	14.4	14	15.5	16	17.7
2.	*Shigella boydii*	14	15.5	2	2.2	12	13.3	15	16.6	17	18.8	19	21.1
3.	*Salmonella typhi-A*	25	27.7	18	20	90	100	90	100	90	100	90	100
4.	*Salmonella typhi-H*	22	24.4	17	18.8	90	100	90	100	90	100	90	100
5.	*Staphylococcus aureus*	24	26.6	3	3.3	13	14.4	15	16.6	17	18.8	20	22.2
6.	*Escherichia coli*	27	30	2	2.2	10	11.1	12	13.3	14	15.5	15	16.6
7.	*Bacillus subtilis*	21	23.3	—	—	—	—	—	—	—	—	—	—
8.	*Pseudomonas aeruginosa*	17	18.8	17	18.8	90	100	90	100	90	100	90	100
9.	*Proteus mirabilis*	19	21.1	4	4.4	14	15.5	15	16.6	17	18.8	18	20
10.	*Klebsiella pneumoniae*	20	22.2	—	—	11	12.2	12	13.3	15	16.6	17	18.8

Table 7.

S. No.	Human pathogens	Concentration of compound **30**											
		A 50 μL		5 μL		50 μL		100 μL		150 μL		200 μL	
		I	%*I*	*I*	%*I*	*I*	%*I*	*I*	%*I*	*I*	%*I*	*I*	%*I*
1.	*Shigella dysenteriae*	28	31.1	2	2.2	12	13.3	13	14.4	15	16.6	16	17.7
2.	*Shigella boydii*	14	15.5	2	2.2	10	11.1	12	13.3	13	14.4	15	16.6
3.	*Salmonella typhi-A*	25	27.7	18	20	90	100	90	100	90	100	90	100
4.	*Salmonella typhi-H*	22	24.4	17	18.8	90	100	90	100	90	100	90	100
5.	*Staphylococcus aureus*	24	26.6	2	2.2	11	12.2	12	13.3	14	15.5	15	16.6
6.	*Escherichia coli*	27	30	—	—	9	10	11	12.2	12	13.3	14	15.5
7.	*Bacillus subtilis*	21	23.3	—	—	—	—	—	—	—	—	—	—
8.	*Pseudomonas aeruginosa*	17	18.8	18	20	90	100	90	100	90	100	90	100
9.	*Proteus mirabilis*	19	21.1	—	—	9	10	10	11.1	13	14.4	15	16.6
10.	*Klebsiella pneumoniae*	20	22.2	2	2.2	10	11.1	11	12.2	12	13.3	14	15.5

Table 8.

S. No.	Human pathogens	Concentration of compound **43**											
		A 50 μL		5 μL		50 μL		100 μL		150 μL		200 μL	
		I	%*I*	*I*	%*I*	*I*	%*I*	*I*	%*I*	*I*	%*I*	*I*	%*I*
1.	*Shigella dysenteriae*	28	31.1	2	2.2	12	13.3	14	15.5	16	17.7	17	18.8
2.	*Shigella boydii*	14	15.5	17	18.8	90	100	90	100	90	100	90	100
3.	*Salmonella typhi-A*	25	27.7	18	20	90	100	90	100	90	100	90	100
4.	*Salmonella typhi-H*	22	24.4	19	21.1	90	100	90	100	90	100	90	100
5.	*Staphylococcus aureus*	24	26.6	—	—	—	—	10	11.1	12	13.3	14	15.5
6.	*Escherichia coli*	27	30	3	3.3	13	14.4	15	16.6	16	17.7	18	20
7.	*Bacillus subtilis*	21	23.3	—	—	—	—	—	—	—	—	—	—
8.	*Pseudomonas aeruginosa*	17	18.8	17	18.8	90	100	90	100	90	100	90	100
9.	*Proteus mirabilis*	19	21.1	—	—	—	—	12	13.3	13	14.4	15	16.6
10.	*Klebsiella pneumoniae*	20	22.2	—	—	—	—	13	14.4	14	15.5	16	17.7

Antifungal activity

The results of the antifungal screening for the compounds **9**, **10**, **12** were given in Tables 18–20. From these data, of all the compounds **9**, **10**, **12** tested, the compound **12** showed 100% inhibitory activity where the growth was nil in all dermatophytes. Thus the introduction of chlorine substituent in aryl ring enhances the antifungal activity.

The results of the antifungal screening for the compounds **25**, **26**, **29**, **30** were given in Tables 21–24. From these data, the compounds **25**, **26** showed 100% inhibitory action against *Microsporum gypseum,* and *Trichophyton rubrum* while with other dermatophytes showed moderate activity. The compounds **29**, **30** showed 100% inhibitory action against all dermatophytes except *Microsporum nanum*. The compound **29** showed good activity compared to **25**, **26**, **30**. It is clear that, introduction of electron releasing group $N(Me)_2$ enhances the antifungal activity in this series.

The results of the antifungal screening for the compounds **43**, **44**, **46**, **48** were given in Tables 25–28. From

Table 9.

S. No.	Human pathogens	Concentration of compound **44**											
		A 50 μL		5 μL		50 μL		100 μL		150 μL		200 μL	
		I	%*I*	*I*	%*I*	*I*	%*I*	*I*	%*I*	*I*	%*I*	*I*	%*I*
1.	*Shigella dysenteriae*	28	31.1	2	2.2	10	11.1	13	14.4	15	16.6	17	18.8
2.	*Shigella boydii*	14	15.5	18	20	90	100	90	100	90	100	90	100
3.	*Salmonella typhi-A*	25	27.7	18	20	90	100	90	100	90	100	90	100
4.	*Salmonella typhi-H*	22	24.4	17	18.8	90	100	90	100	90	100	90	100
5.	*Staphylococcus aureus*	24	26.6	—	—	—	—	9	10	11	12.2	14	15.5
6.	*Escherichia coli*	27	30	2	2.2	10	11.1	12	13.3	13	14.4	16	17.7
7.	*Bacillus subtilis*	21	23.3	—	—	—	—	—	—	—	—	—	—
8.	*Pseudomonas aeruginosa*	17	18.8	17		90	100	90	100	90	100	90	100
9.	*Proteus mirabilis*	19	21.1	—	—	—	—	9	10	10	11.1	11	12.2
10.	*Klebsiella pneumoniae*	20	22.2	—	—	—	—	10	11.1	11	12.2	13	14.4

Table 10.

S. No.	Human pathogens	Concentration of compound **46**											
		A 50 μL		5 μL		50 μL		100 μL		150 μL		200 μL	
		I	%*I*	*I*	%*I*	*I*	%*I*	*I*	%*I*	*I*	%*I*	*I*	%*I*
1.	*Shigella dysenteriae*	28	31.1	2	2.2	12	13.3	14	15.5	15	16.6	16	17.7
2.	*Shigella boydii*	14	15.5	17	18.8	90	100	90	100	90	100	90	100
3.	*Salmonella typhi-A*	25	27.7	16	17.7	90	100	90	100	90	100	90	100
4.	*Salmonella typhi-H*	22	24.4	18	20	90	100	90	100	90	100	90	100
5.	*Staphylococcus aureus*	24	26.6	—	—	—	—	12	13.3	14	15.5	17	18.8
6.	*Escherichia coli*	27	30	—	—	11	12.2	13	14.4	15	16.6	18	20
7.	*Bacillus subtilis*	21	23.3	—	—	—	—	—	—	—	—	—	—
8.	*Pseudomonas aeruginosa*	17	18.8	18	20	90	100	90	100	90	100	90	100
9.	*Proteus mirabilis*	19	21.1	—	—	—	—	11	12.2	14	15.5	17	18.8
10.	*Klebsiella pneumoniae*	20	22.2	—	—	—	—	13	13.3	15	16.6	16	17.7

Table 11.

S. No.	Human pathogens	Concentration of compound **48**											
		A 50 μL		5 μL		50 μL		100 μL		150 μL		200 μL	
		I	%*I*	*I*	%*I*	*I*	%*I*	*I*	%*I*	*I*	%*I*	*I*	%*I*
1.	*Shigella dysenteriae*	28	31.1	3	3.3	13	14.4	14	15.5	16	17.7	17	18.8
2.	*Shigella boydii*	14	15.5	18	20	90	100	90	100	90	100	90	100
3.	*Salmonella typhi-A*	25	27.7	16	17.7	90	100	90	100	90	100	90	100
4.	*Salmonella typhi-H*	22	24.4	18	20	90	100	90	100	90	100	90	100
5.	*Staphylococcus aureus*	24	26.6	—	—	—	—	10	11.1	11	12.2	13	14.4
6.	*Escherichia coli*	27	30	3	3.3	14	15.5	16	17.7	17	18.8	19	21.1
7.	*Bacillus subtilis*	21	23.3	—	—	—	—	—	—	—	—	—	—
8.	*Pseudomonas aeruginosa*	17	18.8	17	18.8	90	100	90	100	90	100	90	100
9.	*Proteus mirabilis*	19	21.1	—	—	—	—	12	13.3	14	15.5	15	16.6
10.	*Klebsiella pneumoniae*	20	22.2	—	—	—	—	11	12.2	13	14.4	14	15.5

these data, all compounds **43**, **44**, **46**, **48** showed good activity against all dermatophytes except *Microsporum nanum*. The compounds **43** and **48** showed equal activity. Since these compounds possess unsaturation moiety that causes for good activity against various dermatophytes.

The results of the antifungal screening for the compounds **55**, **56**, **58**, **60** were given in Tables 29–32. From these data, the compounds **55**, **56**, **58** showed equal and 100% activity against all dermatophytes except *Microsporum nanum*. The compound **60** showed 100% activity against all dermatophytes except *Trichophyton mentagrophytes*.

The results of the antifungal screening for the compounds **67**, **68** were given in Tables 33 and 34. From these data, both the compounds **67**, **68** showed 100% activity against all dermatophytes except *Microsporum nanum*. The compound **67** possessed greater activity as compared to the compound **68**.

Table 12.

S. No.	Human pathogens	Concentration of compound **55**											
		A 50 μL		5 μL		50 μL		100 μL		150 μL		200 μL	
		I	%*I*	*I*	%*I*	*I*	%*I*	*I*	%*I*	*I*	%*I*	*I*	%*I*
1.	*Shigella dysenteriae*	28	31.1	17	18.8	90	100	90	100	90	100	90	100
2.	*Shigella boydii*	14	15.5	18	20	90	100	90	100	90	100	90	100
3.	*Salmonella typhi-A*	25	27.7	18	20	90	100	90	100	90	100	90	100
4.	*Salmonella typhi-H*	22	24.4	19	21.1	90	100	90	100	90	100	90	100
5.	*Staphylococcus aureus*	24	26.6	2	2.2	12	13.3	13	14.4	15	16.6	17	18.8
6.	*Escherichia coli*	27	30	18	20	90	100	90	100	90	100	90	100
7.	*Bacillus subtilis*	21	23.3	—	—	—	—	—	—	—	—	—	—
8.	*Pseudomonas aeruginosa*	17	18.8	3	3.3	13	14.4	15	16.6	16	17.7	18	20
9.	*Proteus mirabilis*	19	21.1	—	—	11	12.2	13	14.4	15	16.6	17	18.8
10.	*Klebsiella pneumoniae*	20	22.2	2	2.2	13	14.4	14	15.5	17	18.8	19	21.1

Table 13.

S. No.	Human pathogens	Concentration of compound **56**											
		A 50 μL		5 μL		50 μL		100 μL		150 μL		200 μL	
		I	%*I*	*I*	%*I*	*I*	%*I*	*I*	%*I*	*I*	%*I*	*I*	%*I*
1.	*Shigella dysenteriae*	28	31.1	18	20	90	100	90	100	90	100	90	100
2.	*Shigella boydii*	14	15.5	17	18.8	90	100	90	100	90	100	90	100
3.	*Salmonella typhi-A*	25	27.7	18	20	90	100	90	100	90	100	90	100
4.	*Salmonella typhi-H*	22	24.4	17	18.8	90	100	90	100	90	100	90	100
5.	*Staphylococcus aureus*	24	26.6	—	—	—	—	10	11.1	12	13.3	15	16.6
6.	*Escherichia coli*	27	30	18	20	90	100	90	100	90	100	90	100
7.	*Bacillus subtilis*	21	23.3	—	—	—	—	—	—	—	—	—	—
8.	*Pseudomonas aeruginosa*	17	18.8	3	3.3	11	12.2	13	14.4	15	16.6	17	18.8
9.	*Proteus mirabilis*	19	21.1	2	2.2	10	11.1	11	12.2	12	13.3	14	15.5
10.	*Klebsiella pneumoniae*	20	22.2	2	2.2	10	11.1	12	13.3	13	14.4	15	16.6

Table 14.

S. No.	Human pathogens	Concentration of compound **58**											
		A 50 μL		5 μL		50 μL		100 μL		150 μL		200 μL	
		I	%*I*	*I*	%*I*	*I*	%*I*	*I*	%*I*	*I*	%*I*	*I*	%*I*
1.	*Shigella dysenteriae*	28	31.1	19	21.1	90	100	90	100	90	100	90	100
2.	*Shigella boydii*	14	15.5	17	18.8	90	100	90	100	90	100	90	100
3.	*Salmonella typhi-A*	25	27.7	18	20	90	100	90	100	90	100	90	100
4.	*Salmonella typhi-H*	22	24.4	18	20	90	100	90	100	90	100	90	100
5.	*Staphylococcus aureus*	24	26.6	—	—	11	12.2	14	15.5	15	16.6	16	17.7
6.	*Escherichia coli*	27	30	14	15.5	78	86.6	90	100	90	100	90	100
7.	*Bacillus subtilis*	21	23.3	—	—	—	—	—	—	—	—	—	—
8.	*Pseudomonas aeruginosa*	17	18.8	2	2.2	12	13.3	14	15.5	16	17.7	17	18.8
9.	*Proteus mirabilis*	19	21.1	—	—	10	11.1	12	13.3	13	14.4	15	16.6
10.	*Klebsiella pneumoniae*	20	22.2	—	—	11	12.2	12	13.3	14	15.5	17	18.8

Table 15.

S. No.	Human pathogens	Concentration of compound **60**											
		A 50 μL		5 μL		50 μL		100 μL		150 μL		200 μL	
		I	%*I*	*I*	%*I*	*I*	%*I*	*I*	%*I*	*I*	%*I*	*I*	%*I*
1.	*Shigella dysenteriae*	28	31.1	18	20	90	100	90	100	90	100	90	100
2.	*Shigella boydii*	14	15.5	16	17.7	90	100	90	100	90	100	90	100
3.	*Salmonella typhi-A*	25	27.7	18	20	90	100	90	100	90	100	90	100
4.	*Salmonella typhi-H*	22	24.4	17	18.8	90	100	90	100	90	100	90	100
5.	*Staphylococcus aureus*	24	26.6	—	—	10	11.1	12	13.3	14	15.5	15	16.6
6.	*Escherichia coli*	27	30	17	18.8	90	100	90	100	90	100	90	100
7.	*Bacillus subtilis*	21	23.3	—	—	—	—	—	—	—	—	—	—
8.	*Pseudomonas aeruginosa*	17	18.8	—	—	10	11.1	11	12.2	14	15.5	17	18.8
9.	*Proteus mirabilis*	19	21.1	—	—	11	12.2	12	13.3	15	16.6	18	20
10.	*Klebsiella pneumoniae*	20	22.2	2	2.2	12	13.3	15	16.6	16	17.7	19	21.1

Table 16.

S. No.	Human pathogens	Concentration of compound **67**											
		A 50 μL		5 μL		50 μL		100 μL		150 μL		200 μL	
		I	%*I*	*I*	%*I*	*I*	%*I*	*I*	%*I*	*I*	%*I*	*I*	%*I*
1.	*Shigella dysenteriae*	28	31.1	18	20	90	100	90	100	90	100	90	100
2.	*Shigella boydii*	14	15.5	17	18.8	90	100	90	100	90	100	90	100
3.	*Salmonella typhi-A*	25	27.7	19	21.1	90	100	90	100	90	100	90	100
4.	*Salmonella typhi-H*	22	24.4	17	18.8	90	100	90	100	90	100	90	100
5.	*Staphylococcus aureus*	24	26.6	—	—	10	11.1	13	14.4	15	16.6	17	18.8
6.	*Escherichia coli*	27	30	19	21.1	90	100	90	100	90	100	90	100
7.	*Bacillus subtilis*	21	23.3	—	—	—	—	—	—	—	—	—	—
8.	*Pseudomonas aeruginosa*	17	18.8	16	17.7	87	100	90	100	90	100	90	100
9.	*Proteus mirabilis*	19	21.1	3	3.3	14	15.5	16	17.7	17	18.8	19	21.1
10.	*Klebsiella pneumoniae*	20	22.2	—	—	11	12.2	14	15.5	15	16.6	16	17.7

Table 17.

S. No.	Human pathogens	Concentration of compound **68**											
		A 50 μL		5 μL		50 μL		100 μL		150 μL		200 μL	
		I	%*I*	*I*	%*I*	*I*	%*I*	*I*	%*I*	*I*	%*I*	*I*	%*I*
1.	*Shigella dysenteriae*	28	31.1	17	18.8	90	100	90	100	90	100	90	100
2.	*Shigella boydii*	14	15.5	18	20	90	100	90	100	90	100	90	100
3.	*Salmonella typhi-A*	25	27.7	17	18.8	90	100	90	100	90	100	90	100
4.	*Salmonella typhi-H*	22	24.4	18	20	90	100	90	100	90	100	90	100
5.	*Staphylococcus aureus*	24	26.6	—	—	—	—	10	11.1	11	12.2	14	15.5
6.	*Escherichia coli*	27	30	18	20	90	100	90	100	90	100	90	100
7.	*Bacillus subtilis*	21	23.3	—	—	—	—	—	—	—	—	—	—
8.	*Pseudomonas aeruginosa*	17	18.8	17	18.8	90	100	90	100	90	100	90	100
9.	*Proteus mirabilis*	19	21.1	—	—	9	10	12	13.3	13	14.4	16	17.7
10.	*Klebsiella pneumoniae*	20	22.2	2	2.2	11	12.2	13	14.4	14	15.5	17	18.8

Table 18. A—Antibiotic griseofulvin; *I*—inhibition zone; %*I*—Percentage of inhibition

S. No.	Dermatophytes	Concentration of compound **9**											
		A 50 μL		5 μL		50 μL		100 μL		150 μL		200 μL	
		I	%*I*	*I*	%*I*	*I*	%*I*		%*I*	*I*	%*I*	*I*	%*I*
1.	*Microsporum gypseum*	10	88.8	9	90	—	100	—	100	—	100	—	100
2.	*Microsporum nanum*	18	80	27	70	12	86.6	11	87.7	10	88.8	—	100
3.	*Trichophyton mentagrophytes*	16	82.2	15	83.3	—	100	—	100	—	100	—	100
4.	*Trichophyton rubrum*	12	86.6	12	86.6	—	100	—	100	—	100	—	100

Table 19.

S. No.	Dermatophytes	Concentration of compound 10											
		A 50 μL		5 μL		50 μL		100 μL		150 μL		200 μL	
		I	%*I*	*I*	%*I*	*I*	%*I*		%*I*	*I*	%*I*	*I*	%*I*
1.	*Microsporum gypseum*	10	88.8	12	86.6	—	100	—	100	—	100	—	100
2.	*Microsporum nanum*	18	80	30	66.6	13	85.5	11	87.7	10	88.8	9	90
3.	*Trichophyton mentagrophytes*	16	82.2	11	87.7	—	100	—	100	—	100	—	100
4.	*Trichophyton rubrum*	12	86.6	10	88.8	—	100	—	100	—	100	—	100

Table 20.

S. No.	Dermatophytes	Concentration of compound **12**											
		A 50 μL		5 μL		50 μL		100 μL		150 μL		200 μL	
		I	%*I*	*I*	%*I*	*I*	%*I*		%*I*	*I*	%*I*	*I*	%*I*
1.	*Microsporum gypseum*	10	88.8	10	88.8	—	100	—	100	—	100	—	100
2.	*Microsporum nanum*	18	80	20	77.7	—	100	—	100	—	100	—	100
3.	*Trichophyton mentagrophytes*	16	82.2	13	85.5	—	100	—	100	—	100	—	100
4.	*Trichophyton rubrum*	12	86.6	15	83.3	—	100	—	100	—	100	—	100

Table 21.

S. No.	Dermatophytes	Concentration of compound 25											
		A 50 μL		5 μL		50 μL		100 μL		150 μL		200 μL	
		I	%*I*	*I*	%*I*	*I*	%*I*		%*I*	*I*	%*I*	*I*	%*I*
1.	*Microsporum gypseum*	10	88.8	11	87.7	—	100	—	100	—	100	—	100
2.	*Microsporum nanum*	18	80	40	55.5	23	74.4	17	81.1	14	84.4	11	87.7
3.	*Trichophyton mentagrophytes*	16	82.2	31	65.5	14	84.4	12	86.6	10	88.8	—	100
4.	*Trichophyton rubrum*	12	86.6	15	83.3	—	100	—	100	—	100	—	100

Table 22.

S. No.	Dermatophytes	Concentration of compound **26**											
		A 50 μL		5 μL		50 μL		100 μL		150 μL		200 μL	
		I	%*I*	*I*	%*I*	*I*	%*I*	*I*	%*I*	*I*	%*I*	*I*	%*I*
1.	*Microsporum gypseum*	10	88.8	10	88.8	—	100	—	100	—	100	—	100
2.	*Microsporum nanum*	18	80	34	62.2	15	83.3	13	85.5	11	87.7	9	90
3.	*Trichophyton mentagrophytes*	16	82.2	28	68.8	10	88.8	9	90	8	91.1	—	100
4.	*Trichophyton rubrum*	12	86.6	9	90	—	100	—	100	—	100	—	100

Table 23.

S. No.	Dermatophytes	Concentration of compound **29**											
		A 50 μL		5 μL		50 μL		100 μL		150 μL		200 μL	
		I	%*I*	*I*	%*I*	*I*	%*I*	*I*	%*I*	*I*	%*I*	*I*	%*I*
1.	*Microsporum gypseum*	10	88.8	13	85.5	—	100	—	100	—	100	—	100
2.	*Microsporum nanum*	18	80	25	72.2	12	86.6	10	88.8	—	100	—	100
3.	*Trichophyton mentagrophytes*	16	82.2	11	87.7	—	100	—	100	—	100	—	100
4.	*Trichophyton rubrum*	12	86.6	10	88.8	—	100	—	100	—	100	—	100

From the results tabulated, it was known that most of the compounds showed good activity against the tested dermatophytic fungi.

Antifungal activity testing by poison plate method

All the compounds were dissolved separately in the chloroform solvent for screening of their antifungal activity. The compounds were poured into the sterile petri-dishes at varying concentrations ranging from 5, 50, 100, 150 and 200 μL using micropipette. The sterilized Sabourads agar medium, 20 mL was poured into each petri-dishes. After solidifying of the medium, the fungal mycelia, 8 mm in diameter, was pluged from the fresh culture plate and inoculated in the center of the plate. The antibiotic griseofulvin was used as a standard for comparative study instead of the compound. The plate with the solvent alone and the mycelia was kept as the control. The plates were incubated at room temperature and after 21 days, the growth of the mycelia was measured. The percentage of inhibition was calculated by the following formula.

$$\% \text{ inhibition} = \frac{\text{Diameter of the inhibition zone–Total diameter of the plate } (I\text{-}90) \times 100}{\text{Total diameter of the plate in mm}}$$

Table 24.

S. No.	Dermatophytes	Concentration of compound **30**											
		A 50 μL		5 μL		50 μL		100 μL		150 μL		200 μL	
		I	%*I*	*I*	%*I*	*I*	%*I*	*I*	%*I*	*I*	%*I*	*I*	%*I*
1.	*Microsporum gypseum*	10	88.8	15	83.3	—	100	—	100	—	100	—	100
2.	*Microsporum nanum*	18	80	28	68.8	14	84.4	12	86.6	10	88.8	—	100
3.	*Trichophyton mentagrophytes*	16	82.2	12	86.6	—	100	—	100	—	100	—	100
4.	*Trichophyton rubrum*	12	86.6	14	84.4	—	100	—	100	—	100	—	100

Table 25.

S. No.	Dermatophytes	Concentration of compound **43**											
		A 50 μL		5 μL		50 μL		100 μL		150 μL		200 μL	
		I	%*I*	*I*	%*I*	*I*	%*I*	*I*	%*I*	*I*	%*I*	*I*	%*I*
1.	*Microsporum gypseum*	10	88.8	10	88.8	—	100	—	100	—	100	—	100
2.	*Microsporum nanum*	18	80	37	58.8	16	82.2	14	84.4	11	87.7	10	88.8
3.	*Trichophyton mentagrophytes*	16	82.2	9	90	—	100	—	100	—	100	—	100
4.	*Trichophyton rubrum*	12	86.6	12	86.6	—	100	—	100	—	100	—	100

Table 26.

S. No.	Dermatophytes	Concentration of compound **44**											
		A 50 μL		5 μL		50 μL		100 μL		150 μL		200 μL	
		I	%*I*	*I*	%*I*	*I*	%*I*	*I*	%*I*	*I*	%*I*	*I*	%*I*
1.	*Microsporum gypseum*	10	88.8	14	84.4	—	100	—	100	—	100	—	100
2.	*Microsporum nanum*	18	80	40	55.5	25	72.2	21	76.6	17	81.1	15	83.3
3.	*Trichophyton mentagrophytes*	16	82.2	10	88.8	—	100	—	100	—	100	—	100
4.	*Trichophyton rubrum*	12	86.6	11	87.7	—	100	—	100	—	100	—	100

Table 27.

S. No.	Dermatophytes	Concentration of compound **46**											
		A 50 μL		5 μL		50 μL		100 μL		150 μL		200 μL	
		I	%*I*	*I*	%*I*	*I*	%*I*	*I*	%*I*	*I*	%*I*	*I*	%*I*
1.	*Microsporum gypseum*	10	88.8	9	90	—	100	—	100	—	100	—	100
2.	*Microsporum nanum*	18	80	38	57.7	18	80	15	83.3	13	85.5	12	86.6
3.	*Trichophyton mentagrophytes*	16	82.2	8	91.1	—	100	—	100	—	100	—	100
4.	*Trichophyton rubrum*	12	86.6	11	87.7	—	100	—	100	—	100	—	100

Table 28.

S. No.	Dermatophytes	Concentration of compound **48**											
		A 50 μL		5 μL		50 μL		100 μL		150 μL		200 μL	
		I	%*I*	*I*	%*I*	*I*	%*I*	*I*	%*I*	*I*	%*I*	*I*	%*I*
1.	*Microsporum gypseum*	10	88.8	11	87.7	—	100	—	100	—	100	—	100
2.	*Microsporum nanum*	18	80	29	67.7	16	82.2	14	84.4	12	86.6	10	88.8
3.	*Trichophyton mentagrophytes*	16	82.2	10	88.8	—	100	—	100	—	100	—	100
4.	*Trichophyton rubrum*	12	86.6	13	85.5	—	100	—	100	—	100	—	100

Table 29.

S. No.	Dermatophytes	Concentration of compound **55**											
		A 50 μL		5 μL		50 μL		100 μL		150 μL		200 μL	
		I	%*I*	*I*	%*I*	*I*	%*I*	*I*	%*I*	*I*	%*I*	*I*	%*I*
1.	*Microsporum gypseum*	10	88.8	11	87.7	—	100	—	100	—	100	—	100
2.	*Microsporum nanum*	18	80	35	61.1	16	82.2	14	84.4	12	86.6	10	88.8
3.	*Trichophyton mentagrophytes*	16	82.2	12	86.6	—	100	—	100	—	100	—	100
4.	*Trichophyton rubrum*	12	86.6	14	84.4	—	100	—	100	—	100	—	100

Table 30.

S. No.	Dermatophytes	Concentration of compound **56**											
		A 50 μL		5 μL		50 μL		100 μL		150 μL		200 μL	
		I	%*I*	*I*	%*I*	*I*	%*I*	*I*	%*I*	*I*	%*I*	*I*	%*I*
1.	*Microsporum gypseum*	10	88.8	12	86.6	—	100	—	100	—	100	—	100
2.	*Microsporum nanum*	18	80	38	57.7	16	82.2	14	84.4	12	86.6	10	88.8
3.	*Trichophyton mentagrophytes*	16	82.2	11	87.7	—	100	—	100	—	100	—	100
4.	*Trichophyton rubrum*	12	86.6	10	88.8	—	100	—	100	—	100	—	100

Table 31.

S. No.	Dermatophytes	Concentration of compound **58**											
		A 50 μL		5 μL		50 μL		100 μL		150 μL		200 μL	
		I	%*I*	*I*	%*I*	*I*	%*I*	*I*	%*I*	*I*	%*I*	*I*	%*I*
1.	*Microsporum gypseum*	10	88.8	12	86.6	—	100	—	100	—	100	—	100
2.	*Microsporum nanum*	18	80	37	58.8	16	82.2	13	85.5	11	87.7	10	88.8
3.	*Trichophyton mentagrophytes*	16	82.2	13	85.5	—	100	—	100	—	100	—	100
4.	*Trichophyton rubrum*	12	86.6	11	87.7	—	100	—	100	—	100	—	100

Table 32.

S. No.	Dermatophytes	Concentration of compound **60**											
		A 50 μL		5 μL		50 μL		100 μL		150 μL		200 μL	
		I	%*I*	*I*	%*I*	*I*	%*I*	*I*	%*I*	*I*	%*I*	*I*	%*I*
1.	*Microsporum gypseum*	10	88.8	13	85.5	—	100	—	100	—	100	—	100
2.	*Microsporum nanum*	18	80	32	64.4	—	100	—	100	—	100	—	100
3.	*Trichophyton mentagrophytes*	16	82.2	11	87.7	13	85.5	12	86.6	10	88.8	—	100
4.	*Trichophyton rubrum*	12	86.6	12	86.6	—	100	—	100	—	100	—	100

Table 33.

S. No.	Dermatophytes	Concentration of compound 67											
		A 50 μL		5 μL		50 μL		100 μL		150 μL		200 μL	
		I	%*I*	*I*	%*I*	*I*	%*I*	*I*	%*I*	*I*	%*I*	*I*	%*I*
1.	*Microsporum gypseum*	10	88.8	11	87.7	—	100	—	100	—	100	—	100
2.	*Microsporum nanum*	18	80	34	62.2	16	82.2	14	84.4	13	85.5	11	87.7
3.	*Trichophyton mentagrophytes*	16	82.2	13	85.5	—	100	—	100	—	100	—	100
4.	*Trichophyton rubrum*	12	86.6	10	88.8	—	100	—	100	—	100	—	100

Table 34.

S. No.	Dermatophytes	Concentration of compound 68											
		A 50 μL		5 μL		50 μL		100 μL		150 μL		200 μL	
		I	%*I*	*I*	%*I*	*I*	%*I*	*I*	%*I*	*I*	%*I*	*I*	%*I*
1.	*Microsporum gypseum*	10	88.8	12	86.6	—	100	—	100	—	100	—	100
2.	*Microsporum nanum*	18	80	37	58.8	15	83.3	12	86.6	10	88.8	—	100
3.	*Trichophyton mentagrophytes*	16	82.2	10	88.8	—	100	—	100	—	100	—	100
4.	*Trichophyton rubrum*	12	86.6	11	87.7	—	100	—	100	—	100	—	100

Conclusion

A series of novel spiro pyrrolidines have been synthesized and screened for antibacterial and antifungal activity. These compounds showed good to moderate activity against various pathogens and dermatophytic fungi. The biological activity of these compounds will trigger more interest in the synthesis of such compounds from the easily available starting materials.

Experimental

The starting materials **4–8**,[21–23] **19–24**,[24] **37–42**,[25–27] and **63–66**[28] were prepared as per the literature procedures.

General procedure for the cycloaddition reactions

A mixture of 2-arylidene-1-cyclohexanones **4–8** (0.5 mmol), isatin **1** (0.5 mmol) and sarcosine **2** (0.5 mmol) was refluxed in methanol or 1,4-dioxan until the disappearance of the starting materials as evidenced by the TLC. After the completion of reaction, the solvent was removed in vacuo and the residue was chromatographed on silica gel using hexane-ethylacetate mixture (10:1) as eluent to give 1-*N*-Methyl-spiro[2.3^1]oxindole-spiro[3.2^{11}]1^{11}-cyclohexanone-4-aryl-pyrrolidines **9–13.**

A mixture of 2-arylidene-1,2,3,4-tetrahydronaphthalen-1-ones **19–24** (0.5 mmol), isatin **1** (0.5 mmol) and sarcosine **2** (0.5 mmol) was refluxed in methanol or 1,4-dioxan until the disappearance of the starting materials as evidenced by the TLC. After the completion of reaction, the solvent was removed in vacuo and the residue was chromatographed on silica gel using hexane–ethylacetate mixture (10:1) as eluent and recrystallized from ethanol to give 1-*N*-methyl-spiro[2.3^1]oxindole-spiro[3.2^{11}]1^{11},2^{11},3^{11},4^{11}-tetrahydronaphthalen-1^{11}-ones-4-aryl-pyrrolidines **25–30.**

A mixture of 2,6-bis(arylmethylidene)cyclohexanones **37–42** (0.5 mmol), isatin **1** (0.5 mmol) and sarcosine **2** (0.5 mmol) was refluxed in methanol until the disappearance of the starting materials as evidenced by the TLC. After the completion of reaction, the solvent was removed in vacuo and the residue was chromatographed on silica gel using hexane–ethylacetate mixture (5:1) as eluent and recrystallized from ethanol to give 1-*N*-methyl-spiro[2.3^1]oxindole-spiro[3.2^{11}]6^{11}-aryl-methylidenecyclohexanone-4-aryl-pyrrolidines **43–48**.

A mixture of **43–48** (0.5 mmol) and 80% hydrazine hydrate (2 mL) was refluxed in ethanol (15 mL) for about 1 h. The reaction mixture was cooled, filtered and then the product was recrystallized from ethanol to give 1-*N*-methyl-spiro[2.3^1]oxindole-spiro[3.7^{11}](3^{11}-aryl)$\Delta^{1_{11}}$,7^{11}a-hexahydro-2*H*-indazole-4-aryl-pyrrolidines **55–60**.

A mixture of (*E*)-3-arylidene -5-phenyl butenolides **63–66** and *cis*-3-benzoyl-1-cyclohexyl-2-phenylaziridine **61** was refluxed in toluene under nitrogen atmosphere for about 36–48 h until the disappearance of the starting materials as evidenced by the TLC. After the reaction was over the solvent was evaporated in vacuo and the resulting product was purified by column chromatography using a hexane-benzene mixture (3:2) as eluent. The product was crystallized from ethanol to give 3-aryl-5-benoyl-1-cyclohexyl-2-phenyl-pyrrolidine-spiro[4.3^1]5^1-phenylbutenolides **67–70**.

C,H,N Analysis (elemental analyses) for the synthesized compounds.[1–4] **(9)** Calcd for $C_{23}H_{24}N_2O_2$: C, 76.64; H, 6.71; N, 7.77; found: C, 76.39; H, 6.78; N, 7.75. (**10**) Calcd for $C_{24}H_{26}N_2O_2$: C, 76.98; H, 7.00; N, 7.48;

found: C, 76.95; H, 7.05; N, 7.50. (**11**) Calcd for $C_{24}H_{26}N_2O_3$: C, 73.82; H, 6.71; N, 7.17; found: C, 73.79; H, 6.68; N, 7.57. (**12**) Calcd for $C_{23}H_{23}ClN_2O_2$: C, 69.95; H, 5.87; N, 7.09; found: C, 69.97; H, 5.54; N, 7.11. (**13**) Calcd for $C_{26}H_{30}N_2O_5$: C, 69.31; H, 6.71; N, 6.22; found: C, 69.37; H, 6.68; N, 6.27. (**25**) Calcd for $C_{27}H_{24}N_2O_2$: C, 79.39; H, 5.92; N, 6.86; found: C, 79.26; H, 5.98; N, 6.70. (**26**) Calcd for $C_{28}H_{26}N_2O_2$: C, 79.59; H, 6.20; N, 6.63; found: C, 79.71; H, 6.25; N, 6.72. (**27**) Calcd for $C_{28}H_{26}N_2O_3$: C, 76.69; H, 5.98; N, 6.39; found: C, 76.89; H, 5.84; N, 6.47. (**28**) Calcd for $C_{27}H_{23}ClN_2O_2$: C, 73.21; H, 5.23; N, 6.32; found: C, 73.43; H, 5.41; N, 6.18. (**29**) Calcd for $C_{29}H_{29}N_3O_2$: C, 77.13; H, 6.47; N, 9.31; found: C, 77.22; H, 6.58; N, 9.47. (**30**) Calcd for $C_{27}H_{23}N_3O_4$: C, 71.51; H, 5.11; N, 9.27; found: C, 71.79; H, 5.02; N, 9.38. (**43**) Calcd for $C_{30}H_{28}N_2O_2$: C, 80.33; H, 6.29; N, 6.25; found: C, 80.24; H, 6.32; N, 6.24. (**44**) Calcd for $C_{32}H_{32}N_2O_2$: C, 80.64; H, 6.77; N, 5.88; found: C, 80.61; H, 6.75; N, 5.89. (**45**) Calcd for $C_{32}H_{32}N_2O_4$: C, 75.57; H, 6.34; N, 5.51; found: C, 75.60; H, 6.32; N, 5.54. (**46**) Calcd for $C_{30}H_{26}N_2O_2Cl_2$: C, 69.63; H, 5.06; N, 5.41; found: C, 69.91; H, 5.03; N, 5.44. (**47**) Calcd for $C_{34}H_{38}N_4O_2$: C, 76.38; H, 7.16; N, 10.48; found: C, 76.32; H, 7.14; N, 10.51. (**48**) Calcd for $C_{30}H_{26}N_4O_6$: C, 66.91; H, 4.87; N, 10.40; found: C, 66.95; H, 4.84; N, 10.43. (**55**) Calcd for $C_{30}H_{30}N_4O$: C, 77.89; H, 6.54; N, 12.11; found: C, 77.89; H, 6.52; N, 12.09. (**56**) Calcd for $C_{32}H_{34}N_4O$: C, 78.34; H, 6.98; N, 11.42; found: C, 78.36; H, 6.95; N, 11.47. (**57**) Calcd for $C_{32}H_{34}N_4O_3$: C, 73.54; H, 6.56; N, 10.72; found: C, 73.54; H, 6.52; N, 10.76. (**58**) Calcd for $C_{30}H_{28}N_4OCl_2$: C, 67.82; H, 5.31; N, 10.54; found: C, 67.81; H, 5.33; N, 10.51. (**59**) Calcd for $C_{34}H_{40}N_6O$: C, 74.42; H, 7.35; N, 15.32; found: C, 74.45; H, 7.35; N, 15.30. (**60**) Calcd for $C_{30}H_{28}N_6O_5$: C, 65.21; H, 5.10; N, 15.21; found: C, 65.21; H, 5.08; N, 15.24. (**67**) Calcd for $C_{38}H_{35}NO_3$: C, 82.46; H, 6.33; N, 2.53; found: C, 82.72; H, 6.48; N, 2.15. (**68**) Calcd for $C_{39}H_{37}NO_3$: C, 82.54; H, 6.53; N, 2.47; found: C, 82.42; H, 6.15; N, 2.69. (**69**) Calcd for $C_{39}H_{37}NO_4$: C, 80.27; H, 6.35; N, 2.40; found: C, 80.39; H, 6.54; N, 2.02. (**70**) Calcd for $C_{38}H_{34}NClO_3$: C, 77.68; H, 5.79; N, 2.38; found: C, 77.43; H, 5.91; N, 2.51.

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