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Synthesis and antifungal activity of N-bromonicotinamide (NBN)

M.N. ABUBACKER, L. PUSHPALATHA AND K. VIVEKANANDAN

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SUMMARY

N-Bromonicotinamide (nbn) was synthesized and screened for antifungal activity against *Aspergillus restrictus, Candida albicans, Cladosporium herbarum, Fusarium oxysporum, Penicillium chrysogenum* and *Rhizoctonia solani*. N-Bromonicotinamide showed total inhibition to all these fungi at 1000 ppm concentration, whereas it showed 75 per cent growth inhibition for *A. restrictus, C. herbarum, F. oxysporum, P. chrysogenum* and *R. solani* and 50 per cent growth inhibition for *C. albicans* at 500 ppm concentration. At lower concentration (250 ppm) of NBN *A. restrictus, C. herbarum, F. oxysporum* and *R. solani* showed 50 per cent growth inhibition, whereas in *C. albicans* with 75 per cent growth inhibition was observed. For *P. chrysogenuus*, the lower concentration do not show any inhibition. Hence, NBN at 1000 ppm concentration can be utilized as antifungal agent against these fungi.

Key words : Antifungal activity, Antifungal agent, Growth inhibition, N-Bromonicotinamide (NBN)

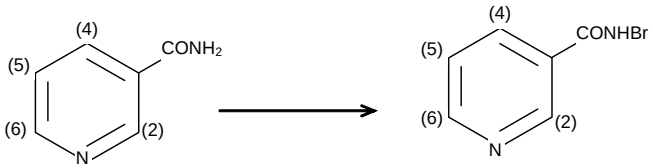
N-Bromo compounds have been used as versatile reagents in kinetic studies and organic synthesis. This compound offers many advantages like easy method of synthesis, low cost, easy handling, low toxicity and mild nature with appreciable stability (Pushpalatha and Vivekanandan, 2007). The antimicrobial activities of N-Bromo related work was carried out by Rittich *et al*. (1992), Sh.El-Sharief *et al*. (2001), Aytemir *et al*. (2003), Hida *et al*. (2005), Fatima *et al*. (2007) and Indira and Abubacker (2008). The synthesis and antimicrobial activities of similar compounds have been carried out by Zelenak *et al*. (2002), Turan-Zitonni *et al*. (2005), De La Fuente *et al*. (2006), Tao Zhao and Gang Sun (2006) and Patel *et al*. (2007).

The present work deals with testing the N-Bromonicotinamide for its antifungal activity against *Aspergillus restrictus, Candida albicans, Cladosporium herbarum, Fusarium oxysporum, Penicillium chrysogenum* and *Rhizoctonia solani*.

MATERIALS AND METHODS

Synthesis and characterisation of N-Bromonicotinamide:

N-Bromonicotinamide (m.p. 210^0 C) was prepared by the standard method described by Hauser and Renfrow (1923). Ten gram of nicotinamide was added to 150 ml of an ice-cold solution of sodium hypobromite, freshly prepared from 14.4 g (0.09 mol) of bromine, and 9.0 g (0.23 mol) of sodium hydroxide. After shaking for ten minutes, the mixture was filtered rapidly with suction into a cold solution of 9 ml of glacial acetic acid and 25 ml of iced-water. The bromomide which precipitated was filtered off, washed and recrystallized from ethanol (yield 70%).



The melting point was found to be 210^0 C with molecular formula as $C_6H_5ON_2Br$. The NBN was found to be soluble in water, acetic acid, sparingly soluble in ethanol, but insoluble in CCl_4, CH_2Cl_2 and dioxane. The stock solution of NBN was prepared in 50% acetic acid and water mixture and kept in amber coloured bottle (to prevent interaction with light). It showed no appreciable change in concentration and appearance over a period of one month indicating a pair degree of stability. The stock solution was used for screening antifungal activity.

Fungal cultures:

Aspergillus restrictus NCBT-131, *Candida albicans* NCBT-140, *Cladosporium herbarum* NCBT-145, *Fusarium oxysporum* NCBT-156, *Penicillium chrysogenum* NCBT-181 and *Rhizoctonia solani* NCBT-194 cultures maintained in immobilized condition in the Department of Botany, Microbiology Laboratory, National College were used in this work.

Correspondence to:
M.N. ABUBACKER, Post Graduate and Research Department of Botany, National College, TIRUCHIRAPPALLI (T.N.) INDIA
Authors' affiliations:
L. PUSHPALATHA AND K. VIVEKANANDAN, Post Graduate and Research Department of Chemistry, National College, TIRUCHIRAPPALLI (T.N.)

Experimental procedure:

Potato-dextrose-agar (PDA) medium was prepared (Harrigan and McCance, 1969) and streptomycin (50 mg) was added to 250 ml medium to control the bacterial growth. Different concentrations of N-Bromonicotinamide (250, 500 and 1000 ppm) were prepared from the stock in distilled water and sterilized.

The sterile medium (20 ml) and the sterile solution (5 ml of 1250 ppm) of NBN were mixed under sterile condition to obtain the effective concentration of NBN in the medium as 250 ppm. Similarly 5 ml of 2500 ppm solution of NBN and 20 ml of the medium were mixed in another Petridish so that the effective concentration was 500 ppm. In the same way 5 ml of 5000 ppm solution of NBN and 20 ml of the medium were mixed in a Petridish to get an effective concentration of 1000 ppm. Control (C) was prepared by pouring 20 ml of the medium and 5 ml of sterile distilled water. Another positive control (C_1) was prepared by using commercial antifungal compound Bavistin at 50 mg/l concentration, for this the sterile medium (20 ml) and the sterile solution (5 ml of 250 mg) of Bavistin were mixed under sterile condition to obtain the effective concentration of Bavistin in the medium as 50 mg. After the medium solidified at room temperature, each Petridish was inoculated with the spores from a pure culture of *Aspergillus restrictus* (NCBT-131), *Candida albicans* (NCBT-140), *Cladosporium herbarum* (NCBT-145), *Fusarium oxysporum* (NCBT-156), *Penicillium chrysogenum* (NCBT-181) and *Rhizoctonia solani* (NCBT-194) fungal strains and incubated for five days at a temperature of 28 ± 2^0C in the dark.

RESULTS AND DISCUSSION

N-Bromonicotinamide was screened for antifungal activity revealed a total inhibition to all the fungal strain tested in this work at 1000 ppm concentration. This total inhibition can be comparable with the commercial antifungal compound Bavistin 50 mg/l (Fig.1). The fungal strains *Aspergillus restrictus* (NCBT-131), *Cladosporium herbarum* (NCBT-145), *Fusarium oxysporum* (NCBT-156), *Penicillium chrysogenum* (NCBT-181) and *Rhizoctonia solani* (NCBT-194) have shown 75% inhibition for NBN. At lower concentration of 250 ppm *A. restrictus, C. herbarum, F. oxysporum* and *R. solani* revealed 50% growth inhibition, whereas *C. albicans* showed 75% inhibition. *P. chrysogenum* fungal strains do not show inhibition at lower concentration of NBN (Table 1).

NBN can serve as an effective reagent for oxidation and halogenation, recently considerable attention has been focused on the N-halogeno compounds due to their ability to act as sources of halogenonium cations, hypohlalite species and nitrogen anions which act as bases and nucleophiles. The halogenated compounds such as NBN can also be utilised for antimicrobial activities as reported by Pushpalatha and Vivekanandan (2007).

The compound Sulfamethoxazole significantly inhibits the growth of *Aspergillus* spp. because of its sulfa compound which acts as a competitive antifungal agent interfering folate metabolism of the fungus (Hida *et al*., 2005). The NBN compound tested for this work have shown a best result as antifungal agent and such mechanisms might have acted as limiting factor with reference to *A. restrictus*.

The antifungal activity of Nia=nicotinamide against *Candida albicans* was reported by Zelenak *et al*. (2002). The antifungal activity of acetamide of thio-4H-1,2,4-triazole derivatives against *Candida albicans* and *C. glabrata* was observed by Turan-Zitonni *et al.* (2005).

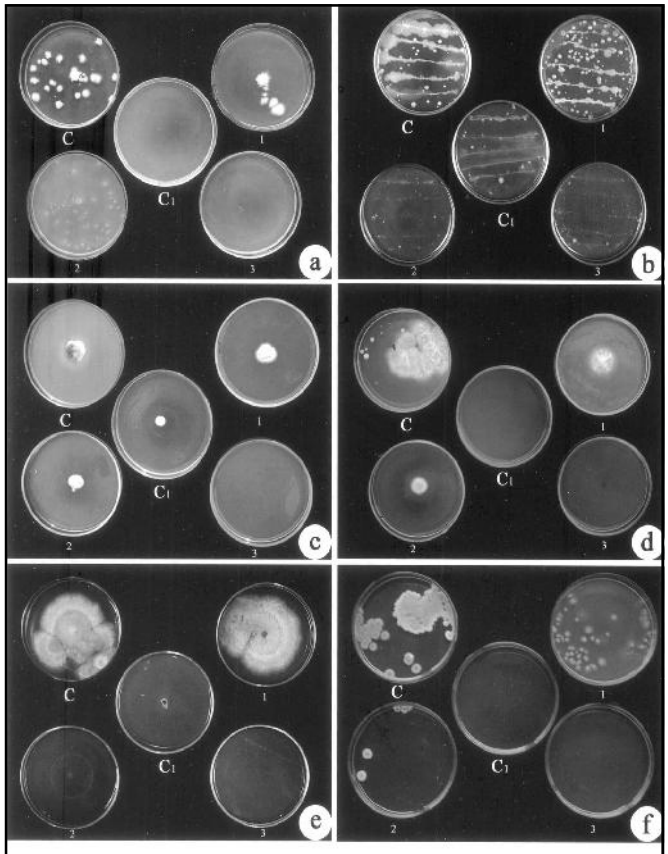


Fig. 1 : Antifungal activity of N-Bromo nicotinamide (NBM)

Table 1 : Antifungal activity of N-Bromonicotinamide (NBN)

Fungal strains	Control (C)	Control-1 (C1)	Concentration of NBN (ppm)		
			250	500	1000
Aspergillus restrictus NCBT-131	++++	–	++	+	–
Candida albicans NCBT-140	++++	+	+++	++	–
Cladosporium herbarum NCBT-145	++++	–	++	+	–
Fusarium oxysporum NCBT-156	++++	–	++	+	–
Penicillium chrysogenum NCBT-181	++++	–	++++	+	–
Rhizoctonia solani NCBT-194	++++	–	++	+	–

C : Control - Medium without N-Bromonicotinamide, C1 : Control-1 - Medium with commercial antifungal agent Bavinstin 50 mg/l
++++ : Normal (100%) growth of fungus, +++ : 25% growth inhibition, ++ : 50% growth inhibition, + : 75% growth inhibition
– : Total growth inhibition of fungus

Antifungal activity of amide derivatives of quinolone compounds against *C. albicans* who reported by Patel *et al*. (2007). The 3-Hydroxy-6-methyl-4-oxo-4H-pyran-2-carboxamide derivatives were tested for antifungal activity against *Candida albicans*, *C. krusei* and *C. parapsilosis* (Aytemir *et al*., 2003). These are the supporting evidences that NBN and related compounds have a strong antifungal effect especially for *Candida albicans*.

The organic compounds *viz.*, m-chloronitrobenzene, 2,3-dichloro-5,6-dicyno-1, 4-benzoquinone (DDQ), p-bromobenzophenone and p-chloroanaline tested for antifungal activity have shown the highest activity at 250 ppm concentration for *Cladosporium cladosporoides* fungi (Pathak and Sharma, 1984). The NBN compound was another best source of antifungal agent for *C. herbarum*.

Ionized form of aliphatic acids have shown antifungal activity to *Fusarium moniliforme* CCMF-180 (Rittich *et al*., 1992). The antifungal oxindole alkaloids isatinone A and B, trisindoline compounds isolated from plant source (*Isatis costata*, Brassicaceae) was found to show antifungal activity against *Fusarium solani* (ATCC-36031) (Fatima *et al*., 2007). The NBN compound tested for the present study is yet another source of antifungal compound for *F. oxysporum.*

Triazinoquinazolinones, triazepinoquinazolinones and triazocinoquin-azolinones obtained via nucleophilic interaction of 3-aminoquinazolinone derivatives were found to show antifungal activity against *Penicillium chrysogenum* (Sh. El-Sharief *et al.*, 2001). The present work tested with NBN compound as one more source of antifungal agent for *P. chrysogenum.*

According to Fatima *et al*. (2007), the oxindole alkaloids isatinone A and B isolated from the plant *Isatis costata,* Brassicaceae as antifungal compound inhibited the growth of *Rhizoctonia solani*. The NBN compound was also a promising antifungal agent for *R. solani*.

NBN and its derivatives are interesting ligands because of their potential binding sites (Isab and Hussain, 1985). The fungal cell wall polymers provide a multitude of chemical groups such as hydroxyl, cabonyl, carboxyl, sulfhydryl, thioether, sulfonate, amide, imine, amide, imidazole, phosphonate and phosphodiester (Schiewer and Volesky, 2000). These chemical groups of biopolymers in turn harbour the binding sites, which provide the ligand atoms to form complexes with metal ions which lead to inhibition of fungal growth (Hunt, 1986; Gadd, 1992). Such factors were perhaps involved in the inhibition of growth against all the fungi tested for this study. The mechanism of antifungal activity may be ionic binding of the fungal cell membrane (Remade, 1990) and absorption and microprecipitation of NBN to the fungal cell wall (Gadd, 1990).

The increasing numbers of pathogen bacteria and fungi that are resistant to the commonly used therapeutic drugs agent is a major worldwide health problem. For these reasons the search for new antimicrobial agents with novel modes of action represents a major target in chemotherapy.

Conclusion:

The NBN compound proved to be a promising antifungal agent. This study would suggest that a more detached structure activity study with this class of compounds could be useful against the human pathogenic fungus *C. albicans* and plant pathogenic fungi *F. oxysporum* and *R. solani.*

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