European Journal of Medicinal Chemistry 46 (2011) 4062–4070

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Synthesis of novel 1,2,5-trisubstituted benzimidazoles as potential antitumor agents

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ARTICLE INFO

Article history:
Received 17 March 2011
Received in revised form
28 May 2011
Accepted 6 June 2011
Available online 14 June 2011

Keywords:
Nucleophilic aromatic substitution
Pyrazole derivatives
Trisubstituted benzimidazoles
Solvent free reaction conditions
Antitumor agents

ABSTRACT

Novel methyl 1-(5-*tert*-butyl-1*H*-pyrazol-3-yl)-2-(aryl)-1*H*-benzo[*d*]imidazole-5-carboxylates **11** were synthesized by following a four-step strategy involving a nucleophilic aromatic displacement (S_NAr) and a solvent free approach as key steps for the formation of the desired products. Structure of intermediates and products were confirmed by X-ray diffraction as well as the tautomeric rearrangement suffered by the pyrazole moiety during the curse of the final cyclization process. Several of the obtained compounds were screened by the US National Cancer Institute (NCI) for their ability to inhibit 60 different human tumor cell lines. Products **11b** and **11n** exhibited the highest activity against a range of cancer cell lines with remarkable values in panels of *Non-Small Cell Lung Cancer*, *Melanoma* and *Leukemia*, with GI_{50} range of 1.15–7.33 μM and 0.167–7.59 μM, respectively, and suitable LC_{50} with values greater than 100 μM.

1. Introduction

The imidazole core is a common moiety in a large number of natural products and pharmacologically active compounds [1]. Particularly, the benzimidazoles may be considered as structural isosters of nucleotides owing to the fused heterocyclic nuclei in their structures, interact easily with biopolymers and possess potential activity for chemotherapeutic applications [2]. The benzimidazole moiety itself is a crucial pharmacophore in modern drug discovery [3], owing to the broad-spectrum of important biological and pharmacological properties exhibited for a large number of benzimidazole-containing compounds, such as anti-ulcerative and antihypertensive [4a], antifungal [4b], antitumor [4c,d], topoisomerase inhibitors [4e], antiallergic [5a] and selective neuropeptide YY1 receptor antagonists [5b]. In addition, some benzimidazole derivatives are effective against several human viruses such as cytomegalovirus (HCMV) [6a], HIV [6b], RNA [6c] and herpes (HSV-1) [6d].

Fig. 1 shows some benzimidazole derivatives with biological and practical activities. For example Omeprazole **1** is used for the treatment of dyspepsia, peptic ulcer disease (PUD) and gastro-esophageal reflux disease [7], the Lansoprazole **2** is used as a proton-pump inhibitor (PPI) which prevents the stomach from producing gastric acid [8], the Albendazole **3** is used for the treatment of a variety of worm infestations [9] and compound **4** recently synthesized showed an interestingly activity against several human tumor cell lines [10].

The demonstrated practical applications of the benzimidazole derivatives have prompted extensive studies for their synthesis. Mainly, there are two general approaches for the synthesis of benzimidazole derivatives. The first method involves the heating of *o*-phenylenediamines and carboxylic acids [11] or their derivatives (nitriles, chlorides, or orthoesters) [12] in the presence of a mineral acid. The second one starts also with *o*-phenylenediamines but using an aldehyde instead of the carboxylic acid. This reaction proceeds in acidic media but in addition, an oxidative reagent is required [13]. More recently, alternative Lewis acid-based catalysts and environmentally friendly reaction conditions have been used for the synthesis of a large number of benzimidazole derivatives [14]. The pyrazole core is frequently found being part of the structure of compounds of biological interest [15]. Some pyrazole-oxime ethers were prepared and examined as cytotoxic agents and particularly the activity of the 5-phenoxypyrazole derivative was comparable to doxorubicin [15c]. Similarly, a series of amino-pyrazoles were synthesized showing some of them significant anticonvulsant activity [15d].

We have recently reported a one-pot procedure mediated by Amberlyst®-15 for the synthesis of compound **4** (Fig. 1), and several

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doi:10.1016/j.ejmech.2011.06.006

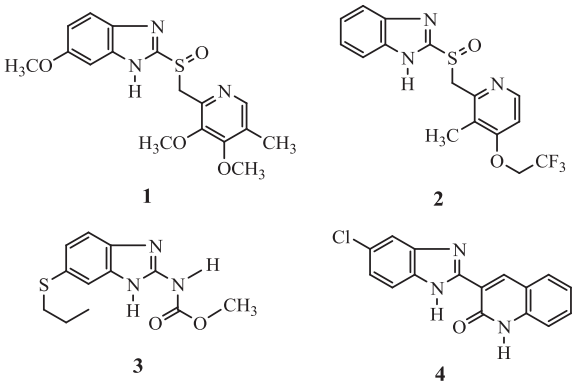


Fig. 1. Some benzimidazole derivatives of biological interest.

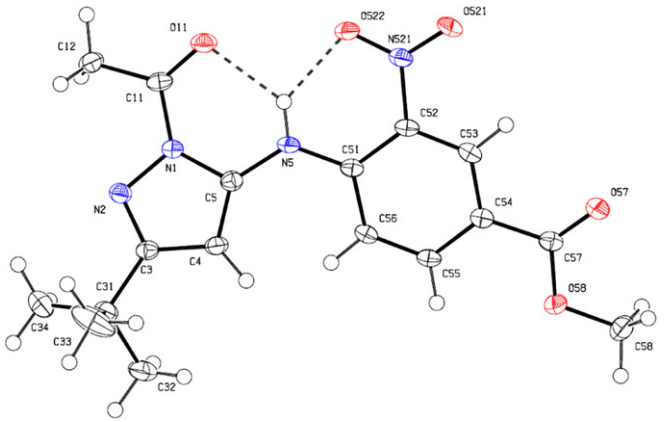


Fig. 2. ORTEP drawing of the structure of compound **8**-(*N*-acetyl) with 50% probability ellipsoids.

new benzimidazole analogs bearing the quinolin-2-one pharmacophore in position 2 to be studied for antitumor activity [10]. Continuing with our current studies on the synthesis of potential antitumor agents [10,15a,16], we felt that substituted benzimidazoles are worthy of further studies and therefore we planned the synthesis of the new trisubstituted benzimidazole derivatives **11**, bearing a pyrazole residue at N-1, with the aim that the presence of such substituent might improve the antitumor activity displayed by compound **4**.

2. Results and discussion

2.1. Chemistry

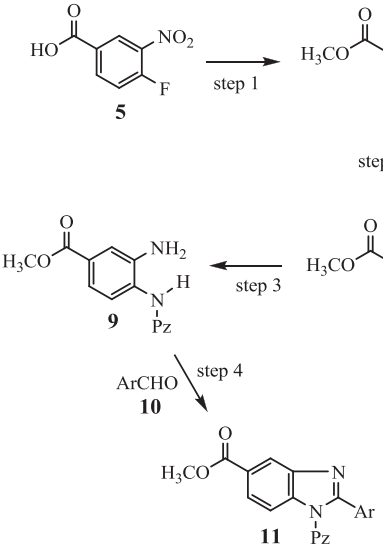
The design of our target compounds **11** implied that one of these three substituents were a pyrazole residue (Pz) owing to the aforementioned activity displayed by this moiety and aimed for a positive synergistic effect by the presence of both pyrazole and benzimidazole pharmacophores forming part of the same structure. The strategy used for the synthesis of the new benzimidazoles **11** is outlined in Scheme 1.

To start with, the methylation of the commercially available 4-fluoro-3-nitrobenzoic acid **5** by treatment with dry methanol in the presence of concentrated sulfuric acid afforded the corresponding methyl ester **6**. Formation of compound **6** was confirmed by the disappearing of the broad (CO_2H) IR band (present in compound **5** in the range of 3500-2500 cm^{-1}), and the appearing of a new singlet at 3.99 ppm (3H, OCH_3) in the ^1H NMR spectrum. This functional group interconversion was carried out to improve the solubility and handling of the starting material **5** in addition to setting the first substituent for the target products **11**.

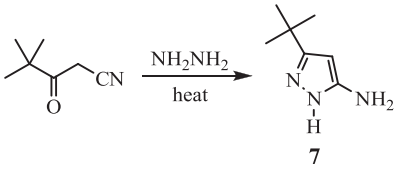
Treatment of the methyl ester **6** with the 5-amino-3-*tert*-butylpyrazol ($PzNH_2$) **7** in DMSO at room temperature afforded the corresponding amino-ester **8** *via* a nucleophilic aromatic displacement (S_NAr) of the fluorine atom. The presence of two N–H absorption bands in the IR spectrum at 3371 and 3316 cm^{-1} respectively, along with the full spectroscopic analysis confirmed the insertion of the aminopyrazole fragment. At this point, the second planed substituent for the target products **11** has been inserted. Additionally, the N-acetylation of compound **8** by treatment with acetic anhydride and growing single-crystals of this product from a solution in EtOH and the study by X-ray diffraction confirmed unambiguously the assigned structure for compound **8**, Fig. 2 [17].

Due to the starting pyrazole **7** was not commercially available it required being synthesized by a solvent-free heating of a mixture of 4,4-dimethyl-3-oxopentanonitrile and hydrazine hydrate as described in Scheme 2 [18].

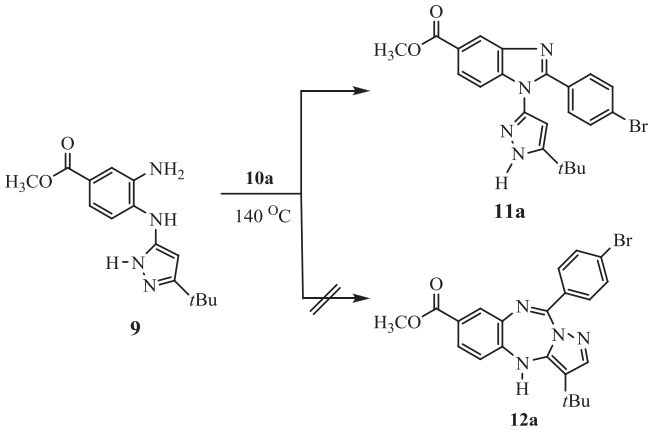
Subsequently, reduction of the nitro group performed by treatment of the amino-ester **8** with the Ni-Raney/hydrazine hydrate reagent led to the formation of the key diamine derivative **9**, Scheme 1. The disappearing of the two absorption nitro bands in the IR spectrum at 1541 and 1352 cm^{-1} along with the full spectroscopic analysis confirmed this process. To complete the synthesis of our target compounds **11**, a mixture of the diamine **9** (1 mmol) and 4-bromobenzaldehyde **10a** (1 mmol) was initially refluxed in ethanol. After 4 h of heating the desired product **11a** was obtained in only 40% yield. Pursuing for a yield improvement, the reaction was repeated by heating the starting materials for 1 h at 140 °C without solvent. The crude was purified by column



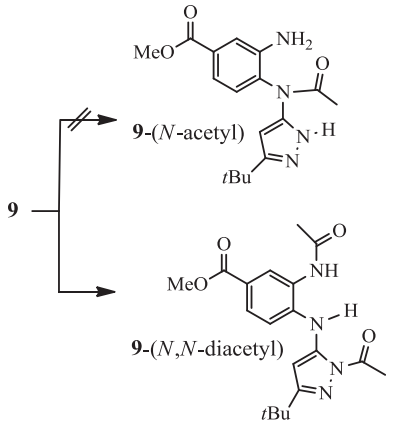
Scheme 1. Four-step synthesis of 1,2,5-trisubstituted benzimidazoles **11**.



Scheme 2. Synthesis of pyrazole **7**.



Scheme 3. Synthesis of benzimidazole **11a** from the reaction of diamine **9** and 4-bromobenzaldehyde **10a**.



Scheme 4. Obtention of the **9**-(*N*,*N*-diacetyl) derivative from treatment of diamine **9** with Ac_2O.

chromatography on silica gel using a mixture $CHCl_3/CH_3OH$ as eluent affording the desired compound **11a**. This apparently simple change of the reaction conditions improved the yield up to 92%.

The main spectroscopic feature of the structure of compound **11a** corresponded to the presence of only one N—H functionality with IR and ^1H NMR signals at 3374 cm^{-1} and 13.03 ppm, respectively. Although the presence of the free NH functionality in the pyrazolic moiety of the diamine **9** might suppose a possible competence to form the isomeric seven-membered diazepine ring **12a** as showed in Scheme 3, single-crystals were obtained from a solution in EtOH and the study by X-ray diffraction unambiguously confirmed the assigned structure for compound **11a**, Fig. 3 [19].

In an attempt to block the 1-NH functionality of diamine **9** to try to address the reaction selectively toward the formation of the triazepine system type **12**, diamine **9** was treated with acetic anhydride at reflux. Unfortunately the undesired **9**-(*N*,*N*-diacetyl) derivative was obtained as unique product but not the expected **9**-(*N*-acetyl) intermediate as shown in Scheme 4.

According to the crystalline structure withdrawn from X-ray diffraction and depicted in Fig. 3, it is remarkable that the pyrazole moiety of the imidazole **11a** suffered a tautomeric rearrangement during the course of the cyclization process. Comparing the position of the pyrazole NH functionality in **7** and the **8**-(*N*-acetyl) derivative with the structure of the Fig. 3, clearly it is observed that the NH shifted toward the adjacent nitrogen atom (N13) in the final product **11a**. A spatial PzNH/NH_2 correlation found in the NOESY spectrum of the diamine **9** also indicates that isomerization have not proceeded yet and confirms that the tautomerization proceeded during the cyclization toward product **11**, probably caused by the temperature and the acidic conditions. This kind of isomerization in pyrazoles has previously been reported [20]. Once established the best reaction conditions and the correct structure

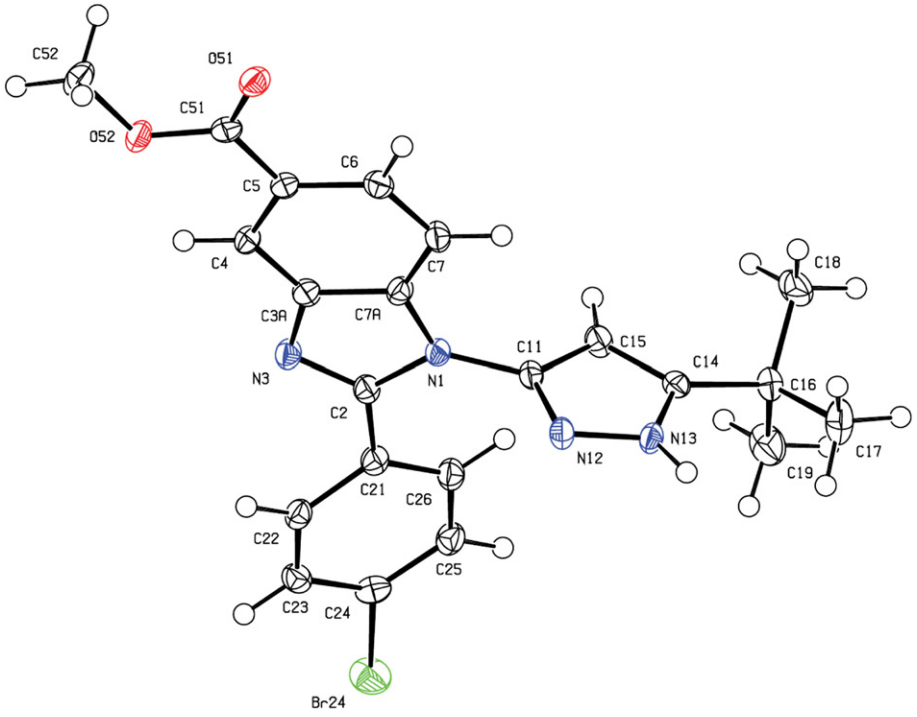


Fig. 3. ORTEP drawing of the structure **11a** with 50% probability ellipsoids.

Table 1
Analytical data for products **11a**–**n**.

Entry	Ar	Compounds **11**	
		Yield[a] (%)	Mp °C
a	4-BrC_6H_4	92	261–262
b	4-ClC_6H_4	94	248–249
c	4-$CH_3C_6H_4$	85	258–259
d	4-$CH_3OC_6H_4$	91	244–245
e	4-FC_6H_4	90	261–262
f	3,4,5-$(CH_3O)_3C_6H_2$	89	209–210
g	2-HOC_6H_4	81	224–225
h	2-HO–4-$OCH_3C_6H_3$	87	252–253
i	4-$(CH_3)_2NC_6H_4$	83	291–292
j	3,4-$(CH_3O)_2C_6H_3$	88	211–212
k	C_6H_5	89	257–258
l	4-$CF_3C_6H_4$	65	221–222
m	1,3-Benzodioxol-5-yl	64	254–255
n	2-Oxo-1,2-dihydroquinolin-3-yl	87	254–256

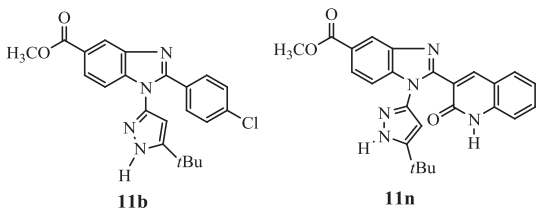
[a] Isolated yield.

for benzimidazole **11a**, this procedure was extended to other aldehydes **10b**–**n** to evaluate the generality of this methodology. In all cases reactions proceeded with the same behavior and yields in the range of 64–94%. Table 1 summarizes the main analytical data for products **11a**–**n**.

2.2. Anticancer activity

The two-stage screening process started with the evaluation of six of the obtained compounds (i.e. **9**, **9**-(diacetyl), **11b**, **11d**, **11h** and **11n**) selected by NCI, against 60 cell lines at a single dose of 1.0 μM. The output from the single dose screen was reported as a mean graph available for analysis by the COMPARE program [21]. The results of the primary assay suggested that compounds **11b** and **11n** (Fig. 4), were declared as active.

Subsequently, a secondary screening process was performed in order to determine the cytostatic activity of compounds **11b** and **11n** against the 60 cell panel representing leukemia, melanoma and cancers of the lung, colon, brain, ovary, breast, prostate and kidney. The testing results were expressed according to the following three parameters: GI_{50} which is the molar concentration of the compounds required to inhibit the growth of that cell lines by 50% (relative to untreated cells). TGI as the molar concentration that causes total growth inhibition, and LC_{50} which is a parameter of cytotoxicity and reflects the molar concentration needed to kill 50% of the cells [22]. Compounds **11b** and **11n** were evaluated at five concentration levels (100, 10, 1.0, 0.1 and 0.01 μM). The test consisted of a 48 h continuous drug exposure protocol using a sulforhodamide B (SRB) protein assay to estimate cell growth. Details of this evaluation method and the complementary information related to the activity pattern over all cell lines have been published [23]. Table 2 shows that compound **11b** displayed the most remarkable activity against 58 human tumor cell lines, being HOP-92 (*Non-Small Cell Lung Cancer*; GI_{50} = 1.15 μM; LC_{50} > 100 μM) and MDA-MB-435 (*Melanoma*; GI_{50} = 1.19 μM; LC_{50} = 0.505 μM) the



11b **11n**

Fig. 4. Active compounds **11b** and **11n** after screening performed by the NCI.

Table 2
In vitro testing expressed as growth inhibition of cancer cells by compounds **11b** and **11n**.

Panel/cell line	Compounds[a]					
	11b			**11n**		
	GI_{50}[b] (μM)	TGI (μM)	LC_{50}[c] (μM)	GI_{50}[b] (μM)	TGI (μM)	LC_{50}[c] (μM)
Leukemia						
RPMI-8226	3.93	19	>100	0.167	0.987	>100
SR	3.23	>100	>100	10.0	>100	>100
CCRF-CEM	4.91	>100	>100	0.585	>100	>100
HL-60(TB)	4.03	11.2	>100	0.322	—	>100
K-562	4.20	>100	>100	1.73	>100	>100
MOLT-4	5.48	>100	>100	0.445	>100	>100
Non-small cell lung cancer						
EKVX	3.06	>100	>100	5.8	>100	>100
HOP-62	3.41	21	>100	0.996	>100	>100
NCI-H226	4.09	44	>100	7.00	>100	>100
NCI-H23	3.42	36	>100	2.78	>100	>100
NCI-H460	2.95	8.81	>100	0.630	>100	>100
NCI-H522	3.21	20.3	>100	0.928	>100	>100
HOP-92	1.15	8.02	>100	—	—	—
NCI-H322M	10.4	86.4	>100	0.929	>100	>100
A549/ATCC	4.03	94.5	>100	>100	>100	>100
Colon						
COLO 205	2.29	6.04	0.463	—	>100	>100
HCT-116	3.47	12.3	0.563	1.74	>100	>100
HCT-15	4.36	>100	>100	—	>100	>100
HT29	3.65	15.4	>100	1.34	>100	>100
KM12	2.12	6.07	>100	1.29	—	>100
SW-620	3.24	>100	>100	—	>100	>100
HCC-2998	3.90	19.8	>100	—	—	>100
CNS						
SF-295	2.48	8.98	>100	1.23	25.5	>100
SF-539	2.54	8.95	0.521	4.12	>100	>100
U251	4.13	22.7	>100	0.652	>100	>100
SF-268	3.29	17.8	>100	2.13	91.9	>100
SNB-19	4.31	>100	>100	7.59	>100	>100
SNB-75	2.14	12.6	>100	5.42	41.5	>100
Melanoma						
LOX IMVI	3.70	14.5	0.429	1.24	—	>100
MALME-3M	3.25	15.6	>100	2.63	>100	>100
MDA-MB-435	1.19	5	0.505	—	>100	>100
SK-MEL-5	2.02	5.1	0.186	2.30	>100	>100
UACC-257	4.97	22.9	0.967	—	>100	>100
UACC-62	2.01	7.78	0.463	—	>100	>100
SK-MEL-2	3.89	18.1	>100	2.25	>100	>100
M14	2.90	8.22	0.758	—	>100	>100
SK-MEL-28	3.38	15.5	0.552	—	>100	>100
Ovarian						
IGROV1	3.64	25	>100	0.965	>100	>100
OVCAR-3	2.35	6.13	0.915	6.27	>100	>100
OVCAR-4	3.40	>100	>100	—	>100	>100
NCI/ADR-RES	2.72	7.35	>100	5.29	>100	>100
OVCAR-8	7.33	58.2	>100	1.79	>100	>100
OVCAR-5	12.9	60.1	>100	—	>100	>100
SK-OV-3	3.71	31	>100	28.5	>100	>100
Renal						
A498	2.26	9.76	0.858	13.6	76.9	>100
ACHN	3.00	9.34	>100	>100	>100	>100
CAKI-1	2.25	12.8	>100	0.476	90.5	>100
RXF 393	1.57	5.77	0.569	2.16	8.86	>100
SN12C	4.34	27.7	>100	5.61	>100	>100
TK-10	6.94	>100	>100	16.9	55.8	>100
UO-31	3.35	44.1	>100	0.533	>100	>100
786-0	3.62	32	>100	5.81	>100	>100
Prostate						
DU-145	3.70	25.8	>100	2.37	>100	>100

(*continued on next page*)

Table 2 (*continued*)

Panel/cell line	Compounds[a]					
	11b			**11n**		
	GI_{50}[b] (μM)	TGI (μM)	LC_{50}[c] (μM)	GI_{50}[b] (μM)	TGI (μM)	LC_{50}[c] (μM)
PC-3	2.93	15.7	>100	2.27	>100	>100
Breast						
MCF7	2.79	12.3	>100	>100	>100	>100
MDA-MB-231/ATCC	4.62	27	>100	4.13	>100	>100
BT-549	3.35	11.2	0.595	4.88	66.2	>100
T-47D	3.64	>100	>100	>100	>100	>100
MDA-MB-468	2.40	7.98	>100	20.5	>100	>100
HS 578T	1.72	7.86	>100	0.619	34.1	>100

[a] Data obtained from NCI's in vitro disease-oriented human tumor cell lines screen [22].

[b] GI_{50} was the drug concentration resulting in a 50% reduction in the net protein increase (as measured by SRB staining) in control cells during the drug incubation. Determined at five concentration levels (100, 10, 1.0, 0.1 and 0.01 μM).

most sensitive strains. Likewise, compound **11n** displayed the most remarkable activity against 40 human tumor cell lines, thirteen of them with GI_{50} values under 1.0 μM. The HL-60(TB) and RPMI-8226 of the *Leukemia* panel (GI_{50} = 0.167 and GI_{50} = 0.322 μM, respectively) were the most sensitive strains with LC_{50} values greater than 100 μM. Products **11b** and **11n** showed significant activity against other cell lines from the different panels of cancer types, with GI_{50} range of 1.15–7.33 μM and 0.167–7.59 μM, respectively.

In general, compound **11n** showed higher activity than compound **11b** for most cell lines. Moreover, compound **11n** has a framework closer to the active imidazole **4** (Fig. 1), than compound **11b**. These findings are probably related to the presence of the 2-oxo-1,2-dihydroquinolin-3-yl moiety in the structure of both compounds **11n** and **4**, which have recently been recognized as convenient pharmacophore for development of new compounds with antitumor properties [24]. The cytotoxic effects associated with compounds **11b** and **11n** were measured as LC_{50} displaying values greater than 100 μM for most of the cell lines evaluated, indicating a low toxicity of these compounds for normal human cell lines, as required for development of potential antitumor agents.

3. Conclusion

The design of a four-step synthesis of novel 1,2,5-trisubstituted benzimidazoles **11**, and studies on their cytotoxic activity have been performed. The X-ray diffraction analysis confirmed not only the formation of the expected products **11** but also revealed that the pyrazole moiety suffered a tautomeric rearrangement during the course of the cyclization process. The antitumor assays showed that, among the six compounds selected and evaluated by the NCI, benzimidazoles **11b** and **11n** exhibited the highest activity against a range of cancer cell lines with remarkable values in panels of *Non-Small Cell Lung Cancer*, *Melanoma* and *Leukemia*. The relative activity values for the different evaluated strains also showed that imidazole **11n** presented a higher activity than imidazole **11b**, probably due to the presence of the 2-oxo-1,2-dihydroquinolin-3-yl pharmacophore in its structure. Studies conducting to evaluate the presence of this pharmacophore on the activity of some other of our structures are currently in progress by the NCI.

4. Experimental

Melting points were determined on a Büchi melting point apparatus and are uncorrected. IR spectra were recorded on a Shimadzu FTIR 8400 spectrophotometer in KBr disks. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance 400 spectrophotometer operating at 400 MHz and 100 MHz respectively, using $CDCl_3$ and DMSO-d_6 as solvents and tetramethylsilane as internal standard. Mass spectra were run on an SHIMADZU-GCMS 2010-DI-2010 spectrometer (equipped with a direct inlet probe) operating at 70 eV. Microanalyses were performed on an Agilent elemental analyzer and the values are within ±0.4% of the theoretical values. Silica gel aluminum plates (Merck 60 F_{254}) were used for analytical TLC. The starting aldehydes **10a**–**n** and 4-fluoro-3-nitrobenzoic acid **5** were purchased from Aldrich, Fluka and Acros (analytical reagent grades) and were used without further purification.

4.1. Synthesis of methyl 4-fluoro-3-nitrobenzoate (*6*)

A mixture of acid **5** (1.85 g, 1 mol), methanol (15 mL) and 96% H_2SO_4 (1 mL) was heated under reflux with stirring for 2 h. The reaction progress was monitored by TLC and after complete disappearance of the starting material **5** the solvent was concentrated under vacuum to one half of the original volume. The solid formed was collected by filtration and washed with methanol (2 × 0.5 mL), to afford compound **6**. White solid, 88% yield; m.p: 97–98 °C. FTIR ν (cm^{-1}): 3065, 2961, 1716 (C=O), 1619 (C=C), 1541 and 1352 (NO_2), 1126 (C–O); ^{1}H NMR ($CDCl_3$) δ 3.99 (s, 3H, OCH_3), 7.40 (bt, 1H, J = 8.7 Hz), 8.34 (m, 1H), 8.75 (bd, 1H, J = 7.0 Hz) ppm; ^{13}C NMR ($CDCl_3$) δ 52.9 (OCH_3), 118.7 (d, J = 21.3 Hz), 127.2 (d, J = 4.1 Hz, Cq), 127.8 (d, J = 1.3 Hz), 136.5, 136.6 (Cq), 158.1 (d, J = 269.9 Hz, Cq), 164.1 (C=O) ppm. MS (EI) m/z (%): 199 (100, M^+), 184 (42), 168 (14, M − OCH_3). Anal. Calcd. for $C_8H_6FNO_4$: C, 48.25; H, 3.04; N, 7.03. Found: C, 48.50; H, 3.17; N, 6.99.

4.2. Synthesis of methyl 4-[(3-tert-butyl-1H-pyrazol-5-yl)amino]-3-nitrobenzoate (*8*)

A mixture of 3-*tert*-butyl-1*H*-pyrazol-5-amine **7** (0.139 g, 1 mmol), methyl ester **6** (0.199 g, 1 mmol) and DMSO (2 mL) was stirred at ambient temperature during 2 h. After complete disappearance of the starting materials (monitored by TLC), the solid formed was collected by filtration and washed with MeOH/H_2O (1/2) (3 × 2 mL), to afford compound **8**. Orange solid, 76% yield; m.p: 241–242 °C. FTIR ν (cm^{-1}): 3371 (NH), 3316 (NH), 3117, 2955, 1705 (C=O), 1625 (C=N), 1532 and 1323 (NO_2), 1122 (C–O); ^{1}H NMR ($CDCl_3$) δ 1.39 (s, 9H, *t*Bu), 3.95 (s, 3H, OCH_3), 5.98 (s, 1H, Pz–CH), 8.06 (d, 1H, J = 9.1 Hz), 8.13 (dd, 1H, J = 9.1 Hz, J = 1.8 Hz), 8.94 (d, 1H, J = 1.8 Hz), 10.07 (bs, 1H, Pz–NH) ppm, the aniline NH is missing; ^{13}C NMR ($CDCl_3$) δ 30.0 (*t*Bu), 31.2 (Cq, *t*Bu), 52.2 (OCH_3), 94.9 (Pz–CH), 117.2, 119.5 (Cq), 128.9, 132.1 (Cq), 136.2, 144.3 (Cq), 148.0 (Cq), 154.6 (Cq), 165.5 (C=O) ppm. MS (EI) m/z (%): 318 (100, M^+), 287 (15, M − CH_3). Anal. Calcd. for $C_{15}H_{18}N_4O_4$: C, 56.60; H, 5.70; N, 17.60. Found: C, 56.54; H, 5.83; N, 17.46.

4.3. Synthesis of methyl 3-amino-4-[(3-tert-butyl-1H-pyrazol-5-yl) amino]benzoate (*9*)

A mixture of methyl ester **8** (0.477 g, 1.5 mmol), hydrazine hydrate (0.225 g, 4.5 mmol), Ni-Raney (0.070 g) and methanol (15 mL) was heated to reflux with stirring for 1 h. Then the hot mixture was filtered and the solvent was concentrated under vacuum to one quarter of the original volume. After cooling the solid formed was collected by filtration and recrystallized from methanol, to afford compound **9**. White solid, 94% yield; m.p: 190–191 °C. FTIR ν (cm^{-1}): 3366 broad (NH_2), 3293 (NH), 2963, 1702 (C=O), 1601 (C=N), 1574, 1494, 1307, 1211 (C–O), 763; ^{1}H NMR (DMSO-d_6) δ 1.25 (s, 9H, *t*Bu), 3.73 (s, 3H, OCH_3), 4.97 (s, 2H, NH_2), 5.70 (s, 1H, Pz–CH), 7.18 (d, 1H, J = 8.2 Hz), 7.26 (s, 1H), 7.44 (s, 1H, NH), 7.65 (bs, 1H), 11.81 (s, 1H, Pz–NH) ppm; ^{13}C NMR (DMSO-d_6) δ 30.40 (*t*Bu), 31.07 (Cq,

*t*Bu), 51.66 (OCH_3), 91.61 (Pz–CH), 113.6, 115.5, 119.6 (Cq), 120.0, 135.5 (Cq), 135.9 (Cq), 150.5 (Cq), 153.1 (Cq), 167.1 (C=O) ppm. MS (EI) *m*/*z* (%): 288 (100, M^+), 272 (21), 257 (15), 246 (17), 215 (26), 190 (17), 121 (15), 107 (15). Anal. Calcd. for $C_{15}H_{20}N_4O_2$: C, 62.48; H, 6.99; N, 19.43. Found: C, 62.33; H, 7.07; N, 19.60.

4.4. Obtention of methyl 3-acetamido-4-(1-acetyl-3-tert-butyl-1H-pyrazol-5-ylamino)benzoate (**9**-N,N-diacetyl)

A mixture of the diamino-ester **9** (0.288 g, 1 mmol) and acetic anhydride (0.5 mL) was heated at 45 °C during 5 min. After complete disappearance of the starting material **9** (monitored by TLC), the excess of acetic anhydride was removed under vacuum. The solid formed was collected by filtration and washed with cold ethanol (2 × 0.5 mL), to afford the 9-(*N,N-diacetyl*) derivative as white solid, 91% yield; m.p: 208–210 °C. FTIR ν (cm^{-1}): 3493 (AcNH), 3237 (NH), 2981, 2951, 1711 (C=O), 1671 br (2× C=O), 1602 (C=N, C=C), 1561, 1532, 1290, 1286, 1223 (C–O), 762; ^{1}H NMR ($CDCl_3$) δ 1.30 (s, 9H, *t*Bu), 2.27 (s, 3H, O=CCH_3), 2.68 (s, 3H, O=CCH_3), 3.90 (s, 3H, OCH_3), 5.82 (s, 1H, Pz–CH), 7.15 (bs, 1H, NH), 7.39 (d, 1H, J = 8.4 Hz), 7.94 (d, 1H, J = 8.4 Hz), 8.15 (s, 1H), 9.62 (s, 1H, Ac–NH) ppm; ^{13}C NMR ($CDCl_3$) δ 23.4 [(C=O)**CH_3**], 23.6 [(C=O)**CH_3**], 29.5 (*t*Bu), 32.7 (Cq, *t*Bu), 52.0 (OCH_3), 88.1 (Pz–CH), 117.5, 124.3 (Cq), 126.6 (Cq), 128.1, 129.0, 139.1 (Cq), 146.0 (Cq), 165.7 (Cq), 166.2 (C=O), 169.4 (C=O), 175.0 (C=O) ppm. MS (EI) *m*/*z* (%): 372 (56, M^+), 341 (6), 330 (63), 313 (39), 312 (40), 288 (100), 272 (26), 246 (20), 190 (63). Anal. Calcd. for $C_{19}H_{24}N_4O_4$: C, 61.28; H, 6.50; N, 15.04. Found: C, 61.50; H, 6.21; N, 14.93.

4.5. General procedure for the synthesis of imidazoles (**11**)

A mixture of the diamino-ester **9** (0.288 g, 1 mmol) and the corresponding arylaldehyde **10** (1.2 mmol) was heated at 140 °C in absence of solvent for 1–2 h. After complete disappearance of the starting material **9** (monitored by TLC), the mixture was cooled at ambient temperature and the residue was purified from column chromatography on silica gel by using a $CHCl_3/CH_3OH$ (40/1) mixture as eluent, to afford pure compounds **11** after evaporation of the solvent.

4.5.1. Methyl 1-(5-tert-butyl-1H-pyrazol-3-yl)-2-(4-bromophenyl)-1H-benzo[d]imidazole-5-carboxylate (**11a**)

White solid. FTIR ν (cm^{-1}): 3374 (NH), 3315 (broad), 2964, 1697 (C=O), 1650 (C=C), 1561 (C=N), 1545, 1278, 763; ^{1}H NMR (DMSO-d_6) δ 1.30 (s, 9H, *t*Bu), 3.87 (s, 3H, OCH_3), 6.18 (s, 1H, Pz–CH), 7.39 (d, 1H, 7-H, J = 8.5 Hz), 7.53 (d, 2H, Ar–H, J = 8.5 Hz), 7.61 (d, 2H, Ar–H, J = 8.3 Hz), 7.92 (d, 1H, 6-H, J = 8.5 Hz), 8.33 (s, 1H, 4-H), 13.03 (s, 1H, NH) ppm; ^{13}C NMR (DMSO-d_6) δ 30.2 (*t*Bu), 31.5 (Cq, *t*Bu), 52.5 (OCH_3), 98.5 (Pz–CH), 111.8 (C-7), 121.4 (C-4), 124.3 (Cq), 125.1 (C-6), 125.2 (Cq), 129.0 (Cq), 131.2, 131.8, 140.5 (Cq), 142.3 (Cq), 143.6 (Cq), 153.4 (Cq), 155.6 (Cq), 167.0 (C=O) ppm. MS (EI) *m*/*z* (%): 454/452 (100/98, M^+), 439/437 (24/24, M – CH_3), 423/421 (25/25, M – OCH_3), 397 (11), 395 (14). Anal. Calcd. for $C_{22}H_{21}BrN_4O_2$: C, 58.29; H, 4.67; N, 12.36. Found: C, 58.20; H, 4.75; N, 12.29.

4.5.2. Methyl 1-(5-tert-butyl-1H-pyrazol-3-yl)-2-(4-chlorophenyl)-1H-benzo[d]imidazole-5-carboxylate (**11b**)

White solid. FTIR ν (cm^{-1}): 3292 (NH), 2960, 2925, 2850, 1715 (C=O), 1614 (C=C), 1565 (C=N), 1470, 1292, 1227, 1091, 830; ^{1}H NMR (DMSO-d_6) δ 1.31 (s, 9H, *t*Bu), 3.88 (s, 3H, OCH_3), 6.19 (s, 1H, Pz–CH), 7.40 (d, 1H, 7-H, J = 8.5 Hz), 7.48 (d, 2H, Ar–H, J = 8.0 Hz), 7.61 (d, 2H, Ar–H, J = 8.0 Hz), 7.92 (d, 1H, 6-H, J = 8.5 Hz), 8.34 (s, 1H, 4-H), 13.02 (s, 1H, NH) ppm; ^{13}C NMR (DMSO-d_6) δ 30.3 (*t*Bu), 31.5 (Cq, *t*Bu), 52.5 (OCH_3), 98.5 (Pz–CH), 111.8 (C-7), 121.4 (C-4), 125.1 (Cq), 125.1 (C-6), 128.7 (Cq), 128.9, 131.0, 135.5 (Cq), 140.6 (Cq), 142.4 (Cq), 143.7 (Cq), 153.3 (Cq), 155.6 (Cq), 167.0 (C=O) ppm. MS (EI) *m*/*z* (%): 410/408 (37/100, M^+), 397/395 (5/16), 393 (31), 379/377 (14/35), 353/351 (5/14), 299 (20). Anal. Calcd. for $C_{22}H_{21}ClN_4O_2$: C, 64.62; H, 5.18; N, 13.70. Found: C, 64.70; H, 5.00; N, 13.59.

4.5.3. Methyl 1-(5-tert-butyl-1H-pyrazol-3-yl)-2-(4-methylphenyl)-1H-benzo[d]imidazole-5-carboxylate (**11c**)

Pale yellow solid. FTIR ν (cm^{-1}): 3394 (NH), 3143, 2965, 2221, 2861, 1679 (C=O), 1614 (C=C), 1562 (C=N), 1508, 1472, 1440, 1307, 1227, 820, 747; ^{1}H NMR (DMSO-d_6) δ 1.30 (s, 9H, *t*Bu), 2.32 (s, 3H, CH_3), 3.88 (s, 3H, OCH_3), 6.16 (s, 1H, Pz–CH), 7.20 (d, 2H, Ar–H, J = 7.2 Hz), 7.36 (d, 1H, 7-H, J = 8.0 Hz), 7.51 (d, 2H, Ar–H, J = 7.2 Hz), 7.90 (d, 1H, 6-H, J = 8.4 Hz), 8.31 (s, 1H, 4-H), 12.98 (s, 1H, NH) ppm; ^{13}C NMR (DMSO-d_6) δ 21.3 (CH_3), 30.3 (*t*Bu), 31.4 (Cq, *t*Bu), 52.5 (OCH_3), 98.5 (Pz–CH), 111.6 (C-7), 121.2 (C-4), 124.8 (C-6 and Cq), 127.0 (Cq), 129.2, 129.3, 140.4 (Cq), 140.7 (Cq), 142.4 (Cq), 144.0 (Cq), 154.5 (Cq), 155.4 (Cq), 167.0 (C=O) ppm. MS (EI) *m*/*z* (%): 388 (100, M^+), 387 (48), 373 (37, M – CH_3), 357 (19, M – OCH_3), 331 (14, M – *t*Bu), 271 (5), 171 (6). Anal. Calcd. for $C_{23}H_{24}N_4O_2$: C, 71.11; H, 6.23; N, 14.42. Found: C, 71.00; H, 6.40; N, 14.31.

4.5.4. Methyl 1-(5-tert-butyl-1H-pyrazol-3-yl)-2-(4-methoxyphenyl)-1H-benzo[d]imidazole-5-carboxylate (**11d**)

White solid. FTIR ν (cm^{-1}): 3302 (NH), 3122, 3079, 2963, 2837, 1713 (C=O), 1614 (C=C), 1562 (C=N), 1509, 1481, 1434, 1362, 1290, 1257, 1232, 1182, 837; ^{1}H NMR ($CDCl_3$) δ 1.29 (s, 9H, *t*Bu), 3.82 (s, 3H, OCH_3), 3.96 (s, 3H, OCH_3), 5.88 (s, 1H, Pz–CH), 6.87 (d, 2H, Ar–H, J = 9.0 Hz), 7.46 (d, 1H, 7-H, J = 8.0 Hz), 7.64 (d, 2H, Ar–H, J = 9.0 Hz), 8.02 (d, 1H, 6-H, J = 8.0 Hz), 8.54 (s, 1H, 4-H), 11.05 (bs, 1H, NH) ppm; ^{13}C NMR ($CDCl_3$) δ 29.9 (*t*Bu), 31.3 (Cq, *t*Bu), 52.0 (OCH_3), 55.3 (OCH_3), 98.6 (Pz–CH), 111.0 (C-7), 113.7, 121.6 (C-4), 121.9 (Cq), 124.8 (C-6), 125.1 (Cq), 130.9, 140.3 (Cq), 142.5 (Cq), 145.4 (Cq), 154.3 (Cq), 155.4 (Cq), 161.0 (Cq), 167.8 (C=O) ppm. MS (EI) *m*/*z* (%): 404 (100, M^+), 403 (27), 389 (27, M – CH_3), 373 (11, M – OCH_3), 347 (13, M – *t*Bu), 179 (7). Anal. Calcd. for $C_{23}H_{24}N_4O_3$: C, 68.30; H, 5.98; N, 13.85. Found: C, 68.54; H, 5.80; N, 13.92.

4.5.5. Methyl 1-(5-tert-butyl-1H-pyrazol-3-yl)-2-(4-fluorophenyl)-1H-benzo[d]imidazole-5-carboxylate (**11e**)

White solid. FTIR ν (cm^{-1}): 3208 (NH), 3121, 2966, 1722 (C=O), 1616 (C=C), 1569 (C=N), 1511, 1484, 1436, 1363, 1287, 1230, 1083, 991, 844; ^{1}H NMR ($CDCl_3$) δ 1.30 (s, 9H, *t*Bu), 3.97 (s, 3H, OCH_3), 5.87 (s, 1H, Pz–CH), 7.05 (bt, 2H, Ar–H, J = 8.8 Hz), 7.49 (d, 1H, 7-H, J = 8.4 Hz), 7.69 (dd, 2H, Ar–H, J = 8.0 Hz, J = 5.2 Hz), 8.06 (d, 1H, 6-H, J = 8.8 Hz), 8.55 (s, 1H, 4-H), 11.08 (bs, 1H, NH) ppm; ^{13}C NMR ($CDCl_3$) δ 29.9 (*t*Bu), 31.3 (Cq, *t*Bu), 52.1 (OCH_3), 98.5 (Pz–CH), 111.1 (C-7), 115.4 (d, J = 22 Hz) 122.0 (C-4), 125.2 (C-6), 125.3 (Cq), 125.7 (d, Cq, J = 3 Hz), 131.4 (d, J = 8 Hz), 140.1 (Cq), 142.4 (Cq), 145.2 (Cq), 153.3 (Cq), 155.5 (Cq), 164.0 (d, Cq, J = 249 Hz), 167.7 (C=O) ppm. MS (EI) *m*/*z* (%): 392 (100, M^+), 391 (32), 377 (26, M – CH_3), 361 (33, M – OCH_3), 335 (10, M – *t*Bu), 173 (5). Anal. Calcd. for $C_{22}H_{21}FN_4O_2$: C, 67.33; H, 5.39; N, 14.28. Found: C, 67.54; H, 5.43; N, 14.09.

4.5.6. Methyl 1-(5-tert-butyl-1H-pyrazol-3-yl)-2-(3,4,5-trimethoxyphenyl)-1H-benzo[d]imidazole-5-carboxylate (**11f**)

White solid. FTIR ν (cm^{-1}): 3310 (NH), 3194, 3135, 3106, 2963, 2864, 1716 (C=O), 1616 (C=C), 1568 (C=N), 1504, 1433, 1359, 1302, 1211, 1088, 771, 694; ^{1}H NMR ($CDCl_3$) δ 1.30 (s, 9H, *t*Bu), 3.74 (s, 6H, OCH_3), 3.87 (s, 3H, OCH_3), 3.97 (s, 3H, OCH_3), 6.00 (s, 1H, Pz–CH), 6.97 (s, 2H, Ar–H), 7.40 (d, 1H, 7-H, J = 8.0 Hz), 8.03 (dd, 1H, 6-H, J = 8.4 Hz, J = 1.6 Hz), 8.55 (bd, 1H, 4-H, J = 1.2 Hz), 11.10 (bs, 1H, NH) ppm; ^{13}C NMR ($CDCl_3$) δ 30.0 (*t*Bu), 31.3 (Cq, *t*Bu), 52.1 (OCH_3), 56.0 (2× OCH_3), 60.9 (OCH_3), 98.8 (Pz–CH), 106.7, 110.8 (C-7), 121.9 (C-4), 124.6 (Cq), 125.1 (C-6), 125.3 (Cq), 139.7 (Cq), 140.4 (Cq), 142.3 (Cq), 145.5 (Cq), 152.9 (Cq), 154.0 (Cq), 155.6 (Cq), 167.7 (C=O) ppm. MS (EI) *m*/*z* (%): 464 (100, M^+),

463 (15), 449 (32, M − CH_3), 433 (9, M − OCH_3), 407 (13, M − *t*Bu), 403 (8), 389 (15), 333 (8), 181 (11). Anal. Calcd. for $C_{25}H_{28}N_4O_5$: C, 64.64; H, 6.08; N, 12.06. Found: C, 64.72; H, 5.96; N, 12.20.

4.5.7. Methyl 1-(5-tert-butyl-1H-pyrazol-3-yl)-2-(2-hydroxyphenyl)-1H-benzo[d]imidazole-5-carboxylate (**11g**)

Pale yellow solid. FTIR ν (cm^{-1}): 3324 (broad, OH and NH), 3138, 2969, 1697 (C═O), 1624 (C═C), 1568 (C═N), 1485, 1317, 1301, 1231, 804, 759; ^{1}H NMR ($CDCl_3$) δ 1.37 (s, 9H, *t*Bu), 4.00 (s, 3H, OCH_3), 6.14 (s, 1H, Pz−CH), 6.65 (td, 1H, Ar−H, J = 8.4 Hz, J = 1.2 Hz), 7.04 (dd, 1H, 7-H, J = 8.0 Hz, J = 1.6 Hz), 7.13 (dd, 1H, Ar−H, J = 8.4 Hz, J = 1.2 Hz), 7.30 (bt, 2H, Ar−H, J = 8.4 Hz), 8.05 (dd, 1H, 6-H, J = 8.0 Hz, J = 1.6 Hz), 8.51 (bd, 1H, 4-H, J = 0.8 Hz), 12.48 (s, 1H, NH), (signal for OH is missing) ppm; ^{13}C NMR ($CDCl_3$) δ 29.9 (*t*Bu), 31.5 (Cq, *t*Bu), 52.2 (OCH_3), 99.3 (Pz−CH), 110.7 (C-7), 112.0 (Cq), 118.0, 118.2, 120.9 (C-4), 125.5 (C-6), 125.7 (Cq), 127.2, 132.1, 139.4 (Cq), 139.8 (Cq), 145.2 (Cq), 152.9 (Cq), 156.2 (Cq), 159.6 (Cq), 167.5 (C═O) ppm. MS (EI) *m*/*z* (%): 390 (100, M^+), 389 (41), 375 (24, M − CH_3), 373 (48, M − OH), 359 (6, M − OCH_3), 333 (6, M − *t*Bu), 317 (15), 299 (23), 172 (54). Anal. Calcd. for $C_{22}H_{22}N_4O_3$: C, 67.68; H, 5.68; N, 14.35. Found: C, 67.75; H, 5.54; N, 14.49.

4.5.8. Methyl 1-(5-tert-butyl-1H-pyrazol-3-yl)-2-(3-hydroxy-4-methoxyphenyl)-1H-benzo[d]imidazole-5-carboxylate (**11h**)

Pale yellow solid. FTIR ν (cm^{-1}): 3301 (broad OH and NH), 2963, 1712 (C═O), 1608 (C═C), 1502 (C═N), 1435, 1302, 1287, 1221, 1127, 760; ^{1}H NMR (DMSO-d_6) δ 1.33 (s, 9H, *t*Bu), 3.63 (s, 3H, OCH_3), 3.89 (s, 3H, OCH_3), 6.24 (s, 1H, Pz−CH), 6.82 (d, 1H, Ar−H, J = 8.0 Hz), 7.10 (d, 1H, Ar−H, J = 2.0 Hz), 7.25 (dd, 1H, Ar−H, J = 8.4 Hz, J = 2.0 Hz), 7.30 (d, 1H, 7-H, J = 8.4 Hz), 7.89 (dd, 1H, 6-H, J = 8.4 Hz, J = 1.6 Hz), 8.30 (bd, 1H, 4-H, J = 1.6 Hz), 9.61 (s, 1H, OH), 13.08 (s, 1H, NH) ppm; ^{13}C NMR (DMSO-d_6) δ 30.3 (*t*Bu), 31.5 (Cq, *t*Bu), 52.5 (OCH_3), 55.6 (OCH_3), 98.8 (Pz−CH), 111.3 (C-7), 112.8, 115.9, 120.5 (Cq), 120.8 (C-4), 123.0, 124.5 (C-6), 124.7 (Cq), 141.0 (Cq), 142.4 (Cq), 144.2 (Cq), 147.5 (Cq), 149.1 (Cq), 154.6 (Cq), 155.5 (Cq), 167.1 (C═O) ppm. MS (EI) *m*/*z* (%): 420 (0.53, M^+), 405 (0.12, M − CH_3), 389 (0.1, M − OCH_3), 363 (0.11, M − *t*Bu), 288 (0.15), 32 (28), 28 (100). Anal. Calcd. for $C_{23}H_{24}N_4O_4$: C, 65.70; H, 5.75; N, 13.33. Found: C, 65.64; H, 5.60; N, 13.49.

4.5.9. Methyl 1-(5-tert-butyl-1H-pyrazol-3-yl)-2-(4-(dimethylamino) phenyl)-1H-benzo[d]imidazole-5-carboxylate (**11i**)

White solid. FTIR ν (cm^{-1}): 3131 (NH), 3100, 2964, 2922, 2851, 1711 (C═O), 1609 (C═C), 1567 (C═N), 1513, 1483, 1433, 1291, 1235, 1203, 826; ^{1}H NMR ($CDCl_3$) δ 1.30 (s, 9H, *t*Bu), 2.99 [s, 6H, $N(CH_3)_2$], 3.96 (s, 3H, OCH_3), 5.94 (s, 1H, Pz−CH), 6.61 (d, 2H, Ar−H, J = 9.2 Hz), 7.37 (d, 1H, 7-H, J = 8.4 Hz), 7.58 (d, 2H, Ar−H, J = 9.2 Hz), 7.96 (dd, 1H, 6-H, J = 8.4 Hz, J = 1.6 Hz), 8.50 (d, 1H, 4-H, J = 1.2 Hz), (signal for PzNH is missing) ppm; ^{13}C NMR ($CDCl_3$) δ 30.0 (*t*Bu), 31.3 (Cq, *t*Bu), 40.1 [$N(CH_3)_2$], 52.0 (OCH_3), 98.7 (Pz−CH), 110.6, 111.2 (C-7), 116.5 (Cq), 121.1 (C-4), 124.3 (C-6), 124.7 (Cq), 130.3, 140.6 (Cq), 142.6 (Cq), 145.3 (Cq), 151.3 (Cq), 155.1 (Cq), 155.4 (Cq), 167.9 (C═O) ppm. MS (EI) *m*/*z* (%): 417 (100, M^+), 416 (22), 403 (15), 402 (22, M − CH_3), 386 (6, M − OCH_3), 360 (13, M − *t*Bu). Anal. Calcd. for $C_{24}H_{27}N_5O_2$: C, 69.04; H, 6.52; N, 16.77. Found: C, 68.94; H, 6.65; N, 16.69.

4.5.10. Methyl 1-(5-tert-butyl-1H-pyrazol-3-yl)-2-(3,4-dimethoxy-phenyl)-1H-benzo[d]imidazole-5-carboxylate (**11j**)

White solid. FTIR ν (cm^{-1}): 3195 (NH), 3137, 2965, 2850, 1716 (C═O), 1650, 1617 (C═C), 1566 (C═N), 1504, 1474, 1439, 1359, 1305, 1218, 1089, 765, 695; ^{1}H NMR ($CDCl_3$) δ 1.32 (s, 9H, *t*Bu), 3.79 (s, 3H, OCH_3), 3.91 (s, 3H, OCH_3), 3.97 (s, 3H, OCH_3), 5.96 (s, 1H, Pz−CH), 6.83 (d, 1H, Ar−H, J = 8.4 Hz), 7.28 (bd, 1H, Ar−H, J = 2.0 Hz), 7.30 (dd, 1H, Ar−H, J = 8.4 Hz, J = 2.0 Hz), 7.42 (d, 1H, 7-H, J = 8.4 Hz), 8.03 (dd, 1H, 6-H, J = 8.4 Hz, J = 1.6 Hz), 8.55 (bd, 1H, 4-H, J = 0.8 Hz), 11.26 (bs, 1H, NH) ppm; ^{13}C NMR ($CDCl_3$) δ 30.0 (*t*Bu), 31.3 (Cq, *t*Bu), 52.1 (OCH_3), 55.7 (OCH_3), 55.9 (OCH_3), 98.7 (Pz−CH), 110.6 (C-7), 110.8 (Cq), 112.1, 121.6 (C-4), 122.0 (Cq), 122.6, 124.8, 125.1 (C-6), 140.4 (Cq), 142.4 (Cq), 145.5 (Cq), 148.5 (Cq), 150.6 (Cq), 154.2 (Cq), 155.5 (Cq), 167.7 (C═O) ppm. MS (EI) *m*/*z* (%): 434 (100) [M^+], 419 (24) [M − CH_3], 403 (25) [M − OCH_3], 299 (2) [M − 135]. Anal. Calcd. for $C_{24}H_{26}N_4O_4$: C, 66.34; H, 6.03; N, 12.89. Found: C, 66.50; H, 6.10; N, 12.73.

4.5.11. Methyl 1-(5-tert-butyl-1H-pyrazol-3-yl)-2-phenyl-1H-benzo [d]imidazole-5-carboxylate (**11k**)

Pale yellow solid. FTIR ν (cm^{-1}): 3190 (NH), 3135, 3105, 2963, 2865, 1715 (C═O), 1646, 1616 (C═C), 1568 (C═N), 1503, 1476, 1434, 1359, 1299, 1251, 1211, 1088, 772, 695; ^{1}H NMR ($CDCl_3$) δ 1.35 (s, 9H, *t*Bu), 3.99 (s, 3H, OCH_3), 5.88 (s, 1H, Pz−CH), 7.36−7.46 (m, 3H, Ar−H), 7.52 (d, 1H, 7-H, J = 8.5 Hz), 7.72 (d, 2H, Ar−H, J = 8.5 Hz), 8.06 (dd, 1H, 6-H, J = 8.5 Hz, J = 1.6 Hz), 8.59 (bd, 1H, 4-H, J = 1.0 Hz), 10.24 (bs, 1H, NH) ppm; ^{13}C NMR ($CDCl_3$) δ 29.9 (*t*Bu), 31.3 (Cq, *t*Bu), 51.9 (OCH_3), 98.6 (Pz−CH), 111.1 (C-7), 122.1 (C-4), 125.0 (C-6), 125.3 (Cq), 128.1, 129.4, 129.9, 140.1 (Cq), 142.7 (Cq), 145.7 (Cq), 150.4 (Cq), 154.3 (Cq), 155.1 (Cq), 167.6 (C═O) ppm. MS (EI) *m*/*z* (%): 374 (100, M^+), 373 (54), 359 (36, M − CH_3), 343 (27, M − OCH_3), 317 (19, M − *t*Bu), 179 (13), 164 (80), 136 (24). Anal. Calcd. for $C_{22}H_{22}N_4O_2$: C, 70.57; H, 5.92; N, 14.96. Found: C, 70.54; H, 6.00; N, 15.07.

4.5.12. Methyl 1-(5-tert-butyl-1H-pyrazol-3-yl)-2-(4-(trifluoro-methyl)phenyl)-1H-benzo[d]imidazole-5-carboxylate (**11l**)

White solid. FTIR ν (cm^{-1}): 3284 (NH), 3135, 2970, 1692 (C═O), 1615 (C═C), 1564 (C═N), 1511, 1434, 1321, 1163, 1127, 848, 753; ^{1}H NMR ($CDCl_3$) δ 1.32 (s, 9H, *t*Bu), 3.99 (s, 3H, OCH_3), 5.94 (s, 1H, Pz−CH), 7.50 (d, 1H, 7-H, J = 8.6 Hz), 7.62 (d, 2H, Ar−H, J = 8.3 Hz), 7.85 (d, 2H, Ar−H, J = 8.3 Hz), 8.10 (dd, 1H, 6-H, J = 8.5 Hz, J = 1.2 Hz), 8.60 (bd, 1H, 4-H, J = 0.8 Hz), 11.17 (bs, 1H, NH) ppm; ^{13}C NMR ($CDCl_3$) δ 29.9 (*t*Bu), 31.4 (Cq, *t*Bu), 52.2 (OCH_3), 98.5 (Pz−CH), 111.3 (C-7), 122.3 (C-4), 125.1 (q, CH, J = 4 Hz), 125.6 (C-6), 128.9 (2× CH and 1Cq), 129.0 (q, CF_3, J = 209 Hz), 131.9 (q, Cq−F, J = 38 Hz), 133.1 (Cq), 140.2 (Cq), 142.4 (Cq), 144.8 (Cq), 152.6 (Cq), 155.8 (Cq), 167.6 (C═O) ppm. MS (EI) *m*/*z* (%): 442 (100, M^+), 441 (51), 427 (25, M − CH_3), 411 (50, M − OCH_3), 385 (9, M − *t*Bu), 198 (19), 188 (64), 171 (20), 156 (37), 129 (28). Anal. Calcd. for $C_{23}H_{21}F_3N_4O_2$: C, 62.44; H, 4.78; N, 12.66. Found: C, 62.53; H, 4.64; N, 12.69.

4.5.13. Methyl 2-(benzo[d] [1,3]dioxol-5-yl)-1-(5-tert-butyl-1H-pyrazol-3-yl)-1H-benzo[d]imidazole-5-carboxylate (**11m**)

Pale yellow solid. FTIR ν (cm^{-1}): 3316 (NH), 2961, 1725 (C═O), 1618 (C═C), 1566 (C═N), 1507, 1473, 1437, 1300, 1241, 1029; ^{1}H NMR ($CDCl_3$) δ 1.29 (s, 9H, *t*Bu), 3.97 (s, 3H, OCH_3), 5.91 (s, 1H, Pz−CH), 5.99 (s, 2H, OCH_2O), 6.78 (d, 1H, Ar−H, J = 8.4 Hz), 7.19 (bd, 1H, Ar−H, J = 1.6 Hz), 7.21 (dd, 1H, Ar−H, J = 8.4 Hz, J = 1.6 Hz), 7.46 (d, 1H, 7-H, J = 8.4 Hz), 8.03 (dd, 1H, 6-H, J = 8.4 Hz, J = 1.6 Hz), (bd, 1H, 4-H, J = 1.2 Hz), 11.54 (bs, 1H, NH) ppm; ^{13}C NMR ($CDCl_3$) δ 8.53 (*t*Bu), 31.3 (Cq, *t*Bu), 52.2 (OCH_3), 98.6 (Pz−CH), 101.5 (OCH_2O), 108.2, 109.6, 111.1(C-7), 121.7 (C-4), 123.2 (Cq), 124.1, 124.9 (C-6), 125.1 (Cq), 140.3 (Cq), 142.3 (Cq), 145.0 (Cq), 147.6 (Cq), 149.2 (Cq), 154.0 (Cq), 155.6 (Cq), 167.8 (C═O) ppm. MS (EI) *m*/*z* (%): 418 (100, M^+), 417 (41), 403 (42, M − CH_3), 387 (21, M − OCH_3), 361 (22, M − *t*Bu), 194 (22), 179 (27). Anal. Calcd. for $C_{23}H_{22}N_4O_4$: C, 66.02; H, 5.30; N, 13.39. Found: C, 65.94; H, 5.17; N, 13.50.

4.5.14. Methyl 1-(5-tert-butyl-1H-pyrazol-3-yl)-2-(2-oxo-1,2-dihydroquinolin-3-yl)-1H-benzo[d]imidazole-5-carboxylate (**11n**)

Pale yellow solid. FTIR ν (cm^{-1}): 3290 (broad 2× NH), 3173, 2963, 2866, 1721 (C═O), 1662 C═O, 1614 (C═C), 1568 (C═N), 1516, 1434, 1310, 1285, 1216, 1086, 751; ^{1}H NMR (DMSO-d_6) δ 1.26 (s, 9H, *t*Bu), 3.92 (s, 3H, OCH_3), 6.23 (s, 1H, Pz−CH), 7.25 (t, 1H, Ar−H, J = 8.5 Hz),

7.33 (d, 1H, Ar–H, $J = 8.4$ Hz), 7.58 (t, 1H, Ar–H, $J = 8.5$ Hz), 7.78 (d, 1H, 7-H, $J = 8.4$ Hz), 7.81 (d, 1H, Ar–H, $J = 8.8$ Hz), 8.00 (dd, 1H, 6-H, $J = 8.4$ Hz, $J = 1.6$ Hz), 8.36 (bd, 1H, 4-H, $J = 1.6$ Hz), 8.42 (s, 1H, Ar–H), 12.05 (s, 1H, NH), 12.72 (s, 1H, NH) ppm; ^{13}C NMR (DMSO-d_6) δ 30.3 (*t*Bu), 31.3 (Cq, *t*Bu), 52.6 (OCH_3), 95.6 (Pz–CH), 112.2 (C-7), 115.4, 119.0 (Cq), 121.4 (C-4), 122.7, 124.6 (Cq), 124.7 (Cq), 125.1 (C-6), 129.2, 132.1, 138.6 (Cq), 139.9 (Cq), 142.5 (Cq), 142.9, 144.4 (Cq), 151.7 (Cq), 154.3 (Cq), 160.1 (C=O), 167.1 (C=O) ppm. MS (EI) *m*/*z* (%): 441 (0.2, M^+), 440 (0.6), 424 (18), 423 (62), 422 (21), 409 (28), 408 (99), 407 (12), 381 (14), 368 (13), 309 (18), 189 (100), 169 (79), 155 (31), 141 (27). Anal. Calcd. for $C_{25}H_{23}N_5O_3$: C, 68.01; H, 5.25; N, 15.86. Found: C, 67.94; H, 5.37; N, 15.92.

Acknowledgments

The authors wish to credit The Developmental Therapeutics Program (DTP) of the National Cancer Institute of the United States (U.S.) for performing the screening of compounds. This work was supported financially by Colciencias, Universidad del Valle, Consejería de Innovación, Ciencia y Empresa (Junta de Andalucía, Spain) and The Universidad de Jaén. Authors thank "Servicios Técnicos de Investigación de la Universidad de Jaén" and the staff for data collection.

AppendixSupplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ejmech.2011.06.006.

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