DOI:10.7598/cst2019.1566
ISSN:2278-3458

Chemical Science Transactions
2019, 8**(2),** 228-236

RESEARCH ARTICLE

A Facile Protocol for Synthesis of Some Novel 2-phenethyl-1*H*-benzimidazole Derivatives and Screening of *In-Vitro* Anti-inflammatory and Antimicrobial Activities

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Received 16 October 2018 / Accepted 20 November 2018

Abstract: A convenient and efficient method for the preparation of some novel 2-phenethyl-1*H*-benzimidazole derivatives **(4a-h)** has been achieved by the condensation of phenyl propionic acids **(2a-h)** with *o*-phenylenediamine **(3)** in $POCl_3$. The advantages of this method is excellent yields, short reaction time, no side reaction and operational simplicity and ease product isolation The structure of all the synthesized compounds were characterized by FT-IR, ^{1}H NMR and MS data. Furthermore, compounds **(4a-h)** were screened for their antibacterial activity against gram negative (*E. coli*) and gram positive (*B.subtilis*) bacteria, antifungal activity against *Aspergillusniger* and *Penicillium chrysogenum* and anti-inflammatory activities. Some of the compounds exhibited promising antibacterial, antifungal and anti-inflammatory activities.

Keywords: Phenyl propionic acid, *o*-phenylenediamine, Benzimidazole, Anti-inflammatory, *In-vitro*, Antibacterial, Antifungal activity

Introduction

Now a days infectious microbial diseases are causing health problems world-wide, because of resistance to number of antimicrobial agents. A variety of clinically significant species of microorganisms has become an important health problem throughout the world[1]. To fight with this challenge is the appropriate usage of the available marketed antibiotics; the other is the development of new anti-microbial agents[2]. Hence, there will always be a vital need of new strategies in drug designing and drug re-designing in order to improve ADME profile.

Due to the structural similarity to purine, antibacterial ability of benzimidazoles are explained by their competition with purines resulting in inhibition of the synthesis of bacterial nucleic acids and proteins[3,4].

Benzimidazole is a versatile pharmacophore producing a diverse range of biological activities and is used as selective neuropeptide YY1 receptor antagonists[5], factor Xainhibitors[6], smooth muscle cell proliferation inhibitors[7], antitumor[8], antiviral[9], and antimicrobial agents[10] and for HIV[11], herpes (HSV-1)[12], RNA[13], influenza[14] and human cytomegalovirus (HCMV) inhibitor[7]. They are also used in diverse areas of chemistry[15]and are very important intermediates in various organic reactions[16].

The benzimidazole is also a core structure is found in a variety of commercial drugs such as Atacand, Nexium, Micardis, Protonix and Vermox. The extensively used drugs such as protonpumpinhibitor[17] (Omeprazole), Antihelmenthetics[18] (Albendazole), anti-dopaminergic[19] (Domperidone). Specifically, the 2-substituted analogs of benzimidazoles are known to be potent biologically active compounds. Therefore preparation of benzimidazole has considerable attention in recent years. It is still valuable to synthesis new derivatives of benzimidazole due to its importance in organic and medicinal chemistry. This is the era of multidrug therapy, if in the same drug antibacterial, antifungal and anti-inflammatory activity exists, then it will be in the interest of medicinal apothecary and evolution of new lead structures.

Several methods have been reported for the synthesis of benzimidazoles[20]. The traditional synthesis of benzimidazole involves the reaction between *o*-phenylenediamine and a carboxylic acid or their derivatives at elevated temperature in the presence of strong acids such as polyphosphoric acid[21]or mineral acids[22] and thermal or acid promoted cyclization of *N*-(*N*-arylbenzimidoyl)-1, 4-benzoquinoneimines[23]. In the last ten years, a large number of scientific publications have appeared in the literature describing the synthesis of 2-arylbenzimidazoles). This indicated clearly the importance of 2-arylbenzimidazoles for a Chemist, a researcher or an Industrialist.

Heterogeneous catalyzed reactions are gaining importance owing to the inexpensive nature and special catalytic attributes due to higher selectivity, milder conditions and easy experimental procedures. In view of the influence of green chemistry to maintain greenness in synthetic pathways synthesis of phenyl propionic acids which is a key step especially in the preparation of 2-phenethyl-1*H*-benzimidazole derivatives is synthesized in presence of Pd/C catalyst. All substituted phenyl propionic acids are novel except hydrocinnamic acid. Literature survey revealed that *o*-phenylenediamine condenses with aliphatic and aromatic acids such as benzoic acids, phenyl acetic acids in PPA or PPE to give benzimidazole, but there is no method for the condensation of phenyl propionic acids with o-phenylenediamine. Hence, it was considered worthwhile to develop a simple and efficient method for the condensation of *o*-phenylenediamine with phenyl propionic acids which forms the subject matter of this paper.

Experimental

Chemicals and solvents required were from Merck and SD fine mock. All melting points were determined in open capillaries in paraffin bath and are uncorrected. The progress of the reactions was monitored by TLC on silica F_{254} coatedaluminum plates (Merck) as adsorbent and UV light and iodine as visualizing agents.The products were characterized by their spectral data. IR Spectra were recorded on Perkin-Elmer FTIR spectrophotometer in KBr disc. ^{1}H NMR spectra were recorded on Broker Advance at 400 MHz in $CDCl_3$ as solvent and chemical shift values are recorded in ppm relative to tetramethylsilane as an internal slandered. Mass spectra were recorded on VG 7070H micro mass spectrometer.

*General procedure for the synthesis of substituted phenyl propionic acid (**2a-h**)*

To a stirred solution of cinnamic acid (1 mmole) in tetralin (10 mL), 1 g of 5% palladium on carbon was added. The mixture was heated on heating mantle at refluxing temperature for two hours. The progress of the reaction was monitored bythin layer chromatography on Merck plates (silica gel F_{254}) using solvent *N*-hexane-ethyl acetate (9:1) after completion of the reaction,the mixture was cooled to room temperature, 15 mL of diethyl ether was added and mixture was filtered (catalyst was removed)filtrate was treated with 5%NaOH (10 mL), the aqueous layer was neutralized with hydrochloric acid,obtained precipitate was washed with water and recrystallized from aqueous ethanol. The physical data of synthesized compounds are given in Table 1.

*General procedure for the synthesis of 2-phenethyl-1H-benzimidazole derivatives (**4a-h**)*

A mixture of substituted phenyl propionic acid (1 mmole) and *o*-phenylenediamine (1 mmole) was dissolved in 1.5 mL of $POCl_3$ and heated in oil bath at temperature 120 °C for 120 minutes. The progress of the reaction was monitored bythin layer chromatography on Merck plates (silica gel F_{254}) using solvent *N*-hexane-ethyl acetate (9:1) and after completion of reaction, mixture was allowed to cool and poured on ice cold water. The solidwas filtered, washed with water and solid was suspended in 5%NaOH for 10-20 minutes to remove the unreacted acid. The product was filtered washed with water and recrystallized from aqueous ethanol.

Spectral data

*2-(Phenethyl)-1H-benzimidazole***(4a)**

Yield 80%, mp: 162-65 °C, Elemental analysis Calcd for($C_{15}H_{14}N_2$):C, 81.05; H, 6.35; N, 12.60;found: C, 81.01; H, 6.30; N, 12.52%, IR (KBr,ν (cm^{-1})): 2911, 2845 (C-H aliph), 3125 (C-H aroma), 3615(N-H stretching),1612 (N-H bending). ^{1}H NMR ($CDCl_3$): δ (ppm): 3.24(s,4H,CH_2),7.02-7.59(m,9H,Ar-H),7.65(s,1H,NH) Es-MI. 222(M^+)100% .

*2-(4-flurophenethyl)-1H-benzimidazole***(4b)**

Yield 78%, mp: 188-90 °C, Elemental analysis Calcd for($C_{15}H_{13}FN_2$): C, 74.98; H, 5.45; N, 11.66;found: C, 74.92; H, 5.40; N, 11.61 %,IR (KBr,ν (cm^{-1})): 2911, 2845(C-H aliph), 3120 (C-H aroma), 3610(N-H stretching),1611 (N-H bending).^{1}H NMR ($CDCl_3$): δ (ppm): 3.22(s,4H,CH_2),7.02-7.55(m,8H,Ar-H),7.60(s,1H,NH) Es-MI. 241(M^+)100%.

*2-(4-Chlorophenethyl)-1H-benzimidazole***(4c)**

Yield 80%, mp: 228-29 °C, Elemental analysis Calcd for($C_{15}H_{13}ClN_2$): C, 70.18; H, 5.10; N, 10.91;found: C, 70.16; H, 5.04; N, 10.85%, IR (KBr,ν (cm^{-1})): 2981(C-H aliph), 3109 (C-H aroma), 3611(N-H stretching),1612 (N-H bending).^{1}H NMR ($CDCl_3$): δ (ppm): 3.20(s,4H,CH_2),7.06-7.55(m,8H,Ar-H),7.56(s,1H,NH) Es-MI. 245(M^+)100% .

*2-(4-Nitrophenethyl)-1H-benzimidazole***(4d)**

Yield 75%, mp: 279-80 °C, Elemental analysis Calcd for($C_{15}H_{13}N_3O_2$):C, 67.40; H, 4.90; N, 15.72;found:C, 67.34; H, 4.84; N, 15.65%,IR (KBr,ν (cm^{-1})): 2911,2845(C-H aliph), 3120 (C-H aroma), 3605(N-H stretching),1593 (N-H bending).^{1}H NMR (CDCl3): δ (ppm): 3.20-3.27(t,2H,CH_2), 3.31-3.38(t,2H,CH_2), 7.25-7.55(m,8H,Ar-H),8.12(s,1H,NH), Es-MI. 256(M^+) 100%.

*2-(4-Methoxyphenethyl)-1H-benzimidazole***(4e)**

Yield 72%, mp: 200-01 °C, Elemental analysis Calcd for($C_{16}H_{16}N_2O$):C, 76.16; H, 6.39; N, 11.10;found:C, 76.10; H, 6.32; N, 11.05%,IR (KBr,ν (cm^{-1})): 2986,2906(C-H aliph), 3120 (C-H aroma), 3742(N-H stretching), 1607 (N-H bending), ^{1}H NMR ($CDCl_3$): δ (ppm): 3.11-3.14(t,2H,CH_2), 3.19-3.23(t,2H,CH_2),3.78(s,3H,OCH_3),7.06-7.55(m,8H,Ar-H), 7.56(s,1H,NH) Es-MI. 245(M^+)100%.

*2-(4-Bromophenethyl)-1H-benzimidazole***(4f)**

Yield 78%, mp: 263-64 °C, Elemental analysis Calcd for($C_{15}H_{13}BrN_2$): C, 59.82; H, 4.35; N, 9.30;found: C, 59.74; H, 4.28; N, 9.22%,IR (KBr,ν (cm^{-1})): 2985, 2886(C-H aliph), 3120 (C-H aroma), 3556(N-H stretching), 1640 (N-H bending).^{1}H NMR ($CDCl_3$): δ (ppm): 2.50-2.51(s,4H,CH_2),)7.13-7.62(m,8H,Ar-H),8.06(s,1H,NH) Es-MI. 300(M^+)100, 221(M^+-Br)

*2-(2-Chlorophenethyl)-1H-benzimidazole***(4g)**

Yield 80%, mp: 218-20 °C, Elemental analysis Calcd for($C_{15}H_{13}ClN_2$): C, 70.18; H, 5.10; N, 10.91;found: C, 70.12; H, 5.04; N, 10.84%, IR (KBr,ν (cm^{-1})): 2990(C-H aliph), 3129 (C-H aroma), 3601(N-H stretching),1612(N-H bending).^{1}H NMR ($CDCl_3$): δ (ppm): 3.25(s,4H,CH_2),7.00-7.65(m,8H,Ar-H),7.60(s,1H,NH) Es-MI. 245(M^+)100% .

*2-(3-Nitrophenethyl)-1H-benzo[d]imidazole***(4h)**

Yield 75%, mp: >300 °C, Elemental analysis Calcd for($C_{15}H_{13}N_3O_2$): C, 67.40; H, 4.90; N, 15.72;found: C, 67.32; H, 4.85; N, 15.65%, IR (KBr,ν (cm^{-1})): 2911,2845(C-H aliph),.3120 (C-H aroma), 3602(N-H stretching),1596 (N-H bending).^{1}H NMR ($CDCl_3$): δ (ppm): 3.21-3.28(t,2H,CH_2), 3.32-3.39(t,2H,CH_2), 7.25-7.55(m,8H,Ar-H),8.14(s,1H,NH) Es-MI. 256(M^+)100% .

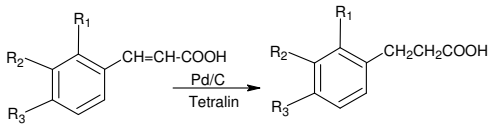
Reusability of catalyst (Pd/C)

We investigated the reusability and recycling of Pd/C.At the end of reaction, the catalyst could be recovered, by a simple filtration. The recovered catalyst could be washed with diethyl ether and subjected to a second run of reaction process. To ensure that catalyst were not dissolved in diethyl ether, the catalyst weighed after filtration and before using. The efficiency of reused catalyst was shown to be the same as fresh catalyst yielding the same product. In the second and third run also it is same.

Results and Discussion

In continuation of our research to develop methods for various transformations[24,25], we herein report protocol for the synthesis of new 2-phenethyl-1*H*- benzimidazole derivatives. Palladium supported catalyst (Pd/C) is the most widely used tool in industries for the selective hydrogenation of α-β unsaturated compounds. The key starting compound, substituted phenyl propionic acids **(2a-h)** required for this conversion were synthesized by the selective hydrogenation of substituted Cinnamic acid **(1a-h)** with palladium on carbon in the presence of tetralin at refluxing temperature. (Scheme 1), the results are summarized in Table 1.

Synthesized phenyl propionic acid **(2a-h)** on reacting with *o*-phenylenediamine in the presence of $POCl_3$, (Scheme 2) yielded **(4a-h)**. A systematic study of phenyl propionic acid **(2a)** and *o*-phenylenediamine **(3)** condensation was carried under various conditions of temperature, heating time period.



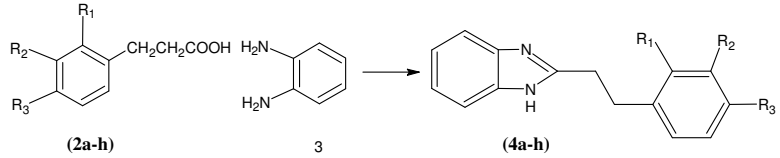
(1a-h) **(2a-h)**

Scheme 1. Synthesis of substituted phenyl prop ionic acids (**2a-h**)

(***Reagents and condition****: Cinnamic acid, tetralin, 1 g of 5% pd/c, refluxing temperature, 2 h*)

Table 1. Synthesis of substituted phenyl propionic acids **(2a-h)**

Entry	R_1	R_2	R_3	Yield%	M.P.°C
2a	H	H	H	85	49-51
2b	H	H	F	80	88-90
2c	H	H	Cl	80	107-10
2d	H	H	NO_2	82	165-67
2e	H	H	OCH_3	84	105-07
2f	H	H	Br	78	127-28
2g	Cl	H	H	80	95-96
2h	H	NO_2	H	75	215-17



(2a-h) 3 **(4a-h)**

Scheme 2. Synthesis of 2-phenethyl-1*H*- benzo[*d*]imidazole derivatives (**4a-h**)

(***Reagents and condition****: Phenyl propionic acid, o-phenylenediamine, $POCl_3$, oil bath, 120 °C, 120 minutes*)

Table 2 depicts 120 °C is found to be effective temperature to give high yield. In order to further optimize the reaction w.r.t. reaction time the reaction was carried out for various periods, Table 3 indicates 120 minutes is optimum time.

Table 2. Effect of temperature on yields of the model reaction

Entry	Temperature, 0 °C	Yield,%
1	80	Trace
2	10	25-30
3	120	75-77
4	140	75-80

Table 3. Effect of heating time on yields of the model reaction

Entry	Time, min	Yield,%
1	60	Trace
2	90	50-55
3	120	75-77

After optimizing the conditions, the generality of this method was examined with different kinds of aryl propionic acids and *o*-phenylenediamine in presence of $POCl_3$. The structures of target compounds **(4a-h)** were deduced from their spectral data (IR, ^{1}H NMR, EI-MS). In the ^{1}H NMR spectra of compounds **4d, 4e** and **4h** having electron withdrawing group

(NO_2) and electron releasing group (OCH_3) group in phenyl ring displayed as triplet at δ 3.21-3.28and at δ 3.32-3.39 with coupling constant J=7Hz for-CH_2-CH_2- linkage. When phenyl ring contains Cl,Br, F in the spectra of **4b, 4c, 4f, 4g** and **4h** -CH_2-CH_2-group displayed as singlet. The NH proton appeared as singlet at δ 7.5-8.15. Aromatic region showed the presence of protons from δ 7.25-7.55 in accordance with the structure.The EI-MS of compounds **4a-h** revealed the existence of their molecular ion peaks which were in accordance with the given structure of product.

We observed the effect of substituent on the benzene ring of propionic acid. Benzene ring substituted by electron donating and electron withdrawing groups were well tolerated providing good yield of the desired product (**4a-h**).

Biological activity

Antibacterial and Antifungal studies

The synthesized 2-phenethyl-1*H*-benzimidazole **(4a-h**).were screened for the antibacterial activity against two gram-positive bacteria *viz. Bacillussubtilis* and gram-negative bacteria *viz*., *Escherichia coli* by using the disc diffusion method[26] penicillium was used as reference standard for comparing the results and DMSO as a control solvent. Compounds **(4a-h)** were screened for antifungal activity against *aspergillusniger, penicilliumchrysogenum,* by standard poison plate method[27]. Using Griseofulvin as reference standard and DMSO as control solvent. Results of the antibacterial and antifungal activity of the 2-phenethyl-1*H*-benzimidazoles derivatives were represented in Table 4 and 5 respectively.

Table 4. Antibacterial activity for compounds (**4a-h)**

Compounds	*Bacillus subtilis*	*Escherichia coli*
4a	16	09
4b	22	14
4c	18	15
4d	14	07
4e	14	12
4f	20	14
4g	24	16
4h	Not tested	Not tested
Penicillin	30	20
DMSO	-ve	-ve

Table 5. Antifungal activity for compounds (**4a-h)**

Compounds	*Aspergillus niger*	*Penicillumchrysogenum*
4a	-ve	-ve
4b	-ve	-ve
4c	-ve	-ve
4d	-ve	RG
4e	+ve	-ve
4f	-ve	-ve
4g	-ve	-ve
4h	Not tested	Not tested
Griseofulvin	-ve	-ve
DMSO	+ve	+ve

-ve No growth Antifungal activity
+ve Growth Antifungal activity absent
RG Reduced Growth

Investigation of the structure activity relationship study revealed that compounds **4b, 4d, 4f and 4g** having electron withdrawing (fluoro, bromo and chloro) group on phenyl rings showed significant activity against both Gram-negative and Gram-positive bacteria. Whereas compounds **4a, 4e** having no substitution and electron releasing methoxy group on phenyl rings showed moderate activity.

The investigation of antifungal activity data revealed that compounds **4a, 4b, 4d, 4f** and **4g,** show inhibitory effect against all fungal steins and compound **4e** show inhibitory effect against *Aspergillusniger.* Compound **4d** Showed reduced growth against Penicillum *chrysogenum.*

In-vitro anti-inflammatory activity

The synthesized compounds are screenedfor anti-inflammatory activity by using inhibition ofalbumin denaturation technique, which was studied according to Muzushima and Kabayashi with slight modification[28]. The Ibuprofen was used as standard drugs. Results are tabulated in Table 6.

Table 6. *In-vitro* Anti-inflammatory activity of synthesized compounds **(4a-h)**

S. No.	Compounds	Mean absorbance value ± SEM	Inhibition of denaturation, %
1	Control	0.0810	-
2	Ibuprofen	0.154±0.004	90.12
3	**4a**	0.115±0.003	41.97
4	**4b**	0.138±0.007	70.37
5	**4d**	0.102±0.002	25.92
6	**4e**	0.112±0.003	38.27
7	**4f**	0.134±0.002	65.54
8	**4g**	0.130±0.005	60.49

Anti-inflammatory activity data of compound **(4a-h)** revealed that compound **4b, 4f and 4g** with electron withdrawing fluoro, bromo and chloro substituent exhibited very good anti-inflammatory activity. Whereas, compounds **4a**, **4d** and **4e** showed moderateactivity.

Conclusion

In this study we have reported an effective and convenient synthesis of new 2-phenethyl-1 *H*-benzimidazole derivatives **(4a-h)**. Synthesized compounds were evaluated for their antibacterial, antifungal and anti-inflammatory activities. The results of these studies proved that many of the synthesized derivatives having substitutions with the electron withdrawing (fluoro, bromo, chloro) group on the phenyl ring exhibited potential antibacterial, antifungal and anti-inflammatory activity.

Acknowledgement

Author thanks to SAIF, Chandigarh for providing IR and ^{1}H NMR spectral facilities and CDRI Lucknow for providing mass spectral facilities.

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