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Synthesis of novel naphtho[1,2-e][1,3]oxazines bearing an arylsulfonamide moiety and their anticancer and antifungal activity evaluations

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Abstract

A series of novel naphtho[1,2-e][1,3]oxazines bearing arylsulfonamide moiety have been synthesized via a one-pot approach and in a green reaction medium. These new naphtho[1,2-e][1,3]oxazine derivatives have been characterized by their ¹H NMR, ¹³C NMR and the X-ray single crystallography method for compound **7a**. All the newly synthesized compounds were examined for their *in vitro* anticancer activity against breast (MCF-7), colon (HCT116), and B-CLL (Waco3-CD5) cancers. Some of these compounds such as **7j** and **7l** showed remarkable activities against MCF-7 (breast) and HCT116 (colon) cancers with comparable IC₅₀ (The half maximal inhibitory concentration) values as that of known drugs such as 5-fluorouracil (5-FU). *In vitro* antimicrobial activities of all compounds were also evaluated against five human pathogenic fungi strains and two bacteria (one gram positive and one gram negative). The best MICs (Minimum Inhibitory Concentrations) were found against the *C. albicans*.

Keywords

Anticancer

Naphthoxazine

Sulfonamide

Synthesis

Antifungal

1. Introduction

The development of environmentally friendly and economically inexpensive processes for the synthesis of biologically active heterocycles using readily available and cheap reagents is an important goal of current organic synthesis (Laszlo, 1995). 1,3-Oxazine derivatives condensed with aromatic rings have gained much attention due to varied pharmacological and biological properties such as anticancer (Urbański et al., 1956, Chylińska et al., 1963, Verma et al., 2012, Bouaziz et al., 1991, Thaler et al., 2013, Benameur et al., 1996), antibacterial (Mathew et al., 2010, Kategaonkar et al., 2010, Mayekar et al., 2011, Didwagh and Piste, 2013, Abou-Elmagd and Hashem, 2013, Verma et al., 2012), antifungal (Verma et al., 2012, Bouaziz et al., 1991, Mathew et al., 2010, Kategaonkar et al., 2010, Mayekar et al., 2011), anti-HIV (Pedersen and Pedersen, 2000, Cocuzza et al., 2001), and anti-inflammatory (Akhter et al., 2011) activities. Naphtho[1,2-e][1,3]oxazine derivatives are also included in this category.

Owing to their medicinal importance, several methods have been reported for the preparation of these compounds in the literature. The most widely adopted route has been benefited from a Mannich-type condensation reaction between phenols, primary amines, and formaldehyde through either direct or indirect pathway. The multi-component condensation of phenols or naphthols with primary amines (or ammonia) and two equivalents of aldehydes leads to these target molecules directly (Burke, 1949, Burke et al., 1952, Burke et al., 1954, Burke and Reynolds, 1954, Dhakane et al., 2014, Sadaphal et al., 2010), whereas a Betti base as starting material represents an indirect approach to the synthesis of 1,3-naphthoxazines (Shi et al., 2010, Turgut et al., 2007, Sapkal et al., 2009, Verma et al., 2012). Another method includes using the condensation reaction of salicylaldehyde with a primary amine followed by reduction and

subsequent cyclization reaction with a suitable aldehyde (Tang et al., 2011, Tang et al., 2012). Furthermore, miscellaneous approaches such as reaction of oxygen-containing dihalo compounds with primary amines (Colin and Loubinoux, 1982), rhodium-catalyzed reaction of 2-(alkenyloxy)benzylamines with a mixture of H₂/CO (Campi et al., 1994, Campi et al., 1996), neutral redox reaction of cyclic amines and 2-hydroxy aromatic aldehydes or ketones (Richers et al., 2014), and direct reaction of ortho-lithiated phenols or thiophenols with *N,N*-bis[(benzotriazol-1-yl)methyl]amines (Katritzky et al., 2002) have also been reported.

The prominent roles of this heterocyclic skeleton in pharmaceutical chemistry inspired chemists to develop novel synthetic methods for efficient preparation of these biologically important compounds. On the other hand, the value of sulfonamide moiety in medicinal chemistry cannot be disregarded, as it consists of a large class of drugs used broadly as pharmaceutical agents. Various biological properties such as antibacterial (Bekdemir et al., 2008), antiviral, (Romines et al., 1997) and carbonic anhydrase (CA) inhibitory (Duffel et al., 1986, Modak et al., 2016) have been reported for the sulfonamide structures as lead (Jain et al., 2013).

Over the past years, dithioimidocarbonates have been identified as useful synthons in organic synthesis and in construction of diverse Heterocycles (Zali-Boeini et al., 2015, Maybhate et al., 1991, Servi et al., 2005, Sato et al., 1981, Alvarez Ibarra et al., 1985, Sauter et al., 1995, Sullivan et al., 1976).

Meanwhile, our preliminary plan was to extend the synthetic utility of 1-(amino(aryl)methyl)naphthalen-2-ol derivatives (Betti bases) by examining their applications in the synthesis of novel 1,3-naphthoxazine derivatives. We intended to develop a novel one-step and efficient method for synthesis of new 1-aryl-2,3-dihydro-1H-naphtho[1,2-e][1,3]oxazine derivatives bearing a sulfonamide moiety via the reaction of *N*-sulfonyldithioimidocarbonates and Betti base salts in the presence of a base in aqueous EtOH as a green solvent. After characterization of all synthesized compounds, their anticancer and antimicrobial activities were evaluated.

2. Experimental

2.1. Chemistry

Reagents and solvents were obtained from commercial suppliers and used as received unless otherwise indicated. Melting points were determined with a Stuart Scientific SMP-2 apparatus and

are uncorrected. Reactions were monitored by thin-layer chromatography (TLC Silica gel 60 F254). ^1H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded on a Bruker Avance 400 MHz NMR spectrometer referenced to residual solvent protons and signals are reported in ppm (δ). The singlet peak at 3.35 ppm in ^1H NMR spectra corresponds to protons of H_2O . X-ray crystallographic data was obtained using a Bruker AXS Kappa APEX II single crystal X-ray diffraction instrument.

2.1.1. General procedure for the synthesis of 1-(amino(aryl)methyl)naphthalen-2-ol hydrochlorides (Betti base hydrochlorides, 3a-e)

The Betti bases (**2a-e**) required for the synthesis of [1,3]naphthoxazines were obtained by the Betti reaction through a known method (Betti, 1941) as follows: In a 50 mL round-bottomed flask was placed a cold solution of β -naphthol (**1**) (7.2 g, 50 mmol) in 95 per cent alcohol (10 mL). To this solution was added benzaldehyde derivative (100 mmol) and EtOH (95%, 10 mL) saturated with ammonia at room temperature. The flask was stoppered and allowed to stand for 2 h. Then the excess ammonia was removed. After about 12 h, the condensation product (**2a-e**), which separated as white needle crystals, was filtered with suction and washed with EtOH (95%, 50 mL). The condensation product thus obtained was introduced into a 250 mL round-bottomed flask arranged for steam distillation and treated four times its volume with a solution of HCl (20%). The mixture was steam distilled about two hours. Meanwhile, an abundant flocculent precipitate of light pink or white needles was separated. The mixture in the flask was cooled thoroughly and filtered with suction. The yields of **3a-e** were 84–91%.

2.1.2. General procedure for the synthesis of arylsulfonamides (5a-d)

Arylsulfonyl chloride (50 mmol) and chloroform (20 mL) were placed in a 100 mL round-bottomed flask. To the mixture an aqueous solution of ammonia (25%, 20 mL) was added and the reaction mixture was stirred for 2 h at room temperature. Then, the precipitated product was filtered and washed with cold water. The crude product was recrystallized using EtOH to give pure sulfonamides (**5a-d**) as white crystals in excellent yields (91–98%).

2.1.3. General procedure for the synthesis of dimethyl arylsulfonylcarbonimidodithioates (6a-d)

Dimethyl arylsulfonylcarbonimidodithioates (**6a-d**) were synthesized according to a reported procedure (Maybhate et al. 1991) starting from aryl sulfonamides **5a-d** with slight modifications as follows: A solution of arylsulfonamide **5a-d** (14 mmol) in DMF (10 mL) was cooled in an ice-bath. To this, a solution of NaOH (20 M, 0.8 mL, 16 mmol) was added with vigorous stirring

followed by addition of CS₂ (0.7 g, 9 mmol). After 20 min a second lot of NaOH solution (20 M, 0.4 mL, 8 mmol) and CS₂ (0.35 g, 4.5 mmol) was added to the reaction mixture followed by a third portion of NaOH solution (20 M, 0.4 mL, 8 mmol) and CS₂ (0.35 g, 4.5 mmol) after an interval of 10 min. The ice-bath was removed and the mixture was stirred at r.t. for 2 h. The mixture was cooled in an ice-bath and dimethyl sulfate (3.73 g, 29 mmol) was added dropwise during 10 min. The reaction mixture was stirred for 2 h at r.t. and poured into H₂O (50 mL). The precipitated solid was filtered, washed with H₂O, and dried under vacuum. The crude product was recrystallized where necessary from a suitable solvent to obtain compounds **6a-d** as odoriferous white solids in good yields (75–86%).

2.1.4. General procedure for the synthesis of (E)-N-(1-aryl-1H-naphtho[1,2-e][1,3]oxazin-3(2H)-ylidene)benzenesulfonamide derivatives (7a–o)

A mixture of 1-(amino(aryl)methyl)naphthalen-2-ol hydrochloride **3** (1 mmol), dimethyl arylsulfonylcarbonimidodithioate **6** (1 mmol) and Na₂CO₃ (0.318 g, 3 mmol) in H₂O/EtOH (1: 3; 5 mL) was refluxed for 2 h. The reaction mixture was cooled to room temperature and neutralized by addition of aqueous HCl (10%). After some dilution with water, the liquor was decanted and the residue after purification by column chromatography using silica gel and a mixture of EtOAc-Hexane (1:1) as eluent gave pure compounds **7a–o** as white or off-white solids in moderate to good yields (43–78%).

2.1.5. Spectral data (¹H-NMR and ¹³C-NMR spectra) of the synthesized compounds

2.1.5.1. (E)-N-(1-phenyl-1H-naphtho[1,2-e][1,3]oxazin-3(2H)-ylidene)benzenesulfonamide (7a)

M.p.: 247–249 °C (decomp.); yield: 62%; ¹H NMR (400 MHz, DMSO-*d*₆): δ 10.18 (s, 1H), 8.03 (d, *J* = 9.0 Hz, 1H), 8.00–7.96 (m, 1H), 7.91 (d, *J* = 7.1 Hz, 2H), 7.80 (d, *J* = 8.7 Hz, 1H), 7.61–7.47 (m, 5H), 7.35–7.25 (m, 4H), 7.22 (d, *J* = 7.4 Hz, 2H), 6.34 (s, 1H); ¹³C NMR (100 MHz, DMSO-*d*₆): δ 152.1, 145.0, 143.2, 141.1, 131.9, 131.0, 130.7, 129.1, 128.8, 128.7, 128.4, 127.8, 126.9, 126.3, 125.8, 123.0, 115.8, 114.1, 52.0; IR (cm⁻¹, KBr): 3294, 3059, 1630, 1415, 1350, 1272, 1084; Anal. Calcd. for C₂₄H₁₈N₂O₃S: C, 69.55; H, 4.38; N, 6.76; S, 7.74 Found: C, 62.59; H, 4.41; N, 6.71; S, 7.77.

2.1.5.2. (E)-4-methyl-N-(1-phenyl-1H-naphtho[1,2-e][1,3]oxazin-3(2H)-ylidene)benzenesulfonamide (7b)

M.p.: 260–262 °C (decomp.); yield: 78%; ¹H NMR (400 MHz, DMSO-*d*₆): δ 10.08 (s, 1H), 8.04 (d, *J* = 9.0 Hz, 1H), 8.00–7.97 (m, 1H), 7.81–7.76 (m, 3H), 7.56–7.47 (m, 2H), 7.36 (d, *J* = 9.0 Hz,

1H), 7.34–7.25 (m, 5H), 7.21 (d, $J=7.1$, 2H), 6.33 (s, 1H), 2.35 (s, 3H); ^{13}C NMR (100 MHz, DMSO- d_6): δ 152.1, 145.1, 142.1, 141.1, 140.3, 130.9, 130.7, 129.2, 129.0, 128.7, 128.40, 128.36, 127.8, 126.9, 126.4, 125.8, 123.0, 115.8, 114.1, 52.0, 20.9; IR (cm^{-1} , KBr): 3240, 3140, 1643, 1431, 1311, 1288, 1153; Anal. Calcd. for $\text{C}_{25}\text{H}_{20}\text{N}_2\text{O}_3\text{S}$: C, 70.07; H, 4.70; N, 6.54; S, 7.48 Found: C, 70.09; H, 4.68; N, 6.51; S, 7.47.

2.1.5.3. (E)-4-chloro-N-(1-phenyl-1H-naphtho[1,2-e][1,3]oxazin-3(2H)-ylidene)benzenesulfonamide (7c)

M.p.: 244–246 °C; yield: 70%; ^1H NMR (400 MHz, DMSO- d_6): δ 10.27 (s, 1H), 8.04 (d, $J=9.0$ Hz, 1H), 8.00–7.96 (m, 1H), 7.92 (d, $J=8.6$ Hz, 2H), 7.81–7.77 (m, 1H), 7.60 (d, $J=8.6$ Hz, 2H), 7.55–7.47 (m, 2H), 7.37 (d, $J=9.0$ Hz, 1H), 7.34–7.26 (m, 3H), 7.21 (d, $J=7.2$ Hz, 2H), 6.34 (s, 1H). ^{13}C NMR (100 MHz, DMSO- d_6): δ 152.1, 145.1, 142.0, 141.2, 136.6, 131.0, 130.7, 129.0, 128.9, 128.7, 128.5, 128.4, 128.3, 127.7, 126.9, 125.8, 123.1, 115.8, 114.0, 52.1; IR (cm^{-1} , KBr): 3225, 3085, 1643, 1477, 1431, 1219, 1157; Anal. Calcd. for $\text{C}_{24}\text{H}_{17}\text{ClN}_2\text{O}_3\text{S}$: C, 64.21; H, 3.82; N, 6.24; S, 7.14 Found: C, 64.27; H, 3.80; N, 6.21; S, 7.11.

2.1.5.4. (E)-N-(1-(p-tolyl)-1H-naphtho[1,2-e][1,3]oxazin-3(2H)-ylidene)benzenesulfonamide (7d)

M.p.: 271–273 °C (decomp.); yield: 54%; ^1H NMR (400 MHz, DMSO- d_6): δ 10.10 (s, 1H), 8.02 (d, $J=9.0$ Hz, 1H), 7.99–7.95 (m, 1H), 7.90 (d, $J=7.0$ Hz, 2H), 7.79–7.75 (m, 1H), 7.61–7.47 (m, 5H), 7.32 (d, $J=9.0$ Hz, 1H), 7.13–7.07 (m, 4H), 6.29 (s, 1H), 2.23 (s, 3H); ^{13}C NMR (100 MHz, DMSO- d_6): δ 152.1, 145.0, 143.2, 138.2, 137.8, 131.9, 131.0, 130.6, 129.5, 128.8, 128.7, 128.4, 127.7, 126.8, 126.3, 125.8, 123.0, 115.7, 114.2, 51.8, 20.6; IR (cm^{-1} , KBr): 3245, 3078, 1650, 1446, 1423, 1223, 1130; Anal. Calcd. for $\text{C}_{25}\text{H}_{20}\text{N}_2\text{O}_3\text{S}$: C, 70.07; H, 4.70; N, 6.54; S, 7.48 Found: C, 70.10; H, 4.73; N, 6.50; S, 7.45.

2.1.5.5. (E)-4-methyl-N-(1-(p-tolyl)-1H-naphtho[1,2-e][1,3]oxazin-3(2H)-ylidene)benzenesulfonamide (7e)

M.p.: 203–205 °C; yield: 43%; ^1H NMR (400 MHz, DMSO- d_6): δ 10.08 (s, 1H), 8.01 (d, $J=9.0$ Hz, 1H), 7.96 (d, $J=7.1$ Hz, 1H), 7.76 (d, $J=8.0$ Hz, 3H), 7.53–7.46 (m, 2H), 7.33 (d, $J=9.0$ Hz, 1H), 7.29 (d, $J=8.0$ Hz, 2H), 7.11–7.06 (m, 4H), 6.25 (s, 1H), 2.34 (s, 3H), 2.22 (s, 3H); ^{13}C NMR (100 MHz, DMSO- d_6): δ 151.9, 145.3, 141.7, 140.6, 138.6, 137.5, 130.8, 130.4, 129.4, 129.0, 128.7, 128.5, 127.6, 126.8, 126.5, 125.6, 123.0, 115.9, 114.4, 52.0, 20.9, 20.6; IR

(cm^{-1} , KBr): 3244, 3067, 1651, 1435, 1219, 1161, 1134; Anal. Calcd. for $\text{C}_{26}\text{H}_{22}\text{N}_2\text{O}_3\text{S}$: C, 70.57; H, 5.01; N, 6.33; S, 7.25 Found: C, 70.61; H, 5.03; N, 6.30; S, 7.21.

2.1.5.6. (E)-4-chloro-N-(1-(p-tolyl)-1H-naphtho[1,2-e][1,3]oxazin-3(2H)-ylidene)benzenesulfonamide (7f)

M.p.: 224–226 °C (decomp.); yield: 55%; ^1H NMR (400 MHz, $\text{DMSO-}d_6$): δ 10.22 (s, 1H), 7.89 (d, $J=8.7$ Hz, 2H), 7.79–7.72 (m, 3H), 7.47–7.38 (m, 2H), 7.34 (d, $J=8.5$ Hz, 2H), 7.23 (d, $J=8.9$ Hz, 1H), 6.99–6.92 (m, 4H), 6.07 (s, 1H), 2.21 (s, 3H); ^{13}C NMR (100 MHz, $\text{DMSO-}d_6$): δ 150.8, 146.9, 143.4, 140.9, 136.2, 135.1, 130.4, 129.2, 129.1, 128.9, 128.6, 128.5, 127.8, 127.0, 126.7, 124.7, 122.9, 116.3, 115.3, 53.6, 20.6; IR (cm^{-1} , KBr): 3290, 3074, 1651, 1423, 1335, 1219, 1011; Anal. Calcd. for $\text{C}_{25}\text{H}_{19}\text{ClN}_2\text{O}_3\text{S}$: C, 64.86; H, 4.14; N, 6.05; S, 6.93 Found: C, 64.80; H, 4.11; N, 6.07; S, 6.98.

2.1.5.7. (E)-N-(1-(4-isopropylphenyl)-1H-naphtho[1,2-e][1,3]oxazin-3(2H)-ylidene)-4-methylbenzenesulfonamide (7g)

M.p.: 255–257 °C; yield: 51%; ^1H NMR (400 MHz, $\text{DMSO-}d_6$): δ 10.07 (s, 1H), 8.03 (d, $J=9.0$ Hz, 1H), 8.00–7.96 (m, 1H), 7.82–7.77 (m, 3H), 7.56–7.47 (m, 2H), 7.38–7.31 (m, 3H), 7.18 (d, $J=8.3$ Hz, 2H), 7.12 (d, $J=8.3$ Hz, 2H), 6.29 (s, 1H), 2.81 (sep, $J=6.9$ Hz, 1H), 2.35 (s, 3H), 1.13 (dd, $J=6.9$ Hz, 1.9 Hz, 6H); ^{13}C NMR (100 MHz, $\text{DMSO-}d_6$): δ 152.1, 148.6, 145.1, 142.0, 140.4, 138.6, 130.9, 130.6, 129.2, 128.7, 128.4, 127.8, 126.94, 126.87, 126.4, 125.8, 123.0, 115.8, 114.3, 51.7, 33.0, 23.7, 23.6, 20.9; IR (cm^{-1} , KBr): 3240, 3090, 1643, 1419, 1304, 1161, 1134; Anal. Calcd. for $\text{C}_{28}\text{H}_{26}\text{N}_2\text{O}_3\text{S}$: C, 71.46; H, 5.57; N, 5.95; S, 6.81 Found: C, 71.49; H, 5.52; N, 5.97; S, 6.85.

2.1.5.8. (E)-4-chloro-N-(1-(4-isopropylphenyl)-1H-naphtho[1,2-e][1,3]oxazin-3(2H)-ylidene)benzenesulfonamide (7h)

M.p.: 262–264 °C; yield: 55%; ^1H NMR (400 MHz, $\text{DMSO-}d_6$): δ 10.22 (s, 1H), 8.05 (d, $J=9.0$ Hz, 1H), 8.01–7.97 (m, 1H), 7.92 (d, $J=8.6$ Hz, 2H), 7.81 (d, $J=7.7$ Hz, 1H), 7.61 (d, $J=8.6$ Hz, 2H), 7.56–7.48 (m, 2H), 7.37 (d, $J=9.0$ Hz, 1H), 7.18 (d, $J=8.3$ Hz, 2H), 7.12 (d, $J=8.3$ Hz, 2H), 6.31 (s, 1H), 2.81 (sep, $J=6.9$ Hz, 1H), 1.13 (dd, $J=6.9$ Hz, 1.6 Hz, 6H); ^{13}C NMR (100 MHz, $\text{DMSO-}d_6$): δ 152.3, 148.6, 145.0, 142.0, 138.6, 136.7, 131.0, 130.6, 128.9, 128.7, 128.5, 128.4, 127.8, 126.95, 126.88, 125.8, 123.1, 115.7, 114.2, 51.8, 33.0, 23.65, 23.57; IR (cm^{-1} , KBr): 3225, 3080, 1643, 1477, 1311, 1219, 1134; Anal. Calcd. for $\text{C}_{27}\text{H}_{23}\text{ClN}_2\text{O}_3\text{S}$: C, 66.05; H, 4.72; N, 5.71; S, 6.53 Found: C, 66.01; H, 4.76; N, 5.67; S, 6.57.

2.1.5.9. (E)-N-(1-(4-isopropylphenyl)-1H-naphtho[1,2-e][1,3]oxazin-3(2H)-ylidene)-4-methoxybenzenesulfonamide (7i)

M.p.: 228–230 °C; yield: 46%; ¹H NMR (400 MHz, DMSO-*d*₆): δ 10.01 (s, 1H), 8.04 (d, *J* = 9.0 Hz, 1H), 8.00–7.97 (m, 1H), 7.84 (d, *J* = 8.9 Hz, 2H), 7.82–7.79 (m, 1H), 7.56–7.48 (m, 2H), 7.38 (d, *J* = 9.0 Hz, 1H), 7.18 (d, *J* = 8.2 Hz, 2H), 7.12 (d, *J* = 8.2 Hz, 2H), 7.05 (d, *J* = 8.9 Hz, 2H), 6.28 (s, 1H), 3.81 (s, 3H), 2.81 (sep, *J* = 6.9 Hz, 1H), 1.13 (dd, *J* = 6.9 Hz, 1.8 Hz, 6H); ¹³C NMR (100 MHz, DMSO-*d*₆): δ 161.7, 152.0, 148.5, 145.1, 138.7, 135.0, 130.9, 130.6, 128.7, 128.5, 128.4, 127.8, 126.9, 126.8, 125.8, 123.0, 115.9, 114.4, 113.8, 55.5, 51.7, 33.0, 23.65, 23.61; IR (cm⁻¹, KBr): 3244, 3086, 1651, 1427, 1292, 1257, 1184; Anal. Calcd. for C₂₈H₂₆N₂O₄S: C, 69.11; H, 5.39; N, 5.76; S, 6.59 Found: C, 69.08; H, 5.36; N, 5.79; S, 6.54.

2.1.5.10. (E)-N-(1-(4-chlorophenyl)-1H-naphtho[1,2-e][1,3]oxazin-3(2H)-ylidene)benzenesulfonamide (7j)

M.p.: 226–228 °C; yield: 63%; ¹H NMR (400 MHz, DMSO-*d*₆): δ 10.20 (s, 1H), 8.03 (d, *J* = 9.0 Hz, 1H), 8.00–7.96 (m, 1H), 7.90–7.87 (m, 2H), 7.78–7.74 (m, 1H), 7.59–7.47 (m, 5H), 7.37 (d, *J* = 8.5 Hz, 2H), 7.32 (d, *J* = 9.0 Hz, 1H), 7.21 (d, *J* = 8.5 Hz, 2H), 6.35 (s, 1H); ¹³C NMR (100 MHz, DMSO-*d*₆): δ 151.9, 145.4, 143.2, 140.3, 132.9, 131.8, 130.9, 130.7, 129.0, 128.8, 128.75, 128.69, 128.4, 127.8, 126.4, 125.8, 122.9, 115.9, 113.7, 51.5; IR (cm⁻¹, KBr): 3240, 3082, 1643, 1473, 1284, 1184, 1084; Anal. Calcd. for C₂₄H₁₇ClN₂O₃S: C, 64.21; H, 3.82; N, 6.24; S, 7.14 Found: C, 64.25; H, 3.82; N, 6.22; S, 7.13.

2.1.5.11. (E)-N-(1-(4-chlorophenyl)-1H-naphtho[1,2-e][1,3]oxazin-3(2H)-ylidene)-4-methylbenzenesulfonamide (7k)

M.p.: 249–251 °C (decomp.); yield: 47%; ¹H NMR (400 MHz, DMSO-*d*₆): δ 10.05 (s, 1H), 8.04 (d, *J* = 8.9 Hz, 1H), 7.98 (d, *J* = 7.2 Hz, 1H), 7.77 (d, *J* = 7.8 Hz, 2H), 7.75–7.71 (m, 1H), 7.55–7.46 (m, 2H), 7.39–7.34 (d, *J* = 8.4 Hz, 3H), 7.30 (d, *J* = 7.8 Hz, 2H), 7.21 (d, *J* = 8.2 Hz, 2H), 6.36 (s, 1H), 2.34 (s, 3H); ¹³C NMR (100 MHz, DMSO-*d*₆): δ 152.1, 145.2, 142.2, 140.0, 139.9, 133.0, 130.95, 130.91, 129.2, 129.1, 128.9, 128.8, 128.3, 127.9, 126.4, 125.9, 122.9, 115.9, 113.6, 51.3, 20.9; IR (cm⁻¹, KBr): 3294, 3047, 1635, 1411, 1377, 1234, 1080; Anal. Calcd. for C₂₅H₁₉ClN₂O₃S: C, 64.86; H, 4.14; N, 6.05; S, 6.93 Found: C, 64.82; H, 4.13; N, 6.08; S, 6.95.

2.1.5.12. (E)-4-chloro-N-(1-(4-chlorophenyl)-1H-naphtho[1,2-e][1,3]oxazin-3(2H)-ylidene)benzenesulfonamide (7l)

M.p.: 242–244 °C (decomp.); yield: 75%; ¹H NMR (400 MHz, DMSO-*d*₆): δ 10.25 (s, 1H), 7.85 (d, *J* = 7.7 Hz, 1H), 7.82 (d, *J* = 8.9 Hz, 1H), 7.72 (d, *J* = 8.2 Hz, 1H), 7.66 (d, *J* = 8.5 Hz, 2H), 7.45–7.33 (m, 2H), 7.20 (d, *J* = 8.5 Hz, 2H), 7.15 (m, 1H), 7.12 (d, *J* = 8.5 Hz, 2H), 6.96 (d, *J* = 8.5 Hz, 2H), 5.97 (s, 1H); ¹³C NMR (100 MHz, DMSO-*d*₆): δ 149.9, 148.4, 144.6, 144.5, 134.1, 130.7, 130.0, 129.6, 128.8, 128.6, 128.5, 128.4, 127.8, 127.1, 126.7, 124.0, 122.6, 116.8, 115.5, 54.3; IR (cm⁻¹, KBr): 3290, 3067, 1635, 1488, 1250, 1153, 1080; Anal. Calcd. for C₂₄H₁₆Cl₂N₂O₃S: C, 59.64; H, 3.34; N, 5.80; S, 6.63 Found: C, 59.62; H, 3.31; N, 5.85; S, 6.61.

2.1.5.13. (E)-N-(1-(4-chlorophenyl)-1H-naphtho[1,2-e][1,3]oxazin-3(2H)-ylidene)-4-methoxybenzenesulfonamide (7m)

M.p.: 235 °C (decomp.); yield: 65%; ¹H NMR (400 MHz, DMSO-*d*₆): δ 10.10 (s, 1H), 8.00 (d, *J* = 9.0 Hz, 1H), 7.96 (d, *J* = 7.6 Hz, 1H), 7.80–7.73 (m, 3H), 7.53–7.45 (m, 2H), 7.36–7.30 (m, 3H), 7.17 (d, *J* = 8.2 Hz, 2H), 6.96 (d, *J* = 8.6 Hz, 2H), 6.28 (s, 1H), 3.79 (s, 3H); ¹³C NMR (100 MHz, DMSO-*d*₆): δ 161.4, 151.6, 145.9, 141.0, 135.4, 132.5, 130.7, 130.4, 128.8, 128.7, 128.6, 128.5, 127.6, 125.5, 122.8, 116.1, 114.1, 113.5, 55.4, 51.9; IR (cm⁻¹, KBr): 3290, 3070, 1643, 1497, 1338, 1257, 1084; Anal. Calcd. for C₂₅H₁₉ClN₂O₄S: C, 62.69; H, 4.00; N, 5.85; S, 6.69 Found: C, 62.66; H, 4.04; N, 5.81; S, 6.73.

2.1.5.14. (E)-4-chloro-N-(1-(4-methoxyphenyl)-1H-naphtho[1,2-e][1,3]oxazin-3(2H)-ylidene)benzenesulfonamide (7n)

M.p.: 193–195 °C; yield: 48%; ¹H NMR (400 MHz, DMSO-*d*₆): δ 10.20 (s, 1H), 8.01 (d, *J* = 9.0 Hz, 1H), 7.99–7.95 (m, 1H), 7.89 (d, *J* = 8.5 Hz, 2H), 7.76 (d, *J* = 7.5 Hz, 1H), 7.55 (d, *J* = 8.5 Hz, 2H), 7.53–7.45 (m, 2H), 7.34 (d, *J* = 9.0 Hz, 1H), 7.08 (d, *J* = 8.7 Hz, 2H), 6.83 (d, *J* = 8.7 Hz, 2H), 6.24 (s, 1H), 3.69 (s, 3H); ¹³C NMR (100 MHz, DMSO-*d*₆): δ 158.9, 151.9, 145.3, 142.3, 136.3, 133.7, 130.9, 130.3, 128.7, 128.52, 128.49, 128.2, 127.6, 125.6, 123.0, 115.8, 114.4, 114.2, 55.0, 51.9; IR (cm⁻¹, KBr): 3240, 3086, 1643, 1438, 1254, 1161, 1080; Anal. Calcd. for C₂₅H₁₉ClN₂O₄S: C, 62.69; H, 4.00; N, 5.85; S, 6.69 Found: C, 62.65; H, 4.02; N, 5.87; S, 6.66.

2.1.5.15. (E)-4-methoxy-N-(1-(4-methoxyphenyl)-1H-naphtho[1,2-e][1,3]oxazin-3(2H)-ylidene)benzenesulfonamide (7o)

M.p.: 182–184 °C; yield: 55%; ¹H NMR (400 MHz, DMSO-*d*₆): δ 10.02 (s, 1H), 8.01–7.97 (d, *J* = 9.0 Hz, 1H), 7.97–7.94 (m, 1H), 7.79 (d, *J* = 8.8 Hz, 2H), 7.78–7.74 (m, 1H), 7.53–7.47 (m,

2H), 7.33 (d, $J=9.0$ Hz, 1H), 7.08 (d, $J=8.6$ Hz, 2H), 6.98 (d, $J=8.8$ Hz, 2H), 6.82 (d, $J=8.6$ Hz, 2H), 6.21 (s, 1H), 3.79 (s, 3H), 3.68 (s, 3H); ^{13}C NMR (100 MHz, DMSO- d_6): δ 161.4, 158.8, 151.7, 145.6, 135.4, 134.0, 130.8, 130.2, 128.64, 128.60, 128.5, 128.2, 127.5, 125.4, 123.0, 116.0, 114.7, 114.1, 113.6, 55.4, 55.0 51.9; IR (cm^{-1} , KBr): 3271, 3067, 1643, 1496, 1261, 1153, 1084; Anal. Calcd. for $\text{C}_{26}\text{H}_{22}\text{N}_2\text{O}_5\text{S}$: C, 65.81; H, 4.67; N, 5.90; S, 6.76 Found: C, 65.85; H, 4.63; N, 5.96; S, 6.72.

3. Biology

3.1. *In vitro* investigation of antimicrobial activity

3.1.1. Microorganisms

The antimicrobial activities of the synthetic compounds against four American Type Culture Collection (ATCC) microorganisms, including *Candida albicans* (ATCC 10261), *A. fumigatus* (ATCC 14110), *Staphylococcus aureus* (ATCC 700698), and *Escherichia coli* (ATCC 25912) were determined. Moreover, three clinical isolates of dermatophytes including *Epidermophyton floccosum* (SUCC 93-552), *Microsporiumcanis* (SUCC 93-560) and *Trichophytonrubrum* (SUCC 93-565) were also tested in this study. The susceptibility of all isolated bacteria and fungi against selected antibiotics were examined by microdilution methods (Clinical and Laboratory Standards Institute, 2006a, Clinical and Laboratory Standards Institute, 2006b, Clinical and Laboratory Standards Institute, 2006c).

3.1.2. Determination of minimum inhibitory concentration

MICs were determined using broth microdilution method recommended by the CLSI with some modifications (Clinical and Laboratory Standards Institute, 2006a, 2006b, 2006c). Briefly, for determination of antifungal activities against fungi, serial dilutions of the synthesized compounds 7a-o (0.25–256.0 $\mu\text{g}/\text{ml}$) were prepared in 96-well microtiter plate using RPMI-1640 media (Sigma, St. Louis, USA) buffered with MOPS (Sigma, St. Louis, USA). To determine the antibacterial activities, serial dilutions of the synthesized compounds **7a–o** (0.25–256.0 $\mu\text{g}/\text{ml}$) were prepared in Muller-Hinton Broth media (Merck, Darmstadt, Germany). The test fungi or bacteria strains were suspended in the media and the cell densities were adjusted to 0.5 McFarland

standards at 530 nm wavelength using a spectrophotometric method (this yields stock suspension of $1-5 \times 10^6$ cells/ml for yeast and $1-1.5 \times 10^8$ cells/ml for bacteria). One hundred microliters of the working inoculums was added to the microtiter plates which were incubated in a humid atmosphere at 30 °C for 24–48 h (fungi) or at 37 °C for 24 h (bacteria). Two hundred microliters of the uninoculated medium was included as a sterility control (blank). In addition, growth controls (medium with inoculums but without drugs) were also included. The growth in each well was compared with that of the growth control well. MICs were visually determined and defined as the lowest concentration of the synthetic compounds or antibiotics that prevents any visible growth (MIC₉₀) of microorganisms (both bacteria and fungi). For fungi MIC₅₀ were also determined and defined as the lowest concentration of the compounds that inhibits about half, or 50% growth of fungal cells than that of the controls. Each experiment was performed in triplicate. The reference antibiotics, fluconazole (Sigma, St. Louis, MO, USA), griseofulvin (sigma) and ciprofloxacin (sigma) were used as positive controls against saprophytes, dermatophytes and bacteria, respectively.

In addition, media from wells with fungi showing no visible growth were further cultured on Sabouraud Dextrose Agar (Merck, Darmstadt, Germany) and from wells with bacteria showing no visible growth on Muller-Hinton agar (Merck, Darmstadt, Germany) to determine the minimum fungicidal concentration (MFC) and minimum bactericidal concentration (MBC). MBCs and MFCs were determined as the lowest concentration yielding no more than 4 colonies, which corresponds to a mortality of 99.9% of the microbes in the initial inoculums.

3.2. Determination of cytotoxicity

3.2.1. Cell line and culture conditions

The human breast (MCF-7), human colon (HCT116) and human B-CLL (Waco3-CD5) cancer cell lines were used in the study. We used RPMI-1640 medium supplemented with 10% fetal bovine serum and 1% penicillin for cell growth under the condition of 37 °C in 5% CO₂.

Peripheral blood mononuclear cells (PBMC) were obtained by venipuncture from healthy adult donors and mononuclear cells were isolated by Ficoll-Hypaque gradient separation. Lymphocytes were washed and resuspended in RPMI medium supplemented with 10% fetal bovine serum. RPMI-1640 medium was used for B-CLL and DMEM medium for others. 5-Fluorouracil (5-FU)

and Rituximab were employed as positive control drugs. Rituximab (Mabthera) drug was obtained from Roche Company and 5-FU was purchased from Merck Company.

3.2.2. Cytotoxic activity

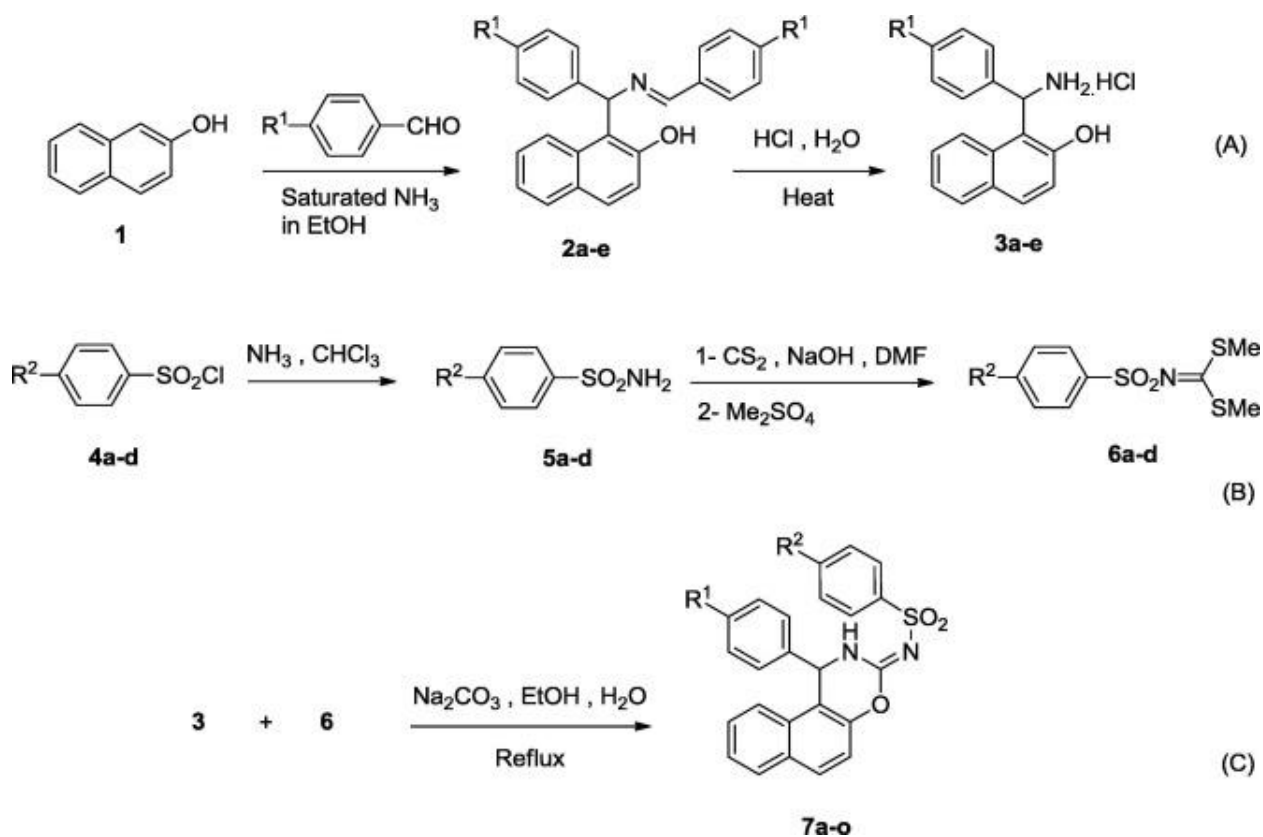
3.2.2.1. MTT assay

The MTT assay was used to evaluate the cytotoxicity of novel synthesized derivatives (**7a–o**) on cell viability (Liu et al., 1997). When cells were in exponentially growing phase, seeded at a concentration of 10,000 cells/well on 96 tissue culture plate overnight. After the culture medium replaced with a fresh one, a solution of synthesized compounds in DMSO was added to reach various concentrations. After 48 h, the drug solutions discarded and cells were washed with PBS. Then 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (5 mg/ml, in PBS) was added into each well and cultured for another 4 h. Subsequently, the supernatant replaced with DMSO to soften the formed formazan. In parallel, we used negative control, blank and positive control (5-FU and Rituximab) in the same conditions. At the final step, absorbance at the wavelength of 540 nm was read by a microplate reader. Data was calculated and expressed as the 50% inhibitory concentrations (IC₅₀) and presented as mean ± SD, n = 3. The MTT assay was also carried out on the non-cancer cells of PBMC as described for the cancer cell lines. As for the PBMC cells, lymphocytes were washed, resuspended in RPMI medium supplemented with 10% fetal bovine serum and plated (1×10^6 cells/mL) in the presence of phytohemagglutinin. After incubation for 24 h at 37 °C and 5% CO₂, the cells were treated with a solution of synthesized compounds in DMSO in various concentrations. (0.1–20 mmol L⁻¹) for 48 h. Cytotoxicity of violacein was determined by the MTT assay.

4. Results and discussion

4.1. Chemistry

A new route for the synthesis of novel 1-aryl-2,3-dihydro-1*H*-naphtho[1,2-*e*][1,3]oxazine derivatives bearing a sulfonamide moiety was accomplished in five steps starting from readily available chemicals in EtOH-H₂O as an environmentally benign medium with good to excellent yields. The synthetic approach is outlined in [Scheme 1](#).



Scheme 1. Synthetic approach for the preparation of Betti bases (A), *N*-sulfonyldithioimidocarbonates (B), and 1-aryl-2,3-dihydro-1*H*-naphtho[1,2-*e*][1,3]oxazine derivatives (C).

The Betti bases **3a-e** required for the synthesis of 1,3-naphthoxazines were obtained by the Betti reaction through a known method (Betti, 1941). Also, *N*-sulfonyldithioimidocarbonates **6a-d** were synthesized according to a reported procedure starting from arylsulfonamides **5a-d** (Maybhate et al., 1991). Finally, in the reaction of bis-nucleophiles **3a-e** and *N*-sulfonyldithioimidocarbonates **6a-d** in the presence of Na_2CO_3 and a mixture of EtOH- H_2O as a reaction medium, 1-aryl-2,3-dihydro-1*H*-naphtho[1,2-*e*][1,3]oxazine derivatives **7a-o** containing an arylsulfonamide moiety were obtained in moderate to good yields (43–78%). The results are summarized in Fig. 1.

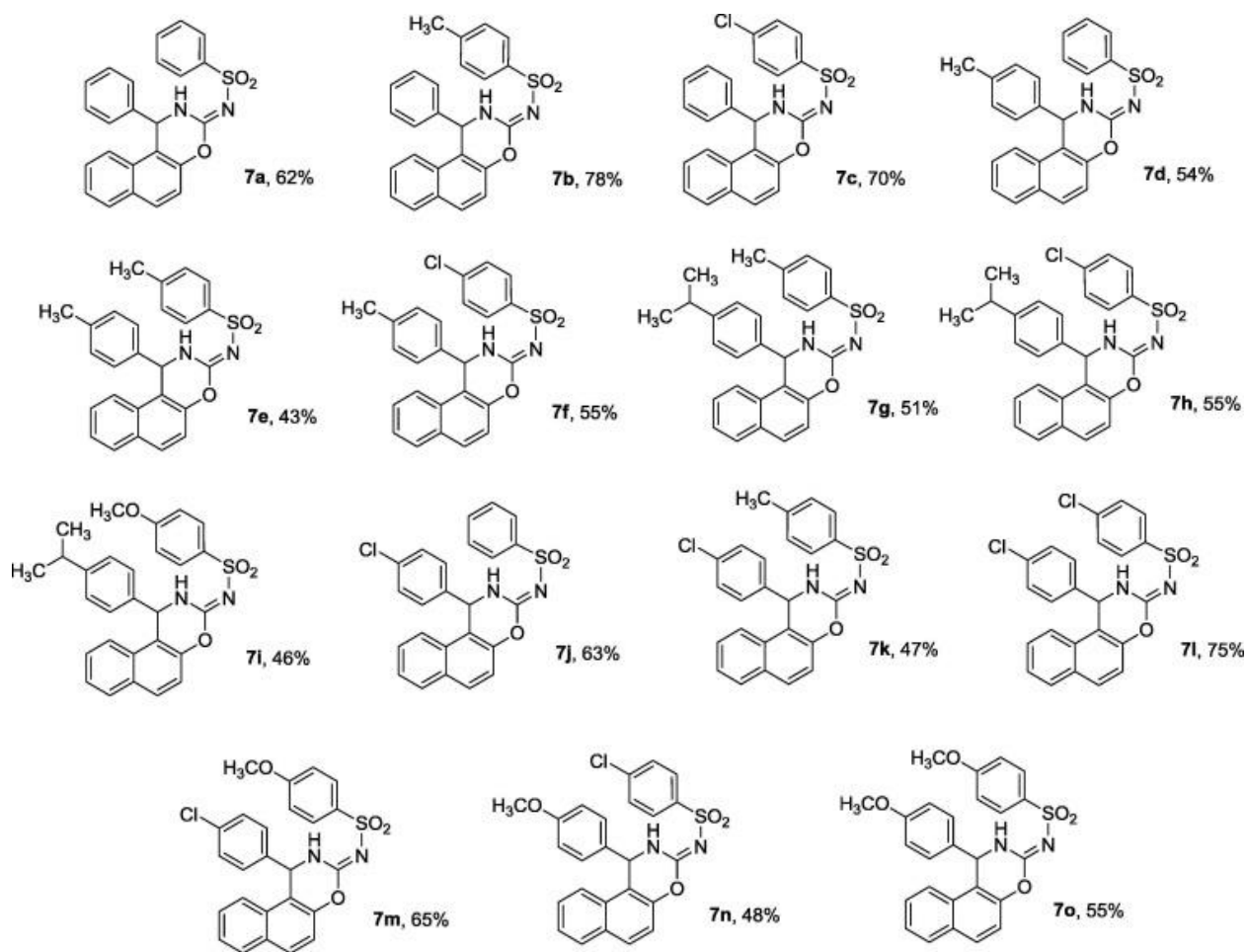


Fig. 1. One-step preparation of 1-aryl-2,3-dihydro-1H-naphtho[1,2-e][1,3]oxazine derivatives. (^aIsolated yields;) (^bReaction conditions: 1-(amino(aryl)methyl)naphthalen-2-ol hydrochloride **3** (1 mmol), dimethyl arylsulfonylcarbonimidodithioate **6** (1 mmol) and Na₂CO₃ (3 mmol) in H₂O/EtOH (1: 3, 5 mL), reflux condition, 2 h.)

The structure of compounds **7a–o** were proved by ¹H NMR, and ¹³C NMR spectra, alongside the X-ray single crystallography method. ¹H NMR spectrum of **7a** revealed the presence of a singlet at 10.17 ppm which could be assigned to NH and a singlet at 6.34 ppm attributed to the only aliphatic C H of the compound **7a**. Absolute assignment of compound **7a** was achieved by single-crystal X-ray crystallography (Fig. 2, CCDC 1405842). Single crystals of

compound **7a** was obtained thru slow evaporation of a solution of **7a** in acetonitrile.

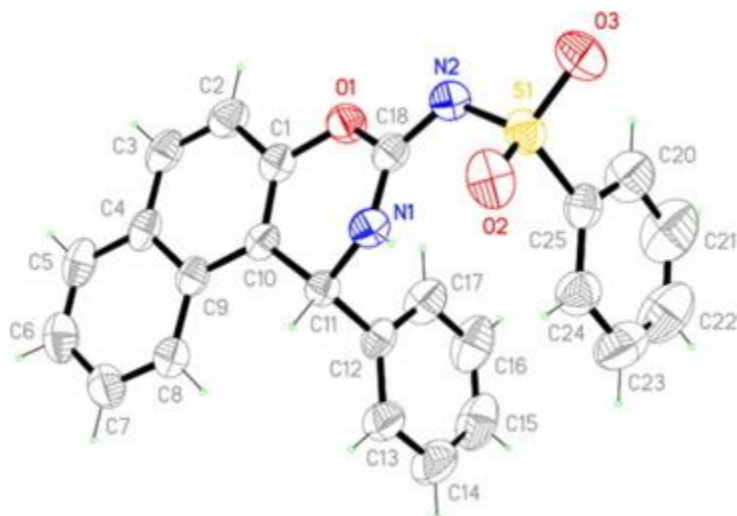


Fig. 2. ORTEP representation of **7a**. Displacement ellipsoids are drawn at the 50% probability level and H atoms are shown as small spheres of arbitrary radii.

The crystallographic data reveal that compound **7a** crystallized in a monoclinic system, space group $P2_1/n$. The asymmetric unit of **7a** contains a whole molecule (Fig.3). All bond lengths and angles are in the normal ranges and comparable with the reported ones (Gelbrich et al., 2007, Selig et al., 2009, Pfaffenrot et al., 2012).

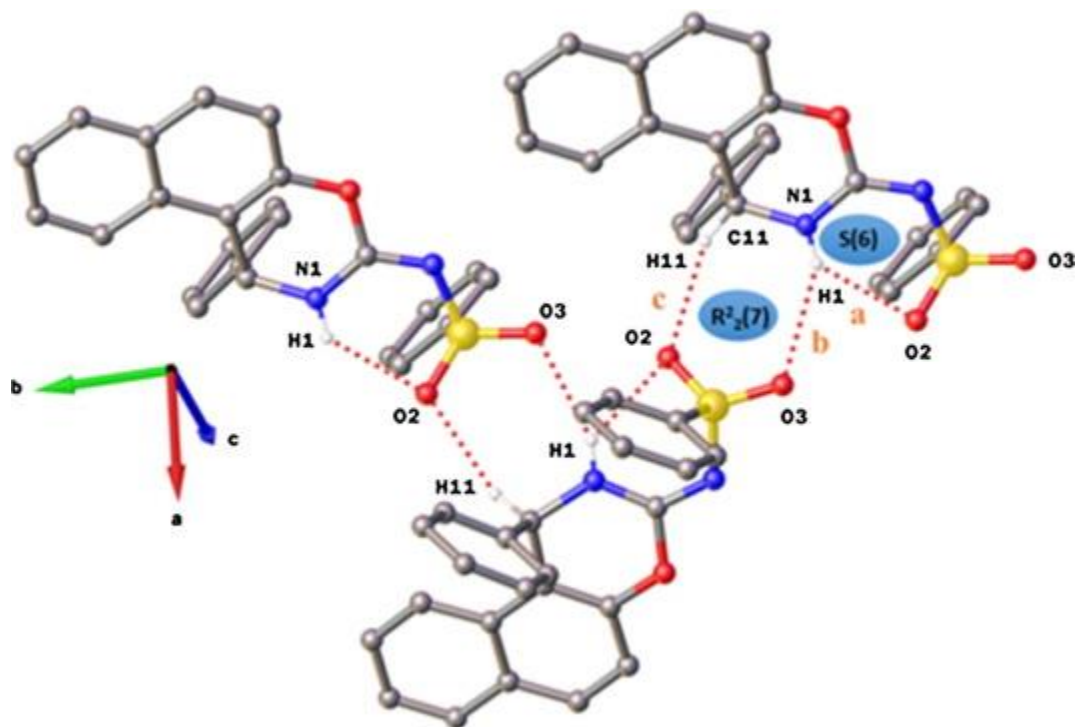


Fig. 3. A representation of intramolecular and intermolecular hydrogen bonds in molecule of **7a**. H atoms have been omitted for clarity except hydrogens that participate in hydrogen bonds.

The hydrogen-bonding pattern of compound **7a** can be described using graph theory (Bernstein et al., 1995, Grell et al., 1999). For this structure, there are a intramolecular hydrogen bond of the N

H...O type (dashed line a in Fig. 3), which is described as S(6) ring in the first level graph set (Fig. 3).

There are also two intermolecular hydrogen bonds of O...H C and O...H N types in the structure that create a dimer (dashed line b and c in Fig. 2) (Table 1). According to graph theory, a R²₂(7) ring is formed by two intermolecular hydrogen bonds (Fig. 3). According to the hydrogen bonding classification (Steiner, 2002, Desiraju and Steiner, 1999), these intramolecular and intermolecular hydrogen-bonding are moderate electrostatic interactions.

Table 1. Intramolecular and intermolecular hydrogen bond geometries (Å, °) for **7a**.

D	H...A	D	H	H...A	D...A	D	H...A
N(1)	H(1)...O(3) [#]	0.800(18)		2.543(18)	3.2175(19)		142.9(16)
N(1)	H(1)...O(2)	0.800(18)		2.235(17)	2.8462(18)		133.6(16)
C(11)	H(11)...O(2) [#]	0.98		2.38	3.2780(18)		151.6

#

Symmetry transformations used to generate equivalent atoms: $-x - 1/2, y + 1/2, -z + 3/2$.

The selected crystal structure data are presented in Table 2.

Table 2. Selected bond lengths (Å) and angles (°) for **7a**.

Bond		Bond length (Å)	Bonds			Bond angle (°)
S(1)	O(3)	1.4294(13)	C(18)	N(1)	C(11)	124.49(12)
S(1)	O(2)	1.4401(12)	C(18)	N(2)	S(1)	119.35(11)
S(1)	N(2)	1.6109(15)	N(1)	C(11)	C(10)	107.43(12)
S(1)	C(25)	1.7691(17)	N(1)	C(11)	C(12)	111.12(12)
N(1)	C(18)	1.3213(19)	C(18)	O(1)	C(1)	119.35(11)
N(1)	C(11)	1.4723(19)	N(2)	C(18)	O(1)	112.49(13)
N(2)	C(18)	1.307(2)	N(2)	S(1)	C(25)	105.26(7)
O(1)	C(18)	1.3532(18)	N(1)	C(18)	O(1)	117.78(14)
O(1)	C(1)	1.4003(19)				

5. Biology

5.1. Antimicrobial activity

In the present study, the antibacterial and antifungal effects of newly synthesized compounds on two bacteria (one gram positive and one gram negative) and various fungi were analyzed (Table 2).

Although Verma et al. (2012) reported antibacterial activity for their naphthoxazine derivatives, none of the examined compounds in this study exhibited antibacterial activity against the tested bacteria at the concentrations up to 256 µg/mL. The difference in antibacterial activities between their compounds and ours might be attributed to presence of an aryl group in their compounds instead of an arylsulfonamide moiety. Antibacterial inactivity of our synthesized compounds **7a**–

o also might be due to lack of an essential *p*-amino substituent in the aromatic ring at R² position, which is quite necessary for the antibacterial role of arylsulfonamides.

Among several tested fungi, the compounds **7a–o** only exhibited notable inhibitory activities against *Candida albicans* (yeasts) with MICs ranging from 32-256 µg/mL. Compounds **7c** and **7l** with a chloro substituent as an electron withdrawing group at the R² position exhibited the most anti *Candida* activity with MIC of 32 µg/mL. A comparison between **7c**, **7f**, **7l**, and **7n** which have a chloro substituent at R² position and respectively an H, CH₃, Cl and/or OCH₃ at R¹ position, revealed that existence of an electron withdrawing group at R¹ position enhances antifungal activity of the final product compared to electron donating groups. Moreover, comparison of **7b** and **7g** as well as **7c** and **7h** revealed that placement of a bulky group such as isopropyl at R¹ position might decrease the anti-*Candida* activity of the title compounds. We found that, placement of a halogen such as chloro as an electron withdrawing group in R² position leads to more effective compounds in terms of antifungal activity. As shown in [Table 3](#), lack of a substituent at para position of arylsulfonamide moiety (compounds **7a**, **7d**, and **7j**) results in lessening the inhibitory activity against the *Candida albicans* and the best result was obtained when there is a methyl group at this position (**7e**). These data show that, this class of compounds could be regarded as fungistatic antifungal agents.

Table 3. Investigation of antimicrobial activities of compounds **7a–o**.

Empty Cell		Minimum inhibitory concentration (MIC) (µg/ml sample)												
		<i>Microorganisms</i>												
		<i>E. coli</i>		<i>S. aureus</i>		<i>C. albicans</i>			<i>A. fumigatus</i>			<i>E. floccos</i>		
Entry	R ¹	R ²	MIC ₉₀	MBC	MIC ₉₀	MBC	MIC ₅₀	MIC ₉₀	MFC	MIC ₅₀	MIC ₉₀	MFC	MIC ₅₀	MFC
7a	H	H	G	G	G	G	32	128	G	G	G	G	G	G
7b	H	CH ₃	G	G	G	G	128	256	G	G	G	G	G	G
7c	H	Cl	G	G	G	G	16	32	G	G	G	G	G	G

Empty Cell			Minimum inhibitory concentration (MIC) (µg/ml sample)												
			<i>Microorganisms</i>												
			<i>E. coli</i>		<i>S. aureus</i>		<i>C. albicans</i>			<i>A. fumigatus</i>			<i>E. floccos</i>		
Entry	R ¹	R ²	MIC ₉₀	MBC	MIC ₉₀	MBC	MIC ₅₀	MIC ₉₀	MFC	MIC ₅₀	MIC ₉₀	MFC	MIC ₅₀	M	
7d	Me	H	G	G	G	G	256	G	G	G	G	G	G	G	
7e	Me	CH ₃	G	G	G	G	8	64	G	G	G	G	G	G	
7f	Me	Cl	G	G	G	G	16	64	G	G	G	G	256	G	
7g	<i>i</i> -Pr	CH ₃	G	G	G	G	256	G	G	G	G	G	G	G	
7h	<i>i</i> -Pr	Cl	G	G	G	G	256	G	G	G	G	G	G	G	
7i	<i>i</i> -Pr	OCH ₃	G	G	G	G	32	64	G	G	G	G	G	G	
7j	Cl	H	G	G	G	G	32	256	G	G	G	G	256	G	
7k	Cl	CH ₃	G	G	G	G	64	64	G	G	G	G	G	G	
7l	Cl	Cl	G	G	G	G	16	32	G	G	G	G	G	G	
7m	Cl	OCH ₃	G	G	G	G	32	64	G	G	G	G	G	G	
7n	OMe	Cl	G	G	G	G	32	64	G	G	G	G	256	G	
7o	OMe	OCH ₃	G	G	G	G	32	128	G	G	G	G	256	G	
FCZ			–	–	–	–	2	4	G	2	8	G	–	–	

Empty Cell		Minimum inhibitory concentration (MIC) ($\mu\text{g/ml}$ sample)													
		<i>Microorganisms</i>													
		<i>E. coli</i>		<i>S. aureus</i>		<i>C. albicans</i>			<i>A. fumigatus</i>			<i>E. floccos</i>			
Entry	R ¹	R ²	MIC ₉₀	MBC	MIC ₉₀	MBC	MIC ₅₀	MIC ₉₀	MFC	MIC ₅₀	MIC ₉₀	MFC	MIC ₅₀	M	
GF			–	–	–	–	–	–	–	–	–	–	–	0.5	1
CIPX			0.025	G	0.5	G	–	–	–	–	–	–	–	–	–

G: >256 $\mu\text{g/ml}$; FCZ: Fluconazole; GF: Griseofulvin; CIPX: Ciprofloxacin.

5.2. Cytotoxic activity

All the compounds were tested *in vitro* against a 3-cell line panel consisting of HCT116, ATCC No.: CCL-247 (human colon cancer), MCF-7, ATCC No.: HTB-22 (human breast cancer), and Waco3-CD5, NCBI No.: C547 (human chronic lymphocytic leukemia) and their IC₅₀ values were obtained. The MTT assay was also carried out on the non-cancer cells of PBMC as described for the cancer cell lines. Results of IC₅₀ experiment demonstrated that all of the compounds prepared in this study showed more inhibitory effect on colon and breast cancer cell lines compared to B-CLL cell line. Among the tested compounds, **7j** and **7l** showed the most cytotoxic effects on breast and colon cancer cell lines whereas their cytotoxicity on Waco3-CD5 was negligible. The lowest cytotoxic effects were achieved by the compound **7f** which showed the lowest toxicity on all the cell lines tested. The presence of electron withdrawing chloro group at either R¹ or R² position improved the anticancer activity in these structures, but the combination of CH₃ and Cl at R¹ and R² positions might decrease the cytotoxicity of these compounds (Table 4). The results of cytotoxicity assay on the non-cancer cells of PBMC demonstrated that the compounds prepared in this investigation had less toxic effects on non-cancer cells in comparison with the cancer cell lines. The compounds of **7g** and **7i** exhibited the lowest toxic effects on PBMC cells whereas the **7a**, **7n** and **7o** showed the highest toxicity on PBMC cells. However, all of the compounds prepared in this study showed higher toxicity on PBMC cells compared with 5FU. The high toxicity of

rituximab on PBMC cell could be explained by the fact that the phytohemagglutinin used for the preparation of the cells results in the over expression of CD20 protein on the surface of the cells which acts as the receptor for rituximab. Therefore, the normal cells with the receptor for the cytotoxic drug could be affected.

Table 4. Cytotoxicity evaluation of new compounds **7a-o** against three different cancer cell lines and PBMC cells.

Empty Cell	Empty Cell	PBMC	Breast	Colon	B-CLL
Empty Cell	Empty Cell	Normal	(MCF-7)	(HCT116)	(Waco3-CD5)
Compd.	R¹	Empty Cell	IC₅₀(μM)		
7a	H	62.20 \pm 2.9	35.11 \pm 4.3	28.56 \pm 2.4	53.56 \pm 3.3
7b	H	80.56 \pm 1.7	47.85 \pm 0.6	54.93 \pm 1.0	91.29 \pm 0.6.0
7c	H	94.01 \pm 3.4	38.37 \pm 2.3	34.36 \pm 2.3	88.11 \pm 1.8
7d	CH ₃	78.62 \pm 1.8	37.11 \pm 3.1	21.18 \pm 1.8	76.15 \pm 4.6
7e	CH ₃	83.31 \pm 3.9	37.14 \pm 2.5	22.14 \pm 1.1	78.12 \pm 0.1
7f	CH ₃	106.74 \pm 2.8	96.31 \pm 7.0	93.05 \pm 1.7	95.57 \pm 0.9
7g	Isopropyl	127.92 \pm 3.6	62.25 \pm 6.1	71.92 \pm 0.2	92.97 \pm 0.2.7
7h	Isopropyl	93.47 \pm 4.2	46.87 \pm 3.8	68.28 \pm 4.6	89.96 \pm 3.6
7i	Isopropyl	129.1 \pm 2.9	46.06 \pm 0.5	60.41 \pm 2.4	108.39 \pm 5.9
7j	Cl	82.02 \pm 3.7	16.14 \pm 1.4	19.72 \pm 2.6	49.11 \pm 2.9

Empty Cell	Empty Cell	PBMC	Breast	Colon	B-CLL
Empty Cell	Empty Cell	Normal	(MCF-7)	(HCT116)	(Waco3-CD5)
Compd.	R¹	Empty Cell	IC₅₀(μM)		
7k	Cl	91.13 \pm 3.1	81.21 \pm 2.3	80.04 \pm 1.9	94.07 \pm 3.8
7l	Cl	79.29 \pm 4.6	15.22 \pm 1.7	18.72 \pm 1.5	51.01 \pm 1.0
7m	Cl	72.51 \pm 2.9	36.02 \pm 2.2	21.61 \pm 4.6	57.36 \pm 2.4
7n	OCH ₃	58.70 \pm 2.6	34.79 \pm 0.1	26.73 \pm 3.8	76.11 \pm 2.0
7o	OCH ₃	53.52 \pm 3.8	35.90 \pm 2.4	64.19 \pm 3.1	82.18 \pm 2.4
5-FU^a	–		14.24 \pm 1.2	18.21 \pm 0.7	–
Rituximab^a	–	30.16 \pm 3.7	–	–	17.36 \pm 2.1

a

5-FU and Rituximab were used as standard anticancer drugs.

As shown in Table 4, the cytotoxic effects of **7j** and **7l** against breast cancer cell line as well the **7d**, **7e**, **7j**, **7l**, and **7m** against colon cancer cells were comparable with the standard anticancer drug 5-FU. However, cytotoxic activities of the compounds **7a–o** against human B-CLL (Waco3-CD5) were lower than the corresponding standard drug Rituximab (with minimal IC₅₀ approximately 3 folds higher than the positive control).

6. Conclusion

In this study we reported the synthesis, characterization and anticancer activities evaluation of new 1-aryl-2,3-dihydro-1*H*-naphtho[1,2-*e*][1,3]oxazine derivatives bearing an arylsulfonamide moiety. The target compounds were obtained from the reaction of *N*-

sulfonyldithioimidocarbonates and Betti bases in the presence of Na₂CO₃ as a cheap base and in aqueous ethanol as a green medium. To study the potential anticancer and antifungal activities of the synthesized compounds, the screening tests were performed for three cancer cell lines and five fungi strains. The best anticancer activity were presented by **7j** and **7l** against all three kinds of cancer cell lines [HCT116, ATCC No.: CCL-247 (human colon cancer), MCF-7, ATCC No.: HTB-22 (human breast cancer), and Waco3-CD5, NCBI No.: C547 (human B-CLL)]. The results showed that, all the compound were less toxic on the normal cells of PBMC rather than the cancer cell lines which make them great candidates for further investigations. However, The MTT test could not be able to show the mechanism by which the compounds induce cytotoxicity on different cells including normal or cancer cell lines. Therefore, it is suggested to carry out the investigations on the mechanism of cytotoxic activity of the compounds to determine the potential role of different functional groups on the cytotoxic mechanism.

In vitro evaluations of the compounds **7a–o** revealed that they presented only notable antifungal activities against *Candida albicans* (yeasts). The most of compounds **7a–o** showed moderate antifungal activities but among them compounds **7c**, **7e**, **7f**, **7i**, **7l**, **7m**, and **7n** exhibited the highest activities. These results are suggesting that the synthesized compounds can be good nominees for future investigations to find new anticancer and antifungal compounds.

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Conflict of interest

The authors have declared no conflicts of interest.

References

Abou-Elmagd, W.S.I., Hashem, A.I., 2013. Synthesis of 1-amidoalkyl-2-naphthols and oxazines derivatives with study of their antibacterial and antiviral activities. *Med. Chem. Res.* 22, 2005–2013. Akhter, M., Habibullah, S., Misbahul, Hasan S., MumtazAlam, M., Akhter, N., Shaquiquzzaman, M., 2011. Synthesis of some new 3,4- dihydro-2H-1,3-benzoxazines under

microwave irradiation in solvent-free conditions and their biological activity. *Med. Chem. Res.* 20, 1147–1153. Alvarez Ibarra, C., Cereceda, J.A., Ortiz, P., Vicente, A., Quiroga, M. L., 1985. A novel synthesis of 2,3-dihydrooxazoles from dimethyl N-ethoxycarbonyl methyliminodithiocarbonate and aromatic aldehydes. *Tetrahedron Lett.* 26, 243–246. Benameur, L., Bouaziz, Z., Nebois, P., Bartoli, M.-H., Boitard, M., Fillion, H., 1996. Synthesis of furnaphth[1,3]oxazine and furo[1,3]oxazinoquinoline derivatives as precursors for an o-quinonemethide structure and potential antitumor agents. *Chem. Pharm. Bull.* 44, 605–608. Bernstein, J., Davis, R.E., Shimoni, L., Chang, N.-L., 1995. Patterns in hydrogen bonding: functionality and graph set analysis in crystals. *Angew. Chem Int. Ed. Engl.* 34, 1555–1573. Betti, M., 1941. b-Naphtholphenylaminomethane. *Org. Synth. Coll.* 1, 381. Bouaziz, Z., Riondel, J., Mey, A., Berlion, M., Villard, J., Fillion, H., 1991. Synthesis of some naphthoxazinecarbolactone derivatives with in vitro cytotoxic and antifungal activities. *Eur. J. Med. Chem.* 26, 469–472. Burke, W.J., 1949. 3,4-Dihydro-1,3,2H-benzoxazines. Reaction of psubstituted phenols with N,N-dimethylolamines. *J. Am. Chem. Soc.* 71, 609–612. Burke, W.J., Reynolds, R.J., 1954. Condensation of 2-naphthol with acetaldehyde ammonia. *J. Am. Chem. Soc.* 76, 1291–1293. Burke, W.J., Kolbezen, M.J., Stephens, C.W., 1952. Condensation of naphthols with formaldehyde and primary amines. *J. Am. Chem. Soc.* 74, 3601–3605. Burke, W.J., Murdock, K.C., Ec, G., 1954. Condensation of hydroxyaromatic compounds with formaldehyde and primary aromatic amines. *J. Am. Chem. Soc.* 76, 1677–1679. Campi, E.M., Jackson, W.R., Mccubbin, Q.J., Trnacek, A.E., 1994. Allylic rearrangements during the rhodium-catalysed reactions of 2-allyloxybenzylamines and 2-(N-allyl-N-benzylamino)benzylamine. *J. Chem. Soc. Chem. Commun.*, 2763 Campi, E.M., Jackson, W.R., Mccubbin, Q.J., Trnacek, A.E., 1996. The stereochemistry of organometallic compounds. XLIII. rhodium-catalysed reactions of 2-(alkenyloxy)benzylamines and 2-(Nallyl-N-benzylamino)benzylamine. *Aus. J. chem.* 49, 219–230. Chylin'ska, J.B., Urban'ski, T., Mordarski, M., 1963. Dihydro-1,3-oxazine derivatives and their antitumor activity. *J. Med. Chem.* 6, 484–487. Clinical and Laboratory Standards Institute, 2006c. Reference method for broth dilution antifungal susceptibility testing of yeasts, Approved standard, CLSI document M27–A3. Clinical and Laboratory Standards Institute, Wayne, PA. Clinical and Laboratory Standards Institute, 2006a. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, Approved standard, 7th edition, CLSI document M7– A7. Clinical and Laboratory Standards Institute, Wayne, PA. Clinical and Laboratory Standards Institute, 2006b. Reference method for broth dilution antifungal

susceptibility testing of filamentous fungi, Approved standard, CLSI document M38-A. Clinical and Laboratory Standards Institute, Wayne, PA. Cocuzza, A.J., Chidester, D.R., Cordova, B.C., Jeffrey, S., Parsons, R. L., Bacheler, L.T., Erickson-Viitanen, S., Trainor, G.L., Ko, S.S., 2001. Synthesis and evaluation of efavirenz (Sustiva) analogues as HIV-1 reverse transcriptase inhibitors: replacement of the cyclopropylacetylene side chain. *Bioorg. Med. Chem. Lett.* 11, 1177–1179. Colin, J.L., Loubinoux, B., 1982. Nouvelle voie d'accès aux dihydro-3,4-dihydro-1,3-benzoxazines-1,3. *Tetrahedron Lett.* 23, 4245–4246. Desiraju, G.R., Steiner, T., 1999. *The Weak Hydrogen Bond in Structural Chemistry and Biology*. Oxford University Press Inc., New York, p. 1999. Dhakane, V.D., Gholap, S.S., Deshmukh, U.P., Chavan, H.V., Bandgar, B.P., 2014. An efficient and green method for the synthesis of [1,3]oxazine derivatives catalyzed by thiamine hydrochloride (VB1) in water. *C. R. Chimie* 17, 431–436. Didwagh, S.S., Piste, P.B., 2013. Novel one-pot synthesis and antimicrobial activity of 6-chloro-2,4-diphenyl 3,4-dihydro-2H-1,3-benzoxazine derivatives. *Int. J. Chem. Tech. Res.* 5, 2199–2203. Duffel, M.W., Ing, I.S., Segarra, T.M., Dixon, J.A., Barfknecht, C.F., Schoenwald, R.D., 1986. N-Substituted sulfonamide carbonic anhydrase inhibitors with topical effects on intraocular pressure. *J. Med. Chem.* 29, 1488–1494. Gelbrich, T., Threlfall, T.L., Bingham, A.L., Hursthouse, M.B., 2007. Polymorph VI of sulfapyridine: interpenetrating two- and three-dimensional hydrogen-bonded nets formed from two tautomeric forms. *Acta Cryst.* 63, 323–326. Genc, Y., Ozkanca, R., Bekdemi, Y., 2008. Antimicrobial activity of some sulfonamide derivatives on clinical isolates of *Staphylococcus aureus*. *Ann. Clin. Microbiol. Antimicrob.* 7, 17–23. Grell, J., Bernstein, J., Tinhofer, G., 1999. Graph-set analysis of hydrogen-bond patterns: some mathematical concepts. *Acta Crystallogr.* 55, 1030–1043. Jain, P., Saravanan, C., Singh, S.K., 2013. Sulphonamides: deserving class as MMP inhibitors? *Eur. J. Med. Chem.* 60, 89–100. Kategaonkar, A.H., Sonar, S.S., Pokalwar, R.U., Kategaonkar, A.H., Shingate, B.B., Shingare, M.S., 2010. An efficient synthesis of 3,4-dihydro-3-substituted-2H-naphtho[2,1-e][1,3]oxazine derivatives catalyzed by zirconyl(IV) chloride and evaluation of its biological activities. *Bull. Korean Chem. Soc.* 31, 1657–1660. Katritzky, A.R., Xu, Y.-J., Jain, R., 2002. A novel dilithiation approach to 3,4-dihydro-2H-1,3-benzothiazines, 3,4-dihydro-2H-1,3-benzoxazines, and 2,3,4,5-tetrahydro-1,3-benzothiazepines. *J. Org. Chem.* 67, 8234–8236. Laszlo, P., 1995. *Organic Reactions: Simplicity and Logic*. Wiley & Sons, New York. Liu, Y.B., Peterson, D.A., Kimura, H., Schubert, D., 1997. Mechanism of cellular 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reduction. *J. Neurochem.* 69, 581–

593. Mathew, B.P., Kumar, A., Sharma, S., Shukla, P.K., Nath, M., 2010. An eco-friendly synthesis and antimicrobial activities of dihydro-2H-benzo- and naphtho-1,3-oxazine derivatives. *Eur. J. Med. Chem.* 45, 1502–1507.

Maybhate, S.P., Rajamohanan, P.P., Rajappa, S., 1991. Regiospecific synthesis of N-sulfonyl derivatives of 3,5-diamino-1H-1, 2,4- triazole and 2,5-diamino-1,3,4-thiadiazole. *Synthesis*, 220–222.

Mayekar, A.N., Yathirajan, H.S., Narayana, B., Sarojini, B.K., SuchethaKumari, N., Harrison, W.T.A., 2011. Synthesis and antimicrobial study of new 8-bromo-1,3-diaryl-2,3-dihydro-1H-naphtho[1,2-e][1,3]oxazines. *Inter. J. Chem.* 3, 74–86.

Modak, J.K., Liu, Y.C., Supuran, C.T., Roujeinikova, A., 2016. Structure-activity relationship for sulfonamide inhibition of *Helicobacter pylori* α -carbonic anhydrase. *J. Med. Chem.* 59, 11098– 11109.

Pedersen, O.S., Pedersen, E.B., 2000. The flourishing syntheses of nonnucleoside reverse transcriptase inhibitors. *Synthesis*, 479–495.

Pfaffenrot, E., Schollmeyer, D., Laufer, S., 2012. N-[(3RS,4SR)-1-Benzyl-4-methylpiperidin-3-yl]-1-(4-methylphenylsulfonyl)-5-nitro-1H-pyrrolo[2,3-b]pyridin-4-amine. *Acta Cryst.* 68, 3000.

Richers, M.T., Breugst, M., Platonova, A.Y., Ullrich, A., Dieckmann, A., Houk, K.N., Seidel, D., 2014. Redox-neutral α -oxygenation of amines: reaction development and elucidation of the mechanism. *J. Am. Chem. Soc.* 136, 6123–6135.

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Sadaphal, S.A., Sonar, S.S., Shingate, B.B., Shingare, M.S., 2010. Water mediated synthesis of various [1,3]oxazine compounds using alum as a catalyst. *Green Chem. Lett. Rev.* 3, 213–216.

Sapkal, S.B., Shelke, K.F., Kategaonkar, A.H., Shingare, M.S., 2009. Dual role of ammonium acetate for solvent-free synthesis of 1,3-disubstituted-2,3-dihydro-1H-naphth-[1,2-e] [1,3]-oxazine. *Green Chem. Lett. Rev.* 2, 57–60.

Sato, M., Fukada, N., Kurauchi, M., Takeshima, T., 1981. New synthesis of dimethyl N-aryloxythiocarbonylthioates and 3-aryl-5-methylthio-1H-1,2,4-triazoles. *Synthesis*, 554–557.

Sauter, F., Frohlich, J., Blasl, K., Gewald, K., 1995. N-[Bis (methylthio)methylene]amino ester (BMMA): Novel reagents for annelation of pyrimidine moieties. *Heterocycles* 40, 851–866.

Selig, R., Schollmeyer, D., Albrecht, W., Laufer, S., 2009. 4-Chloro-1-(4-methylphenylsulfonyl)-1H-pyrrolo[2,3-b]pyridine. *Acta Cryst.* 65, 3018.

Servi, S., Genc, M., Gu' r, S., Koca, M., 2005. The synthesis and antimicrobial activity of some new methyl N-arylthiocarbamates, dimethyl N-aryldithiocarbonylthioates and 2-arylthio-2-imidazolines. *Eur. J. Med. Chem.* 40, 687–693.

Shi, D., Rong, S., Dou, G., Wang, M., 2010. Efficient synthesis of naphtho[1,2-e][1,3]oxazine derivatives via a chemoselective reaction with the aid of low-valent titanium reagent. *J. Comb. Chem.* 12, 25–30.

Skulnick, H.I., Johnson, P.D., Aristoff, P.A., Morris, J.K., Lovasz, K. D., Howe,

W.J., Watenpaugh, K.D., Janakiraman, M.N., Anderson, D.J., Reischer, R.J., Schwartz, T.M., Banitt, L.S., Tomich, P. K., Lynn, J.C., Horng, M.-M., Chong, K.-T., Hinshaw, R.R., Dolak, L.A., Seest, E.P., Schwende, F.J., Rush, B.D., Howard, G. M., Toth, L.N., Wilkinson, K.R., Kakuk, T.J., Johnson, C.W., Cole, S.L., Zaya, R.M., Zipp, G.L., Possert, P.L., Dalga, R.J., Zhong, W.-Z., Williams, M.G., Romines, K.R., 1997. Structurebased design of nonpeptidic HIV protease inhibitors: the sulfonamide-substituted cyclooctylpyranones. *J. Med. Chem.* 40, 1149–1164.

Steiner, T., 2002. The hydrogen bond in the solid state. *Angew. Chem Int. Ed.* 41, 48–76.

Sullivan, D.F., Scopes, D.I.C., Kluge, A.F., Edwards, J.A., 1976. Studies in beta.-lactams. Synthesis of beta.-lactams via cycloaddition of iminodithiocarbonate esters with azidoketene. *J. Org. Chem.* 41, 1112–1117.

Tang, Z., Chen, W., Zhu, Z., Liu, H., 2011. Synthesis of 2,3-diaryl-3,4-dihydro-2H-1,3-benzoxazines and their fungicidal activities. *J. Heterocyclic Chem.* 48, 255–260.

Tang, Z., Zhu, Z., Xia, Z., Liu, H., Chen, J., Xiao, W., Ou, X., 2012. Synthesis and fungicidal activity of novel 2,3-disubstituted-1,3-benzoxazines. *Molecules* 17, 8174–8185.

Thaler, F., Varasi, M., Abate, A., Carezzi, G., Colombo, A., Bigogno, C., Boggio, R., Dal Zuffo, R., Rapetti, D., Resconi, A., Regalia, N., Vultaggio, S., Dondio, G., Gagliardi, S., Minucci, S., Mercurio, C., 2013. Synthesis and biological characterization of spiro[2H-(1,3)-benzoxazine-2,40-piperidine] based histone deacetylase inhibitors. *Eur. J. Med. Chem.* 64, 273–284.

Turgut, Z., Pelit, E., Ko"yucu", A., 2007. Synthesis of new 1,3-disubstituted-2,3-dihydro-1H-naphth[1,2-e][1,3]oxazines. *Molecules* 12, 345–352.

Urban'ski, T., Radzikowski, C., Ledo'chowski, Z., Czamocki, W., 1956. Biological activity of benzoxazine-1,3 derivatives, particularly against experimental sarcoma. *Nature* 178, 1351–1352.

Verma, V., Singh, K., Kumar, D., Klapo"tke, T.M., Stierstorfer, J., Narasimhan, B., Qazi, A.K., Hamid, A., Jaglan, S., 2012. Synthesis, antimicrobial and cytotoxicity study of 1,3-disubstituted-1Hnaphtho[1,2-e][1,3]oxazines. *Eur. J. Med. Chem.* 56, 195–202.

Zali-Boeini, H., Najafi, Z., Abtahi, B., Golshadi Ghalesahi, H., 2015. One-step synthesis ofazole- and benzazole-based sulfonamides in aqueous media. *Mol. Divers.* 19, 283–292.