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Original Article

Synthesis, molecular docking and biological activity of 4-aminoantipyrine dithiocarbamate derivatives

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ABSTRACT

In the present investigation, a series of novel 4-aminoantipyrine dithiocarbamates have been synthesized. The synthesized compounds were characterized by IR, ^{1}HNMR, Mass spectral analysis followed by antimicrobial screening. Furthermore, molecular docking studies is an important tool in computational drug design technique, which is employed to understand the mode of binding and binding affinities, it indicates a very good hydrophobic interactions with target receptors.

Keywords: 4-Aminoantipyrine, Dithiocarbamate, Molecular Docking, Antimicrobial activities.

INTRODUCTON

4-Aminoantipyrine belongs to the class of pyrazolone heterocyclic compounds [1]. Pyrazolone is a five-membered lactum ring, containing two nitrogens and ketone in the same molecule. Molecules with 4-amminoantipyrine nucleus are known to possess analgesic [2], anti-inflammatory [3], anticancer [4], antibacterial [5], antiviral [6] and antifungal [7] properties. Dithiocarbamates[8] are a common class of organic molecules, they form mono and bidentate coordination to transition metal centres. Transition metal complexes present a wide range of biological effects. The most interesting group of dithiocarbamates is brassinin, a phytoalexin first isolated from cabbage had antiproliferative activity in human acute T-lymphoblastic leukaemia cells. Brassinin[9] and its derivatives as inhibitors of indolamine 2,3-dioxygenase (IDO), a new cancer immunosuppression target. SAR studies showed, the dithiocarbamate portion of the brassinin as a crucial for activity, which may be binding to the heme iron of IDO, substitution of the S-methyl group of brassinin with large aromatic groups provides inhibitors that are three times more potent than the most commonly use IDO inhibitor, 1-methyl-tryptophan. 4-Aminoantipyrines and dithiocarbamates as separate entities possess various biological activities [10-17], so we thought it would be interesting to synthesize a series of novel 4-aminoantipyrine derivatives with dithiocarbamate side chain and evaluate for their biological activity inorder to produce synergistic effects. With view new 4-aminoantipyrine derivatives were synthesized molecular modeling studies were carried out and screened for their antimicrobial activity. Molecular docking studies [18] is an important tool in computational drug design technique, which is employed to understand the mode of binding and binding affinities.

MATEIALS AND METHODS:

All reagents were purchased from commercial suppliers like Sigma Aldrich, Merck India Ltd., Sd chemicals. All reagents were of AR grade and were used without purification. Melting points of the synthesized compounds were determined using MELTER FD-51 apparatus and were found uncorrected. The IR spectra of the synthesized compounds were recorded on Perkin Elmer Model 2833 and Nicolet-740 FT-IR instruments and frequencies were recorded in wave numbers were expressed as δ values in ppm, downfield from internal standard TMS. The mass spectrum was recorded on VG Micromass 7070H (ESI and EI) and were given in mass units (m/z). Purity of the compounds were checked by TLC on silica gel 60-254 (Merck) in an appropriate solvent.

Synthesis of 4-aminoantipyrinedithiocarbamtes: A mixture of 4-aminoantipyrine (0.01 mol) was dissolved in 10 ml dimethylformamide, anhydrous

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potassium carbonate (0.01 mol) was added and the mixture was stirred for 30 min, then carbondisulfide (0.02 mol) was added dropwise. The reaction mixture was stirred for additional 20 min, and then appropriate alkyl/aralkyl halides were added and allowed to stir for 10 min. The reaction was monitored with TLC and later the crude compound was subjected to coloumn chromatography by passing through silica gel using mixture of dichloromethane and ethylacetate as eluents to afford the compound 4-aminoantipyrine dithiocarbamate.[19]

Spectral data of the synthesized compounds:

Methyl (1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1*H*-pyrazol-4-yl) carbamodithioate: ^{1}H NMR [$CDCl_3$, 400MHz] δ ppm 2.269 (s, 3H, Ar-CH_3), δ 2.434 (s, 3H, N-CH_3), δ 3.246 (s, 3H, S-CH_3), δ 7.305-7.340 (t, 1H, Ar-H), δ 7.388-7.406 (d, 2H, Ar-H), δ 7.450-7.488 (t, 2H, Ar-H) δ 9.402 (s, 1H, NH). ESI-MS *m/z* [M+1] 294.

Ethyl (1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1*H*-pyrazol-4-yl) carbamodithioate: IR (KBr) v cm^{-1} 3132.69 (N-H), 2953.26 aromatic (CH), 2854.27 aliphatic (CH). ^{1}H NMR [400MHz, DMSO] δ ppm 1.198-1.289 (m, 3H, CH_3), δ 2.176 (s, 3H. Ar-CH_3), δ 3.098-3.182 (m, 3H, N-CH_3), δ 3.200-3.237 (m, 3H, S-CH_2), δ 7.330-7.367 (m, 3H, Ar-H), δ 7.490-7.523 (m, 2H, Ar-H), δ 10.788 (s, 1H, NH). ESI-MS *m/z* [M+1] 308.

Propyl (1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1*H*-pyrazol-4-yl) carbamodithioate: IR (KBr) v cm^{-1} 3135 (N-H), 2960 aromatic (CH), 2927.74 aliphatic (CH). ^{1}H NMR [$CDCl_3$, 400MHz] δ ppm 0.093-1.030 (t, 3H, CH_3), δ 1.632-1.769 (m, 2H, CH_2), δ 2.234(s, 3H, Ar-CH_3), δ 3.149 (s, 3H, N-CH_3), δ 3.209-3.246 (t, 2H, S-CH_2), δ 7.305-7.340 (t, 1H, Ar-H), δ 7.388-7.406 (d, 2H, Ar-H), δ 7.450-7.488 (t, 2H, Ar-H), δ 9.402 (s, 1H, NH). EsI-MS *m/z* [M+1] 322.

Butyl (1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1*H*-pyrazol-4-yl) carbamodithioate: IR (KBr) v cm^{-1} 3140 (N-H), 2920 aromatic (CH), 2870.50 aliphatic (CH). ^{1}H NMR [$CDCl_3$, 400MHz] δ ppm 0.914-0.951 (t, 3H, CH_3), δ 1.383-1.475 (t, 2H, CH_2), δ 1.636-1.711 (m, 2H, CH_2), δ 2.236 (s, 3H, Ar-CH_3), δ 3.151 (s, 3H, N-CH_3), δ 3.231-3.269 (t, 2H, S-CH_2), δ 7.302-7.341 (t, 1H, Ar-H), δ 7.386-7.404 (d, 2H, Ar-H), δ 7.449-7.488 (t, 2H, Ar-H), δ 9.231 (s, 1H, NH). EsI-MS *m/z* [M+1] 336.

2-Methylpropyl (1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1*H*-pyrazol-4-yl) carbamodithioate: IR (KBr) v cm^{-1} 3132 (N-H), 2963 aromatic (CH), 2864.99 aliphatic (CH). ^{1}H NMR [$CDCl_3$, 400MHz] δ ppm 1.132-1.151 (d, 3H, CH_3), δ 1.401-1.436 (d, 3H, CH_3), δ 1.755-1.791 (m, 1H, CH), δ 2.236 (s, 3H, Ar-CH_3), δ 3.151 (s, 3H, N-CH_3), δ 3.231-3.250 (d, 2H, S-CH_2), δ 7.302-7.341 (t, 1H, Ar-H), δ 7.386-7.404 (d, 2H, Ar-H), δ 7.449-7.488 (t, 2H, Ar-H), δ 9.231 (s, 1H, NH). EsI-MS *m/z [*M+1] 336.

Benzyl (1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1*H*-pyrazol-4-yl) carbamodithioate: IR (KBr) v cm^{-1} 3123.54 (N-H), 3051 aromatic (CH), 2925.26 aliphatic (CH). ^{1}H NMR [$CDCl_3$, 400MHz] δ ppm 2.238 (s,3H, Ar-CH_3), δ 3.133 (s, 3H, N-CH_3), δ 4.500 (s, 2H, S-CH_2), δ 7.286-7.464 (m, 10H, Ar-H), δ 9.252(s, 1H, NH). EsI-MS *m/z* [M+1] 370.

MOLECULAR DOCKING

Crystal structure of E.Coli IDH (PDB Code: 4AJA) was download from the protein data bank GLIDE 5.6 was used for molecular docking [20]. Protein was prepared using protein preparation molecule applying the default parameters; a grid was generated around the active site aminoacid by selecting the co-crystalized ligand [21-25]. Receptor Vander Waals scaling for non-polar atoms was kept at 0.9. Molecules were built using Maestro build panel and prepared by ligand Preparation application. Low energy conformation of the ligands were selected and docked into the grid generated for the protein using standard precision (SP) docking mode [26-29]. Dock pose of each ligand was analysed for interactions with the receptor.

BIOLOGICAL ACTIVITY

Antibacterial activity: The synthesized novel compounds were screened for their antibacterial activity against *E.Coli*, a gram negative bacteria and *B.Subtilis*, a *gram* positive bacteria by agar cup plate method [30]. The test compounds were dissolved in methanol to prepare stock solutions. The concentrations of test compounds were 50, 100, 150 and 200 µg/ml in comparision to the standard drug *streptomycin.* Nutrient media was prepared and sterilized by autoclaving at 121ºC for 15 min to which loop full bacteria from 24 hours culture was added, and gently shaken for uniform distribution of organism. The media was poured in petri plates, allowed to solidify, bore wells of 10mm diameter at equal distance were made and a drop of test and standard compound was placed in the plates, evenly distributed with bacteria and incubated at 37ºC for 24-48 hours, for the inhibition of bacteria. Zone of inhibition of compounds were compared with zone of inhibition of standard drug *streptomycin*.

Antifungal activity: The antifungal activity of the synthesized compounds was tested against the fungus *Pencillium Chrysogenum,* by agar cup plate method [31] at a concentrations of 50, 100, 150 and 200µg/ml. Sabouraud-dextrose agar media was prepared and sterilized and incubated with fungi for 2 days. A drop of test and standard compounds was placed in the bore wells made in the plates, and

allowed to incubate for 48 hours, for the inhibition of fungi, the zone of inhibition of test compounds was compared with the standard drug *fluconazole.*

RESULT AND DISCUSSION

All the synthesized compounds **(2a-2f)** were evaluated for anti-bacterial and anti-fungal activities and results were present in Table-1. The zone of inhibition (in mm) value was taken as a parameter for activity. The zone of inhibition of test compounds were compared to that of the standard drugs i.e., *streptomycin* for anti-bacterial and *flucanazole* for anti-fungal activity. In the series most of the compounds were active against gram positive and gram negative organisms. The compounds 2a (methyl) showed very less activity, 2b (ethyl) and 2c (propyl) molecules showed equipotent activity while 2e (isobutyl) and 2f (benzyl) showed moderate activity when compared to streptomycin against gram positive organism. All the compounds showed poor activity except 2b (ethyl) against gram negative organism. Among the series of compounds tested, all of them were inactive against fungal organism up to 200 µg/ml.

Table 1: Anti-bacterial activity of compounds:

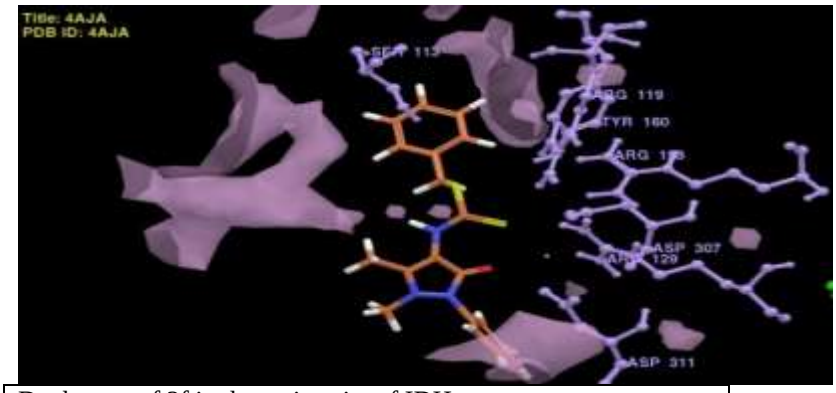
S.No	Compound	Zone of inhibition (in mm)							
		Gram positive organism				Gram negative organism			
		Bacillus subtilis				Escherichia coli			
		50µg	100µg	150µg	200µg	50µg	100µg	150µg	200µg
1.	2a	-	-	-	3	-	-	3	6
2.	2b	8	10	11	12	5	8	10	12
3.	2c	-	6	10	12	-	4	5	8
4.	2d	4	5	8	9	-	3	5	9
5.	2e	9	11	13	15	4	9	10	11
6.	2f	8	11	12	16	3	7	9	10
7.	Streptomycin	18	20	21	22	18	20	22	24

DOCKING STUDIES

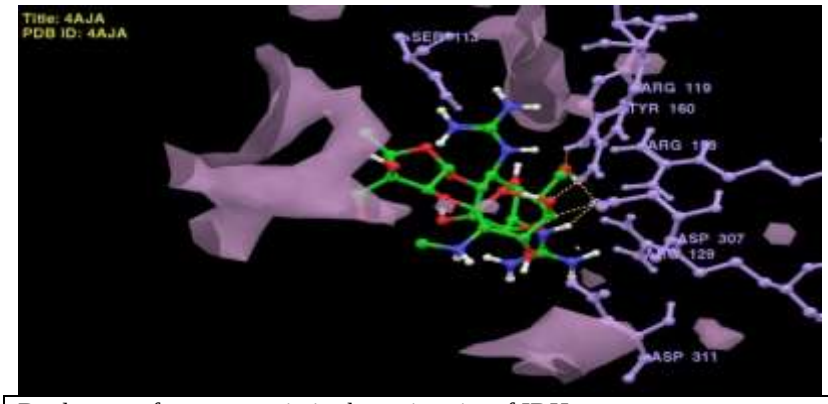
Biological screening of synthesized molecules as antibacterial and antifungal agent showed deprived results. As to find the possible chemotherapeutic target of these molecules a similarity search was performed based on substructure in PubChem structures. The molecules showed purely hydrophobic interactions with the protein active site. The 4-aminoantipyrine and dithiocarbamate moiety occupied hydrophobic groove making significant vanderwaals contacts with the hydrophobic surface having side chain residues Arg-119, Arg-129, and Arg-153. The synthesized molecules were docked into the same grid and when checked for interactions with the receptor active site, the molecules were deeply embedded into the hydrophobic pocket, suggesting that by increasing the hydrophobic substituents at the dithiocarbamate region using aromatic halides etc have increased the hydrophobic interaction with the receptor and the dock score given in the table-2. Most active molecule in the series, 2f showed highest dock score -5.586. In docking protocol, this can be explained in terms of hydrophobic interactions of phenyl rings. Molecules 2a-2c showed dock score ranging from -5.415, -5.494 and -5.491 respectively. Compounds 2d and 2e showed dock score -3.882 and -3.109 respectively. According to the dock score, upto 3carbon atoms (methyl, ethyl, propyl) there was increase in the activity. Furthure increase in the carbon atoms, there was decrease in the activity. From the above observation, it can be proposed that these molecules had better interaction with the receptor active site due to the presence of hydrophobic grooves.

Table 2 : Docking results:

S.No.	Compound	Dock Score(K.cal/mole)	Glide E Model
1.	**2a**	**-5.415**	**-37.826**
2.	**2b**	**-5.494**	**-39.324**
3.	**2c**	**-5.491**	**-40.820**
4.	**2d**	**-3.882**	**-35.161**
5.	**2e**	**-3.109**	**-34.658**
6.	**2f**	**-5.586**	**-41.459**
7.	**Streptomycin**	**-5.464**	**-50.669**

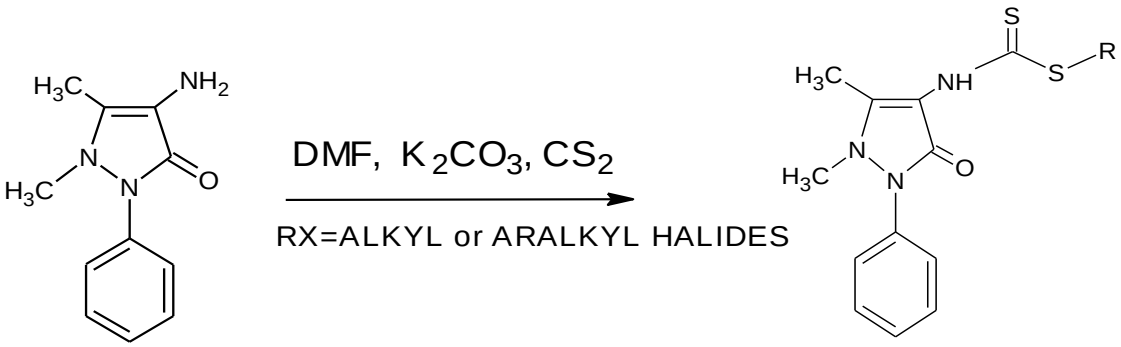


Dock pose of 2f in the active site of IDH enzyme



Dock pose of streptomycin in the active site of IDH enzyme

SCHEME



1 2(a-f)

R	R
2a : CH_3	**2d : n-C_4H_9**
2b : C_2H_5	**2e : iso- C_4H_9**
2c : C_3H_7	**2f : $CH_2C_6H_5$**

CONCLUSION

In conclusion, a series of 4-aminoantipyrine dithiocarbamate derivatives have been synthesised and screened for their antimicrobial activities. Most of the compounds have showed moderate promising activity against gram positive bacteria when compared to gram negative bacteria. More importantly, compounds 2e and 2f exhibited potential antibacterial activity against B.subtilis, indicating that branched alkyl group (isobutyl) and aromatic group (benzyl) as a side chain on dithiocarbamate contributes positively for the activity. Molecular docking studies indicates that hydrophobic interactions are important for antimicrobial activity.

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REFERENCES

1. Acheson RM. An Introduction to Heterocyclic Compound, 3rd ed.; Wiley & Sons: New York, 2009.
2. Turan-Zitouni G et al. Synthesis of some triazolyl-antipyrine derivatives and investigation of analgesic activity. Eur J Med Chem 2001; 36:685–689.
3. Burdulene D et al. Synthesis and antiinflammatory activity of 4-aminoantipyrine derivatives of succinamides. Pharmaceutical Chemistry Journal 1999; 33(4):191–193.
4. Sumit S et al. Design synthesis antimicrobial anticancer evaluation & QSAR studies of 4-(substitutedbenzylidene-amino)-1,5-dimethyl-2-phenyl-1, 2-dihydropyrazol-3-ones. Medicinal chemistry Research 2012; 21:293-307.
5. Yogesh K et al. Synthesis structural determination & antibacterial activity of compounds derived from vanillin and 4-aminoantipyrine. J Serb Chem Soc 2004; 69 (12):991-998.
6. Evstropov A et al. Synthesis and antiviral activity of antipyrine derivative. Pharm Chem J 1992; 26:426-30.
7. Raman N et al. Synthesis spectral characterization of antifungal sensitive Schiff base transition metal complexes. Mycobiology 2007; 35(3):150-153.
8. Schreck R et al. Dithiocarbamates as potent inhibitors of nuclear factor kappa B activation in intact cell. J Exp Med 1992; 175:1181-94.
9. Gaspari P et al. Structure activity relationship of brassinin derivatives as indolamine 2,3-dioxigenase inhibitors. J Med Chem 2006; 49:684-692.
10. Mi-Sun K et al. Antibacterial activity of pyrrolidine dithiocarbamate. FEMS microbiology letters 2008; 280(2): 250-254.
11. Gerhauser C et al. Cancer chemopreventive potential of sulforamate, a novel analogue of sulforaphane that induces phase 2 drug metabolizing enzymes. Cancer Res 1997; 57:272-278.
12. Yong Qian et al. Synthesis Molecular modeling and biological evaluation of dithiocarbamates as novel antitubulin agents. Bioorganic & Medicinal Chemistry 2010; 18:4310-4316.
13. Gaspari P et al. Strucuture Activity study of Brassinin derivatives as Indoleamine 2,3-Dioxygenase Inhibitors. J Med Chem 2006; 49:684-692.
14. Wang et al. Synthesis and in vitro antitumor activity of new butenolide containing dithicarbamates. Bioorganic & Medicinal Chemistry letters 2011; 21(10):3074-3077.
15. Scozzafava A et al. Arylsulfonyl-N, N-diethyl-dithiocarbamates a novel class of antitumor agent. Biorg Med Chem Lett 2000; 10:1887-1891.
16. Guzel O, Salman A. Synthesis antimycobacterial and antitumor activities of new (1,1-dioxido-3-oxo-1,2-benzisothiazol-2(3H)-yl) methyl N,N-disubstitued dithiocarbamate/O-alkyldithiocarbamate derivatives. Biorg med Chem lett 2006; 14:7804-7815.
17. Hou X et al. Dithiocarbamic acid esters as anticancer agent 4-substitued-piperizine-1-carbodithioc acid 3-cyano-3,3-diphenyl-propyl esters Biorg Med Chem Lett 2006; 16:4214-4219.
18. Ooms F. Molecular modeling and computer aided drug design. Examples of their applications in medicinal chemistry. Curr Med Chem 2000; 7:141-158.
19. Alam MS, Lee DU. Synthesis, molecular structure and antioxidant activity of (E)-4[benzylideneamino]-1, 5-dimethyl-2- phenyl-1H-pyrazol-3 (2H)-one a Schiff base ligand of 4- aminoantipyrine. J Chem Crystallogr 2012; 42:93-102.
20. Persidis A. Proteomics. Natural. Biotechnology 1998; 16:393-394.
21. DiMasi JA et al. The price of innovation new estimates of drug development costs J Health Economics 2003; 22:151-185.
22. Jorgensen WL. The many roles of computation in drug discovery Science 2004; 303:1813-1818.
23. Brown D, Superti-Furga G. Rediscovering the sweet spot in drug discovery. Drug Discov Today 2003; 8:1067-1077.
24. Drews J. Strategic trend in the drug discovery. Drug Discov Today 2003; 8:411-420.
25. Lahana R. How many leads from HTS. Drug Discov Today 1999; 4:447-448.
26. Klebe G. Virtual ligand screening Strategies Perspectives and limitation. Drug Discov Today 2006; 11:580-594.
27. Congreve M et al. Structural biology and drug discovery. Drug Discov Today 2005; 10:895-907.
28. Harren J, Leach AR. Structure-based Drug Discovery. Springer Netherlands Dordrecht. 2007; 50.
29. Perutz M. Protein structure New approaches to disease and therapy. Ed. Freeman W.H. Oxford, Newyork. 1992.
30. Neelakantan MA et al. Indian J Pharm Sci 2010; 72: 216.
31. Jayakumarswamy BHM et al. Int J Pharm Tech Res 2011; 3:1864.