Chapter

Benzimidazoles: From Antiproliferative to Multitargeted Anticancer Agents

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Abstract

Benzimidazole derivatives are known to act against a range of biological targets and thus gained clinical applications in a wide spectrum of diseases. Few examples of multitargeted benzimidazole derivatives that were reported during the last decade will be described in this chapter. Multitargeting agents for serving the polypharmacology approach to combat shortcomings of the main one-drug-one target main dogma will be briefly explored. In that context, the multitargeting benzimidazole derivatives gain a special attention. This includes discovery (hit-to-lead), structure-activity relationship (SAR), and binding mode of at least one lead (or hit) in each group. Special attention will be given to two structures dovitinib and AT9283 that are reported to exhibit potent in vitro and in vivo activities against a group of kinases and non-kinase target (as shown recently for dovitinib).

Keywords: benzimidazole, selective, cytotoxic, inhibitor, multitargeting, multikinase, polypharmacology, antiproliferative, quinolinone, carbamate, quinolinone, pyrazole, urea, aniline, anilinobenzimidazolylpyrimidine, chloroacetamide, amidine, binding, mode

1. Introduction

1.1 Antiproliferative action of benzimidazoles

Benzimidazole, a heterocyclic moiety comprising six-membered benzene ring fused with five-membered imidazole ring, containing molecules, was known for its ability to induce antiproliferative effects (named as antineoplastic, anticancer, or antitumor agents). Numerous structures were reported as effective inhibitors of cell growth and division, thus acting as antiviral, antibacterial, antifungal, anthelmintic (or antihelminthics), and anticancer agents. Over the years, several published scripts have reviewed the synthetic approaches, medicinal chemistry, SAR, bioactivities, and preclinical and clinical studies of such "gifted" fragment [1–8].

1.2 Benzimidazoles act on numerous biological targets

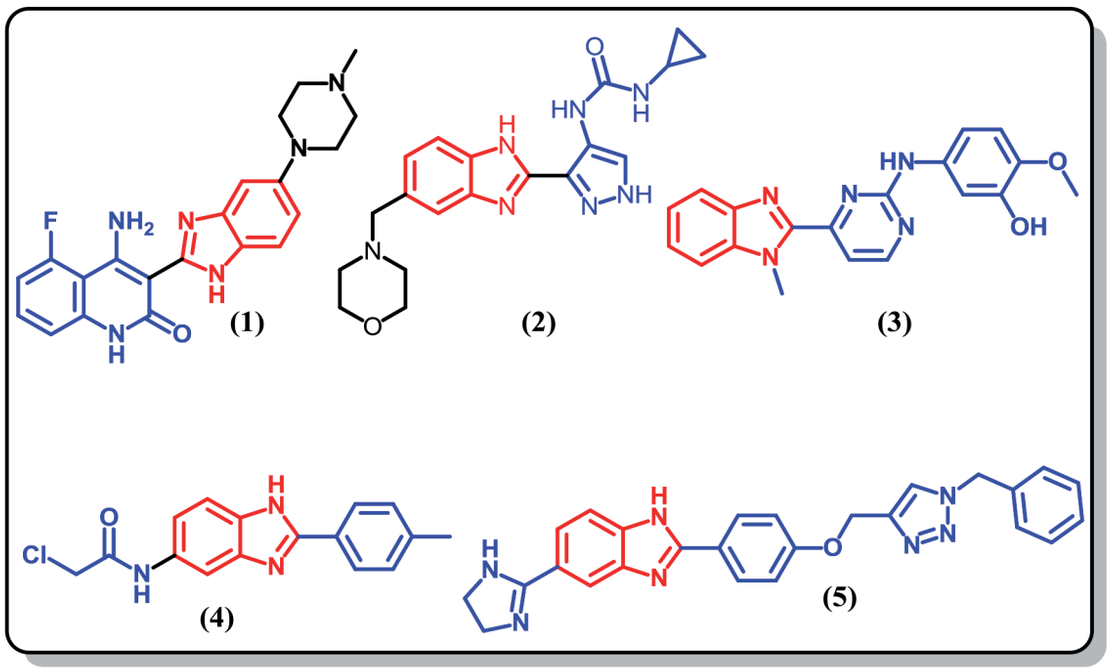
A wide range of activities and medical situations benzimidazole containing compounds have been used for. That includes antihypertensive [9–12], anti-inflammatory [13–15], antibacterial [16–18], antiviral [19–21], antifungal [22–24], anthelmintic [25–28], anticancer [29–32], antiulcer [33–35], antioxidant [36–38], and

psychoactive drugs [39]. And proton pump inhibitors [8, 33], anticoagulants [40, 41], immunomodulators [42], hormone modulators [43, 44], antidepressants [45], lipid level modulators [46–49], and antidiabetics [50–52] are partial list of therapeutic effects of benzimidazole containing comprising compounds. Benzimidazole derivatives exert their actions by interacting with vital biological targets including β-tubulin [52–55], DNA minor groove [56–58], serotonin receptors (5-hydroxytryptamine receptors; 5-HT) [59–62], histamine receptors 4 (H4H) [63], dopamine receptor 2 (D2R) [64], chemokine receptor (CXCR3) [65], interleukin 2-inducible T-cell kinase (ITK) [66], lymphocyte tyrosine kinase (Lck) [67], phosphatidylinositol 3-kinase (PI3K) [68], activated protein kinase (MEK1) [69, 70], anaplastic lymphoma kinase (ALK) [71], polo-like kinase 1 (PLK1) [72, 73], breakpoint cluster region-Abelson kinase (BCR-Abl) [74], casein kinase 2 (CK2) [75], telangiectasia and Rad3-related protein kinase (ATR) [76], tyrosine kinase receptors [fibroblast growth factor receptors (FGFR-1/FGFR-2/FGFR-3)], vascular endothelial growth factor receptor (VEGFR-1/VEGFR-2/VEGFR-3), platelet-derived growth factor receptor (PDGFR-α/PDGFR-β), stem cell factor receptor (c-KIT), FMS-like tyrosine kinase 3 (FLT3) [77], poly(ADP-ribose)polymerase-1 (PARP-1) [78–82], dihydroorotate dehydrogenase (DHODH) [83], topoisomerase 1 (TOPO1) [84], DNA and RNA polymerases [85–89], histone deacetylase 2 (HDAC2) and sirtuin [3, 90], antagonism of angiotensin 1 [2], neuropeptide Y binding [91], inhibition of proton pumps [8], DNA intercalating agents [92], inhibition of cyclin-dependent kinases (CDK) activity [93–96], activation of the p53 protein [97], etc. to mention part of the asserted cellular targets.

1.3 Scope: benzimidazoles as emerging multitargeting agents

The profound success in bringing into clinical application several kinase inhibitors as anticancer drugs made "kinase targeting" a central branch of targetable biomolecules during the past two decades. Nevertheless, the emerging of resistant tumors kinase-directed therapeutics and adverse side effects turned such promising "targeted therapeutics" into challenging field. In addition, it was noticed that lack of response to kinase inhibitors is accompanied by changes in signaling network composition through adaptive kinome reprogramming. Such reprogramming is believed to allow the tumor to escape effects of the drug and manifest resistance. In contrast to the "one-drug-one-target" approach, the "bitopic, that is, two drugs acting on one target" or the "dual, that is, one drug acting on two targets," "polypharmacology" which refers to a novel paradigm that purposes at "simultaneous modulation of more than two biological targets by a single drug" has been emerging as strategy to improve the efficacy and durability of clinical responses to therapies. In cancer treatment, polypharmacology is a result of the ability of "one drug" to simultaneously inhibit multiple cancer-driving targets. However, discovering inhibitors with an appropriate multitarget profile is a challenging task that necessitates a systemic deeper investigation accompanied by major clinical developments. Therefore, a strategy is required to identify single polypharmacological agents with the ability to target multiple cancer-promoting or -sustaining pathways that does not necessarily rely on inhibiting multiple kinases [98]. As a matter of fact, high ratio (~30%) of the FDA-approved kinome-targeting drugs were reported be multitargeted ones [99]. Actually, the first kinase inhibitor imatinib was approved as multitarget agent in a later stage (in addition to its primary target BCR-Abl, it inhibits stem cell factor receptor (c-KIT) and platelet-derived growth factor receptors A and B (PDGFRα and PDGFRβ) tyrosine kinases and human quinone reductase 2

a)



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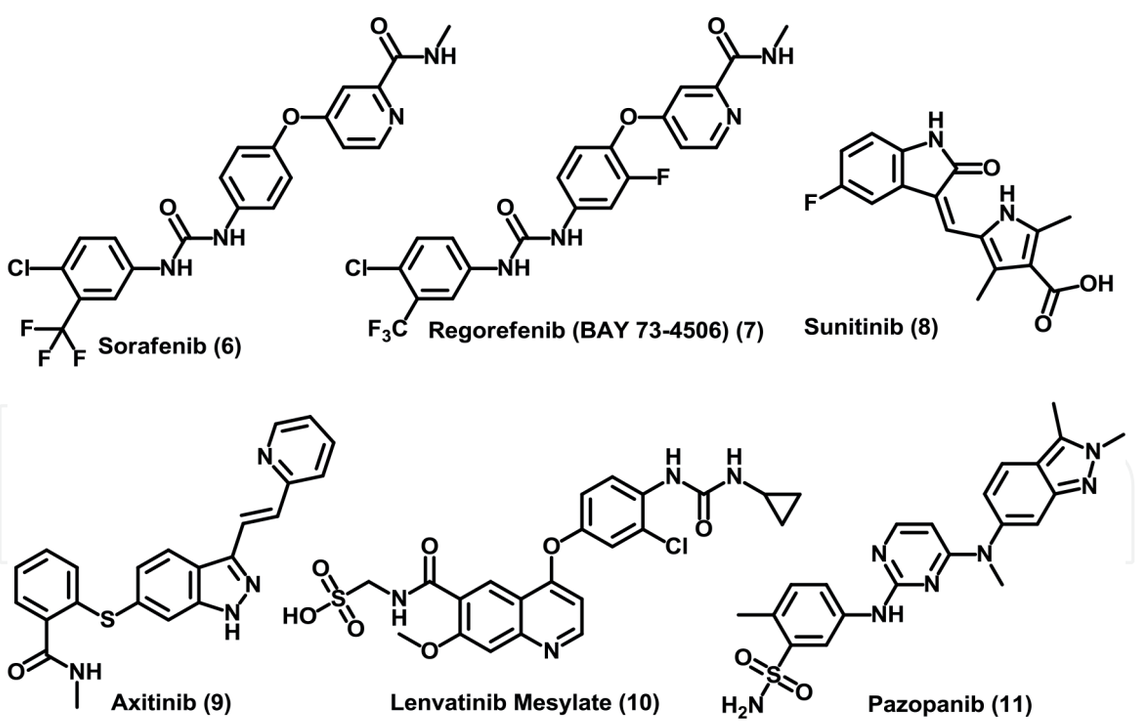


Figure 1.
Multitargeting anticancer agents. (a) Multitargeting cytotoxic benzimidazole-based structures. 3-Benzimidazol-2-ylhydroquinolin-2-one based dovitinib [TKI258, CHIR258; (1)], N-cyclopropyl-N'-[3-[5-(4-morpholinylmethyl)-1H-benzimidazol-2-yl]-1H-pyrazol-4-yl]Urea [AT9283 (2)], 2-anilino-4-(benzimidazol-2-yl)pyrimidine based [2-anilino-4-(benzimidazol-2-yl)-pyrimidine 2-methoxy-5-{[4-(1-methyl-1H-benzimidazol-2-yl)pyrimidin-2-yl]amino}phenol (3)], α-haloacetamidebenzimidazole-based [2-chloro-N-(2-(p-tolyl)-1H-benzo[d]imidazol-5-yl)acetamide (4)], and amidine-benzimidazole based [2-(4-((1-benzyl-1H-1,2,3-triazol-4-yl)methoxy)phenyl)-5-(4,5-dihydro-1H-imidazol-2-yl)-1H-benzo[d] imidazole (5)]; (b) FDA-approved multitargeting anticancer agents [sorafenib (6), regorafenib (7) sunitinib (8), axitinib (9), and lenvatinib (10) and pazopanib (11)].

(NQO2)). Thus the question of how efficacious are selective and specific one-drug-one-target-approved agents in treating advanced and metastatic cancer is still under evaluation [100–102].

This chapter will concisely provide a deeper insight into the benzimidazole-containing structures that exhibit action on multiple cellular targets. Special focus will be drawn to the identification and discovery, the structural activity relationship, proposed binding and interaction, and mechanism of action of each group of compounds. Detailed synthetic procedures and preclinical and clinical studies are out of scope of the current chapter. The focus of this chapter will be on groups of compounds that had been unveiled as concurrently antagonizing multiple targets. Instead, this chapter will focus on five groups of compounds reported to possess cytotoxic activities by acting on multiple (see **Figure 1a**) compounds (1, 2, 3, 4, and 5) holding the potential to be administered as "polytherapies."

2. Benzimidazole scaffold for multitargeting of cancer

Multitargeting agent is defined as "a single chemical entity exerting action as a result of direct interactions on multiple biomolecular targets" [103]. Such agents can be beneficial in overcoming single (or dual)-targeting limitations including compromised effectiveness, severe side effects, emergence of resistant target mutants, and target non-related mutations. In addition, the efficacy of single-molecular-targeted FDA-approved agents in treating brutal and mortal cancers (breast, colorectal lung, pancreatic, and prostate) is limited. Most tumors escape from the inhibition of any single chemotherapeutic agent, and thus one possible therapeutic strategy could be in (1) administering cocktails of highly selective inhibitors (combinational therapy) or (2) development of multitarget inhibitors that act on inhibiting concurrently multiple validated target in cancer cell initiating a concerted molecular response, leading to cell death. Multitargeting chemotherapeutics hold the potential of exhibiting synergistic or at least additive effects when compared to single-targeted ones. It is believed that advances in signaling cascades, networks and crosstalk, chemo- and bioinformatics, detailed three-dimensional structural information of target proteins, computational chemistry tools, proteomics, etc. will allow for designing novel multitarget inhibitors.

It has been realized that molecular targeted therapeutics are facing acquired resistance. Multitargeting approach is gaining increased attention especially when combating resistant cancer cells. Accumulated evidence showed that drug treatment aggravates "selective pressure" of evolutionary force exerted on tumor cells that leads to resistance.

Benzimidazole fragment is reported to be an integral part of multitargeted inhibitors. Such inhibitors challenge the dominant paradigm in drug discovery which deemed to design and develop bioactive agent with maximum selectivity and specificity to individual drug target. Such compounds hold the hope for a new avenue of combating disease cases that could not be cured with one inhibitor acting on single target such as cancer [104, 105].

2.1 Benzimidazolylquinolinone: a scaffold for targeting multiple biomolecules

2.1.1 Discovery of dovitinib (TKI258, CHIR258)

Dovitinib [(TKI258, CHIR258); 4-amino-5-fluoro-3-(5-(4-methylpiperazin-1-yl)-1*H*-benzo[d]imidazol-2-yl)quinolin-2(1*H*)-one (1)] was first designed and synthesized as vascular endothelial growth factor receptor (FEGFR) inhibitor in the

context of developing targeted antiangiogenic treatments [106]. The compound was later reported as a multitargeted kinase inhibitor (by [107]) following the realization that its potent inhibitory effects on cancer cells are associated with action on other multiple key players in oncogenesis, development, and proliferation of cancer [107].

The commercially available 3-benzimidazol-2-ylhydroquinolin-2-one scaffold [benzimidazolylquinolinone for short from now on, **Figure 2** (13)] was identified using high-throughput screening (HTS) method and reported by Renhowe et al. (Novartis) as a potent (IC_{50} values < 0.1 μM) reversible ATP competitive inhibitor of VEGFR-2, FGFR-1, and PDGFRβ [106]. Due to desirable properties as low-molecular-weight compound exhibiting submicromolar activity, (12) was considered a good hit to start with. To overcome the undesirable physicochemical properties of (13) (low aqueous solubility), further optimization was needed that ended up with a drug-like compound (1). Determining the key structural features required for potent kinase inhibition, molecular modeling was employed. The assumption was that in quinolinone portion, both NH at position 1 and the carbonyl group, together with benzimidazole NH, form a donor-acceptor-donor motif that would most probably bind to the hinge region of the RTKs and should be preserved.

To test this hypothesis, a systematic study was conducted through which hydrogen bond donors were masked by methyl group (CH_3-) as shown in **Figure 3** (13a–c) and 14a and 14b. These changes led to significant loss in the potency against all three receptor tyrosine kinases (VEGFR-2, FGFR-1, and PDGFRβ RTKs). The dimethylated analogue (**Figure 3**, 14b) showed no kinase activity at a concentration as high as 25 μM. Interestingly, it was noticed that monomethylation seemed to affect the kinase selectivity profile as well. Introduction of a methyl on the benzimidazole NH (13b) had a more dramatic effect on VEGFR-2 affinity than the methylation at NH in position 1 of the hydroquinolin-2-one (13a). This underlies the importance

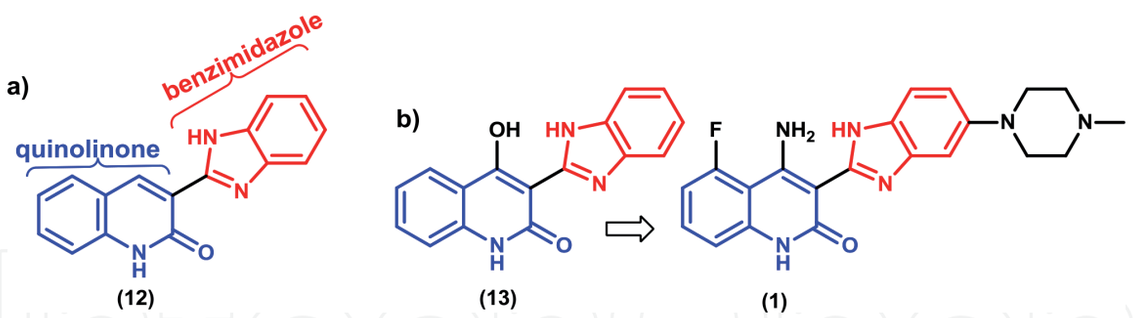


Figure 2.
Benzimidazolquinolinone-based multitargeting scaffold. (a) The basic skeleton of dovitinib (TKI258, CHIR258), 3-(1H-benzimidazol-2-yl)quinolin-2(1H)-one (12) with the two fragments quinolinone (blue) and benzimidazole (red) is indicated, (b) structures of commercially available starting "hit" (13) identified using HTS, and the "lead" approved as a multitargeting drug dovitinib (1).

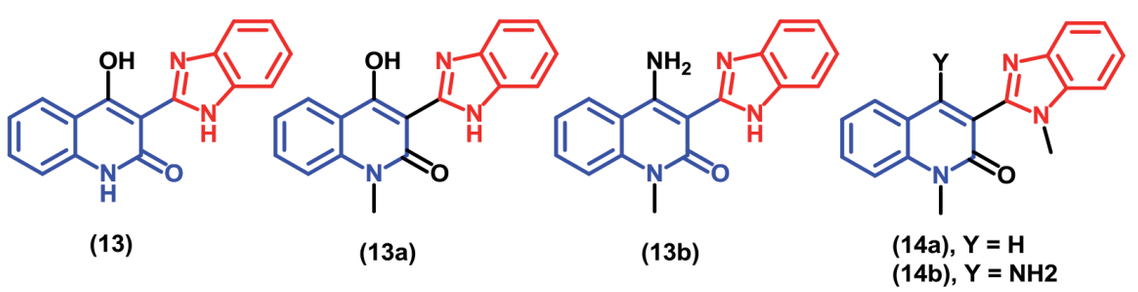


Figure 3.
Assessing the effect of the HBD and HBA on the activity of derivative of 3-benzimidazol-2-ylhydroquinolin-2-one. Methylated analogues of (13 and 14). The monomethylation caused a significant drop in the potency toward RTKs, while dimethylation aborted the RTKs' activity [106].

of preventing the hydrogen bond donor (HBD). An opposite effect was noticed for FGFR-1, which indicates that despite the high homology of the two ATP-binding sites in the tow targets, selectivity opportunities still exist that are likely due to small changes in the shape of binding site. Such change in the shape can influence the accessibility of alternate binding poses of the monomethylated ligands (13a–13b and 14a in **Figure 1**) [106].

2.1.1.1 Structure–activity relationship (SAR)

The scaffold (13) was annotated by four rings (A–D). Modifications were introduced in a systemic manner. Once the basic structural components needed for affinity to targets of interest were understood, a study of the structure–activity relationship around the periphery of central 3-benzimidazol-2-ylhydroquinolin-2-one (13) scaffold was undertaken. Besides electrophilicity, nucleophilicity, bulkiness, steric hindrance, HBD versus HBA, and basicity, C4 of ring A was used for incorporation of moieties that might impart favorable physicochemical properties.

SAR of ring B (C4): While removal of the hydroxyl group reduced the activity, its replacement with amine improved significantly affinity to RTK and also cell potency [EC_{50} of 0.078 μM (NH_2 > OH > H)], suggesting an importance of the HBD at C4 of the hydroquinolin-2-one fragment. Thus, incorporation of larger substituents on the C4-NH of the hydroquinolin-2-one was explored and found to be tolerated (see compounds 15b and 15c, **Figure 4**). Not only substantial improvement in the solubility was attained when the substituents carried an additional basic nitrogen were introduced to this position, it was noticed that this position modulates the selectivity profile of this class of compounds. It was reported that both derivatives (12a) and (12b) exhibited enhanced potency against PDGFR than VEGFR-1 (3000-fold) and FGFR (>1500-fold). Large basic amines like aminoquinuclidine potentiate the derivative (15d) against CHK-1 and GSK-3.

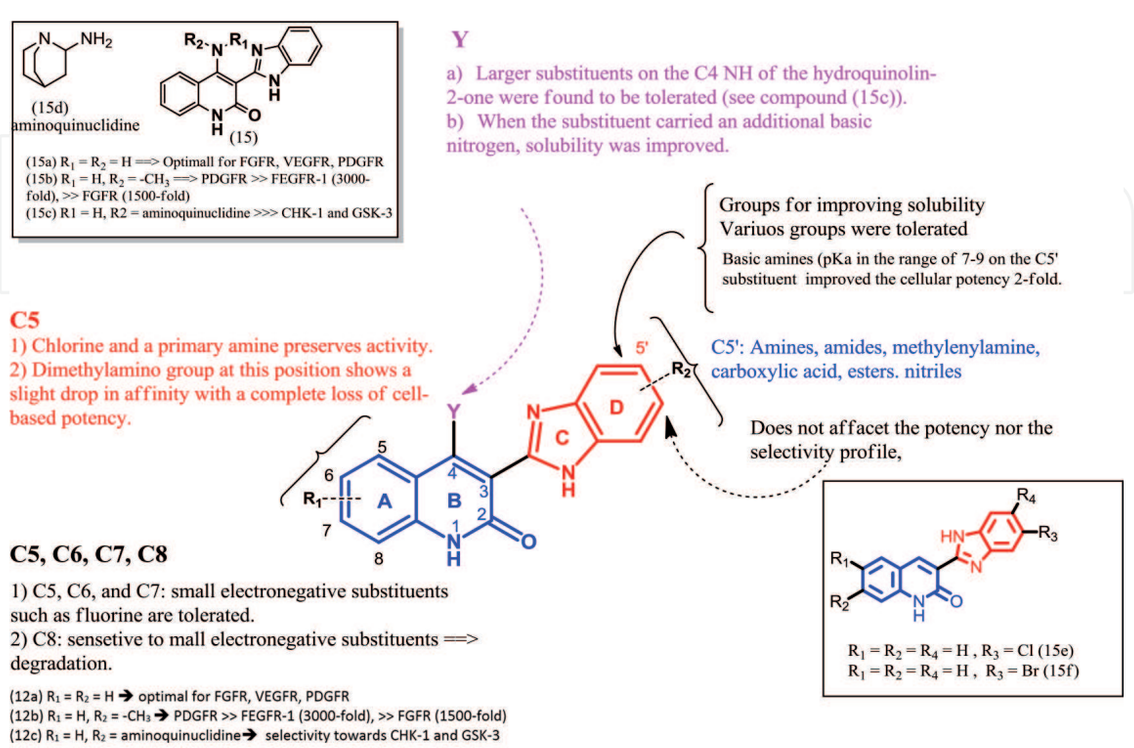


Figure 4.
Summary of structure–activity relationship (SAR) of 3-benzimidazol-2-ylhydroquinolin-2-one (1) [106].

In conclusion, substitution at C4 position was revealed as critical to the activity of the benzimidazolylhydroquinolinone scaffold; however for RTK inhibitor program, the NH_2 group was the optimal substituent at C4 as it avoided inhibition of these additional serine threonine kinases, which could complicate the pharmacological application of these agents.

SAR of ring D: The overall structure–activity relationship (SAR) is summarized in **Figure 4**. Medicinal chemistry efforts were concluded in the selection of compound (1) as a candidate for further development. The compound (1) displayed exceedingly potent inhibitory effect when assessed against receptor protein kinases VEGFR-2, FGFR-1 and FGFR-3, PDGFRβ, VEGFR-1, VEGFR-2 and VEGFR-3, c-KIT, CSF-1R, and FLT-3 with IC_{50} values between 3 and 27 nM. Such activity is translated into antiproliferative action on cells that are VEGF-, FGF-, SCF-, CSF-, or PDGF-driven. Mechanistically, it was also indicated that VEGF-mediated ERK phosphorylation was dipped in endothelial cells treated with (1).

In summary, dovitinib (1), an antineoplastic benzimidazolylquinolinone derivative, inhibits multiple growth factor receptor tyrosine kinases important for tumor angiogenesis and tumor growth. Dovitinib is well established as type III–V receptor tyrosine kinase (RTK) inhibitor. Though it potently inhibits fibroblast growth factor receptors (FGFR-1/FGFR-2/FGFR-3), the compound also inhibits vascular endothelial growth factor receptor (VEGFR-1/VEGFR-2/VEGFR-3), platelet-derived growth factor receptor (PDGFR-α/β), stem cell factor receptor (c-KIT), FMS-like tyrosine kinase 3 (FLT3), and colony-stimulating factor receptor 1 (CSFR-1) emphasizing the nonspecific action of the drug [108]. The orally bioavailable lactate salt of (1) strongly binds to fibroblast growth factor receptor 3 (FGFR3) and inhibits its phosphorylation, which may result in the inhibition of tumor cell proliferation and the induction of tumor cell death. The activation of the abovementioned RTK in singularity or together is associated with cell proliferation and survival in all cancer cell types.

Dovitinib (TKI258, 1) is a highly potent, novel multitargeting receptor tyrosine kinase inhibitor with IC_{50} of 1, 2, 10, 8, 27, and 36 nM for FLT3, c-KIT, VEGFR-1/VEGFR-2/VEGFR-3, PDGFRß, and CSFR −1, respectively. Due to its inhibitory effect of VEGFR1/VEGFR2, the compound displayed both antitumor and antiangiogenic activities in vivo.

Trudel and colleagues reported that in addition to inhibiting the abovementioned TRKs (types II, IV, V), (1) potently inhibits wild-type (WT) FGFR3, F384 L-FGFR3 (IC_{50} = 25 nM), and FGFR3 mutants (IC_{50} = 70–90 nM for the various mutations) driven by B9 cells [107]. Additionally, same group reported that (1) inhibited the proliferation of multiple myeloma (MM) cells. When assessing its antiproliferative effect against U266 and 8226 cells, (1) displayed a potent inhibitory effect (IC_{50} ~ 90 nM) against KMS11 (FGFR3-Y373C), OPM2 (FGFR3-K650E) cells and IC_{50} ~ 550 nM KMS18 (FGFR3-G384D) [109]. (1) Exhibited exceedingly potent antiproliferative effect against acute myelogenous leukemia (AML) cells MV4;11 (mutant FLT3-ITD) compared to AML RS4;11(FLT3 WT) cells [EC_{50} = 13 nmol/L and EC_{50} = 315 nmol/L for MV4;11 and RS4;11, respectively, i.e., (~24-fold decrease in potency for FLT3 WT cells)]. Such results indicated that (1) exhibited far more potent activity against cells that are dependent on constitutively active FLT3-ITD. A similar conclusion was affirmed by Heise et al. by the notion that (CHIR258, 1) inhibited the proliferation of MOLM13 and MOLM14 that are FLT3-ITD mutant cells with EC_{50} ~ 6 nmol/L similar to the ones with MV4;11 [109].

Besides the potent action of (1) against a wide range of RTK, its inhibitors' effect ion fibroblast growth factor receptors in a variety of tumor xenograft models in athymic mice, including acute myeloid leukemia, multiple myeloma, and colon- and prostate-derived models was promising.

Recent studies reported the comparative activities of dovitinib against 16 colorectal cancer (CRC) cell lines (among them, 10 were KRAS or BRAF mutants). Results showed the affectivity of the drug in inhibiting the proliferation of majority of the cell lines excluding the ones harboring KRAS or BRAF mutants. However, when assessing the efficacy of the drug in vivo, it reduced the tumor growth in vivo regardless of the KRAS and BRAF mutation status. The drug exerted significant reduction of the xenograft size of both resistant cell lines (KRAS mutant LoVo cells but not in BRAF mutant HT-29) but without a detectable effect in the resistant mutant cell BRAF mutant HT-29 in vitro on s. Such results were explained by the multitarget action of the drug in which by acting on FGFR and FGFR together with VEGFR has been able to interfere with resistance mechanisms emerging from the synergistic interaction between the various signaling cascades in promoting neovascularization that is believed to be one resistance factor in renal cell carcinoma or pancreatic cancer [110, 111].

Dovitinib was selected to proceed ahead for preclinical and clinical trials. Several clinical trials have been conducted, and others are also underway with the drug and alone or in combination with several chemotherapeutic agents [112–118].

2.1.2 Binding mode of dovitinib (CHIR258, 1) to FGFR-1

Based on FGFR-1 crystal structure (PDB 2FGI) in conjunction with the information received from the X-ray structure of (1) with CHK1, a homology model for (1) complexed with VEGRF2 was constructed [106]. The model was helpful in guiding for the important interactions of (1) with active site. It was concluded that (1) participated in three hydrogen bonds to the hinge domain (Glu917 and Cys919). In addition A-ring makes a VDW interaction with the hydrophobic gatekeeper Val916 and was engaged in an S-H/π interaction with Cys1045. Leu840, Val848 (both in the P-loop and ceiling of the purine pocket), Ala866 (ceiling of the purine pocket), Val 899 (floor of the purine pocket), Phe918 (part of the hinge), Lys920, Gly922 (both in the lower hinge region), and Leu1035 (floor of the purine pocket) took part in hydrophobic interaction with (1). In the following studies, the X-ray structures of (1) complexed with native and with mutant FGFR1 and with FGFR4 were reported [119–122].

2.1.2.1 Going beyond kinases

Although dovitinib binds to several kinases at nanomolar concentrations, recent studies reported its inhibitory effect against cancer-related targets including topoisomerase I and II (Topo I and II) [123] and human recombinant bone morphogenetic protein (BMP)-2, indicating that the cell growth inhibitory activity and the anticancer activity of dovitinib may result, in part, from its ability to target Topo I and II in addition to the ability to inhibit multiple kinases [124]. A study disclosed dovitinib inhibition of BMP-2 enhanced alkaline phosphatase (ALP) induction, which is a representative marker of osteoblast differentiation. Dovitinib also stimulated the translocation of phosphorylated Smad1/Smad5/Smad8 into the nucleus and phosphorylation of mitogen-activated protein kinases, including extracellular signal-regulated protein kinases 1 and 2 (ERK1/2) and p38 **Figure 5**. An increase in the expression of mRNA of BMP-4, BMP-7, ALP, and osteocalcin (OCN) was noticed following treatment with (1). It was also noted that the potent stimulating

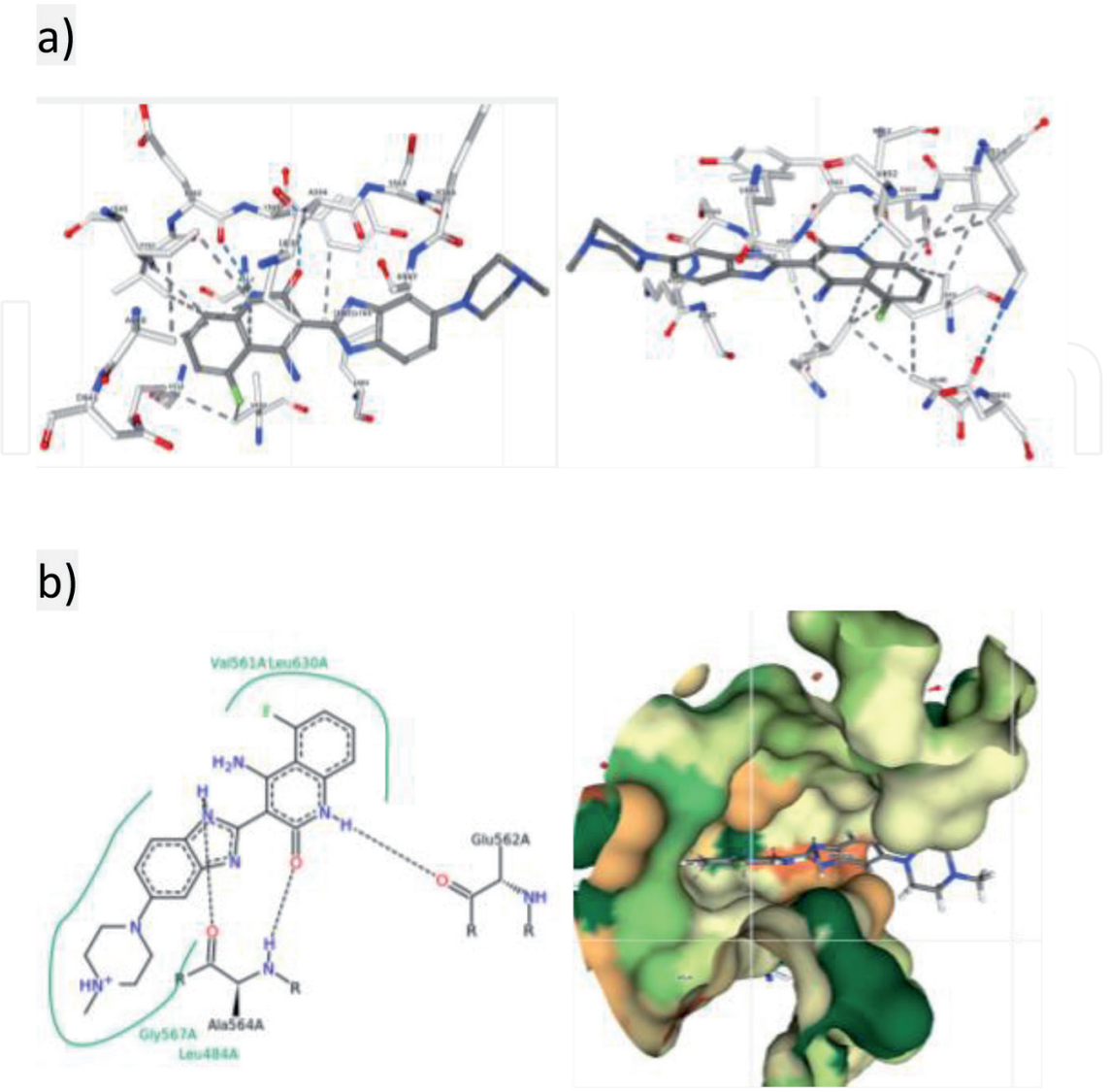


Figure 5.
(a) Cartoon representation of the crystallographic structure of complex of (1) to FGFR-1 (PDB 5 AM6); the binding site is depicted showing the kinase with residues interacting with the ligand in stick model, (b left) the main interactions between (1) and the kinase domain and (b right) the surface representation, with the surface colored by atom type (red, oxygen; blue, nitrogen; yellow, sulfur; gray, carbon/hydrogen). The donor-acceptor-donor motif is shown to interact with the hinge region while the 1-methylpiperazine substituent on C5' points into solution [119].

effect of (1) on BMP-2-induced osteoblast differentiation suggests a potential repositioning for the use of (1) treatment of bone-related disorders [124]. In a recent study, Ye Zhang **et al**. initially used the central scaffold 3-(1*H*-benzimidazol-2-yl)quinolin-2(1*H*)-one (12) to explore that potential diversification of functional groups decorating (12). The compounds synthesized were assessed against HepG2 (human liver cancer cells), SK-OV-3 (human ovarian cancer cells), NCI-H460 (human large cell lung cancer cells), BEL-7404 (human liver cancer cells), and HL-7702 (human liver normal cells) cell lines. Initial studies showed that halogenated derivative [3-(6-chloro-1*H*-benzo[d]imidazol-2-yl)quinolin-2(1*H*)-one (15e) and 3-(6-bromo-1*H*-benzo[d]imidazol-2-yl)quinolin-2(1*H*)-one (15f) (see **Figure 4**)] exhibited better activity than 5-FU and cisplatin when assessed against HepG2, SKOV-3, NCI-H460, and BEL-7404 but not HL-7702. The authors postulated that (15e) and (15f) inhibit HepG2 proliferation by blocking the cells in G2/M stage through activation of p53 protein.

2.2 Pyrazolbenzimidazols as multikinase inhibitors

2.2.1 Discovery of AT9283a

In developing a selective potent aurora kinase inhibitor by employing fragment-based discovery method, the pyrazol-4-yl urea benzimidazole derivative (AT9283, 21) was identified as a multitargeting kinase inhibitor. The pyrazolebenzimidazole-based clinical candidate (21) was optimized by Steven Howard and his colleagues following efficient structure-guided fragment to hit IC_{50} as low as 3 nM activity as a dual potent inhibitor toward Aurora A/Aurora B [125]. AT9283 was identified starting from the pyrazole-benzimidazole fragment (16) that was previously identified during the endeavor of developing cyclin-dependent kinase (CDK) inhibitors. Subsequent structure-based approach using CDK2 crystallographic structure led to the identification of the benzamide analogue (18). Throughout the process of developing CDK2 inhibitors, pyrazole-benzimidazole derivative was identified to act with high potency toward Aurora A. Starting with fragment, (18) demonstrated superior ligand efficiency (LE = 0.59) for Aurora A compared to CDK1 and CDK2 and also sufficient potency to allow detection in a conventional enzyme bioassay [125].

Aiming at optimizing, the "hit" (18) on the way to end up with a lead SAR is performed on the benzamide analogues. The team was aided by polyploid phenotype in HCT116 cells, as a functional assay that differentiates for Aurora A and Aurora B inhibition, combined with potency when screening for further analogues. Guided by the hypothesis that introducing a basic motif into fragment (18) will improve the potency of the compound, modifications were introduced successfully to 5- or 6-position of the benzimidazole without causing any clashes with the protein. In a further step, the morpholinomethyl motif was functionalized at position 5. Details grasped from the X-ray crystal structure of (19) complexed with Aurora A revealed that the pyrazole-benzimidazole motif is positioned in an excellent complementarity with the narrow region of the ATP pocket. A result directed the steps to follow in the design of the optimized structure (**Figure 6**). While retaining the 5-morpholinomethyl on the pyrazole-benzimidazole motif, the benzamide portion was subjected to modifications. Keeping in mind the need to keep the molecular weight while introducing increased flexibility on the glycine region, the amide was converted to urea (20). This strategy was fruitful when comprehending that the urea analogue (20) exhibited reduced plasma protein binding while maintaining in vitro activity against Aurora kinases.

In the following step, the X-ray structure of (20) complexed with Aurora A was solved and iterated a similar binding mode to the hinge region. To resolve a twisted conformation of the phenyl plane in regard to pyrazole-benzimidazole portion of the molecule, a fully reduced cyclohexyl and difluorophenyl groups were also introduced (compound (20a) and (20b), respectively). Adsorption, disposition, metabolism, and excretion (ADME) considerations lead to proposing cyclopropyl derivative (21). As an alternative to introducing additional heterocyclic moiety, aiming at reducing the lipophilicity of (20a) for improving the ADME, the size of cyclohexyl ring was reduced to cyclopropyl analogue resulting in compound (21) that exhibits high enzyme and cellular potency still with reduced both the molecular weight (MW) and lipophilicity (log D7.4 = 2.1, MW = 381). Compound (21) demonstrated potent inhibition of HCT116 colony formation (IC_{50} = 12 nM), a clean CYP450 profile (IC_{50} > 10 μM for CYP3A4, 2D6, 1A2, 2C9, 2C19), acceptable mouse plasma protein binding (81.5%), and good thermodynamic solubility (2.0 mg/mL at pH = 7.0 and 13 mg/mL at pH = 5.5).

Later, AT9283 (21) was shown to bind and potently inhibit a number of kinases including the Aurora kinases A and B (serine–threonine kinases that are known to play essential roles in mitotic checkpoint control during mitosis at IC_{50} ~ 3 nM),

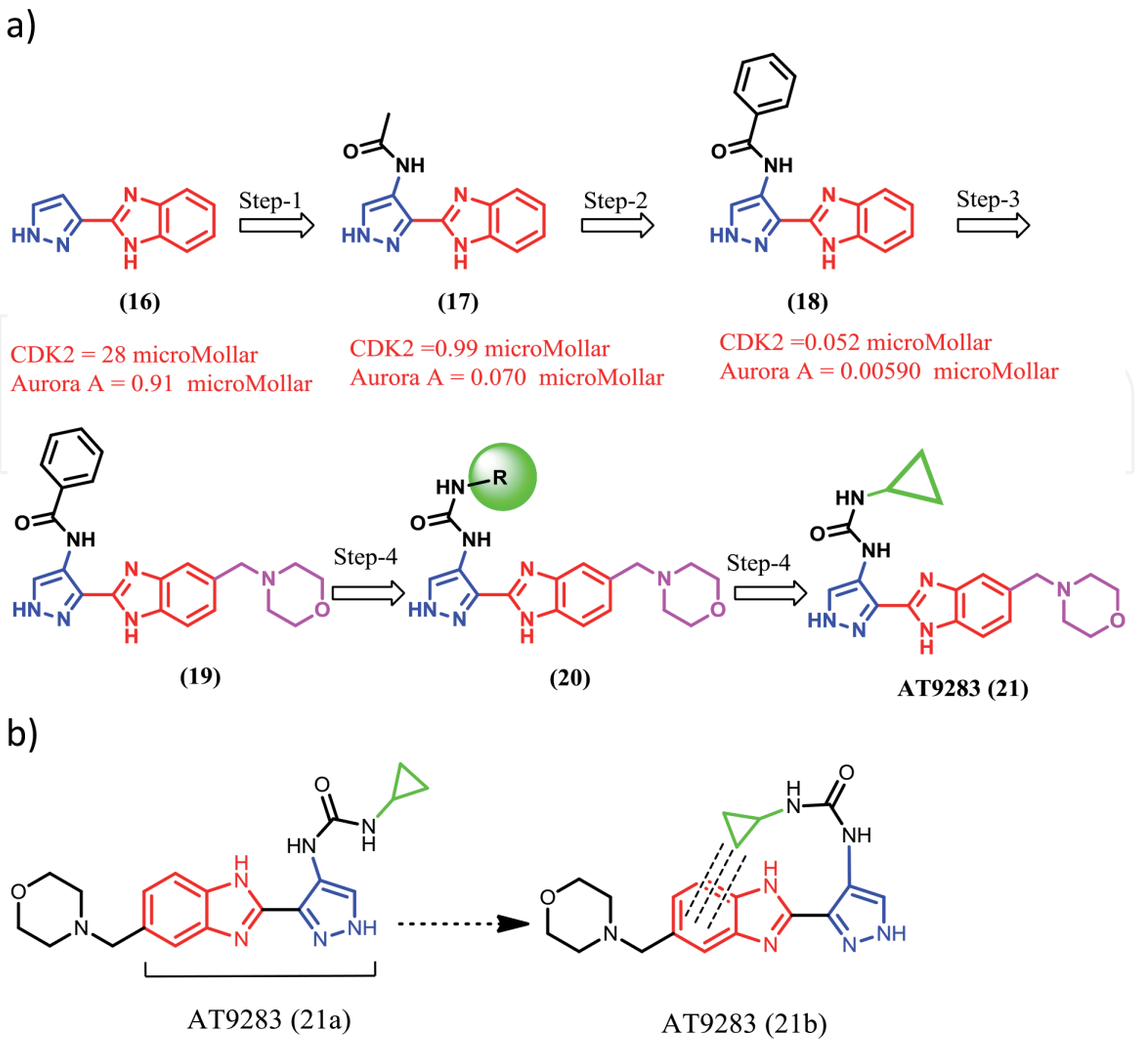


Figure 6.
(a) Main steps in the identification and discovery of pyrazolebenzimidazole-based multitargeting agent AT9283 (21) using fragment-based identification starting from fragment (16). (b) The structure N-cyclopropyl-N'-[3-[5-(4-morpholinylmethyl)-1H-benzimidazol-2-yl]-1H-pyrazol-4-yl] urea [AT9283 (21a)]. The "folded conformation" of (21b). Dotted lines to indicate the hydrophobic interaction between the cyclopropyl and benzimidazole motifs [125].

Janus kinase 2 (JAK2) and JAK3 (1.2 and 1.1 nM, respectively), breakpoint cluster region-Abelson (BCR-Abl) T315I (4 nM), and mitogen-activated protein kinase kinase kinase 2 (MEKK2) with IC_{50} values of lower nanomolar (4.7–18 nM). This set of known kinases is known to play key roles in mitotic progress in cell cycle, induction of proliferation, evasion of apoptosis and tumor growth and thus considered vital targets to chemotherapeutic agents (see **Table 1**). Therefore, AT9283 (21) is defined as multikinase (multitargeting) inhibitor [126].

AT-9283 inhibits effective proliferation of cancer cells both in vitro and in vivo with and its effect is enhanced by with other agents (see **Table 2**) [127]. Henceforth T9283 proceeded to clinical trials including in children with relapsed or refractory acute leukemia, imatinib-resistant BCR-Abl-positive leukemic cells, and patients with relapsed or refractory multiple myeloma. Accumulative results indicate a need for optimizing the pharmacological profile on the way to overcome faced challenges in clinical application of the compound [127, 128].

The activity in imatinib-resistant BCR-Abl chronic myelogeneous leukemia (CML) explained based on modeling which reiterated the assumption that AT-9283 is bound to the kinase domain in the "folded conformation" which allows the needed interactions with the hinge region without a clash between the cyclopropyl group and the isoleucine residue in the T315I mutant. The results obtained in

Enzyme	IC_{50} (nM)
Aurora A	52% I at 3.0 nM
Aurora B	58% I at 3.0 nM
JAK3	1.1
JAK2	1.2
Abl (T315I)	4
GSK3-β, FGFR2, VEGFR3 (Flt4), Mer, Ret, Rsk2, Rsk3, Tyk2, Yes	1–10
Abl(Q252H), DRAK1, FGFR1, FGFR1(V561 M), FGFR2(N549H), FGFR3, VEGFR1(Flt1), Flt-3, PDGFR-α(D842V), PDK1, PKCμ, Rsk4, SRC(T341 M), VEGFR2	10–30

Table 1.
The inhibitory concentration 50% (IC50 of the "lead" (21)) [126].

Inhibitory effect of AT9283 on tumor cell colony formation after 10–14 days treatment			
Origin	**Cell line**	**IC_{50} (nM)**	**p53 status**
Colon	HCT116	13	+
	HT-29	11	—
	SW620	14	—
Ovarian	A2780	7.7	+
Lung	A549	12	+
Breast	MCF7	20	+
Pancreatic	MIA-Pa-Ca-2	7.8	—

+ indicates expression of wild-type p53; – indicates no expression of p53 or that p53 is nonfunctional [126].

Table 2.
IC_{50}s are the mean of two or more independent determinations.

refractory CML suggest that AT-9283 can be efficient in Ph + acute lymphoblastic leukemia (Ph + ALL). It is the distinct binding mode that allows AT-9283 in similar manner to MK-0457 and PHA-739358 to exhibit potent activity against imatinib-resistant T315I mutant [127, 129].

2.2.2 Binding mode of AT-9283 (21) to kinases

Currently, there exist 11 X-ray resolved crystallographic structures of AT-9283 complexed with target proteins that are documented at the Protein Data Bank. They include aurora A, aurora B, mutant of aurora B, JAK2, and protein kinase A mutants as surrogate model for Aurora B. A closer look clarifies that in a similar manner to the binding of dovitinib, the benzene portion in benzimidazole fragment is pointing in an orientation toward the solvents' exposed opening of the binding site. The pyrazole and urea fragments took part in multiple HBA and HBD interactions with the hinge region of the enzyme. The morpholine basic amine is oriented toward the solvent and enhanced significantly the solubility of the compound in physiological pH.

The crystal structure of compound (21) complexed with Aurora A is shown in **Figure 7** [130]. The molecule is positioned at the ATP-binding site of the kinase. It is revealed the urea linker adopts a *cis*/*trans* configuration that results in the molecule having a "folded conformation." This same conformation was also observed in the

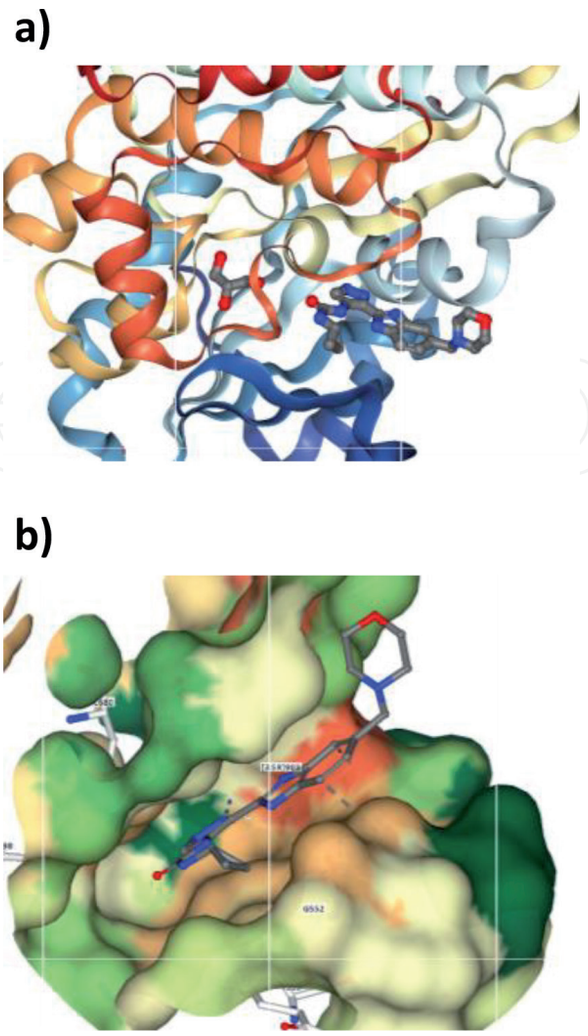


Figure 7.
(a) Cartoon representation of the crystallographic structure of complex pyrazol-4-yl urea AT9283 (21) complexed with JAK2 JH2 (PDB 5UT0); (b) the surface representation, with main interactions between (21) and the kinase domain, colored by atom type (red, oxygen; blue, nitrogen; yellow, sulfur; gray, carbon/hydrogen). The donor-acceptor-donor motif is shown to interact with the hinge region, while morpholine substituent on C5 points toward the solution [130].

crystal structure of (21B) alone (**Figure 8**) and in DMSO. Such "folded conformation" was confirmed by NMR measurement. An NOE was observed between H3b/H3b′ of the cyclopropyl ring and the H4 and H7 protons of the benzimidazole ring. This "folded conformation" was explained by the occurrence of additional stabilization due to a hydrophobic interaction between these two groups.

The crystallographic structures of complexes both dovitinib-FGFR-1 and AT-9283 –Aurora A, revealed that there is a co-planarity between the benzimidazole and the quinolin-2-one of dovitinib, and pyrazole motif in AT-9283. A tautomeric rearrangement of the double bond induces a restriction on the rotation around the connection between the two fragments in each case (see **Figure 8**). This indicate the favorite binding to the less rotatable conformer (21B).

Recently AT-9283 was phase I/phase II trial of AT9283, a selective inhibitor of Aurora kinase in children with relapsed or refractory acute leukemia: challenges to run early phase clinical trials for children with leukemia [131–137].

2.3 α-Haloacetamidebenzimidazole derivatives as multitargeting agents

Employing virtual screening methods of PubChem database as a first step, selected support vector machine (SVM) virtual hits were evaluated by Lipinski's

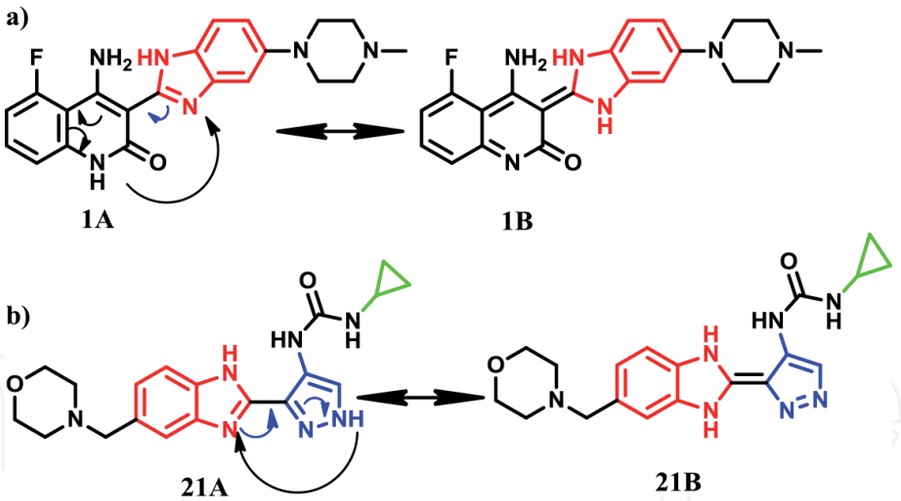


Figure 8.
Tautomeric rearrangement of multitarget inhibitors (1) and (21). (a) benzimidazole quinolin-2-one heterocyclic, and (b) benzimidazole pyrazole derivatives.

rule of five. The compounds which passed Lipinski's rule of five were subject to further and more refined screening by using molecular docking. This sequential refinement led to the identification of 2-aryl benzimidazole group of derivatives as multitarget "EGFR, VEGFR-2, and PDGFR" inhibitors [138]. A mechanistic study reported by Jiang and colleagues displayed that (22) exhibited low to moderate micromolar IC_{50} against nine established breast cancer cell lines that are known to have variable expressing EGFR and HER2 (MDA-MB-468, BT-549, MDA-MB-231, HCC1937, T-47D, BT-474, MDA-MB-453, ZR-75-1, MCF-7, and MCF-10 A). Using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay, (24 and 25) exerts moderate inhibitory effect on growth of panel of breast cancer cell lines (IC_{50} values of 2–9 μM) and was reported to be more potent than lapatinib against MDA-MB-468, BT-549, MDA-MB-231, ZR-75-1, and MCF-7. A correlation was observed between the level of HER2 and EGFR amplification and expression and the sensitivity toward (22). IC_{50} = 3.58 μM against BT-474 (high expression of HER2), whereas against MDA-MB-453 (lower levels of HER2 expression) IC_{50} = 4.91 μM. The activity against lower EGFR and HER2 expressing cell lines (ZR-75-1 and MCF-7), IC_{50} = 1.81–2.99 μM was explained by the assumption that (22) is able to act via other targets of EGFR and HER2 [139].

Docking the compounds into kinase domains revealed that (22) occupies the ATP-binding site of EGFR (PDB: 2J6M). The compound was able to form a hydrogen bond with amino acid MET 793 (N–H···O:2.485 Å), claimed to be an important binding site of EGFR. The difference in the activity between the two compounds against VEGFR2 was explained by the difference in hydrogen bonding using docking into VEGFR-2 kinase. It was shown that (22) formed two hydrogen bonds with amino acids CYS917 (N–H···Cl:2.484 Å) and ASP1044 (N–H···O:2.429 Å), whereas compound (23) formed only one hydrogen bond with ASP1044 (N–H···O:2.419 Å) [140].

The authors concluded that electron-withdrawing substituent residing at 2-aryl ring together with shorter aliphatic chain contributed to the cytotoxic potency and to the induction of apoptosis by such group of compounds in HepG-2 cell lines. Though reported as multitargeting agent, the activity of 2-chloro-N-(2-*p*-tolyl-1*H*-benzo[d]imidazol-5-yl)acetamide (22) exhibiting most potency could not be explained explicitly by docking alone. (22) encompasses a reactive alphahaloacetamide (see **Figure 9**) that is vulnerable to nucleophilic substitution by biological

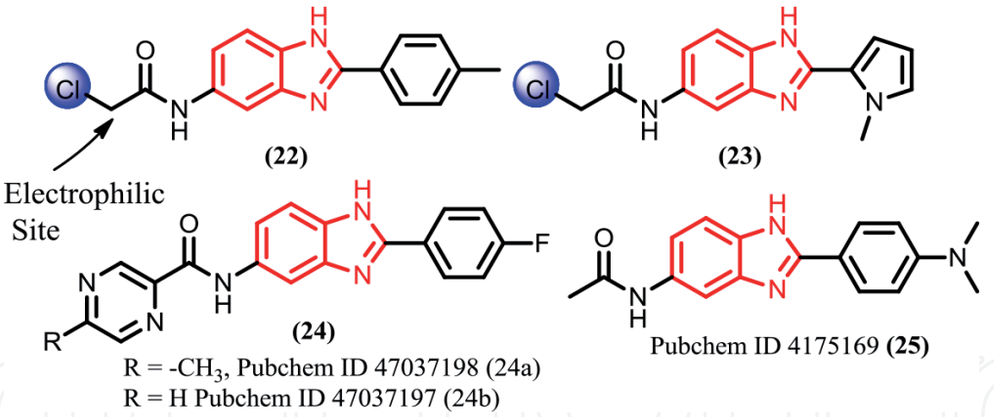


Figure 9.
α-Haloacetamidebenzimidazole derivatives as multitargeting agents. 2-chloro-N-(2-p-tolyl-1H-benzo[d] imidazol-5-yl)acetamide (21), a novel 2-arylbenzimidazole derivative exhibited remarkable activity toward breast cancer. In a study reported by Jiang et al. (22 and 23) were virtually identified as multikinase EGFR and VEGFR inhibitor while (22) was identified as EGFR inhibitor and (23) as PDGFR inhibitor [140, 142].

nucleophiles like thiols (-SH). Thus, a study to explore the formation of irreversible adducts with cellular proteins like kinases is recommended and hoped to uncover the principal mechanism of its wide action.

2.4 2-Anilino-4-(benzimidazol-2-yl)pyrimidines: a multikinase inhibitor scaffold

Anilinopyrimidines (**Figure 10**) displays a wide range of bioactivities. Asymmetric 2-anilinopyrimidines bearing 3-aminopropamides exhibit activity against epidermal growth factor receptor EGFR) [141]. 2-anilinopyrimidine derivatives bearing 4-piperidino substituents exhibited improved and selective activity against triple-negative breast cancer cell line MDA-MB-468 believed to be due to EGFR inhibition. Decorating the pyrimidine nucleus with different substituents at position 4 endowed the final derivatives (4-substituted-2-anilinopyrimidine) with activity as well as selective toward corticotropin-releasing factor (CRF) antagonists [142]. Having the anilino fragment at 2- together with thiazolyl at 4- of the pyrimidine core was reported to exert antagonistic effect of cyclin-dependent kinase-2 (CKD2) [143], and improved inhibitory activity toward CDK9 and (CDK2) [143–145].

Bis-anilinopyrimidine was reported as potent and selective PAK1 inhibitor and as highly selective group I p21-activated kinase (PAK1) inhibitor [146]. Additionally, N-phenyl-N′-[4-(pyrimidin-4-ylamino)phenyl] urea derivatives (see (27) at **Figure 10**) exhibit selective inhibition to class III receptor tyrosine kinase subfamily [147]. Other symmetric 4,6-dianilinopyrimidines induce selective EGFR inhibitions [148].

Notably, introducing the benzimidazolyl moiety at position 4 of the 2-anilinopyrimidine core to produce 2-anilino-4-(benzimidazol-2-yl)-pyrimidines renders such group of compounds' activity against a wider range of kinases (see **Figure 10**).

Renate Determann et al. reported the synthesis and in vitro activity of a small library of compounds that are based on the 2-anilino-4-(benzimidazol-2-yl)-pyrimidine scaffold (**Figure 10**, (30)) [142]. The most potent derivative exhibited antiproliferative activity for several cancer cell lines of the NCI panel in submicromolar concentrations. SAR study was concluded in indicating a basic correlation with the anilinopyrimidine fragment and the substitution pattern at the aniline moiety. It is worth mentioning that 2-anilinopyrimidine fragment (**Figure 10**, (30)) is found in a range of kinase inhibitors.

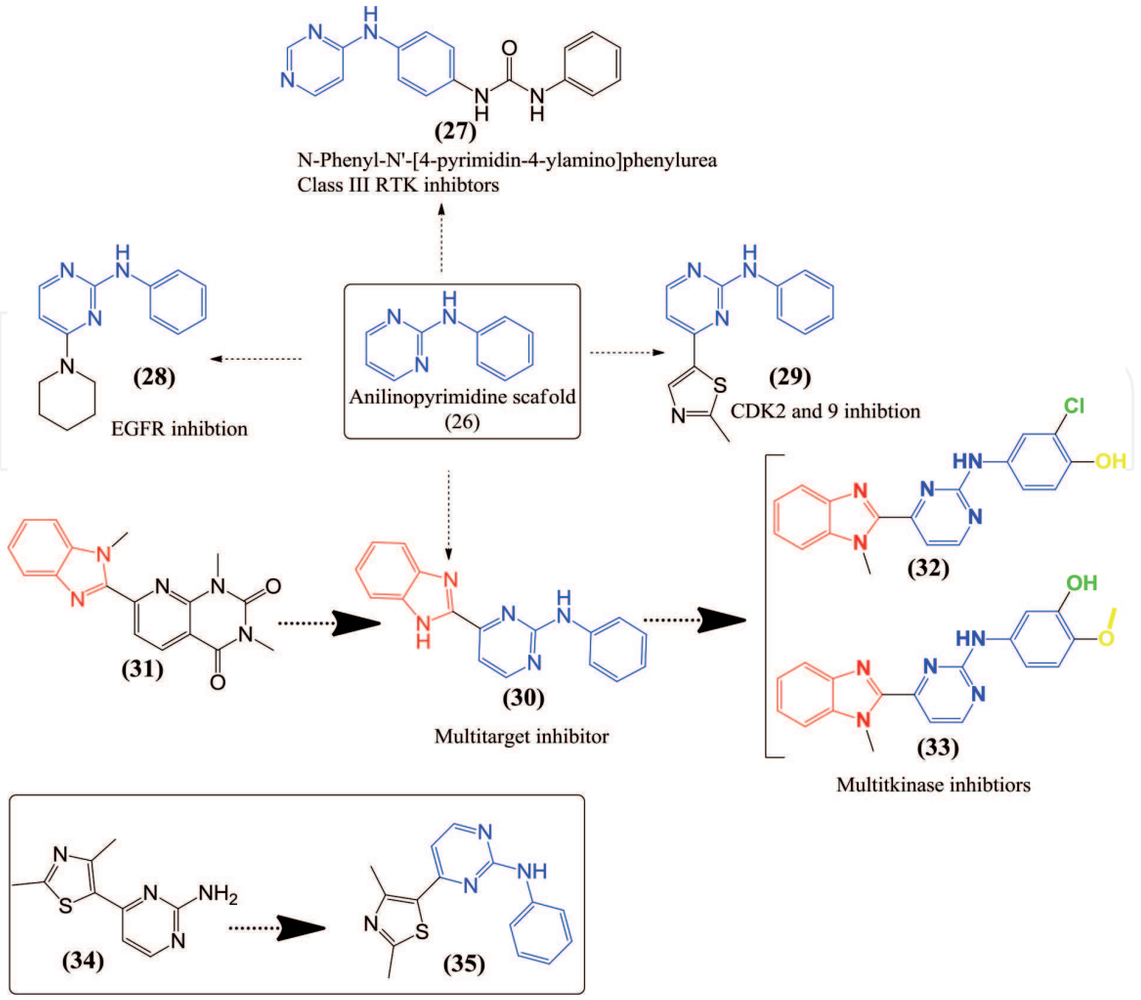
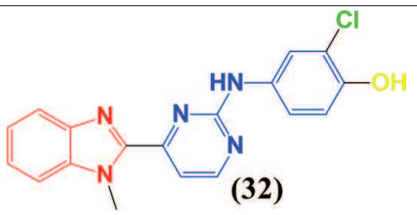
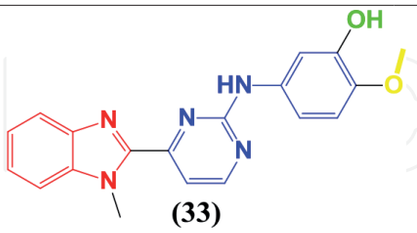
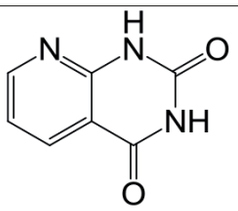


Figure 10.
Development of multitargeting 2-anilino-4-(benzimidazol-2-yl)-pyrimidine scaffold (30) starting from hinge binding compound 1,3-dimethyl-7-(1-methyl-1H-benzimidazol-2-yl)pyrido[2,3-d]pyrimidine-2,4(1H,3H)-dione (31). 2-anilino-4-(benzimidazol-2-yl)-pyrimidine derivatives 2-methoxy-5-{[4-(1-methyl-1H-benzimidazol-2-yl)pyrimidin-2-yl]amino}phenol (33) most potent compound and 2-anilino-4-(benzimidazol-2-yl)-pyrimidine derivatives 2-hydroxy-5-{[4-(1-methyl-1H-benzimidazol-2-yl) pyrimidin-2-yl]amino}phenol (32))), 4-(2,4-dimethyl-thiazol-5-yl)pyrimidin-2-ylamine (35), and 2-anilino-4-(thiazol-5-yl)pyrimidine (29).

Based on high-throughput screening method radiometric protein kinase assay (33PanQinase® Activity Assay) [149], 11 recombinant cancer-related protein kinases (AKT1, ARK5, Aurora B, AXL, FAK, IGF1-R, MET, PLK1, PRK1, SRC, VEGF-R2) were screened by a library of compounds. Interestingly, four kinases (Aurora B, FAK, PLK1, and VEGF-R2) proved to be of particular sensitivity to the tested compounds (**Table 3**). This group of four kinases is involved in oncogenesis and maintenance of vital processes of cancer. Thus it is believed that their concerted inhibition could be useful in the treatment of various malignancies. It is worth noting the infectivity of most of tested compounds, including the active ones against AKT1 (shown in **Table 3**).

2.4.1 2-Anilino-4-(benzimidazol-2-yl)pyrimidine-target interactions

Though the authors did not report a prudent SAR, however, docking compound (33) to ATP-binding pocket of PLK1 (PDB 2OWB) helped rationalize the initial observations [142]. One main reflection highlighted the positioning of the anilinopyrimidine fragment in the hinge region, forming a pair of hydrogen bonds to Cys133. Methoxy (CH_3O-) group at the position 2 of the aniline moiety forms a

	AKT1	Aurora B	FAK	PLK1	VEGF-R2
 (32)	>100	7 ± 2.3	10.4 ± 2.7	6.0 ± 0.1	7.5 ± 2.0
 (33)	>100	6.0 ± 0.2	3.4 ± 0.8	1.2 ± 0.2	7.2 ± 0.3
 (39)	>100	>100	92	>100	85
Sorafenib	>10	1.8	>10	>10	0.022
Sunitinib	>10	1.5	1.6	>10	0.070

Compound (33) exhibited activities that range between IC_{50} = 1.2 and 7.2 μM [142].

Table 3.
Protein kinase inhibition by (32 and 33) compared pyrido[2,3-d]pyrimidine-2,4(1H,3H)-dione (39) to standard multitargeting FDA-approved agents sorafenib and sunitinib.

hydrogen bonding with the guanidine of Arg136 residing at the opening of the PLK1 ATP-binding pocket. The inactivity of derivatives with substituents bulkier than methoxy group (CH_3O-) was explained partially by the clash with Leu59 and Arg136 at the pocket entrance indicating limited tolerance to variation at this region.

2.5 Benzimidazolylamidines as multitargeting agents

Silvana Raić-Malića and colleagues reported the synthesis of a group of benzimidazole amidine derivatives [150]. Specifically, compound (**Figure 11**, (36)) abrogated the activity of several protein enzymes including tissue transglutaminase (TGM2) and kinases like CDK9, sphingosine kinase 1(SK1), and p38 mitogen-activated protein kinase (p38 MAPK), whereas compound (37) did not have profound effect on CDK9 and TGM2 but showed moderate downregulation of SK1 and significant reduction in p38 MAPK.

A small library comprising 27 compounds was screened for the potency. Two of them, *p*-chlorophenyl-substituted 1,2,3-triazolyl derivatives of amidine *N*-isopropyl amidine (36) and imidazoline amidine (37), exhibited remarkable antiproliferative activities with IC_{50} of 0.05 and 0.06 μM in non-small cell lung cancer cells A54 and was defined as multitarget inhibitors.

In their endeavor to look for potent inhibitors for treatment of non-small cell lung cancer, Silvana Raić-Malić and her team developed a group of benzimidazole amidine derivative that showed an inhibitory effect on several key players in cancer

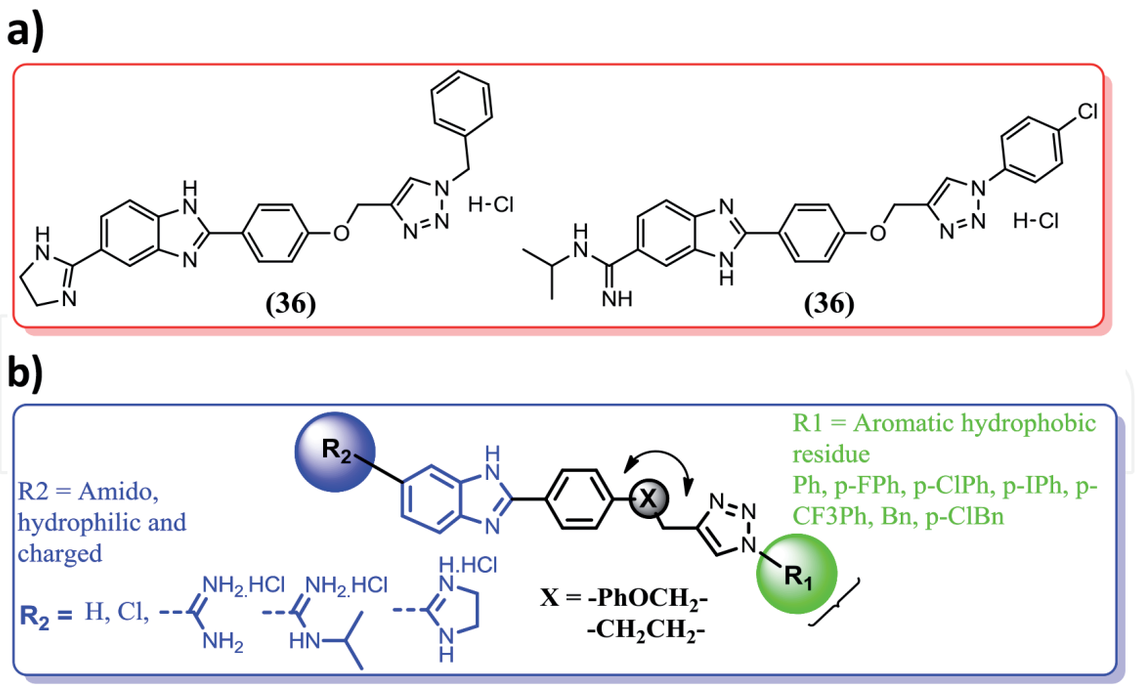


Figure 11.
(a) Hit compounds prepared and screened for multitarget action 2-(4-((1-Benzyl-1H-1,2,3-triazol-4-yl)methoxy)phenyl)-5-(4,5-dihydro-1H-imidazol-2-yl)-1H-benzo[d]imidazole hydrochloride (36),2-(4-((1-(4-chlorophenyl)-1H-1,2,3-triazol-4-yl)methoxy)phenyl)-N-isopropyl-1H-benzo[d]imidazole-6-carboximidamide (37); (b) summary of structure–activity relationship of benzimidazolylamidines [150].

proliferation [150]. A recent study reported that synthesis of amidino 2-substituted benzimidazoles linked to 1,4-disubstituted 1,2,3-triazoles by applying microwave and ultrasound irradiation in click reaction and subsequent condensation of thus obtained 4-(1,2,3-triazol-1-yl)benzaldehyde with *o*-phenylenediamines. The study concluded the improved cytotoxic effect (within the nanomolar range; IC_{50} of 50 and 60 nM) against hepatocellular carcinoma cells. A follow-up study affirms the conclusion that when benzimidazole is conjugated to 1,2,3-triazole moiety, the hybrid exerts potent and selective antiproliferative effect against a panel of cell lines [non-small cell lung cancer (A549), ductal pancreatic adenocarcinoma (CFPAC-1), cervical carcinoma (HeLa), and metastatic colorectal adenocarcinoma (SW620) as well as on normal human lung fibroblasts (WI38)] with 5-fluorouracil (5FU) as a positive control. Two hits (36) and (37) (**Figure 11a**) demonstrated a potent activity at nM range (IC_{50} of 50 and 60 nM) against non-small cell lung cancer (A549). Interestingly, benzyl-substituted 1,2,3-triazolyl analogue of imidazoline (36) exhibited a remarkable and selective activity (IC_{50} = 0.07 μM) on A549 cell line. A mechanistic study performed on A549 cell line using Western blotting reinforced the belief that nature of aromatic substituent of 1-(1,2,3-triazolyl) and amidino moiety at C-5 position of benzimidazole ring is critical to the cytostatic activity of this group of compounds. In silico analysis supported the conception that (36) is bound slightly better than (37) to ATP-binding site of p38 MAPK, which correlates with observed decrement in the expression level of phospho-p38 MAPK displayed by (36). The importance of triazole was referred to its ability to form one H-bond with Met109 in the hinge region. Aminobenzimidazole group forms a number of HB with polar amino acids Glu71, Hid148, and Asp168 in the linker region. Phenyl moieties found on the hybrid both are placed in the hydrophobic environment. The phenyl connected to the triazole is assumed to participate in a π-π stacking with Phe169 (see **Figure 11**). The study reported (36) as a multitarget inhibitor since it abrogated the activity of several protein kinases including TGM2, CDK9, SK1, and p38 MAPK.

3. Conclusion

3.1 Multitargeting and polypharmacology

According to the definition of Richard Morphy, the multitarget drugs are defined as "compounds that are designed to modulate multiple targets of relevance to a disease, with the overall goal of enhancing efficacy and/or improving safety" (Morphy, Rankovic, 2005) [151].

Modulating the function of numerous biological molecules is a well-established pharmacological approach in medicine practice. Paracetamol, a traditional therapeutic used worldwide, is believed to induce its effects via action on multiple targets. Several psychoactive, serotonergic, cholinergic, and adrenergic agonists or antagonists exercise their actions on a wider range of singular biomolecular target.

Apart from the alphachloroacetamidobenimidzoles (22), the groups of compounds reported so far in the literature as multitarget agents act in most cases on receptor tyrosine kinases (RTKs) as competitive ATP inhibitors. Those by virtue occupy the vicinity of ATP with the heteroaromatic system interactively buried in the purine portion pocket and interact with the hinge region of the kinase domain. The thiol (-SH)- π and the stacking π-π together with the hydrophobic interaction with the floor and the ceiling of the purine-binding regions are believed to do the required binding adjustment as kinase inhibitors. Crystallographic structure of dovitinib human FGFR1 revealed the occupancy of the purine-binding regions (part of the ATP-binding site) with the quinolinone-benzimidazole fragment, while the N-methylpiperazine attached to C5' at the phenyl part of the benzimidazole is pointing toward the opening and is exposed to the solution. Thus, it seems that benzimidazole portion is not interacting directly with the hinge region of the enzyme. Similar binding is noticed with AT9382. The pyrazolylbenzimidazole and the benzamide motif take part in HBD-HBA bridging with the hinge of the kinase domain.

In the case of 2-anilino-4-(benzimidazole-2-yl)pyrimidine, the benzimidazole portion looks immersed deep in the purine-binding regions of the ATP-binding site participating in direct interactions via hydrogen bonding and hydrophobic interactions, while the hydroxymethoxyaniline portion points towards the solvent exposed area.

3.2 Lessons learnt

3.2.1 Discovery methods

Despite the imbedded potential, the multitarget activity of the reported benzimidazole-based scaffolds was identified serendipitously. In other words, none of the benzimidazole anticancer multitargeting agents seem to be identified in unforeseen manner, and in many ways they emerge with no intention to be designed initially. While adhering to the development of selective and specific agents, results accumulated afterword revealed multitarget action. For example, 3-benzimidazol-2-ylhydroquinolin-2-one scaffold [benzimidazolylquinolinone (**Figure 4**, (12)] was identified using high-throughput screening (HTS). AT9283 (**Figure 6**, (21)) was identified following fragment-based structural approach with the initial aim to develop an Aurora selective inhibitor, and later it was reported to act as multitargeting agent.

It is hoped that the identification, discovery, and optimization of benzimidazole-based multitargeting anticancer agent will benefit from the "big data era" fueled by data available from public repositories.

3.2.2 Shift in the paradigm

Multitargeting can occur via three possible ways: acting on the same target, on different targets of the same pathway, or on different targets of different pathways. So far the benzimidazole derivatives that have been explored are reported to act as the third category "acting on different targets of different pathways." The focus has been so far on the kinome-relevant signaling key player with dovitinib widening the landscape to non-kinase targets. Broadening "multitargeting" concept to identify novel inhibitors with potency against key targets outside the human kinome necessitates treating complex diseases using "polypharmacology" gains special interest in resistant mutated spreadable cancers [151].

Despite the initial enthusiasm for the efficacy of molecular targeted therapeutics following the approval of imatinib, a small tyrosine kinase inhibitor targeting BCR-Abl, in chronic myeloid leukemia (CML) and trastuzumab, a monoclonal antibody against HER2, for treatment of metastatic breast cancer, scientists and clinicians were challenged by recurrent relapse due to cancer patients who developed drug resistance. In the case of RTKi, resistance can emerge as a result of selection for mutant sin in the target that renders the binding site inaccessible, reduced influx accompanied by enhance efflux, shift in metabolism and excretion of the drug, and the activation of alternative signaling pathways. Thus, the rationale for targeting drugs is shifting. In the last two decades, the main effort was aimed at developing highly specific inhibitors acting on single target. Now, there is a general agreement that molecules interfering simultaneously with multiple RTKs might be more effective than single-target agents. With the recent approval by the FDA of sorafenib, regorafenib, sunitinib, lenvatinib, and axitinib-targeting VEGFR, PDGFR, FLT-3, and c-KIT—more attention is drawn to broad-spectrum anticancer properties multikinase targeting drugs. Thus it is anticipated that more multitargeting agents will be getting into clinical trials and making their way to clinical application. It is hoped that identification, discovery, and optimization of benzimidazole-based multitargeting agents will benefit from the "big data era" fueled by the availability of big data, advances in technology, and artificial intelligence.

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Conflict of interest

No conflict of interest exists.

Notes/thanks/other declarations

I would like to express my special thanks, gratitude and truthful appreciation to my dearest family: my wife Muna, my daughters Aseel and Layan and my sons

Mulham and Majd for all the love, compassion, and support I received from them all along.

This chapter is dedicated to the respectable memories of my mother Jaleelah and father Salem who died of old age and to the reminiscence of my dearest brother Mohammed who left this world due to leukemia. Peace Be Upon Them All.

Acronyms and abbreviations

MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide
PRK1	actin-regulating kinase
MEK1	activated protein kinase
AML	acute myeloid leukemia
ADME	adsorption, disposition, metabolism, excretion
ARK5	AMPK-related kinase 5
ALK	anaplastic lymphoma kinase
ALP	alkaline phosphatase
AXL	"anexelekto" receptor tyrosine kinase
BCR-Abl	breakpoint cluster region-Abelson kinase
CK2	casein kinase 2
CXCR3	chemokine receptor
CML	chronic myelogeneous leukemia
DHODH	dihydroorotate dehydrogenase
D2R	dopamine receptor 2
FGFR-1/FGFR-2/FGFR-3	fibroblast growth factor receptors 1, 2, 3
FAK	focal adhesion kinase
IC_{50}	half maximal inhibitory concentration
HGF or MET	hepatocyte growth factor
H4H	histamine receptors 4
HDAC2	histone deacetylase 2
MDA-MB-468	human breast carcinoma cell lines
HER2	human epidermal growth factor receptor 2
HB	hydrogen bind
HBD	hydrogen bond donor
HBA	hydrogen bond acceptor
5-HTR	hydroxytryptamine receptors
CDK	inhibition of cyclin-dependent kinases
IGF-1R	insulin-like growth factor 1 receptor
ITK	interleukin 2-inducible T-cell kinase
JAK-1/JAK-2/JAK-3	Janus kinase-1/2/3
LE	ligand efficiency
Lck	lymphocyte tyrosine kinase
MEKK2	mitogen-activated protein kinase kinase kinase 2
nM	nanomolar
μM	micromolar
PAK1	p21-activated kinase
p38 MAPK	p38 mitogen-activated protein kinase
Ph + ALL	Ph + acute lymphoblastic leukemia
PI3K	phosphatidylinositol 3-kinase
PDGFR-α/β	platelet-derived growth factor receptor-α/β
PLK-1	polo-like kinase 1
PARP-1	poly(ADP-ribose)polymerase-1

ATP	adenosine triphosphate
PDB	Protein Data Bank
AKT1	RAC-alpha serine/threonine-protein kinase
PTKs	protein tyrosine kinase
PTP	protein tyrosine phosphatases
c-KIT	stem cell factor receptor
FLT3	FMS-like tyrosine kinase 3
FLT3-ITD	FMS-like tyrosine kinase 3 internal tandem duplication
SAR	structure–activity relationship
SVM	support vector machines
ATR	telangiectasia and Rad3-related protein kinase
TOPO1/TOPO2	topoisomerase 1/2
TKRs	tyrosine kinase receptors
VEGFR-1/VEGFR-2/VEGFR-3	vascular endothelial growth factor receptor-1, 2, 3
SRC	v-src sarcoma (Schmidt-Ruppin A-2) viral onco gene homolog (avian)

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