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PII: S0960-894X(15)00506-5
DOI: http://dx.doi.org/10.1016/j.bmcl.2015.05.044
Reference: BMCL 22734

To appear in: *Bioorganic & Medicinal Chemistry Letters*

Received Date: 16 April 2015
Revised Date: 18 May 2015
Accepted Date: 20 May 2015

Please cite this article as: Emmadi, N.R., Bingi, C., Kotapalli, S.S., Ummanni, R., Nanubolu, J.B., Atmakur, K., Synthesis and evaluation of novel fluorinated pyrazolo-1,2,3-triazole hybrids as antimycobacterial agents, *Bioorganic & Medicinal Chemistry Letters* (2015), doi: http://dx.doi.org/10.1016/j.bmcl.2015.05.044

Bioorganic & Medicinal Chemistry Letters
journal homepage: www.elsevier.com

Synthesis and evaluation of novel fluorinated pyrazolo-1,2,3-triazole hybrids as antimycobacterial agents

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ARTICLE INFO

Article history:
Received
Revised
Accepted
Available online

Keywords:
Antimycobacterial activity
Cytotoxicity
Click chemistry
3-Trifluoromethyl pyrazolo-1,2,3-triazole hybrids

ABSTRACT

A library of novel 3-trifluoromethyl pyrazolo-1,2,3-triazole hybrids (**5-7**) were accomplished starting from 5-phenyl-3-(trifluoromethyl)-1H-pyrazol-4-amine (**1**) *via* key intermediate 2-azido-N-(5-phenyl-3-(trifluoromethyl)-1H-pyrazol-4-yl)acetamide (**3**) through Click Chemistry approach. Thus obtained compounds in **5-7** series were evaluated for *in vitro* antimycobacterial activity against *Mycobacterium smegmatis* (MC^2 155) and also verified the cytotoxicity. These studies engendered promising lead compounds **5q**, **7b** and **7c** with MIC (μg/mL) values 15.34, 16.18 and 16.60 respectively. Amongst these three compounds, 2-(4-(4-methoxybenzoyl)-1H-1,2,3-triazol-1-yl)-N-(5-phenyl-3-(trifluoromethyl)-1H-pyrazol-4-yl) acetamide (**5q**) emerged as the most promising antitubercular agent with lowest cytotoxicity against the A549 cancer cell line. This is the first report to demonstrate the pyrazolo triazole hybrids as potential antimycobacterial agents.

Tuberculosis (TB) is a potentially serious infectious disease caused by Mycobacterium tuberculosis which affects mainly the lungs (pulmenory TB) apart from other vital organs.[1] The World Health Organization (WHO 2013) estimated that there are 8.6 million TB cases which includes 1.1 million co-infected with HIV. Tuberculosis is one of the leading cause for mortality and in the year 2012 alone, there were 4,10,000 deaths of women affected by TB including 1,60,000 associated with HIV positive cases.[2] Additionally, multi drug resistant tuberculosis (MDR-TB) and extremely drug resistant tuberculosis (XDR-TB) has become a major threat to human kind.[3] In these circumstances, development of hybrid molecules through the combination of different pharmacophores in a single frame work with novel mechanism of action is one of the best way to achieve effective TB control.[4]

Meanwhile, 1,2,3-triazoles have gained enormous interest in recent years owing to their broad spectrum pharmaceutical and therapeutic applications such as antimicrobial activity against gram-positive bacteria, therapeutic fungicides of second generation, anti-inflammatory agents, inhibitors of tumor proliferation, invasion, metastasis and anti-HIV activity etc (**Fig. 1**).[5]

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Further, triazole based antitubercular agents regarded as a new class of molecules that provide truly effective lead candidates reported to inhibit bacteria.[6] Compound **II** (I-A09) (**Fig. 1**) comes under this category is presently in pre-clinical trials.[7]

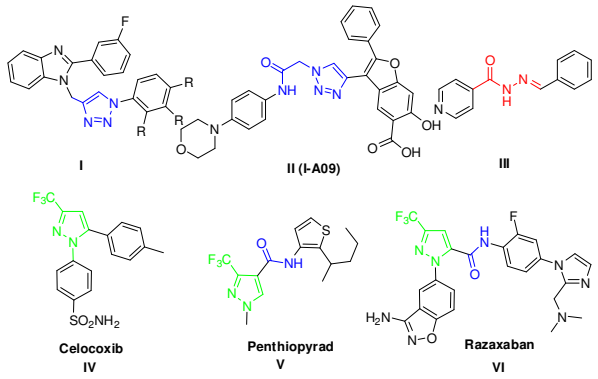


Figure 1. Bioactive triazole and 3-trifluoromethyl containing pyrazole moieties

Furthermore, pyrazole compounds are known to possess good biological activities such as potent aurora A/B kinase inhibitors, calcium (CRAC) channel inhibitors, antitumor and Mycobacterium tuberculosis.[8] Additionally, it is well documented that incorporation of trifluoromethyl group into

organic molecules can lead to profound changes in physical, chemical, and especially biological properties of the molecule.

Specially, insertion of trifluoromethyl group at 3rd position of the pyrazole ring lead to good biologically active moieties including those used as inhibitors of the measles virus RNA polymerase complex,[9] inhibitors of CRAC channel,[10] modulators of AMPA receptor.[11] Selective COX-2 inhibitors such as celecoxib (**IV**),[12] fungicide penthiopyrad (**V**)[13] and factor Xa inhibitor razaxaban(**VI**)[14] (**Fig. 1**).

Based on the biological significance of triazole and 3-trifluoromethyl pyrazole moieties and also as a part of our ongoing research programme on the bioactive heterocyclic compounds,[15] we envisaged the integration of 3-trifluoromethyl pyrazole and triazole pharmacophore units with acetamide linkage in one molecular platform to generate a new pyrazolo triazole hybrid frame work and to determine the anti-TB activity. In this context, the literature survey revealed that there are no reports available on the combination of pyrazole, triazole frame work with anti-tuberculosis properties. With the fact that 1,2,3-triazoles were efficiently made through Cu(I) catalyzed click chemistry,[16] we herein report for the first time an efficient synthesis of a series of novel 3-trifluoromethyl pyrazole-1,2,3-triazole hybrids (**5a–v)** in excellent yields (**Scheme 2**). Further the compound **5i** converted into hydrazone derivatives of 3-trifluoromethyl pyrazole-1,2,3-triazole hybrids (**7a-e)** and all the compounds in **5** to**7** series were subjected to in vitro activity studies against *Mycobacterium smegmatis* (MC^2 155) and also verified the cytotoxicity.

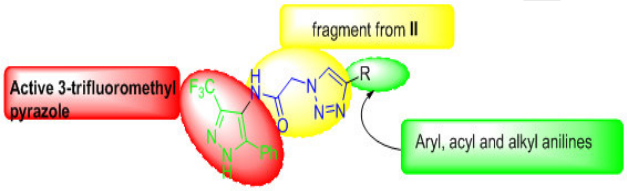
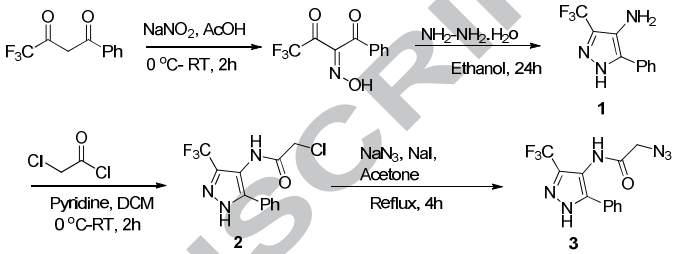


Figure 2. Design strategy for new 3-trifluoromethyl pyrazolo-1,2,3-triazole hybrids.

The designed 3-trifluoromethyl pyrazolo-1,2,3-triazole frame work (**Fig. 2**) made into three parts: N-substituted 1,2,3-triazole as a mainstay, 5-phenyl-3-(trifluoromethyl)-1H-pyrazol-4-amine to intensify the desired pharmacophoric behavior with drug like properties and aliphatic or aromatic groups and aryl amino methyl and aroyl groups adjoined to other side of 1,2,3-triazole moiety. Distinctions in the proposed scaffold also accomplished with the choice of aliphatic or aromatic alkynes **4a–v** (**Fig. 4**). Synthesis of 1,2,3-triazole moiety was through the Huisgen 1,3-dipolar cycloaddition reaction[17] (click reaction) between azide **3** and alkynes **4a–v**.

Initially, synthesis of 5-phenyl-3-(trifluoromethyl)-1H-pyrazol-4-amine (**1**)[18] was started from 4,4,4-trifluoro-1-phenylbutane-1,3-dione on reaction with $NaNO_2$ in acetic acid followed by with hydrazine hydrate in ethanol. Structure of pyrazol-4-amine (**1**) was unambiguously confirmed by single crystal X-ray diffraction analysis and the data deposited at the CCDC 1051815 (**Fig. 3**). Further, compound **1** was treated with chloroacetyl chloride[19] using pyridine as a base in DCM and the resulting compound **2** was reacted with sodium azide using catalytic amount of NaI to give 2-azido-N-(5-phenyl-3-(trifluoromethyl)-1H-pyrazol-4-yl)acetamide in 98% yield (**Scheme 1**). The azide **3** was fully characterized by ^1H, ^{13}C NMR and mass (ESI and HR-MS) spectral data. On the other hand, alkynes **4a-j** were procured from commercially available sources, whereas, **4l-p** were prepared by the reaction of substituted anilines with proparzyl bromide, K_2CO_3 in DMF[20] and **4q-v** were obtained by the reaction of substituted aldehydes with ethynyl magnesium bromide in Dry THF and the resulting alcohols were further oxidised with IBX.[21]



Scheme 1. Synthesis of 2-Azido-N-(5-phenyl-3-(trifluoromethyl)-1H-pyrazol-4-yl)acetamide **3**

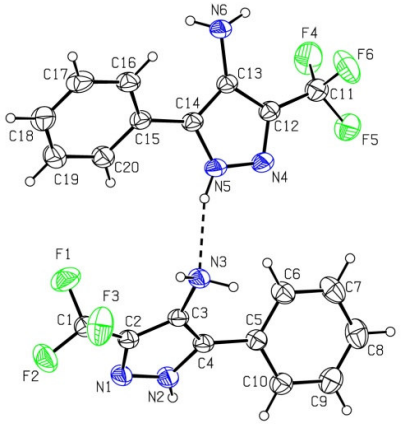
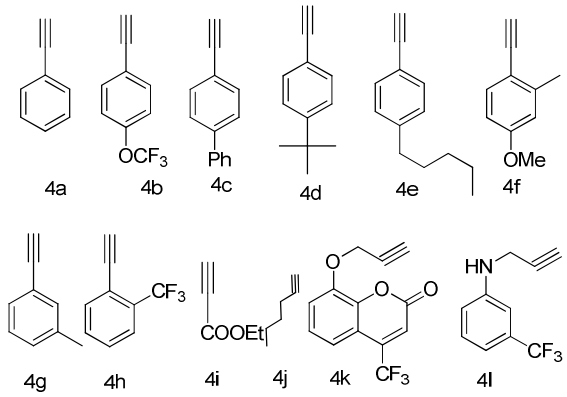
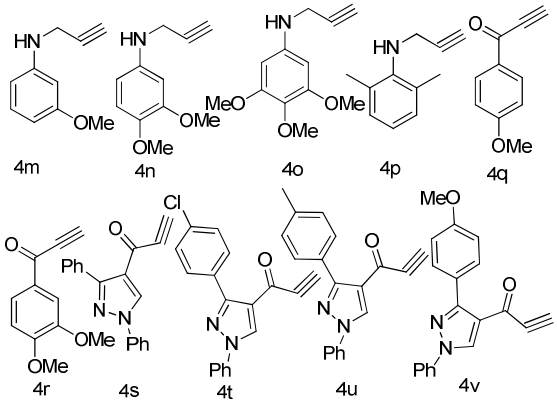


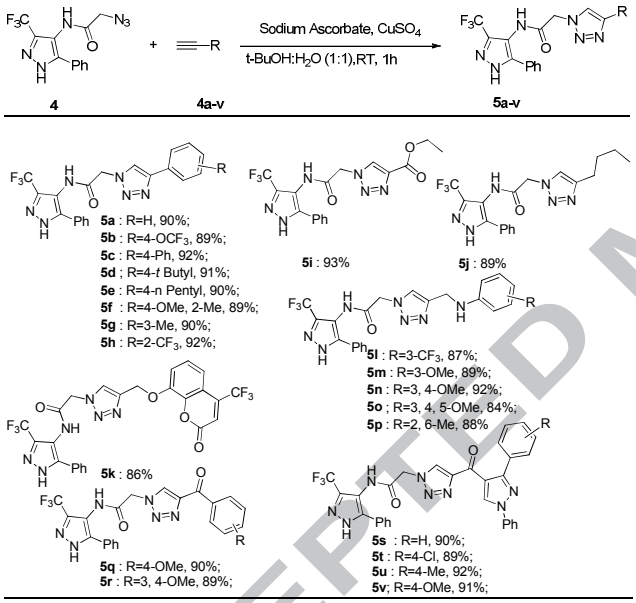
Figure 3. ORTEP Diagram of 5-phenyl-3-(trifluoromethyl)-1H-pyrazol-4-amine (**1**).

Figure 4. Alkynes used for synthesis of pyrazolo triazole hybrids.



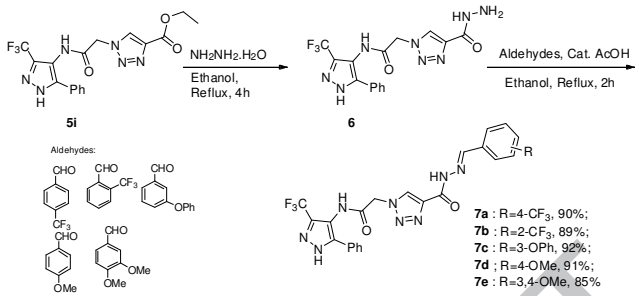


Having both azide **3** and alkynes **4a–v** on hand, we next subjected them to Huisgen's (3+2) cycloaddition reaction in the presence of $CuSO_4$, sodium ascorbate in *t*-Butanol and water (1:1, v/v) to get pyrazole-1,2,3,-triazole hybrids (**5a-v)** in excellent yields (**Scheme 2**) and were characterized by ^1H, ^{13}C NMR and mass spectral data.



Scheme 2. Synthesis of 3-trifluoromethyl pyrazolo1,2,3-triazole hybrids**5a-v**

Further compound **5i** was reacted with hydrazine hydrate to get triazolo carbonyl hydrazine (**6)** and further it was condensed with aromatic aldehydes in ethanol to give triazolo hydrazones[22] (**7a-e**) (**Scheme 3**). Thus obtained hydrazones **7a-e** was well characterized by ^1H, ^{13}C NMR and mass (ESI and HRMS) spectral data.



Scheme 3. Synthesis of 3-trifluoromethyl pyrazolo1,2,3-triazolo hydrazones **7a-e**

All the compounds in **5** to **7** series were evaluated for antimycobacterial activity against *Mycobacterium smegmatis* (MC^2 155). Outcome of the screening study showed that some of the compounds (**Fig. 5**) exhibited promising antimycobacterial activity with MIC values ranging from 15 to 95 μg/mL. Specifically, compounds **5q, 7b** and **7c** showed remarkable MIC (μg/mL) values 15.34, 16.18 and 16.60 respectively. Other compounds such as **5a, 5b, 5g, 5c, 5e** and **5i** showed moderate antimycobacterial activity with MIC (μg/mL) values 35.93, 35.41, 29.94, 31.75, 35.37 and 27.73 respectively.

Structure activity correlation of compounds in **5** to **7** series with respect to their antitubercular activity revealed that, compounds in **5** series bearing 4-OCF_3, Ph and pentyl (**5b**, **c** & **e**) groups on phenyl ring showed moderate activity with MIC values ranging from 31.75-35.41 μg/mL. However, 3-methyl substitution **5g** attributed to increase in activity (MIC 29.94 μg/mL). Whereas, presence of 4-*t*-butyl group on phenyl ring **5d** showed very poor activity in contrast to **5g**. Replacement of phenyl group in **5** series with N-methyl aniline moiety, irrespective of substitution on phenyl group **5l-p** induced decrease in activity. However, replacing the phenyl group with ester functional **5i** attributed to enhanced activity (MIC 27.73 μg/mL). Subsequent replacement of ester functional in **5i** with 4-methoxy benzoyl group **5q** lead to tremendous increase in activity with MIC 15.34 μg/mL (high activity compound in the series), whereas 3,4-dimethoxy benzoyl group **5r** receded the activity. Furthermore, conversion of ester functional in **5i** into hydrazide (**6**) proved to be not effective. In order to get the enhanced activity, hydrazide (**6**) was converted to hydrazones by reacting with substituted aldehydes **7a-e** and screened for antimycobacterial activity. Compound **7b-c** has exhibited very good activity with MIC 16.18 and 16.60 μg/mL (**Fig. 5**). In order to check the cytotoxicity of the compounds in **5** to**7** series, they were screened against lung cancer cell line A549 and outcome of the study presented in **Fig 6**. Interestingly, the compounds **5q** and **7b-c** which have shown high antimycobacterial activity were showed IC_{50} values 80.74 for **5a** and >100 for **7b-c** which signifies that they are not cytotoxic.

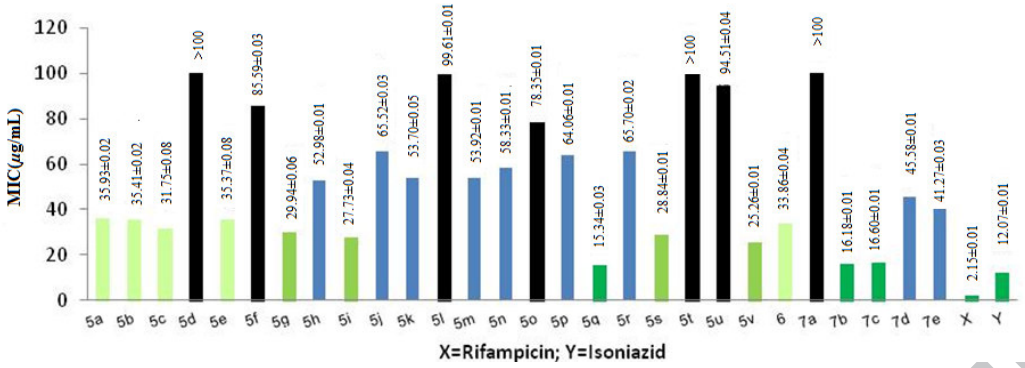


Figure 5. Antimycobacterial activity of compounds in **5-7** series against *M. smegmatis*

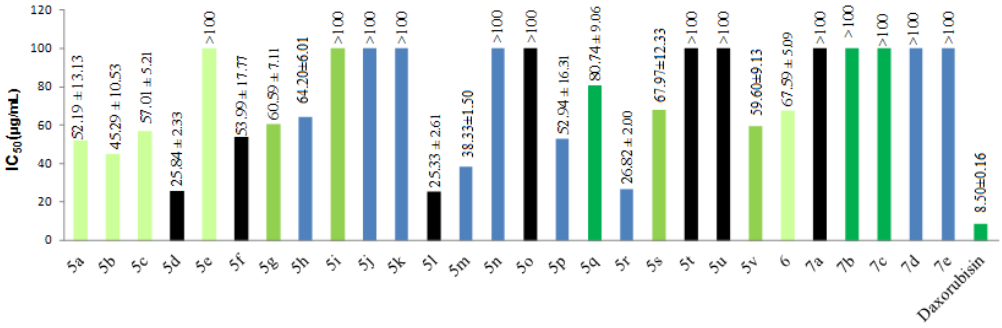


Figure 6. Cytotoxicity values of **5-7** series against A549 cells.

The compounds **5a, 5b, 5g, 5c, 5e** and **5i** which showed moderate antimycobacterial activity against *M. smegmatis* were also found to be not cytotoxic with IC_{50} values 52.19, 45.29, 60.59, 57.01, >100 and >100 respectively. However compounds **5l**, **5d** and **5r** are moderately cytotoxic to A549 lung cancer cells with IC_{50} values of 25.33, 25.84 and 26.82 respectively (**Fig. 6**).

In conclusion, we have synthesized a series of novel -(4-aryl -1H-1,2,3-triazol-1-yl)-N-(5-phenyl-3-(trifluoromethyl)-1H pyrazol-4-yl) acetamide derivatives (**5a-v**) by Huisgen's (3 + 2) cycloaddition reaction of pyrazole azide (**3**) with different alkynes **4a-v** in presence of copper sulphate and sodium ascorbate. Further compound **5i** was converted into triazolo hydrazones (**7a-e**) *via* triazolo carbonyl hydrazine (**6**) and all the compounds were screened against *Mycobacterium smegmatis* (MC^2 155) followed by cytotoxicity against the A549 lung cancer cell line. Outcome of these studies showed that the compounds **5q**, **7b** and **7c** have a very promising antimycobacterial activity (MIC 15.34, 16.18 and 16.60 μg/mL) with less cytotoxicity. Further, compound **5q** emerged as the potential antitubercular candidate with lowest cytotoxicity. This is the first report that has demonstrated the potential utility of 3-trifluoromethyl pyrazole-1,2,3-triazole hybrids as antitubercular agents which can be exploited further.

Acknowledgement

We are grateful to SERB-DST (grant no. SB/EMEQ-075/2013), New Delhi for financial support. ENR is thankful to CSIR New Delhi for SRF.

Supplementary data

Supplementary data (experimental procedures, spectral data, crystallography data and ^{1}H, ^{13}C, mass, IR spectras of representative compounds) associated with this article can be found.

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Graphical abstract