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Research Article

SYNTHESIS OF 1,4-BENZOTHIAZINE COMPOUND CONTAINING ISATIN MOIETIES AS ANTIMICROBIAL AGENT

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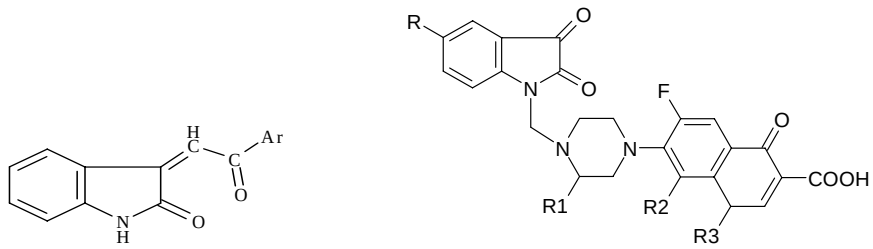
ABSTRACT

A series of novel (3Z)-1-(Secondary amino methyl)-3-(2-(3-methyl-4H-benzo[b][1,4]thiazin-2-yl)-2-oxoethylidene)indolin-2-one have been synthesized and studied on their *in vitro* antimicrobial (*antifungal* and *antibacterial*) activity potency to establish structure-activity relationship. Several compounds demonstrated promising antifungal rather than antibacterial activity; however, other tested compounds exhibited moderate to poor antimicrobial activity with respect to the reference drug against the test strains.

Keywords: 1,4-benzothiazine; Isatin; Antifungal activity; Antibacterial activity

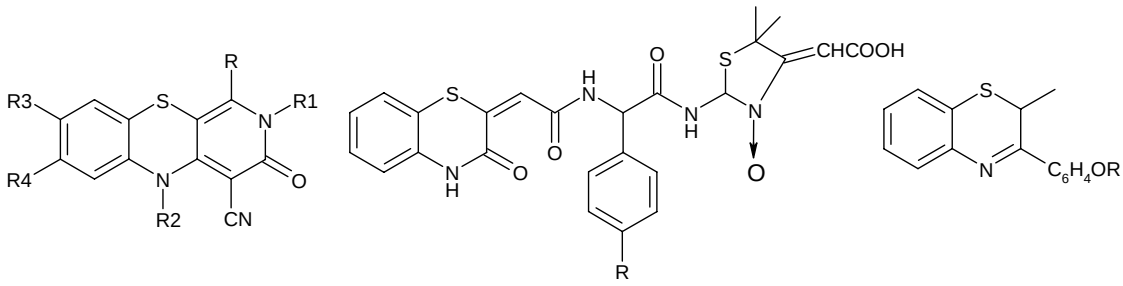
INTRODUCTION

Oxindoles and 2,3-dioxoindols are an endogenous compounds identified in humans, mammalian body fluids and tissues which shows an extensive range of biological activities including CNS depressant activities[1], sedative agents[2], anticonvulsant[3], anticancer[4], anti HIV[5], anxiogenic[6], antimicrobial activity[7] and antitubercular activity[8]. 3-Phenacyclidine-2-indolinone was well known antiseizure agent and active at both 100 and 300 mg kg^{-1} in the maximal electroshock seizure test[9]. (Fig. 1). The isatins with imines of other heterocycles, can be used for hair dyes[10], while azo*bis* isatins have been thoroughly studied as dyes for plastic materials[11]. 4H-1, 4-Benzothiazines are structural analogs of 10H-phenothiazines and possess a wide range of pharmacological activities due to the presence of a fold along the nitrogen and sulfur axis, which is considered to be responsible as one of the structural features to impart their activities [12,13]. The biological activities of the compound containing this basic moiety widely used as antihistaminics[14], antipsychotics[15], antiemetics[16], neuroleptics[17], tranquilizers[18] etc, (Fig. 2).



Antiseizure agent

Fig. 1: Some of Isatin based drugs



Anticonvalsant Antibiotic Blood Cholesterol lowering

Fig. 2: Some of 1,4-benzothiazine based drugs.

Looking at an importance of 1,4-benzothiazine moieties including 2,3-dioxoindoles compounds in biological system, it was thought that it would be worthwhile to design and synthesize compound containing both 1,4-benzothiazine and isatin derivative of mannich

bases to generate a series of new 1,4-benzothiazine derivative and screen them for potential biological activity. We have previously reported the synthesis and *in vitro* antimicrobial activities of some mannich bases of isatin. In continuation of our work on bioactive isatins with the aim of developing a new class of active drugs embracing certain characteristic structural features for active drug–receptor interaction, we now wish to report our result towards the synthesis of some novel 1,4-benzothiazine containing isatin.

MATERIALS AND METHODS

Experimental

Melting points of all the synthesized compounds were determined by open capillary method and are uncorrected. The purity of synthesized compounds were checked by thin-layer chromatography. The homogeneity of the compounds was monitored by ascending thin layer chromatography (TLC) on silica gel (Merck) glass plates, visualized by iodine-vapour. Developing solvents were hexane-ethyl acetate (8:2). The IR spectra were recorded on FTIR spectrophotometer [Perkin Elmer] using nujol mull. H^1 NMR spectra were scanned at 300 MHz on Varian Mercury YH-300 FT NMR spectra in $CDCl_3$/ DMSO-d_6 using tetramethylsilane as an internal standard. Mass spectra were recorded from hp1100 MSD mass spectral instrument (positive and negative APCI ion source, 50-200 V, nitrogen). All the chemicals and solvents used were of synthetic grade (S. d Fine, chemicals, Mumbai, India). The Compound 1 was prepared in the laboratory[19] .

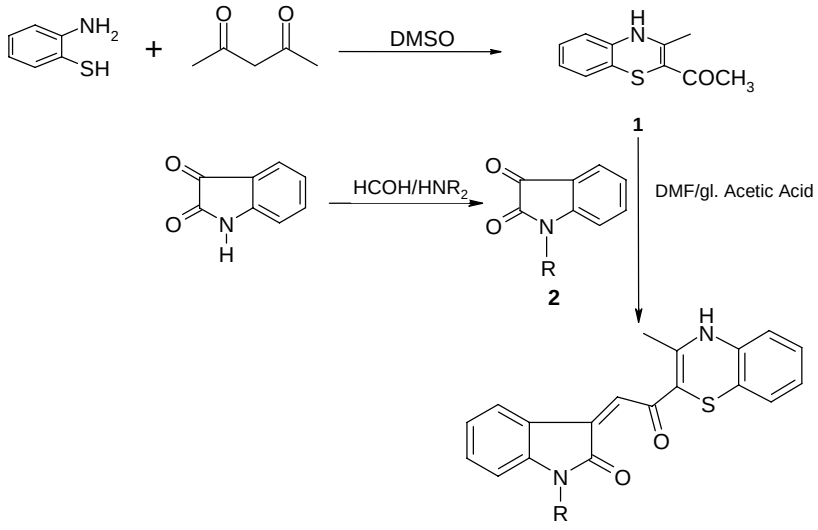
General synthesis of N-mannich bases of Isatin[20] 2 b-l:

Accordingly, Formaldehyde solution (2.5 mmol) and secondary amine (1.0 mmol) were dissolved in ethanol (20 ml) and stirred for 30 min. The iminiun ion formed *in situ* was then refluxed with isatin (1.0 mmol) in ethanol. The reaction was monitored by TLC (ethyl acetate: hexane, 2:8) and cooled at room temperature, refrigerated for 24-48 h to form crystals. The crystalline products were separated by filtration, washed with cold water and vacuum dried. Recrystallization from ethanol rendered desired products in pure form.

General procedure for preparing compounds 3a–l :

The equimolar mixture of Isatin or N-substituted isatin (0.2 g, 1.35 mmol) and compound **1** (0.27 g, 1.35 mmol) containing 1-1.5 mL of glacial acetic acid in dimethylformamide was warm for 2-5 h, the progress of reaction was monitored by TLC. The excess of solvent was removed under vacuum and the obtained semisolid was treated with ice-water then separated solid was filtered, washed with water, vacuum dried and recrystallization from ethanol rendered desired products in pure form.

Scheme 1:



3 a-l

In vitro antimicrobial activity

All the synthesized compounds **3a-l** were tested for *in vitro* antimicrobial activity. The lowest concentration (highest dilution) required to arrest the growth of microorganism was regarded as minimum inhibitory concentration (MIC) by an agar well diffusion method.[21] Normal saline was used to make a suspension of spore of fungal strain for lawning. A loopful particular strain was transferred to 3 mL of saline to get a suspension of corresponding species. Twenty milliliters of agar media were poured into each Petri-dish. Excess of suspension was decanted and the plates were dried in an incubator at 37 . Inhibition zones were measured after 48 h and compared with the control.

For antibacterial studies microorganisms employed were *Escherichia coli (ATCC-25923)* (gram-negative) and *Staphylococcus aureus* NTCC-6571 (gram-positive). For antifungal, Aspergilus niger and *Penicillium marneffei* were used as organism. The compounds whose MIC has to be determined is dissolved in serially diluted DMSO whereas Cloxacillin and Flucanazole were used as a standards for antibacterial and antifungal activity.

RESULTS AND DISCUSSION

The synthesis of (3Z)-1-(Secondary amino methyl)-3-(2-(3-methyl-4H-benzo[b][1,4]thiazin-2-yl)-2-oxoethylidene)indolin-2-one were achieved through the versatile and efficient synthetic route outlined in **Scheme 1**. The 1-(3-methyl-4H-benzo[b][1,4]thiazin-2-yl)ethanone **1** was prepared via the reaction of 2-aminobenzenethiol with acetyl acetone in dimethylsulfoxaide was refluxed for 2h. The progress of reaction was monitored by TLC (ethyl acetate: hexane, 3:7) and excess of solvent was removed under vacuum. The solid obtained was filtered, washed with water and crystallized from ethanol to give **1** and then condensed with isatin and substituted isatin with 4-5 drop of gl. acetic acid in DMF to obtained desire product with good yield **3a-l**.

Physical constants and analytical data of compounds are showed in **Table 1.**

Antibacterial activity

The approximate MIC values of the test derivatives indicate that most compounds exhibit good activity against *E. Coli* and *S. aureus* bacteria.

Antibacterial screening of compounds **3a–l** against *E. Coli* reveals that compounds **3g** and **3j** exhibit the good activity **>100 µg/ml**, whereas compounds **3i, 3k, and 3l** were respectable as compared to standard.

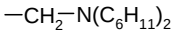
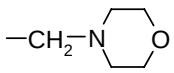
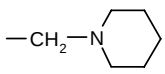
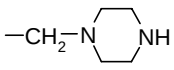
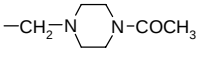
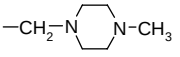
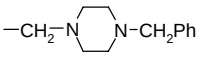
Most tested compounds had appreciable *in-vitro* antibacterial activity against *S. aureus*, the most active compound **3g, 3i, 3k and 3l** shows better activity **>100 µg/ml** as compared to standard. Where as compounds **3h, 3j** shows good activity and compound **3d** did not show any improvement of bacteria activity against *S. aureus*. (fig 3).

Antifungal activity

Antifungal screening of compounds **3a–l** against *A. Nigar* reveals that compounds **3g, 3h, 3i, 3j, 3l** exhibits the excellent activity, whereas compounds **3a-3d,3f** shows less activity as compared to standard.

Whereas the compounds **3j** and **3k** exhibits the excellent activity and **3g,3h,3j** and **3l** shows appreciable *in-vitro* antifungal activity against *P. marneffei*, whereas the compounds **3a -3f** shows less activity as compared to standard. (fig 4).

Table 1: Physical Constants and Analytical Data of Compounds

COMP.	R	M. P. (^{O}C)	YIELD (%)	MOLECULAR FORMULA	IR cm^{-1}	H^1 NMR (δ PPM)	EI-MS (M+1)
3a	H	152-154	79	$C_{19}H_{14}N_2O_2S$	3400, 3398, 3030, 2800, 1690, 1630, 1590, 1550, 1480, 610.	1.21 (3H, s, CH_3), 4.25 (1H, bs, NH), 5.28 (1H, s, -NH-CO), 6.89-7.20 (4H, m, Ar-H), 7.30-7.95 (4H, m, Ar-H), 7.34 (1H,S,CH)	334.08
3b	-CH_2-N(CH_3)$_2$	228-230	72	$C_{22}H_{21}N_3O_2S$	3410, 3390, 3020, 2810, 1680, 1640, 1590, 1550, 1480, 600.	1.39 (3H, s, CH_3), 2.40 (6H, s, 2CH_3), 3.59 (2H, s, CH_2), 3.90 (1H, bs, NH), 6.90-7.80 (8H, m, Ar-H), 7.34(1H,s,CH).	391.14
3c	-CH_2-N(CH_2CH_3)$_2$	215-218	69	$C_{24}H_{25}N_3O_2S$	3415, 3380, 3020, 2840, 1690, 1660, 1610, 1580, 1495, 640.	1.10 (6H, s, 2CH_3), 1.80 (3H, t, 2CH_3), 2.35-2.40(4H, m, 2CH_2), 4.50 (2H, s, CH_2), 5.00 (1H, bs, NH), , 6.80-7.19 (4H, m, Ar-H), 7.59-7.94 (4H, m, Ar-H). 7. 41 (1H,s, CH).	419.17
3d	-CH_2-N[C(CH_3)$_2$]$_2$	110-120	72	$C_{26}H_{29}N_3O_2S$	3400, 3395, 3030, 2830, 1670, 1660, 1600, 1580, 1490, 620.	1.20 (12H, d, 4CH_3), 1.74 (3H, s, CH_3), 2.80 (2H, m, 2CH), 4.35 (2H, s, CH_2), 5.40 (1H, bs, NH), 6.90-7.22 (4H, m, Ar-H),7.30-7.64(4H,m,Ar-H),7.32 (1H,s,CH)	446.00
3e	-CH_2-N[(CH_2)$_3$$CH_3$]$_2$	232-234	75	$C_{28}H_{33}N_3O_2S$	3410, 3385, 3010, 2890, 1680, 1650, 1600, 1510, 1500, 620.	0.90 (6H, t, 2CH_3), 1.34 (8H, q, 4CH_2), 1.71 (3H, s, CH_3), 2.58 (4H, m, 2CH_2), 4.20 (2H, s, CH_2), 5.10 (1H, bs, NH), 6.59-6.75 (4H, m, Ar-H), 6.90-7.30 (4H, m, Ar-H), 7.42 (1H,S,CH).	475.23
3f		212-214	78	$C_{32}H_{37}N_3O_2S$	3420, 3380, 3020, 2900, 1680, 1660, 1610, 1570, 1450, 640	1.10-1.42(12H, m, 6CH_2), 1.58 (4H, m, 2CH_2), 1.81 (3H, s, CH_3), 1.90 (4H, m, 2CH_2), 2.66 (2H, m, 2-NCH), 4.20 (1H, bs, NH), 4.30 (2H, s, CH_2), 6.90-7.29 (4H, m, Ar-H), 7.36(1H,s,CH), 7.62-7.80 (4H, m, Ar-H).	526.00
3g		182	75	$C_{24}H_{23}N_3O_3S$	3410, 3380, 3030, 2920, 1640, 1600, 1590, 1550, 1410, 1080, 640	1.69 (3H, s, CH_3), 2.59 (4H, t, 2CH_2-N), 3.65 (4H, t, 2CH_2-O), 4.20 (2H, s, CH_2), 4.80 (1H, bs, NH), 6.00 (1H,s,CH), 7.20-7.50 (4H, m, Ar-H), 7.79-7.89 (4H, m, Ar-H).	433.00
3h		132	73	$C_{25}H_{25}N_3O_2S$	3400, 3390, 3040, 2850, 1690, 1630, 1580, 1540, 1480, 690	1.20-1.40 (6H, m, 3CH_2), 1.71 (3H, s, CH_3), 2.49 (4H, t, 2CH_2), 4.80 (2H, s, CH_2), 5.10 (1H, bs, NH), 5.45 (1H,s,CH) 6.64-7.00 (4H, m, Ar-H), 7.38-7.56 (4H, m, Ar-H)	433
3i		110	70	$C_{24}H_{24}N_4O_2S$	3410, 3380, 3030, 2900, 1680, 1660, 1620, 1580, 1490, 670	1.70 (3H, s, CH_3), 2.40 (4H, m, 2CH_2-N), 2.82 (4H, t, 2CH_2), 4.60 (2H, s, CH_2), 5.00 (1H,s,CH), 5.28 (1H, bs, NH), 7.00 -7.30 (4H, m, Ar-H), 7.69-8.00 (4H, m, Ar-H), 8.25 (1H, s, NH).	434
3j		270-272	73	$C_{26}H_{26}N_4O_3S$	3430, 3395, 3040, 2850, 1720, 1690, 1630, 1590, 1550, 1450, 600	1.40 (3H, s, CH_3), 2.19 (3H, s, $COCH_3$) 2.64 (4H, t, 2CH_2-N), 3.00 (4H, t, 2CH_2-N), 4.20 (2H, s, CH_2), 4.89 (1H,s,CH), 5.00(1H,bs, NH), 6.80-7.00 (4H, m, Ar-H), 7.64-7.82 (4H, m, Ar-H)	496
3k		228-230	70	$C_{25}H_{26}N_4O_2S$	3400, 3380, 3010, 2900, 1680, 1660, 1620, 1650, 1600, 700	1.60 (3H, s, CH_3), 2.00 (3H, s,NCH_3), 2.50 (4H, t, 2CH_2-N), 2.82 (4H, t, 2CH_2-N), 4.59 (2H,s, CH_2), 4.34 (1H, s, NH), 5.20 (1H,s,CH) 7.10-7.39 (4H, m, Ar-H), 7.19-7.90 (4H, m, Ar-H).	447
3l		215-218	77	$C_{31}H_{30}N_4O_2S$	3410, 3390, 3030, 2800, 1690, 1640, 1590, 1550, 1500, 650	1.58 (3H, s, CH_3), 2.35 (4H, m, 2CH_2-N), 2.60 (4H, t, 2CH_2-N), 3.25 (2H,s, CH_2), 4.00 (2H,s, CH_2), 4.90 (1H, s, NH), 6.20 (1H,s, CH) , 7.00-7.39 (9H, m, Ar-H), 7.68-7.90 (4H, m, Ar-H),	523

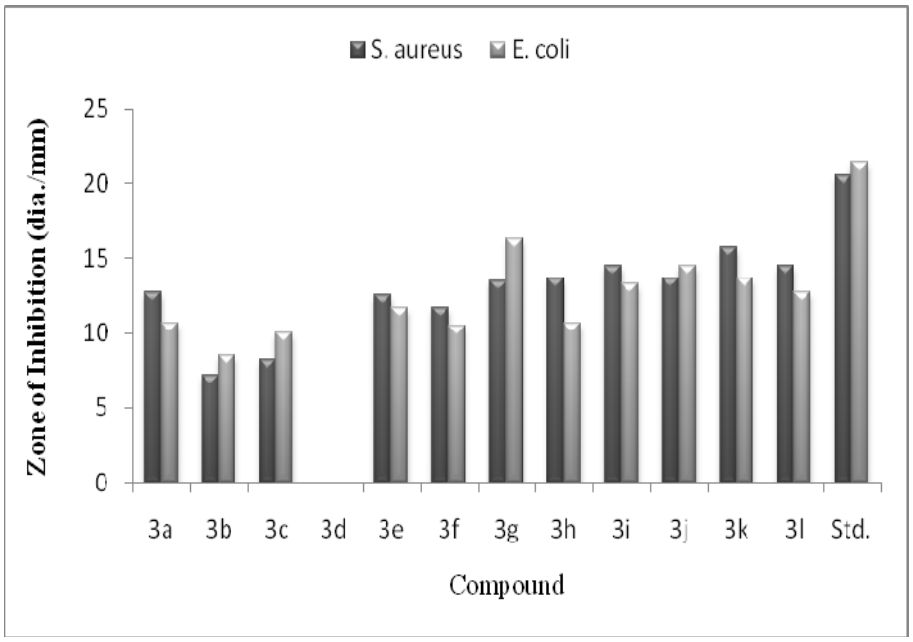


Fig. 3: Graphical representation of antibacterial activity of compounds

3a—3l at mic value 100 µg/ml concentration and cloxacilline used as a standard at mic value 50 µg/ml.

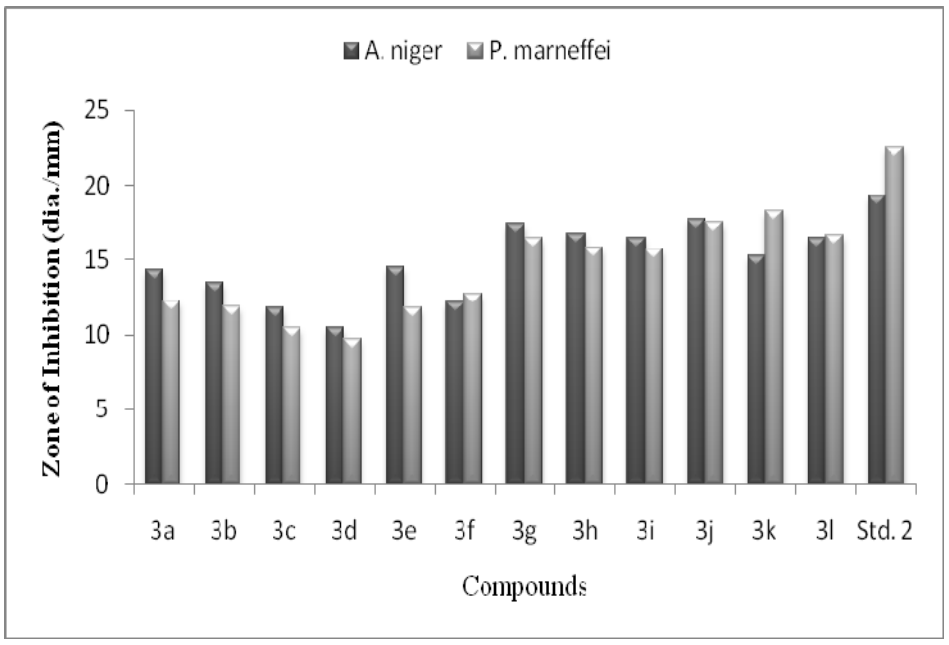


Fig. 4: Graphical representation of antifungal activity of compounds 3a-l

At mic value 100 µg/ml concentration and flucanazole used as a standard at mic 50 µg/ml

According to structure-activity relationships (SAR) for (3Z)-1-(Secondary amino methyl)-3-(2-(3-methyl-4H-benzo[b][1,4]thiazin-2-yl)-2-oxoethylidene)indolin-2-one **3a-l**, the spectrum of antimicribial coverage and the overall pharmacokinetics largely depends upon the substitution.

The structural modification of **3** was found to be more effective towards morpholine, piperazine, *N*-acetyl piperazine, *N*-methyl piperazine, N-benzyl piperazine derivatives **(3f-l)** against tested antifungal species than that of antibacterial. Moreover, the alteration of isatin to a substituted methylene **(3b-e)** group could not improve the overall activity against most strains. Such study of structural activity relationship established on the basis of bioassay could be useful to consolidate the moderately toxic and nontoxic new group.

Table 2: quantitative antimicrobial activity of compounds 3a—l (zone of inhibition in mm)[A]

Compounds	Antibacterial activity		Antifungal activity	
	S. aureus	*E. coli*	*A. niger*	*P. marneffei*
3a	12.7	10.7	14.3	12.3
3b	7.2	8.5	13.5	11.9
3c	8.2	10	11.8	10.5
3d	0	0	10.5	9.7
3e	12.5	11.7	14.5	11.8
3f	11.7	10.5	12.2	12.7
3g	13.5	16.3	17.4	16.5
3h	13.7	10.7	16.8	15.8
3i	14.5	13.3	16.5	15.7
3j	13.7	14.5	17.7	17.5
3k	15.7	13.7	15.3	18.3
3l	14.5	12.7	16.5	16.7
Cloxacilline	20.5	21.5	--	--
Fluconazole	--	--	19.3	22.5

[a] The quantitative antimicrobial data of compounds **3a—l** at MIC value 100 µg/mL concentration and cloxacilline and fluconazole used as a standard for at MIC value 50 µg/mL.

CONCLUSION

In conclusion, we have synthesized a series of isatin derivatives linked to 1,4-benzothiazine moiety and found **3e, 3h-l** compound showed appreciable *in vitro* antimicrobial activity with respect to the reference and modification of the *N-1* substituent of piperazine ring and its derivatives produced relatively major changes in terms of activity. However, other compounds containing morphalin, piperidin exhibited good antibacterial and antifungal activity.

These results are encouraging to better define and optimize the antifungal effect of these compounds. Further investigations are currently in progress to verify the susceptibility of the other fungi to these compounds and to outline their pharmacokinetic profile.

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