Available online at www.sciencedirect.com
SCIENCE DIRECT®
European Journal of Medicinal Chemistry 40 (2005) 1179–1187
EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY
www.elsevier.com/locate/ejmech

Laboratory notes

Design, synthesis and biological activity of acyl substituted 3-amino-5-methyl-1,4,5,7-tetrahydropyrazolo[3,4-b]pyridin-6-ones as potential hypnotic drugs

José L. Falcó [a,*], Maia Lloveras [b], Irma Buira [a], Jordi Teixidó [b], José I. Borrell [b], Eva Méndez [a], José Terencio [a], Albert Palomer [a], Antonio Guglietta [a]

[a] *Medicinal Chemistry Department, R&D Center, Ferrer Internacional S.A., Juan de Sada 32, E-08028 Barcelona, Spain*
[b] *Grup d'Enginyeria Molecular, Institut Químic de Sarriá, Universitat Ramon Llull, Via Augusta 390, E-08017 Barcelona, Spain*

Received 7 February 2005; received in revised form 14 June 2005; accepted 15 June 2005

Available online 10 August 2005

Abstract

Among the known non-benzodiazepinic hypnotic drugs acting on the α1 subunit of the GABA-A receptor, Zolpidem (**2a**), Zaleplon (**1b**) and Indiplon (**1a**) have showed high affinity and selectivity. Following a design methodology including pharmacophoric requirements and ADME-predicted properties, we have synthesized a library of 3-amino-4,5-dihydro-1*H*-pyrazolo[3,4-*b*]pyridin-6(7*H*)-ones (**3**) and their N1-alkyl derivatives (**4**) as new scaffolds for designing non-benzodiazepine BZ receptor ligands.

Keywords: Zolpidem; Zaleplon; Indiplon; Non-benzodiazepinic; GABA-A receptor; Pyrazolo[3,4-*b*]pyridin-6-ones; ADME; Volsurf; Catalyst; Combinatorial chemistry

1. Introduction

The benzodiazepine (BZ) classes of drugs are used clinically for their anxiolytic, hypnotic, muscle-relaxant and anticonvulsant actions. They act allosterically to influence central γ-aminobutyric acid (GABA)-mediated neurotransmission [1].

GABA-A receptors are pentameric assemblies of a large range of subunits (α1-6, β1-3, γ1-3, δ, ϵ, π and θ), of which the α subunit is of particular importance in determining the pharmacology of the BZ binding site [2]. The major BZ-sensitive GABA-A receptor subtypes in the brain are α1βxγ2, α2βxγ2, α3βxγ2, and α5βxγ2 and their distribution in the brain shows distinct regional variations.

Ligands at the BZ site are categorized as agonists, inverse agonists, or antagonists. Agonists act by increasing the frequency of channel opening to give a net hyperpolarization of the neuron and a decreased excitability. BZ inverse agonists have the opposite effect and decrease the frequency of channel openings, resulting in a depolarization and an increased neuronal excitability. Between the two efficacy extremes, there is a continuum of partial agonists and partial inverse agonists that do not alter chloride flow and are functionally silent.

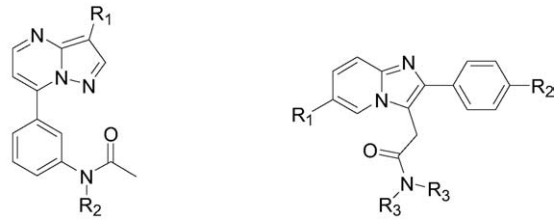
As the effect of BZs is limited to modulate the activity of an endogenous transmitter, they have quite low acute toxicity. Nevertheless, there is considerable clinical concern about undesirable side effects such as sedation, amnesia and, in particular, the ability to induce both physical and psychological dependence [3]. Another problem, particularly with respect to the use of BZs as anticonvulsant, is the quite rapid development of tolerance [4,5].

It is clear that improved understanding of the GABA-A/BZ receptor complex and subtypes of its components may lead to more selective drugs with improved activity and/or fewer side-effects for the treatment of anxiety sleep disorders, convulsions, and memory deficits.

Among the known ligands, the pyrazolo[1,5-a]pyrimidines Indiplon (**1a**) and Zaleplon (**1b**) and the *N,N*-dialkyl-2-phenylacetamidoimidazo[1,2-*a*]pyridines Zolpidem (**2a**) and Alpidem (**2b**) (Scheme 1) showed both high affinity and selectivity toward non-BZ receptors [6].

* Corresponding author. Tel.: +34 93 509 3252; fax: +34 93 411 2764.
E-mail address: jlfalco-research@ferrergrupo.com (J.L. Falcó).

0223-5234/$ - see front matter © 2005 Elsevier SAS. All rights reserved.
doi:10.1016/j.ejmech.2005.06.008



1a Indiplon R^1=thienyl-CO, R^2=Me
1b Zaleplon R^1=CN, R^2=Et

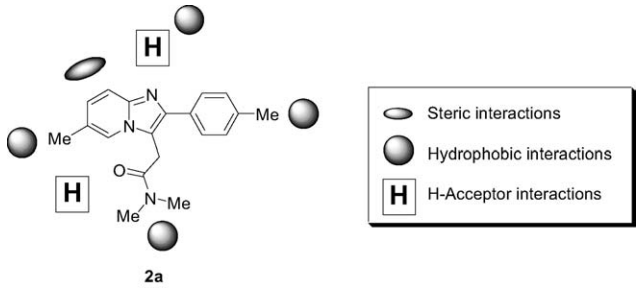
2a Zolpidem R^1=R^2=R^3=Me
2b Alpidem R^1=R^2=Cl, R^3=Pr

Scheme 1.

An examination of the structure-activity relationships (SAR) of affinity and efficacy of these and other compounds [7–9] at the BZ receptor has assisted in development of several pharmacophoric models for ligand–receptor interaction. These models are characterized by a number of points of lipophilic and hydrogen-bonding ligand–receptor interaction [10–13], and in some cases [14–15] areas of steric hindrance have also been defined. According to these models, Scheme 2 shows the hydrophobic, H-acceptor and steric interactions in Zolpidem.

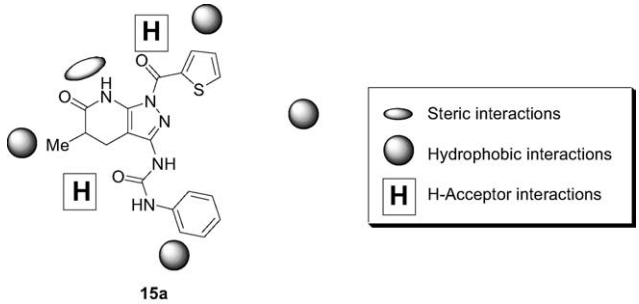
Following this model, we designed a new scaffold according to the previous heterocyclic experience in our group (Scheme 4) with a similar distribution of the different substituents, related to the models Zolpidem (**2a**) and Indiplon (**1a**). Scheme 3 shows such distribution of the substituents of one of the molecules of the library, according to the pharmacophore described above.

We decided to use 3-amino-4,5-dihydro-1*H*-pyrazolo[3,4-*b*]pyridin-6(7*H*)-ones (**3**) and their N1-alkyl derivatives (**4**) [16–17] (Scheme 3 and 4) as new scaffolds for designing non-benzodiazepine BZ receptor ligands. Compounds **3** and **4** are easily obtained by treatment with hydrazine or alkyl substi-



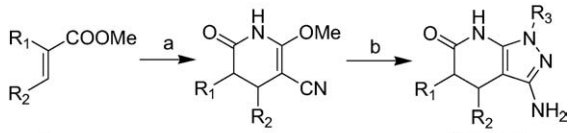
2a

Scheme 2.



15a

Scheme 3.



7 **6** **3**: R_3 = H; **4**: R_3 = alkyl

[a]Reagents: (a) NaOMe/MeOH; (b) R_3NHNH_2/MeOH

Scheme 4. [a]Reagents: (a) NaOMe/MeOH; R_3NHNM_2/MeOH.

tuted hydrazines (R_3NHNH_2) respectively of 2-methoxy-6-oxo-1,4,5,6-tetrahydropyridine-3-carbonitriles (**5**), which in turn are synthesized by reaction of an α,β-unsaturated ester (**7**) and malononitrile in NaOMe/MeOH. In order to fulfill the requirements of the aforementioned pharmacophore we considered the modification of pyrazolopyridines (**3**) and (**4**) by introducing acyl groups either on the NH_2 group or on N1 of the pyrazole ring (when R_3 = H). The present paper deals with the results obtained in such study.

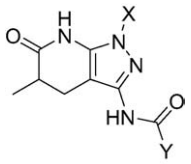
2. Chemistry

In order to mimic the *6*-methyl group present in Zolpidem (**2**) we decided to use 3-amino-4,5-dihydro-5-metyl-1*H*-pyrazolo[3,4-*b*]pyridin-6(7*H*)-one (**8**), which corresponds to general structure **3** with R_1 = Me and R_2 = H (Scheme 4), and its N1-methyl derivative **9** (**4** with R_1 = Me, R_2 = H, and R_3 = Me) as starting materials. On the other hand, in order to mimic the thiophene carbonyl group present in Indiplon (**1a**), we carried out an acylation with acyl chlorides on N1 of the pyrazole ring (when R_3 = H). Further derivatization on the NH_2 group was considered by formation of amides upon treatment with acyl chlorides, or by formation of urea upon treatment with isocyanates.

In this context, we carried out a previous selection of the molecules to synthesize. The first step of the selection process was the construction, using the CombiChem module of Accord for Excel [18], of a virtual library of 500 compounds by varying substituents X and Y attached to N1 or the exocylic nitrogen atom of the 3-amino-4,5-dihydro-5-metyl-1*H*-pyrazolo[3,4-*b*]pyridin-6(7*H*)-one core (see Table 1). All the compounds were filtered by Lipinski's rule of 5 [19] implemented in Accord for Excel. Compounds verifying such rules were submitted to Volsurf [20] in order to calculate predicted ADME properties such as Caco2 (Gastro-Intestinal Barrier) and BBB (Blood–Brain Barrier). Two simultaneous selection criteria were used: $-0.10 < \text{Caco2} < 1.05$ and $-0.10 < \text{BBB} < 1.00$. Afterwards, the last step of the selection process consisted in running under Catalyst [21] the pharmacophore described in the literature [10–15]. Thus, twenty compounds were selected to be synthesized which are summarized in Table 1 together with their MW, ClogP, number of Hbond acceptors and donors, Caco2 and BBB calculated values.

Treatment of methyl methacrylate (**7** with R_1 = Me and R_2 = H) with malononitrile in NaOMe/MeOH at

Table 1
Structure of pyrazolo[3,4-*b*]pyridines synthesized together with their MW, ClogP, number of Hbond acceptors and donors, Caco2 and BBB calculated values, and in vitro inhibitory activity



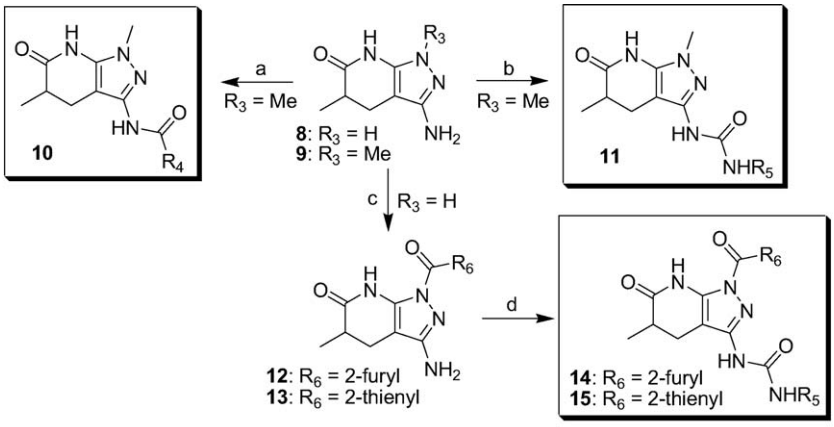
compound	X	Y	Yield (%)	Lipinski's rule of 5				ADME predicted Volsurf		In vitro activity (% inhib)	
				MW	ClogP	N,O[a]	NH,OH[b]	Caco2	BBB	10^{-5} M	10^{-7} M
10a	Me	Me	39	222.25	–0.33	6	2	0.69	0.67	0.0	0.0
10b	Me	Ph	48	284.32	1.27	6	2	0.94	0.73	17.3	10.9
10c	Me	*p*-MeC_6H_4	26	298.35	1.82	6	2	1.01	0.80	0.0	0.0
10d	Me	*p*-ClC_6H_4	36	318.76	1.92	6	2	0.75	0.74	0.0	0.0
10e	Me	$Ph(CH_2)_2$	43	312.37	1.87	6	2	1.03	0.82	0.0	0.0
11a	Me	PhNH	49	299	1.54	7	3	0.91	0.53	20.5	14.7
11b	Me	*p*-MeC_6H_4NH	70	313.36	2.09	7	3	0.97	0.63	0.0	0.0
11c	Me	*p*-ClC_6H_4NH	58	333.78	2.18	7	3	0.72	0.55	0.0	0.0
11d	Me	*p*-$CF_3C_6H_4NH$	87	367.33	2.50	7	3	0.48	0.35	6.1	1.7
11e	Me	(2-thienyl)NH	59	305.36	1.36	7	3	0.87	0.65	5.8	2.9
14a	(2-furyl)CO	PhNH	83	379.38	2.23	9	3	0.06	–0.08	13.5	0.0
14b	(2-furyl)CO	*p*-MeC_6H_4NH	84	393.40	2.78	9	3	0.49	0.26	1.1	0.0
14c	(2-furyl)CO	*p*-ClC_6H_4NH	44	413.82	2.87	9	3	0.53	0.38	0.0	0.0
14d	(2-furyl)CO	*p*-$CF_3C_6H_4NH$	39	447.37	3.19	9	3	0.23	0.28	0.0	0.0
14e	(2-furyl)CO	(2-thienyl)NH	54	385.40	2.05	9	3	0.38	0.34	9.5	0.0
15a	(2-thienyl)CO	PhNH	67	395.44	2.93	8	3	0.58	0.45	10.9	0.6
15b	(2-thienyl)CO	*p*-MeC_6H_4NH	53	409.47	3.47	8	3	0.59	0.32	12.6	0.0
15c	(2-thienyl)CO	*p*-ClC_6H_4NH	51	429.89	3.57	8	3	0.33	0.32	0.0	0.0
15d	(2-thienyl)CO	*p*-$CF_3C_6H_4NH$	19	463.44	3.89	8	3	0.07	0.14	10.1	0.0
15e	(2-thienyl)CO	(2-thienyl)NH	49	401.47	2.75	8	3	0.49	0.30	2.3	0

[a] H bond acceptors.
[b] H bond donors.

reflux afforded 2-methoxy-5-methyl-6-oxo-1,4,5,6-tetrahydropyridine-3-carbonitrile (**6** with R_1 = Me and R_2 = H) in 50% yield [17]b, which was converted to **8** in 42% yield by treatment with hydrazine in methanol at reflux (Scheme 4). On the other hand, the N1-methyl substituted derivative **9** was obtained in 46% yield from pyridone **8** using methylhydrazine in methanol at reflux.

Acylation of the NH_2 group of **9** was accomplished with five acyl chlorides R_4COCl (R_4 = Me, Ph, *p*-MeC_6H_4, *p*-ClC_6H_4, and $Ph(CH_2)_2$) in MeCN using DMAP as base to afford amides **10a–e** in 26–48% yield (Scheme 5). Similarly, **9** was transformed to the corresponding ureas **11a–e** upon treatment with five isocyanates R_5NCO (R_5 = Ph, *p*-MeC_6H_4, *p*-ClC_6H_4, *p*-$CF_3C_6H_4$, and thienyl) in dry pyridine in 49–87% yield (Scheme 5).

The selective acylation of N1 of 3-amino-4,5-dihydro-5-metyl-1*H*-pyrazolo[3,4-b]pyridin-6(7*H*)-one (**8**) was achieved using furan-2-carbonyl chloride and thiophene-2-carbonyl



Scheme 5. [a]Reagents: (a) R_4COCl/MeCN/DMAP; (b) R_5NCO/pyridine; (c) R_6COCl/THF/Et_3N; R_5NNCO/pyridine.

chloride to yield **12** (R_6 = 2-furyl) and **13** (R_6 = 2-thienyl) in 40% and 42% yield, respectively. Compounds **12** and **13** were further derivatized (Scheme 5) upon treatment with five isocyanates R_5NCO (R_5 = Ph, *p*-MeC_6H_4, *p*-ClC_6H_4, *p*-$CF_3C_6H_4$, and thienyl) in dry pyridine to yield ureas **14a**–e and **15a**–**e** in 39–84% and 19–67% yield, respectively.

3. Pharmacology

Male Sprague-Dawley rats were used to obtain membranes containing α_1-$GABA_A$ receptor (from cerebellum). The affinity of compounds **10a**–**e**, **11a**–**e**, **14a**–**e**, and **15a**–**e** for α_1-$GABA_A$ receptors was determined by competitive tests using radiolabeled flumazenil as ligand using 96-well microtiter plate format. Membranes containing the study receptors were incubated with radiolabeled flumazenil (at a final concentration of 1 nM) and ascending concentrations of test compounds. Percentage of specific binding for every concentration of test compound was determined using a scintillation counter.

4. Results and discussion

Results are summarized in Table 1. The results obtained indicated that our acyl substituted 3-amino-4,5-dihydro-5-metyl-1*H*-pyrazolo[3,4-b]pyridin-6(7*H*)-ones are completely devoid of any inhibitory activity, the inhibition being lower than 20% for all cases, while Zolpidem shows an inhibitory activity of 99.4% (at a concentration of $1 \times = 10^{-5}$ M).

5. Conclusions

This lack of activity can be attributed to two possible reasons.

In the aforementioned pharmacophoric model (Scheme 2), Zolpidem (**2a**) shows a hydrophobic interaction of an aryl substituent present in N2 of the pyrazole ring. Such interaction, which is not possible for our molecules, could play a key role for the inhibitory activity.

In addition, a literature search has revealed [22] that Zaleplon (**1b**) is metabolized by oxidation of the pyrimidine ring to afford an inactive metabolite that presents a lactam carbonyl group at the same relative position of the pyridone carbonyl group of our molecules. Consequently, the presence of such carbonyl group, the distinctive feature of our synthetic methodology, could be responsible of the loss of activity. Experiments are being conducted to clarify such assumptions.

6. Experimental protocols

6.1. Chemistry

All melting points were determined with a Büchi 530 capillary apparatus and are uncorrected. Infrared spectra were recorded in a Nicolet Magna 560 FTIR spectrophotometer. ^1H and ^{13}C NMR spectra were determined in a Varian Gemini-300 operating in a field-strength of 300 and 75.5 MHz, respectively. Chemical shifts are given in parts per million (δ) values downfield from Me_4Si as internal standard and coupling constants (J) in Hz. Standard and peak multiplicities are designated as follows: s, singlet; d, doublet; dd, doublet of doublets; t, triplet; q, quadruplet; br, broad signal; m, multiplet. ESI (+) i (–) mass spectra were recorded on a Agilent HP1100 mass spectrometer coupled to a HPLC using the following conditions: Column: X-Terra RP18, 3.5 μm, 4.6 x 50 mm (Waters); Injected volume: 2 μl; Eluent: A: Acetonitrile/B: Ammonium formate 10 mM pH 9.20; Gradient: A from 20% to 60% in 6 min, then maintaining at 60% for 3 min; Flow-rate: 1 ml min^{-1}; Temperature: 30 °C; Detection: 240 nm. Elemental microanalyses were obtained in a Carlo-Erba CHNS-O/EA 1108 analyzer and gave results for the elements stated with ± 0.4% of the theoretical values. 2-Methoxy-5-methyl-6-oxo-1,4,5,6-tetrahydropyridine-3-carbonitrile (**6**) was prepared according to a previously published procedure [17]b.

*6.1.1. 3-Amino-5-methyl-1,4,5,7-tetrahydropyrazolo[3,4-b]pyridin-6-one (**8**)*

To a suspension of 2-methoxy-5-methyl-6-oxo-1,4,5,6-tetrahydropyridine-3-carbonitrile (**6**) (6 g, 36.1 mmol) in MeOH (90 ml) was added hydrazine monohydrate (4.5 g, 90 mmol). The mixture was refluxed under stirring for 2 h and after cooling it was filtered. The white precipitated obtained was washed with cool MeOH and dried in a vacuum dryer to gave 2.53 g (42% yield) of the title compound **8**: mp 261–263 °C; IR (KBr) 3403, 3335, 3227, 1645, 1558, 1539 cm^{-1}; ^1H NMR (DMSO-d_6) d 10.57 (br, 1H, NH pirazole), 9.83 (br, 1H, NH–CO), 4.89 (br, 2H, NH_2), 2.68–2.55 (m, 1H, CH), 2.43–2.38 (m, 1H, CH), 2.24–2.08 (m, 1H, CH), 1.10 (d, J = 7 Hz, 3H, CH_3); ^{13}C NMR d 173.4 (C=O), 148.3, 143.7 (C3, C7a), 82.5 (C3a), 36.0 (C-5), 23.7 (C-4), 16.5 (CH_3); Anal. ($C_7H_{10}N_4O$) C 50.30% (50.58%), H 6.28% (6.07%), N 33.65 (33.72%).

*6.1.2. 3-Amino-1,5-dimethyl-1,4,5,7-tetrahydropyrazolo[3,4-b]pyridin-6-one (**9**)*

To a suspension of 2-methoxy-5-methyl-6-oxo-1,4,5,6-tetrahydropyridine-3-carbonitrile (**6**) (3.2 g, 19.3 mmol) in MeOH (30 ml) was added methylhydrazine (1.1 g, 23.9 mmol). The mixture was refluxed under stirring for 2 h and after cooling it was filtered. The white precipitated obtained was washed with cool MeOH and dried in a vacuum dryer to gave 1.6 g (46% yield) of the title compound **9**: mp > 290 °C; IR (KBr) 3384, 3336, 3239, 1652, 1539 cm^{-1}; ^1H NMR (DMSO-d_6) δ 9.87 (br, 1H, NH–CO), 5.13 (br, 2H, NH_2), 3.37 (s, 3H, CH_3–N), 2.64 (dd, J_1 = 14.7 Hz, J_2 = 6.9 Hz, 1H, CH_2), 2.47–2.34 (m, 1H, CH), 2.13 (dd, J_1 = 14.7 Hz, J_2 = 9.9 Hz, 1H, CH_2), 1.09 (d, J = 6.6 Hz, 3H, CH_3,); ^{13}C NMR δ 172.8 (C=O), 146.3, 142.4 (C3, C7a), 82.4 (C3a), 35.9 (C-5), 33.8 (CH_3–N), 23.9 (C-4), 16.4 (CH_3);

Anal. ($C_8H_{12}N_4O$) C 53,22% (53,32%), H 6,78% (6,71%), N 31,09 (31,09%).

*6.1.3. General Procedure for preparation of N-(1,5-dimethyl-6-oxo-4,5,6,7-tetrahydro-1H-pyrrolo[2,3-b]pyridin-3-yl)-amide derivatives (**10**)*

A suspension of **9** (200 mg, 1.1 mmol) and 4-dimethylaminopyridine (272 mg, 2.22 mmol) in dry acetonitrile (15 ml) was heated at 85 °C and the appropriate acid chloride was added (2 eq). The mixture was stirred for 30 min at 90 °C and overnight at room temperature. Solvent was evaporated under reduced pressure and the precipitate obtained was crystallized from methanol.

*6.1.4. N-(1,5-Dimethyl-6-oxo-4,5,6,7-tetrahydro-1H-pyrazolo[3,4-b]pyridin-3-yl)-acetamide (**10a**, R_4 = Me)*

Mp 233–235 °C; IR (KBr) 3209, 1689, 1656, 1552, 1535 cm^{-1}; ^1H NMR (DMSO-d_6) δ 10.17 (s, 1H, NH), 9.81 (s, 1H, NH), 3.47 (s, 3H, CH_3N), 2.64 (dd, J_1 = 15.0 Hz, J_2 = 6.9 Hz, 1H, H–CH), 2.45 (m, 1H, CH), 2.20 (dd, J_1 = 10.3 Hz, J_2 = 15.3 Hz, 1H, H–CH), 2.04 (s, 3H, CH_3CO), 1.10 (d, J = 6.9 Hz, 3H, CH_3); ^{13}C NMR δ 172.6 (C=O), 168.3 (C=O), 146.4 (C7a), 131.9 (C3), 95.0 (C3a), 35.5 (CH_3N), 35.0 (C5), 24.5 (C4), 22.7 (CH_3CO), 16.1 (CH_3) ; MS *m/z* 223.1 (MH^+); Anal. ($C_{10}H_{14}N_4O_2$) C 54.06% (54.04%), H 6.28% (6.35%), N 24.98% (25.21%).

*6.1.5. N-(1,5-Dimethyl-6-oxo-4,5,6,7-tetrahydro-1H-pyrazolo[3,4-b]pyridin-3-yl)-benzamide (**10b**, R_4 = Ph)*

Mp 239-241 °C; IR (KBr) 3222, 1673, 1647, 1525 cm^{-1}; ^1H NMR (DMSO-d_6) δ 10.30 (s, 1H, NH), 10.24 (s, 1H, NH), 8.00 (d, J = 7.5 Hz, 2H, H–Ph), 7.61 (m, 3H, H–Ph), 3.54 (s, 3H, CH_3N), 2.70 (dd, J_1 = 15.3 Hz, J_2 = 6.9 Hz, 1H, CH), 2.47 (m, 1H, CH), 2.27 (dd, J_1 = 10.2 Hz, J_2 = 15.0 Hz, 1H, CH), 1.12 (d, J = 6.6 Hz, 3H, CH_3); ^{13}C NMR δ 172.6 (C=O), 165.2 (C=O), 146.5 (C7a), 132.9, 132.0, 131.8 (C3, *ipso*-Ph, *p*-Ph), 128.4 (*m*-Ph), 127.7 (*o*-Ph), 96.0 (C3a), 35.5, 35.2 (CH_3N, C5), 24.4 (C4), 16.1 (CH_3); MS *m/z* 285.1 (MH^+); Anal. ($C_{15}H_{16}N_4O_2$) C 63.28% (63.37%), H 5.58% (5.67%), N 19.75% (19.71%).

*6.1.6. N-(1,5-Dimethyl-6-oxo-4,5,6,7-tetrahydro-1H-pyrazolo[3,4-b]pyridin-3-yl)-4-methyl-benzamide (**10c**, R_4 = p-MeC_6H_4)*

Mp 272–274 °C; IR (KBr) 3291, 1686, 1657, 1529 cm^{-1}; ^1H NMR (DMSO-d_6) δ 10.23 (s, 1H, NH), 10.18 (s, 1H, NH), 7.90 (d, J = 8.1 Hz, 2H, H–Ph), 7.35 (d, J = 8.1 Hz, 2H, H–Ph), 3.53 (s, 3H, CH_3N), 2.68 (dd, J_1 = 15.3 Hz, J_2 = 6.9 Hz, 1H, CH), 2.47 (m, 1H, CH), 2.39 (s, 3H, CH_3Ph), 2.26 (dd, J_1 = 10.5 Hz, J_2 = 15.0 Hz, 1H, CH), 1.11 (d, J = 6.9 Hz, 3H, CH_3); ^{13}C NMR δ 172.6 (C=O), 165.1 (C=O), 146.5 (C7a), 142.1 (*ipso*-Ph), 131.9, 130.1 (C3, *p*-Ph), 128.9 (*m*-Ph), 127.7 (*o*-Ph), 96.0 (C3a), 35.5, 35.1 (CH_3N, C5), 24.4 (C4), 21.1 (CH_3Ph), 16.1 (CH_3); MS *m/z* 299.2 (MH^+); Anal. ($C_{16}H_{18}N_4O_2$) C 64.11% (64.41%), H 5.96% (6.08%), N 18.65% (18.78%).

*6.1.7. 4-Chloro-N-(1,5-dimethyl-6-oxo-4,5,6,7-tetrahydro-1H-pyrazolo[3,4-b]pyridin-3-yl)-benzamide (**10d**, R_4 = p-ClC_6H_4)*

Mp 267–269 °C; IR (KBr) 3316, 1667, 1542, 1523 cm^{-1}; ^1H NMR (DMSO-d_6) δ 10.36 (s, 1H, NH), 10.26 (s, 1H, NH), 8.01 (d, J = 8.4 Hz, 2H, H–Ph), 7.64 (d, J = 8.4 Hz, 2H, H–Ph), 3.53 (s, 3H, CH_3N), 2.69 (dd, J_1 = 15.3 Hz, J_2 = 6.9 Hz, 1H, CH), 2.46 (m, 1H, CH), 2.26 (dd, J_1 = 10.2 Hz, J_2 = 15.0 Hz, 1H, CH), 1.12 (d, J = 6.9 Hz, 3H, CH_3); ^{13}C NMR δ 172.6 (C=O), 164.3 (C=O), 146.5 (C7a), 136.9 (*ipso*-Ph), 131.7, 131.5 (C3, *p*-Ph), 129.7 (*m*-Ph), 128.5 (*o*-Ph), 96.1 (C3a), 35.5, 35.2 (CH_3N, C5), 24.4 (C4), 16.2 (CH_3); MS *m/z* 319.1 (MH^+); Anal. ($C_{15}H_{15}ClN_4O_2$) C 56.41% (56.52%), H 4.61% (4.74%), N 17.49% (17.58%).

*6.1.8. N-(1,5-Dimethyl-6-oxo-4,5,6,7-tetrahydro-1H-pyrazolo[3,4-b]pyridin-3-yl)-3-phenyl-propionamide (**10e**, R_4 = $Ph(CH_2)_2$)*

Mp 208–210 °C; IR (KBr) 3245, 3200, 1689, 1674, 1532 cm^{-1}; ^1H NMR (DMSO-d_6) δ 10.14 (s, 1H, NH), 9.74 (s, 1H, NH), 7.24 (m, 5H, H–Ph), 3.34 (s, 3H, CH_3N), 2.89 (t, J = 7.5 Hz, 2H, CH_2), 2.62 (t, J = 7.5 Hz, 2H, CH_2), 2.52 (m, 1H, CH), 2.40 (m, 1H, CH), 2.27 (dd, J_1 = 9.9 Hz, J_2 = 14.7 Hz, 1H, CH), 1.07 (d, J = 6.6 Hz, 3H, CH_3); ^{13}C NMR δ 172.8 (C=O), 170.7 (C=O), 146.6 (C7a), 140.8 (*ipso*-Ph), 132.0 (C3), 128.4, 128.4 (*o*-Ph, *m*-Ph), 126.1 (*p*-Ph), 95.4 (C3a), 37.0 (CH_2), 35.7, 35.1 (CH_3N, C5), 31.1 (CH_2), 24.7 (C4), 16.4 (CH_3); MS *m/z* 313.1 (MH^+); Anal. ($C_{17}H_{20}N_4O_2$) C 65.16% (65.37%), H 6.35% (6.45%), N 17.94% (17.94%).

*6.1.9. General procedure for preparation of 1-(1,5-dimethyl-6-oxo-4,5,6,7-tetrahydro-1H-pyrrolo[2,3-b]pyridin-3-yl)-urea derivatives (**11**)*

To a suspension of 3-amino-1,5-dimethyl-1,4,5,7-tetrahydropyrazolo[3,4-*b*]pyridin-6-one (**9**) (200 mg, 1.11 mmol) in dry pyridine (10 ml) was added the appropriate isocyanate (1.1 eq), and the reaction mixture was stirred at room temperature overnight. The mixture was filtered and the white precipitate obtained was washed with dichloromethane.

*6.1.10. 1-(1,5-Dimethyl-6-oxo-4,5,6,7-tetrahydro-1H-pyrazolo[3,4-b]pyridin-3-yl)-3-phenyl-urea (**11a**, R_5 = Ph)*

Mp > 280 °C; IR (KBr) 3258, 1687, 1648, 1559 cm^{-1}; ^1H NMR (DMSO-d_6) δ 10.18 (s, 1H, NH), 8.92 (s, 1H, NH), 8.40 (s, 1H, NH), 7.45 (d, J = 7.8 Hz, 2H, H–Ph), 7.27 (t, J = 7.5 Hz, 2H, H–Ph), 6.97 (t, J = 7.5 Hz, 1H, H–Ph), 3.52 (s, 3H, CH_3N), 2.72 (dd, J_1 = 15.3 Hz, J_2 = 6.9 Hz, 1H, CH), 2.45 (m, 1H, CH), 2.26 (dd, J_1 = 10.2 Hz, J_2 = 15.0 Hz, 1H, CH), 1.11 (d, J = 6.9 Hz, 3H, CH_3); ^{13}C NMR δ 172.6 (C=O), 152.1 (C=O), 146.4 (C7a), 139.3 (*ipso*-Ph), 132.3 (C3), 128.6 (*m*-Ph), 121.9 (*p*-Ph), 118.2 (*o*-Ph), 94.9 (C3a), 35.6 (CH_3N), 34.8 (C5), 24.6 (C4), 16.2 (CH_3); MS *m/z* 300.3 (MH^+); Anal. ($C_{15}H_{17}N_5O_2$) C 60.15% (60.19%), H 5.67% (5.72%), N 23.24% (23.40%).

*6.1.11. 1-(1,5-Dimethyl-6-oxo-4,5,6,7-tetrahydro-1H-pyrazolo[3,4-b]pyridin-3-yl)-3-p-tolyl-urea (**11b**, R_5 = p-MeC_6H_4)*

Mp > 280 °C; IR (KBr) 3264, 1686, 1647, 1556, 1513 cm^{-1}; ^1H NMR (DMSO-d_6) δ 10.17 (s, 1H, NH), 8.74 (s, 1H, NH), 8.28 (s, 1H, NH), 7.33 (d, J = 7.8 Hz, 2H, H–Ph), 7.07 (d, J = 7.8 Hz, 2H, H–Ph), 3.52 (s, 3H, CH_3N), 2.71 (dd, J_1 = 14.7 Hz, J_2 = 6 Hz, 1H, CH), 2.44 (m, 1H, CH), 2.42 (m, 1H, CH), 2.23 (s, 3H, CH_3Ph), 1.11 (d, J = 6.6 Hz, 3H, CH_3); ^{13}C NMR δ 172.6 (C=O), 152.2 (C=O), 146.4 (C7a), 136.7 (*ipso*-Ph), 132.3 (C3), 130.7 (*p*-Ph), 128.9 (*m*-Ph), 118.4 (*o*-Ph), 94.9 (C3a), 35.5 (CH_3N), 34.7 (C5), 24.6 (C4), 20.4 (CH_3Ph), 16.2 (CH_3); MS *m/z* 314.2 (MH^+); Anal. ($C_{16}H_{19}N_5O_2$) C 61.20% (61.33%), H 6.00% (6.11%), N 22.07% (22.5%).

*6.1.12. 1-(4-Chlorophenyl)-3-(1,5-dimethyl-6-oxo-4,5,6,7-tetrahydro-1H-pyrazolo[3,4-b]pyridin-3-yl)-urea (**11c**, R_5 = p-ClC_6H_4)*

Mp 278–280 °C; IR (KBr) 3265, 1680, 1649, 1556 cm^{-1}; ^1H NMR (DMSO-d_6) δ 10.19 (s, 1H, NH), 9.03 (s, 1H, NH), 8.40 (s, 1H, NH), 7.49 (d, J = 8.7 Hz, 2H, H–Ph), 7.32 (d, J = 8.7 Hz, 2H, H–Ph), 3.52 (s, 3H, CH_3N), 2.71 (dd, J_1 = 15.0 Hz, J_2 = 6.6 Hz, 1H, CH), 2.46 (m, 1H, CH), 2.26 (dd, J_1 = 10.2 Hz, J_2 = 15.0 Hz, 1H, CH), 1.11 (d, J = 6.9 Hz, 3H, CH_3); ^{13}C NMR δ 172.6 (C=O), 152.1 (C=O), 146.4 (C7a), 138.3 (*ipso*-Ph), 132.1 (C3), 128.4 (*m*-Ph), 125.5 (*p*-Ph), 119.8 (*o*-Ph), 95.1 (C3a), 35.5 (CH_3N), 34.8 (C5), 24.6 (C4), 16.2 (CH_3); MS *m/z* 334.1 (MH^+), 157.1; Anal. ($C_{15}H_{16}ClN_5O_2$) C 54.06% (53.98%), H 4.83% (4.83%), N 20.66% (20.98%).

*6.1.13. 1-(1,5-Dimethyl-6-oxo-4,5,6,7-tetrahydro-1H-pyrazolo[3,4-b]pyridin-3-yl)-3-(4-trifluoromethylphenyl)–urea (**11d**, R_5 = p-$CF_3C_6H_4$)*

Mp > 280 °C; IR (KBr) 3276, 1684, 1657, 1560, 1533 cm^{-1}; ^1H NMR (DMSO-d_6) δ 10.21 (s, 1H, NH), 9.33 (s, 1H, NH), 8.51 (s, 1H, NH), 7.66 (q, J = 9.0 Hz, 4H, H–Ph), 3.53 (s, 3H, CH_3N), 2.72 (dd, J_1 = 15.3 Hz, J_2 = 6.9 Hz, 1H, CH), 2.48 (m, 1H, CH), 2.27 (dd, J_1 = 10.5 Hz, J_2 = 15.3 Hz, 1H, CH), 1.12 (d, J = 6.9 Hz, 3H, CH_3); ^{13}C NMR δ 172.6 (C=O), 152.0 (C=O), 146.4 (C7a), 143.1 (*ipso*-Ph), 131.9 (C3), 125.9 (q, J = 4.0 Hz, *m*-Ph), 124.4 (q, J = 269.3 Hz, CF_3), 121.9 (q, J = 31.9 Hz, *p*-Ph), 118.0 (*o*-Ph), 95.3 (C3a), 35.5 (CH_3N), 34.8 (C5), 24.6 (C4), 16.2 (CH_3) ; MS *m/z* 368.2 (MH^+), 157.1; Anal. ($C_{16}H_{16}F_3N_5O_2$) C 52.51% (52.32%), H 4.41% (4.39%), N 18.94% (19.07%).

*6.1.14. 1-(1,5-Dimethyl-6-oxo-4,5,6,7-tetrahydro-1H-pyrazolo[3,4-b]pyridin-3-yl)-3-thiophen-2-yl-urea (**11e**, R_5 = 2-thienyl)*

Mp 257-259 °C; IR (KBr) 3265, 1684, 1648, 1578, 1536 cm^{-1}; ^1H NMR (DMSO-d_6) δ 10.20 (s, 1H, NH), 9.88 (s, 1H, NH), 8.47 (s, 1H, NH), 6.86 (d, J = 5.1 Hz, 1H, thiophene), 6.80 (m, 1H, thiophene), 6.59 (d, J = 3.0 Hz, 1H, thiophene), 3.51 (s, 3H, CH_3N), 2.69 (dd, J_1 = 15.0 Hz, J_2 = 6.6 Hz, 1H, CH), 2.45 (m, 1H, CH), 2.24 (dd, J_1 = 10.2 Hz, J_2 = 15.0 Hz, 1H, CH), 1.11 (d, = 6.9 Hz, 3H, CH_3); ^{13}C NMR δ 172.6 (C=O), 151.6 (C=O), 146.4 (C7a), 140.8 (C2′), 131.9 (C3), 124.0 (C4′), 116.0 (C5′), 109.9 (C3′), 95.4 (C3a), 35.5 (CH_3N), 34.7 (C5), 24.4 (C4), 16.2 (CH_3); MS *m/z* 306.1 (MH^+); Anal. ($C_{13}H_{15}N_5O_2S$) C 50.87% (51.13%), H 4.81% (4.95%), N 23.00% (22.94%), S 10.45% (10.50%).

*6.1.15. 3-Amino-1-(furan-2-carbonyl)-5-methyl-1,4,5,7-tetrahydropyrazolo[3,4-b]pyridin-6-one (**12**, R_6 = 2-furyl)*

To a solution of furan-2-carbonyl chloride (10 mmol) in dry THF (30 ml) were added triethylamine (1.5 eq) and 3-amino-5-methyl-1,4,5,7-tetrahydropyrazolo[3,4-*b*]pyridin-6-one (**8**) (1 eq) and the reaction mixture was stirred at room temperature overnight. The mixture was filtered and the filtrate was evaporated under reduced pressure. The residue thus obtained was washed with MeOH to gave the title compound **12** in 40% yield: mp 175–177 °C; IR (KBr) 3431, 3378, 3356, 1702, 1672, 1633; ^1H NMR (DMSO-d_6) δ 9.44 (br, 1H, NHCO), 8.09 (m, 1H, furan), 8.00 (dd, J_1 = 3.3 Hz, J_2 = 0.6 Hz, 1H, furan), 6.78 (dd, J_1 = 3.6 Hz, J_2 = 1.8 Hz, 1H, furan), 5.83 (br, 2H, NH_2), 2.74 (m, 2H, CH_2), 2.28 (m, 1H, CH), 1.17 (d, J = 6.9 Hz, 3H, CH_3); ^{13}C NMR δ 171.7 (C=O), 155.3, 154.8 (C=O, C2′), 148.1, 144.2, 141.3 (C5′, C3, C7a), 123.1 (C3′), 112.3 (C4′), 91.7 (C3a), 34.9 (C5), 22.9 (C4), 15.9 (CH_3). Anal. ($C_{12}H_{12}N_4O_3$) C 55.50% (55.38%), H 4.53% (4.65%), N 21.13 (21.53%).

*6.1.16. 3-Amino-5-methyl-1-(thiophene-2-carbonyl)-1,4,5,7-tetrahydropyrazolo[3,4-b]pyridin-6-one (**13**, R_6 = 2-thienyl)*

As stated above for **12** but using thiophene-2-carbonyl chloride (10 mmol) to give **13** in 42% yield: mp 168–170 °C; IR (KBr) 3406, 3378, 1690, 1663, 1610; ^1H NMR (DMSO-d_6) δ 9.46 (br, 1H, NHCO), 8.29 (dd, J_1 = 3.9 Hz, J_2 = 1.2 Hz, 1H, thiophene), 8.08 (dd, J_1 = 4.8 Hz, J_2 = 1.2 Hz, 1H, thiophene), 7.25 (dd, J_1 = 4.8 Hz, J_2 = 3.9 Hz, 1H, thiophene), 5.87 (br, 2H, NH_2), 2.75 (m, 2H, CH_2), 2.30 (m, 1H, CH), 1.18 (d, J = 6.9 Hz, 3H, CH_3); ^{13}C NMR δ 171.7 (C=O), 158.6, 154.9 (C=O, C7a), 141.2 (C2′), 137.3, 136.8 (C3′, C5′), 132.7 (C3), 127.1 (C4′), 92.0 (C3a), 34.9 (C5), 23.0 (C4), 15.9 (CH_3). Anal. ($C_{12}H_{12}N_4O_2S$) C 52.04% (52.16%), H 4.37% (4.38%), N 20.23 (20.28%), S 11.31 (11.60).

*6.1.17. General procedure for preparation of 1-[1-(furan-2-carbonyl)-5-methyl-6-oxo-4,5,6,7-tetrahydro-1H-pyrazolo[3,4-b]pyridin-3-yl]-urea derivatives (**14**)*

To a solution of 3-amino-1-(furan-2-carbonyl)-5-methyl-1,4,5,7-tetrahydropyrazolo[3,4-*b*]pyridin-6-one (**12**) in dry pyridine (10 ml) was added the appropriate isocyanate (1.1 eq) and the reaction mixture was stirred at room temperature overnight. The mixture was filtered and the white precipitate obtained was washed with dichloromethane.

*6.1.18. 1-[1-(Furan-2-carbonyl)-5-methyl-6-oxo-4,5,6,7-tetrahydro-1H-pyrazolo[3,4-b]pyridin-3-yl]-3-phenyl-urea (**14a**, R_5 = Ph)*

Mp 272–274 °C; IR (KBr) 3392, 3128, 1709, 1671, 1644, 1547 cm^{-1}; ^1H NMR (DMSO-d_6) δ 9.79 (s, 1H, NH), 9.42 (s, 1H, NH), 9.28 (s, 1H, NH), 8.19 (s, 1H, furan), 7.99 (d, J = 3.3 Hz, 1H, furan), 7.46 (d, J = 7.8 Hz, 2H, H–Ph), 7.31 (t, J = 7.5 Hz, 2H, H–Ph), 7.03 (t, J = 7.5 Hz, 1H, H–Ph), 6.86 (m, 1H, furan), 2.97 (dd, J_1 = 15.6 Hz, J_2 = 6.9 Hz, 1H, H–CH), 2.71 (m, 1H, CH), 2.44 (dd, J_1 = 11.4 Hz, J_2 = 15.6 Hz, 1H, CH), 1.18 (d, J = 6.6 Hz, 3H, CH_3); ^{13}C NMR δ 171.8 (C=O), 155.0, 151.3, 149.1, 148.1, 143.8, 142.0 (C=O, C=O, C2′, C5′, C7a, C3), 138.8 (*ipso*-Ph), 128.7 (*m*-Ph), 123.7, 122.4 (*p*-Ph, C3′), 118.5 (*o*-Ph), 112.7 (C4′), 94.3 (C3a), 34.8 (C5), 24.3 (C4), 15.7 (CH_3); MS *m/z* 380.2.1 (MH^+), 157.1; Anal. ($C_{19}H_{17}N_5O_4$) C 60.30% (60.15%), H 4.36% (4.52%), N 18.37% (18.46%).

*6.1.19. 1-[1-(Furan-2-carbonyl)-5-methyl-6-oxo-4,5,6,7-tetrahydro-1H-pyrazolo[3,4-b]pyridin-3-yl]-3-p-tolyl-urea (**14b**, R_5 = p-MeC_6H_4)*

Mp 278–279 °C; IR (KBr) 3121, 1710, 1675, 1611, 1548 cm^{-1}; ^1H NMR (DMSO-d_6) δ 9.79 (s, 1H, NH), 9.38 (s, 1H, NH), 9.21 (s, 1H, NH), 8.19 (d, J = 0.9 Hz, 1H, furan), 7.97 (d, J = 3.9 Hz, 1H, furan), 7.35 (d, J = 8.7 Hz, 2H, H–Ph), 7.12 (d, J = 8.4 Hz, 2H, H–Ph), 6.86 (m, 1H, furan), 2.97 (dd, J_1 = 15.9 Hz, J_2 = 6.9 Hz, 1H, CH), 2.70 (m, 1H, CH), 2.43 (dd, J_1 = 11.4 Hz, J_2 = 15.9 Hz, 1H, CH), 2.26 (s, 3H, CH_3Ph), 1.18 (d, J = 6.9 Hz, 3H, CH_3); ^{13}C NMR δ 171.8 (C=O), 155.0, 151.3, 149.1, 148.2, 143.8, 142.0 (C=O, C=O, C2′, C5′, C7a, C3), 136.2 (*ipso*-Ph), 131.3 (*p*-Ph), 129.1 (*m*-Ph), 123.7 (C3′), 118.6 (*o*-Ph), 112.7 (C4′), 94.2 (C3a), 34.8 (C5), 24.2 (C4), 20.4 (CH_3Ph), 15.7 (CH_3); MS *m/z* 394.2 (MH^+); Anal. ($C_{20}H_{19}N_5O_4$) C 61.07% (61.06%), H 4.87% (4.87%), N 17.40% (17.80%).

*6.1.20. 1-(4-Chlorophenyl)-3-[1-(furan-2-carbonyl)-5-methyl-6-oxo-4,5,6,7-tetrahydro-1H-pyrazolo[3,4-b]pyridin-3-yl]-urea (**14c**, R_5 = p-ClC_6H_4)*

Mp 276–278 °C; IR (KBr) 3121, 1710, 1675, 1611, 1548 cm^{-1}; ^1H NMR (DMSO-d_6) δ 9.79 (s, 1H, NH), 9.45 (s, 1H, NH), 9.35 (s, 1H, NH), 8.19 (d, J = 0.9 Hz, 1H, furan), 8.00 (d, J = 3.6 Hz, 1H, furan), 7.50 (d, J = 8.7 Hz, 2H, H–Ph), 7.36 (d, J = 8.7 Hz, 2H, H–Ph), 6.86 (m, 1H, furan), 2.95 (dd, J_1 = 15.9 Hz, J_2 = 7.2 Hz, 1H, CH), 2.70 (m, 1H, CH), 2.43 (dd, J_1 = 11.4 Hz, J_2 = 15.9 Hz, 1H, CH), 1.18 (d, J = 6.9 Hz, 3H, CH_3); ^{13}C NMR δ 171.8 (C=O), 155.0, 151.3, 149.1, 147.9, 143.8, 142.0 (C=O, C=O, C2′, C5′, C7a, C3), 137.8 (*ipso*-Ph), 128.6 (*m*-Ph), 125.9 (*p*-Ph), 123.8 (C3′), 120.0 (*o*-Ph), 112.7 (C4′), 94.4 (C3a), 34.8 (C-5), 24.3 (C4), 15.7 (CH_3); MS *m/z* 414.1 (MH^+), 346.3, 157.1; Anal. ($C_{19}H_{16}ClN_5O_4$) C 55.12% (55.15%), H 3.79% (3.90%), N 16.73% (16.92%).

*6.1.21. 1-[1-(Furan-2-carbonyl)-5-methyl-6-oxo-4,5,6,7-tetrahydro-1H-pyrazolo[3,4-b]pyridin-3-yl]-3-(4-trifluoromethylphenyl)-urea (**14d**, R_5 = p-$CF_3C_6H_4$)*

Mp 276–278 °C; IR (KBr) 3131, 1712, 1677, 1610, 1548 cm^{-1}; ^1H NMR (DMSO-d_6) δ 9.81 (s, 1H, NH), 9.57 (s, 1H, NH), 9.53 (s, 1H, NH), 8.20 (d, J = 0.9 Hz, 1H, furan), 8.02 (d, J = 3.3 Hz, 1H, furan), 7.67 (s, 4H, H–Ph), 6.87 (m, 1H, furan), 2.96 (dd, J_1 = 15.6 Hz, J_2 = 6.9 Hz, 1H, CH), 2.70 (m, 1H, CH), 2.45 (dd, J_1 = 11.4 Hz, J_2 = 15.9 Hz, 1H, CH), 1.18 (d, J = 6.6 Hz, 3H, CH_3); ^{13}C NMR δ 171.8 (C=O), 155.0, 151.3, 149.2, 147.7, 143.7, 142.6 (C=O, C=O, C2′, C5′, C7a, C3), 142.0 (*ipso*-Ph), 126.0 (q, J = 3.5 Hz, *m*-Ph), 124.2 (q, J = 278 Hz, CF_3), 123.9 (C3′), 122.3 (q, J = 31.9 Hz, *p*-Ph), 118.2 (*o*-Ph), 112.7 (C4′), 94.5 (C3a), 34.8 (C5), 24.4 (C4), 15.7 (CH_3); MS *m/z* 448.1 (MH^+); Anal. ($C_{20}H_{16}F_3N_5O_4$) C 53.53% (53.69%), H 3.61% (3.60%), N 15.85% (15.65%).

*6.1.22. 1-[1-(Furan-2-carbonyl)-5-methyl-6-oxo-4,5,6,7-tetrahydro-1H-pyrazolo[3,4-b]pyridin-3-yl]-3-thiophen-2-yl-urea (**14e**, R_5 = 2-thienyl)*

Mp 275–277 °C; IR (KBr) 3395, 3124, 1704, 1668, 1644, 1547 cm^{-1}; ^1H NMR (DMSO-d_6) δ 10,18 (s, 1H, NH), 9.82 (s, 1H, NH), 9.62 (s, 1H, NH), 8.20 (m, 1H, furan), 7.99 (d, J = 3.3 Hz, 1H, furan), 6.94 (dd, J_1 = 5.7 Hz, J_2 = 1.5 Hz, 1H, thiophene), 6.88 (m, 1H, furan), 6.84 (m, 1H, thiophene), 6.59 (dd, J_1 = 3.6 Hz, J_2 = 1.5 Hz, 1H, thiophene), 2.94 (dd, J_1 = 15.9 Hz, J_2 = 7.2 Hz, 1H, CH), 2.70 (m, 1H, CH), 2.42 (dd, J_1 = 11.4 Hz, J_2 = 15.9 Hz, 1H, CH), 1.18 (d, J = 6.9 Hz, 3H, CH_3); ^{13}C NMR δ 171.8 (C=O), 155.0, 150.6, 149.1, 147.8, 143.8, 142.0 (C=O, C=O, C2′(furan), C5′(furan), C7a, C3), 140.0 (C2″(thiophene)), 124.1, 123.7 (C3′(furan), C4″(thiophene)), 116.5 (C5″(thiophene)), 112.7 (C4′(furan)), 110.2 (C3″(thiophene)), 94.2 (C3a), 34.8 (C5), 24.0 (C4), 15.7 (CH_3); MS *m/z* 386.0 (MH^+), 157.1; Anal. ($C_{17}H_{15}N_5O_4S$) C 52.81% (52.98%), H 3.94% (3.92%), N 18.30% (18.17%), S 8.21% (8.32%).

*6.1.23. General procedure for preparation of 1-[5-methyl-6-oxo-1-(thiophene-2-carbonyl)-4,5,6,7-tetrahydro-1H-pyrrolo[2,3-b]pyridin-3-yl]-urea derivatives (**15**)*

To a solution of 3-amino-5-methyl-1-(thiophene-2-carbonyl)-1,4,5,7-tetrahydropyrazolo[3,4-*b*]pyridin-6-one (**13**) (150 mg, 0.54 mmol) in dry pyridine (10 ml) was added the appropriate isocyanate (1.1 eq), and the reaction mixture was stirred at room temperature overnight. The mixture was filtered and the white precipitate obtained was washed with dichloromethane.

*6.1.24. 1-[5-Methyl-6-oxo-1-(thiophene-2-carbonyl)-4,5,6,7-tetrahydro-1H-pyrazolo[3,4-b]pyridin-3-yl]-3-phenyl-urea (**15a**, R_5 = Ph)*

Mp 279-280 °C; IR (KBr) 3284, 3120, 1710, 1688, 1658, 1632, 1547 cm^{-1}; ^1H NMR (DMSO-d_6) δ 9.79 (s, 1H, NH), 9.50 (s, 1H, NH), 9.26 (s, 1H, NH), 8.33 (dd, J_1 = 3.9 Hz, J_2 = 1.5 Hz, 1H, thiophene), 8.20 (dd, J_1 = 4.8 Hz, J_2 = 1.5 Hz,

1H, thiophene), 7.49 (d, J = 7.8 Hz, 2H, H–Ph), 7.32 (m, 3H, 2 H–Ph and 1H thiophene), 7.04 (t, J = 7.5 Hz, 1H, H–Ph), 3.00 (dd, J_1 = 15.9 Hz, J_2 = 7.2 Hz, 1H, CH), 2.72 (m, 1H, CH), 2.45 (dd, J_1 = 11.4 Hz, J_2 = 15.9 Hz, 1H, CH), 1.18 (d, J = 6.9 Hz, 3H, CH_3); ^{13}C NMR δ 171.9 (C=O), 158.9, 151.3, 147.8, 142.0 (C=O, C=O, C7a, C2′), 138.6, 138.0, 137.8 (*ipso*-Ph, C3′, C5′), 131.5 (C3), 128.7 (*m*-Ph), 127.7, 122.6 (*p*-Ph, C4′), 119.0 (*o*-Ph), 94.5 (C3a), 34.8 (C5), 24.2 (C4), 15.7 (CH_3); MS *m/z* 396.1 (MH^+), 157.1; Anal. ($C_{19}H_{17}N_5O_3S$) C 57.66% (57.71%), H 4.36% (4.33%), N 17.76% (17.71%), S 7.83% (8.11%).

*6.1.25. 1-[5-Methyl-6-oxo-1-(thiophene-2-carbonyl)-4,5,6,7-tetrahydro-1H-pyrazolo[3,4-b]pyridin-3-yl]-3-p-tolyl-urea (**15b**, R_5 = p-MeC_6H_4)*

Mp 277–278 °C; IR (KBr) 3115, 1712, 1690, 1660, 1544 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 9.79 (s, 1H, NH), 9.47 (s, 1H, NH), 9.21 (s, 1H, NH), 8.32 (dd, J_1 = 3.9 Hz, J_2 = 1.5 Hz, 1H, thiophene), 8.19 (dd, J_1 = 5.1 Hz, J_2 = 1.5 Hz, 1H, thiophene), 7.37 (d, J = 8.4 Hz, 2H, H–Ph), 7.32 (m, 1H, thiophene), 7.13 (d, J = 8.4 Hz, 2H, H–Ph), 3.00 (dd, J_1 = 15.6 Hz, J_2 = 6.9 Hz, 1H, CH), 2.71 (m, 1H, CH), 2.44 (dd, J_1 = 11.4 Hz, J_2 = 15.6 Hz, 1H, CH), 2.26 (s, 3H, CH_3Ph), 1.18 (d, J = 6.6 Hz, 3H, CH_3); ^{13}C NMR δ 171.8 (C=O), 158.9, 151.3, 147.9, 142.0 (C=O, C=O, C7a, C2′), 138.0, 137.8, 136.0 (*ipso*-Ph, C3′, C5′), 131.5, 131.5 (C3, *m*-Ph), 129.1, 127.6 (*p*-Ph, C4′), 119.1 (*o*-Ph), 94.3 (C3a), 34.8 (C5), 24.1 (C4), 20.4 (CH_3Ph), 15.7 (CH_3); MS *m/z* 410.3 (MH^+); Anal. ($C_{20}H_{19}N_5O_3S$) C 58.61% (58.67%), H 4.68% (4.68%), N 16.89% (17.10%), S 7.69% (7.83%).

*6.1.26. 1-(4-Chlorophenyl)-3-[5-methyl-6-oxo-1-(thiophene-2-carbonyl)-4,5,6,7-tetrahydro-1H-pyrazolo[3,4-b]pyridin-3-yl]-urea (**15c**, R_5 = p-ClC_6H_4)*

Mp 274–276 °C; IR (KBr) 3259, 1713, 1688, 1658, 1546 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 9.80 (s, 1H, NH), 9.54 (s, 1H, NH), 9.34 (s, 1H, NH), 8.33 (dd, J_1 = 4.2 Hz, J_2 = 1.5 Hz, 1H, thiophene), 8.20 (dd, J_1 = 5.1 Hz, J_2 = 1.5 Hz, 1H, thiophene), 7.53 (d, J = 9.0 Hz, 2H, H–Ph), 7.37 (d, J = 9.0 Hz, 2H, H–Ph), 7.32 (m, 1H, thiophene), 2.98 (dd, J_1 = 15.9 Hz, J_2 = 7.2 Hz, 1H, CH), 2.71 (m, 1H, CH), 2.44 (dd, J_1 = 11.4 Hz, J_2 = 15.9 Hz, 1H, CH), 1.18 (d, J = 6.9 Hz, 3H, CH_3); ^{13}C NMR δ 171.8 (C=O), 158.9, 151.3, 147.7, 142.0 (C=O, C=O, C7a, C2′), 138.1, 137.8, 137.7 (*ipso*-Ph, C3′, C5′), 131.5 (C3), 128.6 (*m*-Ph), 127.7, 126.1 (*p*-Ph, C4′), 120.4 (*o*-Ph), 94.5 (C3a), 34.8 (C5), 24.3 (C4), 15.7 (CH_3); MS *m/z* 430.0 (MH^+), 346.3, 157.0; Anal. ($C_{19}H_{16}ClN_5O_3S$) C 52.92% (53.09%), H 3.64% (3.75%), N 16.04% (16.29%), S 7.43% (7.46%).

*6.1.27. 1-[5-Methyl-6-oxo-1-(thiophene-2-carbonyl)-4,5,6,7-tetrahydro-1H-pyrazolo[3,4-b]pyridin-3-yl]-3-(4-trifluoromethylphenyl)-urea (**15d**, R_5 = p-$CF_3C_6H_4$)*

Mp 279-280 °C; IR (KBr) 3263, 1718, 1692, 1666, 1544 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 9.81 (s, 1H, NH), 9.59 (s, 1H, NH), 9.53 (s, 1H, NH), 8.35 (dd, J_1 = 3.6 Hz, J_2 = 1.2 Hz, 1H, thiophene), 8.21 (dd, J_1 = 4.8 Hz, J_2 = 1.2 Hz, 1H, thiophene), 7.69 (d, J = 2.4 Hz, 4H, H–Ph), 7.33 (m, 1H, thiophene), 2.99 (dd, J_1 = 15.9 Hz, J_2 = 7.2 Hz, 1H, CH), 2.72 (m, 1H, CH), 2.46 (dd, J_1 = 11.4 Hz, J_2 = 15.9 Hz, 1H, CH), 1.18 (d, J = 6.6 Hz, 3H, CH_3); ^{13}C NMR δ 171.8 (C=O), 159.0, 151.2, 147.4, 142.5, 142.1 (C=O, C=O, C7a, C2′, *ipso*-Ph), 138.2, 137.9 (C3′, C5′), 131.6 (C3), 127.7 (C4′), 126.0 (q, J = 3.5 Hz, *m*-Ph), 124.1 (q, J = 284.5 Hz, CF_3), 122.4 (q, J = 31.9 Hz, *p*-Ph), 118.5 (*o*-Ph), 94.7 (C3a), 34.8 (C5), 24.4 (C4), 15.7 (CH_3); MS *m/z* 464.4 (MH^+), 279.2, 157.1; Anal. ($C_{20}H_{16}F_3N_5O_3S$) C 51.75% (51.83%), H 3.48% (3.48%), N 15.22% (15.11%), S 7.01% (6.92%).

*6.1.28. 1-[5-Methyl-6-oxo-1-(thiophene-2-carbonyl)-4,5,6,7-tetrahydro-1H-pyrazolo[3,4-b]pyridin-3-yl]-3-thiophen-2-yl-urea (**15e**, R_5 = 2-thienyl)*

Mp 279–280 °C; IR (KBr) 3392, 3239, 1706, 1658, 1544, 1515 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 10,18 (s, 1H, NH), 9.84 (s, 1H, NH), 9.78 (s, 1H, NH), 8.33 (dd, J_1 = 3.9 Hz, J_2 = 1.5 Hz, 1H, thiophene–CO), 8.25 (dd, J_1 = 5.1 Hz, J_2 = 1.2 Hz, 1H, thiophene–CO), 7.35 (m, 1H, thiophene–NH), 6.97 (dd, J_1 = 5.7 Hz, J_2 = 1.2 Hz, 1H, thiophene–NH), 6.86 (dd, J_1 = 3.6 Hz, J_2 = 5.4 Hz, 1H, thiophene–NH), 6.67 (dd, J_1 = 3.9 Hz, J_2 = 1.2 Hz, 1H, thiophene–NH), 2.98 (dd, J_1 = 15.9 Hz, J_2 = 6.9 Hz, 1H, CH), 2.72 (m, 1H, CH), 2.43 (dd, J_1 = 11.4 Hz, J_2 = 15.9 Hz, 1H, CH), 1.18 (d, J = 6.6 Hz, 3H, CH_3); ^{13}C NMR δ 171.8 (C=O), 150.5, 147.6, 142.2 (C=O, C=O, C7a, C2′), 139.9, 138.0, 137.9 (C2″, C3′, C5′), 131.3 (C3), 127.7, 124.2 (C4′, C4″), 116.8 (C5″), 110.6 (C3″), 94.1 (C3a), 34.8 (C5), 23.8 (C4), 15.7 (CH_3); MS *m/z* 402.1 (MH^+); Anal. ($C_{17}H_{15}N_5O_3S_2$) C 50.73% (50.86%), H 3.68% (3.77%), N 17.53% (17.44%), S 15.62% (15.97%).

6.2. Pharmacology. Determination of the affinity of test compounds for α_1-GABA$_A$ receptor

Male Sprague-Dawley rats weighing 200–250 g at the time of experiment were used. After decapitation of the animal, the cerebellum (tissue that mostly contains α_1-GABA$_A$ receptor) were removed. Membranes were prepared according to the method by Lameh et al [23] and Noguchi et al [24] with slight modifications. Once the tissues weighed, they were suspended in 50 mM Tris–HCl (pH 7.4), 1:40 (w/v), homogenized and then centrifuged at 20000 g for 10 min at 7 °C. The resulting pellet was resuspended under the same conditions and centrifuged twice more. The pellet was finally resuspended on a minimum volume and kept at –80 °C overnight. On the next day, the process was repeated until the final pellet was resuspended at a ratio of 1:10 (w/v). Affinity was determined by competitive tests using radiolabeled flumazenil as ligand. The tests were performed according to the methods described by Arbilla et al [25] and Wu et al [26] using 96-well microtiter plates. The membranes containing the study receptors, flumazenil (radiolabeling at a final concentration of 1 nM) and ascending concentrations of test compounds (in a total volume of 230 µl in 50 mM [ph 7.4] Tris·HCl buffer)

were incubated. Simultaneously, the membranes were only incubated with the radiolabeled flumazenil (total binding, 100%) and in the presence of an elevated concentration of unradiolabeled flumazenil (non-specific binding, % estimation of radiolabeled ligand). The reactions started on adding the radiolabeled ligand followed by incubation for 60 min at 4 °C. At the end of the incubation period, 200 μl of reaction were transferred to a multiscreen plate (Millipore) and filtered using a vacuum manifold and then washed three times with cold test buffer. The multiscreen plates were equipped with a GF/B filter that retained the membranes containing the receptors and the radiolabeled ligand, which had been bound to the receptors. After washing, the plates were left till dry. Once dried, scintillation liquid was added and left under stirring overnight. The next day the plates were counted using a Perkin–Elmer Microbeta scintillation counter. For analysis of the results the percentage of specific binding for every concentration of test compound was calculated as follows: % specific binding = $(X–N/T–N) \times 100$ where,

- X: amount of bound ligand for every concentration of compound.
- T: total binding, maximum amount bound to the radiolabeled ligand.
- N: non-specific binding, amount of radiolabeled ligand bound in a non-specific way irrespective of the receptor used.

References

[1] E.N. Peterson, Benzodiazepine receptor pharmacology: new vistas, Drugs Future 12 (1987) 1043–1053.

[2] E.A. Barnard, P. Skolnick, R.W. Olsen, H. Mohler, W. Sieghart, G. Biggio, C. Braestrup, A.N. Bateson, S.Z. Langer, International Union of Pharmacology. XV. Subtypes of γ-aminobutyric acidA receptors: classification on the basis of subunit structure and receptor function, Pharmacol. Rev. 50 (1998) 291–313.

[3] F. McGillion, Aspects of the current status of benzodiazepine therapy, Drugs Today 25 (1987) 27–31.

[4] H.C. Rosenberg, E.I. Tietz, T.H. Chiu, Tolerance to anticonvulsant effects of diazepam, clonazepam, and clobazam in amygdala-kindled rats, Epylepsia 30 (1989) 276–285.

[5] C. Kilpatrick, R. Bury, R. Fullinfaw, R. Moulds, Clobazam in the treatment of epilepsy, Clin. Exp. Neurol. 23 (1987) 139–144.

[6] (a) George, P.; Rossey, G.; Depoortere, H.; Mompon, B.; Allen, J.; Wick, A. Imidazopyridines: towards novel hypnotic and anxiolytic drugs. Il Farmaco 1991, 46, 277–288. (b) Browne, L.J.; Shaw, K.J. New anxiolytics, Annu. Rep. Med. Chem. 26 (1991) 1–10.

[7] G. Trapani, M. Franco, A. Latrofa, G. Genchi, G. Liso, Synthesis and benzodiazepine receptor binding of some 4H-pyrimido[2,1-b]benzothiazol-4-ones, Eur. J. Med. Chem. 27 (1992) 39–44.

[8] G. Trapani, A. Carotti, M. Franco, A. Latrofa, G. Genchi, G. Liso, Structure-affinity relationships of some alkoxycarbonyl-2H- or -4H-pyrimido[2,1-b]benzothiazol-2- or 4-one benzodiazepine receptor ligands, Eur. J. Med. Chem. 28 (1993) 13–21.

[9] V. Colotta, L. Cecchi, F. Melani, D. Catarzi, G. Filacchioni, C. Martini, G. Giannaccini, M. Tonelli, A. Lucacchini, Tricyclic heteroaromatic systems. Synthesis and benzodiazepine binding activity of 1-substituted-3-methyl- and 3-phenylpyrazolo[4,5-c]quinolin-4-ones, Drug Des. Discov. 8 (1992) 325–339.

[10] R.I. Fryer, Compr. Med. Chem. 3 (1990) 539–566 (Pergamon, New York).

[11] M.G. Wong, Structure activity relationships of anxiolytics, Drug Deliv. 3 (1988) 15–33.

[12] P.A. Borea, G. Gili, V. Bertolasi, V. Ferretti, Stereochemical features controlling binding and intrinsic activity properties of benzodiazepine-receptor ligands, Mol. Pharmacol. 31 (1987) 334–344.

[13] P.W. Codding, A.K.S. Muir, Molecular structure of Ro15-1788 and a model for the binding of benzodiazepine receptor ligands. Structural identification of common features in antagonists, Mol. Pharmacol. 28 (1985) 178–184.

[14] H.O. Villar, M.F. Davies, G.H. Loew, P.A. Maguire, Molecular models for recognition and activation at the benzodiazepine receptor: a review, Life Sci. 48 (1991) 593–602.

[15] W. Zhang, K.F. Koehler, B. Harris, P. Skolnick, J.M. Cook, Synthesis of benzo-fused benzodiazepines employed as probes of the agonist pharmacophore of benzodiazepine receptors, J. Med. Chem. 37 (1994) 745–757.

[16] P. Victory, J.M. Jover, R. Nomen, Las 3-ciano-2-metoxi-2,3-dehidropiperidin-6-onas como productos de partida en síntesis. Síntesis de heterociclos. I, Afinidad 38 (1981) 497–500.

[17] a) Victory, P.; Diago, J. Contribución a la síntesis de glutarimidas. I. Afinidad 1978, 35, 154–158. b) Victory, P.; Diago, J. Contribución a la síntesis de glutarimidas. II. Afinidad 1978, 35, 161–165. c) Victory, P.; Jover, J. M.; Sempere, J. Contribución a la síntesis de glutarimidas. III. Afinidad 1981, 38, 491–495. d) Victory, P.; Borrell, J. I. 6-Alkoxy-5-cyano-3,4-dihydro-2-pyridones as starting materials for the synthesis of heterocycles. In: Menon J. (Ed.), Trends in Heterocyclic Chemistry, Vol. 3, Council of Scientific Research Integration, Trivandrum, India, **1993**, pp. 235–247.

[18] CombiChem Module, Accord for Excel. Accelrys (http://www.accelrys.com).

[19] C.A. Lipinski, F. Lombardo, B.W. Dominy, P.J. Feeney, Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings, Adv. Drug Deliv. Rev. 23 (1997) 3–25.

[20] Volsurf, Molecular Discovery (http://www.moldiscovery.com).

[21] Catalyst, Accelrys (http://www.accelrys.com).

[22] a) Kawashima, K.; Hosoi, K.; Naruke, T.; Shiba, T.; Kitamura, M.; Watabe, T. Aldehyde oxidase-dependent marked species difference in hepatic metabolism of the sedative-hypnotic, zaleplon, between monkeys and rats. Drug Metab. Dispos., 1999, 27, 422–428.b) Horstkotter, Ch.; Schepmann, D.; Blaschke, G. Separation and identification of zaleplon metabolites in human urine using capillary electrophoresis with laser-induced fluorescence detection and liquid chromatography–mass spectrometry, J. Chromatogr. A. 1014 (2003) 71–81.

[23] J. Lameh, P. Wang, D. Meredith, S.L. Shafer, G.H. Loew, Characterization of benzodiazepine receptors in the cerebellum, Prog. Neuropsychopharmacol. Biol. Psychiatry 24 (2000) 979–991.

[24] H. Noguchi, K. Kitazumi, M. Mori, T. Shiba, Binding and neuropharmacological profile of zaleplon, a novel nonbenzodiazepine sedative/hypnotic, Eur. J. Pharmacol. 434 (2002) 21–28.

[25] S. Arbilla, J. Allen, A. Wick, S.Z. Langer, High affinity [3H]zolpidem binding in the rat brain: an imidazopyridine with agonist properties at central benzodiazepine receptors, Eur. J. Pharmacol. 130 (1986) 257–263.

[26] Y. Wu, H.C. Rosenberg, T.H. Chiu, Rapid down-regulation of [3H]zolpidem binding to rat brain benzodiazepine receptors during flurazepam treatment, Eur. J. Pharmacol. 278 (1995) 125–132.