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Insilico Design, Synthesis and *In vitro* Antidiabetic and Anti-Inflammatory Activities of 1,3,4-Thiadiazole Substituted 2-Methyl Benzimidazole Derivatives

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ABSTRACT

Novel 1,3,4-thiadiazole substituted 2-methyl benzimidazole derivatives were designed by using various softwares such as ACD Lab ChemSketch 12.0, Molinspiration, PASS and Discovery studio. The designed molecules having required physico-chemical properties, drug likeness and obeying Lipinski's rule of five (BT1, BT2, BT3, BT4, BT5, BT6, BT7, BT8 and BT9) were selected for the synthesis. These compounds were synthesized by conventional methods. All the synthesized compounds were confirmed based on their physicochemical parameters and their characteristic peaks in IR, 1HNMR and Mass spectroscopic studies. Based on the Libdock score, the compound BT6 was selected for *in vitro* antidiabetic and anti-inflammatory evaluation. The compound BT6 showed significant antidiabetic and anti-inflammatory activities.

INTRODUCTION

Benzimidazole derivatives are of wide interest because of their diverse biological activity and clinical applications. Recently, some other types of biological activity besides the antibacterial activity have been reported in compounds containing benzimidazole ring [1-4]. Such biological activities include antifungal, antioxidant, antiulcer, antiviral, anti-histaminic, antimalarial, antitubercular, anticancer, antidiabetic, antitumor, activity [4-12]. Thiadiazole play a prominent role in nature. They exhibit a variety of activity from antimicrobial to antidiabetic activity [13-15]. The 1,3,4-thiadizole heterocycle is an interesting building block in a variety of natural and synthetic compounds found to possess good antibacterial potential. This heterocyclic nucleus is a very important group because of its potent antitumor activity and other significant pharmaceutical utilities, such as treatment of inflammatory diseases, epilepsy, analgesia, viral infections, cancer, and tuberculosis. Our ongoing investigations have been directed toward the insilico design, synthesis and pharmacological evaluation of some novel 1,3,4-thiadiazole substituted 2-methyl benzimidazole derivatives.

MATERIALS AND METHODS

Insilico Molecular Modification

The insilico modelling of all proposed compounds were carried out by using different computational software in order to predict the physiological and biological parameters. The softwares used for in-silico studies include Molinspiration, ACDLAB Chemsketch and PASS.

Nine 1,3,4-thiadiazole containing 2-methyl benzimidazole derivatives were selected for synthesis with the help of these selection parameters. They are

N-{5-[(2-methyl-1H-benzimidazol-1-yl)methyl]-1,3,4-thiadiazol-2-yl}-N'-phenylmethane diamine-BT1

2-{[({5-[(2-methyl-1H-benzimidazol-1-yl)methyl]-1,3,4-thiadiazol-2-yl}amino)methyl] amino}phenol-BT2

4-{[({5-[(2-methyl-1H-benzimidazol-1-yl)methyl]-1,3,4-thiadiazol-2-yl}amino)methyl] amino}phenol-BT3

N-{5-[(2-methyl-1H-benzimidazol-1-yl)methyl]-1,3,4-thiadiazol-2-yl}-N'-(2-nitrophenyl) methanediamine-BT4

N-{5-[(2-methyl-1H-benzimidazol-1-yl)methyl]-1,3,4-thiadiazol-2-yl}-N'-(4-nitrophenyl) methanediamine-BT5

5-[(2-methyl-1H-benzimidazol-1-yl)methyl]-N-[(1Z)-1-phenylethylidene]-1,3,4-thiadiazol-2-amine-B76

N-(diphenylmethylidene)-5-[(2-methyl-1H-benzimidazol-1-yl)methyl]-1,3,4-thiadiazol-2-amine-BT7

N-(4-methoxybenzylidene)-5-[(2-methyl-1H-benzimidazol-1-yl)methyl]-1,3,4-thiadiazol-2-amine-BT8

2-methoxy-4-[({5-[(2-methyl-1H-benzimidazol-1-yl)methyl]-1,3,4-thiadiazol-2-yl}imino) methyl]phenol-BT9

Synthesis of Selected Derivatives

The selected compounds were synthesized by conventional method through a series of six steps.

Step-1

Synthesis of 2-methyl benzimidazole (D1): Placed 10.86 g of o-phenylene diamine in a round bottomed flask and added 40 ml of water and 10.8 g of acetic acid. Heated the mixture on a water bath at 100°C for 2 hours. Cooled and added conc. ammonia solution, with constant rotation of the flask, until the mixture was alkaline to litmus. The solid separated out was filtered, washed with 25 ml of cold water and recrystallized from boiling water [2,16].

Step-2

Synthesis of N-ethylacetate-2-methylbenzimidazole (D2): A mixture of 2-methyl benzimidazole (compound 1) (0.30 mole, 39.60 g) and ethyl chloroacetate (0.30 mole, 36.74 g) with potassium carbonate (6.168 g) in methanol (250 ml) was kept overnight at room temperature. The reaction mixture was refluxed on a steam bath for about 3 hours. It was cooled filtered and solvent was distilled off under reduced pressure and the solid thus obtained was passed through a column of silica gel using chloroform: methanol (5:5 v/v) mixture as eluent. The eluate (250 ml) was concentrated to give a product which was recrystallized with ethanol to furnish colourless needles of compound 2 [17,18].

Step-3

Synthesis of N-acetylthiosemicarbazide-2-methylbenzimidazole (D3): N-ethylacetate-2-methylbenzimidazole (compound 2) (0.15 mole, 32.70 g) and thiosemicarbazide (0.15 mole, 30.67 g) in methanol (200 ml) was refluxed on a steam bath for about 8 hours. It was then cooled, filtered and excess of solvent was removed which gave a product. It was purified over the column of silica gel using acetone: methanol 6:4 (v/v) mixture as an eluent. The eluate (200 ml) was concentrated and product was recrystallized with ethanol to give compound 3 [18].

Step-4

Synthesis of N-(2'-amino-5'-methylene)-1',3',4'-thiadiazole-2methylbenzimidazole (D4): Equimolar solution of N-acetylthiosemicarbazide-2-methylbenzimidazole (compound 3) (0.10 mole, 26.30 g) and concentrated sulphuric acid (0.10 mole, 9.80 g, AR grade) in methanol (150 ml) was kept overnight at room temperature. It was then refluxed on a steam bath for about 10 hours. After cooling the solution was neutralized with concentrated liq. ammonia and filtered. The solvent was removed *in vivo* and the solid thus obtained was dried and purified over the column of silica gel using chloroform:methanol (5:5 v/v) mixture as eluent. The eluate (180 ml) was concentrated to give a product which was recrystallized from ethanol to give compound 4 [18].

Step-5

Synthesis of derivatives using different types of amines (BT-1 to BT-5): A methanolic solution of N-(2'-amino-5'-methylene)-1',3',4'-thiadiazole-2-methylbenzimidazole (compound 4) (2 gm, 0.001 mole) was charged into a three necked flask equipped with a stirrer and dropping funnel. The solution was stirred to dissolve it completely. To this methanolic solution, formaldehyde (7 ml) was added dropwise during 15-20 minutes. The resulting mixture was stirred during half an hour to complete reaction of formaldehyde and to yield methylol derivative. To this reaction mixture, the methanolic solution of amine (1.5 gm, 0.001 mol) was added drop wise with stirring in about half an hour at 30°C and refluxed for two hour at 65-70°C. It was allowed to cool and poured into ice water. The solid obtained was filtered off washed thoroughly with hot water and air dried. The procedure was repeated with other aromatic amines to get different compounds [14].

Step-6

Synthesis of derivatives using different types of aromatic ketones and aldehydes (BT-6 to BT-9): N-(2'-amino-5'-methylene)-1',3',4'-thiadiazole-2-methylbenzimidazole (compound 4) (0.2 mol), ketone or aldehyde (0.2 mol) and (2 ml) glacial acetic acid were refluxed in 50 ml methanol for 8 hours. Solvent was distilled off and product recrystallized from mixture of benzene and chloroform (1:6 V/V) [13].

Characterization of Synthesized compounds by Spectral Study

IR spectrum: IR spectra of the synthesized compounds were recorded in the range of 4000-500 cm^{-1} on Shimadzu FT-IR, Affinity 1 using KBr pellets [19].

1HNMR spectrum: NMR spectra of different compounds were recorded on a Bruker 400 NMR spectrometer. The system was operated at 400 MHz for proton using TMS as an internal standard.

Mass spectrum: Mass spectra were recorded with Bruker Daltonics Flex Analysis by MATRIX method. The peaks were recorded as m/z value.

***In vitro* antidiabetic activity (alpha amylase assay)**

Twenty five microlitres of 10 mg/ml BT6 and 25 µl of 25 mM phosphate buffer pH 6.9, containing porcine α amylase at a concentration of 0.5 mg/ml were incubated at 25°C for 10 min. After pre incubation, 25 µl of 0.5% starch solution in 25 mM phosphate buffer pH 6.9 was added. The reaction mixtures were then incubated at 25°C for 10 min. The reaction was stopped with 50 µl of 96 mM 3,5 dinitrosalicylic acid colour reagent. The micro plate was then incubated in a boiling water bath for 5 min and cooled to room temperature. Absorbance was measured at 540 nm using a microplate reader [20].

$$\% \text{ inhibition} = \frac{\text{OD of control} - \text{OD of test}}{\text{OD of control}} * 100$$

Anti-inflammatory Activity (HRBC Membrane Stabilization Assay)

Fresh whole human blood (5 ml) was collected and transferred to the centrifuged tubes containing heparin or EDTA or sodium citrate to prevent clotting. The tubes were centrifuged at 3000 rpm for 10 min and were washed three times with equal volume of normal saline. The volume of the blood was measured and reconstituted as 10% v/v suspension with normal saline.

The reaction mixture consists of different concentrations of sample BT6 (10 µg/ml, 20 µg/ml, 50 µg/ml, 100 µg/ml, and 250 µg/ml) in normal saline and 0.5 mL of 10% HRBC suspension, 1 ml of 0.2 M phosphate buffer, 1 ml hyposaline were incubated at 37°C for 30 min and centrifuged at 3000 rpm for 20 min and the haemoglobin content of the supernatant solution was estimated spectrophotometrically at 560 nm. Diclofenac was used as standard and a control was prepared without test molecule. The percentage of HRBC haemolysis and membrane stabilization or protection was calculated by using the following formula [21-23].

Percentage Haemolysis=(Optical density of test sample/Optical density of control) × 100

Percentage protection = 100-[(Optical density of test sample/Optical density of control) × 100]

RESULTS

In the present study, insilico molecular modifications of proposed derivatives were done by using different softwares. 3D-drawing, optimizing and calculating various descriptors of proposed derivatives were done by using ACD Lab Chemsketch software. The results are shown in **Table 1.**

Table 1. Molecular descriptors of proposed 1,3,4-thiadiazole substituted 2-methyl benzimidazole derivatives.

Compounds	Molar Refractivity (cm^3)	Molar volume (cm^3)	Parachor (cm^3)	Surface Tension (dynes/cm)	Polarizability(10^{-24} cm^3)
BT1	102.15 ± 0.5	256.2 ± 7.0	705.0 ± 8.0	57.3 ± 7.0	40.49 ± 0.5
BT2	103.00 ± 0.5	253.4 ± 7.0	710.7 ± 8.0	61.7 ± 7.0	40.83 ± 0.5
BT3	103.00 ± 0.5	253.4 ± 7.0	710.7 ± 8.0	61.7 ± 7.0	40.83 ± 0.5
BT4	107.81 ± 0.5	261.5 ± 7.0	750.5 ± 8.0	67.8 ± 7.0	42.74 ± 0.5
BT5	107.81 ± 0.5	261.5 ± 7.0	750.5 ± 8.0	67.8 ± 7.0	42.74 ± 0.5
BT6	103.52 ± 0.5	266.7 ± 7.0	716.3 ± 8.0	52.0 ± 7.0	41.04 ± 0.5
BT7	124.20 ± 0.5	319.3 ± 7.0	862.1 ± 8.0	53.1 ± 7.0	49.24 ± 0.5
BT8	104.91 ± 0.5	273.2 ± 7.0	735.5 ± 8.0	52.5 ± 7.0	41.59 ± 0.5
BT9	105.76 ± 0.5	270.4 ± 7.0	741.2 ± 8.0	56.4 ± 7.0	41.92 ± 0.5

The mol inspiration software was used to study the Log P values, violation of Lipinski's rule of five and drug likeness by comparing with already existing standard drugs. The results are shown in **Tables 2-4.**

Table 2. Smile notations of proposed 1,3,4-thiadiazole substituted 2-methyl benzimidazole derivatives.

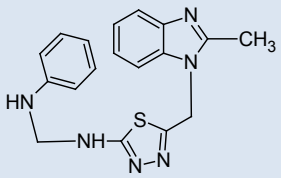
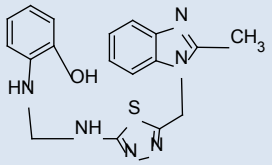
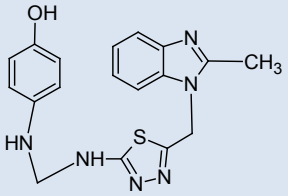
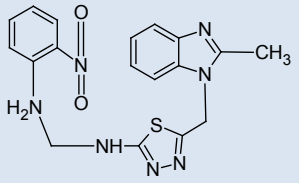
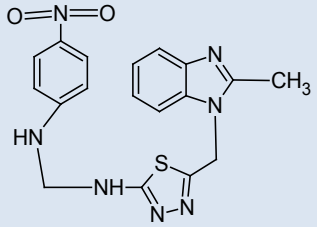
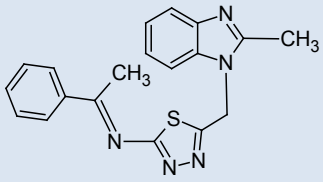
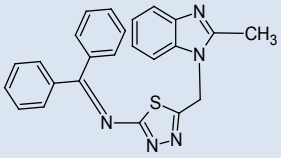
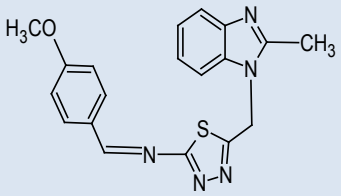
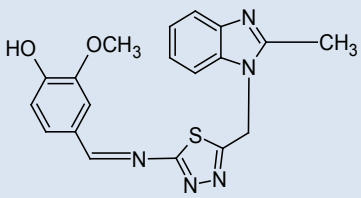
COMPOUNDS	STRUCTURE	SMILE NOTATION
BT1		Cc2nc1ccccc1n2Cc4nnc(NCNc3ccccc3)s4
BT2		Cc2nc1ccccc1n2Cc4nnc(NCNc3ccccc3O)s4
BT3		Cc2nc1ccccc1n2Cc4nnc(NCNc3ccc(O)cc3)s4
BT4		Cc2nc1ccccc1n2Cc4nnc(NCNc3ccccc3N(=O)=O)s4
BT5		Cc2nc1ccccc1n2Cc4nnc(NCNc3ccc(O)cc3)s4
BT6		CC(=Nc3nnc(Cn1c(C)nc2ccccc12)s3)c4ccccc4
BT7		Cc2nc1ccccc1n2Cc5nnc(N=C(c3ccccc3)c4ccccc4)s5
BT8		COc4ccc(C=Nc3nnc(Cn1c(C)nc2ccccc12)s3)cc4
BT9		COc1cc(O)ccc1C=Nc4nnc(Cn2c(C)nc3ccccc23)s4

Table 3. Lipinskis rule analysis of proposed 1,3,4-thiadiazole substituted 2-methyl benzimidazole derivatives.

CODE	miLogP	MW	nON	nOHNH	nviolations	Nrotb
BT1	2.955	350.451	6	2	0	6
BT2	2.688	366.45	7	3	0	6
BT3	2.476	366.45	7	3	0	6
BT4	2.866	395.448	9	2	0	7
BT5	2.914	395.448	9	2	0	7
BT6	3.607	347.447	5	0	0	4
BT7	4.826	409.518	5	0	0	5
BT8	3.217	363.446	6	0	0	5
BT9	2.666	379.445	7	1	0	5

Table 4. Drug likeness analysis of proposed 1,3,4-thiadiazole substituted 2-methyl benzimidazole derivatives.

Compounds	GPCR Ligand	Ion channel modulater	Kinase Inhibitor	Nuclear Receptor Ligand	Protease Inhibitor	Enzyme Inhibitor
BT1	-0.28	-0.73	-0.24	-0.65	-0.26	-0.23
BT2	-0.26	-0.81	-0.23	-0.57	-0.32	-0.21
BT3	-0.23	-0.66	-0.19	-0.49	-0.24	-0.17
BT4	-0.39	-0.75	-0.39	-0.81	-0.40	-0.29
BT5	-0.38	-0.70	-0.35	-0.67	-0.36	-0.30
BT6	-0.42	-0.79	-0.45	-0.50	-0.44	-0.35
BT7	-0.13	-0.42	-0.28	-0.25	-0.21	-0.14
BT8	-0.61	-0.99	-0.53	-0.74	-0.62	-0.42
BT9	-0.53	-0.97	-0.47	-0.59	-0.57	-0.37

The PASS software was used to predict the general biological activities of proposed molecules. The result of prediction is presented as the list of activities with appropriate Pa (Probability to be active) and Pi (Probability to be inactive) sorted in descending order of the difference (Pa-Pi) >0. Pa and Pi are the estimates of probability for the compound to be active or inactive respectively for each type of activity from the biological activity spectrum. Their values vary from 0.000 to 1.000.

If Pa>0.7, the compound is very likely to reveal this activity in experiments, but in this case the chance of being the analogue of the known pharmaceutical agents for this compound is also high.

If 0.5<Pa<0.7, the compound is likely to reveal its activity in experiments, but this probability is less, and the compound is not so similar to the known pharmaceutical agents.

If Pa<0.5, the compound is unlikely to reveal its activity in experiments, but if the presence of this activity is confirmed in the compound, it might be a new chemical entity. The results are shown in **Table 5.**

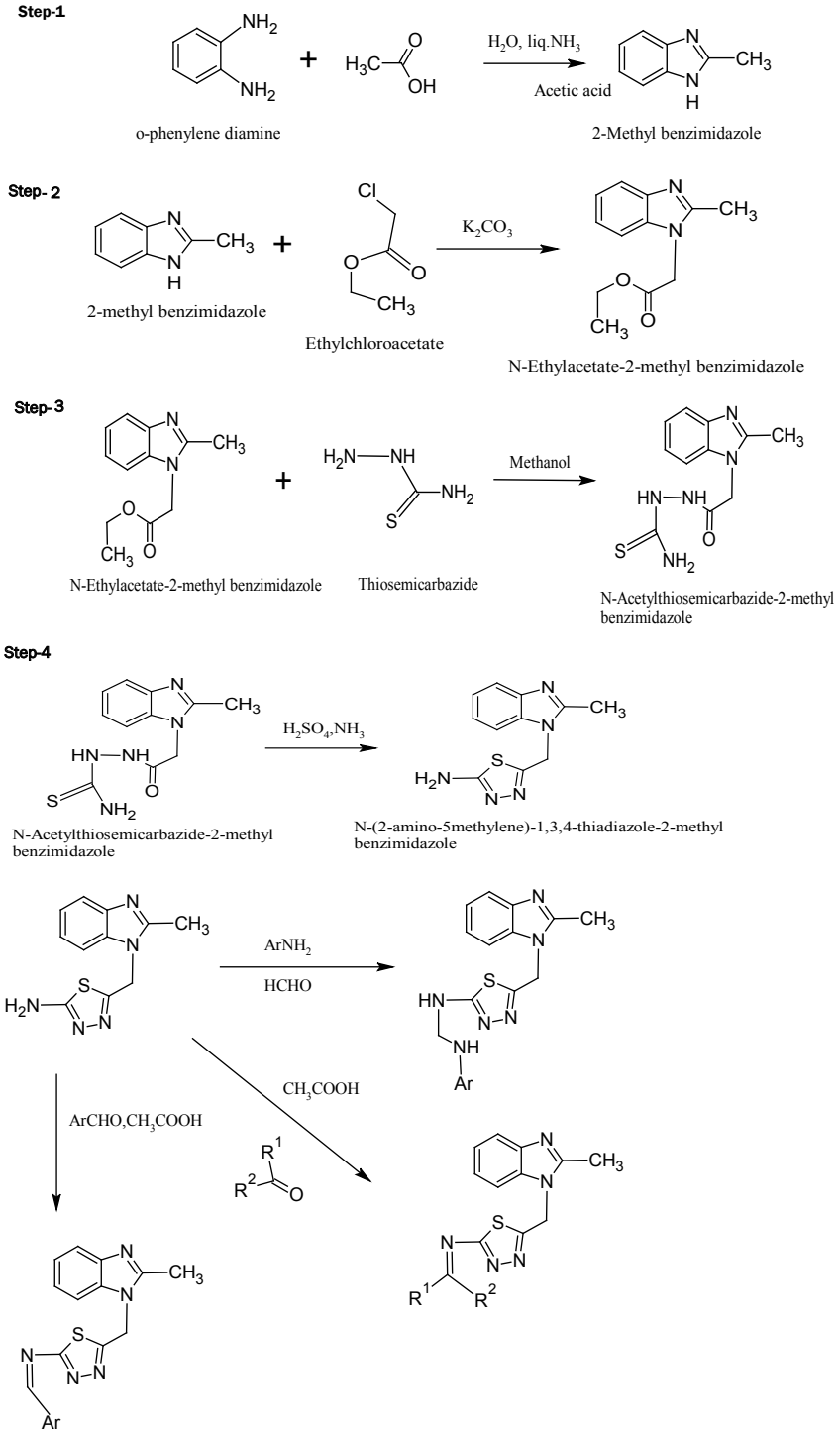
Table 5. PASS of proposed 1,3,4-thiadiazole substituted 2-methyl benzimidazole derivatives for anti-diabetic and anti-inflammatory activity.

Compounds	Antidiabetic activity		Anti-inflammatory activity	
	Pa	Pi	Pa	Pi
BT1	0.283	0.161	0.121	0.036
BT2	0.258	0.048	0.181	0.154
BT3	0.163	0.077	0.189	0.141
BT4	0.181	0.064	0.085	0.068
BT5	0.181	0.064	0.085	0.068
BT6	0.430	0.050	0.378	0.025
BT7	0.480	0.034	0.537	0.007
BT8	0.333	0.117	0.360	0.029
BT9	0.478	0.057	0.340	0.035

With the help of these selection parameters the selected compounds were synthesized by conventional method through a series of six steps. The general scheme for the synthesis is presented in **Figure 1.**

Purity of the synthesized compounds was ascertained by TLC and melting point determination by open capillary tube method. The results are shown in **Table 6.**

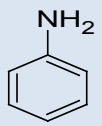
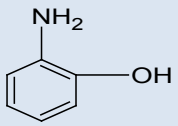
The synthesized compounds were characterized by FTIR, 1HNMR and MASS spectroscopic methods. The FTIR report for the synthesized compounds are shown in **Table 7.**



General scheme for the synthesis of 1,3,4-thiadiazole substituted 2-methyl benzimidazole derivatives.

Figure 1. Synthesis of 1,3,4-thiadiazole substituted 2-methyl benzimidazole derivatives.

Table 6. Physico chemical data of newly synthesized compounds.

Compounds	Ar	Molecular Formula	Molecular Weight	Melting Point	R_f Value
BT1		$C_{18}H_{18}N_6S$	350.44072	60°C	0.562
BT2		$C_{18}H_{18}N_6OS$	366.44012	174°C	0.531

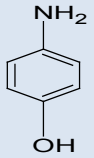
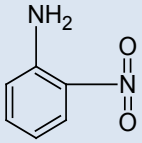
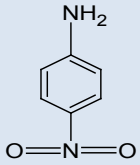
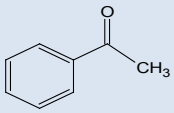
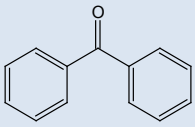
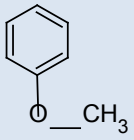
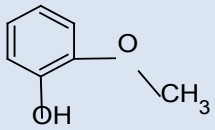
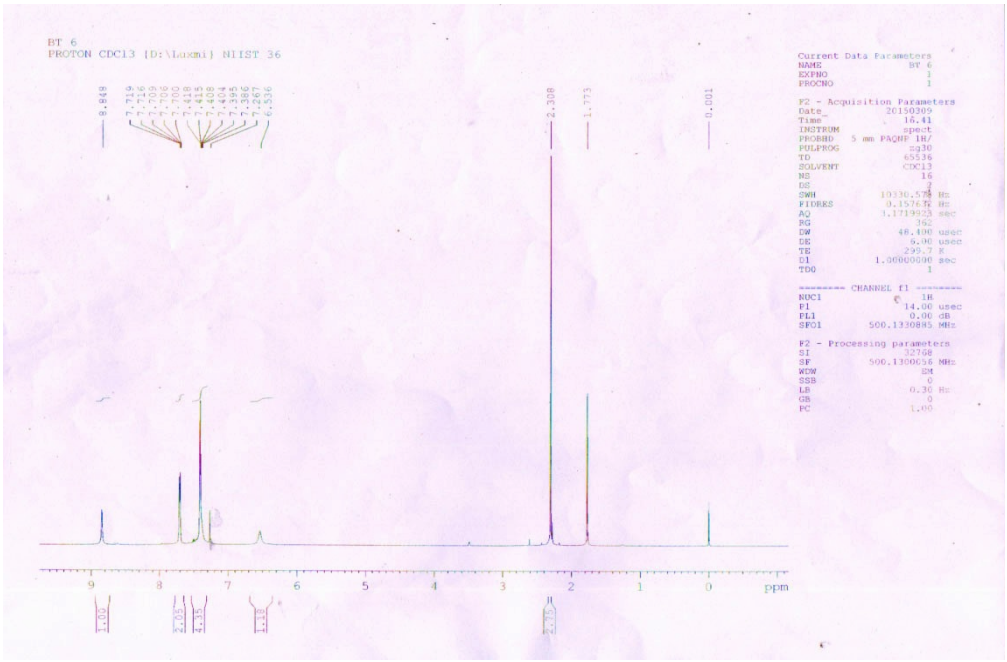
BT3		$C_{18}H_{18}N_6OS$	366.44012	184°C	0.528
BT4		$C_{18}H_{17}N_7O_2S$	395.43828	82°C	0.417
BT5		$C_{18}H_{17}N_7O_2S$	395.43828	136°C	0.402
BT6		$C_{19}H_{17}N_5S$	347.43678	91°C	0.213
BT7		$C_{24}H_{19}N_5S$	409.50616	137°C	0.559
BT8		$C_{19}H_{17}N_5OS$	363.43618	97°C	0.501
BT9		$C_{19}H_{17}N_5O_2S$	379.43558	82°C	0.383

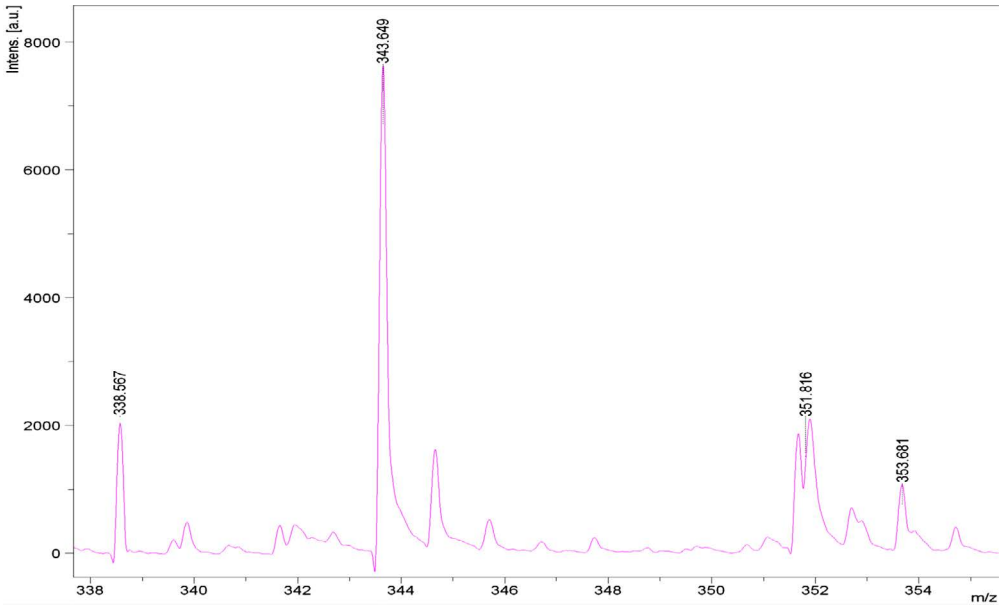
Table 7. IR spectral data of synthesized compounds.

Compounds	IR (KBr cm^{-1})
D1	3034, 1778, 1845, 1880, 1880, 1919, 1996 (Ar-CH), 2912 (-CH_3), 422, 439, 540 (Substituted benzene), 2742, 2912, 3192, 3286, 3375 (-NH), 817, 864, 923, 1029, 1047, 1056, 1120, 1151, 1271 (C-N).
D2	2850, 1456, 1267 (-NCH_2), 2918, 1404, 709 (-CH_2 and -CH_3), 1678, 3350, 1739 (C=O of ester), 1093 (C-O-C), 3124 (Ar-H), 1537, 1573, 1639, 1668 (C=N).
D3	3109 (-NH), 1639 (> CO), 3116 (-NH_2), 2833 (-CH_3), 1008 (> C=S), 1165 (-NCH_2), 572, 474 (Substituted benzene).
D4	3414 (-NH_2), 1093 (-NCH_2), 677 (C-S), 455 (Substituted benzene), 1406 (-CH_3), 3120 (Ar-CH).
BT1	3410 (-NH), 692, 603 (C-S), 447, 511 (Substituted benzene), 2841, 2939 (-NCH_2), 3032, 3061 (Ar-CH), 837, 927, 977, 1031, 1161, 1228 (C-N), 1498 (-CH_3).
BT6	1683, 1587, 1512 (C=N), 3149 (Ar-CH), 3215, 3238, 3406(-NH_2), 518 (Substituted benzene), 763(-CH_3).
BT7	3116 (Ar-CH), 3215 (-NH_2), 451 (Substituted benzene), 1680, 1602 (C=N), 617 (-CH_3).
BT8	3406, 3282 (-NH_2), 3178 (Ar-CH), 2839, 1427, 1244 (-NCH_2), 617 (C-S), 981(C-O Strech), 721 (-CH_3) 516 (Substituted benzene).
BT9	3043 (Ar-CH), 2839, 1278 (-NCH_2), 617 (C-S), 1591, 1546, 1514 (C=N), 455 (Substituted benzene), 3437 (-NH), 3529 (O-H), 1429 (C-O), 702, 779 (-CH_3).

Discovery studio software was used for predicting the protein-ligand binding modes. In this study, the compound having high (-) value is considered as the best one. Based on the Libdock score, BT6 was selected for *in vitro* antidiabetic and anti-inflammatory activities. The results of *in vitro* antidiabetic and anti-inflammatory activities are shown in **Tables 8 and 9.**



Graph 1. HNMR spectrum of compound BT-6.



Graph 2. Mass spectrum of compound BT-6.

Table 8. *In vitro* antidiabetic activity of Compound BT-6.

Concentration (µg)	% inhibition	
	Acarbose	BT6
12.5	51.81	31.85
25	53.56	39.81
50	62.69	44.25
100	68.61	49.25

Table 9. In vitro anti-inflammatory activity of compound BT-6.

Concentration (µg/ml)	Percentage Protection (%)	
	Diclofenac	BT6
10	95.55	65.18
20	97.03	68.66
50	98.50	71.11
100	99.84	73.70
250	99.84	79.62

DISCUSSION

In the present study, the in-silico molecular modeling studies were carried out for the selection of suitable drug candidates prior to wet lab synthesis. In-silico studies were performed by means of ACD Lab ChemSketch 12.0, Molinspiration, PASS, and Discovery studio. These compounds were synthesized by conventional methods. The synthesized compounds were subjected to TLC, melting point determination, IR, 1 HNMR and Mass spectroscopic studies. All these evaluation ensured the synthesized compounds. Of course this compound needs further studies such as toxicity and *in vivo* evaluation.

CONCLUSION

In conclusion, novel analogues of N-[(2-amino-5-methylene)-1,3,4-thiadiazole]-2-methyl benzimidazole are designed and synthesized. The IR, NMR and MASS spectral data confirmed the structure of synthesized compounds. Compound BT6 showed significant antidiabetic and anti-inflammatory activities. This novel compound BT-6 can be subjected to different pharmacological screening for considering it as a new drug candidate.

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