Accepted Manuscript

Attenuation of *Mycobacterium* species through direct and macrophage mediated pathway by unsymmetrical diaryl urea

Anand Babu Velappan, Mamilla R. Charan Raja, Dhrubajyoti Datta, Yi Ting Tsai, Iman Halloum, Baojie Wan, Laurent Kremer, Hugo Gramajo, Scott G. Franzblau, Santanu Kar Mahapatra, Joy Debnath

PII: S0223-5234(16)30817-0

DOI: 10.1016/j.ejmech.2016.09.083

Reference: EJMECH 8949

To appear in: *European Journal of Medicinal Chemistry*

Received Date: 7 June 2016

Revised Date: 23 September 2016

Accepted Date: 25 September 2016

Please cite this article as: A.B. Velappan, M.R. Charan Raja, D. Datta, Y.T. Tsai, I. Halloum, B. Wan, L. Kremer, H. Gramajo, S.G. Franzblau, S.K. Mahapatra, J. Debnath, Attenuation of *Mycobacterium* species through direct and macrophage mediated pathway by unsymmetrical diaryl urea, *European Journal of Medicinal Chemistry* (2016), doi: 10.1016/j.ejmech.2016.09.083.

Attenuation of *Mycobacterium species* through direct and macrophage mediated pathway by unsymmetrical diaryl urea

Anand Babu Velappan[a], Mamilla R. Charan Raja,[b] Dhrubajyoti Datta[c], Yi Ting Tsai[d], Iman Halloum[e], Baojie Wan[f], Laurent Kremer[eg], Hugo Gramajo[d], Scott G. Franzblau[f], Santanu Kar Mahapatra[b*], Joy Debnath[a*]

[a]*Department of Chemistry, School of Chemical and Biotechnology, SASTRA University, Tamilnadu 613401, India.*

[b]*Department of Biotechnology, School of Chemical and Biotechnology, SASTRA University, Tamilnadu 613401, India.*

[c]*Department of Chemistry, Indian Institute of Science Education and Research Pune , Maharashtra 411008, India*

[d]*Instituto de Biología Molecular y Celular de Rosario (IBR-CONICET), Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario, Rosario, Argentina.*

[e]*Centre d'étude des Pathogènes pour la Biotechnologie et la Santé CNRS FRE3689, Université de Montpellier, 34293 Montpellier cedex 5, France.*

[f]*Institute for Tuberculosis Research, College of Pharmacy, University of Illinois at Chicago, 833 S. Wood St. Chicago, Illinois 60612 USA.*

[g]*INSERM, CPBS, 34293 Montpellier Cedex 05, France.*

*Corresponding Author

JD: Tel: +91 4362 264101 (Ext: 3757); Fax: +91 4362 264120;

e-mail: joydebnath@scbt.sastra.edu

joydebnathiitkgp@gmail.com

Highlights:

- Different diaryl ureas are designed and synthesized.
- The antimycobacterial activity has been checked against *M. smegmatis* (mc^2155) and *M. tuberculosis* ($mc^2$6030 and H37Rv strains).
- The EDG and EWG are found to have a preference for the *ortho* and *meta* positions of the first aromatic ring respectively.
- The cytotoxicity values are in the range of 92-232 μg/ml.
- The effective molecules showed selective inhibitory activity on mycolic acid biosynthesis in ^{14}C-radiolabelled assay.
- Effective molecules of this series up regulate the expression level of iNOS2, IFN-γ, IL-12 and down regulate the level of IL-10 to clear the mycobacterium infection.

Abstract: Tuberculosis is a major threat for mankind and the emergence of resistance strain of *Mycobacterium tuberculosis* (*Mtb*) against first line antibiotics makes it lethal for human civilization. In this study, we have synthesized different diaryl urea derivatives targeting the inhibition of mycolic acid biosynthesis. Among the 39 synthesized molecules, compounds **46**, **57**, **58** and **86** showed MIC values ≤ 10 μg/ml against H37Rv and $mc^2$6030 strains. The best molecule with a methyl at *ortho* position of the first aromatic ring and prenyl group at the *meta* position of the second aromatic ring showed the MIC value of 5.2 μg/ml and 1 μg/ml against H37Rv and $mc^2$6030 respectively, with mammalian cytotoxicity of 163.4 μg/ml. The effective compounds showed selective inhibitory effect on mycolic acid (epoxy mycolate) biosynthesis in ^{14}C-radiolabelled assay. At the same time these molecules also executed their potent immunomodulatory activity by up-regulation of IFN-γ and IL-12 and down-regulation of IL-10.

Key words: Tuberculosis, Diaryl urea, Structure-activity relationship, antimycobacterial activity, Cytotoxicity, Mycolic acid, cytokines.

1. Introduction: *Mycobacterium* genus of pathogenic species are responsible for a variety of infectious diseases, e. g., tuberculosis and leprosy which are caused by *M. tuberculosis* (*Mtb*) and *M. leprae* respectively.[1] Altogether, infections caused by this group of species are primarily accountable for millions of death annually among different life forms. In particular, according to the World Health Organization (WHO) report (2015), in the year of 2014, 1.5 million deaths were caused by tuberculosis (TB) along with an estimated 9.6 million new TB incidences.[2] Among the 9.6 million new TB cases 12% people were found to be co-infected with HIV, which brings new challenges for conventional treatment methods that is mostly dependent on antibiotics. The AIDS patients co-infected with TB, are needed to be completely cure from TB in order to avoid the interaction of first line antibiotics (e. g., Rifampacin and Isoniazid) with anti-HIV drugs such as protease inhibitors.[3,4] However, the high tolerance level of *Mtb* towards first line antibiotics along with their ability to survive in the dormant (non-replicating) state is a major concern to provide a complete cure against TB.

There are several targets for the antituberculosis drugs; arguably, the most important one is the cell wall biosynthesis. Early in 1970, Isoxyl or thiocarlide was used as an antituberculosis drug which inhibits the synthesis of mycolic and oleic acids. The Δ9-stearoyl desaturase was identified as the primary target of isoxyl.[5-7] However, the use of isoxyl was restricted due to its interference with mammalian monoamine oxidase.[8] It is also reported that isoxyl get converted to an active form by the monooxygenase EthA.[9] During this bioactivation process, the thiourea gets converted to the urea one.[10] Therefore, different urea derivatives can represent as a suitable scaffold for the development of potent antitubercular and immunomodulatory molecule. Several attempts are also made to achieve this particular purpose.[11-15]

Most TB infections are commenced through the respiratory route. They first get implanted in the alveoli to form the Ghon complex.[16,17] During the progression of TB they thrive inside the host alveolar macrophages and impaired the host protective response. The virulent *Mtb* stain (H37Rv) modulates the host immune response in their favour by up-regulation of the anti-inflammatory cytokines e. g., IL-10, arginase and down-regulation of the proinflammatory cytokines, e. g., iNOS, IL-12, IFN-γ.[18] Therefore,

designing of immunomodulatory molecules also has a great possibility to cure against *Mtb* by re-educating the host immune response.

In this work, we have synthesized several unsymmetrical diaryl ureas to understand the relation between activity and cytotoxicity despite knowing the fact that the minimum inhibitory concentration (MIC) value of the urea analogue of isoxyl is ~100 times higher than isoxyl itself.[19] The key reasons for substituting the thiourea moiety with its bioisostere urea[20-22] are (i) to reduce the possibility of interaction with unspecified target through the sulphur atom and (ii) to find out a cost effective synthetic method for the low-income TB patients.

The first aromatic ring of this series of molecules is substituted with different substitutions having disparate inductive (I) and mesomaric (M) effects, whereas, the second aromatic ring is substituted with three different aliphatic chains at the *meta* and *para* positions (Fig 1). Different combinations of the substitution on both aromatic rings have a great scope to correlate their efficacy with cytotoxicity.

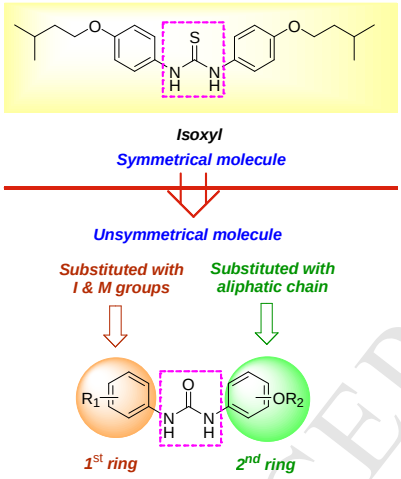


Figure 1: Site of modification at two aromatic rings.

The synthesized molecules were first tested for their direct killing effect on *Mtb* and *M. Smagmatis*, followed by the evaluation of their ramification on fatty acid and mycolic acid biosynthesis by the ^{14}C-radio-labeled assay and it was done with the selected compounds from each different effective prototypes. Finally, the best three molecules of this entire series were investigated for their possible immunomodulatory effect upon proinflammatory and anti-inflammatory cytokines by measuring their mRNA expression level with the help of RT-PCR.

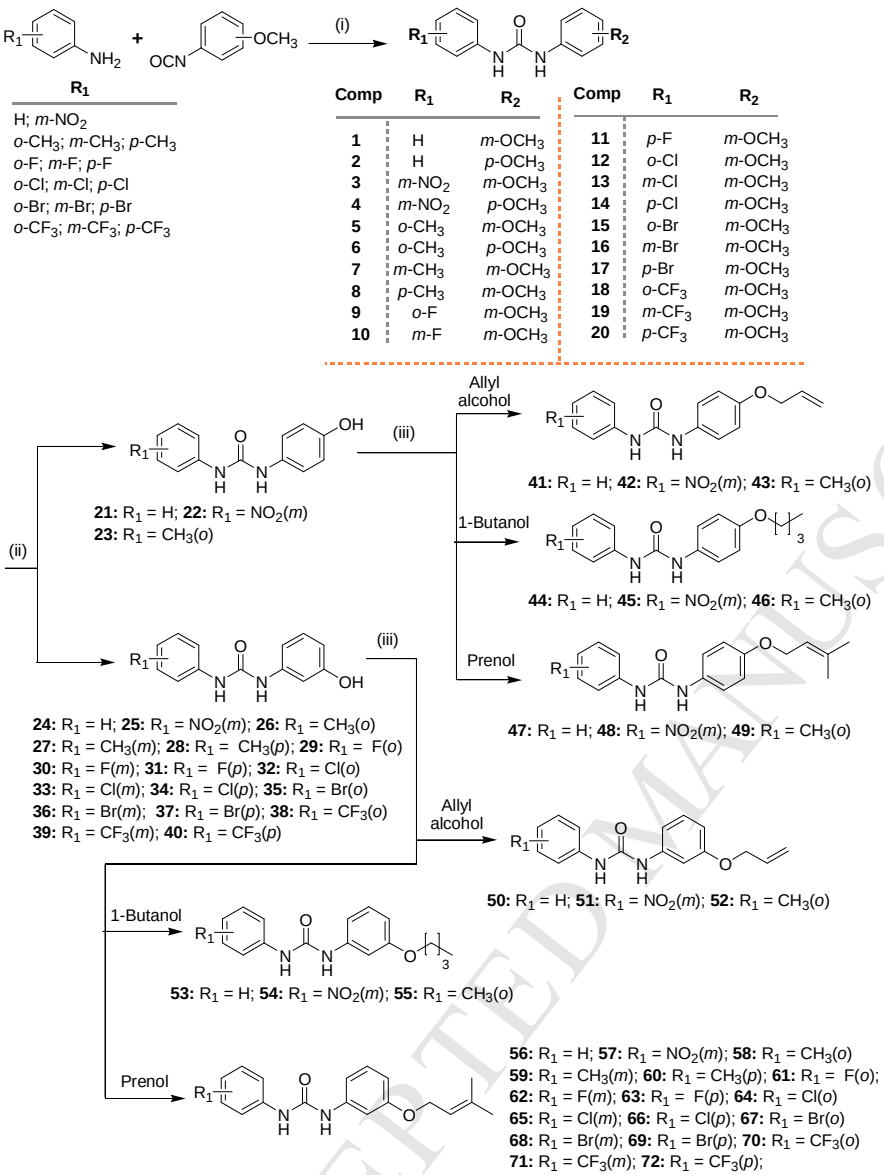
2. Results and Discussion:

2.1 Synthesis of Urea analogues:

In an effort to trounce the involvement of mammalian monoamine oxidase, we have prepared several diaryl ureas. A diverse set of molecules have been synthesized in order to determine the relationship between antitubercular efficiency with its different substitutions. To obtain this specific goal we had introduced several functionalities (with inductive and/or mesomeric effect) in the first aromatic ring, whereas, second aromatic was substituted with three different aliphatic substituents.

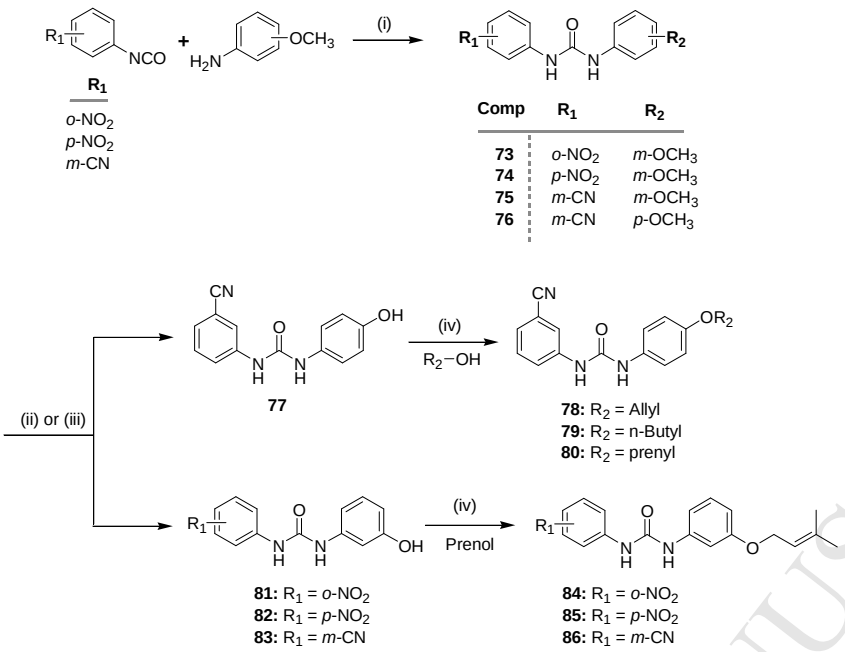
To introduce *+I*, *-I*, *-I* & *-M* and *–I* & *+M* effect in the first aromatic ring at its *ortho*/*meta*/*para* position six groups methyl (*+I*), trifluoromethyl (*-I*), nitro, halides, hydroxyl and amino (*-I* & *-M)* were primarily selected. However, the hydroxyl substitution was ruled out because of its potential participation in Mitsunobu reaction. For the amino substitution although we were able to prepare the monomer using high dilution method but the resulting diarylurea was found to yield benzimidazole (as confirmed by the NMR) during the HBr mediated demethylation step. Again, BBr_3 mediated demethylation for this system was found sluggish in nature (data not shown). Therefore, we were only able to synthesize compounds having methyl, trifluoromethyl, *m*-nitro and halides groups at three different positions of the first aromatic ring.

The diaryl ureas **1** - **20** were synthesized by the reaction of their corresponding amines (un-substituted and substituted) with 3- and 4-Methoxyphenyl isocyanate in dichloromethane (DCM) (Scheme 1)[23]. Aforesaid method was quite successfully utilized for the *m*-Nitroaniline (*-I* & *-M*), however, for *o*-Nitroaniline and *p*-Nitroaniline we opted for the other alternative. The strong *–I* and *–M* effect of the nitro group is presumed to be responsible for diminishing the nucleophilicity of the amine. The demethylation steps were accomplished with HBr (50% in acetic acid) at 70 °C. Thereafter, the three different aliphatic alcohols were coupled with their respective phenol counterpart via Mitsunobu coupling reaction[24] to yield final compounds **41** - **72**.



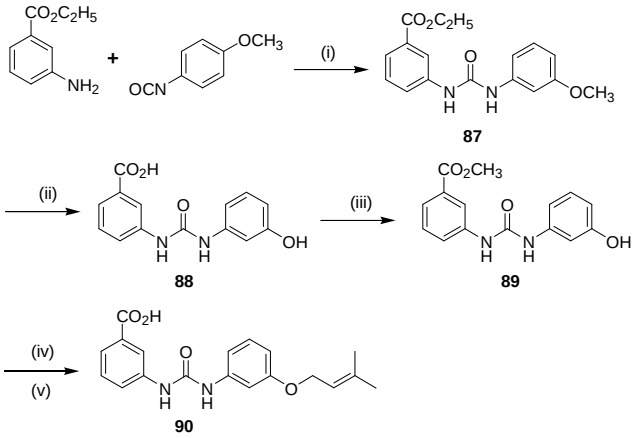
Scheme 1: Reagents and conditions: (i) DCM (dry), 3-5 h, rt; (ii) HBr/AcOH, 10 h, 70 °C; (iii) DIAD/TPP in THF, 6-8 h, rt.

The previous scheme did not work well (yield<5%) because of the poor reactivity of *o* and *p*-Nitroaniline and *m*-Cyanoaniline towards the isocyanates. To overcome the reactivity issue for the groups with *-I* & *-M* effect, we used *o* and *p*-Nitrophenyl isocyanate and *m*-Cyanophenyl isocyanate and reacted them with 3- and 4-Anisidine to obtain **73** – **76**. The use of anisidine increased the nucleophilicity of the amine, whereas, presence of nitro and cyano groups enhanced the electrophilicity of the isocyante. This combination worked well (yield >75%) and made it possible to prepare compounds **78** – **80** and **84** – **86** (Scheme 2).



Scheme 2: Reagents and conditions: (i) DCM (dry), 3-5 h, rt; (ii) HBr/AcOH, 10 h, 70 °C; (iii) BBr_3 in DCM, 10 h, rt; (iii) DIAD/TPP in THF, 6-8 h, rt.

For the carboxylic acid derivative we started with ethyl 3-aminobenzoate and 3-Methoxyphenyl isocyanate. The demethylation step was carried out in presence of HBr at 70 ^{o}C followed by the esterification of the carboxylic group (**89**). The carboxylic group was protected again to resist its participation in Mitsunobu reaction. Thereafter, the phenolic OH group was coupled with prenyl alcohol under Mitsunobu reaction condition followed the de-esterification of the carboxylic group with LiOH (**90**) (Scheme 3).



Scheme 3: Reagents and conditions; (i) DCM (dry), 4 h, rt; (ii) HBr/AcOH, 12 h, 70 °C; (iii) $SOCl_2$/MeOH, reflux 5 h; (iv) DIAD/TPP in THF, 8 h, rt; (v) LiOH/H_2O, 4 h, rt.

For all the synthesized molecules, only one aromatic ring of diaryl urea is substituted with different groups having altered *I* and *M* effect, whereas, the second one bears only an aliphatic chain at *para* and *meta* positions. To compare the effect of different substituents in first aromatic ring, compound **41**, **44**, **47**, **50**, **53** and **56** were prepared (Scheme 1), where the first aromatic ring is un-substituted but the second aromatic ring is substituted by all the three aliphatic chains at the *meta* and *para* positions.

2.2 Antimycobacterial activity:

The antimycobacterial activities and cytoxicities all 39 compounds are given in Tables 1-3. The bactericidal activity was determined against *M. Smegmatis* (mc^2155) and two other *Mtb* strains mc^26030 (non pathogenic strain) and H37Rv (pathogenic). The *Mtb* strain mc^26030 is derived from H37Rv, which is lacking of region of difference 1 (RD1) as well as is an auxotroph for pantothenic acid.[25] MIC values were determined by three different methods, namely, serial dilution method[26] (for mc^2155), agar plate method[27] (for *Mtb* mc^26030) and Microplate Alamer Blue assay (MABA)[28,29] (for *Mtb* H37Rv). Mammalian HepG2 cell line was used to determine their cytoxicity and the corresponding IC_{50} values were calculated from their dose response curve. Thereafter, the best compounds were taken forward to ascertain their direct effect on mycolic and fatty acid biosynthesis by ^{14}C-radiolebelled assay. Finally, their potential immunomodulatory activity was tested by measuring different cytokines levels in infected peritoneal macrophage to probe an alternative pathway for the clearance of mycobacterium species by this series of molecules.

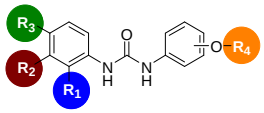
Correlation between +I vs -I effect and activity:

The first set of compounds **41**, **44**, **47**, **50**, **53** and **56** were prepared as a reference to correlate the activity of different substituents on the first aromatic ring. Therefore, for this set of molecules the first aromatic ring was kept un-substituted and the second aromatic ring was substituted with three different aliphatic chains at *meta* and *para* positions. This set of molecules showed moderate activity against mc^2155, $mc^2 6030$ and H37Rv strains except for compound **47**. The MIC values for **47** were found 25 μg/ml, 30 μg/ml and 43.1 μg/ml respectively against mc^2155, $mc^2 6030$ and H37Rv strains. However, for this set of molecules allyl and prenyl substitutions (second aromatic ring) showed better effect against H37Rv strain (>75%) compared to n-butyl one (<60%) at 50 μg/ml concentration. The small discrepancies of the MIC values against $mc^2 6030$ and H37Rv strains can be attributed for the difference in their susceptibility profile and for the methods being used to determine the drug efficacy (solid agar plates versus broth medium). The cytotoxicity for these molecules was found to vary between 182.4 to 124.1 μg/ml. Replacement of the thiourea moiety with its bioisostere (urea) showed a promising effect in order to decrease their cytotoxicity level compared with isoxyl.[13]

To compare only the role of *+I* and *–I* effect, we have prepared another two sets of molecules **43**, **46**, **49**, **52**, **55**, **58**, **59** and **60** (with *+I* effect) and **70 - 72** (with *–I* effect). Initial screening of **43**, **46**, **49**, **52**, **55**, **58**, **59** and **60** on different mycobacterium species showed the preference of *+I* group at *ortho* and *para* positions for their better activity, whereas, out of the six variants of this set the optimum activity was found for compound **58** with a prenyl group at the *meta* position on the second aromatic ring (Table 1). The MIC values were found 12.5 μg/ml, 1 μg/ml and 5.2 μg/ml against mc^2155, $mc^2 6030$ and H37Rv strains respectively. This finding confirmed us about the preferred position of the prenyl chain for second aromatic ring. After optimization of the position and nature of aliphatic chain another two variants **60** and **59** were prepared to find out the effect of methyl group at *meta* and *para* positions. The MIC values were found 32.2 μg/ml and 10.5 μg/ml for **60**and **59** respectively against H37Rv strain. The collective antimycobacterial activity of **58**, **59** and **60** confirmed the perpetuation of the *ortho* position for an *+I* group. Relative activity of **58** and **60** also provided an indication about the effect of steric congestion near the urea bond. Now to validate the above mentioned inference another set of compounds **70 - 72** were synthesized, where the methyl group was substituted by CF_3 having a

strong –*I* effect and the MIC values were found to increased over >50 µg/ml against H37Rv strain but surprisingly improved against mc^{2}155 strain. Comparing the activity of these sets of molecules it can be concluded that the +*I* groups dominate over the –*I* groups for their improved bactericidal activity against H37Rv strain but a reverse effect was encountered against mc^{2}155 strain (Table 1).

Table 1: Role of +*I* and –*I* effect on antimycobacterial activity.



Comp.	R_1	R_2	R_3	R_4	MIC (µg/ml) (H37Rv) (% Inhibition)	MIC (µg/ml) (mc^{2}6030)	MIC (µg/ml) (mc^{2}155)	Cytotoxicity (µg/ml)
44	H	H	H	^{n}Bu(p)	>50 (41%)	>100	>200	132.5
53	H	H	H	^{n}Bu(m)	>50 (57%)	50-100	>200	124.1
41	H	H	H	allyl(p)	>50 (84%)	>100	>200	182.4
50	H	H	H	allyl(m)	>50 (73%)	20	100	150.6
47	H	H	H	prenyl(p)	43.1	30	25	124.7
56	H	H	H	prenyl(m)	>50 (79%)	30	>200	168.2
46	CH_3	H	H	^{n}Bu(p)	7.0	100	100	130.5
55	CH_3	H	H	^{n}Bu(m)	>50 (70%)	>100	>200	175.1
43	CH_3	H	H	allyl(p)	24.5	100	50	99.2
52	CH_3	H	H	allyl(m)	>50 (68%)	>100	>200	102.4
49	CH_3	H	H	prenyl(p)	>50 (61%)	100	50	109.6
58	CH_3	H	H	prenyl(m)	5.2	1	12.5	163.4
59	H	CH_3	H	prenyl(m)	32.2	nd	50	175.3
60	H	H	CH_3	prenyl(m)	10.5	nd	12.5	140.6
70	CF_3	H	H	prenyl(m)	>50 (45%)	nd	12.5	71.4
71	H	CF_3	H	prenyl(m)	40.2	nd	3.12	135.6
72	H	H	CF_3	prenyl(p)	>50 (69%)	nd	6.25	142.2

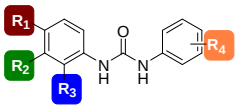
nd = not determined

Correlation between –I & -M vs –I & +M effect and activity:

To understand the outcome of different substitutions having –*I* & –*M* and –*I* & +*M* effect, we synthesized four sets of molecules. For the first three sets the first aromatic ring is substituted with nitro, cyano and carboxylic groups having –*I* & –M effect, whereas, for the fourth set of molecules it is substituted by three different halides (F, Cl and Br) having –*I* & +*M* effect. Among the others, nitro has the strongest –*I* & –*M* effect. Therefore, we

selected this one to optimize the aliphatic side chain and its preferred position in the second aromatic ring. For this reason we had prepared compounds **42**, **45**, **48**, **51**, **54**, **57**, **84** and **85** with the nitro at *meta*, *ortho* and *para* positions respectively. The relative activity of the compounds **42**, **45**, **48**, **51**, **54**, **57** showed a clear preference for the prenyl chain at *meta* position as observed previously for compound **58**. Only compond **57** of this set showed significant effect on three mycobacterium species, the respective MIC value were 6 µg/ml, 2 µg/ml and 3.21 µg/ml against H37Rv, $mc^2$6030 and $mc^2$155 strains. However, a small improvement in activity was observed for *para* nitro substitution over the *ortho* one. The similar trend was observed for compounds **78**, **79**, **80** and **86**, where the first aromatic ring was substituted with a nitrile group (*-I* & *-M*) at *meta* position. Their relative activity also probed the preference of prenyl group at *meta* position, as observed for methyl and nitro substitutions. The best activity was found for compound **86** and the respective MIC values were 9.2 µg/ml and 25 µg/ml against H37Rv and $mc^2$155. Similar to the previous sets of molecules same trend was observed for the molecule (**90**) having a carboxy substitution at *meta* position (Table 2).

Table 2: Role of *-I* and *–M* effect on antimycobacterial activity.

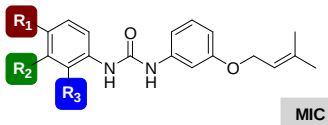


Comp	R_1	R_2	R_3	R_4	MIC (µg/ml) (H37Rv)	MIC (µg/ml) ($mc^2$6030)	MIC (µg/ml) ($mc^2$150)	Cytotoxicity (µg/ml)
49	H	NO_2	H	O-^{n}Bu(m)	>50 (75%)	50-100	>200	92.2
54	H	NO_2	H	O-^{n}Bu(p)	>50 (79%)	50	>200	97.3
51	H	NO_2	H	O-allyl(m)	>50 (76%)	5	50	105.6
42	H	NO_2	H	O-allyl(p)	45.5	10-100	100	111.4
57	H	NO_2	H	O-prenyl(m)	. 6.0	2	3.21	113.7
48	H	NO_2	H	O-prenyl(p)	>50 (69%)	>100	>200	142.6
84	H	H	NO_2	O-prenyl(m)	>50 (43%)	nd	>200	61.4
85	NO_2	H	H	O-prenyl(m)	>50 (94%)	nd	100	91.6
79	H	CN	H	O-^{n}Bu(p)	>50 (22%)	>100	>200	206.7
78	H	CN	H	O-allyl(p)	>50 (66%)	>100	>200	103.5
80	H	CN	H	O-prenyl(p)	.>500 (82%)	5	50	186.4
86	H	CN	H	O-prenyl(m)	9.2	nd	25	172.6
90	H	CO_2H	H	O-prenyl(m)	. 16.5	nd	12.5	132.6

nd = not determined

To find out the amendment of activity with *–I* & *+M* effect we had prepared another three sets of molecules with three different halides **61** – **69**. For these three halids (F, Cl and Br) *–I* effect decreases with the increase of atomic number, whereas, the *+M* effect increases with the increase of atomic number. Compounds **61**, **62** and **63** with respective fluoro substitution at *ortho*, *meta* and *para* positions were found not to be effective against mycobacterium species rather than it increases the cytotoxicity level. However, a trivial improvement was observes for the *meta* substitution (**62**) compared to **61** and **63**. For the chloro series (**64** - **66**) the activity trend was found similar to that of fluoro series (Table 3). Nevertheless, the activity profile was improved for the chloro series along with an improvement in their cytotoxicity level. For the bromo (**67 - 69**) series the activity was improved to a significant amount compared to their fluoro and chloro analogues. Compound **68** showed the best antimycobaterial activity among all other halide derivatives and its MIC values were found was found to improved by two folds against H37Rv and mc^{2}155 strains.

Table 3: Role of *-I* and *+M* effect on antimycobacterial activity.

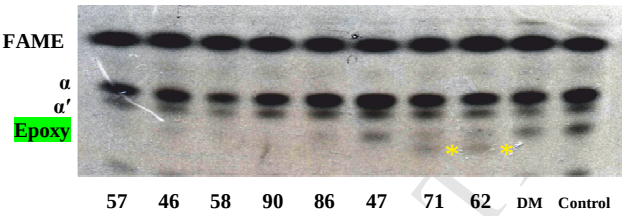


Entry	R_1	R_2	R_3	MIC (µg/ml) (H37Rv)	MIC (µg/ml) (mc^{2}150)	Cytotoxicity (µg/ml)
63	F	H	H	>50 (68%)	100	102.8
62	H	F	H	>50 (89%)	25	94.0
61	H	H	F	>50 (45%)	50	82.4
66	Cl	H	H	>50 (75%)	50-100	122.3
65	H	Cl	H	48.6	25	114.3
64	H	H	Cl	>50 (64%)	50	128.2
69	Br	H	H	>50 (86%)	50-100	135.6
68	H	Br	H	26.5	12.5	148.1
67	H	H	Br	38.7	25	131.9

The comparative activity of these three different halide derivatives clearly showed that the inductive effect (*I*) played a decisive role in enhancing the antimycobaterial activity and decreasing of the cytotoxicity level. Similar to the methyl derivatives a small favourable steric effect near the urea linkage was also observed for these halide series.

2.3 Effect on fatty acid and mycolic acid biosynthesis:

To probe the effect of synthesized molecules on mycobacterium fatty acid (FA) and mycolic acid (MA) biosynthesis, we have selected eight different compounds from each different set. The radio-labeled [1-^{14}C]-acetate was used to determine radiometrically their inhibitory effect on different fatty acids and mycolic acid biosynthesis. Among the eight molecules compound **47** did not showed any inhibitory activity on the biosynthesis of FA and MA as evidenced from the TLC profile (Fig 2), whereas, the other compounds **46**, **57**, **58**, **86** and **90** showed their selective inhibitory activity on MA biosynthesis. For all these five compounds only the epoxy mycolate biosynthesis of *M. smegmatis* was found to get inhibited but the other two α and α' mycolate biosynthesis were remained almost unaffected. This finding clearly showed the importance of the aromatic substitution (first aromatic ring) for inhibition of mycolic acid biosynthesis. However, for compound **62** and **71** weak inhibitory activity was observed for epoxy mycolate biosynthesis, but interestingly an additional band was observe below the epoxy mycolate (Fig 2).



FAME = Fatty acid methyl ester; α = α-mycolic acid
α' = α'-mycolic acid; Epoxy = Epoxy-mycolic acid

Fig 2: Inhibition of Fatty acid and Mycolic acid biosynthesis by the synthesized compounds. Compound concentrations are 5 times to their MIC value.

There are two fatty acid synthetases (FAS I and FAS II) present in *M. smegmatis* (mc^{2}155).[30] Among these two, FAS I is known to be responsible for the synthesis of C_{16} to C_{18} long fatty acid, whereas, further elongation and functionalization takes place in the FAS II module to yield meromycolic acid.[31] However, the synthesized molecules did not show any inhibitory activity upon fatty acid biosynthesis.

2.4 Immunomodulatory activity:

Mtb is an obligatory aerobic, intracellular pathogen, which has a predilection for the lung tissue rich in oxygen supply. Phagocytosis of *Mtb* by alveolar macrophages is the first event in the host-pathogen relationship that decides the outcome of infection. In general, the event of phagocytic killing takes place either by toxic reactive oxygen intermediates (ROI) or reactive nitrogen intermediates (RNI) or by both. However, RNI is known to play a pivotal role during phagocytosis against tubercle bacilli.[32]

In order to determine the production of RNI, we determined the inducible nitric oxide synthase (iNOS2) expression in BALB/c derived peritoneal macrophage (representative of all tissue macrophages) infected by mc^2155 strain, which is primarily responsible for the synthesis of RNI from arginine via NADPH mediated pathway.[32] A significant increase of iNOS2 expression in the infected macrophage treated with compound **58** and **86** compared to infected macrophage alone evidently indicated their immunoprotective role against infection (Fig 3). However, compound **57** showed a little effect on macrophage mediated RNI generation as evidenced from the relative iNOS2 expression level for infected and infected but drug treated macrophage (Fig 4A).

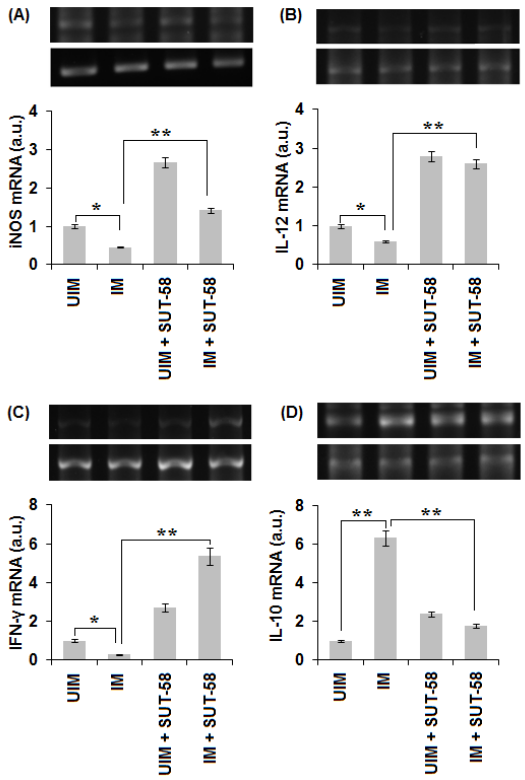


Fig 3: Effect of **58** on iNOS2 expression (A), proinflammatory (IL-12 and IFN-γ) (B-C) and anti-inflammatory (IL-10) (D) cytokines expression by RT-PCR. Data were represents here from one of three independent experiments yielded similar kind of results. Corresponding bars are the mean of the respective densitometry data (mean ± SD). *p<0.05; **p<0.01 indicates significant differences compared with indicated respective control. UIM = Uninfected macrophage; IM = Infected macrophage; SUT-58 = compound ID for molecule **58**.

Proinflammatory cytokines play a decisive role against mycobacterium infection.[33] The IFN-γ is known to trigger the macrophage activation for production of RNI via iNOS2, whereas, IL-12 get induced during phagocytosis of tubercle bacilli as a natural immunoprotection against *Mtb* infection.[34] However, down-regulation of these cytokines is responsible for the disease progression. Therefore, we examined the expression level of interferon-γ (IFN-γ) and interleukin-12 (IL-12) to validate the effect of the synthesized compounds on proinflammatory cytokine oozing during infection. In case of **58** and **86** an increase of IL-12 and IFN-γ expression level between infected and infected with treated macrophage clearly demonstrated their potential to up-regulate the expression of proinflammatory cytokines in infected macrophage (Fig 3B, 3C and Fig 5B, 5C). Unlike the other two compounds, **57** did not show any positive activity on their expression level against infection.

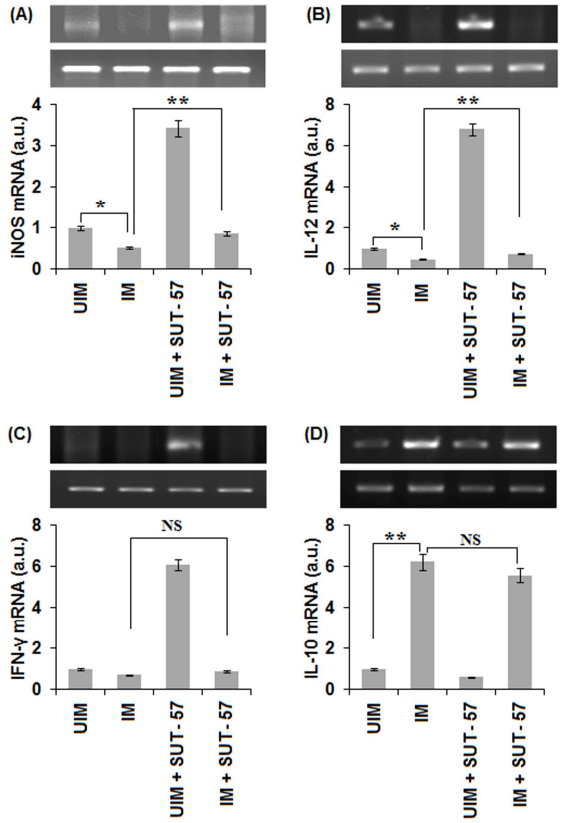


Fig 4: Effect of **57** on $iNOS_2$ expression (A), proinflammatory (IL-12 and IFN-γ) (B-C) and anti-inflammatory (IL-10) (D) cytokines expression by rt-PCR. Data were represents here from one of three independent experiments yielded similar kind of results. Corresponding bars are the mean of the respective densitometry data (mean ± SD). *p<0.05; **p<0.01 indicates significant differences compared with indicated respective control. UIM = Uninfected macrophage; IM = Infected macrophage; SUT-57 = compound ID for molecule **57**.

Interleukin-10 (IL-10) is an anti-inflammatory cytokine and its up-regulation is known to be responsible for the tuberculosis progression.[35] Therefore, the expression of IL-10 was determined in order to find out immunomodulatory activity of the synthesized compounds. In case of compound **57**, **58**, and **86** treated infected macrophage a down-regulation of the IL-10 was observed compared to the up-regulation of IL-10 level in infected macrophage.

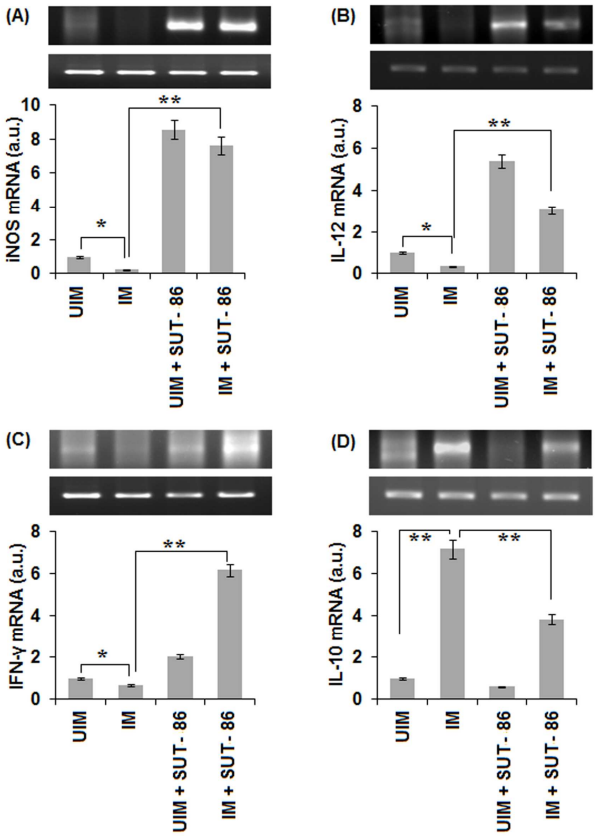


Fig 5: Effect of **86** on $iNOS_2$ expression (A), proinflammatory (IL-12 and IFN-γ) (B-C) and anti-inflammatory (IL-10) (D) cytokines expression by RT-PCR. Data were represents here from one of three independent experiments yielded similar kind of results. Corresponding bars are the mean of the respective densitometry data (mean ± SD). *$p<0.05$; **$p<0.01$ indicates significant differences compared with indicated respective control. UIM = Uninfected macrophage; IM = Infected macrophage; SUT-86 = compound ID for molecule **86**.

The differential expression level of iNOS2, IFN-γ, IL-12 and IL-10 for the infected and treated macrophages compared to uninfected and infected macrophages unambiguously confirms the immunomodulatory activity of these synthesized molecules.

The cytotoxicity (IC_{50}) of the synthesized compounds against peritoneal macrophages was found in the range of 180.9 - 58.1 µg/ml (Table 4) which is almost 16 – 5 higher than the isoxyl (Table 5).

Table 4: Cytotoxicity (IC_{50}) against mammalian cell (Peritoneal Macrophage).

Comp	Cytotoxicity (µg/ml)	Comp	Cytotoxicity (µg/ml)	Comp	Cytotoxicity (µg/ml)	Comp	Cytotoxicity (µg/ml)
41	152.6	**51**	78.4	**61**	68.2	**71**	116.7
42	87.5	**52**	90.3	**62**	83.7	**72**	115.3
43	122.1	**53**	121.7	**63**	87.1	**78**	96.7
44	108.9	**54**	74.3	**64**	105.6	**79**	180.9
45	75.2	**55**	152.9	**65**	96.7	**80**	169.1
46	125.7	**56**	141.8	**66**	115.3	**84**	58.1
47	109.5	**57**	94.1	**67**	119.4	**85**	75.2
48	131.2	**58**	140.5	**68**	131.8	**86**	159.4
49	81.3	**59**	160.3	**69**	140.2	**90**	120.4
50	142.5	**60**	127.6	**70**	69.4		

Table 5: Antimycobacterial activity and cytotoxicity (IC_{50}) values for Isoniazide and Isoxyl.

Comp	MIC (µg/ml) H37Rv	MIC (µg/ml) $mc^2$6030	MIC (µg/ml) $mc^2$155	Cytotoxicity (µg/ml) HepG2	Cytotoxicity (µg/ml) macrophage
Isoniazid	0.11	0.05	6.25	>200	>200
Isoxyl	2.3	nd	25	31.3	12.5

3. Conclusion:

Unsymmetrical diaryl ureas are shown to have a significant effect against *Mtb*. The SAR study of the synthesized compounds demonstrated the positional preference of different functional groups in both of the aromatic rings. For the first aromatic ring *ortho* and *meta* positions were found to be the preferred one for the groups having *+I* and *–I* & *-M* effect respectively. The best activity was found for **58** which has the strongest *+I* effect and for **57** with the strongest *–I* & *–M* effect than the others. However, compounds having *–I* and *–I* & *+M* showed moderate antitubercular activity. On the other hand, for the second aromatic ring, best activity was found for the molecules having prenyl group at the *meta* position, whereas, except compound **46**, other aliphatic substitutions showed moderate activity. In this series it was found that the substitution of thiourea moiety with its bioisostere not only reduces the cytotoxicity by 40 folds (comp. **58** and isoxyl) but also can disengage the participation of FMO (Flavin monooxygenase). Moreover, these series

of molecules are quite distinctive in the sense of their selective inhibitory activity towards mycolic acid biosynthesis.

The *Mtb* is known to suppress the immune response of the host cell during infection. Therefore, rejuvenation of the immune response of macrophage has an important role to clear the bacterium. Interestingly, molecules **58** and **86** showed a significant impact to re-educate the immune response in favour to the host. Therefore, these molecules are not only capable to kill the mycobacterium in a direct killing method by perturbation of the mycolic acid biosynthesis but also able to clear the mycobacterium in a macrophage mediated pathway by upregulation of the proinflammatory and downregulation of the anti-inflammatory cytokines.

Altogether, these findings have a deep impact on the development of potent antimycobacterial molecules with dual killing effect and high selectivity.

4. Experimental:

4.1 Reagents and instrumentation:

All the chemicals were purchased from Sigma–Aldrich, Alfa Aesar and Merck Chemicals. Column chromatographic separations were performed using silica gel (100–200 mesh). Solvents were dried and distilled following standard procedures. TLC was carried out on pre-coated plates (Merck silica gel 60, f_{254}), and the spots were visualized with UV light or by charring the plates dipped in 10% PMA solution in methanol or 5% H_2SO_4/vanillin/EtOH. ^1H NMR (300, 400 MHz) and ^{13}C NMR (75, 100 MHz) spectra were recorded on a Bruker NMR spectrometer (δ scale). UV–vis measurements were made using a Perkin Elmer UV–vis spectrophotometer (Model Lambda 25). Mass spectra had been recorded using Waters Mass Spectrometer (model XevoG2QTof).

4.2 Synthesis of urea derivatives: General method:

Method A: 3- and 4-Methoxyphenyl isocyanate (individually as per the scheme 1) was added drop wise to a stirred solution of the corresponding amine in dichloromethane (DCM) and was allowed to stir at room temperature until the completion of reaction (checked by TLC). The organic solvent was then evaporated under reduced pressure.

Crude residues thus obtained were purified over silica gel column chromatography using hexanes and ethyl acetate as eluent.

Method B: To a stirred solution of *p*-anisidine in DCM (dry) three different phenyl isocyanates were added (individually as per the scheme 2) and the stirring was continued at room temperature until the completion of reaction (checked by TLC). The organic solvent was then evaporated under reduced pressure. Crude residues thus obtained were purified over silica gel column chromatography using hexanes and ethyl acetate.

Demethylation: General method

Method A: 50% Hydrobromic acid in acetic acid was added to a suspension of the methyl ether of urea derivatives dissolved in acetic acid. The clear solution was maintained at 70 °C for 12 hrs. After that the reaction mixture was cooled and quenched with 10% sodium bicarbonate. Then the organic portion was extracted with ethyl acetate. The combined organic layers were washed with water followed by brine solution and then dried over sodium sulphate and evaporated to afford the crude product. The crude thus obtained was purified by silica gel column chromatography using hexanes and ethyl acetate.

Method B: A solution of the methyl ether of urea derivatives in DCM (dry) was maintained at 0-5 °C followed by the addition of 1M solution of Borontribromide (BBr_3) in DCM. The stirring was continued at room temperature for 4-6 h. After completion of the reaction (checked by TLC) it was quenched with 10% sodium bicarbonate and extracted with DCM (3X100 ml). Combined organic layers were then washed with water followed by brine solution and then dried over sodium sulphate and evaporated to afford the crude product. The crude thus obtained was purified by silica gel column chromatography using hexanes and ethyl acetate.

General procedure for the Mitsunobu reaction:

To a solution of alcohol in dry tetrahydrofuran (THF) triphenyl phosphine (PPh_3) and DIAD were added consecutively and stirred at RT for 15 minutes. Thereafter, the corresponding phenolic compound was added slowly to the reaction mixture under nitrogen atmosphere and the stirring was continued for 6-8 hours at room temperature.

After completion of reaction (by TLC), solvent was evaporated and the crude was partitioned between water and ethyl acetate (3X100 ml). The combined organic layers were then washed with water followed by brine solution and dried over sodium sulphate. The crude thus obtained was purified by column chromatography using a mixture of hexanes /ethyl acetate. The purified compounds were dissolved in THF and treated with 5% aqueous Lithium hydroxide solution (THF/H_2O 4:1) for 1 h at room temperature to remove the DIAD by-products (as observed from their ^{1}H-NMR). After that the THF was removed under reduced pressure and the solid was filtered. The solid thus obtained was dried completely and subjected for spectral analysis and bioassay. However, lithium hydroxide treatment was not carried out for the Cyano derivatives **78**, **79**, **80** and **86**.

1-(4-(allyloxy)phenyl)-3-phenylurea (41):

To a solution of Allyl alcohol (0.028 g; 0.48 mmol) in dry THF, TPP (0.149 g; 0.57 mmol) and DIAD (0.115 g; 0.57 mmol) were added consecutively and stirred at RT for 15 minutes. Thereafter, comp. **21** (0.1 g; 0.44 mmol) was added slowly to the reaction mixture under nitrogen atmosphere and the stirring was continued for 8 hours at room temperature. After aqueous workup the crude was purified by column chromatography (20% EtOAc in Hexane). Yield: 59.8%; white solid; m.p. 158-160 ^{o}C. ^{1}H NMR (acetone-d_6) (300 MHz) – δ 4.38-4.40 (m, 2H), 5.09 (dd, *J* = 1.5, 10.3 Hz, 1H), 5.26 (dt, *J* = 1.8, 17.4 Hz, 1H), 5.88-5.94 (m, 1H), 6.73-6.85 (m, 3Ar-H), 7.09-7.14 (m, 2Ar-H), 7.29 (dd, *J* = 1.8, 6.6 Hz, 2Ar-H), 7.37-7.40 (m, 2Ar-H), 7.84 (bs, 1N-H), 7.94 (brs, 1N-H). ^{13}C NMR (acetone-d_6) (75 MHz) - δ 68.7, 114.8, 116.3, 118.4, 184.4, 120.3, 120.4, 121.8, 128.6, 133.2, 134.1, 140.2, 153.0, 154.2. HRMS (ESI+): m/z calcd for $C_{16}H_{17}N_2O_2$ $[M+H]^+$: 269.1290; found: 269.1293.

1-(4-(allyloxy)phenyl)-3-(3-nitrophenyl)urea (42):

To a solution of Allyl alcohol (0.024 g; 0.40 mmol) in dry THF, TPP (0.125 g; 0.48 mmol) and DIAD (0.096 g; 0.46 mmol) were added consecutively and stirred at RT for 15 minutes. Thereafter, comp. **22** (0.1 g; 0.366 mmol) was added slowly to the reaction mixture under nitrogen atmosphere and the stirring was continued for 8 hours at room temperature. After aqueous workup the crude was purified by column chromatography (16% EtOAc in Hexane). Yield: 52.8%; pale yellow solid; m.p. 182-184 ^{o}C. ^{1}H NMR (acetone-d_6) (300 MHz) – δ 4.40-

4.43 (m, 2H), 5.11 (dd, *J* = 1.5, 10.5 Hz, 1H), 5.28 (dd, *J* = 1.8, 17.1 Hz, 1H), 5.88-6.01 (m, 1H), 6.75-6.79 (m, 2Ar-H), 7.31 (dd, *J* = 2.1, 9.0 Hz, 2Ar-H), 7.41 (t, *J* = 8.4 Hz, 1Ar-H), 7.67-7.70 (m, 2Ar-H), 8.00 (brs, 1N-H), 8.50 (brs, 1N-H), 8.51 (t, *J* = 2.1 Hz, 1Ar-H). ^{13}C NMR (acetone-d_6) (75 MHz) – δ 68.7, 112.6, 114.9, 116.2, 116.4, 120.8, 120.9, 124.1, 124.2, 129.8, 132.6, 134.1, 141.7, 148.8, 152.9, 154.6. HRMS (ESI+): m/z calcd for $C_{16}H_{16}N_3O_4$ $[M+H]^+$: 314.1141 ; found: 314.1145.

1-(4-(allyloxy)phenyl)-3-(o-tolyl)urea (43):

To a solution of Allyl alcohol (0.026 g; 0.45 mmol) in dry THF, TPP (0.141 g; 0.54 mmol) and DIAD (0.108 g; 0.54 mmol) were added consecutively and stirred at RT for 15 minutes. Thereafter, comp. **23** (0.1 g; 0.41 mmol) was added slowly to the reaction mixture under nitrogen atmosphere and the stirring was continued for 8 hours at room temperature. After aqueous workup the crude was purified by column chromatography (20% EtOAc in Hexane). Yield: 61.2%; white solid; m.p. 176-178 ^{o}C. ^{1}H NMR (acetone-d_6) (300 MHz) – δ 2.13 (s, 3H), 4.39-4.41 (m, 2H), 5.10 (dd, *J* = 1.8, 10.5 Hz, 1H), 5.27 (dd, *J* = 1.8, 17.4 Hz, 1H), 5.89-5.98 (m, 1H), 6.73-6.83 (m, 3Ar-H), 7.01 (m, 2Ar-H), 7.31 (dd, *J* = 2.4, 6.9 Hz, 3Ar-H), 7.80-7.83 (m, 1N-H), 8.14 (brs, 1N-H). ^{13}C NMR (acetone-d_6 + $CDCl_3$) (75 MHz) – δ 22.8, 74.9, 120.6, 122.7, 126.6, 128.0, 129.9, 132.2, 134.1, 135.9, 137.8, 139.2, 142.7, 159.2, 160.6. HRMS (ESI+): m/z calcd for $C_{17}H_{19}N_2O_2$ $[M+H]^+$: 283.1447; found: 283.1447.

1-(4-butoxyphenyl)-3-phenylurea (44):

To a solution of n-Butyl alcohol (0.036 g; 0.48 mmol) in dry THF, TPP (0.149 g; 0.57 mmol) and DIAD (0.115 g; 0.57 mmol) were added consecutively and stirred at RT for 15 minutes. Thereafter, comp. **21** (0.1 g; 0.44 mmol) was added slowly to the reaction mixture under nitrogen atmosphere and the stirring was continued for 8 hours at room temperature. After aqueous workup the crude was purified by column chromatography (20% EtOAc in Hexane). Yield: 76.6%; white solid; m.p. 168-170 ^{o}C. ^{1}H NMR (acetone-d_6) (300 MHz) – δ 0.83 (t, *J* = 7.5 Hz, 3H), 1.32-1.39 (m, 2H), 1.57-1.62 (m, 2H), 3.82 (t, *J* = 6.3, 2H), 6.72 (dd, *J* = 2.1, 6.6 Hz, 2Ar-H), 6.83 (t, *J* = 7.2 Hz, 1Ar-H), 7.13 (t, *J* = 8.1 Hz, 2Ar-H), 7.28 (dd, *J* = 2.1, 6.9 Hz, 2Ar-H), 7.38-7.41 (m, 2Ar-H), 7.80 (brs, 1N-H), 7.91 (brs, 1N-H). ^{13}C NMR (acetone-d_6) (75 MHz) - δ

13.4, 19.2, 31.5, 67.8, 114.7, 118.6, 120.7, 122.0, 128.8, 133.1, 140.5, 153.0, 155.0. HRMS (ESI+): m/z calcd for $C_{17}H_{21}N_2O_2$ $[M+H]^+$: 285.1603; found: 285.1601.

1-(4-butoxyphenyl)-3-(3-nitrophenyl)urea (45):

To a solution of n-Butyl alcohol (0.030 g; 0.40 mmol) in dry THF, TPP (0.125 g; 0.48 mmol) and DIAD (0.096 g; 0.48 mmol) were added consecutively and stirred at RT for 15 minutes. Thereafter, comp. **22** (0.1 g; 0.37 mmol) was added slowly to the reaction mixture under nitrogen atmosphere and the stirring was continued for 8 hours at room temperature. After aqueous workup the crude was purified by column chromatography (16% EtOAc in Hexane). Yield: 48.2%; pale yellow solid; m.p. 196-198 ^oC. ^1H NMR (acetone-d_6) (300 MHz) – δ 0.83 (t, *J* = 7.5 Hz, 3H), 1.32-1.40 (m, 2H), 1.58-1.63 (m, 2H), 3.83 (t, *J* = 6.6 Hz, 2H), 6.75 (dd, *J* = 2.1, 6.9 Hz, 2Ar-H), 7.30 (d, *J* = 9.3 Hz, 2Ar-H), 7.41 (t, *J* = 8.4 Hz, 1Ar-H), 7.67-7.69 (m, 2Ar-H), 7.99 (brs, 1N-H), 8.45 (brs, 1N-H), 8.51(t, *J* = 2.4 Hz, 1Ar-H). ^{13}C NMR (d_6-DMSO) (100 MHz) – δ 13.7, 18.7, 30.8, 67.3, 112.0, 114.6, 116.0, 120.5, 124.2, 130.0, 132.1, 141.3, 148.1, 152.6, 154.2. HRMS (ESI+): m/z calcd for $C_{17}H_{20}N_3O_4$ $[M+H]^+$: 330.1454; found: 330.1459.

1-(4-butoxyphenyl)-3-(o-tolyl)urea (46):

To a solution of n-Butyl alcohol (0.0336 g; 0.45 mmol) in dry THF, TPP (0.141 g; 0.54 mmol) and DIAD (0.108 g; 0.54 mmol) were added consecutively and stirred at RT for 15 minutes. Thereafter, comp. **23** (0.1 g; 0.41 mmol) was added slowly to the reaction mixture under nitrogen atmosphere and the stirring was continued for 8 hours at room temperature. After aqueous workup the crude was purified by column chromatography (20% EtOAc in Hexane). Yield: 54.6%; off white solid; m.p. 180-182 ^oC. ^1H NMR (acetone-d_6) (300 MHz) – δ 0.97 (t, *J* = 7.2 Hz, 3H), 1.46-1.51 (m, 2H), 1.72-1.74 (m, 2H), 2.27 (s, 3H), 3.96 (t, *J* = 6.0 Hz, 2H), 6.85-6.97 (m, 3Ar-H), 7.15 (brs, 2Ar-H), 7.44 (d, *J* = 7.2 Hz, 2Ar-H), 7.96 (d, *J* = 8.1 Hz, 1N-H), 8.26 (brs, 1N-H). ^{13}C NMR (d_6-DMSO) (100 MHz) – δ 13.7, 17.9, 18.7, 30.8, 67.3, 114.6, 119.7, 120.9, 122.4, 126.1, 127.2, 130.1, 132.8, 137.6, 152.9, 153.8. HRMS (ESI+): m/z calcd for $C_{18}H_{23}N_2O_2$ $[M+H]^+$: 299.1760; found: 299.1764.

1-(4-((3-methylbut-2-en-1-yl)oxy)phenyl)-3-phenylurea (47):

To a solution of Prenyl alcohol (0.0415 g; 0.48 mmol) in dry THF, TPP (0.149 g; 0.57 mmol) and DIAD (0.115 g; 0.57mmol) were added consecutively and stirred at RT for 15 minutes. Thereafter, comp. **21** (0.1 g; 0.44 mmol) was added slowly to the reaction mixture under nitrogen atmosphere and the stirring was continued for 8 hours at room temperature. After aqueous workup the crude was purified by column chromatography (20% EtOAc in Hexane). Yield: 42.3%; off white solid; m.p. 112-114 ^{o}C. ^{1}H NMR (acetone-d_6) (300 MHz) – δ 1.61 (d, *J* = 9.0 Hz, 6H), 4.37 (d, *J* = 6.3 Hz, 2H), 5.31 (t, *J* = 5.1 Hz, 1H), 6.69-6.74 (m, 2Ar-H), 6.82 (t, *J* = 7.2 Hz, 1Ar-H), 7.12 (t, *J* = 8.1 Hz , 2Ar-H), 7.25-7.29 (m, 2Ar-H) 7.38 (d, *J* = 7.8 Hz, 2Ar-H), 7.82 (brs, 1N-H), 7.94 (brs, 1N-H). ^{13}C NMR (acetone-d_6) (75 MHz) – δ 17.4, 25.0, 64.8, 114.8, 118.4, 118.5, 120.4, 120.5, 121.8, 121.9, 128.7, 132.9, 136.6, 140.2, 152.9, 154.6. HRMS (ESI+): m/z calcd for $C_{18}H_{21}N_2O_2$ $[M+H]^+$: 297.1603; found: 297.1606.

1-(4-((3-methylbut-2-en-1-yl)oxy)phenyl)-3-(3-nitrophenyl)urea (48):

To a solution of Prenyl alcohol (0.035 g; 0.40 mmol) in dry THF, TPP (0.125 g; 0.48 mmol) and DIAD (0.096 g; 0.48 mmol) were added consecutively and stirred at RT for 15 minutes. Thereafter, comp. **22** (0.1 g; 0.37 mmol) was added slowly to the reaction mixture under nitrogen atmosphere and the stirring was continued for 8 hours at room temperature. After aqueous workup the crude was purified by column chromatography (16% EtOAc in Hexane). Yield: 53.7%; pale brown solid; m.p. 164-166 ^{o}C. ^{1}H NMR (acetone-d_6) (300 MHz) – δ 1.62 (d, *J* = 7.8 Hz, 6H), 4.39 (d, *J* = 6.6 Hz, 2H), 5.32 (t, *J* = 1.5 Hz, 1H), 6.75 (dd, *J* = 1.2, 9 Hz, 2Ar-H), 7.31 (m, 2Ar-H), 7.41-7.45(m, 1Ar-H), 7.67-7.72 (m, 2Ar-H), 8.01 (brs, 1N-H), 8.46 (brs, 1N-H), 8.46-8.53 (m, 1Ar-H). ^{13}C NMR (acetone-d_6) (75 MHz) – δ 17.3, 24.9, 64.8, 112.6, 114.8, 116.2, 120.4, 120.9, 124.1, 129.7, 132.3, 136.6, 141.6, 148.8, 152.7, 154.9. HRMS (ESI+): m/z calcd for $C_{18}H_{20}N_3O_4$ $[M+H]^+$: 342.1454; found: 342.1456.

1-(4-((3-methylbut-2-ene-1-yl)oxy)phenyl)-3-(o-tolyl)urea (49):

To a solution of Prenyl alcohol (0.039 g; 0.45 mmol) in dry THF, TPP (0.141 g; 0.54 mmol) and DIAD (0.108 g; 0.54 mmol) were added consecutively and stirred at RT for 15 minutes. Thereafter, comp. **23** (0.1 g; 0.41 mmol) was added slowly to the reaction mixture under nitrogen atmosphere and the stirring was continued for 8 hours at room temperature. After aqueous

workup the crude was purified by column chromatography (20% EtOAc in Hexane). Yield: 25.5%; yellow solid; m.p. 52-53 ^{o}C; ^{1}H NMR (300 MHz) ($CDCl_3$) – δ 1.74 (s, 3H), 1.80 (s, 3H), 2.20 (s, 3H), 4.49 (d, *J* = 6.9 Hz, 2H), 5.46-5.50 (m, 1H), 6.28 (s, 1N-H), 6.40 (s, 1N-H), 6.85-6.90 (m, 2Ar-H), 7.11 (t, *J* = 7.5 Hz, 1Ar-H), 7.20-7.26 (m, 4Ar-H), 7.59 (d, *J* = 7.8 Hz, 1Ar-H). ^{13}C NMR (100 MHz) (d_6-DMSO) – δ 17.9, 18.0, 25.4, 64.4, 114.8, 119.7, 120.2, 120.8, 122.4, 126.1, 127.2, 130.1, 132.8, 136.7, 137.6, 152.8, 153.5. HRMS (ESI+): m/z calcd for $C_{19}H_{23}N_2O_2$ $[M+H]^+$: 311.1760; found: 311.1757.

1-(3-(allyloxy)phenyl)-3-phenylurea (50):

To a solution of Allyl alcohol (0.028 g; 0.48 mmol) in dry THF, TPP (0.149 g; 0.57 mmol) and DIAD (0.115 g; 0.57 mmol) were added consecutively and stirred at RT for 15 minutes. Thereafter, comp. **24** (0.1 g; 0.4381 mmol) was added slowly to the reaction mixture under nitrogen atmosphere and the stirring was continued for 8 hours at room temperature. After aqueous workup the crude was purified by column chromatography (20% EtOAc in Hexane). Yield: 45.2%; off white solid; m.p. 142-144 ^{o}C. ^{1}H NMR (acetone-d_6) (300 MHz) – δ 4.40-4.43 (m, 2H), 5.11 (dd, *J* = 1.5, 10.5 Hz, 1H), 5.29 (dd, *J* = 1.5, 17.1 Hz, 1H), 5.90-5.93 (m, 1H), 6.41 (dd, *J* = 2.4, 8.1 Hz, 1Ar-H), 6.85-6.87 (m, 2Ar-H), 7.00-7.03 (m, 1Ar-H), 7.14 (t, *J* = 8.4 Hz, 2Ar-H), 7.22 (t, *J* = 2.1 Hz, 1Ar-H), 7.40 (d, *J* = 7.8 Hz, 2Ar-H), 8.01 (brs, 2N-H). ^{13}C NMR (acetone-d_6) (75 MHz) – δ 68.3, 105.2, 108.2, 111.0, 116.5, 118.5, 122.1, 128.7, 129.4, 133.9, 140.0, 141.3, 152.7, 159.2. HRMS (ESI+): m/z calcd for $C_{16}H_{17}N_2O_2$ $[M+H]^+$: 269.1290; found: 269.1291.

1-(3-(Allyloxy)phenyl)-3-(3-nitrophenyl)urea (51):

To a solution of Allyl alcohol (0.024 g; 0.40 mmol) in dry THF, TPP (0.125 g; 0.48 mmol) and DIAD (0.096 g; 0.48 mmol) were added consecutively and stirred at RT for 15 minutes. Thereafter, comp. **25** (0.1 g; 0.366 mmol) was added slowly to the reaction mixture under nitrogen atmosphere and the stirring was continued for 8 hours at room temperature. After aqueous workup the crude was purified by column chromatography (16% EtOAc in Hexane). Yield: 59.2%; pale yellow solid; m.p. 126-128 ^{o}C; ^{1}H NMR (300 MHz) (acetone-d_6) – δ 4.56-4.59 (m, 2H), 5.26 (dd, *J* = 1.5, 10.8 Hz, 1H), 5.40-5.48 (m, 1H), 6.03-6.16 (m, 1H), 6.64 (dd, *J* = 2.4, 8.4 Hz, 1Ar-H), 7.02-7.05 (m, 1Ar-H), 7.20 (t, *J* = 8.1 Hz, 1Ar-H), 7.37 (t, *J* = 2.1 Hz,

1Ar-H), 7.57 (t, *J* = 8.1 Hz, 1Ar-H), 7.81-7.88 (m, 2Ar-H), 8.35 (brs, 1N-H), 8.67 (t, J = 2.1 Hz, 1N-H, 1Ar-H). ^{13}C NMR (75 MHz) (acetone-d_6 + $CDCl_3$) – δ 73.9, 111.1, 114.8, 116.6, 118.2, 121.3, 122.4, 129.4, 134.9, 138.4, 153.0, 157.4, 162.1, 164.1. HRMS (ESI+): m/z calcd for $C_{16}H_{16}N_3O_4$ $[M+H]^+$: 314.1141 ; found: 314.1138.

1-(3-(allyloxy)phenyl)-3-(o-tolyl)urea (52):

To a solution of Allyl alcohol (0.026 g; 0.45 mmol) in dry THF, TPP (0.141 g; 0.54 mmol) and DIAD (0.108 g; 0.54 mmol) were added consecutively and stirred at RT for 15 minutes. Thereafter, comp. **26** (0.1 g; 0.41 mmol) was added slowly to the reaction mixture under nitrogen atmosphere and the stirring was continued for 8 hours at room temperature. After aqueous workup the crude was purified by column chromatography (20% EtOAc in Hexane). Yield: 69.4; white solid; m.p. 144-146 ^{o}C. ^{1}H NMR (acetone-d_6) (300 MHz) – δ 2.27 (s, 3H), 4.54-4.56 (m, 2H), 5.23-5.27 (m, 1H), 5.43 (dd, *J* = 1.5, 17.1 Hz, 1H), 6.13-6.16 (m, 1H), 6.59 (dd, *J* = 2.4, 8.4 Hz, 1Ar-H), 6.97-7.01(m, 2Ar-H), 7.14-7.19 (m, 3Ar-H), 7.37 (t, *J* = 2.1 Hz, 1Ar-H), 7.53 (brs, 1N-H), 7.94 (d, *J* = 8.4 Hz, 1Ar-H), 8.47 (brs, 1N-H). ^{13}C NMR (d_6-DMSO) (100 MHz) – δ 17.9, 68.0, 104.5, 107.8, 110.5, 117.2, 121.1, 122.7, 126.1, 127.5, 129.5, 130.2, 133.7, 137.3, 141.1, 152.6, 158.6. HRMS (ESI+): m/z calcd for $C_{17}H_{19}N_2O_2$ $[M+H]^+$: 283.1446; found: 283.1451.

1-(3-butoxyphenyl)-3-phenylurea (53):

To a solution of n-Butyl alcohol (0.036 g; 0.48 mmol) in dry THF, TPP (0.149 g; 0.57 mmol) and DIAD (0.115 g; 0.57 mmol) were added consecutively and stirred at RT for 15 minutes. Thereafter, comp. **24** (0.1 g; 0.44 mmol) was added slowly to the reaction mixture under nitrogen atmosphere and the stirring was continued for 8 hours at room temperature. After aqueous workup the crude was purified by column chromatography (20% EtOAc in Hexane). Yield: 56.8%; off white solid; m.p. 138-140 ^{o}C. ^{1}H NMR (acetone-d_6) (300 MHz) – δ 0.98 (t, *J* = 7.2 Hz, 3H), 1.47-1.54 (m, 2H), 1.73-1.76 (m, 2H), 3.98 (t, *J* = 6.3 Hz, 2H), 6.57 (dt, *J* = 0.6, 8.1 Hz, 1Ar-H), 7.01-0.96 (m, 2Ar-H), 7.16 (t, *J* = 8.1 Hz, 1Ar-H), 7.25 (t, *J* = 8.4 Hz, 2Ar-H), 7.35 (t, *J* = 2.1 Hz, 1Ar-H), 7.55 (d, *J* = 8.4 Hz, 2Ar-H), 8.14 (brs, 2N-H). ^{13}C NMR (acetone-d_6) (75 MHz) – δ 13.2, 19.1, 31.3, 67.3, 105.1, 108.2, 110.7, 118.6, 122.1, 128.7, 129.4, 140.0, 141.3, 152.6, 159.9. HRMS (ESI+): m/z calcd for $C_{17}H_{21}N_2O_2$ $[M+H]^+$: 285.1603; found: 285.1608.

1-(3-butoxyphenyl)-3-(3-nitrophenyl)urea (54):

To a solution of n-Butyl alcohol (0.030 g; 0.40 mmol) in dry THF, TPP (0.125 g; 0.48 mmol) and DIAD (0.096 g; 0.48 mmol) were added consecutively and stirred at RT for 15 minutes. Thereafter, comp. **25** (0.1 g; 0.37 mmol) was added slowly to the reaction mixture under nitrogen atmosphere and the stirring was continued for 8 hours at room temperature. After aqueous workup the crude was purified by column chromatography (16% EtOAc in Hexane). Yield: 68.5%; Pale yellow solid; m.p. 108-110 ^{o}C. ^{1}H NMR ($CDCl_3$ + acetone-d_6) (300 MHz) – δ 0.95 (t, *J* = 7.2 Hz, 3H), 1.41-1.48 (m, 2H), 1.69-1.74 (m, 2H), 3.88 (t, *J* = 6.6 Hz, 2H), 6.62 (d, *J* = 7.5 Hz, 1Ar-H), 6.80 (d, *J* = 7.8 Hz, 1Ar-H), 6.99 (s, 1Ar-H), 7.15 (t, *J* = 8.1 Hz, 1Ar-H), 7.36 (t, *J* = 8.4 Hz, 1Ar-H), 7.50 (brs, 1N-H), 7.72 (d, *J* = 8.1 Hz, 1Ar-H), 7.80-7.85 (m, 1Ar-H), 7.85 (brs, 1N-H), 8.1 (s, 1Ar-H). ^{13}C NMR ($CDCl_3$) (75 MHz) – δ 13.8, 19.2, 31.3, 67.8, 107.8, 110.6, 113.2, 114.3, 117.8, 125.5, 129.7, 130.0, 138.5, 139.6, 148.5, 153.4, 160.0 HRMS (ESI+): m/z calcd for $C_{17}H_{20}N_3O_4$ $[M+H]^+$: 330.1454; found: 330.1457.

1-(3-butoxyphenyl)-3-(o-tolyl)urea (55):

To a solution of n-Butyl alcohol (0.0336 g; 0.45 mmol) in dry THF, TPP (0.141 g; 0.54 mmol) and DIAD (0.108 g; 0.54 mmol) were added consecutively and stirred at RT for 15 minutes. Thereafter, comp. **26** (0.1 g; 0.41 mmol) was added slowly to the reaction mixture under nitrogen atmosphere and the stirring was continued for 8 hours at room temperature. After aqueous workup the crude was purified by column chromatography (20% EtOAc in Hexane). Yield: 65.8%; white solid; m.p. 120-122 ^{o}C. ^{1}H NMR (acetone-d_6) (300 MHz) – δ 0.97 (t, *J* = 7.5 Hz, 3H), 1.46-1.54 (m, 2H), 1.72-1.77 (m, 2H), 2.27 (s, 3H), 3.97 (t, *J* = 6.3 Hz, 2H), 6.56 (dd, *J* = 2.4, 8.1 Hz, 1Ar-H), 6.95-6.99 (m, 2Ar-H), 7.12-7.18 (m, 3Ar-H), 7.36 (t, *J* = 1.8 Hz, 1Ar-H), 7.53 (brs, 1N-H), 7.94 (d, *J* = 8.1 Hz, 1Ar-H), 8.45 (brs, 1N-H). ^{13}C NMR (acetone-d_6) (75 MHz) – δ 13.3, 17.3, 19.1, 31.3, 67.3, 104.9, 108.1, 110.6, 121.7, 123.1, 126.2, 128.1, 129.4, 130.2, 137.7, 141.5, 152.9, 159.9. HRMS (ESI+): m/z calcd for $C_{18}H_{23}N_2O_2$ $[M+H]^+$: 299.1759; found: 299.1766.

1-(3-((3-methylbut-2-en-1-yl)oxy)phenyl)-3-phenylurea (56):

To a solution of Prenyl alcohol (0.0415 g; 0.48 mmol) in dry THF, TPP (0.149 g; 0.57 mmol) and DIAD (0.115 g; 0.57 mmol) were added consecutively and stirred at RT for 15 minutes.

Thereafter, comp. **24** (0.1 g; 0.44 mmol) was added slowly to the reaction mixture under nitrogen atmosphere and the stirring was continued for 8 hours at room temperature. After aqueous workup the crude was purified by column chromatography (20% EtOAc in Hexane). Yield: 48.3%; off white solid; m.p. 124-126 ^{o}C. ^{1}H NMR (acetone-d_6) (300 MHz) – δ 1.60 (d, *J* = 9.0 Hz, 6H), 4.40 (d, *J* = 6.6 Hz, 2H), 5.31-5.34 (m, 1H), 6.43 (dd, *J* = 1.8, 7.5 Hz, 1Ar-H), 6.82-6.88 (m, 2Ar-H), 7.02 (t, *J* = 8.1 Hz, 1Ar-H), 7.11-7.19 (m, 3Ar-H), 7.41 (d, *J* = 8.7 Hz, 2Ar-H), 8.0 (brs, 2N-H). ^{13}C NMR (acetone-d_6) (75 MHz) – δ 17.4, 25.0, 64.5, 67.5, 105.2, 108.3, 110.8, 118.6, 120.3, 122.1, 128.7, 129.4, 131.3, 136.8, 140.1, 141.3, 152.7, 159.6. HRMS (ESI+): m/z calcd for $C_{18}H_{21}N_2O_2$ $[M+H]^+$: 297.1603; found: 297.1606.

1-(3-((3-methylbut-2-en-1-yl)oxy)phenyl)-3-(3-nitrophenyl)urea (57):

To a solution of Prenyl alcohol (0.035 g; 0.40 mmol) in dry THF, TPP (0.125 g; 0.48 mmol) and DIAD (0.096 g; 0.48 mmol) were added consecutively and stirred at RT for 15 minutes. Thereafter, comp. **25** (0.1 g; 0.37 mmol) was added slowly to the reaction mixture under nitrogen atmosphere and the stirring was continued for 8 hours at room temperature. After aqueous workup the crude was purified by column chromatography (16% EtOAc in Hexane). Yield: 64.1%; yellow solid; m.p. 82-84 ^{o}C. ^{1}H NMR (acetone-d_6) (300 MHz) – δ 1.63 (d, *J* = 5.8 Hz, 6H), 4.41 (d, *J* = 6.6 Hz, 2H), 5.33 (t, *J* = 1.2 Hz, 1H), 6.46-6.48 (m, 1Ar-H), 6.88-6.91 (m, 1Ar-H), 7.05 (t, *J* = 8.1 Hz, 1Ar-H), 7.18 (t, *J* = 2.1 Hz, 1Ar-H), 7.43 (t, *J* = 8.4 Hz, 1Ar-H), 7.68-7.74 (m, 2Ar-H), 8.19 (brs, 1N-H), 8.52 (s, 1N-H) 8.53 (s, 1Ar-H). ^{13}C NMR ($CDCl_3$) (75 MHz) – δ 18.2, 25.8, 64.8, 106.6, 110.0, 112.3, 113.8, 117.2, 119.6, 125.0, 129.6, 138.2, 139.5, 140.4, 148.6, 152.9, 156.6, 159.6. HRMS (ESI+): m/z calcd for $C_{18}H_{20}N_3O_4$ $[M+H]^+$: 342.1454; found: 342.1455.

1-(3-((3-methylbut-2-en-1-yl)oxy)phenyl)-3-(o-tolyl)urea (58):

To a solution of Prenyl alcohol (0.039 g; 0.45 mmol) in dry THF, TPP (0.141 g; 0.54 mmol) and DIAD (0.108 g; 0.54 mmol) were added consecutively and stirred at RT for 15 minutes. Thereafter, comp. **26** (0.1 g; 0.41 mmol) was added slowly to the reaction mixture under nitrogen atmosphere and the stirring was continued for 8 hours at room temperature. After aqueous workup the crude was purified by column chromatography (20% EtOAc in Hexane). Yield: 51.3%; off white solid; m.p. 130-132 ^{o}C. ^{1}H NMR (acetone-d_6) (300 MHz) – δ 1.62 (d, *J* = 7.8

Hz, 6H), 2.13 (s, 3H), 4.39 (d, *J* = 6.6 Hz, 2H), 5.32 (m, 1H), 6.40 (dd, *J* = 1.8, 7.5 Hz, 1Ar-H), 6.82-6.86 (m, 2Ar-H), 6.98-7.04 (m, 3Ar-H), 7.19 (t, *J* = 2.1 Hz, 1Ar-H), 7.37 (brs, 1N-H), 7.71-7.81 (m, 1Ar-H), 8.29 (brs, 1N-H). ^{13}C NMR (acetone-d_6) (75 MHz) – δ 17.3, 17.4, 25.0, 64.4, 105.0, 108.3, 110.6, 120.4, 121.7, 123.1, 126.3, 127.9, 129.4, 130.2, 136.8, 137.7, 141.4, 152.8, 159.6. HRMS (ESI+): m/z calcd for $C_{19}H_{23}N_2O_2$ $[M+H]^+$: 311.1760; found: 311.1761.

1-(3-((3-methylbut-2-en-1-yl)oxy)phenyl)-3-(m-tolyl)urea (59):

To a solution of Prenyl alcohol (0.039 g; 0.45 mmol) in dry THF, TPP (0.141 g; 0.54 mmol) and DIAD (0.108 g; 0.54 mmol) were added consecutively and stirred at RT for 15 minutes. Thereafter, comp. **27** (0.1 g; 0.41 mmol) was added slowly to the reaction mixture under nitrogen atmosphere and the stirring was continued for 8 hours at room temperature. After aqueous workup the crude was purified by column chromatography (20% EtOAc in Hexane). Yield: 62.5%; off white solid; m.p. 106-108 ^{o}C. ^{1}H NMR (300 MHz) ($CDCl_3$) – δ 1.72 (s, 3H), 1.79 (s, 3H), 2.31 (s, 3H), 4.48 (d, *J* = 6.6 Hz, 2H), 5.45-5.49 (m, 1H), 6.65-6.69 (m, 1Ar-H), 6.76 (d, *J* = 4.5 Hz, 2Ar-H), 6.82-6.85 (m, 1Ar-H), 6.92 (d, *J* = 7.5 Hz, 1Ar-H), 7.04 (t, *J* = 2.4 Hz, 1Ar-H), 7.10-7.12 (m, 1Ar-H), 7.16-7.17 (m, 1Ar-H), 7.19-7.22 (m, 2N-H). ^{13}C NMR (125 MHz) ($CDCl_3$) – δ 18.2, 21.4, 25.8, 64.7, 106.9, 110.4, 112.8, 118.0, 119.6, 121.6, 124.7, 128.9, 129.7, 137.9, 138.2, 139.0, 139.4, 154.1, 159.6. HRMS (ESI+): m/z calcd for $C_{19}H_{23}N_2O_2$ $[M+H]^+$: 311.1760; found: 311.1758.

1-(3-((3-methylbut-2-en-1-yl)oxy)phenyl)-3-(p-tolyl)urea (60):

To a solution of Prenyl alcohol (0.039 g; 0.45 mmol) in dry THF, TPP (0.141 g; 0.54 mmol) and DIAD (0.108 g; 0.54 mmol) were added consecutively and stirred at RT for 15 minutes. Thereafter, comp. **28** (0.1 g; 0.41 mmol) was added slowly to the reaction mixture under nitrogen atmosphere and the stirring was continued for 8 hours at room temperature. After aqueous workup the crude was purified by column chromatography (20% EtOAc in Hexane). Yield: 56.9%; off white solid; m.p. 154-156 ^{o}C. ^{1}H NMR (300 MHz) ($CDCl_3$) – δ 1.69 (s, 3H), 1.76 (s, 3H), 2.27 (s, 3H), 4.42 (d, *J* = 6.9 Hz, 2H), 5.41-5.47 (m, 1H), 6.60-6.63 (m, 1Ar-H), 6.77 (dd, *J* = 1.8, 8.1 Hz, 1Ar-H), 7.01-7.15 (m, *7*Ar-H), 7.26 (s, 1N-H). ^{13}C NMR (100 MHz) ($CDCl_3$) – δ 18.4, 21.0, 26.0, 65.0, 107.3, 110.6, 113.1, 119.7, 121.8, 129.9, 130.0, 134.1, 135.4, 138.5, 139.6,

154.2, 155.4, 156.9, 159.8. HRMS (ESI+): m/z calcd for $C_{19}H_{23}N_2O_2$ $[M+H]^+$: 311.1760; found: 311.1759.

1-(2-fluorophenyl)-3-(3-((3-methylbut-2-en-1-yl)oxy)phenyl)urea (61):

To a solution of Prenyl alcohol (0.0385 g; 0.45 mmol) in dry THF, TPP (0.138 g; 0.53 mmol) and DIAD (0.107 g; 0.53 mmol) were added consecutively and stirred at RT for 15 minutes. Thereafter, comp. **29** (0.1 g; 0.41 mmol) was added slowly to the reaction mixture under nitrogen atmosphere and the stirring was continued for 6 hours at room temperature. After aqueous workup the crude was purified by column chromatography (15% EtOAc in Hexane). Yield: 65.3%; off white solid; m.p. 138-140 ^oC. ^1H NMR (300 MHz) ($CDCl_3$) – δ 1.73 (s, 3H), 1.79 (s, 3H), 4.50 (d, *J* = 6.9 Hz, 2H), 5.48 (t, *J* = 1.5 Hz, 1H), 6.69-6.72 (m, 1Ar-H), 6.83 (bs, 1N-H), 6.87 (dd, *J* = 1.8, 7.8 Hz, 1Ar-H), 6.96-7.07 (m, 3Ar-H and 1N-H), 7.09-7.14 (m, 1Ar-H), 7.23 (t, *J* = 8.1 Hz, 1Ar-H), 8.10 (dt, *J* = 1.5, 8.1 Hz, 1Ar-H). ^{13}C NMR (125 MHz) ($CDCl_3$) – δ 18.2, 25.8, 64.8, 107.5, 111.1, 113.3, 114.9, 119.6, 122.1, 123.8, 124.6, 126.6, 130.0, 152.2, 153.1, 159.7. HRMS (ESI+): m/z calcd for $C_{18}H_{20}FN_2O_2$ $[M+H]^+$: 315.1509; found: 315.1511.

1-(3-fluorophenyl)-3-(3-((3-methylbut-2-en-1-yl)oxy)phenyl)urea (62):

To a solution of Prenyl alcohol (0.0385 g; 0.45 mmol) in dry THF, TPP (0.138 g; 0.53 mmol) and DIAD (0.107 g; 0.53 mmol) were added consecutively and stirred at RT for 15 minutes. Thereafter, comp. **30** (0.1 g; 0.41 mmol) was added slowly to the reaction mixture under nitrogen atmosphere and the stirring was continued for 6 hours at room temperature. After aqueous workup the crude was purified by column chromatography (15% EtOAc in Hexane). Yield: 59.8%; off white solid; m.p. 116-118 ^oC. ^1H NMR (300 MHz) ($CDCl_3$) – δ 1.71 (s, 3H), 1.77 (s, 3H), 4.45 (d, *J* = 6.6 Hz, 2H), 5.43-5.47 (m, 1H), 6.65-6.81 (m, 3Ar-H), 6.96-6.99 (m, 3Ar-H), 7.14-7.18 (m, 2Ar-H and 2N-H). ^{13}C NMR (125 MHz) ($CDCl_3$) – δ 18.1, 25.8, 64.8, 107.5, 107.7, 110.3, 110.7, 113.4, 115.6, 119.4, 129.9, 138.3, 138.8, 139.7, 153.9, 159.6, 162.0, 164.0. HRMS (ESI+): m/z calcd for $C_{18}H_{20}FN_2O_2$ $[M+H]^+$: 315.1509; found: 315.1510.

1-(4-fluorophenyl)-3-(3-((3-methylbut-2-en-1-yl)oxy)phenyl)urea (63):

To a solution of Prenyl alcohol (0.0385 g; 0.45 mmol) in dry THF, TPP (0.138 g; 0.53 mmol) and DIAD (0.107 g; 0.53 mmol) were added consecutively and stirred at RT for 15 minutes.

Thereafter, comp. **31** (0.1 g; 0.41 mmol) was added slowly to the reaction mixture under nitrogen atmosphere and the stirring was continued for 6 hours at room temperature. After aqueous workup the crude was purified by column chromatography (15% EtOAc in Hexane). Yield: 63.4%; off white solid; m.p. 156-158 ^{o}C. ^{1}H NMR (300 MHz) ($CDCl_3$) – δ 1.73 (s, 3H), 1.79 (s, 3H), 4.50 (d, *J* = 6.6 Hz, 2H), 5.46-5.51 (m, 1H), 6.59-6.62 (m, 1Ar-H), 6.94-7.02 (m, 2Ar-H), 7.16 (t, *J* = 8.1 Hz, 1Ar-H), 7.20 (t, *J* = 2.4 Hz, 1Ar-H), 7.36-7.40 (m, 2Ar-H), 7.57 (s, 1N-H), 7.63 (s, 1N-H). ^{13}C NMR (125 MHz) (d_6-DMSO) – δ 18.5, 25.9, 64.6, 105.2, 108.4, 111.0, 115.6, 115.8, 120.5, 129.9, 136.5, 137.3, 141.3, 153.0, 156.6, 156.9, 159.3, 158.8. HRMS (ESI+): m/z calcd for $C_{18}H_{20}FN_2O_2$ $[M+H]^+$: 315.1509; found: 315.1513.

1-(2-chlorophenyl)-3-(3-((3-methylbut-2-en-1-yl)oxy)phenyl)urea (64):

To a solution of Prenyl alcohol (0.036 g; 0.42 mmol) in dry THF, TPP (0.130 g; 0.49 mmol) and DIAD (0.1 g; 0.49 mmol) were added consecutively and stirred at RT for 15 minutes. Thereafter, comp. **32** (0.1 g; 0.38 mmol) was added slowly to the reaction mixture under nitrogen atmosphere and the stirring was continued for 6 hours at room temperature. After aqueous workup the crude was purified by column chromatography (15% EtOAc in Hexane). Yield: 67.3%; off white solid; m.p. 122-124 ^{o}C. ^{1}H NMR (300 MHz) ($CDCl_3$) – δ 1.74 (s, 3H), 1.79 (s, 3H), 4.51 (d, *J* = 6.9 Hz, 2H), 5.46-5.51 (m, 1H), 6.72-6.76 (m, 1Ar-H), 6.78 (s, 1N-H), 6.91 (dd, *J* = 1.2, 7.8 Hz, 1Ar-H), 6.99 (dt, *J* = 1.5, 7.8 Hz, 1Ar-H), 7.04 (t, *J* = 2.1 Hz, 1Ar-H), 7.22-7.23 (m, 1Ar-H), 7.25-7.26 (s, 1N-H), 7.28-7.29 (m, 1Ar-H), 7.33 (dd, *J* = 1.5, 8.1 Hz, 1Ar-H), 8.19 (dd, *J* = 1.5, 8.4 Hz, 1Ar-H). ^{13}C NMR (100 MHz) ($CDCl_3$) – δ 18.2, 25.8, 64.9, 108.0, 111.5, 113.8, 119.4, 121.8, 123.3, 123.9, 127.7, 129.1, 130.1, 135.1, 138.5, 138.8, 152.9, 159.8. HRMS (ESI+): m/z calcd for $C_{18}H_{20}ClN_2O_2$ $[M+H]^+$: 331.1213; found: 331.1217.

1-(3-chlorophenyl)-3-(3-((3-methylbut-2-en-1-yl)oxy)phenyl)urea (65):

To a solution of Prenyl alcohol (0.036 g; 0.42 mmol) in dry THF, TPP (0.130 g; 0.49 mmol) and DIAD (0.1 g; 0.49 mmol) were added consecutively and stirred at RT for 15 minutes. Thereafter, comp. **33** (0.1 g; 0.38 mmol) was added slowly to the reaction mixture under nitrogen atmosphere and the stirring was continued for 6 hours at room temperature. After aqueous workup the crude was purified by column chromatography (15% EtOAc in Hexane). Yield: 73.6%; off white solid; m.p. 126-128 ^{o}C. ^{1}H NMR (300 MHz) ($CDCl_3$) – δ 1.73 (s, 3H), 1.79 (s,

3H), 4.49 (d, *J* = 6.6 Hz, 2H), 5.45-5.49 (m, 1H), 6.70-6.73 (m, 2Ar-H), 6.82-6.86 (m, 2Ar-H), 6.99 (bs, 1N-H), 7.04-7.05 (m, 1Ar-H), 7.20-7.21 (m, 3Ar-H), 7.40 (s, 1N-H). ^{13}C NMR (100 MHz) ($CDCl_3$) – δ 18.3, 25.9, 64.9, 107.9, 111.0, 113.5, 118.4, 119.4, 120.4, 123.8, 130.1, 134.7, 138.5, 138.8, 139.3, 153.6, 159.7. HRMS (ESI+): m/z calcd for $C_{18}H_{20}ClN_2O_2$ $[M+H]^+$: 331.1213; found: 331.1215.

1-(4-chlorophenyl)-3-(3-((3-methylbut-2-en-1-yl)oxy)phenyl)urea (66):

To a solution of Prenyl alcohol (0.036 g; 0.42 mmol) in dry THF, TPP (0.130 g; 0.49 mmol) and DIAD (0.1 g; 0.49 mmol) were added consecutively and stirred at RT for 15 minutes. Thereafter, comp. **34** (0.1 g; 0.38 mmol) was added slowly to the reaction mixture under nitrogen atmosphere and the stirring was continued for 6 hours at room temperature. After aqueous workup the crude was purified by column chromatography (15% EtOAc in Hexane). Yield: 66.4%; off white solid; m.p. 176-178 ^{o}C; ^{1}H NMR (300 MHz) ($CDCl_3$) – δ 1.74 (s, 3H), 1.79 (s, 3H), 4.50 (d, *J* = 6.9 Hz, 2H), 5.46-5.51 (m, 1H), 6.61-6.65 (m, 1Ar-H), 6.85-6.89 (m, 1Ar-H), 7.14-7.15 (m, 1Ar-H), 7.18 (s, 1Ar-H), 7.21-7.26 (m, 2Ar-H), 7.35-7.39 (m, 2Ar-H), 7.40 (s, 1N-H), 7.51 (s, 1N-H). ^{13}C NMR (100 MHz) (Acetone-d_6) – δ 17.3, 24.9, 64.4, 105.3, 108.5, 110.7, 110.8, 119.9, 120.0, 120.3, 126.7, 128.5, 129.3, 136.7, 138.9, 140.9, 152.3, 159.5. HRMS (ESI+): m/z calcd for $C_{18}H_{20}ClN_2O_2$ $[M+H]^+$: 331.1213; found: 331.1213.

1-(2-bromophenyl)-3-(3-((3-methylbut-2-en-1-yl)oxy)phenyl)urea (67):

To a solution of Prenyl alcohol (0.031 g; 0.32 mmol) in dry THF, TPP (0.111 g; 0.42 mmol) and DIAD (0.0856 g; 0.42 mmol) were added consecutively and stirred at RT for 15 minutes. Thereafter, comp. **35** (0.1 g; 0.33 mmol) was added slowly to the reaction mixture under nitrogen atmosphere and the stirring was continued for 6 hours at room temperature. After aqueous workup the crude was purified by column chromatography (15% EtOAc in Hexane). Yield: 63.1 %; off white solid; m.p. 78-80 ^{o}C. ^{1}H NMR (300 MHz) (Acetone-d_6) – δ 1.61 (d, *J* = 6.3 Hz, 6H), 4.40 (d, *J* = 6.3 Hz, 2H), 5.31-5.33 (m, 1H), 6.45 (dd, *J* = 2.1, 7.8 Hz, 1Ar-H), 6.86 (d, *J* = 7.2 Hz, 1Ar-H), 7.00-7.27 (m, 5Ar-H), 7.82 (t, *J* = 1.8 Hz, 1Ar-H), 8.04 (s, 1N-H), 8.14 (s, 1N-H). ^{13}C NMR (100 MHz) (Acetone-d_6) – δ 15.9, 23.5, 63.0, 103.9, 107.2, 109.4, 115.7, 118.9, 119.6, 120.6, 123.3, 127.9, 128.9, 135.3, 139.4, 140.2, 150.8, 158.1. HRMS (ESI+): m/z calcd for $C_{18}H_{20}BrN_2O_2$ $[M+H]^+$: 375.0708; found: 375.0710.

1-(3-bromophenyl)-3-(3-((3-methylbut-2-en-1-yl)oxy)phenyl)urea (68):

To a solution of Prenyl alcohol (0.031 g; 0.36 mmol) in dry THF, TPP (0.111 g; 0.42 mmol) and DIAD (0.0856 g; 0.42 mmol) were added consecutively and stirred at RT for 15 minutes. Thereafter, comp. **36** (0.1 g; 0.33 mmol) was added slowly to the reaction mixture under nitrogen atmosphere and the stirring was continued for 6 hours at room temperature. After aqueous workup the crude was purified by column chromatography (15% EtOAc in Hexane). Yield: 71.2%; off white solid; m.p. 84-86 ^{o}C. ^{1}H NMR (400 MHz) (d_6-DMSO) – δ 1.72 (s, 3H), 1.75 (s, 3H), 4.50 (d, *J* = 6.4 Hz, 2H), 5.41-5.45 (m, 1H), 6.57 (dd, *J* = 2.4, 8.4 Hz, 1Ar-H), 6.83-6.87 (m, 1Ar-H), 7.14-7.24 (m, 5Ar-H), 7.85 (t, *J* = 1.8 Hz, 1Ar-H), 8.71 (s, 1N-H), 8.84 (s, 1N-H). ^{13}C NMR (100 MHz) (d_6-DMSO) – δ 18.5, 25.9, 64.6, 105.3, 108.7, 111.1, 117.5, 120.5, 120.9, 122.2, 124.8, 130.0, 131.2, 137.4, 141.0, 141.8, 152.7, 159.3. HRMS (ESI+): m/z calcd for $C_{18}H_{20}BrN_2O_2$ $[M+H]^+$: 375.0708; found: 375.0709.

1-(4-bromophenyl)-3-(3-((3-methylbut-2-en-1-yl)oxy)phenyl)urea (69):

To a solution of Prenyl alcohol (0.031 g; 0.33 mmol) in dry THF, TPP (0.111 g; 0.42 mmol) and DIAD (0.0856 g; 0.42 mmol) were added consecutively and stirred at RT for 15 minutes. Thereafter, comp. **37** (0.1 g; 0.33 mmol) was added slowly to the reaction mixture under nitrogen atmosphere and the stirring was continued for 6 hours at room temperature. After aqueous workup the crude was purified by column chromatography (15% EtOAc in Hexane). Yield: 62.8%; white solid; m.p. 168-170 ^{o}C. ^{1}H NMR (300 MHz) (d_6-DMSO + $CDCl_3$) – δ 1.76 (s, 3H), 1.80 (s, 3H), 4.59 (d, *J* = 6.6 Hz, 2H), 5.11 (t, *J* = 6.6 Hz, 1H), 6.16-6.66 (m, 1Ar-H), 7.26-7.45 (m, 6Ar-H), 7.51 (dd, J = 2.1, 9.0 Hz, 1 Ar-H), 8.20-8.33 (m, 2N-H). ^{13}C NMR (100 MHz) (Acetone-d_6 + $CDCl_3$) – δ 17.6, 25.1, 65.4, 103.9, 108.5, 111.3, 114.1, 118.6, 119.5, 120.1, 128.3, 131.1, 132.2, 137.4, 138.1, 139.5, 152.0, 155.0. HRMS (ESI+): m/z calcd for $C_{18}H_{20}BrN_2O_2$ $[M+H]^+$: 375.0708; found: 375.0709.

1-(3-((3-methylbut-2-en-1-yl)oxy)phenyl)-3-(2-(trifluoromethyl)phenyl)urea (70):

To a solution of Prenyl alcohol (0.032 g; 0.37 mmol) in dry THF, TPP (0.115 g; 0.44 mmol) and DIAD (0.088 g; 0.44 mmol) were added consecutively and stirred at RT for 15 minutes. Thereafter, comp. **38** (0.1 g; 0.34 mmol) was added slowly to the reaction mixture under nitrogen atmosphere and the stirring was continued for 8 hours at room temperature. After aqueous

workup the crude was purified by column chromatography (18% EtOAc in Hexane). Yield: 72.5%; off white solid; m.p. 118-120 ^{o}C. ^{1}H NMR (300 MHz) ($CDCl_3$) – δ 1.73 (s, 3H), 1.79 (s, 3H), 4.49 (d, *J* = 6.9 Hz, 2H), 5.44-5.50 (m, 1H), 6.74 (dd, *J* = 2.4, 8.4 Hz, 1Ar-H), 6.84-6.87 (m, 2Ar-H), 7.03 (t, *J* = 2.1 Hz, 2Ar-H), 7.14-7.24 (m, 2Ar-H), 7.50-7.58 (m, 1Ar-H, 1N-H), 8.06 (d, *J* = 8.4 Hz, 1Ar-H). ^{13}C NMR (100 MHz) (d_6-DMSO) – δ 18.0, 25.4, 64.1, 104.6, 108.4, 110.4, 122.6, 123.7, 125.7, 125.9, 129.6, 132.9, 136.3, 136.9, 140.6, 152.4, 158.9. HRMS (ESI+): m/z calcd for $C_{19}H_{20}F_3N_2O_2$ $[M+H]^+$: 365.1477; found: 365.1481.

1-(3-((3-methylbut-2-en-1-yl)oxy)phenyl)-3-(3-(trifluoromethyl)phenyl)urea (71):

To a solution of Prenyl alcohol (0.032 g; 0.37 mmol) in dry THF, TPP (0.115 g; 0.44 mmol) and DIAD (0.088 g; 0.44 mmol) were added consecutively and stirred at RT for 15 minutes. Thereafter, comp. **39** (0.1 g; 0.34 mmol) was added slowly to the reaction mixture under nitrogen atmosphere and the stirring was continued for 8 hours at room temperature. After aqueous workup the crude was purified by column chromatography (18% EtOAc in Hexane). Yield: 64.5%; off white solid; m.p. 138-140 ^{o}C. ^{1}H NMR (300 MHz) ($CDCl_3$) – δ 1.72 (s, 3H), 1.78 (s, 3H), 4.48 (d, *J* = 6.9 Hz, 2H), 5.43-5.49 (m, 1H), 6.69-6.73 (m, 1Ar-H), 6.81-6.85 (m, 1Ar-H), 6.87 (bs, 1N-H), 6.99 (t, *J* = 2.4 Hz, 1Ar-H), 7.09 (bs, 1N-H), 7.22 (t, *J* = 8.1 Hz, 1Ar-H), 7.27-7.30 (m, 1Ar-H), 7.37 (t, *J* = 7.8 Hz, 1Ar-H), 7.54 (s, 1Ar-H), 7.57 (s, 1Ar-H). ^{13}C NMR (100 MHz) ($CDCl_3$) – δ 18.3, 26.0, 65.0, 108.1, 111.1, 113.7, 117.0, 119.5, 120.3, 122.1, 123.4, 125.4, 129.7, 130.2, 138.6, 138.9, 153.8, 159.9. HRMS (ESI+): m/z calcd for $C_{19}H_{20}F_3N_2O_2$ $[M+H]^+$: 365.1477; found: 365.1478.

1-(3-((3-methylbut-2-en-1-yl)oxy)phenyl)-3-(4-(trifluoromethyl)phenyl)urea (72):

To a solution of Prenyl alcohol (0.032 g; 0.37 mmol) in dry THF, TPP (0.115 g; 0.44 mmol) and DIAD (0.088 g; 0.44 mmol) were added consecutively and stirred at RT for 15 minutes. Thereafter, comp. **40** (0.1 g; 0.34 mmol) was added slowly to the reaction mixture under nitrogen atmosphere and the stirring was continued for 8 hours at room temperature. After aqueous workup the crude was purified by column chromatography (18% EtOAc in Hexane). Yield: 69.0%; off white solid; m.p. 154-156 ^{o}C. ^{1}H NMR (300 MHz) ($CDCl_3$) – δ 1.72 (s, 3H), 1.78 (s, 3H), 4.48 (d, *J* = 6.9 Hz, 2H), 5.43-5.49 (m, 1H), 6.71-6.75 (m, 1Ar-H), 6.77 (s, 1N-H), 6.83-6.86 (m, 1Ar-H), 6.99 (t, *J* = 2.1 Hz, 1Ar-H), 7.03 (s, 1N-H), 7.22 (d, *J* = 8.1 Hz, 1Ar-H), 7.44-

7.54 (m, 4Ar-H). ^{13}C NMR (100 MHz) ($CDCl_3$) – δ 18.2, 25.8, 64.8, 106.5, 110.0, 112.1, 116.6, 118.6, 119.6, 123.5, 126.1, 126.1, 129.7, 138.3, 139.6, 142.2, 152.8, 159.6. HRMS (ESI+): m/z calcd for $C_{19}H_{20}F_3N_2O_2$ $[M+H]^+$: 365.1477; found: 365.1479.

1-(4-(allyloxy)phenyl)-3-(3-cyanophenyl)urea (78):

To a solution of Allyl alcohol (0.025 g; 0.43 mmol) in dry THF, TPP (0.135 g; 0.51 mmol) and DIAD (0.104 g; 0.51 mmol) were added consecutively and stirred at RT for 15 minutes. Thereafter, comp. **77** (0.1 g; 0.39 mmol) was added slowly to the reaction mixture under nitrogen atmosphere and the stirring was continued for 6 hours at room temperature. After aqueous workup the crude was purified by column chromatography (20% EtOAc in Hexane). Yield: 62.7%; off white solid; m.p. 170-172 ^{o}C. ^{1}H NMR (acetone-d_6) (300 MHz) – δ 4.54-4.57 (m, 2H), 5.25 (dd, *J* = 1.8, 10.8 Hz, 1H), 5.39-5.45 (m, 1H), 6.03-6.07 (m, 1H), 6.90-6.93 (m, 2Ar-H), 7.35-7.38 (m, 1Ar-H), 7.43-7.51 (m, 3Ar-H), 7.72-7.76 (m, 1Ar-H), 8.07 (t, *J* = 1.8 Hz, 1Ar-H), 8.10 (s, 1N-H), 8.41 (s, 1N-H). ^{13}C NMR (d_6-DMSO) (100 MHz) – δ 68.1, 105.0, 108.3, 110.9, 111.6, 117.3, 118.8, 120.8, 122.9, 125.3, 129.6, 130.1, 133.7, 140.5, 140.6, 152.3, 158.6. HRMS (ESI+): m/z calcd for $C_{17}H_{16}N_3O_2$ $[M+H]^+$: 294.1243; found: 294.1241.

1-(4-butoxyphenyl)-3-(3-cyanophenyl)urea(79):

To a solution of n-Butyl alcohol (0.032 g; 0.43 mmol) in dry THF, TPP (0.135 g; 0.51 mmol) and DIAD (0.104 g; 0.51 mmol) were added consecutively and stirred at RT for 15 minutes. Thereafter, comp. **77** (0.1 g; 0.39 mmol) was added slowly to the reaction mixture under nitrogen atmosphere and the stirring was continued for 6 hours at room temperature. After aqueous workup the crude was purified by column chromatography (20% EtOAc in Hexane). Yield: 66.5%; off white solid; m.p. 164-166 ^{o}C. ^{1}H NMR (acetone-d_6) (300 MHz) – δ 0.83 (t, *J* = 7.2 Hz, 3H), 1.30-1.42 (m, 2H), 1.55-1.65 (m, 2H), 3.83 (t, *J* = 6.6 Hz, 2H), 6.74 (dd, *J* = 2.4, 6.9 Hz, 2Ar-H), 7.22 (td, *J* = 1.2,7.5 Hz, 1Ar-H), 7.27-7.37 (m, 3Ar-H), 7.60 (qd, J = 0.9, 8.4 Hz, 1Ar-H), 7.94-7.92 (m, 2Ar-H), 8.24 (brs, 1N-H). ^{13}C NMR (d_6-DMSO) (100 MHz) – δ 13.7, 18.7, 30.7, 67.0, 104.7, 108.2, 110.6, 111.6, 118.8, 120.8, 122.9, 125.3, 129.5, 130.2, 140.4, 140.6, 152.3, 159.1. HRMS (ESI+): m/z calcd for $C_{18}H_{20}N_3O_2$ $[M+H]^+$: 310.1555; found: 310.1561.

1-(3-cyanophenyl)-3-(4-((3-methylbut-2-en-1-yl)oxy)phenyl)urea (80):

To a solution of Prenyl alcohol (0.0374 g; 0.43 mmol) in dry THF, TPP (0.135 g; 0.51 mmol) and DIAD (0.104 g; 0.51 mmol) were added consecutively and stirred at RT for 15 minutes. Thereafter, comp. **77** (0.1 g; 0.39 mmol) was added slowly to the reaction mixture under nitrogen atmosphere and the stirring was continued for 6 hours at room temperature. After aqueous workup the crude was purified by column chromatography (20% EtOAc in Hexane). Yield: 61.5%; off white solid; m.p. 160-162 ^{o}C. ^{1}H NMR (acetone-d_6) (300 MHz) – δ 1.76 (d, *J* = 8.1 Hz, 6H), 4.53 (d, *J* = 6.6 Hz, 2H), 5.44-5.49 (m, 1H), 6.89 (dd, *J* = 2.1, 6.9 Hz, 2Ar-H), 7.35-7.51 (m, 4Ar-H), 7.72-7.76 (m, 1Ar-H), 8.07-8.10 (t, *J* = 1.8 Hz, 1Ar-H), 8.10 (s, 1N-H), 8.42 (s, 1N-H). ^{13}C NMR (acetone-d_6 + $CDCl_3$) (75 MHz) – δ 23.3, 31.0, 70.2, 118.7, 121.1, 123.9, 125.1, 126.9, 127.0, 127.1, 128.2, 130.8, 134.8, 136.4, 142.7, 145.7, 158.6, 160.5. HRMS (ESI+): m/z calcd for $C_{19}H_{20}N_3O_2$ $[M+H]^+$: 322.1556; found: 322.1559.

1-(3-((3-methylbut-2-en-1-yl)oxy)phenyl)-3-(2-nitrophenyl)urea (84):

To a solution of Prenyl alcohol (0.0347 g; 0.40 mmol) in dry THF, TPP (0.125 g; 0.48 mmol) and DIAD (0.096 g; 0.48 mmol) were added consecutively and stirred at RT for 15 minutes. Thereafter, comp. **81** (0.1 g; 0.37 mmol) was added slowly to the reaction mixture under nitrogen atmosphere and the stirring was continued for 8 hours at room temperature. After aqueous workup the crude was purified by column chromatography (16% EtOAc in Hexane). Yield: 73.8%; off white solid; m.p. 60-62 ^{o}C. ^{1}H NMR (300 MHz) ($CDCl_3$) – δ 1.75 (s, 3H), 1.80 (s, 3H), 4.53 (d, *J* = 6.9 Hz, 2H), 5.48-5.52 (m, 1H), 6.77 (d, *J* = 8.4 Hz, 1Ar-H), 6.85 (brs, 1N-H), 6.95 (d, *J* = 8.1 Hz, 1Ar-H), 7.09-7.12 (m, 2Ar-H), 7.28-7.31 (m, 1Ar-H), 7.63 (t, *J* = 8.4 Hz, 1Ar-H), 8.19 (d, *J* = 8.4 Hz, 1Ar-H), 8.67 (d, *J* = 8.7 Hz, 1Ar-H), 10.02 (brs, 1N-H). ^{13}C NMR (100 MHz) ($CDCl_3$ + Acetone- d_6) – δ 18.4, 26.1, 65.1, 108.4, 111.9, 114.1, 119.6, 122.0, 122.2, 126.0, 130.3, 136.1, 136.2, 136.5, 138.4, 138.7, 152.5, 160.0. HRMS (ESI+): m/z calcd for $C_{18}H_{20}N_3O_{42}$ $[M+H]^+$: 342.1454; found: 342.1458.

1-(3-((3-methylbut-2-en-1-yl)oxy)phenyl)-3-(4-nitrophenyl)urea (85):

To a solution of Prenyl alcohol (0.0347 g; 0.40 mmol) in dry THF, TPP (0.125 g; 0.48 mmol) and DIAD (0.096 g; 0.48 mmol) were added consecutively and stirred at RT for 15 minutes. Thereafter, comp. **82** (0.1 g; 0.37 mmol) was added slowly to the reaction mixture under nitrogen

atmosphere and the stirring was continued for 8 hours at room temperature. After aqueous workup the crude was purified by column chromatography (16% EtOAc in Hexane). Yield: 71.5%; yellow liquid. ^{1}H NMR (300 MHz) ($CDCl_3$) - δ 1.73 (s, 3H), 1.79 (s, 3H), 4.48 (d, *J* = 6.6 Hz, 2H), 5.43-5.49 (m, 1H), 6.71 (dd, *J* = 2.1 , 8.4 Hz, 1Ar-H), 6.87 (dd, *J* = 1.8, 8.1 Hz, 1Ar-H), 7.00 (t, *J* = 2.1 Hz, 1Ar-H), 7.16 (brs, 1N-H), 7.23 (t, *J* = 8.1 Hz, 1Ar-H), 7.49-7.54 (m, 2Ar-H), 7.55 (brs, 1N-H), 8.11-8.16 (m, 2Ar-H). ^{13}C NMR (100 MHz) ($CDCl_3$) – δ 18.3, 26.0, 65.1, 108.3, 110.9, 113.6, 118.6, 119.4, 125.4, 130.3, 138.6, 138.9, 142.5, 145.2, 153.1, 159.8. HRMS (ESI+): m/z calcd for $C_{18}H_{20}N_3O_{42}$ $[M+H]^+$: 342.1454; found: 342.1456.

1-(3-cyanophenyl)-3-(3-((3-methylbut-2-en-1-yl)oxy)phenyl)urea (86):

To a solution of Prenyl alcohol (0.0374 g; 0.43 mmol) in dry THF, TPP (0.135 g; 0.51 mmol) and DIAD (0.104 g; 0.51 mmol) were added consecutively and stirred at RT for 15 minutes. Thereafter, comp. **83** (0.1 g; 0.39 mmol) was added slowly to the reaction mixture under nitrogen atmosphere and the stirring was continued for 6 hours at room temperature. After aqueous workup the crude was purified by column chromatography (20% EtOAc in Hexane). Yield: 68.3%; off white solid; m.p. 138-140 ^{o}C. ^{1}H NMR (300 MHz) ($CDCl_3$) – δ 1.72 (s, 3H), 1.78 (s, 3H), 4.47 (d, *J* = 6.9 Hz, 2H), 5.43-5.49 (m, 1H), 6.71 (dd, *J* = 2.4, 8.4 Hz, 1Ar-H), 6.83 (dd, *J* = 1.2, 7.8 Hz, 1Ar-H), 6.93-6.95 (s, 1N-H), 6.99 (t, *J* = 2.1 Hz, 1Ar-H), 7.16-7.18 (m, 1Ar-H), 7.22 (s, 1N-H), 7.28-7.32 (m, 1Ar-H), 7.35-7.38 (m, 1Ar-H), 7.60-7.63 (m, 2Ar-H). ^{13}C NMR (100 MHz) (d_4-MeOH) – δ 18.2, 25.9, 65.8, 107.1, 110.5, 112.6, 113.7, 119.7, 121.2, 122.8, 124.3, 126.9, 130.6, 131.1, 138.5, 141.3, 141.9, 154.8, 160.8. HRMS (ESI+): m/z calcd for $C_{19}H_{20}N_3O_2$ $[M+H]^+$: 322.1556; found: 322.1560.

3-(3-(3-((3-methylbut-2-en-1-yl)oxy)phenyl)ureido)benzoic acid (90):

To a solution of Prenyl alcohol (0.066 g; 0.77 mmol) in dry THF, TPP (0.238 g; 0.91 mmol) and DIAD (0.184 g; 0.91 mmol) were added consecutively and stirred at RT for 15 minutes. Thereafter, comp. **89** (0.2 g; 0.69 mmol) was added slowly to the reaction mixture under nitrogen atmosphere and the stirring was continued for 8 hours at room temperature. After aqueous workup the crude was dissolved in THF/water mixture (4:1) 5 ml and Lithium hydroxide (0.059 g; 1.40 mmol) was added and stirred at RT for 3 hours. After that volatiles evaporated and the aqueous diluted with water and extracted with

ethylacetate. Aqueous acidified with HCl and the solid filtered dried and purified by column chromatography (30% EtOAc in Hexane). Yield: 52.6% (2 steps); off white solid; m.p. 180-182 ^{o}C. ^{1}H NMR (300 MHz) ($CDCl_3$ + acetone-d_6) – δ 1.75 (s, 3H), 1.79 (s, 3H), 4.52 (d, *J* = 6.6 Hz, 2H), 5.46-5.51 (m, 1H), 6.57 (dd, *J* = 1.5, 7.2 Hz, 1Ar-H), 6.94 (dd, *J* = 1.2, 7.8 Hz, 1Ar-H), 7.15 (t, *J* = 8.1 Hz, 1Ar-H), 7.28 (t, *J* = 2.1 Hz, 1Ar-H), 7.38 (t, *J* = 8.1 Hz, 1Ar-H), 7.69-7.72 (m, 1Ar-H), 7.88-7.91 (m, 1Ar-H), 7.96 (bs, 1N-H), 8.00 (t, *J* = 1.8 Hz, 1Ar-H), 8.12 (bs, 1N-H). ^{13}C NMR (100 MHz) (d_6-Acetone + CD_3CN) – δ 17.9, 25.4, 65.0, 105.9, 109.3, 111.6, 120.2, 120.6, 123.6, 123.9, 129.5, 130.0, 131.6, 137.8, 140.6, 141.3, 153.1, 160.0, 167.4. HRMS (ESI+): m/z calcd for $C_{19}H_{21}N_2O_4$ $[M+H]^+$: 341.1501; found: 341.1499.

4.3 Drug susceptibility on *M. Smegmatis* mc^{2}155 strain:

M. smegmatis (mc^{2}155) was cultured in Middlebrook 7H9 broth (HiMedia, India) supplemented with 10% (v/v) albumin–dextrose complex (HiMedia, India) and 0.03% (v/v) Tween80 at 37 °C. For minimum inhibitory concentration (MIC) testing, two fold serial dilutions of compounds were prepared in 360 µl in optically clear, round bottom 96-well plates. An equivalent volume of mid log–phase mc^{2}155 culture (diluted to an optical density at 570 nm of 0.01 was added to achieve a final drug concentration range of 100–0.78 µg/ml in 7H9 broth, with a control. Plates were incubated in ambient air at 37 °C for 48 hours, at which point MICs were recorded at the lowest concentration of compound that prevented visual growth.

4.4 Drug susceptibility on *M. tuberculosis* mc^{2}6030 strain:

M. tuberculosis mc^{2}6030 was grown at 37 °C in Sauton's medium supplemented with 20 µg/ml of pantothenic acid. The susceptibility of *M. tuberculosis* to the various compounds was determined as reported previously.[36] In brief, Middlebrook 7H10 solid medium containing oleic-albumin-dextrose-catalase enrichment (OADC) and 20 µg/ml of pantothenic acid were supplemented with increasing concentrations of the chemical analogues. Stock solutions at 10 mg/ml were diluted in DMSO. Serial 10-fold dilutions of the actively growing culture were plated and incubated at 37 °C for 2-3 weeks. The minimal inhibitory concentration (MIC) was defined as the minimum concentration

required for inhibiting 99% of the growth. Isoniazid was included as an antitubercular drug control.

4.5 Drug susceptibility on *M. tuberculosis* H37Rv strain (MABA):

All compounds were evaluated for MIC against *M. tuberculosis* H37Rv (ATCC 27294) using the microplate Alamar Blue assay (MABA) as previously described[37] except that we used 7H12 media (instead of 7H9 + glycerol + casitone + OADC). In case of compounds exhibiting significant background fluorescence we also used luciferase reporter strains of *M. tuberculosis* H37Rv as well as measurement of intracellular adenosine triphosphate. Cultures were incubated in 200 µl medium in 96-well plates for 7 days at 37 ^{o}C. Alamar Blue and Tween 80 were added and incubation was continued for 24 hours at 37 ^{o}C. Fluorescence was determined at excitation/emission wavelengths of 530/590 nm respectively. The MIC was calculated as the lowest concentration effecting a reduction in fluorescence (or luminescence) of 90% relative to controls. Control compounds were run in each experiment including isoniazid and rifampin.

4.6 Cytotoxicity study:

HepG2 cell line (human liver cancer cell):

Cytotoxicity assays were performed using the HepG2 cell line (HB-8065) obtained from the American Type Culture Collection (ATCC, Manassas, VA). Hep G2 cells were propagated in ATCC-formulated Eagle's Minimum Essential Medium supplemented with 10% fetal bovine serum and maintained in a humidified incubator (37 ^{o}C, 5% CO_2). After scrapping the cells with a cell scraper, they were collected by centrifugation (1000 rpm for 5 min), resuspended in fresh medium at ~1x10^6 cells/mL, dispensed into 96-well microplates (100 µl/well) and incubated for 24 h at 37 ^{o}C before being used for cytotoxicity assays. Test compounds were subsequently added at concentrations ranging from 400 to 0.2 µg/mL and incubation continued for another 72 h before the cytopathic effects of compounds was determined using the MTT Cell Proliferation Assay (ATCC). The cytotoxic IC_{50} defined as the concentration causing 50% reduction in Hep G2 cell viability, was obtained from a dose–response curve plotted from percentage activity versus $\log_{10}$ concentration.

Peritoneal macrophages (Normal mammalian cell):

Cytotoxicity of compounds against peritoneal macrophages was tested by MTT assay described earlier. Briefly, murine peritoneal macrophages (1×10^6 cells/well in 96-well cell culture plates, Genetix) were treated with increasing concentrations of compounds ranging from 0.1 µg/ml to 250 µg/ml were further cultured in RPMI-1640 supplemented with 10% FBS for 48h. Then 10 µl of MTT (5 mg/ml) was added and incubated for 3 h at 37°C. Then HCl-isopropanolic solution (0.04M HCl in isopropanol) was added to each well. After 15 min of incubation at room temperature, absorbance of solubilized MTT formazan product was spectrophotometrically measured at 570 nm. The cytotoxic IC_{50} defined as the concentration causing 50% reduction in cell viability, was obtained from a dose–response curve plotted from percentage activity versus $\log_{10}$ concentration.

4.7 Determination of the effects on mycolic acid biosynthesis:

M. smegmatis was grown at 37 ºC and at OD_{600}=1 the culture was divided into 10 fractions and each of them were treated with a different compound at 5 times its MIC. Two controls were made, one culture was treated with DMSO (solvent used for drug dissolution) and the other one had no treatment (negative control). The treatment lasted for 90 minutes and then the cultures were labelled with 1 µCi/ml [1-^{14}C]-acetate.

Analysis of fatty acids and mycolic acids:

De novo fatty acid and mycolic acid biosyntheses were followed by labeling 5 ml culture aliquots with 1 µCi/ml [1-^{14}C]-acetate (specific activity: 55.3 mCi/mmol; Perkin Elmer) for 1h at 37ºC. Fatty acid and mycolic acid methyl esters were extracted from samples containing equivalent amounts of bacteria.[31] The resulting solution of FAMEs (fatty acid methyl ester) and MAMEs (mycolic acid methyl ester) were assayed for radioactivity in a Beckman liquid scintillation counter and then subjected to TLC using silica gel plates (5735 silica gel 60F254; Merck). Samples were normalized by cpm and developed in hexane:ethyl acetate (9:1, v/v). Autoradiograms were produced by overnight exposure to Kodak X-Omat AR film to reveal ^{14}C-labelled FAMEs and MAMEs.

4.8 Macrophages and mc^{2}155:

Murine peritoneal macrophages were isolated by peritoneal lavage from BALB/c mice (817/04/ac/CPCSEA) after 5 days prior 4% thioglycolate i.p. as described earlier.[38] Macrophages were cultured in RPMI1640 with 10% FBS (Gibco) and antibiotics (Gibco) for 48 hrs prior all experiments.

Fresh culture of bacteria (grown upto mid-log phase) was harvested by centrifugation at 2,500 g for 15 min. The bacteria were then washed with PBS twice using the centrifugal washing method and finally resuspended in PBS at the desired concentration for macrophage infection.

4.9 Infection in macrophages and mRNA expression study:

Isolated peritoneal macrophages (2×10^6 cells/ml) were infected with *M. smegmatis* (mc^{2}155 strain) at macrophage-bacteria ratio of 1:10 for 6hrs followed by non-ingested bacteria were removed by washing three times with fresh RPMI 1640 media.[39] After that, synthesized indicated drug molecules (5μg/mL) were added in the culture medium for another 6 hrs. Macrophages were collected in Trizol (Invitrogen) followed by mRNA isolation according to the manufacturer's instructions. cDNA were prepared from each sample using 1μg of total RNA by cDNA synthesis kit (Thermo Scientific). cDNA from each sample was amplified with green *Taq*DNA Polymerase (Fermetus) in 25 μl reaction volume with following conditions: 95 °C for 90 sec, 94 °C for 45 sec, 60 °C for 1 min, and 72 °C for 1 min for a total of 40 cycles in Thermal cycler (Applied biosystem). Specific primers (Sigma, India) were used for amplification of $iNOS_2$ (forward: 5'-CAG AGG ACC CAG AGA CAA GC-3'; reverse 5'-AAG ACC AGA GGC AGC ACA TC-3';), IL-12 (forward 5'-CAC GCC TGA AGA AGA TGA CA-3'; reverse 5'-GAC AGA GAC GCC ATT CCA CA-3'), IFN-γ (forward 5'- GGA TAT CTG GAG GAA CTG GC-3'; Reverse 5'- CGA CTC CTT TTC CGC TTC CT -3'), IL-10 (forward 5'-TCA CTC TTC ACC TGC TCC AC-3'; reverse 5'- CTA TGC TGC CTG CTC TTA CTC-3'), and *GAPDH* (forward 5'-GAG CCA AAC GGG TCA TCA TC-3'; reverse, 5'-CCT GCT TCA CCA CCT TCT TG-3') to ensure equal cDNA input.[34]

Acknowledgements

A.B. thanks the Department of Science and Technology (DST), India for a fellowship. J. D is grateful to DST, India [Fast track project SB/FT/CS-008/2013] for their financial support. I. H. is grateful to the Labex EpiGenMed for funding her PhD.

References:

1 Brosch, R.; Gordon, S. V.; Eiglmeier, K.; Garnier, T.; Cole, S. T. *Res. Microbiol.* **2000**, *151*, 135.

2 WHO Global Tuberculosis report, 2015. http://www.who.int/tb/publications/global_report/en/

3 Nunn, P.; Kochi, A. *World Health* **1993**, *46*, 7.

4 Debnath, J.; Siricilla, S.; Wan, B.; Crick, D. C.; Lenaerts, A. J.; Franzblau, S. G.; Kurosu, M. *J. Med. Chem.* **2012**, *55*, 3739.

5 Phetsuksiri, B.; Baulard, A. R.; Cooper, A. M.; Minnikin, D. E.; Douglas, J. D.; Besra, G. S.; Brennan, P. J. *Antimicrob Agents Chemother* **1999**, *43*, 1042.

6 Phetsuksiri, B.; Jackson, M.; Scherman, H.; McNeil, M.; Besra, G. S.; Baulard, A. R.; Slayden, R. A.; DeBarber, A. E.; Barry III, C. E.; Baird, M. S.; Crick, D. C.; Brennan, P. J. *J. Biol. Chem.* **2003**, *278*, 53123.

7 Grzegorzewicz, A. E.; Eynard, N.; Quémard, A.; North, E. J.; Margolis, A.; Lindenberger, J. J.; Jones, V.; Korduláková, J.; Brennan, P. J.; Lee, R. E.; Ronning, D. R.; McNeil, M. R.; Jackson, M. *ACS Infect. Dis.* **2015**, *1*, 91.

8 Janin, Y. L. *Bioorg. Med. Chem.* **2007**, *15*, 2479.

9 Dover, L. G.; Alahari, A.; Gratraud, P.; Gomes, J. M.; Bhowruth, V.; Reynolds, R. C.; Besra, G. S.; Kremer, L. *Antimicrob Agents Chemother* **2007**, *51*, 1055.

10 Korduláková, J.; Janin, Y. L.; Liav, A.; Barilone, N.; Dos, V. T.; Rauzier, J.; Brennan, P. J.; Gicquel, B.; Jackson, M. *Antimicrob Agents Chemother* **2007**, *5*, 3824.

11 Brown, C. W.; Liu, S.; Klucik, J.; Berlin, K. D.; Brennan, P. J.; Kaur, D.; Benbrook, D. M. *J. Med. Chem.* **2004**, *47*, 1008.

12 Seth, P. P.; Ranken, R.; Robinson, D. E.; Osgood, S. A.; Risen, L. M.; Rodgers, E. L.; Migawa, M. T.; Jefferson, E. A.; Swayze, E. E. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 5569.

13 Bhowruth, V.; Brown, A. K.; Reynolds, R. C.; Coxon, G. D.; Mackay, S. P.; Minnikin, D. E.; Besra, G. S. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 4743.

14 Nava-Zuazo, C.; Estrada-Soto, S.; Guerrero-Alvarez, J.; León-Rivera, I.; Molina-Salinas, G. M.; Said-Fernández, S.; Chan-Bacab, M. J.; Cedillo-Rivera, R.; Moo-Puc, R.; Mirón-López, G.; Navarrete-Vazquez, G. *Bioorg. Med. Chem.* **2010**, *18*, 6398.

15 Liav, A.; Angala, S. K.; Brennan, P. J.; Jackson, M. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 2649.

16 Ober, W. B. *Pathol Annu.* **1983**, *18*, 79.

17 Grobler, A.; Perez Sierra, Z.; Viljoen, H. J. *J. Theor. Biol.* **2016**, *388*, 85.

18 Das, S.; Bhattacharjee, O.; Goswami, A.; Pal, N. K.; Majumdar, S. *Tuberculosis* **2015**, *95*, 208.

19 Brown, J. R.; North, E. J.; Hurdle, J. G.; Morisseau, C.; Scarborough, J. S.; Sun, D.; Korduláková, J.; Scherman, M. S.; Jones, V.; Grzegorzewicz, A.; Crew, R. M.; Jackson, M.; McNeil, M. R.; Lee, R. E. *Bioorg. Med. Chem.* **2011**, *19*, 5585.

20 Thornber, C. W. *Chem. Soc. Rev.* **1979**, *8*, 563.

21 Burger A. In Medicinal Chemistry, 3rd ed., Burger, A. (Ed.), Wiley, New York, 1970.

22 Korolkovas A. Essentials of Molecular Pharmacology: Background for Drug Design, Wiley, New York, 1970, pp 54-57.

23 Yin, Y.; Zheng, Ke.; Eid, N.; Howard, S.; Jeong, Ji-Hak.; Yi, F.; Guo, J.; Park, C. M.; Bibian, M.; Wu, W.; Hernandez, P.; Park, H.; Wu, Y.; Luo, J. L.; LoGrasso, P. V.; Feng, Y. *J. Med. Chem*. **2015**, *58*, 1846.

24 Mitsunobu, O.; Yamada, Y. *Bulletin of the Chemical Society of Japan* **1967**, *40*, 2380.

25 Sambandamurthy, V. K.; Derrick, S. C.; Hsu, T.; Chen, B.; Larsen, M. H.; Jalapathy, K. V.; Chen, M.; Kim, J.; Porcelli, S. A.; Chan, J.; Morris, S. L.; Jacobs Jr. W. R. *Vaccine* **2006**, *24*, 6309.

26 Wiktor, J.; Lesterlin, C.; Sherratt, D. J.; Dekker, C. *Nucleic Acids Res.* **2016**, pii: gkw214.

27 Panda, S. K.; Mohanta, Y. K.; Padhi, L.; Park, Y. H.; Mohanta, T. K.; Bae, H. *Molecules* **2016**, *21*, pii: E293.

28 Franzblau, S. G.; Witzig, R. S.; McLaughlin, J. C.; Torres, P.; Madico, G.; Hernandez, A.; Degnan, M. T.; Cook, M. B.; Quenzer, V. K.; Ferguson, R. M.; Gilman, R. H. *J Clin Microbiol* **1998**, *36*, 362.

29 Cho, S. H.; Warit, S.; Wan, B.; Hwang, C. H.; Paul, G. F.; Franzblau, S. G. *Antimicrob Agents Chemother* **2007**, *51*, 380.

30 Slayden, R. A.; Lee, R. E.; Armour, J. W.; Cooper, A. M.; Orme, I. M.; Brennan, P. J.; Besra, G. S. *Antimicrob. Agents Chemother.* **1996**, *40*, 2813.

31 Marrakchi, H.; Lanéelle, M. A.; Daffé, M. *Chem. Biol.* **2014**, *21*, 67.

32 Yang, C. S.; Shin, D. M.; Kim, K. H.; Lee, Z. W.; Lee, C. H.; Park, S. G.; Bae, Y. S.; Jo, E. K. *J. Immunol.* **2009**, *182*, 3696.

33 Li, W.; Zhao, Q.; Deng, W.; Chen, T.; Liu, M.; Xie, J. *PLoS One* **2014**; *9*: e94418.

34 Flynn, J. L.; Chan, J. *Cur. Opin. Immunol.* **2003**, *15*, 450.

35 Das, S.; Banerjee, S.; Majumder, S.; Chowdhury, B. P.; Goswami, A.; Halder, K.; Chakraborty, U.; Pal, N. K.; Majumdar, S. *PLoS One* **2014**, *9*, e92477.

36 Kremer, L.; Douglas, J. D.; Baulard, A. R.; Morehouse, C.; Guy, M. R.; Alland, D.; Dover, L. G.; Lakey, J. H.; Jacobs Jr, W. R.; Brennan, P. J.; Minnikin, D. E.; Besra, G. S. *J. Biol. Chem.* **2000**, *275*, 16857.

37 Collins, L. A.; Franzblau, S. G. *Antimicrob. Agents Chemother.* **1997**, *41*, 1004.

38 Mahapatra, S. Kar; Chakraborty, S. P.; Majumdar, S.; Bag, B. G.; Roy, S. *Eur. J. Pharmacol.* **2009**, *623*, 132.

39 Basler, T.; Holtmann, H.; Abel, J.; Eckstein, T.; Baumer, W.; Valentin-Weigand, P.; Goethe, R. *J. Leukoc. Biol.* **2010**, *87*, 173.

Graphical abstract: