SYNTHESIS OF SUBSTITUTED 2-HYDROXYNAPHTHYL ISOXAZOLINE DERIVATIVES AS ANTIBACTERIAL AGENTS

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ABSTRACT

In the present study new series of isoxazolines (3a-3f) were synthesized from 2-hydroxynaphthyl functionalized chalcones and hydroxylamine hydrochloride in 2-ethoxy ethanol. The Synthesized isoxazoline were purified by recrystallization and evaluated for antibacterial activity againstfour pathogenic bacteria, two gram negative and two gram positive.The antibacterial data revealed that electron rich (3f) and halogen disubstituted(3a, 3b, and 3c) isoxazolines showed higher activity against bacterial strain tested. It also revealed that allcompounds showed good to moderate activity compared to standard drug.

Keywords: *Chalcones, hydroxylamine hydrochloride, isoxazolines, antibacterial activity.*

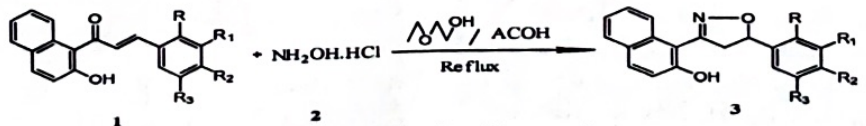
Introduction

In recent years, millions of people in the region of the world are being suffer either Gram-positive or Gram-negative bacterial strains. The result of these microorganism leads to food poisoning, diarrhoea, salmonellosis, rheumatic etc.[1] Thus, antibiotics are the primary solutions for these microbial infections.However, continuous and overuse of these antibiotics has led to multi-drug resistance of several groups of microorganisms[2]. Furthermore, the existing pharmacological drugs are either too expensive or turn to ineffective or have undesirable side effects[3]. Thus, there is an emergency to develop new antibiotic agents with novel targets for the extension of bacterial aggressions in recent years[4].Compounds incorporating heterocyclic ring systems continue to attract considerable interest due to thewide range of biological activities they posses [5-9]. Among the wide range of heterocycles that have been explored for developing pharmacologically important molecule is isoxazolines [10-14], which play a significant role in the field of medicinal chemistry.

In addition, isoxazoline derivatives have medicinal activities such as anti-inflammatory[15], antibacterial[16-18], anticonvulsant [19], antibiotic[20], antituberculer[21], antifungal [22], anxiolytic [23],antidepressant [24], analgesic [25], antioxidant, antituberculosis, anticonvulsant activity [26]. Encouraged by the diverse biological activities of isoxazoline compounds, it prompted us to synthesize new isoxazoline derivatives from 2-hydroxynaphthyl functionalized chalcones and evaluate their antibacterial activity against four pathogenic bacteria.

Material and methods

All the Chemicals used in the synthesis are used were of laboratory grade. Melting points were determined in an open capillary tube and are uncorrected. Purity of compounds and completion of the reaction was monitored by thin layer chromatography using hexane/ethyl acetate (7:3) as the mobile phase on precoated sheets of silica gel-G (Merck, Germeny) using iodine vapour for detection. IR spectra were recorded in KBr on a Perkin-Elmer spectrometer. ^{1}HNMR spectra were recorded on Avance spectrometer (Bruker, Germany) 300 MHz in $CDCl_3$ using TMS as an internal standard and chemical shifts are reported in δ units and Elemental analysis was performed on Perkin-Elmer 240 CHN elemental analyzer.



Scheme 1. Synthesis of Isoxazolines

Table 1. The substituent R, % yield and melting point of synthesized compounds

Sr. No.	Compound	R	R_1	R_2	R_3	% Yield	Mp (°C)
1)	3a	OH	Cl	H	Cl	70	187-189
2)	3b	OH	Br	H	Br	68	196-198
3)	3c	OH	I	H	I	78	202-204
4)	3d	H	H	F	H	76	176-178
5)	3e	H	H	CN	H	74	181-183
6)	3f	H	H	OCH_3	H	70	184-186

General procedure for synthesis of Isoxazolines

To an 2-ethoxy ethanol solution 2-hydroxynaphthyl functionalized chalcones(1)(0.01mol) was added with hydroxylamine hydrochloride(2) (0.02 mol) along with 2-3 drops of acetic acid. The contents were stirred at reflux temperature for 5-6 hrs. After completion of the reaction the mixture was cooled and poured into ice cold water. The resultant solid product (3a-3f) was filtered, washed with sufficient cold water, dried and purified by recrystallization from ethanol[27]. Yields of the products varied between 68 and 78%.

Results and discussion

This paper describes simple method for effective synthesis of isoxazolines from substituted2-hydroxynaphthyl functionalized chalconesand hydroxyamine hydrochloride in 2-ethoxy ethanol as a solvent.All the synthesized compounds (3a-3f) have been characterized by their M.P., Elemental analysis, IR, H1NMR and mass spectra. Scheme-1. All the isoxazolinesshowed absorption band in region 3430-3300 cm^{-1} due to hydroxyl (O-H) stretching vibration. ^{1}HNMR spectra is the best analyzing tool for the structural elucidation, it showed two doublet in the region of 3.60-5.93 δ ppm indicate presence of H_a, H_b and H_x type of protons (isoxazoline ring) and also showed a singlet in range 11.50 to 12.10 ppm due to hydroxyl group.

1-(5-(3,5-dichloro-2-hydroxyphenyl)-4,5-dihydroisoxazol-3-yl)naphthalen-2-ol. (3a)

Yield:70%. M.p.:187-189°C. ^{1}HNMR (300 MHz,$CDCl_3$, ppm): 3.60 (dd, Ha, 1 H), 3.85 (dd, Hb, 1 H),5.93 (dd, Hx,1 H),8.07-7.22 (m, Ar-H, 8 H),11.70 (s,OH, 2H); IR (KBr, cm^{-1}): 3330 (OH), 1498 (C=N), 1382 (C-O), Anal. Calc. for $C_{19}H_{13}Cl_2NO_3$ (374): C 60.98, H 3.50, Cl 18.95, N 3.14, ; Found:C 60.95, H 3.52, Cl 18.93, N 3.12.

1-(5-(3,5-dibromo-2-hydroxyphenyl)-4,5-dihydroisoxazol-3-yl)naphthalen-2-ol. (3b)

Yield:68%. M.p.:196-198°C. ^{1}HNMR (300 MHz,$CDCl_3$, ppm): 3.50 (dd, Ha, 1 H), 3.89 (dd, Hb, 1 H),5.80 (dd, Hx,1 H),8.00-7.30 (m, Ar-H, 8 H),11.50 (s,OH, 2H); IR (KBr, cm^{-1}): 3430 (OH), 1490 (C=N), 1385 (C-O), Anal. Calc. for $C_{19}H_{13}Br_2NO_3$ (463): C 49.28, H 2.83, Br 34.51, N 3.02, ; Found:C 49.20, H 2.80, Br34.50, N 3.00.

1-(5-(3,5-diiodo-2-hydroxyphenyl)-4,5-dihydroisoxazol-3-yl)naphthalen-2-ol. (3c)

Yield:78%. M.p.:202-204°C. ^{1}HNMR (300 MHz,$CDCl_3$, ppm): 3.60 (dd, Ha, 1 H), 3.88 (dd, Hb, 1 H),5.75 (dd, Hx,1 H),8.20-7.35 (m, Ar-H, 8 H), 11.70 (s,OH, 2H); IR (KBr, cm^{-1}): 3420 (OH), 1495 (C=N), 1389 (C-O), Anal. Calc. for$C_{19}H_{13}I_2NO_3$ (557): C 40.96, H 2.35, I 45.56, N 2.51, ; Found:C 40.92, H 2.32, I 45.52, N 2.50.

1-(5-(4-fluorophenyl)-4,5-dihydroisoxazol-3-yl)naphthalen-2-ol.(3d)

Yield:76%. M.p.:176-178°C. ^{1}HNMR (300 MHz,$CDCl_3$, ppm): 3.70 (dd, Ha, 1 H), 3.80 (dd, Hb, 1 H),5.90 (dd, Hx,1 H),7.95-7.35 (m, Ar-H, 10 H),11.90 (s,OH, 1H); IR (KBr, cm^{-1}): 3390 (OH), 1490 (C=N), 1385 (C-O), Anal. Calc. for $C_{19}H_{14}FNO_2$(307): C 74.26, H 4.59, F6.18, N 4.56 ; Found:C 74.22, H 4.57, F6.16, N 4.52.

1-(5-(4-cyanophenyl)-4,5-dihydroisoxazol-3-yl)naphthalen-2-ol. (3e)

Yield:74%. M.p.:181-183°C. ^{1}HNMR (300 MHz,$CDCl_3$, ppm): 3.70 (dd, Ha, 1 H), 3.80 (dd, Hb, 1 H),5.80 (dd, Hx,1 H),7.90-7.30 (m, Ar-H, 10 H),12.10 (s,OH, 1H); IR (KBr, cm^{-1}): 3390 (OH), 2210 (CN), 1485 (C=N), 1380 (C-O), Anal. Calc. for $C_{20}H_{14}N_2O_2$(314): C 76.42,

H 4.49, N 8.91 ; Found:C 76.40, H 4.45, N 8.89.

1-(5-(4-methoxyphenyl)-4,5-dihydroisoxazol-3-yl)naphthalen-2-ol. (3f)

Yield:70%. M.p.:184-186°C. ^{1}HNMR (300 MHz,$CDCl_3$, ppm): 3.49(s, OCH_3, 3H), 3.76 (dd, Ha, 1 H), 3.82 (dd, Hb, 1 H),5.70 (dd, Hx,1 H),7.90-6.90 (m, Ar-H, 10 H),12.00 (s,OH, 1H); IR (KBr, cm^{-1}): 3350 (OH), 1480 (C=N), 1385 (C-O), Anal. Calc. for $C_{20}H_{17}NO_3$(319): C 75.22, H 5.37, N 4.39 ; Found:C 75.20, H 5.35, N 4.37.

Antibacterial activity

All the newly synthesized compounds were screened in vitro antibacterial activity evaluated against 24hr culture of different bacterial strains such as Staphylococcusaureus, Streptococcus pyogenes (Gram +ve) and, Escherichia coli, Pseudomonasaeruginosa (Gram –ve) at a concentration 50 µg ml^{-1} . The cultures were diluted with 5% of autoclaved saline and the final volume was adjusted to a concentration of approximately 105-106 CFU ml^{-1}. The synthesized compounds were diluted with acetone for the antibacterial biological assay for agar disc diffusion method. The liquid form of test compound was soaked on to a disc (5mm) and then allowed to air dry, such that the disc became completely saturated with the test compound. The saturatedchemical discs were introduced onto the upper layer of medium evenly loaded with the bacteria and incubated at 37^0 C for 24 to 48 hrs for better inhibition of bacteria. The zones of inhibition were measured after 24 to 48 hrs. All the experiments were performed in triplicate and the results are expressed as zone of inhibition in mm. The zone of inhibition of the synthesized compounds **(3a-3f)** was comparedwith zone of inhibition of standard antibiotics ofloxacin (50 µg mL^{-1})[27].

From the screening studies (**Table 2**), it is evident that the synthesized isoxazoline derivatives **3a, 3b,3c, 3d** and**3f**showed good antibacterial activity against all the tested organisms. It was further observed that the disubstituted halogen compounds **3a, 3b** and **3c** were better over the monosubstituted halogen compound **3d** this could be due to effective binding in to the active site of the target where as electron rich **3f**with one –OMe substituent, showed best activity near to that of standard drug. This observation leads to conclusion that electron rich and halogen disubstitutedisoxazolines showed higher activity against bacterial strain tested.

Table 2. Antibacterial activity of Compounds

Sr.No	Compounds	Diameter of Zone of inhibition(in mm)			
		Gram +ve bacteria		Gram –ve bacteria	
		S.aureus	S.pygenes	E.coli	P.Aeruginosa
01	3a	22	22	27	24
02	3b	23	24	26	24
03	3c	24	25	28	25
04	3d	21	21	26	23
05	3e	18	20	22	19
06	3f	24	26	26	24
07	Standard	26	28	30	27
08	DMSO	-	-	-	-

Conclusion

In the present work, we synthesized some novel isoxazoline derivatives from different substituted 2-hydroxynaphthyl functionalized chalcones and hydroxylamine hydrochloride. The newly synthesized compounds were obtained in good yield and confirmed byspectral analysis. The antibacterial data revealed that electron rich and halogen disubstitutedisoxazolines showed higher activity against bacterial strain tested.It also

revealed that all compounds showed good to moderate activity compared to standard drug.

Conflict of interest

There is no conflict of interest in the present study.

References

1. Khan, S. A. Kumar, P. Joshi, R. Iqbal, P. F.Saleem, K.(2008).Synthesis and in vitro antibacterial activity of new steroidal thiosemicarbazone derivatives.Eur. J. Med. Chem., 43:2029-2034.
2. Harbottle, H. Thakur, S. Zhao, S. White, D.G.(2006).Genetics of antimicrobial resistance.Anim. Biotechnol., 17:111–124.
3. Berger, W.(1985). Incidence of severe side effects during therapy with sulfonylurease and biguanides.Horm. Metab. Res., 17:111–115.
4. [4]Rangappa, S.Selvam, M.K.Kanekar, S.U.Nagaraja, G. K.(2018). Synthesis, characterization, antibacterial and antioxidantstudies of some heterocyclic compounds fromtriazole-linkedchalconeDerivatives. Chemistry Select, 3:6338- 6343.
5. Ribeiro,C.Praveen Kumar,J.A. RuiMoreira,S. Maria Santos, M.M.(2012).Efficient synthesis of spiroisoxazolineoxindoles. Tetrahedron Lett.,53:281-284.
6. Kamal, E. A. Bharathi, V. Reddy, J. S. Ramaiah, M. J.Dastagiri, D.KashiReddy, M.Viswanath, A. Reddy, T. L. Shaik, T. B. (2011).Synthesis and biological evaluationof 3,5-diaryl isoxazoline/isoxazole linked 2,3-dihydroquinazolinone hybrids asanticancer agents. Eur. J. Med. Chem.,46:691-703.
7. Pinto, A. Conti, P. Grazioso, G.Tamborini, L. Madsen, U. Nielsen, B. Micheli, C. (2011).Synthesis of new isoxazoline-based acidic amino acids and investigation of their affinity and selectivity profile ationotropic glutamate receptors. Eur.J. Med. Chem., 46:787-793.
8. Nonn, M. Kiss, L.EnikoForro, Mucsi, Z. Fulop, F.(2011).Synthesis of novel isoxazoline-fused cyclic β-amino esters by regio- and stereo-selective 1,3-dipolar cycloaddition. Tetrahedron, 67:4079-4085.
9. Pekka, K.Poutiainen, Tuomas, A. Venalainen, Perakyla, M. Juha, M.Matilainen, Vaisanen, S. Honkakoski, P.Laatikainen, R.Pulkkinen, J. T.(2010).Synthesis and biological evaluation of phenolic 4,5-dihydroisoxazoles and 3-hydroxy ketones as estrogen receptor α and β agonists. Bioorg. Med. Chem.,18:3437-3447.
10. Dallanoce, C. Frigerio, F.Martelli, G. Grazioso, G. Matera, C. Pome, Y.D.Pucci, L. Clementi, F.Gotti, C. Amici, M. (2010).Novel tricyclic Δ2-isoxazoline and 3-oxo-2-methyl-isoxazolidine derivatives: Synthesis and binding affinity at neuronal nicotinic acetylcholine receptor subtypes. Bioorg. Med. Chem.,18:4498-4508.
11. Congiu, C.Onnis, V.Vesci, L.Castorina, M. Pisano, C. (2010).Synthesis and in vitro antitumor activity of new 4,5-dihydropyrazole derivatives.Bioorg. Med.Chem., 18:6238-6248.
12. Karthikeyan, K.Seelan, T. V. Lalitha, K. G. Perumal, T. (2009).Synthesis and antinociceptive activity of pyrazolylisoxazolines and pyrazolylisoxazoles. Bioorg. Med. Chem. Lett., 19:3370-3373.
13. [13]Rajendra, P.T. Rakesh, D. S. Budha, N. Robin, E.B. Anne, J.M. Meibohm, B. Richard, E.(2007).Discovery of novel isoxazolines as anti-tuberculosis agents.Bioorg. Med. Chem.Lett., 17:6638-6642.
14. Rakesh, Sun, D. Lee, B. R. Tangallapally, P. R. Lee, R. E. (2009).Synthesis, optimization and structure reactivity relationships of 3,5-disubstituted isoxazolines as newanti-tuberculosis agents. Eur. J. Med.Chem., 44: 460-472.
15. Rojas, J.Paya, M. Dominguez, J.N. Ferrandiz, M. L. (2002). The synthesis and effect of fluorinated chalcone derivatives on nitric oxide production. Bioorg. Med. Chem.Lett.,12:1951-1954.
16. Shah, T.Desai, V. (2007).Synthesis and antibacterial studies of some novel

isoxazolinederivatives. J. Serb. Chem. Soc., 72: 443–449.

17. Bhimwal, R. Sharma, A. K. Jain, A. (2011).Synthesis, characterization and in-vitro antimicrobial evaluation of some novelisoxazoline derivatives.J. Adv. Pharm.Edu. Res.,1:251-258.
18. Kottapalle,G.D. Deshmukh,N.J.Shinde, A.T.(2019).Growth inhibitory properties of synthetic chalcones.Curr. Bioact.Comp.,15:DOI:10.2174/157340721 5666190401202553.
19. Archana, Srivastava, V.K. Chandra, R. Kumar, A. (2002). Synthesis of potential quinazolinylpyrazolines and quinazolinylisoxazolines as anticonvulsant agents. 41B:2371-2375.
20. Patterson, J.W. Cheung, P. S. Ernest, M. J. (1992).3-Carboxy-5-methyl-N-[4-(trifluoromethyl)phenyl]-4-isoxazolecarboxamide, new prodrug for the antiarthritic agent 2-cyano-3-hydroxy-N-[4-(trifluoromethyl)phenyl]-2-butenamide. J.Med.Chem., 35: 507-510.
21. Gupta, R. A. Kaskhedikar, S. G.(2013).Synthesis, antitubercular activity, and QSAR analysisof substituted nitroaryl analogs: chalcone, pyrazole,isoxazole, and pyrimidines.Med Chem Res., 22:3863-3880.
22. Sorthiya, S. D. Patel V.B. Pareikh, A.R. (1997). Synthesis of some novel cyanopyridines and isoxazoles bearing sulphonamide moiety and their antimicrobial activity.Indian J. Chem.,36B:822-825.
23. Wagner, E. Becan, L.Nowakowska, E. (2004).Synthesis and pharmacological assessment of derivatives of isoxazolo[4,5-d]pyrimidine. Bio-org. Med. Chem.,12:265-272.
24. Kumar, J. Chawla, G. Gupta, H. Akhtar, M. Tanwar, O. Bhowmik, M.(2013).Synthesis and neuropharmacological evaluation ofsome new isoxazoline derivatives as antidepressantand anti-anxiety agents.African J. Pharm. And Pharmacology, 7:1523-1530.
25. Rakesh, Sun, D. Lee, R. B. Tangallapally, R.P. Lee R.E. (2009).Synthesis, optimization and structureeactivity relationships of 3,5-disubstituted isoxazolines as newanti-tuberculosis agents. Eur. J. Med.Chem., 44: 460-472.
26. Srivastava, V. K. A. Ramesh, C. Ashok, K. (2002).Synthesis of potential quinazolinonylpyrazolines and quinazolinylisoxazolines as anticonvulsant agents. Ind. J. Chem., 41B:2371-2375.
27. Deshmukh, N.J. Kottapalle, G.D. Shinde, A.T. (2019).Synthesis of some chloro-substituted isoxazoline derivatives as antibacterial agents.Asian J. Pharm.& Pharmacology, 5:49-52.