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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis of 1-amidoalkyl-2-naphthols and oxazine derivatives with study of their antibacterial and antiviral activities

Wael S. I. Abou-Elmagd · Ahmed I. Hashem

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Abstract An efficient and direct protocol for the preparation of 1-amidoalkyl-2-naphthols **1a–f** employing a multi-component, one-pot condensation reaction of 2-naphthol, heterocyclic aldehydes, and amides in the presence of anhydrous zinc chloride under solvent-free conditions is described. The thermal solvent-free offer advantages such as shorter reaction times, simple work-up and excellent yield. Ring closure of **1a–f** gave the pyrazolyl- and indolyl oxazine derivatives **2a–f**. On the other hand, the reaction of 2-naphthol, aldehydes, and ammonia solution gave the dipyrazolyl- and di-indolyl oxazine derivatives **3a**,**b**. Some of the newly synthesized compounds showed promising antibacterial and anti-H_5N_1 activities.

Keywords 1-Amidoalkyl naphthol · Oxazine · Zanamivir · Antibacterial · Anti-H_5N_1

Introduction

The development of simple synthetic routes to widely used organic compounds using readily available reagents is one of the main objectives of organic synthesis. Nitrogen heterocyclic compounds are of special interest. They constitute an important class of natural and non-natural products, many of which exhibit useful biological activities (Turgut *et al*., 2007, Xu and Guo, 2004, Hashem *et al*., 2007). Recently, the use of catalysts and reagents supported on solid supports under solvent-free conditions has been developed. Such reagents not only simplify the purification processes, but also help to prevent the releasing of toxic reaction residues into the environment.

One-pot multi-components condensation of 2-naphthol, aromatic aldehydes, and amide derivatives has been used as a practical synthetic routes toward 1-amidoalkyl-2-naphthols. Several Lewis and Brønsted acids have been applied for this transformation (Shaterian and Yarahmadi, 2008; Das *et al*., 2007; Nagawade and Shinde, 2007; Shaterian *et al*., 2008; Nagarapu *et al*., 2007; Mahdavinia *et al*., 2008; Hajipour *et al*., 2009; Nandi *et al*., 2009; Mahdavinia *et al*., 2009; Kantevari *et al*., 2007; Patil *et al*., 2007; Lei *et al*., 2009; Su *et al*., 2008, Srihari *et al*., 2007; Kumar *et al*., 2009; and Selvam *et al*., 2006). However, many of the reported methods suffer from one or more of the following drawbacks: (i) low product yield, (ii) prolonged reaction time, (iii) the use of large amount of reagents, (iv) the use of toxic reagent, and (v) incompatibility with the green chemistry protocol. Therefore, search for finding a protocol for the synthesis of 1-amidoalkyl-2-naphthols that are not associated with the above disadvantages is still relevant.

1-Amidoalkyl-2-naphthol derivatives are of importance because they can be converted by carbamate hydrolysis into the biologically active 1- aminoalkyl-2-naphthol. The latter products were shown to have hypotensive and bradycardiac effects (Szatmäri and Fülöp, 2004).

The synthesis of 1,3-oxazines has attracted attention in the past because of their potential as antibiotics, antitumor agents, analgesics, and anticonvulsants. 1,3-Oxazines have generated great interest as antipsychotic agents and as possible effectors for serotonin and dopamine receptors. In addition, benzo-1,3-oxazines are known to be biologically active as anti-malarial, anti-anginal, anti-hypertensive, and potent anti-rheumatic agents (Shen *et al*., 1999).

Zanamivir is a neuraminidase inhibitor used in the treatment and prophylaxis of influenza caused by influenza

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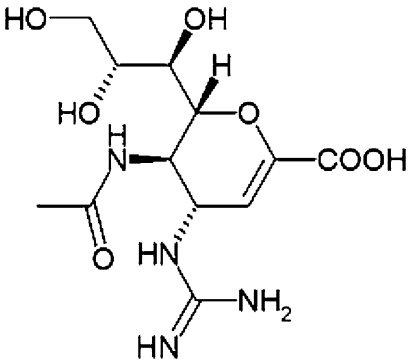
A virus and influenza B virus (Soundararajan *et al*., 2009). The presence of oxine ring with the presence of free hydroxy, amino, amide, and carboxylic acid groups initiated our interest to synthesize the amidoalkyl-2-naphthol and oxazine ring containing one or more of these groups.

We wish to report "Herein" a new, efficient and simple method for the synthesis of 1-amidoalkyl-2-naphthols, pyrazolyl- and indolyl-1,3-oxazine derivatives from 2-naphthol, heterocyclic aldehydes and amides in the presence of anhydrous zinc chloride as catalyst under solvent-free conditions. Selected examples of the compounds obtained were evaluated for their antibacterial and antiviral activities.



Zanamivir

Results and discussion

Chemistry

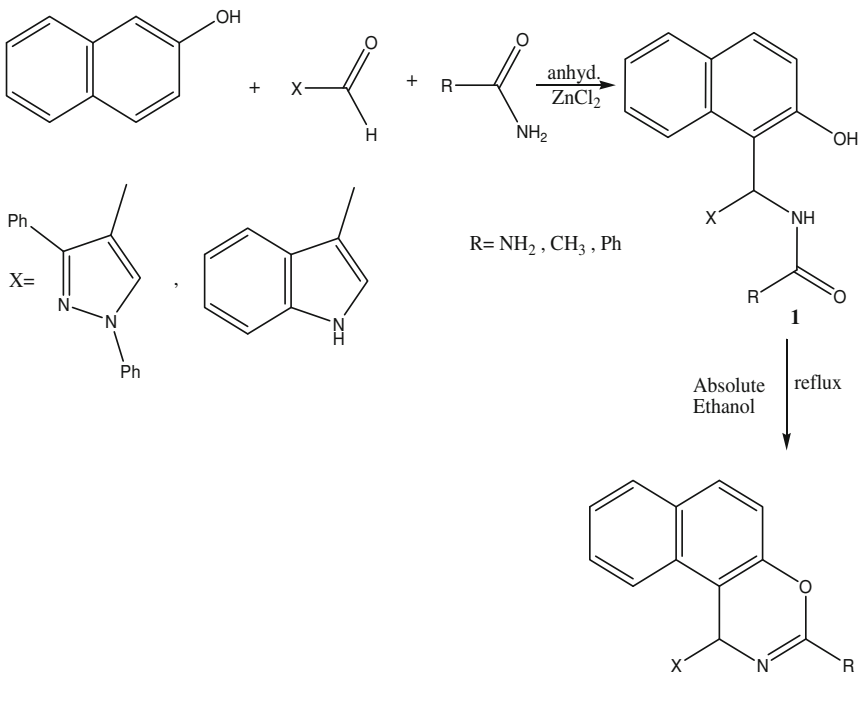
I,3-Diphenylpyrazol-4-carboxaldehyde and/or indole-3-carboxaldehyde were condensed with 2-naphthol and amide (urea, acetamide, benzamide) in the presence of anhydrous zinc chloride at 80–100 °C for 1 h under solvent-free conditions (Scheme 1). The reaction proceeded smoothly and gave the corresponding 1-amidoalkyl-2-naphthols **1a–f** as sole products in 90 % yield.

On heating **1a–f** in absolute ethanol, ring closure occurred with the formation of the corresponding 1,3-oxazine derivatives **2a–f**. It is to be mentioned that, this ring clousure was also affected by refluxing **1a–f** with P_2O_5 in dry toluene (cf., "Experimental" section).

The structures of the 1-amidoalkyl-2-naphthols and 1,3-oxazine derivatives were inferred from their analytical and spectral data (cf., "Experimental" section).

The infrared spectra of **1a–f** revealed the disappearance of the characteristic C=O bands of the aldehydes and absorption bands corresponding to NH groups were observed between 3,219 and 3,321 cm^{-1}, as well as, amide carbonyl group at 1,640–1,650 cm^{-1}. The NCH proton

Scheme 1 Synthesis of 1-amidoalkyl-2-naphthols and 1,3-oxazine derivatives



a, X=1,3-diphenyl pyrazolyl R= NH2
b, X= 1,3-diphenyl pyrazolyl R= CH3
c, X=1,3-diphenyl pyrazolyl R= Ph
d, X= indolyl R= NH2
e, X= indolyl R= CH3
f, X= indolyl R= Ph

singlet between 8.02 and 8.41 ppm were observed in the ^{1}H-NMR spectra in case of **1a–f** and the disappearance of NH and OH groups in case of **2a–f** (Scheme 1).

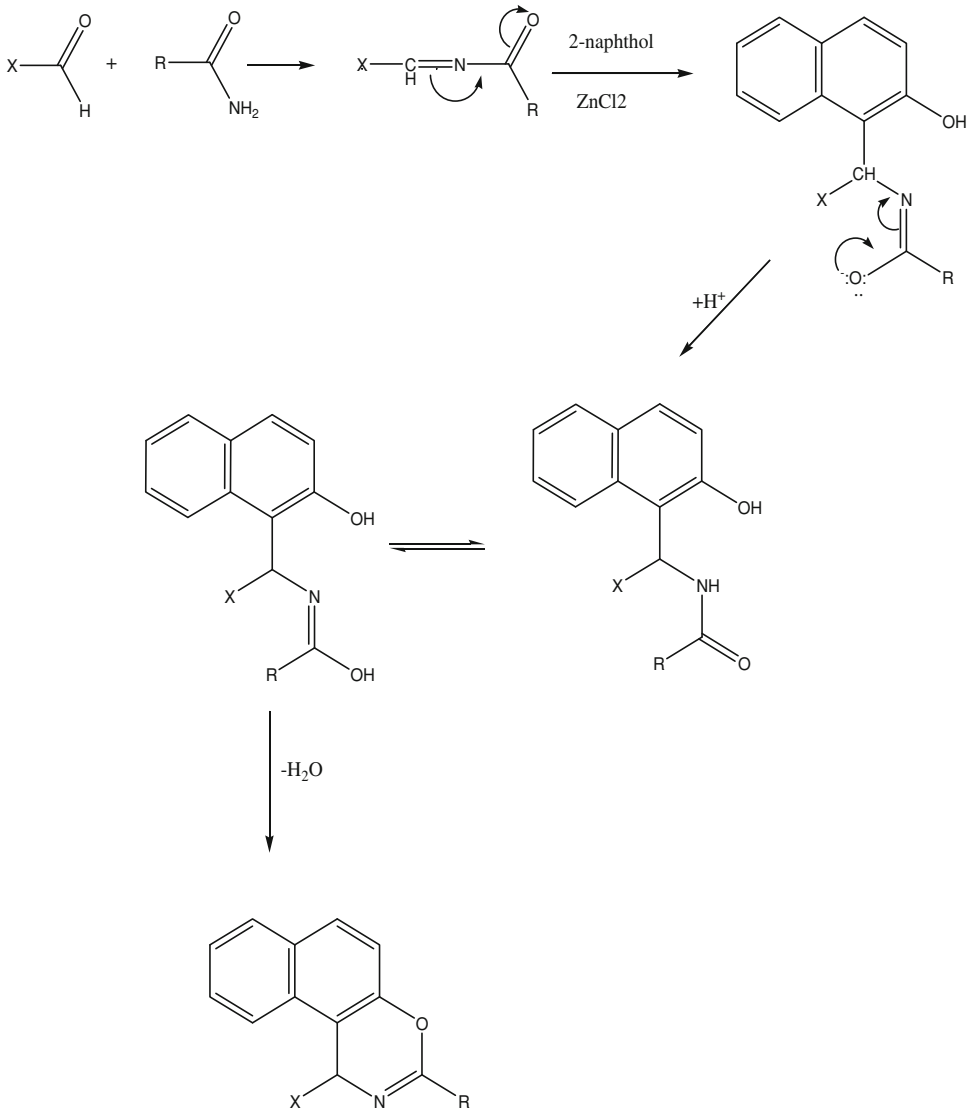
We believe that the formation of **1a–f** is similar to the *Hoesch* reaction in which a chloroimine attacks activated phenols. So, it is possible that the aldehyde reacts firstly with the amide to give the imine X–CH=N–CO–R. The latter intermediate has an α,β—unsaturated carbonyl moiety, and hence can attack position −1 in 2-naphthol via its highly deficient carbon (cf. Scheme 2)

On the other hand, when 1,3-diphenyl-4-carboxaldehyde and/or indole-3-carboxaldehyde were allowed to react with 2-naphthol in ammonia solution according to Mannich type aminoalkylation by applying classical *Betti's* reaction (Scheme 3; Betti, 1942). The reaction proceeded smoothly and gave the corresponding dipyrazolyl oxazine and diindolyl oxazine derivatives **3a**,**b**. The structures of the new compounds obtained have been clarified by spectroscopic data which show the disappearance of the characteristic C=O bands of the aldehydes and OH band of 2-naphthol and the appearance of the saturated oxazine NH at 3,127–3,234 cm^{-1} (cf., "Experimental" section)

Biological evaluation

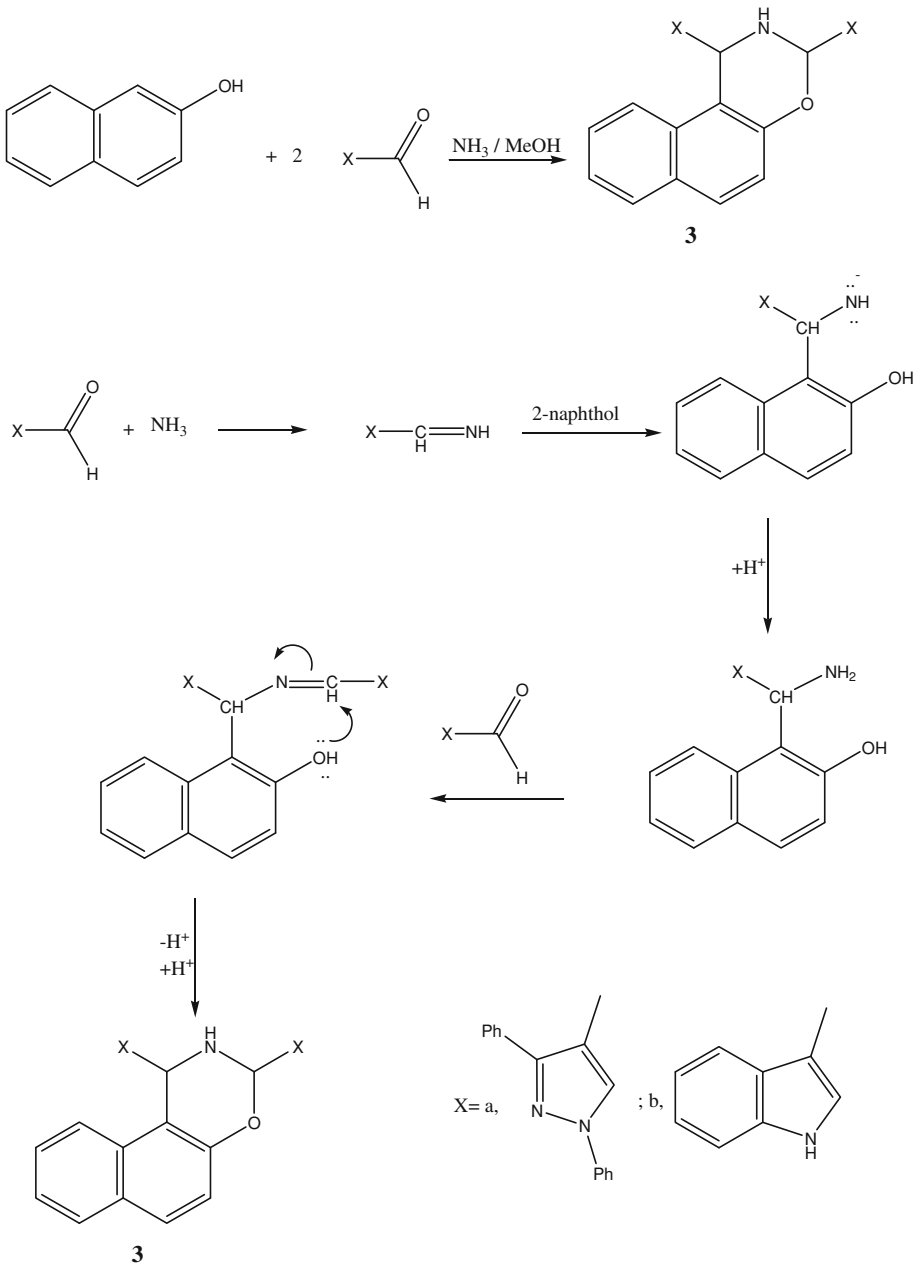
Cytotoxicity and antiviral activity

Selective compounds were evaluated for their cytotoxicity using African green monkey kidney (Vero) cells using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method and were evaluated for their antiviral activity using cytopathogenicity (CPE) assay against avian influenza virus (H_5N_1) taking *Zanamivir* as a control. The results of the assay are summarized in Table 1 and illustrated by Fig. 1. All compounds except **1f** showed better



Scheme 2 A proposed pathway for the formation of 1-amidoalkyl-2-naphthols and 1,3-oxazine derivatives

Scheme 3 A proposed pathway for the formation of diheteryl 1,3-oxazine derivatives



cytotoxicity than the control *Zanamivir*. Among them, compounds **1c**, **2a**, and **3a** showed cytotoxicity against cell line below a concentration of 0.1 μM and **1a**, **1b**, **1e**, **2d**, **2f,** and **3b** showed cytotoxicity below a concentration of 0.2 μM. The antiviral results indicated that compounds **1c** and **1f** were the most active compounds with EC_{50} values 0.018 and 0.019, respectively.

The preliminary structure–activity relationships suggested that compounds with free amino, amide, or hydroxyl groups were generally more potent in cytotoxicity than the cyclic oxazine ring lacking one or more of these groups. But the oxazine ring having a pyrazole moiety was generally more potent in antiviral activity. Finally, the higher effective compound than the control *Zanamivir* was **1f**.

Most of the newly synthesized compounds were screened for their antimicrobial activity against variety of human pathogenic bacteria; the results of the assay are summarized in Table 2.

The results of the assay showed that the amidoalkyl naphthols **1a**, **1b**, **1c**, and **1e** gave high activity (Inhibition zone) and the other compounds showed moderate activities. All compounds showed no activity towards *P. aeroginosa*, *E.coli, and Proteus* Microorganisms.

Table 1 Cytotoxicity and antiviral activity

Compound	LD50 (μM/ml)	EC50 (μM/ml) Avian Influenza Virus (H5N1)	Therapeutic index
1a	0.171	0.025	6.84
1b	0.151	0.021	7.19047619
1c	0.098	0.018	5.444444444
1d	0.217	0.031	7
1e	0.197	0.022	8.954545455
1f	0.307	0.019	16.15789474
2a	0.083	0.0148	5.608108108
2d	0.125	0.061	2.049180328
2f	0.104	0.049	2.12244898
3a	0.089	0.045	1.977777778
3b	0.122	0.168	0.726190476
Zanamivir	0.232	0.015	15.46666667

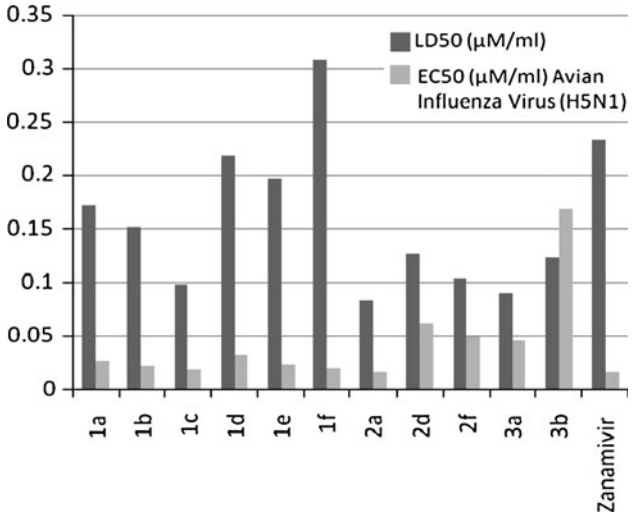


Fig. 1 Cytotoxicity and antiviral activity

Table 2 Antibacterial activity (as inhibition zone in mm diameter)

Sample no./ microorganism	**1a**	**1b**	**1c**	**1d**	**1e**	**1f**	**2a**	**2d**	**2f**	**3a**	**3b**
K. pneumonia	11	18	14	11	–	–	11	13	11	11	12
P. aeroginosa	–	–	–	–	–	–	–	–	–	–	–
E. coli	–	–	–	–	–	–	–	–	–	–	–
Proteus	0	–	–	–	–	–	–	–	–	–	–
S. Aureus	24	–	–	–	20	11	–	–	11	11	–
B. subtilis	–	–	20	–	17	11	12	12	14	11	13

Standard reading using gentamicin antibiotic 0.042 μM/ml. The inhibition zone exhibited by gentamicin 0.042 μM/ml in mm diameter. *K. pneumonia* 19 mm; *P. aeurginosa* 27 mm; *E. coli* 29 mm; *P. mirabilis* 25 mm; *S. aureus* 28 mm; *and B. subtilus* 21 mm

Conclusion

A new, efficient, and simple method for the synthesis of 1-amidoalkyl-2-naphthols, pyrazolyl and indolyl-1,3-oxazine derivatives from 2-naphthol, heterocyclic aldehydes, and amides in the presence of anhydrous zinc chloride as catalyst under solvent-free conditions is reported. On the other hand, the reaction of 2-naphthol, aldehydes, and ammonia solution gave the dipyrazolyl- and di-indolyl oxazine derivatives. Some of the newly synthesized compounds showed promising antibacterial and anti-H_5N_1 activities. Compounds with free amino, amide, or hydroxyl groups were generally more potent in cytotoxicity and antibacterial activities than the cyclic oxazine ring lacking these groups. The oxazine ring having pyrazole moiety was generally more potent in antiviral activity.

Experimental

Melting points are measured on an electrothermal melting point apparatus. Elemental analyses were carried out at the Microanalytical Unit, Cairo University. The IR spectra were measured on a Unicam SP-1200 spectrometer using KBr Wafer technique. The ^{1}H-NMR spectra were measured in DMSO-d_6 on a Varian plus instrument (300 MHz). Mass spectra were recorded on a Shimadzu GC–MS QP- 1000EX instrument operating at 70 eV.

Chemistry

General procedure for the preparation of 1-amidoalkyl-2- naphthols **1a–f**

A mixture of aldehyde (1 mmol), 2-naphthol (1 mmol), amide (1.3 mmol), and anhydrous $ZnCl_2$ (0.25 mmol) were heated at 80–100 °C for 1 h. After completion of the reaction (monitored by TLC), the reaction mixture was diluted with water, and the resulting solid product was collected by filtration and recrystallized from 30 % aqueous ethanol to give 1-amidoalkyl-2-naphthols **1a–f**.

*1-((1,3-Diphenyl-1H-pyrazol-4-yl)(2-hydroxynaphthalen-1-yl)methyl)urea **1a***

Colorless crystals; m.p: 168–170 °C, yield 85 %. IR (KBr) (ν_{max}, cm^{-1}): 3465–3348(br.) (OH), 3321, 3237, 3219 (NH, NH2), 1640 (C=O). ^{1}H-NMR (DMSO-d_6): δ_H (ppm) 6.95–7.80 (m, 17H, ArH), 7.84 (br.s, 1H, OH, exchangeable with D_2O), 8.43 (s, 1H, CH), 8.71 (br.s, 2H, NH_2, exchangeable with D_2O), 9.10 (br.s, 1H, NH, exchangeable

with D_2O). MS, *m/z* (%): 434 ($M^{\cdot+}$, 17), 285 (31), 246 (52), 190 (63), 161 (57), 110 (37), 77 (100). Anal. Calcd. For $C_{27}H_{22}N_4O_2$ (434): C, 74.64; H, 5.10; N, 12.89. Found: C, 75.12; H, 5.33; N, 12.76.

N-((1,3-Diphenyl-1H-pyrazol-4-yl)(2-hydroxynaphthalen-1-yl)methyl)acetamide ***1b***

Colorless crystals; m.p: 230–231 °C, yield 90 %. IR (KBr) (ν_{max}, cm^{-1}): 3470–3380(br.) (OH), 3250 (NH), 1649 (C=O). ^{1}H-NMR (DMSO-d_6): δ_H (ppm) 2.31 (s, 3H, CH_3), 6.90–7.77 (m, 17H, ArH), 7.81 (br.s, 1H, OH, exchangeable with D_2O), 8.43 (s, 1H, CH), 9.14 (br.s, 1H, NH, exchangeable with D_2O). MS, *m/z* (%): 433 ($M^{\cdot+}$, 25), 391(50),285 (27), 246 (34), 190 (50), 161 (43), 110 (39), 77 (100). Anal. Calcd. For $C_{28}H_{23}N_3O_2$ (433): C, 77.58; H, 5.35; N, 9.69. Found: C, 77.72; H, 5.20; N, 9.86.

N-((1,3-Diphenyl-1H-pyrazol-4-yl)(2-hydroxynaphthalen-1-yl)methyl)benzamide ***1c***

Colorless crystals; m.p: 275–278 °C, yield 89 %. IR (KBr) (ν_{max}, cm^{-1}): 3420–3350(br.) (OH), 3226 (NH), 1652 (C=O). ^{1}H-NMR (DMSO-d_6): δ_H (ppm) 7.04–7.82 (m, 22H, ArH), 7.88 (br.s, 1H, OH, exchangeable with D_2O), 8.41 (s, 1H, CH), 9.15 (br.s, 1H, NH, exchangeable with D_2O). MS, *m/z* (%): 495($M^{\cdot+}$, 12), 391(67), 285 (34), 246 (46), 190 (54), 161 (64), 110 (30), 77 (100). Anal. Calcd. For $C_{33}H_{25}N_3O_2$ (495): C, 79.98; H, 5.05; N, 8.48. Found: C, 79.59; H, 5.23; N, 8.66.

1-((2-Hydroxynaphthalen-1-yl)(1H-indol-3-yl))methyl)urea ***1d***

Colorless crystals; m.p: 142-144 °C, yield 88 %. IR (KBr) (ν_{max}, cm^{-1}): 3495–3358(br.) (OH), 3300, 3280, 3267 (NH, NH2), 1647 (C=O). ^{1}H-NMR (DMSO-d_6): δ_H (ppm) 7.05–7.67 (m, 12H, ArH + NH_{indole}), 7.75 (br.s, 1H, OH, exchangeable with D_2O), 8.16 (s, 1H, CH), 8.95 (br.s, 2H, NH_2, exchangeable with D_2O), 9.08 (br.s, 1H, NH, exchangeable with D_2O). MS, *m/z* (%): 331 ($M^{\cdot+}$, 34), 288 (48), 271 (65), 190 (32), 160 (50), 144 (100), 75 (50), 57 (88). Anal. Calcd. For $C_{20}H_{17}N_3O_2$ (331): C, 72.49; H, 5.17; N, 12.68. Found: C, 72.78; H, 5.08; N, 12.97

N-((2-Hydroxynaphthalen-1-yl)(1H-indol-3-yl))methyl)acetamide ***1e***

Colorless crystals; m.p: 180–182 °C, yield 92 %. IR (KBr) (ν_{max}, cm^{-1}): 3495–3350(br.) (OH), 3243, 3220 (NH), 1647 (C=O). ^{1}H-NMR (DMSO-d_6): δ_H (ppm) 2.19 (s, 3H, CH_3), 7.08–7.93 (m, 12H, ArH + NH_{indole}), 7.58 (br.s, 1H, OH, exchangeable with D_2O), 8.02 (s, 1H, CH), 8.97 (br.s, 1H, NH, exchangeable with D_2O). MS, *m/z* (%): 330 ($M^{\cdot+}$, 7), 287(69), 143(37), 98(57), 81 (100), 80 (32), 63 (23). Anal. Calcd. For $C_{21}H_{18}N_2O_2$ (330): C, 76.34; H, 5.49; N, 8.48. Found: C, 76.69; H, 5.24; N, 8.80.

N-((2-Hydroxynaphthalen-1-yl)(1H-indol-3-yl))methyl)benzamide ***1f***

Colorless crystals; m.p: 230–232 °C, yield 91 %. IR (KBr) (ν_{max}, cm^{-1}): 3477–3393(br.) (OH), 3312, 3250 (NH), 1650 (C=O). ^{1}H-NMR (DMSO-d_6): δ_H (ppm) 7.04–7.82 (m, 17H, ArH + NH_{indole}), 7.83 (br.s, 1H, OH, exchangeable with D_2O), 8.08 (s, 1H, CH), 9.02 (br.s, 1H, NH, exchangeable with D_2O). MS, *m/z* (%): 392 ($M^{\cdot+}$, 43), 287(56), 285 (34), 240 (32), 192 (35), 144 (65), 110 (30), 81 (56), 77 (100). Anal. Calcd. For $C_{26}H_{20}N_2O_2$ (392): C, 79.57; H, 5.14; N, 7.14. Found: C, 79.29; H, 5.21; N, 7.06.

General procedure for the cyclization of compounds **1a–f**; formation of 1,3-oxazine derivatives **2a–f**

A solution of **1a–f** in absolute ethanol was heated for 1 h. and then left to cool. The solid obtained was filtered off and recrystallized from absolute ethanol to give the oxazine derivatives **2a–f**.

The same products **2a–f** were obtained when a solution of **1a–f** (1 mmol) and P_2O_5 (1.1 mmol) in dry toluene were refluxed for 1 h. The whole mixture was concentrated and left to cool. The precipitated solid was filtered off, washed with water, dried, and recrystallized from ethanol.

1-(1,3-Diphenyl-1H-pyrazol-4-yl)-1H- naphtho[1,2-e][1,3]oxazin-3-amine ***2a***

Pale yellow crystals; m.p: 125–128 °C, yield 59 %. IR (KBr) (ν_{max}, cm^{-1}): 3227, 3210 (NH_2), 1619 (C=N). ^{1}H-NMR (DMSO-d_6): δ_H (ppm) 4.52 (br.s, 2H, NH_2, exchangeable with D_2O), 5.73 (s, 1H, CH), 6.90–7.67 (m, 17H, ArH). MS, *m/z* (%): 416 ($M^{\cdot+}$, 5), 286(43), 245 (54), 191 (47), 160 (73), 110 (30), 77 (100). Anal. Calcd. For $C_{27}H_{20}N_4O$ (416): C, 77.87; H, 4.84; N, 13.45. Found: C, 77.52; H, 5.13; N, 13.76.

1-(1,3-Diphenyl-1H-pyrazol-4-yl)-3-methyl-1H-naphtho[1,2-e][1,3]oxazin ***2b***

Pale yellow crystals; m.p: 138–139 °C, yield 51 %. IR (KBr) (ν_{max}, cm^{-1}): 3059, 3037 ($CH_{aromatic}$), 2889, 2836, 2758 ($CH_{aliphatic}$), 1622 (C=N). ^{1}H-NMR (DMSO-d_6): δ_H (ppm) 1.93 (s, 3H, CH_3), 5.43 (s, 1H, CH), 7.19–7.82 (m, 17H, ArH). MS, *m/z* (%): 415 ($M^{\cdot+}$, 9), 390(43), 286 (57), 246 (30), 191 (56), 161 (23), 112 (19), 77 (100), 57 (24).

Anal. Calcd. For $C_{28}H_{23}N_3O$ (415): C, 80.94; H, 5.09; N, 10.11. Found: C, 80.70; H, 5.36; N, 9.89.

1-(1,3-Diphenyl-1H-pyrazol-4-yl)-3-phenyl-1H-naphtho[1,2-e][1,3]oxazin ***2c***

Pale yellow crystals; m.p: 142–143 °C, yield 59 %. IR (KBr) (ν_{max}, cm^{-1}): 3038, 3021 ($CH_{aromatic}$), 2878 ($CH_{aliphatic}$), 1620 (C=N). ^{1}H-NMR (DMSO-d_6): δ_H (ppm) 5.77 (s, 1H, CH), 7.19–7.82 (m, 22H, ArH). MS, *m/z* (%): 477($M^{\cdot+}$, 21), 258(32), 219 (63), 143 (78), 77 (100), 57 (30). Anal. Calcd. For $C_{33}H_{23}N_3O$ (477): C, 83.00; H, 4.85; N, 8.80. Found: C, 82.79; H, 5.03; N, 8.66.

1-(1H-indol-3-yl)-1H-naphtho[1,2-e][1,3]oxazin-3-amine ***2d***

Pale yellow crystals; m.p: 107–110 °C, yield 45 %. IR (KBr) (ν_{max}, cm^{-1}): 3302, 3227, 3210 (NH, NH_2), 1620 (C=N). ^{1}H-NMR (DMSO-d_6): δ_H (ppm) 4.39 (br.s, 2H, NH_2, exchangeable with D_2O), 4.91 (s, 1H, CH), 6.84–7.71 (m, 12H, Ar + NH_{indole}). MS, *m/z* (%): 313 ($M^{\cdot+}$, 3), 98 (57), 97 (22), 80 (30), 81 (100), 63 (23). Anal. Calcd. For $C_{20}H_{15}N_3O$ (313): C, 76.66; H, 4.82; N, 13.41. Found: C, 76.97; H, 4.98; N, 13.76.

1-(1H-indol-3-yl) -3-methyl-1H-naphtho[1,2-e][1,3]oxazin ***2e***

Pale yellow crystals; m.p: 122–124 °C, yield 52 %. IR (KBr) (ν_{max}, cm^{-1}): 3243 (NH), 1618 (C=N). ^{1}H-NMR (DMSO-d_6): δ_H (ppm) 1.96 (s, 3H, CH_3), 4.52 (s, 1H, CH), 7.08–7.64 (m, 12H, Ar + NH_{indole}). MS, *m/z* (%): 312($M^{\cdot+}$, 15), 290(42), 195 (51), 143 (100), 116 (67), 57 (54). Anal. Calcd. For $C_{21}H_{16}N_2O$ (312): C, 80.75; H, 5.16; N, 8.97. Found: C, 80.49; H, 5.27; N, 8.83.

1-(1H-indol-3-yl) -3-phenyl-1H-naphtho[1,2-e][1,3]oxazin ***2f***

Pale yellow crystals; m.p: 131–134 °C, yield 51 %. IR (KBr) (ν_{max}, cm^{-1}): 3307 (NH), 1623 (C=N). ^{1}H-NMR (DMSO-d_6): δ_H (ppm) 5.81 (s, 1H, CH), 7.18–7.92 (m, 17H, Ar + NH_{indole}). MS, *m/z* (%): 374($M^{\cdot+}$, 13), 296(25), 219 (59), 143 (82), 76 (100), 57 (76). Anal. Calcd. For $C_{26}H_{18}N_2O$ (374): C, 83.40; H, 4.85; N, 7.48. Found: C, 83.19; H, 5.03; N, 7.19.

General procedure for the preparation of diheteryl-1,3-oxazine derivatives **3a**,**b**

Aldehyde (2 mmol) and (15 ml) ammonia solution (33 %) were added to a solution of 2-naphthol (1 mmol) in absolute MeOH (30 ml). The mixture was left to stand at 0 °C for 2 days, whereby a crystalline product was formed and separated out. The crude crystals were filtered off, washed with cold MeOH, and recrystallized from absolute ethanol to give the diheteryl-1,3-oxazine derivatives **3a**,**b**.

1,3-Bis(1,3-diphenyl-1H-pyrazol-4-yl)-2,3-dihydro-1H-naphtho[1,2-e][1,3] oxazine ***3a***

Creamy powder; m.p: 130–132 °C, yield 88 %. IR (KBr) (ν_{max}, cm^{-1}): 3127 (NH), 3054, 3034, 3019 ($CH_{aromatic}$), 2863, 2836, 2784 ($CH_{aliphatic}$). ^{1}H-NMR (DMSO-d_6): δ_H (ppm) 4.32 (br.s, 1H, NH, exchangeable with D_2O), 5.71 (s, 1H, CH–N), 6.48 (s, 1H, N–CH-O), 6.89–7.82 (m, 28H, ArH). MS, *m/z* (%): 621 ($M^{\cdot+}$, 26), 519 (26), 451 (42), 305 (31), 285 (32), 247(47), 235(26), 190(63), 162(58), 110(36), 84(47), 77(100), 69(84). Anal. Calcd. For $C_{42}H_{31}N_5O$ (621): C, 81.14; H, 5.03; N, 11.26. Found: C, 81.49; H, 4.88; N, 11.50.

1,3-Di(1H-indol-3-yl)-2,3-dihydro-1H-naphtho[1,2-e][1,3] oxazine ***3b***

Creamy powder; m.p: 190–192 °C, yield 80 %. IR (KBr) (ν_{max}, cm^{-1}): 3234, 3169 (NH), 3044 ($CH_{aromatic}$), 2980, 2935, 2989 ($CH_{aliphatic}$). ^{1}H-NMR (DMSO-d_6): δ_H (ppm) 4.02 (br.s, 1H, NH, exchangeable with D_2O), 5.29 (s, 1H, CH–N), 6.21 (s, 1H, N–CH–O), 7.11–7.92 (m, 18H, ArH + $2NH_{indole}$). MS, *m/z* (%): 415 ($M^{\cdot+}$, 9), 360 (33), 216 (33), 200 (50), 186 (33), 172(44), 159(38), 144(100), 115(55), 98(50), 89(83), 73(61), 57(89). Anal. Calcd. For $C_{28}H_{21}N_3O$ (415): C, 80.94; H, 5.09; N, 10.11. Found: C, 81.09; H, 5.28; N, 10.34.

Biology

Cytotoxicity assay

The stock samples were diluted with Dulbecco's Modified Eagle's Medium (DMEM) to desired concentrations. Stock solutions of the test compounds were prepared in DMSO at a concentration of 10 % in D_2O. The cytotoxic activity of the synthetic compounds were tested in African green monkey kidney (Vero) cells using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method (Mossman, 1983) with minor modification. In brief, the cells were seeded in 96 well-plates (100 µl/well at a density of 3×10^5 cells/ml) and treated with various concentrations of the sample solutions. After 24 h, cells were washed with sterile phosphate buffer (PBS) three times and the supernatant was discarded. MTT solution (20 µl of 5 mg/ml) was added to each well and incubated at 37 °C for 4 h then the medium was aspirated. In each well, the formed Formosan crystals were dissolved with 200 µl of acidified 2-propanol (0.04 M HCl in absolute 2-propanol).

An absorbance of Formosan was detected by a dual wavelength UV spectrometer at 540 nm with 620 nm reference wavelength. The percentage of cytotoxicity compared to the untreated cells was determined with the following equation:

$$\% \text{ Cytotoxicity} = \frac{(\text{Absorbance of cell without treatment} - \text{Absorbance of cell with treatment}) \times 100}{\text{Absorbance of cell without treatment}}$$

The plot of % cytotoxicity versus sample concentration was used to calculate the concentration which exhibited 50 % cytotoxicity (LD50).

Antiviral assay

The antiviral activity of the compounds was determined using cytopathicity (CPE) assay against avian influenza virus (H_5N_1) taking *Zanamivir* as a control. Stock solutions of the tested compounds were prepared in DMSO at a concentration of 10 mg/ml. Cells, grown to confluence in 96-well plates, were infected with 100 µl of stock virus. After an adsorption period of 2 h at 37 °C, virus was removed and serial dilutions of the compounds were added. The cultures were further incubated at 37 °C for 3 days, until complete CPE was observed in the infected and untreated virus control. The determination of the anti-influenza activity of the compounds was based on virus-induced cytopathicity (destruction) of LPAI-H_5N_1-infected Vero cells, measured at day 4 post virus infection by the MTT colorimetric method (Pauwels *et al*., 1988). An absorbance of Formosan was detected by a dual wavelength UV spectrometer at 540 nm with 620 nm reference wavelength. The results are expressed as the 50 % effective concentration (EC50). The 50 % effective antiviral concentration (EC50) was defined as the compound concentration required protecting 50 % of the virus-infected cells against viral cytopathicity. The therapeutic index is calculated by dividing LD50 by EC50.

Antibacterial activity

Microorganisms were obtained from our culture collections of Department of Microbiology, Faculty of Science Ain Shams University. Four strains of gram-negative bacteria [*Escherichia coli*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Proteus mirabilis*] and two strains of gram-positive bacteria [*Bacillus subtilis* and *Staphylococcus aureus*] were used. The cultures of bacteria were maintained in their appropriate agar slants at 4 °C throughout the study and used as stock cultures. The inhibition zone was measured for each of the tested compounds taking Gentamycin as a reference.

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