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Original article

An eco-friendly synthesis and antimicrobial activities of dihydro-2*H*-benzo- and naphtho-1,3-oxazine derivatives

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ABSTRACT

A series of 3,4-dihydro-2*H*-benzo[e]-, 2,3-dihydro-1*H*-naphtho[1,2-e]-, 3,4-dihydro-2*H*-naphtho[2,1-e][1,3]oxazine and 1,2-bis(3,4-dihydrobenzo[e][1,3]oxazin-3(4*H*)-yl)ethane derivatives was obtained through an eco-friendly Mannich type condensation–cyclization reaction of phenols or naphthols with formaldehyde and primary amines in water at ambient temperature. Preliminary *in vitro* antimicrobial activity of the synthesized compounds was assessed against six pathogenic fungi, two Gram-negative and two Gram-positive bacteria. Some of the screened compounds have shown significant *in vitro* antimicrobial effect. Cytotoxic activities of the lead compounds (**2m**, **2n**, **3c** and **3d**) against mouse fibroblast cell line (L929) were determined by MTT method. The assay results revealed that these molecules offered remarkable viability (>90%) of L929 cells at concentration of 25 μg/mL.

1. Introduction

Invasive microbial infections with multi-drug resistant bacteria and opportunistic mycoses have become increasingly common worldwide and are collectively a major cause of morbidity and mortality, especially in immunocompromised patients [1]. Although, several new antimicrobial agents have been licensed in the past decades, but some immunosuppressed persons are difficult to treat. The main reason for this include rapid transmission of the systemic fungal pathogens, drug toxicity, drug bioavailability, development of drug resistance and lack of safe oral or intravenous preparations. However, the problem of toxicity is greatly addressed by the development of several broad-spectrum imidazole [2], triazole [3] and echinocandin [4] derivatives for specific use but the problem of drug resistance is still an issue with newly discovered antimicrobial drugs. Therefore, a search for new, safe and highly effective chemotherapeutic agents become inevitable. Thorough literature survey revealed that the compounds containing dihydro-1,3-oxazine ring system exhibited a wide spectrum of pharmacological activities such as anti-tumor [5], anti-bacterial [6], anti-HIV [7] and antimalarial [8] agents. In addition, 6-arylbenzoxazines are reported as potent non-steroidal progesterone receptor agonists [9]. In the course of our search for potent antifungal compounds, we synthesized various dihydro-1,3-oxazine derivatives (**2a**–**n**, **3a**–**d**, **4a**–**j** and **5**) by using environmentally benign process in good to excellent yields. The *in vitro* antimicrobial activities of these compounds were assessed and the results are presented in this paper.

2. Chemistry

The synthesis of various 3,4-dihydro-2*H*-benzo[e]-, 2,3-dihydro-1*H*-naphtho[1,2-e]-, 3,4-dihydro-2*H*-naphtho[2,1-e][1,3]oxazines and 1,2-bis[3,4-dihydrobenzo[e][1,3]oxazin-3(4*H*)-yl]ethanes involves one-pot condensation–cyclization reaction of phenols or naphthols with formaldehyde and primary amines. Various methods have been reported [10–12] for the synthesis of dihydro-1,3-oxazines including the reaction under neat conditions [13]. However, many of these processes have several disadvantages such as the need for a prolonged reaction time, high temperature, use of volatile and toxic organic solvents and occurrence of side products. Thus, the development of simple, efficient and green procedure for the synthesis of these molecules is highly desirable. In continuation of our efforts to develop a simple and economical methodology for the synthesis of target compounds of biological interests, we have successfully developed an efficient and eco-friendly synthetic strategy [14] for the construction of a series of 3,4-dihydro-2*H*-benzo[e][1,3]oxazines (**2a**–**n**), 1,2-bis[3,4-dihydrobenzo-[e][1,3]oxazin-3(4*H*)-yl]ethanes (**3a**–**d**), 2,3-dihydro-1*H*-naphtho-[1,2-e][1,3]oxazines (**4a**–**j**) and 3,4-dihydro-3-phenyl-2*H*-naphtho[2,1-e][1,3]oxazine (**5**) in good to excellent yields via Mannich

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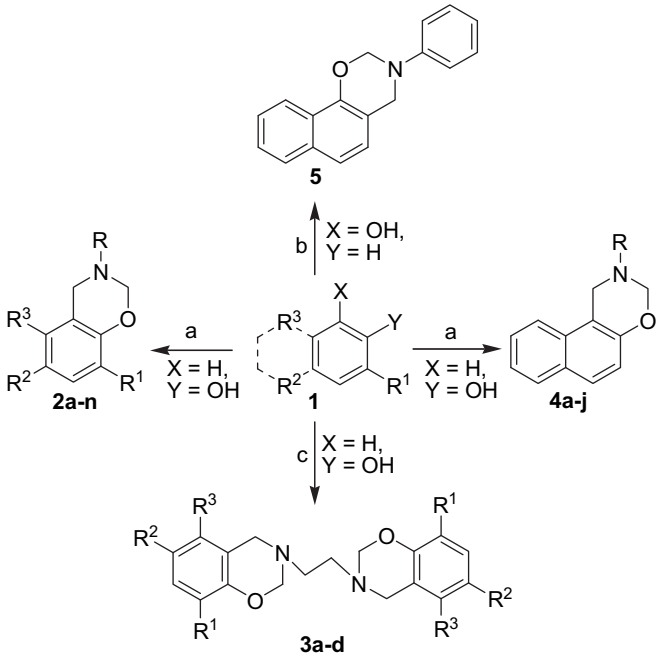
type condensation–cyclization reaction of phenols or naphthols with HCHO and primary amines in water at ambient temperature (Scheme 1). The structures of all the synthesized molecules were confirmed by elemental analyses and spectral (IR, ^{1}H NMR, ^{13}C NMR, and mass) data. The IR spectrum of compound **2i** showed the characteristic absorption peaks of benzoxazine ring structure at 1226 cm^{-1} (asymmetric stretching of C–O–C), and at 1035 cm^{-1} (symmetric stretching of C–O–C). In ^{1}H NMR, characteristic peaks of two methylene groups of 1,3-oxazine ring are observed at δ 4.53 corresponding to Ar–CH_2–N and δ 5.26 corresponding to O–CH_2–N. Further evidence for the formation of 3-(4-fluorophenyl)-6-methyl-3,4-dihydro-2*H*-benzo-[e][1,3]oxazine (**2i**) was obtained by recording its mass spectrum. The mass spectrum of compound (**2i**) showed M^+ ion peak at *m*/*z* 243 for the molecular formula $C_{15}H_{14}FNO$. In addition, differential scanning calorimetry (DSC) thermogram of **2i** showed an endothermic peak at 82.2 °C which was attributed to its melting point. The characteristic exothermic peak was observed due to ring-opening polymerization [10j] with onset at 141.2 °C and maximum at 165.7 °C. The amount of heat of polymerization was found to be 66.4 J/g. The typical synthetic procedure and characterization data of compounds (**2i–j**, **2n**, **3b**, **4b**, and **4d–e**) are presented in the Experimental Section. The physical data of all the known compounds (**2a–h**, **2k–m**, **3a**, **3c–d**, **4a**, **4c**, **4f–j** and **5**) are in agreement with those of reported data [10–14].

3. Pharmacology

3.1. Antifungal studies

The *in vitro* antifungal activity of 3,4-dihydro-2*H*-benzo[e][1,3]oxazines (**2a–n**), 1,2-bis[3,4-dihydrobenzo[e][1,3]oxazin-3(4*H*)-yl]ethanes (**3a–d**), 2,3-dihydro-1*H*-naphtho[1,2-e][1,3]oxazines (**4a–j**) and 3,4-dihydro-3-phenyl-2*H*-naphtho[2,1-e][1,3]oxazine (**5**) was investigated against six pathogenic fungi viz. – *Candida albicans* (Ca), *Cryptococcus neoformans* (Cn), *Sporothrix schenckii* (Ss), *Trichophyton mentagrophytes* (Tm), *Aspergillus fumigatus* (Af) and *Candida parapsilosis* (Cp) by broth micro-dilution technique as per guidelines of National Committee for Clinical Laboratory Standards [15] using RPMI Medium 1640 buffered with MOPS [3-(N-morpholino)propanesulphonic acid] in microtitre plates. The starting inoculums of test culture were 1–5 × 10^3 cfu/mL. Microtitre plates were incubated at 35 °C. Minimal inhibitory concentrations (MICs) were determined by spectrophotometric method at 492 nm after 24–48 h (yeasts) and 72–96 h (mycelial fungi) incubation. Ketoconazole and fluconazole were used as reference fungicides.



Scheme 1. Reagents and conditions: (a) RNH_2, HCHO, H_2O, 25 °C, 0.5–1 h; (b) $C_6H_5NH_2$, HCHO, H_2O, 25 °C, 30 min; (c) $NH_2CH_2CH_2NH_2$, HCHO, H_2O, 25 °C, 1 h.

3.2. Anti-bacterial studies

The anti-bacterial activity of all the prepared compounds was evaluated against two Gram-positive bacteria viz. *Klebsiella pneumonia* (Kp) and *Staphylococcus aureus* (Sa), and two Gram-negative bacteria viz. *Escherichia coli* (Ec) and *Pseudomonas aeruginosa* (Pa) by the NCCLS Method using Mueller Hinton broth in 96-well tissue culture plates. The bacterial strains were grown on nutrient agar at 37 °C. After 24 h of incubation, bacterial cells were suspended in normal saline containing Tween 20 at 0.05% concentration of approximately 1.0–2.0 × 10^7 cells/mL by matching with McFarland standards. Proper growth control, drug control and the negative control were adjusted on to the plate. Compounds were dissolved in dimethyl sulfoxide (DMSO) at a concentration of 1 mg/mL and 20 μL of this solution was added to each well of 96-well tissue culture plate having 180 μL Mueller Hinton broth. The solution was then serially diluted to afford twofold serial dilutions of the test compounds in the subsequent wells. Then McFarland matched bacterial suspension (100 μL) was diluted with 10 mL of media. The diluted bacterial suspension (100 μL) was added to each well and then kept for incubation. The maximum concentration of compounds tested was 50 μg/mL. Microtitre plates were incubated at 35 °C in a moist, dark chamber. MICs were recorded spectrophotometrically after 24 h incubation. Gentamycin and ampicillin were used as reference anti-bacterial agents.

3.3. Cytotoxicity evaluation

The cytotoxic effect of the lead compounds (**2m**, **2n**, **3c** and **3d**) against mammalian cells, mouse fibroblast cell line L929 was determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method [16]. The stock solutions (1.0 mg/mL) of the test compounds were prepared in DMSO. The cells (L929) were grown at 37 °C in RPMI Medium 1640 supplemented with 10% (v/v) foetal bovine serum (FBS) and antimycotic and anti-bacterial solutions (Sigma, USA) in humidified atmosphere having 5% CO_2. 100 μL of the confluent fibroblast stock suspension (1.0 × 10^5 cells/mL) was dispensed in 96-well tissue culture plates. The original medium was replaced from the wells with 100 μL serum-free RPMI Medium 1640 when the cells reached 80% confluence after incubation at 37 °C in humidified incubator with 5% CO_2. L929 cells were exposed to various concentrations (25, 12.5, 6.25, 3.12, 1.56, 0.78, 0.39, 0.19 and 0.09 μg/mL) of test compounds along with control experiment. After incubation for 24 h, cells were washed twice in phosphate-buffered saline (PBS) and 200 μL of MTT solution (0.5 mg/mL of MTT in RPMI Medium 1640) was added to each well and then incubated for 4 h at 37 °C to allow MTT metabolism. An aliquot of 100 μL DMSO solvent was added to each well and the plate was incubated for 30 min at room temperature. Response of L929 cells to the test compounds was determined spectrophotometrically at 570 and 630 nm (reference wavelength). The ratio of differences in absorbance values at these two wavelengths (A570 and A630) for both treated and untreated cultured cells were used to calculate % viability using the equation [(A570–A630)sample/(A570-A630)control] × 100%. Further, the

morphology of cells was observed by using Giemsa stain under phase contrast microscope. After fixation of the cells in the wells of 96-well tissue culture plates, Giemsa stain was added to each well and incubated for 30 min at 37 °C. The excess stain was removed by thorough washing with PBS. The culture plates were dried in air and observed under phase contrast microscope.

4. Results and discussion

Most target compounds showing minimum inhibitory concentration (MIC) values between 0.39 and 50 μg/mL were able to have a significant *in vitro* inhibitory effect against the screened microbes. The activity data are presented in Tables 1 and 2. Among all the synthesized compounds, **2m** was found to be the most active against Ca, Cn, Ss, Tm, Af and Cp with MIC values 12.5, 12.5, 6.25, 3.12, 25 and 25 μg/mL respectively. The other compound **3c** was found equipotent to **2m** except it showed higher MIC value (50 μg/mL) against Af. However, this trend does not translate exactly to anti-bacterial activities. As shown in Table 2, compounds **2m**, **3c** and **3d** were found active against Sa but latter was most promising against Sa and Kp with MIC values 0.39 and 1.56 μg/mL respectively.

As seen in Table 1; although none of the compounds showed more efficacy than fluconazole against tested fungi, but some of the title compounds were found to be significantly potent with low MIC values in the range of 3.12–25 μg/mL. The most active compounds **2m** and **3c** have displayed better fungicidal activity (3.12 μg/mL) than ketoconazole (4 μg/mL) against Tm. The antifungal activity profile of 3,4-dihydro-2*H*-benzo[e][1,3]oxazines (**2a–n**) and 1,2-bis[3,4-dihydrobenzo[e][1,3]oxazin-3(4*H*)-yl]ethanes **(3a–d)** depends mainly on the presence of substituent in the fused aryl ring. The presence of chloro substituent at position 6 and H at 5, 8 positions in **2m** and **3c** displayed significant activity against Ca (12.5 μg/mL), Cn (12.5 μg/mL), Ss (6.25 μg/mL), Tm (3.12 μg/mL) and Cp (25 μg/mL). Introduction of chloro substituent at position 8 in **3d** and 5,8-positions in **2n** did not improve the antifungal activity. As it is evident from the activity data that a mere change of 6-chloro substituent in **2m** or **3c** by electron-donating methyl group as in **2g** or **3b** displayed low order of inhibitory effect against all the tested mycoses.

Structure–activity relationship revealed that the substituent at position 3 in structure **2** is also responsible for antimycotic activity. An exchange of 2-chlorophenyl substituent at position 3 in **2m** by 4-methylphenyl, phenyl or butyl substituent as in **2l**, **2k** and **2j** respectively led to reduce the antifungal activity. Additionally, 2,3-dihydro-1*H*-naphtho[1,2-e][1,3]oxazines (**4a–j)** and 3,4-dihydro-3-phenyl-2*H*-naphtho[2,1-e][1,3]oxazine (**5**) have demonstrated poor antifungal activity compared to 3,4-dihydro-*2H*-benzo[e][1,3]-oxazines.

Besides significant antifungal effect, some of the synthesized compounds indicated potent inhibitory effect against tested Gram-positive and Gram-negative bacteria (Table 2). Among all, the compound **3d** having MIC value 0.39 μg/mL was found to be most active and equipotent to standard drug gentamycin, used against Gram-positive bacterium, *S. aureus*. Surprisingly, the 3,4-dihydro-*2H*-benzo[e][1,3]oxazines (**2d–f**, **2k–l)** were found to be more active against Ec than that of standard drug, ampicillin (MIC 50 μg/mL). In contrast, compounds **2f**, **2h**, **2k–n**, **3c–d** have shown significant activity against Sa (MICs ranged from 0.39 to 25 μg/mL). Similarly, derivatives **2a**, **2c**, **2g**, **2i**, **4a**, and **4f** were found

Table 1
Antifungal activity of 3,4-dihydro-2*H*-benzo[e][1,3]oxazines (**2a–n**), 1,2-bis(3,4-dihydrobenzo[e][1,3]oxazin-3(4*H*)-yl)ethanes (**3a–d**), 2,3-dihydro-1*H*-naphtho[1,2-e][1,3]oxazines (**4a–j)** and 3,4-dihydro-3-phenyl-2*H*-naphtho[2,1-e][1,3]oxazine **(5**).

Compound	R^1	R^2	R^3	R	Minimum inhibitory concentration (MIC) in μg/mL					
					Ca[a]	Cn[a]	Ss[a]	Tm[a]	Af[a]	Cp[a]
2a	H	H	H	C_4H_9	>50	>50	50	50	>50	>50
2b	H	H	H	C_6H_{11}	>50	>50	>50	50	>50	>50
2c	H	H	H	C_6H_5	>50	>50	25	25	50	50
2d	CH_3	H	H	C_6H_5	50	>50	25	25	50	50
2e	H	CH_3	H	C_6H_5	>50	>50	12.5	25	50	50
2f	H	CH_3	H	4-$CH_3C_6H_4$	50	50	12.5	12.5	50	50
2g	H	CH_3	H	2-ClC_6H_4	50	25	12.5	12.5	50	50
2h	H	CH_3	H	4-ClC_6H_4	50	25	12.5	12.5	25	50
2i	H	CH_3	H	4-FC_6H_4	>50	50	25	25	>50	>50
2j	H	Cl	H	C_4H_9	>50	>50	25	50	>50	>50
2k	H	Cl	H	C_6H_5	50	50	6.25	12.5	25	50
2l	H	Cl	H	4-$CH_3C_6H_4$	25	50	6.25	12.5	25	25
2m	H	Cl	H	2-ClC_6H_4	12.5	12.5	6.25	3.12	25	25
2n	Cl	H	Cl	2-ClC_6H_4	>50	50	12.5	12.5	50	50
3a	H	H	H	–	>50	50	25	25	50	>50
3b	H	CH_3	H	–	50	50	12.5	25	50	50
3c	H	Cl	H	–	12.5	12.5	6.25	3.12	50	25
3d	Cl	Cl	H	–	50	25	>50	25	50	50
4a	–	–	–	C_6H_5	50	>50	25	>50	>50	>50
4b	–	–	–	4-ClC_6H_4	>50	50	50	25	>50	>50
4c	–	–	–	4-BrC_6H_4	>50	50	50	50	>50	>50
4d	–	–	–	4-FC_6H_4	>50	50	50	25	>50	>50
4e	–	–	–	2,4-$Cl_2C_6H_3$	>50	>50	>50	50	>50	>50
4f	–	–	–	2,5-$Cl_2C_6H_3$	>50	>50	>50	50	>50	>50
4g	–	–	–	2,4,6-$Cl_3C_6H_2$	>50	>50	>50	50	>50	>50
4h	–	–	–	C_4H_9	>50	50	50	25	>50	>50
4i	–	–	–	C_6H_{11}	50	50	50	25	>50	>50
4j	–	–	–	$CH_2C_6H_5$	>50	>50	50	50	>50	>50
5	–	–	–	–	>50	>50	>50	>50	>50	>50
Ketoconazole	–	–	–	–	0.002	0.001	0.031	4.0	2.0	0.031
Fluconazole	–	–	–	–	0.5	1.0	2.0	1.0	2.0	1.0

[a] Ca – *Candida albicans*; Cn – *Cryptococcus neoformans*; Ss – *Sporothrix schenckii*; Tm – *Trichophyton mentagrophytes*; Af – *Aspergillus fumigatus*; Cp – *Candida parapsilosis* (ATCC 22019).

Table 2
Anti-bacterial activity of the compounds **2a-n**, **3a-d**, **4a-j** and **5**.

a	Minimum inhibitory concentration in μg/mL			
	Kp[a]	Ec[a]	Pa[a]	Sa[a]
2a	>50	50	>50	>50
2b	>50	>50	>50	>50
2c	>50	50	>50	>50
2d	>50	25	>50	50
2e	50	25	>50	50
2f	50	25	>50	25
2g	>50	50	50	>50
2h	25	>50	50	25
2i	50	50	50	50
2j	>50	>50	>50	>50
2k	50	12.5	>50	25
2l	25	6.25	>50	12.5
2m	>50	>50	>50	6.25
2n	6.25	>50	50	12.5
3a	>50	>50	>50	>50
3b	>50	>50	>50	>50
3c	>50	>50	>50	12.5
3d	1.56	>50	>50	0.39
4a	>50	50	50	>50
4b	50	>50	50	>50
4c	>50	>50	50	>50
4d	50	>50	50	>50
4e	>50	>50	>50	>50
4f	>50	50	>50	>50
4g	>50	>50	>50	>50
4h	>50	>50	>50	>50
4i	>50	>50	>50	>50
4j	>50	>50	>50	>50
5	>50	>50	>50	>50
Gentamycin	0.78	0.78	0.78	0.39
Ampicillin	0.39	50	50	0.19

[a] Kp – *Klebsiella pneumoniae*; Ec – *Escherichia coli*; Pa – *Pseudomonas aeruginosa*; Sa – *Staphylococcus aureus*.

equipotent (MIC 50 μg/mL) against Ec, **2g–i**, **2n** and **4a–d** against Pa, to ampicillin.

The structure–activity relationships showed that the anti-bacterial activity of 1,2-bis[3,4-dihydrobenzo[e][1,3]oxazin-3(4*H*)-yl]ethanes (**3a–d)** based on the substituents present in the fused aryl ring and their efficacy is in the order of 6,8-dichloro > 6-chloro > 6-methyl, H. The activity of **4a–j** depends on the nature of N-substituent at position 3. In **4a–d** the N-aryl substituent such as C_6H_5, 4-ClC_6H_4, 4-BrC_6H_4, 4-FC_6H_4 have shown comparable activity (MIC 50 μg/mL) against Pa where as compound **4a** with N–C_6H_5 and **4f** with N–$C_6H_3Cl_2$ (2, 5) demonstrated similar effect against Ec. In addition, compounds **4b** with N–C_6H_4Cl(4) and **4d** with N–C_6H_4F(4) were also found equally potent (MIC 50 μg/mL) against Kp. Other compounds with N-substituents as 2,4-$Cl_2C_6H_3$, 2,4,6-$Cl_3C_6H_2$, C_4H_9, C_6H_{11} and benzyl were found inactive against all the tested bacteria. However, these results could be guide for further anti-bacterial studies.

Finally, the lead compounds (**2m**, **2n**, **3c** and **3d**) were studied for their cytotoxic effects against mammalian L929 cells by MTT assay. Morphological anomalies of L929 cells caused due to the test compounds were evident under phase contrast microscope. In control experiment, L929 cells were found fairly transparent, well spaced and attached to the well's surface of tissue culture plate (Fig. 1A). After exposure of the L929 cells to test compounds, no toxicity was observed at MIC (6.25 μg/mL) and lower concentrations as evident from the normal morphology of cells (Fig. 1B). However, when these cells exposed to higher concentrations (12.5 and 25 μg/mL) of test compounds they lost their normal morphology (Fig. 1C) but the absorbance studies at 570 and 630 nm during MTT assay revealed >90% viability of L929 cells even at higher concentrations of test compounds.

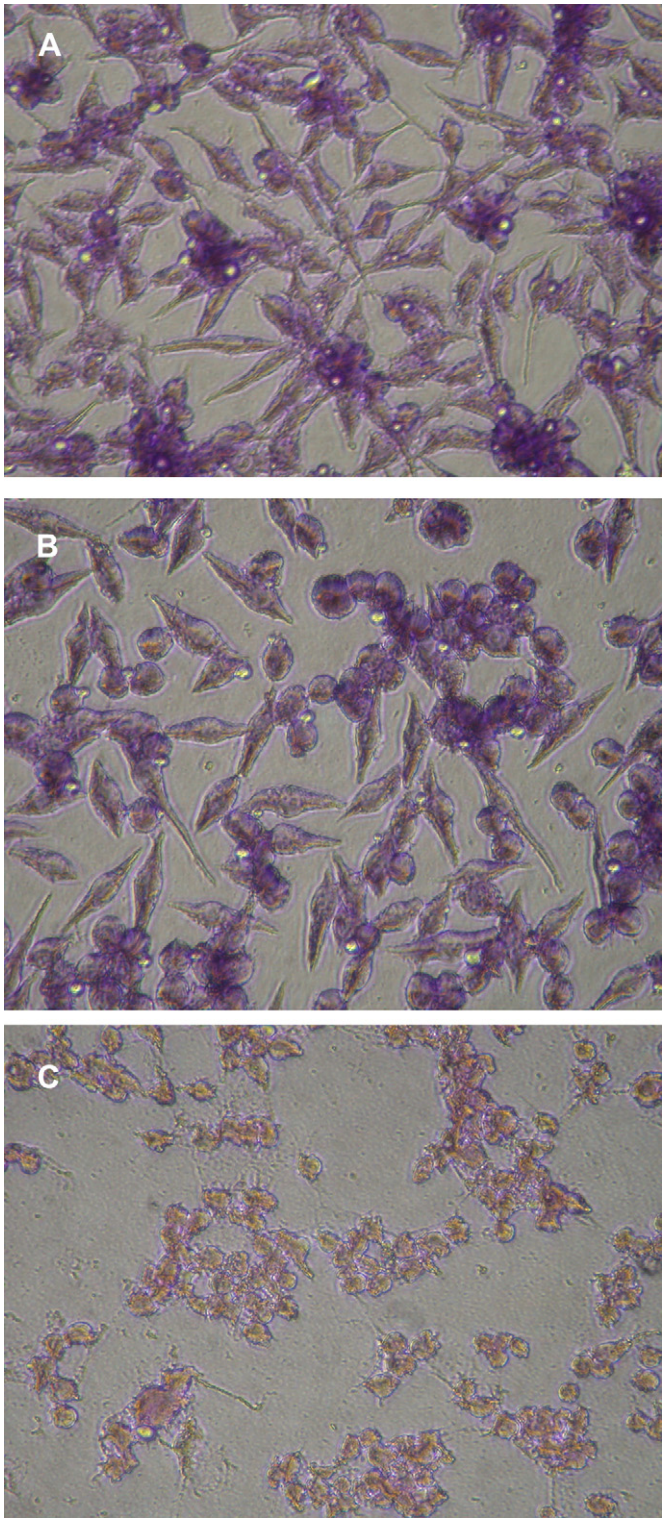


Fig. 1. Morphological changes of L929 cells exposed to compounds **2m, 2n, 3c** and **3d** for 24 h. (A) Control; (B) treated at MIC (6.25 μg/mL); (C) treated at higher concentrations (12.5 and 25 μg/mL).

5. Conclusions

In conclusion, various 3,4-dihydro-2*H*-benzo[e]-, 2,3-dihydro-1*H*-naphtho[1,2-e]- and 3,4-dihydro-2*H*-naphtho[2,1-e][1,3]oxazines have been synthesized by using green methodology and evaluated for their antimicrobial activity. Most of the analogues

have shown significant activity with MIC values in the range of 0.39–50 μg/mL against the tested microbes. The MTT assay results revealed that the compounds **2m**, **2n**, **3c** and **3d** offered remarkable viability (>90%) of L929 cells at 25 μg/mL. Henceforth, these results may be useful for the further development of new antifungal and anti-bacterial agents.

6. Experimental protocols

6.1. Chemistry

Thin-layer chromatography was performed on pre-coated Merck silica 60 F254 plates and spots were detected under UV light (254 nm). All the compounds were purified by column chromatography using silica gel (60–120 mesh, Ranbaxy). Mass spectra were recorded on ESI-MS mass spectrometer in positive ionization mode. Infrared spectra were recorded on Perkin–Elmer IR spectrometer in film; absorption maxima (υ_{max}) are given in cm^{-1}. The ^1H and ^{13}C NMR spectra were measured on Bruker 300 MHz spectrometer in $CDCl_3$. The calibration of spectra was carried out on solvent signal ($CDCl_3$: $\delta_H = 7.26$ and $\delta_C = 77.36$) and chemical shifts (δ) are reported in parts per million (ppm). Coupling constants J are reported in hertz (Hz). DSC traces were recorded on Perkin–Elmer differential calorimetry. Elemental analysis was performed on Elementar Analysensysteme GmbH VarioEL V3.00 for compounds **2i**, **3b**, **4b** and **4d**. Anhydrous sodium sulfate (Na_2SO_4) was used as a drying agent. All chemicals were used without further purification. Solvents were distilled off under reduced pressure on a rotary evaporator.

6.2. General method for the preparation of dihydrobenzo/naphtho[e][1,3]oxazines (**2a–n**, **4a–j** and **5**)

To a mixture of the corresponding phenol or naphthol (10 mmol) and primary amine (10 mmol) in water (20 mL), formalin (37%, w/v, 20 mmol) was added. The reaction mixture was stirred at 25 °C for 30 min to 1 h. After completion of the reaction, the product was extracted with ethyl acetate (20 mL, 2 times). The organic layers were combined and washed with 10% aqueous NaOH solution (30 mL, 2 times) followed by water (50 mL). The organic layer was dried over sodium sulfate and evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel using ethyl acetate/heptane as eluent to afford the desired product.

6.2.1. 3-(4-Fluoro-phenyl)-6-methyl-3,4-dihydro-2H-benzo[e][1,3]oxazine (**2i**)

Dark yellow solid (75%); mp 82.2 °C; υ_{max}(film, cm^{-1}): 2920, 2854, 1618, 1508, 1375, 1226 (asymmetric stretching, C–O–C), 1160, 1035 (symmetric stretching, C–O–C), 971, 948, 915, 821; ^1H NMR (300 MHz, $CDCl_3$): δ: 2.30 (3H, s, CH_3), 4.53 (2H, s, Ar–CH_2–N), 5.26 (2H, s, O–CH_2–N), 6.70 (1H, d, $J = 8.3$ Hz, ArH), 6.80 (1H, s, ArH), 6.90–6.97 (3H, m, ArH), 7.02–7.08 (2H, m, ArH); ^{13}C NMR (300 MHz, $CDCl_3$): δ: 20.6, 51.1, 80.2, 115.8, 115.9, 116.7, 120.2, 120.4, 120.5, 127.0, 128.6, 130.2, 145.0, 152.0, 156.6; MS (ESI): m/z 243 (M^+); Anal. Calcd for $C_{15}H_{14}FNO$ (243.3): C, 74.06; H, 5.80; N, 5.76. Found: C, 74.36; H, 5.49; N, 5.56.

6.2.2. 3-n-Butyl-6-chloro-3,4-dihydro-2H-benzo[e][1,3]oxazine (**2j**)

Brown viscous liquid (84%); bp 186 °C; υ_{max}(film, cm^{-1}) 2957, 2932, 2861, 1578, 1485, 1415, 1377, 1343, 1320, 1226 (asymmetric stretching, C–O–C), 1198, 1170, 1141, 1118, 1092, 1034 (symmetric stretching, C–O–C), 926, 873, 815, 634; ^1H NMR (300 MHz, $CDCl_3$): δ: 0.92 (3H, t, $J = 7.3$ Hz, CH_3), 1.26–1.38 (2H, m, CH_2), 1.48–1.55 (2H, m, CH_2), 2.70 (2H, t, $J = 7.4$ Hz, CH_2), 3.94 (2H, s, Ar–CH_2–N), 4.84 (2H, s, O–CH_2–N), 6.69 (1H, d, $J = 8.7$ Hz, ArH), 6.92 (1H, s, ArH), 7.05 (1H, d, $J = 8.7$ Hz, ArH); ^{13}C NMR (300 MHz, $CDCl_3$): δ: 13.8, 20.3, 29.7, 49.9, 51.1, 82.6, 117.7, 121.7, 124.9, 127.0, 127.2, 152.9; MS (ESI): m/z 225 (M^+).

6.2.3. 5,8-Dichloro-3-(2-chlorophenyl)-3,4-dihydro-2H-benzo[e][1,3]oxazine (**2n**)

Pale yellow viscous liquid (60%); bp 162 °C; υ_{max}(film, cm^{-1}): 2924, 2854, 1588, 1566, 1484, 1454, 1422, 1375, 1240 (asymmetric stretching, C–O–C), 1154, 1051 (symmetric stretching, C–O–C), 975, 937, 799, 758; ^1H NMR (300 MHz, $CDCl_3$): δ: 4.58 (2H, s, Ar–CH_2–N), 5.40 (2H, s, O–CH_2–N), 6.93 (1H, d, $J = 8.6$ Hz, ArH), 7.04–7.10 (1H, m, ArH), 7.15–7.24 (2H, m, ArH), 7.27–7.30 (1H, m, ArH), 7.42 (1H, dd, $J = 1.5$, 6.4 Hz, ArH); ^{13}C NMR (300 MHz, $CDCl_3$): δ: 49.6, 81.2, 111.9, 115.9, 118.6, 119.8, 121.8, 122.7, 125.5, 127.8, 129.5, 142.3, 145.7, 150.9; MS (ESI): m/z 314 (M^+).

6.2.4. 2-(4-Chlorophenyl)-2,3-dihydro-1H-naphtho[1,2-e]-[1,3]oxazine (**4b**)

Pale yellow solid (82%); mp 109.5 °C; υ_{max}(film, cm^{-1}): 3054, 2950, 2900, 1625, 1595, 1495, 1472, 1398, 1230 (asymmetric stretching, C–O–C), 1157, 1095, 1058 (symmetric stretching, C–O–C), 1005, 947, 812, 746; ^1H NMR (300 MHz, $CDCl_3$): δ: 4.89 (2H, s, Ar–CH_2–N), 5.41 (2H, s, O–CH_2–N), 7.00–7.06 (3H, m, ArH), 7.16–7.22 (2H, m, ArH), 7.36 (1H, dd, $J = 7.11$, 7.83 Hz, ArH), 7.50 (1H, dd, $J = 7.05$, 7.92 Hz, ArH), 7.64 (2H, dd, $J = 5.52$, 6.03 Hz, ArH), 7.75 (1H, d, $J = 8.04$ Hz, ArH); MS (ESI): m/z 295 (M^+); Anal. Calcd for $C_{18}H_{14}ClNO$ (295.7): C, 73.10; H, 4.77; N, 4.74. Found: C, 72.66; H, 5.01; N, 4.67.

6.2.5. 2-(4-Fluorophenyl)-2,3-dihydro-1H-naphtho[1,2-e]-[1,3]oxazine (**4d**)

Dark brown solid (77%); mp 56.9 °C; υ_{max}(film, cm^{-1}): 3061, 2921, 2851, 1625, 1598, 1509, 1471, 1401, 1230 (asymmetric stretching, C–O–C), 1157, 1100, 1058 (symmetric stretching, C–O–C), 1005, 945, 813, 746; ^1H NMR (300 MHz, $CDCl_3$): δ: 4.87 (2H, s, Ar–CH_2–N), 5.33 (2H, s, O–CH_2–N), 6.91 (2H, dd, $J = 8.52$, 8.82 Hz, ArH), 7.01–7.10 (3H, m, ArH), 7.36 (1H, dd, $J = 7.08$, 7.80 Hz, ArH), 7.49 (1H, dd, $J = 7.05$, 8.13 Hz, ArH), 7.63 (2H, d, $J = 8.9$ Hz, ArH), 7.76 (1H, d, $J = 8.1$ Hz, ArH); ^{13}C NMR (300 MHz, $CDCl_3$): δ: 48.7, 80.3, 112.3, 115.7, 115.9, 118.8, 120.7, 120.8, 120.9, 123.8, 126.8, 129.1, 129.2, 129.4, 131.2, 145.3, 152.3, 156.7; MS (ESI): m/z 280 (MH^+); Anal. Calcd for $C_{18}H_{14}FNO$ (279.3): C, 77.40; H, 5.05; N, 5.01. Found: C, 77.60; H, 4.85; N, 4.90.

6.2.6. 2-(2,4-Dichlorophenyl)-2,3-dihydro-1H-naphtho[1,2-e]-[1,3]oxazine (**4e**)

Pale yellow viscous liquid (78%); bp 237 °C; υ_{max}(film, cm^{-1}): 3064, 2932, 2890, 2852, 1625, 1599, 1513, 1481, 1436, 1382, 1311, 1265, 1230 (asymmetric stretching, C–O–C), 1159, 1108, 1051 (symmetric stretching, C–O–C), 1004, 948, 813, 747, 686; ^1H NMR (300 MHz, $CDCl_3$): δ: 4.86 (2H, s, Ar–CH_2–N), 5.32 (2H, s, O–CH_2–N), 7.02–7.08 (2H, m, ArH), 7.24 (1H, d, $J = 8.6$ Hz, ArH), 7.35–7.40 (2H, m, ArH), 7.50 (1H, dd, $J = 7.11$, 8.01 Hz, ArH), 7.61–7.69 (2H, m, ArH), 7.78 (1H, d, $J = 8.0$ Hz, ArH); ^{13}C NMR (300 MHz, $CDCl_3$): δ: 48.8, 80.1, 112.2, 118.6, 120.8, 123.7, 123.8, 126.8, 127.7, 128.5, 128.7, 129.1, 129.5, 129.6, 130.1, 130.9, 145.3, 151.8; MS (ESI): m/z 330 (M^+).

6.3. General method for preparation of 1,2-bis(3,4-dihydrobenzo[e][1,3]oxazin-3(4H)-yl)ethanes (**3a–d**)

To a mixture of the corresponding phenol (20 mmol) and ethylene diamine (10 mmol) in water (20 mL), formalin (37%, w/v, 40 mmol) was added. The reaction mixture was stirred at 25 °C for 30 min. After completion of the reaction, the product was extracted with ethyl acetate (20 mL, 2 times). The combined organic layer was washed

successively with 10% aqueous NaOH solution (30 mL, 2 times) and water (50 mL). The organic phase was dried over sodium sulfate and evaporated under reduced pressure. The crude solid isolated was washed with ethanol to obtain sufficiently pure product.

*6.3.1. 1,2-Bis(6-methyl-3,4-dihydro-2H-benzo[e][1,3]oxazin-3(4H)-yl)ethane (**3b**)*

White solid (76%); mp 110 °C; υ_{max}(film, cm^{-1}): 2921, 2857, 1619, 1588, 1501, 1322, 1227, (asymmetric stretching, C–O–C), 1121, 1032 (symmetric stretching, C–O–C), 937, 910, 815, 745; ^{1}H NMR (300 MHz, $CDCl_3$): δ: 2.24 (6H, s, $2CH_3$), 2.96 (4H, s, NCH_2CH_2N), 4.00 (4H, s, $2Ar–CH_2–N$), 4.87 (4H, s, $2O–CH_2–N$), 6.67 (2H, d, $J = 8.24$ Hz, ArH), 6.76 (2H, s, ArH), 6.92 (2H, d, $J = 7.90$ Hz, ArH); ^{13}C NMR (300 MHz, $CDCl_3$): δ: 20.6, 49.6, 50.4, 82.7, 116.2, 119.7, 128.6, 128.9, 129.8, 151.8; MS (ESI): m/z 323 (MH^+); Anal. Calcd for $C_{20}H_{24}N_2O_2.1/2H_2O$ (333.42): C, 72.04; H, 7.56; N, 8.40. Found: C, 72.24; H, 7.61; N, 8.35.

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