Four Component One Pot Synthesis of Spiro Pyrazolo Pyrimidine Derivatives by Using Recyclable Nano Copper Ferrite Catalyst and their Antibacterial Studies

Ravi kumar Ganta[1], Ramgopal A[1], Chatragadda Ramesh[2], Raghu Babu K[1], Murali Krishna Kumar M[3], Venkateswara Rao B[1]

[1]Departmaent of Engineering chemistry, A.U C E (A), Andhra University, Visakhapatnam, India, [2]Department of Ocean Studies and Marine Biology, Pondicherry University, Andaman & Nicobar Islands, India, [3]A U College of pharmaceutical sciences, Andhra University, Visakhapatnam, India

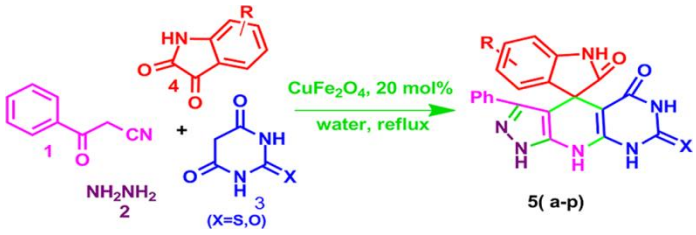
Corresponding Author: Ravi kumar Ganta, E-mail: gantaravichem@gmail.com

Accepted Manuscript

Abstract

A simple, efficient, ecofriendly and cost effective method has been developed for the synthesis of sixteen spiro pyrazolo pyrimidine derivatives (5a-5p) by four component one pot reaction of pyrimidine-2, 4, 6(1H, 3H, 5H)-trione; 3-oxo-3-phenylpropanenitrile; hydrazine and isatins(4a-4f) by using nano copper ferrite catalyst (20 mol%) in water with excellent yields (73-91%). The Isatin with electron withdrawing groups gave products in high yields (5h, 5p). The present methodology offers an environmentally benign, cost effective, high yield w.r.t product formation and recyclable catalyst. The drug likeness or drugability of all the synthesized compounds were tested through rule of five (RO5) parameters. 5f, 5h, 5n and 5p compounds have shown one RO5 violation each. The compounds were screened for their antibacterial activity against human pathogenic bacteria where in two of the synthesized compounds were found to possess significant antibacterial activity. Compounds showing RO5 violations were found to show negligible antibacterial activity.

GRAPHICAL ABSTRACT:



INTRODUCTION

Hetero cyclic compounds are common structural units in marketed drugs and in medicinal chemistry. Several heterocyclic compounds without bearing any substituent possess excellent biological activity, which means that their heterocyclic core is definitely part of the pharmacophore.[1] Even simple aliphatic heterocycles display astonishing biological activities. Pyrazolopyramidine scaffolds are found to show effective biological, pharmacological activities.[2] Multi component reactions (MCR) are important synthetic tools for the synthesis of bio active hetero cyclic compounds.[3] They are ecofriendly, have superior atom economy, less time consuming and involve easy purification processes. MCRs have been broadly employed in the synthesis of heterocyclic compounds due to the range of readily available starting materials, the simplicity of one-pot procedures without the need for isolation of intermediates, and the associated atom economy. The use of water as a green solvent for organic synthesis has recently attracted considerable attention.[4]

Pyrimidines, pyrazoles, and spiro indoles are very important class of heterocyclic compounds. Spiro indoles are key moieties in organic synthesis because of their evident

Accepted Manuscript

pharmacological and biological properties as well as synthetic intermediates for alkaloids and drugs.[5-7] Pyrimidnes are a class of heterocyclic compounds of great importance in biological activities owing to their anti fungal, [8] antimalarial, [9] antitumor, [10] and anti-inflammatory [11] properties. Pyrazole derivatives have been subjected to medicinal research because of their biological, pharmacological properties such as anticancer, [12] anti microbial, [13, 14] antifungal, [15] anti-inflammatory, [16] and analgesic activity. [17] Thus hybrid compounds of these three moieties could potentially lead to a series of structurally and biologically interesting compounds. Spiro pyrazolo pyrimidine derivatives are synthesized earlier [18] by using silica based tin complex. In the present work, a novel green synthetic method is used to synthesize 16 spiro pyrazolo pyrimidine derivatives by using recyclable nano copper ferrite catalyst.

In recent years, magnetic nano particles have emerged as a useful group of heterogeneous catalysts. Separation of magnetic nano particles is simple and an attractive alternative to filtration as it prevents loss of catalyst and enhances reusability without loss of catalytic activity. The use of low-cost and readily available species as catalyst plays a significant role for economical feasibility of the chemical processes. Copper ferrite nanoparticles ($CuFe_2O_4$ NP) have the advantage of recyclability, easy work-up, and cleaner reaction profiles apart from the lack of necessity for external ligands. This minimizes organic waste generation when compared to the conventional catalytic systems. Moreover, they also showed good air stability in various organic transformations. [19]

Accepted Manuscript

Drug development is expensive involving billions of dollars. A lead should a have range of physico chemical properties that are consistent with the previous record of discovery of orally active compounds. Literature survey reveals that the compounds with poorer physico-chemical properties would fail in the pre-clinical trials. Hence a pass in the physico-chemical properties would preserve both money and time. Before going for antibacterial studies, we have explored drug likeness of the synthesized compounds by following rule of five (RO5). Although the primary aim is to synthesize spiro pyrazolo pyrimidine derivatives, we have focused on the physico-chemical properties to test the drug likeness of these compounds so as to set the stage for next phase of development from chemical lead to drug lead.

RO5 has four important parameters that include molecular weight (MW), partition coefficient expressed as MlogP, number of H-bond donors (NH + OH) and number of H-bond acceptors (N + O). [20, 21] Lipophilicity is expressed as log of the ratio of octanol solubility to aqueous solubility as described by Moriguchi et al. [22 -25] An excessive number of H-bond donors may impair permeability of the drug through membranes. [26, 27] An excessive number of H-bond acceptors may prevent permeability across a membrane bi-layer. More than 90% of the oral drugs have a MW < 500, log P < 5, NH + OH < 5 and N + O < 10. [20, 21] Two additional descriptors - total polar surface area (TPSA) and total rotatable bonds are also evaluated. TPSA is a powerful descriptor in the characterization of a drug regarding its absorption including gastro-intestinal tract and bioavailability. [28] TRB is another descriptor that gives information about the conformational flexibility of the molecule during the drug's interaction with receptor

Accepted Manuscript

sites. [29] RO5 parameters combined with TPSA and TRB collectively describe solubility and permeability parameters of a drug.

RESULT AND DISCUSSIONS

Chemistry

In the present work, we began our investigations by screening the reaction of pyrimidine-2, 4, 6(1H, 3H, 5H)-trione, 3-oxo-3-cphenylpropanenitrile, hydrazine and isatin` by using $CuFe_2O_4$ NP catalyst in water. Initially, we have conducted a model reaction (Scheme 1) without catalyst in different solvents at room temperature (30 ^oC), 60 ^oC, 80 ^oC and 100 ^oC. It is observed that no products were obtained even after 6 hr or 12 hr (Table 1 entry 1-10). We have investigated the same reaction with 10 mol% of nano copper ferrite catalyst at room temperature. Products in low yield (less than 10%, Table 1 entry 11-15) were obtained in 12 hr. Increase of catalyst to 15 mol % resulted in slight increase of yield (Table 1, entry 16-19). The yield was considerable at 20 mol% of catalyst. Further increase of catalyst mol% did not result in any increase of yield. There was excellent yield of products (table 1entry 20-23) at 20 mol% of catalyst, when the different solvents are replaced with water and at 100 ^oC temperature (optimized). The model reaction may be summarized as follows: Spiro pyrazolo pyrimidines are synthesized by reacting pyrimidine-2, 4, 6(1H, 3H, 5H)-trione, 3-oxo-3-phenyl propane nitrile, hydrazine and isatin using 20 mol% $CuFe_2O_4$ NP catalyst in water and obtained excellent yields at 100 ^oC. Continuing the success, different isatins were tested in our attempt to synthesize Spiro pyrazolo pyramidine derivatives at the same reaction conditions (Scheme 2) and the results are summarized in Table 2.

Accepted Manuscript

From the results as is evident from Table 2, we can conclude that the isatins with electron withdrawing groups as substituent formed higher yields than isatins having electron releasing substituent. The preferred positions of the substituent being 5- or 7- on the isatin ring of the obtained condensation product. Maximum yields were obtained with nitro group (89 & 91%) and fluorine (88 & 87%) as substituent on isatin (Table 2, entry 5h, 5p, 5b, 5j). The yields (65 & 61%) are lower (Table 2, entry 5g, 5o) for isatins with electron releasing group like methoxy group as substituent. These compounds were confirmed by H^1, C^{13} NMR and Mass spectra.

Over all, the developed method has several advantages like easy work up, cost effectiveness, use of water as solvent, reusability of the catalyst and yields were better than that of earlier reported method.[18]

The plausible mechanism for the formation of spiro pyrazolo pyramidine derivatives from pyrimidine-2, 4, 6(1H, 3H, 5H)-trione, 3-oxo-3-cphenylpropanenitrile, hydrazine and isatin` using CuFeNPS is shown in Figure 1. The reaction proceeds through the formation of highly reactive intermediate6-((3-phenyl-1H-pyrazol-5-yl) amino) pyrimidine-2, 4(1H, 3H)-dione. The above formed intermediate could not be isolated.

Reusability of the Catalyst

The reusability of $CuFe_2O_4$ is one of the most important advantages of this protocol that makes it useful for practical commercial applications. We have examined the

Accepted Manuscript

recyclability of $CuFe_2O_4$ NP catalyst for the model reaction. Interestingly, the recovered catalyst could be reused for up to six cycles (Table 3) under optimized reaction conditions without leaching of the catalyst which is evident from the X-ray powder diffraction (XRD) pattern as shown in Figure 2. The catalyst was separated by using an external magnet after completion of the reaction and washed with water followed by chloroform, dried in oven and reused for the next cycle.

Drug Likeness Through RO5

The drug likeness or drugability of all the synthesized compounds were tested through RO5 parameters. 5f, 5h, 5n and 5p compounds have shown one RO5 violation each (Table 4). The remaining compounds obeyed RO5. These values were correlated with obtained antibacterial activities. It was found that compounds showing RO5 violations have very poor or no antibacterial activity. The descriptors TPSA, TRB were included for the sake of future interpretations and were not detailed here.

Biology (Antibacterial Activity)

Antibacterial activity assay was performed by Kirby-Bauer well diffusion method. Antibacterial activity of synthesized compounds were screened against 10 human pathogenic bacteria: *Pseudomonas aeruginosa* MTCC3216, *Streptococcus pneumonia* MTCC655, *Shigella sonnei* NK4010, *Shigella dysenteriae* type 5NK2440, *Shigella flexneri* type 2a503004, *Salmonella typhi* MTCC733, *Vibrio parahaemolyticus*serovar O3:K6K5030, *Vibrio cholera* MTCC3905, Enterotoxigenic *E. coli* serotype O1151571, *Salmonella enterica*serovar TyphimuriumB12101. Compounds 5l, 5m showed potential

Accepted Manuscript

activity with growth inhibition zones of 9mm, 8mm against *Shigella sonnei*; 12mm, 12mm against *Shigella flexneri* type 2a; 10 mm,12 mm against Enterotoxigenic *E. coli* serotype O115 (Figure 3a, 3b, 3c) and the results were summarized in Table 5.

Structure Activity Relation (Sar)

An examination of antibacterial activity as shown in the Table 5 would reveal that synthesized compounds (5l, 5m) with sulphur atom on pyramidine ring were more active than compounds (5d, 5e) with oxygen atom. Among sulphur containing compounds; -Cl, -Br substituted compounds on 5- position on the isatin were found to be more active. The same was found to be true for oxygen containing compounds.

Simple and –F, -NO_2 group substituted isatins showed no antibacterial activity against the screened human pathogenic bacteria. Whereas –I, -OMe group substituted isatins showed some antibacterial activity. The activity order of the groups was as follows: -Cl ≥ -Br > -OMe > -I.

Over all the synthesized compounds were found to be active against three human pathogenic bacterial cultures of the ten screened cultures.

CONCLUSION

In conclusion, a novel, efficient, four component one pot green synthetic method for the preparation of Spiro pyrazolo pyrimidine derivatives in water by using nano copper ferrite catalyst has been described. The utility of this method is demonstrated in the

Accepted Manuscript

synthesis. The advantages of this method include its simplicity of operation, cleaner reaction, and good yields. The purification of the product is simple involving filtration. The catalyst is easily separated by using external magnet and is reusable up to six cycles. The drug likeness or drugability of all the synthesized compounds were tested through RO5 parameters. 5f, 5h, 5n and 5p compounds have shown one RO5 violation each. Furthermore, the compounds are screened for their antibacterial activity against human pathogenic bacteria where in two of the synthesized compounds were found to possess significant antibacterial activity. Interestingly, compounds showing RO5 violations were found to show negligible antibacterial activity.

EXPERIMENTAL

Materials and Methods

All chemicals and reagents were obtained from Aldrich (Sigma-Aldrich), St. Louis, MO, USA), Lancaster (Alfa Aeser, Johnson Matthey Company, Ward Hill, MA, USA), or Spectrochem Pvt. Ltd. (Mumbai, India) and were used without further purification. Progress of each reaction was monitored by TLC on silica gel glass plate containing 60 GF-254, and visualization was achieved by UV light or iodine indicator. ^{1}H and ^{13}C NMR spectra were determined in DMSO-d_6 by using Varian and Avance instruments. Chemical shifts were expressed in parts per million (δ in ppm) downfield from internal TMS and coupling constants were expressed in Hz. ^{1}H NMR spectroscopic data are reported in the following order: multiplicity (s, singlet; brs, broad singlet; d, doublet; dd, doublet of doublets; t, triplet; m, multiplet). ESI mass spectra were recorded on a Micro mass Quattro LC using ESI+ software with capillary voltage 3.98 kV and an ESI mode

Accepted Manuscript

positive ion trap detector. Melting points were determined with an Electro thermal melting point apparatus, and are uncorrected.

For calculating parameters relating to RO5 the following approaches were adopted: m/z values from mass spectral data was used to arrive at MW; (NH + OH) and (N+O) were arrived at by counting manually from the structures of the synthesized compounds; MlogP values along with TPSA and TRB were exclusively taken from *molinspiration*, an open access package, available on the web. The earlier three parameters were also verified through *molinspiration*.

Typical Synthetic Procedure For Spiropyarazolo Pyramidine(5a-5p) Derivatives

Pyrimidine -2, 4, 6(1H, 3H, 5H)-trione (1mmol), 3-oxo-3-phenylpropanenitrile (1mmol), hydrazine (1mmol), isatin(1mmol), 20 mol% of nano copper ferrite as catalyst and water as solvent were taken in round bottomed flask. The reaction mixture was stirred at 100^{o}c for 6 hours and monitored by Thin Layer chromatography. After the completion of the reaction, catalyst was separated by using external magnet and compounds purified by column chromatography. Structures of compounds were confirmed by H^{1}, C^{13} NMR and Mass spectral analysis.

Spectral Data of 5a & 5m

3'-phenyl-8',9'-dihydrospiro[indoline-3,4'-pyrazolo[4',3':5,6]pyrido[2,3-d]pyrimidine]-2,5',7'(1'H,6'H)-trione **(5a)**

Accepted Manuscript

Cream white solid; mp: >300 ˚C: ^{1}H NMR (300 MHz, DMSO-d_6): δ 6.51 (d, *J* = 7.55 Hz, 1H), 6.61 (d, *J* = 6.98, 2H) 6.84(t, *J* = 8.12 & 14.91 Hz, 1H), 7.00 (d, *J* = 6.98 Hz, 1H), 7.058-7.17(m, 3H), 7.29 (t, *J* = 7.55 & 14.91 Hz, 1H), 8.32(s, 1H), 9.10 (s, 1H), 9.99(s, 1H), 11.70 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 47.99, 79.63, 100.65, 109.39, 121.81, 123.83, 126.10, 128.10, 128.23, 128.79, 129.03, 129.21, 137.54, 139.95, 142.80, 145.30, 147.12, 159.58, 173.89, 178.85. HRMS (ESI): m/z calcd. for $C_{21}H_{14}N_6O_3$: 399.1249 $(M+H)^+$. Found: 399.1243

6-bromo-3'-phenyl-7'-thioxo-6',7',8',9'-tetrahydrospiro[indoline-3,4'-pyrazolo [4', 3' :5, 6] pyrido[2,3-d] pyrimidine]-2,5'(1'H)-dione **(5m)**

White solid; mp: >300 ˚C;^{1}H NMR (300 MHz, DMSO-d_6): δ 6.33(d, *J* = 8.12Hz, 1H), 6.66(d, *J* = 6.98 Hz, 2H), 7.18(t, *J* = 7.36 & 15.10 Hz, 2H), 7.31(m, 2H), 7.39 (dd *J* = 1.88 & 6.23 Hz, 1H), 9.11(s, 1H), 10.12(s, 1H), 11.69(s, 1H),12.04(s, 1H), 12.62(s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 47.43, 83.76, 89.09, 99.68, 111.30, 126.13, 127.79, 128.27, 128.57, 128.66, 130.21, 131.55, 136.08, 139.11, 139.45, 141.52, 142.01, 146.78, 159.15, 173.41, 177.82. HRMS (ESI): m/z calcd. for M.F: $C_{21}H_{13}BrN_6O_2S$: 494.8699 $(M+H)^+$. Found: 494.8694

ACKNOWLEDGEMENT

The Corresponding author is grateful to **CSIR**, New Delhi for supporting through fellowship (JRF&SRF), and Department of Engineering chemistry, AUCE (A), Andhra

Accepted Manuscript

University, and Visakhapatnam for providing general lab facilities. The author is also thankful to **Prof. B. Venkateswara Rao** for his valuable and constant support.

REFERENCES

1. Quin, L.D.; Tyrell, J. Fundamentals of Heterocyclic Chemistry, Wiley-Blackwell, Oxford. **2010.**

2. Bhat, G.A.; Montero, J.G.; Panzica, R.P.; Worting, L.L.; Towsend, L.B. *J. Med. Chem.* **1981** , 4, 1165-1172.

3. (a) Bienayme, H.; Hulme, C.; Oddon, G.; Schmitt, P. *Chem. Eur. J.* **2000**, 6, 3321– 3329; (b) Domling, A.; Ugi, I. *Angew. Chem., Int. Ed*. **2000**, 39, 3168–3210.

4. (a) Umkeherer, M.; Kalinski, C.; Kolb, J.; Burdack, C. *Tetrahedron Lett.* **2006**, 47, 2391. (b) Tejedor, D.; Garcia-Tellado, F. *Chem. Soc. Rev.* **2007**, 36, 484.

5. Maheswari, S.U.; Balamurugan, K.; Perumal, S.; Yogeeswari P.; Sriram, D. *Bioorg. Med. Chem. Lett.* **2010**, 20, 7278–7282.

6. Prasanna, P.; Balamurugan, K.; Perumal, S.; Yogeeswari, P.; Sriram, D. *Eur. J. Med. Chem.* **2010**, 45, 5653–5661.

7. Nandakumar, A.; Thirumurugan, P.; Perumal, P. T.; Vembu, P.; Ponnuswamy M. N.;l Ramesh, P. *Bioorg. Med. Chem. Lett.* **2010**, 20, 4252–4258.

8. (a) Chen, Q.; Zhu, X. L.; Jiang, L. L.; Liu, Z. M.; Yang, G. F, *Eur. J. Med. Chem.* **2008**, 43, 595–603; (b) Hilmy, K. M. H.; Khalifa, M. M. A.; Hawata, M. A. A.; Keshk R. M. A.; El-Torgman, A. A. *Eur. J. Med. Chem.* **2010**, 45, 5243–5250.

9. Davoll, J.; Clarke, J.; Elslager, E. F. *J. Med. Chem.* **1972**, 15, 837–839.

Accepted Manuscript

10. Lin, R.; Johnson, S. G.; Connolly, P. J.; Wetter, S. K.; Binnun, E.; Hughes, T. V.; Murray, W. V.; Pandey, N. B.; Moreno-Mazza, S. J.; Adams, M.; Fuentes-Pesquera A. R.; Middleton, S. A. *Bioorg. Med. Chem. Lett.* **2009**, 19, 2333– 2337.

11. Falcao, E. P. S.; Melo, S. J.; Srivastava, R. M.; Catanho M. T. J. A.; Nascimento, S. C. *Eur. J. Med. Chem.* **2006**, 41, 276.

12. Abdel-Aziz, H. A.; El-Zahabi H. S. A.; Dawood, K. M. *Eur. J. Med. Chem.* **2010**, 45, 2427–2432.

13. Bildirici, I.; Sener, A.; Tozlu, I. *Med. Chem. Res.* **2007**, 16, 418–425.

14. Sridhar, R.; Paramasivam, T. P.; Sundaresan, E.; Guruswamy, S.; Mondikalipudur, N. P.; Vaiyapuri, R. P.; Mathivanan, N. *Bioorg. Med. Chem. Lett.* **2004**, 14, 6035– 6040.

15. Tiwari, R. K.; Verma, A. K.; Chhillar, A. K.; Singh, D.; Singh, J.; Sankar, V. K.; Yadav, V.; Sharma, G. L.; Chandra, R. *Bioorg. Med. Chem.* **2006**, 14, 2747–2752.

16. Bruno, O.; Ranise, A.; Bondavalli, F.; Schenone, P.; D'amico, M. *Farmaco.* **1992**, 47, 1225–1234.

17. Hall, A.; Billinton, A.; Brown, S. H.; Clayton, N. M.; Chowdhury, A.; Giblin, G. M. P.; Goldsmith, P.; Hayhow, T. G.; Hurst, D. N.; Kilford, I. R.; Naylor, A.; Passingham, B.; Winyard, L. *Bioorg. Med. Chem. Lett.* **2008**, 18, 3392–3399.

18. Ghahremanzadeh, R.; Rashid, Z.; Amir-Hassan, Z.; Hossein, N. *Dalton Trans.* **2014**, 42, 15791.

19. (a) Dandia, A.; Jain, A. K.; Sharma, S. *RSC Adv.* **2013**, 3, 2924; (b) Panda, N.; Jena, A. K.; Mohapatra, S.; Rout. S. R. *TetrahedronLett.* **2011**, 52, 1924–1927; (c) Bazgir, A.; Hosseini, G.; Ghahremanzadeh, R. *ACS Comb. Sci.* **2013**, 15, 530–534; (d)

Accepted Manuscript

Tasca, J. E.; Ponzinibbio, A.; Diaz, G.; Bravo, R. D.; Lavat, A.; Gonzalez, M. G. *Top. Catal.* **2010**, 53, 1087–1090.

20. Christopher, A. L.; Franco, L.; Beryl, W.; Paul, J. F. *Advanced Drug Delivery Reviews,* **2001**, 46, 3-25

21. Lipinski, C. A. *Drug Discovery Today: Technologies*, **2004**, **1.4,** 337-341.

22. Bernard, T.; Pierre-Alain, C.; Patrick, G.; Frédéric, B.; Peter, W. *Pharm research.* **1996,** 13. 3, 335

23. Moriguchi, I.; Hirono, S.; Liu, Q.; Nakagome, Y; Matsushita, Y. *Chem. Pharm. Bull.* **1992**, 40, 127-l 30.

24. Moriguchi, I.; Hirono, S.; Nakagome, I; Hiram, H. *Chem. Pharm. Bull.* **1994**, 42, 976-978.

25. Leo, A.J. *Chem. Pharm. Bull*. **1995**, 43, 512-513.

26. Abraham, M.H.; Chadha, S. H.; Whiting, G.S.; Mitchell, R.C. *J. Pharm. Sci.* **1994**, 83, 1085-l 100.

27. Paterson. D.A.; Conradi, R.A.; Hilgers, A.R.; Vidmar, T.J.; Burton, P.S. *Quant. Struct:Act. Relatsh.* **1994**, 13, 4-10.

28. Ertl, P.; Rohde, B.; Selzer, P. *J. Med. Chem*. **2000,** 43, 3714-3717.

29. Veber, D.F.; Johnson, S. R.; Cheng, H.-Y.; Smith, B.R.; Ward, K.W.; Kopple, K.D. *J. Med. Chem.* **2002**, 45, 2615-2623.

Accepted Manuscript

Table 1. Optimized Reaction conditions

Entry	Solvent	Temperature in °C	Catalyst (mol %)	Time (hr)	Yield %
1	–	RT	–	12	–
2	CH_3CN	RT	–	12	–
3	Methanol	RT	–	12	–
4	Ethanol	RT	–	12	–
5	Water	RT	–	12	–
6	–	100	–	12	–
7	CH_3CN	80	–	12	–
8	Methanol	80	–	12	–
9	Ethanol	80	–	12	–
10	Water	100	–	12	–
11	–	RT	10	12	–
12	CH_3CN	RT	10	12	<10
13	Methanol	RT	10	12	<10
14	Ethanol	RT	10	12	<10
15	Water	RT	10	12	<10
16	–	100	15	12	32
17	CH_3CN	80	15	6	42
18	Methanol	80	15	6	60
19	Ethanol	80	15	6	62
20	Water	100	10	4	75
21	Water	100	15	4	83
22	Water	100	20	6	85

Accepted Manuscript

Table 2. Synthesis of spiro pyrazolo pyramidine derivatives in optimized conditions

Entry	R	X	Time	Yield (%)
5a	H	O	6	85
5b	5-F	O	6	88
5c	7-F	O	6	84
5d	Cl	O	6	86
5e	Br	O	6	79
5f	I	O	6	78
5g	OMe	O	6	65
5h	NO_2	O	6	89
5i	H	S	6	76
5j	5-F	S	6	87
5k	7-F	S	6	81
5l	Cl	S	6	86
5m	Br	S	6	76
5n	I	S	6	73
5o	OMe	S	6	61
5p	NO_2	S	6	91

Accepted Manuscript

Table 3. Recyclability of catalyst

Reaction cycle	1st	2nd	3rd	4th	5th	6th
Yield (%)	91	89	86	84	83	81

Accepted Manuscript

Table 4. Solubility and permeability parameters

Compound Code	Different substituents at 5/6/7' positions	no. of atoms	Rule of five parameters					TPSA	TRB
			M log P	MW	N + O	NH + OH	No of violations		
5a	7'-O; 5-H	30	2.04	398.38	9	5	0	135.53	1
5b	7'-O; 5-F	31	2.19	416.37	9	5	0	135.53	1
5c	7'-O; 7-F	31	2.04	416.37	9	5	0	135.53	1
5d	7'-O; 5-Cl	31	2.58	432.83	9	5	0	135.53	1
5e	7'-O; 5-Br	31	2.71	477.28	9	5	0	135.53	1
5f	7'-O; 5-I	31	2.99	524.28	9	5	1*	135.33	1
5g	7'-O;5-OMe	32	1.96	428.41	10	5	0	144.77	2
5h	7'-O; 5-NO_2	33	1.86	443.38	12	5	1*	181.36	2
5i	7'-S; 5-H	30	2.27	414.45	8	5	0	118.46	1
5j	7'-S; 5-F	31	2.41	432.44	8	5	0	118.46	1
5k	7'-S; 7-F	31	2.39	432.44	8	5	0	118.46	1
5l	7'-S; 5-Cl	31	2.93	448.89	8	5	0	118.46	1
5m	7'-S; 5-Br	31	3.06	493.35	8	5	0	118.46	1
5n	7'-S; 5-I	31	3.33	540.35	8	5	1*	118.46	1
5o	7'-S; 5-OMe	32	2.01	446.49	9	5	0	127.69	2
5p	7'-S; 5-NO_2	33	2.21	459.45	11	5	1*	164.28	2

* Nearly 10% of orally active drugs have 1 or more RO5 violations [20]

Accepted Manuscript

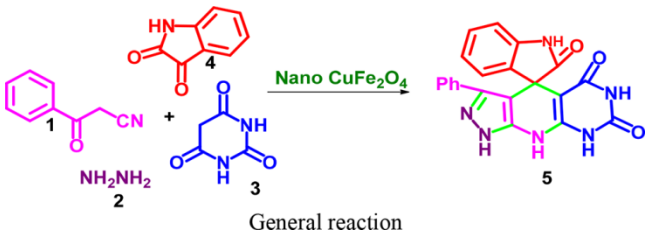
Table 5. Antibacterial studies of synthesized compounds

S. No	Solubi-lity	Volume of sample	Growth inhibition zones (in mm) against human pathogenic bacteria									
			a	b	c	d	e	f	g	h	i	j
5a	Ethanol	100 µl	-	-	-	-	-	-	-	-	-	-
5b	DMSO	100 µl	-	-	-	-	-	-	-	-	-	-
5c	Ethanol	100 µl	-	-	-	-	-	-	-	-	-	-
5d	Ethanol	100 µl	-	-	9	-	11	-	-	-	9	-
5e	Ethanol	100 µl	-	-	7	-	9	-	-	-	11	-
5f	Ethanol	100 µl	-	-	-	-	-	-	-	-	-	-
5g	Ethanol	100 µl	-	-	-	-	-	-	-	-	-	-
5h	Ethyl	100 µl	-	-	-	-	-	-	-	-	-	-
5i	Ethanol	100 µl	-	-	-	-	-	-	-	-	-	-
5j	DMSO	100 µl	-	-	-	-	-	-	-	-	-	-
5k	Ethanol	100 µl	-	-	-	-	-	-	-	-	-	-
5l	Ethanol	100 µl	-	-	9	-	12	-	-	-	10	-
5m	Ethanol	100 µl	-	-	8	-	12	-	-	-	12	-
5n	Ethanol	100 µl	-	-	-	-	-	-	-	-	2	-
5o	Ethanol	100 µl	-	-	-	-	10	-	-	-	4	-
5p	Ethyl	100 µl	-	-	-	-	-	-	-	-	-	-

a).Pseudomonas aeruginosa, b). Streptococcus pneumonia, c).Shigella sonnei, d).Shigelladysenteriae type5, e) Shigella flexneri type 2a, f) Salmonella typhi , g) Vibrio parahaemolyticusserovar O3: K6, h). Vibrio cholera, i) Enterotoxigenic E.coli serotype O115, j) Salmonella entericaserovar Typhimurium

Accepted Manuscript

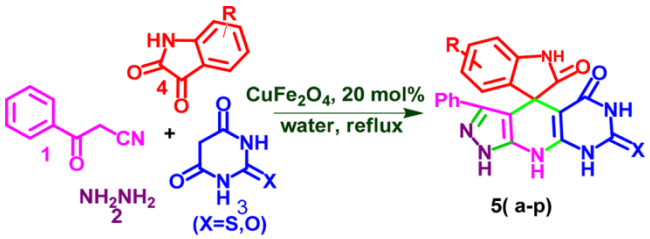
Scheme 1. General Reaction



General reaction

Accepted Manuscript

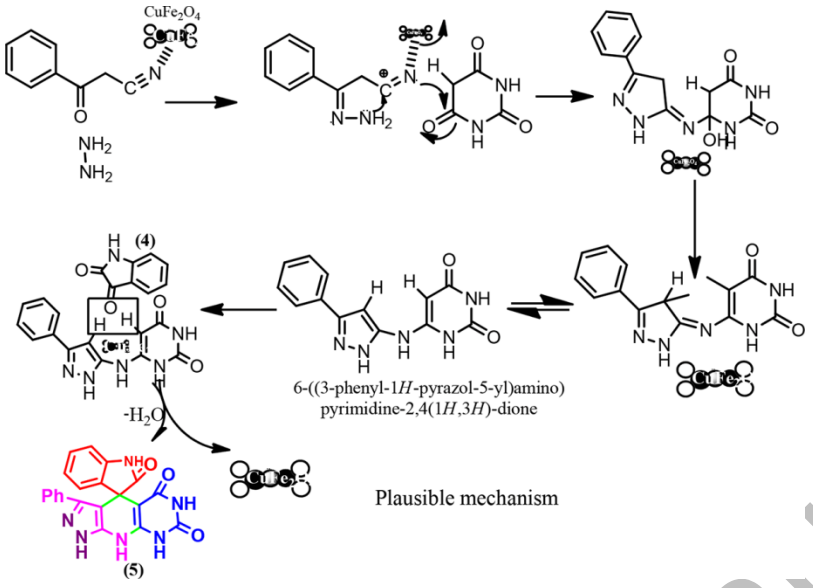
Scheme 2. Model Reaction



Model reaction

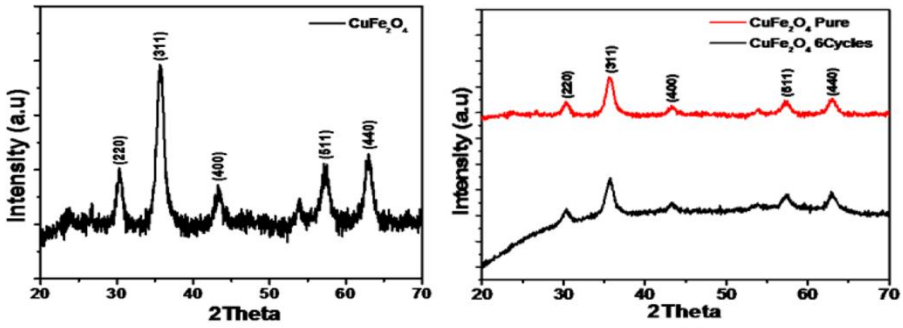
Accepted Manuscript

Figure 1. Reaction pathway for the formation of spiro pyrazolo pyramidine derivatives



Accepted Manuscript

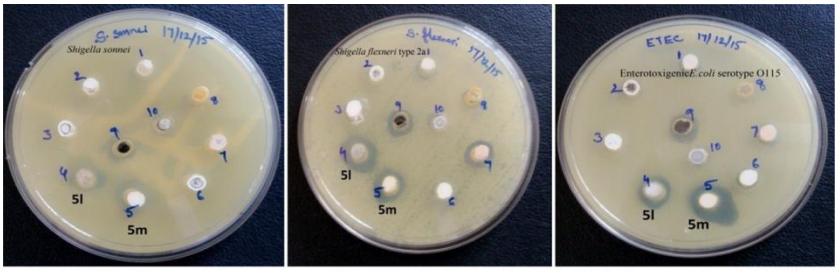
Figure 2. a) XRD of Native form of $CuFe_2O_4$ b) XRD pattern after 6^{th} cycle



(a) (b)

Accepted Manuscript

Figure 3. antibacterial activity



(a) *Shigella sonnei* (b) *Shigella flexneri* type 2a (c) Enterotoxigenic*E.coli* serotypeO115

Accepted Manuscript