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Green And Efficient Microwave One Pot Synthetic Approach to N-Phenyl Piperazinyl-1,3,4-Oxadiazole Derivatives and Evaluation of Their Antioxidant and Anti Inlammatory Activity

KEYWORDS: 1,3,4-oxadiazole, anti inflammatory, antioxidant, N-phenyl piperazine.

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ABSTRACT *A green efficient one pot synthetic protocol has been developed for 2 phenyl-piperazino-5-mercapto-substituted aryl-1,3,4-oxadiazoles. Synthesized compounds were characterized by FTIR, 1H NMR and elemental analysis. Antioxidant activity of methanol solutions of synthesized compounds was determined by Reducing power assay and Hydrogen peroxide scavenging activity at 700 nm and 250 nm respectively. The synthesized compounds were also screened for anti-inflammatory activity. Compound showed good anti-inflammatory and antioxidant activity.*

INTRODUCTION

Microwave assisted synthesis has proved a green and efficient synthetic protocol owing to enhanced rates, higher selectivity, efficiency and higher yield (Pradip et al. 2014, Adnani et al. 2012). A one-pot synthetic strategy includes successive chemical reactions in just one reaction vessel to improve the efficiency of a reaction. This synthetic protocol avoids lengthy separation process and purification of the intermediates and hence increases chemical yield (Bahrami et al. 2007, Huq et al. 2014).

Free radicals are reactive oxygen species (ROS) which are short lived, unstable, highly reactive and capable to destroy structure and function of healthy body cell. Antioxidants are capable of stabilizing or deactivating a free radical before they attack the healthy cell. Some synthesized heterocyclic compounds are reported to show remarkable antioxidant activity (Hossain et al. 2012, Kotaiah et al. 2012). Heterocyclic compounds are also reported to posses anti inflammatory activity by some research groups (Elias et al. 1988, Shaaban et al. 2008).

Moreover 1,3,4-oxadiazole derivatives (Bhardwaj et al. 2009, Sah et al. 2013) and piperazine derivatives have their significance in drug discovery owing to reported pharmacological importance (Savaliya et al.2011, Joshi et al. 2012). In view of above findings 1,3,4-oxadiazole and piperazine moieties are incorporated together evaluated for their anti inflammatory and antioxidant activities.

MATERIAL AND METHODS

Melting points were determined in open capillary tubes in 'Innco' electrical apparatus and are uncorrected. FTIR was carried out on Schimadzu 8101 A. Spectrophotometer in KBr pellets and ^{1}HNMR was recorded on a DPX 300 MHz Brucker Spectrophotometer in DMSO with chemical shift in ppm. MW irradiations were carried out in domestic Samsung microwave oven, model number 310 EMENO 22332. The synthesized products were frequently checked by thin layer chromatography (TLC). Absorbance for antioxidant activity and anti inflammatory activity was determined by ELICO SL 177 scanning mini spec.

One pot Synthesis of 2-N- phenyl piperazino methylene-5-mercapto-1,3,4-oxadiazole (IV):

Equimolar mixture of N-phenyl piperazine and chloro ethyl acetate was irradiated in a microwave oven in 10 ml acetone for 15 minutes in presence of 0.02 mole of anhydrous potassium carbonate. This resulted in formation of ethyl-N-methyl piperazino acetate (II). In the reaction mixture 0.012 mole of hydrazine hydrate was added gradually followed by addition of 10 ml absolute ethanol with continuous stirring and the resultant mixture was irradiated in microwave oven for 15 minutes. N-phenyl piperazino acetyl hydrazide (III) was obtained by above process. 0.01 mole of N-phenyl piperazino acetyl hydrazide and 0.02 mole of potassium hydroxide were dissolved in 10-12 ml of absolute ethanol. 0.015 mole CS_2 was added gradually with continuous stirring. The resultant solution thus obtained was irradiated for 15 minutes and then cooled, diluted with 100 ml water and filtered. The filtrate was acidified with acetic acid. The solid 2 phenyl piperazino-5-mercapto-1,3,4-oxadiazole (IV) thus obtained was filtered, washed with water, dried and recrystallized using ethanol.

Yield 76 %, M.P.-112 ^{0}C, M.W. 276, M. F. $C_{13}H_{16}N_4OS$, Calcd. (Found), N %=20.29 (20.32), FTIR (KBr, cm^{-1}): 3350 (NH), 3020(C-N), 1640(C=N), 1270 (C=S), 1120 (C-O-C), PMR (DMSO, δ ppm, 300 MHz): 2.62-2.78 (m,8H, N-$(CH_2)_4$-N), 3.25 (s,2H,-CH_2-),7.12-8.14 (d, 5H. ArH), 9.12 (s, 1H, NH)

2-N- phenyl piperazino methylene- 4-substituted aryl -5-mercapto-1,3,4-oxadiazoles (Va-Vf):

2-N-phenyl piperazino methylene-5-mercapto-1,3,4-oxadiazole and 4-nitro chloro benzene were taken in equimolar ratio and irradiated under anhydrous conditions in 10 ml acetone and (0.02 g) anhydrous potassium carbonate for 15 minutes. Excess solvent was distilled off. On cooling a solid separated out which was filtered, dried and recrystallised from ethanol. The remaining compounds were synthesized in a similar manner.

2-N- phenyl piperazino methylene-4-(4'-nitro phenyl)-5-mercapto-1,3,4-oxadiazole (Va).

Yield 70 %, M.P.-120 ^{0}C, M.W. 365, M. F. $C_{19}H_{19}5_4O_3S$, Calcd. (Found), N %=19.18 (19.20), FTIR (KBr, cm^{-1}): 3020 (C-N), 1642 (C=N), 1270 (C=S), 1530 (NO_2), PMR (DMSO, δ ppm, 300 MHz): 2.66-2.82 (m,8H, N-$(CH_2)_4$-N), 3.28 (s,2H,-CH_2-),7.16-8.20 (d, 9H. ArH)

2-N- phenyl piperazino methylene-4-(2',4'-dinitro phenyl)-5-mercapto-1,3,4-oxadiazole (Vb).

Yield 72 %, M.P.-120 ^{0}C, M.W. 442, M. F. $C_{19}H_{18}N_6O_5S$, Calcd. (Found), N %=19.00 (19.05), FTIR (KBr, cm^{-1}): 3000 (C-N), 1645 (C=N), 1275 (C=S), 1530 (NO_2), PMR (DMSO, δ

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ppm, 300 MHz): 2.62-2.72 (m,8H, N-$(CH_2)_4$-N), 3.18 (s,2H,-CH_2-),7.18-7.49 (d, 8H. ArH)

2-N- phenyl piperazino methylene-4-(4'-amino, 2'-nitro phenyl)-5-mercapto-1,3,4-oxadiazole (Vc).
Yield 74 %, M.P.-118 ^{0}C, M.W. 412, M. F. $C_{19}H_{20}N_6O_5S$, Calcd. (Found), N %=20.39 (20.42), FTIR (KBr, cm^{-1}): 3290 (NH_2), 2990 (C-N), 1640 (C=N), 1280 (C=S), 1530 (NO_2), PMR (DMSO, δ ppm, 300 MHz): 2.62-2.78 (m,8H, N-$(CH_2)_4$-N), 3.22 (s,2H,-CH_2-),7.18-7.45 (d, 8H. ArH)

2-N- phenyl piperazino methylene-4-(4'-amino phenyl)-5-mercapto-1,3,4-oxadiazole (Vd).
Yield 76 %, M.P.-115 ^{0}C, M.W. 367, M. F. $C_{19}H_{21}N_5OS$, Calcd. (Found), N %=19.07 (19.09), FTIR (KBr, cm^{-1}): 3310 (NH_2), 3010 (C-N), 1640 (C=N), 1275 (C=S), PMR (DMSO, δ ppm, 300 MHz): 2.63-2.78 (m,8H, N-$(CH_2)_4$-N), 3.26 (s,2H,-CH_2-),7.15-7.43 (d, 9H. ArH), 9.15 (s, 2H, NH_2)

2-N- phenyl piperazino methylene-4-(4'-phormyl phenyl)-5-mercapto-1,3,4-oxadiazole (Ve).
Yield 70 %, M.P.-120 ^{0}C, M.W. 380, M. F. $C_{20}H_{20}N_4O_2S$, Calcd. (Found), N %= 14.74 (14.76), FTIR (KBr, cm^{-1}): 3005 (C-N), 1710 (C=O), 1640 (C=N), 1280 (C=S), PMR (DMSO, δ ppm, 300 MHz): 2.64-2.80 (m,8H, N-$(CH_2)_4$-N), 3.24 (s,2H,-CH_2-),7.16-7.54 (d, 9H. ArH), 10.10 (s,1H, CHO)

2-N- phenyl piperazino methylene-4-(4'-carboxy phenyl)-5-mercapto-1,3,4-oxadiazole (Vf).
Yield 80 %, M.P.-125 ^{0}C, M.W. 396, M. F. $C_{20}H_{20}N_4O_3S$, Calcd. (Found), N %= 14.14 (14.17), FTIR (KBr, cm^{-1}): 3010 (C-N), 1690 (C=O), 1644 (C=N), 1280 (C=S), PMR (DMSO, δ ppm, 300 MHz): 2.64-2.74 (m,8H, N-$(CH_2)_4$-N), 3.20 (s,2H,-CH_2-),7.15-7.56 (d, 9H. ArH), 12.10 (s,1H, COOH)

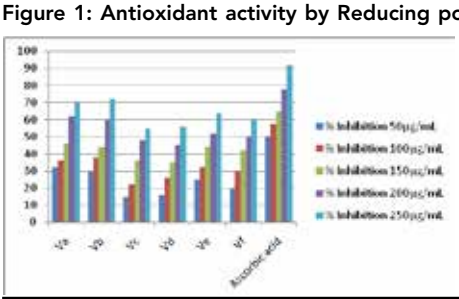
ANTIOXIDANT ACTIVITY

The antioxidant activity of the synthesized N-phenyl-piperazino 1,3,4-oxadiazole derivatives was evaluated using reducing power assay (Oyaizu et al. 1986) and hydrogen peroxide scavenging assay (Ruch et al.1984).

Reducing Power activity by $FeCl_3$

Reducing power (RP) of synthesized compounds was determined according to the method of Oyaizu. Different aliquots of the test sample and ascorbic acid as standard for comparison at concentration of 50 µg/mL, 100 µg/mL, 150 µg/mL, 200 µg/mL and 250 µg/mL were taken in different test tubes. 2.5 mL Phosphate buffer (pH 6.6) and 2.5 mL of 1% $K_3Fe(CN)_6$ were added in each test tube. Test solutions were kept for 20 minutes at 50 ^{0}C in water bath. After 20 minutes 2.5 mL 10% trichloro acetic acid was added in each test solution. An aliquot of 2.5 mL was withdrawn from each test solution and in it 2.5 mL distilled water and 1.0 mL $FeCl_3$ (0.1 %) were added. A blank was also prepared without adding the test compound. Each experiment was carried out in triplicate and mean value was calculated. Finally the antioxidant activity was evaluated by determining the absorbance at 700 nm after 10 minutes.

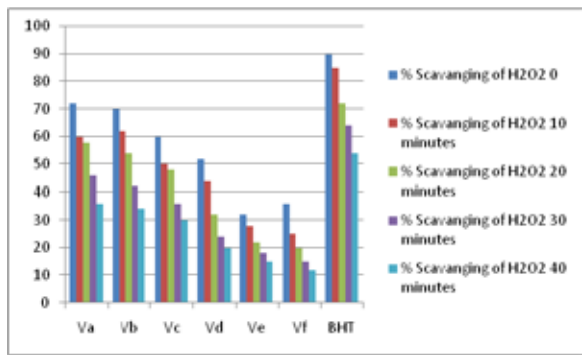
Figure 1: Antioxidant activity by Reducing power assay.



Hydrogen peroxide scavenging activity

The Hydrogen peroxide scavenging activity was determined by the reported method. Synthesized compounds were dissolved in 3.4 mL of 0.1 M phosphate buffer (7.4 p H) and mixed with 600 µL of 43 mM solution of hydrogen peroxide. The absorbance value at 230 nm of the test samples were recorded at 10 minutes intervals between 0 to 40 minutes. BHT was used as standard for comparison.

Figure 2: Antioxidant activity by Hydrogen peroxide scavenging activity.



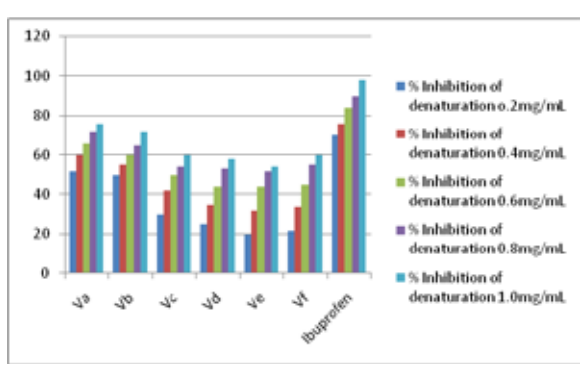
ANTI INFLAMMATORY ACTIVITY

The synthesized compounds were screened for in-vitro anti-inflammatory activity by the reported method (Elias G et.al.1988). Synthesized compounds were dissolved in DMF and diluted with phosphate buffer (0.2 M, pH 7.4) to got solution of 1mg/mL 0.8 mg/mL 0.6 mg/mL 0.4 mg/mL 0.2 mg/mL concentration. Test solution (1 mL) containing different concentrations of compounds was mixed with 1 mL of 1mg/mL albumin solution in phosphate buffer and incubated at 27 ^{0}C for 15 minutes. Denaturation was induced by keeping the reaction mixture at 60 ^{0}C in water bath for 15 minutes. After cooling, the transmittance of the turbid suspension was measured at 660 nm. The percentage inhibition of denaturation was calculated with reference to control where no drug was added and Ibuprofen was used as standard for comparison. The percentage inhibition of denaturation was calculated by using folloving formula.

% of inhibition = 100*(Vt/Vc-1)

Where, Vt =absorbance of test samople, Vc = absorbance of control.

Figure 3: Anti inflammatory activity of synthesized compounds



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RESULT AND DISCUSSION

Synthesized compounds were characterized by FTIR, ^{1}HNMR and elemental analysis. All spectral data were in good agreement with the reported peaks of IR and chemical shift in NMR for the respective groups.

The antioxidant activity in terms of reducing power shows that as the concentration of the test compounds increase there is increase in the reducing power of these derivatives. Among the six derivatives synthesized, maximum reducing potential is observed in compounds Va and Vb. However less activity is observed compared to Ascorbic acid.

The antioxidant activity in terms of hydrogen peroxide scavenging potential shows that oxidation power of the synthesized compounds decreases with increase in time. Hydrogen peroxide scavenging values of 72%, 60%, 588%, 46% and 36% were observed in a span of 0 to 40 minutes for compound Va and 70%, 62%, 54%, 42% and 34% were observed in a span of 0 to 40 minutes for compound Vb, which were maximum among the six tested compounds. It is also known that greater the oxidation power lesser is the reducing capacity. This phenomenon is evident and observed in compound Va and Vb where the oxidation potential is highest while the reducing power is least.

The anti inflammatory activity data reveal that two compounds nnamely Va and Vb show maximum % inhibition of denaturation of albumin and hence are most active towards anti inflammatory potential. Other compounds also show moderate inhibition in comparison to standard drug ibuprofen.

CONCLUSION

Microwave assisted synthesis can be used to reduce the time and increase the yield of reaction. The bioactivity results proved that synthesized N-phenyl piperazino substituted-1,3,4-oxadiazoles may be potential in exploring new anti-oxidant and anti inflammatory lead drugs.

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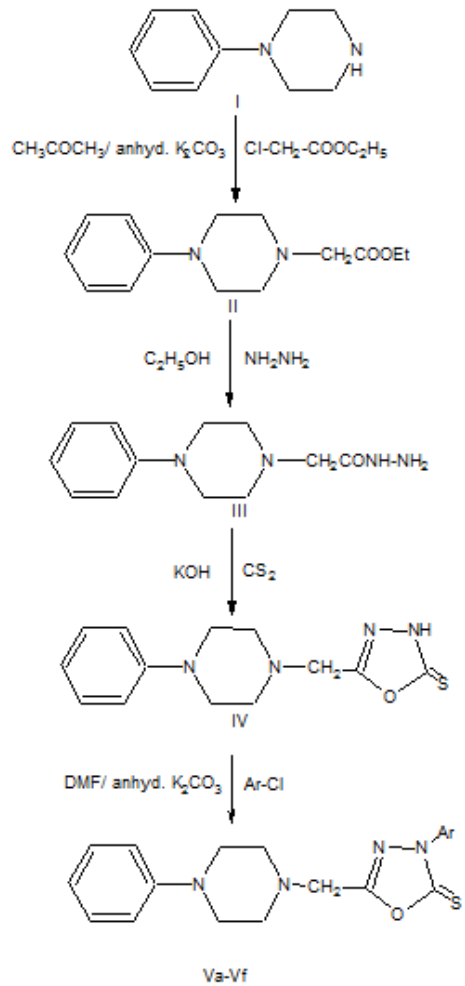
REACTION SCHEME



Ar-Cl= Cl-$C_6H_4NO_2$(p), Cl-$C_6H_3NO_2$(o,p), Cl-$C_6H_4NO_2$(p), Cl-$C_6H_3NO_2$(m),NH_2(p), Cl-$C_6H_4NO_2$(p), Cl-$C_6H_3NH_2$(p), Cl-C_6H_4CHO(p), Cl-C_6H_3COOH(p)

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