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The chemistry and pharmacology of privileged pyrroloquinazolines

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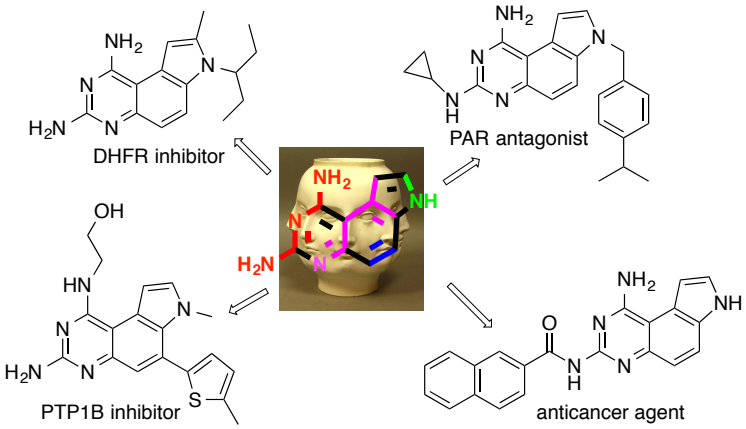
TOC Graphics

Pyrroloquinazoline is a potential privileged scaffold to produce different type of bioactivities resulting from different substitutions.



ABSTRACT

The advent of next-generation sequencing (NGS) technology has plummeted the cost of whole genome sequencing, which has provided a long list of putative drug targets for a variety of diseases ranging from infectious diseases to cancers. The majority of these drug targets are still awaiting high-quality small molecule ligands to validate their therapeutic potential and track their druggability. Screening compound libraries based on privileged scaffolds is an efficient strategy to identify potential ligands to distinct biological targets. 7*H*-Pyrrolo[3,2-*f*]quinazoline (PQZ) is a potential privileged heterocyclic scaffold with diverse pharmacological properties. A number of biological targets have been identified for different derivatives of PQZ. This review summarized the synthetic strategies to access the chemical space associated with PQZ and discussed their unique biological profiles.

1. Introduction

High throughput screening (HTS) has become one of the primary strategies to discover novel chemical modulators of various proteins involved in different disease states.[1] Often these proteins are discovered by genomic and epigenomic studies enabled by next-generation sequencing technology.[2, 3] When the protein targets are not well-characterized for rational drug design, HTS is perhaps the only avenue to identify the small molecule modulators. One of the keys to a successful HTS campaign is to have ready access to high-quality chemical library. Hundreds of thousands of small molecule compounds have been collected in the screening libraries in the public domain. Additional millions of compounds are in the possession of private sector in the large pharmaceutical companies. These libraries have been screened numerous times against multiple targets and pathways in the past two decades. As new biological targets are evolved from genomic studies, these libraries will continue to provide starting points to identify potential chemical modulators. Because many of the newly unveiled targets fall into the category of "undruggable" targets like transcription factors and structural proteins,[4-6] additional library members with unique structural and biological features will be in critical need. In this context, chemical library members from privileged chemical scaffolds will be highly desirable.

As initially put by Evans,[7] a privileged chemical structure is defined as a chemical scaffold with distinct and specific biological activities towards multiple unrelated biological targets. For example, benzodiazepine is a classical privileged scaffold.

Prominent members with benzodiazepine scaffold include widely used sedative drugs diazepam (Figure 1) and nitrazepam.[8] These drugs bind γ-aminobutyric acid ($GABA_A$)-receptors to potentiate the inhibitory neurotransmitter GABA's action.[9] Other members with benzodiazepine scaffold have been shown to be cholecystokinin (CCK) receptor antagonists[7, 10] (e.g. L-364,718) and apoptosis-inducing agents[11, 12] (e.g. Bz-423) (Figure 1). Spirooxindole is another widely studied privileged scaffold that is present in both synthetic and naturally occurring products with drug-like properties. These include MDM2 inhibitor MI-77301,[13] muscarinic M1 and 5-HT_2 receptor modulator pteropodine,[14] and cell cycle inhibitor spirotryprostatin A (Figure 1).[15, 16] Chemical libraries from privileged scaffolds thus represents rich source of chemical ligands to various biological targets. There have been excellent reviews to cover the array of privileged scaffolds.[17, 18] In this review, we will discuss the chemistry and pharmacological activities of 7*H*-pyrrolo[3,2-*f*]quinazoline-1,3-diamine (PQZ). We conclude that PQZ is emerging as a potential privileged scaffold.

2. Chemical Synthesis of PQZs

The synthesis of the tricyclic PQZs, 7*H*-pyrrolo[3,2-*f*]quinazoline-1,3-diamine (**2**) in particular, has attracted much of chemists' attention due to their wide range of pharmacological activities. For example, compound **2** potently inhibited human dihydrofolate reductase (DHFR) with K_i = 1 nM.[19] One core issue of the synthetic pathways is to construct the unique tricyclic skeleton. The other focus of the syntheses is to efficiently and regioselectively introduce different substitutions to different positions on the scaffold. Therefore, diverse methods have been employed to

synthesize functionalized PQZs. The first method to prepare the tricyclic ring system in **2** was reported by Ledig (Scheme 1)[20] and this method remains the method of choice with some variations. In his synthesis, 5-aminoindole hydrochloride (**1**) was chosen as the starting material to condense with sodium dicyanamide under reflux in 1-octanol for 13 h to directly afford PQZ **2**.

To avoid the long-time heating and consequent decomposition of intermediate **3** in Ledig's one-pot procedure, Jones et al isolated intermediate **3** and synthesized **2** through a two-step process as shown in Scheme 1.[21] With the assistance of boron trifluoride (BF_3) as a Lewis acid, the cyclization condition was much milder than Ledig's method. Importantly, the overall yield of **2** was higher and formation of the alternative linear isomer was efficiently suppressed. All of these were beneficial for product isolation and purification. We later found that the currently commercially available 5-aminoindole had to be converted into its corresponding hydrochloride in order to obtain high yield of **2**.[22]

The differential pK_as of the three nitrogen atoms (N^1, N^3 and N^7) is a key factor for regioselective substitution reactions on PQZ.[22] To predict the pK_a difference, Chen et al optimized the structure of **2** using quantum mechanics calculation at HF/6-31G** level of theory and calculated the Mulliken atomic charges.[23] The results suggested that the order of pK_a is $N^7<N^1\leq N^3$. Therefore, it was predicted that N^7–H could be selectively deprotonated for substitution. This hypothesis was consistent with experimental results. For example, regioselective N^7 alkylation[24] and acylation[22] were realized with benzylic halides and acylating reagents (e.g. acid anhydrides and

N-hydroxysuccinimide esters) under basic condition to afford **4** and **5**, respectively (Scheme 2). The minor pK_a difference between N^1 and N^3 was exploited to convert **5** to N^1-acylated analogs **6** with a strong base like NaH in DMF.[22]

To achieve regioselective N^3-acylation of **2**, Chen et al took advantage of the relatively high nucleophilicity of N^3.[22] Therefore, the N^1-amino group was temporarily protected as –OH by acidic hydrolysis[25] to give compound **7**. Nucleophilic acylation of **7** selectively generated **8**. Then, with the aid of BOP reagent,[26, 27] N^1 amine was regenerated using an S_NAr displacement reaction between ammonia (NH_3/MeOH) and **8** to afford N^3-acylated analogs **9**.

To illustrate the generality in exploiting the differential pK_as of the three nitrogen atoms (N^1, N^3 and N^7), Chen et al further demonstrated the synthesis of all the possible combinations of bisacetylated PQZs (Scheme 3).[22] For example, as N^7-H in **6a** and **8a** could be selectively deprotonated by NaH in DMF to react with acetic anhydride to provide bisacetylated PQZs **10** and **11**. Rearrangement of acetyl group from N^7 to N^1 in **11** gave N^1, N^3-biacetylated derivative **12**. Finally, N^1,N^3,N^7-triacetylated compound **13** could be obtained by heating a mixture of **2** and excess acetic anhydride in DMF at 100 °C.[22]

Ahn et al reported a synthesis of N^7-(isopropyl)benzylpyrrolo[3,2-*f*]quinazolines **17** and **18**, which were substituted by different amino groups at C^1 and C^3, respectively (Scheme 4).[24] In this synthesis, 5-nitroindole (**14)** was used as the starting material, which was *N*-alkylated with 4-isopropylbenzyl chloride followed by catalytic hydrogenation to afford substituted 5-aminoindole **15**. Compound **16** was obtained by

chlorination of corresponding hydroxyl intermediate to be obtained by cyclization between **15** and trichloromethyl isocyanate. Based on the differential leaving group ability of the chlorine atoms, different amino groups were sequentially introduced at position 1 and 3.

Kuyper and co-workers synthesized a series of 8-alkyl and 7,8-dialkyl-1,3-diaminopyrrolo[3,2-*f*]quinazolines (Scheme 5).[19] These syntheses were based on the original method developed by Ledig, but using different substituted 5-aminoindoles. The key intermediates, 5-nitroindole derivatives **26**, were obtained by two approaches. In the first one, an S_NAr substitution of 4-fluoronitrobenzene (**19**) or reductive amination of 4-nitroaniline (**20**) was employed to prepare *para*-substituted nitro anilines **21**. After iodination of **21** with ICl, an alkynyl moiety was introduced to the *ortho*-position of the amino group by palladium-catalyzed Sonogashira coupling to yield **22**. Conversion of **22** into **26** was catalyzed by CuI in DMF under reflux. In the alternative method to prepare **26**, the nitro group was introduced after construction of the indole ring in **25**. Finally, the target compounds **28** were prepared from intermediates **27** under the same conditions as reported by Ledig or Jones.[20, 21]

A series of 3-phenyl-pyrroloquinazolinones with platelet aggregation inhibitory activity were discovered by Ferlin et al (Scheme 6).[28] In their synthesis of the target compounds, condensation between **31** and substituted 5-aminoindoles **29** afforded **32**, which were cyclized at high temperature (250 ºC) to give the final products **33**. In an analogous synthesis of tetracyclic pyrimido[4,5-*c*]carbazoles **36**, montmorillonite K-10 clay was used as a Lewis acid to catalyze the cyclization of

N-indole-*N'*-ethoxycarbonylamidine compounds **35** (Scheme 7).[29]

Pyrrolo[3,2-*f*]quinazolinone was a motif in indolo[2,3-*a*]pyrimido[5,4-*c*]carbazoles **42** (Scheme 8), which showed potent inhibitory activity against renal cancer cells. These compounds were synthesized by a key C-C bond ring-closure reaction of **41**. This oxidation step was effected by photocyclization with iodine under continuous UV irradiation.[30]

3. Pharmacological activities of PQZs

A wide range of biological activities have been described for various substituted and fused PQZs. The biochemical targets behind these bioactivities were better understood for some than the others. We divided this section into the biochemical targets that were modulated by different PQZs. Representative members from each class are presented to illustrate their potential medical applications.

3.1 PQZs as dihydrofolate reductase (DHFR) inhibitors

DHFR is an enzyme that catalyzes the reduction of dihydrofolate to tetrahydrofolate in the presence of NADPH as the cofactor.[31] In proliferating cells, this DHFR-catalyzed reaction is the sole source to tetrahydrofolate, which is necessary for the one-carbon transfer reactions to synthesize purine, thymidine and some amino acids. Inhibition of DHFR will lead to blockage of DNA and protein synthesis, both of which are critical for the proliferating cells. Therefore, DHFR inhibitors exhibit activity against various cancers, bacterial infections and parasitic diseases. A large number of PQZs have been evaluated as inhibitors of DHFR from various species.[19] In fact, the original design of **2** was to mimic the known structural

elements required for DHFR inhibition.[19, 20] These structure-activity relationship (SAR) studies showed that a large hydrophobic group appended to N^7 was optimal for DHFR inhibition.[19, 32] Therefore, the majority of structural analogs of **2** in the literature possess an N^7-alkyl group.[19, 20]

3.1.1 Antimicrobial agents

Castaldo et al reported a series of PQZs DHFR inhibitors against *Candida* infections.[33] Among these compounds, **43** and **44** (Figure 2) showed the most potent inhibitory activity against 40 clinical *Candida* isolates, with mean minimal inhibitory concentrations (MICs) of 0.64 μg/mL and 1.39 μg/mL, respectively. In comparison with the treatment with these agents alone, the combination of **44** and sulfamethoxazole (SMX), a structural analog of *para*-aminobenzoic acid (PABA) to suppress the synthesis of folic acid, resulted in synergistic effect in inhibiting the growth of susceptible *Candida* isolates. This synergistic effect was due to the sequential suppression of dihydropteroate synthetase (DHPS) and DHFR, both of which are key enzymes in the synthesis of tetrahydrofolic acid.

Kuyper et al designed and synthesized 7,8-dialkyl-1,3-diaminopyrrolo[3,2-*f*]quinazolines as novel DHFR inhibitors.[19] These compounds were designed based on the X-ray crystal structure of *Candida albicans* DHFR in complex with a PQZ derivative.[34] SAR studies indicated that alkyl substitutions at N^7 were indispensable to retain potent DHFR inhibitory activity. This was consistent with the binding mode that the N^7 substituent pointed toward a large hydrophobic pocket in DHFR.[19] In addition, they found that the inhibitory activity on

fungal DHFR increased significantly with compounds having C^8 substituted with a small group. As one of the most potent inhibitors among these congeners, **45** (Figure 2) not only exhibited potent inhibitory potency against *C. albicans* and human DHFR (K_is 30 pM and 0.3 pM respectively), but also efficiently protected the mice from infections caused by *Pneumocystis carinii* and *Candida albicans*.[19]

3.1.2 Anti-cancer agents

McCormack et al evaluated a series of PQZs as potential anticancer agents.[35] A benzyl group attached to N^7 was crucial to retain excellent inhibitory potency, while the non-substituted or methylated derivatives were almost three orders of magnitude less potent. N^7-Heteroaromatic substituents such as picolyl groups conferred a comparable activity to the N^7-benzyl groups. Consistent with the known roles of DHFR in cancer cells, the potent DHFR inhibitor **46** (Figure 2) significantly suppressed the growth of L1210 leukemia cell line (IC_{50} 40 nM). This was similar to the inhibitory effect of methotrexate, a widely used anticancer DHFR inhibitor.

In addition to the mentioned antimicrobial activity (see section 3.1.1), compound **45** also showed potent inhibitory activity against the growth of HCT-8 colon cancer cells (IC_{50} 0.74 nM) and many other human cancer cell lines.[19] Importantly, **45** did not seem to be a substrate for human P-glycoprotein because it was equipotent in the parental and multiple drug resistance (MDR) cell lines such as KBV-1, MCF-7/ADR and P388/ADR.[19] Furthermore, **45** significantly extended the survival time of the mice with intraperitoneal implants of P388 and P388/ADR tumors *in vivo*.

Compound **45** showed excellent pharmacokinetic profile in male beagle dogs.[36]

After intravenous and oral administration, the corresponding mean plasma half-lives ($t_{1/2}$) were 2.8 ± 0.66 and 4.0 ± 0.76 h. The maximum concentration (C_{max} = 0.17 ± 0.039 μg/mL) was reached in about 1.8 h after oral administration. In comparison with another DHFR inhibitor piritrexim (PTX), **45** exhibited more extensive distribution in tissues, with a volume of distribution (V_d) about 9.3 L/g. The absolute bioavailability in beagle dogs was 49 ± 16%. Moreover, the pharmacokinetic profile of **45** was not significantly affected by co-administration of calcium leucovorin that is often used to prevent the adverse effect of DHFR inhibitors in the clinic. These preclinical data suggested that **45** was a good candidate for further clinical development.

3.2 PQZs as protease-activated receptor (PAR) antagonists

Thrombin is a plasma trypsin-like serine protease and promotes platelet activation and aggregation.[37] The cellular effect of thrombin is partially mediated by G protein-coupled protease-activated receptors (PARs).[38] Activation of PARs is initiated by the cleavage of the receptor's N-terminal exodomain by thrombin. Then the resulting new N-terminus, acting as a tethered ligand, binds to an extracellular loop of the receptor and induces transmembrane signaling.[38, 39] Among the four identified PARs (PAR1, PAR2, PAR3 and PAR4), PAR1 is critical for modulation of thrombin signalling in vascular endothelial cells.[40] Moreover, PAR1, expressed on the surface of human platelets, shows higher affinity to thrombin than PAR4. This higher affinity translates to activation of human platelets by lower concentrations of thrombin.[41, 42]

Since inhibition of platelet aggregation could be a promising treatment strategy

for thrombosis and restenosis,[43] there has been strong interest in developing PAR antagonists. Researchers at then Schering-Plough initiated a HTS campaign to identify non-peptidic PAR1 antagonists.[24] From this screening, PQZ **47** (Figure 3) was identified as a hit with an IC_{50} of 300 nM. A series of related PQZs were designed and synthesized to investigate their SARs. It was found that modifications including alkylation, acylation and sulfonylation at N^7 other than benzylation significantly decreased binding activity to PAR1. Modifications at N^1 and N^3 generated compounds with variable potency. Small alkyl groups at N^3 were favoured while substitution at N^1 was generally detrimental. In the absence of a crystal structure of the complex between PAR1 and PQZs, these SAR results were rationalized by the later-developed 3D-QSAR (three-dimensional quantitative structure-activity relationship) models.[44]

Among the synthesized PQZs as PAR antagonists, N^3-cyclopropylated compounds **48** (SCH79797) and **49** (SCH203099) were the most potent analogs (Figure 3).[24] Both of the compounds significantly blocked the binding between PAR1 on the platelet membranes and a PAR1 peptidic agonist ha-TRAP [Ala-Phe(p-F)-Arg-Cha-HArg-Tyr-NH_2][45] with IC_{50} values of 56 and 52 nM, respectively. Although none of these PAR antagonists were evaluated for DHFR inhibition, **47** would be predicted to be a sub-nM to low nM DHFR inhibitor based on known SAR,[19, 32, 35] suggesting its preferential selectivity towards DHFR. However, it is well-accepted that primary amino groups at N^1 and N^3 are required for optimal DHFR inhibition. This is rationalized by the crystal structure of a PQZ in complex with DHFR where extensive hydrogen-bond networks are involved with the primary

amino groups.[46] Importantly, additional pockets do not exist in DHFR to tolerate substitutions at N^1 and N^3.[46] By this reasoning, compounds **48** and **49** would not be expected to be potent DHFR inhibitors, suggesting that different substitutions in PQZ can impart different target selectivity. This feature is often the case for privileged scaffolds.

When tested in functional assays, compounds **48** and **49** blocked platelet aggregation induced either by ha-TRAP or thrombin.[24] Further biochemical and pharmacological characterization of **48** suggested that it presented high PAR subtype selectivity for PAR1 by using subtype-selective agonists.[24, 47] For example, it did not inhibit platelet aggregation induced by a PAR4-selective agonist (GYPGQV).[24] When the PAR selectivity profile was evaluated in hCASMC (human coronary artery smooth muscle cells), **48** substantially inhibited the transient increase of cytosolic free Ca^{2+} concentration ($[Ca^{2+}]_i$) elicited by thrombin (3 nM) or TFLLRNPNDK-NH_2 (30 μM), a PAR-1-selective agonist. On the other hand, it could not suppress the increase of $[Ca^{2+}]_i$ induced by a PAR2-selective agonist, SLIGKV-NH_2 (10 μM).[47]

In addition to its role in blood clotting, thrombin is also an important factor to mediate myocardial ischemia/reperfusion (I/R) injury.[48] Functional inhibition of thrombin could result in a significant decrease in infarct size following I/R.[49] Based on these findings, PAR1 was hypothesized as a potential target for I/R.[50] Before or during ischemia, treatment with **48** introduced an immediate reduction of infarct size and decrease of myocardial necrosis.[50] If given after ischemia, **48** could also decrease adverse left ventricular (LV) remodelling and preserve LV function through late stage

antifibrotic events.[51] Compound **48** down-regulated the downstream signalling activities of PAR1, which is a crucial initiator of tissue stiffening in cardiac fibroblasts after I/R injury. Therefore, PAR1 inhibitors could be a potential therapy to both myocardial I/R injury and postinfarction cardiomyopathy.

During a study on the fused pyrroloheterocycles, Ferlin et al reported new phenyl pyrroloquinolinones as potential antimitotic agents.[52] They also discovered that a series of 3-phenyl-pyrroloquinazolinones displayed platelet aggregation inhibitory activity.[28] Compound **50** (Figure 3) exhibited the most potent antagonistic activity towards the thrombin-induced platelet aggregation among these derivatives. The comparison between **50** and other analogs showed that both alkyl groups at pyrrolic nitrogen and a methoxy group on the appendant phenyl ring attenuated inhibitory activity. Besides blocking platelet activation, **50** also affected PAR agonist-induced increase of cytosolic [Ca^{2+}] in platelets. Although these biological readouts would be consistent with antagonism of PAR, direct PAR binding data were not established. In fact, its inhibitory effect on platelet aggregation induced by PAR-independent collagen would suggest alternative mechanism of action than PAR inhibition.[28] Overall, the SAR of PQZs as PAR antagonists is distinctly different from that of DHFR inhibition.

3.3 PQZs as protein tyrosine phosphatase 1B (PTP1B) inhibitors

PTP1B is the first protein tyrosine phosphatase to be cloned. Functional studies of PTP1B provided convincing evidence that it is a negative regulator of insulin sensitivity.[53, 54] Therefore, pharmacological inhibitors of PTP1B would represent a

promising strategy to develop therapeutics for type 2 diabetes (T2D).[55] Roche scientists screened their internal collection of compounds for small molecule PTP1B inhibitors to yield a series of substituted PQZs (Figure 4).[56-58] A large number of PQZs, most of which contained an aryl substitution at C^6, were prepared to evaluate their PTP1B inhibitory activity. No detailed SAR studies have been published, but the IC_{50} for these compounds ranged from 1.09-91.79 μM,[58] which is ~1000 fold less potent than the prototypical PQZ DHFR inhibitors. Two representative compounds **51** and **52** are shown in Figure 4. NMR studies showed that these compounds do not directly bind to the phosphate binding pocket, suggesting a unique mechanism of action.[56]

3.4 Miscellaneous targets

Guan et al followed up a series of antimalarial PQZs initially prepared by Ledig[20] and identified WR227825 (Figure 5), which exhibited potent antimalarial activity both *in vitro* and *in vivo*, but with high host toxicity.[25] To reduce the host toxicity, different types of substitution were introduced to the amino groups at C^1 and C^3, such as alkyl and acyl groups. One such modified compound **53** (Figure 5) not only showed excellent antimalarial activity against various clones of *Plasmodium falciparum in vitro*, but also good efficacy in mice and non-human primates. Compound **53** could be rapidly deacetylated in plasma to generate WR227825 and therefore was considered as a prodrug.[59] Although it has not been established if WR227825 inhibits *pf*DHFR biochemically, its structure would predict it was a potent *pf*DHFR inhibitor and its antimalarial activity likely arose from *pf*DHFR inhibition.

The safety profile of **53** improved significantly compared to that of WR227825.[60]

In the adult rat *P. berghei* infection model, the therapeutic index of **53** was 80, while that of WR227825 was only 10. The metabolism of **53** to WR227825 included three steps of sequential hydrolysis of acetamide.[59] Since the metabolic intermediates had similar activity to WR227825, the therapeutic potency of **53** was not affected by such transformations *in vivo*. In fact, the multiple-step metabolic process was the major reason for its decreased host toxicity to allow gradual release of toxic WR227825.[59]

Electrostatic complementarity is a crucial force for binding interactions between small molecule ligands and receptors.[61-63] Chen et al designed and synthesized a series of mono-*N*-acylated PQZs to modulate the electrostatics of PQZ ligands in a different way from the other reported PQZ modifications.[22] These regioselectively mono-*N*-acylated compounds were evaluated in a phenotypic anti-proliferative assay with two triple negative breast cancer cell lines, MDA-MB-231 and MDA-MB-468. It was found that N^7-acylated derivatives dramatically decreased their solubility in DMSO or aqueous buffers, which precluded detailed biological evaluations. On the other hand, the N^1- and N^3-acylated analogs displayed reasonable solubility and they presented variable anti-proliferative activity. In general, the N^1-acylated compounds were less potent than N^3-acylated ones. Within the series of N^3-acylated PQZs, N^3-arylacyl substitution was favoured.[22] Among these compounds, **54** exhibited superior activity in both of the two cell lines (Figure 5). Importantly, **54** was not toxic to normal human mammary epithelial cells. The anti-proliferative activity of **54** was not due to human DHFR inhibition and its biological target(s) to mediate its selective toxicity in cancer cells remain to be identified.[22]

In addition to the diverse biological activities mentioned above, other bioactivities have also been reported for PQZs (Table 1). For example, PQZ **55** was reported to inhibit the replication of herpes simplex virus 1 (HSV-1) with IC_{50} of 15 μM (Figure 5).[64] PQZ **56** was shown to weakly (IC_{50} ~100 μM) inhibit serum paraoxonase (PON), a calcium-dependent esterase to hydrolyze organophosphate (Figure 5).[65] Other more extensively fused PQZs have also been shown to present different biological activities. Tetracyclic pyrimido[4,5-*c*]carbazoles **36** (Scheme 7) showed moderate potency to inhibit growth of leukemia cell line HL60.[29] Through replacement of the lactam ring in the prototypical kinase inhibitor staurosporine (**57**) with a bioisosteric pyrimidone ring, indolo[2,3-*a*]pyrimido[5,4-*c*]carbazole **58** was obtained as a novel anti-cancer agent to inhibit proliferation of various cancer cell lines in the low μM concentration range (Figure 5).[30] Finally, compound **2** is currently collected in the National Institutes of Health (NIH) molecular library and has been screened more 600 times in different assays,[66] and it was found to be active as an inhibitor of dual specificity tyrosine-phosphorylation-regulated kinase 1A (Dyrk1A), DNA repair proteins Rad52 and Rad54 (Table 1). These screening results still await further validation. It is known that compound **2** is brightly fluorescent with excitation at 340 nm and emission at 450 nm[35] and appropriate controls should be included during validation to eliminate fluorescent interference. Interestingly, a survey of screenings performed at NIH suggested that compound **2** does not seem to be a good scaffold for kinases although aminoquinazolines are present in many potent kinase inhibitors.[67-70] Among the 22 screening assays deposited on PubChem against different kinases, only Dyrk1A was

weakly inhibited with an IC_{50} of 5.51 μM.[66]

4. Conclusions and perspectives

Since the initial design and synthesis of PQZ in the 1970s, a large number of PQZs has been prepared from different medicinal chemistry programs. Although PQZs were originally designed as DHFR inhibitors, many new biological activities have been described for the PQZs. These new discoveries would not be possible without the aid of HTS assays. As the PQZs will be continued to be assayed in new screening programs, other bioactivities will undoubtedly be uncovered. These results clearly show that PQZ is emerging as a potential privileged scaffold. Different synthetic strategies have been developed to differentially functionalize the PQZ nucleus, allowing us to access the previously untouched chemical space to fuel further discovery of novel bioactivities. As discussed above, many of the bioactivities associated with PQZs were from phenotypic screening assays without knowing their exact biochemical targets. Identification of their targets and understanding their selectivity profiles will be critical in delivering high quality chemical probes to interrogate the biology and/or therapeutic candidates for various diseases. The recent development in chemical proteomics will definitively contribute to our better understanding of these latter aspects.[71, 72] Further biological studies with compounds derived from PQZ will likely provide novel chemical tools to modulate various targets and potential therapeutics for different diseases.

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References

1. T. Kodadek, *Nat. Chem. Biol.*, 2010, **6**, 162-165.
2. J. C. Mwenifumbo and M. A. Marra, *Nat. Rev. Genet.*, 2013, **14**, 321-332.
3. W. F. Fricke and D. A. Rasko, *Nat. Rev. Genet.*, 2014, **15**, 49-55.
4. T. C. G. A. Network, *Nature*, 2012, **490**, 61-70.
5. J. E. Darnell, *Nat. Rev. Cancer*, 2002, **2**, 740-749.
6. X. Xiao, B. X. Li, B. Mitton, A. Ikeda and K. M. Sakamoto, *Curr. Cancer Drug Targets*, 2010, **10**, 384-391.
7. B. E. Evans, K. E. Rittle, M. G. Bock, R. M. DiPardo, R. M. Freidinger, W. L. Whitter, G. F. Lundell, D. F. Veber and P. S. Anderson, *J. Med. Chem.*, 1988, **31**, 2235-2246.
8. L. H. Sternbach, *J. Med. Chem.*, 1979, **22**, 1-7.
9. E. Sigel, *Curr. Top. Med. Chem.*, 2002, **2**, 833-839.
10. R. S. Chang and V. J. Lotti, *Proc. Natl. Acad. Sci. U. S. A.*, 1986, **83**, 4923-4926.
11. N. B. Blatt, J. J. Bednarski, R. E. Warner, F. Leonetti, K. M. Johnson, A. Boitano, R. Yung, B. C. Richardson, K. J. Johnson, J. A. Ellman, A. W. Opipari, Jr. and G. D. Glick, *J. Clin. Invest.*, 2002, **110**, 1123-1132.
12. A. Boitano, J. A. Ellman, G. D. Glick and A. W. Opipari, Jr., *Cancer Res.*, 2003, **63**, 6870-6876.

13. S. Wang, W. Sun, Y. Zhao, D. McEachern, I. Meaux, C. Barriere, J. A. Stuckey, J. L. Meagher, L. Bai, L. Liu, C. G. Hoffman-Luca, J. Lu, S. Shangary, S. Yu, D. Bernard, A. Aguilar, O. Dos-Santos, L. Besret, S. Guerif, P. Pannier, D. Gorge-Bernat and L. Debussche, *Cancer Res*., 2014, **74**, 5855-5865.

14. T. H. Kang, K. Matsumoto, M. Tohda, Y. Murakami, H. Takayama, M. Kitajima, N. Aimi and H. Watanabe, *Eur. J. Pharmacol*., 2002, **444**, 39-45.

15. C. B. Cui, H. Kakeya and H. Osada, *Tetrahedron*, 1996, **52**, 12651–12666.

16. S. Edmondson, S. J. Danishefsky, L. Sepp-Lorenzino and N. Rosen, *J. Am. Chem. Soc*., 1999, **121**, 2147-2155.

17. M. E. Welsch, S. A. Snyder and B. R. Stockwell, *Curr. Opin. Chem. Biol*., 2010, **14**, 347-361.

18. D. H. Drewry and R. Macarron, *Curr. Opin. Chem. Biol*., 2010, **14**, 289-298.

19. L. F. Kuyper, D. P. Baccanari, M. L. Jones, R. N. Hunter, R. L. Tansik, S. S. Joyner, C. M. Boytos, S. K. Rudolph, V. Knick, H. R. Wilson, J. M. Caddell, H. S. Friedman, J. C. W. Comley and J. N. Stables, *J. Med. Chem*., 1996, **39**, 892-903.

20. K. W. Ledig, US4118561, 1978.

21. M. L. Jones, L. F. Kuyper, V. L. Styles and J. M. Caddell, *J. Heterocycl. Chem*., 1994, **31**, 1681-1683.

22. J. Chen, A. Kassenbrock, B. X. Li and X. Xiao, *MedChemComm*, 2013, **4**, 1275-1282.

23. R. S. Mulliken, *J. Chem. Phys.*, 1955, **23**, 1833-1840.

24. H.-S. Ahn, L. Arik, G. Boykow, D. A. Burnett, M. A. Caplen, M. Czarniecki, M. S. Domalski, C. Foster, M. Manna, A. W. Stamford and Y. Wu, *Bioorg. Med. Chem. Lett.*, 1999, **9**, 2073-2078.

25. J. Guan, Q. Zhang, M. O'Neil, N. Obaldia, A. Ager, L. Gerena and A. J. Lin, *Antimicrob. Agents Chemother.*, 2005, **49**, 4928-4933.

26. Z.-K. Wan, S. Wacharasindhu, E. Binnun and T. Mansour, *Org. Lett.*, 2006, **8**, 2425-2428.

27. Z.-K. Wan, S. Wacharasindhu, C. G. Levins, M. Lin, K. Tabei and T. S. Mansour, *J. Org. Chem.*, 2007, **72**, 10194-10210.

28. M. G. Ferlin, C. Borgo and R. Deana, *Eur. J. Med. Chem.*, 2012, **48**, 275-283.

29. J. Debray, W. Zeghida, B. Baldeyrou, C. Mahieu, A. Lansiaux and M. Demeunynck, *Bioorg. Med. Chem. Lett.*, 2010, **20**, 4244-4247.

30. L. T. Pierce, M. M. Cahill, H. J. Winfield and F. O. McCarthy, *Eur. J. Med. Chem.*, 2012, **56**, 292-300.

31. J. R. Schnell, H. J. Dyson and P. E. Wright, *Annu. Rev. Biophys. Biomol. Struct.*, 2004, **33**, 119-140.

32. J.-C. Chen, L. Qian, Y. Shen, L.-M. Chen and K.-C. Zheng, *Chin. J. Chem* . 2006, **24**, 1531-1537.

33. R. A. Castaldo, D. W. Gump and J. J. McCormack, *Antimicrob. Agents Chemother.*, 1979, **15**, 81-86.

34. D. P. Baccanari, R. L. Tansik, S. S. Joyner, M. E. Fling, P. L. Smith and J. H.

Freisheim, *J. Biol. Chem.*, 1989, **264**, 1100-1107.

35. J. J. McCormack, B. A. Allen, K. W. Ledig and B. L. Hillcoat, *Biochem. Pharmacol.*, 1979, **28**, 3227-3229.

36. S. D. Studenberg, J. L. Woolley, D. V. Deangelis and W. A. Wargin, *Biopharm. Drug Disposition*, 1997, **18**, 433-442.

37. J. M. Stassen, J. Arnout and H. Deckmyn, *Curr. Med. Chem.*, 2004, **11**, 2245-2260.

38. C. Zhang, Y. Srinivasan, D. H. Arlow, J. J. Fung, D. Palmer, Y. Zheng, H. F. Green, A. Pandey, R. O. Dror, D. E. Shaw, W. I. Weis, S. R. Coughlin and B. K. Kobilka, *Nature*, 2012, **492**, 387-392.

39. T.-K. H. Vu, D. T. Hung, V. I. Wheaton and S. R. Coughlin, *Cell*, 1991, **64**, 1057-1068.

40. S. R. Coughlin, *Nature*, 2000, **407**, 258-264.

41. L. Covic, A. L. Gresser and A. Kuliopulos, *Biochemistry*, 2000, **39**, 5458-5467.

42. M. J. Shapiro, E. J. Weiss, T. R. Faruqi and S. R. Coughlin, *J. Biol. Chem.*, 2000, **275**, 25216-25221.

43. D. Baykal, J. F. Schmedtje Jr and M. S. Runge, *Am. J. Cardiol.*, 1995, **75**, 82B-87B.

44. A. Dixit, S. K. Kashaw, S. Gaur and A. K. Saxena, *Biorg. Med. Chem.*, 2004, **12**, 3591-3598.

45. D.-M. Feng, D. F. Veber, T. M. Connolly, C. Condra, M.-J. Tang and R. F.

Nutt, *J. Med. Chem.*, 1995, **38**, 4125-4130.

46. M. Whitlow, A. J. Howard, D. Stewart, K. D. Hardman, L. F. Kuyper, D. P. Baccanari, M. E. Fling and R. L. Tansik, *J. Biol. Chem.*, 1997, **272**, 30289-30298.

47. H.-S. Ahn, C. Foster, G. Boykow, A. Stamford, M. Manna and M. Graziano, *Biochem. Pharmacol.*, 2000, **60**, 1425-1434.

48. P. Raivio, R. Lassila and J. Petäjä, *Ann. Thorac. Surg.*, 2009, **88**, 318-325.

49. A. J. Chong, T. H. Pohlman, C. R. Hampton, A. Shimamoto, N. Mackman and E. D. Verrier, *Ann. Thorac. Surg.*, 2003, **75**, S649-S655.

50. J. L. Strande, A. Hsu, J. Su, X. Fu, G. J. Gross and J. E. Baker, *Basic Res. Cardiol.*, 2007, **102**, 350-358.

51. D. L. Sonin, T. Wakatsuki, K. V. Routhu, L. M. Harmann, M. Petersen, J. Meyer and J. L. Strande, *J. Cardiovasc. Pharmacol. Ther.*, 2013, **18**, 460-475.

52. V. Gasparotto, I. Castagliuolo and M. G. Ferlin, *J. Med. Chem.*, 2007, **50**, 5509-5513.

53. M. Elchebly, P. Payette, E. Michaliszyn, W. Cromlish, S. Collins, A. L. Loy, D. Normandin, A. Cheng, J. Himms-Hagen, C.-C. Chan, C. Ramachandran, M. J. Gresser, M. L. Tremblay and B. P. Kennedy, *Science*, 1999, **283**, 1544-1548.

54. L. D. Klaman, O. Boss, O. D. Peroni, J. K. Kim, J. L. Martino, J. M. Zabolotny, N. Moghal, M. Lubkin, Y.-B. Kim, A. H. Sharpe, A. Stricker-Krongrad, G. I. Shulman, B. G. Neel and B. B. Kahn, *Mol. Cell. Biol.*,

2000, **20**, 5479-5489.

55. A. P. Combs, *J. Med. Chem.*, 2010, **53**, 2333-2344.

56. A. W.-H. Cheung, B. Banner, J. Bose, K. Kim, S. Li, N. Marcopulos, L. Orzechowski, J. A. Sergi, K. C. Thakkar, B.-B. Wang, W. Yun, C. Zwingelstein, S. Berthel and A. R. Olivier, *Bioorg. Med. Chem. Lett.*, 2012, **22**, 7518-7522.

57. S. J. Berthel, A. W.-H. Cheung, K. Kim, K. C. Thakkar and W. Yun, WO 2004101568, 2004.

58. S. J. Berthel and K. C. Thakkar, *US Pat.*, US 7226915 B2, 2004.

59. Q. Li, M. P. Kozar, T. W. Shearer, L. H. Xie, A. J. Lin, K. S. Smith, Y. Si, L. Anova, J. Zhang, W. K. Milhous and D. R. Skillman, *Antimicrob. Agents Chemother.*, 2007, **51**, 2898-2904.

60. L. H. Xie, Q. Li, A. J. Lin, K. Smith, J. Zhang and D. S. Skillman, *Antimicrob. Agents Chemother.*, 2006, **50**, 1649-1655.

61. X. Xiao, S. Antony, Y. Pommier and M. Cushman, *J. Med. Chem.*, 2005, **48**, 3231-3238.

62. N. Muzet, B. Guillot, C. Jelsch, E. Howard and C. Lecomte, *Proc. Natl. Acad. Sci.*, 2003, **100**, 8742-8747.

63. E. Kangas and B. Tidor, *J. Phys. Chem. B*, 2001, **105**, 880-888.

64. I. B. Dicker, J. W. Blasecki and S. Seetharam, *Antiviral Res.*, 1995, **28**, 213-224.

65. M. Aviram, M. Rosenblat, C. L. Bisgaier, R. S. Newton, S. L. Primo-Parmo

and B. N. La Du, *J. Clin. Invest.*, 1998, **101**, 1581-1590.

66. *The PubChem Project*, http://pubchem.ncbi.nlm.nih.gov, Accessed Jun 26, 2014.

67. E. F. DiMauro, J. Newcomb, J. J. Nunes, J. E. Bemis, C. Boucher, J. L. Buchanan, W. H. Buckner, V. J. Cee, L. Chai, H. L. Deak, L. F. Epstein, T. Faust, P. Gallant, S. D. Geuns-Meyer, A. Gore, Y. Gu, B. Henkle, B. L. Hodous, F. Hsieh, X. Huang, J. L. Kim, J. H. Lee, M. W. Martin, C. E. Masse, D. C. McGowan, D. Metz, D. Mohn, K. A. Morgenstern, A. Oliveira-dos-Santos, V. F. Patel, D. Powers, P. E. Rose, S. Schneider, S. A. Tomlinson, Y. Y. Tudor, S. M. Turci, A. A. Welcher, R. D. White, H. Zhao, L. Zhu and X. Zhu, *J. Med. Chem.*, 2006, **49**, 5671-5686.

68. R. Morphy, *J. Med. Chem.*, 2010, **53**, 1413-1437.

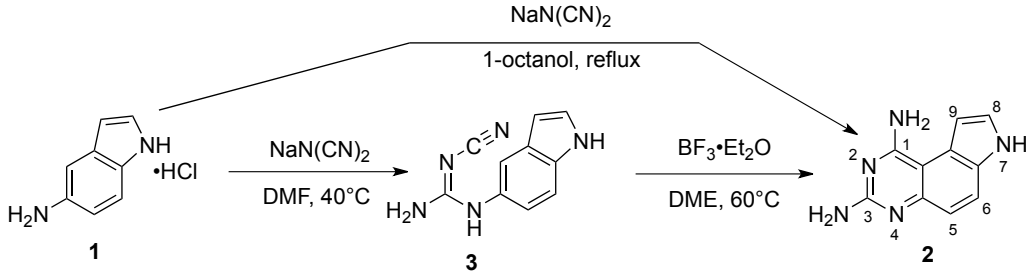
69. L. Ren, K. A. Ahrendt, J. Grina, E. R. Laird, A. J. Buckmelter, J. D. Hansen, B. Newhouse, D. Moreno, S. Wenglowsky, V. Dinkel, S. L. Gloor, G. Hastings, S. Rana, K. Rasor, T. Risom, H. L. Sturgis, W. C. Voegtli and S. Mathieu, *Bioorg. Med. Chem. Lett.*, 2012, **22**, 3387-3391.

70. M. M. Vasbinder, B. Aquila, M. Augustin, H. Chen, T. Cheung, D. Cook, L. Drew, B. P. Fauber, S. Glossop, M. Grondine, E. Hennessy, J. Johannes, S. Lee, P. Lyne, M. Mortl, C. Omer, S. Palakurthi, T. Pontz, J. Read, L. Sha, M. Shen, S. Steinbacher, H. Wang, A. Wu and M. Ye, *J. Med. Chem.*, 2013, **56**, 1996-2015.

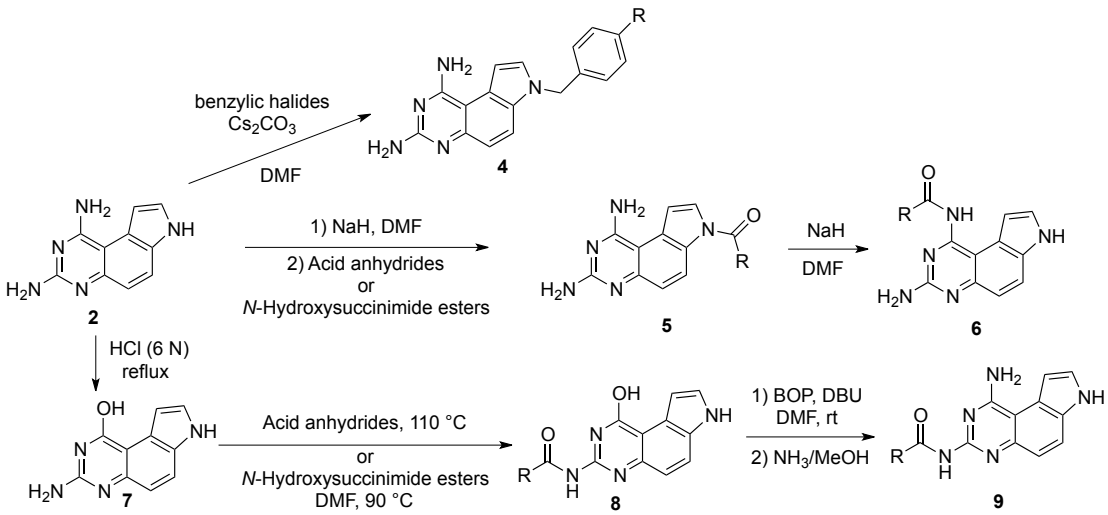
71. L. Burdine and T. Kodadek, *Chem. Biol.*, 2004, **11**, 593-597.

72. M. Schenone, V. Dancik, B. K. Wagner and P. A. Clemons, *Nat. Chem. Biol.*, 2013, **9**, 232-240.

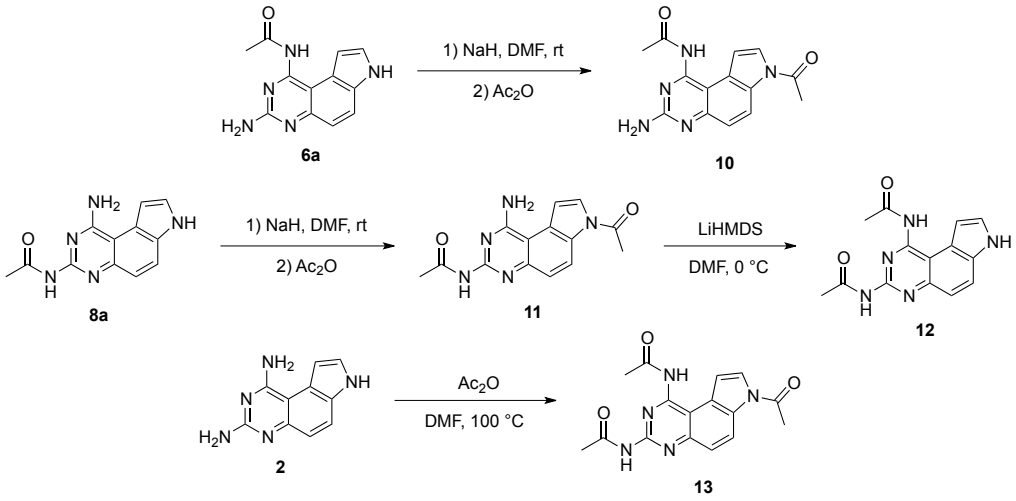
Scheme 1. Synthesis of PQZ **2**.



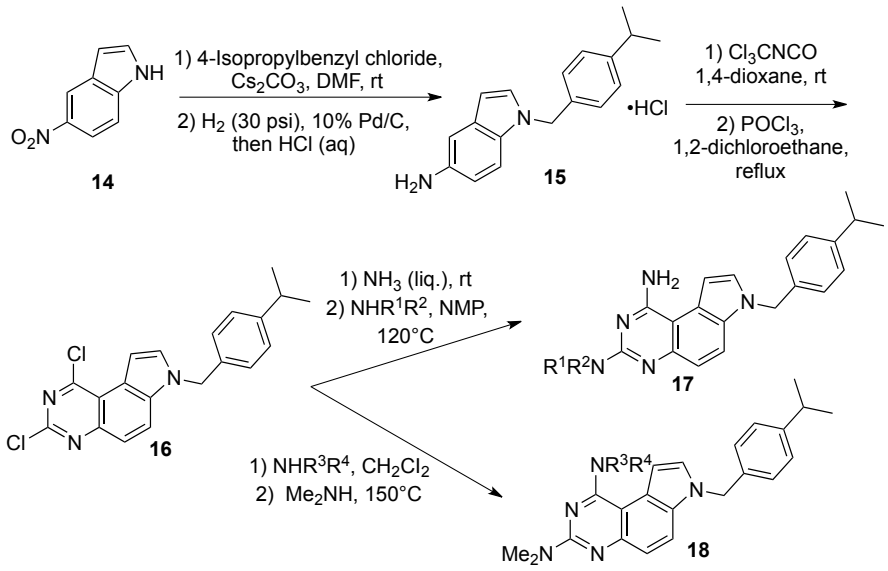
Scheme 2. Regioselectively synthesis of mono-*N*-substituted PQZs **4, 5, 6** and **9**.



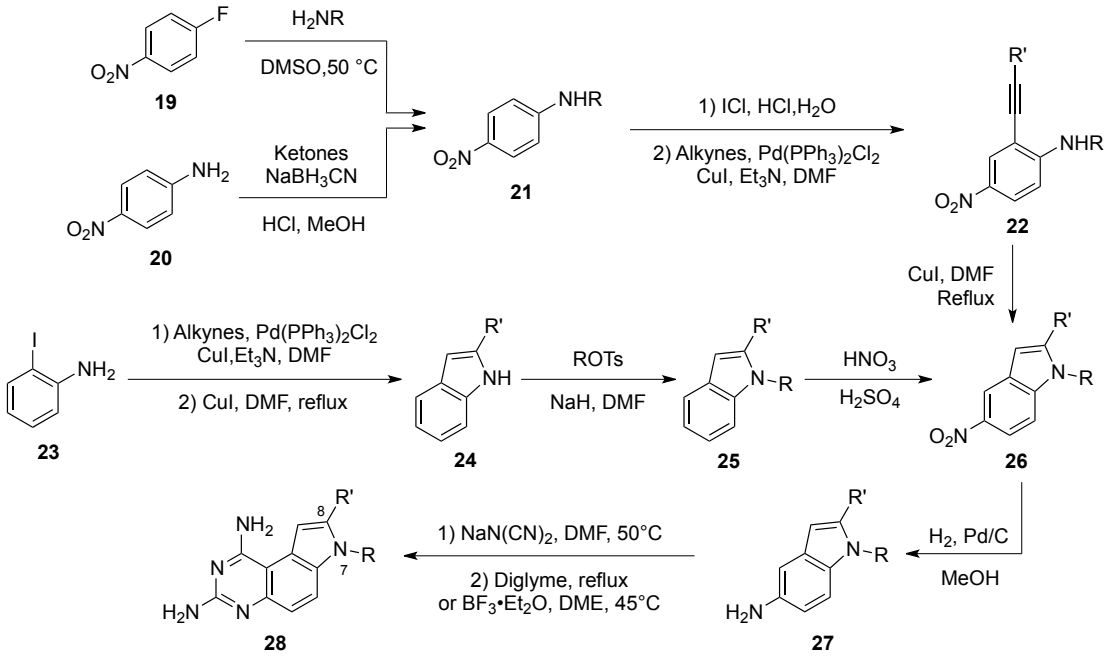
Scheme 3. Synthesis of bis- and tri-acetylated PQZs **10, 11, 12** and **13**.



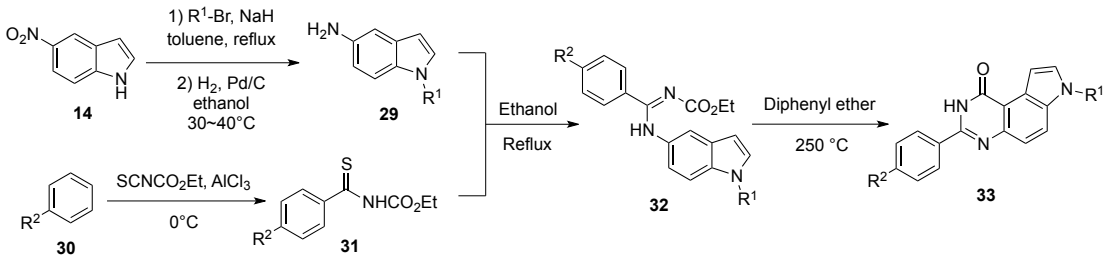
Scheme 4. Synthesis of PQZs **17** and **18**.



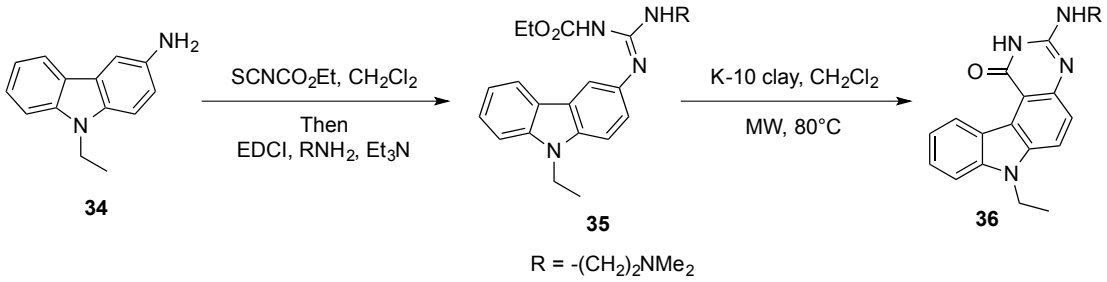
Scheme 5. Synthesis of 8-substituted PQZs **28**.



Scheme 6. Synthesis of 3-phenyl-pyrroloquinazolinones **33**.



Scheme 7. Synthesis of pyrimido[4,5-*c*]carbazole **36**.



Scheme 8. Synthesis of indolo[2,3-*a*]pyrimido[5,4-*c*]carbazoles **42**.

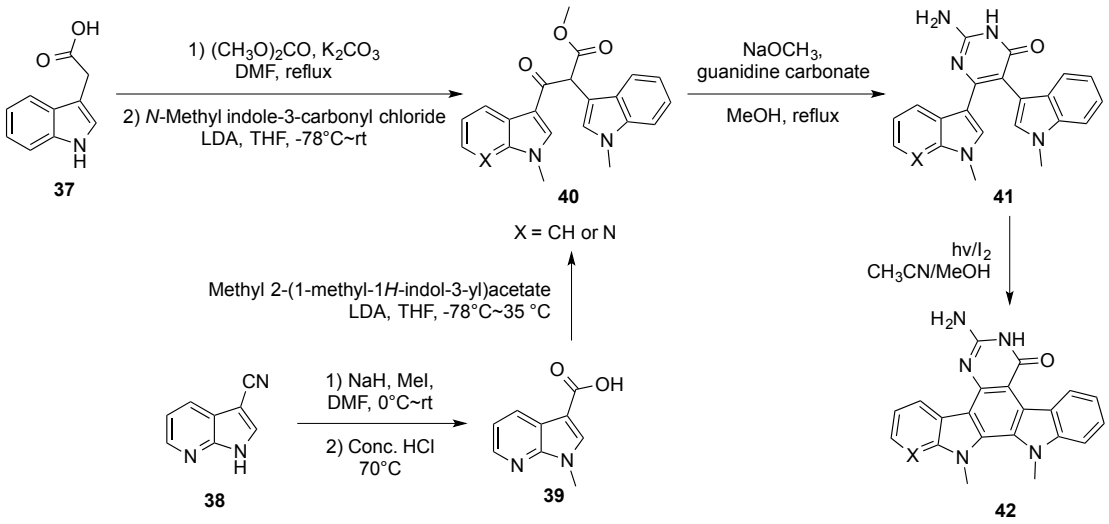


Figure 1. Selected structures with privileged benzodiazepine or spirooxindole.

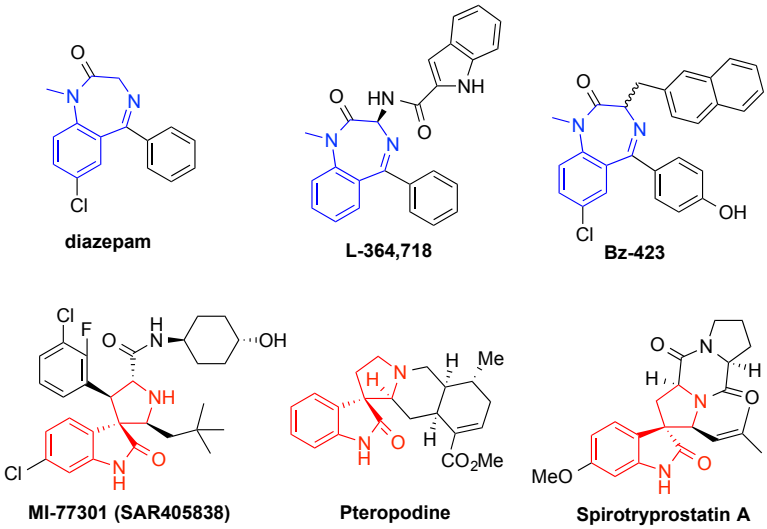
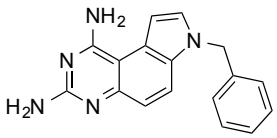
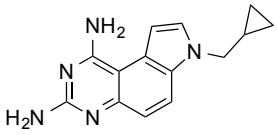


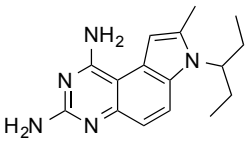
Figure 2. Representative PQZs as DHFR inhibitors.



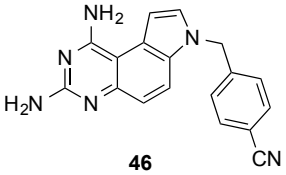
43
C. albicans
MIC: 0.64 μg/mL



44
C. albicans
MIC: 1.39 μg/mL



45
C. albicans DHFR
K_i: 0.030 nM
human DHFR
K_i: 0.3 pM
C. albicans
MIC: 0.025 μg/mL
HCT-8
GI_{50}: 0.74 nM



46
Rat liver DHFR
IC_{50}: 2.1 nM
L1210 DHFR
IC_{50}: 4 nM
C. oncopelti DHFR
IC_{50}: 0.7 nM
L. casei DHFR
IC_{50}: 6.4 nM

Figure 3. Representative PQZs as PAR1 antagonists.

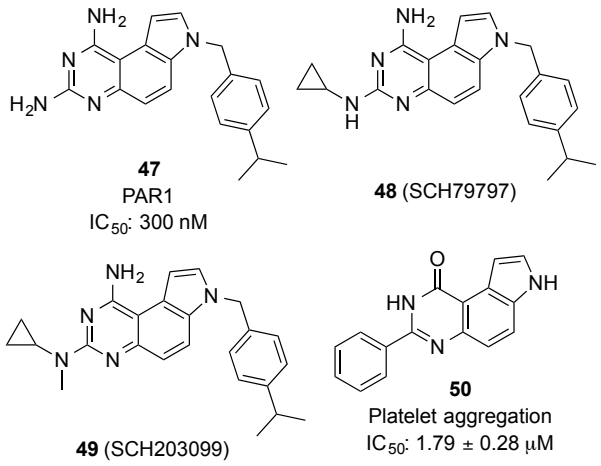
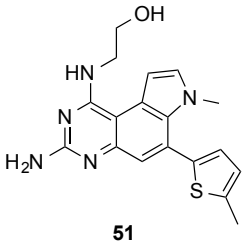
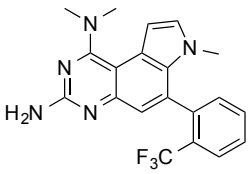


Figure 4. Representative PQZs as PTP1B inhibitors.



51
PTP1B
IC_{50}: 7.10 μM



52
PTP1B
IC_{50}: 9.47 μM

Figure 5. Representative PQZs with miscellaneous pharmacological activities.

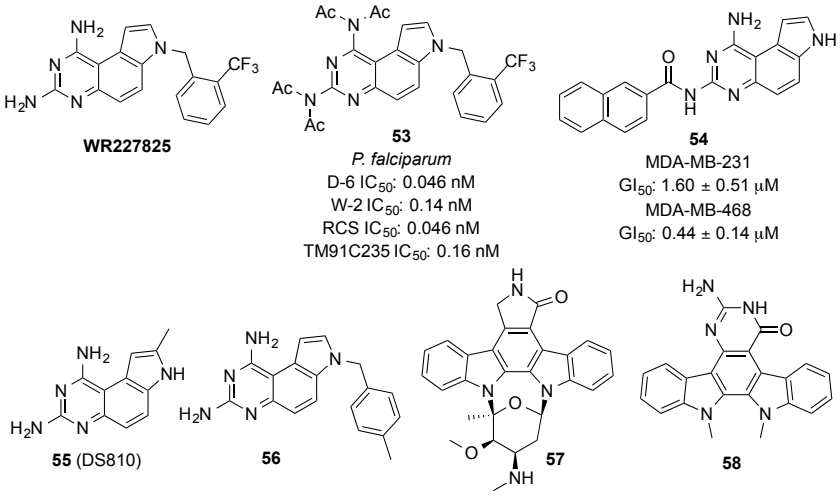


Table 1. Miscellaneous bioactivities of PQZs.

Compound	Targets or cells	IC_{50} (μM)	reference
2	Dyrk1A	5.51	68
2	Rad52	11.97	68
2	Rad54	38.56	68
36	HL60	17	31
55	HSV-1	15	66
56	PON	~100	67
58	cancer cell lines	1.11-9.07	32