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Original article

Synthesis, anti-bacterial, anti-asthmatic and anti-diabetic activities of novel *N*-substituted-2-(4-phenylethynyl-phenyl)-1*H*-benzimidazoles and *N*-substituted 2[4-(4,4-dimethyl-thiochroman-6-yl-ethynyl)-phenyl)-1*H*-benzimidazoles

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Abstract

Synthesis of a series of novel and functionalized benzimidazole derivatives by the condensation of OPDA with 4-bromobenzoic acid and subsequent reactions of the product obtained with phenylacetylene and 6-ethynyl-4,4-dimethylthiochroman utilising Sonogashira coupling has been reported. The Sonogashira coupling products were then alkylated at the benzimidazole −NH with different electrophilic reagents leading to functionalized derivatives. All the compounds synthesized were screened for their potential anti-bacterial, anti-asthmatic and anti-diabetic properties, which exhibited moderate activities in screening studies in vitro.

Keywords: Anti-bacterial; Anti-asthmatic; Anti-diabetic; Alkylations; Acylations; Benzimidazole; Sonogashira coupling

1. Introduction

Benzimidazoles and their analogs are well known biologically active *N*-containing heterocycles [1], widely used as drugs such as proton pump inhibitor Omeprazole [2,3], anti-helmentic Albendazole [4,5], anti-dopaminergic Domperidone [6,7], anti-psychotic Pimozide [8,9], etc. Some of their analogs are the constitutional parts of the marine alkaloids, such as kealiiquinone and anti-tumor agents such as pyrrolo[1,2-*a*] benzimidazole quninone (APBI-A) [10,11] (Fig. 1). Specifically, the 2-substituted analogs of benzimidazoles are known to be potent biologically active compounds [12–14].

Some of the important benzimidazole derivatives have been reported as thyroid receptor agonists [15], gonadotropin releasing hormone receptor antagonists [16], non-nucleoside HIV-1 reverse transcriptase inhibitors [17] and interestingly alkynylbenzimidazoles as modulators of metabotropic glutamate receptors [18]. The biological activities exhibited by compounds containing benzimidazole moiety has prompted chemists to synthesis more and more benzimidazole libraries and screen them for potential activities [19–21]. Owing to the importance and in continuation of our ongoing project on bioactive benzimidazole libraries [22–26], we now wish to describe our efforts towards the synthesis of a novel class of alkynylbenzimidazole derivatives and their biological activity screening studies.

2. Results and discussion

To realize the synthesis of novel alkynylbenzimidazoles, initially we have carried out the condensation of *o*-phenylenediamine (OPDA) (**1**) with 4-bromobenzoic acid (**2**) in

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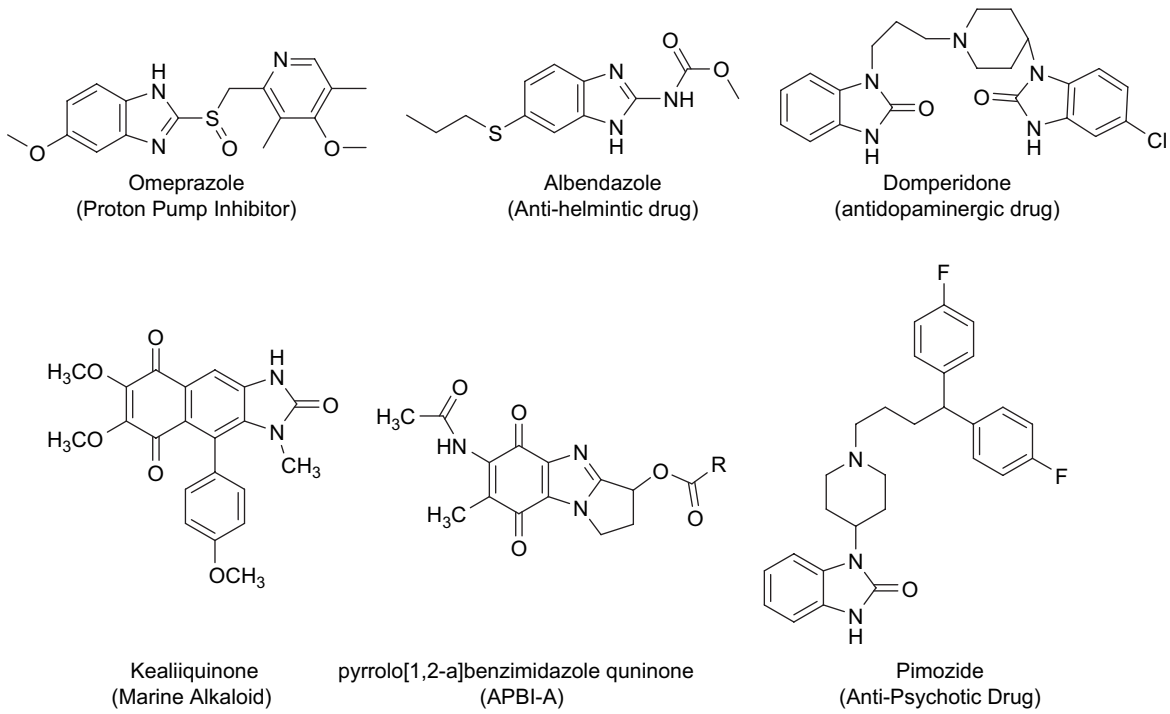


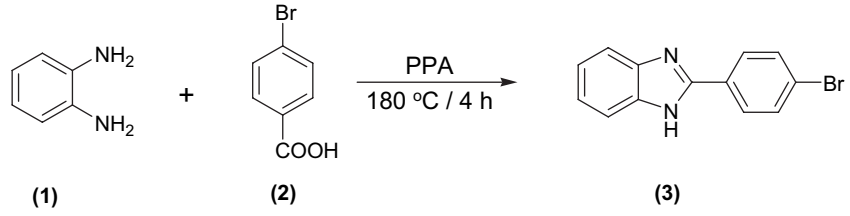
Fig. 1. Some of the benzimidazole based drugs and natural products.

polyphosphoric acid (PPA) at 180 °C for 4 h following a simple work-up to obtain the known 2-(4-bromophenyl)-1*H*-benzimidazole (**3**) [27] (Scheme 1). It is noteworthy to mention here that we have synthesized compound **3** alternatively by a couple of other methods such as by microwave irradiation of a mixture of **1** and **2** in presence of PPA and also *via* Eaton's reagent [28] in comparable yields, which gives scope for the alternate routes either to synthesize benzimidazoles at low temperatures or in less reaction times, unlike conventional methods which involve high temperatures and long reaction times.

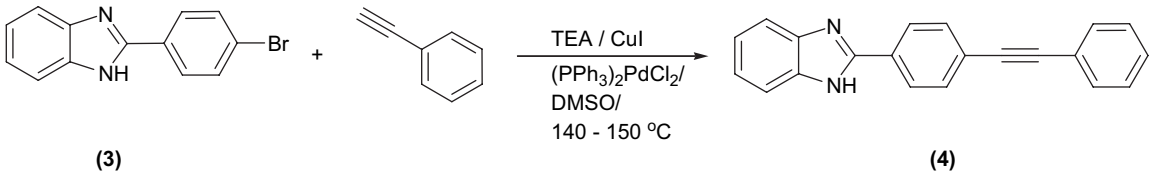
Having obtained compound **3**, we have carried out its reaction with phenylacetylene in the presence of CuI, triethylamine and $Pd(Ph_3P)_2Cl_2$ as catalyst under Sonogashira coupling conditions [29] to obtain 2-(4-(2-phenylethynyl)phenyl)-1*H*-benzimidazole (**4**) based on its spectral data assignments (Scheme 2).

To generate the analogs of **4**, we then carried out the alkylation, acylation and sulfonation reactions of **4** at the benzimidazole −NH and obtained the corresponding *N*-substituted analogs **6a**−**j**, respectively (Scheme 3) (Table 1).

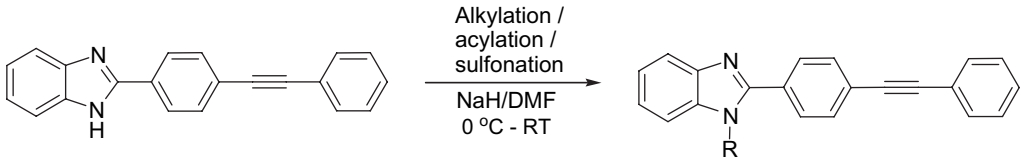
To further extend the synthesis of alkynylbenzimidazoles, we thought that it would be worth trying the Sonogashira coupling of compound **3** with 6-ethynyl-4,4-dimethylthiochroman [30], i.e., the key intermediate of Tazarotene [31], a well



Scheme 1.



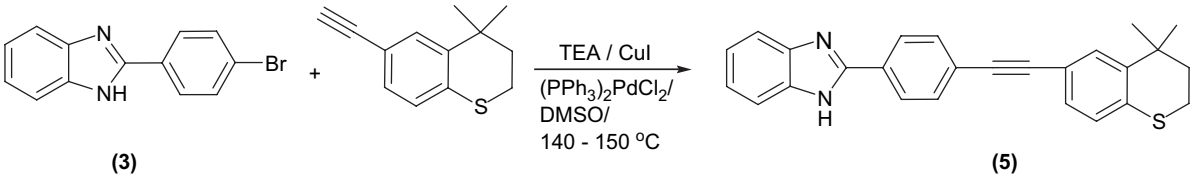
Scheme 2.



(4) **(6a-6j)**

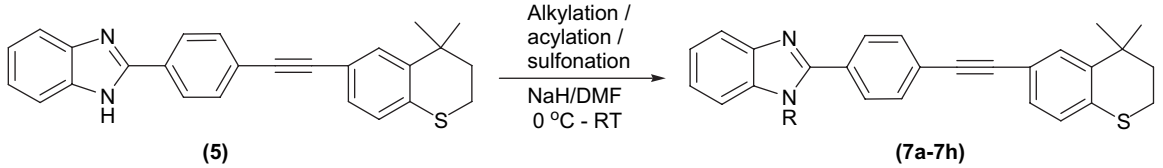
6a, R = Methyl; **6b**, R = Ethyl; **6c**, R = Propyl; **6d**, R = Butyl; **6e**, R = Methyloxycarbonyl
6f, R = Ethyloxycarbonyl; **6g**, R = Butyloxycarbonyl; **6h**, R = Isobutyloxycarbonyl;
6i, R = Trifluoromethanesulfonyl; **6j**, R = 4-Methylphenylsulfonyl.

Scheme 3.



(3) **(5)**

Scheme 4.



(5) **(7a-7h)**

7a, R = Methyl; **7b,** R = Ethyl; **7c**, R = Propyl; **7d**, R = Butyl; **7e**, R = Methyloxycarbonyl;
7f, R = Ethyloxycarbonyl; **7g**, R = Butyloxycarbonyl; **7h**, R = Trifluoromethanesulfonyl.

Scheme 5.

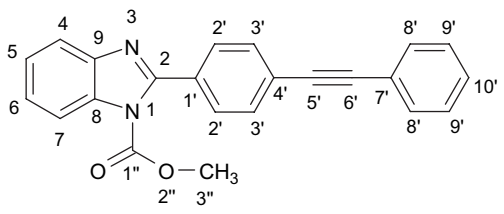
known anti-acne drug used for psoriasis, i.e., to obtain 2-[4-(4,4-dimethylthiochroman-6-yl-ethynyl)-phenyl]-1*H*-benzimidazole (**5**) (Scheme 4).

Compound **5** on alkylation, acylation and sulfonation reactions yielded the corresponding *N*-substituted analogs **7a**–**h**, respectively (Scheme 5) (Table 1).

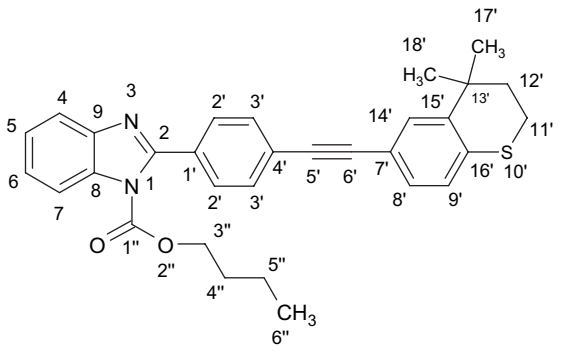
Following numbering has been followed for NMR assignment.

2.1. Biological activity

All the compounds prepared herein were screened for their potential biological activities such as, anti-bacterial, anti-asthmatic and anti-diabetic activities. The anti-bacterial activity was carried out using both *Staphylococcus aureus* (Gram positive) and *Salmonella typhimurium* (Gram negative) bacteria. The compounds were added to the medium as dimethyl



(6e)



(7g)

Table 1
Yields, m.p., ^{1}H NMR, ^{13}C NMR and EI-MS spectroscopic data for the compounds[a]

Compound	Yield (%)	M.p. (°C)	^{1}H NMR (δ, ppm)	^{13}C NMR (δ, ppm)	EI-MS (M + 1)
4[b] $C_{21}H_{14}N_2$ 2-(4-Phenylethynylphenyl)-1*H*-benzimidazole	92	282–283	7.23–7.26 (m, 2H, Ar–H), 7.45–7.48 (m, 3H, Ar–H), 7.60–7.66 (m, 4H, Ar–H), 7.76 (d, $J = 8.1$ Hz, 2H, 3′-Ar–H), 8.26 (d, $J = 8.1$ Hz, 2H, 2′-Ar–H), 13.10 (s, 1H, –NH)	89.29 (acetylenic carbon), 91.30 (acetylenic carbon), 122.29, 122.60, 123.65, 126.85, 129.04, 129.24, 130.33, 131.67, 132.17, 150.62 (aromatic carbons)	295.5
5[b] $C_{26}H_{22}N_2S$ 2-[4-(4,4-Dimethyl-thiochroman-6-yl-ethynyl)-phenyl]-1*H*-benzimidazole	90	272–274	1.30 (s, 6H, 17′ and 18′-CH_3, 1.89–1.91 (m, 2H, 12′-CH_2), 3.06–3.07 (m, 2H, 11′–CH_2), 7.11 (d, $J = 8.1$ Hz, 1H, Ar–H), 7.24–7.26 (m, 3H, Ar–H), 7.63 (bs, 3H, Ar–H), 7.73 (d, $J = 8.1$ Hz, 2H, 3′-Ar–H), 8.25 (d, $J = 8.1$ Hz, 2H, 2′-Ar–H), 13.10 (s, 1H, –NH)	22.66, 29.84, 32.84, 36.79 (aliphatic carbons), 88.24 (acetylenic carbon), 91.91 (acetylenic carbon), 117.60, 122.60, 123.98, 126.75, 126.83, 129.01, 129.84, 130.05, 132.01, 133.78, 142.55, 150.69 (aromatic carbons)	395.5
6a $C_{22}H_{16}N_2$ 1-Methyl-2-(4-phenylethynyl-phenyl)-1*H*-benzimidazole	87	153–155	3.91 (s, 3H, 1″-CH_3), 7.30–7.43 (m, 6H, Ar–H), 7.55–7.58 (m, 2H, Ar–H), 7.69 (d, $J = 9$ Hz, 2H, Ar–H), 7.78–7.85 (m, 3H, Ar–H)	31.76 (–CH_3), 88.73 (acetylenic carbon), 91.29 (acetylenic carbon), 109.62, 119.85, 122.55, 122.82, 122.95, 124.70, 128.38, 128.55, 129.29, 129.73, 131.64, 131.78, 136.61, 142.91, 152.96 (aromatic carbons)	309
6b $C_{23}H_{18}N_2$ 1-Ethyl-2-(4-phenylethynyl-phenyl)-1*H*-benzimidazole	90	98–99	1.50 (t, $J = 7.2$ Hz, 3H, 2″-CH_3), 4.32 (q, $J = 7.2$ Hz, 2H, 1″-CH_2), 7.31–7.46 (m, 6H, Ar–H), 7.55–7.58 (m, 2H, Ar–H), 7.67–7.76 (m, 4H, Ar–H), 7.83–7.86 (m, 1H, Ar–H)	15.23 (–CH_3), 39.63 (–CH_2–), 88.69 (acetylenic carbon), 91.23 (acetylenic carbon), 109.90, 119.97, 122.44, 122.83, 124.69, 128.36, 128.54, 129.09, 130.09, 131.61, 131.82, 135.42, 143.15, 152.61 (aromatic carbons)	323
6c $C_{24}H_{20}N_2$ 1-Propyl-2-(4-phenylethynyl-phenyl)-1*H*-benzimidazole	95	85	0.88 (t, $J = 7.8$ Hz, 3H, 3″-CH_3), 1.83–1.90 (m, 2H, 2″-CH_2). 4.22 (t, $J = 7.8$ Hz, 2H, 1″-CH_2), 7.30–7.45 (m, 6H, Ar–H), 7.55–7.58 (m, 2H, Ar–H), 7.67–7.74 (m, 4H, Ar–H), 7.82–7.85 (m, 1H, Ar–H)	11.06 (–CH_3), 22.99 (–CH_2–), 46.19 (–N–CH_2–), 88.62 (acetylenic carbon), 91.13 (acetylenic carbon), 110.03, 119.80, 122.28, 122.68, 124.48, 128.25, 128.42, 129.06, 130.18, 131.49, 131.69, 135.58, 142.95, 152.77 (aromatic carbons)	337
6d $C_{25}H_{22}N_2$ 1-Butyl-2-(4-phenylethynyl-phenyl)-1*H*-benzimidazole	92	88	0.88 (t, $J = 7.2$ Hz, 3H, 4″-CH_3), 1.25–1.32 (m, 2H, 3″-CH_2), 1.76–1.83 (m, 2H, 2″-CH_2), 4.25 (t, $J = 7.5$ Hz, 2H, 1″-CH_2), 7.26–7.44 (m, 6H, Ar–H), 7.55–7.74 (m, 6H, Ar–H), 7.82–7.85 (m, 1H, Ar–H)	13.50 (–CH_3), 19.85 (–CH_2–), 31.79 (–CH_2–), 44.55 (–N–CH_2–), 88.71 (acetylenic carbon), 91.22 (acetylenic carbon), 110.10, 119.96, 122.43, 122.81, 124.64, 128.38, 128.55, 129.20, 130.24, 131.63, 131.81, 135.63, 143.05, 152.88 (aromatic carbons)	351.1
6e $C_{23}H_{16}N_2O_2$ 2-(4-Phenylethynyl-phenyl)-benzimidazole-1-carboxylicacid methyl ester	89	148–150	3.95 (s, 3H, 3″-CH_3), 7.36–7.43 (m, 5H, Ar–H), 7.55–7.69 (m, 6H, Ar–H), 7.79–7.83 (m, 1H, Ar–H), 8.01–8.04 (m, 1H, Ar–H)	54.21 (–OCH_3), 88.87 (acetylenic carbon), 91.14 (acetylenic carbon), 114.81, 120.26, 122.85, 124.81, 125.32, 128.32, 128.46, 129.29, 130.99, 131.13, 131.59, 133.42, 142.63, 150.60, 153.06 (aromatic carbons and carbonyl carbon)	353
6f $C_{24}H_{18}N_2O_2$ 2-(4-Phenylethynyl-phenyl)-benzimidazole-1-carboxylicacid ethyl ester	90	126–127	1.28 (t, $J = 7.2$ Hz, 3H, 4″-CH_3), 4.40 (q, $J = 7.2$ Hz, 2H, 3″-CH_2), 7.36–7.43 (m, 5H, Ar–H), 7.55–7.69 (m, 6H, Ar–H), 7.79–7.82 (m, 1H, Ar–H), 8.03–8.06 (m, 1H, Ar–H)	13.80 (–CH_3), 64.13 (–O–CH_2–), 88.91 (acetylenic carbon), 91.06 (acetylenic carbon), 114.86, 120.25, 122.88, 124.75, 125.29, 128.34, 128.48, 129.35, 130.95, 131.40, 131.61, 133.56, 142.66, 150.04, 153.10 (aromatic carbons and carbonyl carbon)	367
6g $C_{26}H_{22}N_2O_2$ 2-(4-Phenylethynyl-phenyl)-benzimidazole-1-carboxylicacid butyl ester	91	134–135	0.89 (t, $J = 7.35$ Hz, 3H, 6″-CH_3), 1.19–1.27 (m, 2H, 5″-CH_2), 1.54–1.64 (m, 2H, 4″-CH_2), 4.34 (t, $J = 6.6$ Hz, 2H, 3″-CH_2), 7.35–7.45 (m, 5H, Ar–H), 7.54–7.68 (m, 6H, Ar–H), 7.79–7.82 (m, 1H, Ar–H), 8.04–8.07 (m, 1H, Ar–H)	13.50 (–CH_3), 18.88 (–CH_2–), 30.17 (–CH_2–), 67.99 (–OCH_2–) 88.87 (acetylenic carbon), 91.02 (acetylenic carbon), 114.85, 120.24, 122.90, 124.73, 125.30, 128.33, 128.46, 129.32, 130.96, 131.49, 131.61, 133.61, 142.65, 150.15, 153.07 (aromatic carbons and carbonyl carbon)	395.1

(*continued on next page*)

Table 1 (*continued*)

Compound	Yield (%)	M.p. (°C)	^{1}H NMR (δ, ppm)	^{13}C NMR (δ, ppm)	EI-MS (M + 1)
6h $C_{26}H_{22}N_2O_2$ 2-(4-Phenylethynyl-phenyl)-benzimidazole-1-carboxylicacid isobutyl ester	92	102–103	0.84 (d, $J = 7.2$ Hz, 6H, 5″-CH_3), 1.86–1.95 (m, 1H, 4″-CH), 4.13 (d, $J = 6.6$ Hz, 2H, 3″-CH_2), 7.35–7.45 (m, 5H, Ar–H), 7.55–7.69 (m, 6H, Ar–H), 7.80–7.83 (m, 1H, Ar–H), 8.05–8.08 (m, 1H, Ar–H)	18.76 (–CH_3), 27.43 (–CH–), 74.15 (–O–CH_2–), 88.89 (acetylenic carbon), 91.00 (acetylenic carbon), 114.83, 120.22, 122.84, 124.71, 125.28, 128.29, 128.43, 129.28, 130.99, 131.48, 131.57, 133.60, 142.62, 150.14, 153.04 (aromatic carbons and carbonyl carbon)	395
6i $C_{22}H_{13}F_3N_2O_2S$ 2-(4-Phenylethynyl-phenyl)-1-trifluoromethanesulfonyl-1*H*-benzimidazole	92	188–189	7.36–7.39 (m, 3H, Ar–H), 7.50–7.58 (m, 4H, Ar–H), 7.64–7.70 (m, 4H, Ar–H), 7.85–7.88 (m, 1H, Ar–H), 7.93–7.96 (m, 1H, Ar–H)	88.52 (acetylenic carbon), 91.90 (acetylenic carbon), 114.65, 117.22, 121.18, 121.54, 122.74, 126.27, 126.67, 126.79, 128.05, 128.39, 128.66, 130.43, 131.01, 131.69, 133.00, 142.33, 153.09 (aromatic carbons)	427.5
6j $C_{28}H_{20}N_2O_2S$ 2-(4-Phenylethynyl-phenyl)-1-(toluene-4-sulfonyl)-1*H*-benzimidazole	80	169–170	2.31 (s, 3H, 5″-CH_3–Ar), 7.10 (d, $J = 8.1$ Hz, 2H, Ar–H), 7.32–7.44 (m, 7H, Ar–H), 7.56–7.63 (m, 6H, Ar–H), 7.71–7.74 (m, 1H, Ar–H), 8.20–8.22 (m, 1H, Ar–H)	21.58 (–CH_3), 88.83 (acetylenic carbon), 91.53 (acetylenic carbon), 115.21, 120.39, 122.83, 125.37, 125.58, 126.88, 128.40, 128.61, 129.56, 129.72, 130.76, 130.85, 131.68, 133.90, 134.84, 142.66, 145.78, 153.48 (aromatic carbons)	449.3
7a $C_{27}H_{24}N_2S$ 1-Methyl 2[4-(4,4-dimethyl-thiochroman-6-yl-ethynyl)-phenyl]-1*H*-benzimidazole	86	197–198	1.36 (s, 6H, 17′ and 18′-CH_3), 1.95–1.99 (m, 2H, 12′-CH_2), 3.03–3.08 (m, 2H, 11′-CH_2), 3.90 (s, 3H, 1″-CH_3), 7.08 (d, $J = 8.4$ Hz, 1H, Ar–H), 7.20–7.42 (m, 4H, Ar–H), 7.55–7.56 (m, 1H, Ar–H), 7.67 (d, $J = 8.4$ Hz, 2H, 3′-Ar–H), 7.78 (d, $J = 8.4$ Hz, 2H, 2′-Ar–H), 7.82–7.85 (m, 1H, Ar–H)	23.16, 29.93, 31.75, 32.91, 37.12 (aliphatic carbons), 88.08 (acetylenic carbon), 91.83 (acetylenic carbon), 109.60, 118.05, 119.83, 122.51, 122.90, 124.92, 126.53, 128.95, 129.25, 129.49, 129.70, 131.63, 133.50, 136.62, 142.06, 142.94, 153.01 (aromatic carbons)	409
7b $C_{28}H_{26}N_2S$ 1-Ethyl 2[4-(4,4-dimethyl-thiochroman-6-yl-ethynyl)-phenyl]-1*H*-benzimidazole	90	166–167	1.36 (s, 6H, 17′ and 18′-CH_3), 1.50 (t, $J = 7.2$ Hz, 3H, 2″-CH_3), 1.95–1.99 (m, 2H, 12′-CH_2), 3.04–3.08 (m, 2H, 11′-CH_2), 4.32 (q, $J = 7.2$ Hz, 2H, 1″-CH_2), 7.08 (d, $J = 8.4$ Hz, 1H, Ar–H), 7.20–7.23 (m, 1H, Ar–H), 7.29–7.36 (m, 2H, Ar–H), 7.43–7.46 (m, 1H, Ar–H), 7.55–7.56 (m, 1H, Ar–H), 7.67 (d, $J = 8.4$ Hz, 2H, 3′-Ar–H), 7.73 (d, $J = 8.4$ Hz, 2H, 2′-Ar–H), 7.81–7.85 (m, 1H, Ar–H)	15.19, 23.09, 29.87, 32.84, 37.03, 39.58 (aliphatic carbons), 88.00 (acetylenic carbon), 91.75 (acetylenic carbon), 109.86, 117.98, 119.88, 122.37, 122.77, 124.86, 126.47, 128.88, 129.02, 129.64, 129.76, 131.63, 133.45, 135.37, 141.99, 143.09, 152.60 (aromatic carbons)	423.1
7c $C_{29}H_{28}N_2S$ 1-Propyl 2[4-(4,4-dimethyl-thiochroman-6-yl-ethynyl)-phenyl]-1*H*-benzimidazole	91	118–119	0.88 (t, $J = 7.2$ Hz, 3H, 3″-CH_3), 1.36 (s, 6H, 17′ and 18′-CH_3), 1.80–1.90 (m, 2H, 2″-CH_2), 1.97 (t, $J = 6.0$ Hz, 2H, 12′-CH_2) 3.06 (t, $J = 6.0$ Hz, 2H, 11′-CH_2), 4.21 (t, $J = 7.65$ Hz, 2H, 1″-CH_2), 7.08 (d, $J = 8.1$ Hz, 1H, Ar–H), 7.20–7.36 (m, 4H, Ar–H), 7.41–7.44 (m, 1H, Ar–H), 7.55–7.61 (m, 1H, Ar–H), 7.65–7.73 (m, 3H, Ar–H), 7.81–7.84 (m, 1H, Ar–H)	11.21, 23.17, 29.95, 32.92, 37.13, 46.38 (aliphatic carbons), 88.04 (acetylenic carbon), 91.75 (acetylenic carbon), 110.09, 118.06, 119.95, 122.41, 122.78, 124.87, 126.54, 128.94, 129.18, 129.72, 130.03. 130.78, 131.70, 131.94, 133.48, 135.69, 142.06, 143.07, 153.01 (aromatic carbons)	437.1
7d $C_{30}H_{30}N_2S$ 1-Butyl 2[4-(4,4-dimethyl-thiochroman-6-yl-ethynyl)-phenyl]-1*H*-benzimidazole	88	127–128	0.88 (t, $J = 7.5$ Hz, 3H, 4″-CH_3), 1.25–1.33 (m, 2H, 3″-CH_2), 1.36 (s, 6H, 17′ and 18′-CH_3), 1.76–1.84 (m, 2H, 2″-CH_2), 1.95–1.99 (m, 2H, 12′-CH_2), 3.04–3.08 (m, 2H, 11′-CH_2), 4.25 (t, $J = 7.5$ Hz, 2H, 1″-CH_3), 7.08 (d, $J = 8.1$ Hz, 1H), 7.20–7.35 (m, 4H), 7.41–7.44 (m, 1H), 7.55–7.56 (m, 1H), 7.66–7.74 (m, 3H), 7.82–7.85 (m, 1H)	13.47, 19.85, 23.13, 29.91, 31.75, 32.88, 37.09, 44.50 (aliphatic carbons), 88.03 (acetylenic carbon), 91.75 (acetylenic carbon), 110.07, 118.02, 119.90, 122.38, 122.75, 124.86, 126.51, 128.91, 129.15, 129.68, 129.94, 131.66, 133.48, 135.62, 142.03, 143.01, 152.90 (aromatic carbons)	451.1
7e $C_{28}H_{24}N_2O_2S$ 2[4-(4,4-Dimethyl-thiochroman-6-yl-ethynyl)-phenyl]-benzimidazole-1-carboxylicacid methyl ester	90	108–109	1.34 (s, 6H, 17′ and 18′-CH_3), 1.93–1.97 (m, 2H, 12′-CH_2), 3.01–3.06 (m, 2H, 11′-CH_2), 3.93 (s, 3H, 3″-CH_3), 7.06 (d, $J = 8.1$ Hz, 1H, Ar–H), 7.19–7.22 (m, 1H, Ar–H), 7.36–7.43 (m, 2H, Ar–H), 7.54–7.55 (m, 1H, Ar–H), 7.60–7.68 (m, 4H, Ar–H), 7.78–7.81 (m, 1H, Ar–H), 7.98–8.02 (m, 1H, Ar–H)	23.14, 29.91, 32.89, 37.11 (aliphatic carbons), 54.22 (–O–CH_3), 88.21 (acetylenic carbon), 91.70 (acetylenic carbon), 114.81, 118.09, 120.25, 124.82, 125.07, 125.32, 126.50, 128.93, 129.27, 129.68, 130.87, 133.41, 133.45, 142.02, 142.64, 150.64, 153.13 (aromatic carbons and carbonyl carbon)	453

Table 1 (*continued*)

Compound	Yield (%)	M.p. (°C)	^{1}H NMR (δ, ppm)	^{13}C NMR (δ, ppm)	EI-MS (M + 1)
7f $C_{29}H_{26}N_2O_2S$ 2[4-(4,4-Dimethyl-thiochroman-6-yl-ethynyl)-phenyl]-benzimidazole-1-carboxylicaci ethyl ester	88	102–103	1.26 (t, $J = 7.2$ Hz, 3H, 4″-CH_3), 1.34 (s, 6H, 17′ and 18′-CH_3), 1.92–1.97 (m, 2H, 12′-CH_2), 3.01–3.05 (m, 2H, 11′-CH_2), 4.37 (q, $J = 7.2$ Hz, 2H, 3″-CH_2), 7.06 (d, $J = 8.1$ Hz, 1H, Ar–H), 7.19–7.22 (m, 1H, Ar–H), 7.38–7.41 (m, 2H, Ar–H), 7.54–7.55 (m, 1H, Ar–H), 7.59–7.67 (m, 4H, Ar–H), 7.78–7.81 (m, 1H, Ar–H), 8.02–8.05 (m, 1H, Ar–H)	13.78, 23.12, 29.89, 32.87, 37.09 (aliphatic carbons) 64.10 (O–CH_2–), 88.23 (acetylenic carbon), 91.59 (acetylenic carbon), 114.81, 118.08, 120.19, 124.72, 124.97, 125.25, 126.48, 128.91, 129.30, 129.65, 130.79, 131.08, 133.39, 133.54, 142.00, 142.60, 150.01, 153.13 (aromatic carbons and carbonyl carbon)	467.1
7g $C_{31}H_{30}N_2O_2S$ 2[4-(4,4-Dimethyl-thiochroman-6-yl-ethynyl)phenyl]-benzimidazole-1-carboxylicacid butyl ester	98	110–111	0.89 (t, $J = 7.2$ Hz, 3H, 6″-CH_3), 1.19–1.29 (m, 2H, 5″-CH_2), 1.36 (s, 6H, 17′ and 18′-CH_3), 1.54–1.64 (m, 2H, 4″-CH_2), 1.95–1.99 (m, 2H, 12′-CH_2), 3.03–3.08 (m, 2H, 11′-CH_2), 4.34 (t, $J = 6.6$ Hz, 2H, 3″-CH_2), 7.07 (d, $J = 8.1$ Hz, 1H, Ar–H), 7.19–7.23 (m, 1H, Ar–H), 7.37–7.44 (m, 2H, Ar–H), 7.54–7.55 (m, 1H, Ar–H), 7.59–7.67 (m, 4H, Ar–H), 7.79–7.82 (m, 1H, Ar–H), 8.03–8.07 (m, 1H, Ar–H)	13.53, 18.91, 23.19, 29.95, 30.21, 32.94, 37.17 (aliphatic carbons) 68.03 (–O–CH_2–), 88.24 (acetylenic carbon), 91.59 (acetylenic carbon), 114.88, 118.18, 120.27, 124.77, 125.06, 125.33, 126.54, 128.99, 129.33, 129.70, 130.88, 131.22, 133.41, 133.65, 142.06, 142.67, 150.22, 153.19 (aromatic carbons and carbonyl carbons)	495.1
7h $C_{27}H_{21}F_3N_2O_2S_2$ 2[4-(4,4-Dimethyl-thiochroman-6-yl-ethynyl)-phenyl]-1-trifluoromethanesulfonyl-1*H*-benzimidazole	90	158–159	1.34 (s, 6H, 17′ and 18′-CH_3), 1.93–1.97 (m, 2H, 12′-CH_2), 3.02–3.06 (m, 2H, 11′-CH_2), 7.07 (d, $J = 8.1$ Hz, 1H, Ar–H), 7.19–7.25 (m, 1H, Ar–H), 7.47–7.55 (m, 3H, Ar–H), 7.62–7.69 (m, 4H, Ar–H), 7.84–7.87 (m, 1H, Ar–H), 7.92–7.95 (m, 1H, Ar–H)	23.15, 29.63, 29.90, 32.89, 37.09 (aliphatic carbons) 87.87 (acetylenic carbon), 92.49 (acetylenic carbon), 114.62, 117.19, 117.92, 121.13, 121.50, 126.47, 126.53, 126.61, 126.75, 127.73, 128.96, 129.73, 130.39, 130.83, 132.97, 133.67, 142.04, 142.31, 153.13 (aromatic carbons)	527

[a] All the new compounds prepared gave satisfactory elemental analysis data.
[b] ^{1}H NMR spectra for compounds **4** and **5** were recorded in DMSO-d_6 and for the rest of the compounds in $CDCl_3$.

sulfoxide solutions. No inhibition zone was observed in controls (i.e., for DMSO). The concentrations used were as follows: 500, 200, 100, 10, 1.0 and 0.1 μg/ml. Minimum Inhibitory Concentration values were determined after incubation at 37 °C for 48 h and was determined using tube dilution method according to the standard procedure [32]. Cephalexin was used as the anti-bacterial standard and dimethyl sulfoxide was used both as a solvent and as a control (see Tables 2 and 3).

The anti-asthmatic activity studies were carried out using Phosphodiesterase IV enzyme (PDE-IV) [33] and the primary screening of the compounds was done at 1 nM concentration using human PDE-IV enzyme, where Rolipram and Ariflo were used as standard compounds. (Table 4).

The anti-diabetic activity screening was carried out with dipeptidyl peptidase (DPP-IV) [34] enzyme and the primary screening of the compounds was carried out at 300 nM concentration using recombination human DPP-IV enzyme by the use of 1-(2-amino-3,3-dimethylbutanoyl) pyrrolidine-2-carbonitrile as the standard compound at 100 nM. (see Table 4). Similarly, the PTP-1B (in-house compound, also for anti-diabetic) activity was done using the test compounds at 30 μM with the standard compound *N*-[5-[*N*-acetyl-4-[*N*-(2-carboxyphenyl)-*N*-(2-hydroxyoxalyl)amino]-3-ethyl-DL-phenylalanylamino] pentanoyl]-L-methionine at a concentration of 0.3 μM. (Table 4).

2.1.1. Protocol for PDE-IV inhibition assay

Phosphodiesterase IV enzyme converts [^{3}H] cAMP to the corresponding [^{3}H] 5′-AMP in proportion to the amount of Phosphodiesterase IV present. The [^{3}H] 5′-AMP then was quantitatively converted to free [^{3}H] adenosine and phosphate by the action of snake venom 5′-nucleotidase hence the amount of [^{3}H] adenosine liberated is proportional to Phosphodiesterase IV activity.

The assay was performed at 34 °C in a 200 μl total reaction mixture. The reaction mixture contained 25 mM of tris buffer, 10 mM $MgCl_2$, 1 μM cAMP (cold) and [^{3}H] cAMP (0.1 μCi). Stock solutions of the compounds to be investigated were prepared in dimethyl sulfoxide in concentrations such that the dimethyl sulfoxide content in the test samples did not exceed 0.05% by volume to avoid affecting the Phosphodiesterase IV activity. Compounds were then added in the reaction mixture (25 μl/tube). The assay was initiated by addition of enzyme mix (75 μl) and the mixture was incubated for 20 min at 34 °C. The reaction was stopped by boiling the tubes for 2 min at 100 °C in a water bath. After cooling on ice for 5 min and addition of 50 μg 5′-nucleotidase snake venom from *Crotalus atrox*, incubation was carried out again for 20 min at 34 °C. The unreacted substrate was separated from (^{3}H) adenosine by addition of Dowex AG 1X-8 (400 μl), which was pre-equilibrated in (1:1) water:ethanol. Reaction mixture was then thoroughly mixed, placed on ice for 15 min, vortexed and centrifuged at 14,000 rpm. for 2 min. After centrifugation, a sample of the supernatant (150 μl) was taken and added in 24-well optiplates containing scintillant (1 ml) and mixed well. The samples in the plates were then determined for radioactivity in a Top Counter and the Phosphodiesterase IV activity was calculated. Phosphodiesterase IV enzyme was present in

Table 2
Anti-bacterial activity of compounds against *Staphylococcus aureus*

Compound no.	Concentration						
	0.1 μg/ml	1 μg/ml	10 μg/ml	100 μg/ml	200 μg/ml	500 μg/ml	APP. MIC[a] (μg/ml)
4	++	++	++	P	−	−	200
5	++	++	+	P	−	−	200
6a	++	++	++	+	P	−	500
6b	++	++	++	++	+	−	500
6c	++	++	++	+	P	−	500
6d	++	++	+	+	P	−	500
6e	++	++	+	P	P	−	500
6f	++	++	+	+	P	−	500
6g	++	++	++	++	+	−	500
6h	++	++	++	++	+	−	500
6i	++	++	++	++	P	−	500
6j	++	++	+	+	−	−	200
7a	++	++	+	P	−	−	200
7b	++	++	++	+	−	−	200
7c	++	++	++	+	P	−	500
7d	++	+	+	P	−	−	200
7e	++	++	+	+	−	−	200
7f	++	++	++	+	P	−	500
7g	++	++	+	+	P	−	500
7h	++	++	++	+	+	−	500
Cephalexin	++	++	−	−	−	−	10

[a] Approximate Minimum Inhibitory Concentration.

quantities that yield <30% total hydrolysis of substrate (linear assay conditions). Rolipram and Cilomilast were used as standards in all assays.

2.1.2. Protocol for the DPP-IV assay

DPP-IV inhibition measurement in vitro: DPP-IV activity was determined by the cleavage rate of 7-amino-4-methyl coumarin (AMC) from synthetic substrate Gly-Pro-AMC. In brief, the assay was conducted by adding 10 ng of human recombinant dipeptidyl peptidase IV enzyme (DPP-IV, available commercially from R&D Systems) in 50 μl of the assay buffer (25 mM tris, pH 7.4, 140 mM NaCl, 10 mM KCl, 1% BSA) to 96-well black flat bottom microtiter plates. The reaction was initiated by adding 50 μl of 100 μM substrate Gly-Pro-AMC.

Table 3
Anti-bacterial activity screening results of compounds against *Salmonella typhimurium*

Compound no.	Concentration						
	0.1 μg/ml	1 μg/ml	10 μg/ml	100 μg/ml	200 μg/ml	500 μg/ml	APP. MIC (μg/ml)
4	++	++	++	+	−	−	200
5	++	++	+	P	−	−	200
6a	++	++	++	P	−	−	200
6b	++	++	++	++	+	−	500
6c	++	++	+	+	P	−	500
6d	++	++	+	+	P	−	500
6e	++	++	++	+	P	−	500
6f	++	++	+	P	P	−	500
6g	++	++	++	++	P	−	500
6h	++	++	++	++	+	−	500
6i	++	++	++	+	P	−	500
6j	++	++	++	P	−	−	200
7a	++	++	+	P	−	−	200
7b	++	++	++	P	−	−	200
7c	++	++	++	+	P	−	500
7d	++	++	+	P	−	−	200
7e	++	++	+	+	−	−	200
7f	++	++	++	+	+	−	500
7g	++	++	+	+	P	−	500
7h	++	++	++	P	−	−	200
Cephalexin	++	++	+	P	−	−−	200

Symbols: total inhibition, no growth of organism = −; Poor growth compared to control = P; medium growth compared to controls = +; confluent growth, no inhibition = ++.

Table 4
Anti-diabetic and anti-asthmatic activity screening results of compounds

Compound no.	PTP-1B (30 μM)	PDE-IV (1 μM)	DPP-IV (0.3 μM)
	% Inhibition	% Inhibition	% Inhibition
4	1.64	3.40	0
5	2.42	0	0
6a	8.42	22.29	9
6b	2.2	13.52	0
6c	0	8.91	0
6d	0	19.45	3
6e	29	18.22	0
6f	27.67	16.50	0
6g	5.84	16.25	0
6h	17.43	17.21	0
6i	0	16.88	0
6j	0	22.56	0
7a	0	18.20	0
7b	0	15.84	0
7c	7.38	17.28	0
7d	0.15	19.85	5
7e	0.97	17.89	5
7f	0	16.89	7
7g	1.94	21.03	0
7h	0.52	22.56	9

The incubation was carried out in the kinetic mode at 30 °C for 30 min. Fluorescence was measured using Fluorostar at the excitation filter of 380 nm and the emission filter of 460 nm. Test compounds and solvent controls were added as 1 μl additions. Test compounds were dissolved in DMSO and tested at 300 nM concentration. Percentage inhibition was calculated with respect to the solvent control sample (no test compound added). Dipeptidyl peptidase (i.e., anti-diabetic).

2.1.3. Protocol for PTB-1B assay

In-house generated human recombinant enzyme: ~35 ng in assay.
Paranitrophenyle phosphate (SRL144916): 25 mM.
Buffer: Hepes 25 mM, 3 mM DTT, 0.15 M NaOH, 1 mM EDTA, pH 7.4.
Dilution buffer (for enzyme): 2× reaction buffer (3 mM DTT).
DMSO (Calbiochem).
Test compound in DMSO.
DMSO concentration not to exceed 1% in the assay.
Evaluation of the study observation

Protocol

	Blank (μl)	Control (μl)	Test (μl)
DMSO	1	1	–
Compound	–	–	1
Buffer	89	88	88
Enzyme	–	1	1
PNPP	10	10	10
Incubate and continuously monitor at 30 °C for 30 min at 405 nm			
NaOH	100	100	100
Read at 405 nm			

Calculations: activity = % of control;
% inhibition = 100 − activity

2.1.4. Standard compound assay

1. PTP-1B: *N*-[5-[acetyl]-4-[*N*-(2-carboxypheyl)-*N*-(2-hydroxalyl)amino]-3-ethyl-DL-phenylalanylamino]pentanoyl]-L-methionine is used as a standard in all assays and shows percentage inhibition of 49.09% at a concentration of 0.3 μM.
2. PDE-IV: Rolipram and Cilomilast were used as standards in all assays. Rolipram shows percentage inhibition of 67.41% at a concentration of 2 μM. Cilomilast shows percentage inhibition of 45.28% at a concentration of 0.075 μM.
3. DPP-IV: 1-(2-amino-3,3-dimethylbutyryl) pyrrolidine-2-carbonitrile is used as a standard in all assays and shows percentage inhibition of 96% at a concentration of 0.1 μM.

3. Experimental section

Melting points are uncorrected and were recorded on a MRVIS Series, Lab India Instrument. TLC analysis was done using pre-coated silica gel plates and visualization was done using iodine/UV lamp. IR spectra were recorded on a Perkin–Elmer Spectrum One FT-IR spectrometer. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Varian Mercury Vx SWBB 300 MHz spectrometer. Elemental analysis was carried out on a Perkin–Elmer Series–II CHNS/O Analyzer 2400. The EI-MS spectra were recorded using a Thermo Finnigan UK Navigator Sr. No. 30019. The starting materials *o*-phenylenediamine, alkylating, acylating, aroylating agents and phenylacetylene were obtained from commercial suppliers, 6-ethynyl-4,4-dimethylthiochroman was prepared by using a patented procedure [30].

*3.1. Synthesis of 2-(4-bromo-phenyl)-1H-benzimidazole (**3**)*

A mixture of *o*-phenylenediamine (OPDA) (**1**) (5.40 g, 50 mmol), 4-bromobenzoic acid (**2**) (12.06 g, 60 mmol) and polyphosphoric acid (50 ml) was heated slowly to 180 °C for 4 h. The reaction mixture was then cooled and neutralized with ice-cold concentrated potassium hydroxide solution (200 ml) to obtain neutral pH. The solid separated out was filtered, washed with water (3 × 100 ml) and dried under vacuum to afford an off-white solid (2.45 g, 90%). The crude product was recrystallized from hot aq. ethanol to obtain the pure compound **3**. M.p. 295–297 °C (lit. [27] 296–298 °C).

*3.2. Synthesis of compound **3** via microwave irradiation*

A mixture of *o*-phenylenediamine (1.08 g, 10 mmol), 4-bromobenzoic acid (**2**) (2.01 g, 10 mmol) and polyphosphoric acid (10 ml) was stirred and irradiated in a microwave oven at 100 W for 3 min at 170 °C. The reaction mixture was then

cooled to room temperature and neutralized with ice-cold potassium hydroxide solution (50 ml) to obtain neutral pH. The solid separated out was filtered, washed with water (3 × 25 ml) and dried under vacuum to afford an off-white solid (2.48 g, 91%). The crude product was recrystallized from hot aq. ethanol to obtain the pure compound **3**. M.p. 295–296 °C.

3.3. Synthesis of compound *3* via Eaton's reagent

A mixture of *o*-phenylenediamine (1.08 g, 10 mmol), 4-bromobenzoic acid (**2**) (2.01 g, 10 mmol) and Eaton's reagent (15 ml, 1:10 mixture of P_2O_5–MSA) was heated with stirring at 100 °C for 5 h. The reaction was then quenched with aqueous saturated bicarbonate solution till neutral pH and extracted with ethyl acetate (3 × 50 ml). The organic layer was washed with water (2 × 25 ml), brine (2 × 25 ml) and dried over anhydrous magnesium sulfate and concentrated under vacuum to obtain compound **3** (2.42 g, 89%), which was recrystallized from hot aq. ethanol to obtain pure compound **3** (m.p. 295–296 °C).

3.4. General procedure for the synthesis of *4* and *5* by Sonogashira coupling

A mixture of triphenylphosphine (0.067 g, 0.255 mmol) and palladium chloride (0.022 g, 0.124 mmol) in dimethyl sulfoxide (10 ml) was heated to 140–150 °C for 10 min under argon atmosphere to obtain a clear solution. The reaction mixture was then allowed to cool to room temperature and then slowly added to this solution, a mixture of **3** (1.265 g, 4.65 mmol) and, respectively, acetylene (5.58 mmol), triethylamine (0.79 g, 7.82 mmol), copper iodide (0.032 g, 0.168 mmol) in dimethyl sulfoxide (20 ml). The resulting mixture was heated with stirring at 90 °C for 1–3 h. The reaction mixture was then cooled to room temperature and filtered on a celite pad, followed by washing with ethyl acetate (2 × 50 ml). The filtrate was then washed with water (2 × 25 ml), brine (2 × 10 ml) and dried over anhydrous magnesium sulfate. Evaporation of the solvent under vacuum yielded the crude products, which were recrystallized from acetone to obtain pure products (for details see Table 1).

3.5. General procedure for the synthesis of compounds *6a–6j* and *7a–7j*

To a solution of **4** or **5** (2 mmol) in DMF (10 ml) was added sodium hydride (60%) (2.4 mmol) in small portions at 0 °C. After completion of addition, the temperature of the reaction was slowly raised to room temperature and stirred at this temperature for 1 h. The reaction mixture was again cooled to 0 °C and the respective alkylating agent (2.4 mmol) was added. The temperature of the reaction was then allowed to rise to room temperature and stirred at this temperature for 3 h (as monitored by TLC). After completion of the reaction, DMF was evaporated under vacuum and reaction mixture was quenched with water (50 ml). The reaction mixture was then extracted with ethyl acetate (3 × 25 ml), washed with water (2 × 25 ml), brine (10 ml) and dried over anhydrous magnesium sulfate. Ethyl acetate layer was evaporated under vacuum to yield the corresponding *N*-substituted derivatives. The crude products were purified by silica gel flash column chromatography to obtain the pure products (Table 1).

4. Conclusion

In conclusion, we have demonstrated the synthesis of a series of novel substituted benzimidazole derivatives by the condensation of OPDA with 4-bromobenzoic acid followed by Sonogashira couplings and alkyl, acyl and sulfonylation reactions to obtain corresponding substituted benzimidazole derivatives. All the compounds prepared in the present work have been screened for potential anti-bacterial, anti-asthmatic and anti-diabetic activities. The activity studies showed that the compounds exhibited moderate activities towards the testing bacterial stains and enzymes in vitro. Some of the compounds **4**, **5**, **6j**, **7a**, **7b**, **7d** and **7e** showed complete inhibition against both *S. aureus* and *S. typhimurium*. However, compounds **6a**, **7h** showed complete inhibition only against *S. typhimurium*. These compounds were also tested against PDE-IV for potential anti-asthmatic effect, and against DPP-IV and PTP-1B for potential anti-diabetic effects. Unfortunately, the results were disappointing.

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