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Original article

Synthesis, antimicrobial and cytotoxicity study of 1,3-disubstituted-1*H*-naphtho[1,2-e][1,3]oxazines

Vikas Verma [a], Kuldeep Singh [a], Devinder Kumar [a,*], Thomas M. Klapötke [b], Jörg Stierstorfer [b], Balasubramanian Narasimhan [c], Asif Khurshid Qazi [d], Abid Hamid [d], Sundeep Jaglan [d]

[a] Department of Chemistry, Guru Jambheshwar University of Science & Technology, Hisar 125001, Haryana, India
[b] Department of Chemistry, Ludwig-Maximilian University of Munich, Butenandtstr, 5-13 (D), D-81377 Munich, Germany
[c] Faculty of Pharmaceutical Sciences, M D University, Rohtak 124001, Haryana, India
[d] CSIR-Indian Institute of Integrative Medicine, Jammu 180001, India

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ABSTRACT

A series of new 1,3-disubstituted-1*H*-naphtho[1,2-e][1,3]oxazines (**3** and **7**) was synthesized in good yields and the structure was determined with the help of NMR, 2D-NMR, HRMS studies and X-ray crystallography. These compounds were tested *in vitro* for their antibacterial activity against Gram-positive and Gram-negative bacteria and as well as for antifungal activity. The compounds **3c**, **3e**, **7a**, **7d** and **7k** showed significant antibacterial activity and **7l** showed moderate antifungal activity. The cytotoxicity of 1,3-disubstituted-1*H*-naphtho[1,2-e][1,3]oxazines showed that **3e** and **7e** are more effective against breast, lung and colon cell proliferation.

1. Introduction

There has been a considerable interest in the synthesis of molecules having 1,3-oxazine moiety due to large spectrum of pharmacological activities such as antitumour [1], antimicrobial [2], anti-HIV [3] and antimalarial agent [4]. In particular, naphthoxazine derivatives have exhibited therapeutic potential for the treatment of Parkinson's disease [5]. Further, the growing interest in the utilization of hypervalent iodine reagents [6,7] in the synthesis of new molecules and natural products promoted us to take up the synthesis of naphthoxazine utilizing particularly iodobenzene diacetate. Therefore, herein we report efficient synthesis of 1,3-disubstituted-1*H*-naphtho[1,2-e][1,3]oxazine by the oxidation of N-arylidene-1-(α-aminoarylbenzyl)-2-naphthol with iodobenzene diacetate, antimicrobial and cytotoxicity study. The structure of newly synthesized 1,3-disubstituted-1*H*-naphtho[1,2-e][1,3]oxazine was also confirmed by X-ray crystallography.

2. Results and discussion

2.1. Chemistry

The N-arylidene-1-(α-aminoarylbenzyl)-2-naphthol (**2**) for the synthesis of the target molecules was obtained by condensation of 2-naphthol (**1**) with two equivalents of aromatic aldehydes in presence of ammonia [8]. Reaction of **2** with iodobenzene diacetate furnished the naphthoxazine **3** instead of **4** in good yields. The formation of isomeric naphthoxazine (**4**) was not observed at all. The structure of compound **3** was determined with the help of IR, NMR (^{1}H and ^{13}C), 2D-NMR (COSY and HSQC) and mass spectroscopic data. The IR spectrum of **3b** was devoid of band due OH stretching and NMR spectrum exhibited the singlet at δ 6.32 assigned to C_1-H. The optical purity of the enantiomer of compound **3** due to C-2 chiral centre was also determined by chiral HPLC analysis which shows enantiomeric ratio of 49:51 (Scheme 1).

In order to prove the structure of **3**, the crystals were developed by vapour diffusion hexane into a saturated dichloromethane solution for compound **3b**. The X-ray crystallography of naphthoxazine **3** confirmed the formation of **3** rather than **4**. Bond lengths N1-C1 = 1.263(2) and N1-C2 = 1.470 (2) Å are typical of **3b**. The selected crystal structure data are presented in Tables 1 and 2.

* Corresponding author. Department of Chemistry, Guru Jambheshwar University of Science & Technology, TB#3, Room #102, Hisar 125001, Haryana, India. Fax: +91 1662 276240.
E-mail address: dk_ic@yahoo.com (D. Kumar).

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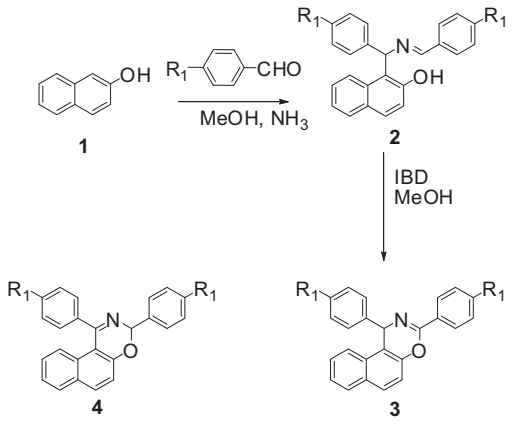


3 (a) R_1 = H; (b) R_1 = CH_3; (c) R_1 = OCH_3; (d) R_1 = Cl; (e) R_1 = F

Scheme 1. Synthesis of naphthoxazine.

Table 1
Selected bond distances (Å) and bond angles (°) of 1,3-di-*p*-tolyl-1*H*-naphtho[1,2-e][1,3]oxazine (**3b**).

O1-C1	1.383(2)	C1-C20	1.473(2)	C3-C4	1.360(3)
O1-C4	1.379(2)	C2-C3	1.513(2)	C3-C8	1.431(2)
N1-C1	1.263(2)	C2-H2	0.999(18)	C4-C5	1.409(3)
N1-C2	1.470(2)	C2-C13	1.526(3)		
C1-O1-C4	117.47(14)	C2-C13-C18	121.95(16)	C4-C3-C8	118.19(16)
C1-N1-C2	120.89(14)	C2-C3-C4	119.64(15)	C4-C5-H5	115.0(12)
C1-C20-C21	121.97(16)	C2-C3-C8	122.17(16)	C4-C5-C6	119.58(18)
C1-C20-C25	119.64(17)	C3-C4-C5	122.96(16)	O1-C1-N1	126.37(16)
N1-C1-C20	122.37(16)	C3-C2-C13	114.75(14)	O1-C1-C20	111.16(15)
N1-C2-C3	113.33(15)	C3-C2-H2	109.1(10)	O1-C4-C3	121.97(15)
N1-C2-C13	106.84(14)	C3-C2-H2	109.1(10)	O1-C4-C5	115.07(16)
N1-C2-H2	107.2(10)	C3-C8-C7	119.61(16)	C13-C2-H2	105.1(10)
C2-C13-C14	119.82(15)	C3-C8-C9	122.49(17)		

Table 2
Crystallographic data of 1,3-di-*p*-tolyl-1*H*-naphtho[1,2-e][1,3]oxazine (**3b**).

Formula	$C_{26}H_{21}NO$
Formula weight	363.44
Temperature (K)	173
Radiation [Å]	MoKa 0.71073
Crystal system	Monoclinic
Space group	P21/c(No. 14)
a [Å]	5.6617(6)
b [Å]	20.311(2)
c [Å]	16.5089(15)
alpha [°]	90
beta [°]	94.551(9)
gamma [°]	90
V [Å^3]	1892.5(3)
Z	4
D(calc) [g/cm^3]	1.276
F(000)	768
Theta range for data collection min–max [°]	4.2–25.8
Nref	3591
Npar	337
Mu(MoKa) [/mm]	0.077
Crystal size [mm]	0.14 × 0.18 × 0.39
Tot., uniq. data, *R*(int)	7182, 3591, 0.029
Dataset	−6: 5; −24: 16; −20: 16
Observed data [$I > 0.0$ sigma(I)]	2463
R, *wR2*, *S*	0.0484, 0.1409, 1.04
$w = 1/[s^2(F_o^2) + (0.0699P)^2]$	where $P = (F_o^2 + 2F_c^2)/3$
Max. and av. shift/error	0.03, 0.00
Min. and max. resd. dens. [e/Å^3]	−0.20, 0.21

Molecules are packed by aromatic ring attractive interactions along the crystallographic *b* and *c* axes (Fig. 1).

In order to generalize the formation of the naphthoxazine by the oxidation of iodobenzene diacetate, other *N*-arylidene-1-(α-aminoarylbenzyl)-2-naphthols (**6**) were prepared. Acidic hydrolysis of **2** resulted in the formation of aminonaphthols (**5**, Betti base) [9] which on condensation with equivalent amount of aromatic aldehydes gave compound **6**. The compound **6** was treated with iodobenzene diacetate to produce naphthoxazine (**7**) in good yields. The structure of **7** was determined by IR, NMR (^{1}H and ^{13}C) and mass spectral data which were in good agreement with the structure of naphthoxazine of the type **3** (Scheme 2).

The NMR spectra of **2** and **6** also showed that in $CDCl_3$ solution the compound exhibited equilibrium with two diastereomeric *trans*- and *cis*-oxazine structures (B and C) formed by tautomeric N,O-proton transfer (Scheme 3) besides the open-chain tautomer

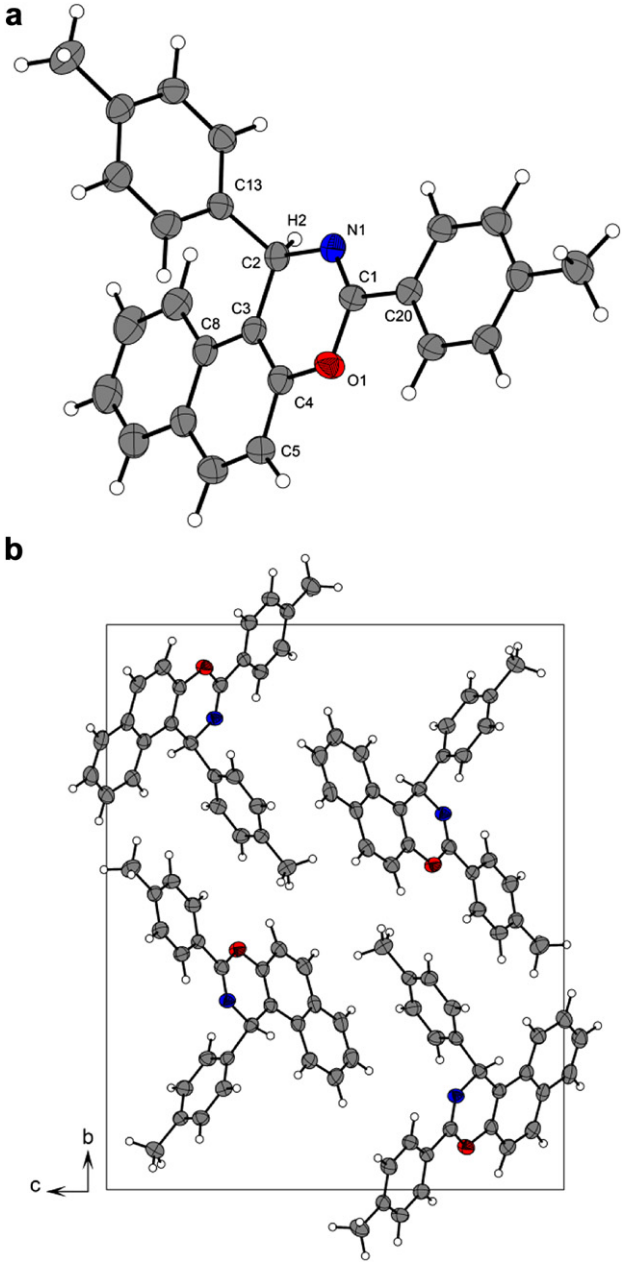
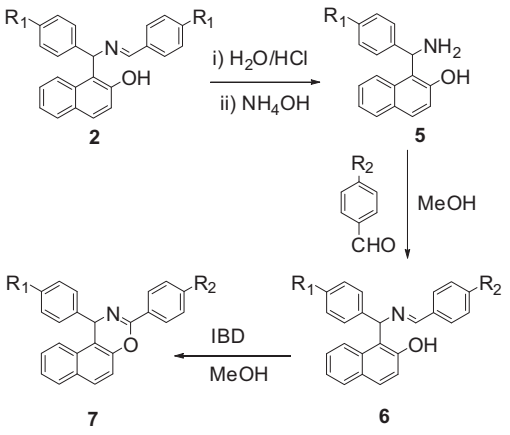


Fig. 1. (a) Molecular structure, (b) packing diagram of **3b.**



Scheme 2. Synthesis of naphthoxazine.

[10]. The major ring forms in all tautomeric equilibria contain the 1,3-diaryl substituent in the *trans* position. The proportions of the chain and distereomeric ring forms of the tautomeric equilibria were determined by integration of well separated O—CHAr—N (ring) and N═CHAr (chain) proton singlets or doublets in the NMR spectra. The azomethine double bond was having E configuration.

The probable mechanism consists of attack of iodobenzene diacetate on **2** or **6** resulting in the formation of intermediate (**8**) that may undergo reductive elimination of iodobenzene to produce either **3/7** or **4**. However, the isomeric compound **4** was not formed probably due to severe steric interaction between two aromatic rings as it will remain in the plane rather there was formation of the compounds **3** as one of the aromatic ring disposed out of plane to overcome this steric interaction in intermediate **8** (Scheme 4).

2.2. Biological section

2.2.1. Antimicrobial & cytotoxic activity

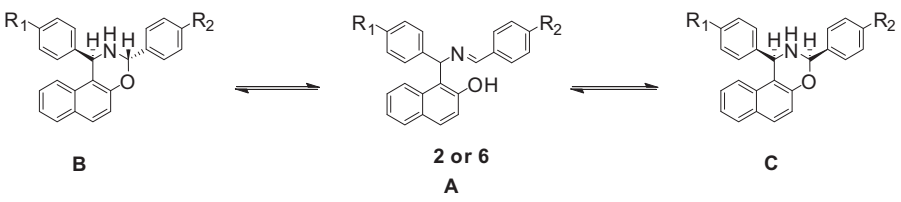
The newly synthesized 1,3-disubstituted-1*H*-naphtho[1,2-e][1,3]oxazines were evaluated for their *in vitro* antibacterial activity against Gram-positive *Bacillus subtilis* [MTCC 2063], *Staphylococcus aureus* [MTCC 2901] and Gram-negative *Escherichia coli* [MTCC 1652] and *in vitro* antifungal activity against *Candida albicans* [MTCC 227] and *Aspergillus niger* [MTCC 8184]. Double strength nutrient broth-I.P. and Sabouraud dextrose broth-I.P. [11] were employed for bacterial and fungal growth respectively. Minimum inhibitory concentrations [MIC] were determined by means of standard serial dilution [12] and the pMIC values in μM are presented in Table 3. All the compounds exhibited appreciable *in vitro* activity against the tested strains compared to reference ciprofloxacin and fluconazole in case of antibacterial and antifungal activity, respectively.

In general, the synthesized compounds were more active against the Gram-negative *E. coli* having $pMIC_{ec}$ value more than 2.07. Among the synthesized compounds **7d** and **7e** were found to be more effective against *E. coli*. Compounds **3c** was found to be more active against *B. subtilis* ($pMIC_{bs} = 2.10$). In case of antibacterial activity against *S. aureus* compound **3e** was found to be more active one. In general the synthesized compounds were moderately active against the fungal species under test and Compound **7l** was found to be the effective one against the *C. albicans* ($pMIC_{ca} = 1.83$) and *A. niger* ($pMIC_{an} = 1.53$) among the synthesized compounds.

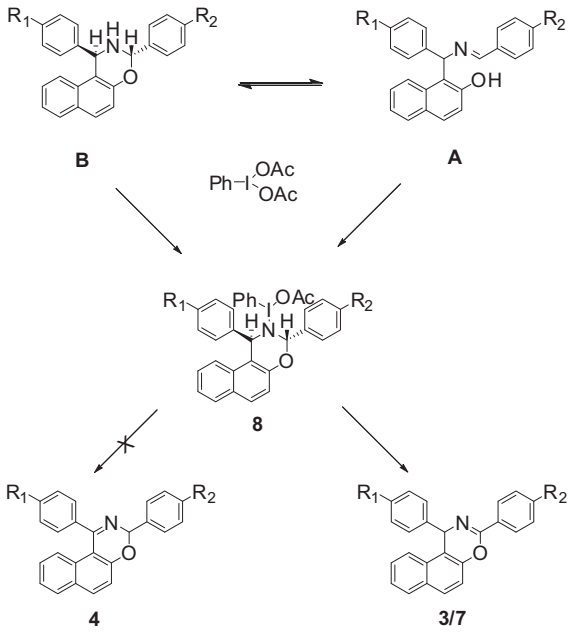
The cytotoxic assay of 1,3-disubstituted-1*H*-naphtho[1,2-e][1,3]oxazines based on MTT was performed on panel of human cancer cell lines (MCF-7, A549, HCT-116 and PC-3). In the present study, **3e** and **7e** produced concentration dependent inhibition of cell proliferation on MCF-7, A549, and HCT-116 cancer cell lines. These results depicted that both **3e** and **7e** showed more effectiveness against breast, lung and colon cell proliferation as reflected by relative percentage growth inhibition values (Table 4).

2.2.2. Structure activity relationship (SAR) studies

1. Compound **3e** having electron withdrawing fluoro group on the phenyl nucleus at position-3 of 1*H*-naphtho[1,2-e][1,3]oxazine was emerged as the most active antibacterial compound against the *S. aureus*. The role of electron withdrawing group in improving antimicrobial activities is supported by the studies of Sharma et al. [13].
2. In case of antibacterial activity against *B. subtilis*, compound **3c** having electron donating *p*-methoxy group on the phenyl nucleus at position-3 of 1*H*-naphtho[1,2-e][1,3]oxazine was found to be the most active one. The role of electron donating methoxy group in improving antibacterial activity against *B. subtilis* is similar to the results obtained by Sharma et al. [14].
3. In case of antibacterial activity against *E. coli*, compounds **7d**, **7e** were found to the most active ones. Here, both **7d**, **7e** requires the presence of electron withdrawing halo groups [Cl-**7d** and F-**7e**] at *para* position of 3-phenyl nucleus and no substitution at the *para* position of 1-phenyl nucleus of 1*H*-naphtho[1,2-e][1,3]oxazine. This indicated a fact that the presence of hydrogen at *para* position of 1-phenyl nucleus is essential for the hydrogen bonding of 1*H*-naphtho[1,2-e][1,3]oxazine at the target site of Gram-negative *E. coli*. This result is in contrast to the antibacterial activity requirements against Gram-positive bacteria where the presence of electron withdrawing/electron donating substituent is essential on the phenyl nucleus at position-3.
4. In case of antifungal activity of 1,3-disubstituted-1*H*-naphtho[1,2-e][1,3]oxazine against *C. albicans* and *A. niger* a totally different structural requirement was observed. The compound, 3-(4-bromophenyl)-1-*p*-tolyl-1*H*-naphtho[1,2-e][1,3] oxazine (**7l**) was emerged as the most active compound against the



Scheme 3. Ring-chain tautomerism of Schiff base.



Scheme 4. Probable mechanism for the formation of naphthoxazine.

both the fungal strains among the synthesized compounds. According to this the presence of electron withdrawing *p*-bromo group (3-phenyl nucleus) and electron donating *p*-methyl group (1-phenyl nucleus) of 1*H*-naphtho[1,2-e][1,3] oxazine is required for a compound to be effective against fungal species.

5. The aforementioned antibacterial and antifungal results indicated a fact that different structural requirements are essential for a compound to be active against different microbial targets. This is similar to the results obtained by Sortino et al. [15].
6. The cytotoxicity screening results indicated that the presence of electron withdrawing fluoro group improved the cytotoxicity activity of synthesized 1,3-disubstituted-1*H*-naphtho[1,2-e][1,3]oxazines (**3e** and **7e**). It is important to note a fact here that the presence of halo substituent at 1-phenyl nucleus of 1*H*-naphtho[1,2-e][1,3]oxazine is not essential for the cytotoxic activity of the synthesized compounds as evidenced by the activity of compound **7e** [3-(4-fluorophenyl)-1-phenyl substituent] which is having cytotoxic activity equal to that of compound **3e** [1,3-bis(*p*-fluorophenyl) substituent].

Table 3
In vitro antimicrobial activity of synthesized 1,3-disubstituted-1*H*-naphtho[1,2-e][1,3]oxazine derivatives.

Comp.	$pMIC_{sa}$	$pMIC_{bs}$	$pMIC_{ec}$	$pMIC_{ca}$	$pMIC_{an}$
3a	1.43	2.03	2.33	1.73	1.43
3b	1.46	2.07	1.46	1.76	1.46
3c	1.50	2.10	2.10	1.80	1.50
3d	1.51	1.81	2.11	1.81	1.51
3e	2.07	1.77	1.47	1.77	1.47
7a	1.77	1.77	2.37	1.77	1.47
7b	1.75	1.45	2.05	1.75	1.45
7c	1.78	1.78	1.78	1.78	1.48
7d	1.77	1.77	2.37	1.77	1.47
7e	1.75	2.05	2.35	1.75	1.45
7f	1.52	1.82	1.52	1.82	1.52
7g	1.45	1.75	2.35	1.75	1.45
7h	1.78	1.78	2.08	1.78	1.48
7i	1.80	1.80	1.80	1.80	1.50
7j	1.49	1.79	0.88	1.79	1.49
7k	1.47	2.07	2.07	1.77	1.47
7l	1.83	1.83	1.83	1.83	1.53
Std.	2.61[a]	2.61[a]	2.61[a]	2.64[b]	2.64[b]

[a] Ciprofloxacin.
[b] Fluconazole.

Table 4
Cytotoxicity of 1,3-disubstituted-1*H*-naphtho[1,2-e][1,3]oxazine against cancer cell lines.

Tissue type		Breast	Lung	Colon	Prostrate
Cell type		MCF-7	A549	HCT-116	PC-3
Entry	Conc(μM)	% Growth inhibition			
3a	50	37	36	22	16
	10	26	19	5	1
3b	50	37	36	24	5
	10	30	17	19	1
3c	50	26	35	29	19
	10	22	22	27	17
3d	50	38	54	10	2
	10	16	28	2	1
3e	50	50	50	51	29
	10	36	20	5	22
7a	50	42	43	14	23
	10	37	19	8	19
7b	50	46	43	38	20
	10	36	19	8	14
7c	50	42	43	14	8.
	10	34	17	4	6
7d	50	39	38	29	19
	10	27	17	3	9
7e	50	51	50	56	39
	10	29	18	12	14
7f	50	39	40	29	11
	10	27	23	6	4
7g	50	32	37	8	3
	10	17	17	2	1
7h	50	39	36	14	10.
	10	36	15	7	1
7i	50	40	41	21	12
	10	35	13	7	8
7j	50	37	28	6	13
	10	21	16	3	8
7k	50	44	44	23	19
	10	20	16	18	5
7l	50	33	32	10	2
	10	26	13	2	0
5-Fluorouracil	20	–	79	78	–
Mitomycin-C	1	82	70	–	70

The aforementioned findings are summarized in Fig. 2.

3. Conclusion

In summary, we have synthesized 1,3-disubstituted-1*H*-naphtho[1,2-e][1,3]oxazine (**3** and **7**) in good yields from Schiff base utilizing iodobenzene diacetate as reagent. The crystal structure was determined and it established the formation of 1,3-disubstituted-1*H*-naphtho[1,2-e][1,3]oxazine instead of 1,3-disubstituted-3*H*-naphtho[1,3-e][1,3]oxazine. All the compounds exhibited appreciable *in vitro* activity against the tested microbial strains. The antibacterial activity of compounds **3a**, **3c**, **3d**, **7a**, **7d**, **7e**, **7g** and **7h** were found to be more active against *E. coli*; compounds **3b**, **3c** and **7k** were found to be more active against *B. subtilis* and compound **3e** was found to be more active against *S. aureus*. Further, the antifungal activity of all the compounds was found to be moderate against *C. albicans* and *A. niger*; compound **7l** was found to be more active. The cytotoxic assay (MTT) of 1,3-disubstituted-1*H*-naphtho[1,2-e][1,3]oxazines indicated that compounds, **3e** and **7e** showed more effectiveness against

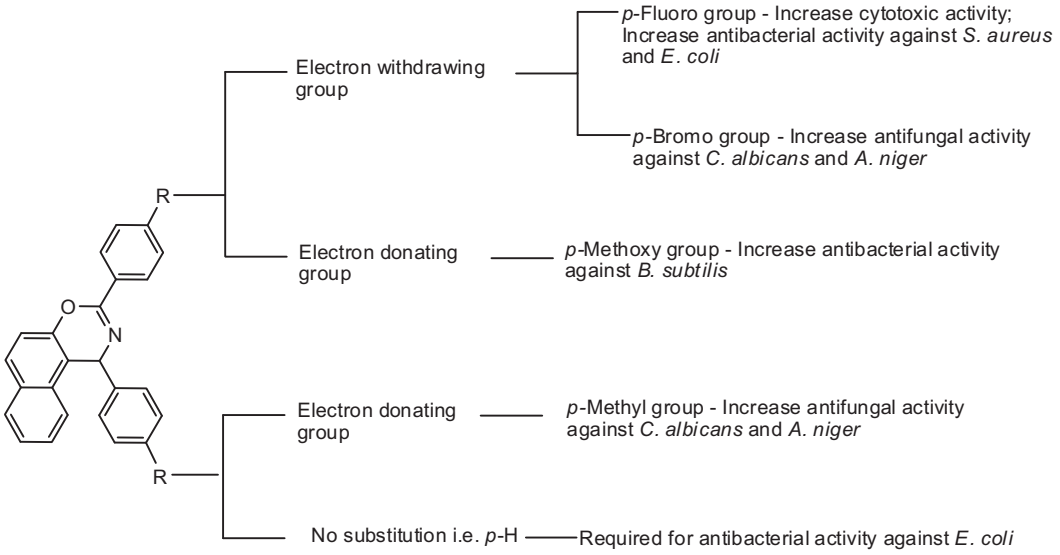


Fig. 2. Structural requirements for antimicrobial and cytotoxic activity of 1,3-disubstituted-1*H*-naphtho[1,2-e][1,3]oxazines (**3** and **7**).

breast, lung and colon cell proliferation as reflected by relative percentage growth inhibition values.

4. Experimental

4.1. General

Melting points were determined in open capillaries and are uncorrected. FTIR spectra were obtained in KBr on IR Affinity-I (Shimadzu) spectrophotometer and are reported in cm^{-1}. ^{1}H, ^{13}C NMR and 2D-NMR, COSY (correlation spectroscopy), HSQC (heteronuclear single-quantum coherence) and HMBC (heteronuclear multiple bond correlation) spectra were scanned on a Bruker Avance II NMR spectrometer operating at 400 MHz in $CDCl_3$ and are expressed as ppm with respect to TMS. X-ray crystallographic data was obtained on a Sapphire CCD diffractometer. The mass spectra were obtained on AB SCIEX 5600 system.

4.2. General procedure for the synthesis of naphthoxazine (**3**)

The compound **2** (prepared by the reaction of 2-naphthol, ammonia and appropriate benzaldehyde) [8] (0.01 mol) was dissolved by warming in methanol (15 mL) and iodobenzene diacetate (0.02 mol) was added into it. The reaction mixture was stirred for 2–3 h at 55–60 °C. The reaction was monitored by TLC using toluene as eluant. The solid so formed was filtered and washed with cold methanol followed by ether and crystallized from dichloromethane.

4.2.1. 1,3-Diphenyl-1H-naphtho[1,2-e][1,3]oxazine (**3a**)

mp 170–171 °C; yield: 73%; IR(KBr)ν: 3061, 3026, 2974, 2933, 1656, 1625, 1597 cm^{-1}; NMR δ_H (400 MHz, $CDCl_3$): 8.15 (d, $J = 7.08$ Hz, 2H, C_3-C_6H_5; ortho protons), 7.86–7.82 (m, 2H, C_7-H, C_9-H). 7.74 (d, $J = 7.88$ Hz, 1H, C_5-H), 7.50–7.38 (m, 8H, ArH), 7.31–7.19 (m, 3H, ArH), 6.32 (s, 1H, C_1-H); NMR δ_C (100 MHz, $CDCl_3$): 151.39, 146.85, 143.54, 132.10, 131.43, 131.06, 130.16, 129.35, 128.73, 128.56, 128.18, 127.86, 127.62, 127.39, 127.01, 124.73, 123.07, 116.58, 114.10, 57.02; HRMS: m/z (M^+) calcd. for $C_{24}H_{17}NO$: 335.1310, found: 336.1397 (M + H).

4.2.2. 1,3-Di-p-tolyl-1H-naphtho[1,2-e][1,3]oxazine (**3b**)

mp 172–173 °C; yield: 80%; IR(KBr)ν: 3039, 3010, 2916, 2872, 1672, 1606, 1512 cm^{-1}; NMR δ_H (400 MHz, $CDCl_3$): 7.92 (d, $J = 8.0$ Hz, 2H, C_3-$C_6H_4CH_3$; ortho protons), 7.73–7.69 (m, 2H, C_7-H, C_9-H). 7.64 (d, $J = 7.96$ Hz, 1H, C_5-H), 7.33–7.25 (m, 3H, C_6-H, C_8-H and C_{10}-H), 7.19 (d, $J = 8.2$ Hz, 2H, C_1-$C_6H_4CH_3$; ortho protons), 7.12 (d, $J = 7.72$ Hz, 2H, C_3-$C_6H_4CH_3$; meta protons), 6.97 (d, $J = 7.72$ Hz, 2H, C_1-$C_6H_4CH_3$; meta protons), 6.32 (s, 1H, C_1-H), 2.29 (s, 3H, $C_{4''}$-CH_3), 2.16 (s, 3H, $C_{4'}$-CH_3); NMR δ_C (100 MHz, $CDCl_3$): 151.42, 146.83, 141.30, 140.83, 136.97, 131.38, 130.20, 129.40 ($C_{3'}$, $C_{5'}$), 129.37, 129.21, 129.17, 128.97, 128.95, 128.87 ($C_{3''}$, $C_{5''}$), 128.82, 128.53, 127.72 ($C_{2''}$, $C_{6''}$), 127.59 ($C_{2'}$, $C_{6'}$), 126.94, 126.05 (C_{10}), 124.64 (C_8), 123.06 (C_6), 116.62 (C_5), 114.43, 56.63, 21.46 ($C_{4'}$), 21.05 ($C_{4''}$); HRMS: m/z (M^+) calcd. for $C_{26}H_{21}NO$: 363.1623, found: 364.1745 (M + H).

4.2.3. 1,3-bis(4-Methoxyphenyl)-1H-naphtho[1,2-e][1,3]oxazine (**3c**)

mp 158–159 °C; yield: 76%; IR(KBr)ν: 3062, 3003, 2958, 2937, 2902, 2839, 1668, 1606, 1504 cm^{-1}; NMR δ_H (400 MHz, $CDCl_3$): 8.08 (d, $J = 8.72$ Hz, 2H, C_3-$C_6H_4OCH_3$; ortho protons), 7.83–7.80 (m, 2H, C_7-H, C_9-H). 7.73 (d, $J = 8.04$ Hz, 1H, C_5-H), 7.44–7.25 (m, 5H, C_6-H, C_8-H, C_{10}-H and C_1-$C_6H_4OCH_3$; ortho protons), 6.93 (d, $J = 8.72$ Hz, 2H, C_3-$C_6H_4OCH_3$; meta protons), 6.80 (d, $J = 8.56$ Hz, 2H, C_1-$C_6H_4OCH_3$; meta protons), 6.39 (s, 1H, C_1-H), 3.84 (s, 3H, $C_{4''}$-OCH_3), 3.72 (s, 3H, $C_{4'}$-OCH_3); NMR δ_C (100 MHz, $CDCl_3$): 161.92, 158.72, 151.06, 146.82, 136.20, 131.34, 130.16, 129.27, 129.13, 128.88, 128.52, 126.90, 124.61, 123.05, 116.59, 114.54, 114.05, 113.48, 56.16, 55.31, 55.13; HRMS: m/z (M^+) calcd. for $C_{26}H_{21}NO_3$: 395.1521, found: 396.1651 (M + H).

4.2.4. 1,3-bis(4-Chorophenyl)-1H-naphtho[1,2-e][1,3]oxazine (**3d**)

mp 202–203 °C; yield: 78%; IR(KBr)ν: 3061, 3024, 2879, 1672, 1624, 1595 cm^{-1}; NMR δ_H (400 MHz, $CDCl_3$): 8.05 (d, $J = 8.44$ Hz, 2H, C_3-C_6H_4Cl; ortho protons), 7.86–7.82 (m, 2H, C_7-H, C_9-H). 7.63–7.61 (m, 1H, C_5-H), 7.44–7.34 (m, 5H, C_6-H, C_8-H, C_{10}-H and C_1-C_6H_4Cl; ortho protons), 7.29 (d, $J = 8.44$ Hz, 2H, C_3-C_6H_4Cl; meta protons), 7.24 (d, $J = 8.28$ Hz, 2H, C_1-C_6H_4Cl; meta protons), 6.41 (s, 1H, C_1-H); NMR δ_C (100 MHz, $CDCl_3$): 150.65, 146.66, 141.82, 137.40, 133.27, 131.46, 130.35, 129.90, 129.71, 129.21, 128.97, 128.94, 128.68, 128.48, 127.21, 124.97, 122.89, 116.45, 113.24, 56.30; HRMS: m/z (M^+) calcd. for $C_{24}H_{15}Cl_2NO$: 403.0531, found: 404.0694 (M + H).

*4.2.5. 1,3-bis(4-Fluorophenyl)-1H-naphtho[1,2-e][1,3]oxazine (**3e**)*

mp 138–139 °C; yield: 71%; IR(KBr)ν: 3062, 2929, 1666, 1600 cm^{-1}; NMR δ_H (400 MHz, $CDCl_3$): 8.12 (dd, J = 8.36, 5.6 Hz, 2H, C_3-C_6H_4F(ortho protons)), 7.86–7.82 (m, 2H, C_7-H, C_9-H), 7.65 (d, J = 8.12 Hz, 1H, C_5-H), 7.44–7.32 (m, 5H, C_6-H, C_8-H, C_{10}-H and C_1-C_6H_4F; ortho protons), 7.10 (t, J = 8.6, 8.56 Hz, 2H, C_3-C_6H_4F; meta protons), 6.96 (t, J = 8.56 Hz, 2H, C_1-C_6H_4F; meta protons), 6.41 (s, 1H, C_1-H); NMR δ_C (100 MHz, $CDCl_3$): 164.70 (d, J = 50.15 Hz), 161.97 (d, J = 44.42 Hz), 150.54, 146.70, 139.32 (d, J = 3.12 Hz), 131.45, 129.96, 129.80 (d, J = 8.74 Hz), 129.59, 129.44 (d, J = 11.30 Hz), 128.66, 128.09 (d, J = 3.01 Hz), 127.15, 124.90, 122.94, 116.48, 115.64 (d, J = 21.33 Hz), 115.27 (d, J = 21.79 Hz), 113.64, 56.17; HRMS: m/z (M^+) calcd. for $C_{24}H_{15}F_2NO$: 371.1122, found: 372.1276 (M + H).

*4.3. General procedure for the synthesis of naphthoxazine (**7**)*

Acidic hydrolysis of appropriate N-arylidene-1-(α-aminophenyl/*p*-tolyl)-2-naphthols (**2**) resulted in the formation of 1-(α-aminophenyl/*p*-tolylbenzyl)-2-naphthols (**5**, Betti base) which on condensation with equivalent amount of aromatic aldehydes gave N-arylidene-1-(α-aminophenyl/*p*-tolylbenzyl)-2-naphthols (**6**) [9]. The compound **6** (0.01 mol) was dissolved by warming in methanol (15 mL) and iodobenzene diacetate (0.02 mol) was added into it. The reaction mixture was stirred for 2–3 h at 55–60 °C. The reaction was monitored by TLC using toluene as eluant. The solid so formed was filtered and washed with cold methanol followed by ether and crystallized from dichloromethane.

*4.3.1. 3-(4-Methoxyphenyl)-1-phenyl-1H-naphtho[1,2-e][1,3] oxazine (**7a**)*

mp 214–215 °C; yield: 75%; IR(KBr)ν: 3068, 3020, 2949, 2924, 2835, 1664, 1602, 1510 cm^{-1}; NMR δ_H (400 MHz, $CDCl_3$): 8.06 (d, J = 8.80 Hz, 2H, C_3-$C_6H_4OCH_3$; ortho protons), 7.84–7.80 (m, 2H, C_7-H, C_9-H). 7.72 (d, J = 7.88 Hz, 1H, C_5-H), 7.42–7.35 (m, 5H, C_6-H, C_8-H, C_{10}-H and C_1-C_6H_5; ortho protons), 7.26–7.25 (m, 2H, C_3-C_6H_5; meta protons), 7.20–7.16 (m, 1H, C_1-C_6H_5; para protons), 6.92 (d, J = 8.76 Hz, 2H, C_1-$C_6H_4OCH_3$; meta protons), 6.42 (s, 1H, C_1-H), 3.84 (s, 3H, $C_{4''}$-OCH_3); NMR δ_C (100 MHz, $CDCl_3$): 162.01, 151.33, 146.92, 143.73, 131.39, 130.22, 129.34, 129.27, 128.71, 128.56, 127.84, 127.33, 126.98, 124.68, 123.07, 116.62, 114.34, 113.54, 56.89, 55.36; HRMS: m/z (M^+) calcd. for $C_{25}H_{19}NO_2$: 365.1416, found: 366.1597 (M + H).

*4.3.2. 1-Phenyl-3-(p-tolyl)-1H-naphtho[1,2-e][1,3]oxazine (**7b**)*

mp 194–195 °C; yield: 72%; IR(KBr)ν: 3064, 3028, 3005, 2918, 2893, 1670, 1606, 1514 cm^{-1}; NMR δ_H (400 MHz, $CDCl_3$): 8.03 (d, J = 8.04 Hz, 2H, C_3-$C_6H_4CH_3$(ortho protons)), 7.85–7.79 (m, 2H, C_7-H, C_9-H). 7.74 (d, J = 7.96 Hz, 1H, C_5-H), 7.44–7.37 (m, 5H, C_6-H, C_8-H, C_{10}-H and C_1-$C_6H_4CH_3$; ortho protons), 7.30–7.18 (m, 5H, ArH), 6.46 (s, 1H, C_1-H), 2.40 (s, 3H, $C_{4'}$-CH_3); NMR δ_C (100 MHz, $CDCl_3$): 151.53, 146.85, 143.62, 141.38, 131.37, 130.15, 129.27, 128.90, 128.66, 128.54, 127.84, 127.58, 127.34, 126.96, 124.67, 123.04, 116.61, 114.21, 56.93, 21.47; HRMS: m/z (M^+) calcd. for $C_{25}H_{19}NO$: 349.1467, found: 350.1647 (M + H).

*4.3.3. 3-(4-Nitrophenyl)-1-phenyl-1H-naphtho[1,2-e][1,3]oxazine (**7c**)*

mp 208–209 °C; yield: 72%; IR(KBr)ν: 3059, 3030, 2864, 1674, 1598, 1519, 1346 cm^{-1}; NMR δ_H (400 MHz, $CDCl_3$): 8.30–8.20 (m, 4H, C_3-$C_6H_4NO_2$), 7.88–7.82 (m, 2H, C_7-H, C_9-H). 7.69–7.67 (m, 1H, C_5-H), 7.44–7.39 (m, 5H, C_6-H, C_8-H, C_{10}-H and C_1-C_6H_5; ortho protons), 7.32–7.21 (m, 3H, C_1-C_6H_5), 6.49 (s, 1H, C_1-H); NMR δ_C (100 MHz, $CDCl_3$): 149.55, 149.38, 146.46, 142.94, 137.84, 131.62, 129.97, 129.71, 128.88, 128.64, 128.57, 127.89, 127.70, 127.23, 125.07, 123.32, 123.12, 116.26, 113.45, 57.31; HRMS: m/z (M^+) calcd. for $C_{24}H_{16}N_2O_3$: 380.1161, found: 381.1334 (M + H).

*4.3.4. 3-(4-Chlorophenyl)-1-phenyl-1H-naphtho[1,2-e][1,3]oxazine (**7d**)*

mp 197–198 °C; yield: 82%; IR(KBr)ν: 3066, 3028, 2899, 1674, 1593, 1496 cm^{-1}; NMR δ_H (400 MHz, $CDCl_3$): 8.07 (d, J = 8.44 Hz, 2H, C_3-C_6H_4Cl; ortho protons), 7.85–7.79 (m, 2H, C_7-H, C_9-H). 7.70 (d, J = 7.56 Hz, 1H, C_5-H), 7.44–7.19 (m, 10H, ArH), 6.45 (s, 1H, C_1-H); NMR δ_C (100 MHz, $CDCl_3$): 150.49, 146.66, 143.66, 137.63, 131.46, 130.55, 130.08, 129.44, 128.97, 128.77, 128.57, 128.42, 127.84, 127.48, 127.07, 124.82, 123.08, 116.43, 113.89, 57.03; HRMS: m/z (M^+) calcd. for $C_{24}H_{16}ClNO$: 369.0920, found: 370.1177 (M + H).

*4.3.5. 3-(4-Fluorophenyl)-1-phenyl-1H-naphtho[1,2-e][1,3]oxazine (**7e**)*

mp 182–183 °C; yield: 74%; IR(KBr)ν: 3070, 3026, 2931, 1666, 1595, 1506 cm^{-1}; NMR δ_H (400 MHz, $CDCl_3$): 8.13 (dd, J = 8.32, 5.8 Hz, 2H, C_3-C_6H_4F; ortho protons), 7.85–7.81 (m, 2H, C_7-H, C_9-H). 7.71 (d, J = 7.60 Hz, 1H, C_5-H), 7.48–7.35 (m, 5H, C_6-H, C_8-H, C_{10}-H and C_1-C_6H_5; ortho protons), 7.29 (t, J = 7.68, 7.56 Hz, 2H, C_3-C_6H_4F; meta protons), 7.25–7.19 (m, 1H, ArH), 7.10 (t, J = 8.56 Hz, 2H, C_1-C_6H_4F; meta protons), 6.48 (s, 1H, C_1-H); NMR δ_C (100 MHz, $CDCl_3$): 165.91, 163.42, 150.56, 146.69, 143.42, 130.09, 129.84 (d, J = 3.16 Hz), 129.42, 128.77, 128.57, 128.20 (d, J = 3.97 Hz), 127.83, 127.46, 127.06, 124.81, 123.07, 116.56, 115.55 (d, J = 3.16 Hz), 115.10, 113.14, 56.96; HRMS: m/z (M^+) calcd. for $C_{24}H_{16}FNO$: 353.1216, found: 354.1467 (M + H).

*4.3.6. 3-(4-Fluorophenyl)-1-phenyl-1H-naphtho[1,2-e][1,3]oxazine (**7f**)*

mp 201–202 °C; yield: 77%; IR(KBr)ν: 3066, 3030, 2899, 2868, 1674, 1624, 1591, 1512 cm^{-1}; NMR δ_H (400 MHz, $CDCl_3$): 7.99 (d, J = 8.44 Hz, 2H, C_3-C_6H_4Br; ortho protons), 7.85–7.80 (m, 2H, C_7-H, C_9-H), 7.69 (d, J = 7.36 Hz, 1H, C_5-H), 7.55 (d, J = 8.40 Hz, 2H, C_3-C_6H_4Br; meta protons), 7.44–7.18 (m, 8H, ArH), 6.44 (s, 1H, C_1-H); NMR δ_C (100 MHz, $CDCl_3$): 150.62, 146.66, 143.32, 142.54, 131.41, 131.38, 129.47, 129.19, 128.79, 128.59, 127.99, 127.85, 127.50, 127.41, 126.66, 125.77, 124.85, 123.09, 116.45, 57.05; HRMS: m/z (M^+) calcd. for $C_{24}H_{16}BrNO$: 413.0415, found: 414.0718 (M + H).

*4.3.7. 3-(Phenyl)-1-p-tolyl-1H-naphtho[1,2-e][1,3]oxazine (**7g**)*

mp 175–176 °C; yield: 76%; IR(KBr)ν: 3039, 3010, 2916, 2872, 1672, 1606, 1512 cm^{-1}; NMR δ_H (400 MHz, $CDCl_3$): 8.15 (d, J = 7.28 Hz, 2H, C_3-C_6H_5; ortho protons), 7.85–7.81 (m, 2H, C_7-H, C_9-H). 7.75 (d, J = 7.96 Hz, 1H, C_5-H), 7.50–7.37 (m, 6H, C_6-H, C_8-H, C_{10}-H and C_3-C_6H_5), 7.30 (d, J = 7.76 Hz, 2H, C_1-$C_6H_4CH_3$; ortho protons), 7.09 (d, J = 7.68 Hz, 2H, C_1-$C_6H_4CH_3$; meta protons), 6.45 (s, 1H, C_1-H), 2.27 (s, 3H, $C_{4'}$-CH_3); NMR δ_C (100 MHz, $CDCl_3$): 151.32, 146.76, 140.69, 137.04, 132.11, 131.41, 131.01, 130.15, 129.42, 129.24, 128.54, 128.15, 127.73, 127.61, 127.30, 126.98, 124.70, 123.06, 116.56, 114.28, 56.68, 21.06; HRMS: m/z (M^+) calcd. for $C_{25}H_{19}NO$: 349.1467, found: 350.1726 (M + H).

*4.3.8. 3-(4-Methoxyphenyl)-1-p-tolyl-1H-naphtho[1,2-e][1,3] oxazine (**7h**)*

mp 176–177 °C; yield: 81%; IR(KBr)ν: 3068, 3020, 2949, 2924, 2835, 1664, 1602, 1510 cm^{-1}; NMR δ_H (400 MHz, $CDCl_3$): 8.08 (d, J = 8.76 Hz, 2H, C_3-$C_6H_4OCH_3$; ortho protons), 7.83–7.80 (m, 2H, C_7-H, C_9-H). 7.74 (d, J = 8.04 Hz, 1H, C_5-H), 7.44–7.35 (m, 3H, C_6-H, C_8-H and C_{10}-H), 7.29 (d, J = 7.92 Hz, 2H, C_1-$C_6H_4CH_3$; ortho protons), 7.08 (d, J = 7.76 Hz, 2H, C_1-$C_6H_4CH_3$; meta protons), 6.93 (d, J = 8.76 Hz, 2H, C_3-$C_6H_4OCH_3$(meta, $C_{3''}$)), 6.41 (s, 1H, C_1-H), 3.84 (s, 3H, $C_{4''}$-OCH_3), 2.27 (s, 3H, $C_{4'}$-CH_3); NMR δ_C (100 MHz, $CDCl_3$): 161.91, 151.16, 146.80, 140.88, 136.94, 131.33, 130.17, 129.39, 129.30,

129.14, 128.52, 127.69, 126.92, 124.61, 124.57, 123.03, 116.59, 114.47, 113.46, 56.54, 55.31, 21.05; HRMS: m/z (M^+) calcd. for $C_{26}H_{21}NO_2$: 379.1572, found: 380.1859 (M + H).

4.3.9. 3-(4-Nitrophenyl)-1-p-tolyl-1H-naphtho[1,2-e][1,3]oxazine (**7i**)

mp 203–204 °C; yield: 75%; IR(KBr)ν: 3064, 3014, 2912, 2873, 1670, 1598, 1519, 1327 cm^{-1}; NMR δ_H (400 MHz, $CDCl_3$): 8.29–8.21 (m, 4H, C_3-$C_6H_4NO_2$), 7.87–7.76 (m, 2H, C_7-H, C_9-H). 7.69 (d, J = 6.72 Hz, 1H, C_5-H), 7.42–7.33 (m, 3H, C_6-H, C_8-H and C_{10}-H), 7.27 (d, J = 7.76 Hz, 2H, C_1-$C_6H_4CH_3$; ortho protons), 7.10 (d, J = 7.76 Hz, 2H, C_1-$C_6H_4CH_3$; meta protons), 6.45 (s, 1H, C_1-H), 2.28 (s, 3H, $C_{4'}$-CH_3); NMR δ_C (100 MHz, $CDCl_3$): 149.41, 146.39, 140.10, 137.88, 137.41, 131.59, 129.97, 129.58, 129.56, 128.60, 128.53, 127.75, 127.33, 127.18, 125.01, 123.28, 123.11, 116.24, 113.63, 56.98, 21.06; HRMS: m/z (M^+) calcd. for $C_{25}H_{18}N_2O_3$: 394.1317, found: 395.1616 (M + H).

4.3.10. 3-(4-Chlorophenyl)-1-p-tolyl-1H-naphtho[1,2-e][1,3]oxazine (**7j**)

mp 170–171 °C; yield: 78%; IR(KBr)ν: 3051, 3016, 2978, 2916, 2879, 2844, 1674, 1597, 1512 cm^{-1}; NMR δ_H (400 MHz, $CDCl_3$): 8.06 (d, J = 8.48 Hz, 2H, C_3-C_6H_4Cl; ortho protons), 7.84–7.80 (m, 2H, C_7-H, C_9-H). 7.71 (d, J = 7.68 Hz, 1H, C_5-H), 7.43–7.34 (m, 5H, C_6-H, C_8-H, C_{10}-H and C_1-$C_6H_4CH_3$; ortho protons), 7.27 (d, J = 7.92 Hz, 2H, C_1-$C_6H_4CH_3$; ortho protons), 7.09 (d, J = 7.92 Hz, 2H, C_1-$C_6H_4CH_3$; meta protons) 6.41 (s, 1H, C_1-H), 2.27 (s, 3H, $C_{4'}$-CH_3); NMR δ_C (100 MHz, $CDCl_3$): 150.41, 146.61, 140.53, 137.19, 137.16, 131.45, 130.61, 130.09, 129.48, 129.34, 128.97, 128.56, 128.40, 127.72, 127.05, 124.80, 123.09, 116.44, 114.09, 56.71, 21.07; HRMS: m/z (M^+) calcd. for $C_{25}H_{18}ClNO$: 383.1077, found: 384.1353 (M + H).

4.3.11. 3-(4-Fluorophenyl)-1-p-tolyl-1H-naphtho[1,2-e][1,3]oxazine (**7k**)

mp 177–178 °C; yield: 74%; IR(KBr)ν: 3057, 3012, 2976, 2918, 2877, 1676, 1600, 1510 cm^{-1}; NMR δ_H (400 MHz, $CDCl_3$): 8.13 (dd, J = 8.52, 5.64 Hz, 2H, C_3-C_6H_4F; ortho protons), 7.84–7.81 (m, 2H, C_7-H, C_9-H). 7.72 (d, J = 7.84 Hz, 1H, C_5-H), 7.44–7.35 (m, 3H, C_6-H, C_8-H and C_{10}-H), 7.28 (d, J = 7.92 Hz, 2H, C_1-$C_6H_4CH_3$; ortho protons), 7.12–7.08 (m, 4H, ArH), 6.41 (s, 1H, C_1-H), 2.27 (s, 3H, $C_{4'}$-CH_3); NMR δ_C (100 MHz, $CDCl_3$): 165.89, 150.43, 146.66, 140.62, 137.13, 131.43, 130.12, 129.84 (d, J = 8.73 Hz), 129.47, 129.31, 128.56, 128.27 (d, J = 2.99 Hz), 127.71, 127.03, 124.77, 123.09, 116.46, 115.29 (d, J = 21.86 Hz), 114.17, 56.66, 21.07; HRMS: m/z (M^+) calcd. for $C_{25}H_{18}FNO$: 367.1372, found: 368.1647 (M + H).

4.3.12. 3-(4-Bromophenyl)-1-p-tolyl-1H-naphtho[1,2-e][1,3]oxazine (**7l**)

mp 156–157 °C; yield: 76%; IR(KBr)ν: 3051, 3024, 2918, 2900, 1676, 1593, 1512 cm^{-1}; NMR δ_H (400 MHz, $CDCl_3$): 8.00 (d, J = 8.40, Hz, 2H, C_3-C_6H_4Br; ortho protons), 7.84–7.79 (m, 2H, C_7-H, C_9-H). 7.72 (d, J = 7.72 Hz, 1H, C_5-H), 7.44–7.34 (m, 5H, C_6-H, C_8-H and C_{10}-H), 7.28 (d, J = 7.84 Hz, 2H, C_1-$C_6H_4CH_3$; ortho protons), 7.09 (d, J = 7.72 Hz, 2H, C_1-$C_6H_4CH_3$; meta protons), 6.41 (s, 1H, C_1-H), 2.27 (s, 3H, $C_{4'}$-CH_3); NMR δ_C (100 MHz, $CDCl_3$): 150.49, 146.58, 140.49, 137.16, 131.45, 131.36, 131.06, 130.08, 129.47, 129.34, 129.18, 128.56, 127.72, 127.04, 125.69, 124.80, 123.08, 116.43, 114.06, 56.72, 21.07; HRMS: m/z (M^+) calcd. for $C_{25}H_{18}BrNO$: 427.0572, found: 428.0901 (M + H).

4.4. In vitro antimicrobial activity

The antimicrobial activity of synthesized compounds was performed against Gram-positive bacteria: *S. aureus* [MTCC 2901], *B. subtilis* [MTCC 2063], Gram-negative bacterium: *E. coli* [MTCC 1652] and fungal strains: *C. albicans* [MTCC 227] and *A. niger* [MTCC 8189] using tube dilution method. Dilutions of test and standard compounds were prepared in double strength nutrient broth – I.P. (bacteria) or Sabouraud dextrose broth I.P. (fungi). The samples were incubated at 37 °C for 24 h (bacteria), 25 °C for 7 days (*A. niger*) and 37 °C for 48 h (*C. albicans*), and the results were recorded in terms of MIC.

4.5. In vitro cytotoxicity assay

The synthesized 1,3-disubstituted-1*H*-naphtho[1,2-e][1,3]oxazine were dissolved in DMSO to form stock solutions (1 mM/mL), which were filter sterilized (0.2 μm) before testing on cell lines. Growth medium RPMI-1640, Minimum Essential Medium (MEM), foetal calf serum (GIBCO), trypsin, penicillin, MTT dye, streptomycin, DMSO and phosphate buffer saline (PBS) chemicals were used for study. Human breast cancer cell line (MCF-7), lung (A-549), Prostate (PC-3) and Colon (HCT-116) were procured from European Collection of cell culture (ECACC), UK. Cells were grown in RPMI-1640 medium supplemented with 10% FCS and 1% penicillin. Penicillin was dissolved in PBS and sterilized by filtering through 0.2 μm filter in laminar air flow hood. Cells were cultured in CO_2 incubator (New Brunswick, Galaxy 170R, Eppendorf) with an internal atmosphere of 95% air and 5% CO_2 gas and the cell lines were maintained at 37 °C. The media was stored at low temperature (2–8 °C). The medium for cryopreservation contained 20% FCS and 10% DMSO in growth medium.

4.5.1. Cell viability assay

The MTT assay was used to assess the effect of 1,3-disubstituted-1*H*-naphtho[1,2-e][1,3]oxazine on cell viability. In each well of a 96-well plate, 3×10^3 cells were grown in 100 μL of medium. After 24 h, 1,3-disubstituted-1*H*-naphtho[1,2-e][1,3]oxazine was added to achieve a final concentration 50–10 μmol/100 μL, respectively. 20 μL of 2.5 mg/mL MTT (Organics Research, Inc.) solution in PBS was added to each well followed by an additional incubation period of at least 4 h to allow for complete bioreduction of MTT to the final formazan product. After 48 h of treatment, supernatant was removed and formazan crystals were dissolved in 200 μL of DMSO. Absorbance was then measured at 570 nm using an absorbance plate reader (Bio-Rad Microplate Reader). Data are expressed as the percentage of viable cells in treated relative to non-treated conditions [16].

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Appendix A. Supplementary data

Crystallographic data for **3b** has been deposited with the Cambridge Crystallographic Data Centre (No. CCDC 874764).

Appendix B. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ejmech.2012.08.018.

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