



## ***In silico* screening of some phytochemicals for treating urinary tract infection (UTI) targeting *fimH* gene**

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### Abstract

**Introduction:** UPEC (Uropathogenic *Escherichia coli*) strains were evaluated to know the prevalence rate of virulence genes, antimicrobial activities of Cd-L<sub>1</sub>, Ni-L<sub>1</sub> and Cu-L<sub>1</sub> transition metal complexes, *in silico* screening of selected phytochemicals and their molecular docking. **Methods:** 140 urine samples were collected to assess the virulence properties of *fimH*, *gafD* and *bmaE* genes by polymerase chain reaction (PCR). *In vitro* antibacterial screening activities of ligand, HL and complexes against UPEC bacterial strains was evaluated by inhibition zone method using well diffusion assay. The *fimH* was docked with the phytochemicals using software FlexX defining the active sites of amino acids and residues with radius showing 10Å were included. **Results:** Presence of urinary tract infection (UTI) infection in 124 (88%) samples of which 61.29% among females and 38.71% among males with overall incidence of UPEC were confirmed in 42 isolates (33.8%). PCR amplification of 16S rRNA gene revealed presence of adhesion genes with its highest prevalence in *fimH* gene (80.9%) while *gafD* and *bmaE* were reported to be negative. *In vitro* antibacterial activities of Cd-L<sub>1</sub>, Ni-L<sub>1</sub> and Cu-L<sub>1</sub> complexes and molecular docking performed by registered docking software FlexX revealed best scores over -20. Highest zone of diameter was observed to be 13 mm by Ni-L<sub>1</sub>- Complex and all the three complexes were reported as good antimicrobial compounds. **Conclusion:** Our findings hold efficient prospects in preventing and formulating new UTI strategies as significant epidemiological research tool in Silchar, India.

**Keywords:** *E. coli*, *fimH*, Molecular docking, Phytochemicals

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## 1. Introduction

Now-a-days UTIs (urinary tract infection) are frequently regarded as one of the most common bacterial infections amongst various groups of patients, particularly females where more than 80% acquires UTI once in their lifetime and more than 20-50% of them exhibit reoccurrence events (Agarwal et al., 2012; and Bush and Macielag, 2010). The infection in female is mostly community based and a large amount of antibiotics to be given orally was prescribed among these patients in outpatient clinics (Lee et al., 2018). The most relevant microorganism

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with lower UTI is found to be *Escherichia coli*, however still now there is no prove of it to be carcinogenic (Russell et al., 2018). It has been observed that these UTIs are often caused by UPEC (Uropathogenic *Escherichia coli*) strains that often require the treatment with antimicrobial therapy. Though the molecular characteristics and virulence functions have been well studied by various workers but the severances of UTI still depicts the higher rates of virulence properties by UTI causing strains within a community. Further contribution to the cause of these virulence factors is observed towards antigenic diversification of proteins that are directly exposed to host defense mechanism (Derakhshandeh et al., 2015; and Emody et al., 2003).

Medicinal inorganic chemistry has been historically rich in metal or metalloid-based drugs. Though the current knowledge based on free radicals and reactive oxygen species (ROS) in biology has revolutionized various treatment procedures in medical history but Paul Erlich's organic arsenic compound still promises a fresh era of health and disease management (Ooms, 2000; and Rice-Evans et al., 1995). Though medicinal plants have great antioxidant potentials due to the presence of phytochemical constituents (Habibi et al., 2015; and Phukan et al., 2014) and can therefore be used as inhibitors for the *in silico* screening using bioinformatics tools. Thus, based upon clinical importance of UTI, the purpose of the study was to investigate whether antimicrobial activity of transition metal complexes along with *in silico* phytochemical screening in presence of molecular characteristics when applied could further discriminate the uropathogenic organisms (Foxman, 2002; and Habibi et al., 2015) which may then be applied to efficiently elucidate the various modes of transmission of community acquired *E. coli* infections.

## 2. Materials and methods

### 2.1. Bacterial strain identification and antimicrobial agents

140 urine samples from community health center of Cachar district in Assam, India were collected within the period of January 2017 to January 2018 and screened for the bacteriological analysis on cysteine lactose electrolyte deficient agar (CLED) agar and eosin methylene blue (EMB) agar. The inoculated test culture Petri plates were incubated in biochemical oxygen demand (BOD) incubator (Orbitek, India) overnight at an incubation temperature of 37 °C. Gram's staining assays of the isolates was performed to study the morphological characteristics of the isolates. Isolates were biochemically analyzed by performing a set of biochemical tests that included IMViC tests (Indole, methyl red, Voges-Proskauer, citrate), oxidase, catalase, triple sugar iron and urease and motility test (Cruickshank et al., 1975).

Antibiotic sensitivity and resistance forming patterns of the test isolates were determined according to disc diffusion method as described by Bauer et al. (1966). The selected colonies of bacteria were freshly inoculated on saline water and the turbidity level was observed by comparing with 0.5 Mc. Farland solution ( $1.5 \times 10^8$  cfu/ml). The Petri plates were kept under incubation at 37 °C for 18-24 h in inverted position and the zones of inhibitions were measured in millimetre with standard chart provided in (CLSI Guidelines, 2016).

### 2.2. DNA extraction and amplification

QIAamp kit (Qiagen, Germany) was used for extraction and purification of all UPEC strains. Presence of adhesion genes (Table 1) that are responsible for the attachment of the organism that is *fimH*, *gafD* and *bmaE* were assessed by multiplex polymerase chain reaction (PCR) (Eppendorf, India) assay.

The reaction mixture was prepared by adding 1  $\mu$ l each of forward and reverse primer (10 picomole concentration) (Table 1). Amplification reactions were carried out in a total volume of 25  $\mu$ l consisting of 2  $\mu$ l of DNA template, 12.5  $\mu$ l of tag green Master Mix 2X DNA polymerase and 8.5  $\mu$ l nuclease free water.

PCR reactions conditions for *fimH* gene were: an initial denaturation at 94 °C for 3 min followed by 32 cycles of denaturation of 94 °C for 40 sec, 64 °C for 40 sec and 72 °C for 2 min, and final step of extension at 72 °C for 7 min followed by *bmaE* and *gafD* reaction conditions as an initial denaturation at 94 °C for 3 min, 32 cycles of denaturation of 94 °C for 40 sec, 64 °C for 40 sec and 72 °C for 2 min, and final extension step at 72 °C for 7 min. All PCR products of 5  $\mu$ l were electrophoresed on 1.5% agarose gel, stained with ethidium bromide and the band sizes were visualized and captured under GelDoc Imager (DNR Image).

Table 1: List of oligonucleotide primers				
Primer set	Targeted genes	Primer Sequences (5'-3')	Size of amplified products (bp)	Reference
bmaE-f bmaE-r	<i>bmaE</i>	F: ATGGCGCTAACTTGCCATGCTG R: AGGGGGACATATAGCCCCCTTC	507	(13)
fimH-f fimH-r	<i>fimH</i>	F: TGCAGAACGGATAAGCCGTGG R: GCAGTCACCTGCCCTCCGGA	508	
gafD-f gafD-r	<i>gafD</i>	F: TGTGGACCGTCTCAGGGCTC R: CTCCCGGAACCTCGCTGTTACT	952	

### 2.3. Antimicrobial activity of Cd-L<sub>1</sub>, Ni-L<sub>1</sub> and Cu-L<sub>1</sub> transition metal complexes

*In vitro* antibacterial screening activities of ligand, HL and complexes against UPEC bacterial strains were performed by using well diffusion Inhibition Zone Method according to previously published studies (Singh et al., 2016; and Nanda and Saravanan, 2009).

The transition metal complexes were synthesized and characterized using UV- mass spectroscopy, <sup>1</sup>H and <sup>13</sup>C NMR.

### 2.4. In silico screening of selected phytochemicals

*In silico* screening of some phytochemicals with selected target as adhesin proteins was docked (Habibi et al., 2015; and Lee et al., 2013). The structure of *fimH* wild type kinase domain was retrieved from RCSB Protein DATA Bank and the protein model with PDB ID: 4auu was chosen for determining the predictions of active sites along with docking process. Protein models were cleaned and optimized by removing ligand and presence of hetero-atoms like water, ions, etc. by using FlexX Software for molecular docking process. The structure of the ligands were downloaded from Pubchem and converted to SMILES and ".smi" format in notepad, converted to ".sdf" format using Open Babel. Further, evaluation of the phytochemicals to study their molecular characteristics and drug likeness was performed by Molsoft database. An ADMET (Absorption, Distribution, Metabolism, Excretion and Toxicity) analysis which is used for detecting drug likeness of compounds was performed using MobyLe@rpbs server. The compounds were loaded in the server in SMILES format.

#### 2.4.1. Molecular docking

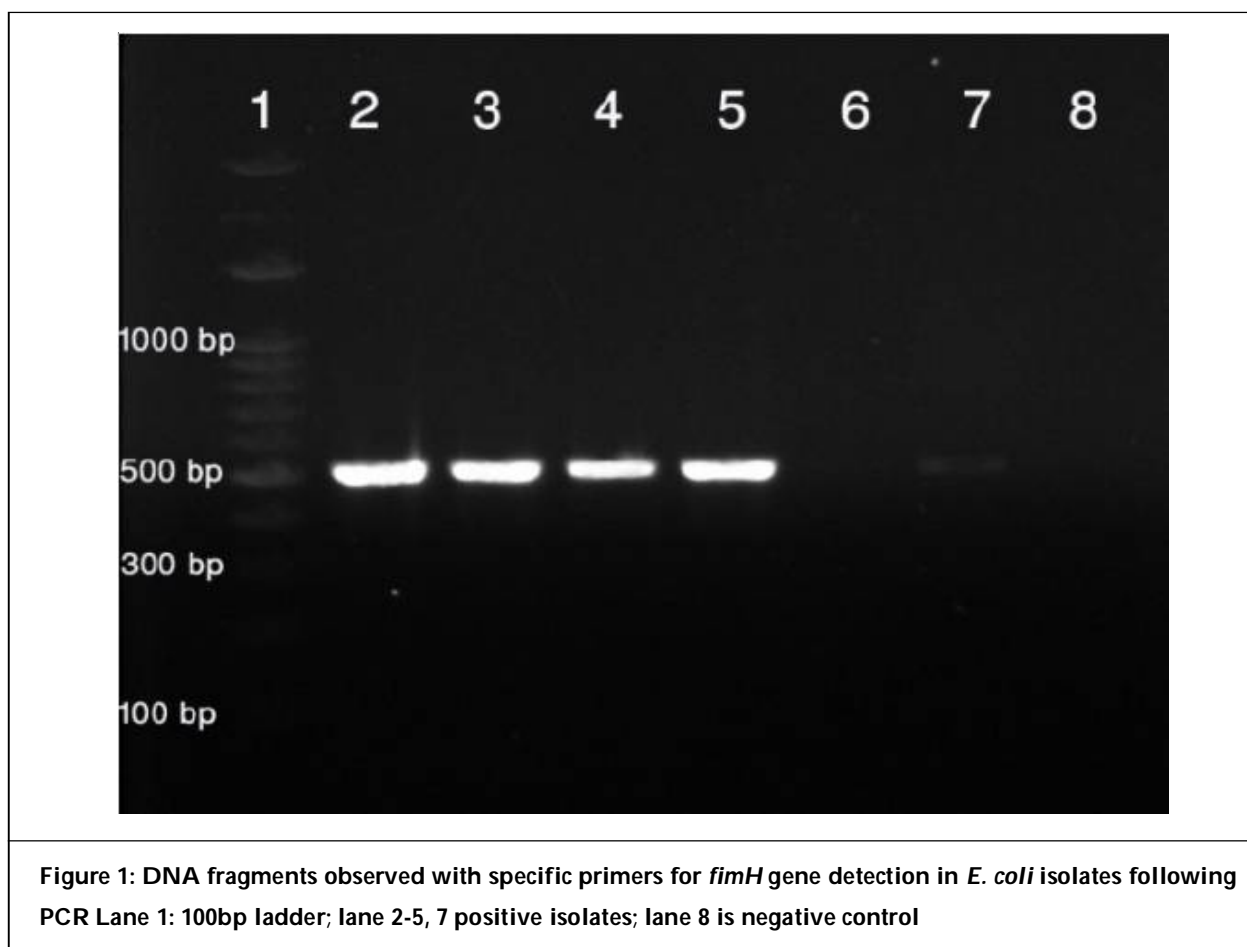
The *fimH* was docked with the phytochemicals using software FlexX. The active site predictions along with the residues of amino acids within a defined radius of 10Å were included within the binding sites and the SDF files of all the compounds were loaded in FlexX to form a docking library. The energy values for each docked molecules were noted and calculated in Kcal/mol from the output files.

#### 2.4.2. Ethical approval

Ethical approval was obtained from Assam University, Silchar, Assam, India (Ref. no. IEC/AUS/2019/32/IS). The protocols of this study were examined and approved by institutional ethical board members to conduct the present study along with informed and written consent from each patient involved with the study which was witnessed by a third party, which also maintained record and names of each participant as he/she enrolled with the study.

## 3. Results

Bacteriological analysis of 140 urine samples revealed the presence of UTI infection in 124 (88%) samples; 61.29% among females (n-76) and 38.71% among males (n-48) and the overall incidence of UPEC were confirmed in 42 isolates (33.8%) by standard biochemical tests. Further molecular confirmation of 16S rRNA gene was performed by using PCR amplification. The adhesion genes that exhibited highest prevalence was *fimH* gene (80.9%; n = 34) (Figure 1) while prevalence of *gafD* and *bmaE* reported to be negative.



Antibiotic susceptibility against amikacin (30  $\mu\text{g}$ ), Amoxycylav (30  $\mu\text{g}$ ), Fosfomycin (200  $\mu\text{g}$ ), Levofloxacin (5  $\mu\text{g}$ ), Netillin (30  $\mu\text{g}$ ), Nitrofluratonin(300  $\mu\text{g}$ ) and Tobramycin(10  $\mu\text{g}$ ) was determined. 90.4% ( $n=38$ ) of UPEC strains were found to be resistant towards fosfomycin; 76.1% ( $n=32$ ) towards tobramycin; 73% ( $n=31$ ) towards levofloxacin; 71.4% ( $n=30$ ) for netillin; 69% ( $n=29$ ) for amoxycylav; 57% ( $n=24$ ) for nitrofluratonin and 30.9% ( $n=13$ ) towards amikacin. Since four strains of UPEC were observed to be resistance against all the above antibiotics used, thus they were further approached for *in vitro* antibacterial screening activity of Cd-L<sub>1</sub>, Ni-L<sub>1</sub> and Cu-L<sub>1</sub> complexes (Table 2). Highest zone of diameter was observed to be 13 mm by Ni-L<sub>1</sub>- Complex and all the three complexes were reported as good antimicrobial compounds.

Sample No.	Cd-L <sub>1</sub> - Complex		Ni-L <sub>1</sub> - Complex		Cu-L <sub>1</sub> -Complex	
	100 $\mu\text{g}$	200 $\mu\text{g}$	100 $\mu\text{g}$	200 $\mu\text{g}$	100 $\mu\text{g}$	200 $\mu\text{g}$
52	+	++	++	++	++	++
61	++	++	+	++	+	++
62	+	++	+	+	++	++
119	+	++	++	++	+	++

Further a total of nine phytochemicals of north eastern-based plants were selected for the molecular docking between a targeted protein and ligands from plant sources (Table 3).

It was observed that two molecules did not reveal any docking signals with any of the selected nine plants based phytochemicals using the LetID software interface; while three phytochemicals revealed best scores over -20 (Table 4).



observed in tobramycin (76.1%), levofloxacin (73.8%) and netillin (71.4%). It is notable that out of 140 urine samples, 124 (88%) UPEC isolates represented community acquired UTIs. Since *E. coli* is the most frequently found pathogen responsible exclusively for more than 85% community and 50% hospital associated UTIs respectively, the following study calculated the overall prevalence rate (Emody et al., 2003; Lee et al., 2013; and Mohajeri et al., 2014) of UTI as 88% (124/140) followed by 61.29% amongst females (76/124) and 38.71% amongst males (48/124). As the strains of UPEC are usually associated with high morbidity, mortality and are genetically more diversified, sharing the virulence determining factors; henceforth 33.8% (42/124) prevalence rate was observed (Derakhshandeh et al., 2015; and Erb et al., 2007).

UPEC are associated with asymptomatic bacteriuria and uncomplicated cystitis as well as severe pyelonephritis. Infection usually occurs following the transmission of UPEC strains since they carry the genetic determinants from the intestinal tract, while in a few patients transmission often occurs from the vagina where they exist as a component of the normal flora, to the periurethral area (Mohajeri et al., 2014). The important step required for the initiation and development of UTI is the attachment of the bacteria to the uroepithelial cells mediated by the bacterial ligands (generally small proteins placed at the tips of bacterial fimbriae) having the capability to bind to the host cell wall possessing carbohydrate residues that works as a receptor (Habibi et al., 2015). Among adhesions, the ability of the UPEC strains possessing the type 1 fimbriae to *fimH* adhesin is one of the major important determinant factors that possesses high tropism for urinary tract receptors which keep the ability to enable the *fimH* adhesion in invasiveness and colonization of different niches of *E. coli* (Derakhshandeh et al., 2015; and Habibi et al., 2015). Thus, in accordance to this, our results showed higher prevalence of *fimH* gene, that is 80.9%, while prevalence for other genes were observed as negative among the UTI samples (Oliveira et al., 2011).

According to previous studies (Bauer et al., 1966; and Ellington et al., 2006), *in vitro* antimicrobial activities performed against *E. coli* along with their metal chelates by agar well-diffusion method showed positive results (Phukan et al., 2014), but in the present study antimicrobial activities of metal complexes Cd-L<sub>1</sub>- Complex, Ni-L<sub>1</sub>- Complex and Cu-L<sub>1</sub>- Complex against UPEC strains exhibited highest zone of diameter of 13 mm by Ni-L<sub>1</sub>- Complex and all the other three complexes thereby reported as good antimicrobial compounds.

Furthermore some plant-based compounds were also used as inhibitors for the *in silico* screening against previously reported potential drug target (Phukan et al., 2014; and Ellington et al., 2006). As detected by the PCR assay, prevalence of *fimH* gene among the UPEC was observed to be high in this region and since protein of the gene is the potential drug target (Ooms, 2000; and Habibi et al., 2015) a molecular docking (Habibi et al., 2015) was performed by registered docking software FlexX in the Bioinformatics Center, Assam University and the best scores for all the three compounds were reported to be over -20.

## 5. Conclusion

As observed in the study, the active metal compounds had a clear and distinct zone of inhibition against selected UPEC strains along with best scores for all the three compounds, and genotyping of *fimH* gene could serve as a highly applicable screening assay for studies based upon epidemiological surveillance. Thus, our findings suggest promising applications in UTI treatment and their preventive methods along with significant future related epidemiological studies in Silchar, Assam.

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## Conflicts of interest

None.

## References

- Agarwal, J., Srivastava, S. and Singh, M. (2012). Pathogenomics of uropathogenic *Escherichia coli*. *Indian Journal of Medical Microbiology*. 30(2), 141.
- Bauer, A. W., Kirby, W. M., Sherris, J. C. and Turck, M. (1966). Antibiotic susceptibility testing by a standardized single disk method. *American Journal of Clinical Pathology*. 45(4\_ts), 493-496.



- Bush, K. and Macielag, M. J. (2010). New  $\beta$ -lactam antibiotics and  $\beta$ -lactamase inhibitors. *Expert Opinion on Therapeutic Patents*. 20(10), 1277-1293.
- CLSI Guideline (2016). *Antimicrobial Susceptibility Testing*.
- Cruickshank, R., Duguid, J. P., Marmion, B. P. and Swain, R. H. A. (1975). *Medical microbiology*. Churchill Living Stone, New York 2(12), 31-57 and 96-218.
- Derakhshandeh, A., Firouzi, R., Motamedifar, M., Motamedi, Boroojeni, A., Bahadori, M., Arabshahi, S., Novinrooz, A. and Heidari, S. (2015). Distribution of virulence genes and multiple drugresistant patterns amongst different phylogenetic groups of uropathogenic Escherichia coli isolated from patients with urinary tract infection. *Letters in Applied Microbiology*. 60(2), 148-154.
- Ellington, M. J., Livermore, D. M., Pitt, T. L., Hall, L. M. and Woodford, N. (2006). Mutators among CTX-M  $\beta$ -lactamase-producing Escherichia coli and risk for the emergence of fosfomycin resistance. *Journal of Antimicrobial Chemotherapy*. 58(4), 848-852.
- Emody, L., Kerényi, M. and Nagy, G. (2003). Virulence factors of uropathogenic Escherichia coli. *International Journal of Antimicrobial Agents*. 22, 29-33.
- Erb, A., Stürmer, T., Marre, R. and Brenner, H. (2007). Prevalence of antibiotic resistance in Escherichia coli: overview of geographical, temporal, and methodological variations. *European Journal of Clinical Microbiology & Infectious Diseases*. 26(2), 83-90.
- Foxman, B. (2002). Epidemiology of urinary tract infections: incidence, morbidity, and economic costs. *The American Journal of Medicine*. July 8, 113(1), 5-13.
- Habibi, M., Karam, M. R. and Bouzari, S. (2015). In silico design of fusion protein of fimH from uropathogenic Escherichia coli and MrpH from Proteus mirabilis against urinary tract infections. *Advanced Biomedical Research*. 4.
- Lee, D. S., Choe, H. S., Lee, S. J., Bae, W. J., Cho, H. J., Yoon, B. I., Cho, Y. H., Han, C. H., Jang, H., Park, S. B. and Cho, W. J. (2013). Antimicrobial susceptibility pattern and epidemiology of female urinary tract infections in South Korea, 2010-2011. *Antimicrobial Agents And Chemotherapy*. 57(11), 5384-5393.
- Lee, D. S., Lee, S. J. and Choe, H. S. (2018). Community-acquired urinary tract infection by Escherichia coli in the era of antibiotic resistance. *BioMed Research International*. Volume 2018, Article ID 7656752, 14 pages <https://doi.org/10.1155/2018/7656752>
- Mohajeri, P., Khademi, H., Ebrahimi, R., Farahani, A. and Rezaei, M. (2014). Frequency distribution of virulence factors in uropathogenic Escherichia coli isolated from Kermanshah in 2011-2012. *International Journal of Applied and Basic Medical Research*. 4(2), 111.
- Nair, G. B., Ramamurthy, T., Bhattacharya, M. K., Krishnan, T., Ganguly, S., Saha, D. R., Rajendran, K., Manna, B., Ghosh, M., Okamoto, K. and Takeda, Y. (2010). Emerging trends in the etiology of enteric pathogens as evidenced from an active surveillance of hospitalized diarrhoeal patients in Kolkata, India. *Gut Pathogens*. 2(1), 4.
- Nanda, A. and Saravanan, M. (2009). Biosynthesis of silver nanoparticles from Staphylococcus aureus and its antimicrobial activity against MRSA and MRSE. *Nanomedicine: Nanotechnology, Biology and Medicine*. 5(4), 452-456.
- Oliveira, F. A., Paludo, K. S., Arend, L. N., Farah, S. M., Pedrosa, F. O., Souza, E. M., Surek, M., Picheth, G. and Fadel-Picheth, C. M. (2011). Virulence characteristics and antimicrobial susceptibility of uropathogenic Escherichia coli strains. *Genet Mol Res*. 10(4), 4114-4125.
- Ooms, F. (2000). Molecular modeling and computer aided drug design. Examples of their applications in medicinal chemistry. *Current Medicinal Chemistry*. 7(2), 141-158.
- Rice-Evans, C., Halliwell, B. and Lunt, G. G. (1995). *Free radicals and oxidative stress: environment, drugs and food additives*. Portland Press Ltd.
- Russell, B., Garmo, H., Beckmann, K., Stattin, P., Adolfsson, J. and Hemelrijck, M. V. (2018). A case-control study of lower urinary-tract infections, associated antibiotics and the risk of developing prostate cancer using PCBaSe 3.0. *PLOS ONE*. <https://doi.org/10.1371/journal.pone.0195690>, April 12.

- Phukan, R., Borgohain, P. and Chutia, Bolin Chetia. (2014). Phytochemical and antimicrobial activity of some medicinal plants of North East India. *Asian Journal of Chemistry*. 26(15), 4752-4754.
- Singh, K., Thakur, R. and Kumar, V. (2016). Co (II), Ni (II), Cu (II), and Zn (II) complexes derived from 4-[(3-(4-bromophenyl)-1-phenyl-1H-pyrazol-4-ylmethylene)-amino]-3-mercapto-6-methyl-5-oxo-1, 2, 4-triazine. *Beni-Suef University Journal of Basic and Applied Sciences*. 5(1), 21-30.

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