Contents lists available at GrowingScience

Current Chemistry Letters

homepage: www.GrowingScience.com

Synthesis, characterization and antimicrobial activity of some new isoxazole derivatives

M. F. Dhaduk[a*] and H. S. Joshi[b]

[a]*Bahauddin Science College, College Road, Junagadh – 362001, Gujrat, India*
[b]*Department of chemistry, Saurashtra University, Rajkot – 362005, Gujrat, India*

C H R O N I C L E

Article history:
Received February 2, 2022
Received in revised form
March 8, 2022
Accepted April 11, 2022
Available online
April 11, 2022

Keywords:
Isoxazoles
Antimicrobial activity
Antofungal activity
Heterocycles

A B S T R A C T

A series of isoxazole derivatives, 3-aryl-5-[5-(4-nitrophenyl)-2-furyl]-2,3-dihydroisoxazoles **(5a-j)** were synthesized by cyclocondensation reaction between 1-aryl-3-[5-(4-nitrophenyl)-2-furyl]prop-2-en-1-ones **(4a-j)** and hydroxylamine hydrochloride in presence of sodium acetate in glacial acetic acid at reflux temperature. Formerly, compounds **(4a-j)** were synthesized by the condensation of 5-(4-nitrophenyl) furan-2-carbaldehyde **(3)** with various aromatic ketones by using alkali as catalyst. The structures of the synthesized various isoxazole have been characterized by using elemental analysis, Infrared,^{1}H-NMR spectroscopy and further supported by Mass spectroscopy. All the products have been screened for their *in-vitro* biological assay like antibacterial activity towards *Gram-positive* and *Gram-negative* bacterial strains and antifungal activity towards *Aspergillus niger* at a concentration of 40 µg/ml. It was exposed that the compounds **5a, 5c, 5e, 5f,** and **5i** showed inspiring antibacterial and antifungal activity compared to the used reference standard.

1. Introduction

Among the wide range of heterocycles that have been discovered for developing pharmacologically significant molecules, isoxazoles play a momentous role in the field of medicinal chemistry. Isoxazole is an azole with an oxygen atom next to the nitrogen. Isoxazole rings are found in some natural products, such as ibotenic acid[1] and also found in a number of drugs, including COX-2 inhibitor valdecoxib.[2]

The presence of the isoxazole nucleus in different structures leads to expanded applications in different areas such as pigments[3] and agriculture.[4] Many isoxazole derivatives has found their applications as marketed drugs such as Zonisamide (antiobecity[5]), Oxacillin (antibacterial[6]), Risperidone (antipsychotic[7]), Acivicin (antitumour and antileishmania[8]), Leflunomide (Antirheumatic[9]), Isocarboxazid (Antidepressant[10]). Furthermore, the chemistry of isoxazoles has been an interesting field of study for decades because of their prominent potential as analgesic,[11] anti-inflammatory,[12] anticancer,[13] antimicrobial[14] and antiviral.[15] The literature survey revealed that the substitution of various groups on the isoxazole ring imparts different activity.

There are different synthetic pathways of synthesis of isoxazoles, using both homogeneous as well as heterogeneous catalysts. Nevertheless, the most broadly researched and reported synthesis of isoxazole derivative is through the [3+2] cycloaddition reaction of an alkyne that acts as a dipolarophile and nitrile N-oxide as the dipole.[16] Other synthetic methods included simultaneous formation of one C-N bond and one C-O bond via [2+3] cycloaddition reaction of nitrile N-oxides with captodative olefins or methyl crotonate derivatives.[17] Also, the [4+1] cycloaddition reaction of isocyanides with *α*-haloketone oxime in the presence of sodium carbonate.[18] One-pot three component condensation of aryl aldehydes,

* Corresponding author. Tel.: +91 9979930862
E-mail address: drdmfk@gmail.com (M. F. Dhaduk)

doi: 10.5267/j.ccl.2022.4.004

hydroxylamine hydrochloride and ketoesters using potassium phthalimide/sodium ascorbate/sodium tetraborate/sodium azide/sodium citrate/sodium saccharin/N-bromo succinimide or boric acid as a catalyst in aqueous medium at room temperature.[19] Recently, The regioselective *zw*-type [3+2] cycloaddition (32CA) reactions of a series of aryl substituted nitrile N-oxides (NOs) with trichloronitropropene (TNP) have been both experimentally and theoretically studied within the Molecular Electron Density Theory (MEDT), these reactions follow a two-stage one-step mechanism in which formation of the O-C(CCl_3) bond takes place once the C-C(NO_2) bond is already formed.[20] Subsequently, many examples of [2+3] cycloadditions are known which are implemented according to stepwise mechanisms with the participation of biradical or zwitterionic intermediates.[21]

From several processes of synthesis for isoxazoles, the most dominant is [3+2] cyclocondensation process. This reaction permits obtaining isoxazoles with "full atomic economy" and retention of the primary stereo-configuration. Most of the synthesis for isoxazoles in a course of [3+2] cyclocondensation is grasped under mild and non-catalytic condition giving high yields. These conditions approach one of the core values of green chemistry.

Taking these considerations into account, herein is reported the synthesis of various 3-aryl-5-[5-(4-nitrophenyl)-2-furyl]-2,3-dihydroisoxazoles **(5a-j)** to study their antimicrobial activity.

2. Results and Discussion

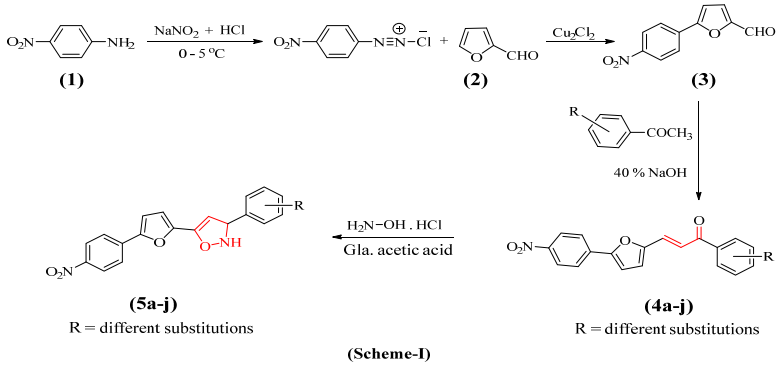
2.1 Chemistry and Spectroscopic discussion

The target compounds 3-aryl-5-[5-(4-nitrophenyl)-2-furyl]-2,3-dihydroisoxazoles **(5a-j)** were synthesized as charted in Reaction **Scheme 1**. The title compounds, 3-aryl-5-[5-(4-nitrophenyl)-2-furyl]-2,3-dihydroisoxazoles **(5a-j)** were synthesized by a four-step procedure starting from 4-nitro aniline **(1)**. Diazotization reaction of 4-nitroaniline **(1)** (0.01mole) in dil. HCl (15%) and water (90 ml) with $NaNO_2$ solution (30%) at 0-5 °C followed by coupling with freshly distilled furfural **(2)** (0.1 mole) in presence of aqueous cupric chloride (2.5 g in 10 ml of water) gives 5-(4-nitrophenyl) furan-2-carbaldehyde **(3)**. The condensation rection of 5-(4-nitrophenyl) furan-2-carbaldehyde **(3)** with various aromatic ketones in methanol + DMF (15+5 ml) solvent in presence of 40% NaOH (1 ml) afforded 1-aryl-3-[5-(4-nitrophenyl)-2-furyl]prop-2-en-1-ones **(4a-j)**. Cyclocondensation reaction of **(4a-j)** and hydroxylamine hydrochloride in presence of sodium acetate in glacial acetic acid gives 3-aryl-5-[5-(4-nitrophenyl)-2-furyl]-2,3-dihydroisoxazoles **(5a-j)**.

The purity of all compounds is checked by thin layer chromatography and their characterization is carried out by means of elemental analysis, Infrared, ^{1}H-NMR spectroscopy and further supported by Mass spectroscopy.

Among all, the IR spectrum of compound **5d** showed strong bands at 3244 cm^{-1} which are due to N-H stretching of isoxazole ring. The compound showed band at 3007 cm^{-1} which are due to aromatic C–H stretching vibration respectively. The band observed at 1599 cm^{-1} and 1514 cm^{-1} is due to aromatic C=C stretching vibration of isoxazole and phenyl ring respectively. Bands observed at 1255 cm^{-1} and 827 cm^{-1} are due to C-N stretching and N-O stretching respectively. Two bands at 1554 cm^{-1} and 1327 cm^{-1} are observed due to N=O asymmetric and symmetric stretching vibrations respectively. The band observed at 1188 cm^{-1} and 1107 cm^{-1} is due to C–O–C asymmetric and symmetric stretching of furyl moiety respectively. The mass spectrum of compound **5i** showed M^+ peak at 350 m/z. The ^{1}H-NMR spectrum of compound **5f** (in $CDCl_3$) showed singlet signal at 2.40 δ due to Ar–CH_3 group. The doublet at 6.61-6.57 δ integrating for one proton is due to C–H of isoxazole ring. A doublet at 7.70-7.66 δ (*J=16.32 Hz*) integrating for one proton is due to =C–H of isoxazole ring. A singlet signal at 11.25 δ for one proton is obtained because of N-H of isoxazole ring. The remaining ten aromatic protons of furyl moiety and phenyl ring appeared between 6.58 δ to 8.26 δ.

All synthesized compounds were screened for their *in-vitro* biological assay like antibacterial activity towards *Gram-positive* and *Gram-negative* bacterial strains and antifungal activity towards *Aspergillus niger* at a concentration of 40 µg/ml.



(Scheme-I)

2.2 biological activity

All the synthesized compounds have been evaluated for antimicrobial activity.

Antibacterial activity

The purified products were screened for their antibacterial activity using cup-plate agar diffusion method. The nutrient agar broth prepared by the usual method was inoculated aseptically with 0.5 ml of 24 hr. old subcultures of *Bacillus coccus*, *Staphylococcus aureus*, *Aerogenes*, *Pseudomonas aeruginosa* in separate conical flasks at 40-50 °C and mixed well by gentle shaking. About 25 ml content of the flask was poured and evenly spreaded in a Petri dish (13 cm diameter) and allowed to set for 2 hr. The cups (10 mm diameter) were formed by the help of borer in agar medium and filled with 0.04ml (40 µg) solution of sample in DMF. The plates were incubated at 37 °C for 24 hrs. and the control was also maintained with 0.04 ml of DMF in a similar manner and the zone of inhibition of the bacterial growth were measured in *millimeter* and recorded in **Table 1**.

Antifungal activity

Aspergillus niger was employed for testing antifungal activity using cup-plate method. The culture was maintained on *subouraud's agar slants*. Sterilized *sabouraud's agar* medium was inoculated with 72 hrs. old 0.5 ml suspension of fungal spores in a separate flask.

About 25 ml of inoculated medium was evenly spreaded in a Petri dish and allowed to set for two hrs. The plates were incubated at 30 °C for 48 hrs. After the completion of incubation period, the zone of inhibition of growth in the form of diameter in *mm* was measured. Along the test solution in each Petri dish one cup was filled with solvent which act as control. Standard drugs like *Amoxicillin*, *Benzyl Penicillin*, *Ciprofloxacin*, *Erythromycin* and *Griseofulvin* were used for comparison purpose. The zones of inhibition are recorded in **Table 1**.

Table 1: Antimicrobial screening results of compounds **5a-j**

Sample ID	R	Zone of inhibition in *mm*				
		Antibacterial activity (%)				Antifungal activity (%)
		B. coccus	*S. aureus*	*Aerogenes*	*Pseudomonas*	*A. niger*
5a	H	24	17	22	16	24
5b	4-Cl	20	18	14	21	20
5c	2-NO_2	18	22	23	19	15
5d	4-NO_2	14	14	15	20	19
5e	4-OCH_3	22	19	19	21	23
5f	4-CH_3	23	20	20	18	14
5g	4-F	19	21	14	12	18
5h	4-Br	12	14	18	17	20
5i	2-OH	22	10	20	16	21
5j	4-OH	20	15	19	13	14
Amoxicillin	-	25	25	20	22	00
Benzyl penicillin	-	18	19	21	21	00
Ciprofloxacin	-	20	15	22	16	00
Erythromycin	-	22	21	19	23	00
Greseofulvin	-	00	00	00	00	26

From activity data, it is observed that compounds **5a** and **5f** were found to be active which is attributed to the presence of simple and 4-CH_3-substituted benzene ring at C_3-position of isoxazole nucleus, while compounds **5b**, **5e**, **5i** and **5j** show moderate activity against *Bacillus coccus* with reference to standard drugs. Compounds **5c** and **5g** were found to be active due to the presence of 2-NO_2 and 4-F substituted benzene ring at C_3-position of isoxazole nucleus, while compounds **5e** and **5f** show moderate activity against *Staphylococcus aureus.* Against *Aerogenes* strain, compound **5a** and **5c** were found to be active which is attributed to the presence of simple and 2-NO_2 substituted benzene ring at C_3-position of isoxazole nucleus, while compounds **5e**, **5f, 5i** and **5j** were found to be moderate active. Against *Pseudomonas aeruginosa,* compounds **5b** and **5e** are active due to the presence of 4-Cl and 4-OCH_3 substituted benzene ring at C_3-position of isoxazole nucleus than others. Compounds **5a** and **5e** were active because of presence of simple and 4-OCH_3 substituted benzene ring at C_3-position of isoxazole nucleus while compounds **5h** and **5i** show moderate activity against *Aspergillus niger* as compare to standard drugs. Among isoxazole derivatives, it was interesting to notice that, the activity depends on the substituents rather than the basic skeleton of the molecule. It was noticed that, groups -CH_3, -OCH_3, -Cl, -NO_2, -F and -H on the benzene ring at C_3-position of isoxazole nucleus, enhanced their antimicrobial activity and are better antimicrobial agents.

3. Conclusions

The synthetic method involves robust synthesis of dihydroisoxazoles **(5a-j)** with furyl moiety, starting from freshly prepared *α, β*-unsaturated ketones and dinucleophiles (hydroxyl amine hydrochlorides) in very good yields. The method is endowed with several unique merits including benign reaction conditions, stereochemical diversity, broad substrate scope etc. The method has successfully applied for synthesis of a diverse library of ten hybrid isoxazoles. Then after, these

synthesized isoxazoles evaluated as anti-microbial agents against a group of antibacterial and antifungal strains. They were found to possess reasonably good antifungal activity and compounds **5a, 5b, 5e, 5f, 5i** and **5j** were found to be the most potent anti-microbial agents. It will be the topic of new research to substitute it with greener reagents and solvents, finding more effective anti-microbial agents. This work confirms the high importance of organic compounds in applied fields as reported before in the previous scientific papers.

Acknowledgements

Authors are thankful to the Department of Chemistry for providing laboratory facilities. The authors are thankful for facilities and grants given under UGC-SAP for Department Research Support (DRS) and Department of Science and Technology (DST), New Delhi for Fund for Improvement of Science and Technology (FIST). Authors are also thankful to RSIC Chandigarh for providing ^{1}H NMR and spectral analysis of the compounds and Department of Bio-chemistry, Saurashtra University for antimicrobial activity of newly synthesized compounds.

4. Experimental

4.1. Materials and Methods

Chemicals and solvents were purchased from Sigma-Aldrich Chemical Co., Merck chemical, Finar, and Spectrochem Ltd. Thin layer chromatography [silica gel G (E Merck) plates] was used to monitor the reactions and purity of newly synthesized compounds. Visualization was achieved with UV light (254 and 365 nm) or with iodine vapor. The melting point were determined by electrothermal apparatus using open capillary tubes and are uncorrected. IR spectra were recorded on a Shimadzu 8400 FTIR instrument in KBr disc and only significant absorbance levels (cm^{-1}) are listed. ^{1}H NMR spectra (400 MHz) were recorded on a "Bruker AVANCE III spectrometer" in $CDCl_3$ solvent using TMS as internal standard. Chemical shift is given in δ ppm. Mass spectra were determined using direct inlet probe on GCMS-QP2010 mass spectrometer (Shimadzu, Kyoto, Japan). Elemental analysis was performed on a Carlo Erba EA1108 elemental analyser.

4.2. General procedure

4.2.1 Procedure for the synthesis of 5-(4-nitrophenyl) furan-2-carbaldehyde **(3)**

A mixture of 4-nitroaniline **(1)** (13.8 g, 0.01 mole), dil. HCl (15%, 60 ml) and water (90 ml) was heated to get a clear solution. The solution was cooled to 0 °C and diazotized with $NaNO_2$ solution (30%, 24 ml) and freshly distilled furfural **(2)** (11.1 ml, 0.1 mole) and aqueous cupric chloride (2.5 g in 10 ml of water) were added with stirring. The stirring was continued for 5 hrs. The separated solid was collected by filtration and washed with cold methanol, crystallized from a mixture of ethanol-DMF (3:7 ratio). Yield 70%, m.p. 187 ^{O}C. IR(KBr): 3052 (C=C-H str.), 2745 and 2830 (C-H str. of aldehyde), 1729 (C=O str. of aldehyde), 1510 (N=O str. asym.), 1490 (C=C str.), 1354 (N=O str. asym.), 1250 (C-O-C str.sym.), 1059 (C-O-C str. asym.) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): 7.42 (d, J = 3.62 Hz, 1H, Furyl-H_e), 7.69 (d, J = 3.69 Hz, 1H, Furyl-H_f), 7.91 (d, J = 8.19 Hz, 2H, Ar-$H_{b,b'}$), 8.33 (d, J = 9.12 Hz, 2H, Ar-$H_{a,a'}$), 9.72 (s, 1H, -CHO); EI-MS: m/z 217. ($C_{11}H_7NO_4$; required: C, 60.83; H, 3.25; N, 6.45%; found: C, 60.79; H, 3.21; N, 6.39%).

4.2.2 Procedure for the synthesis of 1-aryl-3-[5-(4-nitrophenyl)furan-2-yl]prop-2-en-1-ones **(4a-j)**

A solution of substituted acetophenone (0.01 mole) in minimum quantity of methanol (10 ml) was added to a mixture of 5-(4-nitrophenyl) furan-2-carbaldehyde **(3)** (2.17 gm, 0.01 mole) in methanol + DMF (15+5 ml) and 40% NaOH (1 ml) was added to make it alkaline. The reaction mixture was then stirred for 20-24 hrs at room temperature. The product was isolated by filtration and crystallized from suitable solvent to give pure products.

Spectral data of 1-(4-methoxyphenyl)-3-[5-(4-nitrophenyl)furan-2-yl]prop-2-en-1-one **(4e)**
Yield 62%; m.p. 120-22°C; IR (KBr): 3055 (C=C-H str.), 1649 (C=O str.), 1517 (C=C), 1250 (C-O-C str.sym.), 1059 (C-O-C str. asym.) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): 3.91 (s, 3H, Ar-OCH_3), 6.84 (d, J = 3.64 Hz, 1H, Furyl-H_e), 6.99 (d, J = 3.90 Hz, 1H, Furyl-H_f), 7.02 (d, J = 8.84 Hz, 2H, Ar-$H_{d,d'}$), 7.60 (d, 2H, vinyl-H_A, H_B), 7.91 (d, J = 8.92 Hz, 2H, Ar-$H_{b,b'}$), 8.09 (d, J = 8.84 Hz, 2H, Ar-$H_{c,c'}$), 8.31 (d, J = 9.20 Hz, 2H, Ar-$H_{a,a}$; EI-MS: m/z 349. Anal. Calcd. for $C_{20}H_{15}NO_5$; required: C, 68.76; H, 4.33; N, 4.01%; found: C, 68.71; H, 4.29; N, 3.96%).

4.2.3 Procedure for the synthesis of 3-aryl-5-[5-(4-nitrophenyl)-2-furyl]-2,3-dihydroisoxazoles **(5a-j)**

To a solution of 1-aryl-3-[5-(4-nitrophenyl)furan-2-yl]prop-2-en-1-ones **(4a-j)** (0.01 mole) in ethanol + DMF (15+5 ml), anhydrous sodium acetate (0.01 mole) and hydroxylamine hydrochloride (0.01 mole) in minimum quantity of acetic acid were added. The reaction mixture was refluxed on oil bath for 7-12 hrs. The product was isolated and crystallized from suitable solvent to give analytically pure products.

4.3 Physical and Spectral Data

4.3.1 3-phenyl-5-[5-(4-nitrophenyl)-2-furyl]-2,3-dihydroisoxazole (5a)

Yield 54%; m.p. 108-110°C; IR (KBr): 3240 (N-H str.), 3015 (C=C-H str.), 1599 and 1518 (C=C str.), 1242 (C-N str.), 1180 (C-O-C str. asym.), 1106 (C-O-C str. sym.), 827 (N-O str.) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): 6.63-6.57 (d, J = 3.54 Hz, 1H, Furyl-H_d), 6.67-6.63 (d, 1H, Isoxazole-H_g), 6.96 (d, J = 3.90 Hz, 1H, Furyl-H_e), 7.20-7.35 (m, 5H, Ar-H), 7.74-7.62(d, J=16.28, 1H, Isoxazole-H_f), 7.79 (d, J = 7.06 Hz, 2H, Ar-$H_{c,c'}$), 8.22 (d, J=7.10 Hz, 2H, Ar-$H_{d,d'}$), 11.20 (s, 1H, Isoxazole-NH); EI-MS: m/z 334. Anal. Calcd. For $C_{19}H_{14}N_2O_4$; required: C, 68.26; H, 4.19; N, 8.38 %; found: C, 68.23; H, 4.18; N, 8.34%).

4.3.2 3-(4-chlorophenyl)-5-[5-(4-nitrophenyl)-2-furyl]-2,3-dihydroisoxazole (5b)

Yield 62%; m.p. 124-126°C; IR (KBr): 3238 (N-H str.), 3022 (C=C-H str.), 1591 and 1525 (C=C str.), 1236 (C-N str.), 1178 (C-O-C str. asym.), 1102 (C-O-C str. sym.), 821 (N-O str.), 568 (C-Cl str.) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): 6.60-6.56 (d, J = 3.50 Hz, 1H, Furyl-H_d), 6.63-6.60 (d, 1H, Isoxazole-H_g), 6.96 (d, J=3.92 Hz, 1H, Furyl-H_e), 7.24-7.22 (d, J=7.96, 2H, Ar-$H_{a,a'}$), 7.38 (d, J=8.04, 2H, Ar-$H_{b,b'}$), 7.68-7.61 (d, J=16.34, 1H, Isoxazole-H_f), 7.81 (d, J=7.07 Hz, 2H, Ar-$H_{c,c'}$), 8.32 (d, J=7.10 Hz, 2H, Ar-$H_{d,d'}$), 11.20 (s, 1H, Isoxazole-NH); EI-MS: m/z 369. Anal. Calcd. for $C_{19}H_{13}ClN_2O_4$; required: C, 61.78; H, 3.52; N, 7.60%; found: C, 61.73; H, 3.49; N, 7.56%).

4.3.3 3-(2-nitrophenyl)-5-[5-(4-nitrophenyl)-2-furyl]-2,3-dihydroisoxazole (5c)

Yield 58%; m.p. 138-140°C; IR (KBr): 3240 (N-H str.), 3020 (C=C-H str.), 1590 and 1522 (C=C str.), 1220 (C-N str.), 1162 (C-O-C str. asym.), 1110 (C-O-C str. sym.), 822 (N-O str.), cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): 6.58-6.50 (d, J= 3.50 Hz, 1H, Furyl-H_d), 6.68-6.63 (d, 1H, Isoxazole-H_g), 6.97 (d, J=3.92 Hz, 1H, Furyl-H_e), 7.22-7.47 (m, 4H, Ar-H), 7.68-7.65 (d, 1H, J=16.28, Isoxazole-H_f), 7.82 (d, J=7.10 Hz, 2H, Ar-$H_{c,c'}$), 8.31 (d, J=7.14 Hz, 2H, Ar-$H_{d,d'}$), 11.19 (s, 1H, Isoxazole-NH); EI-MS: m/z 379. Anal. Calcd. for $C_{19}H_{13}N_3O_6$; required: C, 60.15; H, 3.43; N, 11.08%; found: C, 60.12; H, 3.42; N, 11.03%).

4.3.4 3-(4-nitrophenyl)-5-[5-(4-nitrophenyl)-2-furyl]-2,3-dihydroisoxazole (5d)

Yield 67%; m.p. 134-136°C; IR (KBr): 3244 (N-H str.), 3007 (C=C-H str.), 1599 and 1514 (C=C str.), 1258 (C-N str.), 1188 (C-O-C str. asym.), 1107 (C-O-C str. sym.), 827 (N-O str.) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): 6.56-6.52 (d, J= 3.51 Hz, 1H, Furyl-H_d), 6.67-6.60 (d, 1H, Isoxazole-H_g), 6.87 (d, J=3.90 Hz, 1H, Furyl-H_e), 7.20-7.26 (d, J=7.92, 2H, Ar-$H_{a,a'}$), 7.32 (d, J=8.00, 2H, Ar-$H_{b,b'}$), 7.70-7.63 (d, J=16.30, 1H, Isoxazole-H_f), 7.86 (d, J=7.11 Hz, 2H, Ar-$H_{c,c'}$), 8.36 (d, J=7.18 Hz, 2H, Ar-$H_{d,d'}$), 11.21 (s, 1H, Isoxazole-NH); EI-MS: m/z 379. Anal. Calcd. for $C_{19}H_{13}N_3O_6$; required: C, 60.15; H, 3.43; N, 11.08%; found: C, 60.123; H, 3.42; N, 11.06%).

4.3.5 3-(4-methoxylphenyl)-5-[5-(4-nitrophenyl)-2-furyl]-2,3-dihydroisoxazole (5e)

Yield 60%; m.p. 158-160°C; IR (KBr): 3234 (N-H str.), 3017 (C=C-H str.), 2970 (C-H str.), 1585 and 1522 (C=C str.), 1224 (C-N str.), 1150 (C-O-C str. asym.), 1106 (C-O-C str. sym.), 821 (N-O str.) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): 3.85 (s, 3H, Ar-OCH_3), 6.62-6.57 (d, J=3.54 Hz, 1H, Furyl-H_d), 6.77-6.63 (d, 1H, Isoxazole-H_g), 6.93 (d, J=3.89 Hz, 1H, Furyl-H_e), 7.26-7.32 (d, J=7.91, 2H, Ar-$H_{a,a'}$), 7.36 (d, J=8.03, 2H, Ar-$H_{b,b'}$), 7.68-7.59 (d, J=16.24, 1H, Isoxazole-H_f), 7.79 (d, J=7.14 Hz, 2H, Ar-$H_{c,c'}$), 8.31 (d, J=7.22 Hz, 2H, Ar-$H_{d,d'}$), 11.26 (s, 1H, Isoxazole-NH); EI-MS: m/z 364. Anal. Calcd. for $C_{20}H_{16}N_2O_5$; required: C, 65.93; H, 4.06; N, 7.69%; found: C, 65.90; H, 4.03; N, 7.65%).

4.3.6 3-(4-methylphenyl)-5-[5-(4-nitrophenyl)-2-furyl]-2,3-dihydroisoxazole (5f)

Yield 49%; m.p. 146-148°C; IR (KBr): 3238 (N-H str.), 3011 (C=C-H str.), 2947 (C-H str.), 1590 and 1518 (C=C str.), 1230 (C-N str.), 1152 (C-O-C str. asym.), 1110 (C-O-C str. sym.), 815 (N-O str.) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): 2.40 (s, 3H, Ar-CH_3), 6.59-6.58 (d, J=3.56 Hz, 1H, Furyl-H_d), 6.61-6.57 (d, 1H, Isoxazole-H_g), 7.00 (d, J=3.96 Hz, 1H, Furyl-H_e), 7.24-7.22 (d, J=7.96, 2H, Ar-$H_{a,a'}$), 7.38 (d, J=8.04, 2H, Ar-$H_{b,b'}$), 7.70-7.66 (d, J=16.32, 1H, Isoxazole-H_f), 7.89 (d, J=7.08 Hz, 2H, Ar-$H_{c,c'}$), 8.26 (d, J=7.12 Hz, 2H, Ar-$H_{d,d'}$), 11.25 (s, 1H, Isoxazole-NH); EI-MS: m/z 348. Anal. Calcd. for $C_{20}H_{16}N_2O_4$; required: C, 68.96; H, 4.59; N, 8.04 %; found: C, 65.90; H, 4.56; N, 8.01%).

4.3.7 3-(4-fluorolphenyl)-5-[5-(4-nitrophenyl)-2-furyl]-2,3-dihydroisoxazole (5g)

Yield 61%; m.p. 163-165°C; IR (KBr): 3244 (N-H str.), 3015 (C=C-H str.), 1582 and 1510 (C=C str.), 1220 (C-N str.), 1145 (C-O-C str. asym.), 1108 (C-O-C str. sym.), 811 (N-O str.), 1327 (C-F str.) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): 6.61-6.55 (d, J = 3.51 Hz, 1H, Furyl-H_d), 6.67-6.62 (d, 1H, Isoxazole-H_g), 6.98 (d, J=3.90 Hz, 1H, Furyl-H_e), 7.26-7.20 (d, J=7.97, 2H, Ar-$H_{a,a'}$), 7.40 (d, J=8.02, 2H, Ar-$H_{b,b'}$), 7.69-7.63 (d, J=16.28, 1H, Isoxazole-H_f), 7.76 (d, J=7.00 Hz, 2H, Ar-$H_{c,c'}$), 8.30

(d, J=7.08 Hz, 2H, Ar-$H_{d,d'}$), 11.22 (s, 1H, Isoxazole-NH); EI-MS: m/z 352. Anal. Calcd. for $C_{19}H_{13}FN_2O_4$; required: C, 64.77; H, 3.69; N, 7.95%; found: C, 64.75; H, 3.67; N, 7.90%).

4.3.8 3-(4-bromolphenyl)-5-[5-(4-nitrophenyl)-2-furyl]-2,3-dihydroisoxazole (5h)

Yield 57%; m.p. 157-159°C; IR (KBr): 3243 (N-H str.), 3016 (C=C-H str.), 1580 and 1513 (C=C str.), 1224 (C-N str.), 1147 (C-O-C str. asym.), 1106 (C-O-C str. sym.), 814 (N-O str.), 622 (C-Br str.) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): 6.62-6.58 (d, J=3.53 Hz, 1H, Furyl-H_d), 6.69-6.64 (d, 1H, Isoxazole-H_g), 6.73 (d, J=3.94 Hz, 1H, Furyl-H_e), 7.28-7.21 (d, J=7.82, 2H, Ar-$H_{a,a'}$), 7.42 (d, J=8.04, 2H, Ar-$H_{b,b'}$), 7.68-7.64 (d, J=16.30, 1H, Isoxazole-H_f), 7.79 (d, J=7.04 Hz, 2H, Ar-$H_{c,c'}$), 8.23 (d, J=7.04 Hz, 2H, Ar-$H_{d,d'}$), 11.24 (s, 1H, Isoxazole-NH); EI-MS: m/z 413. Anal. Calcd. for $C_{19}H_{13}BrN_2O_4$; required: C, 55.20; H, 3.15; N, 6.78%; found: C, 55.23; H, 3.16; N, 6.73%).

4.3.9 3-(2-hydroxylphenyl)-5-[5-(4-nitrophenyl)-2-furyl]-2,3-dihydroisoxazole (5i)

Yield 68%; m.p. 114-117°C; IR (KBr): 3542 (O-H str.), 3240 (N-H str.), 3020 (C=C-H str.), 1590 and 1511 (C=C str.), 1226 (C-N str.), 1140 (C-O-C str. asym.), 1102 (C-O-C str. sym.), 812 (N-O str.), 1125 (C-OH str.) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): 6.58-6.47 (d, J=3.50 Hz, 1H, Furyl-H_d), 6.70-6.62 (d, 1H, Isoxazole-H_g), 6.70 (d, J=3.91 Hz, 1H, Furyl-H_e), 6.96-7.64 (m, 4H, Ar-H), 7.62-7.53 (d, J=16.28, 1H, Isoxazole-H_f), 7.69 (d, J=7.02 Hz, 2H, Ar-$H_{c,c'}$), 8.20 (d, J=7.00 Hz, 2H, Ar-$H_{d,d'}$), 11.20 (s, 1H, Isoxazole-NH), 12.96 (s, 1H, -OH); EI-MS: m/z 350. Anal. Calcd. For $C_{19}H_{14}N_2O_5$; required: C, 65.14; H, 4.00; N, 8.00%; found: C, 65.11; H, 3.99; N, 7.97%).

4.3.10 3-(4-hydroxylphenyl)-5-[5-(4-nitrophenyl)-2-furyl]-2,3-dihydroisoxazole (5j)

Yield 63%; m.p. 124-126°C; IR (KBr): 3552 (O-H str.), 3238 (N-H str.), 3022 (C=C-H str.), 1589 and 1509 (C=C str.), 1219 (C-N str.), 1136 (C-O-C str. asym.), 1100 (C-O-C str. sym.), 810 (N-O str.), 1123 (C-OH str.) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): 6.57-6.46 (d, J=3.48 Hz, 1H, Furyl-H_d), 6.73-6.67 (d, 1H, Isoxazole-H_g), 6.74 (d, J=3.86 Hz, 1H, Furyl-H_e), 6.94 (d, J = 8.31 Hz, 2H, Ar-$H_{a,a'}$); 7.69 (d, J = 8.84 Hz, 2H, Ar-$H_{b,b'}$), 7.65-7.58 (d, J=16.30, 1H, Isoxazole-H_f), 7.65 (d, J=7.05 Hz, 2H, Ar-$H_{c,c'}$), 8.12 (d, J=7.03 Hz, 2H, Ar-$H_{d,d'}$), 11.12 (s, 1H, Isoxazole-NH), 12.94 (s, 1H, -OH); EI-MS: m/z 350. Anal. Calcd. For $C_{19}H_{14}N_2O_5$; required: C, 65.14; H, 4.00; N, 8.00%; found: C, 65.12; H, 3.98; N, 7.97%).

References

1. David A. Warrell, **(2020)** Hunter's Tropical Medicine and Emerging Infectious Diseases, Tenth Edition, pp.1006-1020.
2. Jesse P. W. and Richard C. L., **(2007)** The Synthesis of Highly Substituted Isoxazoles by Electrophilic Cyclization: An Efficient Synthesis of Valdecoxib, *J. Org. Chem,,* 72, 25, 9643–9647.
3. Abdel-Hafiz S. A. and El-Kholy Y. M., **(1995)** Heterocyclic dye synthesis: Synthesis and dye performance of new polyfunctionally isoxazole azo dye, Pigment and Resin Technology, 24 (5), 13-16.
4. Ki-Liang G., Zhao L. and Ze-Whi W., **(2020)** Design, Synthesis and Bioevaluation of Substituted Phenyl Isoxazole Analogs as Herbicide Safeners, *Journal of Agricultural and Food Chemistry*, 68 (39) 10550–10559.
5. Shin J. H., Gadde K. M., Ostbye T., Bray G. A., **(2014)** Weight changes in obese adults 6-months after discontinuation of double-blind Zonisamide or placebo treatment, *Diabetes Obes Metab* 16, 766–768.
6. Lainson J. C., Daly S. M., Triplett K., Johnston S. A., Hall P. R., Diehnelt C. W., **(2017)** Synthetic antibacterial peptide exhibits synergy with Oxacillin against MRSA. *ACS Med Chem Lett*, 8, 853–857.
7. Schoretsanitis G., Spina E., Hiemke C., De Leon J., **(2017)** A systematic review and combined analysis of therapeutic drug monitoring studies for long-acting risperidone, *Expert Rev Clin Pharmacol*, 10, 965–981.
8. Conti P., Roda G., Stabile H., Vanoni M. A., Curti B., De Amici M., **(2003)** Synthesis and biological evaluation of new amino acids structurally related to the antitumor agent Acivicin, *Farmaco,* 58, 683–690.
9. Golicki D., Newada M., Lis J., Pol K., Hermanowski T., Tłustochowicz M., **(2012)** Leflunomide in monotherapy of rheumatoid arthritis: meta-analysis of randomized trials, *Pol Arch Med Wewn*, 122, 22–32.
10. Shader R. I., Greenblatt D. J., **(1999)** The reappearance of a monamine oxidase inhibitor (Isocarboxazid), *J Clin Psychopharmacol*, 19, 105–106.
11. Karthikeyan K., Veenus Seelan T., Lalitha K. G., Perumal P. T., **(2009)** Synthesis and antinociceptive activity of pyrazolyl isoxazolines and pyrazolyl isoxazoles, *Bioorg Med Chem Lett.,* 19, 3370–3373.
12. Rajanarendar E., Rama Krishna S., Nagaraju D., Govardhan Reddy K., Kishore B., Reddy Y. N., **(2015)** Environmentally benign synthesis, molecular properties prediction and anti-inflammatory activity of novel isoxazolo[5,4-d]isoxazol-3-yl-aryl-methanones via vinylogous Henry nitroaldol adducts as synthons, *Bioorg Med Chem Lett*, 25, 1630–1634.
13. Tzanetou E., Liekens S., Kasiotis K. M., Melagraki G., Afantitis A., Fokialakis N., Haroutounian S. A., **(2014)** Antiproliferative novel isoxazoles: Modeling, virtual screening, synthesis, and bioactivity evaluation, *Eur J Med Chem.,* 81, 139–149.
14. Basha S. S., Divya K., Padmaja A., Padmavathi V., **(2015)** Synthesis and antimicrobial activity of thiazolyl pyrazoles and isoxazoles, *Res Chem Intermed,* 41, 10067–10083.

15. Lee Y. S., Park S. M., Kim B. H., **(2009)** Synthesis of 5-isoxazol-5-yl-2′- deoxyuridines exhibiting antiviral activity against HSV and several RNA viruses, *Bioorg Med Chem Lett.*, 19, 1126–1128.
16. Page L. W., Bailey M., Beswick P. J., Frydrych S., Gleave R. J., **(2010)** The acid-mediated intramolecular 1, 3-dipolar cycloaddition of derived 2-nitro-1, 1-ethenediamines for the synthesis of novel fused bicyclic isoxazoles, *Tetrahedron Lett.*, 51, 3388–3391.
17. Lasri J., Mukhopadhyay S., Charmier MAJ., **(2008)** Efficient regioselective synthesis of 4- and 5-substituted isoxazoles under thermal and microwave conditions, *J Heterocycl Chem*., 45, 1385–1389.
18. Buron C., El Kaim L., Uslu A., **(1997)** A new straightforward formation of aminoisoxazoles from isocyanides, *Tetrahedron Lett*., 38, 8027–8030.
19. Agrawal N. and Mishra P., **(2018)** The synthetic and therapeutic expedition of isoxazole and its analogs, *Medicinal Chemistry Research,* 27, 1309–1344.
20. Zawadzinska K., Ríos-Gutierrez M., Kula K., Wolinski P., Miroslaw B., Krawczyk T. and Jasinski R., **(2021)** The participation of 3,3,3-trichloro-1-nitroprop-1-ene in the [3+2] cycloaddition reaction with selected nitrile N-oxides in the light of the experimental and MEDT quantum chemical study, *Molecules*, 26, 6774.
21. Jasinski R. and Dresler E., **(2020)** Question of Zwitterionic Intermediates in the [3+2] Cycloaddition Reactions: A Critical Review, *Organics,* 1, 46-69.