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Synthesis of Novel Nitro Substituted Benzothiazole Derivatives and Antifungal Activity against *Aspergillus niger*

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ABSTRACT: Most alarming of all are the infection that caused by *Aspergillus (A) niger*, it was reported that resistance is developing for all currently available drugs; hence, current trends suggested that there is a requirement to develop a new replacement drug which is effective against resistant fungi having lesser toxicity as well as economical also. In view of the requirement benzothiazole nucleus containing compounds bearing nitro group substitution planned to synthesize and evaluate for antifungal activity against *A. niger*. C-6 nitro-substituted benzothiazole derivatives were synthesized by reaction of 3-chloro-4-nitroaniline with potassium thiocyanate under temperature control and presence of bromine in glacial acetic acid and ammonia. Substituted nitrobenzamides then synthesized by condensation of, 7-chloro-6-nitro-1,3-benzothiazol-2-amine with 2(3or4)-nitrobenzoylchloride acid in presence of dry pyridine and acetone. Finally, newly synthesized derivatives (N-01 to N-09) were synthesized through replacing of chlorine of nitrobenzamide by reaction with 2-nitroaniline, 3-nitroaniline, and 4-nitroaniline in presence of DMF. Analytical characterization was performed by TLC, melting point, IR and NMR spectroscopy. Antifungal activity was performed against *A. niger* by cup plate method (diffusion technique) using griseofulvin as standard. Compound N-03 showed potent antifungal activity against *A. niger* while compound N-05, N-08 and N-09 showed moderate inhibitory activity at both concentrations 50µg/ml and 100µg/ml as compared to standard. In the present work efforts have been made to synthesized C-6 substituted nitro benzothiazole derivatives and screened for antifungal activity. Compound N-03 found as most active against *A. niger*.

Keywords: Benzothiazole; Nitro-benzothiazole; Antibacterial activity; 2-substituted benzothiazole; Benzothiazole synthesis and *Aspergillus niger*.

INTRODUCTION: On the basis of the colour of the conidiospores, in 1965 Raper and Fennel divided the genus *Aspergillus* into *A. niger groups (e.g. A. carbonarius, A. japonicus, A. ellipticus, A. heteromorphus and A. aculeatus*) as brown to black-shaded spores and this group of species is often together called *A. niger van Tieghem*. Most of the brown to black Aspergilli belong to the other group of species, which are difficult to distinguish: *A. ficuum, A. phoenicis, A. niger and A. awamori* being the most prominent. *A. niger* been able to colonise the human body and cause infection e.g. lung infections, ear infections (otomycosis) may be caused by mechanical damage of the skin barrier even it was also reported that *A. nigeris* mainly responsible and consider for the most common and deadly pulmonary fungal infection worldwide through forming a dense colony of filaments embedded in a polymeric extracellular matrix in lugs. Fungi of the genus *Aspergillus* are ubiquitous saprophytic, and widespread presence in the environment, hundreds of *Aspergillus spp*. inhaled may by the average person per day.[1] Mucociliary clearance and phagocytosis by alveolar macrophages, basically involved removing *Aspergillus spp.* from respiratory tract, while polymorphonuclear neutrocytes cleared germinating spores and hyphae through degranulation and the release of oxidants.[2] *Aspergillus spp.* are capable to colonize in respiratory tract, even presence of these effective clearance mechanisms in the body for the elimination of inhaled fungi from the respiratory tracts of healthy individuals. The main target site of colonizing of *Aspergillus spp.* is injured lung tissue and epithelia. Although such colonization often has no clinical consequences, *Aspergillus spp.* can cause a variety of clinical manifestations depending on the immune status of the host.[3] Since the discovery of antifungal drugs has substantially reduced the threats posed by infectious diseases caused by the fungus but it was found that most alarming of all are diseases where resistance is developing for all currently avail-

able drugs. Over the years, antimicrobial has saved the lives and eased the suffering of millions of people. But today's main concern is the emergence and spreads of microbes those are resistant to economic and effective first-line drugs.[4] The fungal infections which contribute most to human diseases are also those in which emerging and fungal resistance are most evident so, there is a requirement to develop new replacement drug immediately which is effective against resistant bacteria having lesser toxicity as well as economical also. In recent years heterocyclic compounds analogues and derivatives especially benzothiazole is among the usually occurring heterocyclic nuclei known to exhibit a wide range of biological.[5-16] During recent years there have been a large number of therapeutic agents are synthesized with the help of benzothiazole nucleus and consider for special significance in the field of medicinal chemistry due to their remarkable pharmacological potentialities because it is used as a starting material for the synthesis of larger, usually bioactive structures to combat resistance developed against currently available drugs.[17-20] The present work concerned with the synthesis of nitro-substituted benzothiazole derivatives followed by the antifungal activity to established structure-activity relationship.

MATERIAL AND METHODS:

Synthesis of substituted benzothiazole (Compound Code 1-NB): Synthesis of substituted benzothiazole nucleus was achieved by adding 8g (0.08mol) of potassium thiocyanate and 1.45g (0.01 mol) of 3-choloro-4-methoxy-aniline into 20 ml cooled glacial acetic acid in such a way that the temperature not exceeded above room temperature. Freezing mixture of ice and salt was used to control the temperature of reaction with continuous mechanical stirring. Again temperature control was maintained during the addition of a solution of 1.6ml of bromine in 6ml of glacial acetic acid using dropping funnel. The time of addition of bromine also considered to take around 105 minute to control temperature. During the addition of bromine, temperature was controlled to never rise beyond the room. As the addition of bromine was completed the solution stirred for 2 hours but below room temperature. After that solution was again stirred at room temperature for 10 hours and allowed to stand overnight to get precipitate followed by heating at 85^0C on a steam bath after addition of 6ml water and filtered hot (Filtrate-01). In the resulting precipitate 10ml of glacial acetic acid was added and heated with at 85^0C and filtered hot (Filtrate 2). Finally, both filtrate combined and cooled at room temperature followed by neutralization with concentrated ammonia solution to pH-6 to get precipitate. The resulting product treated with animal charcoal and recrystalized from benzene, ethanol of (1:1) to get substituted benzothiazole.

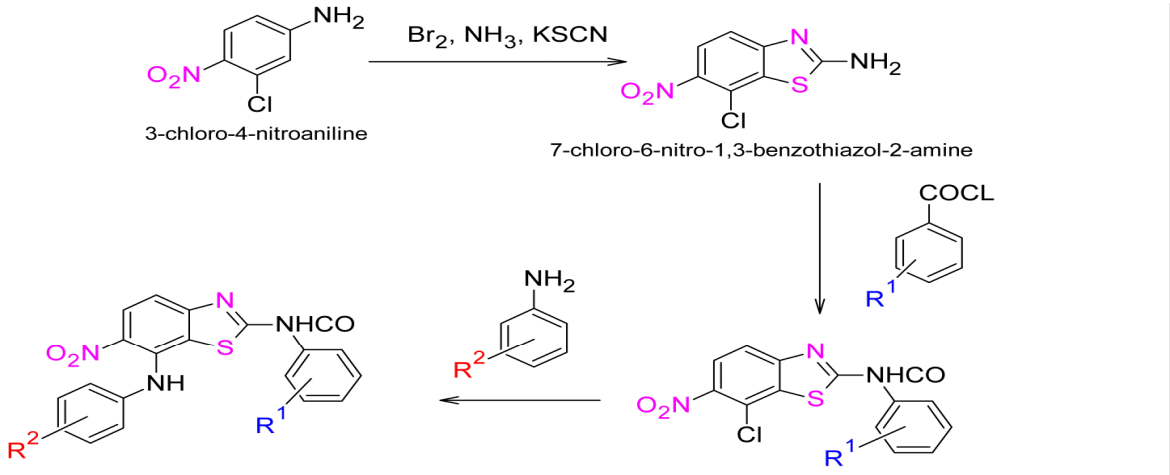
Synthesis of nitrobenzamide (Compound code 2-NB, 3-NB, and 4-NB): 5.36g (0.026mol) of 2-(3 or 4)-nitrobenzoylchloride was dissolved in dry acetone. Product 1-NB separately dissolved in dry pyridine and added drop wise into the solution of 2-(3 or 4)-nitrobenzoylchloride with continuous stirring at room temperature. After complete addition stirring was continued for another 30 minutes then transferred into 200 ml ice cold water. Finally recrystalized with ethanol to get intermediate nitrobenzamide compound 2-NB, 3-NB and 4-NB.

Synthesis of compound N-01 to N-09: 0.008 mol of 2 (3 or 4) nitro-substituted aniline was refluxed with 2.7g (0.0075 mol) of compound 2-NB, 3-NB and 4-NB separately for 2hrs in the presence of DMF. After 2 hrs reflux, mixture cooled at room temperature and poured into crushed ice. The solid was separated, dried and recrystalized with super dry alcohol to get novel benzothiazole derivatives N-01 to N-09 (Figure 1).

Analytical Characterization: Thin layer chromatography (TLC) was used to monitor reaction progress, completion and identification of newly synthesized compounds from starting material using solvent system butanol: ethyl acetate: benzene [1:2:1] and detection performed by exposing them to iodine vapours. The melting point of compounds was determined using open capillaries method. Structure elucidation of compounds was done by IR and ^{1}HNMR spectral study. SHIMADZU (8400S) used for IR spectral study (KBr pellet technique). For the structure elucidation using IR, frequency range for Ar-C=C, C=O, C-S, C-NO_2 were considered. Bruker AM 400 ^{1}H NMR instrument (at 400 MHz) was used using $CDCl_3$ as a solvent and tetramethoxysilane (TMS) as an internal standard. For structure elucidation by ^{1}HNMR, NH proton that characterized benzothiazole was considered.

Antibacterial activity against *Aspergillus niger* using griseofulvin as standard: The synthesized compounds are screened against selected fungal strains *A. niger* by using diffusion method and griseofulvin as a standard drug. Under the aseptic condition, 48 hours old fungal culture was inoculated into the nutrient broth and incubated for 48 hours at 37±2ºC in an incubator. Potato-dextrose agar media (20%) mixed with inoculated culture and poured into

petriplates. Five bores are made at an equal distance by using sterile steel cork borer (8 mm in diameter) after solidification. Different concentrations (50µg/ml and 100µg/ml) of standard drug and synthesized compounds along with control introduced in these plates and placed in a refrigerator at 8-10ºC as cold incubation for two hours that allowed proper diffusion of the drug and synthesized compounds. The petriplates were transferred to the incubator and maintained at 37± 2ºC for 24- 36 hours after cold incubation. Zone of inhibition was observed by using vernier scale. The mean value of the zone of inhibition was measured in millimeter of two preparation of synthesized compounds (N-01 to N-09) and standard drug.



Comp. Code	R^1	R^2	Comp. Code	R^1	R^2	Comp. Code	R^1	R^2
N-01	2-NO_2	2-NO_2	N-04	3-NO_2	2-NO_2	N-07	4-NO_2	2-NO_2
N-02	2-NO_2	3-NO_2	N-05	3-NO_2	3-NO_2	N-08	4-NO_2	3-NO_2
N-03	2-NO_2	4-NO_2	N-06	3-NO_2	4-NO_2	N-09	4-NO_2	4-NO_2

Figure 1: Synthetic scheme for novel C-6 nitro substituted benzothiazole derivatives.

Minimum inhibitory concentration (MIC) by broth dilution method: Nutrient broths (double strength) was prepared in test tubes and labeled them. In first test tube (UT), inoculum is not added which is used for checking the sterility of medium and as a negative control. Other all test tubes, inoculums (three to four drops) is added to reach the final concentration of microorganisms is 106 cells/ml in all test tubes, test antimicrobial compound is added ranging from 0.5 to 5 ml except un-inoculated (negative control) and control (positive) tube. The positive control tube is used to check the suitability of the medium for growth of the test microorganism and the viability of the inoculums. Adjust the final volume (10 ml) in all test tubes by using sterile water. All test tubes are properly shaken and then incubated at 37ºC for two days.

RESULTS AND DISCUSSION: Benzothiazole contains sulphur and nitrogen as heteroatom but imparts biological activity while substitution at C-6, C-2 and C-7 position. In the present work, nitro substituted benzothiazole nucleus while 2-(3 or 4)-arylnitro considered as rotating substitution at C-2 and C-7 position of benzothiazole nucleus derivatives were synthesized (Figure 2).

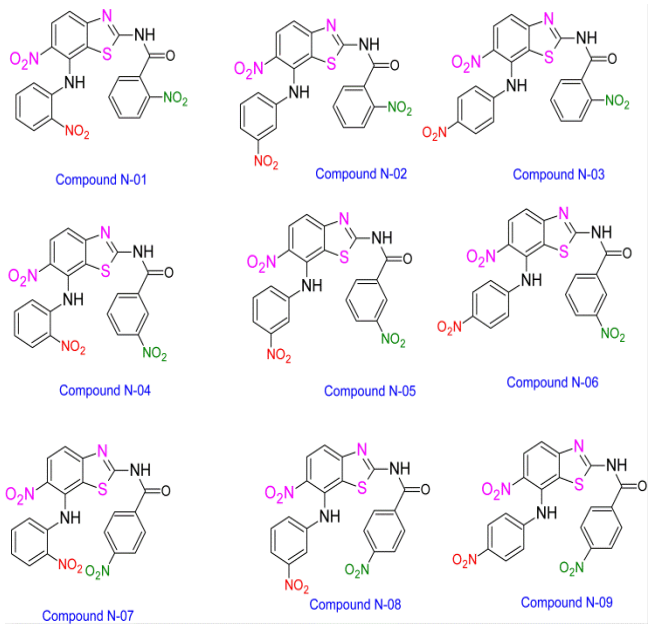


Figure 2: Novel C-6 nitro substituted benzothiazole compounds.

The novel derivatives (N-01 to N-09) evaluated for antifungal activity against *A. niger*. In the present work, nitro group consider as rotating basis on ortho, meta and para position. The reason behind considering the nitro group as a substituent is the fungi rarely acquire resistance. TLC, melting point, IR and 1HNMR were used for analytical characterization. In the TLC, the distance traveled by compound N-01 to N-09 was found to be different from that of the starting compound that proved synthesized compounds were different from parent one, even during TLC performance every time single spot was obtained, hence it also reveals that synthesized compounds were free from impurity as well as reaction was completed. Structure elucidation by IR spectroscopy frequency range for Ar-C=C, C=O, C-S, C-NO2 was considered. In case of structure elucidation of by 1HNMR sharp characteristic signals for 1H, NH; 10H, Ar-H and 1H, CONH was observed and consider as benzothiazole in all the synthesized compounds (Table 1).

Table 1: Analytical characterization of synthesized compounds.

Compound Code	% Yield	Melting point (^{0}C)	TLC (Rf value)	IR Spectral Study	^{1}HNMR Spectral Study (400Hz, DMSO-d6)
N-01	55	275	0.55	1456cm^{-1}ArC=C 1632cm^{-1}C=O 1245cm^{-1}C-S 1544cm^{-1}C-NO$_2$	δ 4.65 (s, 1H, NH) δ 7.15-8.10 (m, 10H, Ar-H) δ 8.77 (s, 1H, CONH)
N-02	63	264	0.51	1454cm^{-1}ArC=C 1640cm^{-1}C=O 1257cm^{-1}C-S 1575cm^{-1}C-NO$_2$	δ 4.58 (s, 1H, NH) δ 7.02-7.89 (m, 10H, Ar-H) δ 8.91 (s, 1H, CONH)
N-03	72	284	0.49	1454cm^{-1}ArC=C 1652cm^{-1}C=O 1241cm^{-1}C-S 1523cm^{-1}C-NO$_2$	δ 4.69 (s, 1H, NH) δ 7.11-7.64 (m, 10H, Ar-H) δ 9.15 (s, 1H, CONH)
N-04	56	256	0.61	1421cm^{-1}ArC=C 1665cm^{-1}C=O 1243cm^{-1}C-S 1537cm^{-1}C-NO$_2$	δ 4.57 (s, 1H, NH) δ 7.18-7.89 (m, 10H, Ar-H) δ 9.22 (s, 1H, CONH)
N-05	59	268	0.53	1443cm^{-1}ArC=C 1626cm^{-1}C=O 1222cm^{-1}C-S 1543cm^{-1}C-NO$_2$	δ 4.41 (s, 1H, NH) δ 7.04-7.96 (m, 10H, Ar-H) δ 9.10 (s, 1H, CONH)
N-06	64	267	0.45	1421cm^{-1}ArC=C 1615cm^{-1}C=O 1212cm^{-1}C-S 1554cm^{-1}C-NO$_2$	δ 4.38 (s, 1H, NH) δ 7.14-7.85 (m, 10H, Ar-H) δ 9.02 (s, 1H, CONH)
N-7	51	278	0.59	1423cm^{-1}ArC=C 1626cm^{-1}C=O 1220cm^{-1}C-S 1540cm^{-1}C-NO$_2$	δ 4.87 (s, 1H, NH) δ 7.32-7.93 (m, 10H, Ar-H) δ 9.24 (s, 1H, CONH)
N-08	62	252	0.42	1421cm^{-1}ArC=C 1615cm^{-1}C=O 1212cm^{-1}C-S 1554cm^{-1}C-NO$_2$	δ 4.53 (s, 1H, NH) δ 7.19-7.86 (m, 10H, Ar-H) δ 9.12 (s, 1H, CONH)
N-09	71	287	0.44	1458cm^{-1}ArC=C 1664cm^{-1}C=O 1244cm^{-1}C-S 1552cm^{-1}C-NO$_2$	δ 4.88 (s, 1H, NH) δ 7.11-7.82 (m, 10H, Ar-H) δ 9.08 (s, 1H, CONH)

Antifungal activity performed at two concentration 50µg/ml and 100µg/ml using griseofulvin as a standard drug against *A. niger*. The result of the zone of inhibition (ZOI) and MIC revealed that compound N-03 showed potent antifungal activity against *A. niger* while compound N-05, N-08 and N-09 showed moderate inhibitory activity at both concentrations 50µg/ml and 100µg/ml as compared to standard (Table-2, Table-3, Figure 3 and Figure 4).The structure-activity relationship of newly synthesized compound

revealed that 2-nitro-N-{6-nitro-7-[(4-nitrophenyl)amino]-1,3-benzothiazol-2-yl}benzamide (Compound N-03) found to be more active than standard while 3-nitro-N-{6-nitro-7-[(3-nitrophenyl)amino]-1,3-benzothiazol-2-yl}benzamide (Compound N-05), 4-nitro-N-{6-nitro-7-[(3-nitrophenyl)amino]-1,3-benzothiazol-2-yl}benzamide (Compound N-08) and 4-nitro-N-{6-nitro-7-[(4-nitrophenyl)amino]-1,3-benzothiazol-2-yl}benzamide (Compound N-09) exhibited prominent inhibitory activity against *A. niger*.

Table 2: Result of antifungal activity for ZOI.

Compound Code	*Aspergillus niger*	
	Zone of Inhibition (mm)*	
	50µg/ml	100µg/ml
Griseoflavin (GF)	24	38
N-01	09	15
N-02	12	16
N-03	32	55
N-04	10	17
N-05	21	34
N-06	05	08
N-07	11	17
N-08	22	36
N-09	22	32

**Each value is the mean of three replicates*

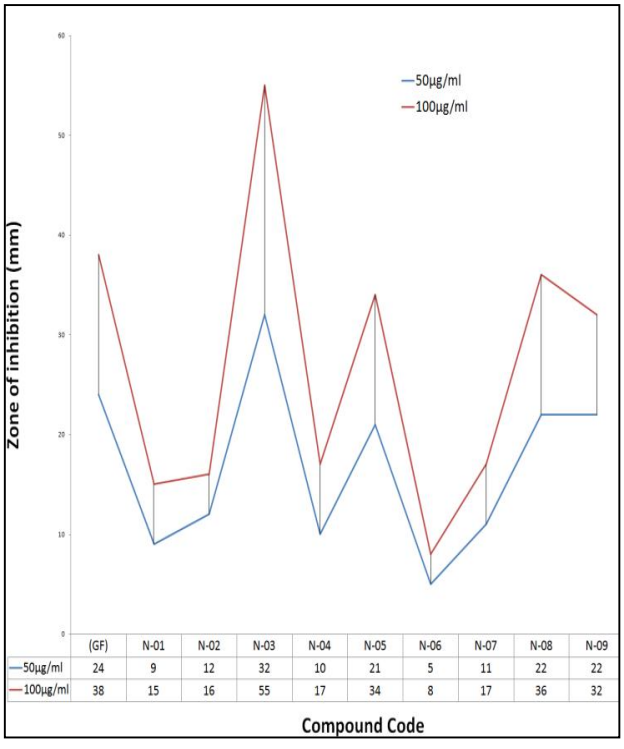


Figure 3: Result of comparative study of novel synthesized derivatives.

Table 3: Result of MIC of synthesized compounds.

Compound Code	Minimum inhibitory concentration (MIC) µg/ml ± SD*	
	Aspergillus niger	
	50µg/ml	100µg/ml
Griseoflavin (GF)	23.80 ± 0.21	35.14 ± 0.47
N-01	07.69 ± 0.28	13.88 ± 0.63
N-02	09.65 ± 0.14	15.22 ± 0.10
N-03	30.57 ± 0.51	52.11 ± 0.68
N-04	09.10 ± 0.74	14.10 ± 0.42
N-05	19.65 ± 0.55	30.25 ± 0.33
N-06	03.96 ± 0.32	05.02 ± 0.21
N-07	10.53 ± 0.47	12.44 ± 0.20
N-08	21.47 ± 0.40	34.05 ± 0.52
N-09	20.21 ± 0.96	28.22 ± 0.78
Control	0	0

**Each value is the mean of three replicates*

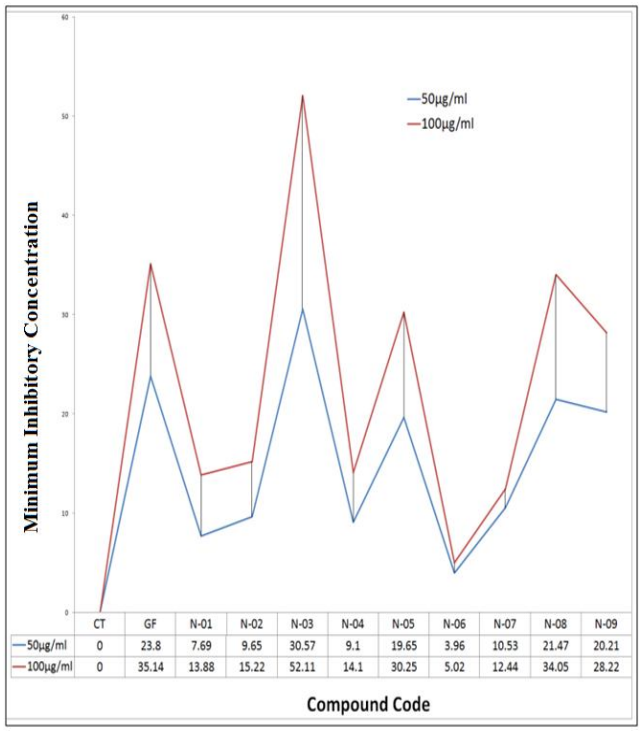


Figure 4: Result of comparative study of MIC of synthesized compounds.

CONCLUSION: In the present work, C-6 nitro-substituted novel benzothiazole derivatives were synthesized and screened for antifungal activity against *A. niger*. C-6 nitrosubstituted aminobenzo-thiazole nucleus was synthesized by one-step process using substituted aniline, potassium thiocyanate and bromine in acidic condition at low temperature (0-5ºC) followed by condensation and reaction in step 2 and 3 to synthesize desired products (N-01 to N-09). The paucity of data showed that compound N-03 exhibited more potent activity as compared to standard.

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