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Synthesis of Fluorinated Heterocyclic Compounds for Pharmacological Screening

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Abstract

A series of derivatives of (E)-6-chloro-5-fluoro-2-styryl-1H-benzo[d]imidazole, and 5-fluoro-2-methyl-N-phenyl-1H-benzo[d]imidazol-6-amine was synthesized. Compounds confirmed by melting point, FT-IR, ^{1}H-NMR, Mass spectral analytical techniques and predicted for their ADME, Pharmacokinetic properties.Synthesized compounds screened for better antibacterial and antiinflammatory activity. To synthesize a series of novel trisubstituted fluorinated benzimidazole derivatives and evaluate the physicochemical, ADME and pharmacokinetic properties and biological activity. The starting material Flouro-chloro-aniline ona series of reaction such as acetylation, nitration, deacetylation followed by reductionto get 4-fluoro-5-chloro-orthophenylene diamine. The di-amino compound cyclized with acetyl chloride to obtain 6-chloro-5-fluoro-2-methyl-1H-benzimidazole which on reaction with various aromatic aldehyde forms series of compounds 31(a-h), and with various anilines forms compounds 32(a-j). Physicochemical, ADME and pharmacokinetic properties predicted Insilco. Antimicrobial activity screened by Agar diffusion method. *In vitro* antiinflammatory activity screened by protein denaturation assay.The compounds synthesized confirmed by melting Point, FT-IR, ^{1}H-NMR, Mass spectral analytical techniques and predicted for their ADME, Pharmacokinetic properties in silico. The compounds screened and confirmed with moderate antimicrobial, and in vitro antiinflammatory activity.

Key words: - fluoro-chloro aniline; benzimidazole; aromatic aldehyde; aniline

Introduction

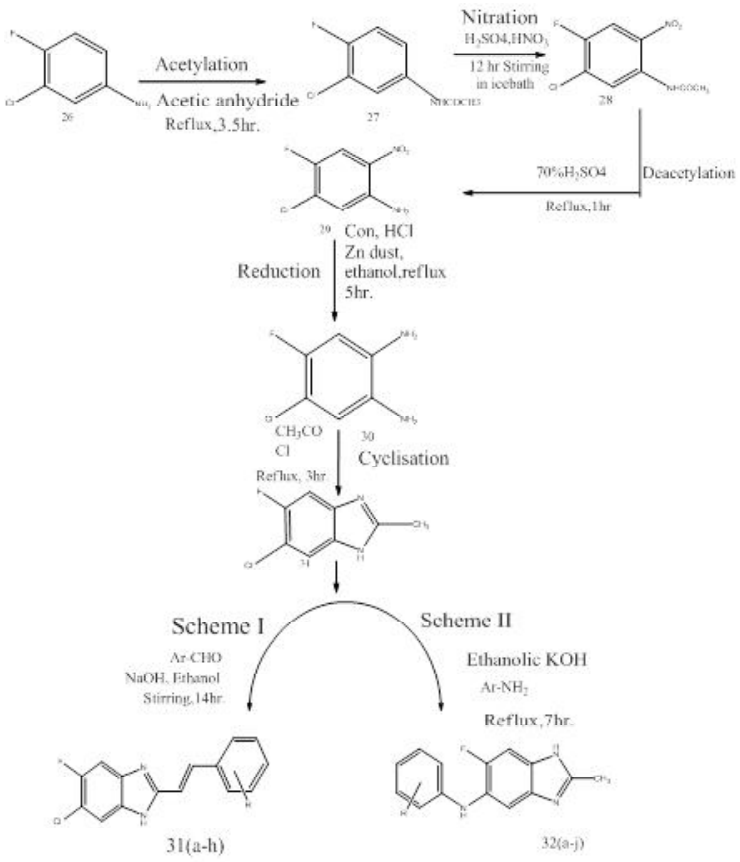
Benzimidazole is a fused heterocyclic ring system of 6-memberd benzene attached with 4, 5 position of a 5-membered imidazole ring. Rapid exchange of proton with the nitrogen atom led to the phenomenon of tautomerism. Benzimidazole is a prominent pharmacophore for the development of therapeutic agents due to the structural similarity or the fact that benzimidazole is a structural isostere of naturally occurring nucleotide in biological system.

It is a well proven fact that, purine involved in the biosynthesis of nucleic acids and proteins in the bacterial cell wall. Benzimidazoles can replace purine as a competitive inhibitor. In such away, it blocks biosynthesis of major components involved in metabolism leads to killing or inhibiting the growth of bacteria.[1]

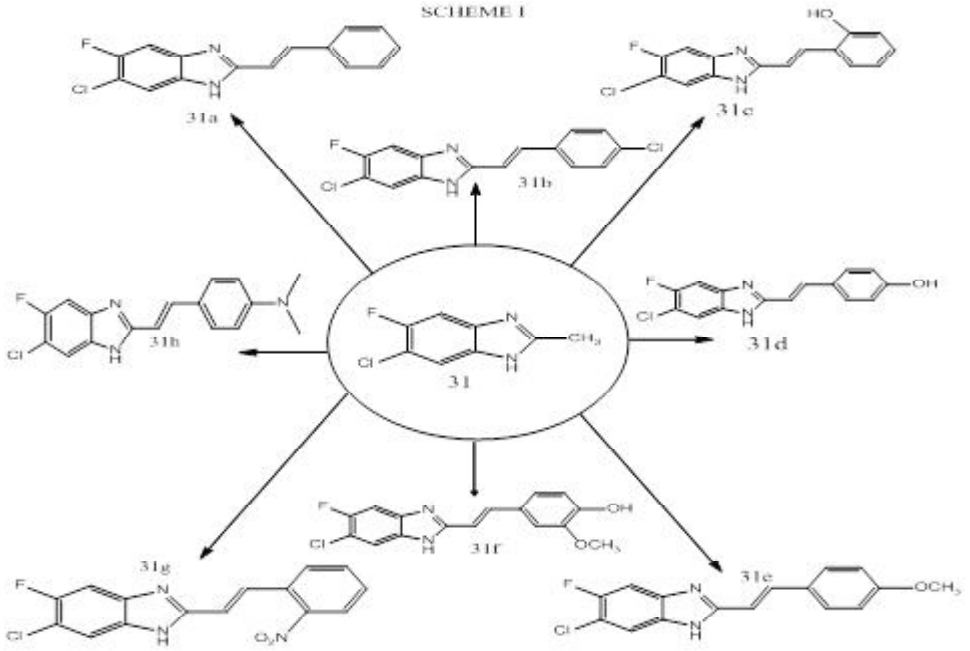
Introducing fluorine atom to the reactive sites of compounds helps to improve lipophilicity, membrane permeability,binding affinity and thus overcome bioavailability concerns. Also, Fluorine atom helps to keep the preferred molecular confirmation[2,3]. By considering the importance of fluorine in biological system aimed development of fluorinated benzimidazole derivatives.Trisubstituted benzimidazole derivatives in 1,2,5 and 2,5,6 position showed antitumor, antitubercular and antimicrobial activity. Substituted benzimidazole derivatives shows a wide range of activities like anticancer, antitubercular, antibacterial, anthelmintic, antifungal, antiviral, immunosuppressive, anti-convulsant, analgesic, anti-inflammatory, antidiabetic, antihypertensive, antioxidant, antidiarrheal and anxiolytic activities.[4-17]

Materials and Methods:

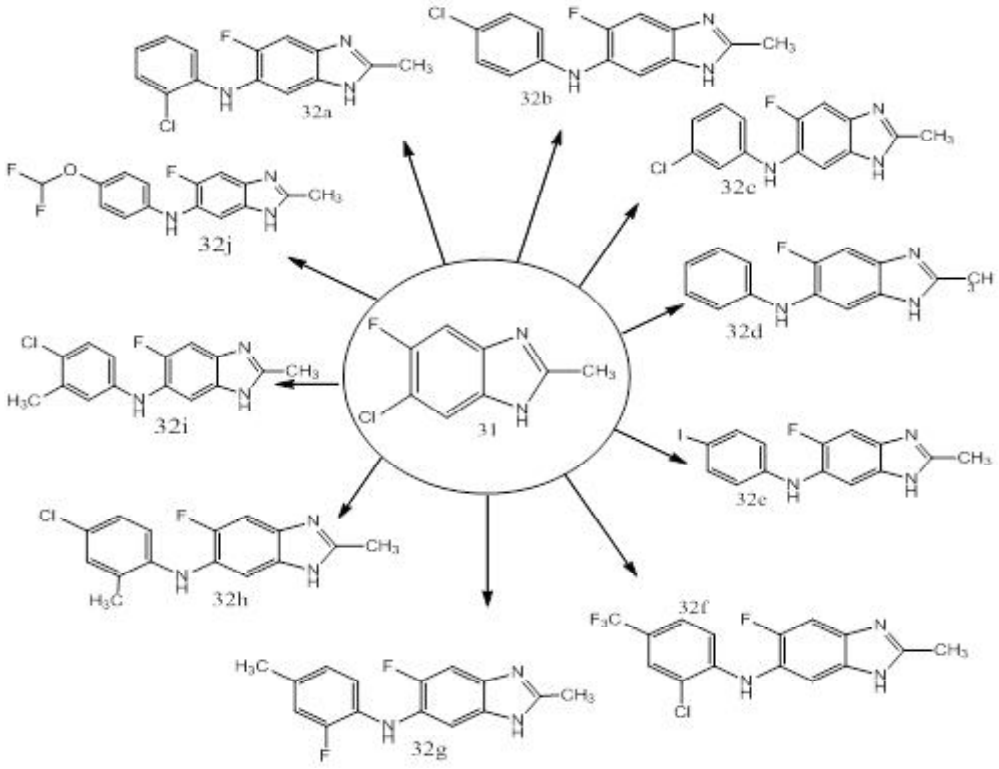
Experimental Scheme[18,19]:



SCHEME I



SCHEME II



Experimental Procedure:

1. Synthesis of N-(3-chloro-4-fluorophenyl) acetamide (27):

5g of 4-Flouro-3-Chloro aniline and 25 ml of Acetic anhydride refluxed for 3.5 h and cooled to room temperature. Reaction mixture poured into ice water to get precipitate of acetyl derivative. This mixture heated to boil for few minutes to get rid of excess unreacted acetic anhydride. Then kept the solution to stand overnight to get the acetyl derivative. R_f: 0.69, Yield: 97%, melting point:120-123 ºC. IR (KBr) cm^{-1} : 3304 (NH- secondary amine split peak), 2929(CH- aliphatic alkane), 3080 (CH- aromatic), 1674 (C=O of amides), 875 (C-F str), 690 cm^{-1} (C-Cl str).

2. Synthesis of N-(5-chloro-4-fluoro-2-nitrophenyl) acetamide (28):

5g N-(3chloro-4-fluorophenyl) acetamide added to RBF holding 15 ml of sulfuric acid cooled to 0ºC in an ice bath with constant stirring followed by slow addition of 11 ml of ice-cold concentrated fuming nitric acid over a period of 1-2hr. Reaction mixture was stirred constantly in an ice bath for 12 hr. The mixture was slowly poured into a beaker having 1 liter of ice water with vigorous stirring until precipitation of yellow solid occurs which was filtered and dried. R_f:0.42;Yield: 89%; melting point: 103-105ºC; IR (KBr) cm^{-1}: 3379 (NH- secondary amine), 2924 (C-H str aromatic), 1585 (NO_2- aromatic str), 887 (C-Fstr), 634(C-Cl str.

3. Synthesis of 5-chloro-4-fluoro-2-nitrobenzenamine (29):

In an RBF 1g of N-(5-chloro-4-fluoro-2-nitrophenyl) acetamide, 5ml of 70% Sulfuric acid added and refluxed for 1.5hr. Cool the reaction mixture and poured into beaker holding ice water with stirring. R_f :0.88; Yield: 86%, melting point;150-155ºC; IR (KBr) cm^{-1}:3493 cm^{-1} (NH_2- primary amine), 1639 cm^{-1} (C=C aromatic), 1502 cm^{-1} (-NO_2), 879 cm^{-1} (C-F), 640 cm^{-1} (C-Cl)

4. Synthesis of 4-fluoro-5-chloro-orthophenylene diamine (30):

0.5g of 4-fluoro-5-chloro-2-nitrobenzenamine placed in an RBF with 10 ml ethanol and 5 g of Zn. To this added concentrated HCl slowly with continuous stirring until all the Zn consumed. The reaction mixture stirred for 5 hr. and poured into ice water. To this mixture added saturated NaOH solution till the pH of 9-11 and the reaction mixture was subsequently extracted with ethyl acetate 3 X 20 ml. The organic layer dried over anhydrous Na_2SO_4, and solvent removed to obtain 4-fluoro-5-chloro-orthophenylene diamine. R_f: 0.39;Yield: 85%; melting point : 98-100ºC; IR (KBr) cm^{-1} : 3344 cm^{-1} (NH_2str primary amine), 3045 cm^{-1} (C-H str aromatic), 1610 cm^{-1} (C=C str aromatic), 860 cm^{-1} (C-F str), 663(C-Cl str), 1219cm^{-1} (C-N str) ; ^{1}H NMR($CDCl_3$, ppm) 6.483-6.694-(s, 2H-CH-aromaticprotons); 3.231-3.516-(s,4H- NH_2 protons).

5. Synthesis of 6-chloro-5-fluoro-2-methyl-1H-benzimidazole (31):

In an RBF 0.32g of 4-fluoro-5-chloro-orthophenylene diamine, add 0.15ml of acetyl chloride, 3ml of Benzene. Reflux for 3 hr. After reflux reaction mixture added to 50 ml of water and extracted with ethyl acetate (3x20ml). The organic layer dried over anhydrous Na_2SO_4, and solvent removed to obtain compound. Recrystallisation done with dil. Ethanol. R_f:0.53; Yield: 95%; melting point : 230-236ºC;IR (KBr) cm^{-1} : 3266 cm^{-1} (NH str secondary amine), 3014 cm^{-1} (C-H str aromatic), 1612 cm^{-1} (C=C str aromatic), 966 cm^{-1} (C-F str),742cm^{-1} (C-Cl str), 1220cm^{-1} (C-N str), 2607m^{-1} (C-CH_3 str); ^{1}H NMR($CDCl_3$, ppm) 2.174-2.191—(s,3H-CH_3), 8.169 (s,1H-NH), 7.351-7.385 (m, 2H-CH aromatic).

6a. Synthesis of 6-chloro-5-fluoro-2-styryl-1H-benzimidazole derivatives(31a-h):

Added 0.001 mol of 6-Chloro-5-Flouro-2-methy-1H-benzimidazole to a solution of 0.3g NaOH in 2 ml of water and 4 ml of absolute ethanol. Stirred reaction mixture for 30 min. Add 0.001 mol of various aromatic aldehydes gradually and the stirring continued for 14 hr. The reaction mixture poured into 50 ml of water and extracted with ethyl acetate. The organic layer dried over anhydrous Na_2SO_4, and solvent removed to obtain the product.

IR (KBr) cm^{-1} : 3448 cm^{-1} (NH str secondary amine), 2943 cm^{-1} (C-H str aromatic), 1246cm^{-1} (C-N str), 1647 cm^{-1} (C=C aromatic str) 2364 cm^{-1} (C=C alkene str), 804 cm^{-1} (C-F str), 684cm^{-1}(C-Cl str)7.325-8.381 (m,7H-aromaticprotons),4.330-(s,1H-NH),2.161-2.216 (s, 2H-aliphatic proton) and confirmed with molecular ion peak of 301.1 (31e) and fragmented ion peak of mass spectral data.

6b. Synthesis of N-(2-chlorophenyl)-6-fluoro-2-methyl-1H-benzo[d]imidazol-5-amine derivatives(32a-j):

To the ethanolic KOH solution of 6-Chloro-5-Flouro-2-methy-1H-benzimidazole (0.001ml), added o-chloro aniline (0.001 mol), refluxed for 7 hours. Hot mixture was poured into crushed ice with constant stirring and extracted with ethyl acetate. The organic layer was dried over anhydrous Na_2SO_4, and solvent removed to obtain the product.

IR (KBr) cm^{-1}: 3441 cm^{-1} (NH str secondary amine), 3047cm^{-1} (C-H str aromatic), 1273cm^{-1} (C-N str), 1627 cm^{-1} (C=C aromatic str), 856 cm^{-1} (C-F str), 2928 (C-CH_3 str) 3.813-3.901 (s-3H-CH_3), 4, 29-4.65 (s-2H-NH), 6.927-8.564-(m,7H Aromatic protons) and confirmed with molecular ionpeak of 276.8 (32b) and fragmented ion peak of mass spectral data.

Screening of Antiinflammatory Activity: In vitro Antiinflammatory screened by protein denaturation assay method (Bovine Serum Albumin Assay). Stock solutions of 1 mg/ml of all the samples and standard (Diclofenac) prepared using methanol as a solvent. Standard drug used was.0.1 ml of each sample (100, 200 mcg/ml) added 5 ml of 0.2% w/v of bovine serum albumin, solutions heated at 72°C for 5 minutes and then cooled for 10 minutes.Absorbance measured at 660 nm.

$$\% \text{ Anti denaturation activity} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

Screening of Antimicrobial Activity: Antimicrobial activity evaluated with Agar Diffusion Method.Media (Beef extract – 2g,Agar-17g, Acid hydrolysate of casein- 17.5g, Starch-1.5g, Distilled water-1000ml) distributed into each of petri plate. Microorganisms, *Staphylococcus aureus* (gram positive) & *E. coli* (Gram negative*)* were inoculated to the Petri plate. The test solution and the standard (Ciprofloxacin) of 0.1 ml in concentrations in chloroform (50 µg/ml) placed and incubated for 24-36 hours at 37 ºC. Zone of inhibition was measured and tabulated.

Results and Discussion

Physico-chemical properties of Synthesized compounds

Table -1.

Comp Code	Molecular Formula	M.W. (g/mol)	R_f value	Physical State	M.P(°C)	Lipophilicity (Log Po/w)	Water Solubility	%Yield
31a	$C_{15}H_{10}ClFN_2$	272.70	0.88	Solid	198-203	4.14	Poorly Soluble	43
31b	$C_{15}H_9Cl_2FN_2$	307.15	0.85	Solid	190-195	4.67	Poorly soluble	43
31c	$C_{15}H_{10}ClFN_2O$	288.70	0.74	Solid	140-145	3.75	Moderately soluble	35
31d	$C_{15}H_{10}ClFN_2O$	288.70	0.72	Solid	160-165	3.72	Moderately soluble	47
31e	$C_{17}H_{15}ClFN_3$	302.73	0.82	Solid	175-170	4.11	Poorly soluble	50
31f	$C_{16}H_{12}ClFN_2O_2$	318.73	0.79	Solid	182-187	3.75	Moderately soluble	52
31g	$C_{15}H_9ClFN_3O_2$	317.70	0.80	Solid	155-160	3.54	Moderately soluble	40

31h	$C_{17}H_{15}ClFN_3$	315.77	0.76	Solid	160-165	4.13	Poorly soluble	45
32a	$C_{14}H_{11}ClFN_3$	275.71	0.43	Semisolid	115-120	3.81	Poorly soluble	40
32b	$C_{14}H_{11}ClFN_3$	275.71	0.40	Semisolid	115-120	3.79	Poorly soluble	32
32c	$C_{14}H_{11}ClFN_3$	275.71	0.42	Semisolid	116-120	3.78	Poorly soluble	35
32d	$C_{14}H_{12}FN_3$	241.26	0.50	Semisolid	117-123	3.21	Poorly soluble	45
32e	$C_{14}H_{11}FIN_3$	367.16	0.32	Semisolid	94-97	3.93	Poorly soluble	30
32f	$C_{15}H_{10}ClF_4N_3$	343.71	0.30	Semisolid	89-92	4.85	Poorly soluble	31
32g	$C_{15}H_{13}F_2N_3$	273.61	0.28	Semisolid	100-105	3.87	Poorly soluble	30
32h	$C_{15}H_{13}ClFN_3$	289.74	0.41	Semisolid	105-109	4.12	Poorly soluble	32
32i	$C_{15}H_{13}ClFN_3$	289.74	0.43	Semisolid	103-106	4.08	Poorly soluble	30
32j	$C_{15}H_{12}F_3N_3O$	307.27	0.27	Semisolid	90-92	3.83	Poorly soluble	30

ADME Properties of Synthesized Compounds

Table-2

Comp Code	GI Absorption	BBB Permeable	CYP1A2 Inhibitor	CYP2C19 Inhibitor	CYP3A4 Inhibitor
30	High	Yes	No	No	No
31	High	Yes	Yes	No	No
31a	High	Yes	Yes	Yes	No
31b	High	Yes	Yes	Yes	No
31c	High	Yes	Yes	Yes	No
31d	High	Yes	Yes	Yes	No
31e	High	Yes	Yes	Yes	No
31f	High	Yes	Yes	Yes	No
31g	High	No	Yes	Yes	No
31h	High	Yes	Yes	Yes	No
32a	High	Yes	Yes	Yes	Yes
32b	High	Yes	Yes	Yes	Yes
32c	High	Yes	Yes	Yes	Yes
32d	High	Yes	Yes	Yes	Yes
32e	High	Yes	Yes	Yes	Yes
32f	Low	No	Yes	Yes	Yes
32g	Low	No	Yes	Yes	Yes
32h	High	Yes	Yes	Yes	Yes
32i	High	Yes	Yes	Yes	Yes
32j	High	Yes	Yes	Yes	Yes

Table 3. Results of Anti-inflammatory Activity

Comp No.	Conc. (µg/ml)	Absorbance at 660 nm	% Anti-inflammatory activity
Control	–	0.041	–
31a	100	0.022	46.34
	200	0.019	53.65
31b	100	0.020	51.29
	200	0.018	56.69
31c	100	0.023	43.95
	200	0.019	53.65
31d	100	0.021	48.78
	200	0.017	58.53
31e	100	0.019	53.65
	200	0.013	68.29
31f	100	0.024	41.46
	200	0.017	58.53
31g	100	0.020	51.29
	200	0.018	56.09
31h	100	0.023	43.91
	200	0.019	53.65
32a	100	0.021	46.34
	200	0.018	56.09
32b	100	0.023	43.90
	200	0.019	53.65
32c	100	0.020	51.29
	200	0.018	56.09
32d	100	0.021	48.78
	200	0.017	58.53
32e	100	0.019	53.65
	200	0.013	68.29
32f	100	0.024	41.46
	200	0.017	58,53
32g	100	0.023	43.91
	200	0.020	51.29
32h	100	0.022	46.34
	200	0.019	53.65
32i	100	0.023	43.90
	200	0.019	53.65
32j	100	0.020	51.29
	200	0.018	56.09
Diclofenac Sodium (Standard)	100	0.014	65.85
	200	0.012	70.73

Results of Antimicrobial Activity

Table 4

Compounds	Gram (+ve) *S.A. Bacilli*	Gram (-ve) *E. coli*
31a	3mm	3mm
31b	4mm	4mm
31c	4mm	4mm
31d	3mm	4mm
31e	4mm	3mm
31f	3mm	2mm
31g	4mm	3mm
31h	4mm	4mm
32a	4mm	4mm
32b	4mm	4mm
32c	4mm	4mm
32d	3mm	3mm
32e	4mm	4mm
32f	4mm	4mm
32g	4mm	3mm
32h	4mm	4mm
32i	4mm	4mm
32j	4mm	4mm
Control	2mm	2 mm
Standard	5mm	6 mm

Results and Discussion

Yield of the synthesized compounds was found to be mild to moderate. Physicochemical, pharmacokinetic and ADME property data were tabulated in Table 1 and 2. The compounds were found to be highly lipophilic. Almost all synthesized compounds are poorly water soluble other than 31c, 31d, 31f, and 31g. which were found moderately soluble in water. GI absorption of all the compounds were found to be high other than 32f, 32g. The synthesized compounds can permeate the BBB (blood-brain barrier) other than 31g, 32f and 32g. All the synthesized compounds inhibit CYP1A2 and CYP2C19 metabolism. The compounds 31a-j found with no CYP3A4 inhibition. Invitro anti-inflammatory study proves that the methanolic solutions of newly synthesized compound inhibited the denaturation of BSA in vitro in a moderate manner, comparable to the reference drug, Diclofenac sodium and the results tabulated in table 3. Anti-microbial activity results tabulated in table No.4 which proves that compounds possess moderate anti-microbial activity compared to the standard.

Conclusion

The derivatives of (E)-6-chloro-5-flouro-2-styryl-1H-Benzo[d]imidazole and 5-fluoro-2-methyl-N-phenyl-1H-benzo[d]imidazol-6-amine was synthesized. The yield of the synthesized compounds was found to be mild to moderate.All the synthesized compounds were analyzed with various spectra and confirmed with the structure. The compounds were evaluated for their ADME and Pharmacokinetic properties Insilco and tabulated the results. Invitro antiinflammatory study suggest thatcompounds have moderate anti-inflammatory activity compared to the standard. The newly synthesized compounds inhibited the microbial growth moderately compared to the reference drug.

Scope for Future Work :

Further work suggested for the optimization of compounds and further pharmacological studies. Need to focus on the computational studies for the structural modification of the synthesized compound to achieve for better biological action.

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