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Synthesis of Novel Trifluorobenzimidazole Derivatives and Their Study of 5-LOX Inhibition and Brine Shrimp Lethal Bioassay (BSLB)

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ABSTRACT

Here in we design and synthesized novel 2-(trifluoromethyl)-5-methyl-1-((5-aryl-1,3,4-oxadiazol-2-yl)methyl)-1*H*-benzimidazoles. All the synthesized compounds were well characterized by advanced analytical and spectrometrical methods (MS, ^{1}H and ^{13}C NMR) and evaluated for their 5-Lipoxygenase (5-LOX) inhibitory activity. Bio evaluation confirms that compound **7d** exhibiting good inhibition with an IC_{50} value of 8.59 µg/mL against 5-LOX. In addition, the same compounds were further screened for *in vitro* cytotoxicity by Brine Shrimp Lethal Bioassay (BSLB). Compounds **7c** and **7a** showed potent cytotoxicity with an ED_{50} values 7.39 µg/mL and 7.54 µg/mL respectively.

KEY WORDS: BSLB, 5-LOX inhibition, 1,3,4-oxadiazole, Trifluorobenzimidazole.

1. INTRODUCTION

Over the years of active research, benzimidazole has evolved as an important heterocyclic system due to its wide range of biological activities includes antiparasitic, anticonvulsant, analgesics, antihistaminic, antiulcers, antihypertensive, antiviral, anticancer, antifungal, anti inflammatory agent, proton pump inhibitors and anticoagulant[1-6]. Among the benzimidazole derivatives, 2-fluoroalkylbenzimidazoles were widely applicable in the syntheses of pharmaceuticals and agrochemicals because of their ability to improve physiochemical properties, metabolic stabilities and binding potencies relative to their non-fluorinated analogs[7,8]. The 2-fluoroalkylbenzimidazoles were considered to be an important chemical synthon possess a variety of biological effects including antiparasitic[9], antiprotozoan, anticarcinomic[10] as well as antihelminthic[11]. Owing to the immense importance and varied bioactivities exhibited by benzimidazoles, efforts have been made from time to time to synthesize these compounds and screened them for potential biological activities. Also it is well documented that the compounds containing 1,3,4-oxadiazole cores have a broad biological activity spectrum including antimicrobial[12], analgesic[13], anti-inflammatory [14], antiviral[15], anticancer[16] , antihypertensive[17] and anticonvulsant[18]. Examples of compounds containing the 1,3,4-oxadiazole unit currently used in clinical medicine are: Raltegravir an antiretroviral drug[19,20] and Zibotentan an anticancer agent[21]. Looking at the importance of benzimidazole and 1,3,4-oxadiazole nucleus, it was thought that it would be worthwhile to design and synthesize new benzimidazole derivatives bearing 1,3,4-oxadiazole moiety and screen them for potential biological activities. In the present work, we have synthesized some 2-(trifluoromethyl)-5-methyl-1-((5-aryl-1,3,4-oxadiazol-2-yl)methyl)-1*H*-benzimidazoles **(7a-e)** and screened for their 5-LOX inhibitory activity and *in vitro* cytotoxicity by BSLB.

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2. MATERIAL AND METHODS

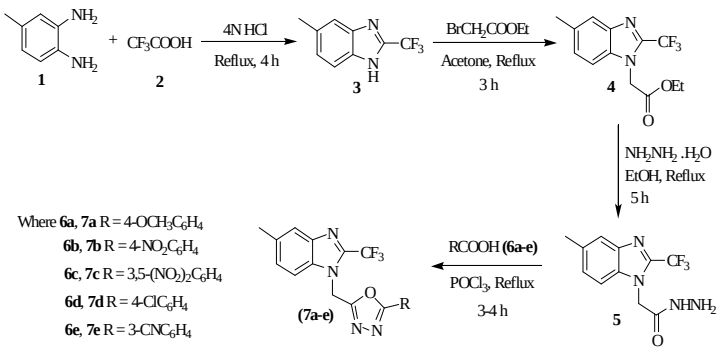
All the chemicals were obtained from Sigma Aldrich and are used without further purification. All melting points were recorded on a Mel-Temp melting point apparatus, in open capillaries and are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded in dimethyl sulfoxide-d_6 or $CDCl_3$ solutions on DZIRE400-vnmrs Spectrometer using TMS as internal standard and the values for chemical shifts (d) being given in ppm and coupling constants (J) in Hertz (Hz). Mass spectra were recorded on Finnigan Matt Mass Spectrometer. All the synthesized compounds were microanalyzed satisfactorily for C, H and N by Elementar Vario EL III elemental analyzer.

3. RESULTS AND DISCUSSION

3.1. Chemistry

The reaction sequence for the synthesized compounds was shown in **Scheme-1**. The compound 2-trifluoromethyl-1*H*-benzimidazole **(3)** was

prepared by the reaction of *o*-phenylenediamine **(1)** with trifluoroacetic acid **(2)**. Nucleophilic substitution of compound **3** yielded ethyl 2-(2-(trifluoromethyl)-5-methyl-1*H*-benzimidazol-1-yl)acetate **(4)**. Which on further treatment with hydrazine hydrate in ethanol at refluxing conditions resulting in the formation of 2-(2-(trifluoromethyl)-5-methyl-1*H*-benzimidazol-1-yl)acetohydrazide **(5)**. Compound **5** on treatment with different aromatic carboxylic acids **(6a-e)** in the presence of phosphoryl chloride at 120 °C yielded 2-(trifluoromethyl)-5-methyl-1-((5-aryl-1,3,4-oxadiazol-2-yl)methyl)-1*H*-benzimidazole **(7a-e)**. The crude products were purified by column chromatography over silica gel. The structures of all the compounds were established on the basis of MS, ^{1}H and ^{13}C



Scheme-1. Synthetic route to 1-[(5-aryl-1,3,4-oxadiazol-2-yl)methyl]-(trifluoromethyl) -5 -methyl-1*H*-benzimidazole (7a-e).

3.2. 5-Lipoxygenase (5-LOX) inhibition activity of compounds (7a-e)

5-lipoxygenase (5- LOX) has been reported to be involved in the biosynthesis of leucotrienes (LTs), This 5- LOX in the presence of 5-LOX-activating protein (FLAP), catalyzing the initial two steps in conversion of arachidonic acid to the key intermediate LTA_4 [22,23]. These LTs are potent lipid mediators of numerous inflammatory diseases and allergic disorders. Inhibition of 5-LOX may lead to the development of new therapeutic treatments for pathologies such as asthma, allergies and other inflammatory disorders[24-26]. Hence the therapeutic potential of 5-LOX inhibition has been widely highlighted in recent years[27-31].

The molecules synthesized were screened *in vitro* for their inhibitory properties against 5-LOX enzyme using the assay described by Reddanna *et al.* [32]. Among the molecules tested, compound **7d** exhibit potent 5-Lipoxygenase inhibition with an IC_{50} of 8.59 µg/mL than standard Curcumin (10.15 µg/mL) and the remaining compounds **7a-c** and **7e** have an IC_{50} of >100 µg/mL. **(Table-1)**

Table-1: IC_{50} values obtained from *in vitro* 5-Lipoxygenase inhibition assay for the compounds (7a-e)

Entry	Compound	IC_{50} (µg/mL)
1	**7a**	>100
2	**7b**	>100
3	**7c**	>100
4	**7d**	**8.59**
5	**7e**	>100
6	**Standard***	10.15

IC_{50} represents the concentration of a drug that is required for 50% inhibition expressed in µg/mL.* Curcumin as Standard

3.3. Cytotoxicity testing using Brine Shrimp Lethality Bioassay

Toxicity to brine shrimps has a good correlation with antitumor, pesticidal[33] and antitrypanosomal activities[34] in man. The brine shrimp larvae (*Artemia salina*) responds similarly to the corresponding mammalian systems[35] since the DNA-dependent RNA polymerases of *A. salina* have been shown to be similar to the mammalian type[36]. This test is not only used for predicting cytotoxicity, but also it is used to predict antitumor, antibacterial and pesticidal activities[37]. Thus, it is possible to evaluate the cytotoxicity of compounds using brine shrimp lethality bioassay rather than the more tedious *in vitro* and *in vivo* antitumor assays.

The synthesized compounds were screened for cytotoxic activity against brine shrimp larvae (*nauplii*) adopting the microplate assay, method of Meyer *et al.*[38] , and using Podophyllotoxin as a reference drug.

Cytotoxic activity of the tested compounds was determined by measuring the ED_{50} (Effective Dose causing death of 50% of brine shrimp nauplii) expressed in µg/mL. The results in **Table-2** reveal that among the tested compounds **7c**, **7a** have good and **7b** exhibit moderate cytotoxic activity against brine shrimp nauplii with an ED_{50} value 7.39, 7.54 and 12.28 µg/mL respectively. The remaining two compounds **7e** and **7d** showed least activity with an ED_{50} value 51.33 and 140.63 µg/mL respectively.

Table-2. *In vitro* cytotoxicity of title compounds 7a-e by Brine Shrimp Lethal Bioassay

Entry	Compounds	ED_{50} (µg/mL)	Degree(s) of freedom	UCL	LCL
1	**7a**	7.54	1.65	16.23	3.25
2	**7b**	12.28	1.75	28.43	5.46
3	**7c**	**7.39**	9.99	16.97	2.86
4	**7d**	140.63	0.36	702.23	48.77
5	**7e**	51.33	2.65	104.24	20.77
6	**Standard** *	1.83	0.32	3.22	0.00

ED_{50} is the Effective Dose causing death of 50% brine shrimps expressed in µg/mL.U.C.L. is the Upper Confident Limits.L.C.L. is the Lower Confident Limits. Podophyllotoxin as positive control.*

3.4. Structure Activity Relationship Studies

To explain the structure activity relationship of the tested compounds **(7a-e)**, the contribution of the substituent should be taken into account, as the substituent can make difference in the activity.

The results shown in **Table-1** demonstrated the effect of different substituents (on the phenyl ring attached to 5th position of 1,3,4-oxadiazole) on 5-LOX inhibition activity. Among the synthesized compounds, **7d** was found to be promising compound with an IC_{50} value of 8.59 µg/mL, which indicates that the hydrophobic substituent (chlorine) is a better substituent against 5-LOX inhibition.

The results obtained from Brine shrimp Lethal Bioassay (BSLB) in **Table-2**, compound **7c** exhibit potent cytotoxicity among the synthesized compounds with an ED_{50} of 7.39 µg/mL.

Concerning the position of the substituents, the compounds **7a**, **7b**, **7d** were *para* substituted. Among the *para* substituted compounds, **7a** which has electron donating methoxy (-OMe) was found to be potent with an ED_{50} of 7.54 µg/mL, **7b** which has electron withdrawing nitro ($-NO_2$) shows moderate activity with an ED_{50} of 12.28 µg/mL and the presence of hydrophobic chlorine is responsible for the least activity of **7d** with an ED_{50} of 140.63 µg/mL. In *meta* substituted compounds, **7e** having electron withdrawing cyano (-CN) was found to be weak with an ED_{50} of 51.33 µg/mL. In case of compound **7c** having two electron withdrawing nitro groups at *meta* positions was found to be potent with an ED_{50} of 7.39 µg/mL.

A closer look into the structure activity relationship (SAR) studies of compounds **7a-e**, indicated that the compound **7d** having hydrophobic substituent chlorine was found to be potent for 5-LOX inhibition and it was weak in case of Brine Shrimp Lethal Bioassay (BSLB). Compound **7c** having two nitro groups exhibit potent cytotoxicity against Brine shrimp Lethal Bioassay (BSLB).

3.5 Experimental section

Synthesis of 2-(trifluoromethyl)-5-methyl-1*H*-benzimidazole (3):

A solution of 3,4-Diaminotoluene **(1)** (0.01 mol), CF_3CO_2H **(2)** (0.02 mol) and HCl (4N; 5 mL) was heated under reflux for 4 h. The progress of the reaction was monitored by TLC. After completion of the reaction, the reaction mixture was allowed to cool to RT. The cooled mixture was neutralized with saturated $NaHCO_3$ solution until pale brown coloured mass precipitated out, which was filtered, washed with water and recrystallized with ethanol. Yield 91 %, MP: 102-104 °C. Anal. Cal. for $C_9H_7F_3N_2$: C, 54.00; H, 3.52; N, 14.00. Found: C, 53.98; H, 3.51; N, 13.97. ^{1}H NMR (400 MHz, DMSO-d_6): *d* 10.23 (s, 1H), 7.59 (d, *J* = 8.4 Hz, 1H), 7.48 (s, 1H), 7.18-7.20 (dd, *J* = 8.4, 1.2 Hz, 1H), 2.43 (s, 3H). ^{13}C NMR (22.4 MHz, DMSO-d_6): *d* 140.58, 137.14, 136.27, 133.58, 125.22, 121.21, 116.15, 114.97, 24.10. ESI-MS: *m/z* 200.88 $[M+H]^+$.

Synthesis of Ethyl 2-(2-(trifluoromethyl)-5-methyl-1*H*-benzimidazol-1-yl)acetate (4):

Ethyl bromoacetate (0.84 ml, 0.0076 mol) was added dropwise to a solution of compound **3** (1 g, 0.0058 mol) and anhydrous potassium carbonate (1.06 g, 0.0076 mol) in 50 ml dry acetone. Then the reaction mixture was refluxed for 3 h. The progress of the reaction was monitored by TLC. After completion of the reaction, acetone was evaporated. The obtained residue was partitioned between EtOAc and water. The collected organic layers were dried over anhy.Na_2SO_4 and evaporated under reduced pressure to get crude product. The obtained crude was recrystallized from ethanol. Yield 89 %, MP: 88-90 °C. Anal. Cal. for $C_{13}H_{13}F_3N_2O_2$: C, 54.55; H, 4.58; N, 9.79. Found: C, 54.53; H, 4.59; N, 9.76. ^{1}H NMR (400 MHz, DMSO-d_6): *d* 7.68 (d, *J* = 8.4 Hz, 1H), 7.42 (s, 1H), 7.19 (d, *J* = 8.4 Hz, 1H), 5.13 (s, 2H), 4.21 (q, *J* = 7.2 Hz, 2H), 2.21 (s, 3H), 1.23 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): *d* 167.02, 140.83, 138.08, 132.94, 124.34, 122.81, 116.49, 116.47, 114.89, 65.43, 47.46, 24.34, 13.98. ESI-MS: *m/z* 287.09 $[M+H]^+$.

Synthesis of 2-(2-(trifluoromethyl)-5-methyl-1*H*-benzimidazol-1-yl)acetohydrazide (5):

To a solution of ethyl 2-(2-(trifluoromethyl)-5-methyl-1*H*-benzimidazol-1-yl)acetate (**4)** (1.32 g, 0.0055 mol) in 25 ml of absolute ethanol, hydrazine hydrate (0.53 ml, 0.011 mol) was added dropwise manner, and the mixture was refluxed for 5 h. The reaction was monitored by TLC. After completion of the reaction, ethanol was evaporated under reduced pressure to get residue. Thus obtained residue washed with excess of water, dried and recrystallized in ethanol. Yield 92%, MP: 179-181 °C. Anal. Cal. for $C_{11}H_{11}F_3N_4O$: C, 48.53; H, 4.07; N, 20.58. Found: C, 48.51; H, 4.05; N, 20.55. ^{1}H NMR (400 MHz, DMSO-d_6): *d* 9.51 (s, 1H), 7.63 (d, *J* = 8.4 Hz, 1H), 7.54 (s, 1H), 7.15 -7.17 (d, *J* = 8 Hz, 1H), 4.97 (s, 2H), 4.26 (s, 2H), 2.44 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): *d* 165.53, 141.08, 138.94, 133.27, 127.21, 125.60, 120.62, 120.46, 111.32, 45.63, 21.99. ESI-MS: *m/z* 294.89 $[M+Na]^+$.

Synthesis of 2-(trifluoromethyl)-5-methyl-1-((5-aryl-1,3,4-oxadiazol-2-yl)methyl)-1*H*-benzimidazole (7a-e)

2-(2-(trifluoromethyl)-5-methyl-1*H*-benzimidazol-1-yl)acetohydrazide **(5)** (0.2 g, 0.00083 mol) and substituted benzoic acids **(6a-e)** (0.001 mol) were refluxed in presence of $POCl_3$ at 120 °C for 3-4 h. The progress of the reaction was monitored by TLC. After completion of the reaction, the mixture was neutralised with saturated $NaHCO_3$ solution and the crude product was extracted with ethyl acetate (3 X 15 mL). The combined organic extracts were washed with water, dried

over anhy. Na_2SO_4 and the solvent was removed under vacuum. The obtained product was purified by column chromatography to get the **target molecules (7a-e)** in good yields.

2-(trifluoromethyl)-1-((5-(4-methoxy phenyl)-1,3,4-oxadiazol-2-yl)methyl)-5-methyl-1*H*-benzimidazole (7a): Yield 85 %, MP: 112-114 °C. Anal. Cal. for $C_{19}H_{15}F_3N_4O_2$: C, 58.76; H, 3.89; N, 14.43. Found: C, 58.74; H, 3.87; N, 14.41. ^{1}H NMR (400 MHz, DMSO-d_6): *d* 7.83 (d, *J* = 8.8 Hz, 2H), 7.67 (d, *J* = 8.8 Hz, 1H), 7.54 (s, 1H), 7.26 (d, *J* = 8.4 Hz, 1H), 7.12 (d, *J* = 8.8 Hz, 2H), 6.05 (s, 2H), 3.83 (s, 3H), 2.46 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6,): *d* 164.68, 163.47, 160.91, 140.62, 138.49, 135.64, 133.56, 128.35, 127.51, 125.82, 115.06, 114.83, 111.38, 111.24, 55.51, 44.02, 21.54. ESI-MS: *m/z* 411.12 $[M+Na]^+$.

2-(trifluoromethyl)-5-methyl-1-((5-(4-nitrophenyl)-1,3,4-oxadiazol-2-yl)methyl)-1*H*-benzimidazole (7b): Yield 78 %, MP: 145-148 °C. Anal. Cal. for $C_{18}H_{12}F_3N_5O_3$: C, 53.60; H, 3.00; N, 17.36. Found: C, 53.58; H, 2.99; N, 17.34. ^{1}H NMR (400 MHz, DMSO-d_6): *d* 8.41 (d, *J* = 8.8 Hz, 2H), 8.16 (d, *J* = 8.8 Hz, 2H), 7.68 (d, *J* = 8.0 Hz, 1H), 7.36 (s, 1H), 7.27 (d, *J* = 8.8 Hz, 1H), 6.12 (s, 2H), 2.46 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): *d* 163.52, 162.42, 149.36, 140.64, 138.49, 135.86, 133.60, 128.36, 127.98, 127.53, 125.85, 111.41, 111.27, 111.04, 45.72, 21.02. ESI-MS: *m/z* 404.10 $[M+H]^+$.

2-(trifluoromethyl)-5-methyl-1-((5-(3,5-dinitrophenyl)-1,3,4-oxadiazol-2-yl)methyl)-1*H*-benzimidazole (7c): Yield 79 %, MP: 153-155 °C.]$^+$. Anal. Cal. For $C_{18}H_{11}F_3N_6O_5$: C, 48.22; H, 2.47; N, 18.75. Found: C, 48.20; H, 2.45; N, 18.72. ^{1}H NMR (400 MHz, DMSO-d_6): *d* 8.99 (s,1H), 8.89 (s, 2H), 7.73 (d, *J* = 7.6 Hz, 1H), 7.66 (s, 1H), 7.27 (d, *J* = 7.6 Hz, 1H), 6.14 (s, 2H), 2.47 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): *d* 162.67, 162.63, 148.68, 140.64, 138.51, 135.84, 135.63, 133.63, 127.47, 126.41, 125.85, 121.25, 111.43, 111.31, 45.37, 21.00. ESI-MS: *m/z* 449.82 [M+H.

1-((5-(4-chlorophenyl)-1,3,4-oxadiazol-2-yl)methyl)-2-(trifluoromethyl)-5-methyl-1*H*-benzimidazole (7d): Yield 82 %, MP: 140-143 °C. Anal. Cal. For $C_{18}H_{12}ClF_3N_4O$: C, 55.04; H, 3.08; N, 14.26. Found: C, 55.02; H, 3.06; N, 14.25. ^{1}H NMR (400 MHz, $CDCl_3$): *d* 7.87 (d, *J* = 8.8 Hz, 2H), 7.54 (d, *J* = 8.4 Hz, 1H), 7.49 (s, 1H), 7.46 (d, *J* = 8.8 Hz, 2H), 7.31 (d, *J* = 8.4 Hz, 1H), 5.78 (s, 2H), 2.51 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): *d* 165.31, 160.08, 141.37, 139.16, 138.76, 137.06, 134.61, 133.13, 129.58, 128.29, 128.09, 121.47, 121.40, 110.17, 39.18, 21.52. ESI-MS: *m/z* 415.06 $[M+Na]^+$.

3-(5-((2-(trifiuoromethyl)-5-methyl-1*H*-benzimidazole-1-yl)methyl)-1,3,4-oxadiazol-2-yl)benzonitrile (7e): Yield 81 %, MP: 184-187 °C. Anal. Cal. For $C_{19}H_{12}F_3N_5O$: C, 59.53; H, 3.16; N, 18.27. Found: C, 59.50; H, 3.14; N, 18.24. ^{1}H NMR (400 MHz, DMSO-d_6): *d* 8.26 (s, 1H), 8.15 (dd, *J* = 8.0, 1.2 Hz, 1H), 8.05 (d, *J* = 7.2 Hz, 1H), 7.72(d, *J* = 8.4 Hz, 1H), 7.62 (d, *J* = 8.4 Hz, 1H), 7.54 (s, 1H), 7.22 (d, J = 8.4 Hz, 1H), 5.25 (s, 2H), 2.45 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): *d* 165.16, 163.59, 140.59, 138.46, 135.39, 135.21, 133.11, 133.02, 132.17, 130.04, 129.98, 126.99, 125.37, 118.10, 111.71, 111.31, 111.23, 45.26, 21.51. ESI-MS: *m/z* 406.96 $[M+Na]^+$.

4. CONCLUSION

From the results of 5-LOX inhibition assay, we can conclude that the presence of chloro substituent at the *para* position (on the phenyl ring attached to 5th position of 1,3,4-oxadiazole) is accountable for the higher activity against 5-LOX enzyme (compound **7d**). On the other hand, the presence of two nitro groups at *meta* positions (compound **7c**) enhances the cytotoxic activity against brine shrimp larvae.

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