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Synthesis, Characterization and Antifungal Evaluation of Novel Bis 1,3,4 Thiadiazole from 4,4'-Diamino-Bibenzyl

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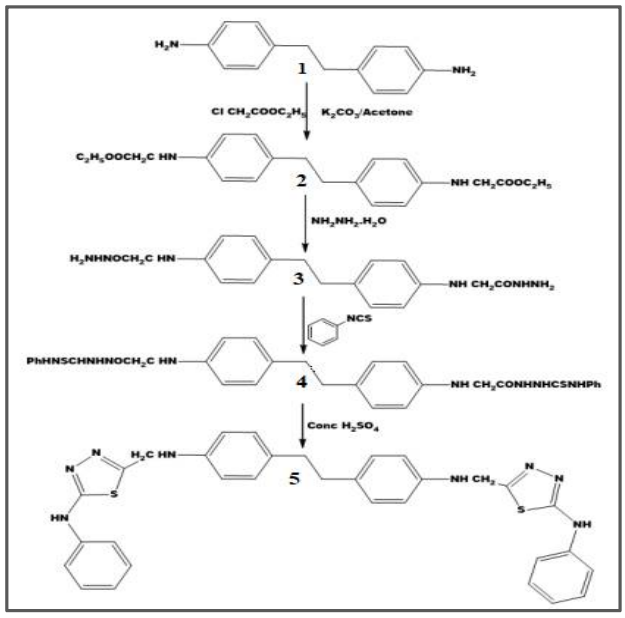
ABSTRACT

*4,4'-diamino-bibenzyl(**1**) in acetone and in the presence of K_2CO_3 is refluxed on a steam bath with mono chloro ethyl acetate to a form a diester **(2)**, this diester on refluxing with hydrazine hydrate forms a dihydrazide**(3)**, this on refluxing with phenyl isothiocyanate forms bis(N-phenylhydrazine-carbothioamide)**(4)**, the bis carbothioamide on warming with conc. H_2SO_4 forms the bis thiadiazole **(5)**. The newly synthesized compounds were characterized by IR, ^1H NMR, spectral data, elemental analysis and evaluated for their in vitro antifungal activity against Penicillium citrinum and Fusarium oxysporum.*

Graphical Abstract



Synthesis of compounds

Keywords: 4,4'-diamino-bibenzyl, Synthesis, Bisthiadiazoles, Antifungal.

INTRODUCTION

Heterocyclic compounds especially those containing sulphur and nitrogen atoms represent an important group of compounds that have diverse bioactivity and can be hold promise for use in the pharmaceutical industry. The 1,3,4-thiadiazole nucleus is one of the most important and well-known heterocyclic nuclei, which is a common and integral feature of a variety of natural products and medicinal agents. The 1,3,4-thiadiazole nucleus containing compounds possess biological [**1**], antimicrobial [**2**], antimalarial [**3, 4**], antiproliferative [**5-7**], anticancer [**8, 9**], analgesic [**10**], antiinflammatory [**11**], antidepressant and anxiolytics [**12**], antihypertensive [**13**], hypoglycemic [**14**], antiglaucoma [**15**], anticonvulsant [**16**], herbicidal [**17**], antiviral [**18**], anti HIV 1 [**19**], anti tuberculosis[**20, 21**], antifungal [**22**], leishamaniacidal [**23**] activities ,they exhibit a very broad spectrum of biological activities which is possibly due to the presence of the toxophoric N-C-S moiety.

MATERIALS AND METHODS

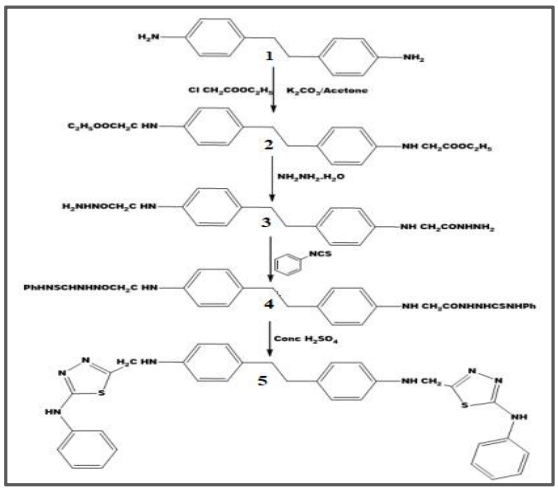
Materials: All the chemicals used were of high purity grade, the solvents were dried and were purified and dried according to the standard procedures. Reactions were monitored by TLC using precoated 0.2 mm plates of silica gel G60 F254 (Merck Germany) as the adsorbent .Visualization of the spots was done using iodine vapors or UV light. Melting points were determined by electro-thermal apparatus using open capillary tubes expressed in °C and are uncorrected. All the compounds were checked by IR, ^{1}HNMR and elemental analysis.

The ^{1}H-NMR and ^{13}C-NMR spectra were recorded on Bruker 400 and 100 MHz, respectively and TMS was used as an internal standard. Chemical shifts relative to TMS as internal standards were given as δ values in ppm. The IR spectra was recorded on a Jasco-FT/IR-4100 spectrophotometer with KBr optics.NMR spectra was recorded on Bruker 400 MHz instrument using d6 -DMSO as solvent at ambient temperature.

4,4'-diamino-bibenzyl**(1)** is treated with mono chloro acetate to a form diester i.e diethyl 2,2'-((ethane-1,2-diylbis(4,1-phenylene))bis(azanediyl))diacetate**(2)**, this diester on treatment with hydrazine hydrate forms a dihydrazide i.e., 2,2'-((ethane-1,2-diylbis(4,1-phenylene)) bis (azanediyl)) di(acetohydrazide)**(3a)**, this dihydrazide on treatment with phenyl isothio cyanate forms 2,2'-(2,2'-((ethane-1,2-diylbis(4,1-phenylene))bis(azanediyl)) bis(acetyl)) bis (N-phenylhydrazinecarbothio-amide)**(3b)** which on heating with conc. H_2SO_4 forms, 5'-(((ethane-1,2-diylbis(4,1-phenylene))bis (azanediyl))bis(methylene))bis(N-phenyl-1,3,4-Thiadiazol-2-amine)**(4)**. The constitution of all the synthesized products has been supported by spectral studies and elemental analysis. The synthesized compounds were screened for their in vitro antifungal activity, some were found to exhibit potent antifungal activity (Scheme 1).

Synthesis of diethyl 2,2'-((ethane-1,2-diylbis (4,1-phenylene)) bis(azanediyl)) diacetate(2): A solution of 4,4'- diamino bibenzyl **(1)** (0.01 mole,2.12 g) in dry acetone (70 cm^3) and ethyl chloro acetate (0.02 mole, 2.8 cm^3)in the presence of anhydrous K_2CO_3 (0.02 mole, 2.76 g) was stirred for 1 h and refluxed on a steam bath for about 16 h. The separated inorganic solid, K_2CO_3 was filtered off and the excess of acetone was evaporated off and the light green solid thus obtained was filtered, washed, dried and crystallized from ethanol to give (**2**).

Synthesis of 2,2'-((ethane-1,2-diylbis(4,1-phenylene))bis(azanediyl))di(aceto-hydrazide) (3): A mixture of the light green ester diethyl 2,2'-((ethane-1,2-diylbis (4,1-phenylene)) bis (azanediyl)) diacetate (2) ,(0.02 mole,7.689 g) and 99% hydrazine hydrate (0.04 mole, 2.002 g) in absolute alcohol (60 cm^3) was stirred for 1 h and refluxed on a steam bath for 8 h .The reaction mixture was distilled under reduced pressure to recover ethanol and cooled and the pale yellow solid separated was filtered. This was purified by crystallization from ethanol furnished pure compound **(3).**



Scheme 1 Synthesis of compounds.

Synthesis of 2,2'-(2,2'-((ethane-1,2-diylbis(4,1-phenyl ene))bis(azanediyl)) bis(acetyl))bis (N-phenylhydrazinecarbothioamide)(4): To 15 cm^3 conc. H_2SO_4 (96%) maintained at 0°C, (0.005 mol, 3.13g) of bis N-phenyl thiosemicarbazide **(4)**was added in small portions over a period of one hour. The reaction mix -ture was allowed to stand at room temperature for 10-12 h .The reaction mixture is then warmed to 50°C on a water bath, cooled and poured into crushed ice with stirring , sodium bicarbonate was added to neutralize the acid. The whitish green solid that separated out was filtered, washed with water and purified by recrystallization from ethanol.

Synthesis of 5,5'-(((ethane-1,2-diylbis(4,1-phenylene))bis(azanediyl)) bis(methylene)) bis (N-phenyl-1,3,4-thiadiazol-2-amine)(5): To 15 cm^3 conc. H_2SO_4 (96%) maintained at 0°C, (0.005 mol, 3.13 g) of bis N-phenyl thiosemicarbazide **(4)** was added in small portions over a period of one hour. The reaction mixture was allowed to stand at room temperature for 10-12 h. The reaction mixture is then warmed to 50 °C on a water bath, cooled and poured into crushed ice with stirring, sodium bicarbonate was added to neutralize the acid. The light green solid that separated out was filtered , washed with water and purified by recrystallization from ethanol.

Biological Assay

The newly synthesized title compounds were screened in vitro for their antifungal activity.

Preparation of test samples: The test sample was prepared by weighing 10 mg of synthesized test compound accurately and dissolving in 10 cm^3 of DMSO solvent, 50µL DMSO was added into one well as solvent control and the observations after incubation were recorded. The antifungal activity of the synthesized test compound was tested against *Fusarium oxysporum* and *Penicillium citrinum* as test micro-organisms by agar well diffusion method. Antifungal activity was tested against *F. oxysporum* and *P. citrinum* using amphotericin B as standard antibiotic. After incubation, zone of inhibition were recorded using zone scale in mm.

RESULTS AND DISCUSSION

The structures of 3,4,5 were established on the basis of elemental analyses, IR, ^{1}H NMR The results of elemental analysis of synthesized compounds were in agreement with the theoretical values within the limits of experimental error (Table 1).

Diethyl 2,2'-((ethane-1,2-diylbis (4,1-phenylene)) bis(azanediyl)) diacetate(2) NMR Spec- tra,^{1}H-NMR [400MHz,δ, ppm,DMSO-d_6]:1.30(s,6H,CH_3),2.88(t,4H,acyclic,CH_2CH_2), 4.36 (q,4H,-CH_2

CO-),6.57-8.77(m,8H,ArH),9.56 (d,2H,NH). C: 68.71; H: 7.30; N, 7.26; O: 16.62; C: 68.73; H: 7.34; N: 7.29; O: 16.65.

Table 1. Molecular weight and physics properties of synthesized compounds

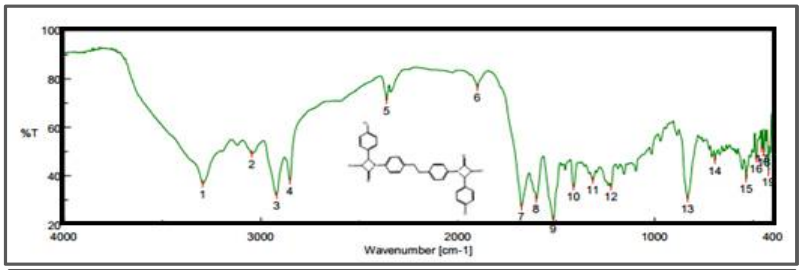
Compound No	Molecular Formula	Mol Wt.	Color	% Yield	Melting Point °C
2	$C_{22}H_{28}N_2O_4$	384.47	Light green	78.24	170
3	$C_{18}H_{24}N_6O_2$	356.42	Pale yellow	69.33	211
4	$C_{32}H_{34}N_8O_2S_2$	626.79	Bluish white	71.01	181
5	$C_{32}H_{28}N_6S_2$	560.73	Whitish green	57.93%	266

2,2'-((ethane-1,2-diylbis(4,1-phenylene))bis(azanediyl))di(aceto-hydrazide)(3)NMR Spec- tra,^{1}H-NMR [400MHz, δ, ppm, DMSO-d_6]: 2.0(s,4H,NH_2),2.81(d,acyclic,-CH_2CH_2), 3.78 (s,4H,CH_2NH), 4.2(s,2H,NH),6.5-7.7(m,8H,ArH),8.12($NHNH_2$): C:60.56; H: 6.72; N: 23.54; O:8.94 ; C:60.66; H: 6.79; N: 23.58; O:8.98.

2,2'-(2,2'-((ethane-1,2-diylbis(4,1-phenylene))bis(azanediyl))bis(acetyl))bis(N-phenylhydrazine carbothioamide)(4) IR Spectra[KBr ν cm^{-1}]: 404,414,422,447(NH, sec amine), 460 ,472, 486, 505, (Ar ring, CH bend), 541 (C-C-C ethyl benzene), 643,692,741 (C-S str), 815 (1,4-disubstituted benzene rings), 933(C-H str), 976,1028(N-N str), 1072(C=S str), 1180, 1281,1380(Ar,C=Cstr,C-Nstr),1449,1520,1596(NHbend),1684,1869(C=Ostr),2098,2341,2358(NH str),3190(C-H str, Ar ring).

NMR Spectra, ^{1}H-NMR[400 MHz, δ, ppm,DMSO-d_6]: 2.0(s,2H,NH),2.81 (d,acyclic,-CH_2 CH_2), 3.78(s,4H,CH_2NH),4.2(s,2H,NH),6.5-7.7.99(m,18H,ArH),8.23(bs,N<u>H</u>NH_2),C,61.38;H, 5.52; N, 17.77;O,5.01;S,10.18,C, 61.32; H, 5.47; N, 17.88; O, 5.11; S, 10.23.

Synthesis of 5,5'-(((ethane-1,2-diylbis(4,1-phenylene))bis(azanediyl))bis(methylene))bis (N-phenyl-1,3,4-thiadiazol-2-amine)(5), IR Spectra[KBr ν cm^{-1}]: 418,444,499, (Ar ring, CH bend), 904(N-C-S);608,690,744,765,1036(C-S-C str in ring), 744,818(1,4-disubstituted benzene rings); 1099(ring vibration including C-S);1206(N-C-S str),1596(C=N & C=C ring str),1293(Ar CH/ C-S def) 1333(C_{Ar}-N str), 1375(N-CH_2CH_2), 1408(C–S–C str.), 1447 (-NHCH_2CH_2,CHdef);1497(N-C-S);1550,1596,(NH str and bend Ar ring),2361,2341(NH str), 1946(C=C str);3050(CH&C=C ring str),3051(CH ,sym);3121,3219,3051,3121,3219 (NH str) (Figure 1).



[Result of Peak Picking]

No.	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity
1	3293.82	36.616	2	3045.05	49.0925	3	2919.7	31.9715
4	2851.24	37.9079	5	2361.41	71.1175	6	1899.54	76.4051
7	1675.84	27.1434	8	1600.63	30.28	9	1514.81	22.0262
10	1411.64	35.4199	11	1314.25	38.1718	12	1220.72	35.4222
13	833.098	30.5921	14	694.248	46.2783	15	535.15	38.8121
16	484.045	47.0968	17	456.082	51.1585	18	444.512	49.7535
19	421.37	41.3901						

Figure 1. I.R.Spectra of (2 a) (KBr in cm-1).

3293 (NH str& bend) 3045, (CH-Ar), 2919,2851(Aliph C-H stretching) ,535, 456,444 (CH_2),2361,1899(CO),1675(CH), 1600(CONH)1514(C=N&C=C ring str), 1411(CH_2),1314, 484 (lactams),1220,421(C-F),833(1,4-disubstituted benzene rings) , 694 (C-Cl).

NMR Spectra, ^{1}H-NMR[400 MHz, δ, ppm,DMSO-d_6]: 2.45(d,4H,acyclic,-CH_2CH_2), 4.29 (bs,4H, CH_2NH),6.9-7.5(m,18H,ArH),8.44(bs,4H,NH-Ph), C, 64.42; H, 5.44; N, 14.03; O, 5.39; S, 10.71, C, 64.40; H, 5.40; N, 14.08; O, 5.36; S, 10.75 (Figure 2)

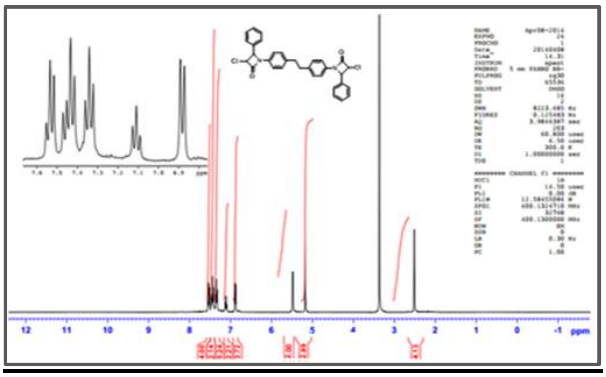


Figure 2. NMR Spectra of (2a) in DMSO

Evaluation of Antifungal Activities: Among the tested compounds 5 showed more potent fungicidal activity against both Fusarium oxysporum and *Penicillium citrinum* than other compounds. The newly synthesized compounds 4 and 5 showed very good to moderate antifungal activity (Figure 3).

Compound 4 showed very good antifungal activity against *Fusarium oxysporum*, it showed 20 mm zone of inhibition. Compound 5 showed a 22 mm zone of inhibition, which is almost the same as exhibited by the antibiotic amphoterin B which has zone of inhibition of 21 mm. Compound 5 showed moderate activity by exhibiting zone of inhibition of 14 mm as compared to 18 mm exhibited by amphoterin B.

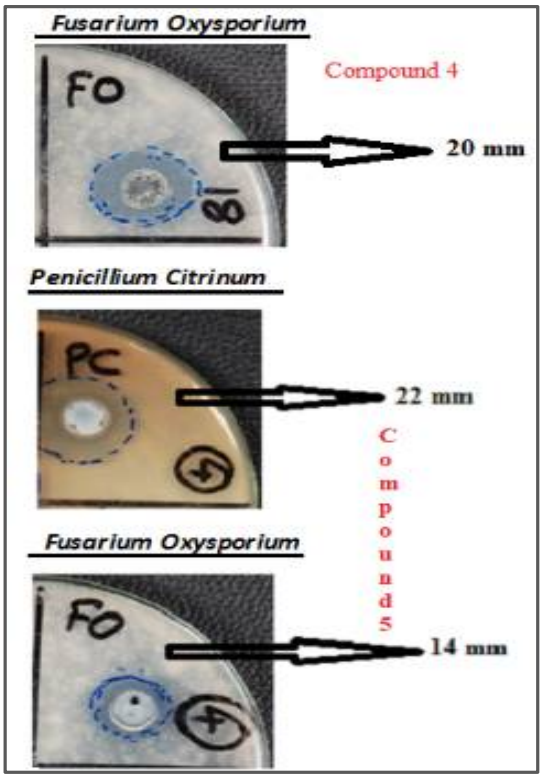


Figure 3. Antifungal activity of synthesized compounds

APPLICATION

The present study has shown that the selected compounds possess remarkable antifungal activity as promising lead molecules for the development of new drugs. This research strongly supports the effectiveness of these compounds against different fungal diseases.

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REFERENCES

[1]. T. K. Ibrahim, Synthesis of 2-Amino-5-(Substituted Phenyl)-1,3,4-Thiadiazole And Evaluation of Biological Activity, *J. Applicable Chem*., **2015**, 4 (3), 847-852.
[2]. M. A. Rahiman, D. R. Mamatha, G. L. Thejashree, M. G. Suresha, Synthesis, Characterization and Antimicrobial Studies of Some Novel Thiadiazoles derived from [1,2,4] Tri azoles, *J.Applicable Chem*., **2017**, 6(1), 1-7.
[3]. A. Bhatt, R. K. Singh, R. Kant, Synthesis, antimicrobial and antimalarial study of novel 1,3,4-thiadiazole derivatives incorporating imidazo[1,2-b]pyridazine and thiazolidinone moieties, *Chemical Biology Letters*, **2017**, 4(2), 73-80.
[4]. A. Tahghighi, F. Babalouei, Thiadiazoles: the appropriate pharmacological scaffolds with leishmanicidal and antimalarial activities: a review, *Iranian Journal of Basic Medical Sciences*, **2017**, 20(6), 613-22.
[5]. J. Matysiak, A. Opolski, Synthesis and antiproliferative activity of N-substituted 2- amino-5-(2,4-dihydroxyphenyl)-1,3,4-thiadiazoles, *Bioorganic and Medicinal Chemistry*, **2006**, 14(13), 4483-9.
[6]. S. M. Gomha, H. M. Abdel-aziz, Synthesis and antitumor activity of 1,3,4-thiadiazole derivatives bearing coumarine ring, *Heterocycles:An International Journal for Reviews and Communications in Heterocyclic Chemistry*, **2015**, 91(3), 583-92.
[7]. J. Zhao, L. Xuan, H. Zhao, J. Cheng, X. Fu, S. Li, F. Jing, Y. Liu, B. Chen, Synthesis and anti tumor activities of 1,3,4-thiadiazole derivatives possessing benzisoselenazolone scaffold, *Chemical Research in Chinese Universities*, **2014**, 30(5),764-9.
[8]. D. Kumar, N. M. Kumar, K. H. Chang, R. Gupta, K. Shah, Synthesis and in-vitro anticancer activity of 3,5-bis (indolyl)-1,2,4-thiadiazoles, *Bioorganic and Medicinal Chemistry Letters*, **2011**, 21(19), 5897-900.
[9]. M. X. Wei, L. Feng, X. Q. Li,X, Z. Zhou, Z. H. Shao, Synthesis of new chiral 2, 5-disubstituted 1,3,4-thiadiazoles possessing γ-butenolide moiety and preliminary evaluation of in vitro anticancer activity, *European Journal of Medicinal Chemistry*, **2009**, 44(8), 3340-4.
[10]. S. Schenone, C.Brullo, O. Bruno, F. Bondavalli, A. Ranise, W. Filippelli, B. Rinaldi, A. Capuano, G. Falcone, New 1,3,4-thiadiazole derivatives endowed with analgesic and anti-inflammatory activities, *Bioorganic and Medicinal Chemistry*, **2006**, 14(6), 1698- 705.
[11]. L. S. Varandas, C. A. Fraga, A. L. Miranda, E. J. Barreiro, Design, synthesis and pharma cological evaluation of new nonsteroidal antiinflammatory 1,3,4-thiadiazole derivatives, *Letters in Drug Design and Discovery*, **2005**, 2(1), 62-7.
[12]. R. Sharma, G. P. Misra, J. Sainy, S. C. Chaturvedi, Synthesis and biological evaluation of 2-amino-5-sulfanyl-1,3,4-thiadiazole derivatives as antidepressant, anxiolytics and anti convulsant agents, *Medicinal Chemistry Research*, **2011**, 20(2), 245-53.
[13]. L.Vio, M. G. Mamolo, A. Laneve, Synthesis and antihypertensive activity of some 1,3, 4-thiadiazole derivatives, *Farmaco (Societachimica italiana*, **1989**, 44(2), 165-72.
[14]. F. L .Chubb, J. Nissenbaum, Some hypoglycemic thiadiazoles, *Canadian Journal of Chemistry*, **1959**, 37(6), 1121-3.

[15]. R. Kasımoğulları, M. Bülbül, B. S. Arslan, B. Gökçe, Synthesis,characterization and anti glaucoma activity of some novel pyrazole derivatives of 5-amino-1,3,4-thia diazole-2-sulfonamide, *European Journal of Medicinal Chemistry*, **2010**, 45(11), 4769 -73.
[16]. P. Mullick, S. A. Khan, S. Verma, O. Alam, Thiadiazole derivatives as potential anti convulsantagents, *Bulletin of the Korean Chemical Society*, **2011**, 32(3), 1011-6.
[17]. H. Kubo, R. Sato, I. Hamura, T Ohi, Herbicidal activity of 1,3,4-thiadiazole derivatives. *Journal of Agricultural and Food Chemistry*, **1970**, 18(1), 60-65.
[18]. M. Tonew, E. Klimk, Antiviral 1,3,4-Thiadiazoles, *Chemotherapy*, **1974**, 20(6), 350-60.
[19]. Y. Hanasaki, H. Watanabe, K. Katsuura, H. Takayama, S. Shirakawa, K. Yamaguchi, S. I. Saka, K.Ijichi, M. Fujiwara,Thiadiazole derivatives: highly potent and specific HIV-1 reverse transcriptase inhibitors, *Journal of Medicinal Chemistry*, **1995**, 38(12), 2038-40.
[20]. E. E.Oruç, S. Rollas, F. Kandemirli, N.Shvets, A. S. Dimogl, 1,3,4-thiadiazole derivatives. Synthesis, structureelucidation and structure-antituberculosis activity relationship investigation, *Journal of Medicinal Chemistry*, **2004**, 47(27), 6760-7.
[21]. P. Kohli, S. K. Srivastava, S. D. Srivastava, Synthesis, Characterization and Evaluation of Some Novel5-(2-aryl-4-oxo-1,3-thiazolidine)- 2-(phenoxazinylmethyl)- 1,3,4- thia diazolazole Derivatives as Antimicrobial and Antitubercular Agents, *J. Applicable Chem*., **2014**, 3(2), 525-34.
[22]. L. J. Zhang, M. Y. Yang, Z. H. Sun, C .X. Tan, J. Q. Weng, H. K. Wu, X. H. Liu, Synthesis and antifungal activity of 1,3,4-thiadiazole derivatives containing pyridine group, *Letters in Drug Design and Discovery*, **2014**, 11(9),1107-11.
[23]. F. Poorrajab, S. K. Ardestani, S, Emami, M. Behrouzi-Fardmoghadam, A. Shafiee, A. Forou madi, Nitroimidazolyl-1,3,4-thiadiazole-based anti-leishmanial agents: synthesis and in vitro biological evaluation, *European Journal of Medicinal Chemistry*, **2009**, 44(4), 1758-62.