Scientific Research Publishing

International Journal of Organic Chemistry, 2016, 6, 157-176
http://www.scirp.org/journal/ijoc
ISSN Online: 2161-4695
ISSN Print: 2161-4687

Design, Synthesis and Biological Evaluation of Novel Antitubercular Agents by Combining Pyrazoline and Benzoxazole Pharmacophores

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How to cite this paper: Soni, H.M., Patel, P.K., Chhabria, M.T., Patel, A.K., Rana, D.N. and Brahmkshatriya, P.S. (2016) Design, Synthesis and Biological Evaluation of Novel Antitubercular Agents by Combining Pyrazoline and Benzoxazole Pharmacophores. *International Journal of Organic Chemistry*, **6**, 157-176.
http://dx.doi.org/10.4236/ijoc.2016.63017

Received: August 23, 2016
Accepted: September 16, 2016
Published: September 19, 2016

Abstract

Various recent reports on Tuberculosis have alarmed an increase in the patient class and subsequent death rates across the globe. Over and above the spread of more dangerous and fatal forms of tuberculosis like MDR-TB *i.e.* multiple-drug resistance tuberculosis, XDR-TB *i.e.* extensively-drug resistance tuberculosis & TDR-TB *i.e.* total-drug resistance tuberculosis has forwarded an urgent need to discover novel antitubercular agents. The current work is aimed at combining two previously well-known pharmacophores (pyrazoline and benzoxazole nucleus) in order to design and synthesize a series of novel benzoxazole-based pyrazoline derivatives. The synthesized target compounds were structurally confirmed by LCMS, ^{1}H-NMR and ^{13}C-NMR analysis. The target compounds were *In vitro* evaluated against *M. tuberculosis* H_{37}Rv strain, multidrug-resistant TB (MDR-TB) and extensively drug-resistant TB (XDR-TB) strains. The *In vitro* screening results depicted that majority of the target compounds displayed potent activity with MIC in a range of ~0.8 to 6.25 μg/mL. Many compounds were found to be more potent than isoniazid against MDR-TB with MIC value 3.12 μg/mL and XDR-TB with MIC value 12.5 μg/mL. Cytotoxicity assay of these active compounds on VERO cell lines also displayed good selectivity index.

Keywords

Antitubercular, Benzoxazole, Pyrazoline, Pharmacophore, Microplate Alamar Blue Assay

1. Introduction

A bacteria called *Mycobacterium tuberculosis* most often affects the lungs and causes TB. TB is infectious and spreads from one person to another through the air. Patients active with TB when cough, sneeze or spit, propel the TB germs into the air and later when a person who inhales only a few of these germs becomes infected. Patients with compromised immune systems, such as those who are victims of HIV, malnutrition or diabetes, or those who use tobacco, have a much higher risk of falling ill. In 2009, as per an estimation presented by the World Health Organization (WHO) (Dye *et al.*) [1], around one-third of the world's then population *i.e.* around 2 billion people were infected with tuberculosis. WHO raised a concern that if the spread of tuberculosis across the globe was left unchecked, than it would be responsible for claiming approximately 36 million lives by 2020, which is a very alarming figure [2]. On an average, yearly more than 8 million people are noted to be victimized by an active form tuberculosis, which subsequently claims nearly 2 million lives. On top of this, various drug-resistant and hence more dangerous forms of tuberculosis have been reported and identified in patients across the globe, like MDR-TB *i.e.* a multi-drug resistant form of TB which doesn't respond to the two first-line of TB drugs, XDR-TB *i.e.* extensively drug resistant form of TB which shows resistance to fluoroquinolones and aminoglycosides. To worsen the situation, recent cases of patients found to be active to TDR-TB *i.e.* a total and most fatal form of TB have been reported [3]. TB has thus become a serious threat, and control of its spread by discovery of novel antitubercular agents acting through newer molecular mechanisms has been the most urgent and un-met need (Udwadia *et al.*, 2012) [4].

Benzoxazole has proved its biological importance in medicinal chemistry since several years. Modifications on the benzoxazole nucleus have resulted in a large number of compounds having diverse pharmacological activities [5]. Over the years, several benzoxazole derivatives have been synthesized and screened for their biological activity and many of them have been found to possess good antitubercular activity (**Figure 1(a)** Vinsova *et al.*, 2006; Özlem *et al.*, 2008; Klimesova *et al.*, 2009; Ileana *et al., 200*6) [6]-[9].

Also, the fact that the living organism finds it difficult to construct of N-N bonds limits the natural abundance of compounds having such bonds, therefore synthesis of compounds with N-N bond has been found to be of primarily importance. Pyrazoline and their derivatives, which is a class of molecules containing the N-N bond, have exhibited a wide range of biological activities including antimycobacterial activity (**Figure 1(b)** Manna and Agrawal, 2010; Shaharyar *et al.*, 2006; Sharma *et al.*, 2011) [10]-[12].

Considering the above facts, we were inspired for combining two previously well-known pharmacophores to design and synthesize [13] [14] chemically novel antimycobacterial agents (**Figure 2**, compound **6, 7a-7i**, **8, 9a-9k, 10 and 11**).

Melting point of all the synthesized intermediates and target compounds were recorded (un-corrected). All the synthesized intermediates were confirmed by MASS analysis while ^{1}H-NMR analysis of few key intermediates were also recorded for structural

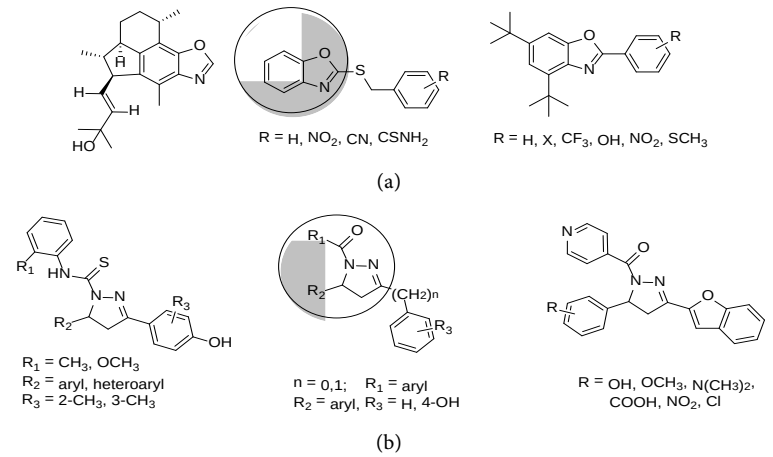


Figure 1. (a) Structures of previously known few representative benzoxazole derivatives (benzoxazole moiety encircled); (b) Structures of previously known few representative pyrazoline, derivatives (pyrazoline moiety encircled).

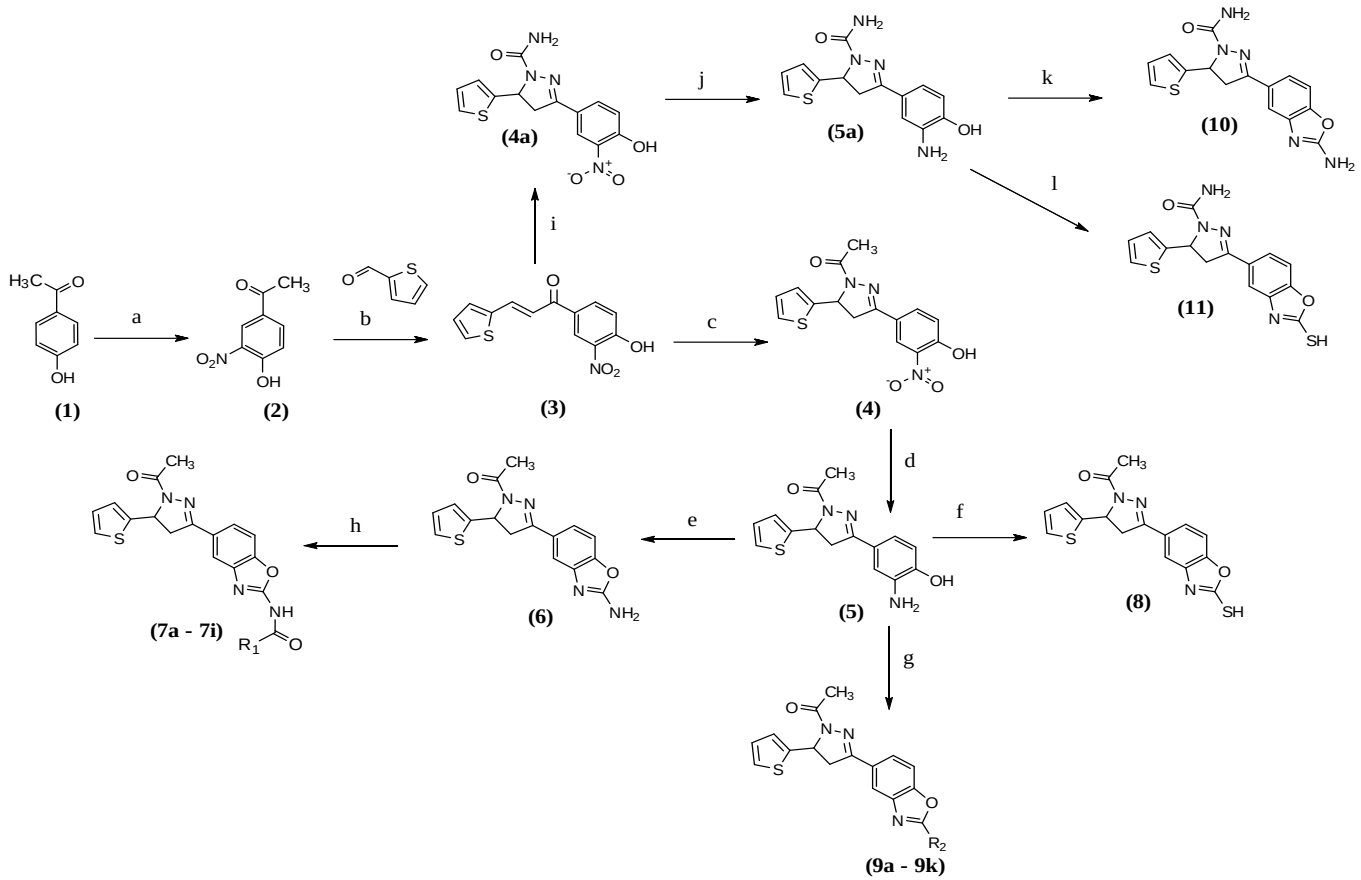


Figure 2. Pathway for the synthesis of the title compounds. (a) H_2SO_4·HNO_3, 5˚C - 10˚C 3 h, then at rt for 2 h; (b) thiophene-2-carbaldehyde, 50% aqueous KOH solution, ethanol, 80˚C 15 h; (c) hydrazine hydrate, glacial acetic acid, reflux 4 h (i) NaOH, semicarbazide hydrochloride, methanol, 80˚C 15 h (d), (j) sodium dithionite, methanol, 100˚C 2 h; (e, k) cyanogen bromide, methanol-water, rt 0.5 h; (f), (l) carbon disulphide, methanol, KOH, 60˚C 3 h; (g) suitable carboxylic acid, T3P, DIPEA, microwave 160˚C, 1.5 to 2.5 h (h) #suitable carboxylic acid, DCC, HOBt, DMAP, DMF, rt 1h, then at 50˚C - 70˚C for 6 - 15 h (h) $^{\$}$acetic anhydride, 80˚C for 0.5 h (h) % chloroacetyl chloride, K_2CO_3, DMF, rt 15 h. #for compounds **7b, 7c, 7e-7i,** $^{\$}$ for compound **7a,** %for compound **7d**.

confirmation. Liquid Chromatography—Mass Spectroscopy (LCMS) analysis of all the target compounds were carried out for purity determination and confirmation of desired molecular weights. Structures all the target compounds were confirmed by ^{1}H-NMR analysis while ^{13}C NMR analysis of selected final target compounds were also recorded.

Development of low-cost, rapid, high-throughput assays for screening of novel drug candidates has also been identified. Recently, a number of new mycobacterial drug susceptibility assays have been an area of prime interest [15]-[19]. However most of these assays are found to lack some of the attributes desired for a mass screening assay, especially like low cost, high throughput, and time efficiency. Microplate Alamar Blue Assay (MABA) has emerged as an excellent technology [20]. MABA is clearly the standard in the field for HTS of compounds against mycobacteria, and is the most widely cited [21]. Besides possessing all the major attributes desired for a mass screening assay, MABA is non-toxic methodology and uses a thermally stable reagent. Further this methodology shows a very good correlation with proportional and BACTEC radiometric methods.

Therefore in response to the need for rapid, inexpensive, high-throughput assays for anti-mycobacterial drug screening all the target compounds synthesized for current work were primarily assessed against *Mycobacterium tuberculosis* H_{37}Rv strain by MABA. As summarized in Table 1 and Table 2, majority of the target compounds were found to possess satisfactory potency. Compounds **6, 8, 10, 7d, 7e** demonstrated potency equivalent to Streptomycin (MIC = 6.25 μg/mL). Compounds **7i, 9f** demonstrated potency **(7i: MIC = 1.6 μg/ml & 9f: MIC = 0.8 μg/ml)** better than all the standard drugs like Streptomycin, Pyrazinamide and Ciprofloxacin against *M. tuberculosis* H_{37}Rv strain.

The *In vitro* antitubercular activity of potent compounds **6, 8, 10, 7d, 7e, 7i, 9f** was further assessed by using Lowenstein-Jensen medium (L. J. medium) where the susceptibility of these compounds against multidrug-resistant TB (MDR-TB) and extensively drug-resistant TB (XDR-TB) strains were also checked. As summarized in Table 3, compounds **7d, 7i, 9f** demonstrated potency better than isoniazid against MDR-TB **(MIC = 3.12 μg/mL)** and XDR-TB **(MIC = 12.5 μg/mL)** strains.

2. Materials and Methods

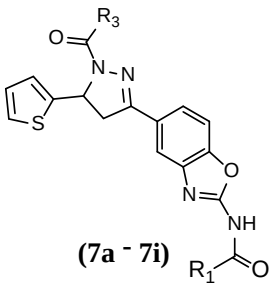
2.1. Chemistry

Analytical grade chemicals and reagents were directly used to perform various experiments. Open glass capillary methodology was used to record the melting points which remain uncorrected. Thin layer chromatography (TLC) was carried out on Merck's Silica Gel G and the spots were visualized under UV, exposure to iodine and with different TLC spray reagents.

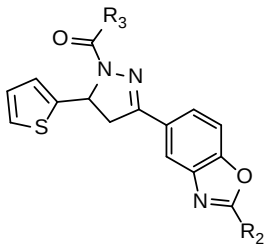
2.2. Analytical

400 MHz BRUKER ULTRASHIELD instrument was used to record ^{1}H NMR and ^{13}C NMR spectra using DMSO-*d*6, CD_3COCD_3, CD_3OD & CF_3COOD as solvents and the chemical shift values were expressed in δ ppm downfield from internal Trimethylsilane

Table 1. *In vitro* antitubercular activity against *Mycobacterium tuberculosis* $H_{37}Rv$ strain by Microplate Alamar Blue Assay (MABA) of target compounds.



(7a - 7i)



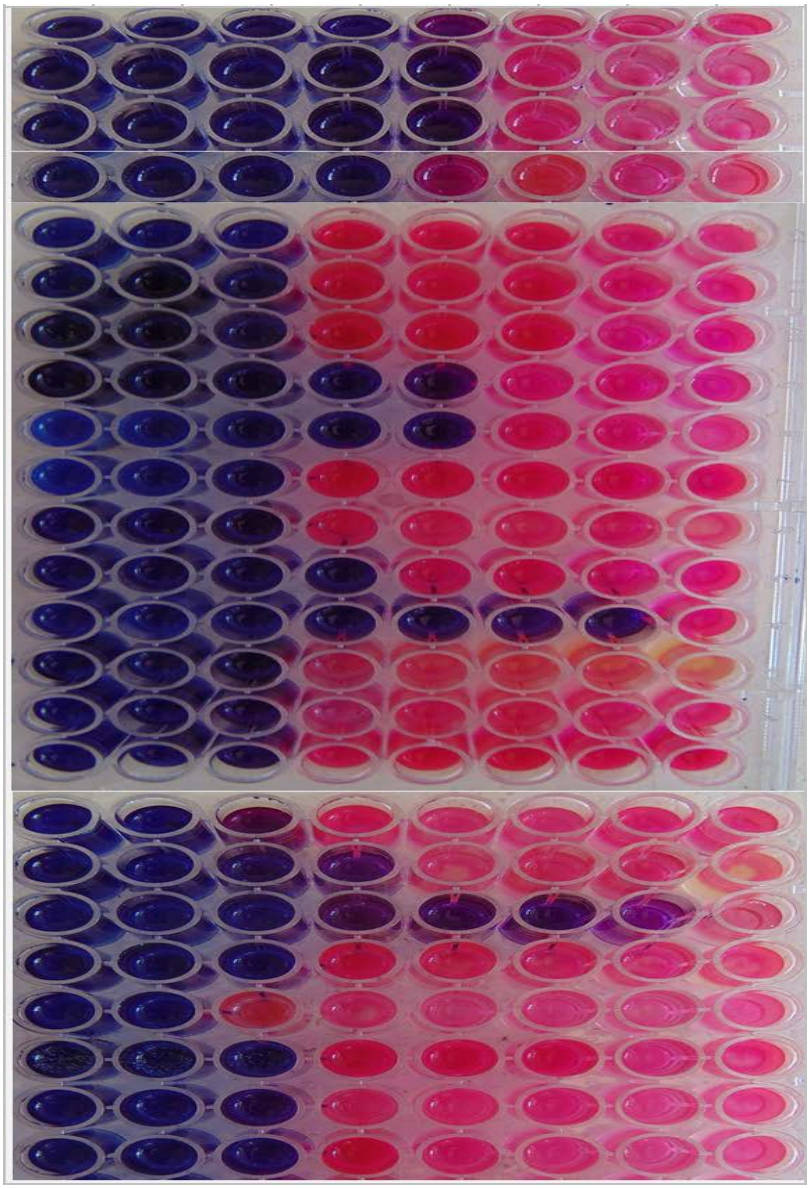
(6, 8, 9a - 9k, 10, 11)

Target Cpd.	R_1	R_2	R_3	MIC (μg/ml)
6	-	NH_2	CH_3	6.25
8	-	SH	CH_3	6.25
10	-	NH_2	NH_2	6.25
11	-	SH	NH_2	12.5
7a	CH_3	-	CH_3	25
7b	CH(CH3)2	-	CH_3	25
7c	$CH_2CH_2CH_3$	-	CH_3	12.5
7d	CH_2Cl	-	CH_3	6.25
7e	C_6H_5	-	CH_3	6.25
7f	2-ClC_6H_4	-	CH_3	25
7g	2-$OMeC_6H_4$	-	CH_3	25
7h	3-ClC_6H_4	-	CH_3	12.5
7i	3-$OMeC_6H_4$	-	CH_3	1.6
9a	-	C_6H_5	CH_3	25
9b	-	2-ClC_6H_4	CH_3	25
9c	-	2-$OMeC_6H_4$	CH_3	25
9d	-	3-$OMeC_6H_4$	CH_3	25
9e	-	4-$OMeC_6H_4$	CH_3	12.5
9f	-	4-OHC_6H_4	CH_3	0.8
9g	-	3,4,5-$(OMe)_3C_6H_2$	CH_3	25
9h	-	$CH_2C_6H_5$	CH_3	50
9i	-	2-Pyridyl	CH_3	25
9j	-	3-Pyridyl	CH_3	25
9k	-	4-Pyridyl	CH_3	25
Pyrazinamide	-	-	-	3.12
Ciprofloxacin	-	-	-	3.12
Streptomycin	-	-	-	6.25

Note: S-Sensitive R-Resistant**,** Strain used: *M. tuberculosis* $H_{37}Rv$; ATCC No: -27294.

Table 2. Target drug's photograph.

Target Cpd.	R_1	R_2	R_3	MIC (μg/ml) 100	50	25	12.5	6.25	3.12	1.6	0.8
6	-	NH_2	CH_3								
8	-	SH	CH_3								
10	-	NH_2	NH_2								
11	-	SH	NH_2								
7a	CH_3	-	CH_3								
7b	$CH(CH_3)_2$	-	CH_3								
7c	$CH_2CH_2CH_3$	-	CH_3								
7d	CH_2Cl	-	CH_3								
7e	C_6H_5	-	CH_3								
7f	2-ClC_6H_4	-	CH_3								
7g	2-$OMeC_6H_4$	-	CH_3								
7h	3-ClC_6H_4	-	CH_3								
7i	3-$OMeC_6H_4$	-	CH_3								
9a	-	C_6H_5	CH_3								
9b	-	2-ClC_6H_4	CH_3								
9c	-	2-$OMeC_6H_4$	CH_3								
9d	-	3-$OMeC_6H_4$	CH_3								
9e	-	4-$OMeC_6H_4$	CH_3								
9f	-	4-OHC_6H_4	CH_3								
9g	-	3,4,5-$(OMe)_3C_6H_2$	CH_3								
9h	-	$CH_2C_6H_5$	CH_3								
9i	-	2-Pyridyl	CH_3								
9j	-	3-Pyridyl	CH_3								
9k	-	4-Pyridyl	CH_3								
Pyrazinamide	-	-	-								
Ciprofloxacin	-	-	-								
Streptomycin	-	-	-								



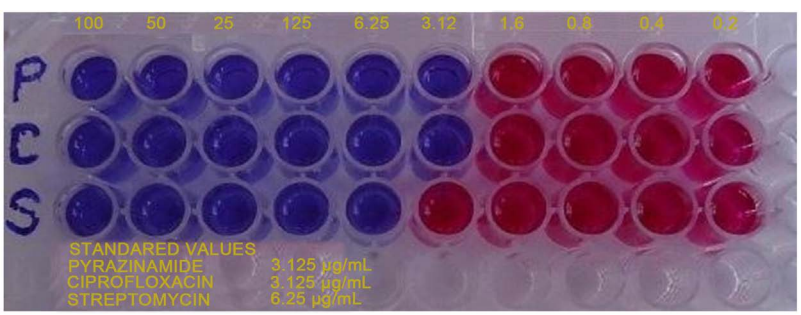
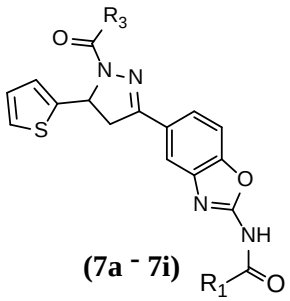
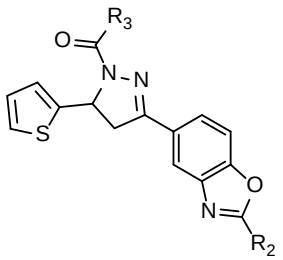


Table 3. Structures of the synthesized target compounds and their *In Vitro* antitubercular activity against *M. tuberculosis* $H_{37}Rv$ strain, MDR-TB and XDR-TB strains by using Löwenstein-Jensen medium (L. J. medium)



(7a - 7i)



(6, 8, 9a - 9k, 10, 11)

Target Compound.	R_1	R_2	R_3	MIC (μg/mL)			Cytotoxicity IC_{50} (μg/mL)
				$H_{37}Rv$	MDR-TB	XDR-TB	
6	-	NH_2	CH_3	6.25	6.25	25	ND
8	-	SH	CH_3	6.25	25	>100	ND
10	-	NH_2	NH_2	6.25	12.5	50	ND
7d	CH_2Cl	-	CH_3	6.25	3.12	12.5	>62.5
7e	C_6H_5	-	CH_3	6.25	12.5	50	ND
7i	3-$OMeC_6H_4$	-	CH_3	1.6	3.12	12.5	>62.5
9f	-	4-OHC_6H_4	CH_3	0.8	3.12	12.5	32.5
Isoniazid	-	-		0.5	6.25	50	ND

ND means not determined.

standard. WATERS LCMS systems were used to record LCMS analysis by following methods

Method-A

Instrument:	Waters Acquity H-Class UPLC, PDA and SQ Detector	
Column:	BEH C 18, 50 * 2.1 mm, 1.7 μm or equivalent	
Mobile Phase:	(A) 5 mM Ammonium Acetate + 0.1% Formic Acid in Water	
	(B) 0.1% Formic Acid in Acetonitrile	
Flow Rate	0.55 mL/min	
Gradient	Time	%B
	0.01	5
	0.40	5
	0.80	35
	1.20	55
	2.50	100
	3.30	100
	3.31	5
	4.00	5

Method-B

Instrument:	Waters LC alliance 2995, PDA 2996 and SQ Detector	
Column:	X-bridge C18, 50*4.6mm, 3.5µm or equivalent	
Mobile Phase:	(A) 0.1% Ammonia in Water	
	(B) 0.1% Ammonia in Acetonitrile	
Flow Rate:	1.0 mL/min	
Gradient:	Time	%B
	0.01	5
	5.00	90
	5.80	95
	7.20	95
	7.21	5
	10.00	5

2.3. Experimental Section

1-(4-hydroxy-3-nitrophenyl)ethanone (2)

To a solution of 1-(4-hydroxyphenyl)ethan-1-one **(1)** (50 g, 367.6 mmol) in H_2SO_4 (500 mL) was added HNO_3 (18.05 mL, 407.35 mmol) drop wise at 5˚C - 10˚C. The reaction mixture was stirred at 5˚C - 10˚C for 3 h and then at room temperature for 2 h. The resulting reaction mixture was poured into ice-water. The obtained yellow solids were filtered off under vacuum and dried well to yield 1-(4-hydroxy-3-nitrophenyl) ethan-1-one **(2)** (48.5g, 267.95 mmol) as pure product. Yield: 72.9%; Melting Point [˚C] [22]: 132 - 133; ^{1}H NMR (CD_3COCD_3): δ 9.50 (s, 1H), 8.65 (d, 1H, J = 2Hz), 8.20 (dd, 1H, J_{HH}" = 2 Hz, J_{HH} = 9.2 Hz), 7.26 (d, 1H, J = 9.2Hz), 2.5 (s, 3H); MS: ES + 182.1 (M + 1).

1-(4-hydroxy-3-nitrophenyl)-3-(thiophen-2-yl)prop-2-en-1-one (3)

To a solution of 1-(4-hydroxy-3-nitrophenyl)ethan-1-one **(2)** (15g, 82.87 mmol) and thiophene-2-carbaldehyde (9.2 g, 82.87 mmol) in ethanol (150 mL) was slowly added 50% aqueous KOH solution (24 mL) whilst maintaining the reaction temperature below 25˚C. The obtained reaction mixture was heated at 80˚C for 15 h. The resulting reaction mixture was allowed to cool to room temperature and acidified by 20% aqueous citric acid solution. The obtained mixture was extracted with Ethyl acetate. The organic phase was washed with brine, dried over Na_2SO_4, filtered and concentrated under reduced pressure to afford 1-(4-hydroxy-3-nitrophenyl)-3-(thiophen-2-yl)prop-2-en-1-one **(3)** (18 g, 65.45 mmol). This material was directly used as such for the next step. Yield: 78.9%; Melting Point [˚C] [23]: 145 - 147; MS: ES + 276.2 (M + 1).

1-(3-(4-hydroxy-3-nitrophenyl)-5-(thiophen-2-yl)-4,5-dihydro-1H-pyrazol-1-yl)ethan-1-one (4)

Hydrazine hydrate (80% w/v) (4.91 g, 98.18 mmol) was added drop wise to a solution of (E)-1-(4-hydroxy-3-nitrophenyl)-3-(thiophen-2-yl)prop-2-en-1-one **(3)** (18 g, 65.45 mmol) in glacial acetic acid (180 mL). After completion of addition, the reaction mixture was refluxed for 4 h. The resulting reaction mixture was allowed to cool to room

temperature and poured into ice-water. The obtained yellow solids were filtered off under vacuum and dried well to get the crude product which was further purified by recrystallization from ethanol to afford 1-(3-(4-hydroxy-3-nitrophenyl)-5-(thiophen-2-yl)-4,5-dihydro-1H-pyrazol-1-yl)ethan-1-one **(4)** (18 g, 54.38 mmol). This material was directly used as such for the next step. Yield: 83.1%; Melting Point [˚C]: 164 - 167; MS: ES + 332.2 (M + 1).

1-(3-(3-amino-4-hydroxyphenyl)-5-(thiophen-2-yl)-4,5-dihydro-1H-pyrazol-1-yl)ethan-1-one (5)

To a solution of 1-(3-(4-hydroxy-3-nitrophenyl)-5-(thiophen-2-yl)-4,5-dihydro-1H-pyrazol-1-yl)ethan-1-one **(4)** (18 g, 54.38 mmol) in methanol (180 mL) was added sodium dithionite (66.27 g, 380.66 mmol) in small portions at 55˚C - 60˚C in order to avoid vigorous frothing. The reaction mixture was heated at 100˚C for 2 h where by a color changed from orange to colorless was observed upon progress of the reaction. Upon completion of the reaction, the resulting reaction mixture was allowed to cool to room temperature and poured into ice-water. The resulting off-white solids were filtered off under vacuum and dried well to get the crude product which was further recrystallized from isopropyl alcohol to yield 1-(3-(3-amino-4-hydroxyphenyl)-5-(thiophen-2-yl)-4,5-dihydro-1H-pyrazol-1-yl)ethan-1-one **(5)** (11 g, 36.54 mmol). This material was immediately for the next step. Yield: 67.4%; MS: ES + 302.2 (M + 1).

1-(3-(2-aminobenzo[d]oxazol-5-yl)-5-(thiophen-2-yl)-4,5-dihydro-1H-pyrazol-1-yl)ethan-1-one (6)

To a suspension of 1-(3-(3-amino-4-hydroxyphenyl)-5-(thiophen-2-yl)-4,5-dihydro-1H-pyrazol-1-yl)ethan-1-one **(5)** (4 g, 13.28 mmol) in 1:1 mixture of methanol and water (40 mL) was added cyanogen bromide (2.11 g, 19.93 mmol) at room temperature. The reaction mixture was stirred at room temperature for next 3 h. The resulting reaction mixture was then neutralized to pH ~8 using sodium bicarbonate. The obtained mixture was stirred at room temperature for 30 min. The resulting mixture was then poured into ice-water and the obtained off-white solids were filtered off under vacuum and dried well to get the crude product which was further recrystallized from ethanol to yield

1-(3-(2-aminobenzo[d]oxazol-5-yl)-5-(thiophen-2-yl)-4,5-dihydro-1H-pyrazol-1-yl)ethan-1-one **(6)** (3.3g, 10.122 mmol). Yield: 76.2%; Melting Point [˚C]: 177 - 180; ^{1}H NMR (DMSO-*d*6): δ 7.603 - 7.628 (m, 3H), 7.395 - 7.485 (m, 3H), 7.031 - 7.038 (d, 1H, J = 2.8 Hz), 6.937 - 6.959 (m, 1H), 5.823 - 5.860 (m, 1H), 3.810 - 3.884 (m, 1H), 3.418 - 3.427 (m, 1H), 2.277 (s, 3H); LCMS: Method A, 1.734min (98.03%), MS: ES + 327.23 (M + 1).

N-(5-(1-acetyl-5-(thiophen-2-yl)-4,5-dihydro-1H-pyrazol-3-yl)benzo[d]oxazol-2-yl)acetamide (7a)

Acetic anhydride (1 mL) was added to 1-(3-(2-aminobenzo[d]oxazol-5-yl)-5-(thiophen-2-yl)-4,5-dihydro-1H-pyrazol-1-yl)ethan-1-one **(6)** (0.1g, 0.30 mmol) at 0˚C. The reaction mixture was heated at 80˚C for 30 min. The resulting reaction mixture was dumped in to saturated sodium bicarbonate solution. The obtained mixture was extracted with Ethyl acetate. The organic phase was washed with brine, dried over

Na_2SO_4, filtered and concentrated under reduced pressure. The resulting residue was purified by flash chromatography (0.9% MeOH in DCM) yielding N-(5-(1-acetyl-5-(thiophen-2-yl)-4,5-dihydro-1H-pyrazol-3-yl)benzo[d]oxazol-2-yl)acetamide **(7a)** (0.03 g, 0.081 mmol). Yield, 26.8%; Melting Point [˚C]: 209 - 211; ^{1}H NMR (DMSO-*d*6): δ 11.793 (s, 1H), 7.980 (s, 1H), 7.709 - 7.799 (m, 2H), 7.404 - 7.416 (d, 1H, J = 4.8 Hz), 7.048 (s, 1H), 6.945 - 6.966 (m, 1H), 5.858 - 5.886 (m, 1H), 3.852 - 3.927 (m, 1H), 3.435 - 3.489 (m, 1H), 2.296 (s, 3H), 2.235 (s, 3H); LCMS: Method A, 1.776 min (100%), MS: ES + 369.23 (M + 1).

N-(5-(1-acetyl-5-(thiophen-2-yl)-4,5-dihydro-1H-pyrazol-3-yl)benzo[d]oxazol-2-yl)-2-chloroacetamide (7d)

To a mixture of 1-(3-(2-aminobenzo[d]oxazol-5-yl)-5-(thiophen-2-yl)-4,5-dihydro-1H-pyrazol-1-yl)ethan-1-one **(6)** (0.2 g, 0.61 mmol) and K_2CO_3 (0.34g, 2.45 mmol) in DMF (7 mL) was added chloroacetyl chloride (0.14 g, 1.226 mmol) at 0˚C. The reaction mixture was stirred for 15 hour at room temperature. The resulting reaction mixture was dumped in to saturated sodium bicarbonate solution. The obtained reaction mixture was extracted with Ethyl acetate. The organic phase was washed with brine, dried over Na_2SO_4, filtered and concentrated under reduced pressure. The resulting residue was purified by flash chromatography (39% Ethyl acetate in n-hexane) yielding N-(5-(1-acetyl-5-(thiophen-2-yl)-4,5-dihydro-1H-pyrazol-3-yl)benzo[d]oxazol-2-yl)-2-chloroacetamide **(7d)** (0.06 g, 0.149 mmol). Yield: 24.4%; Melting Point [˚C]: 197 - 199; ^{1}H NMR (DMSO - *d*6): δ 12.323 (broad s, 1H), 8.006 (s, 1H), 7.735 - 7.822 (m, 2H), 7.403 - 7.414 (d, 1H, J = 4.4 Hz), 7.053 (s, 1H), 6.955 (s, 1H), 5.865 - 5.888 (m, 1H), 4.539 (s, 2H), 3.853 - 3.925 (m, 1H), 3.444 - 3.485 (m, 1H), 2.296 (s, 3H); LCMS: Method A, 1.983 min (100%), MS: ES + 403.30 (M + 1).

General procedure for formation of compounds 7b, 7c, 7e-7i

A mixture of suitable carboxylic acid (both aliphatic and aromatic carboxylic acids were used) (2.45 mmol), DCC (12.28 mmol), HOBT (12.28 mmol) and DMAP (1.22 mmol) in DMF (10 mL) was stirred at room temperature for 1 h. 1-(3-(2-aminobenzo[d]oxazol-5-yl)-5-(thiophen-2-yl)-4,5-dihydro-1H-pyrazol-1-yl)ethan-1-one **(6)** (0.61 mmol) was added in to reaction mixture at room temperature. The reaction mixture was heated at 50˚C - 70˚C for 6 - 15 h. The resulting reaction mixture was allowed to cool to room temperature and dumped in to saturated aqueous sodium bicarbonate solution. The resulting mixture was and extracted with Ethyl acetate. The organic phase was washed with brine, dried over Na_2SO_4, filtered and concentrated under reduced pressure. The resulting residues were purified by flash chromatography (using a suitable gradient of Ethyl acetate in n - hexane) yielding the pure products **7b, 7c, 7e-7i**

N-(5-(1-acetyl-5-(thiophen-2-yl)-4,5-dihydro-1H-pyrazol-3-yl)benzo[d]oxazol-2-yl)isobutyramide (7b)

Yield: 30.8%; Melting Point [˚C]:135 - 137; ^{1}H NMR (DMSO - *d*6): 11.741 (s, 1H), 7.983 (s, 1H), 7.707 - 7.804 (m, 2H), 7.404 - 7.416 (d, 1H, J = 4.8 Hz), 7.050 - 7.056 (d, 1H, J = 2.4 Hz), 6.946 - 6.967 (m, 1H), 5.858 - 5.896 (m, 1H), 3.857 - 3.930 (m, 1H), 3.438 - 3.493 (m, 1H), 2.813 - 2.830 (m, 1H), 2.298 (s, 3H), 1.054 - 1.147 (m, 6H); ^{13}C

NMR (DMSO-*d*6): δ19.41, 22.16, 34.98, 42.33, 55.54, 110.85, 117.05, 122.78, 125.04, 125.45, 127.14, 128.27, 141.70, 145.31, 149.36, 154.86, 156.61, 167.86, 175.13: LCMS: Method B, 2.949 min (94.44%), MS: ES + 396.99 (M + 1).

N-(5-(1-acetyl-5-(thiophen-2-yl)-4,5-dihydro-1H-pyrazol-3-yl)benzo[d]oxazol-2-yl)butyramide (7c)

Yield: 27.5%; Melting Point [˚C]: 178 - 181; ^{1}H NMR (DMSO - *d*6): δ 11.760 (s, 1H), 7.980 (s, 1H), 7.708 - 7.800 (dd, 2H, J_{HH} = 7.6 Hz, J_{HH}' = 28.4 Hz), 7.406 - 7.417 (d, 1H, J = 4.4 Hz), 7.053 (s, 1H), 6.956 - 6.965 (m, 1H), 5.866 - 5.889 (m, 1H), 3.854 - 3.927 (m, 1H), 3.4 - 3.493 (m, 1H), 2.334 (m, 2H), 2.296 (s, 3H), 1.599 - 1.654 (q, 2H, J_{H}'$_{H}$" = 14.8 Hz, J_{HH}' = 7.2 Hz), 0.932 (t, 3H, J = 7.2 Hz); LCMS: Method A, 1.917 min (99.66%), MS: ES + 397.08 (M + 1).

N-(5-(1-acetyl-5-(thiophen-2-yl)-4,5-dihydro-1H-pyrazol-3-yl)benzo[d]oxazol-2-yl)benzamide (7e)

Yield: 26.6%; Melting Point [˚C]: 164 - 168; ^{1}H NMR (CD_3OD): δ 8.437 (broad s, 1H), 8.053 (m, 2H), 7.857 - 7.878 (d, 1H, J = 8.4 Hz), 7.582 - 7.724 (m, 3H), 7.300 - 7.315 (dd, 1H, J_{HH}" = 1.2Hz, J_{HH}' = 5.2 Hz), 7.095 (s, 1H), 6.954 - 6.975 (m, 1H), 5.949 - 5.973 (m, 1H), 3.910 - 3.976 (m, 1H), 3.731 - 3.764 (m, 1H), 3.429 - 3.476 (d, 1H, J = 18.8 Hz), 2.414 (s, 2H), 2.091 - 2.124 (m, 1H), 1.875 - 1.908 (m, 1H); LCMS: Method A, 2.033 min (96.95%), MS: ES + 431.23 (M + 1).

N-(5-(1-acetyl-5-(thiophen-2-yl)-4,5-dihydro-1H-pyrazol-3-yl)benzo[d]oxazol-2-yl)-2-chlorobenzamide (7f)

Yield: 29.2%; Melting Point [˚C]: 139 - 141; ^{1}H NMR (DMSO - *d*6): δ 12.548 (s, 1H), 8.001 (s, 1H), 7.802 - 7.834 (m, 1H), 7.735 - 7.755 (m, 1H), 7.654 - 7.683 (m, 1H), 7.551 - 7.582 (m, 2H), 7.43 - 7.481 (m, 1H), 7.410 - 7.423 (d, 1H, J = 5.2 Hz), 7.056 (s, 1H), 6.961 (s, 1H), 5.866 - 5.900 (m, 1H), 3.863 - 3.937 (m, 1H), 3.444 - 3.494 (m, 1H), 2.302 (s, 3H); LCMS: Method A, 2.026 min (96.67%), MS: ES + 465.33 (M + 1).

N-(5-(1-acetyl-5-(thiophen-2-yl)-4,5-dihydro-1H-pyrazol-3-yl)benzo[d]oxazol-2-yl)-2-methoxybenzamide (7g)

Yield: 30.7%; Melting Point [˚C]: 126 - 129; ^{1}H NMR (DMSO - *d*6): δ 11.797 (s, 1H), 8.005 (s, 1H), 7.730 - 7.824 (m, 2H), 7.617 - 7.636 (d, 1H, J = 7.6 Hz), 7.560 (t, 1H, J = 7.6 Hz), 7.407 - 7.419 (d, 1H, J = 4.8 Hz), 7.184 - 7.205 (d, 1H, J = 8.4 Hz), 7.053 - 7.106 (m, 2H), 6.950 - 6.971 (m, 1H), 5.863 - 5.891 (m, 1H), 3.864 - 3.896 (m, 5H), 2.302 (s, 3H); LCMS: Method A, 2.191 min (99.82%), MS: ES + 460.9 (M + 1).

N-(5-(1-acetyl-5-(thiophen-2-yl)-4,5-dihydro-1H-pyrazol-3-yl)benzo[d]oxazol-2-yl)-3-chlorobenzamide (7h)

Yield: 33.1%; Melting Point [˚C]: 142 - 144; ^{1}H NMR (DMSO - *d*6): δ 12.736 (broad s, 1H), 8.157 (s, 1H), 8.045 - 8.085 (m, 2H), 7.950 (s, 1H), 7.585 - 7.636 (m, 3H), 7.400 - 7.414 (d, 1H, J = 5.6 Hz), 7.011 (m, 1H), 6.946 - 6.967 (m, 1H), 5.839 - 5.849 (m, 1H), 3.810 - 3.884 (m, 1H), 3.418 - 3.427 (m, 1H), 2.244 (s, 3H); LCMS: Method A, 2.242 min (100%), MS: ES + 465.33 (M + 1).

N-(5-(1-acetyl-5-(thiophen-2-yl)-4,5-dihydro-1H-pyrazol-3-yl)benzo[d]oxazol-2-yl)-3-methoxybenzamide (7i)

Yield: 35.8%; Melting Point [˚C]: 122 - 124; ^{1}H NMR (DMSO - *d*6): δ 12.287 (broad s, 1H), 8.030 (s, 1H), 7.757 - 7.836 (m, 2H), 7.581 - 7.649 (m, 2H), 7.479 (t, 1H, J = 8.0 Hz), 7.410 - 7.426 (dd, 1H, J_{HH}" = 1.2 Hz, J_{HH}' = 5.2 Hz), 7.215 - 7.240 (dd, 1H, J_{HH}" = 2.4Hz, J_{HH}' = 7.6 Hz), 7.058 - 7.066 (d, 1H, J = 3.2 Hz), 6.952 - 6.974 (m, 1H), 5.870 - 5.908 (m, 1H), 3.831 (s, 3H), 3.806 - 3.950 (m, 1H), 3.447 - 3.500 (m, 1H), 2.298 (s, 3H). ^{13}C NMR (DMSO - *d*6): δ22.16, 22.19, 42.35, 55.49, 55.83, 55.87, 111.03, 113.68, 119.33, 121.24, 123.22, 125.06, 125.47, 127.16, 128.40, 130.20, 145.30, 154.77, 159.67, 167.88; LCMS: Method A, 2.081min (100%), MS: ES + 461.20 (M + 1).

1-(3-(2-mercaptobenzo[d]oxazol-5-yl)-5-(thiophen-2-yl)-4,5-dihydro-1H-pyrazol-1-yl)ethan-1-one (8)

To a suspension of 1-(3-(3-amino-4-hydroxyphenyl)-5-(thiophen-2-yl)-4,5-dihydro-1H-pyrazol-1-yl)ethan-1-one **(5)** (0.05 g, 0.166 mmol) in methanol (0.5 mL) was added KOH (0.018 g, 0.166 mmol) at room temperature. CS_2 (0.012 g, 0.166 mmol) was added to the reaction mixture at room temperature. The reaction mixture was then heated at 60˚C for next 3 h. The resulting reaction mixture was allowed too cool at room temperature and poured in to water. The obtained mixture was extracted with Ethyl acetate. The organic phase was washed with brine, dried over Na_2SO_4, filtered and concentrated under reduced pressure. The resulting residue was purified by flash chromatography (50% MeOH in DCM) yielding 1-(3-(2-mercaptobenzo[d]oxazol-5-yl)-5-(thiophen-2-yl)-4,5-dihydro-1H-pyrazol-1-yl)ethan-1-one **(8)** (0.035g, 0.102 mmol). Yield: 61.4%; Melting Point [˚C]: 214 - 218; ^{1}H NMR (DMSO - *d*6): δ 7.532 (s, 1H), 7.471 - 7.495 (dd, 1H, J_{HH}" = 1.2 Hz, J_{HH}' = 8.4 Hz), 7.391 - 7.404 (d, 1H, J = 5.2 Hz), 7.306 - 7.327 (d, 1H, J = 8.4 Hz), 7.031 - 7.040 (d, 1H, J = 3.6 Hz), 6.937 - 6.958 (m, 1H), 5.816 - 5.854 (m, 1H), 3.813 - 3.886 (m, 1H), 3.407 - 3.416 (m, 1H), 2.277 (s, 3H). ^{13}C NMR (DMSO - *d*6): δ 22.20, 42.34, 55.35, 108.71, 110.68, 121.11, 124.99, 125.41, 126.76, 127.13, 145.38, 151.96, 155.19, 167.74, 182.67; LCMS: Method A, 1.956min (100%), MS: ES + 344.18 (M + 1).

General procedure for formation of compounds 9a-9k

A mixture of 1-(3-(3-amino-4-hydroxyphenyl)-5-(thiophen-2-yl)-4,5-dihydro-1H-pyrazol-1-yl)ethan-1-one **(5)** (0.996 mmol), suitable aromatic carboxylic acid (0.996 mmol), T3P (0.996 mmol) and DIPEA (1.494 mmol) was heated in a microwave for 1.5 to 2.5 h at 160˚C. The resulting reaction mixture was allowed to cool to room temperature and dumped in to sodium bicarbonate solution. The obtained mixture was extracted with Ethyl acetate. The organic phase was washed with brine, dried over Na_2SO_4, filtered and concentrated under reduced pressure. The resulting residue was purified by flash chromatography (using a suitable gradient of MeOH in DCM) yielding the pure products **9a-9k**

1-(3-(2-phenylbenzo[d]oxazol-5-yl)-5-(thiophen-2-yl)-4,5-dihydro-1H-pyrazol-1-yl)ethan-1-one (9a)

Yield: 45.8%; Melting Point [˚C]: 177 - 179; ^{1}H NMR (DMSO - *d*6): δ 8.214 - 8.246 (m, 3H), 7.975 - 8.000 (m, 1H), 7.902 - 7.924 (m, 1H), 7.628 - 7.705 (m, 3H), 7.412 - 7.427 (dd, 1H, J_{HH}" = 1.2 Hz, J_{HH}' = 4.8 Hz), 7.066 - 7.074 (d, 1H, J = 3.2 Hz), 6.954 -

6.975 (q, 1H, $J_{H'H''}$ = 4.8 Hz, $J_{HH'}$ = 3.6 Hz), 5.884 - 5.922 (m, 1H), 3.893 - 3.967 (m, 1H), 3.527 - 3.538 (m, 1H), 2.316 (s, 3H); LCMS: Method A, 2.567 min (99.71%), MS: ES + 388.23 (M + 1).

1-(3-(2-(2-chlorophenyl)benzo[d]oxazol-5-yl)-5-(thiophen-2-yl)-4,5-dihydro-1H-pyrazol-1-yl)ethan-1-one (9b)

Yield: 53.6%; Melting Point [˚C]: 154 - 156; ^{1}H NMR (DMSO - *d*6): δ 8.286 (s, 1H), 8.185 - 8.208 (dd, 1H, $J_{HH''}$ = 1.6 Hz, $J_{HH'}$ = 8.0 Hz), 8.038 - 8.064 (dd, 1H, $J_{HH''}$ = 1.6 Hz, $J_{HH'}$ = 8.8 Hz), 7.937 - 7.959 (m, 1H),7.746 - 7.766 (m, 1H), 7.657 - 7.699 (m, 1H), 7.597 - 7.634 (m, 1H), 7.417 - 7.429 (d, 1H, J = 8.0 Hz), 7.070 - 7.078 (d, 1H, J = 3.2 Hz), 6.955 - 6.976 (m, 1H), 5.889 - 5.927 (m, 1H), 3.898 - 3.971 (m, 1H), 3.507 - 3.562 (m, 1H), 2.318 (s, 3H); LCMS: Method A, 2.637 min (97.73%), MS: ES + 422.23 (M + 1).

1-(3-(2-(2-methoxyphenyl)benzo[d]oxazol-5-yl)-5-(thiophen-2-yl)-4,5-dihydro-1H-pyrazol-1-yl)ethan-1-one (9c)

Yield: 62.5%; Melting Point [˚C]: 150 - 152; ^{1}H NMR (DMSO - *d*6): δ 8.214 (s, 1H), 8.050 - 8.067 (d, 1H, J = 6.8 Hz), 7.967 - 7.988 (d, 1H, J = 8.4 Hz), 7.874 - 7.895 (d, 1H, J = 8.4 Hz), 7.623 - 7.660 (m, 1H), 7.415 - 7.426 (d, 1H, J = 4.4 Hz), 7.301 - 7.321 (d, 1H, J = 8.0 Hz), 7.171 (t, 1H, J = 7.6 Hz), 7.068 (s, 1H), 6.965 (s, 1H), 5.891 - 5.912 (m, 1H), 3.891 - 3.952 (m, 4H), 3.487 - 3.532 (m, 1H) 2.314(s, 3H). ^{13}C NMR (DMSO - *d*6): δ22.19, 42.32, 55.51, 56.48, 111.75, 113.30, 115.39, 118.95, 121.20, 124.28, 125.11, 125.52, 127.16, 128.38, 131.56, 134.12, 142.23, 145.31, 151.74, 154.77, 158.63, 162.90, 167.92; LCMS: Method B, 5.118 min (91.88%), MS: ES + 417.97 (M + 1).

1-(3-(2-(3-methoxyphenyl)benzo[d]oxazol-5-yl)-5-(thiophen-2-yl)-4,5-dihydro-1H-pyrazol-1-yl)ethan-1-one (9d)

Yield: 59.6%; Melting Point [˚C]: 178 - 180; ^{1}H NMR (DMSO - *d*6): δ 8.210 (s, 1H), 7.977 - 7.995 (m, 1H), 7.901 - 7.921 (m, 1H), 7.804 - 7.824 (d, 1H, J = 8.0 Hz), 7.707 (s, 1H), 7.545 - 7.583 (m, 1H), 7.423 (s, 1H), 7.241 - 7.260 (m, 1H), 7.068 (s, 1H), 6.964 (s, 1H), 5.891 - 5.915 (m, 1H), 3.892 - 3.962 (m, 4H), 3.482 - 3.526 (m, 1H), 2.312 (s, 3H). ^{13}C NMR (DMSO - *d*6): δ22.17, 42.32, 55.53, 55.85, 111.91, 112.41, 118.87, 118.93, 120.20, 124.63, 125.08, 125.49, 127.16, 127.73, 128.66, 131.11, 142.33, 145.29, 151.86, 154.60, 160.12, 163.59, 167.92; LCMS: Method A, 2.690 min (100%), MS: ES + 418.45 (M + 1).

1-(3-(2-(4-methoxyphenyl)benzo[d]oxazol-5-yl)-5-(thiophen-2-yl)-4,5-dihydro-1H-pyrazol-1-yl)ethan-1-one (9e)

Yield: 55.1%; Melting Point [˚C]: 192 - 195; ^{1}H NMR (DMSO - *d*6): δ 8.151 - 8.173 (m, 3H), 7.916 - 7.938 (m, 1H), 7.842 - 7.864 (m, 1H), 7.410 - 7.422 (d, 1H, J = 4.8 Hz), 7.175 - 7.197 (d, 2H, J = 8.8 Hz), 7.061 - 7.068 (d, 1H, J = 2.8 Hz), 6.952 - 6.972 (m, 1H), 5.874 - 5.911 (m, 1Hz), 3.880 - 3.952 (m, 4H), 3.466 - 3.519 (m, 1H), 2.309 (s, 3H); LCMS: Method A, 2.704 min (99.78%), MS: ES + 418.55 (M + 1).

1-(3-(2-(4-hydroxyphenyl)benzo[d]oxazol-5-yl)-5-(thiophen-2-yl)-4,5-dihydro-1H-pyrazol-1-yl)ethan-1-one (9f)

Yield: 66.4%; Melting Point [˚C]: 256 - 258; ^{1}H NMR (DMSO - *d*6): δ 10.429 (s, 1H), 8.130 (s, 1H), 8.050 - 8.072 (d, 2H, J = 8.8 Hz), 7.893 - 7.918 (m, 1H), 7.820 - 7.842 (d,

1H, J = 8.8 Hz), 7.408 - 7.421 (d, 1H, J = 5.2 Hz), 7.058 - 7.065 (d, 1H, J = 2.8 Hz), 6.948 - 6.999 (m, 3H), 5.870 - 5.908 (m, 1H), 3.877 - 3.950 (m, 1H), 3.460 - 3.515 (m, 1H), 2.306 (s, 3H); LCMS: Method A, 2.264 min (99.82%), MS: ES + 404.65 (M + 1).

1-(5-(thiophen-2-yl)-3-(2-(3,4,5-trimethoxyphenyl)benzo[d]oxazol-5-yl)-4,5-dihydro-1H-pyrazol-1-yl)ethan-1-one (9g)

Yield: 68.3%; Melting Point [˚C]: 210 - 214; ^{1}H NMR (DMSO - *d*6): δ 8.182 (s, 1H), 7.961 - 7.983 (m, 1H), 7.895 - 7.916 (m, 1H), 7.498 - 7.508 (d, 2H, J = 4.0 Hz), 7.415 - 7.428 (d, 1H, J = 5.2 Hz), 7.066 - 7.073 (d, 1H, J = 2.8 Hz), 6.957 - 6.978 (m, 1H), 5.888 - 5.924 (m, 1H), 3.935 (s, 6H), 3.783 (s, 3H), 3.508 (m, 1H), 3.462 - 3.472 (m, 1H) 2.335 (s, 3H); LCMS: Method A, 2.423 min (100%), MS: ES + 478.43 (M + 1).

1-(3-(2-benzylbenzo[d]oxazol-5-yl)-5-(thiophen-2-yl)-4,5-dihydro-1H-pyrazol-1-yl)ethan-1-one (9h)

Yield: 56.9%; Melting Point [˚C]: 136 - 140; ^{1}H NMR (DMSO - *d*6): δ 8.101 (s, 1H), 7.894 - 7.914 (d, 1H, J = 8.0 Hz), 7.769 - 7.789 (d, 1H, J = 8.0 Hz), 7.301 - 7.387 (m, 6H), 7.045 (s, 1H), 6.952 (s, 1H), 5.867 - 5.889 (m, 1H), 4.386 (s, 2H), 3.853 - 3.926 (m, 1H), 3.444 - 3.489 (m, 1H), 2.292 (s, 3H). ^{13}C NMR (DMSO - *d*6): δ21.64, 34.08, 41.81, 54.97, 111.08, 118.17, 123.53, 124.55, 124.97, 126.63, 127.09, 127.68, 128.65, 129.03, 134.90, 141.30, 144.78, 151.55, 154.22, 166.56, 167.38; LCMS: Method A, 2.443 min (100%), MS: ES + 402.6 (M + 1).

1-(3-(2-(pyridin-2-yl)benzo[d]oxazol-5-yl)-5-(thiophen-2-yl)-4,5-dihydro-1H-pyrazol-1-yl)ethan-1-one (9i)

Yield: 64.7%; Melting Point [˚C]: 182 - 184; ^{1}H NMR (DMSO - *d*6): δ 8.827 - 8.837 (d, 1H, J = 4.0 Hz), 8.361 - 8.381 (d, 1H, J = 8.0 Hz), 8.265 - 8.268 (d, 1H, J = 1.2 Hz), 8.076 - 8.119 (m, 1H), 8.032 - 8.058 (dd, 1H, J_{HH}" = 1.6 Hz, J_{HH}' = 8.8 Hz), 7.956 - 7.978(d, 1H, J = 8.8 Hz), 7.660 - 7.691 (m, 1H), 7.414 - 7.427 (d, 1H, J = 5.2 Hz), 7.070 - 7.079 (d, 1H, J = 3.6Hz), 6.956 - 6.978 (m, 1H), 5.891 - 5.929 (m, 1H), 3.907 - 3.981 (m, 1H), 3.498 - 3.553 (m, 1H), 2.321(s, 3H); LCMS: Method B, 4.332min (98.45%), MS: ES + 388.90 (M + 1).

1-(3-(2-(pyridin-3-yl)benzo[d]oxazol-5-yl)-5-(thiophen-2-yl)-4,5-dihydro-1H-pyrazol-1-yl)ethan-1-one (9j)

Yield: 52.8%; Melting Point [˚C]: 210 - 210; ^{1}H NMR (CF_3COOD): δ 9.556 - 9.573 (m, 1H), 9.236 - 9.254 (m, 1H), 8.856 - 8.871 (m, 1H) , 8.138 - 8.213 (m, 2H), 8.039 - 8.105 (m, 1H), 7.657 - 7.679 (d, 1H, J = 8.8 Hz), 7.011 - 7.024 (d, 1H, J = 5.2 Hz), 6.858 (s, 1H), 6.693 - 6.714 (m, 1H), 5.924 - 5.950 (m, 1H), 3.837 - 3.908 (m, 1H), 3.437 - 3.482 (m, 1H), 2.519(s, 3H); LCMS: Method B, 4.414min (98.83%), MS: ES + 388.83 (M + 1).

1-(3-(2-(pyridin-4-yl)benzo[d]oxazol-5-yl)-5-(thiophen-2-yl)-4,5-dihydro-1H-pyrazol-1-yl)ethan-1-one (9k)

Yield: 65.9%; Melting Point [˚C]: 162 - 166; ^{1}H NMR (DMSO - *d*6): δ 8.876(s, 2H), 8.262 (s, 1H), 8.105 - 8.120 (d, 2H, J = 6.0 Hz), 8.036 - 8.061 (m, 1H), 7.948 - 7.970 (d, 1H, J = 8.8 Hz), 7.414 - 7.425 (d, 1H, J = 4.4 Hz), 7.067 - 7.075 (d, 1H, J = 3.2Hz), 6.954 - 6.975 (m, 1H), 5.887 - 5.925 (m, 1H), 3.890 - 3.963 (m, 1H), 3.487 - 3.542 (m, 1H),

2.314(s, 3H). ^{13}C NMR (DMSO-*d*6): δ 21.65, 41.76, 55.06, 111.71, 118.96, 120.73, 124.58, 124.99, 126.65, 128.49, 133.03, 141.49, 144.74, 150.84, 151.37, 153.85, 161.19, 167.42; LCMS: Method B, 4.438 min (96.97%), MS: ES + 388.97 (M + 1).

3-(4-hydroxy-3-nitrophenyl)-5-(thiophen-2-yl)-4,5-dihydro-1H-pyrazole-1-carboxamide (4a)

NaOH (0.43g, 10.90 mmol) was added to a suspension of (E)-1-(4-hydroxy-3-nitrophenyl)-3-(thiophen-2-yl)prop-2-en-1-one **(3)** (1g, 3.63 mmol) in methanol (10 mL). Semicarbazide hydrochloride (0.49 g, 4.36 mmol) was added in to the reaction mixture at room temperature in small portions. After completion of addition, the reaction mixture was heated at 80˚C for 15 hours. The resulting reaction mixture was allowed to cool to room temperature and poured into ice-water. The obtained yellow solids were filtered off under vacuum and dried well to get the crude product which was further purified by recrystallization from ethanol to afford 3-(4-hydroxy-3-nitrophenyl)-5-(thiophen-2-yl)-4,5-dihydro-1H-pyrazole-1-carboxamide **(4a)** (0.4 g, 54.38 mmol). Yield: 33.1%; MS: ES + 333.2 (M + 1).

3-(3-amino-4-hydroxyphenyl)-5-(thiophen-2-yl)-4,5-dihydro-1H-pyrazole-1-carboxamide (5a)

The title compound was obtained by following the same procedure as described for **(5)** but using **(4a)** as a starting material instead of **(4)**. The obtained material was directly used as such for further chemistry. Yield: 61.6%; MS: ES + 303.2 (M + 1).

3-(2-aminobenzo[d]oxazol-5-yl)-5-(thiophen-2-yl)-4,5-dihydro-1H-pyrazole-1-carboxamide (10)

The title compound was obtained by following the same procedure as described for **(6)** but using **(5a)** as a starting material instead of **(5)**. Yield: 56.6%; Melting Point [˚C]: 218 - 220; ^{1}H NMR (DMSO-*d*6): δ 7.704 (s, 1H), 7.565 (s, 2H), 7.372 - 7.451 (m, 3H), 6.937 - 7.011 (m, 2H), 6.526 (broad s, 2H), 5.669 - 5.710 (m, 1H) 3.772 - 3.845 (m, 1H), 3.271 - 3.283 (m, 1H); LCMS: Method A, 1.780 min (98.25%), MS: ES + 327.98 (M + 1).

3-(2-mercaptobenzo[d]oxazol-5-yl)-5-(thiophen-2-yl)-4,5-dihydro-1H-pyrazole-1-carboxamide (11)

The title compound was obtained by following the same procedure as described for **(8)** but using **(5a)** as a starting material instead of **(5)**. Yield: 63.9%; Melting Point [˚C]: 270 - 272; ^{1}H NMR (DMSO-*d*6): δ 14.127(s, 1H), 7.682 - 7.705 (m, 2H), 7.573 - 7.593 (d, 1H, J = 8.0 Hz), 7.381 - 7.391 (d, 1H, J = 4.0 Hz), 7.011 - 7.018 (d, 1H, J = 2.8 Hz), 6.940 - 6.982 (m, 1H), 6.621 (broad s, 2H), 5.702 - 5.742 (m, 1H), 3.789 - 3.863 (m, 1H), 3.297 - 3.309 (m, 1H). ^{13}C NMR (DMSO-*d*6): δ42.52, 56.15, 108.33, 110.53, 123.27, 124.55, 125.15, 127.10, 129.25, 132.32, 146.82, 149.47, 150.67, 155.37, 181.15; LCMS: Method A, 1.828 min (95.06%), MS: ES + 345.09 (M + 1).

2.4. Antitubercular Screening

Microplate Alamar Blue Assay (MABA)

The anti-mycobacterial activity of all the synthesized compounds was assessed against *Mycobacterium tuberculosis* H_{37}Rv strain by Microplate Alamar Blue Assay

(MABA) which is a non-toxic methodology and uses a thermally stable reagent. MABA shows a very good correlation with proportional and BACTEC radiometric methods.

200 µl of sterile deionized water was added to all outer perimeter wells of sterile 96 wells plate to minimized evaporation of medium in the test wells during incubation. The 96 wells plate received 100 µl of the Middlebrook 7H9 broth and serial dilution of compounds were made directly on plate. The final drug concentrations tested were 100 to 0.2 µg/ml. Plates were covered and sealed with parafilm and incubated at 37˚C for five days. After this time, 25 µl of freshly prepared 1:1 mixture of Alamar Blue reagent and 10% tween 80 was added to the plate and incubated for 24 hrs. A blue color in the well was interpreted as no bacterial growth, and pink color was scored as growth. The MIC was defined as lowest drug concentration which prevented the color change from blue to pink.

Löwenstein-Jensen medium (L. J. medium)

The *In vitro* antitubercular screening of selected (**potent**) compounds was also carried out by measuring the growth of *M. tuberculosis* $H_{37}R_V$ strain using Löwenstein-Jensen medium (L. J. medium) (Stover *et al*., 2000) [24] where the susceptibility of the compounds against multidrug-resistant TB (MDR-TB) and extensively drug-resistant TB (XDR-TB) strains were also checked. Eggs were broken aseptically to obtain 200 mL of egg solution. The solution was filtered through a sterile muslin cloth into a sterile conical flask containing glass beads. Sterilized mineral salt solution (120 mL) (consisting of 4.0 g potassium phosphate, 0.4 g of magnesium sulfate, 1.6 g magnesium citrate, 6.0 g of asparagine, 20 mL of glycerol, distilled water makeup up to 1000 mL) and 4 mL of sterilized malachite green solution (2.0%) were added to the 200 mL of egg solution. The contents were mixed well to form a uniform medium. Target compounds (10 mg) were dissolved in 10.0 mL of dimethyl sulfoxide (DMSO) and were diluted with DMSO to make 250 and 10 mg/mL stock solutions. An aliquot (0.8 mL) of each concentration was transferred into different McCartney bottles. To this, 7.2 mL of L. J. medium was added and mixed well. Isoniazid was considered as a reference standard for the comparison of antitubercular activity. The drug was dissolved in DMSO and diluted as described above. The bottles were incubated at 75˚C - 80˚C for 3 days for solidification and sterilization.

Procedure for inoculation: A sweep from MDR H_{37}Rv resistant strains of *M. tuberculosis* culture was transferred with the help of 22 S.W. nichrome wire loop of 3 mm external diameter into a sterile bijou bottle containing six 3 mm glass beads and 4 mL of sterile distilled water. Each loop of culture delivered approximately 4 mg of bacilli cells. The bottle was shaken with the help of vertex mixture for 2 min. The suspension was inoculated on the surface of each L. J. medium containing test compounds using 27 S. W. G nichrome wire loop of 3 mm external diameter and L. J. medium containing isoniazid. The medium containing DMSO (control) was inoculated with the test organism for positive and negative controls. Medium without any test compound/DMSO was also inoculated with the test organism to check whether the media supports the growth of the tubercle bacilli or not. The inoculated bottles were incubated at 37˚C for 6 weeks,

at the end of which readings were taken. Bacterial counts were measured and compared with the standard drugs and controls (vehicle - treated).

Cytotoxicity Assay

The selected set of compounds which showed potent activity against MTB ($H_{37}Rv$), MDR-TB and XDR-TB strains were also evaluated for their cytotoxicity on VERO cell lines. The cytotoxicity results have been summarized in Table 3.

3. Results and Discussion

3.1. Chemistry

The target compounds (**6, 7a-7i**, **8, 9a-9k, 10 and 11**) were synthesized in good yields by multistep chemical synthesis as outlined in the Figure 2. Nitration of 1-(4-hydroxyphenyl)ethan-1-one **(1)** provided 1-(4-hydroxy-3-nitrophenyl)ethan-1-one **(2)** in good yields. Aldol condensation of **2** with thiophene-2-carbaldehyde in presence of sodium hydroxide afforded corresponding chalcone 1-(4-hydroxy-3-nitrophenyl)-3-(thiophen-2-yl)prop-2-en-1-one **(3)**. Cyclization of the chalcone derivative **3** was carried out whilst using hydrazine hydrate in glacial acetic acid and the reaction afforded the corresponding pyrazoline derivative 1-(3-(4-hydroxy-3-nitrophenyl)-5-(thiophen-2-yl)-4,5-dihydro-1H-pyrazol-1-yl)ethan-1-one **(4)** in good yields. Reduction of nitro group of **4** whilst using sodium dithionite afforded 1-(3-(3-amino-4-hydroxyphenyl)-5-(thiophen-2-yl)-4,5-dihydro-1H-pyrazol-1-yl)ethan-1-one **(5)**. Cyclization of **5** was carried out in three different ways: ***(a)*** treatment with cyanogen bromide in methanol: water mixture at room temperature to yield corresponding 2-aminobenzoxazole derivative **6**; ***(b)*** treatment with carbon disulphide and potassium hydroxide in methanol at reflux temperature to afford 2-mercaptobenzoxazole derivative **8**; and ***(c)*** reaction with suitable carboxylic acid in presence of T3P, DIPEA, under microwave conditions at 160˚C to afford 2-substituded benzoxazole derivatives **9a-9h**. The 2-aminobenzoxazole derivative **6** was further acylated by reaction with suitable reagents following prior-to-art synthetic methods to afford corresponding amide derivatives **7a-7i**. In order to generate few more interesting molecules **c**yclization of the chalcone derivative **3** was also carried out by reaction with semicarbazide hydrochloride and NaOH in methanol and the reaction afforded 3-(2-amino-1,3-benzoxazol-5-yl)-5-(thiophen-2-yl)- 4,5-dihydro-1H-pyrazole-1-carboxamide **(4a)** in good yields. Reduction of **4a** to get **5a**, followed by cyclization of **5a** to generate 2-aminobenzoxazole derivative **10** and 2-mercaptobenzoxazole derivative **11** were carried out by similar methods described above for getting compounds **5, 6** and **8**, the corresponding products were isolated in average to good yields.

3.2. Antitubercular Screening

Primary *In-Vitro* evaluation of all the synthesized target compounds **6, 7a-7i**, **8, 9a-9k, 10 and 11** for their antitubercular activity against *M. tuberculosis* $H_{37}Rv$ strain was carried out through the rapid, reliable and cost effective MABA assay. Pyrazinamide, Streptomycin and Ciprofloxacin were screened as standard drugs for their antitubercular

activity against *M. tuberculosis* $H_{37}Rv$ strain under similar experimental conditions. Table 1 and Table 2 show results of the biological screening. It was encouraging to see that the front line targets **6**, **8** and **10** displayed satisfactory MIC values and were found to be equipotent to Streptomycin (MIC = 6.25 μg/ml). Further, potency of the target compounds was found to increase in good folds when the space around the second position of benzothiazole nucleus was explored. Target compounds **7d, 7e** were found to be equipotent to Streptomycin (MIC = 6.25 μg/ml) while **7i, 9f** displayed potency **(7i: MIC = 1.6 μg/ml & 9f: MIC = 0.8 μg/ml)** better than all the standard drugs (Streptomycin, Pyrazinamide and Ciprofloxacin) against *M. tuberculosis* $H_{37}Rv$ strain.

Target compounds **6, 8, 10, 7d, 7e, 7i, 9f** which demonstrated good antitubecular activity against *M. tuberculosis* $H_{37}Rv$ strain via MABA were further assessed by using Löwenstein–Jensen medium (L. J. medium) and their susceptibility against multidrug-resistant TB (MDR-TB) and extensively drug-resistant TB (XDR-TB) strains were also checked. As summarized in Table 3 target compounds **7d, 7i, 9f** were found to be better than isoniazid against MDR-TB **(MIC = 3.12 μg/mL)** and **XDR-TB (MIC = 12.5 μg/mL)** strains**.**

Also for rationalization of our "pharmacophore combination-hybridization" hypothesis, it was important to compare the results with the literature activity of pyrazoline and benzoxazole derivatives. Indeed, when compared to some of the literature pyrazoline (*Kuntal and Agrawal 2010; Mohammad et al., 2006*) [25] [26] and benzoxazole (*Vinsova et al*., 2006; *Klimesova et al*., 2009) [6] [8] derivatives we could observe an encouraging and satisfactory gain in the potency of the compounds of current work. Further, as evident from the cytotoxicity results in Table 3, the compounds displayed selectivity index (cytotoxicity IC_{50}/MIC_{MTB}) >10 which implies that the compounds are safe and could be explored as potential lead for further development.

4. Conclusion

In summary, we designed a series of novel target compounds by combining two pharmacophoric motifs—pyrazoline and benzoxazole, for learning their antitubercular activity. The designed target compounds were synthesized, structurally confirmed and *In vitro* screened for their activity against *M. tuberculosis* $H_{37}Rv$, MDR-TB and XDR-TB strains. Potency of the target compounds was found to increase in good folds when the space around the second position of benzothiazole nucleus was explored. Some of the target compounds displayed significant antitubercular activity with a few analogs showing activity even better than the standard drugs against MDR-TB and XDR-TB strains. Overall, the "pharmacophore combination" seems to be a useful strategy for discovery of novel antitubercular agents.

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