Full Paper

Synthesis, Antibacterial and Antifungal Activity of Some Novel 3,5-Disubstituted-1*H*-1,2,4-triazoles

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A rapid and efficient one-pot condensation reaction of long-chain alkyl and alkenyl acid hydrazides and nitriles was carried out to afford 3,5-disubstituted-1*H*-1,2,4-triazoles. The compounds **5a–o** were screened for *in-vitro* antibacterial activity against the representative panel of two Gram-positive and two Gram-negative bacteria. All the synthesized compounds were also tested for their inhibitory action against five strains of fungi. The various compounds show potent inhibitory action against test organisms. The compounds **5a–o** were characterized on the basis of elemental analysis and spectral data.

Keywords: 3,5-Disubstituted-1*H*-1,2,4-triazoles / Long-chain alkyl and alkenyl acid hydrazides / Nitriles

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Introduction

Heterocycles play an important role in all spheres of life including pharmaceuticals, natural resources, veterinary, agriculture products, analytical reagents, and dyes [1]. The development of new approaches for the synthesis of heterocycles decorated with unique functional groups forms the basis of an extensive research activity in synthetic organic chemistry. Justification of much of the chemistry directed to the synthesis of the compounds, possessing nitrogen at the ring fusion, is due to the application of compounds having interesting biological properties in the field of medicinal chemistry. The 1,2,4-triazole moiety is a structural element in certain anti-asthmatic [2], antiviral (ribavirin) [3], antifungal (flucanoazole) [4], antibacterial [5], hypotonic (triazolam) [6] drugs. Certain compounds containing 1,2,4-triazole nucleus have been reported to possess bactericidal [7], antiviral [8], insecticidal [9], anticancer [10], anti-inflammatory [11], anticonvulsant [12, 13] properties. Also, some triazole derivatives have been synthesized as plant-growth regulators [14].

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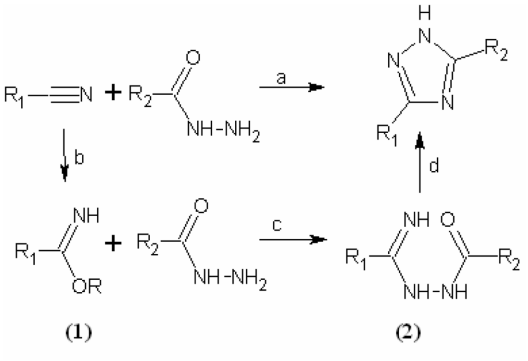
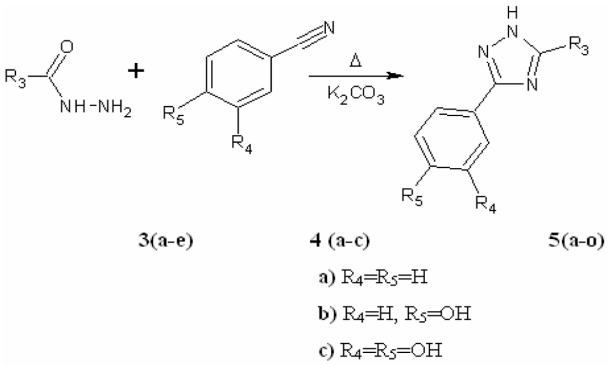
Owing to its broad spectrum of biological activity [15–21], the 1,2,4-triazole ring system represents an attractive target to invent new substrates for their synthesis and production of combinatorial libraries. In several pharmacologically active compounds 3,5-disubstituted-1,2,4-triazoles are found. Recent examples include selective adenosine A_{2A} receptor antagonist [22] and the phosphodiesterase V inhibitor [23]. Previously, 1,2,4-triazoles were synthesized by hydrazides and nitriles either by Pinner reaction and Pellizzari condensation which involve the cyclodehydrative condensation between nitrile and hydrazide. These procedures (Scheme 1; path **b–d**) are usually conducted at elevated temperature and involve the activation of nitrile to acylamidrazone intermediate **2** prior to cyclization. These conventional procedures not only involve high reaction temperature and long reaction time but also results in low yields of the product [24–26]. Herein, we are reporting a simple and scaleable methodology for the one-pot synthesis of 3,5-disubstituted-1,2,4-triazoles.

To the best of our knowledge, 3,5-disubstituted-1*H*-1,2,4-triazoles have not yet been reported from long-chain saturated and olefinic carboxylic acids. The present work is in continuation of our study on the synthesis of heterocycles from such acids. Tetrazoles, [27, 28] pyrazolines [29], tetrazines [30, 31], spiro [oxathiolane-2,2′-dihydrotetrazoles] [32], aziridines [33], and triazines [34] have



Scheme 1. Pathways for synthesis of 3,5-disubstituted-1*H*-1,2,4-triazoles.



Scheme 2. Synthesis of 3,5-disubstituted-1*H*-1,2,4-triazoles **5a–o**.

been previously prepared in our lab. Cyanoethoxy and morpholine derivatives of hydroxy long-chain acids [35] and fatty esters [36] showed significant antifungal and antibacterial activity. In view of the above mentioned pharmacological applications of 1,2,4-triazoles, we considered undertaking the design and synthesis of hitherto unknown 1,2,4-triazoles bearing a long alkyl and alkenyl chain.

Results and discussion

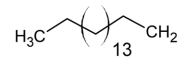
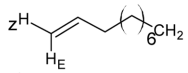
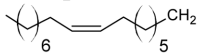
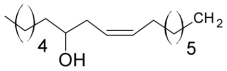
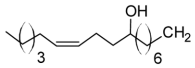
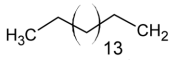
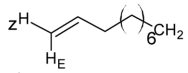
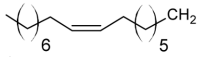
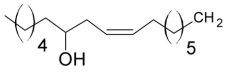
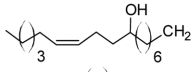
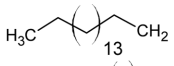
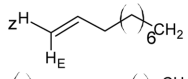
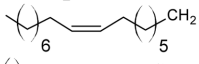
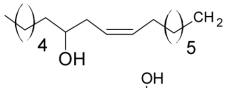
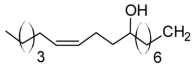
Chemistry

The 3,5-disubstituted-1*H*-1,2,4-triazoles **5a–o** were synthesized by the condensation of long-chain saturated and olefinic carboxylic acid hyrazides **3a–e** with nitriles **4** in presence of a catalytic amount of K_2CO_3 in *n*-BuOH (Scheme 2). The use of a catalytic amount of K_2CO_3 provided the product in reduced reaction time in appreciable yield as compared to methods reported by earlier workers. As can been seen from Table 1, the scope of the reaction using saturated, olefinic (internal and terminal), and hydroxy acid hydrazides was found to be good. The yields of 3,5-disubstituted-1*H*-1,2,4-triazoles did not depend on the length of chain of the acid hydrazide and were found to be appreciable. The synthesized compounds were identified on the basis of elemental analysis, IR, ^{1}H-NMR, ^{13}C-NMR, and mass spectra. The ^{1}H-NMR spectrum of 3-(4′-hydroxyphenyl)-5-(dec-9-enyl)-1*H*-1,2,4-triazole **5g** showed characteristic signals of δ = 10.97 of the -NH proton and multiplets at δ = 7.57–7.53 for four aromatic protons. The methine proton of C-10 showed signals at δ = 5.82. C-11 methylene designated as H_E and Hz displayed two distinct δ values when coupled with the adjacent C-10 methine protons. Thus, the ^{1}H NMR spectrum showed two doublets of a doublet at δ = 5.02 and 4.90 for Hz and H_E protons, respectively. A triplet for two hydrogens was observed at δ = 2.91 for the methylene protons, alpha to the triazole ring. The structure of **5g** was further supported by its mass spectral studies, which showed a molecular ion peak at *m/z* 299, consistent with its molecular formula $C_{18}H_{25}ON_3$. The base peak appears at *m/z* 160. Detailed spectral of the titled compounds are given in Experimental.

Biological studies

All the newly synthesized compounds were evaluated *in vitro* against an assortment of two Gram-positive bacteria *Staphylococcus aureus* MSSA 22 *and Bacillus subtilis* ATCC 6051 and two Gram-negative bacteria *Escherichia coli* K 12 and *Salmonella typhimurium* MTCC 98 at a concentration of 100 μg/mL. Chloramphenicol was used as standard drug for the comparison of the antibacterial results. Screening results are summarized in Table 2. The newly generated compounds **5a–o** have exerted significant inhibitory activity against the growth of the tested bacterial strains. The data pertaining to Table 3 reveal that **5a–o** have significant influence on the antibacterial profile of *S. typhimurium* and *S. aureus*. The synthesized compounds showed good inhibitory results against *B. subtilis* and *E. coli*. In another set of experiments, the above mentioned compounds **5a–o** were also examined for their antifungal activity. Nystatin was used as standard drug for the comparison of the antifungal results. The synthesized compounds showed excellent inhibitory results for *C. albicans* IOA-109 and good results against *Penicillium* sp. (laboratory isolate) and *Helminthosporum oryzae* (2537 laboratory isolate). All compounds showed moderate activity against *Trichoderma. viridae* (laboratory isolate) and *Aspergillus niger* (laboratory isolate) (Table 3). The data also revealed that **5a–o** has produced the marked enhancement in the potency of these analogues as antifungal and antibacterial agents.

Table 1. 3,5-Disubstituted-1*H*-1,2,4-triazoles **5a**–**o**.

Entry	Starting from	R_3	R_4	R_5	Products	Yield (%)
1	**3a, 4a**		H	H	5a	89
2	**3b, 4a**		H	H	5b	85
3	**3c, 4a**		H	H	5c	82
4	**3d, 4a**		H	H	5d	82
5	**3e, 4a**		H	H	5e	80
6	**3a, 4b**		H	OH	5f	81
7	**3b, 4b**		H	OH	5g	88
8	**3c, 4b**		H	OH	5h	85
9	**3d, 4b**		H	OH	5i	83
10	**3e, 4b**		H	OH	5j	80
11	**3a, 4c**		OH	OH	5k	81
12	**3b, 4c**		OH	OH	5l	80
13	**3c, 4c**		OH	OH	5m	79
14	**3d, 4c**		OH	OH	5n	79
15	**3e, 4c**		OH	OH	5o	78

Conclusion

The preparation of 3,5-disubstituted-1*H*-1,2,4-triazole derivatives of long-chain alkyl and alkenyl acid hydrazides is a valuable addition to the heterocyclic chemistry. In conclusion, we have developed a useful procedure for the synthesis of 3,5-disubstituted-1*H*-1,2,4-triazole from fatty acid hydazides which could be scaled up to large quantities. Further biological evaluation of these derivatives may prove potential usefulness.

We are thankful to the SAIF, Panjab University, Chandigarh, and the Central Drug Research Institute, Lucknow, for providing spectral data.

The authors have declared no conflict of interest.

Experimental

Anhydrous conditions were achieved by drying flasks and other equipments in the oven. Reactions were monitored by TLC on silica gel G. Silica gel (60–80 mesh) was used for column chromatography. All reagents and solvents were generally used as received from commercial suppliers and when required, solvents were dried and distilled before use. Undec-10-enoic, (*Z*)-octadec-9-enoic, and octadecanoic acids were obtained commercially from Fluka Chemicals (Switzerland). The eluent was a mixture of petroleum ether / ethyl acetate in different proportions for different compounds and was visualized in an iodine chamber. (9*Z*, 12*R*)-12-Hydroxyoctadec-9-enoic (ricinoleic) and (9*R*, 12*Z*)-9-hydroxyoctadec-12-enoic (isoricinoleic) acids were isolated from the natural sources, *i.e.* from *Ricinus communis* and *Wrightia tinctoria* seed oils, respectively. The concentrate of pure hydroxy acids were obtained by Gunstone's partitioning [37] of freshly prepared fatty acids and further purified by column chromatography. ^{1}H-NMR spectra were recorded in $CDCl_3$ on a

Table 2. Antibacterial screening data for 3,5-disubstituted-1*H*-1,2,4-triazoles **5a** – **o**.

Compound	Diameter of zone of inhibition (mm) at 100 μg/mL			
	Gram-negative		Gram-positive	
	EC[a)]	ST[b)]	BS[c)]	SA[d)]
5a	14.6 ± 0.61	14.6 ± 0.42	19.1 ± 0.66	15.4 ± 0.51
5b	14.2 ± 0.64	15.3 ± 0.42	18.2 ± 0.51	15.5 ± 0.50
5c	13.6 ± 0.53	15.6 ± 0.53	17.1 ± 0.23	16.7 ± 0.31
5d	13.2 ± 0.53	16.5 ± 0.42	16.6 ± 0.53	15.8 ± 0.60
5e	13.2 ± 0.50	16.3 ± 0.46	16.5 ± 0.50	16.6 ± 0.53
5f	16.1 ± 0.98	17.1 ± 0.23	19.1 ± 0.25	16.9 ± 0.83
5g	15.9 ± 0.36	16.7 ± 0.61	18.5 ± 0.46	16.9 ± 0.34
5h	15.4 ± 0.61	16.2 ± 0.29	18.1 ± 0.31	17.8 ± 0.72
5i	13.3 ± 0.75	17.1 ± 0.42	17.1 ± 0.63	18.5 ± 0.42
5j	13.1 ± 0.65	17.4 ± 0.41	16.3 ± 0.50	18.4 ± 0.53
5k	16.6 ± 0.59	18.2 ± 0.53	20.3 ± 0.42	20.3 ± 0.46
5l	16.6 ± 0.60	18.5 ± 0.31	20.2 ± 0.53	20.9 ± 0.90
5m	15.9 ± 0.65	17.1 ± 0.12	19.7 ± 0.31	21.3 ± 0.64
5n	14.0 ± 0.91	16.7 ± 0.61	19.4 ± 0.40	22.5 ± 0.50
5o	14.2 ± 0.53	17.2 ± 0.35	19.2 ± 0.35	22.2 ± 0.81
Chlor amphenicol	25	20	24	26
Control DMSO	–	–	–	–

a) EC: *Escherichia coli*.
b) ST: *Salmonella typhimurium*.
c) BS: *Bacillus subtilis*.
d) SA: *Staphylococcus aureus*.

Table 3. Antifungal screening data for 3,5-disubstituted-1*H*-1,2,4-triazoles **5a** – **o**.

Compound	Diameter of zone of inhibition (mm) at 100 μg/mL				
	CA[a)]	HO[b)]	AN[c)]	TV[d)]	PN[e)]
5a	18.37 ± 0.31	12.3 ± 0.36	15.0 ± 0.25	5.1 ± 0.42	12.5 ± 0.50
5b	18.13 ± 0.32	12.5 ± 0.50	14.7 ± 0.86	5.4 ± 0.46	12.7 ± 0.61
5c	18.3 ± 0.55	11.2 ± 0.49	15.1 ± 0.42	5.6 ± 0.60	12.9 ± 0.80
5d	17.2 ± 0.49	10.7 ± 0.61	14.4 ± 0.40	5.5 ± 0.50	13.2 ± 0.53
5e	17.2 ± 0.35	10.8 ± 0.71	14.8 ± 0.80	5.4 ± 0.58	13.2 ± 0.50
5f	19.03 ± 0.44	13.1 ± 0.57	15.1 ± 0.42	6.5 ± 0.50	14.6 ± 0.55
5g	18.9 ± 0.55	13.2 ± 0.46	14.8 ± 0.91	6.7 ± 0.42	14.7 ± 0.56
5h	18.03 ± 0.68	14.0 ± 0.47	15.1 ± 0.55	6.7 ± 0.61	15.2 ± 0.43
5i	17.6 ± 0.38	14.2 ± 0.47	15.7 ± 0.42	6.8 ± 0.50	15.2 ± 0.58
5j	18.03 ± 0.85	14.2 ± 0.60	15.5 ± 0.50	6.9 ± 0.59	15.6 ± 0.40
5k	18.9 ± 0.40	13.6 ± 0.40	16.1 ± 0.40	8.1 ± 0.50	16.0 ± 0.30
5l	18.2 ± 0.43	13.9 ± 0.75	16.0 ± 0.50	8.2 ± 0.43	16.5 ± 0.55
5m	17.7 ± 0.44	13.9 ± 0.46	15.7 ± 0.80	8.8 ± 0.25	17.2 ± 0.50
5n	18.4 ± 0.40	14.1 ± 0.60	16.1 ± 0.48	8.7 ± 0.50	17.0 ± 0.36
5o	17.9 ± 0.57	14.7 ± 0.55	16.0 ± 0.30	9.2 ± 0.62	17.4 ± 0.43
Nystatin	20	18	18	15	20
Control DMSO	–	–	–	–	–

a) CA: *Candida albicans*.
b) HO: *Helminthosporum oryza*.
c) AN: *Aspergillus niger*.
d) TV: *Trichoderma viridae*.
e) PN: *Penicillium* sp.

Bruker DRX-400 instrument Bruker Bioscience, Billerica, MA, USA). The chemical shifts (δ) were measured relative to TMS as an internal standard. Coupling constants (*J*) are expressed in Hz. Mass spectra were obtained on a Jeol SX-102 (FAB) spectrometer (JEOL, Tokyo, Japan). IR spectra were obtained on Shimadzu 8201 PC FT-IR using KBr pellet with absorption given in cm^{-1} (Shimadzu, Tokyo, Japan).

Synthesis

General procedure for preparation of 3,5-disubstituted-1H-1,2,4-triazoles from fatty acid hydrazides

The hydrazides **3** of corresponding long-chain acids were prepared as reported earlier [34]. A mixture of nitrile (3 mmol) **4**, acid hydrazide **3a-e** (1 mmol), and K_2CO_3 (0.5 mmol) in *n*-BuOH (2 mL) was stirred and refluxed at 150°C for 4 hours. The progress of the reaction was monitored on TLC. After completion of the reaction, the solvent was removed under reduced pressure and the compounds were adsorbed on silica gel and purified by column chromatography. All the compounds **5a – o** were obtained as oily liquids.

3-Phenyl-5-heptadecyl-1H-1,2,4-triazole ***5a***

IR (KBr): 3382 (N-H), 1607 (C=N), 1123 (C-N) cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 10.96 (s, 1H, -N*H*-), 7.69 – 7.67 (m, 2H, Ar-*H*), 7.65 – 7.60 (m, 1H, Ar-*H*), 7.49 – 7.42 (m, 2H, Ar-*H*), 2.96 (t, J = 7.6 Hz, 2H, -CH_2 α to ring), 2.05 (m, 2H, -CH_2 β to ring), 1.72 (brs, 28H, chain CH_2), 0.86 (dist. t, 3H, CH_3). ^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 159.1, 147.9, 133.8, 129.1, 127.3, 32.1, 30.3, 29.9, 28.6, 23.1, 14.8. MS, *m/z* (%): $[M + 1]^+$ 384 (10.5), $[M]^+$ 383 (36.11), 354 (25.3), 270 (27.7), 214 (63.8), 186 (16.6), 58 (100).

3-Phenyl-5-(dec-9-enyl)-1H-1,2,4-triazole ***5b***

IR (KBr): 3392 (N-H), 1594 (C=N), 1122 (C-N) cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 10.98 (s, 1H, -N*H*-), 7.66 – 7.64 (m, 2H, Ar-*H*), 7.62 – 7.58 (m, 1H, Ar-*H*), 7.49 – 7.45 (m, 2H, Ar-*H*), 5.82 (tdd, 1H, $J_{H-^8CH_2}$ = 6.6 Hz, J_{H-H_Z} = 10.2 Hz, J_{H-H_E} = 17.1 Hz, CH_2=C*H*-), 5.02 (dd, 1H, J_{H_Z-H}= 10.2 Hz, $J_{H_Z-H_E}$ = 1.2 Hz, H_Z C=CH-), 4.90 (dd, 1H, J_{H-H_E} = 17.1 Hz, $J_{H_E-H_Z}$= 1.2 Hz, H_E C=CH-), 3.10 (t, 2H, *J* = 7.6 Hz, -CH_2 α to ring), 2.05 (m, 2H, CH_2-CH=CH_2), 1.99 (m, 2H, -CH_2 β to ring), 1.38 (10H, brs, chain CH_2). ^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 158.9, 147.2, 139.8, 133.9, 129.6, 127.6, 114.6, 32.8, 30.8, 29.2, 28.3, 22.9, 14.2. MS, *m/z* (%): $[M + 1]^+$ 284 (14.7), $[M]^+$ 283 (10.8), 270 (28.3), 242 (29.9), 228 (11.8), 214 (18.3), 186 (26.2), 144 (100).

3-Phenyl-5-(heptadec-8-enyl)-1H-1,2,4-triazole ***5c***

IR (KBr): 3382 (N-H), 1597 (C=N), 1133 (C-N) cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) d (ppm): 10.96 (s,1H, -N*H*-), 7.67 – 7.63 (m, 2H, Ar-*H*), 7.60-7.56 (m, 1H, Ar-*H*), 7.49 – 7.44 (m, 2H, Ar-*H*), 5.34 (2H, m, -C*H*=C*H*-), 2.97 (t, 2H, *J* = 7.6 Hz, -CH_2 α to ring), 2.05 (m, 4H, CH_2-CH=CH-CH_2), 1.96 (m, 2H, -CH_2 β to ring), 1.73 (brs, 20H, chain CH_2), 0.86 (dist.t, 3H, CH_3). ^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 158.2, 146.9, 134.1, 131.1, 129.0, 128.1, 33.8, 30.8, 29.9, 28.6, 22.6, 14.6. MS, *m/z* (%): $[M + 1]^+$ 382 (40.5), $[M]^+$ 381 (30.11), 338 (38.3), 310 (27.7), 268 (63.8), 242 (16.6), 158 (100), 144 (10.9).

3-Phenyl-5-[(8Z, 11R)-11-hydroxyheptadec-8-enyl]-1H-1,2,4-triazole ***5d***

IR (KBr): 3386 (N-H), 1590 (C=N), 1119 (C-N) cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 10.99 (s, 1H, -N*H*-), 7.66 – 7.64 (m, 2H, Ar-*H*), 7.60 – 7.57 (m, 1H, Ar-*H*), 7.49 – 7.46 (m, 2H, Ar-*H*), 5.34 (m, 2H, -CH=CH-), 3.88 (m, 1H, C*H*OH), 3.11 (t, 2H, *J* = 7.6 Hz, -CH_2 α to ring), 2.43 (m, 1H, CHO*H*), 2.05 (m, 4H, CH_2-CH=CH-CH_2), 1.89 (m, 2H, -CH_2 β to ring), 1.73 (brs, 18H, chain CH_2), 0.89 (dist.t, 3H, CH_3). ^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 157.9, 146.8, 134.0, 131.6, 129.2, 127.9, 67.8, 39.2, 34.6, 31.2, 29.4, 28.7, 27.1, 22.7, 14.9. MS, *m/z* (%): $[M + 1]^+$ 398 (9.8), $[M]^+$ 397 (21.2), 340 (13.2), 312 (13.1), 282 (26.8), 200 (39.9), 186 (100), 172 (30.0).

3-Pheny-5-[(8R, 11Z)-8-Hydroxyheptadec-11-enyl]-1H-1,2,4-triazole ***5e***

IR (KBr): 3387 (N-H), 1594 (C=N), 1124 (C-N) cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 10.98 (s, 1H, -N*H*-), 7.69 – 7.65 (m, 2H, Ar-*H*), 7.62 – 7.55 (m, 1H, Ar-*H*), 7.49 – 7.45 (m, 2H, Ar-*H*), 5.36 (m, 2H, -C*H*=C*H*-), 3.88 (m, 1H, C*H*OH), 3.11 (t, 2H, *J* = 7.6 Hz, -CH_2 α to ring), 2.42 (m, 1H, CHO*H*), 2.03 (m, 4H, CH_2-CH=CH-CH_2), 1.98 (m, 2H, -CH_2 β to ring), 1.29 (brs, 18H, chain CH_2), 0.88 (dist.t, 3H, CH_3). ^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 157.2, 146.8, 134.7, 131.5, 129.5, 127.2, 67.3, 39.1, 34.6, 31.8, 29.1, 28.7, 27.1, 22.5, 14.7. MS, *m/z* (%): $[M + 1]^+$ 398 (5.8), $[M]^+$ 397 (31.2), 368 (33.2), 326 (13.1), 272 (26.1), 228 (19.9), 214 (100), 172 (30.0).

3-(4′-Hydroxyphenyl)-5-heptadecyl-1H-1,2,4-triazole ***5f***

IR (KBr): 3387 (N-H), 1590 (C=N), 1132 (C-N) cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 10.97 (s, 1H, -N*H*-), 7.57 – 7.53 (m, 4H, Ar-*H*), 6.95 (Ar-O*H*), 2.78 (t, 2H, *J* = 7.6 Hz, -CH_2 α to ring), 1.92 (m, 2H, -CH_2 β to ring), 1.23 (brs, 28H, chain CH_2), 0.88 (dist.t, 3H, CH_3). ^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 159.1, 151.8, 148.7, 138.9, 129.7, 117.3, 32.5, 30.7, 22.8, 21.7, 14.5. MS, *m/z* (%): $[M + 1]^+$ 400 (13.8), $[M]^+$ 399 (41.6), 286 (16.6), 272 (83.3), 258 (16.6) 188 (8.3), 174 (44.4), 160 (100).

3-(4′-Hydroxyphenyl)-5-(dec-9-enyl)-1H-1,2,4-triazole ***5g***

IR (KBr): 3390 (N-H), 1590 (C=N), 1129 (C-N) cm^{-1} ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 10.97 (s, 1H, -N*H*-), 7.57 – 7.53 (m, 4H, Ar-*H*), 6.93 (Ar-O*H*), 5.82 (tdd, 1H, $J_{H-^8CH_2}$ = 6.6 Hz, J_{H-H_Z} = 10.2 Hz, J_{H-H_E} = 17.1 Hz, CH_2=C*H*-), 5.02 (dd, 1H, J_{H_E-H}= 10.2 Hz, $J_{H_E-H_Z}$ = 1.2 Hz, H_Z C=CH-), 4.90 (dd, 1H, J_{H_E-H} = 17.1 Hz, $J_{H_E-H_Z}$= 1.2 Hz, H_E C=CH-), 2.91 (t, 2H, *J* = 7.9 Hz, -CH_2 α to ring), 2.02 (m, 2H, -CH_2-CH=CH_2), 1.93 (m, 2H, -CH_2 β to ring), 1.38 (brs, 10H, chain CH_2). ^{13}C-NMR (100 MHz, $CDCl_3$) J_{H_E-H} (ppm): 158.7, 151.2, 148.1, 139.7, 129.1, 116.2, 114.4, 34.2, 33.7, 29.2, 24.7. MS, *m/z* (%): $[M + 1]^+$ 300 (19.9), $[M]^+$ 299 (12.3), 272 (12.5), 244 (17.3), 230 (3.9), 216 (2.2), 188 (3.9), 174 (4.2), 160 (100).

3-(4′-Hydroxyphenyl)-5-(heptadec-8-enyl)-1H-1,2,4-triazole ***5h***

IR (KBr): 3387(N-H), 1594 (C=N), 1130 (C-N) cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 10.97 (s, 1H, -N*H*-), 7.57 – 7.56 (m, 4H, Ar-*H*), 6.93 (Ar-O*H*), 5.39 (2H, m, -C*H*=C*H*-), 2.79 (t, 2H, *J* = 5.7 Hz, -CH_2 α to ring), 2.02 (m, 4H, -CH_2-CH=CH-CH_2-), 1.96 (m, 2H, -CH_2 β to ring), 1.23 (brs, 20H, chain CH_2), 0.88 (dist.t, 3H, CH_3). ^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 157.7, 151.2, 148.7, 130.8, 129.1, 118.4, 34.2, 31.8, 29.6, 29.1, 27.0, 24.7, 22.5, 14.6. MS, *m/z* (%): $[M + 1]^+$ 398 (13.8), $[M]^+$ 397 (41.6), 284 (16.6), 258 (16.6) 188 (8.3), 174 (100), 160 (44.4).

3-(4′-Hydroxyphenyl)-5-[(8Z, 11R)-11-hydroxyheptadec-8-enyl]-1H-1,2,4-triazole ***5i***

IR (KBr): 3390 (N-H), 1590 (C=N), 1123 (C-N) cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) ((ppm): 10.98 (s, 1H, -N*H*-), 7.58 – 7.52 (m, 4H, Ar-*H*), 6.98 (Ar-O*H*), 5.46 (m, 2H, -C*H*=C*H*-), 3.88 (m, 1H, -C*H*-OH), 3.44 (t, 2H, *J* = 7.5 Hz, -CH_2 α to ring), 2.31 (m, 1H, -CH-O*H*), 2.04 (m, 4H, -CH_2-CH=CH-CH_2-), 1.91 (m, 2H, -CH_2 β to ring), 1.33 (brs, 18H, chain CH_2), 0.86 (dist.t, 3H, CH_3). ^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 157.9, 151.6, 148.5, 130.8, 129.3, 118.7, 67.8, 39.2, 34.1, 31.8, 29.6, 28.9, 27.2, 24.7, 22.5, 14.3. MS, *m/z* (%): $[M + 1]^+$ 414 (12.2), $[M]^+$ 413 (26.13), 370 (75.8), 356 (60.9), 328 (33.4), 202 (69.8), 174 (14.2), 160 (100).

3-(4′-Hydroxyphenyl)-5-[(8R, 11Z)-8-Hydroxyheptadec-11-enyl]-1H-1,2,4-triazole ***5j***

IR (KBr): 3379 (N-H), 1592 (C=N), 1119 (C-N) cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 10.99 (s, 1H, -N*H*-), 7.57-7.53 (m, 4H, Ar-*H*), 6.93 (Ar-O*H*), 5.39 (m, 2H, -C*H*=C*H*-), 3.88 (m, 1H, -C*H*-OH), 3.23 (t, 2H, *J* = 6.4 Hz, -CH_2 α to ring), 2.28 (m, 1H, -CH-O*H*), 2.04 (m, 4H, -CH_2-CH=CH-CH_2-), 1.89 (m, 2H, -CH_2 β to ring), 1.27 (brs, 18H, chain CH_2), 0.86 (dist.t, 3H, CH_3). ^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 158.9, 151.6, 148.7, 131.1, 129.4, 118.6, 67.3, 39.6, 34.1, 31.8, 29.5, 28.7, 27.2, 24.5, 22.1, 14.4. MS, *m/z* (%): $[M + 1]^+$ 414 (12.5), $[M]^+$ 413 (29.1), 342 (51.2), 316 (33.3), 288 (9.8), 188 (100), 174 (18.8), 160 (80.2).

3-(4′,5′-Dihydroxyphenyl)-5-(heptadecyl)-1H-1,2,4-triazole ***5k***

IR (KBr): 3349 (N-H), 1604 (C=N), 1134 (C-N) cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 10.97 (s, 1H, -N*H*-), 7.16 (d, 1H, *J* = 1.6 Hz, Ar-*H*), 7.07 (d, 1H, *J* = 2 Hz, Ar-*H*), 7.03 (d, 1H, *J* = 2 Hz, Ar-*H*), 6.89 (Ar-O*H*), 6.87 (Ar-O*H*), 2.78 (t, 2H, *J* = 7.6 Hz, -CH_2 α to ring), 1.92 (m, 2H, -CH_2 β to ring), 1.23 (brs, 28H, chain CH_2), 0.88 (dist.t, 3H, CH_3). ^{13}C-NMR (100 MHz, $CDCl_3$): 164.5, 145.1, 144.2, 121.9, 115.9, 32.1, 30.3, 28.9, 22.3, 14.5. MS, *m/z* (%): $[M + 1]^+$ 416 (17.0), $[M]^+$ 415 (66.0), 356 (10.4), 314 (2.1), 300 (31.2), 208 (19.2), 190 (12.5), 176 (100).

3-(4′,5′-Dihydroxyphenyl)-5-(dec-9-enyl)-1H-1,2,4-triazole ***5l***

IR (KBr): 3387 (N-H), 1596 (C=N), 1130 (C-N) cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) d (ppm): 10.98 (s, 1H, -N*H*-), 7.11 (d, 1H, *J* = 1.6 Hz, Ar-*H*), 7.04 (d, 1H, *J* = 2 Hz, Ar-*H*), 7.02 (d, 1H, *J* = 2 Hz, Ar-*H*), 6.88 (Ar-O*H*), 6.87 (Ar-O*H*), 5.82 (tdd, 1H, $J_{H-^8CH_2}$ = 6.6 Hz, J_{H-H_Z}= 10.2 Hz, J_{H-H_E}= 17.1 Hz, CH_2=C*H*-), 5.02 (dd, 1H, J_{H_Z-H}= 10.2 Hz, $J_{H_Z-H_E}$ = 1.2 Hz, H_Z C=C*H*-), 4.90 (dd, 1H, J_{H_E-H} = 17.1 Hz, $J_{H_E-H_Z}$= 1.2 Hz, H_E C=C*H*), 3.13 (t, 2H, *J* = 8 Hz, -CH_2 α to ring), 2.02 (m, 2H, -CH_2-CH=CH_2), 1.93 (m, 2H, -CH_2 β to ring), 1.38 (brs, 10H, chain CH_2). ^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 164.9, 145.8, 144.8, 139.9, 121.9, 116.7, 114.6, 34.2, 33.7, 29.2, 28.4, 24.2. MS, *m/z* (%): $[M + 1]^+$ 316 (25.7), $[M]^+$ 315 (23.8), 288 (44.2), 274 (66.6), 243 (17.1), 208 (36.3), 198 (14.6), 176 (100).

3-(4′,5′-Dihydroxyphenyl)-5-(heptadec-8-enyl)-1H-1,2,4-triazole ***5m***

IR (KBr): 3384 (N-H), 1594 (C=N), 1127 (C-N) cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 10.97 (s, 1H, -N*H*-), 7.11 (d, 1H, *J* = 1.6 Hz, Ar-*H*), 7.04 (d, 1H, *J* = 2 Hz, Ar-*H*), 7.02 (d, 1H, *J* = 2 Hz, Ar-*H*), 6.93 (Ar-O*H*), 6.89 (Ar-O*H*), 5.34 (m, 2H, -C*H*=C*H*-), 2.78 (t, 2H, *J* = 7.6 Hz, -CH_2 α to ring), 2.02 (m, 4H, -CH_2-CH=CH-CH_2-), 1.92 (m, 2H, -CH_2 β to ring), 1.23 (brs, 20H, chain CH_2), 0.88 (dist.t, 3H, CH_3). ^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 163.4, 146.8, 144.8, 131.9, 121.6, 119.8, 33.1, 29.9, 25.6, 22.7, 14.9. MS, *m/z* (%): $[M + 1]^+$ 414 (17.0), $[M]^+$ 413 (66.0), 356 (10.4), 314 (12.1), 300 (31.2), 208 (19.2), 190 (100), 176 (57.5).

3-(4′,5′-Dihydroxyphenyl)-5-[(8Z, 11R)-11-hydroxyheptadec-8-enyl]-1H-1,2,4-triazole ***5n***

IR (KBr): 3386 (N-H), 1597 (C=N), 1123 (C-N) cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 10.98 (s, 1H, -N*H*-), 7.13 (d, 1H, *J* = 1.6 Hz, Ar-*H*), 7.04 (d, 1H, *J* = 2 Hz, Ar-*H*), 7.01 (d, 1H, *J* = 2 Hz, Ar-*H*), 6.89 (Ar-O*H*), 6.86 (Ar-O*H*), 5.46 (m, 2H, -C*H*=C*H*-), 3.88 (m, 1H, -C*H*-OH), 3.44 (t, 2H, *J* = 7.5 Hz, -CH_2 α to ring), 2.31 (m, 1H, -CH-O*H*), 2.04 (m, 4H, -CH_2-CH=CH-CH_2-), 1.91 (m, 2H, -CH_2 β to ring), 1.33 (brs, 18H, chain CH_2), 0.86 (dist.t, 3H, CH_3). ^{13}C-NMR (100 MHz, $CDCl_3$) (ppm): 163.9, 147.8, 145.1, 131.8, 121.1, 119.2, 68.2, 39.8, 37.2, 33.1, 29.9, 27.6, 24.1, 22.7, 14.3. MS, *m/z* (%): $[M + 1]^+$ 430 (6.6), $[M]^+$ 429 (28.3), 344 (43.3), 260 (13.8), 253 (41.6), 208 (8.3), 190 (47.2), 176 (100).

3-(4′,5′-Dihydroxyphenyl)-5-[(8R, 11Z)-8-hydroxyheptadec-11-enyl]-1H-1,2,4-triazole ***5o***

IR (KBr): 3392 (N-H), 1600 (C=N), 1123 (C-N) cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 10.98 (s, 1H, -N*H*-), 7.11 (d, 1H, *J* = 1.6 Hz, Ar-*H*), 7.04 (d, 1H, *J* = 2 Hz, Ar-*H*), 7.02 (d, 1H, *J* = 2 Hz, Ar-*H*), 6.89 (Ar-O*H*), 6.87 (Ar-O*H*), 5.39 (m, 2H, -C*H*=C*H*-), 3.88 (m, 1H, -C*H*-OH), 3.23 (t, 2H, *J* = 6.4 Hz, -CH_2 α to ring), 2.28 (m, 1H, *J* = 7.2 Hz, -CH-O*H*), 2.04 (m, 4H, -CH_2-CH=CH-CH_2-), 1.89 (m, 2H, -CH_2 β to ring), 1.27 (brs, 18H, chain CH_2), 0.86 (dist.t, 3H, CH_3). ^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 163.6, 147.5, 145.1, 131.7, 121.2, 119.6, 68.4, 39.8, 37.4, 33.1, 29.5, 27.3, 24.2, 22.4, 14.2. MS, *m/z* (%): $[M + 1]^+$ 430 (55.5), 372 (55.5), $[M]^+$ 429 (8.3), 332 (41.6), 301 (22.2), 280 (13.6), 208 (8.3), 176 (100).

Biological evaluation

Antibacterial activity

The newly synthesized compounds were screened *in vitro* against an assortment of two Gram-positive bacteria *Staphylococcus aureus* MSSA 22 and *Bacillus subtilis* ATCC 6051 and two Gram-negative bacteria *Escherichia coli* K12 and *Salmonella typhimurium* MTCC 98. Screening results are summarized in Table 2. All the synthesized compounds were dissolved in DMSO. The antibacterial activity of test compounds and standard chloramphenicol was done by filter paper disc method [38]. Media with DMSO was set up as control. All cultures were routinely maintained on NA (nutrient agar) and incubated at 37°C. The inoculums of bacteria were performed by growing the culture in NA broth at 37°C overnight. The culture was centrifuged at 1000 rpm, pellets were resuspended, and diluted in sterile NSS to obtain viable count 10^5 CFU/mL. With the help of spreader, 0.1 mL of approximately diluted bacterial culture suspension was spread on NA plates uniformly. Sterile 8-mm discs (Hi-media Pvt. Ltd.) were impregnated with 100 μg/mL concentration of the test compounds. Antibiotic disc, chloramphenicol (30 μg/disc, Hi-Media) was used as control. The disc was placed onto the plate. Each plate had one control disc impregnated with solvent. The plates were then incubated for 24 h at 37°C, and the resulting zones of inhibition (in mm) were measured. Diameters of the zone of inhibition (mm) were measured and the average diameters for the test samples were calculated in triplicate sets.

Antifungal activity

The standard agar disc diffusion method [38] was performed to evaluate the antifungal property of the test compounds and standard nystatin. The newly synthesized compounds were screened for *Aspergillus niger* (laboratory isolate), *Candida albicans* IOA-109, *Penicillium* sp. (laboratory isolate), *Trichoderma viridae* (laboratory isolate), *Helminthosporum oryzae* (2537 ICAR, Jaipur); see Table 3. The synthesized compounds were dissolved in DMSO. Media with DMSO was set up as control. All cultures were routinely maintained on SDA and incubated at 28°C. Spore formation of filamentous fungi was prepared from seven-day old culture in sterile normal solution (8% NaCl) and diluted to

obtain approximately 10^5 CFU/mL. The inoculum of non-sporing fungi *C. albicans* was performed by growing the culture in SD broth at 37°C overnight. The culture was centrifuged at 1000 rpm, pellets was resuspended, and diluted in sterile NSS to obtain a viable count 10^5 CFU/ml. With the help of spreader, 0.1 mL of the approximately diluted fungal culture suspension was spread on SDA plates uniformly. Sterile 8-mm discs (Himedia Pvt. Ltd., Mumbai, India) were impregnated with test compounds. Antibiotic disc, nystatin (30 µg/disc Hi-Media) was used as control. The disc was placed onto the plate. Each plate had one control disc impregnated with solvent. The plates were incubated at 28°C for filamentous fungi for 72 h or more, while for *C. albicans* plates were incubated at 37°C for 18–48 h. Antifungal activity was determined by measuring the diameters of the inhibition zone (mm) in triplicate sets.

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