Account

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LASSBio Chemical Library Diversity and FLT3 New Ligand Identification

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This account describes the analysis of the Laboratory of Evaluation and Synthesis of Bioactive Substances (LASSBio) chemical library as a valuable resource for early drug discovery studies. LASSBio has been using medicinal chemistry strategies for almost 30 years to design new prototype compounds with a focus on pharmacological activity. The LASSBio Chemical Library (LCL) is a collection of more than 2000 compounds, and the aim of this work was to characterize its chemical diversity and to perform a molecular repositioning study to identify ligands of feline McDonough sarcoma (FMS)-like tyrosine kinase 3 (FLT3), a validated target for leukemia treatment. To achieve this, cheminformatic tools were utilized to analyze the chemical diversity of the LCL. The analysis allowed the identification of the most representative compounds of this collection, showing that the *N*-acylhydrazone chemotype is present in approximately 50% of the compounds. Furthermore, the compounds in this chemical library demonstrated remarkable compliance with both the Lipinski's (93% of the compounds) and Veber's (96% of the compounds) rules. In the study on molecular repositioning, 10 compounds were selected through virtual screening to test their enzymatic inhibition of FLT3 kinase. Among them, 4-((6,7-dimethoxyquinazolin-4-yl)amino) benzamide (LASSBio-2166) (**31**) showed a half-maximal inhibitory concentration (IC_{50}) = 194 nM. Optimization of the identified hit and further studies to compare the diversity of the LCL with other libraries are perspectives of this work.

Keywords: medicinal chemistry, chemical library, chemical database, virtual screening, FLT3

1. Introduction

Chemical libraries, also referred to as compound collections, play a key role in drug discovery.[1,2] Since the 1990s, the importance of chemical libraries has increased due to the advances in combinatorial chemistry and high-throughput screening (HTS) techniques. In modern medicinal chemistry, Gallop *et al.*[3] defined chemical libraries as "intentionally created collections of differing molecules which can be prepared either synthetically or biosynthetically and screened for biological activity". Historical perspectives suggest that libraries originating before the period of combinatorial chemistry can be considered classical chemical libraries. Many classical libraries that greatly contributed to medicinal chemistry projects in the 20th century originated from the pharmaceutical industry or academia.[4]

According to Bakken *et al.*,[5] HTS can provide hits in over 90% of screening projects, and 50 to 90% of the hits progress to hit-to-lead optimization steps. However, when considering the number of initial substances in a random HTS, the success rate for identifying hits is very low, between 0.01 and 0.1%. This means that for every 10,000 compounds screened, only 1 to 10 hits will be identified. This scenario explains the high costs associated with HTS, and it has been suggested this is one of the factors behind the crisis in productivity and creativity in the pharmaceutical industry.[6,7] Therefore, combining virtual screening strategies with experimental screening

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has demonstrated a synergistic effect and an increase in the rate of hit identification.[8]

Virtual chemical libraries are increasingly important for supporting screening studies,[9] allowing hit identification after experimental tests. Various names and subdivisions for these collections have been described in the literature, including diversity-oriented, target-oriented, ligand-oriented, peptide-mimetic, fragment-oriented, drug-like, and lead-like chemical libraries.[10] In addition, the term chemical diversity is used to describe the uniformity and similarity in structure of the chemical entities that make up a library of compounds. Therefore, the diversity of a chemical library is related to the scope of the chemical space it occupies. In other words, a more diverse library will be more comprehensive in relation to the possible chemical space. The concept of chemical space aids in both the interpretation and design of compound libraries.[11-13]

Lipinski and Hopkins[14] defined chemical space as analogous to the vastness of the cosmological universe, with chemical compounds populating space instead of stars. Various parameters help interpret large volumes of information, such as the estimation of chemical space described by others.[13,15] Common practices for assessing the chemical diversity of chemical libraries include analysis by substructure, structural similarity, or physicochemical parameters. Demarcations based on physicochemical properties have resulted in defining characteristics that are considered ideal for molecules at different stages of the drug discovery and development process.[16] For instance, the term "lead-like" describes substances with a molecular weight (MW) of 100-350 Da and a clogP (calculated log of the partition coefficient) of 1-3, while the term "drug-like" includes a broader range of variations, including a molecular weight of up to 500 Da and a clogP of –2 to 5.[16-18] In addition, there are general rules that can aid in characterizing and designing chemical libraries, such as the Lipinski's "Rule of 5" (molecular weight < 500 Da; clogP < 5; number of hydrogen bond donors ≤ 5 and number of hydrogen bond acceptors ≤ 10)[19] and Veber's rules (polar surface area ≤ 140 Å^2 and number of rotatable bonds ≤ 10).[20]

In medicinal chemistry projects that involve the use of chemical libraries (Figure 1), the molecular repositioning strategy is a valuable approach for hit identification. Repositioning involves identifying new indications for compounds originally intended for a different therapeutic purpose, which reduces the time and cost of drug discovery and development.[21] Similarly, we suggest using substances with a favorable profile for drug discovery and development, as detailed by assessing the physicochemical properties of the LASSBio Chemical Library, to conduct molecular repositioning studies.

The Laboratory for the Evaluation and Synthesis of Bioactive Substances (LASSBio) uses medicinal chemistry molecular modification strategies to design new prototype compounds. The LASSBio Chemical Library (LCL) contains over 2,000 compounds resulting from the laboratory's research projects. The library has been designed based on medicinal chemistry concepts, prioritizing pharmacological activity and focusing on compounds with an appropriate lead-like and/or drug-like profile. Due to its ideal profile for drug discovery projects, the LCL was the driving force behind the strategic partnership with the pharmaceutical industry Eurofarma, through the National Institute of Science and Technology for Drugs and Medicines (INCT-INOFAR). The institutions signed a technical-scientific cooperation agreement to access the LCL for collaborative research aimed at discovering innovative drugs for the treatment of pain, leishmaniasis, inflammation and depression.[21,22]

As a result of this favorable ecosystem for science and innovation, LASSBio has expanded its medicinal chemistry research programs using the LCL,[22,23] with a focus on characterizing its molecular collection and performing repositioning studies. This work aims to characterize the

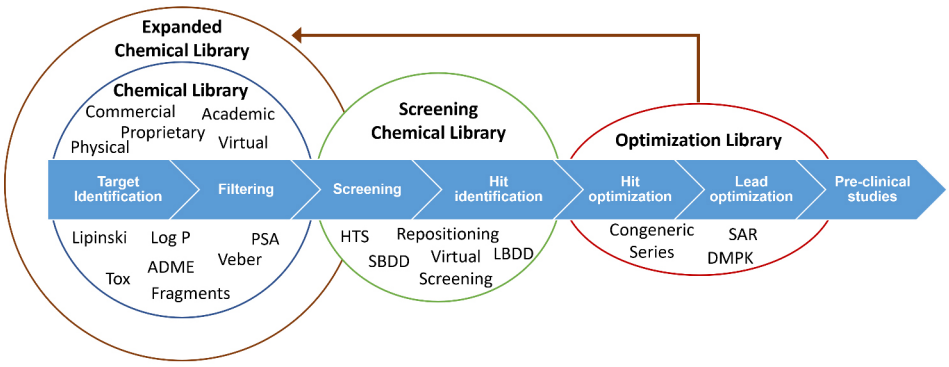


Figure 1. General workflow for using chemical libraries in medicinal chemistry projects. The initial chemical library can be filtered to create a screening compound library, which is suitable for repositioning studies. This stage enables the identification of hits, which can then be optimized to generate leads that can advance to the drug development stages. The compounds from the optimization step can be added to the initial library, expanding it.

LCL in terms of its chemical diversity and molecular profile, from a physicochemical and pharmacodynamic standpoint. In addition, a study was conducted to reposition molecules against feline McDonough sarcoma (FMS)-like tyrosine kinase 3 (FLT3), a validated molecular target for cancer treatment.

2. Methods

2.1. Structures

The LCL currently holds 1811 disclosed compounds and is regularly updated. Chemical structures are represented by a SMILES string and mol format, while 3D conformers are available in mol2 file format.

2.2. Chemical diversity determination

Chemical diversity studies were performed on the KNIME platform.[24] The SMILES descriptors were transformed into Morgan-type fingerprints (1024 bits) using the RDKit program.[25] The bit vectors from the fingerprints were normalized using the Z-score algorithm. Principal component analysis (PCA) was performed to reduce the data to two dimensions. Euclidean distances were calculated from the generated dimensions, and *k*-means clustering was applied based on these distances. *K*-medoids was used to determine the main representative compound for each cluster. The 2D/3D Scatterplot node provided by the Erlwood cheminformatics extension[26] was used to obtain the chemical space plot. To determine the chemical diversity of *N*-acylhydrazones, substructure searches were performed using the "Substructure Search" node[27] provided by the CDK extension.[28]

2.3. Physicochemical and pharmacodynamic profile determination

Physicochemical parameters were analyzed using structures from the LCL in mol format and the RDKit program. The activities associated with compounds from the LCL in the Cortellis Drug Discovery Database were identified by searching for the term 'LASSBio' and extracting all relevant substances from the Drugs & Biologics knowledge area. The Experimental Pharmacology section contains information on pharmacological activities. All activity values with '>' operators and values above 1 mM were excluded before compiling the data. To identify activities associated with compounds of the LCL in ChEMBL database,[29] KNIME was used to extract molecules with a molecular weight less than 1000 Da from the SQL format of ChEMBL31. The molecules were converted into Morgan fingerprints with a radius of 2 (1024 bits). Tanimoto coefficient similarity search was then used to identify LCL compounds from ChEMBL31. Data filtering was based on the ChEMBL31 schema available on the ftp interface. Experimental data for each molecule was extracted using the molregno ID. The data was then curated using assay description and target information, such as protein_class_id and pref_name.

2.4. Virtual screening

The crystal structures of FLT3 (UniProt ID P36888) available in the Protein Data Bank (PDB) database were collected and analyzed in 3D using UCSF Chimera.[30] To generate the protein structure for docking, hydrogen was added using the UCSF Chimera program. The GOLD 5.8.1 program[31] was used to remove water molecules. For the selection of binding site, the native co-crystallized ligand was used as a reference and all amino acid residues in a radius of 6 Å were selected. The molecular docking process was carried out using the GOLD genetic algorithm in default mode, with 30 runs for each ligand, utilizing the structure with PDB ID 4RT7. The redocking procedure was performed using ChemPLP, GoldScore, ChemScore, and ASP scoring functions. The scoring function was selected based on a root-mean-square deviation (RMSD) value lower than the crystal resolution value. To analyze the docking poses of LCL compounds, compounds were selected based on their chemical similarity to FLT3 inhibitors extracted from the ChEMBL database (MW < 1000 Da; half-maximal inhibitory concentration (IC_{50}) < 10 μM). To achieve this, the LCL compounds and FLT3 inhibitors were converted into Morgan fingerprints with a radius of 2 (1024 bits). Only compounds with a Tanimoto coefficient greater than 0.4 were selected for pose analysis. Additional compounds were selected for analysis based on substructures of hinge binding groups of reported inhibitors. These compounds were identified through substructure searches using the CDK package. Additionally, the top 5% ranked poses (ChemScore) were also analyzed. Docking poses were analyzed in Hermes software[32] to determine their interaction with the hinge region and adjacent pockets occupied by the co-crystallized ligand. Compounds for assays were selected based on measurements of distances and angles using the UCSF Chimera software.

2.5. Inhibition of tyrosine kinase FLT3-ITD biochemical tests

Biochemical assays were conducted at Reaction Biology Corp., Malvern, PA, USA, to inhibit the tyrosine kinase activity of FLT3-ITD. The 'HotSpot' assay

platform was used to perform a radiometric assay using ^{33}P-labeled adenosine triphosphate (ATP) at a concentration of 1 μM.[33,34] Compounds were tested in duplicate at a concentration of 10 μM after being resuspended in 10 mM dimethyl sulfoxide (DMSO) stock solutions. The compounds were tested at ten different concentrations (serial dilution starting at 100 μM with a dilution factor of 3) to determine the IC_{50}. The IC_{50} of the control, staurosporine, was determined at 10 different concentrations (serial dilution starting at 20 μM with a dilution factor of 4). The base reaction buffer consisted of 20 mM Hepes (pH 7.5), 10 mM $MgCl_2$, 1 mM egtazic acid (EGTA), 0.01% Brij35, 0.02 mg mL^{-1} BSA, 0.1 mM Na_3VO_4, 2 mM dithiothreitol (DTT) and 1% DMSO. The peptide substrate (EAIYAAPFAKKK, 20 μM) was incubated with the enzyme mixtures and evaluated compounds for 20 min. Next, ^{33}P-ATP was then added to the mixture to initiate the reaction, which was allowed to proceed for 2 h to enable phosphorylation. Finally, the reaction medium was dripped onto a P81 ion exchange paper. Phosphate residues that were not incorporated were removed by washing the paper extensively with 0.75% phosphoric acid. Phosphorescence intensity was used for detection. The percentage of enzyme activity was calculated from the DMSO control.[35,36]

3. Results and Discussion

3.1. Chemical diversity of the LASSBio Chemical Library

In order to visualize the contents of the LCL, a chemical space representation of this library was constructed. In addition, representative compounds from this library were selected to exemplify its chemical diversity. Tools that can select representative compounds with common structural patterns from databases are useful for diversity analysis.[11,37] In order to compare structures, it is necessary to have an appropriate molecular representation.[38-40] Molecular descriptors are representations that characterize structural information.[41] Fingerprint topological descriptors are binary bit vectors used to describe molecular structures.[38,42,43] These descriptors were developed to aid in the search for substructures in chemical libraries.[44] They are also used for analytical tasks such as molecular similarity searches,[45] cluster analysis,[46] and molecule classification.[47] Extended-connectivity fingerprints (ECFPs) are a class of topological descriptors explicitly formulated to capture molecular information relevant to biological activity, and can also be used for similarity searches, clustering studies, and virtual screening.[47-50] The most commonly used methods are ECFP_4 and ECFP_6, as they generally perform best for describing more complex structures.[51]

Once the chemical structures of a library are represented in a computationally tractable manner, cluster analysis is frequently employed to partition a data set into subsets of chemical entities based on their structural similarity. The subsequent selection of compounds that represent each group of molecules can illustrate the structural diversity of the chemical library under study.[38,52,53] To measure molecular diversity, a chemical space is defined with Cartesian axes, and each compound in the collection is given coordinates based on its descriptors.[37] Therefore, to complement the chemical diversity approach, the chemical space of the LCL was illustrated using the PCA method to reduce the fingerprint bit vector to two dimensions.[54] Subsequently, the Euclidean distances between the compounds were then calculated based on the values obtained from the PCA. These distances were then used for the clustering stage using the *k*-means algorithm, followed by determination of the representative compounds of each group by calculating medoids.[55,56] Next, the LCL was successively grouped into increasing numbers of clusters, starting with two groups, represented by LASSBio-1076 (**2**) and LASSBio-596 (**3**), and ending with the division into six groups, where LASSBio-372 (**15**) was identified as the main representative of the most populated cluster (565 molecules) (Figure 2). The dataset was partitioned into six clusters given that LASSBio-372 (**15**) has the main functional group explored by the LCL, an *N*-acylhydrazone (NAH). Furthermore, the NAH is substituted with a phenyl ring in both the acyl and imine groups. This unsubstituted structural pattern is common among most of the NAHs in this library.

Out of the 945 substances that contain the NAH subunit in their structure, 642 have a phenyl ring, functionalized or not, substituting at least one side of the NAH subunit, i.e., either the carbonyl or imine groups. Among them, 437 molecules have the phenyl group as a substituent on the carbonyl group, functionalized or not. Finally, 175 compounds have the phenyl substituent on both the carbonyl and imine groups (Figure 3).

A 2D scatter plot (Figure 4) was generated to illustrate the chemical space of the LCL in two dimensions. The data was divided into six clusters and their representative compounds were highlighted. Each point on the graph represents a chemical structure in the LCL, and each color represents a group of substances.

3.2. Physicochemical and pharmacodynamic profile of the LASSBio Chemical Library

The pharmaceutical industry experienced high attrition rates and low effectiveness in the drug discovery and development process, particularly during the clinical

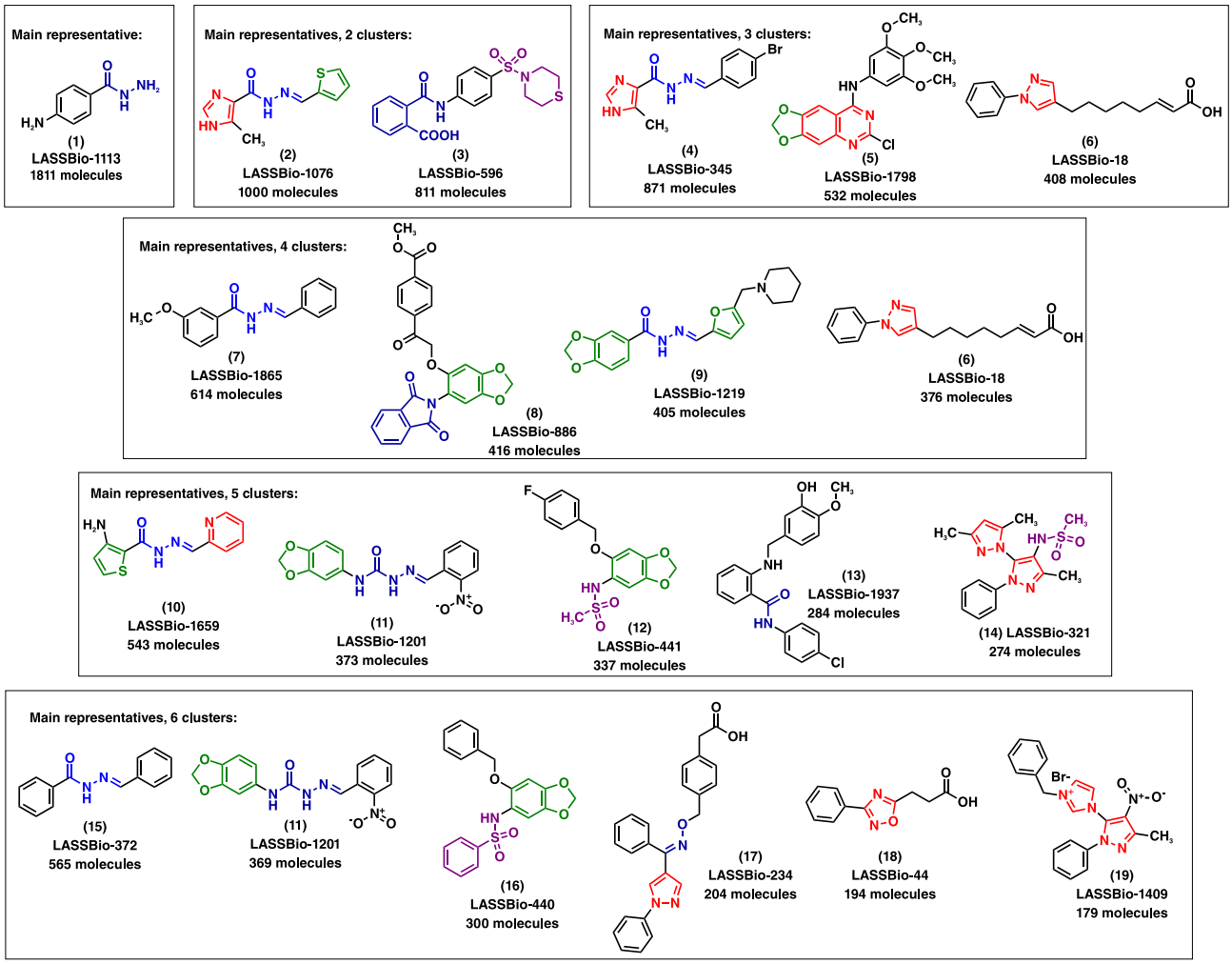


Figure 2. Representative structures of the LASSBio Chemical Library at each clustering step, along with the number of molecules in each cluster.

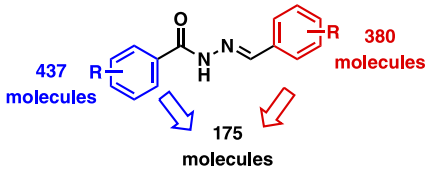


Figure 3. Number of structures that contain phenyl rings, whether functionalized or not, that substitute the carbonyl (blue) and imine (red) groups of the NAHs in the LASSBio Chemical Library. Specifically, 175 molecules have a phenyl ring substituting both ends of the NAH.

stages, due to limitations related to the oral bioavailability of clinical candidates. Lipinski's 'rule-of-five' study and Veber's rules were seminal works that proposed restricting molecular properties to specific ranges to enhance the probability of achieving adequate oral bioavailability.[19,20,57] RDKit was used to calculate descriptors related to these rules for the substances of the LCL (Figure 5). The results showed that approximately 93% of the compounds complied with Lipinski's 'Rule of 5'. Out of the remaining compounds, 102 did not meet the clogP requirement, 29 exceeded the molecular weight limit, and 1 exceeded the limit for the number of hydrogen bond acceptors. Regarding Veber's rules, 96% of the compounds were compliant. However, 63 compounds did not adhere to the rule for the number of rotatable bonds, and 15 were outside the TPSA range.

The analysis of the LCL's structural and physicochemical parameters confirms the presence of both 'lead-like' and 'drug-like' attributes in the compounds of this collection. The data indicate that the compounds have prevalent molecular weights in the range of 300-400, clogP values between 2-3, 5 hydrogen bond acceptors, and 1 hydrogen bond donor. In addition, there are 508 compounds classified as fragments with a molecular weight of less than 300 Da. The majority of these substances have 4 rotatable bonds and a topological polar surface area (TPSA) between 50 and 75 Å^2 (Figure 5). Since the compounds in the LCL were designed as part of medicinal chemistry projects, most of them have demonstrated prior *in vivo* activity, indicating a suitable pharmacokinetic profile. This profile facilitates

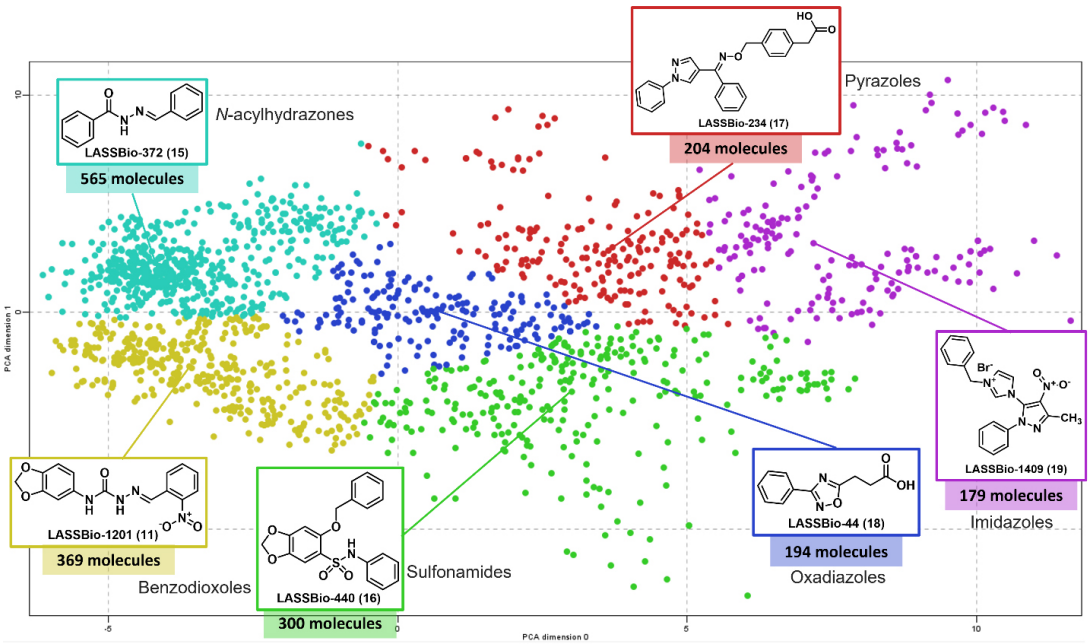


Figure 4. Chemical space of the LASSBio Chemical Library obtained through principal component analysis of the fingerprint descriptor. The library was then clustered using the *k*-means method, and representative compounds were determined for each group using the *k*-medoids method.

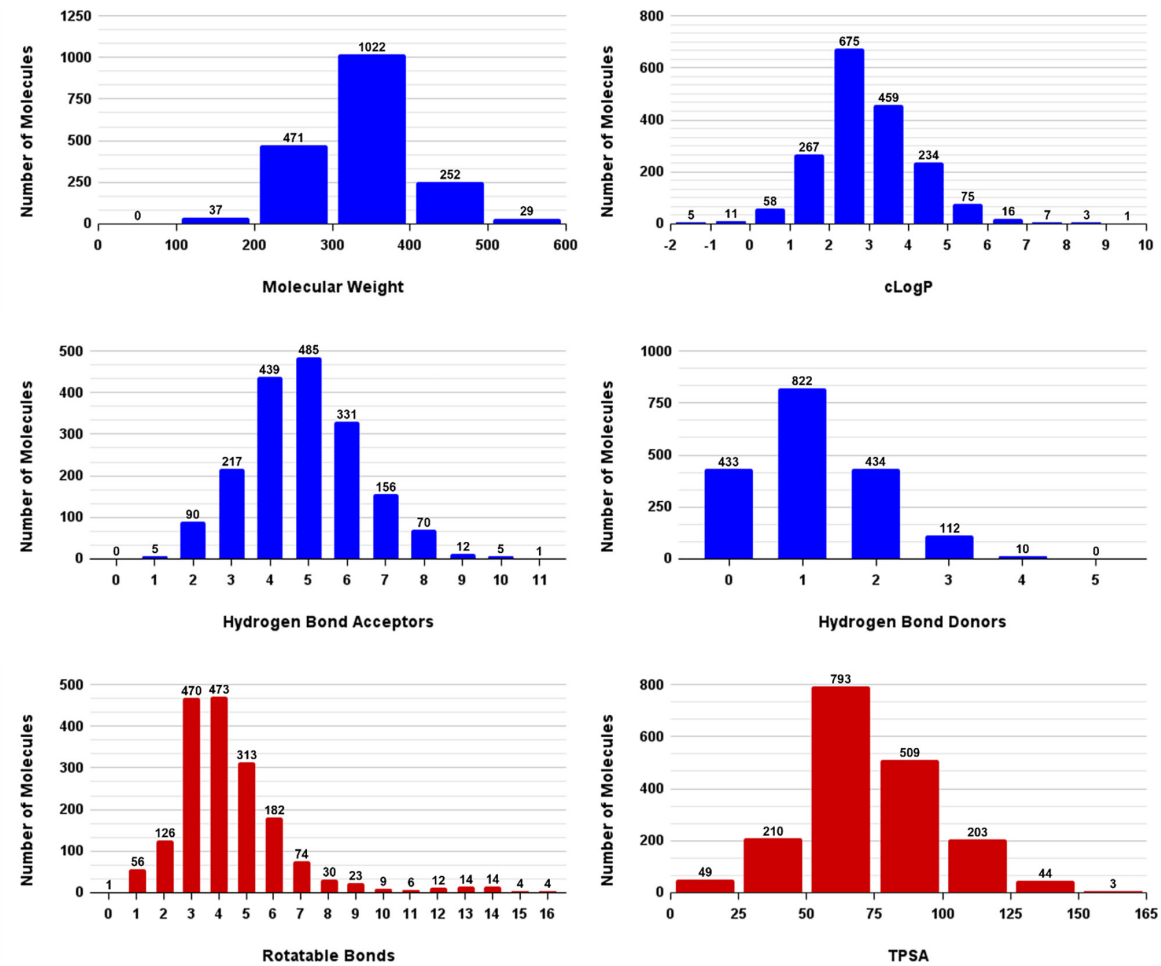
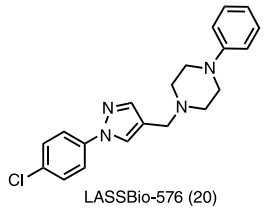


Figure 5. Distribution profile of physicochemical parameters from LASSBio Chemical Library associated with Lipinski's (blue) and Veber's (red) rules. The parameters include molecular weight, clogP, number of hydrogen bond donors and acceptors, number of rotatable bonds, and topological polar surface area (TPSA).

the identification of high-quality hits for drug discovery and development, thereby enabling optimization progress.

To annotate the pharmacological activities of compounds in this library, a search was conducted in the Cortellis Drug Discovery Intelligence database for the activities described for the substances labeled as "LASSBio". The term was used as a query in the "Experimental Pharmacology" knowledge area, and the information extracted information was quantified by the number of occurrences *per* activity described. The search yielded 116 compounds, with 174 types of activity described. This is because some compounds were evaluated in multiple assays. For instance, LASSBio-576 (**20**) was tested against various receptors, including serotoninergic, α-adrenergic, and dopaminergic, with multiple inhibitory constant (K_i) data recorded (Scheme 1).[58-61]



LASSBio-576 (20)
5-HT_{1A} receptor K_i = 90 nM
5-HT_{2A} receptor K_i = 2.32 nM
5-HT_{2C} receptor K_i = 8.63 nM
α_{1B} receptor K_i = 2.60 nM
α_2 receptor K_i = 2.50 nM
D_2 receptor K_i = 110 nM
D_4 receptor K_i = 180 nM

Scheme 1. Chemical structure of LASSBio-576 and its activity profile on serotoninergic, adrenergic and dopaminergic receptors.

The identified compounds represent approximately 5% of the LCL content. This data corresponds to the most active compounds in each published article and not to the entire congeneric series described in each study. This subset provides a general representation of the pharmacological space of the compounds in the library, as shown in Figure 6. These compounds are linked to 53 types of assays. Several enzyme inhibition tests have been described for various targets, particularly kinases (highlighted in red in Figure 6), histone deacetylases, and phosphodiesterases. In addition, there are a significant number of modulators of G protein-coupled receptors (GPCR, highlighted in green in Figure 6). Kinase inhibitors and GPCR represent target classes which are among the most important for recently approved drugs.[62,63]

An additional analysis was conducted to identify LCL compounds in the ChEMBL database.[64] Compounds extracted from the SQL format of ChEMBL31, with a molecular weight of less than 1000 Da, were converted to Morgan fingerprints (radius 2) for Tanimoto coefficient-based similarity search. A total of 412 compounds, with a similarity coefficient of 1.0, associated with 7679 pharmacological activity data points.

After filtering out single-point data, only concentration-response experiments annotated as pChEMBL values were included, such as those corresponding to half-maximal effective concentrations (EC_{50}), dissociation constants (K_d), IC_{50}, and K_i), resulting in 3391 pharmacological activities. Of these, 2298 data points were related to the drug acetazolamide. After removing acetazolamide, 1029 pharmacological activity data points related to 168 compounds remained, which were analyzed by activity range (Figure 7). Protein class classification (Figure 8) was accomplished by filtering out activity data that was not relevant to the modulation of a specific protein target, such as phenotypic assays.

4. FMS-Like Tyrosine Kinase 3 Virtual Screening

A virtual screening was conducted against FMS-like tyrosine kinase 3 (FLT3), a validated target for acute myeloid leukemia (AML), given the favorable profile of the LCL for drug discovery. FLT3 belongs to the family of tyrosine kinase receptors and was first identified and isolated by Rosnet *et al.*[65] in 1991. It is expressed in young hematopoietic cells and lymphoid progenitors and plays a crucial role in stem cell survival and myelocytic differentiation. The enzyme is overexpressed in the majority of AML patients and in up to 50% of blasts from patients with acute lymphoblastic leukemia (ALL). Moreover, about 20% of AML patients exhibit a type of mutation called internal tandem duplications (ITD), which leads to constitutive activation of the enzyme. This mutation is a significant biomarker for the disease and is linked to a negative prognosis.[66]

Two main approaches are used in virtual screening: structure-based virtual screening (SBVS) and ligand-based virtual screening (LBVS). SBVS utilizes the 3D structure of the biological target relevant to the pathophysiology of a given disease, while LBVS is based on substances with described activity in an assay and serves as a reference for carrying out computational studies to predict the activity of new substances. To improve the success rate of identifying hits from compound libraries in virtual screening campaigns, the combination of SBVS and LBVS is an effective strategy.[8,67] An important hypothesis in virtual screening is that structurally similar ligands exhibit similar biological activities. When conducting screening based on ligands, one approach is to use at least one ligand as a reference and establish a similarity index to measure the degree of similarity between pairs of compounds. The Tanimoto-Jaccard T coefficient, also known as the

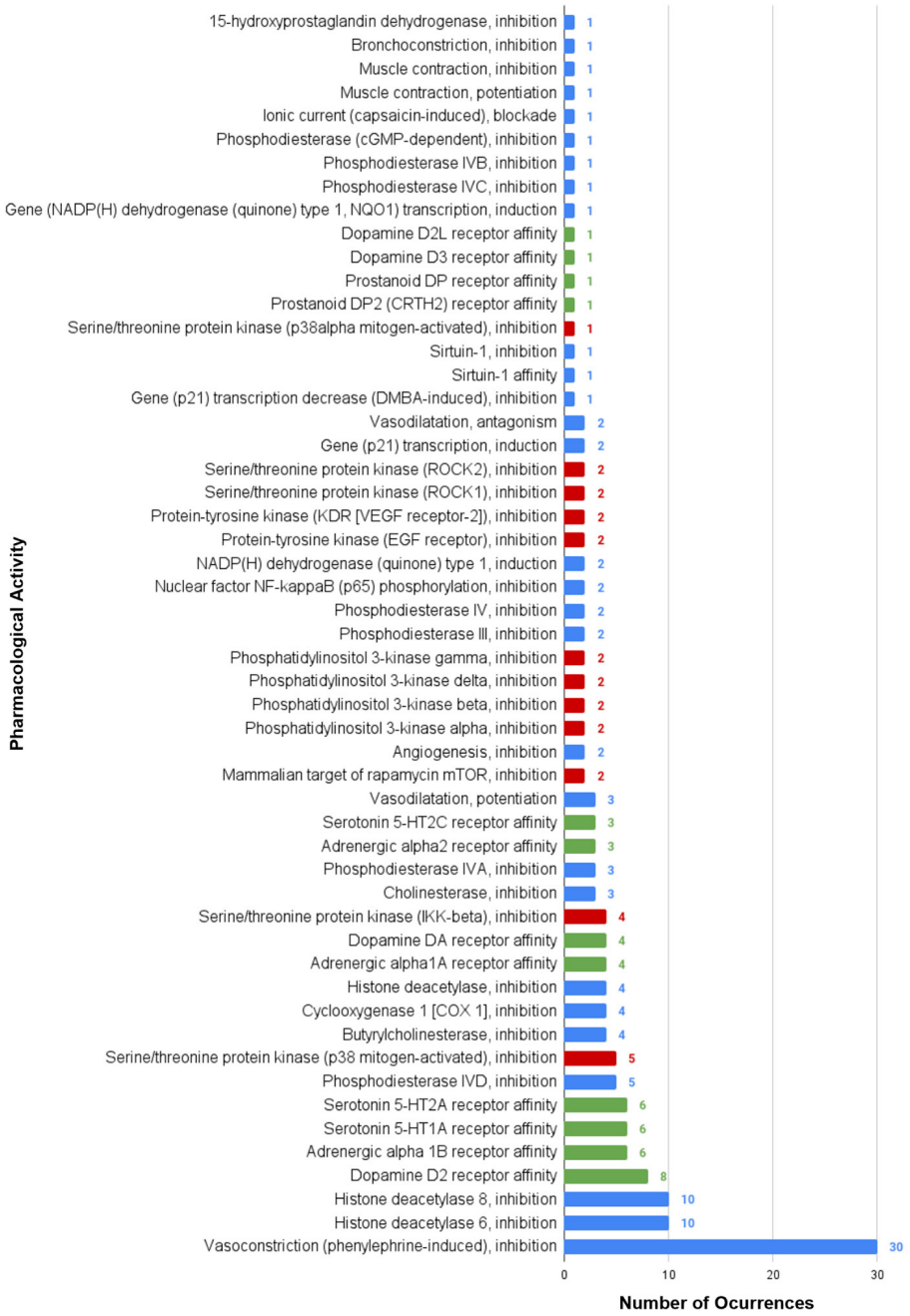


Figure 6. Pharmacological space of the LASSBio Chemical Library. The pharmacological activities are described by the number of occurrences. Assays related to GPCRs and kinases are highlighted in green and red, respectively.

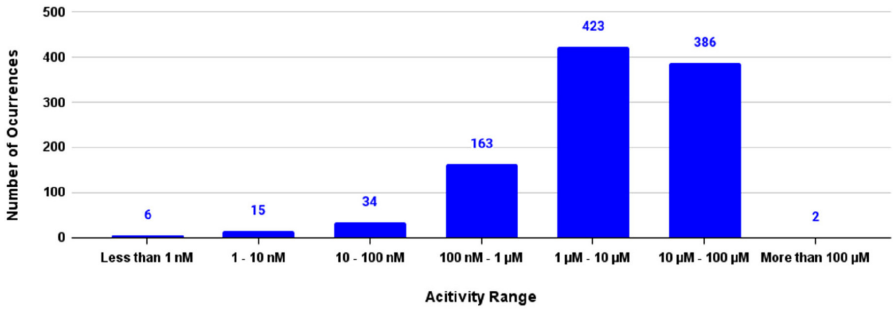


Figure 7. Activity data for compounds in the LASSBio Chemical Library was extracted from ChEMBL31 and grouped by activity range.

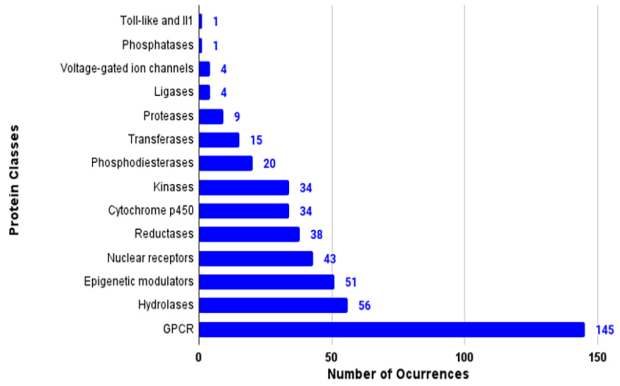


Figure 8. Activity data for compounds in the LASSBio Chemical Library was extracted from ChEMBL31 and grouped by targeted protein classes.

Tanimoto Index,[53,68] is a frequently used similarity index that is based on 2D representations of molecules or substructures. It provides analogous structures, referred to as nearest neighbors of the reference ligands.[69]

A study was conducted to assess the structural similarity between LCL substances and FLT3 inhibitors from the ChEMBL database.[64] Out of 4086 data points, 1686 compounds were obtained as FLT3 inhibitors after removal compounds with IC_{50} > 10 μM or having online single concentration percentage of inhibition data. Using the ECFP_4 binary fingerprint, the structural similarity between compounds of the LCL and FLT3 inhibitors was calculated, identifying 84 compounds from the LCL (Supplementary Information section, Table S1) that were nearest neighbors to 29 FLT3 inhibitors, with a similarity coefficient greater than 0.4.

Substances extracted from the ChEMBL database were classified into specific structural patterns based on their maximum similarity with more than one compound from the LCL. For example, 38 substances in the LCL showed a structural similarity greater than 0.4 with 4 compounds from the FLT3-inhibiting class of thiophene-3-carboxamides (**21**) (Figure 9).[70] This number is due to the presence of the thiophene ring in this class. This ring has been extensively studied by LASSBio in studies involving LASSBio-294 (**38**) and its derivatives.[71-73] In addition, the main compound of the thiophene-3-carboxamide class from ChEMBL contains the benzodioxole ring (**21**) (nearest neighbor to 23 molecules), which is also present in LASSBio-294 (**38**). Additional classes of FLT3 inhibitors have been identified, including disubstituted-1,4-triazole (**22**),[74] quinazoline class (**23**),[75] 1,3-diphenylurea-3-amino-isoxazolo[3,4-*b*]pyridine (**24**),[76] and 1*H*-pyrrolo[2,3-*b*]pyridine (**25**).[77] The substructures of the nearest neighbors of these molecules are highlighted in Figure 9.

The literature FLT3 inhibitors (**21**-**25**) identified in the similarity search interact with the ATP binding site, as suggested by reported molecular docking studies.[74-77] Co-crystallized inhibitors in the PDB include quizartinib (PDB ID: 4RT7)[78] and gilteritinib (PDB ID: 6JQR).[79] These inhibitors interact non-covalently with the ATP binding site and anchor to the hinge region with nitrogenous heterocyclic rings. The compounds from LCL that were nearest neighbors of the FLT3 inhibitors were docked using the FLT3 protein structure, resulting in the selection of ten compounds (**31**-**40**) (Table 1). The compounds were selected for their ability to interact with the hinge region or occupy the adjacent hydrophobic pockets occupied by the co-crystallized reference ligands.

The compounds were evaluated in a radiometric inhibition assay of FLT3-ITD. This assay is based on the conversion of the enzyme substrate to its phosphorylated product using ATP labeled with the radioactive isotope ^{33}P. The labeled phosphate group is transferred to the

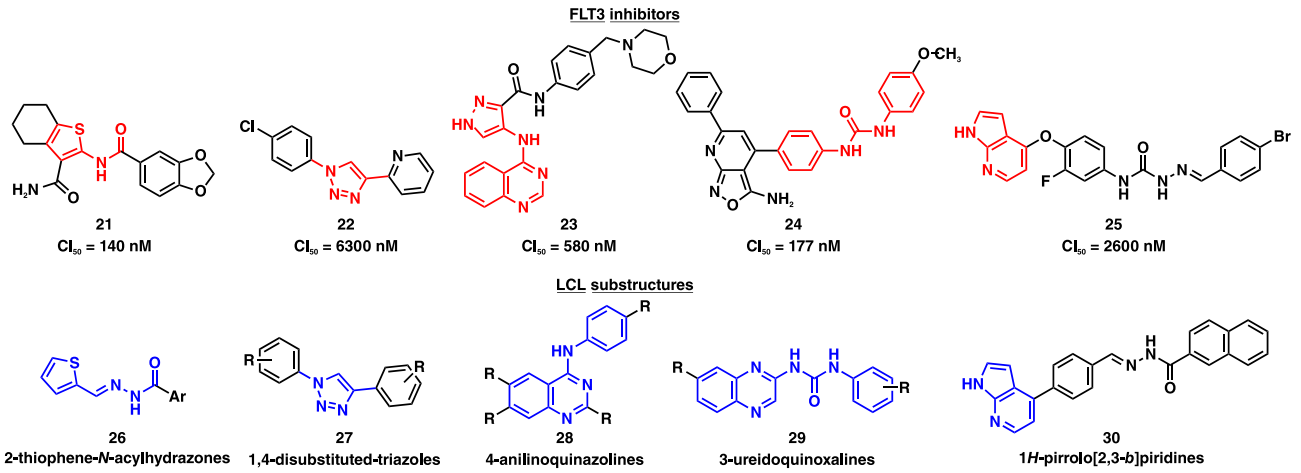
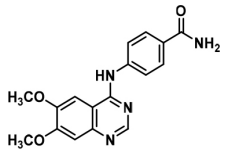
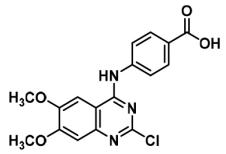
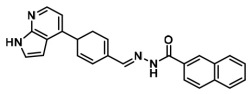
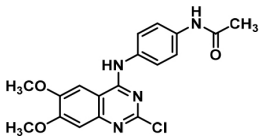
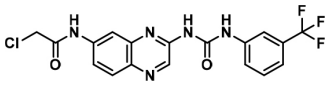
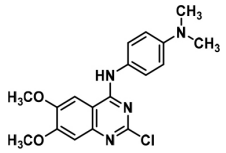
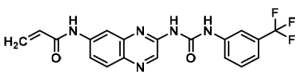
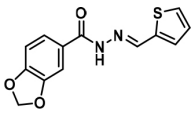
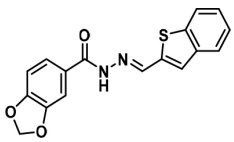
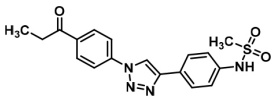


Figure 9. Five substructures belonging to the LCL were identified from similarity search: 2-thiophene-*N*-acylhydrazones (**26**), 1,4-disubstituted-triazoles (**27**), 4-anilinoquinazolines (**28**), 3-ureidoquinoxalines (**29**), and 1*H*-pyrrolo[2,3-*b*]pyridines (**30**). Reference FLT3 inhibitors (**21**-**25**) were extracted from the ChEMBL database.

Table 1. Structures of the substances from the LASSBio Chemical Library evaluated against FLT3-ITD[a]

Compound name	Structure	Inhibition at 10 μM / %
LASSBio-2166 (**31**)[80]		87.1 ± 1.4 (IC_{50} = 194 nM)
LASSBio-1818 (**32**)[81]		48.1 ± 1.0
LASSBio-1829 (**33**)[82]		34.9 ± 1.9
LASSBio-1820 (**34**)[81]		31.7 ± 3.8
LASSBio-1970 (**35**)[83]		31.1 ± 5.3
LASSBio-1812 (**36**)[81]		23.0 ± 5.3
LASSBio-1969 (**37**)[83]		19.9 ± 0.9
LASSBio-294 (**38**)[73]		12.5 ± 0.9
LASSBio-1467 (**39**)[84]		8.9 ± 0.9
LASSBio-1882 (**40**)[85]		6.6 ± 0.9

[a]Staurosporine (IC_{50} = 1.32 nM), a non-selective protein kinase inhibitor, was used as a positive control (Supplementary Information section, Figure S2). IC_{50}: half-maximal inhibitory concentration.

substrate through a reaction catalyzed by FLT3-ITD. After filtration, the ^{33}P-labeled substrate is quantified on filter paper using phosphorescence intensity. Table 1 shows the inhibition percentages of ten compounds selected through virtual screening at a concentration of 10 μM. Based on these results, an assay was conducted to determine the IC_{50} of the most effective compound. LASSBio-2166 (**31**) exhibited 87% inhibition at 10 μM and was tested in a 10-concentration assay, starting at 100 μM with a dilution factor of 3. The IC_{50} of LASSBio-2166 (**31**) was determined to be 194 nM (Table 1).

The docking pose of LASSBio-2166 (**31**) (Chemscore = 32.3) (Figure 10) and the mode of interaction of the cocrystallized drug quizartinib (**41**) (IC_{50} = 1.1 nM) (Chemscore = 50.1; redocking RMSD = 1.37 Å) demonstrate that both compounds bind to the adenine binding region and interact with the hinge region. Both compounds interact with hydrophobic region II, specifically with the Phe830 residue. The amide group of LASSBio-2166 (**31**) occupies a region equivalent to that of the urea group of quizartinib (**41**). However, quizartinib (**41**) interacts with the allosteric site near hydrophobic region II through the isoxazole ring. Therefore, LASSBio-2166 (**31**) could be further optimized to exploit additional interactions and improve its potency.

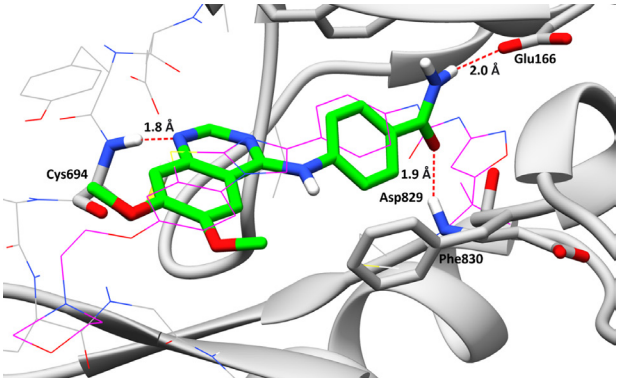


Figure 10. Molecular docking pose of LASSBio-2166 (**31**) (green-colored carbon atoms) and quizartinib (**41**) (magenta-colored carbon atoms) on FLT3. Chemscore for **31** was 32.3, while the Chemscore for **41** was 50.1.

5. Conclusions

Chemical libraries are of growing importance in medicinal chemistry. The methods used in this study allowed for an evaluation of the diversity of LCL, which can be valuable for other groups studying their in-house libraries or *in silico* collections of compounds designed for screening studies. Furthermore, the results of the virtual screening conducted against FLT3 contribute to the development of kinase inhibitors. Since quinazolines are well-known kinase inhibitors, the hit LASSBio-2166 (**31**) can be optimized and used as a starting point for developing FLT3 inhibitors.

Supplementary Information

Supplementary information is available free of charge at http://jbcs.sbq.org.br as PDF file.

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