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# Synthesis, *in silico* study and biological evaluation of some novel 2-mercaptobenzothiazole derivatives containing chalcone moiety

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## Abstract

The emergence of multidrug-resistant (MDR) pathogens has created an urgent need for novel antimicrobial agents. In this study, a series of 2-mercaptobenzothiazole derivatives containing chalcone moieties were synthesized through a multistep reaction pathway and characterized using IR, 1HNMR, and mass spectrometry. The compounds (7a–7e) were evaluated for antibacterial activity against *Escherichia coli* and *Staphylococcus aureus* using the disc diffusion method. Among the derivatives, compound 7d (bromo-substituted) exhibited the highest activity, with inhibition zones comparable to ciprofloxacin at 100 µg/mL, while hydroxyl-substituted derivative 7c showed poor activity. In silico docking studies revealed strong binding affinities of compounds 7a and 7d to DNA gyrase, supported by hydrogen bond interactions with ASN192 and LYS108 residues. SwissADME analysis indicated that compounds 7b, 7c, and 7e complied with Lipinski's rule of five, suggesting favorable drug-likeness. Overall, the integration of synthetic, biological, and computational approaches highlights benzothiazole–chalcone hybrids as promising scaffolds for antimicrobial drug development.

**Keywords:** 2-Mercaptobenzothiazole, chalcone, DNA gyrase, docking, ADMET

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## Introduction

In the past years, health-care associated infections have become an important cause of morbidity and mortality, whilst the incidence of antibiotic-resistant bacteria has increased dramatically and become a serious threat.[1] In fact, the management of bacterial infections is getting increasingly tough due to augmented prevalence of MDR pathogens, which represent a major challenge to antimicrobial therapy. Microbial resistance is now frequently confronted to common antibiotics being used in clinical settings, and there is an imperative demand for newer anti-infective agents to overcome emerging multi-drug resistance. Today, the need for novel antimicrobials has been greater than ever in the face of increasing resistance to the older ones and increasingly tough management of bacterial infections.[2] In spite of urge for such agents, the scientific progression in terms of antimicrobial research and discovery of new antibacterial molecules has declined dramatically in the past few years.[3]

Among heterocyclic frameworks, benzothiazole derivatives have attracted considerable interest due to their wide spectrum of biological activities, including antimicrobial, anticancer, and anti-inflammatory properties.[4-6] The incorporation of chalcone moieties into benzothiazole scaffolds offers a promising strategy, as chalcones themselves are recognized for their diverse pharmacological potential. The combination of these two bioactive motifs may yield compounds with enhanced antimicrobial efficacy and improved drug-like properties.

In this context, the present work focuses on the synthesis of novel 2-mercaptobenzothiazole derivatives containing chalcone moieties. The synthesized compounds were systematically evaluated for antibacterial activity against *Escherichia coli* and *Staphylococcus aureus*. To complement the experimental findings, molecular docking studies targeting DNA gyrase, a key bacterial enzyme essential for DNA replication were performed to elucidate binding interactions. Furthermore, *in silico* ADMET predictions were carried out to assess physicochemical parameters, drug-likeness, and pharmacokinetic suitability. This integrated approach aims to identify promising lead molecules that could serve as candidates for further antimicrobial drug development.

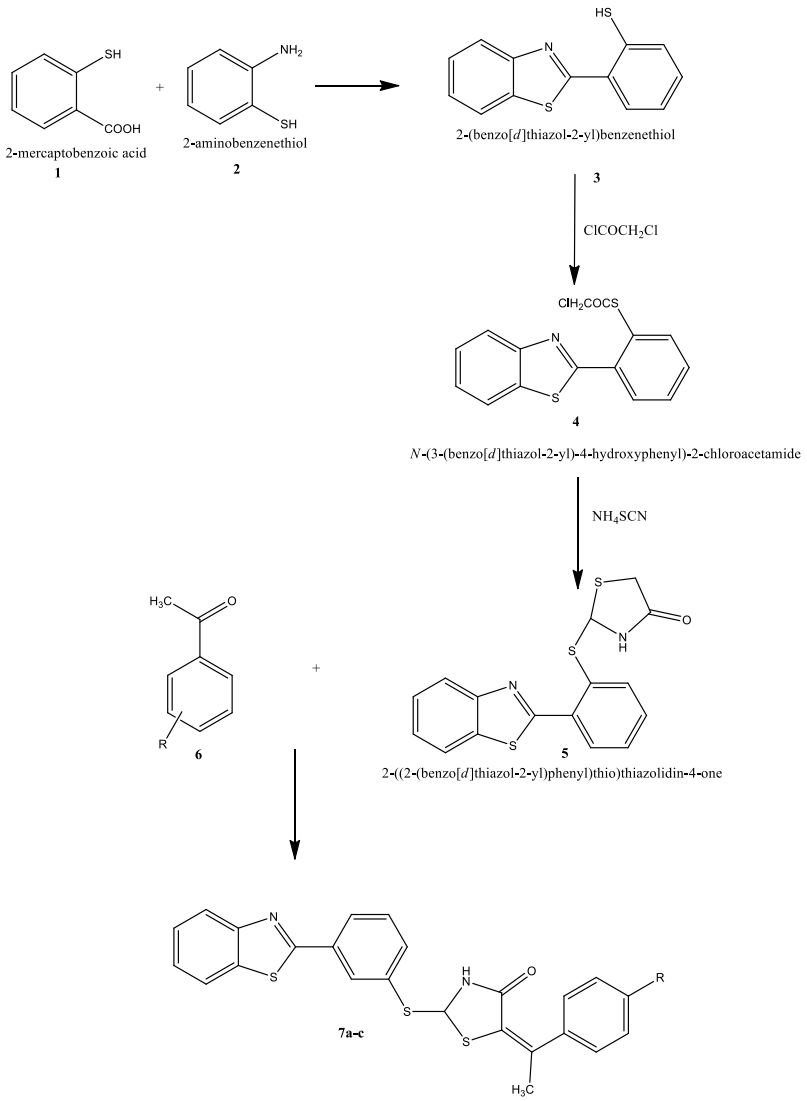
## Material and Methods

### Materials

2-mercaptobenzoic acid, 2-aminobenzenthiol was purchased from Loba, ethanol was obtained from Sigma Aldrich, chloracetylchloride, sulfuric acid, potassium hydroxide, hydrochloric acid, acetophenone, 4-nitroacetophenone, 4-hydroxyacetophenone, 4-aminoacetophenone, 4-bromoacetophenone, and all other chemicals used were of analytical or synthetic grade and purchased from CDH or Loba. Any chemical used in the study was used as received without any further purification. Electronic weighing scale (Wensar), Heating mantle (BioTechnics), Hot air

oven (BioTechnics), melting point apparatus (BioTechnics) were used during the study. Precoated TLC plate on aluminum backing (Silica gel $G_{F254}$) was purchased from Sigma Aldrich.

The experimental scheme was adapted from the previous reports by modification in conditions and optimized for obtaining highest yield (scheme 1).




**Scheme 1.**

**Synthesis of 2-(benzo[d]thiazol-2-yl)benzenethiol**

A mixture of 2-mercaptobenzoic acid (13 mmol) and 2-aminobenzenthiol (13 mmol) in polyphosphoric acid (10 ml) was heated at 200 °C for 4 h with stirring. The reaction mixture was poured into 400 ml of water and the precipitate obtained was filtered, neutralized with a solution of sodium carbonate (10% w/v), washed with water and dried.[7]

**Synthesis of N-(3-(benzo[d]thiazol-2-yl)-4-hydroxyphenyl)-2-chloroacetamide**

To a stirred solution of benzothiazole compound (0.04 mol) and triethylamine ($Et_3N$) (0.02 mol) in dioxane (50 ml), chloro acetyl chloride (0.02 mol) was added dropwise. The reaction mixture

was refluxed for 13 h and the excess of solvent was evaporated using rotary vacuum evaporator. The solid obtained was washed with distilled water, filtered dried and crystallized from ethanol.[8]

**Synthesis of 2-((2-(benzo[d]thiazol-2-yl)phenyl)thio)thiazolidin-4-one**

A well stirred solution of the N-(3-(benzo[d]thiazol-2-yl)-4-hydroxyphenyl)-2-chloroacetamide (0.01 mol) in methanol (50 mL) was added ammonium thiocyanate (0.01 mol) slowly over a period of 10 min. The mixture was refluxed for 7 h and the product obtained on cooling was filtered, washed with cold water and recrystallized from glacial acetic acid.[9]

**General method for synthesis of (E)-2-((3-(benzo[d]thiazol-2-yl)phenyl)thio)-5-(1-phenylethylidene)thiazolidin-4-one**

A well-stirred solution of 2-((2-(benzo[d]thiazol-2-yl)phenyl)thio)thiazolidin-4-one (0.8 g, 4 mmol) in acetic acid (35 mL) was buffered with sodium acetate (8 mmol) and the appropriate arylaldehyde (6 mmol) was added. The solution was refluxed for 4 h and then poured into ice-cold water. The precipitate was filtered and washed with water and the resulting crude product was purified by recrystallisation from Dioxane.[10]

**Antimicrobial activity**

The microorganisms used for the antimicrobial study were procured from Institute of Microbial Technology, Chandigarh (MTCC). *Escherichia coli* (MTCC 40), and *Staphylococcus aureus* (MTCC 3160) were used for the present investigation. The lyophilized cultures obtained from IMT, Chandigarh were revived by adding 0.3 mL of nutrient broth to the culture ampoules to obtain a suspension of the bacteria.

**Preparation of test compounds**

The synthesized Schiff's bases were dissolved in DMSO to obtain the solutions of 25, 50, 75 & 100 μg/mL. These solutions were used as the test samples.

**Determination of zone of inhibition**

About 3 mm thick pre-poured nutrient agar plates were inoculated with a few drops of the bacterial suspension by swabbing on the surface of agar. The antimicrobial action was screened using disc diffusion method.[11] Wells were bored into the agar plate at equal distances using cork borer (10mm) and 200μL of the Schiff's bases (25, 50, 75 & 100 μg/mL) were placed in each hole. The plates were incubated for 24h at 37 ± 0.1°C to allow for microbial growth. The zone of inhibition in each plate was measured in millimeters.

**Docking studies**

The docking study of the synthesized compounds on the active site of DNA gyrase was performed with the help of Autodock software. The DNA gyrase protein structure (4URO) for docking was downloaded from protein database. The macromolecule was prepared for docking by removal of

water molecules, addition of charges and hydrogen. The ligand structure were drawn using Chemdraw ultra 12.0 software and saved as SDF file. The ligand structure for docking was prepared by saving the structure as pdbqt file. Docking study was carried and the binding affinity scores were calculated. The interactions ligand with various amino acid residues in the helix of the enzyme was also observed.[12,13]

### *In silico* ADMET study

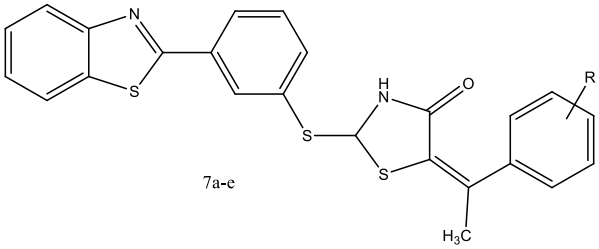
The 2D structures of the ligands were sketched on ChemDraw Ultra 12.0 and then transferred on Chem 3D to create the three-dimensional structures. The canonical SMILES (simplified molecular-input line-entry system) were generated using ChemDraw and then submitted to SwissADME online tools for the ADMET analysis, the prediction of physicochemical parameters and the drug-likeness using the Lipinski rule of five. The so-called Rule-of-five of Lipinski delineated the relationship between pharmacokinetic and physicochemical parameters.[14]

## Results and Discussion

### Synthesis

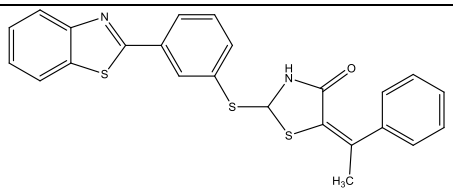
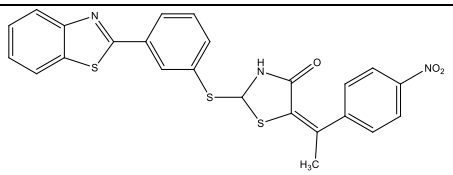
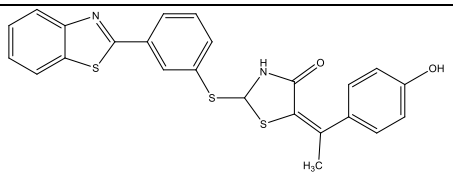
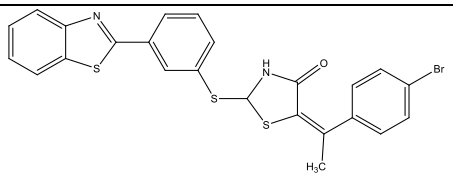
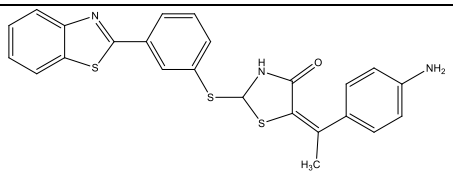
The multistep reaction pathway comprised of synthesizing three intermediate compounds. The third intermediate was reacted with various aromatic aldehydes leading to the formation of the desired substituted product.

The first step involved the cyclization to benzothiazole derivative wherein the amino group of compound (2) attacks the carboxylic acid carbon of compound (1), forming an amide intermediate. Intramolecular cyclization occurs via nucleophilic attack of the thiol (-SH) on the amide carbonyl, leading to ring closure and formation of the benzothiazole nucleus. Dehydration (loss of $H_2O$) completes the heterocyclic ring formation. In the second step acylation with chloroacetyl chloride takes place. Here the phenolic –OH group of compound (3) acts as a nucleophile and attacks the carbonyl carbon of chloroacetyl chloride leading to the formation of and ester intermediate, which rearranges to the chloroacetamide through substitution at the nitrogen site. Thereafter cyclization via nucleophilic substitution and condensation occurs on reaction with ammonium thiocyanate. The thiocyanate ion ($SCN^-$) attacks the chloroacetamide carbon, replacing the chlorine atom. The resulting intermediate undergoes intramolecular cyclization, where the sulfur atom attacks the amide carbonyl, forming a thiazolidin-4-one ring. Proton transfer and ring closure yield the stable heterocycle. In the last step the active methylene group ($–CH_2–$) adjacent to the carbonyl in thiazolidin-4-one undergoes Knoevenagel condensation via nucleophilic attack on the aldehyde carbonyl. This forms a β-hydroxy intermediate, which dehydrates to yield a C=C double bond resulting in the formation of the desired products (7a-e).




The structure, yield, color, retention factor and melting point of all the synthesized compounds are depicted in Table 1.

**Table 1. Physical Parameters of the synthesized compounds**

| Compound | Structure | Yield (%) | Color | Melting Point (°C) | Retention factor ($R_f$ value) |
|---|---|---|---|---|---|
| 7a |  | 71 | Reddish | 233-235 | 0.67 |
| 7b |  | 73 | Dark Yellow | 228-230 | 0.69 |
| 7c |  | 72 | Brown | 225-227 | 0.59 |
| 7d |  | 70 | Dark Yellow | 238-240 | 0.65 |
| 7e |  | 73 | Dark Yellow | 243-245 | 0.63 |

The compounds were soluble in chloroform and DMSO.

**Spectral Studies**

The structural confirmation of the synthesized compounds was done by interpretation of the IR, and $^1$H NMR spectra of the compounds. The IR spectrum is an indicator of the relevant functional

groups present in the compounds, while the proton NMR spectrum indicates the number and position of the hydrogen atoms present in the molecule.

**(E)-2-((3-(benzo[d]thiazol-2-yl)phenyl)thio)-5-(1-phenylethylidene)thiazolidin-4-one, 7a**

IR ($cm^{-1}$): 3228.13 (NH stretch), 3104.67 (CH stretch), 1696.29 (C=N stretch), 1456.90 (C-H bend), 1289.63 (C-N stretch), 1021.18 (C-S stretch); $^1$HNMR (δ ppm): 10.14 (NH), 6.25-7.86 (CH, aromatic), 2.60 ($CH_3$); Mass (m/z): 446.61 ($M^+$ peak)

**(E)-2-((3-(benzo[d]thiazol-2-yl)phenyl)thio)-5-(1-(4-nitrophenyl)ethylidene)thiazolidin-4-one, 7b**

IR ($cm^{-1}$): 3554.99 (NH stretch), 3107.54 (CH stretch), 1718.69 (C=N stretch), 1477.79 (C-H bend), 1288.54 (C-N stretch), 1004.05 (C-S stretch); $^1$HNMR (δ ppm): 10.14 (NH), 6.25-7.97 (CH, aromatic), 8.24 (CH, adj to $NO_2$), 2.60 ($CH_3$); Mass (m/z): 491.61 ($M^+$ peak)

**(E)-2-((3-(benzo[d]thiazol-2-yl)phenyl)thio)-5-(1-(4-hydroxyphenyl) ethylidene)thiazolidin-4-one, 7c**

IR ($cm^{-1}$): 3610.43 (OH stretch), 3112.55 (NH stretch), 3045.31 (CH stretch), 1715.99 (C=N stretch), 1481.33 (C-H bend), 1287.91 (C-N stretch), 1007.43 (C-S stretch); $^1$HNMR (δ ppm): 10.14 (NH), 6.25-7.97 (CH, aromatic), 6.90 (CH, adjacent to OH), 6.02 (OH), 2.60 ($CH_3$); Mass (m/z): 462.61 ($M^+$ peak)

**(E)-2-((3-(benzo[d]thiazol-2-yl)phenyl)thio)-5-(1-(4-bromophenyl)ethylidene)thiazolidin-4-one, 7d**

IR ($cm^{-1}$): 3227.12 (NH stretch), 3100.40 (CH stretch), 1696.43 (C=N stretch), 1456.90 (C-H bend), 1289.17 (C-N stretch), 1023.25 (C-S stretch), 657.84 (C-Br stretch); $^1$HNMR (δ ppm): 10.14 (NH), 6.25-7.97 (CH, aromatic), 2.60 ($CH_3$); Mass (m/z): 525.50 ($M^+$ peak)

**(E)-5-(1-(4-aminophenyl)ethylidene)-2-((3-(benzo[d]thiazol-2-yl)phenyl)thio)thiazolidin-4-one, 7e**

IR ($cm^{-1}$): 3397.17 (NH stretch), 3042.73 (CH stretch), 1676.80 (C=N stretch), 1428.04 (C-H bend), 1267.37 (C-N stretch), 976.85 (C-S stretch); $^1$HNMR (δ ppm): 10.14 (NH), 6.25-7.97 (CH, aromatic), 6.23 (CH, adjacent to $NH_2$), 4.32 ($NH_2$), 2.60 ($CH_3$); Mass (m/z): 461.62 ($M^+$ peak)

**Antibacterial Action**

The antibacterial activity of the synthesized benzothiazole-chalcone derivatives was determined measuring the zone of inhibition in the agar plate. Four concentrations of the synthesized compounds were tested for antibacterial action against ciprofloxacin as the standard drug for antibacterial action. The zone of inhibition of the test compounds is presented in table 2.

**Table 2. Antibacterial activity of synthesized compounds**

| Compound Code | Zone of Inhibition (mm)* | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | *E. coli* | | | | *S. aureus* | | | |
| | 25µg | 50µg | 75µg | 100µg | 25µg | 50µg | 750µg | 100µg |
| **7a** | 10 | 13 | 18 | **21** | 10 | 12 | 15 | **19** |
| **7b** | 10 | 10 | 16 | **19** | 10 | 10 | 12 | 16 |
| **7c** | 10 | 10 | 13 | 13 | 10 | 10 | 11 | 12 |
| **7d** | 10 | 13 | 17 | **22** | 10 | 13 | 16 | **19** |
| **7e** | 10 | 10 | 15 | 17 | 10 | 10 | 13 | 16 |
| **Ciprofloxacin** | **22** | - | - | - | **23** | - | - | - |

* Below 12 mm – poor activity; 13-18 mm – moderate activity & above 18 mm – good activity

The antibacterial activity increases with concentration for all compounds. 7d (Br substituent) shows the highest inhibition at 100µg, nearly matching ciprofloxacin. 7c (OH substituent) shows poor activity, suggesting hydroxyl substitution reduces effectiveness. The unsubstituted compound (7a) itself shows good activity, suggesting the core scaffold is inherently active. Electron-withdrawing group ($NO_2$) gives moderate activity, electron-donating groups (OH, $NH_2$) reduce activity compared to unsubstituted derivative.

***In silico* physicochemical properties**

The SMILES of the synthesized derivatives $PTB_{1-5}$ was generated using chemdraw ultra 12.0 software. The physicochemical properties of the compounds predicted *in silico* by SwissADME are presented in table 3.

**Table 3. Physicochemical properties of 7a-e calculated *in silico***

| Compound Code | HBD | HBA | Log P | NRB | PSA (A) | MR | Log S |
|---|---|---|---|---|---|---|---|
| **7a** | 1 | 2 | 6.15 | 4 | 120.83 | 133.20 | -7.48 |
| **7b** | 1 | 4 | 5.29 | 5 | 166.65 | 142.03 | -7.54 |
| **7c** | 2 | 3 | 5.72 | 4 | 141.06 | 135.23 | -7.34 |
| **7d** | 1 | 2 | 6.82 | 4 | 120.83 | 140.90 | -8.39 |
| **7e** | 2 | 2 | 5.55 | 4 | 146.85 | 137.61 | -7.13 |

Compounds 7b, 7c and 7e obeyed all the five rules of Lipinski's rule of five and were found to be suitable drug candidates. Compounds 7a and 7d violated one and two rules respectively reducing their chances to be potential drug candidates.

**Docking**

The docking study was conducted to predict the binding affinity of the synthesized molecules to DNA gyrase. DNA gyrase is the enzyme responsible for uncoiling of the bacterial DNA which is critical step in replication of the cell.

**Table 4. Binding energy score of 7a-e in active site of DNA gyrase**

| Compound Code | Binding Energy (kcal/mol) |
|---|---|
| 7a | -9.1 |
| 7b | -8.3 |
| 7c | -8.9 |
| 7d | -9.3 |
| 7e | -8.6 |

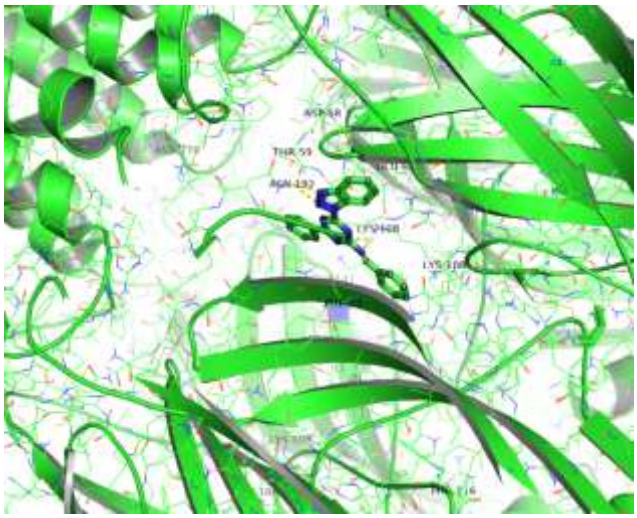


**Figure 1. Binding of 7a to 4URO**

The molecules 7a-e exhibited polar interactions within the active site of the enzyme DNA gyrase to elicit its action. The interaction of 7a and 7d in the active site occurred via two hydrogen bonds with ASN192 and LYS108 residues thus presenting the higher docking score. On the other hand 7b, 7c and 7e formed only one hydrogen bond during interaction with the enzyme.

**Conclusion**

The present investigation demonstrates that benzothiazole–chalcone hybrids possess significant antibacterial potential, particularly against *E. coli* and *S. aureus*. The unsubstituted scaffold (7a) and bromo-substituted derivative (7d) showed strong activity, with 7d nearly matching ciprofloxacin at higher concentrations. Docking studies confirmed favorable interactions of these compounds with DNA gyrase, supporting their mechanism of action. In silico ADMET profiling further suggested that several derivatives (7b, 7c, 7e) meet drug-likeness criteria, making them suitable

candidates for further optimization. Collectively, these findings establish benzothiazole–chalcone hybrids as promising leads for the development of new antimicrobial agents to combat multidrug-resistant pathogens. Future work should focus on expanding the structural diversity of these hybrids and evaluating their efficacy in vivo to validate their therapeutic potential.

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