

SUBSTITUENT SELECTION FOR DESIGN AND SYNTHESIS OF ANTIMICROBIAL 1,3 OXAZINES: A TOPLISS MODIFIED APPROACH

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Abstract

The purpose of the present work was to select substituents by using Topliss modified approach to synthesize new 1,3 oxazines with antimicrobial effect. In the series of 6-[4-substitutedphenyl]-4-phenyl-6H-1,3-oxazin-2-amines and *N*-[6-(4-substitutedphenyl)-4-phenyl-6H-1,3-oxazinyl] acetamides, substituents at fourth position of the phenyl ring were selected according to the Topliss modified approach and the initial set of compounds was synthesized. The antimicrobial screening revealed that compounds with methoxy substituent having negative *sigma* (-0.04) and negative *pi* (-0.27) values are good antimicrobial agents showing low minimum inhibitory concentration (MIC). The hydroxyl group substituent with more negative *sigma* (-0.61) and *pi* (-0.37) values was selected to synthesize final set compounds and were found better antimicrobials than the initial set of compounds. The study revealed that electron donating polar substituents at fourth position of the phenyl ring are required to improve antimicrobial potential in the series of 6-[4-substitutedphenyl]-4-phenyl-6H-1,3-oxazin-2-amines and *N*-[6-(4-substitutedphenyl)-4-phenyl-6H-1,3-oxazinyl] acetamides.

Rezumat

Studiul și-a propus sinteza unor noi 1,3-oxazine cu potențial antimicrobian, utilizând metoda Topliss modificată, pentru alegerea unor substituenți corespunzători, care să potențeze efectul antimicrobian.

Studiul a demonstrat că substituția în poziția 4 a nucleului fenil, cu grupare metoxi (caracterizată prin *sigma* negativ -0,04 și *pi* negativ -0,27) conduce la obținerea unor 1,3 oxazine cu efect antimicrobian marcant. Substituția cu grupare hidroxil (*sigma*=-0,61 și *pi*=-0,37) imprimă un efect antimicrobian mai mare decât compușii descriși anterior.

Keywords: Topliss approach, 1,3-oxazin-2-amine, 1,3-oxazin-2-yl acetamide, antimicrobial activity.

Introduction

Considering that about 40 % of the therapeutic active compounds incorporate an unfused benzene ring, it is essential to search out the optimum substitution on a benzene ring in lead nucleus to maximize the drug potency. The presence of even a single phenyl ring in a drug structure offers many positions for a variety of substituents. All these possible

analogs might not really be worth synthesizing. Hence, a more rational approach needs to be developed which will help select a limited number of substituents having good discrimination between *pi* (π), *sigma* (σ) and steric constant (*E_s*) values. In order to overcome the problem of synthetic difficulty, Topliss has suggested a useful synthesis of new analogs of an active lead to maximize the chances of synthesizing the most potent compounds in the given series, as early as possible [1, 2].

A large number of substituted 1,3 oxazines have been claimed for several biological activities, such as antimicrobial [3, 4], anticancer [5, 6], analgesic, anti-inflammatory [7] and anti-tuberculosis [8]. The present work aimed to select a suitable substituent at fourth position of the phenyl ring to improve antimicrobial potential in the series of 6-[4-substitutedphenyl]-4-phenyl-6H-1,3-oxazin-2-amines and *N*-[6-(4-substitutedphenyl)-4-phenyl-6H-1,3-oxazinyl]acetamides using the Topliss modified approach.

Materials and Methods

Melting points of the synthesized compounds were determined in open capillary tubes and are uncorrected. Purity of final compounds was checked by silica gel G plate using benzene, benzene-methanol as developer. The IR spectra were recorded as KBr discs using a JASCO FTIR 4100 spectrometer. ¹H NMR spectra were registered on a VARIAN-NMR Mercury 300 spectrometer using tetramethylsilane (TMS) as internal standard and CDCl₃ as solvent and are expressed in parts per million (δ , ppm). The structures of the title compounds were established on the basis of elemental analysis and spectral data.

Synthesis of (2E)-3-(4-substitutedphenyl)-1-phenylprop-2-en-1-ones

General procedure [9,10]

A mixture of acetophenone (0.01 mol) and aryl aldehydes (0.01 mol) was stirred in ethanol (30 mL) and an aqueous solution of sodium hydroxide (15 mL) was added. The mixture was kept over night at room temperature and was poured onto crushed ice. The chalcone derivative precipitates out as solid. Then it was filtered, washed with cold water and crystallized from ethanol.

Synthesis of 6-[4-substitutedphenyl]-4-phenyl-6H-1,3-oxazin-2-amines 2a-f

General procedure [11]

A mixture of chalcone (0.02 mol) and urea (0.02 mol) was dissolved in ethanolic sodium hydroxide (10 mL) and stirred for 3 h. To this mixture, it were added poured 400 mL of cold water with continuous stirring for 1 h

and kept over night in the refrigerator. The precipitate obtained was filtered, washed and recrystallized from ethanol.

4, 6-Diphenyl-6H-1,3-oxazin-2-amine 2a

Yield: 65.00 %; m.p.: 242 °C; IR (KBr, cm⁻¹): 3120.37 (1⁰NH₂), 1607.38 (Ar-C=C), 1213.01 (C-N), 1178 (C-O-C); ¹H NMR (300 MHz, CDCl₃) δ: 4.80 (s, 2H, NH₂), 6.093 (d, 1H, CH), 6.29 (d, 1H, CH), 7.22-7.60 (m, 10H, aromatic). Anal. calcd. for C₁₆H₁₄N₂O (mw = 250.29): C, 76.78; H, 5.64; N, 11.19 %. Found: C, 76.58; H, 5.44; N, 11.31 %.

6-[4-(Dimethylamino) phenyl]-4-phenyl-6H-1,3-oxazin-2-amine 2b

Yield: 65.00 %; m.p.: 90 °C; IR (KBr, cm⁻¹): 3051.8 (1⁰NH₂), 1600 (Ar-C=C), 1236 (C-O-C), 1172.51 (C-N); ¹H NMR (300 MHz, CDCl₃) δ: 2.82 [s, 6H, N(CH₃)₂], 4.80 (s, 2H, NH₂), 6.09 (d, 1H, CH), 6.29 (d, 1H, CH), 6.45-7.60 (m, 9H, aromatic). Anal. calcd. for C₁₈H₁₉N₃O (mw = 293.36): C, 73.69; H, 6.53; N, 14.32 %. Found: C, 73.59; H, 6.45; N, 14.20 %.

6-(4-Chlorophenyl)-4-phenyl-6H-1,3-oxazin-2-amine 2c

Yield: 62.00 %; m.p.: 104 °C; IR (KBr, cm⁻¹): 3068.19 (1⁰NH₂), 1602.56 (Ar-C=C), 1218.79 (C-N), 1176 (C-O-C), 1090.55 (Ar-Cl); ¹H NMR (300 MHz, CDCl₃) δ: 4.80 (s, 2H, NH₂), 6.09 (d, 1H, CH), 6.29 (d, 1H, CH), 7.22-7.60 (m, 9H, aromatic). Anal. calcd. for C₁₆H₁₃ClN₂O (mw = 284.74): C, 67.49; H, 4.60; N, 9.84 %. Found: C, 67.39; H, 4.63; N, 9.86 %.

6-(4-Nitrophenyl)-4-phenyl-6H-1,3-oxazin-2-amine 2d

Yield: 66.00 %; m.p.: 112 °C; IR (KBr, cm⁻¹): 3095.19 (1⁰NH₂), 1602.56 (Ar-C=C), 1516 (Ar-NO₂), 1218.79 (C-N), 1101 (C-O-C); ¹H NMR (300 MHz, CDCl₃) δ: 7.22-7.85 (m, 9H, aromatic), 4.80 (s, 2H, NH₂), 6.09 (d, 1H, CH), 6.29 (d, 1H, CH). Anal. calcd. for C₁₆H₁₃N₃O₃ (mw = 295.29): C, 65.08; H, 4.44; N, 14.23 %. Found: C, 65.18; H, 4.30; N, 14.21 %.

6-(4-Methoxyphenyl)-4-phenyl-6H-1,3-oxazin-2-amine 2e

Yield: 78.00 %; m.p.: 78 °C; IR (KBr, cm⁻¹): 3311.18 (1⁰NH₂), 1388.5 (Ar-C-O), 1215.9 (C-N), 1183 (C-O-C), 1159.66 (Ar-C=C); ¹H NMR (300 MHz, CDCl₃) δ: 3.75 (s, 3H, CH₃), 4.80 (s, 2H, NH₂), 6.09 (d, 1H, CH), 6.29 (d, 1H, CH), 6.74-7.60 (m, 9H, aromatic). Anal. calcd. for C₁₇H₁₆N₂O (mw = 280.32): C, 72.84; H, 5.75; N, 9.99 %. Found: C, 72.94; H, 5.70; N, 9.81 %.

2-Amino-4-phenyl-6(4-hydroxyphenyl)-6H-1,3-oxazine 2f

Yield: 53.00 %; m.p.: 86 °C; IR (KBr, cm⁻¹): 2927.41 (Ar-OH), 1593.88 (Ar-C=C), 1215.9 (C-N), 1138 (C-O-C); ¹H NMR (300 MHz, CDCl₃) δ: 4.80 (s, 2H, NH₂), 5.23 (s, 1H, OH), 6.09 (d, 1H, CH), 6.29 (d, 1H, CH),

6.76-7.60 (m, 9H, aromatic). Anal. calcd. for $C_{16}H_{14}N_2O_2$ (mw = 266.29): C, 72.16; H, 5.30; N, 10.52 %. Found: C, 72.03; H, 5.25; N, 10.54 %.

Synthesis of *N*-[6-(4-substitutedphenyl)-4-phenyl-6*H*-1,3-oxazinyl]acetamides 3a-f

General procedure

A mixture of 6-[4-substitutedphenyl]-4-phenyl-6*H*-1,3-oxazin-2-amines (1 mol), acetic anhydride (1.2 mol) and glacial acetic acid (1.2 mol) was refluxed for 10 min. The hot mixture was poured in excess of cold water under stirring where acetamide precipitated out. It was then filtered and washed with cold water.

***N*-(4,6-Diphenyl-6*H*-1,3-oxazin-2-yl)acetamide 3a**

Yield: 64.00 %; m.p.: 164 °C; IR (KBr, cm^{-1}): 3313.11 (NH), 1661.37 (C=O), 1602.56 (Ar-C=C), 1218.79 (C-N), 1101 (C-O-C); 1H NMR (300 MHz, $CDCl_3$) δ : 2.16 (d, 3H, CH_3), 6.12 (d, 1H, CH), 6.30 (d, 1H, CH), 7.22-7.52 (m, 10H, aromatic), 8.62 (m, 1H, NH). Anal. calcd. for $C_{18}H_{16}N_2O_2$ (mw = 292.33): C, 73.95; H, 5.52; N, 9.58 %. Found: C, 73.71; H, 5.38; N, 9.48 %.

***N*-{6-[4-(Dimethylamino) phenyl]-4-phenyl-6*H*-1,3-oxazin-2-yl}acetamide 3b**

Yield: 68.00 %; m.p.: 110 °C; IR (KBr, cm^{-1}): 3287.07 (NH), 1655.59 (C=O), 1564.95 (Ar-C=C), 1341.25 (C-N), 1178 (C-O-C); 1H NMR (300 MHz, $CDCl_3$) δ : 2.16 (d, 3H, CH_3), 2.82 [s, 6H, $N(CH_3)_2$], 6.12 (d, 1H, CH), 6.30 (d, 1H, CH), 6.48-7.53 (m, 9H, aromatic), 8.62 (m, 1H, NH). Anal. calcd. for $C_{20}H_{21}N_3O_2$ (mw = 335.39): C, 71.62; H, 6.31; N, 12.53 %. Found: C, 71.72; H, 6.28; N, 12.77 %.

***N*-[6-(4-Chlorophenyl)-4-phenyl-6*H*-1,3-oxazin-2-yl]acetamide 3c**

Yield: 71.79 %; m.p.: 96 °C; IR (KBr, cm^{-1}): 3313.11 (NH), 1661.37 (C=O), 1602.56 (Ar-C=C), 1218.79 (C-N), 1185 (C-O-C), 1090.55 (Ar-Cl); 1H NMR (300 MHz, $CDCl_3$) δ : 2.16 (d, 3H, CH_3), 6.12 (d, 1H, CH), 6.30 (d, 1H, CH), 7.15-7.52 (m, 9H, aromatic), 8.62 (m, 1H, NH). Anal. calcd. for $C_{18}H_{15}ClN_2O_2$ (mw = 326.77): C, 66.16; H, 10.85; N, 8.57 %. Found: C, 65.11; H, 4.37; N, 8.35 %.

***N*-[6-(4-Nitrophenyl)-4-phenyl-6*H*-1,3-oxazin-2-yl]acetamide 3d**

Yield: 57.87 %; m.p.: 120 °C; IR (KBr, cm^{-1}): 3105.8 (NH), 1658.48 (C=O), 1599.66 (Ar-C=C), 1512.88 (Ar- NO_2), 1215.9 (C-N), 1123 (C-O-C); 1H NMR (300 MHz, $CDCl_3$) δ : 2.16 (d, 3H, CH_3), 6.12 (d, 1H, CH), 6.30 (d, 1H, CH), 7.25-7.88 (m, 10H, aromatic), 8.62 (m, 1H, NH). Anal. calcd. for $C_{18}H_{15}N_3O_4$ (mw = 337.3): C, 64.09; H, 4.48; N, 12.46 %. Found: C, 64.30; H, 4.58; N, 12.58 %.

N-[6-(4-Methoxyphenyl)-4-phenyl-6H-1,3-oxazin-2-yl]acetamide **3e**

Yield: 68.00 %; m.p.: 74 °C; IR (KBr, cm⁻¹): 3308.29 (NH), 1655.59 (C=O), 1602.56 (Ar-C=C), 1341.25 (Ar-C-O), 1213.01 (C-N), 1167 (C-O-C); ¹H NMR (300 MHz, CDCl₃) δ: 2.16 (d, 3H, CH₃), 3.75 (s, 3H, CH₃), 6.12 (d, 1H, CH), 6.30 (d, 1H, CH), 6.77-7.52 (m, 9H, aromatic), 8.62 (m, 1H, NH). Anal. calcd. for C₁₉H₁₈N₂O₃ (mw = 322.35): C, 70.79; H, 5.63; N, 8.69 %. Found: C, 70.51; H, 5.50; N, 8.79 %.

N-[6-(4-Hydroxyphenyl)-4-phenyl-6H-1,3-oxazin-2-yl]acetamide **3f**

Yield: 41.00 %; m.p.: 86 °C; IR (KBr, cm⁻¹): 3313.11 (NH), 2929.34 (Ar-OH), 1661.37 (C=O), 1602.56 (Ar-C=C), 1218.79 (C-N), 1160 (C-O-C); ¹H NMR (300 MHz, CDCl₃) δ: 2.16 (d, 3H, CH₃), 6.05 (s, 1H, OH), 6.12 (d, 1H, CH), 6.30 (d, 1H, CH), 6.80-7.52 (m, 9H, aromatic), 8.62 (m, 1H, NH). Anal. calcd. for C₁₈H₁₆N₂O₃ (mw = 308.33): C, 70.12; H, 5.23; N, 9.09 %. Found: C, 70.00; H, 5.20; N, 9.09 %.

Antimicrobial activity

The synthesized compounds were subjected to antimicrobial screening by serial dilution method [12]. The minimum inhibitory concentration (MIC) for the initial set of compounds was investigated against standard bacterial strains such as gram positive *Staphylococcus aureus* (NCIM-2079), *Bacillus subtilis* (NCIM-2063) and gram negative *Escherichia coli* (NCIM-2089), *Proteus vulgaris* (NCIM-2027) and compared with ampicillin as a standard drug. The stock solutions of the test compounds were prepared in dimethyl sulfoxide. The concentrations of the tested compounds were 1000, 500, 250, 125, 62.5, 31.25, 15.625, 7.8125 µg/mL.

Results and Discussion

For the initial set of compounds (**2a-f**, **3a-f**), the substituents at the fourth position of phenyl ring in the series of 6-[4-substitutedphenyl]-4-phenyl-6H-1,3-oxazin-2-amines and *N*-[6-(4-substitutedphenyl)-4-phenyl-6H-1,3-oxazinyl]acetamides have been selected according to the Topliss modified approach (table I). Selected substituents for the initial set compounds were hydrogen, dimethyl amino, chloro, nitro and methoxy with *sigma* values 00, -0.83, 0.23, 0.78 and -0.27 respectively and *pi* values 00, 0.18, 0.70, -0.28 and -0.04 respectively. These substituents have varied combinations of *sigma* and *pi* values, imparting varied electronic and lipophylic character to the compound.

Table I
Substituents used at the fourth position of the phenyl ring.

No.	Compound	R
Initial set		
1	2a, 3a	H
2	2b, 3b	N(CH ₃) ₂
3	2c, 3c	Cl
4	2d, 3d	NO ₂
5	2e, 3e	OCH ₃
Final set		
6	2f, 3f	OH

The synthesis scheme has three steps (Fig 1). In the first step, acetophenone and the substituted aromatic aldehydes reacted in the presence of sodium hydroxide to give substituted chalcones (Claisen-Schmidt condensation). In the second step, substituted chalcones cyclized by reacting with urea to produce 6-[4-substitutedphenyl]-4-phenyl-6H-1,3-oxazin-2-amines which are further acetylated in the third step to *N*-[6-(4-substitutedphenyl)-4-phenyl-6H-1,3-oxazinyl]acetamides. The progress of the reaction was monitored by thin layer chromatography (TLC) using benzene and benzene-methanol as mobile phase. The single spot on TLC and sharp melting points confirmed the purity of the compounds synthesized. Further, the title compounds were structurally characterized by elemental analysis, IR and NMR spectral data.

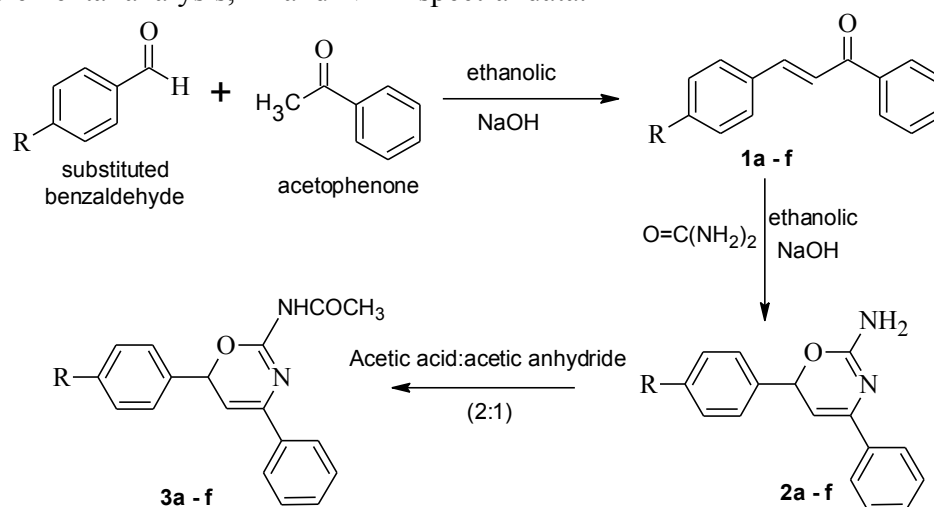


Figure 1
Synthesis scheme

The antimicrobial activity evaluation of the initial set of compounds (**2a-e**, **3a-e**) found that compounds **2e** and **3e** both bearing methoxy substituent at the fourth position of the phenyl ring were good antimicrobial agents with the lowest MIC values in comparison to other test compounds. For the methoxy substituent, both *sigma* and *pi* values are negative. It was suggested that if a new substituent is selected, which means having more negative *sigma* and *pi* values, it could give better antimicrobial agents from this series. There are hydroxy ($\sigma = -0.37$, $\pi = -0.61$) and amino ($\sigma = -0.66$, $\pi = -1.23$) substituents with negative *sigma* and *pi* values. In order to improve the antimicrobial potential in the series of 6-[4-substitutedphenyl]-4-phenyl-6H-1,3-oxazin-2-amines and *N*-[6-(4-substitutedphenyl)-4-phenyl-6H-1,3-oxazinyl]acetamides, hydroxy substituent has been selected to synthesize the second set of compounds (**2e**, **3e**). The antimicrobial screening of the second set of compounds (**2e**, **3e**) has shown a reduction in MIC values (table II) in comparison with the most active compounds of the initial set (**2f**, **3f**). The study reveals that the substituent at the fourth position of the phenyl ring of 6-[4-substitutedphenyl]-4-phenyl-6H-1,3-oxazin-2-amines and *N*-[6-(4-substitutedphenyl)-4-phenyl-6H-1,3-oxazinyl] acetamides should have negative *sigma* and *pi* values for optimum antimicrobial activity.

Table II

Antimicrobial activity (MIC) of the studied compounds

Compound	Minimum Inhibitory Concentration (MIC) ($\mu\text{g/mL}$)			
	Gram + Ve		Gram - Ve	
	<i>Staphylococcus aureus</i>	<i>Bacillus subtilis</i>	<i>Escherichia coli</i>	<i>Proteus vulgaris</i>
Ampicillin	25	12.50	12.50	25
Initial set				
2a	62.50	62.50	62.50	125
2b	500	250	250	500
2c	125	62.50	62.50	125
2d	125	250	500	125
2e	31.25	62.50	125	125
3a	250	250	500	250
3b	125	500	125	125
3c	250	1000	250	250
3d	500	250	250	500
3e	125	125	125	125
Final set				
2f	31.25	31.25	62.50	62.50
3f	62.50	62.50	31.25	62.50

Conclusions

Topliss modified approach can be successfully applied for the selection of substituent at the fourth position of the phenyl ring in the series of 6-[4-substitutedphenyl]-4-phenyl-6H-1,3-oxazin-2-amines and *N*-[6-(4-substitutedphenyl) - 4 - phenyl - 6H - 1,3 - oxaziny] acetamides for the development of better antimicrobial agents. The study revealed that electron donating polar substituents at the fourth position of the phenyl ring from 6-position are required to improve antimicrobial potential in the series of 6-[4-substitutedphenyl] - 4 - phenyl - 6H - 1,3 - oxazin -2- amines and *N*-[6-(4-substitutedphenyl)-4-phenyl-6H-1,3-oxaziny]acetamides.

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Manuscript received: March 9th 2011