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Green and Efficient Protocol for Synthesis of Schiff Bases and Hydroxyl Derivatives of 1,3,4-Thiadiazole Containing N-Phenyl Piperazine Moiety and their Antimicrobial and Antioxidant Potential

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Abstract

A new class of thiadiazole having combination of Schiff base and Mannich base containing N-phenyl piperazine moiety was synthesized by an efficient microwave assisted green synthetic approach. 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 antbacterial activity and were characterized by FTIR, ^{1}H NMR and elemental analysis.

Keywords: Thiadiazole, Schiff bases, Mannich bases, N-phenyl piperazine, antimicrobial activity, antioxidant activity..

Introduction

Microwave assisted synthesis has proved as an efficient synthetic protocol due to enhanced rates, higher selectivity, higher purity, efficiency and higher yield. 1,3,4-Thiadiazole[1-4] and piperazine[6-8] moieties are widely exposed to therapeutic world because of their known Pharmacological importance. Free radicals are produced during oxidation of food in our body. They are capable to destroy the 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[9-11]. Thus there is a need of new class of compounds having antimicrobial and antioxidant potential. In view of above findings thiadiazole and piperazine moieties are incorporated together for evaluating its antimicrobial and antioxidant activities.

Material and Methods

Melting points were determined in open capillary tubes in a '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 was determined by ELICO SL 177 scanning mini spec.

Synthesis of 2-nitro-4-N-phenyll piperazino aniline (Ia): N-phenyl piperazine and p-chloro, o-nitroaniline were taken in equimolar ratio in benzene and were irradiated in a microwave oven for 15 minutes. After the completion of the reaction, the mixture was left to cool to room temperature. The resultant solid was filtered, dried, and recrystallized from ethanol to yield the pure product. The purity of compound was checked by running TLC.

Yield-72 %, M.P. 95 ^{0}C; M.W. 298, M.F. $C_{16}H_{18}N_4O_2$, Calcd. (Found), N %=18.79 (18.81); FTIR (KBr, cm^{-1}): 3350 (NH), 1535 (NO_2), PMR (DMSO, δ ppm, 300 MHz): 2.66-2.74 (br, 8H, N-$(CH_2)_4$-N), 7.55-7.80 (m, 8H. ArH), 9.18 (s, 2H, NH_2).

Synthesis of 2-nitro-4-N-phenyl piperazino aniline thiosemicarbazide (Ib): 0.01 mole of 2-nitro 4-N-phenyl piperazino aniline was dissolved in 20 mL of ammonia solution followed by gradual addition of 0.01 mole CS_2. 20 ml of ethanol was added and stirring was continued till all the CS_2 was dissolved. In an another beaker sodium salt of mono chloro acetic acid was prepared by dissolving sodium hydroxide and mono chloro acetic acid in equimolar ratio in minimum quantity of water to yield sodium salt of mono chloro acetic acid. The solution of the salt was then added to the reaction mixture followed by gradual addition of 10 mL hydrazine hydrate with continuous stirring. The mixture became warm which was cooled. On cooling, a solid separated out which was filtered, dried and recrystallized from ethanol.

Yield 76 %, M.P.-110^{0}C, M.W. 372, M. F. $C_{17}H_{20}N_6O_2S$, Calcd. (Found), N %=22.58 (22.56), FTIR (KBr, cm^{-1}): 3350 (NH), 1530 (NO_2), 1270 (C=S), PMR (DMSO, δ ppm, 300 MHz): 2.62-2.70 (br, 8H, N-$(CH_2)_4$-N), 5.25-5.70 (br, 3H, NH-NH_2), 7.50-7.80 (m, 8H. ArH), 9.10 (s, 1H, NH)

Synthesis of 5-(2'-nitro, 4'-N-phenyl-piperazino anilino)-2-mercapto-1,3,4-thiadiazole (Ic): 2-nitro-4-N-phenyl piperazino

aniline thiosemicarbazide (Ib) and carbon disulphide were taken in equimolar ratioin a beaker and irradiated in microwave oven in presence of DMF (5 mL) for 15 minutes. The solution was cooled and poured into ice cold water. The solid which separated out was filtered, dried and recrystallized from ethanol.

Yield 70 %, M.P. 125 ^{0}C, M.W. 414, M.F. $C_{18}H_{18}N_6O_2S_2$, Calcd. (Found), N %=20.28 (20.30), FTIR (KBr, cm^{-1}): 3360 (NH), 3230 (NH), 1530 (NO_2), 1270 (C=S), 1610 (-C=N), PMR (DMSO, δ ppm, 300 MHz): 2.66-2.72 (br, 8H, N-$(CH_2)_4$-N), 5.20 (s, 1H, NH-C=S), 7.30-7.50 (m, 8H. ArH), 9.15 (s, 1H, NH)

Synthesis of 5-(2'-nitro, 4'-N-phenyl-piperazino anilino)-2-mercapto-3-p-anisidino/morpholino/piperidino methyl-1,3,4-thiadiazole (Id, Ie, If): Equimolar ratio of 5-(2'-nitro, 4'-N-phenyl-piperazino-anilino)-2-mercapto-1,3,4-thiadiazole, p-anisidine and formaldehyde was irradiated in ethanol for 15 minutes. Solid obtained on cooling was filtered, washed with water, dried and recrystallized by ethanol to yield the Mannich base Id. Two other Mannich bases were synthesized by similar manner.

5-(2'-nitro, 4'-N-phenyl-piperazino anilino)-2-mercapto-3-p-anisidino methyl-1,3,4-thiadiazole (Id): Yield 85 %, M.P. 204-206 ^{0}C, M.W. 549, M.F. $C_{26}H_{27}N_7O_3S_2$, Calcd. (Found), N %=17.85 (17.88), FTIR (KBr, cm^{-1}): 3355 (NH), 1630 (-C=N), 1540 (NO_2), 1280 (C=S), PMR (DMSO, δ ppm, 300 MHz): 2.65-2.70 (br, 8H, N-$(CH_2)_4$-N), 3.25 (d, 2H, CH_2), 4.18 (s, 3H, OCH_3), 7.40-7.65 (m, 12H. ArH), 8.25 (t, 1H, NH), 9.12 (s, 1H, NH)

5-(2'-nitro, 4'-N-phenyl-piperazino anilino)-2-mercapto-3-morpholino methyl-1,3,4-thiadiazole (Ie): Yield 75 %, M.P. 200-202 ^{0}C, M.W. 513, M.F. $C_{23}H_{27}N_7O_3S_2$, Calcd. (Found), N %=19.10 (19.12), FTIR (KBr, cm^{-1}): 3360 (NH), 1650 (-C=N) 1540 (NO_2), 1270 (C=S), 1250 (C-O-C), PMR (DMSO, δ ppm, 300 MHz): 2.68-2.74 (br, 8H, N-$(CH_2)_4$-N), 3.22 (s, 2H, CH_2), 3.72-3.75 (br, 8H, N-$(CH_2)_4$-O) 7.40-7.60 (m, 8H. ArH), 9.12 (s, 1H, NH)

5-(2'-nitro, 4'-N-phenyl-piperazino anilino)-2-mercapto-3-piperidino methyl-1,3,4-thiadiazole (If): Yield 70 %, M.P. 199-202 ^{0}C, M.W. 511, M.F. $C_{24}H_{29}N_7O_2S_2$, Calcd. (Found), N %=19.18 (19.22), FTIR (KBr, cm^{-1}): 3360 (NH), 1640 (-C=N), 1540 (NO_2), 1280 (C=S), PMR (DMSO, δ ppm, 300 MHz): 2.50-(br, 10H, N-$(CH_2)_5$), 2.65-2.72 (br, 8H, N-$(CH_2)_4$-N), 3.20 (s, 2H, CH_2), 7.40-7.48 (m, 8H. ArH), 9.12 (s, 1H, NH),

Synthesis of 5-(2'-nitro, 4'-N-phenyl-piperazino anilino)-2-mercapto-3-p-anisidino methylene-4''-hydroxy-3''-methoxy-bezilidene-1,3,4-thiadiazole(Id_a): A mixture of 4-hydroxy,3-methoxy benzaldehyde (0.01 mole), Mannich base 5-(2'-nitro, 4'-N-phenyl-piperazino anilino)-2-mercapto-3-p-anisidino methyl-1,3,4-thiadiazole (Id) (0.01 mole) and anhydrous sodium acetate (0.02 mole) was irradiated in acetic acid for 15 minutes. After cooling solution was poured in to ice cold water and kept overnight. The resulting solid was filtered, washed with hot water, dried and recrystallized from ethanol.

Other Schiff bases are synthesized in similar manner described above.

5-(2'-nitro, 4'-N-phenyl-piperazino anilino)-2-mercapto-3-p-anisidino methylene-4''-hydroxy -3''methoxy benzilidene-1,3,4-thiadiazole (Id_a): Yield 70 %, M.P. 194-196 ^{0}C, M.W. 683, M.F. $C_{34}H_{33}N_7O_5S_2$, Calcd. (Found), N %=14.35 (14.38), FTIR (KBr, cm^{-1}): 3590 (OH), 3360 (NH), 3250 (NH), 1530 (NO_2), 1640 (-C=N), 1280 (C=S), PMR (DMSO, δ ppm, 300 MHz): 2.65-2.70 (br, 8H, N-$(CH_2)_4$-N), 3.72 (s, 1H, =CH), 4.10 (s, 3H, OCH_3), 4.18 (s, 3H, OCH_3), 7.40-7.75 (m, 15H. ArH), 8.25 (s, 1H, NH), 8.80 (s, 1H, NH), 9.18 (s, 1H, NH), 10.12 (s, 1H, OH).

5-(2'-nitro, 4'-N-phenyl-piperazino anilino)-2-mercapto-3-p-anisidino methylene-4''-hydroxy benzilidene-1,3,4-thiadiazole (Id_b): Yield 75 %, M.P. 198-200 ^{0}C, M.W. 667, M.F.$C_{34}H_{33}N_7O_4S_2$, Calcd. (Found), N %=14.69 (14.71), FTIR (KBr, cm^{-1}): 3580 (OH), 3350 (NH), 1530 (NO_2), 1270 (C=S), 1640 (-C=N), PMR (DMSO, δ ppm, 300 MHz): 2.65-2.73 (br, 8H, N-$(CH_2)_4$-N), 4.10 (s, 3H, OCH_3), 7.55-7.85 (m, 16H. ArH), 8.15 (s, 1H, NH), 9.15 (s, 1H, NH), 10.15 (s, 1H, OH).

5-(2'-nitro, 4'-N-phenyl-piperazino anilino)-2-mercapto-3-morpholino methylene-4''-hydroxy 3''methoxy benzilidene-1,3,4-thiadiazole (Ie_a).: Yield 80 %, M.P. 180-184 ^{0}C, M.W. 647, M.F. $C_{31}H_{33}N_7O_5S_2$, Calcd. (Found), N %=15.15 (15.15), FTIR (KBr, cm^{-1}): 3570 (OH), 3350 (NH), 1540 (NO_2), 1630 (-C=N), 1280 (C=S), 1250 (C-O-C), PMR (DMSO, δ ppm, 300 MHz): 2.62-2.70 (br, 8H, N-$(CH_2)_4$-N), 3.72-3.74 (br, 8H, N-$(CH_2)_4$-O), 3.82 (s, 1H, =CH), 4.12 (s, 3H, OCH_3), 7.34-7.40 (m, 11H. ArH), 9.15 (s, 1H, NH), 10.18 (s, 1H, OH).

5-(2'-nitro, 4'-N-phenyl-piperazino anilino)-2-mercapto-3-morpholino methylene-4''-hydroxy benzilidene-1,3,4-thiadiazole (Ie_b): Yield 75 %, M.P. 190-192 ^{0}C, M.W. 631, M.F. $C_{31}H_{33}N_7O_4S_2$, Calcd. (Found), N %=15.53 (15.56), FTIR (KBr, cm^{-1}): 3580 (OH), 3380 (NH), 1640 (-C=N), 1530 (NO_2), 1280 (C=S), 1260 (C-O-C), PMR (DMSO, δ ppm, 300 MHz): 2.20 (s, 3H, $-CH_3$), 2.62 (br, 4H, CH_3-N$(CH_2)_2$), 2.70 (br, 4H, Ar-N$(CH_2)_2$), 3.70-3.76 (br, 8H, N-$(CH_2)_4$-O), 7.25-7.35 (m, 7H. ArH), 9.10 (s, 1H, NH), 10.12 (s, 1H, OH).

5-(2'-nitro, 4'-N-phenyl-piperazino anilino)-2-mercapto-3-piperidino methylene-4''-hydroxy -3''methoxy- benzilidene-1,3,4-thiadiazole (If_a): Yield 82 %, M.P. 192-194 ^{0}C, M.W. 645, M.F. $C_{32}H_{35}N_7O_4S_2$, Calcd. (Found), N %=15.19 (15.21), FTIR (KBr, cm^{-1}): 3580 (OH), 3360 (NH), 1640 (-C=N), 1530 (NO_2), 1270 (C=S), , PMR (DMSO, δ ppm, 300 MHz): 2.50- (br, 10H, N-$(CH_2)_5$), 2.64 (br, 4H, CH_3-N$(CH_2)_2$), 2.70 (br, 4H, Ar-N$(CH_2)_2$), 3.70 (s, 1H, =CH), 4.10 (s, 3H, OCH_3), 7.30-7.44 (m, 6H. ArH), 9.15 (s, 1H, NH), 10.18 (s, 1H, OH).

5-(2'-nitro, 4'-N-phenyl-piperazino anilino)-2-mercapto-3-piperidino methylene-4''-hydroxy methyl benzilidene-1,3,4-thiadiazole (If_b): Yield 70 %, M.P. 202-204 ^{0}C, M.W. 629, M.F. $C_{32}H_{35}N_7O_3S_2$, Calcd. (Found), N %=15.58 (15.59), FTIR (KBr, cm^{-1}): 3580 (OH), 3370 (NH), 1630 (-C=N), 1530 (NO_2), 1280 (C=S), PMR (DMSO, δ ppm, 300 MHz): 2.22 (s, 3H, $-CH_3$), 2.52- (br, 10H, N-$(CH_2)_5$), 2.64 (br, 4H, CH_3-N$(CH_2)_2$), 2.69 (br, 4H, Ar-N$(CH_2)_2$), 7.45-7.58 (m, 7H. ArH), 9.10 (s, 1H, NH), 10.12 (s, 1H, OH)

Antioxidant activity: All the synthesized compounds were screened for antioxidant potential *in vitro* by Reducing power activity and Hydrogen peroxide-scavenging activity.

Reducing Power activity by $FeCl_3$: Reducing power (RP) of synthesized compounds was determined according to the method of Oyaizu[12]. 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. The results are shown as bar diagram (figure 2).

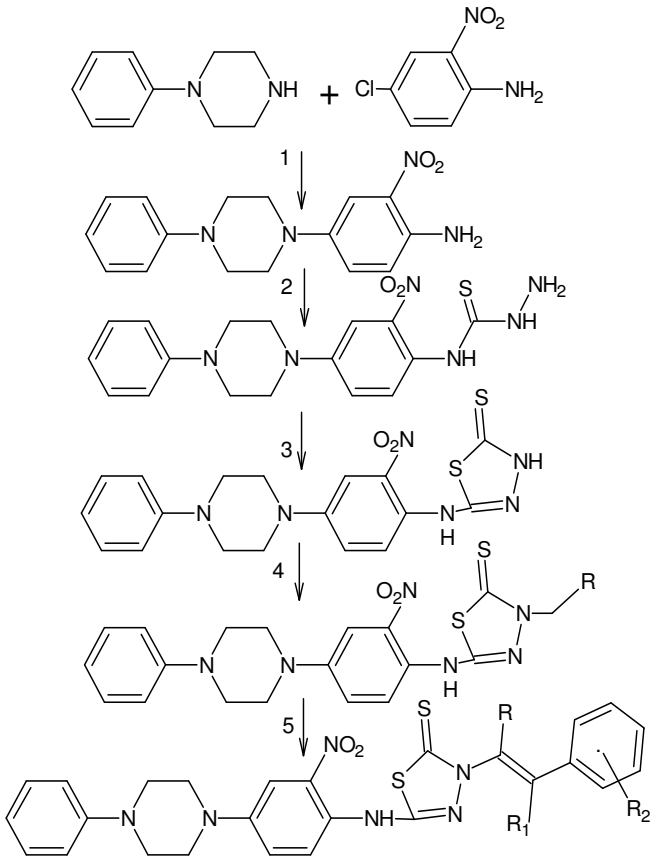
Hydrogen peroxide scavenging activity: The Hydrogen peroxide scavenging activity was determined by the method of Ruch et al.[13] The synthesized compounds were dissolved in 3.4 mL of 0.1 M phosphate buffer (7.4 p H) and mixed with 600 µL of 43 mM solution of hydrogen peroxide. The absorbance value at 230 nm of the test samples were recorded at 10 minutes intervals between 0 to 40 minutes. BHT was used as standard for comparison. The results are shown as bar diagram (figure 3).

Antibacterial activity: The antimicrobial activity of all the synthesized Schiff bases were examined against *S.aureus*, *E.coli, K.pneumoniae* bacterial strains by measuring the zone of inhibition. The antibacterial activity was performed by Agar well diffusion method[14] at a concentration level of 250µg/mL and 500µg/mL following reported procedure. Streptomycin was used as the standard at the same concentration levels. Nutrient agar was used as the culture media for antibacterial activity and DMF was used as control. The results are shown as bar diagram (figure 4).

Results and Discussion

Antioxidant activity: 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 compound Ie_a. 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 70%, 50%, 48%, 36% and 26% was observed in a span of 0 to 40 minutes for compound Ie_a, which were highest 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 Ie_a where the oxidation potential is highest while the reducing power is least.

Antibacterial activity: Among the tested compounds two compound namely Ida and Iea inhibit the growth of all the three tested microbes at higher concentration significantly, while the other synthesized compounds has shown moderate inhibition in comparison of standard drug Steptomysin.



1. Benzene. 2. CS_2, NH_2-NH_2 and $ClCH_2COONa$ in NH_3. 3. CS_2 in DMF. 4. HCHO and Substituted amines in ethanol. 5. Carbonyl compound and anhydrous sodium acetate in acetic acid. R = anisidino/ morpholino/ piperidino, R_1= H, CH_3, R_2 = 4-hydoxy,3-methoxy-benzilidene, 4-hydroxy-benzilide

Figure-1
Systematic reaction scheme of synthesized compounds

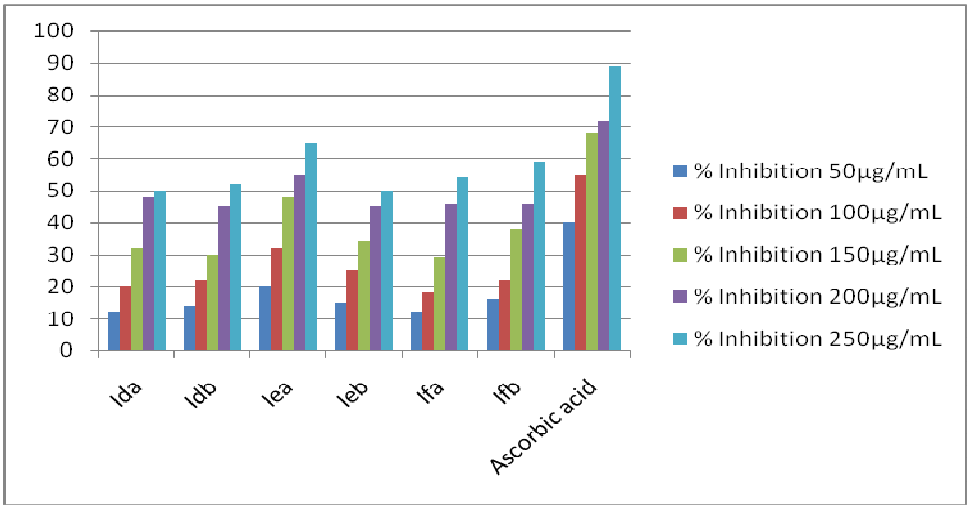


Figure-2
Antioxidant activity by reducing power assay

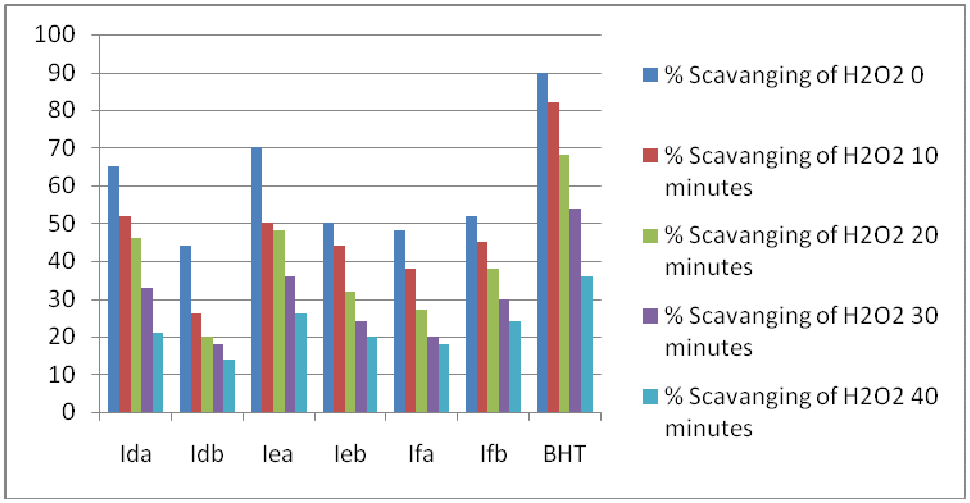


Figure-3
Antioxidant activity by Hydrogen peroxide scavenging activity

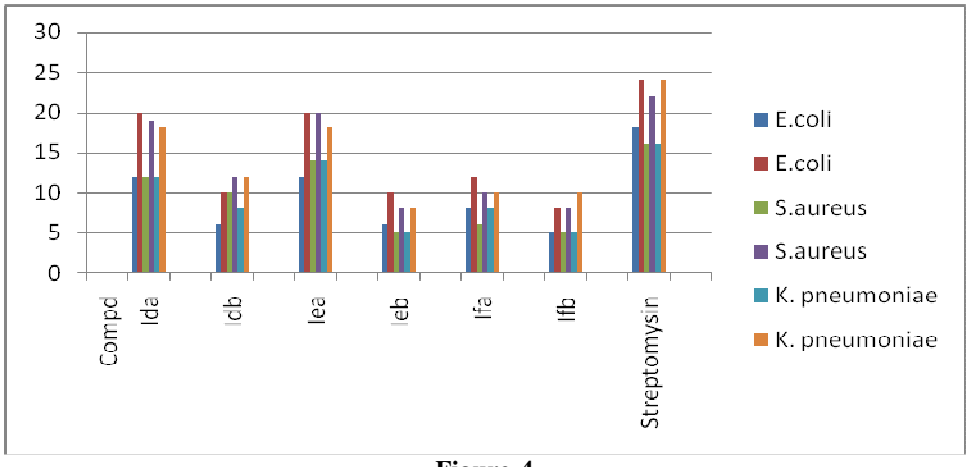


Figure-4
Antibacterial activity of synthesized compounds

Conclusion

Microwave assisted synthesis can be used to reduce the time and increase the yield of reaction. The bioactivity results proved that synthesized Schiff bases can be used for the treatment of diseases caused by microbes and compounds may be potential in exploring new anti-oxidant lead drugs.

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