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RESEARCH ARTICLE

ANTIMICROBIAL POTENT OXAZINE-2-AMINE DERIVATIVES

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ABSTRACT

Objective: To synthesize thirteen effective microbial potent oxazine-2-amine derivatives and evaluate the antimicrobial activities of these compounds. **Material and methods:** The solvent-free solid fly-ash: H_2SO_4 catalyzed cyclization of 4-bromo-1-naphthyl chalcones and urea under microwave irradiation technique was utilized for the synthesis of oxazine derivatives. The synthesized oxazines were characterized by their physical constants and spectroscopic data. The Bauer-Kirby disc-diffusion method of measurement of zone of inhibition was used for evaluation antimicrobial activity of synthesized oxazines**. Results:** The cyclization was effective and this reaction gave more than 80% yield. The catalyst was reused up to five times consecutively without appreciable decreasing of yield. All compounds were active against their microbial strains. **Conclusions:** This solvent-free synthetic method was effective for the synthesis of oxazine derivatives. The amino, dimethylamino, halogens, methoxy and methyl substituted oxazines shows significant antibacterial activity. Chloro, hydroxy and nitro substituted oxazine derivative have shown significant antifungal activity.

Keywords: Microwave-assisted synthesis; oxazines, IR and NMR spectra, antibacterial activity, antifungal activity.

INTRODUCTION

The unsaturated six-membered heterocyclic compounds possess one oxygen and one nitrogen atoms are known as oxazines [1-4]. These oxazine molecules exist many isomeric conformers such as 1, 2 or 1, 3 or 1, 4 oxazines types[5] depend upon the relative position of these tow atoms and the double bond. These oxazines were biologically important due to the presence of oxygen, nitrogen heteroatoms along with double bonds in their structural moieties [6]. The important biological activities of these oxazine derivatives are antibacterial [6-8], anti-fungal [6-8], anti-plasmodial [9], anti-cancer [10], anti-depressants [11], cytotoxicity [12], anti-osteoplastic [13], anti-tumour [14], anti-oxidant [15], anti-tuberculosis [16], anti-neoplastic [17], antagonists [18], anti-inflammatory [19], anti-infectants [20], IKB kinase beta [21] and PTP-1B inhibition [22]. These oxazine derivatives were applied for transcription factor NF-kappaB [23], synthesis of eosinophils [24], identification and separation of neutrophils [25]. Many oxazine derivatives were used as dyes [26]. Reddy et al., have studied the antimicrobial activities of aloe vera extract [27]. The in-vitro antibacterial activity of ethanol, chloroform, hexane and water extracts of leaves and fruits of Aegle marmelos against Methicillin-resistant Staphylococcus aureus was reported by Ganapathy and karpagam [28]. Ukaegbu-Obi et. al. have assessed the antimicrobial activities of aqueous and ethanolic extracts of monodora myristica (ehuru) seeds [29]. Numerous solvent assisted and solvent-free synthetic methods were available for synthesis of oxazine derivatives [30]. Now-a-days scientists, organic chemists are interested for solvent-free synthesis [7, 31-36]. Some reactions such as hetero Diels-alder reaction [6], ring closure [37], Betti base induced condensation [38, 39], Mannich type condensation-cyclization[7] and cyclization of chalcones[8] were used for the synthesis of oxazine derivatives. Verma et. al. [33] have synthesized some benzoxazine/oxazine fused isoquinolines and naphthyridines by the solvent-free method. Elarfi and Al-difar [6] have synthesized some 1, 3-oxazine derivatives by a solvent-assisted method from chalcones and urea. More than 75% yield of dihydro-^{2}H-benzo- and naphtho-1, 3-oxazine derivatives were prepared by Mathew et al.[5] using the eco-friendly method. Efficient synthesis of some 1, 3-oxazine-4-thiones was synthesized by N-methyl imidazole promoted solvent-free conditions. Sapkal et al., have studied the role of ammonium acetate for the solvent-free synthesis of 1,3-disubstituted-2,3-dihydro-^{1}H-naphthyl oxazines [35]. Within the above view, there is no information available in the literature for the solvent-free synthesis the evaluation of biological activities of 4-bromo-1-naphthyl based oxazine-2-amine derivatives. Therefore the author have taken the effort to synthesize some 4-bromo-1-naphthyl based oxazine-2-amines by solvent-free cyclization, characterized by their analytical, spectral data and studied their antimicrobial activities using Bauer-Kirby [40] method.

MATERIAL AND METHODS

General

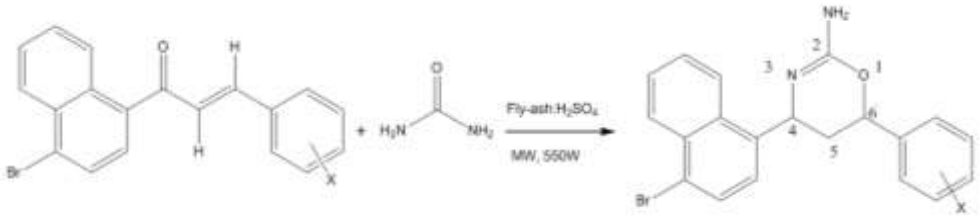
Chemicals were used in this present investigation were purchased from Sigma-Aldrich and Merck Chemical companies. The melting point of oxazine-2-amines were determining in Mettler FP51 apparatus using open glass capillaries and are uncorrected. The infrared spectra of oxazines were recorded in AVATAR-300 Fourier transform spectrophotometer (KBr, 4000-400 cm^{-1}) using KBr disc. The NMR spectra of oxazine-2-amine derivatives were recorded in Bruker AV400 series type NMR spectrometer operating at 400MHz for ^{1}H and100 MHz for ^{13}C spectra in $CDCl_3$ solvent using TMS as an internal standard. Mass spectra of all synthesized oxazine derivatives were recorded on SHIMADZU mass spectrometer using chemical ionization technique.

Preparation of fly-ash:H_2SO_4 catalyst

The fly-ash:H_2SO_4 catalyst was prepared according to a literature procedure[41].

Synthesis of 4-(aryl)-5,6-dihydro-6-(substituted phenyl)-4*H*-1,3-oxazine-2-amines.

An equi-molar quantities of chalcones (2 mmol), urea (2mmol) and 0.4 g of fly-ash:H_2SO_4 were subjected to microwave irradiation for 2-4 minutes at 550W (Scheme 1) (Samsung, Microwave Oven, 100-700W). The completion of the reaction was monitored by TLC. After completion of the reaction, dichloromethane (20 mL) was added, followed by simple filtration. The solution was concentrated; the obtained solid was purified by re-crystallization. The solid catalyst was washed with ethyl acetate, dried in hot air oven at 110°C for 1h, and then it is used for further reaction runs.



Entry	1	2	3	4	5	6	7	8	9	10	11	12	13
X	H	3-NH_2	4-NH_2	3-Br	3-Cl	4-Cl	4-$N(CH_3)_2$	4-OH	4-OCH_3	4-CH_3	2-NO_2	3-NO_2	4-NO_2

Scheme 1: Synthesis of 4-aryl-5,6-dihydro-6-(substituted phenyl)-4H-1,3-oxazine-2-amines by fly-ash:H_2SO_4 catalyzed cyclization of aryl chalcones and urea under microwave irradiation.

Measurement of Antimicrobial activities

Antibacterial activity

The antibacterial activities of all prepared 4-(4-bromo1-naphthyl)-5,6-dihydro-6-(substituted phenyl)-oxazine-2-amines have been evaluated against two gram positive pathogenic strains *Staphylococcus aureus*, *Enterococcus faecalis* and four gram negative strains *Escherichia coli*, *Klebsiella species*, *Psuedomonas,* and *Proteus vulgaris.* The disc diffusion technique was followed using Kirby-Bauer [38] method, at a concentration of 250 µg/mL with Ampicillin and Streptomycin used as the standard drugs.

Antibacterial activities of all 4-(4-bromo1-naphthyl)-5, 6-dihydro-6-(substituted phenyl)-oxazine-2-amines were evaluated using Kirby-Bauer [38] disc diffusion technique. In each Petri plate about 0.5 mL of the test bacterial sample was spread uniformly over the solidified Mueller Hinton agar using sterile glass spreader. Then the discs with 5 mm diameter made up of Whatmann No.1 filter paper, impregnated with the solution of the compound were placed on the medium using sterile forceps. The plates were incubated for 24 h at 37^{o}C by keeping the plates upside down to prevent the collection of water droplets over the medium. After 24 h, the plates were visually examined and the diameter values of the zone of inhibition were measured. Triplicate results were recorded by repeating the same procedure.

Antifungal sensitivity assay

The study of antifungal activities of all 4-(4-bromo-1-naphthyl)-5,6-dihydro-6-(substituted phenyl)-oxazine-2-amines has been done with *Candida albicans* as the fungal strain using the disc diffusion technique and the other two strains *Penicillium* species and *Aspergillus niger*, the dilution method was adopted. The drug dilution was kept as 50µg/mL. *Griseofulvin* has been taken as the standard drug.

Antifungal sensitivity assay all 4-(4-bromo-1-naphthyl)-5, 6-dihydro-6-(substituted phenyl)-oxazine-2-amines were performed using Kirby-Bauer[35] disc diffusion technique. PDA medium was prepared and sterilized as above. It was poured (ear bearing heating condition) in the Petri-plate which was already filled with 1 mL of the fungal species. The plate was rotated clockwise and counter clockwise for uniform spreading of the species. The discs were impregnated with the test solution. The test solution was prepared by dissolving 15 mg of the chalcone in 1mL of DMSO solvent. The medium was allowed to solidify and kept for 24 h. Then the plates were visually examined and the diameter values of the zone of inhibition were measured. Triplicate results were recorded by repeating the same procedure.

RESULTS

The synthesized 4-(4-bromo-1-naphthyl)-5,6-dihydro-6-(substituted phenyl)-oxazine-2-amines were characterized by their physical constants and spectral data. Obtained analytical and mass spectral fragments of synthesized oxazines were presented in **Table. 1.** Further the compounds were characterized by their infrared and NMR spectra. The obtained spectroscopic data of these oxazines were summarized as follows.

4-(4-Bromo-1-naphthyl)-5,6-dihydro-6-phenyl-4H-1,3-oxazine-2-amine (1): FT-IR (KBr): 3427(NH), 1598(C=N), 1253(C-O-C) cm^{-1}. ^{1}H NMR ($CDCl_3$-d_6, TMS) δ: 6.71(s, 2H, NH_2), 5.29(t, 1H, H_4), 2.89 (dd, 1H, H_5), 2.13 (dd, 1H, $H_{5'}$), 4.78(t, 1H, H_6), 7.03-8.13(m, 11H, Ar-H) ppm. ^{13}C NMR ($CDCl_3$-d_6, TMS) δ: 155.19(C_2), 49.75(C_4), 39.83(C_5), 73.19(C_6), 124.39-140.90(Ar-C).

Table 1: Analytical, physical constants, yield and mass fragment of 4-aryl-5,6-dihydro-6(substituted phenyl)-4H-1,3-oxazine-2-amines.

Entry	X	M.W.	Time(m)	Yield(%)	m.p.(ºC)	Mass(m/z)
1	H	380	2.5	90	124-125	380[M^{+}], 382[M^{2+}], 364, 305, 303, 301, 286, 204, 175, 127, 99, 84, 77, 58, 43, 42, 16
2	3-NH_2	396	2	86	103-104	396[M^{+}], 398[M^{2+}], 379, 337, 303, 204 175, 127, 118, 111, 84, 77, 43, 58,16,
3	4-NH_2	396	2	88	113-115	396[M^{+}], 398[M^{2+}], 379, 337, 204 175, 127, 111, 99, 97, 79, 77, 43, 58,16,
4	3-Br	460	3	85	117-118	460[M^{+}], 462[M^{2+}], 464[M^{4+}], 441, 303, 339, 252, 209, 175, 127, 84, 79,77, 58, 43, 35,16
5	3-Cl	415	3	85	99-101	415[M^{+}], 417[M^{2+}], 419[M^{4+}], 397, 379, 303, 335, 225, 209, 175, 127, 99, 77, 58, 43, 35,16,
6	4-Cl	415	3	86	131-132	415[M^{+}], 417[M^{2+}], 419[M^{4+}], 397, 379, 303, 335, 209, 175, 127, 99, 77, 58, 43,41, 35,16,
7	4-$N(CH_3)_2$	424	3.5	88	124-125	424[M^{+}], 426[M^{2+}], 408, 379, 354, 339,290, 263, 218, 205, 204, 165, 127, 120, 107, 91, 77, 58, 43, 42, 31, 16
8	4-OH	397	2.5	90	118-119	397[M^{+}], 399[M^{2+}], 379, 303, 317, 204, 191, 175, 127, 91, 77, 58, 43, 42, 31, 16
9	4-OCH_3	411	2	90	102-103	411[M^{+}], 413[[M^{2+}], 395, 394. 379, 331,205, 191, 175, 148, 127, 107, 91, 77, 58, 43, 42, 31, 16

10	4-CH_3	395	2.5	92	146-147	395[M^+], 397[M^{2+}], 379, 378, 303, 204, 189, 174, 132, 91, 77, 46, 43, 41, 16,
11	2-NO_2	426	4	85	116-117	426[M^+], 428[M^{2+}], 379, 367, 346, 205, 165, 84,77, 46, 43, 41, 16,
12	3-NO_2	426	4	85	105-106	426[M^+], 428[M^{2+}], 3409, 79, 367, 346, 303,232, 220, 205, 204, 165, 136, 127, 122, 88, 84,79, 77, 58, 46, 45, 43, 41, 29, 16,
13	4-NO_2	426	4	85	125-126	426[M^+], 428[M^{2+}], 340, 379, 367, 346, 220, 205, 204, 175, 165, 136, 127, 122, 88, 84,77, 46, 45, 43, 41, 29, 16,

4-(4-Bromo-1-naphthyl)-5, 6-dihydro-6-(3-aminoyphenyl)-4H-1, 3-oxazine-2-amine(2): FT-IR (KBr): 3564(NH), 1612(C=N), 1245(C-O-C) cm^{-1}. ^{1}H NMR ($CDCl_3$-d_6, TMS) δ: 6.685(s, 2H, NH_2), 5.32(t, 1H, H_4), 2.46(dd, 1H, H_5), 2.20(dd, 1H, $H_{5'}$), 4.35(t, 1H, H_6), 6.89-7.25(m, 10H, Ar-H) ppm. ^{13}C NMR ($CDCl_3$-d_6, TMS) δ: 154.82 (C_2), 51.36 (C_4), 47.98 (C_5), 76.25 (C_6), 126.25-139.38 (Ar-C).

4-(4-Bromo-1-naphthyl)-5, 6-dihydro-6-(4-aminophenyl)-4H-1, 3-oxazine-2-amine (3): FT-IR (KBr): 3556(NH), 1602(C=N), 1212(C-O-C) cm^{-1}. ^{1}H NMR ($CDCl_3$-d_6, TMS) δ: 6.29(s, 2H, NH_2), 4.52 (t, 1H, H_4), 2.12(dd, 1H, H_5), 2.32 (dd, 1H, $H_{5'}$), 4.87(t, 1H, H_6), 6.86-7.65 (m, 10H, Ar-H) ppm. ^{13}C NMR ($CDCl_3$-d_6, TMS) δ: 154.84 (C_2), 51.32 (C_4), 47.93(C_5), 76.54 (C_6), 126.11-139.32 (Ar-C).

4-(4-Bromo-1-naphthyl)-5,6-dihydro-6-(3-bromophenyl)-4H-1, 3-oxazine-2-amine(4): FT-IR (KBr): 3552(NH), 1623(C=N), 1209(C-O-C) cm^{-1}. ^{1}H NMR ($CDCl_3$-d_6, TMS) δ: 6.22(s, 2H, NH_2), 5.34 (t, 1H, H_4), 2.32(dd, 1H, H_5), 2.36 (dd, 1H, $H_{5'}$), 4.36(t, 1H, H_6), 6.85-7.28 (m, 10H, Ar-H) ppm. ^{13}C NMR ($CDCl_3$-d_6, TMS) δ: 154.34 (C_2), 51.67 (C_4), 47.76(C_5), 72.43 (C_6), 124.26-139.39 (Ar-C).

4-(4-Bromo-1-naphthyl)-5,6-dihydro-6-(3-chlorophenyl)-4H-1, 3-oxazine-2-amine(5): FT-IR (KBr): 3548(NH), 1614(C=N), 1215(C-O-C) cm^{-1}. ^{1}H NMR ($CDCl_3$-d_6, TMS) δ: 6.21 (s, 2H, NH_2), 5.32(t, 1H, H_4), 2.42(dd, 1H, H_5), 2.65 (dd, 1H, $H_{5'}$), 4.32(t, 1H, H_6), 6.23-7.27 (m, 10H, Ar-H) ppm. ^{13}C NMR ($CDCl_3$-d_6, TMS) δ: 154.33 (C_2), 51.23 (C_4), 47.87(C_5), 66.34 (C_6), 126.23-139.15 (Ar-C).

4-(4-Bromo-1-naphthyl)-5,6-dihydro-6-(4-chlorophenyl)-4H-1, 3-oxazine-2-amine(6): FT-IR (KBr): 3552(NH), 1610(C=N), 1232(C-O-C) cm^{-1}. ^{1}H NMR ($CDCl_3$-d_6, TMS) δ: 6.27 (s, 2H, NH_2), 5.33 (t, 1H, H_4), 2.42(dd, 1H, H_5), 2.83(dd, 1H, $H_{5'}$), 4.38(t, 1H, H_6), 6.24-7.67 (m, 10H, Ar-H) ppm. ^{13}C NMR ($CDCl_3$-d_6, TMS) δ: 154.38 (C_2), 51.27 (C_4), 47.76(C_5), 66.98 (C_6), 126.37-139.87(Ar-C).

4-(4-Bromo-1-naphthyl)-5,6-dihydro-6-(4 dimethylamino-phenyl)-4H-1,3-oxazine-2-amine(7): FT-IR (KBr): 3535(NH), 1611(C=N), 1212(C-O-C) cm^{-1}. ^{1}H NMR ($CDCl_3$-d_6, TMS) δ: 6.53 (s, 2H, NH_2), 5.34 (t, 1H, H_4), 2.31(dd, 1H, H_5), 2.73(dd, 1H, $H_{5'}$), 4.36(t, 1H, H_6), 3.63(s, 6H, $N(CH_3)_2$), 6.78-7.98 (m, 10H, Ar-H) ppm. ^{13}C NMR ($CDCl_3$-d_6, TMS) δ: 154.26 (C_2), 51.27 (C_4), 47.76 (C_5), 66.98 (C_6), 44.87 ($N(CH_3)_2$), 126.67-139.23(Ar-C).

4-(4-Bromo-1-naphthyl)-5,6-dihydro-6-(4-hydroxyphenyl)-4H-1, 3-oxazine-2-amine (8): FT-IR (KBr): 3546(NH), 1601(C=N), 1218(C-O-C) cm^{-1}. ^{1}H NMR ($CDCl_3$-d_6, TMS) δ: 6.22 (s, 2H, NH_2), 5.45 (t, 1H, H_4), 2.41(dd, 1H, H_5), 2.62(dd, 1H, $H_{5'}$), 4.65(t, 1H, H_6), 6.24-7.26(m, 10H, Ar-H) ppm. ^{13}C NMR ($CDCl_3$-d_6, TMS) δ: 154.34 (C_2), 51.76 (C_4), 47.54(C_5), 66.89 (C_6), 44.19 ($N(CH_3)_2$), 126.34-139.80(Ar-C).

4-(4-Bromo-1-naphthyl)-5,6-dihydro-6-(4-methoxyphenyl)-4H-1, 3-oxazine-2-amine (9): FT-IR (KBr): 3540(NH), 1611(C=N), 1223(C-O-C) cm^{-1}. ^{1}H NMR ($CDCl_3$-d_6, TMS) δ: 6.21(s, 2H, NH_2), 5.33 (t, 1H, H_4), 2.44(dd, 1H, H_5), 2.63(dd, 1H, $H_{5'}$), 4.35(t, 1H, H_6), 4.26 (s, 3H, OCH_3), 6.25-7.37(m, 10H, Ar-H) ppm. ^{13}C NMR ($CDCl_3$-d_6, TMS) δ: 154.29 (C_2), 51.28 (C_4), 47.55(C_5), 73.68 (C_6), 62.36(OCH_3), 126.32-139.59(Ar-C).

4-(4-Bromo-1-naphthyl)-5,6-dihydro-6-(4-methylphenyl)-4H-1, 3-oxazine-2-amine (10): FT-IR (KBr): 3534(NH), 1610(C=N), 1231(C-O-C) cm^{-1}. ^{1}H NMR ($CDCl_3$-d_6, TMS) δ: 6.22 (s, 2H, NH_2), 5.39 (t, 1H, H_4), 2.47(dd, 1H, H_5), 2.54(dd, 1H, $H_{5'}$), 4.35(t, 1H, H_6), 2.78 (s, 3H, CH_3), 6.23-7.29(m, 10H, Ar-H) ppm. ^{13}C NMR ($CDCl_3$-d_6, TMS) δ: 154.25 (C_2), 51.36 (C_4), 47.80(C_5), 66.29 (C_6), 26.78(CH_3), 114.98-145.09(Ar-C).

4-(4-Bromo-1-naphthyl)-5, 6-dihydro-6-(2-nitrophenyl)-4H-1, 3-oxazine-2-amine (11): FT-IR (KBr): 3555(NH), 1620(C=N), 1245(C-O-C) cm^{-1}. ^{1}H NMR ($CDCl_3$-d_6, TMS) δ: 6.26 (s, 1H, NH_2), 5.33 (t, 1H, H_4), 2.44(dd, 1H, H_5), 2.64(dd, 1H, $H_{5'}$), 4.46(t, 1H, H_6), 6.24-7.56(m, 10H, Ar-H) ppm. ^{13}C NMR ($CDCl_3$-d_6, TMS) δ: 156.11 (C_2), 51.29 (C_4), 47.36(C_5), 66.23 (C_6), 114.23-145.25(Ar-C).

4-(4-Bromo-1-naphthyl)-5, 6-dihydro-6-(2-nitrophenyl)-4H-1, 3-oxazine-2-amine(12): FT-IR (KBr): 3550(NH), 1625(C=N), 1230(C-O-C) cm^{-1}. ^{1}H NMR ($CDCl_3$-d_6, TMS) δ: 6.23(s, 1H, NH_2), 5.36 (t, 1H, H_4), 2.45(dd, 1H, H_5), 2.63(dd, 1H, $H_{5'}$), 4.59(t, 1H, H_6), 6.52-7.90(m, 10H, Ar-H) ppm. ^{13}C NMR ($CDCl_3$-d_6, TMS) δ: 155.69 (C_2), 51.35 (C_4), 47.42(C_5), 66.36 (C_6), 124.90-145.69(Ar-C).

4-(4-Bromo-1-naphthyl)-5, 6-dihydro-6-(2-nitrophenyl)-4H-1,3-oxazine-2-amine(13): FT-IR (KBr): 3545(NH), 1627(C=N), 1235(C-O-C) cm^{-1}. ^{1}H NMR ($CDCl_3$-d_6, TMS) δ: 6.22 (s, 1H, NH_2), 5.33 (t, 1H, H_4), 2.27(dd, 1H, H_5), 2.57(dd, 1H, $H_{5'}$), 4.39(t, 1H, H_6), 6.57-7.89(m, 10H, Ar-H) ppm. ^{13}C NMR ($CDCl_3$-d_6, TMS) δ: 155.70(C_2), 51.38 (C_4), 47.48(C_5), 66.85 (C_6), 124.58-145.90(Ar-C).

Antibacterial activity

The measured antibacterial activity of synthesized 4-(4-bromo-1-naphthyl)-5, 6-dihydro-6(substituted phenyl)-4H-1, 3-oxazine-2-aminesby disc-diffusion technique was presented in **Table 2**.

Table 2: Antibacterial activities of 4-(4-bromo-1-naphthyl)-5,6-dihydro-6(substituted phenyl)-4H-1,3-oxazine-2-amines.

Entry	X	*E-coli*	*S.aures*	*P.aeruginosa*	*Klebsiella*	*P.vulgaris*	*E. faecalis*
1	H	+	±	+	±	++	++
2	3-NH_2	±	++	+	++	±	±
3	4-NH_2	±	++	+	++	±	±
4	3-Br	++	+	++	++	+	++
5	3-Cl	++	±	++	+	+	+
6	4-Cl	++	+	++	+	++	++
7	4-$N(CH_3)_2$	±	++	+	++	±	++
8	4-OH	±	+	+	±	±	+
9	4-OCH_3	+	++	±	±	++	±
10	4-CH_3	+	+	±	±	++	±

11	2-NO_2	±	+	+	+	±	±
12	3-NO_2	±	+	+	±	+	±
13	4-NO_2	+	±	±	+	+	±

Disc size: 6.35 mm; Duration: 24-45 h; Standard: Ampicillin (30-33 mm) and Streptomycin(20-25 mm); Control: Methanol; ---: No activities; ±: Active(8-12 mm); +: Moderately active(13-19 mm); ++: Active(20-24 mm).

Antifungal activity

The measured antifungal activity of synthesized 4-(4-bromo-1-naphthyl)-5,6-dihydro-6(substituted phenyl)-4H-1,3-oxazine-2-aminesby disc diffusion technique was presented in Table 3.

Table 3: Antifungal activities of 4-(4-bromo-1-naphthyl)-5,6-dihydro-6(substituted phenyl)-4H-1,3-oxazine-2-amines.

Entry	X	Disc diffusion technique (250 µg/mL)	Drug dilution method (50 µg/mL)	
		Candida albicans	***Penicillium***	***Aspergillus niger***
1	H	±	±	+
2	3-NH_2	+	+	+
3	4-NH_2	+	+	+
4	3-Br	±	±	+
5	3-Cl	++	++	+
6	4-Cl	+	++	+
7	4-$N(CH_3)_2$	+	±	±
8	4-OH	++	---	++
9	4-OCH_3	+	+	+
10	4-CH_3	+	+	---
11	2-NO_2	++	++	++
12	3-NO_2	++	++	++
13	4-NO_2	++	++	++

Standard: Griseofulvin and Gentamycin; Duration: 72 h; Control: Methanol; Medium: Potato dextrose agar; ++: No fungal colony; +: One fungal colony; ±: Two-three fungal colonies; ---: Heavy fungal colony.

DISCUSSION

Synthesis

In our organic chemistry research laboratory, attempts made for synthesizing oxazine derivatives by solvent-free cyclization of chalcones possess electron with-drawing as well as electron donating group as substituents and urea in the presence of various solid catalysts including fly-ash:H_2SO_4 using microwave irradiation. Hence the authors have synthesized some substituted 1,3-oxazine-2-amines derivatives by the cyclization of 2 mmole of 4-bromo-1-naphthyl chalcones, 2 mmole of urea and 0.4g of fly-ash:H_2SO_4 catalyst under microwave irradiation at 550W for 4-6 minutes (Samsung Grill, GW73BD Microwave oven, 230V A/c, 50Hz, 2450Hz, 100-750W (IEC-705), (Scheme 1). During the course of this reaction fly-ash:H_2SO_4 catalyzes cyclization between chalcones and urea followed by rearrangement gave the 1, 3-oxazine-2-amines. The yields of the oxazine in this reaction are more than 80%. The chalcone containing an electron donating substituent (OCH_3) gave higher yields than electron-withdrawing (halogens, NO_2) substituents. Further, we have investigated this cyclization reaction with equi-molar quantities of the styryl 4-bromo-1-naphthyl ketone (entry 1) with urea under the same condition as above. In this reaction, the obtained yield was 90%. The effect of a catalyst on this reaction was studied by varying the catalyst quantity from 0.1 g to 1 g. As the catalyst quantity is increased from 0.1 g to 1 g, the percentage of yield of product is increased from 84 to 90%. The synthesized 4-(4-bromo-1-naphthyl)-5,6-dihydro-6(substitutedphenyl)-4H-1,3-oxazine-2-amines were characterized by their physical constants and spectroscopic data.

Infrared spectra

The synthesized 4-(4-bromo-1-naphthyl)-5,6-dihydro-6(substituted phenyl)-4H-1,-oxazine-2-amines(1-13) were characterized by infrared spectral data. The infrared spectra of the parent compound were shown in (**figure 1**). The peaks obtained at 3427, 1598 and 1253 $cm^{-1}$are corresponding stretches of NH, C=N, and C-O-C groups. For all compounds, the NH stretches obtained at the range of 3227-3567 cm^{-1}. The C=N stretches obtained at the range of 1627-1598 cm^{-1}.

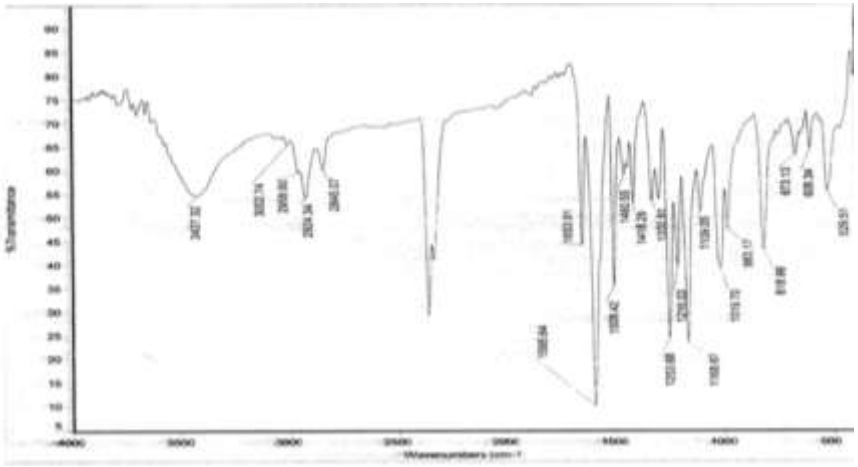


Fig.1: Infrared spectra of 4-(4-bromo-1-naphthyl)-5,6-dihydro-6- phenyl-4H-1,3-oxazine-2-amines(1).

^{1}H NMR spectra

From proton NMR spectra of synthesized 4-(4-bromo-1-naphthyl)-5,6-dihydro-6(substituted phenyl)-4H-1,3-oxazine-2-amines, the NH_2 proton chemical shifts (δ, ppm) obtained in the range of 6.21-6.71. The chemical shifts (δ, ppm) of H_4 proton gave triplet and it obtained in the range of 4.52-5.39. The proton chemical shifts (δ, ppm) H_5 and $_{5'}$ gave doublet of doublet and it obtained in the range of 2.12-2.47 and 2.13-2.73. The proton chemical shifts (δ, ppm) H_6 gave triplet and it falls in the range of 4.35-4.87. These chemical shifts support for the formation of oxazine compounds. The aromatic protons chemical shifts falls in the range of 6.27-8.13 ppm. The parent compound (**1**) of the oxazine also gave the chemical shifts (δ, ppm) values in this range and this is illustrated in (**figure 2**). The chemical shifts are 6.71(s, 2H, NH_2), 5.29(t, 1H, H_4), 2.89 (dd, 1H, H_5), 2.13 (dd, 1H, $H_{5'}$), 4.78(t, 1H, H_6), 7.03-8.13(m, 11H, Ar-H) ppm.

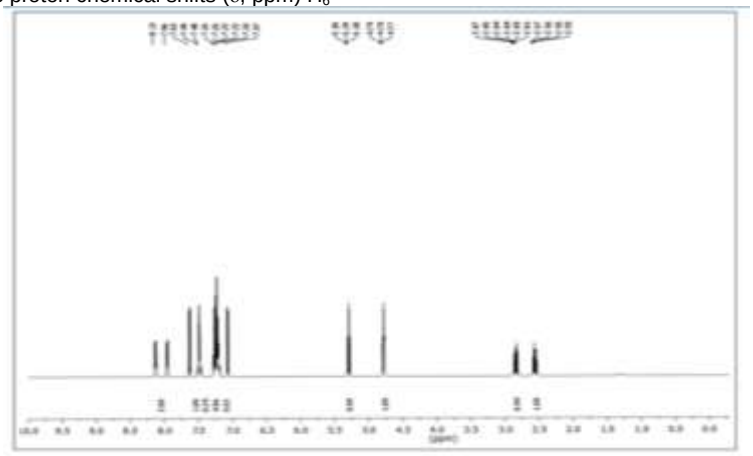


Fig. 2: The ^{1}H NMR spectra of 4-(4-bromo-1-naphthyl)-5,6-dihydro-6-phenyl-4H-1,3-oxazine-2-amines.

^{13}C NMR spectra

The ^{13}C NMR spectral data reveals that the chemical shifts of the ring carbons C_2, C_4, C_5 C_6 and aromatic carbons falls at the range 154.25-155.70, 49.75-51.76, 39.83-47.80, 66.29-73.19 and 114.23-145.90 ppm. The ^{13}C NMR spectra of 4-(4-bromo-1-naphthyl)-5,6-dihydro-6-phenyl-4H-1,3-oxazine-2-amine was shown in (**figure 3**) the corresponding chemical shifts are155.19(C_2), 49.75(C_4), 39.83(C_5), 73.19(C_6), 124.39-140.90(Ar-C). These data are supported for the formation of oxazines.

Further, these oxazines were confirmed by mass spectra. The mass spectral fragments gave the corresponding parent and M^{+1} ion peaks. Similarly, all compounds gave the isotopic peaks of M^{+2} and M^{+4} also obtained with a respective mass of fragments.

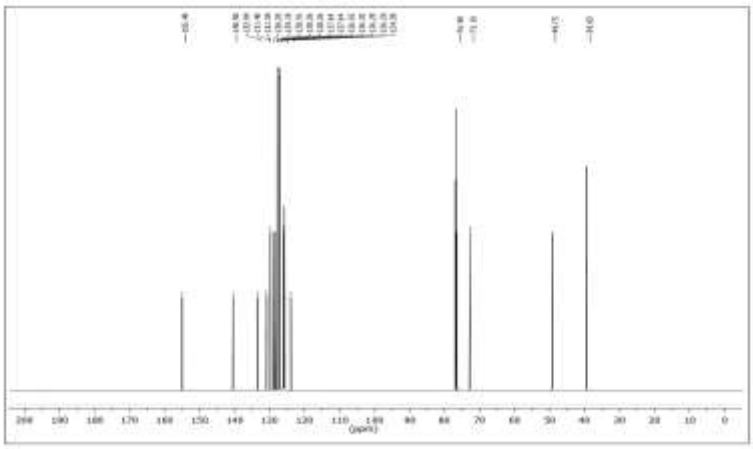


Fig.3: The ^{13}C NMR spectra of 4-(4-bromo-1-naphthyl)-5,6-dihydro-6-phenyl-4H-1,3-oxazine-2-amine(1)

Antibacterial activity assay

From the observed antibacterial activities of the synthesized oxazines by means of measurement of mm of zone of inhibition, the oxazine-2-amines **4-6** showed maximum zone of inhibition against *Escherichia coli*, with greater than 20 mm compared to the oxazines **1, 9, 10** and **13** and they are moderately active within 13-19 mm of zone of inhibition. Compounds **2, 3, 7, 8, 11** and **13** were active within 8-12 mm of the zone of inhibition. The oxazine-2-amine derivatives **2, 3, 7** and **9** were found to be effective against *S. aureus* within 20-24 mm of the zone of inhibition. Compounds **4, 6** and **8** are moderately active within 13-19 mm of the zone of inhibition. The oxazines **1, 5** and **13** were active within 8-12 mm of the zone of inhibition. The oxazine derivatives **4-6** were more active against *Pseudomonas* showing greater than 20 mm zone of inhibition and the other derivatives **1-3, 7, 8, 11** and **12** were showed the zone of inhibitions between 12-19 mm. The oxazines **9, 10** and **13** were showed active within 8-12 mm of the zone of inhibition. The oxazine-2-amines **2-4** and **7** were effective within 20-24 mm of the zone of inhibition against *Klebsiella* . The compounds **5, 6** and **13** were active with 12-12 mm zone of inhibition while the other oxazine showed a moderate activity. The compounds **1, 6, 9** and **10** were active when it is screened against *P. vulgaris* and the other compounds are less effective. The oxazines **1, 4, 6** and **7** showed activities against *E-faecalis* when they are screened with 20-24 mm zone of inhibition.

Antifungal activity assay

The study of antifungal activities of all 4-(1-naphthyl)-5,6-dihydro-6-(substituted phenyl)-oxazine-2-amines against *C. albicans*, showed that the five compounds **5, 8, 11-13** are effective with 20 mm as the zone of inhibition in 250 µg/ disc while oxazine-2-amines **2, 3, 6, 7, 9** and **10** were active with 13-19 mm zone of

inhibition and the compound 1 and 4 were the least active with 8-12 mm zone of inhibitions. The oxazine derivatives 6, 11-13 were more visible against *Penicillum* species, in the development of the fungal colony and 2-3 colonies are recorded for the compounds **1-3, 5, 9** and **10**. The inhibition of oxazine-2-amines against *A.niger* was more active in two compounds **5, 8, 11-13**. The compounds **2-4**, **6** and **9** were active with 8-12 mm zone of inhibitions. The presence of amino-, dimethylamino-, methoxy-, methyl-, dimethyl-, chloro-, bromo- and nitro- substituents are responsible for antimicrobial activities of 4-(4-bromo-1-naphthyl)-5,6-dihydro-6-(substituted phenyl)-oxazine-2-amines.

CONCLUSIONS

Some (4-bromo-1-naphthyl)-5,6-dihydro-6-(substituted phenyl)-oxazine-2-amines have been synthesized by solid fly-ash: H_2SO_4 catalyzed solvent-free cyclization of aryl chalcones and urea using microwave irradiation under solvent-free conditions. The yields of these oxazines were more than 80%. This synthetic methodology offers solvent-free cyclization, non-hazardous, easy-workup procedure, shorter reaction time and better yields. These oxazine derivatives were characterized by their physical constants and spectral data. The antimicrobial activities of these 4-(4-bromo-1-naphthyl)-5, 6-dihydro-6-(substituted phenyl)-oxazine-2-amines have been evaluated. The compounds possess amino, dimethylamino methoxy, methyl, dimethyl, chloro, bromo and nitro substituents were showed antimicrobial activities at 20-24, 13-19 and 8-12 mm of the zone of inhibitions.

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REFERENCES

1. Banerjee A, Ganguly S, Chattopadhyay T, Banu K S, Patra A, Bhattacharya S, Zangrando E and Das D. Metal-assisted oxazolidine/oxazine ring formation in dinuclear zinc (II) complexes: synthesis, structural aspects, and bioactivity. Inorg Chem 2009; 48(18): 8695-8702.
2. Jacob P, Benowitz N L, Yu L and Shulgin A T. Determination of nicotine N-oxide by gas chromatography following thermal conversion to 2-methyl-6-(3-pyridyl)tetrahydro-1,2-oxazine. Anal Chem 1986; 58(11): 2218-2221.
3. Yakovlev I P, Prep'yalov A V and Ivin B A. Unsaturated 4H-1,3-Oxazines. Chem Heterocycl Compd 1994; 30(3):255-271.
4. Manjula M K, Rai K M L, Gaonkar S L, Raveesha K A and Satish S. Synthesis of new series of 5,6-dihydro-4H-1,2-oxazines via hetero Deils-Alder reaction and evaluation of antimicrobial activity. Eur J Med Chem 2009; 44: 280-288.
5. Mathew B P, Kumar A, Sharma S, Shukla P K and Nath M. An eco-friendly synthesis and antimicrobial activities of dihydro-2H-benzo-and naphtho-1, 3-oxazine derivatives. Eur J Med Chem 2010; 45: 502-1507.
6. Elarfi M J and Al-Difar HA. Synthesis of some heterocyclic compounds derived from chalcones. Sci Rev Chem Communn 2012; 2(2): 103-107.
7. Tiwari V, Meshram J, Ali P, Sheikh J and Tripathi U. Novel oxazine skeletons as potential antiplasmodial active ingredients: Synthesis, in vitro and in vivo biology of some oxazine entities produced via cyclization of novel chalcone intermediates. J Enzyme Inhib Med Chem 2011; 26(4): 569-78.
8. Das B C, Madhukumar AV, Anguiano J and Mani S. Design, synthesis and biological evaluation of 2H-benzo[b][1,4] oxazine derivatives as hypoxia targeted compounds for cancer therapeutics. Bioorg Med Chem Lett 2009; 19(15): 4204-4206.
9. Zhou D, Harrison B L, Shah U, Andree T H, Hornby G A and Scerni R. Studies toward the discovery of the next generation of antidepressants. Part 5: 3, 4-Dihydro-2H-benzo [1,4]oxazine derivatives with dual 5-HT1A receptor and serotonin transporter affinity. Bioorg Med Chem Lett 2006; 16(5): 1338-1341.
10. Wang S, Li Y, Liu Y, Lu A and You Q. Novel hexacyclic camptothecin derivatives. Part 1: synthesis and cytotoxicity of camptothecins with an A-ring fused 1, 3-oxazine ring. Bioorg Med Chem Lett 2008; 18(14): 4095-4097.
11. Ando Y, Ando K, Yamaguchi M, Kunitomo J, Koida M and Fukuyama R. A novel oxazine ring closure reaction affording (Z)-((E)-2-styrylbenzo[b]furo [3, 2-d][1,3]oxazin-4-ylideno)acetaldehydes and their anti-osteoclastic bone resorption activity. Bioorg Med Chem Lett 2006; 16(22): 5849-5854.
12. Benameur L, Bouaziz Z, Nebois P, Bartoli MH, Boitard M and Fillion H. Synthesis of furonaphth[1,3]oxazine and furo[1,3]oxazinoquinoline derivatives as precursors for an o-quinonemethide structure and potential antitumor agents. Chem Pharm Bull (Tokyo) 1996; 44(3): 605-608.
13. Roy K, Mitra I and Saha A. Molecular shape analysis of antioxidant and squalene synthase inhibitory activities of aromatic tetrahydro-1, 4-oxazine derivatives. Chem Biol Drug Des 2009; 74(5): 507-516.
14. Blaser A, Palmer B D, Sutherland H S, Kmentova I, Franzblau S G and Wan B. Structure-activity relationships for amide-, carbamate-, and urea-linked analogues of the tuberculosis drug (6S)-2-nitro-6-{[4-(trifluoromethoxy)benzyl]oxy}-6,7-dihydro-5H-imidazo[2,1-b][1,3] oxazine. J Med Chem 2012; 55(1): 312-326.
15. Seal L, Von Hoff D, Lawrence R, Izbicka E and Jamison RM. An in vitro assessment of the antineoplastic potential of 2H-1, 3-oxazine-2, 6(3H)-dione (3-oxauracil), a novel pyrimidine. Invest. New Drugs 1997; 15(4): 289-293.
16. Brudeli B, Moltzau L R, Andressen KW, Krobert K A, Klaveness J and Levy F O. Synthesis and pharmacological properties of novel hydrophilic 5-HT4 receptor antagonists. Bioorg Med Chem 2010; 18(24): 8600-8613.
17. Akhter M, Husain A, Akhter N and Khan MSY. Synthesis, antiinflammatory and antimicrobial activity of some new 1-(3-Phenyl-3, 4-Dihydro-2H-1,3-Benzoxazin-6-yl)-ethanone derivatives. Indian J Pharm Sci 2011; 73: 101-104.
18. Gothi D and Joshi JM. Resistant TB: Newer Drugs and Community Approach. Recent Pat Antiinfect Drug Discov 2011; 6(1): 27-37.
19. Oh K S, Lee S, Choi J K and Lee B H. Identification of novel scaffolds for IκB kinase beta inhibitor via a high-throughput screening TR-FRET assay. Comb Chem High Throughout Screen 2010; 13(9):790-797.
20. Cho SY, Baek J Y, Han S S, Kang S K, Ha J D and Ahn J H. PTP-1B inhibitors: cyclopenta[d][1,2]-oxazine derivatives. Bioorg Med Chem Lett 2006; 16 (3):499-502.
21. Lee S F, Vérolet Q and Fürstenberg A. Improved Super-Resolution Microscopy with Oxazine Fluorophores in Heavy Water. Angew Chem Inter Ed 2013; 52(34): 8948–8951.
22. Kass L. A selective stain for eosinophils using two oxazine dyes applied sequentially. Biotech Histochem 1995; 70(1): 19-23.
23. Schmid J A and Birbach A. IkappaB kinase beta (IKKbeta/IKK2/IKBKB)--a key molecule in signaling to the transcription factor NF-kappa B. Cytokine Growth Factor Rev 2008; 19(2): 157-165.
24. Jung C, Müller B K, Lamb D C, Nolde F, Müllen K and Bräuchle C. A new photostable terrylene diimide dye for applications in single molecule studies and membrane labeling. J Am Chem Soc 2006; 128(15): 5283-5291.
25. MacMillan J H, Washburne S S. Detailed Synthetic Procedure for 4-(4-bromophenyl)-1,3(3H) Oxazine-2,6-Dione and related 4 and 5-aryl substituted -1,3(3H) Oxazine-2,6-Diones. Spectroscopic and analytical data are included. Temple University, 2013. http://www.archive.org.
26. Ziegler E, Kollenz G, Kriwetz G and Ott W. Synthesen von Heterocyclen, CCV. Reaktionen mit cyclischen Oxalylverbindungen, XX Reaktionen von 4-Benzoyl-5-phenyl-2,3-dihydrofuran-2,3-dion mit aliphatischen Nitrilen. Liebigs Ann Chem 1977; 1977: 1751-1757.

27. Harinatha Reddy A, Sri Lakshmi B, Nageswari G, Shobha R and Venkatappa B. Phytochemical screening, tlc fingerprinting and antimicrobial activity of methanolic leaf extract of *aloe vera* against clinical isolates of methicillin resistant *staphylococcus aureus* Mintage J Pharm Med Sci 2016; 5: 16-18.
28. Ganapathy S and Karpagam S. Phytochemical and invitro antibacterial activity of leaf and fruit extracts of *aegle marmelos* against methicillin resistant *staphylococcus aureus* (mrsa). Mintage J Pharm Med Sci 2016; 5: 19-21
29. Ukaegbu-Obi K M, Meribe M O and Odo C E. Assessment of antimicrobial activity of aqueous and ethanolic extracts of *monodora myristica* (ehuru) seeds. Mintage J Pharm Med Sci 2016; 4: 1-3.
30. Thirunarayanan G and Renuka V. Solid SiO_2-H_3PO_4 is an efficient catalyst for cyclization of enones under solvent-free condition: synthesis and antimicrobial activities of some oxazine derivatives. J Chil Chem Soc 2014**;** 59(3): 2574-2581.
31. Thirunarayanan G. Hydroxyapatite catalyzed cyclization of aryl enones: Solvent-free efficient synthesis of some 4-aryl-5, 6-dihydro-6-(substituted phenyl)-4H-1, 3-oxazine-2-amines, Annales-UMCS 2014; 69(1-2): 141-158.
32. Sculnick H I, Smith H W, Smith R J and Wierenga W. *US Pat. No. 4495349, C. A.* 1985, 102, 185093.
33. Verma A K, Chioudhary D, Saunthwal R K,, Rustagi V, Patel M and Tiwari R K. On Water: Silver-catalyzed domino approach for the synthesis of Benzoxazine/Oxazine fused isoquinolines and naphthyridines from o-alkenyl aldehydes. J Org Chem 2013; 78(13): 6657-6669.
34. Khalilzadeh M A, Yavari I, Hossaini Z and Sadeghifar H. N-Methylimidazole promoted efficient synthesis of 1,3-oxazine-4-thiones under solvent-free conditions. Monatsch Chem 2009; 140: 467-471.
35. Sapkal S B, Shelke K F, Kategaonkar A H and Shingare MS. Dual role of ammonium acetate for solvent-free synthesis of 1,3-disubstituted-2,3-dihydro-1H-naphth-[1,2e][1,3]-oxazine. Green Chem Lett 2009; 2(2): 57-60.
36. Turgut Z, Pelit E and Koycil A' synthesis of new 1, 3-disubstituted-2,3-dihydro-1H-naphth-[1,2e][1,3]oxazines. Molecules 2007; 12: 345-352.
37. Capuano L, Fischer W, Scheidt W and Schneider M Synthesen mit 2-Diazo-1,3-diketonen, III. Cyclokondensationen mit H-Nucleophilen. Chem Ber 1978; 111, 2497-2509.
38. Li X A, Livermore D and Nikaido H. Role of efflux pumps(s) in intrinsic resistance of pseudomonas aeruginosa: Resistance to tetracycline, chloramphenicol and norfloxacin. Antimicrob Chemther. 1994: 38; 1732 -1734.
39. Thirunarayanan G. Cyclization of aryl enones by SiO_2-H_3PO_4 catalyst under microwave irradiation: Solvent-free synthesis of some oxazine-2-amine derivatives. Adv Appl Res 2014; 6(2): 135-142.
40. Bauer AW, Kirby WMM, Sherris J C and Truck M. Agar disk diffusion for the quality control testing of Autobac elution disks. Am J Clin Pathol. 1966; 45: 493-496.
41. Thirunarayanan G, Mayavel P and Thirumurthy K. Fly-ash:H_2SO_4 catalyzed solvent free efficient synthesis of some aryl chalcones under microwave irradiation*.* Spectrochim Acta 2012; 91A:18-22.