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SHORT COMMUNICATION

NEW TETRADECANOIC ACID HYDRAZONES IN THE SEARCH FOR ANTIFUNGAL AGENTS: SYNTHESIS AND *IN VITRO* EVALUATIONS

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One of the main objectives of organic and medicinal chemistry is the design, synthesis and production of molecules having a value as human therapeutic agents (1).The clinical relevance of fungal diseases has increased over the past 30 years due to an increasing population of immunocompromised patients who have cancer, AIDS or have received transplant. The wide spread use of antifungal agents has lead to the development of drug resistance. A potential approach to overcome the resistance problem is to design innovative agents with a different mode of action so that no cross resistance with the present therapeutics can occur. So, there is still an existing need for broad spectrum antifungal agents (2). Hydrazide-hydrazones have been demonstrated to possess anticonvulsant (3), antidepressant (4), anti-inflammatory (5), antimalarial (6), antimycobacterial (7), anticancer (8), antimicrobial (9–12), and antiviral (13) activities.

Inspired by the above facts and in continuation of our ongoing research program in the field of synthesis and antimicrobial activity of medicinally important compounds (14–16), we hereby report the synthesis and antifungal activity of tetradecanoic acid hydrazide-hydrazones. All the compounds have been screened for antifungal activity against two fungal strain *C. albicans* and *A. Niger* and some of the synthesized compounds showed good antifungal activity against these strains. The structures of all compounds have been evaluated by elemental and spectral analysis (IR and ^{1}H NMR).

EXPERIMENTAL

Chemistry

The synthesis of target compounds was carried out as depicted in Scheme 1. Tetradecanoic acid was refluxed with methanol in the presence of sulfuric acid to yield its methyl ester. The methyl ester was refluxed with hydrazine hydrate in ethanol to yield the corresponding hydrazide, which was then refluxed with substituted aldehydes to yield the target compounds. The products were recrystallized from methanol. The completion of reaction was monitored by single spot TLC on silica gel G plates. All the compounds were obtained in good yields and physical and analytical data of synthesized compounds are given in Table 1.

Melting points were determined in open capillary tubes and are uncorrected. Infra-red spectra were recorded on Perkin Elmer Spectrum RXI FTIR spectrophotomer in KBr discs. ^{1}H NMR spectra were obtained on BRUKER spectrometer (300 MHz) using TMS as an internal standard. Elemental analyses were done using Carlo Erba 1106 CHN Analyzer. The purity of the synthesized compounds

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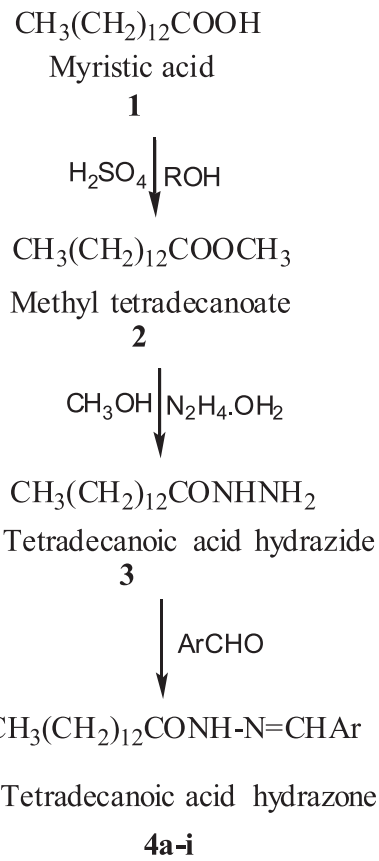
Table 1 Physical data of title compounds

Compound no.	Ar	Molecular formula	Molecular weight	Melting point (°C)	% Yield	R_f*
4a	C_6H_5	$C_{21}H_{34}N_2O$	330.51	82-84	66.34	0.51
4b	3-OHC_6H_4	$C_{21}H_{34}N_2O_2$	346.51	110-112	72.31	0.47
4c	3-ClC_6H_4	$C_{21}H_{33}ClN_2O$	364.95	102-104	77.11	0.42
4d	4-ClC_6H_4	$C_{21}H_{33}ClN_2O$	364.95	98-100	75.44	0.56
4e	3, 4-$(-OCH_3)_2C_6H_3$	$C_{23}H_{38}N_2O_3$	390.56	114-116	79.64	0.68
4f	2-$NO_2C_6H_4$	$C_{21}H_{33}N_3O_3$	375.51	111-113	80.77	0.67
4g	4-FC_6H_4	$C_{21}H_{33}FN_2O$	348.50	112-114	67.28	0.51
4h	3-BrC_6H_4	$C_{21}H_{33}BrN_2O$	409.40	97-99	75.01	0.62
4i	4-$OCH_3C_6H_4$	$C_{22}H_{36}N_2O_2$	360.53	105-107	79.02	0.51

*chloroform : benzene : glacial acetic acid (3:1:1, v/v/v).



Scheme 1. Synthetic pathway for the synthesis of **4a-i**

were checked by thin layer chromatography on silica gel G in various solvent systems using iodine vapors as detecting agent.

General method

The title compounds were prepared in the following steps:

Synthesis of tetradecanoic acid methyl ester (2)

A mixture of myristic acid (0.25 M) (1) and an excess of methanol (250 mL) with 1 mL of sulfuric acid was refluxed for 3–4 h. The solution was cooled and poured onto crushed ice. Sodium bicarbonate was added to remove an excess of acid and then the product was extracted with ether. The ether layer was evaporated on water bath to obtain a thick concentrated ester (**2**). Yield 82%, R_f 0.55, b. p. 250–253°C.

Synthesis of tetradecanoic acid hydrazide (3)

A mixture of tetradecanoic acid methyl ester (0.2 M) and an excess of hydrazine hydrate (0.30 M, 15 mL) in ethanol (250 mL) was refluxed for ca. 3 h and cooled. The solid was separated by filtration and recrystallized from ethanol to afford tetradecanoic acid hydrazide. The purity of compound was checked by single spot TLC using chloroform : benzene : glacial acetic acid (3:1:1, v/v/v). Yield 64%, R_f 0.59, m.p. 112–114°C.

Synthesis of tetradecanoic acid hydrazide hydrazones (4a-i)

A mixture of tetradecanoic acid hydrazide (0.025 M) and required aromatic aldehyde (0.025 M) was refluxed in methanol (50 mL) in the presence of a catalytic amount of glacial acetic acid for 2 h. The mixture was cooled; the solid was separated by filtration and recrystallized from methanol to give the corresponding hydrazide hydrazones. Their purity was checked by single spot TLC using

chloroform : benzene : glacial acetic acid (3:1:1, v/v/v).

Spectral data

Tetradecanoic acid-(benzylidene) hydrazide (4a)

IR (KBr, cm^{-1}): 3312–3224 ($NH-NH_2$), 3022 (C–H arom.), 2887–2834 (C–H), 1651 (C=O), 1644 (C=N), 1625–1423 (C=C). ^1H NMR (300 MHz, DMSO-d_6, δ, ppm): 8.25–7.66 (m, 5H, Ar), 8.11 (s, 1H, CH=N), 8.02 (s, 1H, NH), 1.72 (t, 2H, $COCH_2$), 1.44–1.24 (22 H, m, 11 × $–CH_2$), 0.81 (t, 3H, $-CH_3$). Analysis: calcd. for $C_{21}H_{34}N_2O$: C, 76.31; H, 10.37; N, 8.48%; found C, 76.36; H, 10.41; N, 8.43%.

Tetradecanoic acid-(3-hydroxybenzylidene) hydrazide (4b)

IR (KBr, cm^{-1}): 3316–3246 ($NH-NH_2$), 3147 (O-H swamp with C–H), 3042 (C–H arom.), 2945–2841 (C–H), 1645 (C=O), 1649 (C=N), 1644–1428 (C=C). ^1H NMR (300 MHz, DMSO-d_6, δ, ppm): 8.31–7.55 (t, 4H, Ar), 8.08 (s, 1H, CH=N), 8.02 (s, 1H, NH), 5.86 (s, 1H, -OH), 1.72 (t, 2H, $COCH_2$), 1.41–1.25 (22 H, m, 11 × $-CH_2$), 0.87 (t, 3H, $-CH_3$). Analysis: calcd. for $C_{21}H_{34}N_2O_2$: C, 72.29; H, 9.89; N, 8.08%; found C, 72.24; H, 9.93; N, 8.02%.

Tetradecanoic acid-(3-chlorobenzylidene) hydrazide (4c)

IR (KBr, cm^{-1}): 3456–3341 ($NH-NH_2$), 3028 (C–H arom.), 2937–2852 (C-H), 1662 (C=O), 1654 (C=N), 1639–1455 (C=C), 762 (C-Cl). ^1H NMR (300 MHz, DMSO-d_6, δ, ppm): 8.23–7.77 (m, 4H, Ar), 8.04 (s, 1H, CH=N), 8.02 (s, 1H, NH), 1.89 (t, 2 H, $COCH_2$), 1.42–1.24 (22 H, m, 11 × $-CH_2$), 0.84 (t, 3H, $-CH_3$). Analysis: calcd. for $C_{21}H_{33}ClN_2O$: C, 69.11; H, 9.11; N, 7.68%; found C, 69.15; H, 9.08; N, 7.64%.

Tetradecanoic acid-(4-chlorobenzylidene) hydrazide (4d)

IR (KBr, cm^{-1}): 3367–3277 ($NH-NH_2$), 3047 (C–H arom.), 2931–2842 (C-H), 1661 (C=O), 1634 (C=N), 1611–1423 (C=C), 692 (C-Cl). ^1H NMR (300 MHz, DMSO-d_6, δ, ppm): 8.14–7.57 (d, 4H, Ar), 8.19 (s, 1H, CH=N), 8.00 (s, 1H, NH), 1.81 (t, 2H, $COCH_2$), 1.42–1.28 (22 H, m, 11 × $-CH_2$), 0.81 (t, 3H, $-CH_3$). Analysis: calcd. for $C_{21}H_{33}ClN_2O$: C, 69.11; H, 9.11; N, 7.68%; found C, 69.11; H, 9.13; N, 7.66%.

Tetradecanoic acid-(3,4-dimethoxybenzylidene) hydrazide (4e)

IR (KBr, cm^{-1}): 3365–3243 ($NH-NH_2$), 3029 (C–H arom.), 2944–2823 (C-H), 1644 (C=O), 1631 (C=N), 1614–1433 (C=C), 1253 (C-O-C). ^1H NMR (300 MHz, DMSO-d_6, δ, ppm): 8.12–7.62 (t, 3H, Ar), 8.02. (s, 1H, CH=N), 8.00 (s, 1H, NH), 3.61 (s, 6H, -OCH), 1.89 (t, 2H, $COCH_2$), 1.43–1.22 (22 H, m, 11 × $-CH_2$), 0.81 (t, 3H, $-CH_3$). Analysis: calcd. for $C_{23}H_{38}N_2O_3$: C, 70.73; H, 9.81; N, 7.17%; found C, 70.71; H, 9.85; N, 7.19%.

Tetradecanoic acid-(2-nitrobenzylidene) hydrazide (4f)

IR (KBr, cm^{-1}): 3361–3253 ($NH-NH_2$), 3039 (C–H arom.), 2957–2821 (C–H), 1655 (C=O), 1622 (C=N), 1606–1422 (C=C), 1541 (N–O). ^1H NMR (300 MHz, DMSO-d_6, δ, ppm): 8.21–7.84 (m, 4H, Ar), 8.13. (s, 1H, CH=N), 8.00 (s, 1H, NH), 1.81 (t, 2H, $COCH_2$), 1.47–1.21 (22 H, m, 11 × $-CH_2$), 0.82 (t, 3H, $-CH_3$). Analysis: calcd. for $C_{21}H_{33}N_3O_3$: C, 67.17; H, 8.86; N, 11.19%; found C, 67.21; H, 8.90; N, 11.21%.

Tetradecanoic acid-(4-fluorobenzylidene) hydrazide (4g)

IR (KBr, cm^{-1}): 3287–3222 ($NH-NH_2$), 3035 (C–H arom.), 2993–2842 (C–H), 1657 (C=O), 1625 (C=N), 1607–1420 (C=C), 1235 (C–F). ^1H NMR (300 MHz, DMSO-d_6, δ, ppm): 8.11–7.92 (m, 4H, Ar), 8.07 (s, 1H, CH=N), 8.02 (s, 1H, NH), 1.84 (t, 2H, $COCH_2$), 1.46–1.21 (22 H, m, 11 × $-CH_2$), 0.82 (t, 3H, $-CH_3$). Analysis: calcd. for $C_{21}H_{33}FN_2O$: C, 72.37; H, 9.54; N, 8.04%; found C, 72.40; H, 9.51; N, 8.01%.

Table 2. *In vitro* antifungal activity of the synthesized compounds (**4a-i**)

Compound	Minimum inhibitory concentration (μg/mL)	
	C. albicans (MTCC 8184)	*A. Niger* (MTCC 8189)
4a	12.5	25
4b	12.5	12.5
4c	12.5	6.25
4d	12.5	12.5
4e	12.5	6.25
4f	3.25	1.62
4g	3.25	6.26
4h	6.25	6.25
4i	12.5	6.25
Clotrimazole (standard drug)	0.10	0.30

Tetradecanoic acid-(3-bromobenzylidene) hydrazide (**4h**)

IR (KBr, cm^{-1}): 3344–3230 ($NH\text{-}NH_2$), 3024 (C–H arom.), 2943–2856 (C–H), 1662 (C=O), 1656 (C=N), 1641–1458 (C=C), 549 (C–Br). ^1H NMR (300 MHz, DMSO-d_6, δ, ppm): 8.12–7.93 (m, 4H, Ar), 8.02 (s, 1H, CH=N), 7.92 (s, 1H, NH), 1.82 (t, 2H, $COCH_2$), 1.45–1.26 (22 H, m, 11 × $-CH_2$), 0.86 (t, 3H, $-CH_3$). Analysis: calcd. for $C_{21}H_{33}BrN_2O$: C, 61.61; H, 8.12; N, 6.84%; found C, 61.64; H, 8.16; N, 6.80%.

Tetradecanoic acid-(4-methoxybenzylidene) hydrazide (4i)

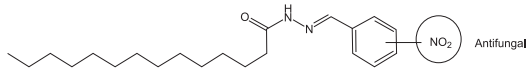
IR (KBr, cm^{-1}): 3421–3332 ($NH\text{-}NH_2$), 3037 (C–H arom.), 2955–2846 (C–H), 1646 (C=O), 1635 (C=N), 1614–1432 (C=C), 1243 (C-O-C). ^1H NMR (300 MHz, DMSO-d_6, δ, ppm): 8.23–7.82 (m, 4H, Ar), 8.08. (s, 1H, CH=N), 8.01 (s, 1H, NH), 3.82 (s, 3H, -OCH), 1.82 (t, 2H, $COCH_2$), 1.44–1.25 (22 H, m, 11 × $-CH_2$), 0.82 (t, 3H, $-CH_3$). Analysis: calcd. for $C_{22}H_{36}N_2O_2$: C, 73.29; H, 10.06; N, 7.77%; found C, 73.31; H, 10.02; N, 7.73%.

Antifungal evaluation

Screening of finally synthesized compounds *in vitro* antifungal activity against fungal strain: *C. albicans* (MTCC 8184) and *A. Niger* (MTCC 8189) was assessed by serial two fold dilution technique. Clotrimazole was used as a standard drug for antifungal activity. All the compounds were dissolved in dimethyl sulfoxide to give a concentration of 10 µg/mL. Twofold dilutions of test and standard compounds were prepared in Sabouraud dextrose broth I.P. (17). The stock solution was serially diluted to give concentrations of 25–0.78 µg/mL in nutrient broth. The inoculum size was approximately 10^6 colony forming units (CFU/mL). The whole batch was incubated for 7 days for fungi at 35°C for *A. Niger* (MTCC 8189) and at 25°C for *C. albicans* (MTCC 8184). After that, the inoculated culture tubes were macroscopically examined for turbidity. The culture tube showing turbidity (lower concentration) and the culture tube showing no turbidity (higher concentration) gave the minimum inhibitory concentration (MIC) for the compounds. The MIC for antifungal is given in Table 2.

RESULTS AND DISCUSSION

In this study nine new compounds incorporating the scaffold of hydrazide-hydrazone have been synthesized and their antifungal activities were evaluated. At the first stage, tetradecanoic methyl ester was prepared. Further, reaction of methyl ester of tetradecanoic acid with hydrazine hydrates gave the corresponding acid hydrazide. The treatment of hydrazide with different substituted aldehydes gave the corresponding title compounds (**4a-i**). All the synthesized compounds were characterized by their physical, analytical and spectral data. The data obtained were found to be in good agreement with the calculated values of proposed structures. The compounds were evaluated for their antifungal properties. In comparison with the control antifungal agent – clotrimazole, compound **4f**:



bearing the nitro group on aromatic ring was the most active compound with MIC value (3.25 µg/mL) against C. *albicans* and MIC value (1.62 µg/ml) against *A. Niger.* In general, the compounds bearing electron withdrawing substituents were found to be more active than the others, indicating probable interaction of such groups with receptor sites. The data reported in this article may be helpful to the medicinal chemists who are working in this area.

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