2013

Evaluation of single electron transfer reduction products of 3-oxoindoles as antibacterial agents

Debasish Bandyopadhyay
The University of Texas Rio Grande Valley, debasish.bandyopadhyay@utrgv.edu

Irani Biswas

Subrata Laskar

Bidyut Basak

Recommended Citation

Debasish Bandyopadhyay, Irani Biswas, Subrata Laskar, Bidyut Basak. “Evaluation of single electron transfer reduction products of 3-oxoindoles as antibacterial agents”, Int. J. Pharm. Pharm. Sci., 2013, 5 (Suppl 3), 447¬–450.

Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 5, Suppl 3, 2013

Research Article

EVALUATION OF SINGLE ELECTRON TRANSFER REDUCTION PRODUCTS OF 3-OXOINDOLES AS ANTIBACTERIAL AGENTS

DEBASISH BANDYOPADHYAY[a], IRANI BISWAS[b], SUBRATA LASKAR[c], BIDYUT BASAK[c]

[a] Department of Chemistry, The University of Texas-Pan American, West University Drive, Edinburg ,TX 78539, USA, [b] Department of Botany, The University of Burdwan, Burdwan 713104, W. Bengal, India, [c]Department of Chemistry, The University of Burdwan, Burdwan - 713104,W. Bengal, India. Email: slaskar01@yahoo.co.in

Received: 03 Jun 2013, Revised and Accepted: 24 Jun 2013

ABSTRACT

Objective: The objective of the present study is to assess the antibacterial properties of six indole derivatives viz, 3-(Naphth-1-y - methyl) –indole (II), 3-(1-Naphthylcarbonyl – indole (III), 3,3' –diformyl-2,2' –bis indole (IV), 1,4- (3,3' –di indolyl) – 1,4-dioxobutane (V), 3-benzoyl indole (VI) and 1,1 – bis – (3-indolyl)- ethane (VII) synthesized by single electron transfer reduction of 3-formylindole (I) against two Gram +ve and two Gram –ve bacteria which usually cause human infections.

Methods: Synthesis of 3-formyl indole from which the indole derivatives were prepared were further characterized by IR and NMR analysis. The six synthesized compounds were screened for their antibacterial activity by agar diffusion method and the activities were further confirmed by determining their MIC values by microdilution technique.

Results: All of the compounds more or less showed activity against different bacterial species except compound IV which showed activity only against *B. subtilis*. Compound II exhibited the most potent activity with lower MIC values against two Gram –ve and one Gram +ve bacteria of which the Gram –ve ones are known to be responsible for nosocomial and community acquired infections.

Conclusion: Compound II being the most potent active compound may serve as leads for further optimization most likely to contribute as a broad spectrum antibiotic.

Keywords: Antibacterial activity, Single electron transfer reduction, Indole-derivatives(.)

INTRODUCTION

Antimicrobial resistance is growing quickly throughout the world due to extensive and indiscriminate use of antibiotics [1]. Development of new class of antibacterial agent with different mode of action is urgently needed to combat this problem. Several pharmaceutical companies intensified their efforts in search for new class of antibacterial agents which resulted in a spectacular advancement in medicinal chemistry.

Reduction of indole derivatives comprises a very important and interesting branch of synthetic organic chemistry as these reactions are utilized in the synthesis of several drugs, dyes, agrochemicals and higher alkaloids. Since the nitrogen heterocycles are of great importance in chemistry and biology, the reduction of these compounds are of vital importance. Synthesized heterocyclic compounds like 3-heterosubstituted indoles, oxindoles and benzimidazole derivatives have attracted considerable interest because of their various biological applications [2,3,4].The reluctance of synthesizing biologically active indole derivatives is explicable in terms of highly electron-rich system of the indole ring, which has little tendency to accept an electron. The tendency to form an indolyl radical anion by accepting an electron can be increased by introducing an electron-withdrawing substituent at C-3 of the indole ring. As a part of our investigation in this field we have recently reported [5] the synthesis of indolo[2,3-a]carbazoles based on the reaction between (**I**) and sodium naphthalenide using the product (**IV**) as the key intermediate (Scheme 1). Indolo[2,3-a]carbazoles can frequently serve as a nucleus of various medicinal important natural products e. g. staurosporine [6], rebeccamycin [7], K-252a [8], alkaloids [9] e.g. arcyriaflavin A, AT2433-B aglycone etc.

The effect of sodium naphthalenide (Na NaPh), a cost-effective and easily available single electron transfer (SET) reagent, on different 3-oxoindoles has been studied. The structural, mechanistic as well as chemical aspects have already been reported [10,11]. The objective of the present study is to evaluate the antibacterial activities of some of the indole derivatives obtained from 3-formyl indole (I) following a schematic synthetic procedure (Scheme 1).

MATERIAIS AND METHODS

Chemistry

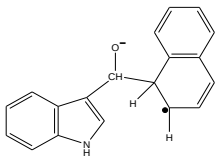
All the chemicals and solvents used in this experiment were of analytical grades and procured from Merck (India).

Synthesis of 3-formyl indole 5 ml of phosphoryl chloride ($POCl_3$) was added dropwise with stirring to N,N dimethyl formamide (16 g) in a flask protected from atmospheric moisture (temperature is maintained between 0 – 20^{0}C). Indole (5.85 g) was then slowly added to 4 g of dry DMF (N,N dimethyl formamide) with stirring (temperature was kept in between 20-30^{0}C). The mixture was then kept at 35^{0}C for 45 min. It was neutralized by aqueous sodium hydroxide solution (9.5 g in 50 ml water) at temperature 20-30^{0}C. Resulting solution was quickly boiled for 1 min. Pale yellow crystals were obtained, filtered, washed with distilled water and dried in air.

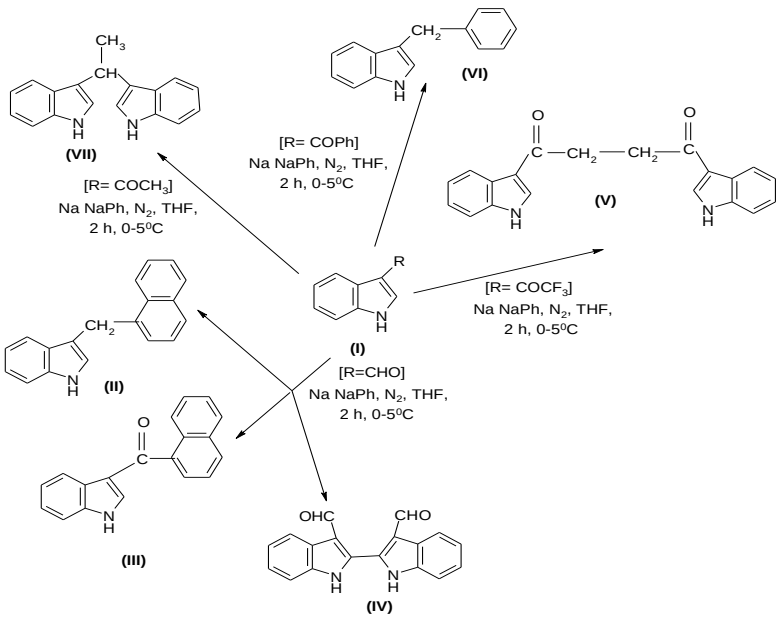
IR spectra were recorded on a Perkin-Elmer FT-IR spectrometer (Model: Spectrum RX1) using solid KBr. Mass spectra were performed on JEOL –AX 500 mass spectrometer. NMR spectra were recorded on a Brooker AV 300 (5 mm probe).

3-formyl indole (I): Yield 86%; m.pt 198^{0}C; Anal. Calcd. for C_9H_7NO: C,74.78; H, 4.83; N,9.65; Found: C, 74.58; H, 4.62; N, 9.51; IR (KBr disc): 3440 cm^{-1} (N –H str.. of a secondary amine), 3115 cm^{-1} (C- H str., hetero aromaticity), 3663 cm^{-1} and 3045 cm^{-1} (coupled vibration with other C- H str.), 2821 cm^{-1}, 2725 cm^{-1} (C – H str. of formyl group), 1633 cm^{-1} (C = O str. in formyl group); ^{1}H NMR (300MHz/ $CDCl_3$): δ 10.1 (1H, s, >NH), 7.55 (1H, s, C^{2}H), 9.73 (1H, s,, -CHO), 8.20,7.19, 7.01, 7.54 (4H, m, Ar<); ^{13}C NMR: δ 138.1, 118.2, 121.3, 119.8, 121.7, 111.1, 126.3, 137.1; MS (70ev) m/z 145 (M$^+$, 62.8%).

The indole derivatives are obtained from 3-formyl indole (I) following the schematic synthetic procedure as given below (Scheme 1).



Radical (A)



Scheme 1: Synthesis of different indole derivatives (II – VII) from 3-formyl indole (I).

Antibacterial screening

a) Agar well diffusion assay

The antibacterial activity of the compounds (**II-VII**) was tested against four bacterial organisms. The test organisms taken were *Bacillus subtilis* AR2, *Escherichia coli* XLI-Blue, *Staphylococcus aureus* (MTCC 1430) and *Pseudomonas aeruginosa* (MTCC 424). The agar-well diffusion method [12] was used to assess the bioactivity of the synthesized compounds. *B. subtilis* and *E. coli* were procured from Bose Institute, Kolkata, India whereas *Staphylococcus aureus* and *Pseudomonas aeruginosa* were procured from Institute of Microbial Technology, Chandigarh, India. Luria-Bertini agar was used as culture medium for *B. subtilis* and *E. coli* species and nutrient agar was used for the remaining two species. The test compounds were dissolved in 50% dioxane (v/v in water) at a concentration of 2 mg/ml. The bacteria were subcultured in their respective liquid media (LB broth and nutrient broth) taken in four different Ehrlenmeyer's flasks of 100 ml capacity containing 50 ml each of sterile media. The flasks were inoculated with loopful of different bacterial strains and incubated on a rotary shaker for 18 hrs at 37°C to activate the strains. The absorbance was read at 530 nm and adjusted with sterile distilled water to match with that of 0.5 Mc Farland standard solution to give a final concentration of 1.0 x 10^6 CFU/ml of bacterial cells. 0.1 ml of bacterial suspensions was taken from each flask and uniformly spread over sterile agar plates by the help of sterilized glass spreader. The plates were kept in refrigerator for 20 minutes to allow the test organisms to get fully embedded in the medium. Then wells (9 mm diameter) were cut out from agar plates using a sterilized cork borer and they were aseptically filled with 100µl of respective test compounds (**II –VII**) with a sterile micropipette. The plates were again kept in refrigerator for 1hr to allow the test compounds to diffuse thoroughly into the agar and finally they were incubated at 37 °C for 48 hrs and the diameter of inhibition zones (in mm) were noted. Same volume of 50% Dioxane and Streptomycin at a concentration of 1mg/ml were used to serve as negative and positive control respectively. All experimental sets were done with three replicates for each and the mean values of the zones were calculated as ± SD (Table 1). The activity indices designated as AI were calculated as the division of zone of inhibition of the test compounds by that of the standard drug [13].

b) Determination of Minimum Inhibitory Concentration (MIC)

For estimating the MIC of the test compounds micro-dilution technique [14] was performed(.) 1ml of of each test compounds at various concentrations ranging from 40 µg/ml to 180µg/ml was added to 3ml of respective sterile broths and 0.1ml of each microorganisms (1.0 x 10^6 CFU/ml) was inoculated into each test tube and mixed thoroughly. The tubes were incubated at 37°C for 24h hrs and the bacterial turbidity in each test tube was measured at 600nm. Streptomycin (10 µg/ml) was used as reference antibiotic and tubes containing only growth medium and microorganisms were used as control. The tubes with lowest dilution showing no turbidity after 24 hrs incubation was considered as the MIC and the results were presented in Table 2.

RESULTS

In our experiment the basic compound, 3 – formyl indole (I) has been synthesized in good yield (86%) , from that other compounds (II – VII) have been synthesized accordingly (Scheme –1) via known methods.

The compounds (**II – VII**) were tested for their antibacterial activities by agar-well diffusion method and further confirmed by their MIC values. The results of inhibition (in mm) are shown in Table 1 and the MIC values are presented in Table 2.

Table 1: Antibacterial activity of different indole derivatives (compounds II-VII) using agar diffusion method (inhibition zones in mm).

Test Samples	*B. subtilis*		*S.aureus*		*E.coli*		*P. aeruginosa*	
	Z.I.	**A.I.**	**Z.I.**	**A.I.**	**Z.I.**	**A.I.**	**Z.I.**	**A.I.**
II	16 ± 0.2	0.64	—	—	15 ± 0.2	0.75	20 ± 0.5	1.1
III	13 ± 0.3	0.52	—	—	16 ± 0.3	0.80	—	—
IV	14 ± 0.6	0.56	—	—	—	—	—	—
V	—	—	15 ± 0.7	0.75	12 ± 0.2	0.60	19 ± 0.7	1.1
VI	13 ± 0.3	0.52	—	—	15 ± 0.5	0.75	—	—
VII	18 ± 0.5	0.72	—	—	18 ± 0.8	0.90	—	—
Streptomycin (+ve control)	25 ± 0.1	NA	20 ± 0.1	NA	20 ± 0.2	NA	18 ± 0.1	NA

Z.I.= zone of inhibition, A.I.= activity index with respect to streptomycin; NA= not applicable;Inhibition zones are mean of three replicates ± SD ; '—' indicates no inhibition.

The results from Table 1 clearly indicates that *B. subtilis* and *E. coli* are more or less susceptible against all the compounds (**II – VII**) in question whereas *S. aureus* showed inhibition only against compound **V** and *P. aeruginosa* showed inhibition against compounds **II** and **V**. All the compounds except compound **V** could inhibit the growth of *B. subtilis*. Compound **IV** showed activity only against *B. subtilis* and thus it may be considered as the least potent bioactive compound amongst the compounds under study.

Table 2: MIC values of indole derivatives (compounds II – VII) against the bacterial species (in µg/ml).

Bacterial Strains	MIC values (µg/ml) of the Indole compounds					
	II	**III**	**IV**	**V**	**VI**	**VII**
B. subtilis (Gram + ve)	90	180	160	--	140	40
S. aureus (Gram + ve)	--	--	--	140	--	--
E. coli (Gram – ve)	70	80	--	180	80	40
P. aeruginosa (Gram – ve)	100	--	--	100	--	--

DISCUSSION

In the present work, effect of a single electron transfer reagent, sodium naphthalenide on a 3-oxoindole compound , 3- formyl indole resulted in production of six different indole derivatives by the following procedure represented as Scheme I. The compounds **II** and **III** are formed from anion radical **(A**) derived from coupling of substrate and naphthalenide radical whereas compound **IV** is a simple dimerisation at 2,2' position of radical anion of 3 – formyl indole. In the formation of Compound VI and Compound VII simple radical ion mechanism was followed. But for Compound V, radical dimerisation followed by stepwise removal of fluorine is the mechanistic pathway. The stepwise defluorination was supported mechanistically by Stocker *et.al.* [15]. Spectral data and detail mechanistic interpretation related to this context have already been reported else where [10,11].

It is obviously interesting to note that most of the compounds have a tendency to be more effective against Gram –ve bacteria as revealed by the comparatively larger dimensions of the inhibitory zones and in this respect compound **II** showed the highest efficacy to combat against two Gram –ve (*P. aeruginosa* and *E. coli*) and one Gram +ve (*S. aureus*) bacteria. Usually the Gram +ve bacteria are more susceptible to antibacterial agents than the Gram – ve one [16] and this is due to the fact that the cell wall of the former possess only a single peptidoglycan layer whereas the cell wall of the latter possess a thi peptidoglycan layer. Thus the efficacy of compounds **II** and **V** and to a certain extent compound **VII** are found quite promising as antimicrobial agent since they could combat against *P. aeruginosa* and *S. aureus* which are reported to be frequently associated with nosocomial and community acquired infection [17,18] and *E. coli* is very much associated with common infections like gastroenteritis[11]. Although the mode of action of these indole derivatives is not known in detail but some studies have shown that the presence of electron withdrawing group have shown influence on bioactivity [19]. Thus the susceptibility of the usually resistant Gram – ve strains may be correlated to two major reasons. One is the electronic effect which infers that the presence of electron withdrawing group withdraw the electron cloud from substituted indole thus acting as nucleophilic centre which caused high level of activity as exhibited by the compounds **II**, **III**, **V** and **VI**. However this is not true in case of Compound **VII** where instead of a nucleophilic centre, it is the substitution of one hydrogen atom of $-CH_2-$ by CH_3 which caused an increase in the number of carbon atoms thus enhancing the maximal binding capacity and potency of the respective compound. The second plausible reason might be that the compounds being heterocyclic and aromatic in nature (property of indole derivatives) are strongly hydrophobic which enable them to partition the lipids of the outer membrane of the Gram – ve bacteria thus disturbing their cellular integrity and rendering them more permeable and vulnerable to cellular leakage [20]. Moreover, in addition to the failure of the outer membrane to act as a barrier to the penetration of these indole derivatives, the enzymes of the periplasmic space were also perhaps unable to degrade those exogenous molecules [21].

The MIC values presented in Table 2 are more or less in conformity with the results obtained in Table 1. However, the differences in diameter of inhibitory zones against different microorganisms may not exactly match with the differences in their corresponding MIC values as noticed in case of compound **II** where *P. areuginosa* showed higher inhibition zone (20mm) than *B. subtilis* (16mm) and *E. coli* (15mm) whereas the MIC values were found to be greater in *P. areuginosa* (100 µg/ml) than those of *B. subtilis* and *E. coli* where inhibition occurred at lower concentrations of 90 µg/ml and 70µg/ml respectively. Such variation in the antibacterial property could be attributed to certain extrinsic factors like variable diffusability in agar medium and inoculum size [22] and certain intrinsic factors like variable permerability of the cell surface of the microorganisms to the antibacterial compounds and the respective chemical structure of the antibacterial compound in itself [23].

CONCLUSION

From the above findings the bioactive potency of the synthesized compounds can be arranged in the following order of decreasing activity:

II > V > VII > VI > III > IV

Moreover, the bacteria like *S. aureus*, *P. aeruginosa* and *E. coli* being responsible for nosocomial and community acquired infections, a further investigation of the potent antimicrobial compounds (**II, V and VII**) needs to be conducted against a number of such strains isolated from hospitals or infected patients in which multidrug resistance might develop along with their mode of action within these microorganisms in order to prove their antibacterial efficacy. Compound **II** being active against both Gram +ve and Gram –ve pathogenic strains at lower MIC values is likely to contribute as a broad spectrum antibiotic. Since the compounds are pure they could be of commercial interest to both pharmaceutical companies and research institutes in the production of new drugs only after thorough checking of their toxicity as well as their efficacy in animal models.

ACKNOWLEDGEMENTS

Authors are indebted to the DSA project, UGC, New Delhi for financial assistances.

REFERENCES

1. Wiley JL, Compton DR, Dai D, Lainton JA, Huffmann JW, Martin BR Structure-activity relationships of indole- and pyrrole-derived cannabinoids. J Pharmacol Exp Ther 1998; 285(3):995-1004.
2. Joule JA, Mills K, Smith GF. Heterocyclic Chemistry(.) 3rd ed. Chapman and Hall; 1995(.) p.305-306.
3. Gawad NMA, Youssef AMA Synthesis of some new oxindole derivatives as potential antipsychotic and anticonvulsant agents. Int J Pharm Pharm Sci 2011; 3 Suppl 3:213-219.
4. Petkar K, Parekh P, Mehta P, Kumari A, Baro A Synthesis & evaluation of 2-chloromethyl-1H-benzimidazole derivatives as antifungal agents. Int J Pharm Pharm Sci 2013; 5(2):115-119.

5. Banerji A, Bandyopadhyay D, Basak B, Biswas PK, Banerji J, Chatterjee A A new route to the synthesis of indolo[2,3-a]carbazoles. Chem Lett 2005; 34(11): 1500-1501.
6. Link JT, Raghavan S, Gallant M, Danishefsky S, Chou TC, Ballas LM Staurosporine and *ent*-staurosporine: the first total syntheses, prospects for a regioselective approach and activity profiles. J Am Chem Soc 1996; 118:2825- 2842.
7. Yamashita Y, Fujii N, Murkata C, Ashizawa T, Okabe M, Nakano H Induction of mammalian DNA topoisomerase I mediated DNA cleavage by antitumour indolecarbazole derivatives. Biochemistry 1992; 31:12069-12075.
8. Kase H, Iwahashi K, Matsuda Y K- 252a, a potent inhibitor of protein kinase C from microbial origin. J Antibiot 1986; 39:1059-1065.
9. Gribble GW, Berthel SJ Synthetic approaches to indole [2,3-a] carbazole alkaloids. Syntheses of arcyriaflavin A and AT 2433-B aglycone. Tetrahedron 1992; 48:8869-8880.
10. Banerji A, Bandyopadhyay D, Basak B Effect of sodium naphthanelide, a key SET reagent, on 3- substituted indoles. Heterocycles 2004; 63:2371-2377.
11. Banerji A, Bandyopadhyay D, Basak B, Prangé T, Neuman A Structure elucidation and X-ray crystallographic study of 3 -(Naph – 1-ylmethyl) indole. J Struct Chem 2005; 46:906-912.
12. Hugo WB, Russel AD(.) Pharmaceutical Microbiology. Oxford (London): Black Well Scientific Publications; 1977. p. 165.
13. Adegako AA, Adebayo-tayo BC Antibacterial activity and phytochemical analysisof leaf extracts of *Lasienthera africanum*. African J Biotech 2009; 8(1): 077-080.
14. McKane L, Kandel J. Microbiology-Essential and Applications. New York: McGraw- Hill Book Company; 1986. p. 335.
15. Stocker JH, Jenevein RM The electrochemical transformation of α, α, α – trifluroacetophenone into acetophenone ; an unusually ready hydrogenolysis of the C-F bond. Chem Com 1968; 934 (b) House HO. In: Modern Synthetic Reactions (2nd ed.), Cummings Publishing Co.; 1972. p. 161.
16. McCutcheon AR, Ellis SM, Hancock REW Towers GHN Antibiotic screening of medicinal plants of the British Columbian native peoples. J Ethnopharmacol 1992; 37:213–223.
17. Indrayan AK, Shama A, Gideria BS, Gupta CP Antibacterial activity of dye from ensalpina sappan (Patang/Brazilworld). In J Microbiol 2002; 42:359- 360.
18. De N, James NE Antibacterial spectrum of extracts of *Ocimum gratissimum* L. (Basil) ND *Xylopia aetiopica* A. Rich (Dunal). Nig J Appl Sci. 2002; 11:165-175.
19. Chakraborty K, Lipton AP, Paluraj R, Chakraborty RD Guaiane sesquiterpines from seaweed *Ulva fasciata* Delile and their antibacterial properties. Eur J MedChem 2010; 45:2237-2244.
20. Sikkema J, De Bont JAM, Poolman BM Interaction of cyclic hydrocarbonswith biological membranes. J Biol Chem 1994; 269(11):8022 - 8028.
21. Tanaka JCA, de Silva CC, de Oliveira AJB, Nakamura CV, Dias Filho BP Antibacterial activity of indole alkaloids from *Aspidosperma ramiflorum*. Brazilian J Med And Biol Res 2006; 39(3):387-391.
22. Gaill W, Jon AW Antimicrobial susceptibility test; dilution and disc diffusion methods. In: Murray PR (ed.). Manuals of Clinical Microbiology. 6th ed. ASM Press; 1995. p. 1327 - 1332.
23. Suzen S, Bozkaya P, Coban T, Dog˘ U Nebiog˘lu, D Investigation of the *in vitro* antioxidant behaviour of some 2-phenylindole derivatives: discussion on possible antioxidant mechanisms and comparison with melatoinin. J Enzym Inhib Med Chem 2006; 21(4):405-411.