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Design, synthesis and *in vitro* evaluation of tetrahydropyrimidine–isatin hybrids as potential antitubercular and antimalarial agents

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Abstract

A series of 5-substituted-3-[{5-(6-methyl-2-oxo/thioxo-4-phenyl-1,2,3,4-tetrahydropyrimidin-5-yl)-1,3,4-oxadiazol-2-yl}-imino]-1,3-dihydro-*2H*-indol-2-one were synthesized, characterized and screened for their anti-tubercular and antimalarial activity.

Keywords: Tetrahydropyrimidines–isatin hybrid; Biginelli reaction; *In vitro* antitubercular; Antimalarial activity

1,3-Dihydro-*2H*-indol-2-one nucleus is used as a versatile lead molecule for designing potential antitubercular [1], antiviral [2], anticonvulsant [3] and anti-tumor [4] agents. Similarly, imine bases of 1,3-dihydro-*2H*-indol-2-ones were reported for various biological activities [5–7]. Literature survey revealed that introduction of electron-withdrawing groups at positions 5, 6, and 7 greatly increased activity from that of 1,3-dihydro-*2H*-indol-2-one, with substitution at the 5th position being most favorable. This is not surprising, as C-5 substitution has been associated with increased biological activity [8,9] and, the presence of substituted aromatic/heteoaromatic ring at 3rd position has been reported to be associated with antimicrobial properties [10,11]. Also 3-substituted indolin-2-ones have been identified as a versatile scaffold for the development of protein kinase inhibitors which exhibit selectivity toward different receptor tyrosine kinases (RTKs) by altering the substituents [12]. Sun et al. reported that several 3-substituted indolin-2-one derivatives containing bulky groups in the phenyl ring at the C-3 position of indolin-2-ones showed high selectivity toward the EGF and Her-2 RTKs [13]. The various substituent at 3rd position of the indolin-2-ones reported, were various substituted phenyl ring [14], heterocyclic ring [15] and aliphatic system [16]. Recently George et al. had also reported some of the pyrimidinone-oxadiazolylindolinones as promising antimicrobials together with antioxidant activities [17]. This observations led us to synthesis 5-substituted-3-[{5-(6-methyl-2-oxo/thioxo-4-phenyl-1,2,3,4 tetrahydropyrimidin-5-yl)-1,3,4-oxadiazol-2-yl}imino]-1,3-dihydro-*2H*-indol-2-ones **4a-l**, using 5-(5-amino-1,3,4-oxadiazol-2-yl)-6-methyl-4-phenyl-3,4-dihydropyrimidin-2(H)-one/thiones and different 5-substituted indoline-2,3-dione.

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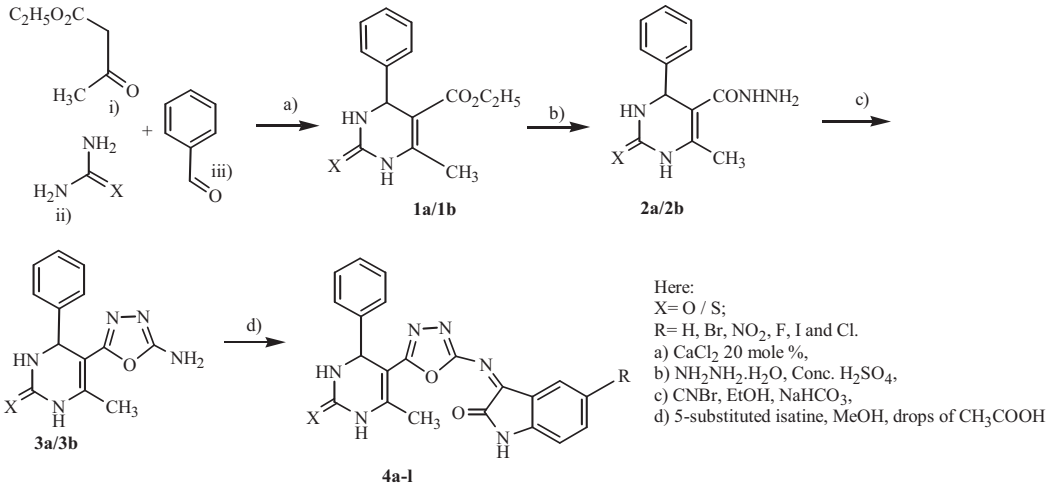

Scheme 1. Synthetic pathway leading to the title compounds **4a-l**.

The synthetic pathway leading to the title compounds **4a-l** is given in Scheme 1. In a typical experimental procedure, a solution of β-ketoester, aldehyde and urea/thiourea in ethanol was heated under reflux in the presence of catalytic amount of $CaCl_2$ to give ethyl 6-methyl-4-phenyl-2-(oxo/thioxo)-1,2,3,4-tetrahydropyrimidine-5-carboxylate **1a/1b** [5], followed by reaction with hydrazine hydrate in ethanol to give 6-methyl-4-phenyl-2-oxo/thioxo-1,2,3,4-tetrahydropyrimidine-5-carbohydrazide **2a/2b** [5]. Treatment of 6-methyl-4-phenyl-2-oxo/thioxo-1,2,3,4-tetrahydropyrimidine-5-carbohydrazide **2a/2b** with ammonium thiocyanate in acidic medium afforded 6-methyl-2-(oxo/thioxo)-4-phenyl-1,2,3,4-tetrahydropyrimidine-5-carbonyl hydrazine-carbothioamide, which on heterocyclization in presence of cyanogen bromide gave 5-(5-amino-1,3,4-oxadiazol-2-yl)-6-methyl-4-phenyl-3,4-dihydropyrimidin-2(1*H*)-one/thione **3a/3b** [5,17]. Finally, compounds **3a/3b** on condensation with various 5-substituted indoline-2,3-dione in acidic medium afforded the title compound 5-substituted-3-[{5-(6-methyl-2-oxo/thioxo-4-phenyl-1,2,3,4 tetrahydropyrimidin-5-yl)-1,3,4-oxadiazol-2-yl}imino]-1,3-dihydro-*2H*-indol-2-one **4a-l** [5,17]. The structure of all the synthesized compounds was established by elemental, IR, (^{1}H and ^{13}C) NMR and mass spectral analysis [18].

All the newly synthesized compounds were evaluated *in vitro* to study its activity against *Mycobacterium tuberculosis H37 Rv*. The primary screening was conducted at concentration 6.25 μg/mL in BACTEC MGIT system [19]. Compounds demonstrating 99% inhibition in the primary screen were described as most potent compounds. The preliminary results indicated that compounds **4c**, **4f**, **4i** and **4l** showed highest inhibition (99%) at a constant concentration level (6.25 μg/mL). This primary bioassay results have driven us to examine the real potency (MIC) of the title compounds against *M. tuberculosis H37 Rv*. The secondary biological screening was performed using Lowenstein–Jensen MIC method and it is worthwhile to note that compound **4c** and **4l** displayed inhibition completely (99%) at the MIC of (3.10–3.12 μg/mL). All the remaining compounds displayed moderate to good activity within 74–99% inhibition at the concentration of (100–500 μg/mL). The secondary antimycobacterial screening for test compounds was obtained by using L.J. (Lowenstein and Jensen) MIC method [20,21] and the observed MIC of compounds are presented in Table 1. Isoniazid, Refampin, Ethambutol and Pyrazinamide were used as the reference drug.

All the synthesized compounds were also evaluated *in vitro* for antimalarial assay against *Plasmodium falciparum 3D7* chloroquine-sensitive strain (Microcare Laboratory & TRC, Surat, Gujarat, India), in 96 well microtitre plates according to the microassay protocol of Rieckmann and co-workers with minor modifications [22–25]. The test concentration which inhibited the complete maturation into schizonts was recorded as the minimum inhibitory concentrations (MIC). Chloroquine was used as the reference drug. Observations of the *in vitro* antimalarial screening are presented in Table 1. The compounds **4a**, **4b**, **4e**, **4f**, **4j** and **4k** have no effect on antimalarial activity (MIC = 10 μg/mL) while compounds **4c**, **4d**, **4g**, **4i**, **4j** and **4l** have remarkable improvement in antimalarial potency with MIC value in the range of 0.035–5.0 μg/mL. Presence of nitro (**4c**, MIC = 0.177 μg/mL) and presence of chloro (**4l**, MIC = 0.035 μg/mL) displayed excellent antimalarial potency. Overall, among the various substitution, the order

Table 1
Biological activity of compounds **4a-l**.

Entry	Antitubercular activity[a]				Antimalarial activity[b]	log *P* value[c]
	BACTEC MGIT method		L.J. MIC method		MIC values (μg/mL)	
	MIC values (μg/mL)	% inhibition	MIC values (μg/mL)	% inhibition		
4a (X = O, R = H)	>6.25	–	200	92	10	3.13 ± 0.83
4b (X = O, R = Br)	>6.25	–	500	74	10	4.11 ± 0.88
4c (X = O,R = NO_2)	6.25	99	3.10	99	0.177	3.04 ± 0.84
4d (X = O, R = F)	>6.25	–	250	84	5	3.39 ± 0.88
4e (X = O, R = I)	>6.25	–	500	93	10	4.37 ± 0.88
4f (X = O, R = Cl)	>6.25	99	100	97	10	3.93 ± 0.84
4g (X = S, R = H)	>6.25	–	500	94	5	3.74 ± 0.85
4h (X = S, R = Br)	>6.25	–	500	75	10	4.71 ± 0.89
4i (X = S, R = NO_2)	>6.25	99	100	97	5	3.65 ± 0.86
4j (X = S, R = F)	>6.25	–	200	89	5	3.99 ± 0.89
4k (X = S, R = I)	>6.25	–	500	94	10	4.97 ± 0.89
4l (X = S, R = Cl)	6.25	99	3.12	99	0.035	4.53 ± 0.86
Isoniazid	0.20	99	0.07	99	–	–
Refampin	0.25	99	0.25	99	–	–
Ethambutol	3.12	99	0.20	99	–	–
Pyrazinamide	6.25	99	–	–	–	–
Chloroquine	–	–	–	–	0.125	–

[a] Against *M. tuberculosis H37Rv* (MTCC – 200).
[b] Against *Plasmodium falciparum 3D7* chloroquine-sensitive strain.
[c] Theoretical values of log *P* were calculated using commercially available ChemDraw program.

of highest antimalarial potency is $NO_2 \geq F > Br \geq H$. It is well known from the literature that the presence of these groups imparts a variety of properties including steric, electronic properties, enhanced binding interactions, metabolic stability, changes in physical properties and selective reactivities [26,27]. This promising antitubercular and antimalarial activity may be due to sufficient hydrogen bonding capacity with desired lipophilicity or with favorable steric hinderance [28].

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References

[1] T.N. Akhaja, J.P. Raval, Chin. Chem. Lett. 23 (2012) 446.
[2] N. Terzioğlu, N. Karalı, A. Gürsoy, et al. ARKIVOC 1 (2006) 109.
[3] G. De Sarro, A. Carotti, F. Campagna, et al. Pharmacol. Biochem. Behav. 65 (3) (2000) 475.
[4] (a) L. Sun, N. Tran, C. Liang, et al. J. Med. Chem. 43 (2000) 2655;
(b) L. Sun, N. Tran, C. Liang, et al. J. Med. Chem. 42 (1999) 5120.
[5] T.N. Akhaja, J.P. Raval, Eur. J. Med. Chem. 46 (2011) 5573.
[6] (a) S.N. Pandeya, P. Yogeeswari, D. Sriram, et al. Chemotherapy 45 (192) (1999);
(b) S.N. Pandeya, S. Smitha, M. Jyoti, S.K. Sridhar, Acta Pharm. 55 (27) (2005);
(c) S.N. Pandeya, D. Sriram, G. Nath, E. De Clercq, Arzneim. Forsch./Drug Res. 50 (2000) 55.
[7] M. Verma, S.N. Pandeya, K. Singh, J.P. Stables, Acta Pharm. 54 (2004) 49.
[8] C.A. Tournaire, M. Barritault, D.M. CrumeyrolleArias, Biochem. Biophys. Res. Commun. 276 (2000) 379.
[9] D. Lee, S.A. Long, J.H. Murray, W.E. DeWolf Jr., J. Med. Chem. 44 (2001) 2015.
[10] R.V. Singh, N. Fahmi, M.K. Biyala, J. Iranian Chem. Soc 2 (2005) 40.

[11] A.K. Padhy, S.K. Sahu, P.K. Panda, et al. Indian J. Chem. 43B (2004) 971.
[12] K. Kiakos, A. Sato, T. Asao, et al. Mol. Cancer Ther. 6 (2007) 2708.
[13] L. Sun, N. Tran, F. Tang, et al. J. Med. Chem. 41 (1998) 2588.
[14] (a) S.N. Pandeya, A.S. Raja, G. Nath, Indian J. Chem. 45B (2006) 494;
(b) B.P. Choudhari, V.V. Mulwad, Indian J. Chem. 44B (2005) 1074.
[15] (a) S.N. Pandeya, D. Sriram, G. Nath, E. De Clercq, Il Farmaco 54 (624) (1999);
(b) R.T. Pardasani, P. Pardasani, D. Sherry, V. Chaturvedi, Indian J. Chem. 40B (1275) (2001);
(c) G.S. Singh, T. Singh, R. Lakhan, Indian J. Chem. 36B (1997) 951.
[16] Y. Teitz, D. Ronen, A. Vansover, et al. Antivir. Res. 24 (1994) 305.
[17] S. George, M.K. Parameswaran, A.R. Chakraborty, et al. Acta Pharm. 58 (2008) 119.
[18] Analytical data of the synthetic compounds. 3a: Yield 72%, m.p. 210–211 °C, Anal. Calcd. for $C_{13}H_{13}N_5O_2$: C, 57.56; H, 4.83; N, 25.82. Found: C, 57.17; H, 4.48; N, 25.58%. IR ((max, cm^{-1}, KBr): 3430, 3067 (C–H, Aromatic), 3384, 3147 (N–H), 1718 (C═O), 1634 (C═N, Iminebase), 1458 (C═C, Aromatic), 1364 (C═N), 1021 (C–O–C), 745, 672, 643 (C–H, Deformation). 1H NMR (400 MHz, DMSO-d6): (8.36, 6.52 (s, 2H, –NH–), 7.42–7.94 (m, 5H, Ar–H), 5.27 (s, 1H, –CH═), 2.37 (s, 3H, –CH3). 13C NMR (100 MHz, DMSO-d6): (161.45 (C═O), 154.13, 152.74 (C–O–C), 142.43 (C–CH3), 109.21, 127.28, 132.81, 136.82 (Ar–C), 111.17 (C–oxadiazole ring), 54.25 (C–furfural ring), 13.98 (–CH3). MS $[M]^+$ $[272.36]^+$. 3b: Yield 79%, m.p. 222–223 °C, Anal. Calcd. for $C_{13}H_{13}N_5OS$: C, 54.34; H, 4.56; N, 24.37. Found: C, 53.89; H, 3.93; N, 23.76%. IR ((max, cm^{-1}, KBr): 3430, 3067 (C–H, Aromatic), 3371, 3154 (N–H), 1624 (C═N, Iminebase), 1462 (C═C, Aromatic), 1429 (C═S), 1372 (C═N), 1027 (C–O–C), 736, 693, 632 (C–H, Deformation). 1H NMR (400 MHz, DMSO-d6): (8.36 (s, 2H, –NH–), 7.12–7.57 (m, 5H, Ar–H), 5.46 (s, 1H, –CH═), 2.18 (s, 3H, –CH3). 13C NMR (100 MHz, DMSO-d6): (173.17 (C═S), 155.23, 153.87 (C–O–C), 142.68 (C–CH3), 110.29,125.27, 132.45, 139.13 (Ar–C), 113.37 (C–oxadiazole ring), 55.76 (C–furfural ring), 14.39 (–CH3). MS $[M]^+$ $[288.57]^+$. 4c: Yield 90%, m.p. 251–253 °C, Anal. Calcd. for ($C_{21}H_{15}N_7O_5$): C, 56.63; H, 3.39; N, 17.96. Found: C, 55.93; H, 3.05; N, 17.61%. IR ((max, cm^{-1}, KBr): 3430, 3067 (C–H, Aromatic), 3371, 3189 (N–H), 1712 (C═O), 1631 (C═N, Iminebase), 1565 (N═O), 1459 (C═C, Aromatic), 1375 (C═N), 1252, 1024 (C–O–C), 741, 685, 657 (C–H, Deformation). 1H NMR (400 MHz, DMSO-d6): (9.05, 6.36 (s, 3H, –NH–), 7.03–7.69 (m, 8H, Ar–H), 5.23 (s, 1H, –CH═), 2.15 (s, 3H, –CH3). 13C NMR (100 MHz, DMSO-d6): (171.29, 168.34 (C═O), 155.45 (C═N–), 153.72, 151.65 (C–O–C), 97.12, 104.37, 110.53, 114.27, 121.37, 126.38, 133.54, 135.65, 142.45, 149.32 (Ar–C), 57.43 (C–furfural), 14.78 (–CH3). MS $[M]^+$ $[447.17]^+$. 4l: Yield 79%, m.p. 245–246 °C, Anal. Calcd. for ($C_{21}H_{15}ClN_6O_2S$): C, 55.94; H, 3.35; N, 18.64. Found: C, 55.71; H, 3.12; N, 18.47%. IR ((max, cm^{-1}, KBr): 3437, 3043 (C–H, Aromatic), 3365, 3183 (N–H), 1718 (C═O), 1643 (C═N, Iminebase), 1457 (C═C, Aromatic), 1367 (C═N), 1246, 1029 (C–O–C), 746, 667, 632 (C–H, Deformation). 710 (C–Cl). 1H NMR (400 MHz, DMSO-d6): δ 11.92, 9.34, 2.67 (s, 3H, –NH–), 7.18–7.93 (m, 8H, Ar–H), 5.12 (s, 1H, –CH═), 2.29 (s, 3H, –CH3). 13C NMR (100 MHz, DMSO-d6): δ 173.67 (C═S), 166.75 (C═O), 155.65 (C═N–), 153.68, 152.05 (C–O–C), 97.12, 107.45, 110.32, 115.53, 121.29, 126.54, 132.48, 136.84, 143.23, 149.19 (Ar–C), 56.96 (C–furfural), 16.19 (–CH3). MS $[M]^+$ $[452.07]^+$.
[19] P. Anargyros, D.S. Astill, I.S. Lim, J. Clin. Microbiol. 28 (1990) 1288.
[20] H.D. Isenberg, Clinical Microbiology Procedures Handbook, vol. 1, American Society for Microbiology, Washington, DC, 1992.
[21] A. Rattan, Antimicrobials in Laboratory Medicine, Churchill B.I. Livingstone, New Delhi, 2000, p. 85.
[22] K.H. Rieckmann, G.H. Campbell, L.J. Sax, J.E. Mrema, Lancet 1 (1978) 221.
[23] R.E. Desjardins, In vitro techniques for antimalarial development and evaluation, in: W. Peters, W.H.G. Richards (Eds.), Handbook of Experimental Pharmacology, Springer-Verlag, Germany, 1984, pp. 179–200.
[24] W. Trager, J.B. Jensen, Science 193 (1976) 673.
[25] C. Lambros, J.P. Vanderberg, J. Parasitol. 65 (1979) 418.
[26] C.G. Wermuth, The Practice of Medicinal Chemistry, 2nd ed., Elsevier, 2010, pp. 303–325 (Chapter 19).
[27] W.K. Hagmann, J. Med. Chem. 5 (2008) 4359.
[28] A. Mälkiä, L. Murtomäki, A. Urtti, K. Kontturi, Eur. J. Pharm. Sci. 23 (2004) 13.