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Synthesis of alkaloids 3,3'-(pyridin-2-yl)methylene)*bis*(1*H*-sustituted-indole) *via* infra red irradiation as heating and their evaluation antifungal against *Candida albicans*, *Cryptococcus neoformans* and *Aspergillus fumigatus*

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Herein is reported a comparative study of a series of new alkaloids structurally related to turbomycin, namely 3,3'-(pyridin-2-yl)methylene)bis(1*H*-indole) **15**, 3,3'-(pyridin-2-yl)methylene)bis(1-methyl-1*H*-indole) **16**, 3,3'-(pyridin-2-yl)methylene)bis(2-methyl-1*H*-indole) **17** and 3,3'-(pyridin-2-yl)methylene)bis(2-phenyl-1*H*-indole) **18**, compounds that have been synthesized through infra red irradiation as an efficient green activation process. In addition, these diindolylmethanes have been evaluated against levaduriform fungi (*Candida albicans* (ATCC 10231) and *Cryptococcus neoformans* (ATCC 36556)) and filamentous fungi (*Aspergillus fumigatus* (ATCC 13073)), with the 3,3'-(pyridin-2-yl) methylene)bis(2-phenyl-1*H*-indole) **15** derivative being the one exhibiting the best activity of the series. The derivatives **15**-**18** exhibit a Minimum Inhibitory Concentrations (MIC) > 900 for *C. albicans* and *C. neoformans* ≤ 600.

Keywords: 3,3'-(Pyridin-2-yl)methylene)bis(1*H*-sustituted-indole), Infra red irradiation, Antifungal activity, *Candida albicans*, *Cryptococcus neoformans*, *Aspergillus fumigatus*

Infra red irradiation as an alternative source of heating to obtain chemical molecules, was studied for the first time by Pool and Teuben who carried out the first investigations using this heating source using Philips IR (infra red) heat lamps as an unconventional and innovative energy source with potential applications in the area of organic synthesis[1,2]. However, its application had a slow acceptance despite being a benign source of heating, being in line with the principles of green chemistry[3]. Infra red light acts under the excitation of vibrations, providing the quanta of energy required to promote chemical reactions according to the principles of photochemistry[4-7].

In time, reactions carried out using infra red energy have resulted in the attaining of various molecules of interest such as 3,4-dihydropyrimidin-2(1*H*)one (Biginelli reaction)[8] 1,4-dihydropyridine (Hantzsch reaction)[9] α-ketothioamide (Willgerodt-Kindler reaction)[10] ε-caprolactam (Beckmann rearrangement reaction)[11] (*Z*)-(aminomethyl) (aryl)phenylhydrazone (palladium-catalyzed Heck reaction)[12] 3,5-diphenyltetrahydrobenzo[*d*]oxazol-2-one (Diels-Alder cycloaddition reaction)[13] biaryls (Suzuki-Miyaura cross-coupling reaction)[14] and benzylidnomalonates (Knoevegenagel reaction)[15], other heterocycles also obtained using the same energy source includes the

synthesis of benzimidazoles-diindolylmethane[16], benzimidazoles[17] and some DIMs using alumina as solid support[18]. Based on the above, we want to explore the synthesis of 3,3'-(pyridin-2-yl)methylene)*bis*(1*H*-substituted-indole) in an acidic ethanolic solution, using infra red energy to build C−Cπ bonds between derivatives of the indole and picolinaldehyde *via* a three component system. Our interest in this type of potentially biologically relevant structures stems from the fact that 3,3′-diindolylmethane (DIM) **1** is a privileged moiety widely used against cancer[19], also exhibiting activity against bacteria and as anticarcinogenic agent[20-,22], anti-HIV, antibiotic, and for its selectivity against COX-2 inhibitors[23] (Fig. 1). So, this scaffold seems to have the essentials to find new potential candidates including DIM that may exhibit antifungal activity against *Candida albicans*, *Cryptococcus neoformans*, and *Aspergillus fumigatus* strains.

Thus, encouraged by the wide range of biological activities exhibited by DIM derivatives, we decided to synthesize a series of derivatives bearing pyridyl as an important antifungal pharmacophore. Thus, in this opportunity we report herein the synthesis of derivatives **15-18** and test them as antifungal agents against three mycotic organisms *Candida albicans* (ATCC 10231), *Cryptococcus neoformans* (ATCC 36556), and *Aspergillus fumigatus* (ATCC 13073). These compounds were obtained from good to excellent yields (74-82%) in short reaction times based on the implementation of three-component reactions, using infra red irradiation as an alternative for heating.

Results and Discussion

The three-component condensation of 2 equivalent of indole with 1 equivalent of pyridincarboxaldehyde in ethanol, irradiated with infra red light (Scheme 1), afforded the series of compounds **15-18** through an efficient unconventional synthetic approach. All compounds were fully characterized by their physical properties and conventional spectroscopic techniques *i.e.*, IR, ^{1}H and ^{13}C NMR spectroscopy, and HRMS method. Analysis by ^{1}H NMR spectroscopy of compounds **15-18** enables us to ascribe the key methine singlet unambiguously between δ 5.96-6.00 ppm due to the magnetic anisotropy of the neighboring aromatic rings. Whereas analysis by ^{13}C NMR techniques affords a series of spectra exhibiting signals in the range of δ 32.5 to 54.8 ppm for the methine carbon. These assignations were further complemented with analysis by IR

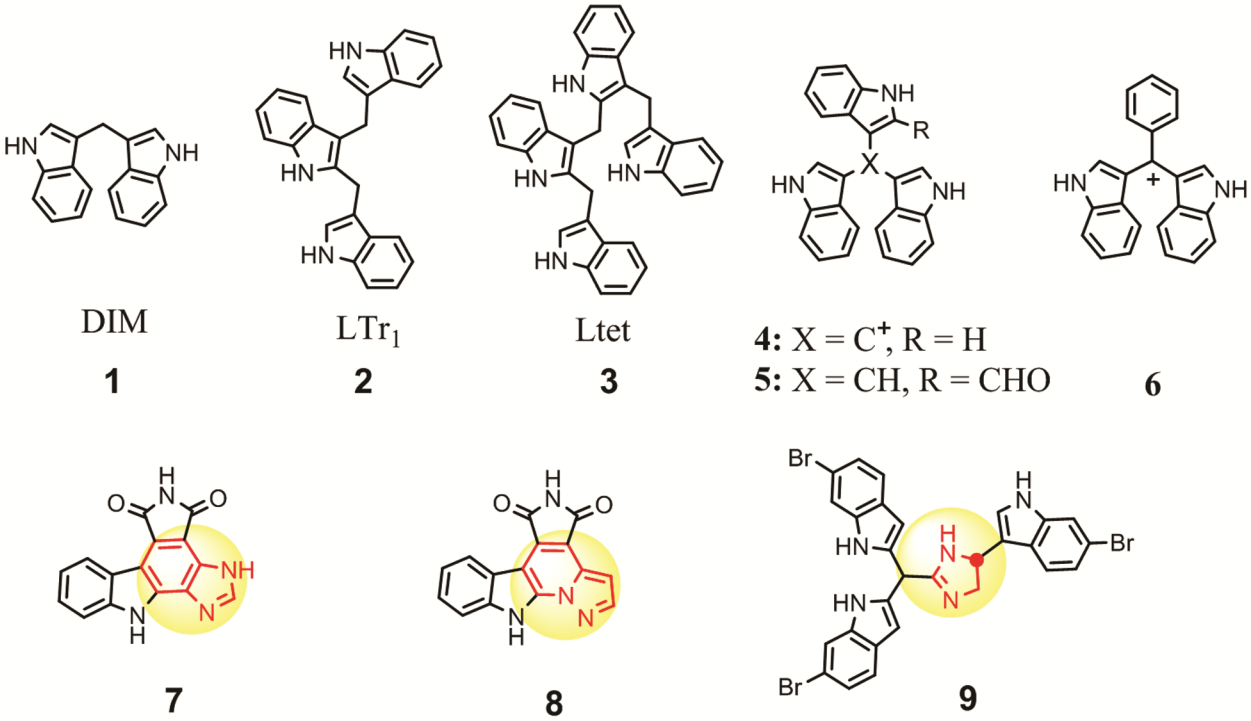
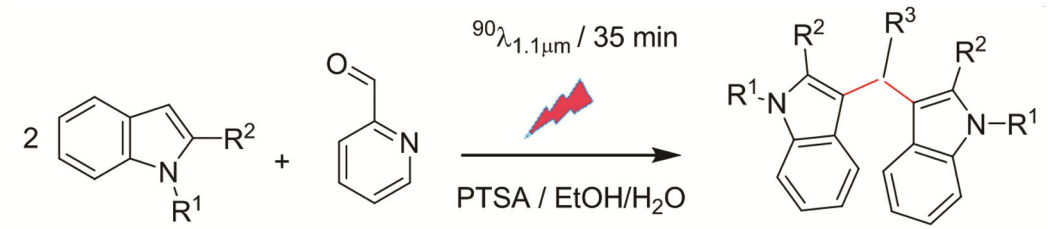


Fig. 1 — DIIM **1**. Compounds featuring indolyl and DIM skeletons. Turbomycin A[24] **2**, trisindolal **3**, turbomycin B **4** anti-microorganisms Gram (+) and Gram (−). LTr1 **5** and Ltet **6** anti-cancer activity. Granulatimide[25] **7**, isogranulatimide[25] **8** natural products against Chk1, and tulongicin A **9** anti-bacterial[26]



10 R^1 = H, R^2 = H
11 R^1 = Me, R^2 = H
12 R^1 = H, R^2 = Me
13 R^1 = H, R^2 = Ph

14 Picolinaldehyde

15 R^1 = H, R^2 = H, R^3 = picolin-2-yl (81%)
16 R^1 = Me, R^2 = H, R^3 = picolin-2-yl (82%)
17 R^1 = H, R^2 = Me, R^3 = picolin-2-yl (80%)
18 R^1 = H, R^2 = Ph, R^3 = picolin-2-yl (74%)

Scheme 1 — Synthesis of 3,3'-(pyridin-2-ylmethylene)bis(1-substituted-indole)

Table 1 — Results of qualitatives test (2 µg/disc)

Compd	Halo (mm) *C. albicans* ATCC 10231	Halo (mm) *C.neoformans* ATCC 36556	Halo (mm) *A. fumigatus* ATCC 13073
15	9	10	No Inhibition
16	7	8	No Inhibition
17	7	9	No Inhibition
18	9	12	No Inhibition

spectroscopy, exhibiting the sp^3 C-H spectral fingerprint around 2910-2937 cm^{-1}. Finally, analysis by mass spectrometry afforded spectra showing peaks with the corresponding molecular mass (*m/z*) for all compounds, thus corroborating these species structural identity and they have been obtained pure (Scheme 1).

Noteworthy is the fact that employing infra red irradiation, steric demanding building blocks as in **16-18** bearing Me− and Ph− groups either on N1 or C2 positions afforded excellent yields. Thus, promoting the C−Cπ bond construction reaction using infra red radiation proved to be successful, emerging as an efficient synthetic tool with potential valuable applications in organic chemistry being worthy to promote for future synthetic developments.

In vitro anti-fungical activity

In order to study the potential antifungal activity of compounds **15-18** they were tested against *C. albicans. C. neoformans* and *A. fumigatus* using the diffusion disc method having as positive control Ketoconazole and as negative control DMSO. The results of these tests, Table 1, indicate that all synthesized compounds have activity against these strains, although this activity is less marked against *C. albicans* in all cases (Fig. 2) and more marked for *C. neoformans* (Fig. 3).

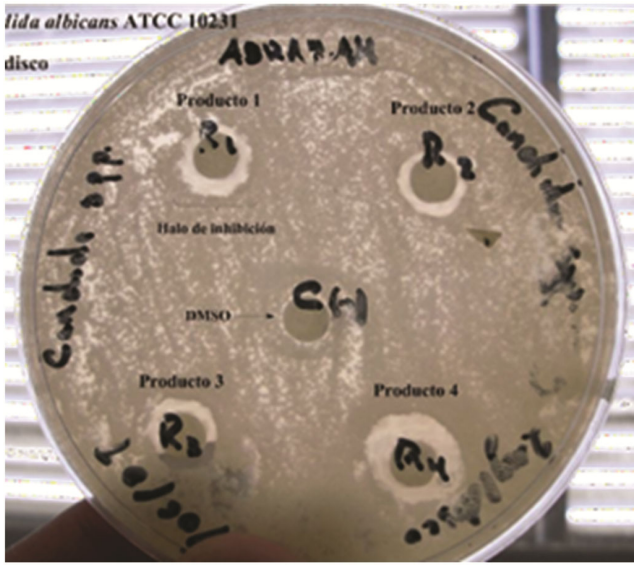


Fig. 2 — *C. albicans* ATCC 10231. Disc diffusion test for the four products at a concentration of 2 µg/disc and as Control (-) DMSO.

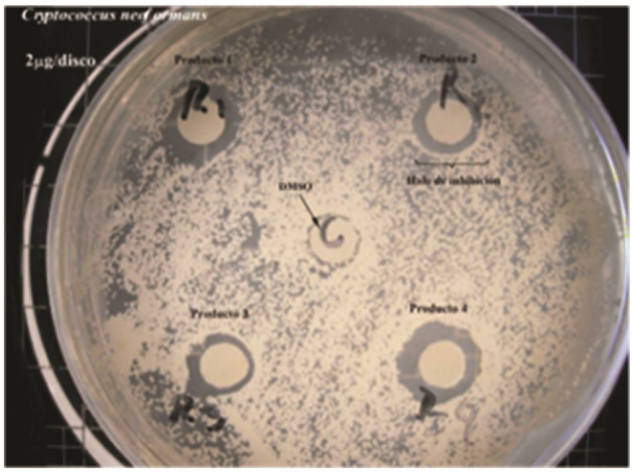


Fig. 3 — *C. neoformans* ATCC 36556. Disc diffusion test for the four products at a concentration of 10 µg/disc. Control (+) Ketoconazole at a concentration of 0.5 µg/disc, Control (-) DMSO.

Compound **18** is more active against yeasts (*C. albicans* and *C. neoformans*), showing the largest inhibition halos. This probably being due to the presence of the phenyl substituents at the indolic groups, while compounds **16** and **17** that have a methyl group on their structures, exhibited similar activity against these yeasts (inhibition halo of both compounds was the same against *C. albicans* and almost the same against *C. neoformans*) and both were less active than compound **15**, which is not substituted at the indolic group, leading to believe that the methyl group present in compounds **16** and **17** may cause some negative effect on their antifungal activity.

To avoid any potential false positive results, the solvents and starting materials used in the synthesis of compounds **15-18** were also evaluated against *C. albicans* and *C. neoformans* not observing any potential inhibition due to these chemicals.

In addition, the radial growth inhibition test was performed with *C. neoformans* at an initial concentration of 2 µg/disc, and subsequently increased to 5 µg and 10 µg/disc. Fig. 4 shows the evaluation of the series of compounds at a concentration of 10 µg, and the positive control, ketoconazole, at a concentration of 0.5 µg/disc.

To further evaluate the activity against filamentous fungi, the method of inhibition of radial growth was performed, with a modification to that made by Wang and Bun[27]. This test seeks the formation of inhibition halos based on the radial growth of the filamentous fungus, and in this work, we used *Aspergillus fumigatus*, as a fungus representative of filamentous fungi which causes aspergillosis. The synthesized compounds were initially evaluated against this strain at a concentration of 2 µg and in which no indication of inhibition was observed, especially in the formation of some halos. Subsequently, the compounds were evaluated at a concentration of 5 and 10 µg, which, like the previous concentration, did not show the formation of half halos. In this test, Control (+) ketoconazole at a concentration of 0.5 µg/10 µL (Fig. 5).

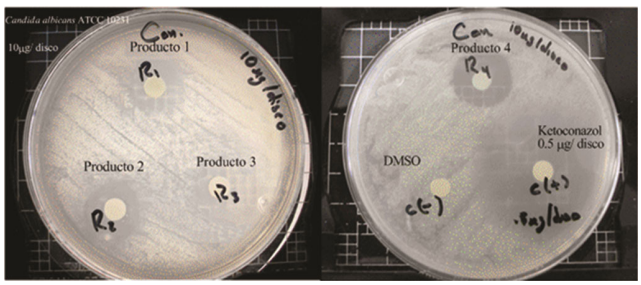


Fig. 4 — *C. albicans* ATCC 10231. Disc diffusion test for the four products at a concentration of 10 µg/disc. Control (+) Ketoconazole at a concentration of 0.5 µg/disc, and as Control (-) DMSO.

Quantitative testing

From the results obtained in the qualitative tests, the Minimum Inhibitory Concentration (MIC) of the products that exhibited antifungal activity was calculated. The products were first evaluated at concentrations comprising the 5 µg at 3.5 µg/10 µL, without obtaining appreciable results. Subsequently, the concentrations of 6 to 9 µg/10 µL were increased in which the growth reduction of *Candida albicans* colonies and total inhibition for *Cryptococcus neoformans* could be observed.

Here Fig. 6, Fig. 7, Fig. 8 and Fig. 9 show the MIC of products at concentrations from 6 to 9 µg/10 µL. While in Fig. 10 we can observe the positive and negative controls. The results of the MIC are shown in Table 2.

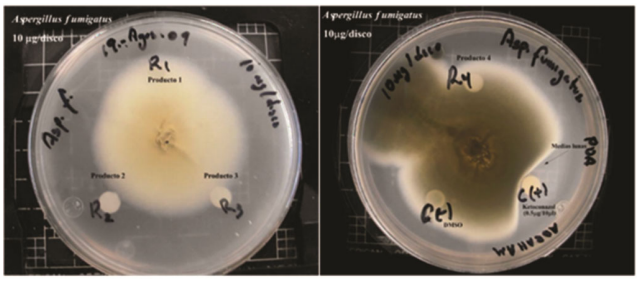


Fig. 5 — Radial growth inhibition test for the four compounds against *A. fumigatus* a (10 µg/disc).

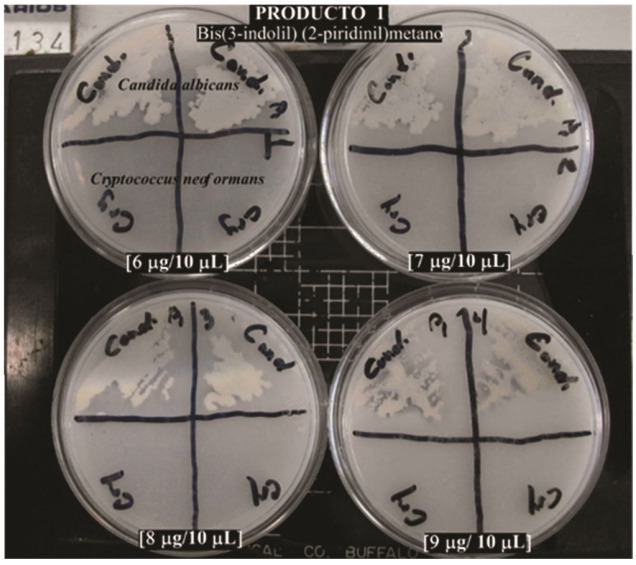


Fig. 6 — Agar diffusion sensitivity test for product **15**, against *C. albicans* ATCC 10231 and *C. neoformans* ATCC 36556 for MIC determination, at concentrations from 6 to 9 µg/10 µL.

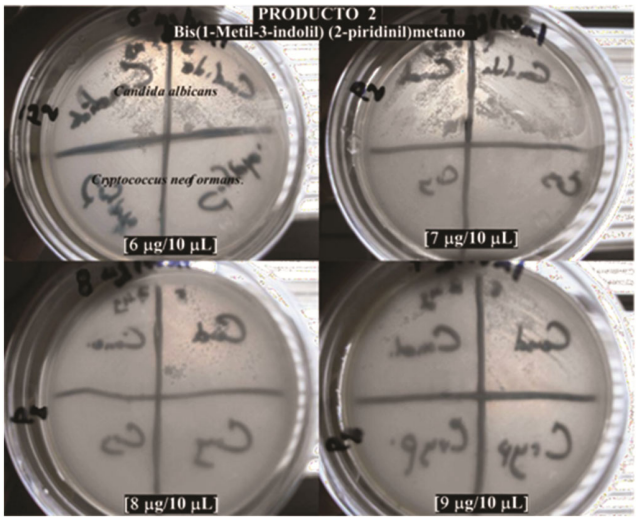


Fig. 7 — Agar diffusion sensitivity test for product **16**, against *C. albicans* ATCC 10231 and *C. neoformans* ATCC 36556 for MIC determination, at concentrations from 6 to 9 µg/10 mL

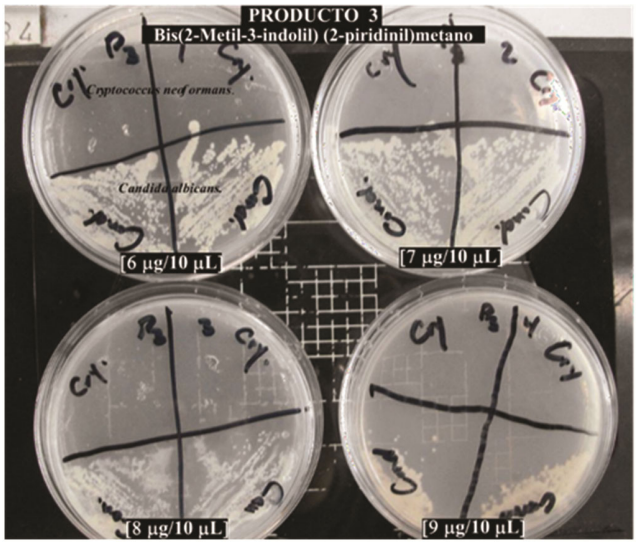


Fig. 8 — Agar diffusion sensitivity test for product **17**, against *C. albicans* ATCC 10231 and *C. neoformans* ATCC 36556 for MIC determination, at concentrations from 6 to 9 µg/10 µL.

Also, the dilution sensitivity test was carried out in agar, which was initially performed at concentrations close to 2 µg (which is the concentration used in the disc diffusion test). The concentrations were varied from 0.5 to 3.5 µg/10 µL, however, no antifungal activity was observed at such concentrations by any of the compounds, nor was observed any decrease in the number of colonies or morphological alteration thereof. But because of these negative results, we increased the concentrations of compounds, however, for the sake of optimize the compounds we had, they were evaluated at concentrations from 6 to 9 g/10 µL.

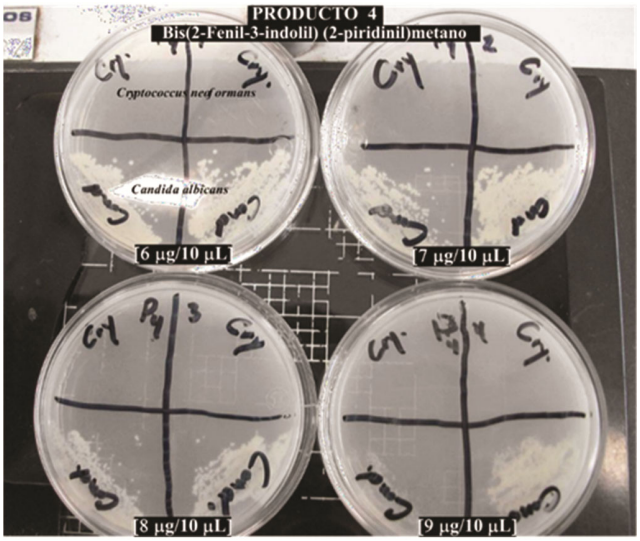


Fig. 9 — Agar diffusion sensitivity test for product **18**, against *C. albicans* ATCC 10231 and *C. neoformans* ATCC 36556 for MIC determination, at concentrations of 6 to 9 µg/10 µL

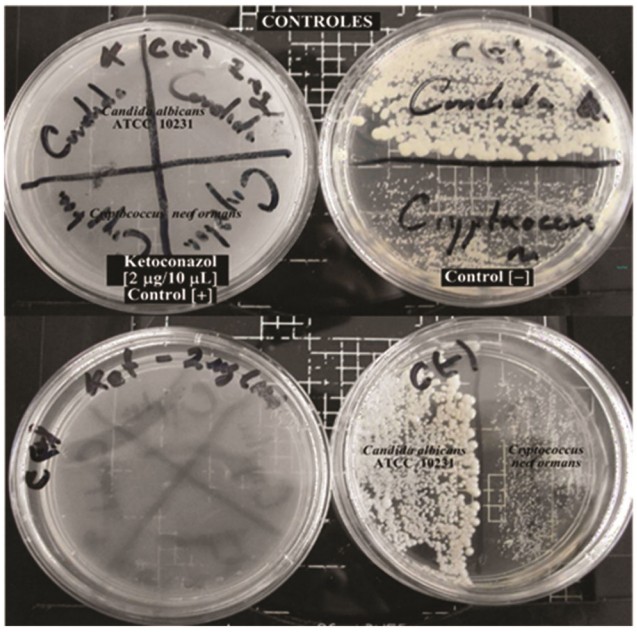


Fig. 10 — Control (+) Ketoconazole 2 µg/10 µL) and Control (-) DMSO, for both yeasts: *Candida albicans* y *Cryptococcus neoformans*.

Table 2 — Results of the quantitative test

Compd	CMI (µg/mL) *C. albicans* ATCC 10231	CMI (µg/mL) *C. neoformans* ATCC 36556
15	>900	≤600
16	>900	≤600
17	>900	≤600
18	>900	≤600

On this basis, compound **15** had a total inhibition at the lowest concentration of 6 µg/10 µL for *C. neoformans*, in contrast, for *C. albicans* only a reduction in growth was observed in comparison with the initial concentration of 6 µg to the final of 9 µg. (Fig. 6). Based on the evaluation described above in which a concentration interval was taken from 0.5 to 3.5 µg and no visible activity was observed, it can be concluded that the MIC of product **15** with *C. neoformans* is found in the range from 400-600 µg/mL, while its MIC with *C. albicans* is above 900 µg/mL.

On the other hand, compound **16**, also showed a total inhibition at the smallest concentration evaluated of 6 µg/10 µL for *C. neoformans*; while for *C. albicans*, an almost total inhibition was observed at the highest concentration of 9 µg/10 µL (Fig. 7). Similarly, the negative result of the concentration interval from 0.5 to 3.5 µg, may indicate that the MIC of compound **16** concerning *C. neoformans*, is in an interval from 400-600 µg/mL, while that for *C. albicans* the MIC is close to 900 µg/mL.

Compound **17** presents a total inhibition against *C. neoformans* at the lowest evaluated concentration of 6 µg /10 µL; whilst for *C. albicans* there is only a gradual decrease in the number of colonies, not observing total inhibition (Fig. 8). Thus, based on the above-mentioned results the MIC of compound **17** concerning *C. neoformans* is established between an interval of concentrations from 400-600 µg/mL, while the MIC with *C. albicans* was the highest concentration evaluated being greater than 900 µg/mL.

Finally, compound **18**, also had a total inhibition for *C. neoformans* at the lowest concentration evaluated at 6 µg/10 µL. And like is analogous derivatives **15-17** MIC values concerning *C. neoformans* were established within an interval from 400–600 µg/mL. Unlike *C. albicans*, which decrease in both the number of colonies and their size, being this visible by comparing the initial and final concentrations evaluated. Thus, the inhibition exhibited by compound **18** was almost complete for *C. albicans* at the highest evaluated concentration of 900 µg/mL, and so the MIC regarding *C. albicans* must be very close to 900 µg/mL (Fig. 9).

Based on the data above, it can be concluded that all synthesized compounds have a similar antifungal activity concerning *C. neoformans*, as they all had a total inhibition at 6 µg/10 µL; while for *C. albicans* they all had a decrease in growth. Compounds **16**, **17**, and **18** had higher activity, which led to a further reduction in colonies growth, and in some cases, a decrease in the size of the colonies was observed (as was the case of compound **18**), which may indicate a morphological change in the structure of the fungus. This difference between the concentration obtained in the disc diffusion test (2 µg/10 µL), compared to those obtained in the agar dilution test (6 µg/10 µL) may be because qualitative tests are not necessarily representative.

Finally, based on the data presented, it can be concluded that the synthetic method used is highly efficient since in all cases the formation of the compounds was reached in higher yields. It must also be noted that the use of infra red radiation caused the reactions to be carried out in short periods of time, reducing the use of solvents and the use of harmful catalysts thus lowering the costs. Hence, the proposed method of synthesis is certainly more empathic with the environment being in line with some of the principles of Green Chemistry.

In this way, the synthesized compounds exhibited activity against yeasts, in contrast to the filamentous fungi in which no activity was shown. This yeast activity does not represent an important antifungal activity compared to existing commercial antifungals. However, these compounds represent an option for the future development of new antifungals, as there is precedent in which metabolites obtained from plants of the cruciferous family *phytoalexins* (mainly indolic derivatives), exhibit antifungal activity with encouraging results[28].

Experimental Section

All commercial reagents and solvents were of reagent grade and purchased from Sigma-Aldrich. All of them were used as received without further treatment. Analytical TLC and preparative chromatography were performed on precoated Kieselgel 60F_{254} plates and Gel silice MN-Kieselgel G/UV_{254} respectively, and spots were located using UV (254 nm). Melting points were determined in a Buchi B-450 melt-point apparatus. ^{1}H and ^{13}C NMR spectra were recorded either in $CDCl_3$ or DMSO-d_6 using a Varian 300 MHz spectrometer. Chemical shifts (δ) are expressed in ppm relative to $Si(Me)_4$. The coupling constant ($^{3}J_{HH}$) values are in Hertz, and the splitting patterns are designated as follows: singlet (*s*), doublet (*d*), triplet (*t*), and multiplet (m). Elemental analysis was obtained using a Thermo

Scientific/Flash 2000. IR spectra were recorded on a Perkin-Elmer 283B spectrophotometer using ATR techniques.

Infra red device

In an electric home-made metallic cylindrical can be fixed and adjusted an IR device adapted with a THERA-TERM OSRAM 250-Watt Red infra red bulb, 125 V which emits a wavelength of 1100 nm (9.09 cm^{-1}), and a thermostat to regulate the power output was used. All reactions were subjected to the reaction after placing the IR source 10 cm away from the reactor flask, (Fig. 11) while monitoring the temperature with an InfraPro® infra red Thermometer.

General procedure for the synthesis of 3,3'-pyrydin-diindolylmethane derivatives, 15-18

In a 25 mL flask charged with a stirring bar was added 4 mmol of the corresponding indol **10-13** derivative and 2 mmol of 2-Pyridinecarboxaldehyde **14** in EtOH (10 mL). The reactant mixture was exposed to IR irradiation using a THERA-TERM OSRAM 250-Watt Red infra red bulb for 35 min (as indicated by *TLC*) at 90 ± 2°C. After this time the crude of the reaction was cooled and poured onto stirred ice water. The precipitate was collected, dried, and purified by preparative chromatography using an eluting system of hexane/ethyl acetate (7:3). The scraping spot was extracted with acetone and dried to obtain the spectroscopically pure compounds. The spectra data of the compounds can be found in the supporting information.

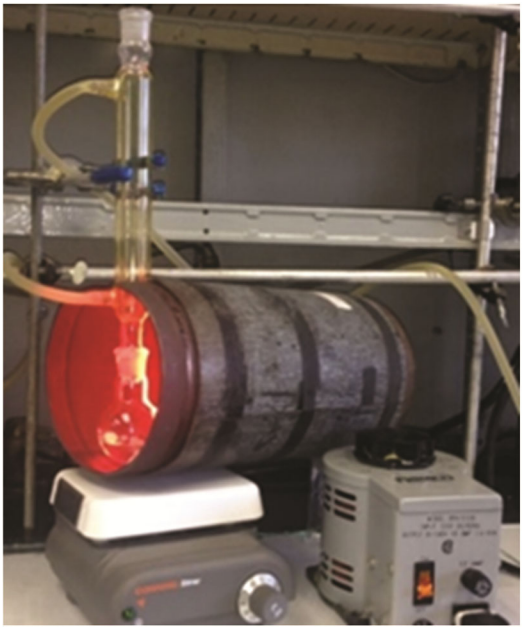


Fig. 11 — Experimental equipment of infra red radiation.

3,3'-(Pyridin-2-yl)methylene)bis(1*H*-indole), 15

Yellow powder. Yield 81%. m.p.184-186°C. IR (ATR): 3444 (NH), 3133, 3099 (CH´s), 1585 (C=N), 1434 (C-N), 733 cm^{-1} (CH's Ar); ^{1}H NMR (DMSO-d_6, 300 MHz): δ 10.88 (2H, s, NH), 7.66 (1H, t, J = 7.5 Hz), 7.34-7.41 (6H, m,), 7.00-7.09 (3H, m), 6.97 (2H, s), 6.89 (2H, t, J = 7.5 Hz), 5.97 (1H, s); ^{13}C NMR (DMSO-d_6, 75 MHz,): δ 149.60, 147.19, 141.02, 137.07, 136.67, 126.82, 124.55, 124.20, 119.42, 118.84, 117.51, 112.05, 56.53; HRMS-DART: *m/z* $[M+1]^+$. Anal. Calcd for $C_{13}H_{12}N_3$: 324.15007. Found: 324.14993.

3,3'-(Pyridin-2-yl)methylene)bis(1-methyl-1*H*-indole), 16

Brown light powder. Yield 82%. m.p.136-138°C. IR (ATR): 3049, 2995 (CH's), 1586 (C=N), 1464 (C-N), 726 cm^{-1} (CH's-Ar); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.51 (1H, s), 7.67 (1H, t, J = 7.2 Hz), 7.36-7.42 (5H, m), 7.197 (1H, t, J = 6.6 Hz), 7.124 (2H, t, J = 7.5 Hz), 6.91-6.97 (4H, m), 6.00 (1H, s), 3.70 (6H, s, CH_3); ^{13}C RMN ($CDCl_3$, 75 MHz): δ 164.16, 149.26, 137.28, 137.07, 128.43, 127.43, 122.95, 121.84, 121.55, 119.54, 118.91, 116.59, 110.09, 32.74; HRMS-DART: *m/z* $[M+1]^+$. Anal. Calcd for $C_{13}H_{12}N_3$: 352.18137. Found: 352.18252.

3,3'-(Pyridin-2-yl)methylene)bis(2-methyl-1*H*-indole), 17

Brown-orange powder. Yield 80%. m.p.92-95°C. IR (ATR): 3392 (NH), 3141, 3060 (CH's), 1587 (C=N), 1432 (C-N), 733 cm^{-1} (CH's-Ar); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 10.81 (2H, s, NH), 8.54 (1H, s), 7.68 (1H, t, J = 7.8 Hz), 7.27 (2H, d, J = 8.1 Hz), 7.20-7.24 (2H, m), 6.94 (2H, t, J = 7.2 Hz), 6.83 (2H, d, J = 7.8 Hz), 6.73 (2H, t, J = 7.5 Hz), 5.03 (1H, s), 2.10 (6H, s); ^{13}C NMR (75 MHz, $CDCl_3$): δ 163.70, 149.05, 136.77, 135.56, 132.61, 128.81, 121.47, 120.06, 118.75, 118.52, 112.17, 110.84, 42.46, 40.53, 40.25, 39.97, 39.69, 39.42, 12.38; HRMS-DART: *m/z* $[M+1]^+$. Anal. Calcd for $C_{13}H_{12}N_3$: 352.18137. Found: 352.18223.

3,3'-(Pyridin-2-yl)methylene)bis(2-phenyl-1*H*-indole), 18

Brown light powder. Yield 74%. m.p.96-99°C. IR (ATR): 3403 (NH), 3049 (CH's), 1589 (C=N), 1429 (C-N), 733 cm^{-1} (CH's-Ar); ^{1}H NMR (DMSO-d_6, 300 MHz): δ 11.39 (2H, s, NH), 7.71 (1H, t, J = 7.2 Hz), 7.40 (2H, d, J = 7.8 Hz), 7.24-7.30 (12H m), 7.031

(2H, t, J = 7.2 Hz), 6.87 (2H, d, J = 7.8 Hz), 6.706 (2H, t, J = 7.5 Hz), 6.04 (1H, s); ^{13}C NMR (75 MHz, DMSO-d_6): δ 165.00, 149.57, 136.85, 136.75, 135.93, 133.24, 128.79, 128.56, 123.63, 121.42, 121.45, 120.86, 119.14, 114.28, 111.86, 43.54; HRMS-DART: *m/z* $[M+1]^+$. Anal. Calcd for $C_{13}H_{12}N_3$: 476.21267. Found: 476.21173.

Antifungal test of yeast and filamentous

The series of compounds **15-18** were screened using the disc diffusion test against *Candida albicans* (ATCC 10231), *Cryptococcus neoformans* (ATCC 36556), and filamentous fungi *Aspergillus fumigatus* (ATCC 13073) obtained from the group of Laboratory 6 of Microbiological Bioprospecting, of the Multidisciplinary Research Unit (UIM) of the Faculty of Higher Studies Cuautitlán, Campo 4. The strains of fungi: *Candida albicans* (ATCC 10231), was with 48 h of incubation at 35°C; *Cryptococcus neoformans* with 72 hours of incubation at 35°C and *Aspergillus fumigatus* with 48 hours of incubation at 25°C. The qualitative antifungal activity technique was carried out according to the next description:

Agar Dextrosa- Sabouraud of BIOXON-Becton Dickinson was used for yeast culture. Agar Dextrosa-papa of DIBICO was used for the cultivation of filamentous fungi; A 2% BD BIOXON-Becton Dickinson and 0.5 g/mL of methylene blue were used for the yeast sensitivity test and 0.5 µg/mL of MERCK's methylene blue Löffler. For the dissolution of the synthesized and antifungal compounds, dimethyl sulfoxide 99.9% (DMSO) was used to mark Fermont, and an electric agitator mark Vortex-Genie 2. For the preparation of physiological saline (NaCl 0.85%). Sodium chloride QP mark REPROQUIFIN was used. The discs used were made from paper Whatman #5, with 5 mm in diameter. For dilutions micropipettes of 1, 10, 100, and 1000 µL mark Rainin were used. The strains were incubated at 35°C by 48 h for *Candida albicans*, at 35°C by 72 h for *Cryptococcus neoformans*, and 25°C for 7 days for *Aspergillus fumigatus*, using incubators Binder BD 23-UL and RIOSSA type E–033. An analytical balance of A&D company limited HR–200 and an analytical balance model BL 3100 of Sartorius AG. All manipulations were performed in a laminar flow hood model CFV–120.

Disc diffusion test for yeast fungus[23,26]

To reactivate yeast strains, they were grown in Petri boxes with dextrose-Sabouraud Agar (SDA) incubating *Candida albicans* (ATCC 10231), for 48 h at 35°C, and *Cryptococcus neoformans* (ATCC 36556) for 72 h at 35°C. Subsequently, yeasts were reseeded in SDA, incubated at 35°C for 24 hours, and inoculums were standardized by suspending them in a saline tube (NaCl 0.85%) to reach a concentration equivalent to the 0.5 tubes of the McFarland nephelometer (1×10^6 UFC/mL). A massive planting was then carried out on the surface of each Mueller–Hinton agar box added with 2% glucose and 0.5 µg/mL of methylene blue with each standardized inoculum, the boxes were allowed to dry for 10 to 15 minutes on a stove, at 35°C. The discs impregnated with 2 µg of each product to be evaluated were placed. Finally, they were incubated at 35°C for 24 h, and it was proceeded to observe if there was the formation of inhibition halos, reincubating the boxes that presented the halo and measuring it again at 48 h.

Radial growth inhibition method, for filamentous fungi

According to Wang & Bun's modified method 2002[11], in Petri plates with dextrose-papa Agar It was sown *Aspergillus fumigatus* (ATCC 13073) and incubated at 25°C for 7 days or until the development of the fungus was observed. Subsequently, an inoculum approximately 5 mm in diameter was taken from the periphery of the culture and placed in an inverted position on Dextrose-papa agar plates. Afterward, the discs impregnated with the product were placed around the inoculum to be evaluated at an initial concentration of 2 µg/disc up to a maximum concentration of 10 µg/disc, and incubated at 25°C for 72 to 96 h or until the surface of the agar was covered by mycelium, and after it was observed in case there was halo inhibition. Both tests applied a negative control consisting of the application of 10 µL of the DMSO used as a solvent to each disc where the compounds were dissolved, and the positive control: Ketoconazole 0.5 µg/10 µL, both tests were performed by triplicate.

Determination of the Minimum Inhibitory Concentration. Dilution sensitivity test in Agar

Dextrose-sabouraud agar was prepared in tubes with 6 mL of the agar and allowed to cool to a temperature of 40–45°C. On the other hand, a stock solution with a sufficient quantity of product to be evaluated was previously prepared to obtain concentrations from 0.5, 1, 1.5, 2, 2.5, 3, and 3.5µg/10µL and 6, 7, 8, 9 µg/10µL and subsequently,

the amount necessary to obtain such concentrations was added to each tube; was homogenized and emptied into 60 mm Petri plates letting themselves be gelified. The inoculum equivalent to the 0.5 tubes of the McFarland nephelometer was standardized for each yeast, in the same way as in the diffusion test. The plates were sown with the respective yeast; the positive control was using ketoconazole to a concentration of 2 µg/10 µL. Subsequently, they were incubated at 35–37°C for 48 hours taking a reading at 24 and 48 h to see if there was colony formation. For both yeasts, DMSO was used as a negative control.

Qualitative testing: Antifungal Assessment

The compounds were evaluated at a concentration of (2 µg/disc), in the case of yeasts (Fig. 3, Fig. 4 and Fig. 5), and in filamentous fungi, these were evaluated from a concentration of (2 µg/disc) to a maximum concentration of (10 µg/disc) (Fig. 6). The results obtained from these tests are shown in Table 1. As a negative control was used DMSO, with 10 µL in each disc.

Conclusion

In conclusion, the synthesis of a series of pyridinildiindolamethanes was achieved using infra red irradiation as an alternate, novel, efficient, rapid and benign source of heating in an ethanolic solution under acidic conditions giving excellent yields in short times of reaction, allowing their straightforwardly assembly *via* a multicomponent approach. The diference between this work and other previously reported could not be compared, because in this work the obtaining of dindolylmethanes by an ethanolic medium (liquid medium) is reported, while in another work the synthesis of diindolylmethanes using a solid support, TAFF bentonite[18], being both different techniques. The rest of the works cited are synthesized different compounds such as derivatives of Biguinelli, Hantzsch, benzimidazoles, *etc*. Therefore, in this work, more and new information has been generated to the existing one, not only in molecular derivatives, but also in terms of methodologies.

It is also worth noting that biological activities of the series of compounds against yeasts *C. albicans*, and *C. neoformans* was determined. However, not observing any activity against filamentous fungi as *A. fumigatus.* Besides, by using dilution techniques in agar the sensitivity tests in MIC against *C. neoformans*, which determined to be less than 600 µg/mL for all compounds, while for *C. albicans*, it could be performed at 900 µg/mL.

Finally, it is proposed to perform morphological alteration studies, as well as studies of sensitivity of the microorganisms studied with the synthesized compounds. This experiments are currently under development in our laboratories.

Supplementary Information

Supplementary information is available in the website http://nopr.niscpr.res.in/handle/123456789/58776.

Conflict of Interests

Authors affirm to no conflicts of interest.

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