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Synthesis and Biological Evaluation of Hydrazide based Sulfonamides

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Abstract

The purpose of present research is to synthesize hydrazide based sulfonamides due to their extraordinary potential in biology and medicine. The characterization of synthesized compounds was done by melting points, elemental analysis and spectroscopic techniques like I.R. Finally their antioxidant, enzyme inhibition and antimicrobial activity have been done. Sulfonamide (3a) shows maximum enzyme inhibition activity, similarly this sulfonamides shows maximum ferric reducing antioxidant power and free radical scavenging activity. Moreover, Synthesized sulfonamides 3a has antibacterial against *E. coli* and *Candida albicans* while compounds 3b shows antibacterial against *Typhimusium Salmonella* and *Staphlococcus aureus* furthermore 3c shows antibaceterial against *Bacillus Subtilis*, *Staphlococcus aureus* and *Candida albicans*.

Keywords: Sulfonamides, Antimicrobial Activity, Antioxidant Assay, FRAP Assay, *E. coli*, Lipoxygenase Enzyme Inhibition

Introduction

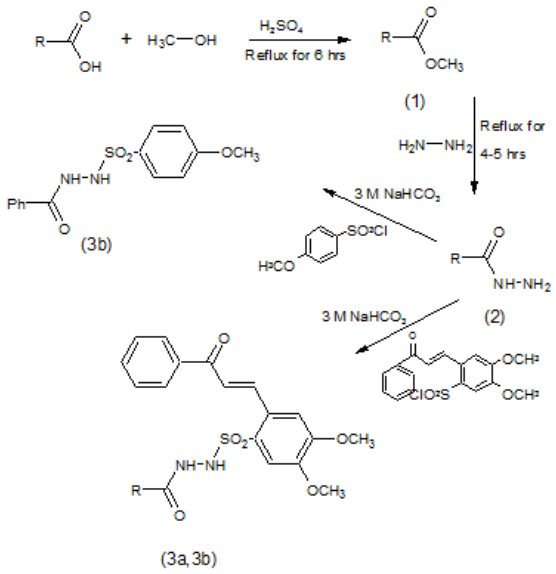
Sulfonamides having functional group (R-SO2-NH2) called sulfonamide group are compounds having potential of antimicrobial activity. They have their familiarity as amide derivatives of sulfonic acid because they are synthesized by introduction of amino group in sulfonic acid after replacing its hydroxyl group. With physical appearance of white colour they are feebly acidic and form water soluble salts with bases. Their sodium salts show PH on higher side and damaging of tissue results from this prominent alkaline behavior, however there are few exceptions.[1] There are compounds present which are not having antimicrobial nature but contain sulfonamide group. Sulthaime, benzothiazide and sulfonyl urease may be presented as examples which are anticonvulsant, diuretic and ant diabetic respectively.[2] From medical point of view sulfonamides have also another recognition as sulfa drugs which are used to treat bacterial type infections as antibiotics in both animals as well as human beings.[3, 4]

The involvement of agriculture and pharma in enjoying various biological activities of sulfonamides is also producing both attention and attraction.[5] Sulfonamides are the main functional portion of many structures of drugs principally because of its stability as well as tolerance in humans.[6, 7] Sulfa drugs are used for treatment of gut infections, conjunctivitis, urinary tract infections, in meningitis, eye lotions, bacillary dysentery, malaria.[8-10]

In the present work we synthesized hydrazide based sulfonamides.

First ester ($RCOOCH_3$) was prepared by reacting substituted aromatic carboxylic acid with methanol. Further this ester was reacted with hydrazine hydrate to get substituted aromatic hydrazides ($RCONHNH_2$); substituted aromatic hydrazides were condensed with Benzohydrazide to get the target compounds. The mode of reaction was shown in scheme no.1. The proposed product was confirmed by Elemental analysis and IR spectrum.

All different samples of sulfonamides were checked for their enzyme inhibition potential, antioxidant activity and antimicrobial activity. Results were found in variable mode.



Where R = Phenyl, 4-nitro Phenyl

Material and Methods

The different instruments, techniques, glass wares, solvents, reagents and methods used in the synthesis of hydrazides and hydrazides based compounds are as follows:

Instruments

- FTIR Spectrometer (MIDAC M 2000)
- Elemental Analyzer (Elementar, Vario micro cube, Germany)
- Microscope
- U.V lamp
- Melting point apparatus (Gallen kemp)
- Rotary evaporator

Glass Apparatus

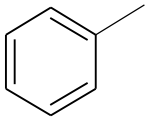
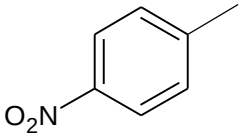
- Round Bottom Flasks of 25 mL, 50 mL and 100 L (Pyrex Made)
- Reflux Condensers
- Micro pipette of 0.1 mL graduation and Macro Pipettes of 1 mL and 5 mL and 10 mL
- Glass Vials
- Beakers

Experimental Work

Synthesis of Substituted Aromatic Ester

About 100 mL of dry methanol allowed reacting with 10 grams of substituted aromatic carboxylic acid in the presence of 2-3 mL conc. H_2SO_4. The mixture was refluxed for 6 hours in round bottom flask. After that evaporate the extra solvent from rotatory evaporator, neutralize the mixture with 3M Na_2CO_3 solution. Extracted the Substituted Aromatic Ester with the help of ether and then evaporate the ether from rotatory evaporator to get the pure product. Process monitored by TLC. Following esters were synthesized (Table 1) -

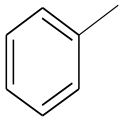
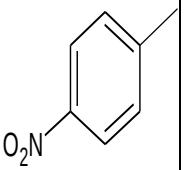
Table 1:

R in Ester	Physical State	% age yield
	Colorless liquid	78 %
	Yellow solid	75 %

Synthesis of Aromatic Hydrazides Substituted

1 mmol of aromatic ester was dissolved in 25 cm3 of dry methanol in 100 cm3 round bottom flask. To the clear solution was then added 2.5 mmol of hydrazine (NH2NH2.H2O). After a reflux of at least 4 hours of reaction mixture, cold water was added to precipitate out the product. After that product was filtered, dried and recrystallized from methanol. Below mentioned hydrazide were synthesized (Table 2).

Table 2:

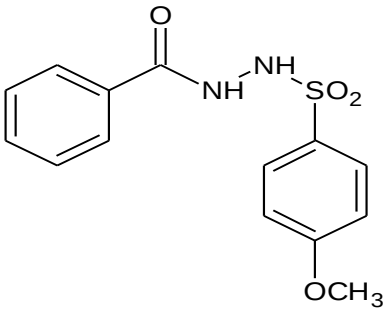
R in Hydrazides	Physical State	Melting Point	% age yield
	White Flaky needles	113 °C	74 %
	Yellow crystalline powder	210 °C	68 %

Synthesis of Substituted Aromatic Hydrazides base Sulfonamides

Benzoyl-4, 5-dimethoxy-2-(3-oxo-3-phenlyporp-1-en-1-yl) benzene sulfonohydrazide (3a)

1 mmol of Benzohydrazide was dissolved in 5 mL dichloromethane taken in 25 ml round bottom flask. Then 3M NaHCO3 and 1 mmol 4, 5-dimethoxy-2-[(1E)-3-oxo-3-phenylprop-1-en-1-yl] benzenesulfonyl chloride were added in the round bottom flask. The reaction mixture was stirred for 5-6 hours at room temperature. On precipitation, sulfonamide was filtered and dried.

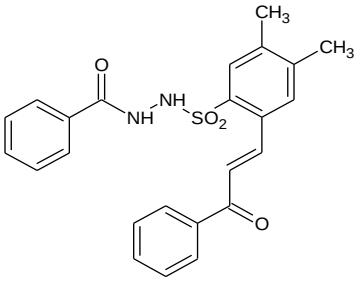
Product was purified by washing with 1M HCL solution and n-hexane respectively.



(3 a)

4- Methoxyphenyl) sulfonyl Benzohydrazide (3b)

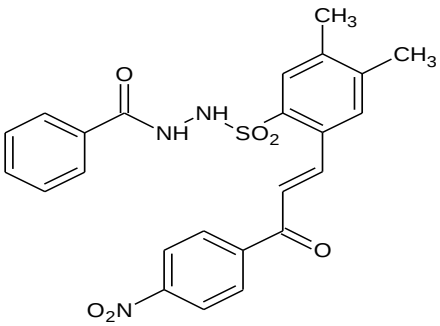
The synthesis of this hydrazide based sulfonamide was carried out by stirring dichloromethane based solution, para methoxy benzene sulfonyl chloride and hydrazide solution in 3 M NaHCO3. The stirring was carried out for at least four hours at room temperature. The white precipitate appeared was separated and washed using 1 M HCl and n-hexane to remove traces of impurities.



(3 b)

Benzoyl-4, 5-dimethoxy-2-(3-(4-nitrophenyl)-3-oxoprop-1-en) benzenesulfonohydrazide (3c)

This hydrazide based sulfonamide was synthesized by just stirring dichloromethane based solution of aryl sufonyl chloride in solution of 4-nitrobenzoyl hydrazide in 3 M NaHCO3 for six hours at room temperature. Precipitate of yellow color appeared which was filtered and washed with HCl and h-hexane to remove the traces of impurities



(3 c)

Results and Discussion

The Hydrazide base sulfonamides are soluble in common organic solvents. The analytical data and physical properties of the Substituted Aromatic Hydrazides base Sulfonamides are listed in Table No. 3

Table 3:

Compound	M.p. °C	Rf Value	% age Yield	Molecular Formula	IR spectrum	Elemental Analyzer
3a	76°C	0.57	76 %	$C_{24}H_{22}N_2O_4S$	3300 N–H St, 2935 C–H St, 1692 C=0, 1500 C=C, 1087, 1141 SO_2 sym and anti sym,	C 59.19% H 4.68% N 5.99% S 6.8%
3b	170°C	0.48	68%	$C_{14}H_{14}N_2O_4S$	3384 N–H St, 2946 C–H Str, 1661 C=O St, 1076, 1169, SO2 sym and anti sym., 1258 C–O Str	C 54.90% H 4.57% N 9.15% S 10.49%
3c	176°C	0.39	73%	$C_{24}H_{21}N_3O_6S$	3069 N–H Str, 2934, 2856 C–H Str, 1714 C=O, 1449, 1515 C=C Str, 1145 SO2 , 1280 C–O Str, C–N (NO2) 863	C 75.5% H 2.9% N 2.7% S 6.24%

Enzyme Inhibition and Antioxidant Assays

3All hydrazide bases sulfonamides were checked for their potential against enzyme inhibition and antioxidant activity. The results of different assays were indicating potential as evident from tables and graphs.

Enzyme Inhibition

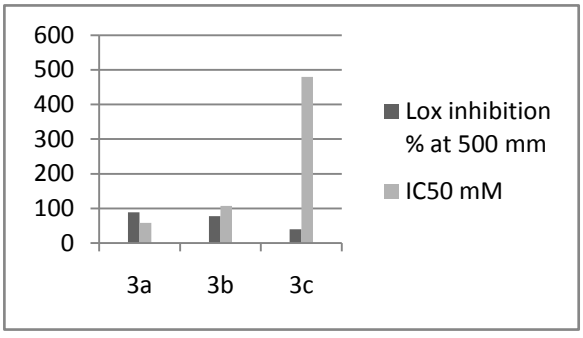
Lipoxygenase Inhibition Assay

The entire chemical which includes linoleic acid and lipoxygenase enzyme (Ec1.13.11.12) were obtained from Sigma (St. Louis, Missouri, USA). In each well of 96 well plate was added 160 ul of sodium phosphate buffer (PH = 8.0 and conc. 100mM) and 10 ul of testing synthetic compound solution in methanol (Conc. 5-500 uM).

For ten minutes 20 uL of Lipoxygenase (LOX) solution (enzyme130 units per well) was added, mixed and incubated at 25^0C. The reaction then was started by 10 ul addition of substrate solution in each well. The substrate solution is linoleic acid 0.5 mM and 0.12% W/V tween - 20 in ratio of 1:2. With the formation of (9Z, 11 E) - 13s) - 13 hydroperoxyo-cta" deca - 9, 11- dienoate the absorption changed and after 15 min was measured at 234 nm. As a standard Baicalein was used and IC50 values were determined by EZ-fit enzyme Kinetic program (pallera Scientific Inc. Amherst, USA). The result of LOX inhibition shown in Table No. 4

Table 4:

Hydrazides base Sulfonamides	LOX inhibition % at 500 μm	IC_{50} μM
3a	89	58.0
3b	78	107.1
3c	40	480



Antioxidant Activity

ABTS Radical Scavenging

ABTS assay is one of different spectrophotometric methods employed to measure the antiosidant activity of solution of pure compound.

The basis of method is generation of ABTS (2, 2 - azinobis (3- ethylbenzothiaziline-6 sulphonic acid) radical cation. The earliar ABTS assay used was based on activation of metmyoglobin with hydrogen peroxide but was criticized on the fact that reduction of ferryl myoglobin radical can also by done by rapidly reacting antioxidants. A suitable way for assay is decolourization process where the radical is produced in stable form.

ABTS radical is generated on reaction of ABTS (7mM) and potassium persulphate (2.5 mM). For 12-16 hours before use at room temperature the mixture was allowed to stand in dark. The stock solution of ABTS was dilute to an absorbance of 0.70 + 0.02 at 734 nm with PBS buffer (PH 7.4). To 2.99 ml of diluted soln. of ABTS radical cation, was added 10 µL of standard antioxidant or sample. Exactly after one minute interual for six minutes the absorbance was noted at 30 ^{0}C. With following formula the percentage inhibition of absorbance was calculated

$$\text{Inhibition \%} = \left(1 - \frac{A}{Ao}\right) \times 100$$

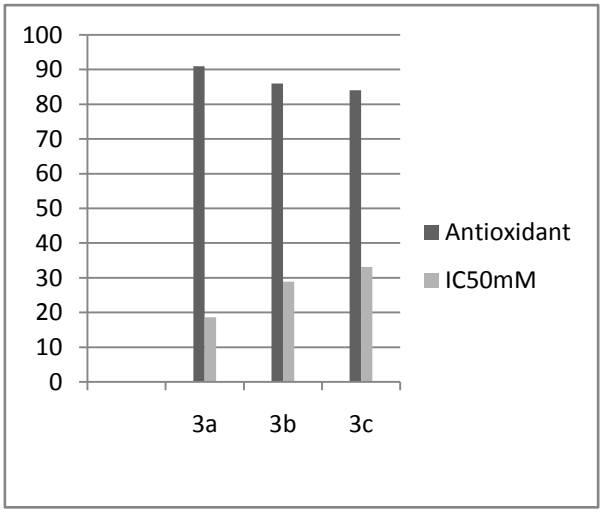
A is the absorbance of mixture with sample at 734 nm.

Ao is the absorbance of mixture without sample at 734nm.

The antioxidant activity results of different hydrazide based sulfonamides are shown in table No. 5.

Table 5:

Hydrazides base Sulfonamides	Antioxidant DPPH % at 500 µM	IC_{50}µM
3a	91	18.6
3b	86	28.9
3c	84	33.1



FRAP ASSAY

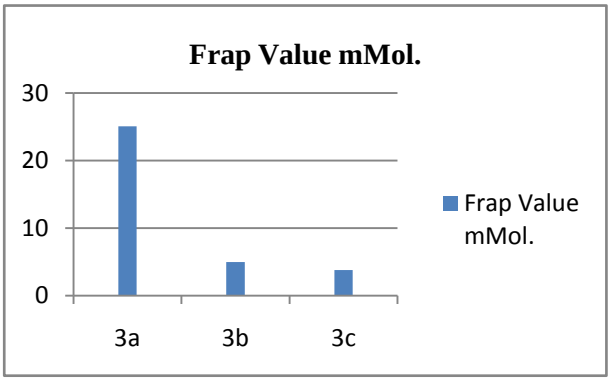
FRAP assay is simple test of determining antioxidant power by measuring the ferric reducing ability of compounds. Reduction of Fe+3 to Fe+2 at low PH results in a colored complex of ferrous-tripyridyltriazine. By comparison of absorbance changes in test reaction mixture and those having Fe+2 in known concentrations at 593 nm FRAP values are obtained. When Ferric-tripyridyltriazine is reduced to ferrous form at low PH an intense blue colour develops at 593 nm.

Freshly prepared FRAP soln. was prepared by mixing 2.5 ml of 10 mM TPTZ solution in 40mM HCL solution, 25 mL of 300 mM acetate buffer (PH3.6) and 2.5 mL of 20mM ferric Chloride solution. The incubation of mixture was done at 37^{0}C. 100 µl of sample, 300 µl of distilled water and 3000ul of FRAP solution were mixed and absorbance noted after 6 min at 593 nm.

$A = \frac{A_o}{0.025}$ A_o is absorbance at 593 nm

Frap value of different hydrazides based sulfonamides are shown in table No. 6

Hydrazides base Sulfonamides	Frap Value mMol.
3a	25.08
3b	4.96
3c	3.792



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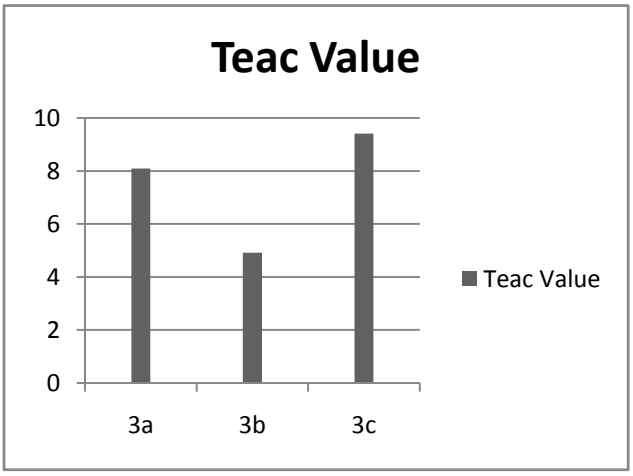
$$\text{Inhibition \%} = \left(1 - \frac{A}{A_o}\right) \times 100$$

A is the absorbance of mixture with sample at 734 nm. Ao is the absorbance of mixture without sample at 734nm.

TEAC value of Hydrazides base Sulfonamides are shown in Table No. 7

Table 7:

Hydrazides base Sulfonamides	TEAC Value
3a	8.09
3b	4.92
3c	9.41



Antimicrobial activity evaluation

All the synthesized compounds i.e; hydrazide based sulfonamides were analyzed for checking their power against

a) Gram positive bacteria

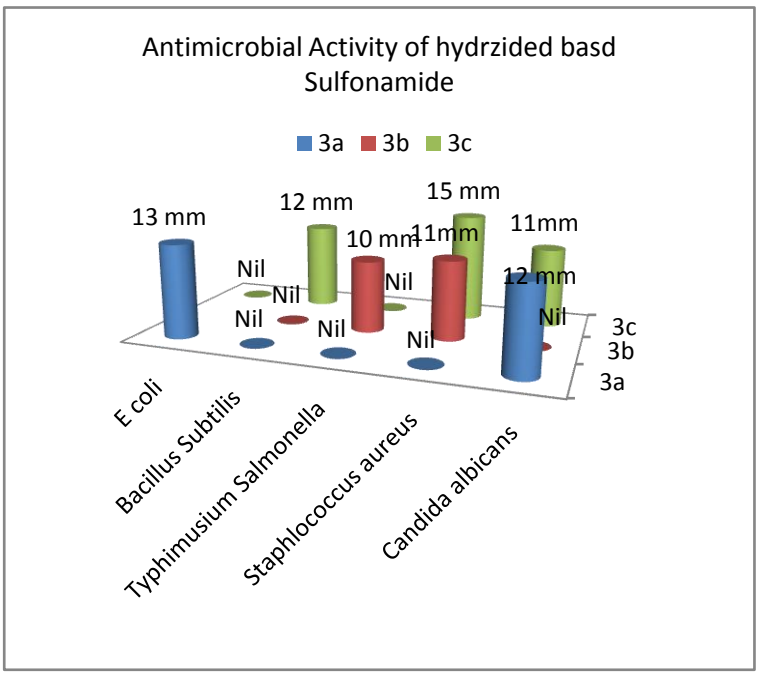
b) Gram negative bacteria

c) Fungus

Agar well method and zone of inhibition mode were used to check the potential. Sample size was 20 µl and solutions were prepared in DMSO. The study indicated different response to different strains but clearly on focal point was that they have some abilities against microbes. The results are indicated in table No. 8

Table 8:

Hydrazides base Sulfonamides	E coli	Bacillus Subtilis	Typhimusium Salmonella	Staphlococcus aureus	Candida albicans
3a	13mm	Nil	Nil	Nil	12mm
3b	Nil	Nil	10mm	11mm	Nil
3c	Nil	12mm	Nil	15mm	11mm



Conclusion

3a and 3b hydrazide based sulfonamides have antioxidant activity. And these synthesized sulfonamides also indicates enzyme inhibition activity against Lox enzymes orderly 3a<3b<3c. Moreover this compound shows antibacterial spectrum best for E coli, Bacillus Subtilis, Typhimusium Salmonella, Staphlococcus aureus and Candida albicans

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