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Sodium Oxalate Catalysed One Pot Synthesis, Characterization And Antimicrobial Activities Of 3-Methyl-4-Arylmethylene-Isoxazol-5(4*H*)-Ones.

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Abstract: We have developed speedy and effective procedure for the synthesised of 3-methyl-4-arylmethylene-isoxazol-5(4H)-ones compounds using sodium oxalate as a catalyst with ethanol as a solvent. The multicomponent one-pot reaction of substituted aldehydes, hydroxylamine hydrochloride and ethyl acetoacetate at room temperature with new catalyst sodium oxalate in alcoholic solvent added in R B flask with stirring prepared 3-methyl-4-arylmethylene-isoxazol-5(4H)-ones with excellent yield. They have been synthesised and characterized by spectroscopic techniques like FT-IR, ^{1}HNMR and LC-MS mass spectra. In this novel methodology, remarkable advantage is less time eco-friendly catalyst using method compare with other few organic solvent used. Additionally, all synthesised compounds were screened for their anti-bacterial and anti-fungal activities.

Keywords: Isoxazole compound, nitrogen containing heterocycles, Anti-microbial activities.

Introduction:

Five-membered nitrogen atom containing aromatic heterocycles with oxygen are Isoxazoles. The isoxazole cyclic structure is found in a various naturally occurring compounds and biologically active molecules [1]. One-pot multicomponent reactions have recently been exposed to be influential synthetic tool used to synthesize heterocyclic compounds, since the products are formed in one-pot and the variety can be completed simply by changeable each component. The easiness procedure of a one-pot, the possible structural distinctions, the atom reduced and convergent atmosphere, working simplicity and the greater number of available organic compounds are among the designated advantages of multicomponent reaction [2-9].

The Isoxazole derivatives are well-known for their extensive biological and pharmacological activities such as antitumor,[10] anti-inflammatory,[11] anti-HIV[12], antimycobacterial,[13] androgen antagonist,[14] antibiotic,[15] antimalarial,[16] antianginal,[17] anti-obesity,[18]

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anti-osteoporotic,[19] antihypertensive,[20] antirheumatic,[21] anticonvulsant,[22] nematicidal agents,[23] antiprotozoal,[24] hypoglycaemic,25 broncho dilating agent,26 analgesic,27 antioxidant,[28] antifungal,[29] antimicrobial,[30] COX-2 inhibitor,[31] and antiviral.[32] Since more application area of 3-Methyl, 4-arylmethylene-Isoxazol-5-(4H)ones compounds have been absorbed on new methodology of its synthesis via novel catalyst time saving lower waste material resolved in one-pot multicomponent synthesised. The synthesised compounds were characterised by using FT-IR, ^{1}HNMR with DMSO solvent, LC-MS spectra with screening antibacterial and antifungal activities of Various synthesised compounds.

Experimental

All chemicals were used for AR grade purchased from reputed chemical Company such as spectrochem and solvents commercially available were purchased from local provider and used as without further purification. The melting point apparatus, Stuart model SMP3, was used for measuring melting points. FT-IR spectra were recorded on a PerkinElmer series II spectrum. ^{1}HNMR spectra were recorded in DMSO-d6 using Bruker Avance Neo 500 MHz NMR Spectrometer and proton chemical shifts were recorded in δ relative to TMS as an internal standard using DMSO as solvent and the LC-MS spectra of synthesised compounds have been carried out with Water Micro mass Q-Tof Micro instrument.

General Procedure for the synthesis of 3-Methyl-4-arylmetheylene-isoxazol-5(4H)-ones.

Ethyl acetoacetate (20 mmol), hydroxylamine hydrochloride (20 mmol) and sodium oxalate solution (20% mmol) in ethanol: water (10 ml) Mixed in Round bottom flask and they were stirred for 10 min, after then substituted aromatic aldehyde (20 mmol) was added in the same slowly with shaking and the mixture was further stirred on magnetic stirrer till the completion of the reaction (monitored by TLC). The prepared compound was filtered off and washed with cold 5% aqueous ethanol (2 × 30 ml), and recrystallized from ethanol (95%) to afford the pure product. The Products were obtained likes 3-Methyl-4-arylmethylene-isoxazol-5(4H)-ones. These synthesized compounds were identified by spectral studies.

Characterization of 3-Methyl-4-arylmetheylene-isoxazol-5(4H)-ones.

1) **4-Benzilidine-3-methyl-isoxazol-5(4H)-ones [N2a]:** Yield 88%. Colour: Yellow. M.P. :141-143 ^{O}C. M.F.: $C_{11}H_9NO_2$. FT-IR (KBr, cm-1): 1495 (C=C), 3141 (H-C=C), 1632 (C=C=O), 1585 &1490 (Ar. C=C), 1399 (C-C), 1326 (C=N), 1148 (C-O-N

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), 840 (N-O). ^{1}HNMR (500 Mz, DMSO-d6), δ: (d, 3H, 2.02), (s, 1H, 2.23), (d, 2H, 7.3), (d, 2H, 7.8). LC-MS (m/z) : Obsv. 188.31.

2) **4(4-Chlorobenzylidine)-3-Methyl-isoxazol-5(4H)-ones [N2b]:** Yield 91 %. Colour: White. M.P.: 118-120 ^{O}C. M.F. $C_{11}H_8NO_2Cl$. FT-IR (KBr, cm-1): 1664 (C=C=O), 1585 & 1515 (Ar.C=C), 1400 (C=N), 1105 (C-O-N), 838 (N-O), 745 (C-Cl). ^{1}HNMR (500 Mz, DMSO-d6), δ: (s, 3H, 1.78), (s, 1H, 2.09), (d, 2H, 7.31), (d, 2H, 7.8). LC-MS (m/z): Obsv. 221.07.

3) **4(4-Nitrobenzylidine)-3-Methyl-isoxazol-5(4H)-ones [N2c]:** Yield 89%. Colour: Yellow. M.P. : 141-143 ^{O}C. M.F. $C_{11}H_8N_2O_4$. FT-IR (KBr, cm-1): 1678 (C=C=O), 1589 & 1515 (Ar.C=C), 1395 (C=N), 1170 (C-O-N), 959 (N=O), 826 (N-O). ^{1}HNMR (500 Mz, DMSO-d6), δ: (s, 3H, 3.36), (s, 1H, 5.63), (d, 2H, 7.88), (d, 2H, 8.3). LC-MS (m/z): Obsv. 232.09.

4) **3-Methyl-4(Fur-2-ylmethylene)-isoxazol-5(4H)-ones [N2d]:** Yield 75%. Colour: White M.P. : 237-241 ^{O}C. M.F. $C_9H_7NO_3$. FT-IR (KBr, cm-1): 1642 (C=C=O), 1570 & 1465 (Ar. C=C), 1326 (C=N), 1110 (C-O-C), 765 (C-O). ^{1}HNMR (500 Mz, DMSO-d6), δ: (s, 3H, 1.87), (s, 1H, 4.12), (d, 2H, 6.7), (d, 1H, 7.1). LC-MS (m/z): Obsv. 177.3.

Bioassay

Protocol for antibacterial activity

The antibacterial activity of the compounds was performed by enumerating feasible number of cells upon in the nutrient broth containing various concentrations of compounds. The feasible number is represented by colony control method. The test organisms on which the antibacterial activity was performed were *Pseudomous aeruginosa, Salmonella typhi, Bacillus subtilis, Escherichia coli and Staphylococcus areas.* In this method, the cells of test organisms were grown in nutrient broth till mid log phase and used as an inoculum for performing antimicrobial test. An approximately, $1*10^6$ cells/ml test organisms were each incubated with 0 to 500 ug/ml concentrations of different compounds, separately, and each incubated for 15 to 17 at 37 ^{o}C. During this incubation cells tend to grow and multiply in number. However, if the compounds interfere with growth of the cells, the number of cells of decrease. After, 15 to 17 hrs. available number of cells were recorded by spreading an aliquot,

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from the broth, inoculated with test organisms and compounds as colony forming units per millilitre.

Protocol for antifungal activity

The Antifungal activity was evaluated against different functional strains such as Aspergillus niger. And Saccharomyces cerevisiae. The medium yeast nitrogen base was dissolved in phosphate buffer pH 7 and it was autoclaved in 110 ^{o}C for 10 min. The suitable concentration of standard was incorporated in medium. With each set of growth control of without the antifungal agents and solvent control DMSO were included.

The fungal strains were freshly sub cultured on to Sabouraud dextrose agar (SDA) and incubated at 25°C for 72 hrs. The fugal cells were deferred in sterile distilled water and dilute to get 10^5 cells/ml. 10 microlitre of uniform suspension was inoculated on to the control plates and the media were combined with the antifungal agents. The inoculated plates were incubated at 25°C for 48 hrs. The readings were taken at the end of 48 hrs and 72 hrs. Minimum inhibitory concentration (MIC) values were determined using standard agar method as per CLSI guidelines.

Results and Discussion

One-pot-multicomponent reaction**,** 4-substitued benzylidine3-Methyl-isoxazol-5(4H)ones compounds synthesized from ethyl acetoacetate (20mmol), Hydroxylamine hydrochloride (20mmol) and sub. Aromatic aldehydes (20mmol) added in R B flask in presence of sodium oxalate as a catalyst(20%) with 5 ml ethanol solvent stirring in room temperature. These synthesised isoxazole-5(4H)ones were agree with their spectral analysed data as per reaction in Figer 1.

Figure 1. synthesis of substituted 3-Methyl-4-arylmethylene-isoxazol-5(4H)-ones

by sodium oxalate as a catalyst in alcoholic medium.

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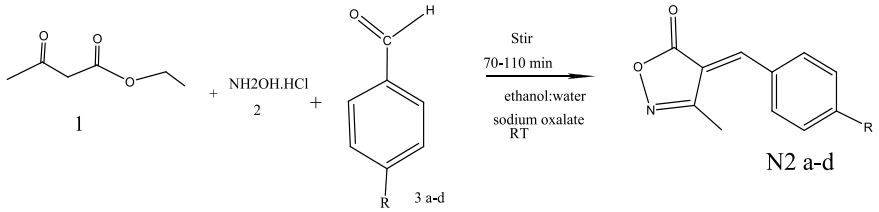

Spectral Characterization

IR Spectra

In the Spectroscopic technique IR spectra of Isoxazole compounds the band at 1678 cm-1 shows presence of conjugated carbonyl group and its frequency were disappeared in the compounds. The 1541 cm-1 region is assigned C=N band, this band of isoxazole undergoes lower frequencies. IR spectra 1170 cm-1 show C-N band and 838 cm-1 for N-O bonding spectra. They also show 767 cm-1 for C-Cl band and 1090 shows for C-O band with the literature data.

^{1}HNMR Spectra

In the ^{1}HNMR Spectra of the synthesized compounds appears δ 2.09 s, with 3H indicates presence of methyl group bonding with conjugated or aromatic compounds. δ 3.02 s, 1H bonded on C=C group & δ 6.90-8.23 s or m indicates presence of Ar-H means presence of phenyl ring in this structure as per the literature.

LC-MS Spectra

In the present investigation, the mass spectrum of 4-Arylidine-3-Methyl-5(4H)ones shows molecular ion peaks at m/z=[188] corresponding to [$C_{11}H_9NO_2$] ion. The spectrum also exhibits peaks for the mass spectra of 4 position sub. Synthesised isoxazole-5(4H)one compounds exhibits molecular ion peaks at m/z [221M+], [232M+], and [177M+] ion respectively that are equivalent to their molecular weight studied with the literature.

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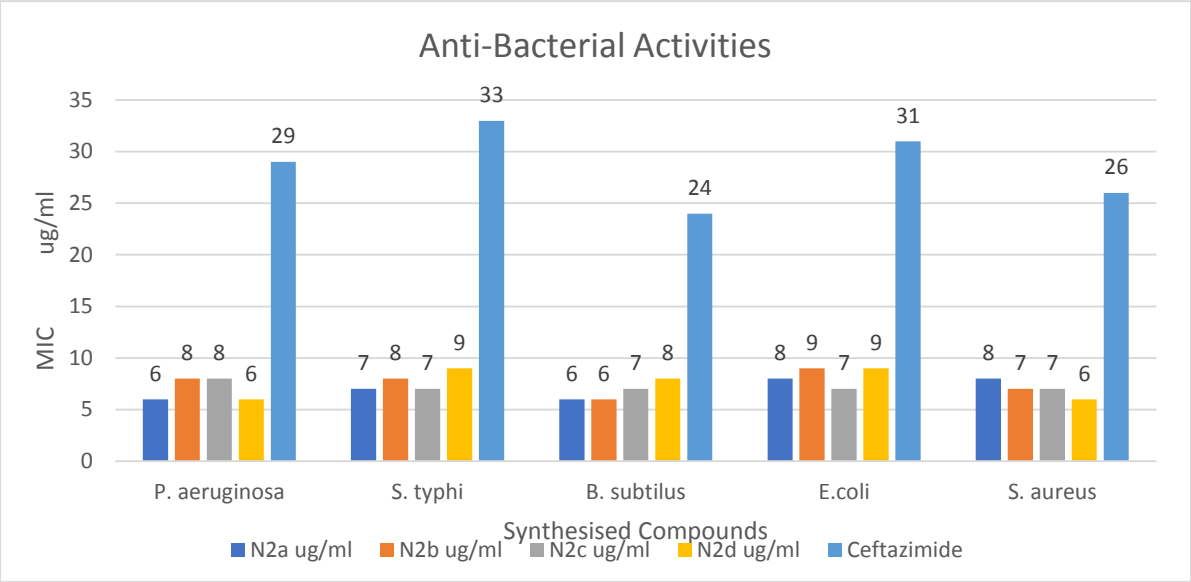
Biological activity



The graphically representation of antibacterial activity

The 4-Benzylidine-3-Methyl-Isoxazol-5(4H)-ones exhibits Minimum inhibitory concentration (MIC) against various bacteria were compare with the MIC values of standard drug Ceftazimide. The MIC values of the synthesised (4-Benzylidine)-3-Methyl-Isoxazol-5(4H)-ones (N2a) were observed to be 06 ug/ml, 07 ug/ml, 06 ug/ml, 08 ug/ml and 08 ug/ml against *Pseudomonas aeruginosa, Salmonella typhi, Bacillus subtilis, Escherichia coli & Staphylococcus aureus* respectively the values exhibit similar activities as compared with standard drug Ceftazimide, they show moderate activity. The MIC values of 4(4-Chlorobenzylidine)-3-Methyl-Isoxazol-5(4H)-ones[N2b] exhibits 08 ug/ml, 08 ug/ml, 06ug/ml, 09 ug/ml & 07 ug/ml against *Pseudomonas aeruginosa, Salmonella typhi, Bacillus subtilis, Escherichia coli & Staphylococcus aureus* respectively the values exhibit good activities as equated with the standard drug Ceftazimide.The MIC values of 4(4-Nitrobenzylidine)-3-Methyl-Isoxazol-5(4H)-ones[N2c] exhibits 08 ug/ml, 07 ug/ml, 07 ug/ml, 07 ug/ml and 07 ug/ml against *Pseudomonas aeruginosa, Salmonella typhi, Bacillus subtilis, Escherichia coli & Staphylococcus aureus* respectively the MIC value compared with standard drug Ceftazimide moderate activities. The MIC values of synthesised compound 3-Methyl-(4-Fur-2-ylmethylene)-5(4H)-ones[N2d] exhibits 06 ug/ml, 09 ug/ml, 08 ug/ml, 09 ug/ml and 6 ug/ml against *Pseudomonas aeruginosa, Salmonella typhi, Bacillus*

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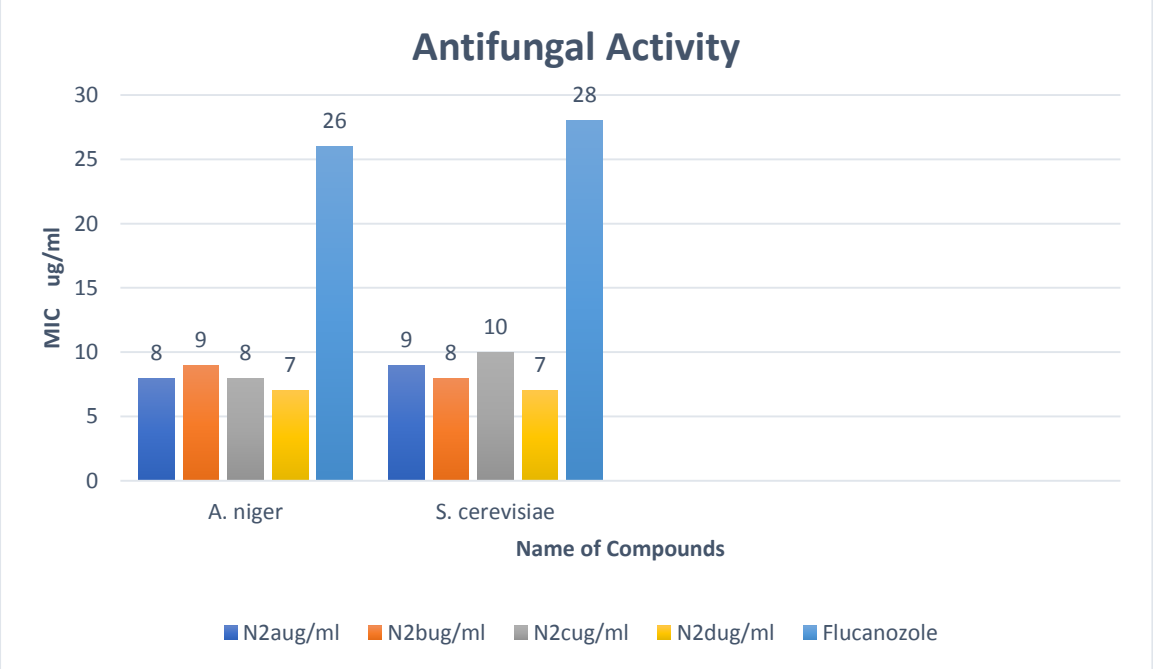
subtilis, Escherichia coli & Staphylococcus aureus respectively the MIC value compared with standard drugs to show moderate activity.

Anti-fungal Activity



The graphically representative of antifungal activity.

In the antifungal activity, the MIC values of the 3-Methyl-4-(arylidene Isoxazol)-5(4H)ones compounds of N2a, N2b, N2c and N2d synthesised compounds exhibits moderate activity as compared to standard drug. The results observed by the analysis for Aspergillus niger 8 ug/ml, 9 ug/ml, 8ug/ml and 7ug/ml and for Saccharomyces cerevisiae shows 9 ug/ml, 8ug/ml, 10 ug/ml and 7 ug/ml equated with standard drug Flucanozole against shows MIC value of 26 ug/ml and 28 ug/ml, they indicate better activities.

Conclusion

One-Pot-multicomponent synthesised 3-methyl-4(4-sub. Arylidene-Isoxazol-)-5(4H)-ones by using new catalyst Sodium oxalate and characterised through various spectral analysis, from this spectroscopic data, structure of the prepared multicomponent 3-Methyl-4(sub. arylmethylene-Isoxazol)-5(4H) ones compounds have been confirmed. These compounds were characterised by various biological activities such as antibacterial and antifungal activities. The *P. aeruginosa*, *S. typhi*, *B. subtilus*, *E. coli* and *S. aureus* for performing

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antibacterial activity, the result indicated that the activity exhibited above isoxazole compounds comparable to Ceftazimide standard drugs. Concurrently, the fungal strains such as A. niger and S. cerevisiae for carrying the antifungal activity as compared to Fluconazole standard drugs and all the compound also exhibited moderate activity compared to standard drugs.

References:

1) Barmade MA et. al. ,[2016] Medicinal chemistry perspective of fused isoxazole derivatives. Curr Top Med Chem 16:2863–2883.
2) Brahmachari, G.et. al. [2014] Facile and One-pot Access to Diverse and Densely Functionalized 2-amino-3-cyano 4H-pyrans and Pyran-annulated Heterocyclic Scaffolds via an Eco-friendly Multicomponent Reaction at Room Temperature Using Urea as a Novel Organo-catalyst. ACS Sustainable Chem. Eng. 2, 411−422.
3) Ramazani, A. et. Al.,[2017] Review on the Synthesis and Functionalization of SiO2 Nanoparticles as Solid Supported Catalysts. Curr. Org. Chem., 21, 908–922.
4) Solhy, A. et. al., [2010], Clean Chemical Synthesis of 2-amino-chromenes in Water Nanostructured Diphosphate Na2CaP2O7. Green Chem. 2010,12, 2261–2267.
5) Khoobi, M. et. al., [2012] One-pot Synthesis of 4H-benzo[b]pyrans and Dihydropyrano[c]chromenes Using Inorganic– Organic Hybrid Magnetic Nanocatalyst in Water. J. Mol. Catal. A Chem.,359, 74–80.
6) Fardood, S.T. et. al. [2017] , Green Synthesis of Ni-Cu-Zn Ferrite Nanoparticles Using Tragacanth Gum and Their Use as An Efficient Catalyst for the Synthesis of Polyhydroquinoline Derivatives. Appl. Organomet. Chem., in press. DOI: 10.1002/aoc.3823.
7) Tabatabaei Rezaei, S.J et. al.,[2017], P. Palladium Nanoparticles Immobilized on Amphiphilic and Hyperbranched Polymer-functionalized Magnetic Nanoparticles: An Efficient Semi-heterogeneous Catalyst for Heck Reaction. Appl. Organomet. Chem., in press. DOI:10.1002/ aoc.3707.
8) Hasanpour, Z. et. al.,[2017] Efficient Multicomponent Synthesis of 1,2,3-triazoles Catalyzed by Cu(II) Supported on PEI@Fe3O4 MNPs in a Water/PEG300 System. Turk. J. Chem.,41, 294–307.
9) Taran, J. et.al., One-pot Three-component Syntheses of α-aminophosphonates from a Primary Amine, Quinoline-4-Carbaldehyde and a Phosphite in the Presence of

ISSN PRINT 2319 1775 Online 2320 7876

 UGC CARE Listed (Group -I) Journal Volume 11, Iss 07, 2022

MCM-41@PEI as an Efficient Nanocatalyst. Phosphorus, Sulfur, Silicon. Relat. Elem. 2017,192, 776–781.

10) Diana P et. al.[2010] Synthesis and antitumor activity of 2,5-bis(3'- indolyl)-furans and 3,5-bis(3'-indolyl) isoxazoles, nortopsentin analogues Med. Chem. 18 4524.

11) Karabasanagouda T,et. al., [2009] Synthesis of some new pyrazolines and isoxazoles carrying 4-methylthiophenyl moiety as potential anal gesic and anti-inflammatory agents Indian J. Chem. 48, 430.

12) Deng B L et. al.,[2006], Synthesis and anti HIV activity of new alkenyl diaryl methane (ADAM) non-nucleoside reverse transcriptase inhibitors (NNRTIs) incorporating benzoxazolone and benzisox azole rings Bioorg. Med. Chem. 14 2366.

13) Changtam C et.al., [2010] Isoxazole analogs of curcuminoids with highly potent multidrug-resistant antimycobacterial activity Eur. J. Med. Chem. 45 4446.

14) Ishioka T et. al., [2002] Novel non-steroidal/non-anilide type androgen antagonists with an isoxazolone moiety Bioorg. Med. Chem. 10 1555.

15) Kusakabe Yet.al.,[1972] Minimycin, a new antibiotic J. Antibiot. 25 44.

16) Ren H et.al.,[2001] Design, synthesis, and biological evaluation of a series of simple and novel potential antimalarial compounds Bioorg. Med. Chem. Lett. 11 1851.

17) Benedini F et. al.,[1995] New antianginal nitro esters with reduced hypotensive activity. Synthesis and pharmaco logical evaluation of 3-[(nitrooxy)alkyl]-2H-1,3-benzox azin-4(3H)-ones J. Med. Chem. 38 130.

18) Kafle B et. al.,[2011] Isoxazol-5(4H)-one derivatives as PTP1B inhibitors showing an anti-obesity effect Chem. Asian J. 6 2073.

19) Chen Y L et. al.,[2013] Synthesis of aminoalkoxy substituted 4,5-diphenylisoxazole derivatives as poten tial anti-osteoporotic agents Med. Chem. 9 748**.**

20) Peglion J L et. al.,[1997] Tetracyclic analogues of [?]-S 14297: Synthesis and determination of affinity and selectivity at cloned human dopamine D3 vs D2 receptors Bioorg. Med. Chem. Lett. 7 881.

21) Matsuoka H et. al.,[1997] Antirheumatic agents: novel methotrexate derivatives bearing a benzoxazine or benzothiazine moiety J. Med. Chem. 40 105.

22) Frolund B et.al., [2002] Novel class of potent 4-arylalkyl substituted 3-isoxazolol GABAA antagonists: synthesis, pharmacology, and molecular modeling J. Med. Chem. 45 2454.

ISSN PRINT 2319 1775 Online 2320 7876

 UGC CARE Listed (Group -I) Journal Volume 11, Iss 07, 2022

23) Srinivas A et.al.,[2010] Synthesis and in vitro study of methylene-bistetrahydro[1,3]thia zolo[4,5-c]isoxazoles as potential nematicidal agents Eur. J. Med. Chem. 45 2353.

24) Patrick D A et. al. [2007] Synthesis and in vitro antiprotozoal activities of dica tionic 3,5-diphenylisoxazoles J. Med. Chem. 50 2468.

25) Kang YK et. al., [2000] Synthesis and antibacterial activity of new carbapenems containing isoxazole moiety Bioorg. Med. Chem. Lett. 10 95.

26) Giustina A et. al., [1995] Effect of two beta 2-agonist drugs, salbutamol and broxaterol, on the growth hormone response to exercise in adult patients with asthmatic bronchitis J. Endocrin. Invest. 18 847.

27) Kano H et. al., [1967] Isoxazoles. XVIII. Synthesis and pharmacological prop erties of 5-aminoalkyl-and 3-aminoalkylisoxazoles and related derivatives J. Med. Chem. 10 411.

28) Padmaja A et. al., [2011] Synthesis and antioxidant activity of oxazolyl/thiazolylsulfonyl methyl pyrazoles and isoxa zoles J. Med. Chem. 46 5034.

29) Santos M M M et. al.,[2010]Reaction of naphthoquinones with substituted nitromethanes facile synthesis and antifungal activity of naphtho Bioorg. Med. Chem. Lett. 20 193.

30) Prashanthi Y et. al.,[2008] Synthesis, potentiometric and antimicro bial studies on metal complexes of isoxazole Schiff bases Spectrochim. Acta A 70 5

31) Talley J J et. al.,[2000] 4-[5-Methyl-3 phenylisoxazol-4-yl] benzenesulfonamide, valdecoxib: a potent and selective inhibitor of COX-2 J. Med. Chem. 43 775.

32) Lee Y S et. al.,[2009] Synthesis of 5-isoxazol-5-yl-20- deoxyuridines exhibiting antiviral activity against HSV and several RNA viruses Bioorg. Med. Chem. Lett. 19 1126.

33) Hamid Beyzaei et.al. [2018] Green multicomponent synthesis, antimicrobial and antioxidant evaluation of novel 5-amino-isoxazole-4-carbonitriles, Chemistry Central Journal, 12:114, 1-8.