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Synthesis, structure–activity relationship of novel substituted 4*H*-chromen-1,2,3,4-tetrahydropyrimidine-5-carboxylates as potential anti-mycobacterial and anticancer agents

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Anti-mycobacterial activity
Anticancer activity

ABSTRACT

Series of 4*H*-chromen-1,2,3,4-tetrahydropyrimidine-5-carboxylate derivatives **7a**–**7zb**, **8a**–**8d** and **9a**–**9d** were synthesized and screened for their in vitro anti-mycobacterial activity against *Mycobacterium tuberculosis* $H_{37}Rv$ (MTB) and cytotoxicity against three human cancer cell lines including A549, SK-N-SH and HeLa. The results indicate that six compounds are more potent and **7za** is most effective anti-mycobacterial derivative compared to the standard drugs Ethambutol and Ciprofloxacin. However, 12 compounds exhibited cytotoxicity against human neuroblastoma cell line; amongst them the compound **7v** is most effective compared to the standard drug Doxorubicin. This is the first report assigning in vitro anti-mycobacterial, anticancer and structure–activity relationship for this new class of 4*H*-chromen-1,2,3,4-tetrahydropyrimidine-5-carboxylates.

Chromone derivatives[1] are an important class of natural, synthetic compounds and pharmacologically active substances displaying a broad range of biological activities including anticancer, monoamine oxidase B (MAO-B) inhibitors, anti-mycobacterial, antimicrobial, human-immunodeficiency virus (HIV-1) and mushroom tyrosinase inhibition.[2] These derivatives are an important intermediates in the manufacture of agrochemicals, pharmaceuticals, and dyestuff industries.[3] 4-Oxo-4*H*-chromen-3-carbaldehyde (3-formylchromone) a useful precursor for the synthesis of several biological active compounds[4] owing to the presence of an unsaturated keto function, a conjugated second carbonyl group at C-3, and an electrophilic centre at C-2. However, there is no report for the synthesis of 4*H*-chromen-1,2,3,4-tetrahydropyrimidine-5-carboxylate derivatives and their biological activities. *Mycobacterium tuberculosis* (MTB), single infectious causative agent of tuberculosis (TB)[5] resulted in the highest number of human death worldwide and the threat factor in current TB problem is the prevalence of multi-drug resistant (MDR) strains and co-infections with AIDS has been responsible for this serious situation. Similarly cancer[6] the uncontrolled, rapid and proliferation of abnormal cells is the leading cause of human death, despite considerable progress of its biology and pharmacology, cancer remains a serious problem, therefore, safe, potent, and selective search for new chemotherapeutic agents are required for tuberculosis and cancer.

Our interest in design and synthesis of diverse range of biologically active heterocyclic compounds with potential activities,[7] and on anti-mycobacterial agents,[8] in this Letter, we report the synthesis, anti-mycobacterial and anticancer activity of new substituted 4*H*-chromen-1,2,3,4-tetrahydropyrimidine-5-carboxylate derivatives. Scheme 1 illustrates the synthesis of 6-substituted 4-oxo-4*H*-chromene-3-carbaldehydes **4a**–**4f**. Substituted phenols **1a**–**1e** were acetylated using acetyl chloride and on subsequent Fries rearrangement with $AlCl_3$ gave **3a**–**3e**. The 1-(2-hydroxy-3-nitrophenyl)ethanone **3f** was prepared according to the literature procedure.[9] The compound **3a** was subjected for Vilsmeier conditions[10] to give **4a** using various reagents such as $POCl_3$/DMF, oxalyl chloride/DMF and triphosgene/DMF, among these oxalyl chloride/DMF was found to be the better reagent in terms of yields. Under similar conditions **4b**–**4f** were synthesized in very good yields (Scheme 1). The reaction of 5-bromoacetophenone

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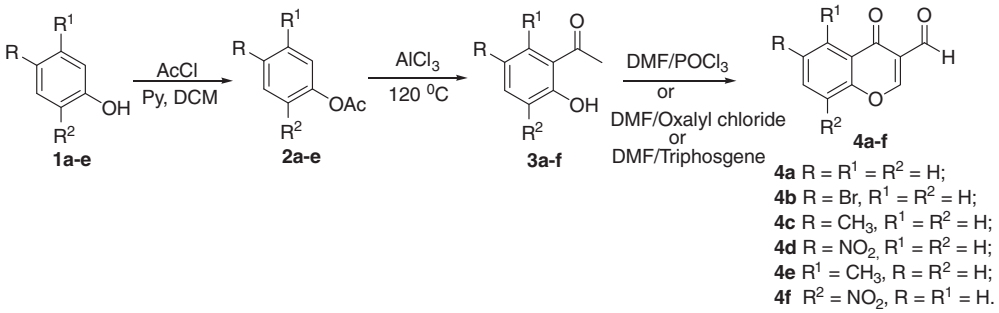
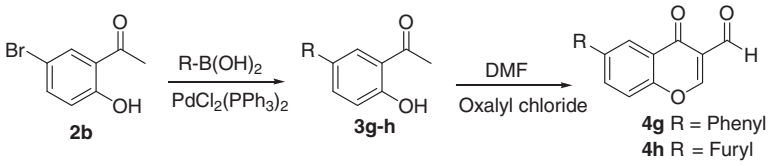


Scheme 1.



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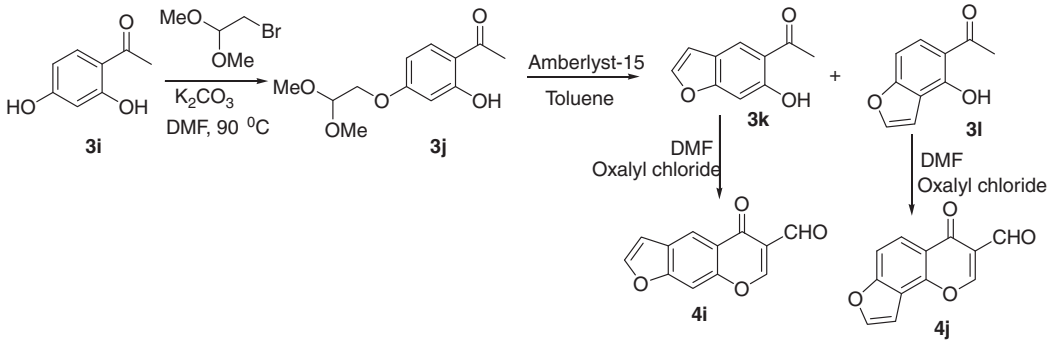

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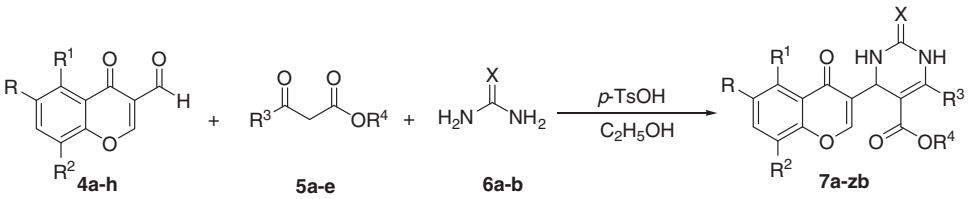

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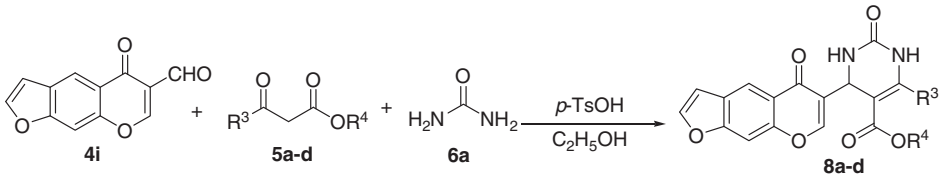
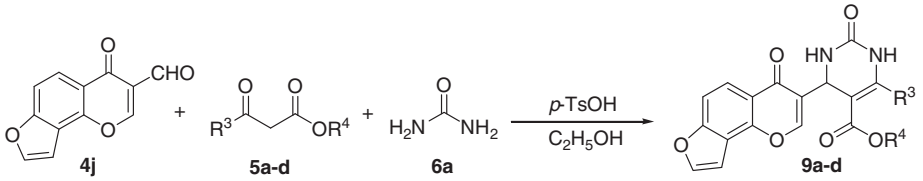


Scheme 5.

2b subjected with phenylboronic acid and furylboronic acid in presence of $PdCl_2(PPh_3)_2$ (20 mol %) in 22–24% aq isopropanol (Suzuki coupling),[7a11] under reflux conditions gave **3g**, **3h** and subsequent Vilsmeier reaction with oxalyl chloride/DMF afforded **4g**, **4h** in good yields (Scheme 2).

Resacetophenone **3i** was reacted with bromoacetaldehyde dimethyl acetal in presence of potassium carbonate in anhydrous DMF yielded 1-(4-(2,2-dimethoxyethoxy)-2-hydroxyphenyl) ethanone **3j**, which upon cyclization using acidic Amberlyst 15 in toluene under reflux conditions yielded mixture of



Scheme 6.

1-(6-hydroxybenzofuran-5-yl)-ethanone **3k** (61%), 1-(4-hydroxybenzofuran-5-yl)ethanone **3l** (39%, Scheme 3).[12] These compounds **3k**, **3l** were separated by column chromatography and subsequent Vilsmeier reaction afforded 5-oxo-5*H*-furo[*3,2-g*]chromene-6-carbaldehyde **4i** and 4-oxo-4*H*-furo[*2,3-h*]chromene-3-carbaldehyde **4j** (Scheme 3). Thus synthesized 4-oxo-4*H*-chromene-3-carbaldehydes **4a**–**4j** were reacted with various β-ketoesters **5a**–**5e** and urea **6a**–**6b** in presence of *p*-TsOH in ethanol under reflux conditions (Biginelli reaction)[13] afforded series of new 4*H*-chromen-1,2,3,4-tetrahydropyrimidine-5-carboxylates **7a**–**7zb**, **8a**–**8d** and **9a**–**9d** in very good yields (Scheme 4–6, Table 1). The synthesized compounds were well characterized by spectral data and documented in Supplementary data.[14]

The compounds **7a**–**7zb**, **8a**–**8d**, and **9a**–**9d** were screened for their in vitro anti-mycobacterial activity against *M. tuberculosis* $H_{37}Rv$ (MTB)[15] by agar dilution method for the determination of MIC and values of the synthesized compounds along with the standard drugs for comparison are presented in Table 2. All the 36 compounds were screened in the present study, MIC's ranging from 3.39–76.21 μM and eight compounds inhibited MTB with less than 10 μM. Six compounds **7t**, **7u**, **7v**, **7za**, **8d** and **9d** shown excellent activity with MIC 3.39–7.38 μM and were more potent than standard drugs Ethambutol (MIC of 7.63 μM), Ciprofloxacin (MIC of 9.44 μM). Two compounds **7d** and **7s** with MIC ranging from 7.86–7.90 μM are potent than Ciprofloxacin and **7za** found to be most active with MIC 3.39 μM. Five compounds **7n**, **7q**, **7zb**, **8b** and **9b** with MIC's 14.53–16.62 μM shown moderate activity in present series. With respect to structure-MTB activity, among 2-oxo-4-(4-oxo-4*H*-chromen-3-yl-1,2,3,4-tetrahydropyrimidine-5-carboxylates, it is interesting to note that in general the

Table 1
Synthesis of substituted 4*H*-chromen-1,2,3,4-tetrahydropyrimidine-5-carboxylates (**7a**–**7zb**, **8a**–**8d** and **9a**–**9d**)

Compds	R	R^1	R^2	R^3	R^4	X	Yield[a] (%)
7a	H	H	H	CH_3	Et	O	97
7b	H	H	H	C_6H_5	Et	O	92
7c	H	H	H	CH_2Cl	Et	O	89
7d	H	H	H	CF_3	Et	O	57 [b]
7e	H	H	H	CH_3	C_2H_4OMe	O	92
7f	H	H	H	CH_3	Et	S	88
7g	H	H	H	C_6H_5	Et	S	80
7h	H	H	H	CH_3	C_2H_4OMe	S	85
7i	Br	H	H	CH_3	Et	O	72
7j	Br	H	H	CH_3	C_2H_4OMe	O	87
7k	Br	H	H	CH_3	Et	S	92
7l	CH_3	H	H	CH_3	Et	O	96
7m	CH_3	H	H	C_6H_5	Et	O	94
7n	CH_3	H	H	CH_2Cl	Et	O	93
7o	CH_3	H	H	CH_3	C_2H_4OMe	O	90
7p	CH_3	H	H	CH_3	Et	S	90
7q	CH_3	H	H	C_6H_5	Et	S	89
7r	CH_3	H	H	CH_3	C_2H_4OMe	S	92
7s	CH_3	H	H	CF_3	Et	O	73[b]
7t	H	CH_3	H	CF_3	Et	O	62[b]
7u	NO_2	H	H	CH_3	Et	O	70[b]
7v	H	H	NO_2	CH_3	Et	O	53[b]
7w	C_6H_5	H	H	CH_3	Et	O	85
7x	C_6H_5	H	H	C_6H_5	Et	O	87
7y	C_6H_5	H	H	CH_3	C_2H_4OMe	O	82
7z	C_6H_5	H	H	CH_3	Et	S	82
7za	C_6H_5	H	H	CF_3	Et	O	40[b]
7zb	Furyl	H	H	CH_3	Et	O	57
8a	––	––	––	CH_3	Et	O	82
8b	––	––	––	Ph	Et	O	80
8c	––	––	––	CH_3	C_2H_4OMe	O	86
8d	––	––	––	CF_3	Et	O	43[b]
9a	––	––	––	CH_3	Et	O	82
9b	––	––	––	Ph	Et	O	82
9c	––	––	––	CH_3	C_2H_4OMe	O	87
9d	––	––	––	CF_3	Et	O	42[b]

[a] Isolated yields.
[b] AcOH was used for the cyclization reaction in stead *p*-TsOH.

Table 2
Anti-mycobacterial and anticancer activities of compounds **7a**–**7zb**, **8a**–**8d** and **9a**–**9d**

Compds	MTB[b] (MIC[a] in μM)	Cytotoxicity[c] (%)		
		A549	SK-N-SH	HeLa
7a	76.21	18.1 ± 0.8	19 ± 2.5	16.6 ± 2.5
7b	64.10	15.4 ± 4.4	15 ± 3.9	5.3 ± 1.60
7c	34.53	30.7 ± 3.1	18.6 ± 1.9	23.3 ± 3.8
7d	7.90	––[d]	23.4 ± 1.9	29.2 ± 0.8
7e	69.83	23.5 ± 0.1	23.9 ± 3	38.5 ± 1.8
7f	72.67	26.3 ± 5.5	32.7 ± 0.3	49.3 ± 7.4
7g	61.57	9.4 ± 3.9	1.9 ± 3.8	33 ± 1.9
7h	66.84	––[d]	––[d]	––[d]
7i	30.78	51.4 ± 0.04	31.1 ± 4.7	32.8 ± 3.8
7j	28.66	29.9 ± 4.5	38.4 ± 1	40.2 ± 2.4
7k	29.69	43.5 ± 0.2	20 ± 2.5	41.1±0.7
7l	36.54	24.8 ± 3.8	1.7 ± 0.2	16.6 ± 0.6
7m	30.94	18.6 ± 4.4	––[d]	29.8 ± 4.5
7n	16.62	46.2 ± 1	55.3 ± 0.9	81.4 ± 1.7
7o	34.15	28.4 ± 5.3	35.6 ± 2.6	40.2 ± 0.9
7p	34.91	18.3 ± 2.3	7 ± 2.4	43.2 ± 5.4
7q	14.88	27.2 ± 0.3	12.1 ± 4	31.5 ± 0.007
7r	32.21	21.2 ± 2.8	22.8 ± 1.2	17.4 ± 1.8
7s	7.86	37.4 ± 2.6	45.5 ± 0.1	33.5 ± 5.3
7t	3.91	22 ± 1.5	35.9 ± 1.7	61.3 ± 5.7
7u	4.18	––[d]	28.7 ± 2.1	54.4 ± 3.3
7v	4.18	47.6 ± 1.5	56 ± 0.9	60.6 ± 1.6
7w	30.71	7.2 ± 1	––[d]	45.3 ± 0.9
7x	25.93	––[d]	––[d]	––[d]
7y	57.60	34.4 ± 0.5	13.4 ± 0.5	33.5 ± 4
7z	59.52	37.4 ± 2.6	25.4 ± 2.1	40.9 ± 0.3
7za	3.39	31.3 ± 2.7	10.7 ± 1.4	28.6 ± 3
7zb	15.86	34.5 ± 2.5	46.4 ± 2.4	35.4 ± 2.6
8a	33.96	29.6 ± 0.1	37.5 ± 2.2	51.5 ± 2.7
8b	14.53	13.1 ± 3.5	––[d]	59.1 ± 3.6
8c	31.40	34.7 ± 1.7	34.7 ± 5.7	37.6 ± 2.4
8d	7.38	21.8 ± 3	6.9 ± 5.1	40.9 ± 0.4
9a	33.96	33.8 ± 3.1	––[d]	42.4 ± 2.7
9b	14.53	33.3 ± 1.8	39.4 ± 0.7	43.3 ± 1.7
9c	31.40	4.5 ± 0.97	8.4 ± 0.04	61.2 ± 5.7
9d	3.67	27.5 ± 1.7	34.3 ± 2.5	32.8 ± 0.04
Isoniazid	0.66			
Ethambutol	7.63			
Ciprofloxacin	9.44			
Doxorubicin		55 ± 3.5	31.8 ± 2.7	86.5 ± 0.09

[a] Minimum inhibitory concentration.
[b] Mycobacterium tuberculosis $H_{37}Rv$.
[c] Cytotoxicity against three cancer cell lines [lung, neuroblastoma, and cervical cancer].
[d] No activity.

compounds having the trifluoromethyl group at 6th position on 1,2,3,4-tetrahydropyrimidine-5-carboxylate has the potential impact in improving the activity compared to methyl, phenyl, chloromethyl substitutions. Nitro substitution on 4*H*-chromene at 6th position **7u** and 8th position **7v** play an important role in enhancing the activity compared to bromo **7i**, methyl **7s**, and phenyl/furyl **7w**/**7zb**. Further methyl at 5th position **7t** on 4*H*-chromen improved the activity over 6th position **7s**.

The in vitro results of anti-mycobacterial activity encouraged us to evaluate its anticancer effects against a panel of three human cancer cell lines including lung (A549), CNS (SK-N-SH), and cervical (HeLa) by using MTT assay[16] for compounds **7a**–**7zb**, **8a**–**8d** and **9a**–**9d**, and the results are presented in Table 2 along with the standard drug Doxorubicin for comparison. Compound **7i** displayed significant, **7k**, **7n**, and **7v** shown moderate anticancer activity against lung cancer cell line (A549). Compounds **7n**, **7s**, **7v** and **7zb** are more potent compared to standard drug Doxorubicin, where as eight compounds **7f**, **7j**, **7o**, **7t**, **8a**, **9b** and **9d** displayed potent activity against CNS cancer cell line (SK-N-SH). Compound **7n** is potent; six compounds **7t**, **7u**, **7v**, **8a**, **8b**, and **9c** are significant against cervical cancer cell line (HeLa). In general the compounds **7n** and **7v** have shown both anti-mycobacterial and anticancer activity on tested cancer cell lines, where as **7s**, **7t**, **7v**, and **9d** shown anti-mycobacterial and selectively on SK-N-SH cancer cell line.

In conclusion, compounds **7a**–**7zb**, **8a**–**8d** and **9a**–**9d** were synthesized applying different synthetic protocols. Suzuki coupling for the synthesis of compounds **7w**–**7zb** has been applied for the first time in synthesizing substituted 4-oxo-4*H*-chromenyl-1,2,3,4-tetrahydropyrimidine-5-carboxylate derivatives. These compounds are not known so far in the literature along with the synthetic methodology applied, and the assignment of the in vitro anti-mycobacterial and anticancer activity to all the 4-oxo-4*H*-chromenyl-1,2,3,4-tetrahydropyrimidine-5-carboxylate derivatives in this report is new aspect. Compounds **7za**, **7t**, **7u**, **7v**, and **9d** are potent in present series in comparison with standard drugs Ethambutol and Ciprofloxacin. Compounds **7n** and **7v** are the most potent against SK-N-SH, cell line. This study further presents substituted 4-oxo-4*H*-chromenyl-1,2,3,4-tetrahydropyrimidine-5-carboxylate derivatives as new class of anti-mycobacterial as well as anticancer and it may serve as a model compounds for design and development of therapeutic based anti-mycobacterial, anticancer agents. The synthesis and biological activity of other heterocycles is currently under investigation.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.03.079.

References and notes

1. Ellis, G. P. Chromenes, Chromanones and Chromones In Weissberger, A., Taylor, E. C., Eds.; John Wiley and Sons: New York, 1977. pp 1–10; (b) Walenzyk, T.; Carola, C.; Buchholz, H.; Ko nig, B. *Tetrahedron* **2005**, *61*, 7366; (c) Boumendjel, A.; Nicolle, E.; Moraux, T.; Gerby, B.; Blanc, M.; Ronot, X.; Boutonnat, J. *J. Med. Chem.* **2005**, *48*, 7275; (d) Lee, K. S.; Seo, S. H.; Lee, Y. H.; Kim, H. D.; Son, M. H.; Chung, B. Y.; Lee, J. Y.; Jin, C.; Lee, Y. S. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 2857.
2. (a) Huang, W.; Liu, M.-Z.; Li, Y.; Tan, Y.; Yamg, G.-F. *Bioorg. Med. Chem.* **2007**, *15*, 5191; (b) Mays, J. R.; Hill, S. T.; Moyers, J. T.; Blagg, B. S. J. *Bioorg. Med. Chem.* **2010**, *18*, 249; (c) Zhang, S.; Ma, J.; Bao, Y.; Yang, P.; Zou, L.; Li, K.; Sun, X. *Bioorg. Med. Chem.* **2008**, *16*, 7127; (d) Nam, D. H.; Lee, K. Y.; Moon, C. S.; Lee, Y. S. *Eur. J.Med. Chem.* **2010**, *45*, 4288; (e) Singh, P.; Kaur, M.; Holzer, W. *Eur. J. Med. Chem.* **2010**, *45*, 4968; (f) Huang, W.; Ding, Y.; Miao, Y.; Liu, M.-Z.; Li, Y.; Yang, G.-F. *Eur. J. Med. Chem.* **2009**, *44*, 3687; (g) Gasper, A.; Reis, J.; Fonseca, A.; Milhazes, N.; Vina, D.; Uriarte, E.; Borges, F. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 707; (h) Piao, L. Z.; Park, H. R.; Park, Y. K.; Lee, S. K.; Park, J. H.; Park, M. K. *Chem. Pharm. Bull.* **2002**, *50*, 309.
3. Harnisch, H. *Liebigs Ann. Chem.* **1972**, *765*, 8.
4. (a) Papafilippou, A.; Terzidis, M. A.; Stephanatou, J. S.; Tsoleridis, C. A. *Tetrahedron Lett.* **2011**, *52*, 1306; (b) Wu, C.; Zeng, H.; Wang, D.; Chen, Y. *Tetrahedron* **2011**, *67*, 1231; (c) Khan, K. M.; Ambreen, N.; Mughal, U. R.; Jalil, S.; Perveen, S. *Eur. J. Med. Chem.* **2010**, *45*, 4058; (d) Lacova, M.; Gasparova, R.; Kois, P.; Bohac, A.; Ei-Shaaer, H. M. *Tetrahedron* **2010**, *66*, 1410; (e) Teridis, M. A.; Stephanatou, J. S.; Tsoleridis, C. A. *J. Org. Chem* **2010**, *75*, 1948; (f) Teridis, M. A.; Stephanatou, J. S.; Tsoleridis, C. A.; Terzis, A.; Raptopoulou, C. P.; Psycharis, V. *Tetrahedron* **2010**, *66*, 947; (g) Ibrahim, M. *Tetrahedron* **2009**, *65*, 7687; (h) Raj, T.; Bhatia, R. K.; Sharma, R. K.; Gupta, V.; Sharma, D.; Ishar, M. P. S. *Eur. J. Med. Chem.* **2009**, *44*, 3209; (i) Khan, K. M.; Ambreen, N.; Hussain, S.; Perveen, S.; Choudhary, M. I. *Bioorg. Med. Chem.* **2009**, *17*, 2983; (j) Waldmann, H.; Khedkar, V.; Duckert, H.; Schurmann, M.; Oppel, I. M.; Kumar, K. *Angew. Chem., Int. Ed.* **2008**, *47*, 6869; (k) Plaskon, A. S.; Ryabukhin, S. V.; Volochnyuk, D. M.; Gavrilenko, K. S.; Shivanyuk, A. N.; Tolmachev, A. A. *J. Org. Chem.* **2008**, *73*, 6010; (l) Kumar, K.; Kapoor, R.; Kapur, A.; Ishar, M. P. *Org. Lett.* **2000**, *2*, 2023.
5. (a)*Tuberculosis and the Tubercle Bacillus*; Cole, S. T., Eisenach, K. D., McMurray, D. N., Jacob, W. R., Eds.; ASM Press: Washington DC, 2005; (b) Lougheed, K. E. A.; Tayler, D. L.; Osborne, S. A.; Bryans, J. S.; Buxton, R. S. *Tuberculosis* **2009**, *89*, 364.
6. (a) Jaracz, S.; Chen, J.; Kuznetsova, L. V.; Ojima, I. *Bioorg. Med. Chem.* **2005**, *13*, 5043; (b) Bruno, R. D.; Njar, V. C. O. *Bioorg. Med. Chem.* **2007**, *15*, 5047; (c) Fang, Z.; Liao, P.-C.; Yang, Y.-L.; Yang, F.-L.; Chen, Y.-L.; Lam, Y.; Hua, K.-F.; Wu, S.-H. *J. Med. Chem.* **2010**, *53*, 7967; (d) Chen, J.; Wang, Z.; Li, C.-M.; Lu, Y.; Vaddady, P. K.; Meibohm, B.; Dalton, J. T.; Miller, D. D.; Li, W. *J. Med. Chem.* **2010**, *53*, 7414.
7. (a) China Raju, B.; Tiwari, A. K.; Kumar, J. A.; Ali, A. Z.; Agwane, A. B.; Saidachary, G.; Madhusudana, K. *Bioorg. Med. Chem.* **2010**, *18*, 358; (b) Kumar, A. J.; Tiwari, A. K.; Ali, A. Z.; Madhusudana, K.; Reddy, B. S.; Ramakrishna, S.; China Raju, B. *J. Enzyme Inhib. Med. Chem.* **2010**, *25*, 80; (c) Gangadasu, B.; Janaki Ram Reddy, M.; Ravinder, M.; Bharath Kumar, S.; China Raju, B.; Pranay Kumar, K.; Murthy, U. S. N.; Jayathirtha Rao, V. *Eur. J. Med. Chem.* **2009**, *44*, 4661; (d) Srinivas, Ch.; Kumar, Ch. N. S. S. P.; China Raju, B.; Rao, V. J.; Naidu, V. G. M.; Ramakrishna, S.; Diwan, P. V. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 5915.
8. (a) Kumar, R. S.; Perumal, S.; Shetty, K. A.; Yogeeswari, P.; Sriram, D. *Eur. J. Med. Chem.* **2010**, *45*, 124; (b) Kumar, R. S.; Rajesh, S. M.; Perumal, S.; Banerjee, D.; Yogeeswari, P.; Sriram, D. *Eur. J. Med. Chem.* **2010**, *45*, 411; (c) Dinakaran, M.; Senthilkumar, P.; Yogeeswari, P.; China, A.; Nagaraja, V.; Sriram, D. *Bioorg. Med. Chem.* **2008**, *16*, 3408.
9. Cushman, M.; Zhu, H.; Geahlen, R. L.; Kraker, A. J. *J. Med. Chem.* **1994**, *37*, 3353.
10. Gangadasu, B.; Narender, R.; Kumar, S. B.; Ravinder, M.; Rao, B. A.; Ramesh, Ch.; China Raju, B.; Rao, V. J. *Tetrahedron* **2006**, *62*, 8398.
11. Wang, Z.; Fan, R.; Wu, J. *Adv. Synth. Catal.* **2007**, *349*, 1943.
12. Dixit, M.; Sharon, A.; Maulik, P. R.; Goel, A. *Synlett* **2006**, *10*, 1497.
13. (a) Biginelli, P. *Ber.* **1891**, *24*, 1317; (b) Biginelli, P. *Ber.* **1893**, *26*, 447; (c) Biginelli, P. *Gazz. Chim. Ital.* **1893**, *23*, 360.
14. *Ethyl-6-(chloromethyl)-2-oxo-4-(4-oxo-4H-chromen-3-yl)-1,2,3,4-tetrahydropyrimidine-5-carboxylate* (**7c**). Yield: 89%; color less solid; mp 240–242 °C. IR (KBr): 3297, 3099, 2939, 1711, 1639, 1231, 1094, 751 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$ + DMSO-d_6): δ 9.50 (br s, 1H, NH), 8.20 (d, 1H, J = 7.9 Hz, aromatic), 7.84 (s, 1H, CH), 7.88–7.69 (m, IH, aromatic), 7.54–7.40 (m, 2H, aromatic), 6.78 (s, 1H, NH), 5.58 (d, 1H, J = 3.1 Hz, CH), 4.85–4.73 (q, 2H, CH_2Cl), 4.22–4.04 (q, 2H, OCH_2), 1.22 (t, 3H, J = 6.9 Hz, CH_3). ^{13}C NMR ($CDCl_3$ + DMSO-d_6): δ 175.6, 163.1, 155.2, 152.5, 151.8, 152.6, 147.1, 133.0, 124.4, 122.8, 122.5, 117.2, 97.0, 59.4, 46.5, 46.3. Mass (ESI-MS): m/z 363, 365 [M^++1], 385, 387 [M^++Na]. *Ethyl 2-oxo-4-(4-oxo-4H-chromen-3-yl)-6-(trifluoromethyl)-1,2,3,4-tetrahydropyrimidine-5-carboxylate* (**7d**). Yield: 57%; pale cream solid; mp 255–257 °C. IR (KBr): 3233, 3100, 1611, 1569, 1438, 1363, 1241, 1093; 957 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$ + DMSO-d_6): δ 8.30–8.15 (m, 1H, aromatic), 8.08 (s, 1H, aromatic), 7.82–7.74 (m, 1H, aromatic), 7.66 (s, 1H, aromatic), 7.56 (d, 2H J = 8.1, Hz, aromatic), 7.52–7.46 (m, 1H, aromatic), 5.30 (d, 1H, J = 4.1 Hz, CH), 3.98–3.75 (m, 2H, OCH_2), 0.88 (t, 3H, CH_3). ^{13}C NMR ($CDCl_3$ + DMSO-d_6): δ 175.5, 174.2, 155.0, 152.4, 149.3, 132.7, 127.0, 124.1, 124.0, 112.6, 94.1, 70.8, 58.4, 46.5, 17.2, 17.0. Mass (ESI-MS): m/z 383 [M^+ +H]. *Ethyl 4-(6-methyl-4-oxo-4H-chromen-3-yl)-6-phenyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate* (**7q**). Yield: 89%; color less solid; mp 220–222 °C. IR (KBr): 3389, 3203, 1662, 1564, 1452, 1199, 1056, 924 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$ + DMSO-d_6): δ 9.32 (br s, 1H, NH), 8.48 (s, 1H, aromatic), 8.02 (s, 1H, aromatic), 7.94 (s, 1H, aromatic), 7.56–7.38 (m, 7H, aromatic), 5.66 (d, 1H, J = 3.3 Hz, CH), 3.98–3.86 (q, 2H, OCH_2), 2.48 (s, 3H, CH_3), 0.90 (t, 3H, J = 7.2 Hz, CH_3). ^{13}C NMR ($CDCl_3$ + DMSO-d_6): δ 175.9, 174.0, 164.0, 153.8, 153.2, 146.5, 134.6, 133.4, 128.9, 127.6, 127.3, 122.9, 122.0, 117.3, 97.6, 59.4, 48.0, 20.2. Mass (ESI-m/z 421 [M^++H]. *Ethyl 6-methyl-4-(6-nitro-4-oxo-4H-chromen-3-yl)-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate* (**7u**). Yield: 71%; brown solid; mp 296–298 °C. IR (KBr): 3226, 3102, 1706, 1645, 1221, 1092, 965, 812 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$ + DMSO-d_6): δ 9.18 (br s, 1H, NH), 8.98 (d, 1H, J = 2.8 Hz, aromatic), 8.54 (dd, 1H, J = 2.6, 9.0 Hz, aromatic), 7.90 (s, 1H, aromatic), 7.74 (d, 1H, J = 9.2 Hz, aromatic), 6.78 (br s, 1H, NH), 5.50 (d,1H, J = 2.8 Hz, CH), 4.14–4.04 (q, 2H, OCH_2), 2.36 (s, 3H, CH_3), 1.18 (t, 3H, J = 6.8 Hz, CH_3), ^{13}C NMR ($CDCl_3$ + DMSO-d_6): δ 174.2, 164.1, 157.8, 153.2,

151.5, 149.6, 143.3, 126.9, 123.7, 122.7, 120.8, 119.3, 93.8, 58.3, 46.5, 17.1, 16.9; Mass (ESI-MS): m/z 374 [M^++H].

15. National Committee for Clinical Laboratory Standards. Anti-mycobacterial susceptibility testing for *Mycobacterium tuberculosis*. Proposed standard M24-T. National Committee for Clinical Laboratory Standards, Villanova, Pa., 1995.
16. Cell proliferation assay using MTT: this assay is a quantitative colorimetric method for determination of cell cytotoxicity.[17] The assessed parameter is the metabolic activity of viable cells. Metabolically active cells reduce pale yellow tetrazolium salt (MTT) to a dark blue water-insoluble formazan, which can be directly quantified after solubilisation with DMSO. The absorbance of the formazan directly correlates with the number of viable cells. The cells were plated in 96-well plates at a density of 2.0×10^4 in 100 μL of medium per well of 96-well plate. Cultures were incubated with the test compounds (10 μM) and incubated for 48 h. The medium was replaced with fresh medium containing 100 μg/mL of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) for 2–3 h. The supernatant was aspirated and MTT-formazan crystals were dissolved in 100 μL DMSO; OD measured at λ 540 nm (reference wavelength, λ 620 nm) on ELISA reader cell cytotoxicity% was calculated by comparing the absorbance of treated versus untreated cells.
17. (a) Slater, T. *Biochem. Biophys. Acta* **1963**, *77*, 383; (b) Van de Loosdrecht, A. A. *J. Immunol. Methods* **1994**, *174*, 311; (c) Alley, M. C. *Cancer Res.* **1988**, *48*, 589.