 doi:10.20944/preprints202109.0188.v1

Article

Synthesis and *in silico* docking of new pyrazolo[*4,3-e*]pyrido[*1,2-a*]pyrimidine-based cytotoxic agents

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Abstract: To explore a new set of anticancer agents, a novel series of pyrazolo[*4,3-e*]pyrido[*1,2-a*]pyrimidine derivatives **7a-l** have been designed and synthesized *via* cyclocondensation reactions of pyrazolo-enaminone **5** with a series of arylidene malononitriles; compound **5** was obtained from 5-amino-4-cyanopyrazole (**3**). The structures of the target compounds **7a-l** were investigated by spectral techniques and elemental analysis (IR, UV-Vis, ^{1}H NMR, ^{13}C NMR and ESI-MS). All compounds were evaluated for their *in vitro* cytotoxicity employing a panel of different human tumor cell lines, A375, HT29, MCF7, A2780, FaDu as well as non-malignant NIH 3T3 and HEK293 cells. It has been found that the conjugate **7e** was the most active towards many cell lines with EC_{50} values ranging between 9.1 and 13.5 µM, respectively. Moreover, *in silico* docking studies of **7e** with six anticancer drug targets, i.e. DHFR, VEGFR2, HER-2/neu, hCA-IX, CDK6 and LOX also was performed, in order to gain some insights into their putative mode of binding interaction and to estimate the free binding energy of this bioactive molecule.

Keywords: pyrazolo-pyrido-pyrimidines; cytotoxicity; tumor cell lines; SAR; in silico docking.

1. Introduction

At present, cancer still constitutes a tremendous frightful disease for numerous patients worldwide. This disease is regarded as one of the foremost causes of mortality, thus endangering health and life of humans. Thereby, cancer cases surpassed 14 million persons in 2012, and it is expected to affect 22 million persons in 2030[1]. Regardless of their state of development and economic prosperity in all countries worldwide, the number of persons suffering from malignant melanoma, colon, breast, human ovarian carcinoma or pharynx carcinoma increased during the last decade. Thereby, besides surgery and radiotherapy, chemotherapy is the most currently used treatment to cure the various types of cancers[2]. Nowadays, chemotherapy applying a set of drugs acting by different mechanisms is one of the most promising techniques being applied to treat cancer.

Thus, in spite of all progress to treat and cure cancer patients there is still need to search for and to develop novel anticancer agents holding superior efficacy but giving rise to minimal side effect. Therefore, the identification of new chemical entities being more reliable and efficient remains a major challenge for medicinal chemists. As a consequence, the synthesis of small molecules still represents a potent and effective strategy to supply novel chemical entities for cancer therapy.

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In this context, heterocyclic compounds, especially those holding more than one non-carbon ring atom have played a key role in the field of medicinal chemistry [3, 4]. Furthermore, the previously reported results from literature reveal that the combination of two or more bioactive heterocyclic pharmacophores into the same molecule appears to be an effective tool for designing new chemical entities of improved activity. Thereby, pyrazole-fused pyrimidines constitute a promising versatile class of heterocyclic scaffolds having always attracted much interest from chemists owing to their outstanding potential, such as antitumor [5], anti-inflammatory [6], anticancer and anti-5-lipoxygenase [7], antibacterial [8], antitubercular [9], and especially cytotoxic activities (Fig. 1 (A, B, C and D)) [10-13].

Pyrido-pyrimidines have, for a long time, attracted the interests of both biological and synthetic researchers alike due to their various biological therapeutic properties such as anticonvulsant [14], antitumor [15], anti-proliferative [16], antifungal [17], adenosine kinase inhibitors [18] in addition to cytotoxic activities (Fig. 1 (E, F and G)) [19, 20].

A review of the literature has shown in several cases that amino and cyano functions can be involved in some interesting interactions towards target enzymes. Indeed, various constructed heterocyclic compounds gained much attention owing to their cytotoxic and anticancer activities especially in cases where the NH_2 group shows conjugation when it is tethered with a pyrimidine (Fig. 2 (A and B)) [21, 22] or the CN group linked in a conjugate way with a pyridine (Fig. 2 (C and D)) [23, 24].

In addition, a review of the literature allowed to notice that several strongly cytotoxic chemical scaffolds contain in their structures amine (Fig. 2 (E and F)) or cyano (Fig. 2 (G and H)) groups directly linked to a heterocycle [25, 26]. This particular structural linkage gave the kick-off to several research teams to design and synthesize amino-pyrimidine and cyano-pyridine scaffolds due to this notable anticancer activity against a wide range of cell lines.

Inspired by the above findings, and in continuation of our previous work on the synthesis of novel fused-pyrimidine scaffolds [27-29] we aimed to design and synthesize new pyrazolo[4,3-e]pyrido[1,2-a]pyrimidine derivatives. Hereby, we are reporting for the first time the cytotoxic activity of these newly synthesized compounds towards five human tumor cell lines, A375, HT29, MCF7, A2780, FaDu as well as non-malignant NIH 3T3 and HEK293 cells. Moreover, their structure-activity relationship (SAR) was investigated.

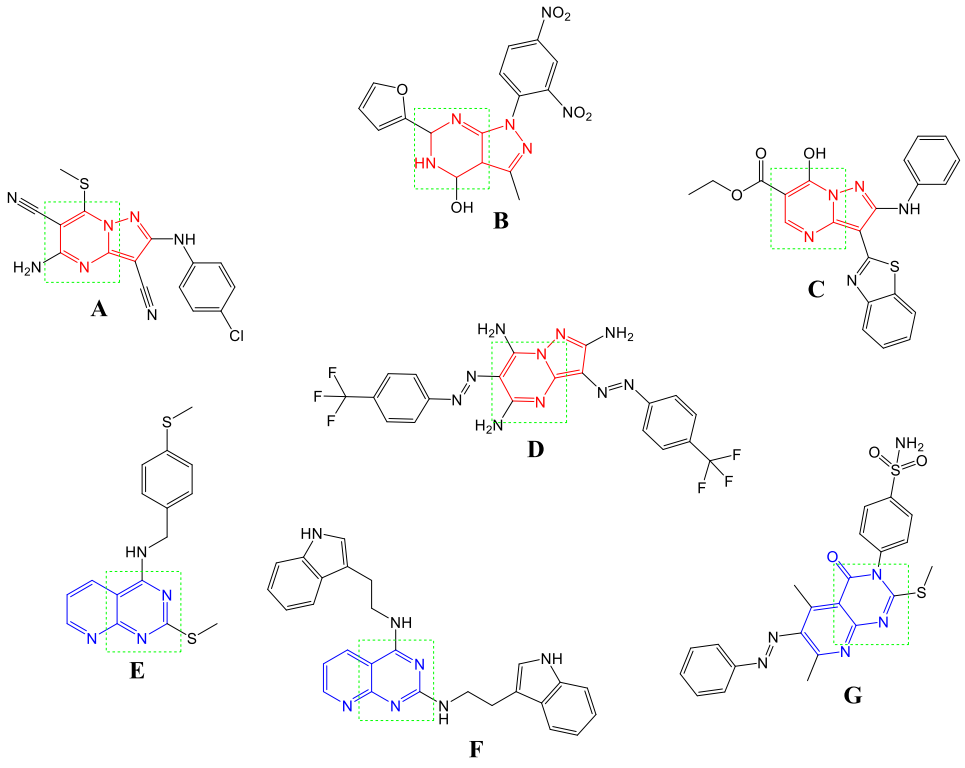

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Figure 1. Previously reported cytotoxic compounds: pyrazole-fused pyrimidines (A–D) and pyridine-fused pyrimidines (E–G).

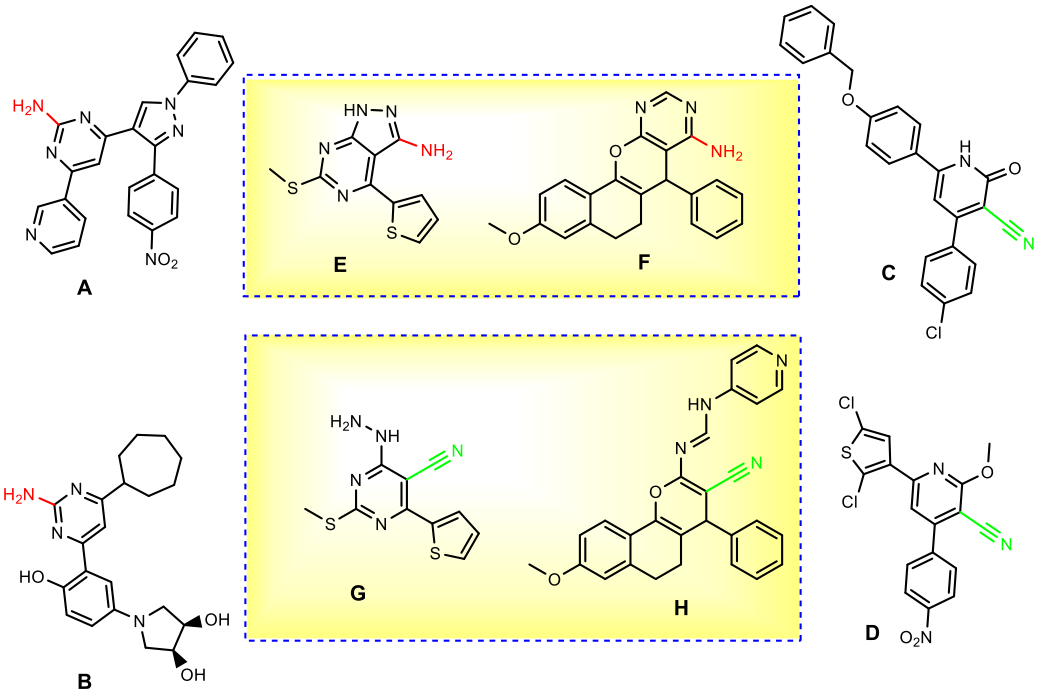

Figure 2. Previously reported cytotoxic compounds tethered with amino or cyano groups.

2. Results and Discussion

2.1. Chemistry

The multi-step synthesis of target compounds **7a-l** (Scheme 1) started from the bi-nucleophilic precursor **3**; the latter was prepared according to a previously published literature procedure [30] starting from 2-(1-ethoxyethylidene)malononitrile (**1**) and phenylhydrazine (**2**). Compound **5**, an enaminone holding a pyrazole moiety, was obtained from the nucleophilic addition reaction of 5-amino-3-methyl-1-phenyl-1*H*-pyrazole-4-carbonitrile (**3**) and acetylacetone (2,4-pentanedione,**4**) in the presence of a catalytic amount of trichloroacetic acid (TCAA) acting as a catalyst under solvent-free conditions. In the next step, cyclo-condensation reactions of **5** and a series of arylidenemalononitriles **6** led to the title polyheterocyclic scaffolds **7a-l** (Scheme 1).

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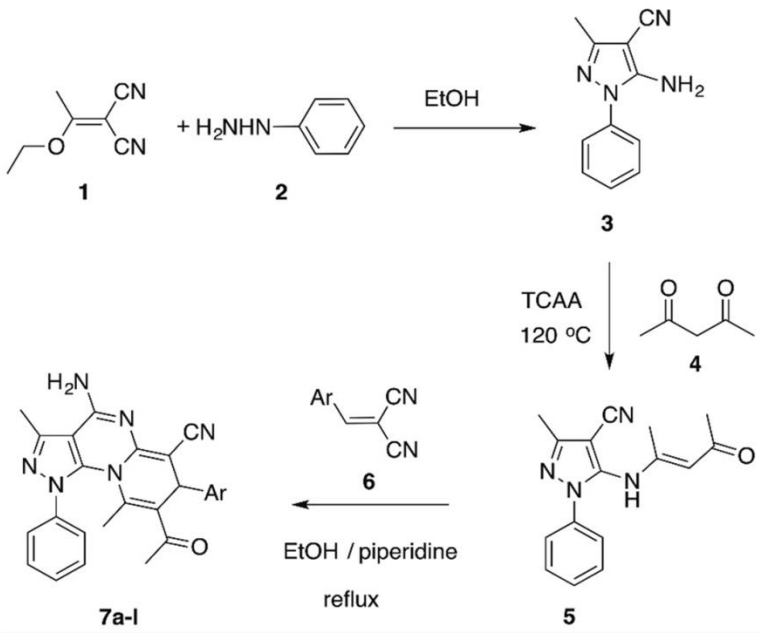
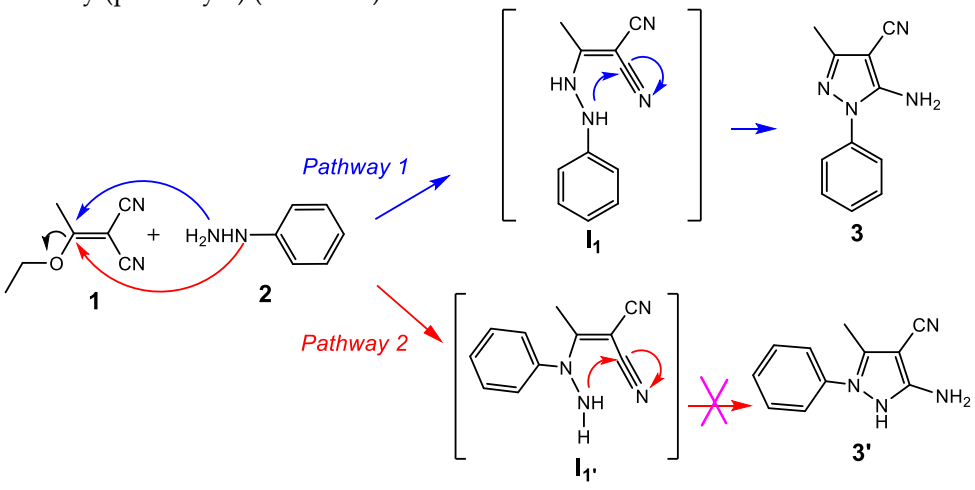


7a-l, Ar = C_6H_nR: R = Cl (a), Me (b), H (c), MeO (d), Br (e), F (f), NO_2 (g), 3,4-MeO_2 (h), 2,4-Cl (i), 5-NO-2-thienyl (j), 2-naphthyl (k) and pyridin-3-yl (l).

Scheme 1. Synthetic pathway for the synthesis of pyrazolo-pyrido-pyrimidine derivatives **7a-l**.

In details, the use of aminopyrazoles as heterocyclizing agents has received considerable attention in recent years. As a result, these molecules have proven to be the building blocks of choice in the preparation of several heterocyclic compounds, they in fact exhibit high reactivity due to the presence of a primary amine function and of a nitrile function in the alpha position. These two functions are capable of reacting as a nucleophilic and electrophilic agent, respectively, and thus subsequently undergo the cyclization reaction. This is why we chose to synthesize this type of amino-cyanopyrazole **3**. Mechanistically, the reaction begins with the attack by the free doublet of the primary amine of phenylhydrazine on the ethylenic quaternary carbon of the ether function in the ethoxyalkylidene **1**, releasing an ethanol molecule, then a second nucleophilic attack on the nitrile function by the secondary amine function of the phenylhydrazine leads to the expected pyrazole compound **3** (pathway 1). The lack of access to aminopyrazole **3'** is explained by the low availability of the free nitrogen doublet directly linked to the aromatic moiety (pathway 2) (Scheme 2).



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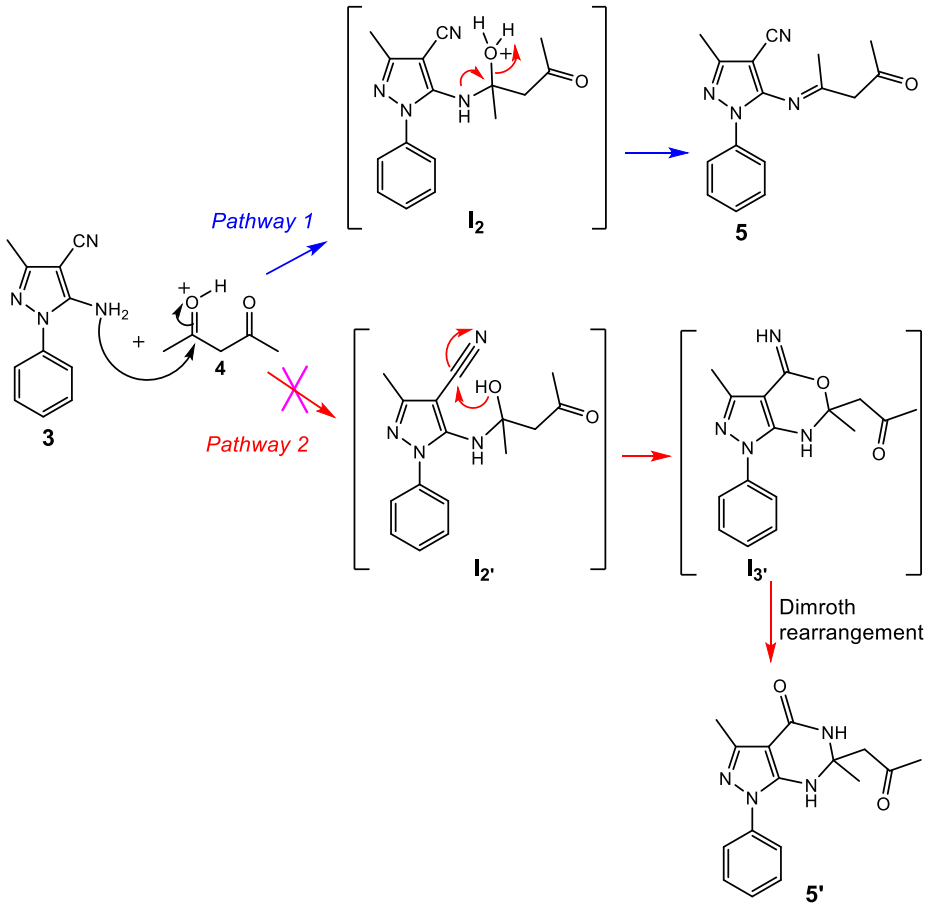
Scheme 2. Plausible mechanistic pathway for the synthesis of target compound **3**

The use of TCAA (pka = 0.77) facilitates the formation of an electrophilic site (C=O) of the acetylacetone **4**, therefore makes easy the attack of the primary amine's free doublet of the starting building block **3** which gives a non-isolable intermediate **I**2. The crucial role of TCAA can be seen again through the facilitation of the dehydration made by a second nucleophilic attack of the free doublet of the same amine function on the same electrophilic site which gives the isolable intermediary **5** (pathway 1). This conditions implie the failure to obtain compound **5'** (pathway 2) (Scheme 3).



Scheme 3. Plausible mechanistic pathway for the synthesis of compound **5**

A putative mechanism (Scheme 4) for the formation of **7a-l** has been depicted in Scheme 2. Thereby, the reaction sequence starts with a nucleophilic attack onto the double bond of the enaminone; this Michael addition forms intermediate **I**3 which subsequently undergoes a proton transfer from one carbon to another one followed by an intramolecular cyclization to afford the non-isolable intermediate **I**5. Upon tautomerization a primary amine is formed which attacks the nitrile function of the pyrazole moiety leading finally to target compounds **7**.

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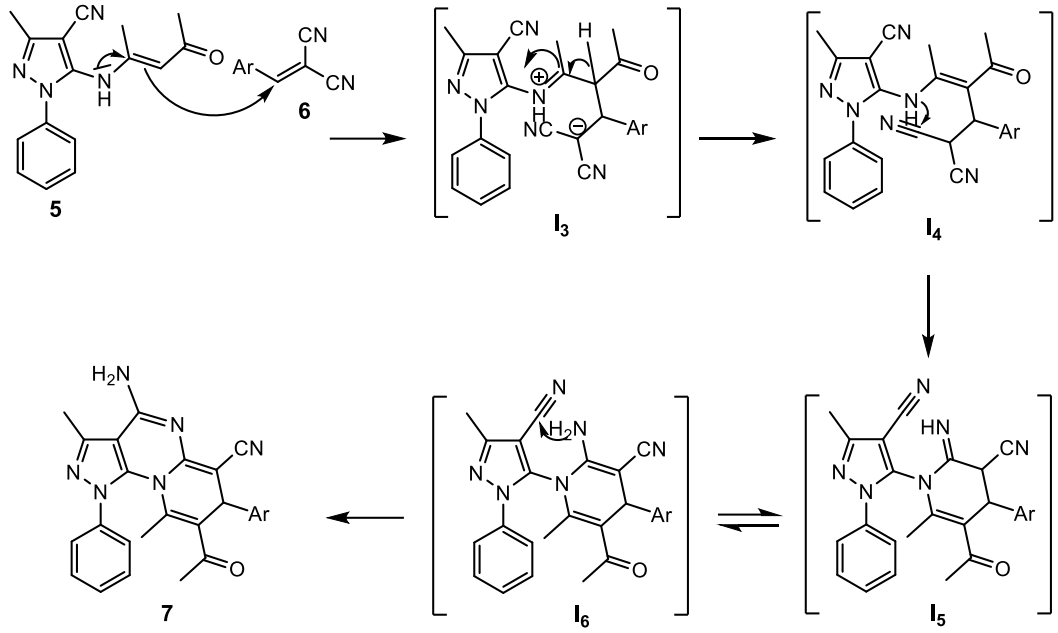

Scheme 4. Putative mechanistic pathway for the synthesis of target compounds **7a–l**.

2.2. Biological Evaluation

The synthesized compounds were evaluated for their cytotoxic activity by photometric rhodamine B assay (SRB) on different human cancer cell lines, malignant melanoma (A375), colon (HT29), breast (MCF-7), human ovarian carcinoma (A2780), and pharynx carcinoma (FaDu); for comparison, non-malignant mouse embryonic fibroblasts (NIH 3T3) and human embryonic kidney cells (HEK293) were included; doxorubicin (**DX**) was used as a positive control [31, 32]. The results are summarized in Table 1.The EC_{50} values in μM from SRB assays were determined after 72h of treatment, and the values are averaged from three independent experiments performed each in triplicate, confidence interval CI = 95%; mean ± standard mean error).

Table 1. Cytotoxicity of compounds **3,5, 7a–7l** against the human cancer cell lines A375, HT29, MCF-7, A2780, FaDu, and non-malignant cells NIH 3T3 and HEK293 (n.d. not determined, n.s. not soluble under the conditions of the assay).

Compound	A375	HT29	MCF-7	A2780	FaDu	NIH 3T3	HEK293
3, 5	> 30	> 30	> 30	> 30	> 30	> 30	> 30
7a	19.3 ± 3.0	29.9 ± 1.4	17.2 ± 1.4	18.0 ± 3.0	> 30	23.0 ±1.4	15.8 ± 2.0
7b	12.9 ± 1.6	17.5 ± 1.8	12.2 ± 1.3	14.6 ± 2.2	22.3 ± 2.7	21.3 ± 0.9	12.4 ± 0.9
7c	19.3 ± 3.8	> 30	19.5 ± 1.1	25.4 ± 2.5	> 30	> 30	7.7 ± 1.1
7d	21.5 ± 3.6	28.3 ± 3.5	18.4 ± 2.3	21.7 ± 3.9	> 30	> 30	16.7 ± 1.2
7e	9.4 ± 1.2	13.3 ± 1.8	9.2 ± 0.7	9.1 ± 1.6	13.5 ± 1.5	12.3 ±0.7	6.6 ± 0.6
7f	18.1 ± 3.3	24.7 ± 4.4	19.2 ± 1.3	19.2 ± 3.9	> 30	24.1 ± 2.8	14.7 ± 1.4
7g	16.2 ± 2.0	25.9 ± 3.4	15.7 ± 2.2	14.5 ± 2.2	> 30	> 30	17.0 ± 1.3
7h	22.1 ± 4.9	> 30	25.1 ± 2.8	27.5 ± 8.1	> 30	> 30	24.0 ± 3.4
7i-l	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
DX	n.d.	0.9 ± 0.01	1.1 ± 0.3	0.01 ± 0.01	n.d.	0.4 ± 0.07	n.d.

The intermediates **3** and **5** were found to be non-cytotoxic (EC_{50} > 30 μM) against all the five cancer cell lines. However, many of the target compounds (**7a-7h**) showed noteworthy cytotoxic effects for all tested human tumor cell lines.

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Our target compounds **7** hold five cyclic rings, A, B, C, D and E (Fig. 3). The values of EC_{50} differ by the group linked to cycle E. Interestingly, the highest activity in A375 cancer cell was determined for compound **7e** with a bromine substituent attached to the aryl moiety E in *para* position. The next molecule in this series was methyl substituted compound **7b** also exhibiting good cytotoxicity (EC_{50} = 12.9 ± 1.6 μM) while nitro-substituted compound **7g** was less cytotoxic (EC_{50} = 16.2 ± 2.0 μM) followed by **7f**, **7a** and **7c**, respectively. On the other hand, the topmost activity in colon cancer cells (HT29) was again determined for **7e** holding an EC_{50} value of 13.3 ± 1.8 μM, followed by **7b**, **7f** (Ar = 4-F-Ph) and **7g** with EC_{50} values of 17.5 ± 1.8, 24.7 ± 4.4, 25.9 ± 3.4 μM, respectively. Towards MCF-7, it is apparent that the same derivative **7e** exhibited the highest activity with an EC_{50} value of 9.2 ± 0.7μM, and compounds **7b** and **7g** (EC_{50} = 12.2 ± 1.3, 15.7 ± 2.2 μM) also exhibited good activities.

The highest cytotoxicity for A2780 cancer cell lines was found again for compound **7e** (EC_{50} = 9.1 ± 1.6 μM). In this series, compounds **7g** and **7b** (EC_{50} = 14.5 ± 2.2, 14.6 ± 2.2 μM) showed noteworthy activity as compared to other analogs, and their EC_{50} values ranged between 18.0 ± 3.0 to 27.5 ± 8.1μM. Unfortunately, compound **7e** also showed significant cytotoxic effects for the non-malignant cell lines.

Since the SRB assays showed **7e** as the most active compound, molecular docking was performed to establish some structure-activity relationships (SAR). The increase in the activity of **7e** might be due to the presence of an electron-withdrawing group attached to the E ring.

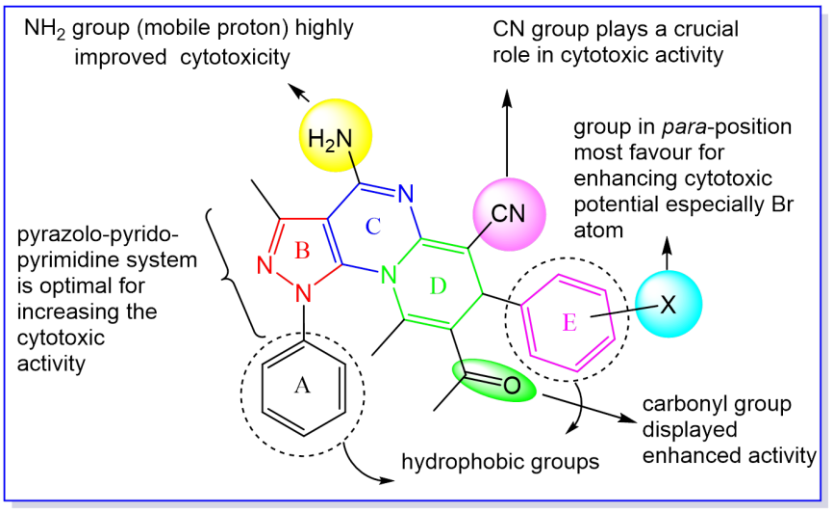

Figure 3. The structure-activity relationship (SAR) of designed scaffolds cytotoxic agents **7**.

To develop a new generation of more potent multi-targeted anticancer agents and motivated by previous research on that topic [28, 32, 33], we took advantage of the reported structure activity relationships (SAR) of pyrazolo-pyrido-pyrimidine analogs. Thus, in order to assess the ability of the top-ranked active compounds to inhibit of six anti-cancer drug targets (DHFR, VEGFR2, HER-2/neu, hCA-IX, CDK6 and LOX), we carried out docking study for complexes consisting of compound **7e** and the target proteins. This ligand fit very well in the active site binding cavity of all target enzymes (Fig. 4).

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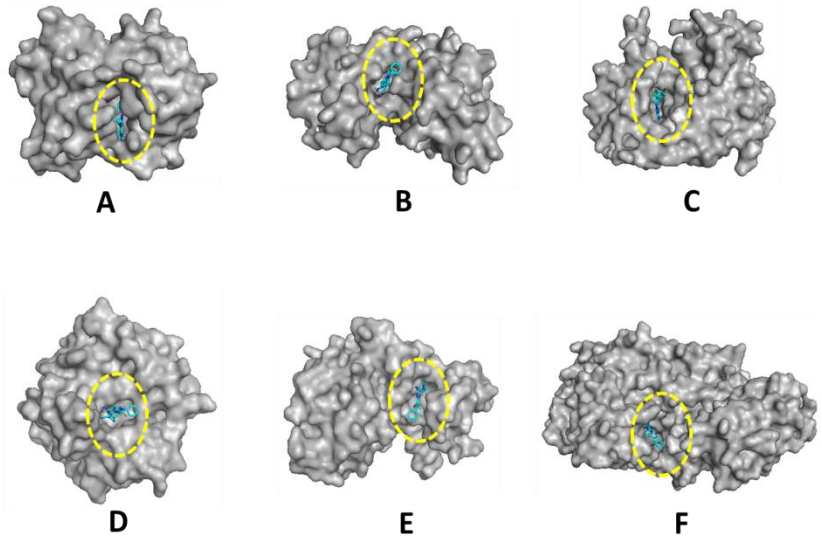

Figure 4. The surface representation of enzyme of pdb: 5HQY (A), 5EW3 (B), 3RCD (C), 5FL4 (D), 3NUP (E) and 3V99 (F) in grey color and the sticks representation (cyan color) of the docked compound **7e** .

The obtained results showed excellent interaction with all the above targets as compared to their respective co-crystallized ligands (Table 2).

Table 2. Free binding energy (Kcal.mol$^{-1)}$ of top-ranked active compound **7e** with the active site of different anti-cancer drug targets studied using Autodock 4.2 software.

Compound	Free binding energy (Kcal.mol^{-1})					
	DHFR (5HQY)	VEGFR2 (5EW3)	HER-2 (3RCD)	hCA-IX (5FL4)	CDK6 (3NUP)	LOX5 (3V99)
7e	-8.5	-6.6	-7.3	-7.2	-7.1	-8.5
Ref. ligand	-8.3	-7.3	-7.2	-7.0	-6.8	-6.0

Considering interactions with all the targets under observation, ligand **7e** fits well inside the pocket; it displayed crucial hydrogen bonds by its (CO) and (NH_2) groups; thereby in the case of the most favorable complex of compound **7e** with DHFR, four H-bonds were formed: three between the amine function and GLY20, ASP21 and SER59 and one through the carbonyl group with THR56. The fused heterocyclic system seems to play a significant binding role through the appearance of pi-sigma interaction with LEU22. In addition, the phenyl groups exhibit many hydrophobic interactions (Fig. 5.A). Moreover, towards active site amino acids of VEGFR2 (Fig. 5.B) **7e** is involved in two H-bonds with SER884 and ARG1027, a pi-sigma interaction with ILE888, a pi-sulfur interaction with CYS1024, as well as alkyl with PRO821 and pi-alkyl with LYS 887 and ILE888. Furthermore, in the case of HER-2/neu, compound **7e** forms some substantial H-bonds, i.e. two through its amine function and one by its carbonyl group, a pi-sigma interaction with LEU852 besides many hydrophobic interactions with the amino acid sequence VAL734, ALA751, LEU800, MET801 and CYS 805 (Fig. 5.C). In the same manner, towards active site amino acids of hCA-IX, ligand **7e** formed a conventional hydrogen bond by its carbonyl group with THR201, showed pi-sigma interaction with LEU199 and hydrophobic interactions with residues HIS68, HIS94, HIS119, VAL121, VAL130, VAL142 and TRP210 (Fig. 5.D). Furthermore, for complex CDK6-**7e**, a hydrogen bond formed between (NH_2) and ASP102, pi-anion with ASP104 and hydrophobic interactions with ILE19, VAL27, LEU152 and ALA162 (Fig. 5.E) were found. Finally, against LOX, **7e** demonstrated three H-bonds through its CO and NH_2 groups with PHE177 and LEU607

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and showed some hydrophobic interactions with the amino acids sequence PHE177, HIS367, LYS409 and LEU607 (Fig. 5.F).

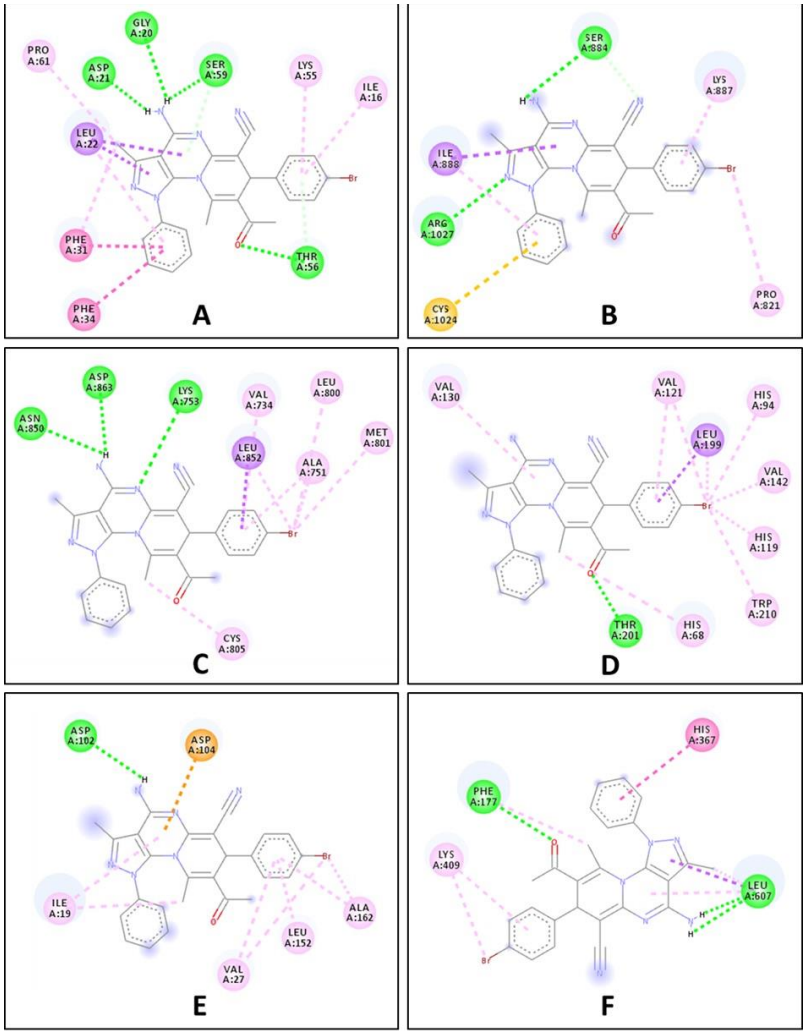

Figure 5. Docking pose (2D binding model) of the most active cytotoxic agent **7e** in the active site of DHFR (PDB: 5HQY). (A), VEGFR2 (PDB: 5EW3). (B), HER-2 (PDB: 3RCD). (C), hCA-IX (PDB: 5FL4). (D), CDK6 (PDB: 3NUP). (E) and LOX5 (PDB: 3V99). (F).

3. Materials and Methods

3.1. General

A detailed description of materials and methods can be found in the supplementary materials file. For molecular docking, the 3D crystal structures of PDB were obtained from the RSCB protein data bank (PDB): DHFR (ID:5HQY[34, 35]), VEGFR2 (ID:5EW3 [34, 36]), HER2/neu (ID:3RCD [34, 37]), hCA-IX (ID:5FL4 [34, 38]), CDK6 (ID:3NUP [34, 39]) and LOX5 (ID:3V99 [34, 40]). All water molecules were removed, and hydrogen atoms were added before docking using discovery studio visualize; Gasteiger charges also were added to the system during the preparation of the receptor input file. Docking studies were performed using AutoDock 4.2 software (Scripps Research; http://autodock.scripps.edu). The structures of the compounds were drawn using ChemDraw [ver. 10.0]. The optimization of all the geometries of scaffolds was performed with ACD (3D viewer) software (http://www.filef acts.com/acd3d-viewer-freeware-info). Co-crystalized ligands in the proteins were taken as reference ligands and re-docked into the active site of proteins for energy comparison. The top-scored conformation was recorded for each compound and used for further analysis and 2D images were captured

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through discovery studio visualizer (2017) developed by Accelrys (BIOvIA, San Diego, USA).^{13}C NMR spectra (Supplementary material) were recorded as APT spectra showing CH and CH_3 groups as positive signals and CH_2 groups and quaternary carbons as negative signals.

3.2. 5-Amino-3-methyl-1-phenyl-1H-pyrazole-4-carbonitrile (3)

To an ice-cold solution of **1** (0.1 mol) in ethanol (100 mL), **2** (0.1 mol) was added, and the mixture was stirred for 3 h. The precipitate was filtered off and re-crystalized from ethanol; yield: 86%; m.p. 132 °C; R_F = 0.69 (SiO_2, $CHCl_3$/MeOH, 95:5); IR (ATR): ν = 3329m, 2216s, 1652m, 1596m, 1563m, 1533s, 1488m, 1444m, 1318w, 996w, 833w, 758s, 722m, 689s, 624m, 554m, 506m, 451w cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.51 – 7.47 (m, 2H, 3-H, 5-H), 7.47 – 7.43 (m, 2H, 2-H, 6-H), 7.42 – 7.38 (m, 1H, 4-H), 4.67 (s, 2H, NH_2), 2.29 (s, 3H, 11-H) ppm; ^{13}C NMR (126 MHz, $CDCl_3$): δ = 151.1 (C-9), 150.3 (C-7), 137.0 (C-1), 130.0 (C-3, C-5), 128.7 (C-4), 124.2 (C-2, C-6), 114.6 (C-10), 76.3 (C-8), 13.0 (C-11) ppm; MS (ESI, MeOH): m/z 199 (100%, $[M+H]^+$), 237 (52%, $[M+K]^+$); analysis calcd for $C_{11}H_{10}N_4$ (198.22): C 66.65, H 5.09, N 28.26; found: C 66.41, H 5.24, N 28.04.

3.3. 3-Methyl-5-{[(1E)-1-methyl-3-oxobut-1-en-1-yl]amino}-1-phenyl-1H-pyrazole-4-carbonitrile (5)

A mixture of aminopyrazole **3** (7 mmol), acetylacetone **4** (7 mmol) and trichloroacetic acid (1 mmol) was stirred at 120 °C for 10 h. The precipitate was filtered off, dissolved in a minimum amount of $CHCl_3$, and precipitated with petrol ether followed by chromate ographic purification (SiO_2, chloroform/ethyl acetate, 9:1): yield: 62%; m.p. 109 °C; R_F = 0.89 (SiO_2, $CHCl_3$/MeOH, 95:5); UV-Vis ($CHCl_3$): λ_{max} (log ε) = 260 nm (3.96), 308 nm (3.96); IR (ATR): ν = 2228m, 1619s, 1574s, 1503m, 1430m, 1357w, 1274s, 1181w, 1125w, 1022w, 908w, 790w, 747s, 693s, 636w, 536w, 512s cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 12.42 (s, 1H, NH), 7.53 – 7.27 (m, 5H, 2-H, 3-H, 4-H, 5-H, 6-H), 5.35 (s, 1H, 13-H), 2.43 (s, 3H, 11-H), 2.09 (s, 3H, 15-H), 1.94 (s, 3H, 16-H) ppm; ^{13}C NMR (126 MHz, $CDCl_3$): δ = 198.7 (C-14), 158.2 (C-12), 152.4 (C-7), 142.4 (C-9), 137.4 (C-1), 129.9 (C-3, C-5), 129.2 (C-4), 124.3 (C-2, C-6), 113.7 (C-10), 101.3 (C-13), 90.6 (C-8), 29.8 (C-15), 19.7 (C-16), 13.6 (C-11) ppm; MS (ESI, MeOH): m/z 281 (32%, $[M+H]^+$), 303 (100%, $[M+Na]^+$); analysis calcd for $C_{16}H_{16}N_4O$ (280.33): C 68.55, H 5,75, N 19.99; found: C 687.37, H 5.98, N 19.75.

3.4. General procedure for the synthesis of compounds 7a-l

A mixture of enaminone **5** (1 mmol) and arylidenemalononitrile **6** (1 mmol) was heated at reflux in ethanol (10 mL) in the presence of a catalytic amount of piperidine for 8 h. The solvent was removed at reduced pressure, and the crude products were purified by chromatography (SiO_2, chloroform/ethyl acetate, 8/2).

3.5. 8-Acetyl-4-amino-7-(4-chlorophenyl)-3,9-dimethyl-1-phenyl-1,7-dihydropyrazolo[4,3-e] pyrido[1,2-a]pyrimidine-6-carbonitrile (7a)

Yield: 78%; m.p. 167 °C; R_F = 0.33 (SiO_2, $CHCl_3$/MeOH, 95:5); UV-Vis ($CHCl_3$):λ_{max} (log ε) = 290 nm (3.97); IR (ATR): ν = 3332w, 2185m, 1653m, 1609s, 1570m, 1540s, 1488m, 1221m, 1192w, 1090w, 1012w, 859w, 764m, 693m, 624w, 509m cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.37 – 7.19 (m, 8H, 2-H, 3-H, 5-H, 6-H, 22-H, 23-H, 25-H, 26-H), 6.73 (m, 1H, 4-H), 4.97 (s, 1H, 15-H), 2.61 (s, 3H, 10-H), 2.36 (s, 3H, 19-H), 1.68 (s, 3H, 17-H) ppm; ^{13}C NMR (126 MHz, $CDCl_3$): δ = 197.9 (C-18), 147.6 (C-12), 146.2 (C-11), 144.1 (C-7), 143.9 (C-13), 141.4 (C-9), 140.3 (C-21), 138.0 (C-1), 133.7 (C-24), 130.1 (C-3, C-5), 129.2 (C-23, C-25), 129.2 (C-2, C-6), 128.4 (C-22, C-26), 125.5 (C-20), 124.0 (C-14), 123.1 (C-4), 100.0 (C-8), 72.2 (C-16), 40.1 (C-15), 31.0 (C-19), 14.5 (C-10) ppm; MS (ESI, MeOH): m/z 467 (100%, $[M-H]^-$); analysis calcd for $C_{26}H_{21}ClN_6O$ (468.94): C 66.59, H 4.51, N 17.92; found: C 66.30, H 4.72, N 17.63.

 doi:10.20944/preprints202109.0188.v1

3.6. 8-Acetyl-4-amino-3,9-dimethyl-7-(4-methylphenyl)-1-phenyl-1,7-dihydropyrazolo[4,3-e] pyrido1,2-a]pyrimidine-6-carbonitrile (7b)

Yield: 66%; m.p. 167 °C; R_F = 0.33 (SiO_2, $CHCl_3$/MeOH, 95:5); UV-Vis ($CHCl_3$): λ_{max} (log ε) = 289 nm (4.27); IR (ATR): ν = 2185m, 1608s, 1542s, 1479m, 1449w, 1357w, 1223m, 1194w, 879w, 810w, 764m, 695m, 624w, 511m cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 7.33 – 7.28 (m, 2H, 2-H, 6-H), 7.23 – 7.18 (m, 2H, 3-H, 5-H), 7.17 (s, 4H, 2-H, 3-H, 5-H, 6-H), 6.78 – 6.67 (m, 1H, 4-H), 4.94 (s, 1H, 15-H), 2.58 (s, 3H, 10-H), 2.38 (s, 3H, 19-H), 2.32 (s, 3H, 27-H), 1.65 (s, 3H, 17-H) ppm; ^{13}C NMR (126 MHz, $CDCl_3$): δ = 197.9 (C-18), 155.8 (C-12), 155.0 (C-11), 145.3 (C-7), 143.2 (C-13), 141.2 (C-9), 138.5 (C-21), 137.8 (C-1), 137.0 (C-24), 129.6 (C-3, C-5), 129.2 (C-23, C-25), 128.4 (C-2, C-6), 126.4 (C-22, C-26), 125.1 (C-20), 122.7 (C-4), 122.5 (C-14), 99.8 (C-8), 71.5 (C-16), 40.0 (C-15), 30.3 (C-19), 21.0 (C-27), 19.1 (C-17), 13.7 (C-10) ppm; MS (ESI, MeOH): m/z449 (100%, $[M+H]^+$); analysis calcd for $C_{27}H_{24}N_6O$ (448.52): C 72.30, H 5.39, N 18.74; found: C 72.07, H 5.51. N 18.55.

3.7. 8-Acetyl-4-amino-3,9-dimethyl-1,7-diphenyl-1,7-dihydropyrazolo[4,3-e] pyrido[1,2-a]pyrimidine-6-carbonitrile (7c)

Yield: 62%; m.p. 198 °C; R_F = 0.31 (SiO_2, $CHCl_3$/MeOH, 95:5); UV-Vis ($CHCl_3$): λ_{max} (log ε) = 289 nm (4.54); IR (ATR): ν = 3148w, 2181m, 1660m, 1601s, 1544s, 1480m, 1447m, 1355w, 1220m, 1197m, 878w, 764m, 722m, 694s, 627w, 511s cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 7.40 – 7.16 (m, 9H, 2-H, 3-H, 5-H, 6-H, 22-H, 23-H, 24-H, 25-H, 26-H), 6.73 – 6.66 (m, 1H, 4-H), 5.00 (s, 1H, 15-H), 2.59 (s, 3H, 10-H), 2.39 (s, 3H, 19-H), 1.65 (s, 3H, 17-H) ppm; ^{13}C NMR (126 MHz, $CDCl_3$): δ = 198.0 (C-18), 155.9 (C-12), 155.2 (C-11), 145.5 (C-7), 143.3 (C-13), 141.6 (C-9), 141.3 (C-21), 137.8 (C-1), 129.7 (C-3, C-5), 128.7 (C-23, C-25), 128.5 (C-2, C-6), 127.5 (C-24), 126.6 (C-22, C-26), 125.3 (C-20), 122.7 (C-4), 122.5 (C-14), 100.0 (C-8), 71.3 (C-16), 40.4 (C-15), 30.5 (C-19), 19.2 (C-17), 13.8 (C-10) ppm; MS (ESI, MeOH): m/z435 (100%, $[M+H]^+$), 457 (22%, $[M+Na]^+$); analysis calcd for $C_{26}H_{22}N_6O$ (434.49): C 71.87, H 5.10, N 19.34; found: C 71.66, H 5.29, N 19.07.

3.8. 8-Acetyl-4-amino-7-(4-methoxyphenyl)-3,9-dimethyl-1-phenyl-1,7-dihydropyrazolo[4,3-e] pyrido[1,2-a]pyrimidine-6-carbonitrile (7d)

Yield: 74%; m.p. 160 °C; R_F = 0.33 (SiO_2, $CHCl_3$/MeOH, 95:5); UV-Vis ($CHCl_3$): λ_{max} (log ε) = 273 nm (4.42); IR (ATR): ν = 2184m, 1607s, 1541s, 1509m, 1478m, 1355w, 1249m, 1174m, 1033m, 825w, 764m, 693m, 621w, 510s cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 7.34 – 7.17 (m, 6H, 2-H, 3-H, 5-H, 6-H, 22-H, 26-H), 6.92 – 6.87 (m, 2H, 23-H, 25-H), 6.73 (m, 1H, 4-H), 4.92 (d, J = 1.0 Hz, 1H, 15-H), 3.77 (s, 3H, 27-H), 2.59 (s, 3H, 10-H), 2.38 (s, 3H, 19-H), 1.65 (s, 3H, 17-H) ppm; ^{13}C NMR (126 MHz, $CDCl_3$): δ = 198.1 (C-18), 159.0 (C-24), 155.7 (C-12), 155.1 (C-11), 145.5 (C-7), 143.2 (C-13), 141.3 (C-9), 137.9 (C-21), 133.5 (C-1), 129.8 (C-3, C-5), 128.6 (C-2, C-6), 127.7 (C-22, C-26), 125.5 (C-20), 122.8 (C-4), 122.5 (C-14), 114.1 (C-23, C-25), 100.0 (C-8), 71.8 (C-16), 55.5 (C-27), 39.8 (C-15), 30.5 (C-19), 19.2 (C-17), 13.8 (C-10) ppm; MS (ESI, MeOH): m/z 465 (100%, $[M+H]^+$), 487 (40%, $[M+Na]^+$); analysis calcd for $C_{27}H_{24}N_6O_2$ (464.52): C 69.81, H 5.21, N 18.09; found: C 69.57, H 5.41, N 17.84.

3.9. 8-Acetyl-4-amino-7-(4-bromophenyl)-3,9-dimethyl-1-phenyl-1,7-dihydropyrazolo[4,3-e] pyrido[1,2-a]pyrimidine-6-carbonitrile (7e)

Yield: 75%; m.p. 192 °C; R_F = 0.40 (SiO_2, $CHCl_3$/MeOH, 95:5); UV-Vis ($CHCl_3$): λ_{max} (log ε) = 272 nm (4.27); IR (ATR): ν = 2186m, 1607s, 1541s, 1485m, 1396w, 1357w, 1223m, 1009m, 879w, 764m, 694m, 622w, 510m cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 7.49 (d, J = 8.5 Hz, 2H, 23-H, 25-H), 7.32 (d, J = 7.4 Hz, 2H, 2-H, 6-H), 7.22 (d, J = 7.8 Hz, 2H, 3-H, 5-H), 7.16 (d, J = 8.5 Hz, 2H, 22-H, 26-H), 6.70 (d, J = 7.7 Hz, 1H, 4-H), 4.94 (s, 1H, 15-H), 2.58 (s, 3H, 10-H), 2.36 (s, 3H, 19-H), 1.67 (s, 3H, 17-H) ppm; ^{13}C NMR (126 MHz, $CDCl_3$): δ = 197.7 (C-18), 156.1 (C-12), 155.3 (C-11), 145.5 (C-7), 143.7 (C-13), 141.2 (C-21), 140.9 (C-9), 137.8 (C-1), 131.7 (C-23, C-25), 129.8 (C-3, C-5), 128.8 (C-2, C-6), 128.5 (C-22, C-26), 125.0 (C-20), 122.7 (C-14), 122.4 (C-4), 121.3 (C-24), 100.1 (C-8), 70.9 (C-16), 39.9 (C-15), 30.7

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(C-19), 19.3 (C-17), 13.8 (C-10) ppm; MS (ESI, MeOH): m/z 513 (100%, $[M+H]^+$); analysis calcd for $C_{26}H_{21}BrN_6O$ (512.39): C 60.83, H 4.12, N 16.37; found: C 60.59, H 4.30, N 16.19.

3.10. 8-Acetyl-4-amino-7-(4-fluorophenyl)-3,9-dimethyl-1-phenyl-1,7-dihydropyrazolo[4,3-e] pyrido[1,2-a]pyrimidine-6-carbonitrile (7f)

Yield: 80%; m.p. 259 °C; R_F = 0.38 (SiO_2, $CHCl_3$/MeOH, 95:5); UV-Vis ($CHCl_3$): λ_{max} (log ε) = 273 nm (4.31), 289 nm (4.30); IR (ATR): ν = 2185m, 1610s, 1542s, 1478m, 1357w, 1223s, 1158m, 1013w, 862w, 764m, 695m, 623w, 513m cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 7.32 (t, J = 7.5 Hz, 2H, 2-H, 6-H), 7.29 – 7.18 (m, 4H, 22-H, 23-H, 25-H, 26-H), 7.06 (t, J = 8.6 Hz, 2H, 3-H, 5-H), 6.72 (d, J = 7.2 Hz, 1H, 4-H), 4.96 (s, 1H, 15-H), 2.59 (s, 3H, 10-H), 2.37 (s, 3H, 19-H), 1.66 (s, 3H, 17-H) ppm; ^{13}C NMR (126 MHz, $CDCl_3$): δ = 197.6 (C-18), 161.9 (d, J = 246.6 Hz C-24), 155.8 (C-12), 155.0 (C-11), 145.3 (C-7), 143.2 (C-13), 141.0 (C-9), 137.6 (C-1), 137.2 (d, J = 3.,1 Hz, C-21), 129.5 (C-3, C-5), 128.4 (C-2, C-6), 128.0 (d, J = 7.9 Hz, C-22, C-26), 128.0 (22, 26), 125.1 (C-20), 122.4 (C-14), 122.2 (C-4), 115.3 (d, J = 21.4 Hz, C-23, C-25), 99.8 (C-8), 71.0 (C-16), 39.5 (C-15), 30.4 (C-19), 19.0 (C-17), 13.5 (C-10) ppm; MS (ESI, MeOH): m/z 453 (100%, $[M+H]^+$), 475 (22%, $[M+Na]^+$); analysis calcd for $C_{26}H_{21}FN_6O$ (452.48): C 69.01, H 4.68,N 18.57; found: C 68.84, H 4.83, N 18.25.

3.11. 8-Acetyl-4-amino-3,9-dimethyl-7-(4-nitrophenyl)-1-phenyl-1,7-dihydropyrazolo[4,3-e] pyrido[1,2-a]pyrimidine-6-carbonitrile (7g)

Yield: 58%; m.p. 164 °C; R_F = 0.31 (SiO_2, $CHCl_3$/MeOH, 95:5); UV-Vis ($CHCl_3$): λ_{max} (log ε) = 270 nm (4.20); IR (ATR): ν = 2187m, 1608s, 1570m, 1542s, 1519m, 1477m, 1450w, 1344s, 1224m, 1191w, 1013w, 873w, 856w, 763m, 727w, 695m, 622w, 510mcm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 8.23 (d, J = 8.8 Hz, 2H, 23-H, 25-H), 7.48 (d, J = 8.2 Hz, 2H, 22-H, 26-H), 7.34 (t, J = 7.5 Hz, 2H, 2-H, 6-H), 7.20 (t, J = 7.8 Hz, 2H, 3-H, 5-H), 6.68 (d, J = 7.5 Hz, 1H, 4-H), 5.11 (s, 1H. 15-H), 2.58 (s, 3H, 10-H), 2.37 (s, 3H, 19-H), 1.70 (s, 3H, 17-H) ppm; ^{13}C NMR (126 MHz, $CDCl_3$): δ = 197.2 (C-18), 156.5 (C-12), 155.4 (C-11), 149.6 (C-21), 147.3 (C-24), 145.6 (C-7), 144.3 (C-13), 141.1 (C-9), 137.8 (C-1), 129.7 (C-3, C-5), 128.9 (C-2, C-6), 127.8 (C-22, C-26), 124.7 (C-20), 123.9 (C-23, C-25), 122.6 (C-14), 122.1 (C-4), 100.2 (C-8), 70.4 (C-16), 40.3 (C-15), 30.9 (C-19), 19.4 (C-17), 13.8 (C-10) ppm; MS (ESI, MeOH): m/z 480 (100%, $[M+H]^+$), 502 (28%, $[M+Na]^+$); analysis calcd for $C_{26}H_{21}N_7O_3$ (479.49): C 65.13, H 4.41, N 20.45; found: C 64.97, H 4.68, N 20.22.

3.12. 8-Acetyl-4-amino-7-(3,4-dimethoxyphenyl)-3,9-dimethyl-1-phenyl-1,7-dihydropyrazolo [4,3-e] pyrido[1,2-a]pyrimidine-6-carbonitrile (7h)

Yield: 72%; m.p. 260 °C; R_F = 0.33 (SiO_2, $CHCl_3$/MeOH, 95:5); UV-Vis ($CHCl_3$): λ_{max} (log ε) = 286 nm (4.23); IR (ATR): ν = 2185m, 1607s, 1542s, 1514s, 1478m, 1253s, 1236s, 1137m, 1025m, 874w, 813w, 764m, 695m, 628w, 511m cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 7.32 – 7.26 (m, 2H, 2-H, 6-H), 7.20 (t, J = 7.8 Hz, 2H, 3-H, 5-H), 6.91 – 6.61 (m, 4H, 4-H, 22-H, 23-H, 26-H), 4.92 (s, 1H, 15-H), 3.87 (s, 3H, 28-H), 3.83 (s, 3H, 27-H), 2.58 (s, 3H, 10-H), 2.38 (s, 3H, 19-H), 1.66 (s, 3H, 17-H) ppm; ^{13}C NMR (126 MHz, $CDCl_3$): δ = 198.4 (C-18), 156.3 (C-12), 155.4 (C-11), 149.7 (C-25), 148.9 (C-24), 145.6 (C-7), 143.4 (C-13), 141.6 (C-9), 138.2 (C-1), 134.5 (C-21), 129.9 (C-3, C-5), 128.9 (C-2, C-6), 125.8 (C-20), 123.0 (C-14), 122.8 (C-4), 118.4 (C-22), 111.1 (C-23), 110.9 (C-26), 100.3 (C-8), 71.9 (C-16), 56.5 (C-27, C-28), 40.3 (C-15), 30.8 (C-19), 19.4 (C-17), 14.0 (C-10) ppm; MS (ESI, MeOH): m/z 495 (100%, $[M+H]^+$), 517 (82%, $[M+Na]^+$); analysis calcd for $C_{28}H_{26}N_6O_3$ (494.55): C 68.00, H 5.30, N 16.99; found: C 67.81, H 5.48, N 16.72.

3.13. 8-Acetyl-4-amino-7-(3,4-dichlorophenyl)-3,9-dimethyl-1-phenyl-1,7-dihydropyrazolo[4,3-e]pyrido[1,2-a]pyrimidine-6-carbonitrile (7i)

Yield: 70%; m.p. 235 °C; R_F = 0.36 (SiO_2, $CHCl_3$/MeOH, 95:5); UV-Vis ($CHCl_3$): λ_{max} (log ε) = 262 nm (4.29), 289 nm (4.25); IR (ATR): ν = 2183m, 1685m, 1600s, 1543s, 1474m, 1382w, 1353m, 1221m, 1183w, 1043w, 956w, 864m, 822w, 770m, 695m, 627m, 512s cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 7.52 (d, J = 2.0 Hz, 1H, 25-H), 7.31 – 7.28 (m, 2H, 3-H, 5-H),

 doi:10.20944/preprints202109.0188.v1

7.21 – 7.12 (m, 4H, 2-H, 6-H, 22-H, 23-H), 6.64 (d, J = 7.5 Hz, 1H, 4-H), 5.03 (s, 1H, 15-H), 2.61 (s, 3H, 10-H), 2.42 (s, 3H, 19-H), 1.50 (s, 3H, 17-H) ppm; ^{13}C NMR (126 MHz, $CDCl_3$): δ = 198.7 (C-18), 157.2 (C-12), 155.3 (C-11), 145.6 (C-7), 141.4 (C-9), 140.5 (C-13), 137.7 (C-1), 136.0 (C-21), 134.7 (C-26), 134.3 (C-24), 130.6 (C-25), 129.7 (C-2, C-6), 129.3 (C-22), 128.9 (C-3, C-5), 127.1 (C-23), 125.0 (C-20), 122.4 (C-4), 121.6 (C-14), 99.8 (C-8), 68.8 (C-16), 40.3 (C-15), 29.9 (C-19), 18.4 (C-17), 13.9 (C-10) ppm; MS (ESI, MeOH): m/z 503 (100%, $[M+H]^+$), 525 (42%, $[M+Na]^+$); analysis calcd for $C_{26}H_{20}Cl_2N_6O$ (502.38): C 62.04, H 4.00, N 16.70; found: C 61.87, H 45.29, N 16.52.

3.14. 8-Acetyl-4-amino-3,9-dimethyl-7-(5-nitro-2-thienyl)-1-phenyl-1,7-dihydropyrazolo[4,3-e] pyrido[1,2-a]pyrimidine-6-carbonitrile (7j)

Yield: 74%; m.p. 262 °C; R_F = 0.60 (SiO_2, $CHCl_3$/MeOH, 95:5); UV-Vis ($CHCl_3$): λ_{max} (logε) = 328 nm (3.86); IR (ATR): ν = 3434m, 2237m, 2195s, 1688m, 1650s, 1578w, 1549m, 1494s, 1420s, 1333s, 1262m, 1197m, 1112w, 955w, 816m, 759m, 688m, 660m, 635m, 562m, 510m cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.69 (d, J = 4.2 Hz, 1H, 23-H), 7.51 – 7.46 (m, 2H, 2-H, 6-H), 7.44 – 7.38 (m, 2H, 3-H, 5-H), 7.28 (d, J = 7.2 Hz, 1H, 24-H), 6.67 (d, J = 4.2 Hz, 1H, 4-H), 4.86 (s, 1H, 15-H), 2.53 (s, 3H, 10-H), 2.23 (s, 3H, 19-H), 2.03 (s, 3H, 17-H) ppm; ^{13}C NMR (126 MHz, $CDCl_3$): δ = 198.0 (C-18), 157.6 (C-12), 157.5 (C-11), 153.2 (C-7), 149.3 (C-21), 142.5 (C-13), 138.0 (C-9), 136.3 (C-1), 131.0 (C-2, C-6), 130.6 (C-3, C-5), 129.5 (C-23), 124.8 (C-24), 123.7 (C-4), 118.9 (C-22), 115.9 (C-14), 95.3 (C-8), 65.1 (16), 36.5 (15), 30.3 (19), 17.7 (17), 13.8 (10) ppm; MS (ESI, MeOH): m/z 507 (100%, $[M+Na]^+$); analysis calcd for $C_{24}H_{19}N_7O_3S$ (485.52): C 59.37, H 3.94, N 20.19, S 6.60; found: C 59.17, H 4.18, N 20.02, S 6.43.

3.15. 8-Acetyl-4-amino-3,9-dimethyl-7-(2-naphthyl)-1-phenyl-1,7-dihydropyrazolo[4,3-e] pyrido[1,2-a]pyrimidine-6-carbonitrile (7k)

Yield: 65%; m.p. 270 °C; R_F = 0.49 (SiO_2, $CHCl_3$/MeOH, 95:5); UV-Vis ($CHCl_3$): λ_{max} (log ε) = 270 nm (4.37); IR (ATR): ν = 3468w, 2181m, 1676m, 1654m, 1611m, 1543s, 1479m, 1357m, 1244m, 1222w, 951w, 879w, 762m, 754m, 695m, 621w, 515m, 479m cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.89 – 7.80 (m, 3H, 23-H, 25-H, 28-H), 7.69 (t, J = 1.5 Hz, 1H, 30-H), 7.51 – 7.41 (m, 3H, 22-H, 26-H, 27-H), 7.22 (tt, J = 7.3, 1.0 Hz, 2H, 2-H, 6-H), 7.04 (t, J = 8.1 Hz, 2H, 3-H, 5-H), 6.60 (d, J = 7.5 Hz, 1H, 4-H), 5.23 – 5.06 (m, 1H, 15-H), 2.60 (s, 3H, 10-H), 2.45 (s, 3H, 19-H), 1.69 (s, 3H, 17-H) ppm; ^{13}C NMR (126 MHz, $CDCl_3$): δ = 198.1 (C-18), 155.7 (C-12), 155.2 (C-11), 145.6 (C-7), 143.7 (C-13), 141.3 (C-9), 139.0 (C-21), 137.8 (C-1), 133.3 (C-29), 132.8 (C-24), 129.7 (C-3, C-5), 128.6 (C-2, C-6), 128.6 (C-23), 128.0 (C-25), 127.8 (C-28), 126.7 (C-26), 126.3 (C-27), 125.3 (C-22), 125.1 (C-20), 124.8 (C-30), 122.6 (C-4), 122.4 (C-14), 99.9 (C-8), 71.5 (C-16), 40.7 (C-15), 30.6 (C-19), 19.4 (C-17), 13.8 (C-10) ppm; MS (ESI, MeOH): m/z 485 (100%, $[M+H]^+$), 606 (24%, $[M+Na]^+$); analysis calcd for $C_{30}H_{24}N_6O$ (484.55): C 74.36, H 4.99, N 17.34; found: C 74.19, H 5.18, N 17.08.

3.16. 8-Acetyl-4-amino-3,9-dimethyl-1-phenyl-7-pyridin-3-yl-1,7-dihydropyrazolo[4,3-e] pyrido[1,2-a]pyrimidine-6-carbonitrile (7l)

Yield: 68%; m.p. 306 °C; R_F = 0.44 (SiO_2, $CHCl_3$/MeOH, 95:5); UV-Vis ($CHCl_3$): λ_{max}(log ε) = 269 nm (4.34); IR (ATR): ν = 3398w, 2193m, 1651m, 1621s, 1570m, 1537s, 1474m, 1325m, 1232m, 1174m, 1025m, 957w, 877w, 759m, 716m, 689m, 623m, 572m, 511m cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.58 (d, J = 4.0 Hz, 1H, 23-H), 8.52 (d, J = 2.4 Hz, 1H, 22-H), 7.79 (d, J = 7.9 Hz, 1H, 25-H), 7.42 – 7.27 (m, 5H, 2-H, 3-H, 5-H, 6-H, 24-H), 6.65 (d, J = 7.4 Hz, 1H, 4-H), 5.08 (s, 1H, 15-H), 2.58 (s, 3H, 10-H), 2.37 (s, 3H, 19-H), 1.70 (s, 3H, 17-H) ppm; ^{13}C NMR (126 MHz, $CDCl_3$): δ = 196.8 (C-18), 156.3 (C-12), 155.1 (C-11), 147.2 (C-23), 147.1 (C-22), 145.3 (C-7), 144.3 (C-13), 140.9 (C-9), 137.5 (C-1), 135.8 (C-25), 129.7 (C-3, C-5), 128.7 (C-2, C-6), 124.2 (C-20), 123.7 (C-21), 122.4 (C-4), 121.8 (C-14), 100.0 (C-8), 69.8 (C-16), 38.1 (C-15), 30.7 (C-19), 19.1 (C-17), 13.5 (C-10) ppm; MS (ESI, MeOH): m/z 436 (100%, $[M+H]^+$); analysis calcd for $C_{25}H_{21}N_7O$ (435.48): C 68.95, H 4.86, N 22.51; found: C 68.80, H 4.97, N 22.31.

 doi:10.20944/preprints202109.0188.v1

4. Conclusions

In summary, a new class of pyrazolo[*4,3-e*]pyrido[*1,2-a*]pyrimidine derivatives was designed, synthesized, characterized and evaluated for their cytotoxic activity towards five human cancer cell lines A375, HT-29, MCF-7, A2780, FaDu, as well as non-malignant NIH 3T3 and HEK293. Compound **7e** displayed a noteworthy cytotoxic effect towards all cancer cell lines. A SAR study demonstrated that fused heterocycles pyrazole, pyridine and pyrimidine and group linked to the aryl moiety E in *para* position especially the presence of a bromine substituent seem to play a crucial role in the cytotoxic activities. Besides, the molecular docking indicate that this class of heterocyclic molecules exhibits important binding energy and interaction with interesting residues of anticancer target such as, DHFR, VEGFR2, HER-2/neu, hCA-IX, CDK6 and LOX.

Supplementary Materials: The following are available online at www.mdpi.com/xxx/s1, details of the SRB assay, and depicted NMR (^{1}H, ^{13}C) and IR spectra of the compounds **3**, **5** and **7a–7l**.

Author Contributions: H.B.J. and R.C. brought the idea, managed the research, and prepared the manuscript; M.H. and A. R. prepared compounds for screening, M.H., A.H.H. and A.R. draft preparation, S.H. and N.H. conducted biological experiments and characterization. All authors have read and agreed to the published version of the manuscript.

Funding: We acknowledge the financial support within the funding program Open Access Publishing by the German Research Foundation (DFG).The authors also express thanks to the Ministry of Higher Education and Scientific Research of Tunisia for financial support (LR11ES39).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Acknowledgments: We like to thank D. Ströhl and his team for the NMR spectra and T. Schmidt for numerous MS spectra; IR, UV/vis spectra and elemental analyses were measured by M. Schneider; the cell lines were kindly provided by Th. Müller (Dep. of Haematology/Oncology, Martin-Luther University Halle-Wittenberg). We are also grateful to Researchers Supporting Project number (RSP-2021/17) at King Saud University, Riyadh, Saudi Arabia.

Conflicts of Interest: The authors declare no conflict of interest.

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