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NEW 3-(2-(2-PHENYL-1H-BENZO [D] IMIDAZOL-1-YL) ACETYL)-2H-CHROMEN-2-ONE ANALOGUES AS POTENTIAL ANTIMICROBIAL AGENTS

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ABSTRACT

The reaction of salicylaldehyde with ethyl acetoacetate yield 3-acetyl coumarin which on bromination yieled 3-(2-bromoacetyl)-2H-chromen-2-one.[1] Various 2-substituted benzimidazoles(2) have been synthesized from o-Phenylenediamine and organic acids, then N-alkylation of compound 2 carried out with 3-(2-bromoacetyl)-2H-chromen-2-one to give some new coumarin induced benzimidazoles. The compounds were synthesized in good yield and the structures of compounds have been established on the basis of their spectral data. Synthesized compounds were screened for antibacterial activities.

KEYWORDS: 3-(2-bromoacetyl)-2H-chromen-2-one, benzimidazole, o-Phenylenediamine, coumarin and biological activity.

INTRODUCTION

Benzimidazole is a privileged bicyclic ring system. Benzimidazole nucleus is a useful structure for further molecular exploration and for the development of new pharmaceutical compounds, has been studied extensively. Synthesis of benzimidazoles has received much attention owing to the varied biological activity exhibited by a number of its derivative.[1,5] The class of these molecules proved to be very important as they possess pharmaceutical properties including antibacterial against different strains of Gram-positive and Gram negative bacteria,[6,7] antifungal,[l8] herbicida,[l9] analgesic,[10] antioxidant,[11,12] antiallergic,[13,14] antitumoral agents,[15] antiparasitic[16] and anthelmintic[17] etc. Various benzimidazole derivatives have been found to posses bioactivity as phosphodiesterase IV inhibitor[18] antagonist[19] of angiotensin II, neuropeptide Y receptors[20] H3-receptor antagonist[21] and neuropeptide Y5-receptor antagonist.[22] Recently, benzimidazole

derivatives have attracted particular interest due to their anticancer activity and may act as in vitro anti-HIV agents.[23-26] On other hand coumarin and its derivatives possess a broad spectrum of biological activities including antibacterial,[27] antifungal,[28] anticoagulant,[29] anti- inflammatory,[30] antitumor,[31] and anti-HIV.[32] Coumarin compounds are also used as additives in food, cosmetic and dyes. Owing to the great biological and synthetic importance of this heterocyclic core, synthesis of benzimidazole and coumarin derivatives has long been an area of intense development, and still constitutes an active domain from academic and industrial points of view. With these consideration in mind we decided to study new coumarin induced benzimidazoles, the main goal of present work is to design, synthesize and evaluate the antibacterial activity of new derivatives of benzimidazole, which were synthesized by N-alkylation of 2-substituted benzimidazole with 3-(2-bromoacetyl)-2H-chromen-2-one.

MATERIALS AND METHODS

All the chemicals and reagents used were of synthetic grade procured from various chemical units like Loba Chemicals, S.D. Fine Chemicals Ltd. Double distilled water was used. The reactions were monitored on silica gel TLC plates by using petroleum ether: ethyl acetate as a mobile phase and visualized in iodine vapour. Melting points of all the synthesized compounds were determined in open capillary tubes and the values were uncorrected. The IR spectra were recorded on Shimadzu FTIR-4200 spectrometer by KBr pellets technique. ^{1}H-NMR spectra were recorded on 300 MHz spectrometer in DMSO-d_6 and $CDCl_3$ as solvent and TMS as internal standard. ^{13}C NMR spectra were recorded in $CDCl_3$ and DMSO-d_6.

Experimental

Preparation of 3-acetyl-2H-chromen-2-one (1)

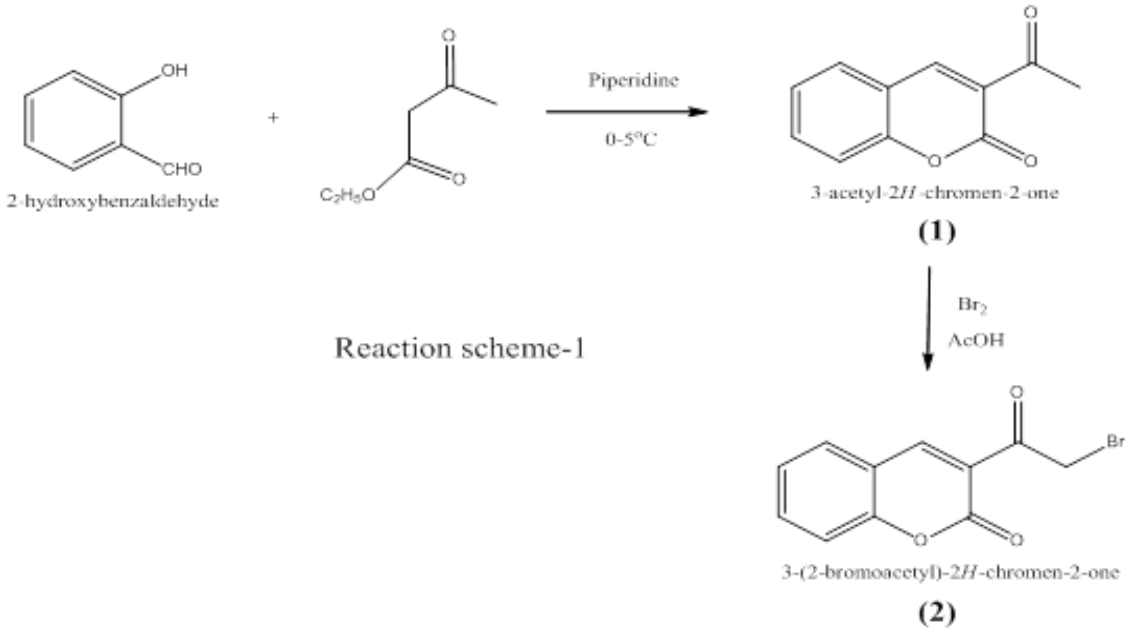
Freshly distilled 2-hydroxybenzaldehyde 11 ml (0.1mol) was mixed with ethyl acetoacetate 13 ml (0.1 mol) in 25ml ethanol; reaction mixture was maintained at 0^{o}C on ice bath and piperidine (1 ml) was added; after two hours mixture had completely solidified into yellow solid; it was then poured into ice cold water,filtered; solid compound obtained was recrystalized from 50% ethanol; yielded yellowish white crystalline compound.[1]

Yield: 87% **M.p.** 118-119^{o}C.

IR (KBr): 3029, 2930, 1739, 1674, 1604, 1555, 1452, 1364, 1264, 1221 cm^{-1}.

^{1}H NMR: (300 MHz, $CDCl_3$): at δ = 2.73 (s, 3H; CH_3), 7.27-7.39 (dd, 2H; ArH), 7.63-7.69 (m, 2H; ArH), 8.51 (s, 1H, Ar H).

^{13}C NMR: ($CDCl_3$): 30.57, 116.70, 118.26, 124.53, 124.99, 130.24, 134.40, 147.47, 155.33, 159.23, and 195.48.



Reaction scheme-1

Preparation of 3-(2-bromoacetyl)-2H-chromen-2-one (2)

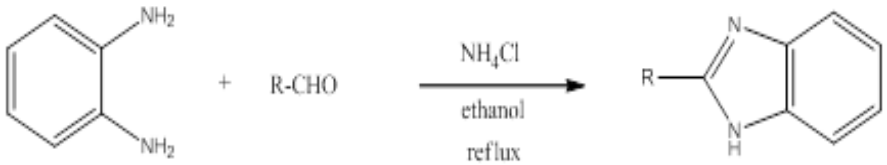
To a stirred solution of 3-acetyl-2H-chromen-2-one; (1 gm; 0.005 mol) in glacial acetic acid (25 ml), and molecular bromine (0.28 ml;0.0016 mol) was added dropwise and the reaction was maintained at 0^{o}C on ice bath. The reaction was allowed to stir at room temperature for 6-8 hrs. Dark brown reaction solution turns to yellow precipitate; it was then poured onto crushed ice, solid compound obtained was recrystalized from 50% ethanol; yielded white crystalline compound (2).

Yield: 76% **M.p.** 160-161^{O}C.

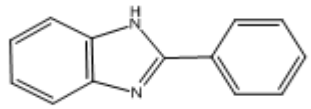
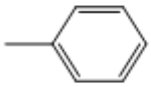
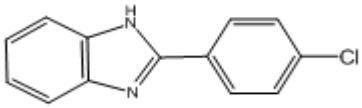
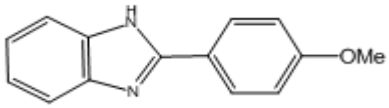
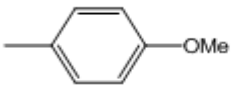
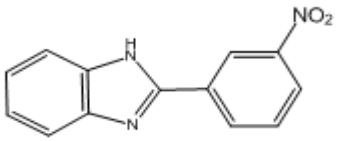
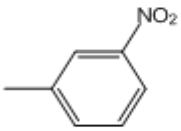
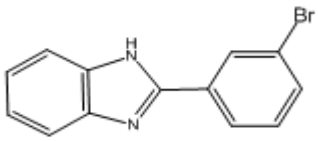
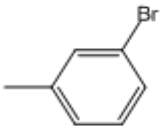
IR (KBr): 3029, 2930, 1739, 1674, 1604, 1555, 1452, 1364, 1264, 1221 cm^{-1}.

^{1}H NMR: (300 MHz, $CDCl_3$): at δ = 4.76 (s, 2H; CH_2), 7.27-7.42 (dd, 2H; ArH), 7.68-7.73 (m, 2H; ArH), 8.64 (s, 1H, Ar H).

^{13}C NMR: ($CDCl_3$): 35.59, 116.89, 118.14, 122.18, 125.30, 130.43, 135.11, 149.52, 155.42, 158.87, and 188.87.



Reaction scheme-2

Compound	R	Yield
3a 		73%
3b 		76%
3c 		75%
3d 		78%
3e 		81%

*Yield refer to purified compounds

Preparation of 2-phenyl-1H-benzo[d]imidazole (3a)

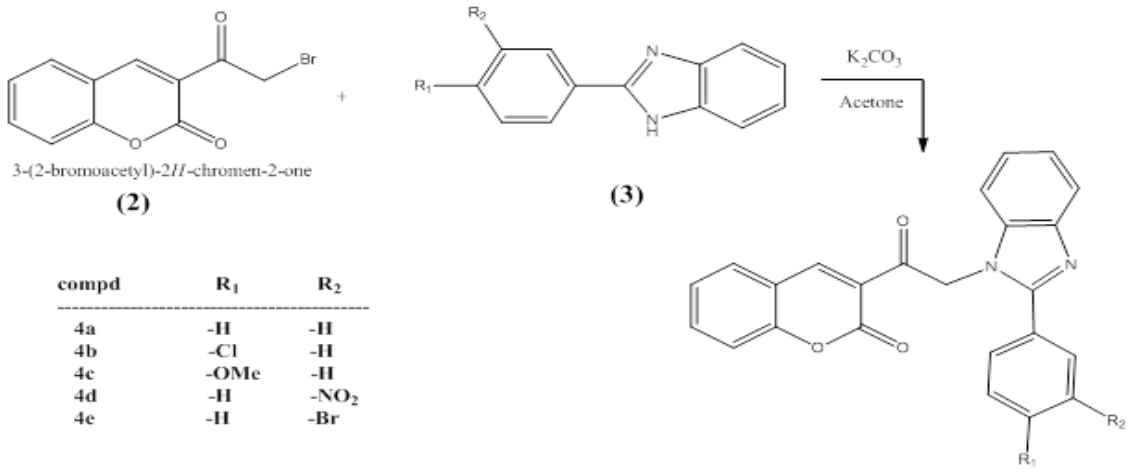
Benzaldehyde (3.9 ml, 0.037 mol) was added to a stirred solution of 1,2-phenylenediamine (4g, 0.037mol) and NH_4Cl in ethanol (60 ml) for five minutes at room temperature. Stirring was continued for four hours at 80^OC. After completion of the reaction (TLC, eluent PET ether :ethyl acetate 30:70), it was poured into ice cold water. Product precipatated as pale yellow solid; solid was filtered, washed with water and recrystalized from ethanol to give pure solid.

Yield: 81% **M.p.** 283^OC. The same procedure was applied for all other compounds.

IR (KBr): 3179**,** 3113, 3038, 2997, 2918, 2725, 2683, 1464, 1387, 1273, 1021, 850, 735 cm^{-1}.

^{1}H NMR: (300 MHz, DMSO-d_6): at δ = 7.47-7.51 (dd, 2H; ArH), 7.03-7.10 (m, 2H; ArH), 7.53 (m, 5H, ArH.) 12.13 (s, 1H, NH).

^{13}C NMR: 151.19, 144.10, 135.93, 130.6, 130.8, 128.3, 127.34, 123.45, 122.89, 120.96, 114.14.



compd	R_1	R_2
4a	-H	-H
4b	-Cl	-H
4c	-OMe	-H
4d	-H	$-NO_2$
4e	-H	-Br

Reaction scheme-3

Preparation of3-(2-(2-phenyl-1H-benzo[d]imidazol-1-yl)acetyl)-2H-chromen-2-one, (4a):

An equimolar proportion of 3-(2-bromoacetyl)-2H-chromen-2-one (2), 0.3gm (0.001mol) and 2-methyl-1H-benzo[d]imidazole (3) (R= Me), 0.14 gm (0.001mol) was taken in acetone with potassium carbonate (K_2CO_3), 0.15gm. The reaction mixture was stirred at 60^OC for 6 hrs. After the complete disappearance of starting material (monitored by TLC, PET: Ethyl acetate); excess solvent was removed by distillation and crude product was washed with water, extracted with ethyl acetate and finally recrystallized from 50% ethanol to give 4a. Similar procedure was employed for the preparation of all other derivatives.

IR (KBr): 3179, 3056, 2923, 2755, 2683, 2544, 1687, 1618, 1593, 1440, 1362, 1220, 1029, 897, 734 cm^{-1}.

^{1}H NMR: (300 MHz, DMSO-d_6): at δ = 5.30 (s, 2H; CH_2), 7.29-7.39 (dd, 2H; ArH), 7.58-7.69 (m, 2H; ArH), 8.57 (s, 1H, Ar H). 7.47-7.51 (dd, 2H; ArH), 7.09-7.25 (m, 2H; ArH), 7.85 (m, 5H, ArH.).

^{13}C NMR: (DMSO-d_6): 51.59, 110.08, 111.85, 116.89, 118.14, 119.82, 122.18, 123.13, 123.46, 125.30, 129.42, 130.43, 130.81, 134.20, 135.11, 142.30, 144.42, 149.30, 149.52, 155.42, 158.87, and 188.87.

Antibacterial bioassay

0.8 % of the MIC (minimum inhibitory concentration, μg/mL) of all the final products were prepare in dimethylformamide solvent and tested against two Gram -ve (Escherichia Coli, Pseudomonas aeruginosa) and two gram +ve bacteria (Staphylococcus Aureus, Staphylococcus epidermidis). The composition of nutrient agar medium was bactotryptone (4g), Broth (3.9 g) less than 2%, NaCl (2.9 g) in 100 ml of water (2.9%). After 18 hrs the

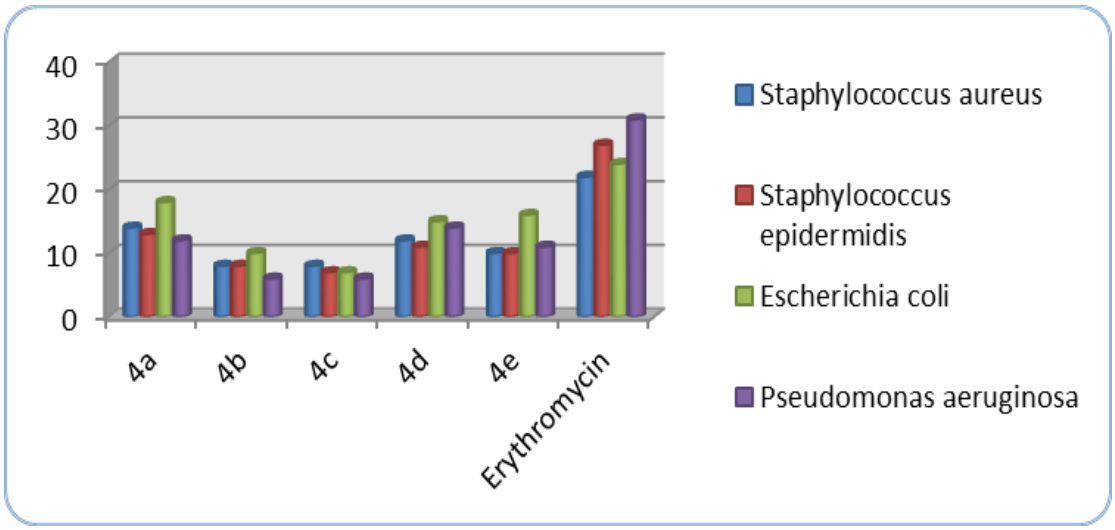
exponentially growing culture of the bacteria in nutrient broth at 37^{o}C were diluted in sterile broth. From each of these diluted culture, 1 ml was added to 100 ml sterilized and cooled nutrient agar media to give a final bacterial culture. The plates were set at room temperature and later dried at 37^{o}C for 20 hrs. Paper discs (6mm, punched from whatmann no. 41 paper) used for the assays. Discs were socked in DMF and placed on the inoculated agar media at regular intervals of 6-7 cm, care was taken to ensure that excess solution was not on the discs. All the samples were taken in triplicates. The plates were incubated at 37^{o}C in an inverted fusion. Activity was determined by zone showing complete inhibition (mm). Growth inhibition was calculated with reference to positive control.

RESULTS AND DISCUSSION

The structure of synthesized compounds was confirmed by IR, ^{1}H NMR, and ^{13}C NMR analysis. Compounds (4a-e) were screened against four pathogenic bacteria. Two Gram negative strains viz., *Escherichia Coli, Pseudomonas aeruginosa* and two Gram positive stains viz., *Staphylococcus Aureus, Staphylococcus epidermidis* following agar well diffusion procedure as per the reference. The antibacterial activity of the synthesized benzimidazole 4a-e was corrected with the zone of inhibition of erythromycin as a standard control. (Table 2). The bacterial test result for the newly synthesized benzimidazole analogues revealed that most of the compounds exhibited moderated to good activity against Gram +ve (*Staphylococcus Aureus, Staphylococcus epidermidis*) and Gram –ve bacteria (*Escherichia Coli, Pseudomonas aeruginosa*). For *Staphylococcus Aureus* compounds 4a and 4d exhibited maximum activity while the other compounds displayed moderate activity. And in case of *Escherichia Coli*, compounds 4a and 4e exhibited good to excellent activity while the remaining compounds displayed moderate and less activity. As all compounds showed antibacterial activity against the bacteria tested. It indicates that this basic moiety can be a potential scaffold for antibacterial drugs. It may be suggested that the coumarin induced benzimidazole derivative with a suitable R group may lead to a good antibacterial agent for all the *Escherichia Coli* and *Staphylococcus Aureus* bacterial strains.

Table 2: Result of antibacterial bioassay of compounds.

Compounds No.	Zone of Inhibition (mm)			
	Gram-Positive		Gram-Negative	
	Staphylococcus aureus	*Staphylococcus epidermidis*	*Escherichia coli*	*Pseudomonas aeruginosa*
4a	14	13	18	12
4b	08	08	10	06
4c	08	07	07	06
4d	12	11	15	14
4e	10	10	16	11
Erythromycin	22	27	24	31



Antibacterial bioassay: Zone of Inhibition (mm)

Computational study

Target prediction and ADME based virtual screening

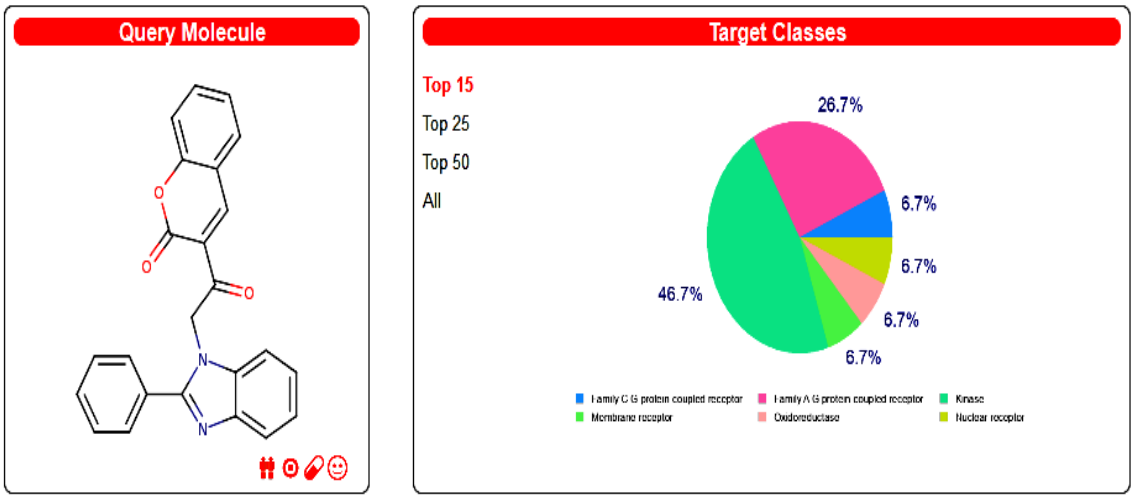


Figure 1: Swiss target prediction approach graph for most active 4a.

Among all the compounds 4a is most active, therefore we have screened it against different targets so as to explore future designing using the tool called Swiss Target Prediction (Gfeller, Wirth, Daina, Michielin, & Zoete, 2014) (http://www.swisstargetprediction.ch/). From Figure:1 it was clear that 4a, would likely to act on kinases having a percentage of prediction 46.7% and may be evolved as future designing, as anticancer agents. Database is displayed in Table: 3. We further explored our study with respect to 4a and carried out ADME based virtual screening.

Table 3:

Target Classes	Target prediction approach (%)
Kinase	46.7.3%
Family A G protein-coupled receptor	26.7%
Family C G protein-coupled receptor	6.7%
Membrane receptor	6.7%
Oxidoreductase	6.7%
Nuclear receptor	6.7%

From our antimicrobial study, we have screened out compound 4a for drug-likeness analysis. The results obtained from drug-likeness values show that there is no violation for popular Lipinski rule of five but some are violating the others. The oral bioavailability score of 0.55 is obtained by using the popular web-based tool called Swiss ADME (Daina, Michielin & Zoete, 2017) (http://www.swissadme. ch/index.php) ('SwissADME: https://www.nature.com/ articles/srep42717Title'). The pharmacokinetic profile data show that all the compound 4a is inhibitors of CYP1A2, CYP2C19 and CYP2C9.

Physicochemical Properties	
Formula	C24H16N2O3
Molecular weight	380.40 g/mol
Num. heavy atoms	29
Num. arom. heavy atoms	25
Fraction Csp3	0.04
Num. rotatable bonds	4
Num. H-bond acceptors	4
Num. H-bond donors	0
Molar Refractivity	112.19
TPSA	65.10 Å²
Lipophilicity	
Log $P_{o/w}$ (iLOGP)	2.33
Log $P_{o/w}$ (XLOGP3)	4.81
Log $P_{o/w}$ (WLOGP)	4.69
Log $P_{o/w}$ (MLOGP)	3.37
Log $P_{o/w}$ (SILICOS-IT)	4.75
Consensus Log $P_{o/w}$	3.99

Pharmacokinetics	
GI absorption	High
BBB permeant	Yes
P-gp substrate	No
CYP1A2 inhibitor	Yes
CYP2C19 inhibitor	Yes
CYP2C9 inhibitor	Yes
CYP2D6 inhibitor	No
CYP3A4 inhibitor	No
Log K_p (skin permeation)	-5.21 cm/s
Druglikeness	
Lipinski	Yes; 0 violation
Ghose	Yes
Veber	Yes
Egan	Yes
Muegge	Yes
Bioavailability Score	0.55
Medicinal Chemistry	
PAINS	0 alert
Brenk	1 alert: cumarine
Leadlikeness	No; 2 violations: MW>350, XLOGP3>3.5
Synthetic accessibility	3.24

Figure 2: Swiss ADME prediction profile data for most active 4a.

CONCLUSION

The coumarin induced benzimidazoles synthesized in the present investigation have been subjected to antimicrobial screening and showed microbial activity against all the organisms under study. Therefore, these compounds may be considered as promising candidates for further antimicrobial investigation. Computational screening showed bioavailability score of 0.55 and it would likely to act on kinases having a percentage of target prediction 33.3% and may be evolved as future designing, as anticancer agents.

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