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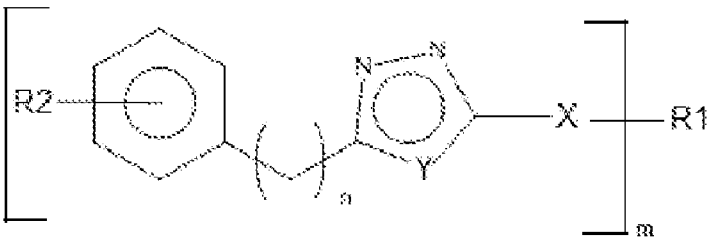
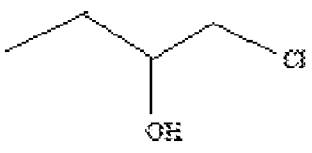
[Continued on next page]

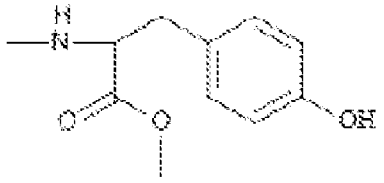
(54) Title: 1,2,4-TRIAZOLE, 1,3,4-OXADIAZOLE, AND 1,3,4-THIADIAZOLE DERIVATIVES AND THEIR ANTIMYCOBACTERIAL ACTIVITY



Formula (I)



(II)



(III)

(57) Abstract: Disclosed herein are novel five membered heterocyclic compounds of Formula (I) wherein, X is S or SO_2; n is 0, 1; m is1 or 2; Y is N, O or S; R2 independently represents H or alkyl or halo selected from -CI, -F; or -CF_3, or -OH or-NH_2 or - NO_2; and R1 independently represents H or C1 to C5 straight or branched chain alkyl, alkenyl, alkynyl or a group -$(CH_2)_5$Br or pyrrolidine or -NHR' wherein R' is H or isopropyl or (II) or (III) which selectively act against dormant pathogenic tuberculi bacilli and exhibit antiproliferative activities and for treatment of a disease or disorder associated with GroEL1/GroEL2 activity. The invention relates to a process for preparation of novel five membered heterocyclic compounds of Formula I and to pharmaceutical compositions thereof.

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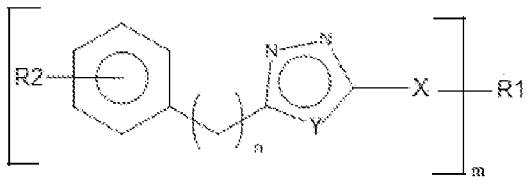
1,2,4-TRIAZOLE, 1,3,4-OXADIAZOLE, AND 1,3,4-THIADIAZOLE DERIVATIVES AND THEIR ANTIMYCOBACTERIAL ACTIVITY

FIELD OF THE INVENTION

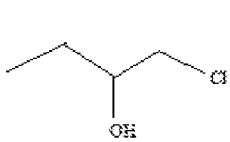
[001] The present invention relates to five membered heterocyclic compounds of Formula I which selectively act against dormant pathogenic tuberculi bacilli and exhibit antiproliferative activities and for treatment of a disease or disorder associated with GroEL1/GroEL2 activity.



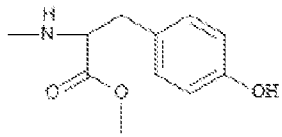
Formula (I)

wherein,

X is S or SO_2; n is 0 or 1; m is 1 or 2; Y is N ,O or S; R2 independently represents H or alkyl or halo selected from -Cl, -F; or $-CF_3$, or -OH or-NH_2 or $-NO_2$; R1 independently represents H or C1 to C5 straight or branched chain alkyl, alkenyl, alkynyl or a group $-(CH_2)_5Br$ or pyrrolidine or –NHR' wherein R' is H or isopropyl or



or



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[002] Particularly, present invention relates to a process for preparation of five membered heterocyclic compounds of Formula I and pharmaceutical composition thereof.

BACKGROUND AND PRIOR ART OF THE INVENTION

[003] Tuberculosis (TB) is a widespread infectious disease caused by various strains of mycobacteria, particularly Mycobacterium Tuberculosis and remains a leading cause of mortality worldwide. Tuberculosis typically infects lungs but can also attack other parts of the body. It is generally spread through the air when people infected with TB cough, sneeze in open public places. Most infections do not have symptoms, known as latent tuberculosis. If,

however, left untreated it eventually progresses to active disease which kills more than 50% of those so infected with TB.

[004] One-third of the world's population is thought to have been infected with latent M. tuberculosis and new infections occur in about 1% of the population each year. Diagnosis of latent TB primarily relies on tuberculin skin test (TST) and/or blood tests. The treatment is difficult and requires administration of multiple antibiotics over a long period of time.

[005]People in the developing world contract tuberculosis more rapidly as compared to developed nations due to overpopulation, malnutrition and low immune system. One of the major reasons of occurrence of TB in immunoaltered individuals is high rate of HIV infection and subsequent development of AIDS.

[006] Treatment of tuberculosis is complicated and requires long term administration of drugs such as Rifampicin, Isoniazid, Ethambutol and Pyrazinamide. The high dosage of these drugs as well as long duration of treatment has adverse side effects and often results in development of multidrug resistant strains and poor compliance from TB patients. The failure to treatment is further related to migration of inhabitants from areas with high incidence of TB to the regions with a favorable epidemiologic situation.

[007] Thus increase in HIV-associated tuberculosis and increase in antibiotic resistance due to prolonged treatments are growing problems in multiple-drug resistant tuberculosis infections. Researchers around the globe are working on alternate medications that can act against multi drug resistant strain as well as against pathogenic tuberculi bacteria in its dormant phase for effective treatment of tuberculosis.

[008] Triazoles are known for their antifungal, antiviral and plant growth regulatory activities but their antimycobacterial potential has gained importance only in recent years. Fluconazole and tebuconazole are known for their antimycobacterial activity but have non-specificity and higher MIC values. Moreover, they are not effective against dormant tubercle bacilli.

[009] 1,3,4-Oxadiazoles form an important class of five-member heterocyclic compounds with a wide range of biological activities. The importance of the oxadiazole nucleus is well established in agricultural and pharmaceutical chemistry as far as its corresponding derivatives are used as antipyretic, analgesic, antidepressant, antimicrobial, antiviral, fungicidal, anti-neoplastic, anti-inflammatory agents, central nervous system stimulants, and anti-convulsive,

anti-cancer, and anti-hypertensive agents. Recently 1,3,4 thiadiazole derivatives have been reported to have anti-microbial/anti-tubercular activity.

[010] The present inventors have reported 1, 2, 4 triazole derivatives with anti-tubercular activity against *Mycobacterium tuberculosis* keeping in view of the biological importance of azoles (WO 2011/111077;D Sarkar et al). Azoles were considered to exert anti-tubercular activity through inhibition of CYP51 and CYP121 by a mechanism in which the heterocyclic nitrogen (N-3 of imidazole or N-4 of 1, 2, 4-triazole) bind to the sixth coordination of heme iron atom of the porphyrin in the substrate-binding site of the enzyme. The lack of any effect of these inhibitors against the non-pathogenic bacilli, *Mycobacterium smegmatis* indicated the probability of targeting other protein(s). Detailed investigation using Biotin linker clearly established that groEL 2 as target for these inhibitors within the bacilli (PCT/IN2012/000184).

[011] Special attention is being given to optimize this novel triazole scaffold having potent anti-mycobacterial activity in an effort to improve the drug like characteristics. Structure-activity relation (SAR) studies shows that the presence of the hydrogen bond acceptor subunit, the position in the aromatic ring, the planarity of heterocyclic and phenyl rings in these compounds is important for exhibiting the anti-tubercular activity.

[012] In light of the above, the present inventors felt that there is a scope to provide novel five membered heterocyclic compounds in addition to the triazole compounds disclosed in their earlier application that can effectively combat the non-replicating dormant phase of *Mycobacterium bovis* BCG and *M. tuberculosis*.

[013] The novel five membered heterocyclic compounds are also found to exhibit effective anti-proliferative activities widening the use of these compounds.

OBJECTS OF THE INVENTION

[014] Main object of the present invention to provide five membered heterocycle scaffold compound of Formula I consisting of three hetero atoms having selective activity against dormant pathogenic tuberculi bacilli and exhibit anti proliferative activities.

[015] Another object of the present invention is to provide a method for the preparation of compound of formula I using simple and economical viable process.

[016] Yet another object of the present invention is to provide a pharmaceutical composition comprising five membered heterocyclic compound of formula I as active ingredient along with suitable excipients to selectively act against both active and dormant pathogenic tuberculi.

[017] Yet another object of the present invention is to provide a pharmaceutical composition comprising five membered heterocyclic compound of formula I as active ingredient along with suitable excipients exhibiting anti proliferative activities against several tumor and normal human cell lines.

[018] Yet another object of the present invention is to provide a pharmaceutical composition comprising five membered heterocyclic compound of formula I as active ingredient along with suitable excipients for treatment of a disease or disorder associated with GroEL1/GroEL2 activity.

[019] Yet another object of the present invention is to provide a method for treating the subject suffering from tuberculosis, cancer and disorders associated with GroEL1/GroEL2 activity.

[020] BRIEF DESCRIPTION OF THE DRAWINGS

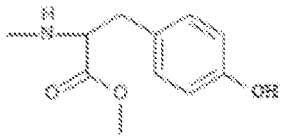
Figure 1 shows protocol for Aqueous Solubility Screening Assay.

Figure 2a: The CoMFA steric molecular interaction fields around molecule 90A. Green contour signify that steric bulk is favored around these positions in the scaffold while yellow contour signify that a disfavored steric substitution.

Figure 2b: The CoMFA electrostatic molecular interaction fields around molecule 90A. Red contour signify that electronegative substitution is favored around these positions in the scaffold while blue contour signify that a electropositive substitution is favored.

Scheme 1 represents process steps for the preparation of compound of formula I wherein Ar represent Nicotinic, Isonicotinic, 2-furyl, 2-thiophenyl or substituted phenyl and reaction conditions are as follow: a)NH_2NH_2,H_2O,reflux 6hrs; b)CS_2,KOH,ethanol,room temperature (rt),12hrs; c)25%NH3, reflux, 8hrs; d) alkylhalide/ ethanol, rt, 12hrs; e)Oxone,THF:Water,12hrs; f) CS_2, KOH, ethanol, reflux, workup in conc. HCl; g)alkyl halide/$NaHCO_3$ h)CS_2,KOH ,ethanol, reflux, workup in conc.H_2SO_4; i) $SOCl_2$, DCM, Cat. DMF, reflux. 4hrs; j) Thiosemicabazide, Pyridine, 15hrs; k)10%KOH, reflux, 8hrs.

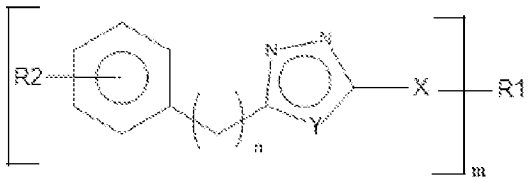
Scheme 2 represents process step for preparation of sulfonamide, wherein R represents H,



straight or branched chain alkyl, pyrolidine or .

SUMMARY OF THE INVENTION

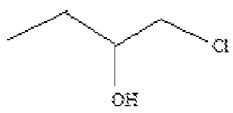
[021] Accordingly, present invention provides compound of formula (I) or its acid addition salts thereof



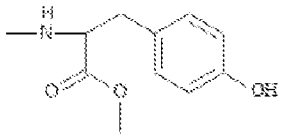
Formula (I)

wherein,

X is S or SO_2; n is 0, 1;m=1 or 2; Y is N ,O or S; R2 independently represents H or alkyl or halo selected from -Cl, -F; or $-CF_3$, or -OH or-NH2 or $-NO_2$; R1 independently represents H or C1 to C5 straight or branched chain alkyl, alkenyl, alkynyl or a group –

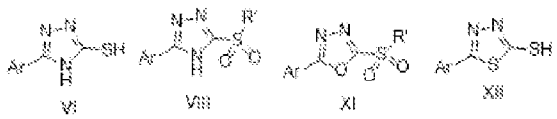


(CH_2)5Br or pyrrolidine or –NHR' wherein R' is H or isopropyl or



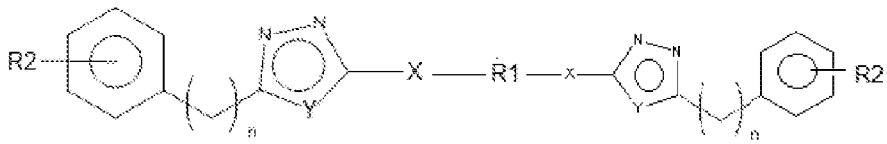
or .

[022] In an embodiment of the present invention, representative compound of formula 1 comprising:



,

formula 1A



Formula 1A

wherein,

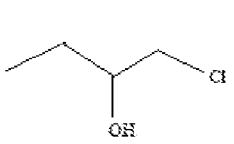
X is S or SO_2;

n is 0, 1;

Y is N ,O or S;

R2 independently represents H or alkyl or halo selected from -Cl, -F; or $-CF_3$, or -OH or-NH2 or $-NO_2$;

R1 independently represents H or C1 to C5 straight or branched chain alkyl, alkenyl, alkynyl or a group $-(CH_2)5Br$ or pyrrolidine or –NHR' wherein R' is H or isopropyl or



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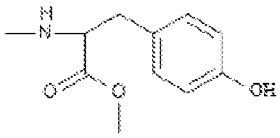


or its acid addition salts thereof.

[023] In another embodiment of the present invention, said compound comprising:

i. 3-(4-chlorophenyl)-1H-1,2,4-triazole-5-thiol **(1);**
ii. 3-(4-nitrophenyl)-1H-1,2,4-triazole-5-thiol **(2);**
iii. 3-(4-fluorophenyl)-1H-1,2,4-triazole-5-thiol **(3);**
iv. 3-(4-(trifluoromethyl) phenyl)-1H-1,2,4-triazole-5-thiol **(4);**
v. 5-(prop-2-yn-1-ylthio)-3-(4-(trifluoromethyl)phenyl)-1H-1,2,4-triazole **(5);**
vi. 5-(allylthio)-3-(4-(trifluoromethyl) phenyl)-1H-1,2,4-triazole **(6);**
vii. 5-(prop-2-yn-1-ylsulfonyl)-3-(4-(trifluoromethyl) phenyl)-1H-1,2,4-triazole **(7);**
viii. 5-(ethylthio)-3-(4-(trifluoromethyl) phenyl)-1H-1,2,4-triazole **(8);**
ix. 5-(butylthio)-3-(4-(trifluoromethyl) phenyl)-1H-1,2,4-triazole**(9);**
x. 3-(pyridin-3-yl)- 1H -1,2,4-triazole**(10);**
xi. 3-(3-(trifluoromethyl)phenyl)-1H-1,2,4-triazole-5-thiol**(11);**
xii. 5-(prop-2-yn-1-ylthio)-3-(3-(trifluoromethyl)phenyl)-1H-1,2,4-triazole **(12);**
xiii. 5-(prop-2-yn-1-ylsulfonyl)-3-(3-(trifluoromethyl) phenyl)-1H-1,2,4-triazole **(13)**;
xiv. 5-(allylthio)-3-(3-(trifluoromethyl) phenyl)-1H-1,2,4-triazole**(14)**;

xv. 5-(ethylthio)-3-(3-(trifluoromethyl) phenyl)-1H-1,2,4-triazole(**15**);
xvi. 5-(butylthio)-3-(3-(trifluoromethyl) phenyl)-1H-1,2,4-triazole(**16**);
xvii. 3-(4-nitro-3-(trifluoromethyl)phenyl)-1H-1,2,4-triazole-5-thiol(**17**);
xviii. 3-(4-nitro-3-(trifluoromethyl)phenyl)-5-(prop-2-yn-1-ylthio)-1H-1,2,4-triazole(**18**);
xix. 3-(4-nitro-3-(trifluoromethyl)phenyl)-5-(prop-2-yn-1-ylsulfonyl)-1H-1,2,4-triazole(**19**);
xx. 5-(allylthio)-3-(4-nitro-3-(trifluoromethyl)phenyl)-1H-1,2,4-triazole(**20**);
xxi. 5-(ethylthio)-3-(4-nitro-3-(trifluoromethyl)phenyl)-1H-1,2,4-triazole(**21**);
xxii. 5-(butylthio)-3-(4-nitro-3-(trifluoromethyl)phenyl)-1H-1,2,4-triazole(**22**);
xxiii. 3-(4-chloro-2-methylphenyl)-1H-1,2,4-triazole-5-thiol(**23**);
xxiv. 3-(2-chloro-5-(trifluoromethyl)phenyl)-1H-1,2,4-triazole-5-thiol(**24**);
xxv. 3-(2-chloro-5-(trifluoromethyl)phenyl)-5-(prop-2-yn-1-ylthio)-1H-1,2,4-triazole(**25**);
xxvi. 3-(2-chloro-5-(trifluoromethyl)phenyl)-5-(prop-2-yn-1-ylsulfonyl)-1H-1,2,4-triazole(**26**);
xxvii. 5-(allylthio)-3-(2-chloro-5-(trifluoromethyl)phenyl)-1H-1,2,4-triazole (**27**);
xxviii. 5-(ethylthio)-3-(2-chloro-5-(trifluoromethyl)phenyl)-1H-1,2,4-triazole(**28**);
xxix. 5-(butylthio)-3-(2-chloro-5-(trifluoromethyl)phenyl)-1H-1,2,4-triazole (**29**);
xxx. 3-(3-chloro-2-methylphenyl)-1H-1,2,4-triazole-5-thiol (**30**);
xxxi. 3-(3-chloro-2-methylphenyl)-5-(butylthio)-1H-1,2,4-triazole (**31**);
xxxii. 3-(5-chloro-2-methylphenyl)-1H-1,2,4-triazole-5-thiol (**32**);
xxxiii. 5-((4-bromobutyl)thio)-3-(4-fluorophenyl)-1H-1,2,4-triazole (**33**);
xxxiv. 3-(2-(trifluoromethyl)phenyl)-1H-1,2,4-triazole-5-thiol (**34**);
xxxv. 5-(prop-2-yn-1-ylthio)-3-(2-(trifluoromethyl)phenyl)-1H-1,2,4-triazole (**35**);
xxxvi. 5-(allylthio)-3-(2-(trifluoromethyl)phenyl)-1H-1,2,4-triazole (**36**);
xxxvii. 5-(ethylthio)-3-(2-(trifluoromethyl)phenyl)-1H-1,2,4-triazole (**37**);
xxxviii. 5-(butylthio)-3-(2-(trifluoromethyl)phenyl)-1H-1,2,4-triazole (**38**);
xxxix. 3-(furan-2-yl)-1H-1,2,4-triazole-5-thiol (**39**);
xl. 3-(4-methoxyphenyl)-1H-1,2,4-triazole-5-thiol (**40**);
xli. 3-(4-chlorophenyl)-5-((cyclopropylmethyl)thio)-1H-1,2,4-triazole (**41**);
xlii. 3-(4-fluorophenyl)-5-((cyclopropylmethyl)thio)-1H-1,2,4-triazole (**42**);
xliii. 3-(4-nitrophenyl)-5-((cyclopropylmethyl)thio)-1H-1,2,4-triazole (**43**);
xliv. 5-(4-chlorophenyl)-1,3,4-thiadiazole-2-thiol (**44**);
xlv. 5-(4-fluorophenyl)-1,3,4-thiadiazole-2-thiol (**45**);
xlvi. 5-(4-nitrophenyl)-1,3,4-thiadiazole-2-thiol (**46**);
xlvii. 3-(2-chlorophenyl)-1H-1,2,4-triazole-5-thiol (**47**);
xlviii. 3-(o-tolyl)-1H-1,2,4-triazole-5-thiol (**48**);
xlix. 5-(allylsulfonyl)-3-(3-(trifluoromethyl)phenyl)-1H-1,2,4-triazole (**49**);
l. 5-(butylsulfonyl)-3-(3-(trifluoromethyl)phenyl)-1H-1,2,4-triazole (**50**);
li. 4-((5-(allylthio)-1H-1,2,4-triazol-3-yl)methyl)phenol (**51**);
lii. 4-((5-(prop-2-yn-1-ylthio)-1H-1,2,4-triazol-3-yl)methyl)phenol(**52**);
liii. 2-(5-mercapto-1H-1,2,4-triazol-3-yl)methyl phenol(**53**);
liv. 5-(allylsulfonyl)-3-(4-(trifluoromethyl)phenyl)-1H-1,2,4-triazole (**54**);
lv. 3-phenyl-1H-1,2,4-triazole-5-thiol (**55**);
lvi. 3-(m-tolyl)-1H-1,2,4-triazole-5-thiol (**56**);
lvii. 2-((5-(allylthio)-1H-1,2,4-triazol-3-yl)methyl)phenol(**57**);

lviii. 3-(4-chlorophenyl)-1H-1,2,4-triazole-5-sulfonamide **(58);**
lix. 3-(4-chlorophenyl)-N-isopropyl-1H-1,2,4-triazole-5-sulfonamide**(59);**
lx. methyl ((5-(4-chlorophenyl)-1H-1,2,4-triazol-3-yl)sulfonyl)tyrosinate **(60);**
lxi. 5-(4-chlorophenyl)-3-(pyrrolidin-1-ylsulfonyl)-1H-1,2,4-triazole **(61);**
lxii. 4-(3-mercapto-1H-1,2,4-triazol-5-yl)phenol **(62);**
lxiii. 4-(3-(butylthio)-1H-1,2,4-triazol-5-yl)phenol **(63);**
lxiv. 4-(3-((cyclopropylmethyl)thio)-1H-1,2,4-triazol-5-yl)phenol **(64);**
lxv. 5-(4-aminophenyl)-1H-1,2,4-triazole-3-thiol **(65);**
lxvi. 4-(3-(butylthio)-1H-1,2,4-triazol-5-yl)aniline **(66);**
lxvii. 3-(butylthio)-5-(o-tolyl)-4H-1,2,4-triazole **(67);**
lxviii. 2-(4-fluorophenyl)-5-(ethylthio)-1,3,4-oxadiazole **(68);**
lxix. 2-(4-fluorophenyl)-5-(butylthio)-1,3,4-oxadiazole **(69);**
lxx. 2-(4-fluorophenyl)-5-(propylthio)-1,3,4-oxadiazole **(70)** ;
lxxi. 5-(sec-butylthio)-3-(p-tolyl)-1H-1,2,4-triazole **(71).**
lxxii. 3-(4-chlorophenyl)-5-methyl-1H-1,2,4-triazole compound with 5-((4-(l1-sulfanyl)butyl)thio)-3-(4-chlorophenyl)-1H-1,2,4-triazole (1:1) **(72);**
lxxiii. 3-(4-fluorophenyl)-5-methyl-1H-1,2,4-triazole compound with 5-((4-(l1-sulfanyl)butyl)thio)-3-(4-fluorophenyl)-1H-1,2,4-triazole (1:1) **(73);**
lxxiv. 3-(4-nitrophenyl)-5-methyl-1H-1,2,4-triazole compound with 5-((4-(l1-sulfanyl)butyl)thio)-3-(4-fluorophenyl)-1H-1,2,4-triazole (1:1) **(74).**

[024] In another embodiment, present invention provides a process for the preparation of compound of formula (I) or its acid addition salts and the said process comprising the steps of:

a) refluxing a mixture of hydrazine hydrate and (un)substituted or substituted aromatic ester (III) to separate hydrazide solid (IV);
b) slurrying hydrazide (IV) as obtained in step (a) in a mixture of ethanolic potassium hydroxide and carbon disulfide at ambient temperature to obtain compound (V);
c) treating compound (V) as obtained in step (b) with 25% ammonia solution followed by acidification to produce corresponding triazolethiol (VI);
d) alkylating triazolethiol (VI) as obtained in step (c) to obtain alkylated triazolethiols (VII); and
e) adding cold solution of oxone dissolved in a mixture of THF and water in the ratio 2:1 to alkylated triazolethiols (VII) as obtained in step (d) to obtain sulfones (VIII).
f) refluxing compound of formula (V) with a mixture of ethanolic potassium hydroxide and carbon disulfide and acidifying with conc. H2SO4 to obtain (XII).

g) alkylating triazolethiol (VI) with a suitable alkylating agent to obtain compounds of formula (II).

h) refluxing mixture of substituted benzoic acid (XIII) and thionyl chloride followed by addition of thiosemicarbazide in pyridine to obtain crude residue (XV);

i) dissolving the crude residue (XV) in 10% aq. KOH followed by neutralizing with 10% aq. HCl to yield 1, 2, 4-triazole 5-thiol (VI).

j) slurrying hydrazide (IV) in a mixture of ethanolic potassium hydroxide and carbon disulfide at ambient temperature followed by acidification with 10% HCl solution to obtain 1,3,4-oxadiazolethiol (IX);

k) alkylating 1,3,4-oxadiazolethiol (IX) in presence of a solvent and base to obtain alkylated compound (X); and

l) adding cold solution of oxone dissolved in a mixture of THF and water in the ratio 2:1 to alkylated compound (X) to obtain sulfones (XI).

[025] In yet another embodiment of the present invention, triazolethiol (VI) is optionally prepared by a process comprising;

(a) refluxing mixture of substituted benzoic acid (XIII) and thionyl chloride followed by addition of thiosemicarbazide in pyridine to obtain crude residue (XV);

(b) dissolving the crude residue (XV) in 10% aq. KOH followed by neutralizing with 10% aq. HCl to yield 1, 2, 4-triazole 5-thiol (VI).

[026] In yet another embodiment of the present invention, the alkylating agent is selected from 3-(4-chlorophenyl)-5-halo methyl-1H-1,2,4-triazole; 3-(4-fluorophenyl)-5-halo methyl-1H-1,2,4-triazole and 3-(4-nitrophenyl)-5-halo methyl-1H-1,2,4-triazole.

[027] In another embodiment, present invention provides a pharmaceutical composition comprising at least one of the novel five membered heterocyclic compounds of formula (I) as an active ingredient along with pharmaceutically acceptable excipients, being present in sufficient amount, to be effective against both active and dormant phase bacilli of *Mycobacterium Bovis* BCG and *Mycobacterium Tuberculosis* H37Ra for preventing the proliferation of HeLa cancer cell lines and for treatment of a disease or disorder associated with GroEL1/GroEL2 activity.

[028] In yet another embodiment of the present invention, the compounds of formula (I) effective against both active and dormant phase bacilli of *Mycobacterium Bovis* BCG and *Mycobacterium Tuberculosis* H37Ra and for treatment of a disease or disorder associated with GroEL1/GroEL2 activity selected from compound (2), (3), (4), (5), (11), (14), (15), (17), (18), (19), (20), (23), (24), (41), (46), (56), (63), (64), (65), (66), (67), (68), (71), (72) and (73).

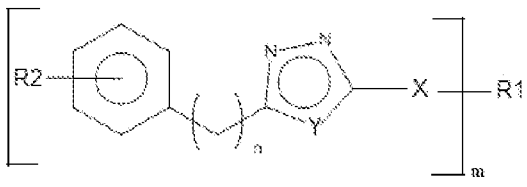
[029] In yet another embodiment of the present invention, the compounds of formula (I) effective for preventing the proliferation of HeLa cancer cell lines selected from (18), (41), (42), (43), (44), (45), (67) and (68).

[030] In another embodiment, present invention provides a method for treating the subject suffering from tuberculosis, cancer and disorders associated with GroEL1/GroEL2 activity comprising administering pharmaceutical composition comprising novel five membered heterocyclic compounds of formula (I) or its acid addition salts thereof either alone or in combination as active ingredient along with pharmaceutically acceptable excipients in therapeutically effective amount.

[031] In another embodiment, present invention provides use of novel five membered heterocyclic compounds of formula (I) or its acid addition salts thereof to selectively act against both active as well as dormant bacilli of *Mycobacterium Bovis* BCG and *Mycobacterium Tuberculi* H37Ra; for treatment of a disease or disorder associated with GroEL1/GroEL2 activity and for preventing the proliferation of HeLa cancer cell lines.

DETAILED DESCRIPTION OF THE INVENTION

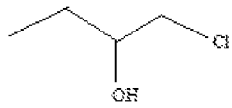
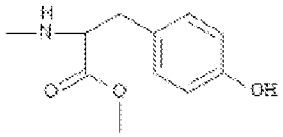
[032] Present invention provides five membered heterocyclic compounds of formula (I) or its acid addition salts



Formula (I)

wherein,

X is S or SO_2; n is 0 or 1; m is 1 or 2; Y is N ,O or S; R2 independently represents H or alkyl or halo selected from -Cl, -F; or $-CF_3$, or -OH or-NH_2 or $-NO_2$; R1 independently represents H or C1 to C5 straight or branched chain alkyl, alkenyl, alkynyl or a group $-(CH_2)_5Br$ or

pyrrolidine or –NHR' wherein R' is H or isopropyl or  or  .

The five membered heterocyclic compounds of formula (I) are active against both active and dormant phase of pathogenic *Mycobacterium tuberculi.*

The compounds of formula (I) comprises

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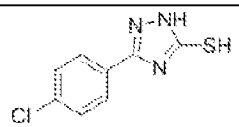
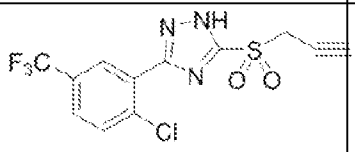
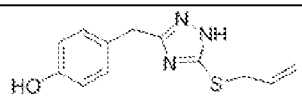
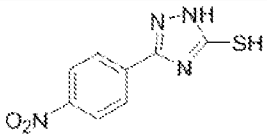
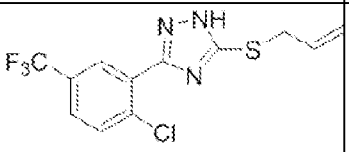
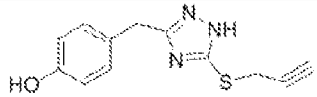
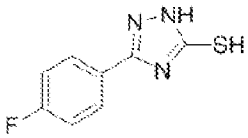
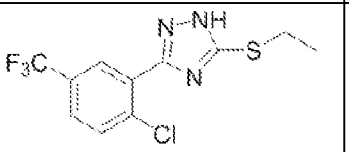
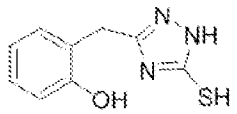
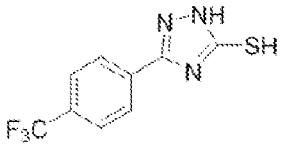
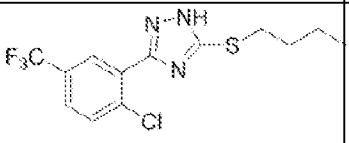
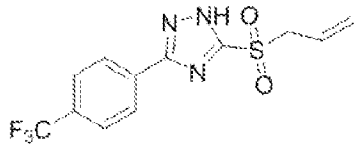
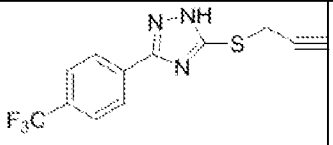
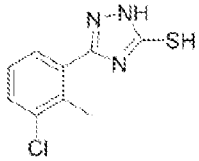
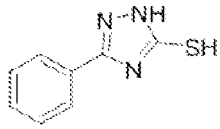
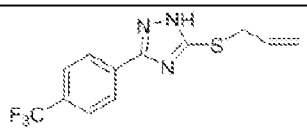
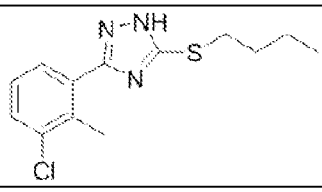
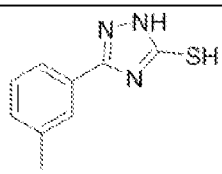
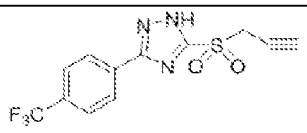
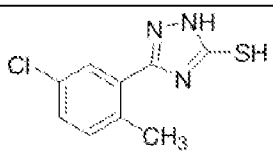
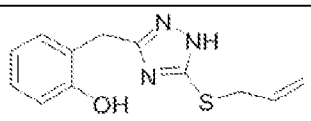
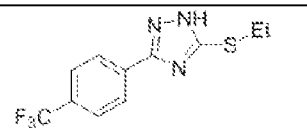
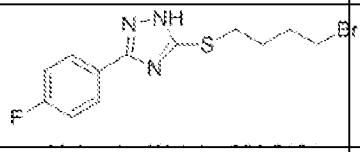
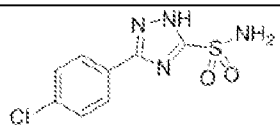
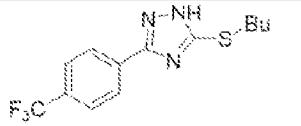
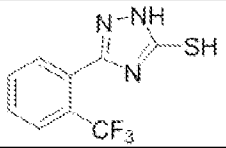
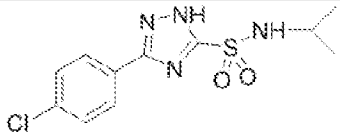
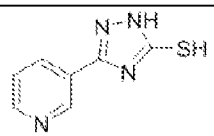
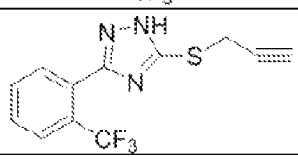
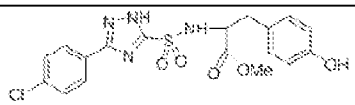
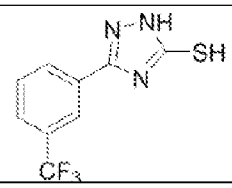
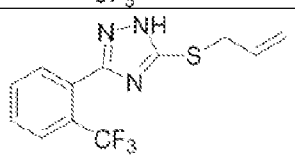
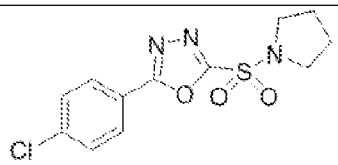
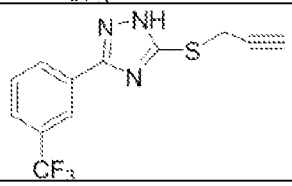
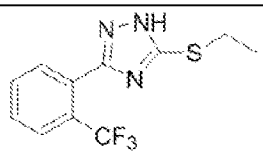
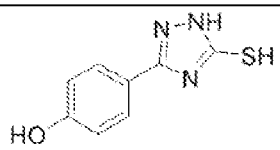
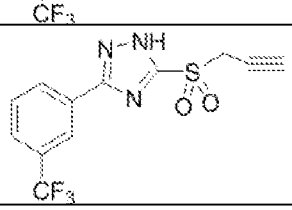
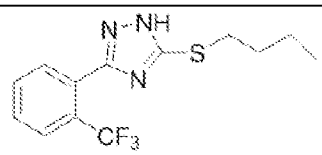
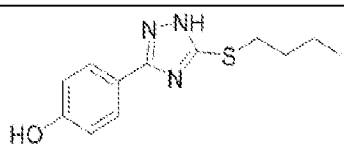
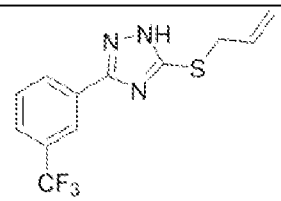
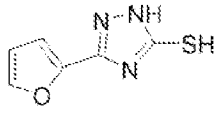
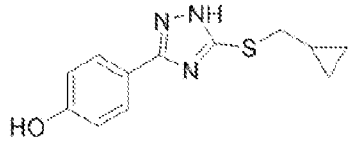
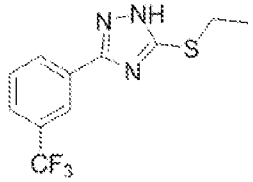
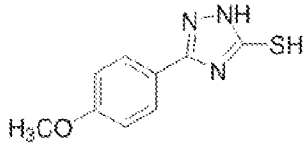
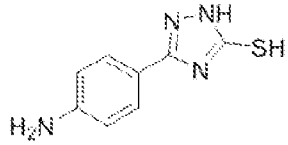
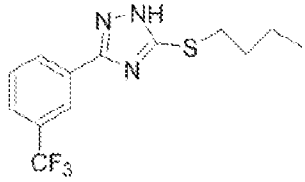
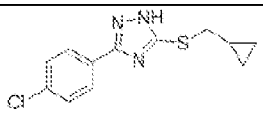
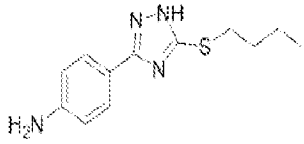
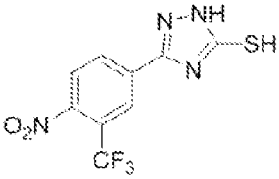
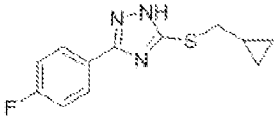
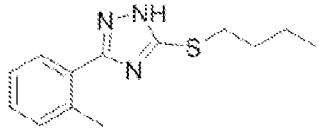
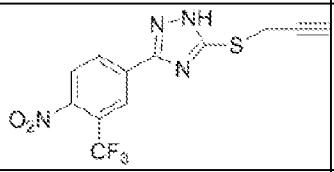
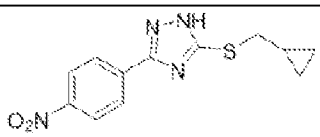
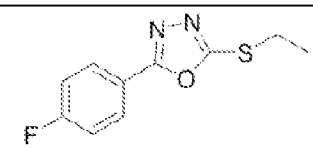
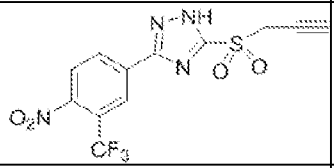
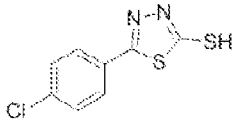
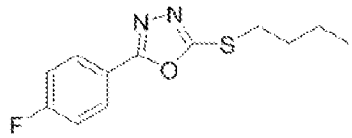
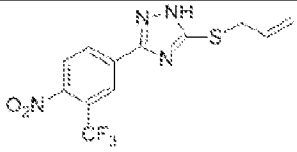
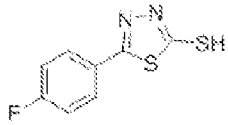
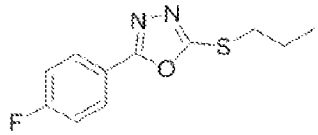
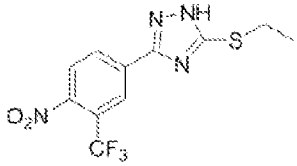
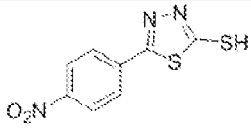
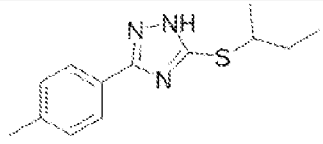
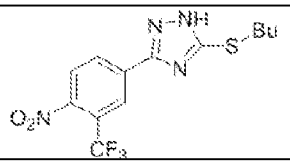
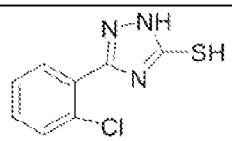
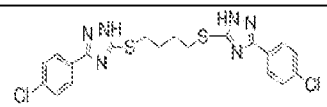
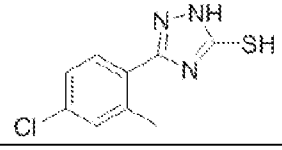
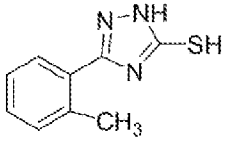
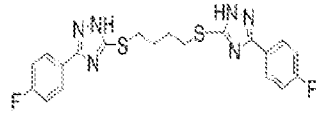
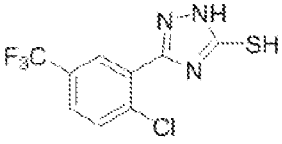
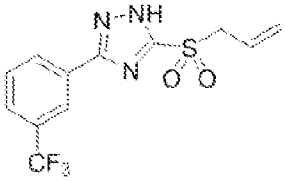
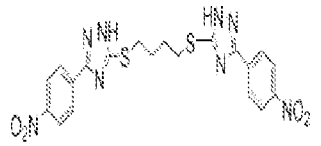
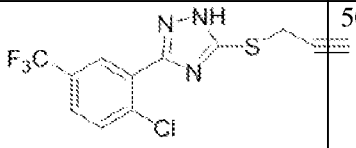
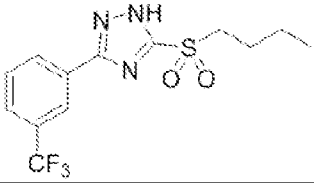
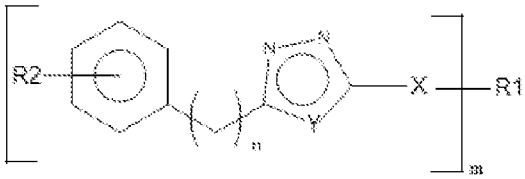
1		26		51	
2		27		52	
3		28		53	
4		29		54	
5		30		55	

6		31		56	
7		32		57	
8		33		58	
9		34		59	
10		35		60	
11		36		61	
12		37		62	
13		38		63	

14		39		64	
		40		65	
16		41		66	
17		42	 Molecular Weight: 249.3074	67	
18		43		68	
19		44		69	
20		45		70	
21		46		71	

22		47		72	
23		48		73	
24		49		74	
25		50			

[033] The present invention provides a process for preparation of compound of formula (I) or its acid addition salts,



wherein;

X is S or SO_2; n is 0, 1;m=1; Y is NH; R2 independently represents H or alkyl or halo selected from -Cl, -F; or -CF3, or -OH or $–NH_2$ or $–NO_2$, R1 independently represents H or C1 to C5 straight or branched chain alkyl, alkenyl, alkynyl or a group $–(CH_2)_5Br$, and the said process comprising the steps of:

a. refluxing the mixture of hydrazine hydrate and (un)substituted or substituted aromatic ester (III) to separate hydrazide solid (IV),
b. slurrying hydrazide (IV) in a mixture of ethanolic potassium hydroxide and carbon disulfide at ambient temperature to obtain compound (V);

c. treating compound (V) with 25% ammonia solution followed by acidification to produce corresponding triazolethiol (VI);

d. alkylating triazolethiol (VI) to obtain alkylated triazolethiols (VII); and

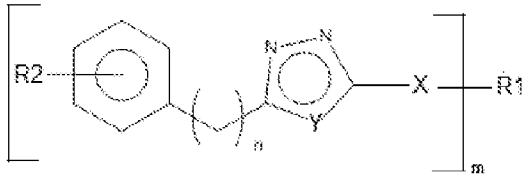
e. adding cold solution of oxone dissolved in a mixture of THF and water in the ratio 2:1 to alkylated triazolethiols (VII) to obtain sulfones (VIII).

[034] The compound triazolethiol (VI) can optionally be prepared by a process comprising:

i. refluxing mixture of substituted benzoic acid (XIII) and thionyl chloride followed by addition of thiosemicarbazide in pyridine to obtain crude residue (XV);

ii. dissolving the crude residue (XV) in 10% aq. KOH followed by neutralizing with 10% aq. HCl to yield 1, 2, 4-triazole 5-thiol (VI).

[035] The present invention provides a process for preparation of compound of Formula (I) or its acid addition salts



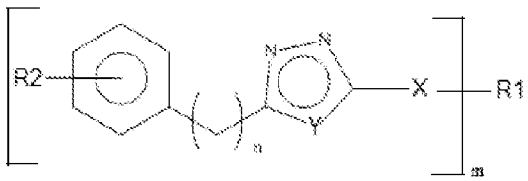
Wherein X is S; n is 0, 1;m=1; Y is S; R2 independently is selected from H, alkyl, -Cl, -F, -CF_3, -OH or $-NO_2$; R1 is H; and the said process comprising the steps of:

a. refluxing the mixture of hydrazine hydrate and (un)substituted or substituted aromatic ester (III) to separate hydrazide solid (IV),

b. slurrying hydrazide (IV) in a mixture of ethanolic potassium hydroxide and carbon disulfide at ambient temperature to obtain compound (V); and

c. refluxing compound of formula (V) with a mixture of ethanolic potassium hydroxide and carbon disulfide and acidifying with conc. H_2SO_4.

[036] The present invention provides a process for preparation of compound of Formula (I) or its acid addition salts,



wherein;

X is S or SO2; n is 0, 1; m=1; Y is O; R2 independently is selected from H, alkyl, -Cl, -F, -CF_3, -OH or $-NO_2$; R1 independently is selected from straight or branched C1-C4 alkyl, comprising the steps of:

(a) refluxing the mixture of hydrazine hydrate and (un)substituted or substituted aromatic ester (III) to separate hydrazide solid (IV);

(b) slurrying hydrazide (IV) in a mixture of ethanolic potassium hydroxide and carbon disulfide at ambient temperature followed by acidification with 10% HCl solution to obtain 1,3,4-oxadiazolethiol (IX);

(c) alkylating 1,3,4-oxadiazolethiol (IX) using a suitable alkylating agent in presence of a solvent and base to obtain alkylated compound (X); and

(d) adding cold solution of oxone dissolved in a mixture of THF and water in the ratio 2:1 to alkylated compound (X) to obtain sulfones (XI).

[037] In another aspect, the present invention provides a process for preparation of compounds of Formula (II) comprising;

i. refluxing the mixture of hydrazine hydrate and substituted aromatic ester (III) to separate hydrazide solid (IV);

ii. slurrying hydrazide (IV) in a mixture of ethanolic potassium hydroxide and carbon disulfide at ambient temperature to obtain compound (V);

iii. treating compound (V) with 25% ammonia solution followed by acidification to produce corresponding triazolethiol (VI); and

iv. alkylating triazolethiol (VI) with a suitable alkylating agent to obtain compounds of formula (II).

[038] In yet another aspect, the present invention provides a pharmaceutical composition comprising at least one of the novel five membered heterocyclic compounds of Formula (I) as an active ingredient along with pharmaceutically acceptable excipients, being present in sufficient amount, to be effective against both active and dormant phase bacilli of Mycobacterium tuberculi for preventing the proliferation of HeLa cancer cell lines and for treatment of a disease or disorder associated with GroEL1/GroEL2 activity.

[039] In another aspect the present invention provides a method for treating the subject suffering from tuberculosis, cancer and disorders associated with GroEL1/GroEL2 activity comprising administering pharmaceutical composition comprising novel five membered

heterocyclic compounds of Formula (I) or its acid addition salts thereof either alone or in combination as active ingredient along with pharmaceutically acceptable excipients in therapeutically effective amount.

[040] The present invention discloses compound of Formula I which exhibit potential activity against dormant pathogenic tuberculi bacilli. The compounds of the present invention can be formulated into a pharmaceutical composition for treatment of a disease or disorder associated with GroEL1/GroEL2 activity.

[041] The compounds of Formula (I) exhibit anti-tubercular and antiproliferative activities. The compounds of the present invention are active against both active and dormant phase of *Mycobacterium tuberculi.*

[042] The tuberculi strains comprise of two Mycobacterium strains viz. *Mycobacterium smegmatis* (ATCC700084), *Mycobacterium bovis* BCG (ATCC35743). For antiproliferative activity, strains were selected from two Gram-negative bacteria: *Pseudomonas fluorescens* (ATCC13525), *Escherichia coli* (ATCC25292) and two Gram positive bacteria: *Staphylococcus aureus* (ATCC29213) and *Bacillus subtilis* (ATCC23857).

[043] The compounds of Formula (I) exhibit anti-tubercular activities against *Mycobacterium tuberculi* H37Ra in dormant phase at minimum inhibitory concentration (MIC) values ranging from 0.11 μg/ml to 30 μg/ml, while at the active state minimum inhibitory concentration (MIC) values ranged from 2.93 μg/ml to 30 μg/ml. The dormant state IC_{50} values ranged from <0.03 μg/ml to 26.54 μg/ml, while as the active state IC_{50} values ranged from <0.03 μg/ml to 30 μg/ml.

[044] Screening of compounds of Formula (I) for anti-proliferative activity was assessed on a panel of four human cancer cell lines at a single concentration of 100 μg/ml. The dose dependent inhibition was further performed at the concentration of 100, 50, 25, 12.5, 6.25, 3.125, 1.5625 and 0.7813 μg/ml to determine the IC_{50} and MIC values. The four human cell lines used were representative of tumors from four types of human tissue including blood, lung, pancreas and cervix tissues. Results are shown in **Table (3)** below. The compounds 6 to 10 significantly prevented the proliferation of HeLa cancer cell lines showing IC_{50}<15.00μg/ml.

[045] The compounds of Formula (I) were selective on THP-1, A549, Panc-1 and HeLa Cells against both active and dormant stage *Mycobacterium tuberculosis* H37Ra as indicated in **Table (4)** below.

[046] The present invention further relates to a pharmaceutical composition of the active ingredient selected from compounds of Formula (I) or formula (II) or their pharmaceutically acceptable salts along with pharmaceutically acceptable excipients. The pharmaceutical composition according to the invention can be in the form of a solid, for example, powders, granules, tablets, capsules or can be present in the liquid form such as solutions, emulsions, suspensions etc or as an injectable composition.

[047] The compounds of the present invention exhibit high solubility and can be formulated as oral drugs in aqueous solutions.

[048] In another aspect, the compounds of formula (I) or formula (II) or their pharmaceutically acceptable salts can be formulated into a pharmaceutical composition along with pharmaceutically acceptable excipients to selectively act against actively growing as well as dormant bacilli of *Mycobacterium Bovis* BCG and *Mycobacterium Tuberculosis* H37Ra.

[049] In another aspect, the compounds of formula (I) or formula (II) or their pharmaceutically acceptable salts can be formulated in to a pharmaceutical composition along with pharmaceutically acceptable excipients for treatment of a disease or disorder associated with GroEL1/GroEL2 activity.

[050] The invention also provides methods for the treatment of the disorder discussed above. The derivatives of Formula I and pharmaceutical compositions containing them may, according to the invention, be administered using any amount, any form of pharmaceutical composition and any route of administration effective for the treatment. After formulation with an appropriate pharmaceutically acceptable carrier in a desired dosage, as known by those of skill in the art, the pharmaceutical compositions of this invention can be administered by any means that delivers the active pharmaceutical ingredient (s) to the site of the body whereby it can exert a therapeutic effect on the patient.

[051] The invention discloses use of compounds of Formula (I) or their pharmaceutically acceptable salts to selectively act against actively growing as well as dormant bacilli of *Mycobacterium Bovis* BCG and *Mycobacterium Tuberculi* H37Ra.

[052] The invention discloses use of compounds of Formula (I) or their pharmaceutically acceptable salts for treatment of a disease or disorder associated with GroEL1/GroEL2 activity.

[053] EXAMPLES

Following examples are given by way of illustration and therefore should not be construed to limit the scope of the invention.

General procedure (A): Method 1: Preparation of 1,2,4- triazole 5-thiols (VI)

To the (un)substituted or substituted aromatic ester (III), hydrazine hydrate was slowly added. The mixture was refluxed for about 5 hrs, when solid hydrazide (IV) separated out. The excess reagent was distilled off. One part of the Hydrazide was slurried in ethanolic potassium hydroxide; carbon disulfide was added drop wise and the reaction mixture was allowed to stir for 24 hr at ambient temperature. After solvent evaporation, the residue was used for next step. To the crude residue, 25 % ammonia solution was added and refluxed for about 10 hrs. The solid mixture was further treated with 10% HCl to produce the corresponding triazolethiol (VI). The process is depicted in steps (a), (b) and (c) in **Scheme 1**.

Method 2

To the cold solution of a substituted benzoic acid (XIII) in dry DCM catalytic amount of DMF, thionylchloride was added; the reaction mixture was refluxed for about 4 hrs. The crude residue of the acid chloride (XIV) obtained after concentration was slowly added to a solution of thiosemicarbazide in pyridine at 0°C over a period of about 10 min and allowed to stir at ambient temperature for about 16 hrs. Pyridine was removed under reduced pressure. The crude residue (XV) was dissolved in 10% aq. KOH and the resulting mixture was allowed to stir at 100°C for about 3 hrs. The reaction mixture was cooled to 0°C and neutralized with 10% aq. HCl. The solid was filtered and dried to get 1, 2, 4-triazole 5-thiol (VI). The process is illustrated in **Scheme 1**, steps (i), (j) and (k).

By using the above procedure the representative 1, 2, 4-triazole 5-thiol compounds 1,2,3,4, 5,6, 8,9, 10, 11,12, 14, 15, 16, 17, 18, 20, 21,22, 23, 24, 25, 27, 28, 29,30, 31, 32, 34, 39, 40, 44, 45, 46, 47, 48, 50 to 53, 55, 56, 57, 62, 65, 67 and 71 were synthesized.

General procedure (B): Alkylation of thiols

Aliphatic straight or branched chain alkyl, alkenyl or alkynyl halide was added to a solution of thiol (VI) in ethanol at ambient temperature and allowed to stir for about 12 hrs. The reaction

mixture was concentrated and partitioned in an organic solvent selected from polar aprotic solvent such as ethyl acetate, acetone, THF, acetonitrile, DMF. The alkylated product thus obtained was purified by column chromatography.

The process step is depicted by step (d) in **Scheme 1**.

By using the above procedure the representative compounds viz., 5, 6, 8, 9, 12, 14, 15, 16, 18, 20, 21, 22, 25, 27, 28, 29, 31, 33, 35, 36, 37,38, 41 to 43, 51, 52, 53, 57, 63, 64, 66, 67, 71, 73 and 74 were synthesized.

General procedure (C): Preparation of alkylated 1,3,4- oxadiazole 2-thiol (X)

To the (un)substituted or substituted aromatic ester (III), hydrazine hydrate was slowly added. The mixture was refluxed for about 5 hrs, when solid hydrazide (IV) separated out. The excess reagent was distilled off. To the slurry of hydrazide in ethanolic potassium hydroxide, carbon disulfide was added drop wise and the reaction mixture was allowed to stir for 24 hrs at ambient temperature. After completion of reaction, the solvent was evaporated. The reaction mixture was acidified with 10% HCl solution. The precipitate was filtered, washed with water and dried to give 1,3,4-oxadiazolethiol (IX).

Further, to a stirred solution of oxadiazolethiol (IX) in acetone was added sodium bicarbonate and alkyl bromide. The mixture was stirred for about 4 hrs. Acetone was evaporated and the reaction mixture was partitioned in ethyl acetate, concentrated and the crude residue was purified by column chromatography to offer a title compound. The process step is depicted in steps (a), (f) and (g) in **Scheme 1**. By using the above procedure the representative compounds viz., 68, 69, 70 were synthesized.

General procedure (D): Preparation of sulfone (VIII and XI)

To the cold solution of oxone (in THF: Water in 2:1 ratio at 0°C), alkylated thiol of formula (VII or X) was added and allowed to stir for 12 hrs at ambient temperature. At the end, the reaction mixture was extracted with ethyl acetate, and was concentrated to offer title compound.

The process steps include step (e) as shown in **Scheme 1**. By using the above procedure the representative compounds viz. 7, 13, 19, 26, 49, 50, 54 were synthesized.

General procedure (E): Preparation of 1,3,4-thiadiazole 3-thiol (XII)

To a solution of (un)substituted or substituted aromatic ester (III), hydrazine hydrate was slowly added. The mixture was refluxed for about 5 hrs until completion of reaction. The

solid product was formed and the excess solvent was removed. To the slurry of hydrazide (IV) in ethanolic potassium hydroxide, carbon disulfide was added slowly and the reaction mixture was refluxed for about 10 hrs. After completion of reaction, solvent was evaporated. The reaction mixture was acidified with conc.H_2SO_4. The precipitate was filtered, washed with water and dried to obtain (XII). The process step is depicted in steps (a), (b) and (f) in scheme 1. By using the above procedure the representative compounds 44, 45 and 46 were prepared.

General procedure (F): Preparation of sulfonamide (XIX)

Thiol VI was stirred in a mixture of dichloromethane and 1M HCl for 10 min at -10°C. Cold sodium hypochlorite was added dropwise with very rapid stirring, maintaining the internal temperature at -10°C.The mixture was stirred for about 15 min at -10°C. After the addition was complete, the reaction mixture was transferred to a separating funnel and the dichloromethane layer was rapidly separated, cooled to -15°C. Required amine was added with stirring, the flask was moved to an ice bath, the suspension was stirred for 20 min at 0°C.The mixture was extracted with dichloromethane and concentrated to yield the product. The process step for preparation of sulfonamide is shown in **Scheme 2**. The compounds 58, 59, 60, 61 were prepared by representative method E. Scheme 2.

[054] Example 1: General procedure (A)

Synthesis of 3-(pyridin-3-yl)-1H-1,2,4-triazole-5-thiol (10)

To the ester III (Ar = 3- pyridyl) (5g, 29.4mmole), hydrazine hydrate (5.2ml, 102.9mmole) was added drop wise. The mixture was refluxed for 5 hrs until completion of reaction. The solid hydrazide (IV) was formed and the excess reagent was removed.

To the slurry of hydrazide (5gm, 36.5mmole),in ethanolic potassium hydroxide (2.45gm, 43.8mmole) carbon disulfide (2.7ml, 43.8mmole) was added drop wise and the reaction mixture was allowed to stir for 24 hrs at ambient temperature. After completion of reaction solvent was evaporated and the residue was used for next step.

To the crude residue 25 % ammonia solution (25 ml) was added and refluxed for 10 hrs .The reaction mixture was acidified with 10% HCl solution. The precipitate was filtered, washed with water and dried.

^{1}H NMR (DMSO-d_6):- δ9.01(s,1H),8.77(dd,1H),8.24(dd,1H),7.62(dd,1H)

mp: 229°C

[055] Example 2: General procedure (B)

Propargyl bromide (0.087g, 1.12mmol) was added to a solution of 3-(pyridin-3-yl)-1H-1,2,4-triazole-5-th (0.2g ,1.12mmol) in ethanol at ambient temperature and stirred for 12 hrs. The reaction mixture was concentrated, extracted with ethyl acetate. The organic layer was washed with brine solution, dried over Na_2SO_4, and concentrated to yield crude product which was purified by column chromatography.

^1H NMR ($CDCl_3$):- δ9.23(s,1H),8.77(dd,1H),8.30(dd,1H),7.48(dd,1H),4.09(d,2H),2.36(t,1H). mp- 185°C

[056] Example 3. General procedure (C)

Synthesisof 3-(furan-2-yl)-1H-1,2,4-triazole-5-thiol (39)

^1H NMR (DMSO-d_6):-δ13.88(bs,1H),13.68(bs,1H),7.87(d,1H),7.10(d,1H),6.67(dd,1H). mp:-285°C.

[057] Example 4. General procedure (B)

Synthesis of 3-(4-chlorophenyl)-5-((cyclopropylmethyl)thio)-4H-1,2,4-triazole (41)

^1H NMR ($CDCl_3$):-δ 7.94(d,2H),7.38(d,2H),3.15(d,2H),1.17(m,1H),0.61(d,2H),0.30(d,2H). mp:- 134°C

[058] Example:-5 General procedure (B)

Synthesis of 4-((5-(allylthio)-1H-1,2,4-triazol-3-yl)methyl)phenol (51)

^1H NMR ($CDCl_3$):-δ 7.02(d,2H),6.71(d,2H),5.84(m,1H),5.06(dd,2H),3.92(s,2H),3.63(d,2H). mp:-138°C

[059] Example:- 6 General procedure (B)

Synthesis of 4-((5-(prop-2-yn-1-ylthio)-1H-1,2,4-triazol-3-yl)methyl)phenol (52)

^1H NMR ($CDCl_3$):-δ 7.01(d,2H),6.69(d,2H),3.93(s,2H),3.75(d,2H),2.21(t,1H). mp:- 161°C

[060] Example:- 7 General procedure(D)

Synthesis of 5-(allylsulfonyl)-3-(4-(trifluoromethyl)phenyl)-1H-1,2,4-triazole (54)

^1H NMR (CD_3OD):-δ 8.11(d,2H),7.74(d,2H),5.82(m,1H),5.36(dd,2H),4.12(d,2H). mp:- 207°C

[061] Example:- 8 General procedure (F)

Synthesis of 5-(4-chlorophenyl)-4H-1,2,4-triazole-3-sulfonamide. (59)

5-(4-chlorophenyl)-4H-1,2,4-triazole-3-thiol(0.2 g, 0.94mmol) was stirred in a mixture of 5 ml of DCM and 5 ml of 1 M HCl for 10 min at -10°C.Cold sodium hypochlorite(4% solution ,5.40 ml, 3.31 mmol) was added dropwise with very rapid stirring ,maintaining the internal

temperature at -10°C.The mixture was stirred for 15 min at -10°C after the addition was complete, the mixture was transferred to a separating funnel and the DCM layer was rapidly separated and collected in a clean flask cooled to -15°C. Isopropyl amine (0.194 ml, 2.36 mmol, 2.5 eq) was added with stirring , the flask was removed to an ice bath, the suspension was stirred for 20 min at 0°C.The mixture was extracted with DCM, dried over Na_2SO_4, and concentrated to yield product.

^{1}H NMR (CD_3OD):- 7.75(d, 2H),7.33(d,2H). mp:176°C.

[062] Example:- 9 General procedure (F)

Synthesis of 5-(4-chlorophenyl)-N-isopropyl-4H-1,2,4-triazole-3-sulfonamide. (61)

^{1}H NMR (CD_3OD):- 7.69(d,2H),7.36(d,2H),6.17(bs,1H),4.25(m,1H),1.24(d,6H). mp:132°C.

[063] Example :-10 General procedure (E)

Synthesis of 5-(4-chlorophenyl)-1,3,4-thiadiazole-2-thiol (44)

A solution of the 4-chloro benzoic acid XIII (5g, 32.05mmole) a catalytic amount of H_2SO_4 and 25 mL anhydrous methanol was refluxed for 4 hrs. After the reaction was complete, the excess alcohol was removed to obtain ester compound.

To a solution of ester (5g, 29.4mmole), hydrazine hydrate (5.2ml, 102.9mmole, 3.5eq) was added dropwise. The mixture was refluxed for 5 hr, after completion of reaction the solid product (hydrazide) was formed and the excess solvent was removed under reduced pressure.

To the slurry of hydrazide (5gm, 29.30mmole) in ethanolic potassium hydroxide (1.97gm, 35.16mmole), carbon disulfide (2.14ml, 35.16mmole) was added slowly followed by reflux for 10 hrs. After completion of reaction solvent was evaporated. The reaction mixture was acidified with conc.H_2SO_4. The precipitate was filtered, washed with water and dried. By using the above procedure the representative compounds viz., 45 ,46 etc were prepared.

[064] Example 11: Anti-tubercular assay

Mycobacterial growth conditions

M. tuberculosis (MTB) H37Ra (ATCC 25177) strain was tested for their susceptibility to five membered heterocycles. *M. tuberculosis* (MTB) H37Ra (ATCC 25177) were grown to logarithmic phase (O.D. 1.0) in a M. phlei medium. The stock culture was maintained at -80^{0}C and sub-cultured once in M. phlei medium before inoculation into experimental culture.

IC_{50} and MIC determination

The anti-tubercular screening of compound library against M.tuberculosis H37Ra was carried out by following a known method disclosed by Khan A. and Sarkar D. (2008) J Microbiological Methods. Five membered heterocyclic compounds of formula I of the present invention were solubilized in 100% dimethyl sulfoxide (DMSO) and stored in aliquots at -20^{0}C. One third serial dilutions were prepared in DMSO in amounts of 100 µl per well in 96-well plate. The above-mentioned five membered heterocycles solutions (2.5 µl) were added to each well of plate in a total volume of 250 µl of M. phlei medium consisting of the *M. tuberculosis*. The final concentration of samples ranged from 0.03-30 µg/ml. The medium without the agents was used as a growth control and the blank control used contained only the medium. Rifampicin and Isoniazid served as the standard drug controls. The microtiter plates were allowed to incubate for 8 and 12 days at 37^{0}C. XTT sodium salt powder (Sigma) was freshly prepared as a 1.25 mM stock solution in sterile 1X PBS. Menadione (Sigma) was prepared as a 6 mM solution in DMSO and was used immediately. Five membered heterocyclic compounds of formula (I) were screened for their inhibitory effect on MTB according to XTT Reduction Menadione Assay (XRMA) protocol. The XRMA was then carried out to estimate viable cells present in different wells of the assay plate. To all well 200 µM XTT was added and incubated at 37^{0}C for another 20 min. It was followed by addition of 60 µM of Menadione and incubated at 37^{0}C for 40 min. The optical density was measured using a microplate reader (Spectramaxplus 384 plate reader, Molecular Devices Inc.) at 470 nm filter against a blank prepared from well free of cells. Absorbance obtained from cells treated with vehicle alone was considered as 100% cell growth. All experiments were performed in triplicates and the quantitative value was expressed as the average ± standard deviation and IC_{50} values were calculated from their dose response curves.The MIC was defined as the lowest concentration of the anti-tubercular agents that prevented visible growth with respect to the growth control.

[065] Anti-bacterial assays

Microbial growth conditions

A total of six microbial strains were tested obtained from the American Type Culture Collection for their susceptibility to five membered heterocycles. These strains comprised of two *Mycobacterium* strains: *Mycobacterium smegmatis* (ATCC700084), *Mycobacterium bovis BCG* (ATCC35743); two Gram-negative bacteria: Pseudomonas fluorescens (ATCC13525),

Escherichia coli (ATCC25292) and two Gram positive bacteria: Staphylococcus aureus (ATCC29213) and Bacillus subtilis (ATCC23857).

Mycobacterium smegmatis strains were grown in a defined *M. phlei* medium containing 0.5 gm KH_2PO_4, 0.2 gm sodium citrate, 60 mg $MgSO_4$, 0.5 gm aspargine and 2 ml glycerol in 100 ml of distilled water at pH 6.6.*M.bovis BCG* (ATCC 35755) was grown in Dubos medium containing 50 mM of Sodium Nitrate was purchased from DIFCO, USA. Other bacterial strains *E. coli, P. aerogenosa* as gram-negative and *B. subtillus, S. aureus* as gram-positive were obtained from NCIM (NCL, Pune) and were grown in Luria Burtony medium from Himedia, India.

The stock culture was maintained at -80°C and subcultured once in a liquid medium before inoculation to an experimental culture.

[066] MIC determination

In order to determine the antimicrobial activity of the synthesized five membered heterocycles compounds of formula (I), two gram positive, two Gram-negative bacteria and two Mycobacterium strains other than tuberculosis were screened using the one third serial dilution technique. All biological results of the compounds are given in **Table 1**.

Mycobacterium bovis BCG (ATCC35743)

The anti-tubercular screening of compound library against M.bovis BCG was carried out by following the known method (Singh U. et al. J. Microbiol Methods, 2011, 84(2), 202-7).

For identifying inhibitors against actively growing bacilli, the incubation was terminated on 8th day of incubation at 37°C to measure the viable cell counts. Briefly, 80 µl of culture from incubated 96 well plate was transferred into another 96 well plate, then added 80 µl of 1% Sulfanilic acid in 20% of conc. HCl; incubated for 10 min at room temperature followed by addition of 80µl of 0.1 % NEDD solution in D/W. Finally, the optical density of the suspension was measured at 540 nm by using a micro plate reader.

For NR assay against hypoxia induced dormant culture, the plates were taken out on the 12th day of incubation to measure the viable cell counts. As mentioned earlier, NR assay method was repeated.

Mycobacterium smegmatis (ATCC700084)

The anti-mycobacterial screening of compound library against M.smegmatis was carried out by following an earlier method (Khan A. and Sarkar D. (2008) J Microbiological Methods).

For XRMA, Optical Density of the culture was measured after 72hrs of incubation before the addition of XTT at 470 nm. 200 µM XTT was added and incubated for 20 min at 37°C after shaking for 1 min. After 20 min incubation, 60 µM menadione was added and mixed for 1 min and then incubated at 37°C for another 20 min. The optical density was measured at 470 nm by using a micro plate reader. For Hypoxia induced XTT reduction microplate assay (HXRMA) on the 7th day of incubation, the plates were taken out and the seal was removed. A similar protocol was repeated as mentioned for aerobically grown *M. smegmatis.*

Other bacteria: ***Pseudomonas fluorescens (ATCC13525), Escherichia coli (ATCC25292), Staphylococcus aureus (ATCC29213) and Bacillus subtilis (ATCC23857)***

The screening of compound library against those above bacteria was carried out by inoculating 0.1% of $1OD_{620nm}$culture along with the compounds in 96 well plate and incubate at 37°C within an incubator for 8hrs (for *P. fluorescence* and *E.coli*) or 12hrs (for *B.subtilis* and *S.aureus*) and then read the absorbance (Cell OD) at 620 nm to monitor cell growth.

[067] Cytotoxic activity assay

Cell lines (THP-1, A549, Panc-1 and HeLa) and treatment

The effect of the five membered heterocyclic compounds of formula (I) on cell growth was determined in a panel of human tumor cells including lung A549 adenocarcinoma, pancreatic PANC-1 adinocarcinoma, cervix adenocarcinoma HeLa, and acute monocytic leukemia cell line THP-1, obtained from The European Collection of Cell Cultures (ECACC), Salisbury, UK. THP-1 was maintained in RPMI 1640 without phenol red, A-549 and Panc-1 cells were maintained in Dulbecco's Modified Eagle Medium (DMEM) whileHeLa was cultured in Eagle's Minimum Essential Medium (EMEM). All media used were supplemented with 10% fetal bovine serum (FBS; Gibco 10082-139), 0.68 µL/mL Gentamicin. The cell lines were maintained under standard cell culture conditions at 37°C and 5% CO_2 in a humidified environment.

Cytotoxic activity by MTT assay

In vitro cytotoxicity against above mentioned four human cancer cell lines was determined using a standard MTT assay as described previously, a widely adopted method of measuring cellular proliferation(G. Ciapetti, E. Cenni, L. Pratelli, A. Pizzoferrato, and Biomaterials 1993, 14, 359-364; Mosmann, T. J. Immunol. Meth., 1983, 65, 55-63).

The cells were harvested in log phase using trypsin (0.05% trypsin, 0.02% EDTA, in PBS). The cell suspensions were diluted with appropriate growth medium to obtain the cell densities depending on the cell line: (10^4 cells/well for HeLa, 10^4 cells/well for A549 and Panc-1, 10^4 cells/well for THP-1).Cell counts were performed by hemocytometry before addition of cells to 96-well plates. An aliquot of 100 µl of each suspension were seeded in 96 wells cell culture plates. The cells were incubated at37°C in an atmosphere of 5% CO2 and 95% relative humidity in a CO_2 incubator. After 24 h incubation, five membered heterocycles (1µl/well) at varying concentrations (100, 50, 25, 12.5, 6.25, 3.13, 1.56 and 0.781 µg/ml) were added to the wells containing cells. Paclitaxel 0.1374 and 5.814µg/ml was used as positive control. Suitable controls with equivalent concentration of DMSO were also included. The plates were further incubated for 48h in a CO_2 incubator after addition of test material.

At the end of the incubation period, the solution containing the unattached cells was discarded and each well containing the test samples was washed thrice with 1ml of PBS. This was followed by addition of 0.01 ml MTT (5 mg/ml in phosphate-buffered saline) to 0.1ml cells in growth medium. After 4h at 37°C for MTT cleavage, the formazan product was solubilized by the addition of 0.1ml 0.04 N HC1 in isopropanol. Optical density was measured on a SPECTRAmax PLUS 384 plate reader, using a reference wavelength of 630 nm and a test wavelength of 570 nm. The MTT assay was completed on both controls and the samples. All experiments were performed in triplicate, and the quantitative value was expressed as the average ± standard deviation.

Growth inhibition was calculated by subtracting mean OD values of respective blank from the mean OD value of experimental set. Percentage growth in presence of test material was calculated considering the growth in absence of any test material as 100% and in turn percentage growth inhibition in presence of test material was calculated. The viability and growth in the presence of test material is calculated by following formula.

% cytotoxicity = (Average of Control-Average of Compound)/ (Average of Control-Average of Blank) X 100)

Where Control is culture medium with cells and DMSO and blank is culture medium without cells.

IC_{50} and MIC value was calculated by plotting the percentage survival versus the concentrations, using OriginPro Software. For all samples, each compound concentration was

tested in triplicates in a single experiment. IC_{50} is the concentration of sample required to inhibit 50% of the cell proliferation and MIC is the concentration of sample required to inhibit 90% of the cell proliferation.

[068] Solubility

The aqueous solubility of selected different compounds was measured using the Millipore protocol as shown in **Figure 1**. Briefly, the Universal Aqueous Buffer solution, pH 7.4, was filtered through a 0.22 μmStericup™ filter unit to remove any particulates, and stored at 4° C. Dispensed 190 μL/well of pH 7.4 buffer at room temperature into a MultiScreen Solubility filter plate with a multi-channel pipettor. Dispensed 10 μL/well of stock compound in triplicate (normally at 10 mM in DMSO, from a 96-well polypropylene, V-bottomed plate), directly into the buffer in the Multiscreen Solubility filter plate with a multi-channel pipettor. The final concentration of test compounds were 100, 200, 500, 1000, 1500, 2000, 2500, 3000, 3500μM. The Multiscreen Solubility filter plate was covered with a lid and mixed with gentle shaking (100 – 300 rpm) at room temperature for 1.5 hours. Parallely, the standard buffer consisting of a solution of 80:20 buffer:acetonitrile (AcN) was prepared to ensure overall compound solubility. Dispensed 192 μL/well of room temperature, pH 7.4, buffer: acetonitrile solution into a UV analysis plate with a multi-channel pipettor. Dispensed 8 μL/well of stock compound directly into the buffer in the UV/Vis analysis plate with a multi-channel pipettor. Covered the standard plate with a lid and mixed with gentle shaking (100 – 300 rpm) at room temperature for 10 minutes. After mixing, read the standard plate with a UV spectrometer plate reader at seven wavelengths: 260, 280, 300, 320, 340, 360, and 800 nm. After mixing the Multiscreen Solubility filter plate for 1.5 hours, vacuum filtered the solutions into a clean polypropylene, 96-well, V-bottomed, collection plate on a vacuum manifold with grid at 10 – 12" Hg. Filtration by vacuum requires that there is liquid in all 96 wells of the Multiscreen Solubility filter plate. After filtration, transferred 160 μL/well of filtrate into a clean UV/Vis analysis plate and diluted with 40 μL/well of acetonitrile. Covered the filtrate plate with a lid, and then mixed with gentle shaking (100 – 300 rpm) at room temperature for 10 minutes. After mixing, read the filtrate plate with a UV/Vis spectrometer plate reader at seven wavelengths: 260, 280, 300, 320, 340, 360, and 800 nm.

[069] Data Collection and Analysis

Data were collected using a Molecular Devices SPECTRAmax® Plus microplate spectrometer. The ratio of filtrate vs. standard absorbance was calculated to quantify the aqueous solubility using the formula below.

$$IF: \frac{(\sum AUat\ 260,280,300,320,340,360nm)-(AUat800nm)Filtrate}{(\sum AUat260,280,300,320,340,360)-(AUat800nm)Standard} \approx 1.0$$

Then Aqueous Solubility ≥ 500 µM

$$IF: \frac{(\sum AUat\ 260,280,300,320,340,360nm)-(AUat800nm)Filtrate}{(\sum AUat260,280,300,320,340,360)-(AUat800nm)Standard} \leq 0.5$$

Then Aqueous Solubility ≤ 100 µM

$$IF: \frac{(\sum AUat\ 260,280,300,320,340,360nm)-(AUat800nm)Filtrate}{(\sum AUat260,280,300,320,340,360)-(AUat800nm)Standard} < 1.0 \text{ and } > 5.0$$

100 µM< Aqueous Solubility < 500 µM

In the present study, the inventors evaluated the anti-microbial and anti-proliferative activity against cell lines with synthetic five membered heterocycles of formula (I) and (II). In a preliminary screening, the anti-tubercular (**Table 1**) and antimicrobial activity (**Table 2**) of five membered heterocycles was assessed at the concentrations of 30, 10, 3 µg/ml, then dose-dependent inhibition was further performed at the concentrations of 100, 30, 10, 3, 1, 0.3, 0.1 and 0.03 to determine IC_{50} and MIC of potential actives (**Table 2**).In order to determine the anti-microbial activity of the synthesized compounds seventy four against two Gram positive, two Gram-negative bacteria and three *Mycobacterium* species were screened. Results of the compounds are given in **Table 2.**

Anti-tubercular activity

All the five membered heterocycles tested in this study exhibited anti-tubercular activities against dormant and active phages of *Mycobacterium tuberculosis* H37Ra.The dormant state minimum inhibitory concentration (MIC) values ranged from 0.11 µg/ml to 30 µg/ml, while as the active state minimum inhibitory concentration (MIC) values ranged from 2.93 µg/ml to 30 µg/ml. The dormant state IC50 values ranged from <0.03 µg/ml to 26.54 µg/ml, while as the active state IC_{50} values ranged from <0.03 µg/ml to 30 µg/ml.

Table. 1 MIC and IC_{50} results for anti-tubercular activity of five membered heterocycles of formula (I) and formula (II) against *Mycobacterium tuberculosis* H37Ra

		Antitubercular activity of heterocycles against *Mycobacterium tuberculosis* H37Ra(µg/ml)							
	Dormant	Active	Dormant	Active	Compound ID	Dormant	Active	Dormant	Active
Compound ID	IC_{50}	MIC	IC_{50}	MIC		IC_{50}	MIC	IC_{50}	MIC
72	0.08	9.12	0.31	3.03	(35)	18.23	>30	>30	>30
(1)	<0.03	28.89	<0.03	13.09	(36)	23.14	>30	>30	>30
(2)	<0.03	0.64	<0.03	9.05	(37)	26.25	>30	>30	>30
(3)	<0.03	3.00	<0.03	15.09	(38)	1.56	>30	28.68	>30
(4)	0.39	4.48	2.69	>30	(39)	0.29	NA	28.94	>30
(5)	0.40	10.00	1.93	30.00	(40)	NA	NA	>30	>30
(6)	0.75	16.91	2.27	26.05	(41)	0.12	8.97	0.90	29.04
(7)	0.85	>30	0.73	14.97	(42)	0.12	NA	28.95	>30
(8)	8.45	>30	3.71	14.03	(43)	0.12	15.62	0.75	29.89
(9)	9.12	>30	6.96	>30	(44)	0.14	NA	>30	>30
(10)	2.75	>30	1.64	22.12	(45)	0.10	NA	7.79	>30
(11)	0.35	13.54	1.98	>30	(46)	0.09	10.88	5.63	>30
(12)	2.89	>30	4.54	>30	(47)	<0.03	>30	>30	>30
(13)	0.63	21.90	1.19	>30	(48)	0.35	23.61	13.29	>30
(14)	0.35	5.34	0.81	29.70	(71)	<0.03	5.49	0.35	21.19
(15)	2.20	13.77	3.25	>30	(71)	19.04	27.85	ND	ND
(16)	0.98	>30	12.35	>30	(49)	34.11	>100	ND	ND
(17)	0.65	10.45	24.54	>30	(50)	8.25	48.71	ND	ND
(18)	1.07	6.28	1.25	>30	(51)	>100	>100	ND	ND
(19)	2.81	8.05	13.25	>30	(52)	>100	>100	ND	ND
(20)	1.81	6.41	2.03	21.32	(53)	>100	>100	ND	ND
(21)	8.80	>30	28.32	>30	(54)	>100	>100	ND	ND
(22)	10.12	>30	17.54	>30	(55)	20.73	29.44	ND	ND
(23)	3.07	12.45	2.13	>30	(56)	0.68	2.47	ND	ND
(24)	0.43	1.93	0.66	20.69	(57)	>100	>100	ND	ND
(25)	8.79	>30	6.58	>30	(58)	>100	>100	ND	ND
(26)	1.21	16.19	3.21	>30	(59)	2.57	24.06	ND	ND
(27)	>30	>30	>30	>30	(60)	61.20	>100	ND	ND
(28)	>30	>30	>30	>30	(61)	45.69	>100	ND	ND
(29)	>30	>30	>30	>30	(62)	17.18	68.64	ND	ND
(30)	>30	>30	8.25	>30	(63)	<0.03	0.44	ND	ND
(31)	8.45	>30	>30	>30	(64)	<0.03	0.58	ND	ND
(32)	1.74	>30	9.21	>30	(65)	0.12	0.46	ND	ND
(68)	0.64	5.74	0.80	19.71	(66)	3.91	12.81	ND	ND
(69)	1.04	16.89	2.98	22.88	(67)	3.45	14.80	ND	ND
(33)	22.12	>30	21.21	>30	Rifampicin	0.0014	0.043	0.0018	0.044
(73)	0.62	1.53	25.63	>30	Isoniazid	0.0023	0.075	0.0019	0.074
(74)	17.14	>30	>30	>30					
(34)	24.25	>30	26.38	>30					

5

ND: Not determined, Dormant state: A reversible state of bacterial metabolic shutdown. (a b) Standard anti-tubercular drugs and positive controls.

The five membered heterocycles including (72) , (2), (3), (4), , (11), (14), (18), (19), (20), (23), , (24), (68), (73), (41), , (46), (71), (56), (63) (64), (65), and (66) showed the highest anti-tubercular activity (both MIC and IC_{50}<14 μg/ml) against dormant phage of *Mycobacterium tuberculosis* H37Ra. Other five membered heterocycles also had significant anti-tubercular activity (both MIC and IC_{50}) against dormant and active phages. Therefore, the overall anti-tubercular activity exhibited in this study varied from weak to significant level.

Although 20 five membered heterocycles including (2), (3), (4), , (24) , (73), (56), (63), (64) and (65) showed activity having an MIC value of <5 μg/ml against dormant phage of *Mycobacterium tuberculosis* H37Ra, they exhibited lower potencies compared to Rifampicin and Isoniazid as control drugs.

Total of 4 five membered heterocycles including (2), (63), (64) (65), were found to be more active than the others at an MIC value of <0.9 μg/ml against dormant phage of *Mycobacterium tuberculosis* H37Ra. As these five membered heterocycles of formula (I) and formula (II) are efficacious against intracellular *M. tuberculosis*, they can be used as potential anti-tubercular drugs.

[070] Anti-bacterial activity

Table. 2. Anti-bacterial activity of five membered heterocycles (μg/ml)

Nos:	*S.aureus*					*B.subtillus*					*E. coli*					*M.smegmatis*					*P.flurescence*				
	Concentration (µg/ml)																								
	100	30	10	3	1	100	30	10	3	1	100	30	10	3	1	100	30	10	3	1	100	30	10	3	1
72	29.46	25.04	32.90	31.62	28.57	64.79	52.28	44.40	39.12	38.28	5.67	-7.62	-1.15	-8.25	-4.90	23.45	20.91	9.88	9.11	29.92	93.74	97.12	98.44	95.81	40.55
(1)	96.30	49.76	17.69	15.71	6.00	62.83	57.58	47.35	45.24	54.84	6.80	-0.66	-4.70	-7.21	-11.11	85.71	31.89	37.39	-106.77	22.05	72.76	3.95	35.26	25.25	52.66
(2)	84.71	17.45	13.26	10.73	4.37	59.99	58.57	57.33	48.13	47.89	-8.45	-1.28	-1.97	-3.70	-5.31	95.54	67.46	44.14	-104.51	8.66	-1.44	50.17	-13.07	-6.42	42.69
(3)	99.00	41.43	18.42	13.12	15.67	97.11	77.93	65.12	46.44	57.72	13.26	-2.74	-8.92	-5.13	-1.48	98.68	78.73	65.96	57.29	53.19	99.48	96.58	25.94	40.21	7.79
(4)	99.24	87.97	30.75	27.21	22.12	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	67.79	98.40	99.66	21.23	24.66
(5)	91.58	34.92	31.92	24.88	19.89	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	99.57	85.84	32.53	10.15	35.54
(6)	98.30	72.42	33.01	31.60	22.61	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	-11.77	21.24	5.22	-21.01	-50.79
(7)	99.46	15.39	14.94	14.66	18.40	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	98.95	92.83	55.74	23.86	8.00
(8)	99.32	18.29	12.11	13.27	17.04	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	99.51	24.51	8.36	44.11	-33.03
(9)	94.	98	98	47	24	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	99.	66	37	27	51

	58	.20	.54	.01	.05																	70	.72	.69	.14	.67
(10)	65.18	48.10	17.78	12.97	17.93	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	11.81	-0.08	-5.31	23.09	17.75
(11)	99.10	98.23	92.12	48.75	24.07	101.20	38.96	29.73	15.98	14.59	-9.57	-13.24	5.70	6.42	10.87	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
(12)	101.20	38.96	29.73	15.98	24.59	102.24	79.55	95.51	-6.78	-4.31	35.05	16.62	10.05	2.70	-2.62	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
(13)	102.24	99.55	95.51	-6.78	-4.31	100.24	98.94	48.41	20.99	17.23	13.98	-7.42	-2.52	1.33	-0.34	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
(14)	100.24	98.94	48.41	20.99	17.23	100.72	74.21	42.13	25.61	37.44	35.73	18.33	12.80	8.27	7.58	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
(15)	100.72	74.21	42.13	25.61	37.44	98.23	99.71	99.93	42.86	39.26	37.94	17.11	10.78	2.70	1.79	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
(16)	100.23	99.71	99.93	42.86	39.26	35.63	24.44	22.90	26.33	28.33	-10.78	-9.09	-1.93	1.30	-0.16	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
(17)	35.63	24.44	22.90	26.33	28.33	100.19	32.65	31.64	25.30	20.58	35.38	15.45	13.97	9.30	8.36	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
(18)	100.19	32.65	31.64	25.30	20.58	99.62	49.21	24.15	20.62	23.27	15.53	1.74	4.89	5.97	6.71	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
(19)	99.62	49.21	24.15	20.62	23.27	100.41	99.75	37.53	30.44	30.20	2.02	4.80	5.01	3.60	-9.53	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
(20	10	99	37	30	30	10	99	21	18	20	9.	11	5.	3.	-1	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

)	0.41	.75	.53	.44	.20	0.52	.14	.75	.44	.03	76	.10	23	09	7.29										
(21)	100.52	99.14	21.75	18.44	20.03	109.22	99.22	99.04	99.85	33.74	12.09	10.24	3.15	6.82	3.46	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
(22)	109.22	99.22	99.04	99.85	33.74	98.73	26.90	19.35	16.11	15.47	-31.23	4.18	6.64	5.21	2.85	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
(23)	-1.63	16.35	12.94	14.62	9.89	45.75	-36.90	-50.20	-46.56	ND	-3.68	-2.80	-0.84	1.52	ND	71.64	ND	ND	ND	ND	99.95	ND	ND	ND	ND
(24)	34.41	62.40	51.52	27.66	17.44	96.32	-0.32	2.85	-5.23	ND	-3.58	-2.67	1.72	1.82	ND	82.33	ND	ND	ND	ND	122.35	ND	ND	ND	ND
(25)	32.05	-10.21	-7.28	11.77	18.90	98.83	3.17	-5.23	2.85	ND	-5.63	3.16	2.77	1.62	ND	96.26	ND	ND	ND	ND	100.98	ND	ND	ND	ND
(26)	95.63	97.70	58.42	48.61	44.53	99.10	98.34	-18.84	-22.09	ND	-4.02	-1.75	-0.90	-0.64	ND	36.79	ND	ND	ND	ND	103.49	ND	ND	ND	ND
(27)	85.10	31.09	31.21	36.48	31.13	101.25	85.99	10.13	-10.69	ND	1.47	2.84	2.57	0.93	ND	90.99	ND	ND	ND	ND	104.77	ND	ND	ND	ND
(28)	93.75	42.71	39.99	35.45	20.35	97.18	12.67	1.27	-5.70	ND	3.03	2.77	10.71	-0.44	ND	99.80	ND	ND	ND	ND	101.38	ND	ND	ND	ND
(29)	101.61	18.33	27.94	25.50	17.77	98.85	98.55	26.56	24.95	ND	3.51	-4.50	-0.23	0.78	ND	101.56	ND	ND	ND	ND	89.16	ND	ND	ND	ND
(30)	-5.0	5.4	14.	8.4	2.9	0.61	-3.	11.	15.	ND	9.5	-11	-8.	-4.	ND	90.5	ND	ND	ND	ND	66.5	ND	ND	ND	ND

	2	3	28	6	2		98	02	40		8	.03	73	79		6					6				
(31)	97.21	36.12	32.90	29.43	26.27	98.61	98.52	33.14	18.03	ND	9.92	-4.40	-1.33	-1.96	ND	94.96	ND	ND	ND	ND	124.47	ND	ND	ND	ND
(32)	1.25	28.94	33.75	32.86	18.56	25.42	22.19	35.44	15.56	ND	-5.41	-5.66	-2.52	-5.94	ND	97.81	ND	ND	ND	ND	96.49	ND	ND	ND	ND
(68)	34.44	95.19	81.45	58.48	42.06	19.75	-3.16	-4.23	-0.91	ND	-3.10	1.94	3.33	0.62	ND	98.13	ND	ND	ND	ND	84.72	ND	ND	ND	ND
(69)	-4.78	55.24	44.25	59.65	34.61	27.57	-3.62	-4.76	-2.63	ND	10.57	3.14	7.19	6.59	ND	76.33	ND	ND	ND	ND	18.77	ND	ND	ND	ND
(33)	38.73	-17.70	16.33	24.60	24.31	22.30	40.84	43.88	30.27	ND	4.62	1.66	4.56	3.91	ND	42.95	ND	ND	ND	ND	65.07	ND	ND	ND	ND
(73)	102.84	97.94	80.91	12.85	9.20	101.62	52.29	24.96	7.09	ND	10.35	0.69	1.66	2.66	ND	93.00	ND	ND	ND	ND	98.58	ND	ND	ND	ND
(74)	44.79	-19.93	2.19	9.77	2.81	33.71	0.47	11.93	-5.36	ND	4.80	5.65	4.88	7.68	ND	98.11	ND	ND	ND	ND	25.35	ND	ND	ND	ND
(34)	36.79	-8.50	19.55	11.69	22.12	21.61	25.89	33.70	27.29	ND	-6.13	0.42	-3.46	4.85	ND	62.57	ND	ND	ND	ND	28.29	ND	ND	ND	ND
(35)	39.40	-12.03	16.54	7.84	28.98	38.45	35.59	36.48	29.29	ND	22.01	-2.75	-4.69	3.59	ND	54.74	ND	ND	ND	ND	53.82	ND	ND	ND	ND
(36)	37.91	-6.4	17.5	19.0	22.6	33.57	34.2	42.9	12.0	ND	-6.0	-0.8	1.00	5.24	ND	61.50	ND	ND	ND	ND	78.97	ND	ND	ND	ND

		6	1	8	1		5	4	8		9	1												
(37)	27.37	-0.42	21.67	21.25	32.56	32.50	25.89	40.83	35.14	ND	-2.72	-0.55	2.43	4.76	ND	30.83	ND	ND	ND	ND	24.62	ND	ND	ND	ND
(38)	99.80	-40.14	5.53	28.51	31.52	99.50	38.82	41.27	20.10	ND	-1.75	0.81	3.66	1.36	ND	102.29	ND	ND	ND	ND	93.89	ND	ND	ND	ND
(39)	ND	ND	ND	ND	ND	ND	38.40	40.33	41.52	ND	ND	2.73	-0.34	9.30	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
(40)	ND	ND	ND	ND	ND	ND	31.61	33.98	33.87	ND	ND	1.32	0.30	7.76	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
(41)	ND	ND	ND	ND	ND	ND	96.84	28.83	-0.59	ND	ND	1.73	0.46	-1.68	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
(42)	ND	ND	ND	ND	ND	ND	10.94	7.46	7.78	ND	ND	0.73	-2.49	-1.38	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
(43)	ND	ND	ND	ND	ND	ND	20.20	17.15	15.52	ND	ND	3.47	0.73	2.54	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
(44)	ND	ND	ND	ND	ND	ND	7.78	10.73	2.09	ND	ND	1.06	-0.68	-2.39	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
(45)	ND	ND	ND	ND	ND	ND	38.62	24.83	8.09	ND	ND	0.46	-1.01	2.74	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
(46)	ND	ND	ND	ND	ND	ND	18.73	31.68	22.46	ND	ND	7.09	6.95	5.15	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
(47)	ND	ND	ND	ND	ND	ND	22.7	21.1	5.83	ND	ND	-1.2	-2.0	1.65	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

							1	3				7	0											
(48)	ND	ND	ND	ND	ND	ND	40.56	36.92	34.97	ND	ND	-2.52	3.72	5.60	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
971)	ND	ND	ND	ND	ND	ND	4.01	11.78	7.47	ND	ND	1.16	3.85	4.81	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
71	ND	ND	-8.7	ND	ND	ND	ND	50.2	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	52.3	ND	ND
(49)	ND	ND	20.9	ND	ND	ND	ND	8.6	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	10.7	ND	ND
(50)	ND	ND	29.7	ND	ND	ND	ND	58.2	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	17.1	ND	ND
(51)	ND	ND		ND	ND	ND	ND		ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND		ND	ND
(52)	ND	ND	8.3	ND	ND	ND	ND	-10.6	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	15.1	ND	ND
(53)	ND	ND	6.8	ND	ND	ND	ND	55.9	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	25.5	ND	ND
(54)	ND	ND	-1.5	ND	ND	ND	ND	45.5	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	27.4	ND	ND
(55)	ND	ND	ND	ND	ND	ND	ND		ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
(56)	ND	ND	99.6	ND	ND	ND	ND	99.7	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	101.8	ND	ND
(57)	ND	ND	2.7	ND	ND	ND	ND	34.6	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	17.0	ND	ND
(58)	ND	ND	-5.6	ND	ND	ND	ND	24.8	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	13.2	ND	ND
(59)	ND	ND	6.	ND	ND	ND	ND	27	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	25	ND	ND

			5					.9																.7		
(60)	ND	ND	2.8	ND	ND	ND	ND	30.4	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	23.4	ND	ND
(61)	ND	ND	-9.8	ND	ND	ND	ND	-31.2	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	11.2	ND	ND
(62)	ND	ND	2.4	ND	ND	ND	ND	26.8	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	13.1	ND	ND
(63)	ND	ND	26.3	ND	ND	ND	ND	33.3	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	30.7	ND	ND
(64)	ND	ND	0.6	ND	ND	ND	ND	31.0	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	6.1	ND	ND
(65)	ND	ND	11.0	ND	ND	ND	ND	25.9	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	4.3	ND	ND
(66)	ND	ND	2.4	ND	ND	ND	ND	21.5	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	20.0	ND	ND
(67)	ND	ND	-24.2	ND	ND	ND	ND	-1.2	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	10.1	ND	ND
a Rifampicin	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
b Isoniazid	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
c Paclitaxel	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Testosterone	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

Furosemide	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

[071] Anti proliferative activity

In initial screening, the anti-proliferative activity of five membered heterocycles of formula (I) and formula (II) was assessed on a panel of four human cancer cell lines at a single concentration of 100 µg/ml; then dose dependent inhibition was further performed at the concentration of 100, 50, 25, 12.5, 6.25, 3.125, 1.5625 and 0.7813 µg/ml to determine the IC_{50} and MIC (**Table 3**). The four human cell lines used were representative of tumors from a four types of human tissue including blood, lung, pancreasand cervix tissues. Results are shown in **table 3**.Table3, where IC_{50} and IC_{90} values of the five membered heterocycles of formula (I) and formula (II) and paclitaxel against human cancer cell lines were calculated. the results given in the table-3 revealed that all the compounds of formula (I) and formula (II) tested shows no significant cytotoxicity up to 100 µg/ml against human cell lines. The anti-tubercular activities against dormant phages of *Mycobacterium tuberculosis* H37Ra and non-toxic properties as well as the selectivity index in THP-1, A549 and Panc-1 cell lines of (2), (63), (64), (65) and prove that these compounds can be used as potential dormant state inhibitors.

Table 3 shows IC_{50} values of the five membered heterocycles of formula (I) and paclitaxel against human cancer cell lines

Compound	**Cytotoxic activity of heterocycles of formula (I) and (II) against human cancer cell lines (THP-1, A549, Panc-1 and HeLa) (ug/ml)**			
	THP-1 (Leukemia)	**A549 (Lung)**	**Panc-1 (Pancreas)**	**HeLa (Cervix)**
	IC_{50}	**IC_{50}**	**IC_{50}**	**IC_{50}**
(1)	>100	43.41	>100	ND
(2)	38.83	>100	>100	40.29
(3)	69.74	>100	>100	33.82
(4)	65.68	81.35	>100	25.92
(5)	49.55	73.21	>100	ND
(11)	>100	44.86	>100	ND
(12)	>100	>100	>100	13.78
(17)	57.40	52.72	>100	ND
(18)	>100	>100	>100	6.08
(19)	>100	>100	>100	>100
(20)	>100	>100	>100	23.22

(23)	98.12	>100	>100	ND
(24)	55.89	>100	>100	ND
(30)	76.44	>100	>100	ND
(32)	90.61	>100	>100	ND
(68)	>100	>100	>100	14.42
(33)	>100	>100	>100	24.79
(34)	38.07	>100	>100	ND
(39)	>100	>100	>100	>50
(41)	>100	>100	>100	5.80
(42)	>100	>100	>100	6.99
(43)	>100	>100	>100	8.50
(44)	>100	>100	>100	13.48
(45)	>100	>100	>100	7.15
(46)	>100	>100	>100	65.30
(48)	>100	>100	>100	18.96
(71)	>100	>100	>100	19.92
(56)	>100	>100	>100	21.11
(59)	>100	>100	>100	>100
(63)	>100	>100	>100	23.56
(64)	>100	>100	>100	31.17
(65)	>100	>100	>100	>100
(67)	>100	>100	>100	12.80
[a]Rifampicin	>100	>100	>100	>100
[b]Isoniazid	>100	>100	>100	>100
[c]Paclitaxel	4.18	1.12	12.73	0.0048

(a b) Standard antitubercular drugs and positive controls.(c) Standard anticancer drugs and positive controls. Data are expressed as the means of triplication. IC_{50}: The concentration of sample required to inhibit 50% of the cell proliferation

Table4. Selectivity Index of five membered heterocycles on THP-1, A549, Panc-1 and HeLa Cells against Active Stage and Dormant stage Mycobacterium *tuberculosis* H37Ra

COMPOUND ID	THP-1 (Leukemia)		A549 (Lung)		Panc-1 (Pancreas)		HeLa (Cervix)	
	SI against Dormant phage	SI against Active Phage	SI against Dormant phage	SI against Active Phage	SI against Dormant phage	SI against Active Phage	SI against Dormant phage	SI against Active Phage
(1)	3333.33	3333.33	1447.00	1447.00	3333.33	3333.33	ND	ND
(2)	1294.33	1294.33	3333.33	3333.33	3333.33	3333.33	3333.33	3333.33
(3)	2324.67	2324.67	3333.33	3333.33	3333.33	3333.33	3333.33	3333.33
(4)	168.41	24.42	208.59	30.24	256.41	37.17	37.17	256.41
(10)	18.02	30.21	26.62	44.64	36.36	60.98	ND	ND
(11)	285.71	50.51	128.17	22.66	285.71	50.51	ND	ND

(14)	285.71	123.15	285.71	123.15	285.71	123.15	44.46	103.14
(17)	88.31	2.34	81.11	2.15	153.85	4.07	ND	ND
(18)	93.46	80.00	93.46	80.00	93.46	80.00	48.92	57.15
(19)	35.59	7.55	35.59	7.55	35.59	7.55	7.55	35.59
(20)	55.25	49.26	55.25	49.26	55.25	49.26	38.88	43.61
(23)	31.96	46.07	32.57	46.95	32.57	46.95	ND	ND
(24)	129.98	84.68	232.56	151.52	232.56	151.52	ND	ND
(30)	2.55	9.27	3.33	12.12	3.33	12.12	ND	ND
(32)	52.01	9.84	57.41	10.86	57.41	10.86	ND	ND
(68)	157.48	125.00	157.48	125.00	157.48	125.00	125.00	157.48
(73)	161.29	3.90	161.29	3.90	161.29	3.90	3.90	161.29
(34)	1.57	1.44	4.12	3.79	4.12	3.79	ND	ND
(39)	350.88	3.46	350.88	3.46	350.88	3.46	ND	ND
(41)	840.34	110.99	840.34	110.99	840.34	110.99	16.18	122.52
(42)	840.34	3.45	840.34	3.45	840.34	3.45	0.92	223.36
(43)	847.46	134.23	847.46	134.23	847.46	134.23	56.19	354.75
(44)	714.29	3.33	714.29	3.33	714.29	3.33	3.33	714.29
(45)	1034.13	12.84	1034.13	12.84	1034.13	12.84	12.84	1034.13
(46)	1118.57	17.77	1118.57	17.77	1118.57	17.77	17.77	1118.57
(48)	285.71	7.52	285.71	7.52	285.71	7.52	7.52	285.71
(71)	3333.33	285.71	3333.33	285.71	3333.33	285.71	156.54	1826.33
(56)	147.06	ND	147.06	ND	147.06	ND	ND	147.06
(59)	38.91	ND	38.91	ND	38.91	ND	ND	38.91
(63)	3333.33	ND	3333.33	ND	3333.33	ND	ND	1404.67
(64)	3333.33	ND	3333.33	ND	3333.33	ND	ND	2972.00
(65)	833.33	ND	833.33	ND	833.33	ND	ND	833.33
(67)	28.99	ND	28.99	ND	28.99	ND	ND	7.99
Rifampicin	71428.57	55555.56	71428.57	55555.56	71428.57	55555.56	55555.56	71428.57
Isoniazid	43478.26	52631.58	43478.26	52631.58	43478.26	52631.58	52631.58	43478.26

[072] Solubility

As determining compound solubility in water has become an essential early measurement in the drug discovery process, the aqueous solubility of five membered heterocycles of formula (I) and formula (II) were tested along with Testosterone, Furosemide as the reference compounds. The solubility of (1) (2), (3), , (4) was in the range 500 µM to 1280 µM, solubility of 24 was in the range 1280 µM to 3200 µM, while solubility of Testosterone, Furosemide were 365 µM and 500 µM respectively. The solubility results prove that these synthetic five membered heterocycles of formula (I) and formula (II) are highly soluble for oral drugs in aqueous solution. The solubility values were quite reproducible.

Table5. Solubility results

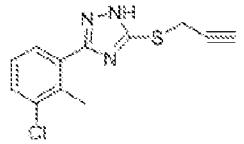
Compound ID	Aqueous Solubility		
	Screening Ratio (SR)	Approximate Solubility (µM) from SR	Solubility from Quantitative Method (µM)
-1	>1.0	>500	>500 to 1280
-2	>1.0	>500	>500 to 1280
-3	>1.0	>500	>500 to 1280
-4	>1.0	>500	>500 to 1280
24	>1.0	>500	>1280 to 3200
Testosterone	0.75	<500	365
Furosemide	0.98	500	500

[073] Computational Studies

Three dimensional Quantitative Structure-Activity relationship (3D-QSAR)study

In order to study and deduce the correlation between structure and biological activity of these five membered heterocyclic compounds, a 3D-QSAR study based on Comparative Molecular Field Analysis (CoMFA) was carried out with the QSAR module integrated in Sybyl 7.1 (Tripos Inc., USA) software. For this, a dataset of 126 compounds (001-126) was segregated into a training set for generating 3D-QSAR models and a test set for validating the models. This was done on the basis of chemical and biological diversity using similarity search techniques viz. D-optimal design, Tanimoto similarity coefficient, and the Euclidian distance matrix criteria. The selection of the training and test sets was carried out such that the molecules in the test set had structural diversityand a range of biological activities similar to that of the training set.

The results of the CoMFA and CoMSIA studies were visualized as 3D 'coefficient contour maps' contoured in terms of contribution (Figure 2a and 2b). One of the most active compounds 90A has been used to demonstrate the areas where a change in the molecular structure may affect its activity. These contour maps are important tools in drug design, as they show regions in 3D space where modifications of steric and electrostatic fields strongly correlate with concomitant changes in biological activity.



Molecular Weight: 263.7430

(90A)

The structural features identified in the 3D-QSAR analysis have been strategically utilized in the design of novel molecules with improved activity (Table 1). Modifications were made to the core 5 membered heterocyclic scaffold as recommended by the CoMFAisopleths. It is noteworthy that the activities of these new molecules are higher than most of the molecules used to build the 3D-QSAR model(**Table 1).** This indicates that the 3D-QSAR model derived in the present investigation is powerful enough to suggest improvement in the existing dataset.

In-silico druggability predictions

A significant number of drug candidates fail in clinical trials owing to their poor ADME (absorption, distribution, metabolism, and excretion) and toxicity related properties.These late-stage failures contribute significantly to the expense incurred on new drug discovery campaign. Therefore, ability to detect problematic candidates in the early phase of drug discovery will significantly reduce the risk of late-stage attrition and the amount of time and resources being wasted on molecules that are doomed to fail and it can also focus lead optimization efforts to arrive at a candidate with the desired ADME and low toxicity properties. With this aim, *in silico* ADME and toxicity predictions were carried out for the molecules of the present invention to gauge their drug-like characteristics. QSAR Toolbox (Version 3.2), software intended to be used by governments, the chemical industry and other research community to fill gaps in toxicity data needed for assessing the hazards of chemicals, was used to carry out these predictions. The properties that were predicted for the compounds discussed herein include: alerts for DNA binding, estrogen receptor binding; bioaccumulation metabolism alerts, alerts for AMES test and mutagenicity. QikProp, (Schrödinger, LLC., New York)integrated in the Schrodinger molecular modeling suite, which is an *in silico* adsorption, distribution, metabolism and excretion (ADME) prediction program was used to carried out the ADME related prediction.**Lipinski's rule of five,** considered as a rule of thumb to gauge drug likeness or determine if a chemical with a certain pharmacological activity has the properties that would make it a potential orally active drug in humans, was also applied to all

molecules investigated in this study. The rule describes molecular properties essential for a drug's pharmacokinetic behavior in the human body, including their absorption, distribution, metabolism and excretion ("ADME"). The rule is important in drug discovery campaign when a pharmacologically active lead structure is optimized step-by-step to increase the activity and selectivity of the compound as well as to ensure drug-like physicochemical properties are maintained as described by Lipinski's rule.Candidate drugs that conform to the **Lipinski's rule of five** have a propensity to have lower attrition rates during clinical trials and therefore have an increased chance of reaching the market. The results for this investigation are also summarized in the supplementary material. The molecules display fairly good oral bioavailability with very low susceptibility to acid hydrolysis in the stomach as reflected from the % Human oral absorption data. Furthermore the predicted apparent Caco-2 cell permeability data accounting for the gut-blood barrier also supports these predictions. Inspection of the partition coefficient values (logPo/w) and prediction of binding to human serum albumin (logKhsa) shows that most of these compounds are hydrophobic in nature. Low values predicted for brain/blood partition coefficient suggest that these molecules will have very low propensity to cross the brain/blood brain barrier thereby eliminating the chances of CNS related toxicity. This is also justified by the predicted apparent MDCK cell permeability data which is considered to be a good mimic for the blood brain barrier. Therefore, the overall *in silico* predictions appear to be favorable for hit/lead optimization.

[074] Summary

Five membered heterocycles of formula (I) and formula (II) with codes (2), (63), (64), (65) and which inhibit the survival of dormant *Mycobacterium tuberculosis* H37Ra (MIC value of <0.9 μg/ml) are highly soluble in aqueous phase. the compounds (2), (3), (4), (24), (73), (56), (63) (64), (65) showed MIC<5ug/ml; the compounds (72), (5), (14), (18), (19), (20), (68), (41), (71), showed MIC >=5 and <10ug/ml while the compounds (11), (15), (17), (23),(46), (66) and (67)) showed MIC >10 and <15 ug/ml against dormant M.Tb. It comes out from this study that these scaffolds have low cytotoxicity in human cancer cell lines, gram +ve and gram –ve bacteria. These prove that five membered heterocycles of formula (I) can be used as potential anti-tubercular inhibitors.These synthetic five membered heterocycles of formula (I) could potentially be pursued for the development of therapeutic agents against the

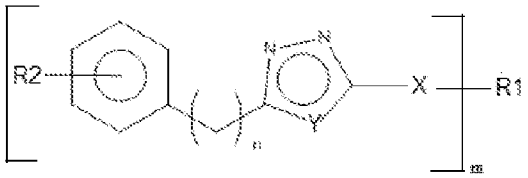
said bacterial infections. These five membered heterocycles of formula (I) are good candidates for further activity-guided fractionation in the search for new active therapeutic compounds. the study reveals that the, (18), , (68), , (41), (42), (43), , (44), (45), and (67) significantly prevent the proliferation of HeLa cancer cell lines showing IC_{50}<15.00µg/ml.

[075] ADVANTAGES OF THE INVENTION

Synthesis of novel five membered heterocycles and their derivatives starting from simple, easily available raw materials having promising antimycobacterial activity specifically in dormant stage.

We claim

1. A compound of formula (I) or its acid addition salts thereof



Formula (I)

wherein,

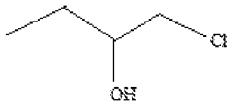
X is S or SO_2;

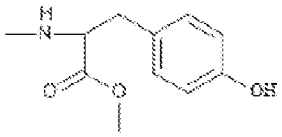
n is 0, 1;

m is 1 or 2;

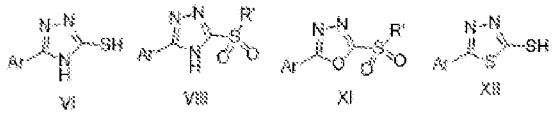
Y is N ,O or S;

R2 independently represents H or alkyl or halo selected from -Cl, -F; or $-CF_3$, or -OH or-NH2 or $-NO_2$;

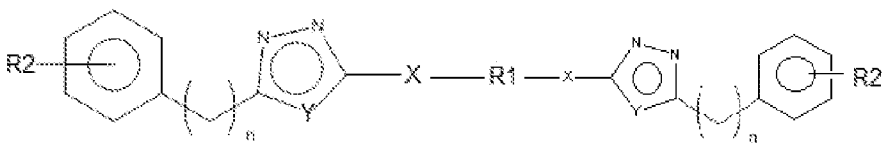
R1 independently represents H or C1 to C5 straight or branched chain alkyl, alkenyl, alkynyl or a group $-(CH_2)5Br$ or pyrrolidine or –NHR' wherein R' is H or isopropyl or  or



2. The compound as claimed in claim 1, wherein representative compound of formula 1 comprising:



, Formula IA



Formula IA

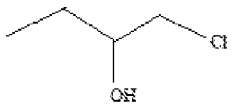
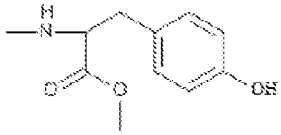
wherein,

X is S or SO_2;

n is 0, 1;

Y is N ,O or S;

R2 independently represents H or alkyl or halo selected from -Cl, -F; or $-CF_3$, or -OH or-NH2 or $-NO_2$;

R1 independently represents H or C1 to C5 straight or branched chain alkyl, alkenyl, alkynyl or a group $-(CH_2)5Br$ or pyrrolidine or –NHR' wherein R' is H or isopropyl or  or  .

or its acid addition salts thereof.

3. The compound as claimed in claim 1 and 2, wherein said compound comprising:

i. 3-(4-chlorophenyl)-1H-1,2,4-triazole-5-thiol **(1);**
ii. 3-(4-nitrophenyl)-1H-1,2,4-triazole-5-thiol **(2);**
iii. 3-(4-fluorophenyl)-1H-1,2,4-triazole-5-thiol **(3);**
iv. 3-(4-(trifluoromethyl) phenyl)-1H-1,2,4-triazole-5-thiol **(4);**
v. 5-(prop-2-yn-1-ylthio)-3-(4-(trifluoromethyl)phenyl)-1H-1,2,4-triazole **(5);**
vi. 5-(allylthio)-3-(4-(trifluoromethyl) phenyl)-1H-1,2,4-triazole **(6);**
vii. 5-(prop-2-yn-1-ylsulfonyl)-3-(4-(trifluoromethyl) phenyl)-1H-1,2,4-triazole **(7);**
viii. 5-(ethylthio)-3-(4-(trifluoromethyl) phenyl)-1H-1,2,4-triazole **(8);**
ix. 5-(butylthio)-3-(4-(trifluoromethyl) phenyl)-1H-1,2,4-triazole**(9);**
x. 3-(pyridin-3-yl)- 1H -1,2,4-triazole**(10);**
xi. 3-(3-(trifluoromethyl)phenyl)-1H-1,2,4-triazole-5-thiol**(11);**
xii. 5-(prop-2-yn-1-ylthio)-3-(3-(trifluoromethyl)phenyl)-1H-1,2,4-triazole **(12);**
xiii. 5-(prop-2-yn-1-ylsulfonyl)-3-(3-(trifluoromethyl) phenyl)-1H-1,2,4-triazole **(13)**;
xiv. 5-(allylthio)-3-(3-(trifluoromethyl) phenyl)-1H-1,2,4-triazole**(14)**;
xv. 5-(ethylthio)-3-(3-(trifluoromethyl) phenyl)-1H-1,2,4-triazole**(15)**;
xvi. 5-(butylthio)-3-(3-(trifluoromethyl) phenyl)-1H-1,2,4-triazole**(16)**;
xvii. 3-(4-nitro-3-(trifluoromethyl)phenyl)-1H-1,2,4-triazole-5-thiol**(17)**;
xviii. 3-(4-nitro-3-(trifluoromethyl)phenyl)-5-(prop-2-yn-1-ylthio)-1H-1,2,4-triazole**(18)**;
xix. 3-(4-nitro-3-(trifluoromethyl)phenyl)-5-(prop-2-yn-1-ylsulfonyl)-1H-1,2,4-triazole**(19)**;
xx. 5-(allylthio)-3-(4-nitro-3-(trifluoromethyl)phenyl)-1H-1,2,4-triazole**(20)**;
xxi. 5-(ethylthio)-3-(4-nitro-3-(trifluoromethyl)phenyl)-1H-1,2,4-triazole**(21)**;
xxii. 5-(butylthio)-3-(4-nitro-3-(trifluoromethyl)phenyl)-1H-1,2,4-triazole**(22)**;
xxiii. 3-(4-chloro-2-methylphenyl)-1H-1,2,4-triazole-5-thiol**(23);**
xxiv. 3-(2-chloro-5-(trifluoromethyl)phenyl)-1H-1,2,4-triazole-5-thiol**(24);**
xxv. 3-(2-chloro-5-(trifluoromethyl)phenyl)-5-(prop-2-yn-1-ylthio)-1H-1,2,4-triazole**(25);**
xxvi. 3-(2-chloro-5-(trifluoromethyl)phenyl)-5-(prop-2-yn-1-ylsulfonyl)-1H-1,2,4-triazole**(26);**
xxvii. 5-(allylthio)-3-(2-chloro-5-(trifluoromethyl)phenyl)-1H-1,2,4-triazole **(27);**

xxviii. 5-(ethylthio)-3-(2-chloro-5-(trifluoromethyl)phenyl)-1H-1,2,4-triazole**(28);**
xxix. 5-(butylthio)-3-(2-chloro-5-(trifluoromethyl)phenyl)-1H-1,2,4-triazole **(29);**
xxx. 3-(3-chloro-2-methylphenyl)-1H-1,2,4-triazole-5-thiol **(30);**
xxxi. 3-(3-chloro-2-methylphenyl)-5-(butylthio)-1H-1,2,4-triazole **(31);**
xxxii. 3-(5-chloro-2-methylphenyl)-1H-1,2,4-triazole-5-thiol **(32);**
xxxiii. 5-((4-bromobutyl)thio)-3-(4-fluorophenyl)-1H-1,2,4-triazole **(33);**
xxxiv. 3-(2-(trifluoromethyl)phenyl)-1H-1,2,4-triazole-5-thiol **(34);**
xxxv. 5-(prop-2-yn-1-ylthio)-3-(2-(trifluoromethyl)phenyl)-1H-1,2,4-triazole **(35);**
xxxvi. 5-(allylthio)-3-(2-(trifluoromethyl)phenyl)-1H-1,2,4-triazole **(36);**
xxxvii. 5-(ethylthio)-3-(2-(trifluoromethyl)phenyl)-1H-1,2,4-triazole **(37);**
xxxviii. 5-(butylthio)-3-(2-(trifluoromethyl)phenyl)-1H-1,2,4-triazole **(38);**
xxxix. 3-(furan-2-yl)-1H-1,2,4-triazole-5-thiol **(39);**
xl. 3-(4-methoxyphenyl)-1H-1,2,4-triazole-5-thiol **(40);**
xli. 3-(4-chlorophenyl)-5-((cyclopropylmethyl)thio)-1H-1,2,4-triazole **(41);**
xlii. 3-(4-fluorophenyl)-5-((cyclopropylmethyl)thio)-1H-1,2,4-triazole **(42);**
xliii. 3-(4-nitrophenyl)-5-((cyclopropylmethyl)thio)-1H-1,2,4-triazole **(43);**
xliv. 5-(4-chlorophenyl)-1,3,4-thiadiazole-2-thiol **(44);**
xlv. 5-(4-fluorophenyl)-1,3,4-thiadiazole-2-thiol **(45);**
xlvi. 5-(4-nitrophenyl)-1,3,4-thiadiazole-2-thiol **(46);**
xlvii. 3-(2-chlorophenyl)-1H-1,2,4-triazole-5-thiol **(47);**
xlviii. 3-(o-tolyl)-1H-1,2,4-triazole-5-thiol **(48);**
xlix. 5-(allylsulfonyl)-3-(3-(trifluoromethyl)phenyl)-1H-1,2,4-triazole **(49);**
l. 5-(butylsulfonyl)-3-(3-(trifluoromethyl)phenyl)-1H-1,2,4-triazole **(50);**
li. 4-((5-(allylthio)-1H-1,2,4-triazol-3-yl)methyl)phenol **(51);**
lii. 4-((5-(prop-2-yn-1-ylthio)-1H-1,2,4-triazol-3-yl)methyl)phenol**(52);**
liii. 2-(5-mercapto-1H-1,2,4-triazol-3-yl)methyl phenol**(53);**
liv. 5-(allylsulfonyl)-3-(4-(trifluoromethyl)phenyl)-1H-1,2,4-triazole **(54);**
lv. 3-phenyl-1H-1,2,4-triazole-5-thiol **(55);**
lvi. 3-(m-tolyl)-1H-1,2,4-triazole-5-thiol **(56);**
lvii. 2-((5-(allylthio)-1H-1,2,4-triazol-3-yl)methyl)phenol**(57);**
lviii. 3-(4-chlorophenyl)-1H-1,2,4-triazole-5-sulfonamide **(58);**
lix. 3-(4-chlorophenyl)-N-isopropyl-1H-1,2,4-triazole-5-sulfonamide**(59);**
lx. methyl ((5-(4-chlorophenyl)-1H-1,2,4-triazol-3-yl)sulfonyl)tyrosinate **(60);**
lxi. 5-(4-chlorophenyl)-3-(pyrrolidin-1-ylsulfonyl)-1H-1,2,4-triazole **(61);**
lxii. 4-(3-mercapto-1H-1,2,4-triazol-5-yl)phenol **(62);**
lxiii. 4-(3-(butylthio)-1H-1,2,4-triazol-5-yl)phenol **(63);**
lxiv. 4-(3-((cyclopropylmethyl)thio)-1H-1,2,4-triazol-5-yl)phenol **(64);**
lxv. 5-(4-aminophenyl)-1H-1,2,4-triazole-3-thiol **(65);**
lxvi. 4-(3-(butylthio)-1H-1,2,4-triazol-5-yl)aniline **(66);**
lxvii. 3-(butylthio)-5-(o-tolyl)-4H-1,2,4-triazole **(67);**
lxviii. 2-(4-fluorophenyl)-5-(ethylthio)-1,3,4-oxadiazole **(68);**
lxix. 2-(4-fluorophenyl)-5-(butylthio)-1,3,4-oxadiazole **(69);**
lxx. 2-(4-fluorophenyl)-5-(propylthio)-1,3,4-oxadiazole **(70)** ;
lxxi. 5-(sec-butylthio)-3-(p-tolyl)-1H-1,2,4-triazole **(71).**
lxxii. 3-(4-chlorophenyl)-5-methyl-1H-1,2,4-triazole compound with 5-((4-(11-sulfanyl)butyl)thio)-3-(4-chlorophenyl)-1H-1,2,4-triazole (1:1) **(72);**
lxxiii. 3-(4-fluorophenyl)-5-methyl-1H-1,2,4-triazole compound with 5-((4-(11-sulfanyl)butyl)thio)-3-(4-fluorophenyl)-1H-1,2,4-triazole (1:1) **(73);**

lxxiv. 3-(4-nitrophenyl)-5-methyl-1H-1,2,4-triazole compound with 5-((4-(l1-sulfanyl)butyl)thio)-3-(4-fluorophenyl)-1H-1,2,4-triazole (1:1) **(74).**

4. A process for the preparation of compound of formula (I) or its acid addition salts according to claim 1 and 2 and the said process comprising the steps of:

a) refluxing a mixture of hydrazine hydrate and (un)substituted or substituted aromatic ester (III) to separate hydrazide solid (IV);

b) slurrying hydrazide (IV) as obtained in step (a) in a mixture of ethanolic potassium hydroxide and carbon disulfide at ambient temperature to obtain compound (V);

c) treating compound (V) as obtained in step (b) with 25% ammonia solution followed by acidification to produce corresponding triazolethiol (VI);

d) alkylating triazolethiol (VI) as obtained in step (c) to obtain alkylated triazolethiols (VII); and

e) adding cold solution of oxone dissolved in a mixture of THF and water in the ratio 2:1 to alkylated triazolethiols (VII) as obtained in step (d) to obtain sulfones (VIII).

f) refluxing compound of formula (V) with a mixture of ethanolic potassium hydroxide and carbon disulfide and acidifying with conc. H2SO4 to obtain (XII).

g) alkylating triazolethiol (VI) with a suitable alkylating agent to obtain compounds of formula (II).

h) refluxing mixture of substituted benzoic acid (XIII) and thionyl chloride followed by addition of thiosemicarbazide in pyridine to obtain crude residue (XV);

i) dissolving the crude residue (XV) in 10% aq. KOH followed by neutralizing with 10% aq. HCl to yield 1, 2, 4-triazole 5-thiol (VI).

j) slurrying hydrazide (IV) in a mixture of ethanolic potassium hydroxide and carbon disulfide at ambient temperature followed by acidification with 10% HCl solution to obtain 1,3,4-oxadiazolethiol (IX);

k) alkylating 1,3,4-oxadiazolethiol (IX) in presence of a solvent and base to obtain alkylated compound (X); and

l) adding cold solution of oxone dissolved in a mixture of THF and water in the ratio 2:1 to alkylated compound (X) to obtain sulfones (XI).

5. The process as claimed in claim 4, wherein triazolethiol (VI) is optionally prepared by a process comprising;

(a) refluxing mixture of substituted benzoic acid (XIII) and thionyl chloride followed by addition of thiosemicarbazide in pyridine to obtain crude residue (XV);

(b) dissolving the crude residue (XV) in 10% aq. KOH followed by neutralizing with 10% aq. HCl to yield 1, 2, 4-triazole 5-thiol (VI).

6. The process as claimed in claim 5, wherein alkylating agent used for alkylation is selected from 3-(4-chlorophenyl)-5-halo methyl-1H-1,2,4-triazole; 3-(4-fluorophenyl)-5-halo methyl-1H-1,2,4-triazole and 3-(4-nitrophenyl)-5-halo methyl-1H-1,2,4-triazole.

7. A pharmaceutical composition comprising at least one of the five membered heterocyclic compounds of formula (I) as an active ingredient along with pharmaceutically acceptable excipients, being present in sufficient amount, to be effective against both active and dormant phase bacilli of *Mycobacterium Bovis* BCG and *Mycobacterium Tuberculosis* H37Ra for preventing the proliferation of HeLa cancer cell lines and for treatment of a disease or disorder associated with GroEL1/GroEL2 activity.

8. The pharmaceutical composition according to claim 8, wherein the compounds of formula (I) effective against both active and dormant phase bacilli of *Mycobacterium Bovis* BCG and *Mycobacterium Tuberculosis* H37Ra and for treatment of a disease or disorder associated with GroEL1/GroEL2 activity selected from compound (2), (3), (4), (5), (11), (14), (15), (17), (18), (19), (20), (23), (24), (41), (46), (56), (63), (64), (65), (66), (67), (68), (71), (72) and (73).

9. The pharmaceutical composition according to claim 8, wherein the compounds of formula (I) effective for preventing the proliferation of HeLa cancer cell lines selected from (18), (41), (42), (43), (44), (45), (67) and (68).

10. A method for treating the subject suffering from tuberculosis, cancer and disorders associated with GroEL1/GroEL2 activity comprising administering pharmaceutical composition comprising five membered heterocyclic compounds of formula (I) or its acid addition salts thereof either alone or in combination as active ingredient along with pharmaceutically acceptable excipients in therapeutically effective amount.

11. Use of novel five membered heterocyclic compounds of formula (I) or its acid addition salts thereof according to claim 1 or claim 8 to selectively act against both active as well as dormant bacilli of *Mycobacterium Bovis* BCG and *Mycobacterium Tuberculi* H37Ra; for treatment of a disease or disorder associated with GroEL1/GroEL2 activity and for preventing the proliferation of HeLa cancer cell lines.

NO

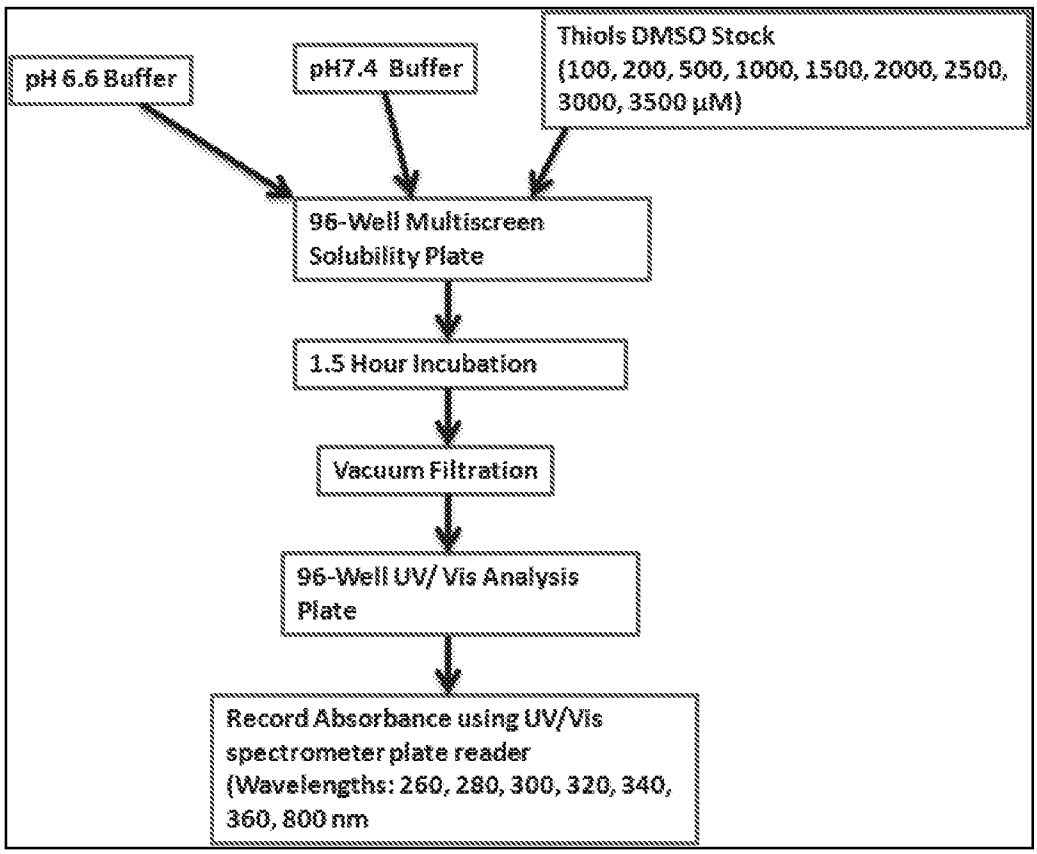


Figure 1

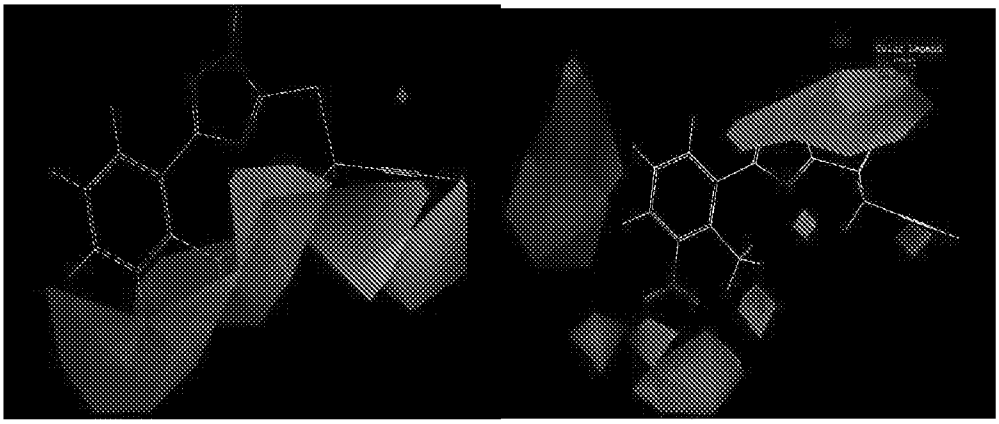
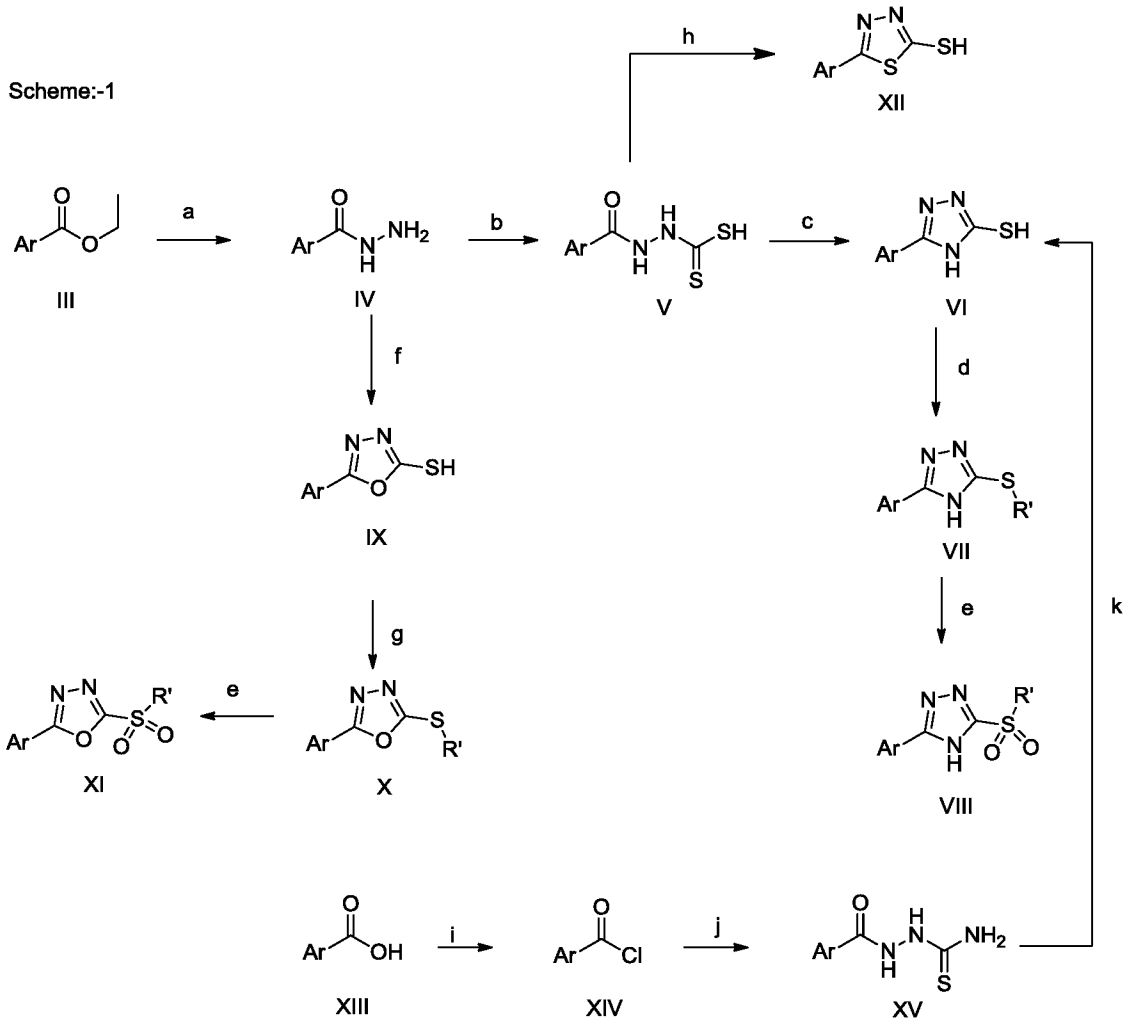
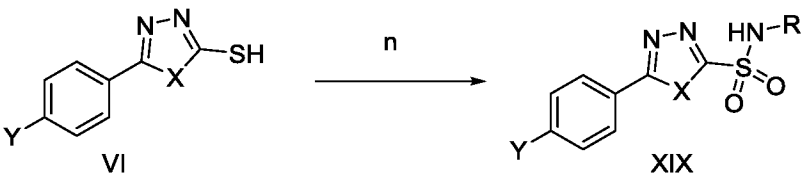


Figure 2a **Figure 2b**

Scheme:-1



Scheme 1



Scheme 2

INTERNATIONAL SEARCH REPORT

International application No
PCT/IN2015/050221

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07D249/12 C07D271/113 C07D285/125 A61K31/196 A61K31/4245
A61K31/433 A61P31/04 A61P31/06 A61P35/00
ADD.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, CHEM ABS Data, WPI Data, BEILSTEIN Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2011/111077 A1 (COUNCIL SCIENT IND RES [IN]; SARKAR DHIMAN [IN]; DESHPANDE SUNITA RANJ) 15 September 2011 (2011-09-15) cited in the application the whole document; in particular, claim 1 and the compounds 18.20 and 36-38 of the table 1	1-3,7,8, 11

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[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
7 March 2016	18/04/2016

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Fink, Dieter

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Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WESTWOOD ISAAC M ET AL: "Identification of arylamine N-acetyltransferase inhibitors as an approach towards novel anti- tuberculars", PROTEIN & CELL, SPRINGER ASIA, BEIJING, CN, vol. 1, no. 1, 18 March 2010 (2010-03-18), pages 82-95, XP009149386, ISSN: 1674-800X the whole document; in particular, page 87, scheme 2 and table 2	1-3,7,8, 11
X	Rajkumar Agrawal ET AL: "Synthesis, characterization and evaluation of antimicrobial activity of a series of 1,2,4-triazoles", Der Pharma Chemica 2011, 3 (6), 1 January 2011 (2011-01-01), pages 32-40, XP055255431, Retrieved from the Internet: URL:http://derpharmachemica.com/vol3-iss6/DPC-2011-3-6-32-40.pdf [retrieved on 2016-03-04] the wole document; in particular, pages 36-37, table 01, the compounds 1-3, 7, and 8	1-3,7
X	US 2009/291967 A1 (GUPTA MUKUR [US] ET AL) 26 November 2009 (2009-11-26) the whole document; in particular, claims 1, 2, and 8; page 39, example 59; and page 42, compounds 69-1 - 69-5, 69-7, and 69-8	1-3,7
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X	MANOHAR V. J J KULKARNI ET AL: "Synthesis and Biological Properties of Some 3-Heterocyclic Substituted Coumarins')", ARCH. PHARM, vol. 314, 1 January 1981 (1981-01-01), pages 435-439, XP055254186, the 3 phenyl-1H-1,2,4-triazole-5-thiol compounds according to scheme A on page 436	1-3

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Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	SØREN EBDRUP ET AL: "Synthesis and Structure-Activity Relationship for a Novel Class of Potent and Selective Carbamoyl-Triazole Based Inhibitors of Hormone Sensitive Lipase", JOURNAL OF MEDICINAL CHEMISTRY, vol. 47, no. 2, 12 December 2003 (2003-12-12), pages 400-410, XP055254292, US ISSN: 0022-2623, DOI: 10.1021/jm031004s page 407, column 1, line 10 - line 15	3
X	LIU W Y ET AL: "The synthesis, X-ray crystal structure and optical properties of novel 1-ferrocenyl-2-(3-phenyl-1H-1,2,4-triazol-5-ylthio)ethanone derivatives", SPECTROCHIMICA ACTA. PART A: MOLECULAR AND BIOMOLECULAR SPECTROSCOPY, ELSEVIER, AMSTERDAM, NL, vol. 76, no. 5, 1 September 2010 (2010-09-01), pages 531-536, XP027066692, ISSN: 1386-1425, DOI: 10.1016/J.SAA.2010.04.019 [retrieved on 2010-04-22] page 532, figure 1, the compounds 4b-4g	1-3
X	WO 2011/126903 A2 (VERSEON INC [US]; SHORT KEVIN MICHAEL [US]; PHAM SON MINH [US]; WILLIA) 13 October 2011 (2011-10-13) pages 85-86; the preparation of the intermediate 17	3
X	HERAVI MAJID M ET AL: "Sodium Hydroxide: a Mild and Inexpensive Catalyst for the Regioselective Synthesis of 2-Substituted 5-Methylthiazolo[3,2-b]-s-triazoles", JOURNAL OF CHEMICAL RESEARCH - SYNOPSES, SCIENCE REVIEWS LTD, GB, no. 8, 1 January 1998 (1998-01-01), pages 488-489, XP009149358, ISSN: 0308-2342, DOI: 10.1039/A802038H see, in particular, the compounds 1c, 1d, 1f and 1g, and the compounds 2c - 2g	1-3

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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WEI-MING XU ET AL: "Inhibition of Tobacco Bacterial Wilt with Sulfone Derivatives Containing an 1,3,4-Oxadiazole Moiety", JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY, vol. 60, no. 4, 1 February 2012 (2012-02-01), pages 1036-1041, XP055254351, US ISSN: 0021-8561, DOI: 10.1021/jf203772d page 1037, scheme 1, the compounds 3k, 4/4' (see, in particular, the 1,3,4-oxadiazole wherein R = 4-fluorophenyl and R' = Et), and 5/5'	1-3
X	XIN JIAN SONG ET AL: "Facile synthesis of novel fluorinated thieno[2,3-]pyrimidine derivatives containing 1,3,4-thiadiazole", CHINESE CHEMICAL LETTERS, vol. 23, no. 5, 29 March 2012 (2012-03-29) , pages 549-552, XP028421609, ISSN: 1001-8417, DOI: 10.1016/J.CCLET.2012.02.007 [retrieved on 2012-02-28] page 550, scheme 1, see, in particular, the compounds 4d and 4j	1-3
X	WEI M X ET AL: "Synthesis of new chiral 2,5-disubstituted 1,3,4-thiadiazoles possessing gamma-butenolide moiety and preliminary evaluation of in vitro anticancer activity", EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY, EDITIONS SCIENTIFIQUE ELSEVIER, PARIS, FR, vol. 44, no. 8, 27 March 2009 (2009-03-27) , pages 3340-3344, XP026108454, ISSN: 0223-5234, DOI: 10.1016/J.EJMECH.2009.03.023 [retrieved on 2009-03-27] page 3341, scheme 2, see, in particular, the compounds 8b and 8e	1-3
X	WO 2006/103345 A1 (GALDERMA RES & DEV [FR]; DIAZ PHILIPPE [FR]; RAFFIN CATHERINE [FR]; PE) 5 October 2006 (2006-10-05) pages 10-12, the table A, see, in particular, the compounds 8-10, 12, and 14	1-3

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Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	I. LALEZARI ET AL: "Synthesis of 1,3,4-thiadiazoles containing the trifluoromethyl group", JOURNAL OF HETEROCYCLIC CHEMISTRY, vol. 3, no. 3, 1 September 1966 (1966-09-01), pages 336-337, XP055254713, US ISSN: 0022-152X, DOI: 10.1002/jhet.5570030320 page 337, right column, penultimate paragraph	1-3
X	GB 767 452 A (AMERICAN CYANAMID CO) 6 February 1957 (1957-02-06) page 2; example IV	1-3
X	SANJAY SAMANTA ET AL: "Synthesis and in vitro Evaluation of West Nile Virus Protease Inhibitors Based on the 2-{6-[2-(5-Phenyl-4 H -{1,2,4]triazol-3-ylsulfanyl)acetylamino]benzothiazol-2-ylsulfanyl}acetamide Scaffold", CHEMMEDCHEM, vol. 8, no. 6, 25 April 2013 (2013-04-25), pages 994-1001, XP055254746, DE ISSN: 1860-7179, DOI: 10.1002/cmdc.201300114 page 995, scheme 1, the compound 2a12 and the compound 2a wherein R1 is p-OH and R2 is H (cf. the target compound 1a34 of table 1 on page 996)	1-3
X	F. LAZRAK ET AL: "SYNTHESE DE NOUVEAUX SYSTEMES CONDENSES RENFERMANT LE 1,2,4-TRIAZOLE, LA 1,3-THIAZINE, LA 1,3-THIAZEPINE ET LA 1,3,5,7-DITHIADIAZOCINE", PHOSPHORUS, SULFUR AND SILICON AND THE RELATED ELEMENTS, vol. 179, no. 9, 1 September 2004 (2004-09-01), pages 1799-1808, XP055255523, US ISSN: 1042-6507, DOI: 10.1080/10426500490466526 see, in particular, page 1806, the compound 6d	1,2

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Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE REGISTRY [Online] CHEMICAL ABSTRACTS SERVICE, COLUMBUS, OHIO, US; 20 August 2003 (2003-08-20), chemical library; supplier: AsInEx: XP002755091, Database accession no. 569651-61-2 abstract ----- DATABASE REGISTRY [Online] CHEMICAL ABSTRACTS SERVICE, COLUMBUS, OHIO, US; 18 February 2014 (2014-02-18), Chemical catalog; Supplier: Aurora Fine Chemicals: XP002755092, Database accession no. 1548540-68-6 abstract ----- DATABASE REGISTRY [Online] CHEMICAL ABSTRACTS SERVICE, COLUMBUS, OHIO, US; 7 August 2012 (2012-08-07), Chemical library; Supplier: Ukrorgsyntez ltd.: XP002755093, Database accession no. 1387137-69-0 abstract ----- DATABASE REGISTRY [Online] CHEMICAL ABSTRACTS SERVICE, COLUMBUS, OHIO, US; 7 June 2009 (2009-06-07), Chemical catalog; Supplier: UkrOrgSynthesis: XP002755094, Database accession no. 1152859-84-1 abstract ----- DATABASE REGISTRY [Online] CHEMICAL ABSTRACTS SERVICE, COLUMBUS, OHIO, US; 1 May 2007 (2007-05-01), Chemical library; Supplier: TimTec, Inc.: XP002755095, Database accession no. 933953-99-2 abstract ----- -/--	1-3 1-3 1-3 1-3 1-3

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X	DATABASE REGISTRY [Online] CHEMICAL ABSTRACTS SERVICE, COLUMBUS, OHIO, US; 25 May 2004 (2004-05-25), Chemical library; Supplier: Maybridge plc: XP002755096, Database accession no. 685541-46-2 abstract -----	1-3
X,P	RODE NAVNATH D ET AL: "First regioselective iodocyclization reaction of 3-aryl-5-(prop-2-ynylthio)-1H-1,2,4-triazoles", TETRAHEDRON LETTERS, PERGAMON, GB, vol. 56, no. 36, 7 July 2015 (2015-07-07), pages 5140-5144, XP029249247, ISSN: 0040-4039, DOI: 10.1016/J.TETLET.2015.07.006 the compounds 2 (see, in particular, the entry 3) of the tables 3 and 4 on page 5142 -----	1-3
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X,P	DATABASE REGISTRY [Online] CHEMICAL ABSTRACTS SERVICE, COLUMBUS, OHIO, US; 1 May 2015 (2015-05-01), Chemical catalog; Supplier: Ukrorgsyntez ltd.: XP002755098, Database accession no. 1696333-93-3 abstract -----	1-3

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/IN2015/050221

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