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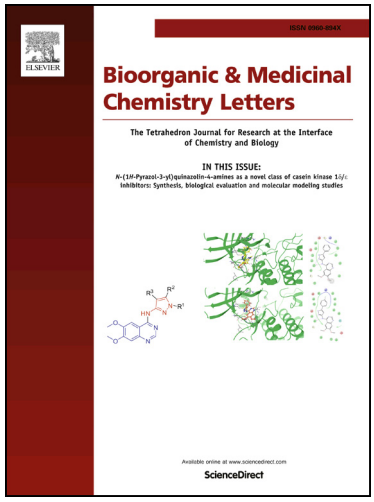

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PII: S0960-894X(17)30664-9
DOI: http://dx.doi.org/10.1016/j.bmcl.2017.06.062
Reference: BMCL 25096

To appear in: *Bioorganic & Medicinal Chemistry Letters*

Received Date: 28 March 2017
Revised Date: 1 June 2017
Accepted Date: 23 June 2017

Please cite this article as: Tiwari, S., Pathak, P., Pratap Singh, K., Sagar, R., One-Pot Two-step Facile Synthesis of 2,3,4,5-Tetra Substituted dihydrooxazoles and their Antimicrobial activity, *Bioorganic & Medicinal Chemistry Letters* (2017), doi: http://dx.doi.org/10.1016/j.bmcl.2017.06.062

One-Pot Two-step Facile Synthesis of 2,3,4,5-Tetra Substituted dihydrooxazoles and their Antimicrobial activity

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Abstract

New 2,3,4,5-tetra substituted dihydrooxazoles derivatives were efficiently synthesized starting from benzaldehyde, aryl thiosemicarbazide and benzoin using designed synthetic route. Newly synthesized 2,3,4,5-tetra substituted dihydrooxazole derivatives were screened for their antibacterial and antifungal activities against different strains of pathogenic bacteria and fungi. The minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) were determined for the test compounds using positive and negative control. Compounds **4b, 4d, 4f, 4i, 4k** and **4m,** have shown good antibacterial activity whereas compounds **4e, 4g, 4h, 4j, 4l** and **4n** have displayed better antifungal activity.

Keywords

2,3,4,5-tetra substituted dihydrooxazoles, aryl thiosemicarbazide, benzoin, thiourea, schiff base, antimicrobial antibacterial activity and antifungal activity.

One pot synthesis of drug-like small molecules has been interest for medicinal chemists and chemical biologists because, this provide the important scaffolds in fewer steps and these molecules play very important role in drug discovery processes.[1] Several bacterial infections such as diarrhea, food poisoning, rheumatic solmonellosis, extraintestinal and intestinal wall infections are caused by gram-positive and gram-negative pathogens.[2] The resistance of pathogens bacteria towards available antibiotics is rapidly becoming a major threat to human health world- wide.[3] In addition, fungal infections continue to increase dramatically because of growing number of immunocompromised hosts such as AIDS patients or those undergoing

anticancer chemotherapy and transplantation.[4-6] Resistance to known antibiotics is becoming great concern in scientific community and big challenge to develop new scaffold as biologically active molecules. Therefore, design of new antimicrobial compounds to deal with these problems is of prime interest.

Oxazoles are class of compounds that are believed to occur in nature from post-translational modification of serine and threonine residue in peptides. They are the key building blocks of natural products, pharmaceuticals and synthetic intermediates. Oxazoles have not only attracted great interest due to their appearance as subunit of various biologically active natural products but also because of their appearance as subunit of valuable precursors in many useful synthetic transformations. Among the numerous heterocyclic moieties of biological and pharmacological interests, the oxazole ring is endowed with a vital role in the manufacture of various active drugs as brain-derived neurotropic factor induced,[7] analgesic,[8] trypanocidal activity,[9] antimitonic agents with pro-apoptotic activity,[10] antifungal activity,[11] anti-inflammatory,[12] antidepressant,[13] pesticidal,[14] and antimicrobial activity.[15]

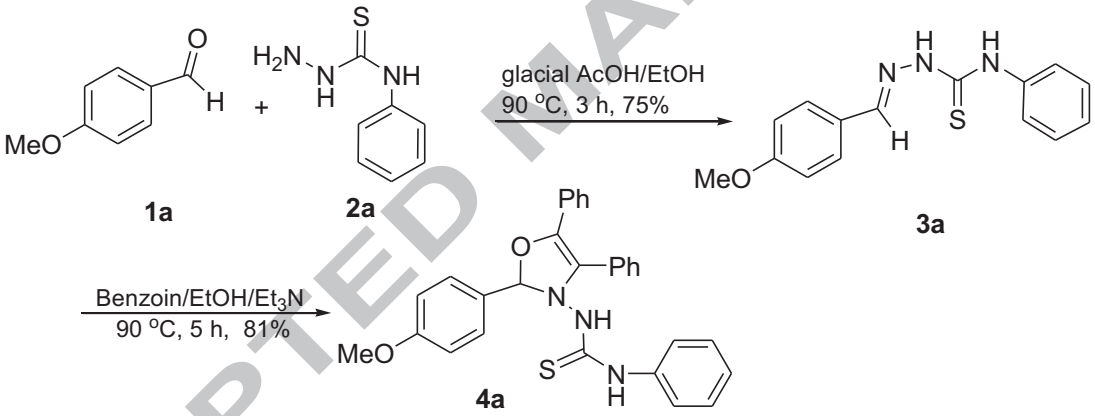
Thiourea and its derivatives have found extensive applications in the field of medicine, agriculture and analytical chemistry. They are known to exhibit a wide variety of biological activities such as antiviral, antibacterial, antifungal,[16] The union of heterocyclic ring with thiourea linkage often results compounds with enhanced biological performance.[17]

In light of the above literature and abundance on bio-potentials of oxazoles and thiourea analogues, we designed 2,3,4,5-tetra substituted dihydrooxazoles having thiourea as one of the appendages and were confident that these framework would provide the important structural motifs for the discovery of new antimicrobial agents. In continuation of our research on efficient synthesis of biologically active small molecules,[18] we developed one-pot synthesis of 2,3,4,5-tetra substituted dihydrooxazole derivatives and demonstrated their antimicrobial activity.

Chemistry

For the development of efficient and facile synthesis of new 2,3,4,5-tetra substituted dihydrooxazole derivatives, we identified substituted benzaldehyde **1a** and 4-phenyl thiosemicarbazide **2a** and benzoin as the key starting materials that can be transferred to our

designed molecules in two step via Schiff's base intermediate **3a.** We initiated our synthetic protocol with 4-methoxy benzaldehyde **1a** refluxing with 4-phenyl thiosemicarbazide **2a** in ethanol in the presence of catalytic amount of acetic acid which furnished corresponding Schiff's bases **3a** as reported in literature in good yields.[19-22] The Schiff's bases **2a** were purified by crystallization and characterized using spectroscopic data. All the NMR and IR data of the Schiff's bases **2a** were found consistent with the reported analytical data. After obtaining Schiff's base **2a**, it was refluxed with benzoin under various reaction conditions using different solvents (MeOH, EtOH Toluene, THF etc) under reflux for 4-5 h, where ethanol in basic condition was found superior with the formation of clean product **4a** (TLC). Reaction mixture was cooled to room temperature, after work-up and purification it furnished compound **4a** in very good (81%) isolated yield (Scheme 1).

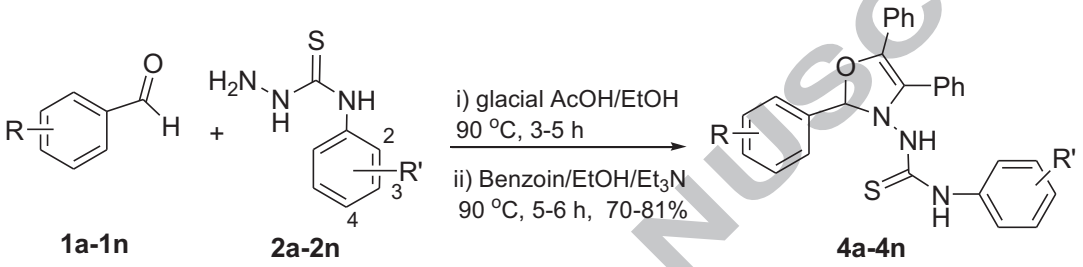


Scheme 1. Synthesis of **4a.**

Once reaction condition was standardized, it was decided to perform same transformation in one-pot two-step manner which may furnish the designed 2, 3, 4, 5-tetra substituted dihydrooxazole derivatives **4a**. Thus 4-methoxy benzaldehyde **1a** was refluxed with 4-phenyl thiosemicarbazide **2a** in ethanol in the presence of catalytic amount of acetic acid for 5 h with careful reaction monitoring (TLC) then benzoin was added to same reaction mixture along with little excess of triethyl amine. Resulting mixture was stirred continuously at high temperature (90 °C) for another 5 h which shows the formation of desired product **4a** (TLC). The reaction mixture was cooled to room temperature and after usual workup compound **4a** was obtained in overall 80%

isolated yields (Table 1). On optimization of one-pot two-step condition a series of 4-aryl thiosemicarbazides **2b-2n** were successfully transformed into corresponding 2, 3, 4, 5-tetra substituted dihydrooxazole derivatives **4b-4n** using similar reaction protocol in good to very good yields (Table 1). Two different benzaldehydes, viz 4-methoxy benzaldehyde and 4-hydroxy benzaldehyde, along with seven different 4-aryl thiosemicarbazides **2a-2n** were used in this synthetic study and results are summarized in table 1.

Table 1. Synthesis of 2,3,4,5-tetra substituted dihydrooxazoles derivatives.



Compounds	R	R'	Yield %	Compounds	R	R'	Yield %
4a	4-OMe	H	80	**4h**	4-OH	H	79
4b	4-OMe	2-OMe	75	**4i**	4-OH	2-OMe	76
4c	4-OMe	4-OMe	76	**4j**	4-OH	4-OMe	74
4d	4-OMe	2-Me	81	**4k**	4-OH	2-Me	80
4e	4-OMe	4-Me	70	**4l**	4-OH	4-Me	69
4f	4-OMe	2-Cl	65	**4m**	4-OH	2-Cl	63
4g	4-OMe	4-Cl	68	**4n**	4-OH	4-Cl	62

The mechanism of this transformation can be postulated as follows. The hydroxyl group of benzoin (in the presence of triethyl amine at elevated temperature) attacked on imine carbon moving imine double bond towards nitrogen to form an unstable intermediate **I** (Figure 1). Intramolecular attack of thiosemicarbazide N-atom to carbonyl carbon of benzoin formed intermediate **II** which under high temperature reaction conditions lost a water molecule and furnished stable 2,3,4,5- tetra substituted dihydrooxazoles derivatives **4a**. All the compounds and intermediates were confirmed by spectral analysis.[23]

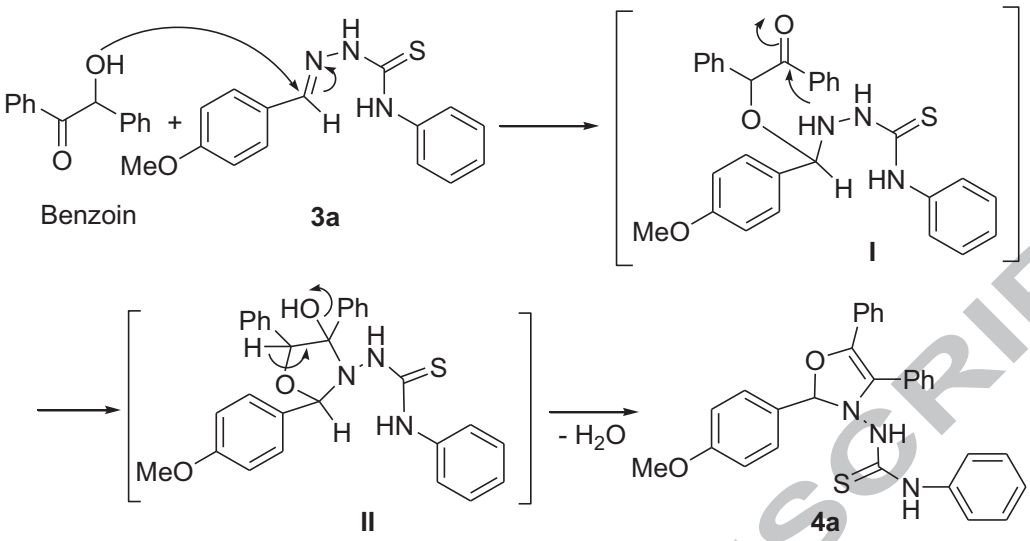


Figure 1. Proposed Mechanism

Biology

The synthesized pure compounds were screened for antibacterial and antifungal activities adopting standard protocols.[24] The antibacterial activity, of prepared final pure compounds **4a-4n** was performed against *Staphylococcus aureus, Bacillus subtilis, Escherichia coli and Klebsiella pneumoniae* using Ciprofloxacin as positive and DMSO as negative control. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined and the activity was reported in µg/mL. The nutrient broth, which contained logarithmic serially two fold diluted amount of test compounds and controls were inoculated with approximately 5×10^5 c.f.u/mL of activity dividing bacteria cells. The cultures were incubated for 24 h at 37 ºC and the growth was monitored visually and spectrophotometrically. The antibacterial results are summarized in Table 2, only for those compounds which were found active against any tested strain of bacteria. It is inferred from Table 2 that compounds **4b, 4d** and **4m** showed moderate activity against gram-positive bacteria *S. aureus* and *B. subtilis* where as **4d**, **4i** and **4k** were active against gram-negative *K. pneumoniae* ranging from 30-50 µg/mL concentration and only one compound **4f** was found active against *E. coli* 25 µg/mL concentration (Table 2). MBC/MIC ratio of all active compounds is ranging from 2.0 to 3.3 suggesting these compounds are bactericidal not bacteriostatic. Antimicrobial agent is considered bacteriostatic when the minimal

MBC/MIC ratio is greater than or equal to 8 whereas it is considered bactericidal if MBC/MIC ratio is less than or equal to 4.

TABLE 2. Antibacterial activity of 2,3,4,5- tetra substituted dihydrooxazoles derivatives

Compd	Gram-positive bacteria				Gram-negative bacteria			
	S. aureus		*B. subtilis*		*E. coli*		*K. pneumonia*	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
4b	40	100	30	50	n.a.	n.a.	n.a.	n.a.
4d	30	100	20	50	n.a.	n.a.	25	50
4f	n.a.	n.a.	n.a.	n.a.	25	50	n.a.	n.a.
4i	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	25	50
4k	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	50	100
4m	30	50	40	100	n.a.	n.a.	n.a.	n.a.
Ciprofloxacin	6.5	12.5	10.0	25	6.25	25	6.25	10.25
DMSO	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.

MIC (μg/mL), minimum inhibitory concentration i.e., the lowest concentration of the compound to inhibit growth of bacteria completely: MBC (μg/mL) minimum bacterial concentration i.e., the lowest concentration of the compound for killing the bacteria completely; MBC/MIC ratio are against- *S. aureus* (**4b** 2.5, **4d** 3.3, **4m** 1.6), *B. subtilis* (**4b** 1.6, **4d** 2.5, **4m** 2.5), *E. coli* (**4f** 2.0), and *K. pneumonia aureus* (**4d** 2.0, **4i** 2.0, **4k** 2.0), n.a.-NO activity detected.

The antifungal activity, of the prepared pure compounds was performed against *Tilletia indica, Trichoderma, P. cubensis, S. fuliginea and P. infestans* using Griseofulvin as positive and DMSO as negative control. Minimum inhibitory concentration (MIC) was determined and reported in μg/mL. Antifungal activity was carried out through disk diffusion method.[25] All fungal cultures were routinely maintained on sabouraud dextrose agar (SDA) and incubated at 28 ^{o}C. The antifungal activities are summarized in Table 3, only for those compounds which were found active against any of these strains of fungi. It is inferred from Table 3 that compounds, **4e**, **4g, 4h, 4j, 4l** and **4n** showed antifungal activity against *T. indica, Trichoderma, P. cubensis, S. fuliginea and P. infestans* strain of fungi ranging from 10-20 μg/mL in concentration which is comparable to Griseofulvin even superior in cases of **4g, 4h** and **4l**.

TABLE 3. Antifungal activity of 2,3,4,5-tetra substituted dihydrooxazoles derivatives.

Compounds	Fungal species and MIC (µg/ml)				
	T. indica	*Trichoderma*	*P. cubensis*	*S. fuliginea*	*P. infestans*
4e	20	16	12	16	14
4g	18	12	14	12	16
4h	12	10	10	10	10
4j	14	12	16	14	16
4l	16	14	12	10	14
4n	18	16	14	18	16
Griseofulvin	20	18	16	18	20
DMSO	n.a.	n.a.	n.a.	n.a.	n.a.

MIC(µg/ml), minimum inhibitory concentration i.e. the lowest concentration of the compound to inhibit the growth of fungi. n.a.(NO activity detected).

The structure- activity relationship (SAR) of the tested compounds for antibacterial activity can be summarized as follows: i) In the series of the 2,3,4,5-tetra substituted dihydrooxazoles derivatives the compounds prepared from *o*-substituted aryl thiosemicarbazide in this series has shown better antibacterial activity than the *p*-substituted groups. ii) Presence of thiourea linkage at position 3 with substituted at phenyl ring is an important scaffold for better antibacterial activity. iii) Most of the antibacterial compounds have MBC/MIC ratio below 4, it means they are bactericidal not bacteriostatic. Antifungal activity of these compounds was similar or even better in some cases as compare to Griseofulvin, a known antifungal agent. The possible mechanism for the antibacterial activity of examined compounds is not known at the moment and investigations are being done to investigate the mechanism of antibacterial action, and to synthesize more effective compounds.

Conclusion

In summary, an efficient, clean and convenient one-pot three components synthesis of 2,3,4,5-tetra substituted dihydrooxazole derivatives has been developed. The procedure offers several advantages including simple reaction conditions and simple experimental and product isolation procedures. Moreover, the synthesis of dihydrooxazole derivatives were designed through the

recombination of cheap and readily available starting materials, benzoin, 4-substituted benzaldehyde and aryl thiosemicarbazides. A preliminary anti-bacterial and anti-fungal screenings were performed on these compounds which revealed that newly generated compounds possess antimicrobial activity and these can be elaborated as potential antimicrobial agents.

Acknowledgements

We are thankful to the Head, Department of Chemistry, University of Allahabad, Allahabad for necessary laboratory facilities. The authors would also like to thank SAIF, Punjab University, Chandigarh and SAIF, Central Drug Research Institute, Lucknow, for spectral and analytical data and centre of biotechnology, University of Allahabad, Allahabad for antimicrobial and antifungal activity data.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://.

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23. Compound **4a**. Isolated as yellowish amorphous solid (yield, 81 %). IR (KBr): 3150 (N–H Str), 3019 (=C–H Str), 1602 (C=C Str), 1156 (>C=S Str); ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.46 (s, 1H, N-H), 8.00 (d, 2H, *J* = 7.5 Hz, ArH), 7.94 (d, 2H, *J* = 7.5 Hz, ArH), 7.76 (t, 1H, *J* = 7.5 Hz, ArH); 7.62-7.51 (m, 3H, ArH), 7.45-7.41 (m, 4H, ArH), 7.37-7.20 (m, 4H, ArH), 6.90-6.86 (m, 2H, ArH), 6.08 (s, 1H, CH), 3.72 (s, 3H, OCH_3); ^{13}C NMR (100 MHz,

DMSO-d_6): δ 194.6 (C=S), 156.4 (ArqC), 139.6, 135.2, 134.6, 133.0, 132.2, 132.1, 129.4, 129.2, 128.7, 128.4, 128.3, 127.5, 127.1, 126.0, 118.3, 113.4, 75.7 (CH), 55.0 (OCH_3); FAB HRMS *m*/*z*: calcd for $C_{29}H_{25}N_3O_2S$ [M + H]+, 480.1746; found, 480.1745. Anal. Calcd for $C_{29}H_{25}N_3O_2S$: C, 72.63; H, 5.25; N, 8.76. Found: C, 72.57; H, 5.18; N, 8.66.

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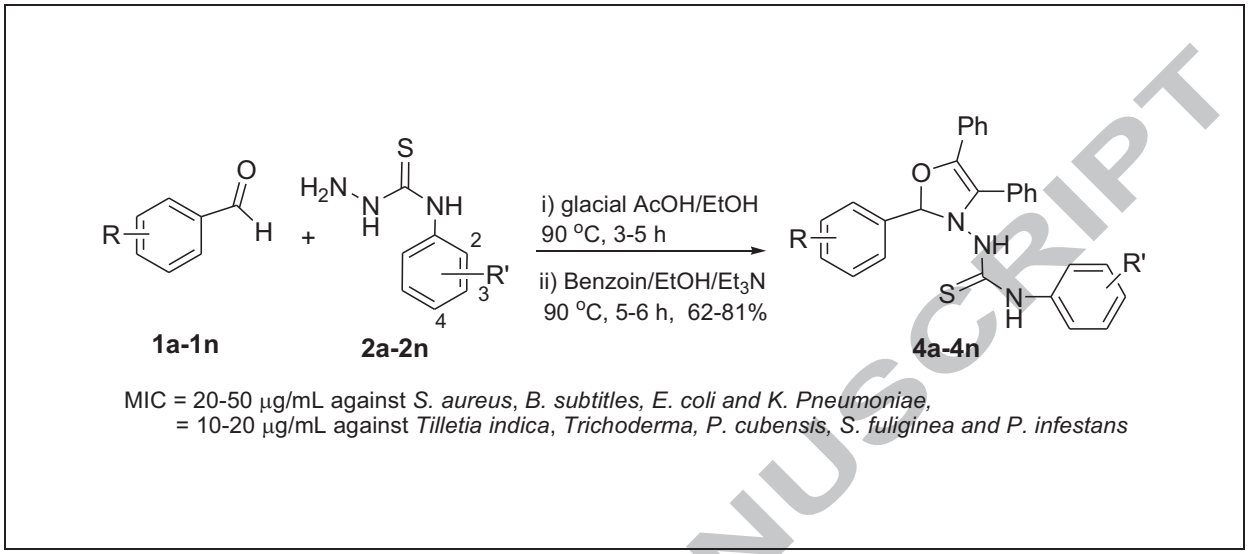
Graphical Abstract



MIC = 20-50 μg/mL against *S. aureus*, *B. subtitles, E. coli and K. Pneumoniae,*
= 10-20 μg/mL against *Tilletia indica*, *Trichoderma, P. cubensis, S. fuliginea and P. infestans*