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ONE-POT GREEN METHOD FOR THE SYNTHESIS, CHARACTERIZATION AND ANTI-MICROBIAL EVALUATION OF SOME 1H-1,3-BENZIMIDAZOLE ANALOGUES BY UTILIZING POWDER OF POMEGRANATE PEEL AS A PLANT BASED CATALYST.

Kusum P. Baser[1] and Yogesh S. Rane[2]
[1]Faculty & Research Guide, Department of Chemistry, N.G. Acharya & D.K. Marathe College Research Centre. Chembur, Mumbai-400071
[2]Research Scholar, Department of Chemistry, N.G. Acharya & D.K. Marathe College Research Centre. Chembur, Mumbai-400071

ABSTRACT
This study reports synthesis of 1H-1,3-benzimidazole analogues. These compounds have been prepared by condensing 1,2-phenylenediamine with halogen group substituted benzaldehydes; and by utilizing the powder prepared by the peels of pomegranate (PGP) as a better green catalyst. The synthesized derivatives were characterized utilizing different spectroscopic techniques. Furthermore, compounds have been determined for their antibacterial and antifungal activities, and the results indicated moderate biological activity against the tested microorganisms.

Keywords: *pomegranate peel, biological activity, 1,2-phenylenediamine, benzimidazole.*

INTRODUCTION
Hoebrecker is the pioneer in the field of synthesis of benzimidazole. In 1943, 2-benzimidazole carboxylic acids and 2-benzimidazole acetic acids were synthesized by Copeland, R., and Day, A. [2]. In 1949 King F. E. & Acheson R. M. synthesized 1H-1,3-benzimidazoles from benzene 1,2-diamine and imidate [3]. The application of benzimidazole dates back several decades, and since the 1990s, numerous benzimidazole analogues have been synthesized. Several well-known therapeutically active drugs containing the benzimidazole nucleus include omeprazole, bendamustine, albendazole and mebendazole. [4]. Benzimidazole's C-2 position is reactive to both electrophilic and nucleophilic substrates because of the tautomerization of the N-H proton with the other nitrogen atom in ring [5].

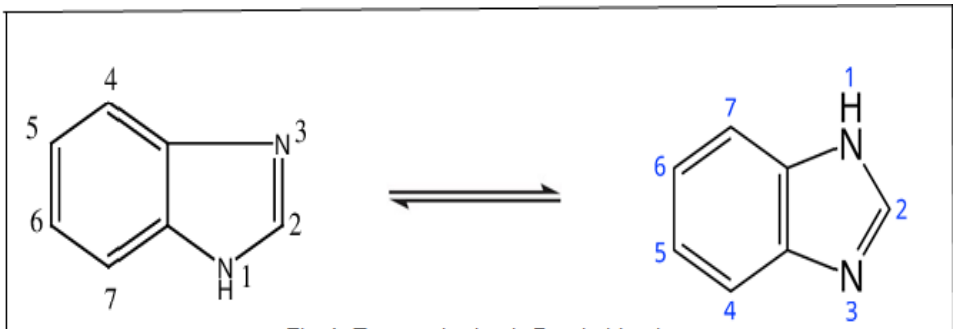

Fig. 1: Tautomerization in Benzimidazole.

Green techniques align with 12 principles of green chemistry, highlighting sustainability, intermediates, final products, energy efficiency, reduced toxicity of reagents, minimal harm to environment and human health, lower contributions to global warming, and more efficient and responsible utilize of natural resources as well as agricultural waste [6].

Benzimidazoles are typically synthesized by condensation of 1, 2-phenylenediamine and benzaldehydes using conventional methods. These conventional methods are time- and energy-intensive, produce significant byproducts, have low atom economies, and rely on hazardous solvents [7]. To overcome these limitations, various green techniques have been employed for the synthesis of benzimidazoles, such as utilization of PGP [8] and banana peel ash extract [9].

Benzimidazole is an immensely important compound in medicinal and heterocyclic chemistry, exhibiting different types of therapeutic uses, comprising antiviral, antiulcer, analgesic, antihypertensive, antifungal, antibacterial, anticancer, and antihistaminic effects [10].

Herein, we describe the method of preparation for 1H-1,3-benzimidazole analogues having substitution at second position; synthesized via condensation of 1,2-phenylenediamine with different halogen group substituted benzaldehydes and powder obtained from pomegranate peel is used as a plant-based catalyst.

Pomegranates exhibit strong antioxidant activity owing to the high flavonoid, anthocyanin, and catechin content in various plant parts, including the fruit, seeds, and peels. The peel constitutes major portion of the fruit, accounting for nearly 60% of fruit, and contains numerous components, namely calcium, phosphorus, potassium, sodium, magnesium [11]. Numerous alkaloids have been identified in pomegranate peels, including ellagic, protocatechuic, chlorogenic, cinnamic, gallic, and hydroxybenzoic acids [12].

From both economic and environmental perspectives, recent research has increasingly emphasized synthesis of organic compounds utilizing natural catalysts. Such catalysts offer several benefits, including easy availability, safety, and eco-friendliness. In this work, we describe green technique of one-pot formation for 1H-1,3-benzimidazole analogues by reacting halogen substituted benzaldehydes with 1,2-phenylenediamine at 28ºC-35ºC using ethanol as the solvent and PGP as a natural catalyst, producing products in lesser time as compared to conventional methods.

MATERIALS AND METHODS:

All reagents have been obtained from commercial suppliers (LOBA Chem, Spectrochem, Merck, Sigma-Aldrich). Equiptronics EQ-730 apparatus was used to measure melting points. On silica gel plates (ordered from Merck), “thin-layer chromatography (TLC)” has been performed, and the corresponding spots have been visible under UV illumination at 254 or 365nm. Proton(^{1}H) NMR and Carbon(^{13}C) NMR spectroscopy peaks have been observed on Bruker 400 MHz NMR spectrometer utilizing hexadeuterodimethyl sulfoxide-d6 as the solvent and tetramethylsilicane (TMS) the internal reference. Infrared spectra have been obtained using Bruker Alpha FTIR spectrometer. Mass spectra have been observed on Shimadzu LC-2010 mass analyser, and elemental (C, H, N) analyses have been performed using PerkinElmer PE 2400 analyser.

EXPERIMENTAL:

Preparation of Pomegranate Peel Powder:

1. The peels have been collected and cleaned
2. The Peels were cut
3. The Peels were dried: Sun Drying (traditional method)
4. Dried Peels were ground
5. Sieved
6. Stored Properly: The powder was transferred into airtight container.

Kept away from moisture and sunlight in a cold, dry location.

Fig. 2: Preparation of Pomegranate Peel Powder.

Synthesis of Benzimidazole Derivatives [8]:

A solution of halogen substituted benzaldehydes (1 millimole) and 1,2-phenylenediamine (1 millimole) in 10 to 15 mL of ethanol has been mixed with 10wt% PGP and stirred for 80-90 minutes. TLC has been used to observe reaction's progress, with n-hexane: ethyl acetate (7:3) serving as mobile phase. Reaction mixture has been placed into ice-cold water and filtered after ethanol has been eliminated by evaporation. Resulting solid

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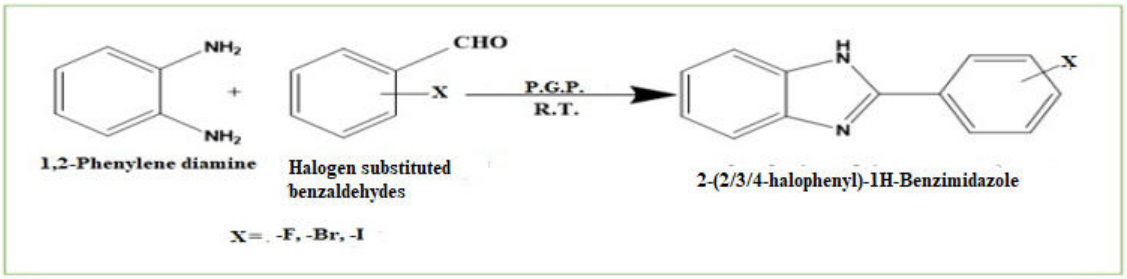
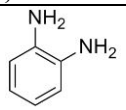
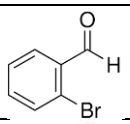
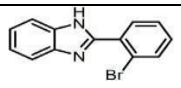
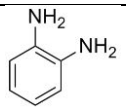
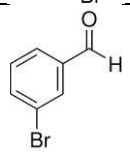
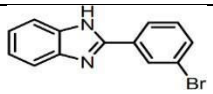
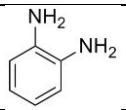
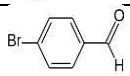
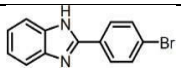
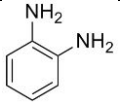
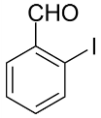
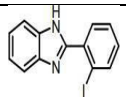
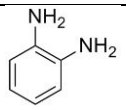
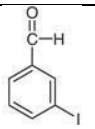
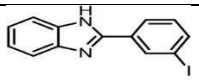
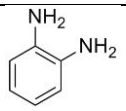
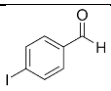
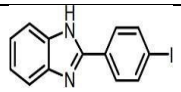
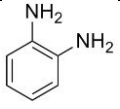
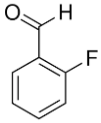
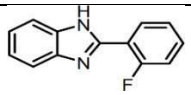
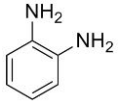
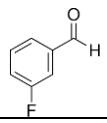
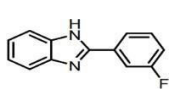
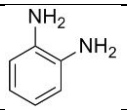
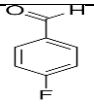
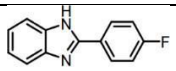
was further purified by chromatographic column technique. In this technique silicic acid gel and mixture of ethyl ethanoate (1 part) and benzine (20 part) is used.



Scheme I: Reaction showing formation of 2-(2/3/4-halophenyl)-1H-Benzimidazole

Table No. I: A Table Displaying Reactants, Products, Colour of products, Yield of products, and Melting Point of the products.

Comp. No.	Benzene-1,2-diamine	Aldehyde	Product	Colour	Yield (%)	M.P. (^{0}C)
1.				White Solid	69	235^{0}C to 240^{0}C
2.				Yellow Solid	**72**	250^{0}C to 255^{0}C
3.				White Solid	71	290^{0}C to 295^{0}C
4.				White Solid	60	120^{0}C to 125^{0}C
5.				White Solid	65	175^{0}C to 180^{0}C
6.				White Solid	68	185^{0}C to 190^{0}C
7.				White Solid	60	245^{0}C to 250^{0}C
8.				White Solid	60	210^{0}C to 215^{0}C

Comp. No.	Benzene-1,2-diamine	Aldehyde	Product	Colour	Yield (%)	M.P. (^{0}C)
9.				Yellow solid	70	255^{0}C to 258^{0}C

Spectral Analysis of the compounds [13]:

Compound No. 01:

2-(2-Bromophenyl)-1H-benzimidazole:

IR(KBR): 3443 (N-H), 3050 (C-H), 745 (C-Br) cm^{-1}.

Proton (^{1}H) NMR (400 Megahertz, hexadeuterodimethyl sulfoxide-d6): δ 12.76 (singlet, 1H), 8.13 (doublet, J = 8.0 Hertz, 1H), 8.06 (doublet, J = 7.9 Hertz, 1H), 7.90 (doublet, J = 7.9 Hertz, 1H), 7.76 (triplet, J = 7.7 Hertz, 2H), 7.67(triplet, J = 7.6 Hertz, 1H), 7.40 – 7.50 (multiplet,2H).

Carbon (^{13}C) NMR (100 Megahertz, hexadeuterodimethyl sulfoxide-d6): δ 149.99, 142.78, 135.13, 133.87, 132.86, 132.75, 131.87, 128.29, 123.15, 122.08, 122.02, 119.59, 112.05.

Mass spectra: Theoretical: C13 H9 Br N2 273.0022, Experimental: 273.0019.

C13 H9 Br N2: Theoretical: C: 57.22, H: 3.32, N: 10.27; Experimental: C: 57.04, H: 3.20, N: 10.21.

Compound No. 02:

2-(3-Bromophenyl)-1H-benzimidazole:

IR(KBR): 3350 (N-H), 3010 (C-H), 650 (C-Br) cm^{-1}.

Proton (^{1}H) NMR (400 Megahertz, hexadeuterodimethyl sulfoxide-d6): δ 13.07 (singlet, 1H), 7.98 (singlet, 1H), 7.80 (doublet, J = 7.8 Hertz, 1H), 7.68 (doublet, J = 7.7 Hertz, 2H), 7.48 – 7.28 (multiplet, 2H), 6.99 (singlet, 2H).

Carbon (^{13}C) NMR (100 Megahertz, hexadeuterodimethyl sulfoxide-d6): δ 150.06, 143.98, 135.46, 132.91, 132.85, 131.68, 129.33, 125.83, 123.44, 122.74, 122.42, 119.54, 111.97.

Mass spectra: Theoretical: C13 H9 Br N2: 273.0022, Experimental: 273.0021.

C13 H9 Br N2: Theoretical: C: 57.22, H: 3.32, N: 10.27; Experimental: C: 57.14, H: 3.22, N: 10.16.

Compound No. 03:

2-(4-Bromophenyl)-1H-benzimidazole:

IR(KBR): 3300 (N-H), 3070 (C-H), 600 (C-Br) cm^{-1}.

Proton (^{1}H) NMR (400 Megahertz, hexadeuterodimethyl sulfoxide-d6): δ 13.12 (singlet, 1H), 8.12 (doublet, J = 8.3 Hertz, 2H), 7.87 (doublet, J = 7.9 Hertz, 2H), 7.41 (singlet, 2H), 6.92 (doublet of doublets, J = 5.6, 3.1 Hertz, 2H).

Carbon (^{13}C) NMR (100 Megahertz, hexadeuterodimethyl sulfoxide-d6): δ 150.68, 132.46, 129.83, 128.82, 123.74, 122.82.

Mass spectra: Theoretical: C13 H9 Br N2 273.0022, Experimental: 273.0018.

C13 H9 Br N2: Theoretical: C: 57.22, H: 3.32, N: 10.27; Experimental: C: 57.34, H: 3.18, N: 10.23.

Compound No. 04:

2-(2-Iodophenyl)-1H-benzimidazole:

IR(KBR): 3430 (N-H), 3010 (C-H), 580 (C-I) cm^{-1}.

Proton (^{1}H) NMR (400 Megahertz, hexadeuterodimethyl sulfoxide-d6): δ 11.98 (singlet, 1H), 7.96 (doublets of doublets, J = 7.9, 0.9 Hertz, 1H), 7.59 (doublet, J = 7.5 Hertz, 1H), 7.52 (doublets of doublets, J = 7.4, 1.6 Hertz, 1H), 7.38 – 7.28 (multiplet, 2H), 7.11 – 6.98 (multiplet, 3H).

Carbon (^{13}C) NMR (100 Megahertz, hexadeuterodimethyl sulfoxide-d6): δ 153.05, 144.06, 140.13, 136.96, 135.03, 132.03, 131.68, 129.01, 123.03, 121.99, 119.61, 111.95, 97.84.

Mass Spectra: Theoretical: C_{13} H_9 I N_2 320.9883, Experimental: 320.9856.

C_{13} H_9 I N_2: Theoretical: C: 48.78, H: 2.83, N: 8.75; Experimental: C: 49.07, H: 3.02, N: 9.01

Compound No. 05:

2-(3-Iodophenyl)-1H-benzimidazole:

IR(KBR): 3443 (N-H), 3060 (C-H), 515 (C-I) cm^{-1}.

Proton (^{1}H) NMR (400 Megahertz, hexadeuterodimethyl sulfoxide-d6): δ 13.07 (singlet, 1H), 8.60 (singlet, 1H), 7.94 (doublet, J = 7.8 Hertz, 1H), 7.69 (doublet, J = 7.6 Hertz, 1H), 7.45 (singlet, 2H), 7.20 (triplet, J = 7.2 Hertz, 1H), 6.97 (singlet, 2H).

Carbon (^{13}C) NMR (100 Megahertz, hexadeuterodimethyl sulfoxide-d6): δ 150.01, 138.60, 135.14, 133.06, 132.08, 130.06, 126.18, 123.14, 122.70, 95.81.

Mass Spectra: Theoretical: C_{13} H_9 I N_2 320.9883, Experimental: 320.9856.

C_{13} H_9 I N_2: Theoretical: C: 48.78, H: 2.83, N: 8.75; Experimental: C: 48.70, H: 3.09, N: 9.08

Compound No. 06:

2-(4-Iodophenyl)-1H-benzimidazole:

IR(KBR): 3355 (N-H), 3010 (C-H), 580 (C-I) cm^{-1}.

Proton (^{1}H) NMR (400 Megahertz, hexadeuterodimethyl sulfoxide-d6): δ 13.14 (singlet, 1H), 7.99 – 7.86 (multiplet, 4H), 7.44 – 7.22 (multiplet, 2H), 6.98 (singlet, 2H).

Carbon (^{13}C) NMR (100 Megahertz, hexadeuterodimethyl sulfoxide-d6): δ 149.98, 139.97, 137.38, 128.80, 128.06, 122.25, 121.36, 97.18.

Mass Spectra: Theoretical: C_{13} H_9 I N_2 320.9883, Experimental: 320.9856.

C_{13} H_9 I N_2: Theoretical: C: 48.78, H: 2.83, N: 8.75; Experimental: C: 49.11, H: 3.01, N: 9.13

Compound No. 07:

2-(2-Fluorophenyl)-1H-benzimidazole:

IR(KBR): 3320 (N-H), 3048 (C-H), 1095 (C-F) cm^{-1}.

Proton (^{1}H) NMR (400 Megahertz, hexadeuterodimethyl sulfoxide-d6): δ 11.98 (singlet, 1H), 8.24 (triplet, J = 8.1, 1.8 Hertz, 3H), 7.56 – 7.44 (multiplet, 3H), 7.33 – 7.22 (multiplet, 2H), 6.94 (d, J = 3.5 Hertz, 2H).

Carbon (^{13}C) NMR (100 Megahertz, hexadeuterodimethyl sulfoxide-d6): δ 159.93 (doublet, JC-F = 249.1 Hertz), 147.08, 132.32 (doublet, JC-F = 8.9 Hertz), 130.70 (doublet, JC-F = 2.6 Hertz), 121.15 (d, JC-F = 2.9 Hertz), 118.56 (doublet, JC-F = 11.8 Hertz), 117.08.

Mass Spectra: Theoretical: C_{13} H_9 F N_2 213.0823, Experimental: 213.0848.

C_{13} H_9 F N_2: Theoretical: C: 73.57, H: 4.27, N: 13.20; Experimental: C: 73.5, H: 4.21, N: 13.28

Compound No. 08:

2-(3-Fluorophenyl)-1H-benzimidazole:

IR(KBR): 3307 (N-H), 3021 (C-H), 1075 (C-F) cm^{-1}

Proton (^{1}H) NMR (400 Megahertz, hexadeuterodimethyl sulfoxide-d6): δ 13.05 (singlet, 1H), 7.99 (doublet, J = 7.9 Hertz, 1H), 7.91 (doublet, J = 10.4, 2.4 Hertz, 1H), 7.60 – 7.31 (multiplet, 3H), 7.16 (triplet, J = 7.3, 1.9 Hertz, 1H), 6.96 (doublets of doublets, J = 5.9, 3.1 Hertz, 2H).

Carbon (^{13}C) NMR (100 Megahertz, hexadeuterodimethyl sulfoxide-d6): δ 159.93 (doublet, JC-F = 240.3 Hz), 147.08, 132.32 (doublet, JC-F = 8.9 Hertz), 130.70 (doublet, JC-F = 2.5 Hertz), 119.55 (doublet, JC-F = 3.4 Hertz), 118.56 (doublet, JC-F = 11.4 Hertz), 109.98.

Mass Spectra: Theoretical: C_{13} H_9 F N_2 213.0823, Experimental: 213.0826.

C_{13} H_9 F N_2: Theoretical: C: 73.57, H: 4.27, N: 13.20; Experimental: C: 72.98, H: 4.11, N: 12.98

Compound No. 09:

2-(4-Fluorophenyl)-1H-benzimidazole:

IR(KBR): 3429 (N-H), 3060 (C-H), 964 (C-F) cm^{-1}.

Proton (^{1}H) NMR (400 Megahertz, hexadeuterodimethyl sulfoxide-d6): δ 7.95 (doublets of doublets, J = 7.9, 5.5 Hertz, 2H), 7.50 (doublets of doublets, J = 5.8, 3.2 Hertz, 2H), 7.15 (triplet, J = 8.8 Hertz, 2H), 6.96 (doublets of doublets, J = 6.0, 3.3 Hertz, 2H).

Carbon (^{13}C) NMR (100 Megahertz, hexadeuterodimethyl sulfoxide-d6): δ 164.04 (doublet, JC-F = 247.3 Hertz), 150.89, 129.2 (doublet, JC-F = 8.6 Hertz), 119.98 (doublet, JC-F = 2.9 Hertz), 112.61, 107.07 (doublet, JC-F = 21.9 Hertz).

Mass Spectra: Theoretical: C_{13} H_9 F N_2: 213.0823, Experimental: 213.0818.

C_{13} H_9 F N_2: Theoretical: C: 73.57, H: 4.27, N: 13.20; Experimental: C: 74.01, H: 4.21, N: 12.98

ANTIMICROBIAL ACTIVITY:

Diderm (Gram negative) bacteria: *Salmonella typhi* (Typhoid bacillus).

Monoderm (Gram positive) bacteria: *Staphylococcus aureus* (Staph aureus).

Culture of fungi: *Candida albicans* (Candida).

All above mentioned microorganisms were tested in vitro using synthetic substances.

Method: Tube Dilution Method **[14]**

Bacterial Culture Medium: Mueller-Hinton (M.H.) broth was used as the culture medium.

Fungal Culture Medium: Sabouraud's Agar Slants as a culture medium.

Standard Control: Ampicillin (M.I.C = 0.01µg/mL) against gram-positive bacteria, Staphylococcus aureus.

Trimethoprim (M.I.C = 1µg/mL) against gram-negative bacteria Salmonella typhi.

Miconazole (M.I.C = 0.625 µg/mL) was used against Candida albicans.

Preparation of Drug Solution: 0.01gm (10mg) of drug sample dissolved in 10mL dimethyl sulfoxide (D.M.S.O) to produce stock solution of 1000µg/mL

Table No. 2: Serial Dilutions of drug samples.

Drug stock solution in (mL)	M.H. broth/Sabouraud's broth in (mL)	Concentration (µg / mL)
2	8	200
1	9	100
0.5	9.5	50
0.4	9.6	40
0.2	9.8	20
0.1	9.9	10

Table No. 3: Result of Anti-Microbial Activity. (N.A.: Not Active at/below 200 µg/mL).

Comp. No.	Name of the Product	Staph aureus (µg. mL^{-1})	Typhoid bacillus (µg. mL^{-1})	Candida (µg. mL^{-1})
01.	2-(2-Bromophenyl)-1H-benzimidazole	200	200	200
02.	2-(3-Bromophenyl)-1H-benzimidazole	100	200	200
03.	2-(4-Bromophenyl)-1H-benzimidazole	100	100	200
04.	2-(2-Iodophenyl)-1H-benzimidazole	50	100	100
05.	2-(3-Iodophenyl)-1H-benzimidazole	50	100	200
06.	2-(4-Iodophenyl)-1H-benzimidazole	100	100	100
07.	2-(2-Fluorophenyl)-1H-benzimidazole	50	50	50
08.	2-(3-Fluorophenyl)-1H-benzimidazole	50	100	50
09.	2-(4-Fluorophenyl)-1H-benzimidazole	100	100	50

RESULT AND DISCUSSION

2-Substituted aryl benzimidazoles were synthesized through an environmentally friendly method using PGP as an effective natural catalyst at room temperature via a mechanical stirring technique. This approach is simple, does not require sophisticated equipment, is energy-efficient, and is cost-effective. The observed antimicrobial activity was found to be moderate. Enhanced antimicrobial results might be achieved by employing substituted o- phenylenediamines (OPDA) or aldehydes bearing a greater number of stronger electron- withdrawing groups.

CONCLUSION

The green synthesis of benzimidazoles described herein afforded good product yields of approximately 60–70%. Replacing the mechanical stirring with sonication could potentially enhance the yield and reduce the reaction time. Furthermore, microwave-assisted synthesis using PGP as an efficient and natural catalyst might achieve an even more significant reduction in the reaction time with improved efficiency.

Based on the above discussion, it is concluded that biological activity is enhanced by or dependent on:

1 A greater number of electron-withdrawing functional groups

2. Highly substituted OPDA

3. Biologically active substitutions

4. Elevated Hyperconjugation.

CONFLICT OF INTEREST:

NIL.

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